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# [1,2]OXAZOLE DERIVATIVES: SYNTHESIS AND BIOLOGICAL EVALUATION AGAINST MULTIPLE MALIGNANT CELL TYPES

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**Summary**

**Abbreviations**

| | |
|---|---|
| *ABC* | Activated B-cell |
| *ADCs* | Antibody-drug conjugate |
| *ALCLs* | Anaplastic large-cell lymphoma |
| *ATC* | Anaplastic thyroid cancer |
| *auto-HSCT* | Autologous stem-cell transplantation |
| *BET* | Bromodomain and Extra-terminal domain |
| *CA-4* | Combretastatin A-4 |
| *CI* | Combination index |
| *DIPEA* | *N*-ethyldiisopropylamine |
| *DLBCL* | Diffuse large B-cell lymphoma |
| *DMADMA* | *N*,*N*-dimethylacetamide dimethylacetal |
| *DMF* | *N*,*N*-dimethylformamide |
| *DMFDEA* | *N*,*N*-Dimethylformamide diethylacetal |
| *DMFDMA* | *N*,*N*-dimethylformamide dimethyl acetal |
| *DMPM* | Diffuse malignant peritoneal mesothelioma |
| *DMSO* | Dimethyl sulfoxide |
| *FACS* | Fluorescent-activated cell sorter |
| *FITC* | Fluorescein isothiocyanate conjugated |
| *GCB* | Germinal center B-cell |
| *GCPs or TUBGPC* | ɣ-tubulin complex components |
| *GSK-3β* | Serine-threonine kinase glycogen synthase kinase-3β |
| *γ-TuRCs* | ɣ-tubulin ring complexes |
| *HL* | Hodgkin’s lymphomas |
| *LRRK2* | Leucine-rich repeat kinase 2 |
| *MAPs* | Microtubule-associated proteins |

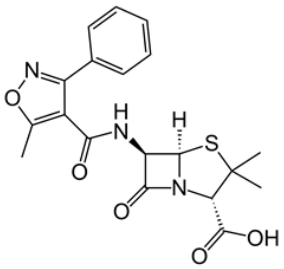
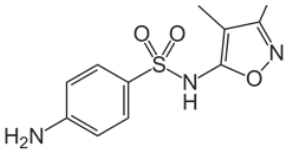
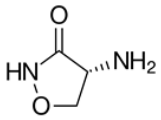
| | |
|---|---|
| *MCL* | Mantle cell lymphoma |
| *MD* | Molecular dynamics |
| *MDR* | Multi-drug resistance |
| *MG_MID* | Mean graph midpoint |
| *MMAE* | Monomethyl auristatin E |
| *moAbs* | Monoclonal antibody |
| *MsCl* | Methanesulfonyl chloride |
| *MTs* | Microtubules |
| *MZL* | Marginal zone lymphoma |
| *NHL* | Non-Hodgkin's lymphomas |
| *PARP* | Poly(ADP-ribose) polymerase |
| *PDGFR* | Platelet-derivative growth factor receptor |
| *PI* | Propidium iodide |
| *PS* | Phosphatidylserine |
| *RTKs* | Receptor tyrosine kinases |
| *SARs* | Structure-activity relationships |
| *SMZL* | Splenic marginal zone lymphoma |
| *TBAI* | Tetrabutylammonium iodide |
| *TBDMAM* | *Tert*-butoxy bis(dimethylamino)methane |
| *TFAA* | Trifluoroacetic anhydride |
| *THF* | Tetrahydrofuran |
| *TosH* | *p*-Toluenesulfonic acid |
| *TosMIC* | Toluenesulfonylmethyl isocyanide |
| *TTL* | Tubulin tyrosine ligase |
| *VEGFR* | Vascular endothelial growth receptor |

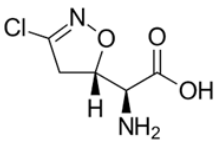
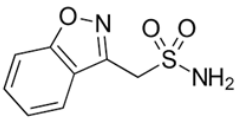
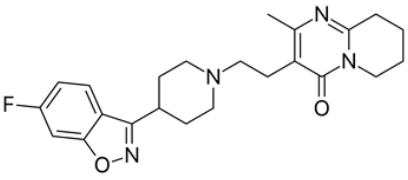
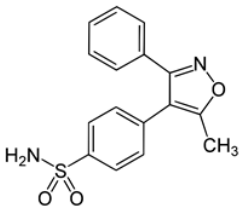
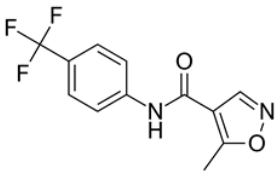
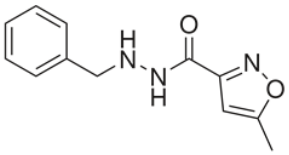
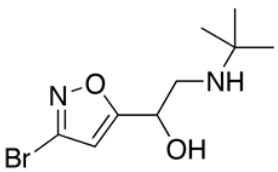
# 1 Introduction

Nitrogen heterocycles represent the *core* structure of many drug candidates with a broad spectrum of pharmaceutical applications and high therapeutic potency. Isoxazoles or [1,2]oxazoles are five-membered heterocycles containing one oxygen and one nitrogen atom. The chemistry of isoxazoles attracts considerable attention in medicinal chemistry because of the broad potential applications as analgesic, anticancer, antimicrobial, antiviral, anticonvulsant, antidepressant, antituberculosis and immunosuppressant drugs. Several marketed drugs contain the isoxazole core and belong to diverse pharmacological classes with diverse therapeutic activities as listed in Table 1.

The chemistry of isoxazoles dates back to 1888, when Claisen recognized the cyclic structure of 3-methyl-5-phenylisoxazole as product from the reaction of hydroxylamine and benzoylacetone [1]. Another relevant contribution was given by Quilico in 1946 who described the formation of isoxazoles from N-oxides and acetylenic compounds [2]. Since then, various substituted isoxazoles have been prepared using different synthetic approaches.

**Table 1 – Marketed drugs containing the [1,2]oxazole moiety**

| Drug | Structure | Pharmacological class |
|---|---|---|
| Oxacillin |  | Antibacterial (Lainson et al. 2017) |
| Sulfisoxazole |  | Antibacterial (Gene et al. 2008) |
| Cycloserine |  | Antitubercolar, Antileprotic (Desjardins et al. 2016) |

| | | |
|---|---|---|
| Acivicin |  | Antitumor, Antileishmania (Conti et al. 2003) |
| Zonisamide |  | Anticonvulsant (Buoli et al. 2017), Antiobesity (Shin et al. 2014) |
| Risperidone |  | Antipsycotic (Schoretsanitis et al. 2017) |
| Valdecoxib |  | COX-2 inhibitor (Bartzatt et al. 2014) |
| Leflunomide |  | Antirheumatic (Golicki et al. 2012) |
| Isocarboxazid |  | Antidepressant (Shader et al. 2012) |
| Broxaterol |  | Bronchodilatory agent (Giustina et al. 1995) |

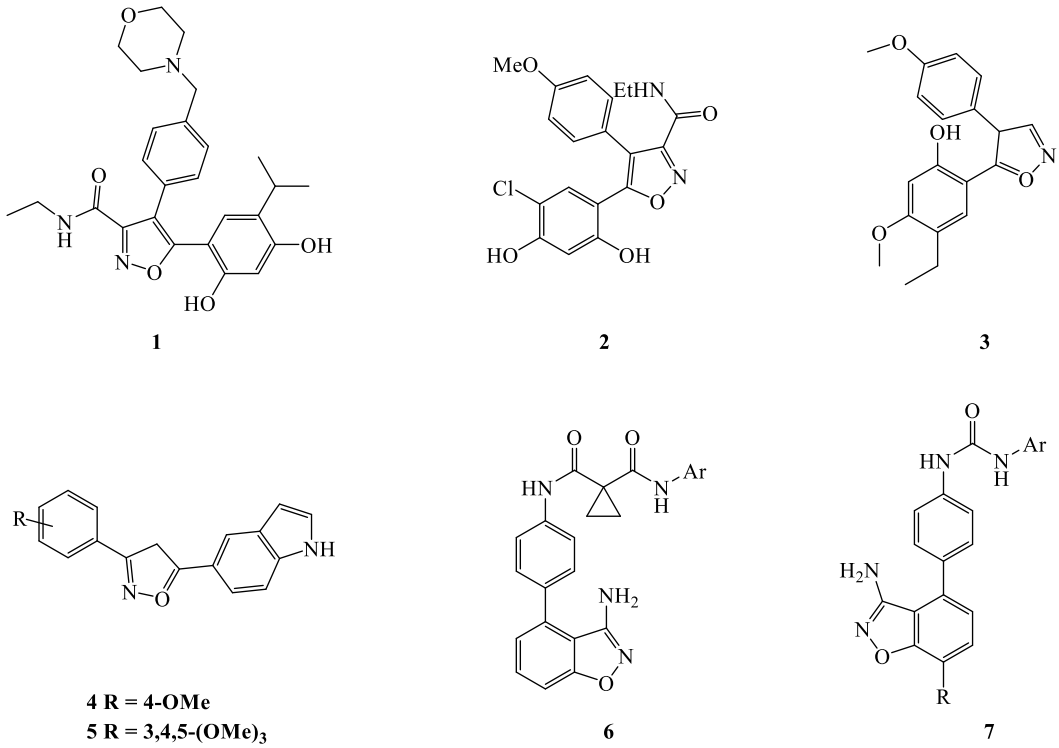
Thanks to its relatively easy synthesis, the isoxazole ring is a popular scaffold for the development of new drugs and is a common feature in numerous anticancer agents. Widely used isoxazole ring modifications include the connection with other aromatic, heteroaromatic or non-aromatic rings and the bound to different alkyl groups. The diversity of isoxazole derivatives structures is associated with a variety of mechanism of actions responsible of tumor suppression. Main targets are: heat shock proteins, caspases, kinases and microtubules (Figure 1).

Resorcinyl isoxazole amide **1** (Figure 1) is a heat shock protein 90 (Hsp90) inhibitor targeting the $NH_2$-terminal nucleotide-binding site of human Hsp90 with a Kd of 1.7 ± 0.5 nmol/L. Since Hsp90 is responsible of the correct conformation, activation and folding of 'client' proteins, many of which implicated in tumor progression, its inhibition has an interesting clinical potential. Compound **1** potently inhibited proliferation in a wide variety of human tumor xenografts models with $GI_{50}$ values ranging from 2.3 to 49.6 nM, inducing G1-G2 arrest and apoptosis. Thanks to these data, it has entered clinical trial [3]. Other isoxazoles Hsp90 inhibitors have been described by Sharp et al. Compared to the pyrazole analogue, the diaryl resorcinylic isoxazole derivative **2** exhibited stronger binding affinity, improved cellular uptake and antiproliferative potency nine times higher ($GI_{50}$ 78±15 nM vs 685±119 nM), demonstrating the higher potency of isoxazole moiety in causing cell death [4]. High expression of the Hsp27 is also observed in different cancers, generally associated with increased metastatic effect. In order to inhibit tumor cell migration, Shin et al. synthesized isoxazole derivatives among which compound **3** (Figure 1) proved to bind Hsp27, blocking migration and invasion of human breast cancer cell line MDA-MB-231 ($IC_{50}$ = 0.15 μM) [5]. The development of new indole-containing diarylisoxazoles, accomplished by Tohid et al., led to the identification of pro-apoptotic antitumor agents targeting caspases. Strong activity was observed for compounds **4** and **5** (Figure 1) with $IC_{50}$ values in the low micromolar range against human colon cancer cell line (Colo320) and lung cancer cell line (Calu-3). Evaluation of the mechanism of action confirmed their capability to increase expression of apoptotic effectors caspase-3 and caspase-7 by two to four times compared to control cells [6].

Several isoxazole derivatives have also been reported as kinase inhibitors. 3-Amino-benzo[d]isoxazoles of type **6** were synthesized and evaluated as c-Met inhibitors. Many of them exhibited $IC_{50}$ lower than 10 nM both at enzymatic and cellular levels [7]. The activity of 3-amino-benzo[d]isoxazoles as inhibitors of receptor tyrosine kinases (RTKs) was also investigated. Extensive SAR studies of benzoisoxazoles demonstrated that *N,N'*-diphenyl urea derivatives of type **7** potently inhibit both vascular endothelial growth receptor (VEGFR) and

platelet-derivative growth factor receptor (PDGFR) families of RTKs. Several benzoisoxazoles with promising in vitro enzymatic inhibitory activity were further tested for their pharmacokinetic properties and they displayed high oral bioavailability and excellent in vivo efficacy [8].

**Figure 1 – Isoxazole-containing anticancer drugs**



## 2 Isoxazoles as antitubulin agents

One of the most attractive pharmacological target for the development of new anticancer drugs are microtubules (MTs) because even minor alteration of their dynamics can engage the spindle assembly, inhibiting chromosome segregation during mitosis, arresting cell cycle progression and ultimately leading to apoptotic cell death. In literature, many examples highlighted the isoxazole ring as a valuable pharmacophore for potent antitubulin agents.

Microtubules are highly dynamic, cytoskeletal protein-polymer with a diameter of approximately 25 nm and length vary from 200 nm to 25 μm involved in many fundamental cell functions such as cellular architecture maintenance, motility, intracellular trafficking, cell division and signalling [9]. They are formed by the polymerization of two globular proteins, α- and β-tubulin, with a molecular weight of 50 kDa each [10]. In human cells, eight isotypes of each α- and β-tubulin have been identified and some isoforms are expressed in specific cells and tissues. For example, β-III is prominent in neurons and testicular cells, whilst β-I is widespread in other cells [11]. ɣ-Tubulin, identified by Oakley and colleagues, does not assemble into microtubules and appears to be localized in the centrosomes and spindle pole bodies. In association with ɣ-tubulin complex components (GCPs or TUBGPC), ɣ-tubulin forms ɣ-tubulin ring complexes (ɣ-TuRCs) that promote the growth of microtubule [12].

In analogy with ɣ-tubulin, δ, ε, ζ and η-tubulin are localized in the centrosome and they are involved in the construction of complex centrioles or basal bodies.

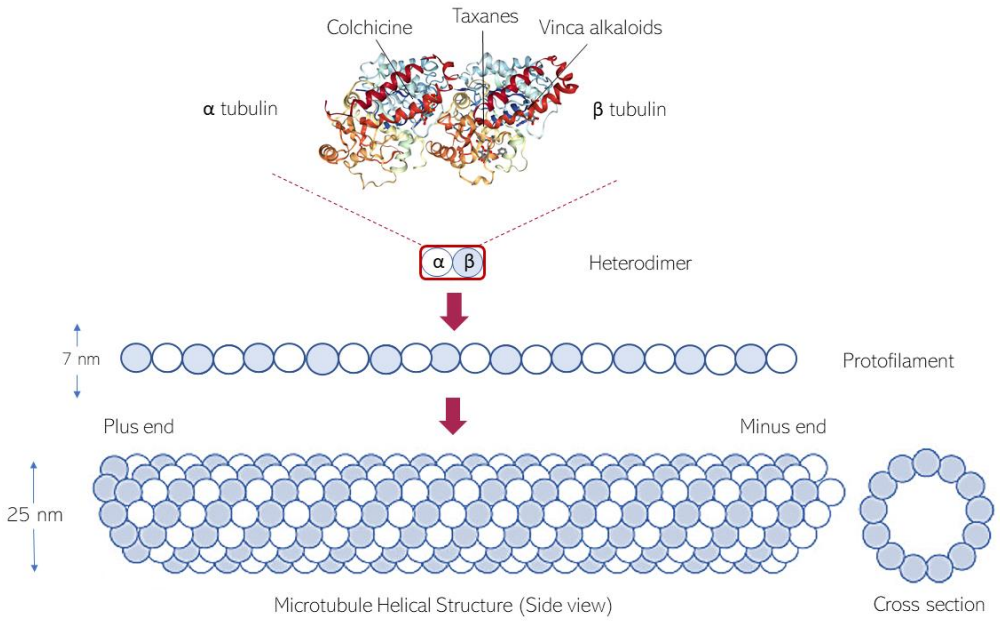
The bond between the N-terminal domain of α-tubulin and the C-terminal domain of β-tubulin creates dimers that assemble to form protofilaments. 11-13 Protofilaments then arrange in parallel into a pipe-like structure, generating a microtubule (Figure 2).

The microtubule end in which α-tubulin is exposed has a negative charge; the other side, exposing β-tubulin, is positively charged. By attaching new heterodimers, both ends are involved in alternative cycling of lengthening and shortening (polymerization and depolymerization), maintaining the microtubule in a state of “dynamic instability”; however, the plus end is kinetically more dynamic and grows more rapidly than the minus one [13]. Both α- and β-tubulin bind GTP in their N-terminal region but only the β-tubulin-bound GTP is accessible and provides the energy needed for polymerization. The GTP linked to α-tubulin is hidden in the interior of the heterodimer and is not hydrolysed in GDP and phosphate during the process. The microtubule growth rate is comprised between 5 and 20 μm/min.

Another dynamic behavior, called "treadmilling", is the addition of monomers at one microtubule end and the removal at the opposite end. Cytoskeletal interactions and kinetics of these processes are regulated by microtubule-associated proteins (MAPs) such as MAP-1A,

MAP-1B, MAP-1C, MAP2, MAP4 and τ proteins. Mutations of MAPs and altered expression of tubulin isotypes are implicated in drug resistance.

**Figure 2 - Microtubule structure**

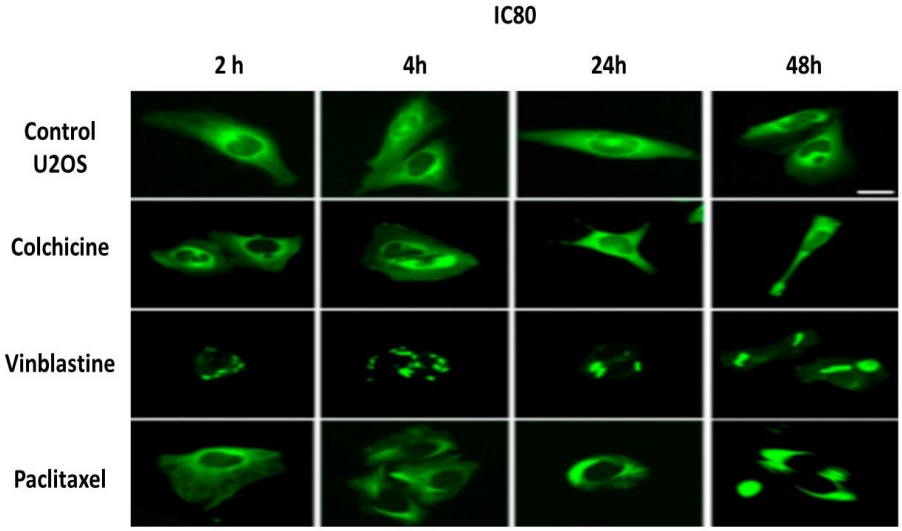



Drugs which interfere with the mitotic apparatus by targeting microtubules are called antitubulin agents.

Based on the interference with dynamic equilibrium of polymerization or depolymerization of microtubules, antitubulin agents are classified in destabilizing or stabilizing agents. Furthermore, tubulin inhibitors can be distinguished depending on their binding domain. Destabilizing agents (such as vincristine, vinblastine, colchicine and combretastatins) target the vinca binding domain or colchicine binding domain whereas stabilizing agents (such as paclitaxel, docetaxel and epothilones) the taxol binding domain. The vinca binding domain is situated in the inter-dimer interface between two longitudinally aligned heterodimers; the colchicine binding site is located in the intra-dimer longitudinal interface of the same heterodimer; taxane binding domain is in the luminal area of the β-tubulin subunit.

Even though their effects on microtubule dynamic are different, both destabilizing and stabilizing agents interfere with the mitotic spindle apparatus, blocking cells in mitosis (M-phase) (Figure 3). As consequence, tumor cells are unable to proceed in anaphase and direct to apoptosis.

**Figure 3 - Antitubulin agents affect the mitotic spindle**




Since the discover of combretastatin A-4 (CA-4) as potent antitubulin agent, medicinal chemists have focused on the development of new analogues with enhanced activity. Among them, several derivatives containing the [1,2]oxazole moiety demonstrated higher antitubulin activity towards tumor cells without affecting normal tissues.

Combretastatins are natural stilbenoid phenols structurally related to colchicine, isolated from the African willow tree *Combretum caffrum* by Pettit et al. in 1989 [14]. CA-4 is the most active compound within combretastatins family and one of the most potent inhibitors of colchicine binding site. Its structure consists of two substituted aromatic rings (trimethoxyphenyl ring and tropone ring) linked by an ethylene bridge. The 3,4,5-trimethoxy moiety on ring A and the *p*-methoxy moiety on ring B are required for optimal cytotoxic activity and to mimic the structure of colchicine, hence combretastatins are also known as 'colchicinoids'. The hydroxy group is optional and can be replaced by an amino group or a bioisosteric boronic acid to improve the solubility [15].

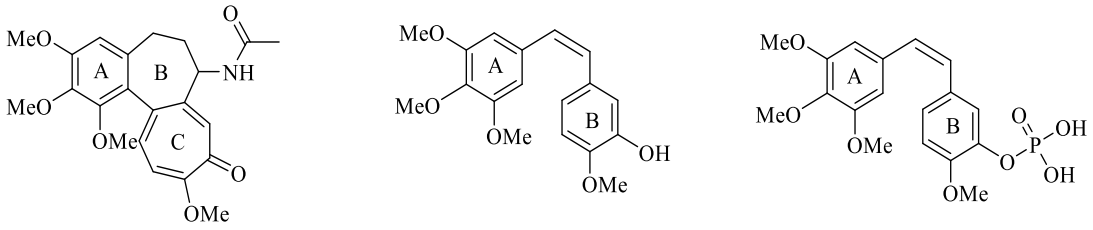
CA-4 exists as two stereoisomers: *cis*-stilbene and *trans*-stilbene but only *cis*-stilbene CA-4 exhibits good activity as anti-tubulin, anti-vascular and anti-angiogenic agent. The *cis*-olefin conformation is a prerequisite for potent cytotoxicity because it maintains the two aromatic rings with an angle of 66°. In addition, CA-4 is not substrate of efflux pumps involved in the multi-drug resistance (MDR) and has superior activity against MDR positive cancer cell lines [16].

At concentration of 1 μM, CA-4 inhibits tubulin polymerization by 35% and block it almost completely at 10 μM, reaching 70% of binding capacity to colchicine domain. A broad spectrum of cancer cell lines (MDA-MB-231, A549, Hela, HL-60, SF295, HCT-8, MDA-

MB435, PC3M, OVCAR-8, NCI-H358M, and lymphocyte cells) proved to be highly sensitive to CA-4 with $IC_{50}$ between 0.53 and 3 μM [17][18].

Despite the encouraging results *in vitro*, the poor water solubility and the short half-life of CA-4 precluded its advanced development. To address these issues, a water-soluble sodium phosphate prodrug (CA-4 phosphate) that is converted to CA-4 by endogenous non-specific phosphatases has been synthesized (Figure 4). Similarly to the parent compound, CA-P induces tubulin depolymerization at nanomolar activity and thanks to its anti-vascular effect, it damages blood supply to tumors causing shrinkage [19]. In 2003, CA-P was approved by FDA and European Medicines Agency as orphan drug for the treatment of anaplastic thyroid cancer (ATC). By 2017 it entered numerous clinical trials as part of combination chemotherapy against resistant ovarian cancer, non-small cell lung cancer and hepatic tumor burden (Clinicaltrials.gov).

**Figure 4 - Colchicine and combretastatins**




Thanks to its simple structure and high activity, CA-4 became the most representative ligand of the colchicine site and an important lead compound in the design of new antitubulin agents. An increasing number of CA-4 derivatives as potent tubulin polymerization inhibitors have been reported in the past few years. Promising compounds were obtained introducing heterocyclic bridges in place of the double bond, such as indole, indazole, triazole, imidazole, pyrazoline, furazan, isoxazole, cyclopentanone, maleimide and thiadiazole. Among them, the five-membered rings and in particular isoxazoles derivatives showed higher cytotoxicity and affinity to tubulin compared to CA-4 (Figure 5).

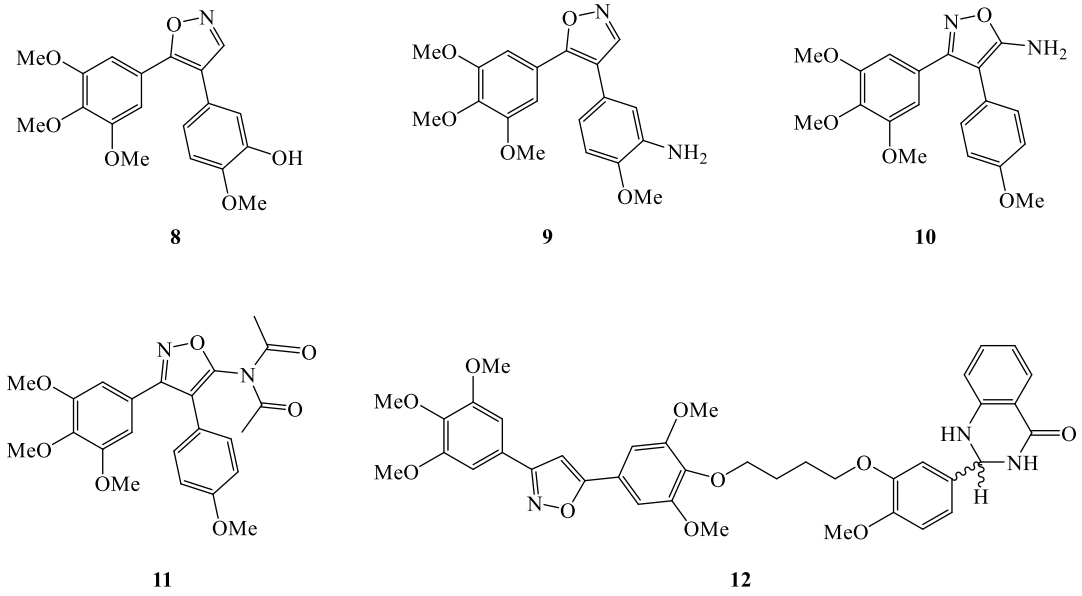
The isoxazole ring is suitable to replace the ethylene bridge because it maintains the two aromatic rings in the active *cis* conformation, preventing *cis-trans* isomerization. Kaffy et al. reported the synthesis of CA-4 analogues with five-membered heterocycles (isoxazole, isooxazoline, oxadiazole) as linker of rings A and B. All compounds were evaluated for their ability to inhibit tubulin assembly and the 4,5-diarylisoxazole **8** exhibited the best activity as depolymerizing agent ($IC_{50}$ = 0.75 μM), demonstrating almost twice more potent than CA-4

($IC_{50}$ = 1.2 μM) [20]. Better cytotoxic profile was obtained by replacing the hydroxy group of 4,5-diarylisoxazole with an amino group (**9**), reaching $IC_{50}$ values as low as 0.022 and 0.065 μM in HeLa and HepG2 cell lines, respectively [21].

Furthermore, the activity of 3,4-diaryl-5-aminoisoxazoles against five different human tumor cell lines (myeloid leukemia cells, esophageal carcinoma cells, non-small lung cancer cells, hepatocellular carcinoma cells and prostate cancer cells) was investigated. The 5-aminoisoxazole **10** bearing a trimethoxyphenyl group on ring A and 4-methoxyphenyl on ring B showed high cytotoxicity with $IC_{50}$ values 0.04-12 μM. Acetylation of 5-aminoisoxazoles gave derivative **11** with similar cytotoxic profile to CA-4. Both compounds **10** and **11** were able to induce disruption of microtubules ($IC_{50}$ values 1.8 and 2.1 μM) [22].

To improve the synergy between CA-4 structure and isoxazole moiety, Kamal et al. developed new diaryl substituted isoxazole derivatives. 3,5-Diarylisoxazolines/isoxazoles were linked to 2,3-dihydroquinazolinones using different alkane spacers, since quinazolinone-based anticancer agents are associated with the inhibition of tubulin polymerization [23]. All the hybrid compounds displayed anticancer activity with $IC_{50}$ ranging from <0.1 to 2.15 μM but the most active compound was the isoxazole derivative **12** with $GI_{50}$ less than 0.1 μM and the ability to induce microtubule disruption as well as fragmentation of nuclei at concentration similar to that of CA-4. Furthermore, **12** caused inhibition of B1 and CDK1 and increased level of cleaved PARP (Poly(ADP-ribose) polymerase) [24].

**Figure 5 – Isoxazole based combretastatin A-4 analogues**

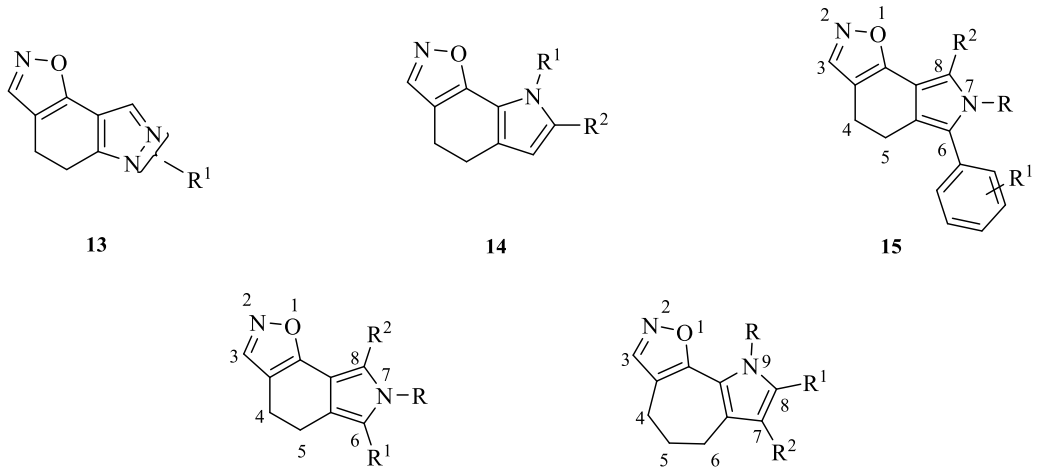


The combination of isoxazoles with other heterocycles is a common approach to design new drug-like molecules, achieving better pharmacological profiles, efficacy and toxicity profile.

Through the years, many efforts have been devoted by our research group to the synthesis of new polycondensed heterocyclic systems incorporating the isoxazole ring as chemotherapeutics agents. Tricyclic compounds bearing the [1,2]oxazole ring fused with an indazole or an indole scaffold, namely [1,2]oxazole[5,4-*e*]indazoles **13** and [1,2]oxazole[4,5-*g*]indoles **14**, have been described as promising antitumor agents, highlighting the isoxazole moiety as valuable pharmacophore. In particular, two derivatives of the latter series emerged for their *in vitro* nanomolar growth inhibitory effect across the National Cancer Institute (NCI; Bethesda) 60 cancer cell line panel with mean graph mid-points (MG_MID) of 0.25 and 0.47 μM [25] [26].

In the attempt to explore further chemical modifications of the tricyclic [1,2]oxazole nucleus, [1,2]oxazolo[5,4-*e*]isoindoles **15** and **16** were synthesized as positional isomers of compounds **14** and they emerged as promising inhibitors of tubulin polymerization (Figure 6) [27] [28].

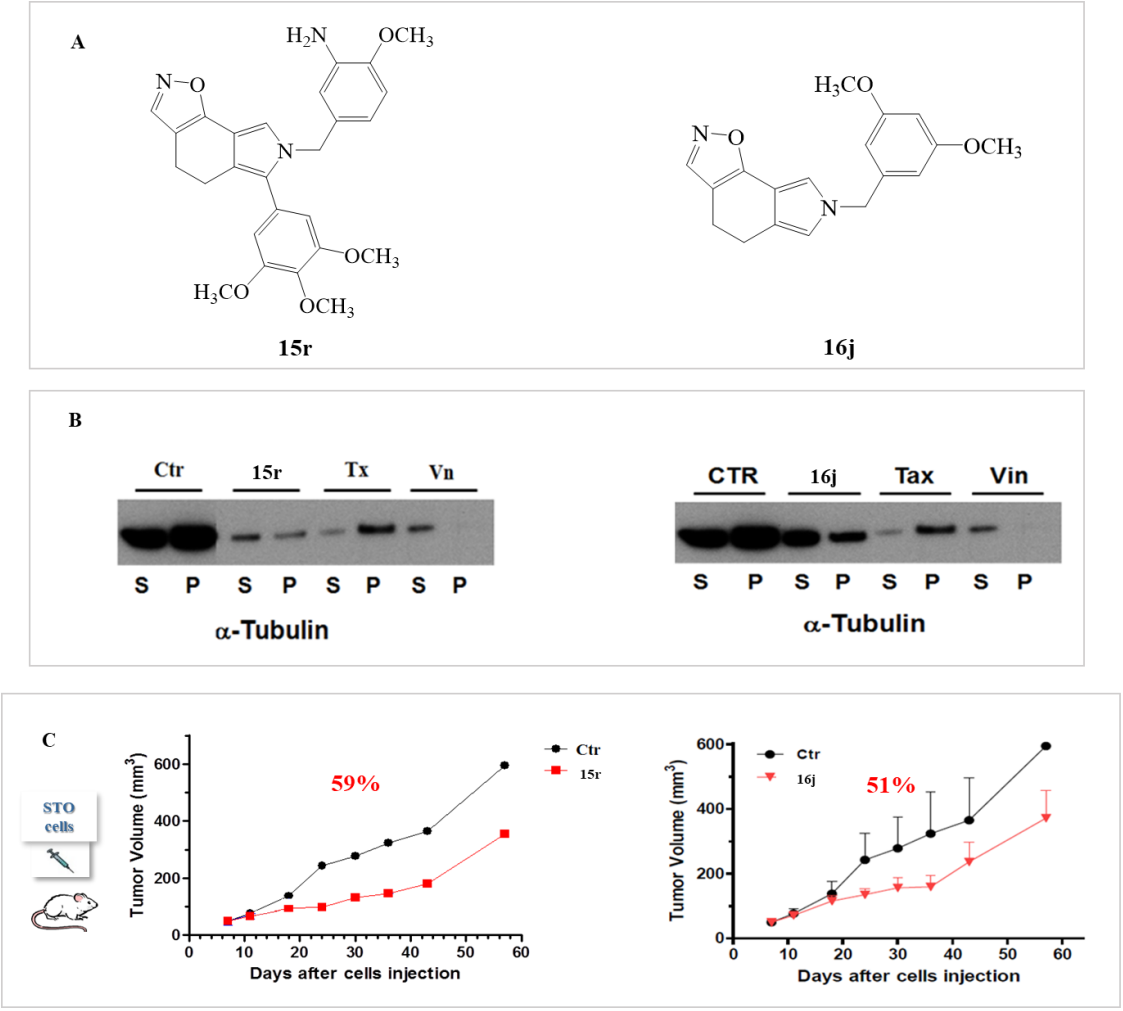
**Figure 6 – Tricyclic [1,2]oxazole derivatives**




Almost all new compounds screened in the NCI panel showed antiproliferative activity against tested cell lines with $GI_{50}$ values in the micromolar - nanomolar range. In particular, derivative **15r** (Figure 7), bearing the 3,4,5-trimethoxyphenyl group and the 3-amino 4-methoxybenzyl substitution at the pyrrole nitrogen, reached nanomolar inhibitory activity in 46 out of 56 cell lines. Since other derivatives of the same class bearing the 4-methoxybenzyl group exhibited remarkable antiproliferative activity, this substitution is to be considered crucial for appreciable cell growth inhibition.

In order to extend the study to additional histotypes, the *in vitro* cytotoxicity of synthesized compounds was tested in three DMPM (diffuse malignant peritoneal mesothelioma) cell lines, STO, MP4 and MP8, which were established from surgical specimens of patients hospitalized at the National Cancer Institute of Milan. DMPM is an aggressive and rapidly lethal tumor, highly chemo-resistant and frequently caused by asbestos. After 72 h exposure to increasing concentrations (0.01 – 100 μM) of each compound, **15r** markedly impaired DMPM cell growth in a dose- and time-dependent manner. The highest sensitivity was displayed by STO cells whose growth was inhibited by 50% at concentration of 0.07±0.02 μM whilst MP4 and MP8 were slightly less responsive with $IC_{50}$s of 0.16±0.05 μM and 0.13±0.04 μM, respectively. Interestingly, compound **16j**, bearing a 3,5-dimethoxybenzyl group at the pyrrole nitrogen and not selected by NCI for further screening at five concentration levels ($10^{-4}$ – $10^{-8}$ M), proved to be one of the most promising compounds at the National Cancer Institute of Milan, reducing the growth of DMPM STO and MP8 cell lines with $IC_{50}$ of 0.06±0.01 μM and 0.07±0.02 μM, respectively (Figure 7). Neither **15r** nor **16j** affected the growth of normal human lung fibroblast (WI38) and adult human breast (MCF10A) cell lines ($IC_{50}$ > 100 μM), demonstrating a preferential activity against cancer cells.

**Figure 7 – A) [1,2]oxazolo[5,4-*e*]isoindoles 15r and 16j structures. B) Effect of 15r and 16j on tubulin polymerization. C) Antitumor activity of 15r and 16j on STO cells xenotransplanted on athymic nude mice.**




As described above, many compounds structurally related to [1,2]oxazolo[5,4-*e*]isoindoles proved to inhibits tubulin polymerization. Thus, the ability of **15r** and **16j** to interfere with microtubule assembly in both STO and MP8 cells was investigated. After 24 h exposure to each compound at concentrations corresponding to the $IC_{50}$ at 72 h, western blot results showed that both **15r** and **16j** markedly diminished the polymerized compared to free soluble fraction of tubulin in a vinca-alkaloid like manner, opposite to that of taxol. Specifically, the cell cycle distribution analysis, assessed by flow cytometry, revealed the accumulation of cells in the G2/M phase and the net reduction of cells in G1 phase. The depletion of tubulin polymerization promoted cell cycle arrest and apoptosis.

Furthermore, the *in vivo* antitumor activity of **15r** and **16j** was evaluated in STO cells, following subcutaneous xenotransplantation on athymic nude mice. The intraperitoneal administration of the compounds, starting 10 days after cell *inoculum*, resulted in a statistical significant tumor growth delay of 59% and 51% respectively, compared to control mice.

Later on, we evaluated the effect of the enlargement of the cycloalkyl ring on the biological activity of the tricyclic derivatives pyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazoles **17** (Figure 6). The synthetic strategy is outlined in Scheme 1 and is reported in our reference [29]. We started from cyclohepta[b]pyrrol-8-one ketones as the α position to the carbonyl is appropriate for the introduction of the second electrophilic site, essential for the subsequent cyclization with dinucleophiles. Enaminoketones were obtained by reaction of ketones with *N*,*N*-dimethylformamide dimethyl acetal (DMFDMA) or tert-butoxy bis(dimethylamino)methane (TBDMAM). Alternatively, we explored a formylation step using ethyl formate, leading to the corresponding hydroxymethyl derivatives. Anellation of the [1,2]oxazole ring was achieved by reaction with hydroxylamine hydrochloride, as a 1,3-dinucleophile.

**Scheme 1 – Reagents and conditions for the synthesis of pyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazoles 17**

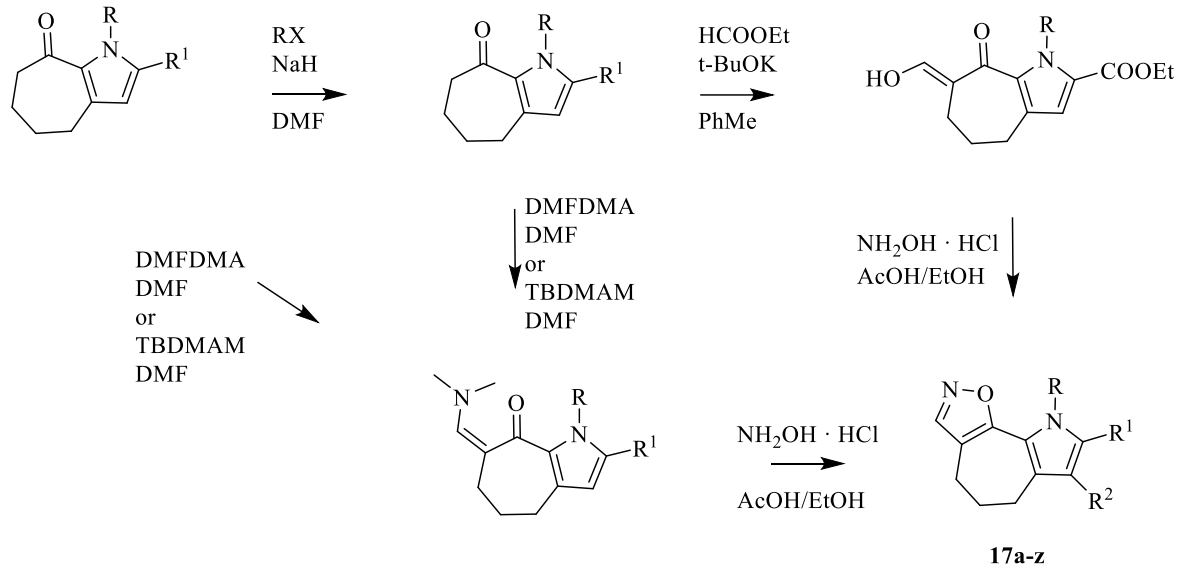



**Table 2 - Pyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazoles 17a-z**

| [1,2-oxazole] | R | $R^1$ | $R^2$ |
|---|---|---|---|
| **17a** | $SO_2Ph$ | H | H |
| **17b** | Me | H | H |
| **17c** | Bn | H | H |
| **17d** | 2-OMeBn | H | H |
| **17e** | 3-OMeBn | H | H |
| **17f** | 4-OMeBn | H | H |
| **17g** | 2,5-$(OMe)_2$Bn | H | H |
| **17h** | 3,5-$(OMe)_2$Bn | H | H |

| | | | |
|---|---|---|---|
| **17i** | 3,4,5-$(OMe)_3$Bn | H | H |
| **17j** | Me | COOEt | H |
| **17k** | Bn | COOEt | H |
| **17l** | 2-OMeBn | COOEt | H |
| **17m** | 3-OMeBn | COOEt | H |
| **17n** | 4-OMeBn | COOEt | H |
| **17o** | 2,5-$(OMe)_2$Bn | COOEt | H |
| **17p** | 3,5-$(OMe)_2$Bn | COOEt | H |
| **17q** | 3,4,5-$(OMe)_3$Bn | COOEt | H |
| **17r** | Bn | COOMe | H |
| **17s** | Me | COOEt | Cl |
| **17t** | Bn | COOEt | Cl |
| **17u** | 2-OMeBn | COOEt | Cl |
| **17v** | 3-OMeBn | COOEt | Cl |
| **17w** | 4-OMeBn | COOEt | Cl |
| **17x** | 2,5-$(OMe)_2$Bn | COOEt | Cl |
| **17y** | 3,5-$(OMe)_2$Bn | COOEt | Cl |
| **17z** | Me | Cl | H |

All the synthesized compounds **17a-y** (Table 2) were tested for their anti-tumor activity on the full NCI-60 panel and six compounds (**17l, 17m, 17p, 17q, 17t, 17y**) were selected for further screening on the same panel at five concentrations at 10-fold dilutions ($10^{-4}$-$10^{-8}$ M).

Among them, several compounds exhibited growth inhibitory effect against the entire NCI cancer cell panel with $GI_{50}$ reaching the micromolar-nanomolar level.

**Table 3 - Overview of the results of the NCI *in vitro* human tumor cell line screening for derivatives 17l, 17m, 17p, 17q, 17t, 17y**

| [1,2-oxazole] | N[b] | N[c] | $GI_{50}$[a] | MG_MID[d] |
|---|---|---|---|---|
| **17l** | 56 | 50 | 0.30 – 46.2 | 4.47 |
| **17m** | 56 | 56 | 0.15 – 18.7 | 1.45 |
| **17p** | 56 | 55 | 0.01 – 13.4 | 0.08 |
| **17q** | 55 | 55 | 0.01 – 64.9 | 0.20 |
| **17t** | 55 | 46 | 1.29 – 5.89 | 7.08 |
| **17y** | 57 | 57 | 0.03 – 27.0 | 0.41 |

[a]$GI_{50}$ = concentration that inhibits 50% net cell growth (μM)

[b]Number of cell lines investigated.

[c]Number of cell lines giving positive $GI_{50}$ values.

[d]MG_MID = mean graph midpoint (μM); the arithmetic mean value for all tested cancer cell lines. If the indicated effect was not attainable under the concentration range used, the highest tested concentration was used for the calculation.

The most potent compound was **17p**, which has a 3,5-dimethoxybenzyl substitutent at the pyrrole nitrogen, showed a mean graph mid-point (MG_MID) of 0.08 μM (Table 3) and particular efficacy against the melanoma ($GI_{50}$ 0.09 – 0.01 μM), prostate ($GI_{50}$ 0.04 μM) and renal ($GI_{50}$ 0.07 – 0.02 μM) cancer subpanels, maintaining nanomolar activity against all the tested cell lines. The second best in potency was compound **17q**, a 3,4,5-trimethoxybenzyl substituted derivative, which demonstrated high selectivity against the leukemia ($GI_{50}$ 0.39 – 0.04 μM), colon cancer ($GI_{50}$ 0.30 – 0.04 μM), CNS cancer ($GI_{50}$ 0.56 – 0.07 μM), melanoma ($GI_{50}$ 0.41 – 0.02 μM), ovarian cancer ($GI_{50}$ 0.72 – 0.03 μM) and breast cancer ($GI_{50}$ 0.74 – 0.04 μM) subpanels, with $GI_{50}$ values at submicromolar-nanomolar levels (Table 4).

**Table 4 - In vitro $GI_{50}$ ($\mu$M) values of compounds 17l, 17m, 17p, 17q, 17t and 17y in individual tumor cell lines**

| Cell lines | 17l | 17m | 17p | 17q | 17t | 17y | Cell lines | 17l | 17m | 17p | 17q | 17t | 17y |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Leukemia** | | | | | | | M14 | 3.67 | 0.54 | **0.05** | 0.15 | 3.78 | 0.26 |
| CCRF-CEM | 2.87 | 2.17 | **0.05** | 0.30 | 2.21 | 0.25 | MDA-MB-435 | 0.35 | 0.15 | **0.01** | 0.02 | 1.40 | 0.03 |
| HL-60(TB) | 3.04 | 0.53 | **0.03** | 0.19 | 3.32 | 0.31 | SK-MEL-2 | - | 0.56 | **0.04** | - | - | 0.22 |
| K-562 | 1.59 | 0.39 | **0.04** | 0.13 | 3.68 | 0.29 | SK-MEL-28 | >100 | 5.12 | **0.09** | 0.30 | - | 0.66 |
| MOLT-4 | 5.68 | 2.30 | **0.26** | 0.39 | 3.58 | 0.58 | SK-MEL-5 | 2.61 | 0.75 | **0.04** | 0.17 | 3.53 | 0.49 |
| RPMI-8226 | 4.11 | 1.89 | **0.05** | 0.38 | 3.27 | 0.39 | UACC-257 | - | 7.61 | - | - | - | - |
| SR | 0.43 | 0.28 | **0.03** | 0.04 | 1.80 | 0.07 | UACC-62 | 3.37 | 0.51 | **0.07** | 0.05 | 4.01 | 0.11 |
| **Non-Small Cell Lung Cancer** | | | | | | | **Ovarian Cancer** | | | | | | |
| A549/ATCC | - | 0.76 | **0.06** | - | - | - | IGROV1 | 1.99 | 1.16 | **0.06** | 0.06 | 4.39 | 0.34 |
| EKVX | - | - | - | - | - | - | OVCAR-3 | 0.87 | 0.30 | **0.02** | 0.03 | 3.13 | 0.19 |
| HOP-62 | 1.89 | 4.34 | - | 0.17 | 4.35 | 0.29 | OVCAR-4 | 9.35 | 18.7 | **13.4** | 0.72 | - | 0.85 |
| HOP-92 | 0.30 | 4.21 | - | - | - | 0.46 | OVCAR-5 | >100 | 5.94 | **0.18** | 0.53 | >100 | - |
| NCI-H226 | >100 | 21.0 | **7.98** | 1.21 | >100 | 9.71 | OVCAR-8 | 3.85 | 3.13 | **0.08** | 0.24 | - | 0.41 |
| NCI-H23 | 8.52 | - | - | 0.37 | - | 0.68 | NCI/ADR-RES | 1.36 | 0.43 | **0.03** | 0.06 | 2.83 | 0.20 |
| NCI-H322M | 5.23 | - | - | 0.32 | >100 | 0.51 | SK-OV-3 | 2.70 | 2.83 | **0.05** | 0.13 | >100 | 0.24 |
| NCI-H460 | 3.88 | 3.53 | **0.04** | 0.23 | 3.62 | 0.35 | **Renal Cancer** | | | | | | |
| NCI-H522 | 0.35 | 0.23 | **0.02** | 0.01 | 1.29 | 0.03 | 786-0 | >100 | 6.74 | **0.05** | 0.97 | - | 4.24 |
| **Colon Cancer** | | | | | | | A498 | 3.46 | 0.44 | **0.02** | 0.11 | 4.38 | 0.17 |
| COLO 205 | 0.87 | 0.58 | **0.03** | 0.04 | 3.53 | 0.17 | ACHN | 16.6 | 3.40 | **0.06** | 0.29 | 5.89 | 0.94 |
| HCC-2998 | >100 | 4.18 | **0.23** | 0.30 | - | - | CAKI-1 | 3.51 | 2.31 | **0.05** | 0.07 | 3.51 | 0.28 |
| HCT-116 | 3.79 | 0.47 | **0.04** | 0.19 | 4.02 | 0.43 | RXF 393 | 2.08 | 0.67 | **0.02** | 0.12 | - | 0.25 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HCT-15 | 2.24 | 0.45 | **0.04** | 0.16 | 3.39 | 0.37 | SN12C | >100 | 3.51 | **0.07** | 0.76 | - | 0.83 |
| HT29 | 0.91 | 0.36 | **0.03** | 0.07 | 2.95 | 0.27 | TK-10 | 67.8 | 11.2 | **>100** | 64.9 | >100 | 10.3 |
| KM12 | 2.10 | 0.46 | **0.03** | 0.05 | 3.70 | 0.32 | UO-31 | 6.53 | 3.37 | **0.05** | 0.08 | - | 0.78 |
| SW-620 | 2.02 | 0.48 | **0.04** | 0.14 | 3.81 | 0.32 | **Prostate Cancer** | | | | | | |
| **CNS cancer** | | | | | | | PC-3 | 2.48 | 2.35 | **0.04** | 0.17 | 3.16 | 0.29 |
| SF-268 | 46.2 | 6.71 | **0.05** | 0.52 | >100 | 1.91 | DU-145 | 5.27 | 2.18 | **0.04** | 0.28 | >100 | 0.35 |
| SF-295 | 1.38 | 0.62 | **0.03** | 0.44 | >100 | 0.06 | **Breast Cancer** | | | | | | |
| SF-539 | 2.48 | 1.14 | **0.03** | 0.12 | - | 0.24 | MCF7 | 0.71 | 0.37 | **0.03** | 0.04 | 3.21 | 0.10 |
| SNB-19 | 64.7 | 8.23 | **0.15** | 0.56 | >100 | 0.52 | MDA-MB-231/ ATCC | 9.12 | 1.84 | **0.24** | 0.48 | 3.51 | 0.84 |
| SNB-75 | 1.97 | 1.60 | **0.03** | 0.07 | - | 0.22 | HS 578T | 3.38 | 1.60 | **0.04** | 0.34 | - | 0.45 |
| U251 | 3.78 | - | - | 0.14 | - | 0.31 | BT-549 | 7.73 | 0.98 | **1.72** | 0.29 | - | 27.0 |
| **Melanoma** | | | | | | | T-47D | 2.46 | 2.00 | - | 0.10 | 2.69 | 0.44 |
| LOX IMVI | 7.64 | 1.75 | **0.05** | 0.41 | 5.26 | 0.88 | MDA-MB-468 | 2.95 | 0.34 | **0.03** | 0.74 | - | 0.73 |
| MALME-3M | 3.08 | 0.89 | - | 0.05 | 3.68 | 0.20 | | | | | | | |

These results provided a structure-based rationale for the drug development of new [1,2]oxazole derivatives.

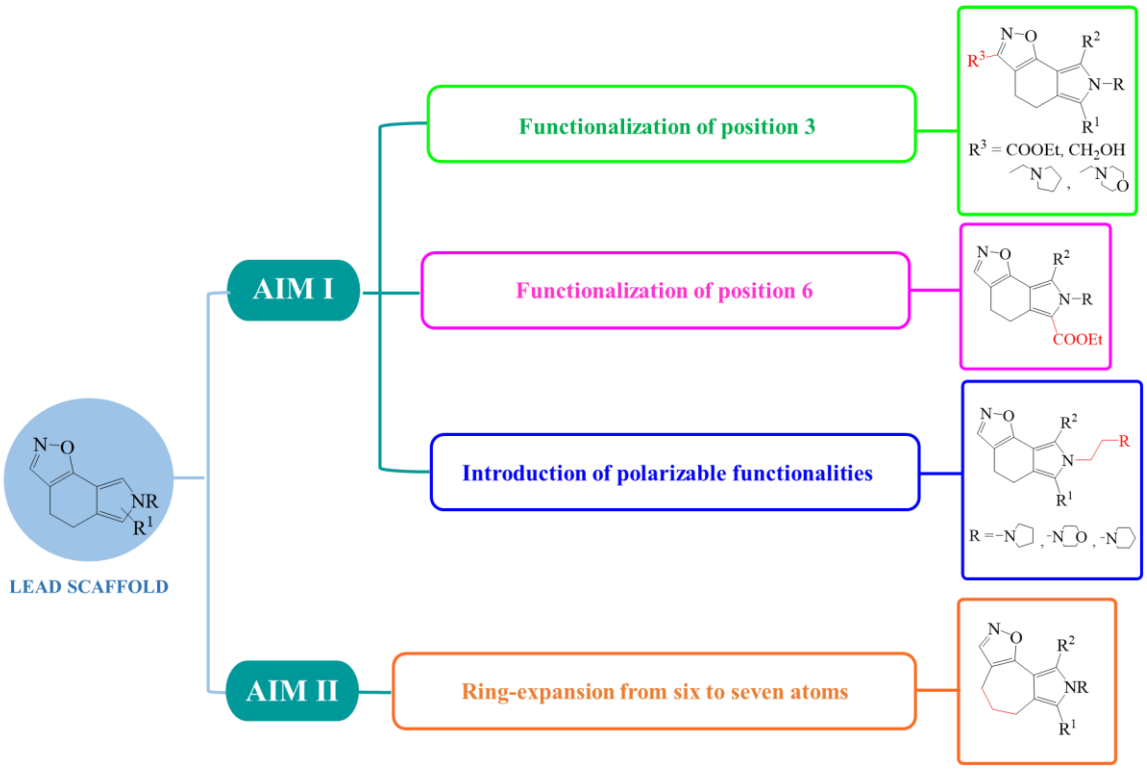
## 3 Aims of the project

[1,2]Oxazolo[5,4-*e*]isoindoles are characterized by a large degree of structural variability and allow the introduction of different functionalizations in the tricyclic core.

Based on the efficacy that isoxazole condensed systems exhibited as antitumor agents and the promising activity of [1,2]oxazolo[5,4-*e*]isoindoles as tubulin polymerization inhibitors, it was planned the *lead* optimization of compounds **15r** and **16j** in order to obtain derivatives with optimal pharmacokinetic profile and drug-like properties. In particular, two main aims were established for my PhD thesis:

- AIM I: synthesis and biological evaluation of new series of [1,2]oxazolo[5,4-*e*]isoindoles;
- AIM II: synthesis and biological evaluation of new scaffolds as analogues of the previous series of [1,2]oxazolo[5,4-*e*]isoindoles;

**Figure 8 – Aims of the project**



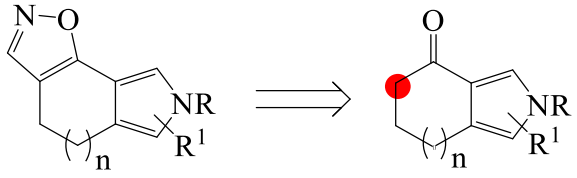

**AIM I**. In order to explore the chemical space around the [1,2]oxazolo[5,4-*e*]isoindole scaffold and to establish structure-activity relationships (SARs), structural modifications were rationalized either in the isoindole moiety and in the [1,2]oxazole ring of lead compounds (Figure 8). These included:

- functionalization of the [1,2]oxazole ring ($R^3$) with biocompatible groups such as ethoxycarbonyl, alcoholic, ethyl-morpholinic or ethyl-pyrrolidinic group. The ethoxycarbonyl functionality is well tolerated in biological environments and take part to many biological reactions. It can be easily converted into alcohol, from which can also be obtained primary and secondary amines. The approach involves the conversion of alcohol into sulfamate esters and the rearrangement to amines.
- introduction of biologically important substrate ethoxycarbonyl group in position 6 of the tricyclic nucleus;
- attachment of aminoalkyl chains bearing polarizable groups such as morpholine, pyrrolidine and piperidine in the pyrrole nitrogen. These modifications have been designed to improve the pharmacokinetic/pharmacodynamic profile of the molecules. In particular, hydrophilic functionalization can balance the moderate lipophilicity enhancing bioavailability and targeted delivery to specific tissues.

**AIM II**. The ring-expansion of the isoindole moiety from six to seven atoms was explored, leading to the synthesis of a new ring system. The enlargement of the central ring system was designed on the basis of the relevant results shown by pyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazoles **17**, whose effects on tubulin dynamics and efficacy in treating hematological malignancies were further investigated.

Ideal *building blocks* for the synthesis of new isoxazoles were ketones of type **18**, easily functionalizable in α-position to the carbonyl with an enamine or a hydroxymethyl group. The introduction of a second electrophilic centre, together with the carbonyl group, created a versatile intermediate for the anellation of the [1,2]oxazole (Scheme 2).

**Scheme 2 - Retrosynthetic scheme for [1,2]oxazolo[5,4-*e*]isoindoles**

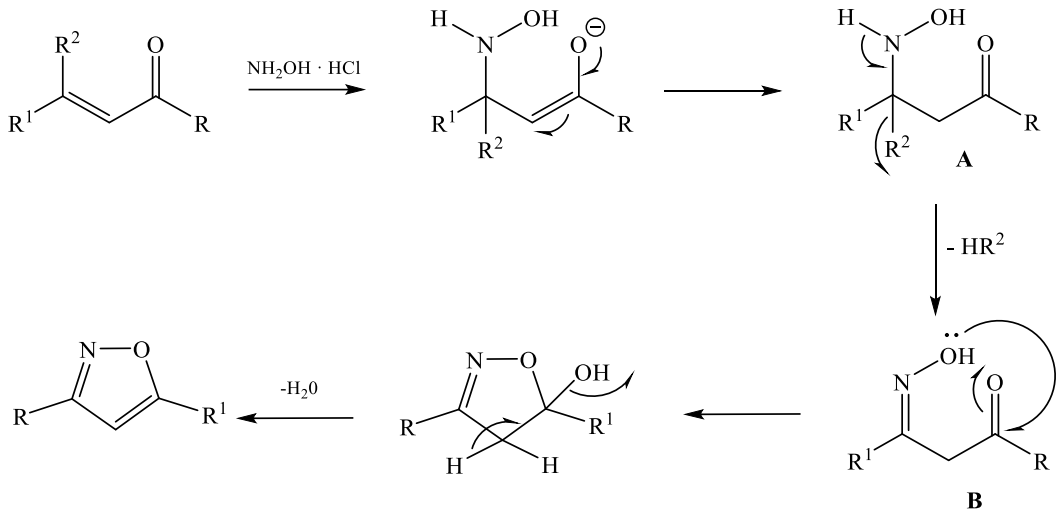



n = 1,2 **18** n = 1,2

The isoxazole ring can be obtained by different type of reactions, among which condensation, 1,3-dipolar cycloaddition and cycloisomerization are the most common. The synthetic approach for our purpose consisted in the reaction of the vinyl ketones, bearing two vicinal electrophilic centres, with hydroxylamine hydrochloride under typical Claisen reaction conditions as presented in Scheme 3. The detailed mechanism consisted of an initial Michael

addition of vinyl ketones and hydroxylamine, giving the intermediate **A**, followed by intramolecular nucleophilic substitution at the vicinal carbonyl group to obtain intermediate **B**. The subsequent intramolecular cyclization and dehydration of intermediate **B** led to the anellation of [1,2]oxazole moiety.

**Scheme 3 - Condensation mechanism for the cyclization of isoxazoles**

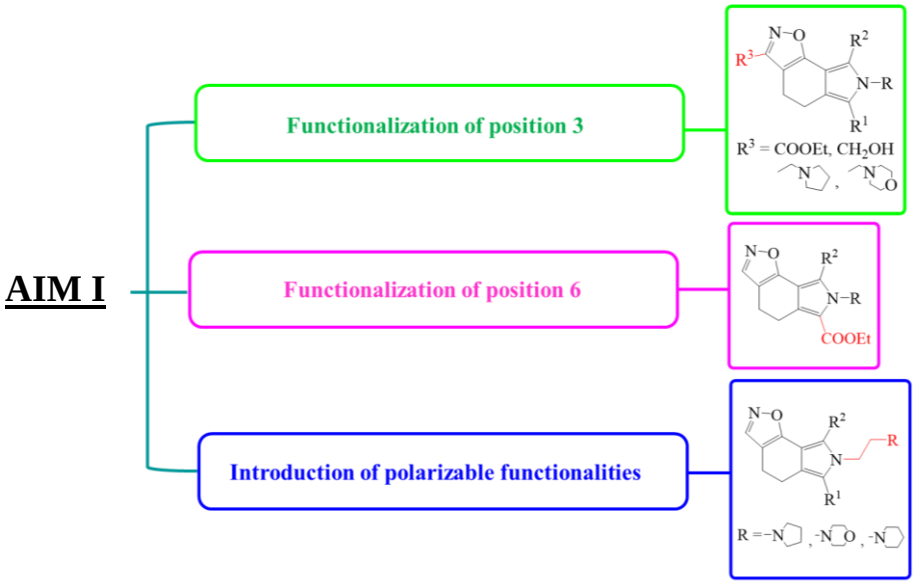



In search of potential therapeutic effects for cancer, the new compounds were evaluated on the 60 human tumor cell lines panel, according to the NCI screening protocol.

Furthermore, considering the wide use of antitubulin agents in the treatment of lymphoma and the absence of lymphoma cell lines in the NCI panel, purpose of the project was to investigate the efficacy of newly synthesized isoxazoles in 4 different subtypes of lymphoma. The biological assays on lymphoma were performed during my 8 months period of research at the Institute of Oncology Research in Bellinzona (Switzerland).

Enzymatic assays were performed at the Friederick National Laboratory for Cancer Research under the supervision of Prof. Ernest Hamel to elucidate the mechanism of action. In addition, X-ray crystallographic studies of tubulin-ligand interactions were conducted by the group of Prof. Michel Steinmetz at the Paul Scherrer Institute.

These data will be submitted for publications as soon as the entire set of biological testing will be available.

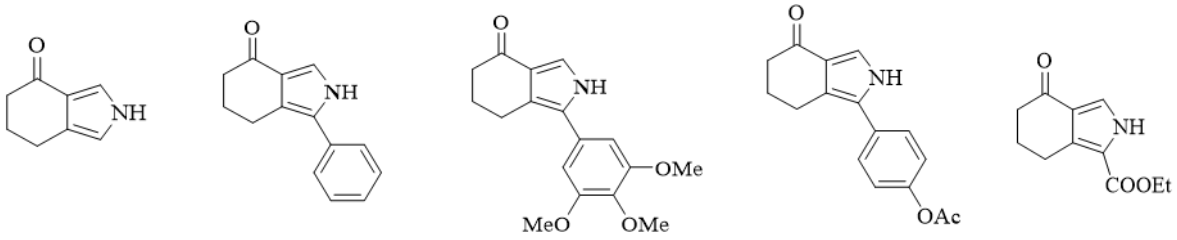



# 4 [1,2]Oxazolo[5,4-*e*]isoindoles

## 4.1 Synthesis of building blocks

The synthesis of tetrahydroisoindole-4-one derivatives (Figure 9), suitable precursor for the new series of [1,2]oxazolo[5,4-*e*]isoindoles, was carried out according to the following synthetic schemes.

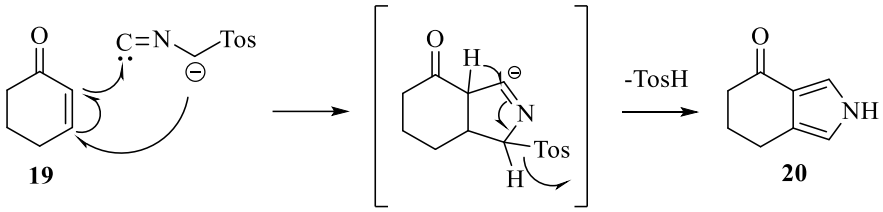
**Figure 9 – Structures of building blocks: tetrahydroisoindole-4-ones**



**20** **25a** **25b** **25d** **28**

In particular, the synthesis of the unsubstituted tetrahydroisoindole-4-one **20** was afforded in only one step and good yield (85%) using 2-cyclohexen-1-one **19**, a good Michael acceptor, and toluenesulfonylmethyl isocyanide (TosMIC), frequently used to obtain heterocyclic systems. Deprotonation of TosMIC at the α-position with potassium *t*-butoxide results in the conjugate anion that reacts with a variety of electrophiles. Reaction between anion of TosMIC, generated *in situ*, and **19** in the presence of *t*-BuOK at room temperature generates a polarized intermediate that, after elimination of *p*-toluenesulfonic acid (TosH), undergoes cyclization to form the tetrahydroisoindole-4-one **20** (Scheme 4) [30].

**Scheme 4 – Synthesis of tetrahydroisoindole-4-one 20**




Several other tetrahydroisoindoles were prepared by a multistep synthetic approach, starting from commercially available 1,3-cyclohexanedione **21**, which was converted into the corresponding enamino derivative **22** in excellent yield (96%) by heating under reflux in *N*,*N*-dimethylformamide dimethylacetal (DMFDMA) used as solvent. The subsequent reaction of 2-[(dimetylamino)methylidene]–cyclohexane-1,3-dione **22** with phenylglycine, 3,4,5-trimethoxy phenylglycine, in turn prepared by Streacker reaction from 3,4,5-trimethoxybenzaldehyde [31], or 4-hydroxy phenylglycine in refluxing ethanol gave the corresponding enamino acids **23a-c** (83-95%) (Scheme 5).

Their cyclization process in acetic anhydride and triethylamine led to the expected dihydroisoindoles **24a-c** (70-75%). Isolation of 4-(4-acetoxy-2-acetyl-6,7-dihydro-2H-isoindol-1-yl)phenyl acetate **24c** ($R^1$= 4-Ac) indicates that the cyclization to isoindole occurs together with the acetylation of the 4-hydroxy group. Upon deacetylation of derivatives **24a,b,** in aqueous acetic acid (80%) and HCl at 60°C, tetrahydroisoindole-4-ones **25a,b** were obtained in 92 and 70% yields, respectively (Scheme 5, Table 5). Instead, deacetylation of compound **24c** was carried out at room temperature. A careful monitoring of reaction time allowed in 55 minutes to obtain a mixture of four derivatives **25f** (51%) and **25d** (23%) with traces of **25e** (5%) and **25c** (9%). Among them, the two compounds useful for our synthetic purpose were: **25d** and **25f**. Hydrolysis of compound **25f** to **25d** was quickly obtained by using an aqueous $K_2CO_3$ (10%) solution, furnishing **25d** in 93% yield and traces of derivative **25c** (Scheme 5, Table 5).

For the synthesis of [1,2]oxazolo[5,4-*e*]isoindoles bearing an ethoxycarbonyl group in position 6 of the tricyclic *core*, the tetrahydro-*2H*-isoindole-1-carboxylate **28** was used as building block. It was prepared following a multistep synthetic pathway (Scheme 5) starting from the enamino derivative **22**. Reaction of **22** with glycine ethyl ester hydrochloride in glacial acetic acid under reflux led to compound **26**, which reacted with DMFDMA in refluxing acetonitrile giving the intermediate **27**. The latter was used as crude product in the next step of dehydration with trifluoroacetic anhydride (TFAA) to yield the desired compound **28** according to a proposed mechanism of cyclization reported in literature [32].

**Scheme 5 - Synthesis of tetrahydroisoindole-4-ones**

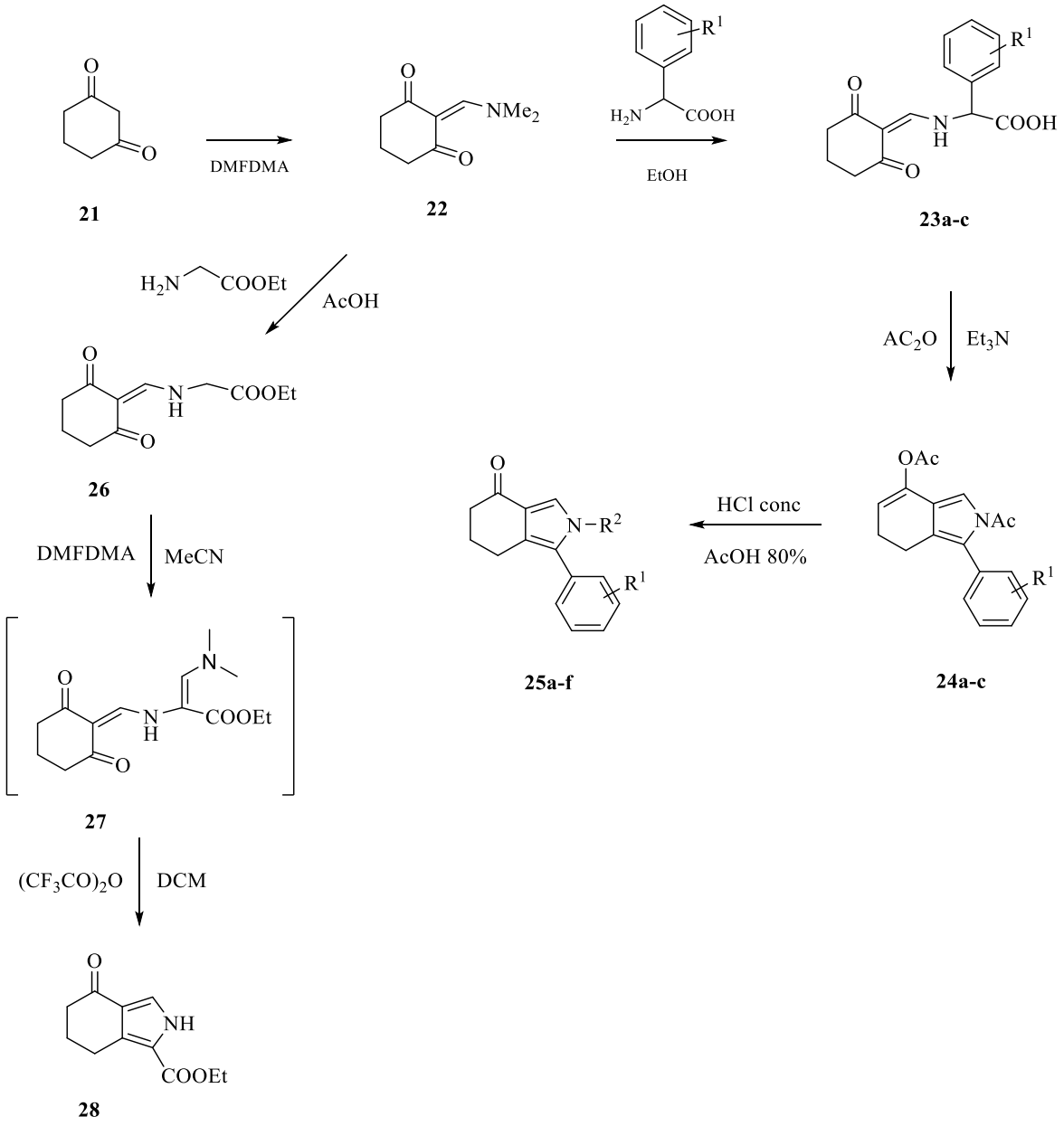



**Table 5 - Tetrahydro-4*H*-isoindol-4-ones 25a-f**

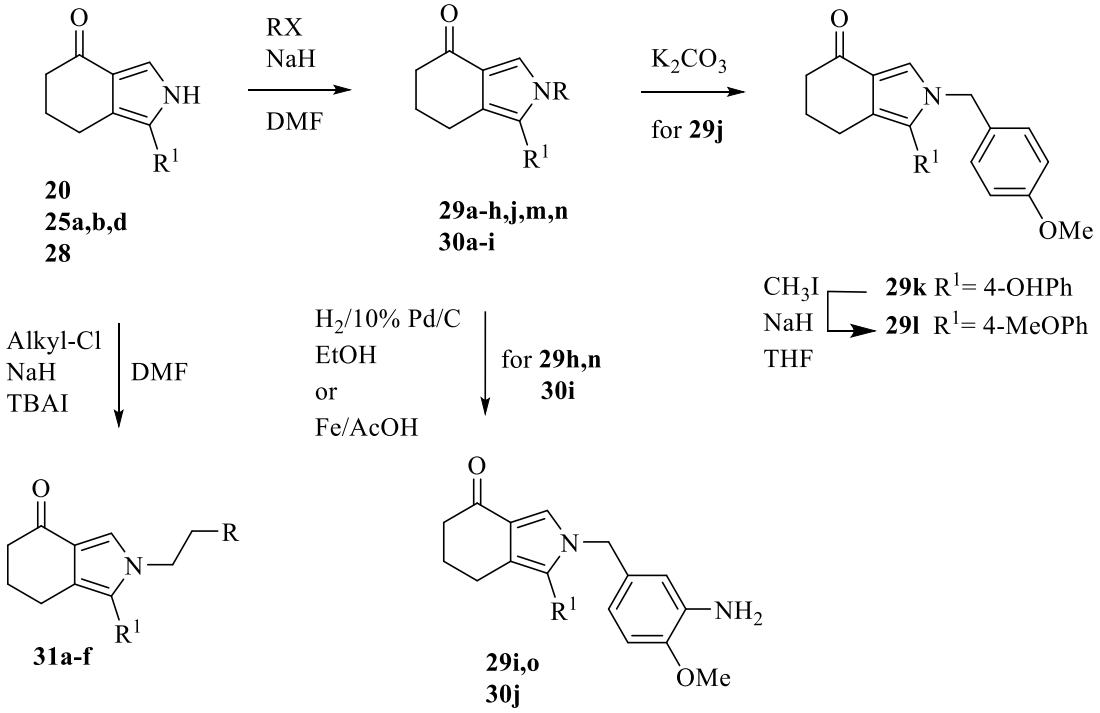
| CPD | $R^1$ | $R^2$ | Yields[a] (%) |
|---|---|---|---|
| **25a** | H | H | 92 |
| **25b** | 3,4,5-$(OMe)_3$ | H | 70 |
| **25c** | 4-OH | H | 9 |
| **25d** | OAc | H | 93 |
| **25e** | 4-OH | Ac | 5 |
| **25f** | OAc | Ac | 51 |

[a] Figures represent the yield obtained at the final reaction step.

Ketones **20**,**25a,b,d** and **28** were then converted in the corresponding *N*-functionalized ketones **29a-h,j,m,n,30a-i** by using benzyl bromide or 3-methoxybenzyl, 4-methoxybenzyl, 3,4-dimethoxybenzyl, 3,5-dimethoxybenzyl, 3,4,5-trimethoxybenyl, 3-nitro-4-methoxybenzyl chlorides in *N*,*N*-dimethylformamide (DMF) in the presence of sodium hydride. The 3-nitro 4-methoxybenzyl substituted derivatives **29h**,**n**,**30i** were subjected to catalytic reduction with 10% Pd/C or Fe and glacial acetic acid, furnishing the corresponding amino derivatives **29i,o**,**30j** (70-98%) (Scheme 6, Table 6).

Furthermore, basic hydrolysis of the 4-acethoxy group of **29j** with $K_2CO_3$ led to the isolation of 4-hydroxyphenyl derivative **29k** (96%), which was subsequently methylated by using iodomethane in tetrahydrofuran and sodium hydride, as base, to obtain the 4-methoxysubstituted compound **29l** in good yield (78%) (Scheme 6, Table 6).

**Scheme 6 – Functionalizations of ketones 20,25a,b,d,28**




One of the main aspect in drug development is the pharmacokinetic/pharmacodynamic profile of the molecule to determine the kinetics of ADME and to assess the drug levels at the biological site of action. In order to increase the dynamic movements of products during their passage through the body, enhancing bioavailability and targeted delivery to specific tissues. For this purpose, we planned the attachment of aminoalkyl chains bearing polarizable groups such as morpholine, pyrrolidine and piperidine at the pyrrole nitrogen. Thus, phenyl or 3,4,5-(trimethoxyphenyl) substituted ketones **25a,b** were subjected to *N*-alkylation of pyrrole by

reaction with suitable aminoalkyl chlorides (such as ethyl-morpholine, -pyrrolidine or -piperidine), in the presence of sodium hydride and tetrabutylammonium iodide (TBAI) as a catalyst in DMF. The aminoethyl chlorides, commercially available as hydrochlorides, were previously treated with 5% NaOH in aqueous solution to have the free basis available just before their use. The *N*-aminoethyl derivatives **31a-f** were obtained in good yields (40-63%) (Scheme 6, Table 6).

**Table 6 – 2,5,6,7-Tetrahydro-4*H*-isoindol-4-ones**

| CPD | R | $R^1$ | Yields[a] | CPD | R | $R^1$ | Yields[a] |
|---|---|---|---|---|---|---|---|
| **29a** | Bn | H | 65 | **30a** | Bn | COOEt | 67 |
| **29b** | 3-OMeBn | H | 69 | **30b** | 4-OMeBn | COOEt | 74 |
| **29c** | 3,4-$(MeO)_2$Bn | H | 60 | **30c** | 3,5-$(OMe)_2$Bn | COOEt | 94 |
| **29d** | 3,5-$(MeO)_2$Bn | H | 63 | **30d** | 3,4,5-$(OMe)_3$Bn | COOEt | 64 |
| **29e** | 3,4,5-$(MeO)_3$Bn | H | 60 | **30e** | 3,4-$(OMe)_2$Bn | COOEt | 68 |
| **29f** | Bn | Ph | 90 | **30f** | 2,3-$(OMe)_2$Bn | COOEt | 35 |
| **29g** | 4-OMeBn | Ph | 85 | **30g** | 2,5-$(OMe)_2$Bn | COOEt | 60 |
| **29h** | 3-$NO_2$-4-OMeBn | Ph | 83 | **30h** | 4-MeBn | COOEt | 55 |
| **29i** | 3-$NH_2$-4-OMeBn | Ph | 98 | **30i** | 3-$NO_2$-4-OMeBn | COOEt | 87 |
| **29j** | 4-OMeBn | 4-AcOPh | 64 | **30j** | 3-$NH_2$-4-OMeBn | COOEt | 70 |
| **29k** | 4-OMeBn | 4-HOPh | 96 | **31a** |  | Ph | 55 |
| **29l** | 4-OMeBn | 4-MeOPh | 78 | **31b** |  | Ph | 40 |
| **29m** | 4-OMeBn | 3,4,5-$(OMe)_3$Ph | 88 | **31c** |  | Ph | 43 |
| **29n** | 3-$NO_2$-4-OMeBn | 3,4,5-$(OMe)_3$Ph | 74 | **31d** |  | 3,4,5-$(OMe)_3$Ph | 63 |
| **29o** | 3-$NH_2$-4-OMeBn | 3,4,5-$(OMe)_3$Ph | 95 | **31e** |  | 3,4,5-$(OMe)_3$Ph | 57 |
| | | | | **31f** |  | 3,4,5-$(OMe)_3$Ph | 40 |

## 4.2 Annelation of [1,2]oxazolo ring

Introduction of an exocyclic double bond as electrophilic site would have led to a versatile building block for further annelation upon reaction with dinucleophiles to give the corresponding isoxazole derivatives.

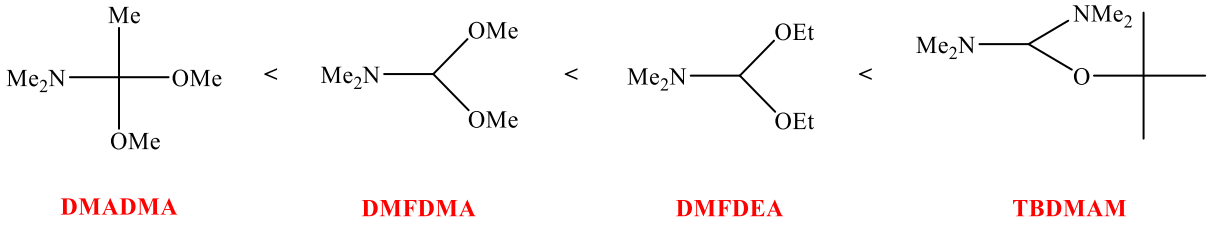
Ketones **29a-g,i,k,l,m,o** were treated with diethyl oxalate and potassium *t*-butoxide in toluene under nitrogen atmosphere to obtain, after a quick work-up, derivatives **32a-d,f,g,j,k** bearing a diethoxycarbonyl group in α-position to the carbonyl (Scheme 7, Table 7).

In the case of derivatives **32e,h,i,l,** it was not possible to isolate them as pure compounds for characterization, thus they were used in the following step without further purifications.

For ketones **30a-j**, two different strategies were carried out to accomplish the α-functionalization. In the first method, a formylation step was explored using ethyl formate as formylating agent in the presence of potassium *t*-butoxide in toluene under nitrogen atmosphere to obtain the hydroxymethyl intermediates **33a-g** in good yields (Scheme 7). Alternatively, direct introduction of an enamine functionality was performed. The latter generally involves the use of commercially available amide acetals, such as *N*,*N*-dimethylacetamide dimethylacetal (DMADMA), *N*,*N*-dimethylformamide dimethylacetal (DMFDMA) and *N*,*N*-dimethylformamide diethylacetal (DMFDEA) which can be selected on the basis of their reactivity with a specific substrate [33] (Figure 10). We decided to use the most reactive *t*-butoxy bis(dimethylamino)methane (TBDMAM), also called Bredereck's reagent, in 1:3 ratio with the substrate, for the preparation of β-enaminoketones **34a-c** in excellent yields. Due to poor stability of some enaminoketone intermediates to chromatographic purification, derivatives **34a-c** were used as crude products in the following step.

The same reaction conditions were used for *N*-aminoethyl derivatives **31a-f**, which were converted into enaminoketones **35a-f**, used as crude products in the following step.

**Figure 10 – Reactivity of amide acetals**




The isoxazoles **36,37,38** were obtained in moderate to excellent yields (40-97%) by the reaction of key intermediates **32,33,34,35** with hydroxylamine hydrochloride, as dinucleophile, in ethanol heated under reflux in the presence of catalytic amounts of acetic acid (Scheme 7, Table 7).

**Scheme 7 – Synthesis of [1,2]oxazolo[5,4-*e*]isoindoles 36,37,38,41**

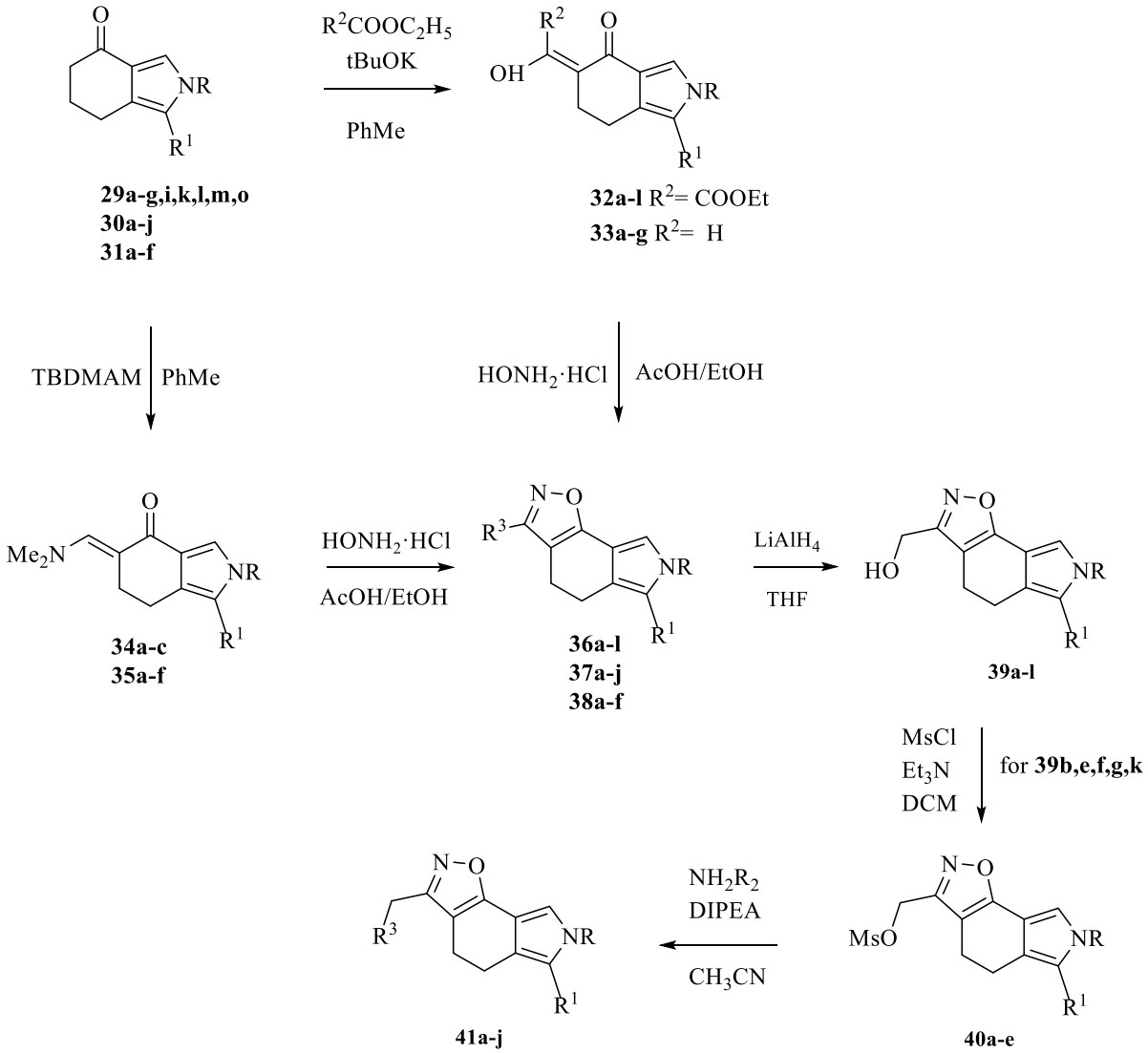
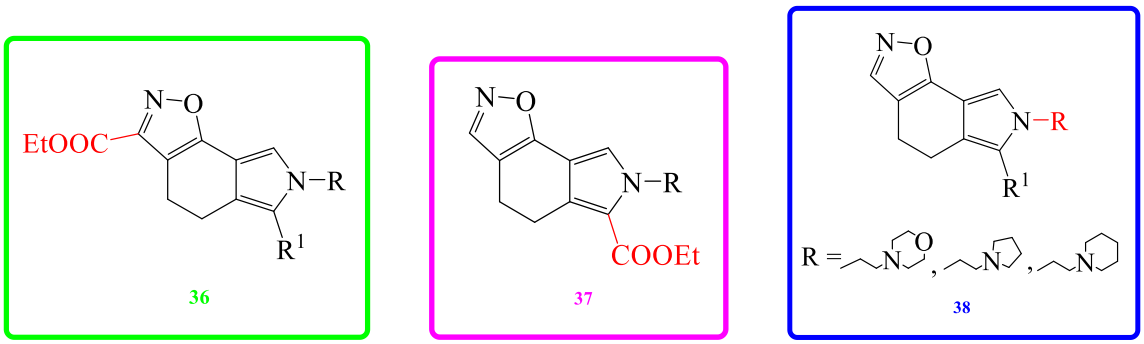
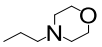
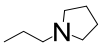
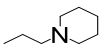
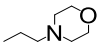
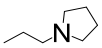
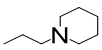



**Table 7 - [1,2]Oxazolo[5,4-*e*]isoindoles 36a-l, 37a-j, 38a-f**




| [1,2-oxazole] | SBT | R | $R^1$ | $R^3$ | Yields[a] (%) |
|---|---|---|---|---|---|
| **36a** | **32a** | Bn | H | COOEt | 82 |
| **36b** | **32b** | 3-OMeBn | H | COOEt | 88 |
| **36c** | **32c** | 3,4-$(OMe)_2$Bn | H | COOEt | 40 |

| | | | | | |
|---|---|---|---|---|---|
| **36d** | **32d** | 3,5-$(OMe)_2$Bn | H | COOEt | 44 |
| **36e** | **32e** | 3,4,5-$(OMe)_3$Bn | H | COOEt | 76 |
| **36f** | **32f** | Bn | Ph | COOEt | 55 |
| **36g** | **32g** | 4-OMeBn | Ph | COOEt | 76 |
| **36h** | **32h** | 3-$NH_2$,4-OMeBn | Ph | COOEt | 43 |
| **36i** | **32i** | 4-OMeBn | 4-OHPh | COOEt | 40 |
| **36j** | **32j** | 4-OMeBn | 4-OMePh | COOEt | 42 |
| **36k** | **32k** | 4-OMeBn | 3,4,5-$(OMe)_3$Ph | COOEt | 51 |
| **36l** | **32l** | 3-$NH_2$,4-OMeBn | 3,4,5-$(OMe)_3$Ph | COOEt | 74 |
| **37a** | **33a** | Bn | COOEt | H | 81 |
| **37b** | **33b** | 4-OMeBn | COOEt | H | 75 |
| **37c** | **33c** | 3,5-$(OMe)_2$Bn | COOEt | H | 51 |
| **37d** | **33d** | 3,4,5-$(OMe)_3$Bn | COOEt | H | 97 |
| **37e** | **33e** | 3,4-$(OMe)_2$Bn | COOEt | H | 77 |
| **37f** | **33f** | 2,3-$(OMe)_2$Bn | COOEt | H | 63 |
| **37g** | **33g** | 2,5-$(OMe)_2$Bn | COOEt | H | 74 |
| **37h** | **34a** | 4-MeBn | COOEt | H | 61 |
| **37i** | **34b** | 3-$NO_2$-4-OMeBn | COOEt | H | 57 |
| **37j** | **34c** | 3-$NH_2$-4-OMeBn | COOEt | H | 85 |
| **38a** | **35a** |  | Ph | H | 44 |
| **38b** | **35b** |  | Ph | H | 64 |
| **38c** | **35c** |  | Ph | H | 40 |
| **38d** | **35d** |  | 3,4,5-$(OMe)_3$Ph | H | 77 |
| **38e** | **35e** |  | 3,4,5-$(OMe)_3$Ph | H | 83 |
| **38f** | **35f** |  | 3,4,5-$(OMe)_3$Ph | H | 51 |

[a] Figures represent the yield obtained at the final reaction step.

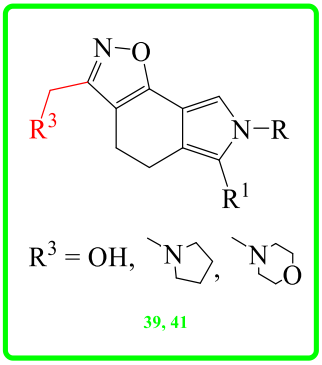
Esters **36a-l** were converted into the corresponding alcohols **39a-l** (43-99%) by reduction with $LiAlH_4$ in tetrahydrofuran (THF) at room temperature (Scheme 7, Table 8).

In order to extend the substitution pattern to the isoxazole ring, the hydroxymethyl derivatives previously prepared as final products, were then converted into secondary amines by using a two-step synthetic pathway reported in literature [34]. In particular, we decided to introduce a morpholine/pyrrolidine end-group as ionizable groups at physiological pH, increasing the hydrophilic character for an easy distribution over body compartments.

In the first step a mesyl (methane sulfonyl) intermediate was formed by reaction of the alcoholic group with methanesulfonyl chloride (MsCl) and triethylamine in DMF under nitrogen atmosphere, thus obtaining intermediates **40a-e**. The latter were used in the next step without further purification and were treated with the appropriate amine (morpholine or

pyrrolidine) and *N*-ethyldiisopropylamine (DIPEA) in acetonitrile under nitrogen atmosphere for 3 hours at room temperature, leading to the isoxazolo[5,4-*e*]isoindoles **41a-j** (40-90%) (Scheme 7, Scheme 8).

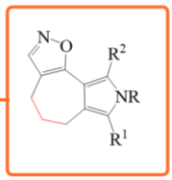
**Table 8 – [1,2]Oxazolo[5,4-*e*]isoindoles 39, 41**




| [1,2-oxazole] | SBT | R | $R^1$ | $R^3$ | Yields[a] (%) |
|---|---|---|---|---|---|
| **39a** | **36a** | Bn | H | OH | 82 |
| **39b** | **36b** | 3-OMeBn | H | OH | 99 |
| **39c** | **36c** | 3,4-$(OMe)_2$Bn | H | OH | 99 |
| **39d** | **36d** | 3,5-$(OMe)_2$Bn | H | OH | 73 |
| **39e** | **36e** | 3,4,5-$(OMe)_3$Bn | H | OH | 85 |
| **39f** | **36f** | Bn | Ph | OH | 77 |
| **39g** | **36g** | 4-OMeBn | Ph | OH | 71 |
| **39h** | **36h** | 3-$NH_2$,4-OMeBn | Ph | OH | 52 |
| **39i** | **36i** | 4-OMeBn | 4-OHPh | OH | 43 |
| **39j** | **36j** | 4-OMeBn | 4-OMePh | OH | 80 |
| **39k** | **36k** | 4-OMeBn | 3,4,5-$(OMe)_3$Ph | OH | 99 |
| **39l** | **36l** | 3-$NH_2$,4-OMeBn | 3,4,5-$(OMe)_3$Ph | OH | 60 |
| **41a** | **40a** | 3-OMeBn | H |  | 65 |
| **41b** | **40a** | 3-OMeBn | H |  | 70 |
| **41c** | **40b** | 3,4,5-$(OMe)_3$Bn | H |  | 42 |
| **41d** | **40b** | 3,4,5-$(OMe)_3$Bn | H |  | 40 |
| **41e** | **40c** | Bn | Ph |  | 47 |
| **41f** | **40c** | Bn | Ph |  | 90 |
| **41g** | **40d** | 4-OMeBn | Ph |  | 78 |
| **41h** | **40d** | 4-OMeBn | Ph |  | 63 |
| **41i** | **40e** | 4-OMeBn | 3,4,5-$(OMe)_3$Ph |  | 44 |
| **41j** | **40e** | 4-OMeBn | 3,4,5-$(OMe)_3$Ph |  | 41 |

[a] Figures represent the yield obtained at the final reaction step.

**AIM II** — Ring-expansion from six to seven atoms

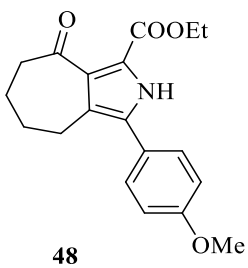



# 5 4,5,6,8-Tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-*d*]isoxazoles

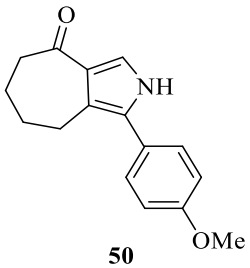
## 5.1 Synthesis of building blocks

Based on the interesting antitubulin activity of [1,2]oxazolo[5,4-*e*]isoindoles **15,16** and pyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazoles **17** recently reported by our research group, we designed a new seven-membered ring system to explore whether the enlargement of the central ring in the tricyclic scaffold could enhance biological properties. Seven-membered ring is a recurrent structural feature in other antitubulin agents such as colchicine. The approach to the synthesis of tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-*d*]isoxazole ring system was based on the preparation of 5,6,7,8-tetrahydrocyclohepta[*c*]pyrrol-4(2*H*)-ones **48, 50** and **55a,b** (Figure 11) as proper building blocks to achieve the tricyclic framework.

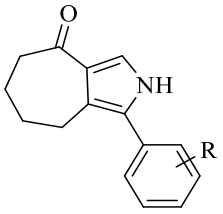
**Figure 11 – Building blocks based on cyclohepta system**




**48**




**50**




**55**

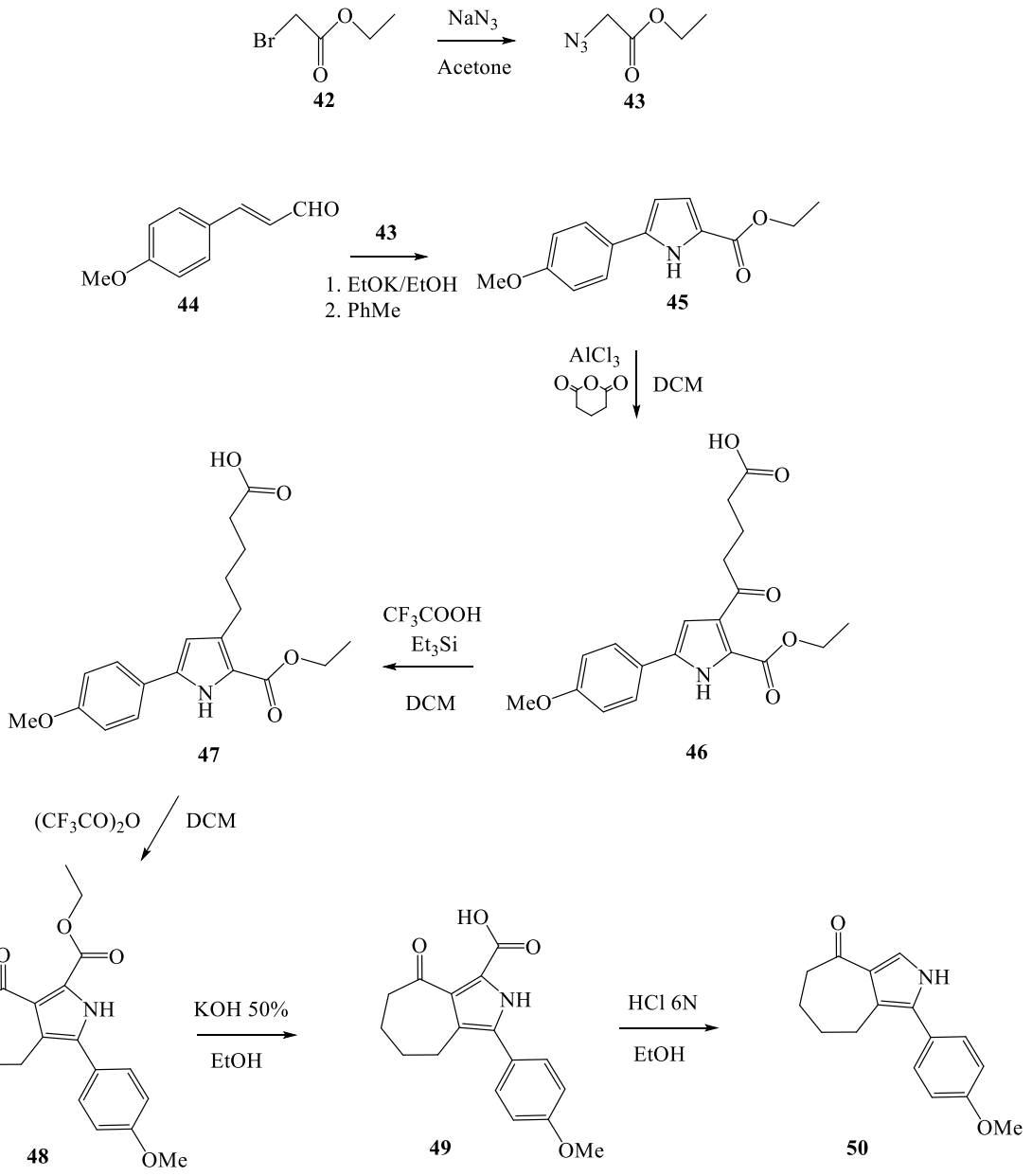
Ketones **48** and **50** have been synthesized using a multistep sequence developed by us, starting from ethyl 5-(4-methoxyphenyl)-1*H*-pyrrole-2-carboxylate **45**, which is not commercially available and it was prepared by reaction of 3-(4-methoxyphenyl)acrylaldehyde **44** with ethyl 2-azidoacetate **43**. The latter was obtained by treating a solution of ethyl 2-bromoacetate **42** with an excess of sodium azide in refluxing acetone. A colourless oil was obtained in good yields after 4 hours and used in the next step without further purifications.

Reaction of ethyl 2-azidoacetate **43** and 3-(4-methoxyphenyl)acrylaldehyde **44** was conducted in presence of potassium ethoxide under a nitrogen atmosphere in anhydrous ethanol at -20°C to give an intermediate which was cyclized in refluxing toluene to the pyrrole derivative **45** in good yield (74%). Compound **45** then was subjected to Friedel–Crafts reaction with glutaric anhydride, as acylating reagent, and $AlCl_3$, as Lewis acid, to introduce an acyl group in the 3-position of the pyrrole ring, leading to compound **46** in good yield (57%). The obtained

derivate was subjected to reduction of the carbonyl group in position-5 of the acyl chain to methylene, through the use of triethylsilane in trifluoroacetic acid, leading to intermediate **47** (61%). The latter was subsequently cyclized by dehydration process with an excess of trifluoroacetic anhydride to achieve the seven membered ring. Thus, the ethyl 3-(4-methoxyphenyl)-8-oxo-2,4,5,6,7,8-hexahydrocyclohepta[c]pyrrole-1-carboxylate **48** was obtained in good yields.

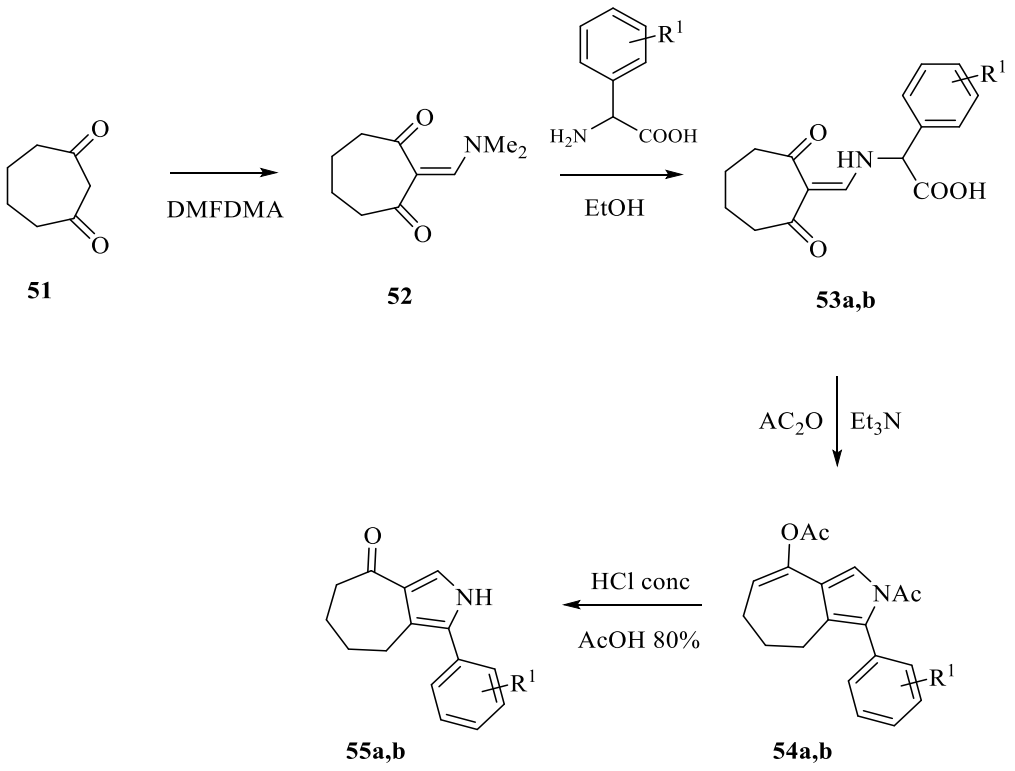
Basic hydrolysis reaction of the ethoxycarbonyl group with 50% potassium hydroxide solution in refluxing ethanol and subsequent decarboxylation with HCl 6 M in ethanol at reflux condition, allowed the isolation of derivative **50** in 60% yield (Scheme 8).

**Scheme 8 – Synthesis of cyclohepta building blocks 48 and 50**



The synthesis of ketones **55a,b** was achieved using the multistep synthetic approach described in the Scheme 9, which was optimized by us on the basis of the one previously described for isoindole derivatives **25a,b** (Scheme 5). Cycloheptane-1,3-dione **51** was used, instead of 1,3-cyclohexanedione, to achieve the seven-membered ring. In this case, reaction of **51** in refluxing DMFDMA used in large excess allowed the introduction of the enamino functionality, giving the derivative **52**. The latter was refluxed in ethanol with phenylglycine or 3,4,5-trimethoxyphenylglycine leading to intermediates **53a,b**, which were used in the following step without any further purification and cyclized by using acetic anhydride and triethylamine, heating to reflux, to obtain compounds **54a,b** (73 and 53%). Hydrolysis of acetyl groups with concentrated HCl and acetic acid (80%) at 60°C led to desired ketones **55a,b** (75 and 81%) (Scheme 9).

**Scheme 9 – Synthesis of cyclohepta building blocks 55a,b**




**a** $R^1$= H, **b** $R^1$= 3,4,5-$(OMe)_3$

## 5.2 Synthesis of tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-*d*]isoxazoles

Ketones **48, 50** and **55a,b**, bearing a free NH, were subjected to reactions with substituted benzyl halides (benzyl bromide or 4-methoxybenzyl, 3,4-dimethoxybenzyl, 3,5-dimethoxybenzyl, 2,3-dimethoxybenzyl, 2,5-dimethoxybenzyl, 3,4,5-trimethoxybenyl, 4-methylbenzyl, 3-nitro-4-methoxybenzyl chlorides), sodium hydride as a base and dry DMF as

solvent to give *N*-substituted derivatives **56a-m,o-y** in 60-98% yields. The 3-nitro 4-methoxybenzyl substituted derivatives **56m,y** were subjected to catalytic reduction with ammonium formate and 10% Pd/C in ethyl acetate, furnishing the corresponding amino derivatives **56n,z** (86% and 71%) (Scheme 10).

The *N*-substituted derivatives were properly converted into the α-enaminoketones **57a-z** using the Bredereck's reagent in refluxing toluene. Finally, anellation of the [1,2]oxazole ring on cyclohepta system was achieved reacting the intermediates **57a-z** with hydroxylamine hydrochloride as dinucleophile and stoichiometric amounts of acetic acid in refluxing ethanol. 4,5,6,8-Tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-*d*]isoxazoles **58a-z** were obtained in moderate to excellent yields (Scheme 10, Table 9).

**Scheme 10 - Synthesis of 4,5,6,8-tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-*d*]isoxazoles 58a-z**

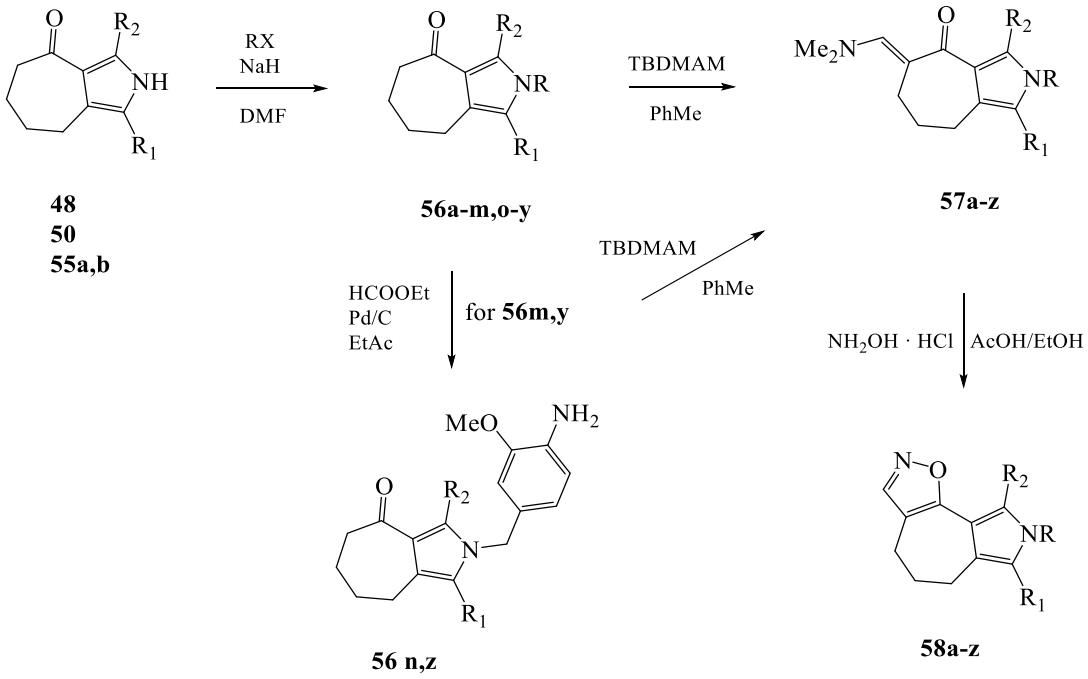
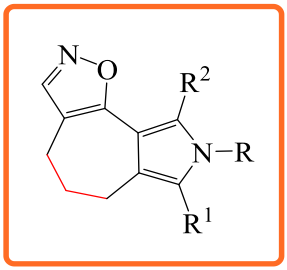



**Table 9 - 4,5,6,8-tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-*d*]isoxazoles 58a-z**



| [1,2-oxazole] | SBT | R | $R^1$ | $R^2$ | Yields[a] (%) |
|---|---|---|---|---|---|
| **58a** | **57a** | Bn | OMe-Ph | COOEt | 93 |
| **58b** | **57b** | 4-OMeBn | OMe-Ph | COOEt | 60 |
| **58c** | **57c** | 3-OMeBn | OMe-Ph | COOEt | 75 |
| **58d** | **57d** | 2-OMeBn | OMe-Ph | COOEt | 83 |
| **58e** | **57e** | 4-OMeBn | OMe-Ph | H | 70 |
| **58f** | **57f** | 3-OMeBn | OMe-Ph | H | 44 |
| **58g** | **57g** | 2-OMeBn | OMe-Ph | H | 40 |
| **58h** | **57h** | 3,4,5-$(OMe)_3$Bn | OMe-Ph | H | 50 |
| **58i** | **57i** | Bn | Ph | H | 73 |
| **58j** | **57j** | 4-OMeBn | Ph | H | 83 |
| **58k** | **57k** | 3-OMeBn | Ph | H | 68 |
| **58l** | **57l** | 2-OMeBn | Ph | H | 78 |
| **58m** | **57m** | 3-$NO_2$,4-OMeBn | Ph | H | 40 |
| **58n** | **57n** | 3-$NH_2$,4-OMeBn | Ph | H | 51 |
| **58o** | **57o** | 3,4-$(OMe)_2$Bn | Ph | H | 80 |
| **58p** | **57p** | 3,4,5-$(OMe)_3$Bn | Ph | H | 49 |
| **58q** | **57q** | 2,5-$(OMe)_2$Bn | Ph | H | 80 |
| **58r** | **57r** | Bn | 3,4,5-$(OMe)_3$Ph | H | 46 |
| **58s** | **57s** | 4-OMeBn | 3,4,5-$(OMe)_3$Ph | H | 51 |
| **58t** | **57t** | 3-OMeBn | 3,4,5-$(OMe)_3$Ph | H | 49 |
| **58u** | **57u** | 2-OMeBn | 3,4,5-$(OMe)_3$Ph | H | 60 |
| **58v** | **57v** | 3,4,5-$(OMe)_3$Bn | 3,4,5-$(OMe)_3$Ph | H | 71 |
| **58w** | **57w** | 2,5-$(OMe)_2$Bn | 3,4,5-$(OMe)_3$Ph | H | 77 |
| **58x** | **57x** | 3,4-$(OMe)_2$Bn | 3,4,5-$(OMe)_3$Ph | H | 51 |
| **58y** | **57y** | 3-$NO_2$,4-OMeBn | 3,4,5-$(OMe)_3$Ph | H | 47 |
| **58z** | **57z** | 3-$NH_2$,4-OMeBn | 3,4,5-$(OMe)_3$Ph | H | 60 |

[a] Figures represent the yield obtained at the final reaction step.

# 6 National Cancer Institute (NCI) biological evaluation

In search of potential therapeutics for cancer, the new series of compounds were evaluated on a panel of 60 cell lines, according to the National Cancer Institute (NCI; Bethesda, MD) screening protocol. Cell lines are divided in 9 subpanels that represent leukemia, melanoma, non-small-cell lung carcinoma and brain, ovary, breast, colon, kidney and prostate cancers. Due to the diversity of cell lines, it is possible to test and compare the action of molecules both on solid and hematological human tumors. All compounds submitted to the NCI 60 cell screen are initially subjected to a single high dose screen ($10^{-5}$ M). The one-dose data are reported as average of the growth percent of treated cells compared to the no-drug control, allowing to identify growth inhibition (values between 0 and 100) and lethality (values less than 0). Only compounds which exhibit significant growth inhibition in the one-dose screen progress to the full 5-doses assay, in which compounds are evaluated against the 60 cells panel at five concentration levels ($10^{-8}$ - $10^{-4}$ M).

For each experimental agent, three dose response parameters are calculated: $GI_{50}$, TGI and $LC_{50}$. $GI_{50}$ is the concentration that causes 50% of growth inhibition and is used by NCI in place of $IC_{50}$ to emphasize the strict correlation between screening results and inhibition of cell proliferation. $GI_{50}$ is calculated from [(T-T0)/(C-T0)] x 100 = 50, in which T is the optical density of the test well after a 48-h period of exposure to test drug, T0 is the optical density at time zero and C is the control optical density. TGI is the concentration that induces total growth inhibition, thus expressing a cytostatic effect. It is calculated from 100 × (T - T0)/(C - T0) = 0. $LC_{50}$ (or lethal concentration 50) is the amount of drug required to kills 50% of cell population after treatment, calculated from 100 × (T - T0)/T0 = -50. It signifies a cytotoxic effect.

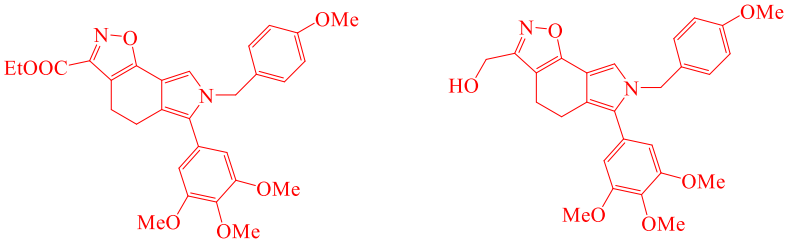
Furthermore, to give the average activity parameter over all cell lines, for each compound is calculated a mean graph midpoint (MG_MID), the middle point of a line segment created between the lowest and the highest $GI_{50}$ values plotted in a xy-axis.

All new compounds synthetized during my PhD thesis were submitted to the NCI Development Therapeutics Programm service. All derivatives were selected for one-dose screening against NCI full panel. Compounds **41a-j** and **58a-z** are currently under evaluation, whilst results have already been collected for the **36**, **37**, **38** and **39** series.

Among the screened derivatives, compounds **36k** and **39k** satisfied the threshold inhibition criteria of the one-dose screening, proceeding to the five-dose testing ($10^{-4} - 10^{-8}$ M) on 60 human cancer cell lines (Figure 12). Results of the screening are presented in Table 10. The

activities of compounds **36k** and **39k** are represented by the percentage of growth altered due to treatment ($GI_{50}$).

**Figure 12 – Selected compounds for five-dose screening**




**36k** **39k**

**Table 10 - *In vitro* $GI_{50}$ values of compounds 36k and 39k in individual tumor cell lines**

| Cell lines | 36k | 39k |
|---|---|---|
| **LEUKEMIA** | | |
| CCRF-CEM | 1.04 | 0.32 |
| HL-60(TB) | 0.45 | 0.20 |
| MOLT-4 | 2.70 | 0.44 |
| RPMI-8226 | 2.85 | 0.41 |
| SR | 2.09 | 0.21 |
| **NON-SMALL CELL LUNG CANCER** | | |
| A549/ATCC | 3.80 | 0.41 |
| EKVX | 3.92 | 0.51 |
| HOP-62 | 2.53 | 0.29 |
| HOP-92 | 2.72 | 3.39 |
| NCI-H226 | - | 15.0 |
| NCI-H23 | 3.04 | 0.51 |
| NCI-H322M | 5.46 | 0.64 |
| NCI-H460 | 1.00 | 0.34 |
| NCI-H522 | 0.37 | 0.14 |
| **COLON CANCER** | | |
| COLO 205 | 1.77 | 0.30 |
| HCC-2998 | 5.17 | 1.34 |
| HCT-116 | 0.39 | 0.26 |
| HCT-15 | 0.49 | 0.25 |
| HT29 | 0.84 | 0.32 |
| KM12 | 0.91 | 0.27 |
| SW-620 | 0.49 | 0.37 |

| Cell lines | 36k | 39k |
|---|---|---|
| **CNS CANCER** | | |
| SF-268 | 8.07 | 1.06 |
| SF-295 | 3.02 | 0.18 |
| SF-539 | 1.84 | 0.23 |
| SNB-19 | 0.66 | 0.39 |
| SNB-75 | 3.65 | 0.17 |
| U251 | 0.54 | 0.33 |
| **RENAL CANCER** | | |
| 786-0 | 3.65 | 0.36 |
| A498 | 2.77 | 0.27 |
| ACHN | 3.51 | 0.61 |
| RXF 393 | 2.17 | 0.15 |
| SN12C | 3.92 | 0.71 |
| TK-10 | - | 17.7 |
| UO-31 | 3.44 | 0.58 |
| **OVARIAN CANCER** | | |
| IGROV1 | 2.45 | 0.49 |
| OVCAR-3 | 0.37 | 0.22 |
| OVCAR-4 | 20.5 | 5.44 |
| OVCAR-5 | 4.72 | 0.70 |
| OVCAR-8 | 3.04 | 0.38 |
| NCI/ADR-RES | 3.52 | 0.25 |
| SK-OV-3 | 5.23 | 0.37 |

| Cell lines | 36k | 39k |
|---|---|---|
| **BREAST CANCER** | | |
| MCF7 | 0.34 | 0.09 |
| MDA-MB-231/ATCC | 2.70 | 0.63 |
| HS 578T | 2.99 | 0.40 |
| BT-549 | 1.47 | 0.48 |
| T-47D | - | - |
| MDA-MB-468 | 5.62 | 0.32 |
| **MELANOMA** | | |
| LOX IMVI | 0.64 | 0.56 |
| MALME-3M | - | 13.5 |
| M14 | 0.41 | 0.15 |
| MDA-MB-435 | 0.57 | 0.03 |
| SK-MEL-2 | 4.29 | 0.45 |
| SK-MEL-28 | 1.90 | 3.60 |
| SK-MEL-5 | 0.49 | 0.21 |
| UACC-257 | 85.8 | 14.7 |
| UACC-62 | 2.74 | 0.25 |
| **PROSTATE CANCER** | | |
| PC-3 | 2.90 | 0.27 |
| DU-145 | 2.32 | 0.38 |

Both **36k** and **39k** exhibited significant dose-dependent patterns of activity against most cancer cell lines, reaching $GI_{50}$ values in the micromolar to nanomolar range (0.03-85.8 µM). The MG_MID for compound **36k** and **39k** were 2.45 µM and 0.49 µM, respectively.

For **36k**, the NCI-60 $GI_{50}$ values ranged from the micromolar to the low micromolar in almost all cell lines. In particular, the most sensitive cell lines were those belonging to the colon cancer subpanel where **36k** maintained $GI_{50}$ values in the sub-micromolar activity. The only exceptions were COLO205 and HCC-2998 cell lines with low micromolar $GI_{50}$ values of 1.77 and 5.17 μM, respectively. The second-best selectivity was for melanoma subpanel, showing antiproliferative activity in the sub-micromolar range with the exception of SK-MEL-2, SK-MEL-28 and UACC-62 cell lines in which **36k** reached low micromolar concentrations ($GI_{50}$ 4.29 and 1.90 μM, respectively). Comparable results were obtained from the remaining subpanel that showed 50% growth inhibition at micromolar concentrations for almost all cell lines, with the exception for leukemia HL-60(TB) cell line ($GI_{50}$ = 0.45 μM), non-small cell lung cancer NCI-H522 cell line ($GI_{50}$ = 0.37 μM), CSN cancer SNB-19 and U251 cell lines ($GI_{50}$ = 0.66 and 0.54 μM), ovarian cancer OVCAR-3 cell line ($GI_{50}$ = 0.37 μM) and breast cancer MCF7 cell line ($GI_{50}$ = 0.34 μM).

Better results were obtained for derivative **39k**, whose $GI_{50}$ values reached the nanomolar range. The highest selectivity was expressed against the breast cancer subpanel with $GI_{50}$ values in the low micromolar to nanomolar range ($GI_{50}$ 0.09-0.63 μM), followed by leukemia and prostate cancer subpanels where **39k** exerted antiproliferative activity at sub-micromolar concentrations against all tested cell lines ($GI_{50}$ 0.20-0.44 μM and 0.27-0.38 μM, respectively).

Furthermore, sub-micromolar activity was maintained against all cell lines in other subpanels, excluding non-small cell lung cancer HOP-92 and NCI-H226 cell lines ($GI_{50}$ = 3.39 and 15 μM), colon cancer HCC-2998 cell line ($GI_{50}$ = 1.34 μM), CSN cancer SF-268 cell line ($GI_{50}$ = 1.06 μM), ovarian cancer OVCAR-4 cell line ($GI_{50}$ = 5.44 μM), melanoma MALME-3M, SK-MEL-28 and UACC-257 cell lines ($GI_{50}$ = 13.5, 3.60 and 14.7 μM, respectively). The least sensitive was the renal cancer TK-10 cell line for which **39k** showed $GI_{50}$ of 17.7 μM.

Of all the cell lines, melanoma MD-MBA-435 cell line and breast cancer MCF-7 cell line were the most sensitive to **39k**, which inhibited cell growth by 50% at nanomolar concentrations ($GI_{50}$ = 0.03 μM and 0.09 μM, respectively).

In the following dose-response curves, the relationship between the logarithm of **39k** concentration (X-axis) and cellular growth percentage after treatment (Y-axis) are reported. Drug concentration is plotted on a base 10 logarithmic scale, highlighting the region where drug response is changing rapidly (Figure 13). Moreover, mean graphs of compound **39k** are reported in Figure 14.

**Figure 13 – Dose-response curves of compound 39k**

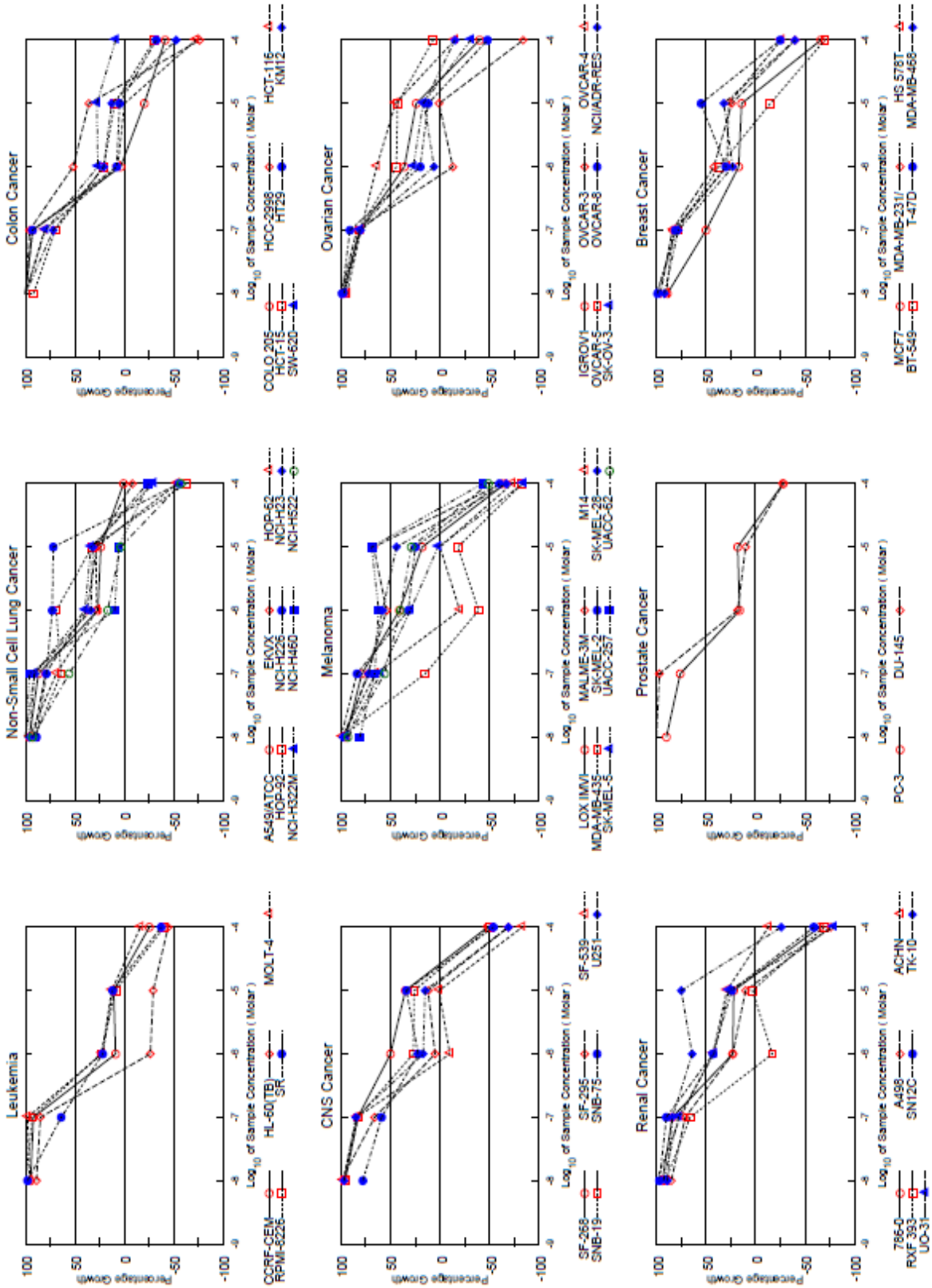
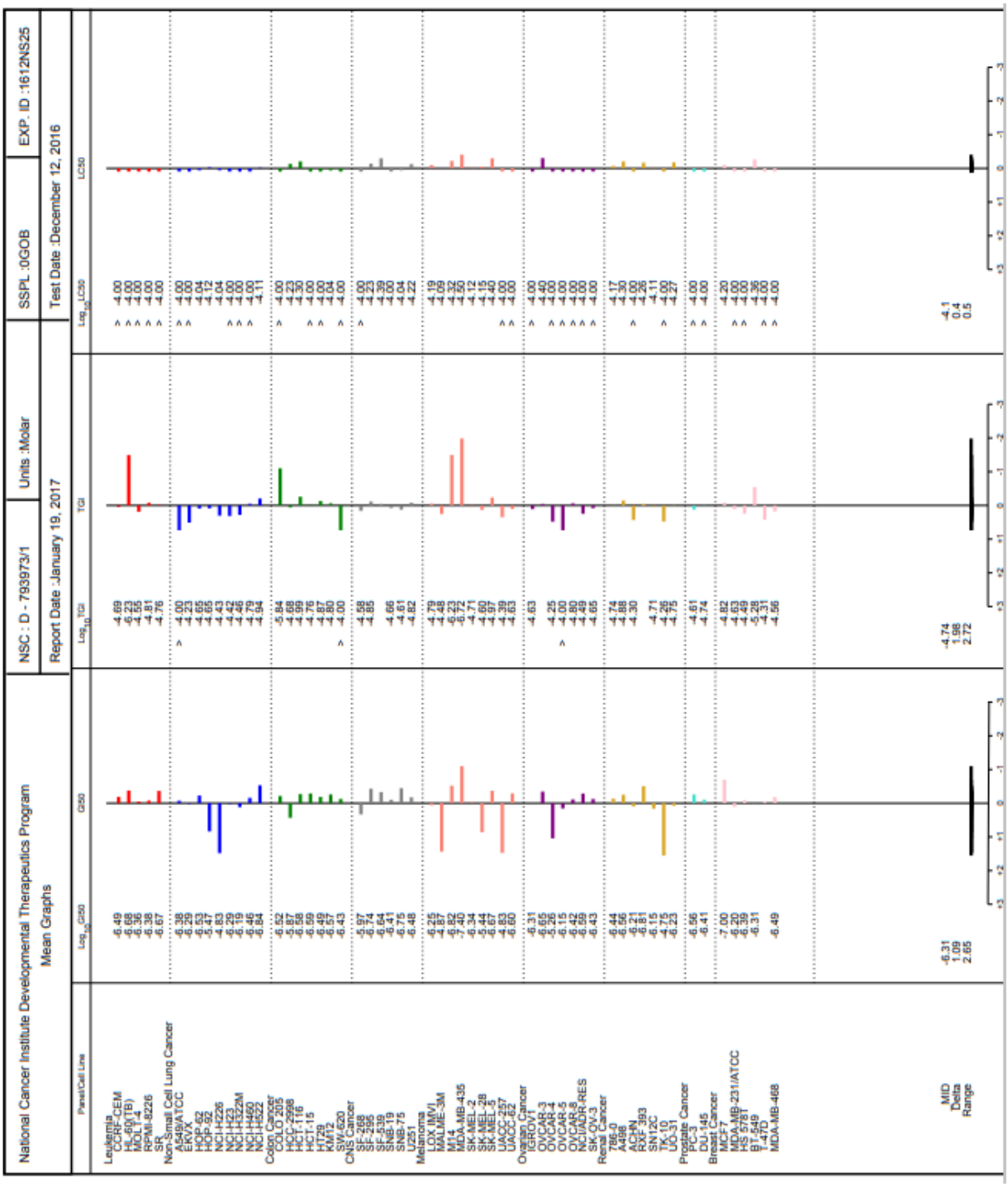


**Figure 14 – Mean graph of compound 39k**




Based on NCI-60 human tumor cell lines screen results, interesting structure-activity relationships (SAR) can be deducted. Compounds **36k** and **39k** share a 4-methoxybenzyl group at the pirrole nitrogen and a 3,4,5-trimethoxyphenyl group in position 6 of the tricyclic core. Thus, both functionalizations are crucial in conferring good activity to [1,2]oxazole derivatives. The importance of these substitutions, in accordance to the CA-4 structure and to

that of the lead compound **15r** identified within the starting set of derivatives, confirms our starting rational drug design.

Moreover, the presence of the hydroxymethyl group bonded to the isoxazole ring seems to be relevant for increased activity, since **39k** induced stronger growth inhibitory effect than **36k**.

# 7 Project in collaboration with the Institute of Oncology Research (IOR)

## 7.1 Introduction

According to the PhD operative program Sicily 2020, I spent nine months of the project (September 2019 - May 2020) at the Institute of Oncology Research (IOR; Bellinzona - Switzerland) in the Lymphoma Genomics group led by Prof. Francesco Bertoni, under the supervision of Dr. Eugenio Gaudio.

The research work was also supported by an additional grant awarded upon selection of the project in the foreign institution by the Short Term Scientific Mission (STSM) program within COST Action CA15135 - Multi-target paradigm for innovative ligand identification in the drug discovery process (Mu.Ta.Lig), whose aim is to support researchers' mobility, to establish and strengthen international collaborations between teams devoted to the multi-target issue in medicinal chemistry.

In order to investigate the efficacy of newly synthesized compounds on lymphoma cell lines, thus expanding the set of potential targeted cells, both [1,2]oxazolo[5,4-*e*]isoindoles and 4,5,6,8-tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-*d*] isoxazoles were tested on a panel of four different subtypes of Non-Hodgkin's lymphoma at the single dose of 1 mM. Selected compounds were further treated with a wider range of concentrations (0-10 μM) to calculate $IC_{50}$ values, according to the in vitro protocol established at the Institute of Oncology Research (IOR). Encouraged by the excellent results previously obtained against different tumor cell lines, screenings were extended to the series of [1,2]oxazolo[5,4-*e*]isoindoles **15**, **16** and pyrrolo [2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazoles **17** that had never been studied before on lymphoma models.

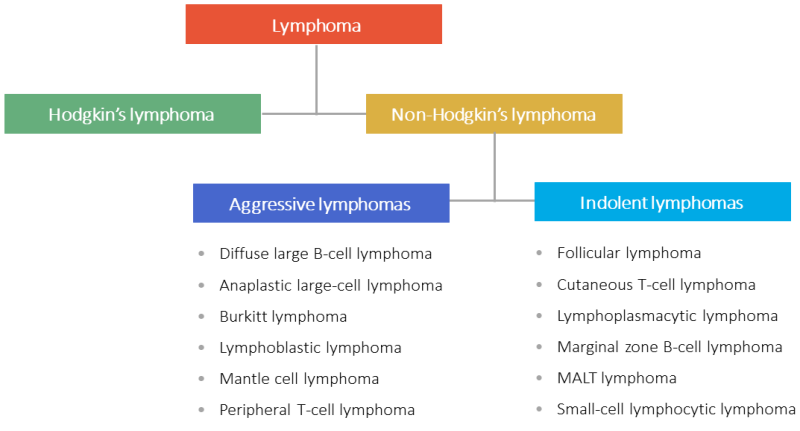
Additionally, I joined several Lymphoma Genomics' research projects that included: development and testing of new drugs against lymphoma, application of genomics techniques to identify new genes or targets involved in lymphoma progression and follow-up studies for national lymphoma clinical trials (SAKK). In particular, many efforts have been devoted to the project supported by San Salvatore foundation aimed at identifying kinase inhibitors to combine with the BET-BRDs inhibitor Birabresib, in order to improve its anti-lymphoma activity. The research work done at the Institute of Oncology Research improved my knowledge on hematologic malignancies enabling me to write an overview on anti-tubulin agents for the treatment of lymphoma patients [35].

Furthermore, it gave me new molecular biology laboratory skills, including: cell culture growth, western blotting, DNA and proteins extraction, agarose gel electrophoresis of DNA, DNA quantitation, PCR amplification and electrophoresis.

## 7.2 Lymphoma

Lymphoma is a cancer that affects the lymphatic system and develops from the lymphocytes, white blood cells of the immune system that are carried throughout the body in the lymph and help fighting infections. The lymph passes through lymph nodes (glands) that act as filters, destroying bacteria and viruses to prevent the spread of infections. Lymphoma occurs when healthy lymphocytes grow out of control, generally in lymph nodes in armpits, neck or groin.
More than 70 cancer types are classified as lymphomas, broadly divided into Hodgkin's (HL) and non-Hodgkin's lymphomas (NHL). They take their names from pathologist Thomas Hodgkin who first described malignant lymphoid disorder in 1832.
The primary difference between these two tumors is the type of lymphocyte affected. HL is characterized by the presence of Reed-Sternberg cells, distinctive large lymphocytes (mature B cells) that may contain more than one nucleus and can be identified using light microscopy. These abnormal cells are not present in NHL patients. Additionally, HL is less common than NHL, it mostly affects young adults and is considered one of the most treatable cancers since is often diagnosed in early stage and spreads in a fairly orderly way from one lymph node to another [36]. Conversely, NHL is more diffuse and aggressive because it is diagnosed at more advanced stages and can involve any part of the body. More than 30 subtypes of NHL have been identified, involving both B lymphocytes (B cells) or T lymphocytes (T cells). One of their classification includes whether they are aggressive (fast-growing) or indolent (slow-growing) (Figure 15) [37].

**Figure 15 – Lymphoma classification**



Depending on the type of lymphoma, the age of the patients and the cancer stage, different treatments can be used. Chemotherapy, radiotherapy, steroids and monoclonal antibody (moAbs) are very frequently used to treat lymphoma, either as single cure or in combination.
Since single-agent chemotherapy have only demonstrated palliative benefits, curative intents require the combination of several drugs, often with the addition of monoclonal antibody (combination immuno-chemotherapy). Their efficacy can be improved by increasing doses, adding further cytotoxic drugs, reducing intervals between cycles or administering as continuous infusion.
Alkylating agents were the first agents to show activity against lymphomas but anthracycline and vinca alkaloids were those that revolutionised lymphoma treatment. Other drugs are generally added to the anthracycline-vinca alkaloids standard regimen backbone depending on single-agent anti-lymphoma activity and non-cross toxicities. The cytotoxic agents active against lymphoma are reported in Table 11.

**Table 11 - Lymphoma chemotherapeutics**

| Drugs | Drug class |
|---|---|
| Prednisone, methylprednisone | Corticosteroids |
| Chlorambucil, cyclophosphamide, ifosfamide, procarbazine, thiotepa | Alkylating agents |
| Doxorubicin, epirubicin | Anthracyclines |
| Bleomycin | Cytotoxic antibiotics |
| Vincristine, vinblastine | Vinca alkaloids |
| Monomethyl auristatin E | Dolastatins |
| Methotrexate, cytarabine, gemcitabine, fludarabine | Antimetabolites |
| Cisplatin, carboplatin, oxaliplatin | Platin derivatives |
| Lenalidomide | Immunoderivatives |
| Idelalisib | PI3K inhibitor |
| Ibrutinib | Bruton tyrosin kinase inhibitor |
| ABT-199 | BCL-2 inhibitor |
| Pidilizumab, nivolumab, pembrolizumab | Checkpoint inhibitors |

The most common chemotherapy regimens used are CHOP for NHL and ABVD for HL. The CHOP regimen consist of cyclophosphamide (750 mg/m$^2$, given IV on day 1), doxorubicin (50 mg/m$^2$ given IV on day 1), vincristine (1.4 mg/m$^2$, given IV on day 1) and prednisone (100mg, PO on day 1 to 5), repeated every 3 weeks for a usual total of 6 to 8 cycles depending on the stage of disease. During the years, numerous variations of this regimen have been tested, either through the addition of other drugs or the intensification of the schedule but the major improvement in the outcome resulted from adding the monoclonal antibody Rituximab, thus making R-CHOP the worldwide first-line treatment. Rituximab was the first genetically engineered chimeric murine-human monoclonal antibody approved for the treatment of cancer and several factors contributed to its success: specificity for the target, efficacy and safety. Rituximab binds the tetra-transmembrane protein CD20 expressed on the surface of B lymphocytes and acts as a vector for drugs, ensuring a highly specific therapy. Furthermore, it can be given as single agent as maintenance therapy.

ABVD is the abbreviation for the chemotherapy combination composed by doxorubicin (25 mg/m$^2$, given IV on day 1 and 15), bleomycin (10 units/m$^2$, given IV on day 1), vinblastine (6 mg/m$^2$, given IV on day 1 and 15) and dacarbazine (375 mg/m$^2$, given IV on day 1 and 15) every four weeks for a usual total of 6-8 cycles (Table 12).

**Table 12 – Frontline regimens in treating lymphoma**

| Drugs | Drug class | Major side effects |
|---|---|---|
| Cyclophospamide (C) | Alkylating | Hematologic, nausea |
| Doxorubicin (H) | Antracycline | Hematologic, cardiac |
| Vincristine (O) | Vinca-alkaloids | Neurophaty, nausea |
| Prednisone (P) | Cortico-steroid | Endocrine (diabetes) |
| Doxorubicin (A) | Antracycline | Hematologic, cardiac |
| Bleomycin (B) | Cytotoxic antibiotic | Pulmonary |
| Vinblastine (V) | Vinca-alkaloids | Hematologic |
| Dacarbazine (D) | Alkylating | Hematologic |

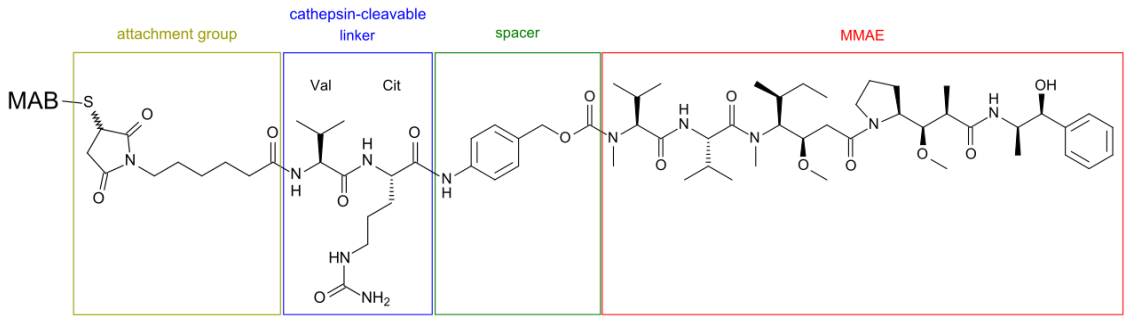
Important and more recent therapies used in treating lymphoma are antibody-drug conjugate (ADCs), immunoconjugates composed of chimeric monoclonal antibody tethered to several units of drug (payload) via a chemical linker. Once the antibody recognises the targeted cell surface antigen, the ADC–target complex is internalised via receptor-mediated endocytosis and the linker is rapidly cleaved to release intracellularly the effector payload.

An ideal target epitope should be homogeneously expressed in the surface of targeted tumor cells and have a limited expression in healthy tissues, avoiding off-target toxicity. An ideal linker should maintain the stability of the ADCs in the systemic circulation and reach the target site without premature cleavage. Linkers currently used are classified as either cleavable or non-cleavable. The first are sensitive to several intracellular conditions such as acid pH environment (hydrazone linkers), proteolysis (dipeptide sequences valine-citrulline and valine-alanine) or high intracellular glutathione concentrations (disulphide linkers). Non-cleavable linkers are more stable in the bloodstream and are degraded by the lysosomes (thioether linkers). The payloads are highly potent cytotoxic agents with $IC_{50}$ value in the subnanomolar range that target DNA or tubulin [38].

To date, only few ADCs received market approval, two of which are indicated for the treatment of lymphoma: Brentuximab vedotin and Polatuzumab vedotin. Both are composed of chimeric monoclonal antibodies (anti-CD30 and anti-CD79, respectively) covalently linked, via a protease-cleavable linker, to the tubulin inhibitor monomethyl auristatin E (MMAE). An average of four MMAE molecules are linked to each moAb (Figure 16) [39][40]. MMAE is a synthetic antineoplastic agent belonging to the family of dolastatins, marine cytotoxic pseudopeptides that inhibit cell division by blocking the polymerisation of tubulin, thus disrupting mitotic function and subsequently inducing cellular apoptosis [41].

Brentuximab vedotin was approved for the treatment of patients with relapsed or refractory systemic anaplastic large-cell lymphoma (ALCLs) and relapsed or refractory CD30-positive HL after autologous stem-cell transplantation (auto-HSCT) or after at least two prior ineffective multi-agent chemotherapy regimens in patients who are not auto-HSCT candidates. It was also recently approved in the front-line setting for HL in combination with doxorubicin, vinblastine, and dacarbazine. Polatuzumab vedotin, approved in June 2019, is indicated in combination with bendamustine and rituximab for adult patients with relapsed or refractory diffuse large B-cell lymphoma (DLBCL).

**Figure 16 – Brentuximab vedotin**



## 7.3 Lymphoma screening

Since antimitotic agents (vinca alkaloids and dolastatins) are widely used as frontline chemotherapeutics in treating lymphoma, no lymphoma cell line is included in the NCI panel and our lead compounds **15r** and **16j** showed to markedly interfere with tubulin polymerization, we investigated whether the antiproliferative activity of [1,2]oxazolo[5,4-*e*]isoindoles and 4,5,6,8-tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-*d*]isoxazoles could significantly interfere with lymphoma cell growth. Compounds **41f,i,j** and **58a,i,m-z** are currently under evaluation, whilst results have already been collected for derivatives reported in Table 13.

*Cell lines*

Experiments were performed on NHL cell lines. NHL is the most common and aggressive category of lymphomas, hence the development of new drugs and new approaches for treatment is increasingly challenging. Four different subtypes of NHL lymphoma were selected, so as to ensure maximum heterogeneity and test all the most severe forms affecting patients.

Established human cell lines derived from activated B-cell (ABC) and germinal center B-cell (GCB) diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL) and splenic marginal zone lymphoma (SMZL) were used.

- Diffuse large B-cell lymphoma (DLBCL) is the most common type of NHL and is characterized by large abnormal B cells that appear diffuse in the tissue rather than grouped together. Based on cell of origin profile, gene expression analysis identified two different DLBCL subgroups with distinct oncogenic mechanisms and responses to therapies: activated B-cell (ABC) and germinal center B-cell (GCB). ABC cells arise from postgerminal center B cells that are in the plasmacytic differentiation process, whilst GCB cells derive from normal germinal center B cells. ABC lymphomas have a worse prognosis than GCB subtype. The ABC cell line HBL1 and the GCB cell line SU-DHL-10 were selected for the screening.
- Mantle cell lymphoma (MCL) develops from small to medium-sized lymphoid B-cells with irregular nuclei that surrounds normal germinal center follicles. MCL cells generally carries the t(11;14)(q13;q32) translocation and constitutively overexpresses cyclin D1. Among all B-cell lymphomas, MCL has one of the worst

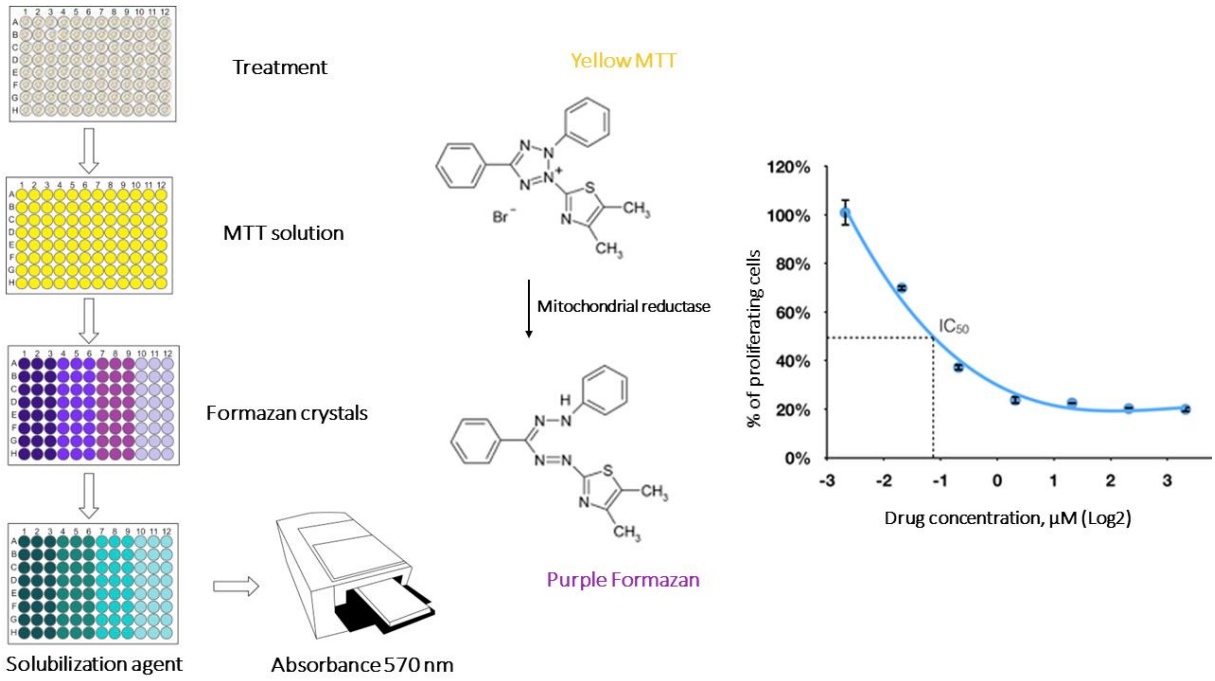
outcomes and is considered incurable. In the screening, MCL was represented by MINO cell line.

- Splenic marginal zone lymphoma (SMZL) arises from defective mature B lymphocytes that originate in secondary lymphoid follicles and then migrate to the marginal zones of mucosa-associated lymphoid tissue (MALT; small portions of lymphoid tissue in submucosal membranes of gastrointestinal tract, mouth, nose, pharynx, thyroid, breasts, lung, salivary glands, eye, skin), spleen, bone marrow and rarely lymph nodes. The SMZL cell line VL51 was used in the study.

*Cell proliferation assay*

The anti-proliferative activity of all compounds was assessed by using the MTT cell proliferation assay, a sensitive colorimetric test that measure cellular metabolic activity as an indicator of cell viability and cytotoxicity (Figure 17). The assay is based on the reduction of the yellow water-soluble 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) into insoluble purple-colored formazan crystals by the mitochondrial succinate dehydrogenase of viable cells. Since the total mitochondrial activity is related to the number of living cells after treatment, the amount of formazan product (detected at a wavelength of 570 nm) is directly proportional to cell viability [42].

**Figure 17 – MTT proliferation assay**



The activity of compounds was represented by the percentage of growth altered due to treatment. The percentage inhibition of proliferation was initially calculated for all investigated compounds by using the single dose of 1 μM. After a 72h incubation, compounds **36k** and **39k** showed the highest inhibitory activity against all four tested cell lines. The lowest percentage of proliferating cells was observed in VL51 cell line, where **39k** reduced growth by 51,3% compared to the control-treated cells. Its second-best proliferation inhibition was against SU-DHL-10 cell line with a percentage of proliferating cells down to 62%. Hence, the least responsive cell lines were HBL1 (80%) and MINO (82%). **36k** had the highest efficacy against MINO cell line with 66% of proliferation, followed by SU-DHL-10 with 79% (Table 13). Other derivatives induced a milder alteration of cell viability, with percentage growths reduced between 80 and 90%.

**Table 13 - Proliferating cells (%) after treatment with [1,2]oxazolo[5,4-*e*]isoindoles 36, 37, 38, 39,41 and 4,5,6,8-tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-*d*]isoxazoles 58**

| CPD | VL51 (MZL) | MINO (MCL) | HBL1 (ABC-DLBCL) | SU-DHL-10 (GCB-DLBCL) |
|---|---|---|---|---|
| **36a** | 86.11 | 101.14 | 82.16 | 75.31 |
| **36b** | 102.5 | 99.38 | 92.61 | 106.91 |
| **36c** | 98.33 | 106.03 | 103.3 | 110.31 |
| **36d** | 101.80 | 103.05 | 97.68 | 114.89 |
| **36e** | 106.52 | 101.52 | 101.67 | 116.91 |
| **36f** | 100.13 | 92.97 | 103.49 | 88.08 |
| **36g** | 100.69 | 100.07 | 103.69 | 123.93 |
| **36h** | 104.86 | 97.09 | 107.38 | 124.68 |
| **36i** | 92.08 | 101.67 | 110.8 | 145.85 |
| **36j** | 83.33 | 91.22 | 99.65 | 83.08 |
| **36k** | 99.72 | **66.33** | 90.14 | **79.04** |
| **36l** | 101.38 | 104.27 | 98.32 | 104.14 |
| **37a** | 94.02 | 100.15 | 103.15 | 205.31 |
| **37b** | 101.11 | 99.54 | 99.21 | 164.89 |
| **37c** | 106.25 | 96.18 | 102.01 | 150.95 |
| **37d** | 96.94 | 96.25 | 82.90 | 86.91 |
| **37e** | 116.80 | 95.03 | 89.31 | 113.61 |

| | | | | |
|---|---|---|---|---|
| **37f** | 109.02 | 98.32 | 94.28 | 158.08 |
| **37g** | 118.75 | 97.78 | 97.68 | 128.19 |
| **37h** | 103.05 | 86.18 | 97.33 | 164.46 |
| **37i** | 107.22 | 96.79 | 97.68 | 176.70 |
| **37j** | 105.83 | 98.09 | 106.05 | 168.51 |
| **38a** | 89.43 | 105.03 | 101.43 | 140.32 |
| **38b** | 87.98 | 83.21 | 90.65 | 92.83 |
| **38c** | 100.31 | 76.45 | 87.13 | 99.01 |
| **38d** | 95.13 | 109.06 | 102.83 | 88.17 |
| **38e** | 94.26 | 93.24 | 96.78 | 75.45 |
| **38f** | 89.74 | 102.14 | 79.15 | 91.03 |
| **39a** | 91.80 | 98.24 | 101.33 | 110.74 |
| **39b** | 99.16 | 103.20 | 102.66 | 112.12 |
| **39c** | 96.66 | 100.45 | 106.50 | 114.14 |
| **39d** | 122.91 | 99.77 | 104.13 | 115.10 |
| **39e** | 96.80 | 98.93 | 105.02 | 129.14 |
| **39f** | 97.08 | 101.29 | 105.12 | 136.70 |
| **39g** | 93.05 | 97.93 | 111.62 | 173.29 |
| **39h** | 106.11 | 103.12 | 97.38 | 125.31 |
| **39i** | 115.41 | 101.98 | 103.34 | 132.23 |
| **39j** | 105.69 | 100.53 | 99.45 | 130.74 |
| **39k** | **51.38** | 82.13 | 80.39 | **62.12** |
| **39l** | 102.36 | 99.69 | 100.44 | 157.02 |
| **41a** | 101.33 | 87.06 | 97.60 | 96.44 |
| **41b** | 105.79 | 92.14 | 100.81 | 99.27 |
| **41c** | 94.58 | 93.65 | 91.23 | 106.93 |
| **41d** | 87.38 | 91.98 | 97.12 | 98.41 |
| **41e** | 87.91 | 76.94 | 79.00 | 86.72 |
| **41g** | 93.04 | 79.12 | 96.18 | 108.65 |
| **41h** | 101.48 | 83.51 | 95.70 | 107.57 |
| **58b** | 102.04 | 95.93 | 133.83 | 127.53 |

| | | | | |
|---|---|---|---|---|
| **58c** | 98.41 | 96.63 | 118.27 | 117.08 |
| **58d** | 91.67 | 97.69 | 113.97 | 117.90 |
| **58e** | 88.53 | 96.44 | 97.41 | 98.95 |
| **58f** | 91.94 | 100.45 | 94.65 | 86.26 |
| **58g** | 80.92 | 91.84 | 88.99 | 89.51 |
| **58h** | 99.51 | 89.13 | 88.69 | 88.70 |
| **58j** | 98.71 | 96.61 | 107.30 | 106.50 |
| **58k** | 80.69 | 102.78 | 114.49 | 99.88 |
| **58l** | 95.51 | 97.78 | 109.70 | 107.28 |

MZL, marginal zone lymphoma; MCL, mantle cell lymphoma; ABC DLBCL, activated B-cell like diffuse large B cell lymphoma; GCB DLBCL, germinal center B-cell type diffuse large B cell lymphoma

Compounds that induced a more pronounced decrease of cell proliferation at 1 μM were further tested with a wider range of concentrations to establish the exact half maximal inhibitory concentration ($IC_{50}$) values. They were determined by a point-to-point analysis made by plotting the percentage of proliferating cells against the increasing drug concentrations used in the treatment. Seven different doses of each compound were used (0.156 – 0.312 – 0.625 – 1.25 – 2.5 – 5 - 10 μM). Thus, a linear regression line passing through all the data points was built and the intercept and slope of the line were used to estimate the $IC_{50}$ values of the single compound against every lymphoma cell line.

**36k** and **39k** showed good growth inhibitory effects on all lymphoma histotypes with $IC_{50}$ values in the low micromolar range (Table 14). The MINO cell line was more sensitive than the others, with $IC_{50}$ values of 1.4 μM for both compounds. Initially, the percentage of proliferation decreased drastically in a dose-dependent manner but once the concentration of 2.5 μM was achieved, cell growth did not exceed 30% for none of the two compounds. Both **36k** and **39k** reached $IC_{50}$ values of 1.7 μM against HBL1 cell line. VL51 and SU-DHL-10 cell viability was markedly reduced by the compounds, enough to reset the percentage of proliferation at concentrations higher than 5 μM. Concentrations that gave 10% or less of proliferation were discarded for further analyses due to the excessive cytotoxicity (Figure 18).

**Figure 18 - Linear regression analysis to determine the $IC_{50}$ values**

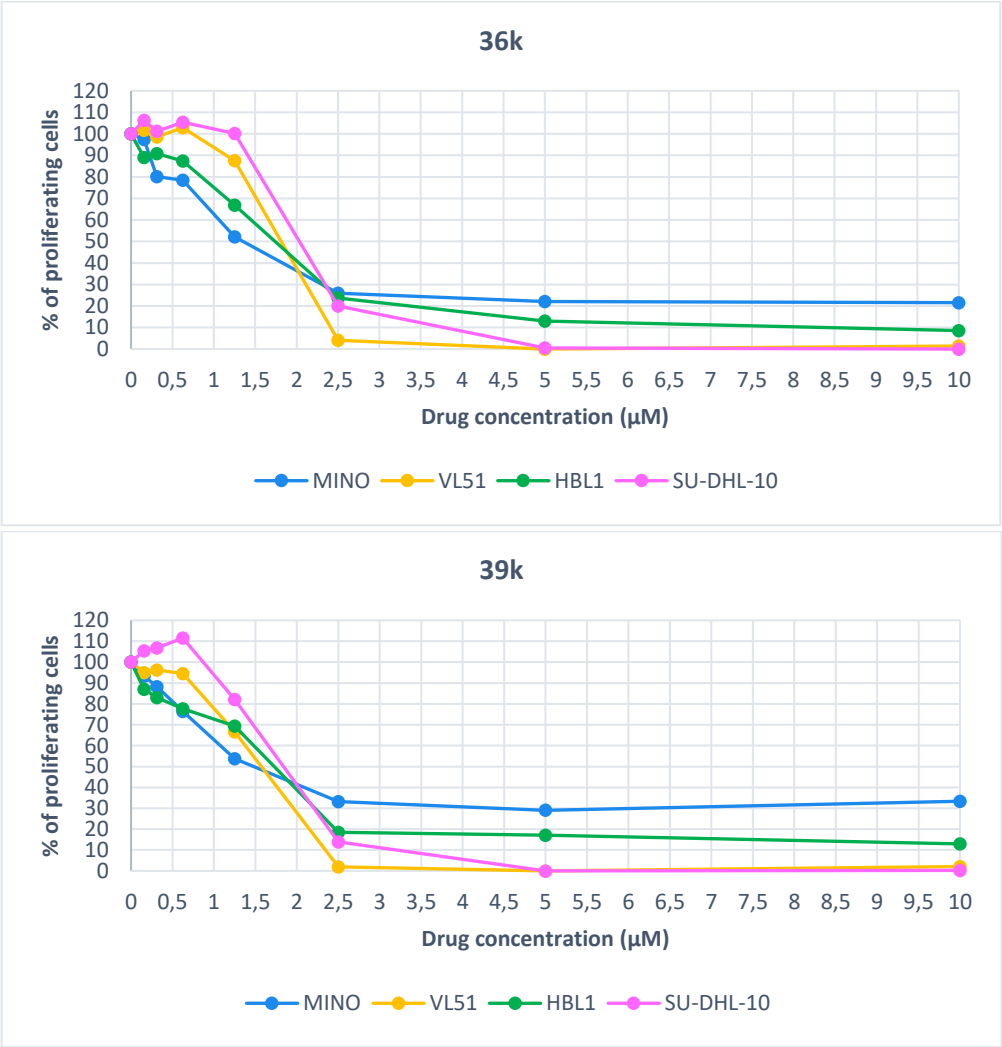



**Table 14 - $IC_{50}$ (µM) values of 36k and 39k**

| CPD | VL51 (MZL) | MINO (MCL) | HBL1 (ABC DLBCL) | SU-DHL-10 (GCB DLBCL) |
|---|---|---|---|---|
| **36k** | 1.8 | 1.4 | 1.7 | 2 |
| **39k** | 1.6 | 1.4 | 1.7 | 1.8 |

The *$IC_{50}$* values are expressed as micromolar

MZL, marginal zone lymphoma; MCL, mantle cell lymphoma; ABC DLBCL, activated B-cell like diffuse large B cell lymphoma; GCB DLBCL, germinal center B-cell type diffuse large B cell lymphoma

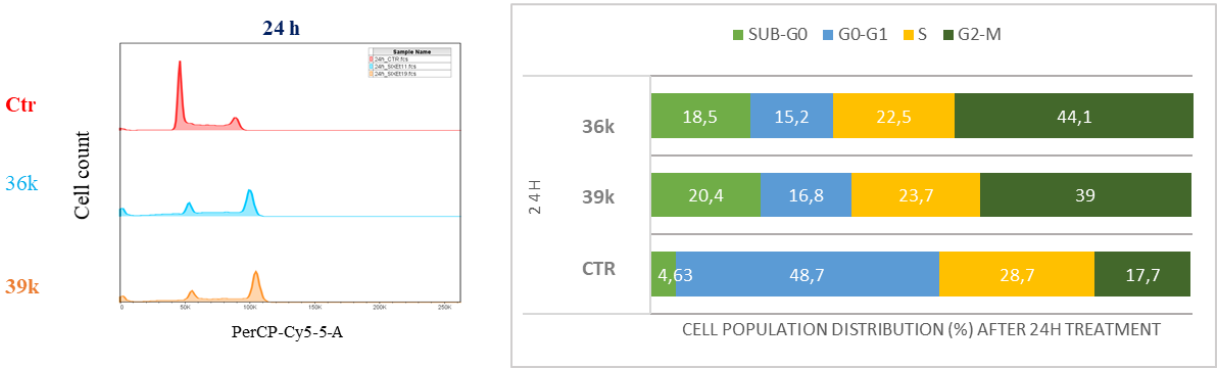
*FACS cell cycle assay*

In order to characterize the mechanism of action of the molecules and to determine whether they affected the cell cycle, cell number and cell cycle phase distribution was determined by flow cytometric analysis, following propidium iodide (PI) staining of the cells. Cell cycle

progression was analysed trough measurement of cellular DNA content, since its alterations during mitotic phases allows discrimination between G1, S, G2 and M phases. The assay was performed using MINO cells which were treated with concentrations of **36k** and **39k** corresponding to the double of their $IC_{50}$. This cell line was selected because it was the most sensitive to the compounds during the MTT proliferation assay. Cell population distribution was studied using a 24 h time point.

The treatment of MINO cells with **39k** induced a time and dose-dependent accumulation of cells in the G2/M phase, with 44.1% of cells arrested in G2/M. Compound **36k** exhibited a similar behavior, with accumulation of 39% of the cells. In both cases, the G2/M arrest was accompanied by a concomitant reduction of the percentage of cells in the G1 and S phase compared to the control (Figure 19). Such an effect is typical of antitubulin agents and comparable to that observed after exposure to taxol and vincristine.

**Figure 19 – Effect of 36k and 39k on cell cycle phase distribution**




*Annexin V apoptosis assay*

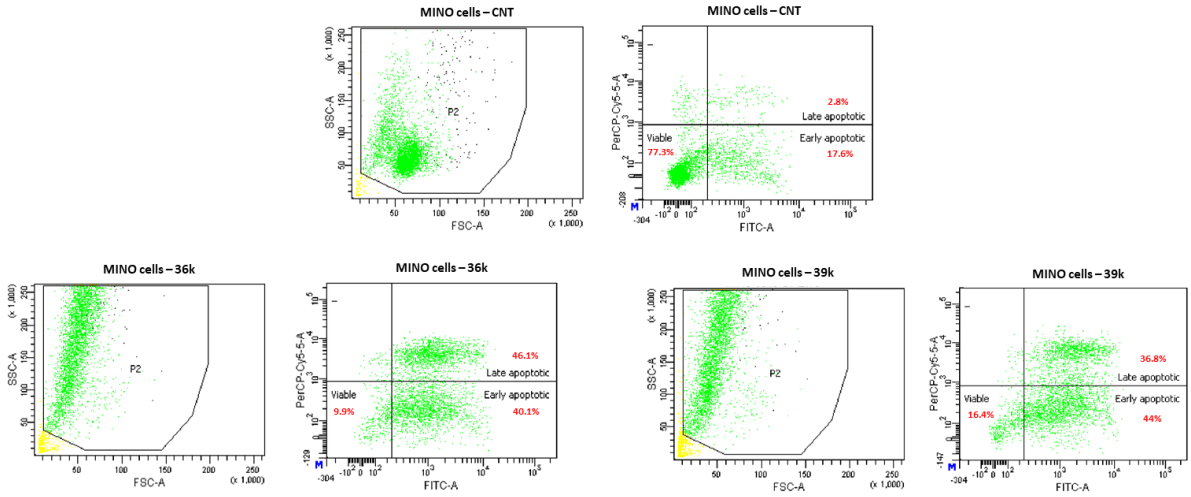
Cells undergo different morphological changes depending on their distinct death pathways: apoptosis (or programmed cell death) and necrosis. Annexin V was used to quantitatively analyze apoptotic cells after treatment with **36k** and **39k**, by detecting an early event in apoptosis, independent of the cell type: the loss of plasma membrane asymmetry and the resulting exposure of phosphatidylserine (PS) at the outer leaflet. Annexin V, which shows strong and specific affinity with PS residues, allows to distinguish apoptosis from necrosis.

MINO cells were incubated for 72 h with each compound using the double of $IC_{50}$ as concentration, then stained with Annexin V-FITC (fluorescein isothiocyanate conjugated) and also with propidium iodide (PI) to analyse secondary necrotic cells. Dual staining for annexin-V and with PI permits discrimination between live cells (annexin-V-/PI-), early apoptotic cells

(annexin-V+/PI-), late apoptotic cells (annexin-V+/PI+) and necrotic cells (annexin-V-/PI+). Cells were sorted by fluorescence-activated cell sorter (FACS).

As shown in Figure 20, the cells treated with **36k** and **39k** showed a significant accumulation of annexin-V positive cells with a marked reduction of vitality compared to control cells. In particular, for **36k** the percentage of apoptotic cells was 40.1% in early apoptosis and 46.1% in late apoptosis. Similarly, **39k** induced cell death by early apoptosis in the 44% of the cells and late apoptosis in the 36.8%. A very few percentages of cells (around 2%) are annexin-V-/PI+, confirming that both compounds induce cell death by apoptosis rather than necrosis.

**Figure 20 - Flow cytometric analysis of apoptotic cells after treatment of MINO cell line with 36k and 39k for 72 h**




Moreover, the best compounds among the [1,2]oxazolo[5,4-*e*]isoindoles **15**, **16** and the pyrrolo [2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazoles **17**, previously synthesized by our research group and widely studied in numerous cancer cell lines, were explored as anti-lymphoma agents. The same screening method described above was used. Derivatives showing promising results in the single dose screening (1μM), were selected for further analysis at different increasing concentrations (0-5μM). Considering the excellent antiproliferative activity data collected about these series, two VL51 cell lines with secondary resistance were added to the screening: the PI3K-delta inhibitor idelalisib resistant clone [43] and the BTK inhibitor ibrutinib resistant clone [44]. These models were generated by exposing the cells to $IC_{90}$ concentration of idelalisib and ibrutinib for several weeks until they acquired specific drug resistance [45].

After 72h treatment at the concentration of 1μM, several compounds showed potent growth inhibitory effects against all tested cell lines, with a percentage of proliferating cells down to

7.3 – 61% of the control-treated cells (Table 15). Among the [1,2]oxazolo[5,4-*e*]isoindoles **15** and **16**, compound **16k** and **15r** were the most potent proliferation inhibitors, reducing cell viability of more than 70%. The most striking effect was the cell growth reduction induced by **16k** against HBL1 cell line, with a percentage of proliferating cells down to 7.3%. A slightly higher growth rate was obtained against MINO cell line (8.6%), followed by SU-DHL-10 (13.8%), VL51 Idelalisib-resistant clone (22.7) and both VL51 and VL51 Ibrutinib-resistant clone (28.1%). The percentages of proliferating cells after treatment with **15r** ranged from 9.31 to 39.7%. In particular, the most sensitive cell lines were MINO (9.31%), SU-DHL-10 (12.0%) and HBL1 (12.9%), whilst the least responsive were VL51 cell lines with a percentage growth by approximately 35%.

Interestingly, many other derivatives induced a remarkable decrease in MINO cell growth, enough to say that this was the most affected cell line after the treatment with isoindoles **15** and **16**. Cell proliferation was by only 8.4% for **15c**, 8.7% for **15p**, 8.9% for **16e**, 10.5% for **15l** and 11.2% for **15t**. The second most sensitive cell line was SU-DHL-10 with percentages of proliferating cells of 12.7% for **15p**, 13% for **15l** and **15c**, 14% for **16e**.

Equally satisfying results were obtained with pyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazoles **17**. The treatment with the single dose of **17g**, **17p**, **17q**, **17u**, **17x** and **17y** showed a slower proliferation rate in all lymphoma histotypes and percentages of proliferating cells lower than 60%. The most pronounced activity was observed against MINO cell lines, to which **17g** reduced viability to 8.5%, **17q** to 8.9%, **17p** to 9.1%, **17y** and **17x** up to 9.5 and 9.8%, respectively. This confirmed the higher sensitivity of MINO cell line to our compounds, as already seen for the previous series.

**Table 15 - Proliferating cells (%) after treatment with [1,2]oxazolo[5,4-*e*]isoindoles of type 15,16 and pyrrolo [2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazoles of type 17**

| CPD | VL51 (MZL) | VL51 Idelalisib-resistant | VL51 Ibrutinib-resistant | MINO (MCL) | HBL1 (ABC DLBCL) | SU-DHL-10 (GCB DLBCL) |
|---|---|---|---|---|---|---|
| **15c** | **40.9** | **38.2** | **41.9** | **8.4** | **21.5** | **13.0** |
| **15g** | 68.0 | 54.2 | 64.3 | 83.4 | 94.4 | 72.1 |
| **15l** | **37.6** | **33** | **45.7** | **10.5** | **27.6** | **13.0** |
| **15p** | **37.9** | **35.7** | **43.2** | **8.7** | **30.7** | **12.7** |
| **15q** | 62.7 | 62.5 | 73.1 | 89.8 | 101.0 | 97.0 |
| **15r** | **34.7** | **32.3** | **39.7** | **9.31** | **12.9** | **12.0** |

| | | | | | | |
|---|---|---|---|---|---|---|
| **15t** | **45.8** | **40.8** | **52.6** | **11.2** | **36.0** | **27.5** |
| **15u** | **49.4** | **43.6** | **53.5** | **29.9** | **57.1** | **47.4** |
| **16b** | 70.4 | 77.4 | 93.9 | 85.8 | 88.9 | 88.6 |
| **16e** | **44.7** | **44.7** | **53.4** | **8.9** | **37.9** | **14.0** |
| **16f** | 82.3 | 92.4 | 95.9 | 90.6 | 104.7 | 83.2 |
| **16i** | **52.2** | **48.3** | **61.0** | **30.3** | **54.2** | **50.1** |
| **16k** | **28.1** | **22.7** | **28.1** | **8.6** | **7.3** | **13.8** |
| **17a** | 88.1 | 118.2 | 120.8 | 104.5 | 118.1 | 110.2 |
| **17b** | 87.4 | 106.7 | 100.3 | 104.3 | 101.4 | 70.2 |
| **17c** | 103.1 | 128.0 | 125.0 | 109.3 | 108.9 | 70.2 |
| **17d** | 119.9 | 135.7 | 112.0 | 112.1 | 122.2 | 141.4 |
| **17e** | 112.3 | 133.0 | 124.6 | 115.2 | 127.3 | 80.3 |
| **17f** | 110.3 | 126.8 | 109.8 | 108.9 | 123.0 | 74.6 |
| **17g** | **36.5** | **34.3** | **37.0** | **8.5** | **29.6** | **11.0** |
| **17h** | 84.0 | 78.4 | 73.0 | 101.4 | 113.6 | 72.4 |
| **17i** | 97.6 | 111.5 | 92.1 | 99.0 | 114.0 | 94.6 |
| **17j** | 107.7 | 115.2 | 125.0 | 111.5 | 146.2 | 61.7 |
| **17k** | 93.3 | 116.8 | 117.7 | 106.3 | 123.9 | 65.2 |
| **17l** | 71.3 | 63.6 | 80.3 | 103.8 | 127.6 | **51.1** |
| **17m** | 66.7 | 62.3 | 64.9 | 86.3 | 117.1 | **51.5** |
| **17n** | 87.4 | 116.4 | 102.0 | 112.3 | 125.1 | 66.3 |
| **17o** | 86.2 | 96.4 | 94.0 | 107.0 | 111.0 | 71.6 |
| **17p** | **40.1** | **34.3** | **40.5** | **9.1** | **24.1** | **11.3** |
| **17q** | **40.5** | **36.6** | **40.4** | **8.9** | **24.2** | **11.2** |
| **17r** | 94.5 | 122.7 | 120.4 | 98.8 | 109.1 | 78.9 |
| **17s** | 93.8 | 121.4 | 109.7 | 115.9 | 102.6 | 78.5 |
| **17t** | 108.1 | 111.4 | 104.4 | 104.1 | 104.2 | 69.5 |
| **17u** | **58.8** | **52.2** | **56.2** | **55.9** | 97.0 | **58.2** |
| **17v** | 106.8 | 127.8 | 102.9 | 110.2 | 105.1 | 76.9 |
| **17w** | 113.3 | 126.2 | 101.5 | 99.5 | 106.9 | 87.4 |
| **17x** | **40.4** | **37.7** | **44.4** | **9.8** | **29.0** | **13.3** |

| | | | | | | |
|---|---|---|---|---|---|---|
| **17y** | **38.1** | **40.2** | **40.8** | **9.5** | **20.7** | **11.5** |

MZL, marginal zone lymphoma; MCL, mantle cell lymphoma; ABC DLBCL, activated B-cell like diffuse large B cell lymphoma; GCB DLBCL, germinal center B-cell type diffuse large B cell lymphoma

Using a wider range of concentrations (0-5μM), selected derivatives showed potent growth inhibitory effects on some or all of the lymphoma cell lines with $IC_{50}$ values included between the low micromolar and the nanomolar range. In particular, **15c**, **16k**, **15l**, **15p**, **15r** and **17p** reached $IC_{50}$ values lower than 500 nM toward the full panel (Table 16). The major antiproliferative effect among all screened compounds was reached by derivative **16k**, whose $IC_{50}$ values ranged from 0.07 μM to 0.12 μM. The most sensitive cell lines were MINO and SU-DHL-10, both showing nanomolar $IC_{50}$ values of 0.07 μM, followed by the HBL1 cell line with a slightly higher $IC_{50}$ value (0.08 μM). The least growth inhibitory activity was towards the VL51 cell line ($IC_{50}$ = 0.12 μM). Similarly, **15r** exhibited a significant dose-dependent potent pattern of activity, which is evident from the low $IC_{50}$ values against all histotypes. Two out of four of the tested cell lines (MINO and HBL1) showed nanomolar $IC_{50}$ values as small as 0.08 and 0.09 μM, respectively. VL51 and SU-DHL-10 cell lines showed sub-micromolar values ($IC_{50}$ = 0.1 μM). Moreover, remarkable sub-micromolar activity was observed for the compounds **15c**, **15l** and **15p**.

Among the pyrrolo [2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazoles, **17p** was particularly effective against VL51, MINO and SU-DHL-10 cell lines, to which exhibited the same $IC_{50}$ value of 0.25 μM. The activity towards the HBL1 was slightly lower ($IC_{50}$ = 0.30 μM). Compound **17g** was the second best in potency and demonstrated high selectivity against the VL51 cell line ($IC_{50}$ = 0.2 μM), followed by SU-DHL-10 ($IC_{50}$ = 0.3 μM), MINO ($IC_{50}$ = 0.4 μM) and HBL1 cell line ($IC_{50}$ = 0.6 μM). In addition, submicromolar $IC_{50}$ were maintained towards all the cell lines by **17q** and **17y**.

Collectively, these results confirm the ability of our compounds to inhibit the proliferation of different lymphoma histotypes, some of them with excellent growth inhibitory activity.

**Table 16 - $IC_{50}$ (μM) values of selected [1,2]oxazolo[5,4-*e*]isoindoles of type 15,16 and pyrrolocyclohepta[1,2-*d*][1,2]oxazoles of type 17**

| CPD | VL51 (MZL) | MINO (MCL) | HBL1 (ABC DLBCL) | SU-DHL-10 (GCB DLBCL) |
|---|---|---|---|---|
| **15c** | 0.27 | 0.23 | 0.25 | 0.28 |
| **15l** | 0.25 | 0.23 | 0.27 | 0.26 |

| | | | | |
|---|---|---|---|---|
| **15p** | 0.27 | 0.37 | 0.47 | 0.5 |
| **15r** | **0.1** | **0.07** | **0.09** | **0.1** |
| **15t** | 0.5 | 0.65 | 0.95 | 1 |
| **15u** | 1.1 | 1.1 | 1.2 | 1.7 |
| **16e** | 1 | 0.75 | 0.73 | 0.83 |
| **16i** | 1.2 | 0.9 | 1 | 1 |
| **16k** | **0.12** | **0.07** | **0.08** | **0.07** |
| **17g** | **0.2** | **0.4** | **0.6** | **0.3** |
| **17l** | 3.2 | 4 | >5 | 3.6 |
| **17m** | 2.3 | 2.6 | >5 | 3 |
| **17p** | **0.25** | **0.25** | **0.3** | **0.25** |
| **17q** | 0.5 | 0.6 | 0.9 | 0.6 |
| **17u** | 2.4 | 3.4 | >5 | 2.2 |
| **17x** | 0.5 | 0.8 | 1 | 0.7 |
| **17y** | 0.8 | 0.9 | 0.9 | 0.9 |

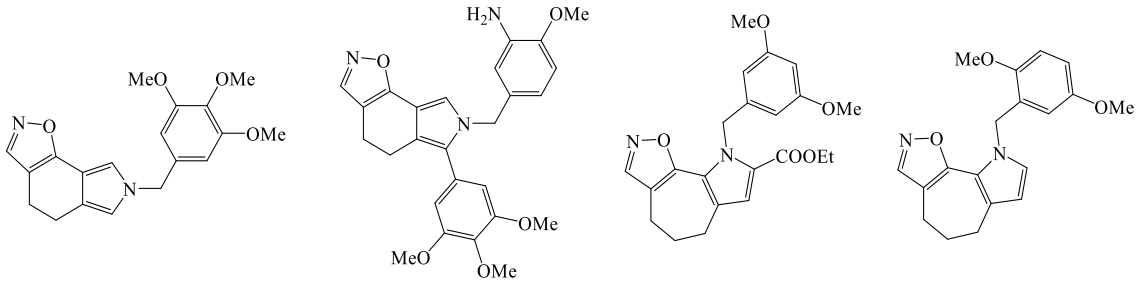
The $IC_{50}$ values are expressed as micromolar

MZL, marginal zone lymphoma; MCL, mantle cell lymphoma; ABC DLBCL, activated B-cell like diffuse large B cell lymphoma; GCB DLBCL, germinal center B-cell type diffuse large B cell lymphoma

Structure-activity relationships (SAR) suggest that the 3,4,5-trimetoxy substituent is crucial for the antiproliferative activity of [1,2]oxazolo[5,4-*e*]isoindoles of type **15** and **16**, with the most potent compound being **16k**, bearing a 3,4,5-trimetoxybenzyl group at the pyrrole nitrogen. Furthermore, the second-best candidate **15r** has a 3,4,5-trimetoxyphenyl substitution in position 6 of the tricyclic core. It is noteworthy that all the compounds selected for the multi-dose screening and therefore with the highest reduction of percentage of proliferation, have a 4-methoxy group at the benzyl bound to the pyrrole nitrogen, except for **16e** bearing a 3-methoxy.

Data analysis show that the enlargement of the cycloalkyl ring maintains a good effect on the biological activity, although pyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazoles **17** are not more potent compared to [1,2]oxazolo[5,4-*e*]isoindoles **15**,**16**. The presence of an ethoxycarbonyl group at position 8 is crucial for activity. In fact, compound **17p** exhibits the lowest $IC_{50}$. Instead, the presence of chlorine in position 7 generally reduces activity compared to the corresponding parent compound (e.g., **17p** and its chlorinated compound **17y**). A dimethoxybenzyl group at the pyrrole nitrogen seems important to obtain a remarkable antiproliferative activity, especially when one of them is in position 3 (Figure 21).

**Figure 21 – Most potent compounds among [1,2]oxazolo[5,4-*e*]isoindoles and pyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazoles in lymphoma cell lines**




**16k** **15r** **17p** **17g**

In the light of these results, [1,2]oxazole derivatives are confirmed as effective candidates in reducing the growth of four different histotypes of lymphoma, suggesting a possible therapeutic potential of the compounds for the management of this disease.

## 7.4 Evaluation of BET inhibitor birabresib in combination with LRRK2 inhibitor in lymphoma models

The Bromodomain and Extra-terminal domain (BET) family of proteins (BRD2/3/4) are transcriptional regulators involved in several signaling cascades, including the expression of essential growth-promoting genes in a number of hematological and solid tumors [46]. Therefore, in the last few years, BET proteins have emerged as novel targets for cancer treatment. Several BET inhibitors demonstrated promising preclinical antitumor activity, leading to clinical studies mostly focused on the treatment of leukemia and lymphoma. Among them, the BET inhibitor birabresib (MK-8628/OTX015) showed relevant results in different lymphoma models, both as a single agent and in combination [47][48][49][50][51] and became the first in class to undergo early clinical activity few years ago [52].

In order to identify agents to combine with birabresib to increase its therapeutic efficacy against lymphoma, a pharmacological screening of 348 compounds, mostly FDA approved, in combination with birabresib was developed. AKT, SRC, JAK and LRRK2 inhibitors came out from the *in vitro* screening as the most interesting and active agents in combination with birabresib. While the first targets had a well-defined role in tumorogenesis, LRRK2 protein was associated with lymphoma treatment for the first time. Leucine-rich repeat kinase 2 (LRRK2), also known as dardarin, is a kinase enzyme largely diffuse in the cytoplasm and in the mitochondrial outer membrane. The role of LRRK2 is not fully elucidated but increasing evidence suggests that is implicated in different cellular functions such as autophagy, cellular proliferation, cytoskeletal dynamics, intracellular trafficking, neurotransmission and

inflammatory response. To date, more than 100 different mutations in the LRRK2 gene have been reported, with the G2019S being one of the most common genetic causes of Parkinson's disease [53][54][55]. Since these alterations increase LRRK2 kinase activity, they are classified as gain-of-function mutations [56][57]. Moreover, the cell types that most highly express LRRK2 are immune cells, including monocytes, macrophages, B lymphocytes, dendritic cells and microglia, hence it's a putative target for lymphoma treatment.

The combination of LRKK2-IN with OTX015 was synergistic in 6/6 lymphoma cell lines: MCL (REC1), CLL cell line (MEC1) and GCB-DLBCL cell lines (OCI-LY-19, WSU-DLCL2, FARAGE, SU-DHL-8). Additionally, it performed relevant *in vivo* synergistic effect, delaying tumor growth in female NOD-Scid mice. The synergism was assesses using the Chou-Talalay method based on the median-effect equation. Data were expressed as combination index (CI): strong synergism (<0.3), synergism (<0.9), additive (0.9-1.1), antagonism/no benefit (> 1.1).

Based on the encouraging *in vitro* and *in vivo* results, we decided to investigate the mechanism of action of the drug combination. The rationale for combining BET and LRRK2 inhibitors in lymphoma therapy could be explained by the interaction with the serine-threonine kinase glycogen synthase kinase-3 beta (GSK-3β), one of the main downstream targets of PI3K pathway involved in cellular metabolism, inflammation, mitochondrial dysfunction and cell survival. BET inhibitors proved to induce GSK-3β (S9) inhibitory phosphorylation, thus enhancing antiproliferative effects in a panel of diffuse large B cell lymphoma (DLBCL) and Burkitt lymphoma cell lines [58]. Furthermore, LRRK2 demonstrated a stimulatory effect on GSK-3β kinase activity, which induces high level of phosphorylation of tau protein. The contribution of LRRK2 to tau abnormal phosphorylation may play a role in pathogenesis [59]. Another signal transduction molecule implied in cellular growth and considered a convincing substrate of LRRK2 is AKT1. The LRRK2-mediated phosphorylation at Ser473 and activation of Akt1 might insure cell survival and protection of cells from apoptosis.

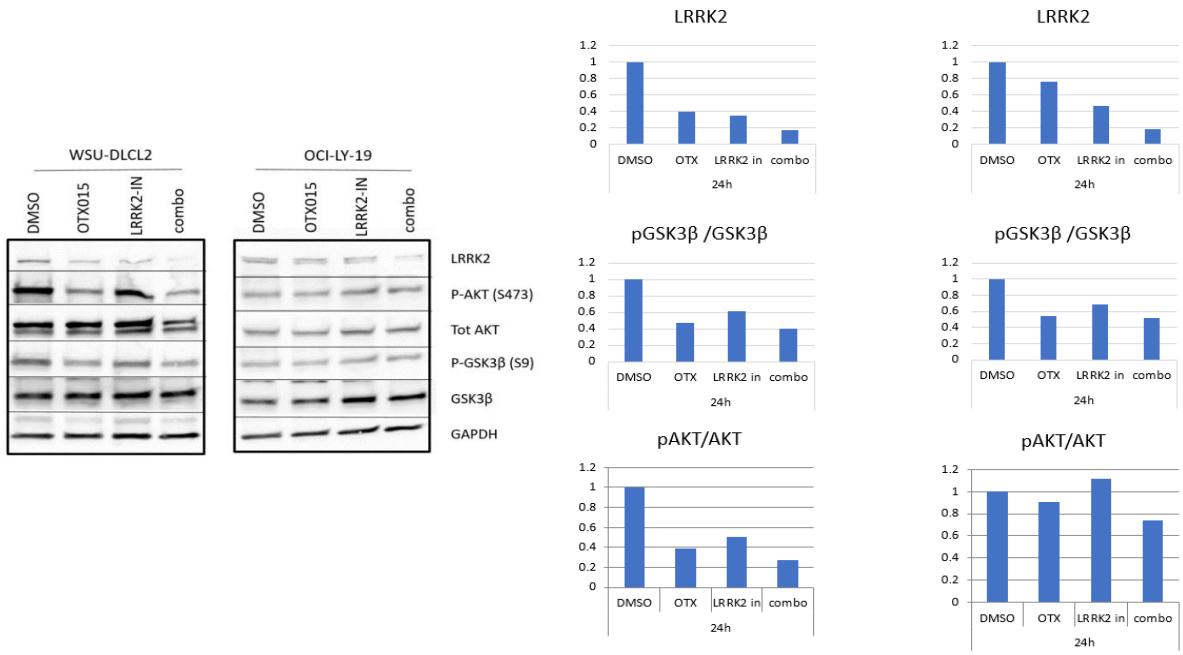
*Western blotting analysis*

To clarify the molecular mechanisms by which the combination of OTX015 and LRRK2-IN induces lymphoma suppression, western blot analysis were performed using antibodies against LRRK2, non-phosphorylated Akt1, phosphorylated Akt1, non-phosphorylated GSK3β (S9) and phosphorylated GSK3β (S9).

Two distinct types of GCB-DLBCL cell lines were used in the study: OCI-LY-19 and WSU-DLCL2. The latter was the same cell line injected into the mice for *in vivo* experiments. Birabresib/OTX015 and LRRK2-IN were purchased from Selleckchem (TX, USA). OCI-LY-19 and WSU-DLCL2 were exposed to OTX015 (500nM) and LRRK2-IN (2000 nM) both as single agents and in combination for 24h.

At molecular level, the combination of OTX015 with LRRK2-IN implied a significant down-regulation of p-AKT (Ser 473) and LRRK2 in both GCB-DLBCL cell lines investigated (Figure 22). In particular, LRRK2 expression was reduced by 80% in both OCI-LY-19 and WSU-DLCL2 by the combination of the two drugs when compared to control (DMSO). Compared to OTX015 and LRRK2-IN single treatments, the combination therapy down-regulated LRRK2 to about 50% in WSU-DLCL2 and a little more than 60% in OCI-LY-19. The phosphorylation of AKT was more affected in WSU-DLCL2, where its expression after combinatory treatment was reduced by 70% compared to the control cells and by 40% and 50% compared to cells that received single OTX015 and LRRK2-IN respectively. The efficacy of the compounds in inducing pAKT down-regulation was less pronounced in OCI-LY-19. OTX015/LRRK2-IN treatment compared to single OTX015 reduced by only 22% the expression of pAKT. Controversially, the treatment with LRRK2-IN alone increased the phosphorylation of AKT.

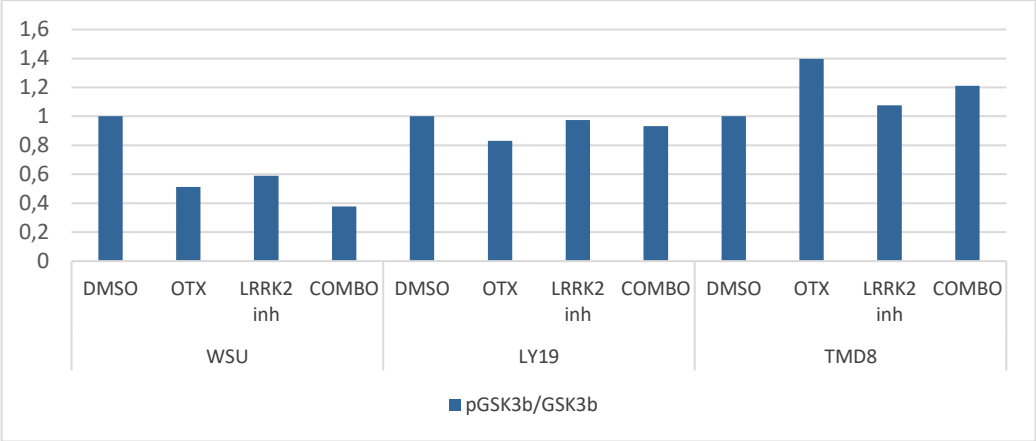
**Figure 22 – Birabresib combination with LRRK2-IN induces down-regulation of p-AKT (S473) and LRRK2**



The down-regulation of p-GSK3β (S9) induced by the combo was higher than the single agents. Compared to control cells, 50% of reduction after OTXO15 alone treatment, 40% after LRRK2-IN alone treatment and 60% after combination treatment were obtained in WSU-DLCL2 cell line; 45% of reduction after OTXO15 alone treatment, 30% after LRRK2-IN alone treatment and 50% after combination treatment were observed in OCY-LY-19 cell line. Figure 22 is representative of two independent experiments and the expression of the proteins were normalized to the respective counterpart and to the housekeeping GAPDH.

Moreover, we decided to evaluate the efficacy of OTX015/LRRK2-IN treatment in a different lymphoma sub-type, since data reported in literature suggested opposite behaviours in the ABC-DLBCL TMD8 cell line by using BET inhibitors (JQ1 and CPI-203). As in the previous experiment, cells were treated with OTX015 at 500nM and LRRK2-IN at 2000 nM for 24h. The modulation of proteins, in particular p-GSK3β (S9), was confirmed to be cell line dependent. In fact, while GCB-DLBCL WSU-DLCL2 showed some down-regulation, in the ABC-DLBCL TMD8 the protein expression was not reduced by single agents or their combination. On the contrary, an up-regulation of p-GSK3β (S9) expression was reported in the ABC histotype (Figure 23).

**Figure 23 - Birabresib induces down-regulation of p-GSK3β (S9) in a cell line dependent manner**




These results propose to consider the LRRK2 inhibitors in combination with drugs with epigenetic action, in particular BET inhibitors, as putative therapy for some lymphoma sub-types thanks to their efficacy in reducing expression of LRRK2, pAKT and p-GSK3β.

## 8 Tubulin assays

Within the classes of [1,2]oxazolo[5,4-*e*]isoindoles **15,16**, lead compounds **15r** and **16j** showed potent antimitotic effect with G2/M phase cell cycle arrest as result of tubulin polymerization impairment. In order to assess if the entire class of [1,2]oxazolo[5,4-*e*]isoindoles **15,16** and pyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazoles **17** were able to bind to tubulin, they were tested for their antitubulin activity in comparison with reference compound CA-4, which potently inhibits tubulin assembly by interacting with the colchicine site on β-tubulin.

The Friederick National Laboratory for Cancer Research evaluated:

- The cytotoxicity against human breast cancer MCF-7 cells;
- the inhibition of tubulin assembly;
- the inhibition of colchicine binding.

The cytotoxicity using human breast cancer MCF-7 was performed since it has excellent correlation with tubulin assembly $IC_{50}$ values, extent that $IC_{50}$ over 1 μM in the MCF-7 cells generally indicates low or no activity as a tubulin assembly inhibitor. Reaction mixtures in the assembly assay contained 9 μM (0.9 mg/mL) of tubulin and compounds that reached $IC_{50}$'s < 6 μM were further evaluated for their ability to compete the colchicine-tubulin interaction. The colchicine assay was performed with 0.5 μM tubulin, 5.0 μM [$^3$H]colchicine and 5.0 μM inhibitor.

Although some compounds showed $IC_{50}$ values > 6 μM in the tubulin assembly assay, several others, shown in Table 17, were more active with $IC_{50}$ values between 1.5 and 5.7 μM. Compounds with $IC_{50}$ < 2 μM proved to be strong inhibitors of tubulin assembly. In particular, compound **15k** reached $IC_{50}$ value of 1.5 μM whilst both **15l** and **15r** showed $IC_{50}$ value of 1.7 μM. Compounds **17y** and **15u** induced inhibition of tubulin assembly with slightly superior $IC_{50}$ values (1.9 and 2 μM, respectively). A value of 1.2 μM was obtained for CA-4.

Strong inhibition of colchicine binding was also obtained by several compounds that showed > 50% inhibition in the colchicine assay. The nine most active compounds as inhibitors of colchicine inhibited binding by 55- 88% versus 97 % for CA-4. Overall, the most powerful compound was **16j**, which displayed 88% inhibition of colchicine binding. Despite no compound was as active as CA-4 in any assay, these results confirm the antitubulin activity proposed in the starting rational.

Studies investigating the effects of the newly synthesized [1,2]oxazolo[5,4-*e*]isoindoles and 4,5,6,8-tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-*d*]isoxazoles are currently ongoing.

**Table 17 - Inhibition of tubulin assembly and colchicine binding by [1,2]oxazolo[5,4-*e*]isoindoles and pyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazoles**

| CPD | MCF-7 cytotoxicity | Inhibition of tubulin assembly | Inhibition of colchicine binding |
|---|---|---|---|
| | $IC_{50}$ (μM) ± SD | $IC_{50}$ (μM) ± SD | % Inhibition ± SD 5 μM inhibitor |
| **CA-4** | 18 ± 4 | 1.2 ± 0.08 | 97 ± 0.9 |
| **15b** | >5 | 4.6 ± 0.1 | 21 ± 3 |
| **15c** | 0.38 ± 0.1 | 2.1 ± 0.2 | 77 ± 0.5 |
| **15d** | 4.5 ± 0.9 | 3.4 ± 0.6 | 34 ± 0.4 |
| **15k** | 0.34 ± 0.05 | 1.5 ± 0.0007 | 55 ± 0.4 |
| **15l** | 0.37 ± 0.08 | 1.7 ± 0.2 | 72 ± 2 |
| **15p** | 0.6 ± 0.03 | 3.2 ± 0.1 | 34 ± 4 |
| **15r** | 0.24 ± 0.06 | 1.7 ± 0.06 | 57 ± 2 |
| **15u** | 5 | 2.0 ± 0.3 | 55 ± 1 |
| **16e** | 0.55 ± 0.07 | 5.2 ± 0.7 | 69 ± 1 |
| **16j** | 0.29 ± 0.02 | 2.8 ± 0.4 | 88 ± 2 |
| **16k** | 0.21 ± 0.08 | 2.3 ± 0.3 | 80 ± 0.6 |
| **17g** | 0.32 ± 0.04 | 3.2 ± 0.06 | 38 ± 0.7 |
| **17h** | 5 | 5.7 ± 1 | 25 ± 3 |
| **17p** | 0.37 ± 0.08 | 2.6 ± 0.03 | 62 ± 2 |
| **17q** | 0.61 ± 0.04 | 4.6 ± 0.8 | 25 ± 0.01 |
| **17y** | 0.62 ± 0.04 | 1.9 ± 0.1 | 42 ± 5 |

# 9 Computational studies

## 9.1 Molecular modeling

To further investigate the binding mode of the best active compounds in the colchicine site, molecular modelling simulations were carried out using the Schrödinger Suite version 2018. Docking studies of the new derivatives were performed by using crystal structures of tubulin downloaded by protein data bank (PDB). Models having PDB codes 4O2B and 1Z2B were selected as colchicine-bound and vinblastine-bound co-crystal structures, respectively. All the compounds belonging to class of pyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazoles **17** were docked into the colchicine and vinblastine binding sites, by selecting for each of them the pose with the best G-Score (Kcal/mol). As shown in Table 18, a good affinity for the colchicine site was observed for all compounds, thus confirming their specificity for this binding pocket.

Molecular modeling studies were performed on the 3N2G model, which displays two additional neighboring pockets (zones 2 and 3) in addition to the main site (zone 1) of the tubulin colchicine domain.

**Table 18 - G-Score (Kcal/mol) values for the best poses of all compounds complexed with both 4O2B and 1Z2B crystallographic structures**

| Compound | Gscore *(Kcal/mol)* | |
|---|---|---|
| | **4O2B** | **1Z2B** |
| **Colchicine** | -9.79 | -4.85 |
| **17a** | -7.06 | -4.01 |
| **17b** | -4.92 | -3.10 |
| **17c** | -6.59 | -4.30 |
| **17d** | -7.10 | -4.08 |
| **17e** | -7.03 | -4.36 |
| **17f** | -7.48 | -3.60 |
| **17g** | -7.55 | -4.57 |
| **17h** | -7.83 | -4.81 |
| **17i** | -7.60 | -4.90 |
| **17j** | -6.43 | -3.81 |
| **17k** | -7.30 | -4.13 |
| **17l** | -7.16 | -4.82 |
| **17m** | -7.53 | -4.47 |
| **17n** | -7.25 | -4.35 |
| **17o** | -7.84 | -5.61 |
| **17p** | -7.28 | -4.78 |
| **17q** | -8.35 | -4.66 |

| | | |
|---|---|---|
| **17s** | -7.21 | -5.04 |
| **17t** | -7.11 | -5.39 |
| **17u** | -7.07 | -5.90 |
| **17v** | -7.45 | -4.42 |
| **17w** | -6.71 | -4.79 |
| **17x** | -7.21 | -3.44 |
| **17y** | -7.31 | -5.01 |
| **17z** | -5.21 | -3.48 |

The best docking poses of active compounds with tubulin structure 4O2B, containing zones 1 and 2 of the colchicine site, showed strong hydrophobic interactions with β-tubulin residues L248, A250, A354, I318, A316 and L255 (Figure 24). In particular, **17p** and **17y**, displayed a binding geometry similar to that of colchicine in zones 1 and 2 of the pocket, by directing their methoxybenzyl groups towards the C241 residue as shown in Figure 24D and 24F). Furthermore, compound **17y** established an H-bond between the oxazole moiety and the backbone of N249and a halogen bond between its chlorine and the backbone of V181.

Unfavorable steric contacts were formed with an additional hydrophobic pocket placed in the β subunit, formed by residues E200, L255, A316, A317, A354, C241 and T179 (Figure 25).

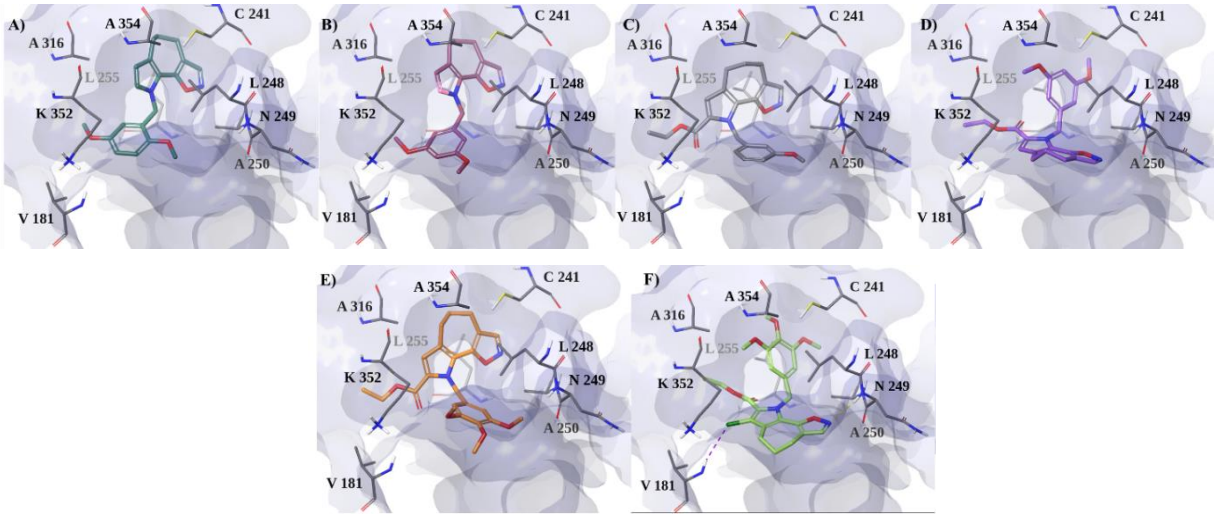



**Figure 24 - The best docked-poses of A) 17g, B) 17h, C) 17m, D) 17p, E) 17q and F) 17y with the 4O2B crystal structure of tubulin, depicting zones 1 and 2 of the colchicine site. Tubulin is represented as a faded blue surface, while ligands and residues, involved in the most important interactions, are shown as sticks. Halogen bond and π-cation interactions are indicated as dashed violet and dark-green lines, respectively.**

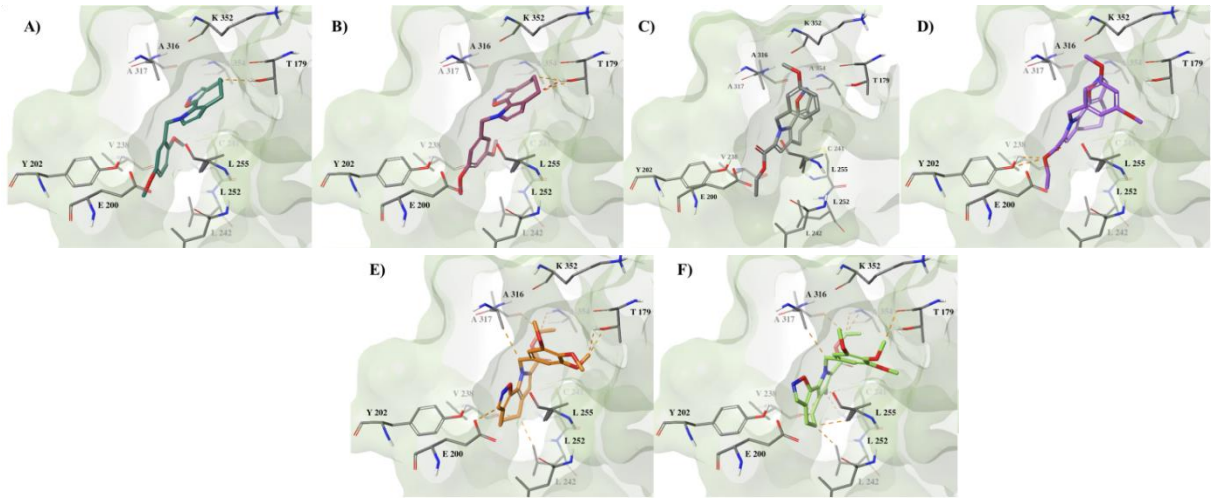
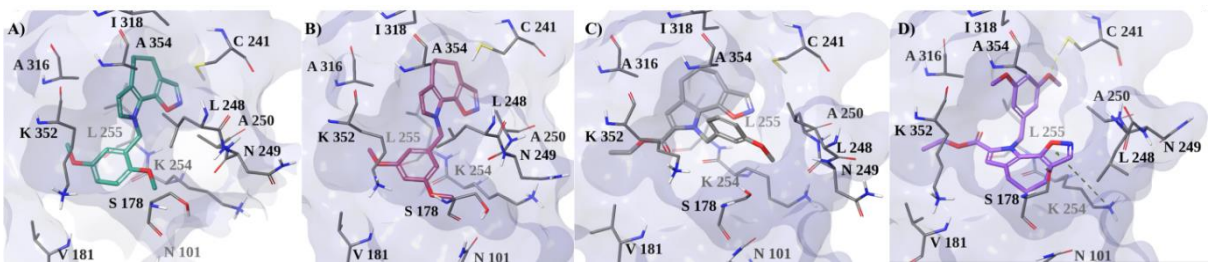



**Figure 25 - The best docked pose of A) 17g, B) 17h, C) 17m, D) 17p, E) 17q and F) 17y against the crystal structure of tubulin with the PDB code 3N2G, depicting zones 2 and 3 of the colchicine binding site. Tubulin is shown in the yellow-green surface, while ligands and residues, involved in the major electrostatic interactions, are shown as sticks. Poor contacts are indicated as dashed orange and red lines.**

In order to deepen the analysis and to investigate the possibility of induced-fit phenomena in the tubulin recognition process of the ligands, the best docking poses of **17g**, **17h**, **17m, 17p, 17q** and **17y** against the 4O2B model were submitted to explicit water solvent molecular dynamics (MD) simulations. The X-ray model of 4O2B, containing colchicine in its binding pocket, was included in similar calculations as reference.

The most representative MD structure for **17p** showed the establishment of an H-bond between the methoxy group of the tubulin molecule and the side chain of C241. Tubulin also adjusted its residues to permit a π-cation between its oxazole ring and K254 (Figure 26D). Further hydrophobic interactions with β-tubulin K254, A250, L255, A316 and A354 stabilized the complex. Similarly, **17y** engaged an H-bond between its 4-methoxy group and C241 and several hydrophobic interactions with L248, A250, L255, I318 and A354 (Figure 26F). Compared to its docking pose (Figure 24F), the absence of the halogen bond and the H-bond with N249 was observed, indicating these interactions as useful in the recognition process, but not in complex stabilization. Moreover, during MDs **17g** and **17h** increased their hydrophobic interactions with the L255, A316, I318, K352 and A354 residues (Figure 26).



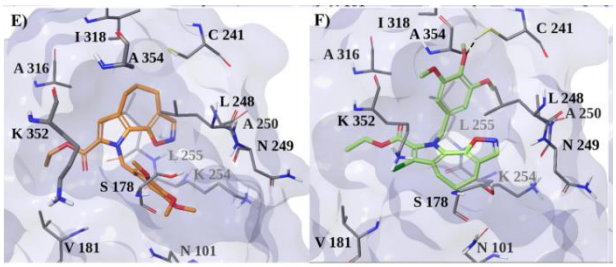



**Figure 26 - The most representative MD structure of tubulin (PDB code 4O2B) complexed with A) 17g, B) 17h, C) 17m, D) 17p, E) 17q and F) 17y. Tubulin is depicted as a pale faded blue surface, while ligands and residues are shown as sticks format. Hydrogen bond and π-cation interactions are indicated as dashed black and dark-green lines, respectively.**

Since several compounds from series **15** and **16** had a good activity in the tubulin binding assays, their binding modes were investigated through molecular modelling studies as well. Preliminary data were collected for compounds **15c, 15l, 15r, 16e, 16j** and **16k.** They were docked into the colchicine site, and, for each of them, the pose with the best thermodynamic profile (ΔGbind) was selected and analyzed (Table 19). According to inhibition of tubulin assembly assays, no compound exhibited better binding free energy than the reference compounds. The values of binding free energy for the compounds ranged between -79.04 and -61.99 kcal/mol, by showing the complex of **16k** with tubulin as the best one, mainly thanks to the ΔGbind vdW component. Moreover, except for the **15c**, we observed that all the complexes of the analyzed compounds were characterized by an improvement of the Columbian (ΔGbind Coul) term than colchicine. Conversely, the lipophilic (ΔGbind_vdW and ΔGbind Lipo) and solvation (ΔGbind SolvGB) components, which play an important role in the tubulin binding of both reference compounds, owned higher values for new synthetized ligands.

**Table 19 - For each best complex, free binding energy values, obtained after MM-GBSA calculation, are shown. Binding free energy and related components are indicated as ΔGbind, ΔGbind Coul, ΔGbind Lipo, ΔGbind SolvGB and ΔGbind vdW and are expressed as Kcal/mol**

| Compound | $\Delta G_{bind}$ | $\Delta G_{bind\ Coul}$ | $\Delta G_{bind\ Lipo}$ | $\Delta G_{bind\ SolvGB}$ | $\Delta G_{bind\ vdW}$ |
|---|---|---|---|---|---|
| **Colchicine** | -113.75 | -9.92 | -49.08 | 14.91 | -68.05 |
| **CSA-4** | -90.69 | -13.13 | -44.18 | 19.07 | -55.85 |
| **15c** | -67.13 | -1.46 | -38.61 | 19.74 | -48.95 |
| **15l** | -64.88 | -9.36 | -39.31 | 22.58 | -39.55 |
| **15r** | -61.99 | -13.06 | -38.21 | 22.82 | -45.33 |

| | | | | | |
|---|---|---|---|---|---|
| **16e** | -62.01 | -18.20 | -25.21 | 27.43 | -44.24 |
| **16j** | -68.71 | -10.90 | -34.57 | 27.69 | -51.39 |
| **16k** | -79.04 | -10.55 | -39.08 | 29.72 | -58.41 |

Regarding the series of compounds **15** (Figure 27), we observed different kind of binding mode for the three analysed ligands. In particular, although **15c** and **15l** place their benzyl moiety in the same region of tubulin active site, they showed an inverted accommodation of the tricyclic ring and the phenyl group (Figure 27A-B). As shown in Figure 27A-B, **15c** established only hydrophobic interactions with the tubulin pocket, according to the energy analysis (Table 19) which showed its $\Delta G_{bind\ vdW}$ value better than the **15l**. Conversely, the better electrostatic term of the **15l** is related to the presence of the meta amino group in the benzyl group and the inverted position of the tricycle ring, which allowed the establishment of two H-bonds with the backbone of the residues S178 and V181, respectively. Finally, the compound **15r** showed a peculiar binding mode, characterized by the tricyclic ring facing the GTP residue and the 3,4,5,-trimethoxyphenyl moiety involved in a π-cation interaction with the side chain of the K352. Thus, the compound is stabilized by a lot of hydrophobic interactions with the tubulin pocket, as shown by the $\Delta G_{bind\ Lipo}$ and $\Delta G_{bind\ vdW}$ terms.

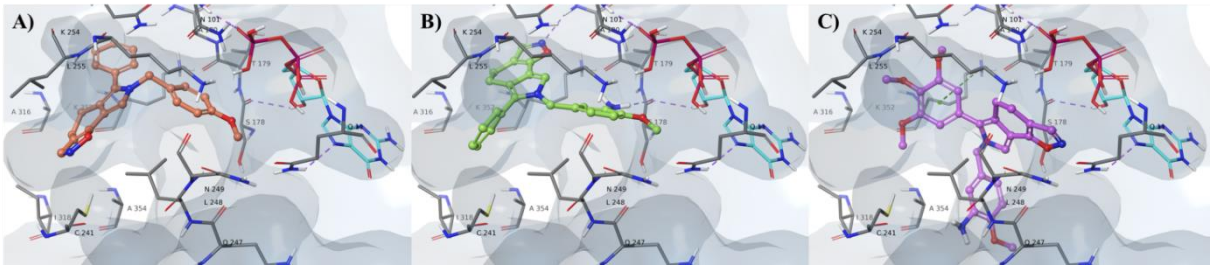



**Figure 27 - The best thermodynamic structure of the tubulin complexed with A) 15c, B) 15l and C) 15r, after docking and MMGB-SA calculation. Tubulin is represented as faded azure surface, GTP and residues, involved in the major electrostatic interactions, are shown as sticks and ligands are depicted as ball and sticks. Hydrogen bond and π-cation interactions are indicated as purple and green dashed lines, respectively.**

Regarding compounds of type **16** (Figure 28), we observed a very similar binding mode for **16j** and **16k** (Figure 28B-C), with the tricyclic portion well orienting towards GTP residue, capable of making numerous good contacts with the side chains of K254, N258 and K352. Interestingly, these compounds share with colchicine the same position of methoxylic mojeties, facing to the side chain of the C241 with the correct distances to dynamically interact by means the establishment of one or more H-bonds.

Conversely, **16e** (Figure 28A) showed a different pattern of interactions, although it is different from **16j** and **16k** only for the methoxylic portion. Indeed, the smaller steric hindrance allows the isoxazole ring to insert deeper into the binding pocket, thus allowing the formation of an H-bond with the N101 side chain but turning away the only methoxy group in meta to the benzyl ring from the right interaction with the key residue C241.

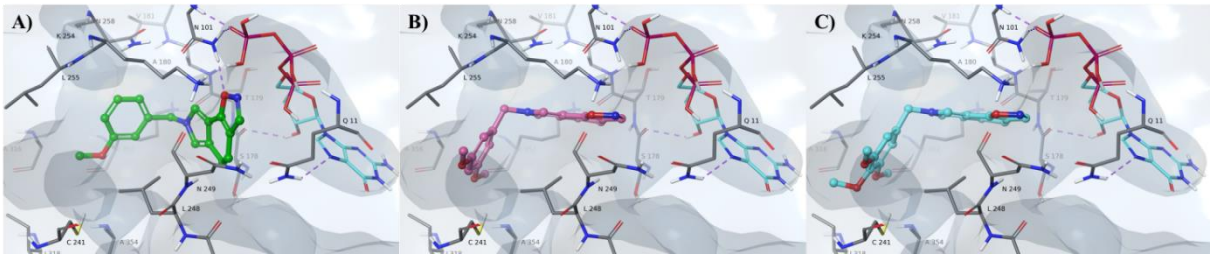



**Figure 28 - The best thermodynamic structure of the tubulin complexed with A) 16e, B) 16j and C) 16k, after docking and MMGB-SA calculation. Tubulin is represented as faded azure surface, GTP and residues, involved in the major electrostatic interactions, are shown as sticks and ligands are depicted as ball and sticks. Hydrogen bond interactions are indicated as dashed purple lines.**

## 9.2 Crystallographic studies

To better explore the interaction of [1,2]oxazolo[5,4-*e*]isoindoles, their new derivatives and 4,5,6,8-tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-*d*]isoxazoles with the colchicine-binding site and obtain a detailed description of how the bond occurs, X-ray crystallographic studies are currently ongoing at the Paul Scherrer Institute, Villigen PSI, Switzerland, for the most representative compounds of the series **15, 16, 17, 36, 37, 38, 39, 41** and **58**. So far, preliminary data have been collected for series **15, 16** and **17**.

A technique that has been gaining utility for crystal-based fragment screening is crystal soaking. This method involves the screening of a protein target with library of small molecules (generally under 300 Da in size), whose probabilities of binding are increased thanks to their smaller and less complex structures. To this end, ligands are soaked into pre-grown crystals formed by a $T_2$R-TTL protein complex composed of two bovin brain αβ-tubulin heterodimers ($T_2$), the rat stathmin-like protein RB3 (R) and the chicken tubulin tyrosine ligase (TTL). High-resolution structures of tubulin in complex with each ligand are then solved by X-ray crystallography, since the weak binding nature of the fragments requires a screening method with high sensitivity [60].

Crystals of the $T_2$R-TTL complex were grown as described by Prota et al. [61][62] over a few days at 20 °C by vapor-diffusion. The crystallization buffer contained 4% PEG 4K, 8% glycerol, 30 mM $MgCl_2$, 30 mM $CaCl_2$, 5 mM tyrosine and 100 mM MES/Imidazole pH 6.5.

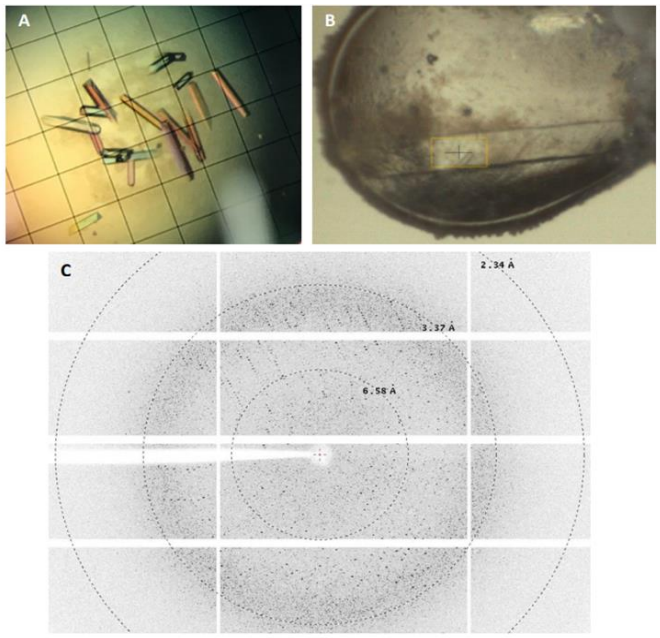



**Figure 29 – A) $T_2$R-TTL crystals; B) Cryo-cooled crystal mounted at X06DA for data collection. The yellow rectangle indicates the position and size of the X-Ray beam; C) Diffraction image of $T_2$R-TTL crystal soaked with 16j.**

The compounds **16j**, **15r** and **17p** were soaked into the crystals for 5h at a final concentration of 5 mM. The soaked crystals were then consecutively transferred into two cryo-protectants which consist of crystallization buffer with increased PEG 4K (10%) and glycerol (16% and 20%) concentrations. The crystals were then flash-cooled in liquid nitrogen. Data collection was done at 100K at beamline X06DA (Figure 29).

Several datasets for $T_2$R-TTL-ligand complexes with three compounds **15r**, **16j** and **17p** were collected. The best resolutions for the individual tubulin-compound complexes are the following: **15r** 2.5 Å, **16j** 2.3 Å and **17p** 2.3 Å. The collected data were merged using XDS [63].

$T_2$R-TTL structures were determined by the difference Fourier method, using the phases of a model in the absence of ligands and solvent as a starting point for refinement. Each tubulin dimer in the complex contains an accessible colchicine site, termed β1 and β2. One cycle of rigid body refinement was followed by three cycles of restrained refinement in PHENIX [64]. After the initial refinement step, the density at the colchicine site on β1-tubulin was inspected (Figure 30).

A large, compound-shaped difference density within the binding site was observed for **16j** and **17p**, showing that these ligands are bound. For **15r**, the binding site was perturbed only to a much smaller extent, thereby suggesting a much lower occupancy of the ligand in the binding site. Accordingly, this latter dataset was not considered for further refinement.

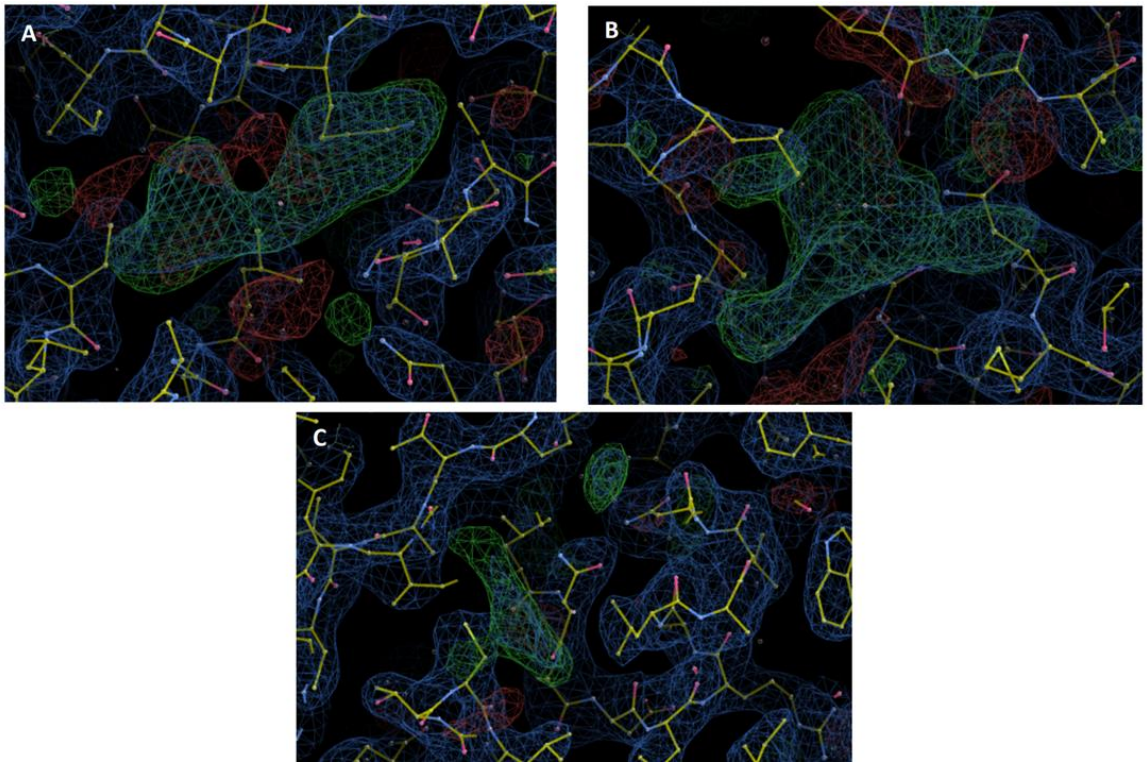



**Figure 30 - Difference density at the colchicine site after rigid body refinement. The direct electron density (blue) is contoured at 1 σ, the difference map mFo-DFc (green/red) is contoured at ± 3 σ. Carbon atoms are colored in yellow, oxygen and nitrogen atoms are in red and blue, respectively. A) $T_2$R-TTL-16j; B) $T_2$R-TTL-17p; C) $T_2$R-TTL-15r.**

Moreover, for **16j** additional density was observed in the second colchicine site on β2 tubulin, which described the identical pose to the one observed in β1 tubulin. Models for the compounds **16j** and **17p** were energy-minimized in Moloc [65] and restraints were generated using the eLBOW software, which is part of the PHENIX suite [64]. The molecules were positioned into the difference density and refined with five cycles of restrained, occupancy and B-factor refinement including solvent addition. Both molecules refined with excellent fit into the electron density (Figure 31). The crystallographic R values for both the current $T_2$R-TTL-16j and $T_2$R-TTL-17p models are $R_{work}$ 21.6%/$R_{free}$ 25.0% and $R_{work}$ 21.7%/$R_{free}$ 25.0%, respectively.

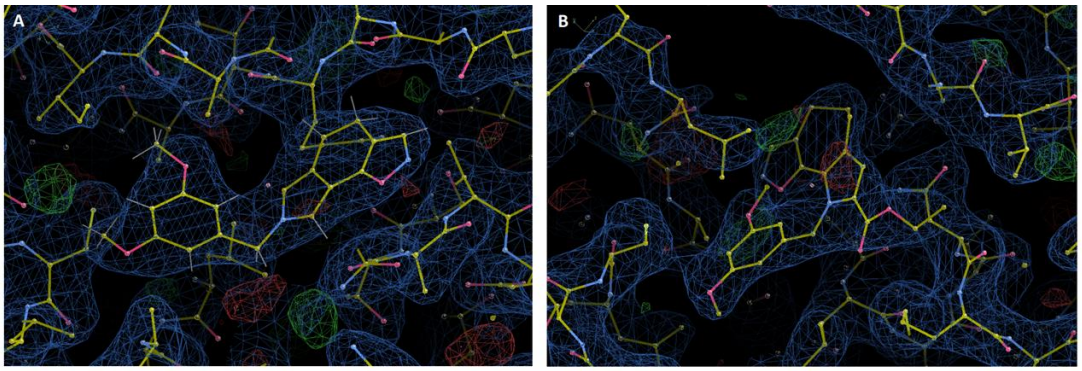



**Figure 31 - Binding pose of 16j (A) and 17p (B) within the β1 colchicine binding site. The direct electron density (blue) is contoured at 1 σ, the difference map mFo-DFc (green/red) is contoured at ± 3 σ.**

In both obtained $T_2$R-TTL-ligand structures the βT7 and αT5 loops are flipped to accommodate the ligands, as was already observed for the coordination of colchicine (PDB ID: 4O2B). Both compounds show a similar binding pose for the methoxy-phenyl moiety. Interestingly, the large size of the three-membered ring system of 17p is causing this moiety into a 180° flipped position compared to the slightly smaller ring system of 16j (Figure 32).

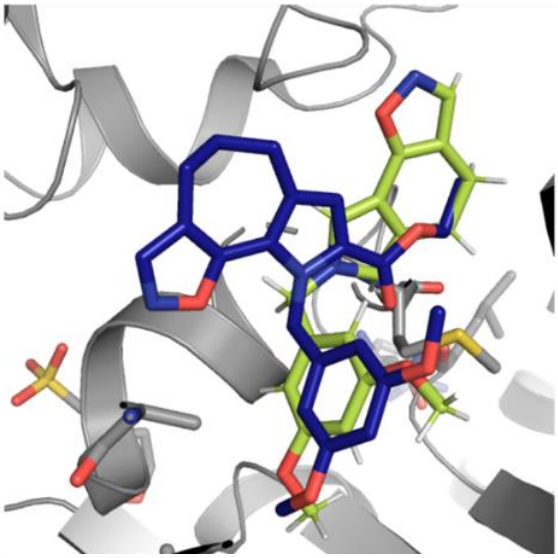


**Figure 32 - Superposition of the binding pose of 16j (green) and 17p (blue). The ring systems of the two compounds adopt two opposing positions within the colchicine site.**

In conclusion, **16j** and **17p** were unambiguously determined to bind to the colchicine site of tubulin. By refinement of both ligands the binding pose was determined revealing an interesting flip in the three membered ring system of the ligands. The strongest colchicine-inhibitor **16j** could also be modeled and refined in the colchicine-site of the second tubulin dimer with identical binding-pose. Despite the low $IC_{50}$ values observed for both MCF-7

cytotoxicity and inhibition of tubulin assembly, compound **15r** was found to occupy the colchicine binding site only partially, and its position could not be refined. This could be due to a poorer solubility in the crystallization buffer compared to the other two ligands, or to a slightly lower affinity for tubulin binding, which is also reflected in the poorer inhibition of colchicine-binding compared to **16j** and **17p**. The refined binding-poses of the current models are unambiguous. However, further steps of refinements are needed to finalize the models, which will include minor adjustments for geometry optimization and manual addition of individual water molecules. Overall, we do not expect any changes to the ligand poses.

These preliminar results demonstrates the high affinity of our compounds for tubulin and their role as colchicine-site ligands, providing interesting prospects for the future.

## 10 Evaluation of *druglike* properties

*In silico* ADME (absorption, distribution, metabolism and elimination) and toxicity studies of the entire class of [1,2]oxazoles were calculated by using QikProp software (version 6.2, Schrödinger, LLC, New York, NY, 2019) and allowing to compare the properties of each compound with those of 95% of the known drugs. Violations of the 95% range are represented by #stars, where a large number of stars suggests that a molecule is less drug-like. Molecular descriptors were computed to predict pharmaceutically relevant properties of the most interesting [1,2]oxazole derivatives. The parameters considered to evaluate ADMET properties are summarized in Table 20.

Among the parameters that most characterize the molecules, we report the SASA values which suggested that all molecules have a good ability to permeate cell membranes, the high intestinal absorption capacity by passive diffusion (PCaco), a good ability to permeate the blood-brain barrier by passive diffusion (logBB and PMDCK) but with low neurotoxicity (CNS), a good skin permeability (logKp), and a high oral absorption. Finally, the logHERG values within the limits and a low ability to bind to human serum albumin (logKha) show a good safety profile of all molecules.

With few exceptions, selected compounds complied with Lipinski's rule of five since they showed MW<500, logPo/w <5, donorHB ≤ 5 and acceptorHB ≤ 10, indicating that the molecules are considered to be drug-like.

**Table 20 – Drug-likeness [1,2]oxazoles**

| CPD | MW | SASA | FOSA | FISA | Donor HB | Accpt HB | logPo/w | logS | PCaco | logBB | CNS | Human Oral Abs | logHERG | logKhsa | Rule of Five | #stars |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **15c** | 356.423 | 623.699 | 211.331 | 60.744 | 0 | 2.250 | 5.613 | -6.370 | 2629.440 | -0.184 | 0 | 1 | -5.717 | 1.191 | 1 | 0 |
| **15k** | 401.421 | 648.530 | 211.932 | 139.327 | 0 | 3.250 | 4.920 | -6.154 | 472.779 | -0.989 | -1 | 3 | -5.511 | 1.060 | 0 | 0 |
| **15l** | 371.438 | 644.107 | 211.383 | 123.503 | 1.5 | 3.250 | 4.668 | -6.085 | 667.922 | -0.864 | -1 | 3 | -5.709 | 0.962 | 0 | 0 |
| **15p** | 446.502 | 672.856 | 409.475 | 60.747 | 0 | 4.500 | 5.526 | -5.635 | 2629.304 | -0.340 | 0 | 3 | -4.692 | 0.989 | 1 | 0 |
| **15r** | 461.516 | 714.003 | 440.417 | 101.026 | 1.5 | 5.500 | 5.016 | -6.069 | 1091.104 | -0.825 | -1 | 3 | -5.027 | 0.954 | 1 | 1 |
| **15t** | 372.423 | 632.481 | 212.430 | 114.870 | 1 | 3.000 | 4.870 | -6.053 | 806.478 | -0.753 | -1 | 3 | -5.514 | 1.032 | 0 | 0 |
| **15u** | 386.449 | 654.282 | 297.440 | 60.759 | 0 | 3.000 | 5.630 | -6.394 | 2628.592 | -0.255 | 0 | 1 | -5.572 | 1.147 | 1 | 0 |
| **16e** | 280.326 | 550.906 | 233.720 | 60.974 | 0 | 2.750 | 3.950 | -4.729 | 2616.301 | -0.192 | 0 | 3 | -5.328 | 0.477 | 0 | 0 |
| **16i** | 310.352 | 567.744 | 303.286 | 60.970 | 0 | 3.500 | 3.950 | -4.492 | 2616.478 | -0.236 | 0 | 3 | -4.873 | 0.424 | 0 | 0 |
| **16j** | 310.352 | 584.024 | 322.917 | 60.950 | 0 | 3.500 | 4.000 | -4.801 | 2617.664 | -0.261 | 0 | 3 | -5.169 | 0.447 | 0 | 0 |
| **16k** | 340.378 | 612.375 | 378.937 | 61.022 | 0 | 4.250 | 4.090 | -4.782 | 2613.519 | -0.322 | 0 | 3 | -5.033 | 0.422 | 0 | 0 |
| **17g** | 324.379 | 570.926 | 326.061 | 35.924 | 0 | 3.000 | 4.544 | -4.814 | 4520.950 | 0.001 | 1 | 3 | -4.738 | 0.633 | 0 | 0 |
| **17p** | 396.442 | 667.567 | 448.707 | 70.907 | 0 | 5.000 | 4.568 | -5.273 | 2106.152 | -0.481 | 0 | 3 | -4.961 | 0.597 | 0 | 0 |
| **17q** | 426.468 | 696.102 | 511.559 | 67.785 | 0 | 5.750 | 4.621 | -5.258 | 2254.719 | -0.521 | 0 | 3 | -4.903 | 0.547 | 0 | 0 |
| **17u** | 370.835 | 609.630 | 270.215 | 71.720 | 0 | 3.500 | 4.759 | -5.514 | 2069.094 | -0.214 | 0 | 3 | -5.015 | 0.722 | 0 | 0 |
| **17x** | 400.861 | 654.636 | 371.582 | 63.954 | 0 | 4.250 | 4.947 | -5.795 | 2451.456 | -0.237 | 0 | 3 | -5.084 | 0.731 | 0 | 0 |
| **17y** | 430.887 | 629.784 | 382.208 | 70.579 | 0 | 5.000 | 4.733 | -4.783 | 2121.283 | -0.277 | 0 | 3 | -4.054 | 0.593 | 0 | 0 |
| **36k** | 518.565 | 823.768 | 546.214 | 101.897 | 0 | 6.500 | 5.856 | -7.122 | 1070.570 | -1.001 | -2 | 1 | -5.733 | 1.090 | 2 | 1 |
| **39k** | 476.528 | 786.742 | 470.555 | 154.097 | 1 | 6.200 | 5.012 | -6.963 | 342.452 | -1.551 | -2 | 1 | -5.651 | 1.032 | 1 | 1 |

**Principal Descriptors. MW**: molecular weight of the molecule [130.0 – 725.0]. **SASA**: total solvent accessible surface area [300.0 – 1000.0]. **FOSA**: hydrophobic component of the SASA (saturated carbon and attached hydrogen) [0.0 – 750.0]. **FISA**: hydrophilic component of the SASA (N, O, and H on

heteroatoms) [7.0 – 330.0]. **HB Donor**: estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution [0.0 – 6.0]. **HB Acceptor**: estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution [2.0 – 20.0]. **Properties Predictions. LogPo/w**: predicted octanol/water partition coefficient [-2.0 – 6.5]. **LogS**: predicted aqueous solubility [-6.5 – 0.5]. **PCaco**: Predicted apparent Caco-2 cell permeability in nm/sec; Caco-2 cells are a model for the gut-blood barrier. Predictions are for non-active transport [<25 poor, >500 great]. **LogBB**: predicted brain/blood partition coefficient [-3.0 – 1.2]. **CNS**: predicted central nervous system activity on a –2 (inactive) to +2 (active) scale. **Human Oral Absorption:** predicted qualitative human oral absorption**:** 1, low; 2, medium; 3, high. **LogHERG:** predicted $IC_{50}$ value for blockage of HERG K+ channels [concern below -5]. **LogKhsa**: prediction of binding to human serum albumin [-1.5 – 1.5]. **Lipinski Rule or Rule of Five**: number of violations of Lipinski's rule of five. The four rules are: mol_MW < 500, cLogPo/w < 5, HB donor ≤ 5, HB acceptor ≤ 10. Compounds that satisfy these rules are considered drug-like [max 4]. **#Stars**: number of property or descriptor values that are outside the 95% range of similar values for known drugs. A large number of stars suggests that a molecule is less drug-like than molecules with few stars [0 – 5].

## 11 Conclusions

In conclusion, the synthesis of a wide family of [1,2]oxazolo derivatives has been reported: three new series of [1,2]oxazolo[5,4-*e*]isoindoles and the 4,5,6,8-tetrahydropyrrolo [3',4':3,4]cyclohepta[1,2-*d*]isoxazoles. Screened against the NCI panel, the new compounds showed significant dose-dependent patterns of activity against most cancer cell lines with $GI_{50}$ values in the micromolar to nanomolar range. In particular, compounds **36k** and **39k** showed the best antiproliferative effects reaching MG_MID of 2.45 μM and 0.49 μM, respectively. Excellent results were observed for **39k** towards the melanoma MD-MBA-435 cell line and the breast cancer MCF-7 cell line with $GI_{50}$ of 0.03 μM and 0.09 μM.

The antitumor efficacy of newly synthesized compounds was also extended to four different subtypes of Non-Hodgkin's lymphoma (activated B-cell and germinal center B-cell diffuse large B-cell lymphoma, mantle cell lymphoma and splenic marginal zone lymphoma), thus expanding the set of potential targeted cells. Compounds **36k** and **39k** showed good growth inhibitory effects against all lymphoma histotypes with $IC_{50}$ values in the low micromolar range (1.4 - 2 μM), causing G2/M arrest and a concomitant reduction of the percentage of cells in the G1 and S phases. Furthermore, annexin V-FITC assay determined a clear apoptotic effect in MINO cell line.

The lymphoma screening was also extended to the [1,2]oxazolo[5,4-*e*]isoindoles **15**, **16** and the pyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazoles **17**, previously synthesized by our research group, in order to deepen their role as anticancer agents and explore their possible activity as anti-lymphoma agents. Several compounds reached $IC_{50}$ values lower than 500 nM toward the full panel, with a major antiproliferative effect exerted by derivative **16k**, whose $IC_{50}$ values ranged from 0.07 μM to 0.12 μM.

Results obtained so far indicate strong potentialities of these classes of small molecules based on pyrrole[1,2]oxazole structure which deserves further insight on the mechanism of action. Crystallographic data will guide new rounds of chemical synthesis to achieve optimization of binding affinity to the colchicine site which could drive to further increase of activity. Our final goal is to generate optimized lead compounds, which could be eligible for further advancements.

During the process of chemical optimization of pyrrole[1,2]oxazoles, we will also pay attention to try to the drug-like properties. For this purpose, we will outsource to a CRO, Lead discovery Siena, the evaluation of a small selection of compounds representative of the different classes. We will determine aqueous solubility, chemical and microsomal stability

and permeability through artificial membranes. If needed, further synthesis will be undertaken to improve ADMET properties while preserving the structural features required for optimal activity.

Finally, several scientific publications have been produced during my PhD course:

- Spanò, V.; Rocca, R.; **Barreca, M**.; Giallombardo, D.; Montalbano, A.; Carbone, A.; Raimondi, M. V.; Gaudio, E.; Bortolozzi, R.; Bai, R.; Tassone, P.; Alcaro, S.; Hamel, E.; Viola, G.; Bertoni, F.; Barraja, P. *Pyrrolo[2',3':3,4]cyclohepta[1,2-d][1,2]oxazoles, a New Class of Antimitotic Agents Active against Multiple Malignant Cell Types*. J. Med. Chem. (2020), 63, 12023-12042.
- Spanò, V.; **Barreca, M**.; Rocca, R.; Bortolozzi, R.; Bai, R.; Carbone, A.; Raimondi, M. V.; Palumbo Piccionello, A.; Montalbano, A.; Alcaro, S.; Hamel, E.; Viola, G.; Barraja, P. *Insight on [1,3]thiazolo[4,5-e]isoindoles as tubulin polymerization inhibitors.* E. J. Med. Chem. (2020), 113122.
- **Barreca, M**.; Spanò, V.; Montalbano, A.; Cueto, M.; Díaz Marrero, A. R.; Deniz, I.; Erdogan, A.; Bilela, L. L.; Moulin, C.; Taffin-de-Givenchy, E.; Spriano, F.; Perale, G.; Mehiri, M.; Rotter, A; Thomas, O. P.; Barraja, P.; Gaudêncio, S. P.; Bertoni, F. *Marine Anticancer Agents: An Overview with a Particular Focus on Their Chemical Classes*. Mar. Drugs (2020), 18, 619.
- **Barreca, M**.; Spanò, V.; Raimondi, M. V.; Montalbano, A.; Bai, R.; Gaudio, E.; Rocca, R.; Alcaro, S.; Hamel, E.; Bertoni, F.; Barraja, P. *Evaluation of [1,2]oxazolo[5,4-e]isoindoles in lymphoma cells.* European Journal of Cancer 138S2 (2020) S1–S62.
- Spriano, F.; **Barreca, M**.; Gordo, M.; O'Brien, S.; Arribas, A.; Jennings, L.; Thoma, O.; Bertoni, F. *Screening of fractions from marine sponges and other invertebrates to identify new lead compounds with anti-tumor activity in lymphoma models.* European Journal of Cancer 138S2 (2020) S1–S62.
- Arribas, A.; Napoli, S.; Cascione, L.; Gaudio, E.; Bordone-Pittau, R.; **Barreca, M**.; Sartori, G.; Tarantelli, C.; Spriano, F.; Rinaldi, A.; Stathis, A.; Stussi, G.; Rossi, D.; Emanuele, Z.; Bertoni, F. *Secondary resistance to the PI3K inhibitor copanlisib in marginal zone lymphoma.* European Journal of Cancer 138S2 (2020) S1–S62.
- Spanò, V.; Venturini, A.; Genovese, M.; **Barreca, M**.; Raimondi, M. V.; Montalbano, A.; Galietta, L. J. V.; Barraja, P. *Current development of CFTR potentiators in the last decade.* Eur. J. Med. Chem., (2020), 180, 112631.

- **Barreca, M**.; Stathis, A.; Barraja, P.; Bertoni. F. *An overview on anti-tubulin agents for the treatment of lymphoma patients.* Pharmacology & Therapeutics 211, (2020), 107552.

Furthermore, several conference contributions have been presented:

- **Barreca, M**.; Spanò, V.; Montalbano, A.; Genovese, M.; Renda, M.; Guidone, D.; Galietta, L. J. V.; Barraja, P. *Discovery of new correctors based on nitrogen heterocyclic systems.* EFMC-YMCS Virtual Event 2020, September 2020 (Poster).
- **Barreca, M**.; Spanò, V.; Raimondi, M. V.; Montalbano, A.; Bai, R.; Gaudio, E.; Rocca, R.; Alcaro, S.; Hamel, E.; Bertoni, F.; Barraja, P. *Screening of [1,2]oxazolo[5,4-e]isoindoles in lymphoma models.* Italian Young Medicinal Chemistry Virtual Meeting, July 2020 (Poster).
- **Barreca, M**.; Spanò, V.; Carbone, A.; Raimondi, M. V.; Montalbano, A.; Bai, R.; Gaudio, E.; Alcaro, S.; Bertoni, F.; Hamel, E.; Barraja, P. *[1,2]oxazolo[5,4-e]isoindoles: new derivatives and biological aspects.* 4$^{th}$ WG meeting – MuTaLig COST Action, Izmir, March 2020 (Poster).
- Spanò, V.; **Barreca, M**.; Carbone, A.; Montalbano, A.; Gaudio, E.; Bertoni, F.; Hamel, E.; Barraja, P. *Lead optimization of [1,2]oxazolo[5,4-e]isoindoles as tubulin polymerization inhibitors.* XXXVI National Meeting in Medicinal Chemistry, Milano, July 2019 (Oral comunication).
- Spanò, V.; **Barreca, M**.; Montalbano, A.; Carbone, A.; Barraja, P. *Lead optimization di sistemi [1,2]ossazolo[5,4-e]isoindolici.* Congresso congiunto delle Sezioni Sicilia e Calabria, Palermo, March 2019 (Oral comunication).

# 12 Experimental

## 12.1 Chemistry

All melting points were taken on a Buchi-Tottoli capillary apparatus and were uncorrected. IR spectra were determined with a Shimadzu IR Affinity-1 spectrophotometer. 1H and 13C NMR spectra were measured in DMSO-d6 or CDCl3 solutions, unless otherwise specified, at 200 and 50.3 MHz respectively, using a Bruker AC series 200 MHz spectrometer (TMS as internal reference). Column chromatography was performed with Merck silica gel 230-400 Mesh ASTM or with a SEPACORE BÜCHI chromatography apparatus or with BIOTAGE 40i chromatography apparatus. Elemental Analysis (C, H, N) were within ± 0.4% of the theoretical values.

**12.1.1 Preparation of 2,5,6,7-tetrahydro-4H-isoindol-4-ones (20).** To a solution of *p*-toluenesulfonylmethyl isocyanide (TOSMIC) (40 mmol) in anhydrous THF (50 mL), 2-cyclohexen1-one **19** (40 mmol) was added at room temperature, followed by the dropwise addition of a solution of *t*-BuOK (49 mmol) in the same solvent (50 mL). The reaction was stirred for 1 hour at room temperature. Water was added and the solution was extracted with ethyl acetate. The organic extracts were dried over $Na_2SO_4$ and evaporated under reduced pressure. The residue was purified by chromatography (dichloromethane : ethyl acetate 9 : 1). Yellow oil; yield: 85%; IR 3238 (NH), 1647 (CO) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) (ppm): 1.85–1.94 (2H, m, $CH_2$), 2.31 (2H, t, *J*= 6.1 Hz, $CH_2$), 2.62 (2H, t, *J*= 6.1 Hz, $CH_2$), 6.61 (1H, s, H-1), 7.27 (1H, s, H-3), 11.37 (1H, bs, NH); $^{13}C$ NMR (DMSO-$d_6$) (ppm): 21.3 (t), 24.9 (t), 38.1 (t), 114.1 (d), 115.4 (s), 119.1 (d), 125.3 (s), 194.0 (s). Anal. Calcd. for $C_8H_9NO$: C, 71.09; H, 6.71; N, 10.36. Found: C, 70.69; H, 7.11; N, 9.96.

**12.1.2 Preparation of 2-[(dimethylamino)methylidene]cyclohexane-1,3-dione (22).** A solution of 1,3-cyclohexanedione **21** (70 mmol) in *N*,*N*-dimethylformamide dimethyl acetal (20 mL) was refluxed for 1 hour. After reaching room temperature, the solvent was evaporated under reduced pressure. Residue was triturated with diethyl ether and filtered off. Brown solid; yield: 96%; mp: 101.7 – 102.4 °C; IR ($cm^{-1}$): 1660 (CO), 1585 (CO); $^1H$ NMR (DMSO-$d_6$) (ppm): 1.90 – 2.03 (2H, m, $CH_2$), 2.46 (4H, s, 2 x $CH_2$), 3.18 (3H, s, $CH_3$), 3.41 (3H, s, $CH_3$), 8.05 (1H, s, CH); $^{13}C$ NMR (DMSO-$d_6$) (ppm): 19.33 (t), 37.92 (2 x q), 44.42 (t), 48.32 (t), 109.11(d), 162.05 (s), 162.12 (s), 195.83 (s). Anal Calcd. for $C_9H_{13}NO_2$: C, 64.65; H, 7.84; N, 8.38. Found: C, 64.78; H, 7.52; N, 8.60.

**12.1.3 Procedure for the synthesis of [[(2,6Dioxocyclohexylidene)methyl]amino} arylacetic acid (23a-c).** To a solution of **22** (18 mmol) in ethanol (42 mL), a solution of the suitable phenylglycine (21.6 mmol) and sodium acetate trihydrate (2.94 g) in ethanol was added, heating under reflux up to completeness (TLC). After cooling, the reaction mixture was filtered and the filtrate was evaporated at reduced pressure. To the residue, ice and water were added and the resulting solution was acidified with HCl 6 M. The solid obtained was filtered and dried.

**Data for 2-(((2,6-dioxocyclohexylidene)methyl)amino)-2-phenylacetic acid (23a).** This compound was obtained after reaction of **22** with phenylglycine after 3h. Pale yellow solid; yield: 83%; mp: 180–181 °C; IR ($cm^{-1}$): 3207 (NH), 2968 (OH), 1728 (CO), 1662 (CO), 1579 (CO); $^{1}H$ NMR ($CDCl_3$) (ppm): 1.90–2.04 (2H, m, $CH_2$), 2.50 (4H, s, 2 x $CH_2$), 5.25 (1H, d, J = 9.5 Hz, CH), 7.27–7.50 (5H, m, Ar), 8.17 (1H, d, J = 14.0 Hz, CH), 10.09 (1H, s, OH), 11.85–11.97 (1H, m, NH); $^{13}C$ NMR ($CDCl_3$) (ppm): 19.5 (t), 36.8 (t), 37.3 (t), 109.3 (s), 127.4 (2 x d), 129.3 (2 x d), 135.1 (d), 158.4 (s), 170.3 (s), 198.3 (s), 200.4 (s). Anal Calcd. for $C_{15}H_{15}NO_4$: C, 65.92; H, 5.53; N, 5.13. Found: C, 65.52; H, 5.93; N, 5.53.

**Data for {[(2,6-Dioxocyclohexylidene)methyl]amino}(3,4,5-trimethoxyphenyl)acetic acid (23b).** This compound was obtained from reaction of **22** with 3,4,5-trimethoxyphenylglycine after 2 h. Pale yellow solid; yield: 95%; mp 183−184 °C. IR ($cm^{-1}$): 3182 (NH), 2939 (OH), 1714 (CO), 1697 (CO), 1651 (CO). $^{1}H$ NMR (DMSO-$d_6$) (ppm): 1.72−1.89 (2H, m, $CH_2$), 2.32−2.40 (4H, m, 2 x $CH_2$), 3.52 (1H, s, OH), 3.67 (3H, s, $CH_3$), 3.78 (6H, s, 2 x $CH_3$), 5.55 − 5.58 (1H, m, CH), 6.68 (2H, s, H-2′ and H-6′), 8.09 (1H, d, J = 13.8 Hz, CH), 11.59−11.69 (1H, m, NH). $^{13}C$ NMR (DMSO-$d_6$) (ppm): 19.4 (t), 37.0 (t), 37.3 (t), 55.9 (2 x q), 60.0 (q), 63.6 (d), 104.7 (2 x d), 108.6 (s), 132.3 (s), 137.7 (s), 153.3 (2 x s), 156.9 (d), 170.6 (s), 195.2 (s), 198.9 (s). Anal. Calcd for $C_{18}H_{21}NO_7$: C, 59.50; H, 5.83; N, 3.85. Found: C, 59.35; H, 5.99; N, 4.01.

**Data for {[(2,6-Dioxocyclohexylidene)methyl]amino}(4-hydroxyphenyl)acetic Acid (23c).** This compound was obtained from reaction of **22** with 4-hydroxyphenylglycine after 1 h. Light-yellow solid; yield 90%; mp 210−211 °C. IR ($cm^{-1}$): 3252 (NH), 2943 (OH), 2883 (OH), 1730 (CO), 1664 (CO), 1606 (CO). $^{1}H$ NMR (DMSO-$d_6$) (ppm): 1.76−1.88 (2H, m, $CH_2$), 2.30 (2H, t, J = 6.2 Hz, $CH_2$), 2.39 (2H, t, J = 6.2 Hz, $CH_2$), 3.49 (1H, s, OH), 5.54 (1H, d, J = 6.9 Hz, CH), 6.81 (2H, d, J = 8.5 Hz, H-3′ and H-5′), 7.16 (2H, d, J = 8.5 Hz, H-2′ and H-6′), 8.04 (1H, d, J = 14.2 Hz, CH), 9.70 (1H, s, OH), 11.58−11.68 (1H, m, NH). $^{13}C$ NMR (DMSO- $d_6$) (ppm): 19.3 (t), 37.0 (t), 37.3 (t), 62.9 (d), 108.5 (s), 115.9 (2 x d), 126.9 (s),

128.7 (2 x d), 156.8 (d), 157.8 (s), 171.0 (s), 195.1 (s), 198.8 (s). Anal. Calcd for $C_{15}H_{15}NO_5$: C, 62.28; H, 5.23; N, 4.84. Found: C, 62.01; H, 5.42; N, 4.28.

**12.1.4 Procedure for the synthesis of 2-acetyl-1-substituted-6,7tetrahydro-2H-isoindol-4-yl acetate (24a-c).** To a solution of **23a-c** (10 mmol) in acetic anhydride (30 mL), triethylamine was added (7.17 mmol, 10 mL). The reaction mixture was heated under reflux up to completeness (TLC). After cooling, the reaction mixture was poured into water and ice and formed a rubbery solid, which was decanted and then stirred with a saturated solution of $Na_2CO_3$ (50 mL). The solid obtained was filtered and dried.

**Data for 2-acetyl-1-phenyl-6,7-dihydro-2H-isoindol-4-yl acetate (24a).** This compound was obtained from reaction of **23a** after 30 min. Brown solid; yield: 70%; mp: 69–70 °C; IR ($cm^{-1}$): 1761 (CO) 1718 (CO); $^1$H NMR (DMSO-*d*$_6$) (ppm): 2.17 (3H, s, $CH_3$), 2.27 (3H, s, $CH_3$), 2.42–2.52 (4H, m, 2 x $CH_2$), 5.55 (1H, s, CH), 7.13–7.43 (6H, m, H-3 and Ar); $^{13}$C NMR (DMSO-*d*$_6$) (ppm): 18.7 (t), 20.8 (q), 23.4 (t), 24.9 (q), 113.1 (d), 114.4 (d), 117.7 (s), 119.4 (s), 124.4 (s), 127.8 (d), 128.1 (2 x d), 129.7 (2 x d), 132.8 (s), 141.9 (s), 168.5 (s), 169.1 (s). Anal. Calcd for $C_{18}H_{17}NO_3$: C, 73.20; H, 5.80; N, 4.74. Found: C, 72.80; H, 6.20; N, 4.34.

**Data for 2-acetyl-1-(3,4,5-trimethoxyphenyl)-6,7-dihydro-2H-isoindol-4-yl acetate (24b).** This compound was obtained from reaction of **23b** after 30 min. Brown solid; yield 75%; mp 147−148 °C. IR ($cm^{-1}$): 1708 (CO), 1658 (CO). $^1$H NMR (DMSO-*d*$_6$) (ppm): 2.25 (3H, s, $CH_3$), 2.35 (3H, s, $CH_3$), 2.38−2.47 (4H, m, 2 x $CH_2$), 3.69 (3H, s, $CH_3$), 3.76 (6H, s, 2 x $CH_3$), 5.50 (1H, t, J = 4.1 Hz, CH), 6.61 (2H, s, H-2′ and H-6′), 7.31 (1H, s, H-3). $^{13}$C NMR (DMSO-*d*$_6$) (ppm): 18.5 (t), 20.7 (q), 22.9 (t), 24.3 (q), 55.9 (2 x q), 56.0 (d), 60.0 (q), 107.1 (2 x d), 114.0 (d), 119.0 (s), 123.5 (s), 128.0 (s), 128.9 (s), 136.8 (s), 141.7 (s), 152.4 (2 x s), 168.6 (s), 168.7 (s). Anal. Calcd for $C_{21}H_{23}NO_6$: C, 65.44; H, 6.02; N, 3.63. Found: C, 65.32; H, 6.13; N, 3.77.

**Data for 4-[2-acetyl-4-(acetyloxy)-6,7-dihydro-2H-isoindol-1-yl]phenyl acetate (24c).** This compound was obtained from reaction of **23c** after 20 min. Brown solid; yield: 71%; mp 86−87 °C. IR ($cm^{-1}$): 1749 (CO), 1712 (CO), 1662 (CO). $^1$H NMR (DMSO-*d*$_6$) (ppm): 2.25 (3H, s, $CH_3$), 2.28 (3H, s, $CH_3$), 2.34−2.47 (7H, m, $CH_3$, 2 x $CH_2$), 5.51 (1H, t, J = 4.3 Hz, CH), 7.12 (2H, d, J = 8.6 Hz, H-3′ and H5′), 7.10−7.35 (3H, m, H-2′, H-6′ and H-3). $^{13}$C NMR (DMSO-*d*$_6$) (ppm): 18.4 (t), 20.7 (q), 20.9 (q), 22.9 (t), 24.1 (q), 114.0 (d), 114.7 (d), 119.2 (s), 121.0 (2 x d), 123.8 (s), 128.1 (s), 130.1 (s), 130.4 (2 x d), 141.6 (s), 149.4 (s), 168.6 (s),

168.7 (s), 169.1 (s). Anal. Calcd for $C_{20}H_{19}NO_5$: C, 67.98; H, 5.42; N, 3.96. Found: C, 68.15; H, 5.64; N, 3.62.

**12.1.5 Procedure for the synthesis of 1-substituted-2,5,6,7tetrahydro-4H-isoindol-4-one (25a,b).** To a solution of **24a** or **24b** (10 mmol) in AcOH (80%, 62 mL), HCl (37%, 5 mL) was added dropwise. The reaction mixture was heated at 60 °C for 15 min. After cooling, the reaction mixture was poured into water and ice. The solid obtained was filtered and dried.

**Data for 1-phenyl-2,5,6,7-tetrahydro-4H-isoindol-4-one (25a).** The compound was obtained from **24a** after 15 min. White solid; yield: 92%; mp: 171–172 °C; IR ($cm^{-1}$): 3253 (NH), 1653 (CO); $^1$H NMR (DMSO-$d_6$) (ppm): 2.03–2.12 (2H, m, $CH_2$), 2.49 (2H, t, J = 5.7 Hz, $CH_2$), 2.87 (2H, t, J = 5.7 Hz, $CH_2$), 7.23–7.47 (6H, m, H-3 and Ar), 9.95 (1H, s, NH); $^{13}$C NMR (DMSO-$d_6$) (ppm): 22.6 (t), 24.9 (t), 39.1 (t), 120.3 (s), 123.1 (s), 125.7 (2 x d), 126.5 (d), 127.3 (s), 128.8 (2 x d), 132.2 (s), 197.0 (s). Anal. Calcd for $C_{14}H_{13}NO$: C, 79.59; H, 6.20; N, 6.63. Found: C, 79.19; H, 6.60; N, 6.23.

**Data for 1-(3,4,5-trimethoxyphenyl)-2,5,6,7-tetrahydro-4H-isoindol-4-one (25b).** This compound was obtained from reaction of **24b**. Brown solid; yield 70%; mp 185−186 °C. IR ($cm^{-1}$): 3251 (NH), 1658 (CO). $^1$H NMR (DMSO-$d_6$) (ppm): 1.92−2.04 (2H, m, $CH_2$), 2.38 (2H, t, J = 6.3 Hz, $CH_2$), 2.87 (2H, t, J = 6.3 Hz, $CH_2$), 3.68 (3H, s, $CH_3$), 3.83 (6H, s, 2 x $CH_3$), 6.79 (2H, s, H2′ and H-6′), 7.46 (1H, s, H-3), 11.88 (1H, s, NH). $^{13}$C NMR (DMSO-$d_6$) (ppm): 22.4 (t), 24.7 (t), 38.8 (t), 55.8 (2 x q), 60.0 (q), 103.0 (2 x d), 119.8 (d), 122.3 (s), 122.5 (s), 126.7 (s), 127.9 (s), 135.8 (s), 153.0 (2 x s), 194.2 (s). Anal. Calcd for $C_{17}H_{19}NO_4$: C, 67.76; H, 6.36; N, 4.65. Found: C, 67.64; H, 6.22; N, 4.76.

**12.1.6 Procedure for the synthesis of 1-substituted-2,5,6,7tetrahydro-4H-isoindol-4-one (25c-f).** HCl (37%, 5 mL) was added dropwise to a solution of **24c** (10 mmol) in AcOH (80%, 62 mL) and the reaction mixture was stirred at room temperature for 55 min. The reaction mixture was poured into water and ice. The solid obtained was filtered and dried. The crude product was purified using chromatography column (dichloromethane : ethyl acetate 95:5).

**Data for 1-(4-hydroxyphenyl)-2,5,6,7-tetrahydro-4H-isoindol-4one (25c).** Pale-yellow solid; yield: 9%; mp 243−244 °C. IR ($cm^{-1}$): 3356 (NH), 2951 (OH), 1643 (CO). $^1$H NMR (DMSO-$d_6$) (ppm): 1.89−2.02 (2H, m, $CH_2$), 2.36 (2H, t, J = 6.1 Hz, $CH_2$), 2.76 (2H, t, J = 6.1 Hz, $CH_2$), 6.84 (2H, d, J = 8.6 Hz, H-3′ and H-5′), 7.30−7.34 (3H, m, H-2′, H-6′ and H-3), 9.49 (1H, s, OH), 11.70 (1H, s, NH). $^{13}$C NMR (DMSO-$d_6$) (ppm): 22.8 (t), 25.3 (t), 39.3 (t), 116.0 (2 x d), 119.5 (d), 121.3 (s), 122.8 (s), 124.0 (s), 127.4 (s), 127.5 (2 x d), 156.4 (s),

194.7 (s). Anal. Calcd for $C_{14}H_{13}NO_2$: C, 73.99; H, 5.77; N, 6.16. Found: C, 74.07; H, 5.99; N, 5.92.

**Data for 4-(4-Oxo-4,5,6,7-tetraidro-2*H*-isoindol-1-yl)phenyl acetate (25d).** Brown solid; yield: 21%; mp: 217 – 218 °C; IR ($cm^{-1}$): 3289 (NH), 1736 (CO), 1655 (CO); $^1H$ NMR (DMSO-$d_6$) (ppm) 1.91 – 2.03 (2H, m, $CH_2$), 2.28 (3H, s, $CH_3$), 2.38 (2H, t, J = 6.1 Hz, $CH_2$), 2.82 (2H, t, J = 6.1 Hz, $CH_2$), 7.20 (2H, d, J = 8.7 Hz, H-3' and H-5'), 7.44 (1H, s, H-3), 7.54 (2H, d, J = 8.7 Hz, H-2' and H-6'), 11.92 (1H, s, NH); $^{13}C$ NMR (DMSO-$d_6$) (ppm): 20.8 (q), 22.3 (t), 24.7 (t), 39.3 (t), 120.1 (d), 122.1 (2 x d), 122.6 (s), 122.7 (s), 125.9 (s), 126.5 (2 x d), 130.0 (s), 148.6 (s), 169.3 (s), 194.1 (s). Anal. Calcd for $C_{16}H_{15}NO_3$: C, 71.36; H, 5.61; N, 5.20. Found: C, 70.96; H, 5.21; N, 5.60.

**Data for 2-Acetyl-1-(4-hydroxyphenyl)-2,5,6,7-tetraidro-4*H*-isoindol-4-one (25e).** Brown solid; yield: 5%; mp: 184 – 185 °C; IR ($cm^{-1}$): 2950 (OH), 1718 (CO), 1645 (CO); $^1H$ (DMSO-$d_6$) (ppm): 1.84 – 1.96 (2H, m, $CH_2$), 2.41 – 2.52 (7H, m, 2 x $CH_2$ and $CH_3$), 6.77 (2H, d, J = 8.5 Hz, H-3' and H-5'), 7.10 (2H, d, J = 8.5 Hz, H-2' and H-6'), 7.95 (1H, s, H-3), 9.59 (1H, s, OH); $^{13}C$ NMR (DMSO-$d_6$) (ppm): 20.8 (t), 22.3 (t), 24.7 (q), 38.7 (t), 115.2 (2 x d), 122.3 (d), 122.2 (s), 122.7 (s), 125.9 (s), 130.0 (s), 131.2 (2 x d), 148.6 (s), 169.3 (s), 194.2 (s). Anal. Calcd for $C_{16}H_{15}NO_3$: C, 71.36; H, 5.61; N, 5.20. Found: C, 70.96; H, 5.21; N, 5.60.

**Data for 4-(2-Acetil-4-oxo-4,5,6,7-tetraidro-2*H*-isoindol-1-yl)phenyl acetate (25f).** Yellow solid; yield: 51%; mp: 160 – 161 °C; IR ($cm^{-1}$): 1734 (CO), 1674 (CO), 1576 (CO); $^1H$ NMR (DMSO-$d_6$) (ppm): 1.85 – 1.97 (2H, m, $CH_2$), 2.29 (3H, s, $CH_3$), 2.43 – 2.55 (4H, m, 2 x $CH_2$), 2.60 (3H, s, $CH_3$), 7.14 (2H, d, J = 8.6 Hz, H-3', H-5'), 7.34 (2H, d, J = 8.6 Hz, H-2' and H-6'), 8.05 (1H, s, H-3); $^{13}C$ NMR (DMSO-$d_6$) (ppm): 20.9 (q), 21.0 (t), 23.7 (t), 24.1 (q), 39.2 (t), 121.1 (2 x d), 122.9 (d), 123.0 (s), 127.8 (s), 127.9 (s), 129.6 (s), 130.3 (2 x d), 149.5 (s), 169.1 (s), 169.8 (s), 194.9 (s). Anal. Calcd for $C_{18}H_{17}NO_4$: C, 69.44; H, 5.50; N, 4.50. Found: C, 69.04; H, 5.10; N, 4.90.

### 12.1.7 Procedure for the synthesis of ethyl 4-oxo-4,5,6,7-tetrahydro-2H-isoindoles-1-carboxylate (28).

**Preparation of ethyl {[(2,6-dioxocyclohexylidene)methyl]amino}acetate (26).** To a solution of **22** (50 mmol) in glacial acetic acid (40 mL), glycine ethyl ester hydrochloride (55 mmol) was added and the reaction was heated under reflux for 2 hours. After cooling, the solvent was evaporated under reduced pressure. Residue was triturated with ethanol and filtered off. Yellow solid; yield: 90%. mp: 58.8 – 59.4 °C; IR ($cm^{-1}$): 3434 (NH), 1747 (CO), 1713 (CO), 1667 (CO); $^1H$ NMR (DMSO-$d_6$) (ppm): 1.21 (3H, t, J = 7.1 Hz, $CH_3$), 1.74 –

1.91 (2H, m, $CH_2$), 2.27 – 2.48 (4H, m, 2 x $CH_2$), 4.14 (2H, q, J = 7.1 Hz, $CH_2$), 4.37 (2H, d, J = 5.6 Hz, $CH_2$), 8.07 (1H, d, J = 14.2 Hz, CH), 10.79 – 10.98 (1H, m, NH) ; $^{13}$C NMR (DMSO-$d_6$) (ppm): 14.0 (q), 19.4 (t), 37.0 (t), 37.4 (t), 50.0 (t), 61.0 (t), 108.1 (s), 159.5 (d), 169.0 (s), 195.2 (s), 198.3 (s). Anal Calcd. for $C_{11}H_{15}NO_4$: C, 58.66; H, 6.71; N, 6.22. Found: C, 58.98; H, 6.56; N, 6.43.

**Preparation of ethyl 4-oxo-4,5,6,7-tetrahydro-2H-isoindole-1-carboxylate (28).** To a solution of **26** (10 mmol) in anydrous acetonitrile (15 mL), *N*,*N*-dimethylformamide dimethyl acetal (6.7 mL, 50 mmol) was added. After refluxing for 2 hours, the solvent was evaporated under reduced pressure and the oily residue of ethyl-3-(dimethylamino)-2-{[(2,6dioxocyclohexylidene)methyl]amino}prop-2-enoate **27** was directly used for the next step without further purification. The crude was solubilised in anhydrous dichloromethane (5 mL), trifluoroacetic anhydride (11 mmol) was added dropwise at 0° C and the reaction mixture was stirred for 16 hours at room temperature. The solvent was evaporated under reduced pressure and the oily residue was neutralized with a saturated solution of $NaHCO_3$ added dropwise at 0° C. The aqueous solution was extracted with ethyl acetate and the organic phase was dried over $Na_2SO_4$. The solvent was evaporated under reduced pressure. The crude product was purified using chromatography column (cyclohexane : ethyl acetate 50 : 50) and further crystallization from ethyl acetate. White solid; yield: 50%; mp: 184.1 – 184.9 $^{o}$C; IR ($cm^{-1}$): 3435 (NH), 1687 (CO), 1662 (CO); $^1$H NMR (DMSO-$d_6$) (ppm): 1.28 (3H, t, J = 7.0 Hz, $CH_3$), 1.82 – 2.08 (2H, m, $CH_2$), 2.30 – 2.45 (2H, m, $CH_2$), 2.85 – 2.98 (2H, m, $CH_2$), 4.26 (2H, q, J = 7.0 Hz, $CH_2$), 7.44 (1H, s, H-3), 12.42 (1H, s, NH); $^{13}$C NMR (DMSO-$d_6$) (ppm): 13.4 (q), 22.0 (t), 24.0 (t), 38.5 (t), 59.7 (t), 118.0 (s), 122.7 (s), 123.6 (d), 134.0 (s), 160.5 (s), 193.8 (s). Anal. Calcd. for $C_{11}H_{13}NO_3$ C, 63.76; H, 6.32; N, 6.76. Found: C, 63.62; H, 6.35; N, 6.66.

**12.1.8 Procedure for the synthesis of 2-substituted-2,5,6,7-tetrahydro-4H-isoindol-4-ones (29a-h,j,m,n,30a-i).** To a solution of **20**,**25a,b,d,28** (1.35 g, 10 mmol) in anhydrous DMF (15 mL), NaH (0.24 g, 10 mmol) was added at 0 °C and the reaction was stirred for 1 h at room temperature. The suitable benzyl chloride (15 mmol) was added at 0 °C and the reaction mixture was stirred at room temperature up to completeness (TLC). The reaction mixture was poured into ice and brine, then the aqueous solution was extracted with dichloromethane (3 × 50 mL). The organic phase was dried over $Na_2SO_4$ and the solvent evaporated at reduced pressure. The crude product was purified using chromatography column (dichloromethane : ethyl acetate 98:2).

**Data for 2-benzyl-2,5,6,7-tetrahydro-4H-isoindol-4-one (29a).** The compound was obtained from the reaction of **20** with benzyl bromide after 5 h. White solid; yield: 65%; m.p.: 78–79°C; IR ($cm^{-1}$) 1651 (CO); $^{1}H$ NMR (DMSO-$d_6$) (ppm): 1.86 – 1.95 (2H, m, $CH_2$), 2.29 (2H, t, J = 5.8 Hz, $CH_2$), 2.57 (2H, t, J = 5.8 Hz, $CH_2$), 5.10 (2H, s, $CH_2$), 6.67 (1H, s, H-1), 7.30–7.34 (5H, m, Ar), 7.43 (1H, s, H-3); $^{13}C$ NMR (DMSO-$d_6$) (ppm): 21.1 (t), 24.8 (t), 38.8 (t), 52.6 (t), 117.3 (d), 121.3 (s), 122.0 (d), 126.4 (s), 127.6 (2 x d), 127.7 (d), 128.6 (2 x d), 137.8 (s), 193.6 (s). Anal Calcd. for $C_{15}H_{15}NO$ C, 79.97; H, 6.71; N, 6.22. Found: C, 80.37; H, 6.31; N, 6.62.

**Data for 2-(3-methoxybenzyl)-2,5,6,7-tetrahydro-4H-isoindol-4-one (29b).** This compound was obtained from reaction of **20** with 3-methoxybenzylchloride after 1 h and 30 min. White solid; yield 69%; m.p.:120–121 °C; IR ($cm^{-1}$): 1649 (CO); $^{1}H$ NMR (DMSO-$d_6$) (ppm): 1.83–1.96 (2H, m, $CH_2$), 2.29 (2H, t, J = 6.0 Hz, $CH_2$), 2.57 (2H, t, J = 6.0 Hz, $CH_2$), 3.73 (3H, s, $CH_3$), 5.06 (2H, s, $CH_2$), 6.67 (1H, s, H-1), 6.82–6.88 (3H, m, Ar), 7.26 (1H, t, J = 8.0 Hz, Ar), 7.43 (1H, s, H-3); $^{13}C$ NMR (DMSO-$d_6$) (ppm): 21.1 (t), 24.8 (t), 38.8 (t), 52.6 (t), 55.0 (q), 112.9 (d), 113.6 (d), 117.3 (d), 119.8 (d), 121.3 (s), 122.1 (d), 126.4 (s), 129.7 (d), 139.3 (s), 159.4 (s), 193.6 (s). Anal calcd for $C_{16}H_{17}NO_2$ C, 75.27; H, 6.71; N, 5.49. Found: C, 75.43; H, 6.90; N 5.15.

**Data for 2-(3,4-dimethoxybenzyl)-2,5,6,7-tetrahydro-4H-isoindol-4-one (29c).** This compound was obtained from reaction of **20** with 3,4-dimethoxybenzylchloride after 1 h. Light brown solid; yield 60%; m.p.: 102–103 °C; IR ($cm^{-1}$): 1651 (CO); $^{1}H$ NMR (DMSO-$d_6$) (ppm): 1.82–1.95 (2H, m, $CH_2$), 2.28 (2H, t, J = 6.1 Hz, $CH_2$), 2.56 (2H, t, J = 6.1 Hz, $CH_2$), 3.72 (3H, s, $CH_3$), 3.74 (3H, s, $CH_3$), 4.99 (2H, s, $CH_2$), 6.67 (1H, s, H-1), 6.81 – 7.01 (3H, m, Ar), 7.41 (1H, s, H-3); $^{13}C$ NMR (DMSO-$d_6$) (ppm): 21.1 (t), 24.8 (t), 38.8 (t), 52.5 (t), 55.4 (q), 55.5 (q), 111.8 (d), 112.0 (d), 117.1 (d), 120.3 (d), 121.1 (s), 121.8 (d), 126.3 (s), 130.0 (s), 148.4 (s), 148.7 (s), 193.5 (s). Anal calcd for $C_{17}H_{19}NO_3$ C, 71.56; H, 6.71; N, 4.91. Found: C, 71.69; H, 6.58; N, 5.15.

**Data for 2-(3,5-dimethoxybenzyl)-2,5,6,7-tetrahydro-4H-isoindol-4-one (29d).** This compound was obtained from reaction of **20** with 3,5-dimethoxybenzylchloride after 1 h. White solid; yield 63%; m.p.: 90–91°C; IR (cm-$^{1}$): 1649 (CO); $^{1}H$ NMR (DMSO-$d_6$) (ppm): 1.84–1.96 (2H, m, $CH_2$), 2.29 (2H, t, J = 6.1 Hz, $CH_2$), 2.58 (2H, t, J = 6.1 Hz, $CH_2$), 3.72 (6H, s, 2 x $CH_3$), 5.01 (2H, s, $CH_2$), 6.44 (1H, s, H-1), 6.45–6.47 (2H, m, H-2$'$ and H-6$'$), 6.68 (1H, s, H-4$'$), 7.43 (1H, s, H-3); $^{13}C$ NMR (DMSO-$d_6$) (ppm): 21.1 (t), 24.8 (t), 38.9 (t), 52.6 (t), 55.2 (2 x q), 99.1 (d), 105.9 (2 x d), 117.4 (d), 121.2 (s), 122.1 (d), 126.3 (s), 139.9 (s),

160.6 (2 s), 193.6 (s). Anal calcd for $C_{17}H_{19}NO_3$ C, 71.56; H, 6.71; N, 4.91. Found: C, 71.67; H, 6.55; N, 5.09.

**Data for 2-(3,4,5-trimethoxybenzyl)-2,5,6,7-tetrahydro-4H-isoindol- 4-one (29e).** This compound was obtained from reaction of **20** with 3,4,5-trimethoxybenzylchloride after 24 h. White solid; yield 60%; m.p.: 101–102 °C; IR ($cm^{-1}$): 1657 (CO); $^1$H NMR (DMSO-$d_6$) (ppm): 1.83–1.95 (2H, m, $CH_2$), 2.29 (2H, t, J = 6.4 Hz, $CH_2$), 2.57 (2H, t, J = 6.4 Hz, $CH_2$), 3.62 (3H, s, $CH_3$), 3.75 (6H, s, 2 x $CH_3$), 4.98 (2H, s, $CH_2$), 6.73 (3H, s, H-2', H-6' and H-1), 7.45 (1H, s, H-3); $^{13}$C NMR (DMSO-$d_6$) (ppm): 21.1 (t), 24.8 (t), 38.8 (t), 52.9 (t), 55.9 (2 x q), 59.9 (q), 105.7 (2 d), 117.2 (d), 121.1 (d), 121.9 (s), 126.3 (s), 133.1 (s), 137.0 (s), 152.9 (2 x s), 193.6 (s). Anal calcd for $C_{18}H_{21}NO_4$ C, 68.55; H, 6.71; N, 4.44. Found: C, 68.36; H, 6.87; N, 4.32.

**Data for 2-benzyl-1-phenyl-2,5,6,7-tetrahydro-4H-isoindol-4-one (29f).** This compounds was obtained from reaction of **25a** whit benzyl bromide after 1 h. White solid; yield: 90%; m.p.: 128–129°C; IR ($cm^{-1}$): 1655 (CO); $^1$H NMR (DMSO-$d_6$) (ppm): 1.88–1.99 (2H, m, $CH_2$), 2.37 (2H, t, J = 6.1 Hz, $CH_2$), 2.56 (2H, t, J = 6.1 Hz, $CH_2$), 5.19 (2H, s, $CH_2$), 6.88–6.93 (2H, m, Ar), 6.95–7.45 (8H, m, Ar), 7.54 (1H, s, H-3); $^{13}$C NMR (DMSO-$d_6$) (ppm): 21.4 (t), 24.7 (t), 39.9 (t), 50.5 (t), 120.8 (s), 123.3 (d), 124.6 (s), 126.8 (2 x d), 127.4 (d), 127.5 (d), 128.4 (2 x d), 128.5 (2 x d), 128.9 (s), 129.6 (2 x d), 130.9 (s), 137.7 (s), 193.8 (s, CO). Anal calcd for $C_{21}H_{19}NO$ C, 83.69; H, 6.35; N, 4.65. Found: C, 83.29; H, 6.75; N, 4.25.

**Data for 2-(4-methoxybenzyl)-1-phenyl-2,5,6,7-tetrahydro-4H-isoindol-4-one (29g).** This compound was obtained from reaction of **25a** with 4-methoxybenzil chloride after 1 h and 30 min. White solid; yield: 85%; m.p.: 135–136 °C; IR ($cm^{-1}$): 1655 (CO); $^1$H NMR (DMSO-$d_6$) (ppm): 1.85–1.97 (2H, m, $CH_2$), 2.36 (2H, t, J = 6.1 Hz, $CH_2$), 2.55 (2H, t, J = 6.1 Hz, $CH_2$), 3.68 (3H, s, $CH_3$), 5.10 (2H, s, $CH_2$), 6.79 (2H, d, J = 8.9 Hz, H-3' and H-5'), 6.87 (2H, d, J = 8.9 Hz, H-2' and H-6'), 7.28–7.47 (5H, m, Ar), 7.49 (1H, s, H-3); $^{13}$C NMR (DMSO-$d_6$) (ppm): 21.4 (t), 24.7 (t), 38.9 (t), 50.0 (t), 55.0 (q), 113.8 (2 x d), 120.7 (s), 123.0 (d), 124.6 (s), 127.5 (d), 128.4 (2 x d), 128.5 (2 x d), 128.7 (s), 129.4 (s), 129.6 (2 x d), 131.0 (s), 158.6 (s), 193.7 (s). Anal calcd for $C_{22}H_{21}NO_2$ C, 79.73; H, 6.39; N, 4.23. Found: C, 79.33; H, 6.79; N, 3.83.

**Data for 2-(4-methoxy-3-nitrobenzyl)-1-phenyl-2,5,6,7-tetrahydro-4H-isoindol-4-one (29h).** This compound was obtained from reaction of **25a** with 3-nitro-4-methoxybenzyl chloride after 1 h and 30 min. Brown solid; yield 83%; m.p.: 196−197 °C. IR (cm−1) 1647 (CO), 1529 ($NO_2$); $^1$H NMR (DMSO-$d_6$) (ppm): 1.86−1.98 (2H, m, $CH_2$), 2.37 (2H, t, J = 6.6 Hz, $CH_2$), 2.54 (2H, t, J = 6.6 Hz, $CH_2$), 3.86 (3H, s, $CH_3$), 5.21 (2H, s, $CH_2$), 7.11−7.49 (8H,

m, Ar), 7.63 (1H, s, H-3). $^{13}$C NMR (DMSO-$d_6$) (ppm): 21.3 (t), 24.6 (t), 38.9 (t), 49.3 (t), 56.6 (q), 114.5 (d), 120.9 (s), 123.3 (d), 123.8 (d), 124.9 (s), 127.7 (d), 128.5 (2 x d), 128.6 (s), 129.6 (2 x d), 129.8 (s), 130.8 (s), 133.2 (d), 138.6 (s), 151.3 (s), 193.9 (s). Anal. Calcd for $C_{22}H_{20}N_2O_4$ C, 70.20; H, 5.36; N, 7.44. Found: C, 70.09; H, 5.48; N, 7.32.

**Data for 4-(2-(4-methoxybenzyl)-4-oxo-4,5,6,7-tetrahydro-2H-isoindol-1-yl)phenyl acetate (29j).** This compound was obtained from reaction of **25d** with 4-methoxybenzyl chloride after 24 h. Brown solid; yield: 64%; mp 84 – 85 °C; IR (cm$^{-1}$) 1655 (CO), 1620 (CO); $^1$H NMR (DMSO-$d_6$) (ppm): 1.85 – 1.98 (2H, m, $CH_2$), 2.29 (3H, s, $CH_3$), 2.36 (2H, t, J = 6.1 Hz, $CH_2$), 2.55 (2H, t, J = 6.1 Hz, $CH_2$), 3.68 (3H, s, $CH_3$), 5.10 (2H, s, $CH_2$), 6.79 (2H, d, J = 8.9 Hz, Ar), 6.87 (2H, d, J = 8.9 Hz, Ar), 7.20 (2H, d, J = 8.6 Hz, Ar), 7.35 (2H, d, J = 8.6 Hz, Ar), 7.50 (1H, s, H-3); $^{13}$C NMR (DMSO-$d_6$) (ppm): 20.9 (q), 21.4 (t), 24.7 (t), 39.3 (t), 50.1 (t), 55.0 (q), 113.9 (2 x d), 120.6 (s), 122.0 (2 x d), 123.1 (d), 124.8 (s), 127.9 (s), 128.5 (2 x d), 129.3 (s), 129.6 (s), 130.7 (2 x d), 149.7 (s), 158.6 (s), 169.1 (s), 193.8 (s). Anal. Calcd for $C_{24}H_{23}NO_4$ C, 74.02; H, 5.95; N, 3.60. Found: C, 74.42; H, 5.45; N, 3.20.

**Data for 2-(4-methoxybenzyl)-1-(3,4,5-trimethoxyphenyl)-2,5,6,7-tetrahydro-4H-isoindol-4-one (29m).** This compound was obtained from reaction of **25b** with 4-methoxybenzil chloride after 1 h. White solid; yield: 88%; mp: 160–161 °C; IR (cm$^{-1}$): 1691 (CO); $^1$H NMR (DMSO-$d_6$) (ppm): 1.87–1.99 (2H, m, $CH_2$), 2.36 (2H, t, J = 6.1 Hz, $CH_2$), 2.59 (2H, t, J = 6.1 Hz, $CH_2$), 3.68 (6H, s, 2 x $CH_3$), 3.69 (3H, s, $CH_3$), 3.70 (3H, s, $CH_3$), 5.12 (2H, s, $CH_2$), 6.50 (2H, s, H-2' and H-6'), 6.84 (2H, d, J = 8.9 Hz, H-3'' and H-5''), 6.92 (2H, d, J = 8.9 Hz, H-2'' and H-6''), 7.49 (1H, s, H-3); $^{13}$C NMR (DMSO-$d_6$) (ppm): 21.5 (t), 24.7 (t), 38.9 (t), 50.3 (t), 55.1 (q), 55.8 (2 x q), 60.0 (q), 107.0 (2 x d), 113.9 (2 x d), 120.5 (s), 122.6 (d), 124.4 (s), 126.4 (s), 128.3 (2 x d), 128.9 (s), 129.8 (s), 136.8 (s), 152.7 (2 x s), 158.5 (s), 193.8 (s). Anal. Calcd for $C_{25}H_{27}NO_5$ C, 71.24; H, 6.46; N, 3.32. Found: C, 71.64; H, 6.06; N, 3.72.

**Data for 2-(4-methoxy-3-nitrobenzyl)-1-(3,4,5-trimethoxyphenyl)-2,5,6,7-tetrahydro-4H-isoindol-4-one (29n).** This compound was obtained from reaction of **25b** with 4-methoxy-3-nitro-benzyl chloride after 1 h. Brown solid; yield 74%; mp 192−193 °C. IR (cm$^{-1}$): 1697 (CO), 1531 ($NO_2$); $^1$H NMR (DMSO-$d_6$) (ppm): 1.86−1.99 (2H, m, $CH_2$), 2.36 (2H, t, J = 6.0 Hz, $CH_2$), 2.57 (2H, t, J = 6.0 Hz, $CH_2$), 3.69 (3H, s, $CH_3$), 3.71 (6H, s, 2 x $CH_3$), 3.86 (3H, s, $CH_3$), 5.21 (2H, s, $CH_2$), 6.50 (2H, s, H-2′ and H-6′), 7.27 (2H, s, Ar), 7.37 (1H, s, Ar), 7.62 (1H, s, H-3). $^{13}$C NMR (DMSO-$d_6$) (ppm): 21.3 (t), 24.6 (t), 39.0 (t), 49.5 (t), 55.8 (2 x q), 56.7 (q), 60.0 (q), 107.1 (2 x d), 114.5 (d), 120.7 (s), 123.0 (d), 123.9 (d), 124.7 (s), 126.2 (s),

128.7 (s), 130.1 (s), 133.4 (d), 137.0 (s), 138.6 (s), 151.3 (s), 152.9 (2 x s), 193.8 (s). Anal. Calcd for $C_{25}H_{26}N_2O_7$: C, 64.37; H, 5.62; N, 6.01. Found: C, 64.44; H, 5.79; N, 5.93.

**Data for ethyl 2-benzyl-4-oxo-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylate (30a).** This compound was obtained from reaction of **28b** with benzyl bromide after 10 h. White solid; yield: 67%; mp: 85 – 86 ºC; IR ($cm^{-1}$): 1702 (CO), 1695 (CO); $^1H$ NMR ($CDCl_3$) (ppm): 1.32 (3H, t, J = 7.1 Hz, $CH_3$), 2.03 - 2.15 (2H, m, $CH_2$), 2.48 (2H, t, J = 6.9 Hz, $CH_2$), 3.00 (2H, t, J = 6.9 Hz, $CH_2$), 4.27 (2H, q, J = 7.1 Hz, $CH_2$), 5.54 (2H, s, $CH_2$), 7.12 – 7.17 (2H, m, H-2' and H-6'), 7.27 – 7.35 (3H, m, H-3', H-4' and H-5'), 7.43 (1H, s, H-3); $^{13}C$ NMR ($CDCl_3$) (ppm): 13.3 (q), 23.5 (t), 24.3 (t), 38.8 (t), 53.3 (t), 60.2 (t), 118.7 (s), 121.6 (s), 127.4 (2 x d), 127.9 (d), 128.6 (d), 128.8 (2 x d), 136.7 (s), 137.2 (s), 161.3 (s), 195.2 (s). Anal. Calcd. for $C_{18}H_{19}NO_3$: C, 72.71; H, 6.44; N, 4.71. Found: C, 72.83; H, 6.37; N, 4.65.

**Data for ethyl 2-(4-methoxybenzyl)-4-oxo-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylate (30b).** This compound was obtained from reaction of **28** with 4-methoxybenzyl chloride after 12 h. Brown solid; yield: 74%; mp: 65 – 66 ºC; IR ($cm^{-1}$): 1698 (CO), 1670 (CO); $^1H$ NMR ($CDCl_3$) (ppm): 1.35 (3H, t, J = 7.1 Hz, $CH_3$), 2.01 – 2.14 (2H, m, $CH_2$), 2.47 (2H, t, J = 6.3 Hz, $CH_2$), 2.98 (2H, t, J = 6.3 Hz, $CH_2$), 3.79 (3H, s, $CH_3$), 4.29 (2H, q, J = 7.1 Hz, $CH_2$), 5.46 (2H, s, $CH_2$), 6.85 (2H, d, J = 8.7 Hz, H-3' and H-5'), 7.14 (2H, d, J = 8.7 Hz, H-2' and H-6'), 7.39 (1H, s, H-3); $^{13}C$ NMR ($CDCl_3$) (ppm): 14.4 (q), 23.5 (t), 24.3 (t), 38.8 (t), 52.8 (t), 55.3 (q), 60.2 (t), 114.2 (2 x d), 118.6 (s), 121.5 (s), 128.3 (d), 128.5 (s), 129.2 (2 x d), 137.2 (s), 159.3 (s), 161.4 (s), 195.2 (s). Anal. Calcd. for $C_{19}H_{21}NO_4$: C, 69.71; H, 6.47; N, 4.28. Found: C, 69.63; H, 6.39; N, 4.42.

**Data for ethyl 2-[(3,5-dimethoxyphenyl)methyl]-4-oxo-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylate (30c).** This compound was obtained from reaction of **28** with 3,5-dimethoxybenzyl chloride after 9 h. Light yellow solid; yield: 94%; mp: 116 – 117 ºC; IR ($cm^{-1}$): 1698 (CO), 1663 (CO); $^1H$ NMR ($CDCl_3$) (ppm): 1.34 (3H, t, J = 7.1 Hz, $CH_3$), 2.03 – 2.15 (2H, m, $CH_2$), 2.48 (2H, t, J = 6.2 Hz, $CH_2$), 3.00 (2H, t, J = 6.2 Hz, $CH_2$), 3.75 (6H, s, 2 x $CH_3$), 4.28 (2H, q, J = 7.1 Hz, $CH_2$), 5.47 (2H, s, $CH_2$), 6.28 (2H, d, J = 2.2 Hz, H-2' and H-6'), 6.37 (1H, t, J = 2.2 Hz, H-4'), 7.41 (1H, s, H-3); $^{13}C$ NMR ($CDCl_3$) (ppm): 14.4 (q), 23.5 (t), 24.3 (t), 38.8 (t), 53.3 (t), 55.3 (2 x q), 60.2 (t), 99.4 (d), 105.5 (2 x d), 118.7 (s), 121.6 (s), 128.6 (d), 137.1 (s), 139.0 (s), 161.1 (2 x s), 161.3 (s), 195.2 (s). Anal. Calcd. for $C_{20}H_{23}NO_5$: C, 67.21; H, 6.49; N, 3.92. Found: C, 67.14; H, 6.67; N, 4.06.

**Data for ethyl 4-oxo-2-[(3,4,5-trimethoxyphenyl)methyl]-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylate (30d).** This compound was obtained from reaction of **28** with 3,4,5-trimethoxybenzyl chloride after 12 h. Light yellow solid; yield: 64%; mp: 125 - 126 ºC; IR

($cm^{-1}$): 1700 (CO), 1670 (CO)[1]; $^{1}H$ NMR ($CDCl_3$) (ppm): 1.36 (3H, t, J = 7.1 Hz, $CH_3$), 2.05 – 2.15 (2H, m, $CH_2$), 2.49 (2H, t, J = 6.4 Hz, $CH_2$), 3.00 (2H, t, J = 6.4 Hz, $CH_2$), 3.81 (6H, s, 2 x $CH_3$), 3.83 (3H, s, $CH_3$), 4.27 (2H, q, J = 7.1 Hz, $CH_2$), 5.46 (2H, s, $CH_2$), 6.44 (2H, s, H-2' and H-6'), 7.42 (1H, s, H-3); $^{13}C$ NMR ($CDCl_3$) (ppm): 14.4 (q), 23.6 (t), 24.3 (t), 38.8 (t), 53.5 (t), 55.7 (q), 56.1 (2 x q), 60.3 (t), 105.0 (2 x d), 121.6 (s), 128.3 (d), 132.0 (s), 136.2 (s), 137.2 (s), 151.5 (s), 153.5 (2 x s), 161.5 (s), 195.3 (s). Anal. Calcd. for $C_{21}H_{25}NO_6$: C, 65.10; H, 6.50; N, 3.62. Found: C, 64.97; H, 6.38; N, 3.79.

**Data for ethyl 2-[(3,4-dimethoxyphenyl)methyl]-4-oxo-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylate (30e).** This compound was obtained from reaction of **28** with 3,4-dimethoxybenzyl chloride after 16 h. White solid; yield: 68%; mp: 116 – 117 °C; IR ($cm^{-1}$): 1699 (CO), 1666 (CO); $^{1}H$ NMR ($CDCl_3$) (ppm): 1.36 (3H, t, J = 7.1 Hz, $CH_3$), 2.02 – 2.14 (2H, m, $CH_2$), 2.48 (2H, t, J = 6.2 Hz, $CH_2$), 2.99 (2H, t, J = 6.2 Hz, $CH_2$), 3.84 (3H, s, $CH_3$), 3.86 (3H, s, $CH_3$), 4.30 (2H, q, J = 7.1 Hz, $CH_2$), 5.46 (2H, s, $CH_2$), 6.72 – 6.95 (3H, m, H-2', H-5' and H-6'), 7.40 (1H, s, H-3); $^{13}C$ NMR ($CDCl_3$) (ppm): 14.4 (q), 23.6 (t), 24.3 (t), 38.8 (t), 53.1 (t), 55.8 (q), 55.9 (q), 60.2 (t), 111.1 (d), 111.2 (d), 118.6 (s), 120.4 (d), 121.5 (s), 128.3 (d), 128.9 (s), 137.2 (s), 148.8 (s), 149.1 (s), 161.4 (s), 195.2 (s). Anal. Calcd. for $C_{20}H_{23}NO_5$: C, 67.21; H, 6.49; N, 3.92. Found: C, 67.33; H, 6.58; N, 3.76.

**Data for ethyl 2-[(2,3-dimethoxyphenyl)methyl]-4-oxo-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylate (30f).** This compound was obtained from reaction of **28** with 2,3-dimethoxybenzyl chloride after 10 h. White solid; yield: 35%; mp: 117 – 118 °C; IR ($cm^{-1}$): 1694 (CO), 1662 (CO); $^{1}H$ NMR ($CDCl_3$) (ppm): 1.34 (3H, t, J = 7.1 Hz, $CH_3$), 2.02 – 2.15 (2H, m, $CH_2$), 2.47 (2H, t, J = 6.0 Hz, $CH_2$), 3.00 (2H, t, J = 6.0 Hz, $CH_2$), 3.83 (3H, s, $CH_3$), 3.87 (3H, s, $CH_3$), 4.29 (2H, q, J = 7.1 Hz, $CH_2$), 5.57 (2H, s, $CH_2$), 6.51 – 6.56 (1H, m, Ar), 6.85 – 7.02 (2H, m, Ar), 7.37 (1H, s, H-3); $^{13}C$ NMR ($CDCl_3$) (ppm): 14.4 (q), 23.6 (t), 24.3 (t), 38.8 (t), 48.4 (t), 55.8 (q), 60.1 (t), 60.6 (q), 112.5 (d), 118.9 (d), 120.6 (s), 121.4 (s), 124.2 (d), 128.8 (d), 130.4 (s), 136.9 (s), 146.8 (s), 152.7 (s), 161.4 (s), 195.1 (s). Anal. Calcd. for $C_{20}H_{23}NO_5$: C, 67.21; H, 6.49; N, 3.92. Found: C, 67.13; H, 6.64; N, 3.82.

**Data for ethyl 2-[(2,5-dimethoxyphenyl)methyl]-4-oxo-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylate (30g).** This compound was obtained from reaction of **28** with 2,5-dimethoxybenzyl chloride after 12 h. White solid; yield: 60%; mp: 92 – 93 °C; IR ($cm^{-1}$): 1695 (CO), 1660 (CO); $^{1}H$ NMR ($CDCl_3$) (ppm): 1.33 (3H, t, J = 7.1 Hz, $CH_3$), 2.02 – 2.14 (2H, m, $CH_2$), 2.47 (2H, t, J = 6.2 Hz, $CH_2$), 3.00 (2H, t, J = 6.2 Hz, $CH_2$), 3.71 (3H, s, $CH_3$), 3.80 (3H, s, $CH_3$), 4.29 (2H, q, J = 7.1 Hz, $CH_2$), 5.51 (2H, s, $CH_2$), 6.56 – 6.85 (3H, m, H-3', H-4' and H-6'), 7.39 (1H, s, H-3); $^{13}C$ NMR ($CDCl_3$) (ppm): 14.3 (q), 23.6 (t), 24.3 (t), 38.8

(t), 48.4 (t), 55.7 (q), 55.8 (q), 60.1 (t), 111.2 (d), 113.1 (d), 115.7 (d), 118.8 (s), 121.3 (s), 126.0 (s), 128.9 (d), 137.0 (s), 151.3 (s), 153.6 (s), 161.4 (s), 195.3 (s). Anal. Calcd. for $C_{20}H_{23}NO_5$: C, 67.21; H, 6.49; N, 3.92. Found: C, 67.41; H, 6.69; N, 3.65.

**Data for ethyl 2-[(4-methylbenzyl)methyl]-4-oxo-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylate (30h).** This compound was obtained from reaction of **28** with 4-methylbenzyl chloride after 12 h. Yellow oil; yield: 55%; IR ($cm^{-1}$): 1691 (CO), 1664 (CO); $^1$H NMR ($CDCl_3$) (ppm): 1.23 (3H, t, J= 6.9 Hz, $CH_3$), 1.97 (2H, m, $CH_2$), 2.25 (3H, s, $CH_3$), 2.34 – 2.51 (2H, m, $CH_2$), 2.88 (2H, t, J= 4.88 Hz, $CH_2$), 4.13 – 4.23 (2H, m, $CH_2$), 5.52 (2H, s, $CH_2$), 7.02 – 7.15 (4H, m, Ar), 7.84 (1H, s, H-3). $^{13}$C NMR ($CDCl_3$) (ppm): 14.1 (t), 20.6 (q), 22.9 (t), 23.8 (t), 38.3 (t), 51.8 (t), 59.8 (t), 118.0 (s), 121.0 (s), 126.9 (2 x d), 129.0 (2 x d), 129.3 (d), 134.8 (s), 135.0 (s), 136.7 (s), 160.5 (s), 193.5 (s). Anal. Calcd. for $C_{19}H_{21}NO_3$: C, 73.29; H, 6.80; N, 4.50. Found: C, 73.69; H, 7.2; N, 4.1.

**Data for ethyl 2-[(3-nitro-4-methoxybenzyl)methyl]-4-oxo-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylate (30i).** This compound was obtained from reaction of **28** with 3-nitro,4-methoxybenzyl chloride after 16 h. Yellow solid; yield: 87%; mp: 110-111 °C; IR ($cm^{-1}$): 1685 (CO), 1661 (CO), 1534 ($NO_2$); $^1$H NMR (DMSO) (ppm): 1.24 (3H, t, J = 7.0 Hz, $CH_3$), 1.97 – 2.00 (2H, m, $CH_2$), 2.37 (2H, t, J = 5.7 Hz, $CH_2$), 2.89 (2H, t, J = 5.5 Hz, $CH_2$), 3.89 (3H, s, $CH_3$), 4.21 (2H, q, J = 7.0 Hz, $CH_2$), 5.54 (2H, s, $CH_2$), 7.46 (2H, d, J = 8.7 Hz, H-5' and H-6'), 7.80 (1H, s, H-2'), 7.69 (1H, s, H-3); $^{13}$C NMR (DMSO) (ppm): 14.1 (t), 22.9 (t), 23.8 (t), 38.3 (t), 50.7 (t), 56.7 (q), 59.9 (t), 114.6 (d), 117.4 (s), 121.3 (s), 123.9 (d), 129.4 (d), 130.2 (s), 133.4 (d), 136.7 (s), 138.8 (s), 151.4 (s), 160.5 (s), 193.5 (s). Anal. Calcd. for $C_{19}H_{20}N_2O_6$: C, 61.28; H, 5.41; N, 7.52. Found: C, 60.88; H, 5.81; N, 7.12.

### 12.1.9 Procedure for the synthesis of 2,5,6,7-tetrahydro-4H-isoindol-4-one (25d, 29k).

To a solution of **25f, 29j** (1 mmol) in ethanol (40 mL) an aqueous solution of $K_2CO_3$ (10%, 0.7 mL) was added dropwise. The mixture was stirred at room temperature up to completeness (TLC). Water was added to the reaction mixture and the solution was extracted with ethyl acetate (3 x 10 mL). The organic phase was dried over $Na_2SO_4$ and the solvent evaporated at reduced pressure. The crude product was purified using chromatography column (dichloromethane:ethyl acetate 95:5).

**Data for 4-(4-Oxo-4,5,6,7-tetraidro-2*H*-isoindol-1-yl)phenyl acetate (25d).** This compound was obtained from reaction of **25f** after 2 minutes. Yield 93%.

**Data for 1-(4-hydroxyphenyl)-2-(4-methoxybenzyl)-2,5,6,7-tetraidro-4*H*-isoindol-4-one (29k).** This compound was obtained from reaction of **29j** after 12h. Yellow oil; yield 96%; IR

($cm^{-1}$) 2956 (OH), 1641 (CO); $^{1}H$ NMR ($CDCl_3$) (ppm) 1.84 – 1.99 (2H, m, $CH_2$), 2.34 (2H, t, J = 6.4 Hz, $CH_2$), 2.53 (2H, t, J = 6.4 Hz, $CH_2$), 3.69 (3H, s, $CH_3$), 5.03 (2H, s, $CH_2$), 6.79 – 6.90 (6H, m, Ar), 7.09 (2H, d, J = 8.5 Hz, Ar), 7.42 (1H, s, H-3), 9.67 (1H, s, OH); $^{13}C$ NMR ($CDCl_3$) (ppm): 21.4 (t), 24.7 (t), 39.0 (t), 49.8 (t), 55.0 (q), 113.8 (2 x d), 115.3 (2 x d), 120.5 (s), 121.4 (s), 122.2 (d), 123.7 (s), 128.4 (2 x d), 129.0 (s), 129.6 (s), 131.0 (2 x d), 156.9 (s), 158.5 (s), 193.8 (s). Anal. Calcd for $C_{22}H_{21}NO_3$ C, 79.06; H, 6.09; N, 4.03. Found: C, 76.46; H, 6.49; N, 4.43.

**12.1.10 Procedure for the synthesis of 2-(4-methoxybenzil)1-(4-methoxyphenyl)-2,5,6,7-tetraidro-4*H*-isoindol-4-one (29l).**

To a solution of **29k** (10 mmol) in anhydrous THF (15 mL), NaH (0.24 g, 10 mmol) was added at 0 °C and the reaction mixture was stirred at room tempreature for 1 h. Iodomethane (0.3 mL, 10 mmoli) was then added at 0 °C and the reaction mixture was stirred at room temperature for 20 h. The reaction mixture was poured into ice and brine, then the aqueous solution was extracted with dichloromethane (3 x 50 mL). The organic phase was dried over $Na_2SO_4$ and the solvent evaporated under reduced pressure. The crude product was purified using chromatography column (dichloromethane:ethyl acetate 98:2). Yellow oil; yield 78%; IR ($cm^{-1}$) 1647 (CO); $^{1}H$ NMR (DMSO-$d_6$) (ppm): 1.85 – 1.99 (2H, m, $CH_2$), 2.35 (2H, t, J = 6.4 Hz, $CH_2$), 2.51 (2H, t, J = 6.4 Hz, $CH_2$), 3.69 (3H, s, $CH_3$), 3.79 (3H, s, $CH_3$), 5.05 (2H, s, $CH_2$), 6.81 (2H, d, J = 9.0 Hz, Ar), 6.87 (2H, d, J = 9.0 Hz, Ar), 6.99 (2H, d, J = 8.7 Hz, Ar), 7.22 (2H, d, J = 8.7 Hz, Ar), 7.45 (1H, s, H-3); $^{13}C$ NMR (DMSO-$d_6$) (ppm): 21.4 (t), 24.7 (t), 38.9 (t), 49.8 (t), 55.0 (q), 55.1 (q), 113.9 (2 x d), 114.0 (2 x d), 120.6 (s), 122.4 (d), 123.1 (s), 124.1 (s), 128.4 (2 x d), 128.6 (s), 129.5 (s), 131.0 (2 x d), 158.5 (s), 158.6 (s), 193.8 (s). Anal. Calcd for $C_{23}H_{23}NO_3$ C, 76.43; H, 6.41; N, 3.88. Found: C, 76.03; H, 6.01; N, 4.28.

**12.1.11 Procedure for the synthesis of 2-(3-amino-4-methoxybenzyl)-1substituted-2,5,6,7-tetrahydro-4H-isoindol-4-one (29i,o).**

To a solution of **29h,n** (1.5 mmol) in ethanol, palladium 10% on carbon was added and the reaction mixture was stirred under hydrogen atmosphere for 24 h. The solution was filtered, and the filtrate was evaporated at reduced pressure. The crude product was purified using chromatography column using (dichloromethane:ethyl acetate 90:10).

**Data for 2-(3-Amino-4-methoxybenzyl)-1-phenyl-2,5,6,7-tetrahydro-4H-isoindol-4-one (29i).** This compound was obtained from reaction of **29h**. White solid; yield 98%; mp 168−169 °C. IR ($cm^{-1}$): 3458−3375 ($NH_2$), 1649 (CO); $^{1}H$ NMR (DMSO-$d_6$) (ppm):

1.87−1.99 (2H, m, $CH_2$), 2.36 (2H, t, J = 6.6 Hz, $CH_2$), 2.56 (2H, t, J = 6.6 Hz, $CH_2$), 3.70 (3H, s, $CH_3$), 4.72 (2H, s, $NH_2$), 4.96 (2H, s, $CH_2$), 6.10 (1H, dd, J = 8.1, 2.1 Hz, Ar), 6.32 (1H, d, J = 2.1 Hz, Ar), 6.65 (1H, d, J = 8.1 Hz, Ar), 7.29−7.44 (6H, m, H-3, Ar). $^{13}$C NMR (DMSO-*d*$_6$) (ppm): 21.5 (t), 24.7 (t), 38.9 (t), 50.4 (t), 55.2 (q), 110.2 (d), 112.1 (d), 114.8 (d), 120.6 (s), 123.0 (d), 124.3 (s), 127.4 (d), 128.5 (2 x d), 128.9 (s), 129.6 (2 x d), 129.7 (s), 131.0 (s), 137.7 (s), 145.7 (s), 193.7 (s). Anal. Calcd for $C_{22}H_{22}N_2O_2$: C, 76.28; H, 6.40; N, 8.09. Found: C, 76.39; H, 6.61; N, 7.92.

**Data for 2-(3-Amino-4-methoxybenzyl)-1-(3,4,5-trimethoxyphenyl)-2,5,6,7-tetrahydro-4H-isoindol-4-one (29o).** This compound was obtained from reaction of **29n**. White solid; yield 95%; mp 164− 165 °C. IR (cm$^{-1}$): 3452−3373 ($NH_2$), 1650 (CO). $^1$H NMR (DMSO-*d*$_6$) (ppm): 1.90−1.99 (2H, m, $CH_2$), 2.35 (2H, t, J = 5.8 Hz, $CH_2$), 2.61 (2H, t, J = 5.8 Hz, $CH_2$), 3.67 (6H, s, 2 x $CH_3$), 3.68 (3H, s, $CH_3$), 3.71 (3H, s, $CH_3$), 4.76 (2H, s, $NH_2$), 4.97 (2H, s, $CH_2$), 6.15−6.20 (1H, m, Ar), 6.36 (1H, s, Ar), 6.51 (2H, s, H-2′ and H-6′), 6.70 (1H, d, J = 8.1 Hz, Ar), 7.41 (1H, s, H-3). $^{13}$C NMR (DMSO-*d*$_6$) (ppm): 21.5 (t), 24.8 (t), 39.2 (t), 50.7 (t), 55.3 (q), 55.7 (2 x q), 60.0 (q), 106.9 (2 x d), 110.9 (d), 111.4 (d), 111.9 (d), 112.1 (s), 114.5 (d), 114.7 (s), 120.6 (s), 124.2 (s), 130.4 (s), 136.9 (s), 137.8 (s), 145.7 (s), 152.7 (2 x s), 194.0 (s). Anal. Calcd for $C_{25}H_{28}N_2O_5$: C, 68.79; H, 6.47; N, 6.42. Found: C, 68.65; H, 6.39; N, 6.60.

### 12.1.12 Procedure for the synthesis of ethyl 2-[(3-amino,4-methoxybenzyl)methyl]-4-oxo-2,4,5,6,7,7a-hexahydro-1H-isoindole-1-carboxylate (30j).

To a solution of **30i** (0.5 mmol) in glacial acetic acid (5 mL), iron powder (0.28g, 5mmol) was added. The reaction mixture was heated at 45°C for 12 h. The mixture was poured into ice and brine and then filtered to remove the excess of iron powder. The aqueous solution was extracted with ethyl ether (3 x 50 mL). The organic phase was dried over $Na_2SO_4$ and the solvent evaporated at reduced pressure. The crude product was purified using chromatography column (dichloromethane : ethyl acetate 93 : 7).

White solid; yield: 70%; mp: 103-104 °C; IR (cm$^{-1}$): 3454 – 3371 ($NH_2$), 1687 (CO), 1659 (CO); $^1$H NMR (DMSO) (ppm): 1.26 (3H, t, J = 7.0 Hz, $CH_3$), 1.94 – 2.00 (2H, m, $CH_2$), 2.37 (2H, t, J = 5.9 Hz, $CH_2$), 2.90 (2H, t, J = 5.7 Hz, $CH_2$), 3.71 (3H, s, $CH_3$), 4.15 – 4.26 (2H, m, $CH_2$), 4.75 (2H, s, $NH_2$), 5.37 (2H, s, $CH_2$), 6.4 (2H, d, J = 2.0 Hz, H-5’ and H-6’), 6.73 (1H, s, H-3’), 7.72 (1H, s, H-3); $^{13}$C NMR (DMSO) (ppm): 14.6 (t), 23.4 (t), 24.4 (t), 50.3 (t), 52.4 (q), 55.7 (q), 60.3 (t), 110.7 (s), 112.9 (s), 115.6 (d), 118.2 (s), 121.3 (s), 129.5 (s), 130.4 (d),

136.9 (d), 138.1 (d), 146.3 (s), 161.0 (s), 194.2 (s). Anal. Calcd. for $C_{19}H_{22}N_2O_4$: C, 66.65; H, 6.48; N, 8.18. Found: C, 67.05; H, 6.88; N, 7.78.

**12.1.13 Procedure for the synthesis of 2-substituted-2,5,6,7-tetrahydro-4H-isoindol-4-ones (31a-f).**

To a solution of **25a,b** (10 mmol) in anhydrous DMF (15 mL), NaH (0.24 g, 10 mmol) was added at 0 °C and the reaction was stirred for 1 h at room temperature. The suitable chloride (40 mmol) was added at 0 °C, followed by the addition of TBAI (11 mmol). The reaction mixture was stirred at room temperature up to completeness (TLC) and then, poured into ice and brine. The aqueous solution was extracted with dichloromethane (3 x 50 mL) and the organic phase was dried over $Na_2SO_4$ and evaporated at reduced pressure. The crude product was purified using chromatography column (dichloromethane : methanol 98 : 2).

**Data for 2-(2-morpholinoethyl)-1-phenyl-2,5,6,7-tetrahydro-4H-isoindol-4-one (31a).** This compound was obtained from reaction of **25a** after 6 h. Yellow oil; yield: 55%; IR ($cm^{-1}$): 1659 (CO); $^1$H NMR (DMSO) (ppm): 1.89 – 1.98 (2H, m, $CH_2$), 2.21 (2H, t, J = 4.6 Hz, $CH_2$), 2.32 – 2.57 (8H, m, 4 x $CH_2$), 3.46 (4H, t, J= 4.6 Hz, 2 x $CH_2$), 4.04 (2H, d, J = 6.6 Hz, $CH_2$), 7.36 – 7.54 (5H, m, Ar), 7.96 (1H, s, Ar); $^{13}$C NMR (DMSO) (ppm): 21.4 (t), 24.7 (t), 38.9 (t), 43.9 (t), 53.1 (2 x t), 58.5 (t), 66.00 (2 x t), 120.5 (s), 123.1 (d), 124.0 (s), 127.5 (d), 128.6 (2 x d), 128.7 (s), 129.7 (2 x d), 131.1 (s), 162.3 (s), 193.7 (s). Anal. Calcd. for $C_{20}H_{24}N_2O_2$: C, 74.05; H, 7.46; N, 8.63. Found: C, 73.65; H, 7.06; N, 9.03.

**Data for 1-phenyl-2-(2-(pyrrolidin-1-yl)ethyl)-2,5,6,7-tetrahydro-4H-isoindol-4-one (31b).** This compound was obtained from reaction of **25a** after 5 h. Yellow oil; yield: 40%; IR ($cm^{-1}$): 1656 (CO); $^1$H NMR (DMSO) (ppm): 1.58 (4H, d, J = 3.1 Hz, 2 x $CH_2$), 1.91 (2H, t, J = 5.7 Hz, $CH_2$), 2.26 – 2.38 (6H, m, 3 x $CH_2$), 2.51 – 2.57 (4H, m, 2 x $CH_2$), 4.03 (2H, t, J = 6.6 Hz, $CH_2$), 7.36 – 7.53 (6H, m, Ar and H-3); $^{13}$C NMR (DMSO) (ppm): 21.4 (t), 23.00 (2 x t), 24.7 (t), 38.9 (t), 39.7 (t), 45.9 (t), 53.4 (2 x t), 55.9 (s), 120.4 (s), 123.0 (d), 124.0 (s), 127.5 (d), 128.6 (2 x d), 129.6 (2 x d), 131.1 (s), 193.7 (s). Anal. Calcd. for $C_{20}H_{24}N_2O$: C, 77.89; H, 7.84; N, 9.08. Found: C, 78.29; H, 8.24; N, 9.48.

**Data for 1-phenyl-2-(2-(piperidin-1-yl)ethyl)-2,5,6,7-tetrahydro-4H-isoindol-4-one (31c).** This compound was obtained from reaction of **25a** after 5 h. Yellow oil; yield: 43%; IR ($cm^{-1}$): 1655 (CO); $^1$H NMR (DMSO) (ppm): 1.34 – 1.38 (6H, m, 3 x $CH_2$), 1.91 (2H, t, J = 5.5 Hz, $CH_2$), 1.99 (4H, s, 2 x $CH_2$), 2.18 – 2.42 (4H, m, 2 x $CH_2$), 2.51 – 2.73 (2H, m, $CH_2$), 4.00 (2H, t, J = 6.7 Hz, $CH_2$), 7.35 – 7.53 (6H, m, Ar and H-3); $^{13}$C NMR (DMSO) (ppm): 21.8 (t), 24.1 (t), 25.1 (t), 25.8 (2 x t), 39.4 (t), 45.1 (t), 54.6 (2 x t), 59.6 (t), 121.3 (s), 122.6 (d), 124.9

(s), 127.7 (d), 128.6 (2 x d), 130.0 (2 x d), 131.4 (s), 162.3 (s), 195.8 (s). Anal. Calcd. for $C_{21}H_{26}N_2O$: C, 78.22; H, 8.13; N, 8.69. Found: C, 78.62; H, 8.53; N, 8.29.

**Data for 2-(2-morpholinoethyl)-1-(3,4,5-trimethoxyphenyl)-2,5,6,7-tetrahydro-4H-isoindol-4-one (31d).** This compound was obtained from reaction of **25b** after 5 h. Yellow oil; yield: 63%; IR ($cm^{-1}$): 1659 (CO); $^1H$ NMR (DMSO) (ppm): 1.98 – 2.09 (2H, m, $CH_2$), 2.38 – 2.48 (6H, m, 3 x $CH_2$), 2.59 (2H, t, J = 4.6 Hz, $CH_2$), 2.86 (2H, t, J= 4.6 Hz, $CH_2$), 3.74 – 3.81 (13H, m, 2 x $CH_2$ and 3 x $CH_3$), 3.98 – 4.09 (2H, m, $CH_2$), 6.55 (1H, s, Ar), 7.10 (2H, s, Ar); $^{13}C$ NMR (DMSO) (ppm): 22.3 (t), 23.7 (t), 37.0 (t), 47.7 (t), 53.2 (2 x t), 55.9 (t), 56.8 (2 x q), 60.7 (q), 66.8 (2 x t), 106.2 (2 x d), 113.3 (s), 115.4 (s), 128.6 (d), 130.8 (s), 131.5 (s), 141.0 (s), 153.0 (2 x s), 189.6 (s). Anal. Calcd. for $C_{23}H_{30}N_2O_5$: C, 66.65; H, 7.30; N, 6.76. Found: C, 66.25; H, 7.70; N, 6.36.

**Data for 2-(2-(pyrrolidin-1-yl)ethyl)-1-(3,4,5-trimethoxyphenyl)-2,5,6,7-tetrahydro-4H-isoindol-4-one (31e).** This compound was obtained from reaction of **25b** after 8 h. Yellow oil; yield: 57%; IR ($cm^{-1}$): 1656 (CO); $^1H$ NMR (DMSO) (ppm): 1.59 – 1.73 (4H, m, 2 x $CH_2$), 1.97 – 2.09 (2H, m, $CH_2$), 2.25 – 2.32 (2H, m, $CH_2$), 2.38 – 2.48 (4H, m, 2 x $CH_2$), 2.58 – 2.65 (2H, m, $CH_2$), 2.85 – 2.92 (2H, m, $CH_2$), 3.81 (3H, s, $CH_3$), 3.82 (6H, s, 2 x $CH_3$), 3.99 – 4.12 (2H, m, $CH_2$), 6.56 (1H, s, Ar), 7.08 (2H, s, Ar); $^{13}C$ NMR (DMSO) (ppm): 22.4 (t), 23.6 (t), 24.9 (2 x t), 37.2 (t), 47.5 (t), 55.9 (t), 54.2 (2 x t), 56.6 (2 x q), 60.4 (q), 106.4 (2 x d), 113.4 (s), 115.6 (s), 128.4 (d), 130.6 (s), 131.7 (s), 141.2 (s), 153.1 (2 x s), 189.9 (s). Anal. Calcd. for $C_{23}H_{30}N_2O_4$: C, 69.32; H, 7.59; N, 7.03. Found: C, 69.72; H, 7.19; N, 7.43.

**Data for 2-(2-(piperidin-1-yl)ethyl)-1-(3,4,5-trimethoxyphenyl)-2,5,6,7-tetrahydro-4H-isoindol-4-one (31f).** This compound was obtained from reaction of **25b** after  h. Yellow oil; yield: 40%; $^1H$ NMR (DMSO) (ppm):1.39 – 1.52 (10H, m, 5 x $CH_2$), 2.01 – 2.10 (2H, m, $CH_2$), 2.43 – 2.65 (6H, m, 3 x $CH_2$), 3.91 (9H, s, 3 x $CH_3$), 4.00 (2H, t, J = 7.3 Hz, $CH_2$), 6.51 (2H, s, H-2' and H-6'),  7.41 (1H, s, H-3); $^{13}C$ NMR (DMSO) (ppm): 21.9 (t), 24.1 (t), 25.1 (t), 25.8 (2 x t), 39.4 (t), 45.2 (t), 54.8 (2 x t), 56.2 (2 x q), 59.8 (t), 61.0 (q), 101.0 (s), 107.3 (2 x d), 117.0 (s), 122.4 (d), 124.7 (s), 127.0 (s), 137.0 (s), 151.0 (s), 153.2 (2 x s). Anal. Calcd. for $C_{24}H_{32}N_2O_4$: C, 69.88; H, 7.82; N, 6.79. Found: C, 70.28; H, 7.42; N, 7.19.

### 12.1.14 Procedure for the synthesis of ethyl (2-substituted-4-oxo-2,4,6,7-tetrahydro-5H-isoindol-5-ylidene)(hydroxy)ethanoate (32a-l)

To a solution of *t*-BuOK (3.7 g, 36 mmol) in anhydrous toluene (30 mL), a solution of proper ketone **29** (12 mmol) in anhydrous toluene (40mL) was added dropwise under $N_2$ atmosphere at 0 °C. After stirring for 2 h and 30 minutes at room temperature, the reaction was cooled at

0 °C and a solution of diethyl oxalate (36 mmol) in anhydrous toluene (20mL) was added. The reaction mixture was stirred for 2 h at room temperature, then the solvent was evaporated under reduced pressure. The residue was dissolved in water and the solution was washed with diethyl ether. The aqueous solution was solution was acidified with HCl 6 M and the solid obtained was filtered and dried. The crude product was purified using chromatography column (dichloromethane).

**Data for ethyl 2-(2-benzyl-4-oxo-2,4,6,7-tetrahydro-5H-isoindol-5-ylidene)-2-hydroxyacetate (32a).** This product was obtained from reaction of **29a**. Brown oil; yield 58%; IR ($cm^{-1}$): 3449 (OH), 1731 (CO), 1645 (CO); $^{1}H$ NMR ($CDCl_3$) (ppm): 1.39 (3H, t, J = 7.1 Hz, $CH_3$), 2.65 (2H, t, J = 7.3 Hz, $CH_2$), 2.96 (2H, t, J = 7.3 Hz, $CH_2$), 4.35 (2H, q, J = 7.1 Hz, $CH_2$), 5.04 (2H, s, $CH_2$), 6.42 (1H, s, H-1), 7.20 – 7.35 (6H, m, H-3, H-2', H-3', H-4', H-5' and H-6'), 15.87 (1H, s, OH); $^{13}C$ NMR ($CDCl_3$) (ppm): 14.1 (q), 20.4 (t), 24.7 (t), 54.2 (t), 61.8 (t), 110.6 (s), 117.4 (d), 120.6 (s), 123.7 (d), 126.5 (d), 127.6 (2 x d), 128.4 (s), 129.0 (2 x d), 136.0 (s), 161.5 (s), 163.5 (s), 188.5 (s). Anal. Calcd for $C_{19}H_{19}NO_4$: C, 70.14; H, 5.89; N, 4.31. Found: C, 70.44; H, 5.49; N, 4.71.

**Data for ethyl 2-hydroxy-2-(2-(3-methoxybenzyl)-4-oxo-2,4,6,7-tetrahydro-5H-isoindol-5-ylidene)acetate (32b).** This compound was obtained from reaction of **29b**. Brown oil; yield 74%; IR ($cm^{-1}$): 3445 (OH), 1738 (CO), 1658 (CO); $^{1}H$ NMR ($CDCl_3$) (ppm): 1.39 (3H, t, J = 7.1 Hz, $CH_3$), 2.65 (2H, t, J = 7.3 Hz, $CH_2$), 2.96 (2H, t, J = 7.3 Hz, $CH_2$), 3.79 (3H, s, $CH_3$), 4.35 (2H, q, J = 7.1 Hz, $CH_2$), 5.01 (2H, s, $CH_2$), 6.42 (1H, s, H-1), 6.70 – 6.88 (3H, m, H-2', H-4' and H-6'), 7.24 – 7.34 (2H, m, H-3 and H-5'), 15.85 (1H, s, OH); $^{13}C$ NMR ($CDCl_3$) (ppm): 14.1 (q), 20.4 (t), 24.7 (t), 54.1 (t), 55.3 (q), 61.8 (t), 110.6 (s), 113.4 (d), 113.5 (d), 117.4 (d), 119.8 (d), 120.6 (s), 123.7 (d), 126.5 (s), 130.1 (d), 137.6 (s), 160.1 (s), 161.5 (s), 163.5 (s), 188.5 (s). Anal. Calcd for $C_{20}H_{21}NO_5$: C, 67.59; H, 5.96; N, 3.94. Found: C, 67.19; H, 5.59; N, 3.54.

**Data for ethyl 2-(2-(3,4-dimethoxybenzyl)-4-oxo-2,4,6,7-tetrahydro-5H-isoindol-5-ylidene)-2-hydroxyacetate (32c).** This compound was obtained from reaction of **29c**. Brown oil; yield 42%; IR ($cm^{-1}$): 3450 (OH), 1732 (CO), 1658 (CO); $^{1}H$ NMR ($CDCl_3$) (ppm): 1.39 (3H, t, J = 7.1 Hz, $CH_3$), 2.64 (2H, t, J = 7.2 Hz, $CH_2$), 2.95 (2H, t, J = 7.8 Hz, $CH_2$), 3.85 (3H, s, $CH_3$), 3.88 (3H, s, $CH_3$), 4.35 (2H, q, J = 7.1 Hz, $CH_2$), 4.97 (2H, s, $CH_2$), 6.42 (1H, s, H-1), 7.33 (1H, s, H-3), 6.69 – 6.83 (3H, m, H-2', H-5' and H-6'), 15.87 (1H, s, OH); $^{13}C$ NMR ($CDCl_3$) (ppm): 14.1 (q), 20.4 (t), 24.7 (t), 54.0 (t), 55.9 (q), 56.0 (q), 61.8 (t), 110.6 (d), 110.8 (s), 111.2 (d), 117.2 (d), 120.4 (d), 123.5 (d), 126.5 (s), 128.2 (s), 131.7 (s), 149.1 (s),

149.3 (s), 161.4 (s), 163.5 (s), 188.6 (s). Anal. Calcd for $C_{21}H_{23}NO_6$: C, 65.44; H, 6.02; N, 3.63. Found: C, 65.04; H, 6.42; N, 3.23.

**Data for ethyl 2-(2-(3,5-dimethoxybenzyl)-4-oxo-2,4,6,7-tetrahydro-5H-isoindol-5-ylidene)-2-hydroxyacetate (32d).** This compound was obtained from reaction of **29d**. Brown oil; yield 68%; IR ($cm^{-1}$): 3445 (OH), 1732 (CO), 1612 (CO); $^1H$ NMR ($CDCl_3$) (ppm): 1.40 (3H, t, J = 7.1 Hz, $CH_3$), 2.65 (2H, t, J = 7.3 Hz, $CH_2$), 2.96 (2H, t, J = 7.3 Hz, $CH_2$), 3.77 (6H, s, 2 x $CH_3$), 4.35 (2H, q, J = 7.1 Hz, $CH_2$), 4.96 (2H, s, $CH_2$), 6.31 (2H, d, J = 2.2 Hz, H-2' and H-6'), 6.40 – 6.42 (2H, m, H-1 and H-4'), 7.33 (1H, d, J = 2.0 Hz, H-3), 15.87 (1H, s, OH); $^{13}C$ NMR ($CDCl_3$) (ppm): 14.1 (q), 20.4 (t), 24.7 (t), 54.2 (t), 55.4 (2 x q), 61.8 (t), 99.7 (d), 105.7 (2 x d), 110.6 (s), 117.4 (d), 120.6 (s), 123.7 (d), 126.4 (s), 138.3 (s), 161.3 (2 x s), 161.5 (s), 163.5 (s), 188.5 (s). Anal. Calcd for $C_{21}H_{23}NO_6$: C, 65.44; H, 6.02; N, 3.63. Found: C, 65.04; H, 6.42; N, 4.03.

**Data for ethyl 2-(2-(3,4,5-trimethoxybenzyl)-4-oxo-2,4,6,7-tetrahydro-5H-isoindol-5-ylidene)-2-hydroxyacetate (32e).** This compound was obtained from reaction of **29e** and used in the next step without purification.

**Data for ethyl 2-(2-benzyl-4-oxo-1-phenyl-2,4,6,7-tetrahydro-5H-isoindol-5-ylidene)-2-hydroxyacetate (32f).** This compound was obtained from reaction of **29f**. Brown oil; yield 76%; IR ($cm^{-1}$): 3450 (OH), 1730 (CO), 1654 (CO); $^1H$ NMR ($CDCl_3$) (ppm): 1.39 (3H, t, J = 7.1 Hz, $CH_3$), 2.64 (2H, t, J = 7.3 Hz, $CH_2$), 2.97 (2H, t, J = 7.3 Hz, $CH_2$), 4.35 (2H, q, J = 7.1 Hz, $CH_2$), 5.07 (2H, s, $CH_2$), 6.68 – 7.02 (2H, m, Ar), 7.20 – 7.43 (9H, m, H-3 and Ar), 15.90 (1H, s, OH); $^{13}C$ NMR ($CDCl_3$) (ppm): 14.1 (q), 20.4 (t), 24.7 (t), 54.4 (t), 61.6 (t), 110.6 (s), 118.0 (s), 120.0 (s), 120.5 (s), 123.8 (d), 125.5 (d), 127.2 (2 x d), 128.5 (2 x d), 128.6 (d), 129.0 (2 x d), 129.2 (2 x d), 136.5 (s), 137.7 (s), 155.1 (s), 165.0 (s), 187.0 (s). Anal. Calcd for $C_{25}H_{23}NO_4$: C, 74.80; H, 5.77; N, 3.49. Found: C, 74.40; H, 5.37; N, 3.89.

**Data for ethyl 2-hydroxy-2-(2-(4-methoxybenzyl)-4-oxo-1-phenyl-2,4,6,7-tetrahydro-5H-isoindol-5-ylidene)acetate (32g).** This compound was obtained from reaction of **29g**. Brown oil; yield 76%; IR ($cm^{-1}$): 3450 (OH), 1732 (CO), 165 (CO); $^1H$ NMR ($CDCl_3$) (ppm): 1.39 (3H, t, J = 7.1 Hz, $CH_3$), 2.62 (2H, t, J = 7.3 Hz, $CH_2$), 2.96 (2H, t, J = 7.3 Hz, $CH_2$), 3.78 (3H, s, $CH_3$), 4.35 (2H, q, J = 7.1 Hz, $CH_2$), 4.99 (2H, s, $CH_2$), 6.81 (2H, d, J = 8.7 Hz, H-3' and H-5'), 6.94 (2H, d, J = 8.7 Hz, H-2' and H-6'), 7.26 – 7.39 (6H, m, H-3, H-2", H-3", H-4", H-5" and H-6"), 15.90 (1H, s, OH); $^{13}C$ NMR ($CDCl_3$) (ppm): 14.1 (q), 20.4 (t), 24.7 (t), 52.1 (t), 55.3 (q), 61.8 (t), 110.8 (s), 114.2 (2 x d), 120.0 (s), 123.8 (d), 124.0 (s), 128.0 (d), 128.5 (s), 128.6 (2 x d), 128.7 (2 x d), 130.1 (2 x d), 130.7 (s), 159.3 (s), 161.6 (s), 163.5 (s),

188.5 (s), 191.0 (s). Anal. Calcd for $C_{26}H_{25}NO_5$: C, 72.37; H, 5.84; N, 3.25. Found: C, 72.77; H, 5.44; N, 3.65.

**Data for ethyl ethyl 2-hydroxy-2-(2-(3-amino,4-methoxybenzyl)-4-oxo-1-phenyl-2,4,6,7-tetrahydro-5H-isoindol-5-ylidene)acetate (32h).** This compound was obtained from reaction of **29i** and used in the next step without purification.

**Data for ethyl 2-hydroxy-2-(1-(4-hydroxyphenyl)-2-(4-methoxybenzyl)-4-oxo-2,4,6,7-tetrahydro-5H-isoindol-5-ylidene)acetate (32i).** This compound was obtained from reaction of **29j** and used in the next step without purification.

**Data for ethyl 2-hydroxy-2-(2-(4-methoxybenzyl)-1-(4-methoxyphenyl)-4-oxo-2,4,6,7-tetrahydro-5H-isoindol-5-ylidene)acetate (32j).** This compound was obtained from reaction of **29l**. Brown oil; yield 62%; IR ($cm^{-1}$): 3450 (OH), 1732 (CO), 1617 (CO); $^1H$ NMR ($CDCl_3$) (ppm): 1.39 (3H, t, J = 7.1 Hz, $CH_3$), 2.59 (2H, t, J = 7.3 Hz, $CH_2$), 2.92 (2H, t, J = 7.3 Hz, $CH_2$), 3.78 (3H, s, $CH_3$), 3.84 (3H, s, $CH_3$), 4.35 (2H, q, J = 7.1 Hz, $CH_2$), 4.95 (2H, s, $CH_2$), 6.81 (2H, d, J = 8.8 Hz, Ar), 6.94 (4H, d, J = 8.4 Hz, Ar), 7.15 (2H, d, J = 8.8 Hz, Ar), 7.34 (1H, s, H-3), 15.91 (1H, s, OH); $^{13}C$ NMR ($CDCl_3$) (ppm): 14.1 (q), 20.4 (t), 24.7 (t), 51.0 (t), 55.3 (q), 55.4 (q), 61.8 (t), 110.8 (s), 114.1 (2 x d), 114.2 (2 x d), 119.8 (s), 122.9 (s), 123.5 (s), 128.6 (s), 128.7 (2 x d), 129.3 (d), 129.8 (s), 131.4 (2 x d), 143.6 (s), 159.3 (s), 161.6 (s), 163.5 (s), 188.5 (s). Anal. Calcd for $C_{27}H_{27}NO_6$: C, 70.27; H, 5.90; N, 3.04. Found: C, 70.67; H, 5.50; N, 3.44.

**Data for ethyl 2-hydroxy-2-(2-(4-methoxybenzyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)-2,4,6,7-tetrahydro-5H-isoindol-5-ylidene)acetate (32k).** This compound was obtained from reaction of **29m**. Brown oil; yield 63%; IR ($cm^{-1}$): 3450 (OH), 1726 (CO), 1617 (CO); $^1H$ NMR ($CDCl_3$) (ppm): 1.40 (3H, t, J = 7.1 Hz, $CH_3$), 2.65 (2H, t, J = 7.3 Hz, $CH_2$), 2.98 (2H, t, J = 7.3 Hz, $CH_2$), 3.73 (6H, s, 2 x $CH_3$), 3.78 (3H, s, $CH_3$), 3.88 (3H, s, $CH_3$), 4.36 (2H, q, J = 7.1 Hz, $CH_2$), 5.01 (2H, s, $CH_2$), 6.38 (2H, s, H-2" and H-6"), 6.84 (2H, d, J = 8.8 Hz, H-3' and H-5'), 6.97 (2H, d, J = 8.8 Hz, H-2' and H-6'), 7.40 (1H, s, H-3), 15.89 (1H, s, OH); $^{13}C$ NMR ($CDCl_3$) (ppm): 14.1 (q), 20.5 (t), 24.7 (t), 51.2 (t), 55.4 (q), 56.0 (2 x q), 60.9 (q), 61.8 (t), 107.2 (2 x d), 110.7 (s), 114.3 (2 x d), 119.9 (s), 123.6 (s), 124.0 (d), 126.0 (s), 128.3 (2 x d), 128.9 (s), 130.0 (s), 137.8 (s), 153.2 (2 x s), 159.3 (s), 161.7 (s), 163.4 (s), 188.5 (s). Anal. Calcd for $C_{29}H_{31}NO_8$: C, 66.78; H, 5.99; N, 2.69. Found: C, 66.38; H, 5.59; N, 2.29.

**Data for ethyl ethyl 2-hydroxy-2-(2-(3-amino,4-methoxybenzyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)-2,4,6,7-tetrahydro-5H-isoindol-5-ylidene)acetate (32l).** This compound was obtained from reaction of **29o** and used in the next step without purification.

**12.1.15 Procedure for the synthesis of ethyl 5-(hydroxymethylidene)-2-substituted-4-oxo-2,4,5,6,7,7a-hexahydro-1H-isoindole-1-carboxylate (33a-g)**

To a solution of *t*-BuOK (3.7 g, 36 mmol) in anhydrous toluene (30 mL), a solution of proper ketone **30a-g** (12 mmol) in anhydrous toluene (40mL) was added dropwise under $N_2$ atmosphere at 0 °C. After stirring for 2 h and 30 minutes at room temperature, the reaction was cooled at 0 °C and a solution of ethyl formate (36 mmol) in anhydrous toluene (20mL) was added. The reaction mixture was stirred for 24 h at room temperature, then the solvent was evaporated under reduced pressure. The residue was dissolved in water and the aqueous solution was acidified with HCl 6 M and the solid obtained was filtered and dried. The crude product was purified by chromatography column using (dichloromethane).

**Data for ethyl 2-benzyl-5-(hydroxymethylidene)-4-oxo-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylate (33a).** This compound was obtained from reaction of **30a.** Brown oil; yield: 57%; IR ($cm^{-1}$): 3485 (OH), 1703 (CO), 1696 (CO); $^1$H NMR ($CDCl_3$) (ppm): 1.32 (3H, t, J = 7.1 Hz, $CH_3$), 2.54 (2H, t, J = 6.9 Hz, $CH_2$), 3.00 (2H, t, J = 6.9 Hz, $CH_2$), 4.26 (2H, q, J = 7.1 Hz, $CH_2$), 5.55 (2H, s, $CH_2$), 7.14 – 7.17 (2H, m, H-2' and H-6'), 7.28 – 7.36 (3H, m, H-3', H-4' and H-5'), 7.44 (1H, s, H-3), 7.52 (1H, s, CH), 14.12 (1H, s, OH); $^{13}$C NMR ($CDCl_3$) (ppm): 14.3 (q), 22.7 (t), 24.9 (t), 53.4 (t), 60.3 (t), 108.9 (s), 119.0 (s), 120.7 (s), 127.3 (2 x d), 127.9 (d), 128.6 (d), 128.8 (2 x d), 135.3 (s), 136.7 (s), 161.2 (s), 165.9 (d), 186.0 (s). Anal. Calcd. for $C_{19}H_{19}NO_4$: C, 70.14; H, 5.89; N, 4.31. Found: C, 70.03; H, 5.98; N, 4.45.

**Data for ethyl 5-(hydroxymethylidene)-2-(4-methoxybenzyl)-4-oxo-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylate (33b).** This compound was obtained from reaction of **30b.** Brown oil; yield: 60%; IR ($cm^{-1}$): 3428 (OH), 1701 (CO), 1694 (CO); $^1$H NMR ($CDCl_3$) (ppm): 1.34 (3H, t, J = 7.1 Hz, $CH_3$), 2.53 (2H, t, J = 6.9 Hz, $CH_2$), 2.99 (2H, t, J = 6.9 Hz, $CH_2$), 3.79 (3H, s, $CH_3$), 4.28 (2H, q, J = 7.1 Hz, $CH_2$), 5.47 (2H, s, $CH_2$), 6.86 (2H, d, J = 8.7 Hz, H-3' and H-5'), 7.14 (2H, d, J = 8.7 Hz, H-2' and H-6'), 7.40 (1H, s, H-3), 7.51 (1H, s, CH), 14.11 (1H, s, OH); $^{13}$C NMR ($CDCl_3$) (ppm): 14.4 (q), 22.7 (t), 24.9 (t), 52.9 (t), 55.3 (q), 60.3 (t), 108.9 (s), 114.2 (2 x d), 118.9 (s), 120.6 (s), 128.3 (d), 128.5 (s), 129.1 (2 x d), 135.4 (s), 159.4 (s), 161.3 (s), 165.8 (d), 186.0 (s). Anal. Calcd. for $C_{20}H_{21}NO_5$: C, 67.59; H, 5.96; N, 3.94. Found: C, 67.41; H, 6.06; N, 4.05.

**Data for ethyl 2-[(3,5-dimethoxyphenyl)methyl]-5-(hydroxymethylidene)-4-oxo-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylate (33c).** This compound was obtained from reaction of **30c.** Brown oil; yield: 73%; IR ($cm^{-1}$): 3501 (OH), 1700 (CO), 1696 (CO); $^1$H NMR ($CDCl_3$) (ppm): 1.33 (3H, t, *J* = 7.1 Hz, $CH_3$), 2.54 (2H, t, J = 6.9 Hz, $CH_2$), 3.00 (2H, t, J = 6.9 Hz, $CH_2$), 3.75 (6H, s, 2 x $CH_3$), 4.28 (2H, q, J = 7.1 Hz, $CH_2$), 5.48 (2H, s, $CH_2$), 6.28

(2H, d, J = 2.2 Hz, H-2' and H-6'), 6.37 (1H, t, J = 2.2 Hz, H-4'), 7.41 (1H, s, H-3), 7.52 (1H, s, CH), 14.11 (1H, s, OH); $^{13}$C NMR ($CDCl_3$) (ppm): 14.3 (q), 22.7 (t), 24.9 (t), 53.3 (t), 55.3 (2 x q), 60.3 (t), 99.4 (d), 105.4 (2 x d), 108.9 (s), 119.0 (s), 120.7 (s), 128.7 (d), 135.3 (s), 139.1 (s), 161.1 (2 x s), 161.3 (s), 166.0 (d), 185.9 (s). Anal. Calcd. for $C_{21}H_{23}NO_6$: C, 65.44; H, 6.02; N, 3.63. Found: C, 65.56; H, 5.92; N, 3.77.

**Data for ethyl 5-(hydroxymethylidene)-4-oxo-2-[(3,4,5-trimethoxyphenyl)methyl]-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylate (33d).** This compound was obtained from reaction of **30d.** Light brown solid; yield: 43%; mp: 102 – 103 $^{o}$C; IR (cm$^{-1}$): 3393 (OH), 1700 (CO), 1695 (CO); $^{1}$H NMR ($CDCl_3$) (ppm): 1.36 (3H, t, J = 7.1 Hz, $CH_3$), 2.55 (2H, t, J = 6.9 Hz, $CH_2$), 3.01 (2H, t, J = 6.9 Hz, $CH_2$), 3.82 (6H, s, 2 x $CH_3$), 3.83 (3H, s, $CH_3$), 4.31 (2H, q, J = 7.1 Hz, $CH_2$), 5.47 (2H, s, $CH_2$), 6.44 (2H, s, H-2' and H-6'), 7.43 (1H, s, H-3), 7.51 (1H, s, CH), 14.10 (1H, s, OH); $^{13}$C NMR ($CDCl_3$) (ppm): 14.4 (q), 22.7 (t), 24.9 (t), 53.5 (t), 56.1 (2 x q), 60.3 (t), 60.9 (q), 104.9 (2 x d), 108.9 (s), 119.0 (s), 120.7 (s), 128.4 (d), 132.0 (s), 135.3 (s), 137.7 (s), 153.5 (2 x s), 161.4 (s), 165.8 (d), 186.1 (s). Anal. Calcd. for $C_{22}H_{25}NO_7$: C, 63.60; H, 6.07; N, 3.37. Found: C, 63.72; H, 5.98; N, 3.29.

**Data for ethyl 2-[(3,4-dimethoxyphenyl)methyl]-5-(hydroxymethylidene)-4-oxo-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylate (33e).** This compound was obtained from reaction of **30e.** Brown oil; yield: 67%; IR (cm$^{-1}$): 3502 (OH), 1700 (CO), 1696 (CO); $^{1}$H NMR ($CDCl_3$) (ppm): 1.35 (3H, t, *J* = 7.1 Hz, $CH_3$), 2.54 (2H, t, J = 6.9 Hz, $CH_2$), 3.00 (2H, t, J = 6.9 Hz, $CH_2$), 3.84 (3H, s, $CH_3$), 3.86 (3H, s, $CH_3$), 4.30 (2H, q, J = 7.1 Hz, $CH_2$), 5.47 (2H, s, $CH_2$), 6.72 – 6.84 (3H, m, H-2', H-5' and H-6'), 7.41 (1H, s, H-3), 7.51 (1H, s, CH), 14.12 (1H, s, OH); $^{13}$C NMR ($CDCl_3$) (ppm): 14.4 (q), 22.7 (t), 24.9 (t), 53.2 (t), 55.8 (q), 55.9 (q), 60.3 (t), 108.9 (s), 111.0 (d), 111.2 (d), 118.9 (s), 120.3 (d), 120.6 (s), 128.3 (d), 128.9 (s), 135.3 (s), 148.8 (s), 149.1 (s), 161.4 (s), 165.8 (d), 186.0 (s). Anal. Calcd. for $C_{21}H_{23}NO_6$: C, 65.44; H, 6.02; N, 3.63. Found: C, 65.51; H, 5.85; N, 3.51.

**Data for ethyl 2-[(2,3-dimethoxyphenyl)methyl]-5-(hydroxymethylidene)-4-oxo-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylate (33f).** This compound was obtained from reaction of **30f.** Light brown solid; yield: 30%; mp: 129 – 130 $^{o}$C; IR (cm$^{-1}$): 3433 (OH), 1695 (CO), 1635 (CO); $^{1}$H NMR ($CDCl_3$) (ppm): 1.33 (3H, t, J = 7.1 Hz, $CH_3$), 2.54 (2H, t, J = 6.9 Hz, $CH_2$), 3.01 (2H, t, J = 6.9 Hz, $CH_2$), 3.84 (3H, s, $CH_3$), 3.87 (3H, s, $CH_3$), 4.29 (2H, q, J = 7.1 Hz, $CH_2$), 5.58 (2H, s, $CH_2$), 6.53 – 6.57 (1H, m, Ar), 6.86 – 7.03 (2H, m, Ar), 7.33 (1H, s, H-3), 7.51 (1H, s, CH), 14.12 (1H, s, OH); $^{13}$C NMR ($CDCl_3$) (ppm): 14.4 (q), 22.7 (t), 25.0 (t), 38.8 (t), 48.5 (t), 55.8 (q), 60.2 (t), 60.6 (q), 108.9 (s), 112.5 (d), 119.2 (s), 120.5 (d), 124.3 (d), 128.9 (d), 129.6 (s), 130.4 (s), 135.1 (s), 146.8 (s), 152.6 (s), 161.3 (s), 165.8 (d), 186.0

(s). Anal. Calcd. for $C_{21}H_{23}NO_6$: C, 65.44; H, 6.02; N, 3.63. Found: C, 65.32; H, 5.79; N, 3.77.

**Data for ethyl 2-[(2,5-dimethoxyphenyl)methyl]-5-(hydroxymethylidene)-4-oxo-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylate (33g).** This compound was obtained from reaction of **30g**. Yield: 63%; light brown solid; mp: 68 – 69 ºC; IR ($cm^{-1}$): 3422 (OH), 1696 (CO), 1636 (CO); $^1H$ NMR ($CDCl_3$) (ppm): 1.33 (3H, t, J = 7.1 Hz, $CH_3$), 2.53 (2H, t, J = 6.9 Hz, $CH_2$), 3.00 (2H, t, J = 6.9 Hz, $CH_2$), 3.71 (3H, s, $CH_3$), 3.80 (3H, s, $CH_3$), 4.28 (2H, q, J = 7.1 Hz, $CH_2$), 5.52 (2H, s, $CH_2$), 6.56 (1H, s, H-6'), 6.75 – 6.85 (2H, m, H-3' and H-4'), 7.41 (1H, s, H-3), 7.49 (1H, s, CH), 14.12 (1H, s, OH); $^{13}C$ NMR ($CDCl_3$) (ppm): 14.3 (q), 22.7 (t), 25.0 (t), 48.4 (t), 55.7 (q), 55.8 (q), 60.2 (t), 108.9 (s), 111.2 (d), 113.1 (d), 115.6 (d), 119.0 (s), 120.4 (s), 126.1 (s), 128.9 (d), 135.2 (s), 151.2 (s), 153.6 (s), 161.4 (s), 165.6 (d), 186.2 (s). Anal. Calcd. for $C_{21}H_{23}NO_6$: C, 65.44; H, 6.02; N, 3.63. Found: C, 65.75; H, 6.14; N, 3.45.

### 12.1.16 Procedure for the synthesis of ethyl 5-((dimethylamino)methylene)-4-oxo-4,5,6,7-tetrahydro-2H-isoindole-1-carboxylate (34a-c) and 5-((dimethylamino)methylene)-2,5,6,7-tetrahydro-4H-isoindol-4-one (35a-f).

To a solution of ketones **30h-j** or **31a-f** (1 mmol) in anhydrous toluene (2.5 mL), TBDMAM (3 mmol) was added and the reaction mixture was heated under reflux for 12 h. After cooling, the solvent was removed under reduced pressure. The residue was used in the next step without further purification.

### 12.1.17 Procedure for the synthesis of [1,2]oxazolo[5,4-*e*]isoindoles (36a-l,37a-j,38a-f)

To a solution of **32-35** (5 mmol) in ethanol (15 mL) and acetic acid (3 mL) the hydroxylamine hydrochloride was added (7.5 mmol) and the reaction mixture was heated under reflux for 1 h. After cooling, the solvent was removed under reduced pressure. The crude product was poured into water and ice. The solid obtained was filtered, dried and purified using chromatography column (dichloromethane).

**Data for ethyl 7-benzyl-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindole-3-carboxylate (36a).** This compound was obtained from reaction of **32a**. White solid; yield 82%; mp 129 – 130 ºC; IR ($cm^{-1}$): 1726 (CO); $^1H$ NMR ($CDCl_3$) (ppm): 1.43 (3H, t, J = 7.1 Hz, $CH_3$), 2.78 (2H, t, J = 7.1 Hz, $CH_2$), 2.97 (2H, t, J = 7.1 Hz, $CH_2$), 4.44 (2H, q, J = 7.1 Hz, $CH_2$), 5.03 (2H, s, $CH_2$), 6.51 (1H, d, J = 1.9 Hz, H-6), 6.99 (1H, d, J = 1.9 Hz, H-8), 7.15 – 7.20 (2H, m, H-2' and H-6'), 7.30 – 7.39 (3H, m, H-3', H-4' and H-5'); $^{13}C$ NMR ($CDCl_3$) (ppm): 14.2 (q), 20.2 (t),

20.3 (t), 53.7 (t), 61.7 (t), 110.4 (s), 110.8 (s), 116.1 (d), 118.2 (d), 121.2 (s), 127.3 (2 x d), 128.1 (d), 128.9 (2 x d), 137.1 (s), 153.0 (s), 161.1 (s), 166.2 (s). Anal. Calcd for $C_{19}H_{18}N_2O_3$: C, 70.79; H, 5.63; N, 8.69. Found: C, 70.39; H, 5.23; N, 8.29.

**Data for ethyl 7-(3-methoxybenzyl)-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindole-3-carboxylate (36b).** This compound was obtained from reaction of **32b**. White solid; yield 88%; mp 79 – 80 ºC; IR ($cm^{-1}$); 1726 (CO); $^1H$ NMR ($CDCl_3$) (ppm): 1.43 (3H, t, J = 7.1 Hz, $CH_3$), 2.78 (2H, t, J = 7.2 Hz, $CH_2$), 2.96 (2H, t, J = 7.2 Hz, $CH_2$), 3.78 (3H, s, $CH_3$), 4.44 (2H, q, J = 7.1 Hz, $CH_2$), 5.00 (2H, s, $CH_2$), 6.51 (1H, s, H-6), 6.70 – 6.87 (3H, m, H-2', H-4' and H-6'), 7.99 (1H, s, H-8), 7.27 (1H, t, J = 7.9 Hz, H-5'); $^{13}C$ NMR ($CDCl_3$) (ppm): 14.2 (q), 20.2 (t), 20.3 (t), 53.6 (t), 55.3 (q), 61.8 (t), 110.5 (d), 110.8 (s), 113.1 (s), 113.2 (d), 116.2 (d), 118.3 (d), 119.5 (d), 121.2 (s), 130.0 (d), 138.7 (s), 153.0 (s), 160.0 (s), 161.1 (s), 166.2 (s). Anal. Calcd for $C_{20}H_{20}N_2O_4$: C, 68.17; H, 5.72; N, 7.95. Found: C, 68.57; H, 5.32; N, 7.55.

**Data for ethyl 7-(3,4-dimethoxybenzyl)-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindole-3-carboxylate (36c).** This compound was obtained from reaction of **32c**. White solid; yield 40%; mp 103 – 104 ºC; IR ($cm^{-1}$): 1724 (CO); $^1H$ NMR ($CDCl_3$) (ppm): 1.36 (3H, t, J = 7.1 Hz, $CH_3$), 2.71 (2H, t, J = 7.3 Hz, $CH_2$), 2.89 (2H, t, J = 7.3 Hz, $CH_2$), 3.78 (3H, s, $CH_3$), 3.81 (3H, s, $CH_3$), 4.37 (2H, q, J = 7.1 Hz, $CH_2$), 4.90 (2H, s, $CH_2$), 6.44 (1H, d, J = 1.9 Hz, H-6), 6.64 – 6.80 (3H, m, H-2', H-5' and H-6'), 6.92 (1H, d, J = 1.9 Hz, H-8); $^{13}C$ NMR ($CDCl_3$) (ppm): 14.2 (q), 20.2 (t), 20.3 (t), 53.6 (t), 55.9 (q), 56.0 (q), 61.8 (t), 110.4 (s), 110.6 (s), 110.7 (d), 111.2 (d), 116.0 (d), 118.0 (d), 120.0 (d), 121.1 (s), 129.3 (s), 148.9 (s), 149.3 (s), 153.0 (s), 161.1 (s), 166.2 (s). Anal. Calcd for $C_{21}H_{22}N_2O_5$: C, 65.96; H, 5.80; N, 7.33. Found: C, 65.56; H, 5.40; N, 7.73.

**Data for ethyl 7-(3,5-dimethoxybenzyl)-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindole-3-carboxylate (36d).** This compound was obtained from reaction of **32d**. Brown oil; yield 44%; IR ($cm^{-1}$): 1732 (CO); $^1H$ NMR ($CDCl_3$) (ppm): 1.43 (3H, t, J = 7.1 Hz, $CH_3$), 2.77 (2H, t, J = 7.2 Hz, $CH_2$), 2.95 (2H, t, J = 7.2 Hz, $CH_2$), 3.76 (6H, s, 2 x $CH_3$), 4.44 (2H, q, J = 7.1 Hz, $CH_2$), 4.95 (2H, s, $CH_2$), 6.31 (2H, d, *J* = 2.1 Hz, H-2' and H-6'), 6.39 (1H, t, J = 2.1 Hz, H-4'), 6.51 (2H, d, J = 1.9 Hz, H-6), 6.98 (1H, d, J = 1.9 Hz, H-8); $^{13}C$ NMR ($CDCl_3$) (ppm): 14.2 (q), 20.2 (t), 20.3 (t), 53.7 (t), 55.4 (2 x q), 61.7 (t), 99.4 (d), 105.4 (2 x d), 110.4 (s), 110.7 (s), 116.2 (d), 118.3 (d), 121.1 (s), 139.5 (s), 153.0 (s), 161.1 (s), 161.2 (2 x s), 166.2 (s). Anal. Calcd for $C_{21}H_{22}N_2O_5$: C, 65.96; H, 5.80; N, 7.33. Found: C, 65.56; H, 5.40; N, 6.93.

**Data for ethyl 7-(3,4,5-trimethoxybenzyl)-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindole-3-carboxylate (36e).** This compound was obtained from reaction of **32e**. White solid; yield 76%; mp 134 – 135 $^{o}$C; IR (cm$^{-1}$): 1717 (CO); $^{1}$H NMR ($CDCl_3$) (ppm): 1.43 (t, 3H, J = 7.1 Hz, $CH_3$), 2.79 (t, 2H, J = 7.2 Hz, $CH_2$), 2.97 (t, 2H, J = 7.2 Hz, $CH_2$), 3.83 (s, 6H, 2 x $CH_3$), 3.84 (s, 3H, $CH_3$), 4.45 (q, 2H, J = 7.1 Hz, $CH_2$), 4.96 (s, 2H, $CH_2$), 6.40 (s, 2H, H-2' and H-6'), 6.52 (d, 1H, J = 2.0 Hz, H-6), 7.00 (d, 1H, J = 2.0 Hz, H-8); $^{13}$C NMR ($CDCl_3$) (ppm): 14.2 (q), 20.2 (t), 20.3 (t), 54.0 (t), 56.2 (2 x q), 60.9 (q), 61.8 (t), 104.5 (2 x d), 110.5 (s), 110.8 (s), 116.1 (d), 118.2 (d), 121.2 (s), 132.5 (s), 137.7 (s), 153.1 (s), 153.6 (2 x s), 161.1 (s), 166.1 (s). Anal. Calcd for $C_{22}H_{24}N_2O_6$: C, 64.07; H, 5.87; N, 6.79. Found: C, 64.47; H, 5.47; N, 6.39.

**Data for ethyl 7-benzyl-6-phenyl-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindole-3-carboxylate (36f).** This compiund was obtained from reaction of **32f**. White solid; yield 55%; mp 115 – 116 $^{o}$C; IR (cm$^{-1}$): 1726 (CO); $^{1}$H NMR (DMSO-$d_6$) (ppm): 1.32 (t, 3H, J = 7.1 Hz, $CH_3$), 2.69 (t, 2H, J = 7.1 Hz, $CH_2$), 2.85 (t, 2H, J = 7.1 Hz, $CH_2$), 4.35 (q, 2H, J = 7.1 Hz, $CH_2$), 5.17 (s, 2H, $CH_2$), 6.92 – 6.95 (m, 2H, Ar), 7.22 – 7.51 (m, 9H, H-8 and Ar); $^{13}$C NMR (DMSO-$d_6$) (ppm): 13.9 (q), 19.6 (t), 19.7 (t), 50.3 (t), 61.4 (t), 109.1 (s), 110.0 (s), 117.6 (d), 118.5 (s), 126.5 (2 x d), 127.3 (d), 127.7 (d), 128.5 (2 x d), 128.6 (2 x d), 129.5 (2 x d), 130.3 (s), 130.9 (s), 138.1 (s), 152.8 (s), 160.1 (s), 165.5 (s). Anal. Calcd for $C_{25}H_{22}N_2O_3$: C, 75.36; H, 5.57; N, 7.03. Found: C, 75.76; H, 5.17; N, 7.43.

**Data for ethyl 7-(4-methoxybenzyl)-6-phenyl-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindole-3-carboxylate (36g).** This compound was obtained from reaction of **32g**. White solid; yield 76%; mp 81 – 82 $^{o}$C; IR (cm$^{-1}$): 1730 (CO); $^{1}$H NMR ($CDCl_3$) (ppm): 1.43 (t, 3H, J = 7.1 Hz, $CH_3$), 2.77 (t, 2H, J = 7.3 Hz, $CH_2$), 2.95 (t,2H, J = 7.3 Hz, $CH_2$), 3.78 (s, 3H, $CH_3$), 4.44 (q, 2H, J = 7.1 Hz, $CH_2$), 4.98 (s, 2H, $CH_2$), 6.81 (d, 2H, J = 8.7 Hz, H-3' and H-5'), 6.95 (d, 2H, J = 8.7 Hz, H-2' and H-6'), 7.03 (s, 1H, H-8), 7.25 – 7.42 (m, 5H, H-2", H-3", H-4", H-5" and H-6"); $^{13}$C NMR ($CDCl_3$) (ppm): 14.2 (q), 20.1 (t), 20.2 (t), 50.6 (t), 55.3 (q), 61.8 (t), 110.3 (s), 110.8 (s), 114.1 (2 x d), 116.4 (d), 118.9 (s), 127.8 (d), 128.4 (2 x d), 128.6 (2 x d), 129.6 (s), 130.0 (2 x d), 130.9 (s), 131.4 (s), 153.0 (s), 159.1 (s), 161.1 (s), 166.3 (s). Anal. Calcd for $C_{26}H_{24}N_2O_4$: C, 72.88; H, 5.65; N, 6.54. Found: C, 72.48; H, 5.25; N, 6.14.

**Data for ethyl 7-(3-amino-4-methoxybenzyl)-6-phenyl-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindole-3-carboxylate (36h).** This compound was obtained from reaction of **32h**. White solid; yield 43%; mp 227 – 228 $^{o}$C; IR (cm$^{-1}$): 3456 – 3374 ($NH_2$), 1716 (CO); $^{1}$H NMR (DMSO-$d_6$) (ppm): 1.30 (t, 3H, J = 7.1 Hz, $CH_3$), 1.76 (t, 2H, J = 7.3 Hz, $CH_2$), 2.39 (t, 2H, J

= 7.3 Hz, $CH_2$), 3.82 (s, 3H, $CH_3$), 4.28 (q, 2H, J = 7.1 Hz, $CH_2$), 5.09 (s, 2H, $CH_2$), 6.72 (d, 1H, J = 8.5 Hz, Ar), 7.00 (d, 1H, J = 8.5 Hz, Ar), 7.30 – 7.42 (m, 5H, Ar), 7.67 (d, 2H, J = 5.5 Hz, Ar), 9.65 (s, 1H, NH), 10.42 (s, 1H, NH); $^{13}$C NMR (DMSO-*d*$_6$) (ppm): 13.8 (q), 24.2 (t), 29.6 (t), 49.5 (t), 56.0 (q), 62.6 (t), 111.2 (d), 112.4 (s), 119.6 (d), 119.9 (s), 124.1 (d), 125.4 (s), 125.8 (d), 127.1 (d), 127.8 (s), 128.4 (2 x d), 128.5 (s), 129.6 (2 x d), 130.6 (s), 131.5 (s), 147.5 (s), 148.7 (s), 154.6 (s), 160.4 (s). Anal. Calcd for $C_{26}H_{25}N_3O_4$: C, 70.41; H, 5.68; N, 9.47. Found: C, 70.81; H, 5.28; N, 9.87.

**Data for ethyl 6-(4-hydroxyphenyl)-7-(4-methoxybenzyl)-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindole-3-carboxylate (36i).** This compound was obtained from reaction of **32i**. Brown oil; yield 40%; IR (cm$^{-1}$): 3405 (OH), 1728 (CO); $^1$H NMR ($CDCl_3$) (ppm): 1.41 (t, 3H, J = 7.1 Hz, $CH_3$), 2.73 (t, 2H, J = 7.4 Hz, $CH_2$), 2.94 (t, 2H, J = 7.4 Hz, $CH_2$), 3.78 (s, 3H, $CH_3$), 4.44 (q, 2H, J = 7.1 Hz, $CH_2$), 4.94 (s, 2H, $CH_2$), 5.96 (s, 1H, OH), 6.81 (d, 2H, J = 8.8 Hz, H-3' and H-5'), 6.90 (d, 2H, J = 8.7 Hz, H-3" and H-5"), 6.95 (d, 2H, J = 8.8 Hz, H-2' and H-6'), 7.01 (s, 1H, H-8), 7.12 (d, 2H, J = 8.7 Hz, H-2" and H-6"); $^{13}$C NMR ($CDCl_3$) (ppm): 14.2 (q), 20.1 (t), 20.2 (t), 50.5 (t), 55.3 (q), 61.9 (t), 110.0 (s), 110.8 (2 x d), 114.1 (2 x d), 115.6 (s), 116.0 (d), 118.6 (s), 123.5 (s), 128.4 (2 x d), 129.7 (s), 130.7 (s), 131.5 (2 x d), 152.9 (s), 155.8 (s), 159.1 (s), 161.1 (s), 166.5 (s). Anal. Calcd for $C_{26}H_{24}N_2O_5$: C, 70.26; H, 5.44; N, 6.30. Found: C, 70.66; H, 5.04; N, 6.70.

**Data for ethyl 7-(4-methoxybenzyl)-6-(4-methoxyphenyl)-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindole-3-carboxylate (36j).** This compound was obtained from reaction of **32j**. Brown oil; yield 42%; IR (cm$^{-1}$): 1732 (CO); $^1$H NMR ($CDCl_3$) (ppm): 1.43 (t, 3H, J = 7.1 Hz, $CH_3$), 2.74 (t, 2H, J = 7.4 Hz, $CH_2$), 2.95 (t, 2H, J = 7.4 Hz, $CH_2$), 3.78 (s, 3H, $CH_3$), 3.84 (s, 3H, $CH_3$), 4.44 (q, 2H, J = 7.1 Hz, $CH_2$), 4.95 (s, 2H, $CH_2$), 6.82 (d, 2H, J = 8.8 Hz, H-3' and H-5'), 6.92 – 6.98 (m, 4H, H-2', H-6', H-3" and H-5"), 7.01 (s, 1H, H-8), 7.19 (d, 2H, J = 8.8 Hz, H-2" and H-6"); $^{13}$C NMR ($CDCl_3$) (ppm): 14.2 (q), 20.1 (t), 20.2 (t), 50.5 (t), 55.3 (2 x q), 61.7 (t), 110.1 (s), 110.7 (s), 114.0 (2 x d), 114.1 (2 x d), 115.9 (d), 118.6 (s), 123.7 (s), 128.3 (2 x d), 129.7 (s), 130.6 (s), 131.3 (2 x d), 153.0 (s), 159.1 (s), 159.3 (s), 161.1 (s), 166.3 (s). Anal. Calcd for $C_{27}H_{26}N_2O_5$: C, 70.73; H, 5.72; N, 6.11. Found: C, 70.33; H, 5.32; N, 6.51.

**Data for ethyl 7-(4-methoxybenzyl)-6-(3,4,5-trimethoxyphenyl)-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindole-3-carboxylate (36k).** This compund was obtained from reaction of **32k**. White solid; yield 51%; mp 155 – 156 $^o$C; IR (cm$^{-1}$): 1721 (CO); $^1$H NMR ($CDCl_3$) (ppm): 1.44 (t, 3H, J = 7.1 Hz, $CH_3$), 2.79 (t, 2H, J = 7.3 Hz, $CH_2$), 2.98 (t, 2H, J = 7.3 Hz, $CH_2$), 3.73 (s, 6H, 2 x $CH_3$), 3.78 (s, 3H, $CH_3$), 3.89 (s, 3H, $CH_3$), 4.45 (q, 2H, J = 7.1 Hz,

$CH_2$), 5.00 (s, 2H, $CH_2$), 6.42 (s, 2H, H-2" and H-6"), 6.84 (d, 2H, J = 8.7 Hz, H-3'and H-5'), 6.99 (d, 2H, J = 8.7 Hz, H-2' and H-6'), 7.07 (s, 1H, H-8); $^{13}$C NMR ($CDCl_3$) (ppm): 14.2 (q), 20.1 (t), 20.3 (t), 50.7 (t), 55.4 (q), 56.0 (2 x q), 60.9 (q), 61.8 (t), 107.2 (2 x d), 110.2 (s), 110.8 (s), 114.2 (2 x d), 116.4 (d), 118.7 (s), 126.7 (s), 128.0 (2x d), 130.0 (s), 130.8 (s), 137.7 (s), 153.0 (s), 153.1 (2 x s), 159.1 (s), 161.1 (s), 166.2 (s). Anal. Calcd for $C_{29}H_{30}N_2O_7$: C, 67.17; H, 5.83; N, 5.40. Found: C, 67.57; H, 5.43; N, 5.0.

**Data for ethyl 7-(3-amino-4-methoxybenzyl)-6-(3,4,5-trimethoxyphenyl)-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindole-3-carboxylate (36l).** This compound was obtained from reaction of **32l**. White solid; yield 74%; mp 90 – 91 $^{o}$C; IR ($cm^{-1}$): 3461 – 3378 ($NH_2$), 1709 (CO); $^1$H NMR ($CDCl_3$) (ppm): 1.42 (t, 3H, J = 7.1 Hz, $CH_3$), 2.80 (t, 2H, J = 7.3 Hz, $CH_2$), 2.98 (t, 2H, J = 7.3 Hz, $CH_2$), 3.77 (s, 6H, 2 x $CH_3$), 3.89 (s, 3H, $CH_3$), 3.91 (s, 3H, $CH_3$), 4.45 (q, 2H, J = 7.1 Hz, $CH_2$), 5.03 (s, 2H, $CH_2$), 6.51 (s, 2H, H-2" and H-6"), 6.67 – 6.88 (m, 3H, J = 8.7 Hz, H-2', H-5' and H-6'), 7.02 (s, 1H, H-8), 8.24 (s, 1H, NH), 9.47 (s, 1H, NH); $^{13}$C NMR ($CDCl_3$) (ppm): 14.2 (q), 20.1 (t), 20.3 (t), 50.7 (t), 56.0 (q), 56.1 (2 x q), 60.9 (q), 61.7 (t), 107.2 (2 x d), 110.2 (d), 110.4 (s), 110.8 (s), 116.3 (d), 118.4 (d), 118.7 (s), 123.5 (d), 126.4 (s), 126.7 (s), 130.6 (d), 131.0 (s), 137.7 (s), 147.9 (s), 153.1 (2 x s), 153.7 (s), 160.7 (s), 161.0 (s), 166.1 (s). Anal. Calcd for $C_{29}H_{31}N_3O_7$: C, 65.28; H, 5.86; N, 7.88. Found: C, 65.68; H, 5.46; N, 7.48.

**Data for ethyl 7-benzyl-5,7-dihydro-4*H*-[1,2]oxazolo[5,4-*e*]isoindole-6-carboxylate (37a).** This compound was obtained from reaction of **33a**. White solid; yield: 81%; mp: 116 – 117 $^{o}$C; IR ($cm^{-1}$): 1695 (CO); $^1$H NMR ($CDCl_3$) (ppm): 1.32 (3H, t, J = 7.1 Hz, $CH_3$), 2.79 (2H, t, J = 7.8 Hz, $CH_2$), 3.15 (2H, t, J = 7.8 Hz, $CH_2$), 4.26 (2H, q, J = 7.1 Hz, $CH_2$), 5.55 (2H, s, $CH_2$), 7.12 – 7.16 (3H, m, Ar), 7.26 – 7.33 (3H, m, Ar), 8.10 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 14.3 (q), 19.3 (t), 22.1 (t), 52.9 (t), 60.2 (t), 109.2 (s), 111.3 (s), 119.5 (s), 122.3 (d), 126.9 (2 x d), 127.7 (d), 128.7 (2 x d), 130.6 (s), 137.7 (s), 148.7 (d), 161.3 (s), 162.0 (s). Anal. Calcd. for $C_{19}H_{18}N_2O_3$: C, 70.79; H, 5.63; N, 8.69. Found: C, 70.91; H, 5.77; N, 8.56.

**Data for ethyl 7-(4-methoxybenzyl)-5,7-dihydro-4*H*-[1,2]oxazolo[5,4-*e*]isoindole-6-carboxylate (37b).** This compound was obtained from reaction of **33b**. Dark yellow solid; yield: 75%; mp: 109 – 110 $^{o}$C; IR ($cm^{-1}$): 1695 (CO); $^1$H NMR ($CDCl_3$) (ppm): 1.34 (3H, t, J = 7.1 Hz, $CH_3$), 2.77 (2H, t, J = 7.8 Hz, $CH_2$), 3.13 (2H, t, J = 7.8 Hz, $CH_2$), 3.77 (3H, s, $CH_3$), 4.28 (2H, q, J = 7.1 Hz, $CH_2$), 5.46 (2H, s, $CH_2$), 6.84 (2H, d, J = 8.7 Hz, H-3' and H-5'), 7.10 – 7.14 (3H, m, H-2', H-6' and H-8), 8.09 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 14.4 (q), 19.3 (t), 22.1 (t), 52.3 (t), 55.3 (q), 60.2 (t), 109.1 (s), 111.2 (s), 114.1 (2 x d), 119.4

(s), 122.0 (d), 128.7 (2 x d), 129.6 (s), 130.5 (s), 148.7 (d), 159.1 (s), 161.4 (s), 162.0 (s). Anal. Calcd. for $C_{20}H_{20}N_2O_4$: C, 68.17; H, 5.72; N, 7.95. Found: C, 68.05; H, 5.61; N, 8.09.

**Data for ethyl 7-[(3,5-dimethoxyphenyl)methyl]-5,7-dihydro-4*H*-[1,2]oxazolo[5,4-*e*]isoindole-6-carboxylate (37c).** This compound was obtained from reaction of **33c**. Dark yellow solid; yield: 51%; mp: 120 – 121 ºC; IR ($cm^{-1}$): 1695 (CO); $^1H$ NMR ($CDCl_3$) (ppm): 1.33 (3H, t, J = 7.1 Hz, $CH_3$), 2.78 (2H, t, J = 7.8 Hz, $CH_2$), 3.15 (2H, t, J = 7.8 Hz, $CH_2$), 3.75 (6H, s, 2 x $CH_3$), 4.27 (2H, q, J = 7.1 Hz, $CH_2$), 5.48 (2H, s, $CH_2$), 6.27 (2H, d, *J* = 2.2 Hz, H-2' and H-6'), 6.36 (1H, t, *J* = 2.2 Hz, H-4'), 7.12 (1H, s, H-8), 8.10 (1H, s, H-3); $^{13}C$ NMR ($CDCl_3$) (ppm): 14.4 (q), 19.3 (t), 22.1 (t), 52.8 (t), 55.3 (2 x q), 60.2 (t), 99.1 (d), 105.0 (2 x d), 109.3 (s), 111.3 (s), 119.5 (s), 122.3 (d), 130.5 (s), 140.2 (s), 148.7 (d), 161.1 (2 x s), 161.3 (s), 162.0 (s). Anal. Calcd. for $C_{21}H_{22}N_2O_5$: C, 65.96; H, 5.80; N, 7.33. Found: C, 66.07; H, 5.95; N, 7.21.

**Data for ethyl 7-[(3,4,5-trimethoxyphenyl)methyl]-5,7-dihydro-4*H*-[1,2]oxazolo[5,4-*e*]isoindole-6-carboxylate (37d).** This compound was obtained from reaction of **33d**. Light yellow solid; yield: 97%; mp: 140 – 141 ºC; IR ($cm^{-1}$): 1697 (CO); $^1H$ NMR ($CDCl_3$) (ppm): 1.36 (3H, t, J = 7.1 Hz, $CH_3$), 2.79 (2H, t, J = 7.8 Hz, $CH_2$), 3.15 (2H, t, J = 7.8 Hz, $CH_2$), 3.81 (6H, s, 2 x $CH_3$), 3.83 (3H, s, $CH_3$), 4.27 (2H, q, J = 7.1 Hz, $CH_2$), 5.47 (2H, s, $CH_2$), 6.42 (2H, s, H-2' and H-6'), 7.14 (1H, s, H-8), 8.11 (1H, s, H-3); $^{13}C$ NMR ($CDCl_3$) (ppm): 14.4 (q), 19.3 (t), 22.1 (t), 53.0 (t), 56.1 (2 x q), 60.3 (t), 60.9 (q), 104.4 (2 x d), 109.3 (s), 111.3 (s), 119.5 (s), 122.0 (d), 130.5 (s), 133.1 (s), 137.5 (s), 148.8 (d), 153.5 (2 x s), 161.4 (s), 162.0 (s). Anal. Calcd. for $C_{22}H_{24}N_2O_6$: C, 64.07; H, 5.87; N, 6.79. Found: C, 64.15; H, 5.99; N, 6.63.

**Data for ethyl 7-[(3,4-dimethoxyphenyl)methyl]-5,7-dihydro-4*H*-[1,2]oxazolo[5,4-*e*]isoindole-6-carboxylate (37e).** This compound was obtained from reaction of **33e**. Light yellow solid; yield: 77%; mp: 134 – 135 ºC; IR ($cm^{-1}$): 1695 (CO); $^1H$ NMR ($CDCl_3$) (ppm): 1.35 (3H, t, J = 7.1 Hz, $CH_3$), 2.78 (2H, t, J = 7.8 Hz, $CH_2$), 3.14 (2H, t, J = 7.8 Hz, $CH_2$), 3.84 (3H, s, $CH_3$), 3.85 (3H, s, $CH_3$), 4.30 (2H, q, J = 7.1 Hz, $CH_2$), 5.47 (2H, s, $CH_2$), 6.69 – 6.83 (3H, m, H-2', H-5' and H-6'), 7.12 (1H, s, H-8), 8.10 (1H, s, H-3); $^{13}C$ NMR ($CDCl_3$) (ppm): 14.4 (q), 19.3 (t), 22.1 (t), 52.6 (t), 55.8 (q), 55.9 (q), 60.2 (t), 109.2 (s), 110.7 (d), 111.1 (s), 111.2 (d), 119.4 (s), 119.8 (d), 121.9 (d), 130.0 (s), 130.5 (s), 148.6 (s), 148.7 (d), 149.1 (s), 161.4 (s), 162.0 (s). Anal. Calcd. for $C_{21}H_{22}N_2O_5$: C, 65.96; H, 5.80; N, 7.33. Found: C, 65.85; H, 5.89; N, 7.47.

**Data for ethyl 7-[(2,3-dimethoxyphenyl)methyl]-5,7-dihydro-4*H*-[1,2]oxazolo[5,4-*e*]isoindole-6-carboxylate (37f).** This compound was obtained from reaction of **33f**. White

solid; yield: 63%; mp: 213 – 214 $^{o}$C; IR ($cm^{-1}$): 1694 (CO); $^{1}$H NMR ($CDCl_3$) (ppm): 1.35 (3H, t, J = 7.1 Hz, $CH_3$), 2.78 (2H, t, J = 7.7 Hz, $CH_2$), 3.15 (2H, t, J = 7.7 Hz, $CH_2$), 3.86 (3H, s, $CH_3$), 3.87 (3H, s, $CH_3$), 4.29 (2H, q, J = 7.1 Hz, $CH_2$), 5.60 (2H, s, $CH_2$), 6.50 (1H, d, J = 7.5 Hz, Ar), 6.84 – 7.02 (2H, m, Ar), 7.11 (1H, s, H-8), 8.09 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 14.4 (q), 19.4 (t), 22.1 (t), 47.9 (t), 55.8 (q), 60.1 (t), 60.6 (q), 109.0 (s), 111.1 (s), 112.1 (d), 119.6 (s), 120.0 (d), 122.4 (d), 124.2 (d), 130.2 (s), 131.5 (s), 146.5 (s), 148.7 (d), 152.6 (s), 161.4 (s), 162.1 (s). Anal. Calcd. for $C_{21}H_{22}N_2O_5$: C, 65.96; H, 5.80; N, 7.33. Found: C, 66.08; H, 5.92; N, 7.24.

**Data for ethyl 7-[(2,5-dimethoxyphenyl)methyl]-5,7-dihydro-4*H*-[1,2]oxazolo[5,4-*e*]isoindole-6-carboxylate (37g).** This compound was obtained from reaction of **33g**. White solid; yield: 74%; mp: 126 – 127 $^{o}$C; IR ($cm^{-1}$): 1684 (CO); $^{1}$H NMR ($CDCl_3$) (ppm): 1.32 (3H, t, J = 7.1 Hz, $CH_3$), 2.78 (2H, t, J = 7.8 Hz, $CH_2$), 3.15 (2H, t, J = 7.8 Hz, $CH_2$), 3.70 (3H, s, $CH_3$), 3.83 (3H, s, $CH_3$), 4.28 (2H, q, J = 7.1 Hz, $CH_2$), 5.53 (2H, s, $CH_2$), 6.49 (1H, d, J = 2.6 Hz, H-6'), 6.77 – 6.84 (2H, m, H-3' and H-4'), 7.13 (1H, s, H-8), 8.09 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 14.3 (q), 19.4 (t), 22.1 (t), 48.0 (t), 55.7 (q), 55.8 (q), 60.1 (t), 109.0 (s), 111.0 (d), 111.1 (s), 112.5 (d), 115.1 (d), 119.5 (s), 122.5 (d), 127.3 (s), 130.1 (s), 148.7 (d), 151.0 (s), 153.7 (s), 161.4 (s), 162.2 (s). Anal. Calcd. for $C_{21}H_{22}N_2O_5$: C, 65.96; H, 5.80; N, 7.33. Found: C, 65.88; H, 5.71; N, 7.52.

**Data for ethyl 7-[(4-methylbenzyl)methyl]-5,7-dihydro-4*H*-[1,2]oxazolo[5,4-*e*]isoindole-6-carboxylate (37h).** This compound was obtained from reaction of **34a**. Yellow oil; yield: 61%; IR ($cm^{-1}$): 1696 (CO); $^{1}$H NMR (DMSO) (ppm): 1.23 (3H, t, J = 7.1 , $CH_3$), 2.24 (3H, s, $CH_3$), 2.73 (2H, t, J = 7.8 Hz, $CH_2$), 3.04 (2H, t, J = 7.8 Hz, $CH_2$), 4.16 – 4.19 (2H, m, $CH_2$), 5.49 (2H, s, $CH_2$), 7.06 (4H, d, J = 10.1 Hz, H-2', H-3', H-5' and H-6'), 7.72 (1H, s, H-8), 8.45 (1H, s, H-3); $^{13}$C NMR (DMSO) (ppm): 14.1 (q), 18.6 (t), 20.6 (t), 21.5 (q), 51.5 (t), 59.8 (t), 99.5 (s), 109.1 (s), 110.21 (s), 118.6 (s), 123.2 (d), 126.6 (2 x d), 129.0 (2 x d), 129.9 (s), 135.4 (s), 136.5 (s), 149.1 (d), 160.4 (s). Anal. Calcd. for $C_{21}H_{23}N_2O_3$: C, 71.77; H, 6.60; N, 7.97. Found: C, 72.17; H, 6.2; N, 7.57.

**Data for ethyl 7-[(3-nitro-4-methoxylbenzyl)methyl]-5,7-dihydro-4*H*-[1,2]oxazolo[5,4-*e*]isoindole-6-carboxylate (37i).** This compound was obtained from reaction of **34b**. Yield: 57%; Yellow oil; IR ($cm^{-1}$): 1697 (CO), 1536 ($NO_2$); $^{1}$H NMR (DMSO) (ppm): 1.25 (3H, t, J = 7.0 Hz, $CH_3$), 2.74 (2H, t, J = 7.8 Hz, $CH_2$), 3.05 (2H, t, J = 7.7 Hz, $CH_2$), 3.89 (3H, s, $CH_3$), 4.15 – 4.26 (2H, m, $CH_2$), 5.53 (2H, s, $CH_2$), 7.35 (1H, s, H-8), 7.4 (1H, d, J = 2.0 Hz, H-5'), 7.77 – 7.82 (2H, m, H-2' and H-6'), 8.47 (1H, s, H-3); $^{13}$C NMR (DMSO) (ppm): 14.1 (q), 18.6 (t), 21.5 (t), 50.5 (t), 56.7 (q), 60.0 (t), 109.3 (s), 110.5 (s), 114.5 (d), 118.4 (s), 123.2

(d), 123.6 (d), 130.1 (s), 130.7 (s), 133.1 (d), 138.8 (s), 149.2 (d), 151.3 (s), 160.5 (s), 160.9 (s). Anal. Calcd. for $C_{21}H_{22}N_3O_6$: C, 61.16; H, 5.38; N, 10.19. Found: C, 60.76; H, 5.78; N, 10.59.

**Data for ethyl 7-[(3-amino-4-methoxybenzyl)methyl]-5,7-dihydro-4*H*-[1,2]oxazolo[5,4-*e*]isoindole-6-carboxylate (37j).** This compound was obtained from reaction of **34c**. Yield: 5%; Yellow oil; IR ($cm^{-1}$): 3451 – 3365 ($NH_2$), 1696 (CO); $^1$H NMR (DMSO) (ppm): 1.32 (3H, t, J = 6.9 Hz, $CH_3$), 2.78 (2H, t, J = 7.3 Hz, $CH_2$), 3.10 (2H, t, J = 7.4 Hz, $CH_2$), 3.76 (3H, s, , $CH_3$), 4.21 – 4.32 (2H, m, $CH_2$), 4.79 (2H, s, $NH_2$), 5.42 (2H, s, $CH_2$), 6.39 – 6.93 (3H, m, H-2', H-5' and H-6'), 7.69 (1H, s, H-8), 8.51 (1H, s, H-3). $^{13}$C NMR (DMSO) (ppm): 14.6 (q), 19.1 (t), 22.0 (t), 55.7 (t), 60.3 (q), 66.3 (t), 109.5 (s), 110.8 (d), 112.7 (d), 114.3 (s), 115.3 (d), 116.9 (s), 119.2 (s), 123.4 (s), 130.2 (s), 131.1 (d), 138.0 (d), 146.2 (s), 149.6 (s), 161.0 (s). Anal. Calcd. for $C_{21}H_{24}N_3O_4$: C, 65.95; H, 6.33; N, 10.99. Found: C, 65.55; H, 5.93; N, 11.39.

**Data for 7-(2-morpholinoethyl)-6-phenyl-5,7-dihydro-4H-isoxazolo[5,4-e]isoindole (38a).** This compound was obtained from reaction of **35a**. Yellow oil; yield 44%; $^1$H NMR ($CDCl_3$) (ppm): 2.32 (4H, t, J = 4.4 Hz, 2 x $CH_2$), 2.55 (2H, t, J = 6.8 Hz, $CH_2$), 2.81 (4H, t, J = 4.5 Hz, 2 x $CH_2$), 3.63 (4H, t, J = 4.5 Hz, 2 x $CH_2$), 3.99 (2H, t, J = 6.8 Hz, $CH_2$), 7.11 (1H, s, H-8), 7.27 – 7.49 (5H, m, Ar), 8.09 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 20.0 (t), 38.63 (t), 44.4 (t), 53.7 (2 x t), 59.5 (t), 66.8 (2 x t), 100.0 (d), 109.1 (s), 115.8 (s), 119.0 (s), 127.7 (d), 128.6 (2 x d), 130.0 (2 x d), 130.4 (s), 131.7 (s), 148.8 (d), 163.4 (s). Anal. Calcd. for $C_{21}H_{23}N_3O_2$: C, 72.18; H, 6.63; N, 12.03. Found: C, 71.78; H, 7.03; N, 12.43.

**Data for 6-phenyl-7-(2-(pyrrolidin-1-yl)ethyl)-5,7-dihydro-4H-isoxazolo[5,4-e]isoindole (38b).** This compound was obtained from reaction of **35b**. Yellow oil; yield 64%; $^1$H NMR ($CDCl_3$) (ppm): 1.76 – 1.98 (6H, m, 3 x $CH_2$), 2.55 – 3.00 (8H, m, 4 x $CH_2$), 4.11 (2H, d, J = 7.06 Hz, $CH_2$), 7.10 (1H, s, H-8), 7.30 – 7.56 (5H, m, Ar), 8.06 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 20.0 (t), 20.5 (t), 23.2 (2 x t), 45.4 (t), 53.8 (2 x t), 56.2 (t), 109.2 (s), 116.0 (d), 117.7 (s), 118.5 (s), 124.7 (s), 127.8 (d), 128.4 (2 x d), 128.9 (2 x d), 131.4 (s), 148.8 (d), 163.3 (s). Anal. Calcd. for $C_{21}H_{23}N_3O$: C, 75.65; H, 6.95; N, 12.60. Found: C, 75.25; H, 6.55; N, 13.00.

**Data for 6-phenyl-7-(2-(piperidin-1-yl)ethyl)-5,7-dihydro-4H-isoxazolo[5,4-e]isoindole (38c).** This compound was obtained from reaction of **35c**. Yellow oil; yield 40%; $^1$H NMR ($CDCl_3$) (ppm): 1.41 – 1.65 (6H, m, 3 x $CH_2$), 2.31 (4H, s, 2 x $CH_2$), 2.54 (2H, t, J = 7.1 Hz, $CH_2$), 2.71 (4H, s, 2 x $CH_2$), 3.99 (2H, t, J = 7.4 Hz, $CH_2$), 7.11 (1H, s, H-8), 7.31 – 7.46 (5H, m, Ar), 8.09 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 21.8 (t), 24.2 (t), 24.5 (t), 25.9 (2 x t),

45.7 (t), 56.8 (2 x t), 57.6 (t), 100.5 (s), 112.0 (d), 112.2 (s), 118.6 (s), 127.5 (2 x d), 128.7 (d), 129.2 (2 x d), 133.0 (s), 140.8 (s), 150.0 (d), 166.1 (s). Anal. Calcd. for $C_{22}H_{25}N_3O$: C, 76.05; H, 7.25; N, 12.09. Found: C, 76.45; H, 7.65; N, 11.69.

**Data for 7-(2-morpholinoethyl)-6-(3,4,5-trimethoxyphenyl)-5,7-dihydro-4H-isoxazolo[5,4-e]isoindole (38d).** This compound was obtained from reaction of **35d**. Yellow oil; yield 77%; $^1$H NMR ($CDCl_3$) (ppm): 2.37 – 2.40 (4H, m, 2 x $CH_2$), 2.61 (2H, t, J = 6.9 Hz, $CH_2$), 2.74 (4H, s, 2 x $CH_2$), 3.01 – 3.02 (2H, m, $CH_2$), 3.67 (4H, m, 2 x $CH_2$), 3.90 (6H, s, 2 x $CH_3$), 3.92 (3H, s, $CH_3$), 6.54 (2H, s, H-2'' and H-6''), 7.12 (1H, s, Ar), 8.11 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 20.6 (t), 33.7 (t), 37.1 (t), 53.8 (2 x t), 56.3 (2 x t), 59.6 (t), 61.0 (q), 66.7 (2 x q), 104.9 (s), 107.4 (2 x d), 109.1 (s), 110.6 (s), 115.6 (2 x s), 118.8 (s), 127.1 (s), 130.4 (s), 148.8 (d), 153.3 (d), 163.2 (s). Anal. Calcd. for $C_{24}H_{29}N_3O_5$: C, 65.59; H, 6.65; N, 9.56. Found: C, 65.19; H, 6.25; N, 9.96.

**Data for 7-(2-(pyrrolidin-1-yl)ethyl)-6-(3,4,5-trimethoxyphenyl)-5,7-dihydro-4H-isoxazolo[5,4-e]isoindole (38e).** This compound was obtained from reaction of **35e**. Yellow oil; yield 83%; $^1$H NMR ($CDCl_3$) (ppm): 2.92 (2H, d, J = 5.4 Hz, $CH_2$), 3.01 (14H, d, J = 1.9 Hz, 7 x $CH_2$), 3.89 (3H, s, $CH_3$), 3.91 (6H, s, 2 x $CH_3$), 6.54 (2H, s, H-2'' and H-6''), 7.54 (1H, s, H-8), 8.10 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 14.1 (s), 19.1 (s), 20.2 (s), 22.7 (s), 23.4 (t), 27.7 (s), 29.3 (s), 29.7 (2 x t), 31.9 (t), 33.7 (2 x t), 36.2 (s), 37.0 (2 x q), 54.3 (q), 56.3 (t), 61.0 (t), 107.4 (2 x d), 128.8 (d), 130.9 (d), 153.4 (2 x s), 165.1 (s). Anal. Calcd. for $C_{24}H_{29}N_3O_4$: C, 68.06; H, 6.90; N, 9.92. Found: C, 68.46; H, 6.50; N, 9.52.

**Data for 7-(2-(piperidin-1-yl)ethyl)-6-(3,4,5-trimethoxyphenyl)-5,7-dihydro-4H-isoxazolo[5,4-e]isoindole (38f).** This compound was obtained from reaction of **35f**. Yellow oil; yield 51%; $^1$H NMR (DMSO-$d_6$) (ppm): 1.21 – 1.97 (6H, m, 3 x $CH_2$), 2.65 – 2.75 (6H, s, 3 x $CH_2$), 2.42 – 2.60 (4H, m, 2 x $CH_2$), 3.73 (3H, s, $CH_3$), 3.82 (6H, s, 2 x $CH_3$), 4.18 – 4.34 (2H, m, $CH_2$), 6.66 (2H, s, H-2'' and H-6''), 7.42 (1H, s, H-8), 8.44 (1H, s, H-3); $^{13}$C NMR (DMSO-$d_6$) (ppm): 19.8 (t), 20.4 (t), 21.5 (t), 22.4 (2 x t), 41.8 (t), 53.8 (2 x t), 56.6 (2 x q), 61.0 (q), 64.0 (t), 107.0 (2 x d), 109.7 (s), 111.3 (s), 116.1 (d), 120.2 (s), 126.1 (s), 129.8 (s), 138.2 (s), 148.8 (2 x s), 153.7 (d), 162.7 (2 x s). Anal. Calcd. for $C_{25}H_{31}N_3O_4$: C, 68.63; H, 7.14; N, 9.60. Found: C, 68.23; H, 7.54; N, 10.00.

### 12.1.18 Procedure for the synthesis of [1,2]oxazolo[5,4-e]isoindol-3-yl)methanol (39a-l)

To a suspension of $LiALH_4$ (0.46 mmol) in anhydrous THF (4 mL) under $N_2$ atmosphere, a solution of proper compound **36** in anhydrous THF (14 mL) was added at 0 °C. After stirring

for 1 h at 0°C, the reaction mixture was poured into water and ice and the solid obtained was filtered and dried.

**Data for (7-benzyl-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindol-3-yl)methanol (39a).** This compound was obtained from reaction of **36a**. White solid; yield 82%; mp > 410ºC IR ($cm^{-1}$): 3251 (OH); $^{1}H$ NMR ($CDCl_3$) (ppm): 2.67 (4H, s, 2 x $CH_2$), 4.5 (2H, d, J= 5.8 Hz, $CH_2$), 5.10 (2H, s, $CH_2$), 5.35 (1H, t, J= 5.8 Hz, OH), 6.76 (1H, s, Ar), 7.22 – 7.41 (6H, m, Ar); $^{13}C$ NMR ($CDCl_3$) (ppm): 19.0 (t), 19.9 (t), 52.4 (t), 54.4 (t), 107.9 (s), 110.3 (s), 115.5 (d), 118.4 (d), 120.0 (s), 127.4 (2 x d), 127.5 (d), 128.6 (2 x d), 138.4 (s), 160.9 (s), 163.0 (s). Anal. Calcd for $C_{17}H_{16}N_2O_2$: C, 72.84; H, 7.75; N, 9.99. Found: C, 72.44; H, 8.15; N, 9.59.

**Data for [7-(3-methoxybenzyl)-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindol-3-yl]methanol (39b).** This compound was obtained from **36b**. White solid; yield 99%; mp > 410ºC IR ($cm^{-1}$): 3248 (OH); $^{1}H$ NMR ($CDCl_3$) (ppm): 2.67 (4H, s, 2 x $CH_2$), 3.74 (3H, s, $CH_3$), 4.5 (2H, d, J= 5.8 Hz, $CH_2$), 5.05 (2H, s, $CH_2$), 5.36 (1H, t, J= 5.9 Hz, OH), 6.77 – 6.88 (4H, m, Ar), 7.23 – 7.31 (2H, m, Ar); $^{13}C$ NMR ($CDCl_3$) (ppm): 14.1 (t), 19.4 (t), 20.5 (t), 22.7 (t), 53.6 (s), 55.3 (q), 56.6 (s), 107.9 (s), 111.3 (s), 113.1 (d), 115.8 (d), 118.1 (d), 119.5 (d), 121.14 (s), 129.9 (d), 138.9 (d), 160.0 (s), 160.4 (s). Anal. Calcd for $C_{18}H_{18}N_2O_3$: C, 69.66; H, 5.85; N, 9.03. Found: C, 70.06; H, 5.45; N, 9.43.

**Data for [7-(3,4-dimethoxybenzyl)-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindol-3-yl]methanol (39c).** This compound was obtained from **36c**. White solid; yield 99%; mp > 410ºC IR ($cm^{-1}$): 3232 (OH); $^{1}H$ NMR ($CDCl_3$) (ppm): 2.66 (4H, s, 2 x $CH_2$), 3.73 (3H, s, $CH_3$), 3.75 (3H, s, $CH_3$), 4.50 (2H, d, J= 5.8 Hz, $CH_2$), 4.99 (2H, s, $CH_2$), 5.34 (1H, t, J= 5.9 Hz, OH), 6.75 – 7.00 (4H, m, Ar), 7.21 (1H, s, Ar); $^{13}C$ NMR ($CDCl_3$) (ppm): 19.1 (t), 19.9 (t), 52.2 (t), 54.4 (t), 55.5 (2 x q), 99.5 (d), 107.8 (s), 110.1 (s), 111.8 (d), 115.3 (d), 118.1 (d), 119.8 (s), 120.0 (d), 130.6 (s), 148.3 (s), 148.7 (s), 160.9 (s), 163.1 (s). Anal. Calcd for $C_{19}H_{20}N_2O_4$: C, 67.05; H, 5.92; N, 8.23. Found: C, 66.65; H, 5.52; N, 8.63.

**Data for [7-(3,5-dimethoxybenzyl)-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindol-3-yl]methanol (39d).** This compound was obtained from reaction of **36d**. White solid; yield 73%; mp > 410ºC IR ($cm^{-1}$): 3242 (OH); $^{1}H$ NMR ($CDCl_3$) (ppm): 2.67 (4H, s, 2 x $CH_2$), 3.72 (6H, s, 2 x $CH_3$), 4.50 (2H, d, J= 5.8 Hz, $CH_2$), 5.00 (2H, s, $CH_2$), 5.36 (1H, t, J= 5.9 Hz, OH), 6.44 (3H, d, J= 3.0 Hz, Ar), 6.77 (1H, s, Ar), 7.23 (1H, s, Ar); $^{13}C$ NMR ($CDCl_3$) (ppm): 19.1 (t), 19.9 (t), 52.5 (t), 54.4 (t), 55.2 (2 x q), 99.0 (d), 99.5 (s), 105.7 (2 x d), 107.9 (s), 110.2 (s), 115.6 (d), 118.4 (d), 119.9 (s), 140.6 (s), 160.6 (2 x s), 160.9 (s), 163.0 (s). Anal. Calcd for $C_{19}H_{20}N_2O_4$: C, 67.05; H, 5.92; N, 8.23. Found: C, 66.65; H, 6.32; N, 8.63.

**Data for [7-(3,4,5-trimethoxybenzyl)-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindol-3-yl]methanol (39e).** This compound was obtained from reaction of **36e**. White solid; yield 85%; mp > 410ºC IR ($cm^{-1}$): 3238 (OH); $^{1}H$ NMR ($CDCl_3$) (ppm): 2.67 (4H, s, 2 x $CH_2$), 3.63 (3H, s, $CH_3$), 3.76 (6H, s, 2 x $CH_3$), 4.50 (2H, d, J= 5.8 Hz, $CH_2$), 4.98 (2H, s, $CH_2$), 5.35 (1H, t, J= 5.9 Hz, OH), 6.70 (2H, s, Ar), 6.80 (1H, s, Ar), 7.27 (1H, s, Ar); $^{13}C$ NMR ($CDCl_3$) (ppm): 19.1 (t), 19.9 (t), 52.7 (t), 54.4 (t), 55.9 (2 x q), 59.9 (q), 99.5 (s), 105.4 (2 x d), 107.9 (s), 110.1 (s), 115.4 (d), 118.2 (d), 119.8 (s), 133.8 (s), 136.9 (s), 152.9 (2 x s), 160.9 (s), 163.1 (s). Anal. Calcd for $C_{20}H_{22}N_2O_5$: C, 64.85; H, 5.99; N, 7.56. Found: C, 64.45; H, 5.59; N, 7.96.

**Data for (7-benzyl-6-phenyl-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindol-3-yl)methanol (39f).** This compound was obtained from reaction of **36f**. White solid; yield 77%; mp > 410ºC IR ($cm^{-1}$): 3251 (OH); $^{1}H$ NMR ($CDCl_3$) (ppm): 2.69 (4H, s, 2 x $CH_2$), 4.52 (2H, d, J= 5.8 Hz, $CH_2$), 5.17 (2H, s, $CH_2$), 5.38 (1H, t, J= 5.8 Hz, OH), 6.90 – 6-95 (2H, m, Ar), 7.20 – 7.47 (9H, m, Ar); $^{13}C$ NMR ($CDCl_3$) (ppm): 19.0 (t), 20.0 (t), 50.2 (t), 54.4 (t), 99.5 (s), 108.6 (s), 110.0 (s), 116.8 (s), 118.5 (s), 126.5 (2 x d), 127.2 (d), 127.5 (2 x d), 128.5 (2 x d), 129.5 (2 x d), 130.1 (s), 131.1 (s), 138.4 (d), 150.6 (d), 161.0 (s). Anal. Calcd for $C_{23}H_{20}N_2O_2$: C, 77.51; H, 5.99; N, 7.86. Found: C, 77.91; H, 5.59; N, 7.46.

**Data for [7-(4-methoxybenzyl)-6-phenyl-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindol-3-yl]methanol (39g).** This compound was obtained from reaction of **36g**. White solid; yield 71%; mp > 410ºC IR ($cm^{-1}$): 3244 (OH); $^{1}H$ NMR ($CDCl_3$) (ppm): 2.68 (4H, s, 2 x $CH_2$), 3.69 (3H, s, $CH_3$), 4.52 (2H, d, J= 5.8 Hz, $CH_2$), 5.08 (2H, s, $CH_2$), 5.38 (1H, t, J= 5.8 Hz, OH), 6.78 – 6.89 (4H, m, Ar), 7.33 – 7.46 (6H, m, Ar); $^{13}C$ NMR ($CDCl_3$) (ppm): 19.0 (t), 20.0 (t), 49.7 (t), 54.5 (t), 55.0 (q), 108.5 (s), 109.9 (s), 113.8 (2 x d), 116.5 (d), 118.5 (s), 127.5 (s), 128.1 (2 x d), 128.5 (2 x d), 129.6 (2 x d), 130.0 (s), 130.2 (s), 131.2 (s), 158.4 (d), 161.0 (s), 162.9 (s). Anal. Calcd for $C_{24}H_{22}N_2O_3$: C, 74.59; H, 5.74; N, 7.25. Found: C, 74.19; H, 6.14; N, 7.65.

**Data for [7-(3-amino-4-methoxybenzyl)-6-phenyl-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindol-3-yl]methanol (39h).** This compound was obtained from reaction of **36h**. White solid; yield 52%; mp > 410ºC IR ($cm^{-1}$): 3443 – 3365 ($NH_2$), 3239 (OH); $^{1}H$ NMR ($CDCl_3$) (ppm): 2.72 – 2.76 (4H, m, 2 x $CH_2$), 3.86 (3H, s, $CH_3$), 4.23 (2H, s, $CH_2$), 4.75 (2H, s, $NH_2$), 4.98 (2H, s, $CH_2$), 6.68 (1H, t, J= 5.9 Hz, OH), 6.81 (1H, s, Ar), 7.20 – 7.47 (6H, m, Ar), 8.22 (1H, s, CH Ar), 8.77 (1H, s, H-3); $^{13}C$ NMR ($CDCl_3$) (ppm): 19.4 (t), 20.8 (t), 50.0 (t), 54.9 (t), 55.8 (q), 111.1 (d), 111.9 (s), 119.8 (d), 120.3 (s), 124.5 (d), 125.7 (s), 125.8 (d), 127.6 (s), 128.1 (2 x d), 128.5 (d), 129.3 (2 x d), 130.7 (s), 131.1 (s), 131.7 (s), 155.1 (s), 161.3 (s),

163.0 (s). Anal. Calcd for $C_{24}H_{23}N_3O_3$: C, 71.80; H, 5.77; N, 10.47. Found: C, 71.40; H, 5.37; N, 10.07.

**Data for [7-(4-methoxybenzyl)-6-(4-hydroxyphenyl)-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindol-3-yl]methanol (39i).** This compound was obtained from **36i**. White solid; yield 43%; mp > 410ºC IR ($cm^{-1}$): 3241 (OH); $^1$H NMR ($CDCl_3$) (ppm): 2.89 (4H, s, 2 x $CH_2$), 2.97 (2H, s, $CH_2$), 3.78 (3H, s, $CH_3$), 4.76 (2H, s, $CH_2$), 4.93 (1H, s, OH), 6.78 – 7.13 (9H, m, Ar and H-3), 8.02 (1H, s, OH); $^{13}$C NMR ($CDCl_3$) (ppm): 20.4 (t), 31.5 (t), 36.6 (t), 55.3 (t), 56.6 (q), 112.2 (s), 114.1 (2 x d), 115.5 (2 x d), 118.6 (s), 125.6 (s), 128.3 (2 x d), 129.6 (s), 131.43 (2 x d), 140.8 (s), 150.0 (s), 156.0 (d), 157.6 (s), 158.5 (s), 162.7 (s), 166.1 (s). Anal. Calcd for $C_{24}H_{22}N_2O_4$: C, 71.63; H, 5.51; N, 6.96. Found: C, 71.23; H, 5.11; N, 6.56.

**Data for [7-(4-methoxybenzyl)-6-(4-methoxyphenyl)-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindol-3-yl]methanol (39j).** This compound was obtained from **36j**. White solid; yield 80%; mp > 410ºC IR ($cm^{-1}$): 3236 (OH); $^1$H NMR ($CDCl_3$) (ppm): 2.66 (4H, t, J= 3.8 Hz, 2 x $CH_2$), 3.70 (3H, s, $CH_3$), 3.79 (3H, s, $CH_3$), 4.50 (2H, d, J= 5.8 Hz, $CH_2$), 5.03 (2H, s, $CH_2$), 5.38 (1H, t, J= 5.9 Hz, OH), 6.80 – 6.91 (4H, m, Ar), 7.00 (2H, d, J= 8.7 Hz, Ar), 7.27 (3H, m, Ar); $^{13}$C NMR ($CDCl_3$) (ppm): 19.0 (t), 20.0 (t), 39.7 (t), 54.5 (t), 54.9 (q), 55.1 (q), 99.5 (s), 108.4 (s), 109.7 (s), 113.8 (2 x d), 114.0 (2 x d), 118.0 (d), 123.3 (s), 128.0 (2 x d), 129.8 (s), 130.3 (s), 130.9 (2 x d), 158.4 (s), 158.6 (s), 161.0 (s), 163.0 (s). Anal. Calcd for $C_{25}H_{24}N_2O_3$: C, 72.10; H, 5.81; N, 6.73. Found: C, 72.5; H, 5.41; N, 7.13.

**Data for [7-(4-methoxybenzyl)-6-(3,4,5-trimethoxyphenyl)-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindol-3-yl]methanol (39k).** This compound was obtained from reaction of **36k**. White solid; yield 99%; mp > 410ºC IR ($cm^{-1}$): 3252 (OH); $^1$H NMR ($CDCl_3$) (ppm): 2.70 (4H, s, 2 x $CH_2$), 3.68 (6H, s, 2 x $CH_3$), 3.69 (3H, s, $CH_3$), 3.70 (3H, s, $CH_3$), 4.52 (2H, d, J= 5.8 Hz, $CH_2$), 5.09 (2H, s, $CH_2$), 5.39 (1H, t, J= 5.9 Hz, OH), 6.53 (2H, s, Ar), 6.83 – 6.97 (4H, m, Ar), 7.33 (1H, s, Ar); $^{13}$C NMR ($CDCl_3$) (ppm): 19.0 (t), 20.1 (t), 49.9 (t), 54.5 (t), 55.1 (q), 55.8 (2 x q), 60.0 (q), 99.5 (s), 107.1 (2 x d), 108.4 (s), 109.7 (s), 113.9 (2 x d), 116.4 (d), 118.3 (s), 126.6 (s), 128.0 (2 x d), 130.0 (s), 130.5 (s), 136.9 (s), 152.7 (2 x s), 158.4 (s), 161.0 (s), 162.9 (s). Anal. Calcd for $C_{27}H_{28}N_2O_6$: C, 68.05; H, 5.92; N, 5.88. Found: C, 68.45; H, 5.52; N, 5.48.

**Data for [7-(3-amino-4-methoxybenzyl)-6-(3,4,5-trimethoxyphenyl)-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindol-3-yl]methanol (39l).** This compound was obtained from reaction of **36l**. White solid; yield 60%; mp > 410ºC IR ($cm^{-1}$): 3454 – 3369 ($NH_2$), 3246 (OH); $^1$H NMR (DMSO-$d_6$) (ppm): 2.79 – 2.75 (4H, m, 2 x $CH_2$), 3.77 (s, 6H, 2 x $CH_3$), 3.78 (s, 3H, $CH_3$), 3.81 (s, 3H, $CH_3$), 4.23 (2H, s, $CH_2$), 4.77 (2H, s, $NH_2$), 4.98 (2H, s, $CH_2$), 6.70 (1H, t,

J= 5.9 Hz, OH), 6.56 (s, 2H, H-2" and H-6"), 6.98 – 7.41 (m, 3H, J = 8.7 Hz, H-2', H-5' and H-6'), 7.66 (s, 1H, H-8); $^{13}$C NMR (DMSO-$d_6$) (ppm): 22.5 (t), 22.8 (t), 54.9 (t), 56.8 (2 x q), 57.0 (q), 60.7 (q), 101.1 (s), 106.2 (2 x d), 111.1 (s), 111.9 (d), 117.4 (d), 120.0 (d), 121.4 (s), 127.2 (s), 128.3 (s), 131.5 (s), 135.4 (s), 135.8 (d), 137.7 (s), 141.0 (s), 146.4 (s), 153.0 (2 x s), 169.5 (s). Anal. Calcd for $C_{27}H_{29}N_3O_6$: C, 65.98; H, 5.95; N, 8.55. Found: C, 65.58; H, 6.35; N, 8.95.

### 12.1.19 Procedure for the synthesis of 5,7-dihydro-4H-isoxazolo[5,4-e]isoindol-3-yl)methyl methanesulfonate (40a-e)

To a solution of compound **39b,e,f,g,k** (9,6 mmol) and $Et_3N$ (5.6 mmol) in anhydrous DCM (42 mL) stirred at 0°C was added dropwise methylsulfonyl chloride (9.6 mmol) and stirred for 2h at room temperature. The resulting mixture was treated with an aquesous saturated solution of $NaHCO_3$ and extracted with DCM (3 x 20 mL). The organic phase was dried over $Na_2SO_4$ and evaporated at reduced pressure. The crude product was used in the following step without further purification.

### 12.1.20 Procedure for the synthesis of 3-(substituted-1-ylmethyl)-5,7-dihydro-4H-isoxazolo[5,4-e]isoindole (41a-j)

Compound **40** (0.9 mmol) was added to a stirred solution of morpholine or piperidine (1.08 mmol) and DIPEA (1.35 mmol) in anhydrous $CH_3CN$ (10 mL). The reaction mixture was stirred at room temperature up to completeness (TLC). The reaction mixture was concentrated under reduced pressure and the residue was purified using column chromatography (dichloromethane : ethyl acetate 8:2).

**Data for 7-(3-methoxybenzyl)-3-(morpholinomethyl)-5,7-dihydro-4H-isoxazolo[5,4-e]isoindole (41a).** This compound was obtained from reaction of **40a** after 3 h. Yellow oil; yield 65%; $^1$H NMR ($CDCl_3$) (ppm): 2.53 (4H, d, J= 3.9 Hz, 2 x $CH_2$), 2.76 (4H, s, 2 x $CH_2$), 3.62 (2H, s, $CH_2$), 3.70 – 3.73 (4H, m, 2 x $CH_2$), 3.80 (3H, s, $CH_3$), 5.01 (2H, s, $CH_2$), 6.51 (1H, s, Ar), 6.71 (1H, s, Ar), 6.78 (1H, d, J= 7.5 Hz, Ar), 6.85 (1H, d, J= 6.7 Hz, Ar), 6.96 (1H, s, Ar), 7.28 (1H, t, J= 7.8 Hz, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 19.9 (t), 20.5 (t), 36.5 (t), 53.0 (t), 53.6 (2 x t), 55.3 (2 x t), 67.0 (q), 109.1 (s), 111.5 (s), 113.0 (d), 113.2 (d), 115.2 (d), 118.0 (d), 119.5 (d), 121.1 (s), 129.9 (d), 138.9 (s), 157.9 (s), 160.0 (s), 178.9 (s). Anal. Calcd. for $C_{22}H_{25}N_3O_3$: C, 69.64; H, 6.64; N, 11.07. Found: C, 70.04; H, 6.24; N, 10.67.

**Data for 7-(3-methoxybenzyl)-3-(pyrrolidin-1-ylmethyl)-5,7-dihydro-4H-isoxazolo[5,4-e]isoindole (41b).** This compound was obtained from reaction of **40a** after 2 h. Yellow oil;

yield 70%; $^{1}$H NMR ($CDCl_3$) (ppm): 1.81 (4H, s, 2 x $CH_2$), 2.65 (4H, s, 2 x $CH_2$), 2.70 (4H, s, 2 x $CH_2$), 3.76 (2H, s, $CH_2$), 3.80 (3H, s, $CH_3$), 5.01 (2H, s, $CH_2$), 6.50 (1H, d, J= 5.40 Hz, Ar), 6.71 (1H, s, Ar), 6.77 (1H, d, J= 7.4 Hz, Ar), 6.85 (1H, d, J= 8.1 Hz, Ar), 6.95 (1H, s, Ar), 7.27 (1H, t, J= 7.9 Hz, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 19.8 (t), 20.6 (t), 23.6 (2 x t), 49.5 (t), 53.5 (t), 53.9 (2 x t), 55.3 (q), 109.1 (s), 111.6 (s), 113.1 (d), 115.7 (d), 118.0 (s), 119.5 (d), 121.2 (d), 129.9 (d), 138.7 (d), 158.5 (s), 160.0 (s), 163.7 (s), 207.5 (s). Anal. Calcd. for $C_{22}H_{25}N_3O_2$: C, 72.70; H, 6.93; N, 11.56. Found: C, 73.1; H, 6.53; N, 11.16.

**Data for 3-(morpholinomethyl)-7-(3,4,5-trimethoxybenzyl)-5,7-dihydro-4H-isoxazolo[5,4-e]isoindole (41c).** This compound was obtained from reaction of **40b** after 2 h. Yellow oil; yield 42%; $^{1}$H NMR ($CDCl_3$) (ppm): 2.51 – 2.54 (4H, m, 2 x $CH_2$), 2.77 (4H, t, J= 3.9 Hz, 2 x $CH_2$), 3.62 (2H, s, $CH_2$), 3.70 – 3.73 (4H, m, 2 x $CH_2$), 3.84 (6H, s, 2 x $CH_3$), 3.85 (3H, s, $CH_3$), 4.96 (2H, s, $CH_2$), 6.41 (2H, s, Ar), 6.52 (1H, s, H-1), 6.96 (1H, d, J= 1.8 Hz, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 19.9 (t), 20.6 (t), 53.0 (t), 53.5 (2 x t), 53.9 (t), 56.2 (2 x t), 60.9 (q), 67.0 (2 x q), 104.5 (2 x d), 109.1 (s), 111.5 (s), 115.6 (s), 117.9 (d), 121.2 (s), 132.7 (2 x s), 137.8 (s), 153.6 (d), 157.4 (s), 168.9 (s). Anal. Calcd. for $C_{24}H_{29}N_3O_5$: C, 65.59; H, 6.65; N, 9.56. Found: C, 65.19; H, 7.05; N, 9.96.

**Data for 3-(pyrrolidin-1-ylmethyl)-7-(3,4,5-trimethoxybenzyl)-5,7-dihydro-4H-isoxazolo[5,4-e]isoindole (41d).** This compound was obtained from reaction of **40b** after 2 h. Yellow oil; Yield 40%; $^{1}$H NMR ($CDCl_3$) (ppm): 1.27 (2H, s, $CH_2$), 1.81 (4H, s, 2 x $CH_2$), 2.66 (4H, s, 2 x $CH_2$), 2.76 (4H, t, J= 7.9 Hz, 2 x $CH_2$), 3.78 (3H, s, $CH_3$), 3.84 (6H, s, 2 x $CH_3$), 4.96 (2H, s, $CH_2$), 6.41 (2H, s, Ar), 6.51 (1H, s, Ar), 6.96 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 23.6 (2 x t), 25.9 (t), 30.1 (t), 40.7 (t), 44.9 (t), 53.7 (2 x t), 53.9 (q), 64.2 (2 x q), 104.6 (2 x d), 106.2 (s), 112.6 (s), 116.4 (s), 135.6 (s), 139.7 (s), 153.6 (d), 158.8 (s), 160.8 (s), 165.6 (d), 189.6 (s), 200.2 (s). Anal. Calcd. for $C_{24}H_{29}N_3O_4$: C, 68.06; H, 6.90; N, 9.92. Found: C, 68.46; H, 7.3; N, 9.52.

**Data for 7-benzyl-3-(morpholinomethyl)-6-phenyl-5,7-dihydro-4H-isoxazolo[5,4-e]isoindole (41e).** This compound was obtained from reaction of **40c** after 3 h. Yellow oil; yield 47%; $^{1}$H NMR ($CDCl_3$) (ppm): 2.53 (4H, d, J= 3.8 Hz, 2 x $CH_2$), 2.76 (4H, s, 2 x $CH_2$), 3.62 (2H, s, $CH_2$), 3.71 (4H, m, 2 x $CH_2$), 5.06 (2H, s, $CH_2$), 7.02 (3H, s, Ar), 7.27 (5H, m, Ar), 7.38 – 7.42 (3H, m, Ar and H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 19.9 (t), 20.5 (t), 51.0 (t), 53.0 (t), 53.5 (2 x t), 67.0 (2 x t), 109.5 (s), 111.1 (s), 116.1 (s), 116.2 (s), 119.0 (s), 126.8 (2 x d), 127.6 (d), 127.7 (d), 128.5 (2 x d), 128.7 (2 x d), 130.0 (2 x d), 130.9 (d), 131.6 (s), 137.9 (d), 158.0 (s). Anal. Calcd. for $C_{27}H_{27}N_3O_2$: C, 76.21; H, 6.40; N, 9.87. Found: C, 75.61; H, 6.8; N, 9.47.

**Data for 7-benzyl-6-phenyl-3-(pyrrolidin-1-ylmethyl)-5,7-dihydro-4H-isoxazolo[5,4-e]isoindole (41f).** This compound was obtained from reaction of **40c** after 3 h. Yellow oil; yield 90%; $^{1}$H NMR ($CDCl_3$) (ppm): 1.79 – 1.82 (4H, m, 2 x $CH_2$), 2.64 – 2.67 (4H, m, 2 x $CH_2$), 2.72 – 2.79 (4H, m, 2 x $CH_2$), 3.78 (2H, s, $CH_2$), 5.03 (2H, s, $CH_2$), 6.50 (1H, d, J= 1.70 Hz, Ar), 6.94 (1H, d, J= 1.3 Hz, Ar), 7.17 (3H, d, J= 7.0 Hz, Ar), 7.30 – 7.36 (5H, m, Ar), 8.12 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 19.2 (t), 20.6 (t), 23.6 (2 x t), 49.2 (t), 53.6 (t), 53.7 (2 x t), 109.1 (d), 111.6 (s), 115.7 (2 x d), 118.0 (d), 121.1 (d), 127.2 (2 x d), 127.9 (2 x d), 128.8 (2 x d), 129.5 (s), 134.3 (s), 137.4 (s), 158.3 (s), 163.7 (s), 166.0 (s), 176.0 (s). Anal. Calcd. for $C_{27}H_{27}N_3O$: C, 79.19; H, 6.65; N, 10.26. Found: C, 79.59; H, 6.25; N, 10.66.

**Data for 7-(4-methoxybenzyl)-3-(morpholinomethyl)-6-phenyl-5,7-dihydro-4H-isoxazolo[5,4-e]isoindole (41g).** This compound was obtained from reaction of **40d** after 1.30 h. Yellow oil; yield 78%; $^{1}$H NMR ($CDCl_3$) (ppm): 2.52 (4H, s, 2 x $CH_2$), 2.76 (4H, s, 2 x $CH_2$), 3.63 (2H, s, $CH_2$), 3.72 (4H, d, J= 3.8 Hz, 2 x $CH_2$), 3.79 (3H, s, $CH_3$), 4.99 (2H, s, $CH_2$), 6.82 (2H, d, J= 8.6 Hz, Ar), 6.94 -7.00 (3H, m, Ar), 7.29 (2H, d, J= 6.9 Hz, Ar), 7.36 – 7.45 (3H, m, Ar and H-3). $^{13}$C NMR ($CDCl_3$) (ppm): 19.9 (t), 20.5 (t), 30.1 (t), 50.5 (t), 53.0 (s), 53.4 (2 x t), 55.3 (q), 66.9 (2 x t), 109.5 (s), 114.1 (2 x d), 116.0 (s), 118.9 (d), 127.7 (d), 128.2 (d), 128.5 (d), 130.0 (d), 131.3 (s), 131.7 (s), 133.8 (s), 159.1 (s), 167.0 (s), 182.1 (s). Anal. Calcd. for $C_{28}H_{29}N_3O_3$: C, 73.82; H, 6.42; N, 9.22. Found: C, 74.22; H, 6.82; N, 8.82.

**Data for 7-(4-methoxybenzyl)-6-phenyl-3-(pyrrolidin-1-ylmethyl)-5,7-dihydro-4H-isoxazolo[5,4-e]isoindole (41h).** This compound was obtained from reaction of **40d** after 1 h. Yellow oil; yield 63%; $^{1}$H NMR ($CDCl_3$) (ppm): 1.74 – 1.81 (6H, m, 3 x $CH_2$), 2.59 - 2.76 (8H, m, 4 x $CH_2$), 3.77 (3H, s, $CH_3$), 4.97 (2H, s, $CH_2$), 6.78 – 7.01 (4H, m, Ar), 7.23 – 7.44 (6H, m, Ar and H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 19.8 (t), 20.6 (t), 23.6 (2 x t), 49.6 (t), 50.5 (t), 54.0 (2 x t), 55.3 (q), 109.4 (s), 111.1 (s), 114.1 (2 x d), 115.9 (d), 119.0 (s), 127.6 (2 x d), 128.2 (d), 128.5 (2 x d), 129.9 (s), 130.0 (2 x d), 130.6 (s), 131.7 (s), 158.8 (s), 159.0 (s), 163.7 (s). Anal. Calcd. for $C_{29}H_{32}N_3O_2$: C, 76.62; H, 7.10; N, 9.24. Found: C, 76.22; H, 6.7; N, 9.64.

**Data for 7-(4-methoxybenzyl)-3-(morpholinomethyl)-6-(3,4,5-trimethoxyphenyl)-5,7-dihydro-4H-isoxazolo[5,4-e]isoindole (41i).** This compound was obtained from reaction of **40e** after 2 h. Yellow oil; yield 44%; $^{1}$H NMR ($CDCl_3$) (ppm): 1.27 – 1.50 (4H, m, 2 x $CH_2$), 2.52 – 2.55 (4H, m, 2 x $CH_2$), 2.78 (2H, s, $CH_2$), 3.64 (2H, s, $CH_2$), 3.70 (3H, s, $CH_3$), 3.73 (6H, s, 2 x $CH_3$), 3.79 (3H, s, $CH_3$), 3.90 (2H, s, $CH_2$), 5.01 (2H, s, $CH_2$), 6.43 (2H, s, H-2'' and H-6''), 6.84 (2H, d, J= 6.84 Hz, Ar), 6.97 – 7.03 (2H, m, Ar), 8.11 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 19.9 (t), 20.6 (t), 29.0 (t), 30.6 (t), 53.4 (2 x t), 55.3 (2 x t), 56.0 (2 x q), 60.9

(q), 66.9 (q), 107.2 (2 x d), 109.4 (s), 110.9 (s), 114.1 (2 x d), 116.0 (s), 118.7 (s), 127.0 (s), 127.9 (2 x d), 129.5 (s), 130.2 (2 x s), 130.6 (s), 137.6 (s), 153.1 (d), 159.1 (s), 163.9 (s). Anal. Calcd. for $C_{31}H_{35}N_3O_6$: C, 68.24; H, 6.47; N, 7.70. Found: C, 68.64; H, 6.07; N, 7.30.

**Data for 7-(4-methoxybenzyl)-3-(pyrrolidin-1-ylmethyl)-6-(3,4,5-trimethoxyphenyl)-5,7-dihydro-4H-isoxazolo[5,4-e]isoindole (41j).** This compound was obtained from reaction of **40e** after 2 h. Yellow oil; yield 41%; $^1$H NMR ($CDCl_3$) (ppm): 1.27 – 1.48 (4H, m, , 2 x $CH_2$), 1.82 (4H, s, 2 x $CH_2$), 2.72 (6H, d, J= 30.7 Hz, 3 x $CH_2$), 3.75 (3H, s, $CH_3$), 3.77 (3H, s, $CH_3$), 3.90 (3H, s, $CH_3$), 5.01 (2H, s, $CH_2$), 6.42 (2H, s, H-2'' and H-6''), 6.85 (2H, d, J= 8.4 Hz, Ar), 6.98 – 7.03 (2H, m, Ar), 8.12 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 19.0 (t), 19.9 (t), 22.8 (2 x t), 48.7 (t), 49.8 (t), 53.0 (2 x t), 54.6 (q), 55.2 (2 x q), 60.1 (q), 106.4 (2 x d), 108.6 (s), 110.3 (s), 113.4 (2 x d), 118.0 (s), 126.2 (s), 127.1 (2 x d), 128.7 (d), 129.5 (s), 129.8 (s), 136.8 (s), 152.3 (s), 157.8 (s), 158.3 (s), 162.9 (s). Anal. Calcd. for $C_{31}H_{35}N_3O_5$: C, 70.30; H, 6.66; N, 7.93. Found: C, 70.70; H, 6.26; N, 7.53.

### 12.1.21 Procedure for the synthesis of ethyl 3-(4-methoxyphenyl)-8-oxo-2,4,5,6,7,8-hexahydrocyclohepta [c]pyrrole-1-carboxylate (48)

**Preparation of ethyl 2-azidoacetate (43).** To a solution of ethyl bromoacetate **42** (1.510 g, 9.04 mmol) in acetone was added $NaN_3$ (0.88 g, 13.56 mmol) and the mixture was heated at reflux for 4 hours. Then the reaction mixture was diluted with ethyl acetate and washed with water. The organic layer was dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. The azide was used for the next step without further purification. Pale yellow oil; yield: 83%. The spectroscopic data are in agreement with the literature [66].

**Preparation of ethyl 5-(4-methoxyphenyl)-1H-pyrrole-2-carboxylate (45).** To a solution of **43** (7g, 54 mmol) in anhydrous ethanol (10 mL), a solution of **44** (1.62 g, 10 mmol) in anhydrous ethanol (30 mL) was added at -20°C, followed by the dropwise addition of a solution of potassium ethoxide (52 mmol) in the same solvent (50 mL). The reaction was stirred for 4 hour and 30 minutes at -20 °C. Once reached room temperature, the solvent was evaporated under reduced pressure and the residue was added of water and extracted with ethyl acetate. The organic layer was dried over anhydrous $Na_2SO_4$, evaporated under reduced pressure. The residue was dissolved in toluene and the reaction mixture was heated under reflux for 24 hours. The solvent was evaporated under reduced pressure and the crude product was purified using chromatography column (dichloromethane). Yellow solid; yield 74%; IR ($cm^{-1}$): 3421 (NH), 1679 (CO); $^1$H NMR (DMSO-$d_6$) (ppm): 1.30 (3H, t, J= 7.4 Hz, $CH_3$), 3.78 (3H, s, $CH_3$), 4.20 – 4.31 (2H, m, $CH_2$), 6.5 (1H, d, J= 3.4 Hz, Ar), 6.8 (1H, d, J= 3.6 Hz,

Ar), 6.9 (2H, d, J= 6.9 Hz, H-3' and H-5'), 7.8 (2H, d, J= 8.8 Hz, H-2' and H-6'), 11.97 (1H, s, NH); $^{13}$C NMR (DMSO-$d_6$) (ppm): 14.4 (q), 55.0 (q), 59.4 (t), 106.7 (d), 114.0 (2 x d), 116.6 (d), 122.4 (s), 124.0 (s), 126.54 (2 x d), 137.1 (s), 158.5 (s), 160.3 (s). Anal Calcd. for $C_{14}H_{15}NO_3$ C, 68.56; H, 6.16; N, 5.71. Found: C, 68.16; H, 5.76; N, 6.11

**Preparation of 5-(2-(ethoxycarbonyl)-5-(4-methoxyphenyl)-1H-pyrrol-3-yl)-5 oxopentanoic acid (46).** A suspension of $AlCl_3$ (6.56 g, 49mmol) and glutaric anhydride (1.86 g, 16 mmol) in anhydrous dichloromethane (30 mL) was stirred at room temperature. After 1 hour, a solution of **45** (2 g, 8,2 mmol) in anhydrous dichloromethane was added dropwise at 0°C and the reaction mixture was stirred for 24 h. The reaction mixture was poured into water and ice and formed a rubbery solid, which was extracted with ethyl acetate. The organic layer was dried over anhydrous $Na_2SO_4$ and the solvent was evaporated under reduced pressure. The crude product was used in the next step without further purifications. Brown oil; Yield 57%; IR (cm$^{-1}$): 3449 (NH), 3344 (OH), 1702 (CO), 1682 (CO), 1644 (CO); $^1$H NMR (DMSO-$d_6$) (ppm): 1.30 (3H, t, J= 7.0 Hz, $CH_3$), 1.63 – 1.80 (4H, m, 2 x $CH_2$), 2.18 – 2.28 (2H, m, $CH_2$), 2.50 – 2.52 (2H, m, $CH_2$), 3.81 (3H, s, $CH_3$), 4.22 - 4.32 (2H, m, $CH_2$), 6.9 (2H, d, J=8.8 Hz, H-3' and H-5'), 7.33 (1H, s, Ar), 7.5 (2H, d, J=8.8 Hz, H-2' and H-6'), 12.12 (1H, s, OH), 12.46 (1H, s, NH); $^{13}$C NMR (DMSO-$d_6$) (ppm): 14.3 (q), 19.9 (t), 32.7 (t), 32.8 (t), 55.2 (q), 59.9 (t), 113.0 (2 x d), 117.6 (d), 121.2 (s), 121.7 (s), 123.2 (s), 131.2 (2 x d), 139.9 (s), 159.5 (s), 160.0 (s), 174.1 (s), 195.1 (s). Anal Calcd. for $C_{19}H_{21}NO_6$ C, 63.50; H, 5.89; N, 3.90. Found: C, 63.91; H, 6.29; N,4.32

**Preparation of 5-(2-(ethoxycarbonyl)-5-(4-methoxyphenyl)-1H-pyrrol-3-yl)pentanoic acid (47).** To a solution of **36** (4.15 g, 12 mmol) in trifluoroacetic anhydride (28 mL), triethylsilane (6.6 mL) was added at 0°C. The reaction mixture was stirred at room temperature for 24 hours. The solvent was evaporated under reduced pressure and the residue was added of water and ice. The formed solid was filtered, dried and purified using chromatography column (dichloromethane : ethyl acetate 84 : 16). Brown oil; yield 61%; IR (cm$^{-1}$): 3434 (NH), 3355 (OH), 1700 (CO), 1675 (CO); $^1$H NMR (DMSO-$d_6$) (ppm): 1.28 (3H, t, J= 7.0 Hz, $CH_3$), 1.52 – 1.53 (4H, m, 2 x $CH_2$), 2.19 – 2.23 (2H, m, $CH_2$), 2.50 – 2.51 (2H, m, $CH_2$), 3.79 (3H, s, $CH_3$), 4.17 – 4.27 (2H, m, $CH_2$), 6.71 (1H, s, Ar), 6.99 (2H, d, J= 8.7 Hz, H-2' and H-6'), 7.43 (2H, J=8.7 Hz, H-3' and H-5'), 11.68 (1H, s, OH), 12.00 (1H, s, NH); $^{13}$ C NMR (DMSO-$d_6$) (ppm): 14.4 (q), 24.3 (t), 25.5 (t), 29.7 (t), 33.4 (t), 55.1 (q), 59.3 (t), 113.8 (2 x d), 116.2 (d), 120.7 (s), 121.4 (s), 124.4 (s), 129.2 (2 x d), 133.9 (s), 158.4 (s), 160.3 (s), 174.5 (s). Anal Calcd. for $C_{19}H_{23}NO_5$ C, 66.07; H, 6.71; N, 4.06. Found: C, 66.47; H, 6.31; N, 4.46

**Preparation of ethyl 3-(4-methoxyphenyl)-8-oxo-2,4,5,6,7,8-hexahydrocyclohepta [c]pyrrole-1-carboxylate (48).** To a solution of **47** (4 g, 12 mmol) in anhydrous dichloromethane, the trifluoacetic anhydride was added (10 mL) at 0°C. The reaction mixture was stirred at room temperature for 1h. The solvent was evaporated under reduced pressure and the residue was purified using column chromatography (petroleum ether : ethyl acetate 9 :1). Brown solid; yield 53%; mp: 108 – 109°C; IR ($cm^{-1}$): 3438 (NH), 1681 (CO), 1667 (CO); $^{1}H$ NMR (DMSO-$d_6$) (ppm): 1.24 (3H, t, J= 7.0, $CH_3$), 2.50 (4H, t, J= 1.7, 2 x $CH_2$), 2.60 – 2.70 (4H, m, 2 x $CH_2$), 3.80 (3H, s, $CH_3$), 4.14 – 4.25 (2H, m, $CH_2$), 7.01 (2H, d, J=8.7 Hz, H-3' and H-5'), 7.42 (2H, d, J=8.7 Hz, H-2' and H-6'), 12.14 (1H, s, NH); $^{13}C$ NMR (DMSO-$d_6$) (ppm): 14.0 (q), 23.3 (t), 23.6 (t), 26.1 (t), 42.2 (t), 55.1 (q), 59.9 (t), 113.8 (2 x d), 120.1 (s), 121.0 (s), 123.3 (s), 130.0 (s), 130.1 (2 x d), 131.8 (s), 158.8 (s), 160.2 (s), 199.3 (s). Anal Calcd. for $C_{19}H_{21}NO_4$ C, 69.71; H, 6.47; N, 4.28. Found: C, 69.31; H, 6.87; N, 4.68

### 12.1.22 Procedure for the synthesis of 1-(4-methoxyphenyl)-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one (50)

**Preparation of 3-(4-methoxyphenyl)-8-oxo-2,4,5,6,7,8-hexahydrocyclohepta[c]pyrrole-1-carboxylic acid (49)** To a solution of **48** (0.73 g, 2.2 mmol) in ethanol (31 mL), 60% potassium hydroxide aqueous solution (1,74 mL) was added. The reaction mixture was heated under reflux for 3 hours. After cooling, the solvent was evaporated under reduced pressure. The residue was poured into water and ice and acidified with HCl 6 M. The formed solid was filtered and dried. Brown solid; yield: 85%; mp: 129 – 130°C; IR ($cm^{-1}$): 3441 (NH), 3339 (OH), 1682 (CO), 1670 (CO); $^{1}H$ NMR (DMSO-$d_6$) (ppm): 1.70 – 2.05 (4H, m, 2 x $CH_2$), 2.91 (4H, t, J= 5.9 Hz, 2 x $CH_2$), 3.87 (3H, s, $CH_3$), 7.0 (2H, d, J=8.7 Hz, H-3' and H-5'), 7.34 (2H, d, J=8.8 Hz, H-2' and H-6'), 9.92 (1H, s, NH), 15.12 (1H, s, OH); $^{13}C$ NMR (DMSO-$d_6$) (ppm): 21.2 (t), 23.2 (t), 24.8 (t), 40.3 (t), 55.4 (q), 114.6 (2 x d), 122.5 (s), 124.8 (s), 125.4 (s), 125.9 (s), 129.6 (2 x d), 133.2 (s), 160.0 (s), 160.7 (s), 204.3 (s). Anal Calcd. for $C_{17}H_{17}NO_4$ C, 68.22; H, 5.72; N, 4.68. Found: C, 68.62; H, 5.32; N, 4.28.

**Preparation of 1-(4-methoxyphenyl)-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one (50)** A solution of **49** (0.47 g, 1.6 mmol) in ethanol (22 mL) was heated almost to boiling and HCl 6 M (10 mL) was then added. The reaction mixture was heated under reflux for 1 hour. The solvent was evaporated under reduced pressure and the residue was poured into water and ice. The solution was extracted with ethyl acetate, dried on $Na_2SO_4$ and evaporated at reduced pressure. The crude product is used in the following step without further purification. Brown solid; mp: 135 – 136°C; Yield: 50%; IR ($cm^{-1}$): 3425 (NH), 1668 (CO); $^{1}H$ NMR ($CDCl_3$)

(ppm): 1.81 – 1.91 (4H, m, 2 x $CH_2$), 2.70 (2H, t, J= 5.8 Hz, $CH_2$), 2.85 (2H, t, J= 5.7 Hz, $CH_2$), 6.95 (2H, d, J=8.6 Hz, H-2' and H-6'), 7.33 (3H, t, J=11.6 Hz, H-3', H-5' and H-3), 9.17 (1H, s, NH); $^{13}$C NMR ($CDCl_3$) (ppm): 22.4 (t), 23.9 (t), 26.3 (t), 41.6 (t), 55.4 (q), 114.2 (2 x d), 120.5 (s), 121.9 (d), 125.1 (s), 126.9 (s), 129.0 (2 x d), 129.4 (s), 158.7 (s), 200.3 (s). Anal Calcd. for $C_{16}H_{17}NO_2$ C, 75.27; H, 6.71; N, 5.49. Found: C, 74.87; H, 7.11; N, 5.89

**12.1.23 Procedure for the synthesis of 2-((dimethylamino)methylene)cycloheptane-1,3-dione (52).** A solution of cycloheptane-1,3-dione **51** (1 g, 8 mmol) in anhydrous *N*,*N*-dimethylformamide dimethyl acetal (2,6 mL) was heated under reflux for 1 hour. After cooling, the solvent was evaporated at reduced pressure and the oily residue was tritured with diethylether and filtered off. Brown solid; yield: 99%; mp: 102 – 103°C; IR ($cm^{-1}$): 1660 (CO) 1585 (CO); $^1$H NMR ($CDCl_3$) (ppm): 1.84 – 1.88 (4H, m, 2 x $CH_2$), 2.59 (4H, t, J= 6.2 Hz, 2 x $CH_2$), 2.80 (3H, s, $CH_3$), 3.30 (3H, s, $CH_3$), 7.72 (1H, s, CH); $^{13}$C NMR ($CDCl_3$) (ppm): 22.2 (2 x q), 40.5 (2 x t), 43.2 (t), 47.9 (t), 112.8 (d), 159.6 (2 x s), 200.2 (s). Anal Calcd. for $C_{10}H_{15}NO_2$ C, 66.27; H, 8.34; N, 7.73. Found: C, 65.87; H, 7.94; N, 8.13.

**12.1.24 Procedure for the synthesis of 2-**{[(**2,7-dioxocycloheptylidene)methyl]amino}-arylacetic acid (53a,b).** To a solution of **52** (16 mmol) in ethanol (30 mL), a solution of suitable phenylglycine (19 mmol) and sodium acetate trihydrate (0.26 g) in ethanol was added and reaction mixture was heated under reflux up to completeness (TLC). After cooling, the reaction mixture was filtered and the filtrate was evaporated under reduced pressure. To the residue, ice and water were added and the resulting solution was acidified with HCl 6 M. The solid obtained was filtered and dried.

**Data for 2-**{[(**2,7-dioxocycloheptylidene)methyl]amino}-2-phenylacetic acid (53a)** This compound was obtained from reaction of **52** with phenylglycine after 1 h and 30 min. Brown oil; yield: 80%; IR ($cm^{-1}$): 3422 (NH), 3287 (OH), 1703 (CO), 1658 (CO), 1621 (CO); $^1$H NMR ($CDCl_3$) (ppm): 1.70 (4H, s, 2 x $CH_2$), 2.50 – 2.60 (5H, m, 2 x $CH_2$ and OH), 5.63 (1H, d, J= 7.2 Hz, CH), 7.33 – 7.46 (5H, m, Ar), 7.92 (1H, d, J= 14.0 Hz, CH), 11.44 – 11.51 (1H, m, NH); $^{13}$C NMR ($CDCl_3$) (ppm): 21.5 (t), 21.6 (t), 63.8 (t), 74.0 (t), 111.8 (d), 127.8 (2 x d), 129.1 (d), 129.7 (2 x d), 137.7 (s), 158.4 (s), 171.3 (d), 198.9 (s), 201.1 (s), 207.5 (s). Anal Calcd. for $C_{16}H_{17}NO_4$ C, 66.89; H, 5.96; N, 4.88. Found: C, 66.49; H, 5.56; N, 4.48.

**Data for 2-**{[(**2,7-dioxocycloheptylidene)methyl]amino}-2-(3,4,5-trimethoxyphenyl)acetic acid (53b)** This compound was obtained from reaction of **52** with 3,4,5-trimethoxyphenylglycine after 1 h and 30 min. Brown solid; yield: 82%; mp: 102-103 °C; IR

($cm^{-1}$): 3401 (NH), 3299 (OH), 1698 (CO), 1652 (CO), 1633 (CO); $^{1}H$ NMR ($CDCl_3$) (ppm): 1.82 (4H, s, 2 x $CH_2$), 2.66 (4H, s, 2 x $CH_2$), 3.81 – 3.90 (9H, m, 3 x $CH_3$), 5.08 (1H, d, J= 6.8 Hz, CH), 6.59 (2H, s, Ar), 8.03 (2H, d, J= 14.1 Hz, Ar), 11.57 – 11.64 (1H, m, NH); $^{13}C$ NMR ($CDCl_3$) (ppm): 21.4 (t), 21.5 (t), 40.1 (t), 40.6 (t), 56.2 (2 x q), 60.8 (q), 66.2 (d), 104.5 (2 x d), 112.2 (s), 130.7 (s), 138.6 (s), 153.8 (s), 159.1 (d), 170.7 (s), 201.7 (s), 202.1 (2 x s). Anal Calcd. for $C_{19}H_{23}NO_7$ C, 60.47; H, 6.14; N, 3.71. Found: C, 60.07; H, 6.54; N, 3.31.

**12.1.25 Procedure for the synthesis of 2-acetyl-1-substituted-2,6,7,8-tetrahydrocyclohepta[c]pyrrol-4-yl acetate (54a,b).**

To a solution of **53a,b** (8 mmol) in acetic anhydride (25 mL), triethylamine was added (5.7 mmol, 8 mL). The reaction mixture was heated under reflux up to completeness (TLC). After cooling, the reaction mixture was poured into water and ice and formed a rubbery solid, which was decanted and then stirred with a saturated solution of $Na_2CO_3$ (50 mL). The solid obtained was filtered and dried. The solid was purified using chromatography column (dichloromethane).

**Data for 2-acetyl-1-phenyl-2,6,7,8-tetrahydrocyclohepta[c]pyrrol-4-yl acetate (54a).** This compound was obtained from reaction of **53a** after 30 min. Brown solid; yield: 73%; mp: 120–121 °C; IR ($cm^{-1}$): 1769 (CO) 1711 (CO); $^{1}H$ NMR ($CDCl_3$) (ppm): 1.75 – 1.86 (2H, m, $CH_2$), 2.08 (3H, s, $CH_3$), 2.28 (3H, s, $CH_3$), 2.47 - 2.53 (4H, m, 2 x $CH_2$), 5.49 (1H, t, J= 5.2 Hz, CH), 7.22 - 7.28 (3H, m, Ar and H-3), 7.39 - 7.43 (3H, m, Ar); $^{13}C$ NMR ($CDCl_3$) (ppm): 21.1 (q), 24.7 (t), 25.1 (q), 26.6 (t), 28.5 (t), 117.2 (d), 119.6 (d), 121.8 (s), 125.9 (s), 128.2 (d), 128.3 (2 x d), 130.3 (s), 130.6 (2 x d), 133.0 (s), 141.0 (s), 168.8 (s), 170.1 (s). Anal Calcd. for $C_{20}H_{23}NO_3$ C, 73.82; H, 7.12; N, 4.30. Found: C, 74.22; H, 7.52; N, 3.90.

**Data for 2-acetyl-1-(3,4,5-trimethoxyphenyl)-2,6,7,8-tetrahydrocyclohepta[c]pyrrol-4-yl acetate (54b).** This compound was obtained from reaction of **53b** after 30 min. Brown oil; yield: 53% IR ($cm^{-1}$): 1753 (CO) 1722 (CO); $^{1}H$ NMR ($CDCl_3$) (ppm): 1.85 – 1.87 (2H, m, $CH_2$), 2.12 (3H, s, $CH_3$), 2.28 (3H, s, $CH_3$), 2.44 –2.48 (2H, m, $CH_2$), 2.52 – 2.56 (2H, m, $CH_2$), 3.85 (6H, s, 2 x $CH_3$), 3.91 (3H, s, $CH_3$), 5.50 (1H, t, J= 5.0 Hz, CH), 6.45 – 6.49 (3H, m, Ar and H-3); $^{13}C$ NMR ($CDCl_3$) (ppm): 20.9 (q), 24.6 (t), 26.7 (q), 28.3 (t), 30.1 (t), 56.0 (2 x q), 61.0 (q), 65.4 (s), 107.8 (2 x d), 116.9 (s), 119.5 (2 x s), 121.5 (s), 125.9 (d), 128.2 (s), 130.0 (s), 141.0 (s), 153.2 (d), 168.6 (s), 169.9 (s). Anal Calcd. for $C_{22}H_{25}NO_6$ C, 66.15; H, 6.31; N, 3.51. Found: C, 66.55; H, 6.71; N, 3.91.

**12.1.26 Procedure for the synthesis of 1-substituted-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one (55a,b).** To a solution of **54a,b** (3,3 mmol) in AcOH (80%, 20 mL), HCl (37%, 1,7 mL) was added dropwise. The reaction mixture was heated at 60 °C up to completeness (TLC). After cooling, the reaction mixture was poured into water and ice. The solid obtained was filtered and dried. The solid was purified using chromatography column (dichloromethane : ethyl acetate 95:5).

**Data for 1-phenyl-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one (55a).** This compound was obtained from **54a** after 15 min. Brown solid; yield: 65%; mp: 103 – 104 °C; IR ($cm^{-1}$): 3248 (NH), 1651 (CO); $^{1}H$ NMR ($CDCl_3$) (ppm): 1.72 – 2.04 (4H, m, 2 x $CH_2$), 2.73 (2 H, t, J= 6.0 Hz, $CH_2$), 2.91 (2H, t, J= 5.9 Hz, $CH_2$), 7.29 - 7.47 (6H, m, Ar and H-3), 8.80 (1H, s, NH); $^{13}C$ NMR ($CDCl_3$) (ppm): 22.3 (t), 23.9 (t), 26.3 (t), 41.6 (t), 99.2 (s), 121.3 (s), 122.2 (d), 127.2 (d), 127.6 (2 x d), 128.9 (2 x d), 129.5 (s), 132.4 (s), 200.0 (s). Anal Calcd. for $C_{15}H_{15}NO$ C, 79.97; H, 6.71; N, 6.22. Found: C, 80.37; H, 6.31; N, 6.62.

**Data for 1-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one (55b).** This compound was obtained from **54b** after 15 min. Brown solid; yield 81%; IR ($cm^{-1}$): 3258 (NH), 1657 (CO); $^{1}H$ NMR ($CDCl_3$) (ppm): 1.90 (4H, s, 2 x $CH_2$), 2.79 (2H, d, J= 9.4 Hz, $CH_2$), 2.91 – 2.95 (2H, m, $CH_2$), 3.89 (3H, s, $CH_3$), 3.90 (6H, s, 2 x $CH_3$), 6.61 (2H, s, Ar), 7.52 (1H, s, H-3), 9.26 (1H, s, NH); $^{13}C$ NMR ($CDCl_3$) (ppm): 22.4 (t), 24.1 (t), 26.3 (t), 41.6 (t), 56.2 (2 x q), 61.0 (q), 105.2 (2 x d), 120.9 (s), 122.2 (s), 126.8 (s), 127.9 (s), 129.6 (s), 137.3 (s), 153.5 (2 x s), 199.7 (s). Anal Calcd. for $C_{18}H_{21}NO_4$ C, 68.55; H, 6.71; N, 4.44. Found: C, 68.95; H, 6.31; N, 7.02.

**12.1.27 Procedure for the synthesis of (4-methoxyphenyl)-8-oxo-2,4,5,6,7,8-hexahydrocyclohepta[c]pyrrole-1-carboxylate (56a-m,o-y).**

To a solution of **48,50,55** (10 mmol) in anhydrous DMF (15 mL), NaH (0.24 g, 10 mmol) was added at 0 °C and the reaction was stirred for 1 h at room temperature. The suitable benzyl chloride (20 mmol) was added at 0 °C and the reaction mixture was stirred at room temperature up to completeness (TLC). The reaction mixture was poured into ice and brine, then the aqueous solution was extracted with dichloromethane (3 x 50 mL). The organic phase was dried over $Na_2SO_4$ and the solvent evaporated at reduced pressure. The crude product was purified using chromatography column (petroleum ether : ethyl acetate 9 : 1).

**Data for ethyl 2-benzyl-3-(4-methoxyphenyl)-8-oxo-2,4,5,6,7,8-hexahydrocyclohepta[c]pyrrole-1-carboxylate (56a).** This compound was obtained from reaction of **48** with benzyl bromide after 3 h. Yellow oil; yield 63%; IR ($cm^{-1}$): 1688 (CO),

1661 (CO); $^{1}$H NMR ($CDCl_3$) (ppm): 1.16 – 1.23 (3H, m, $CH_3$), 1.78 – 1.84 (4H, m, 2 x $CH_2$), 2.54 – 2.58 (2H, m, $CH_2$), 2.76 – 2.80 (2H, m, $CH_2$), 3.82 (3H, d, J = 8.0 Hz, $CH_3$), 4.16 – 4.23 (2H, m, $CH_2$), 5.28 (2H, s, $CH_2$), 6.83 – 6.92 (4H, m, Ar), 7.09 (2H, d, J =. 8.7 Hz, Ar), 7.21 – 7.28 (3H, m, Ar); $^{13}$C NMR ($CDCl_3$) (ppm): 13.7 (q), 23.9 (t), 24.5 (t), 26.7 (t), 42.9 (t), 49.1 (t), 55.3 (q), 61.0 (t), 114.0 (2 x d), 122.0 (s), 122.7 (s), 126.2 (2 x d), 127.1 (d), 128.4 (s), 128.4 (2 x d), 129.8 (s), 132.2 (2 x d), 134.9 (s), 138.1 (s), 159.8 (s), 162.1 (s), 200.6 (s). Anal. Calcd. for $C_{26}H_{27}NO_4$: C, 74.80; H, 6.52; N, 3.35. Found: C, 74.40; H, 6.92; N, 3.75.

**Data for ethyl 2-(4-methoxybenzyl)-3-(4-methoxyphenyl)-8-oxo-2,4,5,6,7,8-hexahydrocyclohepta[c]pyrrole-1-carboxylate (56b).** This compound was obtained from reaction of **48** with 4-methoxybenzyl choride after 4 h. Yellow oil; yield 75%; IR ($cm^{-1}$): 1690 (CO), 1662 (CO); $^{1}$H NMR ($CDCl_3$) (ppm): 1.28 (3H, t, J = 3.6 Hz, $CH_3$), 1.74 – 1.95 (4H, m, 2 x $CH_2$), 2.53 (2H, t, J = 5.8 Hz, $CH_2$), 2.75 (2H, t, J = 6.1 Hz, $CH_2$), 3.74 (3H, s, $CH_3$), 3.83 (3H, s, $CH_3$), 4.10 – 4.26 (2H, m, $CH_2$), 5.19 (2H, s, $CH_2$), 6.75 (4H, m, Ar), 7.05 – 7.11 (2H, m, Ar), 7.27 (2H, m, Ar); $^{13}$C NMR ($CDCl_3$) (ppm): 13.8 (q), 23.8 (t), 24.4 (t), 26.7 (t), 42.9 (t), 48.5 (t), 55.2 (q), 55.3 (q), 61.1 (t), 64.99 (s), 113.8 (2 x d), 113.9 (2 x d), 122.1 (s), 122.8 (s), 127.7 (2x d), 128.7 (s), 130.2 (s), 132.2 (2 x d), 134.7 (s), 158.7 (s), 159.7 (s), 162.2 (s), 200.6 (s). Anal. Calcd. for $C_{27}H_{29}NO_5$: C, 72.46; H, 6.53; N, 3.13. Found: C, 72.06; H, 6.93; N, 3.53.

**Data for ethyl 2-(3-methoxybenzyl)-3-(4-methoxyphenyl)-8-oxo-2,4,5,6,7,8-hexahydrocyclohepta[c]pyrrole-1-carboxylate (56c).** This compound was obtained from reaction of **48** with 3-methoxybenzyl choride after 6 h. Yellow oil; yield 76%; IR ($cm^{-1}$): 1692 (CO), 1667 (CO); $^{1}$H NMR ($CDCl_3$) (ppm): 1.13 – 1.30 (3H, m, $CH_3$), 1.75 – 1.87 (2H, m, $CH_2$), 1.90 – 1.99 (2H, m, $CH_2$), 2.54 (2H, t, J = 5.9 Hz, $CH_2$), 2.75 (2H, t, J = 3.2 Hz, $CH_2$), 3.71 (3H, s, $CH_3$), 3.82 (3H, s, $CH_3$), 5.24 (2H, s, $CH_2$), 6.37 – 6.44 (2H, m, Ar), 6.69 – 6.75 (1H, m, Ar), 6.87 – 6.96 (2H, m, Ar), 7.06 – 7.17 (3H, m, Ar); $^{13}$C NMR ($CDCl_3$) (ppm): 13.8 (q), 23.9 (t), 24.5 (t), 26.8 (t), 42.9 (t), 49.0 (t), 55.1 (q), 55.3 (q), 61.1 (t), 111.9 (d), 112.6 (d), 114.0 (2 x d), 118.6 (d), 122.0 (s), 122.7 (s), 122.9 (s), 129.5 (d), 129.9 (s), 132.2 (2 x d), 134.9 (s), 139.8 (s), 159.6 (s), 159.8 (s), 162.0 (s), 200.7 (s). Anal. Calcd. for $C_{27}H_{29}NO_5$: C, 72.46; H, 6.53; N, 3.13. Found: C, 72.86; H, 6.93; N, 3.53.

**Data for ethyl 2-(2-methoxybenzyl)-3-(4-methoxyphenyl)-8-oxo-2,4,5,6,7,8-hexahydrocyclohepta[c]pyrrole-1-carboxylate (56d).** This compound was obtained from reaction of **48** with 2-methoxybenzyl choride after 5 h. Yellow oil; yield 71%; IR ($cm^{-1}$): 1691 (CO), 1665 (CO); $^{1}$H NMR ($CDCl_3$) (ppm): 1.11 – 1.30 (3H, m, $CH_3$), 1.76 – 1.94 (4H, m, 2 x

$CH_2$), 2.55 (2H, t, J = 5.9 Hz, $CH_2$), 2.75 – 2.81 (2H, m, $CH_2$), 3.70 (3H, s, $CH_3$), 3.81 (3H, s, $CH_3$), 4.07 – 4.21 (2H, m, $CH_2$), 5.23 (2H, s, $CH_2$), 6.47 (1H, d, J = 7.5 Hz, CH), 6.73 – 6.88 (4H, m, Ar), 7.03 – 7.21 (3H, m, Ar); $^{13}$C NMR ($CDCl_3$) (ppm): 13.6 (q), 23.9 (t), 24.5 (t), 26.8 (t), 42.9 (t), 44.9 (t), 55.1 (q), 55.3 (q), 60.9 (t), 109.6 (d), 113.8 (2 x d), 120.5 (d), 121.9 (s), 122.8 (s), 123.3 (s), 126.5 (d), 126.8 (s), 128.0 (d), 129.6 (s), 132.0 (2 x d), 135.0 (s), 155.8 (s), 159.6 (s), 161.8 (s), 200.81 (s). Anal. Calcd. for $C_{27}H_{29}NO_5$: C, 72.46; H, 6.53; N, 3.13. Found: C, 72.86; H, 6.93; N, 2.73.

**Data for 2-(4-methoxybenzyl)-1-(4-methoxyphenyl)-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one (56e).** This compound was obtained from reaction of compound **50** with 4-methoxybenzyl choride after 12 h. Yellow oil; yield 78% IR ($cm^{-1}$): 1659 (CO); $^1$H NMR ($CDCl_3$) (ppm): 1.71 – 1.91 (4H, m, 2 x $CH_2$), 2.59 – 2.71 (4H, m, 2 x $CH_2$), 3.77 (3H, s, $CH_3$), 3.84 (3H, s, $CH_3$), 4.84 (2H, s, $CH_2$), 6.81 (2H, t, J = 5.5 Hz, Ar), 6.88 – 6.95 (4H, m, Ar), 7.11 (2H, t, J = 4.4 Hz, Ar), 7.33 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 22.6 (t), 24.7 (t), 26.4 (t), 42.0 (t), 50.9 (t), 55.3 (2 x q), 113.8 (2 x d), 114.1 (2 x d), 122.7 (s), 123.6 (s), 124.8 (d), 127.7 (s), 128.7 (2 x d), 129.1 (s), 131.3 (s), 132.2 (2 x d), 159.2 (s), 159.6 (s), 200.0 (s). Anal. Calcd. for $C_{24}H_{25}NO_3$: C, 76.77; H, 6.71; N, 3.73. Found: C, 77.17; H, 7.11; N, 4.13.

**Data for 2-(3-methoxybenzyl)-1-(4-methoxyphenyl)-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one (56f).** This compound was obtained from reaction of compound **50** with 3-methoxybenzyl choride after 6 h. Yellow oil; yield 62%; IR ($cm^{-1}$): 1657 (CO); $^1$H NMR ($CDCl_3$) (ppm): 1.77 – 2.00 (4H, m, 2 x $CH_2$), 2.60 – 2.72 (4H, m, 2 x $CH_2$), 3.67 – 3.88 (6H, m, 2 x $CH_3$), 4.88 (2H, s, $CH_2$), 6.48 – 6.58 (2H, m, Ar), 6.74 – 6.93 (3H, m, Ar), 7.09 – 7.45 (4H, m, Ar and H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 22.4 (t), 24.3 (t), 26.3 (t), 41.9 (t), 51.2 (t), 55.2 (q), 55.3 (q), 101.0 (s), 112.9 (d), 113.0 (d), 113.9 (2 x d), 119.4 (d), 122.6 (s), 123.5 (s), 125.0 (d), 129.8 (d), 131.4 (s), 132.2 (2 x d), 138.8 (s), 159.4 (s), 159.8 (s), 199.5 (s). Anal. Calcd. for $C_{24}H_{25}NO_3$: C, 76.77; H, 6.71; N, 3.73. Found: C, 76.37; H, 7.11; N, 4.13.

**Data for 2-(2-methoxybenzyl)-1-(4-methoxyphenyl)-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one (56g).** This compound was obtained from reaction of compound **50** with 2-methoxybenzyl choride after 7 h. Yellow oil; yield 70%; IR ($cm^{-1}$): 1658 (CO); $^1$H NMR ($CDCl_3$) (ppm): 1.77 – 2.06 (4H, m, 2 x $CH_2$), 2.60 – 2.72 (4H, m, 2 x $CH_2$), 3.75 (3H, s, $CH_3$), 3.83 (3H, s, $CH_3$), 4.92 (2H, s, $CH_2$), 6.68 – 6.95 (5H, m, Ar), 7.12 – 7.36 (4H, m, Ar and H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 22.5 (t), 24.3 (t), 26.3 (t), 41.9 (t), 46.3 (t), 55.3 (2 x q), 110.2 (d), 113.8 (2 x d), 120.6 (d), 122.4 (s), 123.8 (s), 124.7 (s), 125.2

(d), 125.45 (s), 128.7 (d), 129.0 (d), 131.5 (s), 132.1 (2 x d), 156.6 (s), 159.2 (s), 199.5 (s). Anal. Calcd. for $C_{24}H_{25}NO_3$: C, 76.77; H, 6.71; N, 3.73. Found: C, 77.17; H, 7.11; N, 4.13.

**Data for 1-(4-methoxyphenyl)-2-(3,4,5-trimethoxybenzyl)-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one (56h).** This compound was obtained from reaction of compound **50** with 3,4,5-trimethoxybenzyl choride after 12 h. Yellow oil; yield 63%; IR ($cm^{-1}$): 1655 (CO); ); $^1H$ NMR ($CDCl_3$) (ppm): 1.73 – 1.83 (4H, m, 2 x $CH_2$), 2.47 – 2.52 (4H, m, 2 x $CH_2$), 3.80 (6H, s, 2 x $CH_3$), 3.81 (3H, s, $CH_3$), 3.82 (3H, s, $CH_3$), 5.18 (2H, s, $CH_2$), 6.51 (2H, m, H-2" and H-6"), 7.04 (2H, d, J = 7.1 Hz, H-3' and H-5'), 7.11 (1H, s, H-3), 7.53 (2H, d, J = 7.1 Hz, H-2' and H-6'); $^{13}C$ NMR ($CDCl_3$) (ppm): 22.4 (t), 24.2 (t), 26.2 (t), 41.6 (t), 49.2 (t), 56.0 (2 x q), 56.1 (q), 60.8 (q), 104.8 (2 x d), 105.2 (s), 107.8 (s), 113.8 (2 x d), 122.8 (s), 125.2 (s), 130.8 (s), 131.8 (2 x d), 136.0 (s), 136.2 (d), 153.1 (2 x s), 159.4 (s), 200.0 (s). Anal. Calcd. for $C_{24}H_{25}NO_3$: C, 76.77; H, 6.71; N, 3.73. Anal. Calcd. for $C_{26}H_{29}NO_5$: C, 71.70; H, 6.71; N, 3.22. Found: C, 71.30; H, 6.31; N, 3.62.

**Data for 2-benzyl-1-phenyl-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one (56i).** This compound was obtained from reaction of compound **55a** with benzyl bromide after 3 h. Yellow oil; yield 90%; IR ($cm^{-1}$): 1661 (CO); $^1H$ NMR ($CDCl_3$) (ppm): 1.81 – 1.93 (4H, m, 2 x $CH_2$), 2.66 – 2.74 (4H, m, 2 x $CH_2$), 4.96 (2H, s, $CH_2$), 6.98 (2H, t, J= 6.98 Hz, Ar), 7.20 – 7.28 (4H, m, Ar), 7.36 – 7.40 (5H, m, Ar and H-3); $^{13}C$ NMR ($CDCl_3$) (ppm): 22.4 (t), 24.3 (t), 26.3 (t), 41.9 (t), 51.4 (t), 122.8 (s), 125.1 (s), 125.3 (d), 127.2 (d), 127.7 (d), 128.0 (2 x d), 128.4 (2 x d), 128.7 (2 x d), 130.9 (2 x d), 131.5 (s), 131.7 (s), 137.1 (s), 199.4 (s). Anal. Calcd. for $C_{22}H_{21}NO$: C, 83.78; H, 6.71; N, 4.44. Found: C, 84.18; H, 6.31; N, 4.84.

**Data for 2-(4-methoxybenzyl)-1-phenyl-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one (56j).** This compound was obtained from reaction of compound **55a** with 4-methoxybenzyl choride after 6 h. Yellow oil; yield 67%; IR ($cm^{-1}$): 1658 (CO); $^1H$ NMR ($CDCl_3$) (ppm): 1.66 – 1.91 (4H, m, 2 x $CH_2$), 2.61 – 2.72 (4H, m, 2 x $CH_2$), 3.77 (3H, s, $CH_3$), 4.87 (2H, s, $CH_2$), 6.70 – 6.92 (4H, m, Ar), 7.15 – 7.38 (6H, m, Ar); $^{13}C$ NMR ($CDCl_3$) (ppm): 22.4 (t), 24.2 (t), 26.2 (t), 41.9 (t), 50.9 (t), 55.3 (q), 114.1 (2 x d), 122.8 (s), 124.9 (s), 125.1 (d), 128.0 (d), 128.4 (2 x d), 128.7 (2 x d), 128.9 (s), 130.9 (2 x d), 131.4 (s), 131.6 (s), 159.0 (s), 199.50 (s). Anal. Calcd. For $C_{23}H_{23}NO_2$: C, 79.97; H, 6.71; N, 4.05. Found: C, 79.57; H, 7.11; N, 3.65.

**Data for 2-(3-methoxybenzyl)-1-phenyl-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one (56k).** This compound was obtained from reaction of compound **55a** with 3-methoxybenzyl choride after 5 h. Yellow oil; yield 78%; IR($cm^{-1}$): 1657 (CO); $^1H$ NMR ($CDCl_3$) (ppm): 1.78 – 1.92 (4H, m, 2 x $CH_2$), 2.62 – 2.73 (4H, m, $CH_2$), 3.73 (3H, s, $CH_3$), 4.92 (2H, s, $CH_2$), 6.47 – 6.58 (2H, m, Ar), 6.75 – 6.80 (2H, m, Ar), 7.14 – 7.24 (3H, m, Ar), 7.32 – 7.42 (3H, m, Ar);

$^{13}$C NMR ($CDCl_3$) (ppm): 22.4 (t), 24.2 (t), 26.2 (t), 41.9 (t), 51.3 (t), 55.2 (q), 112.9 (d), 113.0 (d), 119.5 (d), 122.8 (s), 125.1 (s), 125.3 (d), 128.0 (d), 128.5 (2 x d), 129.8 (d), 130.9 (2 x d), 131.3 (s), 131.7 (s), 138.6 (s), 159.8 (s), 199.5 (s). Anal. Calcd. for $C_{23}H_{23}NO_2$: C, 79.97; H, 6.71; N, 4.05. Found: C, 80.37; H, 7.11; N, 3.65.

**Data for 2-(2-methoxybenzyl)-1-phenyl-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one (56l).** This compound was obtained from reaction of compound **55a** with 2-methoxybenzyl choride after 8 h. Yellow oil; yield 60%; IR ($cm^{-1}$): 1656 (CO); $^1$H NMR ($CDCl_3$) (ppm): 1.66 – 1.85 (4H, m, 2 x $CH_2$), 2.33 – 2.48 (4H, m, $CH_2$), 3.81 (3H, s, $CH_3$), 5.11 (2H, s, $CH_2$), 6.81 – 6.91 (2H, m, Ar), 7.09 – 7.17 (2H, m, Ar), 7.31 – 7.48 (3H, m, Ar), 7.57 – 7.67 (3H, m, Ar); $^{13}$C NMR ($CDCl_3$) (ppm): 22.6 (t), 24.3 (t), 26.4 (t), 41.7 (t), 46.3 (t), 55.3 (q), 110.2 (d), 120.6 (d), 122.4 (s), 123.8 (s), 124.7 (s), 125.2 (d), 125.45 (s), 128.1 (2 x d), 128.5 (d), 128.7 (d), 129.0 (d), 129.2 (2 x d), 131.2 (s), 156.7 (s), 159.4 (s), 199.5 (s). Anal. Calcd. for $C_{23}H_{23}NO_2$: C, 79.97; H, 6.71; N, 4.05. Found: C, 80.37; H, 7.11; N, 3.65.

**Data for 2-(4-methoxy-3-nitrobenzyl)-1-phenyl-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one (56m).** This compound was obtained from reaction of compound **55a** with 3-nitro,4-methoxybenzyl choride after 6 h. Brown solid; yield 90%; mp: 194 – 195 °C; IR ($cm^{-1}$): 1656 (CO), 1535 ($NO_2$); $^1$H NMR ($CDCl_3$) (ppm): 1.68 – 175 (4H, m, 2 x $CH_2$), 2.51 – 2.58 (4H, m, 2 x $CH_2$), 3.85 (3H, s, $CH_3$), 5.10 (2H, s, $CH_2$), 7.12 (1H, d, J = 8.5 Hz, Ar), 7.23 (3H, d, J= 8.4 Hz, Ar), 7.34 – 7.45 (4H, m, Ar), 7.59 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 22.1 (t), 23.9 (t), 261 (t), 41.7 (t), 49.7 (t), 57.1 (q), 115.0 (d), 122.9 (s), 124.3 (d), 124.9 (s), 126.0 (d), 128.4 (d), 129.0 (2 x d), 130.3 (s), 131.0 (2 x d), 131.1 (s), 131.3 (s), 133.8 (d), 139.1 (s), 151.8 (s), 197.8 (s). Anal. Calcd. for $C_{23}H_{22}N_2O_4$: C, 70.75; H, 5.68; N, 7.18. Found: C, 70.35; H, 6.08; N, 7.58.

**Data for 2-(3,4-dimethoxybenzyl)-1-phenyl-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one (56o).** This compound was obtained from reaction of compound **55a** with 3,4-dimethoxybenzyl choride after 4 h. Yellow oil; yield 73%; IR ($cm^{-1}$): 1660 (CO); $^1$H NMR ($CDCl_3$) (ppm): 1.80 – 1.89 (4H, m, 2 x $CH_2$), 2.66 – 2.70 (4H, m, 2 x $CH_2$), 3.74 (3H, s, $CH_3$), 3.84 (3H, s, $CH_3$), 4.89 (2H, s, $CH_2$), 6.40 (1H, s, Ar), 6.54 (1H, d, J= 6.55 Hz, Ar), 6.75 (1H, d, J= 6.75 Hz, Ar), 6.86 – 6.96 (1H, m, Ar), 7.23 (1H, d, J=7.23 Hz, Ar), 7.39 (4H, s, Ar); $^{13}$C NMR ($CDCl_3$) (ppm): 22.4 (t), 24.2 (t), 26.2 (t), 41.8 (t), 51.3 (t), 55.8 (q), 55.9 (q), 110.4 (s), 110.6 (d), 110.9 (s), 111.0 (s), 111.2 (d), 119.3 (s), 119.9 (d), 120.5 (s), 125.2 (d), 127.9 (d), 128.5 (2 x d), 129.3 (s), 130.9 (2 x d), 131.5 (s), 199.5 (s). Anal. Calcd. for $C_{24}H_{25}NO_3$: C, 76.77; H, 6.71; N, 3.73. Found: C, 76.37; H, 7.11; N, 4.13.

**Data for 2-(3,4,5-trimethoxybenzyl)-1-phenyl-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one (56p).** This compound was obtained from reaction of compound **55a** with 3,,4,5-trimethoxybenzyl choride after 6 h. Yield 98%; oil; IR ($cm^{-1}$): 1662 (CO); $^{1}H$ NMR ($CDCl_3$) (ppm): 1.79 – 1.92 (4H, m, 2 x $CH_2$), 2.64 – 2.73 (4H, m, 2 x $CH_2$), 3.81 (3H, s, $CH_3$), 3.86 (3H, s, $CH_3$), 3.88 (3H, s, $CH_3$), 4.89 (2H, s, $CH_2$), 6.13 (2H, s, Ar), 6.62 (1H, s, Ar), 7.36 – 7.45 (5H, m, Ar); $^{13}C$ NMR ($CDCl_3$) (ppm): 17.00 (t), 22.3 (t), 24.1 (t), 41.8 (t), 51.8 (t), 56.0 (q), 56.1 (q), 60.8 (q), 103.7 (s), 104.5 (2 x d), 123.1 (s), 125.0 (s), 125.2 (d), 128.0 (d), 128.5 (2 x d), 131.0 (2 x d), 131.4 (s), 132.4 (2 x s), 153.3 (s), 199.5 (s). Anal. Calcd. for $C_{25}H_{27}NO_4$: C, 74.05; H, 6.71; N, 3.45. Found: C, 74.45; H, 6.31; N, 7.11.

**Data for 2-(2,5-dimethoxybenzyl)-1-phenyl-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one (56q).** This compound was obtained from reaction of compound **55a** with 2,5-dimethoxybenzyl choride after 4 h and 30 min. Yield 82%; oil; IR ($cm^{-1}$): 1655 (CO); $^{1}H$ NMR ($CDCl_3$) (ppm): 1.80 – 1.92 (4H, m, 2 x $CH_2$), 2.66 – 2.73 (4H, m, 2 x $CH_2$), 3.71 (6H, t, J= 3.71 Hz, 2 x $CH_3$), 4.94 (2H, s, $CH_2$), 6.30 (1H, s, Ar), 6.75 (2H, s, Ar), 7.29 – 7.42 (6H, m, Ar and H-3); $^{13}C$ NMR ($CDCl_3$) (ppm): 22.4 (t), 24.2 (t), 26.3 (t), 41.9 (t), 46.3 (t), 55.6 (q), 55.8 (q), 111.3 (d), 113.2 (d), 115.2 (d), 122.6 (s), 124.9 (s), 125.6 (d), 126.5 (s), 127.8 (d), 128.4 (2 x d), 130.9 (2 x d), 131.6 (s), 132.7 (s), 151.0 (s), 153.6 (s), 199.3 (s). Anal. Calcd. for $C_{24}H_{25}NO_3$: C, 76.77; H, 6.71; N, 3.73. Found: C, 77.17; H, 6.31; N, 4.13.

**Data for 2-benzyl-1-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one (56r).** This compound was obtained from reaction of compound **55b** with benzyl bromide after 3 h. Yellow oil; yield 68%; IR ($cm^{-1}$): 1659 (CO); $^{1}H$ NMR ($CDCl_3$) (ppm): 1.87 (4H, t, J = 1.88 Hz, 2 x $CH_2$), 2.68 – 2.75 (4H, m, 2 x $CH_2$), 3.69 (6H, s, 2 x $CH_3$), 3.89 (3H, s, $CH_3$), 4.98 (2H, s, $CH_2$), 6.33 (2H, s, H-2'' and H-6''), 7.01 (2H, t, J = 7.01 Hz, Ar), 7.29 (3H, d, J= 7.29 Hz, Ar), 7.43 (1H, s, H-3); $^{13}C$ NMR ($CDCl_3$) (ppm): 22.3 (t), 24.1 (t), 26.2 (t), 30.1 (t), 41.8 (t), 51.4 (2 x q), 56.0 (q), 107.8 (2 x d), 122.7 (d), 125.0 (s), 125.4 (s), 126.5 (s), 126.8 (2 x d), 127.7 (s), 128.7 (2 x d), 131.6 (s), 137.5 (s), 137.9 (s), 152.9 (2 x s), 199.2 (d). Anal. Calcd. for $C_{25}H_{27}NO_4$: C, 74.05; H, 6.71; N, 3.45. Found: C, 74.45; H, 6.31; N, 3.85.

**Data for 2-(4-methoxybenzyl)-1-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one (56s).** This compound was obtained from reaction of compound **55b** with 4-methoxybenzyl choride after 6 h. Yield 66%; Yellow oil; IR ($cm^{-1}$): 1658 (CO); $^{1}H$ NMR ($CDCl_3$) (ppm): 1.81 – 1.93 (4H, m, 2 x $CH_2$), 2.66 – 2.73 (4H, m, 2 x $CH_2$), 3.67 – 3.79 (9H, m, 3 x $CH_3$), 3.90 (3H, d, J = 3.3 Hz, $CH_3$), 4.90 (2H, s, $CH_2$), 6.36 (2H, s, H-2'' and H-6''), 6.81 (2H, d, J = 8.7 Hz, Ar), 6.93 (2H, d, J = 8.6 Hz, Ar),

7.39 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 22.4 (t), 24.4 (t), 26.3 (t), 30.1 (s), 41.9 (t), 51.1 (t), 55.3 (q), 56.0 (2 x q), 60.9 (q), 105.7 (s), 108.1 (2 x d), 114.1 (2 x d), 122.7 (s), 124.8 (s), 125.2 (s), 126.7 (s), 128.4 (2 x d), 129.3 (s), 131.5 (s), 137.8 (s), 153.0 (2 x s), 159.2 (d). Anal. Calcd. for $C_{26}H_{29}NO_5$: C, 71.70; H, 6.71; N, 3.22. Found: C, 71.30; H, 6.31; N, 3.62.

**Data for 2-(3-methoxybenzyl)-1-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one (56t).** This compound was obtained from reaction of compound **55b** with 3-methoxybenzyl choride after 6 h. Yellow oil; yield 82%; IR ($cm^{-1}$): 1656 (CO); $^1$H NMR ($CDCl_3$) (ppm): 1.83 – 1.94 (4H, m, 2 x $CH_2$), 2.68 – 2.74 (4H, m, 2 x $CH_2$), 3.71 – 3.75 (9H, m, 3 x $CH_3$), 3.89 (3H, s, $CH_3$), 4.94 (2H, s, $CH_2$), 6.36 (2H, s, H-2'' and H-6''), 6.53 (2H, s, Ar), 6.78 (1H, d, J = 2.0 Hz, Ar), 6.81 (1H, d, J= 2.1 Hz, Ar), 7.42 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 22.4 (t), 24.4 (t), 26.3 (t), 41.9 (t), 51.5 (t), 55.2 (q), 55.9 (2 x q), 60.9 (q), 108.1 (2 x d), 112.7 (d), 112.8 (d), 119.1 (d), 122.7 (s), 125.0 (s), 125.4 (s), 129.8 (d), 131.6 (s), 137.8 (s), 139.1 (s), 153.0 (d), 160.0 (2 x s), 199.4 (s). Anal. Calcd. for $C_{26}H_{29}NO_5$: C, 71.70; H, 6.71; N, 3.22. Found: C, 72.10; H, 6.31; N, 3.62.

**Data for 2-(2-methoxybenzyl)-1-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one (56u).** This compound was obtained from reaction of compound **55b** with 2-methoxybenzyl choride after 7 h. Yellow oil; yield 68%; IR ($cm^{-1}$): 1659 (CO); $^1$H NMR ($CDCl_3$) (ppm): 1.68 – 1.84 (4H, m, 2 x $CH_2$), 2.33 – 2.56 (4H, m, $CH_2$), 3.78 – 3.82 (12H, m, 4 x $CH_3$), 5.20 (2H, s, $CH_2$), 6.81 – 6.92 (2H, m, Ar), 7.01 (1H, s, Ar), 7.10 (2H, s, Ar), 7.14 – 7.21 (2H, m, Ar); $^{13}$C NMR ($CDCl_3$) (ppm): 22.9 (t), 24.6 (t), 26.7 (t), 41.0 (t), 46.3 (t), 55.3 (q), 56.8 (3 x q), 60.7 (t), 106.2 (2 x d), 110.8 (d), 120.2 (d), 122.6 (s), 123.9 (s), 124.5 (s), 125.8 (d), 125.6 (s), 128.9 (d), 129.1 (d), 131.6 (s), 156.9 (2 x s), 159.1 (s), 199.5 (s). Anal. Calcd. for $C_{26}H_{29}NO_5$: C, 71.70; H, 6.71; N, 3.22. Found: C, 71.30; H, 6.33; N, 3.62.

**Data for 2-(3,4,5-trimethoxybenzyl)-1-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one (56v).** This compound was obtained from reaction of compound **55b** with 3,4,5-trimethoxybenzyl choride after 12 h. Yellow oil; yield 64%; IR ($cm^{-1}$): 1661 (CO); $^1$H NMR ($CDCl_3$) (ppm): 1.82 – 1.89 (4H, m, 2 x $CH_2$), 2.69 (4H, d, J = 2.69 Hz, 2 x $CH_2$), 3.71 (6H, s, 2 x $CH_3$), 3.72 (6H, s, 2 x $CH_3$), 3.88 (3H, s, $CH_3$), 3.89 (3H, s, $CH_3$), 4.81 (2H, s, $CH_2$), 6.15 (2H, s, Ar), 6.27 (1H, s, Ar), 6.34 (2H, s, Ar). $^{13}$C NMR ($CDCl_3$) (ppm): 22.3 (t), 24.2 (t), 26.3 (t), 41.8 (t), 56.0 (t), 56.1 (2 x q), 60.8 (2 x q), 60.8 (q), 60.9 (q), 104.8 (2 x d), 107.8 (2 x d), 122.9 (s), 124.2 (s), 124.9 (s), 125.0 (s), 126.5 (s), 131.1 (s), 131.5 (s), 136.1 (s), 142.0 (s), 152.4 (s), 153.1 (s), 153.1 (d), 199.3 (s). Anal. Calcd. for $C_{28}H_{33}NO_7$: C, 67.86; H, 6.71; N, 2.83. Found: C, 68.26; H, 7.11; N, 2.43.

**Data for 2-(2,5-dimethoxybenzyl)-1-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one (56w).** This compound was obtained from reaction of compound **55b** with 2,5-dimethoxybenzyl choride after 8 h. Yellow oil; yield 61%; IR ($cm^{-1}$): 1659 (CO); $^{1}H$ NMR ($CDCl_3$) (ppm): 1.81 – 1.93 (4H, m, 2 x $CH_2$), 2.68 – 2.74 (4H, m, 2 x $CH_2$), 3.68 (3H, s, $CH_3$), 3.72 (9H, s, 3 x $CH_3$), 3.88 (3H, s, $CH_3$), 4.94 (2H, s, $CH_2$), 6.32 (1H, s, Ar), 6.40 (2H, s, H-2'' and H-6''), 6.75 (2H, s, Ar), 7.41 (1H, s, H-3); $^{13}C$ NMR ($CDCl_3$) (ppm): 21.7 (t), 23.6 (t), 25.5 (t), 41.0 (t), 45.9 (t), 54.9 (q), 55.0 (q), 55.2 (2 x q), 60.1 (q), 107.2 (2 x d), 110.4 (d), 112.0 (d), 114.4 (d), 121.9 (s), 123.8 (s), 125.2 (d), 126.0 (s), 130.9 (s), 149.9 (s), 150.0 (s), 152.2 (s), 152.9 (2 x s), 156.7 (s), 197.6 (s). Anal. Calcd. for $C_{27}H_{31}NO_6$: C, 69.66; H, 6.71; N, 3.01. Found: C, 69.26; H, 6.31; N, 3.41.

**Data for 2-(3,4-dimethoxybenzyl)-1-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one (56x).** This compound was obtained from reaction of compound **55b** with 3,4-dimethoxybenzyl choride after 6 h. Yellow oil; yield 61%; IR ($cm^{-1}$): 1656 (CO); $^{1}H$ NMR ($CDCl_3$) (ppm): 1.68 – 1.85 (4H, m, 2 x $CH_2$), 2.34 – 2.65 (4H, m, 2 x $CH_2$), 3.77 (6H, s, 2 x $CH_3$), 3.79 (9H, s, 3 x $CH_3$), 5.15 (2H, s, $CH_2$), 6.77 – 7.01 (5H, m, Ar), 7.20 (1H, s, Ar); $^{13}C$ NMR ($CDCl_3$) (ppm): 22.4 (t), 24.2 (t), 26.2 (t), 41.8 (t), 51.3 (t), 55.8 (q), 55.9 (q), 106.2 (2 x d), 110.1 (s), 110.4 (d), 111.1 (s), 111.7 (s), 112.2 (d), 119.1 (s), 119.7 (d), 120.7 (s), 124.8 (d), 129.9 (s), 131.7 (s), 133.2 (s), 149.1 (2 x s), 199.4 (s). Anal. Calcd. for $C_{27}H_{31}NO_6$: C, 69.66; H, 6.71; N, 3.01. Found: C, 69.26; H, 6.31; N, 3.41.

**Data for 2-(4-methoxy-3-nitrobenzyl)-1-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one (56y).** This compound was obtained from reaction of compound **55b** with 3-nitro,4-methoxybenzyl choride after 3 h. Yellow solid; yield 65%; mp: 198 – 199°C; IR ($cm^{-1}$): 1660 (CO), 1531 ($NO_2$); $^{1}H$ NMR ($CDCl_3$) (ppm): 1.79 – 1.87 (4H, m, 2 x $CH_2$), 2.62 – 2.70 (4H, m, 2 x $CH_2$), 3.75 (6H, s, 2 x $CH_3$), 3.86 (3H, s, $CH_3$), 3.89 (3H, s, $CH_3$), 4.94 (2H, s, $CH_2$), 6.34 (2H, s, Ar), 6.97 (1H, d, J= 8.7 Hz, Ar), 7.13 (1H, s, Ar), 7.34 (2H, s, Ar); $^{13}C$ NMR ($CDCl_3$) (ppm): 22.3 (t), 24.3 (t), 26.2 (t), 41.9 (t), 50.2 (t), 56.1 (2 x q), 56.6 (q), 60.9 (q), 108.0 (2 x d), 113.8 (d), 123.2 (s), 124.4 (d), 124.8 (s), 125.3 (s), 126.4 (s), 126.5 (s), 129.7 (s), 131.4 (s), 132.8 (d), 138.1 (s), 139.5 (s), 152.3 (s), 153.2 (d). Anal. Calcd. for $C_{26}H_{28}N_2O_7$: C, 64.99; H, 5.87; N, 5.83. Found: C, 64.59; H, 5.47; N, 5.43.

### 12.1.28 Procedure for the synthesis of 2-(3-amino-4-methoxybenzyl)-1-substituted-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one (56n and 56z).

To a solution of **56m** or **56y** (1 mmol) in ethyl acetate (12 mL), ammonium formate (1 mmol) and Pd/C were added. The reaction mixture was stirred at room temperature for 12 h. Pd/C was removed by filtration through celite using ethyl acetate as eluent. The solvent was evaporated under reduced pressure, giving the desired compound.

**Data for 2-(3-amino-4-methoxybenzyl)-1-phenyl-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one (56n).** This compound was obtained from reaction of compound **56m**. Yellow oil; Yield 86%; IR ($cm^{-1}$): 3461−3389 ($NH_2$), 1651 (CO); $^1$H NMR ($CDCl_3$) (ppm): 1.80 – 1.92 (4H, m, 2 x $CH_2$), 2.66 – 2.73 (4H, m, 2 x $CH_2$), 3.83 (3H, s, $CH_3$), 4.78 (2H, s, $NH_2$), 4.81 (2H, s, $CH_2$), 6.36 – 6.39 (2H, m, Ar), 6.61 – 6.70 (1H, m, Ar), 7.23 – 7.43 (6H, m, Ar); $^{13}$C NMR ($CDCl_3$) (ppm): 22.4 (t), 24.2 (t), 26.2 (t), 41.5 (t), 51.1 (t), 55.5 (q), 110.2 (d), 113.9 (d), 117.5 (s), 122.7 (d), 125.2 (s), 127. (d), 128.1 (s), 128.4 (d), 129.4 (2 x d), 130.9 (2 x d), 130.9 (s), 130.1 (s), 131.4 (s), 136.4 (s), 199.5 (s). Anal. Calcd. for $C_{23}H_{24}N_2O_2$: C, 76.64; H, 6.71; N, 7.77. Found: C, 76.24; H, 7.11; N, 8.17.

**Data for 2-(3-amino-4-methoxybenzyl)-1-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one (56z).** This compound was obtained from reaction of compound **56y**. Yellow oil; yield 71%; IR ($cm^{-1}$): 3444−3361 ($NH_2$), 1655 (CO); $^1$H NMR ($CDCl_3$) (ppm): 1.68 – 1.85 (4H, m, 2 x $CH_2$), 2.34 – 2.58 (4H, m, 2 x $CH_2$), 3.77 (6H, s, 2 x $CH_3$), 3.78 (3H, s, $CH_3$), 3.80 (3H, s, $CH_3$), 4.96 (2H, s, $NH_2$), 5.13 (2H, s, $CH_2$), 6.42 – 6.46 (1H, m, Ar), 6.58 – 6.65 (2H, m, Ar), 6.97 (2H, s, Ar), 7.17 (1H, s, Ar); $^{13}$C NMR ($CDCl_3$) (ppm): 22.1 (t), 24.0 (t), 26.5 (t), 41.7 (t), 51.3 (t), 55.7 (q), 55.9 (2 x q), 56.2 (q), 105.9 (2 x d), 110.1 (d), 114.2 (d), 117.8 (s), 122.2 (d), 125.0 (s), 127.2 (d), 128.2 (s), 131.1 (s), 131.5 (s), 131.8 (s), 133.2 (s), 136.2 (s), 149.1 (2 x s), 199.3 (s). Anal.Calcd. for $C_{26}H_{20}N_2O_5$: C, 69.31; H, 6.71; N, 6.22. Found: C, 69.71; H, 6.31; N, 6.62.

### 12.1.29 Procedure for the synthesis of 5-((dimethylamino)methylene)-1-substituted-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one (57a-z).

To a solution of ketones **56a-z** (1 mmol) in anhydrous toluene (2.5 mL), TBDMAM (3 mmol) was added and the reaction mixture was heated under reflux for 12 h. After cooling, the solvent was removed under reduced pressure. The residue was used in the following step without further purification.

### 12.1.30 Procedure for the synthesis of 4,5,6,8-tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-d]isoxazoles (58a-z).

To a solution of **57a-z** (5 mmol) in ethanol (15 mL) and acetic acid (3 mL) hydroxylamine hydrochloride was added (7.5 mmol) and the reaction mixture was heated under reflux for 1 h. After cooling, the solvent was removed under reduced pressure. The crude product was poured into water and ice. The solid obtained was filtered, dried and purified using chromatography column (dichloromethane).

**Data for ethyl 8-benzyl-7-(4-methoxyphenyl)-4,5,6,8-tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-d]isoxazole-9-carboxylate (58a).** This compound was obtained from reaction of compound **57a**. Yellow oil; Yield 93%; $^{1}$H NMR ($CDCl_3$) (ppm): 1.25 (3H, t, J = 7.1 Hz, $CH_3$), 1.97 – 2.01 (2H, m, $CH_2$), 2.58 – 2.61 (2H, m, $CH_2$), 2.80 (2H, t, J = 6.4 Hz, $CH_2$), 3.84 (3H, s, $CH_3$), 4.29 (2H, d, J = 7.1 Hz, $CH_2$), 5.32 (2H, d, J = 10.1 Hz, $CH_2$), 6.86 (1H, s, Ar), 6.88 (1H, d, J = 1.5 Hz, Ar), 6.91 (1H, d, J = 2.1 Hz, Ar), 6.93 (1H, d, J = 2.1 Hz, Ar), 7.11 (1H, d, J = 2.2 Hz, Ar), 7.13 (1H, d, J = 2.1 Hz, Ar), 7.19 – 7.28 (3H, m, Ar), 8.08 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 14.0 (q), 24.3 (t), 25.1 (t), 25.7 (t), 49.1 (t), 55.3 (t), 61.2 (q), 114.0 (2 x d), 114.2 (s), 115.0 (s), 116.9 (s), 120.8 (s), 122.5 (s), 122.7 (s), 126.1 (2 x d), 127.0 (d), 128.4 (2 x d), 132.2 (2 x d), 135.4 (s), 138.5 (d), 159.8 (s), 160.3 (s), 162.4 (s). Anal. Calcd. for $C_{27}H_{26}N_2O_4$: C, 73.28; H, 5.92; N, 6.33. Found: C, 73.68; H, 5.52; N, 6.73.

**Data for ethyl 8-(4-methoxybenzyl)-7-(4-methoxyphenyl)-4,5,6,8-tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-d]isoxazole-9-carboxylate (58b).** This compound was obtained from reaction of compound **57b**. White solid; yield 60%; mp 131-132°C; $^{1}$H NMR ($CDCl_3$) (ppm): 1.26 (3H, t, J = 7.12 Hz, $CH_3$), 1.89 – 2.10 (2H, m, $CH_2$), 2.54 – 2.59 (2H, m, $CH_2$), 2.77 (2H, t, J = 6.28 Hz, $CH_2$), 3.71 (3H, s, $CH_3$), 3.82 (3H, s, $CH_3$), 4.18 – 4.35 (2H, m, $CH_2$), 5.24 (2H, s, $CH_2$), 6.71 – 6.81 (4H, m, Ar), 6.88 – 6.93 (2H, m, Ar), 7.07 – 7.13 (2H, m, Ar), 8.06 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 14.1 (q), 24.3 (t), 25.1 (t), 25.7 (t), 48.5 (t), 55.2 (q), 55.3 (q), 65.08 (t), 113.8 (2 x d), 113.9 (2 x d), 114.1 (s), 114.9 (s), 120.7 (s), 122.5 (s), 122.8 (s), 127.5 (2 x d), 128.7 (s), 130.6 (s), 132.2 (2 x d), 135.3 (s), 151.9 (d), 158.6 (s), 159.7 (s), 162.6 (s). Anal. Calcd. for $C_{28}H_{28}N_2O_5$: C, 71.17; H, 5.97; N, 5.93. Found: C, 70.77; H, 6.37; N, 6.33.

**Data for ethyl 8-(3-methoxybenzyl)-7-(4-methoxyphenyl)-4,5,6,8-tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-d]isoxazole-9-carboxylate (58c).** This compound was obtained from reaction of compound **57c**. Yellow solid; yield 75%; mp 106-107°C; $^{1}$H NMR ($CDCl_3$) (ppm): 1.21 – 1.29 (3H, m, $CH_3$), 1.90 – 2.10 (2H, m, $CH_2$), 2.54 – 2.59 (2H, m, $CH_2$), 2.78 (2H, t, J = 6.3 Hz, $CH_2$), 3.71 (3H, s, $CH_3$), 3.82 (3H, s, $CH_3$), 4.07 – 4.34 (2H, m, $CH_2$), 5.29 (2H, s, $CH_2$), 6.44 (2H, d, J = 7.6 Hz, Ar), 6.69 – 6.74 (1H, m, Ar),

6.87 – 6.94 (2H, m, Ar), 7.08 – 7.17 (3H, m, Ar), 8.06 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 14.1 (q), 24.3 (t), 25.1 (t), 25.7 (t), 49.1 (t), 55.1 (q), 55.3 (q), 61.2 (t), 111.8 (d), 112.5 (d), 114.0 (2 x d), 114.2 (s), 115.1 (s), 118.4 (d), 120.80 (s), 122.6 (s), 122.7 (s), 129.5 (d), 132.2 (2 x d), 135.4 (s), 140.1 (s), 151.9 (d), 159.2 (s), 160.4 (s), 160.5 (s), 162.5 (s). Anal. Calcd. for $C_{28}H_{28}N_2O_5$: C, 71.17; H, 5.97; N, 5.93. Found: C, 71.57; H, 6.37; N, 6.33.

**Data for ethyl 8-(2-methoxybenzyl)-7-(4-methoxyphenyl)-4,5,6,8-tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-d]isoxazole-9-carboxylate (58d).** This compound was obtained from reaction of compound **57d**. Yellow oil; yield 83%; $^1$H NMR ($CDCl_3$) (ppm): 1.17 – 1.28 (3H, m, $CH_3$), 1.94 – 2.09 (2H, m, $CH_2$), 2.50 – 2.64 (2H, m, $CH_2$), 2.71 – 2.82 (2H, m, $CH_2$), 3.72 (3H, s, $CH_3$), 3.80 (3H, s, $CH_3$), 4.20 – 4.30 (2H, m, $CH_2$), 5.29 (2H, s, $CH_2$), 6.74 – 6.88 (4H, m, Ar), 6.97 – 7.20 (4H, m, Ar), 8.07 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 13.9 (q), 24.3 (t), 25.2 (t), 25.7 (t), 44.9 (t), 55.2 (q), 55.3 (q), 61.1 (t), 109.7 (d), 113.8 (2 x d), 114.1 (s), 114.7 (s), 120.6 (d), 121.0 (s), 122.4 (s), 122.8 (s), 126.2 (d), 127.2 (s), 127.9 (d), 132.0 (2 x d), 135.4 (s), 151.9 (d), 155.8 (s), 159.6 (s), 160.5 (s), 162.2 (s). Anal. Calcd. for $C_{28}H_{28}N_2O_5$: C, 71.17; H, 5.97; N, 5.93. Found: C, 70.77; H, 5.57; N, 6.33.

**Data for 8-(4-methoxybenzyl)-7-(4-methoxyphenyl)-4,5,6,8-tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-d]isoxazole (58e).** This compound was obtained from reaction of compound **57e**. Yellow oil; yield 70%; $^1$H NMR ($CDCl_3$) (ppm): 1.84 – 1.95 (2H, m, $CH_2$), 2.72 (4H, t, J = 5.5 Hz, 2 x $CH_2$), 3.78 (3H, s, $CH_3$), 3.84 (3H, s, $CH_3$), 4.88 (2H, s, $CH_2$), 6.8 (2H, d, J = 8.7 Hz, Ar), 6.92 (4H, d, J = 8.5 Hz, Ar), 7.12 – 7.26 (3H, m, Ar), 7.99 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 24.4 (t), 24.6 (t), 27.2 (t), 50.5 (t), 55.3 (2 x q), 111.0 (s), 111.7 (s), 113.8 (2 x d), 114.0 (2 x d), 118.6 (d), 119.6 (s), 123.8 (s), 128.4 (2 x d), 129.8 (s), 131.3 (s), 132.1 (2 x d), 151.7 (d), 159.0 (s), 159.2 (s), 162.2 (s). Anal. Calcd. for $C_{25}H_{24}N_2O_3$: C, 74.98; H, 6.04; N, 7.00. Found: C, 75.38; H, 5.64; N, 6.6.

**Data for 8-(3-methoxybenzyl)-7-(4-methoxyphenyl)-4,5,6,8-tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-d]isoxazole (58f).** This compound was obtained from reaction of compound **57f**. Yellow oil; yield 44%; $^1$H NMR ($CDCl_3$) (ppm): 1.89 – 2.07 (2H, m, $CH_2$), 2.73 – 2.76 (4H, m, 2 x $CH_2$), 3.76 (3H, s, $CH_3$), 3.85 (3H, s, $CH_3$), 4.96 (2H, s, $CH_2$), 6.53 (1H, s, Ar), 6.61 (1H, d, J = 7.6 Hz, Ar), 6.78 – 6.81 (1H, m, Ar), 6.93 (2H, d, J = 8.7 Hz, Ar), 7.14 – 7.23 (4H, m, Ar), 8.01 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 21.1 (t), 23.5 (t), 27.6 (t), 43.2 (t), 55.2 (q), 55.3 (q), 92.23 (s), 105.8 (s), 112.8 (s), 113.1 (d), 113.8 (d), 114.0 (2 x d), 114.5 (d), 121.5 (d), 127.4 (d), 127.6 (d), 129.2 (s), 129.7 (s), 132.0 (d),

139.5 (s), 150.9 (s), 159.4 (s), 159.9 (s). Anal. Calcd. for $C_{25}H_{24}N_2O_3$: C, 74.98; H, 6.04; N, 7.00. Found: C, 75.38; H, 5.64; N, 7.4.

**Data for 8-(2-methoxybenzyl)-7-(4-methoxyphenyl)-4,5,6,8-tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-d]isoxazole (58g).** This compound was obtained from reaction of compound **57g**. Yellow oil; yield 40%; $^1$H NMR ($CDCl_3$) (ppm): 1.90 – 1.97 (2H, m, $CH_2$), 2.74 – 2.77 (4H, m, 2 x $CH_2$), 3.80 (3H, s, $CH_3$), 3.82 (3H, s, $CH_3$), 4.98 (2H, s, $CH_2$), 6.72 (1H, d, J = 7.3 Hz, Ar), 6.80 – 6.97 (4H, m, Ar), 7.17 – 7.28 (4H, m, Ar), 8.01 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 21.4 (t), 23.5 (t), 27.5 (t), 38.1 (t), 55.4 (q), 55.7 (q), 91.9 (s), 110.1 (s), 110.8 (d), 113.7 (d), 114.2 (2 x d), 118.3 (s), 119.1 (s), 120.6 (s), 121.8 (s), 127.4 (d), 128.3 (s), 128.8 (d), 129.1 (d), 129.3 (d), 132.0 (d), 132.6 (s), 150.6 (s). Anal. Calcd. for $C_{25}H_{24}N_2O_3$: C, 74.98; H, 6.04; N, 7.00. Found: C, 75.38; H, 5.64; N, 6.6.

**Data for 8-(3,4,5-trimethoxybenzyl)-7-(4-methoxyphenyl)-4,5,6,8-tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-d]isoxazole (58h).** This compound was obtained from reaction of compound **57h**. Yellow oil; yield 50%; $^1$H NMR ($CDCl_3$) (ppm): 1.89 (2H, s, $CH_2$), 2.72 (2H, s, $CH_2$), 2.84 (2H, s, $CH_2$), 3.69 (3H, s, $CH_3$), 3.79 (3H, s, $CH_3$), 3.84 (3H, s, $CH_3$), 3.89 (3H, s, $CH_3$), 4.82 (2H, s, $CH_2$), 6.16 (3H, d, J = 14.0 Hz, Ar), 6.86 (2H, d, J = 8.3 Hz, Ar), 7.04 (2H, m, Ar), 8.00 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 24.5 (t), 27.2 (t), 31.4 (t), 48.9 (t), 55.2 (q), 55.9 (2 x q), 60.8 (q), 104.8 (d), 107.5 (s), 113.8 (2 x d), 118.7 (s), 119.8 (s), 123.3 (s), 123.8 (s), 131.4 (s), 131.7 (2x d), 131.9 (s), 136.1 (d), 141.7 (s), 152.3 (d), 152.4 (s), 153.1 (2 x d), 159.2 (s). Anal. Calcd. for $C_{27}H_{28}N_2O_5$: C, 70.42; H, 6.13; N, 6.08. Found: C, 70.82; H, 5.73; N, 6.48.

**Data for 8-benzyl-7-phenyl-4,5,6,8-tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-d]isoxazole (58i).** This compound was obtained from reaction of compound **57i**. White solid; yield 73%; mp: 107 – 108°C; $^1$H NMR ($CDCl_3$) (ppm): 2.02 (2H, s, $CH_2$), 2.86 (4H, s, 2 x $CH_2$), 5.11 (2H, s, $CH_2$), 7.10 – 7.49 (11H, m, Ar), 8.12 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 24.5 (t), 24.7 (t), 27.3 (t), 51.2 (t), 111.2 (s), 112.1 (s), 119.3 (d), 120.0 (s), 127.0 (2 x d), 127.7 (d), 127.9 (d), 128.5 (2 x d), 128.8 (2 x d), 130.9 (2 x d), 131.5 (s), 131.8 (s), 137.9 (s), 151.9 (d), 162.1 (s). Anal. Calcd. for $C_{23}H_{20}N_2O$: C, 81.15; H, 5.92; N, 8.23. Found: C, 80.75; H, 5.52; N, 8.63.

**Data for 8-(4-methoxybenzyl)-7-phenyl-4,5,6,8-tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-d]isoxazole (58j).** This compound was obtained from reaction of compound **57j**. White solid; yield 83%; mp: 194 – 195°C; $^1$H NMR ($CDCl_3$) (ppm): 1.85 – 1.96 (2H, m, $CH_2$), 2.70 – 2.77 (4H, m, 2 x $CH_2$), 3.77 (3H, s, $CH_3$), 4.91 (2H, s, $CH_2$), 6.77 – 6.94 (4H, m, Ar), 7.16 – 7.41 (6H, m, Ar), 7.99 (1H, s, H-3); $^{13}$C NMR

($CDCl_3$) (ppm): 24.4 (t), 24.6 (t), 27.2 (t), 50.6 (t), 55.3 (q), 111.1 (s), 111.9 (s), 114.0 (2 x d), 119.0 (d), 119.9 (s), 127.8 (2 x d), 128.4 (2 x d), 129.7 (d), 130.8 (2 x d), 131.5 (s), 151.8 (d), 153.1 (s), 156.4159.0 (s), 162.1 (s. Anal. Calcd. for $C_{24}H_{22}N_2O_2$: C, 77.81; H, 5.99; N, 7.56. Found: C, 78.21; H, 5.59; N, 7.96.

**Data for 8-(3-methoxybenzyl)-7-phenyl-4,5,6,8-tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-d]isoxazole (58k).** This compound was obtained from reaction of compound **57k**. White solid; yield 68%; mp: 125 – 126°C; $^1$H NMR ($CDCl_3$) (ppm): 1.86 – 1.98 (2H, m, $CH_2$), 2.70 – 2.78 (4H, m, 2 x $CH_2$), 3.73 (3H, s, $CH_3$), 4.96 (2H, s, $CH_2$), 6.50 – 6.61 (2H, m, Ar), 6.75 – 6.80 (1H, m, Ar), 7.15 – 7.26 (4H, m, Ar), 7.30 – 7.44 (3H, m, Ar), 8.00 (1,s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 24.4 (t), 24.6 (t), 27.2 (t), 51.0 (t), 55.2 (q), 111.2 (s), 112.1 (s), 112.6 (d), 112.8 (d), 119.2 (d), 119.3 (d), 119.9 (s), 127.8 (d), 128.4 (2 x d), 129.8 (d), 130.8 (2 x d), 131.4 (s), 131.7 (s), 139.4 (s), 151.8 (d), 159.8 (s), 162.1 (s). Anal. Calcd. for $C_{24}H_{22}N_2O_2$: C, 77.81; H, 5.99; N, 7.56. Found: C, 78.21; H, 6.39; N, 7.96.

**Data for 8-(2-methoxybenzyl)-7-phenyl-4,5,6,8-tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-d]isoxazole (58l).** This compound was obtained from reaction of compound **57l**. White solid; yield 78%; mp: 114 – 115°C; $^1$H NMR ($CDCl_3$) (ppm): 1.85 – 1.96 (2H, m, $CH_2$), 2.69 – 2.78 (4H, m, 2 x $CH_2$), 3.76 (3H, s, $CH_3$), 4.99 (2H, s, $CH_2$), 6.69 – 6.87 (3H, m, Ar), 7.19 – 7.39 (7H, m, Ar), 7.98 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 24.4 (t), 24.6 (t), 27.3 (t), 46.2 (t), 55.3 (q), 110.1 (d), 11111.0 (s), 111.7 (s), 119.5 (d), 119.6 (s), 120.6 (d), 126.2 (s), 127.6 (d), 128.3 (d), 128.4 (2 x d), 128.8 (d), 130.8 (2 x d), 131.6 (s), 131.7 (s), 151.7 (d), 156.5 (s), 162.3 (s). Anal. Calcd. for $C_{24}H_{22}N_2O_2$: C, 77.81; H, 5.99; N, 7.56. Found: C, 77.41; H, 6.39; N, 7.96.

**Data for 8-(4-methoxy-3-nitrobenzyl)-7-phenyl-4,5,6,8-tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-d]isoxazole (58m).** This compound was obtained from reaction of compound **57m**. White solid; yield 40%; mp: 173 – 174°C IR ($cm^{-1}$): 1533 ($NO_2$); $^1$H NMR ($CDCl_3$) (ppm): 1.93 (2H, t, J= 5.6 Hz, $CH_2$), 2.75 (4H, m, 2 x $CH_2$), 3.94 (3H, s, $CH_3$), 4.99 (2H, s, $CH_2$), 6.99 (1H, d, J= 8.7 Hz, Ar), 7.11 – 7.22 (4H, m, Ar), 7.37 – 7.42 (4H, m, Ar), 8.03 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 24.2 (t), 24.6 (t), 27.1 (t), 49.9 (t), 56.6 (q), 111.5 (s), 112.5 (s), 113.8 (d), 113.8 (s), 118.9 (d), 120.5 (s), 124.3 (d), 124.5 (s), 128.1 (d), 128.4 (s), 128.6 (2 x d), 128.7 (s), 130.1 (s), 130.7 (2 x d), 131.1 (s), 132.6 (d), 151.8 (d). Anal. Calcd. for $C_{24}H_{21}N_3O_4$: C, 69.39; H, 5.10; N, 10.11. Found: C, 68.99; H, 5.5; N, 10.51.

**Data for 8-(4-methoxy-3-aminobenzyl)-7-phenyl-4,5,6,8-tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-d]isoxazole (58n).** This compound was obtained from reaction of compound **57n**. Yellow oil; yield 51%; ($cm^{-1}$): 3441−3354 ($NH_2$); $^1$H NMR ($CDCl_3$) (ppm): 1.89 – 1.96 (2H, m, $CH_2$), 2.72 – 2.79 (4H, m, 2 x $CH_2$), 3.83 (3H, s, $CH_3$), 4.86 (2H, s, $CH_2$), 5.23 (2H, s, $NH_2$), 6.39 (2H, d, J= 6.3 Hz, Ar), 6.68 (1H, d, J= 8.7 Hz, Ar), 7.19 – 7.45 (6H, m, Ar), 8.02 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 24.4 (t), 24.6 (t), 27.2 (t), 50.8 (t), 55.5 (q), 110.3 (d), 111.0 (s), 111.1 (s), 111.7 (d), 113.7 (d), 113.8 (d), 119.7 (s), 127.7 (d), 128.3 (2 x d), 130.3 (s), 130.8 (2 x d), 131.6 (s), 136.2 (s), 146.8 (s), 151.7 (d), 162.2 (s), 176.4 (s). Anal. Calcd. for $C_{24}H_{23}N_3O_2$: C, 74.78; H, 6.01; N, 10.90. Found: C, 74.38; H, 6.41; N, 10.5.

**Data for 8-(3,4-dimethoxybenzyl)-7-phenyl-4,5,6,8-tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-d]isoxazole (58o).** This compound was obtained from reaction of compound **57o**. White solid; yield 80%; mp 114 – 115°C; $^1$H NMR ($CDCl_3$) (ppm): 1.84 – 1.90 (4H, m, 2 x $CH_2$), 2.69 – 2.71 (4H, m, 2 x $CH_2$), 3.76 (3H, s, $CH_3$), 3.86 (3H, s, $CH_3$), 4.90 (2H, s, $CH_2$), 6.41 (1H, s, Ar), 6.56 (1H, d, J= 7.8 Hz, Ar), 6.76 (1H, d, J= 7.8 Hz, Ar), 7.28 (1H, s, Ar), 7.41 – 7.43 (6H, m, Ar); $^{13}$C NMR ($CDCl_3$) (ppm): 22.4 (t), 24.2 (t), 26.2 (t), 30.1 (s), 41.9 (t), 51.4 (q), 55.9 (q), 110.3 (d), 111.2 (d), 119.9 (d), 123.0 (s), 124.9 (s), 125.2 (d), 127.9 (d), 128.4 (2 x d), 129.3 (d), 131.0 (2 x d), 131.4 (s), 131.5 (s), 148.6 (s), 149.0 (s), 199.5 (s), 207.5 (s). Anal. Calcd. for $C_{25}H_{24}N_2O_3$: C, 74.98; H, 6.04; N, 7.00. Found: C, 75.38; H, 5.64; N, 7.4.

**Data for 8-(3,4,5-trimethoxybenzyl)-7-phenyl-4,5,6,8-tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-d]isoxazole (58p).** This compound was obtained from reaction of compound **57p**. White solid; yield 49%; mp 155 – 156°C; $^1$H NMR ($CDCl_3$) (ppm): 1.91 – 1.96 (2H, m, $CH_2$), 2.73 – 2.78 (4H, m, 2 x $CH_2$), 3.76 (6H, d, J = 5.0 Hz, 2 x $CH_3$), 3.88 (3H, d, J = 5.0 Hz, $CH_3$), 4.93 (2H, s, $CH_2$), 6.18 (2H, s, Ar), 7.25 – 7.28 (3H, m, Ar), 7.38 – 7.45 (3H, m, Ar), 8.02 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 24.4 (t), 24.6 (t), 27.2 (t), 51.5 (t), 56.0 (2 x q), 60.9 (q), 104.2 (2 x d), 111.2 (s), 112.0 (s), 119.2 (d), 120.2 (s), 127.8 (d), 128.4 (2 x d), 130.8 (2 x d), 131.5 (s), 131.6 (s), 133.2 (s), 137.3 (s),153.3 (2 x s), 151.8 (d), 162.0 (s). Anal. Calcd. for $C_{26}H_{26}N_2O_4$: C, 72.54; H, 6.09; N, 6.51. Found: C, 72.14; H, 6.49; N, 6.11.

**Data for 8-(2,5-dimethoxybenzyl)-7-phenyl-4,5,6,8-tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-d]isoxazole (58q).** This compound was obtained from reaction of compound **57q**. White solid; yield 80%; mp 174 – 175°C; $^1$H NMR ($CDCl_3$) (ppm): 2.03 (2H, s, $CH_2$), 2.85 (4H, s, 2 x $CH_2$), 3.79 (3H, s, $CH_3$), 3.88 (3H, s,

$CH_3$), 5.10 (2H, s, $CH_2$), 6.42 (1H, s, Ar), 6.86 (2H, s, Ar), 7.33 – 7.49 (6H, m, Ar), 8.12 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 24.5 (t), 24.7 (t), 27.3 (t), 46.2 (t), 55.7 (q), 55.9 (q), 111.2 (d), 112.8 (d), 114.2 (s), 114.9 (d), 119.6 (d), 119.8 (s), 127.4 (s), 127.7 (d), 128.4 (2 x d), 130.8 (2 x d), 131.7 (s), 144.6 (s), 150.9 (s), 151.8 (s), 153.5 (d), 153.7 (s), 162.3 (s). Anal. Calcd. for $C_{25}H_{24}N_2O_3$: C, 74.98; H, 6.04; N, 7.00. Found: C, 75.38; H, 5.64; N, 6.6.

**Data for 8-benzyl-7-(3,4,5-trimethoxyphenyl)-4,5,6,8-tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-d]isoxazole (58r).** This compound was obtained from reaction of compound **57r**. Yield 46%; White solid; mp 122 – 123°C; $^1$H NMR ($CDCl_3$) (ppm): 1.96 – 1.99 (2H, m, $CH_2$), 2.75 – 2.82 (4H, m, 2 x $CH_2$), 3.69 (6H, s, 2 x $CH_3$), 3.89 (3H, s, $CH_3$), 5.02 (2H, s, $CH_2$), 6.36 (2H, s, H-2'' and H-6''), 7.03 – 7.06 (2H, m, Ar), 7.25 – 7.33 (4H, m, Ar), 8.02 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 24.4 (t), 24.6 (t), 27.3 (t), 29.7 (s), 51.2 (t), 55.9 (2 x q), 60.9 (q), 107.9 (2 x d), 111.2 (s), 111.9 (s), 115.0 (s), 118.0 (s), 119.3 (s), 119.8 (s), 126.5 (2 x d), 127.5 (d), 128.7 (2 x d), 137.7 (s), 138.3 (s), 151.7 (d), 152.9 (d), 162.0 (s). Anal. Calcd. for $C_{26}H_{26}N_2O_4$: C, 72.54; H, 6.09; N, 6.51. Found: C, 75.14; H, 6.49; N, 6.11.

**Data for 8-(4-methoxybenzyl)-7-(3,4,5-trimethoxyphenyl)-4,5,6,8-tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-d]isoxazole (58s).** This compound was obtained from reaction of compound **57s**. Yellow oil; yield 51%; $^1$H NMR ($CDCl_3$) (ppm): 1.93 – 1.98 (2H, m, $CH_2$), 2.74 – 2.80 (4H, m, 2 x $CH_2$), 3.74 (6H, s, 2 x $CH_3$), 3.79 (3H, s, $CH_3$), 3.90 (3H, s, $CH_3$), 4.94 (2H, s, $CH_2$), 6.39 (2H, s, H-2'' and H-6''), 6.82 – 6.84 (2H, m, Ar), 6.96 (2H, d, J = 8.8 Hz, Ar), 7.21 (1H, s, H-8), 8.01 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 24.4 (t), 24.6 (t), 27.3 (t), 50.8 (t), 55.3 (q), 56.0 (2 x q), 60.9 (q), 108.0 (2 x d), 111.1 (s), 111.8 (s), 113.3 (s), 114.1 (2 x d), 119.1 (d), 119.7 (s), 126.9 (s), 128.1 (2 x d), 130.1 (s), 131.5 (s), 137.7 (s), 151.7 (s), 152.9 (d), 159.1 (2 x s). Anal. Calcd. for $C_{27}H_{28}N_2O_5$: C, 70.42; H, 6.13; N, 6.08. Found: C, 70.02; H, 6.53; N, 6.48.

**Data for 8-(3-methoxybenzyl)-7-(3,4,5-trimethoxyphenyl)-4,5,6,8-tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-d]isoxazole (58t).** This compound was obtained from reaction of compound **57t**. Yellow oil; yield 49%; $^1$H NMR ($CDCl_3$) (ppm): 1.92 – 1.98 (2H, m, $CH_2$), 2.74 – 2.81 (4H, m, 2 x $CH_2$), 3.78 (6H, s, 2 x $CH_3$), 3.76 (3H, s, $CH_3$), 3.89 (3H, s, $CH_3$), 4.98 (2H, s, $CH_2$), 6.38 (2H, s, H-2'' and H-6''), 6.57 (1H, d, J = 1.8 Hz, Ar), 6.64 (1H, dd, J = 7.6, 0.6 Hz, Ar), 6.79 (1H, dd, J = 8.0, 2.4 Hz, Ar), 7.20 – 7.24 (2H, m, Ar), 8.01 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 24.4 (t), 24.6 (t), 27.3 (t), 51.2 (t), 55.2 (q), 55.9 (2 x q), 60.9 (q), 107.9 (2 x d), 111.2 (s), 111.9 (s), 112.5 (d), 112.7 (d), 118.9 (d), 119.4 (d), 119.7 (s), 126.7 (s), 129.8 (s), 129.9 (d), 131.6 (s), 137.7 (s), 139.9 (s), 151.7 (s), 152.9 (d),

160.0 (s), 162.0 (s). Anal. Calcd. for $C_{27}H_{28}N_2O_5$: C, 70.42; H, 6.13; N, 6.08. Found: C, 70.82; H, 6.53; N, 6.48.

**Data for 8-(2-methoxybenzyl)-7-(3,4,5-trimethoxyphenyl)-4,5,6,8-tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-d]isoxazole (58u).** This compound was obtained from reaction of compound **57u**. Yellow oil; yield 60%; $^1$H NMR ($CDCl_3$) (ppm): 1.94 – 2.00 (2H, m, $CH_2$), 2.74 – 2.84 (4H, m, 2 x $CH_2$), 3.69 (6H, s, 2 x $CH_3$), 3.80 (3H, s, $CH_3$), 3.89 (3H, s, $CH_3$), 5.01 (2H, s, $CH_2$), 6.41 (2H, s, H-2'' and H-6''), 6.78 – 6.89 (3H, m, Ar), 7.22 – 7.27 (2H, m, Ar), 8.02 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 24.4 (t), 24.6 (t), 27.4 (t), 29.7 (t), 46.5 (s), 55.3 (q), 55.9 (2 x q), 60.9 (q), 108.0 (2 x d), 110.1 (d), 111.0 (s), 111.7 (s), 119.5 (s), 119.6 (d), 120.6 (d), 126.6 (s), 126.9 (s), 127.9 (d), 128.7 (d), 131.6 (s), 137.5 (s), 151.7 (2 x s), 152.9 (d), 156.3 (s), 162.2 (s). Anal. Calcd. for $C_{27}H_{28}N_2O_5$: C, 70.42; H, 6.13; N, 6.08. Found: C, 70.82; H, 6.53; N, 5.68.

**Data for 8-(3,4,5-trimethoxybenzyl)-7-(3,4,5-trimethoxyphenyl)-4,5,6,8-tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-d]isoxazole (58v).** This compound was obtained from reaction of compound **57v**. Yellow oil; yield 71%; $^1$H NMR ($CDCl_3$) (ppm): 1.88 – 1.96 (2H, m, $CH_2$), 2.73 – 2.78 (4H, m, 2 x $CH_2$), 3.70 (6H, d, J = 5.0 Hz, 2 x $CH_3$), 3.75 (3H, s, $CH_3$), 3.79 (3H, s, $CH_3$), 3.88 (6H, d, J = 3.6 Hz, 2 x $CH_3$), 4.86 (2H, s, $CH_2$), 6.18 (2H, s, H-2' and H-6'), 6.36 (2H, s, H-2'' and H-6''), 7.07 (1H, s, H-8), 8.01 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 24.4 (t), 24.6 (t), 27.3 (t), 31.5 (t), 49.1 (q), 53.3 (q), 56.0 (2 x q), 56.0 (2 x q), 104.9 (2 x d), 107.6 (s), 107.7 (2 x d), 111.2 (s), 111.9 (s), 119.0 (s), 119.9 (s), 124.0 (s), 126.6 (s), 131.9 (s), 141.8 (s), 151.8 (2 x s), 152.4 (2 x s), 153.0 (d), 153.2 (d). Anal. Calcd. for $C_{29}H_{32}N_2O_7$: C, 66.91; H, 6.20; N, 5.38. Found: C, 66.51; H, 6.60; N, 5.78.

**Data for 8-(2,5-dimethoxybenzyl)-7-(3,4,5-trimethoxyphenyl)-4,5,6,8-tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-d]isoxazole (58w).** This compound was obtained from reaction of compound **57w**. Yellow oil; yield 77%; $^1$H NMR ($CDCl_3$) (ppm): 1.94 – 1.99 (2H, m, $CH_2$), 2.74 – 2.83 (4H, m, 2 x $CH_2$), 3.68 (3H, s, $CH_3$), 3.72 (6H, s, 2 x $CH_3$), 3.75 (3H, s, $CH_3$), 3.89 (3H, s, $CH_3$), 4.98 (2H, s, $CH_2$), 6.37 (1H, s, Ar), 6.43 (2H, s, H-2'' and H-6''), 6.72 – 6.79 (2H, m, Ar), 7.23 (1H, s, H-8), 8.01 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 24.4 (t), 24.6 (t), 27.4 (t), 46.4 (t), 55.7 (q), 55.8 (q), 55.9 (2 x q), 60.9 (q), 107.8 (2 x d), 111.0 (d), 111.7 (s), 112.4 (d), 114.8 (d), 119.5 (s), 119.6 (s), 126.9 (s), 127.7 (s), 127.7 (s), 131.5 (s), 137.5 (s), 150.6 (s), 151.7 (d), 152.9 (d), 153.7 (2 x s), 162.2 (s). Anal. Calcd. for $C_{28}H_{30}N_2O_6$: C, 68.56; H, 6.16; N, 5.71. Found: C, 68.16; H, 6.56; N, 5.31.

**Data for 8-(3,4-dimethoxybenzyl)-7-(3,4,5-trimethoxyphenyl)-4,5,6,8-tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-d]isoxazole (58x).** This compound was obtained

from reaction of compound **57x**. Yellow oil; yield 51%; $^{1}$H NMR ($CDCl_3$) (ppm): 1.94 – 1.99 (2H, m, $CH_2$), 2.74 – 2.83 (4H, m, 2 x $CH_2$), 3.69 (3H, s, $CH_3$), 3.72 (6H, s, 2 x $CH_3$), 3.76 (3H, s, $CH_3$), 3.89 (3H, s, $CH_3$), 4.98 (2H, s, $CH_2$), 6.37 (1H, s, Ar), 6.43 (2H, s, H-2'' and H-6''), 6.76 (2H, s, Ar), 7.24 (1H, s, H-8), 8.02 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 24.4 (t), 24.6 (t), 27.4 (t), 30.1 (s), 46.4 (t), 53.4 (s), 55.7 (q), 55.8 (q), 55.9 (2 x q), 60.9 (q), 107.8 (2 x d), 111.0 (d), 111.7 (s), 112.4 (d), 114.8 (d), 119.6 (s), 119.7 (s), 126.9 (s), 127.7 (s), 131.5 (s), 137.5 (s), 150.6 (s), 151.7 (d), 152.9 (d), 153.7 (2 x s), 162.2 (s). Anal. Calcd. for $C_{28}H_{30}N_2O_6$: C, 68.56; H, 6.16; N, 5.71. Found: C, 68.16; H, 6.56; N, 5.31.

**Data for 8-(4-methoxy-3-nitrobenzyl)-7-(3,4,5-trimethoxyphenyl)-4,5,6,8-tetrahydropyrrolo[3',4':3,4]cyclohepta[1,2-d]isoxazole (58y).** This compound was obtained from reaction of compound **57y**. Yellow oil; yield 47%; IR ($cm^{-1}$): 1532 ($NO_2$); $^{1}$H NMR ($CDCl_3$) (ppm): 1.93 – 1.98 (2H, m, $CH_2$), 2.73 – 2.78 (4H, m, 2 x $CH_2$), 3.79 (6H, s, 2 x $CH_3$), 3.90 (3H, s, $CH_3$), 3.93 (3H, s, $CH_3$), 5.00 (2H, s, $CH_2$), 6.40 (2H, s, H-2'' and H-6''), 7.02 (1H, s, Ar), 7.18 – 7.22 (2H, m, Ar), 7.41 (1H, d, J = 2.1 Hz, Ar), 8.02 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$) (ppm): 24.3 (t), 24.5 (t), 27.2 (t), 30.1 (s), 50.0 (t), 56.1 (2 x q), 56.7 (q), 60.9 (q), 107.91 (2 x d), 111.51 (d), 112.48 (s), 113.75 (d), 118.67 (s), 120.40 (s), 124.18 (d), 126.50 (s), 130.4 (s), 131.5 (s), 132.5 (d), 138.0 (s), 139.6 (s), 151.8 (s), 152.3 (2 x s), 153.2 (d), 161.7 (s). Anal. Calcd. for $C_{27}H_{27}N_3O_7$: C, 64.15; H, 5.38; N, 8.31. Found: C, 64.55; H, 5.78; N, 8.71.

**Data for 2-methoxy-5-((7-(3,4,5-trimethoxyphenyl)-5,6-dihydropyrrolo[3',4':3,4]cyclohepta[1,2-d]isoxazol-8(4H)-yl)methyl)aniline (58z).** This compound was obtained from reaction of compound **57z**. Yellow oil; yield 60%; IR ($cm^{-1}$): 3463−3381 ($NH_2$); $^{1}$H NMR ($CDCl_3$) (ppm): 1.94 (2H, d, J = 4.5 Hz, $CH_2$), 2.71 – 2.81 (4H, m, 2 x $CH_2$), 3.73 – 3.78 (6H, m, 3 x $CH_3$), 3.85 (3H, d, J = 6.5 Hz, $CH_3$), 3.89 (3H, s, $CH_3$), 4.94 (2H, s, $CH_2$), 6.44 (2H, d, J = 24.4 Hz, Ar), 6.72 – 6.83 (2H, m, $NH_2$), 7.12 (1H, s, H-8), 7.89 (1H, s, Ar), 8.00 (1H, d, J = 6.5 Hz, Ar), 8.17 (1H, s, Ar), 8.41 (1H, s, H-8); $^{13}$C NMR ($CDCl_3$) (ppm): 24.4 (t), 24.6 (t), 27.3 (t), 38.6 (t), 55.9 (q), 56.1 (2 x q), 60.9 (q), 108.0 (2 x d), 110.1 (d), 111.1 (s), 111.9 (s), 118.9 (s), 119.2 (d), 119.6 (s), 127.7 (d), 126.8 (s), 127.0 (s), 130.6 (s), 131.7 (s), 147.2 (s), 151.7 (2 x s), 153.0 (d), 158.8 (d), 162.1 (s). Anal. Calcd. for $C_{27}H_{29}N_3O_5$: C, 68.19; H, 6.15; N, 8.84. Found: C, 68.59; H, 6.55; N, 8.44.

## 12.2 Biology

### 12.2.1 Cell lines

Lymphoma cell lines were cultured according to the recommended conditions, using RPMI-1640 medium, supplemented with 20% (v/v) fetal bovine serum (FBS), Penicillin-Streptomycin-Neomycin (~5,000 units penicillin, 5 mg streptomycin and 10 mg neomycin/mL, Sigma) and L-glutamine (1%). Cell lines identities were validated by CellCheck test (IDEXX, BioResearch) or with the Promega GenePrint 10 System kit and all the experiments were performed within one month from being thawed. Cells were periodically tested to confirm Mycoplasma negativity using the MycoAlert Mycoplasma Detection Kit (Lonza). Cells were incubated at 37°C with 5% $CO_2$ and were subcultured every three days.

### 12.2.2 Preparation of compounds for *in vitro* screening

All the compounds (solids or oils) were dissolved in dimethyl sulfoxide (DMSO) to obtain a stock concentration of 10 mM and were stored frozen at 4°C. For each experiment, fresh dilutions of compounds were made from the stock solutions in order to obtain the indicated concentrations. The DMSO concentration didn't exceed 0,1% in any experiment.

### 12.2.3 Cell proliferation analysis

For each screening experiment, cells were seeded in 96-well plates (non-tissue culture treated) at a density ranging from 5,000 to 10,000 cells/well depending on doubling time of individual cell lines, using the VIAFLO 96 hand-held electronic channel pipette (Integra Biosciences) or manually with 12-channel pipet. Cells were initially treated with a single dose of 1 μM for 72 h. Selected compounds, that reached proliferation inhibition about 60%, were further tested in the appropriate tissue culture medium with increasing doses of the molecules at concentrations ranging from 0 to 10 μM, using 1:2 dilution, in order to calculate the $IC_{50}$ values. In this case, assays were performed in triplicate. To 100 μL of cells suspended in medium, 100 μL of drug suspension were added, for a total seeding volume of 200 μL/well. After cells treatment, microplates were incubated at 37°C, 5% $CO_2$, 95% air and 100% relative humidity for 72h. Wells containing medium only were included on each plate and used as blanks for absorbance readings.

MTT (Sigma, Buchs) was prepared as a 5 mg/mL stock in phosphate-buffered saline (PBS) and filter-sterilized. At the end of the incubation period, 20 μL of MTT solution was added to

each well and microplates were incubated at 37°C for 4 hours. Cells were then lysed with 50 µL per well of 25% sodium dodecyl sulfate lysis buffer and absorbance was read at 570 nm using a Beckman Coulter-AD340 plate reader. The % of proliferation cells was obtained quantifying the linear relationship between alive cells and signal produced.

### 12.2.4 Cell cycle assay

MINO cells were treated with concentrations corresponding to the double of their $IC_{50}$ for 24 h. After the incubation period, $3\times10^6$ cells were harvested, washed twice and resuspended in 1 mL of PBS containing 1% fetal bovine serum (FBS). Cells were fixed with 4 mL of ice-cold 70% ethanol and after two washes with 9 mL of PBS+FBS, were treated with lysis buffer containing 50 µL of RNase A (10mg/mL), 425 µL of PBS+FBS and then stained with 25 µL of PI (1mg/mL). Samples were analyzed by using Fortessa flow cytometer.

### 12.2.5 Annexin V apoptosis assay

MINO cells were seeded in 6-well plates (Eppendorf) and were exposed to compounds with concentrations corresponding to the double of their $IC_{50}$ for 72 h. After the incubation period, cell suspension culture with cell density of $2–5x10^5$/mL was washed in PBS, resuspend in 200 µL of binding buffer (1x) and treated with Annexin V-FITC (5 µL to 195 µL cell suspension). After incubation for 10 min at room temperature, washing in 500 µL of binding buffer (1x) and resuspension in 190 µL of binding buffer (1x), 10 µL of propidium iodide (20 µg/mL) were added. FACS analysis was performed by Fortessa flow cytometer, measuring the surface exposure of phosphatidyl serine on apoptotic cells according to the manufacturer's instructions (Invitrogen eBioscience).

### 12.2.6 Western blot analysis

OCI-LY-19 and WSU-DLCL2 were seeded in 6-well plates (Eppendorf) and were exposed to OTX015 (500nM) and LRRK2-IN (2000 nM) both as single agents and in combination for 24h. After treatment, cells were resuspended, collected in a 15 mL Falcon tube and centrifuged at 1200 rpm for ~5 min at room temperature. Supernatant was exchanged with 1 mL of PBS and the resuspended pellet was transferred into 1.5 mL Eppendorf tubes. After centrifuging at 1200 rpm for 10 min at room temperature, supernatant was removed from the pellet and cells were solubilized in sodium dodecyl sulfate (SDS) lysis buffer. The protein

content was determined using the BCA protein assay (Pierce Chemical Co). Samples were stored at -80°C.

Before electrophoresis, the lysates were heated to 95 °C for five minutes in order to disrupt the secondary and tertiary structures of the protein and stretch the molecules. Equal amounts of proteins (10 μL) were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and bromophenol blue (7 μL) was used as marker. Since the use of SDS and polyacrylamide gel largely eliminates the influence of the structure and charge, proteins are separated solely based on polypeptide chain mass. The voltage (120 V) applied for separation caused an easy migration of small proteins through the mesh of the gel while larger proteins were more retained and migrated slowly. After sorting all proteins by size, they were transferred to a polyvinylidene difluoride (PVDF) membrane (Bio-Rad) by elettroblotting. To detect the uniformity of protein transfer and to avoid noise of signal, Ponceau S stain was used.

Membranes were blocked with a bovine serum albumin solution (5% in Tween PBS 1X) and were gently rotated for 1 hour at room temperature. Later on, they were incubated with primary antibodies anti-LRRK2 (Ab 133474) from Abcam, anti-AKT (CST 9272), anti-p-AKT (Ser 473) (CST 4060), anti-GSK3β (CST 9832), anti-GSK3β (Ser 9) (CST 9322) from Cell Signaling Technology or anti-a–glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (FF26A) from eBioscience overnight at 4°C, using 1:1000 diluition. Membranes were next incubated with the appropriate horseradish peroxidase–conjugated secondary mouse (NA931V) and rabbit (NA934V) antibodies from GE healthcare for 1 h, using 1:2000 diluition. All membranes were visualized using ECL Select (Westar Cyanagen) and images were acquired using BioID system. Equal loading of samples was confirmed by probing with GAPDH.

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