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Research paper

Identification of pyrrolo[3′,4’:3,4]cyclohepta[1,2-*d*][1,2]oxazoles as promising new candidates for the treatment of lymphomas

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ABSTRACT

Unsatisfactory outcomes for relapsed/refractory lymphoma patients prompt continuing efforts to develop new therapeutic strategies. Our previous studies on pyrrole-based anti-lymphoma agents led us to synthesize a new series of twenty-six pyrrolo[3′,4':3,4]cyclohepta[1,2-*d*] [1,2]oxazole derivatives and study their antiproliferative effects against a panel of four non-Hodgkin lymphoma cell lines. Several candidates showed significant antiproliferative effects, with IC_{50}'s reaching the sub-micromolar range in at least one cell line, with compound **3z** demonstrating sub-micromolar growth inhibitory effects towards the entire panel. The VL51 cell line was the most sensitive, with an IC_{50} value of 0.10 μM for **3z**.

Our earlier studies had shown that tubulin was a prominent target of many of our oxazole derivatives. We therefore examined their effects on tubulin assembly and colchicine binding. While **3u** and **3z** did not appear to target tubulin, good activity was observed with **3d** and **3p**.

Molecular docking and molecular dynamics simulations allowed us to rationalize the binding mode of the synthesized compounds toward tubulin. All ligands exhibited a better affinity for the colchicine site, confirming their specificity for this binding pocket. In particular, a better affinity and free energy of binding was observed for **3d** and **3p**. This result was confirmed by experimental data, indicating that, although both **3d** and **3p** significantly affected tubulin assembly, only **3d** showed activity comparable to that of combretastatin A-4, while **3p** was about 4-fold less active.

Cell cycle analysis showed that compounds **3u** and especially **3z** induced a block in G2/M, a strong decrease in S phase even at low compound concentrations and apoptosis through the mitochondrial pathway. Thus, the mechanism of action of **3u** and **3z** remains to be elucidated.

Very high selectivity toward cancer cells and low toxicity in human peripheral blood lymphocytes were observed, highlighting the good potential of these agents in cancer therapy and encouraging further exploration of this compound class to obtain new small molecules as effective lymphoma treatments.

1. Introduction

Lymphomas represent one of the most common hematological malignancies worldwide, affecting both children and adults. Lymphomas represent almost 5% of all cancers, with slow-growing forms not currently curable, and an annual incidence that has gradually increased in recent years [1,2]. Bone marrow/stem cell transplantation, radiation therapy and immunotherapy/targeted therapy, involving monoclonal

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antibodies, antibody drug conjugates or chimeric antigen receptor (CAR) T-cell therapy are among current treatment options. However, combination chemotherapy regimens, such as R–CHOP, ABVD or BEACOP, remain the cornerstone in the treatment of lymphomas [3,4], encouraging medicinal chemists to search for new agents with improved efficacy, tolerability, and specificity and that are not MDR substrates.

Pyrrole-based compounds have attracted much attention as bioactive molecules [5–12], and in this field, we have been involved in the synthesis of small molecules as anti-proliferative agents [13–17]. At the beginning of our studies, we identified a class of [1,2]oxazole isoindoles **1** (Chart 1) with potent activity in mesothelioma models. When these compounds were further explored, in terms of structure – activity relationships [18], we found that they had antitubulin activity and were active in refractory lymphoma models. In particular, micromolar – nanomolar IC_{50} values were obtained against four different lymphoma subtypes, and the compounds induced cell cycle arrest in the G2/M phase and caused apoptosis. These activities were confirmed by transcriptome analysis [18–20]. The insights gained regarding the chemical space led to the class of pyrrolo[2′,3':3,4]cyclohepta[1,2-*d*] [1,2]oxazoles **2** (Chart 1), which showed potent antitumor activity against the full NCI 60 cell line panel, with GI_{50} values at the nanomolar level and mean graph mid-points (MG_MID) of 0.08–0.41 μM. Moreover, they exhibited potent growth inhibitory activities against six lymphoma cell lines not included in the NCI panel, with IC_{50} values at the micromolar – submicromolar level. The most active compounds were able to induce cell cycle arrest in the G2/M phase, confirming the mechanism of the former class **1**, along with apoptosis, mitochondrial depolarization, ROS generation and PARP cleavage activation [21]. The promising results obtained in refractory lymphoma models encouraged further exploration of the tricyclic pyrrole oxazole system, considering that so far only a few classes of small molecules have been reported as effective lymphoma treatments [2]. In this study, we examined the new class of pyrrolo[3′,4':3,4]cyclohepta[1,2-*d*] [1,2]oxazoles **3**, positional isomers of class **2**, to determine whether they would enhance the antitumor effects of [1,2]oxazoles in lymphomas (Chart 1). This structural manipulation combines the main structural features of compounds **1** and **2**, based on the condensation of the pyrrole moiety in the tricyclic cyclohepta scaffold to yield a new chemical class of compounds **3** (Chart 1).

2. Results and discussion

2.1. Chemistry

Our synthetic approach to the pyrrolo[3′,4':3,4]cyclohepta[1,2-*d*] [1,2]oxazole ring system began with the preparation of 5,6,7,8-tetrahydrocyclohepta[*c*]pyrrol-4(2*H*)-ones **8a-d** as appropriate building blocks to achieve the tricyclic framework.

Based on our previous experience [19,22,23], we obtained ketones **8a,b** by a multistep sequence described in Scheme 1. Commercially available cycloheptane-1,3-dione **4** was converted into the enamino derivative **5** in refluxing *N,N*-dimethylformamide dimethyl acetal (DMFDMA). The latter was reacted with phenylglycine or 3,4,5-trimethoxyphenylglycine, leading to intermediates **6a,b** (80–82%), which were used in the following step without purification. Cyclization of compounds **6a,b** in acetic anhydride and triethylamine yielded compounds **7a,b** (53–73%) (Scheme 1), which were subjected to hydrolysis of acetyl groups in a (1:12) mixture of HCl (37%) and acetic acid (80%) at 60 °C, leading to compounds **8a,b** (75–81%) (Scheme 1).

Ketones **8c,d** were synthesized using a multistep sequence (Scheme 2). Ethyl 5-(4-methoxyphenyl)-1*H*-pyrrole-2-carboxylate **10** was obtained (74%) by reaction of 3-(4-methoxyphenyl)acrylaldehyde **9** with ethyl 2-azidoacetate. Friedel–Crafts acylation of compound **10** in the presence of glutaric anhydride and $AlCl_3$, as acylating reagent and Lewis acid, respectively, led to compound **11** (60%) (Scheme 2). The subsequent reduction of the carbonyl group at the 2-position of the pyrrole ring and then cyclization by dehydration with an excess of trifluoroacetic anhydride gave the ethyl 3-(4-methoxyphenyl)-8-oxo-2,4,5,6,7,8-hexahydrocyclohepta[*c*]pyrrole-1-carboxylate **8c** (60%) (Scheme 2). Basic hydrolysis of the ethoxycarbonyl group and subsequent decarboxylation in the presence of 6 M HCl led to **8d** in satisfactory yield (60%) (Scheme 2).

Ketones **8a-d** were properly functionalized at the pyrrole nitrogen by reaction with benzyl halides and sodium hydride, as a base, to give the corresponding *N*-substituted derivatives **14a-h,j-y** (60–98%) (Scheme 3). The 3-nitro,4-methoxybenzyl substituted derivatives **14h,y** were subjected to catalytic reduction with ammonium formate and 10% Pd/C in ethyl acetate, furnishing the corresponding amino derivatives **14i,z** (71–86%) (Scheme 3).

The *N*-substituted derivatives **14** were converted into the α-enaminoketones **15a-z** using *tert*-butoxybis(dimethylamino)methane (TBDMAM), which upon reaction with hydroxylamine hydrochloride as dinucleophile yielded pyrrolo[3′,4':3,4]cyclohepta[1,2-*d*] [1,2]oxazoles **3a-z** in good to excellent yields (60–93%) (Scheme 3, Table 1).

2.2. Antiproliferative activity

Pyrrolo[3′,4':3,4]cyclohepta[1,2-*d*] [1,2]oxazoles **3a-z** were submitted to the NCI and initially tested at a 10^{-5} M dose in the full panel of 60 human cell lines derived from nine human cancer cell types (leukemia, non-small-cell lung, colon, central nervous system, melanoma, ovarian, renal, prostate and breast) (Tables S1–S3). Two compounds **3u** and **3z** were then selected for further screening over a five-dose concentration range (10^{-4}–10^{-8} M) in each of the 60 tumor cell lines, defining their antiproliferative activity in terms of GI_{50} values. Both compounds showed growth inhibitory effects against all tested human tumor cell lines, with GI_{50} values in the low micromolar to submicromolar range (Table 2).

The 3,4,5-trimethoxyphenyl substituted derivative **3z**, bearing a 3-amino, 4-methoxybenzyl group at the pyrrole nitrogen, emerged as the most potent candidate, with a mean graph_midpoint (MG_MID) of 0.69 μM on the full NCI panel. The analysis of the GI_{50} values listed in Table 3 showed that leukemia and prostate cell lines were particularly responsive to treatment with **3z**, with GI_{50} values of 0.30–0.65 μM and 0.43–0.84 μM, respectively, maintaining submicromolar activity against all the tested cell lines. Comparable potency was also exerted against the renal (GI_{50} 0.36–0.84 μM) and colon (GI_{50} 0.38–0.56 μM) cancer subpanels, with the exception of the TK-10 (GI_{50} 96.6 μM) and HCC-2998 (GI_{50} 1.66 μM) cell lines, respectively. The best antiprolifera-

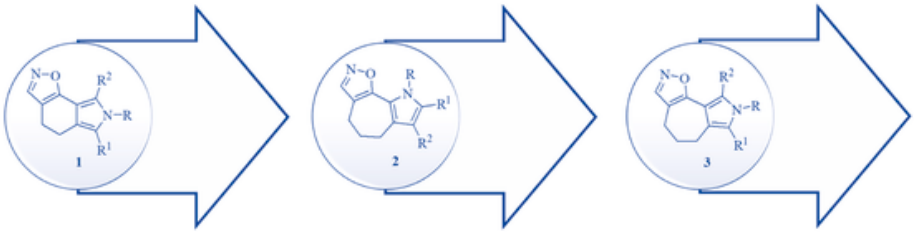
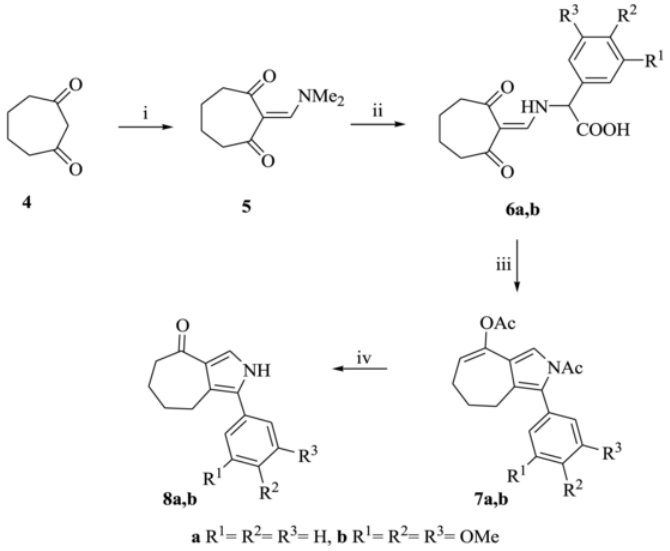


Chart 1. [1,2]Oxazolo[5,4-*e*]isoindoles (**1**), pyrrolo[2′,3':3,4]cyclohepta[1,2-*d*] [1,2]oxazoles (**2**), pyrrolo[3′,4':3,4]cyclohepta[1,2-*d*] [1,2]oxazoles (**3**).



Scheme 1. Synthesis of 1-phenyl-5,6,7,8-tetrahydrocyclohepta[*c*]pyrrol-4(2*H*)-one (**8a**) and 1-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydrocyclohepta[*c*]pyrrol-4(2*H*)-one (**8b**). Reagents and conditions: (i) DMFDMA, reflux, 1 h, 99%; (ii) phenylglycine or 3,4,5-trimethoxy phenylglycine, $AcONa{\cdot}3H_2O$, ethanol, reflux, 90 min, 80–82%; (iii) Et_3N, Ac_2O, 30 min, reflux, 30 min, 53–73%; (iv) 80% acetic acid/37% HCl (1:12), 15 min, 60 °C, 75–81%.

tive effect in specific subpanels was observed for the melanoma MDA-MB-435 cell line (GI_{50} 0.24 μM), the breast cancer line BT-549 (GI_{50} 0.25 μM), and the non-small cell lung line NCI–H522 (GI_{50} 0.26 μM).

Compound **3u**, a 3,4,5-trimethoxyphenyl derivative with a 4-methoxybenzyl group at the pyrrole nitrogen, was second in overall potency with high selectivity against the leukemia and colon cancer subpanels (GI_{50} values of 0.35–1.56 μM and 0.39–1.61 μM, respectively). The calculated MG_MID for these two subpanels were 0.65 and 0.88 μM, respectively, much lower than the overall cell line MG_MID value of 1.41 μM. For compound **3u**, the most sensitive cell lines were MDA-MB-435, with a GI_{50} of 0.23 μM, and the two leukemic cell lines HL-60(TB) and SR, with GI_{50} values of 0.35 and 0.36 μM, respectively.

2.3. Screening results in lymphoma models

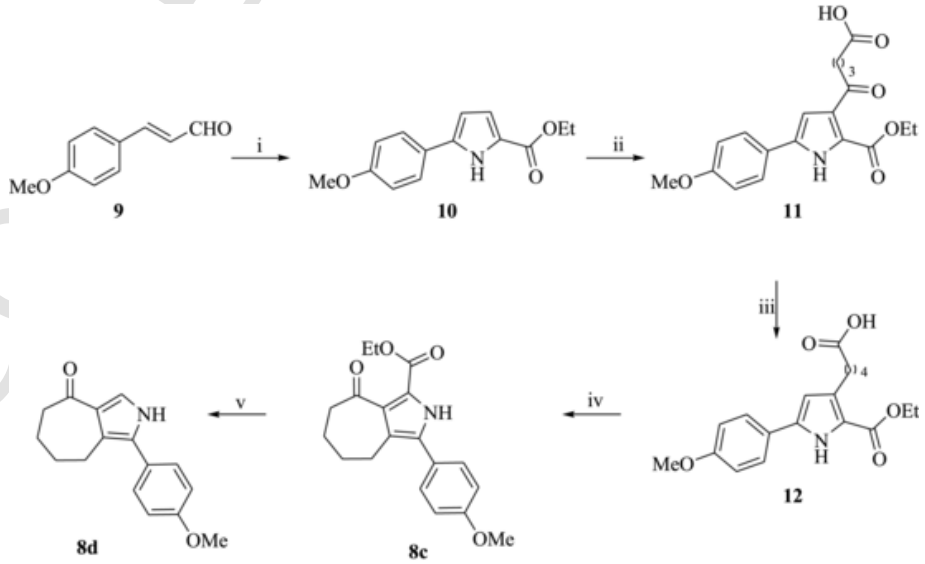
Because of our interest in lymphoma models, the pyrrolo[3′,4’:3,4]cyclohepta[1,2-*d*] [1,2]oxazoles **3a-z** were evaluated against non-Hodgkin lymphoma (NHL) cells. Cell viability was assessed in cultures of HBL1 (activated B cell-like diffuse large B cell lymphoma, ABC-DLBCL), SU-DHL-10 (germinal center B cell-like diffuse large B cell lymphoma, GCB-DLBCL), MINO (mantle cell lymphoma, MCL) and VL51 (marginal zone lymphoma, MZL) cells by means of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assays. In order to select compounds with the highest activity, a concentration of 1 μM was chosen for the initial assay. After a 72 h incubation, compounds **3u** and **3z** showed a reduction in the percentage of cellular proliferation higher than 50% towards each cell line, and they were therefore tested in a wider range of concentrations (0.15–10 μM) to establish IC_{50} values. These are shown in Table 4, with IC_{50} values all in the low micromolar – submicromolar range. Among the four NHL cell lines, VL51 was the most sensitive to the two compounds, with IC_{50} values < 500 nM. In all cell lines, **3z** was more active than **3u**, with IC_{50} values ranging from 0.1 to 0.5 μM.

From a structure-activity relationship point of view, the presence of a 3,4,5-trimethoxyphenyl ring and a 4-methoxybenzyl group at the pyrrole nitrogen were structural requirements for the higher antiproliferative activity of this new class of [1,2]oxazoles. Furthermore, the amino group at position 3 of the benzene ring seemed crucial to obtain the best growth inhibitory effect. The presence of an ethoxycarbonyl group in position 9 was not essential and generally reduced activity compared to the corresponding parent compound.

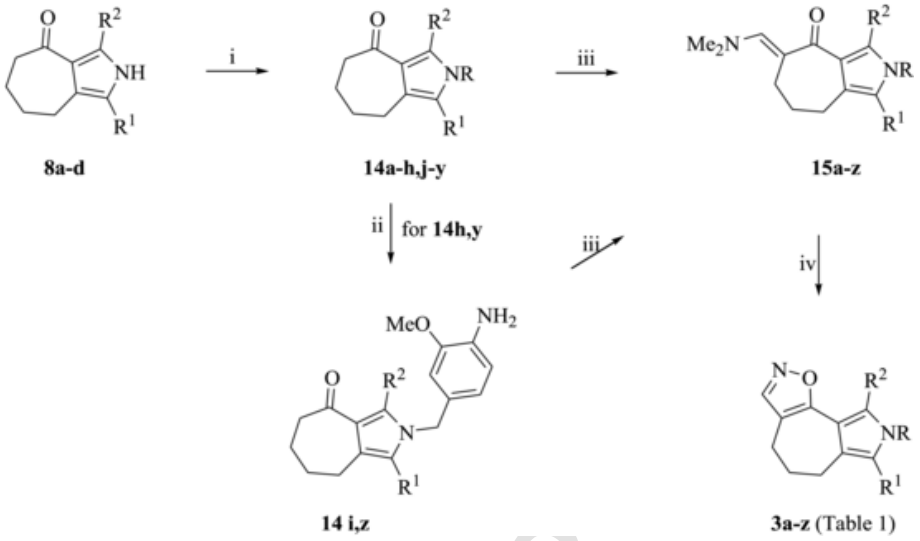
Compared to the previously reported classes of compounds **1** and **2**, the derivatives of group **3** displayed interesting antiproliferative activity, which was, however, slightly reduced with respect to the class **1** compound bearing a cyclohexyl central ring, indicating that the enlargement of the central ring results in reduction of activity.

2.4. Effects of compounds in human peripheral blood lymphocytes (PBLs)

With the aim of obtaining a preliminary indication of the cytotoxic potential of these derivatives in non-tumoral human cells, three representative compounds (**3d**, **3u** and **3z**) were evaluated in vitro against PBLs from healthy donors. As shown in Table 5, all three compounds



Scheme 2. Synthesis of 3-(4-methoxyphenyl)-8-oxo-2,4,5,6,7,8-hexahydrocyclohepta[*c*]pyrrole-1-carboxylate (**8c**) and 1-(4-methoxyphenyl)-5,6,7,8-tetrahydrocyclohepta[*c*]pyrrol-4(2*H*)-one (**8d**). Reagents and conditions: (i) a) ethyl azidoacetate, EtOK, ethanol, −20 °C, 4.5 h; b) toluene, reflux, 24 h, 74%; (ii) $AlCl_3$, glutaric anhydride, DCM, rt, 1 h then **10**, rt, 24 h, 60%; (iii) triethylsilane, trifluoroacetic acid, rt, 24 h, 61%; (iv) trifluoroacetic anhydride, rt, 1 h, 60%; (v) a) 50% KOH, ethanol, reflux, 3 h; b) HCl 6 M, ethanol, reflux, 1 h, 60%.



Scheme 3. Synthesis of pyrrolo[3′,4':3,4]cyclohepta[1,2-*d*] [1,2]oxazoles **3a-z**. Reagents and conditions: (i) NaH, DMF, 0 °C to rt, 1 h then benzyl halides at 0 °C to rt, 3–12 h, 60–98%; (ii) ammonium formate, 10% Pd/C, ethyl acetate, rt, 12 h, 71–86%; (iii) TBDMAM, toluene, reflux, 12 h; (iv) $NH_2OH{\cdot}HCl$, ethanol, reflux, 1 h, 60–93%.

showed IC_{50} values greater than 10 μM both in resting lymphocytes and in lymphocytes in an active phase of proliferation induced by phytohematoagglutinin (PHA), a mitogenic stimulus. These results suggest that these compounds have very high selectivity toward cancer cells and low toxicity in normal cells.

2.5. Tubulin binding assay

Since [1,2]oxazolo[5,4-*e*]isoindoles **1**, pyrrolo[2′,3':3,4]cyclohepta [1,2-*d*] [1,2]oxazoles **2** and other derivatives structurally related to this new class of compounds were previously shown to affect tubulin polymerization [18,21,24], we investigated their antitubulin activity in comparison with reference compound combretastatinA-4 (CA-4), which potently inhibits tubulin assembly by interacting with the colchicine site on tubulin. The inhibition of tubulin assembly was assessed for all compounds in a reaction mixture containing 10 μM (1.0 mg/mL) tubulin. Those compounds having IC_{50} values < 5 μM in the assembly assay were further evaluated for their ability to compete with 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. We found that only **3d** and **3p** significantly affected tubulin assembly, with **3d** having activity similar to that of CA-4, while **3p** was about 4-fold less active. Neither compound had significant activity inhibiting colchicine binding at 5 μM, but weak inhibition was observed when the concentration of **3d** was increased to 25 μM. Neither **3u** nor **3z** caused significant inhibition of tubulin assembly at low concentrations, thus excluding tubulin as their intracellular target, unless they undergo intracellular conversion to a more active agent.

2.6. Molecular modeling

Computational studies were focused on compounds **3d**, **3p**, **3u**, and **3z** to elucidate their potential interactions with tubulin [25]. Thus, we performed docking studies directed toward the colchicine (4O2B PDB code) [26] and vinblastine (1Z2B PDB code) [27] sites by selecting the pose with the best G-Score (kcal/mol) for each compound. The ligands showed insufficient affinity for being accommodated at the vinblastine site, as demonstrated by their G-scores ranging between −4.16 and −5.07 kcal/mol, due to the formation of numerous poor contacts and steric hindrances. Conversely, all ligands exhibited a better affinity for the colchicine site (Table S4), confirming their specificity for this binding pocket.

The experimental data indicate that none of the compounds have a better affinity than colchicine for tubulin, since inhibition of colchicine binding was relatively weak with **3d** and **3p**, while **3u** and **3z** were poor inhibitors of tubulin assembly. The best interactions with tubulin were thus observed with **3d** and **3p**. The docking studies were also performed on the 3N2G PDB model [28] to investigate the binding mode of our ligands towards two additional neighboring pockets (zones 2 and 3) in the tubulin colchicine domain. Thus, we obtained results only for **3d**, while no pose was generated for the other ligands (Table S4). Although zone 1 is still preferred, **3d** showed a better affinity than colchicine for zones 2 and 3. As shown in Fig. S1, **3d** established a hydrogen bond (H-bond) acceptor between its isoxazole ring and the backbone of βA250. Moreover, we observed good contact with an additional hydrophobic pocket of the β subunit, formed by residues L255, A316, A317, A354, C241, and K352. For comparison, in the best docking pose of **3d** with tubulin structure 4O2B [26], containing zones 1 and 2 of the colchicine site, we observed strong hydrophobic interactions with β-tubulin residues V181, L248, A250, L255, A354, and A316 (Fig. 1A). Most importantly, **3d** displayed a binding geometry characterized by the isoxazole moiety facing the C241 and the benzene ring being accommodated in zone 1 and interacting with N258, V181, and A316. The other tubulin-active compound **3p** shared with **3d** the same binding geometry and the same hydrophobic contribution within the colchicine site (Fig. 1B). In contrast, the best poses for **3u** and **3z** placed the tricyclic system more planarly than the **3d** and **3p** poses, with the isoxazole ring facing L255 and the pyrrole ring interacting with the side chain of K352 through a π-cation bond (Fig. 1C–D). In addition, **3z** established two H-bonds between its isoxazole ring and aniline group with N258 and Q247, respectively. Despite the ability of **3u** and **3z** to establish π-cation interactions and hydrogen bonds with tubulin, their lower affinity for the binding site could be explained by several steric hindrances in their docking poses. Specifically, we observed a significant clash between the cycloheptane ring of the **3u** tricyclic system and βA354, while **3z** showed a poor contact that involved the side chain of βS178 and its methylene group linking the pyrrole ring to the 2-methoxy-5-aniline moiety. Furthermore, we observed that the trimethoxyphenyl group was not well located in zone 1, leading to the loss of hydrophobic interactions with β-tubulin residues V181, L248, A250, L255, A354, and A316.

Table 1
Pyrrolo[3′,4′:3,4]cyclohepta [1,2-d] [1,2]oxazoles 3a-z.

[1,2-oxazole]	Starting ketone	R	R^1	R^2	Yields[a] (%)
3a	**14a**	Bn	Ph	H	73
3b	**14b**	2-OMeBn	Ph	H	78
3c	**14c**	3-OMeBn	Ph	H	68
3d	**14d**	4-OMeBn	Ph	H	83
3e	**14e**	2,5-$(OMe)_2$Bn	Ph	H	80
3f	**14f**	3,4-$(OMe)_2$Bn	Ph	H	80
3g	**14g**	3,4,5-$(OMe)_3$Bn	Ph	H	69
3h	**14h**	3-NO_2,4-OMeBn	Ph	H	60
3i	**14i**	3-NH_2,4-OMeBn	Ph	H	63
3j	**14j**	Bn	OMe-Ph	COOEt	93
3k	**14k**	2-OMeBn	OMe-Ph	COOEt	83
3l	**14l**	3-OMeBn	OMe-Ph	COOEt	75
3m	**14m**	4-OMeBn	OMe-Ph	COOEt	66
3n	**14n**	2-OMeBn	OMe-Ph	H	60
3o	**14o**	3-OMeBn	OMe-Ph	H	64
3p	**14p**	4-OMeBn	OMe-Ph	H	70
3q	**14q**	3,4,5-$(OMe)_3$Bn	OMe-Ph	H	64
3r	**14r**	Bn	3,4,5-$(OMe)_3$Ph	H	66
3s	**14s**	2-OMeBn	3,4,5-$(OMe)_3$Ph	H	60
3t	**14t**	3-OMeBn	3,4,5-$(OMe)_3$Ph	H	68
3u	**14u**	4-OMeBn	3,4,5-$(OMe)_3$Ph	H	71
3v	**14v**	2,5-$(OMe)_2$Bn	3,4,5-$(OMe)_3$Ph	H	77
3w	**14w**	3,4-$(OMe)_2$Bn	3,4,5-$(OMe)_3$Ph	H	71
3x	**14x**	3,4,5-$(OMe)_3$Bn	3,4,5-$(OMe)_3$Ph	H	71
3y	**14y**	3-NO_2,4-OMeBn	3,4,5-$(OMe)_3$Ph	H	67
3z	**14z**	3-NH_2,4-OMeBn	3,4,5-$(OMe)_3$Ph	H	68

[a] Obtained at the final reaction step.

Table 2
Overview of the NCI in vitro human tumor cell line screening for derivatives 3u,3z.

Cpd	N[b]	N[c]	GI_{50}[a]	MG_MID[d]
3u	58	55	0.23–4.97	1.41
3z	56	56	0.24–96.6	0.69

[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.

MDs in water as solvent were performed on the best docking poses of **3d**, **3p**, **3u**, and **3z** against the 4O2B model to better assess their binding stability and to evaluate the presence of induced-fit phenomena in the tubulin recognition process of these ligands. The 3N2G model complexed with **3p** was also investigated through MDs, and colchicine was used as a reference compound. Thus, we analyzed the geometric behavior of all MDs and we computed the related binding free energy and the global number of contacts for the MDs most representative structures (Table S5).

Table 3
In vitro GI_{50} (μM) values for compounds 3u and 3z in the full NCI panel.

Cell lines	3u	3z	Cell lines	3u	3z
Leukemia			M14	0.39	0.48
CCRF-CEM	0.41	0.44	MDA-MB-435	0.23	0.24
HL-60(TB)	0.35	0.30	SK-MEL-2	1.61	–
K-562	0.42	0.39	SK-MEL-28	–	2.93
MOLT-4	0.80	0.65	SK-MEL-5	2.00	1.13
RPMI-8226	1.56	0.61	UACC-257	–	0.58
SR	0.36	0.36	UACC-62	0.91	0.43
Non-Small Cell Lung Cancer			**Ovarian Cancer**		
A549/ATCC	2.34	0.95	IGROV1	0.56	1.56
EKVX	3.00	2.67	OVCAR-4	4.97	–
HOP-62	2.22	0.69	OVCAR-5	3.72	1.44
HOP-92	1.27	0.52	OVCAR-8	4.30	1.57
NCI–H226	1.99	2.54	NCI/ADR-RES	0.54	0.60
NCI–H23	2.62	1.34	SK-OV-3	4.18	0.68
NCI–H322 M	3.28	0.84	**Renal Cancer**		
NCI–H460	0.50	0.40	786–0	0.67	0.52
NCI–H522	0.98	0.26	A498	0.69	0.36
Colon Cancer			ACHN	0.90	0.69
COLO 205	1.45	0.38	CAKI-1	0.49	0.58
HCC-2998	1.61	1.66	RXF 393	0.66	0.45
HCT-116	0.39	0.40	SN12C	4.60	0.84
HCT-15	0.48	0.53	TK-10	–	96.6
HT29	1.13	0.42	UO-31	0.92	0.69
KM12	0.69	0.56	**Prostate Cancer**		
SW-620	0.47	0.43	PC-3	0.85	0.43
CNS cancer			DU-145	4.11	0.84
SF-268	4.72	1.45	**Breast Cancer**		
SF-295	0.51	0.50	MCF7	0.44	0.42
SF-539	0.70	0.40	MDA-MB-231/ATCC	2.54	1.15
SNB-19	1.53	0.81	HS 578T	1.54	0.40
SNB-75	0.87	0.38	BT-549	0.48	0.25
U251	4.47	0.51	T-47D	1.85	0.39
Melanoma			MDA-MB-468	0.56	0.65
LOX IMVI	0.99	0.70			

Table 4
IC_{50} values (μM) of 3u and 3z against NHL cell lines. Cell lines were exposed to the compounds at 0.15–10 μM for 72 h.

CPD	VL51 (MZL)	MINO (MCL)	HBL1 (ABC DLBCL)	SU-DHL-10 (GCB DLBCL)
3u	0.4 ± 0.07	0.9 ± 0.4	1 ± 0.3	1.8 ± 0.7
3z	0.1 ± 0.06	0.4 ± 0.02	0.5 ± 0.7	0.5 ± 0.05

Marginal zone lymphoma (MZL); mantle cell lymphoma (MCL); activated B cell-like diffuse large B cell lymphoma (ABC-DLBCL); germinal center B cell-like diffuse large B cell lymphoma (GCB-DLBCL).

Table 5
Cytotoxicity of compounds 3d, 3u and 3z in human PBLs.

	IC_{50} (μM)[a]		
	3d	**3u**	**3z**
$PBL_{resting}$[b]	>10	>10	>10
PBL_{PHA}[c]	>10	>10	>10

[a] Compound concentration required to inhibit cell growth by 50%.
[b] PBL not stimulated with PHA.
[c] PBL stimulated with PHA.

Interestingly, the most active compounds **3d** and **3p** during MDs improved their interactions with the colchicine site with respect to their docking pose (Fig. 1A–B), thanks to their ability to engage in further electrostatic contacts, such as H-bond and π-cation interactions (Fig. 2A–B). Specifically, **3d** established a π-cation interaction between its phenolic ring and the side chain of βK352 (Fig. 2A), while **3p** exhibited an H-bond between its pyrrole moiety and the backbone of βN249 (Fig. 2B). Conversely, for **3u** (Fig. 2C), the loss of the π-cation interaction,

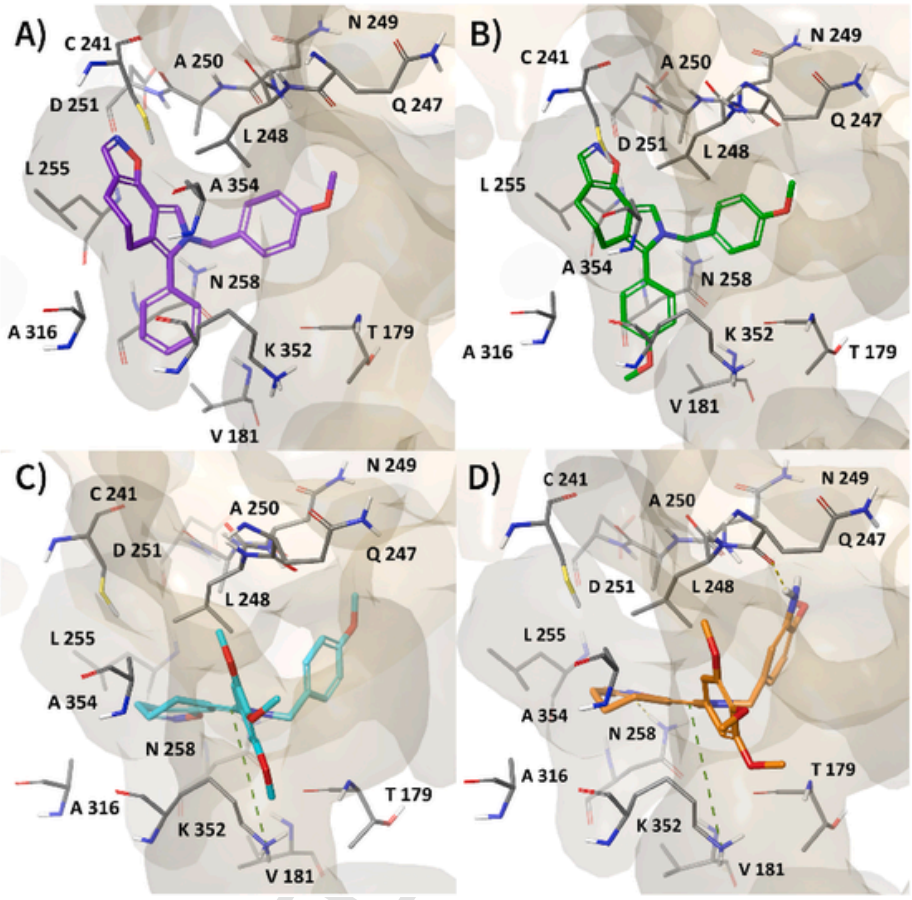


Fig. 1. Best docked pose of **A) 3d**, **B) 3p**, **C) 3u**, and **D) 3z** with the 4O2B crystal structure of tubulin, depicting zones 1 and 2 of the colchicine site. Tubulin is shown in a faded yellow surface, while ligand and residues, involved in the most important interactions, are shown as sticks. H-bond and π-cation interactions are indicated as dashed yellow and green lines, respectively.

previously reported in the docking pose (Fig. 1C), along with the reduction of hydrophobic contacts, could explain its lower activity in the tubulin experiments. Finally, in the most representative MDs structure of **3z** (Fig. 2D), we observed two different H-bonds compared to its docking pose (Fig. 1D). Specifically, the trimethoxyphenyl group and the isoxazole ring formed two H-bonds with the backbone of T179 and of D251, respectively. Despite these favorable interactions, its reduced affinity for the colchicine site may be explained by a higher solvation energy penalty and the lower number of good contacts, as reported in the Supplementary Material.

Finally, the MDs confirmed the binding mode predicted by the docking calculation for **3d** (Fig. S1A) towards the 3N2G model, with the sole exception being the residue engaged in the H-bond with the isoxazole ring. Indeed, in the most representative MDs structure, an induced-fit process allowed the interaction with the backbone of βN249 (Fig. S1B). This finding highlights the possibility that **3d** interacts with tubulin through two distinct, stable binding modes.

Regarding the *in-silico* ADME (absorption, distribution, metabolism, excretion) assessment [29], the most active new derivatives (**3d**, **3p**, **3u** and **3z**) exhibited a pharmacokinetics profile similar to those of colchicine, with the only exception of the logP (QPlogPo/w). As shown in Table S7, all new compounds showed a logP value > 5, violating Lipinski's rule (RO5) but achieving better oral bioavailability.

At the end, we performed a target prediction using the Molinspiration virtual screening engine tool (Table S8) [30], with the aim of exploring possible *off-target* effects for our best compounds. Based on the drug-likeness score, the bioactivity of the ligand molecules can be divided into three categories, such as active (> 0.0), moderate (from −5.0 to 0.0), and inactive (< -5.0). All the most effective compounds exhibited active drug-likeness scores as nuclear receptor and GPCR ligands, especially **3d**. They also showed good scores as kinase and enzyme inhibitors. Conversely, they did not exhibit a significant potential active profile as either an ion channel modulator or a protease inhibitor.

2.7. Cell cycle analysis

To investigate the mechanism of action of the new derivatives, we evaluated their influence on the cell cycle in three cell lines, A549, CCRF-CEM and VL51. As shown in Fig. 3 (Panel A–C), compound **3d**, although endowed with significant activity as an inhibitor of tubulin polymerization (Table 6), induced in the three cell lines only a modest increase in G2/M phase, which was observed only at the maximum concentration used (5 μM for A549 and VL51 and 10 μM for CCRF-CEM). Conversely compounds **3u**, and even more markedly **3z**, both of which did not show significant activity in the tubulin assay, induced a block in G2/M accompanied by a strong decrease in S phase cells even at low concentrations (1.0 μM).

In order to determine whether these compounds were able to block cells at the mitotic phase (M), cells were stained with an immunofluorescent antibody to phospho-histone H3 [31], a well-known mitotic marker, as well as propidium iodide (PI), and analyzed by flow cytometry. As shown in Fig. 3 (Panel D), VL51 cells arrested in M phase, represented by p-histone H3 positive cells, which increased in a concentration dependent manner only for compounds **3u** and **3z**. In particular, the percentage of mitotic cells increased from about 1.5% observed in untreated cells to about 18% at the concentration of 1 μM for both compounds. In good agreement with the cell cycle analysis, we also did not observe a significant increase of mitotic cells with compound **3d**.

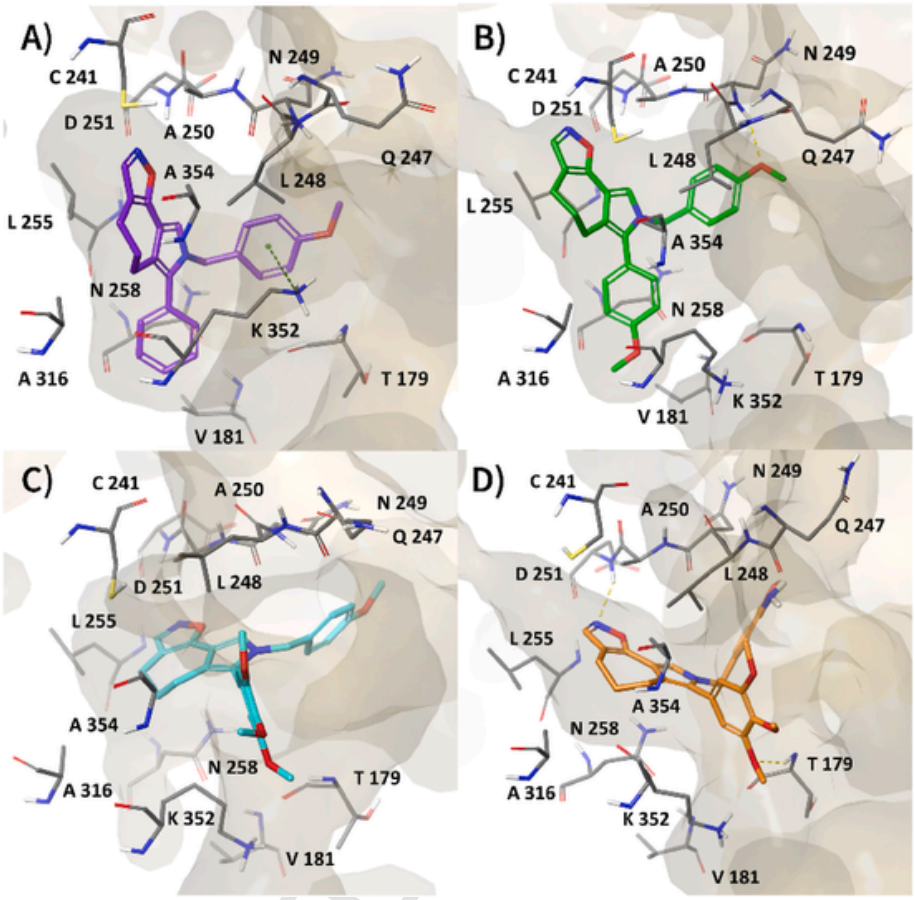


Fig. 2. Most representative MDs structure of tubulin (PDB code 4O2B) complexed with **A) 3d**, **B) 3p**, **C) 3u**, and **D) 3z**. Tubulin is depicted as a pale yellow surface, while ligand and residues, involved in the most important interactions, are shown as sticks. H-bond and π-cation interactions are indicated as dashed yellow and green lines, respectively.

2.8. The new derivatives induce apoptosis through the mitochondrial pathway

With the goal of studying the mode of cell death induced by the new derivatives, we evaluated the induction of apoptosis using double labeling of treated cells with annexin-V conjugated with FITC and with PI. Annexin-V binds to the phosphatidylserine exposed on the outer surface of the cytoplasmic membrane during the process of apoptosis, while PI binds to DNA, indicating cells undergoing necrosis.

In excellent agreement with the cytotoxicity data, the results shown in Fig. 4 (Panels A–C) demonstrate that the more active **3u** and **3z** induced, after a 48 h incubation, massive apoptosis in a dose-dependent manner, while **3d** induced apoptosis to a lesser extent. It should be emphasized that apoptosis occurred in all three cell lines examined, but to a greater extent in the VL51 cells, suggesting a particular tropism of these compounds towards lymphomas.

One of the early events that precede apoptosis is the decrease of the membrane mitochondrial potential [32]. To determine if this happened with our compounds, we used the JC-1 fluorescent dye and analyzed the VL51 cells after treatment for 24 h with compounds **3d**, **3u** and **3z**. The results shown in Fig. 4D demonstrate that **3d** caused a slight depolarization of the mitochondrial membrane, while depolarization was extensive following treatment of the cells with **3u** or **3z**, in excellent agreement with their relative abilities to induce apoptosis. This suggests that apoptosis follows the mitochondrial pathway.

3. Conclusions

In the current study, twenty-six derivatives were evaluated for their antiproliferative activity in the NCI-60 cell panel and in four different NHL histotypes (ABC-DLBCL, GCB-DLBCL, MCL and MZL). All tested compounds showed antiproliferative activity in the low micromolar – submicromolar range, with the greatest growth inhibition activity observed with **3u** and **3z**. Overall, the new structural modification confirmed a promising antiproliferative effect, although reduced compared to the [1,2]oxazolo[5,4-*e*]isoindoles **1**. Contrary to our hypothesis, based on the results obtained from our previous series of compounds, the best candidates **3u** and **3z** probably have a different primary target, as they were found to have modest activity as inhibitors of tubulin polymerization.

Nevertheless, **3u** and **3z** block the cell cycle in metaphase as demonstrated by the increase of p-histone H3 positive cells. In this context, further experiments are needed to verify whether the action of these compounds is linked to an inhibition of proteins which regulate the cell cycle, in particular for those proteins involved in the regulation of spindle associated events. In addition, these compounds induce apoptosis by a mechanism that follows the mitochondrial pathway. We should also note that we cannot exclude metabolic conversion of **3u** and/or **3z** to a tubulin-active compound.

Overall our results provide new perspectives for pyrrolo[3′,4':3,4] cyclohepta [1,2-d] [1,2]oxazoles.

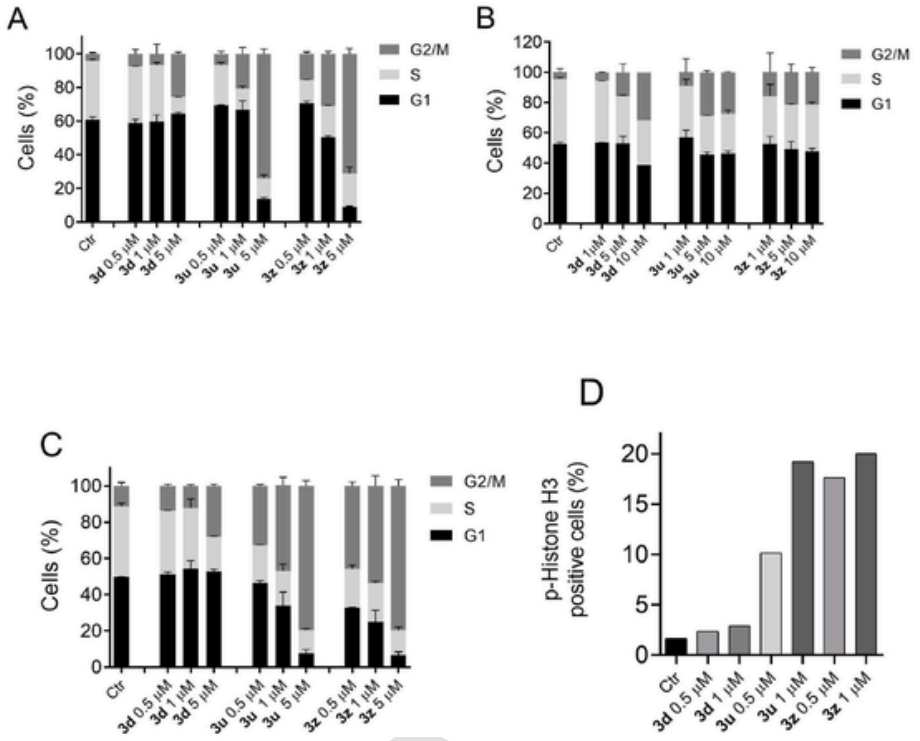


Fig. 3. Cell cycle analysis of A549 (A), CCRF-CEM (B) and VL51 (C) cells treated for 24 h at the indicated concentrations with **3d**, **3u** and **3z**. Cells were fixed and labeled with PI and analyzed by flow cytometry as described in the Experimental section. Data are presented as mean of two independent experiments ± SEM. (D) Percentage of p-histone H3 positive cells (mitotic cells) obtained from flow cytometric analysis of VL51 cells immunofluorescently labeled with an antibody to p-histone H3, following treatment with the indicated concentrations of compounds for 24 h.

Table 6
Inhibition of tubulin assembly and colchicine binding by compounds 3d,3p,3u,3z.

CPD	Inhibition of tubulin assembly	Inhibition of colchicine binding	
	IC_{50} (μM) ± SD	% Inhibition ± SD 5 μM inhibitor	% Inhibition ± SD 25 μM inhibitor
CA-4	0.75 ± 0.06	98 ± 2	–
3d	1.1 ± 0.09	1.8 ± 4	19 ± 3
3p	4.6 ± 0.2	7.7 ± 5	–
3u	>20	–	–
3z	18 ± 3	–	

4. Experimental section

4.1. Chemistry. Synthesis and characterization

All melting points were taken on a Büchi melting point M – 560 apparatus. IR spectra were determined in bromoform with a Shimadzu FT/IR 8400S spectrophotometer. ^{1}H and ^{13}C NMR spectra were measured at 200 and 50.0 MHz, respectively, in DMSO-d_6 or $CDCl_3$ solution using a Bruker Avance II series 200 MHz spectrometer. Column chromatography was performed with Merck silica gel (230–400 mesh ASTM) or a Büchi Sepacor chromatography module (prepacked cartridge system). Elemental analyses (C, H, N) were within ±0.4% of theoretical values and were performed with a VARIO EL III elemental analyzer. The purity of all the tested compounds was >95%, determined by HPLC (Agilent 1100 series).

4.1.1. Procedure for the preparation of 2-((dimethylamino)methylene) cycloheptane-1,3-dione **(5)**

A solution of cycloheptane-1,3-dione **4** (1 g, 8 mmol) in anhydrous DMFDMA (2.6 mL) was heated under reflux for 1 h. After cooling, the solvent was evaporated at reduced pressure, and the oily residue was triturated with diethyl ether with the solvent removed by filtration. Brown solid; yield: 99%; mp: 102–103 °C; IR (cm^{-1}): 1660 (CO) 1585 (CO); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.84–1.88 (m, 4H, 2 x CH_2), 2.59 (t, 4H, J = 6.2 Hz, 2 x CH_2), 2.80 (s, 3H, CH_3), 3.30 (s, 3H, CH_3), 7.72 (s, 1H, CH); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 22.2 (2 x C), 40.5 (2 x C), 43.2, 47.9, 112.8, 159.6 (2 x C), 200.2. Anal Calcd. for $C_{10}H_{15}NO_2$: C, 66.27; H, 8.34; N, 7.73. Found: C, 65.98; H, 8.12; N, 7.89.

4.1.2. General procedure for the preparation of 2-{[(2,7-dioxocycloheptylidene)methyl]amino}-arylacetic acid **(6a,b)**

To a solution of **5** (16 mmol) in ethanol (30 mL), a solution of the appropriate phenylglycine (19 mmol) and sodium acetate trihydrate (0.26 g) in ethanol was added, and the reaction mixture was heated under reflux until the reaction was complete (TLC). After cooling, the reaction mixture was filtered, and the filtrate was dried under reduced pressure. To the residue, ice and water were added, and the resulting solution was acidified with 6 M HCl. The solid obtained was filtered and dried.

*4.1.2.1. 2-{[(2,7-Dioxocycloheptylidene)methyl]amino}-2-phenylacetic acid **(6a)**.* This compound was obtained from reaction of **4** with phenylglycine after 1-1/2 h. Brown oil; yield: 80%; IR (cm^{-1}): 3422 (NH), 3287 (OH), 1703 (CO), 1658 (CO), 1621 (CO); ^{1}H NMR (DMSO-d_6, 200 MHz, ppm): δ 1.70 (s, 4H, 2 x CH_2), 2.55–2.60 (m, 4H, 2 x CH_2), 3.50 (s, 1H, OH), 5.63 (d, 1H, J = 7.2 Hz, CH), 7.33–7.46 (m, 5H, Ar), 7.92 (d, 1H, J = 14.0 Hz, CH), 11.44–11.51 (m, 1H, NH); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): δ 21.5, 21.6, 30.1 (2 x C), 63.8,

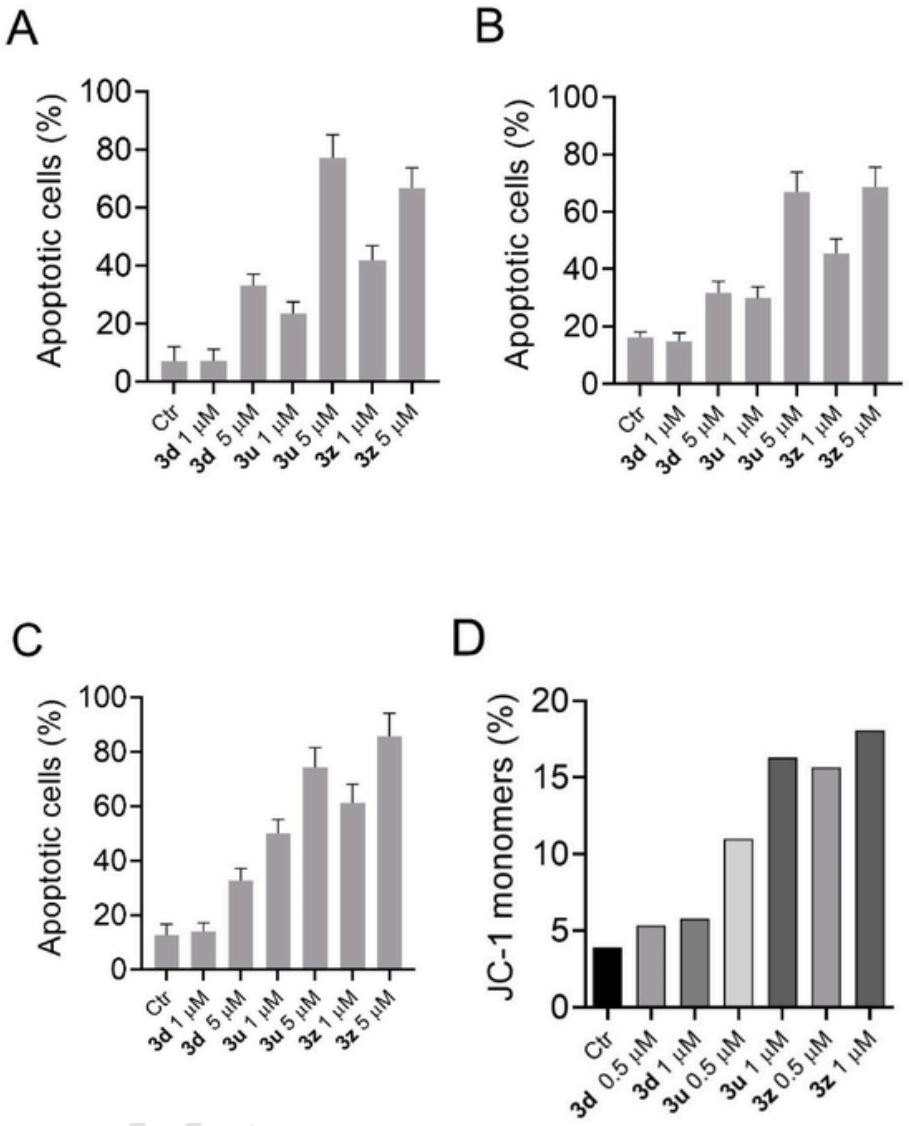


Fig. 4. Compounds **3d**, **3u** and **3z** induced apoptosis in A549 (A), CCRF-CEM (B) and VL51 (C) cells. Cells were treated with the compounds for 48 h at the indicated compound concentrations. The cells were then harvested and labeled with annexin-V-FITC and PI and analyzed by flow cytometry. Data are presented as mean ± S.E.M. for three independent experiments. The percentage of apoptotic cells refers to the sum of annexin-V positive and Annexin-V and PI double positive cells. (D) Assessment of mitochondrial membrane potential by flow cytometry with the fluorescent probe JC-1 after treatment for 24 h of VL51 cells with the indicated compounds at 0.5 and 1.0 μM.

111.8, 127.8 (2 x C), 129.1, 129.7 (2 x C), 137.7, 158.4, 171.3, 198.9, 201.1. Anal Calcd. for $C_{16}H_{17}NO_4$: C, 66.89; H, 5.96; N, 4.88. Found: C, 66.74; H, 5.81; N, 4.99.

4.1.2.2. 2-{[(2,7-Dioxocycloheptylidene)methyl]amino}-2-(3,4,5-trimethoxyphenyl)acetic acid ***(6b)***. This compound was obtained from reaction of **4** with 3,4,5-trimethoxyphenylglycine after 1-1/2 h. Brown solid; yield: 82%; mp: 102–103 °C; IR (cm^{-1}): 3401 (NH), 3299 (OH), 1698 (CO), 1652 (CO), 1633 (CO); ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.82 (s, 4H, 2 x CH_2), 2.66 (s, 4H, 2 x CH_2), 3.53 (s, 1H, OH), 3.83 (s, 3H, CH_3), 3.84 (m, 6H, 2 x CH_3), 5.08 (d, 1H, J = 6.8 Hz, CH), 6.59 (s, 2H, Ar), 8.03 (d, 1H, J = 14.1 Hz, Ar), 11.57–11.64 (m, 1H, NH); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 21.4, 21.5, 40.1, 40.6, 56.2 (2 x C), 60.8, 65.2, 104.5 (2 x C), 112.2, 130.7, 138.6, 153.8 (2 x C), 159.1, 170.7, 201.7, 202.1. Anal Calcd. for $C_{19}H_{23}NO_7$: C, 60.47; H, 6.14; N, 3.71. Found: C, 60.19; H, 6.38; N, 3.56.

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

To a solution of **6a,b** (8 mmol) in acetic anhydride (25 mL), triethylamine was added (5.7 mmol, 8 mL). The reaction mixture was heated under reflux until the reaction was complete (TLC). After cooling, the reaction mixture was poured into water and ice and formed a rubbery solid. The liquid phase was decanted, and the remaining solid was stirred with a saturated solution of Na_2CO_3 (50 mL). The solid obtained was filtered and dried. The solid was dissolved in dichloromethane and purified using a chromatography column (dichloromethane).

4.1.3.1. 2-Acetyl-1-phenyl-2,6,7,8-tetrahydrocyclohepta[c]pyrrol-4-yl acetate ***(7a)***. This compound was obtained from reaction of **6a** after 30 min. Brown solid; yield: 73%; mp: 120–121 °C; IR (cm^{-1}): 1769 (CO) 1711 (CO); ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.75–1.86 (m, 2H, CH_2), 2.08 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 2.41–2.53 (m, 4H, 2 x CH_2), 5.49 (t, 1H, J = 5.1 Hz, CH), 7.22–7.28 (m, 3H, Ar and H-3), 7.35–7.47 (m, 3H, Ar); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 21.1, 24.7, 25.1, 26.6, 28.5, 117.2, 119.6, 121.8, 125.9, 128.2, 128.3 (2 x C),

130.3, 130.6 (2 x C), 133.0, 141.0, 168.8, 170.1. Anal Calcd. for $C_{19}H_{19}NO_3$: C, 73.82; H, 7.12; N, 4.30. Found: C, 74.03; H, 7.33; N, 3.99.

*4.1.3.2. 2-Acetyl-1-(3,4,5-trimethoxyphenyl)-2,6,7,8-tetrahydrocyclohepta[c]pyrrol-4-yl acetate **(7b)***. This compound was obtained from reaction of **6b** after 30 min. Brown oil; yield: 53% IR (cm^{-1}): 1753 (CO) 1722 (CO); ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.80–1.87 (m, 2H, CH_2), 2.12 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 2.44–2.48 (m, 2H, CH_2), 2.52–2.56 (m, 2H, CH_2), 3.85 (s, 6H, 2 x CH_3), 3.91 (s, 3H, CH_3), 5.50 (t, 1H, J = 5.0 Hz, CH), 6.45–6.49 (m, 3H, Ar and H-3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 21.0, 24.6, 24.8, 26.7, 28.3, 56.0 (2 x C), 61.0, 108.0 (2 x C), 117.1, 119.5, 121.7, 125.9, 128.3, 129.7, 130.1, 141.1, 153.2 (2 x C), 168.6, 169.9. Anal Calcd. for $C_{22}H_{25}NO_6$: C, 66.15; H, 6.31; N, 3.51. Found: C, 66.27; H, 6.45; N, 3.28.

*4.1.4. General procedure for the preparation of 1-substituted-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one **(8a,b)***

To a solution of **7a,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 until the reaction was complete (TLC). After cooling, the reaction mixture was poured into water and ice. The solid obtained was filtered and dried. The solid was purified using column chromatography (dichloromethane: ethyl acetate 95 : 5).

*4.1.4.1. 1-Phenyl-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one **(8a)***. This compound was obtained from **7a** after 15 min. Brown solid; yield: 75%; mp: 103–104 °C; IR (cm^{-1}): 3248 (NH), 1651 (CO); ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.84–1.94 (m, 4H, 2 x CH_2), 2.73 (t, 2 H, J = 5.9 Hz, CH_2), 2.91 (t, 2H, J = 5.9 Hz, CH_2), 7.29–7.47 (m, 6H, Ar and H-3), 8.80 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 22.3, 23.9, 26.3, 41.6, 121.3, 122.2, 127.1, 127.2, 127.6 (2 x C), 128.9 (2 x C), 129.5, 132.4, 200.0. Anal Calcd. for $C_{15}H_{15}NO$: C, 79.97; H, 6.71; N, 6.22. Found: C, 80.12; H, 6.47; N, 6.39.

*4.1.4.2. 1-(3,4,5-Trimethoxyphenyl)-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one **(8b)***. This compound was obtained from **7b** after 15 min. Brown solid; yield 81%; IR (cm^{-1}): 3258 (NH), 1657 (CO); ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.86–1.95 (s, 4H, 2 x CH_2), 2.77 (t, 2H, J = 6.4 Hz, CH_2), 2.92 (t, 2H, J = 6.4 Hz, CH_2), 3.89 (s, 3H, CH_3), 3.90 (s, 6H, 2 x CH_3), 6.61 (s, 2H, H-2″ and H-6"), 7.52 (s, 1H, H-3), 9.26 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) (ppm): 22.4, 24.1, 26.3, 41.6, 56.2 (2 x C), 61.0, 105.2 (2 x C), 121.0, 128.5, 129.5, 129.6, 129.7, 137.5, 153.5 (2 x C), 199.9. Anal Calcd. for $C_{18}H_{21}NO_4$: C, 68.55; H, 6.71; N, 4.44. Found: C, 68.67; H, 6.46; N, 4.18.

*4.1.5. Procedure for the preparation of ethyl 5-(4-methoxyphenyl)-1H-pyrrole-2-carboxylate **(10)***

To a solution of ethyl azidoacetate (7g, 54 mmol) in anhydrous ethanol (10 mL), a solution of **9** (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 ethanol (50 mL). The reaction was stirred for 4–1/2 h at −20 °C. The reaction mixture was then allowed to reach room temperature, and the solvent was evaporated under reduced pressure. The residue was dissolved in water and was extracted with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was dissolved in toluene, and the reaction mixture was heated under reflux for 24 h. The solvent was evaporated under reduced pressure, and the crude product was purified using a chromatography column (dichloromethane). Yellow solid; yield 74%; IR (cm^{-1}): 3421 (NH), 1679 (CO); ^1H NMR (DMSO-d_6, 200 MHz, ppm): δ 1.30 (t, 3H, J = 7.1 Hz, CH_3), 3.78 (s, 3H, CH_3), 4.26 (q, 2H, J = 7.1 Hz, CH_2), 6.53 (d, 1H, J = 3.5 Hz, Ar), 6.84 (d, 1H, J = 3.5 Hz, Ar), 6.96 (d, 2H, J = 8.8 Hz, H-3′ and H-5′), 7.80 (d, 2H, J = 8.8 Hz, H-2′ and H-6'), 11.97 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): δ 14.4, 55.1, 59.4, 106.7, 114.0 (2 x C), 116.6, 122.5, 124.1, 126.5 (2 x C), 137.2, 158.6, 160.3. Anal Calcd. for $C_{14}H_{15}NO_3$: C, 68.56; H, 6.16; N, 5.71. Found: C, 68.39; H, 5.82; N, 6.01.

*4.1.6. Procedure for the preparation of 5-(2-(ethoxycarbonyl)-5-(4-methoxyphenyl)-1H-pyrrol-3-yl)-5 oxopentanoic acid **(11)***

A suspension of $AlCl_3$ (6.56 g, 49 mmol) and glutaric anhydride (1.86 g, 16 mmol) in anhydrous dichloromethane (30 mL) was stirred at room temperature. After 1 h, a solution of **10** (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. Brown oil; Yield 60%; IR (cm^{-1}): 3449 (NH), 3344 (OH), 1702 (CO), 1682 (CO), 1644 (CO); ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.30 (t, 3H, J = 7.1 Hz, CH_3), 1.63–1.80 (m, 2H, CH_2), 2.25 (t, 2H, J = 7.2 Hz, CH_2), 77 (t, 2H, J = 7.2 Hz, CH_2), 3.81 (s, 3H, CH_3), 4.27 (q, 2H, J = 7.1 Hz, CH_2), 6.97 (d, 2H, J = 8.8 Hz, H-3′ and H-5′), 7.33 (s, 1H, Ar), 7.50 (d, 2H, J = 8.8 Hz, H-2′ and H-6'), 12.12 (s, 1H, OH), 12.46 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 14.3, 19.9, 32.7, 49.4, 55.2, 59.9, 113.0 (2 x C), 117.6, 121.2, 121.7, 123.2, 131.2 (2 x C), 139.9, 159.5, 160.0, 174.1, 195.1. Anal Calcd. for $C_{19}H_{21}NO_6$: C, 63.50; H, 5.89; N, 3.90. Found: C, 63.31; H, 6.08; N 4.02.

*4.1.7. Procedure for the preparation of 5-(2-(ethoxycarbonyl)-5-(4-methoxyphenyl)-1H-pyrrol-3-yl)pentanoic acid **(12)***

To a solution of **11** (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 h. The solvent was evaporated under reduced pressure, and the residue was added to water and ice. The solid that formed was filtered, dried and purified using column chromatography (dichloromethane: ethyl acetate 84 : 16). Brown oil; yield 61%; IR (cm^{-1}): 3434 (NH), 3355 (OH), 1700 (CO), 1675 (CO); ^1H NMR (DMSO-d_6, 200 MHz, ppm): δ 1.28 (t, 3H, J = 7.1 Hz, CH_3), 1.47–1.57 (m, 4H, 2 x CH_2), 2.19 (t, 2H, J = 6.5 Hz, CH_2), 2.46–2.52 (m, 2H, CH_2), 3.79 (s, 3H, CH_3), 4.22 (q, 2H, J = 7.1 Hz, CH_2), 6.71 (s, 1H, Ar), 6.99 (d, 2H, J = 8.7 Hz, H-2′ and H-6′), 7.43 (d, 2H, J = 8.7 Hz, H-3′ and H-5'), 11.68 (s, 1H, OH), 12.00 (s, 1H, NH); ^{13} C NMR (DMSO-d_6, 200 MHz, ppm): δ 14.4, 24.3, 25.5, 29.7, 33.4, 55.1, 59.3, 113.8 (2 x C), 116.2, 120.7, 121.4, 124.4, 129.2 (2 x C), 133.9, 158.4, 160.3, 174.5. Anal Calcd. for $C_{19}H_{23}NO_5$: C, 66.07; H, 6.71; N, 4.06. Found: C, 66.23; H, 6.54; N, 4.18.

*4.1.8. Procedure for the preparation of ethyl 3-(4-methoxyphenyl)-8-oxo-2,4,5,6,7,8-hexahydrocyclohepta [c]pyrrole-1-carboxylate **(8c)***

To a solution of **12** (4 g, 12 mmol) in anhydrous dichloromethane, trifluoroacetic anhydride was added (10 mL) at 0 °C. The reaction mixture was stirred at room temperature for 1 h. The solvent was evaporated under reduced pressure, and the residue was purified using column chromatography (petroleum ether: ethyl acetate 9 : 1). Brown solid; yield 61%; mp: 108–109 °C; IR (cm^{-1}): 3438 (NH), 1681 (CO), 1667 (CO); ^1H NMR (DMSO-d_6, 200 MHz, ppm): δ 1.24 (t, 3H, J = 7.1, CH_3), 1.69–1.84 (m, 4H, 2 x CH_2), 2.60–2.73 (m, 4H, 2 x CH_2), 3.80 (s, 3H, CH_3), 4.20 (q, 2H, J = 7.1, CH_2), 7.02 (d, 2H, J = 8.7 Hz, H-3′ and H-5′), 7.38 (d, 2H, J = 8.7 Hz, H-2′ and H-6'), 12.14 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 200 MHz, ppm): δ 14.0, 23.3, 23.6, 26.1, 42.2, 55.1, 59.9, 113.8 (2 x C), 120.1, 121.0, 123.3, 130.0 (2 x C), 130.1, 131.8, 158.8, 160.2, 199.3. Anal Calcd. for $C_{19}H_{21}NO_4$: C, 69.71; H, 6.47; N, 4.28. Found: C, 69.56; H, 6.59; N, 4.39.

*4.1.9. Procedure for the preparation of 1-(4-methoxyphenyl)-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one **(8d)***

To a solution of **8c** (0.73 g, 2.2 mmol) in ethanol (31 mL), 50% aqueous KOH (1.74 mL) was added. The reaction mixture was heated under reflux for 3 h. After cooling, the solvent was evaporated under reduced pressure. The residue was poured into water and ice and acidified with 6 M HCl. The formed solid was filtered and dried. A solution of this solid (0.47 g, 1.6 mmol) in ethanol (22 mL) was heated almost to boiling and 6 M HCl (10 mL) was then added. The reaction mixture was heated under reflux for 1 h. The solvent was evaporated under reduced pressure, and the residue was poured into water and ice. The solution was extracted with ethyl acetate and dried on Na_2SO_4, and the solvent evaporated at reduced pressure. Brown solid; mp: 135–136 °C; yield: 60%; IR (cm^{-1}): 3425 (NH), 1668 (CO); ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.81–1.91 (m, 4H, 2 x CH_2), 2.70 (t, 2H, J = 5.8 Hz, CH_2), 2.85 (t, 2H, J = 5.8 Hz, CH_2), 3.83 (s, 3H, CH_2), 6.95 (d, 2H, J = 8.6 Hz, H-3′ and H-5′), 7.33 (d, 3H, J = 8.6 Hz, H-2′ and H-6’), 7.40 (s, 1H, H-3), 9.17 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 22.4, 23.9, 26.3, 41.6, 55.4, 114.2 (2 x C), 120.5, 121.9, 125.1, 126.9, 129.0 (2 x C), 129.4, 158.7, 200.3. Anal Calcd. for $C_{16}H_{17}NO_2$: C, 75.27; H, 6.71; N, 5.49. Found: C, 74.48; H, 6.97; N, 5.63.

*4.1.10. General procedure for the preparation of (4-methoxyphenyl)-8-oxo-2,4,5,6,7,8-hexahydrocyclohepta[c]pyrrole-1-carboxylate **(14a-h,j-y)***

To a solution of **8a-d** (10 mmol) in anhydrous DMF (15 mL), NaH (0.24 g, 10 mmol) was added at 0 °C, and the reaction mixture was stirred for 1 h at room temperature. The appropriate benzyl halide (20 mmol) was added at 0 °C, and the reaction mixture was stirred at room temperature until the reaction was complete (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 (petroleum ether: ethyl acetate 9 : 1).

*4.1.10.1. 2-benzyl-1-phenyl-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one **(14a)***. This compound was obtained from reaction of compound **8a** with benzyl bromide after 3 h. Yellow oil; yield 90%; IR (cm^{-1}): 1661 (CO); ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.81–1.93 (m, 4H, 2 x CH_2), 2.66–2.74 (m, 4H, 2 x CH_2), 4.96 (s, 2H, CH_2), 6.98 (t, 2H, J = 7.0 Hz, Ar), 7.20–7.28 (m, 4H, Ar), 7.36–7.40 (m, 5H, Ar and H-3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 22.4, 24.3, 26.3, 41.9, 51.4, 122.8, 125.1, 125.3, 127.2, 127.7, 128.0 (2 x C), 128.4 (2 x C), 128.7 (2 x C), 130.9 (2 x C), 131.5, 131.7, 137.1, 199.4. Anal. Calcd. for $C_{22}H_{21}NO$: C, 83.78; H, 6.71; N, 4.44. Found: C, 84.02; H, 6.49; N, 4.72.

*4.1.10.2. 2-(2-methoxybenzyl)-1-phenyl-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one **(14b)***. This compound was obtained from reaction of compound **8a** with 2-methoxybenzyl chloride after 8 h. Yellow oil; yield 60%; IR (cm^{-1}): 1656 (CO); ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.66–1.85 (m, 4H, 2 x CH_2), 2.33–2.48 (m, 4H, 2 x CH_2), 3.81 (s, 3H, CH_3), 5.11 (s, 2H, CH_2), 6.81–6.91 (m, 2H, Ar), 7.09–7.17 (m, 2H, Ar), 7.31–7.48 (m, 3H, Ar), 7.57–7.67 (m, 3H, Ar); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 22.6, 24.3, 26.4, 41.7, 46.3, 55.3, 110.2, 120.6, 122.4, 123.8, 124.7, 125.2, 128.1 (2 x C), 128.5, 128.7, 129.0, 129.2 (2 x C), 131.2, 156.7, 159.4, 199.5. Anal. Calcd. for $C_{23}H_{23}NO_2$: C, 79.97; H, 6.71; N, 4.05. Found: C, 80.12; H, 6.92; N, 3.87.

*4.1.10.3. 2-(3-methoxybenzyl)-1-phenyl-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one **(14c)***. This compound was obtained from reaction of compound **8a** with 3-methoxybenzyl chloride after 5 h. Yellow oil; yield 78%; IR (cm^{-1}): 1657 (CO); ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.78–1.92 (m, 4H, 2 x CH_2), 2.62–2.73 (m, 4H, CH_2), 3.73 (s, 3H, CH_3), 4.92 (s, 2H, CH_2), 6.47–6.58 (m, 2H, Ar), 6.75–6.80 (m, 2H, Ar), 7.14–7.24 (m, 3H, Ar), 7.32–7.42 (m, 3H, Ar); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 22.4, 24.2, 26.2, 41.9, 51.3, 55.2, 112.9, 113.0, 119.5, 122.8 (s), 125.1 (s), 125.3, 128.0, 128.5 (2 x C), 129.8, 130.9 (2 x C), 131.3, 131.7, 138.6, 159.8, 199.5. Anal. Calcd. for $C_{23}H_{23}NO_2$: C, 79.97; H, 6.71; N, 4.05. Found: C, 80.18; H, 7.02; N, 3.88.

*4.1.10.4. 2-(4-methoxybenzyl)-1-phenyl-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one **(14d)***. This compound was obtained from reaction of compound **8a** with 4-methoxybenzyl chloride after 6 h. Yellow oil; yield 67%; IR (cm^{-1}): 1658 (CO); ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.77–1.91 (m, 4H, 2 x CH_2), 2.61–2.72 (m, 4H, 2 x CH_2), 3.77 (s, 3H, CH_3), 4.87 (s, 2H, CH_2), 6.70–6.92 (m, 4H, Ar), 7.15–7.41 (m, 6H, Ar); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 22.4, 24.2, 26.2, 41.9, 50.9, 55.3, 114.1 (2 x C), 122.8, 124.9, 125.1, 128.0, 128.4 (2 x C), 128.7 (2 x C), 128.9, 130.9 (2 x C), 131.4, 131.6, 159.0, 199.5. Anal. Calcd. For $C_{23}H_{23}NO_2$: C, 79.97; H, 6.71; N, 4.05. Found: C, 79.71; H, 6.98; N, 3.87.

4.1.10.5. 2-(2,5-Dimethoxybenzyl)-1-phenyl-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one **(14e).** This compound was obtained from reaction of compound **8a** with 2,5-dimethoxybenzyl chloride after 4–1/2 h. Yield 82%; oil; IR (cm^{-1}): 1655 (CO); ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.80–1.92 (m, 4H, 2 x CH_2), 2.66–2.73 (m, 4H, 2 x CH_2), 3.68 (s, 3H, CH_3), 3.71 (s, 3H, CH_3), 4.94 (s, 2H, CH_2), 6.30 (s, 1H, Ar), 6.75 (s, 2H, Ar), 7.29–7.42 (m, 6H, Ar and H-3); ^{13}C NMR ($CDCl_3$, 200 MHz, ppm): δ 22.4, 24.2, 26.3, 41.9, 46.3, 55.6, 55.8, 111.3, 113.2, 115.2, 122.6, 124.9, 125.6, 126.5, 127.8, 128.4 (2 x C), 130.9 (2 x C), 131.6, 132.7, 151.0, 153.6, 199.3. Anal. Calcd. for $C_{24}H_{25}NO_3$: C, 76.77; H, 6.71; N, 3.73. Found: C, 77.03; H, 6.47; N, 3.95.

*4.1.10.5. 2-(3,4-Dimethoxybenzyl)-1-phenyl-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one **(14f)***. This compound was obtained from reaction of compound **8a** with 3,4-dimethoxybenzyl chloride after 4 h. Yellow oil; yield 73%; IR (cm^{-1}): 1660 (CO); ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.80–1.89 (m, 4H, 2 x CH_2), 2.66–2.70 (m, 4H, 2 x CH_2), 3.74 (s, 3H, CH_3), 3.84 (s, 3H, CH_3), 4.89 (s, 2H, CH_2), 6.40 (s, 1H, Ar), 6.55 (d, 1H, J = 8.0 Hz, Ar), 6.75 (d, 1H, J = 8.0 Hz, Ar), 7.23 (d, 2H, J = 6.6 Hz, Ar), 7.37–7.44 (m, 4H, Ar); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 22.4, 24.2, 26.2, 41.8, 51.3, 55.8, 55.9, 110.4, 110.6, 110.9, 111.0, 111.2, 119.3, 119.9, 120.5, 125.2, 127.9, 128.5 (2 x C), 129.3, 130.9 (2 x C), 131.5, 199.5. Anal. Calcd. for $C_{24}H_{25}NO_3$: C, 76.77; H, 6.71; N, 3.73. Found: C, 76.65; H, 6.49; N, 4.01.

*4.1.10.6. 2-(3,4,5-Trimethoxybenzyl)-1-phenyl-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one **(14g)***. This compound was obtained from reaction of compound **8a** with 3,4,5-trimethoxybenzyl chloride after 6 h. Yield 98%; oil; IR (cm^{-1}): 1662 (CO); ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.79–1.92 (m, 4H, 2 x CH_2), 2.64–2.73 (m, 4H, 2 x CH_2), 3.73 (s, 6H, 2 x CH_3), 3.81 (s, 3H, CH_3), 4.89 (s, 2H, CH_2), 6.13 (s, 2H, H-2″ and H-6”), 6.62 (s, 1H, Ar), 7.36–7.45 (m, 5H, Ar); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 22.3, 24.1, 26.2, 41.8, 51.8, 56.0 (2 x C), 60.8 (q), 104.5 (2 x C), 123.1, 125.0, 125.2, 128.0, 128.5 (2 x C), 131.0 (2 x C), 131.4, 131.5, 132.3, 132.4, 153.3 (2 x C), 199.5. Anal. Calcd. for $C_{25}H_{27}NO_4$: C, 74.05; H, 6.71; N, 3.45. Found: C, 74.23; H, 6.48; N, 3.61.

*4.1.10.7. 2-(4-Methoxy-3-nitrobenzyl)-1-phenyl-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one **(14h)***. This compound was obtained from reaction of compound **8a** with 3-nitro,4-methoxybenzyl chloride after 6 h. Brown solid; yield 90%; mp: 194–195 °C; IR (cm^{-1}): 1656 (CO), 1535 (NO_2); ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.68–175 (m, 4H, 2 x CH_2), 2.51–2.58 (m, 4H, 2 x CH_2), 3.85 (s, 3H, CH_3), 5.10 (s, 2H, CH_2), 7.12 (d, 1H, J = 8.5 Hz, Ar), 7.23 (d, 3H,

J = 8.4 Hz, Ar), 7.34–7.45 (m, 4H, Ar), 7.59 (s, 1H, H-3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 22.1, 23.9, 26.1, 41.7, 49.7, 57.1, 115.0, 122.9, 124.3, 124.9, 126.0, 128.4, 129.0 (2 x C), 130.3, 131.0 (2 x C), 131.1, 131.3, 133.8, 139.1, 151.8, 197.8. Anal. Calcd. for $C_{23}H_{22}N_2O_4$: C, 70.75; H, 5.68; N, 7.18. Found: C, 70.51; H, 5.89; N, 7.36.

4.1.10.8. Ethyl 2-benzyl-3-(4-methoxyphenyl)-8-oxo-2,4,5,6,7,8-hexahydrocyclohepta[c]pyrrole-1-carboxylate ***(14j)***. This compound was obtained from reaction of **8c** with benzyl bromide after 3 h. Yellow oil; yield 63%; IR (cm^{-1}): 1688 (CO), 1661 (CO); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.19 (t, 3H, J = 7.1 Hz, CH_3), 1.76–1.84 (m, 2H, CH_2), 1.90–1.99 (m, 2H, CH_2), 2.56 (t, 2H, J = 6.0 Hz, CH_2), 2.78 (t, 2H, J = 6.0 Hz, CH_2), 3.84 (s, 3H, CH_3), 4.19 (q, 2H, J = 7.1 Hz, CH_2), 5.28 (s, 2H, CH_2), 6.83–6.86 (m, 2H, Ar), 6.90 (d, 2H, J = 8.7 Hz, Ar), 7.09 (d, 2H, J = 8.7 Hz, Ar), 7.21–7.28 (m, 3H, Ar); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 13.7, 23.9, 24.5, 26.7, 42.9, 49.1, 55.3, 61.0, 114.0 (2 x C), 122.0, 122.7, 122.9, 126.2 (2 x C), 127.1, 128.4 (2 x C), 129.8, 132.2 (2 x C), 134.9, 138.1, 159.8, 162.1, 200.6. Anal. Calcd. for $C_{26}H_{27}NO_4$: C, 74.80; H, 6.52; N, 3.35. Found: C, 74.68; H, 6.74; N, 3.48.

4.1.10.9. Ethyl 2-(2-methoxybenzyl)-3-(4-methoxyphenyl)-8-oxo-2,4,5,6,7,8-hexahydrocyclohepta[c]pyrrole-1-carboxylate ***(14k)***. This compound was obtained from reaction of **8c** with 2-methoxybenzyl chloride after 5 h. Yellow oil; yield 71%; IR (cm^{-1}): 1691 (CO), 1665 (CO); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.14 (t, 3H, J = 7.1 Hz, CH_3), 1.72–1.85 (m, 2H, CH_2), 1.88–1.97 (m, 2H, CH_2), 2.55 (t, 2H, J = 5.9 Hz, CH_2), 2.78 (t, 2H, J = 5.9 Hz, CH_2), 3.70 (s, 3H, CH_3), 3.81 (s, 3H, CH_3), 4.16 (q, 2H, J = 7.1 Hz, CH_2), 5.23 (s, 2H, CH_2), 6.47 (d, 1H, J = 7.5 Hz, CH), 6.73–6.88 (4H, m, Ar), 7.03–7.21 (3H, m, Ar); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 13.6, 23.9, 24.5, 26.8, 42.9, 44.9, 55.1, 55.3, 60.9, 109.6, 113.8 (2 x C), 120.5, 121.9, 122.8, 123.3, 126.5, 126.8, 128.0, 129.6, 132.0 (2 x C), 135.0, 155.8, 159.6, 161.8, 200.8. Anal. Calcd. for $C_{27}H_{29}NO_5$: C, 72.46; H, 6.53; N, 3.13. Found: C, 72.61; H, 6.67; N, 2.95.

4.1.10.10. Ethyl 2-(3-methoxybenzyl)-3-(4-methoxyphenyl)-8-oxo-2,4,5,6,7,8-hexahydrocyclohepta[c]pyrrole-1-carboxylate ***(14l)***. This compound was obtained from reaction of **8c** with 3-methoxybenzyl chloride after 6 h. Yellow oil; yield 76%; IR (cm^{-1}): 1692 (CO), 1667 (CO); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.19 (t, 3H, J = 7.2 Hz, CH_3), 1.70–1.83 (m, 2H, CH_2), 1.87–1.99 (m, 2H, CH_2), 2.54 (t, 2H, J = 5.9 Hz, CH_2), 2.76 (t, 2H, J = 5.9 Hz, CH_2), 3.71 (s, 3H, CH_3), 3.82 (s, 3H, CH_3), 4.19 (q, 2H, J = 7.2 Hz, CH_2), 5.24 (s, 2H, CH_2), 6.37–6.44 (m, 2H, Ar), 6.69–6.75 (m, 1H, Ar), 6.90 (d, 2H, J = 8.7 Hz, Ar), 7.06–7.17 (m, 3H, Ar); ^{13}C NMR ($CDCl_3$, 200 MHz, ppm): δ 13.8, 23.9, 24.5, 26.8, 42.9, 49.0, 55.1, 55.3, 61.1, 111.9, 112.6, 114.0 (2 x C), 118.6, 122.0, 122.7, 122.9, 129.5, 129.9, 132.2 (2 x C), 134.9, 139.8, 159.6, 159.8, 162.0, 200.7. Anal. Calcd. for $C_{27}H_{29}NO_5$: C, 72.46; H, 6.53; N, 3.13. Found: C, 72.32; H, 6.41; N, 3.29.

4.1.10.11. Ethyl 2-(4-methoxybenzyl)-3-(4-methoxyphenyl)-8-oxo-2,4,5,6,7,8-hexahydrocyclohepta[c]pyrrole-1-carboxylate ***(14m)***. This compound was obtained from reaction of **8c** with 4-methoxybenzyl chloride after 4 h. Yellow oil; yield 75%; IR (cm^{-1}): 1690 (CO), 1662 (CO); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.21 (t, 3H, J = 7.1 Hz, CH_3), 1.74–1.95 (m, 4H, 2 x CH_2), 2.53 (t, 2H, J = 5.8 Hz, CH_2), 2.75 (t, 2H, J = 5.8 Hz, CH_2), 3.74 (s, 3H, CH_3), 3.83 (s, 3H, CH_3), 4.20 (q, 2H, J = 7.1 Hz, CH_2), 5.19 (s, 2H, CH_2), 6.70–6.79 (m, 4H, Ar), 6.90 (d, 2H, J = 8.8 Hz, Ar), 7.27 (d, 2H, J = 8.8 Hz, Ar); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 13.8, 23.8, 24.4, 26.7, 42.9, 48.5, 55.2, 55.3, 61.1, 113.8 (2 x C), 113.9 (2 x C), 122.1, 122.8, 127.7 (2 x C), 128.7, 129.7, 130.2, 132.2 (2 x C), 134.7, 158.7, 159.7, 162.2, 200.6. Anal. Calcd. for $C_{27}H_{29}NO_5$: C, 72.46; H, 6.53; N, 3.13. Found: C, 72.29; H, 6.82; N, 3.02.

4.1.10.12. 2-(2-Methoxybenzyl)-1-(4-methoxyphenyl)-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one ***(14n)***. This compound was obtained from reaction of compound **8d** with 2-methoxybenzyl chloride after 7 h. Yellow oil; yield 70%; IR (cm^{-1}): 1658 (CO); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.77–1.94 (m, 4H, 2 x CH_2), 2.60–2.72 (m, 4H, 2 x CH_2), 3.75 (s, 3H, CH_3), 3.83 (s, 3H, CH_3), 4.92 (s, 2H, CH_2), 6.68–6.95 (m, 5H, Ar), 7.12–7.27 (m, 3H, Ar), 7.33 (s, 1H, H-3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 22.5, 24.3, 26.3, 41.9, 46.3, 55.3 (2 x C), 110.2, 113.8 (2 x C), 120.6, 122.4, 123.8, 124.7, 125.2, 125.45, 128.7, 129.0, 131.5, 132.1 (2 x C), 156.6, 159.2, 199.5. Anal. Calcd. for $C_{24}H_{25}NO_3$: C, 76.77; H, 6.71; N, 3.73. Found: C, 77.02; H, 7.03; N, 3.58.

4.1.10.13. 2-(3-Methoxybenzyl)-1-(4-methoxyphenyl)-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one ***(14o)***. This compound was obtained from reaction of compound **8d** with 3-methoxybenzyl chloride after 6 h. Yellow oil; yield 62%; IR (cm^{-1}): 1657 (CO); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.77–2.00 (m, 4H, 2 x CH_2), 2.60–2.77 (m, 4H, 2 x CH_2), 3.73 (s, 3H, CH_3), 3.83 (s, 3H, CH_3), 4.88 (s, 2H, CH_2), 6.43–6.62 (m, 2H, Ar), 6.74–6.98 (m, 3H, Ar), 7.09–7.26 (m, 3H, Ar), 7.35 (s, 1H, H-3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 22.4, 24.3, 26.3, 41.9, 51.2, 55.2, 55.3, 101.0, 112.9, 113.0, 113.9 (2 x C), 119.4, 122.6, 123.5, 125.0, 129.8, 131.4, 132.2 (2 x C), 138.8, 159.4, 159.8, 199.5. Anal. Calcd. for $C_{24}H_{25}NO_3$: C, 76.77; H, 6.71; N, 3.73. Found: C, 76.49; H, 6.98; N, 3.85.

4.1.10.14. 2-(4-Methoxybenzyl)-1-(4-methoxyphenyl)-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one ***(14p)***. This compound was obtained from reaction of compound **8d** with 4-methoxybenzyl chloride after 12 h. Yellow oil; yield 78% IR (cm^{-1}): 1659 (CO); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.71–1.91 (m, 4H, 2 x CH_2), 2.59–2.71 (m, 4H, 2 x CH_2), 3.77 (s, 3H, CH_3), 3.84 (s, 3H, CH_3), 4.84 (s, 2H, CH_2), 6.78 (d, 2H, J = 8.8 Hz, Ar), 6.88–6.95 (m, 4H, Ar), 7.12 (d, 2H, J = 8.8 Hz, Ar), 7.33 (s, 1H, H-3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 22.4, 24.3, 26.2, 42.0, 50.89, 55.3 (2 x C), 113.8 (2 x C), 114.1 (2 x C), 122.7, 123.6, 124.7, 124.8, 128.7 (2 x C), 129.1, 131.3, 132.2 (2 x C), 159.2, 159.3, 199.5. Anal. Calcd. for $C_{24}H_{25}NO_3$: C, 76.77; H, 6.71; N, 3.73. Found: C, 76.89; H, 6.64; N, 3.98.

4.1.10.15. 1-(4-Methoxyphenyl)-2-(3,4,5-trimethoxybenzyl)-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one ***(14q)***. This compound was obtained from reaction of compound **8d** with 3,4,5-trimethoxybenzyl chloride after 12 h. Yellow oil; yield 63%; IR (cm^{-1}): 1655 (CO); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.73–1.83 (m, 4H, 2 x CH_2), 2.47–2.52 (m, 4H, 2 x CH_2), 3.80 (s, 6H, 2 x CH_3), 3.81 (s, 3H, CH_3), 3.82 (s, 3H, CH_3), 5.18 (s, 2H, CH_2), 6.51 (m, 2H, H-2″ and H-6”), 7.04 (d, 2H, J = 7.1 Hz, H-3′ and H-5′), 7.11 (s, 1H, H-3), 7.53 (d, 2H, J = 7.1 Hz, H-2′ and H-6’); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 22.4, 24.2, 26.2, 41.6, 49.2, 56.0 (2 x C), 56.1, 60.8, 104.8 (2 x C), 105.2, 107.8, 113.8 (2 x C), 122.8, 125.2, 130.8, 131.8 (2 x C), 136.0, 136.2 153.1 (2 x C), 159.4, 200.0. Anal. Calcd. for $C_{26}H_{29}NO_5$: C, 71.70; H, 6.71; N, 3.22. Found: C, 71.46; H, 6.51; N, 3.49.

4.1.10.16. 2-Benzyl-1-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one ***(14r)***. This compound was obtained from reaction of compound **8b** with benzyl bromide after 3 h. Yellow oil; yield 68%; IR (cm^{-1}): 1659 (CO); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.80–1.97 (m, 4H, 2 x CH_2), 2.68–2.75 (m, 4H, 2 x CH_2), 3.69 (s, 6H, 2 x CH_3), 3.89 (s, 3H, CH_3), 4.98 (s, 2H, CH_2), 6.33 (s, 2H, H-2″ and H-6″), 6.99–7.02 (m, 2H, Ar), 7.25–7.32 (m, 3H, Ar), 7.43 (s, 1H, H-3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 22.3, 24.1, 26.2, 30.1, 41.8,

51.4 (2 x C), 56.0, 107.8 (2 x C), 122.7, 125.0, 125.4, 126.5, 126.8 (2 x C), 127.7, 128.7 (2 x C), 131.6, 137.5, 137.9, 152.9 (2 x C), 199.2. Anal. Calcd. for $C_{25}H_{27}NO_4$: C, 74.05; H, 6.71; N, 3.45. Found: C, 74.27; H, 6.56; N, 3.32.

4.1.10.17. 2-(2-Methoxybenzyl)-1-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one ***(14s)***. This compound was obtained from reaction of compound **8b** with 2-methoxybenzyl chloride after 7 h. Yellow oil; yield 68%; IR (cm^{-1}): 1659 (CO); ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.68–1.84 (m, 4H, 2 x CH_2), 2.33–2.56 (m, 4H, CH_2), 3.78 (s, 6H, 2 x CH_3), 3.80 (s, 3H, CH_3), 3.82 (s, 3H, CH_3), 5.20 (s, 2H, CH_2), 6.81–6.92 (m, 2H, Ar), 7.01 (1H, s, Ar), 7.10 (s, 2H, Ar), 7.14–7.21 (m, 2H, Ar); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 22.9, 24.6, 26.7, 41.0, 46.3, 55.3, 56.8 (3 x C), 60.7, 106.2 (2 x C), 110.8, 120.2, 122.6, 123.9, 124.5, 125.8, 125.6, 128.9, 129.1, 131.6, 153.9 (2 x C), 156.9, 159.1, 199.5. Anal. Calcd. for $C_{26}H_{29}NO_5$: C, 71.70; H, 6.71; N, 3.22. Found: C, 71.55; H, 6.37; N, 3.48.

4.1.10.18. 2-(3-Methoxybenzyl)-1-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one ***(14t)***. This compound was obtained from reaction of compound **8b** with 3-methoxybenzyl chloride after 6 h. Yellow oil; yield 82%; IR (cm^{-1}): 1656 (CO); ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.83–1.94 (m, 4H, 2 x CH_2), 2.68–2.74 (m, 4H, 2 x CH_2), 3.71 (s, 6H, 2 x CH_3), 3.75 (s, 3H, CH_3), 3.89 (s, 3H, CH_3), 4.94 (s, 2H, CH_2), 6.36 (s, 2H, H-2″ and H-6″), 6.53 (s, 1H, Ar), 6.60 (1H, d, J = 7.5 Hz, Ar), 6.79 (1H, dd, J = 8.1, 2.1 Hz, Ar), 7.22 (t, 1H, J = 7.9 Hz, Ar), 7.42 (s, 1H, H-3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 22.4, 24.4, 26.3, 41.9, 51.5, 55.2, 55.9 (2 x C), 60.9, 108.1 (2 x C), 112.7, 112.8, 119.1, 122.7, 125.0, 125.4, 129.8, 131.6, 135.4, 137.8, 139.1, 153.0 (2 x C), 160.0, 199.4. Anal. Calcd. for $C_{26}H_{29}NO_5$: C, 71.70; H, 6.71; N, 3.22. Found: C, 72.01; H, 6.39; N, 3.45.

4.1.10.19. 2-(4-Methoxybenzyl)-1-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one ***(14u)***. This compound was obtained from reaction of compound **8b** with 4-methoxybenzyl chloride after 6 h. Yield 66%; yellow oil; IR (cm^{-1}): 1658 (CO); ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.81–1.93 (m, 4H, 2 x CH_2), 2.66–2.73 (m, 4H, 2 x CH_2), 3.74 (s, 6H, 2 x CH_3), 3.78 (s, 3H, CH_3), 3.90 (s, 3H, CH_3), 4.90 (s, 2H, CH_2), 6.36 (s, 2H, H-2″ and H-6″), 6.81 (d, 2H, J = 8.7 Hz, Ar), 6.93 (d, 2H, J = 8.7 Hz, Ar), 7.39 (s, 1H, H-3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 22.4, 24.4, 26.3, 41.9, 51.1, 55.3, 56.0 (2 x C), 60.9, 108.1 (2 x C), 114.1 (2 x C), 122.7, 124.8, 125.2, 126.7, 128.4 (2 x C), 129.3, 131.5, 137.8, 153.0 (2 x C), 159.2, 199.3. Anal. Calcd. for $C_{26}H_{29}NO_5$: C, 71.70; H, 6.71; N, 3.22. Found: C, 71.47; H, 6.56; N, 3.39.

4.1.10.20. 2-(2,5-Dimethoxybenzyl)-1-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one ***(14v)***. This compound was obtained from reaction of compound **8b** with 2,5-dimethoxybenzyl chloride after 8 h. Yellow oil; yield 61%; IR (cm^{-1}): 1659 (CO); ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.81–1.93 (m, 4H, 2 x CH_2), 2.68–2.74 (m, 4H, 2 x CH_2), 3.68 (s, 3H, CH_3), 3.72 (s, 9H, 3 x CH_3), 3.88 (s, 3H, CH_3), 4.94 (s, 2H, CH_2), 6.32 (s, 1H, Ar), 6.40 (s, 2H, H-2″ and H-6″), 6.75 (s, 2H, Ar), 7.41 (s, 1H, H-3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 21.7, 23.6, 25.5, 41.0, 45.9, 54.9, 55.0, 55.2 (2 x C), 60.1, 107.2 (2 x C), 110.4, 112.0, 114.4, 121.9, 123.8, 125.2, 126.0, 130.9, 149.9, 150.0, 152.2 (2 x C), 152.9, 156.7, 199.0. Anal. Calcd. for $C_{27}H_{31}NO_6$: C, 69.66; H, 6.71; N, 3.01. Found: C, 69.51; H, 6.88; N, 3.29.

4.1.10.21. 2-(3,4-Dimethoxybenzyl)-1-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one ***(14w)***. This compound was obtained from reaction of compound **8b** with 3,4-dimethoxybenzyl chloride after 6 h. Yellow oil; yield 61%; IR (cm^{-1}): 1656 (CO); ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.68–1.85 (m, 4H, 2 x CH_2), 2.34–2.65 (m, 4H, 2 x CH_2), 3.77 (s, 6H, 2 x CH_3), 3.79 (s, 9H, 3 x CH_3), 5.15 (s, 2H, CH_2), 6.77–7.01 (m, 5H, Ar), 7.20 (s, 1H, Ar); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 22.4, 24.2, 26.2, 41.8, 51.3, 55.4 (2 x C), 55.8, 55.9, 60.3, 106.2 (2 x C), 110.1, 110.4, 111.1, 111.7, 112.2, 119.1, 119.7, 120.7, 124.8, 129.9, 131.7, 133.2, 153.0 (2 x C), 199.4. Anal. Calcd. for $C_{27}H_{31}NO_6$: C, 69.66; H, 6.71; N, 3.01. Found: C, 69.47; H, 6.92; N, 3.54.

4.1.10.22. 2-(3,4,5-Trimethoxybenzyl)-1-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one ***(14x)***. This compound was obtained from reaction of compound **8b** with 3,4,5-trimethoxybenzyl chloride after 12 h. Yellow oil; yield 64%; IR (cm^{-1}): 1661 (CO); ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.80–1.91 (m, 4H, 2 x CH_2), 2.66–2.72 (m, 4H, 2 x CH_2), 3.71 (s, 6H, 2 x CH_3), 3.72 (s, 6H, 2 x CH_3), 3.88 (s, 3H, CH_3), 3.89 (s, 3H, CH_3), 4.81 (s, 2H, CH_2), 6.15 (s, 2H, Ar), 6.34 (s, 2H, Ar), 7.23 (s, 1H, Ar); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 22.3, 24.2, 26.3, 41.8, 56.0 (2 x C), 56.1 (2 x C), 60.7, 60.8, 60.9, 104.8 (2 x C), 107.8 (2 x C), 122.9, 124.2, 124.9, 125.0, 126.5, 131.1, 136.1, 142.0, 153.1 (2 x C), 153.2 (2 x C), 199.3. Anal. Calcd. for $C_{28}H_{33}NO_7$: C, 67.86; H, 6.71; N, 2.83. Found: C, 68.03; H, 6.99; N, 2.61.

4.1.10.23. 2-(4-Methoxy-3-nitrobenzyl)-1-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one ***(14y)***. This compound was obtained from reaction of compound **8b** with 3-nitro,4-methoxybenzyl chloride after 3 h. Yellow solid; yield 65%; mp: 198–199 °C; IR (cm^{-1}): 1660 (CO), 1531 (NO_2); ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.77–1.87 (m, 4H, 2 x CH_2), 2.62–2.70 (m, 4H, 2 x CH_2), 3.75 (s, 6H, 2 x CH_3), 3.86 (s, 3H, CH_3), 3.89 (s, 3H, CH_3), 4.94 (s, 2H, CH_2), 6.34 (s, 2H, H-2″ and H-6″), 6.97 (d, 1H, J = 8.7 Hz, Ar), 7.14 (dd, 1H, J = 8.7, 2.0 Hz, Ar), 7.34 (s, 2H, Ar); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 22.3, 24.3, 26.2, 41.9, 50.2, 56.1 (2 x C), 56.6, 60.9, 108.0 (2 x C), 113.8, 123.2, 124.4, 124.8, 125.3, 126.4, 129.7, 131.4, 132.8, 138.1, 139.5, 152.3, 153.2 (2 x C), 199.3. Anal. Calcd. for $C_{26}H_{28}N_2O_7$: C, 64.99; H, 5.87; N, 5.83. Found: C, 64.74; H, 5.99; N, 5.67.

4.1.11. General procedure for the preparation of 2-(3-amino-4-methoxybenzyl)-1-substituted-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one ***(14i,z)***

To a solution of **14h,y** (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.

4.1.11.1. 2-(3-Amino-4-methoxybenzyl)-1-phenyl-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one ***(14i)***. This compound was obtained from reaction of compound **14h**. Yellow oil; yield 86%; IR (cm^{-1}): 3461–3389 (NH_2), 1651 (CO); ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.80–1.92 (m, 4H, 2 x CH_2), 2.66–2.73 (m, 4H, 2 x CH_2), 3.83 (s, 3H, CH_3), 4.78 (s, 2H, NH_2), 4.81 (s, 2H, CH_2), 6.36–6.39 (m, 2H, Ar), 6.61–6.70 (m, 1H, Ar), 7.23–7.43 (m, 6H, Ar); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 22.4, 24.2, 26.2, 41.5, 51.1, 55.5, 110.2, 113.9, 117.5, 122.7, 125.2, 127.3, 128.1, 128.4 (2 x C), 129.4, 130.9 (2 x C), 130.9, 130.1, 131.4, 136.4, 199.5. Anal. Calcd. for $C_{23}H_{24}N_2O_2$: C, 76.64; H, 6.71; N, 7.77. Found: C, 76.33; H, 7.04; N, 7.98.

4.1.11.2. 2-(3-Amino-4-methoxybenzyl)-1-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one ***(14z)***. This compound was obtained from reaction of compound **14y**. Yellow oil; yield 71%;

IR (cm^{-1}): 3444–3361 (NH_2), 1655 (CO); ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.80–1.91 (m, 4H, 2 x CH_2), 2.66–2.72 (m, 4H, 2 x CH_2), 3.73 (s, 6H, 2 x CH_3), 3.81 (s, 3H, CH_3), 3.89 (s, 3H, CH_3), 4.76 (s, 2H, NH_2), 4.81 (s, 2H, CH_2), 6.33–6.37 (m, 4H, Ar), 6.66 (d, 1H, J = 8.0 Hz, Ar), 7.38 (s, 1H, Ar); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 21.6, 23.6, 25.5, 41.1, 50.5, 54.8, 55.2 (2 x C), 60.1, 107.4 (2 x C), 109.5, 112.6, 116.2, 116.9, 121.8, 123.9, 124.6, 125.9, 129.1, 130.8, 135.8, 146.0, 152.1 (2 x C), 198.7. Anal.Calcd. for $C_{26}H_{30}N_2O_5$: C, 69.31; H, 6.71; N, 6.22. Found: C, 69.54; H, 6.43; N, 6.39.

General procedure for the preparation of 5-((dimethylamino)methylene)-1-substituted-5,6,7,8-tetrahydrocyclohepta[c]pyrrol-4(2H)-one ***(15a-z).*** To a solution of ketone **14a-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.

4.1.12. General procedure for the preparation of 4,5,6,8-tetrahydropyrrolo [3′,4′:3,4]cyclohepta [1,2-d] [1,2]oxazoles ***(3a-z)***

To a solution of **15a-z** (5 mmol) in ethanol (15 mL) and acetic acid (3 mL), hydroxylamine hydrochloride was added (7.5 mmol). 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 formed was obtained by filtration, dried and purified using a chromatography column (dichloromethane).

4.1.12.1. 8-Benzyl-7-phenyl-4,5,6,8-tetrahydropyrrolo[3′,4′:3,4] cyclohepta [1,2-d] [1,2]oxazole ***(3a)***. This compound was obtained from reaction of compound **15a**. White solid; yield 73%; mp: 107–108 °C; ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 2.02 (s, 2H, CH_2), 2.86 (s, 4H, 2 x CH_2), 5.11 (s, 2H, CH_2), 7.10–7.49 (m, 11H, Ar), 8.12 (s, 1H, H-3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 24.5, 24.7, 27.3, 51.2, 111.2, 112.1, 119.3, 120.0, 127.0 (2 x C), 127.7, 127.9, 128.5 (2 x C), 128.8 (2 x C), 130.9 (2 x C), 131.5, 131.8, 137.9, 151.9, 162.1. Anal. Calcd. for $C_{23}H_{20}N_2O$: C, 81.15; H, 5.92; N, 8.23. Found: C, 80.89; H, 5.67; N, 8.39.

4.1.12.2. 8-(2-methoxybenzyl)-7-phenyl-4,5,6,8-tetrahydropyrrolo [3′,4′:3,4]cyclohepta [1,2-d] [1,2]oxazole ***(3b)***. This compound was obtained from reaction of compound **15b**. White solid; yield 78%; mp: 114–115 °C; ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.85–1.96 (m, 2H, CH_2), 2.69–2.78 (m, 4H, 2 x CH_2), 3.76 (s, 3H, CH_3), 4.99 (s, 2H, CH_2), 6.69–6.87 (m, 3H, Ar), 7.19–7.39 (m, 7H, Ar), 7.98 (s, 1H, H-3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 24.4, 24.6, 27.3, 46.2, 55.3, 110.1, 111.0, 111.7, 119.5, 119.6, 120.6, 126.2, 127.6, 128.2, 128.3 (2 x C), 128.8, 130.8 (2 x C), 131.6, 131.7, 151.7, 156.5, 162.3. Anal. Calcd. for $C_{24}H_{22}N_2O_2$: C, 77.81; H, 5.99; N, 7.56. Found: C, 77.56; H, 6.08; N, 7.78.

4.1.12.3. 8-(3-methoxybenzyl)-7-phenyl-4,5,6,8-tetrahydropyrrolo [3′,4′:3,4]cyclohepta [1,2-d] [1,2]oxazole ***(3c)***. This compound was obtained from reaction of compound **15c**. White solid; yield 68%; mp: 125–126 °C; ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.86–1.97 (m, 2H, CH_2), 2.70–2.78 (m, 4H, 2 x CH_2), 3.73 (s, 3H, CH_3), 4.96 (s, 2H, CH_2), 6.50–6.61 (m, 2H, Ar), 6.77 (dd, 1H, J = 8.2, 2.4 Hz, Ar), 7.15–7.26 (m, 4H, Ar), 7.34–7.44 (m, 3H, Ar), 8.00 (s, 1H, H-3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 24.4, 24.6, 27.2, 51.0, 55.2, 111.2, 112.1, 112.7, 112.8, 119.2, 119.3, 119.9, 127.8, 128.4 (2 x C), 129.8, 130.8 (2 x C), 131.4, 131.7, 139.4, 151.8, 159.8, 162.1. Anal. Calcd. for $C_{24}H_{22}N_2O_2$: C, 77.81; H, 5.99; N, 7.56. Found: C, 78.02; H, 6.08; N, 7.34.

4.1.12.4. 8-(4-Methoxybenzyl)-7-phenyl-4,5,6,8-tetrahydropyrrolo [3′,4′:3,4]cyclohepta [1,2-d] [1,2]oxazole ***(3d)***. This compound was obtained from reaction of compound **15d**. White solid; yield 83%; mp: 194–195 °C; ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.85–1.96 (m, 2H, CH_2), 2.70–2.77 (m, 4H, 2 x CH_2), 3.77 (s, 3H, CH_3), 4.91 (s, 2H, CH_2), 6.79 (d, 2H, J = 8.7, H-3′ and H-5′), 6.92 (d, 2H, J = 8.7, H-2′ and H-6′), 7.16–7.26 (m, 3H, Ar), 7.35–7.41 (m, 3H, Ar), 7.99 (s, 1H, H-3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 24.4, 24.6, 27.2, 50.6, 55.3, 111.1, 111.9, 114.0 (2 x C), 119.0, 119.9, 127.8, 128.4 (4 x C), 129.7, 130.8 (2 x C), 131.5, 131.6, 151.8, 159.0, 162.1. Anal. Calcd. for $C_{24}H_{22}N_2O_2$: C, 77.81; H, 5.99; N, 7.56. Found: C, 78.11; H, 5.78; N, 7.87.

4.1.12.5. 8-(2,5-Dimethoxybenzyl)-7-phenyl-4,5,6,8-tetrahydropyrrolo [3′,4′:3,4]cyclohepta [1,2-d] [1,2]oxazole ***(3e)***. This compound was obtained from reaction of compound **15e**. White solid; yield 80%; mp 174–175 °C; ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 2.03 (2H, s, CH_2), 2.86 (4H, s, 2 x CH_2), 3.79 (3H, s, CH_3), 3.85 (3H, s, CH_3), 5.10 (2H, s, CH_2), 6.34–6.42 (1H, m, Ar), 6.74–6.92 (2H, m, Ar), 7.25–7.55 (6H, m, Ar and H-9), 8.12 (1H, s, H-3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 24.5, 24.7, 27.3, 46.2, 55.7, 55.9, 111.2, 112.8, 114.3, 114.9, 119.6, 119.8, 127.4, 127.7, 128.4 (2 x C), 130.8 (2 x C), 131.7, 150.8, 150.9, 151.8, 153.6, 153.7, 162.3. Anal. Calcd. for $C_{25}H_{24}N_2O_3$: C, 74.98; H, 6.04; N, 7.00. Found: C, 75.21; H, 5.77; N, 7.15.

4.1.12.6. 8-(3,4-Dimethoxybenzyl)-7-phenyl-4,5,6,8-tetrahydropyrrolo [3′,4′:3,4]cyclohepta [1,2-d] [1,2]oxazole ***(3f)***. This compound was obtained from reaction of compound **15f**. White solid; yield 80%; mp 114–115 °C; ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.80–1.93 (2H, m, CH_2), 2.65–2.76 (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), 7.25–7.43 (7H, m, Ar, H-9 and H-3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 22.4, 24.2, 26.2, 51.4, 55.8, 55.9, 110.3, 111.2, 119.9 (2 x C), 123.0, 124.9 125.2 (2 x C), 127.9 (2 x C), 128.4 (2 x C), 129.3, 131.0 (2 x C), 131.4, 131.5, 148.6, 149.0. Anal. Calcd. for $C_{25}H_{24}N_2O_3$: C, 74.98; H, 6.04; N, 7.00. Found: C, 74.79; H, 6.22; N, 7.12.

4.1.12.7. 8-(3,4,5-trimethoxybenzyl)-7-phenyl-4,5,6,8-tetrahydropyrrolo [3′,4′:3,4]cyclohepta [1,2-d] [1,2]oxazole ***(3g)***. This compound was obtained from reaction of compound **15g**. White solid; yield 69%; mp 155–156 °C; ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.91–1.96 (2H, m, CH_2), 2.73–2.78 (4H, m, 2 x CH_2), 3.75 (6H, s, 2 x CH_3), 3.88 (3H, s, 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 ($CDCl_3$, 50 MHz, ppm): δ 24.4, 24.6, 27.2, 51.5, 56.0 (2 x C), 60.9, 104.2 (2 x C), 111.2, 112.0, 119.2, 120.2, 127.8, 128.4 (2 x C), 130.8 (2 x C), 131.5, 131.6, 133.2, 137.3, 151.8 (d), 153.3 (2 x C), 162.0. Anal. Calcd. for $C_{26}H_{26}N_2O_4$: C, 72.54; H, 6.09; N, 6.51. Found: C, 72.35; H, 6.27; N, 6.80.

4.1.12.8. 8-(4-Methoxy-3-nitrobenzyl)-7-phenyl-4,5,6,8-tetrahydropyrrolo[3′,4′:3,4]cyclohepta [1,2-d] [1,2]oxazole ***(3h)***. This compound was obtained from reaction of compound **15h**. White solid; yield 60%; mp: 173–174 °C IR (cm^{-1}): 1533 (NO_2); ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.89–1.96 (m, 2H, CH_2), 2.73–2.77 (m, 4H, 2 x CH_2), 3.94 (s, 3H, CH_3), 4.99 (s, 2H, CH_2), 6.99 (d, 1H, J = 8.7 Hz, Ar), 7.13 (dd, 1H, J = 8.7, 1.8, Ar), 7.19–7.22 (m, 3H, Ar), 7.37–7.42 (m, 4H, Ar), 8.03 (s, 1H, H-3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 24.2, 24.6, 27.1, 49.9, 56.6, 111.5, 112.5, 113.8, 118.9, 120.5, 124.3, 128.1, 128.4, 128.6 (2 x C), 130.1, 130.7 (2 x C), 131.1, 131.6, 132.6, 151.8, 152.3, 161.7. Anal. Calcd. for $C_{24}H_{21}N_3O_4$: C, 69.39; H, 5.10; N, 10.11. Found: C, 69.12; H, 5.26; N, 10.27.

4.1.12.9. 8-(4-Methoxy-3-aminobenzyl)-7-phenyl-4,5,6,8-tetrahydropyrrolo[3′,4′:3,4]cyclohepta [1,2-d] [1,2]oxazole ***(3i)***. This compound was obtained from reaction of compound **15i**. Yellow oil;

yield 63%; (cm^{-1}): 3441–3354 (NH_2); ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.89–1.96 (m, 2H, CH_2), 2.72–2.79 (m, 4H, 2 x CH_2), 3.83 (s, 3H, CH_3), 4.85 (s, 2H, CH_2), 5.23 (s, 2H, NH_2), 6.38–6.40 (m, 2H, Ar), 6.68 (d, 1H, J = 8.7 Hz, Ar), 7.19 (s, 1H, Ar), 7.25–7.28 (m, 2H, Ar), 7.37–7.45 (m, 3H, Ar), 8.02 (s, 1H, H-3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 24.4, 24.6, 27.2, 50.8, 55.5, 110.3, 111.0, 111.7, 113.7, 117.3, 119.2, 119.7, 127.7, 128.3 (2 x C), 130.3, 130.8 (2 x C), 131.5, 131.6, 136.2, 146.8, 151.7, 162.2. Anal. Calcd. for $C_{24}H_{23}N_3O_2$: C, 74.78; H, 6.01; N, 10.90. Found: C, 74.65; H, 6.33; N, 10.54.

*4.1.12.10. Ethyl 8-benzyl-7-(4-methoxyphenyl)-4,5,6,8-tetrahydropyrrolo [3′,4′:3,4]cyclohepta [1,2-d] [1,2]oxazole-9-carboxylate **(3j)**.* This compound was obtained from reaction of compound **15j**. Yellow oil; Yield 93%; ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.25 (t, 3H, J = 7.1 Hz, CH_3), 1.95–2.01 (m, 2H, CH_2), 2.58–2.61 (m, 2H, CH_2), 2.80 (t, 2H, J = 6.4 Hz, CH_2), 3.84 (s, 3H, CH_3), 4.29 (q, 2H, J = 7.1 Hz, CH_2), 5.34 (s, 2H, CH_2), 6.87 (d, 2H, J = 6.7 Hz, Ar), 6.92 (d, 2H, J = 8.8 Hz, Ar), 7.12 (d, 2H, J = 8.8 Hz, Ar), 7.17–7.26 (m, 3H, Ar), 8.08 (s, 1H, H-3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 14.0, 24.3, 25.1, 25.7, 49.1, 55.3, 61.2, 114.0 (2 x C), 114.2, 115.0, 120.8, 122.5, 122.7, 126.1 (2 x C), 127.0, 128.4 (2 x C), 132.2 (2 x C), 135.4, 138.5, 151.8, 159.8, 160.3, 162.4. Anal. Calcd. for $C_{27}H_{26}N_2O_4$: C, 73.28; H, 5.92; N, 6.33. Found: C, 73.59; H, 5.71; N, 6.45.

*4.1.12.11. Ethyl 8-(2-methoxybenzyl)-7-(4-methoxyphenyl)-4,5,6,8-tetrahydropyrrolo[3′,4′:3,4]cyclohepta [1,2-d] [1,2]oxazole-9-carboxylate **(3k)**.* This compound was obtained from reaction of compound **15k**. Yellow oil; yield 83%; ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.21 (t, 3H, J = 7.1 Hz, CH_3), 1.94–2.09 (m, 2H, CH_2), 2.54–2.61 (m, 2H, CH_2), 79 (t, 2H, J = 6.3 Hz, CH_2), 3.72 (s, 3H, CH_3), 3.80 s, (3H, CH_3), 25 (q, 2H, J = 7.1 Hz, CH_2), 5.29 (2H, s, CH_2), 6.49 (d, 1H, J = 7.4 Hz, Ar), 6.74–6.88 (4H, m, Ar), 7.05–7.20 (3H, m, Ar), 8.07 (1H, s, H-3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 13.9, 24.3, 25.2, 25.7, 44.9, 55.2, 55.3, 61.1, 109.7, 113.8 (2 x C), 114.1, 118.4, 120.6, 121.0, 122.4, 122.8, 126.2, 127.2, 127.9, 132.0 (2 x C), 135.4, 151.9, 155.8, 159.6, 160.5, 162.2. Anal. Calcd. for $C_{28}H_{28}N_2O_5$: C, 71.17; H, 5.97; N, 5.93. Found: C, 70.89; H, 6.11; N, 6.18.

*4.1.12.12. Ethyl 8-(3-methoxybenzyl)-7-(4-methoxyphenyl)-4,5,6,8-tetrahydropyrrolo[3′,4′:3,4]cyclohepta [1,2-d] [1,2]oxazole-9-carboxylate **(3l)**.* This compound was obtained from reaction of compound **15l**. Yellow solid; yield 75%; mp 106–107 °C; ^1H ($CDCl_3$, 200 MHz, ppm): δ 1.25 (t, 3H, J = 7.1 Hz, CH_3), 1.91–2.01 (m, 2H, CH_2), 2.54–2.59 (m, 2H, CH_2), 2.78 (t, 2H, J = 6.3 Hz, CH_2), 3.71 (s, 3H, CH_3), 3.82 (s, 3H, CH_3), 4.29 (q, 2H, J = 7.1 Hz, CH_2), 5.29 (s, 2H, CH_2), 6.43 (d, 2H, J = 7.6 Hz, Ar), 6.72 (dd, 1H, J = 8.0, 2.4 Hz, Ar), 6.87–6.94 (m, 2H, Ar), 7.08–7.17 (m, 3H, Ar), 8.06 (s, 1H, H-3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 14.1, 24.3, 25.1, 25.7, 49.1, 55.1, 55.3, 61.2, 111.8, 112.5, 114.0 (2 x C), 114.2, 115.0, 118.4, 120.7, 122.6, 122.7, 129.5, 132.2 (2 x C), 135.4, 140.2, 151.9, 159.7, 159.8, 160.3, 162.4. Anal. Calcd. for $C_{28}H_{28}N_2O_5$: C, 71.17; H, 5.97; N, 5.93. Found: C, 71.31; H, 6.08; N, 5.72.

*4.1.12.13. Ethyl 8-(4-methoxybenzyl)-7-(4-methoxyphenyl)-4,5,6,8-tetrahydropyrrolo[3′,4′:3,4]cyclohepta [1,2-d] [1,2]oxazole-9-carboxylate **(3 m)**.* This compound was obtained from reaction of compound **15m**. White solid; yield 60%; mp 131–132 °C; ^1H NMR($CDCl_3$, 200 MHz, ppm): δ 1.26 (t, 3H, J = 7.1 Hz, CH_3), 1.89–2.01 (m, 2H, CH_2), 2.54–2.59 (m, 2H, CH_2), 2.77 (t, 2H, J = 6.3 Hz, CH_2), 3.74 (s, 3H, CH_3), 3.83 (s, 3H, CH_3), 4.30 (q, 2H, J = 7.1 Hz, CH_2), 5.24 (s, 2H, CH_2), 6.71–6.81 (m, 4H, Ar), 6.88–6.94 (m, 2H, Ar), 7.09–7.13 (m, 2H, Ar), 8.06 (s, 1H, H-3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 14.1, 24.3, 25.1, 25.7, 48.5, 55.2, 55.3, 65.1, 113.8 (2 x C), 113.9 (2 x C), 114.1, 114.9, 120.7, 122.5, 122.8, 127.5 (2 x C), 130.6, 132.2 (2 x C), 135.3, 151.9, 158.6, 159.7, 160.3, 162.6. Anal. Calcd. for $C_{28}H_{28}N_2O_5$: C, 71.17; H, 5.97; N, 5.93. Found: C, 70.96; H, 6.18; N, 5.81.

*4.1.12.14. 8-(2-Methoxybenzyl)-7-(4-methoxyphenyl)-4,5,6,8-tetrahydropyrrolo[3′,4′:3,4]cyclohepta [1,2-d] [1,2]oxazole **(3n)**.* This compound was obtained from reaction of compound **15n**. Yellow oil; yield 60%; ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.90–1.97 (m, 2H, CH_2), 2.73–2.77 (m, 4H, 2 x CH_2), 3.80 (s, 3H, CH_3), 3.85 (s, 3H, CH_3), 4.98 (s, 2H, CH_2), 6.72 (d, 1H, J = 7.3 Hz, Ar), 6.83–6.89 (m, 2H, Ar), 6.93 (d, 2H, J = 8.6 Hz, Ar), 7.17–7.28 (m, 4H, Ar), 8.01 (s, 1H, H-3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 21.4, 23.5, 27.5, 38.1, 55.4, 55.7, 110.1, 110.8, 113.7, 114.2 (2 x C), 118.3, 119.1, 120.6, 121.8, 127.4, 128.3, 128.8, 129.1, 129.3 (2 x C), 132.0, 150.6, 160.0, 160.8. Anal. Calcd. for $C_{25}H_{24}N_2O_3$: C, 74.98; H, 6.04; N, 7.00. Found: C, 75.11; H, 5.89; N, 6.69.

*4.1.12.15. 8-(3-Methoxybenzyl)-7-(4-methoxyphenyl)-4,5,6,8-tetrahydropyrrolo[3′,4′:3,4]cyclohepta [1,2-d] [1,2]oxazole **(3o)**.* This compound was obtained from reaction of compound **15o**. Yellow oil; yield 64%; ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.89–1.96 (m, 2H, CH_2), 2.73–2.76 (m, 4H, 2 x CH_2), 3.76 (s, 3H, CH_3), 3.85 (s, 3H, CH_3), 4.95 (s, 2H, CH_2), 6.53 (s, 1H, Ar), 6.61 (d, 1H, J = 7.6 Hz, Ar), 6.79 (dd, 1H, J = 8.2, 2.3 Hz, Ar), 6.93 (d, 2H, J = 8.7 Hz, Ar), 7.14–7.23 (m, 4H, Ar), 8.01 (s, 1H, H-3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 21.1, 23.5, 27.6, 43.2, 55.2, 55.3, 92.23, 105.8, 112.8, 113.1, 114.0 (2 x C), 114.5, 121.5, 127.4, 127.6, 129.2, 129.7, 132.0, 139.5, 150.9, 151.7, 159.4, 159.9, 161.9. Anal. Calcd. for $C_{25}H_{24}N_2O_3$: C, 74.98; H, 6.04; N, 7.00. Found: C, 74.73; H, 6.26; N, 7.39.

*4.1.12.16. 8-(4-Methoxybenzyl)-7-(4-methoxyphenyl)-4,5,6,8-tetrahydropyrrolo[3′,4′:3,4]cyclohepta [1,2-d] [1,2]oxazole **(3p)**.* This compound was obtained from reaction of compound **15p**. Yellow oil; yield 70%; ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.84–1.95 (m, 2H, CH_2), 2.72 (t, 4H, J = 5.5 Hz, 2 x CH_2), 3.78 (s, 3H, CH_3), 3.84 (s, 3H, CH_3), 4.88 (s, 2H, CH_2), 6.80 (d, 2H, J = 8.7 Hz, Ar), 6.92 (d, 4H, J = 8.5 Hz, Ar), 7.12–7.16 (m, 3H, Ar), 7.98 (s, 1H, H-3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 24.4, 24.6, 27.2, 50.5, 55.3 (2 x C), 111.0, 111.7, 113.8 (2 x C), 114.0 (2 x C), 118.6, 119.6, 123.8, 128.4 (2 x C), 129.8, 131.3, 132.1 (2 x C), 151.7, 159.0, 159.2, 162.2. Anal. Calcd. for $C_{25}H_{24}N_2O_3$: C, 74.98; H, 6.04; N, 7.00. Found: C, 74.77; H, 5.83; N, 6.71.

*4.1.12.17. 8-(3,4,5-trimethoxybenzyl)-7-(4-methoxyphenyl)-4,5,6,8-tetrahydropyrrolo[3′,4′:3,4]cyclohepta [1,2-d] [1,2]oxazole **(3q)**.* This compound was obtained from reaction of compound **15q**. Yellow oil; yield 64%; ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.89 (s, 2H, CH_2), 2.72 (s, 2H, CH_2), 2.84 (s, 2H, CH_2), 3.69 (s, 6H, 2 x CH_3), 3.79 (s, 3H, CH_3), 3.84 (s, 3H, CH_3), 4.82 (s, 2H, CH_2), 6.13 (s, 2H, Ar), 6.86 (d, 2H, J = 8.3 Hz, Ar), 7.02–7.07 (m, 3H, Ar and H-9), 8.00 (s, 1H, H-3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 24.5, 27.2, 31.4, 48.9, 55.2, 55.9 (2 x C), 60.8, 104.8 (2 x C), 107.5, 113.8 (2 x C), 118.7, 119.8, 123.3, 123.8, 131.4, 131.7 (2 x C), 131.9, 136.1, 152.3, 152.4, 153.1 (2 x C), 159.2. Anal. Calcd. for $C_{27}H_{28}N_2O_5$: C, 70.42; H, 6.13; N, 6.08. Found: C, 70.56; H, 5.88; N, 6.34.

*4.1.12.18. 8-Benzyl-7-(3,4,5-trimethoxyphenyl)-4,5,6,8-tetrahydropyrrolo[3′,4′:3,4]cyclohepta [1,2-d] [1,2]oxazole **(3r)**.* This compound was obtained from reaction of compound **15r**. Yield 66%; white solid; mp 122–123 °C; ^1H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.93–1.99 (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″), 7.03–7.05 (2H, m, Ar), 7.25–7.33 (4H, m, Ar and H-9), 8.02 (1H, s, H-

3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 24.4, 24.6, 27.3, 51.2, 55.9 (2 x C), 60.9, 107.9 (2 x C), 111.2, 111.9, 119.3, 119.8, 126.5 (2 x C), 126.7, 127.5, 128.7 (2 x C), 131.6, 137.7, 138.3, 151.7, 152.9 (2 x C), 162.0. Anal. Calcd. for $C_{26}H_{26}N_2O_4$: C, 72.54; H, 6.09; N, 6.51. Found: C, 72.27; H, 5.87; N, 6.79.

4.1.12.19. 8-(2-Methoxybenzyl)-7-(3,4,5-trimethoxyphenyl)-4,5,6,8-tetrahydropyrrolo[3′,4′:3,4]cyclohepta [1,2-d] [1,2]oxazole ***(3s)***. This compound was obtained from reaction of compound **15s**. Yellow oil; yield 60%; ^{1}H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.93–2.00 (m, 2H, CH_2), 2.74–2.84 (m, 4H, 2 x CH_2), 3.69 (s, 6H, 2 x CH_3), 3.80 (s, 3H, CH_3), 3.89 (s, 3H, CH_3), 5.01 (s, 2H, CH_2), 6.41 (s, 2H, H-2″ and H-6″), 6.77 (d, 1H, J = 7.5 Hz, Ar), 6.76–6.92 (m, 2H, Ar), 7.22–7.27 (m, 2H, Ar), 8.02 (s, 1H, H-3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 24.4, 24.6, 27.4, 46.5, 55.3, 55.9 (2 x C), 60.9, 107.8 (2 x C), 110.1, 111.0, 111.7, 119.5, 119.6, 120.6, 126.6, 126.9, 127.9, 128.7, 131.6, 137.5, 151.7, 152.9 (2 x C), 156.3, 162.2. Anal. Calcd. for $C_{27}H_{28}N_2O_5$: C, 70.42; H, 6.13; N, 6.08. Found: C, 70.63; H, 6.29; N, 5.72.

4.1.12.20. 8-(3-Methoxybenzyl)-7-(3,4,5-trimethoxyphenyl)-4,5,6,8-tetrahydropyrrolo[3′,4′:3,4]cyclohepta [1,2-d] [1,2]oxazole ***(3t)***. This compound was obtained from reaction of compound **15t**. Yellow oil; yield 68%; ^{1}H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.92–1.98 (2H, m, CH_2), 2.74–2.81 (4H, m, 2 x CH_2), 3.70 (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 ($CDCl_3$, 50 MHz, ppm): δ 24.4, 24.6, 27.3, 51.2, 55.2, 55.9 (2 x C), 60.9, 107.9 (2 x C), 111.9, 112.5, 112.7, 118.9, 119.4, 119.7, 126.7, 129.8, 129.9, 131.6, 137.7, 139.9, 151.7, 152.9 (2 x C), 160.0, 162.0. Anal. Calcd. for $C_{27}H_{28}N_2O_5$: C, 70.42; H, 6.13; N, 6.08. Found: C, 70.33; H, 6.38; N, 5.83.

4.1.12.21. 8-(4-Methoxybenzyl)-7-(3,4,5-trimethoxyphenyl)-4,5,6,8-tetrahydropyrrolo[3′,4′:3,4]cyclohepta [1,2-d] [1,2]oxazole ***(3u)***. This compound was obtained from reaction of compound **15u**. Yellow oil; yield 51%; ^{1}H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.92–1.98 (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″), 6.83 (2H, d, J = 8.8 Hz, H-3′ and H-5′), 6.96 (2H, d, J = 8.8 Hz, H-2′ and H-6'), 7.21 (1H, s, H-9), 8.01 (1H, s, H-3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 24.4, 24.6, 27.3, 50.8, 55.3, 56.0 (2 x C), 60.9, 108.0 (2 x C), 111.1, 111.8, 114.1 (2 x C), 119.1, 119.7, 126.9, 128.1, 130.1 (2 x C), 131.5, 137.7, 151.7, 152.9 (2 x C), 159.1, 162.0. Anal. Calcd. for $C_{27}H_{28}N_2O_5$: C, 70.42; H, 6.13; N, 6.08. Found: C, 70.23; H, 6.41; N, 6.27.

4.1.12.22. 8-(2,5-Dimethoxybenzyl)-7-(3,4,5-trimethoxyphenyl)-4,5,6,8-tetrahydropyrrolo[3′,4′:3,4]cyclohepta [1,2-d] [1,2]oxazole ***(3v)***. This compound was obtained from reaction of compound **15v**. Yellow oil; yield 77%; ^{1}H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.92–1.99 (m, 2H, CH_2), 2.74–2.83 (m, 4H, 2 x CH_2), 3.68 (s, 3H, CH_3), 3.72 (s, 6H, 2 x CH_3), 3.75 (s, 3H, CH_3), 3.89 (s, 3H, CH_3), 4.98 (s, 2H, CH_2), 6.37 (s, 1H, Ar), 6.43 (s, 2H, H-2″ and H-6″), 6.75–6.76 (m, 2H, Ar), 7.23 (s, 1H, H-9), 8.01 (s, 1H, H-3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 24.4, 24.6, 27.4, 46.4, 55.7, 55.8, 55.9 (2 x C), 60.9, 107.8 (2 x C), 111.0, 111.7, 112.4, 114.8, 119.5, 119.6, 126.9, 127.7, 127.8 (s), 131.5, 137.5, 150.6, 151.7, 152.9 (2 x C), 153.7, 162.2. Anal. Calcd. for $C_{28}H_{30}N_2O_6$: C, 68.56; H, 6.16; N, 5.71. Found: C, 68.31; H, 6.42; N, 5.55.

4.1.12.23. 8-(3,4-Dimethoxybenzyl)-7-(3,4,5-trimethoxyphenyl)-4,5,6,8-tetrahydropyrrolo[3′,4′:3,4]cyclohepta [1,2-d] [1,2]oxazole ***(3w)***. This compound was obtained from reaction of compound **15w**. Yellow oil; yield 71%; ^{1}H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.92–1.99 (m, 2H, CH_2), 2.74–2.83 (m, 4H, 2 x CH_2), 3.69 (s, 3H, CH_3), 3.72 (s, 6H, 2 x CH_3), 3.76 (s, 3H, CH_3), 3.89 (s, 3H, CH_3), 4.98 (s, 2H, CH_2), 6.37 (s, 1H, Ar), 6.43 (s, 2H, H-2″ and H-6″), 6.76 (s, 2H, Ar), 7.24 (s, 1H, H-9), 8.02 (s, 1H, H-3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 24.4, 24.6, 27.4, 46.4, 55.7, 55.8, 55.9 (2 x C), 60.9, 107.8 (2 x C), 111.0 (2 x C), 111.7, 112.4, 114.8, 119.6, 119.7, 126.9, 127.7, 131.5, 137.5, 150.6, 151.7, 152.9, 153.7 (2 x C), 162.2. Anal. Calcd. for $C_{28}H_{30}N_2O_6$: C, 68.56; H, 6.16; N, 5.71. Found: C, 68.32; H, 5.88; N, 5.92.

4.1.12.24. 8-(3,4,5-Trimethoxybenzyl)-7-(3,4,5-trimethoxyphenyl)-4,5,6,8-tetrahydropyrrolo[3′,4′:3,4]cyclohepta [1,2-d] [1,2]oxazole ***(3x)***. This compound was obtained from reaction of compound **15x**. Yellow oil; yield 71%; ^{1}H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.90–1.95 (m, 2H, CH_2), 2.73–2.78 (m, 4H, 2 x CH_2), 3.70 (s, 6H, 2 x CH_3), 3.71 (s, 6H, 2 x CH_3), 3.75 (s, 3H, CH_3), 3.79 (s, 3H, CH_3), 4.86 (s, 2H, CH_2), 6.18 (s, 2H, Ar), 6.36 (s, 2H, Ar), 7.07 (s, 1H, H-9), 8.01 (s, 1H, H-3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 24.4, 24.6, 27.3, 49.1 (q), 56.0 (2 x C), 56.1 (2 x C), 60.8, 60.9, 104.9 (2 x C), 107.6, 107.7 (2 x C), 111.2, 111.9, 119.0, 119.9, 124.0, 126.6, 131.9, 136.2, 151.8, 153.0 (2 x C), 153.2 (2 x C), 161.9. Anal. Calcd. for $C_{29}H_{32}N_2O_7$: C, 66.91; H, 6.20; N, 5.38. Found: C, 67.09; H, 5.99; N, 5.57.

4.1.12.25. 8-(4-Methoxy-3-nitrobenzyl)-7-(3,4,5-trimethoxyphenyl)-4,5,6,8-tetrahydropyrrolo[3′,4′:3,4]cyclohepta [1,2-d] [1,2]oxazole ***(3y)***. This compound was obtained from reaction of compound **15y**. Yellow oil; yield 67%; IR (cm^{-1}): 1532 (NO_2); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.90–1.98 (m, 2H, CH_2), 2.73–2.78 (m, 4H, 2 x CH_2), 3.79 (s, 6H, 2 x CH_3), 3.90 (s, 3H, CH_3), 3.93 (s, 3H, CH_3), 5.00 (s, 2H, CH_2), 6.40 (s, 2H, H-2″ and H-6″), 7.01 (d, 1H, J = 8.7 Hz, Ar), 7.18–7.22 (m, 2H, Ar), 7.41 (d, 1H, J = 2.1 Hz, Ar), 8.02 (s, 1H, H-3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 24.3, 24.5, 27.2, 50.0, 56.1 (2 x C), 56.7, 60.9, 107.91 (2 x C), 111.5, 112.5, 113.8, 118.7, 120.4, 124.2, 126.5, 130.4, 131.5, 132.5, 138.0, 139.6, 151.8, 152.3, 153.2 (2 x C), 161.7. Anal. Calcd. for $C_{27}H_{27}N_3O_7$: C, 64.15; H, 5.38; N, 8.31. Found: C, 64.31; H, 5.54; N, 8.11.

4.1.12.26. 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 ***(3z)***. This compound was obtained from reaction of compound **15z**. Yellow oil; yield 60%; IR (cm^{-1}): 3463–3381 (NH_2); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm): δ 1.90–1.97 (m, 2H, CH_2), 2.71–2.81 (m, 4H, 2 x CH_2), 3.78 (s, 6H, 2 x CH_3), 3.86 (s, 3H, CH_3), 3.89 (s, 3H, CH_3), 4.94 (s, 2H, CH_2), 6.48 (s, 2H, Ar), 6.80 (s, 2H, NH_2), 7.12 (s, 1H, Ar), 7.89 (s, 1H, Ar), 8.00 (d, 1H, J = 6.5 Hz, Ar), 8.17 (s, 1H, Ar), 8.41 (s, 1H, H-3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): δ 24.4, 24.6, 27.3, 50.8, 55.9, 56.1 (2 x C), 60.9, 108.0 (2 x C), 110.1, 111.1, 111.9, 118.9, 119.2, 119.6, 122.7, 126.8, 127.0, 130.6, 131.7, 147.2, 151.7, 153.0 (2 x C), 158.8, 162.1. Anal. Calcd. for $C_{27}H_{29}N_3O_5$: C, 68.19; H, 6.15; N, 8.84. Found: C, 68.48; H, 5.87; N, 8.63.

4.2. Biology

4.2.1. Cell lines

All the cell lines used in this paper were of human origin and purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Non-small cell lung carcinoma (A549) and T-acute lymphoblastic leukemia (CCRF-CEM) cells were grown in DMEM or RPMI (A549) medium (Gibco, Milano, Italy). Both media were supplemented with 115 units/mL of penicillin G (Gibco, Milano, Italy), 115 μg/mL of streptomycin (Invitrogen, Milano, Italy) and 10% fetal bovine serum (Invitrogen, Milano, Italy).

Lymphoma cell lines were cultured as recommended, using RPMI-1640 medium, supplemented with 20% (v/v) fetal bovine serum, Penicillin-Streptomycin-Neomycin (~5000 units penicillin, 5 mg strepto-

mycin and 10 mg neomycin/mL, Sigma) and L-glutamine (1%). Cell line identities were validated by CellCheck test (IDEXX, BioResearch) or with the Promega GenePrint 10 System kit, and all experiments were performed within one month after the cells were 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.

4.2.2. Preparation of compounds for in vitro screening

All 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 to obtain the indicated concentrations. The DMSO concentration did not exceed 0.1% in any experiment.

4.2.3. Cell proliferation analysis

For each screening experiment, cells were seeded in 96-well plates (non-tissue culture treated) at a density ranging from 5000 to 10,000 cells/well, depending on the doubling time of the specific cell line. For distributing cells into wells of the plates either a VIAFLO 96 hand-held electronic channel pipette (Integra Biosciences) or manual 12-channel pipet was used. Cells were initially treated with a single dose of 1 μM compound for 72 h. Selected compounds, which reached proliferation inhibition of about 60%, were further tested in the appropriate tissue culture medium with increasing compound doses ranging from 0 to 10 μM, using 1:2 dilution in series to obtain IC_{50} values. These assays were performed in triplicate. To 100 μL of cells suspended in medium, 100 μL of drug suspension was added, for a total seeding volume of 200 μL/well. After preparation of the microplates, they were incubated at 37 °C in a 5% CO_2, 95% air atmosphere with 100% relative humidity for 72 h. 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 and filter-sterilized, as we previously performed also for solid cell lines [33]. 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 h. 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 by the cells was obtained by quantifying the linear relationship between live cells and the A_{570} signal produced.

4.2.4. Antiproliferative activity in PBLs

Additional experiments were carried out with PBLs from healthy donors obtained as described previously [34]. For cytotoxicity evaluations in proliferating PBL cultures, non-adherent cells were resuspended at 5×10^5 cells/mL in a growth medium containing 2.5 g/mL PHA (Irvine Scientific). The same cellular density was used also for resting PBL cultures but without the addition of PHA. Scalar concentrations of the test compounds were added, and viability was determined after a 72 h incubation by the MTT test.

4.2.5. Analysis of cell cycle by flow cytometry

For these experiments, the A549, CCRF-CEM and VL51 cell lines were used. They were seeded in 6-well plates at a density of 5×10^4, 2.5×10^5 and 2.0×10^5, respectively, in a final volume of 2 mL culture medium. The cells were then treated with the test compounds for 24 h at the indicated concentrations. After this incubation period, the cells were detached with trypsin-EDTA (A549) and harvested by centrifugation. The pellet thus obtained was fixed in 70% ice cold ethanol. Then the cells were processed and analyzed as described previously [35], except that for the acquisition of data with labeled cells, a Cytomics FC500 flow cytometer (Beckman Coulter) was used.

Further experiments were performed to distinguish cells in G2 from those in metaphase by combining cell cycle analysis with phosphohistone H3 (p-H3, Cell Signalling) staining. This assay specifically identifies cells in metaphase.

4.2.6. Apoptosis assay

The quantification of apoptosis induced by the test compounds was carried out by flow cytometric analysis using the Annexin-V Fluos kit (Roche Diagnostics) following the manufacturer's instructions. The cells treated with different concentrations of the test compounds after a 48 h incubation were labeled with annexin V/FITC and PI and analyzed with a Coulter Cytomics FC500 instrument (Beckman Coulter) in the FL1 and FL3 channels, respectively.

4.2.7. Analysis of mitochondrial potential

The analysis of the mitochondrial potential was carried out by flow cytometric analysis. Briefly, cells treated with the test compound were labeled with the JC-1 dye as previously described [35]. The labeled cells were analyzed using the Coulter Cytomics FC500 instrument (Beckman Coulter) in the FL1 and FL2 channels, respectively.

4.2.8. Molecular modeling

All molecular modeling simulations were carried out using the Schrödinger Suite version 2018 [36]. For compounds **3d**, **3p**, **3u** and **3z,** we performed docking, molecular dynamics simulations, and thermodynamics evaluations by following the previously described protocol [21].

QikProp software [37] was applied for calculating the drug-like properties of the active derivatives (**3d**, **3p**, **3u** and **3z**) and for evaluating their pharmacokinetic properties, by considering their absorption, distribution, metabolism and excretion (ADME) profile [38].

Finally, we used the Molinspiration virtual screening engine v2018.08 to explore potential off-target effects, by predicting the biological activity of the given ligands quickly and efficiently towards other targets [39]. In particular, this tool provides a druglikeness score of our ligands towards GPCR ligands, ion channel modulators, kinase inhibitors, nuclear receptor ligands, protease inhibitors and other enzyme targets based on Molinspiration technology [40,41].

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Francesco Bertoni reports financial support was provided by ADC Therapeutics, Bayer AG, Cellestia, Helsinn, HTG Molecular Diagnostics, ImmunoGen, Menarini Ricerche, NEOMED Therapeutics 1, Nordic Nanovector ASA, Helsinn, Menarini. Eugenio Gaudio reports a relationship with Helsinn Healthcare SA that includes: employment.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2023.115372.

Abbreviations used

NHL	non-Hodgkin's lymphoma
DLBCL	activated B-cell like diffuse large B cell lymphoma
ABC	activated B-cell
GCB	germinal center B-cell
FL	follicular lymphoma
MCL	mantle cell lymphoma
MZL	marginal zone lymphoma
NCI	National Cancer Institute
MG_MID	mean graph mid-points
DMFDMA	*N,N*-dimethylformamide dimethyl acetal
TFAA	trifluoroacetic anhydride
DCM	dichlomethane
DM0F	*N,N*-dimethylformamide
THF	tetrahydrofuran
TBDMAM	*tert*-butoxy *bis*(dimethylamino)methane
CA-4	combretastatin A-4
PBLs	peripheral blood lymphocytes
DMSO	dimethyl sulfoxide
$CDCl_3$	deuterated chloroform
s	singlet
d	doublet
t	triplet
q	quartet
m	multiplet

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