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FUSED NITROGEN HETEROCYCLES AS PROMISING SMALL MOLECULES FOR THE TREATMENT OF CANCER

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1. Introduction

The use of light for therapeutic purposes has a very ancient origin; in fact, sunlight has been an important light source utilized in many old cultures to treat different diseases varying from vitiligo to psychosis. Photodynamic therapy (PDT) was discovered more than 100 years ago, when Niels Finsen demonstrated the usefulness of sunlight in the treatment of different skin diseases like *lupus vulgaris*, an infection caused by *Mycobacterium tuberculosis*. In 1900, Oscar Raab, a medical student, accidentally discovered that microorganism such as paramecia, a protozoa, could be killed when exposed to light, but not in the dark, after incubation with acridine dye [1] [2]. Raab's group was the first to introduce the photodynamic therapy into the clinic, reaching excellent results in the treatment of skin tumors [3] [4]. The modern PDT was born in 1960 with both the discovery of the photosensitizing properties of porphyrin derivatives (Figure 1) [5] and with the studies of Dougherty and his colleagues at Roswell Park Cancer Institute [6].

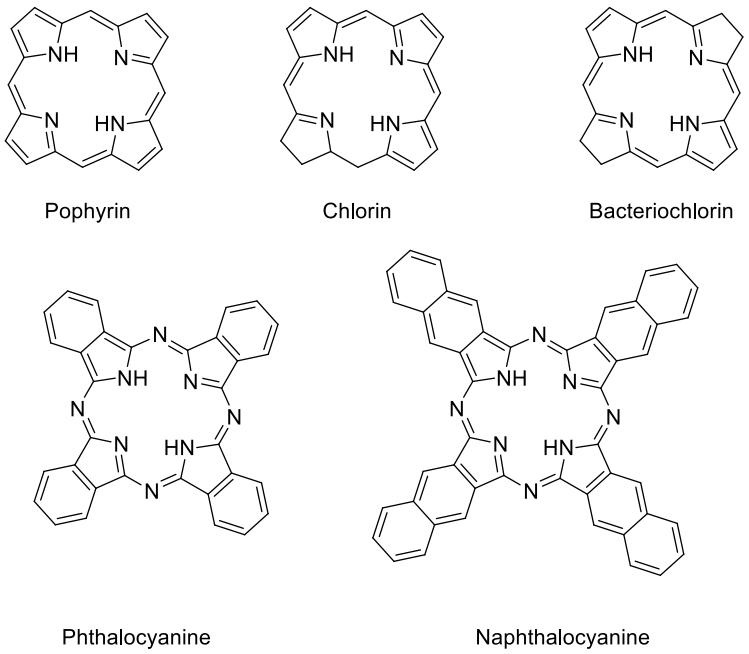


Figure 1

They reported the first large scale trial of PDT in human cancers through a water-soluble mixtures of porphyrins, under the name Photofrin [7], which is still used in the treatment of skin cancer and in 1993 it was also approved in Canada for the treatment of bladder cancer. Unlike conventional therapies, PDT has many advantages in fact it results non-invasive and induces selective cytotoxicity against malignant cells. PDT requires topical or systemic administration of a light-activated drug called photosensitizer (PS), which is localized in particular intracellular sites (plasma membrane, mitochondria, lysosomes, endoplasmic reticulum), followed by activation with light of proper wavelength in accordance with the absorption spectrum of the PS. This light reaction produces reactive oxygen species (ROS) among which singlet oxygen ($^1O_2$), an excited state of molecular oxygen. ROS can induce cell death by interaction with proteins, lipids, and nucleic acids. Generally, PSs are molecules containing highly conjugated double bond system able to absorb light. According to the Jablonsky's diagram the PS molecule is in a singlet state in its ground state. When a photon of light of the specific wavelength is absorbed, the electron moves from its ground state with low energy to its excited singlet state which is at a higher energy level. At this level the PS in the excited singlet state can undergo to "*intersystem crossing*" leading to a more stable excited triplet state with a longer lifetime than the excited singlet state (microseconds compared with nanoseconds), (Figure 2).

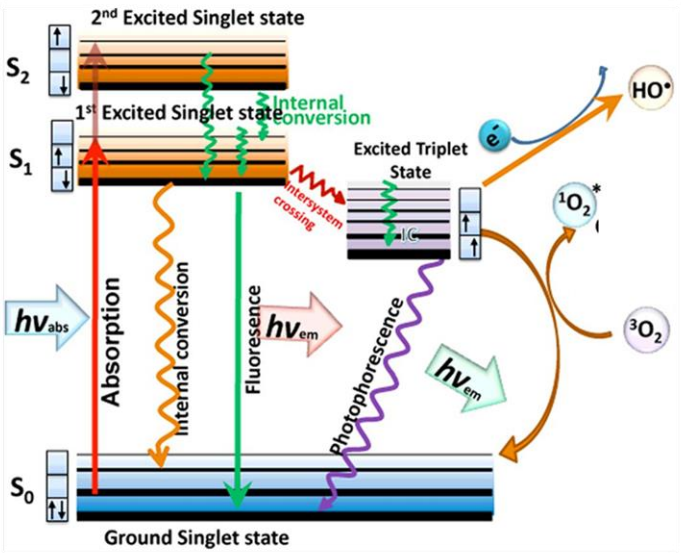


Figure 2

In this state, PS has enough time to transfer its energy colliding with molecular oxygen, which is in a triplet state in its ground state, generating either superoxide anion ($O_2^{\cdot -}$) and hydroxyl radicals ($OH^{\cdot}$) (Type I reactions) or singlet oxygen ($^1O_2$) (Type II reactions) (Figure 2).

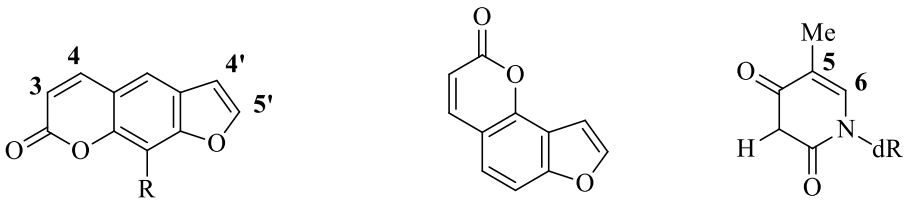
Interaction of ROS with cellular targets induces damage to cellular organelles such as plasma membrane, mitochondria, lysosomes, endoplasmic reticulum leading to cell death through apoptosis or necrosis. Many PSs used in PDT act according to this oxygen dependent mechanism (Type I and II reactions). Some PSs, can instead act with an oxygen independent mechanism. In this case the PS in its triplet excited state reacts directly with the biological substrates (Type III reactions).

A good PS should posses a favorable combination of following factors:

- absorption of light at wavelengths which can penetrate the biological tissue;
- a high quantum yield for singlet oxygen generation;
- selective accumulation in malignant tumor tissue and a fast clearance from normal cells;
- low toxicity

Among PSs, acting with an oxygen independent mechanism, there are furocumarins, a class of natural compounds. Furocumarins are planar tricyclic aromatic compounds in which a cumarin moiety is condensed to a furan ring and depending on the position in which the condensation occurs, different isomers can be obtained, generating linear and angular furocumarins.

Psoralen belongs to the first group, the second one is represented by Angelicin (Figure 3).



Psoralen (R= H)
8-Methoxypsoralen (R=OMe)

Angelicin

Thymine

Figure 3

Currently, they are studied for the treatment of skin disease such as psoriasis, mycosis fungoides, vitiligo and for treatment of T-cell lymphoma and autoimmune diseases such as lupus eritematosus. Parrish and collaborators introduced in clinical practice the use of psoralen in combination with UVA light under the name of PUVA therapy (psoralen plus UVA) [8]. The treatment consists in the oral administration of 8-methoxypsoralen (8-MOP) followed by irradiation of the patient with artificial UVA light.

Interesting results in this field have been obtained with the use in clinical practice of Extracorporeal photopheresis (ECP) a variant of leukapheresis. At the end of 80's, Edelson proposed it for the treatment of T-cell skin Lymphoma (CTCL). In 1988 it was approved by the US Food and Drug Administration [9] and is currently used in the treatment of autoimmune disease (Systemic Sclerosis and artritis reumatoides) as well as in the GVHD (Graft Versus Host Disease) [10]. Recently, it has also shown promising results in the treatment of several T-cell-mediated disorders, including Crohn's disease [11].

The treatment consists of three steps (Figure 4):

1. Oral administration of the 8-methoxypsoralen (8-MOP);
2. Separation by apheresis of the red blood cells from the white blood cells. Addition of 8-MOP to white blood cells and irradiation with UVA light.
3. Reinfusion of red and white blood cells treated to the patient.

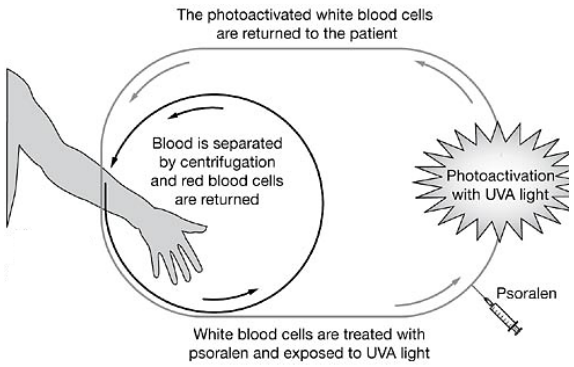


Figure 4

ECP has many advantages as patient is not directly exposed to UVA light, as in the classic PUVA therapy [8], so he suffers from fewer side effects, such as a mild fever and a slight

reddening of the skin associated with itching. Moreover, ECP does not cause suppression of patient's immune system and normally does not damage sensible organs such as liver, heart and lungs.

The main target of psoralen is DNA. In fact, psoralen and angelicin, due to their planar and aromatic structure, can intercalate in the "dark" between DNA base pairs and, after photoactivation upon UVA light irradiation, can give photocycloaddition with DNA bases, in particular with thymine, leading to mono- and bis-adducts. Irradiation with light of proper wavelenghts, provides the necessary energy for the photocycloaddition reaction which involves 3,4 and 4',5'psoralen's double bonds and 5,6 thymine's double bond. This reaction generates two monoadducts: 4',5' mono-adduct and the 3,4 mono-adduct (Figure 5).

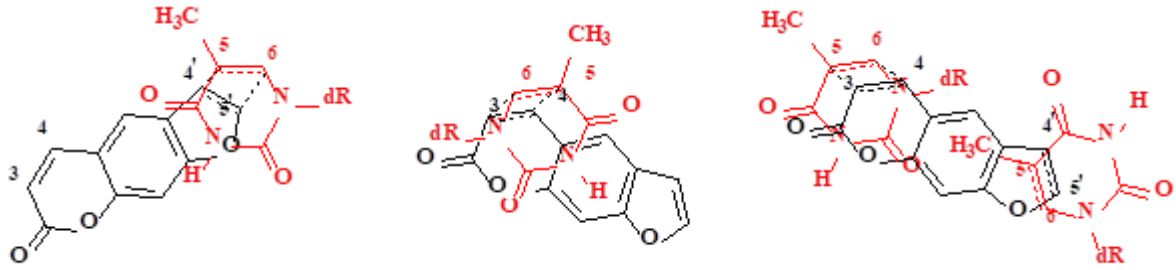
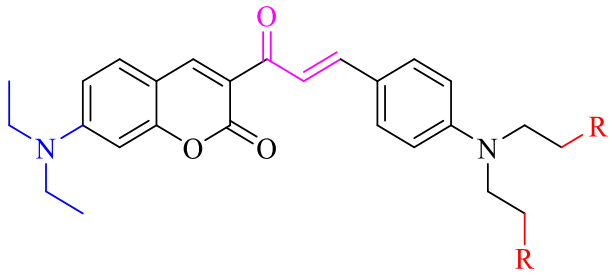


Figure 5

Absorption of a second photon of light, only the 4',5' mono-adduct can react with a second thymine base of the complementary strand of the DNA leading to the formation of a bis-diadduct or interstrand crosslink (ISCs), (Figure 5). The formation of bis-adduct prevents the normal progression of DNA-polymerase and replication of DNA representing the cause of long-term side effects such as mutagenesis and carcinogenesis. Instead the formation of monoadducts causes only short-term side effects such as itching and, nausea. To date, the most common drugs used in photochemotherapy are 5-methoxypsoralen, 8-methoxypsoralen and 4,5',8-trimethylpsoralen which, due to their linear structures, induce long-term side effects. This explains the large amount of studies towards the class of angular furanocumarines leading to the synthesis of new derivatives of angelicin, such as 6,4,4' trimethylangelicin (TMA) which used in clinical tests for psoriasis, mycosis

fungoides and other skin diseases, showed significant therapeutic activity, less mutagenicity and risk of skin cancer and absence of phototoxicity.

The limitation of the use of small molecules in PDT is represented by the absorption wavelength. For this reason their use has been limited to superficial tumors and skin diseased. Near infrared wavelength are generally required to reach deep seated tumors. Recently it was demonstrated the possibility to exciting cumarin derivatives, which normally absorb light at 300-400 nm, by the absorption of two photons (2PA). This new technique, called *two-photon excited photodynamic therapy* (2PE-PDT), which would allow to enlarge the therapeutic window of PDT, is a non linear optical process, in which two photons are absorbed simultaneously. In particular, the two photons's energy is equivalent to the energy of a photon with half of their wavelength when they are simultaneously absorbed by the PS. As a consequence PSs, absorbing light in the UV-Visible spectra could be excited by long wavelength upon 2PE-PDT. Crucial requirement, for the application of 2PE-PDT, is that the compound used has an adequate value of 2PE cross section (σ2). In fact, σ2 describes the ability of PS to absorbs the two photons making it appropriate for 2PE-PDT. However, the σ2 values of coumarin derivatives is too low for their use in 2PE-PDT. Some authors have recently reported the possibility to increase the cross section value of coumarin derivatives by introducing strongly electron donor groups or increasing conjugation in the molecule (Figure 6) [12].



R= $O(CH_2CH_2O)_3CH_3$

Figure 6

The research group, where I developed my PhD work, upon studying heterocyclic systems containing the pyrrole ring, came across a series of compounds, in mainly pyrroloquinolinones, aza-isosters of angelicin, which were mostly inactive in the 'dark',

but which surprisingly showed all the properties of photosensitizers as they are capable of being activated by light (Figure 7).

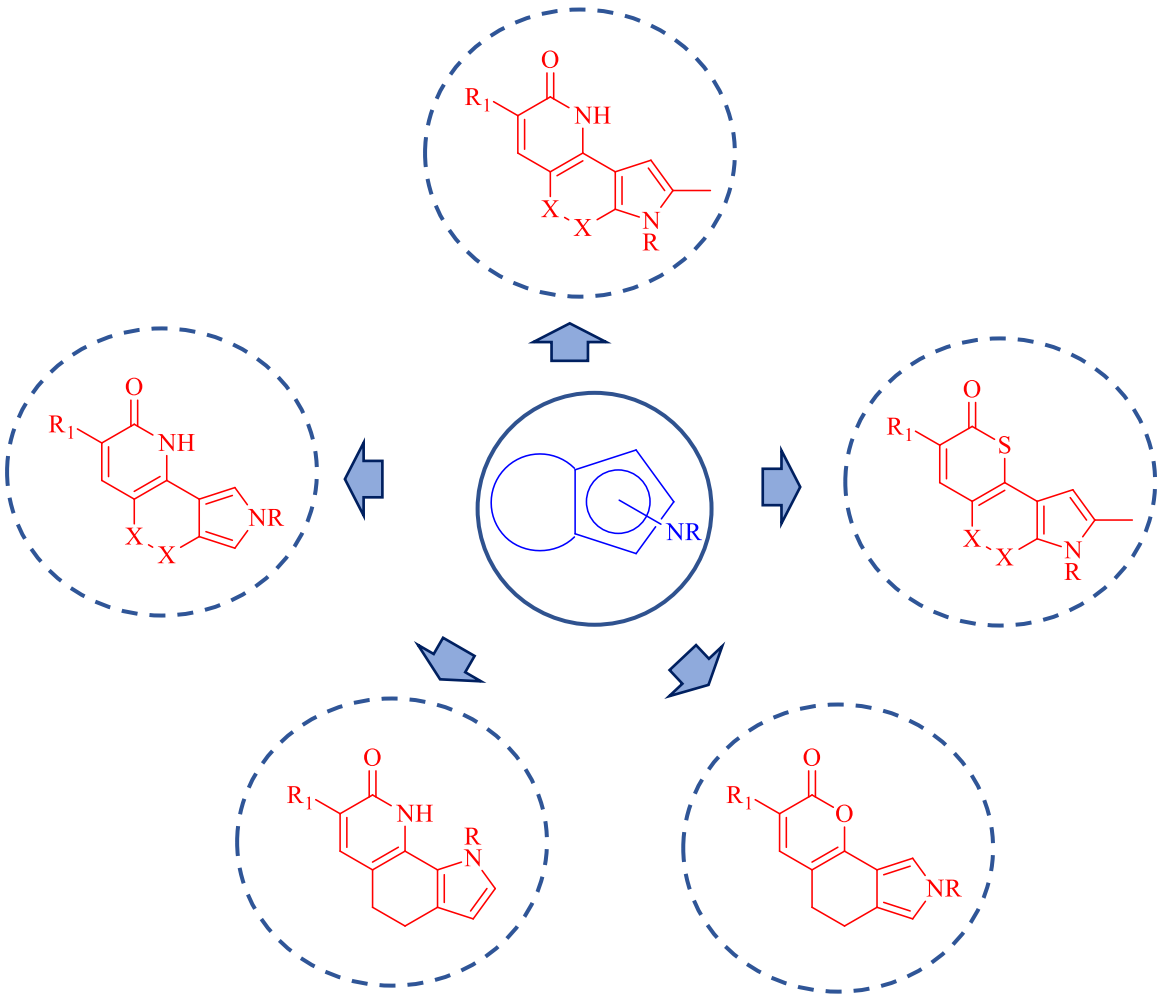


Figure 7

At the beginning, few derivatives of the tricyclic system, *Pyrrole[2,3-h]quinolinonone*, bearing different substitutions on the pyrrole nitrogen and on the 3 position of the pyridone ring were synthesized (Figure 8) [13] [14]. The analysis of their chemical-physical properties showed a marked lipophilicity and ability to localize into intracellular organelles such as mitochondria and lysosomes. Moreover, derivatives **1** showed absorbance in the UV-visible region with a marked bathochromic shift compared to angelicin. In particular, compound **1a** emitted in the blue region, an event due to the conjugation of the tricyclic system with the substituents in positions 3 and 7.

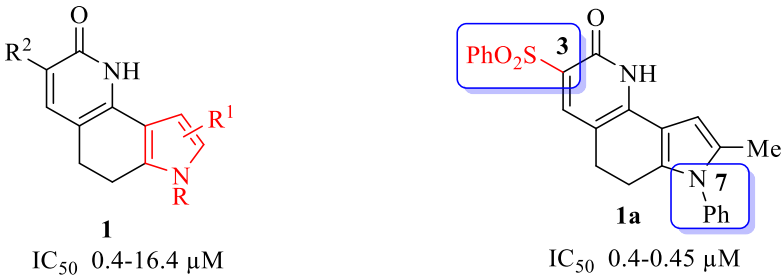


Figure 8

All the tested derivatives resulted inactive "in the dark", in the absence of irradiation, against three different cell lines (HL-60 “human promyelocytic leukemia”, HT-1080 “human fibrosarcoma”, LoVo “human intestinal adenocarcinoma”). After irradiation of the same cell lines at three different UVA doses (2.6, 3.2, 6.5 J/cm^2) derivatives **1** (IC_{50} 0.4-16.4μM) showed antiproliferative activity in the submicro-micromolar range resulting in some case more active than the reference drug, angelicin. Structure-activity relationship (SAR) studies indicated that the presence of phenylsulfonyl group in position 3 increased the phototoxic activity, probably due to the conjugation of the tricyclic system with the phenylsulfonyl group. However, the study of the mechanism of action revealed a different behaviour from that of furocumarin. In fact, due to the fact that the scaffold is not a full aromatic system they were not able to intercalate into DNA, and it turned out that phototoxicity was related to a massive production of ROS.

Then, it was decided to investigate the phototoxic activity of isomers of pyrroquinolinones **1** synthesizing the *pyrrole[3,2-h]quinolinones* **2** [15] and *pyrrole[3,4-h]quinolinones* **3** [16] (Figure 9) which maintain the structural requirements that were crucial to obtain the best phototoxicity, i.e. an unsaturated central ring, the presence of phenylsulfonyl group in position 3 and a lipophilic substituent on the nitrogen pyrrole. It was also evaluated the effect of the presence of an ethoxycarbonyl group on the pyrrole ring and of the methylation of the amide function.

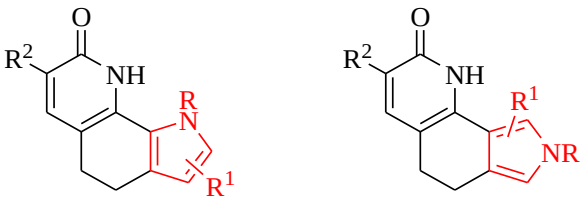


Figure 9

Both classes of compounds were generally inactive in the 'dark'. After irradiation they showed good phototoxic activity against different cell lines, such as HL-60 “human promyelocytic leukemia”, Jurkat “human T-cell leukemia”, MCF-7 “human breast adenocarcinoma”, Lovo “human intestinal adenocarcinoma”, NCTC-2544 “human skin keratinocyte”, K-562 “ human erythroleukemia”, which it is in both concentration and UV dose dependent, with GI_{50} values reaching the submicromolar level.

The presence of the ethoxycarbonyl group appears to be crucial for the activity of these two classes of compounds. In fact, the most active pyrroloquinolinones **2** are **2j**, **2k** and **2m** that, in addition to the phenylsulfonyl group in position 3, have an ethoxycarbonyl group on the pyrrole ring and on the pyrrole nitrogen a methyl, benzyl and 4-methoxybenzyl group respectively (Figure 10).

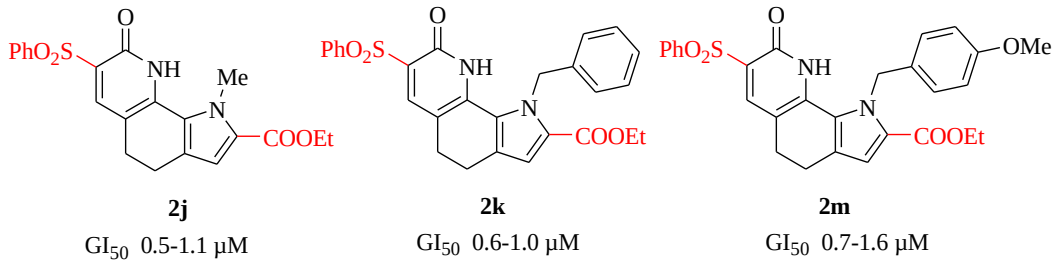


Figure 10

Among the pyrroloquinolinones **3** the best results were obtained with derivatives **3g** and **3q**, in which the quinoline moiety is in its amidic form and an ethoxycarbonyl group is present on the pyrrole ring. Derivative **3s**, which presents a 2-methoxy substituent in position 2 of the tricyclic system, showed activity comparable and in some cases higher

activity to that of angelicin (Figure 11).

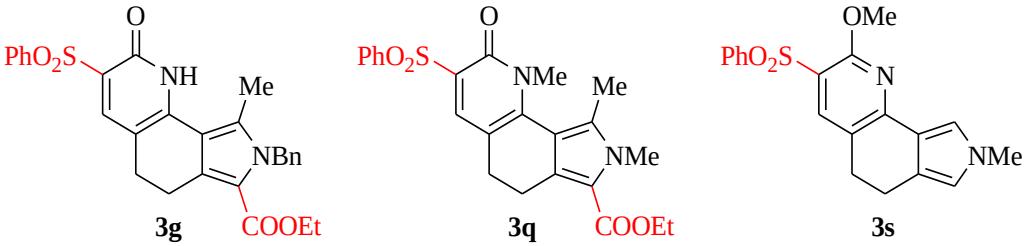


Figure 11

The study of the mechanism of action on the most active compounds of the 2 classes showed that they photoinduced cell death by apoptosis, as demonstrated by the appearance of the typical sub-G1 peak, which indicates apoptotic fragmentation of the DNA, and confirmed by the Annexin test assay. They easily penetrate the cell and accumulate into mitochondria and lysosomes, indicating them as possible targets. Both classes of compounds induced a high production of ROS. Linear dichroism (LD) studies confirmed, in analogy to the previous series, that DNA is not a target for these compounds. Finally, the evaluation of the possible DNA oxidative damage showed that only the pyrrole[3,2-*h*]quinolinones **2** do not cause any breakage of the circular and supercoiled DNA, used in the assay, without formation of (open circular) OC-DNA or (linear) L-DNA indicating absence of phototoxicity towards the macromolecule. This result was considered of extraordinary importance for the modulation of long-term side effects related to the administration of furocumarins. For this reason pyrroloquinolinones of type 2 were covered by patent [17].

On the basis of these results, in order to better understand the behaviour of these small molecules as photosensitizing agents and to cover the chemical space occupied by the former class of compounds, some modifications of the pyrroloquinolinone scaffold were investigated. These included **a**) the replacement of the pyrrole ring with a pyrazole ring, **b**) the enlargement of the central ring of the best class of pyrroloquinolones and **c**) the introduction of a second nitrogen atom, replacing the pyridine ring with the pyrimidine ring (Figure 12). Introduction of a heteroatom is the so called “heavy atom

effect" which is generally related to an increase of photochemical properties.

Thus, a good number of *pyrazolo[3,4-h]quinolinones* of type **4** and **5** were synthesized (Figure 12), which showed the ability to absorb and emit at wavelength in the UV-near visible, with a shift towards red compared to angelicin [18].

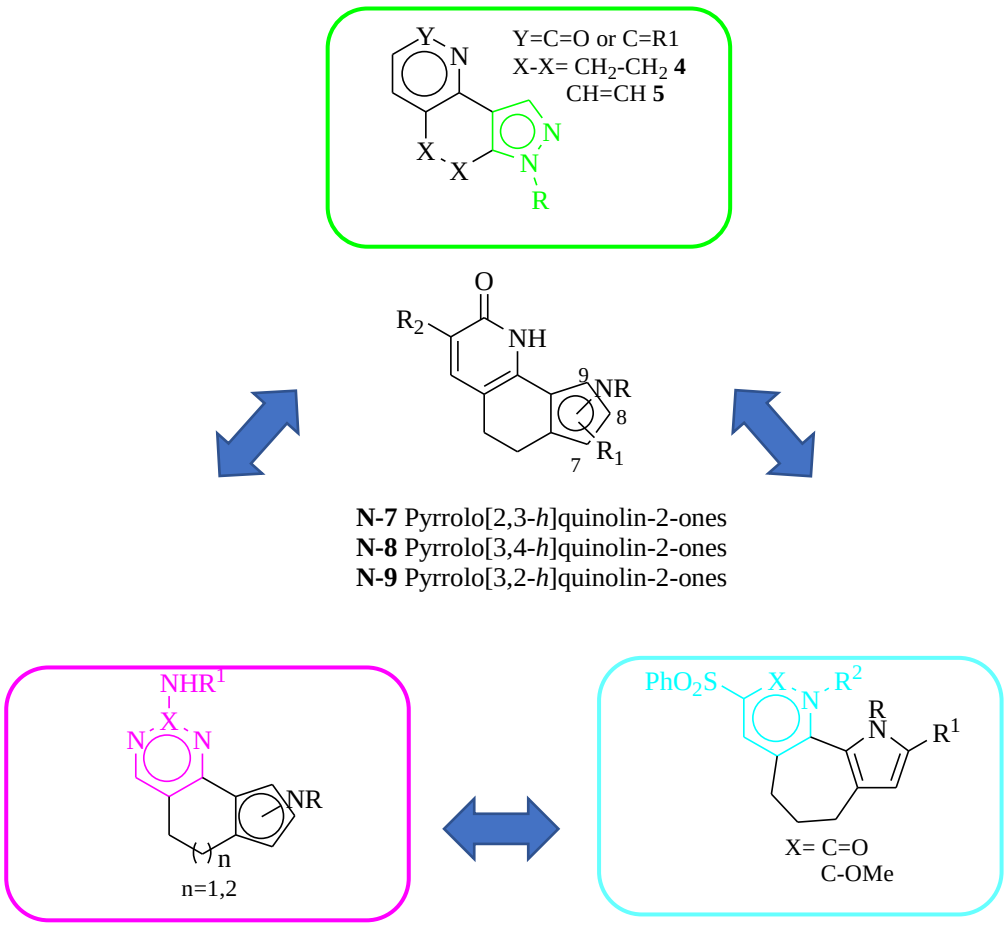


Figure 12

The antiproliferative activity of compounds **4**,**5** was evaluated at two different doses of UVA light on five different cell lines: two liquid tumors cell lines (Jurkat, human T-cell leukemia, and HL-60, human promyelocytic leukemia) and three of solid tumors cell lines (LoVo, human intestinal adenocarcinoma, MCF-7, human breast adenocarcinoma, and A-549, human lung carcinoma). Pyrazoloquinolinones showed an antiproliferative activity in the micro-nanomolar range (GI_{50} 0.04-15.5 μM), resulting in some cases more active than the reference drugs. Among the dihydro derivatives **4** the most active

compounds were those bearing a 2-methoxy substituent in position 2 of the tricyclic system which showed phototoxic activity in the micro-submicromolar range (GI_{50} 0.43-12.68 µM). The best results were obtained with derivative **4u** (GI_{50} 0.43-1.69 µM) and **4v** (GI_{50} 0.53-1.52 µM) bearing a 4-chlorophenyl and 4-methoxyphenyl group respectively on the pyrazole nitrogen and a phenylsulfonyl group in position 3 of the tricyclic system (Figure 13). Among the aromatic derivatives **5**, the best results were obtained with **5m** and **5n** belonging to the 1-methyl substituted series and bearing on the pyrazole nitrogen the same substitution of the dihydro derivatives **4u** and **4v** (Figure 13), which showed a phototoxicity in the submicro to nanomolar range (**5m** GI_{50} 0.04-0.31 µM, **5m** GI_{50} 0.08-0.44 µM).

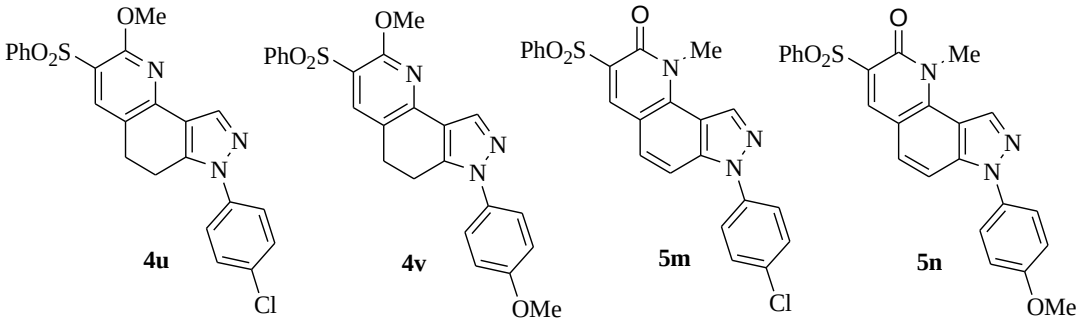
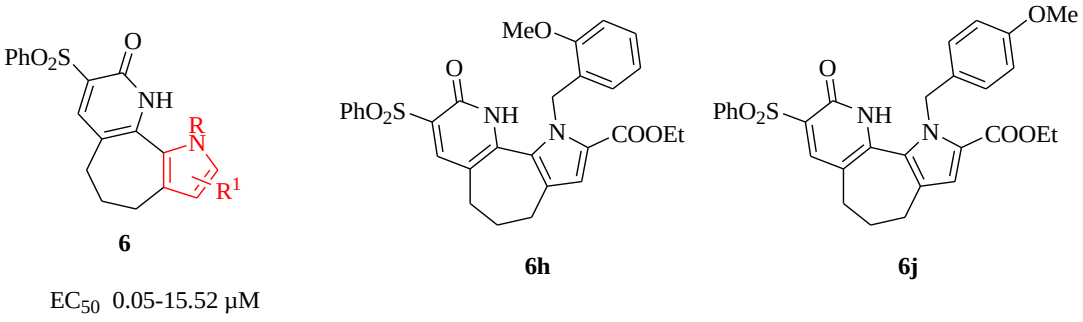


Figure 13

The study of the mechanism of action once again highlighted the ability of these compounds to induce cell death by apoptosis with the involvement of mitochondria and lysosomes, and to induce ROS production. As for derivatives of type **2** the new compounds of type **4** and **5** were not able to induce DNA photo-damage. The enlargement of the central ring of pyrroloquinolinones **2** led to the synthesis of *pyrrole[3',2':6,7]cyclohepta[1,2-b]pyridinones* **6** (Figure 14) [19].

The new synthesized compounds showed no cytotoxic activity, in the absence of irradiation, against MCF-7 “human breast adenocarcinoma”, Jurkat “human T-cell leukemia”, K-562 “human erythroleukemia”, and, after irradiation they showed antiproliferative effect in the micro-submicromolar range (EC_{50} 0.05-15.52 µM) and UVA dose dependent activity. From a structure-activity point of view the 2-ethoxycarbonyl substituted derivatives showed the highest activity underlining the importance of the

presence of this substitution on the pyrrole ring and confirming the results obtained in the previous series. In fact, compounds **6a-e**, lacking of such substituent, were less active than the ethoxycarbonyl analogues **6f-j**, although they maintain an activity in the micro-submicromolar range. Derivatives **6h**, **6j**, and **6p** were the most active of the series with phototoxicity generally at the nanomolar level (EC_{50} 0.05-0.09 μM).



6

EC_{50} 0.05-15.52 μM

6h

6j

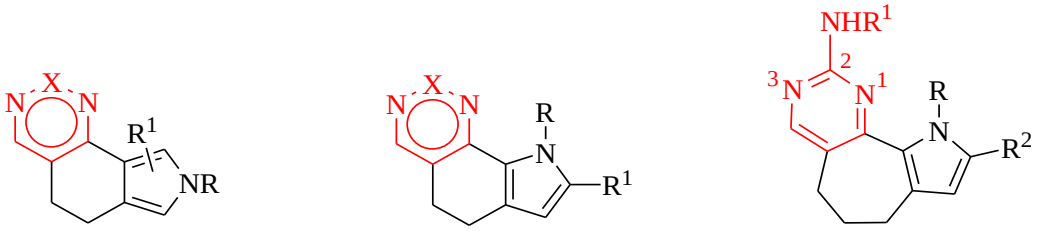
Figure 14

Studies carried out to elucidate the mechanism of action have demonstrated that the most active compound **6j** was able to generate in vitro production of singlet oxygen and superoxide anion. Furthermore, their ability to oxidate specific intracellular dyes and the ability of many scavengers to reduce their phototoxicity confirm the involvement of ROS production in the photoinduced cell death and the involvement of mitochondria and lysosomes. A strong lipid peroxidation of cell membranes was also detected. All these data indicate that pyrrolocycloeptapyridones were able to induce phototoxicity through multiple photosensitizing mechanisms.

Finally, the pyridine ring of pyrroloquinolinones was replaced with the pyrimidine ring. This modification was also supported by a strong rationale considering that the pyrimidines and their benzocondensed derivatives, quinazolines, constitute the core of many anticancer drugs acting for example as kinase inhibitors. An example are *pyrazole[4,3-hquinazolin-3-carboxamides* derivatives which showed the ability to inhibit kinase activity. In particular, the presence of a cyclohexylamine group in position 2 of the tricyclic system generated selective inhibitors of cyclin-dependent kinases (CDK) [20] [21].

This structural modification led to the synthesis of three different positional isomers with

based on the pyrroloquinazoline core structure such as *pyrrolo[3,4-h]quinazolines* **7**, p*yrrolo[3,2-h]quinazolines* **8** and the *pyrrolocyclohepta[1,2-d]pyrimidine* **9** (Figure 15) [22] [23] [24]. Pyrroloquinazolines of type **7** were already active in the 'dark' unlike the other two series of compounds **8** and **9,** thus phototoxicity studies were not carried on further.



7 **8** **9**

GI_{50} 0.2-15.2 µM EC_{50} 0.06-4.96 µM

Figure 15

Pyrroloquinazolines **8** demonstrated generally active with a GI_{50} values range from micro- to submicromolar (GI_{50} 0.21-15.20 µM). In particular, the most active derivatives of the series were **8d** (GI_{50} 0.21-0.48 µM) and **8e** (GI_{50} 0.23-0.41 µM) bearing a benzyl and a 4-methoxybenzyl group respectively, on the pyrrole nitrogen, an amino group in position 2 of the pyrimidine ring and an ethoxycarbonyl group in α-position to the pyrrole nitrogen (Figure 16). Derivatives of type **9** showed a high phototoxic activity with EC_{50} values in micro-nanomolar range (EC_{50} 0.06-4.96 µM). The most active compounds, resulted: **9g** (EC_{50} 0.08-0.09 µM) and **9h** (EC50 0.06-0.10 µM) showing EC_{50} equal to or below 0.1 µM against all tested cell lines.

SAR analysis indicated that a cyclohexyl amino group in position 2 of the pyrimidine ring and an ethoxycarbonyl group near to the pyrrole nitrogen were crucial for the activity.

These two new classes of compounds **8**,**9** showed a good antiproliferative activity after irradiation with values reaching the nanomolar range; in particular the most active compounds of the two series show again an ethoxycarbonyl group in position 2 of the pyrrole ring.

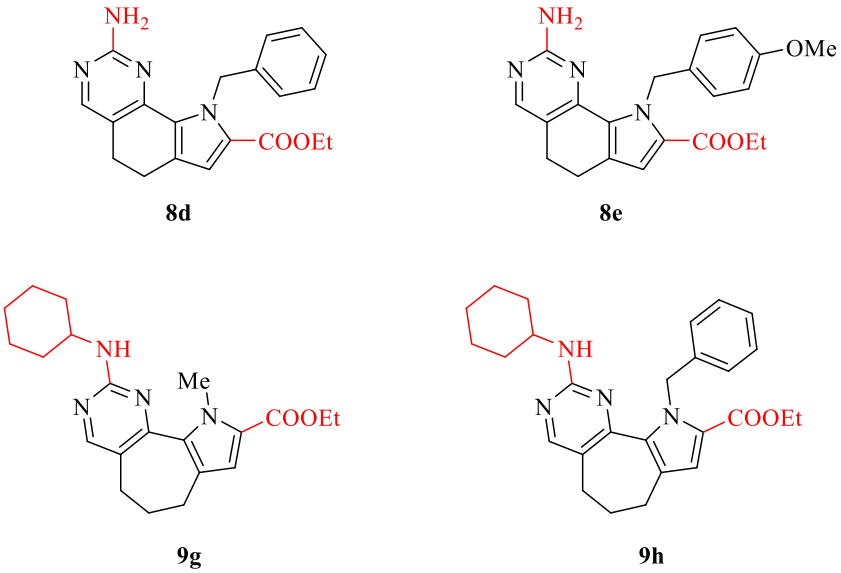


Figure 16

Also, in this case the study of the mechanism of action on the most active compounds demonstrated that they were able to induce cell death by apoptosis with the involvement of mitochondria and lysosomes and a remarkable production of ROS. Furthermore, derivatives of type **9** showed a fairly long triplet state ($\tau \sim 7$ µs), absorbance in the near UV-visible and, more importantly they did not induced DNA photo-damage.

2. Project

Considered the interesting results obtained in the field, it was thought for my PhD project, to synthesize new photoactivable systems, in which the pyrrole ring is rotated, leading to the synthesis of pyrimidoindolyzines (n=1) and pyrimidopyrroloazepines (n=2) as well as pyrrolonaphtyridinones (n=1) and pyridopyrroloazepinones (n=2) (Figure 17) with the aim to evaluate the effect on the phototoxicity.

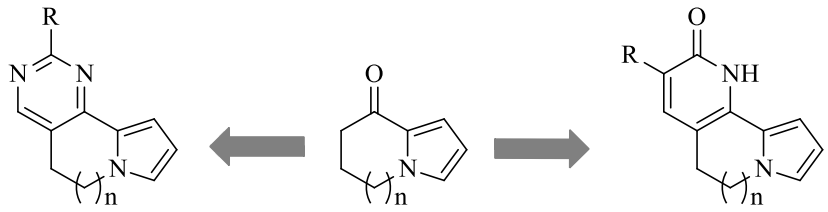


Figure 17

3. Synthesis of the *building blocks*

At the beginning, considering the relevant phototoxic activity of pyrimidine compounds **9** and their long lived triplet state (microseconds), a favorable condition for interaction with tissue oxygen and biological macromolecules, I focused my attention on the synthesis of the two new tricyclic systems *Pyrimido[5,4-g]indolizin-2-amine* **38,40,42** and *Pyrimido[4,5-c]pyrrolo[1,2-a]azepin-2-amine* **39,41** (Figure 18) as positional isomers of the previous type of pyrimidine derivatives **8** and **9** shown in the Figure 15.

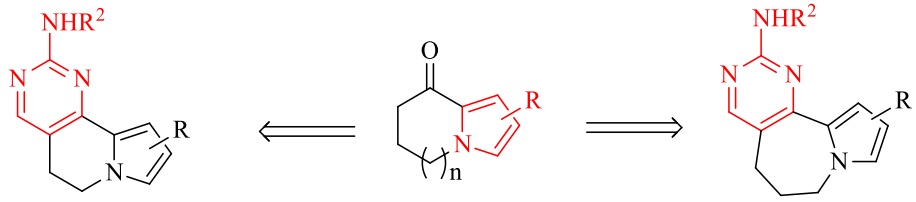
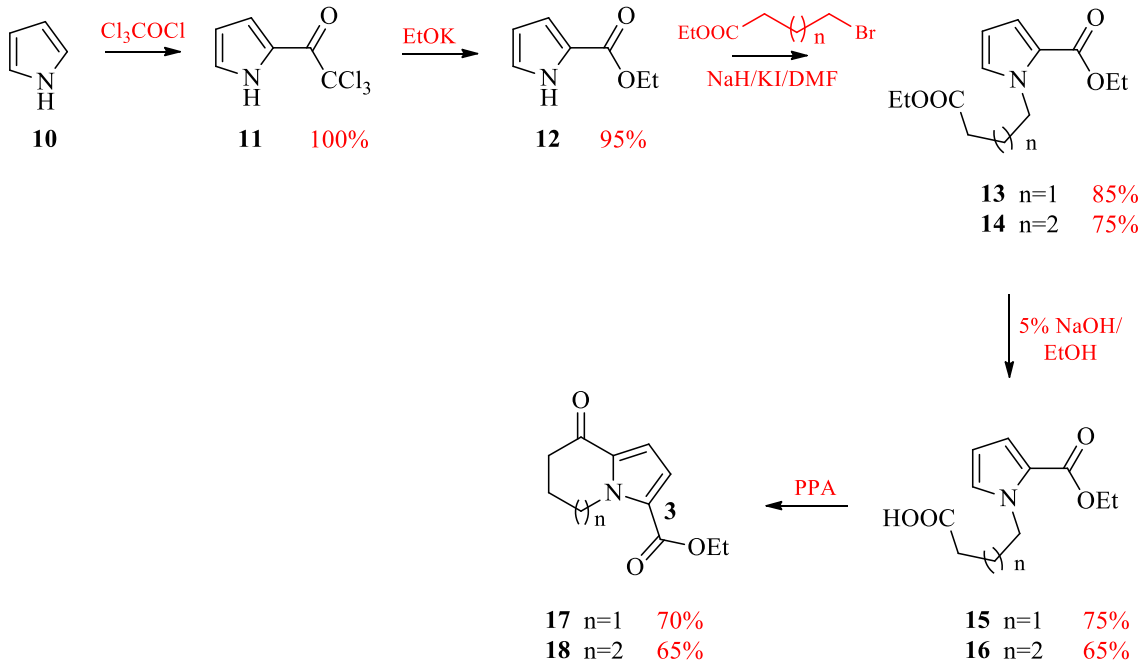


Figure 18

Ideal building blocks for the synthesis of the new heterocyclic systems would have been ketones *6,7-dihydroindolizin-8(5H)-one* **17,24,29** (n=1) (Scheme 1) and *5,6,7,8-tetrahydro-9H-pyrrole[1,2-a]azepin-9-one* **18,25** (n=2) (Scheme 2).

The presence of the annular carbonyl in fact make the α position available for the introduction of a second electrophilic center as second reactive center, which together with the carbonyl group, easily react with dinucleophiles leading to further annelations.

My study started with ethoxycarbonyl derivatives **17**,**18** to mimic the decoration of the most powerful derivatives of the previous series. For the synthesis of ketone **17** a suitable intermediate was found to be the pyrrole derivative **15** which can be cyclized by activation of the carboxylic group of γ-pyrrolic acid with polyphosphoric acid according to the modified Taylor conditions (Scheme 1) [12].



Scheme 1

The intermediate **15** can be obtained in turn, starting from ethyl pyrrole-2-carboxylate **12** by introduction of an ethyl butyric chain on the nitrogen atom and subsequent basic hydrolysis of the ester functionality (Scheme 1). Ethyl pyrrole-2-carboxylate **12** can be synthesized starting from pyrrole **10**, by reaction with trichloroacetyl chloride, to give intermediate **11**, and subsequent nucleophilic substitution in the presence of potassium ethoxide which allowed, the isolation of ethyl pyrrole-2-carboxylate **12** in 95% yield in two steps (Scheme 1).

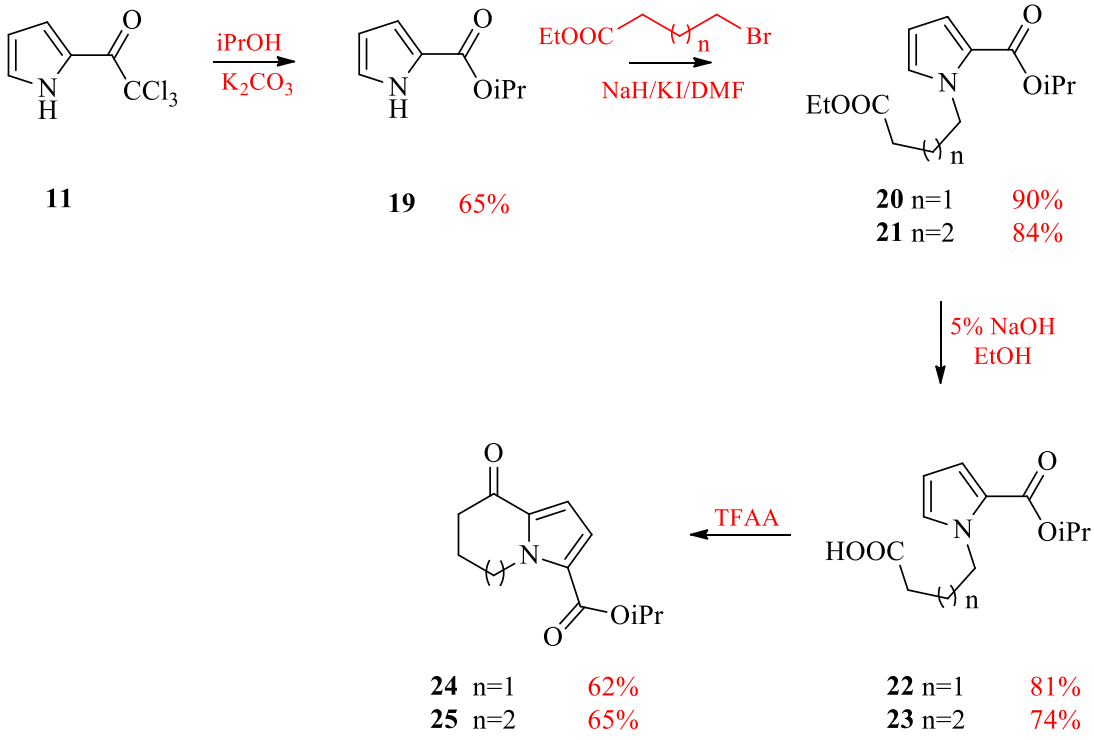
The alkylation of the pyrrole nitrogen was carried out using a base and ethyl 4-bromobutyrate as an alkylating agent (Scheme 1). Numerous attempts have been done involving the use of different bases, such as K_2CO_3, *t*-BuOK or NaH, in DMF or THF, at

different temperatures. The best results were obtained using NaH, as a base, in DMF and stoichiometric amount of KI, often used in the alkylation reactions as a catalyst, at reflux. First the reaction was heated at 40°C for 6 h after adding the base to promote the formation of the anion, then KI and the alkyl chain were added and the reaction was heated at reflux for 24 h. This expedient has allowed the completion of the reaction and the isolation of the desired intermediate **13** with a 85% yield. This latter **13** was then subjected to basic hydrolysis of the ethoxycarbonyl function in the side chain, using a 5% aqueous solution of NaOH, at room temperature. The use of mild conditions and long reaction times were necessary to obtain the selective hydrolysis of the ethoxycarbonyl function of the alkyl chain to give derivatives **15** (75%) (Scheme 1). The reaction of compound **15** in polyphosphoric acid finally allowed the isolation of ketone **17** with a 70% yield (Scheme 1).

Similarly, using ethyl 5-bromovalerate as alkyl chain we succeeded in the isolation of ethyl 9-oxo-6,7,8,9-tetrahydro-5*H*-pyrrole[1,2-*a*]azepino-3-carboxylate **18**. The introduction of the ethyl 5-bromovaleric chain on the nitrogen pyrrole led to the isolation of intermediate **14** (75%) which was subjected to basic hydrolysis to give compound **16** (65%) which was cyclized in polyphosphoric acid leading to the ketone **18** with a 65% yield (Scheme 1).

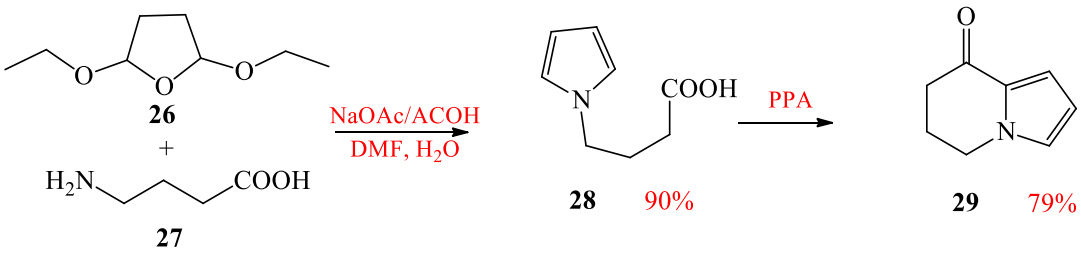
In order to increase the number of derivatives two other series of products have been synthesized bearing an isopropyl carboxy group or a hydrogen in position 3. The synthesis of ketones **24** and **25** were obtained following the same synthetic pathway of ketones **17,18** starting from isopropyl pyrrole-2-carboxylate **19**. The synthesis, as for ethyl pyrrole-2-carboxylate, started from the trichloroacetyl derivative **11** which reacted in presence of isopropanol and K_2CO_3, as base, at 60ºC to give derivative **19** with a 65% yield (Scheme 2).

The alkylation of the pyrrole nitrogen was carried out in DMF in the presence of NaH, KI, and ethyl-4-bromobutyrate or ethyl 5-bromovalerate under the same operating conditions used for ethoxycarbonyl ketones (90-84%) (Schema 2). Derivatives **20**,**21** were hydrolyzed using 5% NaOH to give intermediates **22,23** (81-74%) which were converted into ketones **24,25** using an excess of trifluoroacetic anhydride in DCM (62-65%).



Scheme 2

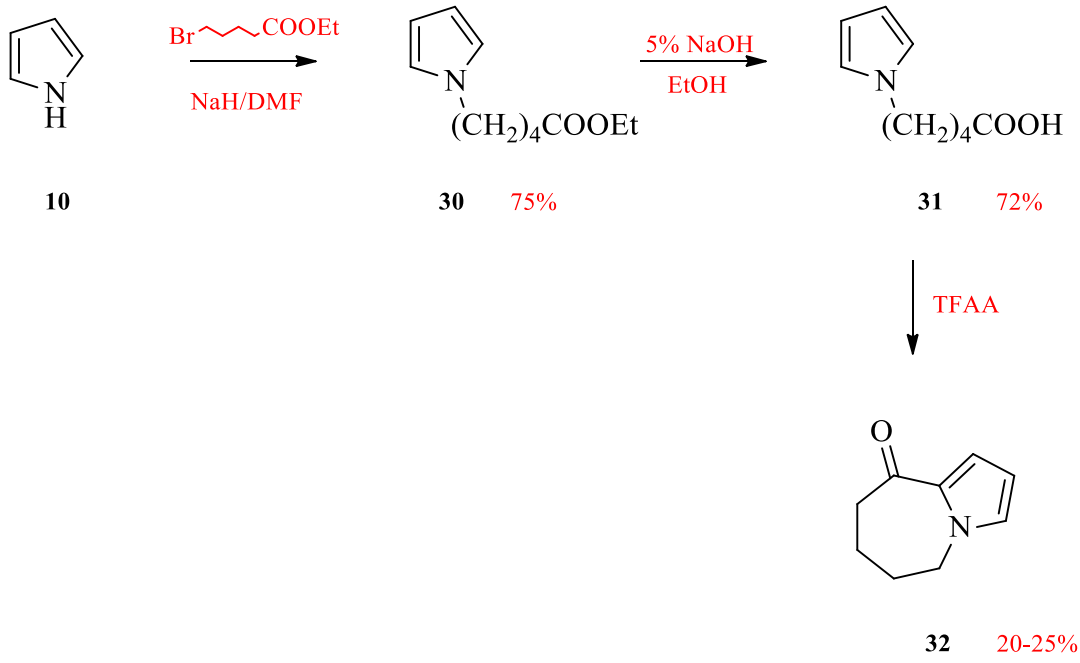
As reported in literature, ketone **29** , bearing a hydrogen in position 3, can be obtained by condensation of γ-amino butyric acid (GABA) **27** with 2,3,4,5-tetrahydro-2,5-diethoxytetrahydrofuran **26** followed by activation of the carboxylic group of γ-pyrrolic acid **28** with polyphosphoric acid (Scheme 3) [25], [26].



Scheme 3

Compounds **26** and **27** were reacted using anhydrous sodium acetate in a mixture of H_2O:AcOH:DMF (3:1:3) at 90°C. After a careful and long work up it was possible to isolate intermediate **28** from the reaction mixture in 90% yield. Subsequent reaction of the intermediate **28** in polyphosphoric acid led to the isolation of the desired ketone **29** (90%) (Scheme 3).

To obtain ketone **32**, the commercially available pyrrole **10** was functionalized to the nitrogen using ethyl 5-bromovalerate, NaH, as base, KI, as catalyst, in DMF (Scheme 4). Then intermediate **30** was subjected to basic hydrolysis followed by cyclization with PPA to give ketone **32** with poor yield (20%). Also, cyclization of derivative **31** in the presence of trifluoroacetic anhydride allowed the isolation of ketone **32** with very poor yield (25%) (Scheme 4).



Scheme 4

The so obtained ketones **17,18,24,25,29** were subjected to the next step in order to introduce a second electrophilic center to have the access to versatile building blocks for the final annelation of pyrimidine ring.

In particular the enamino derivatives represent versatile intermediates having two vicinal electrophilic centers: the carbonyl, and the exocyclic carbon bonded to α position to the carbonyl. In fact α-*N*,*N*-disubstituted-aminomethylenketones (Figure 19), constitute a system in which the resonance of charges, offers numerous possibilities of reactions, also thanks to the intervention of the nitrogen atom.

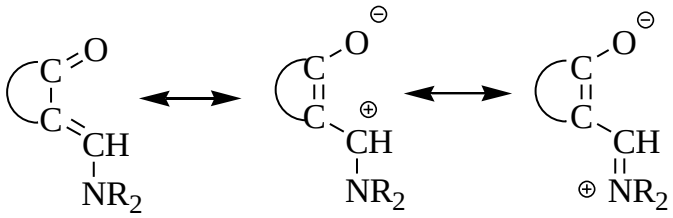


Figure 19

The introduction of the enamino functionality can be obtained in two steps through the formation of a hydroxymethylene intermediate or in one step by direct introduction of the enamino group. In the two steps route, ketones were first subjected to the introduction of a hydroxymethylene group into α position to the carbonyl, using *t*-BuOK as a base, toluene as solvent, and ethyl formate as a formilating agent allowing the isolation of derivatives **33**,**34**,**35** (65-68%) (Scheme 5). Hydroxymethylene derivatives **33**, **34**,**35** were further reacted with diethylamine in toluene, and after evaporation of the solvent, it was possible to the obtain the desired enamino derivatives **36, 37, 38** (77-87%) (Scheme 5). Direct introduction of the enamino functionally generally involves the use of commercially available amide acetals, such as *N*,*N*-dimethylacetamide dimethylacetal (DMADMA), *N*,*N*-dimethylformamide dimethylacetal (DMFDMA) and *N*,*N*-dimethylformamide diethylacetal (DMFDEA). These react in solvents such as toluene or benzene at reflux leading to quantitative yields of the dialkylamino derivative.

In a review of 2004 [27], it was reported a scale of reactivity among amide acetals (Figure 20), placing the DMFDMA as the less reactive and the *tert*-butoxy bis(dimethylamino)methane (TBDMAM), as the most reactive.

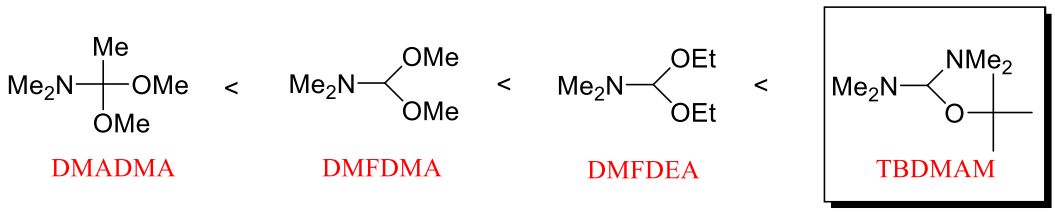
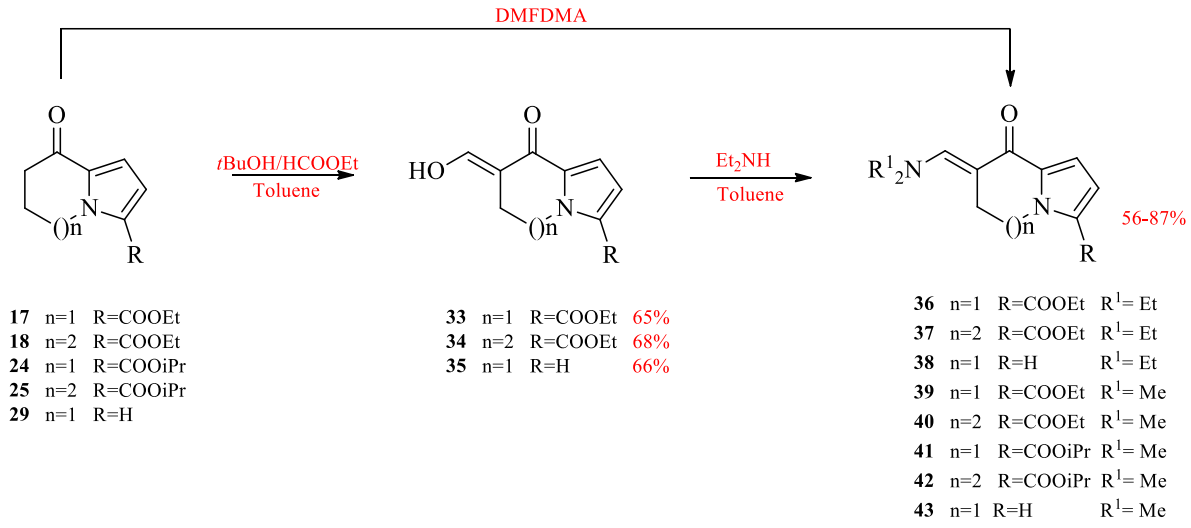


Figure 20

TBDMAM, also called Bredereck's reagent, seemed to be the most efficacious reagent; however a disadvantage was the high cost. For this reason, we started our studies using DMFDMA available at low cost.

So, ketones **17,18,24,25,29**, have been reacted in the presence of DMFDMA and DMF, as a solvent, both under conventional and microwave-assisted heating, using a CEM Discover LabmateTM apparatus. The use of microwaves not only allows to reduce reaction times but also to obtain cleaner reactions and higher yields. In fact taking advantage of the ability of some liquids or solids to transform electromagnetic energy into heat, microwave are able to cross the container by heating only the reagents and the solvent, leading to a rapid and uniform increase in temperature thus avoiding the formation of by-products and decomposition products. Moreover, temperatures higher than the boiling point of the solvent used can be reached with the use of pressurized vial.

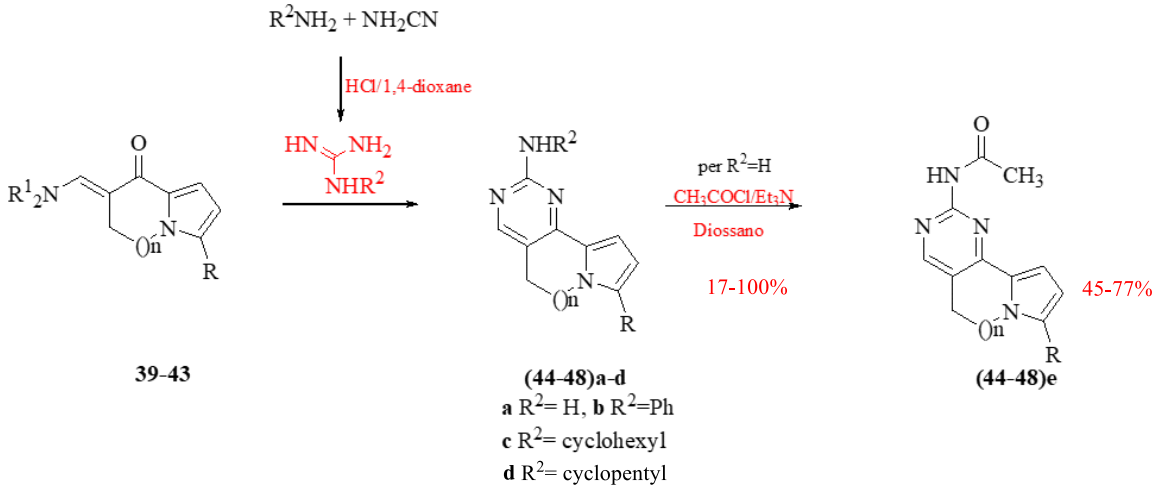


Scheme 5

In our case the microwave reactions were conducted at the temperature (T) of 120°C and power (PW) of 50 watts for 30 minutes. In both case the desired enaminoketones **39**,**40**,**41**,**42**,**43** were isolated after pouring the reaction mixture onto crushed ice (56-87%) (Scheme 5). So ketones **17,18,24,25,29** resulted much more reactive, allowing the use of DMFDMA, compared to previous classes in which it was necessary to use TBDMAM to obtain the isolation of the desired enaminoketones. To achieve the closure of the pyrimidine ring we had to react enaminoketones with the suitable guanidine.

4. Pyrimido[5,4-*g*]indolizin-2-aminine and Pyrimido[4,5-*c*]pyrrolo[1,2-*a*]azepin-2-aminine

Based on the results of the antiproliferative activity obtained with the previous series of pyrimidine compounds of type **9** we selected guanidine, phenyl guanidine, cyclopentyl and cyclohexyl guanidines to have pyrimidines bearing an amino, aniline, cyclohexyl, cyclopentyl substituents. Guanidines which were not commercially available were prepared starting from the corresponding amine by reaction with cyanamide in presence of HCl in 1,4-dioxane (Scheme 6).



Scheme 6

The reaction of intermediates **39**,**40**,**41**,**42**,**43** with guanidine nitrate was carried out in the presence of sodium methoxide in ethanol by reflux leading to the isolation of 2-amino derivatives **(44-48)a** with a good yield (41-100%). Reaction of enaminoketones **39**,**40**,**41**,**42**,**43** with phenyl, cyclopenthyl and cyclohexyl guanidines was carried out in the absence of base, using DMF, as solvent, at 100°C. After pouring the reaction mixture onto crushed ice, it was possible isolate the desired pyrimidine derivatives **(44-48)c-d** bearing an aniline, a cyclohexyl and a cyclopenthyl substitution on the nitrogen in 2 position of the tricyclic system (17-98%) (Scheme 6). Furthermore, the amino derivatives **(44-48)a** were subjected to acetylation of the amino group using acetyl chloride, as acetylating agent, triethylamine as base, in dioxane, leading to the isolation of the acetyl substituted derivatives **(44-48)e** (45-77%) (Scheme 6, Table 1).

CDP	n	R	R^2	Yield	CDP	n	R	R^2	Yield
44a	1	COOEt	H	42%	**46d**	1	COOiPr	Cyclopentyl	73%
44b	1	COOEt	Ph	30%	**46e**	1	COOiPr	-	63%
44c	1	COOEt	Cyclohexyl	17%	**47a**	2	COOiPr	H	100%
44d	1	COOEt	Cyclopentyl	32%	**47b**	2	COOiPr	Ph	80%
44e	1	COOEt	-	77%	**47c**	2	COOiPr	Cyclohexyl	98%
45a	2	COOEt	H	41%	**47d**	2	COOiPr	Cyclopentyl	82%
45b	2	COOEt	Ph	23%	**47e**	2	COOiPr	-	48%
45c	2	COOEt	Cyclohexyl	44%	**48a**	1	H	H	74%
45d	2	COOEt	Cyclopentyl	61%	**48b**	1	H	Ph	34%
45e	2	COOEt	-	45%	**48c**	1	H	Cyclohexyl	56%
46a	1	COOiPr	H	71%	**48d**	1	H	Cyclopentyl	70%
46b	1	COOiPr	Ph	79%	**48e**	1	H	-	60%
46c	1	COOiPr	Cyclohexyl	86%					

Table 1

5. Biology

All the new synthesized compounds of type **(44-48)a-e** (Table 1) were submitted to the National Cancer Institute for a first evaluation of the antitumor properties "in the dark" against a panel of about 60 human tumor cell lines divided into 9 subpanels (breast, ovaries, lung, colon, CNS, melanoma, leukemia, kidney, prostate). All pyrimidine derivatives did not show antiproliferative activity on the NCI panel at a dose of 10^{-5} M, which is desirable for a photo-chemotherapeutic compound.

The new derivatives were also sent to the University of Padua to assess their photoantiproliferative activity. Initially, spectrophotometric properties, such as the maximum wavelength (λ_{max}), absorbance and molar extinction coefficient (ε) at λ_{max} and at 365nm (wavelength used in therapy for cutaneous diseases), were evaluated. All compounds absorbed UV-Vis light, a fundamental requirement for a photosensitizer, and presented bands in the UV-A region. With some exceptions, **(44-48)a-e** displayed high molar extinction coefficients both at their λ_{max} and at 365 nm (Table 2).

CDP	λ_{max} (nm)	Absorbance (λ_{max})	Absorbance (365 nm)	$\varepsilon_{\lambda max}$	$\varepsilon_{365\ nm}$	CDP	λ_{max} (nm)	Absorbance (λ_{max})	Absorbance (365 nm)	$\varepsilon_{\lambda max}$	$\varepsilon_{365\ nm}$
44a	337	0.463	0.081	9260	1628	**46d**	389	0.527	0.351	21080	14040
44b	309	0.263	0.063	5260	1256	**46e**	346	0.656	0.139	26240	5560
44c	307	0.462	0.3	9240	6000	**47a**	337	0.709	0.096	14180	1924
44d	388	0.633	0.418	25320	16720	**47b**	291	0.847	0.408	16940	8160
44e	332	0.512	0.077	20480	3072	**47c**	374	0.946	0.877	18920	17540
45a	337	0.899	0.293	17980	5860	**47d**	375	0.799	0.725	15980	14500
45b	/	/	/	/	/	**47e**	329	0.501	0.021	20040	852
45c	245	0.753	0.558	15060	11160	**48a**	350	0.946	0.473	18920	9460
45d	376	0.633	0.576	25320	23040	**48b**	277	0.649	0.406	12980	8120
45e	329	0.504	0.172	20160	6880	**48c**	355	0.472	0.416	9440	8320
46a	350	0.842	0.613	16840	12260	**48d**	376	0.617	0.537	86402	75200
46b	292	0.162	0.069	3240	1386	**48e**	342	0.559	0.136	22360	5440
46c	388	0.459	0.311	18360	12440						

Table 2

In a preliminary screening, cell viability was determined after irradiation with a UV-A light dose of 2.0 J/cm^{2}HCC1954 breast cancer cell line and T24 bladder cancer cell line in presence of the new derivatives.

Cells were seeded into 96-well plates (Eppendorf, Milan, Italy) overnight before treatment. Then they were incubated with different concentrations of the title compounds (0 - 20.0 µM) for 1 h and irradiated with 2.0 J/cm^{2}of UV-A light. For UV-A irradiation, a Philips HPW 125 lamp was used, mainly emitting at 365 nm. The total energy hitting the sample was monitored by means of a radiometer (Variocontrol,Waldmann, Villingen-Schwenningen, Germany), equipped with a Variocontrol UV Sensor (Waldmann). The radiant power emitted by the UV-A lamp was about 8 mW cm^{-2}. The samples were maintained at room temperature during irradiation (total UVA dose 2.0 J/cm^2). Cell viability was determined using resazurin assay. Briefly, 10 µL of a 2.5 mM solution of resazurin were added to each well of the treat 96-well plates and incubated for 4 h. Fluorescence measurements were carried out using Victor 3 multimodal plate reader (Ex/Em = 555/610 nm, Perkin Elmer, Massachusetts, United States). The results have been expressed as IC_{50} values, i.e. the concentration of compound able to induce 50% cell death with respect to control culture (untreated cells).

Preliminary results on **44c**, **45c**, **46c** and **47c** showed the ability of our compounds to decrease cell viability with IC_{50} values in the micromolar range. Three out of four displayed photoantiproliferative activity lower than 5 µM against HCC1954 cell line (Figure 21).

HCC1954 + 2 J/cm^2 UVA		HCC1954 + 2 J/cm^2 UVA	
IC_{50} (µM)		IC_{50} (µM)	
44c	2.28	**46c**	> 20
45c	1.15	**47c**	3.63

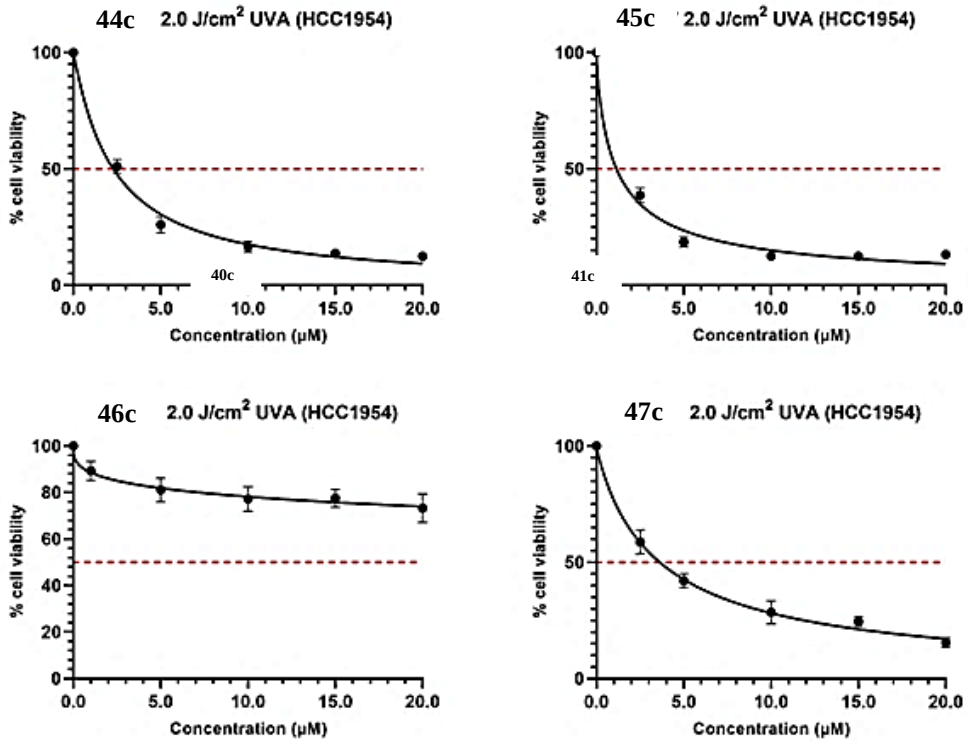
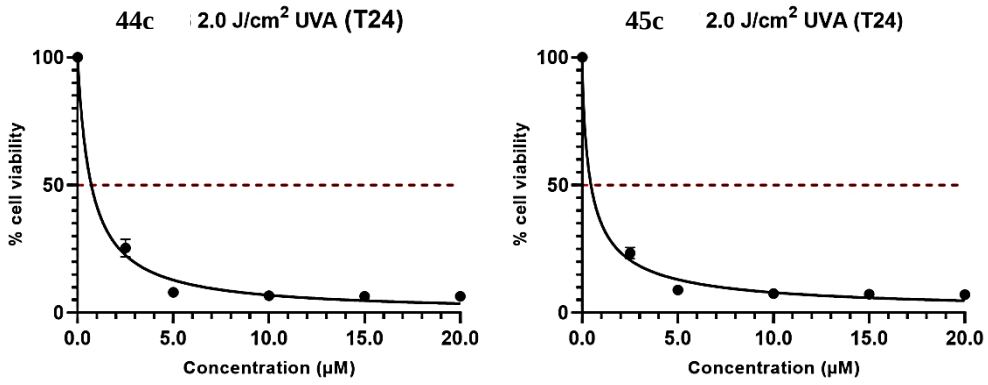


Figure 21

Moreover preliminary results of **44c** and **45c** showed that bladder cancer cell line T24 was more sensitive than breast cancer cell line HCC1954. In particular, both **44c** and **45c** were able to completely abrogate T24 cell viability at 5 μM concentration (Figure 22).



T24 + 2 J/cm^2 UVA	
IC_{50} (µM)	
44c	0.72
45c	0.75

Figure 22

The best results were obtained with compound **45c** which reached IC_{50} value in the submicromolar-low micromolar range (1.15 µM against HCC1954 cell line and 0.75 µM against T24 cell line).

For derivative **45c** was also evaluate the antiproliferaty activity after irradiation with two different UV-A doses (2.0 J/cm^2 and 4.0 J/cm^2). As can be seen in Figure 23, the trend of the two graphics is almost identical, in fact derivatives **45c** showed IC_{50} value of 0.75 µM at 2.0 J/cm^2 dose and 0.83 µM at 4.0 J/cm^2 dose, indicating an antiproliferative activity UV-A dose-independent.

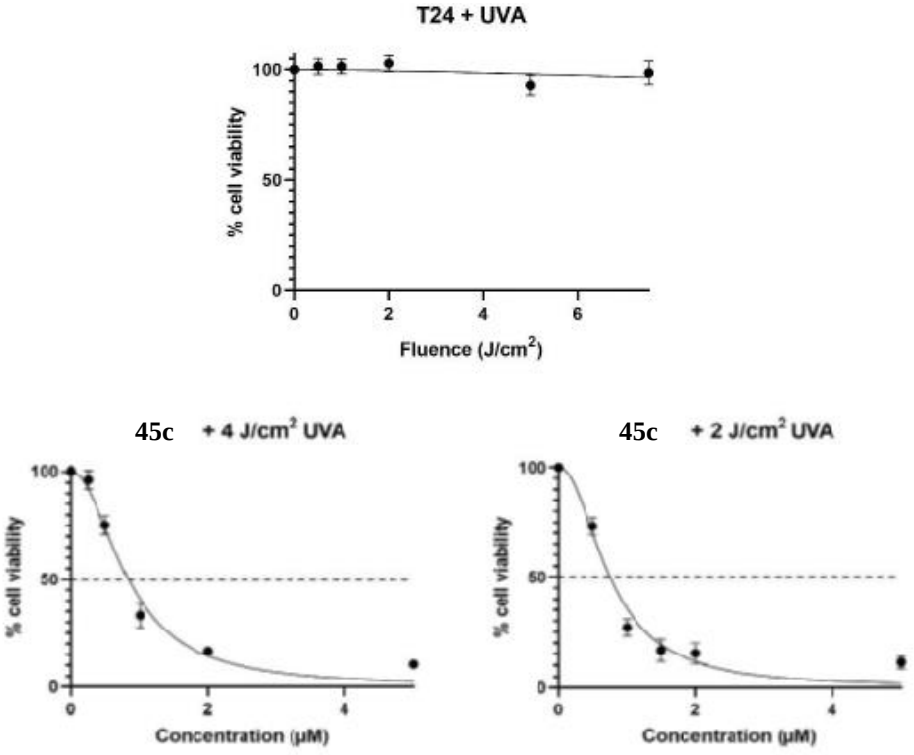


Figure 23

The production of singlet oxygen ($^1O_2$) plays an important role in the activity of a PS, so it was evaluated the ability of these four derivatives to induce production of oxygen species after irradiation at different UV-A doses. Samples containing the test compounds, *p*-nitrosodimethylaniline (RNO) and imidazole in 0.02 M phosphate buffer (pH 7.3) were irradiated with increasing UV-A radiant exposures and their absorbance at 440 nm was then measured. The data were expressed as percentage of RNO bleaching. In fact it has been observed that singlet oxygen acceptors (like imidazole derivatives) induce the bleaching of RNO, which can be followed spectrophotometrically at 440 nm. Since $^1O_2$ does not react chemically with RNO, this bleaching is a consequence of $^1O_2$ capture by the imidazole ring which results in the formation of a trans-annular peroxide intermediate able to induce the photodegradation of RNO.

As shown in Figure 24, derivative **45c** induces the highest percentage of photobleaching (about 60%) followed by compound **44c** with a maximum photobleaching rate of about 36%, while derivatives **46c** and **47c** produce a significantly smaller quantity of singlet oxygen. In this latter case the minimum increase in the photobleaching percentage showed from the two derivatives when the dose of light applied increases is due to the degradation of the RNO and not to the actual production of singlet oxygen by the compounds. In fact even though **46c** and **47c** have a higher absorbance value at 365 nm than **44c** and **45c**, they do not appear to be the pyrimidine derivatives that produce the highest level of $^1O_2$. This aspect was indeed confirmed by the lower photoantiproliferative activity of **46c** and **47c** on tested tumour cell lines compared to the other two derivatives. Furthermore, the higher production of singlet oxygen by **45c** is perfectly correlated with its high photoantiproliferative activity.

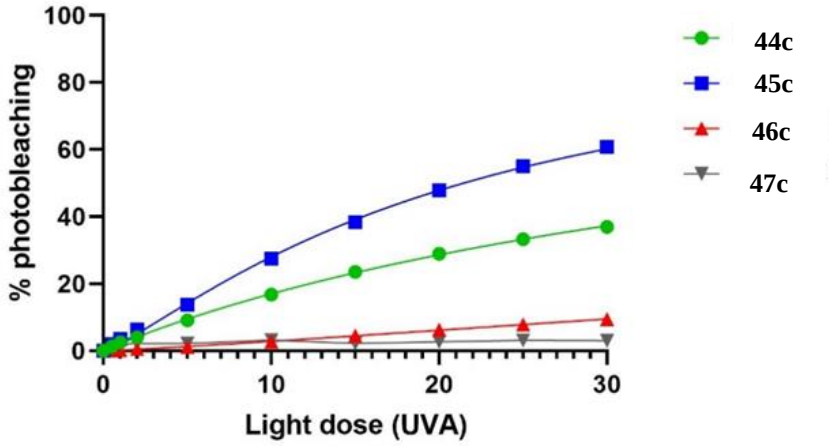


Figure 24

It was also evaluated the ability of our compounds to induce the production of superoxide anion. So samples containing the compounds under examination and nitroblue tetrazolium in10 mM carbonate buffer (pH 10) were irradiated with increasing UV-A radiant exposures, and their absorbance at 560 nm was measured (Figure 25). In fact the superoxide anion induces the reduction of the nitroblue tetrazolium to blue insoluble formazan salts which produce an increase in absorbance at 560 nm.

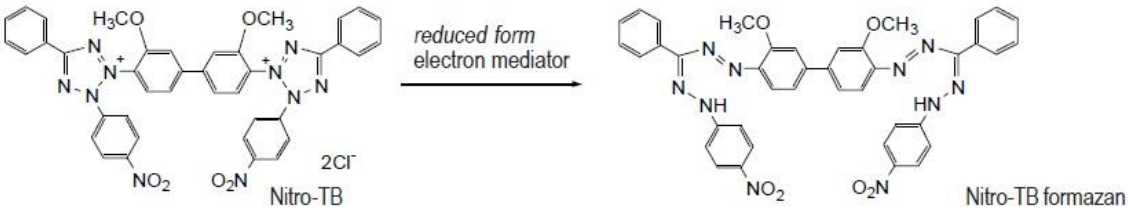


Figure 25

As can be seen in the Figure 26, even in this case, the compound that induced the greatest increase in absorbance was derivative **45c**.

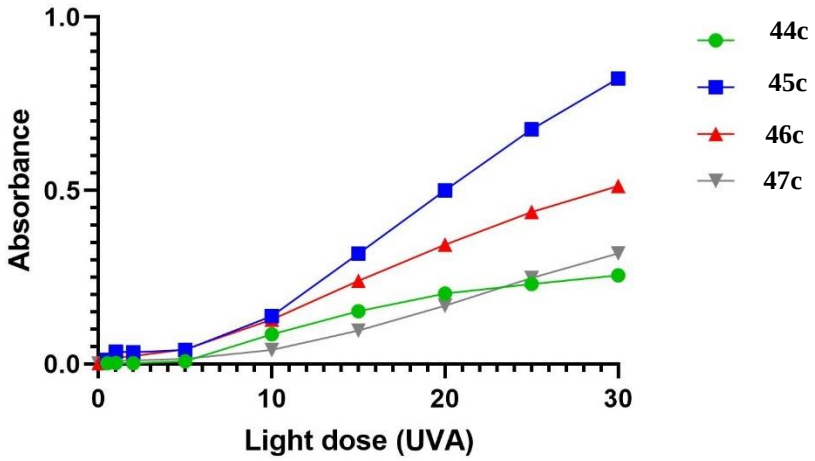


Figure 26

Some experiments were carried out in order to determine whether the new derivatives were able to photosensitize DNA strand break activity. Their ability to photodamage DNA was evaluated using supercoiled circular DNA because it allowed the detection of structural alterations such as strand breaks. Double-stranded supercoiled (SC or I form) plasmid is sensitive to damage by a variety of photosensitizers. Cleavage of one strand produces a relaxed, but still double-stranded, open circular (OC or II form) DNA (Figure 27). It is possible to separate the two different forms with horizontal electrophoresis thanks to their different hydrodynamic properties.

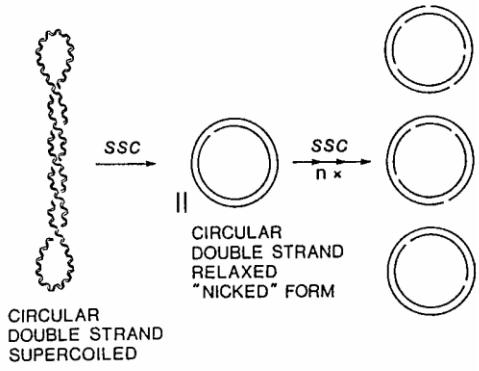


Figure 27

In this case, supercoiled pBR322 plasmid, dissolved in phosphate buffer 10 mM, was used. Plasmid solutions were irradiated with increasing UV-A doses (0 J/cm^2, 2 J/cm^2, 7.5 J/cm^2) in the presence of **45c** at 12.5:1 drug/DNA molar ratio. As control were used non-treated supercoiled DNA (SC), linear DNA (L) and supercoiled DNA irradiated the higest UV-A dose used (7.5 J/cm^2) (SC+UVA).

As can be seen in the electrophoretic gel in Figure 28, compound **45c** did not induce a significant increase of OC form respect to the control.

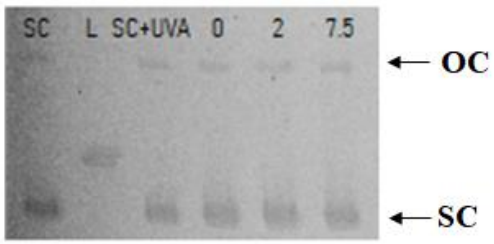


Figure 28

Finally it was also evaluated if DNA bases were involved in the oxidative damage to DNA using base excision repair enzyme Endonuclease III (Endo III). Supercoiled pBR322 plasmid solutions were irradiated with increasing UV-A doses (0 J/cm^2, 7.5 J/cm^2, 15 J/cm^2) in the presence of **45c** at 12.5:1 drug/DNA molar ratio and the enzyme. As control were used non-treated supercoiled DNA (SC), linear DNA (L) and supercoiled DNA irradiated the higest UV-A dose used (15 J/cm^2) (SC+UVA).

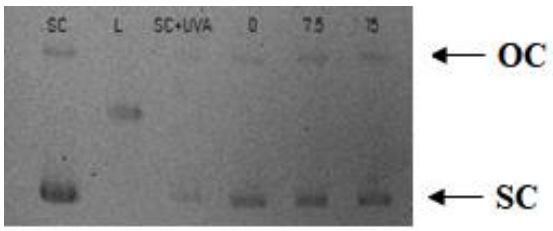


Figure 29

As showed in the electrophoretic gel in Figure 29 and in Table 3, compound **45c** induced an increase of the percentage of OC form respect to the control in a UV-A dose dependant manner. Thus we can concluded that this compound is able to produce of oxidative damage to DNA, even if the damage is not so significant that it can be said that DNA represents a target.

UVA dose	% oc	% sc
SC	12.1	87.9
0 J/cm^2	13.8	86.2
7.5 J/cm^2	23.9	76.1
15 J/cm^2	25.3	74.7

Table 3

From these preliminary data it emerged derivative **45c** which showed a good phototoxic activity mediated by the production of singlet oxygen and superoxide anion and DNA does not seem to be the preferred target of this new compound.

To evaluate the possible mechanism by which cell death is induced by derivative **45c**, a flow cytofluorimetric experiment was performed on T24 cells in the presence of Annexin V-FITC, able to detect phosphatidylserine translocation from the inner face to the outer surface of plasma membrane during apoptosis, and DNA-specific dye propidium iodide, able to intercalate into DNA after damage to the cellular membrane.

The dot-plot is constructed by placing the fluorescence of the Annexin V-FITC marker in the abscissa while in the ordinate the fluorescence deriving from the PI marker, both on a logarithmic scale. It is possible to divide the dot-plot into four quadrant which allow to identify the changes in distribution of the percentage of the cell population. Q1 quadrant, Annexin V-negative/PI-positive cells, indicates necrotic cells in which the damaged cellular membrane allow to PI to reach DNA and to intercale; Q2 quadrant, Annexin V-positive/PI-positive cells, indicates cells in late apoptosis; Q3 quadrant,

Annexin V-negative/PI-negative cells, indicates viable cells; Q4 quadrant, Annexin V-positive/PI-negative cells, indicates cells in early apoptosis. The cells were incubated with compound 4**5c** at its IC_{50} concentration (0.75 μM) and irradiated with a UV-A dose of 2 J/cm^2. As showed in Figure 30, a good part of the cell population (about 38%) is present in the Q2 quadrant related to late apoptosis followed by about 5% present in the Q4 quadrant corresponding to the initial apoptosis; while about 4% of the total population is in the Q1 quadrant (necrotic population). So it can be concluded that compound **45c** was able to induce cell death through apoptosis.

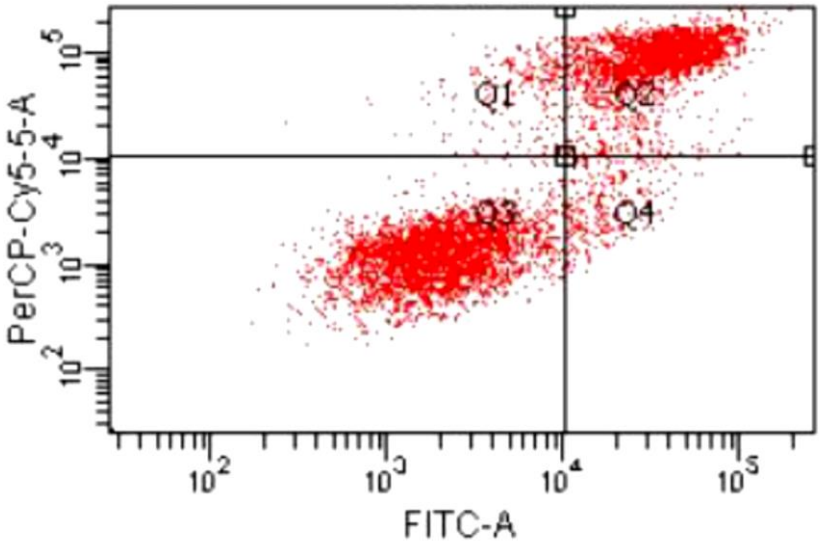
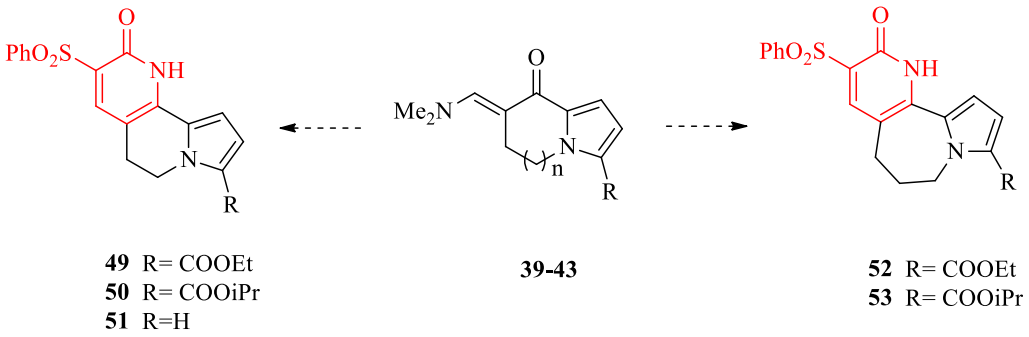


Figure 30

Further studies are currently underway with the aim to identify possible targets of the new compounds.

6. Pyrrolo[1,2-*h*][1,7]naphthyridinones and pyrido[2,3-*c*]pyrrolo[1,2-*a*]azepinones

Continuing our studies, starting from enamino intermediates of type **39-43** we decided to synthesized the two new tricyclic systems containing the pyridine ring pyrrolo[1,2-*h*][1,7]naphthyridinones **49-51** and pyrido[2,3-*c*]pyrrolo[1,2-*a*]azepinones **52,53** (Figure 31).



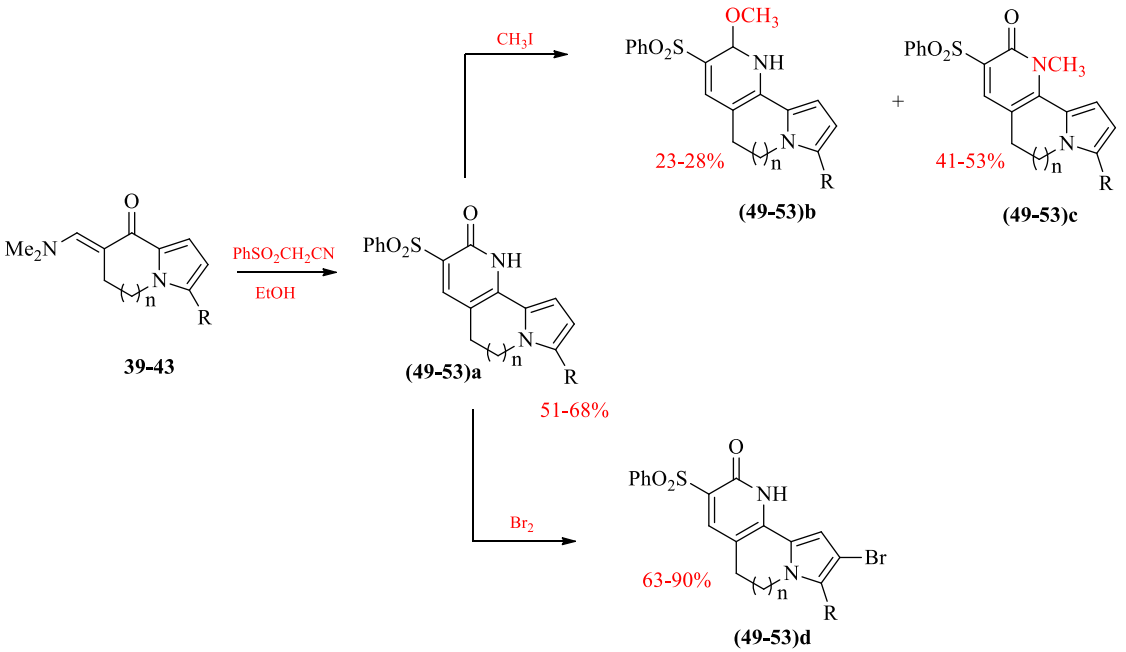
49 R= COOEt
50 R= COOiPr
51 R=H

39-43

52 R= COOEt
53 R= COOiPr

Figure 31

To obtain the new tricyclic systems, we could react our enaminoketons **39-43** with dinucleophiles having a C-C-N structure, such as cyanoacetamide, ethyl cyanoacetate, phenylsulfonylacetonitrile. After an evaluation of the results of the previous class of quinolinones it was clear the importance of the presence of a phenylsulfonyl group in position 3 of the pyridine ring to achieve the best antiproliferative activity. The reaction between enaminoketons **39-43** and phenylsulfonylacetonitrile was carried out in refluxing EtOH, under N_2 atmosphere. After 24 h, TLC monitoring of the reaction showed its completeness and the formation of the desired tricyclic derivatives **(49-53)a** (51-68%) (Scheme 7) which separate from cold reaction mixture and were recovered by filtration as a pure solid. Similarly, to some of the previous class of pyridine compounds the new derivatives **(49-53)a** were subjected to methylation. The reaction was carried out in a presence of NaH, as a base, in DMF and CH_3I, as a methylating agent (Scheme 7). After completeness, from the reaction mixture it was possible to isolate two different products, the *O*-methyl derivatives **(49-53)b** (23-28%) and the *N*-methyl derivatives **(49-53)c** (41-53%), due to the tautomeric keto-enol equilibrium (Table 4). Finally compounds **(49-53)a** were subjected to bromination with the aim of evaluating the influence of the presence of bromine atom on pyrrole ring on antiproliferative activity. The bromination of the pyrrole ring was achieved using by the DCM, as a solvent, and Br_2 as brominating agent, leading to bromo derivatives **(49-52)d** (63-90%)(Table 4). From the reaction of derivative **51a**, it was not possible to isolate the desired bromo derivative as pure product.



Scheme 7

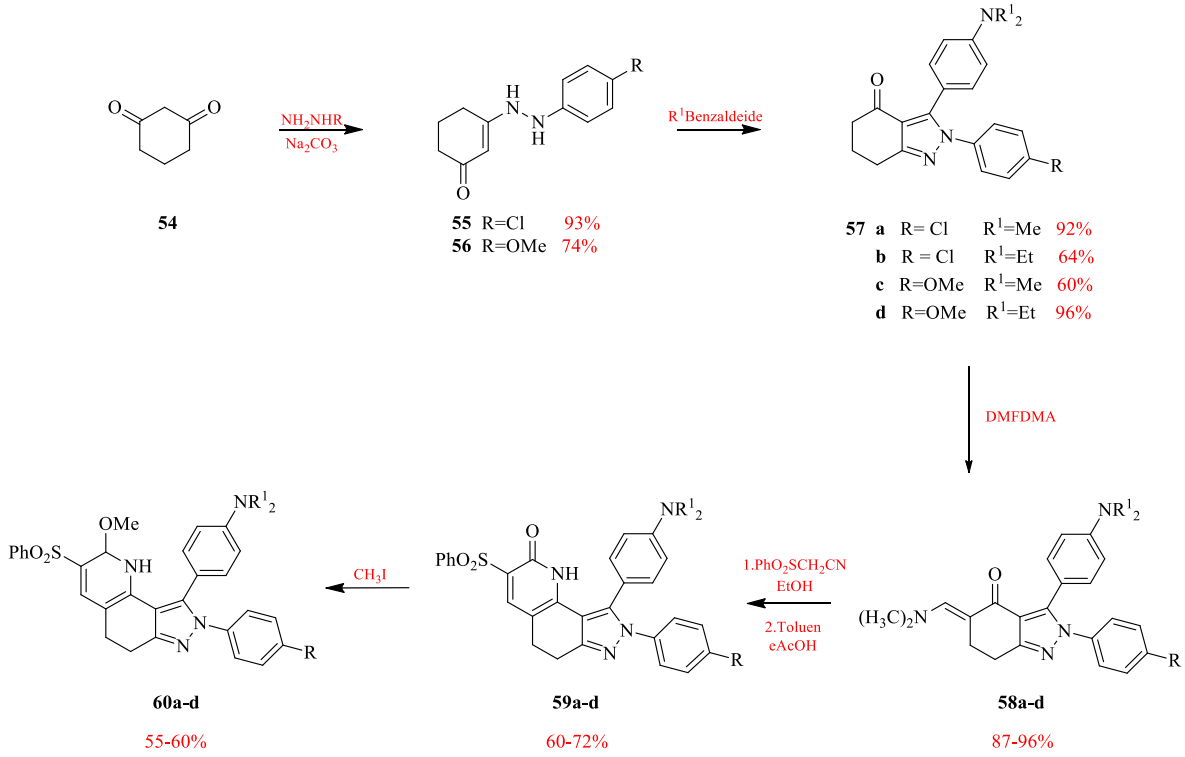
CDP	n	R	Yield	CDP	n	R	Yield
49a	1	COOEt	68%	**51c**	1	H	47%
49b	1	COOEt	25%	**52a**	2	COOEt	51%
49c	1	COOEt	53%	**52b**	2	COOEt	23%
49d	1	COOEt	63%	**52c**	2	COOEt	45%
50a	1	COOiPr	61%	**52d**	2	COOEt	90%
50b	1	COOiPr	24%	**53a**	2	COOiPr	54%
50c	1	COOiPr	45%	**53b**	2	COOiPr	28%
50d	1	COOiPr	69%	**53c**	2	COOiPr	41%
51a	1	H	64%	**53d**	2	COOiPr	84%
51b	1	H	27%				

Table 4

All new derivatives **(49-53)a,b,c,d** (Table 4) will be screened to evaluate their antiproliferative activity.

7. Pyrazole[3,4-*h*]quinolinones

During my PhD, I also approached the synthesis of a focused library of compounds for possible application in 2PE-PDT. In particular, I worked on the synthesis of a new class of *pyrazole[3,4-h]quinolinones* bearing special two photon absorption chromophores (PA). The synthesis of the new compounds **59** started from commercially available 1,3-cyclohexanedione **54** which, by reacting with the suitable hydrazine, 4-methoxy or 4-chloro phenyl hydrazine, in the presence of Na_2CO_3, led to the isolation of intermediates **55,56**. The closure of the pyrazole ring was obtained by reaction of derivatives **55,56** with the appropriate benzaldehyde, 4-dimethylamino or 4-diethylamino benzaldehyde, in DMF in the presence of catalytic amounts of piperidine and acetic acid at 40°C (74-93%), (Scheme 8).



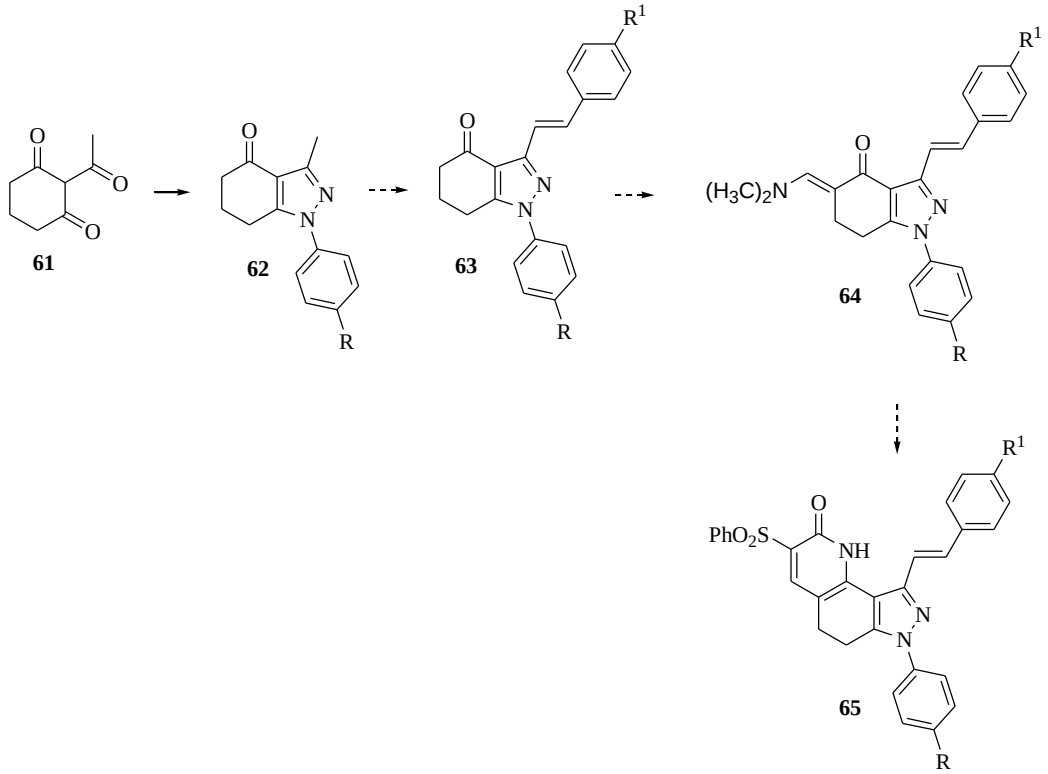
Scheme 8

Ketones **57a-d** were then converted into the key intermediates **58a-d** by reaction with DMFDMA, in 1:10 ratio, in DMF, as a solvent, under a microwave assisted heating, with a power (PW) of 150 Watts and at temperature (T) of 150°C. After 15 minutes, the reaction was completed and the desired product was collected by filtration after pouring into crush ice (87-96%), (Scheme 8). Once the necessary key intermediates were synthesized, we proceeded with the cyclization reactions of the pyridone ring. The dimethylamino derivatives **58a-d** were reacted with phenylsulfonylacetonitrile in anhydrous EtOH, under N_2, under reflux for 24 h. TLC monitoring showed the disappearance of enaminone derivatives **58a-d** and the formation of a series of intermediates as well as a small amount of desired cyclic products. It had already been observed that several hours of reflux are required for the intermediate to close by hydrolysis of the CN group to $CONH_2$. The latter is responsible of the nucleophilic attack to the annular carbonyl, to give the desired pyridine ring. To speed up dehydration and thus the closure of the pyridone ring, it was decided to use the Dean-Stark apparatus which favoured the removal of water by forming an azeotropic water / toluene mixture. So, after 24 h of reflux in ethanol, the reaction mixtures were evaporated and the residues added with toluene and catalytic amounts of acetic acid and refluxed, in the presence of the Dean-Stark apparatus. After further 24 h, it was possible to isolate the desired pyridone derivatives **59a-d** with good yields. The pyrazolquinolinone derivatives **59a-d** thus obtained were reacted with CH_3I, in anhydrous DMF and NaH as a base. In this case from the reaction mixture, it was possible to isolate only the *O*-methyl derivatives, probably due to the hindrance created by the presence of the 4-dialkylaminophenyl group on position 3 of the pyrazole ring.

Additionally, it was hypothesized to modify the most active derivatives of pyrazolo[3,4-h]quinolinones of type **4** and **5** through the introduction of chromophore groups. This structural modification would allow to increase the conjugation in the molecule, fundamental condition for a possible application in 2PE-PDT. To obtain the new tricyclic systems it was supposed to start the synthesis from the commercially available 2-acetylcyclohexane-1,3-dione **61** to give the ketones **62**, which present a methyl group on position 3 of the pyrazole ring. The methyl group could be undergo to aldol condensation reaction in presence of the suitable aldehyde to give a new ketone with increased conjugation. Then the conversion of this latter one into enaminoketones by reaction with

DMFDMA or TBDMAM and cyclization with phenylsulfonylacetonitrile would allow to obtain the desired tricyclic derivatives (Scheme 9).

All the new derivatives will undergo to evaluation of physicochemical properties and phototoxicity studies forthe possible application in 2PE-PDT at the laboratory of Prof Zhao Yuxia, Tsinghua University of Beijing.



Scheme 9

8. Project in collaboration with the University of Paris Descartes

During my internship at Paris Descarts University under the supervision of Prof Hervè Galons, I worked on the synthesis of potential cyclin-dependant kinases (CDK) inhibitors. CDKs is a family of kinases involved in the regulation of cell cycle.

8.1 The cell cycle and its phases

The cell cycle can be defined as the series of events that leads to duplication of DNA (DNA replication) and division of cytoplasm and organelles to produce two daughter

cells. The duration of the cell cycle depends on the type of cell and on the growth conditions; the duration of the cell cycle of a human cell is about 24 hours.
The cell cycle can be divided into two phases (Figure 32):

- **Interphase (G1, S and G2)**

- **Mitosis phase (M)**

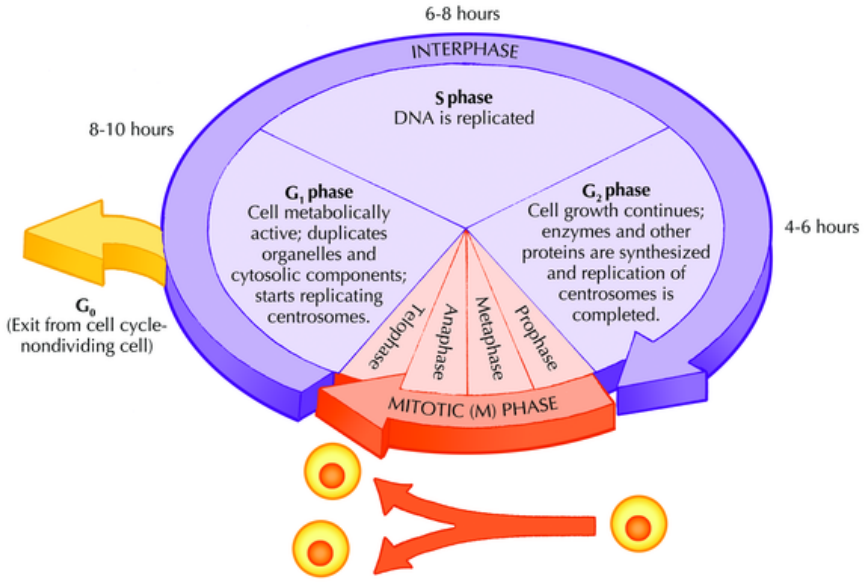


Figure 32

While the *interphase* describes the time when the cell accumulates material and nutrients and replicates its genome, the *mitotic phase*, instead, summarizes the processes during which the cell actually splits into two distinct but identical daughter cells.
The *Interphase* is the period that, in the cell cycle, describes the series of events between a mitosis and the next. In this time frame the process of duplication of the genetic material (DNA), of some cellular organelles, such as centrioles and mitochondria, and in general the increase of the mass and of the cellular dimensions take place. The interphase can be divided into (Figure 32):

- **G1 phase (Gap 1)**: it is the interval between the completion of phase M and the beginning of phase S; the cell monitors its size and the external environment and grows by synthesizing RNA and proteins;

- **S phase (Synthesis):** in this phase DNA replication occurs;

- **G2 phase (Gap 2):** it is the interval between the end of phase S and the beginning of phase M. This phase is a period of protein synthesis and rapid cell growth to prepare the cell for mitosis.

Mitosis can be described as the phase of the cell cycle in which the eukaryotic cell produces two daughter cells. Chromosomes duplicated during the phase S are divided so to ensure that each daughter cell receives a copy of each chromosome. Mitosis can be divided into the following four/five phases (Figure 33):

- **Prophase:** it occupies over half of mitosis. The nuclear membrane breaks down to form a number of small vesicles and the nucleolus disintegrates. Centrosomes are duplicated to form two child centrosomes that migrate to the opposite poles of the cell. Centrosomes organize the production of microtubules that form the spindle fibers which will constitute the mitotic spindle. Each replicated chromosome can now be seen as consisting of two identical chromatids (or sister chromatids) held together by a structure known as centromere;

- **Prometaphase/Metaphase:** the chromosomes, through their centromeres, migrate to the equatorial plane of cell. The spindle fibers bind to a structure associated with the centromere of each chromosome called *kinetochore*. The chromosomes line up along the equatorial plate of the spindle apparatus;

- **Anaphase:** it is the shortest stage of mitosis. The centromers divide and the brother chromatids are separated and moved towards the opposite poles of the cell, driven by the mitotic spindle;

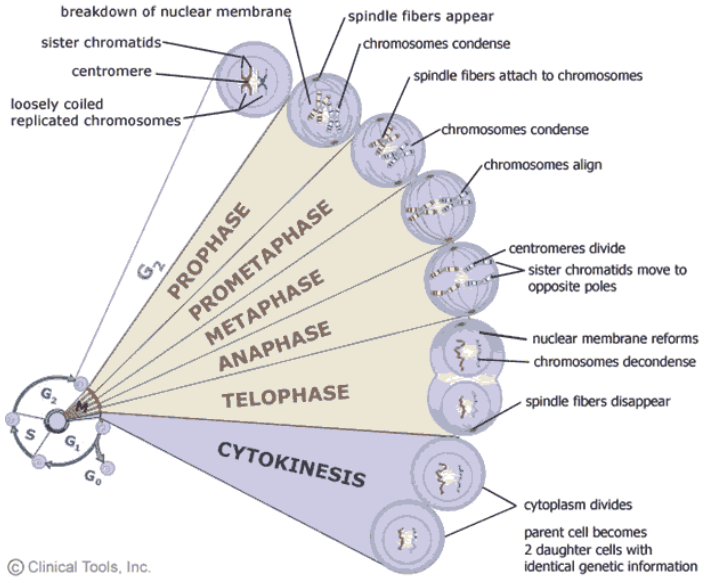


Figure 33

- **Telophase:** it is the final stage of mitosis. The chromosomes grouped on both poles of the cell and the mitotic spindle disappears.

The mitotic phase ends with cytokinesis, which divides the nuclei, cytoplasm, organelles and cell membrane into two cells containing roughly equal shares of these cellular components.

8.2 Cyclin and Cyclin-depended kinase (CDK)

The progression of the cell cycle is supported by the sequential and transient expression of proteins called cyclins, whose levels change according to the phase of the cell cycle. They play a key role in the activation of specific serine / threonine kinases, called cyclin-dependent kinases, (**CDK**). Deregulation of CDKs activity is a component of many diseases, including cancer [28].

Cyclins are a family of proteins, ranging in size from 35 to 90 kDa, which regulate the progression of the cellular cycle. They present a central domain of 100-150 a.a. called "Cyclin Box" [29]. This is essential for the association of the cyclins with their respective CDKs and for their activation [30]. Cyclins can be divided into general classes, each

defined by the stage of the cell cycle in which they are associated with the CDKs. The three classes that are essential in eukaryotic cells are:

- G1/S-phase-cyclins: they bind CDKs at the end of G1 and lead the cell to the replication of its DNA;

- S-phase-cyclins: they are essential for the activation of DNA replication, and

- M-phase cyclins: they promote and drive the process of mitosis [31].

At the beginning of the G1 phase, the first complex that CDKs and cyclins form is that between cyclin D and, depending on the cell cycle, CDK4 or CDK6. The formation of these complexes is stimulated by particular signals that include the initial inhibitory phosphorylation of the retinoblastoma protein (pRb) and the sequestration of $p21^{Cip1}$ and $p27^{kip1}$, which also inhibit CDK2, thus promoting activation of the CDK2 / cyclin E complex.

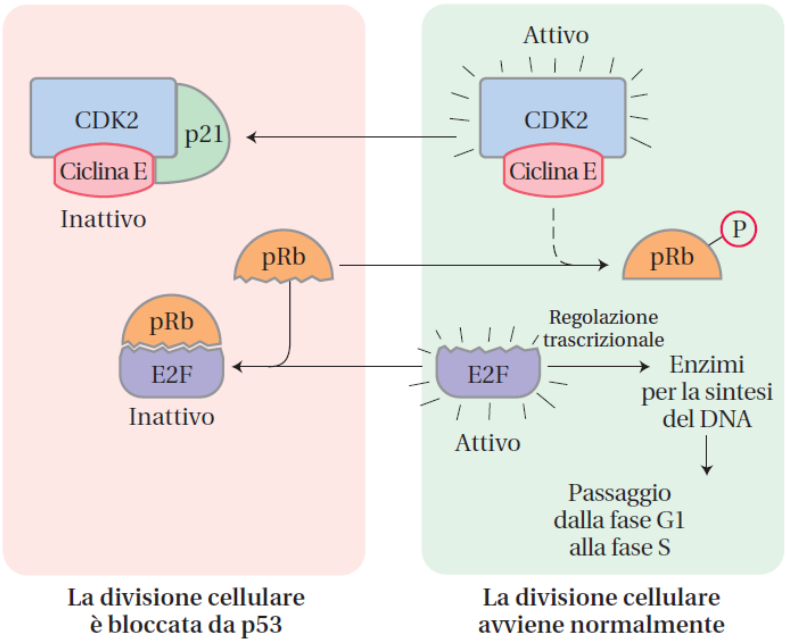


Figure 34

At the end of the G1 phase, CDK2 / cyclin E complex completes the phosphorylation and therefore the inactivation of the Rb protein, which in turn releases the E2F transcription factors. E2F promotes the transcription of cyclin E, necessary for the G1 / S transition (Figure 34) [32].

The activity of CDK2 is crucial for DNA duplication; in this phase its activity is supported by the association with cyclin A. Cyclin A begins to be synthesized at the end of G1phase and its concentration progressively increases in S phase until reaching a maximum at the beginning of mitosis. In addition to CDK2, cyclin A can bind and activate CDK1; the CDK1 / cyclin A complex contribute to the preparation of mitosis in the G2 phase. It is believed, however, that the activation of CDK1 occurs physiologically by association with the cyclins B [30]. The activity of CDK1 / cyclin B is strictly regulated by phosphorylation of a threonine residue (Thr 160/161) by the activating CDK-kinase (CAK) (a multisubunit protein that includes CDK7, cyclin H and MAT1) (Figure 35).

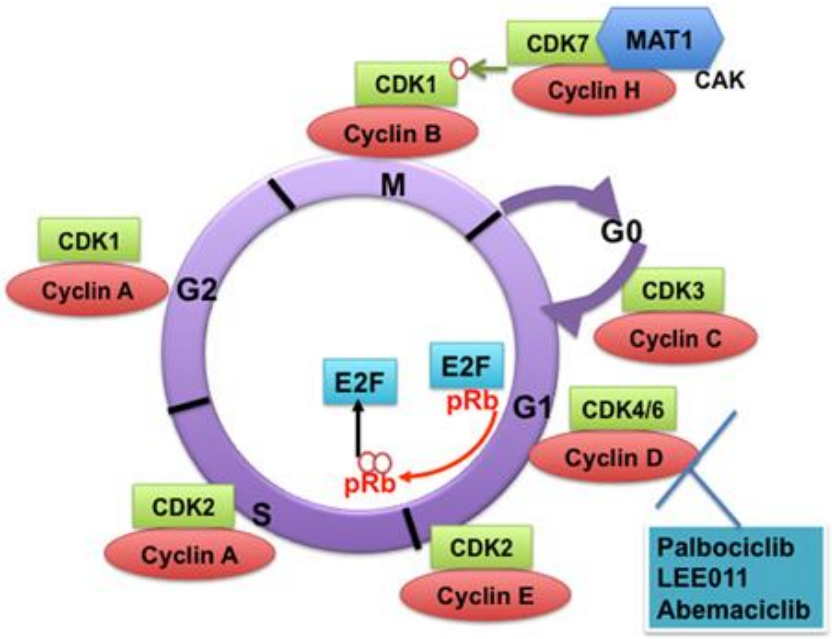


Figure 35

Inactivation of the cyclin / CDK complexes is responsible for the beginning of mitosis; this occurs either through the intervention of a specific phosphatase (**CDC25C**), which

removes the phosphate on the Thr160 / 161 of the CDK1 / cyclin B complex, or through the addition of a second phosphate on a Tyr15 residue by action of a kinase called **WEE1**. The cyclin-CDK complexes can also be inhibited by a second class of proteins: the cyclin kinase inhibitors (**CDKI**). CDKIs are small proteins that act as negative regulators of the cell cycle. The dependent cyclin kinase inhibitors inactivate the cyclin-CDK dimers forming non-covalent complexes, thus making possible a negative control of cell proliferation. CDK inhibitors can be divided into two large families: INK4 and Cip / Kip, based on common sequences and mechanism of action [33]. Three structurally similar polypeptides $p15^{ink4b}$, $p18^{ink4c}$, $p19^{ink4d}$ and $p16^{ink4a}$ are part of the INK4 family. These proteins inhibit the CDK4 / 6 complexes with cyclin D. The proteins $p21^{cip1}$, $p27^{kip1}$ and $p57^{kip2}$ belong to the Cip / Kip family (*kinase inhibitor protein*). So deregulation events on these protein may play an important role in carcinogenesis.

The importance of protein kinases in physiological processes has stimulated an active search for possible "small" molecules able to inhibit the activity of these molecules with a protein structure.

Another key factor in cell cycle regulation is the p53 protein. The p53 tumor suppressor gene has anti-proliferative activity and may cause cell cycle arrest in G1 or induce apoptosis. The preferred transcriptional target of p53 is the p21 protein which inhibits the activity of the cyclin E-CDK2 complex causing blockade of the G1 phase / S phase transition. Moreover p21 binds to the cyclin D/CDK4 complex preventing phosphorylation of pRb and thus suppressing the pRb / E2F pathway [34]. In addition to their direct role in the regulation of mitosis, some classical CDK / cyclin complexes have an essential function in meiosis, such as CDK2, in transcription and / or DNA repair. Some CDKs, for example, influence transcription by phosphorylation of the carboxy-terminal domain (CTD) of ribonucleic acid polymerase II (RNAPolII) during DNA transcription.

In addition to their direct role in the regulation of cell mitosis, some classical CDK / cyclin complexes have an essential function in meiosis, such as CDK2, in transcription and / or DNA repair. Some CDKs, for example, influence transcription by phosphorylation of the carboxy-terminal domain (CTD) of ribonucleic acid polymerase II (RNAPolII) during DNA transcription.

8.3 Background on CDKIs

Over the years, the functional and structural study on CDK and the discovery that their deregulation is often at the basis of many disorders, including cancer, has led to the synthesis of possible molecules able to inhibit the activity of CDK. Since the inhibition of CDK would lead to the arrest of tumor cell division. The first CDK pharmacological inhibitors were neither particularly active nor selective. However, they provided the first data on the essential components to have molecules capable of inhibiting CDKs and the starting point for the search for more powerful and selective inhibitors. In fact, despite the remarkable chemical diversity, all CDK inhibitors share some common properties:

- they have low molecular weights (<600);
- they are hydrophobic heterocycles;
- they act in competition with ATP for binding to the kinase ATP binding site;

they are bound mainly by hydrophobic interactions and hydrogen bonds with the kinase [35].

The first CDK inhibitors were natural or semi-synthetic products, including *flavopyridol*, *butyrolactone*, *staurosporin* and its *7-hydroxy derivative*. The flavopyridol, one of the most characterized CDK inhibitors, is a semisynthetic flavonoid deriving from the natural indolithic alkaloid Roituchina isolated from the leaves and the stalk of *Amoora rohituka*. The flavopyridol was initially developed as inhibitor for Epithelial Growth Factor Receptor (EGFR) and protein kinase A [36]. However, it was discovered that the compound inhibits CDK at concentrations much lower than those required for inhibition of EGFR or protein kinase A. In particular, it was observed that flavopyridol induces cell cycle arrest in G1 in vivo and in vitro, possibly through inhibition of CDK1 and CDK2 [37]. Another well-characterized CDK inhibitor is 7-hydroxyurysurine (UCN-01). UCN-01 is an alkaloid derived from staurosporin, isolated from Streptomyces bacteria. Initially discovered as CDK1 and CDK2 inhibitor, it is now known to have inhibitory activity on almost all CDKs, as well as promoting cell apoptosis having as target the protein kinases Chk1 and Chk2 [38]. The search for specific CDK inhibitors has led to the discovery of the 6-aminopurine, semi-specific but not very potent CDK inhibitors [39], described for the first time in 1973. On the basis of these results, several studies have focused on the research of new CDKIs, through the screening of compounds previously developed on

the basis of structure / activity relationship, in order to identify new molecules with a purine structure [40] [41]. From these studies emerged 2,6,9 trisubstituted purine derivatives Olomoucine and (R)-Roscovitina. In particular, (R)-Roscovitina, which resulted 10-fold more activity than Olomoucine but with the same selectivity, is in clinical stage II-b against non-small cell lung cancer (NSCLC), breast cancer and pharyngeal carcinoma [42] and, moreover, has activity against myeloma [43]. Through the use of combinatorial chemistry, other purine molecules such as purvalanol A and purvalanol B were obtained and they showed inhibition constants against CDK2 greater than that of (R)-Roscovitine but a less appropriate pharmacological profile (Figure 36) [44].

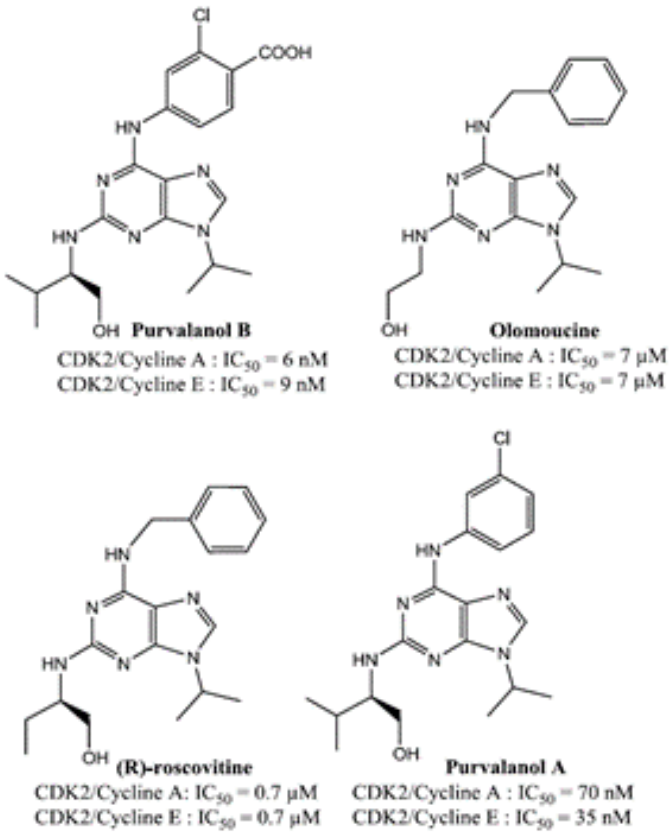


Figure 36

The isolation of co-crystallizate structures of Olomoucine and (R)-Roscovitine with CDK2 allowed to identify the essential functional groups needed for the bond between the protein and the purine derivative [45]. It was found that substituents in 2,6 and 9

positions have the greatest impact on the activity and the selectivity of purine derivatives as they are located outside the active site of CDK and can, therefore, form hydrogen bonds with the amino acids surrounding the protein binding site. The best biological results were obtained when an alkyl or cyclic group having a hydroxyl function (OH) [46] were present on C-2, an amino-aryl function that could be substituted in meta or in para by electron group attractor or donor was present on C-6; while the C-9 position tolerates only very few groups. The best selectivity and activity were obtained when aliphatic groups (from C1 to C4), in particular an isopropyl group, are present in this position, however some derivatives having a cyclic group in this position showed a strong inhibition on the CDK2 [47].

8.4 Aim of the thesis and synthetic strategies

During my Internship at the University of Paris Descartes, I focused my attention on the synthesis of new molecules with purine structure. My study originates from the consideration that in the metazoans the CDK7 / Cyclin H / MAT1 complex is able to initiate the progression of the entire cell cycle. Thus, it is expected that inhibition of CDK7 could inhibit all CDKs downstream of the cell cycle. CDK7 is also a component of the general transcription factor H (TFIIH), responsible for the phosphorylation of Polymerase II, involved in DNA trascription. Therefore, specific CDK7 inhibitors would have the potential to inhibit not only the progression of the cell cycle but also the transcription of DNA, making them attractive anticancer drugs.

To date, a considerable amount of work has been done to examine the selectivity of potential inhibitors towards different CDKs, in particular CDK2, CDK4 and CDK9. On the contrary, the selectivity towards CDK7 is much less understood. In order to explore the structure / activity relationship of possible inhibitors against CDK7, structural, thermodynamic and modelling studies have recently been carried out. For this purpose, three inhibitors were considered: BS-194 (A), ICEC-0942(B) and ICEC-0943(C), an enantiomer of the previous one, all three belonging to the same library (Figure 37).

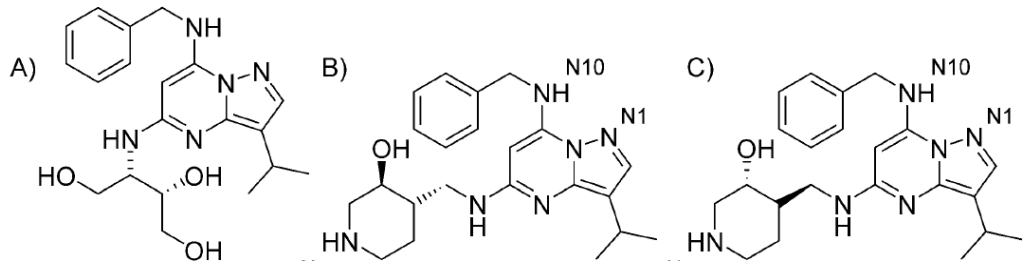


Figure 37

The study started from the kinase assays, which showed a higher activity of SB-194 on CDK2 compared to CDK7, of ICEC0942 on CDK7 compared to CDK2 while ICEC-0943 did not showed considerable activity towards both kinase. The binding affinity for each compound against CDK2 and CDK7 was then evaluated using isothermal titration calorimetry (ITC). Through ITC it was highlighted that all three tested compounds bind more closely their targets when these latter are in dimeric form, ie linked to their own cyclin, and that binding affinities reflect the activity obtained in the kinase assays (ICEC0942 and ICEC0943 were bound, in fact, more firmly to CDK7 rather than CDK2). Thermodynamic data also showed that the binding of all three inhibitors to their CDK monomers was enthalpically driven, however, the binding of ICEC0942 and ICEC0943 against the CDK7 / Cyclin H dimer was guided both enthalpically and entropically; this probably attributable to the structural changes occurring at the ATP binding site, following the formation of the CDK7 / cyclin H complex. ICEC0942 and ICEC0943 were then involved in molecular dynamics (MD) studies that allowed to explain the reason why these two inhibitors were more selective and more similar to CDK7 compared to CDK2. For this study, high-resolution crystallographic structures of CDK2 and CDK7 (at a lower resolution) linked to ICEC0942 were observed and compared. During MD simulations of CDK7 with ICEC0942, the inhibitor remained bound in a conformation similar to that of CDK2. The hydrogen bonds between the inhibitors and the Met94 were maintained in all the simulations. However, various specific interactions have been observed, in particular with the Gly21 (of the G-rich loop) and the side chains of Asp155 and Asp137. It should however be emphasized that at the beginning of the acquisition it seemed impossible that the inhibitor could reach the Asp155 either because it was placed too deep in the ATP binding pocket and because it was cluttered by the Lys41. However, protein motions

during CDK7 MD simulations show that the polar interactions between the inhibitors and Asp155 are possible and have actually been observed for substantial periods of simulation time. This suggests that the binding site for ATP is particularly flexible in CDK7 and less in CDK2. The importance of Asp155 in the two CDKs was also evaluated by substitution with alanine. This substitution allowed to understand that the asparagine residue is very important for the binding of CDK7 to ICEC0942 and not very influential for CDK2. Finally, it was observed that the binding site for ATP on CDK7 is more open thus allowing entry of sterically more clogged compounds [28]. Currently, ICEC0942 is in clinical trials under the name of CT7001 proving effective against breast cancer.

The constant effort in the research of CDK inhibitors led to the synthesis of two new purine derivatives, LDC3140 and LDC4297, which have proved to be interesting CDK7 inhibitors. In particular, LDC3140 showed, in kinase assays in vitro, both remarkable selectivity and potency unlike LDC4297 which proved to be potent but much less selective towards CDK7 (Figure 38).

	$IC_{50}(\mu M)$[a]					
Compound name	**CDK1**	**CDK2**	**CDK4**	**CDK6**	**CDK7**	**CDK9**
Flavopiridol	<0.005	0.0147±0.00	0.0376±0.01	0.305±0.02	0.1031±0.02	<0.005
BS-181	>10*	4.58±2.90†	>10	>10	0.0571±0.04†	1.94±0.58
LDC043140	>10	3.897±0.37	>10	>10	<0.005	7.45±3.14*
LDC044297	0.0537±0.01	0.0064±0.00*	>10	>10	<0.005	1.7113±0.12

In all cases except as noted, the number of replicates (n)=3. *, n=2; †, n=4.

Figure 38

The two new purine derivatives were able to inhibit phosphorylation of Ser5 and Ser7 residues of the RNA Pol II, block transcription, and arrest cell cycle progression in G1 / S phase [48].

Recently, *SY-1365* was discovered as anticancer agent. It showed not only potent activity but also selectivity towards CDK7 to which it is covalently bound (Figure 39).

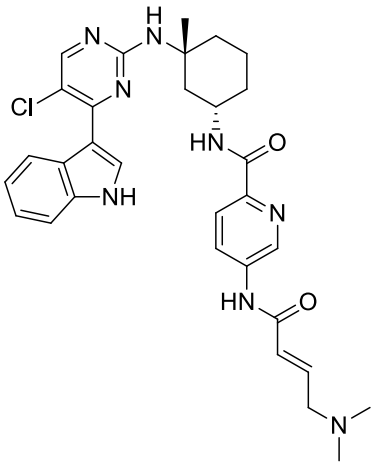


Figure 39

Preclinical studies on solid tumors and haematological malignancies showed that treatment with SY-1365 led to antitumor activity, inducing apoptosis in vitro and complete regressions in xenographic models (Figure 40).

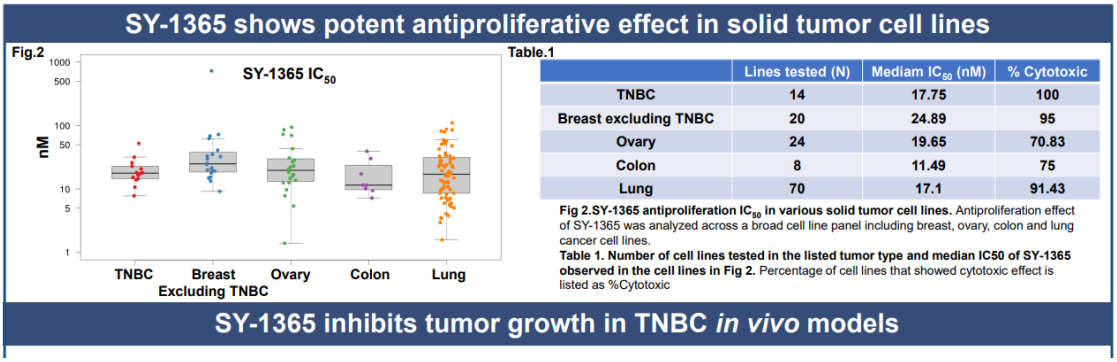


Figure 40

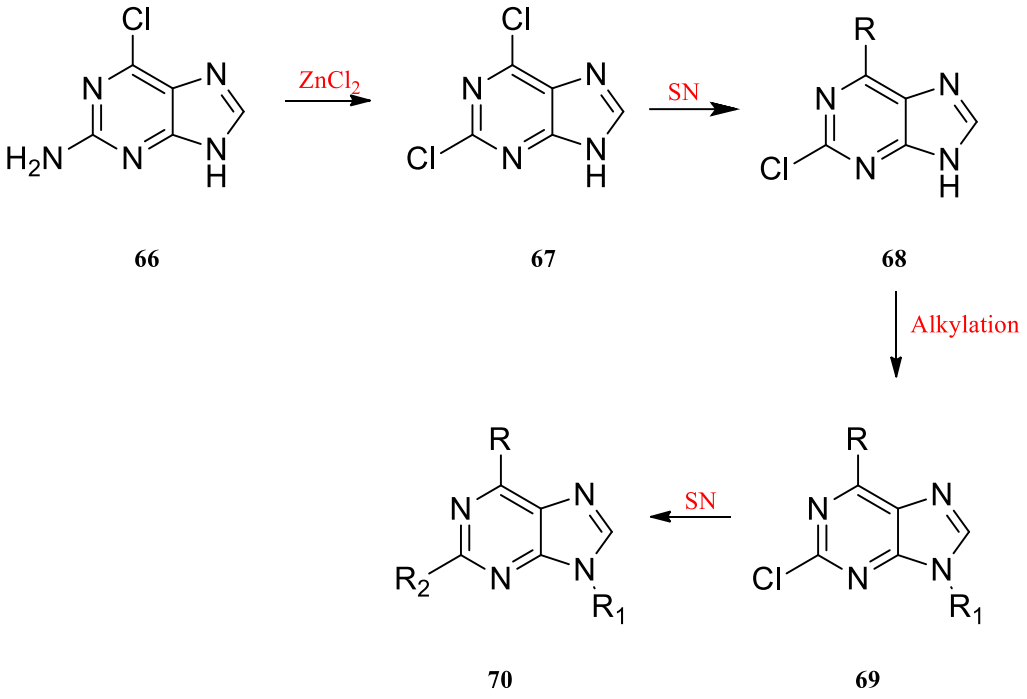
Currently, SY-1365 is involved, either in mono therapy or in combination with *carboplatin* or *fulvestrant*, in phase I studies of patients with advanced solid-phase cancer.

8.5 Synthesis of purine and pyrazolopyrimidine

On this basis, I worked on the synthesis of new molecules having purine and pyrazolopyrimidine structures with potential inhibitory activity towards CDK7.

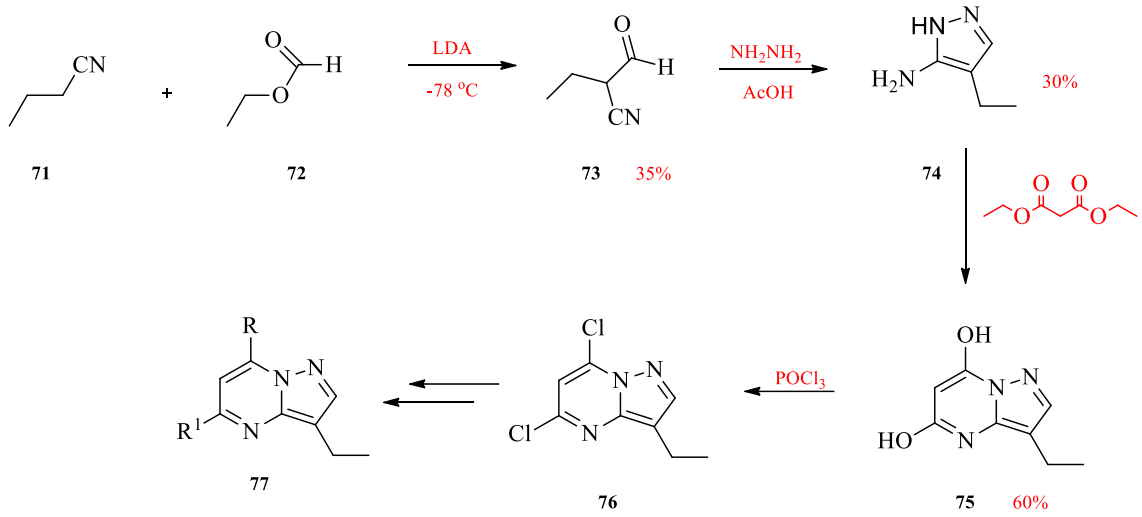
For confidentiality reasons the full details of the synthetic sequence are not presented, as a patent application is pending.

The synthesis of the new *2,6,9-trisubstituted* purine derivatives started from the commericially available *6-chloro-9H-purin-2-amine* derivative **66**. The intermediate **67** it was obtained by reaction of halogenation with $ZnCl_2$, $NaNO_2$, in HCl concentrated, as a solvent, at -5°C. Then compound **67** was transformed into derivatives of type **68** by nucleophilic substitution. The latter were converted into alkyl derivative by introduction of an alkyl chain on the nitrogen atom. Finally, the intermediates of type **69** were subjected to a second nucleophilic substitution in position 2 of the tricyclic system to give compounds of type **70** (Scheme 10).



Scheme 10

For the synthesis of *pyrazolopyrimidine* compounds it was used a similar synthetic strategy (Scheme 11). Compound **76** was synthesized by reaction of butyronitrile **71** and ethyl formiate **72** using potassium *tert*-butoxide, as abase. The closure of the pyrazole ring was obtained by reaction of derivative **73** with hydrazine using EtOH as a solvent. Then, aminopyrazole **74** was converted into pyrazolopyrimidine system **75**, using diethyl malonate in the presence of Na, as base, and EtOH, as solvent [49].



Scheme 11

Finally, compound **75** was chlorinated using $POCl_3$ and subsequently subjected to two nucleophilic substitutions to give compounds of type **77** (Scheme 11).

	CPT	**70l**	**70w**	**70x**	**70y**
HUVEC	0.12±0.13	2.88±0.46	7.28±1.65	26.03±5.29	>100
HEK293	>100	16.46±9.41	3.61±0.45	28.40±3.49	25.51±8.46
K562	0.09±0.04	1.76±1.73	2.72±0.50	19.51±4.27	13.64±5.49
HepG2	0.06±0.01	3.79±0.47	1.62±0.29	31.00±27.07	12.72±9.01
HCT116	0.04±0.02	2.77±1.56	2.95±0.22	29.53±7.27	26.95±3.94
MCF-7	2.83±0.79	2.56±0.37	18.76±5.34	>100	59.90±15.01
MGC803	>100	11.18±3.72	4.98±2.46	63.90±24.88	>100
A549	1.93±0.57	3.02±0.24	5.02±0.56	49.64±36.17	40.22±15.05
H1299	>100	6.31±1.48	4.14±1.00	>100	>100
HT-29	0.19±0.06	3.35±0.04	6.50±1.38	>100	43.37±13.36
PC-3	>100	4.13±0.83	4.03±0.44	65.19±11.69	30.29±5.43
MDA-MB-231	**23.37±4.25**	**4.27±0.28**	14.12±6.53	65.19±11.69	49.02±5.97
MDA-MB-468	**0.05±0.01**	**3.69±0.34**	2.97±0.58	46.97±5.88	26.04±8.06

Table 5

The antiproliferative activity of all the new synthesized compounds were tested against different tumor cell lines in the laboratory of Prof. P. Yu in China. The new compounds showed antiproliferative activity in the micromolar range. In particular, compound **70l** showed considerable activity against two breast cancer cell lines (Table 5). The evaluation of the inhibitory kinase activity is currently ongoing.

9. Experimental Section

All melting points were taken on a Buchi-Tottoli capillary apparatus and were uncorrected. IR spectra were determined with a Shimadzu IR Affinity-1 spectrophotometer. ^{1}H and ^{13}C NMR spectra were measured in DMSO-d_6 or $CDCl_3$ solutions, unless otherwise specified, at 200 and 50.3 MHz respectively, using a Bruker AC series 200 MHz spectrometer (TMS as internal reference). Column chromatography was performed with Merck silica gel 230-400 Mesh ASTM or with a SEPACORE BÜCHI chromatography apparatus or with BIOTAGE 40i chromatography apparatus. Microwave experiments were carried out using a CEM Discover Labmate^{TM}.

Preparation of 2,2,2-Trichloro-1-(1*H*-pyrrol-2-yl)ethanone (11).

To 8 mL of diethyl ether, 5.5 mL of trichloroacetyl chloride (49.2 mmol) were added, and the solution was stirred at room temperature for 3 h. A solution of pyrrole **10** (3 g, 44.7 mmol) in diethyl ether (25 mL) was added and the mixture was stirred for 1 h. Very slowly the reaction mixture was neutralized with a solution of K_2CO_3 (3.9 g in 11.7 mL of H_2O). The two phases were separated and the organic fraction was filtered on celite. The phase was recovered, dried on Na_2SO_4 and the solvent was removed under reduced pressure. Quantitative yield. The spectroscopic data are in agreement with the literature [50].

Preparation of Ethyl 1*H*-pyrrole-2-carboxylate (12).

To a solution of potassium ethylate (4 g, 49.2 mmol) in ethanol (120 mL), a solution of **11** (9.5 g, 44.7 mmol) in dichloromethane (75 mL) was added. After 30 min. at room temperature, the solvent was removed under reduced pressure. The residue was dissolved with HCl 2 N (45 mL) and diethyl ether (105 mL). The two phases were separated and the aqueous fraction was extracted with diethyl ether (2 x 80 mL). The organic phase was washed with a satured solution of Na_2CO_3 (100 mL). The organic layer was dried on Na_2SO_4 and the solvent was removed under reduced pressure to give the crude product that was purified by chromatography column (DCM). Yield 95%. The spectroscopic data are in agreement with the literature [50].

Synthesis of 1-substituted pyrrole-2-carboxylate (13,14).

To a solution of compound **12** (3.6 mmol) in anhydrous DMF (15 mL), NaH (7.2 mmol) was added at 0 ^{o}C and the reaction mixture was stirred at 40°C for 3 h. After cooling, KI (0.64 g, 4.0 mmol), ethyl 4-bromobutyrate or ethyl 5-bromovalerate (7.2 mmol) were added and the reaction mixture was heated at reflux for 16 h. After cooling, the reaction mixture was poured onto crushed ice and the aqueous solution was extracted with ethyl acetate (3 x 50 mL). The organic layers were dried over Na_2SO_4 and the solvent removed under reduced pressure. The crude product was purified by chromatography column (Petroleum ether/AcOEt 9:1).

*Ethyl 1-(4-ethoxy-4-oxobutyl)-1H-pyrrole-2-carboxylate (**13**)*. This compound was obtained by reaction of **12** with ethyl 4-bromobutyrate. Yellow oil; yield: 85%; IR: 1718 (CO), 1684 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.22-1.38 (6H, m, 2 x CH_3), 2.02-2.16 (2H, m, CH_2), 2.28 (2H, t, J = 6.8 Hz, CH_2), 4.07-4.41 (6H, m, 3 x CH_2), 6.10- 6.13 (1H, m, Ar), 6.80-6.85 (1H, m, Ar), 6.94-6.96 (1H, m, Ar);. ^{13}C nmr (DMSO-d_6) (ppm): 14.2 (q), 14.4 (q), 26.7 (t), 31.0 (t), 48.1 (t), 59.8 (t), 60.6 (t), 108.0 (d), 118.2 (d), 121.9 (s), 128.8 (d), 161.1 (s), 172.9 (s).

*Ethyl 1-(5-ethoxy-5-oxopentyl)-1H-pyrrole-2-carboxylate (**14**)*. This compound was obtained by reaction of **12** with ethyl 5-bromovalerate. Yellow oil; yield: 75%; IR: 1715 (CO), 1698 (CO) cm^{-1}; ^{1}H nmr ($CDCl_3$) (ppm): 1.21-1.38 (6H, m, 2 x CH_3), 1.54-1.69 (2H, m, CH_2), 1.73-1.87 (2H, m CH_2), 2.31 (2H, t, J = 7.3 Hz, CH_2), 4.06-4.35 (6H, m, 3 x CH_2), 6.09- 6.13 (1H, m, Ar), 6.82-6.84 (1H, m, Ar), 6.94-6.97 (1H, m, Ar); ^{13}C nmr ($CDCl_3$) (ppm): 14.2 (q), 14.4 (q), 22.0 (t), 31.0 (t), 33.8 (t), 48.8 (t), 59.7 (t), 60.3 (t), 107.9 (d), 118.1 (d), 121.8 (s), 128.6 (d), 161.1 (s), 173.3 (s).

General procedure for the synthesis of 4-[2-(ethoxycarbonyl)-1*H*-pyrrol-1-yl]butanoic acid (15) and 5-[2-(ethoxycarbonyl)-1*H*-pyrrol-1-yl]pentanoic acid (16).

To a solution of **13,14** (0.440 g, 1.65 mmol) in EtOH (14 mL) was added a solution of 5% NaOH (1.42 mL, 1.65 mmol). The mixture was stirred at room temperature up to completeness (TLC). Then the solvent was removed under reduced pressure and water and crushed ice were added. The solution was acidified with 6 M HCl and the aqueous solution was extracted with ethyl acetate (3 x 50 mL). The organic layers were dried over

Na_2SO_4 and the solvent removed under reduced pressure. The crude product was purified by chromatography column (DCM/AcOEt 8:2).

*4-[2-(Ethoxycarbonyl)-1H-pyrrol-1-yl]butanoic acid (**15**)*. This compound was obtained by reaction of **13** after 24 h. Yellow oil; yield: 75%; IR: 3123 (OH), 1716 (CO); 1675 (CO)cm^{-1}; ^{1}H nmr ($CDCl_3$) (ppm): 1.34 (3H, t, *J* = 7.2 Hz, CH_3), 1.90-2.20 (2H, m, CH_2), 2.31(2H, t, *J* = 6.5 Hz, CH_2), 4.21-4.41 (2H, m, 2 x CH_2), 6.11 (1H, s, Ar), 6.84 (1H, s, Ar), 6.96 (1H, s, Ar), 9.30 (1H, s, OH); ^{13}C nmr ($CDCl_3$) (ppm): 14.4 (q), 26.5 (t), 30.7 (t), 47.9 (t), 59.9 (t), 108.1 (d), 118.4 (d), 121.8 (s), 128.9 (d), 161.2 (s), 178.2 (s).

*5-[2-(Ethoxycarbonyl)-1H-pyrrol-1-yl]pentanoic acid (**16**)*. This compound was obtained by reaction of **14** after 72 h. Yellow oil; yield: 65%; IR: 3123 (OH), 1702 (CO); 1681 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.26 (3H, t, *J* = 7.0 Hz, CH_3), 1.35-1.49 (2H, m, CH_2), 1.60-1.74 (2H, m, CH_2), 2.21 (2H, t, *J* = 7.3 Hz, CH_2), 4.15-4.31 (4H, m, 2 x CH_2), 5.96-6.20 (1H, m, Ar), 6.69-6.93 (1H, m, Ar), 7.12-7.17 (1H, m, Ar), 12.04 (1H, s, OH); ^{13}C nmr (DMSO-d_6) (ppm): 14.2 (q), 21.4 (t), 30.6 (t), 33.1 (t), 47.9 (t), 59.3 (t), 107.6 (d), 117.7 (d), 120.9 (s), 129.6 (d), 160.2 (s), 174.3 (s).

General procedure for the synthesis of ethyl 8-oxo-5,6,7,8-tetrahydroindolizine-3-carboxylate (17) and ethyl 9-oxo-6,7,8,9-tetrahydro-5*H*-pyrrolo[1,2-*a*]azepine-3-carboxylate (18).

The suitable acid derivative **22a,b** (8.37 mmol) was stirred in polyphosphoric acid (13 g) at 35°C for 16 h. The reaction mixture was quenched with water and crushed ice and the resulting solution was extracted with ethyl acetate (3 x 50 mL). The organic layer were dried over Na_2SO_4, and the solvent removed under reduced pressure. The crude product was purified by chromatography column (DCM).

*Ethyl 8-oxo-5,6,7,8-tetrahydroindolizine-3-carboxylate (**17**)*. This compound was obtained by reaction of **15**. Yellow oil; yield: 70%; IR: 1704 (CO), 1687 (CO) cm^{-1}; ^{1}H nmr ($CDCl_3$) (ppm): 1.37 (3H, t, *J* = 6.7 Hz, CH_3), 2.16.2.41 (2H, m, CH_2), 2.61 (2H, t, *J* = 6.6 Hz, CH_2), 4.31 (2H, q, *J* = 6.7 Hz, CH_2), 4.55 (2H, t, *J* = 6.6 Hz, CH_2), 5.32 (1H, s, Ar), 6.90 (1H, s, Ar); ^{13}C nmr ($CDCl_3$) (ppm): 14.2 (q), 23.3 (t), 36.0 (t), 44.5 (t), 60.5(t), 112.4 (d), 116.8 (d), 126.0 (s), 135.5 (s), 160.6 (s), 188.3 (s).

*Ethyl 9-oxo-6,7,8,9-tetrahydro-5H-pyrrolo[1,2-a]azepine-3-carboxylate (**18**)*. This compound was obtained by reaction of **16**. Yellow oil; yield: 65%; IR: 1708 (CO), 1679 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.37 (3H, t, J = 7.1 Hz, CH_3), 1.82-1.94 (2H, m, CH_2), 2.00-2.13 (2H, m, CH_2), 2.77 (2H, t, J = 6.0 Hz, CH_2), 4.32 (2H, q, J = 7.1 Hz, CH_2), 4.79 (2H, t, J = 6.0 Hz, CH_2), 6.85-6.92 (2H, m, Ar); ^{13}C nmr (DMSO-d_6) (ppm): 14.3 (q), 19.4 (t), 26.1 (t), 39.6 (t), 44.0 (t), 60.7 (t), 114.6 (d), 116.9 (d), 139.8 (s), 161.2 (s), 193.8 (s).

Synthesis of propan-2-yl 1*H*-pyrrole-2-carboxylate (19).

A suspension of K_2CO_3 (3.96 g) in 2-propanol (12 mL) was stirred at room temperature for 16 h. Then a solution of 2,2,2-trichloro-1-(1*H*-pyrrol-2-yl)ethanone **11** (1.5 g, 7.2 mmol) in 2-propanol was added dropwise and the reaction mixture was heated at 60°C for 1 h and 30 min. After cooling, the solvent was removed under reduced pressure. The residue was added with water and the solution was acidified with HCl 6M and then extracted with ethyl acetate (1 x 40 mL). The organic phase was dried on Na_2SO_4 and the solvent was removed under reduced pressure to give the desired compound **19**. Colorless oil; yield 65%; IR: 1699 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.27 (6H, d, J = 6.2 Hz, 2 x CH_3), 1.28 (3H, s, CH_3), 4.97-5.15 (1H, m, CH), 6.12- 6.18 (1H, m, Ar), 6.72-6.79 (1H, m, Ar), 6.97-7.02 (1H, m, Ar); ^{13}C nmr (DMSO-d_6) (ppm): 21.8 (2 x q), 66.6 (d), 109.3 (d), 114.7 (d), 121.1 (s), 123.7 (d), 159.9 (s), 171.9 (s).

Synthesis of 1-substituted pyrrole-2-carboxylate (20,21).

To a solution of compound **19** (3.6 mmol) in anhydrous DMF (15 mL), NaH (7.2 mmol) was added at 0 °C and the reaction mixture was stirred at room temperature for 16 h. Then KI (0.64 g, 4.0 mmol), ethyl 4-bromobutyrate or ethyl 5-bromovalerate (7.2 mmol) were added at 0°C and the reaction mixture was stirred at room temperature for 3 h. After cooling, the reaction mixture was poured onto crushed ice and the aqueous solution was extracted with ethyl acetate (3 x 50 mL). The organic layers were dried over Na_2SO_4 and the solvent removed under reduced pressure. The crude product was purified by chromatography column (Petroleum ether/AcOEt 9:1).

*Propan-2-yl 1-(4-ethoxy-4-oxobutyl)-1H-pyrrole-2-carboxylate (**20**)*. This compound was obtained by reaction of **19** with ethyl 4-bromobutyrate. Colorless oil; yield: 90%; IR:

1729 (CO), 1696 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.13-1.31 (9H, m, 3 x CH_3), 1.89-99 (2H, m, CH_2), 2.21 (2H, t, *J* = 7.3 Hz, CH_2), 4.03 (2H, q, *J* = 7.1 Hz, CH_2), 4.29 (2H, t, *J* = 7.3 Hz, CH_2), 4.95- 5.12 (1H, m, CH), 6.08- 6.11 (1H, m, Ar), 6.81-6.84 (1H, m, Ar), 7.09-7.15 (1H, m, Ar); ^{13}C nmr (DMSO-d_6) (ppm): 14.0 (q), 21.7 (2 x q), 26.4 (t), 30.5 (t), 47.4 (t), 59.9 (t), 66.6 (d), 107.7 (d), 117.8 (d), 121.3 (s), 129.5 (d), 159.7 (s), 172.1 (s).

*Propan-2-yl 1-(5-ethoxy-5-oxopentyl)-1H-pyrrole-2-carboxylate (**21**)*. This compound was obtained by reaction of **19** with ethyl 5-bromovalerate. Colorless oil; yield: 84%; IR: 1724 (CO), 1695 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.12-1.27 (9H, m, 3 x CH_3), 1.40-1.51 (2H, m, CH_2), 1.59-1.73 (2H, m, CH_2), 2.28 (2H, t, *J* = 7.2 Hz, CH_2), 4.03 (2H, q, *J* = 7.1 Hz, CH_2), 4.26 (2H, t, *J* = 7.2 Hz, CH_2), 4.97-5.10 (1H, m, CH), 6.06-6.09 (1H, m, Ar), 6.80-6.85 (1H, m, Ar), 7.11-7.13 (1H, m, Ar); ^{13}C nmr (DMSO-d_6) (ppm): 14.0 (q), 21.4 (t), 21.7 (2 x q), 30.5 (t), 32.9 (t), 47.9 (t), 59.6 (t), 66.5 (d), 107.6 (d), 117.7 (d), 121.3 (s), 129.5 (d), 159.7 (s), 172.6 (s).

General procedure for the synthesis of 4-{2-[(propan-2-yloxy)carbonyl]-1H-pyrrol-1-yl}butanoic acid (22) and 5-{2-[(propan-2-yloxy)carbonyl]-1H-pyrrol-1-yl}pentanoic acid (23).

To a solution of **20,21** (1.65 mmol) in EtOH (14 mL) was added a solution of 5% NaOH (1.42 mL, 1.65 mmol). The mixture was stirred at 70°C up to completeness (TLC). Then the solvent was removed under reduced pressure and water and crushed ice were added. The solution was acidified with 6 M HCl and the aqueous solution was extracted with ethyl acetate (3 x 50 mL). The organic layers were dried over Na_2SO_4 and the solvent removed under reduced pressure. The crude product was purified by chromatography column (DCM/AcOEt 8:2).

*4-{2-[(Propan-2-yloxy)carbonyl]-1H-pyrrol-1-yl}butanoic acid (**22**)*. This compound was obtained by reaction of **20** after 12 h. colorless oil; yield: 81%; IR: 3113 (OH), 1706 (CO); 1685 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.26 (6H, d, *J* = 6.2 Hz, 2 x CH_3), 1.82-1.92 (2H, m, CH_2), 2.14 (2H, t, *J* = 7.3 Hz, CH_2), 4.28 (2H, t, *J* = 7.3 Hz, CH_2), 4.95-5.13 (1H, m, CH), 6.008-6.11 (1H, m, Ar), 6.81-6.84 (1H, m, Ar), 7.09-7.11 (1H, m, Ar),

12.15 (1H, s, OH); ^{13}C nmr (DMSO-d_6) (ppm): 21.7 (2 x q), 26.6 (t), 30.5 (t), 47.5 (t), 66.6 (d), 107.7 (d), 117.8 (d), 121.3 (s), 129.5 (d), 159.7 (s), 173.8 (s).

*5-{2-[(Propan-2-yloxy)carbonyl]-1H-pyrrol-1-yl}pentanoic acid (**23**)*. This compound was obtained by reaction of **21** after 24 h. Colorless oil; yield: 74%, IR: 3131 (OH), 1704 (CO), 1696 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.27 (6H, d, J = 6.4 Hz, 2 x CH_3), 1.59-1.67 (2H, m, CH_2), 1.71-1.76 (2H, m, CH_2), 2.21 (2H, t, J = 7.4 Hz, CH_2), 4.26 (2H, t, J = 7.4 Hz, CH_2), 5.01-5.10 (1H, m, CH), 6.06-6.12 (1H, m, Ar), 6.80-6.85 (1H, m, Ar), 7.12-7.16 (1H, m, Ar), 12.05 (1H, s, OH); ^{13}C nmr (DMSO-d_6) (ppm): 21.4 (t), 21.7 (2 x q), 30.7 (t), 33.1 (t), 47.9 (t), 59.6 (t), 66.5 (d), 107.6 (d), 117.7 (d), 121.3 (s), 129.5 (d), 159.8 (s), 174.2 (s).

General procedure for the synthesis of propan-2-yl 8-oxo-5,6,7,8-tetrahydroindolizine-3-carboxylate (24) and propan-2-yl 9-oxo-6,7,8,9-tetrahydro-5*H*-pyrrolo[1,2-*a*]azepine-3-carboxylate (25).

To a solution of **22,23** (4.32 g, 18 mmol) in anhydrous DCM (52 mL) trifluoroacetic anhydride (16.6 mL, 217 mmol) was added and the reaction mixture was stirred at room temperature for 24 h. The solvent was removed under reduced pressure and water and crushed ice were added. The solution was neutralized with a saturated solution of $NaHCO_3$. Then the aqueous phase was extracted with dichloromethane (3 x 50 mL). The organic layers were dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by chromatography column (Cyclohexane/AcOEt 8:2).

*Propan-2-yl 8-oxo-5,6,7,8-tetrahydroindolizine-3-carboxylate (**24**)*. This compound was obtained by reaction of **22**. Pale yellow solid; yield 65%; m.p.: 66-67 °C; IR: 1703 (CO), 1669 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.30 (6H, d, J = 6.2 Hz, 2 x CH_3), 2.15-2.27 (2H, m, CH_2), 2.57 (2H, t, J = 6.0 Hz, CH_2), 4.48 (2H, t, J = 6.0 Hz, CH_2), 5.04-5.17 (1H, m, CH), 6.80-6.88 (2H, m, Ar); ^{13}C nmr (DMSO-d_6) (ppm): 21.6 (2 x q), 22.8 (t), 35.5 (t), 44.4 (t), 67.9 (d), 111.5 (d), 116.3 (d), 125.7 (s), 134.3 (s), 159.6 (s), 188.4 (s).

*Propan-2-yl 9-oxo-6,7,8,9-tetrahydro-5H-pyrrolo[1,2-a]azepine-3-carboxylate (**25**)*. This compound was obtained by reaction of **23**. Yellow oil; yield: 62%; IR: 1703 (CO), 1658 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.29 (6H, d, J = 6.2 Hz, 2 x CH_3), 1.68-1.80

(2H, m, CH_2), 1.88-1.99 (2H, m, CH_2), 2.71-2.77 (2H, m, CH_2), 4.72-4.78 (2H, m, CH_2), 5.06-5.16 (1H, m, CH), 6.71-6.86 (2H, m, Ar); ^{13}C nmr (DMSO-d_6) (ppm): 18.8 (t), 21.6 (2 x q), 25.5 (t), 39.0 (t), 43.5 (t), 67.8 (d), 113.5 (d), 116.1 (d), 126.0 (s), 139.3 (s), 159.8 (s), 193.1 (s).

Synthesis of 4-(Pyrrol-1-yl)butanoic acid (28).

A solution of 4-aminobutyric acid **27** (10.0 g, 97.0 mmol) and NaOAc (8.0 g, 97.5 mmol) in a mixture of H_2O (144 mL), AcOH (48 mL) and DMF (144 mL) was heated at 90°C. Then 2,5-dimethoxytetrahydrofuran **26** (12.9 g, 97.2 mmol) was added and the reaction mixture was stirred at 90°C for 16 h. After cooling, the reaction mixture was poured on crushed ice and the resulted solution was extracted with DCM (3 x 40 mL). The combined organic layers were washed with water (2 x 200 mL), dried on Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was dissolved in DCM (20 mL) and extracted repeatedly with a saturated aqueous $NaHCO_3$ solution. The combined basic layers were made acidic with aqueous HCl solution and extracted with DCM (3 x 30 mL). The organic layers were dried on Na_2SO_4 and the solvent was removed under reduced pressure. Yield 90%. The spectroscopic data are in agreement with the literature [26].

Synthesis of 6,7-dihydroindolizin-8(5*H*)-one (29).

The acid derivative **28** (8.37 mmol) was stirred in polyphosphoric acid (13 g) at 35°C for 16 h. The reaction mixture was quenched with water and crushed ice and the resulting solution was extracted with ethyl acetate (3 x 50 mL). The organic layer were dried over Na_2SO_4, and the solvent removed under reduced pressure. The crude product was purified by chromatography column (DCM). Yield 79%. The spectroscopic data are in agreement with the literature [26].

Synthesis of ethyl 5-(1*H*-pyrrol-1-yl)pentanoate (30).

To a solution of compound **10** (3.6 mmol) in anhydrous DMF (15 mL), NaH (7.2 mmol) was added at 0 ^{o}C and the reaction mixture was stirred at room temperature for 3 h. Then KI (0.64 g, 4.0 mmol), ethyl 5-bromovalerate (7.2 mmol) were added at 0°C and the reaction mixture was stirred at room temperature for 16 h. After cooling, the reaction mixture was poured onto crushed ice and the aqueous solution was extracted with ethyl acetate (3 x 50 mL). The organic layers were dried over Na_2SO_4 and the solvent removed

under reduced pressure. The crude product was purified by chromatography column (Petroleum ether/AcOEt 9:1). Colorless oil; yield: 75%; IR: 1725 (CO) cm^{-1}; ^{1}H nmr ($CDCl_3$) (ppm): 1.24 (3H, t, *J* = 7.1 Hz, CH_3), 1.53-1.88 (4H, m, 2 x CH_2), 2.30 (2H, t, *J* = 7.0 Hz, CH_2), 3.89 (2H, t, *J* = 7.0 Hz, CH_2), 4.12 (2H, q, *J* = 7.1 Hz, CH_2), 6.13 (2H, t, *J* = 7.1 Hz, Ar), 6.64 (2H, t, *J* = 2.1 Hz, Ar); ^{13}C nmr ($CDCl_3$) (ppm): 14.3 (q), 22.2 (t), 31.0 (t), 33.7 (t), 49.2 (t), 60.4 (t), 108.0 (2 x d), 120.5 (2 x d), 173.3 (s).

Synthesis of 5-(1*H*-pyrrol-1-yl)pentanoic acid (31).

To a solution of **40** (1.65 mmol) in EtOH (14 mL) was added a solution of 5% NaOH (1.42 mL, 1.65 mmol). The mixture was stirred at room temperature for 48 h. Then the solvent was removed under reduced pressure and water and crushed ice were added. The solution was acidified with 6 M HCl and the aqueous solution was extracted with ethyl acetate (3 x 50 mL). The organic layers were dried over Na_2SO_4 and the solvent removed under reduced pressure. The crude product was purified by chromatography column (DCM/AcOEt 8:2). Colorless oil; yield: 72%; IR: 3125 (OH), 1707 (CO), cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.34-1.49 (2H, m, CH_2), 1.61-1.76 (2H, m, CH_2), 2.21 (2H, t, *J* = 7.3 Hz, CH_2), 3.86 (2H, t, *J* = 7.3 Hz, CH_2), 5.97 (2H, t, *J* = 2.1 Hz, Ar), 6.73 (2H, t, *J* = 2.1 Hz, Ar), 12.06 (1H, s, OH); ^{13}C nmr (DMSO-d_6) (ppm): 21.6 (t), 30.5 (t), 33.1 (t), 48.2 (t), 107.4 (2 x d), 120.4 (2 x d), 174.3 (s).

Synthesis of 5,6,7,8-tetrahydro-9*H*-pyrrolo[1,2-*a*]azepin-9-one (32).

Method A. The acid derivative **31** (8.37 mmol) was stirred in polyphosphoric acid (13 g) at 35°C for 16 h. The reaction mixture was quenched with water and crushed ice and the resulting solution was extracted with ethyl acetate (3 x 50 mL). The organic layer were dried over Na_2SO_4, and the solvent removed under reduced pressure. The crude product was purified by chromatography column (DCM).

Method B. To a solution of **31** (4.32 g, 18 mmol) in anhydrous DCM (52 mL) trifluoroacetic anhydride (16.6 mL, 217 mmol) was added and the reaction mixture was stirred at room temperature for 24 h. The solvent was removed under reduced pressure and water and crushed ice were added. The solution was neutralized with a saturated solution of $NaHCO_3$. Then the aqueous phase was extracted with dichloromethane (3 x 50 mL). The organic layers were dried over Na_2SO_4, and the solvent was removed under

reduced pressure. The crude product was purified by chromatography column (DCM). Colorless oil; yield: Method A 20%, Method B 25%; IR: 1667 (CO) cm^{-1}; ^{1}H nmr ($CDCl_3$) (ppm): 1.85-2.12 (4H, m, 2 x CH_2), 2.75 (2H, t, *J* = 6.2 Hz, CH_2), 4.22 (2H, t, *J* = 6.2 Hz, CH_2), 6.14-6.17 (1H, m, Ar), 6.79-6.81 (1H, m, Ar), 7.01-7.04 (1H, m, Ar); ^{13}C nmr ($CDCl_3$) (ppm): 20.4 (t), 26.5 (t), 39.8 (t), 48.1 (t), 109.0 (d), 117.4 (d), 128.4 (d), 134.4 (s), 191.8 (s).

General procedure for the synhesis of 7-(hydroxymethylidene)-indolizinones (33,35) and 8-(hydroxymethylidene)-pyrrolo[1,2-*a*]azepinone (34).

To a suspension of *t*-BuO^-K^+ (13.5 mmol) in anhydrous toluene (12 mL), at 0°C under N_2 atmosphere, a solution of the appropriate ketone **17,18,29** (4.5 mmol) in anhydrous toluene (40 mL) was added and the reaction mixture was stirred at room temperature for 3 h. Then a solution of ethyl formate (1.09 mL, 13.5 mmol) in anhydrous toluene (12 mL) was added, and the reaction mixture was stirred at room temperature for 16 h. The solvent was removed under reduced pressure and the residue was added water (50 mL). The aqueous phase was acidified with HCl 6M and extracted with DCM (2 x 60 mL). The organic layers were dried on Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by chromatography column (DCM).

*Ethyl 7-(hydroxymethylidene)-8-oxo-5,6,7,8-tetrahydroindolizine-3-carboxylate (**33**)*. This compound was obtained by reaction of **17**. Yellow oil; yield: 65%; IR: 3405 (OH), 1708 (CO), 1673 (CO) cm^{-1}; ^{1}H nmr ($CDCl_3$) (ppm): 1.38 (3H, t, *J* = 6.3 Hz, CH_3), 2.75 (2H, t, *J* = 5.5 Hz, CH_2), 4.33 (2H, q, *J* = 6.3 Hz, CH_2), 4.59 (2H, t, *J* = 5.5 Hz, CH_2), 6.81-6.98 (2H, m, Ar), 7.93 (1H, s, CH), 14.16 (1H, s, OH); ^{13}C nmr ($CDCl_3$) (ppm): 14.3 (q), 23.8 (t), 43.6 (t), 60.8 (t), 105.3 (s), 112.7 (d), 117.9 (d), 126.7 (s), 132.8 (s), 160.8 (s), 172.2 (d), 177.3 (s).

*Ethyl 8-(hydroxymethylidene)-9-oxo-6,7,8,9-tetrahydro-5H-pyrrolo[1,2-a]azepine-3-carboxylate (**34**)*. This compound was obtained by reaction of **18**. Yellow oil; yield: 68%; IR: 3381 (OH), 1703 (CO), 1629 (CO) cm^{-1}; ^{1}H nmr ($CDCl_3$) (ppm): 1.37 (3H, t, *J* = 7.1 Hz, CH_3), 2.09-2.22 (2H, m, CH_2), 2.33 (2H, t, *J* = 6.4 Hz, CH_2), 4.32 (2H, q, *J* = 7.1 Hz, CH_2), 4.56 (2H, t, *J* = 6.4 Hz, CH_2), 6.81-6.77 (1H, d, *J* = 4.2 Hz, Ar), 6.95 (1H, d, *J* = 4.2 Hz, Ar), 8.16 (1H, s, CH), 14.76 (1H, s, OH); ^{13}C nmr ($CDCl_3$) (ppm): 14.3 (q), 24.0

(t), 29.8 (t), 44.7 (t), 60.6 (t), 110.7 (s), 113.3 (d), 117.2 (d), 126.8 (s), 137.7 (s), 160.9 (s), 178.3 (d), 180.9 (s).

*7-(Hydroxymethylidene)-6,7-dihydroindolizin-8(5H)-one (**35**)*. This compound was obtained by reaction of **29**. Yellow oil; yield: 66%; IR: 3439 (OH), 1669 (CO) cm^{-1}; ^{1}H nmr ($CDCl_3$) (ppm): 2.74 (2H, t, *J* = 6.4 Hz, CH_2), 4.07 (2H, t, *J* = 6.4 Hz, CH_2), 6.28-6.31 (1H, m, Ar), 6.87-6.89 (1H, m, Ar), 7.02-7.04 (1H, m, Ar), 7.65 (1H, s, CH), 14.40 (1H, s, OH); ^{13}C nmr ($CDCl_3$) (ppm): 24.6 (t), 45.1 (t), 105.0 (s), 111.0 (d), 114.4 (d), 126.7 (d), 128.8 (s), 167.9 (d), 179.3 (s).

General procedure for the synhesis of 7-[(diethylamino)methylidene]-indolizinones (36,38) and 8-[(diethylamino)methylidene]-pyrrolo[1,2-*a*]azepinone (37).

To a solution of **33-35** (1.3 mmol) in anhydrous toluene (2.5 mL), diethylamine (2 mmol) was added and the reaction mixture was stirred at room temperature for 16 h. The solvent was removed under reduced pressure and the residue was used in the next step without further purification.

*Ethyl 7-[(diethylamino)methylidene]-8-oxo-5,6,7,8-tetrahydroindolizine-3-carboxylate (**36**)*. This compound was obtained by reaction of **33**. Yellow oil; yield: 77%; IR: 1705 (CO), 1675 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.15-1.32 (9H, m, 3 x CH_3), 2.81-3.11 (2H, m, CH_2), 3.39 (2H, q, *J* = 6.7 Hz, 2 x CH_2), 4.25 (2H, q, *J* = 6.6 Hz, CH_2), 4.33-4.59 (2H, m, CH_2), 6.63 (1H, s, Ar), 6.84 (1H, s, Ar), 7.55 (1H, s, CH); ^{13}C nmr (DMSO-d_6) (ppm): 14.2 (q), 14.6 (2 x q), 23.7 (t), 43.0 (t), 47.3 (2 x t), 59.9 (t), 97.7 (s), 110.2 (d), 116.5 (d), 123.0 (s), 136.6 (s), 147.0 (d), 160.3 (s), 176.5 (s).

*Ethyl 8-[(diethylamino)methylidene]-9-oxo-6,7,8,9-tetrahydro-5H-pyrrolo[1,2-a]azepine-3-carboxylate (**37**)*. This compound was obtained by reaction of **34** and used in the next step without further purification.

*7-[(Diethylamino)methylidene]-6,7-dihydroindolizin-8(5H)-one (**38**)*. This compound was obtained by reaction of **35**. Yellow oil; yield: 87%; IR: 1641 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.17 (6H, m, *J* = 7.1 Hz, 2 x CH_3), 2.92 (2H, t, *J* = 6.2 Hz, CH_2), 3.35 (2H, q, *J* = 7.1 Hz, 2 x CH_2), 4.02 (2H, t, *J* = 6.2 Hz, CH_2), 6.10-6.13 (1H, m, Ar), 6.58-6.60 (1H, m, Ar), 6.93-6.95 (1H, m, Ar), 7.46 (1H, s, CH); ^{13}C nmr (DMSO-d_6) (ppm):

14.6 (2 x q), 24.1 (t), 43.9 (t), 47.2 (2 x t), 98.2 (s), 108.6 (d), 110.9 (d), 123.7 (d), 131.4 (s), 146.1 (d), 176.8 (s).

General procedure for the synthesis of 7-(dimethylamino)methylene)-8-oxo-5,6,7,8-tetrahydroindolizine (39,41,43) and 8-(dimethylamino)methylene)-9-oxo-6,7,8,9-tetrahydro-5*H*-pyrrolo[1,2-*a*]azepine (40,42).

To a solution of the suitable ketones (0.44g, 2 mmol) in anhydrous DMF (2.6 mL) was added DMFDMA (2.64 mL, 20 mmol). The reaction mixture was stirred at reflux up to completeness (TLC). The reaction mixtures was poured onto crushed ice. The precipitate was filtered off and dried, in absence the solution was extracted with ethyl acetate (3 x 30 mL). The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure.

*Ethyl 7-[(dimethylamino)methylidene]-8-oxo-5,6,7,8-tetrahydroindolizine-3-carboxylate (**39**)*. This compound was obtained by reaction of **17** after 2 h and used in the next step without further purification.

*Ethyl 8-(dimethylamino)methylene)-9-oxo-6,7,8,9-tetrahydro-5H-pyrrolo[1,2-a]azepine-3-carboxylate (**40**).* This compound was obtained by reaction of **18** after 3 h: yellow solid; yield: 87%, mp: 115-116 °C; IR: 1697 (CO), 1641 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.28 (3H, t, J = 7.1 Hz, CH_3), 1.86-199 (2H, m, CH_2), 2.32 (2H, t, J = 6.3 Hz, CH_2), 3.11 (6H, s, 2 x CH_3), 4.25 (2H, q, J = 7.1 Hz, CH_2), 4.51 (2H, t, J = 6.3 Hz, CH_2), 6.44 (1H, d, J = 4.0 Hz, Ar), 6.82 (1H, d, J = 4.0 Hz, Ar), 7.53 (1H, s, CH); ^{13}C nmr (DMSO-d_6) (ppm): 14.2 (q), 21.7 (t), 30.6 (t), 42.9 (t), 43.1 (2 x q), 59.8 (t), 102.4 (s), 110.6 (d), 116.1 (d), 122.3 (s), 142.1 (s), 150.1 (d), 160.4 (s),184,9 (s).

*Propan-2-yl 7-[(dimethylamino)methylidene]-8-oxo-5,6,7,8-tetrahydroindolizine-3-carboxylate (**41**)*. This compound was obtained by reaction of **24** after 3 h: yellow solid; yield: 56%, mp: 126-127°C; IR: 1691 (CO), 1603 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.28 (6H, d, J = 6.2 Hz, 2 x CH_3), 3.04-3.12 (8H, m, CH_2 and 2 x CH_3), 4.41 (2H, t, J = 6.6 Hz, CH_2), 5.01-5.14 (1H, m, CH), 6.61 (1H, d, J = 4.1 Hz, Ar), 6.81 (1H, d, J = 4.1 Hz, Ar),7.49 (1H, s, CH); ^{13}C nmr (DMSO-d_6) (ppm): 21.7 (2 x q), 23.7 (t), 23.8 (t), 43.2 (2 x q), 67.3 (d), 98.5 (s), 110.1 (d), 116.5 (d), 123.4 (s), 136.6 (s), 149.2 (d), 159.9 (s), 176.5 (s).

*Propan-2-yl 8-[(dimethylamino)methylidene]-9-oxo-6,7,8,9-tetrahydro-5H-pyrrolo[1,2-a]azepine-3-carboxylate (**42**)*. This compound was obtained by reaction of **25** after 2 h and used in the next step without further purification.

*7-[(dimethylamino)methylidene]-6,7-dihydroindolizin-8(5H)-one (**43**)*. This compound was obtained by reaction of **29** after 16 h and used in the next step without further purification.

General procedure for the synthesis of 2-aminopyrimido[5,4-*g*]indolizine (44a,46a,48a) and 2-aminopyrimido[4,5-*c*]pyrrolo[1,2-*a*]azepine (45a,47a).

To a suspension of MeONa (0.81 g, 15 mmol) in anhydrous ethanol (20 mL), guanidine nitrate (0.92 g, 7.5 mmol) and a solution of the suitable enaminoketons (2 mmol) in anhydrous ethanol (20 mL) were added. The reaction mixture was heated at reflux up to completeness (TLC). Then the reaction mixture was poured onto crushed ice and the precipitate was filtered off, dried and purified by chromatography (DCM/AcOEt 9:1).

*Ethyl 2-amino-5,6-dihydropyrimido[5,4-g]indolizine-8-carboxylate (**44a**)* This compound was obtained by reaction of **39** after 7 h. White solid; yield: 62%,; mp: 222-223°C; IR: 3270-3160 (NH_2), 1663 (CO) cm^{-1}; ^1H nmr (DMSO-d_6) (ppm): 1.29 (3H, t, J=7.1 Hz, CH_3), 2.91 (2H, t, J = 6.8 Hz, CH_2), 4.26 (2H, q, J = 7.1 Hz, CH_3), 4.54 (2H, t, J = 6.8 Hz, CH_2), 6.53 (2H, s, NH_2), 6.76 (1H, d, J = 4.1 Hz, Ar), 6.94 (1H, d, J = 4.1 Hz, Ar), 8.17 (1H, s, H-4); ^{13}C nmr (DMSO-d_6) (ppm): 14.2 (q), 23.4 (t), 42.3 (t), 59.9 (t), 108.0 (d), 112.1 (s), 117.5 (d), 124.0 (s), 134.1 (s), 152.9 (s), 156.7 (d), 160.2 (s), 162.9 (s).

*Ethyl 2-amino-6,7-dihydro-5H-pyrimido[4,5-c]pyrrolo[1,2-a]azepine-9-carboxylate (**45a**)*. This compound was obtained by reaction of **40** after 3 h. White solid; yield: 41%; mp: 180-181°C; 3411-3320 (NH_2), 1696 (CO) cm^{-1}; ^1H nmr (DMSO-d_6) (ppm): 1.30 (3H, t, J = 7.1 Hz, CH_3), 2.14-2.20 (2H, m, CH_2), 2.42 (2H, t, J = 6.3 Hz, CH_2), 4.20-4.36 (4 H, m, 2 x CH_2), 6.54 (1H, d, J = 4.0 Hz, Ar), 6.59 (2H, s, NH_2) 6.93 (1H, d, J = 4.0 Hz, Ar), 8.18 (1H, s, H-4); ^{13}C nmr (DMSO-d_6) (ppm): 14.2 (q), 24.8 (t), 30.5 (t), 43.6 (t), 59.8 (t), 109.7 (d), 116.8 (d), 118.3 (s), 123.8 (s), 139.8 (s), 157.6 (s), 158.5 (d), 160.2 (s), 162.9 (s).

*Propan-2-yl 2-amino-5,6-dihydropyrimido[5,4-g]indolizine-8-carboxylate (**46a**)*. This compound was obtained by reaction of **41** after 1 h. White solid; yield: 71%; mp: 211-212°C; IR: 3414-3332 (NH_2), 1695 (CO) cm^{-1}; ^1H nmr ($CDCl_3$) (ppm): 1.35 (6H, d, *J* = 6.2 Hz, 2 x CH_3), 2.96 (2H, t, *J* = 6.8 Hz, CH_2), 5.14-4.65 (2H, t, *J* = 6.8 Hz, CH_2), 5.12-5.25 (3H, m, CH and NH_2), 6.91-7.03 (2H, m, Ar), 8.15 (1H, s, H-4); ^{13}C nmr ($CDCl_3$) (ppm): 22.1 (2 x q), 24.4 (t), 42.5 (t), 67.9 (d), 109.3 (d), 113.6 (s), 118.1 (d), 125.6 (s), 133.8 (s), 154.4 (s), 156.1 (d), 160.7 (s), 162.5 (s).

*Propan-2-yl 2-amino-6,7-dihydro-5H-pyrimido[4,5-c]pyrrolo[1,2-a]azepine-9-carboxylate (**47a**)*. This compound was obtained by reaction of **42** after 1 h. White solid; yield: 86%; mp: 176-177°C; IR: 3514-3411 (NH_2), 1692 (CO) cm^{-1}; ^1H nmr (DMSO-d_6) (ppm): 1.30 (6H, d, *J* = 6.0 Hz, 2 x CH_3), 2.09-2.24 (2H, m, CH_2), 2.41-2.51 (2H, m, CH_2), 4.24-4.32 (2H, m, CH_2), 5.06-5.12 (1H, m, CH), 6.51-6.61 (2H, m, Ar and NH_2), 6.92 (1H, s, Ar), 8.18 (1H, s, H-4); ^{13}C nmr (DMSO-d_6) (ppm): 22.16 (2 x q), 27.9 (t), 31.1 (t), 47.3 (t), 68.9 (d), 108.7 (d), 117.7(s), 126.9 (d), 121.1 (s), 128.5 (s), 148.2 (s), 154.6 (d), 161.1 (s), 162.2 (s).

*5,6-Dihydropyrimido[5,4-g]indolizin-2-amine (**48a**)*. This compound was obtained from **43** after 6 h. Brown solid; yield: 74%; mp: 238-239 °C; IR: 3307-3188 (NH_2), 1664 (CO) cm^{-1}; ^1H nmr (DMSO-d_6) (ppm): 2.85 (2H, t, *J* = 6.6 Hz, CH_2), 4.08 (2H, t, *J* = 6.6 Hz, CH_2), 6.16-6.19 (1H, m, Ar), 6.35 (2H, s, NH_2), 6.68-6.71 (1H, m, Ar), 7.00-7.02 (1H, m, Ar), 8.05 (1H, s, H-4); ^{13}C nmr (DMSO-d_6) (ppm): 24.1 (t), 43.6 (t), 108.3 (d), 109.0 (d), 111.2 (s), 124.5 (d), 128.1 (s), 154.0 (s), 156.0 (d), 162.8 (s).

General procedure for the synthesis of 2-substituted-pyrimido[5,4-*g*]indolizine (44b-d,46b-d,48b-d) and 2-substituted-pyrimido[4,5-*c*]pyrrolo[1,2-*a*]azepine (45b-d,47b-d).

A solution of the suitable enaminoketons (1.5 mmol) and phenylguanidine (R^2= Ph), cyclohexylguanidine (R^2= cyclohexyl) or cyclopentylguanidine (R^2= cyclopentyl) (4.5 mmol) in anhydrous DMF (8 mL) was heated at 100 °C up to completeness (TLC). Then the reaction mixture was poured onto crushed ice and the precipitate was filtered off, dried and purified by chromatography (DCM).

*Ethyl 2-anilino-5,6-dihydropyrimido[5,4-g]indolizine-8-carboxylate (**44b**)*. This compound was obtained by reaction of **39** with phenylguanidine after 1 h. Yellow solid; Yield: 55%; mp: 177-178°C; IR: 3417 (NH), 1698 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.31 (3H, t, J = 7.1 Hz, CH_3), 3.02 (2H, t, J = 6.8 Hz, CH_2), 4.28 (2H, q, J = 7.1 Hz, CH_2), 4.60 (2H, t, J= 6.8 Hz, CH_2), 6.89-7.01 (2H, m, Ar), 77.24-7.33 (3H, m, Ar), 7.83 (2H, d, J = 7.7 Hz, Ar), 8.41 (1H, s, H-4), 9.58 (1H, s, NH); ^{13}C nmr (DMSO-d_6) (ppm): 14.2 (q), 23.4 (t), 42.2 (t), 60.0 (t), 108.6 (d), 114.3 (s), 117.7 (d), 118.4 (2 x d), 121.0 (d), 128.4 (2 x d), 133.8 (s), 140.8 (s), 152.7 (s), 156.5 (d), 159.2 (s), 159.8 (s), 160.1 (s).

*Ethyl 2-(cyclohexylamino)-5,6-dihydropyrimido[5,4-g]indolizine-8-carboxylate (**44c**)*. This compound was obtained by reaction of **39** with cyclohexylguanidine after 6 h. White solid; yield: 52%; mp: 130-131°C; IR: 3433 (NH), 1697 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.09-1.33 (13H, m, CH_3 and 5 x CH_2), 2.92 (2H, t, J = 6.6 Hz, CH_2), 3.70-3.73 (1H, m, CH), 4.25 (2, q, J=7.1 Hz, CH_2), 4.54 (2H, t, J = 6.6 Hz, CH_2), 6.76 (1H, d, J= 4.1 Hz, Ar), 6.89-6.96 (2H, m, Ar and NH), 8.19 (1H, s, H-4); ^{13}C nmr (DMSO-d_6) (ppm): 14.2 (q), 23.4 (t), 24.8 (2 x t), 25.4 (t), 32.4 (2 x t), 42.4 (t), 49.3 (d), 59.9 (t), 108.0 (d), 111.6 (s), 117.5 (d), 124.0 (s), 134.3 (s), 152.8 (s), 156.4 (d), 160.2 (s), 161.1 (s).

*Ethyl 2-(cyclopentylamino)-5,6-dihydropyrimido[5,4-g]indolizine-8-carboxylate (**44d**)*. This compound was obtained by reaction of **39** with cyclopentylguanidine after 2 h. Pale yellow solid; yield: 52%; mp: 95-96°C; IR: 3405 (NH), 1699 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.28 (3H, t, J = 7.1 Hz, CH_3), 1.48-1.67 (6H, m, 3 x CH_2), 1.87-1.99 (2H, m, CH_2), 2.64-274 (2H, m, CH_2), 3.72-3.85 (1H, m, CH), 4.24 (2H, q, J = 7.1 Hz, CH_2), 4.33-4.51(2H, m, CH_2), 6.59-6.62 (1H, m, Ar), 6.81-6.90 (1H, m, Ar), 7.31-7.35 (1H, m, Ar), 10.01-10.11 (1H, m, NH); ^{13}C nmr (DMSO-d_6) (ppm): 14.2 (q), 23.0 (2 x t), 26.4 (t), 33.5 (2 x t), 43.9 (t), 59.1 (d), 59.9 (t), 96.5 (s), 109.4 (d), 116.8 (d), 123.2 (s), 136.8 (s), 137.0 (s), 151.9 (d), 160.3 (s), 176.7 (s).

*Ethyl 2-anilino-6,7-dihydro-5H-pyrimido[4,5-c]pyrrolo[1,2-a]azepine-9-carboxylate (**45b**)*. This compound was obtained by reaction of **40** with phenylguanidine after 5 h. White solid; yield: 56%; mp: 134-135°C; IR: 3443 (NH), 1697 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.31 (3H, t, J=7.1 Hz, CH_3), 2.20-2.26 (2H, m, CH_2), 2.50-2.57 (2H, m, CH_2), 4.22-4.42 (4H, m, 2 x CH_2), 6.71 (1H, d, J = 4.1 Hz, Ar), 6.89-7.00 (2H, m, Ar),

7.28 (2H, t, *J* = 8.3 Hz, Ar), 7.81 (2H, d, *J* = 7.6 Hz, Ar), 8.43 (1H, s, H-4), 9.67 (1H, s, NH); ^{13}C nmr (DMSO-d_6) (ppm): 14.2 (q), 25.1 (t), 30.6 (t), 43.8 (t), 59.9 (t), 110.4 (d), 116.3 (d), 118.5 (2 x d), 120.6 (s), 121.0 (d), 124.4 (s), 128.4 (2 x d), 139.4 (s), 140.7 (s), 157.3 (s), 158.4 (d), 159.1 (s), 160.2 (s).

*Ethyl 2-(cyclohexylamino)-6,7-dihydro-5H-pyrimido[4,5-c]pyrrolo[1,2-a]azepine-9-carboxylate (**45c**)*. This compound was obtained by reaction of **40** with cyclohexylguanidine after 2 h. Yellow solid; yield: 61%; mp: 98-99°C; IR: 3422 (NH), 1693 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.09-1.90 (13H, m, CH_3 and 5 x CH_2), 2.10-2.24 (2H, m, CH_2), 2.41 (2H, t, *J* = 6.5 Hz, CH_2), 3.72-3.82 (1H, m, CH), 4.20-4.37 (2H, m, 2 x CH_2), 6.39 (1H, d, *J* = 4.0 Hz, Ar), 6.92-7.03 (2H, m, Ar and NH), 8.19 (1H, d, H-4); ^{13}C nmr (DMSO-d_6) (ppm): 14.2 (q), 24.8 (3 x t), 25.4 (t), 30.4 (t), 32.4 (2 x t), 43.6 (t), 49.2 (d), 59.8 (t), 109.5 (d), 116.8 (d), 123.8 (s), 124.0 (s), 139.9 (s), 141.6 (s), 158.3 (d), 160.2 (s), 161.0 (s).

*Ethyl 2-(cyclopentylamino)-6,7-dihydro-5H-pyrimido[4,5-c]pyrrolo[1,2-a]azepine-9-carboxylate (**45d**)*. This compound was obtained by reaction of **40** with cyclopentylguanidine after 3 h. Yellow solid; yield: 61%; mp: 98-99°C; IR: 3405 (NH), 1696 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.31 (3H, t, *J* = 7.1 Hz, CH_3), 1.49-1.68 (6H, m, 3 x CH_2), 1.90-2.24 (4H, m, 2 x CH_2), 3.72-3.82 (1H, m, CH), 4.23 (2H, q, *J* = 7.1 Hz, CH_2), 4.38-4.47 (2H, m, CH_2), 6.39-6.41 (1H, m, Ar), 6.80.6.83 (1H, m, Ar), 7.454-7.61 (1H, m, Ar), 10.20-10.30 (1H, m, NH); ^{13}C nmr (DMSO-d_6) (ppm): 14.2 (q), 23.0 (2 x t), 25.8 (t), 30.4 (t), 33.5 (2 x t), 43.1 (t), 59.2 (d), 59.8 (t), 101.4 (s), 109.5 (d), 116.1 (s), 116.2 (d), 122.4 (s), 142.5 (s), 147.6 (s), 153.6 (d), 160.3 (s).

*Propan-2-yl 2-anilino-5,6-dihydropyrimido[5,4-g]indolizine-8-carboxylate (**46b**)*. This compound was obtained by reaction of **41** with phenylguanidine after 3 h. White solid; yield: 79%; mp: 184-185°C; IR: 3422 (NH), 1695 (CO) cm^{-1}; ^{1}H nmr ($CDCl_3$) (ppm): 1.30 (6H, d, *J* = 6.2 Hz, 2 x CH_3), 3.01 (2H, t, *J* = 6.8 Hz, CH_2), 4.68 (2H, t, *J* = 6.8 Hz, CH_2), 5.14-5.26 (1H, m, CH), 6.98-7.07 (3H, m, Ar and NH), 7.21-7.39 (3H, m, Ar), 7.68 (1H, d, *J* = 7.7 Hz, Ar), 8.26 (1H, s, H-4); ^{13}C nmr ($CDCl_3$) (ppm): 22.1 (2 x q), 24.4 (t), 42.5 (t), 67.9 (d), 109.3 (d), 114.4 (s), 118.1 (d), 118.9 (2 x d), 122.2 (d), 125.6 (s), 128.9 (2 x d), 134.0 (s), 139.8 (s), 154.0 (s), 156.1 (d), 159.5 (s), 160.1 (s).

*Propan-2-yl 2-(cyclohexylamino)-5,6-dihydropyrimido[5,4-g]indolizine-8-carboxylate (**46c**)*. This compound was obtained by reaction of **41** with cyclohexylguanidine after 2 h. Yellow solid; yield: 86%; mp: 123-124°C; IR: 3399 (NH), 1694 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.27-1.86 (16H, m, 2 x CH_3 and 5 x CH_2), 2.64-2.73 (2H, m, CH_2), 3.12-3.22 (1H, m, CH), 4.38-4.44 (2H, m, CH_2), 5.01-5.13 (1H, m, CH), 6.59 (1H, d, *J* = 4.1 Hz, Ar), 6.82 (1H, d, *J* = 4.1 Hz, Ar), 7.27 (1H, s, H-4), 10.04-10.14 (1H, m, NH); ^{13}C nmr (DMSO-d_6) (ppm): 21.7 (2 x q), 24.0 (2 x t), 24.7 (t), 26.4 (t), 33.4 (2 x t), 33.7 (t), 55.8 (d), 67.3 (d), 96.4 (s), 109.8 (d), 116.5 (d), 123.5 (s), 136.8 (s), 136.9 (s), 151.4 (d), 159.9 (s).

*Propan-2-yl 2-(cyclopentylamino)-5,6-dihydropyrimido[5,4-g]indolizine-8-carboxylate (**46d**)*. This compound was obtained by reaction of **41** with cyclopentylguanidine after 3 h. White solid; yield: 73%; mp: 124-125°C; IR: 3406 (NH), 1692 (CO) cm^{-1}; ^{1}H nmr ($CDCl_3$) (ppm): 1.33 (6H, d, *J* = 6.3 Hz, 2 x CH_3), 1.64-1.99 (8H, m, 4 x CH_2), 2.70 (2H, t, *J*= 6.4 Hz, CH_2), 3.64-3.73 (1H, m, CH), 4.52 (2H, t, *J*=6.4 Hz, CH_2), 5.11-5.23 (1H, m, *J*= 6.2 Hz, CH), 6.79-6.93 (3H, m, Ar), 10.09-10.18 (1H, m, NH); ^{13}C nmr ($CDCl_3$) (ppm): 22.0 (2 x q), 23.5 (2 x t), 27.4 (t), 34.1 (2 x t), 44.2 (t), 60.2 (d), 67.7 (d), 97.1 (s), 110.1 (d), 117.4 (d), 124.5 (s), 132.5 (s), 137.0 (s), 151.1 (d), 160.9 (s), 178.2 (s).

*Propan-2-yl 2-anilino-6,7-dihydro-5H-pyrimido[4,5-c]pyrrolo[1,2-a]azepine-9-carboxylate (**47b**)*. This compound was obtained by reaction of **42** with phenylguanidine after 2 h. White solid; yield: 80%; mp: 101-102°C; IR: 3421 (NH), 1691 (CO) cm^{-1}; ^{1}H nmr ($CDCl_3$) (ppm): 1.36 (6H, d, *J* = 6.1 Hz, 2 x CH_3), 2.30-2.39 (2H, m, CH_2), 2.59 (2H, t, *J* = 7.0 Hz, CH_2), 4.46 (2H, t, *J* = 7.0 Hz, CH_2), 5.14-5.29 (1H, m, CH), 6.75 (1H, d, *J* = 3.6 Hz, Ar), 6.69-7.06 (2H, m, Ar and NH), 7.26-7.37 (3H, m, Ar), 7.66 (1H, d, *J* = 7.8 Hz, Ar),8.28 (1H, s, H-4); ^{13}C nmr ($CDCl_3$) (ppm): 22.3 (2 x q), 27.8 (t), 31.0 (t), 46.9 (t), 68.9 (d), 108.7 (d), 121.0 (s), 122.0 (2 x d), 123.9 (d), 122.1 (s), 126.9 (d), 128.5 (s), 129.9 (2 x d), 139.9 (s), 147.7 (s), 156.1 (d), 159.7 (s), 161.4 (s).

*Propan-2-yl 2-(cyclohexylamino)-6,7-dihydro-5H-pyrimido[4,5-c]pyrrolo[1,2-a]azepine-9-carboxylate (**47c**)*. This compound was obtained by reaction of **42** with cyclohexylguanidine after 4 h. White solid; yield: 98%; mp: 108-109°C; IR: 3381 (NH), 1685 (CO) cm^{-1}; ^{1}H nmr ($CDCl_3$) (ppm): 1.03-1.35 (10H, m, 2 x CH_3 and 2 x CH_2), 1.59-

1.80 (4H, m, 2 x CH_2), 1.93-2.10 (4H, m, 2 x CH_2), 2.15-2.21 (2H, m, CH_2), 3.10-3.16 (1H, m, CH), 4.53 (2H, t, , *J* = 6.3 Hz, CH_2), 5.10-5.23 (1H, s, CH), 6.56 (1H, d, *J* = 4.1 Hz, Ar), 6.86-6.92 (2H, m, Ar), 10.28-10.37 (1H, m, NH); ^{13}C nmr ($CDCl_3$) (ppm): 22.0 (2 x q), 24.5 (2 x t), 25.2 (t), 26.9 (t), 31.1 (t), 34.1 (2 x t), 43.5 (t), 57.4 (d), 67.5 (d), 102.4 (2 x s), 110.2 (d), 116.7 (d), 123.9 (s), 142.6 (s), 152.3 (d), 160.9 (s), 185.1 (s).

*Propan-2-yl 2-(cyclopentylamino)-6,7-dihydro-5H-pyrimido[4,5-c]pyrrolo[1,2-a]azepine-9-carboxylate (**47d**)*. This compound was obtained by reaction of **42** with cyclopentylguanidine after 2 h. Brown solid; yield: 82%; mp: 140-141°C; IR: 3416 (NH), 1690 (CO) cm^{-1}; ^{1}H nmr ($CDCl_3$) (ppm): 1.34 (6H, d, *J* = 6.2 Hz, 2 x CH_3), 1.60-1.78 (6H, m, 3 x CH_2), 1.82-2.22 (6H, m, 3 x CH_2), 3.65-3.77 (1H, m, CH), 4.53 (2H, t, *J*= 6.2 Hz, CH_2), 5.10-5.23 (1H, m, CH), 6.55 (1H, d, *J*= 4.0 Hz, Ar), 6.84-6.95 (2H, m, Ar), 10.32-10.36 (1H, m, NH); ^{13}C nmr ($CDCl_3$) (ppm): 22.0 (2 x q), 23.5 (2 x t), 26.8 (t), 31.0 (t), 34.1 (2 x t), 43.5 (t), 60.2 (d), 67.5 (d), 102.5 (2 x s), 110.2 (d), 116.7 (d), 124.0 (s), 142.6 (s), 152.9 (d), 160.9 (s), 185.1 (s).

*N-phenyl-5,6-dihydropyrimido[5,4-g]indolizin-2-amine (**48b**)*.This compound was obtained by reaction of **43** with phenylguanidine after 2 h. White solid; yield: 54%; mp: 181-182 °C; IR: 3422 (NH) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 2.96 (2H, t, *J* = 6.6 Hz, CH_2), 4.15 (2H, t, *J* = 6.6 Hz, CH_2), 6.23-6.26 (1H, m, Ar), 6.83-6.90 (2H, m, Ar), 7.07-7.09 (1H, m, Ar), 7.27 (2H, t, *J* = 7.4 Hz, Ar), 7.83 (2H, d, *J* = 7.7 Hz, Ar), 8.28 (1H, s, H-4), 9.42 (1H, s, NH); ^{13}C nmr (DMSO-d_6) (ppm): 24.1 (t), 43.4 (t), 109.0 (d), 109.4 (d), 113.4 (s), 118.3 (2 x d),120.7 (d), 125.1 (d), 127.8 (s), 128.4 (2 x d), 141.1 (s), 153.9 (s), 155.7 (d), 159.2 (s).

*N-cyclohexyl-5,6-dihydropyrimido[5,4-g]indolizin-2-amine (**48c**)*. This compound was obtained by reaction of **43** with cyclohexylguanidine after 5 h. Brown solid; yield: 56%; mp: 145-146°C; IR: 3427 (NH) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.09-1.90 (10H, m, 5 x CH_2), 2.84 (2H, t, *J* = 6.3 Hz, CH_2), 3.69 (1H, m, CH), 4.07 (2H, t, *J* =6.3 Hz, CH_2), 6.17-6.19 (1H, m, Ar), 6.68-6.72 (2H, m, Ar), 7.01 (1H, s, NH), 8.07 (1H, s, H-4); ^{13}C nmr (DMSO-d_6) (ppm): 24.1 (t), 24.9 (2 x t), 25.4 (t), 32.5 (2 x t), 43.6 (t), 49.1 (d), 108.2 (d), 109.0 (d), 110.7 (s), 124.4 (d), 128.1 (s), 154.0 (s), 155.7 (d), 161.0 (s).

*N-cyclopentyl-5,6-dihydropyrimido[5,4-g]indolizin-2-amine (**48d**)*. This compound was obtained by reaction of **43** with guanidine after 2 h. Yellow solid; yield: 70%, mp: 105-106°C; IR: cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.46-1.64 (6H, m, 3 x CH_2), 1.90-1.92 (2H, m, CH_2), 2.62 (2H, t, J = 6.3 Hz, CH_2), 3.66-3.76 (1H, m, CH), 3.98 (2H, t, J = 6.3 Hz, CH_2), 6.11 (1H, d, J = 62.2 Hz, Ar), 6.55 (1H, t, J = 2.2 Hz, Ar), 6.94 (1H, s, Ar), 7.08 (1H, s, H-4), 9.76-9.85 (1H, m, NH); ^{13}C nmr (DMSO-d_6) (ppm): 23.0 (t), 27.2 (t), 33.1 (t), 33.6 (t), 43.4 (t), 45.0 (t), 58.8 (d), 108.7 (d), 110.1 (d), 124.0 (d), 131.6 (s), 131.7 (s), 150.3 (d), 175.6 (s), 177.8 (s).

General procedure for the synthesis of 2-acetamido-5,6-dihydropyrimido[5,4-*g*]indolizine (44e,46e,48e) and 2-acetamido-pyrimido[4,5-*c*]pyrrolo[1,2-*a*]azepine (45e,47e).

To a solution of compounds of type (**44-48)a** (0.15 g, 0.52 mmol) in anhydrous dioxane (20 mL) acetyl chloride (0.057 mL, 0.78 mmol) and triethylamine (0.08 mL, 0.57 mmol) were added and the reaction mixture was stirred at reflux up to completeness (TLC). Then the reaction mixture was poured onto crushed ice and the precipitate was filtered off, dried and purified by chromatography (DCM).

*Ethyl 2-acetamido-5,6-dihydropyrimido[5,4-g]indolizine-8-carboxylate (**44e**)*. This compound was obtained by reaction of **44a** after 4 h. White solid; yield: 77%, mp: 249-250°C; IR: 3403 (NH), 1663 (CO), 1651 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.31 (3H, t, J = 7.1 Hz, CH_3), 2.22 (3H, s, CH_3), 3.08 (2H, t, J = 6.8 Hz, CH_2), 4.28 (2H, q, J = 7.1 Hz, CH_2), 4.62 (2H, t, J = 6.8 Hz, CH_2), 6.88 (1H, d, J = 4.1 Hz, Ar), 6.99 (1H, d, J = 4.1 Hz, Ar), 8.54 (1H, s, H-4), 10.46 (1H, s, NH); ^{13}C nmr (DMSO-d_6) (ppm): 14.2 (q), 23.5 (t), 24.6 (q), 41.9 (t), 60.1 (t), 109.1 (d), 117.7 (d), 118.5 (s), 124.8 (s), 133.2 (s), 153.1 (s), 156.6 (d), 156.8 (s), 160.1 (s), 169.2 (s).

*Ethyl 2-acetamido-6,7-dihydro-5H-pyrimido[4,5-c]pyrrolo[1,2-a]azepine-9-carboxylate (**45e**)*. This compound was obtained by reaction of **45a** after 2 h. White solid; yield: 79%, mp: 211-212°C; IR: 3387 (NH), 1700 (CO), 1695 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.30 (3H, t, J = 7.1 Hz, CH_3), 2.21-2.29 (5H, m, CH_3 and CH_2), 2.61 (2H, t, J = 6.8 Hz, CH_2), 4.27 (2H, q, J = 7.1 Hz, CH_2), 4.39 (2H, t, J = 6.8 Hz, CH_2), 6.71 (1H, d, J = 4.1 Hz, Ar), 6.98 (1H, d, J = 4.1 Hz, Ar), 8.57 (1H, s, H-4), 10.54 (1H,

s, NH); ^{13}C nmr (DMSO-d_6) (ppm): 14.2 (q), 24.6 (q), 25.5 (t), 30.1 (t), 44.0 (t), 67.9 (d), 111.1 (d), 116.9 (d), 124.8 (s), 138.7 (s), 145.1 (s), 156.6 (s), 157.4 (s), 158.7 (d), 160.2 (s), 169.1 (s).

*Propan-2-yl 2-acetamido-5,6-dihydropyrimido[5,4-g]indolizine-8-carboxylate (**46e**)*. This compound was obtained by reaction of **46a** after 4 h. White solid; yield: 63%; mp: 242-243°C; IR: 3199 (NH), 1693 (CO), 1675 (CO) cm^{-1}; ^{1}H nmr ($CDCl_3$) (ppm): 1.36 (6H, d, *J* = 6.2 Hz, 2 x CH_3), 2.59 (3H, s, CH_3), 3.08 (2H, t, *J* = 6.8 Hz, CH_2), 4.71 (2H, t, *J* = 6.8 Hz, CH_2), 5.17-5.27 (1H, m, CH), 6.95-7.05 (2H, m, Ar), 8.47 (1H, s, H-4), 8.92 (1H, s, NH); ^{13}C nmr ($CDCl_3$) (ppm): 22.0 (2 x q), 24.5 (t), 25.4 (q), 42.2 (t), 68.1 (d), 110.5 (d), 117.4 (d), 117.5 (s), 124.8 (s), 126.4 (s), 138.7 (s), 156.5 (d), 156.7 (s), 160.6 (s), 161.1 (s).

*Propan-2-yl 2-acetamido-6,7-dihydro-5H-pyrimido[4,5-c]pyrrolo[1,2-a]azepine-9-carboxylate (**47e**)*. This compound was obtained by reaction of **47a** after 5 h. White solid; yield: 58%; mp: 211-212°C; IR: 3388 (NH), 1689 (CO), 1673 (CO) cm^{-1}; ^{1}H nmr ($CDCl_3$) (ppm): 1.36 (6H, d, *J* = 6.2 Hz, 2 x CH_3), 2.34-2.44 (2H, m, CH_2), 2.54 (3H, s, CH_3), 2.67 (2H, t, *J* = 6.9 Hz, CH_2), 4.46 (2H, t, *J* = 6.9 Hz, CH_2), 5.14-5.27 (1H, m, CH), 6.74 (1H, d, *J* = 4.1 Hz, Ar), 7.02 (1H, d, *J* = 4.1 Hz, Ar), 8.47 (1H, s, H-4), 8.62 (1H, s, NH); ^{13}C nmr ($CDCl_3$) (ppm): 22.0 (2 x q), 25.3 (q), 26.2 (t), 30.8 (t), 44.1 (t), 67.9 (t), 111.5 (d), 117.4 (d), 117.5 (s), 124.9 (s), 126.4 (s), 138.7 (s), 156.5 (s), 158.4 (d), 158.7 (s), 160.6 (s).

*N-(5,6-dihydropyrimido[5,4-g]indolizin-2-yl)acetamide (**48e**)*. This compound was by reaction of **48a** after 2 h. Green solid; yield: 60%; mp: 250-251°C; IR: 3188 (NH) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 2.21 (3H, s, CH_3), 3.01 (2H, t, *J* = 6.6 Hz, CH_2), 4.16 (2H, t, *J* = 6.6 Hz, CH_2), 6.25 (1H, m, Ar), 6.82 (1H, m, Ar), 7.11 (1H, m, Ar), 8.40 (1H, s, H-4), 10.32 (1H, s, NH); ^{13}C nmr (DMSO-d_6) (ppm): 24.1 (t), 24.6 (q), 43.1 (t) 109.6 (2 x d), 117.4 (s), 125.6 (d), 127.3 (s), 154.2 (s), 155.8 (d), 156.7 (s), 169.2 (s).

General procedure for the synthesis of pyrrolo[1,2-*h*][1,7]naphthyridinones (49-51a) and pyrido[2,3-*c*]pyrrolo[1,2-*a*]azepinones (52-53a).

To a solution of the suitable enamiketones (2.76 mmol) in anhydrous ethanol (20 mL) under nitrogen atmosphere phenylsulfonylacetonitrile (0.75 g, 4.14 mmol) was added.

The reaction mixture was heated under reflux for 24 h. The solvent was removed under reduced pressure. The crude product was recrystallized from diethyl ether.

*Ethyl 3-(benzenesulfonyl)-2-oxo-1,2,5,6-tetrahydropyrrolo[1,2-h][1,7]naphthyridine-8-carboxylate (**49a**)*. This compound was obtained by reaction of **39**. Brown solid; yield: 68%; mp: 145-146°C; IR: 3365 (NH), 1718 (CO), 1646 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.29 (3H, t, J = 7.1 Hz, CH_3), 3.01 (2H, t, J = 7.0 Hz, CH_2), 4.27 (2H, q, J = 7.1 Hz, CH_2), 4.54 (2H, t, J = 7.0 Hz, CH_2), 6.65 (1H, d, J = 4.0 Hz, Ar), 7.16 (1H, d, J = 4.0 Hz, Ar), 7.55-7.73 (3H, m, Ar), 7.96-8.01 (2H, m, Ar), 8.29 (1H, s, H-4), 12.58 (1H, s, NH); ^{13}C nmr (DMSO-d_6) (ppm): 14.2 (q), 24.8 (t), 42.1 (t), 60.3 (t), 108.4 (s), 110.4 (d), 117.6 (d), 118.7 (s), 120.1 (s), 125.5 (s), 128.0 (2 x d), 128.8 (2 x d), 133.3 (d), 138.9 (d), 140.3 (s), 144.1 (s), 157.1 (s), 159.9 (s).

*Propan-2-yl 3-(benzenesulfonyl)-2-oxo-1,2,5,6-tetrahydropyrrolo[1,2-h][1,7]naphthyridine-8-carboxylate (**50a**)*. This compound was obtained by reaction of **41**. White solid; yield: 61%, mp: 332-333°C; IR: 3125 (NH), 1703 (CO), 1652 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.29 (6H, d, J = 6.2 Hz, 2 x CH_3), 3.00 (2H, t, J = 6.7 Hz, CH_2), 4.54 (2H, t, J = 6.7 Hz, CH_2), 5.07-5.13 (1H, m, CH), 6.91 (2H, d, J = 4.1 Hz, Ar), 7.14 (1H, d, J = 4.1 Hz, Ar), 7.56-7.69 (3H, m, Ar), 7.99 (2H, d, J = 7.5 Hz, Ar), 8.29 (1H, s, H-4), 12.57 (1H, s, NH); ^{13}C nmr (DMSO-d_6) (ppm): 22.1 (2 x q), 25.3 (t), 42.6 (t), 68.3 (d), 107.5 (s), 110.8 (d), 118.0 (d), 123.9 (s), 126.3 (s), 128.5 (2 x d), 129.2 (2 x d), 133.8 (d), 138.7 (d), 140.8 (s), 145.8 (s), 149.7 (s), 157.5 (s), 160.0 (s).

*3-(benzenesulfonyl)-5,6-dihydropyrrolo[1,2-h][1,7]naphthyridin-2(1H)-one (**51a**)*. This compound was obtained by reaction of **43**. Yellow solid; yield: 64%; mp: 317-318°C; IR: 3119 (NH), 1641 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) (ppm): 2.95 (3H, t, J = 6.7 Hz, CH_2), 4.11 (2H, t, J = 6.7 Hz, CH_2) 6.22-6.25 (1H, m, Ar), 7.13-7.16 (2H, m, Ar), 7.54-7.71 (3H, m, H-Ar), 7.95-8.00 (2H, m, Ar), 8.20 (1H, s, H-4), 12.38 (1H, s, NH). ^{13}C NMR (DMSO-d_6) (ppm): 25.4 (t), 43.4 (t), 106.2 (s), 110.1 (d), 111.7 (d), 121.7 (s), 127.0 (d), 127.8 (2 x d), 128.7 (2 x d), 133.0 (d), 135.5 (s), 140.8 (s), 141.9 (s), 143.7 (d), 157.2 (s).

*Ethyl 3-(benzenesulfonyl)-2-oxo-1,5,6,7-tetrahydro-2H-pyrido[2,3-c]pyrrolo[1,2-a]azepine-9-carboxylate (**52a**)*. This compound was obtained by reaction of **40**. Yellow solid; yield: 51%, mp: 340-341°C; IR: 3131 (NH), 1703 (CO), 1652 (CO) cm^{-1}; ^{1}H nmr

(DMSO-d_6) (ppm): 1.29 (3H, t, J = 7.1 Hz, CH_3), 2.23-2.26 (2H, m, CH_2), 2.32-2.59 (2H, s, CH_2), 4.27 (2H, q, J = 7.1 Hz, CH_3), 4.38 (2H, t, J = 6.1 Hz, CH_2), 6.64 (1H, d, J = 4.2 Hz, Ar), 6.94 (1H, d, J = 4.2 Hz, Ar), 7.57-7.70 (3H, m, Ar), 8.00 (2H, d, J = 6.9 Hz, Ar), 8.33 (1H, s, H-4), 12.54 (1H, s, NH); ^{13}C nmr (DMSO-d_6) (ppm): 14.7 (q), 27.2 (t), 31.8 (t), 44.0 (t), 60.6 (t), 112.7 (d), 117.0 (d), 117.7 (s), 125.7 (s), 126.5 (s), 128.7 (2 x d), 129.3 (2 x d), 133.3 (s), 133.8 (d), 140.7 (s), 145.2 (s), 146.0 (d), 157.6 (s), 160.5 (s).

*Propan-2-yl 3-(benzenesulfonyl)-2-oxo-1,5,6,7-tetrahydro-2H-pyrido[2,3-c]pyrrolo[1,2-a]azepine-9-carboxylate (**53a**)*. This compound was obtained by reaction of **42**. White solid; yield: 54%, mp: 237-238°C; IR: 3131 (NH), 1697 (CO), 1646 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.30 (6H, d, J = 6.1 Hz, 2 x CH_3), 2.12-2.34 (2H, m, CH_2), 2.42-2.51 (2H, m, CH_2), 4.29-4.43 (2H, m, CH_2), 5.04-5.16 (1H, m, CH), 6.63 (1H, d, J = 4.1 Hz, Ar), 6.91 (1H, d, J = 4.1 Hz, Ar), 7.57-7.73 (3H, m, Ar), 8.01 (2H, d, J = 6.9 Hz, Ar), 8.33 (1H, s, H-4), 12.53 (1H, s, NH); ^{13}C nmr (DMSO-d_6) (ppm): 21.7 (2 x q), 26.7 (t), 31.3 (t), 43.6 (t), 67.6 (d), 102.8 (s), 112.1 (d), 116.4 (d), 121.4 (s), 125.6 (s), 128.2 (2 x d), 128.8 (2 x d), 133.4 (d), 137.0 (d), 140.1 (s), 145.5 (s), 155.7 (s), 157.2 (s), 159.7 (s).

General procedure for the synthesis of *O*-methyl derivatives (49-53b) and *N*-methyl derivatives (49-53c).

To a solution of the suitable derivatives of type **(49-53)a** (15 mmol) in anhydrous DMF (20 mL), NaH (0.64 g, 16 mmoli) was added at 0 °C and the reaction mixture was stirred at room temperature. After 6 h, iodomethane (16 mmol) was added at 0°C and the reaction mixture was stirred at room temperature for 24 h. Then the reaction mixture was poured onto crushed ice. The precipitate was filtered off and dried, in absence the solution was extracted with ethyl acetate (3 x 30 mL). The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product, containing *O*-methyl (**26**) and *N*-methyl substituted derivatives (**27**), was purified by chromatography column (DCM/AcOEt 95:5).

*Ethyl 3-(benzenesulfonyl)-2-methoxy-5,6-dihydropyrrolo[1,2-h][1,7]naphthyridine-8-carboxylate (**49b**)*. This compound was obtained by reaction of **49a**. Light yellow solid; yield: 25%, mp: 157-158°C; IR: 1697 (CO) cm^{-1}; ^{1}H nmr ($CDCl_3$) (ppm): 1.37 (3H, t, J

= 7.1 Hz, CH_3), 3.13 (2H, t, *J* = 6.9 Hz, CH_2), 3.96 (3H, s, CH_3), 4.32 (2H, q, *J* = 7.1 Hz, CH_2), 4.67 (2H, t, *J* = 6.9 Hz, CH_2), 6.88 (1H, d, *J* = 4.1 Hz, Ar), 7.01 (1H, d, *J* = 4.1 Hz, Ar), 7.46-7.64 (3H, m, 3H-Ar), 7.98-8.03 (2H, m, Ar), 8.23 (1H, s, H-4); ^{13}C nmr ($CDCl_3$) (ppm): 14.4 (q), 26.9 (t), 42.2 (t), 54.0 (q), 60.4 (t), 109.5 (d), 118.4 (d), 119.2 (s), 121.0 (s), 124.9 (s), 128.5 (2 x d), 128.7 (2 x d), 133.3 (d), 134.61 (s), 138.5 (d), 140.6 (s), 149.2 (s), 159.11 (s), 161.1 (s).

*Propan-2-yl 3-(benzenesulfonyl)-2-methoxy-5,6-dihydropyrrolo[1,2-h][1,7]naphthyridine-8-carboxylate (**50b**)*. This compound was obtained by reaction of **50a**. Yellow solid; yield: 24%, mp: 168-169 °C; IR: 1692 (CO) cm^{-1}; ^{1}H nmr ($CDCl_3$) (ppm): 1.35 (6H, d, *J* = 6.2 Hz, 2 x CH_3), 3.31 (2H, t, *J* = 6.9 Hz, CH_2), 3.97 (3H, s, CH_3), 4.67 (2H, t, *J* = 6.9 Hz, CH_2), 5.13-5.26 (1H, m, CH), 6.88 (1H, d, *J* = 4.1 Hz Ar), 6.99 (1H, d, *J* = 4.1 Hz, Ar), 7.47-7.63 (3H, m, Ar), 8.03 (2H, d, *J* = 6.6 Hz Ar), 8.22 (1H,s, H-4); ^{13}C nmr ($CDCl_3$) (ppm): 22.0 (2 x q), 26.9 (t), 42.2 (t), 54.0 (q), 67.9 (d), 109.4 (d), 118.2 (d), 119.1. (s), 120.9 (s), 125.4 (s), 128.5 (2 x d), 128.7 (2 x d), 133.3 (d), 134.5 (s), 138.5 (d), 140.7 (s), 149.2 (s), 159.1 (s), 160.7 (s).

*2-methoxy-3-(phenylsulfonyl)-5,6-dihydropyrrolo[1,2-h][1,7]naphthyridine (**51b**)*. This compound was obtained by reaction of **51a**. White solid; yield: 27%; mp: 210-211°C; ^{1}H NMR ($CDCl_3$) (ppm): 3.11 (2H, t, *J* = 6.6 Hz, CH_2), 3.95 (3H, s, CH_3), 4.12 (2H, t, *J* = 6.6 Hz, CH_2), 6.25-6.28 (1H, m, Ar), 6.81 (1H, s, Ar), 6.88-6.90 (1H, m, Ar), 7.44- 7.61 (3H, m, Ar), 7.97-8.01 (2H, m, Ar), 8.15 (1H, s, H-4); ^{13}C NMR ($CDCl_3$) (ppm): 27.4 (t), 43.9 (t), 53.9 (q), 108.2(s), 110.0 (d), 110.3 (d), 117.5 (s), 118.8 (s), 124.4 (d), 128.4 (2xd), 128.6 (2xd), 129.3 (s), 133.0 (d), 138.3 (d), 141.1 (s), 150.4 (s), 159.2 (s).

*Ethyl 3-(benzenesulfonyl)-2-methoxy-6,7-dihydro-5H-pyrido[2,3-c]pyrrolo[1,2-a]azepine-9-carboxylate (**52b**)*. This compound was obtained by reaction of **52a**. White solid; yield: 23%, mp: 157-158°C; IR: 1697 cm^{-1}; ^{1}H nmr ($CDCl_3$) (ppm): 1.38 (3H, t, *J* = 7.1 Hz, CH_3), 2.31-2.45 (2H, m, CH_2), 2.70 (2H, t, *J* = 7.0 Hz, CH_2), 3.96 (3H, s, CH_3), 4.27-4.45 (4H, m, 2 x CH_2), 6.62 (1H, d, *J* = 4.1 Hz, Ar), 7.01 (1H, d, *J* = 4.1 Hz, Ar), 7.49-7.65 (3H, m, Ar), 8.01-8.06 (2H, m, Ar), 8.27 (1H,s, H-4); ^{13}C nmr ($CDCl_3$) (ppm): 14.4 (q), 28.7 (t), 31.5 (t), 43.8 (t), 51.1 (q), 60.3 (t), 111.0 (d), 117.4 (d), 121.6 (s), 125.0

(s), 126.5 (s), 128.6 (2 x d), 128.7 (2 x d), 133.4 (d), 140.0 (s), 140.5 (s), 140.6 (d), 153.4 (s), 158.4 (s), 161.1 (s).

*Propan-2-yl 3-(benzenesulfonyl)-2-methoxy-6,7-dihydro-5H-pyrido[2,3-c]pyrrolo[1,2-a]azepine-9-carboxylate (**53b**)*. This compound was obtained by reaction of **53a**. White solid; yield: 28%, mp: 211-212°C; IR: 1696 (CO) cm^{-1}; ^{1}H nmr ($CDCl_3$) (ppm): 1.35 (6H, d, *J* = 6.2 Hz, 2 x CH_3), 2.35-2.44 (2H, m, CH_2), 2.64-2.73 (2H, m, CH_2), 3.95 (3H, s, CH3), 4.41 (2H, t, *J* = 6.5 Hz, CH_2), 5.16 (1H, m, CH), 6.62 (1H, d, *J* = 4.1 Hz, Ar), 7.00 (1H, d, *J* = 4.1 Hz, Ar), 7.49-7.65 (3H, m, Ar), 8.0 (2H, d, *J* = 6.8 Hz, Ar), 8.27 (1H,s, H-4); ^{13}C nmr ($CDCl_3$) (ppm): 22.1 (2 x q), 28.7 (t), 31.5 (t), 43.7 (t), 51.1 (q), 67.7 (d), 109.9 (d), 117.3 (d), 121.6 (s), 125.5 (s), 126.5 (s), 128.6 (2 x d), 128.7 (2 x d), 133.4 (d), 139.9 (s), 140.5 (d), 153.4 (s), 158.4 (s), 159.8 (s), 160.7 (s).

*Ethyl 3-(benzenesulfonyl)-1-methyl-2-oxo-1,2,5,6-tetrahydropyrrolo[1,2-h][1,7]naphthyridine-8-carboxylate (**49c**)*. This compound was obtained by reaction of **49a**. Yellow solid; yield: 53%, mp: 250-251°C; IR: 1709 (CO), 1658 (CO) cm^{-1}; ^{1}H nmr ($CDCl_3$) (ppm): 1.38 (3H, t, *J* = 7.1 Hz, CH_3), 2.93 (2H, t, *J* = 6.4 Hz, CH_2), 3.77 (3H, s, CH_3), 4.35 (2H, q, *J* = 7.1 Hz, CH_2), 4.68 (2H, t, *J* = 6.4 Hz, CH_2), 6.76 (1H, d, *J* = 4.4 Hz, Ar), 7.03 (1H, d, *J* = 4.4 Hz, Ar), 7.47-7.64 (3H, m, 3H-Ar), 8.12-8.18 (2H, m, 2H-Ar), 8.27 (1H, s, H-4); ^{13}C nmr ($CDCl_3$) (ppm): 14.4 (q), 27.7 (t), 35.2 (q), 41.8 (t), 60.9 (t), 110.7 (s), 113.9 (d), 117.3 (d), 125.4 (s), 125.5 (s), 127.1 (s), 128.6 (2 x d), 129.0 (2 x d), 133.3 (d), 139.9 (s), 141.5 (d), 143.1 (s), 157. 8 (s), 160.5 (s).

*Propan-2-yl 3-(benzenesulfonyl)-1-methyl-2-oxo-1,2,5,6-tetrahydropyrrolo[1,2-h][1,7]naphthyridine-8-carboxylate (**50c**).* This compound was obtained by reaction of **50a**. Yellow solid; yield: 45%, mp: 207-208°C; IR: 1703 (CO), 1658 (CO) cm^{-1}; ^{1}H nmr ($CDCl_3$) (ppm): 1.36 (6H, d, *J* = 6.2 Hz, 2 x CH_3), 2.92 (2H, t, *J* = 7.2 Hz, CH_2), 3.77 (3H, s, CH_3), 4.68 (2H, t, *J* = 7.2 Hz, CH_2), 5.18-5.24 (1H, m, CH), 6.75 (1H, d, *J* = 4.4 Hz, Ar), 7.01 (1H, d, *J* = 4.4 Hz, Ar), 7.47-7.63 (3H, m, Ar), 8.11-8.17 (2H, m, Ar), 8.27 (1H,s, H-4); ^{13}C nmr ($CDCl_3$) (ppm): 21.9 (2 x q), 27.7 (t), 35.1 (q), 41.8 (t), 68.5 (d), 110.7 (s), 113.8 (d), 117.2 (d), 125.4 (s), 125.8 (s), 126.9 (s), 128.6 (2 x d), 129.1 (2 x d), 133.3 (d), 139.9 (s), 141.5 (d), 143.2 (s), 157.8 (s), 160.1 (s).

*1-Methyl-3-(benzenesulfonyl)-5,6-dihydropyrrolo[1,2-h][1,7]naphthyridin-2(1H)-one (**51c**)*. This compound was obtained by reaction of **51a**. Yellow solid; yield: 47%, mp: 268-269°C; IR: 1658 (CO) cm^{-1}; ^{1}H nmr ($CDCl_3$) (ppm): 3.11 (2H, t, *J* = 6.7 Hz, CH_2), 3.95 (3H, s, CH_3), 4.14 (2H, t, *J* = 6.7 Hz, CH_2), 6.31-6.35 (1H, m, Ar), 6.81-6.91 (1H, m, Ar), 6.92-6.95 (1H, m, Ar), 7.46-7.58 (3H, m, Ar), 8.11-8.16 (2H, m, Ar), 8.21 (1H, s, H-4); ^{13}C nmr ($CDCl_3$) (ppm): 29.7 (t), 34.6 (q), 44.6 (t), 108.2 (s), 110.0 (d), 115.7 (d), 122.0 (s), 122.5 (s), 126.1 (d), 128.6 (2xd), 128.8 (2xd), 133.0 (d), 140.3 (s), 141.5 (d), 144.5 (s), 157.8 (s).

*Ethyl 3-(benzenesulfonyl)-1-methyl-2-oxo-1,5,6,7-tetrahydro-2H-pyrido[2,3-c]pyrrolo[1,2-a]azepine-9-carboxylate (**52c**)*. This compound was obtained by reaction of **52a**. Yellow solid; yield: 45%, mp: 238-239 °C; IR: 1697 (CO), 1658 (CO) cm^{-1}; ^{1}H nmr ($CDCl_3$) (ppm): 1.39 (3H, t, *J* = 7.1 Hz, CH_3), 2.05-2.19 (2H, m, CH_2), 2.37-2.62 (2H, m, CH_2), 3.60 (3H, s, CH_3), 4.34(2H, q, *J* = 7.1 Hz, CH_2), 5.35-5.45 (2H, m, CH_2), 6.37 (1H, d, *J* = 4.2 Hz, Ar), 7.04 (1H, d, *J* = 4.2 Hz, Ar), 7.49-7.65 (3H, m, Ar), 8.11-8.18 (2H, m, Ar), 8.28 (1H,s, H-4); ^{13}C nmr ($CDCl_3$) (ppm): 14.4 (q), 28.3 (t), 31.9 (t), 35.3 (q), 43.3 (t), 60.6 (t), 112.6 (d), 116.3 (s), 116.8 (d), 124.4 (s), 127.6 (s), 128.6 (2 x d), 129.1 (2 x d), 130.6 (s), 133.4 (d), 139.7 (s), 143.3 (d), 145.8 (s), 157.6 (s), 160.7 (s).

*Propan-2-yl 3-(benzenesulfonyl)-1-methyl-2-oxo-1,5,6,7-tetrahydro-2H-pyrido[2,3-c]pyrrolo[1,2-a]azepine-9-carboxylate (**53c**)*. This compound was obtained by reaction of **53a**. Yellow solid; yield: 41%, mp: 253-254°C; IR: 1697 (CO), 1658 (CO) cm^{-1}; ^{1}H nmr ($CDCl_3$) (ppm): 1.37 (6H, d, *J* = 6.2 Hz, 2 x CH_3), 2.08-2.61 (4H, m, 2 x CH_2), 3.60 (3H, s, CH_3), 5.15-5.44 (3H, m, CH_2 and CH), 6.35 (1H, d, *J* = 4.1 Hz, Ar), 7.02 (1H, d, *J* = 4.1 Hz, Ar), 7.54-7.62 (3H, m, Ar), 8.14-8.19 (2H, m, Ar), 8.29 (1H,s, H-4); ^{13}C nmr ($CDCl_3$) (ppm): 22.0 (2 x q), 28.4 (t), 31.8 (t), 35.3 (q), 43.3 (t), 68.2 (d), 112.5 (d), 116.3 (s), 117.8 (d), 124.9 (s), 127.7 (s), 128.6 (2 x d), 129.2 (2 x d), 130.5 (s), 133.4 (d), 139.7 (s), 143.3 (d), 145.9 (s), 157.6 (s), 160.2 (s).

General procedure for the synthesis of 9-bromo-pyrrolo[1,2-*h*][1,7]naphthyridine (49d,50d) and 10-bromo-pyrido[2,3-*c*]pyrrolo[1,2-a]azepine (52d,53d).

To a solution of suitable tricyclic compounds of type **(49-53)a** (0.22 mmol) in anhydrous DCM (20 ml), Br_2 (0.44 mmol, 0.02 mL) was added at 0°C and the reaction mixture was

stirred at room temperature for 24 h. Then the reaction mixture was evaporated under reduced pressure. The crude product was recrystallized from diethyl ether.

*Ethyl 3-(benzenesulfonyl)-9-bromo-2-oxo-1,2,5,6-tetrahydropyrrolo[1,2-h][1,7]naphthyridine-8-carboxylate (**49d**)*. This compound was obtained by reaction of **49a**. Yellow solid; yield: 63%; mp: 196-197°C; IR: 3342 (NH), 1700 (CO), 1669 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.37 (3H, t, J = 7.1 Hz, CH_3), 2.99 (2H, t, J = 7.0 Hz, CH_2), 4.33 (2H, q, J = 7.1 Hz, CH_2), 4.67 (2H, t, J = 7.0 Hz, CH_2), 7.03 (1H, s, Ar), 7.49-7.65 (3H, m, Ar), 8.09-8.17 (2H, m, Ar), 8.31 (1H, s, H-4), 10.02 (1H, s, NH); ^{13}C nmr (DMSO-d_6) (ppm): 14.3 (q), 23.8 (t), 42.8 (t), 61.3 (t), 97.5 (s), 108.2 (s), 120.8 (d), 122.3 (s), 126.2 (s), 127.9 (s), 128.7 (2 x d), 129.0 (2 x d), 133.6 (d), 138.8 (s), 139.6 (s), 143.8 (d), 156.0 (s), 159.6 (s).

*Propan-2-yl 3-(benzenesulfonyl)-9-bromo-2-oxo-1,2,5,6-tetrahydropyrrolo[1,2-h][1,7]naphthyridine-8-carboxylate (**50d**)*. This compound was obtained by reaction of **50a**. White solid; yield: 69%, mp: 174-175°C; IR: 3336 (NH), 1705 (CO), 1669 (CO) cm^{-1}; ^{1}H nmr ($CDCl_3$) (ppm): 1.40 (6H, d, J = 6.1 Hz, 2 x CH_3), 2.94-3.18 (2H, m, CH_2), 4.59-4.83 (2H, m, CH_2), 5.15-5.36 (1H, m, CH), 7.02 (1H, s, Ar), 7.45-7.69 (3H, m, Ar), 8.11-8.22 (2H, m, Ar), 8.37 (1H, s, H-4), 9.25 (1H, s, NH); ^{13}C nmr ($CDCl_3$) (ppm): 22.1 (2 x q), 26.3 (t), 43.7 (t), 69.2 (d), 98.0 (s), 102.7 (s), 109.3 (s), 120.9 (d), 122.4 (s), 124.9 (s), 126.8 (s), 129.1 (2 x d), 129.5 (2 x d), 133.7 (d), 144.6 (d), 156.3 (s), 158.7 (s), 159.1 (s).

*Ethyl 3-(benzenesulfonyl)-10-bromo-2-oxo-1,5,6,7-tetrahydro-2H-pyrido[2,3-c]pyrrolo[1,2-a]azepine-9-carboxylate (**52d**)*. This compound was obtained by reaction of **52a**. White solid; yield: 90%, mp: 172-173°C; IR: 3399 (NH), 1705 (CO), 1652 (CO) cm^{-1}; ^{1}H nmr ($CDCl_3$) (ppm): 1.33 (3H, t, J = 7.1 Hz, CH_3), 2.18-2.21 (2H, m, CH_2), 2.38-2.46 (2H, m, CH_2), 4.21-4.38 (4H, m, 2 x CH_2), 7.05 (1H, s, Ar), 7.58-7.75 (3H, m, Ar), 8.02 (2H, d, J = 6.9 Hz, Ar), 8.37 (1H, s, H-4), 12.48 (1H, s, NH); ^{13}C nmr ($CDCl_3$) (ppm): 14.0 (q), 26.0 (t), 31.4 (t), 46.7 (t), 61.1 (t), 98.0 (s), 106.7 (s), 119.2 (d), 123.0 (s), 124.6 (s), 128.3 (2 x d), 128.8 (2 x d), 134.0 (d), 140.3 (s), 140.4 (s), 147.9 (d), 157.3 (s), 159.1 (s), 159.7 (s).

*Propan-2-yl 3-(benzenesulfonyl)-10-bromo-2-oxo-1,5,6,7-tetrahydro-2H-pyrido[2,3-c]pyrrolo[1,2-a]azepine-9-carboxylate (**53d**)*. This compound was obtained by reaction of **53a**. White solid; yield: 84%, mp: 167-168°C; IR: 3422 (NH), 1702 (CO), 1646 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.28-1.35 (6H, m, 2 x CH_3), 2.18-2.41 (2H, m, CH_2), 2.44 (2H, s, CH_2), 4.14-4.40 (2H, m, CH_2), 5.03-5.15 (1H, m, CH), 7.00 (1H, s, H-Ar), 7.58-7.72 (3H, m, 3 x H-Ar), 8.01 (2H, d, 2H-Ar), 8.37 (1H, s, 1H-Ar), 12.11-12.81 (1H, m, NH); ^{13}C nmr (DMSO-d_6) (ppm): 22.6 (2 x q), 25.4 (t), 28.8 (t), 48.6 (t), 69.9 (d), 98.6 (s), 121.2 (d), 124.4 (s), 125.3 (s), 129.1 (2 x d), 129.5 (2 x d), 133.8 (d), 134.0 (s), 135.5 (s), 138.0 (s), 139.9 (s), 145.1 (d), 157.1 (s), 161.5 (s).

General procedure for the synthesis of 3-[2-(4-chlorophenyl)hydrazinyl]cyclohex-2-en-1-one (55) and 3-[2-(4-methoxyphenyl)hydrazinyl]cyclohex-2-en-1-one (56).

To a solution of cyclohexane-1,3-dione **54** (2.8 g, 25 mmol) in (100 mL) a solution of suitable hydrazine (25 mmol) in water (70 mL) was added at 0°C and the reaction mixture was stirred at rt up to completeness (TLC). From the reaction mixture a solid was separated. Then it was filtered off and dried.

*3-[2-(4-chlorophenyl)hydrazinyl]cyclohex-2-en-1-one (**55**)*. This compound was obtained by reaction with 4-chlorophenylhydrazine hydrochloride after 24 h. Pink solid; yield: 93%, mp: 148.149°C; IR: 1663 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.79-1.91 (2H, m, CH_2), 2.11 (2H, t, J= 6 Hz, CH_2), 2.38 (2H, t, J= 6 Hz, CH_2), 4.96 (1H, s, CH), 6.69 (2H, d, *J= 8* Hz, 2 x CH), 7.20 (2H, d, *J= 8* Hz, 2 x CH), 8.04 (1H, s, NH), 8.79 (1H, s, NH); ^{13}C nmr (DMSO-d_6) (ppm): 21.6 (t), 25.6 (t), 36.6 (t), 95.7 (d), 113.3 (2 x d), 121.9 (s), 128.7 (2 x d), 147.0 (s), 164.9 (s), 195.0 (s).

*3-[2-(4-methoxyphenyl)hydrazinyl]cyclohex-2-en-1-one (**56**).* This compound was obtained by reaction with 4-methoxyphenylhydrazine hydrochloride in the presence of equimolar Na_2CO_3 after 10 min. White solid; yield: 74%; mp: 176-177 °C; IR 3232 (NH), 3230 (NH), 1618 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6): 1.78-1.89 (2H, m, CH_2), 2.10 (2H, t, *J*=5.9 Hz, CH_2), 2.37 (2H, t, *J*=5.9 Hz, CH_2), 3.65 (3H, s, CH_3), 5.02 (1H, s, CH), 6.65 (2H, d, *J*=9.0 Hz, H-3' and H-5'), 6.79 (2H, d, *J*=9.0 Hz, H-2' and H-6'), 7.54 (1H, s, NH), 8.72 (1H, s, NH); ^{13}C nmr (DMSO-d_6): 21.7 (t), 25.8 (t), 36.7 (t), 55.2 (q), 95.5 (d), 113.3 (2 x d), 114.4 (2 x d), 141.8 (s), 152.7 (s), 165.2 (s), 194.8 (s).

General procedure for the synthesis of 2-substituted-3-(4-(dimethylamino)phenyl)-6,7-dihydro-2*H*-indazol-4(5*H*)-one (57a,c) and 2-substituted-3-(4-(diethylamino)phenyl)-6,7-dihydro-2*H*-indazol-4(5*H*)-one (57b,d).

To a solution of the suitable intermediates **55,56** (2.11 mmol) in anhydrous DMF (5 mL), AcOH (0.01 mL), piperidine (0.42 mL) and 4-dimethylaminobenzaldehyde or 4-diethylaminobenzaldehyde (2.11 mmol) were added and the reaction mixture was stirred at 40-90^{o}C up to completeness (TLC). After cooling, the mixture was poured onto crushed ice, the solid was filtrated off and recrystallized from diethyl ether.

*2-(4-chlorophenyl)-3-(4-(dimethylamino)phenyl)-6,7-dihydro-2H-indazol-4(5H)-one **(57a)***. This compound was obtained by reaction of **55** with 4-dimethylaminobenzaldehyde at 40°C after 8 h. White solid; yield: 92%, mp: 201-202°C; IR: 1667 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 2.01-2.13 (2H, m, CH_2), 2.42-2.51 (2H, m, CH_2), 2.83-2.93 (8H, m, CH_2 and 2 x CH_3), 6.63 (2H, d, J = 8.6 Hz, Ar), 7.05 (2H, d, J = 8.6 Hz, Ar), 7.25 (2H, d, J = 8.6 Hz, Ar), 7.47 (2H, d, J = 8.6 Hz, Ar); ^{13}C nmr (DMSO-d_6) (ppm): 22.8 (2 x t), 39.5 (t), 39.6 (2 x q), 110.9 (2 x d), 114.1 (s), 115.7 (s), 127.3 (2 x d), 129.0 (2 x d), 131.1 (2 x d), 132.2 (s), 138.2 (s), 143.8 (s), 150.4 (s), 156.8 (s), 192.9 (s).

*2-(4-chlorophenyl)-3-(4-(diethylamino)phenyl)-6,7-dihydro-2H-indazol-4(5H)-one **(57b)***. This compound was obtained by reaction of **55** with 4-diethylaminobenzaldehyde at 40°C after 6 h. Yellow solid; yield: 64%, mp: 148-149°C; IR: 1667 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.08 (6H, t, J = 6.8 Hz, 2 x CH_3), 1.99-2.13 (2H, m, CH_2), 2.45 (2H, t, J = 5.4 Hz, CH_2), 2.84 (2H, t, J = 5.4 Hz, CH_2), 3.29-3.36 (4H, m, 2 x CH_2), 6.58 (2H, d, J = 8.6 Hz, Ar), 7.08 (2H, d, J = 8.6 Hz, Ar), 7.26 (2H, d, J = 8.6Hz, Ar), 7.48 (2H, d, J = 8.6 Hz, Ar); ^{13}C nmr (DMSO-d_6) (ppm): 12.4 (2 x q), 22.9 (t), 39.5 (t), 43.4 (2 x t), 110.0 (2 x d), 113.1 (s), 115.6 (s), 127.2 (2 x d), 129.0 (2 x d), 131.5 (2 x d), 132.2 (s), 138.3 (s), 143.9 (s), 147.8 (s), 156.8 (s), 192.8 (s).

*3-(4-(dimethylamino)phenyl)-2-(4-methoxyphenyl)-6,7-dihydro-2H-indazol-4(5H)-one **(57c)***. This compound was obtained by reaction of **56** with 4-dimethylaminobenzaldehyde at 90°C after 1 h. White solid; yield: 60%; mp: 181-182°C; IR: 1663 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 2.02-2.13 (2H, m, CH_2), 2.44 (2H, t, J =

5.9 Hz, CH_2), 2.83 (2H, t, *J* = 5.9 Hz, CH_2), (6H, s, 2 x CH_3), 3.76 (3H, s, CH_3), 6.61 (2H, d, *J* = 8.9 Hz, Ar), 6.91 (2H, d, *J* = 9.0 Hz, Ar), 7.07-7.17 (4H, m, Ar); ^{13}C nmr (DMSO-d_6) (ppm): 22.8 (t), 22.9 (t), 39.5 (t), 39.6 (2 x q), 55.3 (q), 110.8 (2 x d), 114.0 (2 x d), 114.6 (s), 115.3 (s), 127.0 (2 x d), 131.1 (2 x d), 132.4 (s), 143.6 (s), 150.2 (s), 156.2 (s), 158.5 (s), 192.8 (s).

3-(4-(diethylamino)phenyl)-2-(4-methoxyphenyl)-6,7-dihydro-2H-indazol-4(5H)-one ***(57d)***. This compound was obtained by reaction of **56** with 4-dimethylaminobenzaldehyde at 90°C after 3 h. White solid; yield: 90%; mp: 118-119°C; IR: 1669 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.06 (6H, t, *J* = 6.9 Hz, 2 x CH_3), 2.04-2.13 (2H, m, CH_2), 2.43 (2H, t, *J* = 5.7 Hz, CH_2), 2.82 (2H, t, *J* = 5.7 Hz, CH_2), 3.32 (4H, q, *J* = 6.9 Hz, 2 x CH_2), 3.75 (3H, s, CH_3), 6.55 (2H, d, *J* = 8.5 Hz, Ar), 6.93 (2H, d, *J* = 8.6 Hz, Ar), 7.06 (2H, d, *J* = 8.5 Hz, Ar), 7.15 (2H, d, *J* = 8.6 Hz, Ar); ^{13}C nmr (DMSO-d_6) (ppm): 12.9 (2 x q), 23.3 (t), 23.5 (t), 39.6 (t), 44.0 (2 x t), 55.8 (q), 110.5 (2 x d), 114.5 (2 x d), 114.2 (s), 115.6 (s), 127.5 (2 x d), 131.9 (2 x d), 132.9 (s), 144.2 (s), 148.0 (s), 156.8 (s), 159.0 (s), 193.4 (s).

General procedure for the synthesis of 3-substituted-2-(4-chlorophenyl)-5-((dimethylamino)methylene)-6,7-dihydro-2*H*-indazol-4(5*H*)-one (58a,b).

To a solution of ketones **57a,b** (0.1 g, 0.27 mmol) in anhydrous DMF (2mL), DMFDMA (0.36 mL, 2.7 mmol) was added and the mixture was stirred under microwave irradiation (Power 150 W; Time 20 min; Pressure 100 psi; Temperature (max) 150^{o}C). Then the reaction mixture was poured onto crushed ice and the precipitate was filtered off and dried.

2-(4-chlorophenyl)-5-((dimethylamino)methylene)-3-(4-(dimethylamino)phenyl)-6,7-dihydro-2H-indazol-4(5H)-one ***(58a)***. This compound was obtained by reaction of **57a**. Brown solid; yield: 96%, mp: 205-206°C; IR: 1644 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 2.71-2.77 (2H, m, CH_2), 2.91 (8H, s, CH_2 and 2 x CH_3),3.07 (6H, s, 2 x CH_3), 6.61 (2H, d, *J* = 8.6 Hz, Ar), 7.12 (2H, d, *J* = 8.6 Hz, Ar), 7.22 (2H, d, *J* = 8.6 Hz, Ar), 7.33 (1H, s, CH), 7.44 (2H, d, *J* = 8.6 Hz, Ar); ^{13}C nmr (DMSO-d_6) (ppm): 22.8 (t), 23.4 (t), 39.9 (2 x q), 43.2 (2 x q), 102.9 (s), 110.8 (2 x d), 115.3 (s), 116.8 (s), 127.0 (2 x d),

128.8 (2 x d), 131.4 (2 x d), 131.6 (s), 138.7 (s), 143.1 (s), 148.2 (d), 150.1 (s), 155.0 (s), 181.8 (s).

*2-(4-chlorophenyl)-3-(4-(diethylamino)phenyl)-5-((dimethylamino)methylene)-6,7-dihydro-2H-indazol-4(5H)-one (**58b**)*. This compound was obtained by reaction of **57b**. Green solid; yield: 87%; mp: 148-149°C; IR: 1643 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.08 (6H, t, *J* = 6.7 Hz, 2 x CH_3), 2.73-2.77 (2H, m, CH_2), 2.92 (2H, t, *J* = 6.8 Hz, CH_2), 3.07 (6H, s, 2 x CH_3), 3.28-3.38 (4H, m, 2 x CH_2), 6.55 (2H, d, *J* = 8.6 Hz, Ar), 7.09 (2H, d, *J* = 8.6 Hz, Ar), 7.23 (2H, d, *J* = 8.6 Hz, Ar), 7.33 (1H, s, CH), 7.44 (2H, d, *J* = 8.6 Hz, Ar); ^{13}C nmr (DMSO-d_6) (ppm): 12.4 (2 x q), 22.8 (t), 23.4 (t), 43.2 (2 x q), 43.5 (2 x t), 102.9 (s), 109.9 (2 x d), 114.0 (s), 116.8 (s), 127.0 (2 x d), 128.8 (2 x d), 131.6 (s), 131.7 (2 x d), 138.8 (s), 143.2 (s), 147.5 (s), 148.2 (d), 155.0 (s), 181.6 (s).

General procedure for the synthesis of 3-substituted-5-((dimethylamino)methylene)-2-(4-methoxyphenyl)-6,7-dihydro-2*H*-indazol-4(5*H*)-one (58c,d).

To a solution of ketones **57c,d** (2 mmol) in anhydrous DMF (2.6 mL) was added DMFDMA (2.64 mL, 20 mmol). The reaction mixture was stirred at reflux up to completeness (TLC). The reaction mixtures was poured onto crushed ice. The precipitate was filtered off and dried, in absence the solution was extracted with ethyl acetate (3 x 30 mL). The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure.

*(3-(4-(dimethylamino)phenyl)-5-((dimethylamino)methylene)-2-(4-methoxyphenyl)-6,7-dihydro-2H-indazol-4(5H)-one (**58c**)*. This compound was obtained by reaction of **57c** after 16 h and used in the next step without further purification.

*(3-(4-(diethylamino)phenyl)-5-((dimethylamino)methylene)-2-(4-methoxyphenyl)-6,7-dihydro-2H-indazol-4(5H)-one (**58d**)*. This compound was obtained by reaction of **57d** after 24 h. Yellow solid; yield: 70%; mp: 201-202°C; IR: 1651 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.07 (6H, t, *J* = 6.9 Hz, 2 x CH_3), 2.72 (2H, t, *J* = 6.3 Hz, CH_2), 2.96 (2H, t, *J* = 6.3 Hz, CH_2), 3.06 (6H, s, 2 x CH_3), 3.27-3.35 (4H, m, 2 x CH_2), 3.75 (3H, s, CH_3), 6.51 (2H, d, *J* = 9.0 Hz, Ar), 6.91 (2H, d, *J* = 9.0 Hz, Ar), 7.05-7.16 (4H, m, Ar), 7.31 (1H, s, CH). ^{13}C nmr (DMSO-d_6) (ppm): 12.4 (2 x q), 22.8 (t), 23.5 (t), 43.2 (2 x q),

43.4 (2 x t), 55.3 (q), 103.0 (s), 109.8 (2 x d), 113.9 (2 x d), 114.6 (s), 116.2 (s), 126.9 (2 x d), 131.6 (2 x d), 132.9 (s), 143.0 (s), 147.2 (s), 147.9 (d), 154.3 (s), 158.1 (s) 181.7 (s).

General procedure for the synthesis 3-(phenylsulfonyl)-5,6-dihydro-1*H*-pyrazolo[3,4-*h*]quinolin-2(8*H*)-one (59a-d).

To a solution of the suitable enaminoketones **58a-d** (2 mmol), in anhydrous ethanol (40 mL) under N_2 atmosphere, phenylsulfonylacetonitrile (3 mmol) was added. The reaction mixture was heated at reflux for 48 h. The solvent was removed under reduced pressure, and the residue was dissolved in anhydrous toluene (40 mL). Acetic acid (2 mmol) was added and the reaction mixture was heated at reflux with the Dean-Stark apparatus for 24 h. The solvent was removed under reduced pressure and the residue was recrystallized from the suitable solvent or purified by chromatography column (DCM/AcOEt 8:2).

*8-(4-chlorophenyl)-9-(4-(dimethylamino)phenyl)-3-(phenylsulfonyl)-5,6-dihydro-1H-pyrazolo[3,4-h]quinolin-2(8H)-one (**59a**)*. This compound was obtained by reaction of **58a** and recrystallized from toluene. Yellow solid; yield: 72%, mp: 272-273°C; IR: 3340 (NH), 1663 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 2.82-2.94 (10H, m, 2 x CH_3 and 2 x CH_2), 6.73 (2H, d, *J*=8.5 Hz, Ar), 7.11-7.25 (4H, m, Ar), 7.38-7.73 (5H, m, Ar), 7.95 (2H, d, *J* = 7.2 Hz, Ar), 8.24 (1H, s, H-4), 10.04 (1H, s, NH); ^{13}C nmr (DMSO-d_6) (ppm): 21.2 (t), 25.7 (t), 39.6 (2 x q), 112.0 (2 x d), 113.4 (s), 123.0 (s), 125.4 (s), 126.8 (2 x d), 128.0 (2 x d), 128.7 (2 x d), 129.0 (2 x d), 130.5 (2 x d), 132.0 (s), 133.2 (d), 135.6 (s), 137.9 (s), 138.0 (s), 140.5 (s), 141.0 (s), 142.8 (d), 150.7 (s), 156.4 (s), 162.3 (s).

*8-(4-chlorophenyl)-9-(4-(diethylamino)phenyl)-3-(phenylsulfonyl)-5,6-dihydro-1H-pyrazolo[3,4-h]quinolin-2(8H)-one (**59b**)*. This compound was obtained by reaction of **58b** and recrystallized from diethyl ether. Yellow solid; yield: 61%, mp: 255-256°C; IR: 3347 (NH), 1668 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.08 (6H, t, *J* = 6.6 Hz, 2 x CH_3), 2.83-2.99 (2H, m, CH_2), 2.35-2.36 (2H, m, CH_2), 3.30-3.40 (4H, m, 2 x CH_2), 6.68 (2H, d, *J* = 8.6 Hz, Ar), 7.09 (2H, d, *J* = 8.6 Hz, Ar), 7.24 (2H, d, *J* = 8.6Hz, Ar), 7.44-7.66 (5H, m, Ar), 7.94 (2H, d, *J* = 7.3 Hz, Ar), 8.27 (1H, s, H-4), 11.50 (1H, s, NH); ^{13}C nmr (DMSO-d_6) (ppm): 12.3 (2 x q), 21.1 (t), 25.7 (t), 43.5 (2 x t), 111.3 (2 x d), 122.0 (s), 125.3 (s), 126.8 (2 x d), 128.0 (2 x d), 128.7 (2 x d), 128.9 (s), 129.0 (2 x d), 130.6 (s),

130.7 (2 x d), 132.1 (s), 133.2 (d), 140.5 (s), 141.2 (s), 143.3 (d), 144.2 (s), 147.3 (s), 148.2 (s), 152.8 (s), 161.9 (s).

*9-(4-(dimethylamino)phenyl)-8-(4-methoxyphenyl)-3-(phenylsulfonyl)-5,6-dihydro-1H-pyrazolo[3,4-h]quinolin-2(8H)-one (**59c**)*. This compound was obtained by reaction of **58c** and purified by chromatography column. White solid; yield: 60%; mp: 282-283°C; IR: 3327 (NH), 1665 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 2.86-2.94 (10H, m, 2 x CH_3 and 2 x CH_2), 3.75 (3H, s, CH_3), 6.71 (2H, d, *J* = 8.8 Hz, Ar), 6.92 (2H, d, *J* = 9.0 Hz, Ar), 7.08-7.17 (4H, m, Ar), 7.53-7.70 (3H, m, Ar), 7.94 (2H, d, *J* = 6.9 Hz, Ar), 8.26 (1H, s, H-4), 9.56 (1H, s, NH); ^{13}C nmr (DMSO-d_6) (ppm): 21.1 (t), 25.6 (t), 39.6 (2 x q), 55.3 (q), 108.5 (s), 111.9 (2 x d), 113.8 (s), 114.0 (2 x d), 122.4 (s), 126.7 (2 x d), 128.0 (2 x d), 128.7 (2 x d), 130.4 (2 x d), 132.1 (s), 133.2 (d), 137.7 (s), 138.5 (s), 140.5 (s), 140.9 (s), 142.6 (s), 146.7 (d), 150.6 (s), 152.0 (s), 158.4 (s).

*9-(4-(diethylamino)phenyl)-8-(4-methoxyphenyl)-3-(phenylsulfonyl)-5,6-dihydro-1H-pyrazolo[3,4-h]quinolin-2(8H)-one (**59d**)*. This compound was obtained by reaction of **58d** and purified by chromatography column. White solid; yield: 65%; mp: 225-226°C; IR: 3396 (NH), 1664 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) (ppm): 1.08 (6H, t, *J* = 6.8 Hz, 2 x CH_3), 2.80-3.01 (4H, m, 2 x CH_2), 3.29-3.41 (4H, m, 2 x CH_2), 3.75 (3H, s, CH_3), 6.66 (2H, d, *J* = 8.6 Hz, Ar), 6.92 (2H, d, *J* = 8.9 Hz, Ar), 7.05-7.18 (4H, m, Ar), 7.52-7.69 (3H, m, Ar), 7.95 (2H, d, *J* = 6.9 Hz, Ar), 8.26 (1H, s, H-4), 9.56 (1H, s, NH); ^{13}C nmr (DMSO-d_6) (ppm): 12.3 (2 x q), 21.2 (t), 25.6 (t), 43.4 (2 x t), 55.3 (q), 106.6 (s), 111.1 (2 x d), 112.7 (s), 114.0 (2 x d), 125.1 (s), 126.8 (2 x d), 128.0 (2 x d), 128.7 (2 x d), 130.6 (2 x d), 132.1 (s), 133.2 (d), 140.5 (s), 141.0 (s), 142.9 (d), 144.7 (s), 148.1 (s), 152.2 (s), 155.9 (s), 158.5 (s), 159.2 (s).

General procedure for the synthesis of 2-methoxy-3-(phenylsulfonyl)-6,8-dihydro-5*H*-pyrazolo[3,4-*h*]quinolin-9-yl)-*N*,*N*-disubstitutedaniline (60a-d).

To a solution of **59a-d** (0.36 mmol) in anhydrous DMF (20 mL), NaH (0.011 g, 0.47 mmol) was added at 0ºC and the reaction mixture was stirred at rt for 2 h. Then iodomethane (0.03 mL, 0.39 mmol) was added at 0ºC and the reaction mixture was stirred at rt for 24 h. The reaction mixture was poured onto crushed ice and the precipitate was filtered off, dried and purified by chromatography column (Etp/AcOEt 95:5).

*4-(8-(4-chlorophenyl)-2-methoxy-3-(phenylsulfonyl)-6,8-dihydro-5H-pyrazolo[3,4-h]quinolin-9-yl)-N,N-dimethylaniline (**60a**)*. This compound was obtained by reaction of **59a**. Yellow solid; yield: 60%; mp: 218-219°C; ^{1}H nmr ($CDCl_3$) (ppm): 2.94-3.10 (10H, m, 2 x CH_2 and CH_3), 3.52 (3H, s, CH_3), 6.55-6.61 (2H, m, Ar), 7.16-7.28 (6H, m, Ar), 7.42-7.54 (3H, m, Ar), 7.94-8.00 (2H, m, Ar), 8.17 (1H, s, H-4); ^{13}C nmr ($CDCl_3$) (ppm): 21.9 (t), 27.9 (t), 40.3 (2 x q), 54.2 (q), 111.2 (2 x d), 115.8 (s), 116.3 (s), 118.9 (s), 122.9 (s), 126.2 (2 x d), 128.4 (2 x d), 128.5 (2 x d), 128.9 (2 x d), 131.7 (2 x d), 132.7 (s), 133.0 (d), 138.1 (d), 138.5 (s), 141.0 (s), 141.9 (s), 150.4 (s), 153.5 (s), 153.7 (s), 158.5 (s).

*4-(8-(4-chlorophenyl)-2-methoxy-3-(phenylsulfonyl)-6,8-dihydro-5H-pyrazolo[3,4-h]quinolin-9-yl)-N,N-diethylaniline (**60b**)*. This compound was obtained by reaction of **59b**. Yellow solid; yield: 56%; mp: 196-197°C; ^{1}H nmr (DMSO-d_6) (ppm): 1.00-1.12 (6H, m, 2 x CH_3), 2.90 (2H, t, J = 7.0 Hz, CH_2), 3.10 (2H, t, J = 7.0 Hz, CH_2), 3.25-3.40 (4H, m, 2 x CH_2), 3.47 (3H, s, CH_3), 6.58 (2H, d, J = 8.8 Hz, Ar), 7.13 (2H, d, J = 8.7 Hz, Ar), 7.28 (2H, d, J = 8.8 Hz, Ar), 7.44 (2H, d, J = 8.7 Hz, Ar), 7.55-7.72 (3H, m, Ar), 7.90 (2H, d, J = 6.8 Hz, Ar), 8.20 (1H, s, H-4); ^{13}C nmr (DMSO-d_6) (ppm): 12.2 (2 x q), 21.1 (t), 26.8 (t), 43.5 (2 x t), 53.8 (q), 110.4 (2 x d), 114.4 (s), 115.1 (s), 118.3 (s), 123.2 (s), 126.5 (2 x d), 127.8 (2 x d), 128.8 (2 x d), 129.0 (2 x d), 131.5 (s), 131.7 (2 x d), 133.5 (d), 138.0 (d), 138.5 (s), 140.5 (s), 141.8 (s), 147.3 (s), 152.9 (s), 153.1 (s), 157.6 (s).

*4-(8-(4-methoxyphenyl)-2-methoxy-3-(phenylsulfonyl)-6,8-dihydro-5H-pyrazolo[3,4-h]quinolin-9-yl)-N,N-dimethylaniline (**60c**)*. This compound was obtained by reaction of **59c**. Yellow solid; yield: 58%; mp: 197-198°C; ^{1}H nmr ($CDCl_3$) (ppm): 2.84-2.99 (13H, m, 2 x CH_2 and 3 x CH_3), 3.82 (3H, s, CH_3), 6.57 (2H, d, J = 8.6 Hz, Ar), 6.85 (4H, d, J = 8.6 Hz, Ar), 7.15 (2H, d, J = 8.7 Hz, Ar), 7.45-7.59 (3H, m, Ar), 8.10-8.19 (2H, m, Ar), 8.33 (1H, s, H-4); ^{13}C nmr ($CDCl_3$) (ppm): 29.0 (t), 29.7(t), 37.1 (q), 40.0 (2 x q), 55.5 (q), 103.7 (s), 112.1 (2 x d), 114.1 (2 x d), 113.7 (s), 116.7 (s), 118.4 (s), 122.5 (s), 127.1 (2 x d), 127.8 (s), 128.5 (2 x d), 129.0 (2 x d), 130.1 (2 x d), 132.8(d), 140.7 (s), 141.9 (d), 149.0 (s), 150.9 (s), 158.4 (s), 159.6 (s), 162.6 (s).

*4-(8-(4-methoxyphenyl)-2-methoxy-3-(phenylsulfonyl)-6,8-dihydro-5H-pyrazolo[3,4-h]quinolin-9-yl)-N,N-diethylaniline (**60d**)*. This compound was obtained by reaction of **59d**. Light yellow solid; yield: 55%; mp: 238-239°C; ^{1}H nmr (DMSO-d_6) (ppm): 1.01

(6H, t, J = 6.8 Hz, 2 x CH_3), 2.88 (2H, t, J = 7.1 Hz, CH_2), 3.09 (2H, t, J = 7.0 Hz, CH_2), 3.23-3.37 (4H, m, 2 x CH_2), 3.46 (3H, s, CH_3), 3.74 (3H, s, CH_3), 6.55 (2H, d, J = 8.5 Hz, Ar), 6.90 (2H, d, J = 8.9 Hz, Ar), 7.08-7.21 (4H, m, Ar), 7.55-7.72 (3H, m, Ar), 7.90 (2H, d, J = 6.9 Hz, Ar), 8.18 (1H, s, H-4); ^{13}C nmr (DMSO-d_6) (ppm): 12.3 (2 x q), 21.1 (t), 26.9 (t), 43.5 (2 x t), 53.7 (q), 55.3 (q), 110.3 (2 x d), 115.1 (s), 115.8 (s), 118.4 (s), 122.7 (s), 113.9 (2 x d), 126.6 (2 x d), 127.8 (2 x d), 129.1 (2 x d), 131.7 (2 x d), 133.2 (s), 133.5 (d), 137.9 (d), 139.0 (s), 141.2 (s), 142.0 (s), 147.5 (s), 152.9 (s), 154.1 (s), 158.5 (s).

Synthesis of 2,6-Dichloropuine (67).

To a solution of $ZnCl_2$ (7.0g, 110 mmol) in conc. hydrochloric acid (9.2 mL) finely powdered compound **66** (2.3g, 13.6 mmol) was added at 10°C. The reaction mixture was cooled at -5°C and $NaNO_2$ was added (1.27g, 18.4 mmol) over a period of 30 min keeping the temperature below 5°C. The reaction mixture was stirred for additional 30 min and then diluted with 12 mL of water. The aqueous solution was extracted with ethyl acetate (3 x 30 mL) and the organic layers were dried on Na_2SO_4 and the solvent was removed under reduced pressure. The spectroscopic data are in agreement with the literature [51].

Synthesis of 2-formylbutanenitrile (73).

To solution of potassium *tert*-butoxide (11.29 g, 100.6 mmol) in THF (76 mL), butyronitrile (4.00 mL, 45.7 mmol) and ethyl formate (3.86 mL, 48.0 mmol) in THF (7.60 mL) was added dropwise at room temperature. Then the reaction mixture was stirred at room temperature for 16 h. The crude reaction mixture was concentrated under reduced pressure and the residual solid was suspended in Et_2O, filtered, and the solid was washed with Et_2O (50 mL). Then the solid was dissolved in water and acidified to pH 4 with 2 M HCl and extracted with DCM (3 x 30 mL). The organic layers were dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude material was used in the next step without further purification (Yield: 35%).

Synthesis of 4-ethyl-1H-pyrazol-5-amine (74).

To a solution compound **73** (12.93 g, 133 mmol) in EtOH (26.3 mL) hydrazine hydrate was added (2.03 mL, 41.9 mmol) and the reaction mixture was heated at 80 °C for 3 h. After cooling, the solvent was removed under reduced pressure and the crude product

was purified by chromatography column (DCM/MeOH 98:2).Yield:30%.The spectroscopic data are in agreement with the literature [49].

Synthesis of 3-ethylpyrazolo[1,5-*a*]pyrimidine-5,7-diol (75).

To a solution of Na (0.170 g, 7.38 mmol) in anhydrous EtOH (6.0 mL), a solution of compound **74** (0.41 g, 3.69 mmol) in EtOH (6.0 mL) was added dropwise at 0 °C. After stirring at 0 °C for 10 min, diethyl malonate (0.619 mL, 4.06 mmol) was added. The reaction mixture was heated at 85 °C for 4 h. The reaction mixture was cooled to room temperature and the formed solid was filtered off and washed with Et_2O. The solid was dissolved in water and the pH was adjusted to 1 with 6 M HCl. The solid was filtered off, dried and used in the next step without further purification. Yield:60%.

Synthesis of 5,7-dichloro-3-ethylpyrazolo[1,5-*a*]pyrimidine (76).

A solution of compound **75** (7.4 g, 41 mmol) and dimethylaniline (20 mL) in $POCl_3$ (100 mL) heated at reflux for 24 h. After cooling, the reaction mixture was poured onto crushed ice. The aqueous solution was extracted with ethyl acetate (2 x 500 mL). The organic layer was washed with H_2O, dried on Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by chromatography column (Cyclohexane/EtOAc 95:5), yield 90% [52].

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