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Cycloheptapyrrolo-Fused Systems of Pharmaceutical Interest

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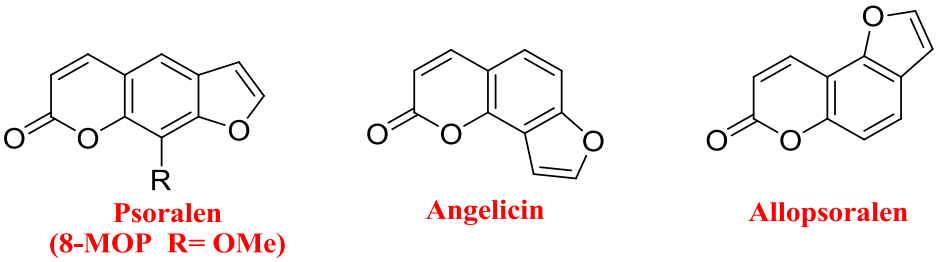
INDEX

1. INTRODUCTION

Furocoumarins are a class of planar tricyclic aromatic compounds produced by a wide variety of plants, whose chemical structures originate from the condensation of a coumarin nucleus with a furan ring. Depending on the position in which the condensation occurs, different isomers can originate, including linear and angular furocoumarins. Leads of these two main structures are respectively, psoralen and angelicin (Figure 1). [1]

Such molecules have attracted great attention for their photobiological and phototherapeutic activity. Since ancient times, in fact, these properties have been recognized by the Turks, Hindu and Egyptian, and have been used for the treatment of vitiligo (skin disease of autoimmune origin). Nowadays, they are object of study in the treatment of skin diseases (psoriasis, mycosis fungoides, vitiligo), T-cell lymphoma and autoimmune diseases (lupus erythematosus).

Figure 1



Psoralen (8-MOP R= OMe) **Angelicin** **Allopsoralen**

Psoralen and angelicin, due to their planar and aromatic structures, can intercalate in the "dark" between DNA bases and can be photoactivated after irradiation by UV-A light; this molecules are in fact classified as photosensitizing agents, because, if activated by light of a suitable wavelength, give a biological photodynamic effect. The photodynamic effect induces destruction of cell membranes, or the block of the replication and transcription of nucleic acids, and in both cases, leads to cell death. [2]

The photodynamic therapy (PDT) represents an interesting therapeutic option for the treatment of several cancers, including carcinomas of the esophagus and lung. It requires the systemic administration of a photosensitizing agent (PS), followed by irradiation of the tumor with visible light of a wavelength compatible with the absorption spectrum of the PS.

The interest for PDT in oncology is due to the tendency of the PS to accumulate in neoplastic tissues, where they are selectively activated by illuminating only the area of interest, that means an high therapeutic index. Furthermore, the use of PDT is not precluded by previous radio or chemotherapy treatments, and do not appear to be present forms of cross-resistance with these treatment modalities.

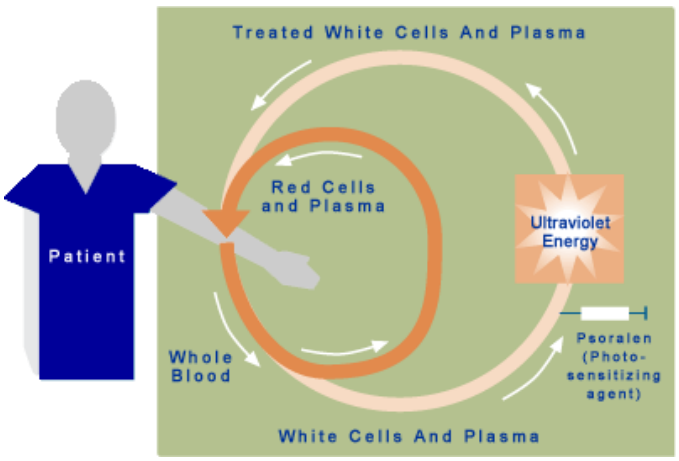
The use of psoralens in the PDT in combination with the UV-A light, was introduced by Parrish and collaborators in clinical practice under the name of PUVA (psoralen plus UV-A). [3] This type of treatment, consists in the oral administration of 8-methoxypsoralen (8-MOP) followed by irradiation of the patient with artificial ultraviolet light (UV-A), and has been used for the first time in the treatment of psoriasis, a disease characterized by hyperproliferation of skin cells. Very interesting results in the field of PUVA therapy, have been achieved with the introduction of a new clinical treatment: the extracorporeal photopheresis (ECP), a variant of the leukapheresis.

At the end of the 80's Edelson proposed it for the treatment of cutaneous T cell lymphoma (CTCL) and in 1988 it was approved by the U.S. Food and Drug Administration. [4] Currently, the ECP is also used in the treatment of autoimmune diseases (systemic sclerosis and rheumatoid arthritis) as well as in the prevention of rejection in transplantation (GVHD, Graft Versus Host Disease). [5]

The treatment is carried out in three successive stages (Figure 2):

1. Oral administration of the photoactivatable drug, psoralen or 8-methoxypsoralen, by the patient.
2. Formation of an extracorporeal blood circuit, in which the white blood cells are separated from the red blood cells. For both components are added heparin and saline. At this point is generally added other 8-methoxypsoralen, and the leukocyte portion is subjected to irradiation with UV-A light.
3. After treatment, the blood, containing the photoactivated drug, is reinfused to the patient.

Figure 2



The duration of the treatment is about 4 hours and is repeated for 2 consecutive days every 3-4 weeks. The advantages of ECP are numerous, in fact the patient is not directly exposed to UV-A irradiation, as in the classic PUVA therapy, and suffers fewer side effects. Patients subjected to ECP in fact, suffer from a mild fever in a time interval between 2-12 hours after the end of the treatment,

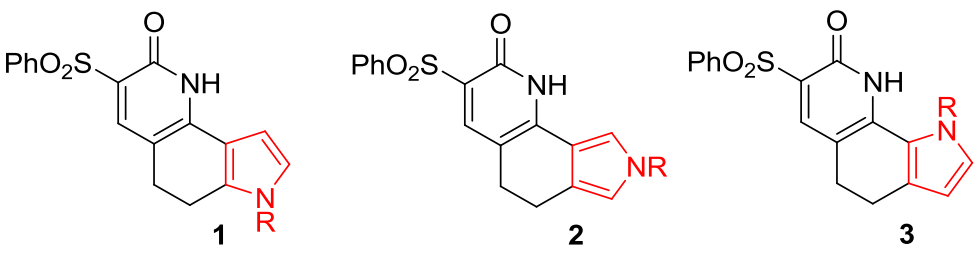
and a slight reddening of the skin associated with itching. A further advantage is that the ECP does not lead to suppression of the immune system of the patient, and normally doesn't harm susceptible organs such as liver, heart and lungs.

The drugs currently most used in photochemotherapy are the 5-methoxypsoralen, the 8-methoxypsoralen, and the 4,5,8-trimethylpsoralen which, however, as all linear furanocoumarins, showed severe side effects. Indeed UV-A inducing photoactivaction, produces covalent bonds between the drug and the DNA bases, through photocycloaddition reactions [2+2]. Psoralens, due to their planarity can form covalent bonds with two nucleotides of the two complementary strands of DNA (ISC inter-strand crosslinks) whereas angelicin, due to its geometry, can only form mono-adducts. Indeed, the formation of crosslinks which bind covalently the two chains of DNA, can lead to long-term in mutagenesis and carcinogenesis, whereas the formation of mono-adducts, is associated with less severe side effects only in short-term (itching, nausea and vomiting).

This has given importance to the study of monofunctional furanocoumarins, and has led to the synthesis of derivatives of angelicin. These drugs, are not phototoxic and therefore may also be administered topically, because they do not form inter-strand crosslink, thus the side effects in the long term, peculiar of the bifunctionals, are missing. For example, the trimethylangelicin (TMA) used in clinical trials for psoriasis, mycosis fungoides and other cutaneous diseases, has relevant therapeutic activity and show less mutagenicity, less risk of skin cancer, and absence of phototoxicity. [6]

In regard of this knowledge, in recent years, the research group in which I'm doing my PhD project, interested in the synthesis of new polycondensated heterocyclic systems that possessed the features of drugs already used as photochemotherapeutic agents, such as the presence of photoreactive sites in suitable position for the photo-conjugation to DNA. Based on experience gained on the chemistry of indoles and pyrroles, for example, new heterocyclic systems such as the *pyrrolo[2,3-h]quinolin-2-one* **1**, nitrogenous bioisoster of angelicin, in which both oxygens are replaced by nitrogen atoms, and its positional isomers, the *pyrrolo[3,4-h]quinolin-2-one* **2** and *pyrrolo[3,2-h]quinolin-8-one* **3** were synthesized (Figure 3).

Figure 3



For the first class of pyrroloquinolinones **1** (Table 1), the phototoxic activity was tested *in vitro* using three human tumor cell lines: HL-60 leukemic line, HT-1080, solid tumor from fibrosarcoma and LoVo intestinal adenocarcinoma. [7 , 8]

Table 1

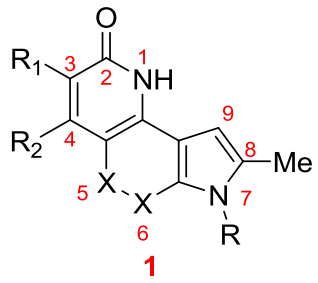
	Cpd	R	R_1	R_2	X-X
	**1a**	Ph	SO_2Ph	H	CH_2-CH_2
	1b	Ph	COPh	H	CH_2-CH_2
	1c	Ph	CN	H	CH_2-CH_2
	1d	Ph	COOEt	H	CH_2-CH_2
	1e	Me	SO_2Ph	H	CH_2-CH_2
	1f	Me	COPh	H	CH_2-CH_2
	1g	Bn	SO_2Ph	H	CH_2-CH_2
	1h	Bn	COPh	H	CH_2-CH_2
	1i	H	H	Me	CH=CH

Table 1 Pyrrolo[2,3-*h*]quinolin-2-ones **1**

All derivatives have not showed cytotoxic effects "in the dark", (in the absence of irradiation), while after irradiation at three different UV-A doses (2.6, 3.2, 6.5 J/cm^{-2}), it is evident the high anti-proliferative activity (IC_{50} 0.4-17.5 μM), which is concentration and UV dose dependent, and it is comparable, and even in some cases superior, to the reference drug angelicin (Table 2). In particular the derivative **1a** bearing the phenylsulfonyl group in position three, seems to be on all the cell lines, the most active derivative of the series (IC_{50} 0.4 - 3.6 μM).

Table 2

	HL-60 (IC_{50}, μM)			HT-1080 (IC_{50}, μM)			LoVo (IC_{50}, μM)		
Dose UVA (J/cm2)	**2.5**	**3.75**	**6.5**	**2.5**	**3.75**	**6.5**	**2.5**	**3.75**	**6.5**
1a	1.2±0.2	0.82±0.02	0.45±0.04	3.6±0.5	2.3±0.8	0.4±0.04	1.2±0.2	0.6±0.1	0.41±0.04
1b	8.5±0.3	3.9±0.5	0.5±0.08	6.6±1.6	4.6±0.9	1.7±0.2	4.1±0.6	2.8±0.3	1.3±0.2
1c	5.4±1.1	4.7±0.5	1.3±0.7	>20	13.1±1.5	11.1±1.4	9.8±1.1	8.9±0.3	8.3±1.1
1d	7.7±3.0	5.5±2.2	1.3±0.5	12.2±2.1	7.9±0.9	2.1±0.6	6.9±1.2	3.1±1.3	1.1±0.9
1e	>20	13.2±2.1	2.8±0.8	>20	>20	16.4±1.4	>20	17.5±4.7	6.6±1.1
1f	8.9±0.1	6.2±0.01	0.8±0.06	>20	>20	>20	>20	>20	8.9±0.9
1g	12.4±1.2	6.9±0.2	1.8±0.7	>20	15.1±1.2	1.7±0.2	>20	10.7±1.3	2.9±0.3
1h	4.0±0.2	2.2±0.2	1.1±0.1	14.7±0.9	9.0±1.1	1.9±0.02	6.5±0.7	3.6±1.3	0.6±0.1
1i	>20	>20	>20	>20	>20	>20	>20	>20	>20
8-MOP	**1.4±0.1**	**1.2±0.1**	**0.7±0.1**	**7.8±0.7**	**2.1±0.3**	**1.5±0.2**	**1.1±0.4**	**0.7±0.1**	**0.4±0.1**
Ang	**1.5±0.2**	**0.9±0.1**	**0.6±0.1**	**15.7±1.9**	**2.6±0.2**	**2.5±0.3**	**1.6±0.2**	**0.9±0.1**	**0.8±0.1**

Table 2 Photocytotoxicity of compounds **1**

From an examination of the biological results of pyrroloquinolinones **1**, in fact, it was evident the importance of the substituent in position three on the pyridone ring in modulating the antiproliferative activity, as the bond in position 3-4 together with the 8-9, is one of two photoreactive sites of the photoactivable drug.

For this reason, it was proposed to synthesize the pyrrolo[3,4-*h*]quinolin-2ones **2** still bearing the phenylsulfonyl group in position three (Table 3).[9]

Table 3

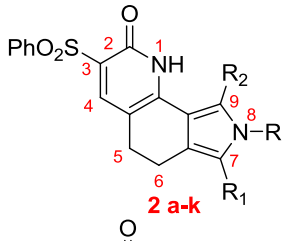
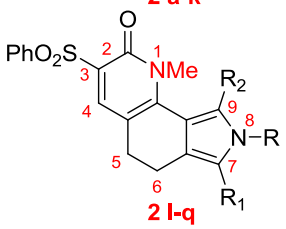
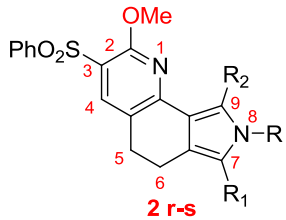
	Cpd	R	R_1	R_2
 **2 a-k**	**2a**	Me	H	H
	2b	Bn	H	H
	2c	Ph	H	H
	2d	H	COOEt	Me
	2e	Me	COOEt	Me
	2f	Bn	COOEt	Me
 **2 l-q**	**2g**	Me	H	Me
	2h	Bn	H	Me
	2i	H	COOH	Me
	2j	Me	COOH	Me
	2k	Bn	COOH	Me
	2l	Me	H	H
 **2 r-s**	**2m**	Bn	H	H
	2n	Ph	H	H
	2o	H	COOEt	Me
	2p	Me	COOEt	Me
	2q	Bn	COOEt	Me
	2r	Me	H	H
	2s	Bn	H	H

Table 3 Pyrrolo[3,4-*h*]quinolin-2-ones **2**

Such molecules, assayed on five tumor cell lines HL-60 (leukemia line), Jurkat (lymphoblastoid leukemia), LoVo (intestinal carcinoma), MCF-7 (breast adenocarcinoma) and NCTC-2544 (immortalized human keratinocytes) showed still a good photochemotherapic activity (IC_{50} 0.2-17.5 μM) reaching submicromolar levels at 72 hours after UV-A irradiation, even in this case UV-A dose dependent (Table 4).

The best results were achieved on leukemic lines rather than on solid tumors, and have been obtained from derivatives bearing a carboxyethyl function in adjacent position to the nitrogen of pyrrole ring. In some cases the results are better than angelicin, used as reference drug.

Table 4

	HL-60 (IC_{50}, μM)		Jurkat (IC_{50}, μM)		MCF-7 (IC_{50}, μM)		LoVo (IC_{50}, μM)		NCTC-2544 (IC_{50}, μM)	
Dose UVA (J/cm2)	**2.5**	**3.75**	**2.5**	**3.75**	**2.5**	**3.75**	**2.5**	**3.75**	**2.5**	**3.75**
2a	› 20	5.6±0.6	› 20	› 20	› 20	› 20	› 20	› 20	› 20	› 20
2b	› 20	14.5±1.5	› 20	› 20	› 20	› 20	› 20	› 20	› 20	› 20
2c	12.2±1.0	8.0±1.0	› 20	7.4±0.7	› 20	› 20	› 20	› 20	› 20	› 20
2d	13.7±1.0	9.1±0.8	18.0±2.0	10.0±1.0	› 20	› 20	17.5±1.5	13.6±0.5	› 20	15.0±1.4
2e	3.6±0.5	1.8±0.2	2.0±0.2	1.4±0.2	10.0±1.0	5.1±0.6	7.9±0.8	4.0±0.4	10.1±1.0	6.2±0.6
2f	1.2±0.2	0.8±0.02	2.9±0.3	1.9±0.2	3.3±0.4	1.4±0.1	4.0±0.4	2.3±0.2	4.0±0.5	2.5±0.3
2g	› 20	› 20	› 20	› 20	› 20	› 20	› 20	› 20	› 20	› 20
2h	4.0±0.4	2.5±0.3	13.0±1.5	3.0±0.5	› 20	7.7±0.8	› 20	› 20	› 20	14.4±0.8
2i	› 20	› 20	› 20	› 20	› 20	› 20	› 20	› 20	› 20	› 20
2j	› 20	› 20	› 20	› 20	› 20	› 20	› 20	› 20	› 20	› 20
2k	› 20	› 20	› 20	› 20	› 20	› 20	› 20	› 20	› 20	› 20
2l	› 20	› 20	› 20	› 20	› 20	› 20	› 20	› 20	› 20	› 20
2m	4.4±0.4	2.8±0.3	3.6±0.4	2.5±0.3	6.6±0.7	3.7±0.4	3.9±0.4	2.8±0.3	8.2±0.9	5.8±0.6
2n	› 20	› 20	› 20	› 20	› 20	› 20	› 20	› 20	› 20	› 20
2o	› 20	5.8±0.6	› 20	12.5±1.3	› 20	› 20	› 20	13.2±1.3	› 20±	› 20
2p	1.2±0.1	0.9±0.02	1.2±0.1	0.8±0.1	1.7±0.1	1.3±0.1	0.7±0.05	0.4±0.03	2.4±0.2	1.3±0.2
2q	5.4±0.6	2.6±0.3	2.9±0.3	1.3±0.1	› 20	14.4±1.5	12.1±1.2	8.7±0.8	› 20	14.3±1.5
2r	1.6±0.1	0.9±0.1	1.0±0.08	0.2±0.01	2.5±0.3	2.0±0.1	1.9±0.2	0.9±0.09	4.9±0.5	1.1±0.1
2s	8.3±0.8	7.3±0.7	8.5±0.5	6.3±0.6	10.0±1.2	7.5±0.8	9.9±0.1	8.7±0.6	15.3±1.4	9.6±0.5
Ang	**1.2±0.1**	**0.9±0.2**	**1.0±0.2**	**0.9±0.1**	**4.4±0.5**	**1.5±0.2**	**4.0±0.4**	**1.1±0.4**	**4.2±0.5**	**0.9±0.1**

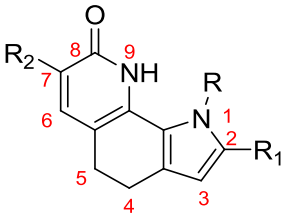
Table 4 Photocytotoxicity of compounds **2**

The class of pyrrolo[3,2-*h*]quinolin-8-ones **3** (Table 5), was suitably synthesized with the aim to evaluate the effect of different condensation of pyrrole in the tricyclic system.

Cytotoxicity studies were undertaken "in the dark", namely in the absence of UV-A irradiation, of different human tumor cell lines such as a line of keratinocytes K-562 (chronic myelogenous leukemia), Jurkat cells (lymphoblastoid leukemia), LoVo (intestinal carcinoma), MCF-7 (breast adenocarcinoma) and NCTC-2544 (immortalized human keratinocytes).

On the same cells, tests in the presence of two different UV-A doses, 2.5 and 3.75 J/cm^{-2},were conducted using lamps that emit at 365 nm. These compounds showed high photocytotoxicity with IC_{50} values of 0.5-9.3 μM; phototoxicity also in this case was concentration and UV-A dose dependent (Table 6). [10]

Table 5

	Cpd	R	R_1	R_2
	**3a**	SO_2Ph	H	SO_2Ph
	3b	H	H	SO_2Ph
	3c	SO_2Ph	H	CN
	3d	Me	H	SO_2Ph
	3e	Bn	H	SO_2Ph
	3f	Ph	H	SO_2Ph
	3g	Bn*p*Me	H	SO_2Ph
	3h	Bn*p*OMe	H	SO_2Ph
	3i	H	COOEt	SO_2Ph
	3j	Me	COOEt	SO_2Ph
	3k	Bn	COOEt	SO_2Ph
	3l	Bn*p*Me	COOEt	SO_2Ph
	3m	Bn*p*OMe	COOEt	SO_2Ph

3

Table 5 Pyrrolo[3,2-*h*]quinolin-8-ones **3**

Table 6

	Jurkat (IC_{50}, µM)		K-562 (IC_{50}, µM)		LoVo (IC_{50}, µM)		MCF-7 (IC_{50}, µM)		NCTC (IC_{50}, µM)	
Dose UVA (J/cm2)	**2.5**	**3.75**	**2.5**	**3.75**	**2.5**	**3.75**	**2.5**	**3.75**	**2.5**	**3.75**
3a	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20
3b	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20
3c	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20
3d	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20
3e	1,3±0,2	1,1±0,1	4,8±0,5	3,3±0,5	2,6±0,2	2,2±0,2	4,0±0,6	1,7±0,7	7,5±0,8	5,5±0,6
3f	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20
3g	1,6±0,1	1,2±0,2	2,7±0,2	2,4±0,1	2,7±0,1	2,3±0,2	3,8±0,5	2,4±0,2	3,3±0,3	2,6±0,5
3h	1,6±0,2	1,2±0,1	3,1±0,3	2,5±0,2	2,8±0,3	2,0±0,2	4,2±0,3	1,8±0,1	4,3±0,3	2,7±0,2
3i	3,5±0,2	2,5±0,2	6,1±0,2	5,3±0,3	5,5±0,2	4,6±0,4	6,1±0,2	4,3±0,3	9,3±0,6	7,2±0,7
3j	0,7±0,1	0,5±0,1	1,0±0,1	0,9±0,1	1,1±0,1	1,0±0,1	1,2±0,1	1,0±0,1	1,2±0,1	1,1±0,1
3k	0,8±0,1	0,6±0,1	0,9±0,1	0,6±0,1	0,9±0,1	0,8±0,1	0,9±0,1	0,8±0,1	1,1±0,1	1,0±0,1
3l	1,5±0,1	1,2±0,1	1,3±0,1	1,0±0,1	1,6±0,2	1,0±0,1	1,7±0,1	1,4±0,1	2,7±0,2	2,2±0,2
3m	0,8±0,1	0,7±0,1	1,0±0,1	0,9±0,1	1,1±0,1	1,0±0,1	1,6±0,2	1,3±0,1	1,9±0,1	1,6±0,1
Ang	**1,0±0,2**	**0,9±0,1**	**1,2±0,1**	**1,0±0,1**	**3,6±0,4**	**1,5±0,3**	**4,4±0,5**	**1,5±0,2**	**4,2±0,5**	**0,9±0,1**

Table 6 Photocytotoxicity of compounds **3**

Studies on the mechanism of action on the best of the three classes of pyrroloquinolinones **1-3** have shown that:

- Each class of compounds is able to photonduce cell death by apoptosis, demonstrated by the appearance of the typical peak subG1, indicating an apoptotic degradation of DNA, which was confirmed by the test of "Annexin".

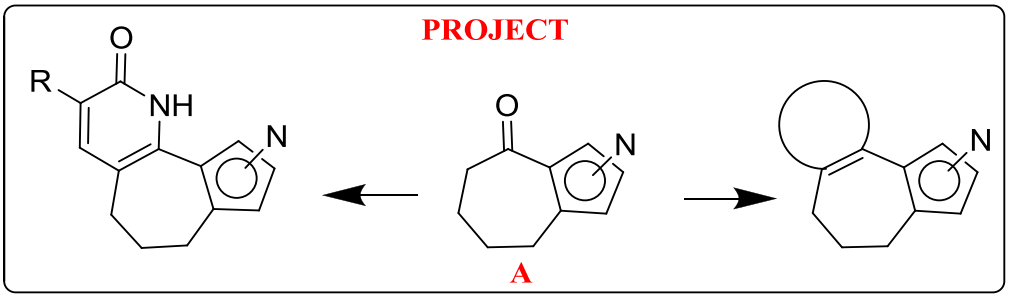
- Easily penetrate into the cell and accumulates into subcellular organelles. Experiments using specific "probes" for mitochondria (JC-1) and lysosomes (Acridine orange), which have the property of selectively accumulate in these organelles, highlight a specific photo-induced damage against them, indicating them as targets for pyrroloquinolinones.
- In addition it was studied the possible interaction of pyrroloquinolinones with the DNA, since this is the preferential target for psoralen and angelicin. Therefore linear dichroism spectra (LD) which allow to study the presence of a bond between an organic compound and the nucleic acid (DNA), were performed, and it was found that pyrroloquinolinones derivatives exclude the DNA as target. In fact, in the spectrum of LD are not observed absorption bands between 300 and 500 nm, which is the region where the DNA absorbs.

Unlike the first pyrroloquinolinone classes **1** and **2**, the pyrrolo[3,2-*h*]quinolinones **3**, do not cause any DNA damage with formation of OC-DNA or L-DNA, indicating an absence of phototoxicity towards the macromolecule. This result is of great importance, in fact this class of pyrroloquinolinone **3** were covered by patent, since the absence of interactions with DNA may be a determining factor in the modulation of the long term toxic effects related to the administration of psoralens, such as mutagenesis and carcinogenesis. [11]

2. PROJECT

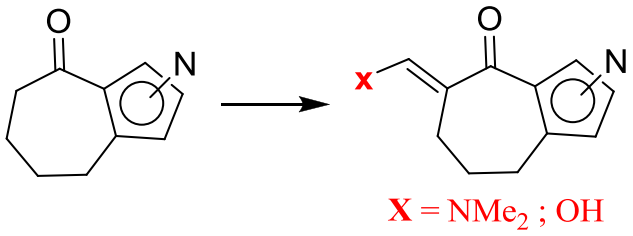
In light of the promising results already illustrated by the classes of pyrroloquinolinones **1-3**, it was thought for my PhD project (Scheme 1), to study the analogous tricyclic systems always containing the pyridin-2-one ring, in which the six terms central ring was enlarged to seven, with the aim to evaluate the effect on the phototoxicity.

Scheme 1



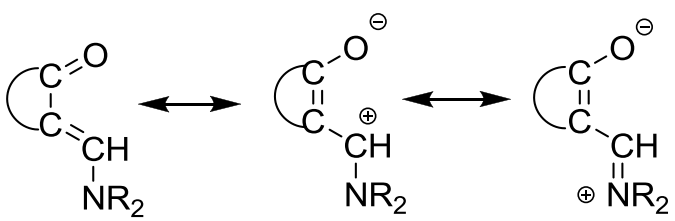
The ideal building blocks, were cyclohepta[*b*]pyrrolones (A), easily functionalizable in position α to the carbonyl with an enamine or formylic group (Scheme 2):

Scheme 2



This latter in fact appear to be versatile intermediates having two vicinal electrophilic centers: the carbonyl, and the exocyclic carbon bonded to α position to the carbonyl. In particular α-*N*,*N*-disubstituted-aminomethylenketones (Figure 4), constitute a resonant system in which the relocation of charges, offers numerous possibilities of reactions, also thanks to the intervention of the nitrogen atom.

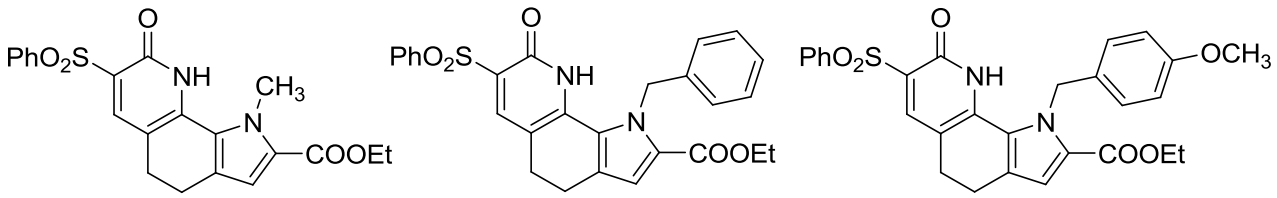
Figure 4



In our case, they could lead to the formation of a variety of tricyclic systems by reaction with dinucleophiles as urea, guanidine, cyanomethylene compounds, hydroxylamines, thus offering the possibility to extend the project to the synthesis of several new ring systems.

In particular, we thought to start from the synthesis by **pyrrolo[3',2':6,7]cyclohepta[1,2-*b*]pyridin-9-one**, analogues of pyrroloquinolinones of type **3**, by planning two series of derivatives bearing the ethoxycarbonyl group and an hydrogen as substituents in *ortho* position to pyrrole nitrogen. Furthermore, since the lead coumpounds (Figure 5) of the series of type **3** are ethoxycarbonyl derivatives, bearing a methyl, benzyl, or 4-methoxy-benzyl group at the pyrrole nitrogen, we decided to start our own synthesis with the same type of substitution at the pyrrole.

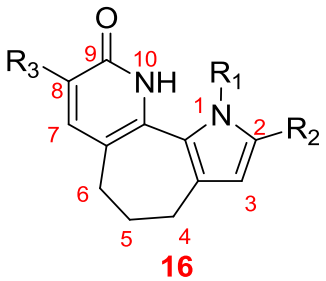
Figure 5



3j
IC_{50} 0.5-1.2 (μM)

3k
IC_{50} 0.6-1.1 (μM)

3m
IC_{50} 0.7-1.9 (μM)



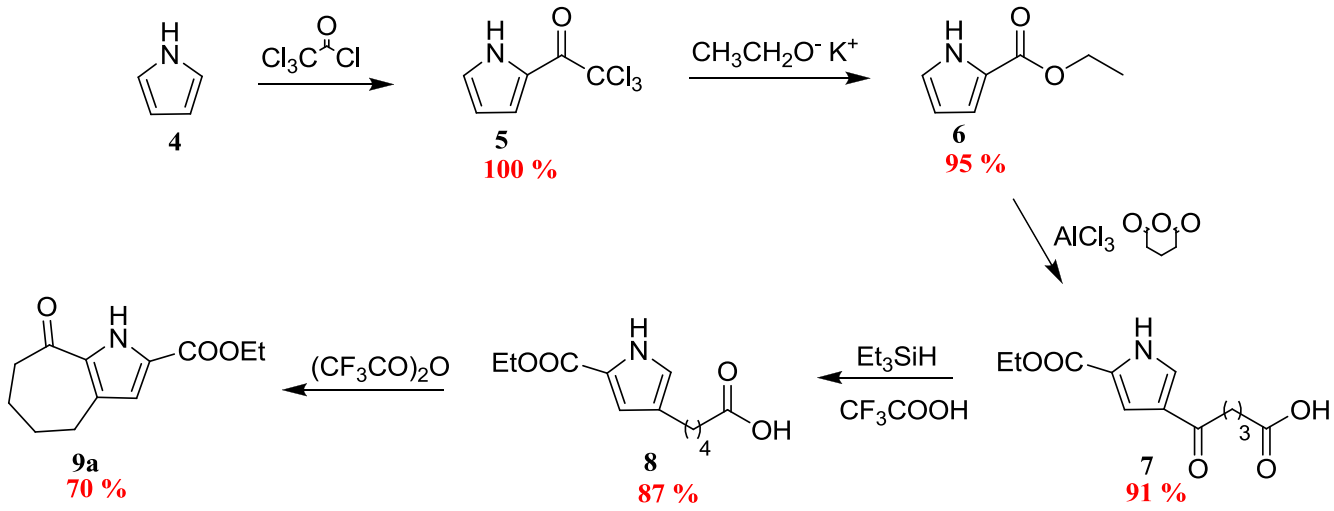
16
pyrrolo[3',2':6,7]cyclohepta[1,2-*b*]pyridin-9-one

3. SYNTHESIS OF BUILDING BLOCKS

The synthesis of cyclohepta[*b*]pyrrol-8-one ketones of type **9**, suitable precursors for the new ring systems, was achieved with the following synthetic schemes (Schemes 3 and 4).

By reaction of pyrrole **4** with trichloroacetyl chloride, the trichloroacetyl pyrrole **5** was obtained in quantitative yield, which in turn undergoes nucleophilic substitution with potassium ethylate giving the ethyl 1*H*-pyrrole-2-carboxylate **6**.[12] The three position of the pyrrole was acylated by a Friedel-Crafts acylation using $AlCl_3$ as Lewis acid, and glutaric anhydride as acylating agent. The obtained derivate **7**, was subsequently subjected to reduction of the carbonyl group to methylene, through the use of triethylsilane in trifluoroacetic acid to give **8**, which in turn was cyclized by dehydration reaction with an excess of trifluoroacetic anhydride, with the subsequent ring closure to the seven members ring. Thus the ethyl 8-oxo-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2-carboxylate **9a** was obtained with a high yield.

Scheme 3



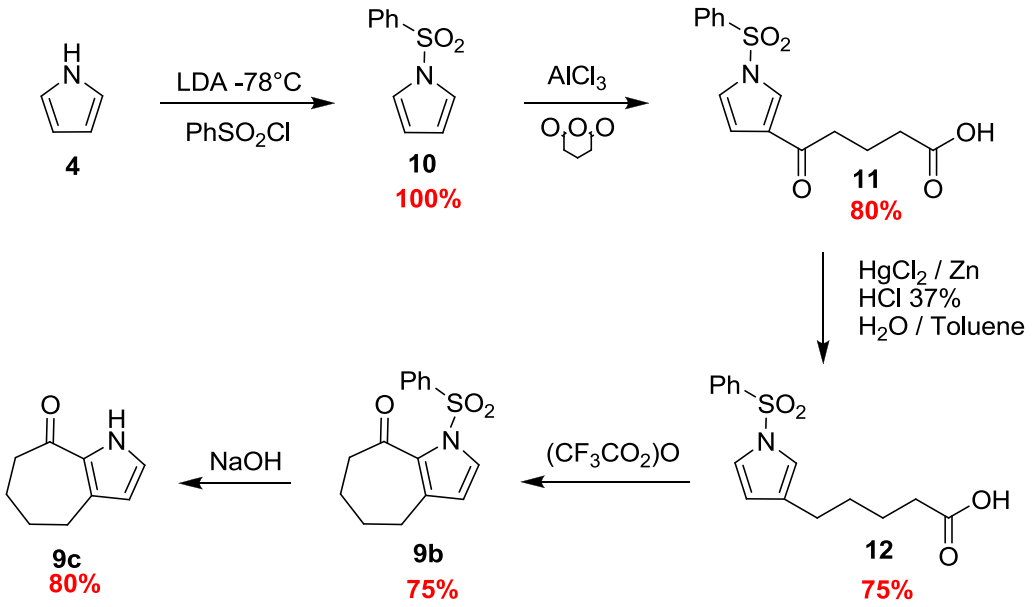
In order to increase the number of derivatives, it has been synthesized a second series of products bearing an hydrogen as a substituent in position two. In this regard, the new scaffold, 2,3*H*-pyrrole, was obtained starting from 1-(phenylsulfonyl)-1*H*-pyrrole **10**, which although it is commercially available, was prepared in quantitative yield according to the methos used in the past in our group (Scheme 4).

The pyrrole **4** dissolved in anhydrous THF at -78 ° C, was deprotonated with LDA, and subsequently reacted with benzenesulfonyl chloride to give **10** in quantitative yield.

Similarly to the ethoxycarbonyl series, the second step was a Friedel Crafts acylation with glutaric anhydride and $AlCl_3$. Also in this case the reaction proceeds with excellent yields. The reduction step with trifluoroacetic acid and triethylsilane, probably not activated due to the absence of ethoxycarbonyl group, has led to a secondary product of ring opening of the pyrrole ring. Also other attempts of reduction with $NaBH_4$ or with H_2/Pd, did not lead to the desired product too.

The Clemmensen reduction, which involves the use of an amalgam of zinc and mercuric chloride in concentrated HCl and toluene at reflux condition, allowed to obtain the desired product **12** in 75% yield. The dehydration reaction with trifluoroacetic anhydride, led to the isolation of **9b**, which in turn was deprotected with NaOH at reflux in ethanol to obtain the NH derivative **9c**.

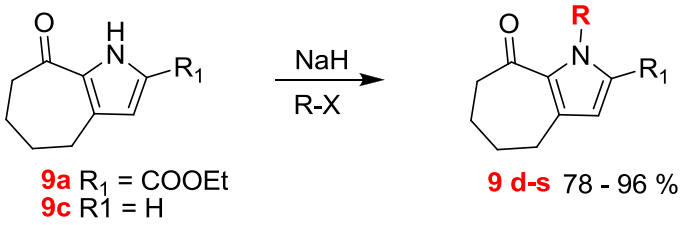
Scheme 4



At this point we proceeded to the subsequent functionalization of the NH of the pyrrole ring, by reacting the ketones of type **9a** and **9c** in DMF with sodium hydride as a base, and then with various alkyl or alkylaryl halides, in order to obtain a good number of new ketones with a good pattern of substitution (Scheme 5).

In many cases the halides that we used, were prepared starting from the corresponding alcohols, in concentrated HCl used as solvent.

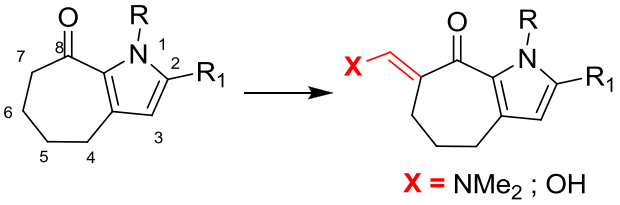
Scheme 5



Cpd	R	R_1	Yield	Cpd	R	R_1	Yield
9d	Me	COOEt	94 %	**9l**	Me	H	88 %
9e	Bn	COOEt	96 %	**9m**	Bn	H	92 %
9f	2-OMe-Bn	COOEt	78 %	**9n**	2-OMe-Bn	H	93 %
9g	3-OMe-Bn	COOEt	81 %	**9o**	3-OMe-Bn	H	84 %
9h	4-OMe-Bn	COOEt	79 %	**9p**	4-OMe-Bn	H	96 %
9i	2,5-$(OMe)_2$-Bn	COOEt	81 %	**9q**	2,5-$(OMe)_2$-Bn	H	90 %
9j	3,5-$(OMe)_2$-Bn	COOEt	83 %	**9r**	3,5-$(OMe)_2$-Bn	H	90 %
9k	3,4,5-$(OMe)_3$-Bn	COOEt	80 %	**9s**	3,4,5-$(OMe)_3$-Bn	H	89 %

Having obtained a good number of derivatives, the ketones of type **9**, were subjected to the next step (Scheme 6) in order to introduce in the molecule a second electrophilic center, to constitute versatile building blocks for further annelations according to the synthetic scheme anticipated in the presentation of the project (see scheme 2) and here proposed again.

Scheme 6

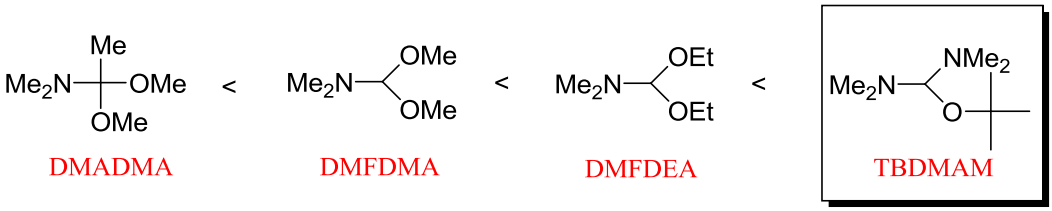


We decided to start our studies with the direct introduction of the enamine functionality according to previous works carried out on the analogues series of compounds in our laboratory.

Direct introduction of the enamine functionality, 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 readily react in solvents such as toluene or benzene at reflux leading to quantitative yields of the dialkylamino derivative.

In a review of Stanovnick in 2004 [13] it was indicated a scale of reactivity among amide acetals (Figure 6), placing the DMFDMA as the less reactive and the *tert*-Butoxy bis(dimethylamino)methane (TBDMAM), as the most reactive.

Figure 6



TBDMAM, also called Bredereck's reagent, seemed to be the most efficacious reagent; however a disadvantage was the high cost, and so for this reason we investigated also the use of DMFDMA available at low cost, but also less reactive.

Thus the two series of products, ethoxycarbonyl and unsubstitued series, were reacted with the DMFDMA, but showed, different results. In fact, by reacting substrates of type **9d-k**, bearing the ethoxycarbonyl group in position two, with an excess of DMFDMA used as solvent with conventional heating at reflux, we observed the introduction of the enamine functionality, but at the same time also a transesterification to carboxymethyl derivatives occurred.

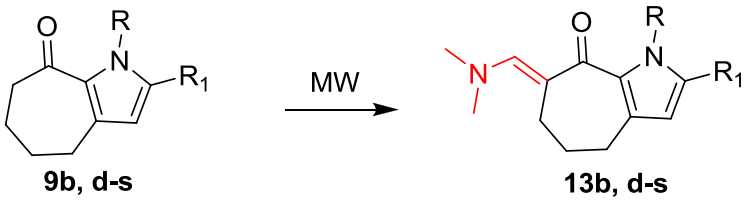
So we decided to carry out this reactions with the aid of microwaves apparatus. Initially, we used a power of 150 W for 15 minutes and temperature of 150°C, dissolving the starting material in anhydrous DMF and using a ratio of 10 to 1 of DMFDMA with the substrate, but also in this case, we isolated the transesterificated product obtaining the intermediate **13t** (Table 7).

In less drastic conditions, reducing the power of the instrument from 150 to 50 W, increasing the reaction time from 15 to 80 minutes, and using the amide acetal in stoichiometric ratio with the substrate, the 2-carboxyethyl substituted aminomethylenketones, are obtained with excellent yields as oily products (Scheme 7). In this case it is observed the stabilization of the temperature around 100 ° C, which might be responsible of the undesired reaction.

Alternatively the more reactive TBDMAM was used. In this case the desired products **13** were obtained as crystals with shorter reaction times and excellent yields.

With regards to the ketones **9b,l-s** of the unsubstituted series, missing the ethoxycarbonyl group as a substituent in the 2-position, no side reactions occurred. However they proved to be less reactive, and for this reason a ratio 10 to 1 of DMFDMA with the substrate was required to obtain the desired enaminoketones **13**.

Scheme 7



9b, d-s **13b, d-s**

Table 7

	R	R_1	TBDMAM	DMFDMA
13d	Me	COOEt	99 % [a]	------
13e	Bn	COOEt	100 % [a]	73 % [b]
13f	2-OMe-Bn	COOEt	97 % [a]	70 % [b]
13g	3-OMe-Bn	COOEt	92 % [a]	87 % [b]
13h	4-OMe-Bn	COOEt	95 % [a]	75 % [b]
13j	3,5-$(OMe)_2$-Bn	COOEt	94% [a]	77 % [b]
13k	3,4,5-$(OMe)_3$-Bn	COOEt	99 % [a]	-----
13b	SO_2-Ph	H	-----	90 % [c]
13l	Me	H	-----	89 % [c]
13m	Bn	H	-----	95 % [c]
13n	2-OMe-Bn	H	-----	96 % [c]
13o	3-OMe-Bn	H	-----	91 % [c]
13p	4-OMe-Bn	H	-----	96 % [c]
13q	2,5-$(OMe)_2$-Bn	H	-----	93 % [c]
13r	3,5-$(OMe)_2$-Bn	H	-----	90 % [c]
13s	3,4,5-$(OMe)_3$-Bn	H	-----	86 % [c]
13t	Bn	COOMe	-----	65 % [c]

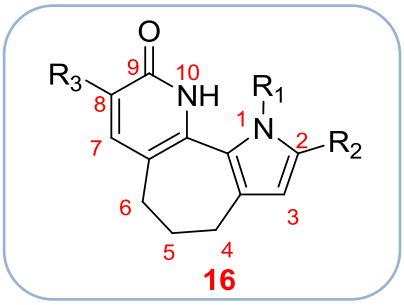
a TBDMAM / Starting Material (1.5 eqv: 1 eqv) 50 W, 20-40min
b DMFDMA / Starting Material (1 eqv : 1 eqv) 50W, 40-120 min
c DMFDMA / Starting Material (10 eqv: 1 eqv) 150 W, 40-120 min

Table 7: yield of the reactions with the use of the two different amide acetals.

4. PYRROLO[3',2':6,7] CYCLOHEPTA[1,2-*b*]PYRIDIN-9(1*H*)-ONE

4.1 Synthesis

As widely anticipated in the introduction, the pyrrolo[3',2':6,7]cyclohepta[1,2-*b*]pyridin-9(1*H*)-one system **16** was the first annelation considered.



16

To achieve the tricyclic system **16**, we could react our dimethylaminomethylenketones **13** with dinucleophiles having a C-C-N structure, such as cyanoacetamide, ethyl or methyl cyanoacetate, and phenylsulfonylacetonitrile.

From an evaluation of results of the classes of pyrroloquinolinones **1-3**, it was evident the importance of the substituent in position eight of the pyridone ring in the modulation of the antiproliferative activity. In particular, it appeared that pyrroloquinolinones **1-3** bearing a phenylsulfonyl group in position eight, were the most active compounds, on all the tested cell lines. For this reason we planned to begin our studies on the N-methyl derivative **13d**, belonging to the ethoxycarbonyl series, using phenylsulfonylacetonitrile as dinucleophile to afford the corresponding tricyclic phenylsulphonyl derivative.

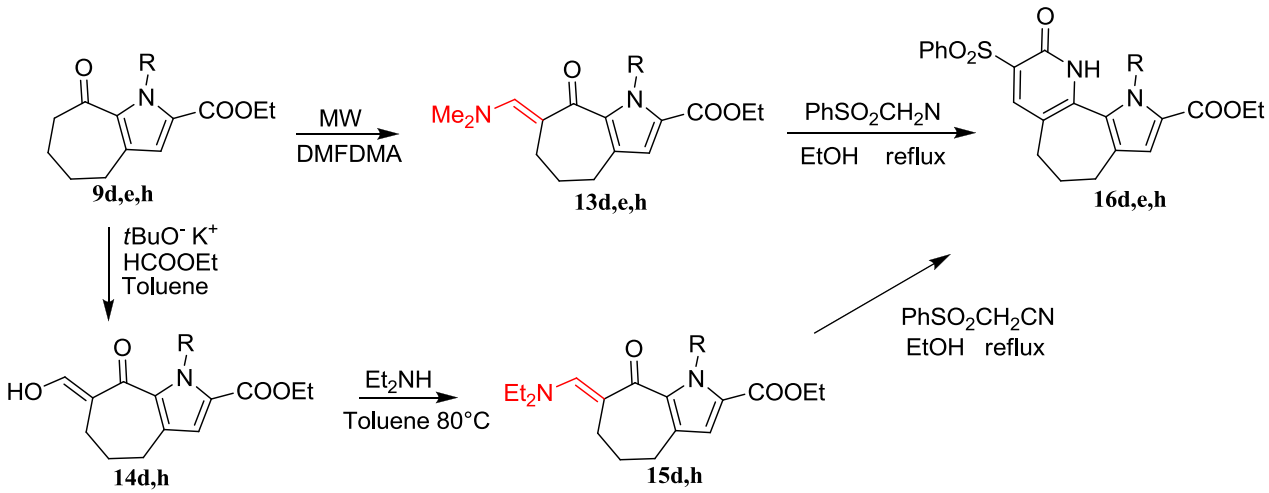
The reaction was carried out in ethanol, under nitrogen atmosphere at reflux, but contrary to our expectations, applying the same operating conditions previously used in the pyrroloquinolinones **1-3** series, we isolated the desired product **16d** in very low yields (15%). The same behaviour was also observed for the N-benzyl **13e** (8%) and 4-OMe-benzyl derivatives **13h** (13%).

Disappointed by the low reactivity exhibited by these derivatives, we decided to investigate the reactivity of diethylenaminoketones **15d,h**, obtainable in two steps starting from the appropriate ketones **9** through hydroxymethylenketones intermediates of type **14**, in order to compare their reactivity with the corresponding dimethylenaminoketones **13d,h** (Scheme 8).

Thus reacting ketones **9d**,**h** in toluene with potassium *tert*-butoxide and ethyl formate, the hydroxymethylenketones **14d,h** are obtained in good yields (73-80%). The subsequent reaction with diethylamine at 80° C, leads to the desired diethylenaminoketones derivatives **15d**,**h** in quantitative yields.

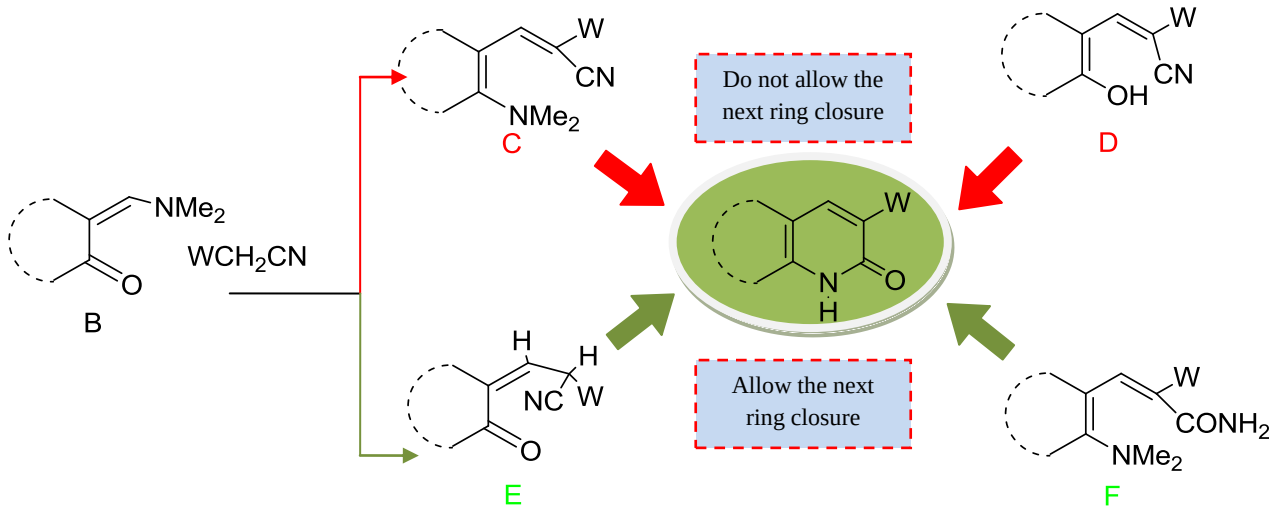
However, reactions of this latter in the same conditions used for the compounds of type **13** ($PhSO_2CH_2CN$, EtOH reflux), gave even more complex reaction mixtures from which it was not possible isolate the desired pyridone derivatives **16**.

Scheme 8



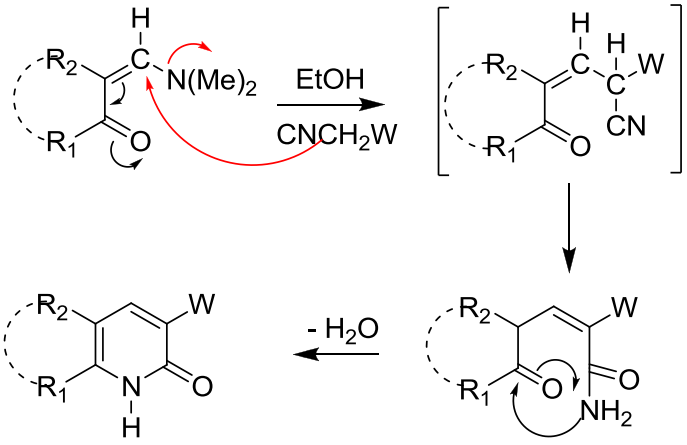
In a paper by Mosti et al. [14] it was described, a study in benzofuran series, concerning the isolation of pyridone derivatives starting from enaminoketones. Four types of possible intermediates were reported, among which only **E** and **F,** gave the subsequent ring closure (Scheme 9).

Scheme 9



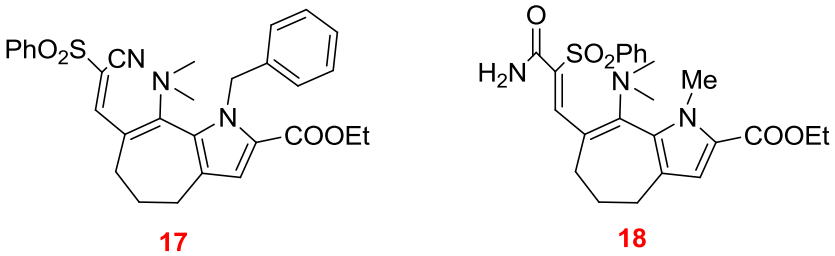
The proposed mechanism of cyclization, involves the initial nucleophilic attack to the enaminic carbon, followed by formation of an intermediate in which the nitrile was hydrolyzed to carboxamide, and the subsequent nucleophilic attack to the annular carbonyl by the nitrogen of the amide, leads to the desired cyclization (Figure 7).

Figure 7



Thus, taking in mind these studies, we were able to isolate from two different reaction mixtures the intermediates of type **17** and **18** (Figure 8).

Figure 8



These intermediates confirm the direct intervention of the dimethylamine, which may act as a nucleophile on the carbonyl group. In particular, from reaction of benzyl substituted derivative enaminone **13e**, we isolated the reaction product between the dimethylamine released *in situ* (from the dimethylenaminoketones) with the annular carbonyl groups, as a consequence of Michael addition of phenylsulfonylacetonitrile to the enamine group (intermediate of type C **17**). Moreover, intermediate **18** was isolated from reaction of the N-Methyl substituted enaminone **13d** as a consequence of the reaction of dimethylamine with the carbonyl group, in which the cyano group was hydrolyzed to carboxamide (intermediate of type F **18**).

Since that the intermediate of type **17** was supposed to fail in the ring closure, so we thought to extend the time of reflux in ethanol to help the hydrolysis of the cyano group. However, the reaction did not seem to proceed and the same behaviour was observed for the other type of intermediate (**18**) which was supposed to give the desired ring.

Thus we thought to encourage the ring closure by prolonging the reaction time and by favouring the dehydration process removing the water through the formation of an azeotropic mixture water / toluene using Dean-Stark apparatus.

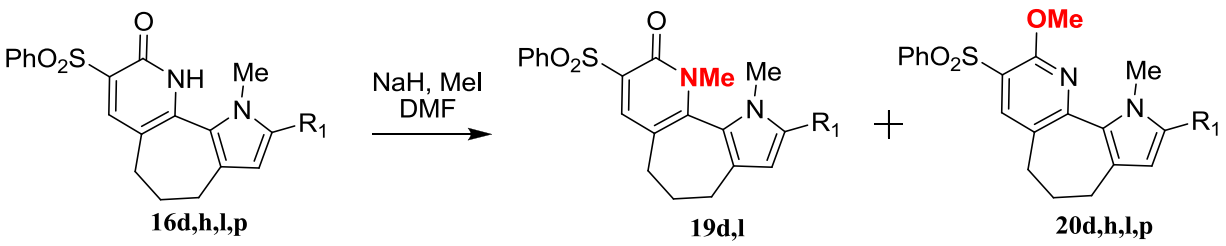
So at first, the mixture was reacted at reflux in ethanol for 24-48 hours, and it was possible to notice, by TLC monitoring, that the starting material **13** disappeared to give the intermediates **17**, and a small amount of desired product **16**. The long reflux helped the hydrolysis of the cyano intermediate **17** in to the carboxamide. At this point the reaction mixture was evaporated, and at the residue were added toluene and a catalytic amount of acetic acid, and was reacted with a Dean-Stark apparatus. After several hours of reflux the desired pyridone was isolated from the corresponding reaction mixtures with discrete yields (37 – 51%).

Once obtained the best reaction conditions, we applied the method in two steps to prepare all the other pyrrolo[3',2':6,7]cyclohepta[1,2-*b*]pyridin-9(1*H*)-one derivatives **16d-p**.

A better reactivity was observed for compounds belonging to the pyrrolo unsubstitued series. In fact the desired compounds, precipitated from the reaction mixture upon cooling and were recrystallized from ethanol. Moreover after evaporation of the ethanolic solution, the residue was reacted in the second step, improving the total yield of the desired tricyclic derivatives. Unfortunately, when we used cyanoacetamide, which already bears the carboxyamide function readily available for the cyclization, the yield still remains modest.

In addition, by exploiting the tautomeric keto-enol equilibria, the N-methyl and the 4-OMe-benzyl substituted derivatives of the two series, **16d,h,l,p** obtained in best yield, were further functionalizated by methylation (Scheme 10). But from the reaction mixture, only in the case of N-methyl derivatives **16d,l** it was possible to obtain both products **19d,l** and **20d,l**, whereas for the 4-OMe-benzyl substituted derivatives **16h,p,** it was possible to obtain, only the 9-methoxy derivatives **20h,p**.

Scheme 10



16d,h,l,p **19d,l** **20d,h,l,p**

A final series of sixteen compounds was completed and submitted to biological laboratory of "Dipartimento di Scienze Farmaceutiche" University of Padova in collaboration with professor Dall'Acqua with the aim of evaluating the photo-antiproliferative activity.

Table 8

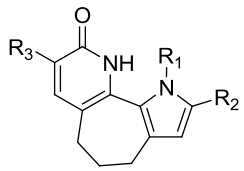
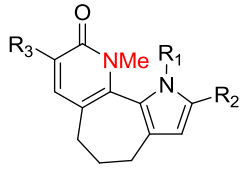
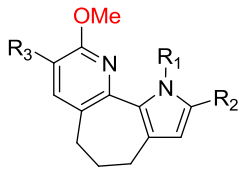
		R_1	R_2	R_3	Yield
 **16**	**16d**	Me	COOEt	SO_2Ph	51%
	16e	Bn	COOEt	SO_2Ph	40%
	16f	2-OMe-Bn	COOEt	SO_2Ph	46%
	16g	3-OMe-Bn	COOEt	SO_2Ph	39%
	16h	4-OMe-Bn	COOEt	SO_2Ph	48%
	16l	Me	H	SO_2Ph	49%
 **19**	**16m**	Bn	H	SO_2Ph	40%
	16n	2-OMe-Bn	H	SO_2Ph	43%
	16o	3-OMe-Bn	H	SO_2Ph	37%
	16p	4-OMe-Bn	H	SO_2Ph	48%
	19d	Me	COOEt	SO_2Ph	30%
	19l	Me	H	SO_2Ph	20%
 **20**	**20d**	Me	COOEt	SO_2Ph	59%
	20h	4-OMe-Bn	COOEt	SO_2Ph	73%
	20l	Me	H	SO_2Ph	74%
	20p	4-OMe-Bn	H	SO_2Ph	68%

Table 8: 4,5,6,10-tetrahydropyrrolo[3',2':6,7] cyclohepta[1,2-*b*]pyridin-9(1*H*)-ones **16**, **19-20**

4.2 Pyrrolo[3',2':6,7]cyclohepta[1,2-*b*]pyridine-9(1*H*)-one Photochemoterapeutic Activity

4.2.1 METHODS

1) SPECTROPHOTOMETRIC DETERMINATIONS

All spectrophotometric measures were performed using UV-Vis Perkin Elmer instrument (Lambda12, double beam). For all the compounds, absorption spectra, molar extinction coefficients (ε) and determination of maxima peaks (λ_{max}) were carried out in DMSO.

2) IRRADIATION PROCEDURES

HPW 125 Philips lamps, mainly emitting at 365 nm, were used for irradiation experiments. The spectral irradiance of the source was 4.0 mW cm^{-2} as measured, at the sample level, by a Cole-Parmer Instrument Company radiometer (Niles, IL), equipped with a 365-CX sensor.

3) CELL CULTURES

For experiments of cellular viability, 3 human cell lines were used: Jurkat, K-562 and A431.

Jurkat were human lymphoblastoid cells taken from 14 years old boy in 1976. They were grown in complete RPMI 1640 medium. Cells were kept at 37°C in 5% CO_2 humidified atmosphere and re-seeded into fresh medium three times a week.

K-562 were obtained from a 53 years old woman with chronic myelogenous leukemia in terminal blast crisis. Cells were kept at 37°C in 5% CO_2 humidified atmosphere and re-seeded into fresh medium (complete RPMI) three times a week.

A431 derived from a EGFR-overexpressing carcinoma. They were grown in complete DMEM medium. Cells were kept at 37°C in 5% CO_2 humidified atmosphere, trypsinized and re-seeded into fresh medium twice a week.

4) CELLULAR CYTOTOXICITY AND PHOTOTOXICITY

Individual wells of a 96-well tissue culture microtiter plate were inoculated with 100 μl of complete medium containing $5x10^3$ cells. Plates were harvested at 37 °C in a humidified 5% CO_2 incubator for 24 hours prior to the cell viability experiments. Drugs were dissolved in DMSO and then were diluted with Hank's Balanced Salt Solution (HBSS pH=7,2) for phototoxicity experiments or in the appropriated complete medium for the cytotoxicity ones.

In cytotoxicity tests after medium removal, 100 μl of the drug solution at different concentration were added to each well and incubated at 37 °C for 72 hours.

In phototoxicity experiments after medium removal, 100 μl of the drug solution were put into each well and incubated at 37 °C for 30 minutes and then irradiated (2.5 and 3.75 J/cm^2). After irradiation, drug solution was replaced by cellular medium and plates were incubated for 72 hours.

After the period of incubation, in both cases cell viability was assayed by the MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide)] test. In each well, 10 μl of MTT (5 mg/ml in PBS) (PBS = phosphate buffer 10 mM, NaCl 9g/L, pH=7,2) were added and plates were put in incubator for 3 or 4 hours. MTT is a yellow dye that can be absorbed by viable cell and reduced by mitochondrial dehydrogenases, producing insoluble blue crystals. After incubation, crystals were solubilized by adding 100 μl of HCl 0.08 N in isopropanol and plates were detected through microplate Biorad reader at 570 nm. The absorbance of each sample was corrected by instrument which subtracted the mean value of blanks, i.e. wells in which there were all the reactives except cells.

The absorbance is proportional to cellular viability, so the cellular survival was calculated by this equation:

$$\% \text{ cell survival} = \frac{A_{100\%} - A_{sample}}{A_{100\%}}$$

$A_{100\%}$ = mean of controls, i.e. cells without drug nor irradiation exposure, which represents 100% of survival.
A_{sample} = absorbance of various samples in which cells were in contact with drug or irradiated in presence of drug.

For every cellular line, GI_{50} (GI_{50} is the drug concentration at which 50% of treated cells are dead) was measured through SigmaPlot software. In phototoxicity experiments, the protective effect of some scavengers was also evaluated: reduced glutathione (GSH, 1mM in PBS), vitamin E (vit E, 60 μM in ethanol), *N,N'*-dimethyl thiourea (DMTU, 1 mM in ethanol), mannitol (MAN, 10 mM in PBS), sodium azide (NaN_3, 1 mM in PBS). Each experiment was repeated at least three times.

5) REACTIVE OXYGEN SPECIES DETERMINATION

Samples containing the compounds under examination (10 μM), p-nitrosodimethylaniline (40 μM) and imidazole (40 μM) in phosphate buffer (0.02 M, pH 7.3) were irradiated with increasing UVA doses, and their absorbance at 440 nm was then measured for singlet oxygen production. Superoxide anion was determined following Pathak and Joshi:[15] samples containing the compounds under examination (10 μM) and nitroblue tetrazolium (160 μM) in carbonate buffer (pH 10) were irradiated with increasing UVA doses, and their absorbance at 560 nm was measured.

6) DNA PHOTOCLEAVAGE

To evaluate the capability of provoking DNA photodamage, we used supercoiled pBR322 plasmid, dissolved in phosphate buffer 10 mM. Plasmid solutions were irradiated (7.5 J/cm^2) in the presence of the most active compounds in a 96-wells microplate. We used growing [compound]/[DNA] rates. Irradiation with a photosensitizer can induce cleavage of one or both DNA strands and so the formation of a open circular plasmid (OC, II form) or of a linear one (L, III form), respectively.

Figure 9

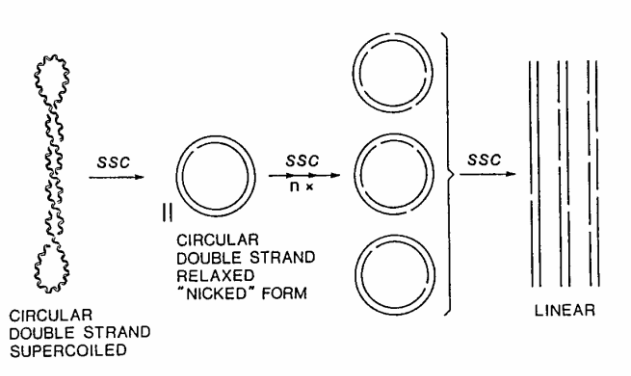


Figure 9*:* The 3 possible plasmidic DNA forms: supercoiled (SC, form I), open circular (OC, form II) and linear (L, form III).

It is possible to separate the three different forms with horizontal electrophoresis thanks to their different hydrodynamic properties.

In addition to frank strand break, we also evaluated if purine and/or pyrimidine bases were involved in the oxidative damage to DNA using base excision repair enzymes Formamido pyrimidin glycosilase (Fpg) and Endonuclease III (Endo III), respectively.

Each pBR322 DNA sample (100 ng) dissolved in TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH = 8.0) was irradiated with UV in the presence of the compounds.

After irradiation, two aliquots of sample were incubated at 37 °C with Fpg (Formamydo pyrimidin glycosilase) and Endo III (Endonuclease III) respectively.

Prior to the run, samples were added of loading buffer (0.25% bromophenol blue, 0.25% xilene cianol, 30% glycerol in water). Samples were loaded on 1% agarose gel and the run was carried out in TAE buffer at 80 V for 2 hrs.

After staining with ethidium bromide solution, gel was washed with water and the DNA bands were detected under UV radiation with a UV transilluminator. Photographs were taken by a digital photocamera Kodak DC256 and the quantification of the bands was achieved by image analyzer software Quantity One.

The fractions of supercoiled DNA (Form I) were calculated:

$$\text{sc-DNA} = \frac{\text{Area}_{sc}}{\text{Area}_{sc} + \text{Area}_{rel}/1.66}$$

Where Area_{sc} is the area of supercoiled DNA and Area_{rel} is the area of relaxed DNA (form II and III) obtained by the densitometric analysis of gels. The presence of the coefficient 1.66 in the formula is due to the fact that ethidium bromide bound to the supercolied pBR322 is 1.66 times less than that bound to the relaxed form.

7) EVALUATION OF LIPIDIC PEROXIDATION

Lipidic peroxidation was monitored by Morlière method or TBARS assay, which checks the formation of malondialdehyde, one of the final product of this oxidation process, as it reacts with thiobarbituric acid.

500.000 jurkat cells were irradiated (2.5 J/cm^2) in the presence of the most active compounds, dissolved in HBSS. Then, the drug solution was replaced by RPMI medium and cells were incubated for 24 hours.

To verify lipidic peroxidation, after cell centrifugation, 900 µl of surnatant were collected and put at 253 K after having added 90 µl of 2,6-di-tert-butyl-*p*-cresol (BHT, 2% in absolute ethanol). Cells were washed, resuspended in 500 µl of water.400 µl of cells were lysed with 400 µl of SDS (1% in water) and vortexed.

This suspension was divided into two aliquots: in 500 µl were added 50 µl of BHT; 300 µl were used for protein quantification with Peterson method. Lipid peroxidation was measured using a thiobarbituric acid assay as described by Morliere *et al*.[16]

A standard curve of 1,1,3,3 tetraethoxypropane was used to quantify the amount of produced malondialdehyde. Data were expressed in terms of nanomoles of TBARS normalized to the total protein content in an aliquot of the cell extract.

8) PROTEIN OXIDATION

Solutions of Bovine serum albumin (BSA) (0.5 mg/ml) in phosphate buffer 10 mM were irradiated in the presence of 20 µM compounds for various times in a quartz cuvette.

At each time, the tryptophan (Trp) content was followed by monitoring the characteristic Trp fluorescence as described by Balasubramanian and Ziegler (1990).

9) PROTEIN EXTRACTION AND WESTERN BLOTTING.

10^6 Jurkat cells were seeded in 24 well-plates; after 24h, they were irradiated in the presence of the most active compounds. After 24h from irradiation, cell extracts were prepared, resuspending cells in lysis buffer. After the cells were lysed on ice for 20 min, lysates were centrifuged at 10000g at 4 °C for 20 min. The protein concentration in the supernatant was determined.

Equal amounts of protein (20 µg) were resolved using 8-16 % gradient polyacrylamide precast gels (Thermo Scientific) and transferred on a nitrocellulose Hybond-p membrane (GE Healthcare). Membranes were blocked with 5% skim milk powder in Tween-PBS for at least 2 h. Membranes were incubated with primary antibodies against caspase-3, Bid and β-actin (all rabbit, 1:1000, Cell Signaling,), for 2 h.

Membranes were next incubated with peroxidase-labeled goat antirabbit IgG (1:3000, Cell Signaling) for 60 min.

All membranes were visualized using ECL Advance (GE Healthcare) and exposed to Hyperfilm MP (GE Healthcare).

4.2.2 RESULTS

1) SPECTROSCOPIC PROPERTIES

The absorption and spectra were collected in DMSO or in phosphate buffer (10 mM, pH = 7,2) and ε were calculated (Table 9).

Table 9

	λ_{MAX}	ε_{MAX}	$\varepsilon_{365\ nm}$
16d	390	15877	14965
16e	359	6504	6415
16f	388	12600	12365
16g	359	8616	8329
16h	362	15758	15578
16l	404	16353	9851
16m	403	17758	13063
16n	403	23583	13308
16o	403	17514	13243
16p	403	18827	13356
19d	384	14678	12547
19l	399	25446	13522
20d	354	35140	29643
20h	351	22871	17872
20l	365	27666	27666
20p	364	23987	23973

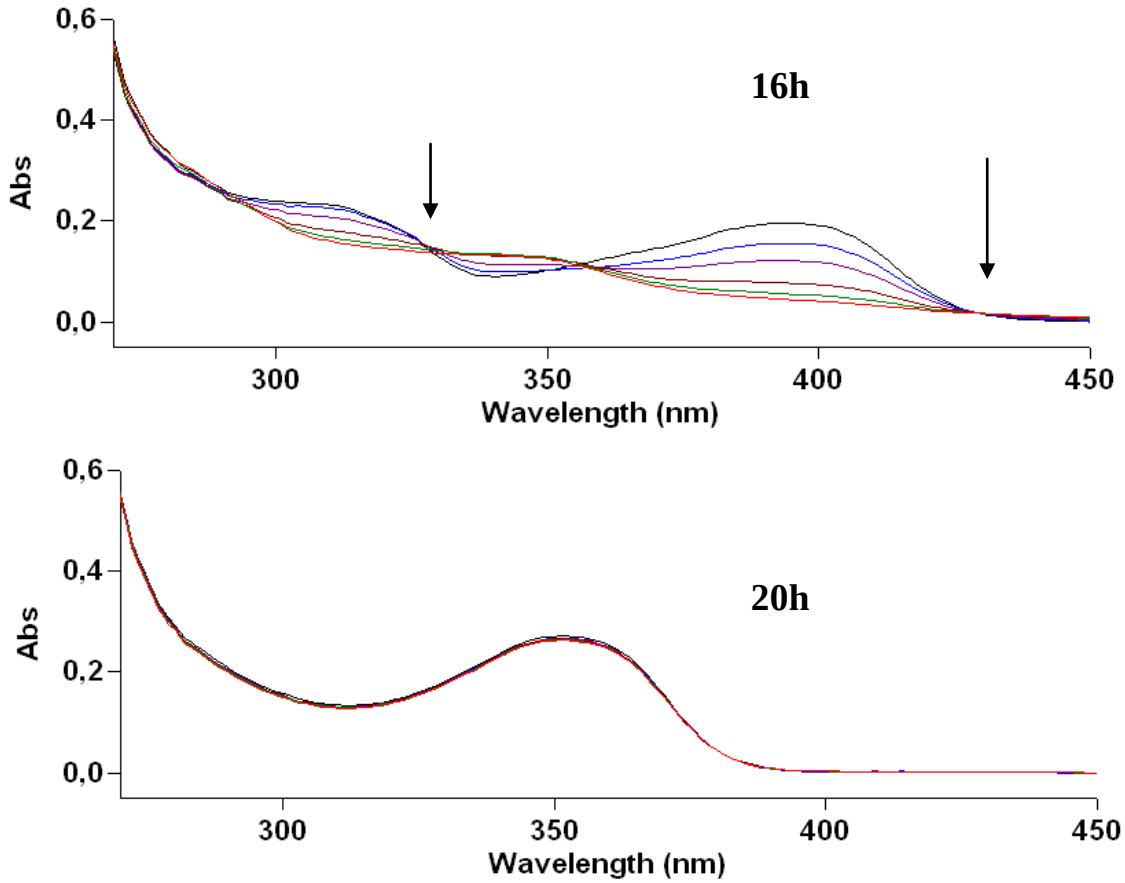
All compounds absorbed solar radiation spectrum and presented bands in UV-A region and this fact is fundamental for a photosensitizer. All compounds demonstrated quite high values of molar extinction coefficients in their λ_{MAX} but also at 365 nm.

2) PHOTOSTABILITY

When irradiated in solution, furocoumarins are subjected to photolysis. Thus, absorption spectra of 20 μM DP in DMSO were recorded after increasing the UV-A doses.

These spectra (Figure 10) gave information about the photostability of compounds and about the formation of other species as a consequence of irradiation. All photobleaching spectra were reported in the figure 10 below: **16h** and **20h** spectra were reported as representative.

Figure 10



16h spectra was deeply modified after increasing UV-A doses, with the reduction of maxima absorption and the onset of a new peak at 340 nm. On the contrary, **20h** was photostable.

3) CYTOTOXICITY AND PHOTOCYTOTOXICITY

The antiproliferative activity of the test compounds was evaluated on a panel of cultured human cell lines: A431 (EGFR-overexpressing carcinoma), Jurkat (T-cell leukemia) and K-562 (chronic myelogenous leukemia) cells.

No cytotoxic activity was found in leukemia cell lines by MTT test after 72 hours from the incubation with these compounds (Table 10). However, some molecules presented an antiproliferative activity on the carcinoma line.

Table 10

GI_{50} (µM)			
	A431	**Jurkat**	**K-562**
16d	2.31 ± 0.56	> 20	> 20
16e	> 20	> 20	> 20
16f	> 20	> 20	> 20
16g	> 20	> 20	> 20
16h	> 20	> 20	> 20
16l	8.29 ± 0.72	> 20	> 20
16m	> 20	> 20	> 20
16n	> 20	> 20	> 20
16o	5.22 ± 0.76	> 20	> 20
16p	> 20	> 20	> 20
19d	> 20	> 20	> 20
19l	> 20	> 20	> 20
20d	12.96 ± 1.50	> 20	> 20
20h	> 20	> 20	> 20
20l	> 20	> 20	> 20
20p	> 20	> 20	> 20

The phototoxicity tests were conducted in the same human tumour cell lines. These experiments were conducted as described in Materials and methods section. Briefly, we incubated cells with the test compounds for 30 min prior to the irradiation. We used two UV-A doses: 2.5 and 3.75 J/cm^2 for the carcinoma cell line and 1.25 and 2.5 J/cm^2 for the leukemia cell lines (Table 11). Then, the irradiated solution was replaced by cellular medium.

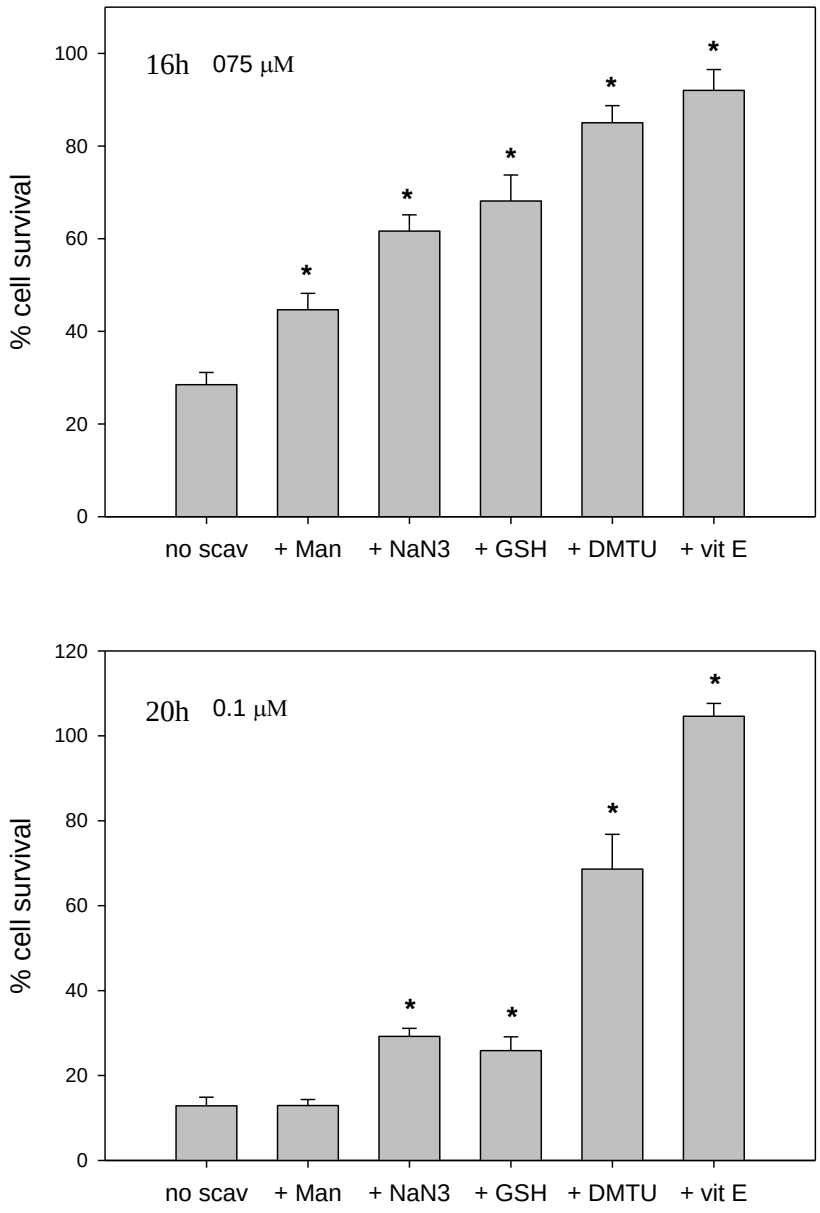
Table 11

GI_{50} (µM)						
	A431		Jurkat		K-562	
	2.5 J/cm^2	3.75 J/cm^2	1.25 J/cm^2	2.5 J/cm^2	1.25 J/cm^2	2.5 J/cm^2
16d	2.25 ± 0.28	1.78 ± 0.20	3.30 ± 0.44	1.00 ± 0.14	10.70 ± 1.87	3.90 ± 0.45
16e	0.47 ± 0.06	0.12 ± 0.02	0.30 ± 0.03	0.09 ± 0.01	0.40 ± 0.03	0.22 ± 0.01
16f	0.26 ± 0.01	0.09 ± 0.01	0.19 ± 0.02	0.09 ± 0.01	0.27 ± 0.02	0.19 ± 0.03
16g	0.28 ± 0.01	0.12 ± 0.02	0.24 ± 0.01	0.11 ± 0.03	0.47 ± 0.05	0.22 ± 0.03
16h	0.30 ± 0.06	0.07 ± 0.01	0.21 ± 0.03	0.05 ± 0.02	0.28 ± 0.03	0.16 ± 0.04
16l	8.75 ± 1.05	6.50 ± 0.50	> 20	> 20	> 20	> 20
16m	2.56 ± 0.25	0.84 ± 0.06	3.59 ± 0.21	0.43 ± 0.13	8.50 ± 0.83	1.85 ± 0.25
16n	4.00 ± 0.90	0.88 ± 0.03	1.90 ± 0.26	0.53 ± 0.04	7.68 ± 0.98	1.80 ± 0.20
16o	> 20	5.90 ± 0.94	> 20	> 20	> 20	> 20
16p	2.74 ± 0.23	0.85 ± 0.05	0.93 ± 0.07	0.47 ± 0.06	2.54 ± 0.35	0.53 ± 0.02
19d	1.95 ± 0.10	0.91 ± 0.08	1.15 ± 0.22	0.51 ± 0.06	3.53 ± 0.45	1.41 ± 0.25
19l	> 20	15.52 ± 0.89	> 20	7.08 ± 0.78	> 20	> 20
20d	1.22 ± 0.10	0.14 ± 0.02	0.25 ± 0.08	0.09 ± 0.02	0.63 ± 0.05	0.37 ± 0.04
20h	0.23 ± 0.02	0.09 ± 0.02	0.25 ± 0.04	0.08 ± 0.02	0.95 ± 0.23	0.19 ± 0.02
20l	2.63 ± 0.22	1.49 ± 0.04	1.96 ± 0.28	0.68 ± 0.07	5.76 ± 0.59	2.61 ± 0.29
20p	3.93 ± 0.78	0.87 ± 0.13	1.25 ± 0.15	0.46 ± 0.15	5.67 ± 0.52	1.64 ± 0.07
Angelicin	**5.09 ± 0.65**	**2.15 ± 0.35**	**n.a.**	**1.0±0,2**	**n.a.**	**1.2±0.1**

Almost all the compounds inhibited the proliferation of cells at submicromolar/micromolar concentrations. Some of them were really very phototoxic and presented GI_{50} in the nanomolar range. The most active compounds brought very lipophilic groups in position 1 and 2. The substitution of the carbonyl group in position 9 with a methoxyl group did not increase or reduce the phototoxic activity. For the following experiments the two most active compounds were chosen: **16h** and **20h**.

Phototoxicity experiments in the presence of some scanvengers can be very useful to identify which reactive species are involved in photosensitization. These tests were performed with the most active compounds using Jurkat cells. The radical scavengers reduced glutathione (GSH) and vitamin E (vit E); the hydroxyl radical scavengers dimethylthiourea (DMTU) and mannitol (MAN), and the singlet oxygen scavenger sodium azide (NaN_3) were employed. Jurkat were irradiated (2,5 J/cm^2) in the presence of 0.075 µM **16h** or 0.1 µM **20h**: we chose drug concentrations closed to GI_{50} values (Figure 11).

Figure 11



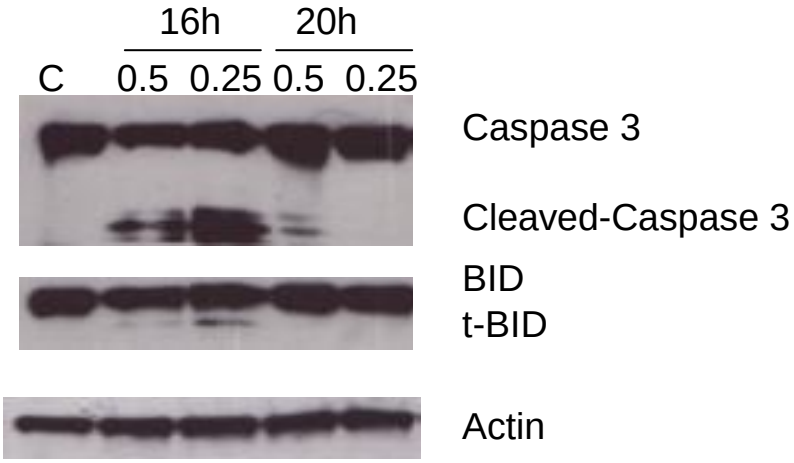
For **16h** and **20h**, the protective effect of scavengers was evident and non-specific. In particular, all scavengers seemed to demonstrate a significant protective effect in **16h** experiments but 80% of cell viability was assessed when the irradiation was conducted in the presence of vit E and DMTU, indicating alkyl and hydroxyl radicals may be involved in its phototoxicity. Irradiating Jurkat cells in the presence of vit E and **20h**, the percentage of cell survival reached the one of control. Significant protection was demonstrated when irradiation was carried out in the presence of sodium azide, glutathione and DMTU. However, from these data, an univocal mechanism of photosensitization was not distinguished but the complexity of the phototoxicity of these compounds was clear.

4) EVALUATION OF CELLULAR DEATH

The activation of caspase 3 is considered as a hallmark of apoptotic process. The levels of cleaved active subunits of executioner caspase-3 were evaluated by immunoblotting Jurkat cell lysates after 24 h from irradiation. No cleaved caspase 3 was detected in control cells, while the apoptotic process is clearly activated after 24 h from irradiation with **16h** at both concentrations and the highest concentration of **20h**. In particular, when irradiation was carried out in the presence of 0.5 μM **16h** the level of active caspase was lower than the one in the presence of 0.25 μM: this fact could be explained by the elevated damage with the highest concentration that could also induce cellular death by necrosis. Moreover, the activation of another important apoptotic protein such as BID, was evaluated. Following TNFα or Fas treatment, BID, a BH3-domain-only molecule is cleaved at its amino terminus. Cleavage of cytosolic p22 BID by caspase-8 generates a p15 carboxy-terminal fragment that translocates to the mitochondria. Truncated p15 BID (tBID) inserts into the membrane and is required for cytochrome *c* release from the mitochondria.

The presence of t-BID was found in the samples irradiated in the presence of **16h** (Figure 12). This can supports the possibility of a involvement of mitochondria in cellular death.

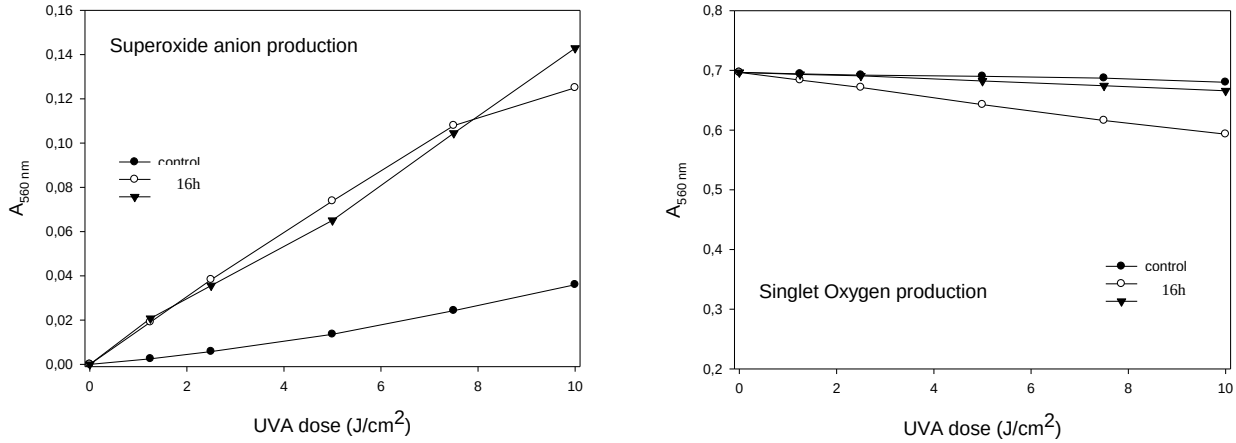
Figure 12



5) DETERMINATION OF REACTIVE OXYGEN SPECIES

Production of reactive oxygen species is one of the mechanisms underlying drug induced photosensitization damage. Both compounds generated the superoxide anion efficiently under UVA irradiation. Singlet oxygen production was very low and was increased in comparison to control only when irradiation was carried out in the presence of **16h** (Figure 13).

Figure 13

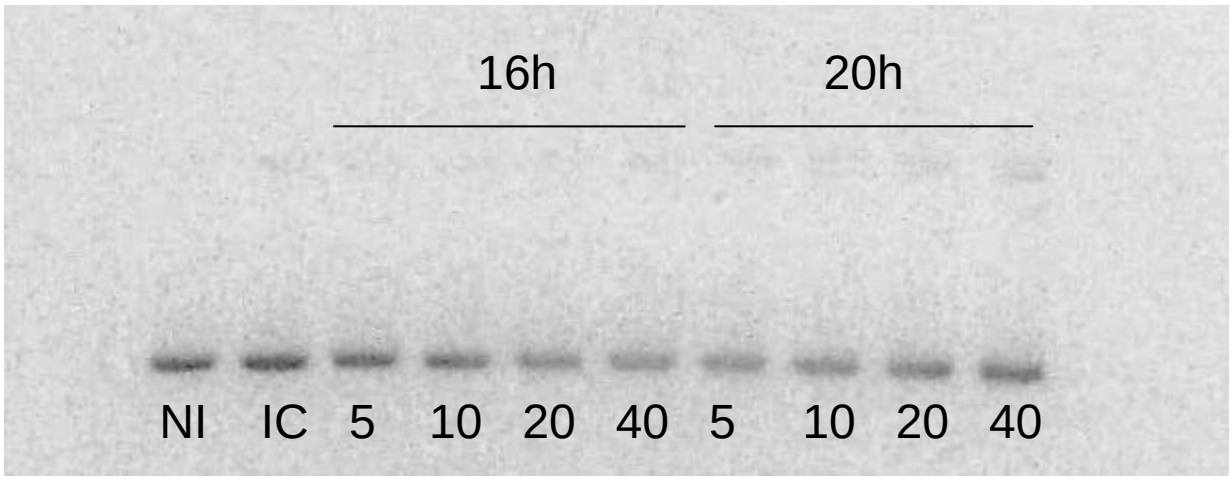


6) DNA PHOTOCLEAVAGE

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. Further cleavage of the other strand within a short distance of the first site of cleavage generates a linear (L or III form) DNA.

The irradiation of supercoiled plasmid pBR322 was carried out in the presence of various rate [DNA]/[DP]. DNA samples were resolved by an electrophoretic run in agarose 1% gel thanks to the different hydrodynamic properties of the plasmidic forms (Figure 14).

Figure 14

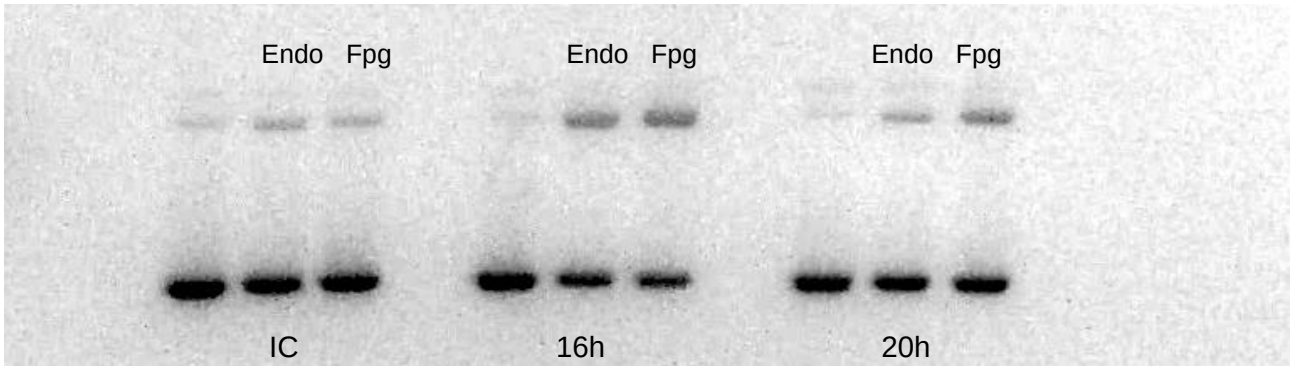


The results pointed out that the new compounds did not provoke any formation of open circular or linear bands, indicating that they did not sensitize single strand breaks.

In addition to frank strand breaks, the oxidative damage of purine and/or pyrimidine bases was also evaluated using the base excision repair enzymes Formamido pyrimidin glycosilase (Fpg) and Endonuclease III (Endo III), respectively. DNA strand breaks can be induced either directly (frank strand breaks) or indirectly using DNA repair enzymes. Fpg demonstrated to recognize 8-hydroxy guanine, purines whose imidazole ring was open (Fapy residues) and sites of base loss (apurinic sites). Endonuclease III recognized 5,6-dihydropyrimidine derivatives, in addition to apurinic sites. Damaged base recognition was followed by an elimination step, resulting in DNA breakage.

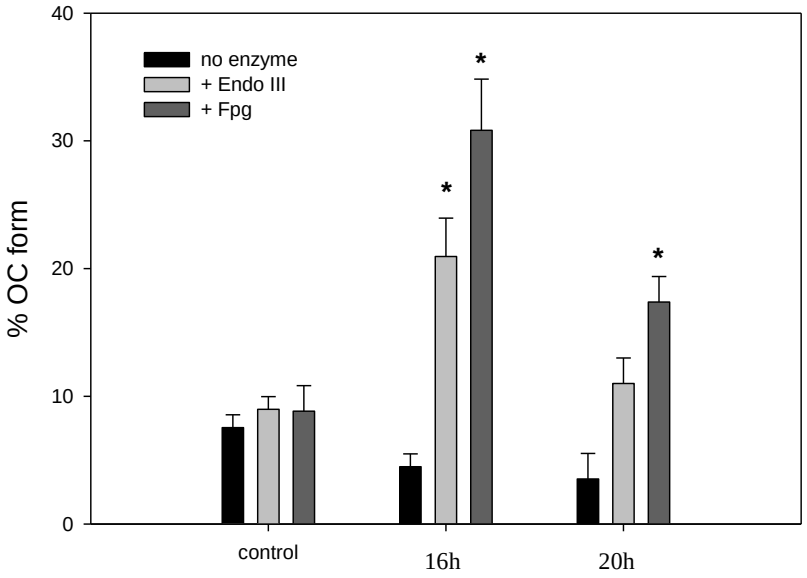
Thus, after the irradiation in the presence of 40 μM **16h** and **20h**, pBR322 was incubated with Fpg or Endo III at 37°C for 30 min. Then, DNA samples were resolved by electrophoretic run as described in Material and Methods section (Figure 15).

Figure 15



The treatment with the two base excision enzymes revealed that they were able to photooxidize DNA bases in a great amount. The percentages of OC form were calculated as suggested by *Ciulla et al.*[17] and were summarized in the following figure 16:

Figure 16

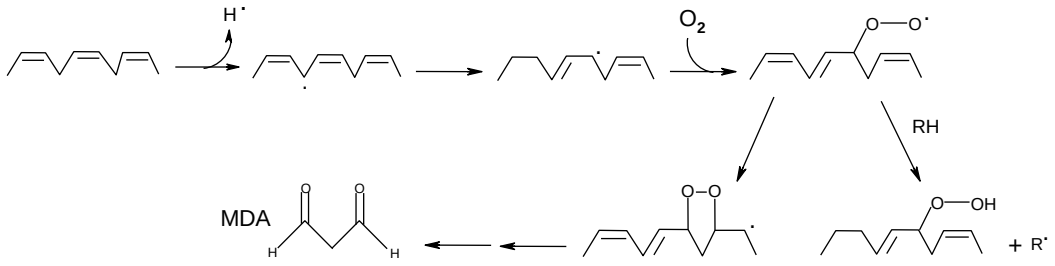


All compounds were able to photoinduce base oxidation but **16h** resulted the most active especially in the presence of Fpg, indicating purinic bases were its preferential targets.

7) LIPID PEROXIDATION

Like nucleic acids and proteins, unsaturated lipids, glycolipids and cholesterol are important targets of $^1O_2$ or free radical attack in cells subjected to photooxidative stress. That photodamage develops as lipid peroxidation. This phenomenon is initiated by the attack of any chemical species that has sufficient reactivity to abstract a hydrogen atom from a methylene carbon in the side chain. The hydrogen atom is a free radical and its removal leaves behind an unpaired electron on the carbon to which it was originally attached. That new radical can give a peroxyl radical after the reaction with oxygen. Peroxyl radicals can combine each other or they can attack membrane proteins, but also are capable of abstracting hydrogen from adjacent fatty acid side chains in a membrane and so propagating the chain reaction of lipid peroxidation (Figure 17).

Figure 17

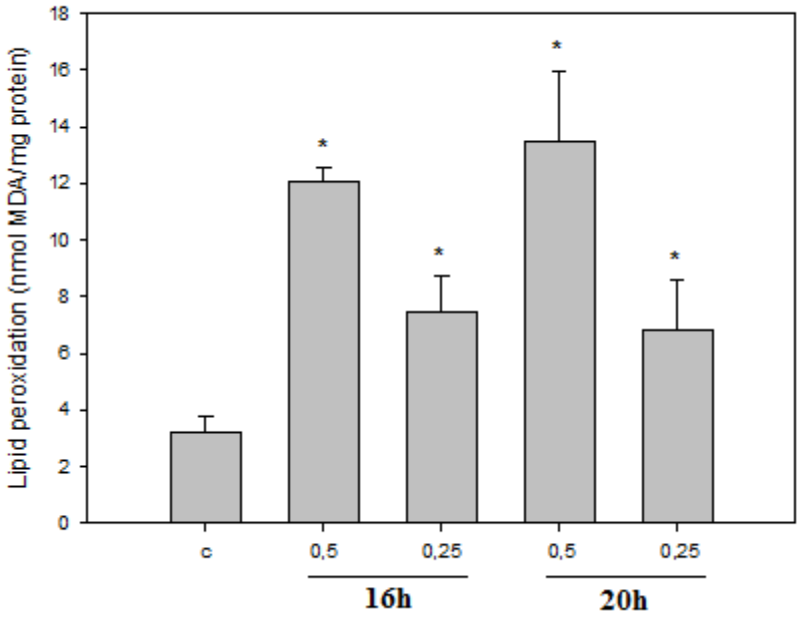


Schematic representation of lipidic peroxidation

Membrane lipids may be a central site of photodamage if sensitizing agents localize in the membrane bilayer. Being hydrophobic, these compounds may be expected to be localized mainly in plasmatic and/or in subcellular membranes, making these structures particularly sensitive to photodamage.

The thiobarbituric assay (TBA test) was used in order to determine a potential lipid peroxidation after irradiation of jurkat cells in the presence of test compounds. The TBA test was performed as described in the experimental section. In brief, this assay used the reaction of one molecule of malondialdehyde (MDA), which is a secondary product of lipid peroxidation, with two molecules of TBA, forming a pink chromogen. Thus, the resulting chromogen was monitored by fluorescence at 553 nm (Figure 18).

Figure 18



Thiobarbituric reactive substances (TBARS) were significantly produced in a concentration-dependent manner when the cells were exposed to the compounds and UV-A. Thus, lipid peroxidation could be important in the mechanism of phototoxicity.

8) PROTEIN PHOTOREACTION

In order to investigate more deeply photosensitizing properties of the title compounds towards proteins, solutions containing bovine serum albumin (BSA) as model and the compounds in phosphate buffer were irradiated several times. The amount of the aromatic aminoacid tryptophan (Trp) was directly analysed by monitoring the characteristic fluorescence of Trp residues. No

significant Trp photoreaction was assessed by fluorescence in comparison to control (data not shown): thus, proteins were likely marginally involved in their phototoxicity.

9) CONCLUSION

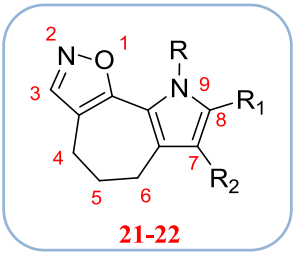
All the molecules showed high photocytotoxicity at the tested concentrations. In particular, **16e-h** and **20h** show the best values of photoactivity, that reach in some cases the nanomolar range, being far more active than Angelicin used as reference drug, and also more active than the lead compounds **3** (compare Table 6 with Table 11). Phototoxicity was find to be UV-A dose and concentration dependent.

SAR studies indicates that all the compounds belonging to the ethoxycarbonyl series are more active than the unsubstituted series (compare **16d-h** with **16l-p**). Moreover, within the two series, the presence of a big lipophilic group like the benzyl or a methoxy benzylgroup, increases the value of activity. Furthermore the further functionalization of carboxamide of 2-pyridone ring, gave a little increase in the value of activity (compare **16d** with **19d** and **20d** and compare **16l** with **20l**). Unfortunaly, biological tests show also that this class of compounds, were able to photoinduce DNA bases oxidation, especially the purinic bases, at difference of the class of compounds **3**[10,11] (also psoralens and angelicin cause photodamage).

5. PYRROLO[2',3':3,4]CYCLOHEPTA[1,2-*d*][1,2]OXAZOLE

5.1 Synthesis

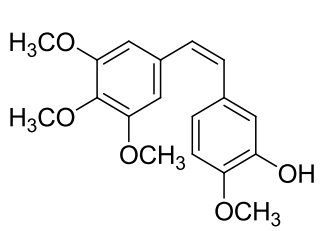
An extension of the project, concerns the synthesis of pyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazoles **21-22**.



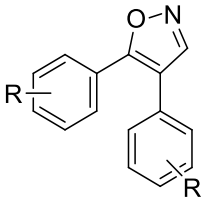
21-22

In the literature, there are many example of compounds bearing the oxazole core that showed interesting biological activity (Figure 19). Combretastatin CA-4 is an inhibitor of the polimerization of tubulin studied as antitumor drug. Combretastatin and its analogues with a diaryl oxazole structure, are significant examples of oxazole active compounds. These were synthesized in order to block the -C=C- bond in the *cis* geometry that is the most active one; some of them showed inhibition activity with IC_{50} in the nanomolar *range* against different tumor cell lines. The mechanism involved G_2/M phase arrest of the cell cycle.[18,19] Furthermore some pteridinic compounds bonded to an oxazole ring, showed inhibition of cellular growth when tested on MCF-7, NCI-H460 and SN-268.[20]

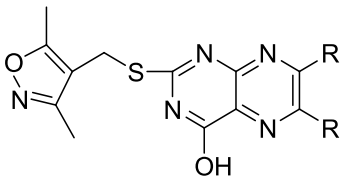
Figure 19



COMBRETASTATIN CA-4



4-5 DIARYLOXAZOLE

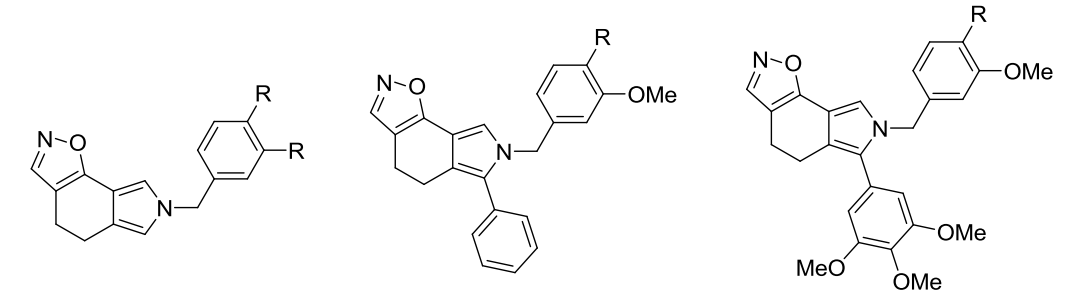


PTERIDINIC COMPOUNDS

In the light of this results, in our research group in the past years, was thought to cyclize the oxazole ring on the isoindole core, obtaining the new heterocyclic system oxazole[5,4-*e*]isoindole (Figure 20). The new derivatives tested by NCI of Bethesda in order to evaluate the antiproliferative activity on a panel of 60 human cell lines divided into 9 subpanels (breast, ovaries, lung, colon, CNS, melanoma, leukemia, kidney, prostate), showed antiproliferative activity from micro to nanomolar range. In particular, the oxazoles **SIX2** (GI_{50} 0.2-17 μM) and **SIX13** (GI_{50} 0.04-48 μM) were the most interesting of the first series of derivatives, active on all the tumor cell lines tested with GI_{50} values from submicro to nanomolar (0.04 - 48 μM) with a Mean Graph Mid-Point (pMG_MID) of

6.68 and 5.80 (Figure 20). Moreover a further extension of this series, led to derivatives with comparable activity such as **SIX 2D** (pMG_MID = 6.62) **SIX 13O** (pMG_MID = 6.61)**,** and **SIX 13S** (pMG_MID = 6.41), or even more active such as **SIX 13U** (pMG_MID = 7.19).

Figure 20



SIX 2 (R = H) MID = 5.80
SIX 2D (R = OMe) MID = 6.62

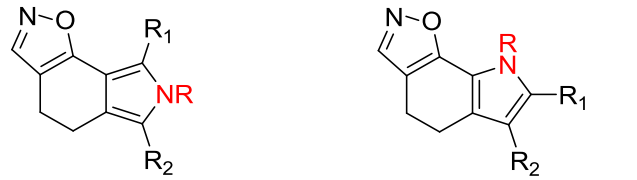
SIX 13 (R = H) MID = 6.68
SIX 13O (R = NH_2) MID = 6.61

SIX 13S (R = H) MID = 6.41
SIX 13U (R = NH_2) MID = 7.19

On derivatives **SIX 2** and **SIX 13** the NCI performed the **Hollow Fiber Assay** (HFA) a *in vivo* assay in which was evaluated the maximum tolerated dose (MTD), and on the derivate **SIX 13** was performed a **COMPARE** analysis in which the compound was compared with a "**standard agent database**" giving an indication of a possible mechanism of action. The results of this analysis, showed a good correlation index with the antimitotic agent taxol. Studies to evaluate the mechanism of action are in fact underway to confirm the possible molecular target.

Given the high efficacy shown by these compounds, the new class of [1,2]oxazolo[4,5-*g*]indole was studied, in which the isoindole scaffold has been replaced by an indole (Figure 21).

Figure 21

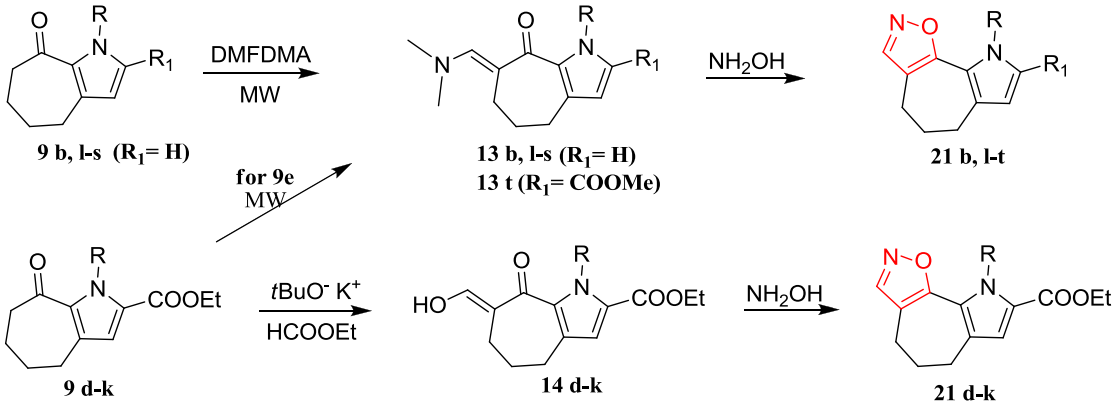


[1,2]oxazolo[5,4-*e*]isoindole [1,2]oxazolo[4,5-*g*]indole

Also in this case the new derivatives tested by NCI of Bethesda, gave positive GI_{50} values against almost all the tested human cell lines, showing GI_{50} values in the micromolar - nanomolar range. [21] An evaluation of the results, indicated that the presence of a 3,5-dimethoxy-substituted benzyl moiety at the indole nitrogen appeared crucial in conferring good activity to the [1,2]oxazolo[4,5-*g*]indole derivatives. In fact, the most active compounds showed mean graph mid-points (pMG_MID) of 6.60 and 6.33. From here the idea to try the synthesis of new oxazole derivatives on our scaffold, using as lead compounds the 3,5-dimethoxybenzyl[1,2]oxazolo[4,5-*g*]indole with the

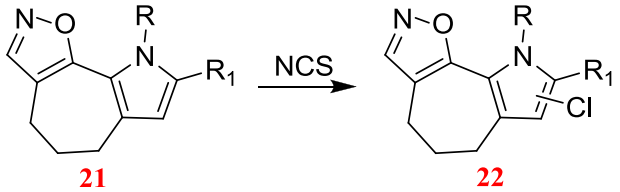
aim of obtaining more potent antiproliferative agents. Depending on the substitution pattern of the pyrrole ring, the new tricyclic derivatives pyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazole **21-22,** were obtained starting from our enaminoketones **13** or hydroxymethylketones **14** by reaction with hydroxylamine as dinucleofile. In fact, ketones of type **9b,l-s** belonging to unsubstituted series, were first converted into enaminoketones **13**, and then subjected to ring closure with hydroxylamine hydrochloride to give the desired oxazole **21b, l-s**. Regarding the ethoxycarbonyl series, it was more convenient to start from hydroxymethylenketones **14** obtained by reaction of the appropriate ketones of type **9d-k**, in toluene, using *tert*-butoxide as a base and then ethyl formate (Scheme 11). The formyl-derivatives **14d-k**, proved to be quite difficult to handle, in fact even small variations in the work up procedure, or in the reaction itself, led to big decrease in yield. Anyway they were obtained with good yields (60-90%). Also in this case the subsequent cyclization was achieved with hydroxylamine in refluxing ethanol to give the desired compounds **21d-k**. Also the derivate **21t** was obtained, using the intermediate **13t** obtained from the reaction of transesterification of **9e** with an excess of DMFDMA under microwave conditions.

Scheme 11



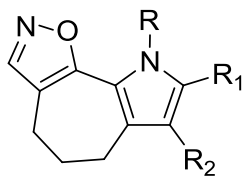
In order to complete the substitution pattern to pyrrole, and to verify the effect on biological activity, the oxazoles **21** were subjected to smooth chlorination with N-chlorosuccinimide in anhydrous DMF for one night at room temperature. Mixed results were obtained from the two series of compounds (Scheme 12).

Scheme 12



In fact, while in the case of products **21d-k** belonging to the ethoxycarbonyl series, only the position 7 was available for the reaction of addition of chlorine, in the case of the products of type **21b, l-s**, both the two pyrrole positions were available, and from the reaction mixture the 7-chloro and 8-chloro derivatives were obtained, showing identical Rf values. Unfortunately also by using high pressure chromatography apparatus (Biotage), we were able to isolate only in the case of the N-Methyl derivative **21l**, the compound bearing the chlorine in position 8 (**22l**), as pure compound and in lower yield.

Table 12



21 - 22

	R	R_1	R_2	Yield
21b	SO_2Ph	H	H	60 %
21d	Me	COOEt	H	90 %
21e	Bn	COOEt	H	60 %
21f	2-OMe-Bn	COOEt	H	60 %
21g	3-OMe-Bn	COOEt	H	82 %
21h	4-OMe-Bn	COOEt	H	81 %
21i	2,5-$(OMe)_2$-Bn	COOEt	H	74 %
21j	3,5-$(OMe)_2$-Bn	COOEt	H	82 %
21k	3,4,5-$(OMe)_3$-Bn	COOEt	H	72 %
21l	Me	H	H	75 %
21m	Bn	H	H	83 %
21n	2-OMe-Bn	H	H	70 %
21o	3-OMe-Bn	H	H	83 %
21p	4-OMe-Bn	H	H	68 %
21q	2,5-$(OMe)_2$-Bn	H	H	78 %
21r	3,5-$(OMe)_2$-Bn	H	H	76 %
21s	3,4,5-$(OMe)_3$-Bn	H	H	74 %
21t	Bn	COOMe	H	72%
22d	Me	COOEt	Cl	50 %
22e	Bn	COOEt	Cl	57 %
22f	2-OMe-Bn	COOEt	Cl	74 %
22g	3-OMe-Bn	COOEt	Cl	65 %
22h	4-OMe-Bn	COOEt	Cl	70 %
22i	2,5-$(OMe)_2$-Bn	COOEt	Cl	68 %
22j	3,5-$(OMe)_2$-Bn	COOEt	Cl	70 %
22l	Me	Cl	H	49 %

Table 12: Pyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazole **21-22**.

5.2 Pyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazole

Anticancer activity

The final series of 26 compounds (Table 12) was submitted to the US National Cancer Institute (NCI) in Bethesda for cell-based antiproliferative screens. Among this, 18 compounds, (with the exception of **21i,n,o,q,r,** and **22f,g,i**), were selected for the one-dose (10^{-5}M) prescreening on the full panel of 60 human tumor cell lines divided into nine subpanels (leukemia, non-small-cell lung, colon, central nervous system, melanoma, ovarian, renal, prostate, and breast cancers).

Six compounds (**21f,g,j,k,** and **22e,j**) were then further selected for evaluation on the full panel at five doses (10^{-4}–10^{-8}M). Three of the selected compounds (**21g,k** and **22j**) gave positive GI_{50} values against all tested human cell lines, while **21f, 21j** and **22e**, were not responsive only against one or few cell lines (Table 13).

Table 13

Cpd	N. of cell investigate	N. of cell with positive GI_{50}	Range [μM]	pMG_MID
21f	56	50	0.30 – 46.2	5.35
21g	56	56	0.15 – 18.7	5.84
21j	56	55	0.01 – 13.4	7.10
21k	55	55	0.01 – 64.9	6.70
22e	55	46	1.29 – 5.89	5.15
22j	57	57	0.03 – 27.0	6.39

Table 13 Overview of the antitumor screenings of **21f,g,j,k,** and **22e,j.**

An evaluation of the data listed in Table 14 indicates a good potential of this class of compounds showing growth inhibitory activity reaching the nanomolar range. In particular, the presence of a 3,5-dimethoxy-substituted benzyl moiety at the indole nitrogen (position 9) appears crucial for conferring good activity to the pyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazole derivatives. In fact, the most active compounds were **21j**, **21k** and **22j**, having mean graph mid-points (pMG_MID) of 7.10, 6.70 and 6.39 respectively. All this compounds belong to the ethoxycarbonyl series. From a structure–activity point of view, the addition of a methoxy group (**21k**) in position 4, led to a slight decrease in activity, while the removal of one methoxy group (**21f, 21g**) produce a bigger decrease in the activity (compare **21j**, pMG_MID = 7,10, **21k**, pMG_MID = 6.70, **21f**, pMG_MID = 5.35, and **21g**, pMG_MID = 5.84). Moreover the presence of chlorine in 7 position, slightly reduce the activity with respect of corresponding non halogenated derivatives (compare **21j**, pMG_MID = 7,10, with **22j**, pMG_MID = 6.39).

Table 14

Cell lines	21f	21g	21j	21k	22e	22j	Cell lines	21f	21g	21j	21k	22e	22j
Leukemia							M14	3.67	0.54	0.05	0.15	3.78	0.26
CCRF-CEM	2.87	2.17	0.05	0.30	2.21	0.25	MDA-MB-435	0.35	0.15	0.01	0.02	1.40	0.03
HL-60(TB)	3.04	0.53	0.03	0.19	3.32	0.31	SK-MEL-2	-	0.56	0.04	-	-	0.22
K-562	1.59	0.39	0.04	0.13	3.68	0.29	SK-MEL-28	>100	5.12	0.09	0.30	-	0.66
MOLT-4	5.68	2.30	0.26	0.39	3.58	0.58	SK-MEL-5	2.61	0.75	0.04	0.17	3.53	0.49
RPMI-8226	4.11	1.89	0.05	0.38	3.27	0.39	UACC-257	-	7.61	-	-	-	-
SR	0.43	0.28	0.03	0.04	1.80	0.07	UACC-62	3.37	0.51	0.07	0.05	4.01	0.11
Non-Small Cell Lung Cancer							**Ovarian Cancer**						
A549/ATCC	-	0.76	0.06	-	-	-	IGROV1	1.99	1.16	0.06	0.06	4.39	0.34
EKVX	-	-	-	-	-	-	OVCAR-3	0.87	0.30	0.02	0.03	3.13	0.19
HOP-62	1.89	4.34	-	0.17	4.35	0.29	OVCAR-4	9.35	18.7	13.4	0.72	-	0.85
HOP-92	0.30	4.21	-	-	-	0.46	OVCAR-5	>100	5.94	0.18	0.53	>100	-
NCI-H226	>100	21.0	7.98	1.21	>100	9.71	OVCAR-8	3.85	3.13	0.08	0.24	-	0.41
NCI-H23	8.52	-	-	0.37	-	0.68	NCI/ADR-RES	1.36	0.43	0.03	0.06	2.83	0.20
NCI-H322M	5.23	-	-	0.32	>100	0.51	SK-OV-3	2.70	2.83	0.05	0.13	>100	0.24
NCI-H460	3.88	3.53	0.04	0.23	3.62	0.35	**Renal Cancer**						
NCI-H522	0.35	0.23	0.02	0.01	1.29	0.03	786-0	>100	6.74	0.05	0.97	-	4.24
Colon Cancer							A498	3.46	0.44	0.02	0.11	4.38	0.17
COLO 205	0.87	0.58	0.03	0.04	3.53	0.17	ACHN	16.6	3.40	0.06	0.29	5.89	0.94
HCC-2998	>100	4.18	0.23	0.30	-	-	CAKI-1	3.51	2.31	0.05	0.07	3.51	0.28
HCT-116	3.79	0.47	0.04	0.19	4.02	0.43	RXF 393	2.08	0.67	0.02	0.12	-	0.25
HCT-15	2.24	0.45	0.04	0.16	3.39	0.37	SN12C	>100	3.51	0.07	0.76	-	0.83
HT29	0.91	0.36	0.03	0.07	2.95	0.27	TK-10	67.8	11.2	>100	64.9	>100	10.3
KM12	2.10	0.46	0.03	0.05	3.70	0.32	UO-31	6.53	3.37	0.05	0.08	-	0.78
SW-620	2.02	0.48	0.04	0.14	3.81	0.32	**Prostate Cancer**						
CNS cancer							PC-3	2.48	2.35	0.04	0.17	3.16	0.29
SF-268	46.2	6.71	0.05	0.52	>100	1.91	DU-145	5.27	2.18	0.04	0.28	>100	0.35
SF-295	1.38	0.62	0.03	0.44	>100	0.06	**Breast Cancer**						
SF-539	2.48	1.14	0.03	0.12	-	0.24	MCF7	0.71	0.37	0.03	0.04	3.21	0.10
SNB-19	64.7	8.23	0.15	0.56	>100	0.52	MDA-MB-231/ ATCC	9.12	1.84	0.24	0.48	3.51	0.84
SNB-75	1.97	1.60	0.03	0.07	-	0.22	HS 578T	3.38	1.60	0.04	0.34	-	0.45
U251	3.78	-	-	0.14	-	0.31	BT-549	7.73	0.98	1.72	0.29	-	27.0
Melanoma							T-47D	2.46	2.00	-	0.10	2.69	0.44
LOX IMVI	7.64	1.75	0.05	0.41	5.26	0.88	MDA-MB-468	2.95	0.34	0.03	0.74	-	0.73
MALME-3M	3.08	0.89	-	0.05	3.68	0.20	The values of GI_{50} are expressed in micromolar.						

Table 14: Inhibition *in vitro* of tumor cell growth by **21f,g,j,k,** and **22e,j.**

Figure 22

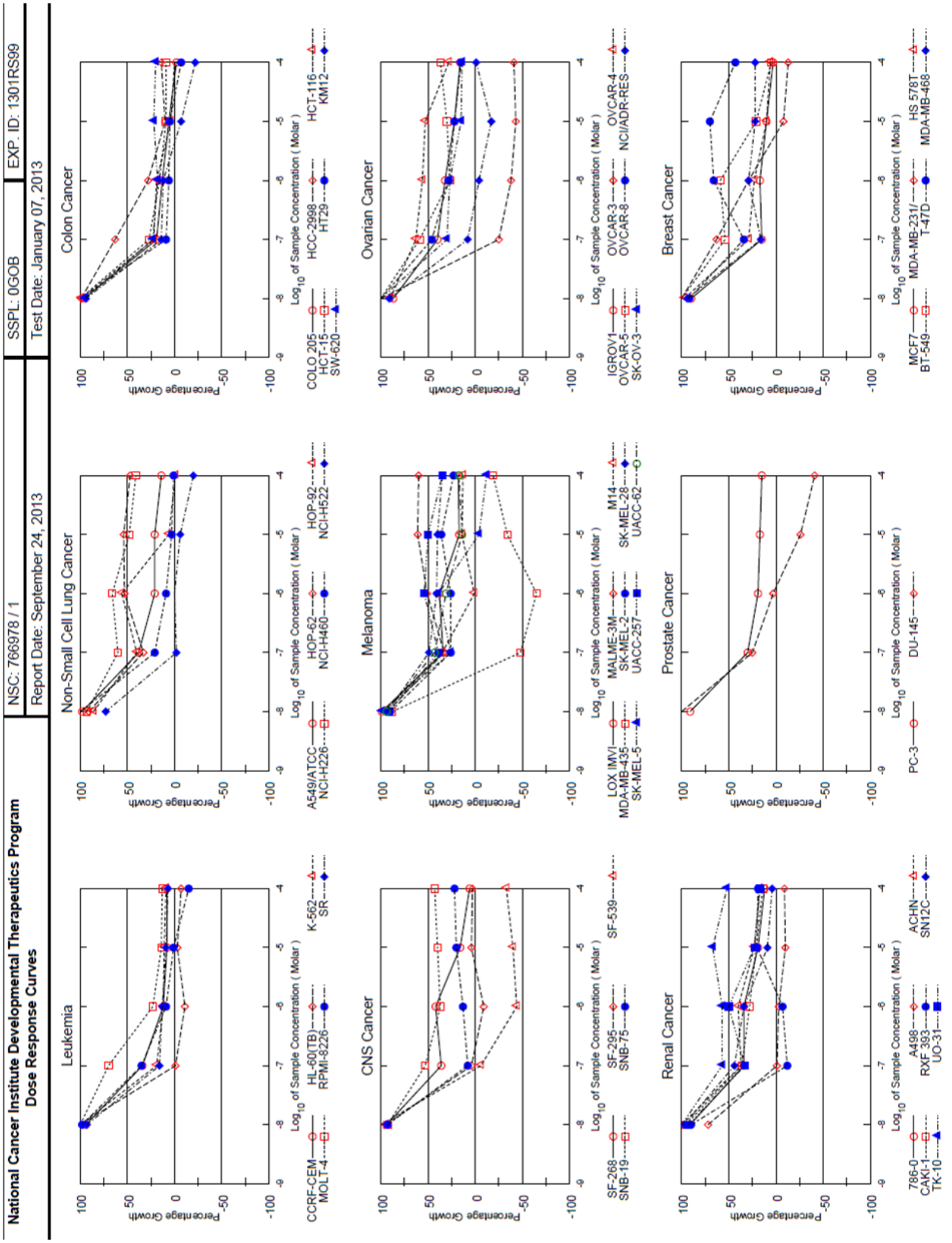


Figure 22 Dose Response Curves of **21j**

Figure 23

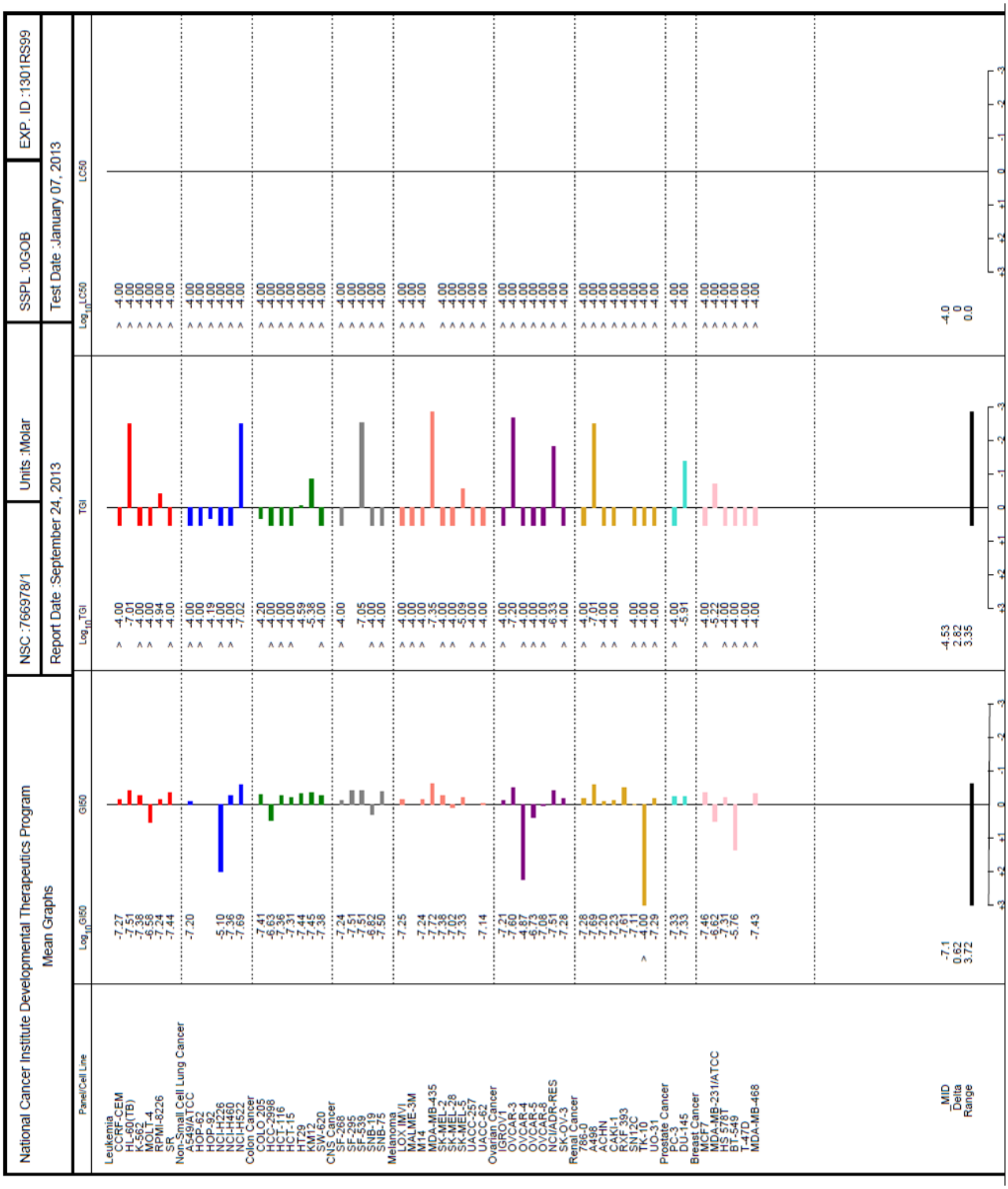


Figure 23 Mean Graphs of **21j**

Figure 24

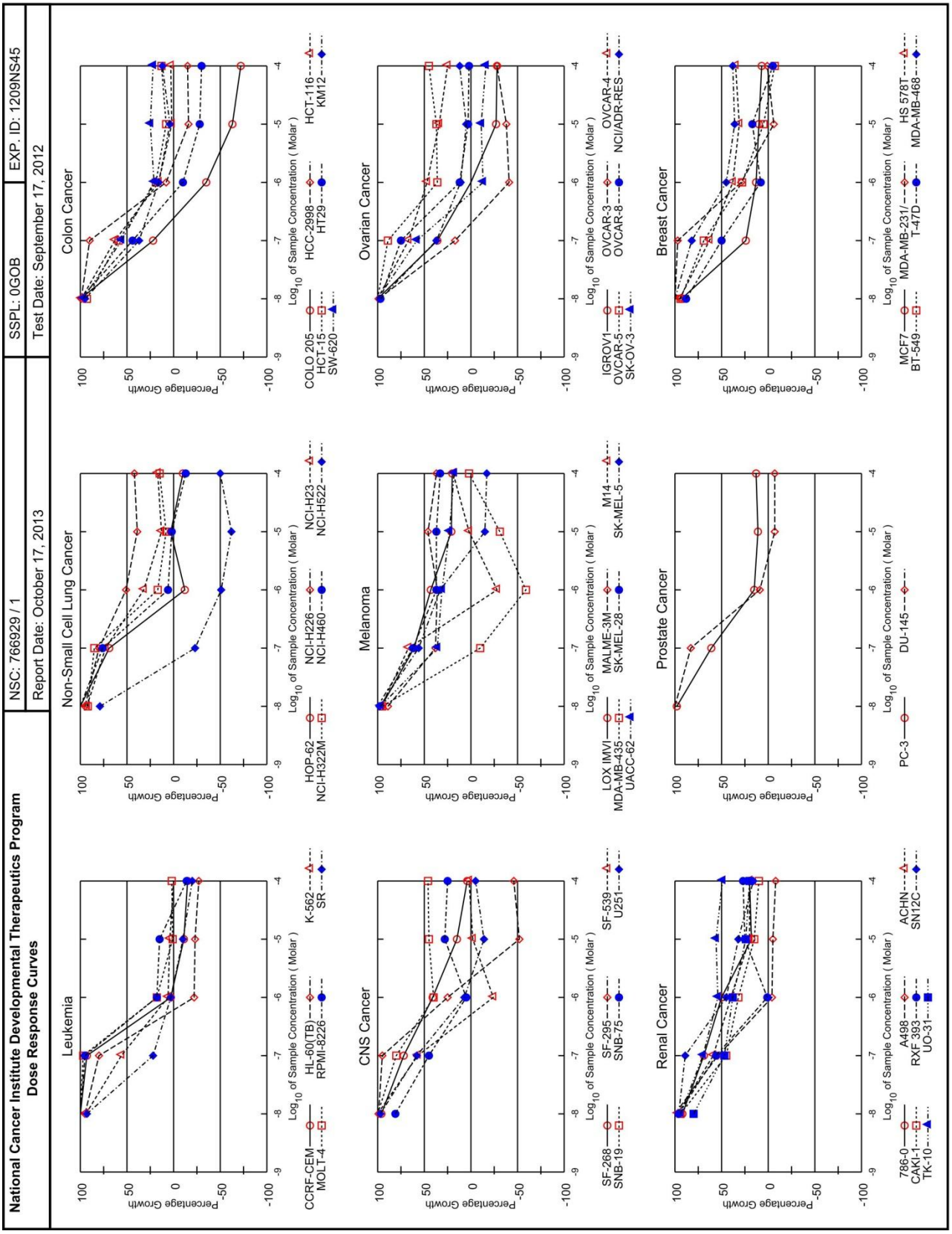


Figure 24 Dose Response Curves of **21k**

Figure 25

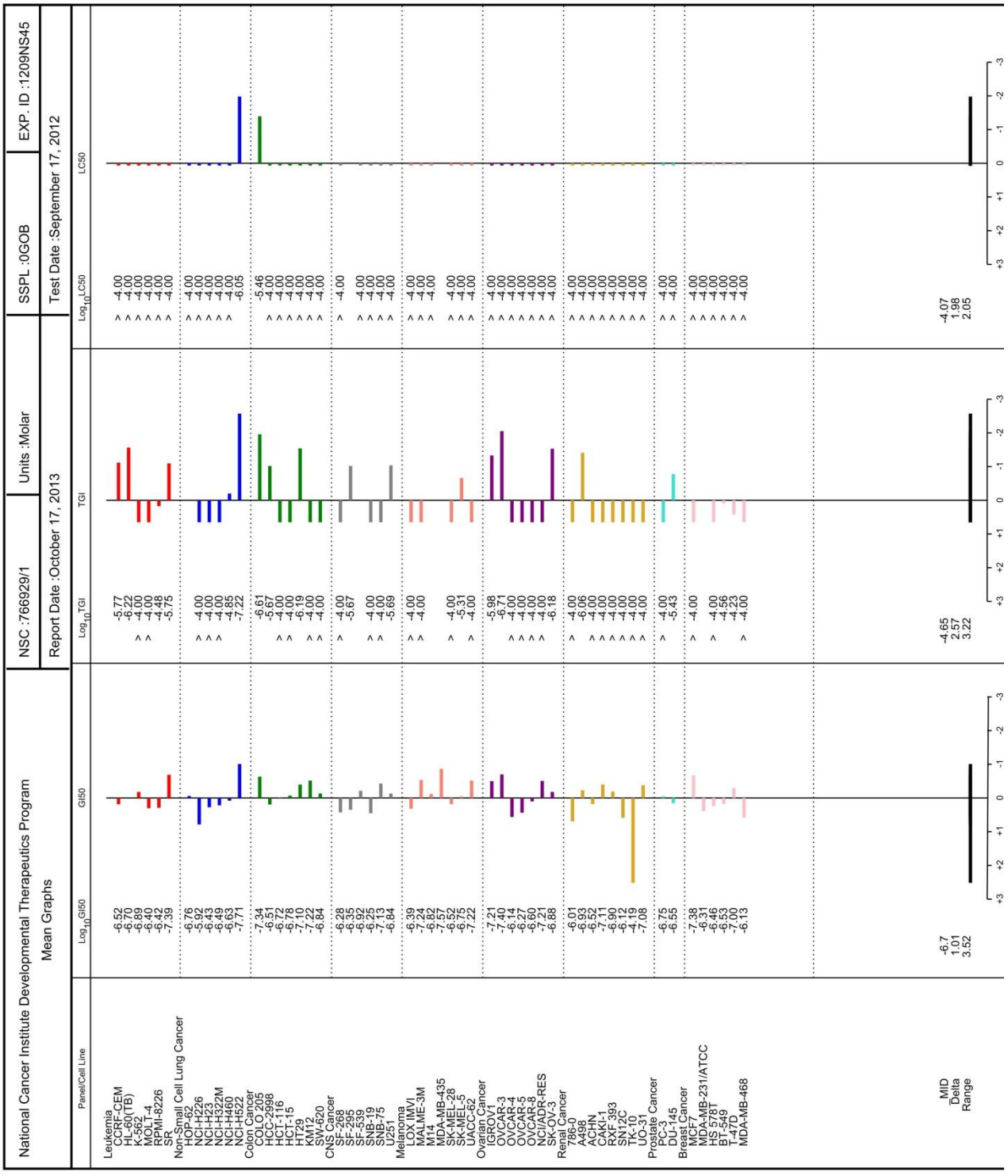


Figure 25 Mean Graphs of **21k**

Analysis of the GI_{50} values listed in Table 14 indicates that the most active compound, **21j**, was particularly effective against the colon cancer, CNS cancer, melanoma, prostate and renal cancer subpanels (Figures 22 and 23). In fact, the calculated pMG_MID value for the single subpanels was in some cases higher than the overall cell lines pMG_MID value (for example prostate 7.33, colon 7.16, CNS 7.21, melanoma7.25).

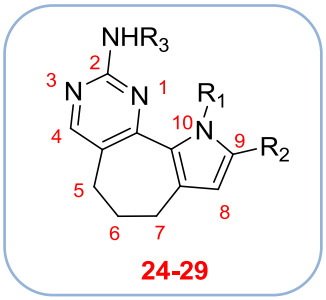
The most sensitive cell lines were MDA-MB-435 of melanoma subpanel and A498 of renal cancer subpanel, having GI_{50} values in the nanomolar range, 19 and 20 nM, respectively. Compound **21k** was also highly selective against the leukemia, colon cancer, CNS cancer, melanoma, ovarian cancer and breast subpanels, having subpanel GI_{50} values at sub-micromolar levels, 0.04 – 0.39 μM, 0.04 – 0.59 μM, 0.07 – 0.56 μM, 0.02 – 0.41 μM, 0.03 – 0.72 μM, 0.04 – 0.74 μM respectively (Figures 24 and 25). The same compounds, however, got responses in the nanomolar range also in other cell lines belonging to different subpanels: just for example the cell line NCI-H522 of the non-small cell lung cancer subpanel (20 nM for **21j** and 19 nM for **21k**), OVCAR 3 of the ovarian cancer subpanel (25 nM for **21j**), MCF7 of breast cancer subpanel (24 nM for **21j**) and CAKI-1 of renal cancer subpanel (78 nM for **21k**) and moreover.

In conclusion, was reported a method for the synthesis of derivatives of the new ring system pyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazole. The antiproliferative activity exhibited by derivatives **21f,g,j,k,** and **22e,j** against the totality of the NCI full panel of human tumor cell lines makes this class of compounds interesting for further studies. In particular, the potent activity of compounds **21j, 21k** and **22j** encourages the synthesis of new derivatives and makes them lead compounds for new classes of compounds with the aim of obtaining more potent antiproliferative agents. The mechanism of action is under investigation by the “Istituto Tumori di Milano” on a big panel of kinases.

6. PYRROLO[3',2':6,7]CYCLOHEPTA[1,2-*d*]PYRIMIDIN-2-AMINE

6.1 Synthesis

Continuing our studies on tricyclic systems, we set the synthesis of pyrrolo[3',2':6,7]cyclohepta[1,2-*d*]pyrimidin-2-amine **24-29**.



24-29

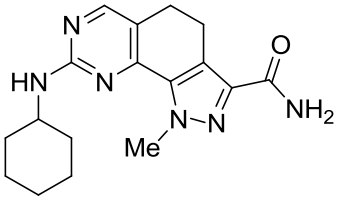
The pyrimidine nucleus is of great interest, being the scaffold of many antitumor drugs, and compounds that incorporate such moiety have recently emerged as inhibitors of cyclin dependent kinases (CDKs) and Polo-like kinase 1 (Plk1). It is well known that compounds targeting the complexes between cyclin-dependent kinases (CDKs) and cyclins (Cy) and inhibiting their activity, are regarded as promising antitumor agents to complement the existing therapies.

CDKs are a family of serine/threonine kinases that specifically form heterodimeric complexes with regulatory subunits named cyclins (Cy), and in concert with cyclins (positive regulators) and natural inhibitors (CDKI), play a crucial role in the cell cycle progression. Deregulation of the activity of CDKs, (due to alterations of expression and/or genetic mutations of cyclins, CDKs, CDKIs, and other components of the retinoblastoma protein (pRB) pathway), has been reported in more than 90 % of human neoplasms. For example, cyclins E and A have been found overexpressed in 50 % of breast and lung cancer whereas decreased levels of the inhibitor p27 indicate a poor prognosis in breast, prostate, colon, gastric, lung and esophageal cancer. The high frequency of alterations found in the core members of this pathway in human tumors led to the suggestion that its deregulation, leading to increased activity of CDK/cyclin complexes, is an obligatory event for the development of all human cancers.

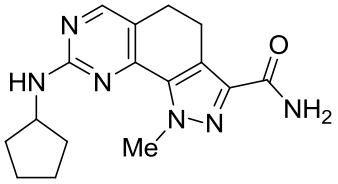
Overexpression of these kinases mediated by disruption of the mechanisms that keep the cell cycle under control is a hallmark of virtually all cancer cells. In fact CDKs not only are recognized to be key components in the control of the cycle cell progression, but they play also very important roles on the mitotic entry, centrosome duplication, bipolar mitotic spindle formation, transition from metaphase to anaphase, cytokinesis and maintenance of genomic stability.

Tricyclic quinazoline system reported in the literature [22] with similar structure to our scaffold, have been reported as potent selective inhibitors of the complex CDK2/CyA (Figure 22). Moreover, recently in our research laboratory, a series of products bearing the pyrimidine core condensed to the indole moiety were synthesized. This class of compounds pyrrolo[3,2-*h*]quinazoline (Table 19) showed significant activity after irradiation with UV light against a panel of different human tumor cell lines [23], demonstrating a very promising class of photosensitizing agents with IC_{50} values of 0.21-15.21 μM.

Figure 22



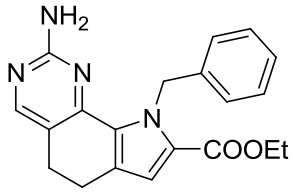
IC_{50} 4nM (CDK2/CyA)



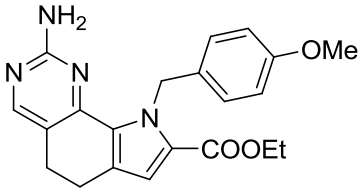
IC_{50} 2nM (CDK2/CyA)

Two of these compounds (Figure 23) showed IC_{50} values lower than angelicin used as reference drug. It was also studied the possible mechanism of action, and it seems that they induce apoptosis mediated by mitochondria and lysosomes. The DNA does not seem to be involved in the process of induction of phototoxicity. This aspect is of great importance in modulating the side effects since that the drugs currently on the market, can cause genotoxicity, mutagenicity and skin cancer.

Figure 23



23i
IC_{50} 0.21-0.44 μM



23j
IC_{50} 0.23-0.41 μM

From the phototoxicity data listed in the table 20, we can note that the substituent in position two plays an important role in inducing phototoxicity. In fact, the antiproliferative activity was maximal when in position two there was an amino group (**23i** and **23j**), and decreased if there were an aniline group (**23l** and **23m**), a carbonyl group (**23d**) or a hydrogen (**23q**). In general the presence of a ethoxycarbonyl group in position eight increases phototoxicity. It passes from inactive compounds such as **23a,b,e** and analogous phototoxic **23c,d,h**.

Table 19

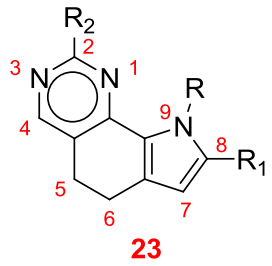
	Cpd	R	R_1	R_2	Cpd	R	R_1	R_2
	**23a**	Me	H	C=O	**23j**	4-OMe-Bn	COOEt	NH_2
	23b	Bn	H	C=O	**23k**	Me	H	NHPh
	23c	Me	COOEt	C=O	**23l**	Bn	H	NHPh
	23d	Bn	COOEt	C=O	**23m**	4-OMe-Bn	H	NHPh
	23e	Me	H	NH_2	**23n**	Me	COOEt	NHPh
	23f	Bn	H	NH_2	**23o**	4-OMe-Bn	COOEt	NHPh
	23g	4-OMe-Bn	H	NH_2	**23p**	Bn	H	H
	23h	Me	COOEt	NH_2	**23q**	Ph	H	H
	23i	Bn	COOEt	NH_2				

Table 19: List of pyrrolo[3-2*h*]quinazolines **23**

Table 20

	Jurkat (IC_{50}, μM)		K-562 (IC_{50}, μM)		LoVo (IC_{50}, μM)		A-431 (IC_{50}, μM)	
Dose UVA (J/cm2)	**2.5**	**3.75**	**2.5**	**3.75**	**2.5**	**3.75**	**2.5**	**3.75**
23a	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20
23b	> 20	> 20	> 20	13,69±1,09	> 20	> 20	> 20	> 20
23c	4.85±0.53	3.47±0.11	5.70±0.76	4.38±0.44	9.72±1.32	6.76±0.57	> 20	8.05±1,69
23d	2.05±0.62	1.08±0.25	7.96±1.46	2.92±0.42	3.07±0.35	2.30±0.24	3.91±0.15	2.45±0.38
23e	> 20	8.42±1.49	> 20	> 20	> 20	> 20	> 20	> 20
23f	0.65±0.13	0.39±0.05	1.44±0.12	0.72±0.28	0.85±0.19	0.54±0.09	1.19±0.03	0.96±0.05
23g	0.43±0.08	0.28±0.08	1.71±0.17	0.53±0.09	1.12±0.23	0.59±0.18	1.31±0.17	0.60±0.16
23h	0.83±0.16	0.46±0.09	2.38±0.70	1.16±0.20	1.57±0.12	1.02±0.09	1.90±0.11	1.34±0.25
23i	0.43±0.08	0.21±0.06	0.74±0.13	048±0.08	0.56±0.07	0.44±0.04	0.74±0.07	0.39±0.10
23j	0.29±0.02	0.23±0.01	0.75±0.18	0.41±0.05	0.47±0.07	0.34±0.03	0.44±0.05	0.26±0.06
23k	0.83±0.08	0.55±0.09	3.51±0.80	1.82±0.39	0.82±0.14	0.55±0.08	2.28±0.18	1.44±0.15
23l	0.81±0.07	0.52±0.11	3.90±0.70	2.85±0.20	2.31±0.17	0.95±0.09	1.77±0.27	1.16±0.12
23m	2.05±0.12	0.68±0.09	4.06±0.60	2.16±0.28	2.80±0.29	1.43±0.15	3.79±0.43	2.53±0.53
23n	> 20	8.53±1.53	> 20	> 20	15.21±1.97	9.61±1.32	> 20	5.69±1.32
23o	0.70±0.11	0.50±0.05	2.63±0.36	1.88±0.35	0.88±0.11	0.48±0.11	1.57±0.11	1.09±0.21
23p	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20
23q	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20
Ang	1.00±0.20	0.90±0.10	1.21±0.11	1.09±0.10	4.08±0.41	1.12±0.44	5.09±0.65	2.15±0.35

Table 20: Phototoxicity of Pyrrolo[3,2-*h*]quinazolines **23**

Given these results of considerable importance, it was planned the synthesis of this new class of derivatives pyrrolo[3',2':6,7]cyclohepta[1,2-*d*]pyrimidin-2-amine **24-29.** Since the lead coumpounds (Figure 23) of the previous series **23** are ethoxycarbonyl derivatives, bearing a benzyl or 4-methoxy-benzyl group at the pyrrole nitrogen, we decided to start our own synthesis with the same type of substitution at the pyrrole.

Starting from our intermediates of type **13**, in only one step, the pyrimidine ring was anellated to our scaffold bearing the proper decoration. With the aim of obtaining the best interactions with the

above mentioned kinases, a further functionalization, was achieved by converting the ester functionality in position nine to carboxamide in two steps. Indeed by using KOH in ethanol at reflux, the ester functionality was hydrolyzed to corresponding acid, and then by using ammonium carbonate in presence of benzotriazole and *N*-Ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride, as activating agents of acid function, the carboxamide derivatives **27 - 29** were obtained with good yields (Scheme 13).

Scheme 13

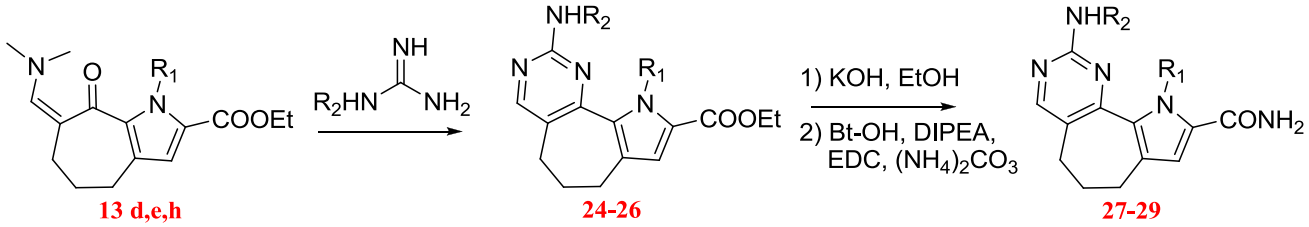


Table 21

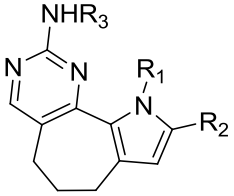
	Cpd	R_1	R_1	R_2	Yield
 24-29	**24d**	Me	COOEt	H	59%
	24e	Bn	COOEt	H	40%
	24h	4-OMe-Bn	COOEt	H	50%
	25d	Me	COOEt	Ph	62%
	25e	Bn	COOEt	Ph	60%
	25h	4-OMe-Bn	COOEt	Ph	43%
	26d	Me	COOEt	Cyclohexyl	76%
	26e	Bn	COOEt	Cyclohexyl	81%
	26h	4-OMe-Bn	COOEt	Cyclohexyl	49%
	27d	Me	$CONH_2$	H	66%
	27e	Bn	$CONH_2$	H	25%
	27h	4-OMe-Bn	$CONH_2$	H	94%
	28d	Me	$CONH_2$	Ph	74%
	28e	Bn	$CONH_2$	Ph	64%
	28h	4-OMe-Bn	$CONH_2$	Ph	54%
	29d	Me	$CONH_2$	Cyclohexyl	82%
	29e	Bn	$CONH_2$	Cyclohexyl	85%
	29h	4-OMe-Bn	$CONH_2$	Cyclohexyl	93%

Table 21: List of pyrrolo[3',2':6,7]cyclohepta[1,2-*d*]pyrimidin-2-amine **24-29**

6.2 Pyrrolo[3',2':6,7]cyclohepta[1,2-*d*]pyrimidin-2-amine

Anticancer activity

The obtained compounds (Table 21), were submitted for the antitumor evaluation at NCI of Bethesda (USA). The compounds also were sent to University of Padova to evaluate their photo antiproliferative activity. Subsequently it will be evaluated the mechanism of action.

Figure 24

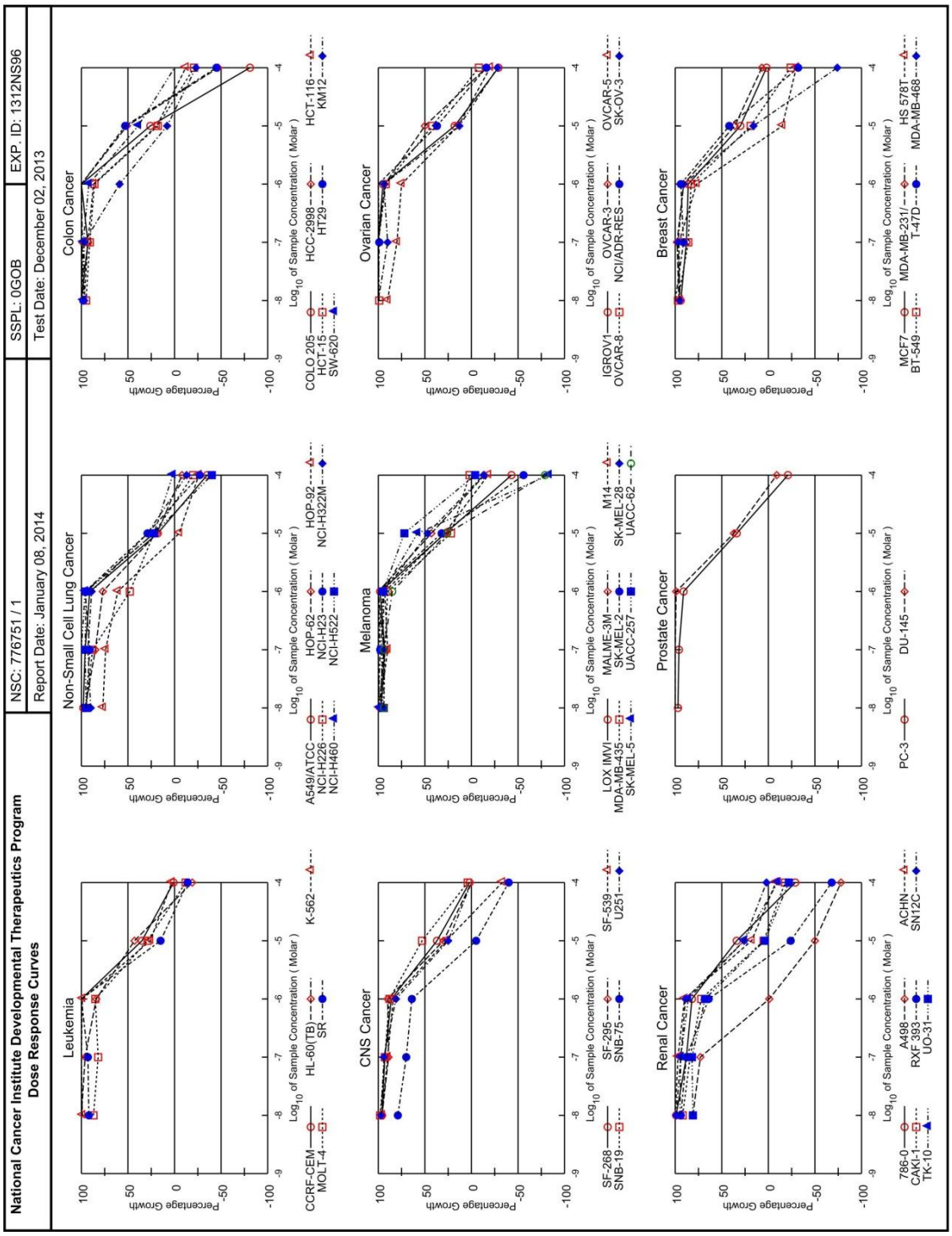


Figure 24 Dose Response Curves of **28d**

Figure 25

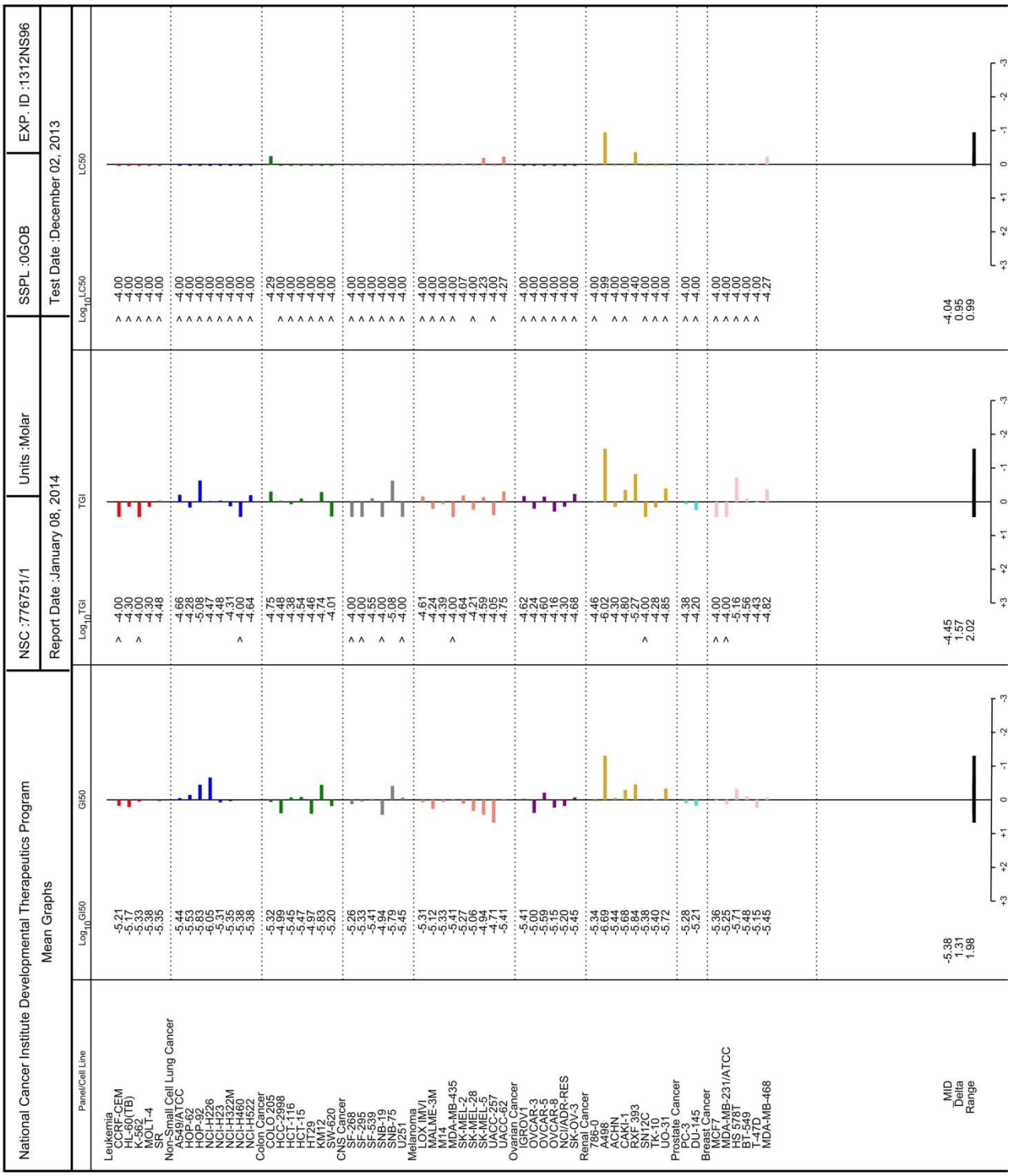


Figure 25 Mean Graph of **28d**

Moreover, only nine of the compounds submitted bearing the caboxamide functionality, were selected by NCI. After the first prescreening, only the compound **28d** was selected for the one-dose (10^{-5}M) screening on the full panel of 60 human tumor cell lines divided into nine subpanels

(leukemia, non-small-cell lung, colon, central nervous system, melanoma, ovarian, renal, prostate, and breast cancers).

In detail (Figures 24 and 25) it can be observed that compound **28d** showed the best selectivity against the renal and the non-small cell lung cancer subpanels. In fact the calculated pMG_MID values for the single subpanels (5.56 and 5.48 respectively) was higher than the overall cell lines pMG_MID value (5.38).

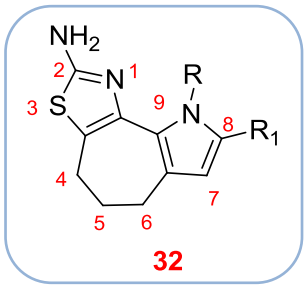
The most sensitive cell lines were A498 of the renal subpanel and NCI-H226 of the non-small cell lung cancer subpanel, having GI_{50} values in the submicromolar range, 0.2 and 0.9 μM respectively. These results in "the dark" could indicate a possible inhibitory effect induced only after photoactivation, in agreement with the previous class of pyrrolo[3-2*h*]quinazolines **23**.

The evaluation of their photoantiproliferative activity is still in progress at the University of Padova.

7. PYRROLO[3',2':6,7]CYCLOHEPTA[1,2-*d*][1,3]THIAZOL-2-AMINE

7.1 Synthesis

Starting from our scaffold, another class of tricyclic compounds was planned. In this case we set the synthesis of the new ring system pyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazol-2-amine **32.**



32

Inositol trisphosphate or inositol 1,4,5-trisphosphate (abbreviated InsP3 or Ins3P or IP3), together with diacylglycerol (DAG), are a secondary messenger molecules used in signal transduction in biological cells. While DAG stays inside the membrane, IP3 is soluble and diffuses through the cell. It originates from hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2), a phospholipid that is located in the plasma membrane, by phospholipase C (PLC).

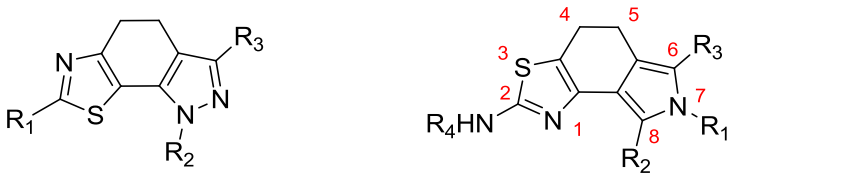
The IP3, binds and activates the inositol triphosphate receptor, a large channel protein that is found on the surface of the endoplasmic reticulum. His bonding with this protein, allows its opening and the release of calcium flowing into the cytoplasm. There are multiple molecular actions resulting therefrom. Just think that through its link with the calmodulin protein, the calcium ion is able to affect more than thirty intracellular enzymes linked to metabolism, signal transduction or production of energy.

The phosphatidylinositide 3-kinases (PI 3-kinases, PI3Ks, PI(3)Ks, or PI-3Ks) are a family of intracellular signal transducer enzymes involved in complex cellular functions such as cell growth, proliferation, differentiation, motility, survival and intracellular trafficking; mechanisms that in turn are also involved in cancer. PI3Ks are capable of phosphorylating the hydroxyl group in position three of the inositol ring of phosphatidylinositol. Alterations in the activity of PI3 kinase are present in a broad spectrum of tumors, so this has stimulated the research and the development of inhibitors of these kinases.

The interest on this class of compounds rised from the similarity of the trycliclic scaffold with compounds of the pyrazole series, such as thiazolyl-dyhydro-indazoles derivatives, [24] which proved potent inhibitory activity of PI3K. On the basis of these data, we had already synthetized the tricyclic 2-amine-[1,3]thiazolo[4,5-*e*]isoindoles derivatives **30** (Figure 26) with the aim of studying their inhibitory activity toward kinases, and the effect of the replacement of the pyrazole with a

pyrrole ring on the biological activity. Among the new derivatives 6 out of 32 reacted the 5-doses NCI in vitro screening, forth evaluation of the growth inhibitory effect against a panel of about 60 tumor cell lines.

Figure 26



thiazolyl-dyhydro-indazoles 2-amine-[1,3]thiazolo[4,5-*e*]isoindoles (**30**)

Table 22

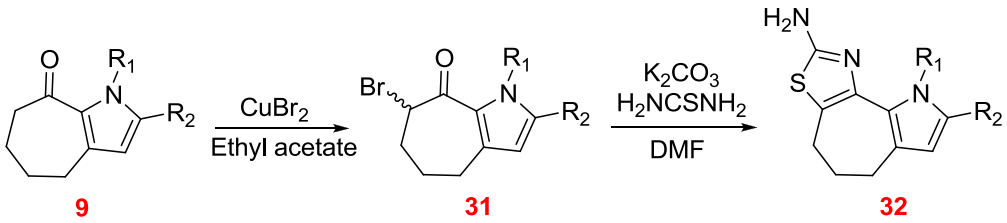
Cpd	R_1	R_2	R_3	R_4	pMG_MID
30-a	4-OMe-Bn	Br	3,4,5-$(OMe)_3$-Ph	H	**6.08**
30-b	SO_2-Ph	H	3,4,5-$(OMe)_3$-Ph	H	**5.99**
30-c	4-OMe-Bn	Br	Br	H	**5.43**
30-d	4-OMe-Bn	Br	3,4,5-$(OMe)_3$-Ph	Acetyl	**5.36**
30-e	Bn	Br	Br	Benzoyl	**5.29**
30-f	Bn	Br	Br	H	**5.09**

An evaluation of the data listed in Table 22 indicates a good potential of this class of compounds showing growth inhibitory activity in the micromolar range with a pMG_MID value between 6 and 5. The most active compounds of the series were **30a** and **30b**, having mean graph mid-points (pMG_MID) of 6.08 and 5.99 respectively. From a structure–activity point of view, it seems that the presence of a 3,4,5-trimethoxyphenyl moiety at position 6, is essential for the activity. Among the 3 derivatives bearing a 3,4,5-$(OMe)_3$-Ph moiety, the addition of a bromine atom in position 8, seems to be important in the modulation of activity, whereas the presence of acetyl groups as substituent on the amine in position 2, produces a slight decrease in the activity (compare **30a** pMG_MID = 6.08 and **30d** pMG_MID = 5.99). Moreover for the N-benzyl derivatives, the presence of the free amine produces a small reduction of activity, with respect to the corresponding benzoyl substituted derivatives (compare **30e**, pMG_MID = 5.29 and **30f** pMG_MID = 5.09).

In this contest we decided to extend the synthesis to new thiazole derivatives bearing the seven member ring in the tryciclyc scaffold, obtaining the new class of compound pyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazol-2-amine **32,** aiming to an improvement of the activity. To achieve the new ring system **32**, we reacted ketones **9,** with copper(II) bromide ($CuBr_2$) obtaining brominated intermediates of type **31,** that upon subsequent reaction with thiourea, gave the desired compounds (Scheme 14).

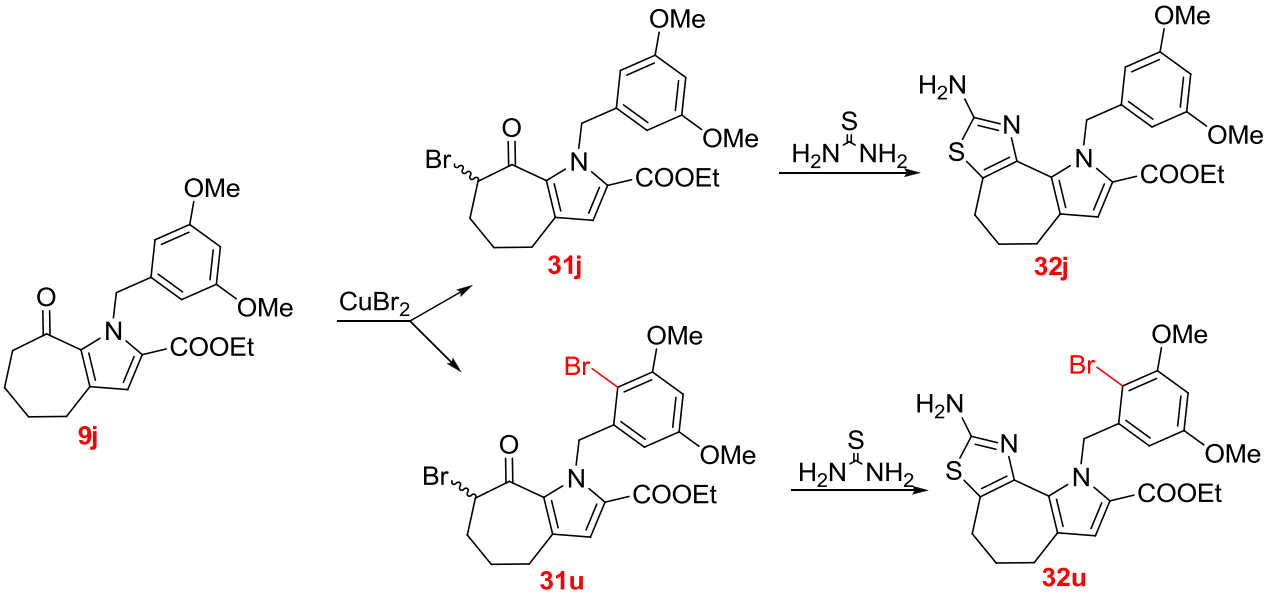
In our firs attempt of obtaining compounds **32** in one pot reaction using ethyl acetate as solvent and $CuBr_2$ as brominated agent, we noted that ketones **9** reacted giving the brominated intermediates **31**. The following addition of potassium carbonate and thiourea to the reaction mixture, yielded the desired thiazole derivatives in modeste yields (30 - 40 %). We tried also the reaction in two steps, using the same reagents and solvent. Thus, when the brominated intermediates were formed, the reaction mixtures were filtered to remove the copper(I) bromide. Mixtures were concentrated and reacted in DMF with potassium carbonate and thiourea obtaining an improvement of the yields up to 50 - 60%. (Table 23)

Scheme 14



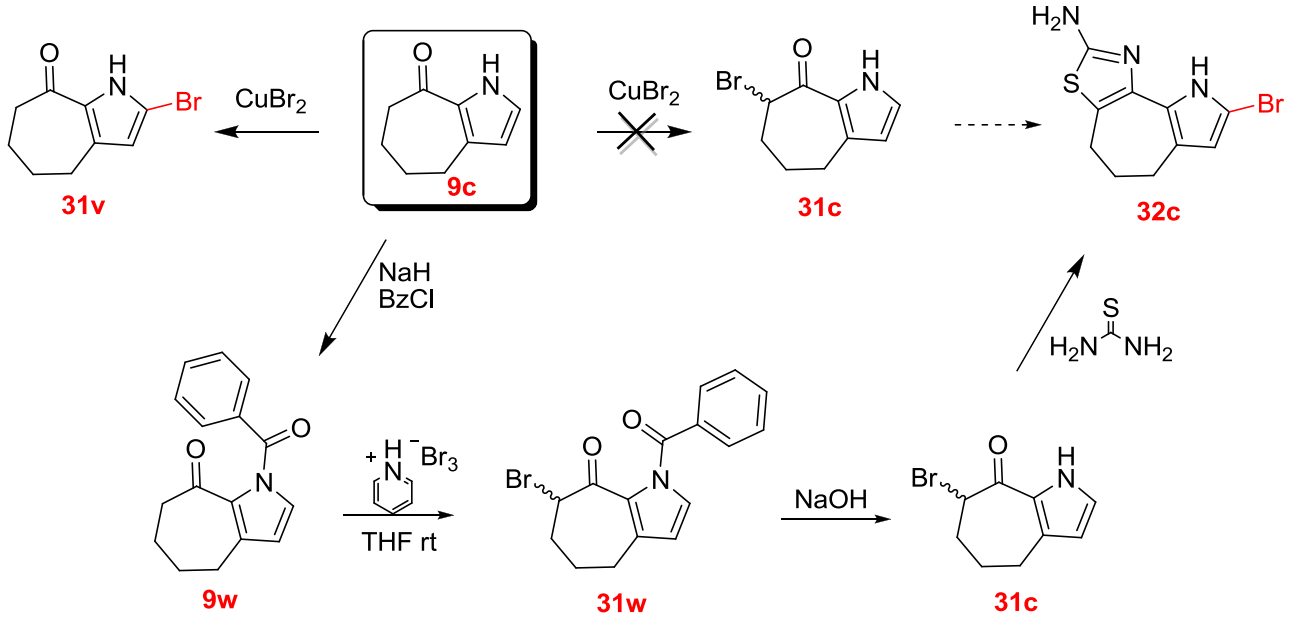
From the reaction of ketone **9j** with copper(II) bromide, beside the main component **31j**, which was successfully cyclized to **32j**, from the reaction mixture a secondary product **31u** was isolated, bearing a second bromine atom to position 2 of the 3,5-$(OMe)_2$-Bn moiety (Scheme 15). This product was also annellated with thiourea to obtain another derivate **32u** to submit for biological screening.

Scheme 15



When the ketone **9c** bearing the free NH, was reacted with copper(II) bromide, the bromine atom was introduced preferentially at the position 2 of the pyrrole ring rather than the α position of the carbonyl (Scheme 16) furnishing **31v**. Thus, in order to obtain the thiazole derivative **32c** bearing the free NH, we tried directly the deprotection of the SO_2Ph group with NaOH starting either from thiazole **32b** and from the brominated intermediate **31b**. However it was not possible obtain the desired product because decomposition of the starting material was observed upon heating.

Scheme 16



So we decided to insert a different protecting group to the pyrrole nitrogen in order to direct the bromination to the desired position, but which could be more easily removed. Thus by reaction with NaH and benzoylchloride, a benzoyl group was introduced to furnishing ketone **9w**, which was subjected to bromination giving **31w**.

The reaction of bromination made with Pyridine hydrobromide perbromide in THF at room temperature, instead the copper(II) bromide in ethyl acetate at reflux, gave **31w** with better yield (65% versus 18%). The deprotection step, also in this case made with NaOH solution, but at room temperature, gave **31c** with good yield, and finally the ring closure with thiourea gave the desired tryciclic derivative **32c**.

Considering that in the pyrazole series, thiazoles bearing an acetylamino group gave interesting results of biological activity, a further functionalization was achieved by acylation of amine group, by reaction of derivates **32** with DIPEA and acetyl chloride at room temperature in DCM as solvent (Scheme 17).

A final series of 14 derivatives has been prepared (Table 23), but further functionalization of the ring system at the pyrrole and/or at the thiazole moiety are still in progress. All the compounds will be submitted to NCI of Bethesda (USA) to evaluate their antiproliferative activity.

Scheme 17

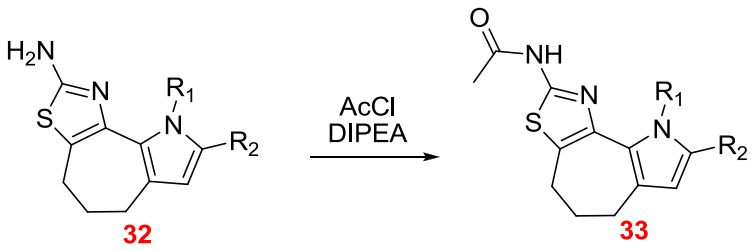
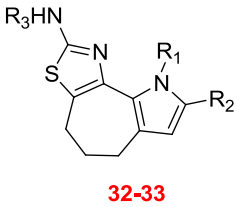


Table 23



Cpd	R_1	R_2	R_3	Yield
32a	H	COOEt	H	52 %
32b	SO_2Ph	H	H	44 %
32c	H	H	H	64 %
32d	Me	COOEt	H	50 %
32h	4-OMe-Bn	COOEt	H	59 %
32j	3,5-$(OMe)_2$-Bn	COOEt	H	38 %
32k	3,4,5-$(OMe)_3$-Bn	COOEt	H	92 %
32u	2-Br-3,5-$(OMe)_2$-Bn	COOEt	H	23%
33b	SO_2Ph	COOEt	Acetyl	73 %
33d	Me	COOEt	Acetyl	51 %
33h	4-OMe-Bn	COOEt	Acetyl	65 %
33j	3,5-$(OMe)_2$-Bn	COOEt	Acetyl	72 %
33k	3,4,5-$(OMe)_3$-Bn	COOEt	Acetyl	39 %
33v	2-Br-3,5-$(OMe)_2$-Bn	COOEt	Acetyl	49 %

Table 23 List of Pyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazol-2-amine **32-33**

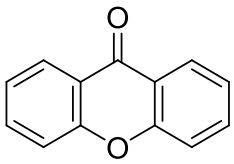
7. PROJECT IN COLLABORATION WITH THE UNIVERSITY OF NOTTINGHAM

7.1 Introduction

During my PhD project, I spent 8 months (May-December 2012) at the School of Chemistry of Nottingham for an international collaboration with Prof. Sir. C. J. Moody. In this period I worked on a project about the synthesis of two natural products belonging to the family of xanthones.

Xanthones are a group of natural products which have been shown to provide various medically beneficial effects and that are all built around the central "xanthone" core. (Figure 27)

Figure 27



Xanthone core

Xanthone-containing fruit (e.g. mangosteen) have long been known to possess medicinal properties, and have been used in traditional Chinese medicine for many years.

The overall benefits of each xanthone derivate can vary; however there are certain properties which are common amongst all natural products possessing this xanthone core. For example, they display anti-inflammatory[25], anti-bacterial[26], and anti-oxidant[27] properties. They are also shown to have anti-cancer potential[28], and, in some cases show activity against chloroquine- resistant strain of malaria[29].

The anti-inflammatory properties of these molecules come from their ability to inhibit the platelet-activating factor (PAF). PAF is a phospholipid activator, and mediator of many things such as platelet aggregation, degranulation, inflammation and anaphylaxis[30,31]. The PAF is produced by the body to attack and eliminate a foreign or unwanted entity in the system, however it can be produced at the wrong times (e.g. allergies and asthma), which can lead to severe health problems and potentially death.

Alongside inhibition of PAF, there are also drugs which inhibit cyclooxygenase (COX) to treat inflammation. In the body, COX produces prostaglandins, which promote the production of a lining to the stomach which protects it from acid, but which also promote the aggregation of platelets and cause inflammation (cf. PAF). But while inhibition of COX, leads to well known side-effects (cf. NSAIDs Non-Steroidal Anti-Inflammatory drugs), using xanthones to inhibit PAF rather than COX,

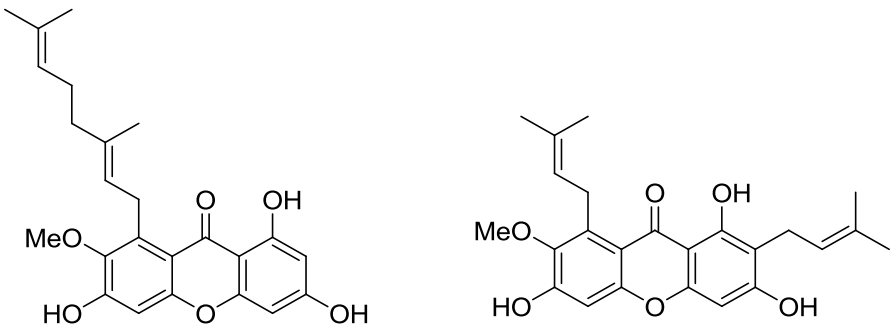
could potentially lead to drugs which could provide relief from inflammation and pain with fewer side-effects[25]. Moreover compounds which inhibit the specific binding between PAF and receptors (found in a variety of cell membranes including those from platelets), have been extensively sought to be used as leads in the development of therapeutic agents in a variety of inflammatory, respiratory, immunological, and cardiovascular disorders.

The anti-cancer properties of these molecules are due to the planar, aromatic nature of the xanthone ring system, which means that is possible for them to intercalate with deoxyribonucleic acid (DNA). This ability to bind DNA has been proven by numerous biological studies to be a key feature in anticancer medicine [32,33] as it is by binding to the DNA that anti-cancer drugs are able to inhibit the growth and cause death of the cancer cells.
The synthesis of a new xanthone compounds might further develop drugs which excel in binding with DNA and might therefore become potent anticancer drugs.

Xanthones have been shown to possess strong antibacterial properties, especially against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Bacillus subtilis*. Indeed, rubraxanthone itself has been shown to be one of the most effective antibacterial and bactericidal molecules in the xanthone family[34]. Rubraxanthone is also active against *Escherichia coli* and *Pseudomonas aureginosa* where most of other xanthones are not. Furthermore, rubraxanthone has been shown to be an anti-oxidant, and therefore may possess yet more biologically beneficial use.

It is also encouraging to note the similarities between rubraxanthone and α-mangostin (Figure 28) when discussing the potential significance and impact that rubraxanthone may have in the medicinal world.

Figure 28



Rubraxantone **α-mangostin**

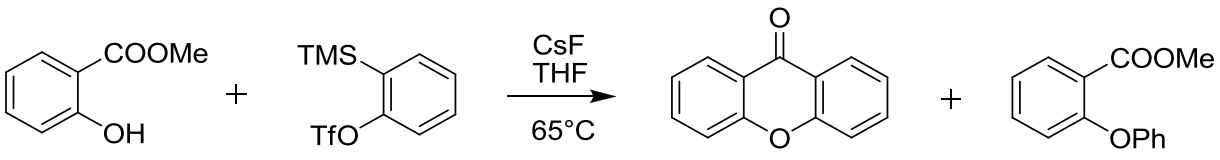
For example, α-mangostin has been shown to have inhibithory effects against HIV[35] and Alzheimer's disease [36]. It has also been shown to exhibit a protective effect on cardiac reperfusion

damage due to the way it can alleviate oxidative stress[37]. It has additionally been shown to work side-by-side with cisplatin and betulinic acid to provide cancer treatment without the common side-effects produced in the absence of α-mangostin[38]. While the tests performed on rubraxanthone and α-mangostin have largely been different, and therefore the similarities are currently hypothetical, it is encouraging that there have been incidences when both compounds have been analysed together[39]. Indeed, where they have been both subjected to the same analytical tests, rubraxanthone has been shown to be of comparable, and sometimes even greater, strength than α-mangostin[40,41].

7.2 General synthesis of xanthones

The very first synthesis of xanthones was obtained from the distillation of a mixture of a phenol, a phenolic acid, and acetic anhydride[42] yet this route is now archaic in both its approach and yield (which was extremely poor). As time progressed, higher yielding routes for the synthesis of xanthones were developed, with better selectivity, yet often requiring the use of strong acids[43], or toxic metal catalysts[44], both of which could cause problems with the successful synthesis of rubraxanthone, not only because they could alter the double bonds in the key geranyl chain[45], but also because they are toxic and therefore not ideal to use in the synthesis of a potential drug molecule. The first documented synthesis of α-mangostin is a prime example of the usage of dangerous chemicals and a long synthetic route to obtain the required xanthone[46]. In 2005, Larock suggested a synthesis of xanthones using mild reagents (Scheme 18) at a reasonable temperature which gave high yield and high selectivity, the most basic example of which is shown in the following scheme[47].

Scheme 18



This method can produce either the desired xanthone, or a by-product in the form of a benzoate. To cause preferential production of the xanthone over the benzoate requires alteration of the reaction conditions and the reagents used.

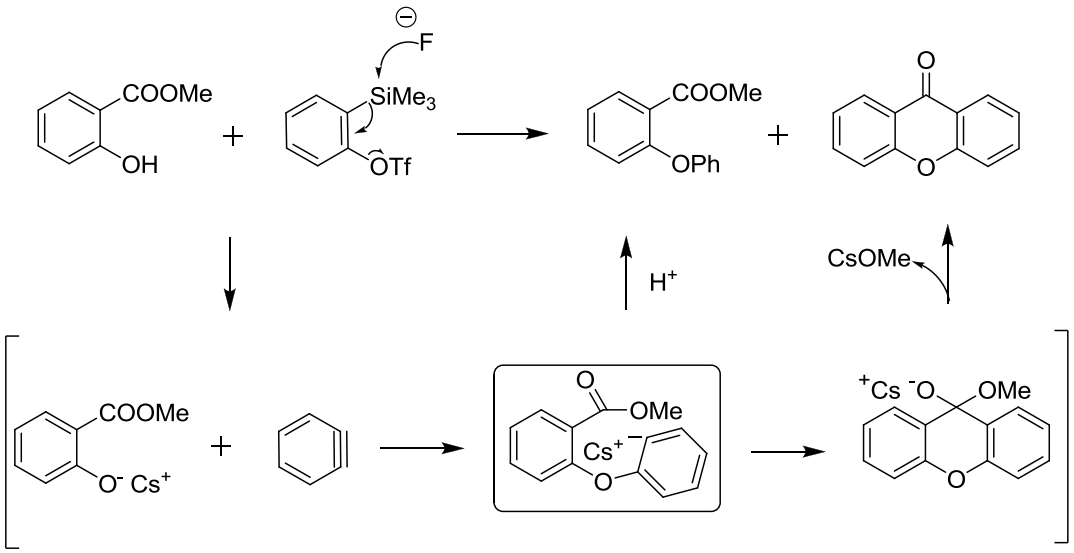
The first step of the Larock reaction is to produce an aryne by the attack of a fluoride anion on the TMS group, which produces a triple bond on the benzene ring by the tandem expulsion of the

highly reactive triflate leaving group (this method of creating an aryne was first described by Kobayashi in 1983)[48].

It was found that, in the simple model shown above, the source of this fluoride anion must be from CsF (rather than TBAF, for example), as CsF gives a far better ratio of the xanthone to benzoate (Scheme 19). It was also discovered that the usage of MeCN as a solvent gave a high yield (80%), but poor selectivity (40:60 benzoate : xanthone). As the route of the reaction is decided by the ease of proton abstraction (readily available protons result in the creation of the benzoate), a less polar solvent was theorized to solve this selectivity issue[47]. However, solvents which were much less polar resulted in very poor yield; thus it was found that a good compromise was to use THF, which, although a slightly less polar solvent, still gave high yields.

Through experimentation, Larock found that the ideal temperature for this reaction was 65°C, as this increased the yield and speed of reactivity. Lower temperatures gave a much poorer yield (20%), with a high selectivity, while higher temperatures gave a good yield, but poor selectivity. When the reaction was performed at 65°C, a 75% yield was obtained with only a trace of by-product.

Scheme 19

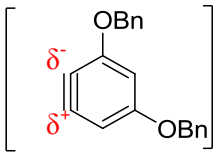


The Larock reaction proceeds via an aryne intermediate. These intermediates are incredibly useful when creating new C-C bonds, as an aryne can be attacked by a nucleophile on one side and attack an electrophile from the other, creating two new neighbouring bonds.

When performing these reactions, it is important to consider the regioselectivity. For example, in our case, it is possible to get a regioisomer where the geranyl chain is at the bottom of the left hand ring rather than the top. The ultimate outcome is governed by the location of charge on the newly

created triple bond in the aryne. To be precise, the point at which the most negative charge resides on the triple bond will be where it attacks the ester group, and the other side, where the most positive charge lies, will be where the alcohol will attack (Figure 29).

Figure 29



There are two effects that may affect the regioselectivity of this reaction, the first of which being the steric hindrance of the benzyl group, and the second being the inductive electronic effects of the oxygen. However, it has been found that when directly in opposition to one another, (the steric control directs the substitution *meta*, while electronics dictate that the addition must be *ortho*), the electronics play a far more influential role than sterics, by stabilizing the aryl-caesium complex[49]. These findings have also been found by Stoltz in a paper discussing regioselecticity in arynes[50]. It was also found by Stoltz in the same paper, that in the aryne shown in Figure 29, the two effects work in tandem to one another, as both electronic and steric effects necessitate that the addition of a nucleophile (which is the first step due to highly electrophilic nature of the triple bond) must be *meta* to the benzyloxy group, as discussed below. The reaction proceeds extremely fast due to the low lying LUMO of the aryne orbital, which means that there is a smaller energy gap between the LUMO and the HOMO of an incoming nucleophile, thus resulting in a faster reactivity than most other systems[51].

When considering how the reaction proceeds and how the regioselectivity is governed, it is of utmost importance to understand that the orbitals of the triple bond in an aryne ring are orthogonal to those of the rest of the ring system, and as such are not influenced by the usual resonance forms seen in reactions on a benzene ring. As shown in Figure 30, any donation of electrons via resonance into the π system (such as the oxygen groups would do in Figure 29) will not be felt by the orthogonal orbitals of the triple bond.

Figure 30

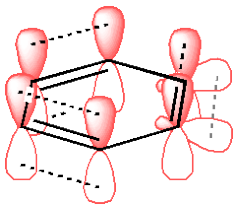


Figure 30 An orbital map of an aryne, showing the plane in which the triple bond sits.

The triple bond, however, is a very easily polarized system (a soft electrophile), which means that an electron withdrawing group *ortho* to the triple bond will polarize the bond effectively and result in the nucleophile attacking at *meta* position. This selectivity is seen because the attack of a nucleophile from that position will cause the temporary negative charge to reside close to the electron withdrawing group, which will stabilize the transition state.

A benzyloxy group would usually be considered an electron donating group on a benzene ring, as resonance forms would stabilize nucleophilic attack at the *ortho* position, because the resultant negative charge would reside farthest from the electron donating group. However, as the triple bond in an aryne ring is orthogonal to the pi-system, this resonance ability does not affect the polarisation of the triple bond, which is governed instead exclusively by inductive effects. This means that as oxygen is an electronegative atom, the benzyloxy group acts to be electron withdrawing, and so polarises the triple bond in such a way that the initial nucleophilic attack occurs at the bottom of the triple bond (as shown in Figure 31), such that the negative charge resides *ortho* to the electron withdrawing site, thus stabilizing the transition state. From here, the resulting anion attacks the electrophilic ester (which is held in a favourable position). This reaction is highly favourable, because not only there is a residual negative charge from the previous reaction which will be fairly unstable until it reacts, the resonance forms of the benzyloxy groups are now accessible. Therefore, in the absence of protons (which can quench the intermediate and produce the benzoate by-product), the reaction will cyclise to form the desired xanthone.

Figure 31

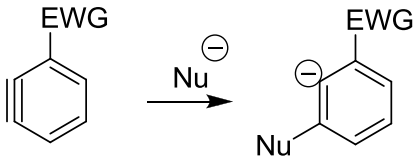
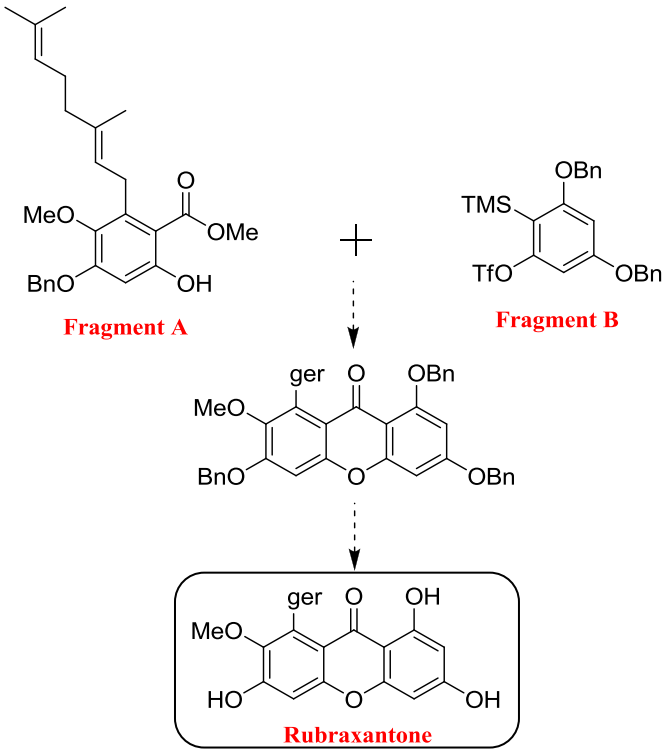


Figure 31. Illustration of how an electon withdrawing group governs the regioselectivity of a nucleophilic attack on an aryne.

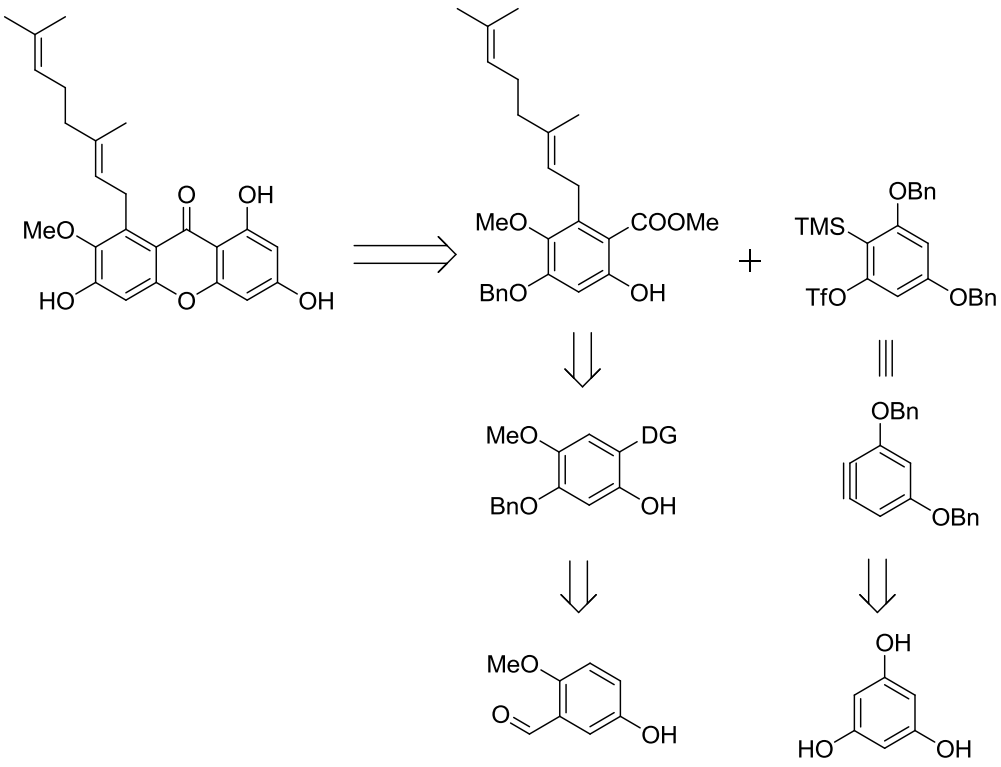
Our retrosynthetic analysis of rubraxanthone employed a Larock xanthone synthesis as a key step, leading to highly functionalised salycilate and silyl aryl triflate fragments A and B (Scheme 20).

Scheme 20



These fragment could be synthetised from 2,5-dimethoxybenzaldehyde and phloroglucinol respectively (Scheme 21).

Scheme 21



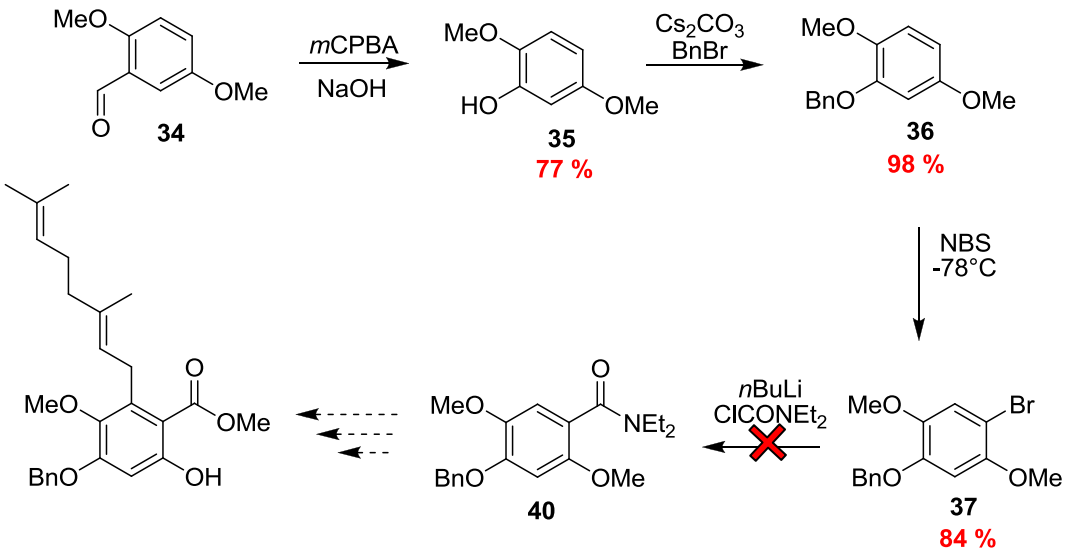
7.3 Synthesis of two fragments:

8.3.1 Fragment A

As regards the fragment A, the first approach followed was that shown in the scheme below (Scheme 22).

The synthesis was started from 2,5-dimethoxybenzaldehyde **34** which underwent Dakin oxidation to give 2,5-dimethoxyphenol with good yield. This was then protected with Cs_2CO_3 and benzyl bromide, to give the product **36**, which in turn was brominated in 4-position with NBS to give the product **37** in excellent yield.

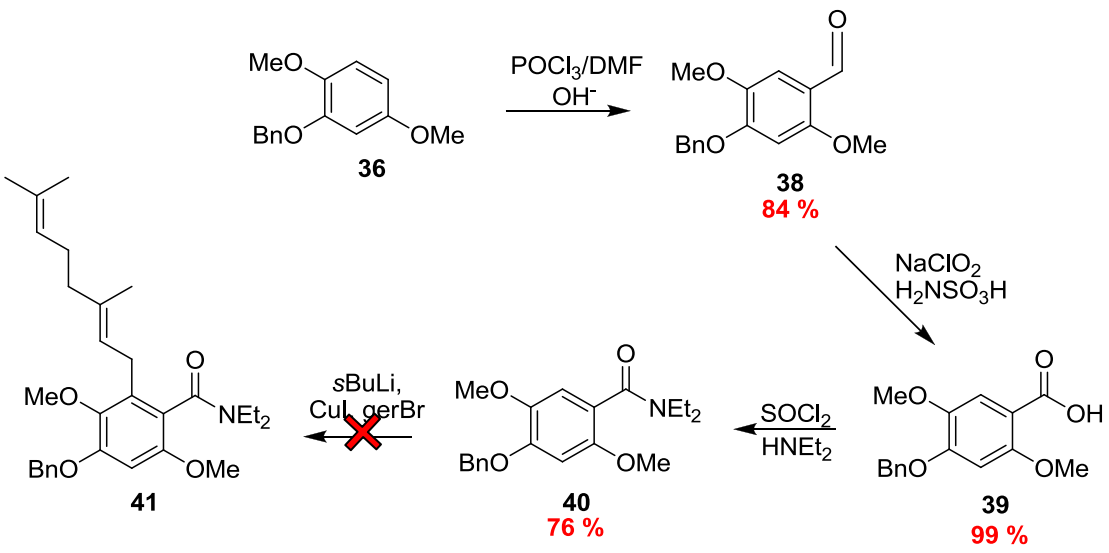
Scheme 22



Unfortunately, however, all attempts to exchange with lithium in order to introduce the carboxy-amide function, didn't lead to the desired product **40**.

Therefore benzyl ether **36** was subjected to Vilsmeier-Haak formylation to give the corresponding aldehyde **38**, which was immediately subjected to further oxidation with $NaClO_2$ obtaining the corresponding acid **39** (Scheme 23). Treatment with $SOCl_2$ at reflux, and then with $HNEt_2$, finally gave the desired product **40**.

Scheme 23



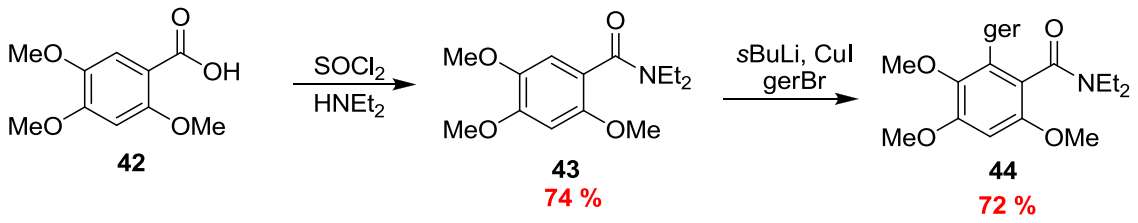
So, the next step would be the introduction of the geranyl chain in the *ortho* position to the carboxy-amide function, but unfortunately, this reaction failed to proceed under several conditions.

Intrigued by this total lack of reactivity shown, it was attempted an exchange with D_2O, in order to understand the reason for the failure of the reaction, but the resulting NMR clearly showed the absence of reactivity of the substrate with the *sec*-BuLi.

Even attempts with *tert*-BuLi showed the same result.

To clarify this issue, we have taken as a model 2,4,5-trimethoxybenzoic acid **42**, and brought upon it the same attempts made previously on the benzyl derivative, but getting completely opposite results.

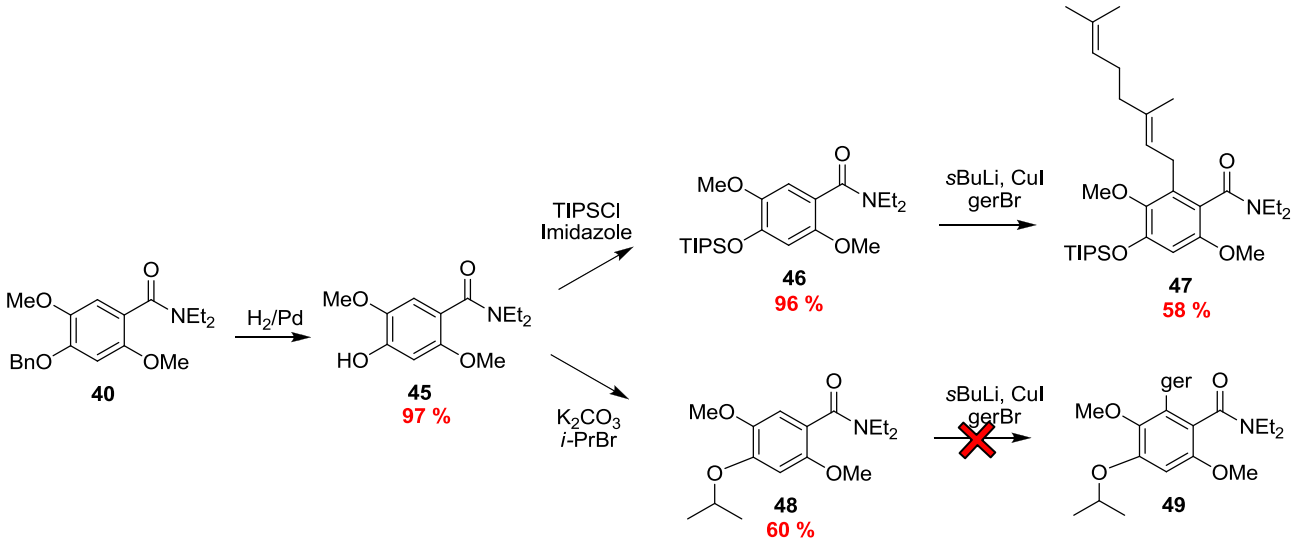
Scheme 24



In fact, trimethoxy derivative **43**, obtained from 2,4,5-trimethoxybenzoic acid **42** under the same conditions as the corresponding benzyl-ether **40** (Scheme 24), not only reacted with *sec*-BuLi, exchanging with D_2O in the desired position, but reacted with geranyl bromide giving the product **44** and in high yield.

As the benzyl group appeared to be the cause of the problem, an alternative protecting group was sought. The triisopropylsilyl ethers **46** and isopropyl **48** were prepared (Scheme 25), and while with the second had the same problems as the benzyl-ether **40** (it does not exchange with D_2O), with the TIPS-ether **46**, we were able to introduce the geranyl chain in the desired position and in good yield.

Scheme 25

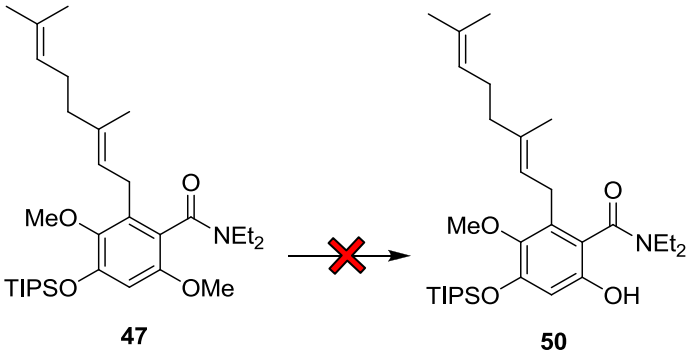


On the derivative **47** thus obtained, it was therefore decided to complete the fragment by selective demethylation of the methoxyl *ortho* to the carboxy-amide, and the conversion of the amide to its methyl ester.

The attempt to demethylation was performed with six different methods in order to choose the one with the best yield, using respectively BCl_3, $AlCl_3$, $MgBr_2$, MgI_2, TMSCl/NaI as Lewis acids, and also with LiOH as nucleophile agent. While attempts with the last three reagents failed, the attempts with the first three Lewis acids gave demethylation, but unfortunately also altered the geranyl chain (Scheme 26), giving an inseparable mixture of isomers.

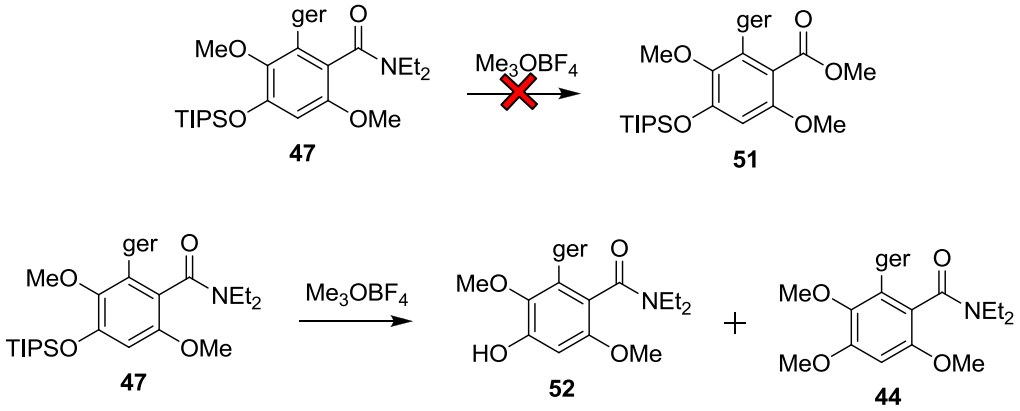
It is likely that the double bond in position 2-3 of the geranyl chain undergoes a shift to position 1-2 moving into conjugation.

Scheme 26



In view of these results, the conversion to the methyl ester **51** was attempted, but again without success.

Scheme 27

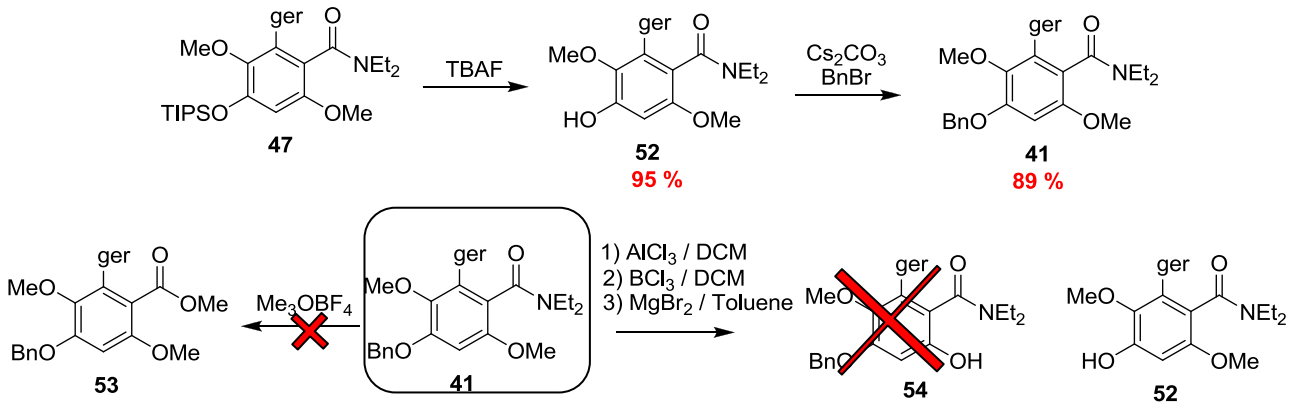


In fact, from the reaction mixture, instead of isolating the desired methyl ester **51** (Scheme 27), we recovered the product TIPS deprotected **52**, and subsequent methylation of the hydroxyl **44**.

View the impossibility to pursue this route with the TIPS protecting group, we decided to re-enter again the benzyl as in our initial synthetic analysis, especially since in the fragment B we had two additional benzyl groups to remove (Scheme 28).

The product **47** was then deprotected with TBAF in THF in quantitative yield, and subsequently benzylated with Cs_2CO_3 as a base and benzyl bromide in acetonitrile (product **41**). The new derivative obtained then was also subjected to demethylation with $AlCl_3$, BCl_3 and $MgBr_2$ and with Me_3OBF_4 in an attempt to convert the amide to ester.

Scheme 28



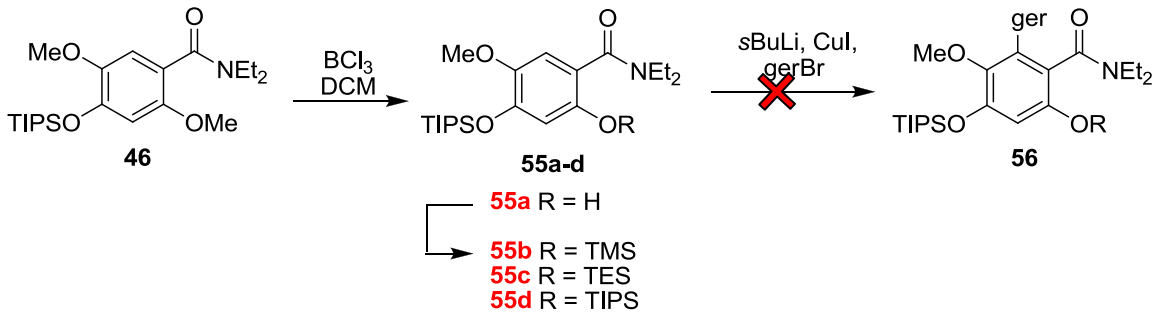
But unfortunately also in this case we did not get the desired results. In fact, while the attempt with trimethyloxonium tetrafluoroborate failed, all three attempts of demethylation lead to the isolation of the product **52** in which the benzyl group is removed, while the methoxy group is unchanged.

A last, an attempt was made by reversing the synthetic sequence trying to do the demethylation even before entering the geranyl chain.

The derivative **46** was then successfully demethylated in quantitative yield with BCl_3 in DCM, and then used for the reaction in order to introduce the geranyl chain passing through the formation of a di-anion. But given the failure of this attempt, we tried to protect the now free hydroxyl group with TMSCl, and then insert the chain. All this was done in one step, since it was impossible to purify the intermediate via chromatography column, but also in this case the desired product was not obtained (Scheme 29).

It was also tried the protection of the hydroxyl with the TES, and the TIPS but even in these cases the new derivative **56** was not recovered. When you protect the hydroxyl with the TIPS is possible to isolate the product via chromatography column, but also in this case the next step failed.

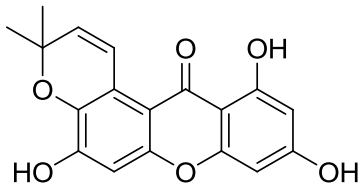
Scheme 29



Considering the great difficulties to continue the synthesis without damage to the geranyl chain, we decided to change and plan an entirely new synthetic route that would start with totally different products, allowing us to form the xanthone core first, and only subsequently introduce the geranyl chain.

Moreover this synthetic route would have allowed us to obtain also a second natural product with the same substituents in the same positions: toxyloxanthone B (Figure 32).

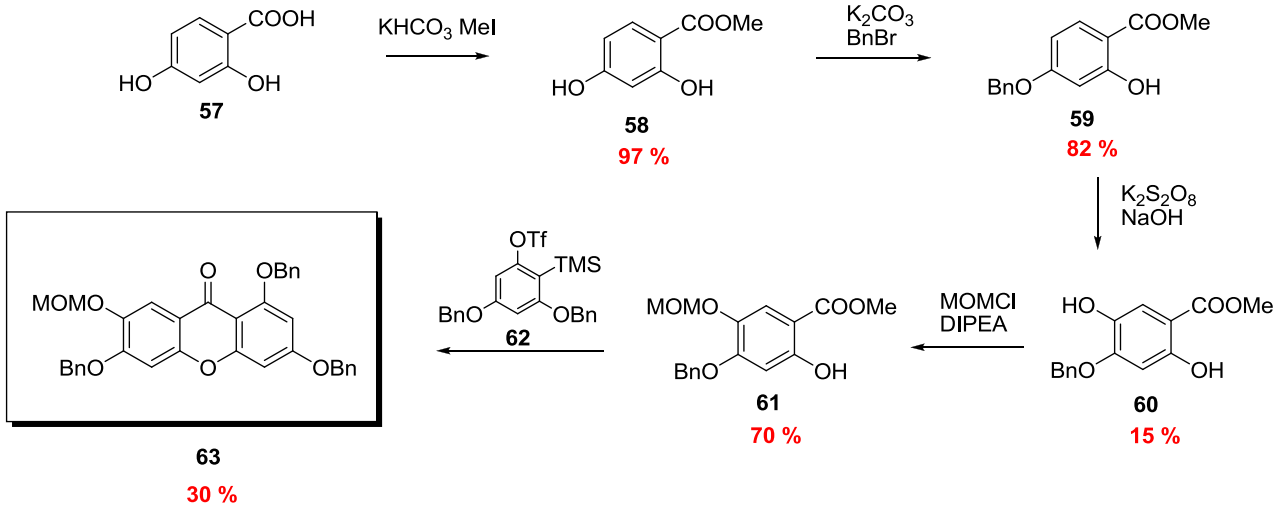
Figure 32



Toxyloxantone B

This new route started from 2,3-dihydroxybenzoic acid **57**, that, was converted in near-quantitative yield to the corresponding methyl-ester **58**, which in turn, undergoes selective benzylation of the hydroxyl in position 3, and subsequent Elbs's oxidation[52], to give the corresponding diphenol **60** (Scheme 30). The yield of this reaction is very low, only 15%, but the possibility to recover 65% of the starting material back with a simple filtration, combined with the simple reagents required, led us also to carry on this path.

Scheme 30



The newly introduced hydroxyl group, was then protected using chloromethyl methyl ether (MOMCl), thus giving the new fragment A **61**, ready to be used in the reaction with the second

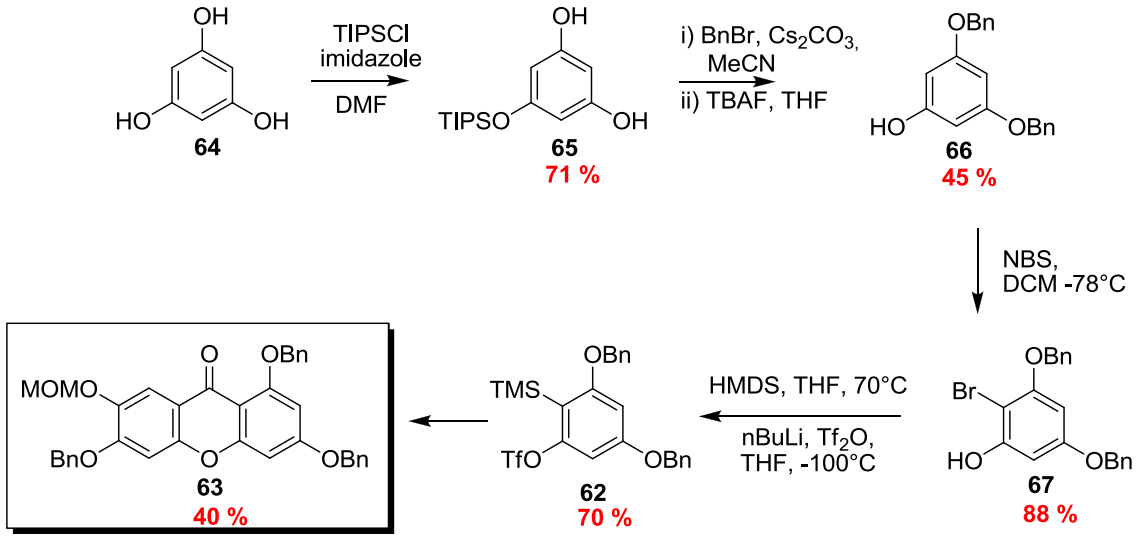
fragment (B), to obtain the xanthone **63** according to the method described by Larock and fully described in the introduction.

8.3.2 Fragment B

The isolation of the fragment B was found to be much easier than to fragment A, as its synthesis was found to be known in the literature.

The synthetic route devised by Stoltz[50], started from phloroglucinol **64** (1,3,5-trihydroxybenzene), which was mono-protected using TIPSCl and imidazole, thus obtaining the silyl ether **65** in good yields. This reaction proceeds by using an excess of phloroglucinol **64** (3 equivalents) in DMF at low concentration. The use of less solvent, stoichiometric amount of base and protecting agent, would lead to obtain a mixture of mono, bis and tris substituted products, as the desired mono-protected product turns out to be more active towards the second protection compared to the starting material itself (Scheme 31). The derivative **65** thus obtained, was then benzylated on the two remaining free hydroxyls, and without further purification, TIPS-deprotected. A bromination with NBS in DCM at -78 ° C, allowed us to obtain in good yield the derivative **67**, which has been subjected to the final step to obtain the silyl-aryl triflate **62**.

Scheme 31



This final reaction proceeds in two steps, first with HMDS in THF at reflux, obtaining an intermediate in which the TMS group protects the free phenolic oxygen, and then, immediately after, with *n*BuLi and triflic anhydride at -100°C.

The initial addition of *n*BuLi causes a retro-Brook reaction in which the TMS is transferred from oxygen to the *ortho* position on the benzene ring. This is due to the fact that, the exchange caused on that position, from *n*BuLi with bromine, creates a situation in which the TMS, can re-arrange in the adjacent position, leaving the negative charge on the oxygen, so that it can form the strong link with the counter-ion litium.

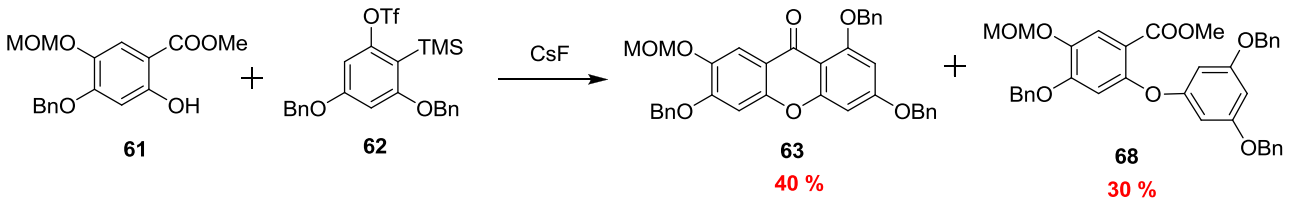
The subsequent addition of triflic anhydride, forms the desired silyl-aryl triflate **62**, in turn ready to be reacted with the previous fragment (A), to obtain the xanthone **63** according to the method described by Larock and fully described in the introduction.

8.4 Towards the synthesis of Rubraxantone and of Toxyloxantone B

Having obtained two fragments **61** and **62**, so next step should be try to fuse them together as described by Larock. But contrary to our expectations, the ratio between the two possible products (the desired xanthone and the open intermediate **68**), was found to be in favour the undesidered product, in disagreement as to what described by Larock (1 to 1 against the 9 to 1 by Larock reported) (Scheme 32).

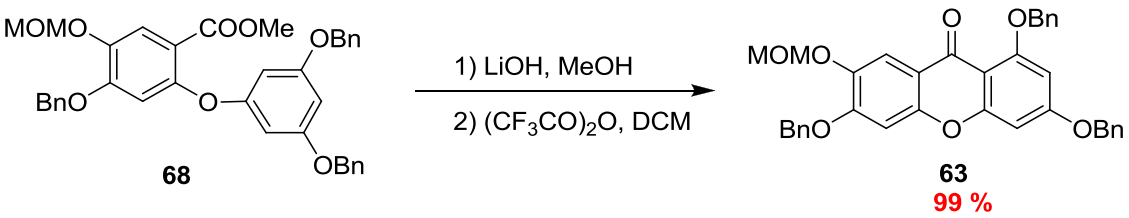
Intrigued by that ratio obtained, contrary to what described Larock, since the only available source of protons is from the reaction of the intermediate with a second molecule of phenol (see Scheme 16), we decided to try to make the fragment A first react with one equivalent of NaH, before adding the CsF and fragment B. Proceeding in this way, the total yield of the reaction was always the same (70%), but it was possible to improve the ratio between the two products obtained, thus passing from a 1 to 1, to a 3 to 2.

Scheme 32



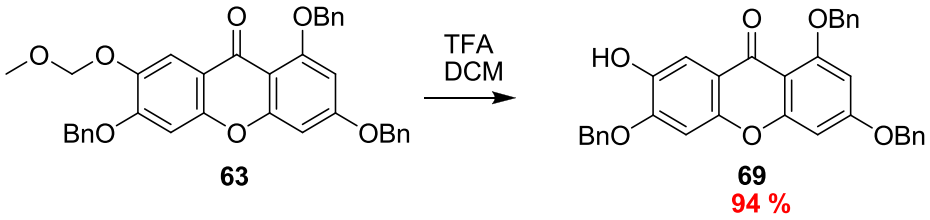
Furthermore the isolated product **68**, was collected, and reacted in two steps, first with LiOH in methanol, to give the corresponding acid, and then with trifluoroacetic anhydride to give xanthone **63** (scheme 33).

Scheme 33



Having obtained the key product **63**, the next step was to remove the MOM, with trifluoroacetic acid in dichloromethane as solvent, giving the product **69** with the free hydroxyl with excellent yield (Scheme 34).

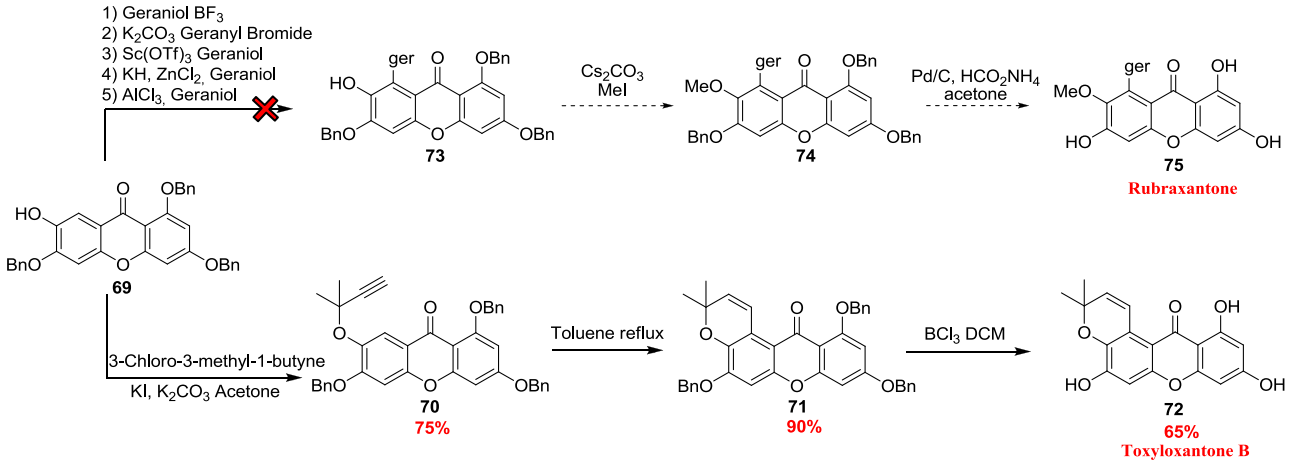
Scheme 34



In the case of toxyloxanthone B, derivative **69**, underwent an alkylation on the phenol, with 3-chloro-3-methyl-1-butyne, to give the product **70** in good yield, followed by a Claisen rearrangement to afford the product of intramolecular cyclization **71** in only toluene to reflux, and finally, a selective debenzylation gave the desired xanthone **72** (Scheme 35).

This final step, the same for both natural products, required more attention, as the conditions to remove the benzyl must not alter the double bonds in the geranyl chain. This means that the common methods used to remove the benzyl such as the use of Lewis acids or H_2-Pd / C, were not convenient. However, in the literature there are several examples in which this reaction is performed in the presence of double bonds susceptible to secondary reactions. Initial attemps used ammonium formate as a hydrogen donor[53], but while working at room temperature the reaction didn't proceed, when we heated the reaction mixture, the starting material was decomposed. A last attempt was made by using BCl_3 and pentamethylbenzene[54], giving the desiderated and final product in good yield.

Scheme 35



In the case of rubraxantone, the derivative **69** should undergo a Friedel Crafts alkylation, with the intent to enter the geranyl in the *ortho* position to the one free hydroxyl. This reaction was tried with different methods reported in literature[55-58], but none of them gave the desired product. In reactions with gerianiol and different Lewis acids, not strong enough to remove the benzyl groups, such as boron trifluoride-diethyl etherate, Scandium triflate and Zinc chloride, and also Aluminum chloride, gave only starting material back. Another attempt was made by using potassium carbonate as base and geranyl bromide, in an attempt to exploit the present conjugation and obtain the C-alkylation[56], but in our case the product obtained, was the product of O-alkylation. Other attempts should be tried, but unfortunately could not be carried out due to time constraints.

8.5 Conclusion and future work

The synthesis of toxyloxanthone B was completed, while few steps remain before the synthesis of rubraxanthone will be completed. In this case, once the geranyl chain is introduced, all that remains is the methylation of phenol, and finally the removal of the three benzyl groups (Scheme 32). This step will require close attention as the conditions required to remove the benzyl groups, must not alter the double bonds in the geranyl chain.

9. EXPERIMENTAL

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}.

9.1.1 Preparation of ethyl 8-oxo-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2-carboxylate (9a)

Preparation of 2,2,2-Trichloro-1-(1*H*-pyrrol-2-yl)ethanone (5).

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 hours. A solution of pyrrole (3 g, 44.7 mmol) in diethyl ether (25 mL) was added and the mixture was stirred for another hour. 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 concentrated under reduced pressure. Quantitative yield. The spectroscopic data are in agreement with the literature.[12]

Preparation of Ethyl 1*H*-pyrrole-2-carboxylate (6).

To a solution of potassium ethylate (4 g, 49.2 mmol) in ethanol (120 mL), a solution of **5** (9.5 g, 44.7 mmol) in dichloromethane (75 mL) was added. After 30 minutes at room temperature, the reaction mixture was evaporated. The residue was dissolved with HCl 2 N (45 mL) and diethyl ether (105 mL). The two phases were separated and the acqueos 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 fraction was dried on Na_2SO_4 and concentrated under reduced pressure to give the crude product that was purified by flash column chromatography (dichloromethane). Yield 95%. The spectroscopic data are in agreement with the literature. [12]

Preparation of 5-[5-(Ethoxycarbonyl)-1*H*-pyrrol-3-yl]-5-oxopentanoic acid (7).

A suspension of $AlCl_3$ (12 g, 90 mmol) and glutaric anhydride (3.40 g, 30 mmol) in dry dichloromethane, (60 mL) was stirred at room temperature for one hour. A solution of **6** (2.1 g, 15

mmol) in dry dichloromethane (30 mL), was added and the reaction mixture was stirred for another hour and 30 minutes. Ice and water were added (80 mL), and the two phases were separated. The acqueous fraction was extracted with ethyl acetate (3 x 60 mL). The organic phases were collected, dried on Na_2SO_4, and concentrated under reduced pressure to give the crude product that was purified by flash column chromatography (dichloromethane : ethyl acetate 1:1). Yield: 91%, light brown solid; mp: 81.4 – 82.3 ^oC; IR: 3337 (OH broad), 1702 (CO), 1696 (CO), 1653 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) (ppm): 1.29 (3H, t, J = 6.7 Hz, CH_3), 1.70 – 1.86 (2H, m, CH_2), 2.27 (2H, t, J = 6.2 Hz, CH_2), 2.80 (2H, t, J = 6.2 Hz, CH_2), 4.26 (2H, q, J = 6.7 Hz, CH_2), 7.13 (1H, s, H-4), 7.72 (1H, s, H-2), 12.07 (1H, s, OH), 12.51 (1H, s, NH); ^{13}C NMR (DMSO-d_6) (ppm): 14.2 (q), 19.6 (t), 32.9 (t), 37.9 (t), 60.1 (t), 114.0 (d), 123.6 (s), 125.8 (s), 127.8 (d), 160.1 (s), 174.2 (s), 194.6 (s).

Preparation of 5-[5-(ethoxycarbonyl)-1*H*-pyrrol-3-yl]pentanoic acid (8).

To a solution of **7** (1.5 g, 6 mmol) in trifluoracetic acid (13 mL), triethylsilane (3.3 mL, 21 mmol) was added and the reaction mixture was stirred at room temperature for one night (16 h). The solvent was evaporated and brine (40 mL) was added to the residue. The solid formed was filtered and purified by flash column chromatography (dichloromethane : ethyl acetate 1:1). Yield: 80%, brown solid; mp: 84.6 – 85.4 ^oC; IR: 3355 (OH broad), 1700 (CO), 1653 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) (ppm): 1.26 (3H, t, J = 7.1 Hz, CH_3), 1.41 – 1.62 (4H, m, CH_2 x 2), 2.21 (2H, t, J = 6.4 Hz, CH_2), 2.39 (2H, t, J = 6.4 Hz, CH_2), 4.20 (2H, q, J = 7.1 Hz, CH_2), 6.60 (1H, s, H-4), 6.79 (1H, s, H-2), 11.55 (1H, s, OH), 11.99 (1H, s, NH); ^{13}C NMR (DMSO-d_6) (ppm): 14.4 (q), 24.1 (t), 25.7 (t), 29.9 (t), 33.5 (t), 59,3 (t), 114.5 (d), 121.5 (s), 121.7 (d), 124.5 (s), 160.4 (s), 174.5 (s).

Preparation of Ethyl 8-oxo-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2-carboxylate (9a).

Trifluoroacetic anhydride (3.5 mL, 25.2 mmol) was added to a solution of **8** (1 g, 4.2 mmol), in dry dichloromethane (10 mL), and the reaction mixture was stirred for three hours at room temperature. The solvent was evaporated and a satured solution of $NaHCO_3$ (40 mL) was added to the residue. The solid formed, was filtered and purified by flash column chromatography (dichloromethane). Yield: 70%, white solid; mp: 76.7 – 76.9 ^oC; IR: 3415 (NH), 1709 (CO), 1633 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) (ppm): 1.28 (3H, t, J = 6.9 Hz, CH_3), 1.60 – 1.97 (4H, m, CH_2 x 2), 2.63 (2H, t, J = 6.6 Hz, CH_2), 2.79 (2H, t, J = 6.6 Hz, CH_2), 4.24 (2H, q, J = 6.9 Hz, CH_2), 6.67 (1H, s, H-3), 11.91 (1H, s, NH); ^{13}C NMR (DMSO-d_6) (ppm): 14.1 (q), 21.5 (t), 25.4 (t), 26.0 (t), 41.2 (t), 60.2 (t), 115.7 (d), 125.9 (s), 131.9 (s), 132.7 (s), 160.0 (s), 192.1 (s).

9.1.2 Preparation of 4,5,6,7-tetrahydrocyclohepta[*b*]pyrrol-8(1*H*)-one (9c)

Preparation of 1-(Phenylsulfonyl)-1*H*-pyrrole (10).

To a solution of pyrrole (2.72 mL, 40 mmol) in dry THF (28 mL), under N_2 atmosphere at -78 ^oC, 24 mL of LDA (2M in THF/heptanes/ethylbenzene, 48 mmol) were added and the mixture was heated until room temperature and stirred for 2 hours. The reaction mixture was then cooled again at -78 ^oC, and a solution of phenylsulfonil chloride (5.6 mL, 48 mmol) in dry THF (25 mL) was added. After 2 hours and half, the reaction was heated to room temperature, and 5 mL of water were added. The reaction mixture was distilled *in vacuo* and the residue was washed with a solution of NH_4Cl (20 g) in HCl 0.1 M (140 mL). The solution was extracted with dichloromethane (3 x 100 mL) and the organic phase was dried on Na_2SO_4 and evaporated. The residue was purified by flash column chromatography (dichloromethane). Quantitative yield. The spectroscopic data are in agreement with the structure of the commercial product.

Preparation of 5-oxo-5-[1-(Phenylsulfonyl)-1*H*-pyrrol-3-yl]pentanoic acid (11)

A suspension of $AlCl_3$ (8 g, 60 mmol) and glutaric anhydride (2.3 g, 20 mmol) in dry dichloromethane, (40 mL) was stirred at room temperature for one hour. A solution of **10** (2 g, 10 mmol) in dry dichloromethane (20 mL), was added and the reaction mixture was stirred for another hour and 30 minutes. Ice and water were added (80 mL), and the two phases were separated. The acqueous fraction was extracted with dichloromethane (3 x 40 mL). The organic phases were collected, and dried on Na_2SO_4, and concentrated under reduced pressure to give the crude product that was purified by flash column chromatography (dichloromethane : ethyl acetate 6:4). Yield: 80%, light brown solid; mp: 111.6 – 111.8 ^oC; IR: 3550 (OH), 1706 (CO), 1675 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) (ppm): 1.72 – 1.83 (2H, m, CH_2), 2.26 (2H, t, J = 7.3 Hz, CH_2), 2.85 (2H, t, J = 7.3 Hz, CH_2), 6.67 (1H, dd, J = 3.3, 1.8 Hz, H-4), 7.45 (1H, dd, J = 3.3, 1.8 Hz, H-5), 7.64 – 7.86 (3H, m, H-3', H-4'and H-5'), 8.06 – 8.13 (2H, m, H-2'and H-6'), 8.21 (1H, s, H-2), 12.07 (1H, s, OH); ^{13}C NMR (DMSO-d_6) (ppm): 19.2 (t), 32.8 (t), 38.0 (t), 112.0 (d), 122.2 (d), 125.4 (d), 127.2 (d x 2), 128.6 (s), 130.1 (d x 2), 135.1 (d), 137.3 (s), 174.1 (s), 194.7 (s).

Preparation of 5-[1-(Phenylsulfonyl)-1*H*-pyrrol-3- yl]pentanoic acid (12).

An amalgam of zinc (8.5 g, 130 mmol) and mercury(II) chloride (2.96 g, 10.9 mmol) in water (12 mL) and HCl 12 M (0.7 mL) was prepared; after 30 minutes the acqueous phase was eliminated and **11** (3.5 g, 10.9 mmol), water (5 mL), toluene (50 mL) and HCl 12 M (12.5 mL) were added. The reaction mixture was heated to reflux for 4 hours, and the two phases were separated. The acqueous phase was extracted with dichloromethane (3 x 30 mL) and the organic fractions were collected,

dried on Na_2SO_4 and evaporated. The residue was purified by flash column chromatography (dichloromethane : ethyl acetate 9:1). Yield: 75%, white solid; mp: 84.0 – 84.2 ^oC; IR: 3548 (OH), 1701 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) (ppm): 1.46 (4H, t, J = 7.2 Hz, CH_2 x 2), 2.19 (2H, t, J = 7.2 Hz, CH_2), 2.34 (2H, t, J = 7.2 Hz, CH_2), 6.24 (1H, dd, J = 2.9, 1.5 Hz, H-4), 7.10 (1H, s, H-2), 7.24 (1H, dd, J = 2.9, 1.5 Hz, H-5), 7.57 – 7.79 (3H, m, H-3', H-4' and H-5'), 7.89 – 7.95 (2H, m, H-2'and H-6'), 11.99 (1H, s, OH); ^{13}C NMR (DMSO-d_6) (ppm): 24.0 (t), 25.7 (t), 28.8 (t), 33.3 (t), 115.1 (d), 117.5 (d), 121.2 (d), 126.5 (d x 2), 129.3 (s), 129.7 (d x 2), 134.3 (d), 138.3 (s), 174.4 (s).

Preparation of 1-(Phenylsulfonyl)-4,5,6,7-tetrahydrocyclohepta[*b*]pyrrol-8(1*H*)-one (9b).

Trifluoroacetic anhydride (5.4 mL, 39 mmol) was added to a solution of **12** (2 g, 6.5 mmol), in dry dichloromethane (20 mL), and the reaction mixture was stirred for three hours at room temperature. The solvent was evaporated and a satured solution of $NaHCO_3$ (40 mL) was added to residue. The solid formed, was filtered and purified by flash column chromatography (dichloromethane). Yield: 75%, white solid; mp: 99.3 – 99.6 ^oC; IR: 1659 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) (ppm): 1.63 – 1.78 (4H, m, CH_2 x 2), 2.53 (2H, t, J = 5.9 Hz, CH_2), 2.77 (2H, t, J = 5.9 Hz, CH_2), 6.39 (1H, d, J = 3.2 Hz, H-3), 7.59 – 7.76 (3H, m, H-3', H-4' and H-5'), 7.81 (1H, d, J = 3.2 Hz, H-2), 7.96 (2H, dd, J = 8.2, 1.6 Hz, H-2' and H-6'); ^{13}C NMR (DMSO-d_6) (ppm): 21.0 (t), 24.4 (t), 25.8 (t), 40.9 (t), 113.3 (d), 127.5 (d x 2), 129.0 (d x 2), 129.1 (d), 131.0 (s), 133.8 (d), 139.0 (s), 139.6 (s), 190.2 (s).

Preparation of 4,5,6,7-Tetrahydrocyclohepta[*b*]pyrrol-8(1*H*)-one (9c).

A solution of NaOH (1.66 g, 41.52 mmol) in ethanol (15 mL) was added to a suspension of **9b** (3 g, 10.4 mmol) in ethanol (15 mL), and the resulting mixture was heated to reflux for 3 hours. The reaction mixture was concentrated under reduce pressure and the residue poured into ice and water (20 mL) and acidified with HCl 6 M. The solution was extracted with dichloromethane (3 x 30 mL) and the organic phases was collected, dried on Na_2SO_4 and evaporated. The residue was purified by flash column chromatography (dichloromethane) Yield: 80%, white solid, mp: 55.1 – 55.3 ^oC; IR: 3438 (NH), 1617 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) (ppm): 1.69 – 1.90 (4H, m, CH_2 x 2), 2.56 (2H, t, J = 6.2 Hz, CH_2), 2.79 (2H, t, J = 6.2 Hz, CH_2), 6.01 (1H, d, J = 2.8 Hz, H-3), 6.95 (1H, d, J = 2.8 Hz, H-2), 11.38 (1H, s, NH); ^{13}C NMR (DMSO-d_6) (ppm): 22.3 (t), 26.0 (t), 27.1 (t), 41.4 (t), 110.6 (d), 124.5 (d), 129.3 (s), 132.1 (s), 190.5 (s).

9.2 Functionalization of Ethyl 8-oxo-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2-carboxylate (9a) and of 4,5,6,7-Tetrahydrocyclohepta[*b*]pyrrol-8(1*H*)-one (9c)

To a solution of the appropriate ketone ethyl 8-oxo-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2-carboxylate (**9a**) or 4,5,6,7-tetrahydrocyclohepta[*b*]pyrrol-8(1*H*)-one (**9c**) (9 mmol) in dry DMF (17 mL), NaH (10 mmol) was added at 0 °C and the reaction was stirred for one hour and half at room temperature. The appropriate alkyl or alkylaryl halide (13.5 mmol) was added at 0 °C, and the reaction mixture was stirred at room temperature or at higher temperature (max 80 °C) up to completeness. Then the reaction was poured into ice and brine, and in the case of formation of a precipitate, the solid was filtered. In the absence of precipitate, the aqueous solution was extracted with dichloromethane (3 x 50 mL). The organic phase was dried over Na_2SO_4 and the solvent evaporated at reduced pressure. The crude product was purified by chromatography column (dichloromethane).

Ethyl 1-methyl-8-oxo-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2-carboxylate (9d). This compound was obtained from reaction of **9a** with iodomethane in DMF in 2 hours at room temperature. Yield: 94%, white solid; mp: 51.3 – 51.5 °C; IR: 1705 (CO), 1642 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) (ppm): 1.28 (3H, t, J = 7.1 Hz, CH_3), 1.68 – 1.77 (4H, m, CH_2 x 2), 2.64 (2H, t, J = 6.6 Hz, CH_2), 2.75 (2H, t, J = 6.6 Hz, CH_2), 4.06 (3H, s, CH_3), 4.25 (2H, q, J = 7.1 Hz, CH_2), 6.74 (1H, s, H-3); ^{13}C NMR (DMSO-d_6) (ppm): 14.1 (q), 20.8 (t), 24.4 (t), 24.8 (t), 34.3 (q), 41.4 (t), 60.2 (t), 116.6 (d), 126.3 (s), 132.8 (s), 133.5 (s), 160.3 (s), 194.1 (s).

Ethyl 1-benzyl-8-oxo-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2-carboxylate (9e). This compound was obtained from reaction of **9a** with benzyl bromide in DMF in 2 hours at room temperature. Yield: 96%, white solid; mp: 46.6 – 47.4 °C; IR: 1710 (CO), 1645 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) (ppm): 1.21 (3H, t, J = 7.1 Hz, CH_3), 1.60 – 1.80 (4H, m, CH_2 x 2), 2.60 (2H, t, J = 6.6 Hz, CH_2), 2.81 (2H, t, J = 6.6 Hz, CH_2), 4.18 (2H, q, J = 7.1 Hz, CH_2), 6.01 (2H, s, CH_2), 6.84 (1H, s, H-3), 6.88 (2H, s, H-2' and H-6'), 7.11 – 7.32 (3H, m, H-3', H-4' and H-5'); ^{13}C NMR (DMSO-d_6) (ppm): 14.0 (q), 20.7 (t), 24.3 (t), 24.7 (t), 41.4 (t), 48.4 (t), 60.3 (t), 117.7 (d), 125.6 (d x 2), 126.1 (s), 126.6 (d), 128.3 (d x 2), 133.0 (s), 133.8 (s), 139.2 (s), 160.1 (s), 194.2 (s).

Ethyl 1-(2-methoxybenzyl)-8-oxo-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2carboxylate (9f). This compound was obtained from reaction of **9a** with 2-methoxybenzylchloride in DMF in a night (16 hours) at room temperature. Yield: 78%, white solid; mp: 121.0 – 121.2 °C; IR: 1709 (CO), 1645 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) (ppm): 1.18 (3H, t, J = 7.1 Hz, CH_3), 1.64 – 1.81 (4H,

m, CH_2 x 2), 2.57 (2H, t, *J* = 5.7 Hz, CH_2), 2.80 (2H, t, *J* = 5.7 Hz, CH_2), 3.83 (3H, s, CH_3), 4.14 (2H, q, *J* = 7.1 Hz, CH_2), 5.92 (2H, s, CH_2), 6.01 (1H, d, *J* = 7.4 Hz, H-3'), 6.75 (1H, t, *J* = 7.4 Hz, H-5'), 6.88 (1H, s, H-3), 6.96 (1H, d, *J* = 7.4 Hz, H-6'), 7.16 (1H, t, *J* = 7.4 Hz, H-4'); ^{13}C NMR (DMSO-d_6) (ppm): 13.9 (q), 20.7 (t), 24.3 (t), 24.8 (t), 41.3 (t), 44.8 (t), 55.2 (q), 60.2 (t), 110.1 (d), 117.5 (d), 120.3 (d), 124.2 (d), 126.5 (s), 127.5 (d), 127.7 (s), 133.4 (s), 133.5 (s), 155.7 (s), 159.9 (s), 194.0 (s).

Ethyl 1-(3-methoxybenzyl)-8-oxo-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2carboxylate (9g). This compound was obtained from reaction of **9a** with 3-methoxybenzylchloride in DMF in 8 hours at room temperature. Yield: 81%, white solid; mp: 65.5 – 65.8 °C; IR: 1709 (CO), 1646 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) (ppm): 1.22 (3H, t, *J* = 7.1 Hz, CH_3), 1.65 - 1.80 (4H, m, CH_2 x 2), 2.60 (2H, t, *J* = 5.6 Hz, CH_2), 2.81 (2H, t, *J* = 5.6 Hz, CH_2), 3.67 (3H, s, CH_3), 4.19 (2H, q, *J* = 7.1 Hz, CH_2), 5.98 (2H, s, CH_2), 6.38 – 6.41 (2H, m, H-2' and H-6'), 6.76 (1H, dd, *J* = 8.1, 2.3 Hz, H-4'), 6.87 (1H, s, H-3), 7.17 (1H, t, *J* = 8.1 Hz, H-5'); ^{13}C NMR (DMSO-d_6) (ppm): 14.0 (q), 20.7 (t), 24.3 (t), 24.7 (t), 41,4 (t), 48.2 (t), 54.8 (q), 60.4 (t), 111.5 (d), 111.6 (d), 117.6 (d), 117.7 (d), 126.2 (s), 129.4 (d), 133.0 (s), 133.8 (s), 140.9 (s), 159.2 (s), 160.1 (s), 194.2 (s).

Ethyl 1-(4-methoxybenzyl)-8-oxo-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2-carboxylate (9h). This compound was obtained from reaction of **9a** with 4-methoxybenzylchloride in DMF in 6 hours at room temperature. Yield: 79%, white solid; mp: 73.2 – 73.6 °C; IR: 1709 (CO), 1645 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) (ppm): 1.23 (3H, t, *J* = 7.1 Hz, CH_3), 1.63 – 1.79 (4H, m, CH_2 x 2), 2.60 (2H, t, *J* = 5.6 Hz, CH_2), 2.79 (2H, t, *J* = 5.6 Hz, CH_2), 3.69 (3H, s, CH_3), 4.20 (2H, q, *J* = 7.1 Hz, CH_2), 5.93 (2H, s, CH_2), 6.78 – 6.81 (4H, m, Ar), 6.83 (1H, s, H-3); ^{13}C NMR (DMSO-d_6) (ppm): 14.0 (q), 20.7 (t), 24.2 (t), 24.7 (t), 41.4 (t), 47.6 (t), 54.9 (q), 60.4 (t), 113.6 (d x 2), 117.7 (d), 126.1 (s), 127.2 (d x 2), 131.0 (s), 132.9 (s), 133.9 (s), 158.0 (s), 160.2 (s), 194.4 (s).

Ethyl 1-(2,5-dimethoxybenzyl)-8-oxo-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2-carboxylate (9i). This compound was obtained from reaction of **9a** with 2,5-dimethoxybenzylchloride in DMF in a night (16 hours) at room temperature. Yield: 81%, white solid; mp: 92.8 – 93.4 °C; IR: 1711 (CO), 1647 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) (ppm): 1.20 (3H, t, *J* = 7.1 Hz, CH_3), 1.65 – 1.81 (4H, m, CH_2 x 2), 2.59 (2H, t, *J* = 5.6 Hz, CH_2), 2.84 (2H, t, *J* = 5.6 Hz, CH_2), 3.54 (3H, s, CH_3), 3.78 (3H, s, CH_3), 4.16 (2H, q, *J* = 7.1 Hz, CH_2), 5.53 (1H, d, *J* = 2.9 Hz, H-6'), 5.90 (2H, s, CH_2), 6.72 (1H, dd, *J* = 8.8, 2.9 Hz, H-4'), 6.89 (1H, d, *J* = 8.8 Hz, H-3'), 6.90 (1H, s, H-3); ^{13}C NMR (DMSO-d_6) (ppm): 13.9 (q), 20.7 (t), 24.4 (t), 24.7 (t), 41.3 (t), 44.8 (t), 55.0 (q), 55.6 (q), 60.3 (t), 110.3 (d), 110.8 (d), 111.6 (d), 117.5 (d), 126.5 (s), 129.1 (s), 133.3 (s), 133.6 (s), 149.9 (s), 153.1 (s), 159.9 (s), 194.0 (s).

Ethyl 1-(3,5-dimethoxybenzyl)-8-oxo-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2-carboxylate (9j). This compound was obtained from reaction of **9a** with 3,5-dimethoxybenzylchloride in DMF in a night (16 hours) at room temperature. Yield: 83%, white solid; mp: 71.8 – 72.0 °C; IR: 1707 (CO), 1647 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) (ppm): 1.22 (3H, t, J = 7.1 Hz, CH_3), 1.72 (4H, m, CH_2 x 2), 2.61 (2H, t, J = 6.9 Hz, CH_2), 2.82 (2H, t, J = 6.9 Hz, CH_2), 3.65 (6H, s, CH_3 x 2), 4.19 (2H, q, J = 7.1 Hz, CH_2), 5.94 (4H, s, CH_2, H-2' and H-6'), 6.32 (1H, s, H-4'), 6.88 (1H, s, H-3); ^{13}C NMR (DMSO-d_6) (ppm): 14.1 (q), 20.8 (t), 24.4 (t), 24.8 (t), 41.4 (t), 48.2 (t), 55.0 (q x 2), 60.4 (t), 97.8 (d), 103.7 (d x 2), 117.8 (d), 126.3 (s), 133.1 (s), 133.9 (s), 141.9 (s), 160.1 (CO), 160.5 (s x 2), 194.3 (s).

Ethyl 1-(3,4,5-trimethoxybenzyl)-8-oxo-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2-carboxylate (9k). This compound was obtained from reaction of **9a** with 3,4,5-trimethoxybenzylchloride in DMF in 5 hours at 80 °C. Yield: 80%, white solid; mp: 70 – 70.2 °C; IR: 1709 (CO), 1646 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) (ppm): 1.24 (3H, t, J = 7.1 Hz, CH_3), 1.66 – 1.80 (4H, m, CH_2 x 2), 2.64 (2H, t, J = 5.6 Hz, CH_2), 2.82 (2H, t, J = 5.6 Hz, CH_2), 3.60 (3H, s, CH_3), 3.64 (6H, s, CH_3 x 2), 4.22 (2H, q, J = 7.1 Hz, CH_2), 5.97 (2H, s, CH_2), 6.16 (2H, s, H-2' and H-6'), 6.88 (1H, s, H-3); ^{13}C NMR (DMSO-d_6) (ppm): 14.0 (q), 20.8 (t), 24.3 (t), 24.7 (t), 41.4 (t), 48.1 (t), 55.6 (q x 2), 59.9 (q), 60.4 (t), 102.9 (d x 2), 117.8 (d), 126.3 (s), 133.1 (s), 133.9 (s), 135.0 (s), 136.2 (s), 152.8 (s x 2), 160.2 (s), 194.4 (s).

1-Methyl-4,5,6,7-tetrahydrocyclohepta[*b*]pyrrol-8(1*H*)-one (9l). This compound was obtained from reaction of **9c** with iodomethane in DMF in 2 hours at room temperature. Yield: 88%, colourless oil; IR: 1639 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) (ppm): 1.69 – 1.75 (4H, m, CH_2 x 2), 2.55 (2H, J = 6.2 Hz, CH_2), 2.74 (2H, t, J = 6.2 Hz, CH_2), 3.79 (3H, s, CH_3), 5.96 (1H, d, J = 2.4 Hz, H-3), 6.98 (1H, d, J = 2.4 Hz, H-2); ^{13}C NMR (DMSO-d_6) (ppm): 21.0 (t), 24.6 (t), 25.7 (t), 36.9 (q), 41.0 (t), 108.7 (d), 128.5 (s), 129.9 (d), 135.3 (s), 191.6 (s).

1-Benzyl-4,5,6,7-tetrahydrocyclohepta[*b*]pyrrol-8(1*H*)-one (9m). This compound was obtained from reaction of **9c** with benzyl bromide in DMF in 4 hours at room temperature. Yield: 92 %, beige solid; mp: 54.2 – 54.4 °C; IR: 1623 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) (ppm): 1.65 – 1.73 (4H, m, CH_2 x 2), 2.50 (2H, t, J = 6.2 Hz, CH_2), 2.77 (2H, t, J = 6.2 Hz, CH_2), 5.51 (2H, s, CH_2), 6.07 (1H, d, J = 2.4 Hz, H-3), 7.04 (2H, d, J = 6.9 Hz, H-2'and H-6'), 7.19 (1H, d, J = 2.4 Hz, H-2), 7.24 – 7.29 (3H, m, H-3', H-4' and H-5'); ^{13}C NMR (DMSO-d_6) (ppm): 20.9 (t), 24.4 (t), 25.5 (t), 40.9 (t), 51.1 (t), 109.6 (d), 126.53 (d x 2), 126.9 (d), 127.9 (s), 128.3 (d x 2), 129.7 (d), 136.1 (s), 139.3 (s), 191.6 (s).

1-(2-Methoxybenzyl)-4,5,6,7-tetrahydrocyclohepta[*b*]pyrrol-8(1*H*)-one (9n). This compound was obtained from reaction of **9c** with 2-methoxybenzylchloride in DMF in a night (16hours) at room temperature. Yield: 93 %, grey solid; mp: 64.5 – 64.9 °C; IR: 1628 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.79 – 1.85 (4H, m, CH_2 x 2), 2.61 (2H, t, *J* = 6.2 Hz, CH_2), 2.82 (2H, t, *J* = 6.2 Hz, CH_2), 3.85 (3H, s, CH_3), 5.55 (2H, s, CH_2), 5.99 (1H, d, *J* = 2.5 Hz, H-3), 6.76 – 6.88 (4H, m, H-3', H-4', H-5' and H-6'), 7.17 (1H, d, *J* = 2.5 Hz, H-2); ^{13}C NMR ($CDCl_3$) (ppm): 21.5 (t), 24.9 (t), 26.4 (t), 41.6 (t), 47.4 (t), 55.3 (q), 109.4 (d), 110.0 (d), 120.6 (d), 127.2 (s), 128.1 (d), 128.4 (d), 128.9 (s), 129.4 (d), 136.4 (s), 156.7 (s), 192.8 (s).

1-(3-Methoxybenzyl)-4,5,6,7-tetrahydrocyclohepta[*b*]pyrrol-8(1*H*)-one (9o). This compound was obtained from reaction of **9c** with 3-methoxybenzylchloride in DMF in 4 hours at room temperature. Yield: 84 %, white solid; mp: 53.1 – 53.4 °C; IR: 1629 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) (ppm): 1.69 – 1.72 (4H, m, CH_2 x 2), 2.50 (2H, t, *J* = 5.9 Hz, CH_2), 2.77 (2H, t, *J* = 5.9 Hz, CH_2), 3.69 (3H, s, CH_3), 5.48 (2H, s, CH_2), 6.07 (1H, d, *J* = 2.4 Hz, H-3), 6.58 – 6.62 (2H, m, H-2' and H-6'), 6.79 (1H, dd, *J* = 7.9, 2.3 Hz, H-4'), 7.16 – 7.24 (2H, m, H-2 and H-5'); ^{13}C NMR (DMSO-d_6) (ppm): 20.9 (t), 24.4 (t), 25.5 (t), 40.9 (t), 51.0 (t), 54.8 (q), 109.6 (d), 112.0 (d), 112.4 (d), 118.6 (d), 127.9 (s), 129.4 (d), 129.8 (d), 136.1 (s), 140.9 (s), 159.2 (s), 191.7 (s).

1-(4-Methoxybenzyl)-4,5,6,7-tetrahydrocyclohepta[*b*]pyrrol-8(1*H*)-one (9p). This compound was obtained from reaction of **9c** with 4-methoxybenzylchloride in DMF in a night (16 hours) at room temperature. Yield: 96 %, white solid; mp: 63.0 – 63.3 °C; IR: 1635 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.77 – 1.83 (4H, m, CH_2 x 2), 2.61 (2H, t, *J* = 6.3 Hz, CH_2), 2.80 (2H, t, *J* = 6.3 Hz, CH_2), 3.77 (3H, s, CH_3), 5.46 (2H, s, CH_2), 6.00 (1H, d, *J* = 2.5 Hz, H-3), 6.78 – 6.86 (3H, m, H-2, H-3' and H-5'), 7.06 – 7.11 (2H, m, H-2' and H-6'); ^{13}C NMR ($CDCl_3$) (ppm): 21.4 (t), 24.9 (t), 26.4 (t), 41.6 (t), 51.9 (t), 55.2 (q), 109.6 (d), 113.9 (d x 2), 128.6 (d), 128.7 (d x 2), 128.8 (s), 130.6 (s), 136.8 (s), 158.8 (s), 192.8 (s).

1-(2,5-Dimethoxybenzyl)-4,5,6,7-tetrahydrocyclohepta[*b*]pyrrol-8(1*H*)-one (9q). This compound was obtained from reaction of **9c** with 2,5-dimethoxybenzylchloride in DMF in 2 hours at room temperature. Yield: 90 %, beige solid; mp: 85.0 – 85.4 °C; IR: 1634 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.79 – 1.85 (4H, m, CH_2 x 2), 2.62 (2H, t, *J* = 6.1 Hz, CH_2), 2.81 (2H, t, *J* = 6.1 Hz, CH_2), 3.67 (3H, s, CH_3), 3.81 (3H, s, CH_3), 5.52 (2H, s, CH_2), 5.99 (1H, d, *J* = 2.6 Hz, H-3), 6.35 (1H, d, *J* = 2.5 Hz, H-6'), 6.73 (1H, d, *J* = 2.6 Hz, H-2), 6.75 – 6.78 (1H, m, H-4'), 6.80 – 6.84 (1H, m, H-3'); ^{13}C NMR ($CDCl_3$) (ppm): 21.5 (t), 24.9 (t), 26.4 (t), 41.6 (t), 47.4 (t), 55.5 (q), 55.9 (q), 109.5 (d), 111.0 (d), 112.3 (d), 114.4 (d), 128.5 (s), 128.9 (s), 129.4 (d), 136.5 (s), 150.9 (s), 153.7 (s), 192.7 (s).

1-(3,5-Dimethoxybenzyl)-4,5,6,7-tetrahydrocyclohepta[*b*]pyrrol-8(1*H*)-one (9r). This compound was obtained from reaction of **9c** with 3,5-dimethoxybenzylchloride in DMF in 2 hours at room temperature. Yield: 90 %, grey solid; mp: 60.8 – 61.7 °C; IR: 1625 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.78 – 1.84 (4H, m, CH_2), 2.61 (2H, t, *J* = 6.0 Hz, CH_2), 2.81 (2H, t, *J* = 6.0 Hz, CH_2), 3.73 (6H, s, CH_3 x 2), 5.48 (2H, s, CH_2), 6.01 (1H, d, *J* = 2.5 Hz, H-3), 6.22 (2H, d, *J* = 2.2 Hz, H-2' and H-6'), 6.32 (1H, t, *J* = 2.2 Hz, H-4'), 6.79 (1H, d, *J* = 2.5 Hz, H-2); ^{13}C NMR ($CDCl_3$) (ppm): 21.5 (t), 24.9 (t), 26.4 (t), 41.6 (t), 52.4 (t), 55.2 (q x 2), 99.0 (d), 104.9 (d x 2), 109.8 (d), 128.8 (s), 129.1 (d), 136.7 (s), 141.1 (s), 160.9 (s x 2), 192.8 (s).

1-(3,4,5-Trimethoxybenzyl)-4,5,6,7-tetrahydrocyclohepta[*b*]pyrrol-8(1*H*)-one (9s). This compound was obtained from reaction of **9c** with 3,4,5-trimethoxybenzylchloride in DMF in a night (16 hours) at room temperature. Yield: 89 %, beige solid; mp: 64.6 – 65.3 °C; IR: 1635 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.79 – 1.85 (4H, m, CH_2 x 2), 2.63 (2H, t, *J* = 6.1 Hz, CH_2), 2.82 (2H, *J* = 6.1 Hz, CH_2), 3.79 (6H, s, CH_3 x 2), 3.81 (3H, s, CH_3), 5.47 (2H, s, CH_2), 6.03 (1H, d, *J* = 2.5 Hz, H-3), 6.33 (2H, s, H-2' and H-6'), 6.81 (1H, d, *J* = 2.5 Hz, H-2); ^{13}C NMR ($CDCl_3$) (ppm): 21.5 (t), 25.0 (t), 26.4 (t), 41.6 (t), 52.5 (t), 56.0 (q x 2), 60.8 (q), 104.0 (d x 2), 109.8 (d), 128.9 (s), 129.0 (d), 134.3 (s), 136.9 (s), 137.0 (s), 153.3 (s x 2), 192.9 (s).

9.3 Preparation of 7-[(dimethylamino)methylidene]-1-substituted-4,5,6,7-tetrahydrocyclohepta[*b*]pyrrol-8(1*H*)one (13)

Different conditions have been used depending on the series.

METHOD A: To a solution of ketones **9d-k** (1.3 mmol) in dry DMF (2.5 mL), TBDMAM (2 mmol) was added and the reaction mixture was irradiated under microwave conditions (Power 50 W; Time 20-40 min; Pressure (max) 100 psi; Temperature (max) 120°C).

METHOD B: To a solution of ketones **9d-k** (1.3 mmol) in dry DMF (2.5 mL), DMFDMA (1.4 mol) was added and the reaction mixture was irradiated under microwave conditions (Power 50 W; Time 40-120 min; Pressure (max) 100 psi; Temperature (max) 120°C).

METHOD C: To a solution of ketones **9b,l-s** (1.3 mmol) in dry DMF (2.5 mL) DMFDMA (13 mmol) was added and the reaction mixture was irradiated under microwave conditions (Power 150 W; Time 40-120 min; Pressure (max) 150 psi; Temperature (max) 130°C).

At the end in all the cases, when the reactions were completed, the reaction mixtures was poured into ice and brine and in the precipitate was filtered off. In the absence of precipitate, the aqueous phase was extracted with AcOEt (3 x 30 mL). The organic layer was dried (Na_2SO_4) and the solvent removed *in vacuo*.

Ethyl 7-[(dimethylamino)methylidene]-1-methyl-8-oxo-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2-carboxylate (13d). This compound was obtained from reaction of **9d** under MW conditions (Method A, 20 min). Yield: 99 %, pale yellow solid; mp: 110.8 – 111.0 °C; IR: 1700 (CO), 1636 (CO), cm^{-1}; ^{1}H NMR (DMSO-d_6) (ppm): 1.27 (3H, t, J = 6.9 Hz, CH_3), 1.70 – 1.80 (2H, m, CH_2), 2.32 (2H, t, J = 6.2 Hz, CH_2), 2.46 (2H, t, J = 6.2 Hz, CH_2), 3.09 (6H, s, CH_3 x 2), 3.96 (3H, s, CH_3), 4.22 (2H, q, J = 6.9 Hz, CH_2), 6.67 (1H, s, H-3), 7.46 (1H, s, CH); ^{13}C NMR (DMSO-d_6) (ppm): 14.2 (q), 22,8 (t), 23.0 (t), 29.6 (t), 33.8 (q), 43.0 (q x 2), 59.6 (t), 105.2 (s), 115.7 (d), 123.9 (s), 125.5 (s), 136.7 (s), 150.0 (d), 160.4 (s), 186.4 (s).

Ethyl 7-[(dimethylamino)methylidene]-1-benzyl-8-oxo-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2-carboxylate (13e). This compound was obtained from reaction of **9e** under MW conditions (Method A, 20 min; Method B, 80 min). Quantitative yield (Method A), 73% (Method B), pale yellow solid; mp: 130.3 - 131 °C; IR: 1704 (CO), 1635 (CO), cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.26 (3H, t, J = 7.1 Hz, CH_3), 1.72 – 1.86 (2H, m, CH_2), 2.36 (2H, t, J = 6.9 Hz, CH_2), 2.62 (2H, t, J = 6.9 Hz, CH_2), 3.07 (6H, s, CH_3 x 2), 4.21 (2H, q, J = 7.1 Hz, CH_2), 6.08 (2H, s, CH_2), 6.78 (1H, s, H-3), 7.10 – 7.22 (5H, m, Ar), 7.54 (1H, s, CH); ^{13}C NMR (DMSO-d_6) (ppm): 14.1 (q), 22.8 (t), 23.0 (t), 29.8 (t), 43.0 (q x 2), 47.4 (t), 59.7 (t), 104.9 (s), 116.7 (d), 123.5 (s), 125.6 (s), 125.7 (d x 2), 126.6 (d), 128.3 (d x 2), 133.8 (s), 139.8 (s), 150.3 (d), 160.2 (s), 186.4 (s).

Ethyl 7-[(dimethylamino)methylidene]-1-(2-methoxybenzyl)-8-oxo-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2-carboxylate (13f). This compound was obtained from reaction of **9f** under MW conditions (Method A, 20 min; Method B, 40 min). Yield: 97% (Method A), 70% (Method B), yellow solid; mp: 139 °C; IR: 1717 (CO), 1646 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) (ppm): 1.17 (3H, t, J = 7.0 Hz, CH_3), 1.73 – 1.78 (2H, m, CH_2), 2.29 (2H, t, J = 6.3 Hz, CH_2), 2.54 (2H, t, J = 6.3 Hz, CH_2), 3.05 (6H, s, CH_3 x 2), 3.80 (3H, s, CH_3), 4.11 (2H, q, J = 7.0 Hz, CH_2), 5.90 (2H, s, CH_2), 6.01 (1H, d, J = 7.1 Hz, H-3'), 6.68 – 7.01 (3H, m, H-3, H-5' and H-6'), 7.14 (1H, t, J = 7.2 Hz, H-4'), 7.39 (1H, s, CH); ^{13}C NMR (DMSO-d_6) (ppm): 14.0 (q), 22.8 (t), 23.1 (t), 29.8 (t), 43.0 (q x 2), 43.8 (t), 55.2 (q), 59.6 (t), 104.9 (s), 110.0 (d), 116.5 (d), 120.2 (d), 123.9 (s), 124.3 (d), 126.1 (s), 127.5 (d), 128.3 (s), 137.0 (s), 150.3 (d), 155.6 (s), 160.0 (s), 186.2 (s).

Ethyl 7-[(dimethylamino)methylidene]-1-(3-methoxybenzyl)-8-oxo-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2-carboxylate (13g). This compound was obtained from reaction of **9g** under MW conditions (Method A, 20 min; Method B, 40 min). Yield: 92% (Method A), 87% (Method B), yellow solid; mp: 87.3 – 88.1 °C; IR: 1707 (CO), 1636 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) (ppm): 1.21 (3H, t, J = 7.0 Hz, CH_3), 1.73 – 1.79 (2H, m, CH_2), 2.28 (2H, t, J = 6.7 Hz, CH_2), 2.57 (2H, t, J = 6.7 Hz, CH_2), 3.08 (6H, s, CH_3 x 2), 3.66 (3H, s, CH_3), 4.16 (2H, q, J = 7.0 Hz, CH_2), 5.95 (2H, s, CH_2), 6.37 – 6.49 (2H, m, H-2' and H-6'), 6.70 – 6.78 (2H, m, H-4' and H-3), 7.15 (1H, t, J = 7.9 Hz, H-5'), 7.46 (1H, s, CH); ^{13}C NMR (DMSO-d_6) (ppm): 14.1 (q), 22.8 (t), 23.0 (t), 29.8 (t), 43.0 (q x 2), 47.3 (t), 54.8 (q), 59.7 (t), 104.9 (s), 111.6 (d), 111.7 (d), 116.7 (d), 117.8 (d), 123.5 (s), 126.3 (s), 129.4 (d), 136.6 (s), 141.4 (s), 150.4 (d), 159.2 (s), 160.2 (s), 186.4 (s).

Ethyl 7-[(dimethylamino)methylidene]-1-(4-methoxybenzyl)-8-oxo-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2-carboxylate (13h). This compound was obtained from reaction of **9h** under MW conditions (Method A, 40 min; Method B, 40 min). Yield: 95% (Method A), 75% (Method B), brown oil; IR: 1706 (CO), 1635 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) (ppm): 1.22 (3H, t, J = 7.1 Hz, CH_3), 1.70 – 1.77 (2H, m, CH_2), 2.25 (2H, t, J = 6.7 Hz, CH_2), 2.54 (2H, t, J = 6.7 Hz, CH_2), 3.08 (6H, s, CH_3 x 2), 3.68 (3H, s, CH_3), 4.17 (2H, q, J = 7.1 Hz, CH_2), 5.87 (2H, s, CH_2), 6.74 (1H, s, H-3), 6.76 – 6.90 (4H, m, Ar), 7.48 (1H, s, CH); ^{13}C NMR (DMSO-d_6) (ppm): 14.2 (q), 22.8 (t), 23.0 (t), 29.8 (t), 43.1 (q x 2), 46.6 (t), 55.0 (q), 59.8 (t), 104.9 (s), 113.7 (d x 2), 116.9 (d), 123.4 (s), 126.4 (s), 127.4 (d x 2), 131.7 (s), 136.5 (s), 150.3 (d), 158.1 (s), 160.3 (s), 186.6 (s).

Ethyl 7-[(dimethylamino)methylidene]-1-(3,5-dimethoxybenzyl)-8-oxo-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2-carboxylate (13j). This compound was obtained from reaction of **9j** under MW conditions (Method A, 20 min; Method B, 110 min). Yield: 94% (Method A), 77% (Method B), pale yellow solid; mp: 139.2 – 140 °C; IR: 1714 (CO), 1698 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.28 (3H, t, J = 7.1 Hz, CH_3), 1.73 – 1.87 (2H, m, CH_2), 2.39 (2H, t, J = 6.8 Hz, CH_2), 2.62 (2H, t, J = 6.8 Hz, CH_2), 3.07 (6H, s, CH_3 x 2), 3.67 (6H, s, CH_3 x 2), 4.22 (2H, q, J = 7.1 Hz, CH_2), 6.06 (2H, s, CH_2), 6.16 (2H, s, H-2' and H-6'), 6.24 (1H, s, H-4'), 6.77 (1H, s, H-3), 7.55 (1H, s, CH); ^{13}C NMR ($CDCl_3$) (ppm): 14.4 (q), 16.6 (t), 23.7 (t), 30.3 (t), 43.3 (q x 2), 48.6 (t), 55.1 (q x 2), 60.0 (t), 99.0 (d), 104.3 (d x 2), 107.1 (s), 117.0 (d), 125.2 (s), 127.6 (s), 136.6 (s), 142.8 (s), 150.3 (d), 160.9 (s x 2), 161.0 (s), 188.7 (s).

Ethyl 7-[(dimethylamino)methylene]-8-oxo-1-(3,4,5-trimethoxybenzyl)-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2-carboxylate (13k). This compound was obtained from reaction of **9k** under MW conditions (Method A, 40 min). Yield: 99%, brown oil; IR: 1707 (CO), 1635 (CO)

cm^{-1}; ^{1}H NMR (DMSO-d_6) (ppm): 1.23 (3H, t, J = 7.0 Hz, CH_3), 1.70 – 1.79 (2H, m, CH_2), 2.30 (2H, t, J = 6.8 Hz, CH_2), 2.51 (2H, t, J = 6.8 Hz, CH_2), 2.91 (6H, s, CH_3 x 2), 3.59 (3H, s, CH_3), 3.62 (6H, s, CH_3 x 2), 4.19 (2H, q, J = 7.0 Hz, CH_2), 5.90 (2H, s, CH_2), 6.20 (2H, s, H-2' and H-6'), 6.77 (1H, s, H-3), 7.51 (1H, s, CH); ^{13}C NMR (DMSO-d_6) (ppm): 14.2 (q), 22.7 (t), 23.1 (t), 29.8 (t), 43.1 (q x 2), 47.2 (t), 55.6 (q x 2), 59.8 (t), 59.9 (q), 103.2 (d x 2), 104.9 (s), 116.8 (d), 123.6 (s), 126.3 (s), 135.5 (s), 136.2 (s), 136.7 (s), 150.6 (d), 152.7 (s x 2), 160.3 (s), 186.5 (s).

7-[(Dimethylamino)methylidene]-1-(phenylsulfonyl)-4,5,6,7-tetrahydrocyclohepta[*b*]pyrrol-8(1*H*)-one (13b). This compound was obtained from reaction of **9b** under MW conditions (Method C, 80 min). Yield: 90%; grey solid; mp: 172.6 – 173 °C; IR: 1653 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) (ppm): 1.74 (2H, t, J = 6.6 Hz, CH_2), 2.30 – 2.49 (4H, m, CH_2 x 2), 3.06 (6H, s, CH_3 x 2), 6.26 (1H, d, J = 2.7 Hz, H-3), 7.33 (1H, s, CH), 7.57 – 7.71 (4H, m, H-2, H-3', H-4' and H-5'), 8.03 (2H, d, J = 7.1 Hz, H-2', H-6'); ^{13}C NMR (DMSO-d_6) (ppm): 22.8 (t), 23.0 (t), 29.2 (t), 42.9 (q x 2), 104.1 (s), 112.3 (d), 125.6 (d), 127.4 (d x 2), 128.8 (d x 2), 131.3 (s), 132.6 (s), 133.6 (d), 139.4 (s), 149.7 (d), 184.1 (s).

7-[(Dimethylamino)methylidene]-1-methyl-4,5,6,7-tetrahydrocyclohepta[*b*]pyrrol-8(1*H*)-one (13l). This compound was obtained from reaction of **9l** under MW conditions (Method C, 80 min). Yield: 89%, brown oil; IR: 1623 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) (ppm): 1.71 – 1.81 (2H, m, CH_2), 2.34 (2H, t, J = 6.6 Hz, CH_2), 2.55 (2H, t, J = 6.6 Hz, CH_2), 3.04 (6H, s, CH_3 x 2), 3.72 (3H, s, CH_3), 5.85 (1H, d, J = 2.1 Hz, H-3), 6.80 (1H, d, J = 2.1 Hz, H-2), 7.24 (1H, s, CH); ^{13}C NMR (DMSO-d_6) (ppm): 23.6 (t), 23.9 (t), 29.6 (t), 35.4 (q), 42.8 (q x 2), 105.8 (s), 107.3 (d), 126.7 (d), 128.0 (s), 130.2 (s), 148.1 (d), 186.9 (s).

7-[(Dimethylamino)methylidene]-1-benzyl-4,5,6,7-tetrahydrocyclohepta[*b*]pyrrol-8(1*H*)-one (13m). This compound was obtained from reaction of **9m** under MW conditions (Method C, 120 min). Yield: 95 %; yellow oil; IR: 1636 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) (ppm): 1.70 – 1.79 (2H, m, CH_2), 2.26 (2H, t, J = 6.5 Hz, CH_2), 2.56 (2H, t, J = 6.5 Hz, CH_2), 3.03 (6H, s, CH_3 x 2), 5.46 (2H, s, CH_2), 5.93 (1H, d, J = 2.3 Hz, H-3), 6.97 – 7.06 (3H, m, H-2, H-2' and H-6'), 7.19 – 7.30 (4H, m, H-3', H-4', H-5' and CH); ^{13}C NMR (DMSO-d_6) (ppm): 23.6 (t), 23.7 (t), 29.8 (t), 42.8 (q x 2), 50.1 (t), 105.5 (s), 108.0 (d), 126.3 (d), 126.4 (d x 2), 126.8 (d), 128.2 (d x 2), 128.6 (s), 129.7 (s), 139.9 (s), 148.4 (d), 186.9 (s).

7-[(dimethylamino)methylidene]-1-(2-methoxybenzyl)-4,5,6,7-tetrahydrocyclohepta[*b*]pyrrol-8(1*H*)-one (13n). This compound was obtained from reaction of **9n** under MW conditions (Method C, 40 min). Yield: 96%, brown oil; IR: 1632 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.79 – 1.86 (2H,

m, CH_2), 2.42 (2H, t, *J* = 6.9 Hz, CH_2), 2.65 (2H, t, *J* = 6.9 Hz, CH_2), 3.06 (6H, s, CH_3 x 2), 3.83 (3H, s, CH_3), 5.54 (2H, s, CH_2), 5.94 (1H, d, *J* = 2.3 Hz, H-3), 6.75 – 6.86 (4H, m, Ar, H-2), 7.13 – 7.21 (1H, m, Ar), 7.39 (1H, s, CH); ^{13}C NMR ($CDCl_3$) (ppm): 24.1 (t), 24.3 (t), 30.3 (t), 43.2 (q x 2), 46.3 (t), 55.2 (q), 107.4 (s), 108.0 (d), 109.9 (d), 120.5 (d), 124.7 (s), 126.7 (d), 128.0 (d), 128.1 (d), 129.9 (s), 130.6 (s), 148.5 (d), 156.7 (s), 188.9 (s).

7-[(dimethylamino)methylidene]-1-(3-methoxybenzyl)-4,5,6,7-tetrahydrocyclohepta[*b*]pyrrol-8(1*H*)-one (13o). This compound was obtained from reaction of **9o** under MW conditions (Method C, 80 min). Yield: 91%, brown oil; IR: 1633 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) (ppm): 1.72 – 1.78 (2H, m, CH_2), 2.28 (2H, t, *J* = 6.5 Hz, CH_2), 2.48 – 2.56 (2H, m, CH_2), 3.03 (6H, s, CH_3 x 2), 3.67 (3H, s, CH_3), 5.43 (2H, s, CH_2), 5.94 (1H, d, *J* = 2.3 Hz, H-3), 6.55 – 6.59 (2H, m, H-2' and H-6'), 6.76 (1H, d, *J* = 2.3 Hz, H-4'), 6.97 (1H, d, *J* = 2.3 Hz, H-2), 7.13 – 7.25 (2H, m, H-5', CH); ^{13}C NMR (DMSO-d_6) (ppm): 23.6 (t), 23.7 (t), 29.8 (t), 42.8 (q x 2), 49.9 (t), 54.8 (q), 105.5 (s), 108.0 (d), 112.0 (d), 112.2 (d), 118.5 (d), 126.4 (d), 128.5 (s), 129.3 (d), 129.8 (s), 141.5 (s), 148.4 (d), 159.2 (s), 186.9 (s).

7-[(dimethylamino)methylidene]-1-(4-methoxybenzyl)-4,5,6,7-tetrahydrocyclohepta[*b*]pyrrol-8(1*H*)-one (13p). This compound was obtained from reaction of **9p** under MW conditions (Method C, 40 min). Yield: 96 %, brown oil; IR: 1633 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.78 – 1.85 (2H, m, CH_2), 2.38 (2H, t, *J* = 6.6 Hz, CH_2), 2.64 (2H, t, *J* = 6.6 Hz, CH_2), 3.07 (6H, s, CH_3 x 2), 3.76 (3H, s, CH_3), 5.42 (2H, s, CH_2), 5.94 (1H, d, *J* = 2.4 Hz, H-3), 6.71 (1H, d, *J* = 2.4 Hz, H-2), 6.78 – 6.84 (2H, m, H-3' and H-5'), 7.02 – 7.11 (2H, m, H-2' and H-6'), 7.39 (1H, s, CH); ^{13}C NMR ($CDCl_3$) (ppm): 24.1 (t), 24.3 (t), 30.2 (t), 43.2 (q x 2), 50.8 (t), 55.2 (q), 107.4 (s), 108.1 (d), 113.8 (d x 2), 126.1 (d), 128.5 (d x 2), 130.3 (s), 130.4 (s), 131.5 (s), 148.6 (d), 158.6 (s), 188.9 (s).

7-[(dimethylamino)methylidene]-1-(2,5-dimethoxybenzyl)-4,5,6,7-tetrahydrocyclohepta[*b*]pyrrol-8(1*H*)-one (13q). This compound was obtained from reaction of **9q** under MW conditions (Method C, 40 min). Yield: 93 %, brown oil; IR: 1633 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.76 – 1.90 (2H, m, CH_2), 2.44 (2H, t, *J* = 6.7 Hz, CH_2), 2.65 (2H, t, *J* = 6.7 Hz, CH_2), 3.06 (6H, s, CH_3 x 2), 3.64 (3H, s, CH_3), 3.79 (3H, s, CH_3), 5.52 (2H, s, CH_2), 5.94 (1H, d, *J* = 2.4 Hz, H-3), 6.31 (1H, d, *J* = 2.4 Hz, H-2), 6.65 – 6.81 (3H, m, H-3', H-4' and H-6'), 7.39 (1H, s, CH); ^{13}C NMR ($CDCl_3$) (ppm): 24.2 (t), 24.4 (t), 30.3 (t), 43.2 (q x 2), 46.4 (t), 55.6 (q), 55.8 (q), 107.4 (s), 108.2 (d), 110.9 (d), 112.3 (d), 114.0 (d), 126.7 (d), 129.3 (s), 130.0 (s), 130.5 (s), 148.6 (d), 150.8 (s), 153.7 (s), 188.8 (s).

7-[(dimethylamino)methylidene]-1-(3,5-dimethoxybenzyl)-4,5,6,7-tetrahydrocyclohepta[*b*]pyrrol-8(1*H*)-one (13r). This compound was obtained from reaction of **9r** under MW conditions (Method C, 40 min). Yield: 90 %, brown oil; IR: 1635 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.76 – 1.90 (2H, m, CH_2), 2.42 (2H, t, *J* = 6.7 Hz, CH_2), 2.65 (2H, t, *J* = 6.7 Hz, CH_2), 3.06 (6H, s, CH_3 x 2), 3.71 (6H, s, CH_3 x 2), 5.46 (2H, s, CH_2), 5.95 (1H, d, *J* = 2.5 Hz, H-3), 6.22 (2H, d, *J* = 2.2 Hz, H-2', H-6'), 6.29 (1H, t, *J* = 2.2 Hz, H-4'), 6.72 (1H, d, *J* = 2.5 Hz, H-2), 7.39 (1H, s, CH); ^{13}C NMR ($CDCl_3$) (ppm): 24.1 (t), 24.3 (t), 30.3 (t), 43.2 (q x 2), 51.3 (t), 55.2 (q x 2), 99.1 (d), 104.7 (d x 2), 107.3 (s), 108.3 (d), 126.5 (d), 130.2 (s), 130.5 (s), 141.9 (s), 148.6 (d), 160.8 (s x 2), 188.8 (s).

7-[(dimethylamino)methylidene]-1-(3,4,5-trimethoxybenzyl)-4,5,6,7-tetrahydrocyclohepta[*b*]pyrrol-8(1*H*)-one (13s). This compound was obtained from reaction of **9s** under MW conditions (Method C, 120 min). Yield: 86 %, brown oil; IR: 1653 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.77 – 1.90 (2H, m, CH_2), 2.41 (2H, t, *J* = 6.6 Hz, CH_2), 2.66 (2H, t, *J* = 6.6 Hz, CH_2), 3.08 (6H, s, CH_3 x 2), 3.77 (6H, s, CH_3 x 2), 3.79 (3H, s, CH_3), 5.46 (2H, s, CH_2), 5.97 (1H, d, *J* = 2.5 Hz, H-3), 6.31 (2H, s, H-2', H-6'), 6.74 (1H, d, *J* = 2.5 Hz, H-2), 7.41 (1H, s, CH); ^{13}C NMR ($CDCl_3$) (ppm): 24.2 (t), 24.3 (t), 30.3 (t), 43.2 (q x 2), 51.4 (t), 55.9 (q x 2), 60.8 (q), 103.7 (d x 2), 107.2 (s), 108.3 (d), 126.4 (d), 130.4 (s), 130.5 (s), 135.2 (s), 136.8 (s), 148.7 (d), 153.2 (s x 2), 188.8 (s).

9.4 Preparation of ethyl 7-(hydroxymethylidene)-1-substituted-8-oxo-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2-carboxylate (14)

To a suspension of *t*-BuO^-K^+ (13.5 mmol) in dry toluene (12 mL), at 0 °C under N_2 atmosphere, a solution of the appropriate ketone **9d-k** (4.5 mmol) in toluene (40 mL) was added and the reaction mixture was stirred at room temperature for one hour and half. A solution of ethyl formate (1.09 mL, 13.5 mmol) in dry toluene (12 mL) was added, and the mixture was stirred up to completeness (1.5 – 4 hours). The solvent was removed in vacuo and to residue was added water (50 mL). The aqueous phase was acidified with HCl 3N and extracted with dichloromethane (2 x 60 mL). The organic phase was dried on Na_2SO_4, and evaporated. The crude product was then purified by chromatography column (dichloromethane).

Ethyl 7-(hydroxymethylidene)-1-methyl-8-oxo-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2-carboxylate (14d). This compound was obtained from reaction of **9d.** Yield: 80%, brown oil. IR:

3415 (OH), 1704 (CO), 1620 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.36 (3H, t, *J* = 7.1 Hz, CH_3), 1.84 – 1.97 (2H, m, CH_2), 2.22 (2H, t, *J* = 6.9 Hz, CH_2), 2.69 (2H, t, *J* = 6.9 Hz, CH_2), 4.11 (3H, s, CH_3), 4.31 (2H, q, *J* = 7.1 Hz, CH_2), 6.70 (1H, s, H-3), 7.76 (1H, d, *J* = 7.9 Hz, CH), 14.93 (1H, d, *J* = 7.9 Hz, OH); ^{13}C NMR ($CDCl_3$) (ppm): 14.3 (q), 25.0 (t), 25.9 (t), 28.5 (t), 35.0 (q), 60.5 (t), 114.0 (s), 117.0 (d), 127.5 (s), 129.7 (s), 132.8 (s), 160.9 (s), 172.3 (d), 186.3 (s).

Ethyl 7-(hydroxymethylene)-1-benzyl-8-oxo-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2-carboxylate (14e). This compound was obtained from reaction of **9e.** Yield: 90%, brown oil. IR: 3391 (OH), 1702 (CO), 1619 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.29 (3H, t, *J* = 7.1 Hz, CH_3), 1.84 – 1.95 (2H, m, CH_2), 2.13 (2H, t, *J* = 6.8 Hz, CH_2), 2.69 (2H, t, *J* = 6.8 Hz, CH_2), 4.24 (2H, q, *J* = 7.1 Hz, CH_2), 5.99 (2H, s, CH_2), 6.83 (1H, s, H-3), 6.92 – 7.00 (2H, m, H-2' and H-6'), 7.16 – 7.29 (3H, m, H-3', H-4' and H-5'), 7.71 (1H, d, *J* = 8.0 Hz, CH), 14.77 (1H, d, *J* = 8.0 Hz, OH); ^{13}C NMR ($CDCl_3$) (ppm): 14.2 (q), 24.5 (t), 25.7 (t), 28.8 (t), 49.2 (t), 60.5 (t), 113.9 (s), 117.9 (d), 126.1 (d x 2), 126.9 (d), 127.0 (s), 128.3 (d x 2), 130.2 (s), 132.7 (s), 139.2 (s), 160.6 (s), 172.5 (d), 186.5 (s).

Ethyl 7-(hydroxymethylene)-1-(2-methoxybenzyl)-8-oxo-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2-carboxylate (14f). This compound was obtained from reaction of **9f.** Yield: 80%, brown oil. IR: 3400 (OH), 1711 (CO), 1638 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.26 (3H, t, *J* = 7.2 Hz, CH_3), 1.86 – 1.99 (2H, m, CH_2), 2.16 (2H, t, *J* = 6.4 Hz, CH_2), 2.70 (2H, t, *J* = 6.4 Hz, CH_2), 3.82 (3H, s, CH_3), 4.21 (2H, q, *J* = 7.2 Hz, CH_2), 5.94 (2H, s, CH_2), 6.40 (1H, d, *J* = 6.7 Hz, H-3'), 6.77 – 6.81 (2H, m, H-5', H-6'), 6.83 (1H, s, H-3), 7.16 (1H, t, *J* = 6.7 Hz, H-4'), 7.70 (1H, d, *J* = 8.1 Hz, CH), 14.72 (1H, d, *J* = 8.1 Hz, OH); ^{13}C NMR ($CDCl_3$) (ppm): 14.2 (q), 24.5 (t), 25.7 (t), 28.9 (t), 45.6 (t), 55.2 (q), 60.4 (t), 109.8 (d), 113.8 (s), 117.6 (d), 120.3 (d), 125.8 (d), 127.6 (s), 127.8 (d), 128.1 (s), 129.8 (s), 133.2 (s), 156.3 (s), 160.5 (s), 172.3 (d), 186.4 (s).

Ethyl 7-(hydroxymethylene)-1-(3-methoxybenzyl)-8-oxo-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2-carboxylate (14g). This compound was obtained from reaction of **9g.** Yield: 68%, colourless oil; IR: 3389 (OH), 1707 (CO), 1622 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.30 (3H, t, *J* = 7.1 Hz, CH_3), 1.88 – 1.98 (2H, m, CH_2), 2.15 (2H, t, *J* = 6.7 Hz, CH_2), 2.69 (2H, t, *J* = 6.7 Hz, CH_2), 3.73 (3H, s, CH_3), 4.24 (2H, q, *J* = 7.1 Hz, CH_2), 5.97 (2H, s, CH_2), 6.52 (1H, d, *J* = 2.3 Hz, H-6'), 6.57 (1H, s, H-2'), 6.72 (1H, dd, *J* = 8.2 *J* = 2.3 Hz, H-4'), 6.82 (1H, s, H-3), 7.15 (1H, t, *J* = 8.2 Hz, H-5'), 7.72 (1H, s, CH), 14.76 (1H, s, OH); ^{13}C NMR ($CDCl_3$) (ppm): 14.2 (q), 24.6 (t), 25.8 (t), 28.8 (t), 49.1 (t), 55.1 (q), 60.5 (t), 111.8 (d), 112.2 (d), 113.9 (s), 117.9 (d), 118.4 (d), 127.1 (s), 129.4 (d), 130.2 (s), 132.7 (s), 140.9 (s), 159.6 (s), 160.6 (s), 172.5 (d), 186.5 (s).

Ethyl 7-(hydroxymethylene)-1-(4-methoxybenzyl)-8-oxo-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2-carboxylate (14h). This compound was obtained from reaction of **9h.** Yield: 73%, pale yellow oil. IR: 3399 (OH), 1706 (CO), 1619 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.31 (3H, t, *J* = 7.1 Hz, CH_3), 1.83 – 1.97 (2H, m, CH_2), 2.12 (2H, t, *J* = 6.6 Hz, CH_2), 2.67 (2H, t, *J* = 6.6 Hz, CH_2), 3.75 (3H, s, CH_3), 4.23 (2H, q, *J* = 7.1 Hz, CH_2), 5.91 (2H, s, CH_2), 6.74 (1H, s, H-3), 6.76 – 6.82 (2H, m, H-3' and H-5'), 6.91 – 7.00 (2H, m, H-2' and H-6'), 7.73 (1H, d, *J* = 7.8 Hz, CH), 14.80 (1H, d, *J* = 7.8 Hz, OH); ^{13}C NMR ($CDCl_3$) (ppm): 14.3 (q), 24.4 (t), 25.7 (t), 28.9 (t), 48.6 (t), 55.2 (q), 60.5 (t), 113.7 (d x 2), 113.9 (s), 117.9 (d), 126.9 (s), 127.7 (d x 2), 130.2 (s), 131.4 (s), 132.6 (s), 158.5 (s), 160.7 (s), 172.5 (d), 186.6 (s).

Ethyl 1-(2,5-dimethoxybenzyl)-7-(hydroxymethylene)-8-oxo-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2-carboxylate (14i). This compound was obtained from reaction of **9i.** Yield: 76%, yellow oil, IR: 3398 (CO), 1709 (CO), 1623 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.27 (3H, q, *J* = 7.1 Hz, CH_2), 1.85 – 1.99 (2H, m, CH_2), 2.17 (2H, t, *J* = 6.6 Hz, CH_2), 2.70 (2H, t, *J* = 6.6 Hz, CH_2), 3.64 (3H, s, CH_3), 3.78 (3H, s, CH_3), 4.22 (2H, q, *J* = 7.1 Hz, CH_2), 5.92 (2H, s, CH_2), 6.02 (1H, d, *J* = 2.7 Hz, H-6'), 6.63 – 6.77 (2H, m, H-3' and H-4'), 6.83 (1H, s, H-3), 7.70 (1H, d, *J* = 8.2 Hz, CH), 14.73 (1H, d, *J* = 8.2 Hz, OH); ^{13}C NMR ($CDCl_3$) (ppm): 14.2 (q), 24.5 (t), 25.8 (t), 28.8 (t), 45.6 (t), 55.5 (q), 55.8 (q), 60.4 (t), 110.6 (d), 111.1 (d), 113.0 (d), 113.8 (s), 117.7 (d), 127.5 (s), 129.4 (s), 129.8 (s), 133.1 (s), 150.6 (s), 153.5 (s), 160.5 (s), 172.2 (d), 186.4 (s).

Ethyl 1-(3,5-dimethoxybenzyl)-7-(hydroxymethylene)-8-oxo-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2-carboxylate (14j). This compound was obtained from reaction of **9j.** Yield: 68%, yellow oil. IR: 3398 (CO), 1706 (CO), 1609 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.30 (3H, t, *J* = 7.1 Hz, CH_3), 1.85 – 1.94 (2H, m, CH_2), 2.15 (2H, t, *J* = 6.7 Hz, CH_2), 2.69 (2H, t, *J* = 6.7 Hz, CH_2), 3.70 (6H, s, CH_3), 4.25 (2H, q, *J* = 7.1 Hz, CH_2), 5.95 (2H, s, CH_2), 6.11 (2H, d, *J* = 2.2 Hz, H-2' and H-6'), 6.28 (1H, t, *J* = 2.2 Hz, H-4'), 6.82 (1H, s, H-3), 7.71 (1H, d, *J* = 7.9 Hz, CH), 14.78 (1H, d, *J* = 7.9 Hz, OH); ^{13}C NMR ($CDCl_3$) (ppm): 14.3 (q), 24.5 (t), 25.8 (t), 28.8 (t), 49.1 (t), 55.2 (q x 2), 60.5 (t), 98.8 (d), 104.1 (d x 2), 113.9 (s), 118.0 (d), 127.1 (s), 130.2 (s), 132.7 (s), 141.8 (s), 160.6 (s), 160.8 (s x 2), 172.4 (d), 186.6 (s).

Ethyl 7-(hydroxymethylene)-8-oxo-1-(3,4,5-trimethoxybenzyl)-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2-carboxylate (14k). This compound was obtained from reaction of **9k.** Yield: 60%, yellow oil. IR: 3410 (OH), 1710 (CO), 1647 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.33 (3H, t, *J* = 7.1 Hz, CH_3), 1.88 – 1.98 (2H, m, CH_2), 2.13 (2H, t, *J* = 6.8 Hz, CH_2), 2.68 (2H, t, *J* = 6.8 Hz, CH_2), 3.74 (6H, s, CH_3 x 2), 3.78 (3H, s, CH_3), 4.28 (2H, q, *J* = 7.1 Hz, CH_2), 5.94 (2H, s, CH_2), 6.28 (2H, s, H-2' and H-6'), 6.81 (1H, s, H-3), 7.74 (1H, d, *J* = 8.0 Hz, CH),

14.83 (1H, d, *J* = 8.0 Hz, OH); ^{13}C NMR ($CDCl_3$) (ppm): 14.3 (q), 24.4 (t), 25.7 (t), 28.9 (t), 48.9 (t), 55.9 (q x 2), 60.6 (t), 60.8 (q), 100.0 (s), 103.7 (d x 2), 114.0 (s) 118.0 (d), 126.7(s), 130.3 (s), 134.9 (s), 136.9 (s), 153.1 (s x 2), 160.8 (s), 172.5 (d), 186.9 (s).

9.5 Preparation of ethyl 7-[(diethylamino)methylidene]-8-oxo-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2-carboxylate (15)

To a solution of **14d,h** (1.3 mmol) in dry toluene (2.5 mL), was added diethylamine (2 mmol) and the reaction mixture was stirred at 80°C for one night (16 hours). The solvent was removed in vacuo and the residue used in the next step.

Ethyl 7-[(diethylamino)methylene]-1-methyl-8-oxo-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2-carboxylate (15d). This compound was obtained from reaction of **14d**. Quantitative yield, red oil; IR: 1700 (CO), 1629 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.22 (6H, t, *J* = 7.1 Hz, CH_3 x 2), 1.35 (3H, t, *J* = 7.1 Hz, CH_3), 1.73 – 1.86 (2H, m, CH_2), 2.34 (2H, t, *J* = 6.8 Hz, CH_2), 2.58 (2H, t, *J* = 6.8 Hz, CH_2), 3.35 (4H, q, *J* = 7.1 Hz, CH_2 x 2), 4.12 (3H, s, CH_3), 4.28 (2H, q, *J* = 7.1 Hz, CH_2), 6.71 (1H, s, H-3), 7.59 (1H, s, CH); ^{13}C NMR ($CDCl_3$) (ppm): 14.4 (q), 14.8 (q x 2), 23.7 (t x 2), 23.9 (t), 29.6 (t), 34.4 (q), 47.7 (t), 60.0 (t), 106.2 (s), 115.8 (d), 125.1 (s), 126.9 (s), 136.8 (s), 148.3 (d), 161.3 (s), 184.4 (s).

Ethyl 7-[(diethylamino)methylene]-1-(4-methoxybenzyl)-8-oxo-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2-carboxylate (15h). This compound was obtained from reaction of **14h**. Quantitative yield, red oil; IR: 1695 (CO), 1627 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.18 – 1.34 (9H, m, CH_3 x 3), 1.73 – 1.82 (2H, m, CH_2), 2.28 (2H, t, *J* = 6.8 Hz, CH_2), 2.60 (2H, t, *J* = 6.8 Hz, CH_2), 3.33 (4H, q, *J* = 7.1 Hz, CH_2 x 2), 3.73 (3H, s, CH_3), 4.22 (2H, q, *J* = 7.1 Hz, CH_2), 5.97 (2H, s, CH_2), 6.74 (2H, d, *J* = 8.5 Hz, H-3' and H-5'), 6.76 (1H, s, H-3), 6.99 (2H, d, *J* = 8.5 Hz, H-2' and H-6'), 7.59 (1H, s, CH); ^{13}C NMR ($CDCl_3$) (ppm): 14.3 (q), 14.7 (q x 2), 23.7 (t), 23.8 (t), 29.6 (t), 47.6 (t x 2), 47.9 (t), 55.2 (q), 60.0 (t), 106.0 (s), 113.6 (d x 2), 116.8 (d), 124.6 (s), 127.4 (s), 127.7 (d x 2), 132.3 (s), 136.6 (s), 148.5 (d), 158.2 (s), 161.0 (s), 188.4 (s).

9.6.1 Preparation of Pyrrolo[3',2':6,7]cyclohepta[1,2-*b*]pyridin-9(1*H*)-ones (16)

To a solution of appropriate enaminone **13** (2 mmol), in dry ethanol (40 mL) under N_2 atmosphere, phenylsulfonylacetonitrile (3 mmol) was added. The reaction mixture was heated to reflux until the disappearance of the starting material (20-48 h). The solvent was evaporated, and the residue was

dissolved in toluene (40 mL). Acetic acid (2 mmol) was added and the reaction mixture was heated to reflux with the Dean-Stark apparatus for 24 hours. The solvent was evaporated and the residue was purified by chromatography column (dichloromethane : ethyl acetate 8:2).

Ethyl 1-methyl-9-oxo-8-(phenylsulfonyl)-1,4,5,6,9,10-hexahydropyrrolo[3',2':6,7] cyclohepta[1,2-*b*]pyridine-2-carboxylate (16d). This compound was obtained from reaction of **13d**. Yield: 51%, yellow solid; mp: 301-302 °C; Rf: (DCM: EtOAc 8:2) 0.19; IR: 3408 (NH), 1701 (CO), 1642 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) (ppm): 1.28 (3H, t, J = 7.0 Hz, CH_3), 2.07 (2H, t, J = 5.9 Hz, CH_2), 2.32 – 2.46 (4H, m, CH_2 x 2), 4.04 (3H, s, CH_3), 4.24 (2H, q, J = 7.0 Hz, CH_2), 6.85 (1H, s, H-3), 7.58 – 7.83 (4H, m, Ar), 8.01 (1H, d, J = 7.0 Hz, Ar), 8.33 (1H, s, H-7), 12.30 (1H, s, NH); ^{13}C NMR (DMSO-d_6) (ppm): 14.2 (q), 22.1 (t), 23.1 (t), 32.4 (t), 34.6 (q), 60.3 (t), 116.4 (d), 128.2 (d x 2), 128.8 (d x 2), 129.0 (d), 129.2 (s), 130.4 (s), 133.4 (d), 137.3 (s), 140.2 (s), 146.2 (s), 157.0 (s), 160.2 (s), 163.8 (s), 186.5 (s).

Ethyl 1-benzyl-9-oxo-8-(phenylsulfonyl)-1,4,5,6,9,10-hexahydropyrrolo[3',2':6,7] cyclohepta[1,2-*b*]pyridine-2-carboxylate (16e). This compound was obtained from reaction of **13e**. Yield: 40%, yellow solid; mp: 256-257 °C; Rf: (DCM: EtOAc 8:2) 0.21; IR: 3407 (NH), 1700 (CO), 1642 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) (ppm): 1.23 (3H, t, J = 7.1 Hz, CH_3), 2.04 – 2.12 (2H, m, CH_2), 2.16 – 2.28 (2H, m, CH_2), 2.35 – 2.46 (2H, m, CH_2), 4.20 (2H, q, J = 7.1 Hz, CH_2), 5.82 (2H, s, CH_2), 6.61 (2H, dd, J = 6.0, 2.1 Hz, H-2' and H-6'), 6.95 (1H, s, H-3), 7.09 (3H, dd, J = 6.0, 2.1 Hz, H-3', H-4' and H-5'), 7.55 – 7.74 (3H, m, H-3'', H-4'' and H-5''), 7.90 – 7.97 (2H, m, H-2'' and H-6''), 8.19 (1H, s, H-7), 12.48 (1H, s, NH); ^{13}C NMR (DMSO-d_6) (ppm): 14.1 (q), 22.0 (t), 28.2 (t), 32.3 (t), 48.5 (t), 60.0 (t), 117.7 (d), 124.5 (s), 125.5 (d x 2), 127.1 (d), 128.1 (d x 2), 128.3 (d x 2), 128.4 (d), 128.9 (d x 2), 133.4 (d), 138.2 (s), 138.4 (s), 140.2 (s), 154.0 (s), 155.7 (s), 157.2 (s), 157.3 (s), 160.2 (s), 170.3 (s).

Ethyl 1-(2-methoxybenzyl)-9-oxo-8-(phenylsulfonyl)-1,4,5,6,9,10-hexahydropyrrolo[3',2':6,7] cyclohepta[1,2-*b*]pyridine-2-carboxylate (16f). This compound was obtained from reaction of **13f**. Yield: 46%, yellow solid; mp: 249-250 °C; Rf: (DCM: EtOAc 8:2) 0.26; IR: 3402 (NH), 1706 (CO), 1646 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) (ppm): 1.24 (3H, t, J = 7.1 Hz, CH_3), 1.99 – 2.06 (4H, m, CH_2 x 2), 2.35 – 2.42 (2H, m, CH_2), 3.45 (3H, s, CH_3), 4.20 (2H, q, J = 7.1 Hz, CH_2), 5.76 (2H, s, CH_2), 6.13 – 6.16 (1H, m, H-3'), 6.59 – 6.73 (2H, m, H-5' and H-6'), 6.94 (1H, s, H-3), 7.09 (1H, t, J = 7.4 Hz, H-4'), 7.58 – 7.75 (3H, m, H-3'', H-4'' and H-5''), 7.96 (2H, d, J = 7.0 Hz, H-2'' and H-6''), 8.17 (1H, s, H-7), 12.56 (1H, s, NH); ^{13}C NMR (DMSO-d_6) (ppm): 14.1 (q), 21.9 (t), 29.0 (t), 32.4 (t), 43.9 (t), 54.9 (q), 59.9 (t), 110.2 (d), 117.3 (d), 117.4 (d), 118.8 (d), 120.0 (d), 125.9 (s),

126.0 (s), 128.1 (d x 2), 128.2 (s), 128.4 (d), 128.5 (s), 128.8 (d x 2), 128.9 (s), 129.1 (s), 133.4 (d), 138.9 (s), 140.0 (s), 142.1 (s), 155.6 (s), 160.3 (s).

Ethyl 1-(3-methoxybenzyl)-9-oxo-8-(phenylsulfonyl)-1,4,5,6,9,10-hexahydropyrrolo[3',2':6,7] cyclohepta[1,2-*b*]pyridine-2-carboxylate (16g). This compound was obtained from reaction of **13g**. Yield: 39%, pale brown solid; mp: 289-290 °C; Rf: (DCM: EtOAc 8:2) 0.21; IR: 3424 (NH), 1720 (CO), 1639 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) (ppm): 1.24 (3H, t, J = 6.8 Hz, CH_3), 2.09 – 2.41 (6H, m, CH_2 x 3), 3.53 (3H, s, CH_3), 4.21 (2H, q, J = 6.8 Hz, CH_2), 5.77 (2H, s, CH_2), 6.18 (1H, s, H-2') 6.22 (1H, d, J = 8.4 Hz, H-6'), 6.68 (1H, dd, J = 8.4, J = 1.8 Hz, H-4'), 6.96 (1H, s, H-3), 7.03 (1H, t, J = 8.4 Hz, H-5'), 7.57 – 7.75 (3H, m, H-3'', H-4'' and H-5''), 7.96 (2H, d, J = 7.3 Hz, H-2'' and H-6''), 8.23 (1H, s, H-7), 12.59 (1H, s, NH); ^{13}C NMR (DMSO-d_6) (ppm): 14.1 (q), 25.2 (t), 29.0 (t), 34.8 (t), 46.4 (t), 54.7 (q), 60.1 (t), 111.1 (d), 117.6 (d), 117.8 (d), 119.5 (s), 121.5 (d), 128.1 (d x 2), 128.9 (d), 129.5 (d x 2), 133.4 (d), 136.4 (d), 140.1 (s), 141.6 (s), 151.8 (s), 154.0 (s), 154.8 (s), 159.1 (s), 160.2 (s), 171.4 (s), 175.0 (s), 182.0 (s).

Ethyl 1-(4-methoxybenzyl)-9-oxo-8-(phenylsulfonyl)-1,4,5,6,9,10-hexahydropyrrolo[3',2':6,7] cyclohepta[1,2-*b*]pyridine-2-carboxylate (16h). This compound was obtained from reaction of **13h**. Yield: 48%, yellow solid; mp: 251-252 °C; Rf: (DCM: EtOAc 8:2) 0.23; IR: 3380 (NH), 1700 (CO), 1653 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.27 (3H, t, J = 7.1 Hz, CH_3), 2.13 – 2.37 (6H, m, CH_2 x 3), 3.71 (3H, s, CH_3), 4.26 (2H, q, J = 7.1 Hz, CH_2), 5.82 (2H, s, CH_2), 6.64 – 6.75 (4H, m, H-2', H-3', H-5' and H-6'), 7.07 (1H, s, H-3), 7.26 – 7.53 (3H, m, H-3''. H-4'' and H-5''), 7.96 (2H, d, J = 7.6 Hz, H-2'' and H-6''), 8.31 (1H, s, H-7), 12.64 (1H, s, NH); ^{13}C NMR ($CDCl_3$) (ppm): 14.3 (q), 22.8 (t), 29.1 (t), 33.2 (t), 49.5 (t), 55.2 (q), 60.7 (t), 113.9 (d x 2), 118.2 (d), 119.7 (s), 126.5 (s), 126.7 (s), 127.2 (d x 2), 128.4 (d x 2), 128.7 (s), 129.0 (d x 2), 129.2 (s), 130.4 (s), 133.3 (d), 139.6 (s), 143.9 (s), 145.8 (d), 158.8 (s), 159.1 (s), 160.6 (s).

1-Methyl-8-(phenylsulfonyl)-4,5,6,10-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*b*]pyridin-9(1*H*)-one (16l). This compound was obtained from reaction of **13l**. Yield: 49%, yellow solid; mp: 331-332 °C; Rf: (DCM: EtOAc 8:2) 0.21; IR: 3418 (NH), 1635 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) (ppm): 1.45 – 1.59 (2H, m, CH_2), 2.01 – 2.11 (2H, m, CH_2), 2.35 – 2.48 (2H, m, CH_2), 3.68 (3H, s, CH_3), 6.06 (1H, d, J = 2.1 Hz, H-3), 6.95 (1H, d, J = 2.1 Hz, H-2), 7.57 – 7.68 (3H, m, H-3'', H-4'' and H-5''), 8.00 (2H, d, J = 6.9 Hz, H-2'' and H-6''), 8.22 (1H, s, H-7), 11.94 (1H, s, NH); ^{13}C NMR (DMSO-d_6) (ppm): 23.9 (t), 28.9 (t), 33.2 (t), 35.1 (q), 108.7 (d), 120.3 (s), 127.6 (d), 128.0 (d x 2), 128.7 (d x 2), 129.3 (s), 133.1 (d), 138.5 (s), 140.6 (s), 142.3 (d), 149.7 (s), 150.6 (s), 157.1 (s).

1-Benzyl-8-(phenylsulfonyl)-4,5,6,10-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*b*]pyridin-9(1*H*)-one (16m). This compound was obtained from reaction of **13m**. Yield: 40%, pale brown solid; mp: 315-316 °C; Rf: (DCM: EtOAc 8:2) 0.26; IR: 3405 (NH), 1641 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) (ppm): 2.03 (2H, t, *J* = 6.1 Hz, CH_2), 2.12 – 2.20 (2H, m, CH_2), 2.43 (2H, t, *J* = 6.1 Hz, CH_2), 5.34 (2H, s, CH_2), 6.14 (1H, d, *J* = 2.4 Hz, H-3), 6.67 – 6.75 (2H, m, H-2' and H-6'), 7.03 – 7.12 (3H, m, H-3', H-4' and H-5'), 7.16 (1H, d, *J* = 2.4 Hz, H-2), 7.56 – 7.70 (3H, m, H-3'', H-4'' and H-5''), 7.87 – 7.96 (2H, m, H-2'' and H-6''), 8.06 (1H, s, H-7), 12.25 (1H, s, NH); ^{13}C NMR (DMSO-d_6) (ppm): 23.1 (t), 28.6 (t), 33.1 (t), 51.2 (t), 109.1 (d), 117.6 (s), 122.5 (s), 126.1 (d x 2), 127.2 (d), 127.7 (d), 127.8 (d x 2), 128.0 (d), 128.1 (d x 2), 128.5 (s), 128.7 (d x 2), 129.8 (s), 130.3 (s), 133.1 (d), 138.5 (s), 166.3 (s), 175.4 (s).

1-(2-Methoxybenzyl)-8-(phenylsulfonyl)-4,5,6,10-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*b*]pyridin-9(1*H*)-one (16n). This compound was obtained from reaction of **13n**. Yield: 43%, yellow solid; mp: 290-291°C; Rf: (8:2 DCM: EtOAc) 0.58; IR: 3419 (NH), 1635 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) (ppm): 2.01 – 2.17 (4H, m, CH_2 x 2), 2.40 (2H, m, CH_2), 3.42 (3H, s, CH_3), 5.21 (2H, s, CH_2), 6.11 (1H, d, *J* = 2.5 Hz, H-3), 6.49 (1H, d, *J* = 7.3 Hz, H-3'), 6.65 – 6.75 (2H, m, H-5' and H-6'), 7.08 (1H, d, *J* = 2.5 Hz, H-2), 7.11 – 7.16 (1H, m, H-4'), 7.55 – 7.73 (3H, m, H-3'', H-4'' and H-5''), 7.96 (2H, d, *J* = 6.7 Hz, H-2'' and H-6''), 8.12 (1H, s, H-7), 12.26 (1H, s, NH); ^{13}C NMR (DMSO-d_6) (ppm): 22.7 (t), 32.9 (t), 46.1 (t), 54.9 (q), 71.0 (t), 109.0 (d), 110.3 (d), 120.0 (d), 126.1 (s), 127.3 (d), 127.4 (s), 127.5 (d), 127.9 (d), 128.0 (d x 2), 128.1 (s), 128.7 (d x 2), 129.0 (s), 130.0 (s), 133.1 (d), 140.5 (s), 143.8 (d), 145.1 (s), 155.7 (s), 157.0 (s).

1-(3-Methoxybenzyl)-8-(phenylsulfonyl)-4,5,6,10-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*b*]pyridin-9(1*H*)-one (16o). This compound was obtained from reaction of **13o**. Yield: 37%, yellow solid; mp: 274-275°C; Rf: (8:2 DCM: EtOAc) 0.24; IR: 3406 (NH), 1634 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) (ppm): 1.96 – 2.25 (6H, m, CH_2 x 3), 3.52 (3H, s, CH_3), 5.32 (2H, s, CH_2), 6.14 (1H, d, *J* = 2.4 Hz, H-3), 6.25 – 6.36 (2H, m, H-2' and H-6'), 6.67 (1H, d, *J* = 8.0 Hz, H-4'), 7.01 (1H, t, *J* = 8.0 Hz, H-5'), 7.16 (1H, d, *J* = 2.4 Hz, H-2), 7.55 – 7.74 (3H, m, H-3'', H-4'' and H-5''), 7.94 (2H, d, *J* = 7.2 Hz, H-2'' and H-6''), 8.10 (1H, s, H-7), 12.29 (1H, s, NH); ^{13}C NMR (DMSO-d_6) (ppm): 33.1 (t), 41.3 (t), 51.2 (t), 54.7 (q), 71.1 (t), 109.1 (d), 111.4 (d), 112.8 (d), 112.9 (s), 118.2 (d), 127.5 (s), 127.8 (d x 2), 128.2 (s), 128.7 (d x 2), 129.3 (d), 129.8 (s), 132.6 (d), 133.1 (d), 133.9 (d), 139.8 (s), 140.6 (s), 157.1 (s), 159.1 (s), 169.1 (s).

1-(4-methoxybenzyl)-8-(phenylsulfonyl)-4,5,6,10-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*b*]pyridin-9(1*H*)-one (16p). This compound was obtained from reaction of **13p**. Yield: 48%,

yellow solid; mp: 265-266°C; Rf: (8:2 DCM: EtOAc) 0.4; IR: 3454 (NH), 1639 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) (ppm): 2.03 – 2.17 (4H, m, CH_2 x 2), 2.38 – 2.46 (2H, m, CH_2), 3.64 (3H, s, CH_3), 5.23 (2H, s, CH_2), 6.11 (1H, d, *J* = 2.3 Hz, H-3), 6.65 – 6.69 (4H, m, Ar), 7.12 (1H, d, *J* = 2.3 Hz, H-2), 7.57 – 7.70 (3H, m, H-3'', H-4'' and H-5''), 7.96 (2H, d, *J* = 6.9 Hz, H-2'' and H-6''), 8.10 (1H, s, H-7), 12.26 (1H, s, NH); ^{13}C NMR (DMSO-d_6) (ppm): 23.1 (t), 28.6 (t), 50.6 (t), 54.9 (q), 71.1 (t), 109.0 (d), 113.5 (d), 123.0 (d), 127.4 (d x 2), 127.5 (d x 2), 127.8 (d x 2), 128.7 (d x 2), 130.1 (s), 133.1 (d), 134.0 (s), 140.5 (s), 140.6 (s), 146.2 (s), 152.6 (s), 157.1 (s), 158.2 (s), 158.3 (s).

9.6.2 Functionalization of Pyrrolo[3',2':6,7]cyclohepta[1,2-*b*]pyridin-9(1*H*)-ones (19-20)

To a solution of the appropriate pyrrolo[3',2':6,7]cyclohepta[1,2-*b*]pyridin-9(1*H*)-one **16d,h,l,p** (0.4 mmol) in dry DMF (8 mL), NaH (0.44 mmol) was added and the reaction mixture was stirred for one hour and half. Iodomethane (0.6 mmol) was added and the reaction mixture was stirred up to completeness. Then the reaction was poured into ice and brine, and the precipitate formed, was filtered and purified by chromatography column (dichloromethane).

Ethyl 1,10-dimethyl-9-oxo-8-(phenylsulfonyl)-1,4,5,6,9,10-hexahydropyrrolo[3',2':6,7]cyclohepta[1,2-*b*]pyridine-2-carboxylate (19d). This compound was obtained from reaction of **16d**. Yield: 30%, yellow solid; mp: 110-111°C; Rf: (8:2 DCM: EtOAc) 0.47; IR: 1700 (CO), 1652 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.37 (3H, t, *J* = 7.2 Hz, CH_3), 2.04 – 2.26 (4H, m, CH_2 x 2), 2.46 – 2.63 (2H, m, CH_2), 3.42 (3H, s, CH_3), 3.71 (3H, s, CH_3), 4.32 (2H, q, *J* = 7.2 Hz, CH_2), 6.90 (1H, s, H-3), 7.54 – 7.63 (3H, m, H-3'', H-4'' and H-5''), 8.19 (2H, d, *J* = 8.2 Hz, H-2'' and H-6''), 8.34 (1H, s, H-7); ^{13}C NMR ($CDCl_3$) (ppm): 14.8 (q), 22.0 (t), 29.7 (t), 32.9 (t), 35.4 (q), 35.7 (q), 60.5 (t), 116.6 (d), 119.6 (s), 126.0 (s), 127.6 (s), 127.7 (s), 128.6 (d x 2), 129.1 (s), 129.2 (d x 2), 133.4 (d), 139.6 (s), 143.6 (d), 145.3 (s), 157.9 (s), 160.8 (s).

Ethyl 9-methoxy-1-methyl-8-(phenylsulfonyl)-1,4,5,6-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*b*]pyridine-2-carboxylate (20d). This compound was obtained from reaction of **16d**. Yield: 59%, pale yellow solid; mp: 153-154°C; Rf: (DCM) 0.15; IR: 1696 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.36 (3H, t, *J* = 7.0 Hz, CH_3), 2.15 – 2.27 (2H, m, CH_2), 2.45 (2H, t, *J* = 6.9 Hz, CH_2), 2.58 (2H, t, *J* = 6.9 Hz, CH_2), 3.93 (3H, s, CH_3), 4.11 (3H, s, CH_3), 4.31 (2H, q, *J* = 7.0 Hz, CH_2), 6.86 (1H, s, H-3), 7.49 – 7.62 (3H, m, H-3'', H-4'' and H-5''), 8.05

(2H, d, *J* = 8.2 Hz, H-2'' and H-6''), 8.29 (1H, s, H-7); ^{13}C NMR ($CDCl_3$) (ppm): 14.4 (q), 23.4 (t), 30.3 (t), 32.6 (t), 34.5 (q), 54.1 (q), 60.0 (t), 116.8 (d), 120.4 (s), 124.8 (s), 126.5 (s), 128.6 (d x 2), 128.7 (d x 2), 130.3 (s), 133.3 (d), 134.0 (s), 140.5 (d), 140.6 (s), 153.9 (s), 157.5 (s), 161.2 (s).

Ethyl 9-methoxy-1-(4-methoxybenzyl)-8-(phenylsulfonyl)-1,4,5,6-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*b*]pyridine-2-carboxylate (20h). This compound was obtained from reaction of **16h**. Yield: 73%, pale yellow solid; mp: 71-72°C; Rf: (DCM) 0.14; IR: 1699 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.28 (3H, t, *J* = 7.1 Hz, CH_3), 2.17 (2H, t, *J* = 6.8 Hz, CH_2), 2.43 – 2.55 (4H, m, CH_2 x 2), 3.61 (3H, s, CH_3), 3.71 (3H, s, CH_3), 4.22 (2H, q, *J* = 7.1 Hz, CH_2), 5.92 (2H, s, CH_2), 6.67 (2H, d, *J* = 8.9 Hz, H-3' and H-5'), 6.75 (2H, d, *J* = 8.9 Hz, H-2' and H-6'), 6.93 (1H, s, H-3), 7.46 – 7.63 (3H, m, H-3'', H-4'' and H-5''), 8.00 (2H, d, *J* = 6.8 Hz, H-2'' and H-6''), 8.26 (1H, s, H-7); ^{13}C NMR ($CDCl_3$) (ppm): 14.3 (q), 23.2 (t), 29.7 (t), 32.9 (t), 48.4 (t), 54.0 (q), 55.1 (q), 60.0 (t), 113.5 (d x 2), 117.7 (d), 120.6 (s), 120.7 (s), 124.2 (s), 126.9 (d x 2), 128.5 (d x 2), 128.6 (d x 2), 130.1 (s), 131.6 (s), 133.3 (d), 133.9 (s), 140.5 (s), 140.6 (d), 154.0 (s), 157.6 (s), 158.2 (s), 160.7 (s).

1,10-Dimethyl-8-(phenylsulfonyl)-4,5,6,10-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*b*]pyridin-9(1*H*)-one (19l). This compound was obtained from reaction of **16l**. Yield: 20%, brown solid; mp: 136-137°C; Rf: (8:2 DCM: EtOAc) 0.44; IR: 1647 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.62 – 1.70 (2H, m, CH_2), 2.04 – 2.15 (2H, m, CH_2), 2.48 – 2.62 (2H, m, CH_2), 3.44 (3H, s, CH_3), 3.49 (3H, s, CH_3), 6.13 (1H, d, *J* = 2.4 Hz, H-3), 6.77 (1H, d, *J* = 2.4 Hz, H-2), 7.52 – 7.59 (3H, m, H-3'', H-4'' and H-5''), 8.15 – 8.22 (2H, m, H-2'' and H-6''), 8.30 (1H, s, H-7); ^{13}C NMR ($CDCl_3$) (ppm): 22.5 (t), 29.7 (t), 33.7 (t), 35.4 (q), 35.7 (q), 109.3 (d), 119.0 (s), 122.7 (s), 125.6 (s), 126.2 (d), 128.5 (d x 2), 129.1 (d x 2), 129.8 (s), 133.2 (d), 140.0 (s), 143.6 (d), 147.3 (s), 158.3 (s).

9-Methoxy-1-methyl-8-(phenylsulfonyl)-1,4,5,6-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*b*]pyridine (20l). This compound was obtained from reaction of **16l**. Yield: 74%, yellow solid; mp: 188-189°C; Rf: (DCM) 0.26; ^{1}H NMR ($CDCl_3$) (ppm): 2.10 – 2.20 (2H, m, CH_2), 2.60 – 2.70 (4H, m, CH_2 x 2), 3.88 (3H, s, CH_3), 3.91 (3H, s, CH_3), 6.05 (1H, d, *J* = 2.5 Hz, H-3), 6.71 (2H, d, *J* = 2.5 Hz, H-2), 7.46 – 7.59 (3H, m, H-3'', H-4'' and H-5''), 8.03 (2H, d, *J* = 8.3 Hz, H-2'' and H-6''), 8.18 (1H, s, H-7); ^{13}C NMR ($CDCl_3$) (ppm): 25.6 (t), 31.0 (t), 31.4 (t), 37.0 (q), 54.0 (q), 108.9 (d), 118.0 (s), 126.9 (d), 127.2 (s), 128.4 (d x 2), 128.5 (d x 2), 128.8 (s), 128.9 (s), 133.0 (d), 140.0 (d), 141.1 (s), 154.6 (s), 157.3 (s).

9-methoxy-1-(4-methoxybenzyl)-8-(phenylsulfonyl)-1,4,5,6-tetrahydropyrrolo[3',2':6,7] cyclohepta[1,2-*b*]pyridine (20p). This compound was obtained from reaction of **16p**. Yield: 68%,

pale yellow solid; mp: 92-93°C; Rf: (DCM) 0.22; ^{1}H NMR ($CDCl_3$) (ppm): 2.18 (2H, t, *J* = 6.0 Hz, CH_2), 2.54 – 2.61 (4H, m, CH_2 x 2), 3.69 (3H, s, CH_3), 3.74 (3H, s, CH_3), 5.45 (2H, s, CH_2), 6.11 (1H, d, *J* = 2.6 Hz, H-3), 6.70 – 6.75 (3H, m, H-2, H-3' and H-5'), 6.88 (2H, d, *J* = 8.7 Hz, H-2' and H-6'), 7.44 – 7.61 (3H, m, H-3'', H-4'' and H-5''), 7.97 – 8.02 (2H, m, H-2'' and H-6''), 8.17 (1H, s H-7); ^{13}C NMR ($CDCl_3$) (ppm): 24.9 (t), 31.1 (t), 32.2 (t), 50.9 (t), 53.8 (q), 55.2 (q), 109.6 (d), 113.8 (d x 2), 118.3 (s), 125.7 (d), 127.3 (s), 127.7 (d x 2), 128.4 (d x 2), 128.5 (d x 2), 128.9 (s), 129.0 (s), 131.0 (d), 133.0 (s), 140.1 (d), 141.0 (s), 154.9 (s), 157.5 (s), 158.7 (s).

9.7.1 Preparation of Pyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazole (21)

To a solution of the appropriate enaminone **13** or hydroxymethylenketones **14** (1.5 mmol) in ethanol (6 mL), and acetic acid (3 mL), hydroxylamine hydrochloride (1.65 mmol) was added and the reaction mixture was heated to reflux for one hour. Then the reaction mixture was poured into ice and brine, and in the case of formation of a precipitate, the solid was filtered. In the absence of precipitate, the aqueous solution was extracted with dichloromethane (3 x 20 mL). The organic phase was dried over Na_2SO_4 and the solvent evaporated at reduced pressure. The crude product was then purified by chromatography column (dichloromethane : ethyl acetate 95 : 5).

9-(Phenylsulfonyl)-4,5,6,9-tetrahydropyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazole (21b). This compound was obtained from reaction of **13b**. Yield: 60%, orange solid; mp: 140.3 – 141.0 °C; ^{1}H NMR ($CDCl_3$) (ppm): 1.88 – 1.98 (2H, m, CH_2), 2.65 – 2.77 (4H, m, CH_2 x 2), 6.20 (1H, d, *J* = 3.3 Hz, H-7), 7.49 – 7.62 (4H, m, H-8, H-3', H-4' and H-5'), 7.96 (1H, s, H-3), 8.06 (2H, d, *J* = 7.9 Hz, H-2' and H-6'); ^{13}C NMR ($CDCl_3$) (ppm): 23.5 (t), 23.7 (t), 28.0 (t), 113.1 (d), 113.9 (s), 118.9 (s), 125.6 (d), 128.1 (d x 2), 128.9 (d x 2), 133.1 (s), 133.8 (d), 138.9 (s), 151.5 (d), 156.1 (s).

Ethyl 9-methyl-4,5,6,9-tetrahydropyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazole-8-carboxylate (21d). This compound was obtained from reaction of **14d**. Yield: 90%, white solid; mp: 91.7 °C IR: 1699 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.36 (3H, t, *J* = 7.1 Hz, CH_3), 1.89 – 2.00 (2H, m, CH_2), 2.74 – 2.85 (4H, m, CH_2 x 2), 4.24 – 4.35 (5H, m, CH_2 and CH_3), 6.76 (1H, s, H-7), 8.08 (1H, s, H-3); ^{13}C NMR ($CDCl_3$) (ppm): 14.4 (q), 23.4 (t), 24.5 (t), 28.1 (t), 35.2 (q), 60.1 (t), 114.8 (s), 114.9 (s), 118.0 (d), 124.2 (s), 126.1 (s), 151.8 (d), 158.3 (s), 161.1 (s).

Ethyl 9-benzyl-4,5,6,9-tetrahydropyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazole-8-carboxylate (21e). This compound was obtained from reaction of **14e**. Yield: 60%, grey solid; mp: 149.4 °C IR: 1695 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.30 (3H, t, *J* = 7.1 Hz, CH_3), 1.92 – 2.00 (2H, m, CH_2),

2.71 – 2.89 (4H, m, CH_2 x 2), 4.24 (2H, q, *J* = 7.1 Hz, CH_2), 6.17 (2H, s, CH_2), 6.87 (1H, s, H-7), 6.99 – 7.07 (2H, m, H-2' and H-6'), 7.13 – 7.30 (3H, m, H-3', H-4' and H-5'), 8.00 (1H, s, H-3); ^{13}C NMR ($CDCl_3$) (ppm): 14.3 (q), 23.4 (t), 24.5 (t), 28.2 (t), 50.1 (t), 60.2 (t), 115.1 (s), 119.0 (d), 124.2 (s), 125.5 (s), 126.1 (d x 2), 126.7 (s), 126.8 (d), 128.3 (d x 2), 139.0 (s), 151.7 (d), 157.8 (s), 160.8 (s).

Ethyl 9-(2-methoxybenzyl)-4,5,6,9-tetrahydropyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazole-8-carboxylate (21f). This compound was obtained from reaction of **14f**. Yield: 60%, white solid; mp: 137.5 – 138.3 °C IR: 1700 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.26 (3H, t, *J* = 7.1 Hz, CH_3), 1.89 – 2.06 (2H, m, CH_2), 2.77 (2H, t, *J* = 6.1 Hz, CH_2), 2.86 (2H, t, *J* = 6.1 Hz, CH_2), 3.89 (3H, s, CH_3), 4.20 (2H, q, *J* = 7.1 Hz, CH_2), 6.11 (2H, s, CH_2), 6.29 (1H, d, *J* = 7.5 Hz, H-3'), 6.68 – 6.88 (3H, m, H-7, H-5', H-6'), 7.13 (1H, t, *J* = 8.1 Hz, H-4'), 7.97 (1H, s, H-3); ^{13}C NMR ($CDCl_3$) (ppm): 14.2 (q), 23.5 (t), 24.5 (t), 28.2 (t), 46.5 (t), 55.3 (q), 60.1 (t), 109.8 (d), 114.9 (s), 118.8 (d), 120.3 (d), 124.7 (s), 125.0 (d), 125.8 (s), 126.5 (s), 127.5 (d), 128.0 (s), 151.6 (d), 156.2 (s), 158.0 (s), 160.6 (s).

Ethyl 9-(3-methoxybenzyl)-4,5,6,9-tetrahydropyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazole-8-carboxylate (21g). This compound was obtained from reaction of **14g**. Yield: 82%, pale yellow solid; mp: 98 – 98.4 °C IR: 1696 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.30 (3H, t, *J* = 7.1 Hz, CH_3), 1.91 – 2.00 (2H, m, CH_2), 2.72 – 2.87 (4H, m, CH_2 x 2), 3.71 (3H, s, CH_3), 4.24 (2H, q, *J* = 7.1 Hz, CH_2), 6.15 (2H, s, CH_2), 6.54 – 6.73 (3H, m, H-2', H-4' and H-6'), 6.86 (1H, s, H-7), 7.14 (1H, t, *J* = 7.9 Hz, H-5'), 8.00 (1H, s, H-3); ^{13}C NMR ($CDCl_3$) (ppm): 14.3 (q), 23.4 (t), 24.5 (t), 28.2 (t), 49.9 (t), 55.0 (q), 60.2 (t), 111.8 (d), 112.0 (d), 115.0 (s), 118.4 (d), 119.0 (d), 124.3 (s), 125.5 (s), 126.7 (s), 129.3 (d), 140.7 (s), 151.7 (d), 157.8 (s), 159.6 (s), 160.8 (s).

Ethyl 9-(4-methoxybenzyl)-4,5,6,9-tetrahydropyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazole-8-carboxylate (21h). This compound was obtained from reaction of **14h**. Yield: 81%, white solid; mp: 94.9 °C IR: 1690 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.32 (3H, t, *J* = 7.1 Hz, CH_3), 1.88 – 1.99 (2H, m, CH_2), 2.72 – 2.86 (4H, m, CH_2 x 2), 3.74 (3H, s, CH_3), 4.26 (2H, q, *J* = 7.1 Hz, CH_2), 6.09 (2H, s, CH_2), 6.76 (2H, d, *J* = 6.8 Hz, H-3' and H-5'), 6.85 (1H, s, H-7), 7.01 (2H, d, *J* = 8.6 Hz, H-2' and H-6'), 8.02 (1H, s, H-3); ^{13}C NMR ($CDCl_3$) (ppm): 14.3 (q), 23.4 (t), 24.5 (t), 28.2 (t), 49.4 (t), 55.1 (q), 60.2 (t), 113.7 (d x 2), 115.0 (s), 119.0 (d), 124.3 (s), 125.5 (s), 126.7 (s), 127.6 (d x 2), 131.1 (s), 151.8 (d), 157.8 (s), 158.4 (s), 160.9 (s).

Ethyl 9-(2,5-dimethoxybenzyl)-4,5,6,9-tetrahydropyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazole-8-carboxylate (21i). This compound was obtained from reaction of **14i**. Yield: 74%,

white solid; mp: 94.9 °C IR: 1690 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.26 (3H, t, *J* = 7.1 Hz, CH_3), 1.93 – 2.02 (2H, m, CH_2), 2.74 – 2.88 (4H, m, CH_2 x 2), 3.60 (3H, s, CH_3), 3.85 (3H, s, CH_3), 4.20 (2H, q, *J* = 7.1 Hz, CH_2), 5.91 (1H, d, *J* = 2.6 Hz, H-6'), 6.08 (2H, s, CH_2), 6.63 (1H, dd, *J* = 8.7, 2.6 Hz, H-4'), 6.76 (1H, d, *J* = 8.7 Hz, H-3'), 6.87 (1H, s, H-7), 7.97 (1H, s, H-3); ^{13}C NMR ($CDCl_3$) (ppm): 14.2 (q), 23.5 (t), 24.5 (t), 28.2 (t), 46.5 (t), 55.4 (q), 55.9 (q), 60.1 (t), 110.4 (d), 110.6 (d), 112.6 (d), 114.9 (s), 118.9 (d), 124.5 (s), 125.6 (s), 126.5 (s), 129.5 (s), 150.6 (s), 151.6 (d), 153.5 (s), 157.9 (s), 160.6 (s).

Ethyl 9-(3,5-dimethoxybenzyl)-4,5,6,9-tetrahydropyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazole-8-carboxylate (21j). This compound was obtained from reaction of **14j**. Yield: 82%, white solid; mp: 114 °C IR: 1701 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.31 (3H, t, *J* = 7.1 Hz, CH_3), 1.92 – 2.00 (2H, m, CH_2), 2.64 – 2.86 (4H, m, CH_2 x 2), 3.70 (6H, s, CH_3 x 2), 4.24 (2H, q, *J* = 7.1 Hz, CH_2), 6.07 – 6.28 (4H, m, CH_2, H-2' and H-6'), 6.26 (1H, s, H-4'), 6.86 (1H, s, H-7), 8.00 (1H, s, H-3); ^{13}C NMR ($CDCl_3$) (ppm): 14.3 (q), 23.4 (t), 24.5 (t), 28.2 (t), 50.0 (t), 55.2 (q x 2), 60.2 (t), 98.6 (d), 100.0 (s), 104.1 (d x 2), 115.1 (s), 119.1 (d), 124.2 (s), 126.7 (s), 141.6 (s), 151.7 (d), 157.8 (s), 160.7 (s x 2), 160.8 (s).

Ethyl 9-(3,4,5-trimethoxybenzyl)-4,5,6,9-tetrahydropyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazole-8-carboxylate (21k). This compound was obtained from reaction of **14k**. Yield: 72%, pale yellow solid; mp: 112.9 – 113.1 °C IR: 1696 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.33 (3H, t, *J* = 7.1 Hz, CH_3), 1.88 – 1.99 (2H, m, CH_2), 2.73 – 2.87 (4H, m, CH_2 x 2), 3.72 (6H, s, CH_3 x 2), 3.77 (3H, s, CH_3), 4.28 (2H, q, *J* = 7.1 Hz, CH_2), 6.11 (2H, s, CH_2), 6.29 (2H, s, H-2' and H-6'), 6.86 (1H, s, H-7), 8.04 (1H, s, H-3); ^{13}C NMR ($CDCl_3$) (ppm): 14.4 (q), 23.6 (t), 24.5 (t), 28.1 (t), 49.8 (t), 55.9 (q x 2), 60.3 (t), 60.8 (q), 100.0 (s), 103.5 (d x 2), 115.1 (s), 119.1 (d), 124.2 (s), 125.5 (s), 126.9 (s), 134.6 (s), 151.8 (d), 153.1 (s x 2), 157.8 (s), 161.0 (s).

9-Methyl-4,5,6,9-tetrahydropyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazole (21l). This compound was obtained from reaction of **13l**. Yield: 75%, white solid; mp: 60.4 °C; ^{1}H NMR ($CDCl_3$) (ppm): 1.89 – 2.00 (2H, m, CH_2), 2.74 (2H, t, *J* = 6.0 Hz, CH_2), 2.87 (2H, *J* = 6.0 Hz, CH_2), 3.95 (3H, s, CH_3), 5.95 (1H, d, *J* = 2.5 Hz, H-7), 6.58 (1H, d, *J* = 2.5 Hz, H-8), 8.01 (1H, s, H-3); ^{13}C NMR ($CDCl_3$) (ppm): 23.2 (t), 24.4 (t), 28.8 (t), 37.6 (q), 99.9 (s), 109.3 (d), 111.1 (s), 125.5 (d), 127.3 (s), 151.4 (d), 159.7 (s).

9-Benzyl-4,5,6,9-tetrahydropyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazole (21m). This compound was obtained from reaction of **13m**. Yield: 83%, pale yellow solid; mp: 61.3°C; ^{1}H NMR ($CDCl_3$) (ppm): 1.88 – 2.00 (2H, m, CH_2), 2.72 (2H, t, *J* = 6.2 Hz, CH_2), 288 (2H, t, *J* = 6.2

Hz, CH_2), 5.52 (2H, s, CH_2), 6.02 (1H, d, *J* = 2.6 Hz, H-7), 6.67 (1H, d, *J* = 2.6 Hz, H-8), 7.12 – 7.33 (5H, m, Ar), 7.94 (1H, s, H-3); ^{13}C NMR ($CDCl_3$) (ppm): 23.1 (t), 24.3 (t), 28.8 (t), 53.0 (t), 110.2 (d), 111.4 (s), 119.1 (s), 125.2 (d), 126.9 (d x 2), 127.3 (d), 127.7 (s), 128.5 (d x 2), 138.3 (s), 151.4 (d), 159.3 (s).

9-(2-Methoxybenzyl)-4,5,6,9-tetrahydropyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazole (21n). This compound was obtained from reaction of **13n**. Yield: 70%, pale yellow solid; mp: 80.4°C; ^{1}H NMR ($CDCl_3$) (ppm): 1.91 – 2.02 (2H, m, CH_2), 2.74 (2H, t, *J* = 5.5 Hz, CH_2), 2.90 (2H, t, *J* = 5.5 Hz, CH_2), 3.87 (3H, s, CH_3), 5.54 (2H, s, CH_2), 6.01 (1H, d, *J* = 2.6 Hz, H-7) 6.65 (1H, d, *J* = 2.6 Hz, H-8), 6.71 – 6.89 (3H, m, Ar), 7.15 – 7.23 (1H, m, Ar), 7.95 (1H, s, H-3); ^{13}C NMR ($CDCl_3$) (ppm): 23.2 (t), 24.4 (t), 28.8 (t), 48.2 (t), 55.3 (q), 109.9 (d), 110.0 (d), 111.2 (s), 119.2 (s), 120.6 (d), 125.3 (d), 127.0 (s), 127.3 (s), 127.5 (d), 128.4 (d), 151.3 (d), 156.5 (s), 159.5 (s).

9-(3-Methoxybenzyl)-4,5,6,9-tetrahydropyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazole (21o). This compound was obtained from reaction of **13o**. Yield: 83%, pale yellow solid; mp: 67.6 °C; ^{1}H NMR ($CDCl_3$) (ppm): 1.89 – 2.00 (2H, m, CH_2), 2.73 (2H, t, *J* = 5.5 Hz, CH_2), 2.88 (2H, t, *J* = 5.5 Hz, CH_2), 3.74 (3H, s, CH_3), 5.50 (2H, s, CH_2), 6.02 (1H, d, *J* = 2.6 Hz, H-7) 6.68 (1H, d, *J* = 2.6 Hz, H-8), 6.71 – 6.78 (3H, m, H-2', H-4' and H-6'), 7.20 (1H, t, *J* = 7.9 Hz, H-5'), 7.95 (1H, s, H-3); ^{13}C NMR ($CDCl_3$) (ppm): 23.2 (t), 24.4 (t), 28.8 (t), 52.9 (t), 55.1 (q), 110.2 (d), 111.4 (s), 112.5 (d), 112.7 (d), 119.1 (s), 119.2 (d), 125.2 (d), 127.7 (s), 129.6 (d), 140.0 (s), 151.4 (d), 159.3 (s), 159.7 (s).

9-(4-Methoxybenzyl)-4,5,6,9-tetrahydropyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazole (21p). This compound was obtained from reaction of **13p**. Yield: 68%, pale yellow solid; mp: 101 °C; ^{1}H NMR ($CDCl_3$) (ppm): 1.88 – 1.99 (2H, m, CH_2), 2.73 (2H, t, *J* = 5.8 Hz, CH_2), 2.88 (2H, t, *J* = 5.8 Hz, CH_2), 3.76 (3H, s, CH_3), 5.45 (2H, s, CH_2), 6.01 (1H, d, *J* = 2.5 Hz, H-7) 6.68 (1H, d, *J* = 2.5 Hz, H-8), 6.82 (2H, d, *J* = 8.5 Hz, H-3' and H-5'), 7.12 (2H, d, *J* = 8.5 Hz, H-2' and H-6'), 7.95 (1H, s, H-3); ^{13}C NMR ($CDCl_3$) (ppm): 23.2 (t), 24.4 (t), 28.8 (t), 52.5 (t), 55.2 (q), 110.0 (d), 111.5 (s), 113.9 (d x 2), 118.9 (s), 124.9 (d), 127.7 (s), 128.5 (d x 2), 130.2 (s), 151.9 (d), 158.8 (s), 156.9 (s).

9-(2,5-Dimethoxybenzyl)-4,5,6,9-tetrahydropyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazole (21q). This compound was obtained from reaction of **13q**. Yield: 78%, white solid; mp: 116.6°C ^{1}H NMR ($CDCl_3$) (ppm): 1.90 – 2.01 (2H, m, CH_2), 2.74 (2H, t, *J* = 6.2 Hz, CH_2), 2.89 (2H, t, *J* = 6.2 Hz, CH_2), 3.63 (3H, s, CH_3), 3.83 (3H, s, CH_3), 5.51 (2H, s, CH_2), 6.00 (1H, d, *J* = 2.6 Hz, H-7), 6.29 (1H, d, *J* = 2.6 Hz, H-8), 6.67 – 6.82 (3H, m, H-3', H-4' and H-6'), 7.95 (1H, s, H-3); ^{13}C

NMR ($CDCl_3$) (ppm): 23.2 (t), 24.4 (t), 28.8 (t), 48.2 (t), 55.5 (q), 55.9 (q), 110.6 (d), 110.9 (d), 111.2 (s), 112.1 (d), 114.0 (d), 119.2 (s), 125.4 (d), 127.4 (s), 128.2 (s), 150.8 (s), 151.3 (d), 153.6 (s), 159.4 (s).

9-(3,5-Dimethoxybenzyl)-4,5,6,9-tetrahydropyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazole (21r). This compound was obtained from reaction of **13r**. Yield: 76%, yellow solid; mp: 118.6°C ^{1}H NMR ($CDCl_3$) (ppm): 1.89 – 2.00 (2H, m, CH_2), 2.73 (2H, t, *J* = 6.0 Hz, CH_2), 2.88 (2H, t, *J* = 6.0 Hz, CH_2), 3.72 (6H, s, 2 x CH_3), 5.46 (2H, s, CH_2), 6.01 (1H, d, *J* = 2.6 Hz, H-7), 6.27 (2H, d, *J* = 2.2 Hz, H-2', H-6'), 6.32 (1H, t, *J* = 2.2 Hz, H-4'), 6.67 (1H, d, *J* = 2.6 Hz, H-8), 7.95 (1H, s, H-3); ^{13}C NMR ($CDCl_3$) (ppm): 23.2 (t), 24.3 (t), 28.8 (t), 53.0 (t), 55.2 (q x 2), 99.1 (d), 104.8 (d x 2), 110.2 (d), 111.4 (s), 119.1 (s), 125.2 (d), 127.7 (s), 140. 8 (s), 151.4 (d), 159.3 (s), 160.9 (s x 2).

9-(3,4,5-Trimethoxybenzyl)-4,5,6,9-tetrahydropyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazole (21s). This compound was obtained from reaction of **13s**. Yield: 74%, white solid; mp: 104.5 °C IR: 1683 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.89 – 2.00 (2H, m, CH_2), 2.74 (2H, t, *J* = 6.0 Hz, CH_2), 2.88 (2H, t, *J* = 6.0 Hz, CH_2), 3.77 (6H, s, CH_3 x 2), 3.80 (3H, s, CH_3), 5.45 (2H, s, CH_2), 6.02 (1H, d, *J* = 2.6 Hz, H-7), 6.39 (2H, s, H-2' and H-6'), 6.69 (1H, d, *J* = 2.6 Hz, H-8), 7.97 (1H, s, H-3); ^{13}C NMR ($CDCl_3$) (ppm): 23.3 (t), 24.4 (t), 28.8 (t), 53.2 (t), 56.0 (q x 2), 60.8 (q), 104.1 (d x 2), 110.2 (d), 111.5 (s), 119.1 (s), 125.1 (d), 127.9 (s), 133.8 (s), 137.1 (s), 151.5 (d), 153.3 (s x 2), 159.3 (s).

Methyl 9-benzyl-4,5,6,9-tetrahydropyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazole-8-carboxylate (21t). This compound was obtained from reaction of **13t** (crude product). Yield: 72%, white solid; mp: 150.3 °C ^{1}H NMR ($CDCl_3$) (ppm): 1.89 – 2.00 (2H, m, CH2), 2.77 (2H, t, *J* = 6.2 Hz, CH2), 2.84 (2H, t, *J* = 6.2 Hz, CH2), 3.78 (3H, s, CH3), 6.17 (2H, s, CH2), 6.86 (1H, s, H-7), 6.98 (2H, s, *J* = 9.7 Hz, H-2' and H-6'), 7.12 – 7.28 (3H, m, H-3', H-4' and H-5'), 8.01 (1H, s, H-3); ^{13}C NMR ($CDCl_3$) (ppm): 23.3 (t), 24.5 (t), 28.1 (t), 50.0 (t), 51.3 (q), 115.1 (s), 119.1 (d), 123.8 (s), 125.6 (s), 126.0 (d x 2), 126.7 (s), 126.8 (d), 128.3 (d x 2), 138.9 (s), 151.7 (d), 157.7 (s), 161.2 (s).

9.7.2 Preparation of chloro-pyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazole (22)

To a solution of **21** (1 mmol) in dry DMF (5 mL), was added a solution of N-chlorosuccinimide (1.5 mmol) in dry DMF (2 mL) and the reaction mixture was stirred for one night at room temperature (16 hours). Then, the reaction mixture was poured into ice and brine, and in the case of formation of a precipitate, the solid was filtered. In the absence of precipitate, the aqueous solution

was extracted with dichloromethane (3 x 30 mL). The organic phase was dried over Na_2SO_4 and the solvent evaporated at reduced pressure. The crude product was then purified by chromatography column (dichloromethane : ethyl acetate 98 : 2).

Ethyl 7-chloro-9-methyl-4,5,6,9-tetrahydropyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazole-8-carboxylate (22d). This compound was obtained from reaction of **21d**. Yield: 50%, white solid; mp: 88.2 °C IR: 1695 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) (ppm): 1.43 (3H, t, *J* = 7.1 Hz, CH_3), 1.94 – 2.04 (2H, m, CH_2), 2.77 – 2.89 (4H, m, CH_2 x 2), 4.29 (3H, s, CH_3), 4.40 (2H, q, *J* = 7.1 Hz, CH_2), 8.08 (1H, s, H-3); ^{13}C NMR ($CDCl_3$) (ppm): 14.4 (q), 23.3 (t), 24.6 (t), 25.4 (t), 36.2 (q), 60.7 (t), 115.7 (s), 119.5 (s), 121.4 (s), 124.1 (s), 124.5 (s), 151.7 (d), 157.7 (s), 160.6 (s).

Ethyl 7-chloro-9-benzyl-4,5,6,9-tetrahydropyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazole-8-carboxylate (22e). This compound was obtained from reaction of **21e**. Yield: 57%, white solid; mp: 141.8 – 142.4 °C; IR: 1696 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) (ppm): 1.32 (3H, t, *J* = 7.1 Hz, CH_3), 1.90 – 2.02 (2H, m, CH_2), 2.73 – 2.89 (4H, m, CH_2 x 2), 4.30 (2H, q, *J* = 7.1 Hz, CH_2), 6.12 (2H, s, CH_2), 6.99 (2H, d, *J* = 7.2 Hz, H-2', H-6'), 7.14 – 7.26 (3H, m, H-3', H-4', H-5'), 8.02 (1H, s, H-3); ^{13}C NMR ($CDCl_3$) (ppm): 14.2 (q), 23.1 (t), 24.4 (t), 25.3 (t), 50.8 (t), 60.8 (t), 116.1 (s), 120.4 (s), 121.2 (s), 124.1 (s), 124.8 (s), 126.0 (d x 2), 127.0 (d), 128.4 (d x 2), 138.5 (s), 151.7 (d), 157.0 (s), 160.3 (s).

Ethyl 7-chloro-9-(2-methoxybenzyl)-4,5,6,9-tetrahydropyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazole-8-carboxylate (22f). This compound was obtained from reaction of **21f**. Yield: 74%, yellow solid; mp: 159.8 °C; IR: 1696 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) (ppm): 1.26 (3H, t, *J* = 7.1 Hz, CH_3), 1.92 – 2.03 (2H, m, CH_2), 2.77 (2H, t, *J* = 5.8 Hz, CH_2), 2.87 (2H, t, *J* = 5.8 Hz, CH_2), 3.85 (3H, s, CH_3), 4.26 (2H, q, *J* = 7.1 Hz, CH_2), 6.05 (2H, s, CH_2), 6.41 (1H, d, *J* = 7.4 Hz, H-3'), 6.71 – 6.85 (2H, m, H-5' and H-6'), 7.14 (1H, t, *J* = 7.4 Hz, H-4'), 8.00 (1H, s, H-3); ^{13}C NMR ($CDCl_3$) (ppm): 14.0 (q), 23.2 (t), 24.4 (t), 25.3 (t), 42.3 (t), 55.3 (q), 60.7 (t), 109.8 (d), 115.8 (s), 120.0 (s), 120.4 (d), 121.5 (s), 124.3 (s), 124.5 (s), 125.4 (d), 127.4 (s), 127.8 (d), 151.6 (d), 156.2 (s), 157.2 (s), 160.1 (s).

Ethyl 7-chloro-9-(3-methoxybenzyl)-4,5,6,9-tetrahydropyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazole-8-carboxylate (22g). This compound was obtained from reaction of **21g**. Yield: 65%, green solid; mp: 92.8 – 93.5 °C; IR: 1695 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) (ppm): 1.33 (3H, t, *J* = 7.1 Hz, CH_3), 1.90 – 2.02 (2H, m, CH_2), 2.73 – 2.89 (4H, m, CH_2 x 2), 3.72 (3H, s, CH_3), 4.30 (2H, q, *J* = 7.1 Hz, CH_2), 6.10 (2H, s, CH_2), 6.53 – 6.61 (2H, m, H-2', H-6'), 6.70 (1H, dd, *J* = 7.9, 2.3 Hz, H-4'), 7.15 (1H, t, *J* = 7.9 Hz, H-5'), 8.03 (1H, s, H-3); ^{13}C NMR ($CDCl_3$) (ppm): 14.2 (q),

23.1 (t), 24.4 (t), 25.3 (t), 50.6 (t), 55.1 (q), 60.8 (t), 111.9 (d), 112.1 (d), 116.1 (s), 118.3 (d), 120.4 (s), 121.1 (s), 124.7 (s), 129.4 (d), 138.4 (s), 140.2 (s), 151.7 (d), 157.0 (s), 159.6 (s), 160.3 (s).

Ethyl 7-chloro-9-(4-methoxybenzyl)-4,5,6,9-tetrahydropyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazole-8-carboxylate (22h). This compound was obtained from reaction of **21h**. Yield: 70%, white solid; mp: 118.1 °C; IR: 1695 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.34 (3H, t, *J* = 7.1 Hz, CH_3), 1.89 – 2.01 (2H, m, CH_2), 2.73 – 2.87 (4H, m, CH_2 x 2), 3.73 (3H, s, CH_3), 4.32 (2H, q, *J* = 7.1 Hz, CH_2), 6.05 (2H, s, CH_2), 6.76 (2H, d, *J* = 8.7 Hz, H-3' and H-5'), 6.97 (2H, d, *J* = 8.7 Hz, H-2' and H-6'), 8.04 (1H, s, H-3); ^{13}C NMR ($CDCl_3$) (ppm): 14.2 (q), 23.1 (t), 24.4 (t), 25.3 (t), 50.1 (t), 55.2 (t), 60.8 (q), 113.8 (d x 2), 116.1 (s), 120.3 (s), 121.1 (s), 123.9 (s), 124.8 (s), 127.6 (d x 2), 130.6 (s), 151.8 (d), 151.1 (s), 158.6 (s), 160.4 (s).

Ethyl 7-chloro-9-(2,5-dimethoxybenzyl)-4,5,6,9-tetrahydropyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazole-8-carboxylate (22i). This compound was obtained from reaction of **21i**. Yield: 68%, white solid; mp: 147.2 °C IR: 1696 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.28 (3H, t, *J* = 7.1 Hz, CH_3), 1.92 – 2.03 (2H, m, CH_2), 2.77 (2H, t, *J* = 6.0 Hz, CH_2), 2.86 (2H, t, *J* = 6.0 Hz, CH_2), 3.62 (3H, s, CH_3), 3.82 (3H, s, CH_3), 4.27 (2H, q, *J* = 7.1 Hz, CH_2), 5.98 – 6.06 (3H, m, CH_2 and H-6'), 6.65 (1H, dd, *J* = 8.8, 2.6 Hz, H-4'), 6.76 (1H, d, *J* = 8.8 Hz, H-3'), 8.01 (1H, s, H-3); ^{13}C NMR ($CDCl_3$) (ppm): 14.0 (q), 23.1 (t), 24.4 (t), 25.3 (t), 47.2 (t), 55.5 (q), 55.8 (q), 60.7 (t), 110.5 (d), 110.7 (d), 113.0 (d), 115.9 (s), 120.2 (s), 121.3 (s), 124.2 (s), 124.5 (s), 128.8 (s), 150.6 (s), 151.6 (d), 153.4 (s), 157.1 (s), 160.0 (s).

Ethyl 7-chloro-9-(3,5-dimethoxybenzyl)-4,5,6,9-tetrahydropyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazole-8-carboxylate (22j). This compound was obtained from reaction of **21j**. Yield: 70%, white solid; mp: 165.5 °C; IR: 1701 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.26 (3H, t, *J* = 7.1 Hz, CH_3), 1.94 – 2.03 (2H, m, CH_2), 2.79 (2H, t, *J* = 6.0 Hz, CH_2), 2.87 (2H, t, *J* = 6.0 Hz, CH_2), 3.58 (3H, s, CH_3), 3.87 (3H, s, CH_3), 4.21 (2H, q, *J* = 7.1 Hz, CH_2), 5.50 (1H, d, *J* = 2.6 Hz, H-2'), 6.13 (2H, s, CH_2), 6.34 (1H, d, *J* = 2.6 Hz, H-6'), 6.91 (1H, s, H-4'), 7.99 (1H, s, H-3); ^{13}C NMR ($CDCl_3$) (ppm): 14.2 (q), 23.4 (t), 24.5 (t), 28.2 (t), 49.1 (t), 55.2 (q), 56.2 (q), 60.2 (t), 97.2 (d), 102.9 (d), 111.7 (s), 115.1 (s), 119.1 (d), 124.5 (s), 125.7 (s), 126.6 (s), 139.2 (s), 151.6 (d), 155.7 (s), 157.6 (s), 158.9 (s), 160.5 (s).

8-Chloro-9-methyl-4,5,6,9-tetrahydropyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazole (22l). This compound was obtained from reaction of **21l**. Yield: 49%, white solid; mp: 66.9 – 67.2 °C; ^{1}H NMR ($CDCl_3$) (ppm): 1.87 – 1.99 (2H, m, CH_2), 2.71 – 2.83 (4H, m, CH_2 x 2), 3.92 (3H, s, CH_3),

5.95 (1H, s, H-7), 8.02 (1H, s, H-3); ^{13}C NMR ($CDCl_3$) (ppm): 23.2 (t), 24.3 (t), 28.6 (t), 33.6 (q), 99.9 (s), 108.4 (d), 111.4 (s), 120.2 (s), 126.2 (s), 151.5 (d), 158.9 (s).

9.8 Preparation of Pyrrolo[3',2':6,7]cyclohepta[1,2-*d*]pyrimidin-2-amine (24-29)

- ✓ General synthesis for the amine derivates **24**

To a suspension of sodium methoxide (20 mmol) in dry ethanol (15 mL), guanidine nitrate (10 mmol) and a solution of appropriate enaminone **13** (2 mmol) in dry ethanol (20 mL) were added. The reaction mixture was heated to reflux for 1 hour and 45 minutes. After completeness the reaction mixture was poured into brine (50 mL), and the precipitate formed, was purified by chromatography column (dichloromethane ethyl acetate 8:2).

- ✓ General synthesis for the substituted amine derivates **25-26**

A solution of appropriate enaminone **13** (1.5 mmol) and phenylguanidine (in the case of derivates **25**) or cycloesilguanidine (in the case of derivates **26**) (4.5 mmol) in dry DMF (8 mL), was heated at 100 °C for 2 hours. After completeness the reaction mixture was poured into brine (20 mL) and the precipitate formed, was purified by chromatography column (dichloromethane).

- ✓ General synthesis for the carboxamide derivates **27-29**

To a solution of NaOH (0.5 mmol), in ethanol (5 mL), a solution of starting material of type **24**, **25** or **26**, in ethanol (8 mL) was added, and the reaction mixture was heated to reflux for 3 hours. After cooling the solution was evaporated. Water (10 mL) was added, and the mixture was acidified with HCl 6M. The solution was extracted with dichloromethane, and the organic phase was collected, dried on Na_2SO_4 and the solvent removed in vacuo, to give the crude acid derivative product which was taken up immediately onto next step.

N,N-diisopropylethylamine (0.55 mmol), 1-hydroxybenzotriazole (0.55 mmol), and EDC (0.55 mmol) were added to a stirred suspension of the above product in anhydrous THF (8 mL). The reaction mixture was stirred at room temperature for ten minutes. Ammonium carbonate (1.5 mmol) was added in one portion, and the resulting suspension was stirred at room temperature overnight (16 hours). The reaction mixture was concentrated, and a oversatured solution of Na_2CO_3 (15 mL) was added, and stirring was continued for other 2 hours. The suspension was filtered and the solid was purified by flash column chromatography (dichloromethane ethyl acetate 8:2).

ethyl 2-amino-10-methyl-5,6,7,10-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*]pyrimidine-9-carboxylate (24d) This compound was obtained from reaction of **13d**. Yield: 59%, white solid; mp: 212 °C; Rf: (DCM : EtOAc 8:2) 0.12; IR: 3520 - 3412 (NH_2), 1696 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) (ppm): 1.36 (3H, t, *J* = 7.1 Hz, CH_3), 2.03 – 2.16 (2H, m, CH_2), 2.42 (2H, t, *J* = 6.9 Hz, CH_2), 2.49 (2H, t, *J* = 6.9 Hz, CH_2), 4.16 (3H, s, CH_3), 4.30 (2H, q, *J* = 7.1 Hz, CH_2), 5.01 (2H, bs, NH_2), 6.85 (1H, s, H-8), 8.17 (1H, s, H-4); ^{13}C NMR ($CDCl_3$) (ppm): 14.4 (q), 23.5 (t), 27.4 (t), 31.7 (t), 34.6 (q), 60.0 (t), 116.8 (d), 123.9 (s), 124.9 (s), 126.6 (s), 133.4 (s), 158.4 (d), 159.5 (s), 161.2 (s), 161.4 (s).

ethyl 2-amino-10-benzyl-5,6,7,10-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*]pyrimidine-9-carboxylate (24e) This compound was obtained from reaction of **13e**. Yield: 40%, white solid; mp: 150 – 151 °C; Rf: (DCM : EtOAc 8:2) 0.23; IR: 3523 - 3411 (NH_2), 1700 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) (ppm): 1.29 (3H, t, *J* = 7.1 Hz, CH_3), 2.07 – 2.14 (2H, m, CH_2), 2.36 (2H, t, *J* = 6.9 Hz, CH_2), 2.51 (2H, t, *J* = 6.9 Hz, CH_2), 4.24 (2H, q, *J* = 7.1 Hz, CH_2), 4.92 (2H, bs, NH_2), 6.13 (2H, s, CH_2), 6.85 (2H, d, *J* = 7.8 Hz, H-2', H-6'), 6.93 (1H, s, H-8), 7.10 – 7.17 (3H, m, H-3', H-4', H-5'), 8.12 (1H, s, H-4); ^{13}C NMR ($CDCl_3$) (ppm): 14.3 (q), 23.5 (t), 27.3 (t), 31.8 (t), 48.7 (t), 60.0 (t), 117.9 (d), 123.6 (s), 124.7 (s), 126.0 (d x 2), 126.6 (d), 127.3 (s), 128.2 (d x 2), 133.4 (s), 139.7 (s), 158.5 (d), 159.5 (s), 160.9 (s), 161.4 (s).

Ethyl 2-amino-10-(4-methoxybenzyl)-5,6,7,10-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*]pyrimidine-9-carboxylate (24h). This compound was obtained from reaction of **13h**. Yield: 50%, white solid; mp: 78 – 79 °C; Rf: (DCM : EtOAc 8:2) 0.19; IR: 3512 - 3411 (NH_2) 1696 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) (ppm): 1.31 (3H, t, *J* = 7.1 Hz, CH_3), 2.06 – 2.16 (2H, m, CH_2), 2.34 (2H, t, *J* = 6.7 Hz, CH_2), 2.49 (2H, t, *J* = 6.7 Hz, CH_2), 3.71 (3H, s, CH_3), 4.25 (2H, q, *J* = 7.1 Hz, CH_2), 4.94 (2H, bs, NH_2), 6.06 (2H, s, CH_2), 6.69 (2H, d, *J* = 8.7 Hz, H-3' and H-5'), 6.83 (2H, d, *J* = 8.7 Hz, H-2' and H-6'), 6.90 (1H, s, H-8), 8.13 (1H, s, H-4); ^{13}C NMR ($CDCl_3$) (ppm): 14.3 (q), 23.4 (t), 27.3 (t), 31.9 (t), 48.0 (t), 55.1 (q), 60.0 (t), 113.6 (d x 2), 117.9 (d), 123.6 (s), 124.6 (s), 127.3 (s), 127.5 (d x 2), 131.8 (s), 133.3 (s), 158.3 (s), 158.6 (d), 159.7 (s), 160.9 (s), 161.4 (s).

Ethyl 2-(phenylamino)-10-methyl-5,6,7,10-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*]pyrimidine-9-carboxylate (25d). This compound was obtained from reaction of **13d**. Yield: 62%, pale yellow solid; mp: 159 °C; Rf: (DCM : EtOAc 8:2) 0.79; IR: 3417 (NH), 1704 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) (ppm): 1.37 (3H, t, *J* = 7.1 Hz, CH_3), 2.10 – 2.17 (2H, m, CH_2), 2.43 – 2.55 (4H, m, CH_2 x 2), 4.20 (3H, s, CH_3), 4.32 (2H, q, *J* = 7.1 Hz, CH_2), 6.87 (1H, s, H-8), 7.02 (1H, t, *J* = 8.4 Hz, H-4'), 7.29 (1H, bs, NH), 7.34 (2H, dd, *J* = 8.4 Hz, *J* = 2.5 Hz, H-2' and H-6'), 7.62 (2H, d, *J* = 8.4 Hz, H-3' and H-5'), 8.29 (1H, s, H-4); ^{13}C NMR ($CDCl_3$) (ppm): 14.4 (q), 23.6 (t), 27.5 (t), 32.0

(t), 34.8 (q), 60.0 (t), 116.8 (d), 119.0 (d x 2), 122.2 (d), 124.8 (s), 124.9 (s), 126.7 (s), 128.9 (d x 2), 133.5 (s), 139.7 (s), 158.3 (d), 158.5 (s), 159.0 (s), 161.3 (s).

Ethyl 2-(phenylamino)-10-benzyl-5,6,7,10-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*]pyrimidine-9-carboxylate (25e). This compound was obtained from reaction of **13e**. Yield: 60%, yellow solid; mp: 147.5 °C; Rf: (DCM : EtOAc 8:2) 0.89; IR: 3417 (NH), 1700 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.29 (3H, t, *J* = 7.1 Hz, CH_3), 2.11 – 2.20 (2H, m, CH_2), 2.39 (2H, t, *J* = 6.9 Hz, CH_2), 2.54 (2H, t, *J* = 6.9 Hz, CH_2), 4.24 (2H, q, *J* = 7.1 Hz, CH_2), 6.18 (2H, s, CH_2), 6.83 (2H, dd, *J* = 8.4, 2.3 Hz, Ar), 6.95 (1H, s, H-8), 7.00 – 7.31 (7H, m, Ar, NH), 7.56 (2H, d, *J* = 8.4 Hz, Ar), 8.23 (1H, s, H-4); ^{13}C NMR ($CDCl_3$) (ppm): 14.3 (q), 23.5 (t), 27.5 (t), 32.0 (t), 48.7 (t), 60.1 (t), 117.9 (d), 119.0 (d x 2), 122.2 (d), 124.6 (s), 124.8 (s), 125.9 (d x 2), 126.6 (d), 127.5 (s), 128.2 (d x 2), 128.9 (d x 2), 133.4 (s), 139.6 (s), 139.7 (s), 158.3 (d), 158.5 (s), 159.1 (s), 160.9 (s).

Ethyl 2-(phenylamino)-10-(4-methoxybenzyl)-5,6,7,10-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*]pyrimidine-9-carboxylate (25h). This compound was obtained from reaction of **13h**. Yield: 43%, pale yellow solid; mp: 132.6 – 133.3 °C; Rf: (DCM : EtOAc8:2) 0.80; IR: 3416 (NH), 1696 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.31 (3H, t, *J* = 7.1 Hz, CH_3), 2.09 – 2.19 (2H, m, CH_2), 2.37 (2H, t, *J* = 6.8 Hz, CH_2), 2.51 (2H, t, *J* = 6.8 Hz, CH_2), 3.70 (3H, s, CH_3), 4.25 (2H, q, *J* = 7.1 Hz, CH_2), 6.12 (2H, s, CH_2), 6.67 (2H, d, *J* = 8.9 Hz, H-3' and H-5'), 6.77 (2H, d, *J* = 8.9 Hz, H-2' and H-6'), 6.92 (1H, s, H-8), 7.00 (1H, t, *J* = 8.3 Hz, H-4''), 7.22 – 7-32 (3H, m, H-2'', H-6'' and NH), 7.57 (2H, d, *J* = 8.3 Hz, H-3'' and H-5''), 8.25 (1H, s, H-4); ^{13}C NMR ($CDCl_3$) (ppm): 14.3 (q), 23.4 (t), 27.4 (t), 32.1 (t), 47.9 (t), 55.1 (q), 60.1 (t), 113.6 (d x 2), 117.9 (d), 119.0 (d x 2), 122.2 (d), 124.2 (s), 124.5 (s), 124.6 (s), 127.4 (d x 2), 127.5 (s), 128.9 (d x 2), 131.7 (s), 133.3 (s), 139.7 (s), 158.3 (d), 158.6 (s), 159.3 (s), 161.0 (s).

Ethyl 2-(cyclohexylamino)-10-methyl-5,6,7,10-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*]pyrimidine-9-carboxylate (26d). This compound was obtained from reaction of **13d**. Yield: 76%, white solid; mp: 140.5 °C; Rf: (DCM : EtOAc 8:2) 0.67; IR: 3427 (NH), 1697 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.13 – 1.39 (7H, m, CH_3, CH_2 x 2), 1.57 – 1.85 (4H, m, CH_2 x 2), 1.95 – 2.12 (4H, m, CH_2 x 2), 2.37 – 2.51 (4H, m, CH_2 x 2), 3.78 – 3.90 (1H, m, CH), 4.18 (3H, s, CH_3), 4.30 (2H, q, *J* = 7.1 Hz, CH_2), 4.97 (1H, d, *J* = 8.3 Hz, NH), 6.84 (1H, s, H-8), 8.13 (1H, s, H-4); ^{13}C NMR ($CDCl_3$) (ppm): 14.4 (q), 23.7 (t), 24.9 (t x 2), 25.7 (t), 27.3 (t), 31.7 (t), 33.4 (t x 2), 34.6 (q), 49.9 (d), 59.9 (t), 116.8 (d), 122.3 (s), 124.6 (s), 126.4 (s), 134.0 (s), 146.3 (s), 158.4 (d), 160.4 (s), 161.3 (s).

Ethyl 2-(cyclohexylamino)-10-benzyl-5,6,7,10-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*]pyrimidine-9-carboxylate (26e). This compound was obtained from reaction of **13e**. Yield: 81%, white solid; mp: 162.1 °C; Rf: (DCM: EtOAc 8:2) 0.70; IR: 3424 (NH), 1700 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.15 – 1.31 (7H, m, CH_3, CH_2 x 2), 1.56 – 1.65 (4H, m, CH_2 x 2), 1.87 – 1.96 (2H, m, CH_2), 2.06 – 2.16 (2H, m, CH_2), 2.34 (2H, t, *J* = 6.9 Hz, CH_2), 2.51 (2H, t, *J* = 6.9 Hz, CH_2), 3.65 – 3.70 (1H, m, CH), 4.21 (2H, q, *J* = 7.1 Hz, CH_2), 4.90 (1H, d, *J* = 8.4 Hz, NH), 6.15 (2H, s, CH_2), 6.87 (1H, s, H-8), 6.90 – 6.94 (2H, m, H-2' and H-6'), 7.13 – 7.20 (3H, m, H-3', H-4' and H-5'), 8.09 (1H, s, H-4); ^{13}C NMR ($CDCl_3$) (ppm): 14.3 (q), 23.4 (t), 24.7 (t x 2), 25.7 (t), 27.3 (t), 32.0 (t), 33.4 (t x 2), 48.7 (t), 49.5 (d), 59.9 (t), 117.9 (d), 122.0 (s), 124.2 (s), 125.9 (d x 2), 126.5 (d), 127.0 (s), 128.2 (d x 2), 133.9 (s), 139.8 (s), 158.5 (d), 159.2 (s), 160.4 (s), 160.9 (s).

Ethyl 2-(cyclohexylamino)-10-(4-methoxybenzyl)-5,6,7,10-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*]pyrimidine-9-carboxylate (26h). This compound was obtained from reaction of **13h**. Yield: 49%, yellow solid; mp: 135.8 °C; Rf: (DCM : EtOAc 8:2) 0.67; IR: 3428 (NH), 1704 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.12 – 1.33 (7H, m, CH_3, CH_2 x 2), 1.55 – 1.68 (4H, m, CH_2 x 2), 1.87 – 2.13 (4H, m, CH_2 x 2), 2.32 (2H, t, *J* = 6.9 Hz, CH_2), 2.49 (2H, t, *J* = 6.9 Hz, CH_2), 3.72 (3H, s, CH_3), 3.75 – 3.78 (1H, m, CH), 4.23 (2H, q, *J* = 7.1 Hz, CH_2), 4.92 (1H, d, *J* = 8.4 Hz, NH), 6.08 (2H, s, CH_2), 6.71 (2H, d, *J* = 8.4 Hz, H-3' and H-5'), 6.85 (2H, d, *J* = 8.4 Hz, H-2' and H-6'), 6.90 (1H, s, H-8), 8.11 (1H, s, H-4); ^{13}C NMR ($CDCl_3$) (ppm): 14.3 (q), 23.4 (t), 24.8 (t x 2), 25.7 (t), 27.2 (t), 32.1 (t), 33.4 (t x 2), 47.9 (t), 49.5 (d), 55.1 (q), 59.9 (t), 113.6 (d x 2), 117.8 (d), 122.0 (s), 124.0 (s), 127.0 (s), 127.4 (d x 2), 131.9 (s), 133.8 (s), 158.3 (s), 158.5 (d), 159.3 (s), 160.5 (s), 160.9 (s).

2-Amino-10-methyl-5,6,7,10-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*]pyrimidine-9-carboxamide (27d). This compound was obtained from reaction of **24d**. Yield: 66%, pale yellow solid; mp: 196.2 – 196.8 °C; Rf: (EtOAc) 0.19; IR: 3502 - 3396 (NH_2), 3309 - 3212 (NH_2) 1662 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 2.04 – 2.14 (2H, m, CH_2), 2.39 – 2.51 (4H, m, CH_2 x 2), 4.14 (3H, s, CH_3), 5.26 (2H, s, NH_2), 6.06 (2H, s, NH_2), 6.51 (1H, s, H-8), 8.14 (1H, s, H-4); ^{13}C NMR ($CDCl_3$) (ppm): 23.7 (t), 27.4 (t), 31.6 (t), 34.7 (q), 112.8 (d), 123.6 (s), 126.6 (s), 127.3 (s), 132.4 (s), 158.1 (d), 159.6 (s), 161.5 (s), 163.9 (s).

2-Amino-10-benzyl-5,6,7,10-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*]pyrimidine-9-carboxamide (27e). This compound was obtained from reaction of **24e**. Yield: 25%, white solid; mp: 191.4 – 192.2 °C; Rf: (EtOAc) 0.32; IR: 3481 (NH_2), 3420 (NH_2), 3313 (NH_2), 3250 (NH_2) 1662 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 2.01 – 2.09 (2H, m, CH_2), 2.33 (2H, t, *J* = 7.1 Hz, CH_2), 2.50 (2H, t, *J* = 7.1 Hz, CH_2), 4.97 (2H, bs, NH_2), 5.65 (2H, bs, NH_2), 6.13 (2H, s, CH_2), 6.55 (1H,

s, H-8), 6.85 – 6.90 (2H, m, H-2’ and H-6’), 7.06 – 7.19 (3H, m, H-3’, H-4’ and H-5’), 8.09 (1H, s, H-4); ^{13}C NMR ($CDCl_3$) (ppm): 23.6 (t), 27.3 (t), 31.8 (t), 48.6 (t), 113.7 (d), 123.4 (s), 126.3 (d x 2), 126.7 (d), 127.1 (s), 127.3 (s), 128.2 (d x 2), 132.4 (s), 139.8 (s), 158.3 (d), 159.6 (s), 161.3 (s), 163.3 (s).

2-Amino-10-(4-methoxybenzyl)-5,6,7,10-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*]pyrimidine-9-carboxamide (27h). This compound was obtained from reaction of **24h**. Yield: 94%, white solid; mp: 193.7 °C; Rf: (EtOAc) 0.29; IR: 3479 - 3420 (NH_2), 3310 - 3246 (NH_2), 1663 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 2.05 – 2.15 (2H, m, CH_2), 2.33 (2H, t, *J* = 6.9 Hz, CH_2), 2.49 (2H, t, *J* = 6.9 Hz, CH_2), 3.71 (3H, s, CH_3), 4.93 (2H, bs, NH_2), 5.55 (2H, bs, NH_2), 6.09 (2H, s, CH_2), 6.53 (1H, s, H-8), 6.70 (2H, d, *J* = 8.7 Hz, H-3’ and H-5’), 6.86 (2H, d, *J* = 8.7 Hz, H-2’ and H-6’), 8.12 (1H, s, H-4); ^{13}C NMR ($CDCl_3$) (ppm): 23.5 (t), 27.3 (t), 31.8 (t), 47.9 (t), 55.1 (q), 113.5 (d x 2), 113.7 (d), 123.4 (s), 127.0 (s), 127.3 (s), 127.8 (d x 2), 130.9 (s), 131.9 (s), 132.3 (s), 158.3 (d), 159.8 (s), 161.3 (s), 163.4 (s).

10-Methyl-2-(phenylamino)-5,6,7,10-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*]pyrimidine-9-carboxamide (28d). This compound was obtained from reaction of **25d**. Yield: 74%, pale yellow solid; mp: 253.6 °C; Rf: (DCM : EtOAc 7:3) 0.21; IR: 3401 (NH), 3254 - 3170 (NH_2), 1635 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 2.10 – 2.17 (2H, m, CH_2), 2.43 – 2.55 (4H, m, CH_2 x 2), 4.21 (3H, s, CH_3), 5.72 (2H, bs, NH_2), 6.52 (1H, s, H-8), 7.02 (1H, t, *J* = 8.3 Hz, H-4’), 7.26 – 7.36 (3H, m, NH, H-2’ and H-6’), 7.63 (2H, dd, *J* = 8.3, 1.2 Hz, H-3’ and H-5’); 8.28 (1H, s, H-4); ^{13}C NMR ($CDCl_3$) (ppm): 23.7 (t), 27.6 (t), 32.0 (t), 34.9 (q), 112.7 (d), 119.1 (d x 2), 122.2 (d), 124.6 (s), 126.6 (s), 127.0 (s), 128.9 (d x 2), 132.7 (s), 139.7 (s), 158.2 (d), 158.5 (s), 159.0 (s), 163.5 (s).

10-Benzyl-2-(phenylamino)-5,6,7,10-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*]pyrimidine-9-carboxamide (28e). This compound was obtained from reaction of **25e**. Yield: 64%, pale yellow solid; mp: 195.7 °C; Rf: (DCM : EtOAc 7:3) 0.42; IR: 3318 (NH), 3273 - 3164 (NH_2), 1661 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 2.10 – 2.18 (2H, m, CH_2), 2.37 (2H, t, *J* = 6.8 Hz, CH_2), 2.53 (2H, t, *J* = 6.8 Hz, CH_2), 5.62 (2H, bs, NH_2), 6.21 (2H, s, CH_2), 6.58 (1H, s, H-8), 6.84 (2H, d, *J* = 7.4 Hz, Ar), 7.01 (1H, t, *J* = 7.4 Hz, Ar), 7.12 – 7.32 (6H, m, NH, Ar), 7.58 (2H, d, *J* = 7.9 Hz, Ar), 8.22 (1H, s, H-4); ^{13}C NMR ($CDCl_3$) (ppm): 23.6 (t), 27.5 (t), 32.0 (t), 48.7 (t), 113.7 (d), 119.1 (d x 2), 122.2 (d), 124.4 (s), 126.2 (d x 2), 126.7 (d), 127.1 (s), 127.4 (s), 128.2 (d x 2), 128.9 (d x 2), 132.5 (s), 139.7 (s), 139.8 (s), 158.1 (d), 158.5 (s), 159.2 (s), 163.4 (s).

10-(4-Methoxybenzyl)-2-(phenylamino)-5,6,7,10-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*]pyrimidine-9-carboxamide (28h). This compound was obtained from reaction of **25h**. Yield:

54%, pale yellow solid; mp: 195.8 – 196.4 °C; Rf: (7:3 DCM: EtOAc) 0.28; IR: 3399 (NH), 3319 (NH_2), 3204 (NH_2), 1668 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) (ppm): 2.08 – 2.18 (2H, m, CH_2), 2.36 (2H, t, *J* = 6.7 Hz, CH_2), 2.50 (2H, t, *J* = 6.7 Hz, CH_2), 3.69 (3H, s, CH_3), 5.72 (2H, bs, NH_2), 6.14 (2H, s, CH_2), 6.55 (1H, s, H-8), 6.66 (2H, d, *J* = 8.7 Hz, H-3' and H-5'), 6.80 (2H, d, *J* = 8.7 Hz, H-2' and H-6'), 7.01 (1H, t, *J* = 7.8 Hz, H-4''), 7.28 – 7.33 (3H, m, H-2'', H-6'' and NH), 7.61 (2H, d, *J* = 7.8 Hz, H-3'' and H-5''), 8.23 (1H, s, H-4); ^{13}C NMR ($CDCl_3$) (ppm): 23.5 (t), 27.5 (t), 32.1 (t), 47.9 (t), 55.1(q), 113.6 (d x 2), 113.7 (d), 119.0 (d x 2), 122.2 (d), 124.3 (s), 127.0 (s), 127.4 (s), 127.7 (d x 2), 128.9 (d x 2), 131.9 (s), 132.4 (s), 139.7 (s), 158.1 (d), 158.3 (s), 158.6 (s), 159.3 (s), 163.4 (s).

2-(Cyclohexylamino)-10-methyl-5,6,7,10-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*] pyrimidine-9-carboxamide (29d). This compound was obtained from reaction of **26d**. Yield: 82%, pale pink solid; mp: 204.9 °C; Rf: (EtOAc) 0.48; IR: 3403 (NH), 3276 - 3183 (NH_2), 1662 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) (ppm): 1.15 – 1.50 (4H, m, CH_2 x 2), 1.57 – 1.91 (4H, m, CH_2 x 2), 1.98 – 2.12 (4H, m, CH_2 x 2), 2.37 – 2.53 (4H, m, CH_2 x 2), 3.75 – 3.84 (1H, m, CH), 4.18 (3H, s, CH_3), 5.04 (1H, d, *J* = 8.1 Hz, NH), 5.70 (2H, bs, NH_2), 6.50 (1H, s, H-8), 8.12 (1H, s, H-4); ^{13}C NMR ($CDCl_3$) (ppm): 23.8 (t), 24.9 (t x 2), 25.7 (t), 27.4 (t), 31.7 (t), 33.4 (t x 2), 34.8 (q), 49.9 (d), 112.7 (d), 122.1 (s), 126.3 (s), 126.8 (s), 133.1 (s), 158.2 (d), 159.1 (s), 160.4 (s), 163.6 (s).

2-(Cyclohexylamino)-10-benzyl-5,6,7,10-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*] pyrimidine-9-carboxamide (29e). This compound was obtained from reaction of **26e**. Yield: 85%, white solid; mp: 226.6 – 227.2 °C; Rf: (EtOAc) 0.61; IR: 3396 (NH), 3209 - 3122 (NH_2), 1696 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) (ppm): 1.10 – 1.25 (4H, m, CH_2 x 2), 1.61 – 1.75 (4H, m, CH_2 x 2), 1.89 – 2.12 (4H, m, CH_2 x 2), 2.32 (2H, t, *J* = 6.8 Hz, CH_2), 2.50 (2H, t, *J* = 6.8 Hz, CH_2), 3.62 – 3.79 (1H, m, CH), 4.91 (1H, d, *J* = 7.8 Hz, NH), 5.53 (2H, bs, NH_2), 6.20 (2H, s, CH_2), 6.55 (1H, s, H-8), 6.89 (2H, d, *J* = 7.0 Hz, H-2' and H-6'), 7.14 – 7.26 (3H, m, H-3', H-4' and H-5'), 8.08 (1H, s, H-4); ^{13}C NMR ($CDCl_3$) (ppm): 23.5 (t), 24.8 (t x 2), 25.7 (t), 27.3 (t), 32.1 (t), 33.4 (t x 2), 48.5 (t), 49.5 (d), 113.7 (d), 121.9 (s), 126.2 (d x 2), 126.5 (s), 126.6 (d), 126.9 (s), 128.2 (d x 2), 133.0 (s), 139.9 (s), 158.4 (d), 159.2 (s), 160.4 (s), 163.2 (s).

2-(Cyclohexylamino)-10-(4-methoxybenzyl)-5,6,7,10-tetrahydropyrrolo[3',2':6,7] cyclohepta[1,2-*d*]pyrimidine-9-carboxamide (29h). This compound was obtained from reaction of **26h**. Yield: 93%, pale yellow solid; mp: 162.9 °C; Rf: (DCM : EtOAc 7:3) 0.30; IR: 3403 (NH), 3270 - 3160 (NH_2), 1663 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) (ppm): 1.20 – 1.38 (4H, m, CH_2 x 2), 1.57 – 1.73 (4H, m, CH_2 x 2), 1.94 – 2.11 (4H, m, CH_2 x 2), 2.31 (2H, t, *J* = 7.0 Hz, CH_2), 2.48 (2H, t, *J* = 7.0 Hz, CH_2), 3.71 (3H, s, CH_3), 3.72 – 3.77 (1H, m, CH), 4.93 (1H, d, *J* = 8.4 Hz, NH), 5.53 (2H,

bs, NH_2), 6.12 (2H, s, CH_2), 6.53 (1H, s, H-8), 6.70 (2H, d, *J* = 8.7 Hz, H-3' and H-5'), 6.87 (2H, d, *J* = 8.7 Hz, H-2' and H-6'), 8.09 (1H, s, H-4); ^{13}C NMR ($CDCl_3$) (ppm): 23.5 (t), 24.8 (t x 2), 25.7 (t), 27.3 (t), 32.1 (t), 33.4 (t x 2), 47.8 (t), 49.6 (d), 55.1 (q), 113.6 (d x 2), 113.7 (d), 121.9 (s), 126.3 (s), 126.9 (s), 127.7 (d x 2), 132.1 (s), 132.8 (s), 158.3 (s), 158.4 (d), 159.4 (s), 160.5 (s), 163.3 (s).

9.9.1 Preparation of Pyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazol-2-amine (32)

To a suspension of $CuBr_2$ (5.4 mmol) in dry ethyl acetate (12 mL) the appropriate ketone **9** (3 mmol) was added and the reaction mixture was heated to reflux for 2 hours. After cooling the reaction mixture was filtered under vacuum to remove the CuBr formed. The solvent was evaporated and the residue dissolved in dry DMF (12 mL). K_2CO_3 (6 mmol) and thiourea (6 mmol) were added and the new mixture was stirred at room temperature for one night (16 h). The reaction was poured into brine (30 mL) and in the case of formation of a precipitate, the solid was filtered. In the absence of precipitate, the aqueous solution was extracted with dichloromethane (3 x 40 mL). The organic phase was dried over Na_2SO_4 and the solvent evaporated at reduced pressure. The crude product was then purified by chromatography column (dichloromethane ethyl acetate 8:2).

Ethyl 2-amino-4,5,6,9-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazole-8-carboxylate (32a) This compound was obtained from reaction of **9a** Yield: 52%, white solid; mp: 190 – 191°C; IR: 3370 (NH), 3202 - 3156 (NH_2) 1692 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) (ppm): 1.27 (3H, t, *J* = 7.1 Hz, CH_3), 1.85 – 1.99 (2H, m, CH_2), 2.75 – 2.85 (4H, m, CH_2 x 2), 4.22 (2H, q, *J* = 7.1 Hz, CH_2), 6.63 (1H, s, H-7), 6.88 (2H, s, NH_2), 9.64 (1H, s, NH); ^{13}C NMR (DMSO-d_6) (ppm): 14.3 (q), 23.9 (t), 27.0 (t), 27.6 (t), 59.5 (t), 116.4 (d), 118.9 (s), 119.3 (s), 121.6 (s), 129.8 (s), 137.8 (s), 159.9 (s), 165.5 (s).

9-(Phenylsulfonyl)-4,5,6,9-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazol-2-amine (32b) This compound was obtained from reaction of **9b** Yield: 44 %, yellow solid; mp: 173 - 174 °C; IR: 3252 - 3143 (NH_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 2.01 – 2.11 (2H, m, CH_2), 2.41 (2H, t, *J* = 6.7 Hz, CH_2), 2.45 (2H, t, *J* = 6.7 Hz, CH_2), 4.68 (2H, s, NH_2), 6.17 (1H, d, *J* = 3.0 Hz, H-7), 7.35 (1H, d, *J* = 3.0 Hz, H-8), 7.40 – 7.55 (3H, m, H-3', H-4' and H-5'), 7.86 (2H, d, *J* = 7.0 Hz, H-2' and H-6'); ^{13}C NMR ($CDCl_3$) (ppm): 24.0 (t), 25.0 (t), 31.8 (t), 113.5 (d), 123.2 (d), 125.3 (s), 126.7 (s), 127.2 (d x 2), 128.4 (d x 2), 130.5 (s), 133.0 (d), 138.3 (s), 140.1 (s), 162.7 (s).

4,5,6,9-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazol-2-amine (32c) This compound was obtained from reaction of **31c** Yield: 64 %, brown solid; mp: 66.4 – 67.2 °C; IR: 3455 – 3416 (NH_2), 3287 (NH) cm^{-1}; ^1H NMR ($CDCl_3$) (ppm): 2.02 – 2.09 (2H, m, CH_2), 2.87 – 2.94 (4H, m, CH_2 x 2), 4.97 (2H, bs, NH_2), 5.99 (1H, d, *J* = 2.9 Hz, H-7), 6.58 (1H, d, *J* = 2.9 Hz, H-8), 9.27 (1H, bs, NH); ^{13}C NMR ($CDCl_3$) (ppm): 24.3 (t), 27.6 (t), 28.5 (t), 110.3 (d), 116.2 (d), 117.0 (s), 120.5 (s), 124.8 (s), 139.2 (s), 144.6 (s).

Ethyl 2-amino-9-methyl-4,5,6,9-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazole-8-carboxylate (32d) This compound was obtained from reaction of **9d** Yield: 50 %, yellow solid; mp: 113 - 114 °C; IR: 3255 - 3188 (NH_2), 1690 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) (ppm): 1.34 (3H, t, *J* = 7.1 Hz, CH_3), 2.05 – 2.09 (2H, m, CH_2), 2.61 (2H, t, *J* = 6.4 Hz, CH_2), 2.76 (2H, t, *J* = 6.4 Hz, CH_2), 4.16 (3H, s, CH_3), 4.27 (2H, q, *J* = 7.1 Hz, CH_2), 4.86 (2H, s, NH_2), 6.79 (1H, s, H-7); ^{13}C NMR ($CDCl_3$) (ppm): 14.5 (q), 25.7 (t), 25.8 (t), 29.9 (t), 35.2 (q), 59.6 (t), 117.3 (d), 121.8 (s), 124.2 (s), 126.2 (s), 132.7 (s), 139.0 (s), 161.5 (s), 163.3 (s)

Ethyl 2-amino-9-(4-methoxybenzyl)-4,5,6,9-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazole-8-carboxylate (32h) This compound was obtained from reaction of **9h** Yield: 59 %, yellow solid; mp: 59°C; IR: 3253 - 3175 (NH_2), 1715 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) (ppm): 1.27 (3H, t, *J* = 7.1 Hz, CH_3), 2.05 – 2.12 (2H, m, CH_2), 2.63 (2H, t, *J* = 6.5 Hz, CH_2), 2.73 (2H, t, *J* = 6.5 Hz, CH_2), 3.73 (3H, s, CH_3), 4.17 (2H, q, *J* = 7.1 Hz, CH_2), 4.73 (2H, s, NH_2), 6.09 (2H, s, CH_2), 6.73 (2H, d, *J* = 8.6 Hz, H-3' and H-5'), 6.86 (1H, s, H-7) 6.89 (2H, d, *J* = 8.6 Hz, H-2' and H-6'); ^{13}C NMR ($CDCl_3$) (ppm): 14.4 (q), 25.5 (t), 25.7 (t), 30.2 (t), 48.6 (t), 55.1 (q), 59.6 (t), 113.5 (d x 2), 118.6 (d), 121.4 (s), 124.7 (s), 126.3 (s), 127.5 (d x 2), 132.5 (s), 132.6 (s), 139.1 (s), 158.1 (s), 161.1 (s), 163.4 (s).

Ethyl 2-amino-9-(3,5-dimethoxybenzyl)-4,5,6,9-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazole-8-carboxylate (32j) This compound was obtained from reaction of **9j** Yield: 38 %, brown solid; mp: 140.6 – 141.0 °C; IR: 3377 – 3253 (NH_2), 1696 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) (ppm): 1.27 (3H, t, *J* = 7.1 Hz, CH_3), 2.03 – 2.13 (2H, m, CH_2), 2.63 (2H, t, *J* = 6.6 Hz, CH_2), 2.73 (2H, t, *J* = 6.6 Hz, CH_2), 3.66 (6H, s, CH_3 x 2), 4.19 (2H, q, *J* = 7.1 Hz, CH_2), 4.77 (2H, s, NH_2), 6.08 (2H, s, CH_2), 6.14 (2H, s, H-2' and H-6'), 6.23 (1H, s, H-4'), 6.87 (1H, s, H-7); ^{13}C NMR ($CDCl_3$) (ppm): 14.4 (q), 25.6 (t), 25.7 (t), 30.2 (t), 49.1 (t), 55.1 (q), 59.6 (t), 98.6 (d), 104.1 (d x 2), 118.6 (d), 121.7 (s), 124.7 (s), 126.1 (s), 132.7 (s), 139.1 (s), 142.9 (s), 160.5 (s x 2), 161.1 (s), 163.5 (s).

Ethyl 2-amino-9-(2-bromo-3,5-dimethoxybenzyl)-4,5,6,9-tetrahydropyrrolo [3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazole-8-carboxylate (32u) This compound was obtained from reaction of **9j** Yield: 23 %, brown solid; mp: 189.3 – 189.9 °C; IR: 3334 – 3227 (NH_2), 1695 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) (ppm): 1.25 (3H, t, *J* = 7.1 Hz, CH_3), 2.00 – 2.09 (2H, m, CH_2), 2.69 – 2.84 (4H, m, CH_2 x 2), 3.56 (3H, s, CH_3), 3.84 (3H, s, CH_3), 4.18 (2H, q, *J* = 7.1 Hz, CH_2), 4.62 (2H, s, NH_2), 5.43 (1H, s, H-4'), 6.21 (2H, s, CH_2), 6.28 (1H, s, H-6'), 6.91 (1H, s, H-7); ^{13}C NMR ($CDCl_3$) (ppm): 14.3 (q), 26.6 (t), 26.7 (t), 28.4 (t), 51.1 (t), 55.1 (q), 56.2 (q), 59.7 (t), 97.2 (d), 101.3 (s), 103.2 (d), 118.7 (d), 121.9 (s), 124.6 (s), 126.0 (s), 132.4 (s), 138.3 (s), 142.4 (s), 156.1 (s), 159.7 (s), 160.9 (s), 162.9 (s).

Ethyl 2-amino-9-(3,4,5-trimethoxybenzyl)-4,5,6,9-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazole-8-carboxylate (32k) This compound was obtained from reaction of **9k** Yield: 92 %, yellow solid; mp: 149.5 – 150.3 °C; IR: 3408 – 3357 (NH_2), 1701 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) (ppm): 1.30 (3H, t, *J* = 7.1 Hz, CH_3), 2.03 – 2.11 (2H, m, CH_2), 2.63 (2H, t, *J* = 6.4 Hz, CH_2), 2.73 (2H, t, *J* = 6.4 Hz, CH_2), 3.70 (6H, s, CH_3 x 2), 3.76 (3H, s, CH_3), 4.22 (2H, q, *J* = 7.1 Hz, CH_2), 4.78 (2H, s, NH_2), 6.14 (2H, s, CH_2), 6.23 (2H, s, H-2' and H-6'), 6.86 (1H, s, H-7); ^{13}C NMR ($CDCl_3$) (ppm): 14.4 (q), 25.5 (t), 25.6 (t), 30.4 (t), 48.8 (t), 55.7 (q x 2), 59.7 (t), 60.7 (q), 103.6 (d x 2), 118.7 (d), 121.6 (s), 124.9 (s), 126.1 (s), 132.6 (s), 135.9 (s), 136.3 (s), 139.3 (s), 152.8 (s x 2), 161.2 (s), 163.6 (s).

9.9.2 General Procedure of 2-(acetylamino)-Pyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazole (33)

To a solution of appropriate 2-amine-thiazole **31** (0.5 mmol) in dry dichloromethane (5 mL), at 0°C were added DIPEA (0.6 mmol) and acetyl chloride (0.55 mmol), and the reaction mixture was added for one night at room temperature (24 h). Water (3 mL) was added and the organic phase was separated. The acqueous phase was extracted with further dichloromethane (2 x 5 mL), and the organic phase, collect dried on Na_2SO_4 and evaporated at reduced pressure. The residue was purified by chromatography column (dichloromethane ethyl acetate 95:5).

***N*-[9-(phenylsulfonyl)-4,5,6,9-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazol-2-yl]acetamide (33b)** This compound was obtained from reaction of **32b** Yield: 73 %, white solid; mp: 222.6 – 223.4 °C; IR: 3408 (NH), 1713 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) (ppm): 1.83 (3H, s, CH_3), 2.04 – 2.10 (4H, m, CH_2 x 2), 2.32 – 2.36 (2H, m, CH_2), 6.27 (1H, s, H-7), 7.30 – 7.36 (3H, m, H-

3', H-4' and H-5'), 7.44 (1H, s, H-8), 7.71 (2H, d, *J* = 6.4 Hz, H-2' and H-6'), 11.95 (1H, s, NH); ^{13}C NMR ($CDCl_3$) (ppm): 21.8 (q), 22.4 (t), 23.6 (t), 34.1 (t), 113.2 (d), 123.1 (d), 125.5 (s), 127.4 (d x 2), 128.7 (d x 2), 130.1 (s), 131.1 (s), 133.6 (d), 136.6 (s), 137.9 (s), 155.8 (s), 168.8 (s).

Ethyl 2-(acetylamino)-9-methyl-4,5,6,9-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazole-8-carboxylate (33d) This compound was obtained from reaction of **32d** Yield: 51 %, pale yellow solid; mp: 193.8 – 194.2 °C; IR: 3399 (NH), 1701 (CO), 1597 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.35 (3H, t, *J* = 7.1 Hz, CH_3), 1.88 (3H, s, CH_3), 2.06 – 2.14 (2H, m, CH_2), 2.61 (2H, t, *J* = 6.3 Hz, CH_2), 2.87 (2H, t, *J* = 6.3 Hz, CH_2), 4.12 (3H, s, CH_3), 4.28 (2H, q, *J* = 7.1 Hz, CH_2), 6.83 (1H, s, H-7), 10.5 (1H, s, NH); ^{13}C NMR ($CDCl_3$) (ppm): 14.4 (q), 22.1 (q), 25.1 (t), 25.5 (t), 30.0 (t), 34.9 (q), 59.8 (t), 117.5 (d), 122.4 (s), 124.5 (s), 130.1 (s), 132.0 (s), 137.4 (s), 155.3 (s), 161.4 (s), 167.8 (s).

Ethyl 2-(acetylamino)-9-(4-methoxybenzyl)-4,5,6,9-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazole-8-carboxylate (33h) This compound was obtained from reaction of **32h** Yield: 65 %, yellow solid; mp: 87.6 – 88.4 °C; IR: 3403 (NH), 1696 (CO), 1557 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.28 (3H, t, *J* = 7.0 Hz, CH_3), 1.92 (3H, s, CH_3), 2.11 – 2.19 (2H, m, CH_2), 2.61 (2H, t, *J* = 6.3 Hz, CH_2), 2.82 (2H, t, *J* = 6.3 Hz, CH_2), 3.69 (3H, s, CH_3), 4.20 (2H, q, *J* = 7.0 Hz, CH_2), 6.00 (2H, s, CH_2), 6.63 (2H, d, *J* = 8.3 Hz, H-3' and H-5'), 6.77 (2H, d, *J* = 8.3 Hz, H-2' and H-6'), 6.91 (1H, s, H-7), 10.11 (1H, s, NH); ^{13}C NMR ($CDCl_3$) (ppm): 14.3 (q), 22.5 (q), 24.7 (t), 25.2 (t), 30.8 (t), 48.5 (t), 55.1 (q), 59.8 (t), 113.5 (d x 2), 118.6 (d), 122.0 (s), 125.0 (s), 127.2 (d x 2), 130.2 (s), 131.7 (s), 132.0 (s), 137.6 (s), 155.2 (s), 158.2 (s), 161.0 (s), 167.8 (s).

Ethyl 2-(acetylamino)-9-(3,5-dimethoxybenzyl)-4,5,6,9-tetrahydropyrrolo[3',2':6,7]cyclohepta [1,2-*d*][1,3]thiazole-8-carboxylate (33j) This compound was obtained from reaction of **32j** Yield: 72 %, brown solid; mp: 70.2 – 71.0 °C; IR: 3264 (NH), 1695 (CO), 1597 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.27 (3H, t, *J* = 7.1 Hz, CH_3), 1.94 (3H, s, CH_3), 2.10 – 2.25 (2H, m, CH_2), 2.62 (2H, t, *J* = 6.3 Hz, CH_2), 2.83 (2H, t, *J* = 6.3 Hz, CH_2), 3.57 (6H, s, CH_3 x 2), 4.18 (2H, q, *J* = 7.1 Hz, CH_2), 5.98 (2H, s, CH_2), 6.04 (2H, s, H-2' and H-6'), 6.17 (1H, s, H-4'), 6.93 (1H, s, H-7), 9.96 (1H, s, NH); ^{13}C NMR ($CDCl_3$) (ppm): 14.3 (q), 22.6 (q), 24.8 (t), 25.3 (t), 30.7 (t), 49.1 (t), 55.0 (q), 59.8 (t), 98.1 (d), 103.9 (d x 2), 118.8 (d), 122.2 (s), 124.9 (s), 130.1 (s), 132.0 (s), 137.8 (s), 142.4 (s), 154.9 (s), 160.6 (s x 2), 161.0 (s), 167.6 (s).

Ethyl 2-(acetylamino)-9-(2-bromo-3,5-dimethoxybenzyl)-4,5,6,9-tetrahydropyrrolo[3',2':6,7] cyclohepta [1,2-*d*][1,3]thiazole-8-carboxylate (33u) This compound was obtained from reaction of **32u** Yield: xx %, white solid; mp: 192.0 – 192.8 °C; IR: 3413 (NH), 1704 (CO), 1593 (CO) cm^{-1};

^{1}H NMR ($CDCl_3$) (ppm): 1.29 (3H, t, *J* = 7.0 Hz, CH_3), 1.94 – 2.12 (5H, m, CH_3, CH_2), 2.73 – 2.77 (2H, m, CH_2), 2.89 – 2.92 (2H, m, CH_2), 3.56 (3H, s, CH_3), 3.82 (3H, s, CH_3), 4.18 (2H, q, *J* = 7.0 Hz, CH_2), 5.44 (1H, s, H-4'), 6.14 (1H, s, CH_2), 6.25 (1H, s, H-6'), 6.95 (1H, s, H-7), 9.16 (1H, s, NH); ^{13}C NMR ($CDCl_3$) (ppm): 14.3 (q), 23.1 (q), 26.2 (t), 26.7 (t), 28.2 (t), 51.0 (t), 55.1 (q), 56.2 (q), 59.8 (t), 97.1 (d), 101.1 (s), 103.4 (d), 118.9 (d), 122.3 (s), 124.8 (s), 129.8 (s), 131.8 (s), 137.2 (s), 142.1 (s), 153.5 (s), 156.1 (s), 159.8 (s), 160.9 (s), 167.5 (s).

Ethyl 2-(acetylamino)-9-(3,4,5-trimethoxybenzyl)-4,5,6,9-tetrahydropyrrolo[3',2':6,7] cyclohepta [1,2-*d*][1,3]thiazole-8-carboxylate (33k) This compound was obtained from reaction of **32k** Yield: 39 %, pale brown solid; mp: 73.1 – 73.8 °C; IR: 3405 (NH), 1703 (CO), 1597 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.30 (3H, t, *J* = 7.1 Hz, CH_3), 2.00 (3H, s, CH_3), 2.13 – 2.17 (2H, m, CH_2), 2.61 – 2.65 (2H, m, CH_2), 2.80 – 2.85 (2H, m, CH_2), 3.61 (6H, s, CH_3 x 2), 3.73 (3H, s, CH_3), 4.23 (2H, q, *J* = 7.1 Hz, CH_2), 6.08 (2H, s, CH_2), 6.13 (2H, s, H-2' and H-6'), 6.91 (1H, s, H-7), 10.41 (1H, s, NH); ^{13}C NMR ($CDCl_3$) (ppm): 14.4 (q), 22.4 (q), 24.9 (t), 25.3 (t), 30.6 (t), 48.8 (t), 55.7 (q x 2), 59.9 (t), 60.7 (q), 103.2 (d x 2), 118.9 (d), 122.0 (s), 125.2 (s), 130.1 (s), 132.1 (s), 135.3 (s), 136.4 (s), 137.8 (s), 152.9 (s x 2), 155.3 (s), 161.2 (s), 168.0 (s).

9.9.3 Preparation of 7-bromo-4,5,6,7-tetrahydrocyclohepta[b]pyrrol-8(1H)-one (31c).

1-(Phenylcarbonyl)-4,5,6,7-tetrahydrocyclohepta[*b*]pyrrol-8(1*H*)-one (9w). To a solution of **9c** (6 mmol) in dry DMF (12 mL), NaH (6.6 mmol) was added at 0 °C and the reaction was stirred for one hour and half at room temperature. Benzoyl Chloride (9 mmol) was added at 0 °C, and the reaction mixture was stirred at room temperature for 3 h. Then the reaction was poured into ice and brine (40 mL), and, the aqueous solution was extracted with dichloromethane (3 x 40 mL). The organic phase was dried over Na_2SO_4 and the solvent evaporated at reduced pressure. The crude product was purified by chromatography column (dichloromethane). Yield: 82 %, colourless oil; IR: 1699 (CO) 1645 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) (ppm): 1.83 – 2.01 (4H, m, CH_2 x 2), 2.59 (2H, t, *J* = 6.1 Hz, CH_2), 2.81 – 2.90 (2H, m, CH_2), 6.13 (1H, d, *J* = 2.4 Hz, H-3), 7.17 (1H, d, *J* = 2.4 Hz, H-2), 7.40 – 7.73 (5H, m, Ar); ^{13}C NMR ($CDCl_3$) (ppm): 22.4 (t), 26.0 (t), 27.6 (t), 42.1 (t), 112.4 (d), 128.2 (d), 128.5 (d x 2), 129.6 (d x 2), 130.1 (s), 133.2 (d), 133.8 (s), 137.2 (s), 169.0 (s), 192.1 (s).

7-Bromo-1-(phenylcarbonyl)-4,5,6,7-tetrahydrocyclohepta[*b*]pyrrol-8(1*H*)-one (31w). To a solution of **9w** (5 mmol) in dry THF (10 mL), pyridine hydrobromide perbromide (5 mmol)

dissolved in dry THF (5 mL), was added and the reaction mixture was stirred at room temperature for 6 hours. The solid formed was filtered and eliminated, and the organic phases was concentrated under reduced pressure. The residue was dissolved in dichloromethane (10 mL), washed with a solution at 5% of $NaHCO_3$ (10 mL), dried on Na_2SO_4 and evaporated at reduced pressure. The residue was purified by chromatoghraphy column (dichloromethane). Yield: 60 %, yellow oil; IR: 1711 (CO) 1636 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) (ppm): 1.93 – 2.02 (2H, m, CH_2), 2.33 – 2.42 (2H, m, CH_2), 2.82 – 3.09 (2H, m, CH_2), 4.63 – 4.70 (1H, m, CH), 6.12 (1H, d, *J* = 2.6 Hz, H-3), 7.24 (1H, d, *J* = 2.6 Hz, H-2), 7.41 – 7.76 (5H, m, Ar); ^{13}C NMR ($CDCl_3$) (ppm): 22.8 (t), 28.5 (t), 32.3 (t), 55.3 (d), 112.5 (d), 128.6 (d), 129.8 (d x 2), 129.9 (d x 2), 130.1 (s), 133.0 (s), 133.4 (d), 136.3 (s), 168.6 (s), 184.6 (s).

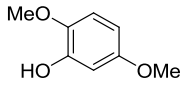
7-bromo-4,5,6,7-tetrahydrocyclohepta[*b*]pyrrol-8(1*H*)-one (31c). To a solution of **31w** (5 mmol) in ethanol (15 mL), sodium hydroxide (10 mmol) was added and the reaction mixture was stirred at room temperature for 4 hours. The solution was concentrated, cooled at 0°C and acidified using HCl 6 M. The acqueous phase was extracted with dichloromethane (3 x 50 mL) and the organic phase was collect, dried on Na_2SO_4 and evaporated at reduced pressure. The residue was purified by chromatoghraphy column (dichloromethane). Yield: 72 %, pale brown solid, mp: 99.9 – 100.5 ; IR: 3425 (NH), 1622 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) (ppm): 1.94 – 2.03 (2H, m, CH_2), 2.33 – 2.39 (2H, m, CH_2), 2.85 – 3.08 (2H, m, CH_2), 4.83 – 4.90 (1H, m, CH), 6.10 (1H, d, *J* = 2.4 Hz, H-3), 7.03 (1H, d, *J* = 2.4 Hz, H-2), 9.45 (1H, bs, NH); ^{13}C NMR ($CDCl_3$) (ppm): 23.3 (t), 28.5 (t), 32.8 (t), 55.0 (d), 112.2 (d), 126.3 (d), 127.0 (s), 133.0 (s), 185.2 (s).

9.10 Total synthesis of Toxyloxanthone B and toward the synthesis of Rubraxanthone

All the IR spectra were determined in chloroform. ^1H and ^{13}C NMR were registered at 400 MHz and 100 MHz using a Bruker spectrometer (TMS as internal standard).

9.10.1 First Approach Fragment A

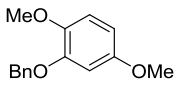
2,5-dimethoxyphenol (35)



To a solution of 2,5-dimethoxybenzaldehyde (**34)** (6 g, 36.1 mmol) in dichloromethane (250 mL),

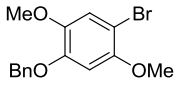
was added at 0°C in small portions *m*CPBA (10.6 g, 61.4 mmol), and the reaction mixture was stirred at room temperature for 18 h. The organic solution was washed with aqueous satured sodium bicarbonate solution (200 mL x 2), and then with aqueous satured sodium thiosulfate solution (200 mL). The organic layer was dried on $MgSO_4$, filtered and concentrated *in vacuo* to give a dark oil that was dissolved in methanol (90 mL), and stirred with aqueous NaOH (4M, 18 mL), for 3h. The reaction mixture was acidified with HCl and extracted with ethyl acetate (100 mL x 3). The organic layer was dried on $MgSO_4$, filtered and concentrated in vacuo to give an oil that was purified by flash chromatography column (6:4 dichlorometane, light petroleum). Yield 77%, clear oil; (Found: [M + H^+], 155.0699. $C_8H_{11}O_3^+$ requires 155.0703); ν_{max} ($CHCl_3$)/cm^{-1}: 3538, 3008, 2958, 2938, 2908, 2838, 2062, 1627, 1599, 1504, 1466, 1336, 1284; δ_H (400 MHz, $CDCl_3$): 6.79 (1 H, d, *J* 8.8, H-3), 6.59 (1 H, d, *J* 2.8, H-6), 6.40 (1 H dd, *J* 8.8, 2.8, H-4), 5.65 (1 H, s, OH), 3.87 (3 H, s, OCH_3), 3.77 (3 H, s, OCH_3); δ_C (100 MHz, $CDCl_3$): 154.5 (C), 146.4 (C), 140.9 (C), 111.4 (CH), 104.2 (CH), 101.7 (CH), 56.6 (Me), 55.6 (Me).

2-(benzyloxy)-1,4-dimethoxybenzene (36)



Potassium carbonate (5.4 g, 39 mmol) and Benzyl bromide (3.1 mL, 26 mmol), were added to a stirred solution of starting material **35** (4 g, 26 mmol) in acetonitrile (120 mL), and the mixture was refluxed for 1hour and 30 minutes. After cooling and filtration of the potassium carbonate, the solvent was removed in vacuo and the residue was dissolved in dichloromethane (50 mL), so washed with water (50 mL x 2), dried on $MgSO_4$, concentrated *in vacuo* and purified by flash chromatography column (9:1 light petroleum, ethyl acetate). Yield 99%, white solid, mp 38 °C; (Found: [M + Na^+], 267.0990. $C_{15}H_{16}NaO_3^+$ requires 267.0992); ν_{max} ($CHCl_3$)/cm^{-1}: 3010, 2937, 2910, 2836, 1597, 1511, 1465, 1429, 1381, 1259, 1239, 1183, 1160, 1136; δ_H (400 MHz, $CDCl_3$): 7.46 – 7.29 (5 H, m, C_6H_5), 6.83 (1 H, d, *J* 8.8, H-3), 6.56 (1 H, d, *J* 2.8, H-5), 6.43 (1 H, dd, *J* 8.8, 2.8, H-4), 5.16 (2 H, s, CH_2), 3.87 (3 H, s, OCH_3), 3.75 (3 H, s, OCH_3); δ_C (100 MHz, $CDCl_3$): 154.2 (C), 149.1 (C), 144.1 (C), 137.0 (C), 128.6 (CH x 2), 127.9 (CH), 127.3 (CH x 2), 112.8 (CH), 103.9 (CH), 102.7 (CH), 71.0 (CH_2), 56.8 (Me), 55.6 (Me).

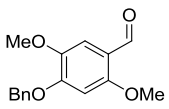
1-(benzyloxy)-4-bromo-2,5-dimethoxybenzene (37)



NBS (882 mg, 4.95 mmol), was added to a stirred solution of starting material **36** (1.10 g, 4.50

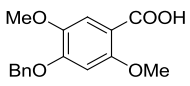
mmol) in dichloromethane (60 mL), and the solution was stirred at room temperature for 18 hours. The reaction was quenched by the addition of satured aqueous sodium bicarbonate solution (30 mL), and diluted with water (150 mL). The product was extracted with dichlorometane (80 mL x 2), the organic extracts were combined, dried on $MgSO_4$, and the solvent removed in *vacuo* to give the crude product as a brown solid that was purified by flash column chromatography (9:1 light petroleum, ethyl acetate). Yield 84%, beige solid, mp 88 °C; (Found: [M + Na^+], 345.0091. $C_{15}H_{15}{}^{79}BrNaO_3{}^+$ requires 345.0097); ν_{max} ($CHCl_3$)/cm^{-1}: 3011, 2937, 1781, 1754, 1722, 1585, 1505, 1464, 1443, 1388, 1362, 1344, 1192, 1160; δ_H (400 MHz, $CDCl_3$): 7.46 - 7.34 (5 H, m, C_6H_5), 7.09 (1 H, s, CH), 6.58 (1 H, s, CH), 5.17 (2 H, s, CH_2), 3.86 (3 H, s, OCH_3), 3.77 (3 H, s, OCH_3); δ_C (100 MHz, $CDCl_3$): 150.1 (C), 148.1 (C), 144.6 (C), 136.7 (C), 128.6 (CH x 2), 128.1 (CH), 127.4 (CH x 2), 117.2 (CH), 107.2 (C), 101.9 (CH), 71.8 (CH_2), 57.0 (Me), 56.9 (Me).

4-(benzyloxy)-2,5-dimethoxybenzaldehyde (38)



Phosphorus oxychloride (1 mL, 11,3 mmol) was added dropwise over 10 minutes, to a stirred solution of starting material **36** (2.5 g, 10.2 mmol) in DMF (10 mL), at 0°C. The solution was left to warm to room temperature and stirred overnight. When complete, aqueous NaOH (1M, 41 ml) was added to the mixture and a solid was formed. The sospension was filtered and the solid washed with water, and than purified by flash cromatography column (8:2 light etroleum, ethyl acetate). Yield 84%, yellow solid, mp 133 °C, (Found: [M + Na^+], 295.0938. $C_{16}H_{16}NaO_4{}^+$ requires 295.0941); ν_{max} ($CHCl_3$)/cm^{-1}: 3008, 2939, 2870, 2836, 1667, 1606, 1511, 1468, 1453, 1414, 1349, 1278, 1177, 1125; δ_H (400 MHz, $CDCl_3$): 10.31 (1 H, s, CHO), 7.48 – 7.40 (5 H, m, C_6H_5), 7.37 (1 H, s, H-2), 6.53 (1 H, s, H-3), 5.27 (2 H, s, CH_2), 3.91 (3 H, s, OCH_3), 3.82 (3 H, s, OCH_3); δ_C (100 MHz, $CDCl_3$): 188.0 (CHO), 158.3 (C), 154.8 (C), 144.0 (C), 135.9 (C), 128.8 (CH x 2), 128.3 (CH), 127.2 (CH x 2), 117.6 (C), 109.5 (CH), 98.3 (CH), 71.1(CH_2), 56.3 (Me), 56.1 (Me).

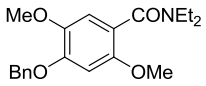
4-(benzyloxy)-2,5-dimethoxybenzoic acid (39)



To a solution of starting material **38** (2 g, 7.3 mmol) in a mixture of acetone (79 mL) and DMSO (32 mL), was added an aqueous solution (24 mL) of sulfamic acid (1.2 g, 12.5 mmol). The solution was cooled at 0°C and an aqueous solution (55 mL) of sodium chlorite (1.3 g, 14.7 mmol) was

added slowly. The reaction was stirred at 0°C for 30 minutes and then at room temperature for one night. Water (150 mL) was added and the solution was extracted with ethyl acetate (200 mL x 3). The organic layer was dried on $MgSO_4$, filtered and concentrated *in vacuo*, to give an oil that was purified by flash cromatography column (8:2 dichloromethane, ethyl acetate). Yield 98 %, colourless solid, mp 135 °C; (Found: [M + H^+], 289.1065. $C_{16}H_{17}O_5^+$ requires 289.1071); ν_{max} ($CHCl_3$)/cm^{-1}: 3297, 3011, 2943, 2840, 1726, 1611, 1513, 1467, 1452, 1418, 1378, 1313, 1279, 1239, 1177, 1167; δ_H (400 MHz, $CDCl_3$): 12.4 (1 H, s, OH), 7.67 (1 H, s, H-6), 7.47 – 7.40 (5 H, m, C_6H_5), 6.59 (1 H, s, H-3), 5.27 (2 H, s, CH_2), 3.95 (3 H, s, OCH_3), 3.93 (3 H, s, OCH_3); δ_C (100 MHz, $CDCl_3$): 165.2 (C), 153.3 (C), 153.2 (C), 144.6 (C), 135.8 (C), 128.8 (CH x 2), 128.4 (CH), 127.2 (CH x 2), 114.9 (CH), 109.3 (C), 98.7 (CH), 71.4 (CH_2), 57.2 (Me), 56.4 (Me).

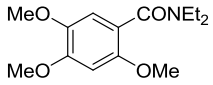
4-(benzyloxy)-N,N-diethyl-2,5-dimethoxybenzamide (40)



To 7 mL of thionyl chloride at 0°C was added starting material **39** (2 g, 7 mmol) and the resulting solution was stirred at reflux for 1 hour. Excess of thionyl chloride was removed by distillation (using several toluene co-evaporations to ensure the complete removal off all thionyl chloride) to give a white solid which was taken up immediately onto next step.

Diethylamine (1.45 mL, 13.9 mmol) was added to a solution of the above product in toluene (50 mL) at 0°C, and the reaction was stirred for one night at room temperature. Upon complete the reaction was washed with diluite HCl (25 mL) and brine, than dried on $MgSO_4$, and concentrated in vacuo to give a brown oil that was purified by flash cromatography column (9:1 light petroleum, ethyl acetate). Yield 76 %, colourless solid, mp 86 °C; (Found: [M + H^+], 344.1856. $C_{20}H_{26}NO_4^+$ requires 344.1856); ν_{max} ($CHCl_3$)/cm^{-1}: 3004, 2937, 1612, 1510,1478, 1463, 1437, 1395, 1381, 1364, 1314, 1280, 1192, 1147; δ_H (400 MHz, DMSO): 7.49 – 7.35 (5 H, m, C_6H_5), 6.84 (1 H, s, CH), 6.73 (1 H, s, CH), 5.14 (2 H, s, CH_2), 3.73 (3 H, s, OCH_3), 3.71 (3 H, s, OCH_3), 3.46 – 3.38 (2H, m, CH_2), 3.09 (2 H, q, *J* 6.8, CH_2), 1.12 (3 H, t, *J* 7.2, CH_3), 0.98 (3 H, t, *J* 6.8, CH_3); δ_C (100 MHz, DMSO): 1675 (C), 149.5 (C), 149.1 (C), 143.4 (C), 137.4 (C), 128.9 (CH x 2), 128.5 (CH), 128.4 (CH x 2), 118.7 (C), 112.2 (CH), 100.3 (CH), 70.7 (CH_2), 56.8 (Me), 56.7 (Me), 42.7 (CH_2), 38.7 (CH_2), 14.4 (Me), 13.4 (Me).

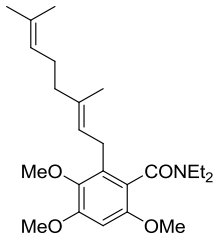
N,N-diethyl-2,4,5-trimethoxybenzamide (43)



To 4 mL of thionyl chloride at 0°C was added 2,4,5-trimethoxybenzoic-acid **(42)** (1 g, 4.7 mmol) and the risultantig solution was stirred at reflux for 1 hour. Excess of thionyl chloride was removed by distillation (using several toluene co-evaporations to ensure the complete removal off all thionyl chloride) to give a white solid which was taken up immediately onto next step.

Diethylamine (0.98 mL, 9.4 mmol) was added to a solution of the above product in toluene (50 mL) at 0°C, and the reaction was stirred for one night at room temperature. Upon complete the reaction was washed with diluted HCl (25 mL) and brine, than dried on $MgSO_4$, and concentrated in vacuo to give a brown oil that was purified by flash cromatography column (8:2 light petroleum, ethyl acetate). Yield 74 %, colourless solid, mp 82 °C, (Found: $[M + H^+]$, 268.1535. $C_{14}H_{22}NO_4^+$ requires 268.1543); ν_{max} ($CHCl_3$)/cm^{-1}: 3003, 2938, 2847, 1612, 1513, 1479, 1463, 1437, 1390, 1364, 1337, 1281, 1150, 1084; δ_H (400 MHz, DMSO): 6.72 (1 H, s, CH), 6.71 (1 H, s, CH), 3.82 (3 H, s, OCH_3), 3.76 (3 H, s, OCH_3), 3.70 (3 H, s, OCH_3), 3.41 (2 H, broad, CH_2), 3.09 (2 H, q, *J* 7.2, CH_2), 1.12 (3 H, t, *J* 7.2, CH_3), 0.98 (3H, t, *J* 7.2, CH_3); δ_C (100 MHz, DMSO): 167.6 (C), 150.2 (C), 149.6 (C), 143.1 (C), 118.2 (C), 112.0 (CH), 98.7 (CH), 56.8 (Me), 56.7 (Me), 56.2 (Me), 42.7 (CH_2), 38.7 (CH_2), 14.4 (Me), 13.3 (Me).

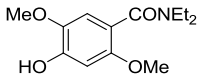
(E)-2-(3,7-dimethylocta-2,6-dienyl)-N,N-diethyl-3,4,6-trimethoxybenzamide (44).



To a solution of starting material **43** (500 mg, 1.87 mmol) in dry THF (15 mL) at -78°C, was added TMEDA (0.42 mL, 2.80 mmol), *sec*BuLi (1M in THF, 2.80 mL), Copper(I)iodide (533 mg, 2.80 mmol) and geranyl bromide (0.55 mL, 2.80 mmol), and the resultant mixture was left to warm to room temperature and stirred overnight (16h). The reaction was quenched by the addition of saturated aqueous ammonium chloride solution (15 mL), and ethyl acetate (15 mL). The aqueous layer was extracted always with ethyl acetate (2 x 30 mL), and then the organic layer was collect, dried and concentrated *in vacuo*, to give the crude product as a brown oil, that was purified by flash column chromatography (9:1 light petroleum, ethyl acetate). Yellow oil, Yield 72%, (Found: [M +

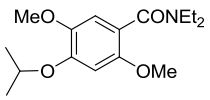
H^+], 404.2796. $C_{24}H_{37}NO_4^+$ requires 404.2723); ν_{max} ($CHCl_3$)/cm^{-1}: 2999, 2937, 2360, 1613, 1461, 1430, 1380, 1364, 1333, 1281, 1241, 1144, 1090; δ_H (400 MHz, $CDCl_3$): 6.40 (1 H, s, CH), 5.19 (1 H, t, *J* 6.4, CH), 5.08 (1 H, t, *J* 6.9, CH), 3.90 (3 H, s, OCH_3), 3.80 (3 H, s, OCH_3), 3.76 (3 H, s, OCH_3), 3.74 (1 H, dq, *J* 14.0, 7.2, CH_2 Et), 3.46 (1 H, dq, *J* 14.6, 7.0, CH_2 Et), 3.40 (1 H, dd, *J* 12.8, 6.9, CH_2), 3.23 (1 H, dd, *J* 12.8, 6.9, CH_2), 3.18 (1 H, dq, *J* 14.6, 7.0, CH_2 Et), 3.06 (1 H, dq, *J* 14.0, 7.2, CH_2 Et), 2.05 (2 H, q, *J* 8.4, CH_2), 1.97 (2 H, t, *J* 8.4, CH_2), 1.72 (3 H, s, CH_3), 1.67 (3 H, s, CH_3), 1.59 (3 H, s, CH_3), 1.24 (3 H, t, *J* 7.2, CH_3), 1.05 (3 H, t, *J* 7.2, CH_3); δ_C (100 MHz, $CDCl_3$): 167.8 (C), 153.3 (C), 151.7 (C), 141.5 (C), 135.0 (C), 133.4 (C), 131.3 (C), 124.4 (CH), 122.7 (CH), 119.0 (C), 95.1 (CH), 60.6 (Me), 55.9 (Me x 2), 42.9 (CH_2), 39.8 (CH_2), 38.5 (CH_2), 26.7 (CH_2), 26.6 (CH_2), 25.6 (Me), 17.6 (Me), 16.2 (Me), 13.6 (Me), 12.7 (Me).

N,N-diethyl-4-hydroxy-2,5-dimethoxybenzamide (45)



Starting material **(40)** (3 g, 8.7 mmol) and Palladium on carbon (10% w/w 300 mg), were suspended in a mixture of ethyl acetate (50 mL) and ethanol (50 mL). An H_2 atmosphere was created and the reaction was left to stirred for 15 hours at room temperature. Upon complete the suspension was filtered on a septum of celite than concentrated *in vacuo* to give the right product that didn't need further purification. Yield 97% clear oil; (Found: [M + H^+], 254.1380. $C_{13}H_{20}NO_4^+$ requires 254.1387); ν_{max} ($CHCl_3$)/cm^{-1}: 3535, 3000, 2939, 2844, 2455, 1726, 1608, 1513, 1479, 1435, 1350, 1303, 1192, 1170; δ_H (400 MHz, DMSO): 9.26 (1 H, s, OH), 6.68 (1 H, s, CH), 6.52 (1 H, s, CH), 3.72 (3 H, s, OCH_3), 3.67 (3 H, s, OCH_3), 3.43 – 3.36 (2 H, m, CH_2), 3.09 (2 H, q, *J* 7.2, CH_2), 1.12 (3 H, t, *J* 7.2, CH_3), 0.98 (3 H, t, *J* 7.2, CH_3); δ_C (100 MHz, DMSO): 167.8 (C) 149.8 (C), 148.2 (C), 141.8 (C), 117.1 (C), 112.65 (CH), 101.0 (CH), 57.0 (Me), 56.2 (Me), 42.8 (CH_2), 38.7 (CH_2), 14.5 (Me),14.4 (Me).

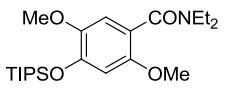
N,N-diethyl-2,5-dimethoxy-4-(propan-2-yloxy)benzamide (48)



2-Bromopropane (0.65 mL, 6.91 mmol) was added to a stirred solution of starting material **45** (500 mg, 1.97 mmol) and potassium carbonate (545 mg, 3.95 mmol) in DMF (8 mL), cooled at 0°C, and the resulting mixture was stirred at room temperature for 48 h. Upon complete the reaction was diluted with diethyl ether (15 mL) and washed with dilute HCl (20 mL), than extracted with ethyl

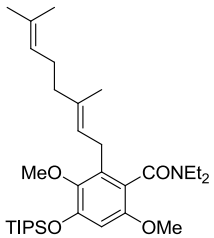
acetate (3 x 20 mL), dried on $MgSO_4$, and concentrated *in vacuo* to give a brown oil that was purified by flash cromatography column (9:1 light petrol, ethyl acetate). Yield 60%, brown oil; (Found: $[M + H^+]$, 296.1851. $C_{16}H_{26}NO_4^+$ requires 296.1856); ν_{max} ($CHCl_3$)/cm^{-1}: 2981, 2936, 2851, 1612, 1509, 1477, 1464, 1439, 1393, 1317, 1280, 1192, 1173, 1150, 1110; δ_H (400 MHz, $CDCl_3$): 6.74 (1 H, s, CH), 6.52 (1 H, s, CH), 4.52 (1 H, septuplet, *J* 6.0, CH), 3.79 (3 H, s, OCH_3), 3.75 (3 H, s, OCH_3), 3.54 (2 H, broad, CH_2), 3.17 (2 H, q, *J* 7.2, CH_2), 1.35 (6 H, d, *J* 6.0, CH_3 x 2), 1.22 (3H, t, *J* 7.2, CH_3), 1.03 (3H, t, *J* 7.2, CH_3); δ_C (100 MHz, $CDCl_3$): 168.5 (C), 149.5 (C), 148.4 (C), 144.8 (C), 119.1 (C), 112.2 (CH), 102.3 (CH), 72.1 (CH), 56.6 (Me), 56.5 (Me), 42.9 (CH_2), 38.9 (CH_2), 22.1 (Me x 2), 14.0 (Me), 12.9 (Me).

N,N-diethyl-2,5-dimethoxy-4-(triisopropylsilyloxy)benzamide (46)



Chlorotriisopropylsilane (1.27 mL, 5.93 mmol) was added to a stirred solution of starting material **(45)** (1.5 g, 5.93 mmol) and imidazole (815 mg, 11.8 mmol) in DMF (15 mL), and the resulting mixture was stirred at room temperature for 15 hours. The reaction was diluted with diethyl ether (20 mL), washed with water and brine (20 mL x 3), and then dried on $MgSO_4$, and concentrated in *vacuo* to give the crude product as clear oil, that didn't need further purification. Yield 96%, Clear oil; (Found: $[M + H^+]$, 410.2728. $C_{22}H_{40}NO_4Si^+$ requires 410.2721); ν_{max} ($CHCl_3$)/cm^{-1}: 2945, 2868, 1610, 1512, 1464, 1446, 1394, 1365, 1332, 1279, 1251, 1174, 1154; δ_H (400 MHz, DMSO): 6.75 (1 H, s, CH), 6.52 (1 H, s, CH), 3.71 (3 H, s, OCH_3), 3.68 (3 H, s, OCH_3), 3.41 (2 H, broad, CH_2), 3.08 (2 H, q, *J* 7.2, CH_2), 1.30 – 1.20 (3 H, m, CH x 3), 1.12 (3 H, t, *J* 7.2, CH_3), 1.07 (18 H, d, *J* 7.2, CH_3 x 6), 0.97 (3 H, t, *J* 7.2, CH_3); δ_C (100 MHz, DMSO): 167.5 (C), 149.3 (C), 145.9 (C), 144.8 (C), 119.7 (C), 112.2 (CH), 105.4 (CH), 56.5 (Me), 56.4 (Me), 42.7 (CH_2), 38.8 (CH_2), 18.2 (Me x 6), 14.4 (Me), 13.4 (Me), 12.5 (CH x 3).

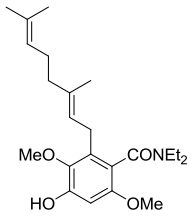
(E)-2-(3,7-dimethylocta-2,6-dienyl)-N,N-diethyl-3,6-dimethoxy-4-(triisopropylsilyloxy)benzamide (47).



To a solution of starting material **(46)** (1g, 2.45 mmol) in THF (30 mL) at -78°C, was added

TMEDA (0.55 mL, 3.67 mmol), *sec*BuLi (1M in THF, 3.67 mL), Copper(I)iodide (697 mg, 3.67 mmol) and geranyl bromide (0.73 mL, 3.67 mmol), and the resultanting mixture was left to warm to room temperature and stirred overnight (16h). The reaction was quenched by the addition of saturated aqueous ammonium chloride solution (30 mL), and ethyl acetate (30 mL). The aqueous layer was extracted always with ethyl acetate (2 x 50 mL), and then the organic layer was collect, dried and concentrated *in vacuo*, to give the crude product as a brown oil, that was purified by flash column chromatography (9:1 light petroleum, ethyl acetate). Yellow oil, Yield 58% (Found: [M + H^+], 546.3980. $C_{32}H_{56}NO_4Si^+$ requires 546.3973); ν_{max} ($CHCl_3$)/cm^{-1}: 2944, 2868, 2461, 1613, 1463, 1444, 1405, 1381, 1346, 1313, 1284, 1241, 1148; δ_H (400 MHz, DMSO): 6.36 (1 H, s, CH), 5.04 (1 H, t, *J* 6.8, CH), 5.02 (1 H, t, *J* 7.5, CH), 3.66 (3 H, s, OCH_3), 3.65 (3 H, s, OCH_3), 3.53 (1 H, dq, *J* 13.6, 7.0, CH_2 Et), 3.26 (1 H, dq, *J* 13.6, 7.0, CH_2 Et), 3.23 (1 H, dd, *J* 14.4, 7.5, CH_2), 3.04 (1 H, dd, *J* 14.4, 7.5, CH_2), 3.00 (2 H, q, *J* 7.2, CH_2), 1.99 (2 H, q, *J* 6.8, CH_2), 1.90 (2 H, t, *J* 6.8, CH_2), 1.62 (3 H, s, CH_3), 1.60 (3 H, s, CH_3), 1.53 (3 H, s, CH_3), 1.30 (3 H, septuplet, *J* 7.5, SiCH x 3), 1.10 – 1.06 (21 H, m, CH_3 x 6, CH_3(Et)), 0.94 (3 H, t, *J* 7.2, CH_3); δ_C (100 MHz, DMSO): 166.7 (C), 151.4 (C), 149.3 (C), 143.2 (C), 134.6 (C), 133.2 (C), 131.1 (C), 124.5 (CH), 123.0 (CH), 120.0 (C), 102.5 (CH), 60.6 (Me), 55.9 (Me), 42.7 (CH_2), 39.6 (CH_2), 38.5 (CH_2), 26.7 (CH_2), 26.4 (CH_2), 25.9 (Me), 18.3 (Me x 6), 17.9 (Me), 16.4 (Me), 13.9 (Me), 13.0 (Me), 12.7 (CH x 3).

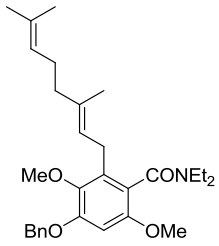
(E)-2-(3,7-dimethylocta-2,6-dienyl)-N,N-diethyl-3,6-dimethoxy-4-hydroxybenzamide (52).



To a solution of starting material **(47)** (910 mg, 1.7 mmol) in THF (20 mL), was added TBAF (1M in THF, 2.5 mL), and the solution was stirred at room temperature for one night (15h). The resulting solution was diluted with ether (100 mL), washed with water and brine (100 mL x 2), dried and concentrated *in vacuo* to give the crude product as a brown oil, that was purified by flash column cromatography (8 : 2 light petroleum; ethyl acetate). Clear oil, Yield 95% (Found: [M + H^+], 390.2640. $C_{23}H_{36}NO_4^+$ requires 390.2639); ν_{max} ($CHCl_3$)/cm^{-1}: 3011, 1602, 1521, 1473, 1430, 1282, 1241, 1164, 1051; δ_H (400 MHz, $CDCl_3$): 6.44 (1 H, s, CH), 5.88 (1 H, broad, OH), 5.20 (1 H, t, *J* 6.0, CH), 5.08 (1 H, t, *J* 6.4, CH), 3.76 (3 H, s, OCH_3), 3.74 – 3.70 (4 H, m, OCH_3, CH_2 Et), 3.45 (1 H, dq, *J* 14.6, 7.0, CH_2 Et), 3.41 (1 H, dd, *J* 12.8, 6.9, CH_2), 3.26 (1 H, dd, *J* 12.8, 6.9, CH_2), 3.20 (1 H, dq, *J* 14.6, 7.0, CH_2 Et), 3.07 (1 H, dq, *J* 14.0, 7.2, CH_2 Et), 2.06 (2 H, t, *J* 7.6, CH_2), 1.99 (2 H, t, *J* 7.6, CH_2), 1.72 (3 H, s, CH_3), 1.67 (3 H, s, CH_3), 1.59 (3 H, s, CH_3), 1.23 (3 H, t, *J* 7.2, CH_3),

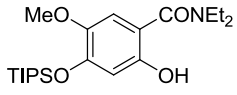
1.06 (3 H, t, *J* 7.2, CH_3); δ_C (100 MHz, $CDCl_3$): 167.7 (C), 152.4 (C), 149.7 (C), 139.3 (C), 135.5 (C), 132.7 (C), 131.4 (C), 124.4 (CH), 122.4 (CH), 118.8 (C), 97.3 (CH), 61.4 (Me), 55.6 (Me), 42.9 (CH_2), 39.7 (CH_2), 38.4 (CH_2), 26.6 (CH_2), 26.5 (CH_2), 25.7 (Me), 17.6 (Me), 16.2 (Me), 13.6 (Me), 12.7 (Me).

(E)-2-(3,7-dimethylocta-2,6-dienyl)-N,N-diethyl-3,6-dimethoxy-4-benzyloxybenzamide (41).



Benzyl bromide (0.23 mL, 1.98 mmol), was added to a stirred solution of starting material **(52)** (700 mg, 1.8 mmol) and Cesium carbonate (1.18 g, 3.6 mmol) in acetonitrile (30 mL), and the resultanting mixture was stirred at room temperature for two hours. The reaction was diluted with ether (150 mL), and washed with water and brine (120 mL). The organic phase was dried on $MgSO_4$, and removed *in vacuo*, to give the crude benzyl-ether product which was purified by flash column cromatography (9 : 1 light petroleum; ethyl acetate). Clear oil, Yield 89% (Found: [M + H^+], 480.3115. $C_{30}H_{42}NO_4^+$ requires 480.3108); ν_{max} ($CHCl_3$)/cm^{-1}: 3091, 2995, 2936, 1614, 1463, 1444, 1431, 1409, 1381, 1332, 1281, 1241, 1144, 1089; δ_H (400 MHz, $CDCl_3$): 7.50 – 7.35 (5 H, m, Ar), 6.45 (1 H, s, CH), 5.20 (1 H, t, *J* 6.2, CH), 5.15 (2 H, d, *J* 4.9, CH_2), 5.09 (1 H, t, *J* 7.0, CH), 3.81 (3 H, s, OCH_3), 3.73 - 3.70 (4H, m, OCH_3, CH_2 Et), 3.45 (1 H, dq, *J* 14.2, 7.2, CH_2 Et), 3.42 (1 H, dd, *J* 14.9, 7.0, CH_2), 3.26 (1 H, dd, *J* 14.9, 7.0, CH_2), 3.18 (1 H, dq, *J* 14.2, 7.2, CH_2 Et), 3.08 (1 H, dq, *J* 14.2, 6.3, CH_2 Et), 2.06 (2 H, t, *J* 8.0, CH_2), 1.98 (2 H, t, *J* 8.0, CH_2), 1.74 (3 H, s, CH_3), 1.67 (3 H, s, CH_3), 1.60 (3 H, s, CH_3), 1.24 (3 H, t, *J* 7.2, CH_3), 1.05 (3H, t, *J* 7.2, CH_3); δ_C (100 MHz, $CDCl_3$): 167.7 (C), 152.2 (C), 151.5 (C), 142.1 (C), 136.9 (C), 135.0 (C), 133.5 (C), 131.3 (C), 128.6 (CH x 2), 128.0 (CH), 127.3 (CH x 2), 124.4 (CH), 122.7 (CH), 119.6 (C), 97.2 (CH), 71.0 (CH_2), 60.7 (Me), 55.8 (Me), 42.9 (CH_2), 39.8 (CH_2), 38.5 (CH_2), 26.9 (CH_2), 26.7 (CH_2), 25.7 (Me), 17.6 (Me), 16.2 (Me), 13.6 (Me), 12.7 (Me).

N,N-diethyl-2-hydroxy-4-((Triisopropylsilyl)oxy)-5-methoxybenzamide (55).

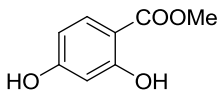


To a solution of starting material **46** (350 mg, 0.85 mmol) in dichloromethane (15 mL), was added

at 0°C boron trichloride (1M solution in hexane, 1.71 mL) and the reaction was left to stirrer at room temperature for 1h. Ice was added, and the reaction was extracted with dichlorometane (2 x 20 mL). The organic phase was dried on $MgSO_4$, and removed *in vacuo*, to give the crude unprotected phenol that didn't need further purification. Yield 94%, beige solid, mp 92 °C; (Found: $[M + H^+]$, 396.2559. $C_{21}H_{38}NO_4Si^+$ requires 396.2565); ν_{max} $(CHCl_3)/cm^{-1}$: 3216, 2946, 2868, 1628, 1587, 1508, 1478, 1464, 1438, 1384, 1350, 1329, 1263, 1242, 1159, 1099; δ_H (400 MHz, $CDCl_3$): 6.80 (1 H, s, CH), 6.55 (1 H, s, CH), 3.77 (3 H, s, OCH_3), 3.53 (4 H, q, *J* 7.2, CH_2 x 2), 1.33 – 1.28 (9 H, m, CH_3 x 2, CH x 3), 1.12 (18 H, d, *J* 7.2, CH_3 x 6); δ_C (100 MHz, $CDCl_3$): 171.9 (C), 155.0 (C), 150.0 (C), 143.3 (C), 112.0 (CH), 109.7 (CH), 109.3 (C), 56.7 (Me), 42.2 (CH_2 x 2), 17.8 (Me x 6), 13.4 (Me x 2), 12.8 (CH x 3).

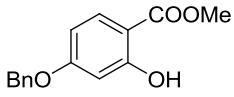
9.10.2 Second Approach Fragment A

methyl 2,4-dihydroxybenzoate (58).



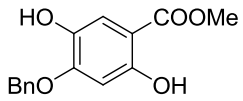
To a solution of 2-3-dihydroxybenzoic acid **57** (4g, 26 mmol) in DMF (39 mL), potassium hydrogen carbonate (3.12g, 31.2 mmol) was added and the solution stirred for several minutes at room temperature. Then, methyl iodide (2.45 mL, 39 mmol) was added, and the reaction mixture was warmed to 40°C and stirred for 2 hours. Water (130 mL) was added and the resulting mixture was extracted with ethyl acetate (4 x 120 mL). The organic layer was subsequently washed with 5 % sodium bicarbonate solution and 5 % brine, and dried over $MgSO_4$, filtered and concentrated under reduced pressure to give the crude product that was purified by flash column chromatography (8 : 2 light petroleum; ethyl acetate). Yield 97%, colourless solid, mp 119 °C; (Found: $[M + H^+]$, 169.0496. $C_{15}H_{14}NaO_4^+$ requires 169.0495); ν_{max} $(CHCl_3)/cm^{-1}$: 3585, 3011, 2956, 1671, 1625, 1600, 1511, 1442, 1346, 1272, 1186, 1143, 1096; δ_H (400 MHz, DMSO): 10.72 (1 H, s, OH), 10.46 (1 H, s, OH), 7.65 (1 H, d, *J* 8.8, CH), 6.39 (1 H, dd, *J* 8.8, 2.3, CH), 6.31 (1 H, d, *J* 2.3, CH), 3.85 (3 H, s, OCH_3); δ_C (100 MHz, DMSO): 170.0 (C), 164.7 (C), 163.1 (C), 132.0 (CH), 108.8 (CH), 104.4 (C), 102.9 (CH), 52.5 (Me).

methyl 4-(benzyloxy)-2-hydroxybenzoate (59).



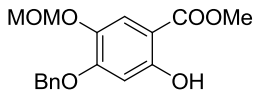
A mixture of **58** (4g, 23.8 mmol) and anhydrous potassium carbonate (4.9g 35.7 mmol) in acetone (22 mL), was stirred at room temperature for 5 minutes. Benzyl bromide (2.83 mL, 23.8 mmol), was added dropwise into the reaction flask and the mixture was stirred at 10-15°C for 3 hours. Water (30 mL) and ethyl acetate (30 mL) were added and two phases were separated. The aqueous phase was extracted with further ethyl acetate (3 x 40 mL). The combined organic layers were washed with brine (40 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure to give the crude product, which was purified by flash column chromatography (9 : 1 light petroleum; ethyl acetate). Yield 82% colourless solid, mp 104 °C, (Found: [M + Na^+], 281.0781. $C_{15}H_{14}NaO_4^+$ requires 281.0784); ν_{max} ($CHCl_3$)/cm^{-1}: 3008, 2956, 1668, 1623, 1583, 1505, 1441, 1382, 1349, 1255, 1183, 1142, 1099, 1014; δ_H (400 MHz, DMSO): 10.78 (1 H, s, OH); 7.74 (1 H, d, *J* 7.2, CH), 7.47 – 7.36 (5 H, m, Ar), 6.64 – 6.61 (2 H, m, CH x 2), 5.18 (2 H, s, CH_2), 3.88 (3 H, s, OCH_3); δ_C (100 MHz, DMSO): 169.8 (C), 164.7 (C), 163.0 (C), 136.8 (C), 131.8 (CH), 128.9 (CH x 2), 128.5 (CH), 128.2 (CH x 2), 108.5 (CH), 105.9 (C), 102.4 (CH), 70.1 (CH_2), 52.7 (Me).

methyl 4-(benzyloxy)-2,5-dihydroxybenzoate (60).



To a stirred mixture of starting material **59** (3.5g 13.5 mmol) in aqueous solution of NaOH (1M 135 mL), was added over 30 minutes, a solution of potassium persulfate (7.7g 28.5 mmol) in water (135 mL), at 0°C. After stirring for 20 hours at room temperature, the reaction mixture was acidified to pH 4 with HCl conc. The mixture was filtered to remove the starting material unreacted (2.25g), and to the aqueous phase was added further HCl conc. (30 mL), and heated to 80 °C for 2 hours. After cooling at room temperature, ethyl acetate (150 mL) was added, and then the aqueous layer was extracted with further ethyl acetate (3 x 200 mL). The organic phases, were collect, dried on $MgSO_4$ and evaporated to give a dark oil that was purified by column cromatography (9 : 1 light petroleum; ethyl acetate). The product obtained was furthermore crystallized with methanol and then filtered to give a colourless solid. Yield 15%, mp 165.8 °C, (Found: [M + Na^+], 297.0716. $C_{15}H_{14}NaO_5^+$ requires 297.0733); ν_{max} ($CHCl_3$)/cm^{-1}: 3684, 3555, 3011, 2956, 2414, 1669, 1635, 1509, 1440, 1399, 1374, 1276, 1239, 1168, 1082, 1000; δ_H (400 MHz, DMSO): 10.33 (1 H, s, OH), 8.94 (1 H, s, OH), 7.50 – 7.35 (5 H, m, Ar), 7.18 (1 H, s, CH), 6.63 (1 H, s, CH), 5.18 (2 H, s, CH_2), 3.86 (3 H, s, OCH_3); δ_C (100 MHz, DMSO): 170.0 (C), 156.1 (C), 154.2 (C), 140.1 (C), 136.9 (C), 128.9 (CH x 2), 128.4 (CH), 128.2 (CH x 2), 114.4 (CH), 103.7 (C), 102.1 (CH), 70.2 (CH_2), 52.6 (Me).

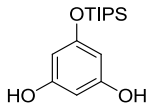
methyl 4-(benzyloxy)-2-hydroxy-5-(methoxymethoxy)benzoate (61).



To a solution of **60** (450 mg, 1.64 mmol) in dichloromethane (18 mL), were added at 0°C, DIPEA (0.51 mL, 2.95 mmol), and after few minutes, MOMCl (0.14 mL, 1.8 mmol). The mixture was stirred for 2 hours at room temperature. Water (15 mL) was then added and the mixture was extracted with ethyl acetate (3 x 15 mL). The organic layer was dried over $MgSO_4$, and evaporated. The residue was purified by flash column cromatography (9:1 light petroleum, ethyl acetate) to give the *title compound* like a colourless solid Yield 78%, mp 104.3 °C, (Found: [M + Na^+], 341.0994. $C_{17}H_{18}NaO_6^+$ requires 341.0996); ν_{max} ($CHCl_3$)/cm^{-1}: 3011, 2955, 1668, 1620, 1511, 1497, 1441, 1355, 1259, 1192, 1162, 1097, 1070, 988; δ_H (400 MHz, DMSO): 10.58 (1 H, s, OH), 7.48 – 7.37 (6 H, m, Ar, CH), 6.73 (1 H, s, CH), 5.20 (2 H, s, CH_2), 5.09 (2 H, s, CH_2), 3.88 (3 H, s, OCH_3), 3.39 (3 H, s, OCH_3); δ_C (100 MHz, DMSO): 169.7 (C), 158.4 (C), 156.3 (C), 139.5 (C), 136.6 (C), 129.0 (CH x 2), 128.6 (CH), 128.3 (CH x 2), 117.8 (CH), 103.9 (C), 102.4 (CH), 96.2 (CH_2), 70.5 (CH_2), 56.2 (Me), 52.7 (Me).

9.10.3 Fragment B

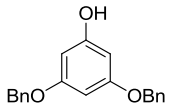
5-((Triisopropylsilyl)oxy)benzene-1,3-diol (65).



Chlorotriisopropylsilane (1.7 mL, 7.93 mmol) was added to a stirred solution of phloroglucinol **64** (3g, 23.8 mmol) and imidazole (540 mg, 7.93 mmol) in DMF (70 mL), and the resulting mixture was stirred at room temperature for one night. The reaction was diluted with diethyl ether (100 mL), washed with water and brine (100 mL x 3), and then dried on $MgSO_4$, and concentrated *in vacuo* to give the crude product as a yellow oil, that was purified by flash column chromatography (light petroleum, ethyl acetate 9:1) to give the *title compound* as a clear oil. Yield 61%; (Found: [M + H^+], 283.1720. $C_{15}H_{27}O_3Si_1^+$ requires 283.1724); ν_{max} ($CHCl_3$)/cm^{-1}: 3324, 3010, 2946, 2868, 1670, 1603, 1498, 1463, 1387, 1145; δ_H (270 MHz, $CDCl_3$): 6.14 (2 H, broad, OH), 6.00 (2 H, d, *J* 2.1, H-2, H-6), 5.96 (1 H, t, *J* 2.1, H-4), 1.27 – 1.14 (3 H, m, CH x 3), 1.04 (18 H, d, *J* 6.8, CH_3 x 6); δ_C

(68 MHz, $CDCl_3$): 158.1 (C), 157.1 (C x 2), 100.5 (CH x 2), 96.4 (CH), 17.9 (Me x 6), 12.7 (CH x 3).

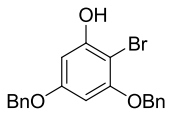
3,5-bis(Benzyloxy)phenol (66).



Benzyl bromide (1.77 mL, 14.9 mmol), was added to a stirred solution of starting material **65** (2g, 7.1 mmol) and cesium carbonate (5.1g, 15.6 mmol) in acetonitrile (50 mL), and the resultanting mixture was stirred at room temperature for two hour. The reaction was diluted with diethyl ether (250 mL), and washed with water and brine (200 mL). The organic phase was dried on $MgSO_4$, and removed in vacuo, to give the crude benzyl protected product which was taken up immediately onto next step.

TBAF (1M in THF, 10.6 mL) was added to a stirred solution of the above product in THF (50 mL), and the reaction was stirred at room temperature for one night. The resulting solution was diluted with ether (250 mL), washed with water and brine (150 mL x 2), dried and concentrated *in vacuo* to give the crude product as a brown oil, that was purified by flash column chromatography (light petroleum, ethyl acetate 9:1) to give the *title compound* as a white solid. Yield on two step 41%; colourless solid, mp 89 °C; (Found: [M + H^+], 307.1319. $C_{20}H_{19}O_3^+$ requires 307.1329); ν_{max} ($CHCl_3$)/cm^{-1}: 3409, 3068, 3010, 2933, 2876, 1601, 1494, 1454, 1375, 1275, 1153; δ_H (400 MHz, $CDCl_3$): 7.44 – 7.35 (10 H, m, Ar), 6.27 (1 H, t, *J* 2.0, H-4), 6.13 (2 H, d, *J* 2.0, H-2, H-6), 5.02 (4 H, s, CH_2 x 2), 4.78 (1 H, s, OH); δ_C (100 MHz, $CDCl_3$): 160.8 (C x 2), 157.2 (C), 136.8 (C x 2), 128.6 (CH x 4), 128.0 (CH x 2), 127.5 (CH x 4), 95.3 (CH x 2), 94.9 (CH), 70.1 (CH_2 x 2).

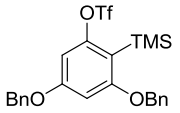
3,5-bis(Benzyloxy)-2-bromophenol (67)



To a solution of **66** (1g, 3.27 mmol) in dichloromethane (50 mL) at -78°C, was added slowly NBS (0.58 g, 3.27 mmol). After three hours the reaction was complete and was quenched with an aqueous solution of potassium carbonate at 10% (25 mL), than warmed at room temperature. The mixture was diluted with water (200 mL), and extracted with dichloromethane (150 mL x 2), than the organic phase was collected, dried on $MgSO_4$, and concentrated *in vacuo* and purified by flash column chromatography (9:1 light petroleum, ethyl acetate). Yield 88%, beige solid, mp 81 °C;

(Found: [M + H^+], 385.0441. $C_{20}H_{18}BrO_3^+$ requires 385.0434); ν_{max} ($CHCl_3$)/cm^{-1}: 3068, 3011, 2878, 1596, 1498, 1483, 1446, 1375, 1307, 1190, 1159; δ_H (400 MHz, $CDCl_3$): 7.48 – 7.33 (10 H, m, Ar x 2), 6.38 (1 H, d, *J* 2.4, H-4), 6.27 (1 H, d, *J* 2.8, H-2), 5.68 (1 H, s, OH), 5.11 (2 H, s, CH_2), 5.02 (2 H, s, CH_2); δ_C (100 MHz, $CDCl_3$): 159.7 (C), 155.9 (C), 153.9 (C), 136.4 (C), 136.3 (C), 128.7 (CH x 2), 128.6 (CH), 128.2 (CH), 128.0 (CH x 2), 127.6 (CH x 2), 127.0 (CH x 2), 94.7 (CH), 94.6 (CH), 92.0 (C), 70.8 (CH_2), 70.4 (CH_2).

3,5-bis(benzyloxy)-2-(trimethylsilyl)phenyl-trifluoromethanesulfonate (62)

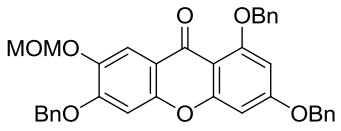


To a solution of bromophenol **67** (250 mg, 0.65 mmol) in THF (2.5 mL), was added HMDS (300 µL, 1.43 mmol), and the solution was heated to 70°C and maintained for 5 h. The reaction was cooled to room temperature and concentrated under vacuum, and the resulting oil was immediately taken on to the next step.

The crude was taken up in THF (9 mL) and cooled to -100°C. *n*-Butyllithium (2.5 M in hexanes, 310 µL, 0.78 mmol) was added slowly and the reaction was allowed to warm to -82°C. The reaction was cooled again to -100°C and maintained between -100°C and -82°C for 30 minutes.
After this period, triflic anhydride (142 µL, 0.84 mmol) was added at -100°C. The reaction was warmed to -80°C, quenched by the addition of saturated aqueous sodium bicarbonate solution (5 mL), and subsequently warmed to room temperature. The reaction was diluted with diethyl ether (20 mL) and washed with brine (15 mL). The organic layer was dried with $MgSO_4$, concentrated under vacuum, and purified by flash column chromatography (98:2 light petroleum, ethyl acetate). Yield 70 %, clear oil, (Found: [M + H^+], 511.1226. $C_{24}H_{26}F_3O_5SSi^+$ requires 511.1217); ν_{max} ($CHCl_3$)/cm^{-1}: 3156, 3011, 2902, 2253, 1794, 1603, 1562, 1466, 1381, 1300, 1140, 1092, 1040; δ_H (400 MHz, $CDCl_3$): 7.43 – 7.39 (10 H, m, Ar x 2), 6.60 (1 H, d, *J* 2.0, H-3), 6.55 (1 H, d, *J* 2.0, H-5), 5.05 (2 H, s, CH_2), 5.04 (2 H, s, CH_2), 0.33 (9 H, s, CH_3 x 3); δ_F (376.5 MHz, $CDCl_3$): -72.62 (3 F, s, CF_3); δ_C (100 MHz, $CDCl_3$): 165.2 (C), 161.5 (C), 155.3 (C), 136.0 (C), 135.9 (C), 128.8 (CH x 2), 128.6 (CH x 2), 128.4 (CH), 128.2 (CH), 127.8 (CH x 2), 127.6 (CH x 2), 118.6 (Me, J_{C-F} = 321 Hz), 112.6 (C), 99.3 (CH), 99.1 (CH), 70.9 (CH_2), 70.5 (CH_2), 1.0 (Me x 3).

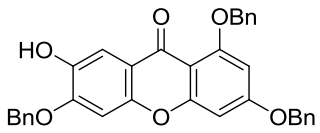
9.10.4 Toward the synthesis of Toxyloxanthone and Rubraxanthone

1,3,6-tri(benzyloxy)-7-(methoxymethoxy)-9H-xanthen-9-one (63).



To a solution of fragment A **61** (409 mg, 1.28 mmol) in dry THF (15 mL), was added sodium hydride (1.29 mmol) and the mixture was left to stirrer for 45 minutes. The mixture was heated to 65°C and was added Caesium fluoride first, and then was added a solution of fragment B **62** (720 mg, 1.41 mmol) in dry THF (10 mL), over 30 minutes. The mixture was stirred for 24 h always at 65°C, then was cooled, and was diluted with ether (100 mL), and washed with brine (100 mL). The aqueous layer was extracted with ethyl acetate (2 x 60 mL), then dried over $MgSO_4$, filtered and evaporated to give a residue that was purified by flash column chromatography (8:2 light petroleum, ethyl acetate) Light brown solid yield 40% mp 149 °C, (Found: [M + H^+], 575.2062. $C_{36}H_{31}O_7^+$ requires 575.2064); ν_{max} ($CHCl_3$)/cm^{-1}: 3691, 3068, 3010, 2931, 1715, 1644, 1624, 1606, 1499, 1453, 1438, 1376, 1268, 1182, 1156, 1122, 1076; δ_H (400 MHz, $CDCl_3$): 8.00 (1 H, s, CH), 7.67 (2 H, d, *J* 7.15, Ar), 7.51 – 7.34 (13 H, m, Ar), 6.87 (1 H, s, CH), 6.55 (1 H, d, *J* 2.3, CH), 6.50 (1 H, d, *J* 2.3, CH), 5.31 (2 H, s, CH_2), 5.27 (2 H, s, CH_2), 5.25 (2 H, s, CH_2), 5.14 (2 H, s, CH_2), 3.56 (3 H, s, OCH_3); δ_C (100 MHz, $CDCl_3$): 174.3 (C), 163.3 (C), 160.7 (C), 159.7 (C), 154.6 (C), 151.6 (C), 143.9 (C), 136.4 (C), 135.9 (C), 135.8 (C), 128.8 (CH x 4), 128.6 (CH x 2), 128.4 (CH), 128.3 (CH), 127.7 (CH), 127.6 (CH x 2), 127.2 (CH x 2), 126.8 (CH x 2), 116.6 (C), 113.2 (CH), 107.5 (C), 101.0 (CH), 97.2 (CH), 96.1 (CH_2), 94.0 (CH), 71.0 (CH_2), 70.8 (CH_2), 70.5 (CH_2), 56.4 (Me).

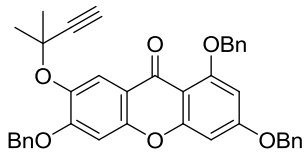
7-(hydroxy)-1,3,6-tri(benzyloxy)-9H-xanthen-9-one (69).



To a solution of starting material **63** (380 mg, 0.66 mmol) in anhydrous dichloromethane (24 mL), was added trifluoroacetic acid (4 mL) at 0 °C, and the reaction mixture was stirred for 30 minutes at room temperature. Saturated sodium hydrogen carbonate (30 mL) was added slowly and the mixtyre extracted with ethyl acetate (3 x 30 mL). The combined organics fractions were washed with saturated brine solution (10 mL), dried over $MgSO_4$, filtered and concentrated in vacuo to give an oil that was purified by flash column chromatography (7:3 light petrol, ethyl acetate). Yield 94%,

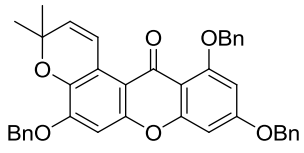
colourless solid, mp 174 °C, (Found: [M + H^+], 531.1814. $C_{34}H_{27}O_6^+$ requires 531.1802); ν_{max} ($CHCl_3$)/cm^{-1}: 3687, 3603, 3011, 2437, 1630, 1602, 1500, 1438, 1272, 1239, 1174, 1118, 1017; δ_H (400 MHz, $CDCl_3$): 7.82 (1 H, s, CH), 7.62 (2 H, d, *J* 7.3, Ar), 7.48 – 7.35 (13 H, m, Ar), 6.91 (1 H, s, CH), 6.54 (1 H, s, CH), 6.48 (1 H, s, CH), 5.27 (2 H, s, CH_2), 5.24 (2 H, s, CH_2), 5.13 (2 H, s, CH_2); δ_C (100 MHz, $CDCl_3$): 175.0 (C), 163.5 (C), 160.7 (C), 159.8 (C), 151.2 (C), 150.2 (C), 143.0 (C), 136.3 (C), 135.7 (C), 135.0 (C), 129.0 (CH x 2), 128.9 (CH), 128.8 (CH x 2), 128.7 (CH x 2), 128.4 (CH), 127.9 (CH x 2), 127.8 (CH), 127.6 (CH x 2), 126.8 (CH x 2), 117.0 (C), 109.9 (CH), 107.3 (C), 99.6 (CH), 97.1 (CH), 94.0 (CH), 71.6 (CH_2), 70.8 (CH_2), 70.5 (CH_2).

7-(2,2-dimethylbut-3-yn-1-yl)-1,3,6-tri(benzyloxy)-9H-xanthen-9-one (70).



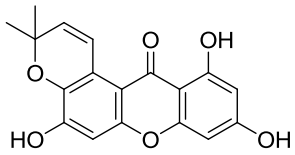
Potassium carbonate (82 mg, 0.594 mmol) and potassium iodide (82 mg) were added to a solution of starting material **69** (150 mg, 0.283 mmol) in acetone (15 mL). Commercially 3-chloro-3-methyl-1-butyne (80 μL, 0.707 mmol) was added in a single portion and the reaction mixture was heated to reflux and stirred for 48 hours. After cooling ether (60 mL) was added, and the ethereal solution was washed with 1N NaOH (3 x 10 mL). The organic layer was dried over $MgSO_4$, and concentrated in vacuo to give a solid that was purified by flash column chromatography (8:2 light petroleum, ethyl acetate). Yield 75% colourless solid, mp 162 °C, (Found: [M + H^+], 597.2287. $C_{39}H_{33}O_6^+$ requires 597.2272); ν_{max} ($CHCl_3$)/cm^{-1}: 3689, 3305, 3011, 1645, 1623, 1605, 1498, 1443, 1379, 1270, 1182, 1119, 1048, 1028; δ_H (400 MHz, $CDCl_3$): 8.28 (1H, s, CH), 7.65 (2H, d, J = 7.3 Hz, Ar), 7.52 (2H, d, J = 7.3 Hz, Ar), 7.46 – 7.34 (11H, m, Ar), 6.89 (1H, s, CH), 6.55 (1H, d, J = 1.8 Hz, CH), 6.49 (1H, d, J = 1.8 Hz, CH), 5.28 (2H, s, CH_2), 5.20 (2H, s, CH_2), 5.13 (2H, s, CH_2), 2.57 (1H, s, CH), 1.72 (6H, s, CH_3 x 2); δ_C (100 MHz, $CDCl_3$): 174.5 (C), 163.2 (C), 160.7 (C), 159.7 (C), 157.4 (C), 152.5 (C), 142.2 (C), 136.4 (C), 136.0 (C), 135.7 (C), 128.8 (CH x 2), 128.7 (CH x 2), 128.6 (CH x 2), 128.4 (CH), 128.1 (CH), 127.7 (CH), 127.6 (CH x 2), 127.2 (CH x 2), 126.8 (CH x 2), 119.9 (CH), 116.4 (C), 107.6 (C), 100.7 (CH), 97.2 (CH), 94.1 (CH), 85.5 (C), 74.9 (C), 74.3 (CH), 70.9 (CH_2), 70.8 (CH_2), 70.5 (CH_2), 29.6 (Me x 2).

5,9,11-tri(benzyloxy)-3,3-dimethylpyrano[3,2-a]xanthen-12(3H)-one (71).



A solution of starting material **70** (60 mg) in toluene (14 mL) was heated under reflux for 2 hours and half. The solvent was evaporated and the residue purified by flash column chromatography (8:2 light petroleum, ethyl acetate). Colourless solid, yield 97% mp 182 °C; (Found: [M + H^+], 597.2283. $C_{39}H_{33}O_6^+$ requires 597.2272); ν_{max} ($CHCl_3$)/cm^{-1}: 3689, 3606, 3011, 2928, 1701, 1615, 1438, 1378, 1274, 1192, 1166, 1125, 1059, 1016; δ_H (400 MHz, $CDCl_3$): 8.14 (1 H, d, *J* 9.8, CH), 7.62 (2H, d, *J* 7.3, Ar), 7.49 – 7.35 (13 H, m, Ar), 6.74 (1 H, s, CH), 6.48 (1 H, d, *J* 2.1, CH), 6.43 (1 H, d, *J* 2.1, CH), 5.85 (1 H, d, *J* 9.8, CH), 5.30 (4 H, s, CH_2 x 2), 5.09 (2 H, s, CH_2), 1.53 (6 H, s, CH_3 x 2); δ_C (100 MHz, $CDCl_3$): 176.9 (C), 162.9 (C), 160.5 (C), 158.8 (C), 152.3 (C), 151.3 (C), 139.8 (C), 136.6 (C), 136.4 (C), 131.8 (CH), 128.8 (CH x 2), 128.7 (CH x 2), 128.6 (CH x 2), 128.4 (CH), 128.0 (CH), 127.6 (CH x 3), 126.9 (CH x 2), 126.7 (CH x 2), 121.6 (CH), 120.9 (C), 111.8 (C), 108.5 (C), 101.1 (CH), 97.3 (CH), 93.5 (CH), 75.3 (C), 70.8 (CH_2), 70.7 (CH_2), 70.4 (CH_2), 27.1 (Me x 2).

5,9,11-trihydroxy-3,3-dimethylpyrano[3,2-a]xanthen-12(3H)-one (Toxyloxanthone B) (72).



To a stirred solution of starting material **71** (20 mg, 0.033 mmol), and pentamethylbenzene (45 mg, 0.3 mmol) in dry dichloromethane (1 mL), was added BCl_3 (0.2 mL, solution 1 M in hexane,) dropwise over 10 min at -78 °C. After 45 min, the reaction mixture was quenched with a mixture chloroform-methanol (10:1, 4mL) at -78 °C and warmed to room temperature. The excess of solvents were removed under reduced pressure. The residue was purified by preparative TLC (8:2:1 cyclohexane, ethyl acetate, methanol). Yield 65% pale yellow solid, mp 240 °C, (Found: [M + Na+], 281.0781. $C_{15}H_{14}NaO_4^+$ requires 281.0784); λ_{max} (MeOH)/nm 243 (log ε 4.21), 262 (4.19), 331 (4.05), 322sh (4.02), 384 (3.67); ν_{max} ($CHCl_3$)/cm^{-1}: 3689, 3605, 3011, 2925, 1601, 1433, 1239, 1117, 930, 826; δ_H (500 MHz, $(CD_3)_2CO$): 14.08 (1 H, s, 11-OH), 8.07 (1 H, d, *J* 10.1, H-1), 6.39 (1 H, s, H-6), 6.23 (1 H, s, H-8), 6.08 (1 H, s, H-10), 5.70 (1 H, d, *J* 10.1, H-2), 1.44 (6 H, s, CH_3 x 2); δ_C (125 MHz, $(CD_3)_2CO$): 180.5 (C-12), 165.3 (C-5), 165.2 (C-9), 163.3 (C-11), 156.9 (C-7a), 155.6 (C-6a), 141.3 (C-4a), 129.6 (C-2), 122.2 (C-1), 118.0 (C-12b), 103.1 (C-6), 101.9 (C-11a), 101.6 (C-12a), 97.6 (C-10), 92.9 (C-8), 74.9 (C-3), 26.2 (Me x 2).

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