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Synthesis of Azole-Heterocycles as Potential Antitumor and/or Antiviral Agents

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INTRODUCTION

Cancer and AIDS are today two of the leading causes of death in the world. Statistical data show about six million new cases of cancer per year in all over the world and about one in four people will get it in some form during their life time. The World Health Organization (WHO) estimated that, in 2009, 33.4 million people of the world population was suffering from AIDS, the new cases of HIV infections were 2.7 million per year and the annual deaths due to AIDS were 2.0 million. These data confirm the importance of these diseases both from the social and the purely scientific points of view, since the research aim to the identification of new efficacious therapeutic strategies.

Cancer belongs to that class of diseases in which a group of cells display uncontrolled growth forming a tumour that can involve also the adjacent tissues and eventually bits of this tumour break off and form new tumours (this is known as metastasis).

These features make possible to differentiate cancer from benign tumors which show a limited growth, do not exhibit invasive properties nor give rise to metastasis.

Most cancers are initially recognized either because signs or symptoms appear or through cancer screening but definitive diagnosis requires histological examination and evaluation of specific markers that can be useful to establish prognosis and individual treatments.

Once diagnosed, patients may undergo surgery and/or radiotherapy and/or chemotherapy. Which treatment is used depends on the type of cancer, the location and grade of the tumor, and the stage of the disease, as well as the general state of a person's health.

Many progress have been made in the development of effective and selective treatments in order to limit the damage over the normal cells.

The most effective treatment used for the complete removal of the cancer is the surgical removal of affected part, even if the effectiveness of treatment is often limited by the ability of the tumor to metastasize.

Radiation and chemotherapy can provide valuable tools to fight the disease, but they can also cause damage to normal tissue and have several side effects such as myelosuppression.

For these reasons the search for new anticancer drugs is one of the main objectives of scientific research.

Anticancer drugs can be generally divided into two broad categories: cytotoxic and cytostatic. Both lead to a reduction in the tumour size by preventing the reproduction of the cancer cells.

The cytostatic drugs act by interfering with DNA replication. Because cancer cells rapidly divide, rapidly synthesize new DNA and if this is damaged the cell will die.

These drugs are divided into three main classes:

• Antimetabolites: molecules, similar to the nucleotides, that fit the normal metabolic pathways causing alterations or block.

• Alkylating agents and intercalators: molecules that bind (not covalently) the DNA, causing changes in its shape.

• Strand breakers: highly reactive species that lead to breakage of the double helix DNA strands.

Among cytotoxic drugs are those that interact with the mitotic spindle that express their action by acting mainly on microtubules. Microtubules are filamentous proteins formed by α- and β-tubulin dimers that by assembling themselves, during the cell division, form the mitotic spindle which cause the separation of sister chromatids by carrying the chromosomes. The role of microtubules is essential for the proper distribution of the genetic material in daughter cells. (Fig.1)

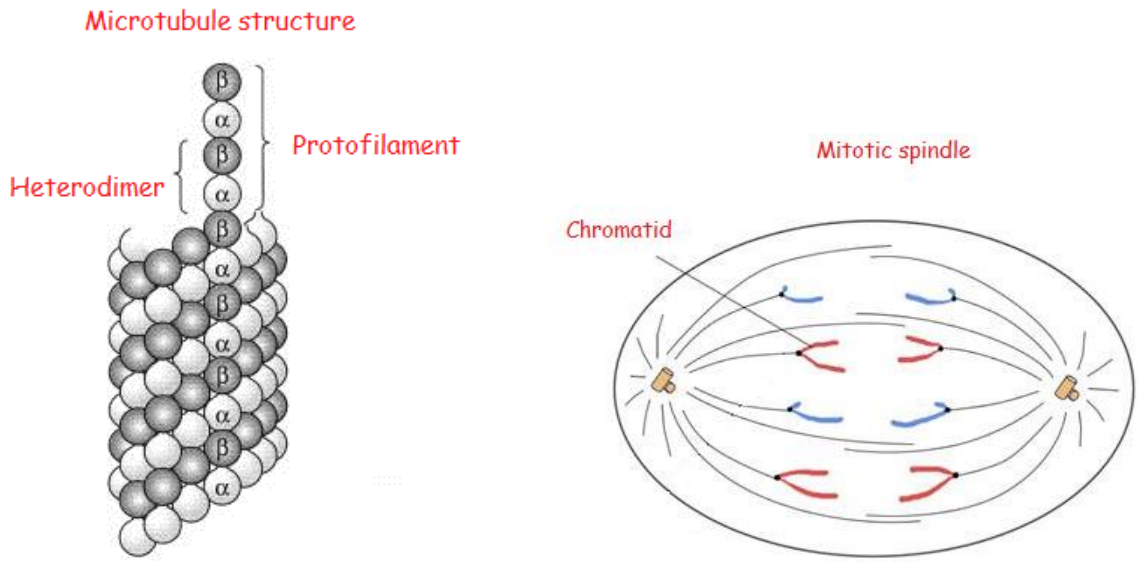
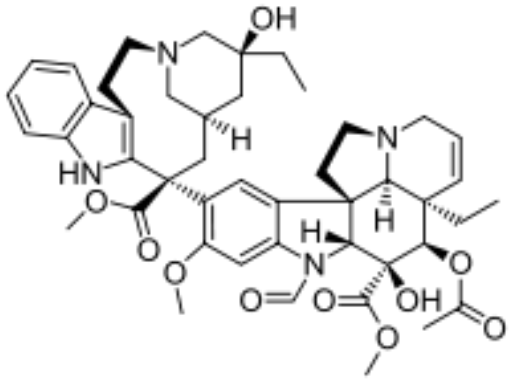
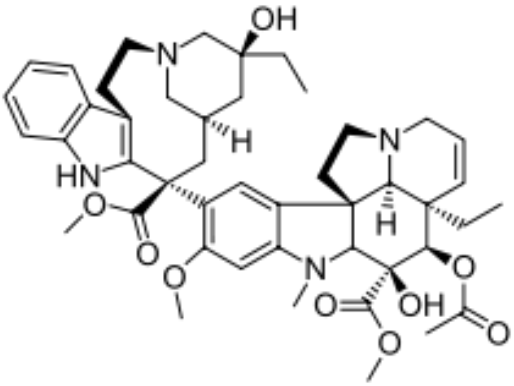


Figure 1

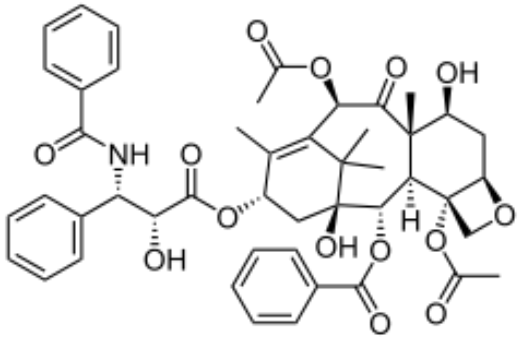
The drugs that interfere with microtubule prevent the proper functioning of the mitotic spindle resulting in a non-cell division and subsequent apoptosis. Reference molecules are definitely the well-known vinca alkaloids (Vincristine **1** e Vinblastine **2**) and taxanes (Paclitaxel **3** e Docetaxel **4**).



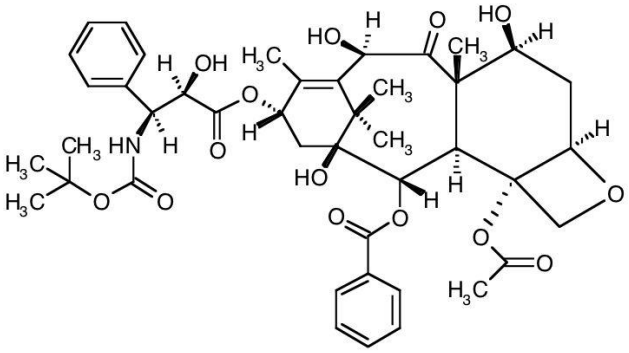
1
Vincristine



2
Vinblastine



3
Paclitaxel



4
Docetaxel

Examples of compounds showing antitumor activity are also polycondensed nitrogen heterocycles. From several years the research group where I carried out my PhD was interested in the synthesis and biological evaluation of polycondensed nitrogen heterocycles. In this regard they already synthesized new heterocyclic systems with antitumor activity, such as pyrrolo[2,1-*c*][1,2,4]triazines **5** [1], indolo[4,3-*a*][1,2,4]triazines **6** [2], indolo[3,2-*c*]cinnolines **7** [3], indolo[1,2-*c*]benzo[1,2,3]triazines **8** [1], pyrrolo[2,1-*d*][1,2,3,5]tetrazines **9** [4] and indolo[5,4-*a*][1,2,3,5]tetrazines **10.** [5] (Fig.2)

All derivatives screened *in vitro* showed a good antineoplastic activity showing IC_{50} values in micromolar range and in some cases from submicromolar to nanomolar range (indole[3,2-*c*]cinnoline IC_{50} = 0.08-7.0 μM; indole[1,2-*c*]benzo[1,2,3]triazine IC_{50} = 0.2-10 μM; pyrrolo[2,1-*d*][1,2,3,5]tetrazine IC_{50} <0.01-56 μM).

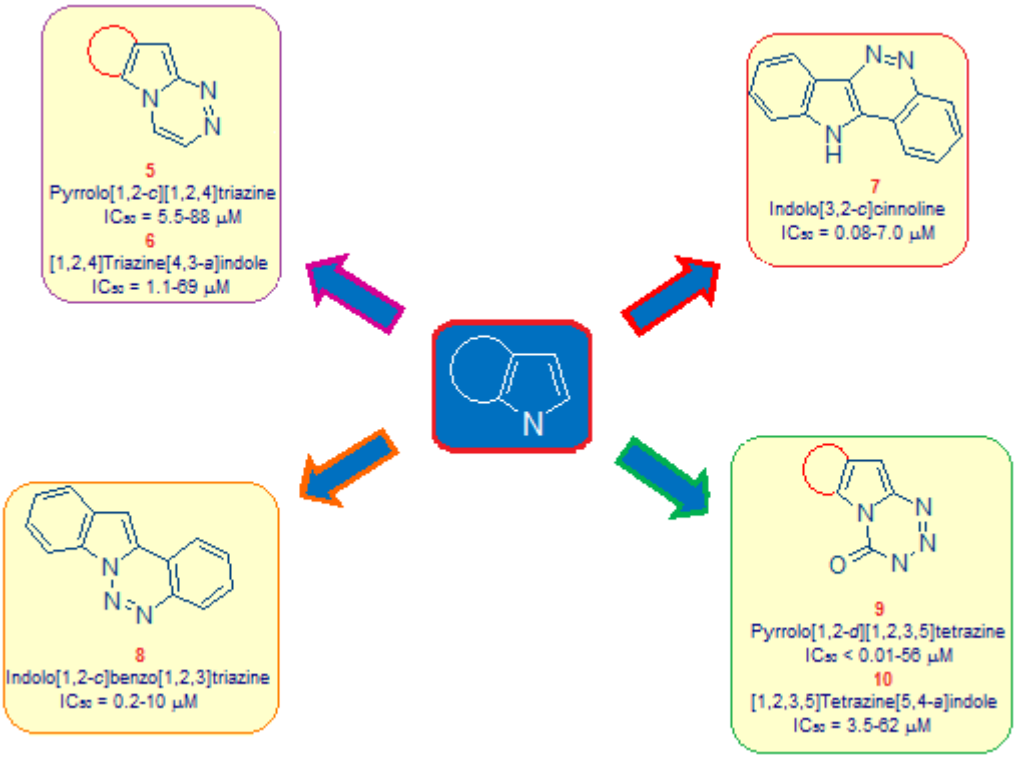
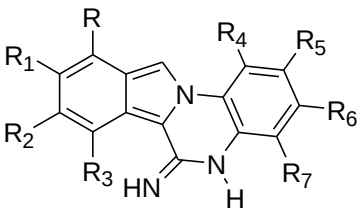
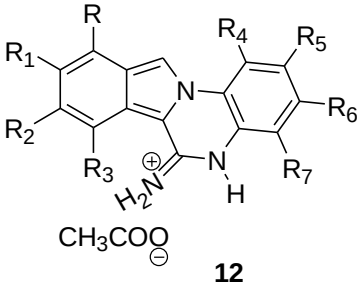


Figure 2

Considering the results obtained with pyrrole and indole compounds, new heterocyclic systems containing the isoindole nucleus were also synthesized and in particular isoindole[2,1-*a*] quinoxaline derivatives **11** and **12** (Tab.1).



11



12

Compd	R	R_1	R_2	R_3	R_4	R_5	R_6	R_7
11a	H	Me	Me	H	H	H	OH	H
11b	F	H	H	H	H	H	OH	H
12a	H	H	H	H	H	OMe	H	H
12b	H	H	H	H	OMe	H	H	OMe
12c	H	Me	Me	H	H	H	OMe	H
12d	H	OMe	OMe	H	H	H	OMe	H
12e	F	H	H	H	H	H	OMe	H
12f	H	OMe	H	H	H	H	OMe	H
12g	H	H	OMe	H	H	H	OMe	H
12h	H	OMe	OMe	H	H	H	OH	H
12i	H	OMe	H	H	H	H	OH	H
12l	H	H	OMe	H	H	H	OH	H

Table 1

Some of these compounds, screened on a panel of approximately 60 human tumor cell lines derived from nine different tumor types (leukemia, melanoma, lung cancer, colon cancer, kidney cancer, ovarian cancer, breast cancer, prostate cancer and tumors of the CNS), showed inhibitory activity against all the lines tested from micro- to nanomolar concentrations (Tab.2).

			pGI_{50}	
Compd	**$N^{[1]}$**	**$N^{[2]}$**	**Range**	**MG_MID**
11a	60	60	>8.00-5.18	7.03
11b	60	60	6.52-5.11	5.73
12a	46	46	6.86-5.81	6.38
12b	59	58	7.14-5.64	5.91
12c	58	56	>8.00-5.42	5.83
12d	59	56	>8.00-4.81	7.75
12e	58	54	7.68-4.97	6.95
12f	58	54	>8.00-5.92	7.87
12g	58	56	>8.00-5.98	7.70
12h	60	57	>8.00-5.49	7.45
12i	59	58	7.49-4.96	6.63
12l	60	59	7.57-4.91	6.75

Table 2

$N^{[1]}$: number of the cell lines investigated

$N^{[2]}$: number of the cell lines giving a positive pGI_{50} values

Test carried out on rats with tumor cells Ovcar-3, showed that the compound **12d** (NSC747526) (it was decided to use this compound that is not the most active because it was the easiest to be prepared) determined a significant reduction of the tumor mass at the dose of 15mg/kg with a concomitant decrease in body weight of the animal. It also showed significant anti-angiogenic effects in Ovcar-3 tumors. It was observed a dose dependent significant reduction of the micro vessel number, micro vessel ratio and size.

Immunohistochemical studies were also performed regarding the expression of tumor markers CD31 and Ki67. Biological results showed that the compound **12d** caused a dose-dependent decrease of the expression of Ki67 and CD31. (Fig.3)

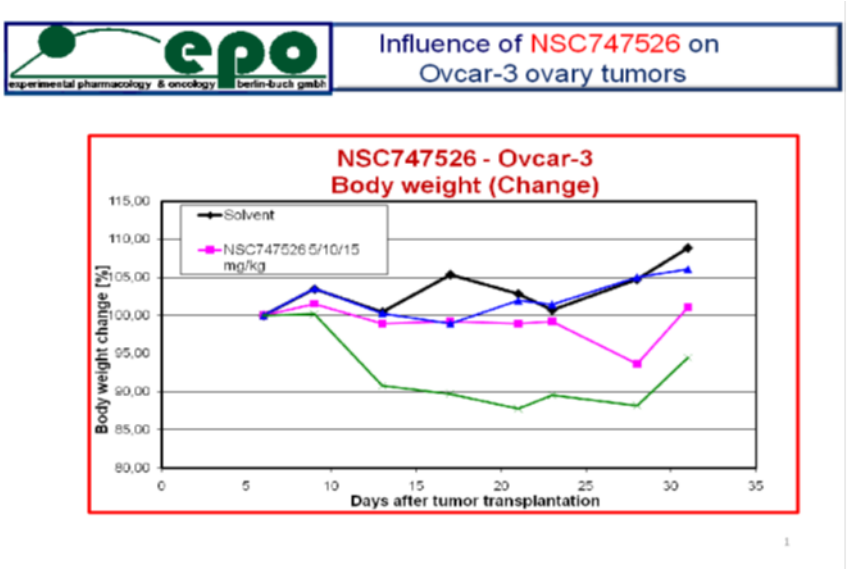


Figure 3

Preliminary tests also showed interactions between isoindolol-quinoxaline derivatives and G-quadruplex telomeric DNA, containing nucleic acid sequences rich in guanine, capable of forming structures with four strands in the telomeres, structures that are located at the end of chromosomes and necessary for their stability and involved in approximately 85% of all forms of cancer. The shortening of telomeres is implicated in cellular senescence. Telomerase is an enzyme that synthesizes the filaments rich in guanine of telomeric DNA. The activity of telomerase is highly correlated with the cancer and may allow cancer cells to avoid senescence. Considering these observations, a promising new approach for the treatment of tumors is based on the identification of

small molecules that can inhibit telomerase having as target DNA G-quadruplex structures involved in the function of telomeres and the related enzyme.
This approach would lead to the discovery of drugs which would affect selectively cancer cells and not normal cells, bypassing the substantial problems of toxicity of current drugs that are not able to discern between healthy cells and cancer cells. The preliminary results of the assays of G-quadruplex telomeric showed that isoindolol-quinoxaline derivatives had a remarkable selectivity against these forms of DNA, suggesting these compounds as " lead compound " to treat a high number of tumor forms.

The agent responsible for Acquired Immunodeficiency Syndrome, or more commonly known as AIDS, that affects the immune system, is the human immunodeficiency virus (HIV). Currently the two viral strains known are HIV-1 and HIV-2. They have a different geographical location, the former, initially called LAV (lymphadenopathy-associated virus), is mainly widespread in Europe, Asia and Africa, and the second one, mostly in West Africa and Asia, causes a syndrome clinically more moderate compared to the previous strain. [6]
The virion has a spherical structure with a diameter of about 100 nm, with two external membranes (pericapsid) produced by the cell; they are a conoid capsid and an envelope in which the glycoproteins *gp120* and *gp41* are located. In the central core there is the RNA genome and different enzymes such as reverse transcriptase, integrase and protease. (Fig. 4)

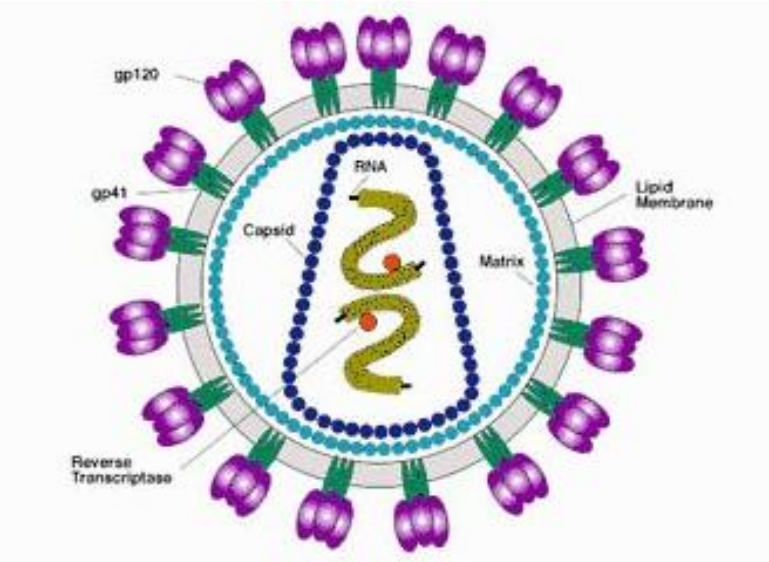


Figure 4

The replicative cycle of HIV-1 or HIV-2 (Fig.5) starts when the *gp120* protein, located on the external membrane of the virus, recognizes the homologue receptor on the surface of target cells, a particular protein known as CD4. Human cells with more CD4 receptors are $CD4^+$ T lymphocytes, which are crucial in the process of immune defense. The virus replication process destroys these cells, in particular when new viruses leave the cell by budding that often causes the lysis of the latter. Once the bond occurred, the fusion of the viral membrane with the host cell starts and a fundamental role is played by the *gp41* protein.

Completed the fusion, inside the host cell the reverse transcriptase transcribes DNA from the viral RNA (Fig.5), and this DNA is then transported within the cell nucleus and inserted, via integrase, into the genome of the host cell to be subsequently transcribed into genomic and messenger RNA, essential for the synthesis of viral proteins necessary for the maturation of the virus.

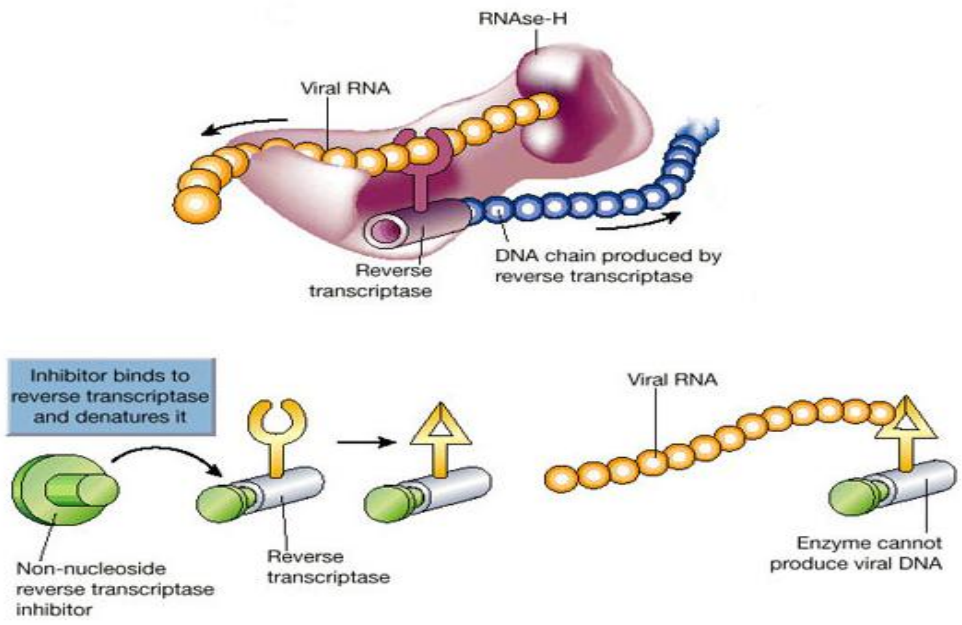


Figure 5

These viral proteins produced are assembled together to proviral RNA to form the internal components of the structure of the virion. After that the membrane of the infected cell forms a lipid bilayer around the viral matrix (containing *gp41* and *gp120*) and new infectious particles are released into the extracellular medium by a process of budding. Lastly the stage of maturation of the virion consists on the acquisition of the ability to infect other cells. (Fig. 6) [7] [8]

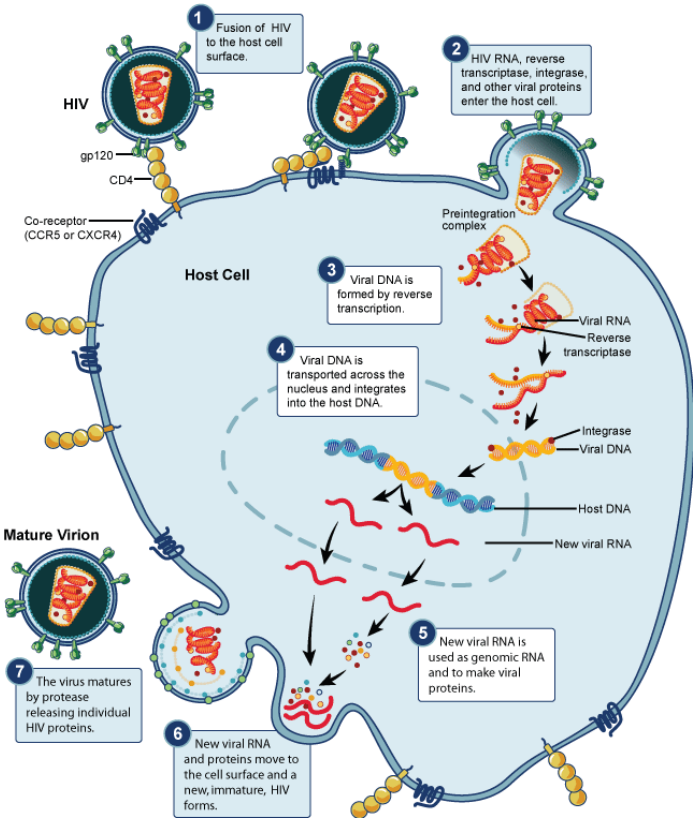


Figure 6

Currently, the anti-AIDS drugs commercially available are different: reverse transcriptase inhibitors, protease inhibitors, integrase inhibitors and fusion inhibitors; although the most used treatment is surely multidrug, which has the purpose of reduce the resistance that commonly occur and delay the progression of the disease. [9]

Considering the key role of reverse transcriptase in this disease, it is not surprising the interest moved over the years to the development of new inhibitors.

HIV reverse transcriptase (RT) is a crucial enzyme responsible for the synthesis of double-stranded DNA from the single-stranded RNA genome.

HIV-1 RT is a protein dimer consisting of two subunits of 66 kDa (p66) and 51 kDa (p51). [10] The p66 and p51 subunits are composed of three subdomains called thumb, palm and fingers. The p66 subunit also contains an RNase H active site. NNRTIs bind to a common hydrophobic site (NNIBP) in the p66 palm subdomain 10 Å away from the polymerase. (Fig. 7) [11] [12]

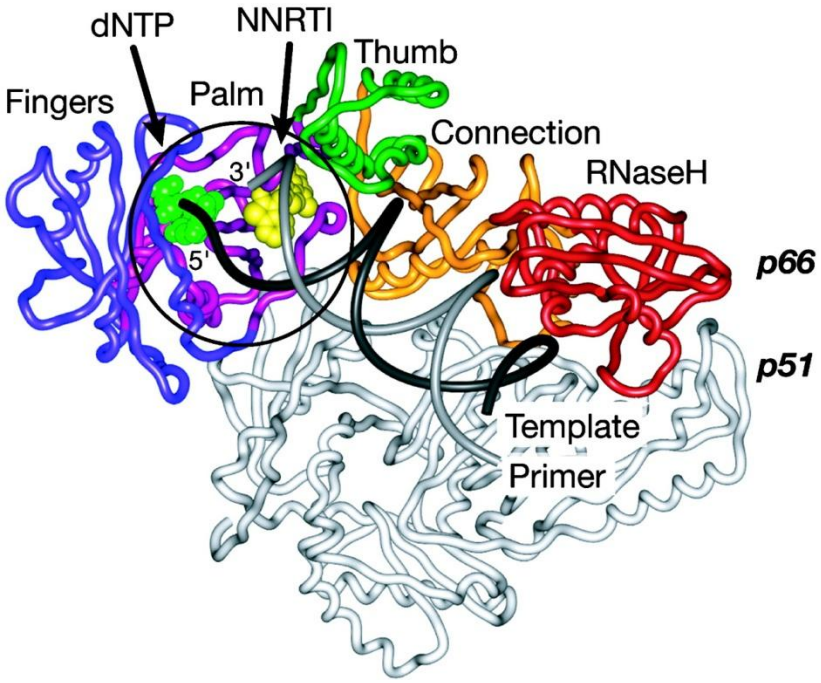


Figure 7

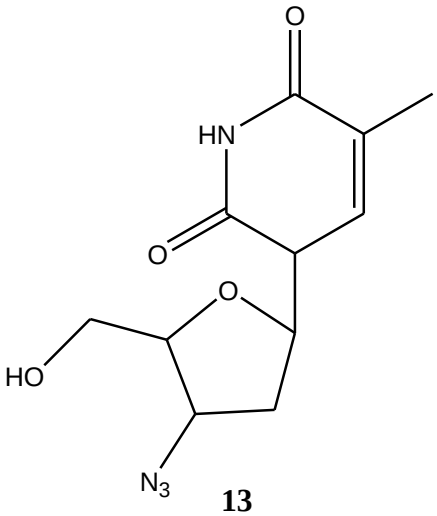
The frequent mutations that occur in this enzyme of the amino acid residues Y181, Y188 and K103 led to the identification of four highly conserved residues of the binding pocket for NNRTIs: F227, W229, L324 and Y318. With the exception of Y318 the other three are part of the "hairpin" β12-β13 responsible for maintaining the correct orientation of the enzyme for the nucleophilic attack on the incoming dNTP. [13]

The mutation in this region of the enzyme compromise the activity of the enzyme, in particular, the mutation of the fragment W229 reduces the activity of reverse transcriptase to less than 2%, highlighting the vital importance of this residue for the enzyme. [14] The mutation of this fragment has never been observed in combination with other, indicating that the compensatory mutations to restore the enzyme activity of reverse transcriptase in the mutated residue W229 does not happen easily. [15]

The reverse transcriptase inhibitors can be classified into two main groups: nucleoside RT inhibitors (NRTIs) and non-nucleoside RT inhibitors (NNRTIs). [16] [17] [18] [19]

NRTIs compete with the natural deoxynucleoside triphosphate (dNTP) substrate for RT binding, and thus inhibit HIV replication by terminating chain elongation. NNRTIs bind to an allosteric site in HIV-RT and indirectly interefere with dNTP incorporation, probably by altering the conformation of residues located in the active site and inducing structural distortion of RT.

A competitive inhibitor (NRTI) currently used in the treatment of AIDS is Zidovudine (AZT) **13**, but its therapeutic usefulness is limited by poor brain penetration, viral resistance, toxicity and low specificity. [20] [21] [22]



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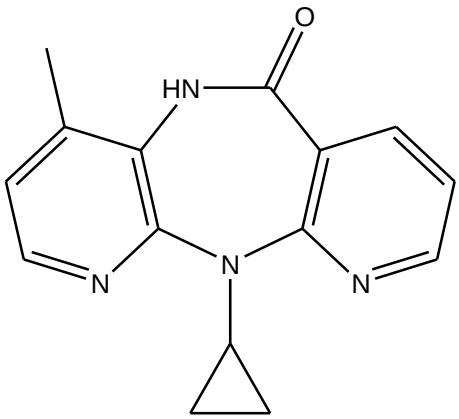

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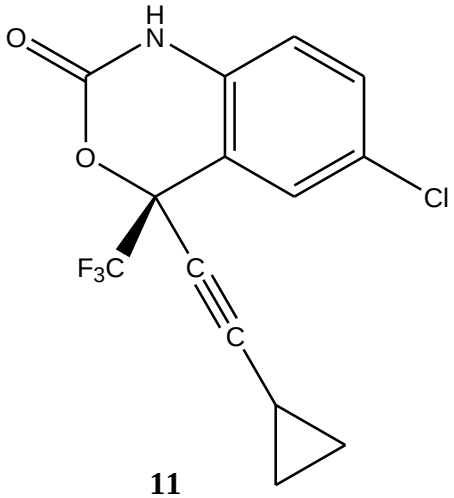


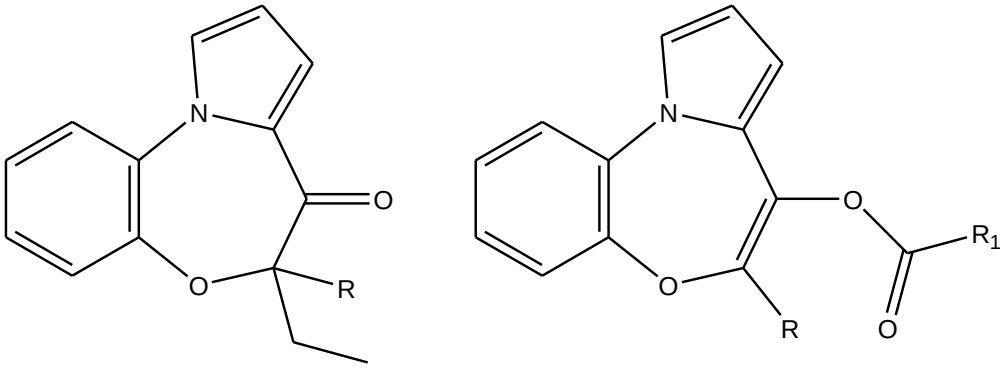
11

NNRTIs can bypass some of these disadvantages inherent to nucleoside inhibitors and the representative compounds of this class are Nevirapine **14** and Efavirenz **15**.

Beacuse of their generally good tollerability and low toxicity, [23] these drugs are essential component of highly active antiretroviral therapy (HAART), with nucleoside reverse transcriptase inhibitors (NRTI) and/or protease inhibitors (PI). [11] The multidrug therapy provides a substantial delay in disease progression compared to mono therapy or dual therapy, allowing significant immune system function restoration and retarding the emergence of resistant viral strains. [23] [24]

Mutation to RT rapidly emerge and they confer resistance to all known NNRTIs, reducing the efficiency of the drugs. It is necessary to understand the interactions of the inhibitor with RT in order to design more effective drugs.

In order to identify new molecules with antiviral and/or antitumor properties, pyrrolo[1,5]benzoxazepine (PBOX) derivatives are reported. In fact, these compounds showed both anti-HIV (derivatives of type **16**) and anticancer (derivatives of type **17**) activity depending on the substitution on the benzoxazepine core.



16 **17**

R = phenyl, naphtyl, *p*-OMephenyl, *p*-tolyl
R_1= Me, $N(Me)_2$, $N(Et)_2$

PBOXs **16** represent a new class of non-nucleoside inhibitors of reverse transcriptase of the immunodeficiency virus type 1 (HIV-1) that is also capable of preventing cytopathogenicity in the T4 lymphocytes. Their activity was influenced by the substituents in the 6-position on the fused aromatic ring. Small lipophilic substituents on the C-6 were preferred, while substitutions on the benzo-fused ring were found to be useless for the activity, compared to not substituted compounds. Changes to the π system to the C-6 were well tolerated, on the contrary the replacement of the benzo-fused ring with a [2,3]naphtho-system led to less active compounds. Finally, the best activity and specificity were obtained when in position 6 of the pyrrole-benzoxazepine system a phenyl group or ethyl alcohol were inserted. Furthermore, through enzyme assays the activity of these derivatives was compared with the activity of nevirapine and it was found that the latter is even less active, bringing an IC_{50} value of 0.5 μM versus 0.25 μM of pyrrole-benzoxazepines. The cell culture assays also showed a specificity of action against HIV-1 than HIV-2. [25]

The same pyrrolo[1,5]benzoxazepines ring **17**, variously substituted, showed antiproliferative activity in a wide range of tumor cell lines at micromolar concentrations. Many pyrrolo[1,5]benzoxazepines caused apoptosis as confirmed by cell shrinkage, by chromatin condensation and by the DNA fragmentation in three tumor cell lines, such as HL-60 promyelocytic cell line, Jurkat T lymphoma and Hut-78 lymphoma. The pro-apoptotic effect appeared to be due to an activation of caspase-3-like. Studies showed that the mitochondrial protein cytochrome *c*

induced apoptosis when it was accumulated in the cytosol in response to different stress inducers. It was also analyzed this aspect on the three tumor cell lines and it was found that this release of cytochrome *c*, which subsequently activates the caspase 3, had an important role in the induction of apoptosis by the PBO. [26] Interesting IC_{50} values (1.7-1.9 μM), related to cytotoxic activity of benzoxazepine derivatives, were found on the cell lines of colon carcinoma HT29 and lung carcinomas NCI-H460 and M109. The cell death process appeared to be triggered by the activation of the proapoptotic MAP kinase JNK, suggesting that the molecular target of the pyrrolo[1,5]benzoxazepines was intracellular, since it was also found that their analogues, unable to penetrate inside the cell, did not induce apoptosis or activation of JNK. [27]

Evidence also showed that the antitumor activity in different tumor cell lines, such as ovarian cancer and chronic myelogenous leukemia, was explicated through targeted action on both the tumor cells and their vasculature. The pro-apoptotic effect, shown for example on the A2780 ovarian carcinoma cell line (IC_{50} = 0.23-3.58 μM) or K562 chronic myeloid leukemia (IC_{50} = 0.37-6.49 μM), was mediated by the cell arrest in G_2/M phase, due to the binding of these compounds to tubulin site not yet characterized, different from the one that binds colchicine and vinca alkaloids. This causes the formation of anomalous microtubules, unable to complete the cell division process resulting in apoptosis. Moreover, PBOXs showed an anti-angiogenic activity targeting cells vasculature. In particular, in the Human Umbilical Vein Endothelial Cells (HUVEC) the formation of capillaries and the migratory activity of the cells was inhibited (IC_{50} = 0.06-0.70 μM). (Fig.8) [28]

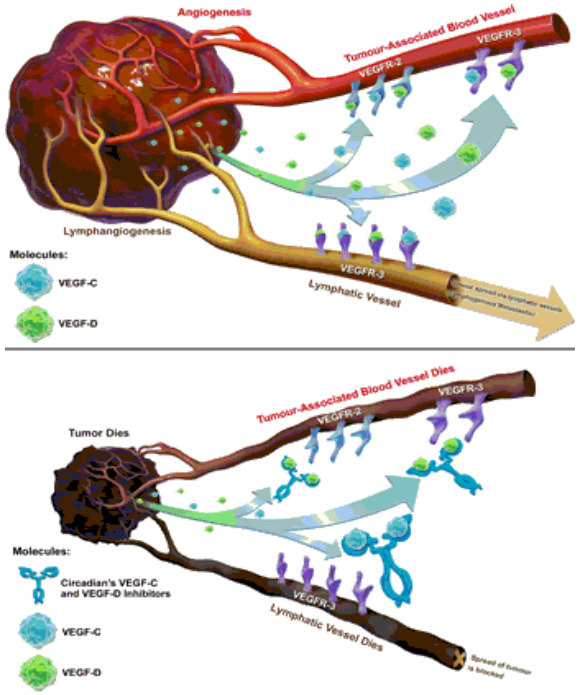
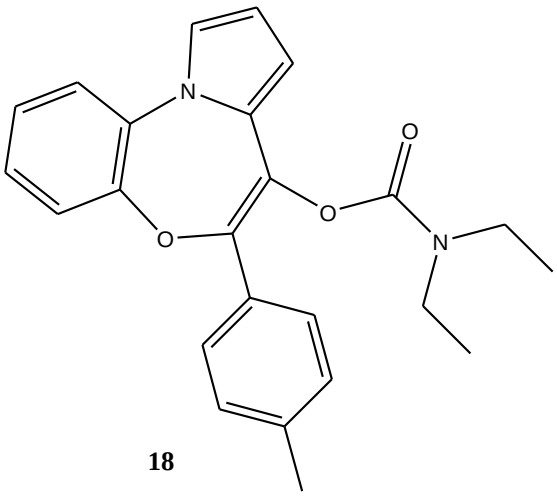


Figure 8

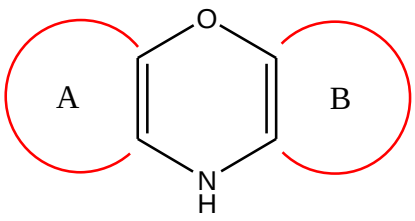
It was also found, in the breast cancer cell line MCF-7 and in the chronic myeloid leukemia K562, an arrest in prometaphase, an accumulation of B_1 cyclin and an activation of B_1 cyclin/CDK1 kinase, similar to the more representative antimicrotubular agents such as nocodazole and paclitaxel. Thanks to indirect immunofluorescence studies, depolymerization of microtubules was in fact detected in the cell lines treated with the benzoxazepine derivatives and, *in vitro,* the inhibition of tubulin assembly was also underlined. [29]

Moreover, compound **18** showed antiproliferative effect by arresting the cell cycle in G_1 phase avoiding cytotoxic effects. It seemed to enhance the apoptotic effectiveness of STI571 (Imatinib) in chronic myeloid leukemia. The induction of apoptosis by PBO/STI751 resulted in the activation of caspase 8, cleavage of PARP (Poly (ADP-ribose) polymerase) and Bcl-2 (antiapoptotic protein), up-regulation of the proapoptotic protein Bim and down-regulation of Bcr-Abl. [30]



18

Moreover, in literature there are some variously condensed oxazines **19** showing an interesting *in vitro* antiproliferative activity against the highly metastatic osteosarcoma LM8G7 and two ovarian cancer cell lines OVSAHO and SHOV-3 by binding some growth factors/cytokines VEGF (vascular endothelial growth factor), TNF-α (tumor necrosis factor), HB-EGF (heparin-binding epidermal growth factor-like growth factor) that appeared to be involved in cancer progression.



19

They also inhibited the proliferation of LM8G7 cells that express VEGF, of OVSAHO cells expressing TNF-α and of SHOV-3 cells that express HB-EGF in a micromolar range concentration. And finally they seemed to be also able to inhibit *in vitro* the LM8G7 migration and invasion, the angiogenic events, such as endothelial cells proliferation and migration, and the formation of capillary-like structures. (Fig.9) [31]

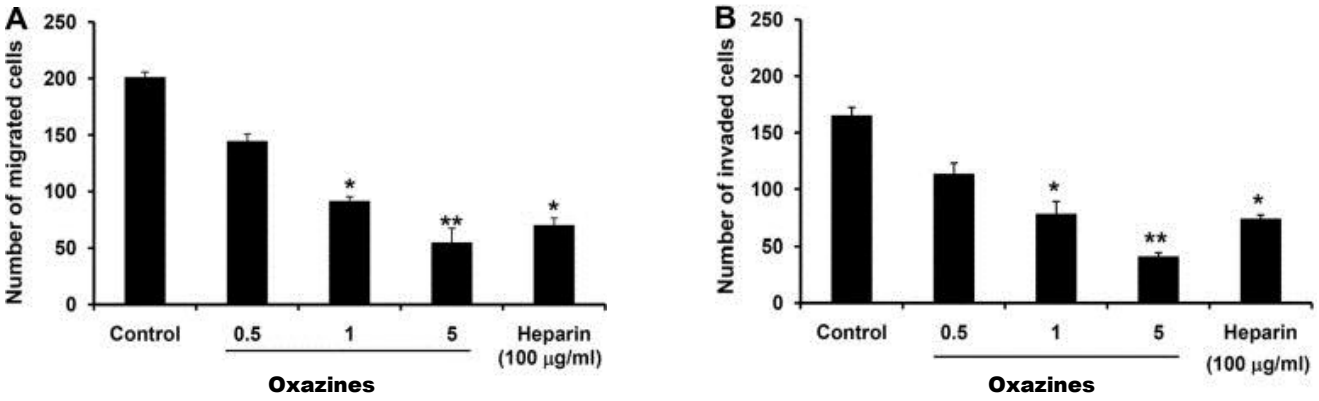
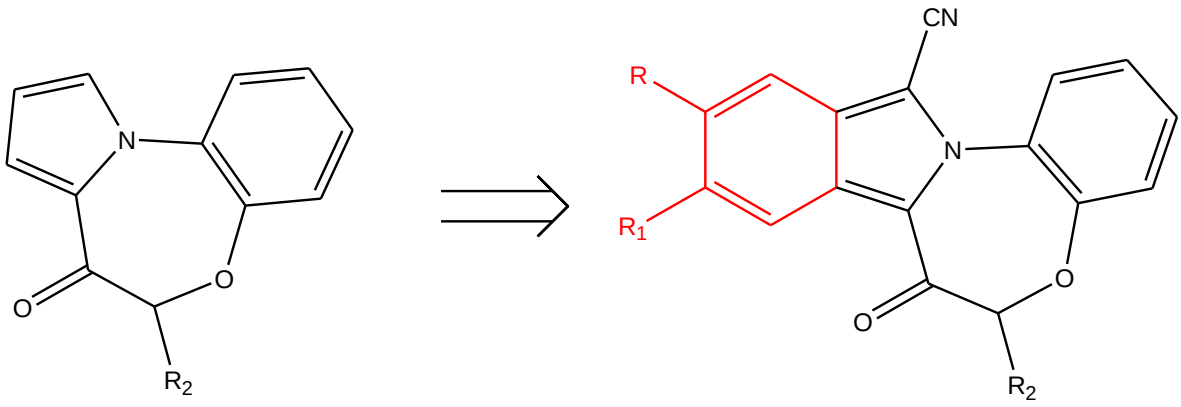


Fig. 9 Oxazines (0.5-5 mM) or heparin effects (100 mg/mL) on migration (A) and invasion (B) of LM8G7 cells. The data shown represent the mean values ± SD of two different experiments.

Considering the interesting results shown by both the pyrrolo[1,2-*d*][1,5]benzoxazepine derivatives and the isoindolo[2,1-*a*]quinoxalines as well as the experience gained in the synthesis of the isoindole nucleus, the purpose of the project was the synthesis of new isoindolo[1,2-*d*][1,5]benzoxazepine heterocycle in order to evaluate if the substitution of the pyrrole ring with a isoindole ring could influence the antitumor and antiviral activities.



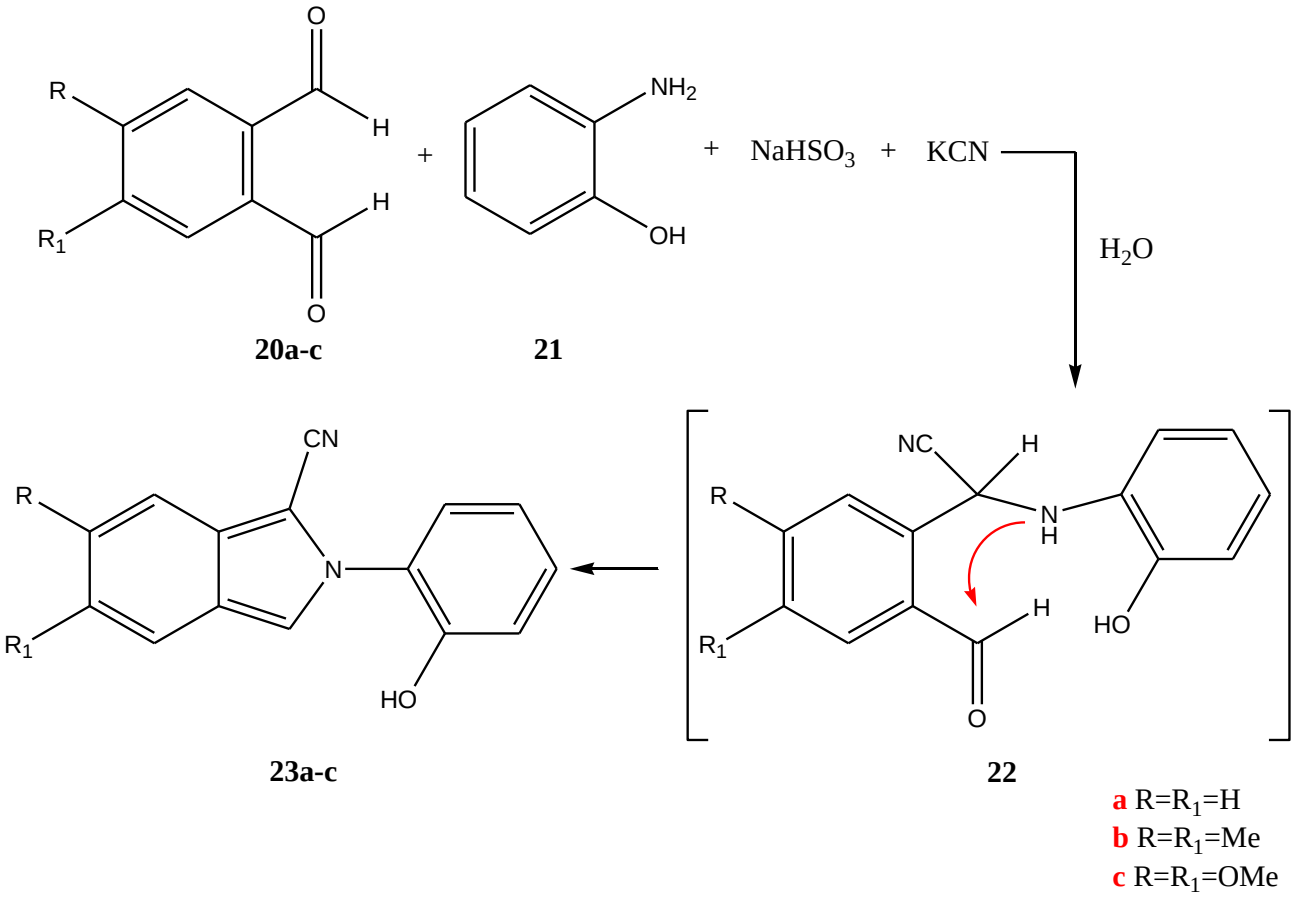
pyrrolo[1,2-*d*][1,5]benzoxazepine

isoindolo[1,2-*d*][1,5]benzoxazepine

R=R_1=H, Me, OMe
R_2= phenyl, *p*-tolyl

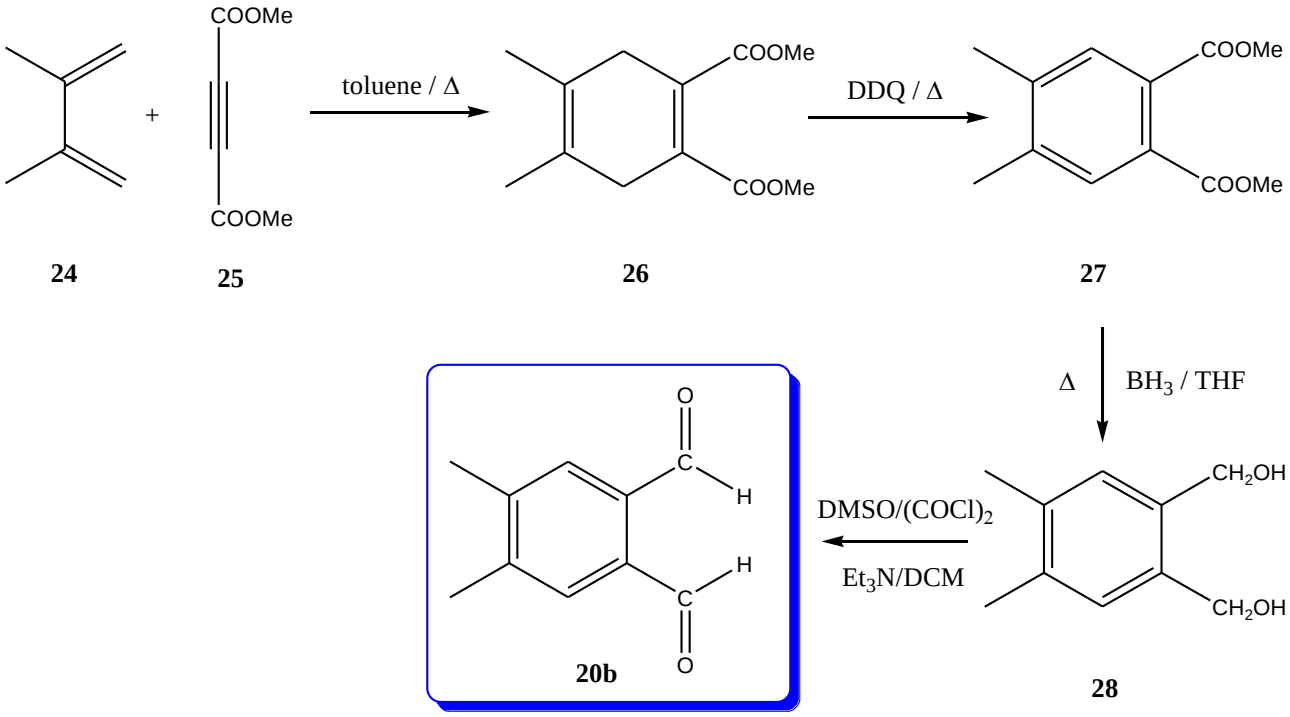
RESULTS AND DISCUSSION

The synthetic pathway involved the synthesis of the 2-(2'-hydroxyphenyl)-2*H*-isoindole-1-carbonitrile intermediates **23a-c**, prepared by Strecker like reaction between different *ortho*-phthaldialdehydes **20a-c** and 2-aminophenol **21** in the presence of sodium hydrogensulfite and potassium cyanide.



We initially focused on the synthesis of *ortho*-phthaldialdehydes **20b-c** not commercially available. A large number of papers reports the oxidation of alcohols to aldehydes using activated dimethyl sulfoxide (DMSO). The most widely used procedure in oxidation reactions is known as "Swern oxidation", it involved the use of oxalyl chloride (CO_2Cl_2) as activating agent of DMSO.

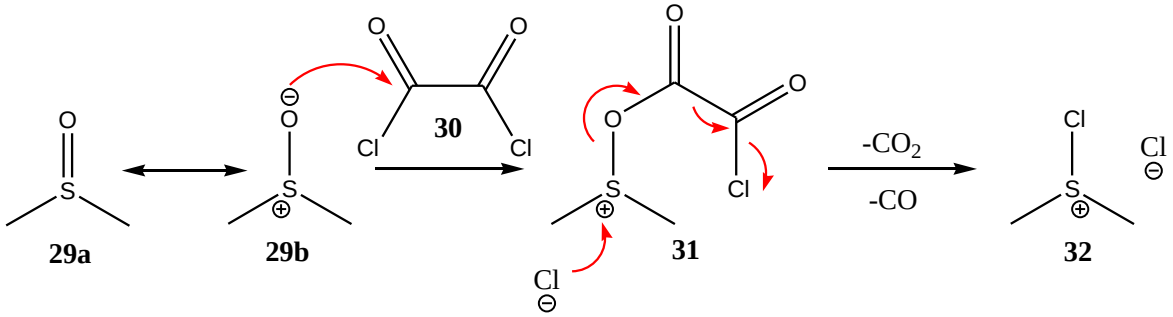
Farooq in a work of 1994 reported the synthesis of the dimethyl-*ortho*-phthaldialdehyde **20b** by oxidation of the corresponding 1,2-dimethanol **28**. [32]



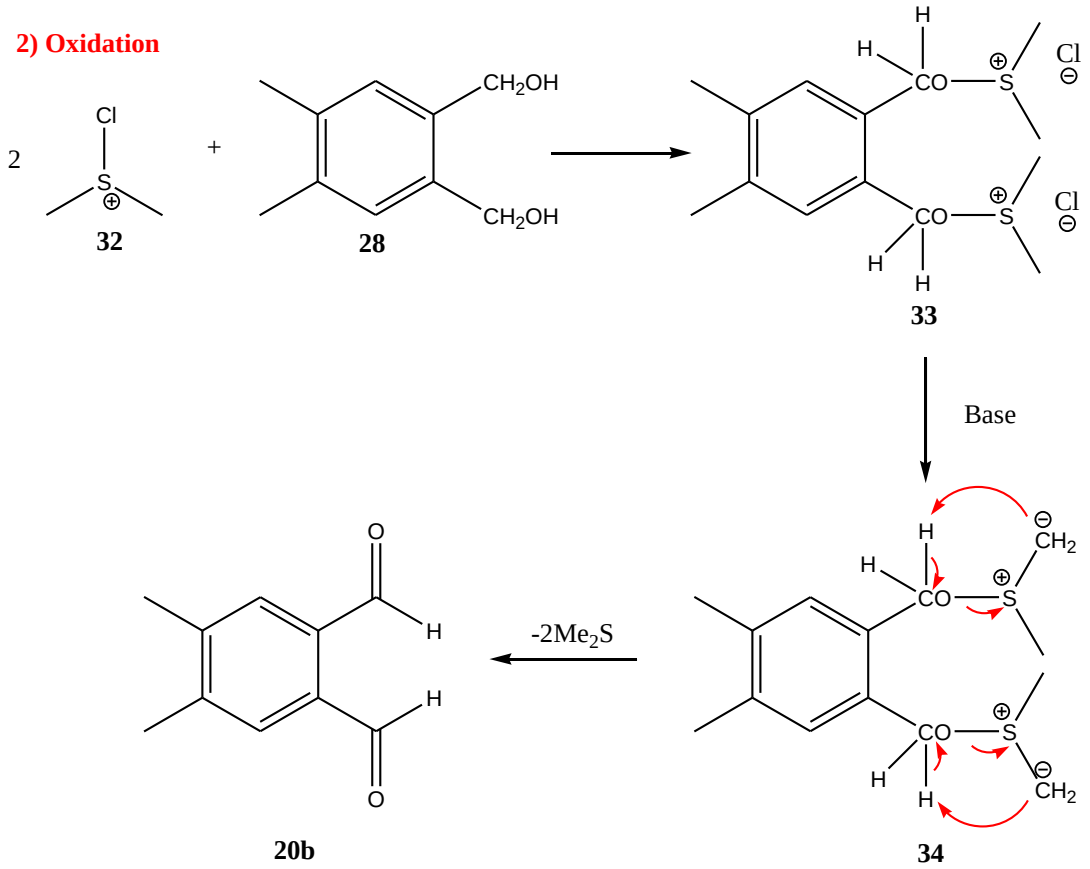
The mechanism of the oxidation could be explained through two primary steps: the activation of DMSO and the following oxidation.

In the first step, the reaction between the DMSO (**29b**) and the CO_2Cl_2 (**30**) led to the formation of the intermediate **31**, which quickly decomposed to form the dimetilclorosulfonil chloride **32**.

1) DMSO Activation



In the second step the formed dimetilclorosulfonil chloride **32** reacted with the 1,2-dimethanol **28** to generate first the alkyl sulfonium ion **33** which, after treatment with a base (triethylamine), underwent deprotonation to give the ylide **34**. The subsequent elimination of two molecules of dimethylsulfide led to the formation of the aldehyde **20b**.

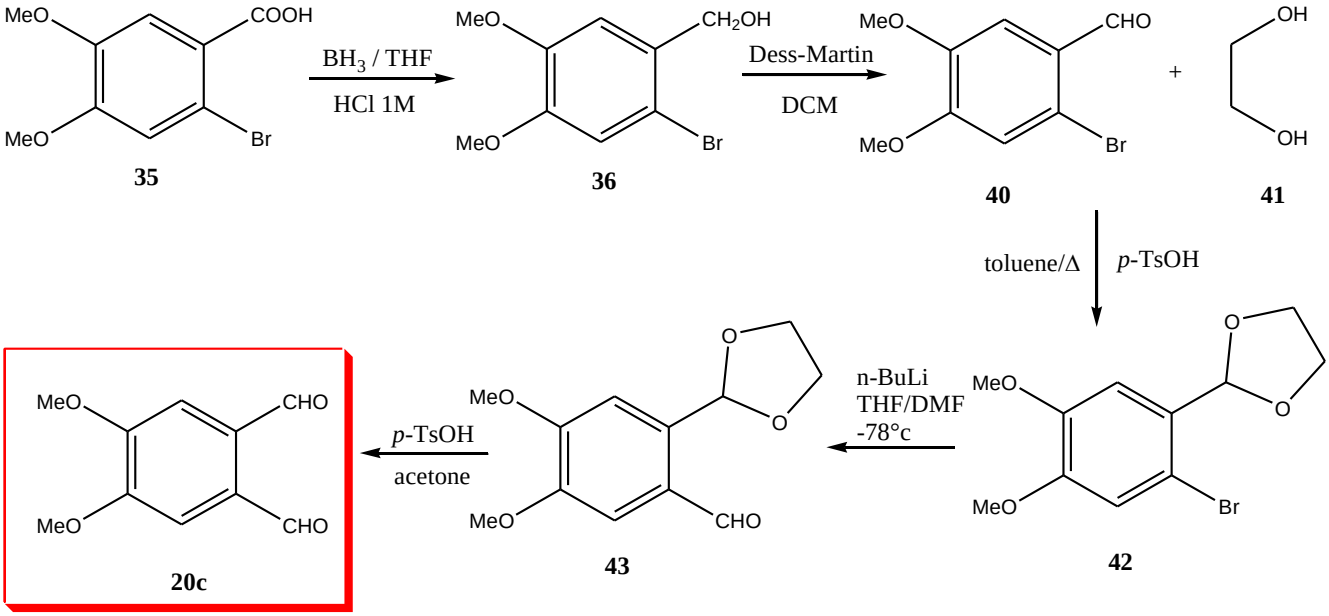


It was therefore necessary to synthesize the 1,2-dimethanol **28** through the reduction of the corresponding dimethylester **27**.

This latter compound **27** was synthesized using 2,3-dimetilbutan-1,3-diene **24** as starting material**.** The Diels Alder reaction between derivative **24** and dimethyl acetylene dicarboxylate **25** in toluene refluxing for 24 hours, led to the isolation, in high yield (95%), of intermediate **26.** The subsequent oxidation of derivatives **26** with DDQ (2,3-dichloro-5,6-dicyano-benzo-1,4-quinone) in chlorobenzene refluxing for 24 hours, led to the isolation of the desired ester **27** in high yield (92%). The reduction of the derivative **27** with borane in tetrahydrofuran refluxing for 20 hours led to the formation of the alcohol **28** with 80% yield.

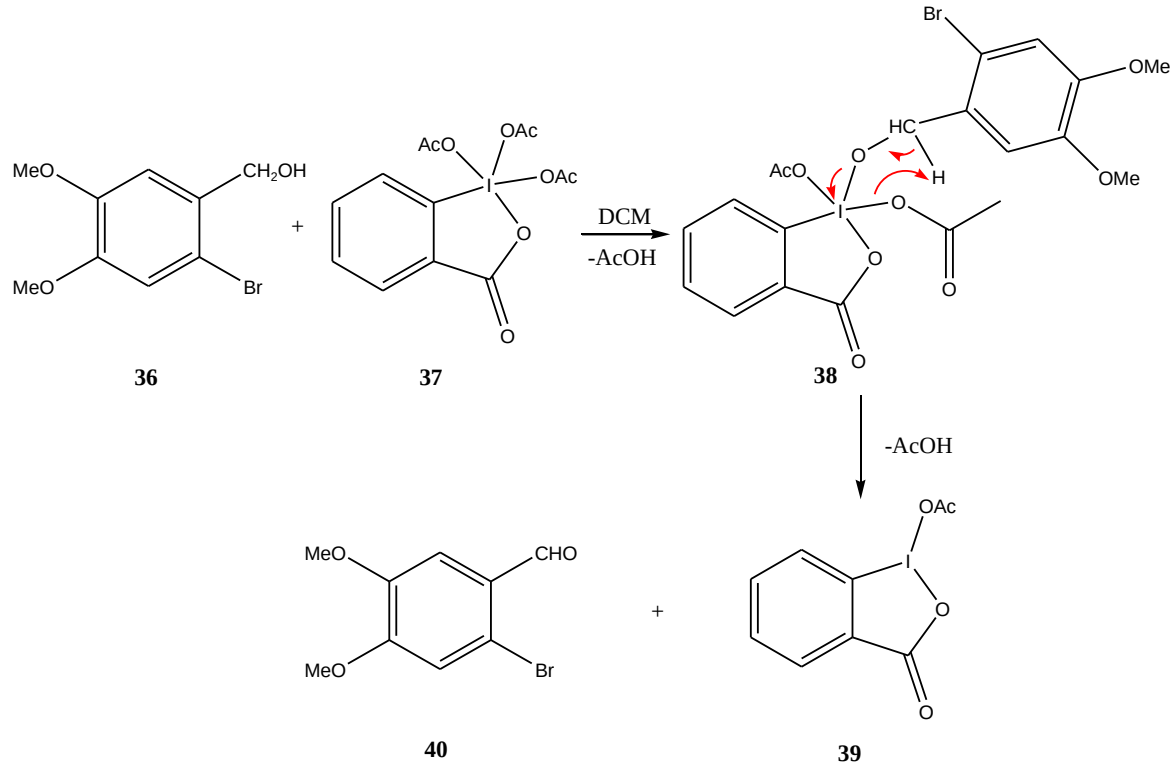
The oxidation of the alcohol group, using dimethyl sulfoxide, oxalyl chloride and triethylamine as base in dichloromethane under an argon atmosphere at -78 ° C gave the 4,5-dimethylphthaldialdehyde **20b**. (87%)

The synthetic route necessary to obtain the 4,5-dimethoxyphthaldialdehyde **20c** involved the reduction of the 2-bromo-4,5-dimethoxy benzoic acid **35** to the corresponding alcohol **36**, followed by its oxidation to give the aldehyde **40**. The aldehyde group of the latter had to be protected, lithiated and deprotected to give the desired *ortho*- phthaldialdehyde **20c**.



Reduction of the suitable bromo-benzoic acid **35** was carried out using a solution of borane in tetrahydrofuran. From the reaction mixture, after stirring at room temperature for 2 hours and following acidification, it was possible to isolate the corresponding alcohol **36** in excellent yield (90%). [33]

The alcohol **36** underwent oxidation using Dess-Martin periodinane (1,1,1-triacetoxy-1,1-dihydro-1 ,2-benziodossol-3(1*H*)-one) in dichloromethane at room temperature for 2 hours under argon atmosphere, to furnish the corresponding aldehyde **40** in excellent yield (90%).



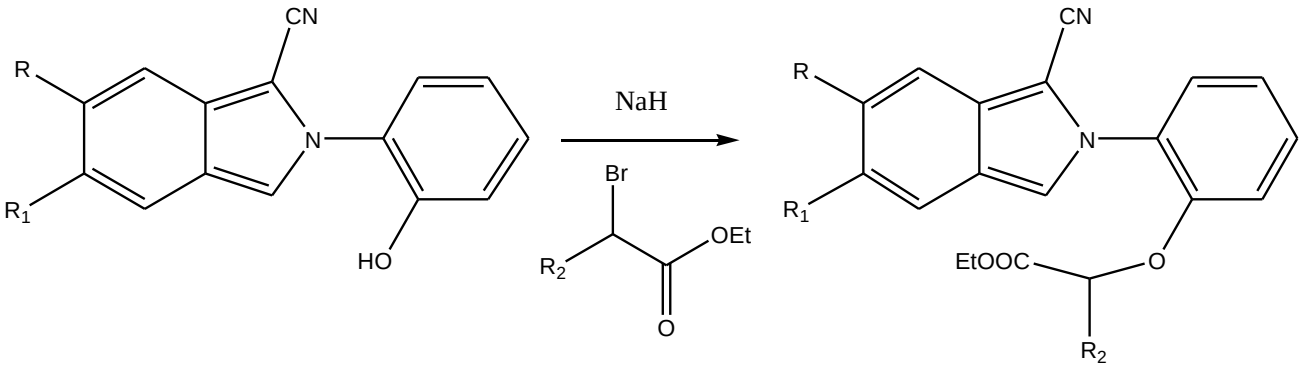
The obtained aldehydes were protected with ethylene glycol in the presence of a catalytic amounts of *p*-toluensulfonic acid in toluene refluxing for 24 hours using the Dean-Stark apparatus that, by removing the water formed in the reaction process, improve the rate of the reaction giving the acetal **42** in high yield (90%). [34]

The subsequent litiation of the derivative **42** with *n*-BuLi (solution 1.6M in *n*-hexane) in presence of dimethylformamide in tetrahydrofuran at -78° C for 1 hour led to the aldehyde **43** in quantitative yield. Deprotection of the aldehyde group of the latter compound with *p*-toluensulfonic acid in acetone at room temperature, afforded the *ortho*-phthaldialdehyde derivative **20c** in excellent yield (88%).

All phthaldialdehydes **20a-c** were reacted with the commercially available 2-aminophenol **21** in the presence of sodium hydrogen sulfite and potassium cyanide to give the corresponding 2-(2'-hydroxyphenyl)-2H-isoindoles-1-carbonitrile **23a-e** in very good to quantitative yields (84-100%).

The reaction mechanism provided that the amino group attacks one of the two aldehyde functions of *ortho*-phthaldialdehydes. The following addition of an aqueous solution of potassium cyanide led the formation of a non-isolable intermediate **22**, that after a further nucleophilic attack and subsequent intramolecular rearrangement, gave the desired isoindole derivatives.

Starting from 2-(2'-hydroxyphenyl)-1-cyano-isoindoles **23a-c** it was possible to obtain the esters **45a-d** by *O*-alkylation using ethyl α-bromoesters **44a,b** in the presence of sodium hydride as base, in tetrahydrofuran refluxing, in good yields (52-76%).



23a-c

a R=R_1=H
b R=R_1=Me
c R=R_1=OMe

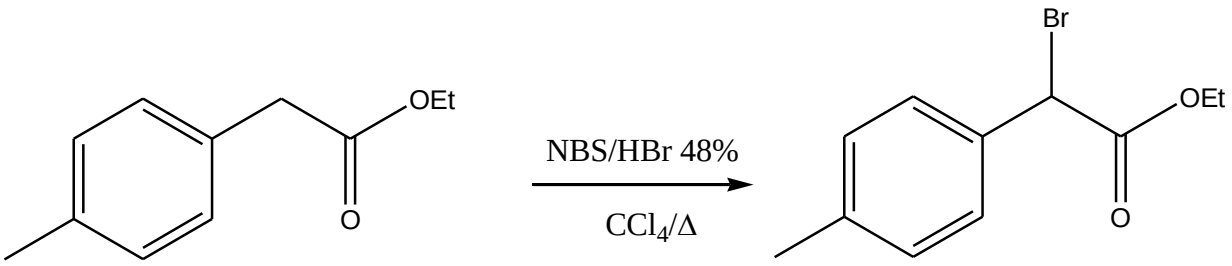
44a,b

a R_2=phenyl
b R_2=*p*-tolyl

45a-d

a R=R_1=H, R_2=phenyl
b R=R_1=Me, R_2=phenyl
c R=R_1=OMe, R_2=phenyl
d R=R_1=H, R_2=*p*-tolyl

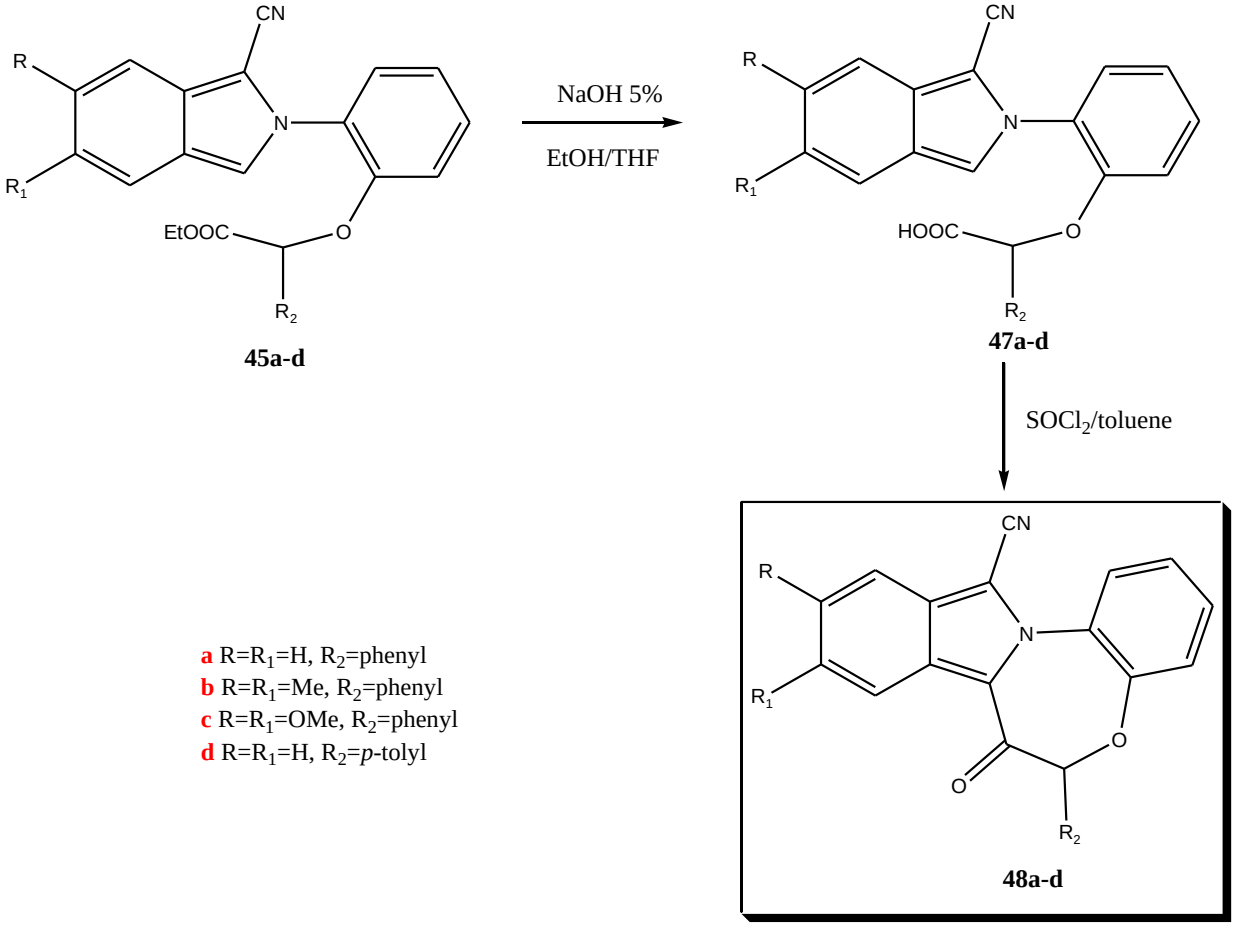
The *p*-tolyl-ethyl α-bromoester **44b,** not commercially available, was prepared starting from the ethyl *p*-tolylacetate **46** by reaction with NBS (*N*-bromosuccinimide) in carbon tetrachloride in the presence of HBr 48% refluxing for 26 hours, with 75% yield.



46 **44b**

The saponification of the ester group of **45a-d** derivatives with an aqueous NaOH 5% solution in a mixture of EtOH/THF (1:1) at room temperature for 15 minutes, allowed the formation of the corresponding acids **47a-d** from good to high yields (73-95%).

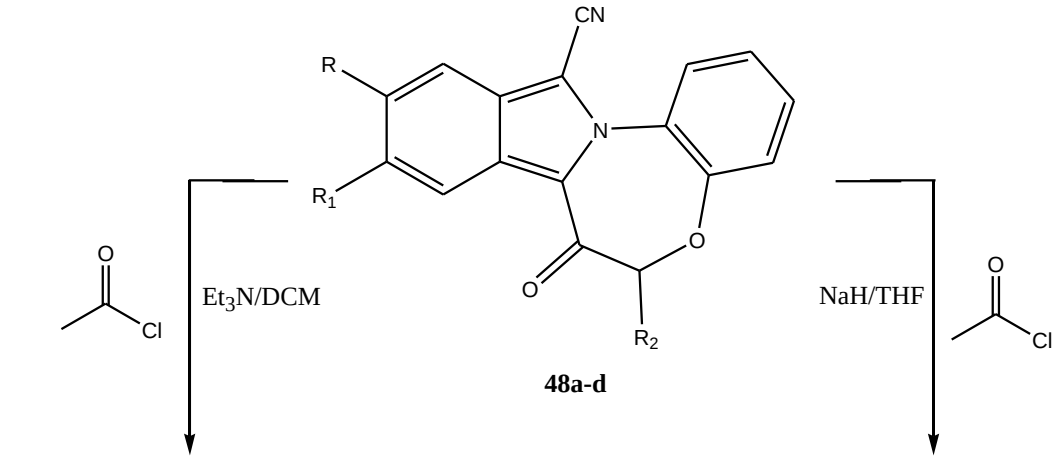
The acids thus obtained were reacted with thionyl chloride in toluene refluxing to generate the benzoxazepine nucleus of derivatives **48a-d**, through intramolecular cyclization in moderate yields (35-44%).

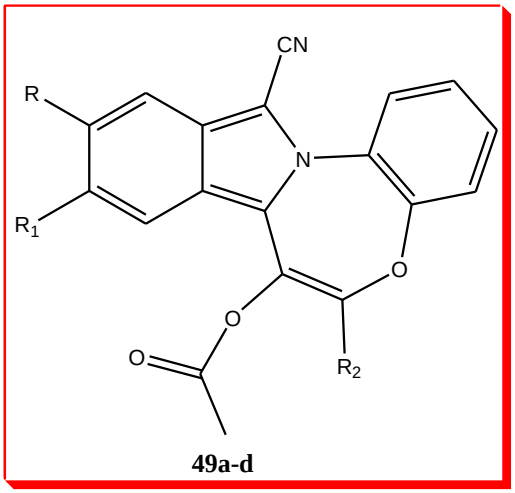


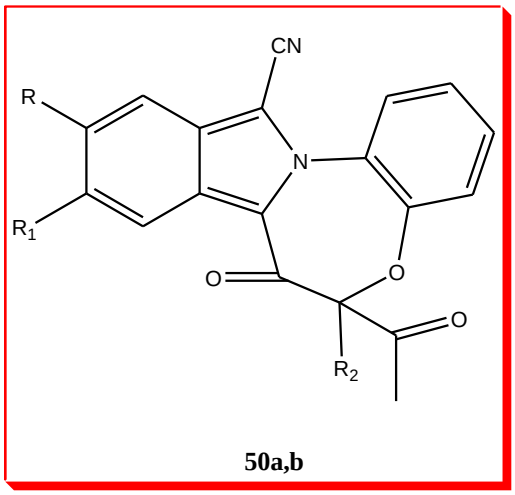
45a-d **47a-d** **48a-d**

a R=R_1=H, R_2=phenyl
b R=R_1=Me, R_2=phenyl
c R=R_1=OMe, R_2=phenyl
d R=R_1=H, R_2=*p*-tolyl

The isondolo[1,5]benzoxazepine derivatives **48a-d** were functionalized by the insertion of an acetyl group by reacting with acetyl chloride and a suitable base at room temperature; to obtain derivatives **49a-d**, the reaction was carried out in the presence of triethylamine as a base (64-90%) while sodium hydride was used for derivatives **50a,b** in tetrahydrofuran (50-54%).

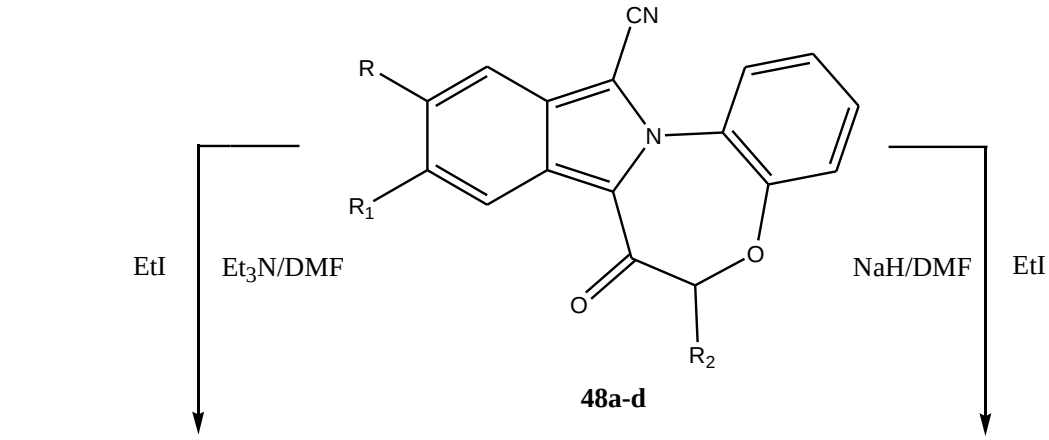




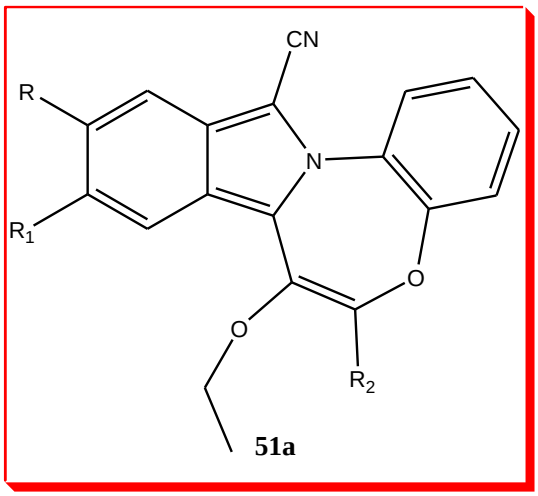


a R=R_1=H, R_2=phenyl
b R=R_1=Me, R_2=phenyl
c R=R_1=OMe, R_2=phenyl
d R=R_1=H, R_2=*p*-tolyl

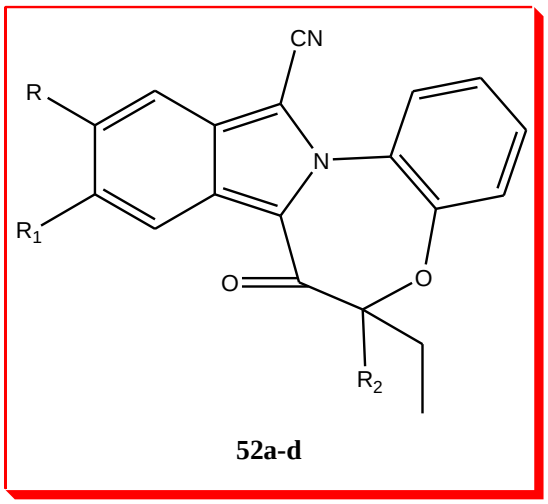
Moreover the same isondolo[1,5]benzoxazepine derivatives **48a-d** were functionalized by the insertion of an ethyl group using ethyl iodide and triethylamine as base for derivative **51a** (30%) and sodium hydride for derivatives **52a-d** (24-55 %).



48a-d



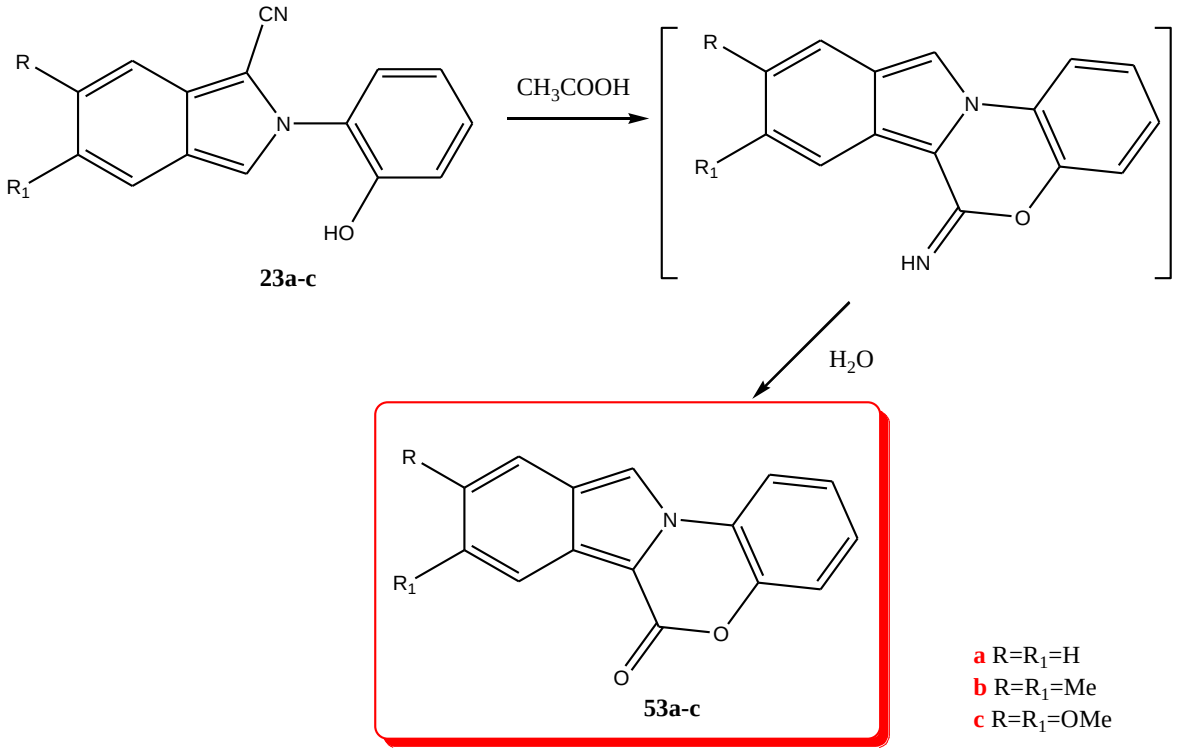
51a



52a-d

a R=R_1=H, R_2=phenyl
b R=R_1=Me, R_2=phenyl
c R=R_1=OMe, R_2=phenyl
d R=R_1=H, R_2=*p*-tolyl

Finally, starting from the 2-(2'-hydroxyphenyl)-2H-isoindoles-1-carbonitrile intermediates **23a-e** it was also possible to obtain the isoindole[1,4]benzoxazinones **53a-c** by refluxing in acetic acid and subsequent hydrolysis (10 -20%).



All derivatives obtained **48a-d, 49a-d, 50a-b, 51a, 52a-d** and **53a-c** were submitted to evaluate their biological activity as antitumor and/or antiviral agents.

Biological screenings were performed on seven isondolo[1,5]benzoxazepines (**48a**, **48b**, **48c**, **50a**, **51a**, **52a**, **52d**), selected by the National Cancer Institute (Bethesda), at one dose concentration (10^{-5} M), for the *in vitro* disease-oriented antitumor screenings against a panel of about 60 human tumor cell lines grouped in disease sub-panel including leukemia, non-small cell lung cancer, colon cancer, central nervous system cancer, melanoma, ovarian cancer, renal cancer, prostate cancer and breast cancer cell lines. **48a**, **48b**, **48c** showed the most interesting results were. These results take into consideration the percentage of growth inhibitory power. The three screened derivatives showed a very good selectivity towards the non-small lung cancer cell line NCI-H522 (range -43.73% - -32.37%) and also a good selectivity towards the BT-549 breast cancer cell line (16.96% - -7.13%) and the SR leukemia cell line (12.51% - -7.43%). (Fig.10, 11, 12)

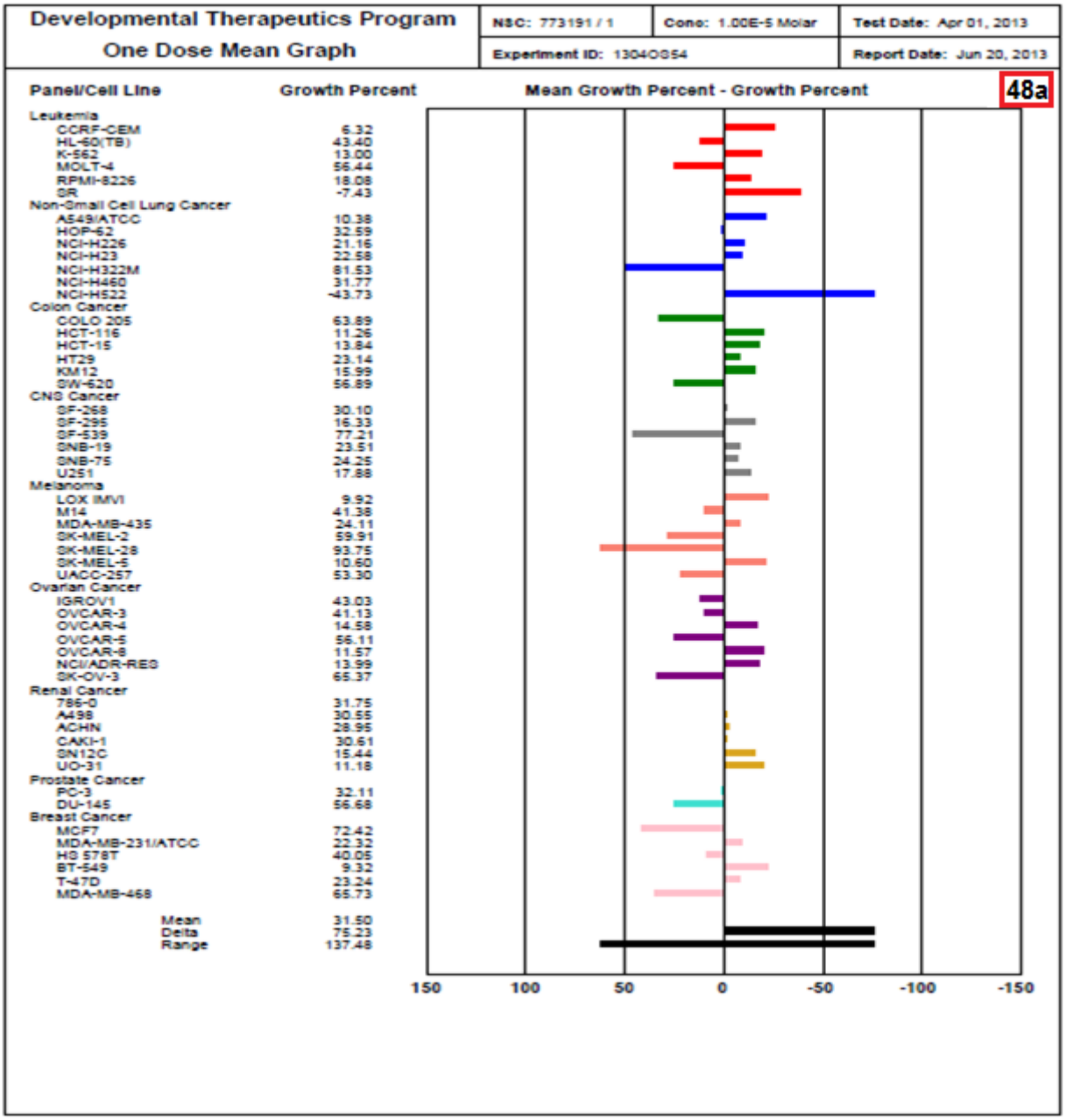

Developmental Therapeutics Program
One Dose Mean Graph
NSC: 773191 / 1
Conc: 1.00E-5 Molar
Test Date: Apr 01, 2013
Experiment ID: 1304OG54
Report Date: Jun 20, 2013
48a
Panel/Cell Line
Growth Percent
Mean Growth Percent - Growth Percent
Leukemia
CCRF-CEM 6.32
HL-60(TB) 43.40
K-562 13.00
MOLT-4 56.44
RPMI-8226 18.08
SR -7.43
Non-Small Cell Lung Cancer
A549/ATCC 10.38
HOP-62 32.59
NCI-H226 21.16
NCI-H23 22.58
NCI-H322M 81.53
NCI-H460 31.77
NCI-H522 -43.73
Colon Cancer
COLO 205 63.89
HCT-116 11.26
HCT-15 13.84
HT29 23.14
KM12 15.99
SW-620 56.89
CNS Cancer
SF-268 30.10
SF-295 16.33
SF-539 77.21
SNB-19 23.51
SNB-75 24.25
U251 17.88
Melanoma
LOX IMVI 9.92
M14 41.38
MDA-MB-435 24.11
SK-MEL-2 59.91
SK-MEL-28 93.75
SK-MEL-5 10.60
UACC-257 53.30
Ovarian Cancer
IGROV1 43.03
OVCAR-3 41.13
OVCAR-4 14.58
OVCAR-5 56.11
OVCAR-8 11.57
NCI/ADR-RES 13.99
SK-OV-3 65.37
Renal Cancer
786-0 31.75
A498 30.55
ACHN 28.95
CAKI-1 30.61
SN12C 15.44
UO-31 11.18
Prostate Cancer
PC-3 32.11
DU-145 56.68
Breast Cancer
MCF7 72.42
MDA-MB-231/ATCC 22.32
HS 578T 40.05
BT-549 9.32
T-47D 23.24
MDA-MB-468 65.73
Mean 31.50
Delta 75.23
Range 137.48
150
100
50
0
-50
-100
-150

Figure 10

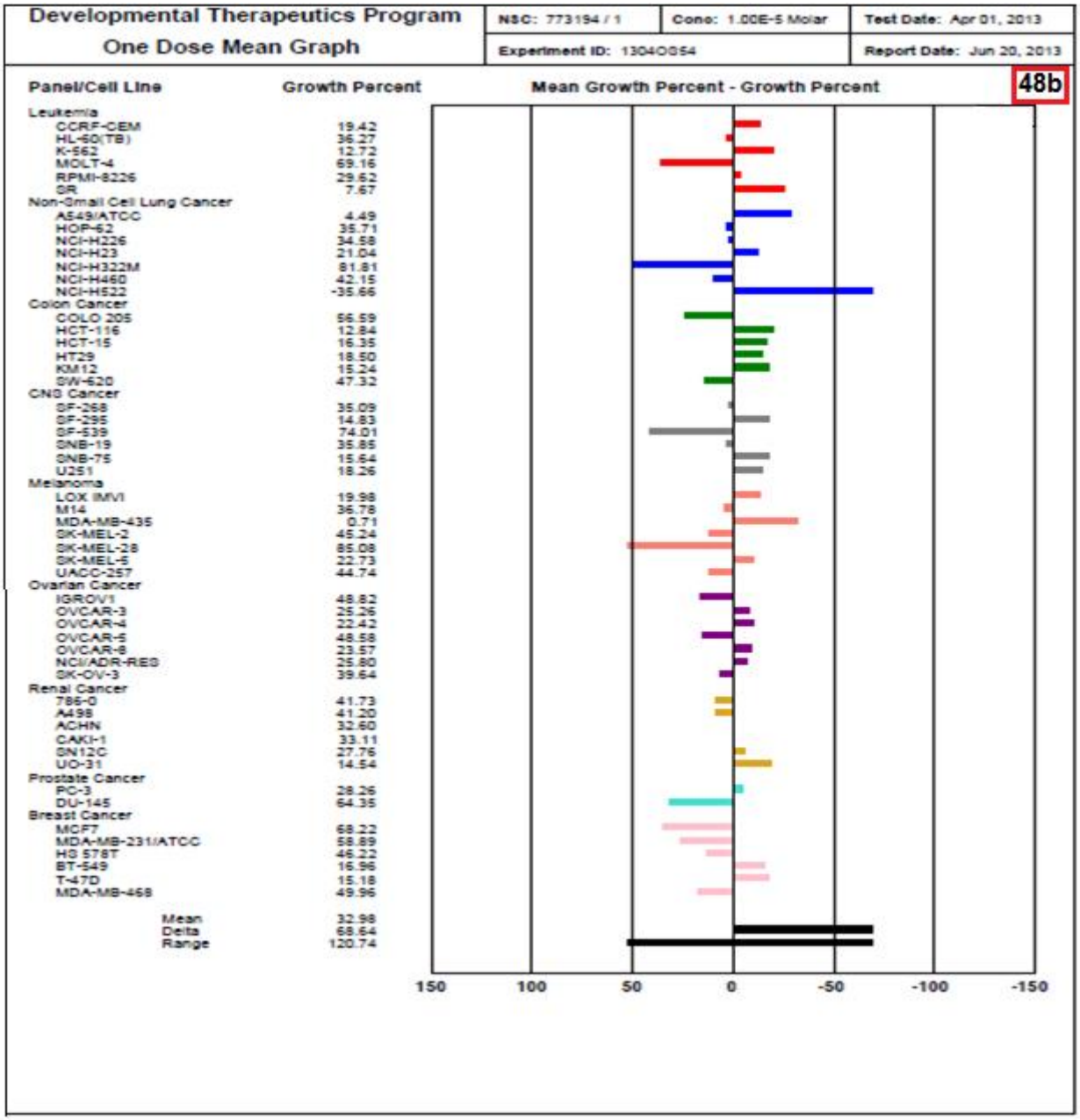

Developmental Therapeutics Program
One Dose Mean Graph
NSC: 773194 / 1
Conc: 1.00E-5 Molar
Test Date: Apr 01, 2013
Experiment ID: 13040064
Report Date: Jun 20, 2013
48b
Panel/Cell Line
Growth Percent
Mean Growth Percent - Growth Percent
Leukemia
CCRF-CEM 19.42
HL-60(TB) 36.27
K-562 12.72
MOLT-4 69.16
RPMI-8226 29.62
SR 7.67
Non-Small Cell Lung Cancer
A549/ATCC 4.49
HOP-62 35.71
NCI-H226 34.58
NCI-H23 21.04
NCI-H322M 81.81
NCI-H460 42.15
NCI-H522 -35.66
Colon Cancer
COLO 205 56.59
HCT-116 12.84
HCT-15 16.35
HT29 18.50
KM12 15.24
SW-620 47.32
CNS Cancer
SF-268 35.09
SF-295 14.83
SF-539 74.01
SNB-19 35.85
SNB-75 15.64
U251 18.26
Melanoma
LOX IMVI 19.98
M14 36.78
MDA-MB-435 0.71
SK-MEL-2 45.24
SK-MEL-28 85.08
SK-MEL-5 22.73
UACC-257 44.74
Ovarian Cancer
IGROV1 48.82
OVCAR-3 25.26
OVCAR-4 22.42
OVCAR-5 48.58
OVCAR-8 23.57
NCI/ADR-RES 25.80
SK-OV-3 39.64
Renal Cancer
786-0 41.73
A498 41.20
ACHN 32.60
CAKI-1 33.11
SN12C 27.76
UO-31 14.54
Prostate Cancer
PC-3 28.26
DU-145 64.35
Breast Cancer
MCF7 68.22
MDA-MB-231/ATCC 58.89
HS 578T 46.22
BT-549 16.96
T-47D 15.18
MDA-MB-468 49.96
Mean 32.98
Delta 68.64
Range 120.74
150
100
50
0
-50
-100
-150

Figure 11

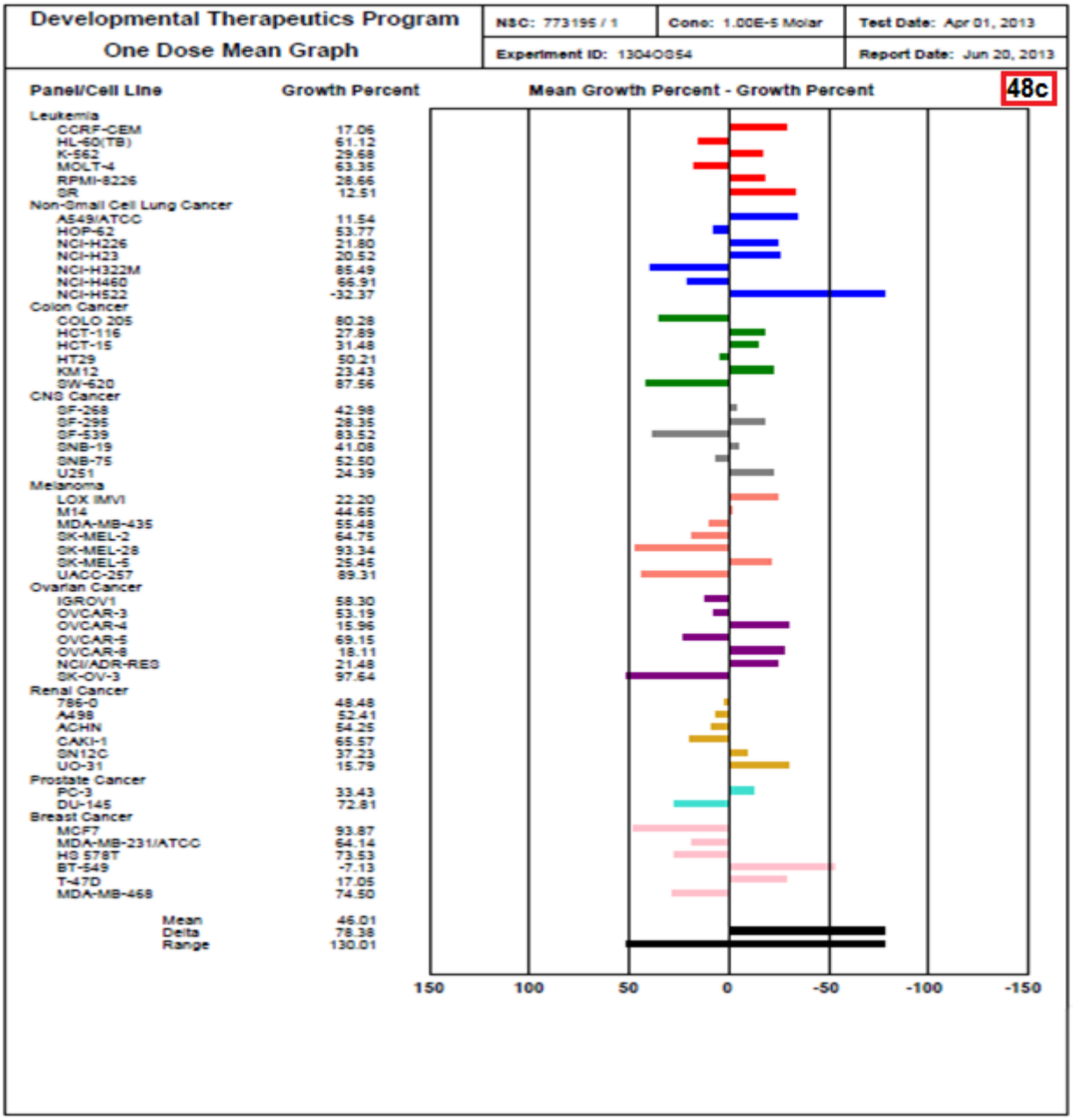


Figure 12

These most interesting compounds **48a, 48b** and **48c** were selected for the total-screening at five dose concentrations (10^{-4}-10^{-8} M).

All of them resulted active at micromolar concentration with a range of IC_{50} value of 1.8-18.2 μM for **48a**, 1.6-5.5 μM for **48b** and 1.6-91 μM for **48c.**

In particular they showed a particular selectivity towards some tumor cell line such as the leukemia cell line CCRF-CEM, the non-small lung cancer cell lines HOP-92 and NCI-H522, the CNS cancer cell line SNB-75 and the breast cancer cell line MDA-MB-2231 with the best activity observed for

the HOP-92 cell line with an IC_{50} value between 1.5-2.0 μM. (Tab.3)

TUMOR CELL LINE		IC_{50} (μM)		
		48a	**48b**	**48c**
CCRF-CEM	(Leukemia Cell Line)	2.7	2.5	2.7
HOP-92	(Non-Small Lung Cancer Cell Line)	2.0	1.6	1.5
NCI-H522	(Non-Small Lung Cancer Cell Line)	2.1	1.6	1.6
SNB-75	(CNS Cancer Cell Line)	2.2	2.3	4.4
MDA-MB-231	(Breast Cancer Cell Line)	2.8	2.6	4.6

Table 3

Moreover, all compounds were submitted to the University of Padova to investigate their antiviral activity.

They were solubilized in dimethylsulfoxide at 10 mM concentration. Initially it was determineted the molar extinction coefficient by spectrophotometric titration. It was used the TN 10/20 (10 mM Tris HCl and 20 mM NaCl) buffer for the analysis and the range of wavelengths of the spectra was between 250 and 550 nm. To evaluate the molar extinction coefficient the compound was added in a increasing way and the spectrum was recorded after each add. The data are shown in table. (Tab.4)

Compound	**EPSILON** $(cm^{-1}M^{-1})$	**λ (nm)**
48a	15343 ± 380	427.00
48b	16646 ± 473	352.53
48d	7025 ± 493	335.31
49c	14822 ± 286	398.92
49d	18079 ± 661	404.12
50a	13495 ± 489	393.76
52b	10854 ± 1476	370.95
53b	6524.3 ± 203	367.50
53c	11427 ± 197	276.69

Table 4

It was not possible to evaluate the molar extinction coefficient of the compounds missing in the table as they tumbled in the cuvette.

It was also examined whether the compounds emitted in the same range of emission of fluorescein as this could compromise the subsequent tests. The analysis was carried out with the multi-plate reader VICTORIII.

96-well plates were used and the final volume for each well was 100 µl adding the buffer TN 10/20, water and the compound tested with a final concentration of 100 µM. The analysis was carried out by exciting the derivatives at 485 nm and observing the possible emission at 535 nm, that is the emission wavelength of fluorescein.

Once demonstrated that all compounds were suitable to the following tests, it was analyzed their activity by FRET using the multi-plate reader VICTORIII.

The purpose of the test was to evaluate the inhibition of the nucleocapsid protein (NC protein) through the analysis of the variation of fluorescence.

The NC protein is a very small, basic protein containing two retroviral zinc finger which are connected by a short basic peptide linker. (Fig.13)

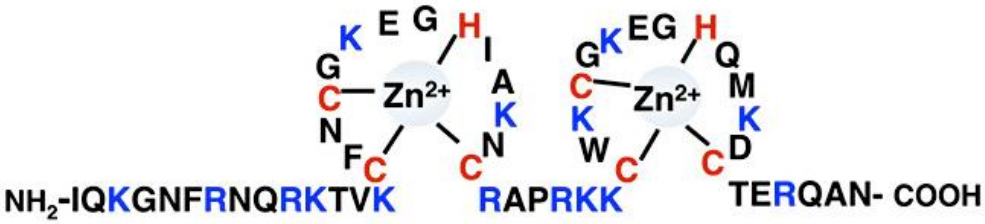


Figure 13

It is initially synthesized as part of part of the Gag polyprotein. It takes part in the selection of the genomic RNA for encapsidation into the new virus particle. [35] It also act as a nucleic chaperone, unfolding a cellular tRNA molecule and promoting the hybridization of a part of the tRNA to the viral genome, where it serves as a primer for reverse transcription, a critical process to the replicative cycle of the virus. [36] [37]

To evaluate the activity of compounds the oligonucleotide cTAR FAM- DAB labeled (Fluorescein and Dabcyl) was used; in his folded conformation the radiation of fluorescein was quenched by the acceptor of fluorescence Dabcyl and no signal could be registered; when the NC protein promoted the unfolding of the oligonucleotide, a process of denaturation of cTAR took place, with a consequent separation of the two markers which resulted in a fluorescence signal. When the protein

inhibitor was added it was possible to notice a decrease in the fluorescence signal due to the inhibition of the process of unfolding of the oligonucleotide.

The analysis was performed with increasing concentrations of all compounds to understand at which concentration the activity began to appear.

The solution of the oligonucleotide was prepared by diluting the cTAR in TNMg (1M Tris HCl, 1M NaCl and $Mg(ClO_4)_2$ 1M) and water to obtain a final concentration in the well of 0.1 μM. The solution was heated for 5 minutes in a water bath and then brought to room temperature to promote the folding of the cTAR.

The protein was diluted in water to a concentration of 0.8 μM, after that the solutions with the increasing concentrations of sample in dimethylsulfoxide were prepared: 0.1 μM, 0.5 μM, 1.0 μM, 5.0 μM, 10 μM, 50 μM and 100 μM.

The results was processed using the following equation:

$$\text{Fluorescence} = (F_x\text{-}F_0)/(F_m\text{-}F_0)$$

- Where F_x is the fluorescence in the presence of the compound, cTAR and NC
- F_0 is the fluorescence of cTAR
- F_m is the fluorescence of cTAR together with NC.

By analyzing the variation of fluorescence as a function of the logarithm of molarity we obtained the curves (for example **48b** Fig.14 and **49b** Fig.15) from which it was possible to obtain the IC_{50} values reported in the table (Tab.5).

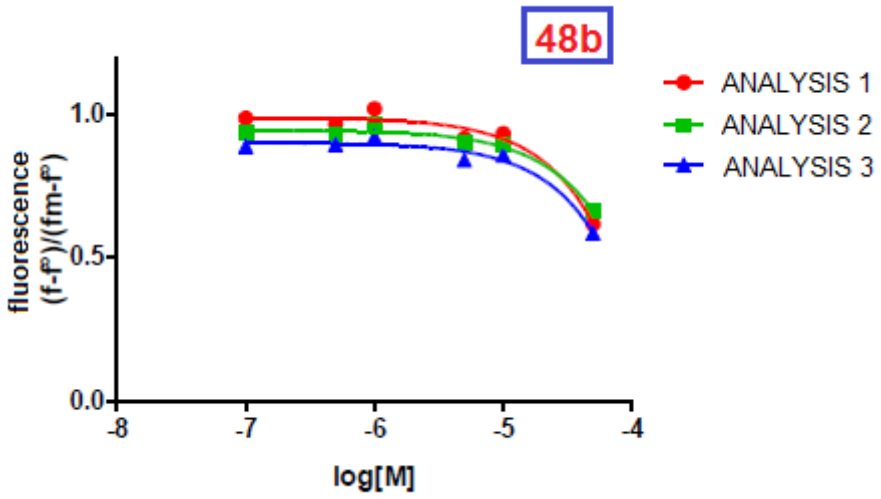


Figure 12

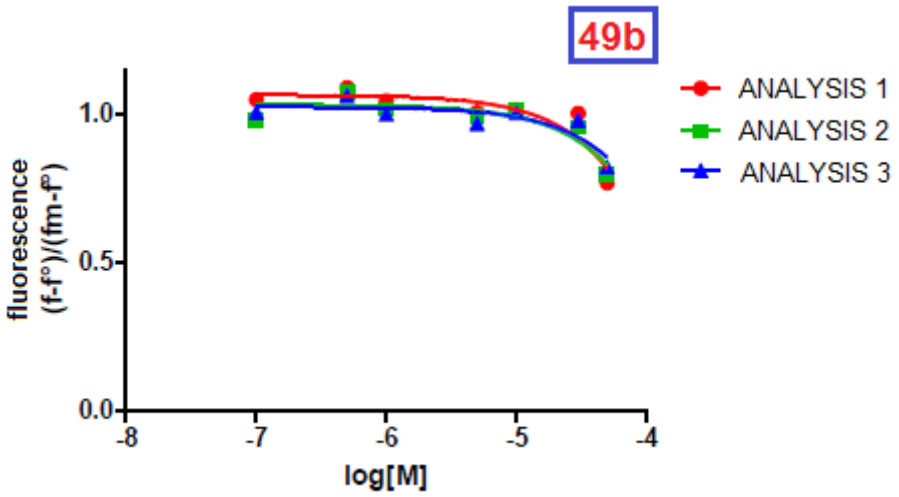


Figure 13

COMPOUND	IC_{50} (μM)
48a	>100
48b	>100
48c	>100
48d	>100
49a	>100
49b	>100
49c	>100
49d	>100
50a	>100
50b	>100
51a	>100
52a	>100
52b	>100
52c	>100
52d	>100
53a	>100
53b	>100
53c	>100

Table 5

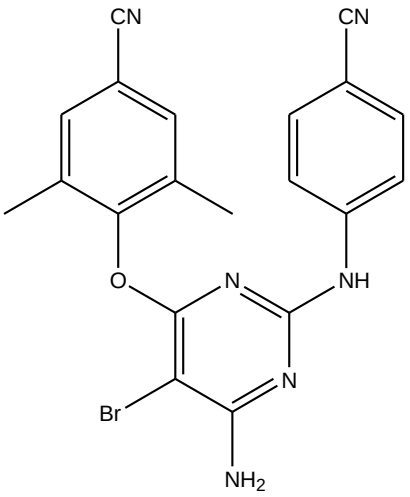
Unfortunately none of the 18 derivatives showed inhibition activity of the cTAR melting by the NC protein of HIV-1 up to a concentration of 100 μM. Further studies are still in progress.

INTRODUCTION

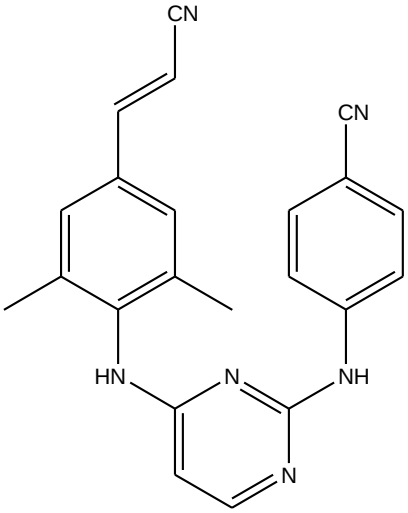
In attempt to overcome drug resistance, lesser toxicity, good patient adherence, and better pharmacokinetic properties of NNRTIs and find more potent inhibitor several cocrystals of HIV-1 RT with NNRTIs have been studied, showing that the structural features that appear to be important for the binding to the allosteric site include the presence of a double plane and the ability of those systems to adopt two conformations which were called “butterfly like” [38] [39] or “horseshoe” [40] conformation.

In particular hypothetical 3-D pharmacophore models were built by using a combined ligand- and structure-based molecular modeling approach to understand which were the main features that an NNRTI should incorporate.

For example in the past years, seven NNRTIs have been marketed or are undergoing clinical trials, etravirine **54**, rilpivirine **55**, RDEA806 **56**, [41] UK-453061 **57**, [42] IDX-899 **58**, [43] MK-4965 **59,** [44] and the drug candidate BILR 355 **60.** [45]



54



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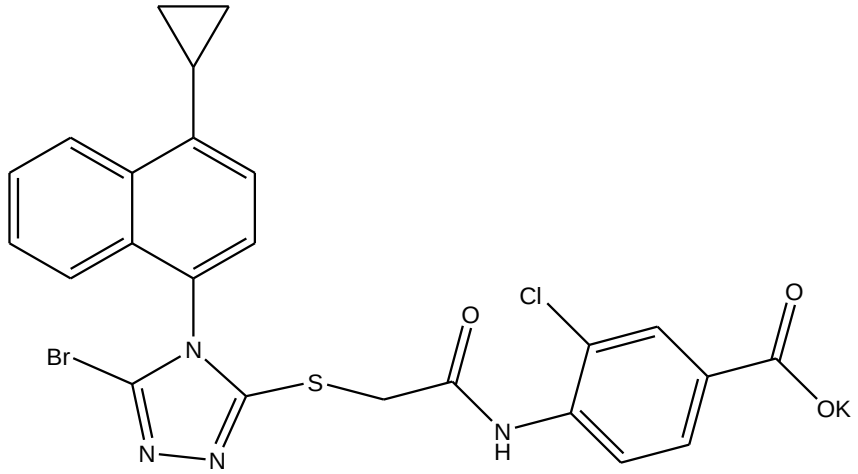
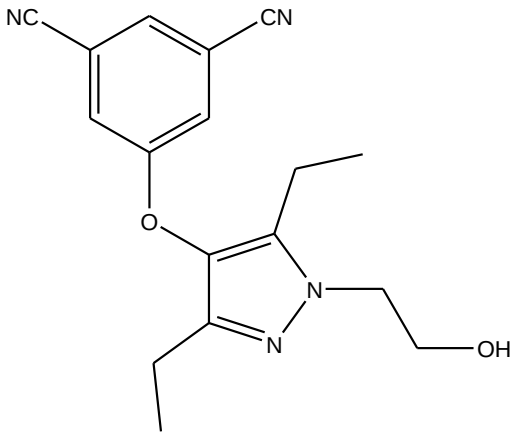
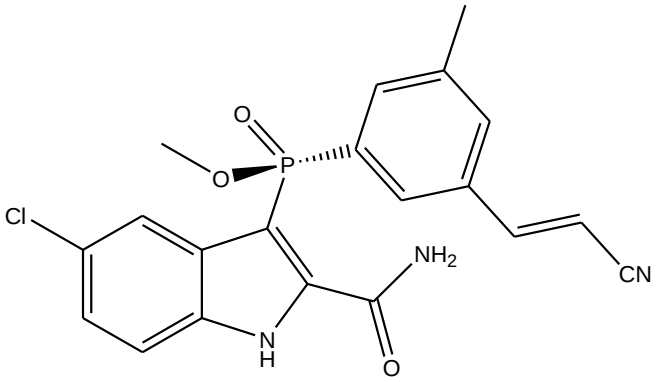

Br
N
N
N
S
O
N
H
Cl
O
OK

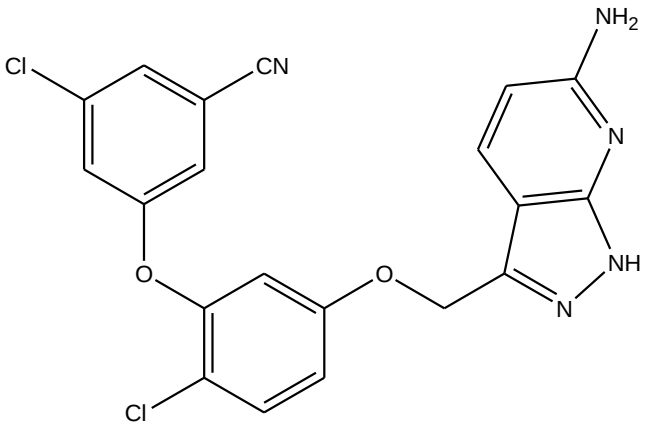
56


NC
CN
O
N
N
OH

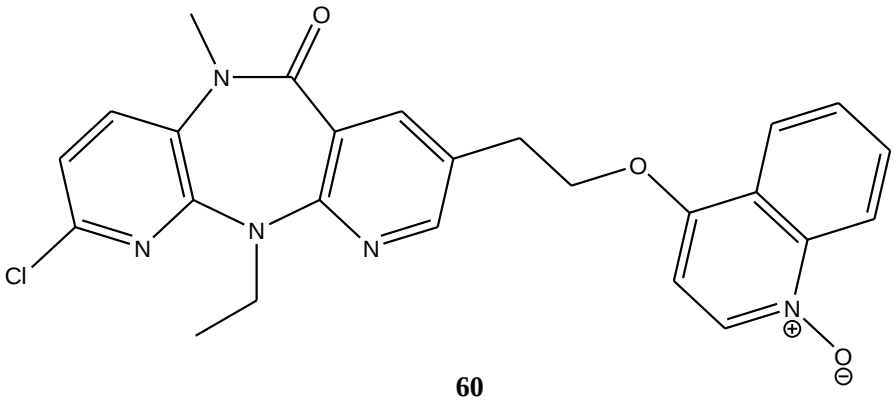
57


O
O
P
Cl
NH2
N
H
O
CN

58


Cl
CN
NH2
N
O
O
NH
N
Cl

59



60

According with the known bind information of these 7 compounds, a pharmacophore model was built. (Fig.14)

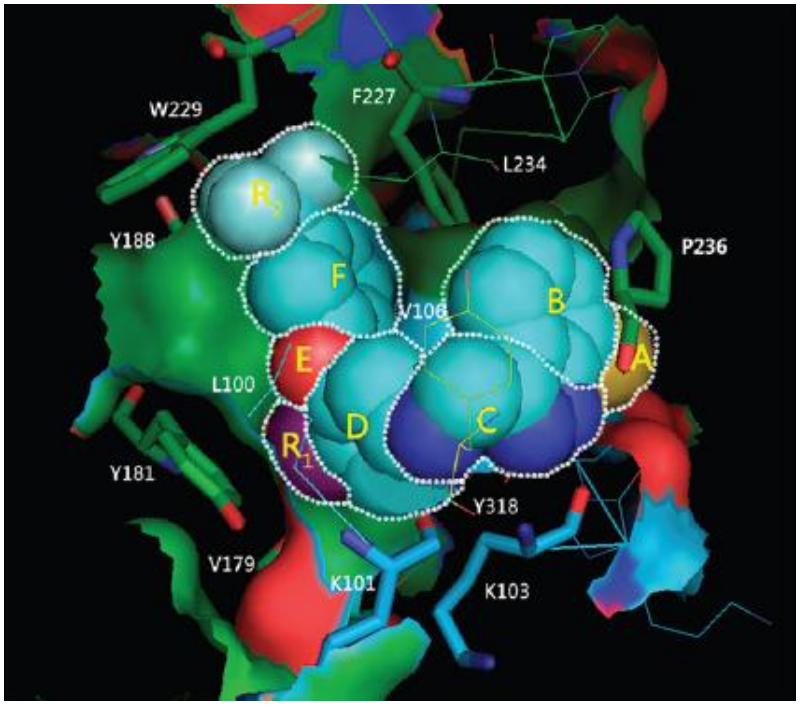


Figure 14

In this model F and B are the hydrophobic region that interact with the the residues Y181 (Tyr), Y188 (Tyr), W229 (Trp), F227 (Phe), V106 (Val), P236 (Pro), L100 (Leu), L234 (Leu), and Y318 (Tyr). D is the region that is responsible for the adaptability, aryl or alkyl groups, single ring or double ring, phosphonate or sulfonyl group for example. C is the part of the molecule responsible of the hydrogen bonding interaction with K101 (Lys), K103 (Lys) and P236 (Pro). A is the polar

moiety that interact with the solvent interface. The linkers between B and F (C and E) are responsible for the molecular flexibility. (Fig.15) [46]

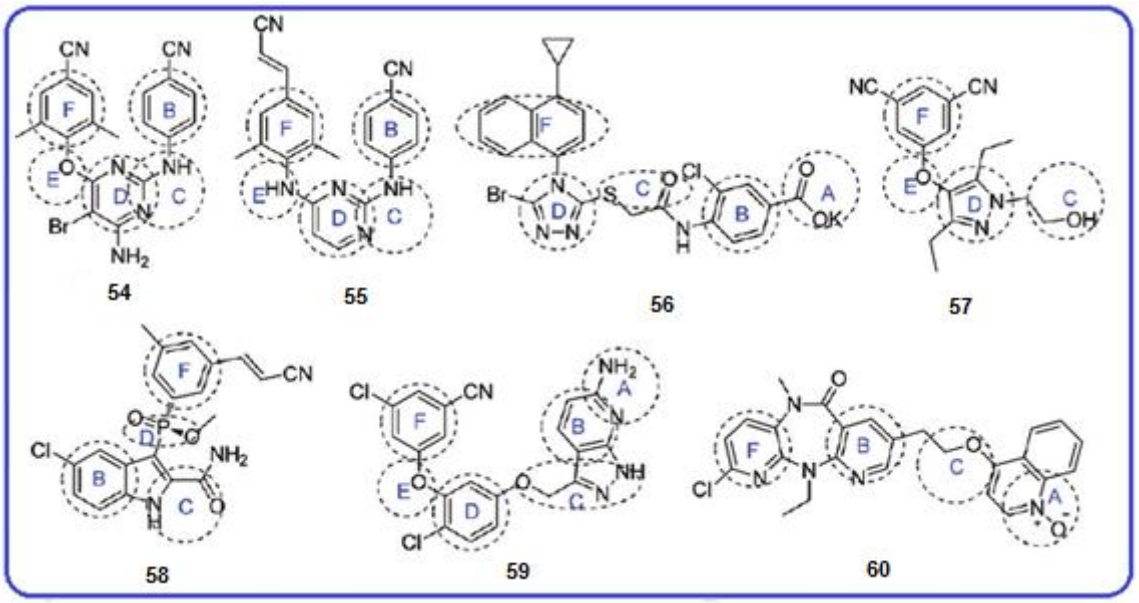
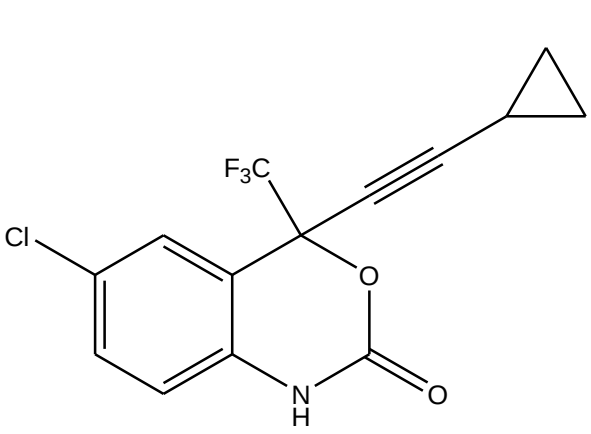
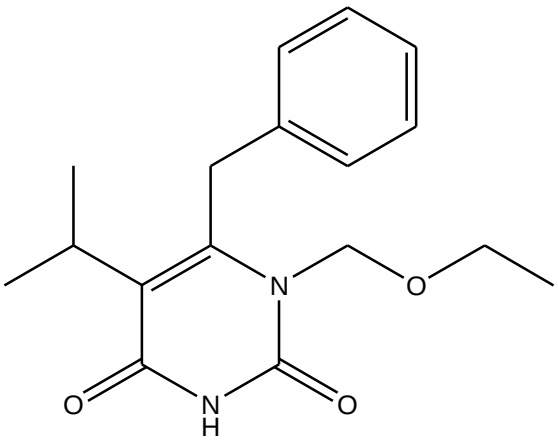


Figure 15

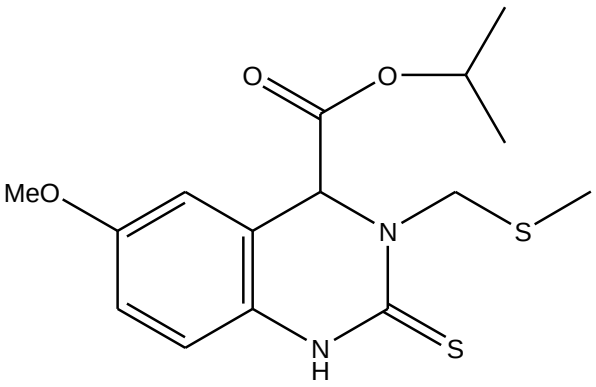
Moreover further information were given by a pharmacophore model built using the crystal structure of RT with 7 different ligands which were able to establish hydrogen bonding interactions with the backbone of the aminoacids K101 and/or K103: efavirenz **61**, emivirine **62**, HBY097 **63**, MSC204 **64**, UC781 **65**, 739W94 **66**, and TMC120 **67**.



61



62



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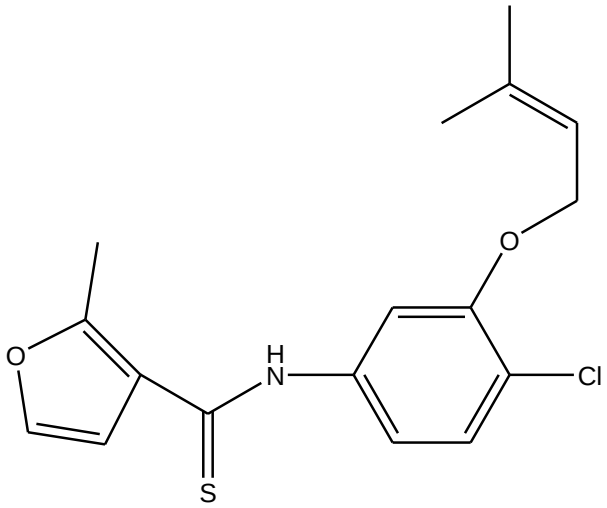

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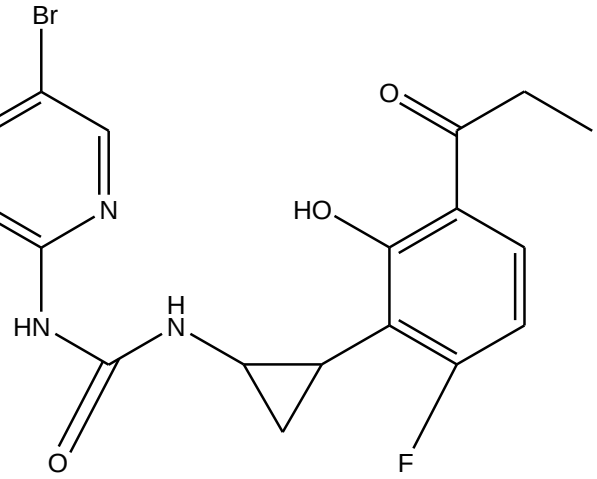

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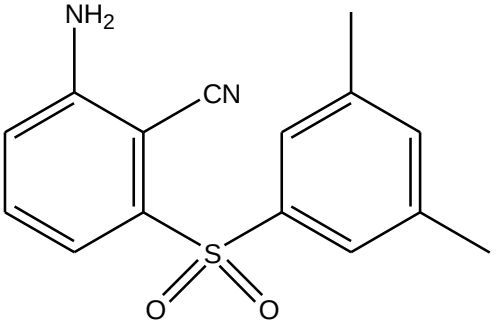

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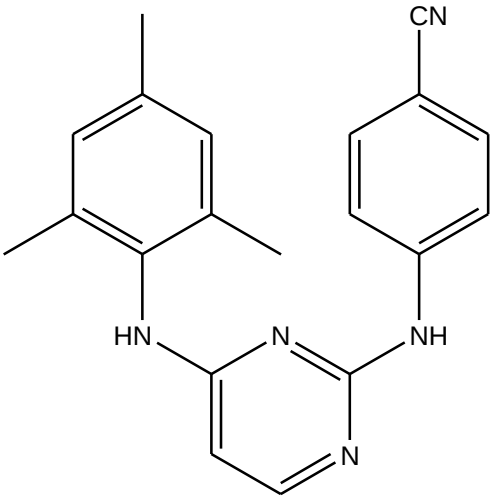


67

The final model contained three lipophilic parts and two hydrophobic region, one hydrogen bond acceptor and one hydrogen bond donor.

The figure 16 showed the alignment of the 3D coordinates of the bound ligand efavirenz **61** onto the five feature hypothesis built.

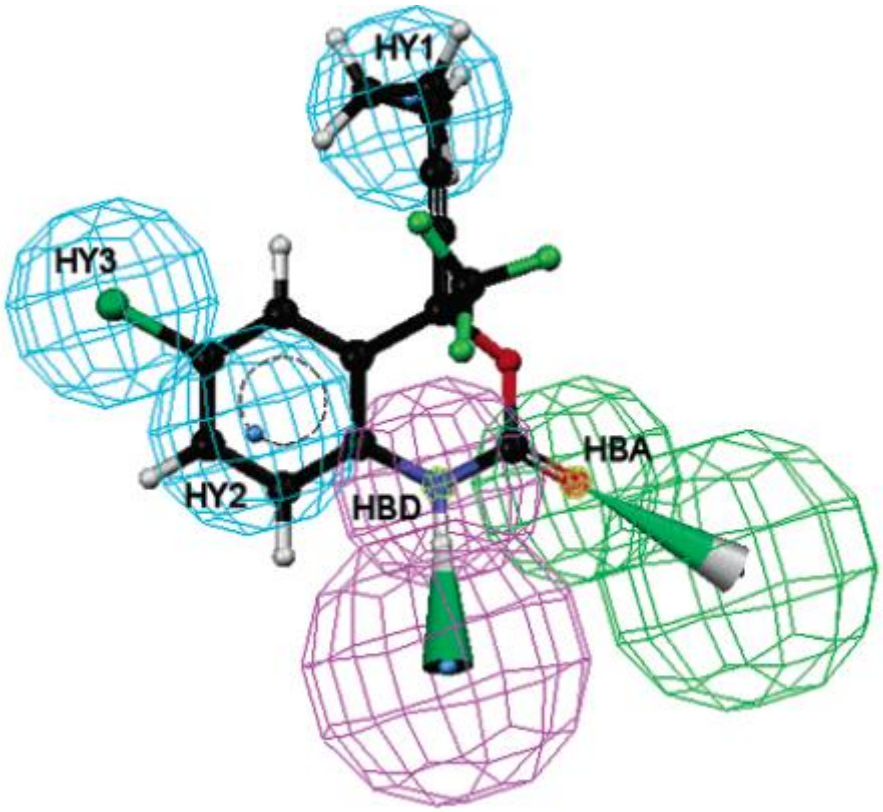
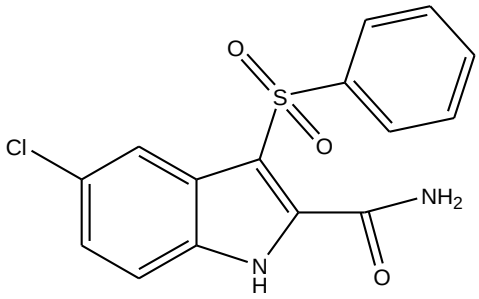


Figure 16

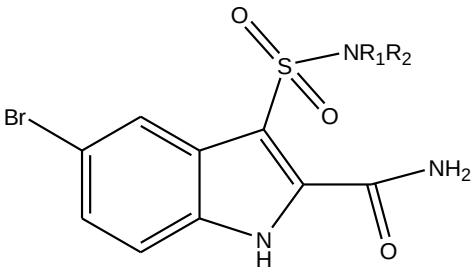
The HBA (hydrogen bond acceptor) and HBD (hydrogen bond donor) are mapped by the carboxiamide group (Lys101), while the three hydrophobic sites HYs are mapped by the cyclopropyl group (HY1, Trp229, Tyr181, Tyr188, Pro95), the benzene-fused ring (HY2, Val106, Phe227, Leu234, Pro236), and the chlorine atom (HY3, Leu100, Val106, Tyr318, Leu234, Pro236). [47]

Considering the necessary features described, a series of compounds that had most of them was observed to be the indole-3-sulfonamides. The 5-chloro-3-(phenylsulfonyl)indole-2-carboxamide **68** has been reported as a potent NNRTI against wild type RT (IC_{50}= 3 nM), it also showed a sub-micromolar activity against K103N (IC_{50}= 116 nM) and Y181C (IC_{50}= 71 nM), two HIV-RT mutants observed clinically with high frequency. [48]



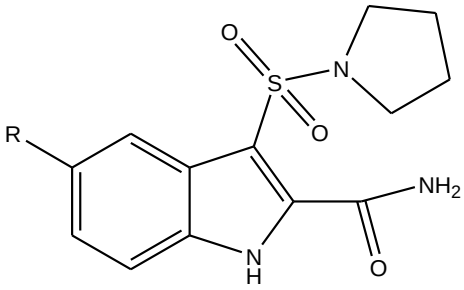
68

Considering these results different series of new indole-3-sulfonamides **69, 70, 71, 72** have been developed showing IC_{50} values at nanomolar concentrations. [49]



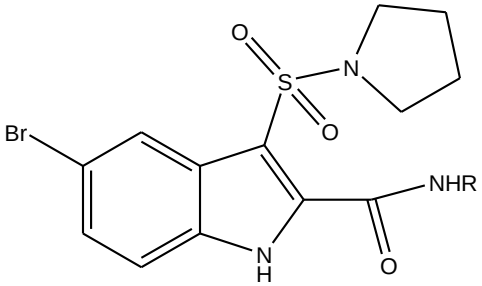
69

IC_{50} = 0.2-19 nM



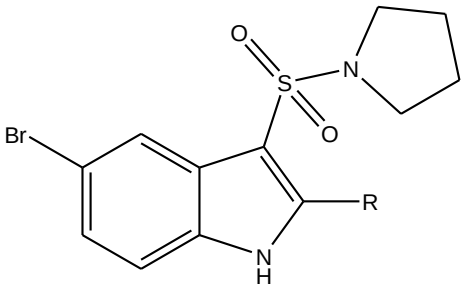
70

IC_{50} = 3.9-2200 nM



71

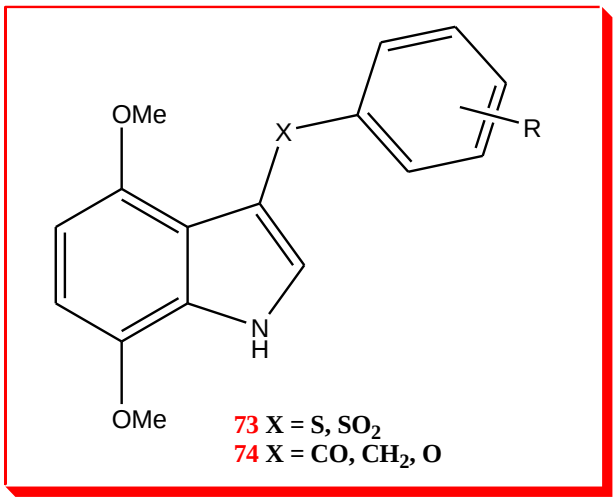
IC_{50} = 2.5-14 nM



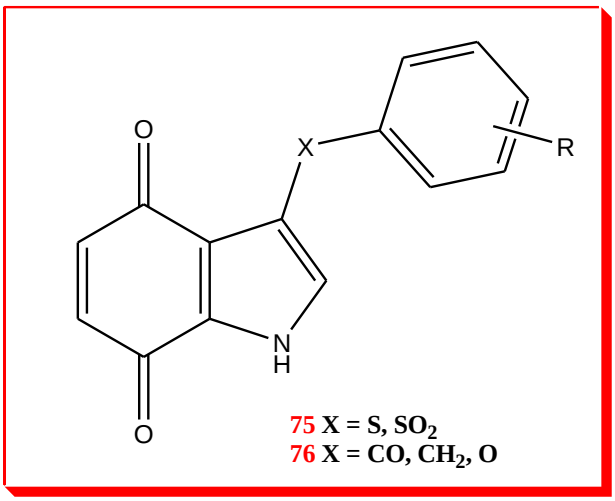
72

IC_{50} = 3.1-45 nM

In light of these results, the purpose of the project was the synthesis of new classes of indole **73, 74, 75** and **76** in order to improve the activity of the indole-3-sulfonamides compounds through the insertion of a group that is able to form hydrogen bonds as it was described in the pharmacophore models.



73 X = S, SO_2
74 X = CO, CH_2, O



75 X = S, SO_2
76 X = CO, CH_2, O

R = H, 2-Br, 2-Cl, 2-F, 2-Me, 2-OMe, 2-NO_2, 3-Me, 3-OMe, 3-NO_2, 4-Cl, 4-OMe, 4-NO_2, 2,6-dimethyl, 2,6-dimethyoxy, 2,6-diCl, 2,6-diF, 2,4-diNO_2, 3,4-diNO_2, 2,4,6-trimethyl, 3,4,5-trimethoxy, 4-Cl-3-NO_2

DOCKING STUDY

With the aim of gaining an insight into the binding mode and interactions of indole derivatives with HIV-1 RT and to improve the development of more active inhibitors, it was performed a docking study of these derivatives with HIV-1 RT.

For docking studies the X-ray crystallographic structure of HIV-1 RT protein with PDB code 1RT2 was selected. In this structure RT is constituted by two different subunits (chain A and B) and is co-crystallized with TNK 651 that bind just one of the two subunits. (Fig.17)

This structure was used to design new NNRTIs in several works present in literature. [50] [51] [52]

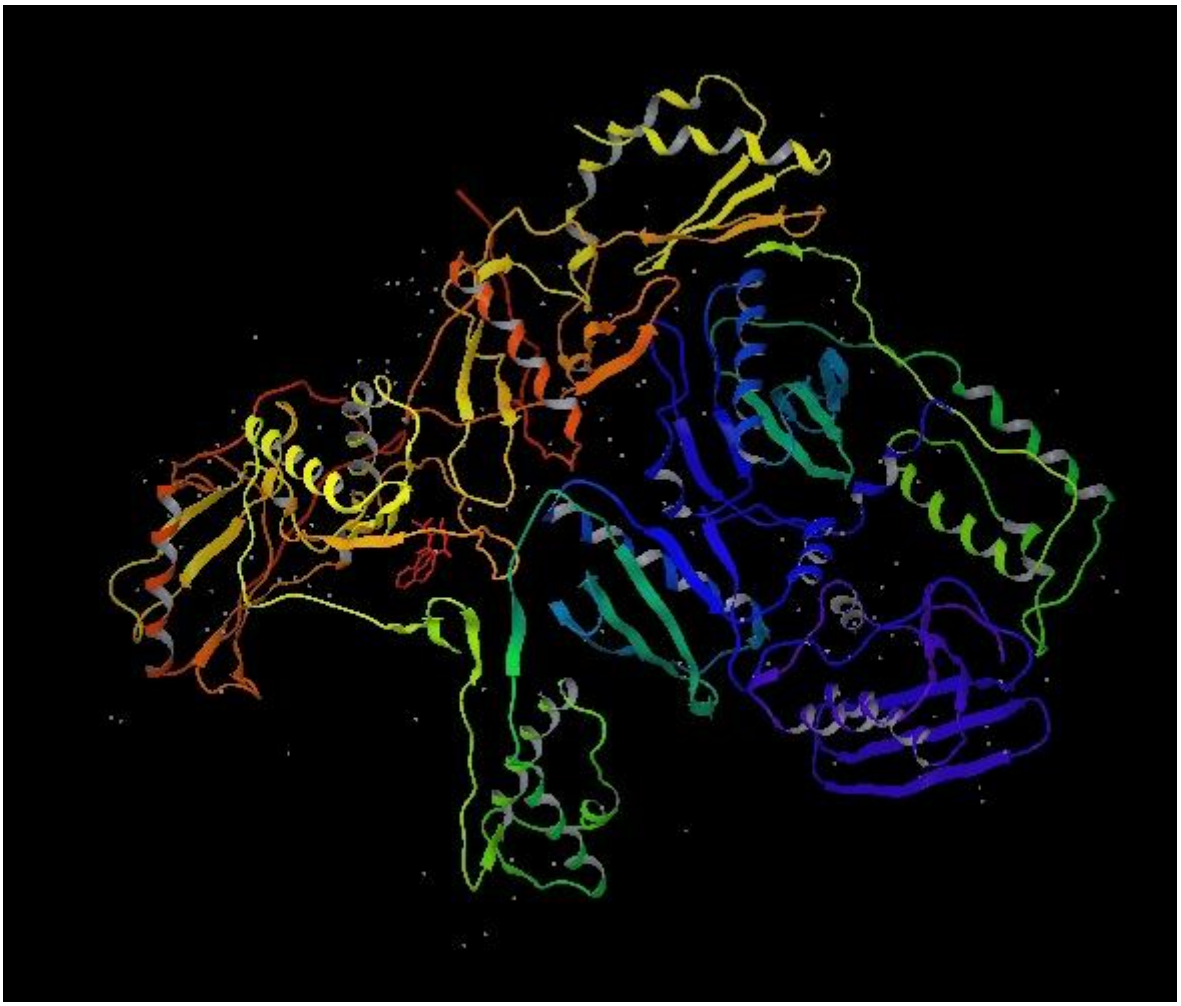


Figure 17

To validate the model, non nucleoside structures, similar to our compounds, with antiviral activity on RT, were docked. [48] [49] [53] [54]

A number of 57 compounds **77a-v, 78a-y, 79a-j** with a molecular scaffold showed in table 6 were docked using different conditions: standard and extra precision mode, using Lys101 as constraints for hydrogen bond an using different crystallographic structures.

The best docking was that one in which the X-ray structure 1RT2 and extra precision Glide mode was used.

34 active compounds showed H-bond interaction between N of indole portion and Lys101 residue (Tab.6) that seems to be very important for antiviral activity as reported in literature. [50] [51] [52] [55]

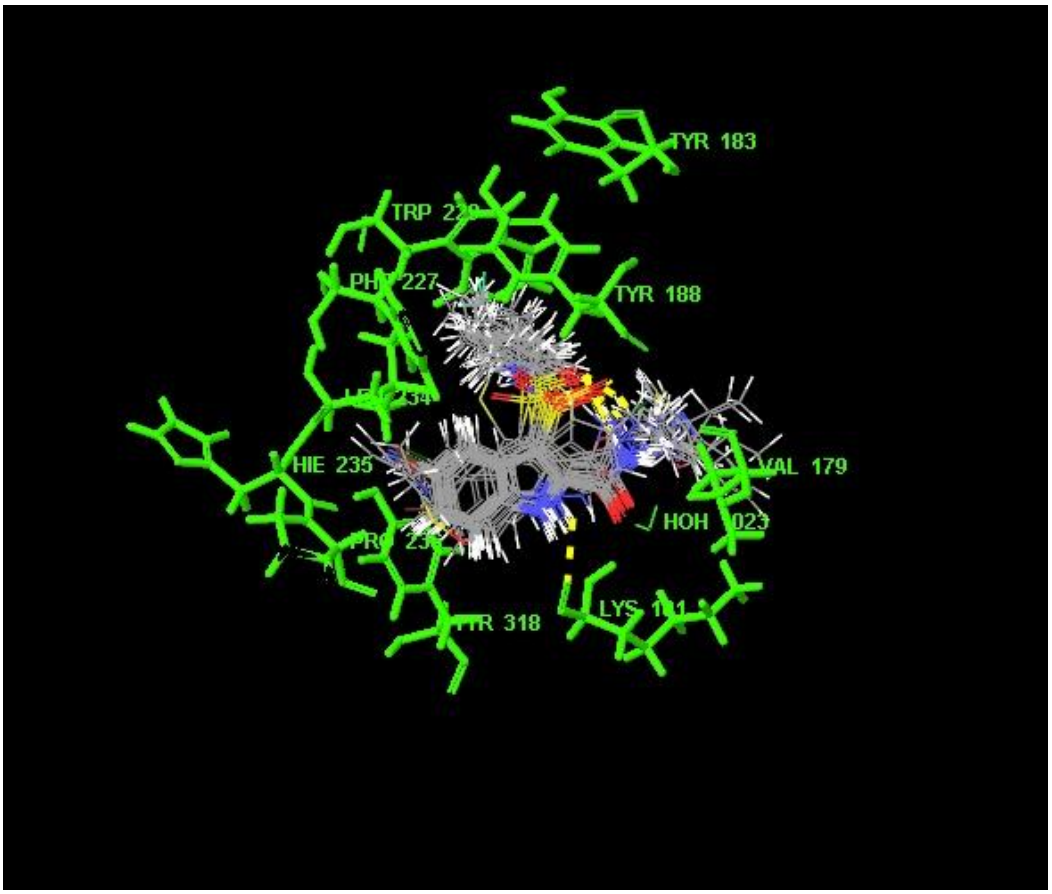
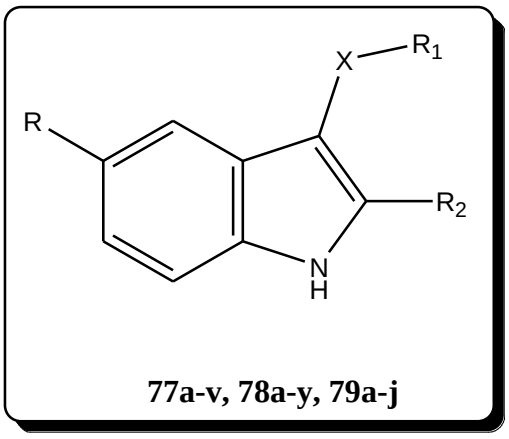
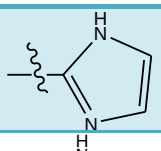
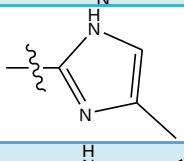
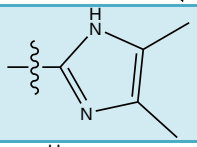
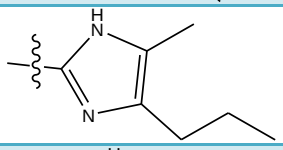
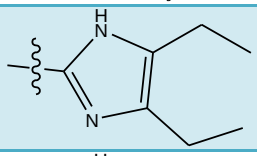
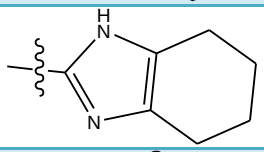
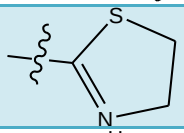
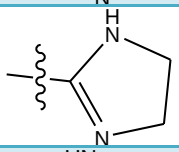
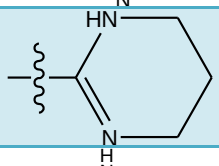
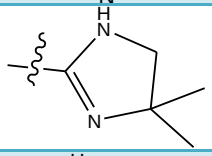
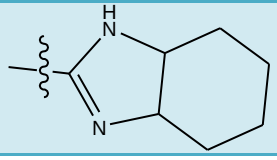


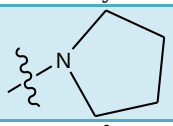
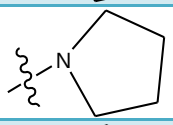
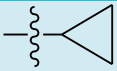
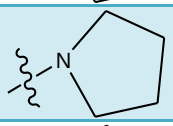
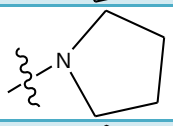
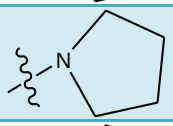
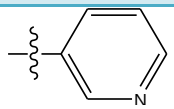
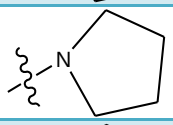
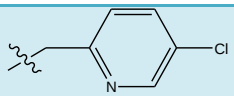
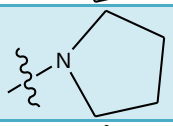
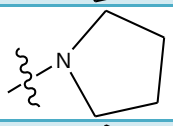
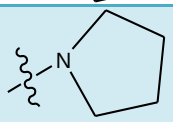
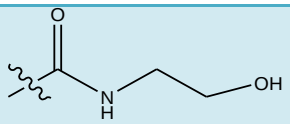
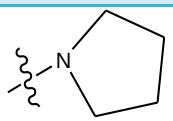
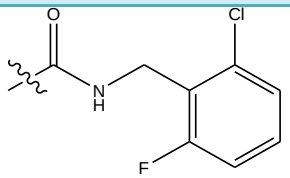
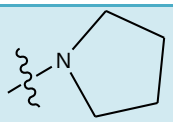
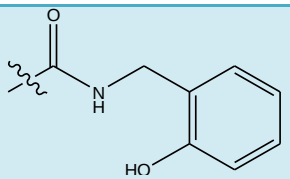
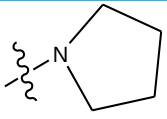
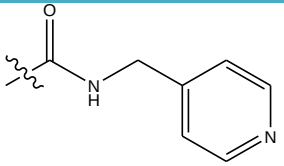
Figure 18

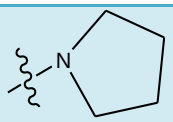
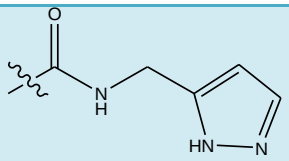
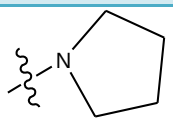
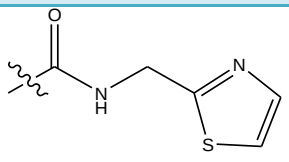
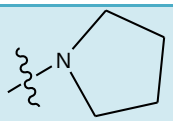
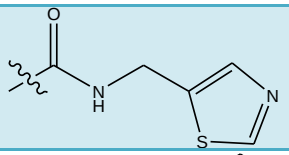
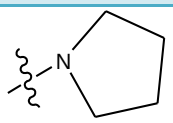
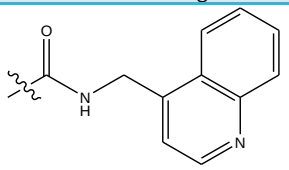
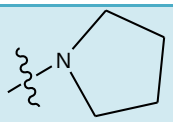
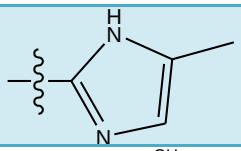
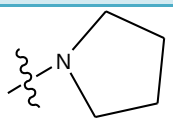
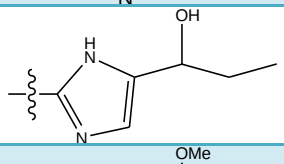
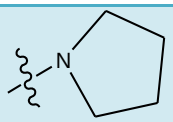
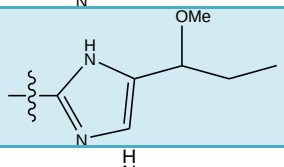
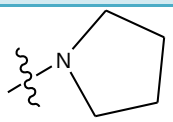
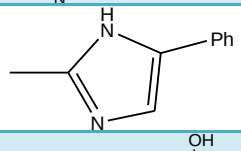
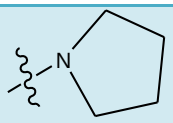
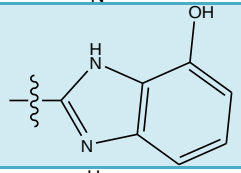
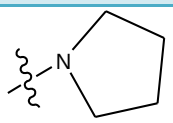
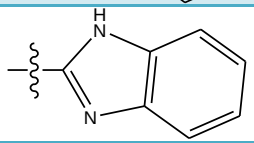
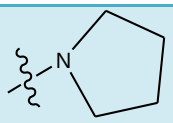
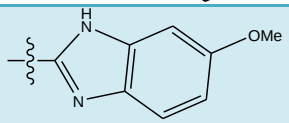
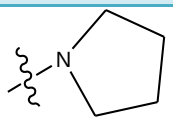
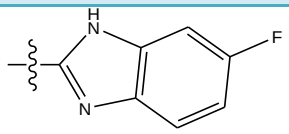
The total number of active compounds showed hydrophobic interactions between R1 substituents and Tyr 181, Trp 229, Phe227, Tyr181 residues. Furthermore the indole portion establish hydrophobic interaction with Leu 234, Pro236, Tyr318 an Leu100 residues. (Fig.18)



77a-v, 78a-y, 79a-j

Compd	X	R	R_1	R_2	Docking Score	H-bond interaction
77a	SO_2	Cl	Phenyl		-10.008	K101
77b	SO_2	Cl	Phenyl		-8.673	-
77c	SO_2	Cl	Phenyl		-8.718	K101
77d	SO_2	Cl	Phenyl		-7.901	-
77e	SO_2	Cl	Phenyl		-8.715	-
77f	SO_2	Cl	Phenyl		-8.583	-
77g	SO_2	Cl	Phenyl		-8.407	-
77h	SO_2	Cl	Phenyl		-8.780	-
77i	SO_2	Cl	Phenyl		-4.931	-
77j	SO_2	Cl	Phenyl		-8.611	-
77k	SO_2	Cl	Phenyl		-	-
77l	SO_2	Cl	Phenyl	$CONH_2$	-12.447	K101
77m	S	H	Phenyl	$CH_2S(O)Ph$	-7.167	K101
77n	S	Cl	Phenyl	$CH_2S(O)Ph$	-10.037	K101
77o	SO	H	Phenyl	$CH_2S(O)Ph$	-12.100	K101
77p	S	H	Phenyl	CO_2CH_3	-11.877	K101
77q	S	Cl	Phenyl	CO_2CH_3	-11.230	K101
77r	S	Cl	Phenyl	$CONH_2$	-12.595	K101
77s	S	H	Phenyl	$CON(CH_3)_2$	-	-

77t	S	H	Phenyl	$CONHCH_3$	-12.218	K101
77u	S	Cl	Phenyl	$CONHCH_3$	-12.420	K101
77v	SO	Cl	Phenyl	$CONH_2$	-12.869	K101
78a	SO_2	Cl		$CONH_2$	-10.464	K101
78b	SO_2	CN		$CONH_2$	-10.203	K101
78c	SO_2			$CONH_2$	-10.847	K101
78d	SO_2			$CONH_2$	-	-
78e	SO_2	Phenyl		$CONH_2$	-	-
78f	SO_2			$CONH_2$	-	-
78g	SO_2			$CONH_2$	-12.436	K101
78h	SO_2	Br		$CONH_2$	-10.344	K101
78i	SO_2	Br			-9.042	K101
78j	SO_2	Br			-8.068	-
78k	SO_2	Br			-8.372	-
78l	SO_2	Br			-8.818	K101

78m	SO_2	Br			-9.837	K101
78n	SO_2	Br			-9.254	K101
78o	SO_2	Br			-9.053	K101
78p	SO_2	Br			-9.250	K101
78q	SO_2	Br			-6.258	-
78r	SO_2	Br			-7.291	K101
78s	SO_2	Br			-8.295	K101
78t	SO_2	Br			-3.028	-
78u	SO_2	Br			-5.761	-
78v	SO_2	Br			-6.185	-
78w	SO_2	Br			-6.301	-
78x	SO_2	Br			-6.339	-

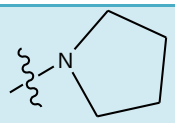
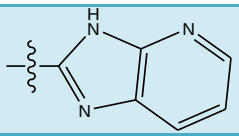
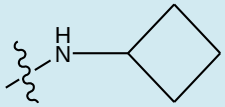
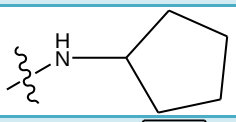
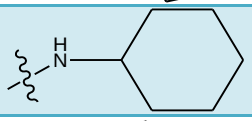
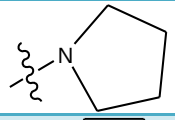
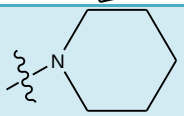
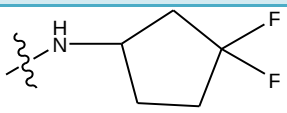
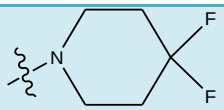
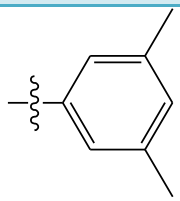
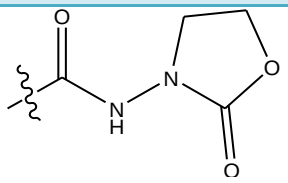
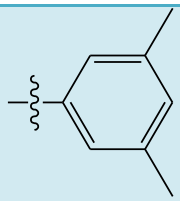
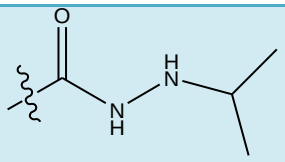
78y	SO_2	Br			-7.161	-
79a	SO_2	Br		$CONH_2$	-9.201	K101
79b	SO_2	Br		$CONH_2$	-9.615	K101
79c	SO_2	Br		$CONH_2$	-11.040	K101
79d	SO_2	Br		$CONH_2$	-10.698	K101
79e	SO_2	Br		$CONH_2$	-10.377	K101
79f	SO_2	Br		$CONH_2$	-11.652	K101
79g	SO_2	Br		$CONH_2$	-12.396	K101
79h	SO_2	Br		$CONH_2$	-12.892	K101
79i	SO_2	Cl			-8.583	-
79j	SO_2	Cl			-11.312	K101

Table 6

The same crystallographic structure with PDB code 1RT2 was used to dock compounds **73-76**.
Also in this case structures were docked in different conditions: standard and extra precision mode, using Lys 101 as constraints for hydrogen bond and using different crystallographic structures.
The best docking was that one in which Lys101 was used as constraint.

All compounds interact with RT in a butterfly like mode showing a very good superimposition (Fig.19) and good docking score values between -12.108 and -7.040. (Tab.7, 8, 9, 10) One of the wings of this butterfly is made of π-electron rich moiety that interact with a hydrophobic pocket formed by the side chains of aromatic amino acids such as Tyr181, Tyr188, Phe227, Trp229, Tyr318.

On the other hand the other wing is constituted by the quinone or indole moiety bearing a donating hydrogen bond with Lys101.

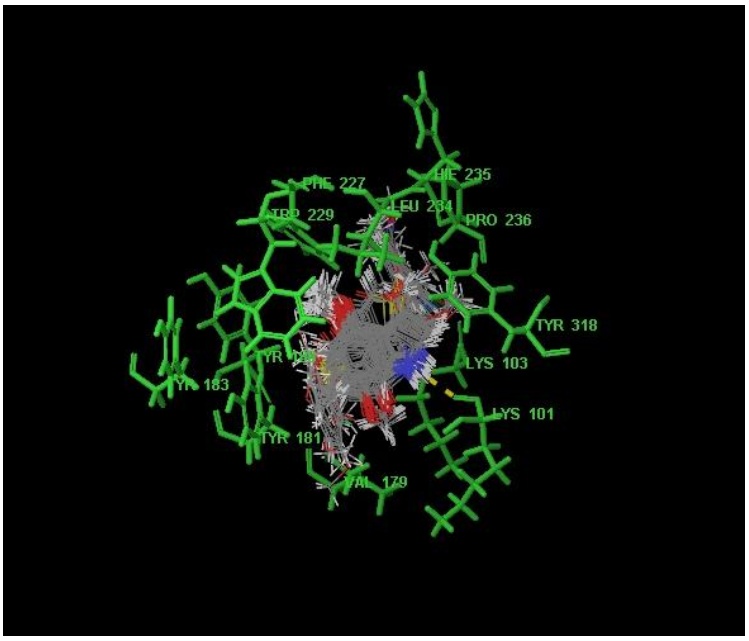
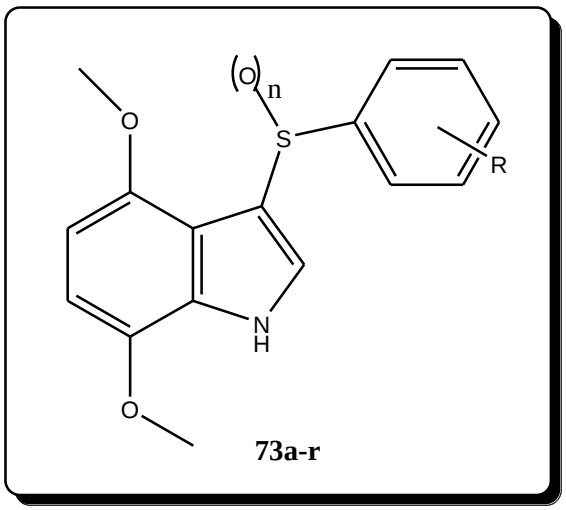


Figure 19

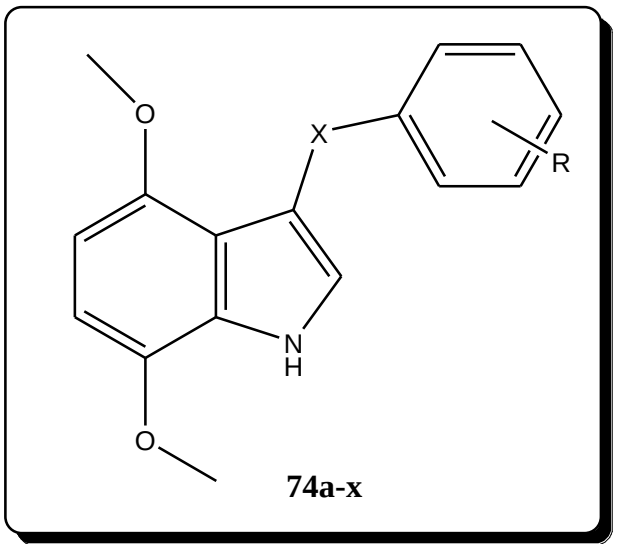
In some cases halogenated atoms on R substituents establish additional H-bond accepting interaction with Lys103 residue. (Tab.8, 9, 10)



73a-r

Compound	n	R	Docking Score	H-bond interaction
73a	0	H	-9.373	K101
73b	0	4-Cl	-9.984	K101
73c	0	3-OMe	-9.388	K101
73d	0	3-Me	-10.272	K101
73e	0	2-Cl	-9.583	K101
73f	0	2-Br	-9.582	K101
73g	0	2-OMe	-9.494	K101
73h	0	2,4,6-triMe	-8.121	K101
73i	2	2,4,6-triMe	-7.221	K101
73j	2	2-Me	-8.455	K101
73k	2	2-F	-7.743	K101
73l	2	2-Br	-7.711	K101
73m	2	2-Cl	-8.542	K101
73m	2	3-Me	-8.455	K101
73o	2	2-OMe	-8.621	K101
73p	2	4-Cl	-8.152	K101
73q	2	3-OMe	-8.929	K101
73r	2	H	-7.986	K101

Table 7

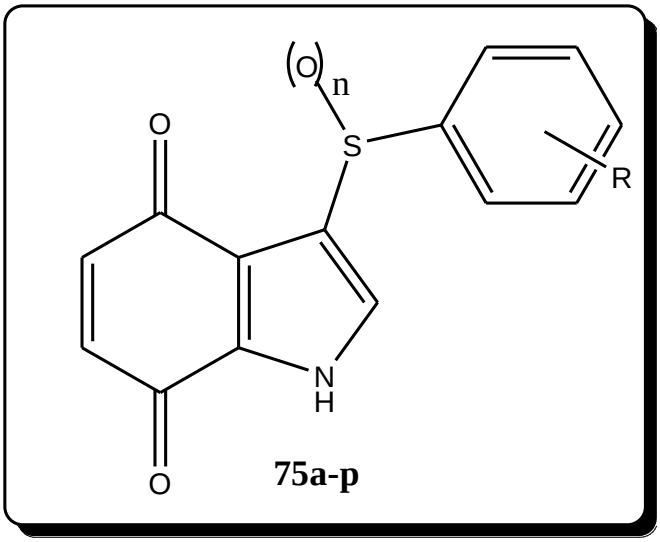


74a-x

Compound	X	R	Docking Score	H-bond interaction
74a	CO	4-OMe	-10.310	K101
74b	CO	4-Cl	-10.736	K101
74c	CO	2-Me	-9.556	K101
74d	CO	3-Me	-9.619	K101
74e	CO	3,4,5-triOMe	-7.350	K101
74f	CO	2,6-diF	-7.870	K101
74g	CH_2	4-Cl	-11.181	K101
74h	CH_2	2,6-diOMe	-8.933	K101
74i	CH_2	H	-10.399	K101
74j	CH_2	4-OMe	-11.418	K101
74k	CH_2	2,6-diF	-9.924	K101

74l	CH_2	3-Me	-11.060	K101
74m	CH_2	2-Me	-10.134	K101
74n	O	2,6-diOMe	-7.448	K101
74o	O	3-OMe	-9.251	K101
74p	O	4-Cl-3-NO_2	-10.280	K101-K103
74q	O	4-OMe	-9.443	K101
74r	O	4-Me-2-NO_2	-10.195	K101
74s	O	2,4-diMe	-9.129	K101
74t	O	H	-9.436	K101
74u	O	3,4-diMe	-10.111	K101
74v	O	3-Me-4-Cl	-9.251	K101
74w	O	3-NO_2	-10.390	K101-K103
74x	O	4-Me	-10.076	K101

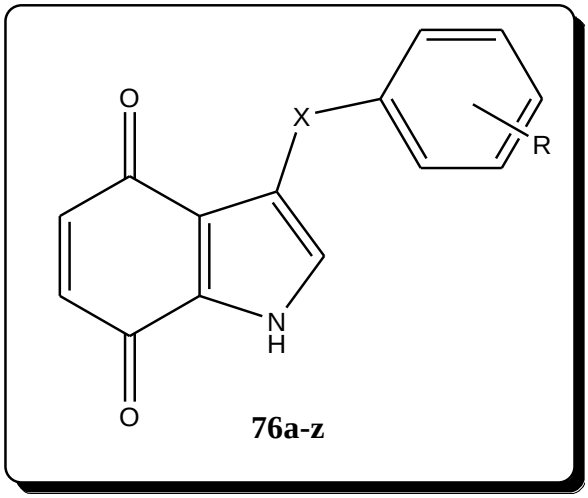
Table 8



75a-p

Compound	n	R	Docking Score	H-bond interaction
75a	0	H	-10.067	K101
75b	0	4-Cl	-10.935	K101
75c	0	3-OMe	-10.246	K101
75d	0	3-Me	-11.113	K101
75e	0	2-Cl	-10.918	K101
75f	0	2-Br	-10.496	K101
75g	0	2-OMe	-9.893	K101
75h	0	2-F	-10.639	K101-K103
75i	0	2-Me	-10.318	K101
75j	2	2-F	-10.623	K101
75k	2	2-Br	-9.330	K101
75l	2	2-Cl	-9.616	K101-K103
75m	2	2-OMe	-9.070	K101-K103
75n	2	3-Me	-10.985	K101
75o	2	3-OMe	-10.453	K101
75p	2	H	-10.441	K101

Table 9



76a-z

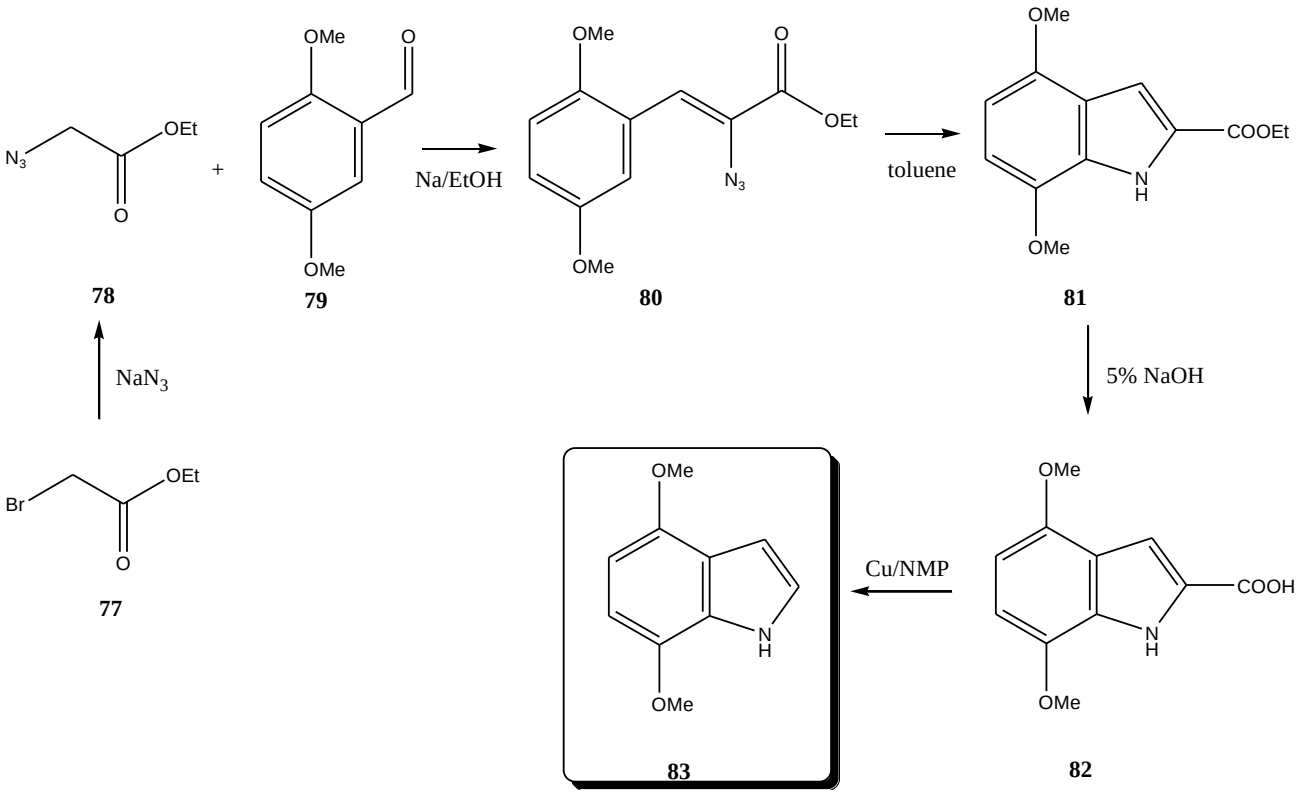
Compound	X	R	Docking Score	H-bond interaction
76a	CO	H	-10.363	K101-K103
76b	CO	4-Cl	-10.741	K101
76c	CO	3-NO_2	-10.038	K101-K103
76d	CO	4-OMe	-11.418	K101
76e	CO	2-NO_2	-10.143	K101-K103
76f	CO	2,6-diMe	-9.523	K101-K103
76g	CO	2,6-diF	-11.575	K101-K103
76h	CO	2,6-diCl	-8.593	K101-K103
76i	CH_2	4-Cl	-11.171	K101
76j	CH_2	2,6-diOMe	-9.081	K101-K103
76k	CH_2	H	-10.641	K101
76l	CH_2	4-NO_2	-11.424	K101
76m	CH_2	4-OMe	-12.108	K101
76n	CH_2	2-NO_2	-9.975	K101-K103
76o	CH_2	2,6-diF	-10.549	K101-K103
76p	O	3-NO_2	-11.180	K101-K103
76q	O	2,6-diOMe	-8.479	K101-K103
76r	O	3,4-diNO_2	-7.040	K101-K103
76s	O	3-OMe	-10.265	K101
76t	O	4-Cl-3-NO_2	-10.199	K101
76u	O	4-OMe	-10.102	K101
76v	O	4-NO_2	-10.056	K101
76w	O	2-NO_2	-10.480	K101
76x	O	4-Cl-2-NO_2	-11.461	K101-K103
76y	O	4-Me-2-NO_2	-11.347	K101-K103
76z	O	5-Me-2-NO_2	-10.166	K101-K103

Table 10

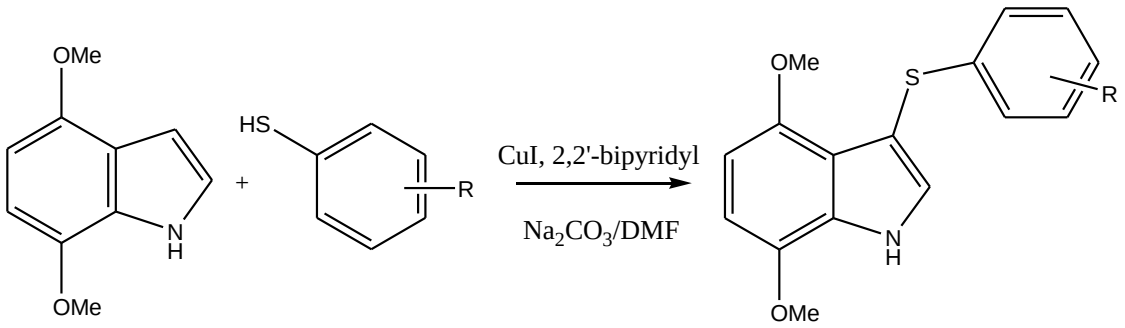
Considering the results showed by both the docking studies and the activity of compounds reported in the literature, we started with the synthesis of **73** and **75** derivatives.

RESULTS AND DISCUSSION

The 4,7-dimethoxyindole **83** is the key intermediate for the synthesis of all compounds desired. Starting from the 2,5-dimethoxybenzaldehyde **79** which reacted in presence of sodium in ethanol with the ethyl azidoacetate **78**, previously prepared from the ethyl bromoacetate **77** with sodium azide in a quantitative yield, it was possible to obtain compound **80** (48%). This latter compound was converted into the the dimethoxyindole **81** through intramolecular cyclization in toluene refluxing with a 66% yield. The hydrolysis of the ester group of compound **81** was performed with an 5% aqueous solution of sodium hydroxide refluxing (74%) [56] and the so formed acid **82** was decarboxylated at high temperature with copper in *N*-methylpyrrolidinone to afford the 4,7-dimethoxyindole **83** in very good yield (78%).



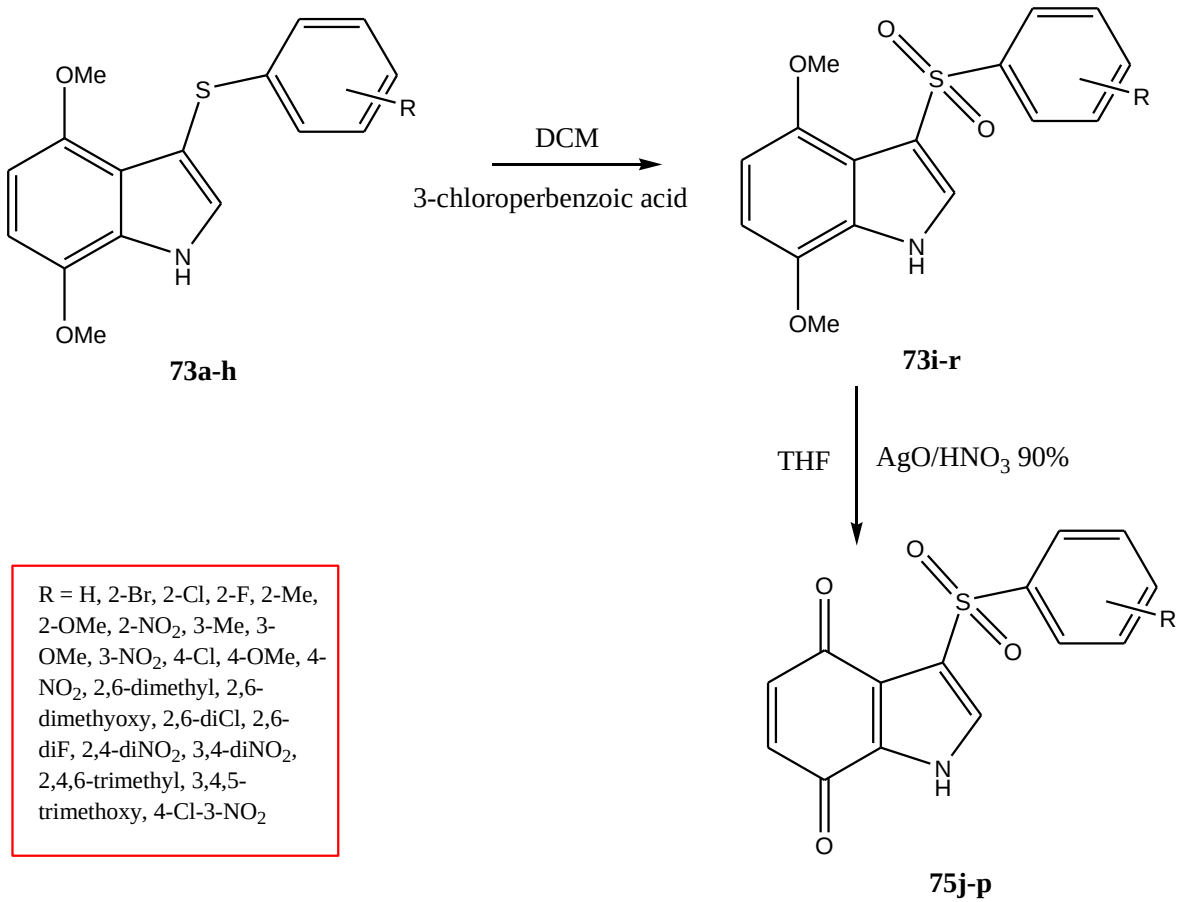
Starting from the 4,7-dimethoxyindole **83** it was possible to obtain compound **73a-h** through C-S cross coupling reaction copper catalyzed with variously substituted tiophenol **84**, using copper iodide, 2,2 '-bipyridyl and sodium carbonate as base in dimethylformamide.



83 **84** **73a-h**

R = H, 2-Br, 2-Cl, 2-F, 2-Me, 2-OMe, 2-NO_2, 3-Me, 3-OMe, 3-NO_2, 4-Cl, 4-OMe, 4-NO_2, 2,6-dimethyl, 2,6-dimethyoxy, 2,6-diCl, 2,6-diF, 2,4-diNO_2, 3,4-diNO_2, 2,4,6-trimethyl, 3,4,5-trimethoxy, 4-Cl-3-NO_2

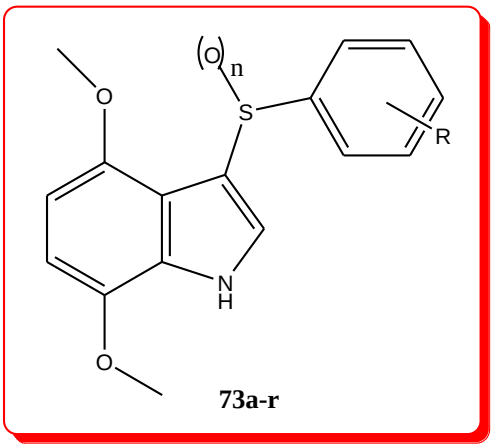
Phenylsulfonylindoles **73a-h** could be oxidized to phenylsulfonylindoles **73i-r** using 3-chloroperbenzoic acid in dichloromethane, these latter compounds could be then oxidized by reacting with 90% solution of nitric acid and silver(II)oxide in tetrahydrofuran to obtain quinones **75j-p**.



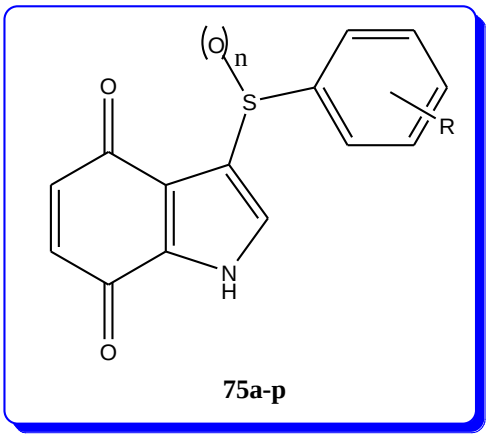
73a-h **73i-r** **75j-p**

R = H, 2-Br, 2-Cl, 2-F, 2-Me, 2-OMe, 2-NO_2, 3-Me, 3-OMe, 3-NO_2, 4-Cl, 4-OMe, 4-NO_2, 2,6-dimethyl, 2,6-dimethyoxy, 2,6-diCl, 2,6-diF, 2,4-diNO_2, 3,4-diNO_2, 2,4,6-trimethyl, 3,4,5-trimethoxy, 4-Cl-3-NO_2

In the table are reported all compounds synthesized at the moment. (Tab.11)



73a-r



75a-p

Compound	n	R
73a	0	H
73c	0	3-OMe
73d	0	3-Me
73f	0	2-Br
73m	2	3-Me
73r	2	H
75n	2	3-Me
75p	2	H

Table 11

All derivatives thus obtained will be biologically tested in order to evaluate their antitumor antiviral activity.

EXPERIMENTAL DATA

All melting points were taken on a Buchi B-540 apparatus. IR spectra were determined in bromoform with a spectrophotometer Shimadzu FT / IR 8400S. ^{1}H and ^{13}C NMR spectra were measured at 200 and 50.3 MHz, respectively, on DMSO-*d6* or $CDCl_3$ ssolution, using a Bruker AC 200 MHz (TMS as internal standard). Chromatography column was performed with MERK silica gel 230-400 mesh ASTM or FLASH40i Biotage chromatography or with Buchi Sepacore chromatography module (prepacked cartridge reference).

CHEMISTRY

Synthesis of dimethyl-4,5-dimethylcycloesa-1,4-diene-1,2-dicarboxylate 26

To a solution of dimethyl acetylene dicarboxylate **25** (25g, 0.17 mol) in toluene (100 ml) 2,3-dimethylbutan-1,3-diene **24** (13.1g, 0.16 mol) was added under nitrogen atmosphere. The reaction mixture was stirred under reflux for 24 hours. The solvent was evaporated under reduced pressure.
Mp 64-65°C; Yield 95%; IR 1732 (CO) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 1.69 (3H, s, CH_3), 3.77 (2H, s, CH_2), 3.84 (3H, s, CH_3).

Synthesis of dimethylbenzene-1,2-dicarboxylate 27

To a solution of compound **26** (30g, 0.13 mol) in chlorobenzene (500 ml) DDQ (2 eq) was added. The mixture was stirred under reflux for 24 hours. After cooling, diethyl ether was added and the mixture was filtered on celite. Evaporated the solvent under reduced pressure, the crude was purified by chromatography using dichloromethane as eluent.
Mp 49-50°C; Yield 92%; IR 1728 (CO) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 2.29 (3H, s, CH_3), 3.81 (3H, s, CH_3), 7.47 (1H, s, CH).

Synthesis of (4,5-dimethyl-1,2-phenylen)dimethanol 28

To a solution of borane (1.5M in THF/diethyl ether, 50ml) at 0°C under argon atmosphere, a solution of ester **27** (24 mmol) in THF (40 ml) was added. The mixture was stirred at 0°C for 2 hours and then under reflux for 20 hours. After cooling methanol was added to the mixture and the solvent evaporated under reduced pressure. The resulting crude was dissolved in ethyl acetate and washed with a saturated solution of sodium hydrogen carbonate. The organic layer was dried on Na_2SO_4 and evaporated.

Mp 103°C; Yield 80%; ^{1}H NMR $CDCl_3$ (ppm): 2.24 (3H, s, CH_3), 3.33 (1H, bs, OH), 4.61 (2H, d, CH_2), 7.08 (1H, s, CH).

Synthesis of 4,5-dimethylphtalaldehyde 20b

To a mixture of dichloromethane (100 ml) and oxalyl chloride (8ml, 88 mol) at -78°C and under argon atmosphere, a mixture of dimethyl sulfoxide (13.6 ml, 0.18 mol) and dichloromethane (25 ml) was added dropwise. After 3-5 min a solution of the 1,2-dimethanol **28** (40 mol) in dichloromethane-dimethyl sulfoxide (10 ml) was added dropwise. After 30 min 100 ml of triethylamine were added dropwise. After stirring for 10 min the mixture was warmed to room temperature and poured in water and ice. It was extracted in dichloromethane and the organic layer dried and evaporated. The crude was purified in column using dichloromethane as eluent.
Mp 95-100°C; Yield 87%; IR 1688 (CHO) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 1.77 (3H, s, CH_3), 7.28 (1H, s, CH), 10.3 (1H, s, CHO).

Synthesis of (2-bromo-4,5-dimethoxyphenyl)methanol 36

To a solution of benzoic acid derivative **35** (20 mmol) in tetrahydrofuran (50 ml), a solution of borane (solution 1.0 M in THF, 28.0 ml, 30 mmol) was added dropwise and the mixture was stirred at room temperature for 2 hours. After cooling at 0°C a solution of hydrochloric acid 1M was added and the mixture was extracted with diethyl ether. The organic layer was washed with water and saturated solution of sodium hydrogen carbonate.
Mp 95°C; Yield 90%; IR 3498 (OH) cm^{-1}; ^{1}H NMR DMSO (ppm): 3.77 (3H, s, CH_3), 3.77 (3H, s, CH_3), 4.45 (2H, d, J = 5.5 Hz, CH_2), 5.35 (1H, t, J = 5.5 Hz, OH), 7.10 (1H, s, Ar-H), 7.12 (1H, s, Ar-H); ^{13}C NMR DMSO-*d6* (ppm): 55.5 (CH_3), 55.9 (CH_3), 62.3 (CH_2), 110.7 (C), 111.6 (CH), 115.2 (CH), 132.9 (C), 148.1 (C), 148.2 (C).

Synthesis of 2-bromo-4,5-dimethoxybenzaldehyde 40

To a solution of the appropriate phenyl methanol derivative **36** (1.7 mmol) in dichloromethane, 2.4 g (1.9 mmol) of Dess-Martin periodinane were added under argon atmosphere. The mixture was stirred for 2 hours. Diethyl ether was added and the organic phase was washed with 5% solution of sodium thiosulfate and saturated solution of sodium hydrogen carbonate. The organic phase was dried using Na_2SO_4 and evaporated. The crude was purified in column using dichloromethane as eluent.
Mp 148-149 °C; Yield 90%; IR (CHO) 1676 cm^{-1}; ^{1}H NMR DMSO-*d6* (ppm): 3.83 (3H, s, CH_3), 3.91 (3H, s, CH_3), 7.31 (1H, s, Ar-H), 7.32 (1H, s, Ar-H), 10.06 (1H, s, CHO); ^{13}C NMR DMSO-

d6 (ppm): 55.6 (CH_3), 56.4 (CH_3), 110.4 (CH), 115.9 (CH), 119.3 (C), 125.7 (C), 148.6 (C), 154.4 (C), 190.1 (CH).

Synthesis of 2(1,3-dioxolan-2-yl)-4,5-dimethoxy-bromobenzene 42

To a solution of 2-bromo-benzaldehyde derivative **40** (3.3 mmol) in toluene, ethylene glicole **41** (9.2 mmol, d= 1.113, 0.6 ml) and a catalytic amount of *p*-toluensulfonic acid were added. The mixture was stirred at reflux for 24 hours using Dean-Stark trap. After cooling, the organic phase was washed with a saturated solution of sodium hydrogen carbonate and dried and evaporated. The crude was purified by BIOTAGE chromatography using cyclohexane:ethyl acetate (98:2) as eluent.

Mp 98-99°C; Yield 90%; ^{1}H NMR DMSO-*d6* (ppm): 3.76 (3H, s, CH_3), 3.79 (3H, s, CH_3), 3.95-4.11 (4H, m, 2xCH_2), 5.85 (1H, s, CH), 7.05 (1H, s, Ar-H), 7.14 (1H, s, Ar-H); ^{13}C NMR DMSO-*d6* (ppm): 56.1 (CH_3), 56.4 (CH_3), 65.3 (2xCH_2), 102.3 (CH), 111.1 (CH), 113.0 (C), 115.9 (CH), 128.5 (C), 148.6 (C), 150.5 (C).

Synthesis of 2-(1,3-dioxolan-2-yl)-4,5-methoxybenzaldehyde 43

To a solution of benzaldehyde derivative **42** (2.5 mmol) in tetrahydrofuran (8ml) at -78°C, *n*-butyl lithium (solution 1.6 M in *n*-hexane, 2.5 mmol, 1.6 ml) was added during 1 hour. The mixture was stirred for 1 hour at -78°C. After that, 0.3 ml of dimethylformamide were added and the mixture was warmed to room temperature during 1 hour. A saturated solution of ammonium chloride was added and the mixture extracted in dichloromethane. The organic layer was dried with Na_2SO_4 and evaporated. The crude was purified in column using dichloromethane as eluent.

Mp 77-78°C; Yield 100%; IR (CHO) 1680 cm^{-1}; ^{1}H NMR DMSO-*d6* (ppm): 3.98 (3H, s, CH_3), 3.99 (3H, s, CH_3), 4.00-4.12 (4H, m, 2xCH_2), 6.33 (1H, s, CH), 7.22 (1H, s, Ar-H), 7.41 (1H, s, Ar-H), 10.30 (1H, s, CHO); ^{13}C NMR DMSO-*d6* (ppm): 55.6 (CH_3), 55.8 (CH_3), 64.8 (2xCH_2), 99.9 (CH), 109.5 (CH), 110.0 (CH), 127.2 (C), 134.0 (C), 149.1 (C), 153.0 (C), 189.9 (CH).

Synthesis of 4,5-dimethoxyphtaldehyde 20c

To a solution of *ortho*-benzylaldehyde derivative **43** (1.5 mmol) in acetone (17 ml) a catalytic amount of *p*-toluensulfonic acid was added. The solution was stirred for 15 minutes at room temperature. Evaporated the solvent, the resulting residue was dissolved in dichloromethane and it was washed with a solution of sodium carbonate and brine. The crude was purified by chromatography using dichloromethane as eluent.

Mp 169,4-170,0; Yield 88%; IR (CHO) 1680 cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 3.94 (3H, s, CH_3), 7.54 (1H, s, CH), 10.5 (1H, s, CHO); ^{13}C NMR $CDCl_3$ (ppm): 11.7 (CH), 131.2 (C), 153.2 (C), 191.7 (CH).

Synthesis of 2-(2-hydroxyphenyl)-2*H*-isoindole-1-carbonitriles 23a-c

General procedure:

To a solution of sodium hydrogen sulfite (0.71 g, 0.004 mol) in water (18 ml), the suitable phthalaldehyde **20a-c** (0.004 mol) was added. The mixture was stirred until the solid was dissolved, and the 2-aminophenol **21** (0.004 mol) was added. The reaction was heated on a steam bath for 30 min at 40°C, then potassium cyanide (0.81 g, 0.012 mol) in water (5 ml) was added, and the mixture was heated for an additional 90 min. The solid formed upon cooling was filtered and used as crude for the subsequent reaction.

2-(2-Hydroxyphenyl)-2H-isoindoles-1-carbonitrile 23a: Mp 143°C; Yield 100%; IR: 3299 (OH), 2256 (CN) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 7.17-7.35 (6H, m, 6xAr-H), 7.68-7.74 (2H, m, 2xAr-H), 7.91 (1H, bs, OH), 8.28 (1H, d, J=8.4 Hz, Ar-H); ^{13}C NMR $CDCl_3$ (ppm): 107.6 (C), 107.8 (CH), 115.4 (CH), 117.6 (CH), 120.0 (CH), 120.9 (CH), 122.4 (C), 123.7 (CH), 123.9 (CH), 125.6 (CH), 126.2 (C), 126.5 (C), 127.6 (CH), 144.2 (C), 152.1 (C).

2-(2-Hydroxyphenyl)-5,6-dimethyl-2H-isoindoles-1-carbonitrile 23b: Mp 146.1-147.5°C; Yield 84%; IR: 3301 (OH), 2256 (CN) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 2.36 (3H, s, CH_3), 2.40 (3H, s, CH_3), 7.24 (4H, m, 4xAr-H), 7.44 (1H, s, Ar-H), 7.68 (1H, d, J=7.3 Hz, Ar-H), 7.76 (1H, s, Ar-H), 8.28 (1H, bs, OH); ^{13}C NMR $CDCl_3$ (ppm): 20.7 (CH_3), 20.9 (CH_3), 107.0 (CH), 115.3 (CH), 117.4 (CH), 118.8 (CH), 119.6 (CH), 119.7 (C), 122.7 (C), 123.6 (CH), 125.8 (C), 126.0 (C), 127.1 (CH), 134.0 (C), 136.2 (C), 141.0 (C), 144.0 (C).

2-(2-Hydroxyphenyl)-5,6-dimethoxy-2H-isoindoles-1-carbonitrile 23c: Mp 131.4-132.7°C; Yield 100%; IR: 3410 (OH), 2257 (CN) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 3.84 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 7.02 (1H, s, Ar-H), 7.19-7.41 (3H, m, 3xAr-H), 7.53 (1H, s, Ar-H), 8.10-8.15 (1H, m, Ar-H), 8.33 (1H, s, Ar-H), 10.39 (1H, bs, OH); ^{13}C NMR $CDCl_3$ (ppm): 55.3 (CH_3), 55.5 (CH_3), 93.4 (C), 95.5 (CH), 99.1 (CH), 116.1 (CH), 116.8 (CH), 118.8 (C), 119.2 (CH), 125.5 (C), 126.9 (CH), 127.4 (C), 127.9 (CH), 148.0 (C), 148.9 (C), 150.9 (C), 152.2 (C).

Synthesis of ethyl-bromo(4-methylphenyl)acetate 44b

To a solution of ethyl *p*-tolylacetate **46** (2g, 0.011 mol) in carbon tetrachloride (29 ml) *N*-bromosuccinimide (2g, 0.011 mol) and a catalytic amount of aqueous HBr 48% solution were added. The mixture was stirred at reflux for 26 hours. After cooling, the excess *N*-bromosuccinimide was removed by filtration. The solvent was evaporated under reduced pressure and the crude product is purified by chromatography using dichloromethane as eluent.

Oil; Yield 75%; IR: 1681 (CO) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm):1.13 (3H, t, J=7.0 Hz, CH_3), 2.30 (3H, s, CH_3) 4.18 (2H, q, J=7.0 Hz, CH_2), 5.87 (1H, s, CH), 7.20 (2H, d, J=7.5 Hz, 2xAr-H), 7.45 (2H, d, J=7.5 Hz, 2xAr-H); ^{13}C NMR $CDCl_3$ (ppm): 13.8 (CH_3), 20.7 (CH_3), 62.1 (CH_2), 128.5 (2xCH), 129.3 (2xCH), 132.3 (C), 138.7 (C), 139.6 (CH), 168.0 (C).

Synthesis of ethyl [2-(1-cyano-2*H*-isoindol-2-yl)phenoxy]acetate compounds 45a-d

General procedure:

To a solution of suitable isoindoles **23a-c** (0.004 mol) in tetrahydrofuran (20 ml) sodium hydride (0.004 mol) was added. The mixture was stirred at room temperature for 1 hour, then ethyl-α-bromoester **44a,b** (0.004 mol) was added. The mixture was stirred at reflux for 4-24 hours. After cooling, the solvent was evaporated at reduced pressure and the crude was purified by chromatography using dichloromethane as eluent.

Ethyl [2-(1-cyano-2*H*-isoindol-2-yl)phenoxy](phenyl)acetate 45a: The mixture was stirred at reflux for 7 hours. Mp 98.6-100°C; Yield 76%; IR: 2200 (CN), 1746 (CO) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 1.12 (3H, t, J=7.1 Hz, CH_3), 4.12 (2H, q, J=7.1 Hz, CH_2), 5.65 (1H, s, CH), 7.04-7.18 (3H, m, 3xAr-H), 7.22-7.32 (6H, m, 6xAr-H), 7.45 (1H, dd, J=1.7, 7.9 Hz, Ar-H), 7.52 (1H, dd, J=1.7, 7.9 Hz, Ar-H), 7.68-7.74 (3H, m, 3xAr-H); ^{13}C NMR $CDCl_3$ (ppm): 13.9 (CH_3), 61.9 (CH_2), 79.3 (CH), 95.1 (C), 114.5 (C), 114.7 (CH), 118.2 (CH), 120.9 (CH), 121.7 (CH), 122.4 (CH), 122.9 (CH), 124.1 (C), 125.7 (CH), 126.7 (2xCH), 128.1 (C), 128.3 (CH), 128.7 (2xCH), 129.0 (CH), 130.7 (CH), 131.9 (C), 134.3 (C), 151.4 (C), 169.1 (C).

Ethyl [2-(1-cyano-5,6-dimethyl-2*H*-isoindol-2-yl)phenoxy](phenyl)acetate 45b: The mixture was stirred at reflux for 4 hours. Mp 131-133°C; Yield 52%; IR: 2196 (CN), 1746 (CO) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 1.13 (3H, t, J=7.1 Hz, CH_3), 2.36 (3H, s, CH_3), 2.40 (3H, s, CH_3), 4.13 (2H, q, J=7.1 Hz, CH_2), 5.63 (1H, s, CH), 7.04 (1H, dd, J=1.1, 8.3 Hz, Ar-H), 7.16 (1H, td, J=1.2, 7.7 Hz, Ar-H), 7.24-7.33 (5H, m, 5xAr-H), 7.36-7.52 (4H, m, 4xAr-H), 7.58 (1H, bs, Ar-H); ^{13}C NMR $CDCl_3$ (ppm): 13.9 (CH_3), 20.6 (CH_3), 20.9 (CH_3), 61.9 (CH), 79.3 (CH), 99.9 (C), 114.7 (CH),

115.0 (C), 117.1 (CH), 119.5 (CH), 120.8 (CH), 122.3 (CH), 123.6 (C), 126.7 (2xCH), 128.3 (C), 128.4 (CH), 128.7 (2xCH), 129.0 (CH), 130.4 (CH), 131.6 (C), 132.8 (C), 134.4 (C), 136.2 (C), 151.4 (C), 169.1 (C).

Ethyl [2-(1-cyano-5,6-dimethoxy-2*H*-isoindol-2-yl)phenoxy](phenyl)acetate 45c: The mixture was stirred at reflux for 6 hours. Mp 114.1-115.6°C; Yield 58%; IR: 2256 (CN), 1731 (CO) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 1.14 (3H, t, J=7.1 Hz, CH_3), 3.94 (3H, s, CH_3), 3.99 (3H, s, CH_3), 4.13 (2H, q, J=7.1 Hz, CH_2), 5.61 (1H, s, CH), 6.94 (2H, d, J=6.2 Hz, 2xAr-H), 7.04 (1H, dd, J=1.1, 8.3 Hz, Ar-H), 7.16 (1H, td, J=1.0, 8.2 Hz, Ar-H), 7.26-7.29 (5H, m, 5xAr-H), 7.35-7.48 (2H, m, 2xAr-H), 7.52 (1H, bs, Ar-H); ^{13}C NMR $CDCl_3$ (ppm): 13.9 (CH_3), 55.9 (CH_3), 56.1 (CH_3), 61.8 (CH_2), 79.4 (CH), 94.4 (C), 98.4 (CH), 100.0 (CH), 114.9 (CH), 115.0 (C), 119.4 (C), 120.8 (CH), 122.4 (CH), 126.7 (2xCH), 128.3 (CH), 128.4 (C), 128.5 (C), 128.7 (2xCH), 129.0 (CH), 130.3 (CH), 134.5 (C), 148.7 (C), 151.1 (C), 151.5 (C), 169.2 (C).

Ethyl [2-(1-cyano-2*H*-isoindol-2-yl)phenoxy](4-methylphenyl)acetate 45d: The mixture was stirred at reflux for 24 hours. Mp 101.4-102.2°C; Yield 60%; IR: 2200 (CN), 1729 (CO) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 1.10 (3H, t, J=7.1 Hz, CH_3), 2.26 (3H, s, CH_3), 4.11 (2H, q, J=7.1 Hz, CH_2), 5.58 (1H, s, CH), 7.00-7.29 (7H, m, 7xAr-H), 7.35-7.53 (3H, m, 3xAr-H), 7.63-7.73 (3H, m, 3xAr-H); ^{13}C NMR $CDCl_3$ (ppm): 14.0 (CH_3), 21.2 (CH_3), 61.8 (CH_2), 79.7 (CH), 100.2 (C), 115.2 (CH), 118.5 (CH), 121.0 (CH), 121.7 (CH), 122.5 (CH), 123.0 (CH), 124.3 (C), 125.7 (CH), 126.9 (2xCH), 127.4 (C), 128.5 (CH), 129.2 (C), 129.5 (2xCH), 130.8 (CH), 131.7 (C), 132.0 (C), 139.0 (C), 151.9 (C), 169.3 (C).

Synthesis of [2-(1-cyano-2*H*-isoindol-2-yl)phenoxy] acetic acids 47a-d

General procedure:

To a solution of proper derivative **45a-d** (0.025 mol) in a mixture of tetrahydrofuran and ethanol (1:1) (30 ml) an aqueous sodium hydroxide 5% solution (25 ml) was added dropwise. The reaction mixture was stirred for 15 minutes at room temperature. The solvent was evaporated under reduced pressure, the remaining aqueous layer was acidified with 6N hydrochloric acid and extracted with ethyl acetate. The organic layer was dried with Na_2SO_4 and evaporated. The product obtained did not require further purification.

[2-(1-Cyano-2*H*-isoindol-2-yl)phenoxy](phenyl)acetic acid 47a: Mp 181.8-183°C; Yield 90%; IR: 3566 (OH), 2211 (CN), 1733 (CO) cm^{-1}; ^{1}H NMR DMSO-*d6* (ppm): 6.05 (1H, s, CH), 7.14-

7.35 (9H, m, 9xAr-H), 7.53-7.67 (3H, m, 3xAr-H), 7.84 (1H, d, J=8.5 Hz, Ar-H), 8.05 (1H, d, J=0.7 Hz, Ar-H), 13.35 (1H, bs, OH); ^{13}C NMR DMSO-*d6* (ppm): 77.7 (CH), 94.1 (C), 114.1 (C), 114.7 (CH), 117.4 (CH), 121.6 (CH), 121.8 (CH), 122.7 (CH), 123.0 (CH), 123.6 (C), 126.0 (CH), 126.9 (2xCH), 127.1 (C), 128.4 (2xCH), 128.5 (CH), 128.8 (CH), 131.0 (CH), 131.1 (C), 134.4 (C), 151.3 (C), 170.2 (C).

[2-(1-Cyano-5,6-dimethyl-2*H*-isoindol-2-yl)phenoxy](phenyl)acetic acid 47b: Mp 212-213.2°C; Yield 95%; IR: 3557 (OH), 2203 (CN), 1728 (CO) cm^{-1}; ^{1}H NMR DMSO-*d6* (ppm): 2.33 (3H, s, CH_3), 2.37 (3H, s, CH_3), 6.04 (1H, s, CH), 7.21 (1H, td, J=0.9, 7.5 Hz, Ar-H), 7.25-7.36 (6H, m, 6xAr-H), 7.42 (1H, bs, Ar-H), 7.51-7.61 (3H, m, 3xAr-H), 7.87 (1H, bs, Ar-H), 13.36 (1H, bs, OH); ^{13}C NMR DMSO-*d6* (ppm): 20.1 (CH_3), 20.4 (CH_3), 77.6 (CH), 92.9 (C), 114.4 (C), 114.6 (CH), 116.2 (CH), 119.9 (CH), 121.7 (CH), 121.8 (CH), 123.0 (C), 126.9 (2xCH), 127.2 (C), 128.3 (CH), 128.5 (2xCH), 128.7 (CH), 130.7 (C), 130.8 (CH), 132.3 (C), 135.4 (C), 136.1 (C), 151.2 (C), 170.2 (C).

[2-(1-Cyano-5,6-dimethoxy-2*H*-isoindol-2-yl)phenoxy](phenyl)acetic acid 47c: Mp 200-201.5°C; Yield 80%; IR: 3021 (OH), 2204 (CN), 1751 (CO) cm^{-1}; ^{1}H NMR DMSO-*d6* (ppm): 2.23 (3H, s, CH_3), 5.98 (1H, s, CH), 7.05-7.36 (8H, m, 8xAr-H), 7.52-7.67 (3H, m, 3xAr-H), 7.83 (1H, d, J=8.5 Hz, Ar-H), 8.03 (1H, s, Ar-H), 12.84 (1H, bs, OH); ^{13}C NMR DMSO-*d6* (ppm): 20.6 (CH_3), 77.6 (CH), 94.1 (C), 114.0 (C), 114.7 (CH), 117.4 (CH), 121.5 (CH), 121.6 (CH), 121.7 (CH), 122.7 (CH), 122.9 (CH), 123.5 (C), 125.9 (C), 126.9 (2xCH), 127.1 (C), 128.3 (CH), 129.0 (2xCH), 131.0 (CH), 132.4 (C), 138.2 (C), 151.3 (C), 170.3 (C).

[2-(1-Cyano-2*H*-isoindol-2-yl)phenoxy](4-methylphenyl)acetic acid 47d: Mp 214.9-216.8°C; Yield 73%; IR: 3020 (OH), 2207 (CN), 1733 (CO) cm^{-1}; ^{1}H NMR DMSO-*d6* (ppm): 3.83 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 6.03 (1H, s, CH), 6.92 (1H, s, Ar-H), 7.15 (1H, s, Ar-H), 7.20-7.38 (7H, m, 7xAr-H), 7.54-7.57 (2H, m, 2xAr-H), 7.78 (1H, s, Ar-H), 13.30 (1H, bs, OH); ^{13}C NMR DMSO-*d6* (ppm): 55.4 (CH_3), 55.5 (CH_3), 77.6 (CH), 93.2 (C), 95.5 (CH), 99.1 (CH), 114.6 (CH), 118.8 (C), 121.7 (CH), 126.6 (CH), 126.9 (2xCH), 127.4 (C), 127.6 (C), 128.1 (C), 128.3 (CH), 128.5 (2xCH), 128.7 (CH), 130.6 (CH), 135.4 (C), 148.2 (C), 151.1 (C), 151.3 (C), 170.2 (C).

Synthesis of 7-oxo-6,7-dihydroisoindolo[1,2-*d*][1,5]benzoxazepine-12-carbonitriles 48a-d

General procedure:

To a suspension of proper **47a-d** (0.0014 mol) in dichloromethane (30 ml) thionyl chloride (0.3 ml, 0.0042 mol) was added and the reaction mixture was stirred at reflux for 30-90 min. After cooling, the mixture was basified with an aqueous sodium hydroxide 5% solution and extracted with dichloromethane. The solvent was evaporated under reduced pressure and the crude was purified by chromatography using dichloromethane as eluent.

7-Oxo-6-phenyl-6,7-dihydroisoindolo[1,2-*d*][1,5]benzoxazepine-12-carbonitrile 48a: The mixture was stirred at reflux for 1 hour. Mp 152.8-154°C; Yield 35%; IR: 2256 (CN), 1652 (CO) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 5.66 (1H, s, CH), 7.23-7.48 (10H, m, 10xAr-H), 7.82-7.94 (2H, m, 2xAr-H), 8.18-8.25 (1H, m, Ar-H); ^{13}C NMR $CDCl_3$ (ppm): 92.9 (CH), 99.4 (C), 113.0 (C), 118.4 (CH), 121.9 (CH), 124.5 (CH), 125.7 (CH), 126.5 (CH), 126.9 (C), 127.4 (CH), 128.1 (2xCH), 128.2 (CH), 128.6 (C), 128.7 (2xCH), 129.2 (CH), 130.9 (CH), 131.4 (C), 131.7 (C), 135.9 (C), 149.9 (C), 190.7 (C).

9,10-Dimethyl-7-oxo-6-phenyl-6,7-dihydroisoindolo[1,2-*d*][1,5]benzoxazepine-12-carbonitrile 48b: The mixture was stirred at reflux for 1 hour. Mp 223.8-225°C; Yield 38%; IR: 2228 (CN), 1660 (CO) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 2.36 (3H, s, CH_3), 2.41 (3H, s, CH_3), 5.60 (1H, s, CH), 7.19-7.24 (1H, m, Ar-H), 7.34-7.40 (7H, m, 7xAr-H), 7.58 (1H, bs, Ar-H), 7.85-7.89 (1H, m, Ar-H), 8.02 (1H, bs, Ar-H); ^{13}C NMR $CDCl_3$ (ppm): 20.7 (CH_3), 20.8 (CH_3), 92.9 (CH), 100.2 (C), 113.3 (C), 117.5 (CH), 121.0 (CH), 124.5 (CH), 125.8 (CH), 126.4 (CH), 128.3 (2xCH), 128.5 (C), 128.6 (C), 128.7 (2xCH), 129.1 (CH), 130.5 (CH), 131.5 (C), 132.0 (C), 136.4 (C), 138.1 (C), 139.2 (C), 150.2 (C), 190.4 (C).

9,10-Dimethoxy-7-oxo-6-phenyl-6,7-dihydroisoindolo[1,2-*d*][1,5]benzoxazepine-12-carbonitrile 48c: The mixture was stirred at reflux for 30 minutes. Mp 195-197°C; Yield 40%; IR: 2256 (CN), 1658 (CO) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 3.95 (3H, s, OCH_3), 4.02 (3H, s, OCH_3), 5.60 (1H, s, CH), 7.04 (1H, s, Ar-H), 7.19-7.24 (1H, m, Ar-H), 7.34-7.41 (7H, m, 7xAr-H), 7.57 (1H, s, Ar-H), 7.81-7.86 (1H, m, Ar-H); ^{13}C NMR $CDCl_3$ (ppm): 56.2 (CH_3), 56.3 (CH_3), 92.2 (CH), 96.1 (CH), 98.9 (C), 99.6 (CH), 113.4 (C), 124.3 (CH), 125.3 (CH), 125.5 (C), 126.1 (C), 126.3 (CH), 128.2 (2xCH), 128.3 (C), 128.7 (2xCH), 129.1 (CH), 130.1 (CH), 131.7 (C), 136.1 (C), 149.8 (C), 152.0 (C), 153.0 (C), 190.3 (C).

6-(4-Methylphenyl)-7-oxo-6,7-dihydroisoindolo[1,2-d][1,5]benzoxazepine-12-carbonitrile 48d: The mixture was stirred at reflux for 1.5 hours. Mp 179.6-181°C; Yield 44%; IR: 2255 (CN), 1653 (CO) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 2.35 (3H, s, CH_3), 5.63 (1H, s, CH), 7.14-7.26 (4H, m, 4xAr-H), 7.40-7.45 (4H, m, 4xAr-H), 7.83-7.92 (2H, m, 2xAr-H) 8.21-8.25 (2H, m, 2xAr-H); ^{13}C NMR $CDCl_3$ (ppm): 21.3 (CH_3), 92.9 (CH), 113.0 (C), 118.4 (CH), 122.0 (CH), 124.6 (CH), 125.7 (CH), 126.4 (CH), 127.0 (C), 127.1 (C), 127.4 (CH), 128.1 (2xCH), 128.2 (CH), 128.6 (C), 129.4 (2xCH), 130.9 (CH), 131.5 (C), 131.7 (C), 132.3 (C), 133.1 (C), 164.5 (C), 191.0 (C).

Synthesis of isoindolo[1,2-*d*][1,5]benzoxazepin-7-yl-12-carbonitrile acetate compounds 49a-d

General procedure:

To a solution of the suitable derivative **48a-d** (0.0006 mol) in dichloromethane (5 ml) triethylamine was added (0.16 ml, 0.0012 mol) and the mixture was stirred for 1 hour at room temperature. After that acetyl chloride (0.0012 mol) was added, the mixture was maintained under stirring at room temperature for 5-24 hours. The mixture was poured onto ice and extracted with ethyl acetate. The organic phase was concentrated and the crude purified by chromatography using dichloromethane as eluent.

6-Phenylisoindolo[1,2-*d*][1,5]benzoxazepin-7-yl-12-carbonitrile acetate 49a: The mixture was stirred at room temperature for 5 hours. Mp 211.8-212.8°C; Yield 90%; IR: 2196 (CN), 1765 (CO) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 2.39 (3H, s, CH_3), 7.30-7.63 (8H, m, 8xAr-H), 7.77-7.95 (4H, m, 4xAr-H), 8.05-8.10 (1H, m, Ar-H); ^{13}C NMR $CDCl_3$ (ppm): 20.5 (CH_3), 94.1 (C), 113.9 (C), 117.9 (CH), 120.4 (CH), 122.2 (CH), 122.4 (C), 124.2 (C), 125.1 (CH), 125.8 (CH), 126.9 (2xCH), 127.0 (CH), 127.4 (CH), 129.1 (2xCH), 129.9 (C), 130.1 (CH), 130.8 (C), 131.2 (CH), 131.6 (C), 131.7 (C), 149.3 (C), 153.8 (C), 169.0 (C).

9,10-Dimethyl-6-phenylisoindolo[1,2-*d*][1,5]benzoxazepin-7-yl-12-carbonitrile acetate 49b: The mixture was stirred at room temperature for 24 hours. Mp 248-249°C; Yield 64%; IR: 2256 (CN), 1775 (CO) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 2.37 (3H, s, CH_3), 2.38 (3H, s, CH_3), 2.39 (3H, s, CH_3), 7.49-7.64 (8H, m, 8xAr-H), 7.87-7.92 (3H, m, 3xAr-H); ^{13}C NMR $CDCl_3$ (ppm): 20.2 (CH_3), 20.3 (CH_3), 20.5 (CH_3), 93.0 (C), 99.5 (CH), 114.2 (C), 116.6 (CH), 118.8 (CH), 121.8 (C), 122.1 (CH), 123.2 (CH), 125.6 (CH), 126.8 (2xCH), 126.9 (C), 129.0 (2xCH), 129.9 (CH), 130.1 (C), 130.9 (CH), 131.2 (C), 131.8 (C), 135.2 (C), 138.0 (C), 148.7 (C), 153.6 (C), 168.9 (C).

9,10-Dimethoxy-6-phenylisoindolo[1,2-*d*][1,5]benzoxazepin-7-yl-12-carbonitrile acetate 49c: The mixture was stirred at room temperature for 24 hours. Mp 246.6-248°C; Yield 64%; IR: 2256 (CN), 1768 (CO) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 2.29 (3H, s, CH_3), 3.93 (3H, s, OCH_3), 4.00 (3H, s, OCH_3), 6.98 (1H, s, Ar-H), 7.05 (1H, s, Ar-H), 7.29-7.47 (6H, m, 6xAr-H), 7.78-7.81 (2H, m, 2xAr-H), 7.89-7.94 (1H, m, Ar-H); ^{13}C NMR $CDCl_3$ (ppm): 20.7 (CH_3), 55.9 (CH_3), 56.2 (CH_3), 94.0 (C), 96.4 (CH), 98.1 (CH), 115.1 (C), 118.4 (C), 122.0 (CH), 123.5 (C), 125.3 (CH), 126.4 (CH), 127.1 (2xCH), 128.6 (2xCH), 129.3 (C), 129.5 (CH), 129.6 (CH), 131.0 (C), 131.9 (C), 132.2 (C), 149.4 (C), 149.6 (C), 151.7 (C), 153.9 (C), 168.3 (C).

6-(4-Methylphenyl)isoindolo[1,2-*d*][1,5]benzoxazepin-7-yl-12-carbonitrile acetate 49d: The mixture was stirred at room temperature for 24 hours. Mp 239°C; Yield 64%; IR: 2196 (CN), 1763 (CO) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 2.28 (3H, s, CH_3), 2.42 (3H, s, CH_3), 7.15-7.46 (7H, m, 7xAr-H), 7.68-7.83 (4H, m, 4xAr-H), 7.94-7.99 (1H, m, Ar-H); ^{13}C NMR $CDCl_3$ (ppm): 20.8 (CH_3), 21.5 (CH_3), 94.5 (C), 100.1 (C), 114.7 (C), 118.5 (CH), 120.4 (CH), 122.1 (CH), 122.9 (C), 124.4 (CH), 124.9 (C), 125.7 (CH), 126.5 (CH), 126.7 (CH), 127.1 (2xCH), 129.0 (C), 129.4 (2xCH), 130.2 (CH), 130.8 (C), 132.6 (C), 140.1 (C), 150.4 (C), 154.5 (C), 168.6 (C).

Synthesis of 6-acetyl-7-oxo-6,7-dihydroisoindolo[1,2-*d*][1,5]benzoxazepine-12-carbonitriles 50a,b

General procedure:

To a solution of the suitable derivative **48a,b** (0.001 mol) in tetrahydrofuran (23 ml) sodium hydride (0.001 mol) was added and the reaction mixture was stirred for 1 hour at room temperature. Acetyl chloride (0.001 mol) was added and the reaction was stirred at room temperature for 1-24 hours. Some drops of methanol were added and the solvent was evaporated under reduced pressure. The residue was dissolved in dichloromethane, washed with a saturated solution of ammonium chloride, dried and evaporated. The crude was purified by chromatography with dichloromethane as eluent.

6-Acetyl-6-phenyl-7-oxo-6,7-dihydroisoindolo[1,2-*d*][1,5]benzoxazepine-12-carbonitrile 50a: The mixture was stirred at room temperature for 24 hours. Mp 126.4-127.9°C; Yield 50%; IR: 2256 (CN), 1775 (CO), 1635 (CO) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 2.02 (3H, s, CH_3), 7.24-7.68 (9H, m, 9xAr-H), 7.81-7.93 (4H, m, 4xAr-H); ^{13}C NMR $CDCl_3$ (ppm): 20.4 (CH_3), 106.2 (C), 111.3 (C), 119.2 (CH), 120.8 (CH), 123.5 (CH), 123.9 (C), 126.6 (CH), 126.9 (CH), 128.4 (CH), 128.6 (CH),

128.7 (C), 128.9 (2xCH), 129.5 (C), 130.0 (2xCH), 130.1 (C), 131.6 (CH), 132.3 (C), 135.0 (CH), 145.8 (C), 167.3 (C), 182.3 (C), 191.8 (C).

6-Acetyl-9,10-dimethyl-6-phenyl-7-oxo-6,7-dihydroisoindolo[1,2-*d*][1,5]benzoxazepine-12-carbonitrile 50b: The mixture was stirred at room temperature for 1 hour. Mp 97-98.6°C; Yield 54%; IR: 2215 (CN), 1771 (CO), 1630 (CO) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 1.95 (3H, s, CH_3), 2.29 (3H, s, CH_3), 2.38 (3H, s, CH_3), 7.32 (1H, d, J = 8.1 Hz, Ar-H), 7.40-7.49 (2H, m, 2xAr-H), 7.55-7.63 (3H, m, 3xAr-H), 7.69 (1H, s, Ar-H), 7.74-7.89 (4H, m, 4xAr-H); ^{13}C NMR $CDCl_3$ (ppm): 19.8 (CH_3), 19.9 (CH_3), 20.8 (CH_3), 99.5 (C), 105.3 (C), 111.4 (C), 117.8 (CH), 118.6 (CH), 122.0 (C), 123.4 (CH), 126.7 (CH), 127.5 (C), 128.7 (CH), 128.9 (C), 129.3 (2xCH), 129.5 (C), 129.6 (2xCH), 131.7 (CH), 135.5 (CH), 137.7 (C), 139.6 (C), 145.6 (C), 167.3 (C), 182.0 (C), 192.2 (C).

Synthesis of 7-ethoxy-6-phenylisoindolo[1,2-*d*][1,5]benzoxazepine-12-carbonitrile 51a

To a solution of **48a** (0.0006 mol) in dimethylformamide (3 ml) triethylamine (0.0006 mol) was added and the mixture was stirred for 1 hour at room temperature. After that ethyl iodide (0.35 ml, 0.0004 mol) was added, the mixture was maintained under stirring at room temperature for 1.5 hours. The mixture was poured onto ice, the resulting precipitate was filtered off and purified by chromatography with dichloromethane as eluent.

Mp 219.4-221°C; Yield 30%; IR: 2256 (CN) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 1.19 (3H, t, J=7.1 Hz, CH_3), 4.25 (2H, q, J=7.1 Hz, CH_2), 7.26-7.37 (4H, m, 4xAr-H), 7.45-7.76 (8H, m, 8xAr-H), 8.13-8.18 (1H, m, Ar-H); ^{13}C NMR $CDCl_3$ (ppm): 14.0 (CH_3), 60.9 (CH_2), 100.0 (C), 118.5 (CH), 119.1 (C), 122.0 (CH), 123.4 (CH), 123.5 (C), 126.2 (CH), 126.4 (CH), 126.6 (CH), 127.2 (C), 127.3 (C), 128.2 (C), 128.3 (CH), 128.5 (2xCH), 130.0 (2xCH), 130.1 (C), 130.5 (C), 131.1 (CH), 133.8 (CH), 146.2 (C), 163.2 (C).

Synthesis of 6-ethyl-7-oxo-6,7-dihydroisoindolo[1,2-*d*][1,5]benzoxazepine-12-carbonitriles 52a-d

General procedure:

To a solution of **48a-d** (0.0006 mol) in dimethylformamide (3 ml) sodium hydride (0.0006 mol) was added and the mixture was stirred for 1 hour at room temperature. After that ethyl iodide (0.04 ml, 0.0006 mol) was added, the mixture was maintained under stirring at room temperaturefor 1-3 hours. The mixture was poured onto ice and extracted with ethyl acetate. The organic phase was concentrated and the crude purified by chromatography using dichloromethane as eluent.

6-Ethyl-7-oxo-6-phenyl-6,7-dihydroisoindolo[1,2-*d*][1,5]benzoxazepine-12-carbonitrile 52a: The mixture was stirred at room temperature for 3 hours. Mp 99.1-100°C; Yield 55%; IR: 2213 (CN), 1747 (CO) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 1.13 (3H, t, J=7.1 Hz, CH_3), 4.23 (2H, q, J=7.1 Hz, CH_2), 7.33-7.44 (4H, m, 4xAr-H), 7.56-7.81 (7H, m, 7xAr-H), 7.88 (1H, dd, J=1.5, 7.7 Hz, Ar-H), 8.04-8.09 (1H, m, Ar-H); ^{13}C NMR $CDCl_3$ (ppm): 13.7 (CH_3), 60.7 (CH_2), 99.5 (CH), 101.8 (C), 112.0 (C), 118.2 (CH), 118.6 (C), 121.4 (CH), 123.5 (CH), 126.3 (C), 126.7 (CH), 126.8 (CH), 127.4 (C), 128.5 (2xCH), 128.9 (CH), 129.2 (2xCH), 129.3 (C), 130.3 (C), 131.3 (CH), 134.3 (CH), 145.6 (C), 158.6 (C), 162.7 (C).

6-Ethyl-9,10-dimethyl-7-oxo-6-phenyl-6,7-dihydroisoindolo[1,2-*d*][1,5]benzoxazepine-12-carbonitrile 52b: The mixture was stirred at room temperature for 1 hour. Mp 116-118°C; Yield 24%; IR: 2211 (CN), 1746 (CO) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 1.16 (3H, t, J=7.1 Hz, CH_3), 2.37 (6H, s, 2xCH_3), 4.23 (2H, q, J=7.1 Hz, CH_2), 7.30 (2H, t, J=7.8 Hz, 2xAr-H), 7.45-7.64 (6H, m, 6xAr-H), 7.74-7.79 (2H, m, 2xAr-H), 7.90 (1H, bs, Ar-H); ^{13}C NMR $CDCl_3$ (ppm): 14.0 (CH_3), 20.7 (CH_3), 20.9 (CH_3), 60.7 (CH_2), 101.6 (C), 112.6 (C), 117.3 (CH), 118.2 (C), 120.7 (CH), 123.4 (CH), 126.2 (CH), 127.0 (C), 128.3 (C), 128.4 (CH), 128.5 (2xCH), 129.8 (C), 130.0 (2xCH), 130.7 (C), 130.8 (CH), 133.7 (CH), 137.0 (C), 137.1 (C), 146.3 (C), 159.6 (C), 163.2 (C).

6-Ethyl-9,10-dimetoxy-7-oxo-6-phenyl-6,7-dihydroisoindolo[1,2-*d*][1,5]benzoxazepine-12-carbonitrile 52c: The mixture was stirred at room temperature for 1 hour. Mp 118.8-120°C; Yield 40%; IR: 2211 (CN), 1681 (CO) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 1.17-1.24 (5H, m, CH_3, CH_2), 4.01 (6H, s, 2xCH_3), 6.58-7.11 (3H, m, 3xAr-H), 7.18-7.79 (8H, m, 8xAr-H); ^{13}C NMR $CDCl_3$ (ppm): 14.5 (CH_3), 56.2 (CH_3), 56.3 (CH_3), 64.0 (CH_2), 96.8 (CH), 100.1 (C), 100.2 (CH), 112.3 (CH), 112.4 (C), 120.2 (CH), 124.7 (C), 125.6 (C), 126.2 (C), 128.4 (2xCH), 129.4 (2xCH), 130.1 (C), 130.9 (C), 132.0 (CH), 132.6 (C), 134.3 (CH), 151.4 (C), 152.9 (C), 154.3 (C), 184.2 (C), 191.6 (C).

6-Ethyl-7-oxo-6-(4-methylphenyl)-6,7-dihydroisoindolo[1,2-*d*][1,5]benzoxazepine-12-carbonitrile 52d: The mixture was stirred at room temperature for 1 hour. Oil; Yield 25%; IR: 2213 (CN), 1740 (CO) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 1.18 (3H, t, J=7.1 Hz, CH_3), 2.33 (3H, s, CH_3), 4.25 (2H, q, J=7.1 Hz, CH_2), 7.08 (2H, d, J=8.0 Hz, 2xAr-H), 7.30-7.35 (2H, m, 2xAr-H), 7.46-7.76 (7H, m, 7xAr-H), 8.14-8.19 (1H, m, Ar-H); ^{13}C NMR $CDCl_3$ (ppm): 14.0 (CH_3), 21.7 (CH_3), 60.9 (CH_2), 102.6 (C), 112.3 (C), 118.5 (CH), 119.1 (C), 122.0 (CH), 123.5 (CH), 125.4 (C), 126.1

(CH), 126.4 (CH), 126.5 (CH), 127.3 (C), 128.2 (CH), 129.2 (2xCH), 130.0 (2xCH), 130.1 (C), 130.5 (C), 131.0 (CH), 144.7 (C), 146.3 (C), 159.4 (C), 163.2 (C).

Synthesis of 6*H*-isoindolo[1,2-*c*][1,4]benzoxazin-6-ones 53a-c

General procedure:

A solution of the intermediates **23a-c** (0.0009 mol) in acetic acid (2 ml) was heated under reflux for 2 hours. After cooling the mixture was poured onto ice and the resulting precipitate filtered off and purified by chromatography using dichloromethane as eluent.

6*H*-isoindolo[1,2-*c*][1,4]benzoxazin-6-one 53a: Mp 213-214.1°C; Yield 20%; IR: 1710 (CO) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 7.29-7.50 (5H, m, 5xAr-H), 7.76-7.85 (2H, m, 2xAr-H), 8.10 (1H, s, Ar-H), 8.34 (1H, dd, J=0.9, 8.4 Hz, Ar-H); ^{13}C NMR $CDCl_3$ (ppm): 106.2 (C), 110.1 (CH), 115.4 (CH), 118.7 (CH), 120.2 (CH), 120.6 (CH), 122.1 (C), 124.6 (CH), 124.7 (CH), 126.6 (C), 127.0 (CH), 128.2 (CH), 129.0 (C), 144.3 (C), 153.4 (C).

8,9-dimethyl-6*H*-isoindolo[1,2-*c*][1,4]benzoxazin-6-one 53b: Mp 244.4-246°C; Yield 10%; IR: 1717 (CO) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 2.37 (3H, s, CH_3), 2.40 (3H, s, CH_3), 7.27-7.46 (3H, m, 3xAr-H), 7.49 (1H, s, Ar-H), 7.74-7.78 (1H, m, 1xAr-H), 7.92 (1H, s, Ar-H), 8.06 (1H, s, Ar-H); ^{13}C NMR $CDCl_3$ (ppm): 20.7 (CH_3), 20.9 (CH_3), 110.3 (CH), 115.2 (CH), 118.6 (CH), 119.1 (CH), 119.5 (CH), 122.3 (C), 124.5 (CH), 124.6 (C), 126.0 (C), 127.7 (CH), 128.6 (C), 134.8 (C), 137.7 (C), 137.8 (C), 144.2 (C).

8,9-dimetoxy-6*H*-isoindolo[1,2-*c*][1,4]benzoxazin-6-one 53c: Mp 212°C; Yield 15%; IR: 1710 (CO) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 3.87 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 7.18 (1H, s, Ar-H), 7.39-7.50 (4H, m, 4xAr-H), 8.34 (1H, dd, J=1.8, 6.1 Hz, Ar-H), 8.63 (1H, s, Ar-H); ^{13}C NMR $CDCl_3$ (ppm): 55.4 ($2xCH_3$), 97.5 (CH), 99.0 (CH), 104.5 (C), 112.7 (CH), 116.1 (CH), 117.8 (CH), 121.5 (C), 122.2 (C), 124.5 (C), 124.8 (CH), 127.4 (CH), 143.2 (C), 149.1 (C), 151.6 (C), 152.8 (C).

Synthesis of ethyl azidoacetate 78:

To a solution of ethyl bromoacetate **77** (6.64 ml, 0.06 mol) in acetone (64 ml) sodium azide (9.8g, 0.14 mol) dissolved in water (50 ml) was added with an ice bath. The reaction mixture was stirred under reflux for 4 hours. The solvent is evaporated under reduced pressure. A saturated solution of sodium hydrogen carbonate was added and the aqueous phase was extracted with ethyl acetate. The

solvent was evaporated under reduced pressure and the product obtained was used without further purification.

Oil; Yield 100%; IR: 2108 (N_3), 1740 (CO) cm^{-1}; ^1H NMR $CDCl_3$ (ppm): 1.32 (3H, t, J=7.1 Hz, CH_3), 3.87 (2H, s, CH_2), 4.27 (2H, q, J=7.1 Hz, CH_2); ^{13}C NMR $CDCl_3$ (ppm): 14.2 (CH_3), 50.4 (CH_2), 61.9 (CH_2), 168.3 (C).

Synthesis of ethyl (2Z)-2-azido-3-(2,5-dimethoxyphenyl)prop-2-enoate 80:

0.55 g of sodium were added, under inert atmosphere, to 23 ml of ethanol and the mixture stirred at room temperature until complete dissolution. 24 mmol of compound **78** (3.1 g), dissolved in ethanol (5 ml) were added dropwise to the sodium ethylate thus formed at -20°C. The mixture stirred at -20°C for 2 hours then the 2,5-dimethoxybenzaldehyde **79** (1 g, 6 mmol) dissolved in ethanol (7 ml) was added. The mixture was stirred at -20°C for additional 2.5 hours, then it was cooled to room temperature, the solvent evaporated under reduced pressure and the residue dissolved in ethyl acetate and washed with water. The organic phase was concentrated and the crude purified by column chromatography using dichloromethane as eluent.

Mp 68.7-70°C; Yield 48%; IR: 2116 (N_3), 1704 (CO) cm^{-1}; cm^{-1}; ^1H NMR $CDCl_3$ (ppm): 1.40 (3H, t, J=7.1 Hz, CH_3), 3.81 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 4.37 (2H, q, J=7.1 Hz, CH_2), 6.78-6.91 (2H, m, 2xAr-H), 7.36 (1H, s, CH), 7.82 (1H, d, J=2.8 Hz, Ar-H); ^{13}C NMR $CDCl_3$ (ppm): 14.3 (CH_3), 55.8 (CH_3), 56.2 (CH_3), 62.2 (CH_2), 111.5 (CH), 115.8 (CH), 116.1 (CH), 119.1 (CH), 122.7 (C), 125.5 (C), 152.2 (C), 153.0 (C), 163.7 (C).

Synthesis of ethyl 4,7-dimethoxy-1*H*-indole-2-carboxylate 81:

A solution of compound **80** (2.6 mmol) in toluene (15 ml) was refluxed for 2 hours. The solvent was evaporated under reduced pressure and the crude purified by column chromatography using dichloromethane as eluent.

Mp 113.6 °C; Yield 66%; IR: 3442 (NH), 1695 (OH) cm^{-1}; ^1H NMR $CDCl_3$ (ppm): 1.33 (3H, t, J=7.1 Hz, CH_3), 3.83 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 4.30 (2H, q, J=7.1 Hz, CH_2), 6.40 (1H, d, J=8.3 Hz, Ar-H), 6.67 (1H, d, J=8.3 Hz, Ar-H), 7.08 (1H, d, J=2.2 Hz, Ar-H), 11.92 (1H, bs, NH); ^{13}C NMR $CDCl_3$ (ppm): 14.2 (CH_3), 55.2 (CH_3), 55.6 (CH_3), 60.2 (CH_2), 98.8 (CH), 104.6 (CH), 105.7 (CH), 119.3 (C), 126.6 (C), 129.2 (C), 141.2 (C), 147.6 (C), 160.8 (C).

Synthesis of 4,7-dimethoxy-1*H*-indole-2-carboxylic acid 82:

An aqueous solution of 5% sodium hydroxide (31 ml) was added to indole **81** (4.1 mmol) and the mixture was refluxed for 1 hour. Then, the mixture was cooled in an ice-bath and neutralized with

6N hydrochloric acid. The precipitate obtained was filtered and it was used without any further purification.

Mp 204 °C; Yield 74%; IR: 3486 (NH), 3480 (OH), 1675 (CO) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 3.83 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 6.39 (1H, d, J=8.3 Hz, Ar-H), 6.65 (1H, d, J=8.3 Hz, Ar-H), 7.03 (1H, d, J=2.2 Hz, Ar-H), 11.71 (1H, bs, NH), 12.80 (1H, bs, OH); ^{13}C NMR $CDCl_3$ (ppm): 55.1 (CH_3), 55.6 (CH_3), 98.6 (CH), 104.4 (CH), 105.4 (CH), 119.4 (C), 127.6 (C), 129.1 (C), 141.2 (C), 147.6 (C), 162.3 (C).

Synthesis of 4,7-dimethoxy-1*H*-indole 83:

The indole-2-carboxylic acid **82** (0.2 g, 0.9 mmol), copper powder (0.2 g, 3.6 mmol), and *N*-methylpyrrolidinone (11 ml) were heated at reflux (250°C) for 2 hours under nitrogen atmosphere. The mixture was poured into ice and water and extracted with ethyl acetate, the crude was purified by column chromatography using dichloromethane as eluent.

Mp 127.7-128.6°C; Yield 78%; IR: 3368 (NH) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 3.91 (6H, s, 2xOCH_3), 6.38 (1H, d, J=8.2 Hz, Ar-H), 6.51 (1H, d, J=8.2 Hz, Ar-H), 6.63 (1H, s, Ar-H), 7.10 (1H, s, Ar-H), 8.39 (1H, bs, NH); ^{13}C NMR $CDCl_3$ (ppm): 55.7 (2x CH_3), 98.7 (CH), 100.3 (CH), 101.4 (CH), 119.9 (C), 122.6 (CH), 127.6 (C), 141.1 (C), 147.7 (C).

Synthesis of 4,7-dimethoxy-3-(phenylsulfanyl)-1*H*-indoles 73a, c, d, f:

General procedure:

To a solution of indole **83** (1.7 mmol) and suitable thiophenol **84** (1.7 mmol) in dimethylformamide (8 ml) copper iodide (1.7 mmol), 2,2 '-bipyridyl (1.7 mmol) and sodium carbonate (4.3 mmol) were added. The mixture was heated at reflux for 1.5-2 hours then the mixture was cooled to room temperature and poured into water and ice. The precipitate obtained was filtered and purified by column chromatography using dichloromethane as eluent.

4,7-Dimethoxy-3-(phenylsulfanyl)-1*H*-indole 73a: The mixture was stirred at reflux for 1.5 hours. Mp 115.2-116.5°C; Yield 68%; IR: 3451 (NH) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 3.65 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 6.40 (1H, d, J=8.3 Hz, Ar-H), 6.55 (1H, d, J=8.3 Hz, Ar-H), 7.04-7.30 (6H, m, 6xAr-H), 8.56 (1H, bs, NH); ^{13}C NMR $CDCl_3$ (ppm): 55.7 (CH_3), 56.2 (CH_3), 100.0 (C), 101.0 (CH), 102.5 (CH), 103.0 (C), 124.6 (CH), 126.4 (2xCH), 128.4 (2xCH), 128.7 (C), 129.3 (CH), 140.6 (C), 140.9 (C), 148.8 (C).

4,7-Dimethoxy-3-[(3-methoxyphenyl)sulfanyl]-1H-indole 73c: The mixture was stirred at reflux for 2 hours. Mp: 194.4-194.9°C; Yield 35%; IR: 3441 (NH) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 3.92 (9H, m, CH_3, 2xOCH_3), 6.40 (1H, d, J = 8.3 Hz, Ar-H), 6.56 (1H, d, J = 8.3 Hz, Ar-H), 6.70-6.84 (4H, m, 4xAr-H), 7.28 (1H, m, Ar-H), 8.61 (1H, bs, NH); ^{13}C NMR $CDCl_3$ (ppm): 55.7 (CH_3), 55.8 (CH_3), 56.2 (CH_3), 100.8 (CH), 100.9 (C), 102.5 (CH), 109.7 (CH), 110.1 (C), 110.3 (C), 120.9 (CH), 125.1 (CH), 126.7 (CH), 129.5 (C), 129.6 (C), 129.7 (C), 129.8 (CH), 141.3 (C).

4,7-Dimethoxy-3-[(3-methylphenyl)sulfanyl]-1*H*-indole 73d: The mixture was stirred at reflux for 2 hours. Mp 111.4-112.3°C; Yield 35%; IR: 3454 (NH) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 2.23 (3H, s, CH_3), 3.67 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 6.40 (1H, d, J = 8.4 Hz, Ar-H), 6.55 (1H, d, J = 8.4 Hz, Ar-H), 6.83 -7.21 (4H, m, 4xAr-H), 7.26 (1H, d, J = 2.6 Hz, Ar-H), 8.55 (1H, bs, NH); ^{13}C NMR $CDCl_3$ (ppm): 21.4 (CH_3), 55.7 (CH_3), 56.2 (CH_3), 101.0 (CH), 102.5 (CH), 103.3 (C), 123.7 (CH), 125.6 (CH), 127.1 (CH), 128.3 (CH), 128.7 (C), 129.3 (CH), 138.1 (C), 140.3 (C), 140.9 (C), 148.9 (C), 177.0 (C).

3-[(2-Bromophenyl)sulfanyl]-4,7-dimethoxy-1*H*-indole 73f: The mixture was stirred at reflux for 2 hours. Mp 126-127°C; Yield 47%; IR: 3447 (NH) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 3.64 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 6.41 (1H, d, J=8.3 Hz, Ar-H), 6.57 (1H, d, J=8.3 Hz, Ar-H), 6.70-7.19 (4H, m, 4xAr-H), 7.34-7.76 (1H, m, Ar-H), 8.61 (1H, bs, NH); ^{13}C NMR $CDCl_3$ (ppm): 55.7 (CH_3), 56.2 (CH_3), 100.0 (CH), 101.2 (CH), 102.0 (C), 102.6 (C), 102.7 (CH), 119.4 (C), 125.4 (CH), 127.2 (CH), 128.9 (C), 130.0 (CH), 132.1 (CH), 140.9 (C), 142.2 (C), 148.8 (C).

Synthesis of 4,7-dimethoxy-3-(phenylsulfonyl)-1*H*-indoles 73m, r:

General procedure:

To a solution of 3-chloroperbenzoic acid (4.2 mmol) in dichloromethane (20 ml) at 0°C a solution of suitable 4,7-dimethoxy-3-(phenylsulfanyl)-1H-indoles compounds (1.05 mmol) in dichloromethane (10 ml) was added dropwise. Immediately a solid separated from the reaction mixture. A saturated solution of sodium hydrogen carbonate (10 ml) was added and aqueous phase was extracted with dichloromethane. The solvent was evaporated under reduced pressure and the product obtained was purified by column chromatography.

4,7-Dimethoxy-3-[(3-methylphenyl)sulfonyl]-1H-indole 73m: The crude was purified using a mixture of dichloromethane and ethyl acetate (99:1) as eluent. Mp: 173.8-174.3°C; Yield 48%; IR: 1141 (SO_2), 3438 (NH) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 2.36 (3H, s, CH_3), 3.62 (3H, s, OCH_3), 3.85

(3H, s, OCH_3), 6.40 (1H, d, J = 8.3 Hz, Ar-H), 6.55 (1H, d, J = 8.3 Hz, Ar-H), 7.39 (1H, s, Ar-H), 7.71 (3H, bs, 3xAr-H), 7.91 (1H, s, Ar-H), 12.40 (1H, bs, NH); ^{13}C NMR $CDCl_3$ (ppm): 20.7 (CH_3), 55.0 (CH_3), 55.7 (CH_3), 101.5 (CH), 103.7 (CH), 116.1 (C), 124.6 (CH), 127.6 (CH), 128.5 (C), 128.7 (C), 131.6 (CH), 132.9 (CH), 138.2 (C), 140.8 (C), 143.2 (C), 146.5 (C).

4,7-Dimethoxy-3-(phenylsulfonyl)-1*H*-indole 73r: The crude was purified using a mixture of dichloromethane and ethyl acetate (95:5) as eluent. Mp 192.6-194°C; Yield 70%; IR: 1141 (SO_2), 3313 (NH) cm^{-1}; ^1H NMR $CDCl_3$ (ppm): 3.69 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 6.40 (1H, d, J = 8.3 Hz, Ar-H), 6.55 (1H, d, J = 8.3 Hz, Ar-H), 7.04-7.46 (3H, m, 3xAr-H), 7.96-8.02 (3H, m,3x Ar-H), 8.98 (1H, bs, NH); ^{13}C NMR $CDCl_3$ (ppm): 55.6 (CH_3), 55.8 (CH_3), 100.0 (C), 102.2 (CH), 103.8 (CH), 117.9 (C), 127.8 (2xCH), 128.3 (2xCH), 128.9 (C), 130.2 (CH), 132.2 (CH), 140.7 (C), 143.2 (C), 147.3 (C).

Synthesis of 3-(phenylsulfonyl)-1*H*-indole-4,7-dione compounds 75n, p:

General procedure:

4,7-Dimethoxy-3-(phenylsulfonyl)-1*H*-indole compound **73m, r** (0.32 mmol) was dissolved in tetrahydrofuran (3 ml) and a 90% solution of nitric acid (0.12 ml) and silver(II)oxide (0.12 g) were added at 0°C. The mixture was stirred at room temperature for 30 minutes, then it was poured into ice and water and extracted with dichloromethane. The solvent is evaporated under reduced pressure and the product obtained was purified by column chromatography using a mixture of dichloromethane and ethyl acetate (95:5) as eluent.

3-[(3-Methylphenyl)sulfonyl]-1H-indole-4,7-dione 75n: Mp: 183.8-184.3°C; Yield 80%; IR: 1142 (SO_2), 1593,1664 (CO), 3557 (NH) cm^{-1}; ^1H NMR $CDCl_3$ (ppm): 2.42 (3H, s, CH_3) 6.64 (2H, m, 2xAr-H), 7.27-7.39 (2H, m, 2xAr-H), 7.87-7.97 (3H, m, 3xAr-H), 10.57 (1H, bs, NH); ^{13}C NMR $CDCl_3$ (ppm): 21.4 (CH_3), 89.2 (CH), 125.7 (CH), 128.7 (CH), 129.5 (CH), 132.9 (C), 133.8 (C), 134.4 (CH), 134.9 (CH), 138.8 (CH), 139.1 (C), 140.0 (C), 140.6 (C), 177.7 (C), 180.0 (C).

3-(Phenylsulfonyl)-1*H*-indole-4,7-dione 75p: Mp 197.6-198.7°C; Yield 65%; IR: 1142 (SO_2), 1662, 1679 (CO), 3313 (NH) cm^{-1}; ^1H NMR $CDCl_3$ (ppm): 6.68 (2H, q, J = 10.2 Hz, 2xAr-H), 7.55-7.70 (3H, m, 3xAr-H), 7.96 (1H, s, Ar-H), 8.06 (2H, d, J = 6.9 Hz, 2xAr-H), 13.64 (1H, bs, NH); ^{13}C NMR $CDCl_3$ (ppm): 119.8 (C), 123.1 (C), 127.6 (2xCH), 128.9 (2xCH), 130.7 (CH), 133.4 (CH), 135.5 (CH), 137.9 (CH), 141.3 (C), 148.0 (C), 177.6 (C), 180.4 (C).

Docking studies were performed for all designed compounds by Glide 8.0 (Schrodinger Inc., 2008) installed on a 3.4 GHz Pentium 4 processor with 1 GB RAM and 250 GB Hard Disk with Windows XP operating system.

The X-ray crystallographic structure of HIV-1 RT protein (PDB code 1RT2) was downloaded from Protein Data Bank (www.rcsb.org/pdb).

For Glide docking studies, chain A was retained and the protein was minimized by the protein preparation wizard. Partial atomic charges were assigned according to the OPLS_AA force field. A radius of 20 Å was selected for active site cavity during receptor grid generation.

All compounds used in the docking study with Glide were built within maestro by using build module of Schrodinger Inc., (2008).

All docking calculations were performed using the Extra Precision (XP) mode of Glide Program 8.0. A grid box was prepared with the center defined by the co-crystallized ligand TNK 651 of 1RT2.

To validate the Glide docking protocol, TNK 651, the co-crystalized ligand was docked into the binding site. The docking structure was compared to the crystal structure showing that this protocol successfully reproduces the crystal TNK 651- RT complex.

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SYNTHESIS OF TRIAZOLES AND OXAZOLES DERIVATIVES AS G-QUADRUPLEX LIGANDS (UCL, SCHOOL OF PHARMACY)

INTRODUCTION

During my PhD, I spent 6 months at the University College of London under the supervision of Professor Stephen Neidle, where I carried out a project based on the synthesis of potential ligand of G-quadruplex DNA.

Telomeres are specialized sequences at the end of chromosomes, comprising tandem repeats of simple DNA sequences, they serve to protect chromosome integrity and ensure complete replication of essential genes.

Human telomeric DNA consist of tandem repeats of the sequence 5'-TTAGGG with single-stranded 3'-end overhang (Fig.1). [1]

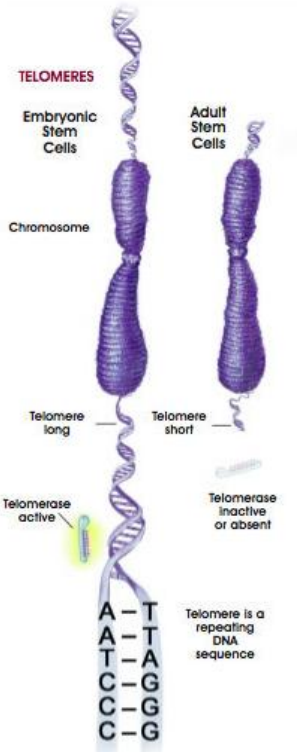


Figure 1 [2]

This single stranded DNA can be recognized as a damage so it is not usually exposed to the cell environment, but is associated with proteins to organize it in a ordered structure, called T-loop, in which the G-rich sequence loops back on itself and is inserted in the duplex telomeric DNA, paring with the C-rich strand replacing its complementary counterpart, which thus forms a D-loop. [3]

This arrangement is maintained by the “Shelterin complex”: a complex of six proteins assembled at the telomeric DNA. [4] Three components of this complex, TRF1 (telomeric-repeat-binding factor 1), TRF2 (telomeric-repeat-binding factor 2) and POT1 (protection of telomeres 1) specifically recognize the sequence TTAGGG, TRF1 and 2 bind the double stranded DNA while POT1 recognizes the single stranded DNA. The structure is stabilized by a bridging protein chain made by the other three subunits TIN2, TPP1 and RAP1 (Fig.2). [5]

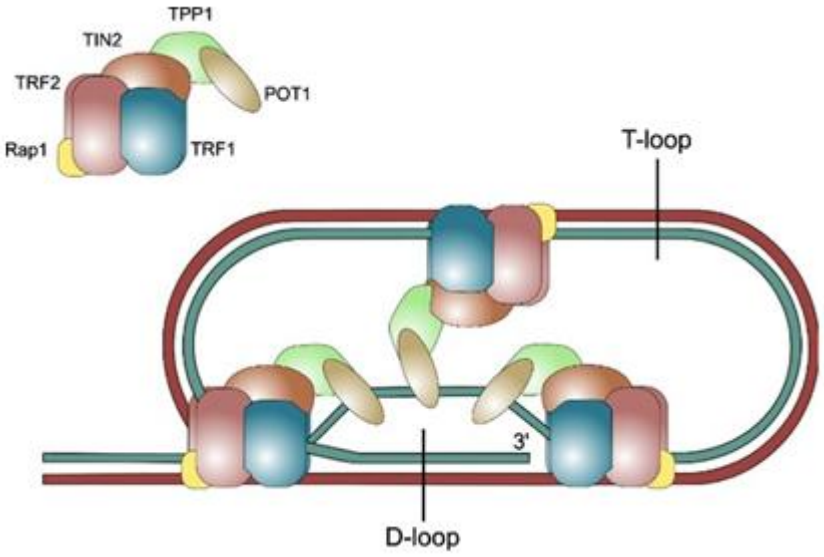


Figure 2 [6]

Due to replication problem after every round of cell division the telomere is shortened by 50-200 nucleotides: DNA polymerase is unable to replicate until the end of the chromosome and after a the loss of a number of nucleotides (known as Hayflick) the telomere becomes critically short causing the cells’ senescence and apoptosis. [7]

In the majority of the tumour cells the telomeres are maintained at a constant length by the activation of the enzyme telomerase causing cell immortalization. [8] This enzyme is up-regulated in 80-85% of human tumours [9] but the enzyme does exist in stem cells. [10]

The telomerase enzyme complex is a reverse transcriptase that adds bases onto the 3’ single stranded telomeric overhang; it consists of a (catalytic subunit hTERT and a ribonucleic acid subunit hTR, which carries a 11-bp RNA template, scaffold for DNA retrosynthesis (Fig.3). [11]

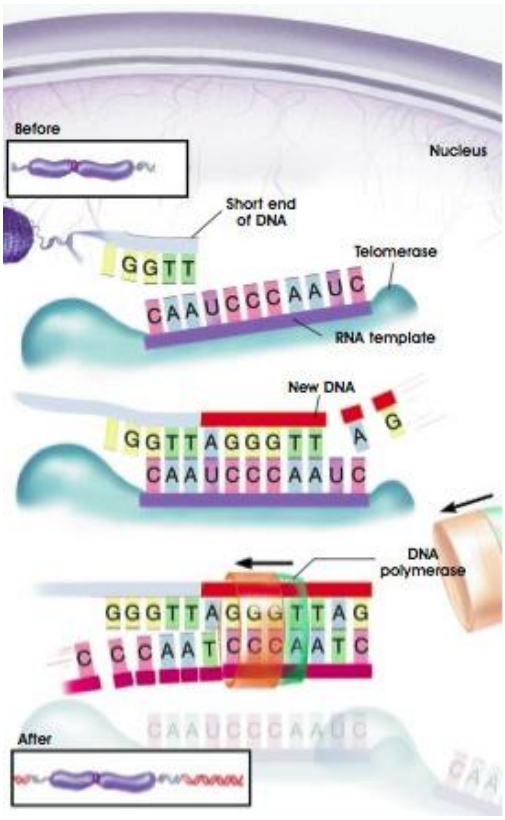


Figure 3 [2]

Zahler and co-workers demonstrated that for the activity of the telomere the single stranded 3' end has to be unfolded, thus it was shown that induction of the telomere folding to form four-stranded quadruplex structures results in inhibition of telomerase activity. [12]

DNA can form other tertiary structures besides the well-known double helix. The G-quadruplex is a stable four-stranded structure characteristic of nucleic acid sequences rich in guanine.

Four coplanar guanine bases can be linked through Hoogsteen hydrogen bonding to generate a square planar arrangement called G-tetrad (o G-quartet): two or more G-tetrads can stack on top of each other by π–π interactions to generate a G-quadruplex, whose structure is stabilized by the presence of cations, such as K^+ or Na^+, which coordinates the carbonyl groups in the centre of the quartets (Fig.4). [13]

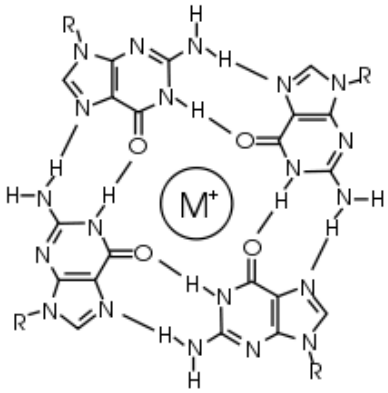


Figure 4

The stacked bases exist as quadruple helix, because of the steric constraints they cannot stay directly on each other but are twisted.

G-quadruplexes can be formed by the association of four DNA strands that can run parallel and anti-parallel: both intra- and intermolecular arrangement are possible, in the second case the quadruplex formed will be dimeric or tetrameric, it depends in how many different strands are involved (Fig.5).

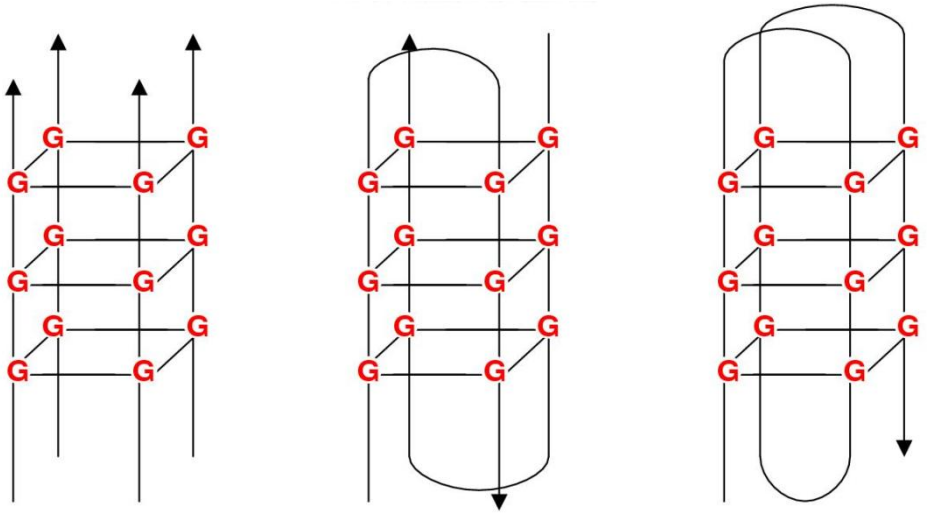


Figure 5 [14]

Depending on whether loops connect strands which are either adjacent or opposite, they can be lateral, diagonal o external and the guanines can have relative *syn* or *anti* conformation (Fig.6). [15]

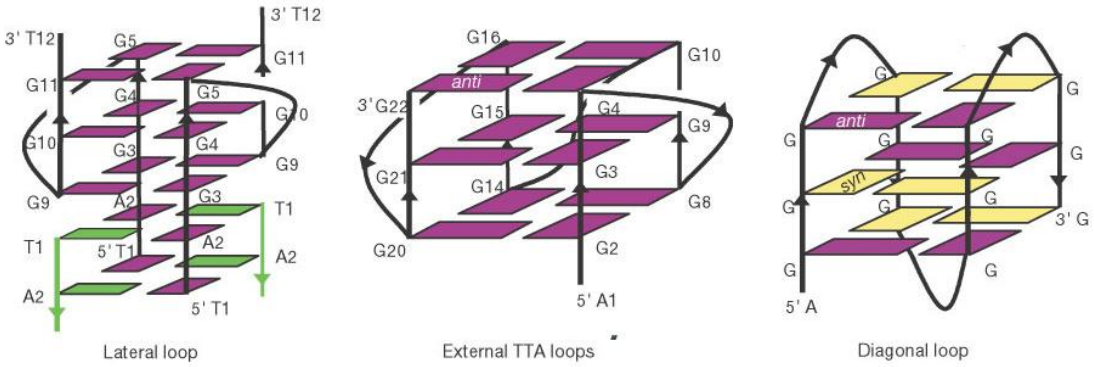


Figure 6 [13]

Regions with G-rich sequences, that can form G-quadruplexes, were found in many different areas of the genome other than telomeres.

It was found that the gene of the human heat shock protein 90 (Hsp 90) contains a guanin-rich sequence upstream of the transcription start site, analogous to the *c*-kit promoter sequence. [16]

The human proto-oncogene *c*-kit encodes a receptor tyrosine kinase that, upon activation by its ligand, stimulates cell proliferation, differentiation and survival. [17]

Activating mutations or overexpression can result in aberrant function and oncogenic cellular transformation.

Two G-rich sequences were found in *c*-kit promoter region, they occur between position -87 and -109 base pairs (Kit 1) and between -140 and -160 base pairs (Kit 2) relative to the transcription start site. Both of them were shown to form stable G-quadruplexes *in vitro*, and the three-dimensional structures was solved for each sequence. [18]

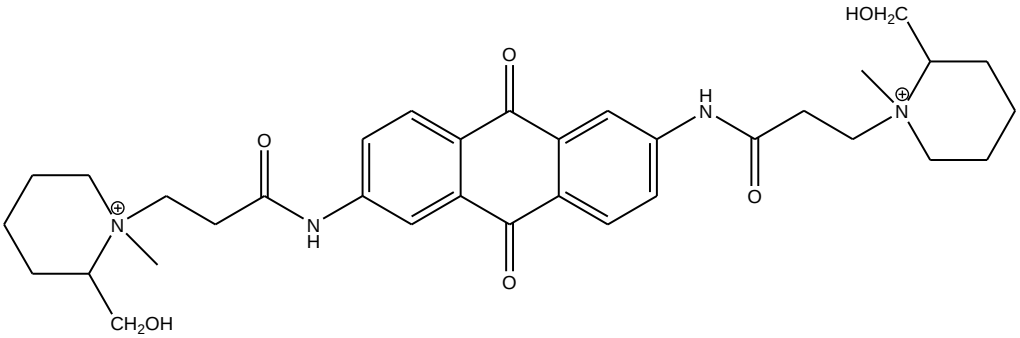
Heat shock protein 90 (Hsp90) is an ATP-dependent molecular chaperone that regulates the late-stage maturation, activation, and stability of different kind of proteins, many of them are involved in signal transduction and other pathways important in the malignancy. [19]

It was found that Hsp90 proteins are required to obtain a functionally active telomerase *in vitro* [20] and the essential sites for the function are the *N*-terminal ATP-binding site [21] and a putative *C*-terminal ATP-binding site, they act cooperatively allowing cross-talk mediated by a central charged domain between the two termini. [22]

G-quadruplex structure can be stabilized by organic molecules through the combination of electrostatic and hydrophobic force and the formation of hydrogen bonds.

Through molecular modeling and crystallographic data, [13] it was possible to identified some features required for the interaction, including a flat aromatic surface, the presence of cationic charges, amino groups which are protonated at physiological pH and the ability to interact with the G-quartets through $\pi-\pi$ force.

The first G-quadruplex binding ligand was the 2,6-diamidoanthraquinone **1,** [23] it was developed after a series of anthraquinones that showed to act as selective DNA triplex interacting compounds, with reduced affinity for duplex DNA. [24] [25]

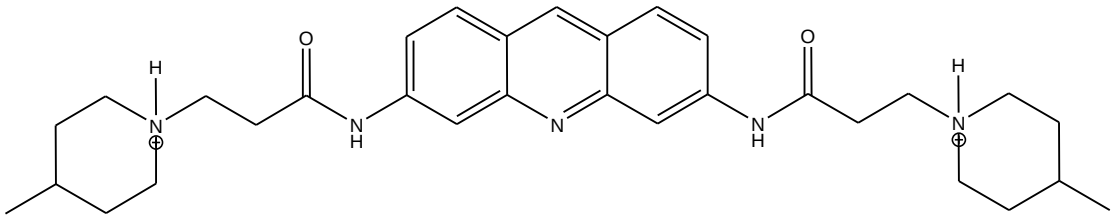


1

This compound demonstrated telomerase inhibitory ability (IC_{50} = 23 µM) but there was an almost complete inhibition of telomerase activity at 100 µM.

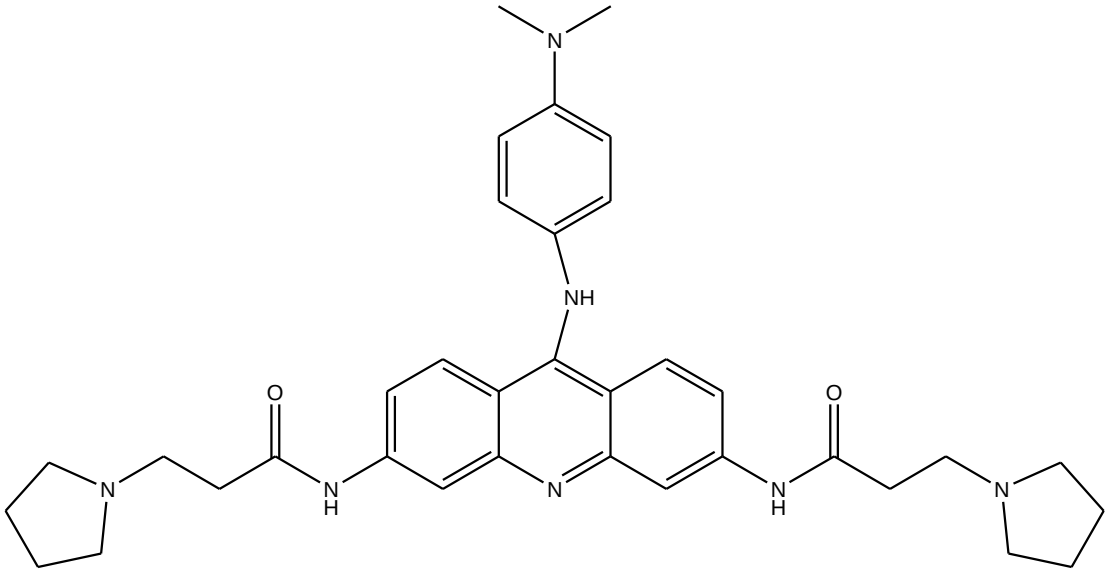
Structure-activity relationship studies on anthraquinone compounds showed the effect of chromophore, chain linker and regioisomerism on telomerase activity. Based on these studies, a series of analogous 3,6-disubstituted acridine derivatives were developed since the acridine moiety is an inherently planar chromophore, but contains a heterocyclic nitrogen atom with the ability to be protonated at physiological pH.

The acridine derivatives examined showed a telomerase inhibitory activity with the most potent inhibitor **2** having a IC_{50} value of 1.35 µM. [26]



2

Ligands based on the acridine skeleton were subsequently developed by derivatizing the 9-position of the 3,6-disubstituted acridine ring system. The lead compound, BRACO-19 **3**, showed an IC_{50} value of 0.06 µM. [27]



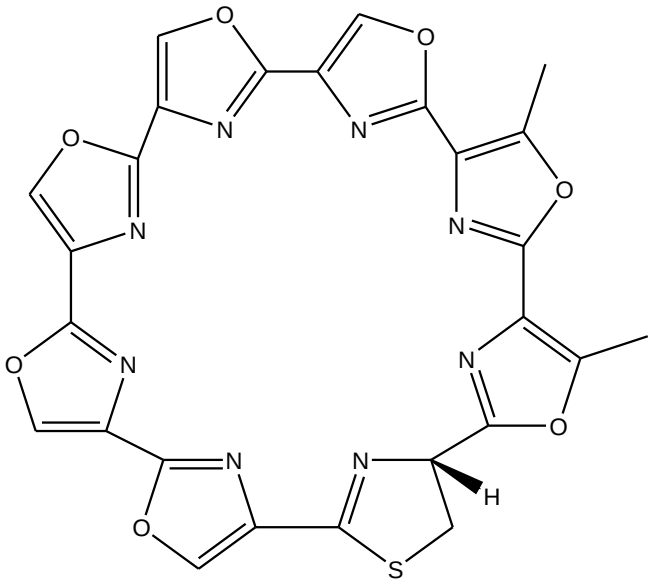
3

This compound inhibited cell growth at subcytotoxic concentrations after days rather than weeks, by inducing senescence and displacement of POT1 from telomeres. [28]

Evidence of telomere shortening in UXF1138L cells (uterine carcinoma cell line) was discovered; with an IC_{50} of 2.5 µM and an IC_{100} of 5 µM, and the concentration that would cause zero growth inhibition was found 1µM, in a 5 day proliferation assay. [29]

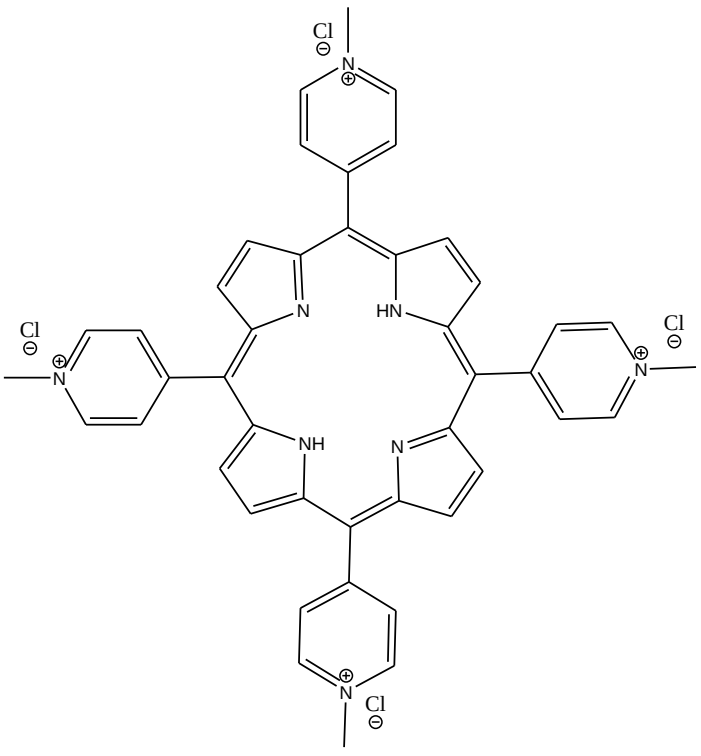
In vivo activity against a human tumor xenograft was also found, where the cytotoxic agent taxol was used to produce tumor regression and BRACO-19, given after debulking, was able to suppress tumor regrowth. [30]

The most potent and specific telomerase inhibitor known is a natural compound produced by *Streptomyces Anulatus* 3533-SV4; Telomestatine **4** showed an IC_{50} value of 0.005 µM and it was proved to be a specific telomerase inhibitor since it showed weak activities against reverese transcriptases such as HIV- and MMLV- (moloney murine leukemia virus), with IC_{50} values of 19.4 µM and 13.4 µM respectively. [31]



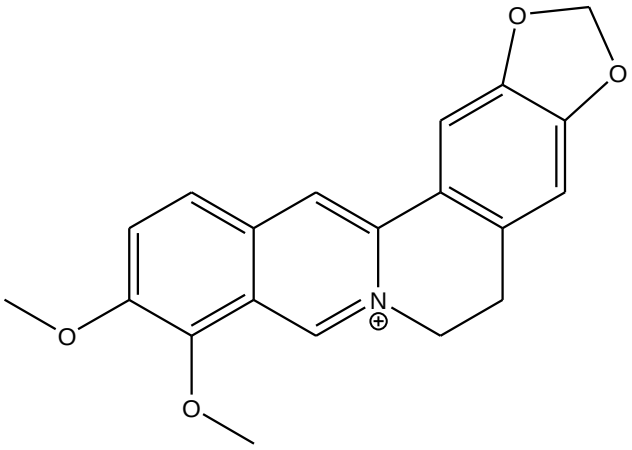
4

In the porphyrine family the most active compound was TMPyP4 **5** (5,10,15,20-tetra-(N-methyl-4-pyridyl)porphine) with an IC_{50} values of 6.5 μM, by stacking on the G-quartet surface setting up π–π interactions. [32]



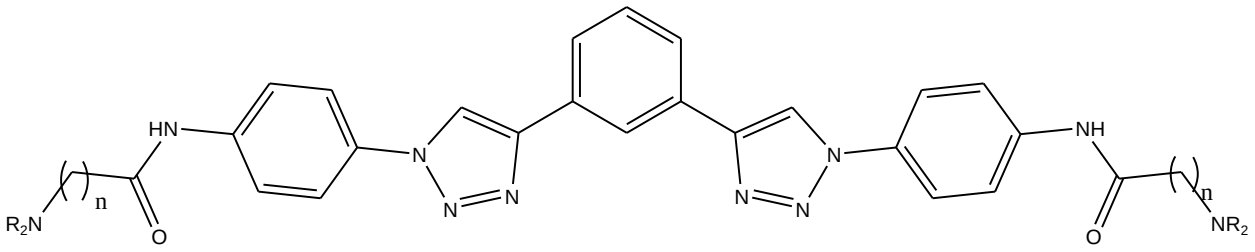
5

Berberine **6** is an antibiotic alkaloid originating from Chinese herbal medicine, [33] it was subsequently screened for anticancer activity showing evidence of antineoplastic properties. [34]
It was after shown to inhibit telomere elongation and to bind G-quadruplex DNA with an IC_{50} values of 35 µM. [35]



6

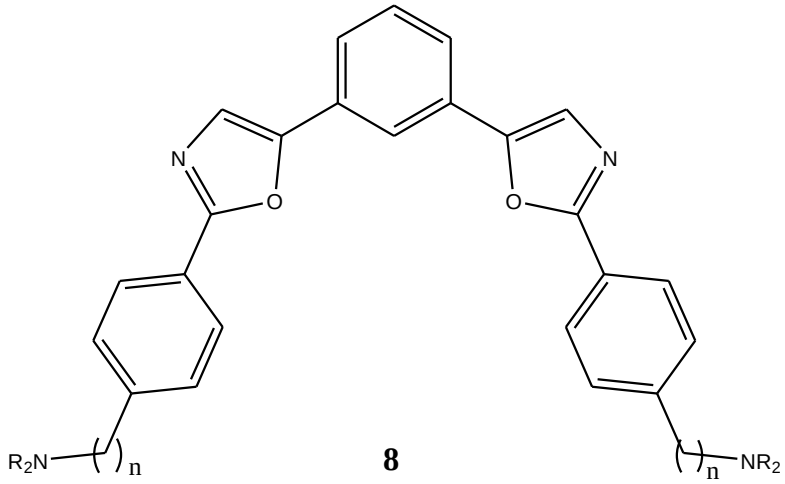
Based on data and *in vitro* and *in vivo* knowledge gathered from previous G-quadruplex ligands and chemotypes, two series of compounds were developed: phenyl bis-triazole and phenyl bis-oxazole derivatives.
The triazole compounds **7** are the first generation of G-quadruplex binding ligand synthesised via "click chemistry" and they showed high affinity for G-quadruplex over duplex DNA, along with some telomerase inhibition *in vitro* (best IC_{50} value is 13.2 µM). [36]



7

n =1, 2
NR_2 = pyrrolidinyl-, piperidinyl-, dimethylamino-, diethylamino-

The phenyl bis-oxazole **8** derivative showed high selectivity for Hsp90A and Hsp90B G-quadruplex DNA.

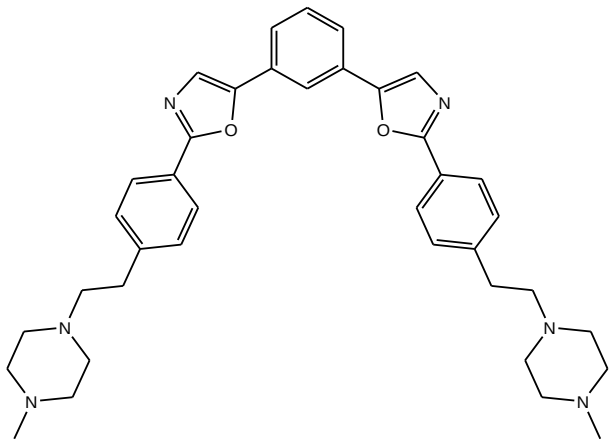


8

n =1, 2

NR_2 = pyrrolidinyl-, piperidinyl-, dimethylamino-, diethylamino-

The lead compound **9** was evaluated in cell-based assays to investigate the cell growth inhibitory ability and gave IC_{50} values in cancer cell lines (Tab.1) [37]

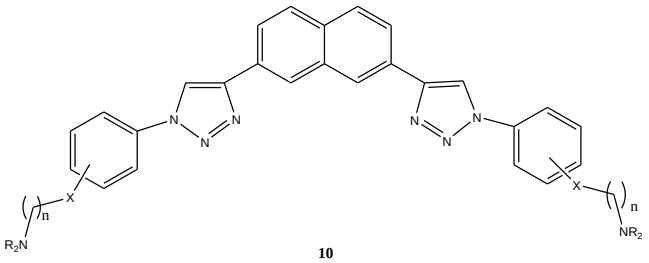


9

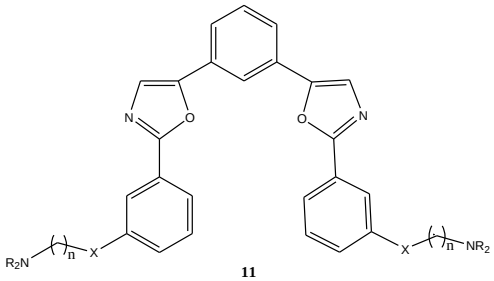
Cell line	IC_{50} (µM)
A549	1.02
MCF7	1.32
RCC4	0.94
786-O	1.33
MiaPaCa	1.25
WI38	2.59

Table 1

Considering the results shown by the former ligands, the purpose of the project was the synthesis of naphthalene bis-triazole derivatives **10**, and phenyl and naphthalene bis-oxazole derivatives **11** and **12** and the derivatisation occurred with the linker of the appropriate length and with a different panel of amines.



10



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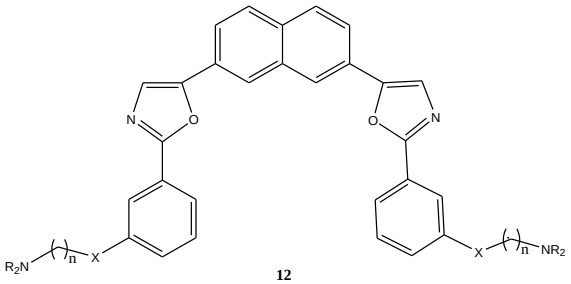


12

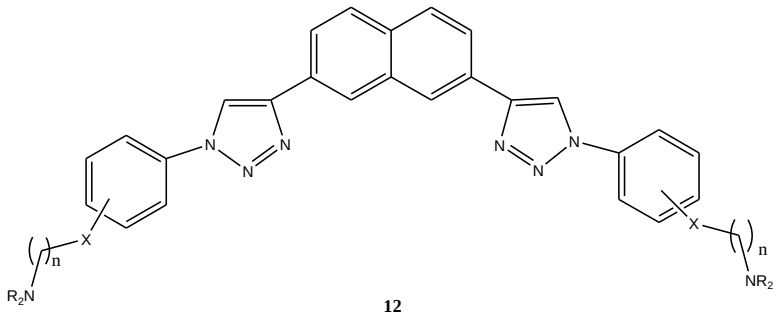
X = O, NH

n = 2,3

NR_2 = diethylamino-, pyrrolidinyl-, N-methyl-piperazinyl-

RESULTS AND DISCUSSION

The synthesis of the bis-triazole compounds was based on a convergent approach of the alkyne **13** and azide **14** building blocks, the final compounds were the result of a formation of two triazole rings via Huisgen 1,3-dipolar cycloaddition.



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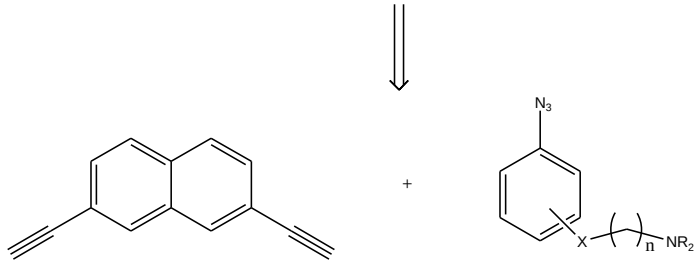
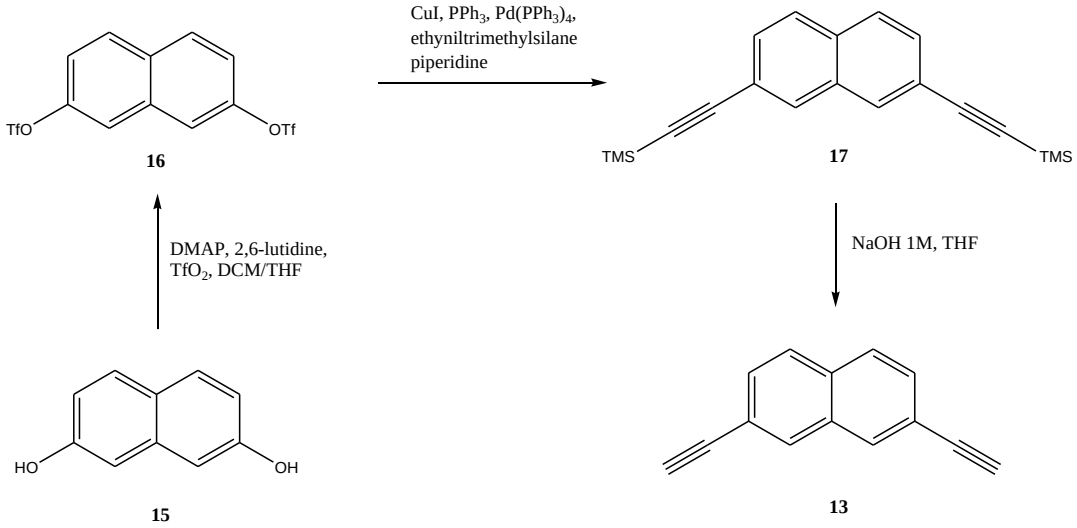


13 **14**

X = O, NH
n = 2,3
NR_2 = diethylamino-, pyrrolidinyl-, N-methyl-piperazinyl-

The alkyne building block was synthesized in three steps.



The first step was the conversion of naphthalene-2,7-diol **15** into the corresponding di-triflate **16** using triflic anhydride, that was substituted by the nuclophilic hydroxyl group of the naphthalene.

The second was the formation of the protected alkyne **17** through Sonogashira coupling reaction using tetrakis(triphenylphosphine)palladium, copper iodide and piperidine as base under reflux overnight to give the desired compound with 90% yield.

The mechanism was based on Pd and Cu cycles; the first one goes through the oxidative insertion of the alkyne, transmetallation, tran-cis isomerisation and reductive elimination with formation of the desired product and regeneration of Pd(0); the Cu cycle provides the formation of a π alkyne-Cu complex, the formation of the alkynyl copper, the transmetallation and the regeneration of copper iodide (Fig.7).

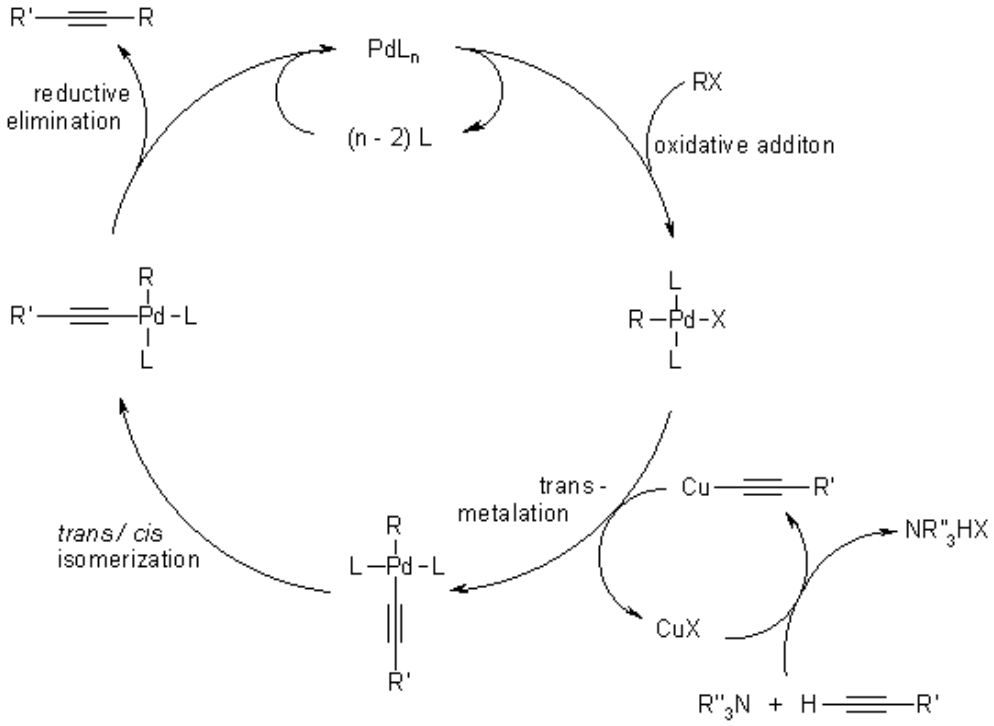
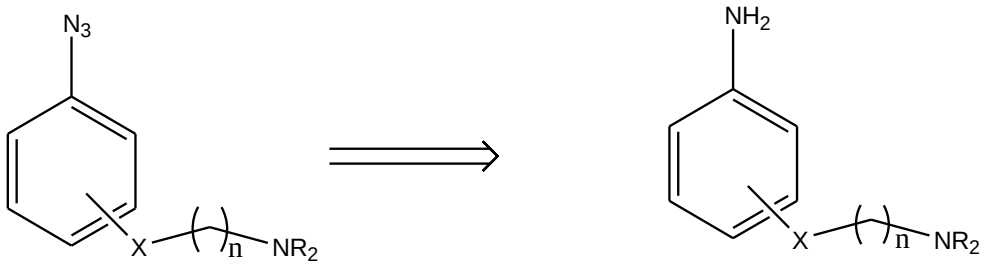


Figure 7 [38]

The final step is an easy deprotection of the trimethylsilane with aqueous sodium hydroxide solution obtaining the compound **13** in a quantitative yield.

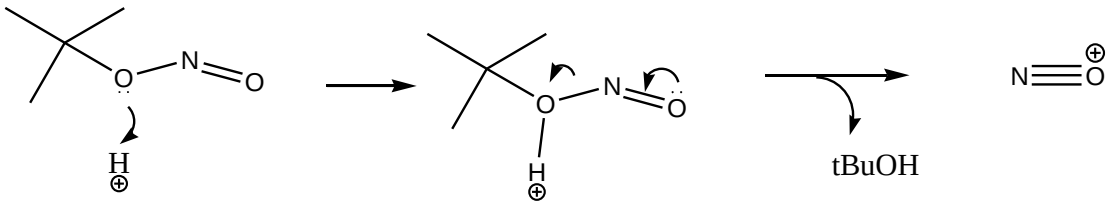
The azide building block **14a-i** was synthesized starting from the corresponding amino-compound **18a-i** through diazotization with one pot azide substitution.



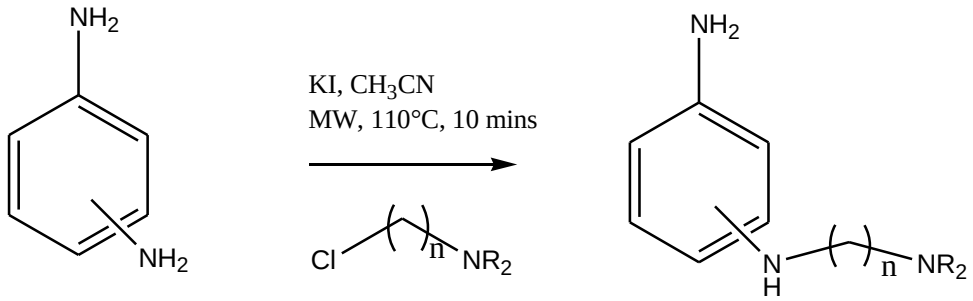
14a-i **18a-i**

a X=NH, n=2, NR_2=diethylamino-, *m*-substituted
b X=NH, n=3, NR_2=diethylamino-, *m*-substituted
c X=NH, n=3, NR_2=N-methyl-piperazinyl-, *p*-substituted
d X=NH, n=3, NR_2=N-methyl-piperazinyl-, *m*-substituted
e X=NH, n=3, NR_2=pyrrolidinyl-, *m*-substituted
f X=NH, n=2, NR_2=pyrrolidinyl-, *m*-substituted
g X=O, n=3, NR_2=N-methyl-piperazinyl-, *m*-substituted
h X=O, n=2, NR_2=diethylamino-, *m*-substituted
i X=O, n=3, NR_2=pyrrolidinyl-, *m*-substituted

The reactive cation NO^+ was formed using *tert*-butyl nitrite and hydrochloric acid, the formed aryl diazonium salt was a good leaving group replaced by the azide, introduced as the sodium salt.



The amino compounds **18a-i** were prepared starting from the *m*- or *p*-phenylendiamine **19a-b** or from the 3-aminophenol **21** depending on whether X is –O- or –NH-. For the preparation of the compound **18a-f** the *m*- or *p*-phenylendiamine **19a-b** was added to a solution of the chlorinated side chains **20a-e** in acetonitrile in the presence of potassium iodide under microwave irradiation for 10 minutes at 110°C to give the desired compound in good yields.



19a-b

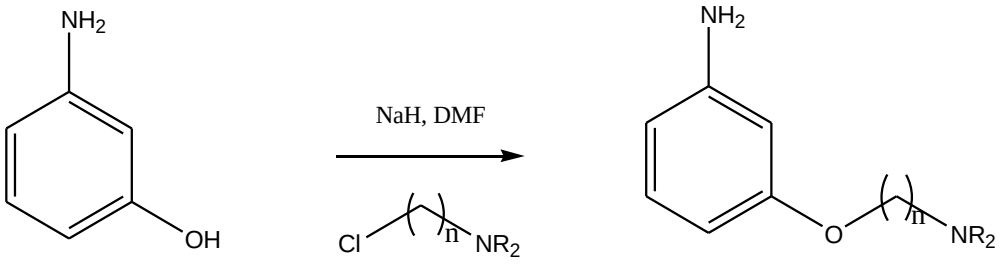
a *m*-substituted
b *p*-substituted

20a-e

a n=3, NR_2=diethylamino-
b n=3, NR_2=N-methyl-piperazinyl-
c n=3, NR_2=pyrrolidinyl-
d n=2, NR_2=diethylamino-
e n=2, NR_2=pyrrolidinyl-

18a-f

If X was –O-, the 3-aminophenol **21** reacted with chlorinated side chains **20b-d** in DMF using sodium hydride as base to give the compounds **18g-i**.

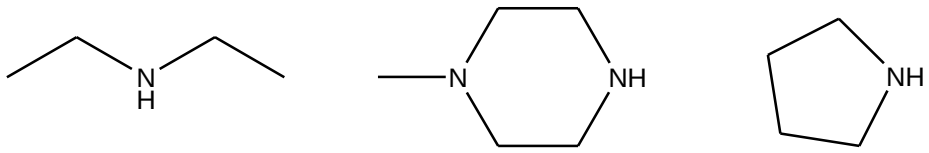


21

20b-d

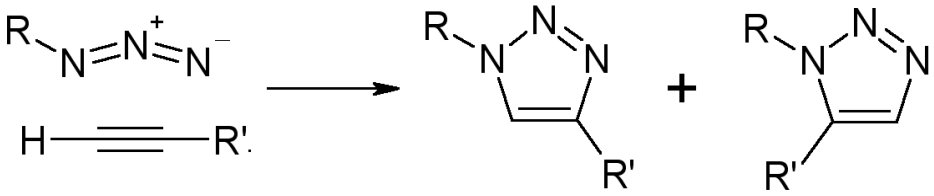
18g-i

The chlorinated side chains **20a-c,** not commercially available, were prepared starting from the corresponding amine **22a-c** by reaction with the iodo- or bromo-chloropropane.



22a **22b** **22c**

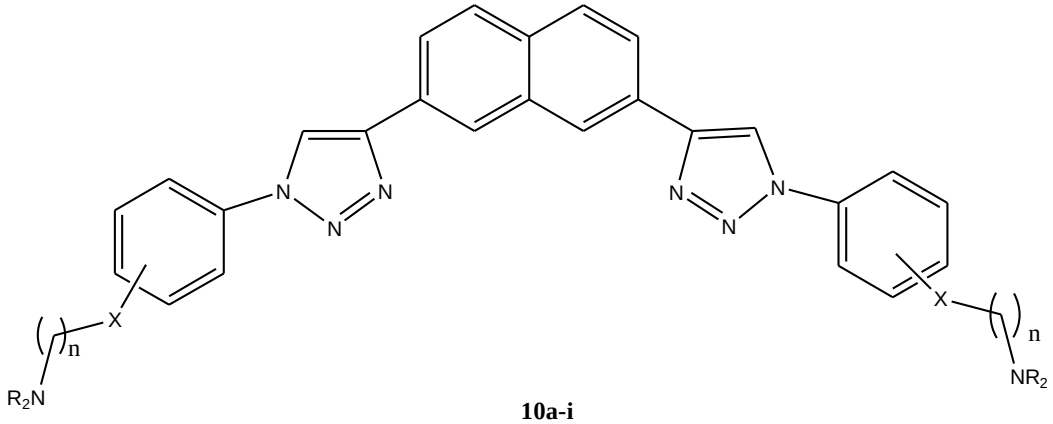
Once the two building blocks were synthesized, the final compounds were obtained via Huisgen 1,3-dipolar cycloaddition, [39] reaction of a dipolarophile with a 1,3-dipolar derivative that led to 5-membered cycle.



The risk of forming both regioisomers was avoided using the so called “click reaction” that was first fully described by K. Barry Sharpless whose purpose was to train a set of small molecules that could generate compounds by joining together heteroatom links. He established some criteria for an ideal click reaction that shloud be modular, wide in scope, work at high yields, produce only harmless byproducts and be stereospecific.

The azide-alkyne Huisgen reaction combined Cu(I) catalysis with the cycloaddition making the reaction regioselective for the 1,4-disubstituted isomer. [40]

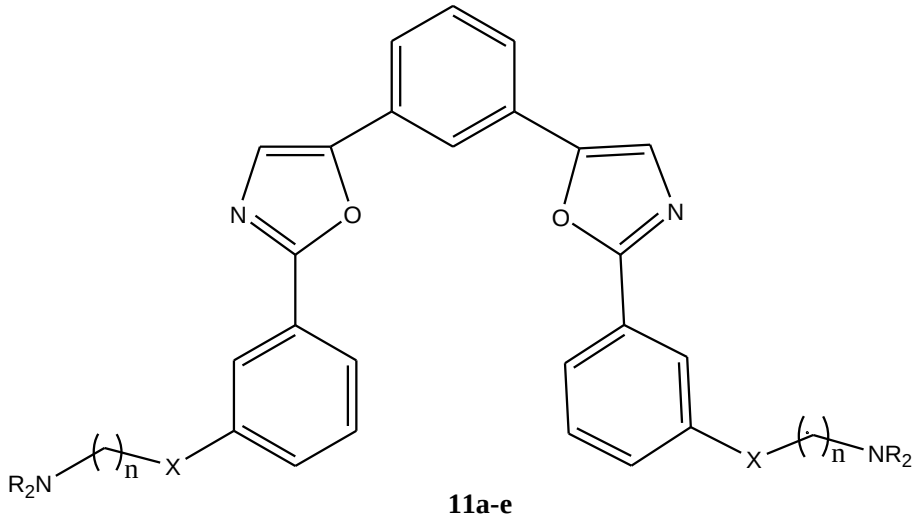
The reaction was performed by producing Cu(I) *in situ* through a mixture of $CuSO_4 \cdot 5H_2O$ and sodium ascorbate in a ratio of 1:10; bathophenanthrolinedisulfonic acid disodium salt hydrate was used for catalysing the multiple click reactions and an excess of the appropriate amine was added in order to avoid elimination of the amino-side chains. The desired compounds **10a-i** were obtained by purification of the crude products using a semi-prep C-18 reversed phase HPLC.



10a-i

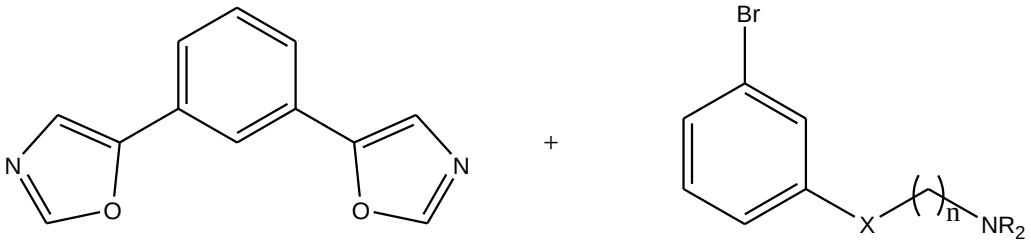
a X=NH, n=2, NR_2=diethylamino-, *m*-substitued
b X=NH, n=3, NR_2=diethylamino-, *m*-substitued
c X=NH, n=3, NR_2=N-methyl-piperazinyl-, *p*-substitued
d X=NH, n=3, NR_2=N-methyl-piperazinyl-, *m*-substitued
e X=NH, n=3, NR_2=pyrrolidinyl-, *m*-substitued
f X=NH, n=2, NR_2=pyrrolidinyl-, *m*-substitued
g X=O, n=3, NR_2=N-methyl-piperazinyl-, *m*-substitued
h X=O, n=2, NR_2=diethylamino-, *m*-substitued
i X=O, n=3, NR_2=pyrrolidinyl-, *m*-substitued

The synthesis of the bis-oxazole compounds was based on an arylation, using the substituted arylbromides, of the phenyl and naphthalene bis-oxazole.

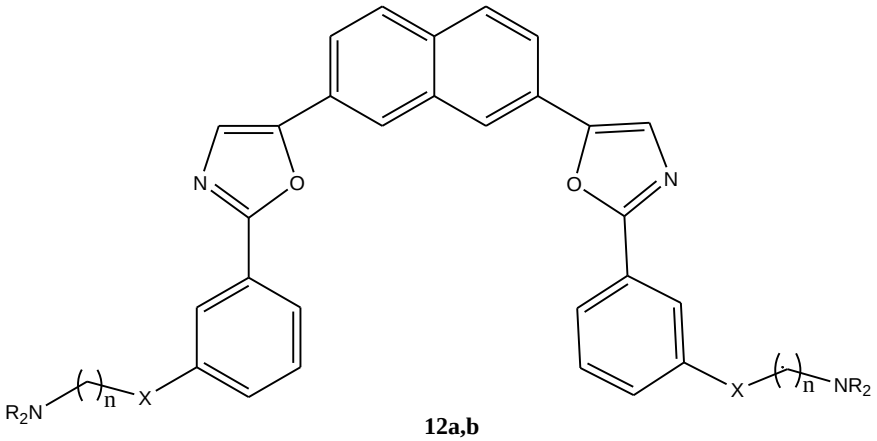


11a-e

11a X=NH, n=2, NR_2=diethylamino-
11b X=NH, n=3, NR_2= N-methyl-piperazinyl-
11c X=NH, n=2, NR_2= pyrrolidinyl-
11d X=O, n=2, NR_2= diethylamino-
11e X=O, n=2, NR_2=pyrrolidinyl-

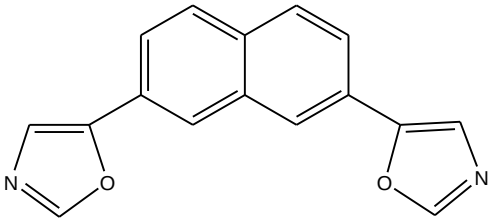


24 **29a-e**

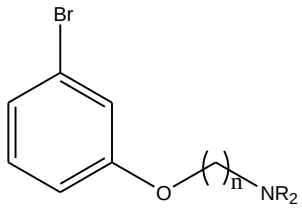


12a,b

12a X=NH, n=2, NR_2=diethylamino-
12b X=NH, n=3, NR_2= N-methyl-piperazinyl-

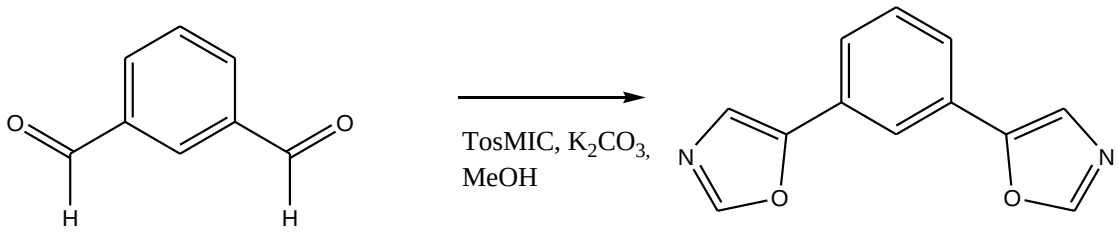


27



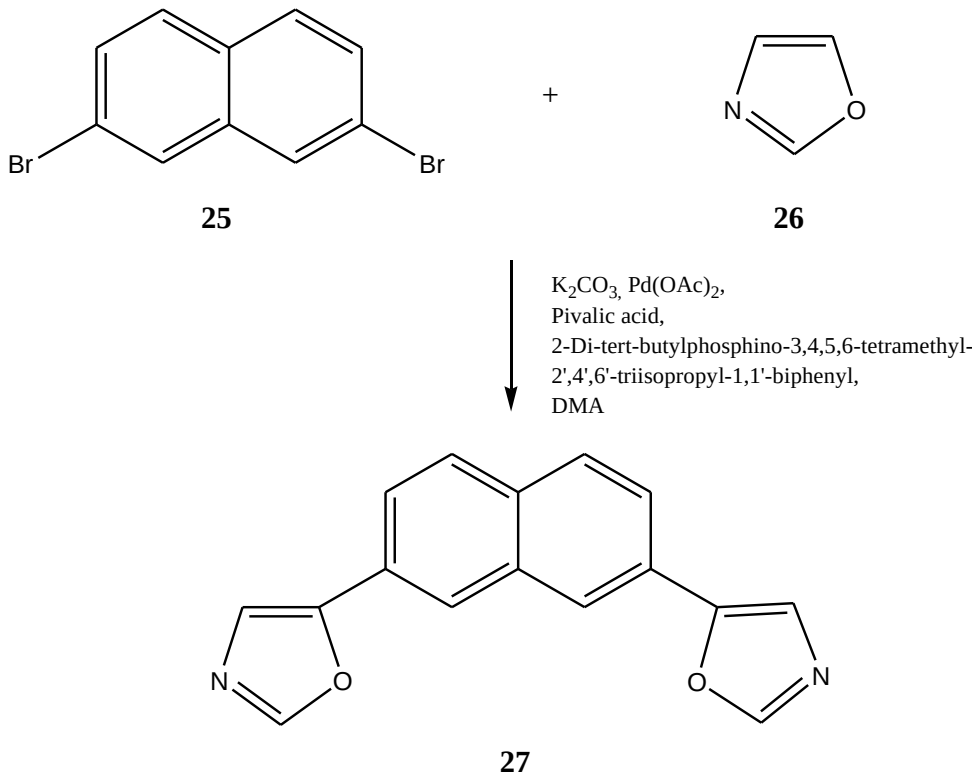
29a,b

Before this step the phenyl bis-oxazole **24** was synthesized from the isophathalaldehyde **23** using the tosylmethylcyanate-procedure described by van Leusen with potassium carbonate in methanol. [41]



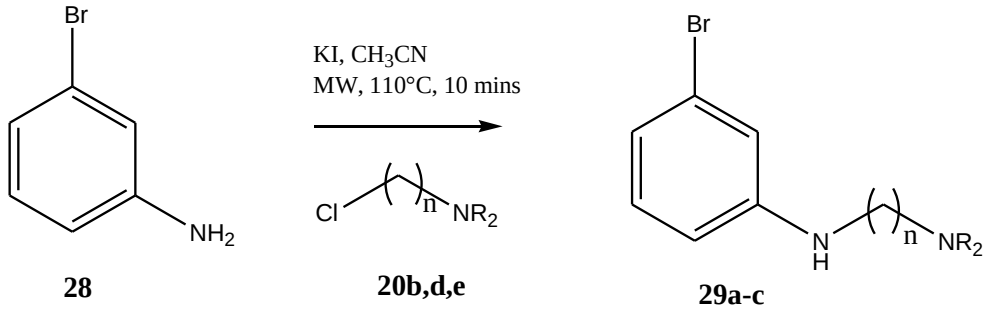
23 **24**

The synthesis of the naphthalene bis-oxazole **27** was based on the Strotman's paper, [42] starting from the 2,7-dibromonaphtalene **25** by reaction with the oxazole **26** in the presence of potassium carbonate, palladium acetate, pivalic acid and the proper ligand (2-di-tert-butylphosphino-3,4,5,6-tetramethyl-2',4',6'-triisopropyl-1,1'-biphenyl) in DMA under reflux for 72 hours.



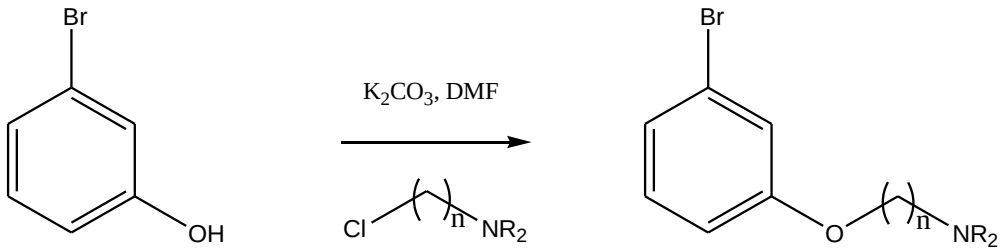
The *meta*-substitued arylbromides **29a-e** were prepared using two different pathways.

If X was –NH-, 3-bromoaniline **28** were added to a solution of the chlorinated side chains **20b,d,e** in acetonitrile in the presence of potassium iodide under microwave irradiation for 10 minutes at 110°C to give the compounds **29a-c.**



29a n=2, NR_2=diethylamino-
29b n=3, NR_2= N-methyl-piperazinyl-
29c n=2, NR_2= pyrrolidinyl-

If instead X was –O- the synthesis started from the 3-bromophenol **30** by reaction with chlorinated side chains **20d,e** using potassium carbonate as base in DMF, stirring at room temperature overnight to give the arylbromides **29d,e**.



30 **20d,e** **29d,e**

29d n=2, NR_2=diethylamino-
29e n=2, NR_2= pyrrolidinyl-

The phenyl and naphthalene bis-oxazole were then arylated twice with the arylbromides under Strotman's conditions using a different ligand (2-dicyclohexylphosphino-2',6'-diisopropoxybiphenyl) in toluene to make the reaction selective for the C-5 arylation of the oxazole obtaining the derivatives **11a-e** and **12a,b**.

All compounds obtained **10a-i**, **11a-e**, **12a,b** were analyzed by FRET (Fluorescence Resonance Energy Transfer), which evaluated the distance-dependent interaction between the electronic excited states of two molecules in which excitation was transferred from a donor molecule to an acceptor molecule through non-radiative dipole–dipole coupling, resulting in the return of the donor to its ground state causing a fluorescent emission (Fig.8). [43]

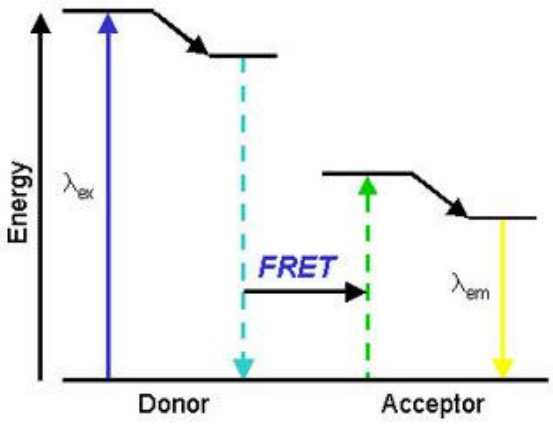


Figure 8

Primary conditions were that donor and acceptor molecules must be in close proximity (typically 10–100 Å) and the absorption spectrum of the acceptor must overlap the fluorescence emission spectrum of the donor (Fig.9).

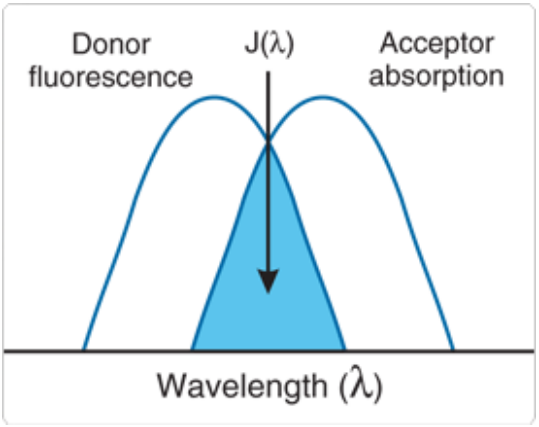


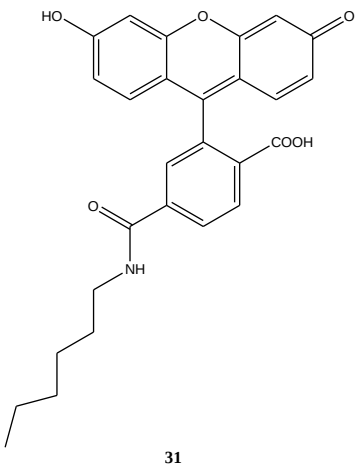
Figure 9 [44]

The FRET *efficiency* (E) was described in the empirical Förster equation and depends on the donor-to-acceptor separation distance (r) with an inverse 6th power law due to the dipole-dipole coupling mechanism:

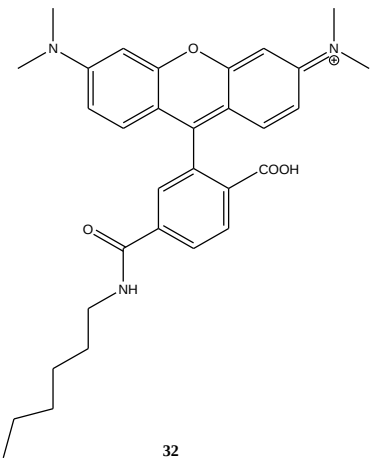
$$E = \frac{1}{1 + (r/R_0)^6}$$

R_0 is the Förster distance of this pair of donor and acceptor, i.e. the distance at which the energy transfer efficiency is 50%.

DNA can be synthesized with its 3' and 5' ends tagged with fluorescent dyes, at the 5' end 6-carboxyfluorescein (FAM) **31** the donor, and tetramethyl-6-carboxyrhodamine (TAM) **32** at the 3' end as the acceptor, using 6 carbon linkers.



31



32

When nucleotides are folded in the G-quadruplex structure, the fluorophores are closer in space and the FRET efficiency is high.

Using this property, by increasing the temperature while measuring the fluorescence intensity, melting experiments could be done on DNA and the melting temperature of the labelled oligonucleotides could be monitored to screen the G-quadruplex stabilising ability of small molecules. [45]

The experiment was performed with four different sequences of labelled DNA:

F21T: 5'-FAM-GGG TTA GGG TTA GGG TTA GGG-TAMRA-3'

C-Kit 2: 5'-FAM-CCC GGG CGG GCG CGA GGG AGG GGA GG-TAMRA-3'

Hsp90A: 5'-FAM-GGG CCA AAG GGA AGG GGT GGG-TAMRA-3'

TLoop: 5'-FAM-TAT AGC TATA TTT TTT TATA GCT ATA-TAMRA-3'

The experiments were all run in potassium cacodylate buffer 60 mM (pH 7.4), in which the DNA sequences have the melting point shown in the table. (Tab.2)

DNA-sequences	Tm (°C)
F21T	**58.4 ± 0.9**
***C*-kit 2**	**53.5 ± 0.5**
Hsp90A	**57.5 ± 0.5**
TLoop	**53.5 ± 0.5**

Table 2

The ΔTm values obtained for all the derivatives (at 1μM and 5 μM concentrations) were reported in the table. (Tab.3)

	ΔTm(°C)	F21T	ΔTm(°C)	*C*-kit 2	ΔTm(°C)	Hsp90A	ΔTm(°C)	TLoop
	1 μM	5 μM	1 μM	5 μM	1 μM	5 μM	1 μM	5 μM
10a	1.7	8	1.5	6.8	1.1	10.9	1	0.7
10b	0.2	3.2	0.5	5.4	1.3	9.5	0	0.5
10c	0.6	5	0.1	3.8	1.9	9	0.2	0.4
10d	2.5	9.4	2.4	7.8	5.8	15.7	0.1	0.7
10e	3.9	15.8	3	17.7	10.8	20.5	0.6	1.6
10f	0.3	21.2	0	16.74	<2	26	0.2	3.2
10g	4	22	4.2	18.5	10.5	24.9	0.3	2.5
10h	4.2	20.1	2.2	22.9	8.8	23.6	0.3	5
10i	0.8	26.5	1.3	29.9	6	28.2	0.1	5.8
11a	0.5	13.3	0	13.6	1.2	19.5	0.1	0.2
11b	0.6	20	0	18.3	<2	25	0.2	1.8
11c	0.6	24.7	0.7	17	<2	26	0.4	6.8
11d	0.7	17.6	0.9	12.8	3	23	0.3	1.3
11e	1	25.9	1	20.3	3.4	27.7	0.1	5.3
12a	1.1	3.4	1	5	2.7	11.3	0.3	0.6
12b	1.5	11.3	1	12.6	2.5	14.6	0	0

Table 3

All the compounds showed a general preference for G-quadruplex DNA over duplex DNA but the best result was obtained with bis triazole compounds **10g** and **10i**, as it is evident from the graphs (Fig.10 and 11), in which the general trend showed a gradual increase of ΔTm increasing the concentration of the ligand.

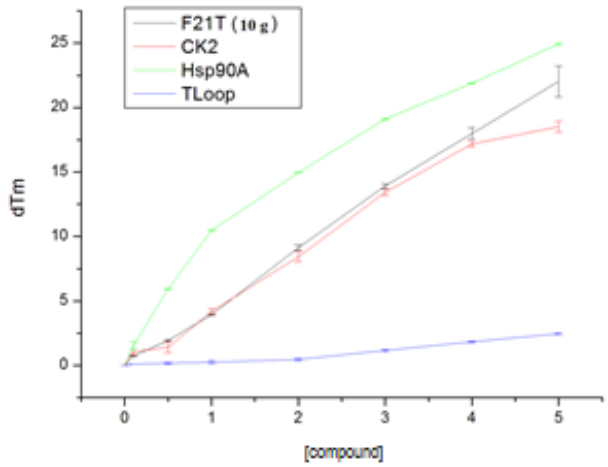


Figure 10

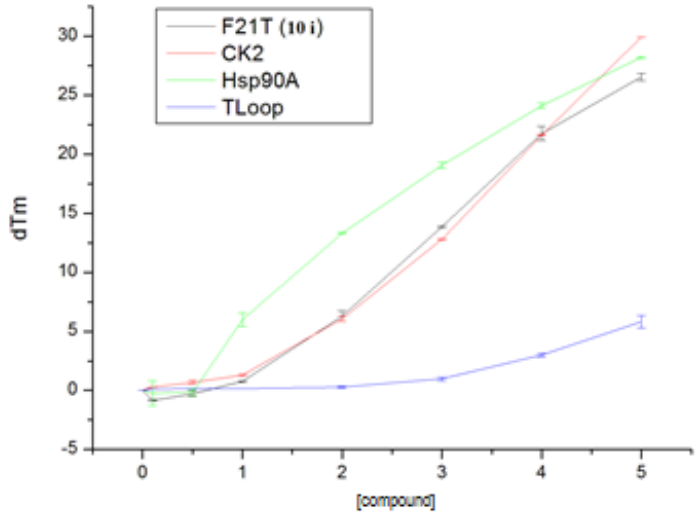


Figure 11

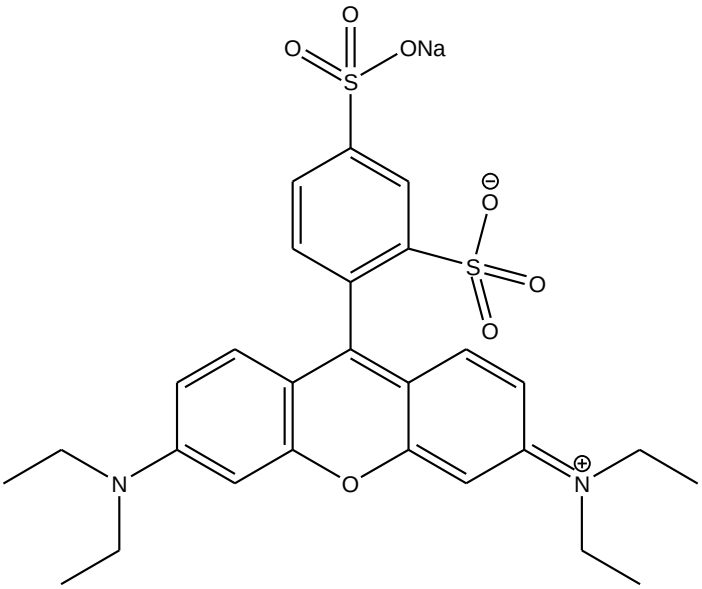
In particular the compound **10g** showed ΔTm values equal to 4°C (1 μM) and 22°C (5 μM) for F21T, 4.2°C (1 μM) and 18.5°C (5 μM) for C-kit 2, 10.5°C (1 μM) and 24.9°C (5 μM) for Hsp90A and 0.3°C (1 μM) and 2.5°C (5 μM) for the duplex DNA.

The naphthalene bis triazole compound **10i** instead showed ΔTm values equal to 0.8°C (1 μM) and 26.5°C (5 μM) for F21T, 1.3°C (1 μM) and 29.9°C (5 μM) for C-kit2, 6°C (1 μM) and 28.2°C (5 μM) for Hsp90A and 0.1°C (1 μM) and 5.8°C (1 μM) for the duplex DNA.

All compounds were screened against a panel of cancer and a non-cancer cell lines (referred to as normal fibroblast cell line, WI38). The cancer-derived cell lines studied were A549 (human lung adenocarcinoma), MCF7 (human breast adenocarcinoma), MiaPaCa (pancreatic cancer) and RCC4 and 786-O (renal cancer cell lines).

The experiment was based on a short cell exposure (up to 96 hours) to different concentrations of ligand in order to evaluate the IC_{50} value of each of them.

The assay used is the sulforhodamine B calorimetric assay (SRB). It is based on the ability of the purple dye SRB **33** to interact with the basic amino acid residues of cellular proteins. This molecule is UV active and the quantification of the dye bound to the cells at 540 nm is an indirect measure of the amount of viable cells.



33

Cells were seeded in a 96-well plate and incubated for 24 hours, and then with different concentrations of ligand for 96 hours. The supernatant was removed and the remaining cells were fixed with trichloroacetic acid and stained with sulforhodamine B.

The results obtained were adjusted by the negative control (cells without ligand and without SRB) and normalized by the positive control (cells only with SRB) to obtain the rate of viable cells. The IC_{50} values were calculated as the concentration responsible for a 50% decrease of cell viability.

The results obtained are shown in the following table. (Tab.4)

	A549 (µM)	MCF7 (µM)	MiaPaCa (µM)	RCC4 (µM)	786-O (µM)	WI38 (µM)
10a	5.8	8.8	5.7	4	8.8	13.4
10b	14.1	>25	16.3	>25	8.9	7.8
10c	>25	>25	>25	>25	>25	>25
10d	>25	>25	15.8	8.4	8.7	15.3
10e	1.7	2.8	5.1	2.5	1.1/2.2	1.7
10f	1.2	1.1	n/a	1.2	2.9/2.3	2.4
10g	3.4	10.6	5.6	>25	10.2	7.2
10h	4.1	>25	5	5	10.1	13.9
10i	2	3.2	2.1	2/2.5	3.6/2.3	2.6
11a	2.5	3.1	1.2	1	1.3	2.8
11b	1.2	1	n/a	1.2	0.9	1.1
11c	0.6	2.1	n/a	1.1	1.1	0.95
11d	2.3	4.1	n/a	2.8	1.5	3.2
11e	0.95	1.2	n/a	1.3	0.7	1
12a	8.3	5	3.6	3	5	4.1
12b	1.9	1.9	1	2.1	1.2	3.1

Table 4

The table showed that, with the exception of the derivative **10c** that appeared to be completely inactive on all the tested cell lines, all the others were found to be cytotoxic for almost all the tumor cell lines, with the IC_{50} values in the micromolar range. In particular, the most active compounds were found to be the bis phenyl oxazoles **11c** and **11e** but they still do not show a particular selectivity for tumor cells over the normal cells.

It was showed that in this series of compounds what could determinanted an anti-proliferative activity were the presence of a central phenyl ring ,two attached phenyl-oxazole rings that should be *meta* attached to the phenyl rings and the length of the linker and nature of the terminal group is of lesser importance.

It is not possible to assume that the cellular mode of action involves G-quadruplex stabilization since only at high concentration there was a strong stabilization in the FRET assay; they could act at other quadruplex loci that are yet to be determined, or that the compounds act by non-quadruplex mechanism. [46]

EXPERIMENTAL DATA

All chemicals, reagents and solvents were purchased from Sigma-Aldrich, Alfa Aesar, Lancaster Synthesis and Fluorochem (UK) and used without further purification. Solvents were supplied by VWR and Fisher scientific. Column chromatography was performed using BDH silica gel (BDH 153325P). HPLC analysis was carried out with a Gilson apparatus combining a 322 PUMP and an Agilent 1100 SERIES detector, using a C18 5µ (100 x 4.6 mm) column (41622271 (W), YMC, Japan), at a flow of 1 mL/min. Preparative HPLC was carried out with a Gilson apparatus combining a 322 PUMP and a UV/VIS-155 detector with detection at 280 nm, using a C18 5µ (100 x 20 mm) column (201022272) (W), YMC, Japan, at a flow of 20 mL/min. Water and methanol with 0.1 % formic acid were used as solvents for HPLC. For the purification of compounds X the following method was used: 100 % aqueous for 2 min after injection, gradually decreased to 50 % aqueous over 20 min to 5% aqueous after 30 min and then increased to 100% aqueous after 33 min. NMR spectra were recorded at 400 MHz (^{1}H NMR) or 500 MHz (^{13}C NMR) on a Bruker spectrometer in $CDCl_3$ (with 0.05 % TMS, Cambridge Isotope Laboratories, USA). NMR spectra were analyzed with MestReC 4.5.6.0 with chemical shifts using TMS as a standard (δ = 0 ppm). NMR multiplicity abbreviations are s (singlet), bs (broad singlet), d (doublet), t (triplet), q (quartet), 5q (quintet), and m (multiplet). Coupling constants J are reported as observed in Hertz (Hz). High Resolution Mass spectra (HRMS) were measured on a Micromass Q-TTOF Ultima Global tandem mass spectrometer run under electrospray ionisation (ESI), and processed using the MassLab 3.2 software.

CHEMISTRY

Synthesis of naphthalene-2,7-diyl bis(trifluoromethanesulfonate)16

A mixture of 2,7-dihydroxynaphtalene **15** (1.35 g, 8.43 mmol), 4-(Dimethylamino)pyridine (0.21 g, 1.69 mmol), and 2,6-lutidine (2.2 ml, 18.53 mmol) was suspended in anhydrous dichloromethane (13.5 ml) and anhydrous tetrahydrofuran (13.5 ml) under argon atmosphere at
-78°C. Triflic anhydride (5 g, 17.7 mmol) was added dropwise to this stirred mixture over 15 minutes and the reaction was stirred at -78°C for 2 hours, then for 5 hours at 0°C. After that time, the reaction was carefully neutralized with saturated aqueous sodium hydrogen carbonate solution, extracted with dichloromethane, dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. The oil thus obtained was purified by column chromatography (dichloromethane) to give the compound **16** as a white solid. Mp 60-62°C; Yield 61%; ^{1}H NMR (ppm): 7.40 (2H, dd, *J*=2.4, 8.8 Hz, 2xAr-

H), 7.73 (2H, d, *J*=2.4 Hz, 2xAr-H), 7.92 (2H, d, *J*= 9.2 Hz, 2xAr-H); ^{13}C NMR (ppm): 118.8 (CF3), 119.5 (2xCH), 121.1 (2xCH), 130.8 (2xCH), 131.3 (C), 133.6 (C), 148.3 (2xC). Spectroscopic data are in accordance with those reported in literature (Yao et al., 1998).

Synthesis of (naphthalene-2,7-diyldiethyne-2,1-diyl)bis(trimethylsilane) 17

A mixture of **16** (1 g, 2.36 mmol), copper iodide (0.046 g, 0.24 mmol), tetrakis(triphenylposphine)palladium(0) (0.28 g, 0.24 mmol), and Triphenylposphine (0.13 g, 0.48 mmol) was dissolved in piperidine (15 ml) under argon atmosphere. Ethynyltrimethylsilane (1.98 ml, 14 mmol) was added and the stirred mixture heated at reflux for 24 hours. The solvent was evaporated *in vacuo* and the resulting brown oil was purified by column chromatography (hexane: ethyl acetate 95:5). Mp 127-129°C; Yield 90%; ^{1}H NMR (ppm): 0.28 (18H, s, 6xCH_3), 7.50 (2H, m, 2xAr-H), 7.71 (2H, d, J=8.4 Hz, 2xAr-H), 7.91 (2H, s, 2xAr-H); ^{13}C NMR (ppm): -0.03 (6xCH_3Si), 95.2 (C), 105.0 (C), 121.2 (2xC), 127.7 (2xCH), 129.5 (2xCH), 131.6 (2xCH), 132.2 (C), 132.3 (C). (Crisp *et al*., 1997).

Synthesis of 2,7-diethynylnaphthalene 13

Compound **17** (0.45 g, 1.39 mmol) was dissolved in tetrahydrofuran (80 ml) and 1M sodium hydroxide solution (80 ml) and the mixture stirred for 2 hours at room temperature. The reaction was extracted with dichloromethane, dried over $MgSO_4$ and concentrated *in vacuo*. The pale yellow solid obtained did not require further purification. Mp 124-126°C; Yield 100%; ^{1}H NMR (ppm): 3.17 (2H, s, 2xCH), 7.54 (2H, dd, J=1.6, 8.4 Hz, 2xAr-H), 7.78 (2H, d, J=8.9 Hz, 2xAr-H), 7.97 (2H, s, 2xAr-H); ^{13}C NMR (ppm): 78.0 (2xCH), 83.6 (2xC), 120.3 (2xC), 127.9 (2xCH), 129.6 (2xCH), 132.0 (2xCH), 132.3 (C), 132.6 (C). (Crisp *et al.*, 1997).

Synthesis of 3-chloro-*N*,*N*-diethylpropan-1-amine 20a

1-Iodo-3-chloropropane **22a** (1.1 ml, 10.5 mmol) were added to 4.26 ml of diethylamine (40.5 mmol). The reaction mixture was stirred at room temperature for 1 hour and after that the same amount of 1-iodo-3-chloropropane (1.1 ml, 10.5 mmol) was added. The reaction was stirred for 1 hour at room temperature and after that 25 ml of sodium hydroxide 5 M were added. The reaction mixture was extracted with dichloromethane, dried on $MgSO_4$ and concentrated *in vacuo*. The oil obtained did not required further purification. Yield 20%; ^{1}H NMR: 1.01 (6H, t, J=7.1 Hz, 2xCH_3), 1.89 (2H, 5q, J=6.6 Hz, CH_2), 2.47-2.56 (6H, m, 3xCH_2), 3.58 (2H, t, J=6.6 Hz, CH_2); ^{13}C NMR (ppm): 5.5 (2xCH_2), 27.9 (CH_2), 41.1 (CH_2), 44.5 (2xCH_3), 51.5 (CH_2).

Synthesis of 1-(3-chloropropyl)-4-methylpiperazine 20b

1-Methylpiperazine **22b** (1g, 10 mmol) was dissolved in acetone (20 ml), 5 ml of 25% sodium hydroxide solution were added and after that 1-bromo-3-chloropropane (2ml, 20 mmol) was added. The mixture stirred for 24 hours at room temperature. The organic layer was evaporated *in vacuo* and the aqueous layer was extracted with ethyl acetate, dried over $MgSO_4$ and concentrated. The oil obtained did not required further purification. Quantitative yield; ^{1}H NMR: 1.91 (2H, 5q, J=6.6 Hz, CH_2), 2.25 (3H, s, CH_3), 2.34-2.47 (10H, m, 5xCH_2), 3.56 (2H, t, J=6.6 Hz, CH_2); ^{13}C NMR (ppm): 24.6 (CH_2), 38.0 (CH_2), 40.8 (CH_2), 48.0 (2xCH_2), 49.9 (2xCH_2), 50.2 (CH_3).

Synthesis of 1-(3-chloropropyl)pyrrolidine 20c

Pyrrolidine **22c** (1.16 ml, 0.014 mol), acetone (10 ml), 5M sodium hydroxide solution (8.4 ml, 0.042 mol) and 1-bromo-3-chloropropane (2.8 ml, 0.028 mol) were stirred together under nitrogen atmosphere for 24 hours. The organic layer was separated, dried over $MgSO_4$, concentrated in vacuo and purified via coloumn chromatography (dichloromethane:methanol 99:1). Yield 90%; ^{1}H NMR (ppm): 1.75-1.79 (4H, m, 2xCH_2), 1.97 (2H, 5q, J=6.7 Hz, CH_2), 2.48-2.51 (4H, m, 2xCH_2), 2.58 (2H, t, J=7.4 Hz, CH_2), 3.60 (2H, t, J=6.7 Hz, CH_2); ^{13}C NMR (ppm): 23.5 (2xCH_2), 32.1 (CH_2), 43.4 (CH_2), 53.6 (CH_2), 54.2 (2xCH_2).

Synthesis of amino compounds 18a-f

General procedure:

To a solution of the chloro-compound **20a-e** (1 eq) in acetonitrile in an oven dried microwave vial, potassium iodide (0.2 eq) and benzendiamine **19a-b** (3 eq) were added.

The vial was heated under microwave irradiation for 10 minutes at 110°C. The cooled reaction mixture was diluted with ethyl acetate, washed with aqueous saturated sodium hydrogen carbonate, dried over $MgSO_4$, concentrated *in vacuo* and purified via coloumn chromatography.

***N*-[2-(diethylamino)ethyl]benzene-1,3-diamine 18a:** The crude was purified by chromatography (dichloromethane:methanol 8:2). Yield 67%; ^{1}H NMR (ppm): 1.06 (6H, t, J=7.0 Hz 2xCH_3), 2.65-2.70 (6H, m, 3xCH_2), 3.09 (2H, t, J=6.5 Hz, CH_2), 4.76 (2H, bs, NH_2), 5.85-5.89 (3H, m, 3xAr-H), 6.77 (1H, t, J=8.2 Hz, Ar-H); ^{13}C NMR (ppm): 11.3 (2xCH_2), 46.6 (2xCH_2), 51.3 (CH_2), 1 CH_2 is hidden underneath DMSO peak, 98.0 (CH), 101.3 (CH), 103.2 (C), 129.2 (CH), 149.2 (CH), 149.5 (C).

***N*-[3-(diethylamino)propyl]benzene-1,3-diamine 18b:** The crude was purified by chromatography (dichloromethane:methanol 8:2). Yield 53%; ^{1}H NMR: 1.24 (6H, t, J=7.0 Hz, 2xCH_3), 1.89-1.93 (2H, m, CH_2), 3.06-3.12 (8H, m, 4xCH_2), 4.78 (2H, bs, NH_2), 5.86-5.89 (3H, m, 3x Ar-H), 6.78 (1H, t, J=7.8 Hz, Ar-H); ^{13}C NMR (ppm): 10.9 (2xCH_2), 25.7 (CH_2), 42.8 (CH_2), 46.7 (2xCH_3), 51.3 (CH_2), 99.4 (CH), 104.0 (CH), 104.7 (CH), 130.0 (CH), 147.5 (C), 149.7 (C).

***N*-[3-(4-methylpiperazin-1-yl)propyl]benzene-1,4-diamine 18c:** The crude was purified by chromatography (dichloromethane:methanol 9:1). Yield 25%; ^{1}H NMR (ppm): 1.79 (2H, 5q, J=6.4 Hz, CH_2), 2.32 (3H, s, CH_3), 2.49-2.52 (10H, m, 5xCH_2), 3.16 (2H, t, J=6.4 Hz, CH_2), 4.35 (2H, bs, NH_2), 6.56-6.58 (2H, m, 2xAr-H), 6.83-6.86 (2H, m, 2xAr-H); ^{13}C NMR (ppm): 26.0 (CH_2), 44.4 (CH_2), 45.8 (CH_3), 52.9 (2xCH_2), 55.0 (2xCH_2), 57.1 (CH_2), 114.5 (2xCH), 117.0 (2xCH_2), 137.6 (C), 141.9 (C).

***N*-[3-(4-methylpiperazin-1-yl)propyl]benzene-1,3-diamine 18d:** The crude was purified by chromatography (dichloromethane:methanol 8:2). Yield 40%; ^{1}H NMR (ppm): 1.64 (2H, 5q, J=6.7 Hz, CH_2), 2.21 (3H, s, CH_3), 2.39 (2H, t, J=5.5 Hz, CH_2), 2.51 (8H, bs, 4xCH_2), 2.91 (2H, t, J=6.5 Hz, CH_2), 5.54-5.46 (2H, m, 2xAr-H), 5.86-5.89 (1H, m, Ar-H), 6.74-6.76 (1H, m, Ar-H); ^{13}C NMR (ppm): 25.9 (CH_2), 43.2 (CH_2), 45.9 (CH_2), 53.0 (2xCH_2), 55.1 (2xCH_2), 57.0 (CH_3), 99.4 (CH), 104.1 (CH), 104.7 (CH), 130.0 (CH), 147.5 (C), 149.9 (C).

***N*-[3-(pyrrolidin-1-yl)propyl]benzene-1,3-diamine 18e:** The crude was purified by cromatography (dichloromethane:methanol 95:5). Yield 35%; ^{1}H NMR (ppm): 1.82-1.88 (6H, m, 3xCH_2), 2.59-2.65 (6H, m, 3xCH_2), 3.17 (2H, t, J=6.6 Hz, CH_2), 3.53 (2H, bs, NH_2), 5.95 (1H, t, J=2.14 Hz, Ar-H), 6.03-6.06 (2H, m, 2xAr-H), 6.94 (1H, t, J=7.9 Hz, Ar-H); ^{13}C NMR (ppm): 23.5 (2xCH_2), 28.0 (CH_2), 42.9 (CH_2), 54.2 (2xCH_2), 54.7 (CH_2), 99.4 (CH), 104.0 (CH), 104.6 (CH), 130.0 (CH), 147.5 (C), 149.8 (C).

***N*-[2-(pyrrolidin-1-yl)ethyl]benzene-1,3-diamine 18f:** The crude was purified by chromatography (dichloromethane:methanol 9:1). Yield 63%; ^{1}H NMR (ppm): 2.00 (4H, 5q, J=3.9 Hz, 2xCH_2), 3.05-3.11 (6H, m, 3xCH_2), 3.46 (2H, t, J=5.9 Hz, CH_2), 6.06-6.08 (3H, m, 3xAr-H), 6.94 (1H, t, J=8.4 Hz, Ar-H); ^{13}C NMR (ppm): 23.3 (2xCH_2), 50.8 (CH_2), 54.2 (2xCH_2), 54.7 (CH_2), 99.8 (CH), 103.5 (CH), 105.3 (CH), 130.2 (CH), 147.7 (C), 148.4 (C).

Synthesis of amino compounds 18g-i

General procedure:

3-Aminophenol **21** (1 eq) was dissolved in dimethylformamide and cooled in an ice bath. Sodium hydride (1.1 eq) was added and the reaction was allowed to warm to room temperature and stirred for 45 mins. Then the chloro-compound **20b-d** (1.1 eq) was added and the mixture stirred at room temperature for 24 hours. Ice and water were added and the mixture was extracted with ethyl acetate, dried over $MgSO_4$, concentrated *in vacuo* and purified via column chromatography.

3-[3-(4-Methylpiperazin-1-yl)propoxy]aniline 18g: The crude was purified by chromatography (dichloromethane:methanol 9:1). Yield 43%; ^{1}H NMR: 1.81 (2H, 5q, J=6.6 Hz, CH_2), 2.20 (3H, s, CH_3), 2.39-2.42 (10H, m, 5xCH_2), 3.87 (2H, t, J=6.6 Hz, CH_2), 4.99 (2H, bs, NH_2), 6.1 (1H, dd, J=2.1, 7.5 Hz, Ar-H), 6.11-6.13 (2H, m, 2xAr-H), 6.87 (1H, t, J=8.4 Hz, Ar-H); ^{13}C NMR (ppm): 26.2 (CH_2), 45.3 (CH_3), 52.3 (2xCH_2), 54.3 (CH_2), 54.4 (2xCH_2), 65.2 (CH_2), 99.9 (CH), 102.0 (CH), 106.7 (CH), 129.4 (CH), 149.6 (C), 159.6 (C).

3-[2-(Diethylamino)ethoxy]aniline 18h: The crude was purified by chromatography (dichloromethane:methanol 9:1). Yield 50%; ^{1}H NMR: 1.08 (6H, t, J=7.2 Hz, 2xCH_3), 2.67 (4H, q, J=7.2 Hz, 2xCH_2), 2.76 (2H, t, J=6.0 Hz, CH_2), 3.20 (2H, t, J=6.0 Hz, CH_2), 4.43 (2H, bs, NH_2), 6.16-6.20 (3H, m, 3xAr-H), 7.00 (1H, t, J=7.9 Hz, Ar-H); ^{13}C NMR (ppm): 10.9 (2xCH_2), 40.8 (CH_2), 46.8 (2xCH_3), 51.5 (CH_2), 100.1 (CH), 104.8 (CH), 105.8 (CH), 130.2 (CH), 149.7 (C), 157.2 (C).

3-[3-(Pyrrolidin-1-yl)propoxy]aniline 18i: The crude was purified by chromatography (dichloromethane:methanol 9:1). Yield 40%; ^{1}H NMR: 1.83 (4H, bs, 2xCH_2), 2.03 (2H, 5q, J=6.4 Hz, CH_2), 2.62 (4H, bs, 2xCH_2), 2.68 (2H, t, J=7.5 Hz, CH_2), 3.65 (2H, bs, NH_2), 3.97 (2H, t, J=6.4 Hz, CH_2), 6.23 (1H, t, J=2.2 Hz, Ar-H), 6.26-6.31 (2H, m, 2xAr-H), 7.03 (1H, t, J=8.1 Hz, Ar-H); ^{13}C NMR (ppm): 23.4 (2xCH_2), 28.5 (CH_2), 53.3 (CH_2), 54.2 (2xCH_2), 66.0 (CH_2), 101.6 (CH), 104.6 (CH), 107.9 (CH), 130.1 (CH), 147.8 (C), 160.1 (C).

Synthesis of azido compounds 14a-i

General procedure:

The amino compound **18a-i** (1 eq) was dissolved in tetrahydrofuran and cooled in an ice bath. The mixture was treated sequentially with concentrated aqueous hydrochloric acid (5.5 eq) and then with *tert*-butyl nitrite (2.5eq). The reaction was stirred in an ice bath for 1.5 hours and after that

sodium azide (3 eq) was added, followed by addition of water until the reaction ceased to effervesse. The reaction was allowed to warm to room temperature and stirred for 24 hours. The mixture was neutralized with saturated aqueous sodium hydrogen carbonate solution and tertrahydrofuran was evaporated *in vacuo.* The aqueous solution was extracted with ethyl acetate, dried on $MgSO_4$, concentrated and purified by column chromatography.

***N'*-(3-azidophenyl)-N,N-diethylethane-1,2-diamine 14a**: The crude was purified by chromatography (dichloromethane:methanol 98:2). Yield 54%; ^{1}H NMR (ppm): 2.52 (6H, t, J=7.1 Hz, 2xCH_3), 2.50-2.58 (6H, m, 3xCH_2), 4.06 (2H, t, J=6.9 Hz, CH_2), 7.01-7.03 (1H, m, Ar-H), 7.36-7.46 (3H, m, 3xAr-H); ^{13}C NMR (ppm): 11.9 (2xCH_3), 43.7 (CH_2), 47.5 (2xCH_2), 48.9 (CH_2), 110.7 (CH), 115.9 (CH), 117.5 (CH), 130.6 (CH), 141.5 (C), 143.5 (C).

***N'*-(3-azidophenyl)-N,N-diethylpropane-1,3-diamine 14b**: The crude was purified by chromatography (dichloromethane:methanol 95:5). Yield 12%; ^{1}H NMR (ppm): 1.06 (6H, t, J=7.2 Hz , 2xCH_3), 1.77 (2H, 5q, J=7.1 Hz, CH_2), 2.52 (2H, t, J=7.1 Hz, CH_2), 2.61 (4H, q, J=7.2 Hz , 2xCH_2), 4.06 (2H, t, J=7.1 Hz, CH_2), 7.02 (1H, ddd, J=0.86, 2.1, 8.0 Hz, Ar-H), 7.29 (1H, t, J=2.1 Hz, Ar-H), 7.33 (1H, ddd, J=0.86, 2.1, 8.0 Hz, Ar-H), 7.44 (1H, t, J=8.0 Hz, Ar-H); ^{13}C NMR (ppm): 10.9 (2xCH_2), 23.8 (CH_2), 41.9 (CH_2), 46.7 (2xCH_3), 50.0 (CH_2), 110.0 (CH), 115.3 (CH), 117.5 (CH), 130.8 (CH), 141.7 (C), 142.9 (C).

4-Azido-*N*-[3-(4-methylpiperazin-1-yl)propyl]aniline 14c: The crude was purified by chromatography (dichloromethane:methanol 95:5). Yield 64%; ^{1}H NMR (ppm): 1.72 (2H, 5q, J=7.0 Hz, CH_2), 2.30-2.35 (5H, m, CH_3,CH_2), 2.48 (8H, bs, 4xCH_2), 4.06 (2H, t, J=7.0 Hz, CH_2), 7.10-7.12 (2H, m, 2xAr-H), 7.56-7.59 (2H, m, 2xAr-H); ^{13}C NMR (ppm): 23.6 (CH_2), 42.3 (CH_2), 45.8 (CH_2), 52.6 (CH_3), 54.9 (2xCH_2), 55.0 (2xCH_2), 120.0 (2xCH), 120.9 (2xCH), 138.7 (C), 139.1 (C).

3-Azido-*N*-[3-(4-methylpiperazin-1-yl)propyl]aniline 14d: The crude was purified by chromatography (dichloromethane:methanol 9:1). Yield 32%; ^{1}H NMR (ppm): 1.70-1.74 (4H, m, 2xCH_2), 2.29-2.33 (5H, m, CH_2, CH_3), 2.45 (8H, bs, 4xCH_2), 7.02-7.04 (1H, m, Ar-H), 7.29 (1H, t, J=2.1 Hz, Ar-H), 7.35-7.38 (1H, m, Ar-H), 7.44 (1H, t, J=8.1 Hz, Ar-H); ^{13}C NMR (ppm): 23.7 (CH_2), 41.9 (CH_2), 45.9 (CH_2), 52.8 (CH_3), 55.0 (4xCH_2), 109.9 (CH), 115.2 (CH), 117.3 (CH), 130.6 (CH), 141.6 (C), 143.2 (C).

3-Azido-*N*-[3-(pyrrolidin-1-yl)propyl]aniline 14e: The crude was purified by chromatography (dichloromethane:methanol 98:2). Yield 55%; ^{1}H NMR (ppm): 1.76-1.81 (6H, m, 3xCH_2), 2.48 (2H, t, J=7.2 Hz, CH_2), 2.55 (4H, bs, 2xCH_2), 4.07 (2H, t, J=7.2 Hz, CH_2), 7.00-7.02 (1H, m, Ar-H), 7.26 (1H, t, J=2.1 Hz, Ar-H), 7.32-7.34 (1H, m, Ar-H), 7.42 (1H, t, J=8.1 Hz, Ar-H); ^{13}C NMR (ppm): 23.4 (2xCH_2), 25.5 (CH_2), 41.9 (CH_2), 53.2 (CH_2), 54.0 (2xCH_2), 110.0 (CH), 115.2 (CH), 117.4 (CH), 130.7 (CH), 141.6 (C), 143.0 (C).

3-Azido-*N*-[2-(pyrrolidin-1-yl)ethyl]aniline 14f: The crude was purified by chromatography (dichloromethane:methanol 95:5). Yield 54%; ^{1}H NMR (ppm): 1.80 (4H, 5q, J=3.3 Hz, 2xCH_2), 2.55 (4H, bs, 2xCH_2), 2.74 (2H, t, J=6.2 Hz, CH_2), 3.18 (2H, q, J=5.5 Hz, CH_2), 4.46 (1H, bs, NH), 6.23 (1H, t, J=2.2 Hz, Ar-H), 6.38 (1H, ddd, J=0.8, 2.1, 8.0 Hz, Ar-H), 6.41 (1H, ddd, J=0.7, 2.2, 8.2 Hz, Ar-H), 7.12 (1H, t, J=8.0 Hz, Ar-H); ^{13}C NMR (ppm): 22.5 (2x CH_2), 41.1 (CH_2), 52.9 (2xCH_2), 53.6 (CH_2), 101.8 (CH), 106.5 (CH), 108.8 (CH), 129.3 (CH), 139.9 (C), 148.9 (C).

1-[3-(3-Azidophenoxy)propyl]-4-methylpiperazine 14g: The crude was purified by chromatography (dichloromethane:methanol 9:1). Yield 63%; ^{1}H NMR (ppm): 1.95 (2H, 5q, J=6.5 Hz, CH_2), 2.31 (3H, s, CH_3), 2.51-2.54 (10H, m, 5xCH_2), 4.00 (2H, t, J=6.5 Hz, CH_2), 6.54 (1H, t, J=2.2 Hz, Ar-H), 6.62 (1H, ddd, J=0.8, 2.2, 8.0 Hz, Ar-H), 6.67 (1H, ddd, J=0.8, 2.2, 8.0 Hz, Ar-H), 7.23 (1H, t, J=8.0 Hz, Ar-H); ^{13}C NMR (ppm): 26.7 (CH_2), 45.9 (CH_2), 53.1 (2xCH_2), 55.0 (CH_3), 55.1 (2xCH_2), 66.4 (CH_2), 105.5 (CH), 111.2 (2xCH), 130.4 (CH), 141.2 (C), 160.2 (C).

2-(3-Azidophenoxy)-*N,N*-diethylethanamine 14h: The crude was purified by chromatography (dichloromethane:methanol 95:5). Yield 54%; ^{1}H NMR (ppm): 1.10 (6H, t, J=7.1 Hz, 2xCH_3), 2.67 (4H, q, J=7.1 Hz, 2xCH_2), 2.89 (2H, t, J=6.3 Hz, CH_2), 4.06 (2H, t, J=6.3 Hz, CH_2), 6.59-6.60 (1H, m, Ar-H), 6.66 (1H, dd, J=1.6, 7.9 Hz, Ar-H), 6.71 (1H, dd, J=1.9, 8.3 Hz, Ar-H), 7.24-7.29 (1H, m, Ar-H); ^{13}C NMR (ppm): 11.9 (2xCH_2), 47.9 (2xCH_3), 51.7 (CH_2), 66.8 (CH_2), 105.6 (CH), 111.2 (CH), 111.3 (CH), 130.4 (CH), 141.2 (C), 160.1 (C).

1-[3-(3-Azidophenoxy)propyl]pyrrolidine 14i: The crude was purified by chromatography (dichloromethane:methanol 95:5). Yield 53%; ^{1}H NMR (ppm): 1.78-1.80 (4H, m, 2xCH_2), 2.00 (2H, 5q, J=6.5 Hz, CH_2), 2.54 (4H, bs, 2xCH_2), 2.63 (2H, t, J=7.4 Hz, CH_2), 4.03 (2H, t, J=6.5 Hz, CH_2), 6.56 (1H, t, J=2.2 Hz, Ar-H), 6.63 (1H, dd, J=1.9, 7.9 Hz, Ar-H), 6.69 (1H, dd, J=2.2, 8.2 Hz, Ar-H), 7.23 (1H, t, J=8.2 Hz, Ar-H); ^{13}C NMR (ppm): 23.5 (2xCH_2), 28.8 (CH_2), 53.0 (CH_2), 54.2 (2xCH_2), 66.6 (CH_2), 105.6 (CH), 111.2 (CH), 111.3 (CH), 130.3 (CH), 141.3 (C), 160.3 (C).

Synthesis of naphthalene bis triazole compounds 10a-i

General procedure:

Compound **13** (1 eq) was dissolved in the appropriate volume of solvent (water:*tert*-butanol 1:1), followed by the addition of required azide **14a-i** (3 eq) and the catalytic mixture of copper sulfate pentahydrate (0.05 eq), sodium ascorbate (0.5 eq) and bathophenanthrolinedisulfonic acid disodium salt hydrate (click catalyst, 0.1 eq). The reaction was performed in excess of the required amine (6 eq), to avoid elimination. The mixture was heated under microwave irradiation for 15 minutes at 120°C. after that the reaction was evaporated *in vacuo* and the crude obtained was purified by HPLC.

***N1,N1'*-[naphthalene-2,7-diylbis(1H-1,2,3-triazole-4,1-diylbenzene-3,1-diyl)]bis(N2,N2-diethylethane-1,2-diamine) 10a:** Diethylamine was used to avoid elimination. Yield 17%; ^{1}H NMR: 1.03 (6H, t, J=7.1 Hz, 2xCH_3), 1.16 (6H, t, J=7.2 Hz, 2xCH_3), 2.56-2.67 (6H, m, 3xCH_2), 2.80 (4H, q, J=7.2 Hz, 2xCH_2), 2.64 (2H, t, J=5.6 Hz, CH_2), 3.37 (2H, t, J=5.6 Hz, CH_2), 4.19 (2H, t, J=6.9 Hz, CH_2), 6.71 (1H, dd, J=1.6, 8.2 Hz, Ar-H), 7.05 (1H, dd, J=1.1, 7.9 Hz, Ar-H), 7.1 (1H, bs, Ar-H), 7.29 (2H, m, 2xAr-H), 7.64 (1H, t, J=8.1 Hz, Ar-H), 7.72-7.74 (1H, m, Ar-H), 7.83-7.85(1H, m, Ar-H), 7.90 (2H, dd, J=3.3, 8.5 Hz, 2xAr-H), 8.00-8.02 (2H, m, 2xAr-H), 8.24 (1H, bs, Ar-H), 8.33 (1H, s, Ar-H), 8.43 (1H, d, J=6.1 Hz, Ar-H), 8.48 (1H, s, Ar-H); ^{13}C NMR (ppm): 10.5 (CH_2), 11.7 (2xCH_2), 40.3 (CH_2), 43.3 (CH_2), 46.8 (2xCH_3), 47.3 (2xCH_3), 48.8 (CH_2), 51.2 (2xCH_2), 104.1 (CH), 108.8 (CH), 111.4 (CH), 113.5 (CH), 118.1 (CH), 118.3 (CH), 118.5 (CH), 119.2 (CH), 124.1 (CH), 124.4 (CH), 124.8 (CH), 125.0 (CH), 128.0 (C), 128.5 (C), 128.6 (CH), 128.7 (CH), 130.4 (CH), 130.8 (CH), 133.0 (C), 133.6 (C), 137.9 (C), 138.1 (C), 143.3 (C), 147.9 (C), 148.5 (C), 149.2 (C).

***N1,N1'*-[naphthalene-2,7-diylbis(1H-1,2,3-triazole-4,1-diylbenzene-3,1-diyl)]bis(N3,N3-diethylpropane-1,3-diamine) 10b:** Diethylamine was used to avoid elimination . Yield 44%; ^{1}H NMR: 1.16 (12H, t, J=7.2 Hz, 4xCH_3), 1.97 (4H, 5q, J=7.4 Hz, 2xCH_2), 2.75-2.84 (12H, m, 6xCH_2), 4.17 (4H, t, J=7.8 Hz, 2xCH_2), 7.68 (2H, t, J=8.1 Hz, 2xAr-H), 7.76 (2H, dd, J=0.8, 8.2 Hz, 2xAr-H), 7.95-7.98 (4H, m, 4xAr-H), 8.08 (2H, dd, J=1.1, 8.5 Hz, 2xAr-H), 8.13-8.14 (2H, m, 2xAr-H), 8.52 (2H, s, 2xAr-H), 8.84 (2H, s, 2xAr-H); ^{13}C NMR (ppm): 9.6 (4xCH_2), 22.8 (2xCH_2), 41.8 (2xCH_2), 45.9 (4xCH_3), 49.3 (2xCH_2), 110.3 (2xCH), 118.5 (2xCH), 118.6 (2xCH), 118.8 (2xCH), 124.4 (2xC), 125.0 (2xCH), 128.2 (2xC), 128.7 (2xCH), 131.1 (2xCH), 133.2 (C), 133.7 (C), 138.2 (2xCH), 142.7 (2xC), 148.6 (2xC).

4,4'-[Naphthalene-2,7-diylbis(1*H*-1,2,3-triazole-4,1-diyl)]bis{*N*-[3-(4-methylpiperazin-1-yl)propyl]aniline} 10c: N-methylpiperazine was used to avoid elimination. Yield 12%; ^{1}H NMR: 1.82-1.88 (8H, m, 4xCH_2), 2.34 (6H, s, 2xCH_3), 2.53-2.56 (16H, m, 8xCH_2), 3.26 (4H, t, J=6.2 Hz, 2xCH_2), 6.69-6.71 (4H, m, 4xAr-H), 7.57-7.59 (4H, m, 4xAr-H), 7.93 (2H, d, J=8.5 Hz, 2xAr-H), 8.03 (2H, dd, J=1.4, 8.5 Hz, 2xAr-H), 8.19 (2H, s, 2xAr-H), 8.44 (2H, s, 2xAr-H); ^{13}C NMR (ppm): 25.2 (2xCH_2), 43.7 (2xCH_2), 46.1 (2xCH_3), 53.2 (4xCH_2), 55.3 (4xCH_2), 57.4 (2xCH_2), 112.6 (4xCH), 118.2 (2xCH), 122.4 (4xCH), 124.2 (2xC), 124.7 (2xCH), 127.2 (2xC), 128.5 (2xCH), 128.6 (2xCH), 132.9 (C), 133.8 (C), 147.8 (2xC), 149.3 (2xC).

3,3'-[Naphthalene-2,7-diylbis(1*H*-1,2,3-triazole-4,1-diyl)]bis{*N*-[3-(4-methylpiperazin-1-yl)propyl]aniline}10d: *N*-methylpiperazine was used to avoid elimination . Yield 60%; ^{1}H NMR: 1.81 (4H, 5q, J=6.6 Hz, 2xCH_2), 2.45 (4H, t, J=6.6 Hz, 2xCH_2), 2.58 (6H, s, 2xCH_3), 2.67 (8H, bs, 4xCH_2), 2.94 (8H, bs, 4xCH_2), 4.16 (4H, t, J=7.3 Hz, 2xCH_2), 7.64-7.68 (2H, m, 2xAr-H), 7.75-7.77 (4H, m, 4xAr-H), 7.93-8.03 (4H, m, 4xAr-H), 8.19 (2H, t, J=1.8 Hz, 2xAr-H), 8.46-8.48 (4H, m, 4xAr-H); ^{13}C NMR (ppm): 23.3 (2xCH_3), 41.7 (2xCH_2), 43.8 (2xCH_2), 50.7 (4xCH_2), 53.3 (4xCH_2), 54.4 (2xCH_2), 110.7 (2xCH_2), 118.1 (2xCH), 118.2 (2xCH), 118.6 (2xC), 124.3 (2xC), 125.0 (2xCH), 128.1 (2xC), 128.8 (2xC), 131.0 (2xCH), 133.3 (C), 133.6 (C), 138.0 (2xCH), 143.2 (2xCH), 148.6 (2xCH).

3,3'-[Naphthalene-2,7-diylbis(1*H*-1,2,3-triazole-4,1-diyl)]bis{*N*-[3-(pyrrolidin-1-yl)propyl]aniline} 10e: Pyrrolidine was used to avoid elimination .Yield 18%; ^{1}H NMR: 1.94 (8H, bs, 4xCH_2), 2.02 (4H, quint, J=7.2 Hz, 2xCH_2), 2.80 (4H, t, J=7.4 Hz, 2xCH_2), 2.87 (8H, bs, 4xCH_2), 4.20 (4H, t, J=7.4 Hz, 2xCH_2), 7.67 (2H, t, J=8.1 Hz, 2xAr-H), 7.75-7.77 (2H, m, 2xAr-H), 7.95 (4H, d, J=8.3 Hz, 4xAr-H), 8.08 (2H, d, J=8.3 Hz, 2xAr-H), 8.14 (2H, s, 2xAr-H), 8.52 (2H, s, 2xAr-H), 8.82 (2H, s, 2xAr-H); ^{13}C NMR (ppm): 23.4 (4xCH_2), 24.5 (2xCH_2), 41.7 (2xCH_2), 52.7 (2xCH_2), 53.6 (4xCH_2), 110.5 (2xCH), 118.5 (2xCH), 118.6 (2xCH), 118.8 (2xCH), 124.3 (2xCH), 125.0 (2xCH), 128.2 (2xC), 128.7 (2xCH), 131.1 (2xCH), 133.2 (C), 133.7 (C), 138.2 (2xC), 142.8 (2xC), 148.6 (2xC).

3,3'-[Naphthalene-2,7-diylbis(1*H*-1,2,3-triazole-4,1-diyl)]bis{*N*-[2-(pyrrolidin-1-yl)ethyl]aniline} 10f: Pyrrolidine was used to avoid elimination .Yield 13%; ^{1}H NMR (ppm): 1.92 (8H, bs, 4xCH_2), 3.00 (8H, bs, 4xCH_2), 3.08 (4H, t, J=5.3 Hz, 2xCH_2), 3.45 (4H, t, J=5.3 Hz, 2x CH_2), 6.63 (2H, d, J=8.1 Hz, 2xAr-H), 6.97-7.02 (4H, m, 4xAr-H), 7.21 (2H, t, J=8.1 Hz, 2xAr-H), 7.82 (2H, d, J=8.5 Hz, 2xAr-H), 7.93 (2H, dd, J=1.1, 8.5 Hz, 2xAr-H), 8.30 (2H, s, 2xAr-H), 8.34

(2H, s, 2xAr-H); ^{13}C NMR (ppm): 23.3 (4xCH_2), 40.4 (2xCH_2), 53.8 (4xCH_2), 54.0 (2xCH_2), 103.6 (2xCH), 108.7 (2xCH), 113.6 (2xC), 118.4 (2xCH), 124.2 (2xC), 124.8 (2xCH), 128.4 (2xCH), 128.5 (2xCH), 130.4 (2xCH), 133.0 (C), 133.7 (C), 138.2 (2xC), 148.0 (2xCH), 148.9 (2xC).

1,1'-[Naphthalene-2,7-diylbis(1*H*-1,2,3-triazole-4,1-diylbenzene-3,1-diyloxypropane-3,1-diyl)]bis(4-methylpiperazine) 10g: *N*-methylpiperazine was used to avoid elimination. Yield 10%; ^{1}H NMR: 2.03 (4H, 5q, J=6.4 Hz, 2xCH_2), 2.34 (6H, s, 2xCH_3), 2.57-2.60 (20H, m, 10xCH_2), 4.12 (4H, t, J=6.4 Hz, 2xCH_2), 6.99 (2H, dd, J=2.4, 8.1 Hz, 2xAr-H), 7.26-7.36 (2H, m, 2xAr-H), 7.42-7.46 (4H, m, 4xAr-H), 7.94 (2H, d, J=8.5 Hz, 2xAr-H), 8.02 (2H, dd, J=1.5, 8.5 Hz, 2xAr-H), 8.31 (2H, s, 2xAr-H), 8.46 (2H, s, 2xAr-H); ^{13}C NMR (ppm): 26.6 (2xCH_2), 45.6 (2xCH_2), 52.8 (2xCH_3), 54.7 (4xCH_2), 54.8 (4xCH_2), 66.7 (2xCH_2), 107.0 (2xCH), 112.4 (2xCH), 115.2 (2xCH), 118.1 (2xCH), 124.3 (2xC), 124.9 (2xCH), 128.3 (2xC), 128.6 (2xC), 130.6 (2xCH), 133.1 (C), 133.7 (C), 138.1 (2xC), 148.2 (2xCH), 160.1 (2xCH).

2,2'-[Naphthalene-2,7-diylbis(1*H*-1,2,3-triazole-4,1-diylbenzene-3,1-diyloxy)]bis(*N*,*N*-diethylethanamine) 10h: Diethylamine was used to avoid elimination . Yield 24%; ^{1}H NMR (ppm): 1.12 (12H, t, J=7.1 Hz, 4xCH_3), 2.71 (8H, q, J=7.1 Hz, 4xCH_2), 2.96 (4H, t, J=6.0 Hz, 2xCH_2), 4.17 (4H, t, J=6.0 Hz, 2xCH_2), 7.01 (2H, dd, J=2.1, 7.9 Hz, 2xAr-H), 7.36-7.38 (2H, m, 2xAr-H), 7.42-7.46 (4H, m, 4xAr-H), 7.94 (2H, t, J=8.4 Hz, 2xAr-H), 8.03 (2H, dd, J=1.2, 8.4 Hz, 2xAr-H), 8.32 (2H, s, 2xAr-H), 8.46 (2H, s, 2xAr-H); ^{13}C NMR (ppm): 11.7 (4xCH_2), 47.8 (4xCH_3), 51.5 (2xCH_2), 66.9 (2xCH_2), 107.0 (2xCH_2), 112.5 (2xCH), 115.1 (2xCH), 118.1 (2xCH), 124.3 (2xCH), 124.9 (2xCH), 128.3 (2xC), 128.6 (2xCH), 130.6 (2xCH), 133.2 (C), 133.7 (C), 138.0 (2xC), 148.2 (2xC), 160.0 (2xC).

4,4'-Naphthalene-2,7-diylbis(1-{3-[3-(pyrrolidin-1-yl)propoxy]phenyl}-1*H*-1,2,3-triazole) 10i: Pyrrolidine was used to avoid elimination . Yield 40%; ^{1}H NMR: 1.91 (8H, bs, 4xCH_2), 2.16 (4H, 5q, J=6.2 Hz, 2xCH_2), 2.81 (8H, bs, 4xCH_2), 2.87 (4H, t, J=7.5 Hz, 2xCH_2), 4.15 (4H, t, J=6.2 Hz, 2xCH_2), 6.97-6.99 (2H, m, 2xAr-H), 7.37 (2H, d, J=7.8 Hz, 2xAr-H), 7.43-7.46 (4H, m, 4xAr-H), 7.94 (2H, d, J=8.5 Hz, 2xAr-H), 8.03 (2H, d, J=8.5 Hz, 2xAr-H), 8.34 (2H, s, 2xAr-H), 8.46 (2H, s, 2xAr-H); ^{13}C NMR (ppm): 23.5 (4xCH_2), 27.7 (2xCH_2), 52.8 (2xCH_2), 53.8 (4xCH_2), 66.4 (2xCH_2), 107.1 (2xCH), 112.5 (2xCH), 115.0 (2xCH), 118.1 (2xCH), 124.3 (2xCH), 124.9 (2xCH), 128.3 (2xC), 128.6 (2xCH), 130.6 (2xCH), 133.1 (C), 133.7 (C), 138.1 (2xC), 148.2 (2xC), 159.9 (2xC).

Synthesis of 5,5'-benzene-1,3-diylbis(1,3-oxazole) 24

Compound **24** was prepared according to literature procedure. [41] Analytical data and ^{1}H and ^{13}C NMR spectra matched literature values.

Synthesis of 5,5'-naphthalene-2,7-diylbis(1,3-oxazole) 27

An oven dried microwave vial was charged with palladium acetate (0.1 eq), potassium carbonate (3 eq), ligand (2-di-tert-butylphosphino-3,4,5,6-tetramethyl-2',4',6'-triisoproppyl-1,1'-biphenyl) (0.2 eq), compound **26** (1 eq) and compound **25** (2.2 eq). The microwave vial was capped, evacuated and backfilled with argon and then pivalic acid (0.4 eq) and dimethylacetamide were added. The vial was heated at 110°C for 72 hours. After cooling to room temperature, the reaction mixture was concentrated and purified via HPLC.

Yield 50%; ^{1}H NMR: 6.81-6.88 (2H, m, 2xAr-H), 7.38 (2H, t, J=7.9 Hz, 2xAr-H), 7.68-7.73 (2H, m, 2xAr-H), 8.01 (2H, s, 2xAr-H), 8.68 (2H, s, 2xAr-H); ^{13}C NMR (ppm): 124.4 (2xCH), 125.4 (2xCH), 126.5 (2xCH), 128.5 (2xCH), 133.6 (2xC), 133.9 (C), 134.6 (2xC), 138.1 (C), 150.0 (2xCH).

Synthesis of *N*-substituted 3-bromoaniline compounds 29a-c

General procedure:

To a solution of the chloro-compound **20b,d,e** (1 eq) in acetonitrile in an oven dried microwave vial, potassium iodide (0.2 eq) and 3-bromoaniline **28** (3 eq) were added.

The vial was heated under microwave irradiation for 10 minutes at 110°C. The cooled reaction mixture was diluted with dichloromethane, washed with aqueous saturated sodium hydrogen carbonate, dried over $MgSO_4$, concentrated *in vacuo* and purified via column chromatography.

N'-(3-bromophenyl)-N,N-diethylethane-1,2-diamine 29a: The crude was purified by chromatography (dichloromethane:methanol 9:1). Yield 83%, ^{1}H NMR (ppm): 1.17 (6H, t, J=7.12 Hz, 2xCH_3), 2.8 (4H, q, J=7.12 Hz, 2xCH_2), 2.91 (2H, t, J=5.7 Hz, CH_2), 3.29 (2H, bs, CH_2), 6.58 (1H, dd, J=2.1, 8.0, Ar-H), 6.76 (1H, t, J=2.1, Ar-H), 6.80 (1H, dd, J=2.1, 8.0, Ar-H), 7.00 (1H, t, J=8.0, Ar-H); ^{13}C NMR (ppm): 10.4 (CH_2), 39.9 (CH_2), 47.0 (2xCH_3), 51.4 (2xCH_2), 111.9 (CH), 115.1 (CH), 120.2 (CH), 123.3 (C), 130.6 (CH), 149.2 (C).

3-Bromo-*N*-[3-(4-methylpiperazin-1-yl)propyl]aniline 29b: The crude was purified by chromatography (dichloromethane:methanol 9:1).Yield 20%, ^{1}H NMR (ppm): 1.79 (2H, 5q, J=6.4 Hz, CH_2), 2.35 (3H, s, CH_3), 2.50-2.55 (10H, m, 5xCH_2), 3.16 (2H, t, J=6.4 Hz, CH_2), 4.88 (1H, bs,

NH), 6.49 (1H, ddd, J=0.75, 2.2, 8.0 Hz, Ar-H), 6.71 (1H, t, J=2.2 Hz, Ar-H), 6.77 (1H, ddd, J=0.75, 2.2, 8.0 Hz, Ar-H), 6.99 (1H, t, J=8 Hz, Ar-H); ^{13}C NMR (ppm): 25.4 (CH_2), 43.3 (CH_2), 45.9 (CH_3), 53.0 (2xCH_2), 55.2 (2x CH_2), 57.1 (CH_2), 111.5 (CH), 114.9 (CH), 119.6 (CH), 123.3 (C), 130.4 (CH), 150.0 (C).

3-Bromo-*N*-[2-(pyrrolidin-1-yl)ethyl]aniline 29c: The crude was purified by chromatography (dichloromethane:methanol 95:5). Yield 46%, ^{1}H NMR (ppm): 1.89 (4H, bs, 2xCH_2), 3.04-3.05 (6H, m, 3xCH_2), 3.36 (2H, q, J=5.65 Hz, CH_2), 6.66 (1H, dd, J=1.7, 8.1 Hz, Ar-H), 6.75 (1H, dd, J=1.0, 7.8 Hz, Ar-H), 6.83 (1H, t. J=1.7 Hz, Ar-H), 7.08 (1H, t, J=8.1 Hz, Ar-H); ^{13}C NMR (ppm): 22.7 (4xCH_2), 53.3 (2xCH_2), 111.2 (CH), 114.1 (CH), 118.3 (C), 122.4 (CH), 130.7 (CH), 149.9 (C).

Synthesis of *O*-substituted 3-bromophenol compounds 29d,e

General procedure:

3-Bromo-phenol **30** (1 equiv), 1-(2-chloro-ethyl)-amino compound **20d,e** (1 equiv) and potassium carbonate (8 equiv) were diluted with dimethylformamide and stirred for 2 days. The contents were poured onto ethyl acetate water mixture and extracted. The organic layer was separated, washed with brine and dried over $MgSO_4$, concentrated *in vacuo* and purified via column chromatography to give the desired compound.

1-[2-(3-Bromophenoxy)ethyl]pyrrolidine 29e: The crude was purified by chromatography (dichloromethane:methanol 98:2). Yield 48 %; ^{1}H NMR: 1.81 (4H, quint, J=3.1 Hz, 2xCH_2), 2.61-2.64 (4H, m, 2xCH_2), 2.90 (2H, t, J=5.9 Hz, CH_2), 4.08 (2H, t, J=5.9 Hz, CH_2), 6.83-6.86 (1H, m, Ar-H), 7.05-7.15 (3H, m, 3x Ar-H); ^{13}C NMR (ppm): 23.5 (2xCH_2), 54.7 (2xCH_2), 54.9 (CH_2), 67.4 (CH_2), 113.6 (CH), 118.0 (CH), 122.8 (C), 123.8 (CH), 130.5 (CH), 159.7 (C).

2-(3-Bromophenoxy)-*N*,*N*-diethylethanamine 29d: The crude was purified by chromatography (dichloromethane:methanol 9:1). Yield 78 %; ^{1}H NMR: 1.06 (6H, t, J=7.2 Hz, 2xCH_3), 2.64 (4H, q, J=7.2 Hz, 2xCH_2), 2.86 (2H, t, J=6.2 Hz, CH_2), 4.02 (2H, t, J=6.2 Hz, CH_2), 6.83 (1H, ddd, J=1.2, 2.3, 8.1 Hz, Ar-H), 7.05-7.07 (2H, m, 2x Ar-H), 7.12 (1H, t, J=8.1 Hz, Ar-H); ^{13}C NMR (ppm): 11.9 (2xCH_3), 47.9 (2xCH_2), 51.7 (CH_2), 66.9 (CH_2), 113.6 (CH), 117.9 (CH), 122.8 (C), 123.7 (CH), 130.5 (CH), 159.7 (C).

Synthesis of benzene bis oxazole compounds 11a-e

General procedure:

An oven dried microwave vial was charged with palladium acetate (0.1 eq), potassium carbonate (3 eq), ligand (2-dicyclohexylphosphino-2',6'-diisopropoxybiphenyl) (0.2 eq), compound **24** (1 eq) and compound **29a-e** (2.2 eq). The microwave vial was capped, evacuated and backfilled with argon and then pivalic acid (0.4 eq) and toluene were added. The vial was heated at 110°C for 72 hours. After cooling to room temperature, the reaction mixture was concentrated and purified.

***N1,N1'*-[benzene-1,3-diylbis(1,3-oxazole-5,2-diylbenzene-3,1-diyl)]bis(*N2,N2*-diethylethane-1,2-diamine) 11a:** The crude was purified by chromatography (dichloromethane:methanol 95:5). Yield quantitative; ^{1}H NMR (ppm): 1.05 (12H, t, J=7.1 Hz, 4xCH_3), 2.64 (8H, q, J=7.1 Hz, 4xCH_2), 2.68 (4H, t, J=6.9 Hz, 2xCH_2), 3.24 (4H, q, J=6.9 Hz, 2xCH_2), 5.85 (2H, bs, 2xNH), 6.81-6.84 (2H, m, 2xAr-H), 7.31 (2H, t, J=7.7 Hz, 2xAr-H), 7.37-7.40 (4H, m, 4xAr-H), 7.69 (1H, t, J=7.9 Hz, Ar-H), 7.87 (2H, dd, J=1.5, 7.9 Hz, 2xAr-H), 8.01 (2H, s, 2x Ar-H), 8.28 (1H, t, J=1.5 Hz, Ar-H); ^{13}C NMR (ppm): 11.6 (2xCH_2), 46.5 (4xCH_3), 51.1 (2xCH_2), 4xCH_2 is hidden underneath DMSO peak, 108.9 (CH), 113.6 (2xC), 114.6 (2xC), 119.1 (2xC), 119.1 (2xC), 123.7 (2xC), 125.0 (CH), 127.3 (2xCH), 128.4 (2xCH), 129.7 (2xCH), 130.0 (CH), 149.2 (CH), 149.8 (2xCH), 161.3 (2xCH).

3,3'-(Benzene-1,3-diyldi-1,3-oxazole-5,2-diyl)bis{*N*-[3-(4-methylpiperazin-1-yl)propyl]aniline} 11b: The crude was purified by prep-HPLC. Yield 12%; ^{1}H NMR (ppm): 1.26 (4H, s, 2xCH_2), 1.65 (4H, bs, 2xCH_2), 1.85 (4H, 5q, J=6.3 Hz, 2xCH_2), 2.31 (6H, s, 2xCH_3), 2.51-2.55 (12H, m, 6xCH_2), 3.30 (4H, t, J=6.3 Hz, 2xCH_2), 6.72 (2H, dd, J=1.6, 8.1 Hz, 2xAr-H), 7.29 (2H, t, J=7.8 Hz, 2xAr-H), 7.35 (2H, t, J=1.6 Hz, 2xAr-H), 7.46 (2H, d, J=7.8 Hz, 2xAr-H), 7.49-7.53 (3H, m, 3xAr-H), 7.69 (2H, dd, J=1.5, 7.8 Hz, 2xAr-H), 8.01 (1H, bs, Ar-H); ^{13}C NMR (ppm): 25.6 (2xCH_3), 43.5 (2xCH_2), 46.0 (2xCH_2), 53.2 (4xCH_2), 55.3 (4xCH_2), 57.2 (2xCH_2), 109.8 (2xCH), 115.2 (2xCH), 115.3 (CH), 119.7 (2xC), 124.0 (2xC), 124.1 (2xC), 128.1 (2xCH), 129.0 (2xCH), 129.6 (2xC), 129.7 (2xCH), 149.1 (2xCH), 150.4 (CH), 162.1 (2xC).

3,3'-(Benzene-1,3-diyldi-1,3-oxazole-5,2-diyl)bis{*N*-[2-(pyrrolidin-1-yl)ethyl]aniline} 11c: The crude was purified by prep-HPLC. Yield 32%; ^{1}H NMR (ppm): 1.83 (8H, bs, 4xCH_2), 2.62 (8H, bs, 4xCH_2), 2.83 (4H, t, J=6.0 Hz, 2xCH_2), 3.32 (4H, t, J=6.0 Hz, 2xCH_2), 6.76 (2H, dd, J=1.7, 8.0 Hz, 2xAr-H), 7.30 (2H, t, J=7.9 Hz, 2xAr-H), 7.39 (2H, bs, 2xAr-H), 7.46-7.54 (5H, m, 5xAr-H), 7.69 (2H, dd, J=1.4, 7.9 Hz, 2xAr-H), 8.0 (1H, s, Ar-H); ^{13}C NMR (ppm): 23.5 (4xCH_2), 42.2 (2xCH_2),

53.9 (4xCH_2), 54.7 (2xCH_2), 110.0 (2xCH), 115.3 (2xCH), 115.4 (2xCH), 119.7 (2xC), 124.0 (2xCH), 124.1 (2xCH), 128.1 (2xC), 128.9 (2xC), 129.6 (2xC), 129.7 (2xCH), 148.9 (CH), 150.4 (CH), 162.1 (2xC).

2,2'-[bBenzene-1,3-diylbis(1,3-oxazole-5,2-diylbenzene-3,1-diyloxy)]bis(*N,N*-diethylethanamine) 11d: The crude was purified by column chromatography (dichloromethane:methanol 8:2). Yield 50%; ^{1}H NMR (ppm): 1.14 (12H, t, J=7.15 Hz, 4xCH_3), 2.75 (8H, q, J=7.15 Hz, 4xCH_2), 3.00 (4H, t, J=5.9 Hz, 2xCH_2), 4.20 (4H, t, J=5.9 Hz, 2xCH_2), 7.02 (2H, dd, J=1.96, 8.1 Hz, 2xAr-H), 7.39 (2H, t, J=8.0 Hz, 2xAr-H), 7.49-7.54 (3H, m, 3xAr-H), 7.65-7.72 (6H, m, 6xAr-H), 7.96 (1H, s, Ar-H); ^{13}C NMR (ppm): 11.4 (4xCH_2), 47.8 (4xCH_3), 51.6 (2xCH_2), 66.2 (2xCH_2), 112.0 (2xCH), 117.3 (CH), 119.1 (CH), 119.7 (2xC), 124.1 (2xCH), 124.2 (2xCH), 128.5 (2xC), 128.8 (2xC), 129.6 (2xC), 130.0 (2xCH), 150.7 (2xCH), 159.0 (2xCH), 161.3 (2xC).

5,5'-Benzene-1,3-diylbis(2-{3-[2-(pyrrolidin-1-yl)ethoxy]phenyl}-1,3-oxazole) 11e: The crude was purified by column chromatography (dichloromethane:methanol 8:2). Yield 75%; ^{1}H NMR (ppm): 1.90 (8H, bs, 4xCH_2), 2.83 (8H, bs, 4xCH_2), 3.08 (4H, t, J=5.6 Hz, 2xCH_2), 4.31 (4H, t, J=5.6 Hz, 2xCH_2), 7.05 (2H, ddd, J=0.75, 2.5, 8.3 Hz, 2xAr-H), 7.41 (2H, t, J=8.1 Hz, 2xAr-H), 7.51-7.55 (3H, m , 3xAr-H), 7.67-7.74 (6H, m, 6xAr-H), 7.98 (1H, t, J=1.42, Ar-H); ^{13}C NMR (ppm): 23.5 (4xCH_2), 54.7 (4xCH_2), 54.8 (2xCH_2), 66.5 (2xCH_2), 112.2 (2xCH), 117.2 (CH), 119.1 (2xC), 119.2 (2xC), 119.8 (2xC), 124.2 (2xCH), 128.5 (2xCH), 128.8 (CH), 129.7 (2xC), 130.1 (2xCH), 150.7 (2xCH), 158.9 (2xCH), 161.3 (2xC).

<u>Synthesis of naphthalene bis oxazole compounds 12a,b</u>

General procedure:

An oven dried microwave vial was charged with palladium acetate (0.1 eq), potassium carbonate (3 eq), ligand (2-dicyclohexylphosphino-2’.6’-diisopropoxybiphenyl) (0.2 eq), compound **27** (1 eq) and compound **29a,b** (2 eq). The microwave vial was capped, evacuated and backfilled with argon and then pivalic acid (0.4 eq) and toluene were added. The vial was heated at 110°C for 72 hours. After cooling to room temperature, the reaction mixture was concentrated and purified.

***N1,N1'*-[naphthalene-2,7-diylbis(1,3-oxazole-5,2-diylbenzene-3,1-diyl)]bis(*N2,N2*-diethylethane-1,2-diamine) 12a:** The crude was purified by column chromatography (dichloromethane:methanol 95:5). Yield 86%; ^{1}H NMR : 1.06 (12H, t, J=7.1 Hz 4xCH_3), 2.61 (8H,

q, J=7.1 Hz, 4xCH_2), 2.68 (4H, t, J=7.3 Hz, 2xCH_2), 3.23 (4H, q, J=7.3 Hz, 2xCH_2), 5.85 (2H, t, J=5.5 Hz, 2xNH), 6.83-6.85 (2H, m, 2xAr-H), 7.33 (2H, t, J=7.9 Hz, 2xAr-H), 7.38-7.42 (4H, m, 4xAr-H), 8.01 (2H, s, 2xAr-H), 8.03 (2H, dd, J=1.6, 8.5 Hz, 2xAr-H), 8.12 (2H, d, J=8.5 Hz, 2xAr-H), 8.5 (2H, s, 2xAr-H); ^{13}C NMR (ppm): 11.8 (4xCH_3), 41.1 (2xCH_2), 46.6 (4xCH_2), 51.3 (2xCH_2), 108.9 (2xC), 113.4 (2xC), 114.6 (2xC), 122.4 (2xCH), 122.6 (2xC), 125.1 (2xCH), 125.8 (2xCH), 127.3 (2xCH), 128.8 (2xC), 129.7 (2xCH), 132.1 (C), 133.1 (C), 149.3 (2xCH), 150.2 (2xCH), 161.4 (2xCH).

3,3'-(Naphthalene-2,7-diyldi-1,3-oxazole-5,2-diyl)bis{*N*-[3-(4-methylpiperazin-1-yl)propyl]aniline} 12b: The crude was purified by HPLC. Yield 10%; ^{1}H NMR: 1.91 (4H, quint, J=6.6 Hz, 2xCH_2), 2.54 (6H, s, 2xCH_3), 2.71 (4H, t, J=6.6 Hz, 2xCH_2), 2.82-2.92 (16H, m, 8xCH_2), 3.31 (4H, t, J=6.6 Hz, 2xCH_2), 6.73 (2H, dd, J=1.6, 8.1 Hz, 2xAr-H), 7.30 (2H, t, J=7.9 Hz, 2xAr-H), 7.38 (2H, bs, 2xAr-H), 7.50 (2H, d, J=7.9 Hz, 2xAr-H), 7.56 (2H, s, 2xAr-H), 7.78 (2H, dd, J=1.3, 8.6 Hz, 2xAr-H), 7.89 (2H, d, J=8.6 Hz, 2xAr-H), 8.25 (2H, s, 2xAr-H), 8.43 (2H, bs, 2xNH); ^{13}C NMR (ppm): 25.4 (2xCH_2), 42.1 (2xCH_2), 44.1 (2xCH_2), 50.9 (4xCH_2), 53.2 (4xCH_2), 55.6 (2xCH_2), 109.7 (2xC), 115.4 (2xC), 115.5 (2xC), 123.0 (2xC), 124.2 (2xC), 126.3 (2xCH), 128.2 (2xCH), 128.7 (2xCH), 129.8 (2xCH), 132.6 (C), 133.5 (C), 148.7 (2xCH), 150.9 (2xCH), 162.0 (2xCH), 167.2 (2xCH).

BIOPHYSICAL STUDIES: FRET

All oligonucleotides and their fluorescent conjugates were purchased from Eurofins. DNA was initially dissolved as a stock 20 µM solution in purified water; further dilutions were carried out in the relevant buffer. The ability of the compounds to stabilise G-quadruplex DNA was investigated using a FRET assay modified to be used as a high-throughput screen in a 96-well format.

The labelled DNA were diluted from stock to the correct concentration in a 60 mM potassium cacodylate buffer and then annealed by heating to 95°C for 10 min, followed by cooling to room temperature in the heating block. Compounds were stored at -20°C as 1 mM stock solution in 90% water and 10% DMSO, further dilutions were carried out using the potassium cacodylate buffer. All experiment values were determinated in triplicate. 96-well plates (MJ Research, Waltham, MA) were prepared by aliquoting 50 µl of the compounds solutions into each well, followed by 50 µl of the annealed DNA. Measurements were made on a DNA Engine Opticon (MJ Research) with excitation at 450-495 nm and detection at 515-545 nm. Fluorescence readings were taken at intervals of 0.5°C over the range 30-100°C, with a constant temperature being maintained for 30

seconds prior to each reading to ensure a stable value. Final analysis of the data was carried out using a script written in the program Origin 7.0 (OriginLab Corp., Northampton, MA). In brief, the raw data from the fluorescence detector was first smoothed using a 10-point running average and subsequently normalised. For the determination of the melting temperature, the first derivative of the smoothed melting curve was then calculated, and the resulting value plot against compound concentration as ΔTm with respect to the native melting profile of the sequences.

IN VITRO CELL ASSAY

The cell lines MCF7, A549, MIA-Pa-Ca-2, RCC4, 786-0 (European Collection of Cell Cultures) and WI38 were maintained in monolayer culture in 75 cm^2 flasks (TPP, Switzerland) under a humidified 5 % CO_2 atmosphere at 37 °C. Incubations were also done under these conditions, unless specified otherwise. For the cell lines MCF7 and A549, the medium Dulbecco's MEM (GIBCO 21969, Invitrogen, UK) supplemented with L-glutamine (2 mM, GIBCO 25030, Invitrogen, UK), essential amino acids (1 %, GIBCO 11140, Invitrogen, UK), foetal bovine serum (10 %, S1810, Biosera, UK) was used. The medium MEM (M2279, Sigma, UK) with added L-glutamine (2 mM), essential amino acids (1 %) and foetal calf serum (10 %) was used for the cell line WI38. Cells were sub-cultured every 3 to 4 days by washing with phosphate buffered saline (Gibco, Invitrogen, UK), followed by tripsinisation (Gibco, Invitrogen, UK) and re-seeding into fresh medium.

In a 96 well plate the required number of cells was seeded in 160 μl of the appropriate media and incubated overnight at 37°C. The drugs were stored at 4°C as 10 mM stock solution in DMSO, this solution was first diluted with a 1 mM aqueous solution of HCl to 1mM concentration and further diluitions were performed in the required media.

Then 40 μl of the stock solution of the drug were added to the cells, which were incubated for 96 hours. For positive and negative controls 40 μl of media was added instead.

After 96 hours the plates were emptied and the cell fixed with trichloroacetic acid solution (10 g TCA in 100 ml H_2O, 160 μl per well; Sigma-Aldrich, UK) for 30 minutes in ice. The supernatant was then removed the plates were washed with water and dried at 60°C for one hour. The plates were then stained with SRB (0.4% stain in 1% acetic acid w/v, 80 μl per well; Sigma-Aldrich, UK) for 15 minutes.

The excess of SRB was removed with 1%5 acetic acid, the plates were wiped and dried at 60°C.

The dye was extracted with 10 mM Tris base (100 μl per well; Sigma-Aldrich, UK) for determination of optical density in a computer-interfaced, 96-well microtiter plate reader (Anthos 2010, Anthos Labtec, Austria) at 540 nm.

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