UNIVERSITÀ DEGLI STUDI DI PALERMO
Dipartimento Scienze e Tecnologie Molecolari e Biomolecolari –(STEMBIO)

PhD in Pharmaceutical Sciences

"Doctor Europeus"

Synthesis and antitumor activity of new tetracyclic systems containing the pyrrole ring

Settore scientifico disciplinare CHIM/08

Dr. Barbara Parrino

TUTOR
Chiar.mo Prof. Girolamo Cirrincione

PhD Coordinator:
Chiar.mo Prof. Girolamo Cirrincione

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INTRODUCTION

Cancer is a growing problem estimated to have about seven million new incidences per year in all over the world. About one in four people will get it in some form during their life time, and at the present time about one in five of all deaths are due to cancer.

Cancer is a term which describes a group of perhaps 120 different diseases which share some broad similarities. In these diseases, a single cell begins to divide uncontrollably 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 differentiate cancer cells from normal cells that are kept under tight control by a number of different biological mechanisms that are still being explored. We do know that cell division is controlled by a relatively small group of enzymes. Some of these operate to form a communication network, relaying growth signals from the surface of the cell to its DNA and telling it when to begin dividing.

Others work as a surveillance team, preventing a cell with damaged DNA from reproducing by first repairing the damage or by instructing it to die. However, sometimes the damage (mutation) occurs in the DNA that codes for these enzymes, so that they are themselves defective. Such a cell will divide uncontrollably, and produce daughter cells that do the same.

However is almost always impossible to prove exactly what caused a cancer in any individual, because most cancers have multiple possible causes; cancers are primarily an environmental disease with 90-95% of cases attributed to environmental factors and 5-10% due to genetics. *Environmental* means any cause that is not genetic, not merely pollution. Common environmental factors that contribute to cancer death include tobacco (25-30%), diet and obesity (30-35%), infections(15-20%), radiation (both ionizing and non-ionizing, up to 10%), stress, lack of physical activity, and environmental pollutants.

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.

Many management options for cancer exist including: chemotherapy, radiation therapy,

surgery, immunotherapy, monoclonal antibody therapy and other methods. Which treatments are used depends upon the type of cancer, the location and grade of the tumour, and the stage of the disease, as well as the general state of a person's health.

Complete removal of the cancer without damage to the rest of the body is the goal of treatment for most cancers. Sometimes this can be accomplished by surgery, but the propensity of cancers to invade adjacent tissue or to spread to distant sites by microscopic metastasis often limits its effectiveness. Surgery often required the removal of a wide surgical margin and the effectiveness of chemotherapy is often limited by toxicity to other tissues in the body. Radiation can also cause damage to normal tissue.

For these reasons cancer research is the intense scientific effort to understand disease processes and discover possible therapies.

The drugs used to combat cancer belong to one of two broad categories. The first is cytotoxic (cell killing) drugs and the second is cytostatic (cell stabilising drugs). Both categories lead to a reduction in the size of the tumour because cancer cells (for various reasons) have such a high mortality rate that simply preventing them from dividing will lead to a reduction in the population.

Cytotoxic drugs work by interfering with DNA replication. Because cancer cells are rapidly dividing, they are rapidly synthesizing new DNA and if this is damaged the cell will die.

There are three main groups of molecules that can be used to interfere with DNA replication:

- antimetabolites: molecules that appear to be nucleotides and so are incorporated into DNA, leading to non-functional DNA.
- alkylating agents: molecules that permanently attach to the DNA, distorting its shape. Unfortunately these also attach to many other molecules in cells.
- DNA-binding agents: molecules that attach to the DNA chain, break it, disengage from the chain and then attach to another chain to repeat the process. These usually function in conjunction with an enzyme.

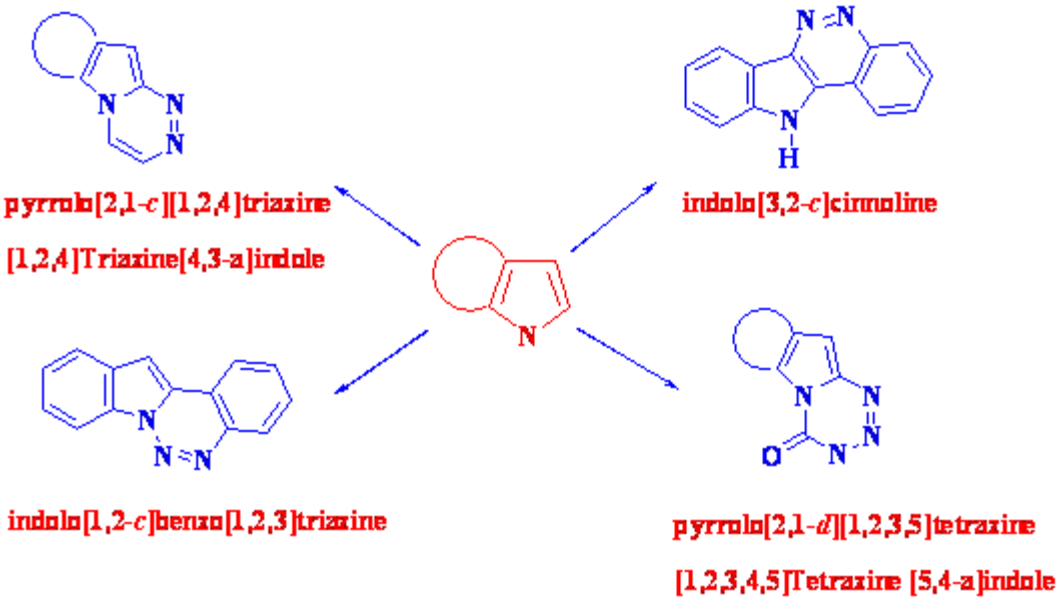
Examples of compounds showing antitumor activity are polycondenced nitrogen heterocycles. They found infact wide range of pharmaceutical applications.

From several years the research group where I carried out my PhD thesis has been interested in the synthesis and biological evaluation of polycondensed nitrogen heterocycles, containing pyrrole or indole moieties, endowed with antineoplastic

activity and, being planar systems, they potentially might intercalate between the base pairs of double-stranded DNA.

Pyrrolo[2,1-*c*][1,2,4]triazine, [02EJMC267] and [1,2,4]triazine[4,3-a]indole, [04ACR3775] showed antiproliferative activity with GI_{50} at low micromolar concentrations against a wide range of human cell lines (panel of about 60 cell lines).

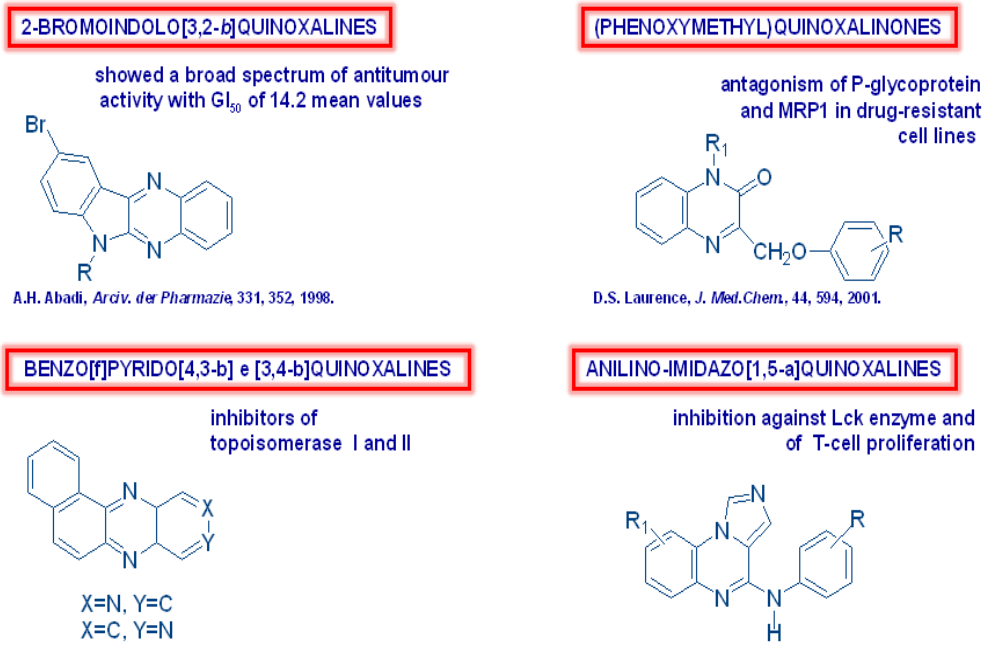
Indolo[1,2-*c*]benzo[1,2,3]triazine, [99JMC2561] and indolo[3,2-*c*]cinnoline, [99BMC1591] exhibited potent antiproliferative activity, reaching GI_{50} values even at nanomolar level against leukaemia cell lines and solid tumours cell lines. They also showed antibacterial activity being two magnitude orders more active than streptomycin. Pyrrolo[2,1-*d*][1,2,3,5]tetrazine, [03BMC2371] and [1,2,3,5]tetrazine[5,4-a]indole, [05BMCL295] showed as well antiproliferative activity from micromolar to nanomolar concentration.



Quinoxalines represent an important class of compounds that are found in a variety of biologically and medicinally useful agents. Infact, the quinoxaline portion is the pharmacophore moiety of a large number of molecules.

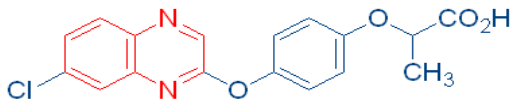
For instance, substituted (phenoxymethyl)quinoxalin-2-ones demonstrated excellent antagonism of P-glycoprotein and MRP1 in drug-resistant cell lines [01JMC594];

quinoxaline ring condensed with a pyridine moiety led to benzo[f]pyrido [4,3-b] and pyrido[3,4-b]quinoxalines that are inhibitors of topoisomerase I and II [95ACDD277]. Incorporation of indole ring gave 5-substituted 2-bromoindolo[3,2-b]quinoxaline that showed a broad spectrum of antitumor activity with two biochemical mechanism-based screens (cdc2 kinase and cdc25 phosphatase) with IC_{50} of 70 and 25 μM respectively [98ADP352]. (Anilinophenyl)imidazo[1,5-a]quinoxalines derivatives showed inhibition against Lck enzyme and T-cell proliferation [02BMC3153].



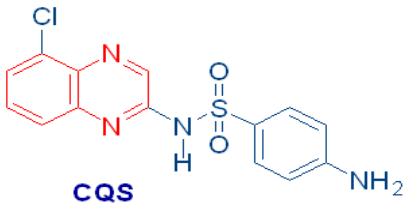
Some quinoxaline derivatives found their way in clinical application. For example the two known antineoplastic quinoxaline topoisomerase II inhibitors, chloroquinoaline-phenoxy-propionic acid (**XK469**) [07IND147], [08EJC1684], [08IND331] and chloroquinoxalinesulfonamide (**CQS**) [06IND343].

2-[4-(7-Chloroquinoxalin-2-yl)-phenoxy-propionic acid



XK469

5-Chloroquinoxaline-2-sulfonamide



CQS

For all these reasons, in the last years, our research group have been interested to the synthesis of isoindolo[1,2-a]quinoxalines.

Isoindole derivatives are quite unstable, in fact, they rapidly darken and resinify at room temperature, especially in the presence of air.

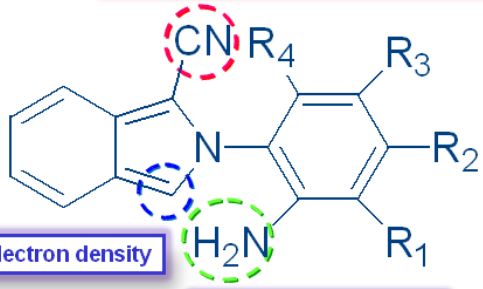
ANNELATED ISOINDOLO WITH ANTITUMOR ACTIVITY

PLANNING ISOINDOLE INTERMEDIATES

STRATEGIC SUBSTITUENTS

- **STABILIZATION**
- **FUNCTIONALIZATION**

Electron-withdrawing and N-phenyl substituents, which stabilize the isoindole nucleus

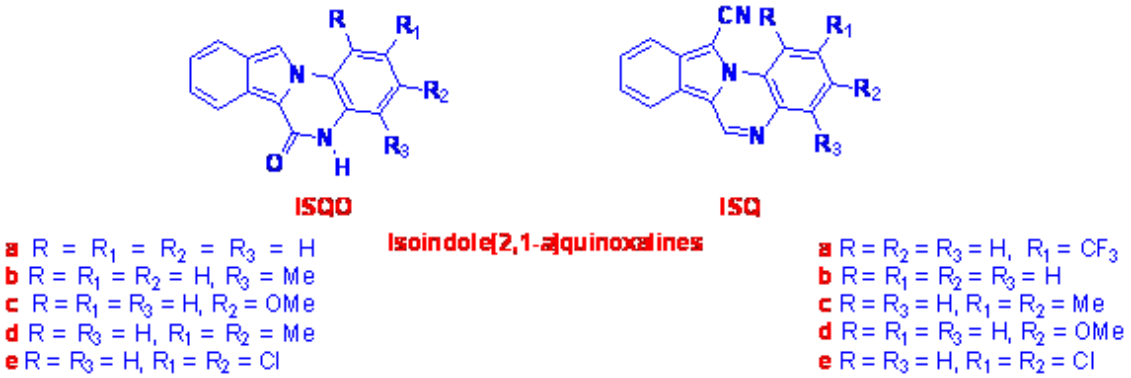


High π-electron density

Versatile amino group

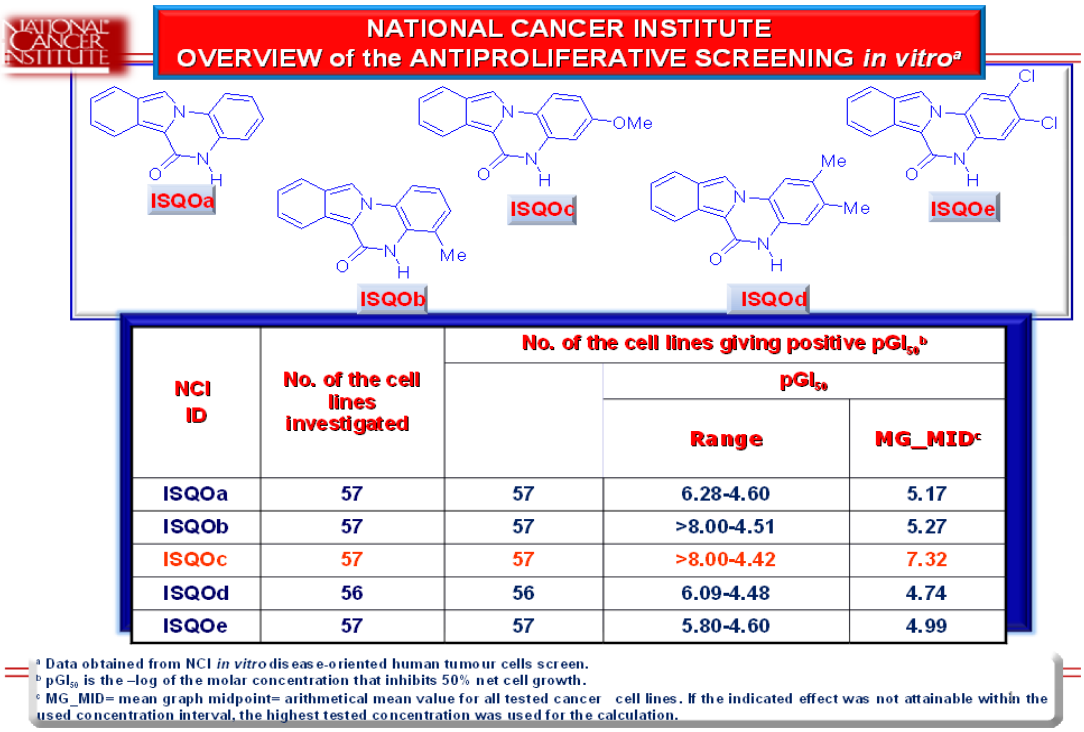
2-(2-AMINOARYL)-1-CYANO-ISOINDOLES

Therefore, isoindolo[1,2-a]quinoxalines. were synthesized from isoindole intermediates which would have strategic substituents: to both stabilize and functionalize the isoindole structure. Were synthesized compounds having an electron-withdrawing substituent at C-1, which stabilize the isoindole structure, high π-electron density at the position 3, also a phenyl substituted moiety on the nitrogen bearing a versatile amine group.



Isoindole[2,1-a]quinoxalines **ISQO** and **ISQ** have been synthesized and all derivatives were evaluated by the National Cancer Institute (NCI, Bethesda, MD) in the 3-cell line (MCF7-breast, NCI-H460-nonsmall cell lung and SF-268-CNS) one dose primary anticancer assay (10^{-4} M) (Table 1) [08JMC2387].

All five isoindoloquinoxalines **ISQO** were selected for evaluation against the full NCI panel of approximately 60 human cancer cell lines and were determined the following parameters: GI_{50},TGI and LC_{50}. An evaluation of the data reported in the table 1 point out that all five derivatives exhibited antineoplastic activity against all the human cell lines. The most active compound was the 3-methoxy derivative **ISQOc** for which the pGI_{50} mean value was 7.32. From the data reported, it was supposed that substituents that enriched the π-electron density of the benzene moiety of the quinoxaline system improved the antineoplastic activity. Derivatives **ISQO**, with the exception of **ISQOe**, were particularly effective against leukaemia subpanels; in fact the calculated pGI_{50} mean value is higher than that of the overall cell lines.



NCI ID	No. of the cell lines investigated	No. of the cell lines giving positive pGI_{50}[b]	pGI_{50}	
			Range	MG_MID[c]
ISQOa	57	57	6.28-4.60	5.17
ISQOb	57	57	>8.00-4.51	5.27
ISQOc	57	57	>8.00-4.42	7.32
ISQOd	56	56	6.09-4.48	4.74
ISQOe	57	57	5.80-4.60	4.99

[a] Data obtained from NCI *in vitro* disease-oriented human tumour cells screen.
[b] pGI_{50} is the –log of the molar concentration that inhibits 50% net cell growth.
[c] MG_MID= mean graph midpoint= arithmetical mean value for all tested cancer cell lines. If the indicated effect was not attainable within the used concentration interval, the highest tested concentration was used for the calculation.

Table 1

The mean graph of the most active compound (table 2), shows that 60% of the treated cell lines are sensitive to compound **ISQOc** at nanomolar concentration. The most sensitive subpanels are leukaemia and colon cancer, for which the average value of pGI_{50} is 8.00.

Interestingly, also the TGI of some cell lines reaches the nanomolar level and in some cell lines the LC_{50} was measured in the low micromolar range.

The antiproliferative effects of isoindolo-quinoxaline derivatives were also evaluated in three human cancer cell lines overexpressing P-glicoprotein drug efflux and showed to be equally resistant to vinblastine, doxorubicin and sublines resistant to different drugs.

In order to clarify the mechanism(s) of action of isoindoloquinoxaline, the effects of different concentration of the most active compound **ISQOc** on cell cycle progression of Jurkat cells (pGI_{50} =8.39) after 24 h of drug exposure were studied.

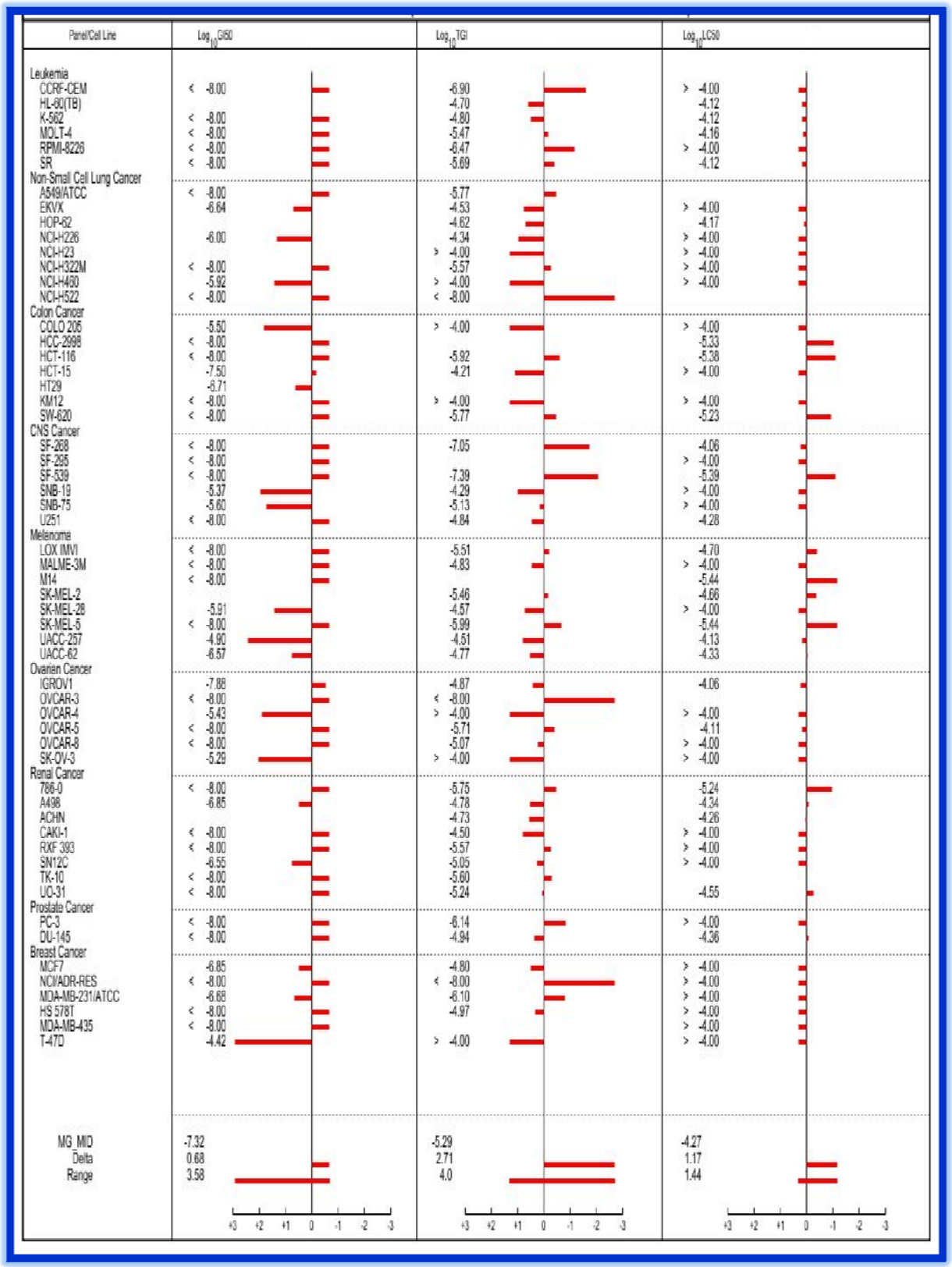


Table 2 Mean graph of compound **ISQOc**

Treatment of Jurkat cells with **ISQOc** at the concentration of 500 nM led to profound changes of the cell cycle profile (fig.1).

Untreated cells showed a classical pattern of proliferating cells proportionally distributed in G1 (48%), S (35%) and G2/M (16%) phases.

On the contrary, a clear G2/M arrest pattern was observed with a concomitant decrease of G1 phase.

It was also interesting to note the appearance of a hypodiploid peak (sub-G1) indicative of apoptosis, which reached the value of 35% at the concentration of 62.5 nM.

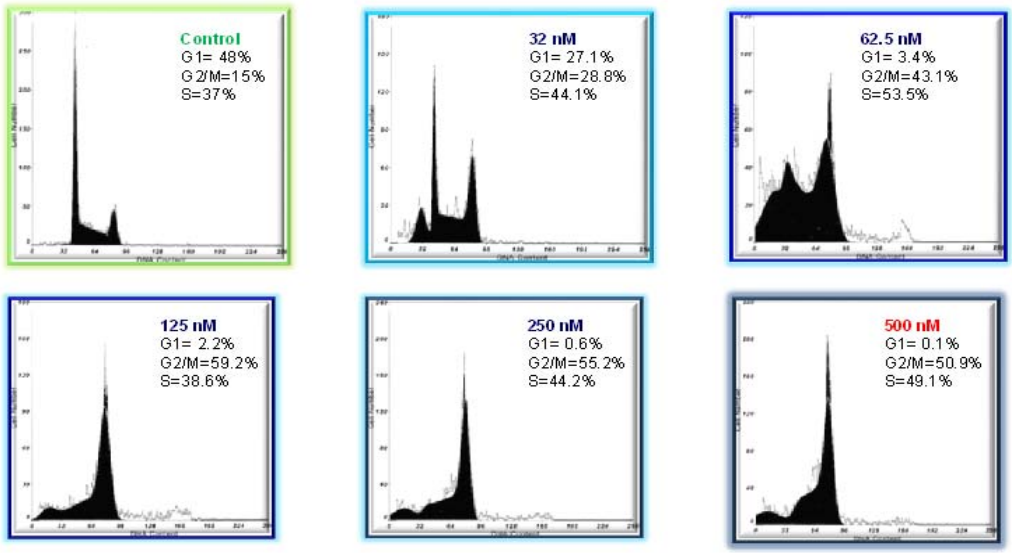


Fig.1

Compound **NSC 734237 (ISQOc)** was also checked by immunofluorescence microscopy to study possible interactions with microtubules.

For these experiments was used a tumor cell line of small lung carcinoma (A-549) which was previously demonstrated to be sensible to these compounds, the microtubule network in control cells exhibited normal organization and arrangement.

On the contrary, compound **NSC 734237 (ISQOc)**, at different concentrations (5 μM and 2.5 μM) disrupted the tubulin network.

Cells showed an evident characteristic "rounded up" morphology caused by disaggregation of microtubule in both interphase and mitotic phases after 24 h of

treatment.

These effects resemble that of vinblastine chosen as reference compound.

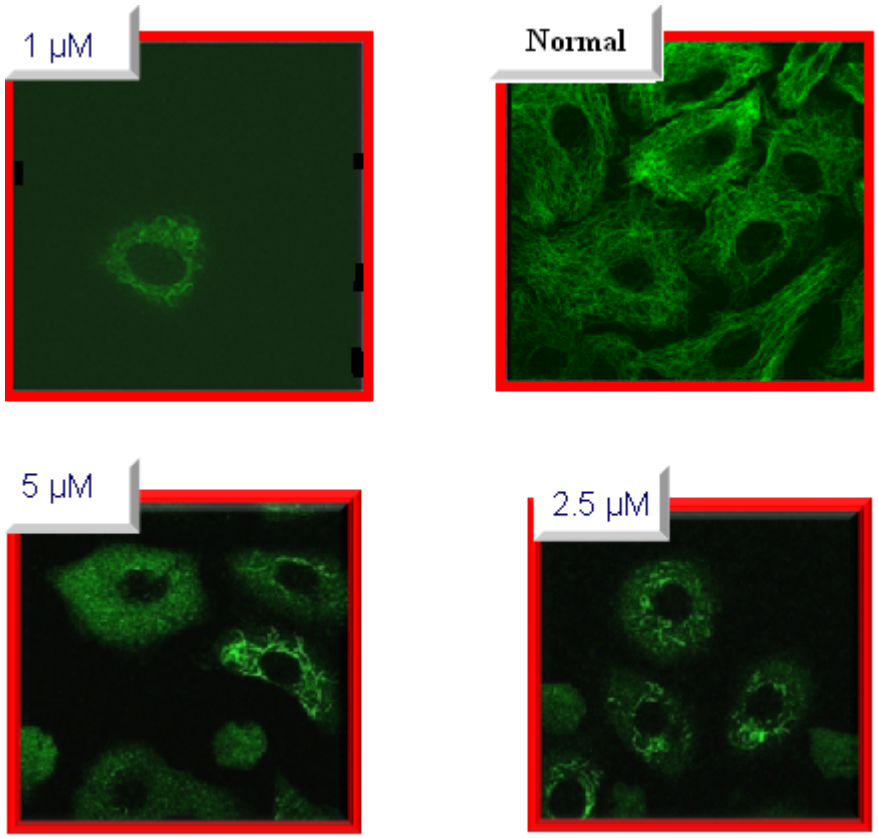


Fig.2

Immunofluorescence microscopy showed that **ISQOc** after 24 h of incubation completely disrupted the tubulin network. Polymerization was followed by fluorescent enhacement because of incorporation of a fluorescent reported into the microtubules as polymerization occurred. **ISQOc** investigated clearly induce a concentration dependent inhibition of tubulin polymerization (fig.2). In particular a complete inhibition of microtubule assembly was observed for **ISQOc** at a concentration similar to that of colchicine

By using DNA relaxation assay, it was determined that isoindoloquinoxalines inhibited topoisomerase I catalytic activity but not topoisomerase II activity.

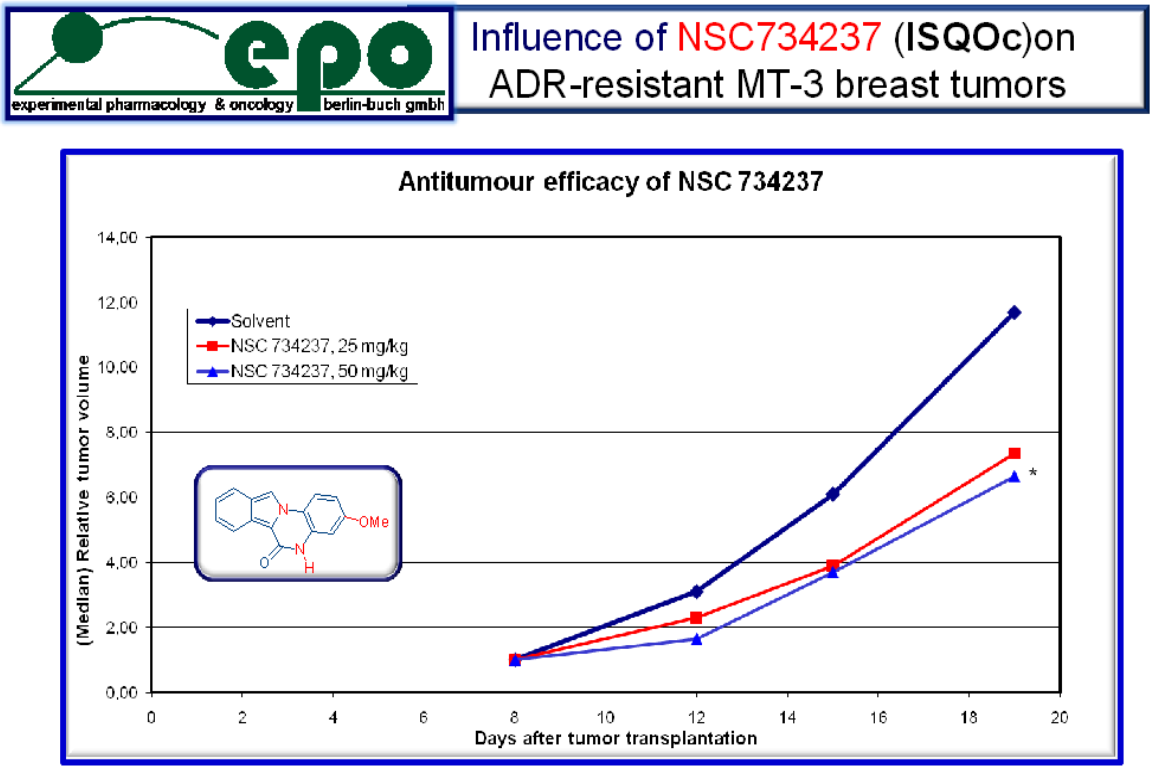
Further studies showed that **ISQOc** is a potent inducer of apoptosis in Jurkat cell line. The induction of apoptosis is associated with dissipation of the mitochondrial transmembrane potential, production of reactive oxygen species and cardiolipin

oxidation and activation of caspase-3 and caspase-9.

Due to these really interesting data the Drug Discover Committee of the European Organization of Research and Treatment of Cancer decided to start the in vivo tests on compound **ISQOc**.

The influence of **ISQOc** on ADR-resistant MT-3 breast tumors was also investigate.

For this study the ADR-resistant, human breast carcinoma MT-3 grown as xenograft in nude mice was used. Mice (eight) bearing palpable MT-3/ADR tumors were treated 5-times weekly, for 2 weeks intra peritoneal with 25 or 50 mg/kg of ISQ3 or with the solvent (DMSO). Results showed that compound **ISQOc** induced a dose-dependent and significant tumor growth inhibition accompanied by a moderate and dose-dependent body weight reduction (fig.3).



1

Fig.3

The expression of CD31 and Ki-67 was also investigated by immunohistochemistry.

ISQOc showed significant anti-angiogenic effects in MT-3/ADR xenografts. Except from micro vessel number a significant reduction of micro vessel ratio and size was

even found for decreased in tumor treated with 50 mg/kg **ISQOc**. The expression of the proliferation marker Ki-67 was also significantly diminished due to treatment with **ISQOc**. This effect was not dose-dependent and found for both doses (fig.4). These findings are concordant with the observed anti-tumoral efficacy of **ISQOc** in MT-3/ADR xenografts.

Isoindole quinoxalines represent potential compounds for the therapy of cancer (probably including such tumours with acquired resistance to conventional cytostatics) with anti-angiogenic and anti-proliferative activity even at low doses and after a relatively short treatment period.

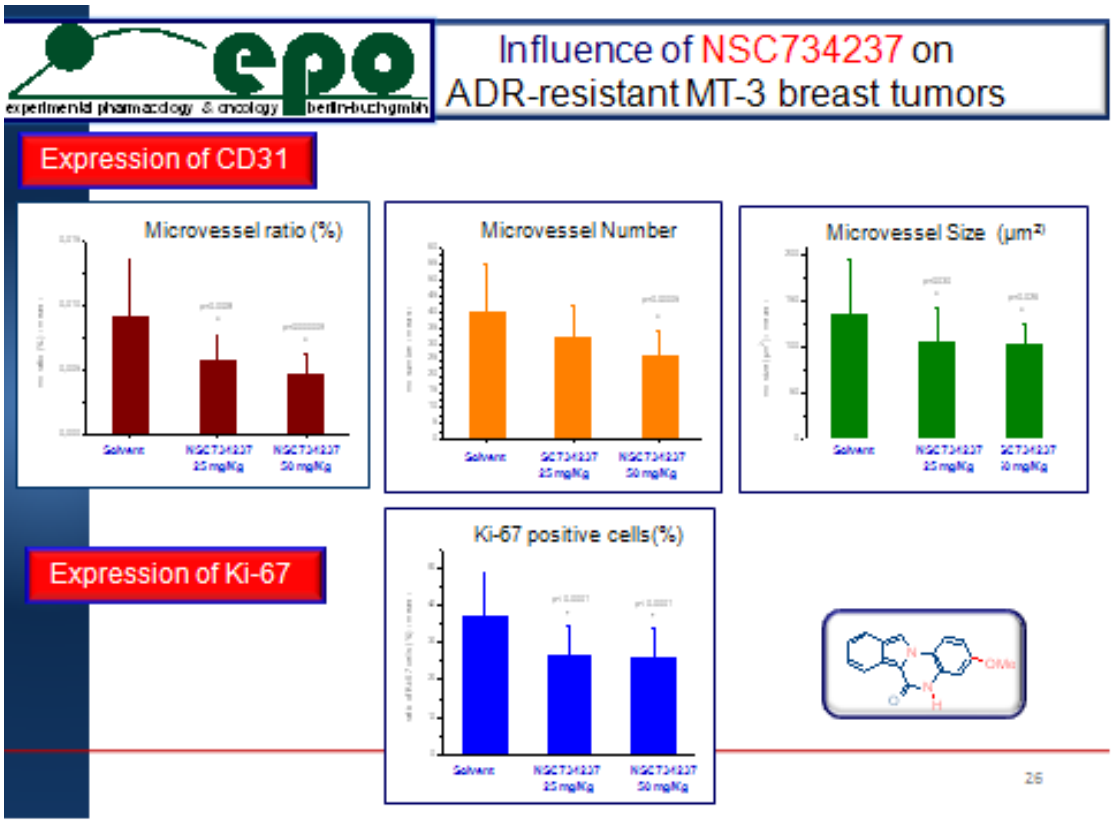


Fig.4

Molecular modeling

Considering the interesting results obtained for **ISQOa-e**, preliminary molecular modeling studies were performed in order to investigate the ability of new quinoxalines to bind Topoisomerase 1 and α, β tubulin, the two principal targets of **ISQO** compounds. To build the modeling, docking studies on literature compounds similar to **ISQO**, with antitubuline activity and with topoisomease 1 inhibition, were performed and the model was applied on new synthesized compounds.

1.Tubulin model

Colchicine is a tubulin assembly inhibitor that interacts with α, β tubulin dimers at distinct interfaces between the two subunits. These interactions cause microtubule destabilization and subsequent cellular apoptosis.

In 2004, Ravelli et al. reported the structure of tubulin in complex with DAMA-colchicine and with the stathmin-like domain (SLD) of RB3 (PDB 1SA0), at 3.5 Å resolution [04NAT198]. In this structure the colchicine binding site is located at the α, β interface (Fig.5).

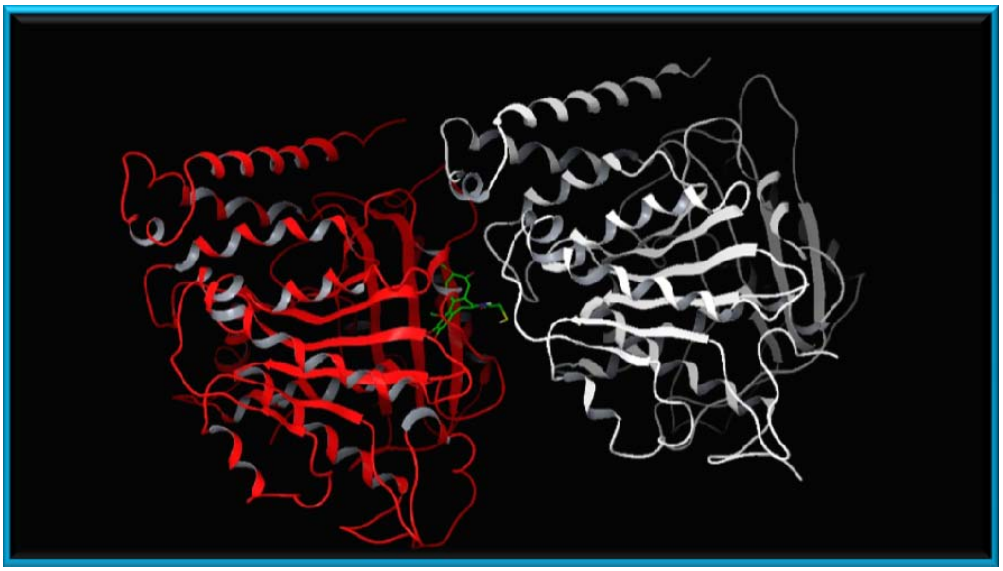


Fig.5

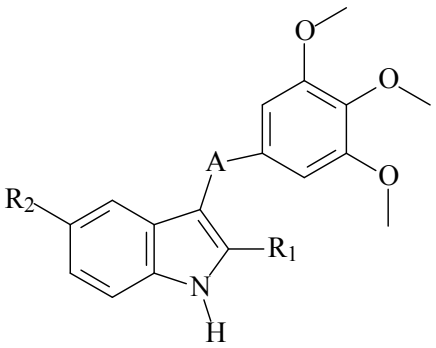
Strands S8 and S9, loop T7 and helices H7 and H8 of β-tubulin surround DAMA-colchicine, which also interacts with loop T5 of the neighbouring α –subunit. This structure was selected to dock new isoindole quinoxaline and **ISQO** compounds and it was useful in other works present in literature [08JMC4620], [05JMC6107], [09BMC6279].

a) Docking on literature compounds

To validate the model, structures with antitubulinic activity for which are reported IC_{50} (μM) for tubulin inhibition were docked [09JMC7512]. These structures are similar to **ISQO.** They have an indole portion linked to an aromatic portion with different substituents.

A number of 43 compounds with a molecular scaffold **I**, were docked using different conditions: using standard conditions, minimum sized Glide box, using different constraints for hydrogen bonds as VAL 181, ASN 101 and CYS 241.

The best docking was that one in which CYS 241 was used as constraint. The 77% of compounds bind the site through hydrogen bonds between indole NH and THF 179 residue and between -OMe groups and CYS 241 (Fig.6-7).



Scaffold I

The rest of other compounds bind the site orienting the indole portion in a different way. This is probably due to the free rotation around A atom (Fig.8).

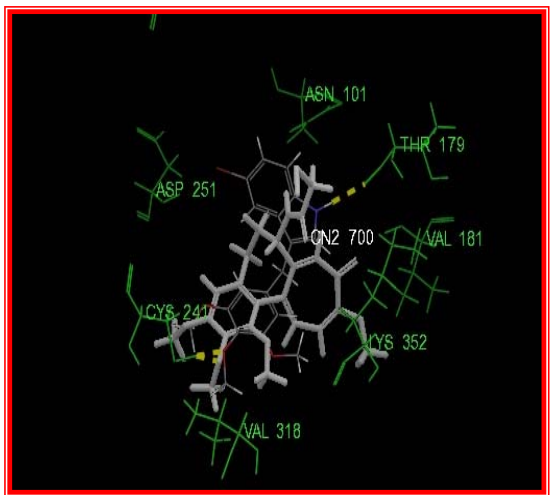


Fig.6

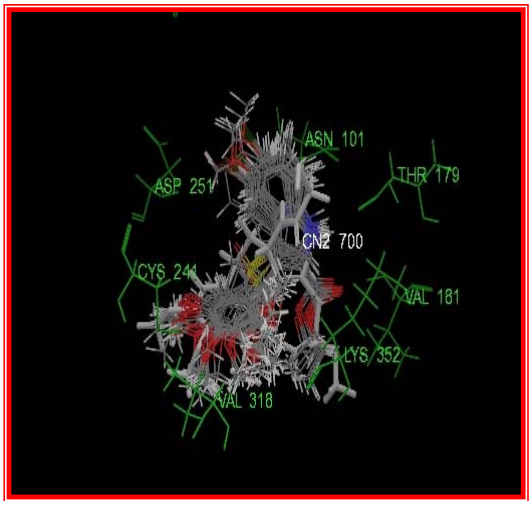


Fig.7

Although most compounds bind the site in the same way and are good fitted, a correlation between IC_{50} and docking score was not found. This is probably due to a imperfect relationship between tubulin inhibition and inhibition of colchicine binding as data reported show [09JMC7512].

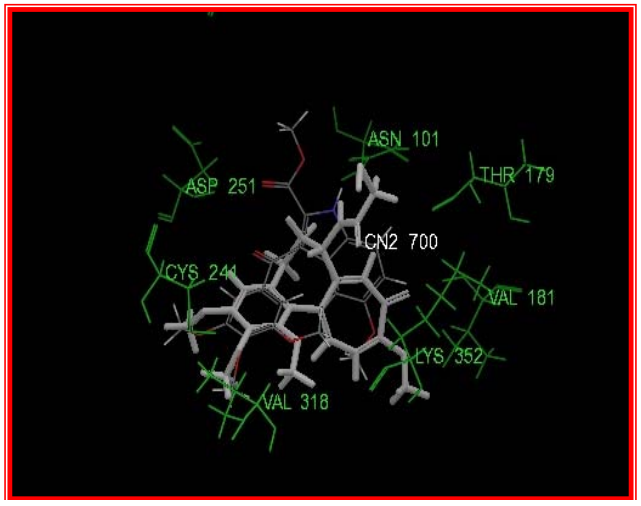


Fig.8

b) Docking on ISQ compounds

The same protein structure 1SA0 was used to dock quinoxaline compounds. Also in this case, structures were docked in different conditions: using standard conditions, minimum sized glide box, maximum sized glide box and using THF 179, VAL 181, ASN 101, and CYS 241 as constraints. A number of 28 compounds were docked.

The best docking was that one in which VAL 181 was used as constraint. Compounds bind the active site depending on their structural features.

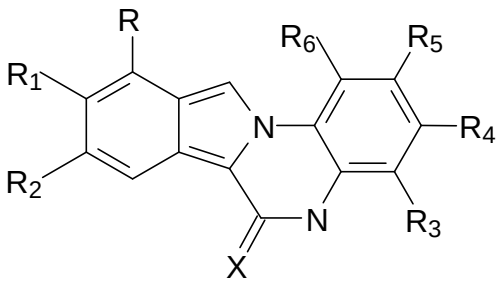


Fig.9

The docked compounds can be divided into 2 groups depending on their poses.

The first group includes compounds with high glide score in which C=X group is involved in H-bonds with VAL 181 (Fig.10.1). Belong to this group compounds bearing the methoxyl group on the quinoxaline potion in different position from R_4.

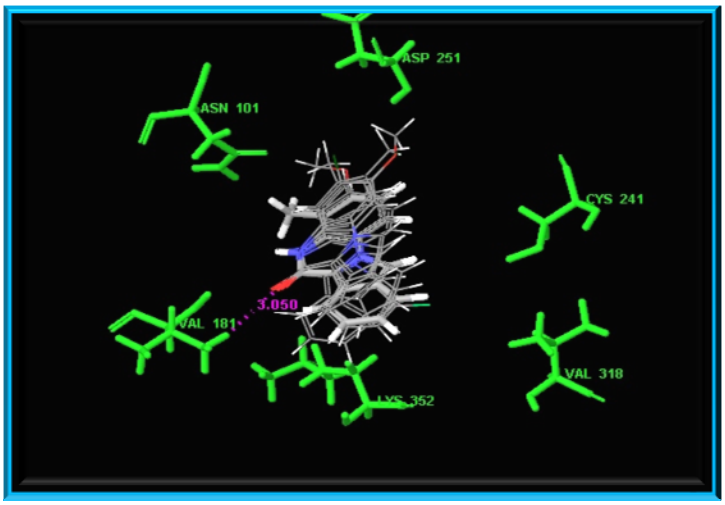


Fig.10

The second group includes compounds with medium-high glide score in which R_4 group is involved in H-bond with VAL 181 and R_1 is near to CYS 241 residue (Fig.11). Belong to this group compounds bearing methoxy or hydroxy group in R_4; Hydroxyl derivatives showed higher docking score values compared to the corresponding methoxyl derivatives

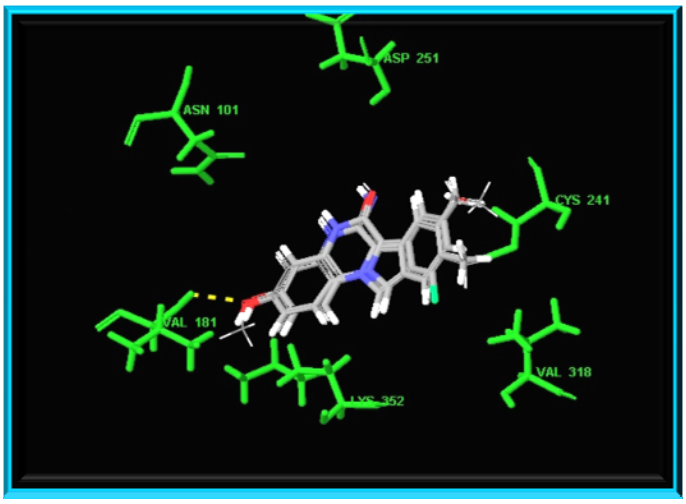


Fig.11

Only for some **ISQO** docked compounds, are known pGI_{50} values [08JMC2387]. For most of these compounds a correlation between glide score and pGI_{50} was found but not for all. This is due to pGI_{50} values that are related to both antitubuline and anti-Topoisomerase 1 activity.

According to the previous docking and to data reported in literature, compounds that bind Val 181 residue and interact in the same time with CYS 241 residue should be more active.

Comparing results, in both docking we can notice that the aromatic portion is often oriented to the CYS 241 residue and this feature could be responsible to confer better antitubuline activity.

ISQ	Sustituents	Docking score
5m	X=O, R_1=R_2=Me,R=R_3=R_5=R_6=H R_4=OH	-7,616457
5q	X=O, R=F R_1=R_2=R_3=R_5=R_6=H R_4=OH	-7,530704
5p	X=O, R=R_1=R_3=R_5=R_6=H R_2=OMe R_4=OH	-7,518082
5g	X=O, R=R_3=R_5=R_6=H R_1=R_2=OMe R_4=OMe	-7,446021
4m	X=NH, R=R_3=R_5=R_6=H R_1=R_2=Me R_4=OH	-7,414586
4q	X=NH, R=F, R_1=R_2=R_3=R_5=R_6=H, R_4=OH	-7,311471
5o	X=O, R=R_2=R_3=R_5=R_6=H R_1=OMe R_4=OH	-7,169742
5k	X=O, R=R_1=R_2=R_3=R_4=R_6=H R_5=OMe	-7,142603
4o	X=NH, R=R_2=R_3=R_5=R_6=H R_1=OMe R_4=OH	-7,072346
5j	X=O, R=R_1=R_3=R_5=R_6=H R_2=OMe R_4=OMe	-7,043955
5n	X=O, R=R_3=R_5=R_6=H R_1=R_2=OMe R_4=OH	-7,035969
5f	X=O, R_1=R_2=Me,R=R_3=R_5=R_6=H R_4=OMe	-6,799753
5h	X=O, R=F, R_1=R_2=R_3=R_5=R_6=H R_4=OMe	-6,622998
ISQOb	X=O, R=R_1=R_2=R_3=R_5=R_6=H R_4=R_5=Me	-6,254501
5i	X=O, R=R_2=R_3=R_5=R_6=H R_1=OMe R_4=OMe	-6,203328
ISQOe	X=O, R=R_1=R_2=R_3=R_5=R_6=H R_4=R_5=Cl	-6,014557
ISQOc	X=O, R=R_1=R_2=R_3=R_5=R_6=H R_4=OMe	-5,887919
5l	X=O, R=R_1=R_2=R_4=R_5=R_6=H R_3=R_6=OMe	-5,815474
ISQOd	X=O, R=R_1=R_2=R_4=R_5=R_6=H R_3=OMe	-5,803996
ISQOa	X=O, R=R_1=R_2=R_3=R_4=R_5=R_6=H	-5,434076

Table 3

2.Topoisomerase 1 model

DNA-topoisomerase 1 is an important enzyme responsible to relax supercoiled DNA for transcription, replication, and mitosis [98SCI1504].

The X-ray ternary human Topoisomerase 1-DNA- Topotecan structure (fig.12) isolated by Staker and co-workers [02PNAS15387] shows that Topotecan intercalates at the site of DNA cleavage and bind the enzyme through H-bond with ASP533 residue.

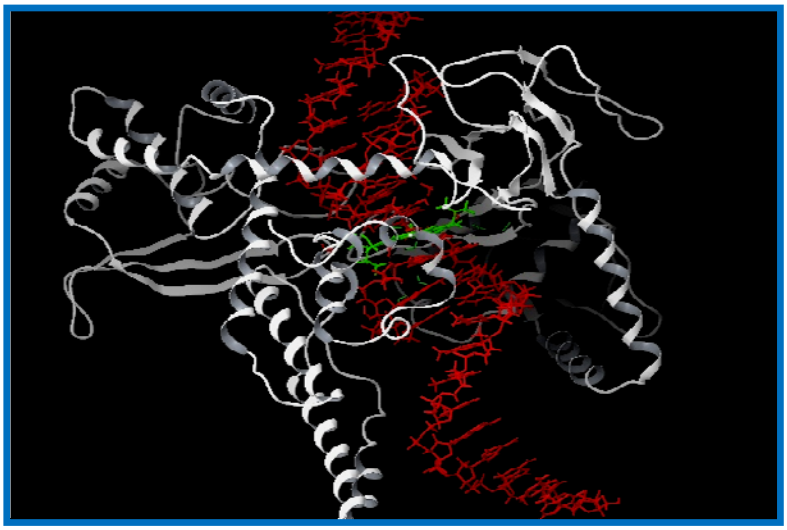


Fig.12

Because of structural analogy with **ISQO** compounds this X-ray structure with PDB code 1K4T was used for docking.

a) Docking on literature structures

To validate the model, structures taken from two different articles [08JMC4609], [06BMCL4395] (group A and B) and with anti-Topoisomerase 1 activity for which are reported qualitative data for topoisomerase 1 inhibition, were docked.
They are very similar to **ISQO** compounds. They contain an aromatic planar system in which a quinoxalinone portion is present as **ISQO** compounds.
The structures were docked in different conditions: using standard conditions, using compound protonated forms, neutral forms, using minimum and maximum sized glide box, using ARG 364 as constraint.
The best docking for group A was that one in which constraint ARG 364 and neutral ligands were used. A total number of 20 compounds were docked. Some molecules (12 of 20 compounds) respond to one way of binding in which pyridine N is oriented and interacts with H-bond with ARG 364. Compounds that no respond to this pose are the ones that have not electron pair available on this atom. Comparing structures with TTG docked structure we can see a very good superimposition (Fig.13).

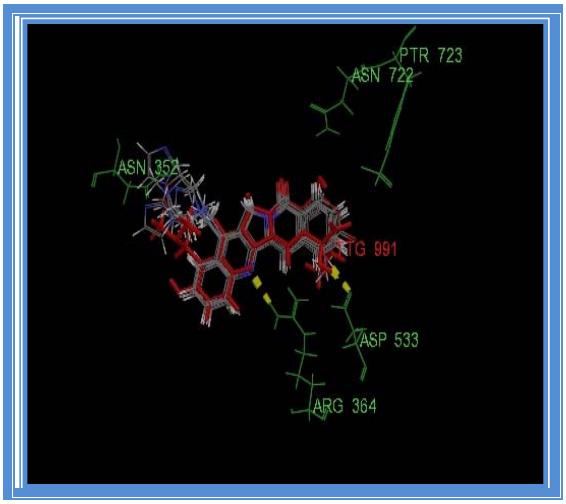


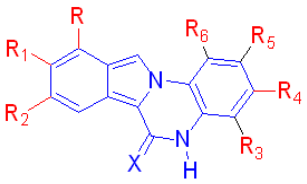
Fig.13

The best docking for group B was that one in which a minimum sized glide box and no ligand protonated forms were used. A total number of 17 compounds were docked.
All molecules interact with ARG 364 through H-bonds but orienting the planar system in different ways.
10 of 17 compounds bind the active site interacting through H-bond between C=O group of five-membered ring and ARG 364 (Fig.14).

b) Docking on **ISQO** compounds

The same protein X-ray structure 1K4T was used for **ISQO** compounds docking.
Also in this case all structures were docked using different conditions: using minimum and maximum sized Glide box, using standard condition and using ARG 364 as constraint.
The best docking was that where ARG 364 was used as constraint.
All compounds bind the active site in very similar ways interacting through C=X group with ARG 364. All molecules are in the same plane but additional interactions are responsible to orient molecules in different ways.

ISQ	Substituents	Docking score
4o	X=NH, R=R_2=R_3=R_5=R_6=H R_1=OMe R_4=OH	-9,684861
5o	X=O, R=R_2=R_3=R_5=R_6=H R_1=OMe, R_4=OH	-9,627385
5n	X=O, R=R_3=R_5=R_6=H R_1=R_2=OMe, R_4=OH	-9,181279
5p	X=O, R=R_1=R_3=R_5=R_6=H R_2=OMe, R_4=OH	-9,142769
5q	X=O, R=F, R_1=R_2=R_3=R_5=R_6=H, R_4=OH	-9,001540
4q	X=NH, R=F, R_1=R_2=R_3=R_5=R_6=H, R_4=OH	-8,993757
5m	X=O, R_1=R_2=Me,R=R_3=R_5=R_6=H, R_4=OH	-8,910252
4m	X=NH, R=R_3=R_5=R_6=H R_1=R_2=Me, R_4=OH	-8,876916
SQOc	X=O, R=R_1=R_2=R_3=R_5=R_6=H, R_4=OMe	-8,799884
5l	X=O, R=R_1=R_2=R_4=R_5=H, R_3=R_6=OMe	-8,760821
SQOb	X=O, R=R_1=R_2=R_3=R_6=H R_4=R_5=Me	-8,510735
5k	X=O, R=R_1=R_2=R_3=R_4=R_6=H R_5=OMe	-8,329139
5f	X=O, R_1=R_2=Me,R=R_3=R_5=R_6=H, R_4=OMe	-8,288526
5h	X=O, R=F, R_1=R_2=R_3=R_5=R_6=H, R_4=OMe	-8,232042
5j	X=O, R=R_1=R_3=R_5=R_6=H R_2=R_4=OMe	-8,212415
SQOd	X=O, R=R_1=R_2=R_4=R_5=R_6=H, R_3=OMe	-8,155822
SQOa	X=O, R=R_1=R_2=R_3=R_4=R_5=R_6=H	-8,078321
SQOe	X=O, R=R_1=R_2=R_3=R_6=H R_4=R_5=Cl	-8,002569
5i	X=O, R=R_2=R_3=R_5=R_6=H R_1=R_4=OMe	-7,957684
5g	X=O, R=R_3=R_5=R_6=H R_1=R_2=R_4=OMe	-6,261761



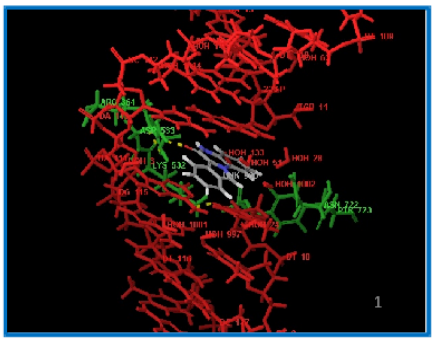


Table 4

c) Comparing results

Comparing docking for ISQ compounds and literature compounds we can see how different structures are able to bind the active site in similar way interacting with ARG 364 residue. Also on this case compounds bearing hydroxyl group in para position had higher docking score value than the corresponding methoxyl derivatives.

AIM OF THE WORK

The aim of my work, considering the interesting results obtained by **ISQOc**, was the synthesis of new tetracyclic systems containing the pyrrole ring in order to study:

1 how the methoxy group position, on the quinoxaline moiety, can influence the anticancer activity;

2 how different substituents on the isoindole ring can influence the anticancer activity maintaining the methoxy group in *para* position;

3 how modifications on isoindole quinoxaline ring system can led to new more hydrophilic compounds reducing the use of DMSO in biological assays. In particular:

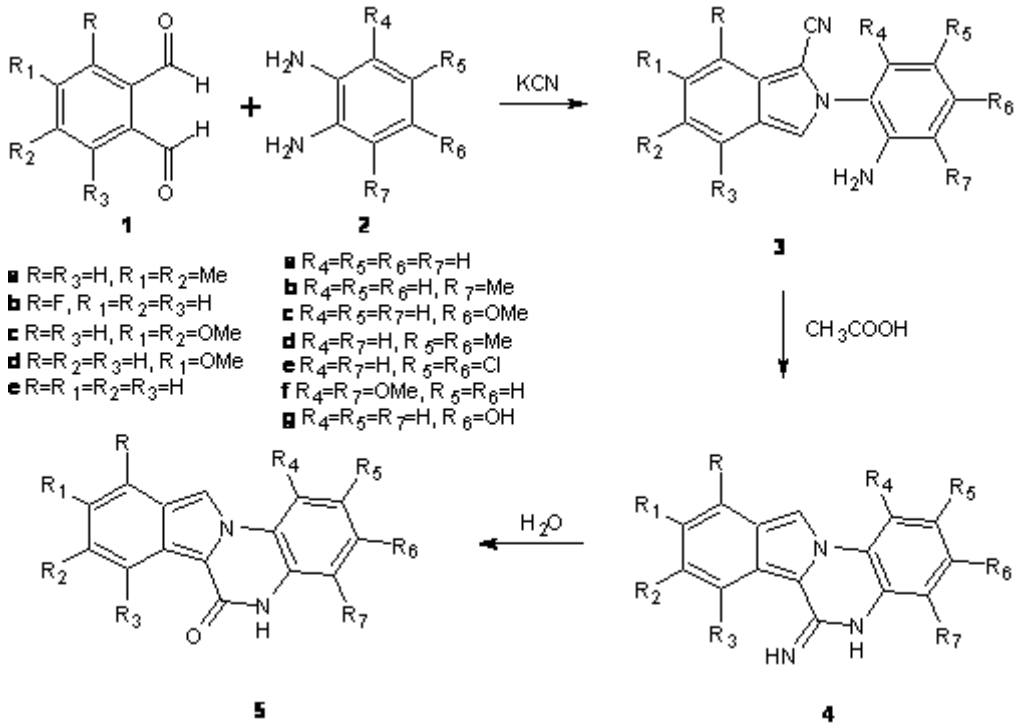
a) it was introduced an hydroxyl group in para position on the aromatic portion and it was studied how different substituents on isoindole ring can influence the anticancer activity;

b) it was introduced a N atom on the isoindole ring that could be protonated at physiological pH and it was studied how different substituents on aromatic portion can influence the anticancer activity;

c) it was introduced a N atom on the aromatic portion that could be protonated at physiological pH and it was studied how different substituents on isoindole ring can influence the anticancer activity;

d) were isolated the more hydrophilic imino forms of the corresponding quinoxalinones compounds synthesized.

SYNTHESIS



Compounds	Subtituents
3,4a	R= R_1=R_2=R_3=R_4=R_5=R_6=R_7=H;
3,4b	R= R_1=R_2=R_3=R_4=R_5=H; R_7=Me;
3,4c	R= R_1=R_2=R_3=R_4=R_5=R_7=H; R_6=OMe;
3,4d	R= R_1=R_2=R_3=R_4=R_7=H;R_5=R_6=Me;
3,4e	R= R_1=R_2=R_3=R_4=R_7=H;R_5=R_6=Cl;
3,4,5f	R=R_3=R_4=R_5=R_7=H; R_1=R_2=Me; R_6=OMe;
3,4,5g	R=R_3=R_4=R_5=R_7=H; R_1=R_2=R_6=OMe;
3,5h	R_1=R_2=R_3=R_4=R_5=R_7=H; R=F;R_6=OMe;
3,5i	R=R_2=R_3=R_4=R_5=R_7=H; R_1=R_6=OMe;
3,5j	R=R_1=R_3=R_4=R_5=R_7=H; R_2=R_6=OMe;
3,4,5k	R=R_1=R_2=R_3=R_4=R_7=H; R_5=OMe;
3,4,5l	R=R_1=R_2=R_3=R_5=R_6=H; R_4=R_7=OMe;
3,4,5m	R=R_3=R_4=R_5=R_7=H; R_1=R_2=Me;R_6=OH;
3,4,5n	R=R_3=R_4=R_5=R_7=H; R_1=R_2=OMe;R_6=OH;
3,4,5o	R=R_2=R_3=R_4=R_5=R_7=H; R_1=OMe;R_6=OH;
3,5p	R=R_1=R_3=R_4=R_5=R_7=H; R_2=OMe;R_6=OH;
3,4,5q	R_1=R_2=R_3=R_4=R_5=R_7=H; R=F;R_6=OH;

Scheme 1

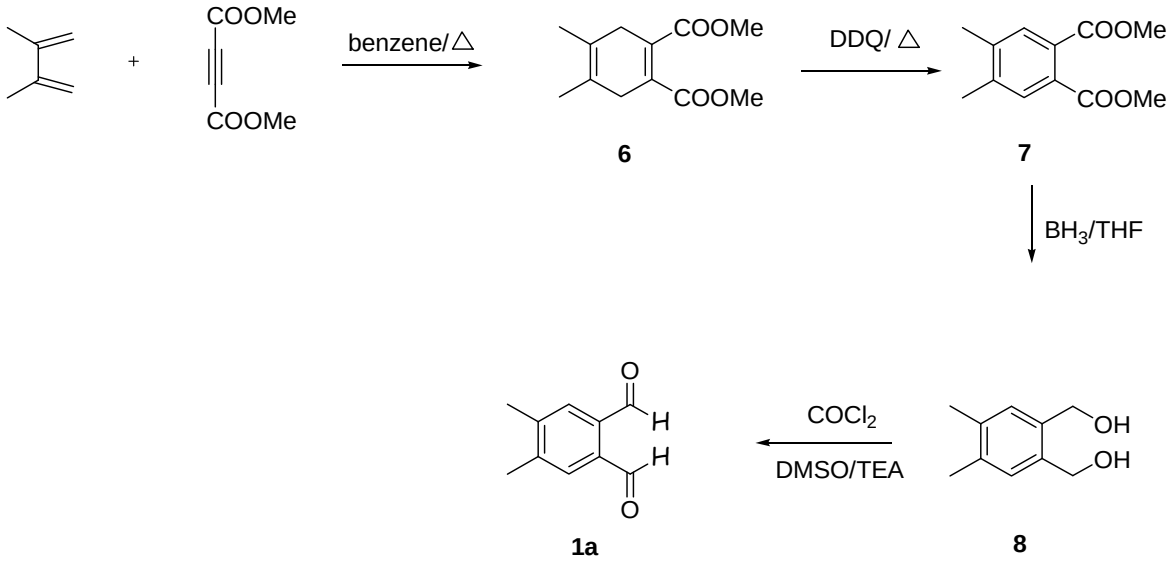
Considering the interesting results obtained in molecular docking studies, first it was planned to synthesize compounds in which the methoxyl group in para position in **ISQOc** compound was maintained in order to study how different substituents on isoindole portion can influence the anticancer activity and some compounds in which the methoxyl group position was changed.

It was also planned to synthesize new quinoxalines in which the methoxyl group in para position was replaced for an hydroxyl group in order to improve the solubility and to limitate the use of DMSO in biological screening. (Scheme 1)

Isoindole quinoxalines **4** and **5** were synthesized from 2-(2-aminoaryl)1-cyano-isoindoles **3**. These intermediates were prepared by a Strecker type synthesis between different phtaloyldicarboxaldehydes **1** and substituted o-phenylendiamines **2** in the presence of potassium cyanide.

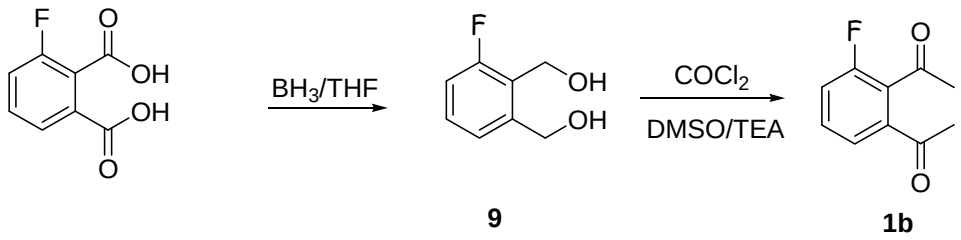
The not commercial available phthaloyldicarboxaldehydes were synthesized

Substituted fluoro-phthaloyldicarboxaldehyde and *ortho*- dimethyl-phthaloyldicarboxaldehyde were prepared by Swern oxidation of the corresponding dimethanols **8** and **9**. The reaction was carried out using oxalyl chloride activated by dimethyl sulfoxide and trietilamine as base in DCM at -78°C; the resulting phthaloyldicarboxaldehydes **1a** and **1b** were obtained in good yields (80-87%) [94S1035]. (Scheme 2-3)



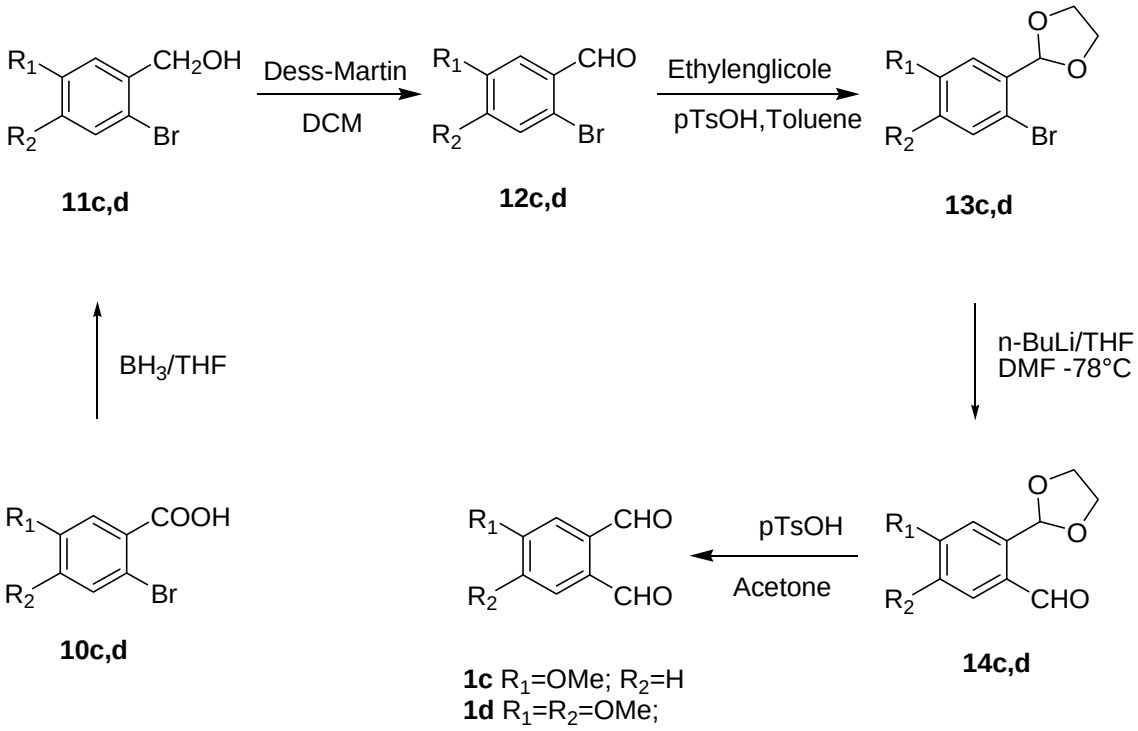
Scheme 2

Derivatives **8** and **9** were prepared in good yields (80-99%) from the reduction either the corresponding dimethyl-ester **7** or the fluoro-carboxylic acid commercial available using BH_3 in THF solution. The dimethyl-ester **7** was previously obtained by a Diels-Alder reaction from dimethyl acetylenedicarboxylate and 2,3-dimethylbuta-1,3-diene in benzene or toluene under reflux to obtain compound **6** in excellent yield (92%); it was then oxidized using DDQ in chlorobenzene (2,3—dichloro-5,6-dicyano-benzo-1,4-quinone). (Scheme 2-3)



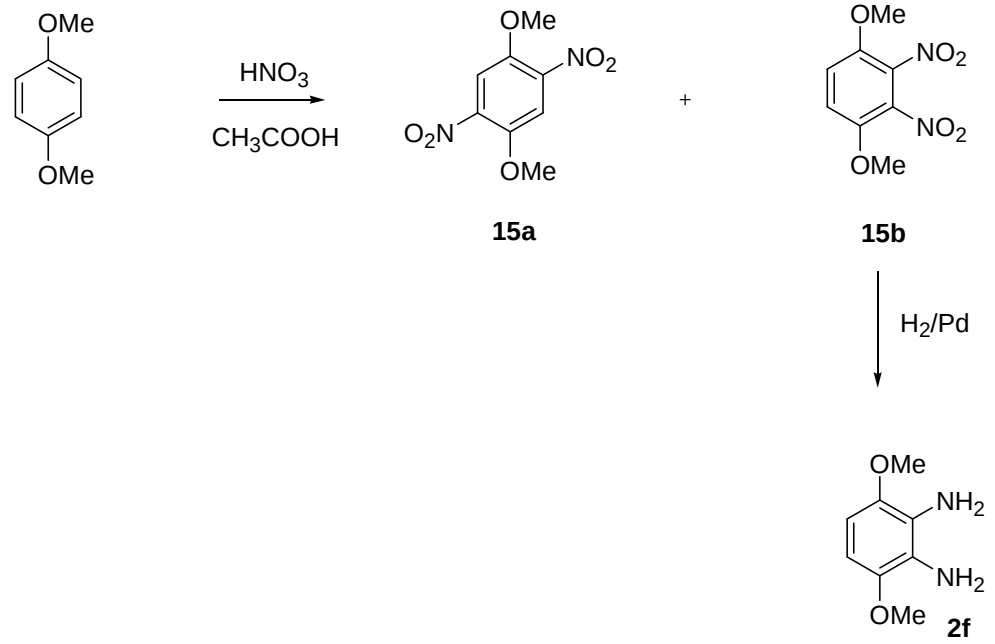
Scheme 3

Mono- and di-methoxy phthaloylcarboxaldehydes **1c** and **1d** were obtained in excellent yields (90-99%) from the suitable bromo-benzoic acids **10**. They were reduced using BH_3 solution in THF at O°C to the corresponding methanol derivatives **11c**. Subsequent Dess-Martin oxidation furnished the phthaloylcarboxaldehydes **12** in very good yields (90-98%). Aldehydes **12** were protected with ethylene glycol using Dean-Stark trap and compounds **13** were obtained in good yields (90%). Compounds **13** were formylated to compounds **14** using nBuLi in THF at -78°C and DMF and then deprotected at rt in acetone using p-TsOH to afford the corresponding phthaloylcarboxaldehydes **1c** and **1d**. (Scheme 4)



Scheme 4

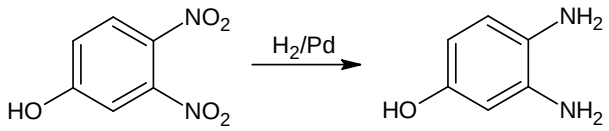
All phthaloylcarboxaldehydes, including the not substituted commercial available, were reacted with different phenilendiamine (**2a-g**); for the synthesis of 3,6-dimethoxy-1,2-phenylendiamine **2f**, the 1,4-dimethoxy-benzene was treated with acetic anhydride and nitric acid 70% at -15°C for few minutes.



Scheme 5

The resulting yellow precipitate, a mixture of the two isomers **15a-b**, was filtered off using acetic acid and purified in column. Isolated compound **15b,** it was reduced in methanol with Pd to the diamine **2f** . (Scheme 5)

The 3,4-diamino phenol **2g** was obtained from reduction of the corresponding dinitro compound using H_2/Pd and in methanol. (Scheme 6)



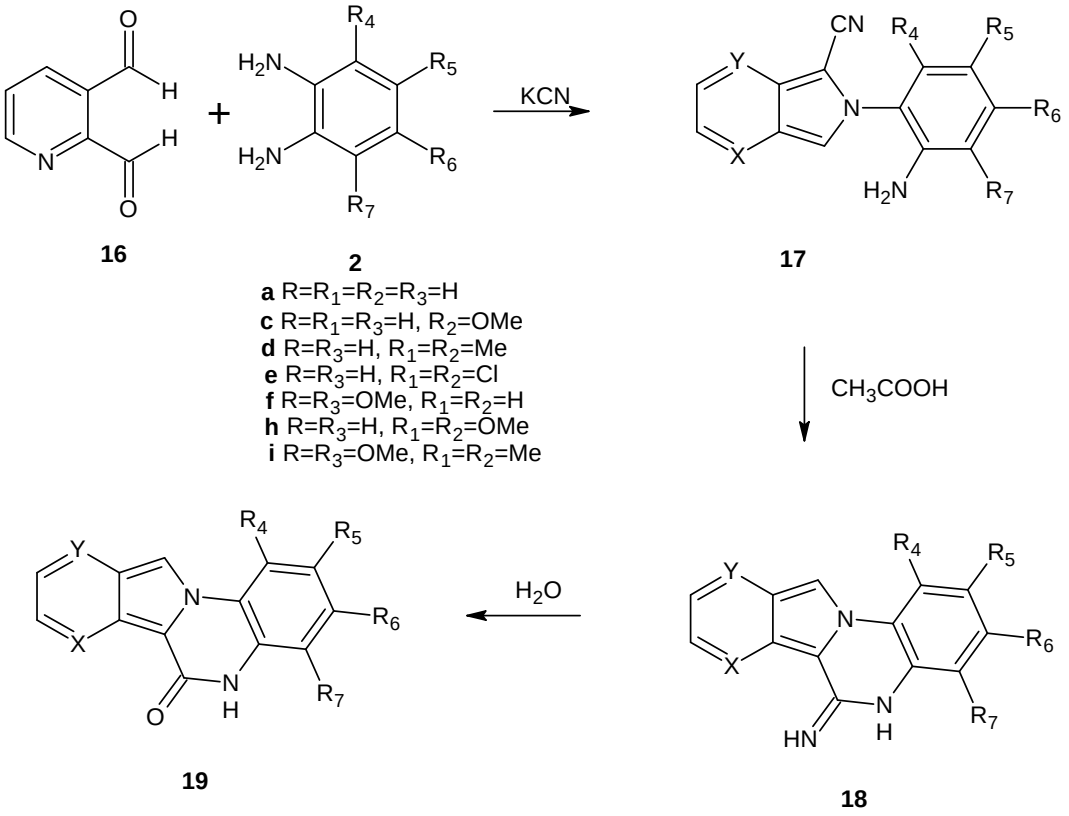
2g

Scheme 6

All the key intermediates were cyclized in acetic acid to give new isoindoloquinoxaline derivatives in good yields (**4a-q** and **5f-q**). (Scheme 1)

Then we planned to synthesize new tetracyclic systems introducing a nitrogen atom that protonated at physiological pH could improve quinoxalines solubility.

Were synthesized compounds **18a-m** and **19a** starting from a reaction between pyridine dicarboxy aldehyde **16** and different phenylendiamines **2** in the presence of potassium cyanide. In almost all cases a mixture of the two cyano intermediate isomers was obtained due to pyridine aldehyde asymmetry.



16

2

a R=R_1=R_2=R_3=H
c R=R_1=R_3=H, R_2=OMe
d R=R_3=H, R_1=R_2=Me
e R=R_3=H, R_1=R_2=Cl
f R=R_3=OMe, R_1=R_2=H
h R=R_3=H, R_1=R_2=OMe
i R=R_3=OMe, R_1=R_2=Me

17

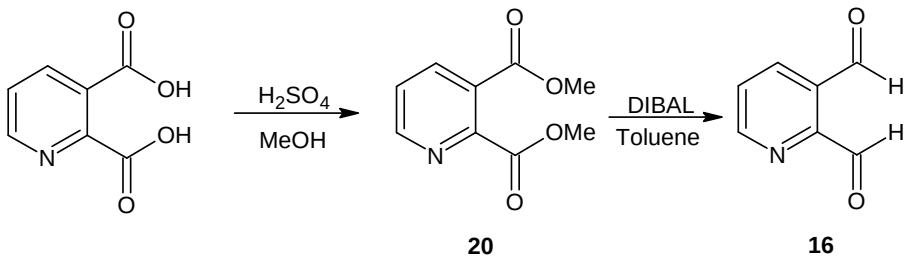
18

19

Compounds	Substituents
19a	R_4=R_7=H; R_5=R_6=Me; Y=CH; X=N
19b	R_4=R_7=H; R_5=R_6=Me; Y=N; X=CH
19c	R_4=R_7=H; R_5=R_6=Cl; Y=CH; X=N
19d	R_4=R_7=H; R_5=R_6=Cl; Y=N; X=CH
19e	R_4=R_7=OMe; R_5=R_6=H; Y=CH; X=N
19f	R_4=R_7=OMe; R_5=R_6=H; Y=N; X=CH
19g	R_4=R_7=H; R_5=R_6=OMe; Y=CH; X=N
19h	R_4=R_7=H; R_5=R_6=OMe; Y=N; X=CH
19i	R_4=R_7=OMe; R_5=R_6=Me; Y=CH; X=N
19j	R_4=R_7=OMe; R_5=R_6=Me; Y=N; X=CH
19k	R_4=R_5=R_6=R_7=H; Y=CH; X=N
19l	R_4=R_5=R_6=R_7=H; Y=N; X=CH
19m	R_4=R_5=R_7=H; R_6=OMe; Y=N; X=CH
18a	R_4=R_5=R_7=H; R_6=OMe; Y=N; X=CH

Scheme 7

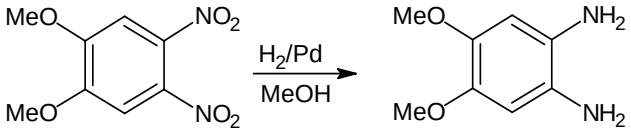
Pyridine-2,3-dicarbaldehyde **16** was prepared according to literature [68BSCF4117] from the corresponding carboxylic acid that was dissolved in methanol and a catalytic amount of sulfuric acid to led to compound **20**. Compound **20** was then reduced to dicarbaldehyde **16** using DIBAL in toluene at -78°C. (Scheme 8)



20 **16**

Scheme 8

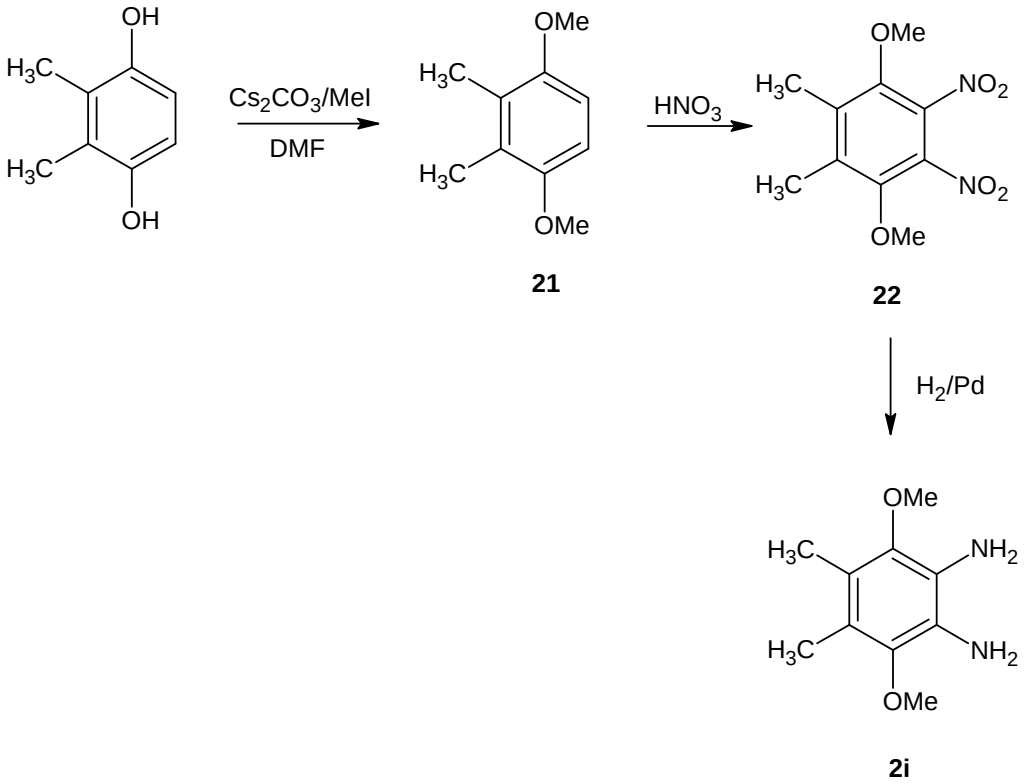
Pyridine-2,3-dicarbaldehyde was reacted with different phenylendiamines (**2a, 2c-f, 2h-i**). For the synthesis of 4,5-dimethoxy-o-phenylendiamine **2h**, not commercial available, the corresponding dinitro compound was reduced in methanol using H_2/Pd. (Scheme 9)



2h

Scheme 9

3,6-dimethoxy-4,5-dimethyl-phenylendiamine **2i** was synthesized from 2,3-dimethylbenzene-1,4-diolo that was methylated in DMF using Cs_2CO_3 as base in good yield (70%). Compound **21** was treated with nitric acid 70% sol. at -15°C and the resulting compound **22** , obtained in 80% yield, reduced in methanol to the diamine **2i** using H_2/Pd. (Scheme 10)

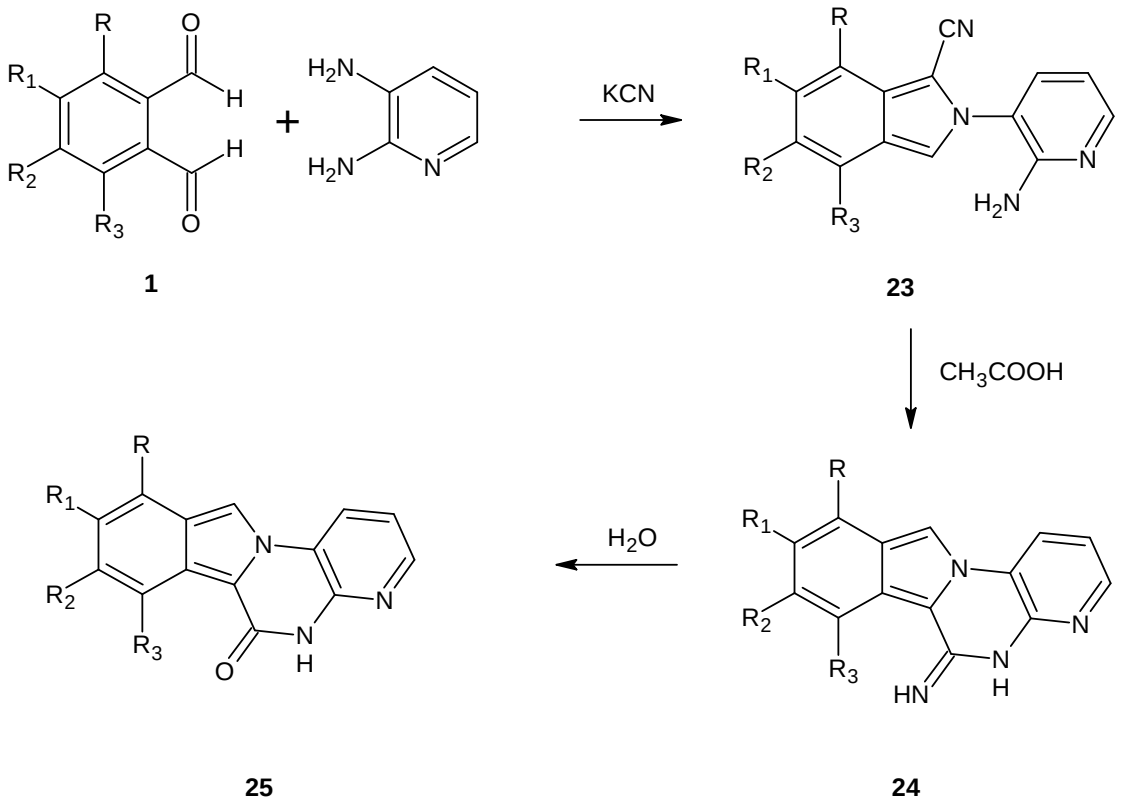


Scheme 10

All the key intermediates **17** were cyclized in acetic acid to give new aza-isoindoloquinoxaline derivatives (**18a-m** and **19a**) in good yields. (Scheme 7)

Then we also try to introduce a nitrogen atom on the aromatic portion to obtain the new pyrido-pyrazino-isoindole system of type **24** and **25.**

In this case different aldehydes **1** were reacted with pyridine-2,3-diamine to afford the intermediates **23** that were cyclized in acetic acid using a catalytic amount of DMF to led compound **24a-c** and **25a-c** in good yields. (Scheme 11)



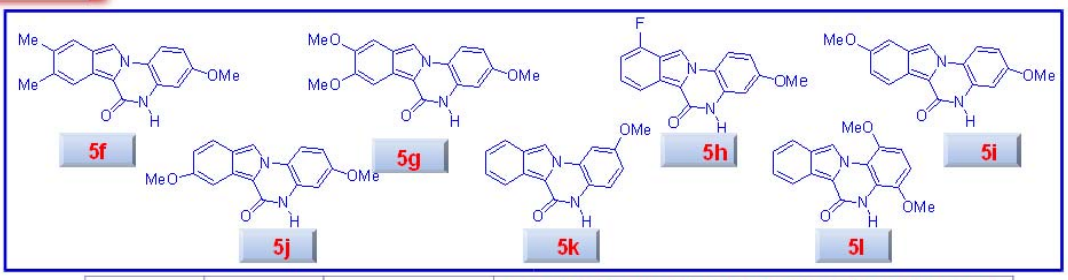
1 **23** **24** **25**

Compounds	Substituents
25a	$R=R_1=R_2=R_3=H$;
24a	$R=R_1=R_2=R_3=H$;
25b	$R=R_3=H$; $R_1=R_2=Me$;
24b	$R=R_3=H$; $R_1=R_2=Me$;
25c	$R=R_3=H$; $R_1=R_2=OMe$;
24c	$R=R_3=H$; $R_1=R_2=OMe$;

Scheme 11

BIOLOGICAL DATA

Compound **5f-l** were submitted to the National Cancer Institute of Bethesda (NCI) and tested in a full panel of about 60 different tumour cell lines.



NSC	Compd.	No. of the cell lines investigated	No. of the cell lines giving positive pGI_{50}[b]	pGI_{50}	
				Range	MG_MID[c]
747520	5f	56	56	>8.00-5.42	7.57
747526	5g	56	56	>8.00-4.81	7.75
747521	5h	54	53	7.86-4.37	6.95
747523	5i	54	54	>8.00-5.92	7.87
747522	5j	56	56	>8.00-5.98	7.70
747524	5k	46	46	7.14-5.70	6.38
747525	5l	58	58	7.14-5.64	5.91

Table 5

All the new derivatives resulted to be more potent than the lead compound **ISQOc**.

They showed potent antitumor activity against the total number of cell lines investigated with pGI_{50} mean values from 5.91 to 7.87.

The most active derivative was compound **5i**, whose pGI_{50} mean value was 7.87.

Is interesting to note that the two derivatives **5k** and **5l**, in which the methoxy group position was changed, are the less active although they showed pGI_{50} of 6.38 and 5.91 respectively (table 5).

In fig.15 is reported the mean graph of the most active compound **5i** (NSC 747523) whose GI_{50} value reached nanomolar level on 89% of the cell lines and 20% of the cell lines at TGI.

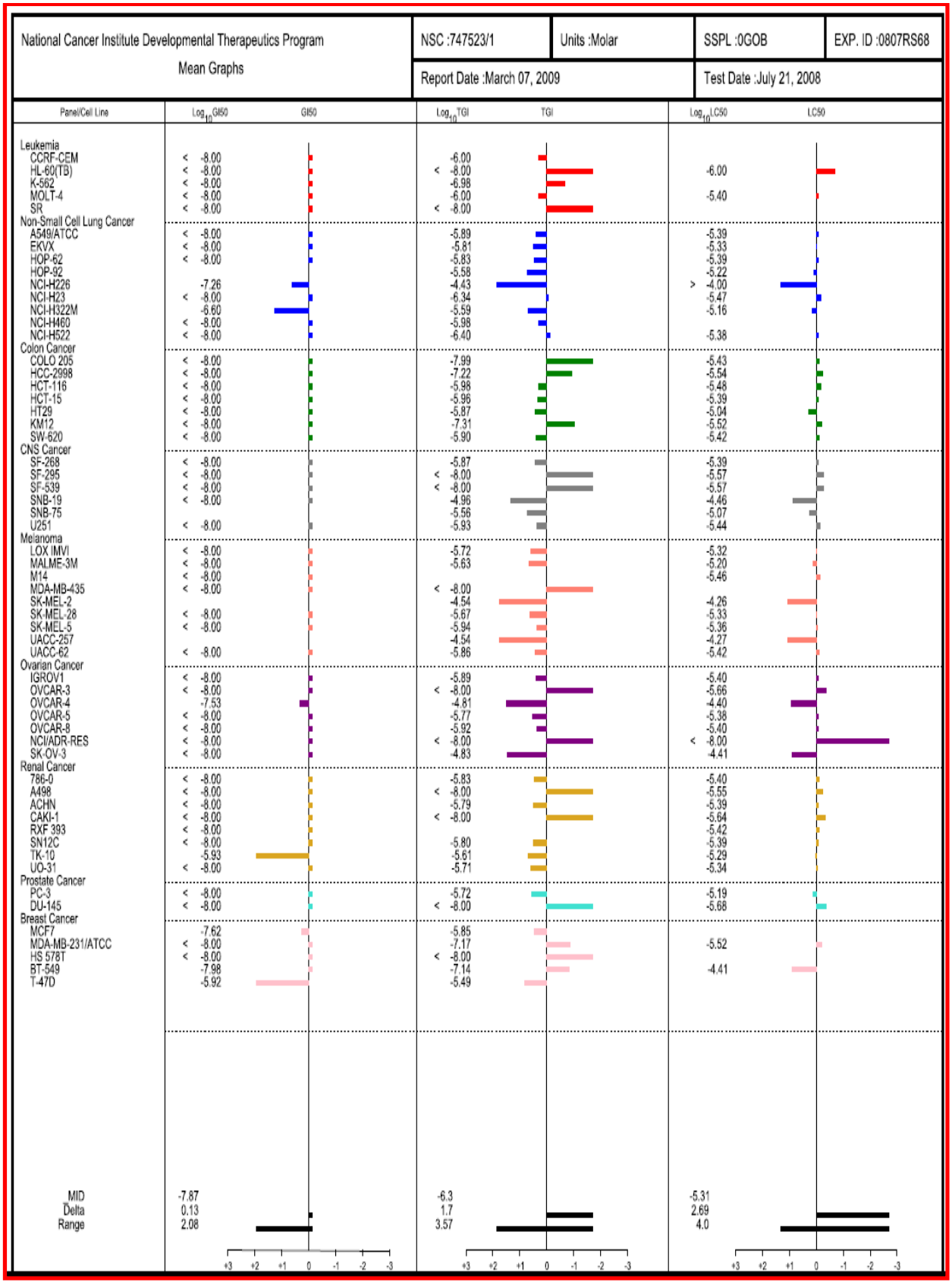

National Cancer Institute Developmental Therapeutics Program
Mean Graphs
NSC :747523/1
Units :Molar
SSPL :0GOB
EXP. ID :0807RS68
Report Date :March 07, 2009
Test Date :July 21, 2008
Panel/Cell Line
Log10GI50
GI50
Log10TGI
TGI
Log10LC50
LC50
Leukemia
CCRF-CEM < -8.00 -6.00
HL-60(TB) < -8.00 < -8.00 -6.00
K-562 < -8.00 -6.98
MOLT-4 < -8.00 -6.00 -5.40
SR < -8.00 < -8.00
Non-Small Cell Lung Cancer
A549/ATCC < -8.00 -5.89 -5.39
EKVX < -8.00 -5.81 -5.33
HOP-62 < -8.00 -5.83 -5.39
HOP-92 -5.58 -5.22
NCI-H226 -7.26 -4.43 > -4.00
NCI-H23 < -8.00 -6.34 -5.47
NCI-H322M -6.60 -5.59 -5.16
NCI-H460 < -8.00 -5.98
NCI-H522 < -8.00 -6.40 -5.38
Colon Cancer
COLO 205 < -8.00 -7.99 -5.43
HCC-2998 < -8.00 -7.22 -5.54
HCT-116 < -8.00 -5.98 -5.48
HCT-15 < -8.00 -5.96 -5.39
HT29 < -8.00 -5.87 -5.04
KM12 < -8.00 -7.31 -5.52
SW-620 < -8.00 -5.90 -5.42
CNS Cancer
SF-268 < -8.00 -5.87 -5.39
SF-295 < -8.00 < -8.00 -5.57
SF-539 < -8.00 < -8.00 -5.57
SNB-19 < -8.00 -4.96 -4.46
SNB-75 -5.56 -5.07
U251 < -8.00 -5.93 -5.44
Melanoma
LOX IMVI < -8.00 -5.72 -5.32
MALME-3M < -8.00 -5.63 -5.20
M14 < -8.00 -5.46
MDA-MB-435 < -8.00 < -8.00
SK-MEL-2 -4.54 -4.26
SK-MEL-28 < -8.00 -5.67 -5.33
SK-MEL-5 < -8.00 -5.94 -5.36
UACC-257 -4.54 -4.27
UACC-62 < -8.00 -5.86 -5.42
Ovarian Cancer
IGROV1 < -8.00 -5.89 -5.40
OVCAR-3 < -8.00 < -8.00 -5.66
OVCAR-4 -7.53 -4.81 -4.40
OVCAR-5 < -8.00 -5.77 -5.38
OVCAR-8 < -8.00 -5.92 -5.40
NCI/ADR-RES < -8.00 < -8.00 < -8.00
SK-OV-3 < -8.00 -4.83 -4.41
Renal Cancer
786-0 < -8.00 -5.83 -5.40
A498 < -8.00 < -8.00 -5.55
ACHN < -8.00 -5.79 -5.39
CAKI-1 < -8.00 < -8.00 -5.64
RXF 393 < -8.00 -5.42
SN12C < -8.00 -5.80 -5.39
TK-10 -5.93 -5.61 -5.29
UO-31 < -8.00 -5.71 -5.34
Prostate Cancer
PC-3 < -8.00 -5.72 -5.19
DU-145 < -8.00 < -8.00 -5.68
Breast Cancer
MCF7 -7.62 -5.85
MDA-MB-231/ATCC < -8.00 -7.17 -5.52
HS 578T < -8.00 < -8.00
BT-549 -7.98 -7.14 -4.41
T-47D -5.92 -5.49
MID -7.87 -6.3 -5.31
Delta 0.13 1.7 2.69
Range 2.08 3.57 4.0
+3 +2 +1 0 -1 -2 -3

Fig.15

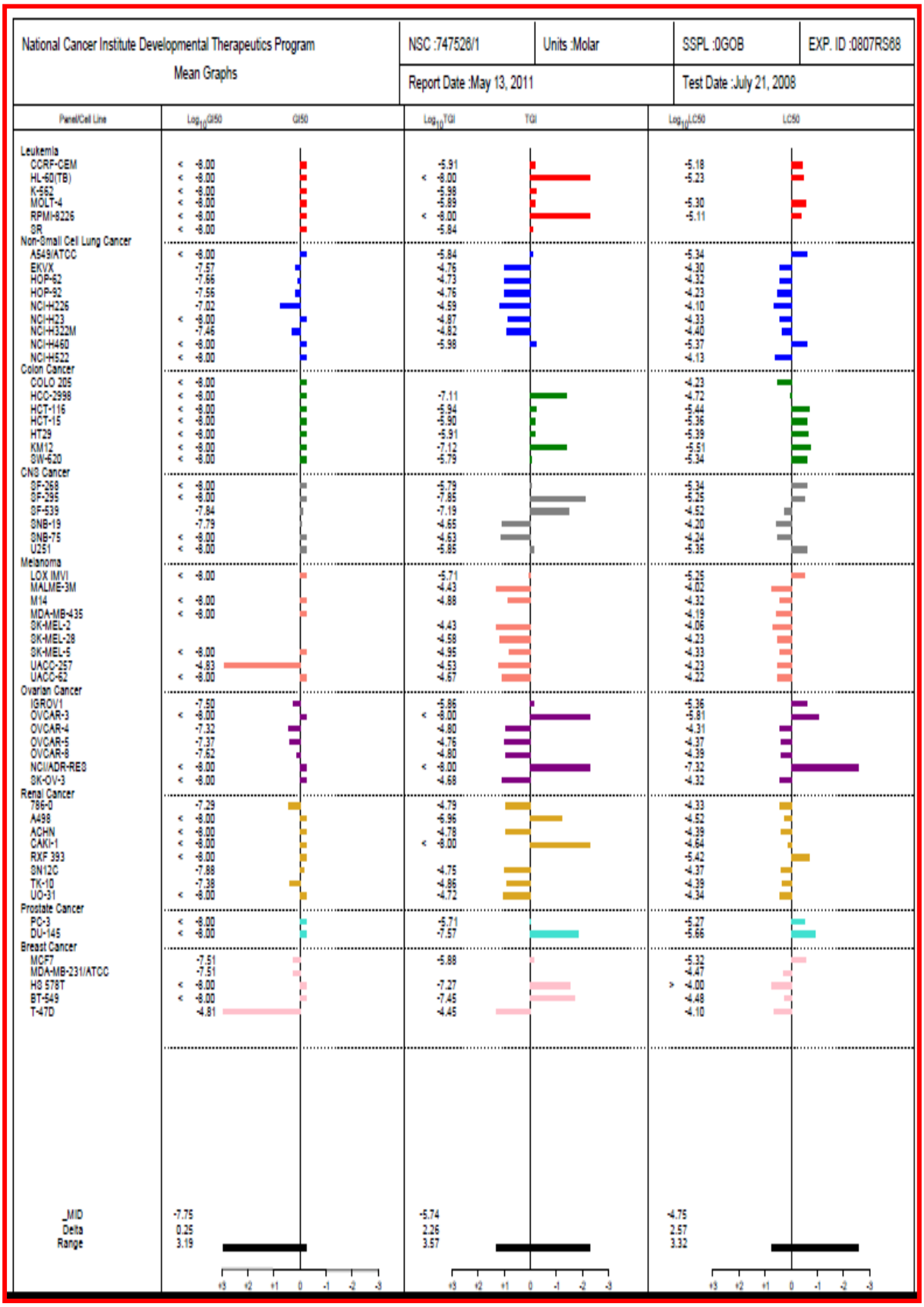

National Cancer Institute Developmental Therapeutics Program
Mean Graphs
NSC :747526/1
Units :Molar
SSPL :0GOB
EXP. ID :0807RS68
Report Date :May 13, 2011
Test Date :July 21, 2008
Panel/Cell Line
Log10GI50
GI50
Log10TGI
TGI
Log10LC50
LC50
Leukemia
CCRF-CEM < -8.00 -5.91 -5.18
HL-60(TB) < -8.00 < -8.00 -5.23
K-562 < -8.00 -5.98
MOLT-4 < -8.00 -5.89 -5.30
RPMI-8226 < -8.00 < -8.00 -5.11
SR < -8.00 -5.84
Non-Small Cell Lung Cancer
A549/ATCC < -8.00 -5.84 -5.34
EKVX -7.57 -4.76 -4.30
HOP-62 -7.66 -4.73 -4.32
HOP-92 -7.56 -4.76 -4.23
NCI-H226 -7.02 -4.59 -4.10
NCI-H23 < -8.00 -4.87 -4.33
NCI-H322M -7.46 -4.82 -4.40
NCI-H460 < -8.00 -5.98 -5.37
NCI-H522 < -8.00 -4.13
Colon Cancer
COLO 205 < -8.00 -4.23
HCC-2998 < -8.00 -7.11 -4.72
HCT-116 < -8.00 -5.94 -5.44
HCT-15 < -8.00 -5.90 -5.36
HT29 < -8.00 -5.91 -5.39
KM12 < -8.00 -7.12 -5.51
SW-620 < -8.00 -5.79 -5.34
CNS Cancer
SF-268 < -8.00 -5.79 -5.34
SF-295 < -8.00 -7.85 -5.35
SF-539 -7.84 -7.19 -4.52
SNB-19 -7.79 -4.65 -4.20
SNB-75 < -8.00 -4.63 -4.24
U251 < -8.00 -5.85 -5.35
Melanoma
LOX IMVI < -8.00 -5.71 -5.25
MALME-3M -4.43 -4.02
M14 < -8.00 -4.88 -4.32
MDA-MB-435 < -8.00 -4.19
SK-MEL-2 -4.43 -4.06
SK-MEL-28 -4.58 -4.23
SK-MEL-5 < -8.00 -4.95 -4.33
UACC-257 -4.83 -4.53 -4.23
UACC-62 < -8.00 -4.67 -4.22
Ovarian Cancer
IGROV1 -7.50 -5.86 -5.36
OVCAR-3 < -8.00 < -8.00 -5.81
OVCAR-4 -7.32 -4.80 -4.31
OVCAR-5 -7.37 -4.76 -4.37
OVCAR-8 -7.62 -4.80 -4.39
NCI/ADR-RES < -8.00 < -8.00 -7.32
SK-OV-3 < -8.00 -4.68 -4.32
Renal Cancer
786-0 -7.29 -4.79 -4.33
A498 < -8.00 -6.96 -4.52
ACHN < -8.00 -4.78 -4.39
CAKI-1 < -8.00 < -8.00 -4.64
RXF 393 < -8.00 -5.42
SN12C -7.88 -4.75 -4.37
TK-10 -7.38 -4.86 -4.39
UO-31 < -8.00 -4.72 -4.34
Prostate Cancer
PC-3 < -8.00 -5.71 -5.27
DU-145 < -8.00 -7.57 -5.66
Breast Cancer
MCF7 -7.51 -5.88 -5.32
MDA-MB-231/ATCC -7.51 -4.47
HS 578T < -8.00 -7.27 > -4.00
BT-549 < -8.00 -7.45 -4.48
T-47D -4.81 -4.45 -4.10
_MID -7.75 -5.74 -4.75
Delta 0.25 2.26 2.57
Range 3.19 3.57 3.32
+3 +2 +1 0 -1 -2 -3

Fig.16

In fig.16 is also reported the mean graph of compound **5g** (NSC 740526) that was selected for in vivo test. For the pharmacologist infact the two compounds are very similar but **5g** was synthesized in better yield and, for this reason, selected for in vivo tests.

In vivo test performed on Ovcar-3 tumor showed that the compound at the dose of 15 mg/kg induced a significant reduction of the tumor although accompanied by a decrease of the body weight which was however recovered (fig.17)

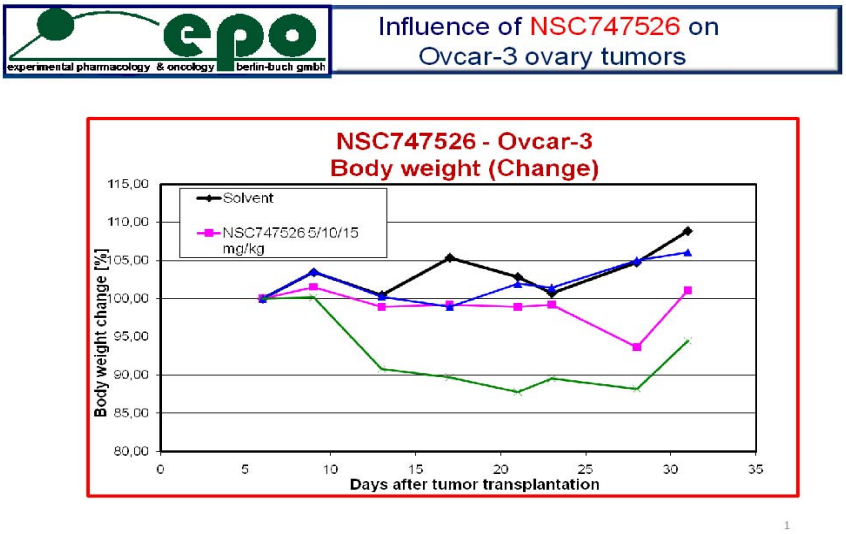


Fig.17

Also for this compound the expression of CD31 and Ki-67 was investigated by immunohystochemistry (fig.18).

Compound **5i** 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.

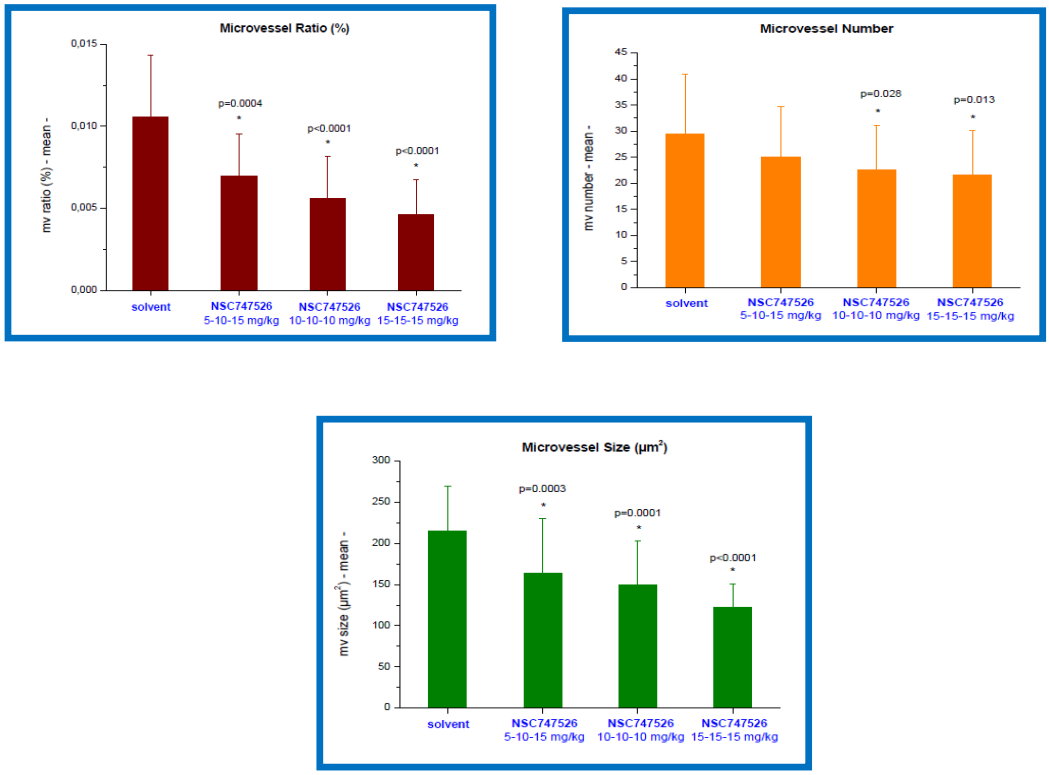


Fig.18

The expression of the proliferation marker Ki-67 was also significantly diminished and in this case was dose-dependent (fig.19)

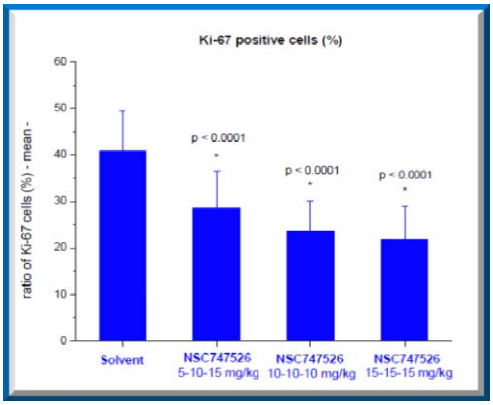
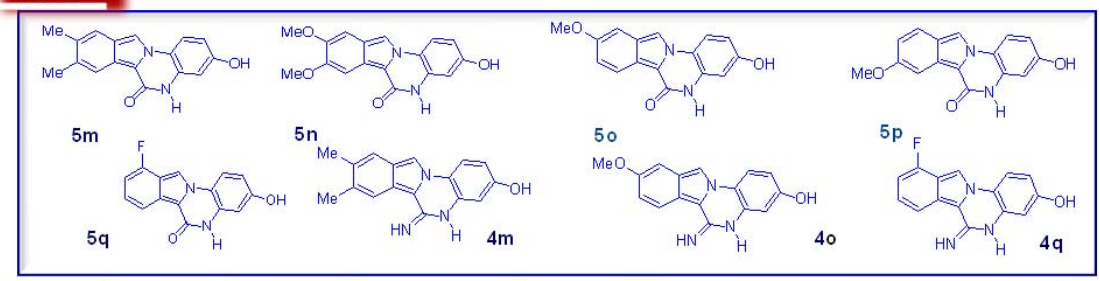


Fig.19

Also compounds **5m-q** and **4m, 4o** and **4q** were selected by the NCI for the total screening on the full tumour cell lines panel.



NSC	Compd.	No. of the cell lines investigated		pGI_{50}	
				Range	MG_MID[c]
753223	5m	60	60	>8.00-6.24	7.38
753224	5n	59	59	>8.00-6.45	7.54
743217	5o	59	59	7.67-5.94	6.61
753219	5p	59	59	7.37-4.89	6.56
753220	5q	59	59	6.66-4.00	5.57
753222	4m	59	59	>8.00-5.44	6.80
743218	4o	59	59	7.62-6.01	6.84
743221	4q	59	59	7.68-4.00	5.60

Table 6

All compounds showed antiproliferative activity on all tested cell lines from micromolar to nanomolar concentrations. The most active compound was **5n** whose pGI_{50} mean value was 7.54 (table 6). The imino forms **4m**, **4q** and **4o** showed antiproliferative activity from micromolar to nanomolar concentrations.

Also the imino **4a-g, 4k-l** and **4n** were isolated and selected for the one-dose pre-screening by the NCI and the most interesting compounds **4g** and **4n** were selected for the total-screening at five dose concentrations.

For compound **4g** (NSC 760286) was calculated a pGI_{50} mean value of 7.44 and its mean graph showed it is very active on colon cancer cell line that are sensitive even at nanomolar concentration (fig.20).

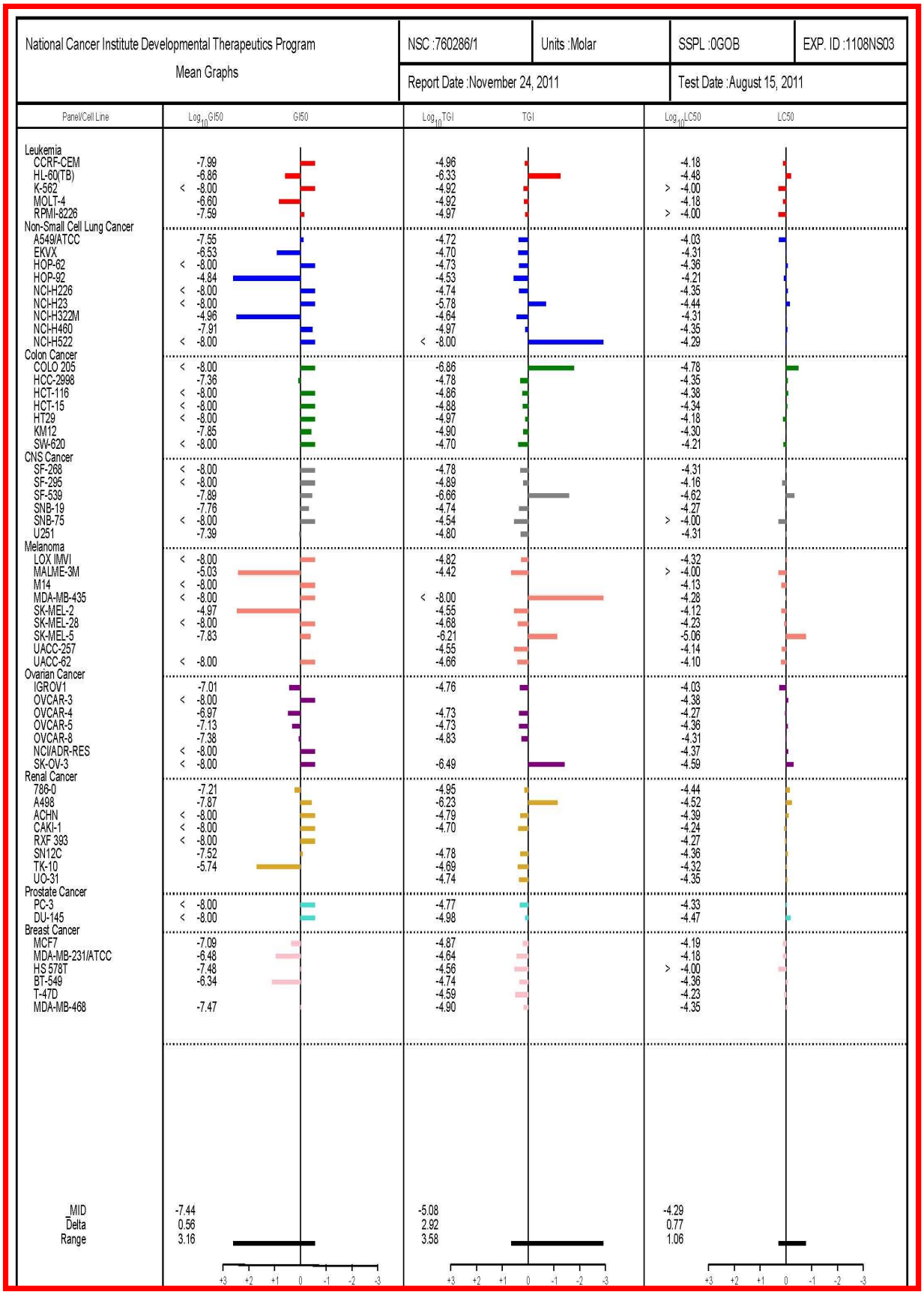

National Cancer Institute Developmental Therapeutics Program
Mean Graphs
NSC :760286/1
Units :Molar
SSPL :0GOB
EXP. ID :1108NS03
Report Date :November 24, 2011
Test Date :August 15, 2011
Panel/Cell Line
Log10GI50
GI50
Log10TGI
TGI
Log10LC50
LC50
Leukemia
CCRF-CEM -7.99 -4.96 -4.18
HL-60(TB) -6.86 -6.33 -4.48
K-562 < -8.00 -4.92 > -4.00
MOLT-4 -6.60 -4.92 -4.18
RPMI-8226 -7.59 -4.97 > -4.00
Non-Small Cell Lung Cancer
A549/ATCC -7.55 -4.72 -4.03
EKVX -6.53 -4.70 -4.31
HOP-62 < -8.00 -4.73 -4.36
HOP-92 -4.84 -4.53 -4.21
NCI-H226 < -8.00 -4.74 -4.35
NCI-H23 < -8.00 -5.78 -4.44
NCI-H322M -4.96 -4.64 -4.31
NCI-H460 -7.91 -4.97 -4.35
NCI-H522 < -8.00 < -8.00 -4.29
Colon Cancer
COLO 205 < -8.00 -6.86 -4.78
HCC-2998 -7.36 -4.78 -4.35
HCT-116 < -8.00 -4.86 -4.38
HCT-15 < -8.00 -4.88 -4.34
HT29 < -8.00 -4.97 -4.18
KM12 -7.85 -4.90 -4.30
SW-620 < -8.00 -4.70 -4.21
CNS Cancer
SF-268 < -8.00 -4.78 -4.31
SF-295 < -8.00 -4.89 -4.16
SF-539 -7.89 -6.66 -4.62
SNB-19 -7.76 -4.74 -4.27
SNB-75 < -8.00 -4.54 > -4.00
U251 -7.39 -4.80 -4.31
Melanoma
LOX IMVI < -8.00 -4.82 -4.32
MALME-3M -5.03 -4.42 > -4.00
M14 < -8.00 -4.13
MDA-MB-435 < -8.00 < -8.00 -4.28
SK-MEL-2 -4.97 -4.55 -4.12
SK-MEL-28 < -8.00 -4.68 -4.23
SK-MEL-5 -7.83 -6.21 -5.06
UACC-257 -4.55 -4.14
UACC-62 < -8.00 -4.66 -4.10
Ovarian Cancer
IGROV1 -7.01 -4.76 -4.03
OVCAR-3 < -8.00 -4.38
OVCAR-4 -6.97 -4.73 -4.27
OVCAR-5 -7.13 -4.73 -4.36
OVCAR-8 -7.38 -4.83 -4.31
NCI/ADR-RES < -8.00 -4.37
SK-OV-3 < -8.00 -6.49 -4.59
Renal Cancer
786-0 -7.21 -4.95 -4.44
A498 -7.87 -6.23 -4.52
ACHN < -8.00 -4.79 -4.39
CAKI-1 < -8.00 -4.70 -4.24
RXF 393 < -8.00 -4.27
SN12C -7.52 -4.78 -4.36
TK-10 -5.74 -4.69 -4.32
UO-31 -4.74 -4.35
Prostate Cancer
PC-3 < -8.00 -4.77 -4.33
DU-145 < -8.00 -4.98 -4.47
Breast Cancer
MCF7 -7.09 -4.87 -4.19
MDA-MB-231/ATCC -6.48 -4.64 -4.18
HS 578T -7.48 -4.56 > -4.00
BT-549 -6.34 -4.74 -4.36
T-47D -4.59 -4.23
MDA-MB-468 -7.47 -4.90 -4.35
MID -7.44 -5.08 -4.29
Delta 0.56 2.92 0.77
Range 3.16 3.58 1.06
+3 +2 +1 0 -1 -2 -3

Fig.20

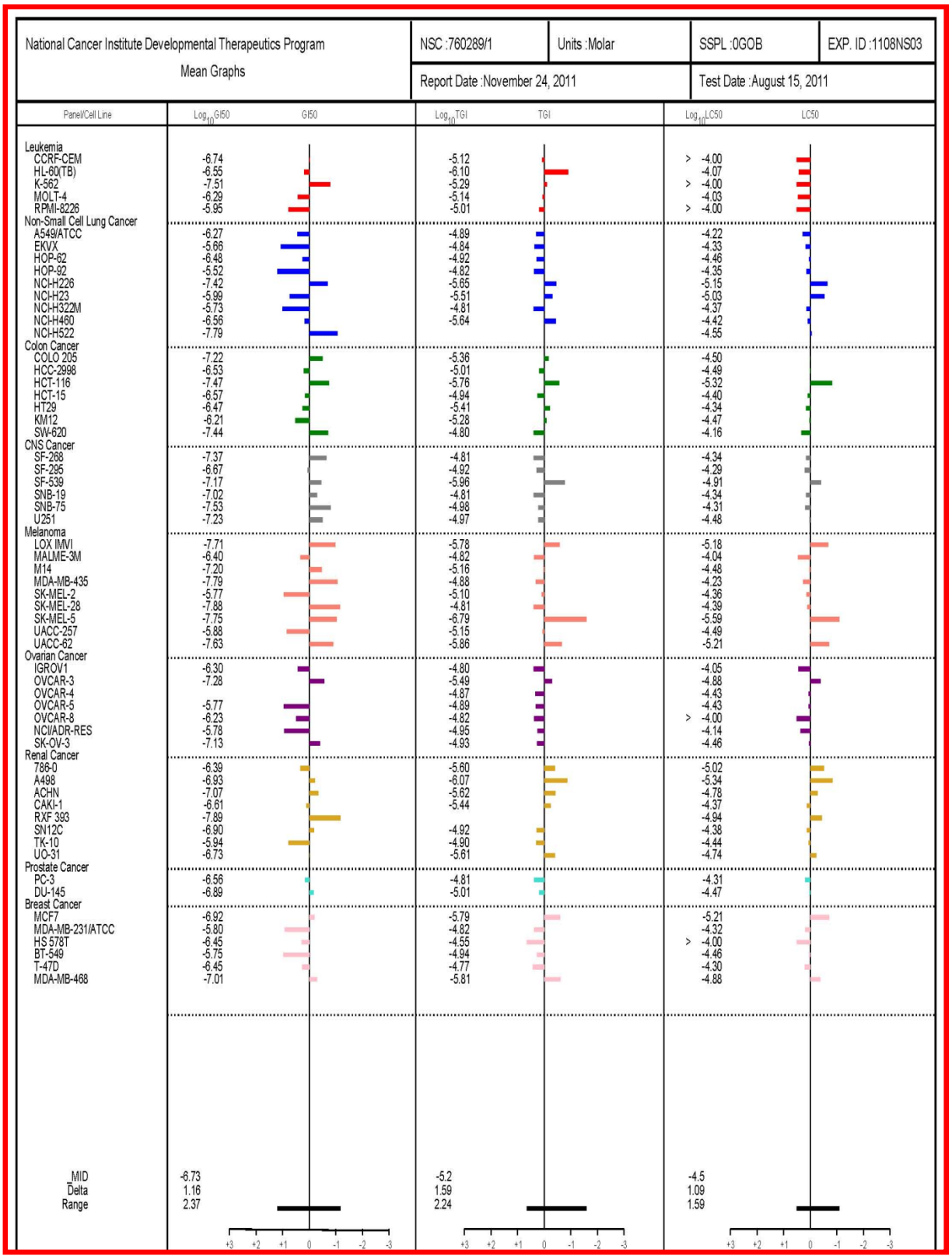

National Cancer Institute Developmental Therapeutics Program
Mean Graphs
NSC :760289/1
Units :Molar
SSPL :0GOB
EXP. ID :1108NS03
Report Date :November 24, 2011
Test Date :August 15, 2011
Panel/Cell Line
Log10GI50
GI50
Log10TGI
TGI
Log10LC50
LC50
Leukemia
CCRF-CEM -6.74 -5.12 > -4.00
HL-60(TB) -6.55 -6.10 -4.07
K-562 -7.51 -5.29 > -4.00
MOLT-4 -6.29 -5.14 -4.03
RPMI-8226 -5.95 -5.01 > -4.00
Non-Small Cell Lung Cancer
A549/ATCC -6.27 -4.89 -4.22
EKVX -5.66 -4.84 -4.33
HOP-62 -6.48 -4.92 -4.46
HOP-92 -5.52 -4.82 -4.35
NCI-H226 -7.42 -5.65 -5.15
NCI-H23 -5.99 -5.51 -5.03
NCI-H322M -5.73 -4.81 -4.37
NCI-H460 -6.56 -5.64 -4.42
NCI-H522 -7.79 -4.55
Colon Cancer
COLO 205 -7.22 -5.36 -4.50
HCC-2998 -6.53 -5.01 -4.49
HCT-116 -7.47 -5.76 -5.32
HCT-15 -6.57 -4.94 -4.40
HT29 -6.47 -5.41 -4.34
KM12 -6.21 -5.28 -4.47
SW-620 -7.44 -4.80 -4.16
CNS Cancer
SF-268 -7.37 -4.81 -4.34
SF-295 -6.67 -4.92 -4.29
SF-539 -7.17 -5.96 -4.91
SNB-19 -7.02 -4.81 -4.34
SNB-75 -7.53 -4.98 -4.31
U251 -7.23 -4.97 -4.48
Melanoma
LOX IMVI -7.71 -5.78 -5.18
MALME-3M -6.40 -4.82 -4.04
M14 -7.20 -5.16 -4.48
MDA-MB-435 -7.79 -4.88 -4.23
SK-MEL-2 -5.77 -5.10 -4.36
SK-MEL-28 -7.88 -4.81 -4.39
SK-MEL-5 -7.75 -6.79 -5.59
UACC-257 -5.88 -5.15 -4.49
UACC-62 -7.63 -5.86 -5.21
Ovarian Cancer
IGROV1 -6.30 -4.80 -4.05
OVCAR-3 -7.28 -5.49 -4.88
OVCAR-4 -4.87 -4.43
OVCAR-5 -5.77 -4.89 -4.43
OVCAR-8 -6.23 -4.82 > -4.00
NCI/ADR-RES -5.78 -4.95 -4.14
SK-OV-3 -7.13 -4.93 -4.46
Renal Cancer
786-0 -6.39 -5.60 -5.02
A498 -6.93 -6.07 -5.34
ACHN -7.07 -5.62 -4.78
CAKI-1 -6.61 -5.44 -4.37
RXF 393 -7.89 -4.94
SN12C -6.90 -4.92 -4.38
TK-10 -5.94 -4.90 -4.44
UO-31 -6.73 -5.61 -4.74
Prostate Cancer
PC-3 -6.56 -4.81 -4.31
DU-145 -6.89 -5.01 -4.47
Breast Cancer
MCF7 -6.92 -5.79 -5.21
MDA-MB-231/ATCC -5.80 -4.82 -4.32
HS 578T -6.45 -4.55 > -4.00
BT-549 -5.75 -4.94 -4.46
T-47D -6.45 -4.77 -4.30
MDA-MB-468 -7.01 -5.81 -4.88
MID -6.73 -5.2 -4.5
Delta 1.16 1.59 1.09
Range 2.37 2.24 1.59
+3 +2 +1 0 -1 -2 -3

Fig. 21

The mean graph of compound **4n** showed a pGI_{50} mean value of 6.73 and that CNS Cancer and melanoma cell line are very sensitive to this compound (fig.21).

For all compounds the possible interaction with nucleic acids was also studied. The thermal DNA denaturation was evaluated in presence of quinoxaline compounds at 2 μM and 10 μM concentration. Compounds were tested on human telomeric sequence (AGGGTTAGGGTTAGGGTTAGGGT), c-Myc promoter sequence (Pu24, TGAGGGTGGGGAGGGTGGGGAAGG) both having a G-quadruplex arrangement, and a double helix random sequence fragment (GGATGTGAGTGTGAGTGTGAGG).

Imino and ketone forms of quinoxalines were compared and results showed that there is not significant difference between the two forms for the interation with G-quadruplex form or doble helix DNA.

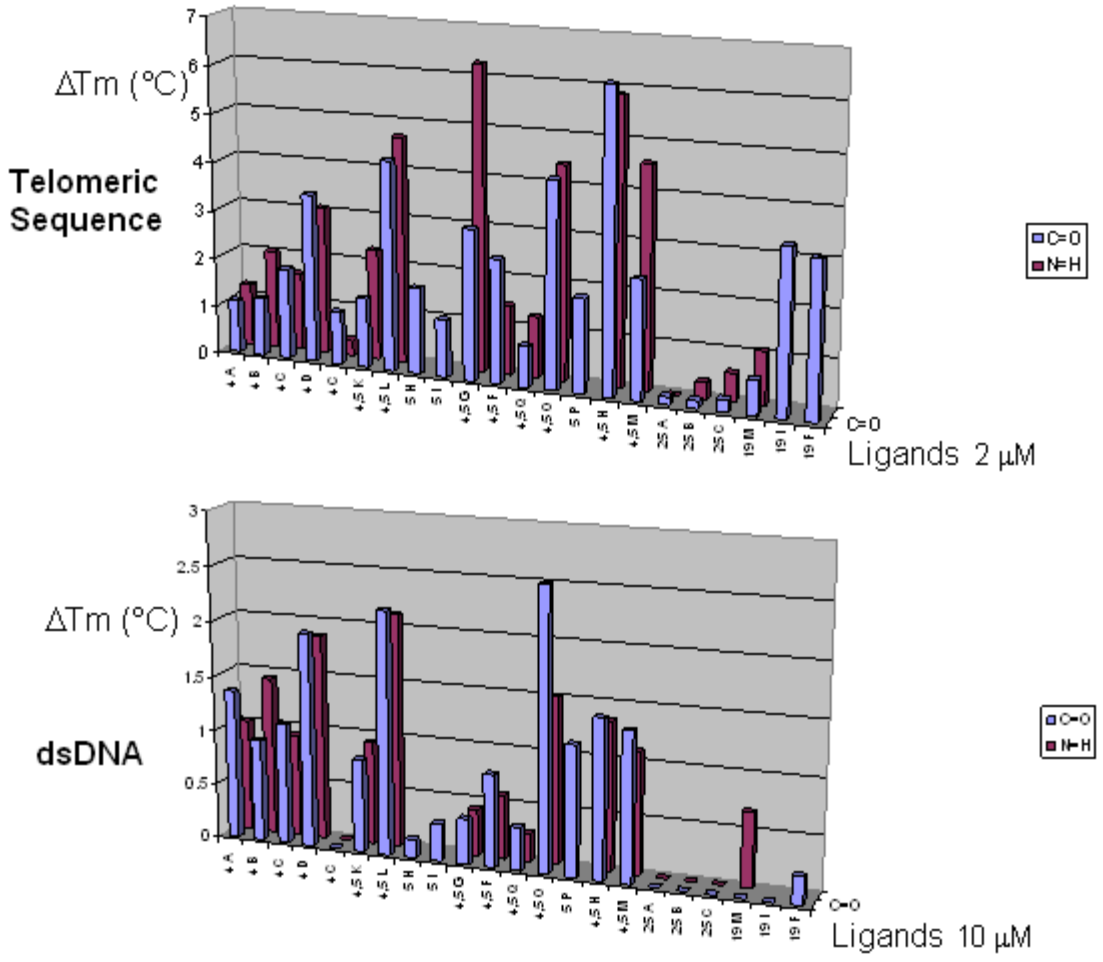


Fig.22

For G-quadruplex interation is very important the presence of substituents in position 8 and 9 bearing an electron-negative atom. Infact compounds **4n** and **5n** showed the best results in terms of G-quadruplex interaction contrary to compound **4m** and **5m** that didn't show excellent resuls. Also the presence of hydroxyl group in para position on the quinoxaline portion seems to be more important than methoxyl group for G-quadruplex interation. Same results were obtained for Pu24 promoter recognition.

For compounds **4n**, **5n**, **5i** was evaluated the DNA denaturation relating to compound concentrations compairing the results with ISQOc.

All compounds are able to induce DNA denaturation in a dose dependent way (Fig.23-24).

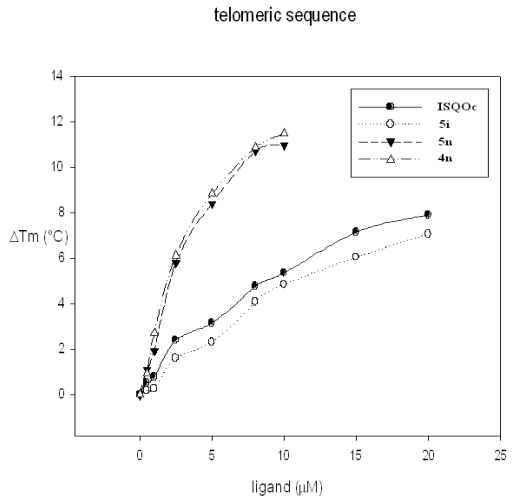


Fig.23

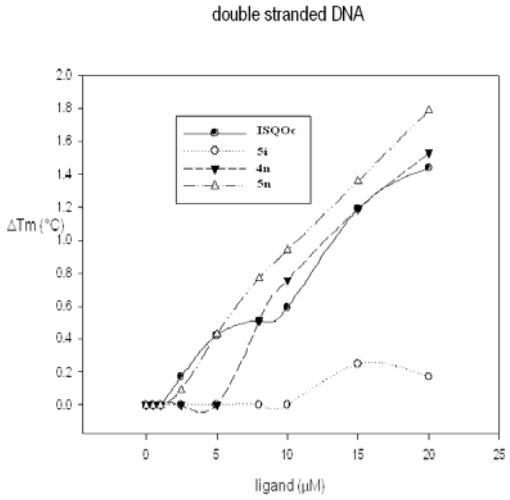


Fig.24

Studies on circular dichroism to understand how quinoxalines are able to modify nucleic acid structures were also performed. Results showed that all compounds are able to increase the signal at 290 nm for the human telomeric sequence showing an effective interaction. Also in this case the best result was related to compound **4n.**

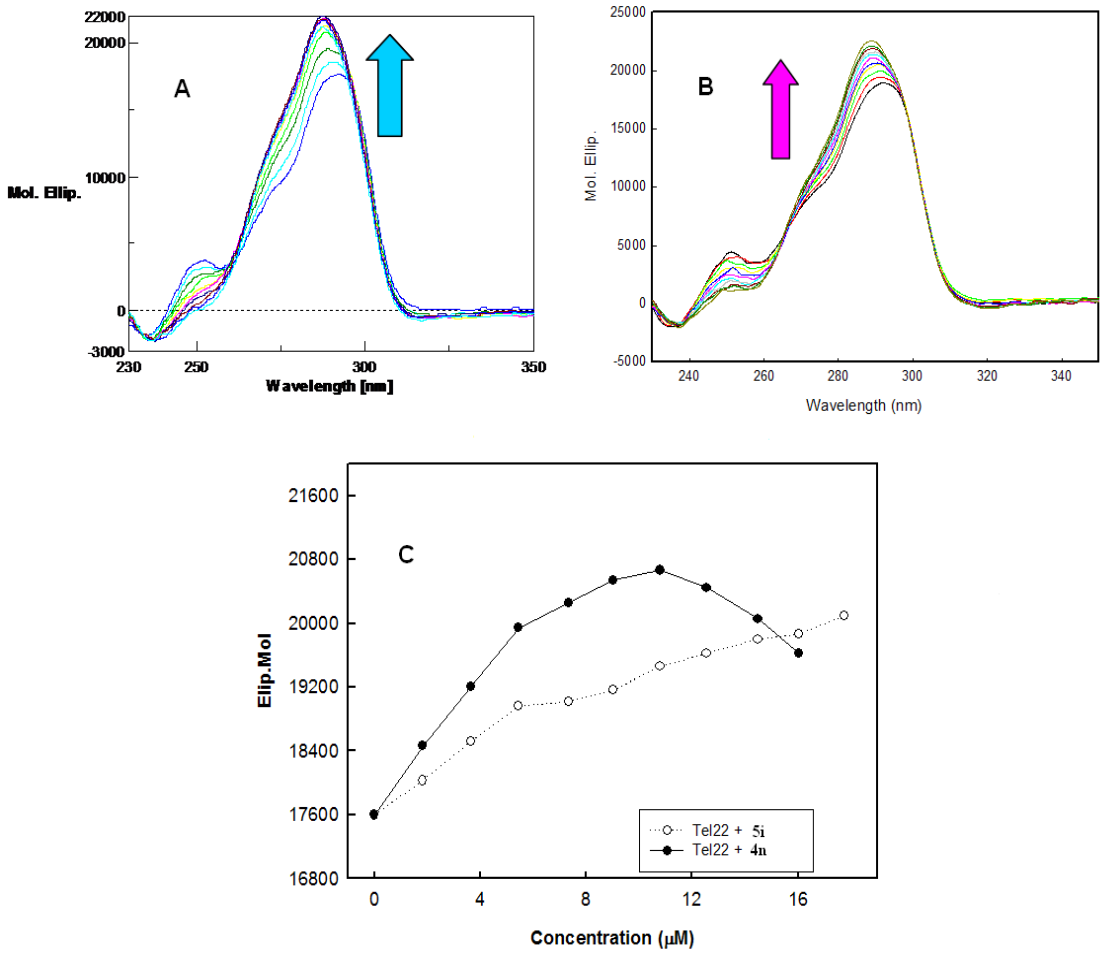


Fig.25. **A)** TeI22 (4μM) in presence of **4n** (0.16 μM) in TK 10/50 pH 7.4; **B)** TeI22 (4μM) in presence of **5i** (0.16 μM) in TK 10/50 pH 7.4; **C)** CD Titration of TeI22 at 292 nm

About interaction with double helix DNA, compound **4n** causes significant dichroism spectrum alteration in a similar way to Mitoxantrone showing better results than compound **5i**.

Same results were obtained for compound **5n.** Compound **5o** causes three-dimensional DNA structure alteration and compound **5i** seems to be no effective.

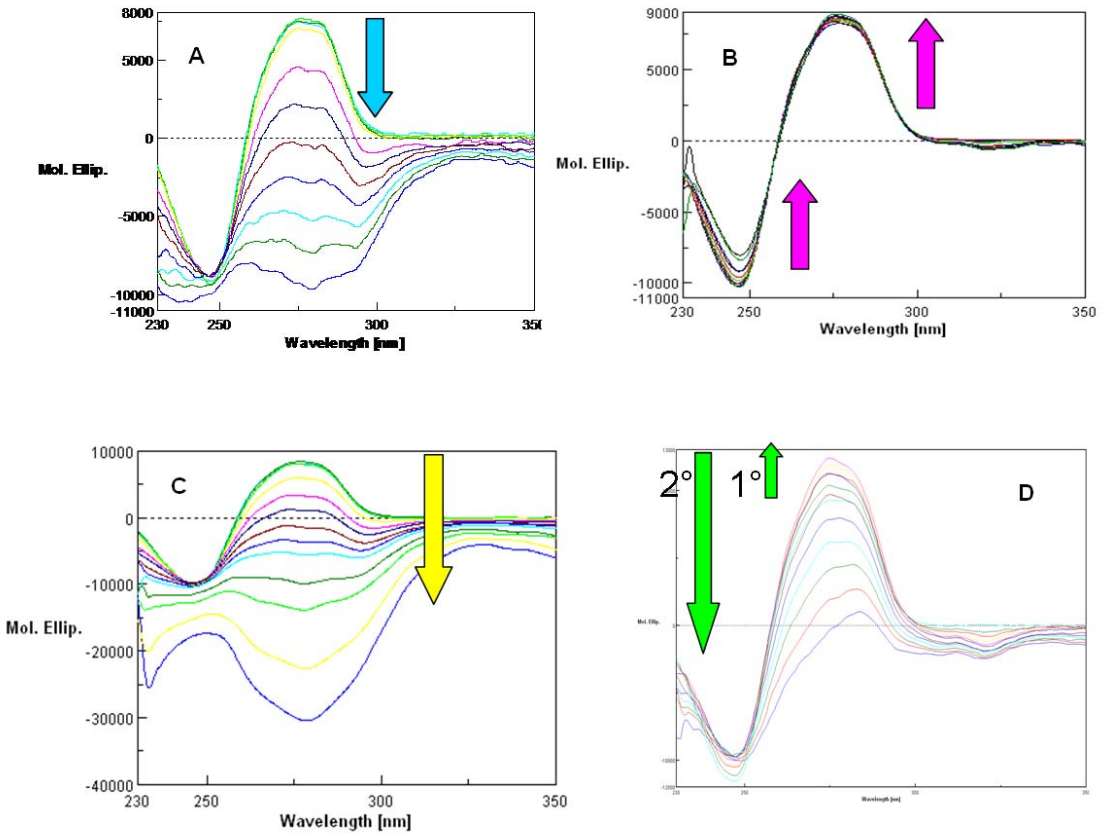


Fig.26. A) ctDNA (96 µM) in presence of **4n** (0-30 µM)in TK 10/50 pH 7.4; B) ctDNA (96 µM) in presence of **5i** (0-30 µM)in TK 10/50 pH 7.4; C) ctDNA (96 µM) in presence of **5n** (0-30 µM)in TK 10/50 pH 7.4; D) ctDNA (96 µM) in presence of **5o** (0-30 µM)in TK 10/50 pH 7.4;

Electrophoresis studies were also performed and showed that compound **5o**, **5i**, **5n** and **4n** are able to interact with nucleic acids confirming dichroism studies results (Fig.27).

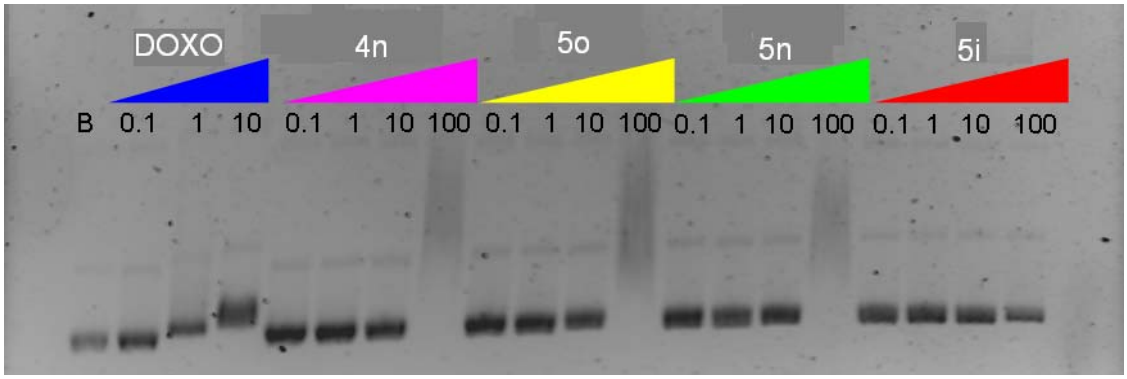
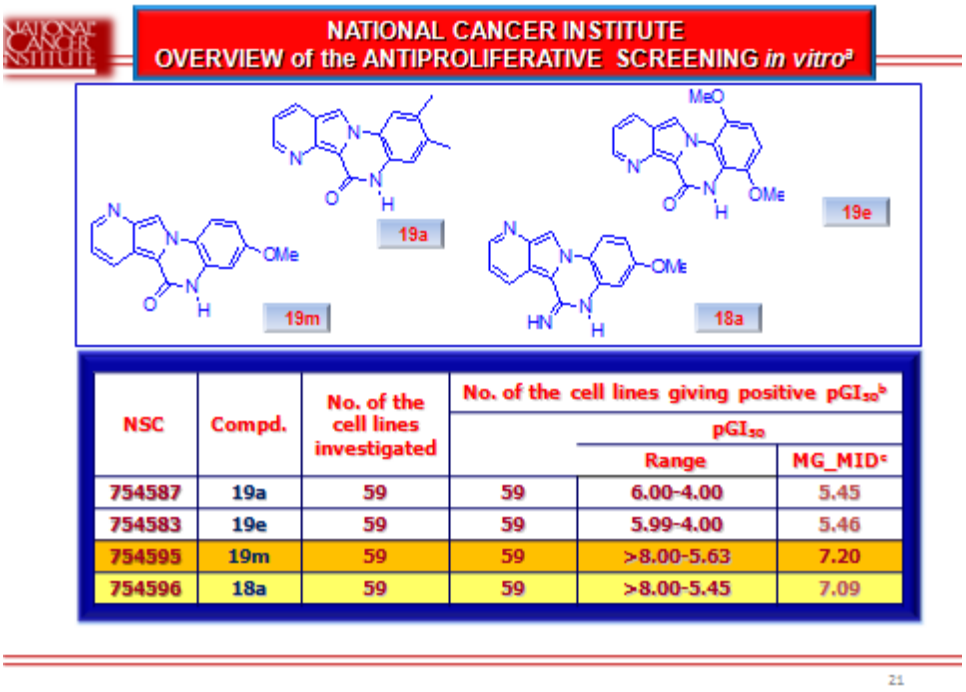


Fig.27



NSC	Compd.	No. of the cell lines investigated	No. of the cell lines giving positive pGI_{50}[b]		
				pGI_{50}	
				Range	MG_MID[c]
754587	19a	59	59	6.00-4.00	5.45
754583	19e	59	59	5.99-4.00	5.46
754595	19m	59	59	>8.00-5.63	7.20
754596	18a	59	59	>8.00-5.45	7.09

Fig.28

Compounds **18a-m** and **19a** were submitted to the NCI and selected for the pre-screening. The most interesting derivatives were **19a, 18a, 19e** and **19m**. For all compounds was calculated the pGI_{50} mean value that was of 5.45, 7.09, 5.46 and 7.20 respectively. The most interesting compounds **19m** and **18a** were active at nanomolar concentration in more than 80% of tumor cell lines.

Also compounds **24a-c** and **25a-c** were selected by the NCI for the pre-screening. The most interesting compound was **25b** that was tested on the full panel and whose pGI_{50} mean value was 5.78.

X- Ray studies were also performed on compound **4c** to know its exact mesomeric form.

Compound **4c** was crystallized in acetic acid and through its crystallographic structure bond distances were calculated and compared to analogue structures for which the same parameters were known.

Results showed that the double bound is probably located outside the quinoxaline ring system, having the shortest bound distance. (Fig.29)

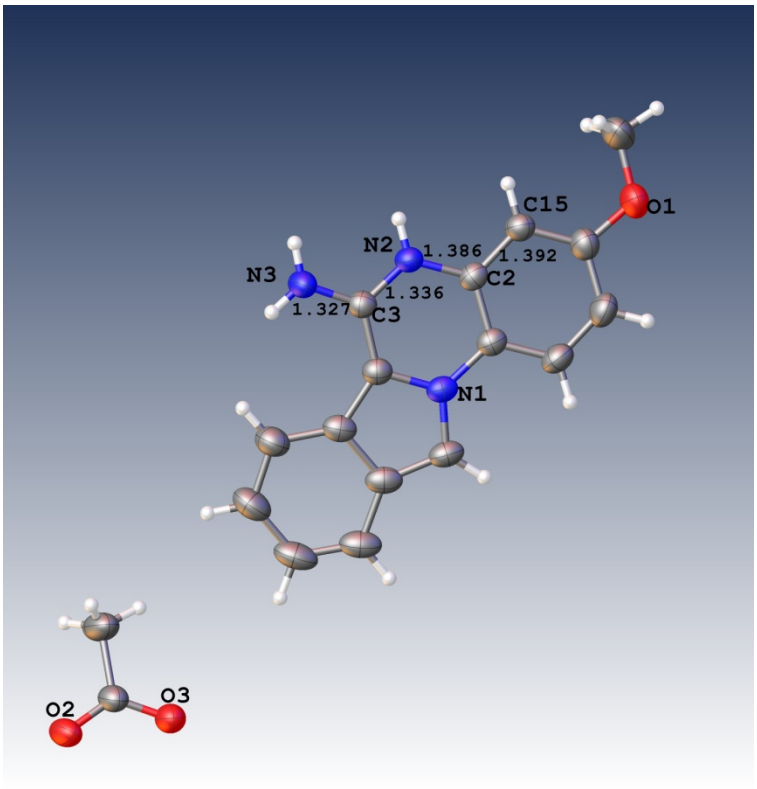


Fig.29

EXPERIMENTAL DATA

All melting points were taken on a Büchi-Tottoli apparatus and are uncorrected; IR spectra were determined in bromoform with a Jasco FT/IR 5300 spectrophotometer. 1H e 13C NMR spectra were measured at 200 and 50.3 MHz, respectively, in DMSO- d6 or $CDCl_3$ solution, using a Bruker AC 200 MHz spectrometer (TMS as internal reference). Chromatography column was performed with Merk silica gel 230-400 mesh ASTM or BIOTAGE FLASH chromatography or with Büchi Sepacore chromatography module (prepacked cartridge system). Elemental analyses (C, H, N) were within ±0.4% of the theoretical values. Microwave experiments were carried out using a CEM Discover LabmateTM microwave apparatus.

Synthesis of dimethyl 4,5-dimethylciclohexa-1,4-diene-1,2-dicarboxilate 6

To a solution of dimethyl acetilene dicarboxilate (25g, 0.17 mol) in toluene (100 ml) 2,3-dimethylbuta-1,3-diene (13.1g, 0.16 mol) was added under nitrogen atmosphere. The mixture was stirred under reflux overnight. The solvent was evaporated under reduced pressure.

m.p. 64.0-65.0°C; Yield 95%; IR 1732 (CO) cm^{-1}; ^1H NMR $CDCl_3$ (ppm): 1.69 (s, 3H, CH_3), 3.77 (s, 2H, CH_2), 3.84 (s, 3H, CH_3).

Synthesis of 4,5-dimethylbenzene-1,2-dicarboxilate 7

To a solution of compound **6** (30g, 0.13 mol) in chlorobenzene (500 ml) 2 equivalents of DDQ were added. The mixture was stirred under reflux for 24h. 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 DCM as eluent.

m.p. 49.0-50.0°C; Yield 92%; IR 1728 (CO) cm^{-1}; ^1H NMR $CDCl_3$ (ppm): 2.29 (s, 3H, CH_3), 3.81 (s, 3H, CH_3), 7.47 (s, 1H, CH).

Synthesis of 1,2-phenylene-dimethanol 8,9

To a solution of boronhydride (sol 1,5M in THF/diethyl ether, 50ml) at 0°C under argon atmosphere, a solution of the appropriate acid or ester (24 mmol) in THF (40 ml) was added. The mixture was stirred at 0°C for 2h and then under reflux for 20h. 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.

(3-Fluoro-1,2-phenylen)dimethanol 9: m.p. 73.0°C; yield 99%; ^{1}H NMR DMSO (ppm): 3.41 (br, 2H, OH), 4.68 (d, 2H, CH_2), 7.05 (m, 3H, Ar), 7.16 (d, 2H, CH_2), 7.30 (t).

(4,5-Dimetil-1,2-phenylen)dimethanol 8: m.p. 103.0°C; yield 80%; ^{1}H NMR $CDCl_3$ (ppm): 2.24 (s, 3H, CH_3), 3.33 (br, 1H, OH), 4.61 (d, 2H, CH_2), 7.08 (s, 1H, CH).

Synthesis of *o*-phtalaldehydes 1a,1b

To a mixture of DCM (100 ml) and oxalyl chloride (8ml, 88 mol) at -78°C and under argon atmosphere, a mixture of DMSO (13.6 ml, 0.176 mol) and DCM (25 ml) was added dropwise. After 3-5 min a solution of the appropriate 1,2-dimethanol **8**,**9** (40 mol) in DCM-DMSO (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 rt and poured in water and ice. It was extracted in DCM and the organic layer dried and evaporated. The crude was purified in column using DCM as eluent.

4,5-Dimethyphtalaldehyde 1a: m.p. 95.0-100.0°C; yield 87%; IR 1688 (CHO) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 1.77 (s, 3H, CH_3), 7.28 (s, 1H, CH), 10.3 (s, 1H, CHO).

3-Fluorophtaladehyde 1b: m.p. 75.0-80.0°C; yield 80%; IR 1700 (CHO) cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 7.75 (d), 7.81 (m), 8.04 (m), 10.5 (CHO, 10.6 (CHO).

Synthesis of (2-bromo-phenylen)methanols 11c,d

To a solution of benzoic acid **10c,d** (20 mmol) in THF (50 ml), a solution of BH_3/THF (solution 1.0M in THF, 28.0 ml, 30 mmol) was added dropwise and the mixture was

stirred at rt for 3h and 30 min. After cooling at 0°C a solution of HCl 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, dried and evaporated.

(2-Bromo-5-methoxyphenyl)methanol 11c: m.p. 45.0-46.0°C; yield 99%; IR 3408 (OH) cm^{-1}; ^{1}H NMR DMSO (ppm): 3.77 (s, 6H, 2xCH_3),4.49 (d, 2H, J = 5.5 Hz, CH_2), 5.47 (d, 1H, J = 5.5 Hz, OH), 6.79 (dd J = 3.1 e 8.7 Hz, H-4), 7.13 (d J = 3.1 Hz, H-6), 7.44 (d J = 8.8 Hz, H-3); ^{13}C NMR DMSO (ppm): 55.2 (q), 62.5 (t), 110.9 (s), 113.6 (d), 113.9 (d), 132.5 (d), 142.2 (s), 158.8 (s).

(2-Bromo-4,5-dimethoxyphenyl)methanol 11d: m.p. 95.0°C; yield 90%; IR 3498 (OH) cm^{-1}; ^{1}H NMR DMSO (ppm): 3.77 (s, 3H, CH_3), 3.77 (s, 3H, CH_3), 4.45 (d, 2H, J = 5.5 Hz, CH_2), 5.35 (d, 1H, J = 5.5 Hz, OH), 7.10 (s, 1H, H-6), 7.12 (s, 1H, H-3); ^{13}C NMR DMSO (ppm): 55.5 (q), 55.9 (q), 62.3 (t), 110.7 (s), 111.6 (d), 115.2 (d), 132.9 (s), 148.1 (s), 148.2 (s).

Synthesis of 2-bromo-benzaldehydes 12c,d

To a solution of the appropriate phenyl methanol **11c,d** (1.7 mmol) in DCM, 2.4 mmol (1.9 mmol) of Dess-Martin periodinane are 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 $Na_2S_2O_3$ and saturated solution of $NaHCO_3$. The organic phase was dried using Na_2SO_4 and evaporated. The crude was purified in column using DCM as eluent.

2-Bromo-5-methoxybenzaldehyde 12c: m.p. 74.0-75.0°C; yield 98%; IR (CHO) 1682 cm^{-1}; ^{1}H NMR DMSO (ppm): 3.83 (s, 3H, CH_3), 7.23 (dd, 1H, J = 3.2 e 8.7 Hz, H-4), 7.34 (d, 1H J = 3.2 Hz, H-6), 7.70 (d, 1H J = 8.7 Hz, H-3), 10.2 (s, 1H, CHO); ^{13}C NMR DMSO (ppm): 55.7 (q), 113.6 (d), 116.3 (s), 122.7 (d), 133.6 (s), 134.8 (d), 158.9 (s), 191.4 (d).

2-Bromo-4,5-dimethoxybenzaldehyde 12d: m.p. 148.0-149.0 °C; yield 90%; IR (CHO) 1676 cm^{-1}; ^{1}H NMR DMSO (ppm): 3.83 (s, 3H, CH_3), 3.91 (s, 3H, CH_3), 7.31 (s, 1H, H-6), 7.32 (s, 1H, H-3), 10.06 (s, 1H, CHO); ^{13}C NMR DMSO (ppm): 55.6 (q), 56.4 (q), 110.4 (d), 115.9 (d), 119.3 (s), 125.7 (s), 148.6 (s), 154.4 (s), 190.1 (d).

Synthesis of 2-(2-bromo-phenyl)1,3-dioxolanes 13c,d

To a solution of 2-bromo-benzaldehyde **12c,d** (3.3 mmol) in toluene, ethyl glicole (9.2 mmol, d= 1.113, 0.6 ml) and a catalytic amount of *p*-toluensulfonic acid are added. The mixture was stirred at reflux for 24h using Dean-Stark trap. After cooling, the organic phase was washed with a saturated solution of $NaHCO_3$ and dried and evaporated. The crude was purified by BIOTAGE chromatography using cycloexane/ethyl acetate (98/2) as eluent.

2-(2-Bromo-5-methoxyphenyl)-1,3-dioxolane 13c: oil, yield 90%; ^{1}H NMR $CDCl_3$ (ppm): 3.79 (s, 3H, CH_3), 4.05-4.16 (m, 4H, 2xCH_2), 6.04 (s, 1H, CH), 6.78 (dd, 1H J = 3.1 e 8.8 Hz, H-4), 7.16 (d, 1H J = 3.1 Hz, H-6), 7.44 (d, 1H J = 8.8 Hz, H-3); ^{13}C NMR $CDCl_3$ (ppm): 55.5 (t), 65.4 (q), 102.4 (d), 113.0 (d), 113.1 (s), 116.7 (d), 133.6 (d), 137.4 (s), 159.0 (s).

2-(2-Bromo-4,5-dimethoxiphenyl)-1,3-dioxolane 13d: m.p. 98.0-99.0°C; yield 90%; ^{1}H NMR DMSO (ppm): 3.76 (s, 3H, CH_3), 3.79 (s, 3H, CH_3), 3.95-4.11 (m, 4H, 2xCH_2), 5.85 (s, 1H, CH), 7.05 (s, 1H, H-6), 7.14 (s, 1H, H-3); ^{13}C NMR DMSO (ppm): 56.1 (q), 56.4 (q), 65.3 (t), 102.3 (d), 111.1 (d), 113.0 (s), 115.9 (d), 128.5 (s), 148.6 (s), 150.5 (s).

Synthesis of 2-(1,3-dioxolan-2-yl)benzaldehydes 14c,d

To a solution of dioxolane **13c,d** (2.5 mmol) in THF (8ml) at -78°C, *n*-butil lithium (solution 1.6 M in *n*-hexanes, 2.5 mmol, 1.6 ml) are added during 1h. The mixture was stirred for 1h at -78°C. After that, 0.3 ml of DMF are added and the mixture was warmed to rt during 1-5h. A saturated solution of NH_4Cl was added and the mixture extracted in DCM. The organic layer was dried with Na_2SO_4 and evaporated. The crude was purified in column using DCM as eluent.

2-(1,3-Dioxolan-2-yl)-5-methoxybenzaldehyde 14c: oil, yield 86%; IR (CHO) 1682 cm^{-1}; ^{1}H NMR DMSO (ppm): 3.88 (s, 3H, CH_3), 3.99-4.10 (m, 4H, 2xCH_2), 6.39 (s, 1H, CH), 7.10-7.20 (m, 2H, H-4 e H-6), 7.89 (d, 1H J = 8.5 Hz, H-3), 10.2 (s, 1H, CHO); ^{13}C NMR DMSO (ppm): 55.7 (q), 64.9 (t), 99.8 (d), 112.2 (d), 114.4 (d), 127.3 (s), 131.7 (d), 141.8 (s), 163.4 (s), 190.3 (d).

2-(1,3-Dioxolan-2-yl)-4,5-methoxybenzaldehyde 14d: m.p. 77.0-78.0°C; yield 100%; IR (CHO) 1680 cm^{-1}; ^{1}H NMR DMSO (ppm): 3.98 (s, 3H, CH_3), 3.99 (s, 3H,

CH_3), 4.00-4.12 (m, 4H, 2xCH_2), 6.33 (s, 1H, CH), 7.22 (s, 1H, H-6), 7.41 (s, 1H, H-3), 10.3 (s, 1H, CHO); ^{13}C NMR DMSO (ppm): 55.6 (q), 55.8 (q), 64.8 (t), 99.9 (d), 109.5 (d), 110.0 (d), 127.2 (s), 134.0 (s), 149. 1(s), 153.0 (s), 189.9 (d).

Synthesis of *orto*-phtaldehydes 1c,d

To a solution of orto-benzylaldehyde **14c, d** (1.5 mmol) in acetone (17 ml) a catalytic amount of *p*-toluensulfonic acid was added. The solution was stirred for 15 minutes at rt. Evaporated the solvent, the resulting residue was dissolved in DCM and it was washed with a solution of Na_2CO_3 and brine. The crude was purified by chromatography using DCM as eluent.

5-Methoxyphtaldehyde 1c: m.p. 37.1°C; yield 60%; IR (CHO) 1695 cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 3.96 (s, 3H, CH_3), 7.23 (dd, 1H J = 2.6 e 8.5 Hz, H-4), 7.44 (d, 1H J = 2.6 Hz, H-6), 7.94 (d, 1H J = 8.5 Hz, H-3), 10.3 (s, 1H, CHO), 10.6 (1H, s, CHO); ^{13}C NMR $CDCl_3$ (ppm): 56.0 (q), 114.9 (d), 118.7 (d), 129.4 (s), 134.6 (d), 138.6 (s), 163,9 (s), 191.1 (d), 192.0 (d).

4,5-Dimethoxyphtaldehyde 1e: m.p. 169.4-170.0; yield 88%; IR (CHO) 1680 cm^{-1}; ^{1}H NMR $CDCl_3$ (ppm): 3.94 (s, 6H, 2xCH_3), 7.54 (1, 2H, 2xCH), 10.5 (s, 2H, 2xCHO); ^{13}C NMR $CDCl_3$ (ppm): 11.7 (d), 131.2 (s), 153.2 (s), 191.7 (d).

Synthesis of 1-Cyano-2-(2-aminophenyl)isoindoles 3

To a solution of sodium hydrogen sulfite (1.56 g, 0.015 mol) in water (38 ml), phthalaldehyde **1e** (2 g, 0.015 mol) was added. The mixture was stirred until the solid was dissolved, and the appropriate 1,2-phenylenediamine **2a-f** (0.015 mol) was added. The reaction was heated on a steam bath for 30 min at 40°C, then KCN (3.39 g, 0.052 mol) in water (8.0 ml) was added, and the mixture was heated for an additional 90 min. The solid formed upon cooling was filtered and purified by chromatography. In the case of derivative **3e**, the 1,2-phenylenediamine **1e** was first dissolved in DMF (15 ml) and then added to the reaction mixture.

1-Cyano-2-(2-aminophenyl)isoindole 3a: this compound was purified by flash chromatography by using DCM as eluent to give a solid: yield 65%; m.p. 150.0-151.0

°C; IR 3473, 3363 (NH_2), 2202 (CN) cm-1; 1H NMR (DMSO-d6) (ppm) 5.19 (2H, bs, NH_2), 6.70 (t, 1H, J=7.2 Hz, H-5′), 6.94 (d, 1H, J=7.2 Hz, H-2), 7.15-7.19 (m, 3H, H-4′, H-5, H-6′), 7.31 (t, 1H, J=8.2 Hz, H-6), 7.66 (d, 1H, J=8.2 Hz, H-4), 7.78 (d, 1H, J=8.2 Hz, H-7), 7.86 (s, 1H, H-3); 13C NMR (DMSO-*d*6) ppm: 93.9 (s), 114.0 (s), 115.8 (d), 116.1 (d), 117.5 (d), 121.5 (d), 122.2 (d), 122.4 (d), 122.5 (s), 124.1 (s), 125.5 (d), 127.8 (d), 130.4 (d), 131.2 (s), 144.1 (s).

1-Cyano-2-(2-amino-3-methylphenyl)isoindole 3b: this compound was purified by chromatography using DCM-cyclohexane (7:3) as eluent to give compound **3b**:

yield 40%; m.p. 62.0-63.0 °C; IR 3481, 3388 (NH_2), 2200 (CN) cm-^{1}; ^{1}H NMR ($CDCl_3$) ppm*:* 2.25 (s, 3H, CH_3), 3.55 (s, 2H, NH_2), 6.80 (t, 1H, J=7.6 Hz, H-5′), 7.10 (d, 1H, J=7.6 Hz, H-6′), 7.14 (d, 1H, J=7.6 Hz, H-4′), 7.23 (t, 1H, J=8.1 Hz, H-5), 7.31 (t, 1H, J=8.1 Hz, H-6), 7.44 (s, 1H, H-3), 7.69 (d, 1H, J=8.1 Hz, H-4), 7.73 (d, 1H, J=8.1 Hz, H-7); 13C NMR (CDCl3) ppm: 17.6 (q), 92.2

(s), 113.0 (s), 117.9 (d), 118.3 (d), 120.5 (d), 120.9 (d), 123.2 (d), 123.8 (s), 124.1 (s), 124.6 (s), 125.5 (d), 125.9 (d), 131.8 (s), 131.9 (d), 140.5 (s).

1-Cyano-2-(22-amino-42-methoxyphenyl)isoindole 3c: this compound was purified by chromatography by using DCM as eluent to give a solid: yield 95%**;** m.p. 116.0-117.0°C; IR 3465, 3375 (NH2), 2198 (CN) cm^{-1}; ^{1}H NMR ($CDCl_3$) ppm: 3.64 (bs, 2H, NH_2), 3.80 (s, 3H, CH_3), 6.38 (bs, 1H, H-3′), 6.42 (d, 1H, J=7.9 Hz, H-5′), 7.12 (d, 1H, J=7.9 Hz, H-6′), 7.14 (t, 1H, J=8.6 Hz, H-5), 7.29 (t, 1H, J=8.6 Hz, H-6), 7.41 (s, 1H, H-3), 7.66 (d, 1H, J=8.6 Hz, H-4), 7.70 (d, 1H, J=8.6 Hz, H-7); 13C NMR (CDCl3) ppm: 55.4 (q), 95.4 (s), 101.4 (d), 104.5 (d), 113.9 (s),

117.5 (s), 118.2 (d), 120.8 (d x 2), 123.1 (d), 124.5 (s), 125.8 (d), 128.7 (d), 131.7 (s), 143.3 (s), 161.5 (s).

1-Cyano-2-(2′-amino-4′,5′-dimethylphenyl)isoindole 3d: this compound was purified by chromatography by using DCM as eluent to give a solid: yield 85%; m.p. 119-120 °C; IR 3465, 3375 (NH_2), 2202 (CN) cm^{-1}; ^{1}H NMR ($CDCl_3$) ppm: 2.17 (s, 3H, CH_3), 2.22 (s, 3H, CH_3), 3.44 (s, 2H, NH_2), 6.64 (s, 1H, H-3′), 6.95 (s, 1H, H-6′), 7.12 (t, 1H,J=7.6 Hz, H-5), 7.27 (t,1H, J=7.6 Hz, H-6), 7.38 (s, 1H, H-3), 7.64 (d, 1H, J=7.6 Hz, H-4), 7.68 (d, 1H, J=7.6 Hz, H-7);

^{13}C NMR ($CDCl_3$) ppm: 18.6 (q), 19.7 (q), 95.1 (s), 114.0 (s), 118.1 (d), 118.2 (d), 120.6 (d), 120.9 (d), 121.8 (s), 123.1 (d), 124.5 (s), 125.8 (d), 127.0 (s), 128.2 (d), 131.7 (s), 139.6 (s), 139.7 (s).

1-Cyano-2-(2′-amino-4′,5′-dichlorophenyl)isoindole 3e: this compound was purified by chromatography by using DCM as eluent to give a solid: yield 77%; m.p. 71.0-72.0 °C; IR 3394, 3340 (NH_2), 2200 (CN) cm^{-1}; ^1H NMR (DMSO-*d*6) ppm: 5.75 (2H, s, NH_2), 7.12 (1H, s, H-3′), 7.16 (1H, t, J=8.4 Hz, H-5), 7.32 (1H, t, J=8.4 Hz, H-6), 7.58 (1H, s, H-6′), 7.66 (1H, d, J= 8.4 Hz, H-4), 7.78 (1H, d, J=8.4 Hz, H-7), 7.91 (1H, s, H-3);

^{13}C NMR (DMSO-*d*6) ppm: 94.0 (s), 113.8 (s), 115.8 (s), 116.2 (d), 117.5 (d), 121.6 (d), 121.7 (s), 122.4 (d), 122.6 (d), 124.2 (s), 125.8 (d), 129.6 (d), 131.2 (s), 132.9 (s), 144.7 (s).

1-Cyano-5,6-dimethyl-2-(2'-amino-4'-methoxiphenyl)-isoindole 3f: m.p. 179.0-180.0°C; yield 55%; IR: 3487, 3392 (NH_2), 2195 (CN) cm^{-1}; ^1H NMR $CDCl_3$ (ppm): 2.34 (3H, s, CH_3), 2.38 (3H, s, CH_3), 3.63 (2H, s, NH_2), 3.79 (3H, s, CH_3), 6.35-6.42 (1H, m), 7.11 (1H, d), 7.25 (1H, s), 7.20 (1H, t, J=7.6 Hz, H-5), 7.40-7.45 (2H, m);

^{13}C NMR $CDCl_3$ (ppm): 20.5 (q), 20.9 (q), 55.5 (q), 94.3 (s), 101.4 (d), 104.4 (d), 114.5 (s), 117.2 (d), 117.7 (s), 119.5 (d), 119.9 (d), 124.0 8s), 128.8 (d), 131.4 (s), 133.2 (s), 136.4 (s), 143.5 (s), 161.4 (s).

1-Cyano-5,6-dimethoxy-2-(2'-amino-4'-methoxyphenyl)-isoindole 3g: m.p. 223.0-224.0°C; yield 60%**;** IR: 3469, 3371 (NH_2), 2200 (CN) cm^{-1}; ^1H NMR $CDCl_3$ (ppm): 3.64 (2H, s, NH_2), 3.82 (3H, s, CH_3), 3.93 (3H, s, CH_3), 3.98 (3H, s, CH_3), 6.39-6.44 (2H, m), 6.93 (1H, d,), 6.90 (1H, s), 6.95 (1H, s), 7.21 (1H, s);

^{13}C NMR $CDCl_3$ (ppm): 55.5 (q), 55.9 (q), 56.1 (q), 94.7 (s), 96.2 (d), 98.3 (d), 101.4 (d), 104.4 (d), 114.5 (s), 117.8 (s), 119.7 (s), 119.8 (s), 128.2 (d), 128.9 (d), 143.5 (s), 148.8 (s), 151.1 (s), 161.3 (s)

Cyano-7-fluoro-2-(2'-amino-4'-methoxyphenyl)isoindole 3h: m.p. 118.0-119.0°C; yield 45%; IR: 3481, 3381 (NH_2), 2212 (CN) cm^{-1} ^1H NMR DMSO (ppm): 3.65 (2H, bs, NH_2), 3.81 (3H, s, CH_3), 6.39-6.45 (2H, m), 6.80-7.46 (5H, m);

^{13}C NMR $CDCl_3$ (ppm): 52.5 (q), 91.0 (s), 102.9 (d), 103.4 (d), 113.9 (s), 116.0 (d), 117.9 8s), 120.5 (d), 123.9 (d), 124.6 (s), 127.8 (d), 130.4 (s), 131.2 (s), 135.2 (s), 140.5 (s), 159.4 (s).

1-Cyano-6-methoxy-2-(2'-amino-4'-methoxyphenyl)- isoindole 3i: m.p. 146.0-147.0°C; yield 42%**;** IR: 3477, 3423 (NH_2), 2200 (CN) cm^{-1}; ^1H NMR $CDCl_3$ (ppm): 3.65 (2H, s, NH_2), 3.82 (3H, s, CH_3), 3.89 (3H, s, CH_3), 6.38-6.45 (2H, m), 6.85 (1H, dd,), 6.93 (1H, d), 6.93 (1H, d), 7.14 (1H, d), 7.32 (1H, d), 7.55 (1H, dd);

^{13}C NMR $CDCl_3$ (ppm): 55.4 (q), 55.5 (q), 95.0 (d), 101.5 (d), 104.5 (d), 114.5 (s), 117.7 (s), 118.4 (d), 120.6 (s), 121.2 (d), 122.2 (d), 128.8 8d), 133.2 (s), 143.4 (s), 158.5 (s), 161.4 (s).

1-Cyano-5-methoxy-2-(2'-amino-4'-methoxyphenyl)- isoindole 3j: m.p. 144.0-145.0°C; yield 40%**;** IR: 3465, 3375 (NH_2), 2198 (CN) cm^{-1}; ^1H NMR $CDCl_3$ (ppm): 3.63 (2H, s, NH_2), 3.83 (3H, s, CH_3), 3.85 (3H, s, CH_3), 6.38-6.45 (2H, m), 6.90 (1H, d,), 7.00 (1H, dd), 7.12-7.27 (3H, m), 7.61 (1H, d);

^{13}C NMR $CDCl_3$ (ppm): 55.3 (q), 55.5 (q), 97.2 (d), 101.4 (d), 104.5 (d), 117.7 (s), 118.1 (s), 119.5 (d), 119.6 (d), 120.8 (d), 124.9 (s), 127.9 (s), 128.8 (d), 143.3 (s), 156.2 (s), 161.4 (s).

2(2-amino-3,6-dimethoxyphenyl-2H-isoindole-1-carbonitrile 3l: m.p. 161.5-162.7°C; yield 20%; IR 3486,3389 (NH2), 2202 (CN) cm^{-1}; ^1H NMR DMSO-d6 (ppm):3.61 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 4.73 (2H, s, NH_2), 6.35 (1H, d, J=8.9 Hz, H-5'), 6.95 (1H, d, J=8.9 Hz, H-4'), 7.12 (1H, t, J=6.3 Hz, H-4), 7.29 (1H, t, J=6.3 Hz, H-3), 7.63 (1H, d, J=8.5 Hz, H-5), 7.73-7.78 (2H, m, H-2, H-6);

^{13}C NMR $CDCl_3$ (ppm): 97.16 (d), 111.37 (d), 111.84 (s), 114.09 (s), 117.59 (d), 121.61 (d), 122.13 (d), 122.58 (d), 124.14 (s), 125.37 (d), 131.00 (s),135.69 (s), 135.75 (s), 141.09 (s), 149.43 (s).

Synthesis of 1-cyano-2-(2'-amino-4'-methixy-phenyl)-isoindole 3k

To a solution of sodium hydrogen sulfite (1.56 g, 0.015 mol) in water (38 ml), phthalaldehyde **1e** (2 g, 0.015 mol) was added. The mixture was stirred until the solid was dissolved, and the appropriate 1,2-phenylenediamine **2c** (0.015 mol) was added. The reaction was heated on a steam bath for 30 min at 40°C, then KCN (3.39 g, 0.052 mol) in water (8.0 ml) was added, and the mixture was heated for an additional 90 min. The solid formed upon cooling was filtered and purified by chromatography in DCM.

1-cyano-2-(2'-amino-4'-methoxy-phenyl)-isoindole 3k: m.p. 105.0-106.0°C; yield 42%**;** IR: 3452, 3365 (NH_2), 2200 (CN) cm^{-1}; ^1H NMR $CDCl_3$ (ppm): 3.38 (2H, bs, NH_2), 3.77 (3H, s, CH_3), 6..82-6.96 (3H, m, H-3', H-4' e H-6'), 7.12 (1H, d, J=7.9 Hz, H-6'), 7.17(1H, t, J=8.0 Hz, H-5), 7.27 (1H, t, J=8.0 Hz, H-6), 7.47 (1H, s, H-3), 7.67.7.74 (2H, m, H-4 e H-7);

^{13}C NMR $CDCl_3$ (ppm): 55.9 (q), 95.0 (s), 112.7 (d), 113.8(s), 117.8 (d), 118.2 (d), 118.3(d), 120.4 (d), 120.9 (d), 123.3 (d), 124.4 (s), 124.5 (s), 126.0 (d), 131.8 (s), 135.7 (s), 152.5 (s).

Synthesis of 1-cyano-2-(2'-amino-4'-hydroxyphenyl)isoindoles 3

To a solution of sodium hydrogen sulfite (1.56 g, 0.015mol) in water (38 ml), phthalaldehyde **1a-b, 1d** (0.015 mol) was added. The mixture was stirred until the solid was dissolved, and the 3,4-diamino phenol **2g** (0.015 mol) was added. The reaction was stirred at rt for 3h, then KCN (3.39 g, 0.052 mol) in water (8.0 ml) was added, and the mixture was stirred at rt for additional 24h. The solid formed upon cooling was filtered and purified by chromatography using DCM as eluent.

1-Cyano-5,6-dimethyl-2-(2'-amino-4'-hydroxyphenyl)-isoindole 3m: m.p. 197.2-197.8°C; yield 10%**;** IR: 3487 e 3392 (NH_2), 3328 (OH), 2196 (CN) cm^{-1}; ^{1}H NMR DMSO-d6 (ppm): 2.30 (3H, s, CH_3), 2.34 (3H, s, CH_3), 4.92 (2H, bs, NH_2), 6.12 (1H, dd, J=2.5,8.5 Hz, H5'), 6.32 (1H, d, J=2.5Hz, H3'), 6.94 (1H, d, J=8.5 Hz, H6'), 7.39 (1H, s, H4), 7.47 (1H, s, H7), 7.57 (1H, s, H3), 9.50 (1H, s, OH);

^{13}C NMR DMSO-d6 (ppm): 20.0 (q), 20.3 (q), 93.2 (s), 101.6 (d), 103.8 (d), 114.6 (s), 115.4 (s), 116.3 (d), 120.0 (d), 121.5 (d), 123.4 (s), 128.6 (d), 130.7 (s), 131.8 (s), 135.5 (s), 145.1 (s), 159.0 (s).

1-Cyano-6-methoxy-2-(2'-amino-4'-hydroxyphenyl)-isoindole 3o: m.p. 211.2-212.0°C; yield 13%**;** IR: 3430 e 3345 (NH_2), 3303 (OH), 2200 (CN) cm^{-1}; ^{1}H NMR DMSO-d6 (ppm): 3.79 (3H, s, OCH_3), 4.92 (2H, bs, NH_2), 6.10 (1H, dd, J=2.5, 8.5 Hz, H5'), 6.30 (1H, d, J=2.5 Hz, H3'), 6.94 (1H, d, J=8.5 Hz, H6'), 6.96 (1H, dd, J=2.2, 9.1Hz, H5), 7.07 (1H, d, J=2.2 Hz, H7), 7.54 (1H, d, J=9.1 Hz, H4), 7.58 (1H, s, H3), 9.49 (1H, s, OH);

^{13}C NMR DMSO-d6 (ppm): 55.0 (q), 94.5 (s), 98.2 (d), 101.6 (d), 103.7 (d), 114.2 (s), 115.3 (s), 118.9 (d), 120.0 (d), 121.3 (d), 124.3 (s), 127.1 (s), 128.6 (d), 145.2 (s), 155.1 (s), 159.0 (s).

1-Cyano-5-methoxy-2-(2'-amino-4'-hydroxyphenyl)-isoindole 3p: m.p. 194.4-195.5°C; yield 10%**;** IR: 3475 e 3372 (NH_2), 3266 (OH), 2198 (CN) cm^{-1}; ^{1}H NMR DMSO-d6 (ppm): 3.84 (3H, s, OCH_3), 4.97 (2H, bs, NH_2), 6.09 (1H, dd, J=2.5, 8.5 Hz, H5'), 6.29 (1H, d, J=2.5 Hz, H3'), 6.79 (1H, dd, J=2.2, 9.0 Hz, H5), 6.89 (1H, d, J=2.2

Hz, H7), 6.93 (1H, d, J=8.5Hz, H6'), 7.64 (1H, d, J=9.0 Hz, H4), 7.66 (1H, s, H3), 9.47 (1H, s, OH) ;

^{13}C NMR DMSO-d6 (ppm): 55.2 (q), 93.8 (s), 94.7 (d), 101.6 (d), 103.7 (d), 114.7 (s), 115.3 (s), 116.9 (d), 120.0 (s), 121.3 (d), 123.0 (d), 128.6 (d), 132.3 (s), 145.2 (s), 157.9 (s), 159.0 (s).

1-Cyano-4-fluoro-2-(2'-amino-4'-hydroxyphenyl)-isoindole 3q: m.p. 203.6-204.7°C; yield 20%**;** IR: 3482 e 3376 (NH_2), 3234 (OH), 2217 (CN) cm^{-1}; ^{1}H NMR DMSO-d6 (ppm): 5.09 (2H, bs, NH_2), 6.10 (1H, dd, J=2.5, 8.5 Hz, H5'), 6.30 (1H, d, J=2.5 Hz, H3'), 6.98 (1H, d, J=8.5 Hz, H6'), 7.02-7.14 (2H, m, H5,H7), 7.58 (1H, dt, J_F=2.5 Hz, J=6.7 Hz, H6), 7.87 (1H, d, J_F=2.5 Hz, H3), 9.52 (1H, s, OH);

^{13}C NMR DMSO-d6 (ppm): 92.1 (d, J_F=2.8 Hz), 101.6 (d), 103.7 (d), 108.1 (dd, J_F=16.7 Hz), 114.0 (s), 114.7 (s), 117.90 (dd, J_F= 4.3 Hz), 121.1 (d, J_F=18 Hz), 122.4 (dd, J_F=6.4 Hz), 123.7 (d), 126.7 (d, J_F=6.7 Hz), 128.7 (d), 145.3 (s), 153.7 (d, J_F=248 Hz), 159.3 (s).

Synthesis of 1-cyano-2-(2'-amino-4'-hydroxyphenyl)-isoindole 3n

To a solution of sodium hydrogen sulfite (1.56 g, 0.015mol) in water (38 ml), phthalaldehyde **1c** (0.015 mol) was added. The mixture was stirred until the solid was dissolved, and the 3,4-diamino phenol **2g** (0.015 mol) was added. The reaction was stirred at rt for overnight, then KCN (3.39 g, 0.052 mol) in water (8.0 ml) was added, and the mixture was stirred at rt for additional 24h. The solid formed upon cooling was filtered and purified by chromatography using DCM- ethyl acetate 8/2 as eluent.

1-Cyano-5,6-dimethoxy-2-(2'-amino-4'-hydroxyphenyl)-isoindole 3n: m.p. 202.4-203.2°C; yield 30%**;** IR: 3467 e 3384 (NH_2), 3326 (OH), 2186 (CN) cm^{-1}; ^{1}H NMR DMSO-d6 (ppm): 3.79 (3H, s, CH_3), 3.86 (3H, s, CH_3), 4.92 (2H, bs, NH2), 6.09 (1H, dd, J=2.5, 8.5 Hz, H5'), 6.30 (1H, d, J=2.5 Hz, H3'), 6.90 (1H, s, H4), 6.92 (1H, d, J=8.5Hz, H6'), 7.05 (1H, s, H7), 7.48 (1H, s, H3), 9.46 (1H, s, OH);

^{13}C NMR DMSO-d6 (ppm): 55.3 (q), 55.5 (q), 93.6 (s), 95.8 (d), 99.3 (d), 101.6 (d), 103.7 (d), 114.8 (s), 115.5 (s), 119.1 (s), 121.4 (d), 127.3 (s), 128.6 (d), 145.2 (s), 148.0 (s), 150.7 (s), 158.9 (s).

Synthesis of isoindolo[2,1*a*]quinoxalin-6-imino 4

A solution of 1-cyano-2-(2'-amino-phenyl)isoindoles **3** (3 mmol) in acetic acid (10 ml) was stirred under reflux for 30 min. After cooling the mixture was evaporated under reduced pressure and the resulting solid crystallized in ethanol. In case of derivatives **4m, 4o, 4q** the precipitate formed after cooling was filtered, collected and crystallized in ethanol.

5,6-dihydroisoindolo[2,1-a]quinoxalin-6-amine 4a: m.p. 248.5-249.9°C; yield quant.; IR 3367, (NH_2) cm^{-1}; ^1H NMR DMSO-d6 (ppm): 6.87 (2H, bs, NH_2), 7.28-7.36 (3H, m, H-4, H-8, H-9), 7.46 (1H, t, J=7.0 Hz, H-3), 7.58 (1H, dd, J=1.2, 8.1 Hz, H-2), 7.86-7.90 (1H, m, H-7), 8.38 (1H, d, J=8.1, H-5), 8.42-8.47 (1H, m, H-10), 8.93 (1H, s, H-6). ^{13}C NMR DMSO-d6 (ppm): 117.66 (d), 108.02 (s), 115.64 (d), 119.43 (d), 119.54 (s), 120.16 (d), 122.14 (d), 122.67 (d), 123.06 (d), 123.60 (s), 125.46 (d), 125.72 (s), 126.92 (d), 138.35 (s), 151.40 (s).

4-methyl-5,6-dihydroisoindolo[2,1-a]quinoxalin-6-amine 4b: m.p. 210.3-211.6°C; yield 95%; IR 3309, 3320 (NH_2) cm^{-1}; ^1H NMR DMSO-d6 (ppm): 2.60 (3H, s, CH_3), 6.81 (2H, bs, NH_2), 7.17-7.37 (4H, m, H-3, H-4, H-8, H-9), 7.85-7.89 (1H, m. H-7), 8.22 (1H, d, J=7.7 Hz, H-5), 8.43-8.48 (1H, m, H-10), 8.89 (1H, s, H-6);
^{13}C NMR DMSO-d6 (ppm): 18.23 (q), 99.49 (d), 107.97 (s), 113.39 (d), 119.36 (d), 119.51 (s), 120.23 (d), 121.58 (d), 122.15 (s), 122.54 (d), 123.05 (d), 123.28 (s), 123.83 (s), 127.59 (d), 133.39 (s), 150.37 (s).

3-methoxy-5,6-dihydroisoindolo[2,1-a]quinoxalin-6-amine 4c: m.p. 242.5-243.0°C; yield quant.; IR 3303, 3500(NH_2) cm^{-1}; ^1H NMR DMSO-d6 (ppm): 3.86 (3H, s, OCH_3), 6.83 (2H, bs, NH_2), 6.93 (1H, dd, J=2.8, 8.9 Hz, H-4), 7.04 (1H, d, J=2.7 Hz, H-2), 7.28 (1H, dd, J=2.9, 6.5 Hz, H-5, H-8), 7.84 (1H, m, H-9), 8.29 (1H, d, J=9.0 Hz, H-7), 8.42 (1H, m, H-10), 8.82 (1H, s, H-6).
^{13}C NMR DMSO-d6 (ppm): 55.28 (q), 107.51 (d), 107.52 (d), 110.40 (d), 116.61 (d), 117.89 (s), 118.58 (s), 119.22 (d), 119.53 (s), 119.98 (d), 122.36 (d), 122.81 (d), 125.67 (s), 129.93 (s), 151.73 (s), 158.24 (s).

Crystallographic Data of compound **4c**

Empirical formula	$C_{18}H_{17}N_3O_3$
Formula weight	323.35
Wavelength (Å) / Temperature (K)	1.54184 / 293(2)
Crystal system	monoclinic
Space group	$P2_1 / c$
a (Å)	12.8001(2)
b (Å)	17.7147(3)
c (Å)	6.91504(11)
β (deg)	101.1176(16)
Volume (Å^3)	1538.56(4)
Z (molecules/unit cell)	4
Calculated density (Mg m^{-3})	1.396
Absorption coefficient, μ (mm^{-1})	0.796
F(000)	680.0
Total reflections	18882
Index ranges (h, k, l)	-15/15, -20/21, -8/8
Independent (unique) reflections / R_{int}	2754 / 0.0242
Observed reflections [$I > 2\sigma(I)$]	2562
Data / parameters / restraints	2754 / 219 / 0
Goodness–of–fit [a] on F^2	1.069
Final R indices [$I > 2\sigma(I)$]	R_1 [b] = 0.0458; wR_2 [c] = 0.1313
Largest difference peak and hole (eÅ^{-3})	0.28 and –0.21

[a] Goodness–of–fit = $[\Sigma (w (F_o^2 - F_c^2)^2] / (N_{obsevns} - N_{params})]^{1/2}$, based on all data;

[b] $R_1 = \Sigma (|F_o| - |F_c|) / \Sigma |F_o|$;

[c] $wR_2 = [\Sigma[w (F_o^2 - F_c^2)^2] / \Sigma[w (F_o^2)^2]]^{1/2}$.

2,3-dimethyl-5,6-dihydroisoindolo[2,1-a]quinoxalin-6-amine 4d: m.p. 238.2-239.0°C; yield 90%; IR 3362, 3401 (NH_2) cm^{-1}; ^{1}H NMR DMSO-d6 (ppm): 2.34 (3H, s, CH_3), 2.40 (3H, s, CH_3), 6.71 (2H, bs, NH_2), 7.25-7.30 (2H, m, H-8, H-9), 7.37 (1H, s, H-2), 7.83-7.88 (1H, m, H-7), 8.11 (1H, s, H-5), 8.39-8.43 (1H, m, H-10), 8.83 (1H, s, H-6);

^{13}C NMR DMSO-d6 (ppm): 19.37 (q), 21.05 (q), 106.91 (d), 108.06 (s), 115.83 (d),

119.26 (d), 119.38 (s), 120.13 (d), 121.52 (s), 122.28 (d), 122.85 (d), 125.70 (s), 125.74 (d), 131.02 (s), 135.46 (s), 136.41 (s), 150.98 (s).

2,3-dichloro-5,6-dihydroisoindolo[2,1-a]quinoxalin-6-amine 4e: m.p. 241.9-242.6°C; yield quant.; IR 3557, 3398 (NH_2) cm^{-1}; ^1H NMR DMSO-d6 (ppm): 7.14 (2H, bs, NH_2), 7.30-7.35 (2H, m, H-8, H-9), 7.69 (1H, s, H-2), 7.83-7.89 (1H, m, H-10), 8.77 (1H, s, H-5), 9.03 (1H, s, H-6);

^{13}C NMR DMSO-d6 (ppm): 99.34 (s), 107.72 (d), 109.23 (s), 117.57 (s), 119.69 (s), 119.91 (d), 120.110 (s), 123.11 (d), 123.34 (s), 123.45 (d), 123.50 (s), 125.88 (s), 128.91 (d), 138.81 (d), 152.31 (d).

3-methoxy-8,9-dimethyl-5,6-dihydroisoindolo[2,1-a]quinoxalin-6-amine 4f: m.p. 243.1-244.0°C; yield 95%; IR 3399, 3398 (NH_2) cm^{-1}; ^1H NMR DMSO-d6 (ppm): 2.38 (3H, s, CH_3), 2.42 (3H, s, CH_3), 3.84 (3H, s, OCH_3), 6.74 (2H, bs, NH_2), 6.89 (1H, dd, J=2.8, 8.9 Hz, H-4), 7.00 (1H, d, J=2.7 Hz, H-2), 7.58 (1H, s, H-7), 8.19-8.23 (2H, m, H-5, H-10), 8.64 (1H, s, H-6);

^{13}C NMR DMSO-d6 (ppm): 20.23 (q), 20.38 (q), 56.24 (q), 106.31 (d), 106.80 (s), 107.47 (d), 110.07 (d), 116.36 (d), 118.09 (d), 118.96 (d), 119.06 (s), 125.16 (s), 132.14 (s), 132.25 (s), 139.75 (s), 151.62 (s), 157.95 (s).

3-methoxy-8,9-dimethoxy-5,6-dihydroisoindolo[2,1-a]quinoxalin-6-amine 4g: m.p. 356.8-358.2°C; yield quant; IR 3439, 3366 (NH_2) cm^{-1}; ^1H NMR DMSO-d6 (ppm): 3.84 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 6.77 (2H, bs, NH_2), 6.87 (1H, dd, J=2.8, 8.9 Hz, H-4), 6.97 (1H, d, J=2.7, H-2), 7.14 (1H, s, H-7), 7.63(1H, s, H-10), 8.13 (1H, d, J=9.0 Hz, H-5), 8.55 (1H, s, H-6);

^{13}C NMR DMSO-d6 (ppm): 55.22 (q), 55.84 (q), 55.98 (q), 97.66 (d), 99.24 (d), 106.45 (d), 107.37 (s), 107.47 (d), 109.81 (d), 114.98 (s), 115.86 (d), 118.39 (s), 121.36 (s), 139.27 (s), 148.26 (s), 148.40 (s), 151.51 (s), 157.61 (s);

2-methoxy-8,9-dimethoxy-5,6-dihydroisoindolo[2,1-a]quinoxalin-6-amine 4k: m.p. 182.0-182.3°C; yield quant.; IR 3500, 3347 (NH_2) cm^{-1}; ^1H NMR DMSO-d6 (ppm): 3.94 (3H, s, OCH_3), 6.70 (2H, bs, NH_2), 7.12 (1H, dd, J=2.7, 8.9 Hz, H-3), 7.28-7.33 (2H, m, H-9, H-9), 7.53 (1H, d, J=8.9 Hz, H-2), 7.86-7.90 (1H, m, H-7), 7.91 (1H, d, J=2.6 Hz, H-4), 8.41-8.45 (1H, m, H-10), 8.98 (1H, s, H-6)

^{13}C NMR DMSO-d6 (ppm): 55.87 (q), 99.16 (d), 108.07 (d), 115.57 (d). 116.73 (s), 119.57 (d), 120.37 (d), 122.59 (d), 123.80 (d), 125.80 (s), 126.33 (d), 131.68 (s), 149.83

(s), 155.22 (s), 155.74 (s), 159.66 (s).

1,4-dimethoxy-5,6-dihydroisoindolo[2,1-a]quinoxalin-6-amine 4l: m.p. 272.4-272.9°C; yield 90%; IR 3352, 3339 (NH_2) cm^{-1}; ^1H NMR DMSO-d6 (ppm): 3.87 (3H, s, OCH_3), 4.06 (3H, s, OCH_3), 6.87-7.02 (4H, m, NH_2, H-3, H-4), 7.25-7.35 (2H, m, H-8, H-9), 7.90-7.95 (1H, m, H-7), 8.41-8.45 (1H, m, H-10);

^{13}C NMR DMSO-d6 (ppm): 55.81 (q), 56.39 (q), 103.27 (d), 107.51 (d), 108.33 (s), 113.91 (d), 114.88 (s), 118.17 (s), 119.71 (d), 119.76 (d), 122.69 (d), 122.89 (d), 125.20 (s), 130.94 (s), 144.58 (s), 147.39 (s), 150.71 (s).

3-idrossi-5*H*-8,9-dimetil-isoindolo[2,1*a*]chinossalin-6-imino 4m: m.p. 289.1-289.4°C, yield 55%, IR: 3390 (OH), 3284 e 3264 (NH) cm^{-1}; ^1H NMR DMSO-d6 (ppm): 2.38 (3H, s, CH_3), 2.42 (3H, s, CH_3), 6.77 (1H, bs, NH), 6.78 (1H, dd, J=2.6, 8.8 Hz, H4), 6.91 (1H, d, J=2.6 Hz, H2), 7.59 (1H, s, H7), 8.10 (1H, d, J=8.8 Hz, H5), 8.14 (1H, s, H10), 8.57 (1H, s, H6), 9.08 (1H, bs, OH), 9.59 (1H, bs, NH).

^{13}C NMR DMSO-d6 (ppm): 20.2 (q), 20.4 (q), 106.3 (d), 106.7 (s), 109.4 (d), 110.9 (d), 116.2 (d), 117.1 (s), 118.1 (d), 119.0 (d), 119.1 (s), 125.1 (s), 132.0 (s), 132.1 (s), 139.6 (s), 151.4 (s), 156.1 (s).

6-amino-8,9-dimethoxy-5,6-dihydroisoindolo[2,1-a]quinoxalin-3-ol, 4n: m.p. 242.5-243.0°C; yield quant.; IR 3677, 3557 (NH_2), 3348 (OH) cm^{-1}; ^1H NMR DMSO-d6: 3.85 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 6.68 (3H, m, NH2, H-4), 6.86 (1H, d, J=2.4 Hz, H-2), 7.13 (1H, s, H-10), 7.61 (1H, s, H-7), 8.02 (1H, d, J= 8.9 Hz, H-5), 8.49 (1H, s, H-6);

^{13}C NMR DMSO-d6 (ppm):55.19 (q), 55.81 (q), 97.65 (d), 99.50 (d), 106.03 (d), 107.32 (s), 109.42 (d), 110.68 (d), 114.86 (d), 115.68 (s), 117.45 (s), 118.66 (s), 121.23 (s), 139.32 (s), 148.12 (s), 148.22 (s), 151.38 (s), 155.73 (s).

3-idrossi-5*H*-8-metossi-isoindolo[2,1*a*]chinossalin-6-imino 4o: m.p. 279.1-280.4 °C, yield 85%, IR: 3306 (OH), 3280 e 3244 (NH) cm^{-1}; ^1H NMR DMSO-d6 (ppm): 3.92 (3H, s, CH_3), 6.72 (1H, dd, J=2.6, 8.8 Hz, H4), 6.87 (1H, bs, NH), 6.88 (1H, d, J=2.6 Hz, H2), 6.93 (1H, dd, J= 1.9, 9.1 Hz, H9), 7.63 (1H, d, J=1.9 Hz, H7), 7.73 (1H, d, J=9.1 Hz, H10), 8.07 (1H, d, J=8.8 Hz, H5), 8.65 (1H, s, H6), 9.05 (1H, bs, OH), 9.96 (1H, bs, NH).

^{13}C NMR DMSO-d6 (ppm): 55.5 (q), 98.2 (d), 107.2 (s), 107.4 (d), 109.3 (d), 110.8 (d), 116.0 (d), 116.9 (d), 117.2 (s), 120.4 (s), 121.7 (d), 139.4 (s), 151.7 (s), 155.9 (s), 156.2 (s), 172.1 (s).

3-idrossi-5*H*-7-fluoro-isoindolo[2,1*a*]chinossalin-6-imino 4q: m.p. 340°C, yield 86 %, IR: 3387 (OH), 3264 e 3252 (NH) cm^{-1}; ^{1}H NMR DMSO-d6 (ppm): 6.78 (1H, bs, NH), 6.81 (1H, dd, J=2.4, 8.9 Hz, H4), 6.93 (1H, d, J=2.4 Hz, H2), 7.03-7.29 (3H, m, H6-H8-H9), 7.69 (1H, d, J=8.2 Hz, H10), 8.20 (1H, d, J=8.9 Hz, H5) 8.92 (1H, bs, OH), 10.60 (1H, bs, NH). ^{13}C NMR DMSO-d6 (ppm): 99.5 (s), 105.8 (d, J_F=4.1 Hz), 106.5 (dd, J_F=22.9 Hz), 108.4 (d), 108.8 (d, J_F=16.8 Hz), 109.5 (d), 111.7 (d), 116.3 (dd, J_F=3.6 Hz), 116.4 (s), 116.8 (d), 123.1 (dd, J_F=8.1 Hz), 128.6 (d, J_F=7.4 Hz), 139.9 (s), 150.2 (s), 154.5 (d, J_F=246.4 Hz).

Synthesis of isoindole[2,1*a*]quinoxalin-6-one 5

A solution of 1-cyano-2-(2'-amino-phenyl)isoindoles **3** (3 mmol) in acetic acid (10 ml) was stirred under reflux for 30 min. After cooling the mixture was poured onto ice. The resulting precipitate was filtered off and crystallized in ethanol.

3-Methoxy-5*H*-8,9-dimethyl-isoindole[2,1*a*]quinoxalin-6-one 5f m.p. >410°C; yield 60%; IR: 3435 (NH), 1722 (CO) cm^{-1}; ^{1}H NMR DMSO (ppm): 2.40 (3H, s, CH_3), 2.45 (3H, s, CH_3), 3.89 (3H, s, CH_3), 7.15 (s, 1H), 7.72 (s, 1H), 8.38-8.46 (m, 3H), 9.11 (s, 1H), 13.46 (1H, bs, NH).

3-Methoxy-5*H*-8,9-dimethoxy-isoindolo[2,1*a*]quinoxalin-6-one 5g: m.p.<410°C; yield 72%**;** IR: 3556 (NH), 1637 (CO) cm^{-1}; ^{1}H NMR DMSO (ppm): 3.89 (3H, s, CH_3), 3.94 (3H, s, CH_3), 3.99 (3H, s, CH_3), 7.11-7.31 (2H, m), 7.82 (1H,s), 8.35-8.67 (2H, m), 9.1 (1H, d) 13.14 (1H, bs, NH).**3-Methoxy-5*H*-10-fluoro-isoindole[2,1*a*]quinoxalin-6-one 5h**: m.p. 246-247°C; yield 58%**;** IR: 3430 (NH), 1710 (CO) cm^{-1}; ^{1}H NMR DMSO (ppm): 3.90 (3H, s, CH_3), 7.16-7.46 (2H, m), 7.85 (1H, d), 8.09 (1H, s), 8.47 (1H, d), 9.40 (1H, s), 13.62 (1H, bs, NH).

3,8-Dimethoxy-5H-isoindole[2,1a]quinoxalin-6-one 5i: m.p.298-299°C; yield 60%; IR: 3280 (NH), 1635 (CO) cm^{-1}; ^{1}H NMR DMSO (ppm): 3.89 (1H, s, OCH_3), 3.95 (3H,s, OCH_3), 7.13-7.30 (3H, m,), 9.08 (1H, s), 13.2 (1H, bs, NH);

3,9-Dimethoxy-5*H*-isoindole[2,1*a*]quinoxalin-6-one 5j: m.p. 283-284°C; yield 60%; IR: 3309 (NH), 1641 (CO) cm^{-1}; ^{1}H NMR DMSO (ppm): ^{1}H NMR DMSO (ppm): 3.89 (1H, s, OCH_3), 3.97 (3H,s, OCH_3), 7.09-7.14 (3H, m, NH_2, H-2'), 7.80-7.93 (3H, m, H-5', H-4', H-6), 8.36 (1H, d J=8.8 Hz, H-5), 8.50 (1H, s, H-3), 9.17 (1H, s, H-2), 12.84 (1H, bs, NH);

4-Methoxy-5*H*-isoindole[2,1*a*]quinoxalin-6-one 5k: m.p. 291°C; yield 90%**;** IR 3360 (NH), 1634 (CO) cm^{-1}; ^1H NMR DMSO (ppm): 3.90 (3H, s, CH_3), 7.06 (1H, s), 7.09 (2H, m,), 7.45 (1H, s), 7.60 (1H, t), 8.00 (1H, s), 8.37 (2H, m), 13.4 (1H, bs, NH).

3-Methoxy-5*H*-8,9-dimethoxy-isoindole[2,1*a*]quinoxalin-6-one 5l: m.p. 282.7-289.0°C; yield 70%**;** IR: 3314 (NH), 1638 (CO) cm^{-1}; ^1H NMR DMSO (ppm): 4.00 (3H, s, OCH_3), 4.10 (3H, s, OCH_3), 7.15-7.31 (2H, m, H-3', H-6'), 7.47 (1H, t, J= 7.5 Hz, H-4), 8.62 (1H, t, J=7.50, H-5), 8.11 (1H, d, J=8.0 Hz, H-3'), 8.55 (1H, d, J=8.4, H-6'), 9.66 (1H, s, H-2), 13.14 (1H, bs, NH).

3-hydroxy-5*H*-8,9-dimethyl-isoindole[2,1*a*]quinoxalin-6-one 5m: m.p. >410°C, yield 88%, %, IR: 3280 (OH), 3189 (NH), 1637 (CO) cm^{-1}; ^1H NMR DMSO-d6 (ppm): 2.38 (3H, s, CH_3), 2.43(3H, s, CH_3), 6.95 (1H, dd, J=2.4, 9.1 Hz, H4), 7.07 (1H, d, J=2.4 Hz, H2), 7.71 (1H, s, H7), 8.30 (1H, d, J=9.1 Hz, H5), 8.32 (1H, s, H10), 9.03 (1H, s, H6), 10.40 (1H, bs, OH), 13.01 (1H, bs, NH).

3-hydroxy-5*H*-8,9-dimethoxy-isoindolo[2,1*a*]quinoxalin-6-one 5n: m.p. 287.2-288°C, yield 50%, IR: 3316 (OH), 3264 (NH), 1640 (CO) cm^{-1}; ^1H NMR DMSO-d6 (ppm): 3.88 (3H, s, CH_3), 3.97 (3H, s, CH_3), 6.93 (1H, dd, J=2.3, 9.0 Hz, H4), 7.04 (1H, d, J=2.3 Hz, H2), 7.26 (1H, s, H7),7.75 (1H, s, H10), 8.21 (1H, d, J=9.0 Hz, H5), 8.44 (1H, s, H6), 8.90 (1H, bs, OH), 13.04 (1H, bs, NH).

3-hydroxy-5*H*-8-methoxy-isoindole[2,1*a*]quinoxalin-6-one 5o: m.p. 335.0-335.7 °C, yield 83%, IR: 3306 (OH), 3280 (NH), 1635 (CO) cm^{-1}; ^1H NMR DMSO-d6 (ppm): 3.89 (3H, s, CH_3), 6.94 (1H, dd, J=2.3, 9.0 Hz, H4), 7.06 (1H, d, J=2.3 Hz, H2), 7.09 (1H, dd, J= 1.7, 9.1 Hz, H9),7.80 (1H, d, J=1.7 Hz, H7), 7.89 (1H, d, J=9.1 Hz, H5), 8.26 (1H, d, J=9.0 Hz, H10), 9.11 (1H, bs, OH), 13.05 (1H, bs, NH).

3-hydroxy-5*H*-9-methoxy-isoindole[2,1*a*]quinoxalin-6-one 5p: m.p.316.4-317.7 °C, yield 89%, IR: 3326 (OH), 3309 (NH), 1641 (CO) cm^{-1}; ^1H NMR DMSO-d6 (ppm): 3.89 (3H, s, CH_3), 7.00 (1H, dd, J=2.4, 9.1 Hz, H2), 7.10 (1H, d, J=2.4 Hz, H2), 7.21 (1H, dd, J= 2.1, 9.1 Hz, H8), 7.32 (1H, d, J=2.1 Hz, H10), 8.33 (1H, d, J=9.1 Hz, H5), 8.45 (1H, s, H6), 8.46 (1H, d, J=9.1 Hz , H7), 9.05 (1H, bs, OH), 13.50 (1H, bs, NH).

3-hydroxy-5*H*-7-fluoro-isoindole[2,1*a*]quinoxalin-6-one 5q: m.p. 362-363°C; yield 87%, IR: 3265 (OH), 3180 (NH), 1642 (CO) cm^{-1}; ^1H NMR DMSO-d6 (ppm): 7.02 (1H, dd, J=2.1, 9.0 Hz, H4), 7.12 (1H, d, J=2.1 Hz, H2), 7.31-7.48 (2H, m, H8-H9), 7.84 (1H, d, J=7.2 Hz, H10), 8.38 (1H, d, J=9.0 Hz, H5), 9.35 (1H, d, J_F=1.9 Hz, H6), 10.69 (1H, bs, OH), 14.11 (1H, bs, NH).

Synthesis of diethyl pyridine-2,3-dicarboxilate 20

Pyridin 2,3- dicarboxilic acid (12 mmol, 2g) was suspended in methanol (8 ml). To the mixture, sulfuric acid conc. (0.8 ml) was added and it was refluxed for 24h. The solvent was evaporated under reduced pressure and to the resulting oil a saturated solution (20 ml) of NaHCO3 was added until neutralization. It was extracted in ethyl acetate, dried and evaporated under reduced pressure.

Dimethyl pyridine-2,3-dicarboxilate, 20: 55.0-56.0°C; yield 78%; IR 1734 (CO) cm^{-1}; ^{1}H NMR DMSO (ppm): 3.88 (6H, s, 2xCH_3), 7.73 (1H, dd, J=4.8, 8.0 Hz, H-5), 8.32 (1H, d, J=8.0 Hz, H-4, 8.83 (1H, d, J=4.8 Hz, H-6); ^{13}C NMR DMSO-d6 (ppm): 52.72 (q), 52.94 (q), 124.88 (s), 125.57 (d), 137.87 (d), 150.57 (s), 152.29 (d), 165.07 (s), 166.37 (s).

Synthesis of pyridine-2,3-dicarbaldehyde 16

To a stirred solution of compound 20 (9.7 mmol, 1.9 g) in toluene (68 ml), cooled at -78°C and under nitrogen atmosphere, DIBAL 1M solution (26 ml) was added drop wise. After 30 min a solution of diethyl ether and acetic acid (10 ml, 1/1) was added and the mixture was stirred for 15 min more at -78°C and then warmed to rt in 1h. The resulting precipitate was filtered and suspended in toluene and filtered again (5 times). The organic layers were evaporated under reduced pressure. It was crystallized in ethanol.

Pyridine-2,3-dicarbaldehyde 16: Yield 60%; IR 1697, 1710 cm^{-1} ; ^{1}H NMR DMSO (ppm): 7.46-9.01 (3H, m, H-4, H-5, H-6), 10.19 (1H, s, CHO); 10.59 (1H, s, CHO).
^{13}C NMR DMSO-d6 (ppm): 127.78 (d), 135.93 (d), 153.27 (d), 191.63 (d), 194.39 (d).

Synthesis of 1,4-dimethoxy-2,3-dimethylbenzene 21

To a stirred solution of 2,3-dimethyl-hydroquinone (3.6 mmol, 0.5 g) in DMF (6 ml), Cs_2CO_3 (10.0 mmol, 3.3 g) and methylen iodide (10 ml) were added. The mixture was stirred at rt overnight. It was poured in water and ice, extracted in DCM, dried and evaporated.

1,4-dimethoxy-2,3-dimethylbenzene, 21: m.p. 78.0°C; yield 70%; ^{1}H NMR DMSO (ppm): 2.07 (6H, s, CH_3), 3.70 (6H, s, OCH_3), 6.71 (2H, s, H-5, H-6); ^{13}C NMR

DMSO-d6 (ppm): 11.83 (q), 55.58 (q), 107.91 (d).

Synthesis of 1,4-dimethoxy-2,3-dimethyl-5,6-dinitrobenzene 22

To a stirred solution of compound **21** (3 mmol, 0.5 g) in acetic anhydride (4ml), cooled at 0°C, nitric acid 90% (0.7 ml) was added drop wise. The mixture was warmed at 90°C for 1h and then cooled to rt and poured onto water and ice. The resulting precipitate was filtered off.

1,4-dimethoxy-2,3-dimethyl-5,6-dinitrobenzene, 22: m.p. 155.0°C; yield 80%; ^{1}H NMR DMSO (ppm): 2.31 (6H, s, CH_3), 3.84 (6H, s, OCH_3); ^{13}C NMR DMSO-d6 (ppm): 13.40 (q), 63.17 (q), 139.14 (s), 146.00 (s).

Synthesis of 3,6-dimethoxy-4,5-dimethyl-1,2-diamine, 2i

Method A: to a mixture of Sn (1.9 g) in HCl conc. compound **22** (2 mmol, 0.51 g) was added and it was stirred at rt for 72h. It was poured onto ice and extracted in ethyl acetate. It was dried, evaporated and purified in column using ethyl acetate as eluent. Yield 30%

Method B: to a solution of compound 22 (3.7 mmol, 0.95g) in methanol (20 ml) a catalytic amount of 10% Pd/C is added to the mixture that is hydrogenated on Paar for 24h at rt. The catalyst is filtered off and the filtrate evaporated under reduced pressure. Yield 96%

3,6-dimethoxy-4,5-dimethyl-1,2-diamine, 2i: mp. 114.0°C; IR 3410, 3332 (NH_2) cm^{-1}; ^{1}H NMR DMSO (ppm): 2.01 (3H, s, CH_3), 3.56 (3H, s, OCH_3), 4.17 (4H, bs, NH_2); ^{13}C NMR DMSO-d6 (ppm): 11.67 (q), 58.72 (q), 116.45 (s), 125.84 (s), 141.68 (s).

Synthesis of 1,4-dimethoxy-2,5-dinitrobenzene and 1,4-dimethoxy-2,3-dinitrobenzene, 15a-b

To a stirring solution of 1,4-dimethoxybenzene (10 mmol, 1.38 g) in acetic anhydride (10 ml) cooled at -10°C, a mixture of nitric acid and acetic anhydride (8.8 ml, 1/1) previously cooled at -15°C was added drop wise. The resulting yellow precipitate was filtered off and purified in column cycloexane-ethyl acetate 9/1,

1,4-dimethoxy-2,5-dinitrobenzene, 15a: m.p. 189.0-190.0°C; ^{1}H NMR DMSO (ppm): 3.95 (6H, s, 2xCH_3), 7.71 (1H, s, H-3, H-6); ^{13}C NMR DMSO-d6 (ppm): 57.80 (q), 119.11 (d), 144.80 (s), 132.65 (s).

1,4-dimethoxy-2,3-dinitrobenzene, 15b: m.p. 210.0-211.0°C; ^{1}H NMR DMSO (ppm): 3.92 (6H, s, 2xCH_3), 7.95 (1H, s, H-5, H-6); ^{13}C NMR DMSO-d6 (ppm): 57.58 (q), 110.93 (d), 141.42 (s), 142.75 (s).

Synthesis of 6-(2-amino)-6H-pyrrolo[3,4-b]pyridine-4-carbonitrile 17:

To a solution of sodium hydrogen sulfite (1.56 g, 0.015 mol) in water (3.8 ml), pyridine-2,3-dicarbaldehyde **16** (0.015 mol) was added. The mixture was stirred until the solid was dissolved, and the appropriate 1,2-phenylenediamine **2a, 2c-i** (0.015 mol) was added. The reaction was heated on a steam bath for 1h, then KCN (3.39 g, 0.052 mol) in water (8.0 ml) was added and the mixture was heated for additional 5h. The solid formed upon cooling was filtered and purified by chromatography using DCM-ethyl acetate 9/1 or 8/2.In the case of derivatives **17c** and **17d,** the 1,2-phenylenediamine **2e** was first dissolved in DMF (15 ml) and then added to the reaction mixture. In the case of derivative **17m** the reaction was performed at rt during all reaction time.

6-(2-amino-4,5-dimethylphenyl)-6H-pyrrolo[3.4-b]pyridine-5-carbonitrile, 17a: m.p. 176.5-177.6°C; yield 10% ; IR 3558, 3386 (NH_2), 2212 (CN) cm^{-1}; ^{1}H NMR DMSO (ppm): 2.13 (3H, s, CH_3), 2.19 (3H, s , CH_3), 4.95 (2H, bs, NH_2), 6.73 (1H, s, H-3'), 6.97 (1H, s, H-6'), 7.18 (1H, dd, J=3.7, 8.1 Hz, H-6), 7.84 (1H, s, H_3), 8.22 (1H, d, J= 8.1 Hz, H-7), 8.65 (1H, d, J= 2.2 Hz, H-5);

^{13}C NMR DMSO-d6 (ppm): 18.03 (q), 19.34 (q), 95.18 (s), 113.35 (s), 117.16 (s), 117.31 (d), 118.36 (d), 120.30 (s), 121.27 (d), 123.68 (s), 128.03 (d), 130.21 (d), 138.95 (s), 141.61 (s), 145.79 (s), 150.81 (d).

6-(2-amino-4,5-dimethylphenyl)-6H-pyrrolo[3.4-b]pyridine-7-carbonitrile, 17b: m.p. 136.0-137.0°C; yield 10% ; IR 3556, 3465 (NH_2), 2208 (CN) cm^{-1} ^{1}H NMR DMSO (ppm): 2.13 (3H, s, CH_3), 2.19 (3H, s, CH_3), 4.96 (2H, bs, NH_2), 6.72 (1H, s, H.3'), 6.97 (1H, s, H-6'), 7.29 (1H, dd, J=4.1, 8.6 Hz, H-5), 8.04 (1H, s, H-3), 8.16 (1H, d, J=8.5 Hz, H-4), 8.59 (1H, dd, J= 1.5, 4.1 Hz, H-6);

^{13}C NMR DMSO-d6 (ppm): 17.99 (q), 19.31 (q), 92.93 (s), 113.26 (s), 117.23 (d),

120.16 (s), 120.58 (d), 122.49 (d), 123.57 (d), 124.11 (s), 126.19 (d), 127.99 (d), 138.93 (s), 139.93 (s), 141.58 (s), 149.43 (d).

6-(2-amino-4,5-dichlorophenyl)-6H-pyrrolo[3,4-b]pyridine-5-carbonitrile, 17c: m.p. 194.5-195.3 °C; yield 10%; IR 3558, 3480 (NH_2), 2210 (CN) cm^{-1}; ^1H NMR DMSO (ppm): 5.78 (2H, bs, NH_2), 7.12 (1H, s, H-3), 7.21 (1H, dd, J=4.0, 8.4 Hz, H-6), 7.64 (1H, s, H6'), 7.96 (1H, s, H-3), 8.25 (1H, d, J=8.4 Hz, H-7), 8.68 (1H, d, J=3.8 Hz, H-5);

^{13}C NMR DMSO-d6 (ppm): 95.19 (s), 113.11 (s), 115.84 (s), 116.28 (d), 117.38 (s), 118.66 (d), 121.58 (d + s), 129.65 (d), 130.46 (d), 133.15 (s), 144.75 (s), 145.82 (s), 151.29 (d).

6-(2-amino-4,5-dichlorophenyl)-6H-pyrrolo[3,4-b]pyridine-7-carbonitrile, 17d: m.p. 348.6-350.4°C; yield 10%; IR 3396, 3330 (NH_2), 2206 (CN) cm^{-1}; ^1H NMR DMSO (ppm): 5.77 (1H, bs, NH2), 7.12 (1H, s, H-3'), 7.32 (1H, dd, J=4.0, 8.7 Hz, H-5), 7.64 (1H, s, H-6'), 8.17-8.21 (2H, m, H-3, H-4), 8.61 (1H, d, J=4, H-6);

^{13}C NMR DMSO-d6 (ppm): 92.96 (s), 112.99 (s), 115.74 (s), 116.20 (d), 121.47 (s), 122.78 (d), 124.35 (s), 126.34 (d), 129.60 (d), 133.07 (s), 137.19 (s), 144.69 (s), 148.77 (d).

6-(2-amino-3,6-dimethoxyphenyl)-6H-pyrrolo[3,4-b]pyridine-5-carbonitrile, 17e: m.p. 214.2-214.7°C; yield 12%; IR 3284, 3189 (NH_2), 2214 (CN) cm^{-1} ^1H NMR DMSO (ppm): 3.62 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 4.84 (2H, bs, NH_2), 6.35 (1H, d, J=8.9 Hz, H-4'), 6.96 (1H, d, J=8.9 Hz, H-5'), 7.16 (1H, dd, J=4.1, 8.5 Hz, H-6), 7.77 (1H, s, H-3), 8.21 (1H, dd, J= 1.5, 8.5 Hz, H-7), 8.64 (1H, dd. J= 1.6 , 4.2 Hz, H-5);

^{13}C NMR DMSO-d6 (ppm): 55.72 (q), 56.06 (q), 95.39 (s), 97.05 (d), 111.54 (d), 111.65 (s), 113.28 (s), 117.31 (s), 118.21 (d), 121.66 (d), 130.32 (d), 135.73 (s), 141.10 (s), 145.61 (s), 149.31 (s), 150.81 (d).

6-(2-amino-3,6-dimethoxyphenyl)-6H-pyrrolo[3,4-b]pyridine-5-carbonitrile, 17f: m.p. 196.8-198.4°C; yield 15%; IR 3486, 3392 (NH_2), 2206 (CN) cm^{-1}; ^1H NMR DMSO (ppm):3.62 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 4.85 (2H, bs, NH_2), 6.36 (1H, d, J=8.9 Hz, H-4'), 6.96 (1H, d, J=9.1 Hz, H-5'), 7.28 (1H, dd, J=4.1, 8.6 Hz, H-5), 7.98 (1H, s, H-3), 8.15 (1H, dt, J=9.5 Hz, H-4), 8.57 (1H, dd, J=1.5, 4.1 Hz, H-6);

^{13}C NMR DMSO-d6 (ppm): 55.73 (q), 56.05 (q), 93.28 (s), 97.07 (d), 99.50 (s), 111.54 (d), 113.25 (s), 120.59 (d), 122.88 (d), 121.10 (s), 126.34 (d), 135.74 (s), 139.25 (s), 141.10 (s), 148.39 (d), 149.27 (s).

6-(2-amino-4,5-dimethoxyphenyl)-6H-pyrrolo[3,4-b]pyridine-5-carbonitrile, 17g: m.p. 178.3-179.2°C; yield 15%; IR: 3218,3116 (NH_2), 2208 (CN) cm^{-1} ^1H NMR DMSO (ppm): 3.65 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 4.85 (2H, bs, NH_2). 6.58 (1H, s, H-3'), 6.89 (1H, s, H-6'), 7.18 (1H, dd, J= 4.17, 8.5 Hz, H-6), 7.86 (1H, s, H-3), 8.22 (1H, dd, J=1.55, 8.5 Hz, H-7), 8.65 (1H, dd, J=4.54, 4.15 Hz, H-5);

^{13}C NMR DMSO-d6 (ppm): 55.39 (q), 56.41 (q), 95.53 (s), 100.23 (d), 112.49 (d), 113.43 (s), 113.88 (s), 117.18 (s), 118.35 (d), 121.61 (d), 130.20 (d), 138.52 (s), 139.68 (s), 145.70 (s), 150.82 (d), 150.97 (s).

6-(2-amino-4,5-dimethoxyphenyl)-6H-pyrrolo[3,4-b]pyridine-7-carbonitrile, 17h: m.p. 216.2-217.3°C; yield 15%; IR 3320, 3207 (NH2), 2202 (CN) cm^{-1}; ^1H NMR DMSO (ppm): 3.65 (3H, s, OCH_3), 3.76 (3H, s, OCH_3), 4.85 (2H, bs, NH_2), 6.57 (1H, s, H-3'), 6.89 (1H, s, H-6'), 7.29 (1H, dd, J= 4.1, 8.6 Hz, H-5), 8.05 (1H, s, H-3), 8.14 (1H, d, J=8.5 Hz, H-4), 8.6 (1H, dd, J=1.3, 4.02 Hz, H-6);

^{13}C NMR DMSO-d6 (ppm): 55.40 (q), 56.40 (q), 95.35 (s), 100.25 (d), 112.50 (d), 113.38 (s), 113.85 (s), 120.64 (d), 122.84 (d), 124.11 (s), 124.21 (d), 138.50 (s), 139.13 (s), 139.69 (s), 148.49 (d), 150.99 (s).

6-(2-amino-3,6-dimethoxy-4,5-dimethylphenyl)-6H-pyrrolo[3,4-b]pyridine-5-carbonitrile, 17i: m.p. 186.0-187.0°C, yield 11%; IR 3457, 3436 (NH_2), 2212 (CN) cm^{-1} ^1H NMR DMSO (ppm): 2.08 (3H, s, CH_3), 2.19 (3H, s, CH_3), 3.28 (3H, s, OCH_3), 3.65 (3H, s, OCH_3), 4.81 (2H, s, NH_2), 7.19 (1H, dd, J=4.1, 7.5 Hz, H-6), 7.88 (1H, s, H-3), 8.24 (1H, dd, J=1.6, 8.6 Hz, H-7), 8.66 (1H, dd, J=1.6, 4.2 Hz, H-5);

^{13}C NMR DMSO-d6 (ppm):11.79 (q), 11.64 (q), 61.07 (q), 95.66 (s), 113.33 (s), 115.08 (s), 115.96 (s), 117.17 (s), 118.35 (d), 122.19 (d), 130.39 (d), 132.10 (s), 136.49 (s), 141.16 (s), 145.64 (s), 149.87 (s), 150.95 (d).

6-(2-amino-3,6-dimethoxy-4,5-dimethylphenyl)-6H-pyrrolo[3,4-b]pyridine-7-carbonitrile, 17j: m.p.180.0°C; yield 26 %; IR 3475, 3374 (NH_2), 2204 (CN) cm^{-1}; ^1H NMR DMSO (ppm): 2.08 (3H, s, CH_3), 2.19 (3H, s, CH_3), 3.28 (3H, s, OCH_3), 3.65 (3H, s, OCH_3), 4.80 (2H, s, NH_2), 7.30 (1H, dd, J=4.1, 8.6 Hz, H-5), 8.09 (1H, s, H-3), 8.18 (1H, d, J= 8.4 Hz, H-4), 8.60 (1H, dd, J=1.4, 4.1 Hz, H-6);

^{13}C NMR DMSO-d6 (ppm):11.79 (q), 13.63 (q), 59.23 (q), 61.11 (q), 93.57 (s), 113.29 (s), 115.07 (s), 115.98 (s), 120.70 (d), 123.38 (d), 124.12 (s), 126.38 (d), 132.11 (s), 136.48 (s), 139.16 (s), 141.17 (s), 148.93 (d), 149.86 (s).

6-(2-aminophenyl)-6H-pyrrolo[3,4-b]pyridine-5-carbonitrile, 17k: m.p. 164.7-165.9°C; Yield 30%; IR 3423, 3311 (NH_2); 2200 (CN) cm^{-1}; ^1H NMR DMSO (ppm):5.28 (2H, bs, NH_2), 6.70 (1H, t, J=7.5 Hz, H-5'), 6.92 (1H, d. J=8.3 Hz, H-6), 7.19-7.23 (2H, m, H-3', H-6'), 7.27 (1H, t, J=7.5 Hz, H-7), 8.67 (1H, d, J=3.9 Hz, H-5); ^{13}C NMR DMSO-d6 (ppm): 95.12 (s), 113.34 (s), 115.73 (d), 116.18 (d), 117.29 (s), 118.48 (d), 121.34 (d), 122.40 (s), 127.91 (d), 130.34 (d), 130.74 (d), 144.21 (s), 145.88 (s), 150.97 (d).

6-(2-aminophenyl)-6H-pyrrolo[3,4-b]pyridine-7-carbonitrile, 17l: m.p. 180.3-181.4°C; yield 40%; IR 3478, 3392 (NH_2), 2208 (CN) cm^{-1} ^1H NMR DMSO (ppm): 5.28 (2H, s, NH2), 6.70 (1H, dt, J=1.2, 7.5 Hz, H5'), 6.92 (1H, dd, J=1.2, 8.5 Hz, H-3'), 7.19-7.34 (3H, m, H-4', H-6', H-5), 8.12 (1H, s, H-3), 8.15-8.20 (1H, dt, J= 1.3, 8.5 Hz, H-4), 8.6 (1H, dd, J=1.5, 4.1 Hz, H-6);

^{13}C NMR DMSO-d6 (ppm): 92.84 (s), 113.18 (s), 115.60 (d), 116.07 (d), 120.66 (d), 122.26 (s), 122.50 (d), 124.20 (s), 126.24 (d), 127.81 (d), 130.64 (d), 139.20 (s), 144.11 (s), 148.50 (d).

6-(2-amino-4-methoxyphenyl)-6H-pyrrolo[3,4-b]pyridine-5-carbonitrile, 17m: m.p. 185.0-186°C; yield 35%; 3308, 3415 (NH_2), 2255 (CN) IR cm^{-1} ^1H NMR DMSO (ppm): 3.76 (3H, s, OCH_3), 5.27 (2H, bs; NH_2), 6.27 (1H, dd, J=2.6, 8.7 Hz, H-4'), 6.45 (1H, d, J=2.6 Hz, H-2'), 7.12 (1H, d, J=8.7 Hz, H-5'). 7.20 (1H, t, J=4.2 Hz, H-6), 7.84 (1H, s, H-3), 8.22 (1H, dd, J=1.3, 8.5 Hz, H-7), 8.65 (1H, dd, J=1.4, 4.1 Hz, H-5);

^{13}C NMR DMSO-d6 (ppm): 55.06 (q), 95.47 (s), 100.04 (d), 102.16 (d), 113.39 (s), 116.14 (s), 117.20 (s), 118.36 (d), 121.66 (d), 128.80 (d), 130.25 (d), 145.33 (s), 145.75 (s), 150.82 (d), 160.95 (s).

Synthesis of 7-azaisoindole[2,1-a]quinoxalin-6(5H)-one 19:

A solution of the intermediates 17a-m (3 mmol) in acetic acid (10 ml) was heated under reflux for 30 min. After cooling the mixture is poured onto ice and the resulting precipitate filtered off. It was crystallized in ethanol. In case of derivatives 17i and 17j the reflux was maintained for 1h and 30 min and 3h respectively. In case of derivatives 17e and 17f the reflux was maintained for 8h and 3h respectively.

2,3-dimethyl-7-azaisoindole[2,1-a]quinoxalin-6(5H)-one, 19a: m.p. 340.0-341.0°C; yield quant.; IR 3079 (NH), 1656 (CN) cm^{-1} ^1H NMR DMSO (ppm): 2.36 (3H, s,

CH_3), 2.39 (3H, s, CH_3); 7.49 (1H, dt, J=6.5, H-8), 8.37 (1H, s, NH), 8.52 (1H, dd, J= 1.2, 8.5 Hz, H-7), 8.87 (1H, dd, J= 1.2, 4,2 H-9), 9.14 (1H, s, H-6).

2,3-dimethyl-4-azaisoindole[2,1-a]quinoxalin-6(5H)-one, 19b: m.p. >410.0 °C; yield 40%; IR 3558 (NH), 1643 (CO) cm^{-1} ^{1}H NMR DMSO (ppm): 2.38 (3H, s, CH_3), 2.42 (3H, s, CH_3), 7.47-7.52 (2H, m, H-5, H-9), 8.45 (1H, s, H-2), 8.81 (1H, d, J= 3.42 Hz, H-10), 9.05 (1H, d, J=8.73 Hz, H-8), 9.39 (1H, s, H-6), 13.65 (1H, bs, NH).

2,3-dichloro-7-azaidoindole[2,1-a]quinoxalin-6(5H)-one, 19c: m.p. 322.0-323.0°C; yield 99%; IR 3343 (NH), 1664 (CO) cm^{-1} ^{1}H NMR DMSO (ppm): 7.51 (1H, dd, J=4.28, 8.56 Hz, H-8), 8.03 (1H, s, H-2), 8.54 (1H, dd, J=1.38, 10.0 Hz, H-7), 8.91 (1H, dd, J=1.38, 4.27 Hz, H-9), 9.01 (1H, s, H-5), 9.30 (1H, s, H-6), 13.60 (1H, bs, NH).

2,3-dichloro-4-azaidoindole[2,1-a]quinoxalin-6(5H)-one, 19d: mp. 348.0-349.0°C; yield 71%; IR 3089 (NH), 1650 (CN) cm^{-1} ^{1}H NMR DMSO (ppm): 7.30-7.36 (2H, m, H-5, H-8), 7.73 (1H, s, H-2), 8.72 (1H, d, J=4.3 Hz, H-7), 8.91 (1H, d, J=2.9 Hz, H-9), 9.25 (1H, s, H-6), 13.80 (1H, bs, NH).

2,3-dimethoxy-4-azaisoindole[2,1-a]quinoxalin-6(5H)-one, 19e: m.p. 284.0-285.0°C; yield 90%; IR 3143 (NH), 1646 (CO) cm^{-1} ^{1}H NMR DMSO (ppm): 3.92 (3H, s, OCH3), 4.00 (3H, s, OCH3), 7.30 (1H, s, H-2), 7.55 (1H, dd, J=4.0, 8.3 Hz, H-9), 8.15 (1H, s, H-5), 8.85 (1H, d, J=3.8 Hz, H-10), 9.10 (1H, d, J=8.8 Hz, H-8), 9.63 (1H, s, H-6), 13.70 (1H, bs, NH).

2,3-dimethoxy-7-azaisoindole[2,1-a]quinoxalin-6(5H)-one, 19f: m.p. 272.0-273.0°C; yield 80%; IR 3305 (NH), 1660 (CO) cm^{-1} ^{1}H NMR DMSO (ppm): 3.91 (3H, s, OCH3), 3.99 (3H, s, OCH3), 7.32 (1H, s, H-2), 7.50 (1H, dd, J=4.3, 8.5 Hz, H-8), 8.09 (1H, s, H-5), 8.53 (1H, dd, J=1.3, 8.5 Hz, H-7), 8.87 (1H, dd, J=1.3, 4.3 Hz, H-9), 9.26 (1H, s, H-6), 13.29 (1H, bs, NH).

1,4-dimethoxy-7-azaisoindole[2,1-a]quinoxalin-6(5H)-one, 19g: m.p. 287-288°C; yield 40%; IR 3018 (NH), 1627 (CO) cm^{-1} ^{1}H NMR DMSO (ppm): 3.87 (3H, s, OCH3), 4.07 (3H, s, OCH3), 6.93 (1H, d, J=9.0 Hz, H-3), 7.04 (1H, d, J=4.2, 8.5 Hz, H-8), 8.40 (1H, d, J=7.5 Hz, H-7), 8.72 (1H, dd, J=4.14, H-9), 9.25 (1H, s, H-6), 13.50 (1H, bs, NH).

1,4-dimethoxy-4-azaisoindole[2,1-a]quinoxalin-6(5H)-one, 19h: m.p. 268-269°C; yield 70%; IR 3288 (NH), 1646 (CO) cm^{-1} ^{1}H NMR DMSO (ppm): 4.01 (3H, s, OCH3), 4.13 (3H, s, OCH3), 7.19 (1H, d, J=9.1 Hz, H-3), 7.31 (1H, dd, J=4.1, 8.6 Hz, H-9), 8.86 (1H, d, J = 3.8 Hz, H-10), 9.03 (1H, d, J= 8.6 Hz, H-8), 13.65 (1H, bs, NH).

1,4-dimethoxy-2,3-dimethyl-7-azaisoindolo[2,1-a]quinoxalin-6(5H)-one, 19i: m.p. 285-286°C; yield 41%; IR 3324 (NH), 1658 (CN) cm^{-1} ^1H NMR DMSO (ppm): 2.34 (3H, s, CH3), 2.36 (3H, s, CH3), 2.86 (3H, s, OCH3), 2.87 (2H, s, OCH3), 7.52 (1H, dd, J= 4.3, 8.5 Hz, H-9), 8.58 (1H, dd, J= 1.2, 8.5 Hz, H-10), 8.93 (1H, dd, J= 1.3, 4.3 Hz, H-8), 9.38 (1H, s, H-6), 12.47 (1H, bs, NH).

1,4-dimethoxy-2,3-dimethyl-4-azaisoindolo[2,1-a]quinoxalin-6(5H)-one, 19j: m.p. 246-247°C; yield 90%; IR 3018 (NH), 1710 (CN) cm^{-1} ^1H NMR DMSO (ppm):2.35 (3H, s, CH3), 2.37 (3H, s, CH3), 3.85 (3H, s, OCH3), 3.88 (3H, s, OCH3), 7.54-7.61 (1H, m, H-8), 8.87 (1H, d, J=3.6 Hz, H-7), 9.03 (1H, d, J=8.7, H-9), 9.50 (1H, s, H-6), 12.70 (1H, bs, NH).

4-azaisoindole[2.1-a]quinoxalin-6(5H)-one, 19k: m.p. 330.0-331.0°C; yield 60%; IR 3322 (NH), 1656 (CO) cm^{-1} ^1H NMR DMSO (ppm): 7.53-7.79 (3H, m, H-2, H-3, H-4), 8.65-8.69 (2H, m, H-9, H-5), 8.87 (1H, d, J=3.7 Hz, H-10), 9.11 (1H, d, J=8.5 Hz, H-8), 9.61 (1H, s, H-6), 13.50 (1H, bs, NH).

7-azaisoindole[2.1-a]quinoxalin-6(5H)-one, 19l: m.p. 322.0-323.0 °C; yield 80%; IR 3073(NH), 1664 (CO) cm^{-1} ^1H NMR DMSO (ppm): 7.51-7.71 (3H, m, H-3, H-4, H-8), 7.81 (1H, d, J=7.3 Hz, H-2), 8.53-8.62 (2H, m, H-5, H-7), 8.91 (1H, d, J=3.3 Hz, H-9), 9.29 (1H, s, H-6), 12.90 (1H, bs, NH).

3-methoxy-4-azaisoindole[2.1-a]quinoxalin-6(5H)-one, 19m: m.p. 267.0-268.0°C; yield 80%; IR 3382 (NH), 1659 (CO) cm^{-1} ^1H NMR DMSO (ppm): 3.90 (3H, s, OCH3), 7.19 (1H, dd, J=2.6, 9.1 Hz, H-5), 7.29 (1H, d, J=2.6 Hz, H-2), 7.50 (1H, dd, J=4.3, 8.5 Hz, H-8), 8.52 (2H, d, J=9.5 Hz, H-5, H-7), 8.88 (1H, dd, J=1.2, 4.3 Hz, H-9), 9.20 (1H, s, H-6), 13.75 (1H, bs, NH).

Synthesis of 6-amino-3-methoxy-7-azaisoindole[2,1-a]quinoxaline, 18a

A solution of the intermediates 17 m (3 mmol) in acetic acid (10 ml) was heated under reflux for 30 min. After cooling the resulting precipitate filtered off. It was crystallized in ethanol. In case of derivatives 17i and 17j the reflux was maintained for 1h and 30 min and 3h respectively. In case of derivatives 17e and 17f the reflux was maintained for 8h and 3h respectively.

6-amino-3-methoxy-7-azaisoindole[2,1-a]quinoxaline, 18a: m.p. 238.0-239.0°C; yield 60%; IR 3399, 3000 (NH_2) cm^{-1} ^1H NMR DMSO (ppm): 3.87 (3H, s, OCH_3),

6.96 (1H, dd, J=2.8, 8.9 Hz, H-5), 7.08 (1H, d, J=2.7 Hz, H-2), 7.30-7.36 (4H, m, H-8, H-4, NH2), 8.34 (1H, dd, J=2.1, 8.8 Hz, H-7), 8.68 (1H, dd, J=1.4, 4.2 Hz, H-9), 8.77 (1H, s, H-6).

^{13}C NMR DMSO-d6 (ppm): 55.36 (q), 104.47 (d), 106.33 (s), 108.01 (d), 110.71 (d), 116.72 (d), 117.61 (s), 118.47 (s), 118.73 (d), 128.03 (d), 135.75 (s), 140.79 (d), 147.25 (s), 151.59 (s), 158.75 (s).

Synthesis of 2-(2-aminopyridin-3-yl)-2H-isoindole-1-carbonitrile 23:

To a solution of sodium hydrogen sulfite (1.56 g, 0.015 mol) in water (3.8 ml), phtalaldehyde **1a, 1c** or **1e** (0.015 mol). The mixture was stirred until the solid was dissolved, and 2,3-diaminopyridine (0.015 mol, 1.64 g) was added. The reaction was heated on a steam bath for 1h, then KCN (3.39 g, 0.052 mol) in water (8.0 ml) was added and the mixture was heated for additional 6h and then cooled to rt it was stirred at rt overnight. The solid formed upon cooling was filtered and purified by chromatography using DCM-ethyl acetate 9/1 as eluent. In the case of derivative 2**3b**, it was purified in column using DCM-ethyl acetate 84/16 as eluent.

2-(2-aminopyridin-3-yl)-2H-isoindole-1-carbonitrile, 23a: : m.p. 284.0-285.0°C ;yield 38%; IR 3500, 3397 (NH2), 2200 (CN); 1H NMR DMSO (ppm): 6.16 (2H, s, NH2), 6.74 (1H, dd, J=4.9,7.6, H-5'), 7.17 (1H, t, J=6.8 Hz, H-3), 7.33 (1H, t, J=6.8, H-4), 7.59-7.69 (2H, m, H-3, H-5), 7.79 (1H, d, J=8.5 Hz, H-4'), 7.94 (1H, s, H-6), 8.16 (1H, dd, J=1.7, 4.9 Hz, H-6'); ^{13}C NMR DMSO-d6 (ppm): 111.91 (d), 113.96 (s), 117.58 (d), 117.83 (s), 121.62 (d), 122.47 (d), 122.55 (d), 124.30 (s), 125.78 (d), 131.38 (s), 136.23 (d), 149.76 (d), 155.13 (s).

2-(2-aminopyridin-3-yl)-5,6-dimethyl-2H-isoindole-1-carbonitrile, 23b: m.p. 175.0-176.0°C; yield 15%; IR 3381, 3313 (NH_2), 2198 (CN) cm^{-1}; ^{1}H NMR DMSO (ppm): 6.07 (2H, bs, NH_2), 6.72 (1H, dd, J=4.9, 7.6 Hz, H-5'), 7.42 (1H, s, H-4), 7.51 (1H, s, H-7), 7.57 (1H, dd, J=1.7, 7.6 Hz, H-4'), 7.74 (1H, s, H-3), 8.12 (1H, dd, J=1.7, 4.9 Hz, H-6');

3C NMR DMSO-d6 (ppm): 20.02 (q), 20.33 (q), 99.50 (s), 111.92 (d), 114.31 (s), 116.39 (d), 117.96 (s), 120.07 (d), 121.35 (d), 123.71 (s), 131.05 (s), 132.17 (s), 136.00 (s), 136.12 (d), 149.55 (d), 155.19 (s).

2-(2-aminopyridin-3-yl)-5,6-dimethoxy-2H-isoindole-1-carbonitrile, 23c: m.p. >410°C; yield 10%; IR 3323, 3321 (NH_2), 2256 (CN) cm-1; ^{1}H NMR DMSO (ppm): 3.80 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 6.06 (2H, bs, NH_2), 6.71 (1H, dd, J=4.9, 7.6 Hz, H-4'), 7.64 (1H, s, H-3), 8.13 (1H, dd, J=1.6, 4.8 Hz, H-6');
^{13}C NMR DMSO-d6 (ppm): 55.37 (q), 55.61 (q), 99.29 (d), 99.55 (d), 112.02 (d), 114.55 (s), 118.13 (s), 119.55 (s), 121.10 (d), 127.86 (s), 136.19 (d), 148.23 (s), 149.40 (d), 151.07 (s), 155.38 (s).

Synthesis of pyrido[2',3':5,6]pyrazino[2,1-a]isoindol-6(5H)-one 25

To a solution of the intermediates 23a-c (2.1 mmol) in acetic acid (7.5 ml) DMF (1ml) was added. The mixture was heated under reflux for 3h. After cooling the mixture is poured onto ice and the resulting precipitate filtered off. It was crystallized in ethanol.

Pyrido[2',3':5,6]pyrazino[2,1-a]isoindol-6(5H)-one, 25a: m.p. 352.5-354.0°C; yield quant.; IR 3079 (NH), 1635 (CO) cm^{-1}; ^{1}H NMR DMSO (ppm): 7.43-7.66 (3H, m, H-5', H-4, H-5), 8.04 (1H, d, J=8.2, H-3, H-6), 8.60 (1H, m, H-4'), 9.02 (1H, d, J=7.1 Hz, H-6'), 9.42 (1H, s, H-2), 13.30 (1H, bs, NH);

8,6-dimethylpyrido[2',3':5,6]pyrazino[2,1-a]isoindol-6(5H)-one, 25b m.p. >410°C; yield 95%; IR 3092 (NH), 1634 (CO) cm^{-1}; ^{1}H NMR DMSO (ppm): 2.46 (3H, s, CH_3), 2.50 (3H, s, CH_3), 7.75 (1H, s, H-2), 7.58 (1H, m, H-5'), 8.37 (1H, s, H-6), 8.60 (1H, d, J=5.3 Hz, H-4'), 8.95 (1H, d, J=7.7 Hz, H-6'), 9.21 (1H, s, H-2), 13.10 (1H, bs, NH);

8,6-dimethoxypyrido[2',3':5,6]pyrazino[2,1-a]isoindol-6(5H)-one, 25c: m.p. 275.5-277.0°C; yield quant.; IR 3350 (NH), 1630 (CO) cm^{-1}; ^{1}H NMR DMSO (ppm): 3.90 (3H, s, OCH_3), 3.99 (3H, s, OCH_3), 7.31 (1H, s, H-6), 7.56 (1H, dd, J=4.9, 8.2 Hz, H-5'), 7.81 (1H, s, H-3), 8.52 (1H, d, J=5.1 Hz, H-4'), 8.90 (1H, d, J=7.4 Hz, H-6'), 9.13 (1H, s, H-2) 13.25 (1H, bs, NH);

Synthesis of 5,6-dihydropyrido[2',3':5,6]pyrazino[2,1-a]isoindol-6-amine, 24

To a solution of the intermediates 23a-c (2.1 mmol) in acetic acid (7.5 ml) DMF (1ml) was added. The mixture was heated under reflux for 3h. After cooling the solvent was evaporated under reduced pressure and it was crystallized in ethanol.

5,6-dihydropyrido[2',3':5,6]pyrazino[2,1-a]isoindol-6-amine, 24a: m.p. 306.2-307.1°C; yield 70%; IR 3405, 3493 (NH_2) cm^{-1}; ^1H NMR DMSO (ppm): 7.27-7.35 (5H, m, NH_2, H-3, H-4, H-6), 7.87-7.91 (1H, m, H-5), 8.46-8.51 (1H, m, H-5'), 8.58 (1H, dd, J= 1.6, 5.0 Hz, H-4'), 8.78 (1H, dd, J=1.6, 8.1 Hz, H-6'), 9.01 (1H, s, H-2).

^{13}C NMR DMSO-d6 (ppm): 99.55 (s), 107.62 (s), 109.43 (d), 117.09 (d), 119.10 (d), 119.63 (d), 119.74 (s), 120.12 (d), 123.37 (d), 123.84 (d), 126.16 (s), 148.27 (d), 150.00 (s), 153.59 (s).

8,6-dimethyl-5,6-dihydropyrido[2',3':5,6]pyrazino[2,1-a]isoindol-6-amine, 24b: m.p. >410.0°C; yield 70%; IR 3423, 3320 (NH_2) cm^{-1}; ^1H NMR DMSO (ppm): 7017 (2H, bs, NH_2), 7.26 (1H, m, H-5'), 7.61 (1H, s, H-3), 8.27 (1H, s, H-6), 8.53 (1H, dd, J=1.5, 4.6 Hz, H-4'), 8.70 (1H, dd, J=1.5, 8.1 Hz, H-6'), 8.82 (1H, s, H-2);

^{13}C NMR DMSO-d6 (ppm): 20.22 (q), 20.40 (q), 106.80 (d), 107.38 (s), 108.48 (d), 116.84 (d), 118.38 (d), 118.91 (d), 119.22 (s), 119.26 (s), 123.42 (d), 125.65 (s), 132.94 (s), 133.30 (s), 147.87 (s), 153.43 (s).

8,6-dimethoxy-5,6-dihydropyrido[2',3':5,6]pyrazino[2,1-a]isoindol-6-amine, 24c: m.p. 233.0 234.0°C; yield 80%; IR 3380, 3445 (NH_2) cm^{-1}; ^1H NMR DMSO (ppm): 3.87 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 7.16-7.26 (4H, m, NH_2, H-6, H-5'), 7.67 (1H, s, H-3), 8.48 (1H, dd, J=7.6, 4.6 Hz, H-4'), 8.62 (1H, dd, J=1.6, 8.1, H-6'), 8.72 (1H, s, H-2);

^{13}C NMR DMSO-d6 (ppm): 55.19 (q), 55.86 (q), 98.80 (d), 99.43 (d), 107.28 (s), 108.28 (d), 115.29 (s), 116.17 (d), 119.42 (s), 121.95 (s), 122.64 (d), 147.01 (d), 148.57 (s), 149.03 (s), 149.69 (s), 153.32 (s).

Molecular modeling

These studies were carried out using GLIDE program from Schrodinger software.

Docking on tubuline

The structure PDB 1SA0 was used for tubulin docking in several works present in literature.

As ISQO compounds inhibit tubulin polymerization in a way similar to colchicine this X-ray structure was used for docking.

The protein was prepared using Protein Preparation Wizard. The stathmin-like domain and the C and D subunits, Mg ions, were removed.

Prime refinement was used to complete residue side chains with missing atoms.

Docking was carried out using Glide program.

To validate the Glide docking protocol, colchicine, the co-crystallized ligand, was docked into the active site. The docked structure was compared with the crystal structure showing that this protocol successfully reproduces the crystal tubulin-colchicine complex.

Docking studies on Topoisomerase 1

The X-ray structure with PDB code 1K4T was used for docking.

The protein was prepared using Protein Preparation Wizard. The TTC coligand, Hg ions and water molecules with a distance longer than 5 A were removed.

Glide program was used to carry out the docking. To validate the Glide protocol, Topotecan (TTG), the co-crystallized coligand, was docked into the active site. The docked structure was compared with the crystal structure showing that this protocol successfully reproduces the crystal tubulin- colchicine complex.

Synthesis of Ventilatone A in collaboration with The University of Nottingham (May-September 2011)

Ventilatone A was isolated from the root bark of Ventilago calyculata in 1985 and belongs to the pyranonaphthoquinone family of natural products [91JCS327], [85P2669]. Naphtho[2,3-c]pyran-5,10-dione is the most common ring system arrangement for pyranonaphthoquinones, however the naphtha[2,3-c]pyran-6,9-dione systems are also known. This configuration is much less common in nature and only a handful of natural products of this type have been synthesized [99NPR267], [00T1937], [08NPR376].

Ventilatone A belongs to this second group and no synthesis of it has yet been reported. Its configuration in not very common in nature and only a handful of natural products of this type have been synthesized [90JOC1466], [92AJC2025], [04THL939], [07THL1545]. An optical rotation is given for it but the absolute configuration at its stereogenic center is not known.

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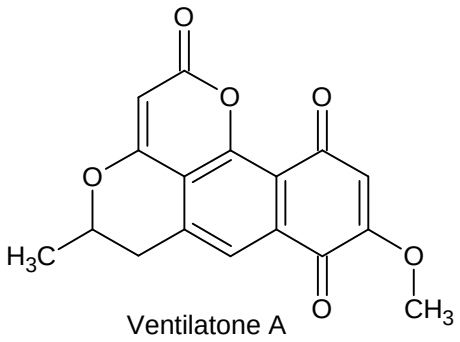


Ventilatone A

Fig.30

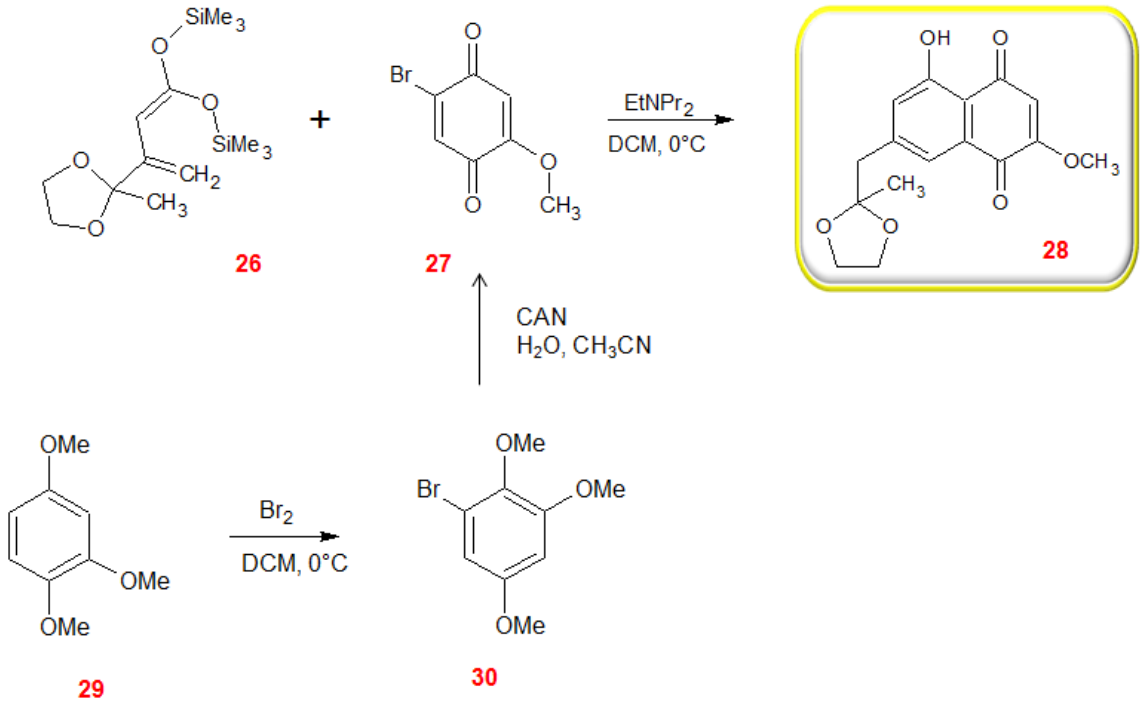
Results and discussion

To synthesize Ventilatone A whose structure is shown in fig.30, we imagine to break down the molecule in order to obtain the smaller unit easier to synthesize.

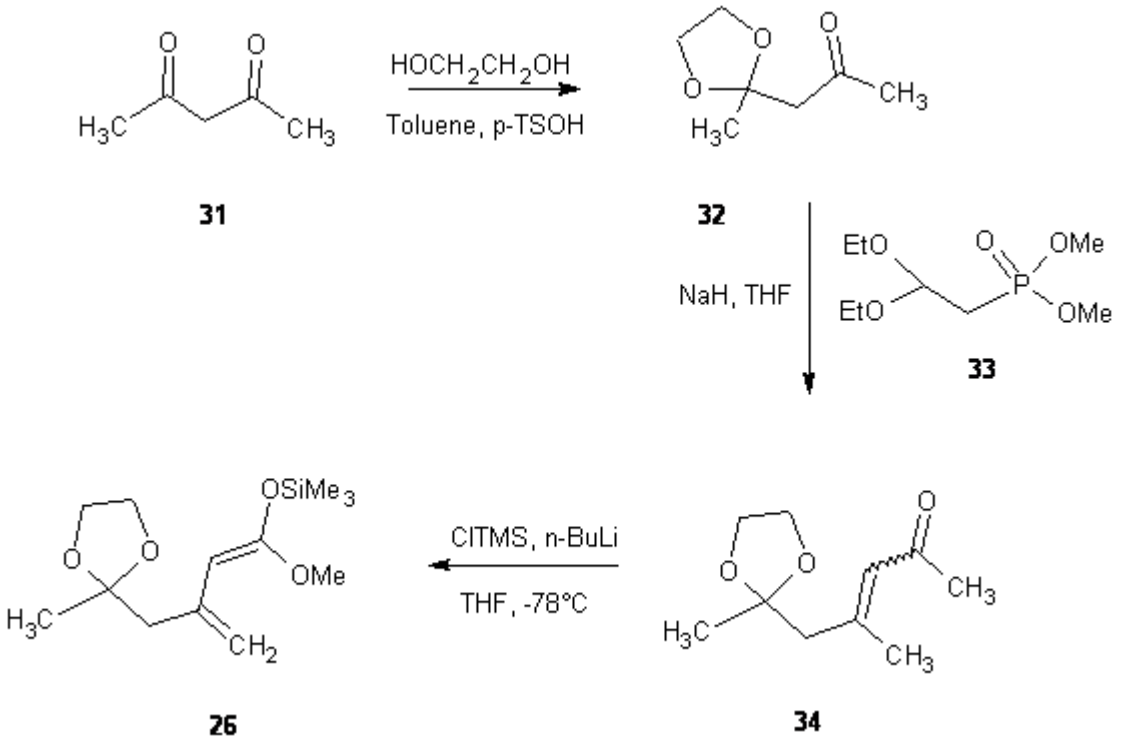
We planned to start our Ventilatone A synthesis from the naphtoquinone intermediate **28**.

Diels-Alder reaction between benzoquinone **27** and diene **26** can give the intermediate **28**. Benzoquinone **27** was obtained by bromination of trimethoxybenzene **29** and then subsequent oxidation of 1-bromo-2,4,5-trimethoxybenzene **30** with cerium ammonium nitrate (CAN).

The position of the bromine in the benzoquinone dienophile dictates the regiochemistry of the halogen directing Diels-Alder reaction with diene **26**.



The diene **26** was synthesized starting from the diketone **31** in which one carbonyl function was protected to give compound **32**. Reaction of this latter with methyl diethylphosphonoacetate **33** afforded compound **34** in a mixture of the two isomers that was directly used for the sequent step with chloro-trimethylsilane and n-BuLi.



31 **32** **33** **26** **34**

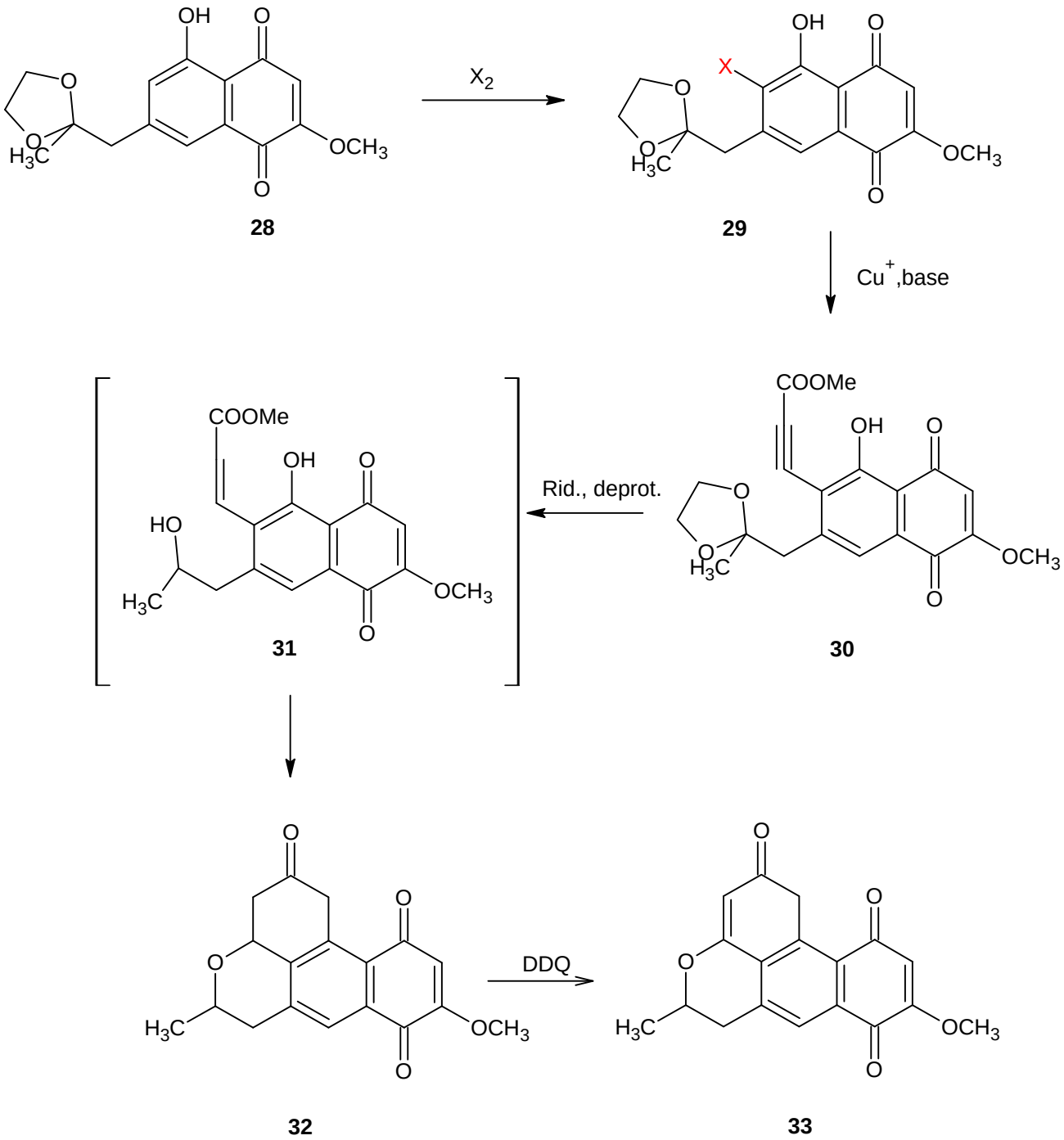
Once we synthesized the naphoquinone intermediate we planned the synthetic route for Ventilatone A starting from this compound.

What we though to do was first try the introduction of an halogen atom into the orto phenolic position to obtain compound **29** that can be useful for Sanogashima coupling and to obtain compound **30**.

Reduction of the triple bond and of the aldehyde function, and then deprotection of the ketone give compound **31** that probably will evolve to compound **32** spontaneously.

Finally, oxidation of compound **32** with DDQ allows to complete the synthesis of Ventilatone A.

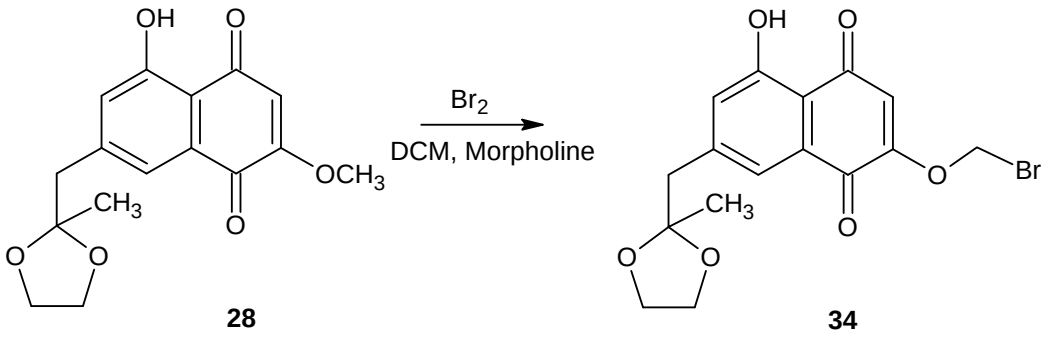
We start to find the best method to introduce the halogen atom on the orto-phenolic position of naphtoquinone intermediate.



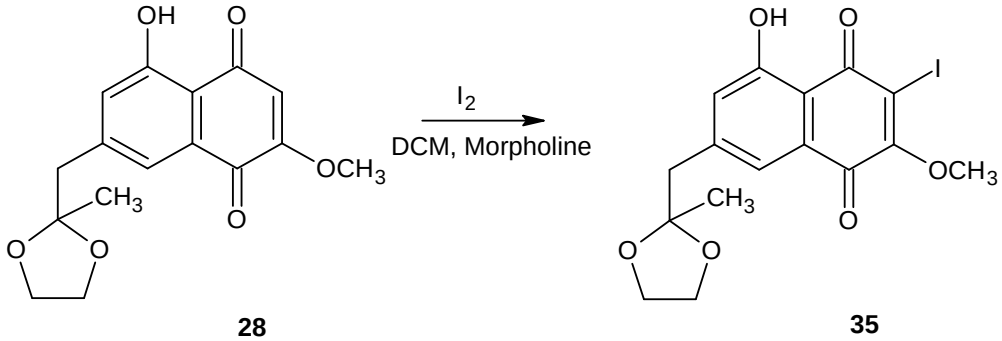
Our first attempt was the halogenation with NBS or NIS with different ratio equivalents and in the presence of diisopropylamine. From this reaction was possible isolate only starting material.

The iodination using I_2 and HIO_3 in water and CH_3CN gave starting material.

The halogenation of compound **28** in DCM and morpholine in presence of Br_2 gave compound **34**; instead when we used I_2 was isolated the corresponding iodinated compound in good yield but not on quinine portion.



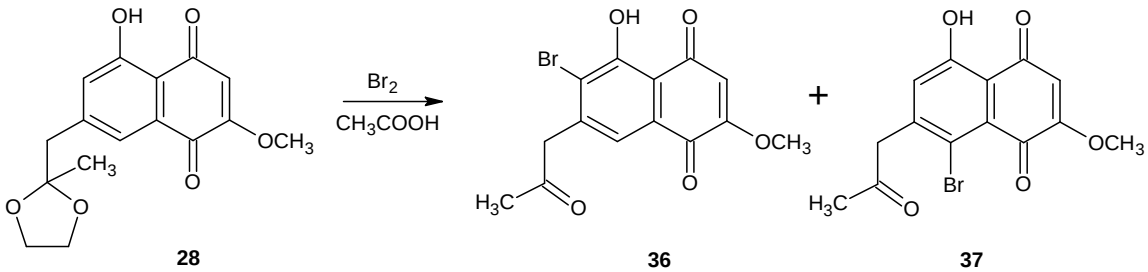
28 **34**



28 **35**

Then we try halogenation in acetic acid with Br_2 and I_2.

In case of Br_2, using 1:1 ratio of quivalentes (Br_2:**28),** we collected just starting material. Using a ratio 2:1 (Br_2:**28)** we obtained a mixture of two isomers **36** and **37** from which was very difficult to isolate the pure compoundsify.



28 **36** **37**

Using I_2 we were finally able to obtain the desired compound although the yield should be improved.

Actually this project is still in process in Nottingham.

EXPERIMENTAL DATA SYNTHESIS VENTILATONE A

Synthesis of 1-bromo-2,4,5-trimethoxybenzene 30

Bromine (3,2 ml, 63.1 mmol) in DCM (50 ml) was added dropwise over 1h to a solution of 1,2,4-trimethoxybenzene (10.1 g, 60.1 mmol) in DCM (200 ml) at 0°C. After addition had completed the resulting brown solution was stirred for 5 min then washed with saturated aqueous sodium thiosulfate solution (200ml), saturated aqueous sodium hydrogen carbonate solution (100 ml), water (100 ml) and brine (100 ml). The organic phase was then dried and concentrated .

Yield 75%; mp 52-53°C; IR 3011, 2938, 2843, 1586, 1506, 1465, 1438, 1379, 1166, 1030; $CDCl_3$ HNMR 7.04 (1H, s, ArH), 6.56 (1H, s, ArH), 3.89 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 3.83 (3H, s, OCH_3); CNMR 150.3 (C), 149.1 (C), 143.8 (C), 116.4 (CH), 101.1 (C), 98.9 (CH), 57.2 (CH_3), 56.6 (CH_3)

Synthesis of 2-bromo-5-methoxy-1,4-benzoquinone 27

CAN (83.2 g, 151.8 mmol) in water (200 ml) was added to 1-bromo-2,4,5-trimethoxybenzene (15 g, 60.7 mmol) in aceto nitrile (200 ml). The dark brown solution was stirred in an open flask at rt for 1h, becoming bright orange. The reaction mixture was diluted with water (500 ml) and filtered to give a yellow solid compound.

Yield 90%; IR 3045, 2941, 2850, 1680, 1661, 1624, 1588, 1192, 1171, 1006; $CDCl_3$ HNMR 7.25 (1H, s, ArH), 6.13 (1H, s, OCH3); CNMR 179.2 (C), 160.0 (C), 139.2 (C), 135.8 (CH), 106.9 (CH), 56.7 (CH_3)

Synthesis of acetylacetone-2,2-(ethylene glycol)monoketal 32

P-toluenesulfonic acid (11 mg, 0.006 mmol) was added to a solution of acetylacetone (5g, 50 mmol) and ethylene glicol (3.15 g, 50mmol) in toluene. The mixture was warmed to reflux overnight with azeotropic removal of water using a Dean-Stark trap. Toluene was removed under reduced pressure and the remaining crude was distillated

twice under reduced pressure.

Yield 70%; bp 115-118 °C; IR $CDCl_3$ 2989, 2888, 1709, 1790, 1361, 1241, 1054 cm^{-1}; $CDCl_3$ HMNR 3.97-3.94 (4H, m, -OCH_2), 2.75 (2H, s, CH_2), 2.20 (3H, s, OCH_3), 1.39 (3H, s, CH_3); CNMR 205.9 (C), 107.8 (C), 64.6 (CH_2), 64.2(CH_2), 52.5 (CH_2), 31.6 (CH_3), 24.3 (CH_3)

Synthesis of 3-methyl-4-(2-methyl-1,3-dioxolan-2-yl)but-2-enoate 34

Sodium hydrade (60% in mineral oil; 8.32 g, 208.1 mmol) was washed with dry n-pentane (30 ml), dried under vacuum, suspended in THF (250 ml) and cooled to 0°C.

Methyl diethylphosphonoacetate (38.2 ml, 208.1 mmol) was added dropwise and the reaction mixture was warmed to reflux over 30 min. Acetylacetone-2,2-(ethylene glycol)monoketal (20g,138.7 mmol) in THF was added dropwise and the suspension stirred for 6 h then cooled to rt. Ether and brine were added and the aqueous phase was extracted with ether three times. The combined ethereal extracts were washed with water and dried under reduced pressure.

The crude product was purified in column eluting with petroleum ether-ethyl acetate 8:2

Yield 65%; IR 2989, 2952, 2888, 1712, 1646, 1437, 1380, 1155, 1055 cm^{-1}; $CDCl_3$ HNMR 5.77 (1H, d, J 0.7, CH), 3.93-3.91 (4H, m, OCH_2OCH_2), 3.69 (3H, s, OCH_3), 2.45 (2H, s, CH_2), 2.07 (3H, s, CH=C-CH_3), 1.35 (3H, s, OCH_3); CNMR 166.8 (C), 154.9 (C), 118.8 (CH), 109.9 (C), 64.5 (CH_2), 50.8 (CH_3), 41.1 (CH_2), 26.5 (CH_3), 24.0 (CH_3)

Synthesis of 1-methoxy-3-[(2-methyl-1,3-dioxolan-2-yl)methyl]buta-1,3-dienyloxytrimethylsilane 26

n-Butyllithium (2.5 in hexane; 0.52 ml, 1.3mmol) was added to a 0°C solution of diidopropylamine (0.17 ml,1.30 mmol) in THF (6 ml). The solution was warmed to rt, stirred for 5 min then cooled to -78°C. Chlorotrimethylsilane (0.20 ml, 1.60 mmol) was added, followed by dropwise addition of methyl 3 –methyl-4-(2-methyl-1,3-dioxolan-2-yl)but-2-enoate (200mg, 1 mmol) in THF (3 ml). The resulting solution was stirred at -78°C for 90 min then warmed to rt and concentrated without heating in vacuo. The residue was diluted with dry n-pentane (10 ml) and filtrated and concentrated.

Yield 75%; IR 3011, 2962, 2888, 1707, 1646, 1598, 1254, 1097, 1050, 676 cm^{-1}; $CDCl_3$ HNMR 5.26 (1H, d, J 2.5, CH), 4.81 (1H, d, J 2.5, H_2C=C), 4.24 (1H, s, H_2C=C), 3.97-3.95 (4H, m, OCH_2OCH_2), 2.50 (2H, s, CH_2), 1.37 (3H, s, CH_3), 0.26 (9H, s, $OSiCH_3$);

CNMR 157.7 (C), 138.3 (C), 110.9 (CH2), 110.2 (C), 79.2 (CH), 64.4 (CH_2), 55.0 (CH), 47.3 (CH_2), 23.8 (CH_3), 20.5 (CH_3)

Synthesis of 5-hydroxy-2-mehoxy-7-[(2-methyl-1,3-dioxolan-2-yl)-methyl]-1,4-naphtoquinone 28

1-methoxy-3-[(2-methyl-1,3-dioxolan-2-yl)methyl]buta-1,3-dienyloxytrimethylsilane (1.88 g, 6.91 mmol) in DCM (25 ml) was added dropwise to a 0°C solution of 2-bromo-5-methoxy-1,4-benzoquinone (500 mg, 2.30 mmol) and diisopropylethylamine (0.48 ml, 2.76 mmol) in DCM (25 ml). The resulting green solution was stirred for 2 h then silica gel (10g) was added and the mixture warmed to rt and stirred for 24h. The mixture was filtrated to remove silica and the filtrate concentrated without heating.

The crude was purified in column eluiting with petroleum ether- ethyl acetate 6:4

Yield 90%; mp 173-174 °C; 3694, 2987,1685, 1635, 1605, 1380, 1346, 1275, 1242, 1115 cm-1; $CDCl_3$ HNMR 12.17 (1H, s, OH), 7.65 (1H, d, J 1.5, ArH), 7.25 (1H, d, J 1.5, ArH), 6.11 (1H, s), 3.99-3.95 (2H, m, OCH_2), 3.94 (3H, s, OCH_3), 3.82-3.86 (2H, m, OCH_2), 3.01 (2H, s, CH_2), 1.36 (3H, s, CH_3); CNMR 190.5 (C), 179.6 (C), 161.1(C), 160.8 (C), 130.5 (C), 126.7 (CH), 122.3 (CH), 112.7 (C), 109.5 (CH), 109.1 (C), 64.9 (CH_2), 56.7 (CH_3), 45.5 (CH_2), 24.5 (CH_3)

Synthesis of 2-(bromomethoxy)-5-hydroxy-7-((2-methyl-1,3-dioxolan-2yl)methyl)naphthalene-1,4-dione 34

To a stirred solution of 5-hydroxy-2-methoxy-7-(2-methyl-[1,3]dioxolan-2-ylmethyl)-[1,4]naphthoquinone (100 mg, 0.3 mmol) in DCM (10 ml) bromine (110 mg, 0.66 mmol) was added at rt followed by morpholine (catalytic amount). The reaction mixture was stirred at rt for 2h and then quenched with $Na_2S_2O_3$ saturated solution. The aqueous layer was extracted with ethyl acetate (10 ml x2) and evaporated under reduced pressure. The crude was purified in column eluting with petroleum ether – ethyl acetate 8:2

Yield 30%; IR 3690, 3607, 2360, 1626, 1674, 1569, 1488, 1239, 1017; mp 172-173°C; HNMR CDCl3: 12.51 (1H, OH), 7.46 (1H, d, ArH), 7.16 (1H, d, ArH), 5.89 (1H, s, ArH), 3.97-3.94 (2H, m), 3.88 (2H, s), 3.86-3.82 (2H, m), 2.97 (2H, s), 1.34 (3H, s); CNMR 188.86 (C), 182.53 (C), 160.29 (C), 154.24 (C), 144.79 (C), 132.21 (C), 126.10 (CH), 121.90 (CH), 112.91 (C), 110.22 (CH), 109.22 (C), 66.41 (CH_2), 64.91 (CH_2),

45.15 (CH_2), 45.44 (CH_2), 24.41 (CH_3)

Synthesis of 5-hydroxy-3-iodo-2-methoxy-7-((2-methyl-1,3-dioxolan-2-yl)methyl)naphthalene-1,4-dione 35

To a stirred solution of 5-hydroxy-2-methoxy-7-(2-methyl-[1,3]dioxolan-2-ylmethyl)-[1,4]naphthoquinone (100 mg, 0.3 mmol) in DCM (10 ml) iodine (167 mg, 0.66 mmol) was added at rt followed by morpholine (catalytic amount). The reaction mixture was stirred for 2h and then quenched with $Na_2S_2O_3$ saturated solution (10 ml). The acqueous layer was estracted with ethyl acetate (10 ml x2) and evaporated under reduced pressure. The crude was purified in column eluiting with petroleum ether ethyl acetate 8:2.

Yield 70%; IR: 3682, 3011, 1627, 1520, 1425, 1239, 1103; mp165-166°C; $CDCl_3$ HNMR: 12.20 (1H,s, ArOH), 7.48 (1H, d, J 1.6, ArH); 7.13 (1H, d, J 1.6, ArH), 3.88 (3H, s, OCH_3), 3.94-3.84 (2H, m, OCH_2), 3.81-3.78 (2H, m, OCH_2), 2.94 (2H, s, CH_2), 1.34 (3H, s, CH_3); CNMR 184.64 (C), 180.43 (C), 160.57 (C), 158.47 (C), 145.73 (C), 130.89 (C), 125.97 (CH), 122.62 (CH), 111.39 (C), 110.47 (C), 109.15 (C), 67.48 (CH_2), 64.93(CH_2), 56.23 (CH_3), 45.44(CH_2), 24.44 (CH_3)

Synthesis bromo-5-hydroxy-7-((E)-2-hydroxyprop-1-enyl)-2-methoxynaphthalene-1,4-dione 36, 37

A solution of hydroxy-2-methoxy-7-(2-methyl-[1,3]dioxolan-2-ylmethyl)-[1,4]naphthoquinone (0.33 mmol, 100 mg) in acetic acid (3ml) was treated at rt with bromine (0.33mmol, 53 mg) and stirred overnight. The cooled reaction mixture was poured into ice and the precipitate filtered and washed with ethanol.

6-bromo-5-hydroxy-7-((E)-2-hydroxyprop-1-enyl)-2-methoxynaphthalene-1,4-dione 36: Yield 35%; IR 3691, 3045, 1636, 1603, 1241, 1114; HNMR $CDCl_3$ 12.82 (1H, s, OH), 7.83 (1H, s, ArH), 5.93 (1H, s), 4.41 (3H, CH_3), 2.50 (1H, s), 2.45 (3H, s); CNMR 183.55 (C), 183.52 (C), 177.44 (C), 159.72 (C), 153.83 (C), 143.33 (C), 129.48 (C), 121.41 (C), 120.42 (CH), 119.46 (C), 113.16 (CH), 63.35 (=CH), 62.45 (OCH_3), 27.49 (CH_3)

8-bromo-5-hydroxy-7-((E)-2-hydroxyprop-1-enyl)-2-methoxynaphthalene-1,4-dione 37: Yield 25%; IR 3607, 2999, 1724, 1686, 1274; HNMR $CDCl_3$ 12.82 (1H, s,

OH), 7.89 (1H, s, ArH), 6.03 (1H, s, ArH), 4.37 (3H, s), 2.54 (1H, s), 2.50 (3H, s); CNMR 183.54 (C), 183.20 (C), 161.00 (C), 157.78 (C), 143.28 (C), 129.34 (C), 122.17 (CH), 121.28 (C), 119.44 (C), 115.93 (C), 113.00 (CH), 62.30 (OCH_3), 52.37 (=CH), 28.07 (OCH_3)

Synthesis of 5-hydroxy-6-iodo-2-methoxy-7-(2-oxopropyl)naphthalene-1,4-dione 38

A solution of hydroxy-2-methoxy-7-(2-methyl-[1,3]dioxolan-2-ylmethyl)-[1,4]naphthoquinone (0.33 mmol, 100 mg) in acetic acid (3ml) was treated at rt with iodine (0.33mmol, 84 mg) and stirred overnight. The cooled reaction mixture was poured into ice and the precipitate filtered and washed with ethanol.

Yield 30%; IR 3607, 1724, 1686, 1636, 1274; HNMR $CDCl_3$ 13.03 (1H, s, OH), 7.53 (1H, s, ArH), 6.15 (1H, s), 4.06 (2H, s, CH_2), 3.96 (1H, s, OCH_3), 2.33 (1H, s, CH_3); CNMR 203.18 (C), 190.10 (C),179.65 (C), 161.02 (C), 158.08 (C), 143.17 (C), 130.25 (C), 121.66 (CH), 113.42 (C), 109.23 (CH),105.00 (C), 56.86 (CH_3), 51.30 (CH_2), 30.18 (CH_3)

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