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Synthesis of Pyrrolo-Condensed Derivatives with Potential Antiviral Activity

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(STEBICEF)

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INTRODUCTION

Acquired Immunodeficiency Syndrome (AIDS) remains one of the world's greatest public health challenges with over 25 million mortalities estimated since it was first recognized in 1981.[1] The highly active antiretroviral therapy (HAART) used in the treatment of AIDS utilizes a combination of antiviral drugs with at least two mechanisms of action to effectively inhibit the replication cycle of HIV.[2] Due to the persistence and development of drug resistance by HIV virus in the infected host cell reservoir and within the tissues, AIDS remains an incurable disease.

HIV, the causative agent of AIDS, is a lentivirus that belongs to the family of Retroviridae. It is genetic information is not encoded as DNA, but as RNA and therefore is reverse-transcribed into DNA.

The HIV virus comprises two distinct viruses HIV-1 and HIV-2, which differ in origin and gene sequence. HIV-1 carries the *vpu* gene whereas HIV-2 carries the *vpx* gene. These strains of HIV share similar molecular structures they contain a single-stranded RNA genome and both viruses cause AIDS with a similar spectrum of symptoms. Like all viruses, HIV-1 is an intracellular parasite and cannot multiply without the host cell.[3]

The structure of HIV-1 is spherical in the shape. It has two membranes, one outer and one inner. The outer membrane of the virus, known as the viral envelope, has a hicosahedral shape with a knobby-looking envelope and it consists of a bilayer lipid acquired from the host cell, in which are located the envelope glycoproteins (gp) 120 and 41.[4] The inner membrane, known as capsid, contains two proteins, *p*24 and *p*17, as matrix proteins surrounding the nucleocapsid that contains the genetic material. Beneath the envelope there is the viral matrix *p*17, which, aside structural maintenance, enables the DNA copy of the viral genome to be transmitted to the host nucleus. Inside there is the *p*24-capsid. (Fig. 1)

The RNA genome consists of at least seven structural landmarks (LTR, TAR, RRE, PE, SLIP, CRS, and INS), and nine genes (*gag*, *pol*, and *env*, *tat*, *rev*, *nef*, *vif*, *vpr*, *vpu*, and sometimes a tenth *tev*, which is a fusion of tat env and rev), encoding 19 proteins. Three of these genes, *gag*, *pol*, and *env*, contain information needed to make the structural proteins for new virus particles. The six remaining genes, *tat*, *rev*, *nef*, *vif*, *vpr*, and *vpu* (or *vpx* in the case of HIV-2), are regulatory genes for proteins that control the ability of HIV to infect cells, produce new copies of virus (replicate), or cause disease.

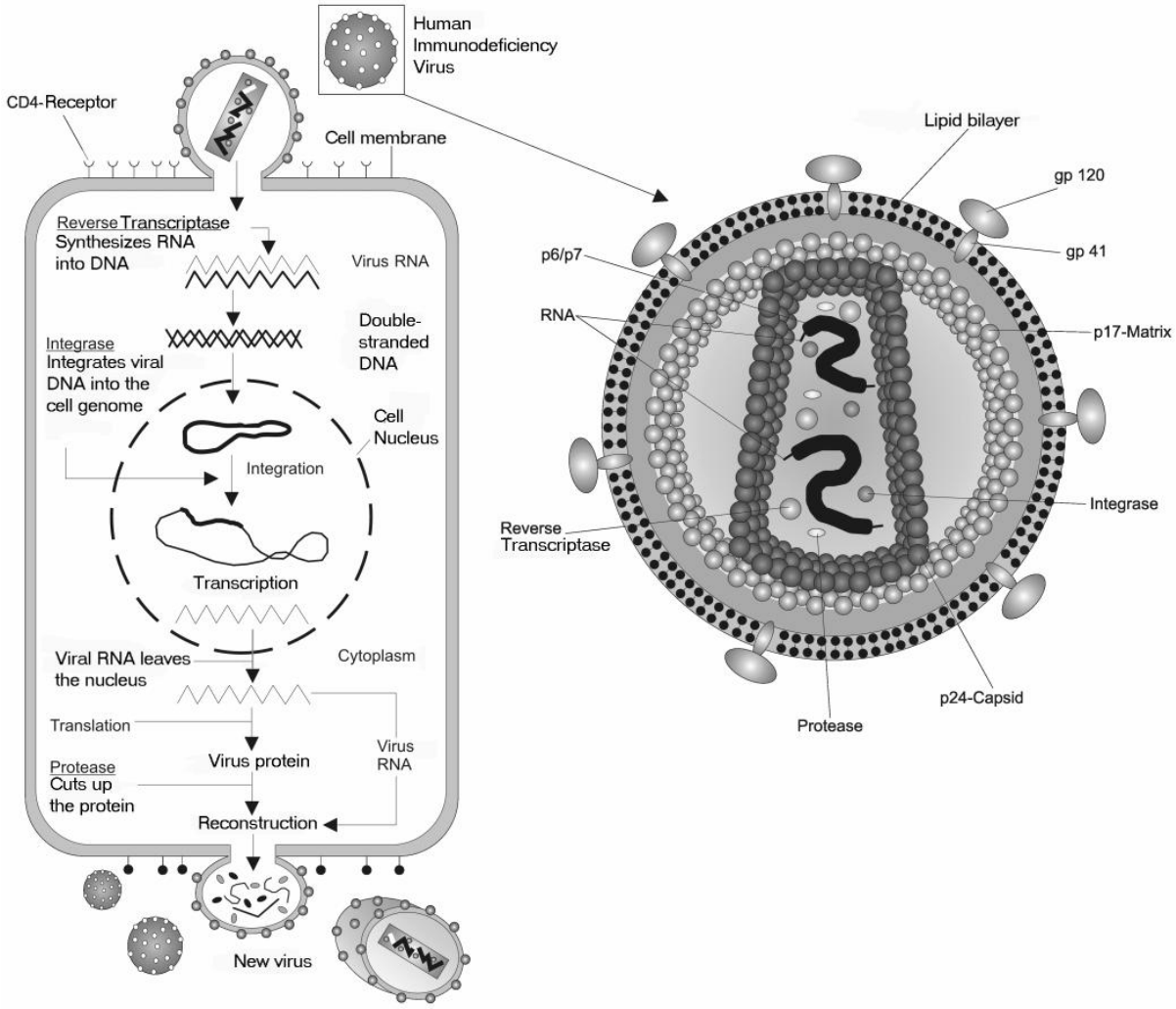


Fig. 1 structure and life cycle of HIV

The HIV virus, for his life cycle, needs to use cells of human immune system. The life cycle starts inside the host cells, in $CD4^+$ cells and macrophages.[5] (Fig. 1-2)

The virus enters in the blood stream of a new host. A protein present on the surface of the virus called gp120 [6], has high affinity for the CD4 receptor found on cells circulating in the blood called CD4+ or T helper cells. The virus attaches the host cell, and this binding is strengthened further by a co-receptor on the cell surface, called chemokine co-receptor type-5 (CCR-5) and CXC chemokine receptor type-4 (CXR-4). Once the virion has been bound to the host cell surface, its next task is to get inside. This is achieved through the fusion of the virus coat and the cell membrane. During the fusion the genetic material of the virus, is released into the cell, along with the viral enzymes, reverse transcriptase and integrase.

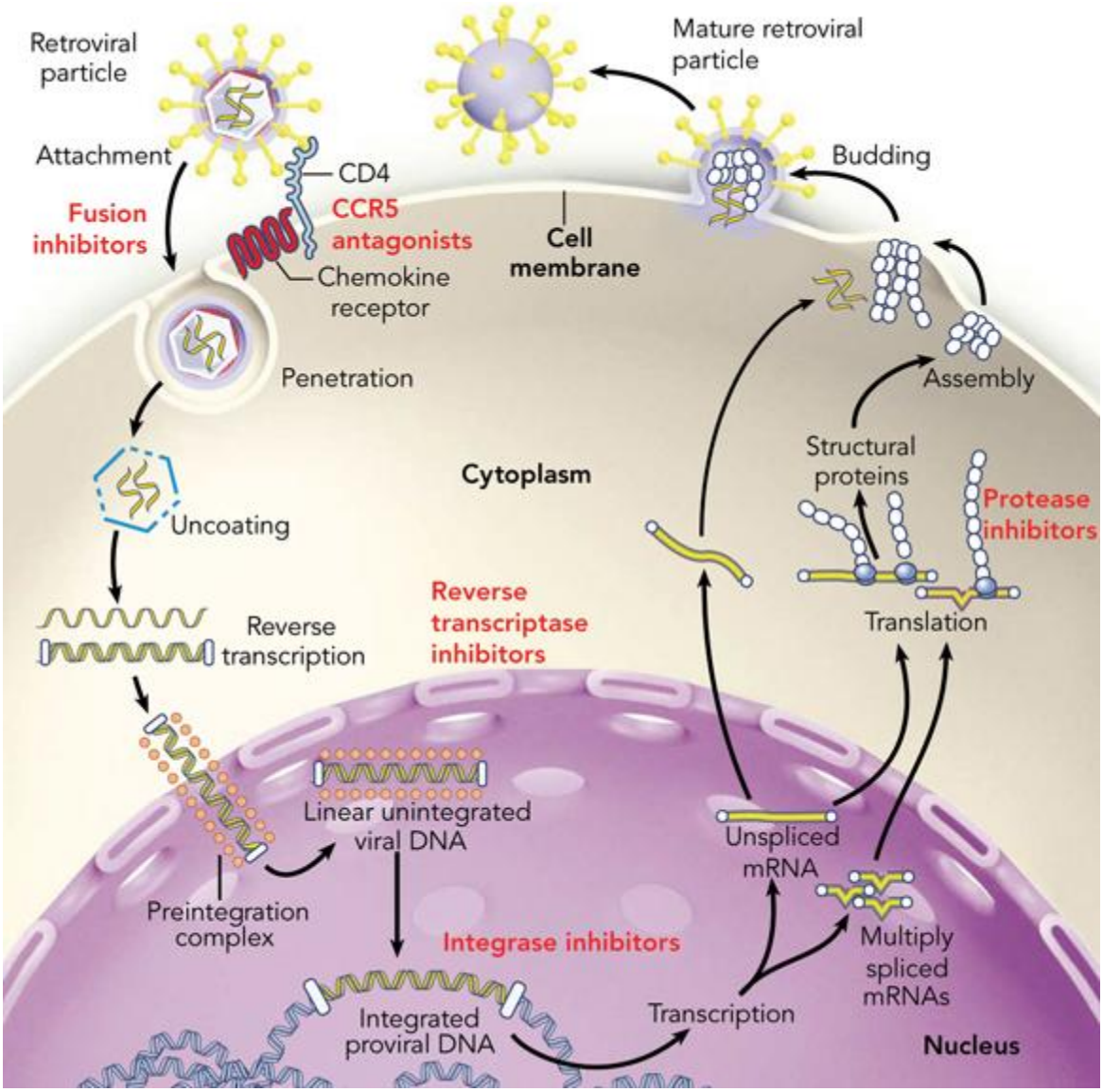


Fig.2 Life Cycle of HIV

Once in the host cell, the Reverse transcriptase reads the viral RNA and builds the corresponding DNA strands. The DNA copy is known as a provirus. Viral DNA then moves to the cell nucleus, where the cell's own genes, also made of DNA, are present. The viral enzyme integrase, splices the strands of DNA into the host cell genome and helps the integration of virus into the host genome. The proviral DNA can remain as it is for many years in a latent state, but when the host cell is in an active form during cell the division, the activated RNA polymerase II transcribes the viral DNA into messenger RNA (mRNA) which is then translated into viral proteins and polyproteins.

Together with the RNA these proteins then migrate inside the host cell membrane, where they assembly into new virions. Among the viral proteins is the enzyme, protease, which

cleaves the polyproteins into smaller, functional proteins, thereby, allowing the new viral particles to mature. After the assembly, the newly formed virions bud from the host cell surface, entering the bloodstream where they encounter uninfected CD4+ cells and begin another cycle . [7]

The RT is the enzyme essential for viral replication because is responsible for converting the genomic single-stranded RNA of HIV into double-stranded DNA (dsDNA), which subsequently becomes integrated into the host genome.

HIV-1 RT is a multifunctional enzyme that exhibits distinct enzymatic activities. In particular, reverse transcription is carried out using the following three catalytic activities. [8]

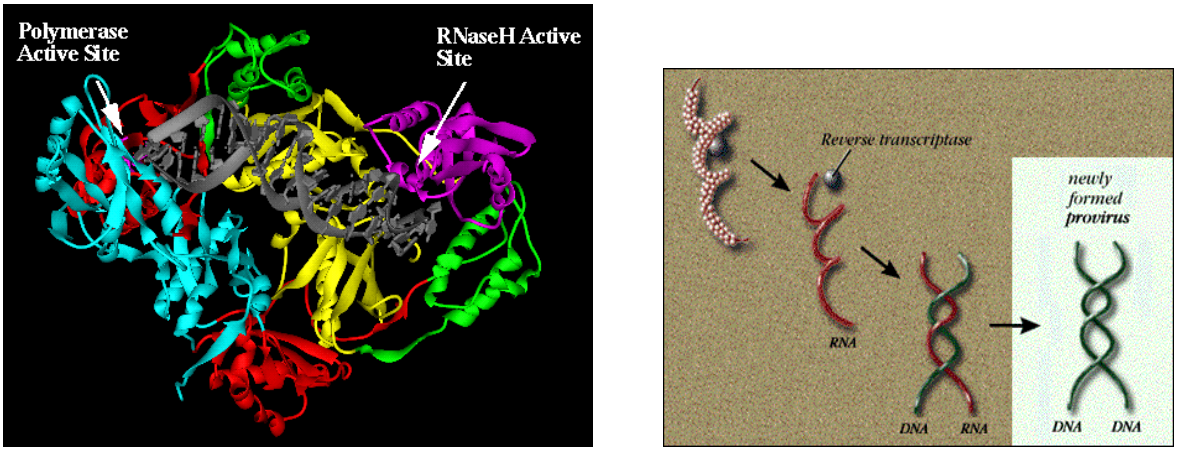
i) RNA-dependent DNA polymerisation to form an RNA-DNA hybrid

ii) RNase H degradation of the RNA strand from the RNA-DNA hybrid

iii) DNA-dependent DNA polymerisation to form a dsDNA.

This enzyme is a dimer of two related chains, a 66-kD subunit (*p*66) and a 51-kD subunit (*p*51), which is derived from *p*66 by proteolytic cleavage. The *p*66 subunit contains the polymerase and RNase H active sites as well as the non-nucleoside reverse transcriptase inhibitors binding pocket (Fig. 3). The *p*51 polypeptide corresponds to the polymerase subdomain of *p*66, and comprises the first 440 aminoacids of *p*66.[8] The subdomains within the polymerase domain of each subunit have been named fingers, palm, thumb and connection. The additional 120 residues at the carboxyl terminus of *p*66 comprise the RNase H domain. The arrangement of the subdomains in the two subunits is dramatically different. In *p*66, the subdomains are arranged to form a cleft in which the template-primer binding cleft and active site residues are buried. There is, therefore, only one functional polymerase active site per *p*66/*p*51 heterodimer. [9]

As a result of its crucial role in the life cycle of HIV, RT has been considered to be a good drug target [10] and in the years several inhibitor of this enzyme have been developed.



(a) (b)

Fig. 3 (a) Structure of the HIV-1 reverse transcriptase enzyme adapted from Huang *et al*. (b) RNA-directed synthesis followed by DNA-directed synthesis. [11]

They are classified into two classes :

a) Nucleoside and nucleotide RT inhibitors (NRTI)

b) Non-nucleoside reverse transcriptase inhibitors (NNRTI)

In the class of Nucleoside Reverse Transcriptase Inhibitors (NRTI) we can find several drugs belonging to the 2',3'-dideoxynucleosides (ddNs), that have been approved by the Food and Drug Administration for the treatment of HIV and are commercially available. They include, Zidovudine **1** (AZT, Retrovir),[12] Stavudine **2** (d4T, Zerit),[13] Zalcitabine **3** (ddC, Hivid),[14] Didanosine **4** (ddI, Videx),[15] Lamivudine **5** (3TC, Epivir),[16] Abacavir [17] and one nucleotide analogue, Tenofovir disoproxil fumarate **6** (PMPA, Viread). [18]

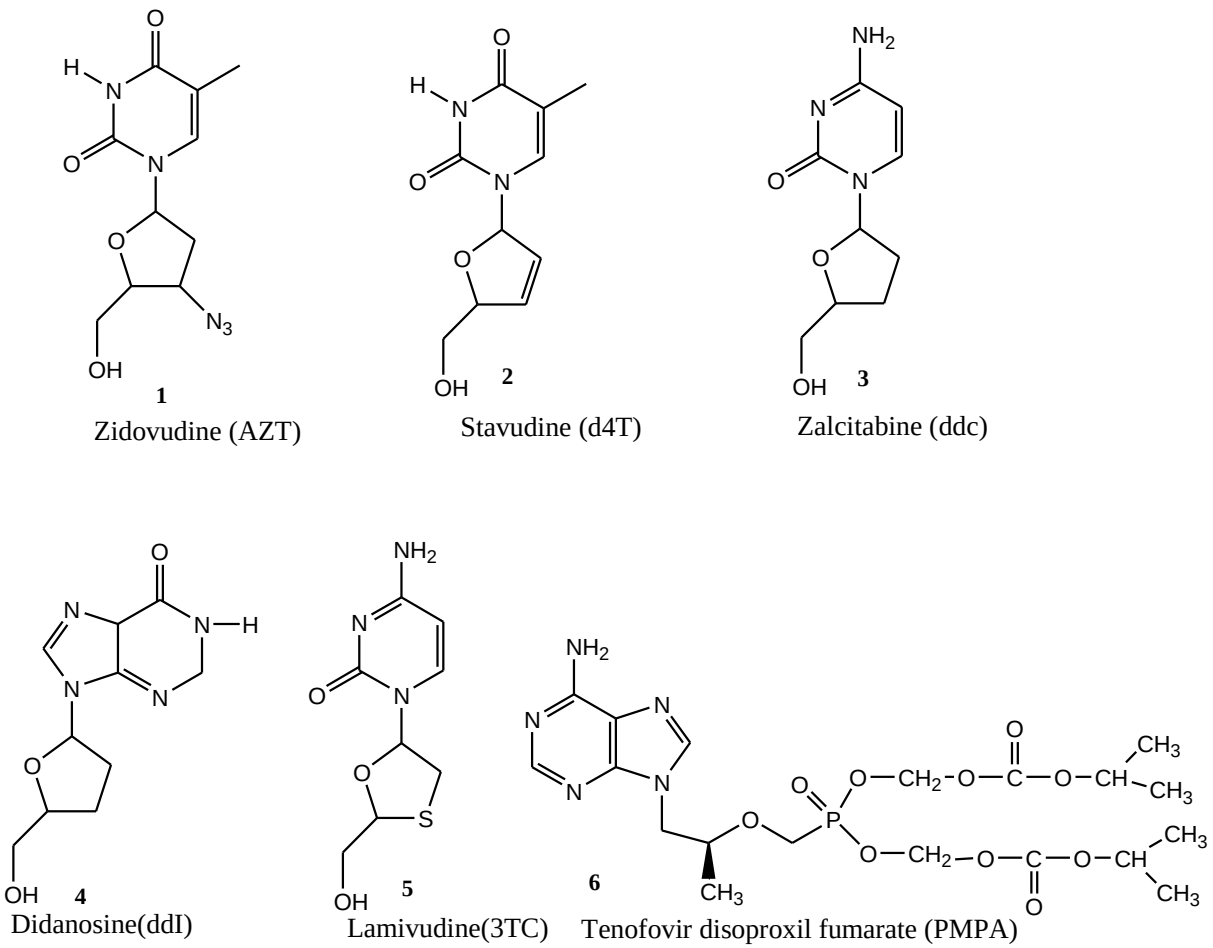


Fig. 4 Nucleoside analogues used to treat AIDS

2',3'-Dideoxynucleosides are the most important class of compounds active against HIV. They act as DNA-chain terminator and competitive inhibitors of viral reverse transcriptase (RT).[19] They block DNA elongation is blocked because the chain terminators lack the 3'-OH functional group essential for incorporation of additional nucleotides.

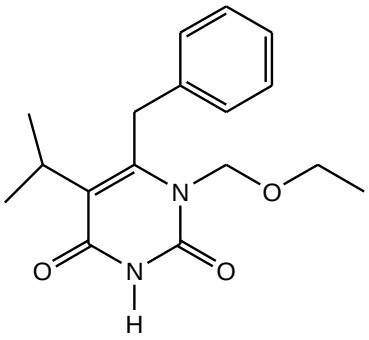
NRTIs are not highly specific and can inhibit normal cellular polymerases, causing serious side effects. They must be phosphorylated intracellularly to their 5'-triphosphate form by cellular kinases before to act as chain terminators in the RNA-directed DNA polymerisation reaction catalysed by HIV-1 RT. In their 5'-triphosphated form these dideoxynucleosides compete with the natural substrate of RT showing selective anti-HIV activity comparable to that of Zidovudine **1** (AZT) *in vitro*. Stavudine **2** (d4T) is less toxic and less inhibitory to mitochondrial DNA Nucleotide analogues binding at the dNTP-binding site, adjacent to the 3' terminus of the primer strand that is located in the palm subdomain of the *p*66 subunit.

NRTIs are subjected to drug resistance and in particular two biochemical mechanism of NRTI drug resistance are known. The first mechanism is mediated by mutations that allows the RT enzyme to discriminate NRTI during DNA synthesis, therefore preventing their addition to the growing DNA chain. The second mechanism is mediated by nucleotide excision mechanism (NEM) that increase the rate of hydrolytic removal of the chain terminator of NRTI and continue DNA synthesis. In the second mechanism the oxygen anion of a nucleoside diphosphate or triphosphate is used as a pyrophosphate nucleophile to attack and cleave the phosphate bond of the primer, producing an unblocked primer and a dinucleoside or tetraphosphate containing the dideoxynucleoside monophosphate from the primer terminus linked through its phosphate group to the distal phosphate of the free nucleoside di or triphosphate . [20]

Due to drug resistance, a considerable effort has be made two find new antiviral molecules.

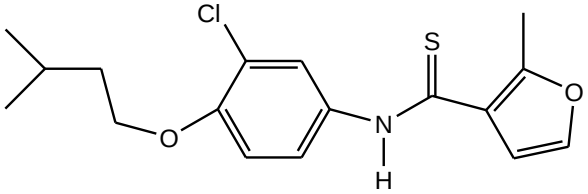
In 1987 when the first NRTI **1** (AZT) was approved for the treatment of HIV infection, the understanding of the replication cycle of the HIV virus was limited and the molecular targets that are known today to be central to HIV replication had not been identified yet.[21] A broad screening of the Janssen compound library in the early nineties led to the discovery of TIBOand α-APAas anti-HIV agents. This was followed by systematic leading optimization, which yield the first generation of NNRTIs such as Tivirapine, a TIBO derivative, and Loviride, an α-APA derivative. These compounds were effective against wild-type HIV-1 but had lower potency when tested against common NNRTI-resistant mutants. Chemical modifications were introduced in these TIBO and α-APA derivatives, resulting in a systematic structure-based molecular modeling study which played a key role in understanding the three dimensional structure-activity relationships in these two chemically distinct series.

The disclosure of TIBO compounds inspired the search for more potent and selective RT inhibitors. To date, more than 30 structurally diffrent NNRTIs have been identified. They include HEPT derivatives **7**, Thiocarboxanilide derivatives **8**,[22] quinoxaline derivatives **9**, [23] and PETT derivatives **10**. [24] (Fig.5)



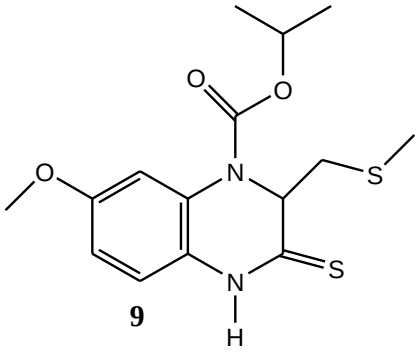
7

Hydroxyethoxymethylphenylthiothymine
(HEPT)



8

Thiocarboxanilide



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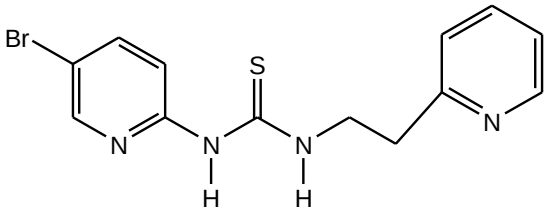
Quinoxaline



10

Trovirdine
(PETT)

Fig. 5 Chemical structures of some selected NNRTI inhibitors

The number of compounds within the NNRTIs rapidly increased and by the end of 1998, the following three compounds had been approved for clinical use by the FDA Nevirapine **11**, [25] Delavirdine **12** [26] and Efavirenz **13**. [27] (Fig.6)

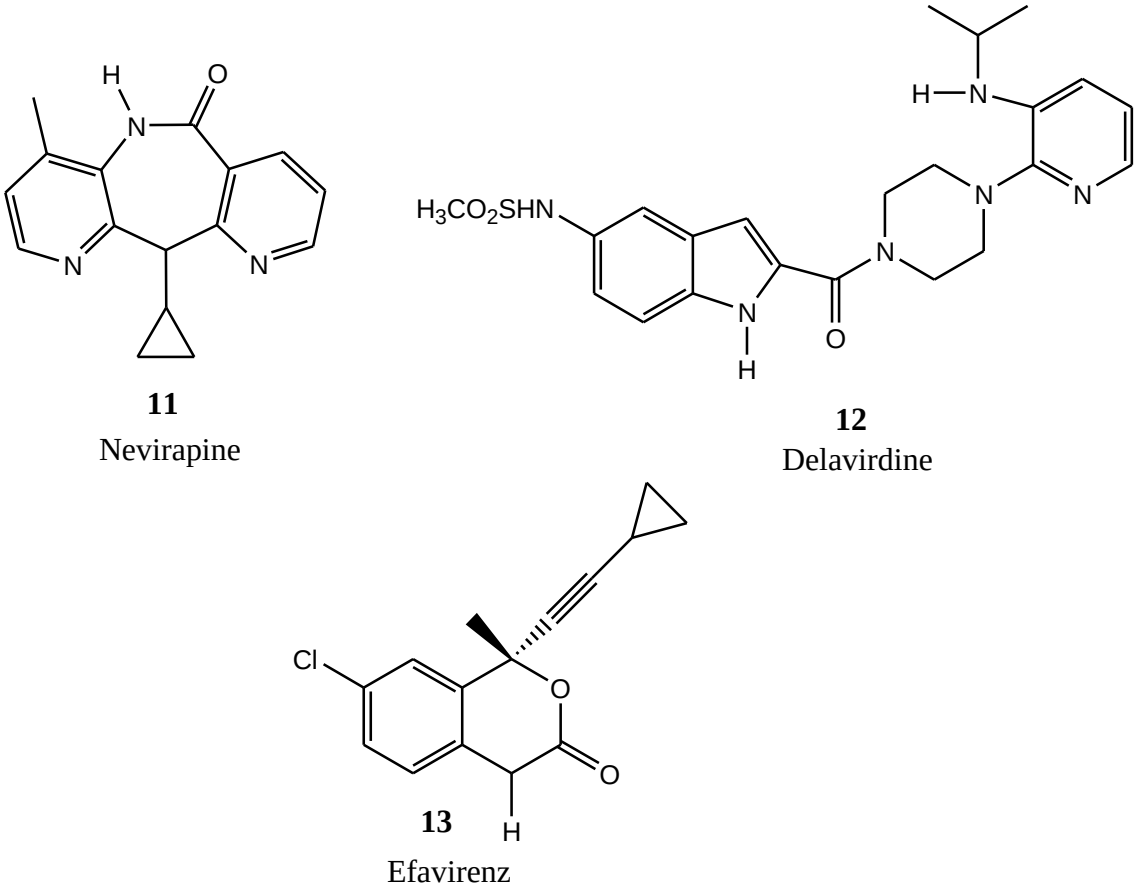


Fig. 6 NNRTIs approved for the treatment of AIDS

Unfortunately also this class of inhibitors was vulnerable to HIV's high mutation rate, which resulted in a rapid selection of resistant strains. This tempered the initial enthusiasm and even led some groups to abandon NNRTI research altogether. [28]

By early 2000, structural activity studies around the α-APAs led to produce imidazoylthiourea (ITU) derivatives. An effort to improve the metabolic stability of the ITUs led to the serendipitous discovery of DATA compounds. These compounds have a triazine ring that replaced the unstable thiourea moiety of ITU (Fig. 7). Molecular modeling of DATA compounds suggested that replacing the central triazine ring with a pyrimidine ring it would be improved the activity. This structural modification led to the discovery of etravirine, a diarylpyrimidine (DAPY) derivative, that is highly potent and effective against wild-type and drug-resistant HIV-1 mutants. [29]

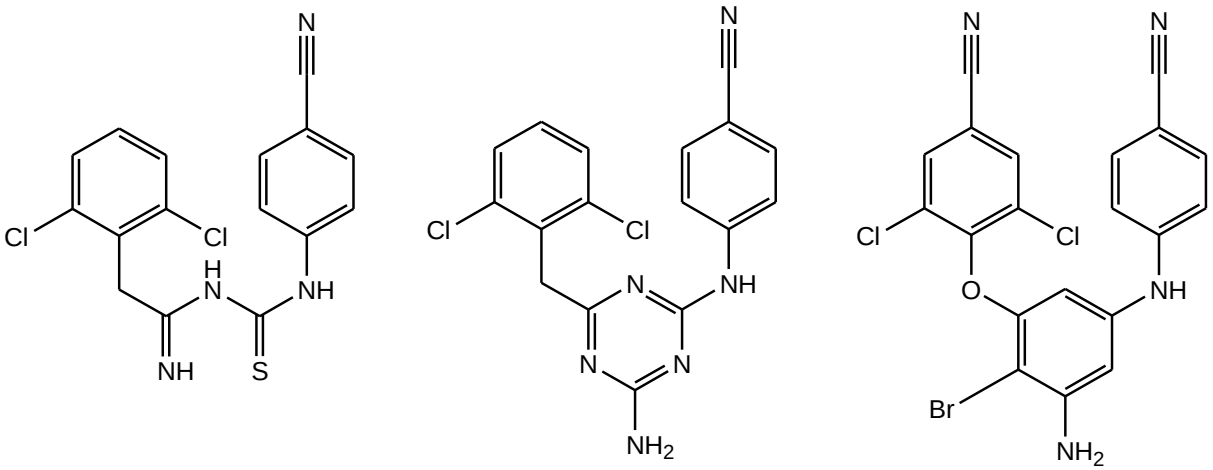


Fig. 7 Chemical structures of second-generation NNRTIs.

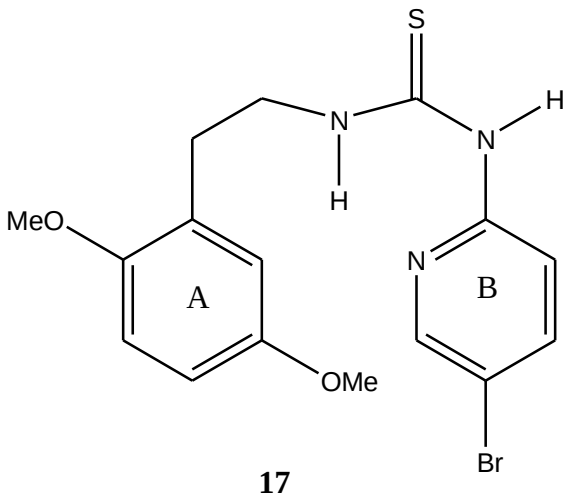
NNRTIs bind at a largely non-conserved site. The NNRTI binding site ('pocket') is located in the *p*66 domain about 10Å away from the substrate binding site. The residues in this pocket are mainly of hydrophobic nature with substantial aromatic character. The pocket region can also contains a few hydrophilic residues and backbone atoms that may form hydrogen bonds to non-nucleoside compounds, which all include hydrogen-bond donor and acceptor groups.

The NNRTIs block the HIV-1 reaction through interaction with an allosterically located, non-substrate binding site. [30] The NNRTI binding in this pocket causes a repositioning of the three-stranded β-sheet in the *p*66 subunit, which contains the catalytic aspartic acid residues 100, 185 and 186. This inhibits HIV-1 RT by locking the active catalytic site into an inactive conformation, reminiscent of the conformation observed in the inactive *p*51 subunit. [31] Steady state kinetic studies carried out have revealed that NNRTI do not prevent the binding of nucleotide triphosphate substrates to the enzyme, but they block the chemical step of NRTI incorporation. [32]

When bound into their pocket, the first-generation NNRTIs maintain a 'butterfly-like' shape. They roughly overlay each other in the binding pocket and appear to function as electron donors to aromatic side-chain residues lining the pocket. [33]

In crystal structures of unliganded HIV-1 RT the non-nucleoside inhibitor binding site (NNIBS) does not exist. During the process of binding significant conformational changes occur in the orientation of the side chains of Tyr181 and Tyr188 leading to the formation of the hydrophobic pocket accommodating the inhibitor. It is evident from a comparison of the various RT structures that the NNIBS has a very flexible structure, and that this flexibility apparently allows the enzyme to accommodate structurally diverse inhibitors with different shapes and sizes. In fact, side-chain residues adapt themselves to each bound inhibitor in a highly specific manner, closing the surface of the drug to make tight van der Waals interactions. [34]

Structural studies around N-(2-phenylethyl)-N'-(2-thiazolyl)thiourea (PETT) led to the discovery of *N*-[2-(2,5-dimethoxyphenylethyl)]-*N*'-[2(5-bromopyridyl)]-thiourea **17** (Fig.8) which was designed to optimize occupancy of the binding pocket. [35] Docking experiments of HI-236 revealed that the 2-methoxy group was situated beneath the ethyl linker and fits favourably into a cavity of the binding pocket, providing additional interactions with the protein residues. [36]



17

Fig. 8 1-(5-bromopyridine-2-yl)-3-[dimethoxyphenyl)ethyl]thiourea (HI-236)

Structure-activity study of ring A showed that the methoxy group substitution is more favourable on the *meta* (to the alkyl side chain) position, compared to the *para* position. Fluorine substitution is favourable on the *ortho* and *meta* positions, whereas chlorine was only favourable at the *ortho* position. Similarly, a hydrophobic group is more desirable than polar group or hydrophilic group at the *para* position. [37]

NNRTIs have been notorious for rapidly triggering the emergence of drug-resistant HIV virus. Resistance usually emerges when an NNRTI is administered as a monotherapy. NNRTI resistance mutations arise rapidly causing changes in the binding of NNRTI to RT. The most common mutations are Lys103 with Asn and Tyr181 with Cys.[38] The mutations cause a loss of aromaticity, by reducing favourable interactions, and indirectly affects NNRTI potency by stabilizing the closed form of the non-nucleoside inhibitor binding pocket (NNIBP) through the formation of a hydrogen bond between the Asn103 side-chain amide and the oxygen of the phenoxy group, reducing the rate of inhibitor entry.[39] The Leu100 mutation cause steric interference between the β-branching isoleucine and a bound NNRTI. The mutation causes resistance through steric hindrance of the methyl side chain and the bound inhibitor. [40]

Mutations associated with resistance to nevirapine involve residues which have van der Waals interaction with the inhibitor. Mutations of these residues lead to the weakening of the inhibitor binding to RT. [41] The most common resistance mutation observed for nevirapine *in vivo* is prevented from emerging by co-administration of AZT. The mutually antagonistic effects of different resistance mutations and the hypersensitivity that is seen under some conditions, argues in favour of the combined use of NNRTIs with NRTIs and different NNRTIs with another one. [42]

Because of resistance, combination of drug therapy is often used to exploit the synergistic and additive activities of individual drugs. “Highly active anti-retroviral therapy” (HAART), is a treatment regime that commonly combines the use of two NRTIs and one NNRTI or protease inhibitor.[43] Combinations of NRTI and NNRTIs have been found to decrease viral load, increase CD4 count, decrease mortality and delay disease progression to AIDS. [44]

Giuseppe Campiani reported a series of quinoxalinylethylpyridylthiourea as potent Non-Nucleoside HIV-1 Reverse Transcriptase (RT) Inhibitors. (Fig. 9) [45],[46]

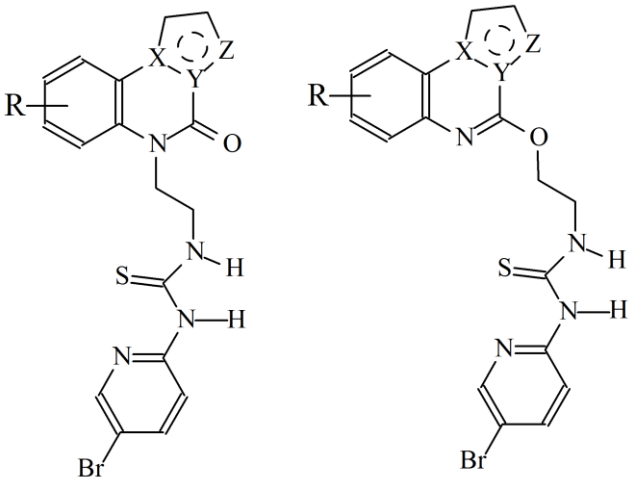


Fig. 9

The derivatives reported showed excellent biological activity as potent Non-Nucleoside HIV-1 Reverse Transcriptase Inhibitors showed in table 1.

Compound	R	A	X	Y	Z	W T $IC_{50}(\mu M)$
18 a	7-F	CH	N	C	CH	0.029
18 b	9-F	CH	N	C	CH	0.04
18 c	7,9-diF	CH	N	C	CH	0.022
18 d	7-Cl	CH	N	C	CH	0.02
18 e	H	N	N	C	CH	0.009
18 f	H	CH	N	C	N	0.25
18 g	H	CH	C	N	N	0.015
19 a	6-F					0.058
19 b	7-Cl					0.053
19 c	7,8-diMe					0.22
Efavirenz						0.03
Nevirapine						0.4

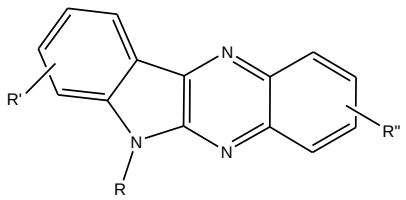
Table 1. Inhibition of HIV-1 Wild type RT Enzymes.

All compounds showed IC_{50} value at nanomolar concentration and in particular compound

18d, **18e** and **18g** showed higher IC_{50} than marketed Efavirenz.

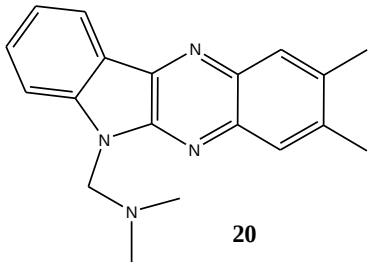
Based on encouraging results shown by quinoxalinylethylpyridylthiourea derivatives it was decided to substitute the five term ring with an isoindol ring in order to verify their antiviral activity, considering the experience gained from my research group on isoindolquinoxaline compounds.

Quinoxalines and structurally related quinoxalinones represents an important class of biologically and medicinally important compounds. A large number of synthetic quinoxalines showed antineoplastic, antibacterial, antiviral, antifungal activity. [47],[48]



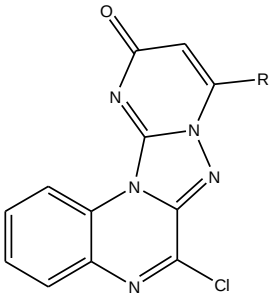
19

6*H*-Indolo[2,3-*b*]quinoxaline



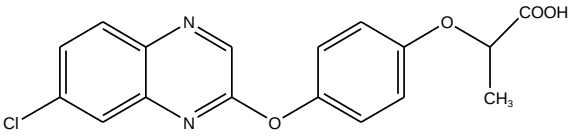
20

(2,3-Dimethyl-indolo[2,3-*b*]quinoxalin-6-ylmethyl)-dimethyl-amine



21

6-Chloro-5,7,7a,11,11b-pentaaza-benzo[*c*]fluoren-10-one



22

2-[4-(7-Chloro-quinoxalin-2-yloxy)-phenoxy]-propionic acid

Fig.10 Common heterocyclic quinoxalines and quinoxalines like compounds with biological activity.

In the last years our research group was interested in the synthesis of quinoxalines and Isoindolequinoxalines derivatives biologically active as anticancer compounds. [49]

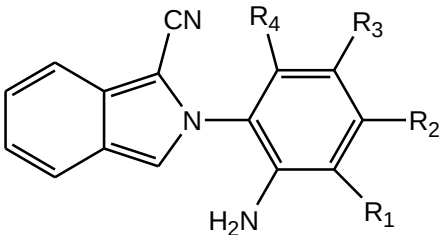
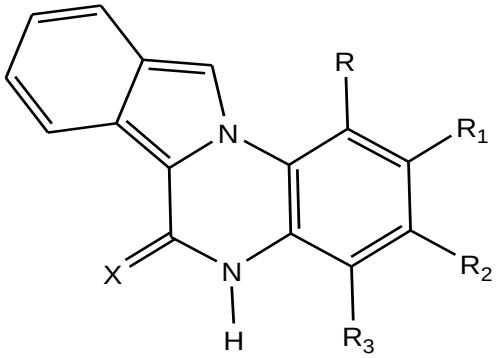


Fig.11 General chemical structer of isoindole intermediates.

Isoindolo[1,2-*a*]quinoxalines compounds were synthesized starting from isoindole intermediates with diffrent substituents for both stabilize and functionalize the isoindole moiety. The synthesized compounds had an electron-withdrawing substituent at C-1, which stabilize the isoindole structure, high electron density at the position 3 and also a phenyl substituted moiety on the nitrogen bearing a important amine group showed in fig 11.



23

X = O
X = NH

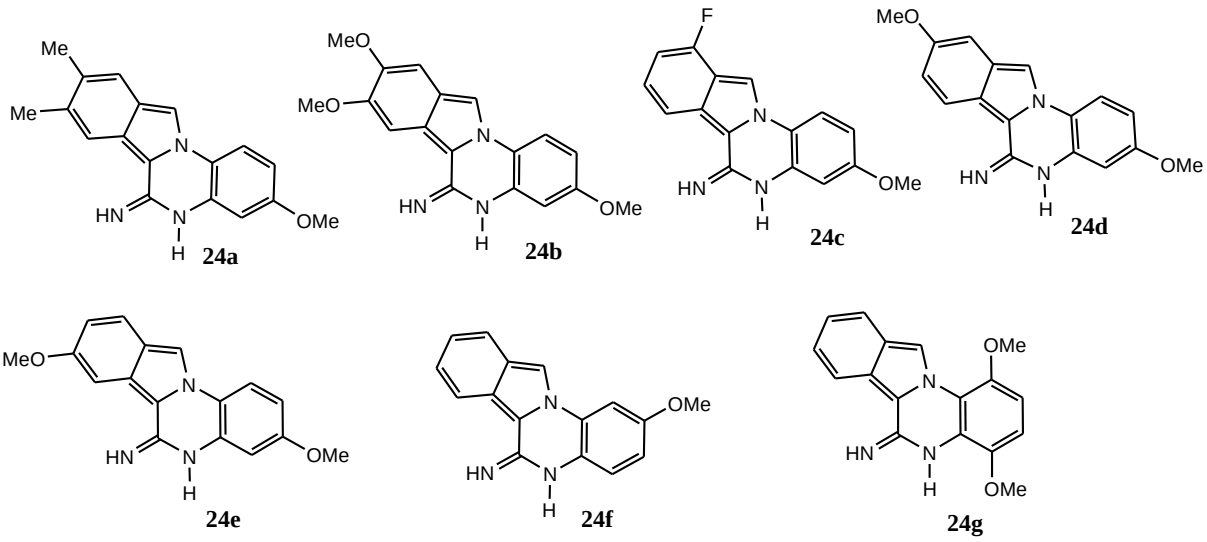
Fig.12 **Isoindole[2,1-*a*]quinoxalines**

a R= R_1 =R_2 = R_3 =H
b R= R_1 = R_2 =H, R_3 =Me
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

All five isoindolequinoxalines synthesised were tested at National Cancer Institute (NCI, Bethesda) and showing high anticancer activity. They were firstly tested against three cancer cell lines (MCF7-breast, NCI-H460-nonsmall cell lung and SF-268-CNS) at one dose concentration (10^{-4} M).

All Isoindole[2,1-*a*]quinoxalines compounds (**ISQ**) **23a-e** were selected for the biological evaluation on the full (NCI) panel of about 60 human cancer cell lines and determined parameters of GI_{50},TGI and $LC_{50.}$ All of them showed antineoplastic activity against all human cancer cell line tested. The biological data reported showed the most active compound is the 3 methoxy-compound **23c** with pGI_{50} of 7.32. After that to increase the water solubility of these compounds a new series of ISQ with a NH group instead a carbonyl one, were synthesized. These latter compounds showed comparable activity with the previous one. In order to study how different substituents can influence the biological activity, more compounds prepared using different kind of substitution on the aromatic rings and changing substituent's position both in benzene fused ring and in the indole portion. The newly synthesised derivatives showed more potent activity against cancer than previously reported isoindolequinoxalines.

These synthesised derivatives **24 a-g** were also submitted to the National Cancer Institute of Bethesda (NCI) and tested in a full panel of about 60 different tumour cell lines.
They showed antitumor activity against the total number of cell lines investigated with pGI_{50} mean values from 5.91 to 7.87 showed in table 2.



NSC	Compd.	No. of the Cell Lines Investigated	No. of cell lines giving positive $_{P}GI_{50}$		
				Range	MG_MID
747520	**24a**	56	56	>8.00 – 5.42	7.57
747526	**24b**	56	56	>8.00 – 4.81	7.75
747521	**24c**	54	53	7.86 – 4.37	6.95
747523	**24d**	54	54	>8.00 – 5.92	7.87
747522	**24e**	56	56	>8.00 – 5.98	7.70
747524	**24f**	46	46	7.14 – 5.70	6.38
477525	**24g**	58	58	7.14 - 5.64	5.91

Table 2

The most active compound was compound **24d**, whose pGI_{50} mean value was 7.87.

It is interesting to note that the two derivatives **24f** and **24g**, in which the methoxy group position was changed, are less active than **24d** they showing pGI_{50} of 6.38 and 5.91 respectively. (Table 2)

In fig.13 is reported the mean graph of the most active compound **24d** (NSC 747523) whose GI_{50} value reached nanomolar concentration on 89% of the cell lines and 20% of the cell lines at TGI.

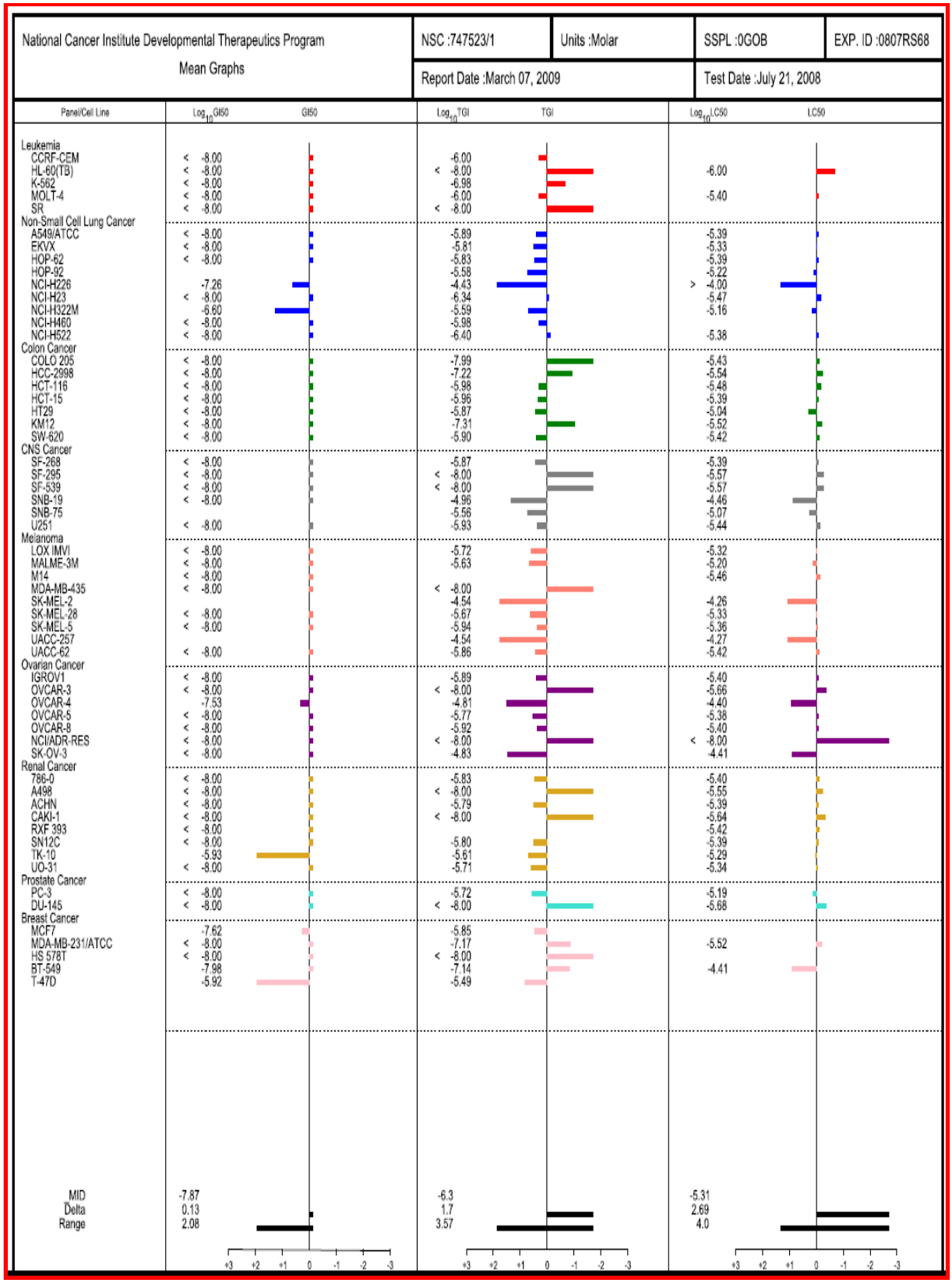

National Cancer Institute Developmental Therapeutics Program
Mean Graphs
NSC :747523/1
Units :Molar
SSPL :0GOB
EXP. ID :0807RS68
Report Date :March 07, 2009
Test Date :July 21, 2008
Panel/Cell Line
Log10GI50
GI50
Log10TGI
TGI
Log10LC50
LC50
Leukemia
CCRF-CEM < -8.00 -6.00
HL-60(TB) < -8.00 < -8.00 -6.00
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. 13

The compound **24d** was selected for *in vivo* screening

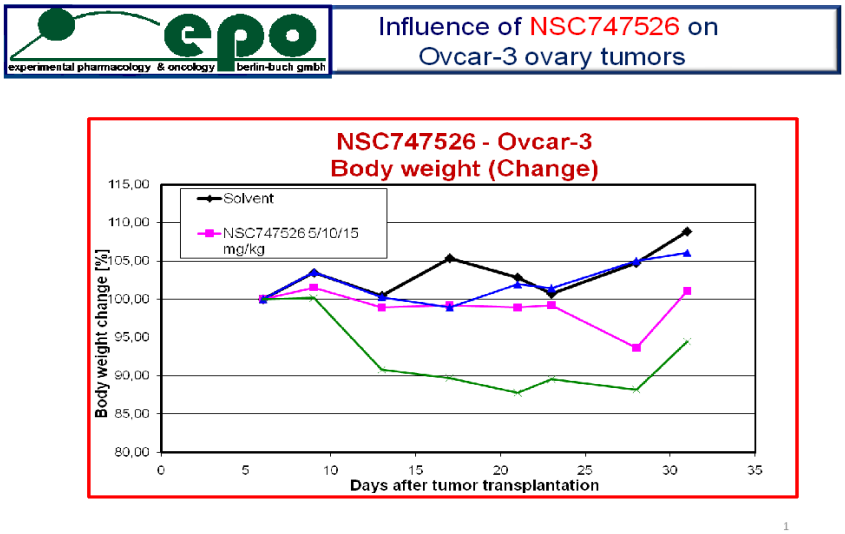
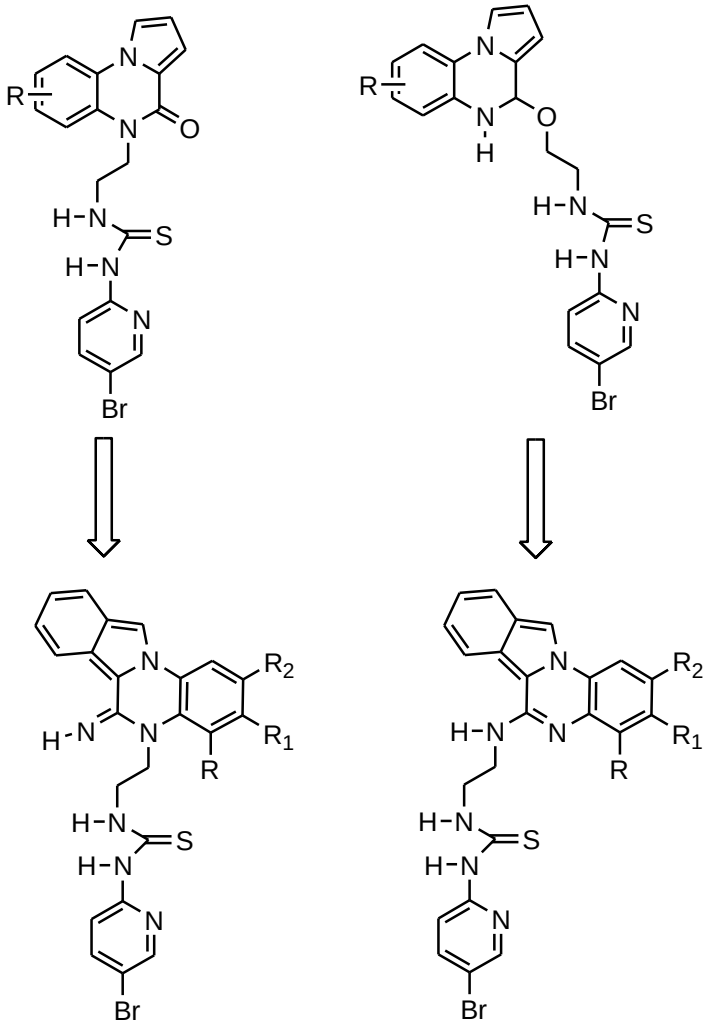


Fig. 14

In vivo test performed on Ovcar-3 tumor showed that at the dose of 15 mg/kg **24d** induced a significant reduction of the tumor although accompanied by a decrease of the body weight which was however recovered. (Fig. 14)



25 **26**

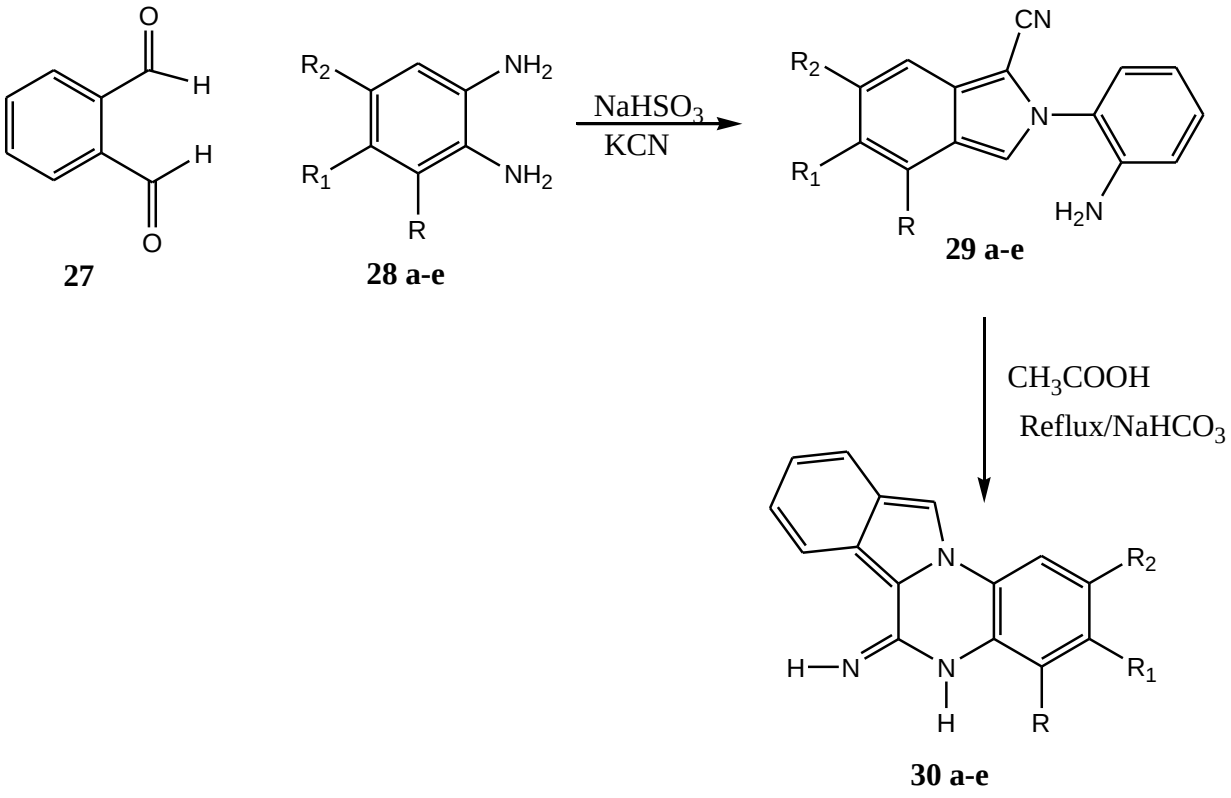
Fig. 15

Considering the good biological activity of ISQ and considering the commercially available starting material used to obtain compounds **23**, it was proposed to synthesize compounds of type **25** and **26** with the insertion of the side chains present in the Campiani's compounds on derivatives **23**. For the synthesis of new derivatives isoindoloqunoxalinethylpyridylthiourea the route involves the preparation of intermediate 5*H*-isoindol[2,1-*a*]quinoxalin-6-ylideneamine, obtained by following previously reported reaction process.

SYNTHESIS

We started the synthetic route from the 5*H*-isoindolo[2,1-*a*]quinoxalin-6-ylideneamine intermediates **30a-e.**

They were synthesized from the commercially available *o*-phtaldehyde **27** and the substituted *o*-phenylenediamines **28a-e.** They were reacted in the presence of sodium hydrogen sulphite and potassium cyanide in water to give 1-cyano-2-(2'aminophenyl) isoindoles **29a-e** in 60-80% yield.



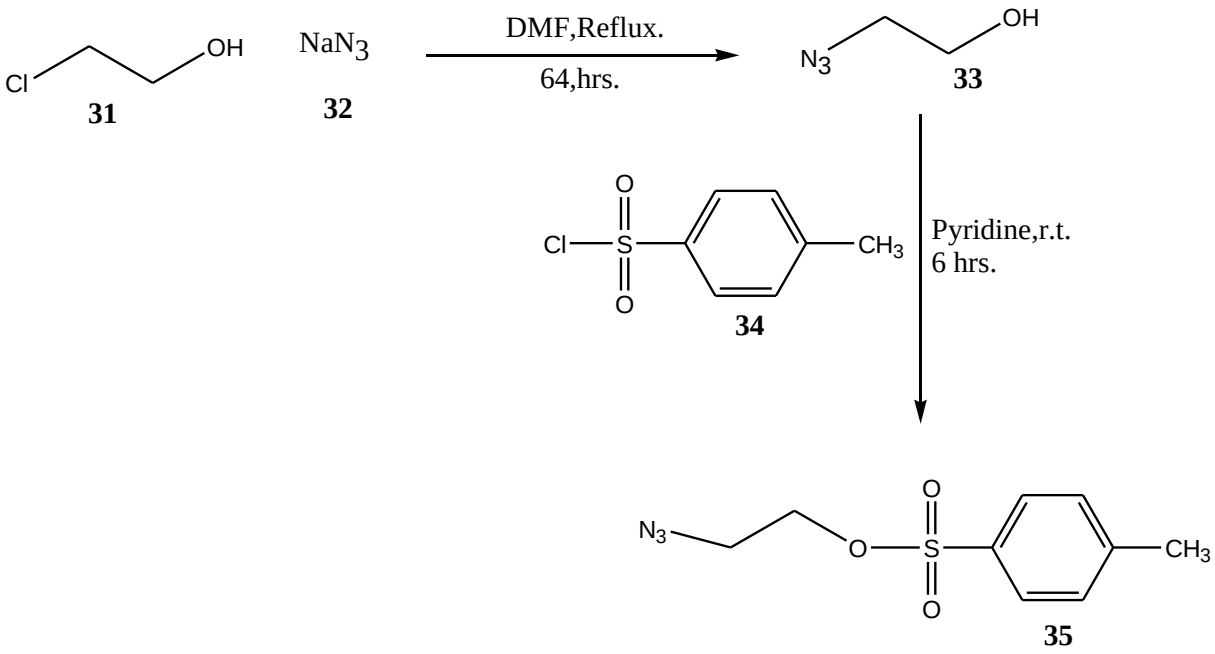
27 **28 a-e** **29 a-e**

30 a-e

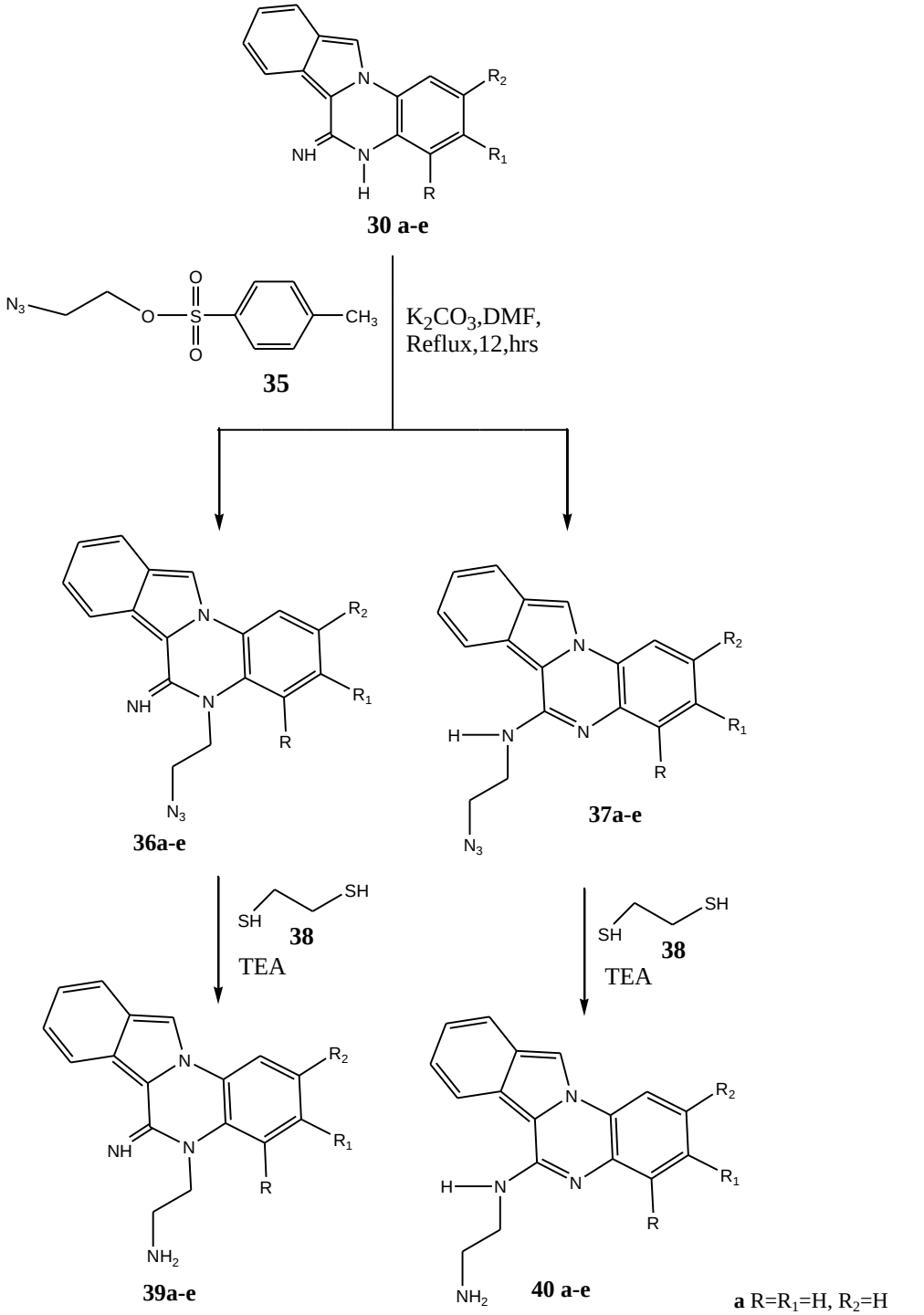
a R=R_1=H, R_2=H
b R=R_2=H, R_1=OMe
c R=R_2=H, R_1=Me
d R= H, R_1=R_2=Cl
e R=H, R_1=R_2=Me

Compounds **29a-e** were then dissolved in acetic acid and refluxed for 30 min. The resulting reaction mixture was neutralized with sodium hydrogen carbonate at 0°C obtaining 5*H*-isoindolo[2,1-*a*]quinoxalin-6-ylideneamine intermediates **30a-e** in 60-90% yield.

Once synthesized the 5*H*-isoindolo[2,1-*a*]quinoxalin-6-ylideneamine intermediates **30a-e** were functionalized using compound **35** synthesized from reaction between 2-chloroethanol **31** and sodium azide **32** in DMF under reflux for 64 hours. The 2-azidoethanol **33** obtained was then treated with *p*-toluenesulfonyl chloride **34** in the presence of pyridine at room temperature for 6 hours to obtain the desired compound **35** in 60% yield.



Isoindolquinoxalines **30a-e** were reacted with tosylethyl azide **35** in the presence K_2CO_3 in DMF at 90°C giving a mixture of ethylazide substituted quinoxaline **36a-e** and **37a-e**. The two ethylazide isomers were separated by column chromatography to give **36a-e** and **37a-e** in 30-50 % yield and 25-30 % yield.

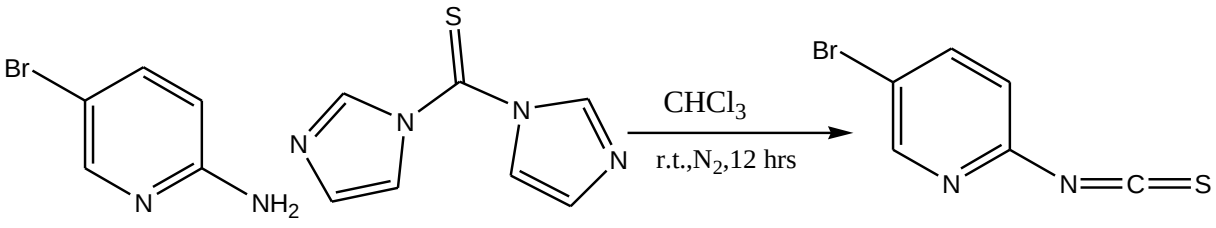


a R=R_1=H, R_2=H
b R=R_2=H, R_1=OMe
c R=R_2=H, R_1=Me
d R= H, R_1=R_2=Cl
e R=H. R_1=R_2=Me

The substituted azide 5-(2-azido-ethyl)-5H-isoindolo[2,1-a]quinoxalin-6-ylideneamine intermediates **36a-e** and (2-azido-ethyl)-isoindolo[2,1-*a*]quinoxalin-6-yl-amine **37a-e** were subjected to reduction using propane-1,3-dithol **38** and triethylamine at room temperature for

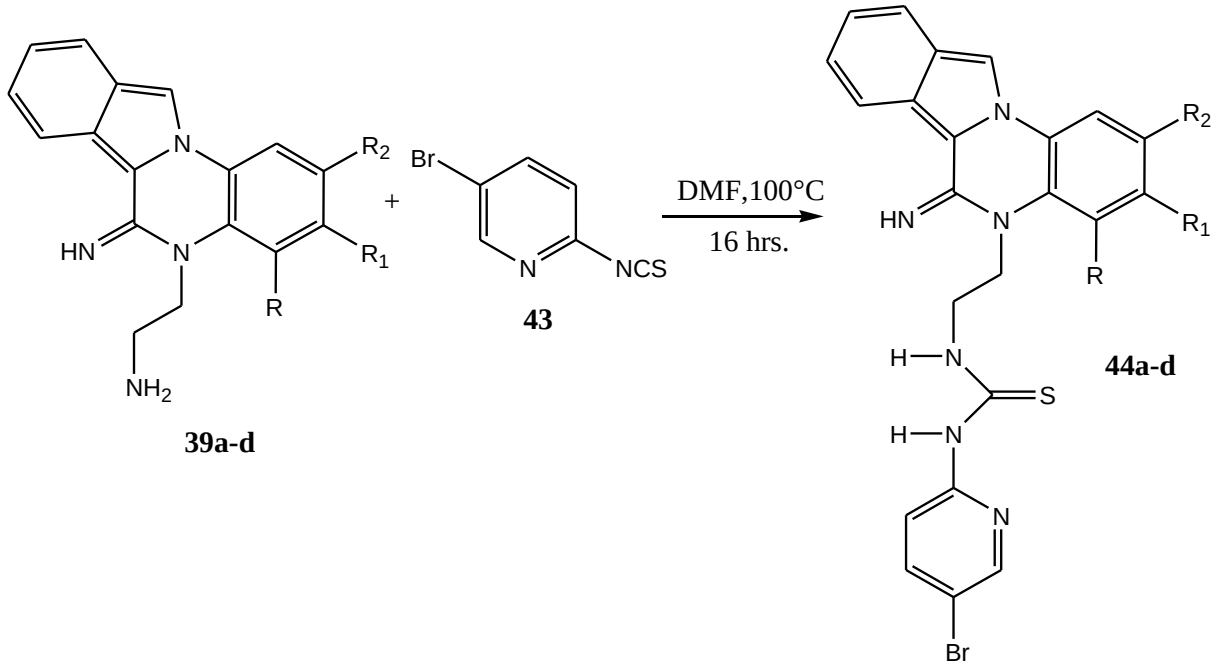
24 hours under argon atmosphere to give the corresponding substituted amines **39a-e** and **40a-e** in 60-80% yield.

The substituted amino intermediates **39a-e** and **40a-e** were functionalized with bromopyridylthiourea **43**. It was synthesized from 2-amino-5-bromo-pyridine **41** and 1,1'-Thiocarbonyldiimidazole **42** in chloroform at room temperature under nitrogen in 74% yield.

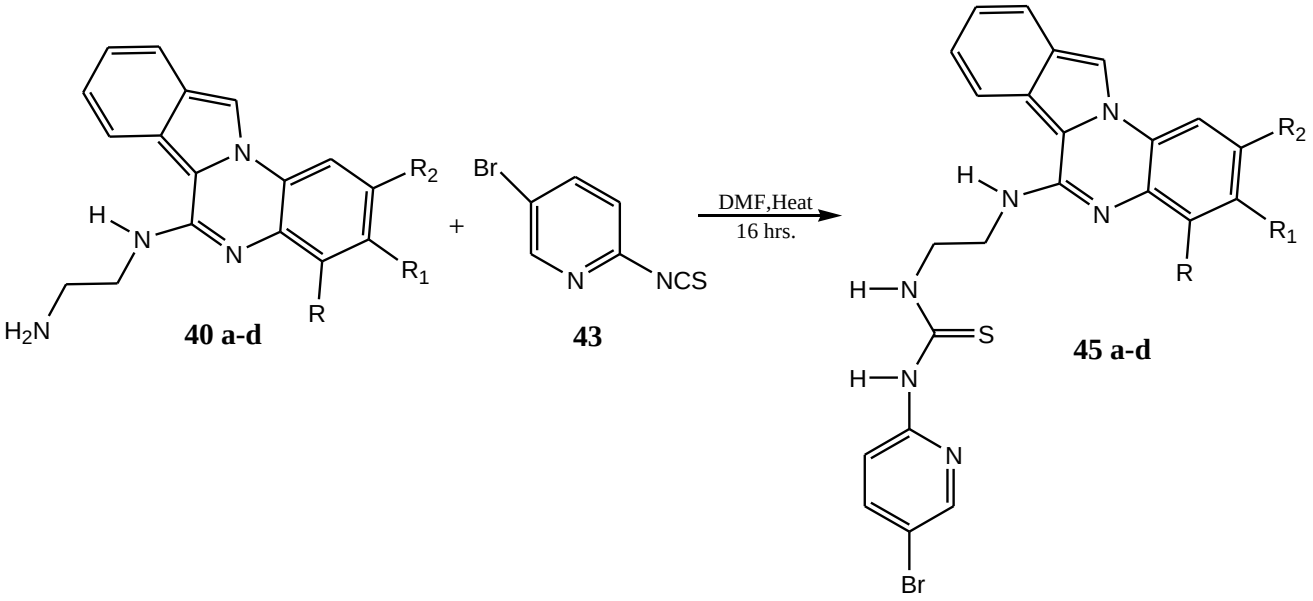


41 **42** **43**

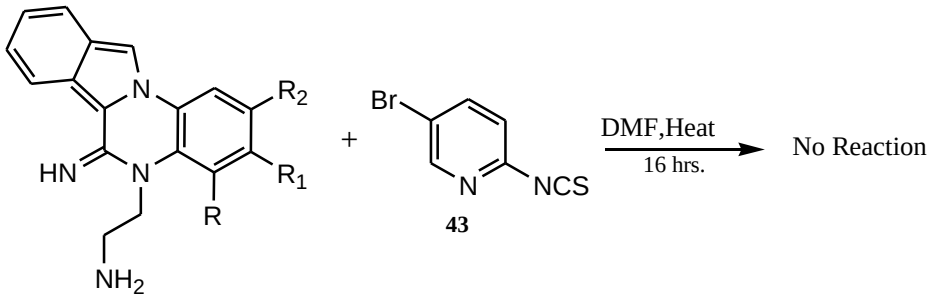
Once synthesised compound **43** was reacted with amino intermediates **39a-e** and **40a-e** in DMF at 100°C to give the desired compounds **44a-d** (20-30%) and **45a-d** (20-35%).

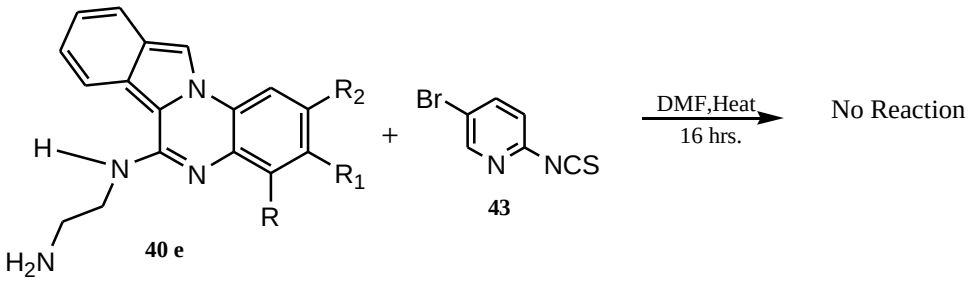


39a-d **43** **44a-d**



In case of compounds **39e** and **40e** the reaction was unsuccessful.



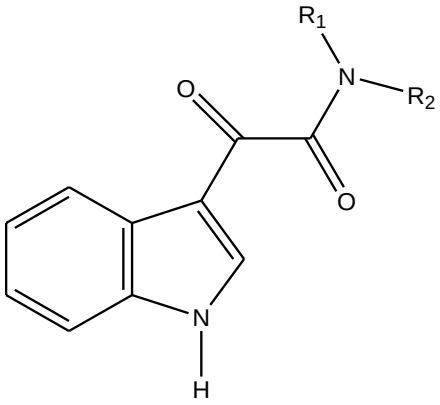


I tried to synthesize compound **44e** and **45e** using different methods and reaction conditions but synthesis was not possible.

INTRODUCTION

Indole (2,3-benzopyrrole, ketole, 1-benzazole C_8H_7N) is an aromatic heterocyclic organic compound that has a bicyclic structure consisting of a six-membered benzene ring fused to a five-membered pyrrole ring. The participation of the nitrogen ione pair in the aromatic ring means that indole is not a base and it does not behave like a simple amine. The name indole come from the words "indigo" and "oleum" since indole was first isolated by treatment of the indigo dye with oleum. Indole and many of its derivatives are the most important structural components in many naturally occurring compounds, having a wide variety of pharmacological and biological properties. Moreover substituted indoles are very important intermediates for the synthesis of classes of biologically important compounds. Likewise, pyrrole derivatives are present in compounds such as bile pigments, vitamin B12, haemin, chlorophyll, and related natural products. In addition several pyrrole derivatives are important intermediates for the synthesis of drugs, pigments and pharmaceuticals. Certain indole derivatives were important dyestuffs until the end of the 19th century. In the 1930, interest in indole increased, when it became known that the indole nucleus is present in many important alkaloids, as well as in tryptophan and auxins, and it remains an active area of research today.[50] The indole moiety is one of the most attractive scaffolds with a wide range of biological and pharmacological activities. This chemically important nucleus is frequently found in therapeutic agents as well as in natural products.

For example indole-3-yl-glyoxylamide derivatives **46** are potent biological active agents, this led to the synthesis of a large number of structural compounds containing indole-2-carboxylic moiety as an invariable ingredient. The diversity and rapid access to small and highly functionalized organic molecules makes this approach of current interest in the drug discovery process. [51]

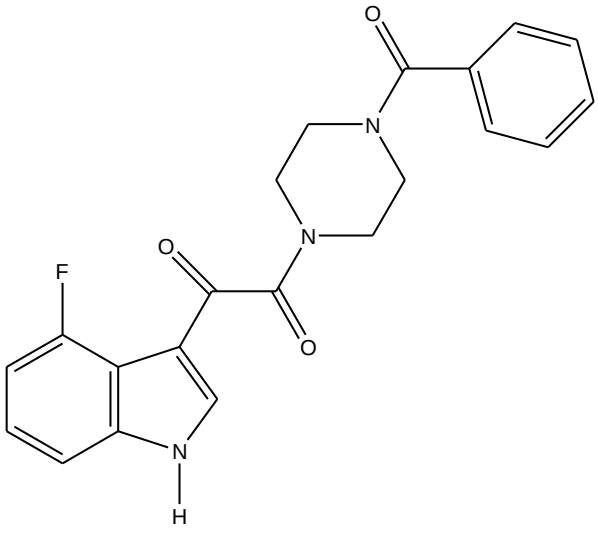


46

Fig. 15 General structure of indole-3-yl-glyoxylamide.

Therapeutic importance of indole glyoxylamide derivatives showed high affinity against the benzodiazepine binding site receptors. Indole-3-yl-glyoxylamide derivatives showed wide spectrum of pharmacological activities as anticancer, anti-HIV, antiviral,[52] antimicrobial,[53] cardiovascular.[54]

It was described [55] the discovery of indole-3-glyoxamide **47** (fig.16) derivative as the first small molecule inhibitor of the *gp*120-CD4 interaction (HIV-1 attachment inhibition) that demonstrate potent antiviral activity in cell culture. [56]

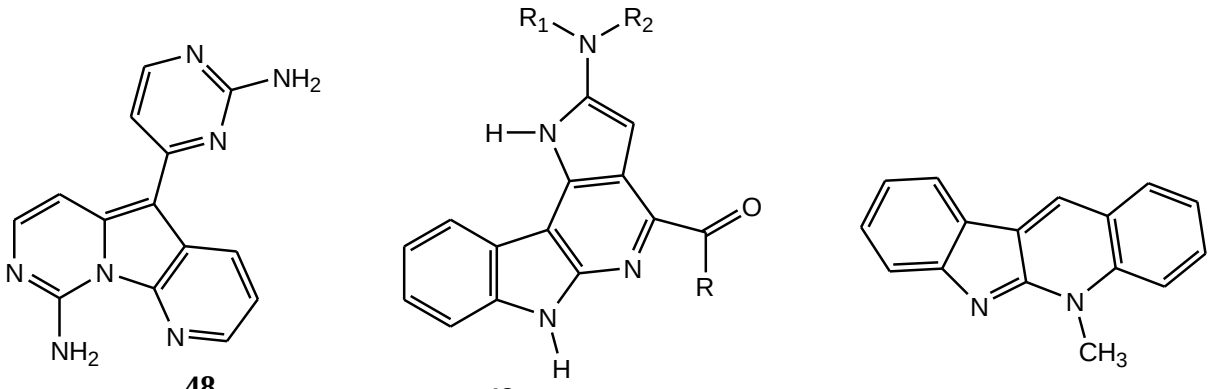


47

Fig. 16 Indole-3-glyoxamide.

Heterocyclic compounds bearing Indole-3-yl-glyoxylamide ring system are endowed with variety of biological activities.

Replacing one of the carbon atoms at positions 4 to 7 in the indole moiety with a nitrogen atom gives the azaindoles [57] which are known as indole bioisosteres [58] and, although some examples exist in the nature, most of they are synthetic products. Indoles and azaindoles belong to the fused [5,6]-membered ring systems. They are often classified as purinomimetics or purine isosteres, and exhibit a wide range of biological activities and pharmacological properties.[59] These rings are part of synthetic products of naturally occurring alkaloids, such as 7-aza-rebeccamycin and 5-aza-ellipticine. Their biological activities are based mostly on their affinity toward DNA, but also as topoisomerase inhibitors and as potential kinase inhibitors.[60]



48 Variolin B

49 Grossularines

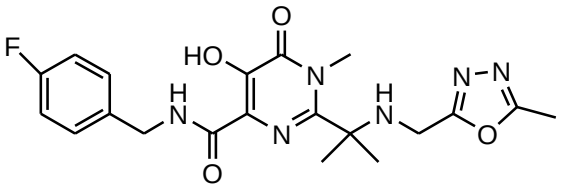
50 Neocryptolepine

Fig. 17 Common chemical structure of natural products containing azaindol moiety.

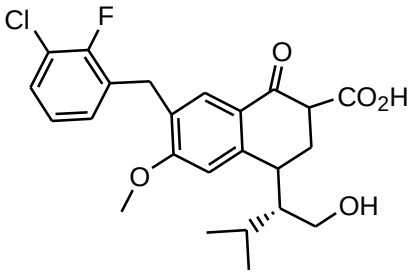
The interest in azaindole compounds increased for their medicinal importance and applications in material synthesis and coordination chemistry, and some azaindole are contained in compounds existing in nature, like variolins,[61] grossularines [62] and neocryptolepine.[63]

Azaindole ring systems have attracted considerable interest from the chemistry as they represent promising building blocks with potential applications in the field of pharmaceuticals, natural product synthesis and also diverse key synthetic intermediates.

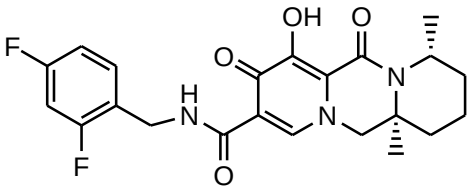
For example a new class of therapeutics for treatment of AIDS as integrase inhibitors is known, by recent approval, to be azaindole compounds as raltegravir **51** and in encouraging phase II clinical trial elvitegravir **52** and S/GSK1349572 **53**. [64]



51 raltegravir

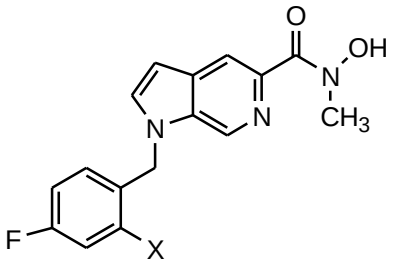


52 elvitegravir



53 S/GSK1349572

Also Tanis et.al. recently reported the discovery of azaindol *N*-methyl hydroxamic acids **54** as potent HIV-1 integrase inhibitors. [65]



54 X=H
X=F

These azaindol *N*-methyl hydroxamic acids showed potent antiviral activity as integrase inhibitors.

Hui Xu reported the synthesis of some *N*-arylindoles with HIV-1 integrase inhibition activity. [66]

They synthesised substituted arylindoles **55a-h** as showed in fig.18 showed potent activity against HIV-1 as integrase inhibirors. All compounds were tested *in vitro* for their HIV-1 integrase activity (EC_{50}) and cytotoxicity (CC_{50}) in cell based assay against HIV-1integrase replication in acutely infected C8166 cells. In addition , the therapeutic index (TI) was also calculated as showed in table 3 and 3'-azido-3'-deoxythymidine (AZT) was used as control.

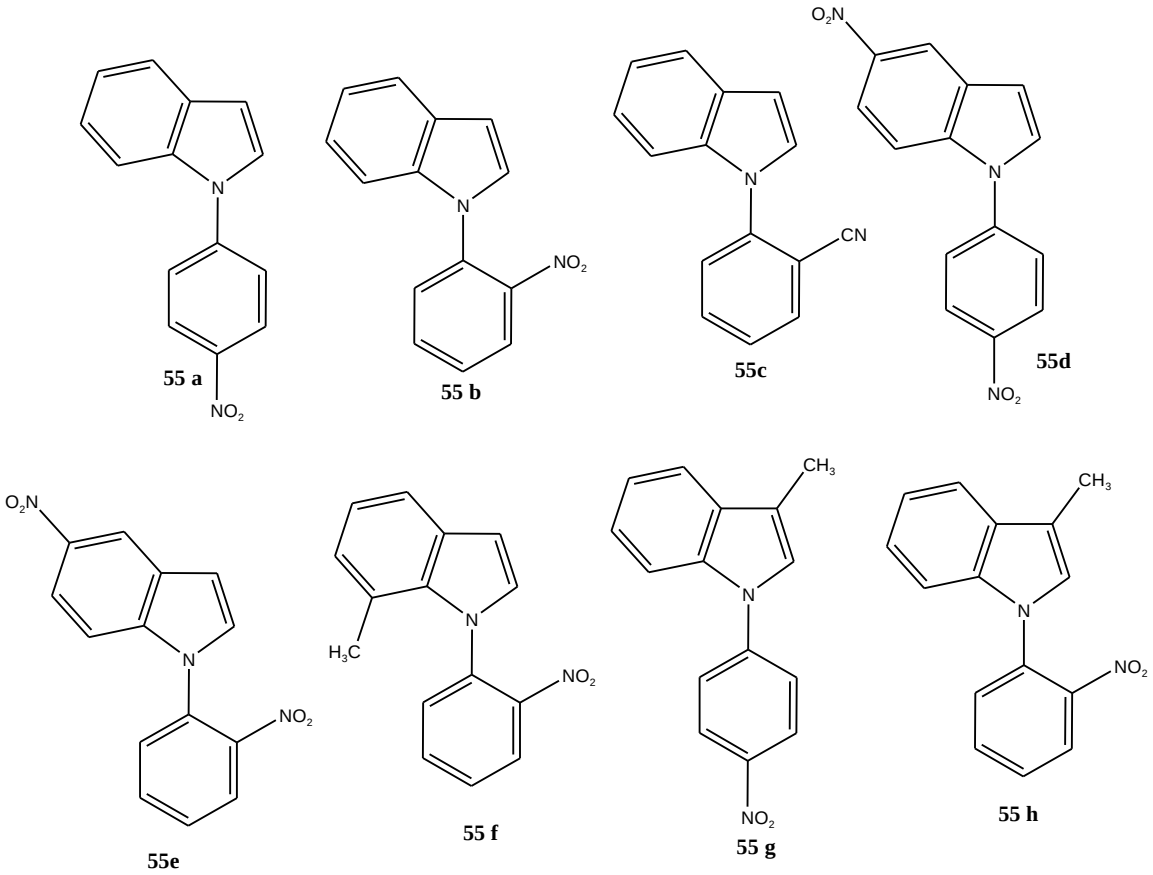


Fig. 18 Structures of different *N*-arylindoles .

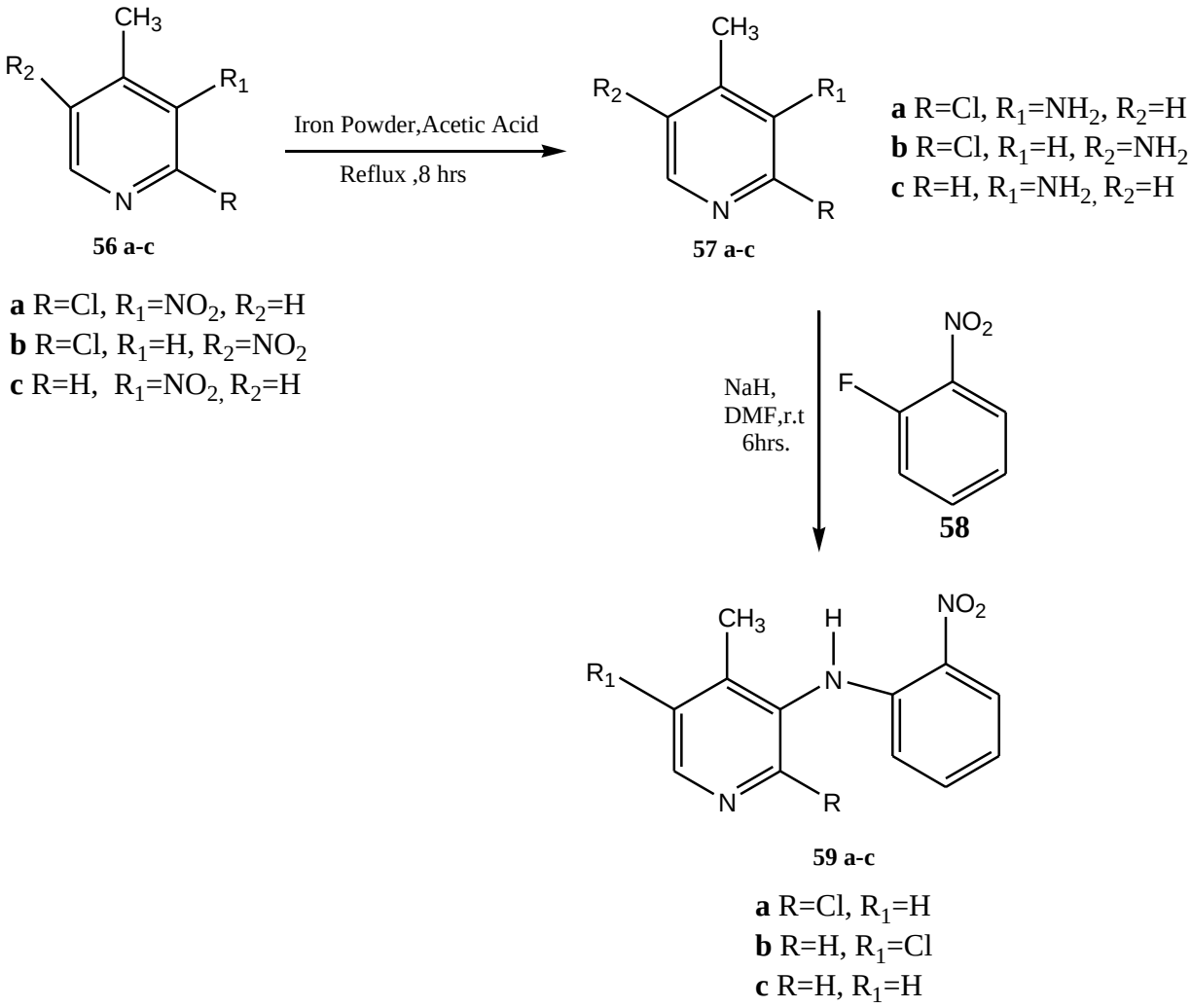
Compounds	CC_{50}(µg/ml)	EC_{50}(µg/ml)	TI
55 a	91.44	35.82	2.55
55 b	191.9	7.88	24.61
55 c	>200	82.28	>2.61
55 d	5.40	2.93	1.90
55 e	99.61	11.24	9.48
55 f	>200	37.76	>6.63
55 g	157.14	19.22	8.26
55 h	14.41	12.09	1.28
AZT	1288.24	0.007	184034.28

Table. 3 Anti HIV-1 integrase inhibition activity of some *N*-arylindoles .

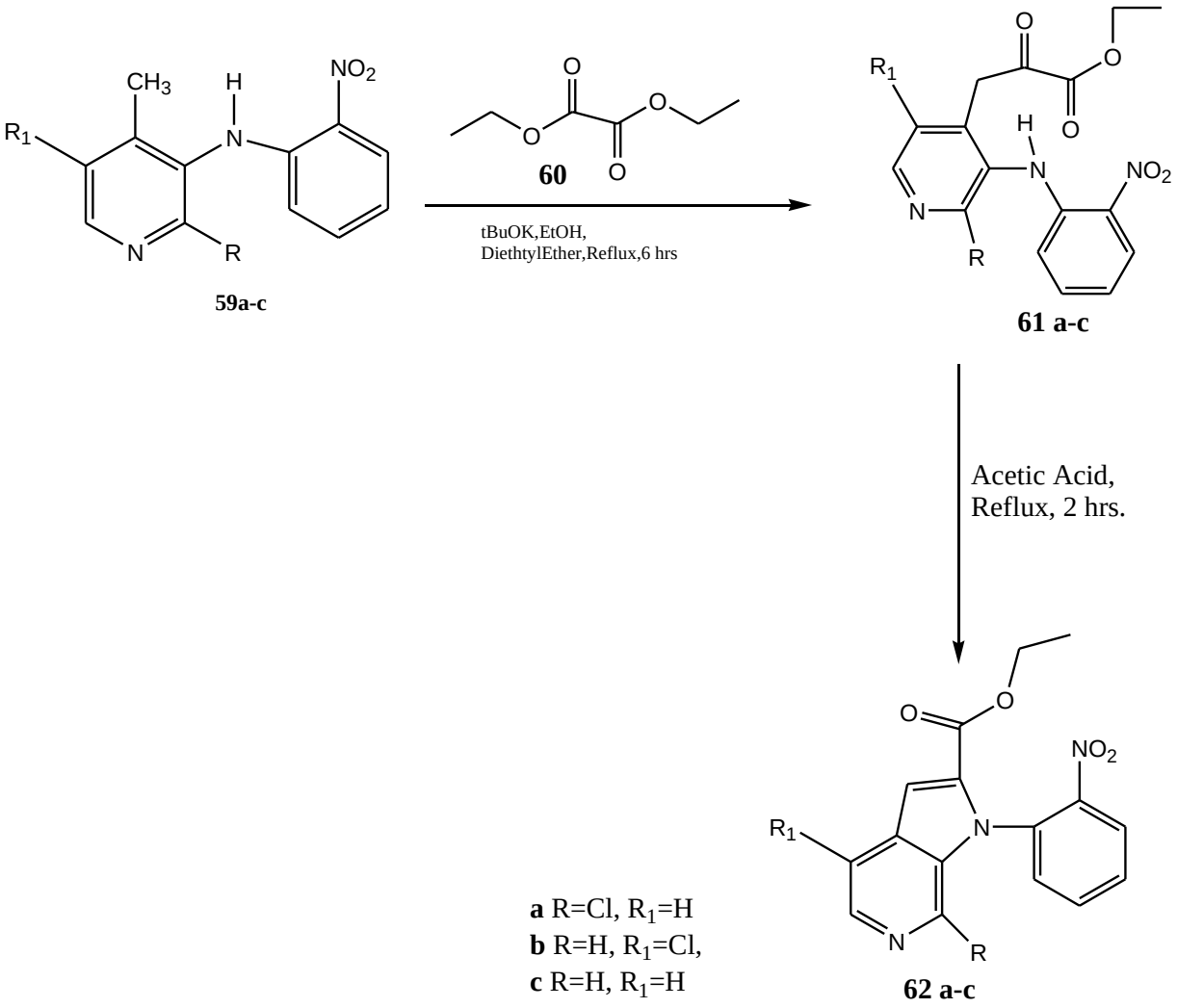
Considering the promising biological activity of these compound . Our research group was interested to insert a nitrobenzene group to the 6-azaindole moiety.

SYNTHESIS

For the synthesis of desired product we started from commercially available 4-methyl-pyridines **56a-c** that were treated with iron powder in acetic acid to give substituted 4-methyl-pyridin-3-ylamine **57a-c,** these latter amine intermediates were reacted with 2-fluoro-1-nitrobenzene **58** in the presence of sodium hydride in dimethylformamide to give the substituted nitro-phenyl-amine products **59a-c** in 70-80% yield.



Compound **59a-c** were reacted with diethyl oxalate **60** in the presence of potassium *tert*-butoxide in ethanol and diethyl ether. The resulting reaction mixture was refluxed for 8 hours and it was concentrated, dissolved in water and acidified with acetic acid to reach pH 4. The precipitate was collected by filtration and purified to obtain compounds **61 a-c** in 50-52% yield.



a R=Cl, R_1=H
b R=H, R_1=Cl,
c R=H, R_1=H

Compounds **61a-c** were dissolved in acetic acid and refluxed for 2 hours then cooled to room temperature, dissolved in water and extracted with ethyl acetate. After concentration under reduced pressure the desired products **62a-c** were obtained in 60-65% yield.

EXPERIMENTAL DATA

Synthesis of substituted 1-cyano-2-(2'-aminophenyl)isoindoles 29a-e .

To a solution of sodium hydrogen sulfite (1g, 0.015mol) in water (38 ml) phthalaldehyde **28** (2g, 0.015mol) was added. The mixture was stirred until the solid was dissolved, and the appropriate 1,2-phenylenediamine **27a-e** (0.015mol) was added. The reaction was heated in a steam bath for 30 min. Then KCN (3.39g, 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 column chromatography. In the case of derivative **29-d**, the 1,2-phenylenediamine **27-d** was first dissolved in DMF (15 ml) and then added to the reaction mixture.

General procedure synthesis of substituted 5H-Isoindolo[2,1-*a*]quinoxalin-6-ylideneamine 30a-e

1-Cyano-2-(2'-aminophenyl) isoindoles **29a-e** (3 mmol) were dissolved in acetic acid (10 ml) and refluxed for 30min. The reaction mixture was poured into ice water (150 ml).The resulting precipitate was collected by filtration and recrystallized from ethanol.

5*H*-Isoindolo[2,1-a]quinoxalin-6-ylideneamine

M.p. 255-265°C, yield-99 %, **IR :** 3390, 3203 (NH), 1685 (C=NH) cm^{-1}.

^{1}**H-NMR (DMSO-*d6*):** 7,75 (1H, dt, J=7.0, 1.6 Hz, Ar-H), 7.78-7.91 (2H, m, 2xAr-H), 7.93 (1H, s, Ar-H), 7.96 (1H, t, J=7.8 Hz, Ar-H), 8.00 (1H, t, J=7.8 Hz, Ar-H), 8.27 (1H, dd, J=7.0, 1.6 Hz, Ar-H), 8.34 (1H, d, J=7.8 Hz, Ar-H), 8.84 (1H, d, J=7.8 Hz, Ar-H), 12.35 (1H, bs, NH).

^{13}**C-NMR (DMSO-*d6*):** 124.1 (d), 127.0 (d), 127.5 (d), 127.8 (d), 127.9 (d), 128.9 (d), 130.9 (d), 131.4 (d), 133.1 (s), 133.4 (d), 135.3 (s), 138.9 (s), 141.0 (s), 143.9 (s), 161.7 (s).

3-Methoxy-5H-isoindolo[2,1-a]quinoxalin-6-ylideneamine

M.p. 293-294°C, yield-75 %, **IR :** 3363, 3280 (NH), 1635 (C=NH) cm^{-1}.

^{1}**H-NMR (DMSO-*d6*):** 3.84 (3H, s, CH_3), 7.04 (1H, s, Ar-H), 7.05 (1H, d, J=9.8 Hz, Ar-H), 7.40 (1H, t, J=7.8Hz, Ar-H), 7.51 (1H, t, J=7.8 Hz, Ar-H), 7.95 (1H, d, J=7.8 Hz, Ar-H), 8.36 (1H, d, J=9.8, Hz, Ar-H), 8.51 (1H, d, J=7.8 Hz, Ar-H), 9.18 (1H, s, Ar-H), 13.50 (1H, bs, NH).

^{13}C-NMR (DMSO-*d6*): 55.7 (q),101.5 (d), 104.6 (s), 112.7 (d), 115.7 (d), 116.2 (s), 118.2 (d), 119.1 (d), 120.9 (d), 124.3 (d), 124.4 (s), 126.2 (d), 128.5 (s), 147.1 (s), 158.9 (s),

4-Methyl-5H-isoindolo[2,1-a]quinoxalin-6-ylideneamine

M.p. 338-339°C, yield-98 %, **IR :** 3360, 3284 (NH), 1632 (C=NH) cm^{-1}.

^{1}H-NMR (DMSO-*d6*): 2.62 (3H, s, CH_3), 7.32-7.40 (2H, m, Ar-H), 7.45 (1H, t, J=6.8 Hz, Ar-H), 7.51 (1H, t, J=8.8 Hz, Ar-H), 7.96 (1H, d, J=8.8 Hz, Ar-H), 8.27 (1H,d, J=6.8 Hz, Ar-H), 8.47 (1H, d, J=8.8 Hz,Ar-H), 9.24 (1H, s, Ar-H), 12.53 (1H, bs, NH).

^{13}C-NMR (DMSO-*d6*): 17.8 (q), 104.8 (s), 114.8 (d), 117.3 (d), 119.2 (d), 121.5 (d), 122.3 (s), 125.0 (d),125.1 (s), 125.2 (d), 125.3 (s), 127.1 (d), 127.3(sx2), 130.0 (d), 147.0 (s).

2,3-Dichloro-5H-isoindolo[2,1-a]quinoxalin-6-ylideneamine

M.p. 340-343°C, yield-87 %, **IR :** 3350, 3302 (NH), 1645 (C=NH) cm^{-1}.

^{1}H-NMR (DMSO-*d6*): 7.43 (1H, t, J=7.8 Hz, Ar-H), 7.54 (1H, t, J=7.8 Hz, Ar-H), 7.86 (1H, s, Ar-H), 7.95 (1H, d, J=7.8 Hz, Ar-H), 8.52 (1H, d, J=7.8 Hz, Ar-H), 8.85 (1H, s, Ar-H), 9.32 (1H, s, Ar-H), 12.46 (1H, bs, NH).

^{13}C-NMR (DMSO-*d6*): 105.1 (s), 117.1 (d), 118.7 (d), 119.2 (d), 119.4 (d), 121.1 (d), 122.0 (s), 124.7 (s), 124.8 (d), 126.5 (s), 126.9 (s), 127.0 (d),127.2 (s), 130.3 (s), 147.0 (s).

2,3-Dimethyl-5H-isoindolo[2,1-a]quinoxalin-6-ylideneamine

M.p. 370-371°C, yield-96 %, **IR :** 3365, 3286 (NH), 1631 (C=NH) cm^{-1}.

^{1}H-NMR (DMSO-*d6*): 2.30 (3H, s, CH_3), 2.35 (3H, s, CH_3), 7.31 (1H, s, Ar-H), 7.46 (1H, t, J=7.8 Hz, Ar-H), 7.57 (1H, t, J=7.8 Hz, Ar-H), 8.00 (1H, d, J=7.8 Hz, Ar-H), 8.09 (1H,s, Ar-H), 8.41 (1H, d, J=7.8 Hz, Ar-H), 9.02 (1H, s, Ar-H), 13.14 (1H, bs, NH).

^{13}C-NMR (DMSO-*d6*): 19.5 (q), 19.6 (q), 116.7 (d), 117.4 (d), 118.7 (s), 119.1 (d), 119.6 (d), 121.0 (s), 122.0 (d), 125.3 (s), 125.4 (d), 125.8 (s), 127.6 (d), 127.9 (d), 135.9 (s), 139.0 (s), 147.2 (s).

Synthesis of 2-Azido-ethanol 33

2-Chloroethanol **31 (**10g ,124.2 mmol) was dissolved in DMF (35 ml) and sodium azide **32** (10.6g, 173.8 mmol) was added. The reaction mixture was heated at 80°C for 64 hrs. Then it was allowed to cool to room temperature and extracted with ethyl acetate .The organic layer was washed with water and dried over sodium sulfate. The solvent was evaporated under

reduced pressure and the crude was purified by chromatography using dichloromethane and ethyl acetate as eluent (9:1. DCM:EA)

Oil, Yield 74 %, **IR:** 3386 (OH), 2105 (N_3) cm^{-1}.

^{1}**H-NMR ($CDCl_3$):** 3.43 (2H, t, J=5 Hz, CH_2), 3.77 (2H, t, J=5Hz, CH_2), 5.00 (1H, t, J=5.1,Hz, OH)

^{13}**C-NMR ($CDCl_3$):** 53.4 (t), 61.3 (t).

Synthesis of toluene-4-sulfonic acid 2-azido-ethyl ester 35

The 2-azido-ethanol **33** (5g, 57.47 mmol) was dissolved in pyridine (35ml) and *p*-toluenesulfonyl chloride **34** (16.37g, 86.20 mmol) was added to the reaction mixture at 0°C.The reactiom mixture warmed to room temperature and stirred for 3 hrs. It was diluted with diethyl ether (100 ml), aqueous hydrochloric acid (2 N, 20 ml) was added slowly and reaction stirred vigorously for 10 min. The aqueous layer was separated and extracted with diethyl ether, the organic layer was dried over sodium sulphate and concentrated under reduced pressure. The crude product was purified by coloumn chromatography using dichloromethane and ethyl acetate as eluent (9:1 DCM:EA) .

Oil, Yield 80 %, **IR:** 2111 (N_3), 1365 (SO_2) cm^{-1}

^{1}**H-NMR ($CDCl_3$):** 2.44 (3H, s, CH_3), 3.56 (2H, t, J=4.7 Hz, CH_2), 4.16 (2H, t, J=4.7 Hz, CH_2), 7.51 (2H, d, J=8 Hz, 2xAr-H), 7.82 (2H, d, J=8 Hz, 2xAr-H).

^{13}**C-NMR ($CDCl_3$):** 21.0 (q), 48.9 (t), 69.2 (t), 127.6 (d x 2), 130.2 (d x 2), 145.1 (s), 147.0 (s).

Synthesis of substituted 5-(2-Azido-ethyl)-5*H*-isoindolo[2,1-*a*]quinoxalin-6-aylideneamine 36-e and (2-Azido-ethyl)-isoindolo[2,1-*a*]quinoxalin-6-yl-amine 37a-e .

A solution of 5*H*-Isoindolo[2,1-*a*]quinoxalin-6-ylideneamine **30a-e** (0.5g, 2.13 mmol) in DMF (7 ml) was stirred at room temperature for 10 min. Then potassium carbonate (0.73g, 5.34mmol) was added to the reaction mixture and stirred for 2 hours at room temperature. Then tosylethyl azide **35** (1.5g, 6.41 mmol) was added and the reaction mixture was heated at 90°C for 12 hours. The reaction mixture was quenched with water and extracted with ethyl acetate. The solvent was evaporated under reduced pressure and the crude was purified by chromatography using dichloromethane and ethyl acetate as eluent (8:2 DCM:EA)

5-(2-Azido-ethyl)-5*H*-isoindolo[2,1-*a*]quinoxalin-6- aylideneamine 36a

M.p. 165°C, yield 30%, **IR:** 3688 (NH), 2256 (N_3), 1718 (C=NH) cm^{-1} .

^{1}H-NMR ($CDCl_3$): 3.83 (2H, t, J=6 Hz, CH_2), 5.05 (2H, t, J=6 Hz,CH_2), 7.80-7.90 (3H, m, 3xAr-H), 7.98 (1H, dd, J=1.5, 8 Hz, Ar-H), 8.05 (1H, dd , J=1.3, 7.2 Hz, Ar-H), 8.2-8.31 (2H, m, 2xAr-H), 8.35 (1H, dd, J=1.5 Hz, Ar-H), 9.26 (1H, dd, J=1.8 Hz, Ar-H).

^{13}C-NMR ($CDCl_3$): 50.2 (t), 66.5 (t), 122.6 (s), 124.7 (d), 125.3(d), 129.1 (d), 129.2 (d), 129.3 (s), 130.5 (d), 130.8 (d), 132.5 (d), 134.9 (s), 137.6 (s), 142.3 (s), 143.2 (s), 140.2 (s), 143.4(s).

5-(2-Azido-ethyl)-3-methoxy-5*H*-isoindolo[2,1-*a*]quinoxalin-6-aylideneamine 36b

M.p.189°C, Yield 34%, **IR:** 3021(NH), 2204 (N_3), 1694 (C=NH) cm^{-1}.

^{1}H-NMR ($CDCl_3$): 3.83 (2H, t, J=6 Hz, CH_2), 4.02 (3H, s, OCH_3), 5.08 (2H, t, J=6 Hz, CH_2), 7.46-7.55 (2H, m, 2xAr-H), 7.80-8.02 (3H, m, 3xAr-H), 8.18 (1H, d, J=10 Hz, Ar-H), 8.41 (1H, d, J=8.4 Hz, Ar-H), 9.19 (1H, d, J=8.4 Hz, Ar-H)

^{13}C-NMR ($CDCl_3$): 29.7 (t), 50.2 (t), 55.8 (q), 99.9 (d), 105.5 (d), 122.0 (s), 123.7 (d), 124.1 (d), 124.4 (s), 125.2 (d), 130.0 (d), 130.3 (d), 132.3 (d), 135.0 (s), 135.3 (s), 137.8 (s), 145.0 (s), 161.4 (s).

5-(2-Azido-ethyl)-3-methyl-5*H*-isoindolo[2,1-*a*]quinoxalin-6- aylideneamine 36c

M.p. 161°C, Yield 30%, **IR:** 3442 (NH), 2256 (N_3), 1729 (C=NH) cm^{-1}

^{1}H-NMR ($CDCl_3$): 2.98 (3H, s, CH_3), 3.82 (2H, t, J=5 Hz, CH_2), 5.08 (2H, t, J=5Hz, CH_2), 7.67-8.04 (5H, m, 5xAr-H), 8.16 (1H, dd, J=2.4, 7.5 Hz, Ar-H), 8.42 (1H, tt, J=0.7, 9.3 Hz, Ar-H), 9.25 (1H, dt, J=0.7, 9.3 Hz, Ar-H).

^{13}C-NMR ($CDCl_3$): 17.9 (q), 50.2 (t), 66.3 (t), 122.6 (d), 124.6 (d), 125.3 (d), 127.2 (d), 129.0 (d), 130.2 (d), 130.6 (d), 132.4 (d), 135.0 (s), 137.0 (s), 137.3 (s) 141.4 (s), 142.6 (s), 143.8 (s), 146.6 (s).

5-(2-Azido-ethyl)-2,3-dichloro-5*H*-isoindolo[2,1-*a*]quinoxalin-6- aylideneamine 36d

M.p. 169°C, Yield 40%, **IR:** 3409 (NH), 2257 (N_3), 1722 (C=NH) cm^{-1}

^{1}H-NMR ($CDCl_3$): 3.83 (2H, t, J=5Hz, CH_2), 5.03 (2H, t, J=5Hz, CH_2), 7.83-8.06 (3H, m, 3x Ar-H), 8.36-8.43 (3H, m, 3xAr-H), 9.18 (1H, d, J=8.6, 2Hz, Ar-H).

^{13}C-NMR ($CDCl_3$): 50.1 (t), 66.7 (t), 122.8 (d), 124.9 (d), 125.5 (d), 129.4 (d), 129.6 (d), 131.4 (d), 132.8 (d), 133.9 (s), 134.4 (s), 135.1 (s), 138.5 (s), 139.7 (s), 141.7 (s), 148.0 (s), 163.5 (s).

5-(2-Azido-ethyl)-2,3-dimethyl-5*H*-isoindolo[2,1-*a*]quinoxalin-6- aylideneamine 36e

M.p. 183°C, Yield 38%, **IR:** 3396 (NH), 2257 (N_3), 1614 (C=NH) cm^{-1}

^{1}H-NMR ($CDCl_3$): 2.57 (6H, s, 2xCH_3), 3.82 (2H, t, J=5 Hz, CH_2), 5.02 (2H, t, J=5 Hz, CH_2), 7.80-8.05 (5H, m, 5xAr-H), 8.40 (1H, dd, J=0.7, 7.9 Hz, Ar-H), 9.21 (1H, dd, J=0.7, 7.9 Hz, Ar-H).

^{13}C-NMR ($CDCl_3$): 20.4 (q), 20.7 (q), 50.2 (t), 66.3 (t), 122.3 (d), 124.4 (d), 125.2(d), 127.9 (d), 128.0 (d), 130.3 (d), 132.2 (d), 135.2 (s), 136.5 (s), 140.1 (s), 140.4 (s), 141.5 (s),142.2 (s), 147.0 (s) 162.1 (s).

6-(2-Azido-ethoxy)-isoindolo[2,1-*a*]quinoxaline 6-yl-amine 37a

M.p. 156°C, Yield 37%, **IR:** 3428 (NH), 2257 (N_3) cm^{-1} .

^{1}H-NMR ($CDCl_3$): 3.77 (2H, t, J=6 Hz, CH_2), 4.80 (2H, t, J=6 Hz, CH_2), 7.45 (1H, d, J=8 Hz, Ar-H), 7.60 (1H, d, J=8 Hz, Ar-H), 7.71-8.08 (4H, m, 4xAr-H), 8.20 (1H, d, J=6 Hz, Ar-H), 8.41 (1H, d, J=8 Hz, Ar-H), 8.91 (1H, d, J=8 Hz, Ar-H).

^{13}C-NMR ($CDCl_3$): 39.5 (t), 48.1 (t), 127.1 (d), 127.5 (d), 127.6 (d), 128.0 (d), 128.6 (d), 128.7 (d), 129.7 (d), 131.1 (d), 131.6 (d), 131.7 (s), 132.3 (s), 133.7 (s), 138.5 (s), 140.2 (s), 143.3 (s).

6-(2-Azido-ethoxy)-3-methoxy-isoindolo[2,1-*a*]quinoxaline 6-yl-amine 37b

M.p. 180°C, Yield 32 %, **IR:** 3480 (NH), 2256 (N_3) cm^{-1}

^{1}H-NMR ($CDCl_3$): 3.78 (2H, t, J=6Hz, CH_2), 3.99 (3H, s, OCH_3), 4.79 (2H, t, J=6Hz, CH_2), 7.40-7.50 (2H, m, 2xAr-H), 7.82-8.08 (4H, m, 4xAr-H), 8.39 (1H, d, J=0.9 Hz, Ar-H), 8.84 (1H, d, J=7.1 Hz, Ar-H).

^{13}C-NMR ($CDCl_3$): 29.7 (t), 50.2 (t), 55.8 (q), 99.9 (d), 105.5 (d), 122.0 (s), 123.7 (d), 124.1 (d), 124.4 (s), 125.2 (d), 130.0 (d), 130.3 (d), 132.3 (d), 135.0 (s), 135.3 (s), 137.8 (s), 145.0 (s), 161.4 (s).

6-(2-Azido-ethoxy)-3-methyl-isoindolo[2,1-*a*]quinoxaline 6-yl-amine 37c

M.p. 147°C , Yield 33 %, **IR** 3391 (NH), 2257 (N_3) cm^{-1}

^{1}H-NMR ($CDCl_3$): 2.80 (3H, s, CH_3), 3.80 (2H, t, J=6.5 Hz, CH_2), 4.95 (2H, t, J=6.5 Hz, CH_2), 7.58-8.06 (6H, m, 6xAr-H), 8.53 (1H, dd, J=1.7, 8 Hz, Ar-H), 8.97 (1H, dd, J=1.7, 8 Hz, Ar-H).

^{13}C-NMR ($CDCl_3$): 17.3 (q), 39.7 (t), 48.4 (t), 124.4 (d), 127.0 (d), 127.3 (d), 128.2 (d), 128.6 (d), 129.6 (s), 130.8 (d), 131.1 (d), 132.9 (s), 133.3 (d), 136.1 (s), 139.5 (s), 139.9 (s).

141.4 (s), 143.8 (s).

6-(2-Azido-ethoxy)-2,3-dichloro-isoindolo[2,1-*a*]quinoxaline 6-yl-amine 37d

M.p. 152°C , Yield 38 %, **IR:** 3427 (NH), 2256 (N_3) cm^{-1}

^{1}**H-NMR (CDCl$_3$):** 3.77 (2H, t, J=6 Hz, CH_2), 4.92 (2H, t, J=6 Hz, CH_2), 7.83-7.93 (3H, m, 3xAr-H), 8.21 (1H, s, Ar-H), 8.33 (1H, s, Ar-H), 8.52 (1H, d, J=7.7 Hz, Ar-H), 8.95 (1H, d, J=7.7 Hz, Ar-H).

^{13}**C-NMR (CDCl$_3$):** 39.9 (t), 47.6 (t), 124.7 (d), 125.1 (s), 127.4 (d), 128.5 (d), 128.8 (d), 129.6 (d), 131.9 (d), 132.4 (s), 133.6 (s), 133.7 (d), 135.5 (s), 139.5 (s), 139.6 (s), 158.6 (s), 161.9 (s).

6-(2-Azido-ethoxy)-2,3-dimethyl-isoindolo[2,1-*a*]quinoxaline 6-yl-amine 37e

M.p. 168°C , Yield 30 %, **IR:** 3475 (NH), 2257 (N_3) cm^{-1}

^{1}**H-NMR (CDCl$_3$):** 2.53 (6H, s, 2xCH_3), 3.77 (2H, t, J=6.4 Hz, CH_2), 4.95 (2H, t, J=6.4 Hz, CH_2), 7.67 (1H, td, J=4.0,2.0 Hz, Ar-H), 7.76-7.95 (4H, m, 4xAr-H), 8.53 (1H, d, J=8Hz, Ar-H), 8.95 (1H, d, J=8Hz, Ar-H)

^{13}**C-NMR (CDCl$_3$):** 20.2 (q), 20.5 (q), 39.7 (t), 48.7 (t), 124.2 (d), 127.0 (d), 127.1 (d), 127.8 (s), 128.1 (d), 128.2 (d), 128.5 (d), 129.6 (s), 130.7 (d), 133.2 (s), 133.3(d),134.4 (s), 138.4 (s), 138.9 (s), 139.7 (s), 141.8 (s).

Synthesis of substituted 5-(2-Amino-ethyl)-6-imino-5,6-dihydro-isoindolo[2,1-*a*]quinoxaline 39a-e and N^1-Isoindolo[2,1-*a*]quinoxalin-6-yl-ethane-1,2-diamine 40a-e

The azide intermediates **39a-e** and **40a-e** (0.1g, 0.3mmol) were dissolved in anhydrous methanol and transferred to the reaction flask under argon atmosphere containing 1-3,propane dithiol. (0.1g, 0.94 mmol) and triehlylamine (1.3g, 0.94 mmol) was added. Reaction mixture was stirred for 24 hrs . The solvent was evaporated under reduced pressure and the crude was purified by chromatography using dichloromethane and ethyl acetate as eluent (8:2. DCM:EA).

5-(2-Amino-ethyl)-6-imino-5,6-dihydro-isoindolo[2,1-*a*]quinoxaline 39a

M.p. 194°C , Yield 72 %, **IR:** 3679, 3556 (NH_2), 3393 (NH), 1719 (C=NH) cm^{-1}

^{1}H-NMR ($CDCl_3$): 3.83 (2H, t, J=5Hz, CH_2), 5.03 (2H, t, J=5Hz, CH_2), 7.80-8.05 (5H, m, 5xAr-H), 8.27-8.35 (2H, m, 2xAr-H), 8.42 (1H, dd, J=0.8,9.2Hz, Ar-H), 9.25 (1H, dd, J=0.8, 9.2 Hz, Ar-H).

^{13}C-NMR ($CDCl_3$): 39.5 (t), 48.1 (t), 127.1 (d), 127.5 (d), 127.6 (d), 128.0 (d), 128.6 (d), 128.7 (d), 129.7 (d), 131.1 (d), 131.6 (d), 131.7 (s), 132.3 (s), 133.7 (s), 138.5 (s), 140.2 (s), 143.3 (s).

5-(2-Amino-ethyl)-6-imino-3-methoxy-5,6-dihydro-isoindolo[2,1-*a*]quinoxaline 39b

M.p. 198.8°C , Yield 62 %, **IR:** 3584, 3437 (NH_2), 3319 (NH), 1721 (C=NH) cm^{-1}

^{1}H-NMR ($CDCl_3$): 3.77 (2H, t, J=6.1Hz, CH_2), 3.98 (3H, s, OCH_3), 4.77 (2H, t, J=6.1 Hz, CH_2), 7.35-8.09 (5H, m, 5xAr-H), 8.37 (1H, d, J=7.2 Hz, Ar-H), 8.79 (1H, d, J=7.2, Hz, Ar-H), 8.59 (1H, d, J=8Hz, Ar-H).

^{13}C-NMR ($CDCl_3$): 18.8 (q), 40.1 (t), 48.0 (t), 105.5 (d), 121.6 (d), 123.5 (d), 126.4 (s), 127.9 (d), 128.6 (s), 129.9 (d), 130.8 (d), 131.6 (d),132.5 (s), 132.6 (s), 133.5 (d), 134.5 (s), 142.1 (s), 143.5 (s).

5-(2-Amino-ethyl)-6-imino-3-methyl-5,6-dihydro-isoindolo[2,1-*a*]quinoxaline 39c

M.p. 189°C , Yield 64%, **IR:** 3604, 3501 (NH_2), 3407 (NH), 1713 (C=NH) cm^{-1}

^{1}H-NMR ($CDCl_3$): 2.82 (3H, s, CH_3), 3.82 (2H, t, J=5Hz, CH_2), 5.07 (2H, t, J=5Hz, CH_2), 7.60-8.19 (5H, m, 5xAr-H), 8.16 (1H, dd, J=2.3,7.4 Hz, Ar-H), 8.40 (1H, d, J=8Hz, Ar-H), 9.25 (1H, dd, J=0.8,8 Hz, Ar-H).

^{13}C-NMR ($CDCl_3$): 18.0 (q), 50.3 (t), 66.4 (t), 122.6 (d), 124.6 (d), 125.3 (d), 127.2 (d), 129.0 (d), 130.2 (d), 130.6 (d), 132.4 (d), 135.0 (s), 137.0 (s), 137.3 (s), 141.4 (s), 142.6 (s), 146.6 (s), 162.6 (s).

5-(2-Amino-ethyl)-2,3-dichloro-6-imino-5,6-dihydro-isoindolo[2,1-*a*]quinoxaline 39d

M.p. 182°C , Yield 78%, **IR:** 3679, 3558 (NH_2), 3393 (NH), 1719 (C=NH) cm^{-1}

^{1}H-NMR ($CDCl_3$): 3.83 (2H, t, J=5Hz, CH_2), 5.03 (2H, t, J=5Hz, CH_2), 7.83-8.06 (3H, m, 3xAr-H), 8.36-8.43 (3H, m, 3xAr-H), 9.18 (1H, d, J=8.6, 2Hz, Ar-H).

^{13}C-NMR ($CDCl_3$): 50.1(t), 66.7 (t), 122.8 (d), 124.9 (d), 125.5 (d), 129.4 (d), 131.4 (d), 132.8 (d), 133.7 (d), 133.9 (s), 134.4 (s), 135.1 (s), 138.5 (s), 139.7 (s), 141.7 (s), 148.0 (s), 163.5 (s).

5-(2-Amino-ethyl)-6-imino-2,3-dimethyl-5,6-dihydro-isoindolo[2,1-*a*]quinoxaline 39e

M.p.186°C , Yield 60 %, **IR:** 3558, 3392 (NH_2), 3219 (NH), 1725 (C=NH) cm^{-1}

^{1}H-NMR($CDCl_3$): 2.57 (6H, s, 2xCH_3), 3.82 (2H, t, J=5Hz, CH_2), 5.03 (2H, t, J=5Hz, CH_2), 7.81-8.07 (5H, m, 5xAr-H), 8,41 (1H, d, J=8Hz, Ar-H), 9.22 (1H, d, J=8Hz, Ar-H).

^{13}C-NMR ($CDCl_3$): 20.2 (q), 20.5 (q), 39.7 (t), 48.7 (t), 124.2 (d), 127.0 (d), 127.1 (d), 127.8 (s), 128.1 (d), 128.5 (d), 129.6 (s), 130.7 (d), 133.2 (s), 133.3 (d), 134.4 (s), 138.4 (s), 138.9 (s), 139.7 (s), 141.8 (s).

N^1-Isoindolo[2,1-*a*]quinoxalin-6-yl-ethane-1,2-diamine 40a

M.p. 177°C , Yield 58%, **IR:** 3688, 3557 (NH_2), 3370 (NH) cm^{-1}

^{1}H-NMR ($CDCl_3$): 3.76 (2H, t, J=6.2 Hz, CH_2), 4.78 (2H, t, J=6.2 Hz, CH_2), 7.77-8.07 (6H, m, 6xAr-H), 8.19 (1H, td, J=1.7, 2Hz, Ar-H), 8.39 (1H, dd, J=1,7.8 Hz, Ar-H)
8.86 (1H, dd, J=1, 7.8 Hz, Ar-H)

^{13}C-NMR ($CDCl_3$): 39.8 (t), 48.8 (t), 124.5 (d), 127.3 (s), 127.8 (d), 128.0 (d), 128.4 (d), 128.6 (d), 129.2 (d), 129.6 (d), 129.7 (s), 130.9 (d), 131.3 (d), 132.9 (s), 140.9 (s), 142.6 (s), 143.5 (s).

N^1-(3-Methoxy-isoindolo[2,1-*a*]quinoxalin-6-yl)-ethane-1,2-diamine 40b

M.p.192.4°C , Yield 60 %, **IR:** 3566, 3446 (NH_2), 3398 (NH) cm^{-1}

^{1}H-NMR($CDCl_3$): 3.76 (2H, t, J=6.2 Hz, CH_2), 3.97 (3H, s, OCH_3), 4.75 (2H, t, J=6.2 Hz, CH_2), 7.32 (1H, d, J=2.6 Hz, Ar-H), 7.42 (1H, dd, J=2.7, 9 Hz, Ar-H), 7.79-8.06 (4H, m, 4xAr-H), 8.35 (1H, dd, J=1, 7.8 Hz, Ar-H), 8.77 (1H, dd, J=1,7.8 Hz, Ar-H).

^{13}C-NMR ($CDCl_3$): 18.8 (q), 39.4 (t), 48.0 (t), 121.6 (d), 123.5 (d), 126.4 (s), 127.9 (d), 128.6 (d), 129.9 (d), 130.8 (d),131.6 (d), 132.5 (s), 132.6 (s),133.6 (d), 134.5 (s), 142.2 (s), 143.5 (s), 161.4 (s).

N^1-(3-Methyl-isoindolo[2,1-*a*]quinoxalin-6-yl)-ethane-1,2-diamine 40c

M.p. 168°C , Yield 64%, **IR:** 3689, 3567 (NH_2), 3372 (NH) cm^{-1}

^{1}H-NMR($CDCl_3$): 2.73 (3H, s, CH_3), 3.78 (2H, t, J=6.3 Hz, CH_2), 4.78 (2H, t, J=6.3 Hz, CH_2), 7.62-8.05 (6H, m,6x Ar-H), 8.39 (1H, dd, J=1,7.7 Hz, Ar-H), 8.84 (1H, dd, J=1,7.7Hz, Ar-H).

^{13}C-NMR($CDCl_3$): 16.7 (q), 39.6 (t), 47.7 (t), 123.9 (d), 126.5 (d), 127.5 (d), 128.0 (d), 128.2.(d), 129.7 (d), 130.7 (d), 131.5 (d), 131.6 (s), 132.2 (s), 133.6 (s),134.9 (s), 135.4 (s)

138.5 (s), 139.1 (s).

N^1-(2,3-Dichloro-isoindolo[2,1-*a*]quinoxalin-6-yl)-ethane-1,2-diamine 40d

M.p. 164°C , Yield 60 %, **IR:** 3671 ,3584 (NH_2), 3419 (NH) cm^{-1}

^{1}H-NMR ($CDCl_3$): 3.76 (2H, t, J=6.3 Hz, CH_2), 4.92 (2H, t, J=6.3Hz. CH_2), 7.78-7.97 (3H, m, 3xAr-H), 8.20 (1H, s, Ar-H), 8.32 (1H, s, Ar-H), 8.53 (1H, dd, J=1.1,6.9 Hz, Ar-H), 8.92 (1H, d, J=0.6 Hz, Ar-H).

^{13}C-NMR ($CDCl_3$): 29.17 (t), 48.72 (t), 119.0 (d), 124.7 (d), 127.5 (s), 128.5 (d), 128.8 (d), 129.6 (d), 131.9 (d), 132.3 (s), 132.8 (s), 133.7 (d), 135.5 (s),138.1 (s), 139.6 (s), 161.8 (s), 191.2 (s).

N^1-(2,3-Dimethyl-isoindolo[2,1-*a*]quinoxalin-6-yl)-ethane-1,2-diamine 40e

m.p.174°C , Yield 68 %, **IR:** 3676, 3558 (NH_2), 3380 (NH) cm^{-1}

^{1}H-NMR ($CDCl_3$): 2.50 (6H, s, 2xCH_3), 3.76 (2H, t, J=6.4 Hz, CH_2), 4.91 (2H, t, J=6.4 Hz, CH_2), 7.70-7.92 (5H, m, 5xAr-H), 8.51 (1H, dd, J=1,7.8 Hz, Ar-H), 8.92 (1H, dd, J=1, 7.8 HZ, Ar-H).

^{13}C-NMR ($CDCl_3$): 20.2 (q), 20.5 (q), 39.7 (t), 48.7 (t), 124.2 (d), 127.0 (d), 127.1 (d), 127.8 (s), 128.1 (d), 128.5 (d), 129.6 (s), 130.7 (d), 133.2 (s), 133.3 (d), 134.4 (s), 138.4 (s), 138.9 (s), 139.7 (s), 141.8 (s).

Synthesis of 5-Bromo-2-isothiocyanato-pyridine 43

1,1'Thiocarbonyldiimidazole **42** (0.5g, 89.11 mmol) was dissolved under nitrogen atmosphere in chloroform (5 ml) and stirred until compound was completely dissolved. Then 2-Amino 5-bromo pyridine **41** (0.49g, 2.83 mmol) was added and the reaction was stirred at room temperature for 12 hrs under nitrogen atmosphere. The resulting precipitate was collected by filtration and the crude was purified by chromatography using dichloromethane as eluent. (8:2. DCM:EA)

M.p. 130°C , Yield 72 %, **IR:** 2005 (NCS) , 1091 (Br) cm^{-1}

^{1}H-NMR ($CDCl_3$): 7.02 (1H, dd, J=0.5,8.4 Hz, Ar-H), 7.82 (1H, dd, J=2.5,8.4 Hz, Ar-H) 8.48 (1H, d, J=2.5 Hz, Ar-H).

^{13}C-NMR ($CDCl_3$): 118.5 (s) , 120.7 (d) , 141.2 (d) , 143.1 (s), 145.1 (s) , 151.08 (d).

Synthesis of substituted 1-(5-Bromo-pyridin-2-yl)-3-[2-(6-imino-6*H*-isoindolo[2,1-a]quinoxalin-5-yl)-ethyl]-thiourea 44 a-d and 1-(5-Bromo-pyridin-2-yl)-3-[2-(isoindolo[2,1-*a*]quinoxalin-6-ylamino)-ethyl]-thiourea 45 a-d.

The amine intermediates **39a-d** and **40a-d** was added to a suspension of 5-Bromo-2-isothiocyanato-pyridine **43** in a DMF and reaction heated to 100°C for 16 hrs. Reaction mixture was cooled to room temperature and poured in ice water then the resulting precipitate was collected by filtration and the crude was purified by chromatography using dichloromethane and ethyl acetate as eluent (9:1 DCM:EA)

1-(5-Bromo-pyridin-2-yl)-3-[2-(6-imino-6*H*-isoindolo[2,1-*a*]quinoxalin-5-yl)-ethyl]-thiourea 44a

M.p. 205°C, Yield 15%, **IR:** 3670 (NH), 3557 (NH), 3367 (NH), 1323 (C=S) cm^{-1}

^{1}**H-NMR (DMSO-*d6*):** 3.77 (2H, t, J=5.9 Hz, CH_2), 4.80 (2H, t, J=5.9 Hz, CH_2), 7.38 (1H, d, J=8 Hz, Ar-H), 7.83-8.25 (8H, m, 8xAr-H), 8.42 (1H, d, J=9.5 Hz, Ar-H), 8.61 (1H, d, J=2.5 Hz, NH), 8.90 (1H, d, J=8 Hz, NH).

^{13}**C-NMR (DMSO-*d6*):** 39.7 (t), 48.0 (t), 118.8 (d),121.6 (s), 124.1 (d), 127.1 (s), 127.5 (s), 127.6 (d), 128.0 (d), 128.6 (d), 128.8 (d), 129.7 (d), 131.1 (d), 131.6 (d), 133.7 (d), 135.5 (s), 138.5 (s), 140.2 (s), 142.0 (d), 143.5 (s), 143.8 (s), 150.7 (d), 151.3 (s).

1-(5-Bromo-pyridin-2-yl)-3-[2-(6-imino-3-methoxy-6*H*-isoindolo[2,1-*a*]quinoxalin-5-yl)-ethyl]-thiourea 44b

M.p. 243°C, Yield 10 % , **IR:** 3680 (NH), 3558 (NH), 3408 (NH), 1297 (C=S) cm^{-1}

^{1}**H-NMR (DMSO-*d6*):** 3.95 (3H, s, OCH_3) 4.20 (2H, m, CH_2), 4.90 (2H, t, J=5Hz,CH_2), 6.77 (1H, d, J=8.6 Hz, Ar-H), 7.01 (1H, d, J=2.7 Hz, Ar-H), 7.35 (1H, dd, J=2,9 Hz,Ar-H), 7.68-8.10 (6H, m, 6xAr-H), 8.31 (1H d, J=8 Hz, Ar-H), 8.82 (1H, d, J=6 Hz, NH), 10.44 (1H, s, NH), 11.22 (1H, t, J=4Hz, NH).

^{13}**C-NMR (DMSO-*d6*):** 40.2 (t), 43.9 (t), 56.7 (q), 106.2 (d),112.4 (d), 114.7 (d), 122.2 (d), 124.4 (d), 127.7 (s), 128.8 (d), 130.5 (d), 131.5 (d), 132.4 (d), 133.4 (s), 133.6.(s), 134.3 (d), 135.1 (s), 142.8 (d), 145.0 (s), 146.0 (s), 152.6 (s),162.0 (s), 162.3 (s), 180.8 (s).

1-(5-Bromo-pyridin-2-yl)-3-[2-(6-imino-3-methyl-6*H*-isoindolo[2,1-*a*]quinoxalin-5-yl)-ethyl]-thiourea 44c

M.p. 241°C, Yield 11%, **IR:** 3679 (NH), 3570 (NH), 3384 (NH), 1297 (C=S) cm^{-1}

^{1}H-NMR (DMSO-*d6*): 2,71 (3H, s, CH_3), 2.24 (2H, d, J=4.0 Hz, CH_2), 4.93 (2H, t, J=4.0Hz, CH_2), 6.77 (1H, d, J=8 Hz, Ar-H), 7.44 (1H, d, J=4 Hz, Ar-H), 7.62 (1H, t, J=2 Hz, Ar-H), 7.67 (1H, d, J=2 Hz, Ar-H), 7.85-8.13 (4H, m, 4xAr-H), 8.37 (1H, dd, J=8 Hz, Ar-H), 8.53 (1H, t, J=2Hz, Ar-H),8,89 (1H, dd, J=2 Hz, Ar-H), 10.47 (1H, s, NH), 11.12 (1H, t, J=6Hz, NH).

^{13}C-NMR (DMSO-*d6*): 17.3 (q), 29.7 (t), 77.2 (t), 115.3 (d), 120.1 (d), 124.7 (d), 127.3 (d), 128.1 (d), 128.3 (d), 130.9 (d), 131.1 (d), 133.6 (d), 133.8 (s), 134.7 (d), 135.9 (s), 140.4 (s), 140.9 (d), 141.8 (s), 149.3 (s), 154.8 (s), 162.1 (s), 165.1 (s), 170.3 (s), 179.8 (s).

1-(5-Bromo-pyridin-2-yl)-3-[2-(2,3-dichloro-6-imino-6*H*-isoindolo[2,1-*a*]quinoxalin-5-yl)-ethyl]-thiourea 44d

M.p. 228°C, Yield 10 %, **IR:** 3676 (NH), 3567 (NH), 3394 (NH), 1297 (C=S) cm^{-1}

^{1}H-NMR (DMSO-*d6*): 4.19 (2H, t, J=8 Hz, CH_2), 4.86 (2H, t, J=8 Hz, CH_2), 6.77 (1H, d, J=8.4 Hz, Ar-H), 7.67-7.75 (2H, m, 2xAr-H), 7,84 (1H, s, Ar-H), 7.85-8,08 (3H, m, 3xAr-H), 8.36 (3H, m, 3xAr-H), 8.82 (1H, d, J=6 Hz, NH), 10.43 (1H, s, NH), 11.18 (1H, t, J=2Hz, NH).

^{13}C-NMR (DMSO-*d6*): 40.1 (t), 42.9 (t), 111.6 (s), 113.7 (d), 116.3 (d), 117.1 (s), 118.3 (s), 124.1 (d), 127.6 (d), 127.8 (d), 128.0 (s), 129.0 (d), 130.5 (s), 131.9 (d), 133.2 (s), 133.5 (d), 136.8 (s), 138.8 (s), 140.7 (d), 145.1 (d), 147.6 (s), 151.1 (s), 161.4 (s),

1-(5-Bromo-pyridin-2-yl)-3-[2-(isoindolo[2,1-*a*]quinoxalin-6-ylamino)-ethyl]-thiourea 45a

M.p. 198°C, Yield 10 %, **IR:** 3678 (NH), 3538 (NH), 3403 (NH),1328 (C=S) cm^{-1}

^{1}H-NMR (DMSO-*d6*): 4.22 (2H, t, J=5 Hz, CH_2), 4.90 (2H, t, J=5 Hz, CH_2), 7.76 (1H, d, J=9 Hz, Ar-H), 7.67-8.13 (8H, m, 8xAr-H), 8.35 (1H, d, J=7.1 Hz, Ar-H), 8.89 (1H, d, J=7.5 Hz, Ar-H), 10.42 (1H, s, NH), 11.21 (1H, t, J=5 Hz,NH).

^{13}C-NMR (DMSO-*d6*): 40.3 (t), 42.8 (t), 111.5 (d), 113.8 (d), 113.9 (d), 123.9 (d), 127.3 (d), 127.9 (d), 128.2 (d), 130.6 (d), 131.4 (d), 132.4 (s), 133.4 (d), 135.4 (s), 135.6 (s), 138.1 (s), 140.0 (s), 140.7 (d), 143.9 (s), 145.2 (d), 151.6 (s), 161.3 (s), 179.9 (s).

1-(5-Bromo-pyridin-2-yl)-3-[2-(3-methoxy-isoindolo[2,1-*a*]quinoxalin-6-ylamino)-ethyl]-thiourea 45b

M.p. 239°C,Yield 16%, **IR:** 3688 (NH), 3584 (NH), 3400 (NH), 1297 (C=S) cm^{-1}

^{1}H-NMR (DMSO-*d6*): 4.37 (3H, s, OCH_3), 4.63 (2H, m, CH_2), 5.34 (2H, t, J=4.7 Hz, CH_2), 7.20 (1H, d, J=8 Hz, Ar-H), 7.58 (1H, d, J=2Hz, Ar-H), 7.77 (1H, dd, J=2,9 Hz, Ar-H), 8.06-8.46 (6H, m, 6xAr-H), 8.77 (1H, dd, J=2,2 Hz, Ar-H), 9.25 (1H, dd, J=2,2 Hz, Ar-H), 10.86 (1H, s, NH), 11.64 (1H, t, J=6Hz, NH).

^{13}C-NMR (DMSO-*d6*): 40.2 (t), 43.9 (t), 56.7 (q), 106.22 (d),112.4 (d), 114.7 (d), 122.2 (d), 124.4 (d), 127.7 (s), 128.8 (d), 130.5 (d), 131.5 (d), 132.4 (d), 133.4 (s), 133.6.(s), 134.3 (d), 135.1 (s), 142.8 (d), 145.0 (s), 146.0 (s), 152.6 (s),162.0 (s), 162.3 (s), 180.8 (s).

1-(5-Bromo-pyridin-2-yl)-3-[2-(3-methyl-isoindolo[2,1-*a*]quinoxalin-6-ylamino)-ethyl]-thiourea 45c

M.p. 230°C ,Yield 15%, **IR:** 3757 (NH), 3570 (NH), 3406 (NH),1297 (C=S) cm^{-1}

^{1}H-NMR (DMSO-*d6*): 2.71 (3H, s, CH_3), 4.24 (2H, m, CH_2), 4.92 (2H, t, J=5.1 Hz, CH_2), 6.77 (1H, d, J=4 Hz, Ar-H), 7.43 (1H, d, J=4Hz, Ar-H), 7.60-8.13 (7H, m, 7xAr-H), 8.37 (1H, dd, J=2,8Hz, Ar-H), 8.89 (1H, dd, J=2,2 Hz, Ar-H), 10.47 (1H, s, NH), 11.12 (1H, t, J=6Hz, NH).

^{13}C-NMR (DMSO-*d6*): 16.7 (q), 42.7 (t), 59.7 (t), 111.5 (d), 113.7 (d), 123.9 (d), 126.2 (d), 127.4 (d),131.3 (d), 132.4 (d),133.4 (d), 134.9 (s), 135.4 (s), 138.2 (s), 138.9 (s), 140.6 (d), 141.3 (s), 142.9 (s), 144.9 (d),145.0 (d), 151.5 (s), 161.3 (s), 170.3 (s), 179.8 (s).

1-(5-Bromo-pyridin-2-yl)-3-[2-(2,3-dichloro-isoindolo[2,1-*a*]quinoxalin-6-ylamino)-ethyl]-thiourea 45d

M.p. 235°C, Yield 21%, **IR:** 3671 (NH), 3558 (NH), 3414 (NH), 1211 (C=S) cm^{-1}

^{1}H-NMR(DMSO-*d6*): 4.19 (2H, t, J=8 Hz, CH_2), 4.86 (2H, t, J = 8 Hz, CH_2), 6.77 (1H, d, J=8.4 Hz, Ar-H), 7.67-7.75 (2H, m, 2xAr-H), 7,84 (1H, s, Ar-H), 7.85-8,08 (3H, m, 3xAr-H), 8.36 (3H, m, 3xAr-H), 8.82 (1H, d, J=6 Hz, NH), 10.43 (1H, s, NH), 11.18 (1H, t, J=2Hz, NH).

^{13}C-NMR (DMSO-*d6*): 40.1 (t), 42.9 (t), 111.6 (d), 113.8 (d), 121.8 (d), 124.1 (d), 127.6 (d), 127.8 (d), 128.0 (d), 129.0 (d), 129.4 (s), 130.5 (d), 131.9 (s), 132.0 (s), 133.2 (s), 133.6 (d), 136.9 (s), 138.8 (s), 140.6 (s), 145.0 (s), 145.1 (s), 147.6 (s), 151.6 (s)

EXPERIMETAL DATA

Synthesis of substituted amine 57a-c

The substituted 4-methylpyridine **56a-c** (1 g, 5.79 mmol) were dissolved in acetic acid (4 ml), and the reaction mixture was stirred at room temperature for 10 minutes. Then iron powder (0.6 g, 35.74 mmol) was added, and the reaction was heated at 90°C for 8 hours. The solvent was evaporated under reduced pressure, the crude was diluted with water , neutralized using a aqueous solution of sodium carbonate and extracted with ethyl acetate The organic layer was dried over sodium sulphate, filtered and the solvent evaporated under reduced pressure. **57a-c** was obtained 60-70% yield as brown coloured solid.

2-Chloro-4-methyl-pyridin-3-ylamine 57a

M.p. 72°C , Yield 60%, **IR:** 3486, 3395 (NH_2) cm^{-1}.

^{1}**HNMR (DMSO-d*6*):** 2.1 (3H, s, CH_3), 5.27 (2H, bs, NH_2), 7.0 (1H, d, J=4.6, Ar-H), 7.5 (1H, d, J=4.6 Hz, Ar-H).

^{13}**C-NMR (DMSO-*d6*):** 17.4 (q), 124.8 (d), 131.4 (d), 134.9 (s), 135.7 (s), 139.4 (s).

6-Chloro-4-methyl-pyridin-3-ylamine 57b

M.p. 75 °C , Yield 65 %, **IR:** 3378, 3307 (NH_2) cm^{-1}.

^{1}**HNMR (DMSO-d*6*):** 2.07 (3H, s, CH_3), 5.7 (2H, bs, NH_2), 7.04 (1H, s, Ar-H), 7.67 (1H, s, Ar-H).

^{13}**C-NMR (DMSO-*d6*):** 16.6 (q), 124.1 (d), 133.3 (s), 134.3 (d), 136.4 (s), 143.0 (s).

4-Methyl-pyridin-3-ylamine 57-c

M.p. 98°C , yield- 80% **IR:** 3390, 3320 (NH_2) cm^{-1}.

^{1}**HNMR(DMSO-d*6*):** 5.06 (3H, s, CH_3), 5.07 (2H, s, NH_2), 6.90 (1H, d, J=4.3 Hz, Ar-H), 7.67 (1H, d, J=4.5 Hz, Ar-H), 7.91 (1H, s, Ar-H).

^{13}**C-NMR (DMSO-*d6*):** 16.5 (q), 124.6 (d), 128.3 (s), 135.8 (d), 137.3 (d), 143.2 (s).

Synthesis of substituted (2-Chloro-4-methyl-pyridin-3-yl)-(2-nitro-phenyl)-amine 59a-c

The amino compounds **57a-c** (0.1g, 0.7 mmol) were dissolved in DMF (3 ml) and stirred at room temperature for 10 minutes, the reaction mixture was cooled at 0°C, and sodium hydride (0.05g, 1.4 mmol) was added. The reaction mixture was warmed to room temperature and stirred for 2 hours 1-fluoro-2-Nitrobenzene **58** (0.07g, 0.70 mmol) was added, and the

reaction mixture was stirred for additional 6 hours at room temperature. The reaction mixture was quenched with saturated ammonium chloride solution, diluted with water, and extracted with ethyl acetate. The organic layer was collected, dried over sodium sulphate and concentrated under reduced pressure. The crude was purified by chromatography coloumn using dichloromethane and ethyl acetate as eluents (6:4 DCM:EA). The amino nitrobenzene intermediates **59a-c** were obtained as a white solids.

(2-Chloro-4-methyl-pyridin-3-yl)-(2-nitro-phenyl)-amine 59a

M.p. 96°C , Yield 74%, **IR:** 3341 (NH), 1495 (NO_2) cm^{-1}.

^{1}**HNMR(DMSO-d*6*):** 2.2 (3H, s, CH_3), 6.33 (1H, dd, J=0.8, 8.5 Hz, Ar-H), 6.87 (1H ,td, J=1, 10.1 Hz, Ar-H), 7.46 (1H, m, Ar-H), 8.17 (1H, dd, J=1.4, 8.5 Hz, Ar-H), 8.3 (1H, d, J=4.8 Hz, Ar-H), 9.38 (1H, s, NH).

^{13}**C-NMR (DMSO-*d6*):** 17.7 (q), 115.2 (d), 117.5 (d), 125.6 (d), 126.2 (d), 132.0 (s), 132.3 (s), 136.5 (d), 142.1 (s), 147.4 (d), 150.0 (s), 150.1 (s).

(6-Chloro-4-methyl-pyridin-3-yl)-(2-nitro-phenyl)-amine 59b

M.p. 115°C , Yield 70%, **IR:** 3345 (NH), 1497 (NO_2) cm^{-1}.

^{1}**HNMR(DMSO-d*6*):** 2.19 (3H, s, CH_3), 6.63 (1H, dd, J=0.9, 8.5 Hz,Ar-H), 6.88 (1H, m, Ar-H), 7.51 (2H, m, 2xAr-H), 8.14 (1H, dd, J=1.4, 8.5 Hz, Ar-H), 8.29 (1H, s, Ar-H), 9.28 (1H, s, NH).

^{13}**C-NMR (DMSO-*d6*):** 16.9 (q), 116.1 (d), 117.8 (d), 125.6 (d), 126.2 (d), 132.9 (s), 134.6 (s), 136.3 (d), 142.5 (s), 147.3 (s), 148.0 (d), 148.4 (s).

(4-Methyl-pyridin-3-yl)-(2-nitro-phenyl)-amine 59c

M.p. 109°C , Yield 78%, **IR:** 3347 (NH), 1506 (NO_2) cm^{-1}.

^{1}**HNMR(DMSO-d*6*):** 2.20 (3H, s, CH_3), 6.57 (1H, d, J=8.6 Hz, Ar-H), 6.85 (1H, t, J=7.1 Hz, Ar-H), 7.40-7.51 (2H, m, 2xAr-H), 8.15 (1H, d, J=8.3 Hz, Ar-H), 8.40-8.44 (2H, m, 2xAr-H), 9.34 (1H, s, NH).

^{13}**C-NMR (DMSO-*d6*):** 16.8 (q), 115.8 (d), 117.4 (d), 125.7 (d), 126.2 (d), 132.5 (s), 134.6 (s), 136.3 (d), 143.0 (s), 144.2 (s), 147.3 (d), 148.3 (d).

Synthesis of substituted 3-[2-Chloro-3-(2-nitro-phenylamino)-pyridin-4-yl]-2-oxo-propionic acid ethyl ester 61a-c

To a mixture diethyl ether (5 ml) and ethanol (1ml), potassium *tert*-butoxide (0.08g, 0.7 mmol) was added, the mixture was stirred for 5 minutes at room temperature, then diethyl oxalate **60** was added (0.05 ml, 0.3mmol) and the resulting reaction mixture was stirred for 30 minutes at room temperature. Then amino nitrobenzene intermediates **59a-c** (0.1g, 0.3 mmol) were added and the reaction was refluxed for 6 hours, after which it was cooled at room temperature. The solvent was evaporated under reduced pressure and the crude was acidified with acetic acid to pH 4 and a precipitation of solid was observed. The solid was filtered and purified by chromatography coloumn using dichloromethane and ethyl acetate as eluents (9:1 DCM:EA). The acrylic acid ethyl ester intermediates **61a-c** were obtained as pale yellow solids.

3-[2-Chloro-3-(2-nitro-phenylamino)-pyridin-4-yl]-2-oxo-propionic acid ethyl ester 61a

M.p. 154°C , Yield 50 %, **IR:** 3415 (NH), 1653 (C=O), 1470 (NO_2) cm^{-1}.

^{1}**HNMR(DMSO-d*6*):** 1.12 (3H, t, J=7 Hz, CH_3), 4.15 (2H, q, J=7.1 Hz , CH_2), 6.32 (2H, m, CH, Ar-H), 6.88 (1H, td, J=1, 4.7 Hz, Ar-H) , 7.48 (td, 1H, J=7, 7.1 Hz, Ar-H), 8.19 (2H, m, 2xAr-H), 8.40 (1H, d, J=5.2 Hz, Ar-H), 9.40 (1H, s, NH), 10.83 (s, 1H, OH).

^{13}**C-NMR (DMSO-*d6*):** 13.7 (q), 61.8 (t), 101.5 (d), 115.9 (d), 117.8 (d), 122.8 (d), 126.1 (d), 129.8 (s), 132.5 (s), 136.5 (d), 142.4 (s), 144.0 (s), 146.6 (s), 147.6 (d), 150.7 (s), 163.4 (s).

3-[2-Chloro-5-(2-nitro-phenylamino)-pyridin-4-yl]-2-oxo-propionic acid ethyl ester 61b

M.p. 158°C , Yield 50 %, **IR:** 3424 (NH), 1720 (C=O), 1490 (NO_2) cm^{-1}.

^{1}**HNMR(DMSO-d*6*):** 1.12 (3H, t, J=7 Hz, CH_3), 4.16 (2H, q, J=7.1 Hz, CH_2), 6.27 (1H, s, CH), 6.61 (1H, d, J=8 Hz, Ar-H), 6.75 (1H , t, J=8 Hz, Ar-H), 7.42-7.50 (1H, m, Ar-H), 8.12-8.20 (2H, m, 2xAr-H), 8.36 (1H, d, J=6 Hz, Ar-H), 9.32 (1H, s, NH), 10.91 (1H, s, OH)

^{13}**C-NMR (DMSO-*d6*):** 13.6 (q), 16.4 (t), 109.6 (d), 115.9 (d), 125.6 (d), 130.1 (d), 130.9 (d), 131.2 (s), 133.1 (s), 133.6 (s), 134.6 (d), 135.1 (d), 135.4 (s), 140.8 (s), 145.9 (s), 159.5 (s).

3-[3-(2-Nitro-phenylamino)-pyridin-4-yl]-2-oxo-propionic acid ethyl ester 61c

M.p. 92°C , Yield 52%, **IR:** 3423 (NH), 1613 (C=O) ,1509 (NO_2) cm^{-1}.

[1]**HNMR(DMSO-d*6*):** 1.16 (3H, t, J=8 Hz, CH_3), 4.15 (2H, q, J=8.1 Hz, CH_2), 6.32 (2H, m, 2xAr-H), 6.88 (1H, t, J=7.5 Hz, Ar-H), 7.46 (1H, t, J=7.3 Hz, Ar-H), 8.20 (2H, m, 2xAr-H), 8.40 (1H, t, J=2.2 Hz, Ar-H), 9.38 (1H, s, NH), 10.80 (1H, s, OH).

[13]**C-NMR (DMSO-*d6*):** 13.7 (q), 61.8 (t), 101.5 (d), 115.8 (d), 117.8 (d), 122.8 (d), 126.1 (d), 129.8 (d), 132.4 (s), 136.5 (d), 142.4 (s), 144.1 (s), 146.5 (s), 147.7 (d), 150.7 (s), 163.3 (s).

Synthesis of substituted 1-(2-nitro-phenyl)-1H-pyrrolo[2,3-c]pyridine-2-carboxylic acid ethyl ester 62 a-c

The acrylic acid ethyl ester intermediates **61a-c** (0.5g, 2.59 mmol) were dissolved in acetic acid (10 ml) and refluxed for 2 hours . The reaction mixture was poured into ice and water (150 ml) and the resulting precipitate was collected by filtration and recrystallized from ethanol. The substituted Chloro-1-(2-nitro-phenyl)-1H-pyrrolo[2,3-c]pyridine-2-carboxylic acid ethyl ester **62a-c** were obtained as white solid.

7-Chloro-1-(2-nitro-phenyl)-1H-pyrrolo[2,3-c]pyridine-2-carboxylic acid ethyl ester 62 a

M.p. 131°C , Yield 65 %, **IR:** 1721 (C=O), 1530 (NO_2) cm^{-1}.

[1]**HNMR(DMSO-d*6*):** 1.12 (3H, t, J=7 Hz, CH_3), 4.15 (2H, q, J=7.1 Hz, CH_2), 7.65 (1H, s, Ar-H), 7.85 (4H, m, 4xAr-H), 8.14 (1H, d, J=5.06 Hz, Ar-H), 8.30 (1H, d, J=5.76 Hz, Ar-H).

[13]**C-NMR (DMSO-*d6*):** 13.6 (q), 61.3 (t), 110.7 (d), 117.1 (d), 124.8 (d), 131.1(d),131.2 (s), 131.4 (s), 132.6 (d), 132.9 (s), 133.7 (s), 133.9 (s), 134.1 (d), 138.9 (d), 146.9 (s), 159.3 (s).

5-Chloro-1-(2-nitro-phenyl)-1H-pyrrolo[2,3-c]pyridine-3-carboxylic acid ethyl ester 62b

M.p. 122°C , Yield 63%, **IR:** 1717 (C=O), 1532 (NO_2) cm^{-1}.

[1]**HNMR(DMSO-d*6*):** 1.13 (3H, t, J=9.5 Hz, CH_3), 4.16 (2H, q, J=10 Hz, CH_2), 7.53 (1H, s, Ar-H), 7.81-8.04 (4H, m, 4xAr-H), 8.30 (1H, s, Ar-H), 8.33 (1H, dd, J=2.3,7.5 Hz, Ar-H).

[13]**C-NMR (DMSO-*d6*):** 13.6 (q), 61.3 (t), 110.7 (d), 117.1 (d), 124.8 (d), 131.1(d),131.2 (s),131.4 (s), 132.6 (d), 132.9 (s), 133.7 (s), 133.9 (s), 134.1 (d), 138.9 (d), 146.9 (s), 159.3 (s).

1-(2-Nitro-phenyl)-1H-pyrrolo[2,3-c]pyridine-2-carboxylic acid ethyl ester 62c

M.p. 137°C , Yield 60%, **IR:** 1725 (C=O) , 1530 (NO_2) cm^{-1}.

[1]**H-NMR (DMSO-*d6*):** 1.12 (3H, t, J=6.9 Hz, CH_3), 4.15 (2H, q, J=6.7 Hz, CH_2), 7.65-7.80 (6H, m, 6xAr-H), 8.14 (1H, d, J=5 Hz, Ar-H), 8.30 (1H, d, J=7 Hz, Ar-H).

^{13}C-NMR (DMSO-*d6*): 13.6 (q), 61.3 (t), 110.7 (d), 117.1 (d), 124.8 (d), 131.14 (d), 131.17 (d), 131.4 (s), 132.6 (d), 132.9 (s), 133.7 (s), 133.9 (s), 134.1 (d), 138.9 (d), 146.9 (s), 159.4 (s).

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