



## Review Article

# Antiviral activity of isoindole derivatives

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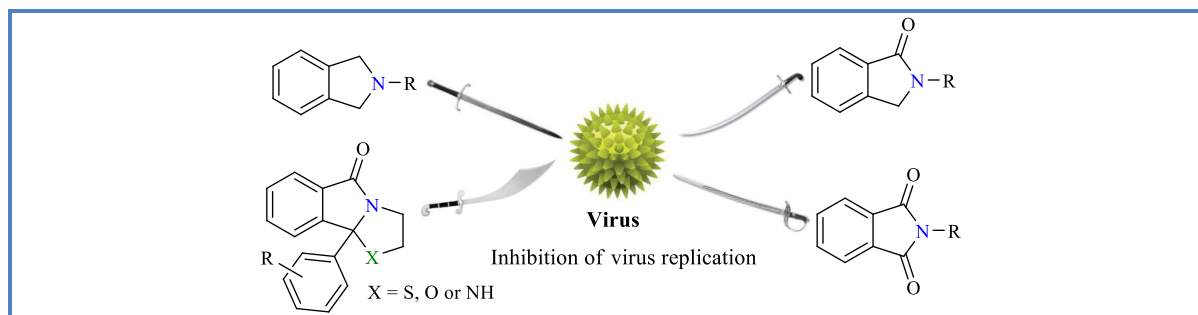
### ABSTRACT

The isoindole scaffolds and their related compounds are very important as they have a wide range of biological activities such as anti-inflammatory, antiarrhythmic, nootropic, anxiolytic, sedative, and antimicrobial activities. The design, synthesis and biological evaluation of novel antiviral agents have seen an increased relevance over the past few years. Several promising and successful results have been collected and reported in this mini review that could be useful for their further development and application in antiviral therapy. Furthermore, the main types of drug targets, the mechanism of actions, and the structure–activity relationship have also been studied to find potent and safe antiviral agents. This review summarises the considerable antiviral activities of isoindoline derivatives against several human viruses. The isoindole framework has and comprises many advantageous physicochemical- and biological properties. Several promising results showed that linkage, fusion, substitution or hybridization of the isoindole ring with various other rings or side chains led to effective antivirals. Results reported refer to versatile mechanism of action of these compounds.

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### Graphical Abstract



## Introduction

The saturated and oxo derivatives of isoindole (isoindoline, isoindolinone or phthalimide) are much more stable than the 2*H*-isoindole ring itself, which has a considerable aromatic character and strong reactivity towards the dienophiles. The isoindoline scaffolds and their related compounds are getting more and more important in medicine because these molecules have a wide range of pharmacological effects including anti-inflammatory (indoprofen) [1], antiarrhythmic (ubisindine) [2, 3], nootropic [4], anxiolytic and sedative (pazinaclone and pagoclone) [5, 6] or diuretic and antihypertensive activities (chlorthalidone) [7].

Other derivatives are potent 5-HT<sub>1A</sub> and 5-HT<sub>2C</sub> receptor antagonists [8, 9], antispasmodic [10], antinociceptive agents (JM-1232) [11] and have hypnotic activities [12], while the *N,N*-phthaloyl derivative of  $\alpha$ -,  $\beta$ - and  $\gamma$ -amino acids showed anticonvulsant activity [13]. Some 1, 2, 4-triazole-fused isoindoles show anti-inflammatory, analgetic, CNS-depressant, and antimicrobial effects [14, 15]. The naphthoquinone derivative (mitoquidone) is the first member of the new pentacyclic quinone group, developed as a potential anticancer agent [16]. The 8-amino-isoindolo [1, 2-*b*]quinazolinone (batracylin), acts in different ways to stop the growth of cancer cells [17]. A phenylethylidene derivative (AKS-186) inhibits the thromboxane A<sub>2</sub> (TXA<sub>2</sub>) analogue-induced vasoconstriction [18, 19]. Finally, some 3-benzylidene-2-(2-diethylaminoethyl)-phthalimidine derivatives are useful as local anesthetics, in this manner compound AL-12 is about 5-6 times more active than lidocaine [20].

This review discusses the antiviral activities of isoindoline derivatives against human immunodeficiency virus (HIV), dengue virus, enterovirus (EV-71), hepatitis B and C virus

(HBV and HCV), respiratory syncytial virus (RSV) and influenza A and B viruses. Considerable effort has been devoted to the design of various isoindoline derivatives that are active against drug-resistant virus variants, with advantageous dosage and fewer side effects. Different scaffolds have been designed through both rational and computer-aided drug design strategies. Researchers are working to extend the range of antiviral agents to other families of pathogens and trying to attack viruses selectively at every stage of their life cycles. The increased understanding to the molecular mechanisms of the viral infection has provided great potential for the discovery of new antiviral agents that target viral proteins or host factors. Virus-targeting agents can inhibit the biological functions of viral proteins, mostly enzymatic activities, or block viral replication mechanism. Nowadays, researchers have extended their knowledge on virology and several possible strategies that are outlined for the design and development of novel and efficient antiviral agents.

## Results and Discussion

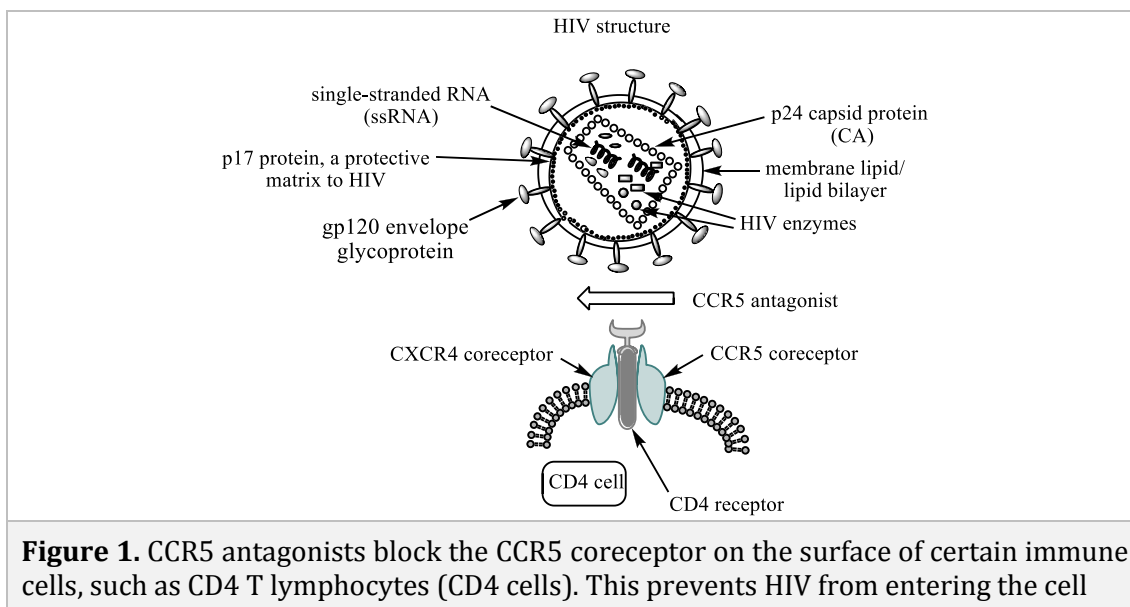
### *Antiviral targets*

#### *Virus entry inhibitors*

Viruses are perfect parasites, HIV and other viruses enter the body, attach to CD4 receptors and co-receptors such as CCR5 or CXCR4, then a membrane fusion occurs. As soon as a virus penetrates to the host cell, the virus opens the external wrapper and releases its genetic material (DNA or RNA) and replication enzymes. Thus cellular procedures are modified and production of protein which is encoded by the virus, afterwards reproduce the RNA or DNA of virus [21]. Thus in the course of infection the second step is the viral entry when the virus attaches to and enters to the host cell. The CCR5

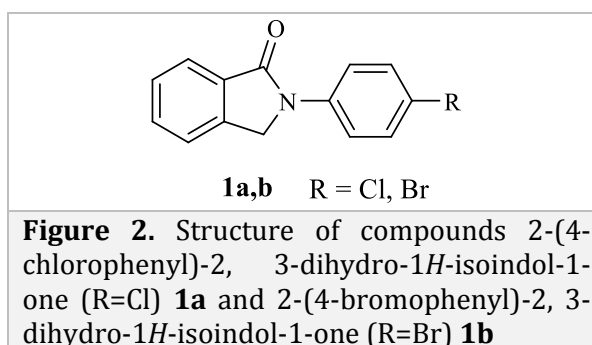
is an important co-receptor for HIV-1 and other viruses, allowing these viruses to enter the cells. CCR5 antagonists block the CCR5 co-receptor on the surface of certain immune cells, such as CD4 T lymphocytes (CD4 cells). This prevents the HIV from entering the cell (Figure 1). The inhibition of virus-receptor interactions was accomplished by maraviroc (8-

azabicyclo[3.2.1]octane derivative), a CCR5 receptor antagonist that has proved to be a successful HIV inhibitor [22]. The prototypical virus-cell fusion inhibitor is the enfuvirtide, a synthetic biomimetic peptide corresponding to the HR2 domain from HIV, which efficiently inhibits infection by preventing completion of six-helix bundle formation [23].



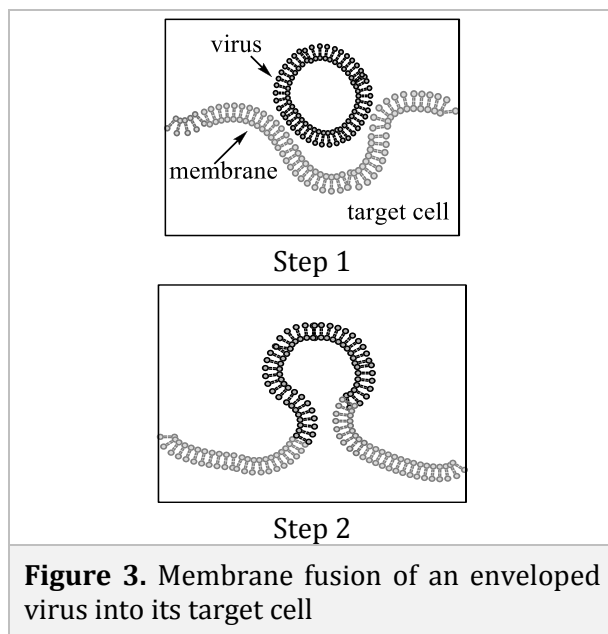
Enterovirus 71 (EV-A71) is a global infectious disease that affects millions of people. The virus is the main aetiological agent for hand, foot, and mouth disease; moreover, the infection can cause severe neurological, cardiac, and respiratory problems in children. Using the fragment-hopping strategy, a series of 2-aryl-isoindolin-1-one compounds were designed, synthesized and investigated for their *in vitro* antiviral activity towards multiple EV-A71 clinical isolates [24]. About 24 compounds were tested, approximately 10 showed significant antiviral activity ( $EC_{50} < 10 \mu\text{M}$ ) towards four EV-A71 strains. Compounds **1a** and **1b** exhibited broad and potent antiviral activity (Figure 2) with  $EC_{50}$  values in the range of 1.23–1.76  $\mu\text{M}$  and the selectivity indices of **1a** and **1b** were significantly higher than those of the

reference compound. In addition, the preliminary mechanism of the action studies revealed that the compound **1a** played an antiviral role in the virus entry stage.



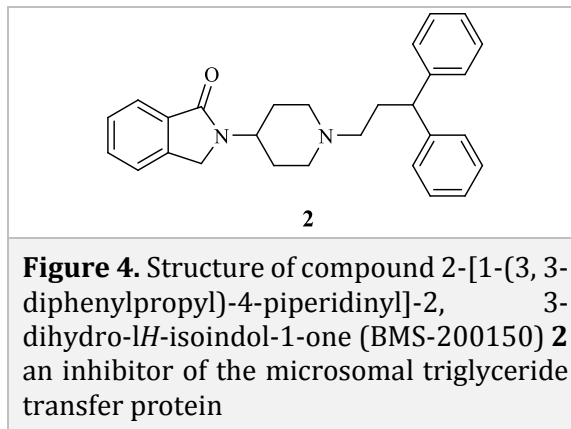
On the other hand, the enveloped viruses may contain a nucleocapsid (genome and the protein coat), that is encompassed by a lipid bilayer. In order to infect a cell, the virus passes

on its genome into the host cell cytoplasm by the fusion with the enclosing endosomal membrane of the host cell (Figure 3). This fusion is mediated by transmembrane proteins (TP) and different glycoproteins.



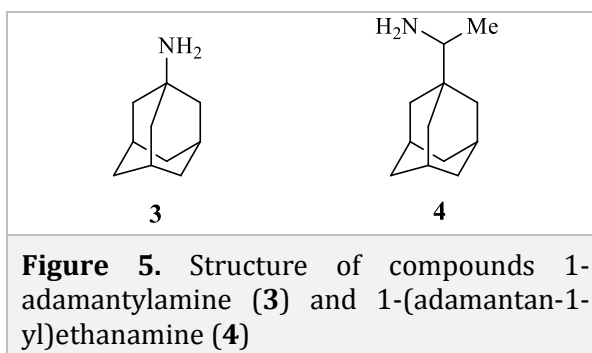
**Figure 3.** Membrane fusion of an enveloped virus into its target cell

Jamil *et al.* reported that the diphenylpropyl-4-piperidinyl-2, 3-dihydro-1*H*-isoindol-1-one derivative (BMS-200150) **2** (Figure 4) inhibits selectively the microsomal transfer protein (MTP)-mediated transfer of triglyceride (TG) and cholesteryl ester (CE) between membranes. Microsomal triglyceride transfer protein (MTP), an endoplasmic reticulum lipid transfer protein critical for apolipoprotein B (apoB) secretion, regulates CD1d antigen presentation. The  $IC_{50}$  for the inhibition of TG transfer was  $0.6 \mu\text{M}$ . BMS-200150 also inhibits human MTP-mediated TG transfer with a similar  $IC_{50}$  of  $2.2 \mu\text{M}$  [25, 26]. Regrettably, compound **2** was identified as a weak ligand for CCR5 (binding  $IC_{50}$  in the low micromolar range); however, further structural modification and optimization by structure–activity relationship (SAR) led to a more efficient phenylacetamide analogue [27].



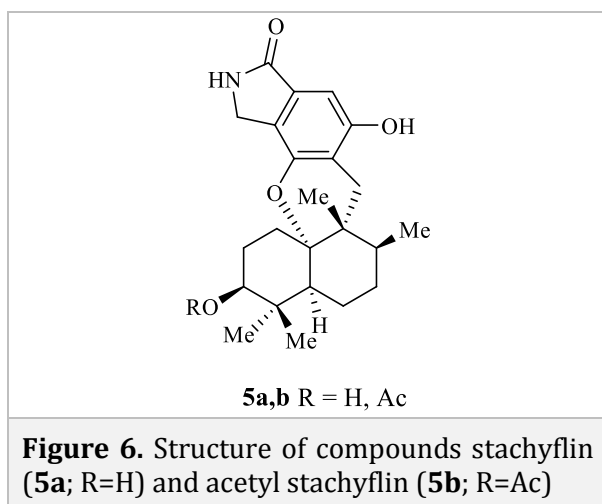
#### Viral uncoating inhibitors

Viral uncoating as a target is an essential step in viral replication, which results in the release of the viral genome into either the cytoplasm (RNA viruses) or the nucleus (DNA viruses and RNA tumor viruses). Uncoating represents one of the early steps in the life cycle of a virus [28]. Amantadine **3** and rimantadine **4**, which are viral uncoating inhibitors, are used to prevent and treat influenza A infections (Figure 5). The antiviral mechanism of the amantadine and its analogue involve interference with the M2 ion channel protein of influenza A viruses. These channel blockers act through the destabilization of the nucleic acid helix by blocking the proton transport across the transmembrane and thus stop the replication process.



Stachyflin (**5a**) and acetylstachyflin (**5b**) are novel anti-influenza A virus agents, produced by *Stachybotrys* fungi, species RF-7260 (Figure 6). The mechanism of the antiviral action of

stachyflin **5a** has been shown to be inhibition of the fusion process between the viral envelope and the host cell membrane. This differs from the above mentioned amantadine and rimantadine [29, 30]. Furthermore stachyflin had potent antiviral activity against influenza viruses of H1 and H2 subtypes and inhibited replication of viruses of H5 and H6 subtypes, as well as the pandemic influenza A virus subtype H1N1/09 virus in Madin-Darby canine kidney (MDCK) cells. Antiviral activity was identified potential binding pocket for stachyflin on the hemagglutinin (HA) [31]. Interference of (+)-stachyflin with the low-pH induced HA conformation change, which is essential for the virus-cell membrane fusion process that occurs during influenza viral infection. Stachyflin also inhibited the virus growth in lungs of infected mice and it was about 1760 times more active than **3** amantadine ( $IC_{50}=5.3 \mu M$ ) [26]. (+)-Stachyflin has shown promising anti-influenza activity both *in vitro* and *in vivo*.



### Inhibition of virus replication

#### Reverse transcriptase inhibitors (RTIs)

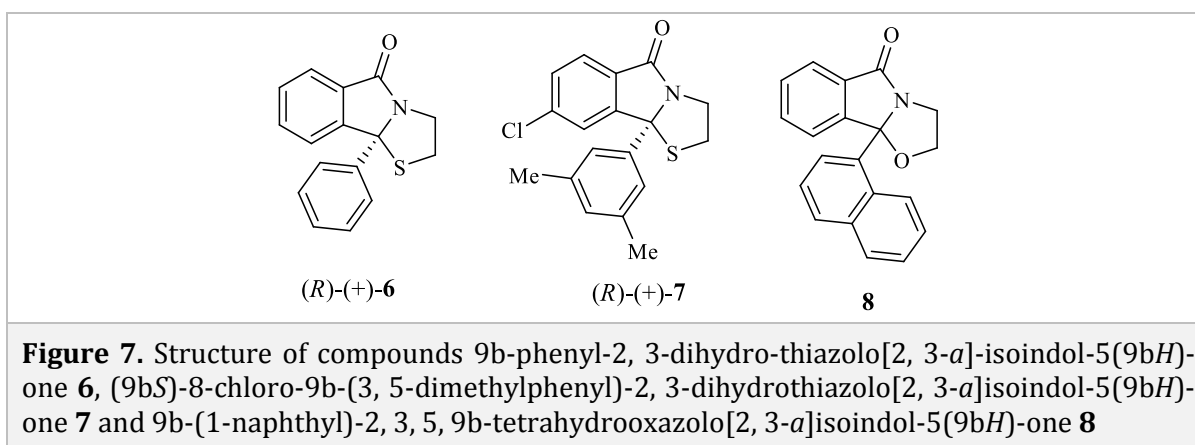
The reverse transcriptase (RT) enzyme, which catalyzes the synthesis of the complementary DNA (cDNA) from a single-

stranded RNA template. The cDNA is a DNA copy of a messenger RNA (mRNA) molecule. The RT inhibitors block this process and include three main types of RTI drugs, namely the nucleoside and nucleotide analogues as well as non-nucleoside inhibitors. Many viruses use reverse transcriptase to copy their genetic material and generate new viruses. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are bound in a hydrophobic pocket proximal to the catalytic site of RT in HIV-1. The X-ray crystallographic studies of NNRTIs in complex with RT have shown that the NNRTIs maintain a very similar conformational shape and appear to function as  $\pi$ -electron donors to aromatic side-chain residues surrounding the binding pocket.

The major problem in the development of new NNRTIs is the rapid emergence of resistant strains of HIV-1 in cell culture and patients [32]. Mertens *et al.* identified 9b-phenyl-2, 3-dihydro-thiazolo[2, 3-*a*]-isoindol-5(9b*H*)-one **6** (BM 21.1298) as a lead compound, when searched selective RT inhibitors against HIV-1 (Figure 7). The compound **6** was further optimized by various substituents relative to the inhibition of the HIV-1 RT screening assay and subsequently in HIV-1-infected MT2 cells [33]. These compounds specifically bind to an allosteric site of HIV-1 RT close to but not identical with the active site of this enzyme. Enantiomers of **6** were separated by chromatography and it was observed that (*R*)-(+)-configuration was about twice as active ( $IC_{50}=0.25 \mu M$ ) as the racemic mixture ( $IC_{50}=0.7 \mu M$ ), whereas the (*S*)-(-)-**6** enantiomer was at least 50 times less active ( $IC_{50}=39.3 \mu M$ ) in the RT assay. Several derivatives confirmed that (*R*)-(+)- enantiomers had more activity at RT inhibition. The presence of the aromatic group in position 9b is absolutely necessary for the inhibition activity; however, the replacement of the phenyl ring by heteroaromatics (thiophene,

furan or pyrrole) led to decreased activity or inefficiency. Substitution of the phenyl ring with alkyl-, alkoxy-, chloro- and other groups also significantly influenced the inhibition values. Replacement of the sulfur by O, NH and CH<sub>2</sub> or the ring enlargement of the same series by a methylene group yielded less active isoindolones. In general, five-membered rings were more potent than six-membered rings, and the antiviral activity decreased in the series S>O>CH<sub>2</sub>>NH. The types of dihydrothiazolo-[2, 3-*a*]isoindolone ring systems were the most

potent inhibitors, namely the compound (9*bR*)-8-chloro-9*b*-(3, 5-dimethyl-phenyl)-2, 3-dihydro-thiazolo [2, 3-*a*] isoindol-5(9*bH*)-one **7** (IC<sub>50</sub>=16 nM), which carries a phenyl ring in position 9*b* and has one or two methyl groups in the *meta* position. The presence of a chlorine atom in position 8 is also favourable. (*R*)-(+)-**6** and (*R*)-(+)-**7** unfortunately produced only moderate serum levels after per os administration to rats due to weak oral bioavailability of these highly lipophilic compounds.

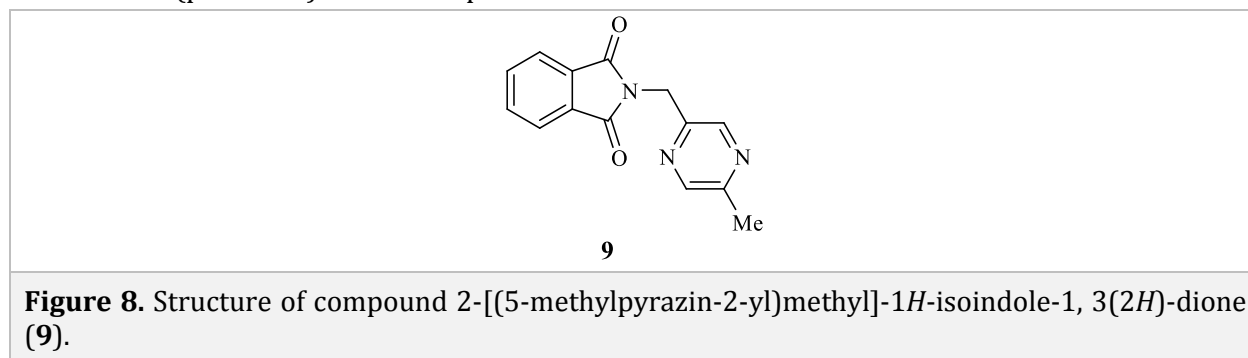


Maas *et al.* [34] evaluated the 3, 5-dimethyl derivative (**7**) of the compound **6** thiazolo[2, 3-*a*]isoindolone and observed that with has with high specificity toward the RT of human HIV-1. The compound was the most potent and inhibited HIV-1 RT at a 50% inhibitory concentration of 90 nM *in vitro*, however no antiviral effect was observed with an HIV-2 isolate. Viral resistance also was investigated with this compound. Ren *et al* determined the crystal structures of two potent thiazolo[2, 3-*a*]isoindolone inhibitors in complex with HIV-1 RT. These inhibitors bind in a mode resembling that of „two-ring” non-nucleoside reverse transcriptase inhibitors (NNRTIs) and on the basis of this similarity some structure-activity were rationalized. The reasonable suggestions for the modification of isoindolinones were also

made with improved resilience to common resistance mutations [35]. Previously, König *et al.* [36] patented the synthesis and evaluation of antiviral oxazolo[2, 3-*a*]isoindole derivatives. Thus antiviral compound 9*b*-(1-naphthyl)-2, 3, 5, 9*b*-tetrahydrooxazolo [2, 3-*a*]isoindol-5(9*bH*)-one **8** (Figure 7) inhibited HIV-RT very potently *in vitro* (IC<sub>50</sub>=1.8 μM). This compound is especially useful for treatment of clinical manifestation of retroviral HIV infections, *i.e.* generalized lymphadenopathy, progressed stages of the AIDS-related complex (ARC) and treatment of infections caused by DNA viruses such as herpes simplex, cytomegalovirus, papilloma, varicella zoster, *etc.* Samee *et al.* subjected to three-dimensional quantitative structure-activity relationship (3D-QSAR) studies using comparative molecular field

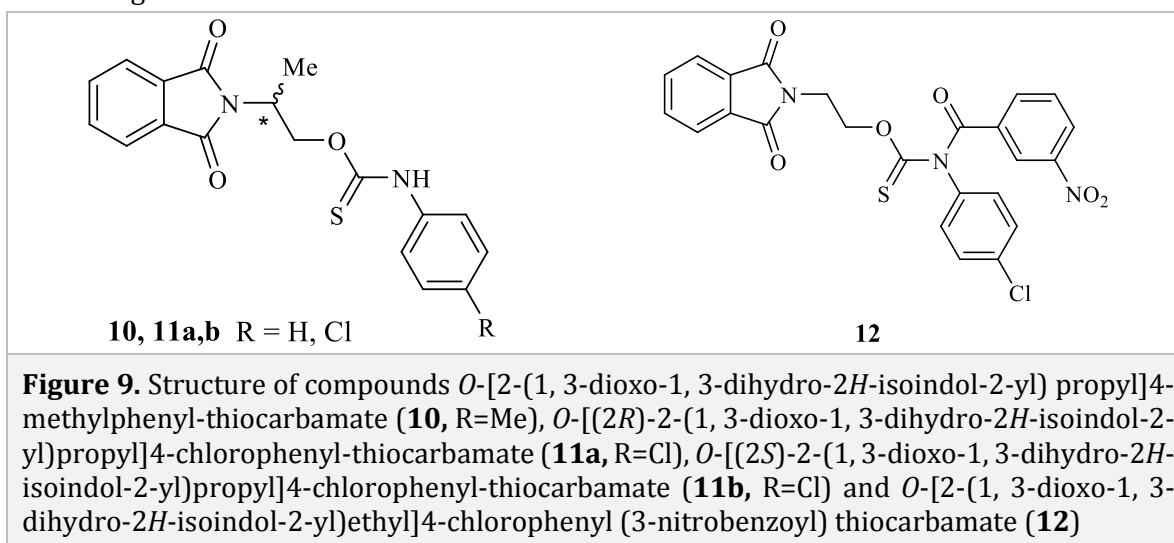
analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA) of a series of phthalimides as possible non-nucleoside reverse transcriptase inhibitors (NNRTIs) [37]. Twelve test compounds were used to determine the predictive values of the models and the calculated (predicted) and experimental

inhibitory activities were well correlated. Compound **9** phthalimide derivative (Figure 8) revealed the highest *in vitro* RT inhibitory activity (84% and  $IC_{50}=120.75 \mu\text{g/mL}$ ) and it was used as template molecule for the superimposition and for field fit methods.



Several *O*-(2-phthalimidoethyl)-*N*-arylthiocarbamates (TCs) analogues were prepared by a convergent two-step solution-phase parallel synthesis (Figure 9). Numerous TC derivatives displayed activity against wild-type HIV-1 and a number of analogues were effective against the Y181C mutant [38]. The 4-tolyl analogue **10** was 7-fold less active than **11a**. Docking simulations carried out on **11a**

and **11b** helped to rationalize the activity gap between the *R* and *S* stereoisomers. The *R* isomers fit the NNRTI binding pocket much better than *S* enantiomers. Compound **11a** proved the highest activity against Y181C mutant ( $EC_{50}=1.3 \mu\text{M}$ ) with good potency against the K103R mutant in a cell-based assay ( $EC_{50}=4.8 \mu\text{M}$ ).

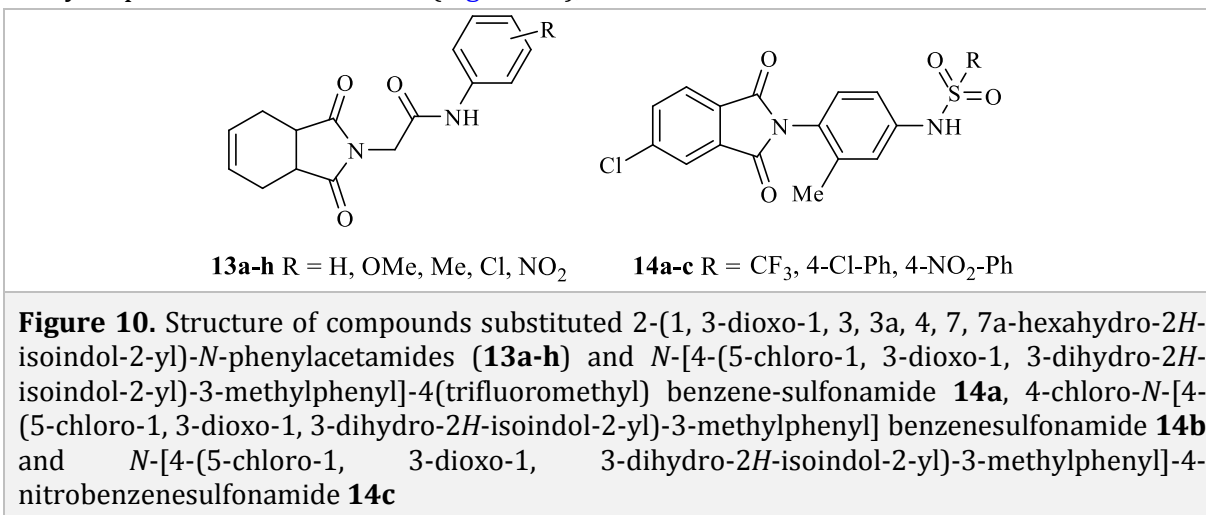


Spallarossa *et al.* studied the structure-activity relationships (SARs) of *N*-acyl

derivatives of *O*-(2-phthalimidoethyl)-*N*-arylthiocarbamates (TCs) and it was observed

that the potency of new TCs was better than that of the first series [39]. Thus 3-nitrobenzoyl analogue **12** (Figure 9) of thiocarbamate **11a, b** was the most potent ATC so far synthesized ( $EC_{50}=1.5$  nM) against HIV-1 strains. The docking model built for the most potent derivative **12** suggested for the inhibitor an extended conformation superimposable with that predicted for lead compound *N*-phenyl-*N*-benzoyl-thiocarbamate. The RT-**12** complex is mainly stabilized by two hydrogen bonds: the former involving the thiocarbonyl sulphur atom and the Lys101 amidic nitrogen (S–NH distance: 2.9 Å), the latter between one of the imidic carbonyls and the Lys103 side chain amino group. The higher potency of **12**, in comparison with that of the lead compound, would be explained by the hydrophobic contacts of the chlorine atom with some amino acids and by the polar interactions of the 3-nitro group. Penta *et al.* designed and prepared several tetrahydrophthalimide derivatives (Figure 10),

which *in vitro* have weak to moderate HIV type 1 reverse transcriptase inhibitory activities [40]. The molecular docking studies were applied for the design using HIV-1 RT enzyme as the target protein and the tetrahydrophthalimide scaffold as one of the hydrophobic wings in butterfly-shape pharmacophore. In the course of development of new NNRTs the Lipinski's rule of five parameters was also considered such as ClogP, molecular weight, hydrogen bond acceptors and donors, druglikeness and drug score. Designed and selected analogues were synthesized in a simple procedure, and then products were evaluated for HIV-1 RT inhibitory activity at concentration 2 and 20  $\mu$ M by use of DNA polymerase assay. Among the synthesized compounds **13e-h** (R=4-NO<sub>2</sub>, 3-NO<sub>2</sub>, 2-NO<sub>2</sub>, 2-Cl-3-Me) showed satisfactory and comparable docking results such as free binding energy and predicted inhibitory constant ( $K_i=10.38$ -20.37 nM).



*In vitro* evaluation of these compounds (**13a-f**, R=H, 4-OMe, 3-Me, 3-Cl, 4-NO<sub>2</sub>, 3-NO<sub>2</sub>) showed weak HIV-1 RT inhibitory activity at 20  $\mu$ M concentration. In this series of compound **13a** (2-(1, 3-dioxo-1, 3, 3a, 4, 7, 7a-hexahydro-2H-isoindol-2-yl)-*N*-phenylacetamide) with an unsubstituted phenyl ring (mentioned as wing

2 in pharmacophore) showed 25% inhibition of HIV-1 RT at tested concentration of 20  $\mu$ M. Several phthalimide derivatives bearing imine, amide, thioamide, and sulfonamide linkages were designed *in silico*, synthesized, and evaluated for their anti-HIV activity by Kumari *et al.* [41]. SAR studies referred to the

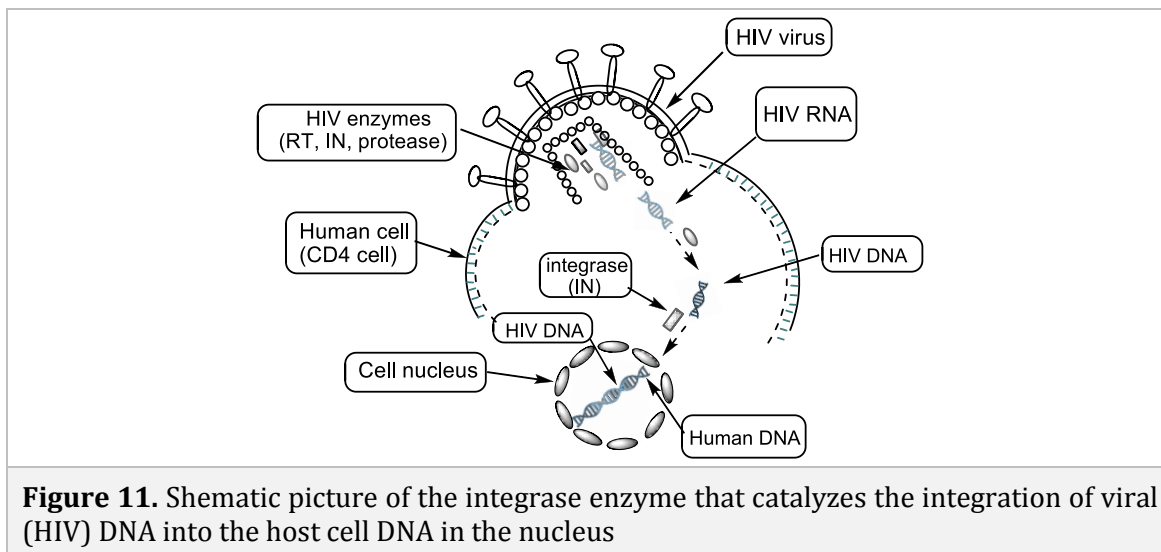


connections affect their anti-HIV activity in these molecules and the presence of sulfonamide linkages (**14a-c**) led to the most potent HIV-RT inhibitors (Figure 10). The S=O bonds of the sulfonamide moiety interacted with Lys103 (NH or carbonyl or both) and Pro236, while the NH part of the sulfonamide chain formed a bond with the carbonyl of Lys101 on the reverse transcriptase. Some of these molecules were effective inhibitors of HIV-1 replication at nanomolar concentration ( $EC_{50}$ =3–4 nM) with selectivity indices (ratio  $CC_{50}/EC_{50}$ ) ranging from 33.75 to 73.33 under *in vitro* conditions. It is interesting that considerably decreased efficiency was observed ( $EC_{50}$ =8 nM) after replacement of the trifluoromethyl substituent (**14a**,  $EC_{50}$ =4 nM) to methyl group. The molecular modeling strategy was employed successfully at the rigid congeneric series of NNRTIs with known SAR as structural probes to develop an all-atom non-nucleoside binding site (NNBS) model [42]. This model was generated using only the coordinates of C-R atoms from a low-resolution crystal structure. On the basis of the receptor-ligand atom contacts, the program HINT (Hydrophobic INTERactions) was used to develop a 3D-QSAR that predicted the rank order of binding affinities for the series of inhibitors. Electronic profiles of the ligands in their docked conformations were characterized using electrostatic potential maps and frontier orbital calculations. These results led to the development of a 3D stereoelectronic pharmacophore, which was used to construct 3D queries for database searches. Vanangamudi *et al.* reported the summary of lead optimization strategies from the 3D-QSARs

studies on NNRTI class in a valuable review [43]. Outside of the structure-activity relationship (SAR) revealed from CoMFA and CoMSIA studies of these drug classes, the molecular docking experiments and calculated protein-ligand interaction fingerprints are also relevant methods. Information extracted from the computational drug design methods such as molecular docking, pharmacophore modeling and improved free energy calculation provided highly valuable strategies for ligand design. Ligand design approaches such as CoMFA and CoMSIA have proven to be the very best ligand based approaches in terms of better understanding the SAR of chemical series and the efficient lead optimization strategy.

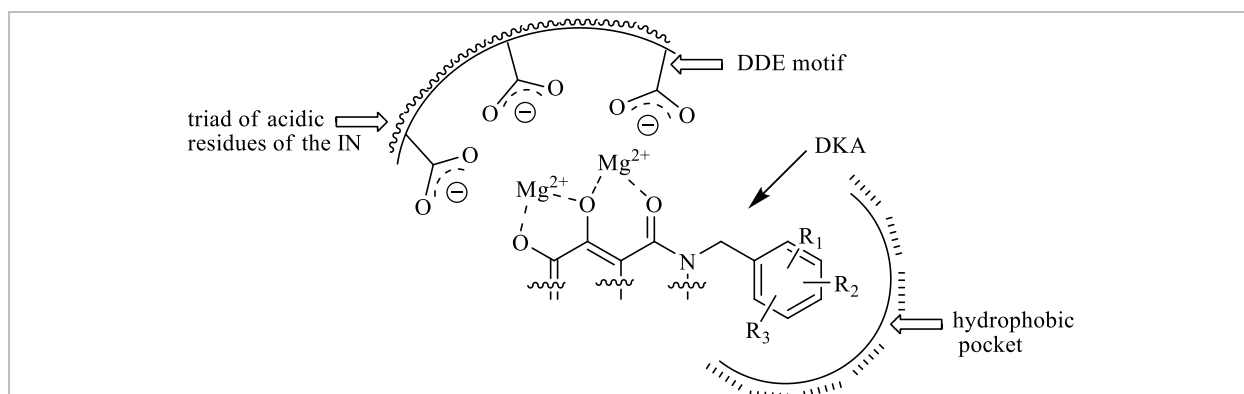
#### *Integrase inhibitors (INIs)*

Integrase (IN) is a key enzyme in the life cycle of virus, which is produced by a retrovirus and catalyzes the integration of virally derived DNA into the host cell DNA in the nucleus, forming a provirus that can be activated to produce viral proteins [44]. HIV and other retroviruses (*e.g.* human T-cell leukemia virus) use the integrase to insert (integrate) their viral DNA into the DNA of the host CD4 cell (Figure 11). Integration is an important step in the retrovirus life cycle and is blocked by a specific type of antiretroviral (ARV) drugs called integrase strand transfer inhibitors (INSTIs). Integrase has two main catalytic activities: an endonucleolytic cleavage at each 3'-OH terminals of the viral genome (3'-processing) and a strand transfer (ST) process when the viral DNA segments are inserted into the target DNA by a transesterification mechanism [45].



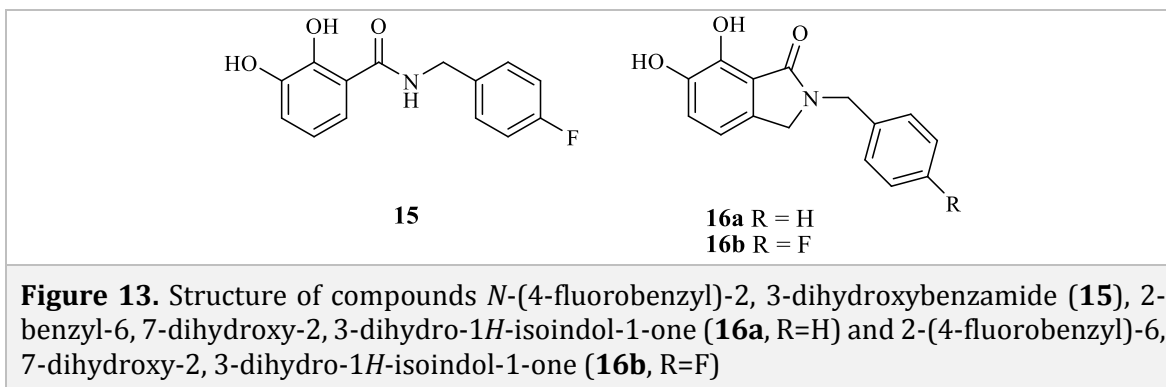
Integrase inhibitors (INIs) are a class of antiretroviral drugs and are consisting of two main groups: integrase strand transfer inhibitors (INSTIs) and integrase binding inhibitors (INBIs), which are still in experimental status. Hajimahdi *et al.* discussed in detail the structural and functional properties of HIV-1 IN and binding modes of IN inhibitors in a review article [46]. Initially, several potent inhibitors were developed against the IN-catalyzed 3'-processing (3'-P) and strand transfer (ST) reactions using *in vitro* IN assays that employ  $Mn^{2+}$  as a metal cofactor. At the same time under physiological conditions  $Mg^{2+}$  serves as the IN cofactor (Figure 12) and

these inhibitors often show failed antiviral potency in HIV-1 infected cells. The retroviral integrases belong to a group of proteins, which have three preserved acidic amino acids, by two aspartic acids (D, D) and a glutamic acid (E), composing the D-D-E motif, to serve as the active center of the integrase (IN) proteins. For the mechanism of inhibition is supposed the chelation two divalent metal ions ( $Mg^{2+}$ ) in association with the catalytically essential integrase residues (D-D-E motif). Additionally, for the optimal integrase (IN) inhibition the pharmacophore requires a regiospecific diketoacid (DKA) with a typical bond distance [47].



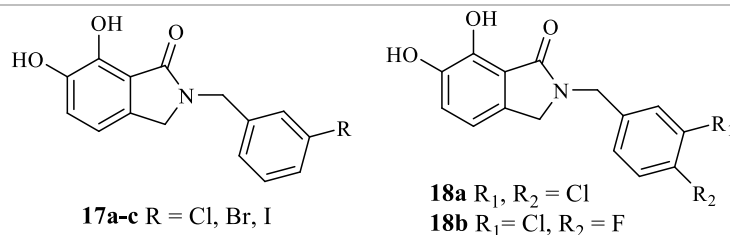
Zhao *et al.* reported that some 2, 3-dihydro-6, 7-dihydroxy-1*H*-isoindol-1-one ring systems (Figure 13) as conformationally constrained 2, 3-dihydroxybenzoyl analogues provided good selectivity for IN-catalyzed strand transfer in contrast to the 3'-processing reactions and antiviral efficiency in cells [48]. Transformation of the rigid diphenolic isoindole hydrazide series to the corresponding amide series

usually resulted in good inhibitory potency in  $Mg^{2+}$  and strand transfer (ST) selectivity. The isoindolinones **16a** ( $IC_{50}=12.3\pm 5.6 \mu M$ ) and **16b** ( $IC_{50}=10\pm 4 \mu M$ ) exhibited 10-fold higher inhibition potency *in vitro* than the non-rigid frame amide **15** ( $IC_{50}=108\pm 12 \mu M$ ). The isoindolinone core offers itself a structurally starting point for the further development of IN inhibitors.



The further investigations of relationship between the efficiency and the halogen substituents on benzyl ring confirmed the hypothesis of the binding of the hydrophobic pocket and the integrase-DNA complex. Generally, it was observed that dihalo-substituted analogues have higher potency than monohalo-substituted compounds, but that further addition of halogens is unfavourable [47]. Antiviral potencies for series isoindolinone compounds (Figure 14) were better than for series phthalimide derivatives. The antiviral efficacy was observed for the 3-halo-substituted analogues (**17a-c**;  $EC_{50}=3-4 \mu M$ ), while the dihalogenated-benzyl derivatives (**18a, b**) were more potent inhibitors ( $EC_{50}=0.1-0.16 \mu M$ ). On the other hand, the mono- and polyfluorated-isoindolinone analogs showed unexpected and surprising anomalies. The 3, 5-difluoro analogue of **16a** has considerable IN inhibitory potency ( $EC_{50}=0.3 \mu M$ ) with the greatest ST-

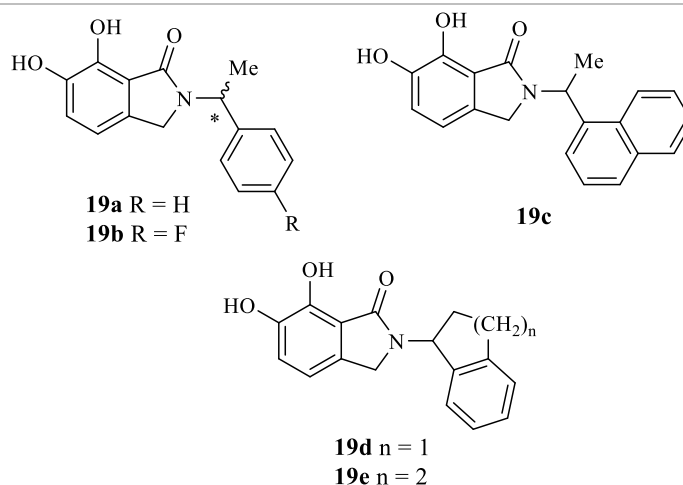
selectivity (700-fold) among all fluoro compounds the 2, 5-difluorobenzyl was the least effective analogue ( $EC_{50}=27 \mu M$ ) in series isoindolinones. The further halogen substituent-activity relationships were examined and described thoroughly by the authors and they established that molecules have a prochiral center at the benzylic methylene group [49] and the IN inhibition activity may also be influenced by the introduction of a substituent. Formation of the chiral center is equally damaging to binding, with the (*R*)-enantiomer being more damaging than the (*S*)-enantiomer. A notable enantiomeric difference in potency is shown by inhibitors that have restricted rotation of the aryl ring, with the larger difference being due to lower activity of the (*R*)-enantiomer rather than higher potency of the (*S*)-enantiomer. The ST inhibitory potency for all analogues tested was greater for the (*S*)-enantiomer than for the corresponding (*R*)-enantiomer.



**Figure 14.** Structure of compounds 2-(3-halobenzyl)-2,3-dihydro-1*H*-isoindol-1-ones **17a-c** and 2-(3,4-dihalobenzyl)-2,3-dihydro-1*H*-isoindol-1-ones **18a, b**

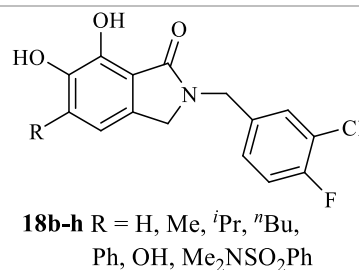
The difference in potencies of enantiomers is approximately twofold for the (*S*)-enantiomer benzyl analogues (**Figure 15**) **19a** (EC<sub>50</sub>=49 μM) and **19b** (EC<sub>50</sub>=12.1 μM), as well as more than

10-fold for naphthalen-1-ylmethyl (**19c**), dihydroinden-1-yl (**19d**, n=1) and tetrahydronaphthalen-1-yl (**19e**, n=2) derivatives (EC<sub>50</sub>=4-9 μM).



**Figure 15.** Structure of compounds 6,7-dihydroxy-2-(1-phenylethyl)-2,3-dihydro-1*H*-isoindol-1-one **19a**, 2-[1-(4-fluoro-phenyl)-ethyl]-6,7-dihydroxy-2,3-dihydro-1*H*-isoindol-1-one **19b**, 6,7-dihydroxy-2-(1-naphthalen-1-ylethyl)-2,3-dihydro-1*H*-isoindol-1-one **19c** and 2-(2,3-dihydro-1*H*-inden-1-yl)-6,7-dihydroxy-2,3-dihydro-1*H*-isoindol-1-one **19d** and 6,7-dihydroxy-2-(1,2,3,4-tetrahydronaphthalen-1-yl)-2,3-dihydro-1*H*-isoindol-1-one **19e**

The effects of the inserted substituent into the 4- and 5-positions of the parent 6,7-dihydroxyoxoisoindolinone ring were examined and reported [50]. Introduction of substituents at the 5-position (**Figure 16**) alike decreased ST inhibitory potency relative to the unsubstituted parent **18b** (ST IC<sub>50</sub>=0.16 μM).



**Figure 16.** Structure of compounds 5-substituted-6,7-dihydroxy-isoindolinones **18b-h**.

Although almost all substituents at the 5-position (**18c-h**) decreased inhibitory potencies

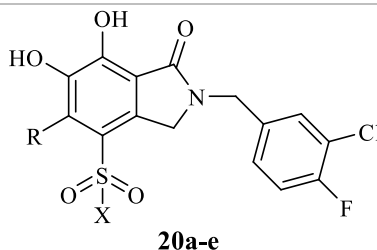
in the ST reactions compared to the parent compound **18b** (Table 1).

**Table 1.** Inhibitory potencies of compounds **18b-h** using an *in vitro* IN assay using a gel-based protocol with Mg<sup>2+</sup> cofactor

Compounds	R	IC <sub>50</sub> values (μM)	
		3'Processing	Standard transfer
<b>18b</b>	H	13.2 ± 3.1	0.16 ± 0.08
<b>18c</b>	Me	> 111	11.5 ± 1.6
<b>18d</b>	<i>i</i> -Pr	> 111	5.4 ± 0.6
<b>18e</b>	<i>n</i> -Bu	> 111	6.8 ± 0.9
<b>18f</b>	Ph	> 111	0.87 ± 0.08
<b>18g</b>	OH	36.0 ± 6	1.5 ± 0.3
<b>18h</b>	Me <sub>2</sub> NSO <sub>2</sub> Ph	> 111	0.4 ± 0.04

These are unrepresentative examples, since the compounds lacked functionalities at the neighbouring 4-position, therefore a series of analogs was prepared that combined sulfonamido functionality at the 4-position with various substituents at the 5-position. Formation of the sulfonamide groups at the 4-position on parent compound **18b** had a

moderate effect on inhibitory potencies in the standard transfer reaction (Table 2). Addition of a 4-sulfonamido group (Figure 17) increased the inhibitory potencies in the ST reaction and the increase was most explicit in the case of **20b** (ST IC<sub>50</sub>=0.19 μM), which has an *i*-propyl group at the 5-position.



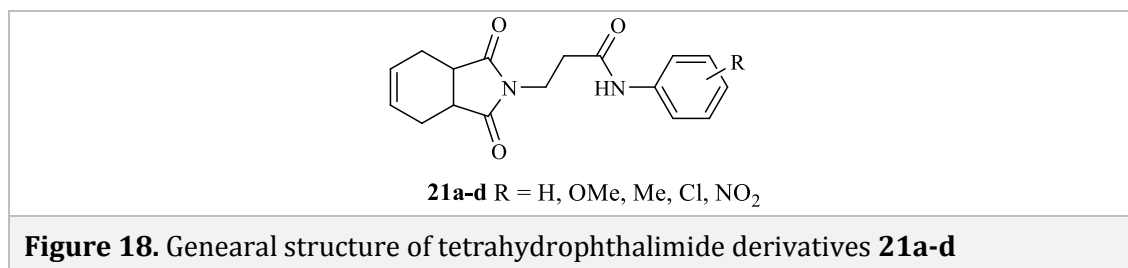
**Figure 17.** Structure of compounds sulfonamide derivatives of 5-substituted-6, 7-dihydroxyoxoisoindolinones **20a-e**

**Table 2.** Inhibitory potencies of compounds **20a-e** using an *in vitro* IN assay using a gel-based protocol with Mg<sup>2+</sup> cofactor

Compounds	R	X	IC <sub>50</sub> values (μM)	
			3'Processing	Standard transfer
<b>20a</b>	Me	NMe <sub>2</sub>	49.7 ± 4.0	2.8 ± 0.6
<b>20b</b>	<i>i</i> -Pr	NMe <sub>2</sub>	> 111	0.19 ± 0.03
<b>20c</b>	<i>i</i> -Pr		> 111	0.41 ± 0.06
<b>20d</b>	<i>n</i> -Bu	NMe <sub>2</sub>	> 111	2.3 ± 0.4
<b>20e</b>	OH	NMe <sub>2</sub>	7.6 ± 0.8	0.26 ± 0.08

Many 3-(1, 3-dioxo-hexahydro-2*H*-isoindol-2-yl)-*N*-phenyl- propanamides (**21a-d**) were prepared (Figure 18) starting from 1, 2, 3, 6-tetrahydrophthalimide and evaluated for their HIV-1 IN inhibitory activity using <sup>32</sup>P-labeled assays [51]. Unfortunately, in this study, none of the compounds showed any significant IN

inhibitory activity (both 3' processing and ST) at tested concentration 100 µg/mL. It correlates to results that Penta *et al.* reported elsewhere [40.]. Similar tetrahydrophthalimide analogs were designed and prepared (Figure 10), these compounds showed weak or moderate *in vitro* RT inhibitory activities at HIV type 1.



Earlier, Verschueren *et al.* reported [52] the design and synthesis of a novel series of tricyclic phthalimides as integrase inhibitors. The tricyclic phthalimide analogues **22** (Figure 19) were synthesized *via* a double Claisen condensation of an suitable *N*-substituted succinimide. Thus products obtained had different fused rings at the ring A, than benzene (X, Y, Z=C), pyridine (X, Y=C; Z=N), pyridazine

(X=C; Y, Z=N) or pyrazine ring (X, Z=N, Y=C). The pyrazine-fused phthalimides were found to be promising inhibitor candidates. The proposed binding hypothesis, based on the presence of a metal-chelating pharmacophore and the docking analysis method have been helpful in the optimization of this class of tricyclic phthalimide analogues. (Figure 13).

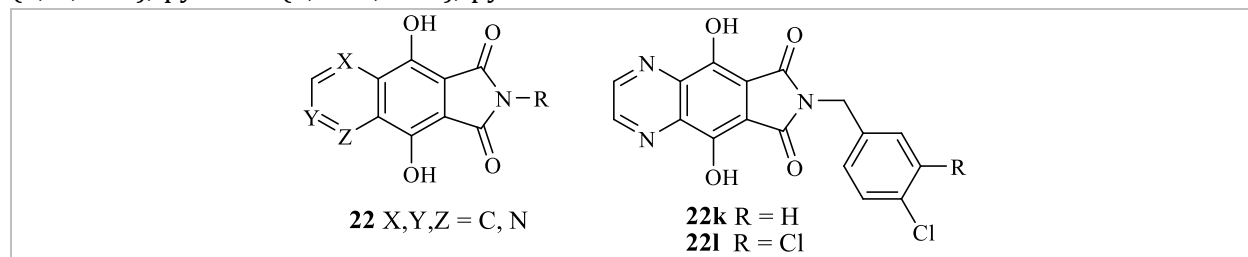


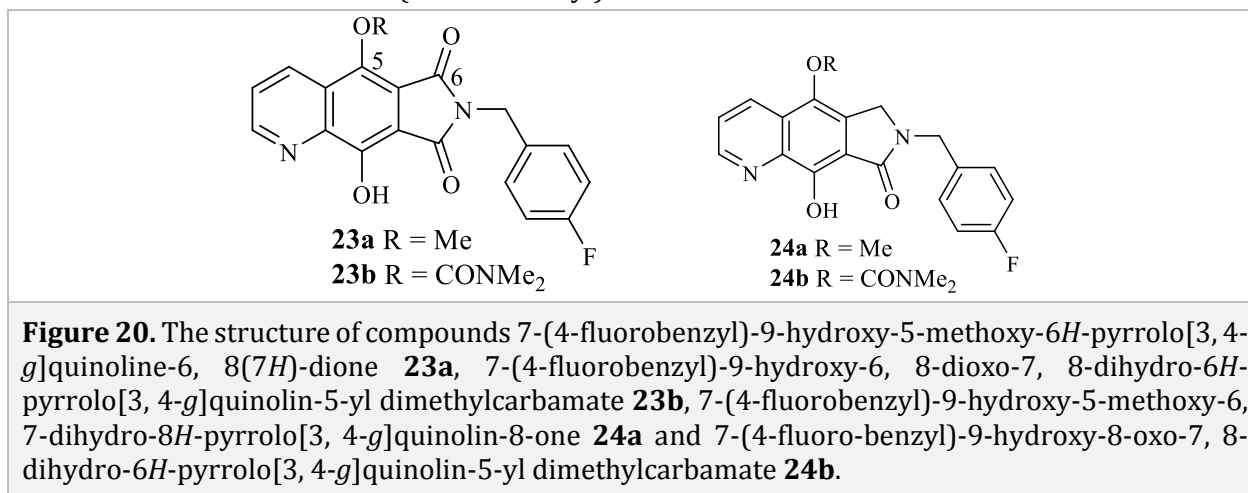
Figure 1. Synthesis of unsymmetric diimines **3a-3c**

Essential structural features, including the carbonyl-hydroxy-aromatic nitrogen motif for the enzymatic activity were confirmed. More than forty tricyclic phthalimide derivatives were screened and enzymatically the most active compound from this series is the pyrazine-fused

compound **22i** with an IC<sub>50</sub> value of 112 nM on the HIV-1 integrase enzyme, while the 4-chlorobenzyl-5, 9-dihydroxy-6*H*-pyrrolo[3, 4-*g*]quinoxaline analogue **22k** showed EC<sub>50</sub>=270 nM value against HIV-1 in a cell-based assay. The replacement of the pyridazine ring A to

pyridine moiety and the change of the part phthalimide to isoindolinone resulted in significantly increased antiviral activity [53-58]. The synthesis of the tricyclic isoindolinone derivatives was achieved by the Dieckmann condensation of the *N*-(4-fluorobenzyl)

succinimide and pyridine-2, 3-dicarboxylic acid dimethyl ester. Compared to **23a** and **23b** compounds **24a, b** exhibited significantly altered efficacy in the cell-based assay (Figure 20/Table 3).



**Table 3.** Strand transfer (ST) inhibition, anti-HIV proliferation and cytotoxicity assay values for compounds **23a, b** and **24a, b**

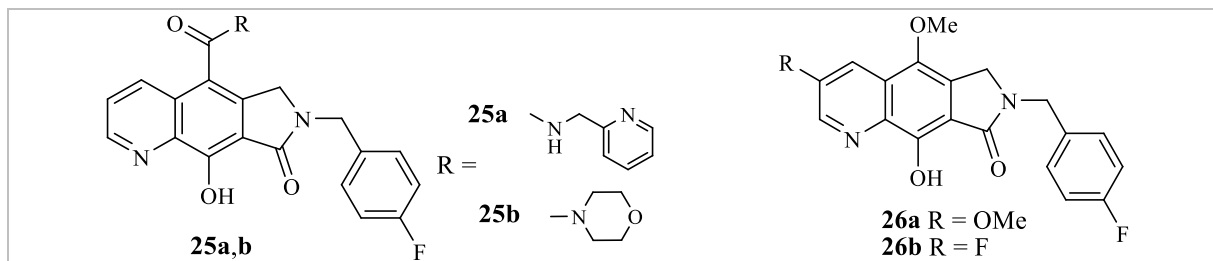
Compound	IC <sub>50</sub> (μM)	EC <sub>50</sub> (μM)	CC <sub>50</sub> (μM)	Solubility <sup>a</sup>
<b>23a</b>	0.08	7.5	19	< 1.4
<b>23b</b>	2.4	1.18	22	2.5
<b>24a</b>	0.05	0.089	5.1	3
<b>24b</b>	0.76	0.003	1.5	nd <sup>b</sup>

<sup>a</sup>Measured by dissolving the solid of testing compound in 20% of MeCN or 20% of EtOH in phosphate buffer (pH 7.3); <sup>b</sup>nd: not determined

The improved potency of **24a** over **23a** could be due to the increased solubility and permeation through the cell membrane. Other modifications of functional groups at C5 position (Figure 21) were also investigated, thus both **25a** and **25b** displayed IC<sub>50</sub> in submicromolar and EC<sub>50</sub> in low nanomolar ranges (IC<sub>50</sub>=0.19 and 0.036 μM; EC<sub>50</sub>=0.0098 and 0.0034 μM). Substitution of the pyridine ring at C3 position with methoxy functional group (**26a**) largely preserved the enzymatic activity (IC<sub>50</sub>=0.093 μM), while improving anti-HIV potency was observed in the cell assay when compared to **24a**. Furthermore insertion

of fluorine atom to the same position (**26b**) resulted in significant enhancement of enzymatic activity (IC<sub>50</sub>=0.0072 μM), but generating significant loss of anti-HIV activity simultaneously [54]. Replacement of 5-methoxy group to dimethyl sulfamate gave the best cell-based antiviral activity (EC<sub>50</sub>=7 nM). Substitution of C6 position of dimethyl sulfamate derivative with small alkyl groups (methyl, methylene, dimethyl and spirocyclopropane) led to also notable antiviral activities, however these changes were associated with the noteworthy decline of ST inhibition (IC<sub>50</sub>=357-815 nM) [55]. Different

and significant results were observed at 5-methoxy-6-alkyl analogues ( $IC_{50}$ =7-210 nM).



**Figure 21.** The structure of compounds **25a** and **25b** 7-(4-fluorobenzyl)-9-hydroxy-8-oxo-7,8-dihydro-6H-pyrrolo[3,4-g]quinolin-5-carboxamides, 7-(4-fluorobenzyl)-9-hydroxy-3,5-dimethoxy-6,7-dihydro-8H-pyrrolo[3,4-g]quinolin-8-one **26a** and 3-fluoro-7-(4-fluorobenzyl)-9-hydroxy-5-methoxy-6,7-dihydro-8H-pyrrolo[3,4-g]quinolin-8-one **26b**.

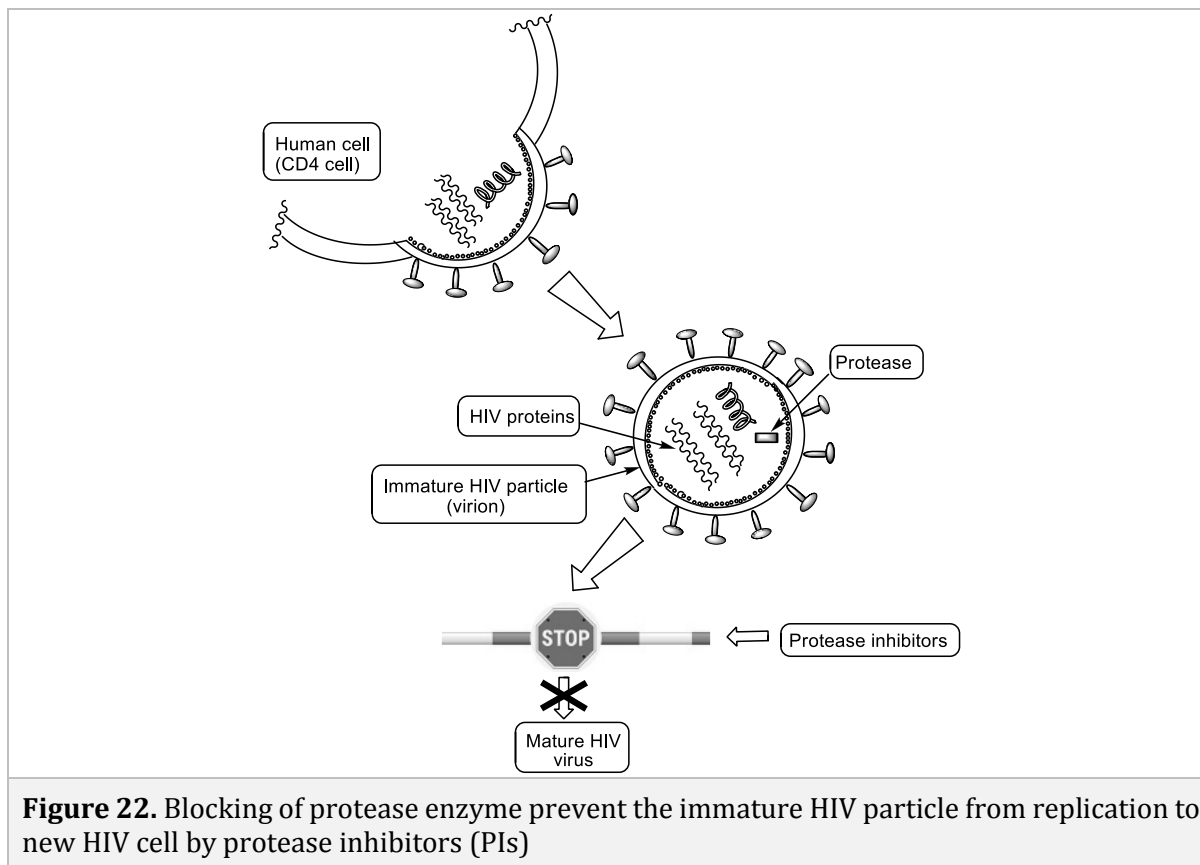
On the basis of previously SAR studies several C5-amine derivatives were prepared and screened for possible HIV integrase inhibitory activity [56]. Especially, the C5 sulfonamide, sulfonylurea and sultam showed remarkable activity. Jin and his research group also examined the effect of various substituents on the *N*-benzyl ring [57] and translocation of the benzyl ring from the N7 to C3 position at the pyridine ring resulting in a lead compound, which showed good oral bioavailability, low *in vivo* clearance and high antiviral activity [58].

#### Protease inhibitors (PIs)

Viral proteases are enzymes encoded by the genetic material (DNA or RNA) of viral pathogens. The function of these enzymes is to catalyze the cleavage of specific peptide bonds in viral polyprotein precursors or in cellular proteins. Proteases can be classified into seven extensive groups (*e.g.* serine, cysteine, aspartic, threonine and glutamic acid). For example the HIV-1 protease is a dimeric enzyme from the

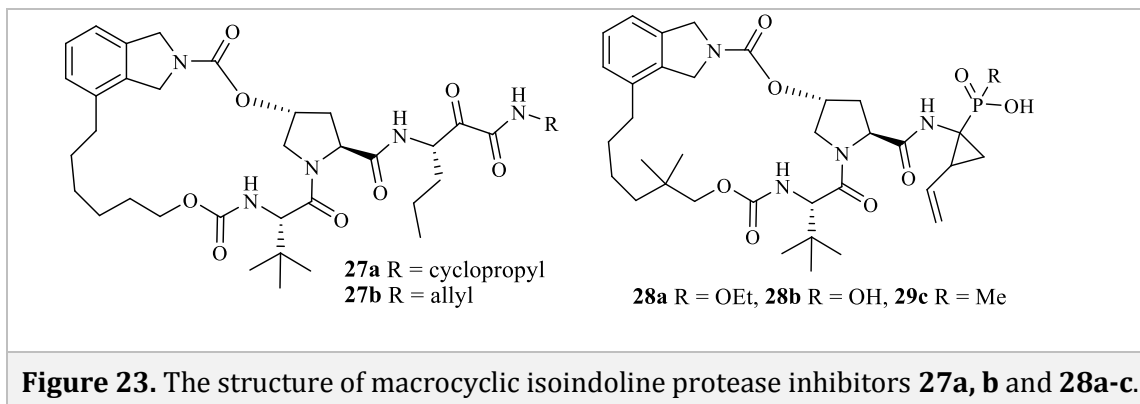
family of aspartic proteases and it cuts up large precursor proteins into smaller protein units. These protein segments combine with genetic material of HIV to form a new HIV virus. This essential enzyme is necessary for the maturation of infectious virions. If HIV protease becomes ineffective, the HIV virions remain uninfected. Another infectious and prevalent virus is the hepatitis C virus (HCV) having a NS2-3 protease enzyme, which is carrying out the proteolytic cleavage between NS2 and NS3 to form part of the HCV virus particle. The NS3/4A is a chymotrypsin-like serine protease that plays an essential role in the HCV viral replication process. Protease inhibitors (PIs) are an important class of antiviral drugs that are mainly used to treat HIV and hepatitis C (HCV). These enzyme inhibitors prevent viral replication by selectively binding to viral proteases and blocking proteolytic cleavage of protein precursors that are necessary for the production of infectious mature HIV-1 particles or other retroviruses (Figure 22).





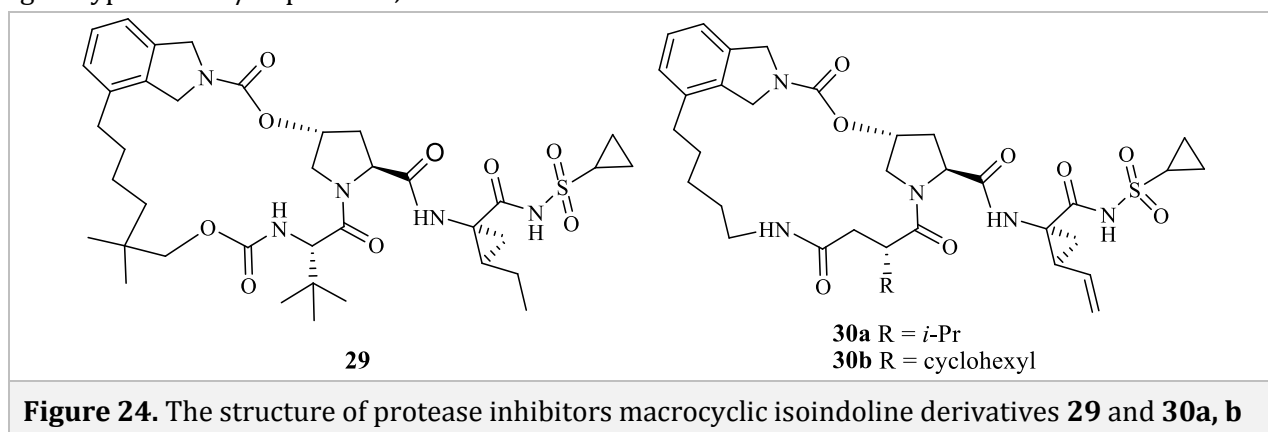
The synthesis and antiviral evaluation of a series of hepatitis C virus (HCV) NS3/4A protease inhibitors bearing a P2-P4 macrocycle and a P1-P1'  $\alpha$ -ketoamide serine trap was reported by Avolio *et al.* [59]. The novel class of protease inhibitors was designed using a molecular-modeling derived strategy, which contains the P2 to P4 macrocyclic constraint. Two compounds **27a** and **27b** (Figure 23) showed improved *in vitro* activity when compared to the clinical candidate lead molecule and in particular, compound **27a** showed a >4-fold increase of the inhibition in the cell based replicon assay. Inhibition of the full-length HCV NS3/4A protease measured by the inhibition constants, which values at compounds **27a** and **27b** were the best with  $K_i=57$  nM and  $K_i=71$  nM respectively. Inhibition

of HCV replication was determined in Huh-7 cell expressing a stably-replicating subgenomic HCV RNA at 37 °C incubating for 96 hours. When these compounds showed good activity ( $EC_{50}=380$  and 550 nM) thus compounds **27a** and **27b** were selected for further evaluation of their properties *in vivo* and they were dosed orally to rats at 5 mg/kg and results compared with reported data for other efficient compounds (VX-950 and SCH 503034). The introduction of an acidic phosphorous moiety to the dioxo side chain in compounds **28a** and **28b** (Figure 23) provided a dramatic increase in activity ( $K_i=0.016$  and 0.070  $\mu$ M, respectively) [60]. The methyl-phosphinate analogues **28c** were very potent in the enzyme inhibition assay ( $K_i=0.0024$   $\mu$ M) with more than 1000-fold increase in activity compared to analogue **28a**.



Thorough investigations of a series of P2-P4 macrocycles containing a hydroxy-proline carbamates led to the identification of 3-linked isindoline as a promising P2 substituent for a new class of HCVNS3/4a protease inhibitors. Optimization of this series, including exploration of linker length and P3 amino acid side chains, showed that a number of different compounds maintained good activity versus the genotype 1b enzyme, but also revealed that rat liver exposure was modest. Further optimization of the fully reduced compounds with regard to linker length and substitution was performed (Figure 24) in an effort to maintain rat liver exposure and achieve balanced activity against both the 1b and 2a genotypes of NS3/4a protease, and these efforts

led to the identification of macrocyclic carbamate **29** (later vaniprevir or MK-7009) as a clinical candidate molecule. Inhibition/binding affinity constants values were excellent ( $K_i=0.05$  nM and 0.9 nM at the 1b and 2a genotypes) and cell based replicon assays  $IC_{50} \sim 5.0-27$  nM were determined in the presence of 10% fetal bovine serum (FBS) and 50% normal human serum (NHS). Compound **29** showed good to excellent liver exposure across species and poor plasma exposure in rats and rhesus following a 5 mg/kg oral dose [61]. Pompei *et al.* exchanged the P3-carbamate moiety for the cyclic amide to give novel macrocyclic isindoline analogues of compound **29** [62].

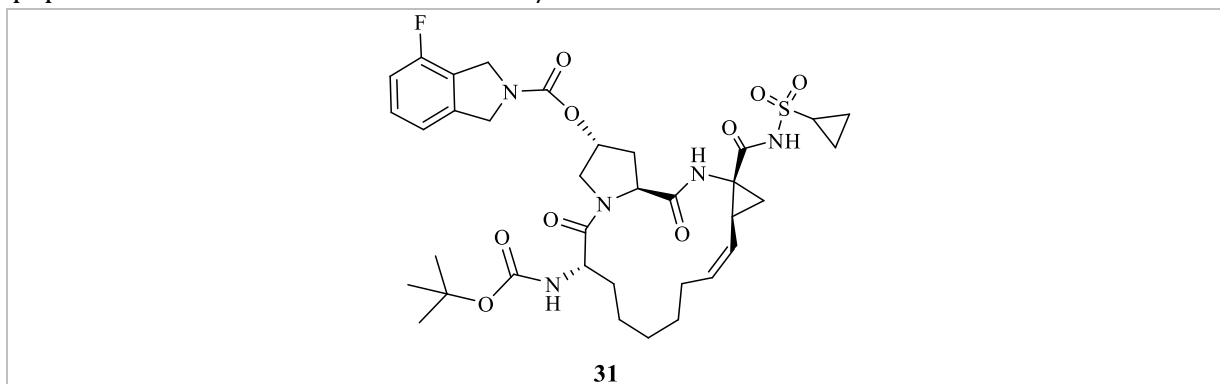


This replacement of the P3-carbamate with the succinamide backbone produced analogues with intrinsic potency in the low nanomolar

concentration range, thus the inhibition constant of compound **30a** (Figure 24) was  $K_i=1.4$  nM with a weak replicon potency

(EC<sub>50</sub>=100 nM). The presence of the cyclohexyl substituent as in **30b** resulted in a slight improvement of the observed inhibition (K<sub>i</sub>=0.76 nM) but significant efficiency was detected in the inhibition of HCV replication (EC<sub>50</sub>=12 nM). Several similar macrocyclic peptidomimetic molecules bearing both a lipophilic P2 isoindoline carbamate and P1/P1'

acylsulfonamide bioisostere were designed and prepared against the NS3 serine protease and compound **31** was selected (Figure 25) as the clinical development candidate for its favorable potency (with IC<sub>50</sub>=0.2-3.5 nM inhibition effect for HCV genotypes) and good *in vitro* ADME profiles.



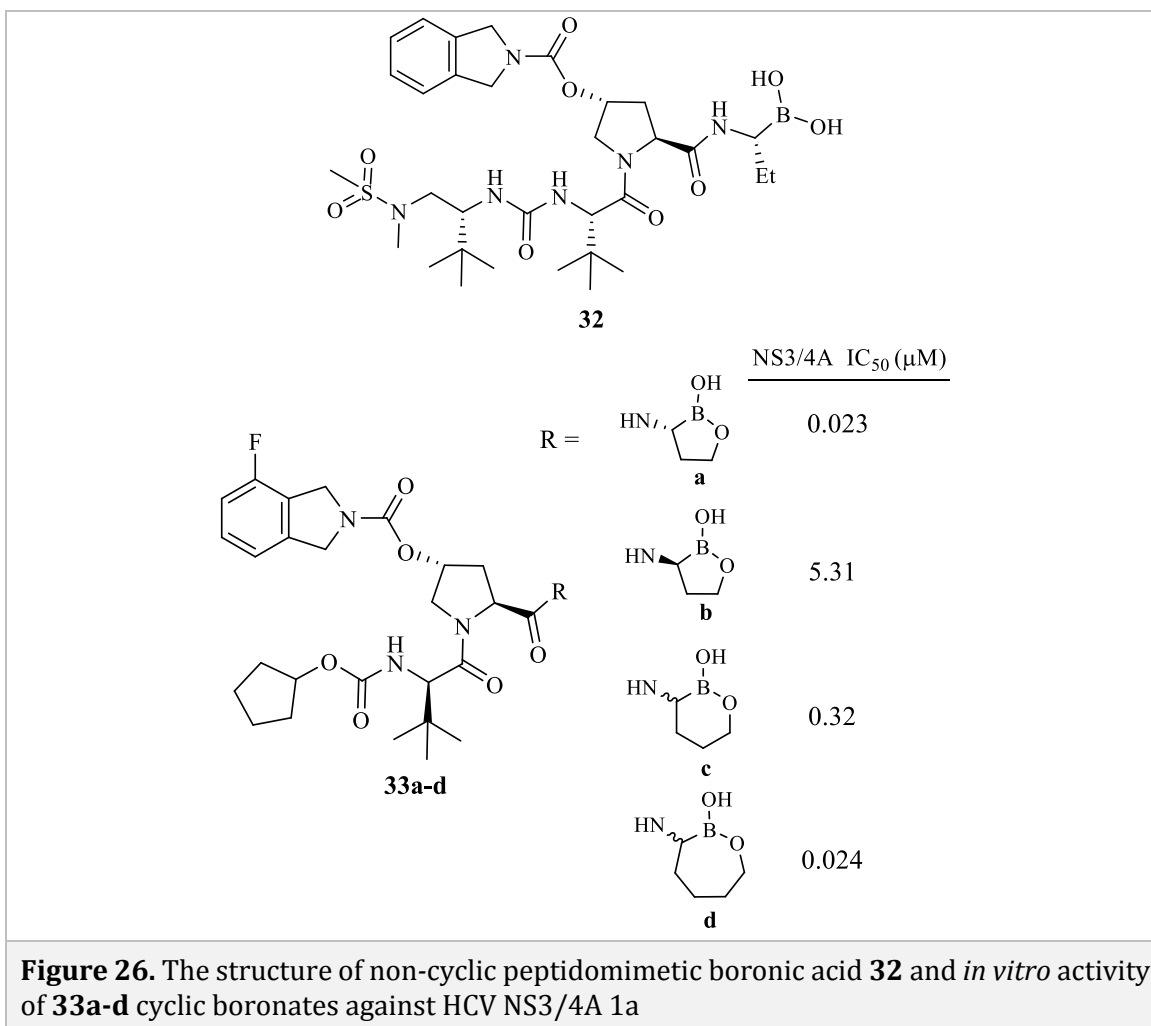
**Figure 25.** The structure of protease inhibitors macrocyclic isoindoline derivative (1*S*, 4*R*, 6*S*, 7*Z*, 14*S*, 18*R*)-14-[[*tert*-butoxy]carbonyl]amino}-4-[[cyclopropanesulfonyl]carbamoyl]-2, 15-dioxo-3, 16-diaza-tricyclo[14.3.0.0<sup>4,6</sup>]nonadec-7-en-18-yl]-4-fluoro-2, 3-dihydro-1*H*-isoindole-2-carboxylate (**31**).

This compound yielded replicon EC<sub>50</sub> values of approximately 2 nM and exhibited stability *in vitro* in rat, dog and human hepatocyte incubation assays, moreover the macrocyclic isoindoline **31** did not display toxicity in rats at 30 mg/kg twice a day for 7 days [63-66]. The usual (INN) name of compound **31** is danoprevir (code name: ITMN-191 or RG7227). Jiang and his coworkers reported a detailed and comprehensive structure-based design, SAR investigation using drug metabolism and pharmacokinetics (DMPK) parameters optimization [64]. These results led to the discovery of the novel HCV NS3/4A protease inhibitor danoprevir (ITMN-191/R7227), which is a macrocyclic noncovalent reversible NS3 inhibitor with a slow-off rate. Rajagopalan *et al.* investigated a two-step binding mechanism of the danoprevir (ITMN-191) **31**, in which an initial complex is rapidly formed

and slowly converts to a more stable form [65]. Kinetic characterization of NS3 protease inhibition by ITMN-191 showed slow and tight binding with an extremely slow dissociation that is unique among macrocyclic NS3 protease inhibitors. Previously, the preclinical characteristics of ITMN-19 as potent NS3/4A protease inhibitor of HCV was described [66]. Under preequilibrium conditions, 0.29 nM ITMN-191 half-maximally inhibited the reference NS3/4A protease, but a 35,000-fold-higher concentration did not appreciably inhibit a panel of 79 proteases, ion channels, transporters, and cell surface receptors. On the basis of the favorable preclinical characteristics observed, the clinical investigation of ITMN-191 for the treatment of chronic hepatitis C was supported. Numerous non-cyclic peptidomimetic boronic acid small molecules were synthesized and evaluated as a new series

of HCV NS3/4A inhibitors, these compounds also bear an isoindoline ring [67]. Compound **32** (Figure 26) bearing an *N*-methyl sulfonamide substituted urea group displayed the best replicon activity of this series ( $EC_{50}$ =25 nM,  $EC_{90}$ =117 nM) and it was remarkable in that it

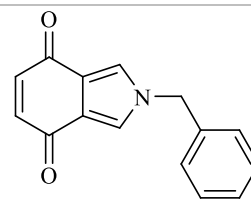
displayed subnanomolar potencies for each of the genotypes tested. The accepted mechanism of protease inhibition by boronic acids is the formation of a tetracoordinate boronate complex between the boronic acid and the active site serine hydroxyl group.



Li *et al.* [68] prepared several  $\alpha$ -amino cyclic boronates and these moieties were integrated favorably into the acyclic part. These compounds are inhibitors of the HCV NS3 serine protease, and structural studies show that they inhibit the NS3 protease by trapping the Ser-139 hydroxyl group in the active site. In general, isoindoline derivatives exhibited better inhibitory activities than isoquinoline series,

suggesting that the isoindoline moiety at the P2\* site contribute more significantly on inhibitor binding. The (*R*)- $\alpha$ -amino oxaborole inhibitor (**33a**) was significantly more potent than the corresponding (*S*)-isomer (**33b**), which validated the specificity of these inhibitors for HCV NS3 protease (Figure 26). The five-membered oxaboroles were replaced with racemic six- and seven-membered cyclic

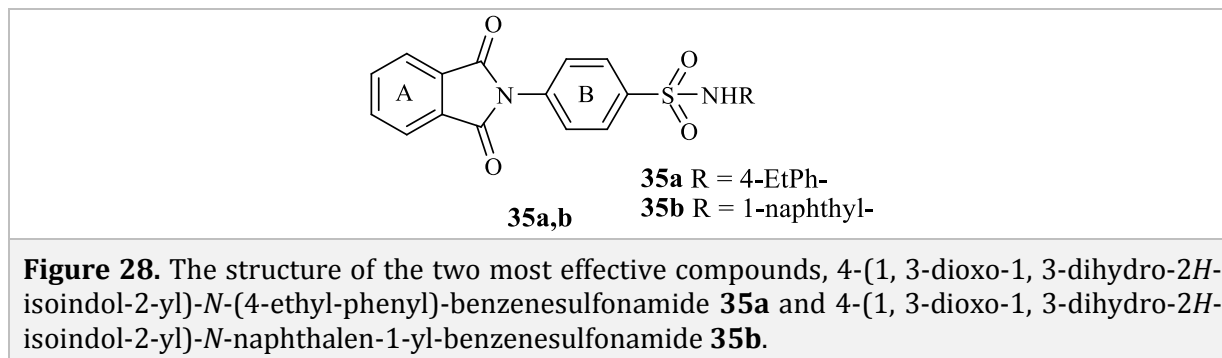
boronates (**33c, d**) to further probe the influence of ring size. Interestingly, the seven-membered boronate inhibitors (**33d**) showed comparable activities as the five-membered boronates (**33a**). Some positive-sense RNA viruses can be classified into the picornavirus-like supercluster, which includes picornaviruses (PV), caliciviruses, and coronaviruses (CoV). These viruses possess 3C or 3C-like proteases, which contain a typical chymotrypsin-like fold and a catalytic triad with a Cys residue as a nucleophile; may serve as useful targets for the design of antiviral drug development [69]. Earlier a novel human coronavirus (CoV) caused severe acute respiratory syndrome (SARS), spread from China to 29 countries in 2003 infecting and killing numerous people. SARS-CoV contains a 3C-like protease, which is analogous to the 3C protease of picornaviruses (PV). Kuo *et al.* identified several novel inhibitors of SARS-CoV 3C-like protease with low  $IC_{50}$  values using high throughput screening against ~6800 small molecules [70]. The five hits that inhibited SARS-CoV, 3CL proteases at 10  $\mu$ M were found and were also evaluated against further members of human coronavirus (CoV) proteases. Among others the isoindole-4, 7-dione **34** (Figure 27) showed smaller  $IC_{50}$  value than 10  $\mu$ M inhibitory activity (7.0  $\mu$ M). Computer modelling study of **34** binding to the proteases was achieved and it was found that molecule is rigid and possesses a planar structure, these two rigid aromatic moieties connect by a small linker in this wise. Based on the computer modelling, each of these aromatic moieties is bound to S1 or S2 site of SARS protease by forming H-bonds and hydrophobic interactions. The Glu166, Gly143 and Cys145 side chains of SARS 3CL protease form H-bonds with this inhibitor.

**34**

**Figure 27.** The structure of 2-benzyl-2H-isoindole-4, 7-dione **34**.

Dengue viruses (DENVs) are single-stranded, positive-sense RNA viruses, which are one of the oldest invasive viral infections. There are four serotypes in dengue virus, DENV1–DENV4 and Timiri *et al.* focused on their study of the inhibition an Asian strain of the dengue virus type 2 (DENV-2) [71]. They found that the NS2B–NS3 protease an ideal target for drug design against dengue infection. Dengue virus 2 (DENV2) protease inhibitors were developed by the synthesis and molecular modeling studies of novel 4-isoindolyl benzenesulphonamide derivatives (Figure 28). After a high throughput virtual screening (HTVS) protocol twenty compounds were tested for their inhibitory activity against DENV2 NS2B–NS3 protease. Two compounds were found to be active (**35a** and **35b**) at the concentration of 100  $\mu$ M and all the other compounds were found inactive. Activity values at serial dilutions  $IC_{50}$ =48.2  $\mu$ M (**35a**) and 121.9  $\mu$ M (**35b**) were observed respectively. Molecular docking of these compounds resulted in several interactions, such as  $\pi$  electron cloud of benzene ring of phthalimide (ring A) interacting with  $\pi$  electron cloud of Tyr200 by  $\pi$ – $\pi$  stacking interaction. Oxygen atom present in carbonyl group of ring A, interact with nitrogen atom present in imidazole group of His90 to have H-bond with a distance of 3.05 Å and 2.95 Å for the compounds. The 4-ethylphenyl and naphthyl groups have hydrophobic interactions with residues Trp89 and Val111. Finally, oxygen atom of sulphonamide group, which is at 2.75 Å

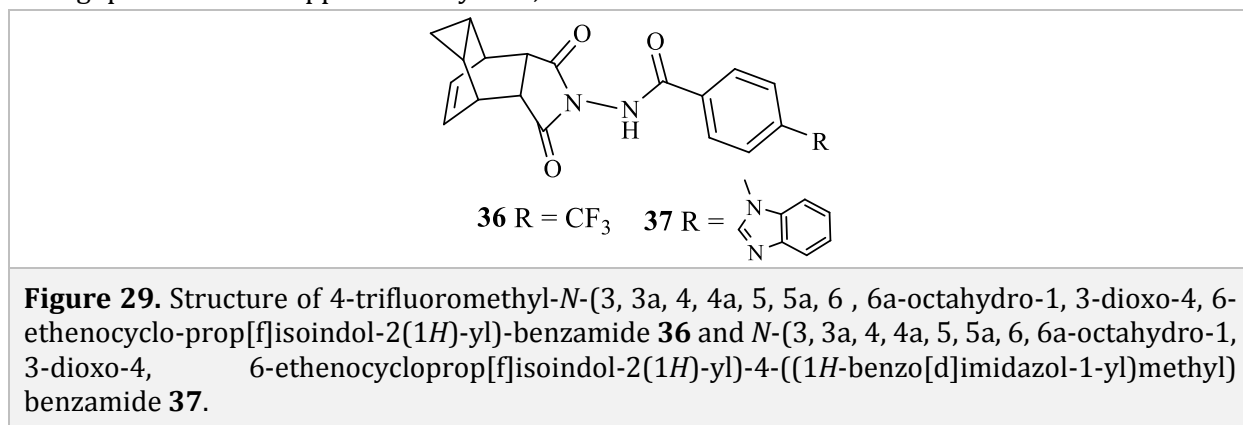
distance to -NH group of side chain of residue Arg93 interacts through H-bond.



#### Other antiviral agents with miscellaneous mechanisms

The genus orthopoxvirus causes the species variola (smallpox) virus and some zoonotic species such as monkeypox virus, cowpox virus, vaccinia virus and camelpox virus also can infect humans. When the viral proteins entry into the host cell, the viral proteins attach to host glycosaminoglycans (GAGs). Then the viral envelope merges with the plasma membrane and the viral core releases into the host cytoplasm. A series of tricyclononene carboxamides were found as hits in a high-throughput screen of approximately 356, 000

compounds in a chemically diverse library and it was tested against vaccinia and cowpox viruses *in vitro* and found to be potently active and specific for orthopoxviruses [72-74]. Prominent and significant virus-induced cytopathic effects (CPE) were observed for ST-246 (tecovirimat) (**36**), which is a small molecule with a bicyclic saturated phthalimide framework (Figure 29), that specifically inhibits formation of extracellular forms of virus ( $EC_{50}=0.04 \mu\text{M}$  and  $0.6 \mu\text{M}$  against vaccinia and cowpox viruses respectively). Bailey *et al.* studies SAR of tricyclononene carboxamides by variation of substituents on aroyl moiety [72].

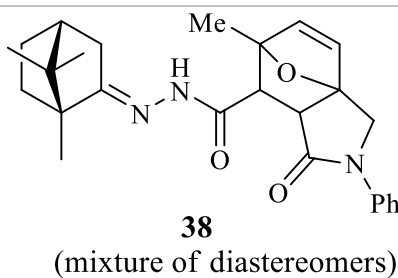


Antipoxvirus compound ST-246 (**36**) inhibits the production of extracellular forms of virus and protects mice against a lethal challenge of orthopoxvirus. The antiviral

mechanism of this compound is to prevent the formation of egress-competent forms of orthopoxviruses and presumably, to interfere and block the wrapping of infectious

orthopoxvirus virions for which F13L (peripheral membrane protein) and a type of I glycoprotein (B5R) are required. The compound **36** was inactive against unrelated DNA- and RNA-containing viruses, demonstrating specificity for the inhibition of orthopoxvirus replication. Some modifications of the chemical structure of anti-orthopoxvirus compound ST-246 (**36**) led to a novel series of tricyclononene carboxamide derivatives, which were tested for anti-HIV-1 activity and cytotoxicity [75]. The benzoimidazol-substituted compound **37** (Figure 29) proved to be highly effective in inhibiting HIV-1 R5 infection with an  $IC_{50}=0.41 \mu M$  value in addition the lowest cytotoxicity ( $CC_{50}=119.6 \mu M$ ). The selectivity index was 292, but it exhibited no significant inhibitory activity on HIV-1 reverse transcriptase, integrase and protease enzymes. To correlate the structure of title compound with the inhibitory activity on HIV-1 replication, a 3D QSAR analysis was performed by the use of a CoMFA module following the standard procedure. In contrast, compounds that have

other substituted amines at *para* position, which contain small hydrophilic or hydrophobic groups, were much less active than compound **37**. Kovaleva *et al.* reported the design and synthesis of a series of novel D-(+)-camphor *N*-acylhydrazones exhibiting inhibitory activity against vaccinia and influenza H1N1 viruses [76]. Compound **38** (Figure 30) containing in its structure a monoterpene (1, 7, 7-trimethylbicyclo[2.2.1]heptane) moiety, *N*-acylhydrazone linker and aromatic substituent, demonstrated low toxicity and the best activity against vaccinia virus ( $IC_{50}=6.2 \mu M$ ). This hexahydro-3a, 6-epoxyisoindole-7-carbohydrazide derivative (**38**) showed moderate activity ( $IC_{50}=22 \mu M$ ) against influenza virus. The biological data presented that some agents were active against vaccinia virus but were practically not or slightly active against the influenza (H1N1) virus, because the structures of surface viral proteins in influenza and vaccinia viruses differ significantly.



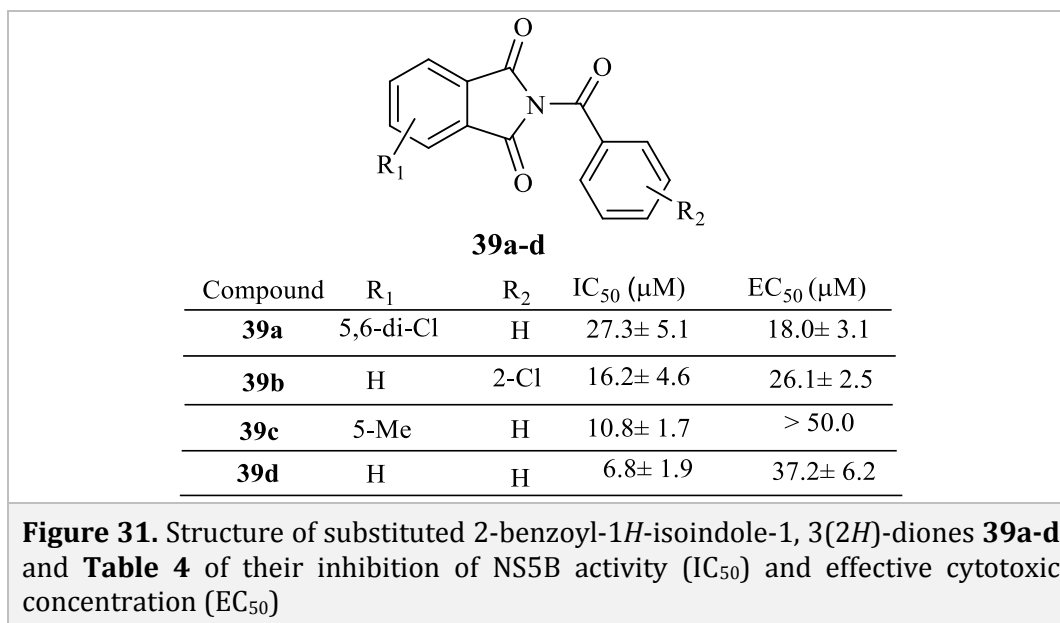
**Figure 30.** Structure of 6-methyl-1-oxo-2-phenyl-*N'*-[(1*R*, 2*E*, 4*R*)-1, 7, 7-trimethylbicyclo[2.2.1]hept-2-ylidene]-1, 2, 3, 6, 7, 7a-hexahydro-3a, 6-epoxyisoindole-7-carbohydrazide **38**.

Hepatitis C virus (HCV) (Figure 32) is an enveloped linear single stranded RNA virus in the hepacivirus genus of the flaviviridae family. The RNA-dependent RNA polymerase of HCV, NS5B, plays a crucial role in viral replication and it has no counterpart in mammalian cells, making it a common target. Integration of 2-*N*-

hydroxyl- and 2-*N*-benzoyl-1, 3-diketo acid moieties into isoindole-1, 3-dione led to the development of a new series of hepatitis C virus NS5B polymerase inhibitors [77]. Compounds **39a** and **39b** (Figure 31) are moderate NS5B inhibitors, but they displayed selective cytotoxic activity against HCV replicon-containing Ava5 cells. The Ava5 cells contain the

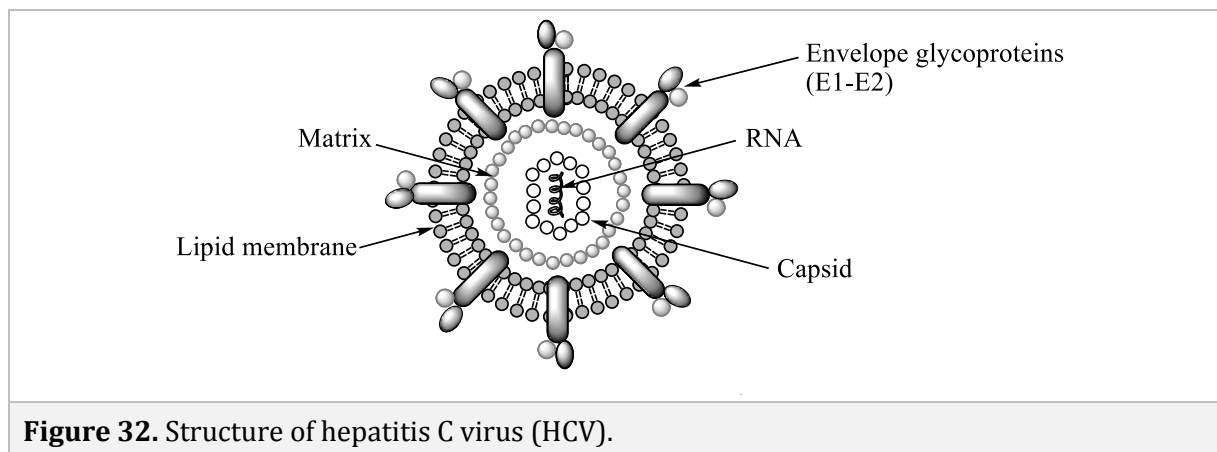
replicon for the most prevalent HCV genotype, 1b. Binding profiles of **39a** strongly suggest that this series of inhibitors target the active site of NS5B. The cytotoxic activity of **39a** may also be attributable to enhanced lipophilicity of the dichloro group. Although the 5-methyl substituted **39c** was active against NS5B

( $IC_{50}=10.8 \mu\text{M}$ ), it was not toxic to either of the cell lines. Structural optimization to translate enzyme inhibitory activity to cellular cytotoxicity yielded compound **39a**, a moderate enzyme inhibitor ( $IC_{50}=27.3 \mu\text{M}$ ) with selective toxicity to hepatitis C virus 1b replicon-containing Ava5 cells ( $EC_{50}=18.0 \mu\text{M}$ ).



Isoindoline-1, 3-dione **39d**, which was the most potent NS5B inhibitor in the *in vitro* inhibition assay, exhibited an excellent concentration-dependent binding profile, with a  $K_d$  value of  $0.85 \mu\text{M}$  and this compound

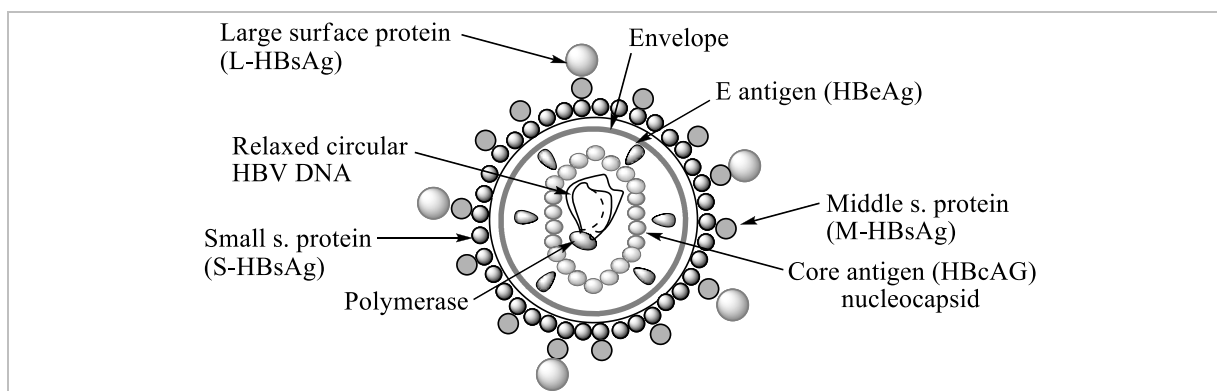
showed stronger interaction for NS5B than the other compounds. **39d** demonstrated moderate cytotoxicity in the HCV replicon-containing Ava 5 cells ( $EC_{50}=37.2 \mu\text{M}$ ), but was not toxic to the parent Huh7 cells.





Hepatitis B virus (HBV) is a partially double-stranded DNA virus, which causes a serious liver infection. It is spread when people come in contact with the blood, open sores or other body fluids of an infected person. Hepatitis B virus is a member of the Hepadnavirus family. The virus particle, called Dane particle (a spherical particle that is the virion), consists of an external lipid envelope and an icosahedral nucleocapsid core composed of protein (Figure

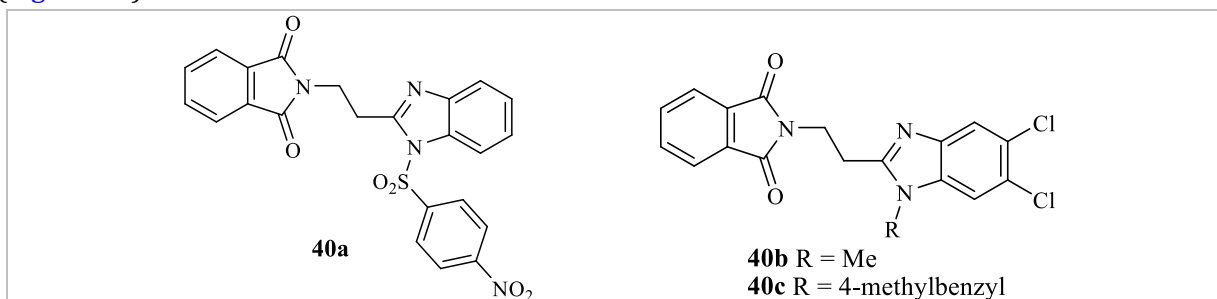
33). The viral DNA and a DNA polymerase are enclosed by a nucleocapsid, that has reverse transcriptase activity. The external envelope of the HBV contains inserted proteins and the external position conforms to a receptor binding action at viral entry into recipient cells. The hepatitis B virus has three envelope proteins, large, middle and small surface proteins, which are necessary for the formation of Dane particles.



**Figure 33.** Structure of hepatitis B virus (HBV).

Several compounds were screened [78] for anti-HBV activity, benzenesulfonyl-benzimidazolyl-ethyl-isoindole-1, 3-dione **40a** (Figure 34) was found as a moderate inhibitor

of HBV, with an  $IC_{50}$  of  $14.2 \mu M$  in inhibiting HBV DNA replication and low cytotoxicity ( $CC_{50}=200 \mu M$ ) *in vitro*.



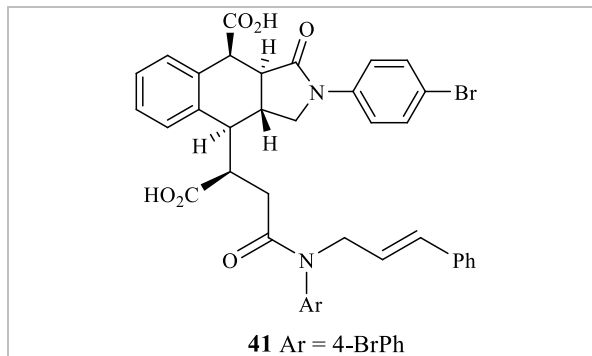
**Figure 34.** Structure of 2-(2-{1-[(4-nitrophenyl)sulfonyl]-1H-benzimidazol-2-yl}ethyl)-1H-isoindole-1, 3(2H)-dione **40a**, 2-[2-(5, 6-dichloro-1-methyl-1H-benzimidazol-2-yl)ethyl]-1H-isoindole-1, 3(2H)-dione **40b** and 2-[2-(5, 6-dichloro-1-(4-methylbenzyl)-1H-benzimidazol-2-yl)ethyl]-1H-isoindole-1, 3(2H)-dione **40c**.

An important advancement was made when the N-1 position of the benzimidazole core was substituted with methyl or 4-methylbenzyl. The

most promising compounds were **40b** and **40c**, with similar high antiviral potency ( $IC_{50}=0.9$  and  $0.7 \mu M$ , respectively) and remarkable

selectivity indices (>1111 and >714, respectively). Replacement of the phthalimide group of **40b** with different types of functional groups led to significant decreases in antiviral potency and SI relative to those of **40b**. In particular, replacing the phthalimide group with phenyl, 2-furanyl, or 2-thiophenyl resulted in a complete loss of inhibitory activity. Small change was observed in the antiviral potency when the phthalimide ring reduced to the corresponding isoindolin-1-one ( $IC_{50}=3.4 \mu M$ ), whereas the latter compound had a small selectivity index ( $SI \sim 7$ ). It suggested that the carbonyl groups of the phthalimide derivatives are important features in conferring relatively low cytotoxicity. Finally, replacement of the phthalimide group with maleimide resulted in a 4-fold improvement in antiviral potency and significantly greater cytotoxicity.

Influenza A viruses have two subtypes according to proteins which are on the surface of the virus: the hemagglutinin (H) and the neuraminidase (N). Influenza (H1N1) virus is the subtype of influenza A virus, it can cause common human influenza (flu). It is worth noting that this virus is resistant to first-generation adamantane-based antiinfluenza drugs, amantadine and rimantadine. Voronov *et al.* prepared *N*-arylbenzo [*f*] isoindole-4-carboxylic acids by intramolecular Diels-Alder reaction of the vinylarenes [79]. The synthesized compounds were tested for cytotoxicity and antiviral activity using the MTT test and virus yield reduction assay, respectively. Generally, the antiviral activity of the prepared compounds was slight, with low selectivity indexes. Surprisingly, compound **41** (Figure 35) showed low toxicity and the highest antiviral activity ( $IC_{50}=17 \mu M$ ) with high selectivity ( $SI=33$ ).

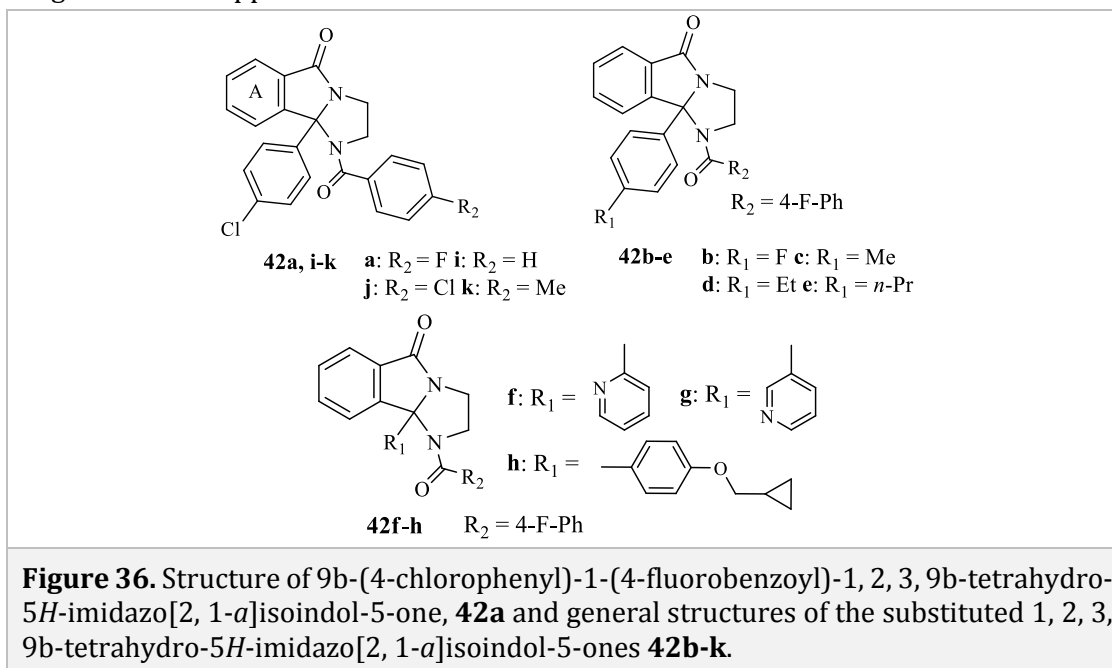


**Figure 35.** Structure of 2-(4-bromophenyl)-3-oxo-2,3,3a,4,9,9a-hexahydro-1H-benzo [*f*]isoindole-4-carboxylic acid derivative **41**.

Human respiratory syncytial virus (HRSV) that is human orthopneumovirus, causes respiratory tract infections in infants and young children. The virus can also infect adults and older but RSV symptoms are mild and similar to the common cold. The agent is an enveloped RNA virus with a non-segmented single-stranded negative-sense genome. The viral genome encodes 8 structural and 2 non-structural proteins. Important structural proteins include the fusion (F) protein and the attachment (G) protein, which are essential for viral penetration and attachment to the host cells. Previously, Bond *et al.* patented an invention related to discovery of certain polycyclic isoindolinones, which exhibited favourable anti-RSV activity [80]. The inventors described the synthesis, spectral data, pharmacological evaluations and use of various drug formulations of several substituted imidazo [2, 1-*a*] isoindol-5-one derivatives in detail. As part of an extensive screen program, a diverse library of compounds was screened for activity against RSV using a cytopathic effect (CPE) assay in Hep2 cells. From this screening process, 9b-(4-chlorophenyl)-1-(4-fluorobenzoyl)-1,2,3,9b-tetrahydro-5H-imidazo[2, 1-*a*]iso-indol-5-one, **42a** (Figure 36) was identified as an RSV inhibitor, displaying

very promising activity against both A and B strains [81]. The antiviral activity of compound **42a** was susceptible to the known mutation D489Y in the F protein of the RSV-A Long strain. This imidazo[2, 1-*a*]isoindol-5-one derivative **42a** acts early in the virus replication cycle providing additional support that functions as a

fusion inhibitor. A program was undertaken to investigate the SAR and improve the drug-like properties of compound **42a**. At first focussing on the variation of substituents at positions 9bC ( $R_1$ ) and 1N ( $R_2$ ) or change of the fused aromatic ring (ring A).

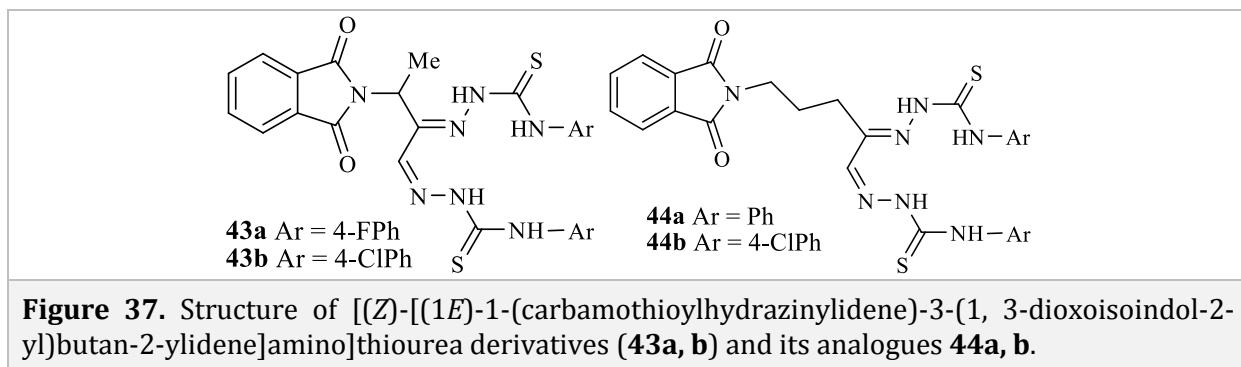


Attempts to replace the chloro substituent on the phenyl ring with a small range of different functional groups (compounds **42b-e**) generally resulted in compounds with similar or slightly reduced potency. Replacement of the phenyl group with 2- or 3-pyridine (compounds **42f** and **42g**) gave compounds with poor or no activity. Relatively, all compounds showed a low cytotoxicity ( $CC_{50} > 17-20 \mu M$ ). The introduction of a cyclopropyl-methoxy group (**42h**) gave a three-fold improvement in activity (RSV inhibitory activity:  $EC_{50} = 0.079-0.11 \mu M$ ) compared to the original hit. Additionally, the presence of the  $R_2$  substituent and carbonyl group at 1N position was found to be critical for activity against RSV, with the tetrahydroimidazole core proved to be inactive. Removal of the fluoro substituent from

compound **42a** resulted in a small loss of activity (**42i**:  $EC_{50} = 0.45-0.54 \mu M$ ), while replacing it with a chloro (**42j**) or methyl (**42k**) group resulted in a greater than 10-fold decrease in potency. A series of new imidazo[2, 1-*a*]isoindol-5-one derivatives as RSV inhibitors was studied by Liu *et al.* [82] using *in silico* methodologies 3D-QSAR (CoMFA and CoMSIA) to find the best efficient compounds. In the CoMSIA contour maps it appears that presence of the wide hydrogen bond acceptor groups at the  $R_2$  (1N) position is important to influence the antiviral activity. Furthermore, the former results verified, that minor and hydrophobic groups (*e.g.* phenyl or ether bond at *para* position) at  $R_1$  (9bC) position may increase the inhibitory activity. Yang *et al.* prepared several bis(thiosemicarbazone)

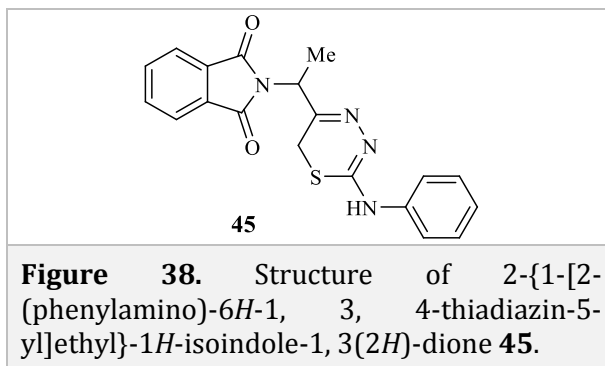
derivatives (phthiobuzone analogues) from phthalimide or phthalic anhydride, then compounds were evaluated for their anti-herpes virus activities [83]. The results were compared with anti-HVS activity of phthiobuzone (TDA) and acyclovir (ACV). Among the candidate molecules, compounds **43a** and **43b**, containing the substituted 4-halogenated phenyl ring at *N*-4', 4'' position (Figure 37), showed increasing antiviral activity compared with TDA against herpes simplex virus 1 ( $IC_{50}$ =8.56 and 2.85  $\mu$ g/mL, respectively) and herpes simplex virus 2 ( $IC_{50}$ =1.75 and 4.11  $\mu$ g/mL). It is noticeable that presence of a halogen atom at the 4-position on

the phenyl ring (**5i-k**) resulted in remarkable anti-HSV-1 activity. The effect of the chain between the phthalimide and bithiosemicarbazone moieties was studied, two alkyl linkers were introduced to produce novel TDA derivatives. The propylene analogues (**44a** and **44b**) were 3-4 fold more potent than the corresponding methylene analogues. Compounds **44a** and **44b** with a propylene linker between the phthalimide and bithiosemicarbazone moieties display similar antiviral potency against herpes simplex virus 1 ( $IC_{50}$ =2.85 and 4.11  $\mu$ g/mL, respectively), moreover **44b** exhibited a high selectivity index (SI=12.9).



The mechanism of the antiviral action of TDA is unique, which might be related to the ribonucleotide reductase and the action is also different from other antiviral nucleotide analogues. Presumably, these new TDA analogues also have a similar antiviral mechanism. A series of novel 1, 3, 4-thiadiazine derivatives were synthesized starting from phthalic anhydride *via* chemical optimization by modification on phthiobuzone [84]. Their anti-herpes simplex virus (HSV) activities *in vitro* were also tested. The most potent anti-HSV compound was **45** (Figure 38), which showed considerable inhibition against HSV-1 ( $IC_{50}$ =77.04  $\mu$ g/mL) and HSV-2 ( $IC_{50}$ =30.00  $\mu$ g/mL) and it had low cytotoxicity ( $CC_{50}$ =1000.00  $\mu$ g/mL) with a high selectivity

index ( $SI=CC_{50}/EC_{50}$ ) against two types of herpes simplex virus.



## Conclusion

In this review we focused on the potent antiviral isoindole derivatives, which might be

efficient and successful against the most prevalent and common human viruses such as HIV, herpes viruses, the hepatitis B and C viruses, influenza A and B viruses. Several promising results were reported where use of connection linkage of isoindole moiety with different substituents; fusion or hybridization of the isoindole ring with various other heterocyclic rings led to effective antiviral molecules. Researchers need to extend the range of antiviral agents to other families of pathogens using rational drug design strategies for developing antivirals to attack viruses at every stage of their life cycles. Usually viruses have one or more special targets (*e.g.* envelope glycoproteins, enzymes), where the isoindole derivatives can attack by inhibition mainly. The isoindole or isoindoline moiety has useful lipophilic and considerable aromatic character, which is a stable and bulky carrier framework of bioactive side chains, chemical groups and substituents. In most of the cases a small modification (*e.g.* halogenation, alkylation) of the molecule lead to unexpected change in the antiviral activity. After comprehensive QSARs studies and the *in vitro* or *in vivo* evaluation of numerous compounds only few molecules were selected as lead compounds or candidate molecule for the additional developments of novel potent and safe antiviral drugs with fewer side effects.

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### Conflict of interest

We have no conflicts of interest to disclose.

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