Antiviral Sulfonamide Derivatives

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Abstract: The sulfonamides constitute an important class of drugs, with several types of pharmacological agents possessing antibacterial, anti-carbonic anhydrase, diuretic, hypoglycemic, antithyroid and anticancer activity among others. A large number of structurally novel sulfonamide derivatives have ultimately been reported to show substantial antiviral activity *in vitro* and *in vivo*. The review summarizes recent classes of sulfonamides and related sulfonyl derivatives disclosed as effective such agents. Thus, at least some HIV protease inhibitors used clinically (amprenavir) or compounds in advanced clinical trials (tipranavir, TMC-126, TMC-114, etc.) possess sulfonamide moieties in their molecules, whereas a very large number of other derivatives are constantly being synthesized and evaluated in order to obtain compounds with less toxicity or activity against drug-resistant viruses. Several non nucleoside HIV reverse transcriptase or HIV integrase inhibitors containing sulfonamido groups were also reported. Another approach to inhibit the growth of retroviruses, including HIV, targets the ejection of zinc ions from critical zinc finger viral proteins, which has as a consequence the inhibition of viral replication in the absence of mutations leading to drug resistance phenotypes. Most compounds with antiviral activity possessing this mechanism of action incorporate in their molecules primary sulfonamide groups. Finally, some small molecule chemokine antagonists acting as HIV entry inhibitors also possess sulfonamide functionalities in their scaffold.

1. INTRODUCTION

Viral infections represent a major medical problem worldwide. Human immunodeficiency virus (HIV) infection affects more than 40 million people, and although much progress has been registered in the treatment of this infection by the introduction of highly active antiretroviral therapy, HAART (a combination of nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and/or aspartic protease inhibitors (PIs) [1-4]) the massive viral replication (with more than 10^9 virions produced daily) and the high error rate of the reverse transcriptase, leads to the emergence of drug resistant strains and the stringent need of new therapeutic approaches. Ultimately several such new approaches have been explored, many of which will be dealt with here [1-4].

In this paper we shall review such antiviral targets ultimately emerged, pointing out several original approaches proposed for developing new types of drugs. The common denominator of all the classes of pharmacological agents dealt with here is the presence of sulfonamide moieties in their molecules. Indeed, the sulfonamides constitute an important class of drugs, with several types of pharmacological agents possessing antibacterial [5], antitumor [6], anti-carbonic anhydrase [7,8], diuretic [7,9], hypoglycemic [10], antithyroid [11], or protease inhibitory activity [12-15] among others. The very simple sulfanilamide **1** lead molecule afforded the development of all these types of pharmacological agents with such a wide variety of biological actions, as exemplified below for the antibacterial agent sulfathiazole **2** [5], the carbonic anhydrase inhibitor acetazolamide **3** (clinically used for over 45 years) [7,8], the widely used diuretic furosemide **4** [9], the hypoglycemic agent glibenclamide **5** [16], the anticancer sulfonamide indisulam **6** (in advanced clinical trials) [6], the aspartic HIV protease inhibitor amprenavir **7** used for the treatment of AIDS and HIV infection [12] or the metalloprotease inhibitors of type **8** [13-15] among others (Fig. **1**).

The different types of pharmacological agents discussed here will be dealt with taking into consideration the molecular targets on which they intervene. Thus, for most of the agents reviewed here the target is well known and the anti-viral activity understood in great details, but in some other cases, especially for very new types of leads reported ultimately, the molecular mechanisms by which the biological activity is achieved, are far less clear at this moment.

Although viral infections are widespread, till recently very few antiviral drugs were available [17]. The emergence of the worldwide AIDS epidemic fostered much research and great progress in this area, and presently more than 30 antiviral drugs are available, most of them for the management of HIV infection and AIDS, but also for the treatment of other viral diseases such as hepatitis B, influenza, herpes simplex, varicella-zoster and cytomegalovirus (HCMV) infections [17]. Some of these drugs that saved the life of millions of people worldwide, contain in their molecules sulfonamide moieties.

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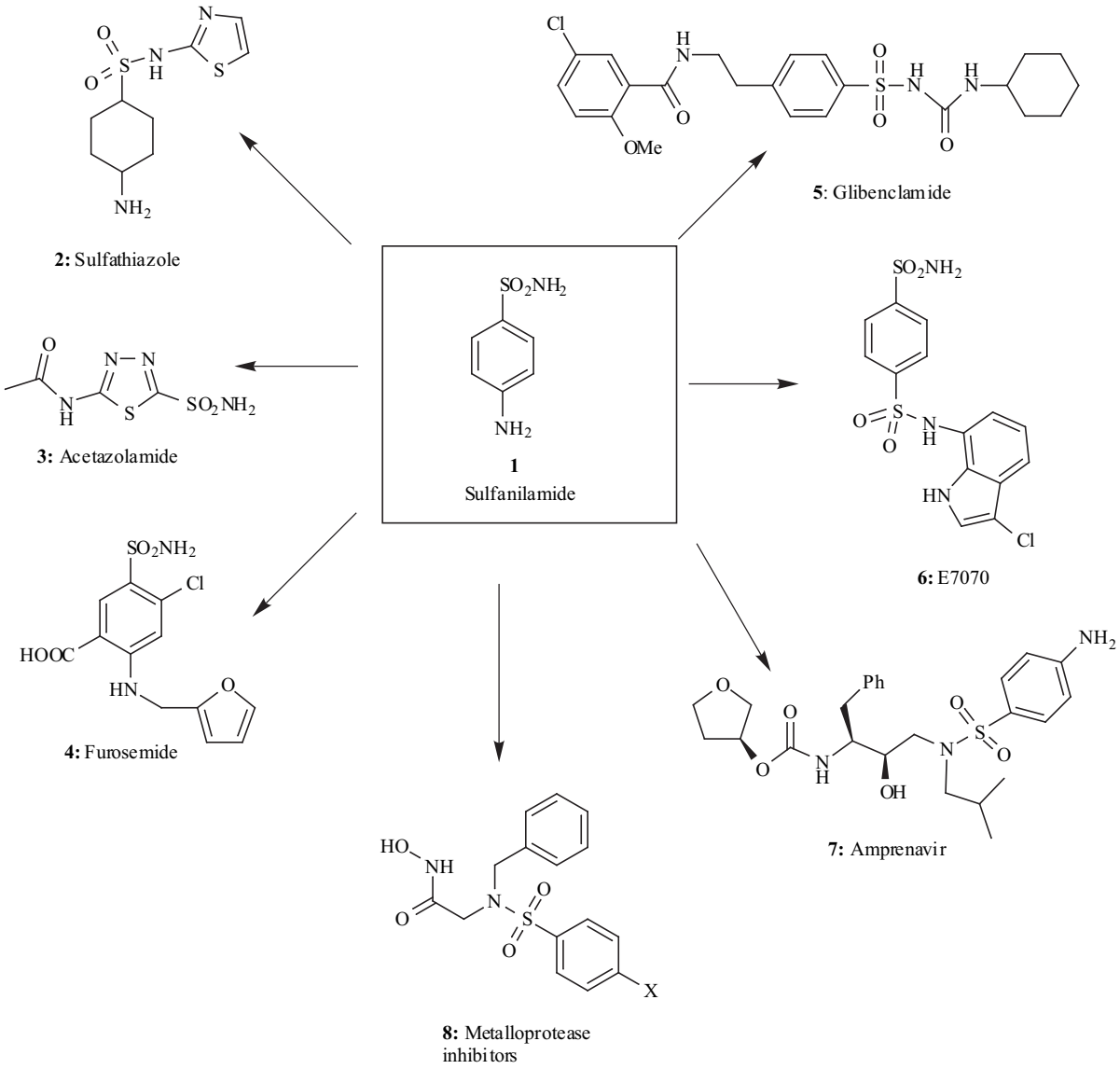


Fig. (1). The main classes of pharmacological agents developed from sulfanilamide **1** as lead molecule: antibacterials (such as sulfathiazole **2**); carbonic anhydrase inhibitors (such as acetazolamide **3**); diuretics (such as furosemide **4**); hypoglycemic agents (such as glibenclamide **5**); anticancer agents (such as indisulam (E7070) **6**, in advanced clinical studies); anti-AIDS agents (such as the HIV protease inhibitor amprenavir **7**) as well as matrix metalloprotease (MMP) inhibitors of type **8**.

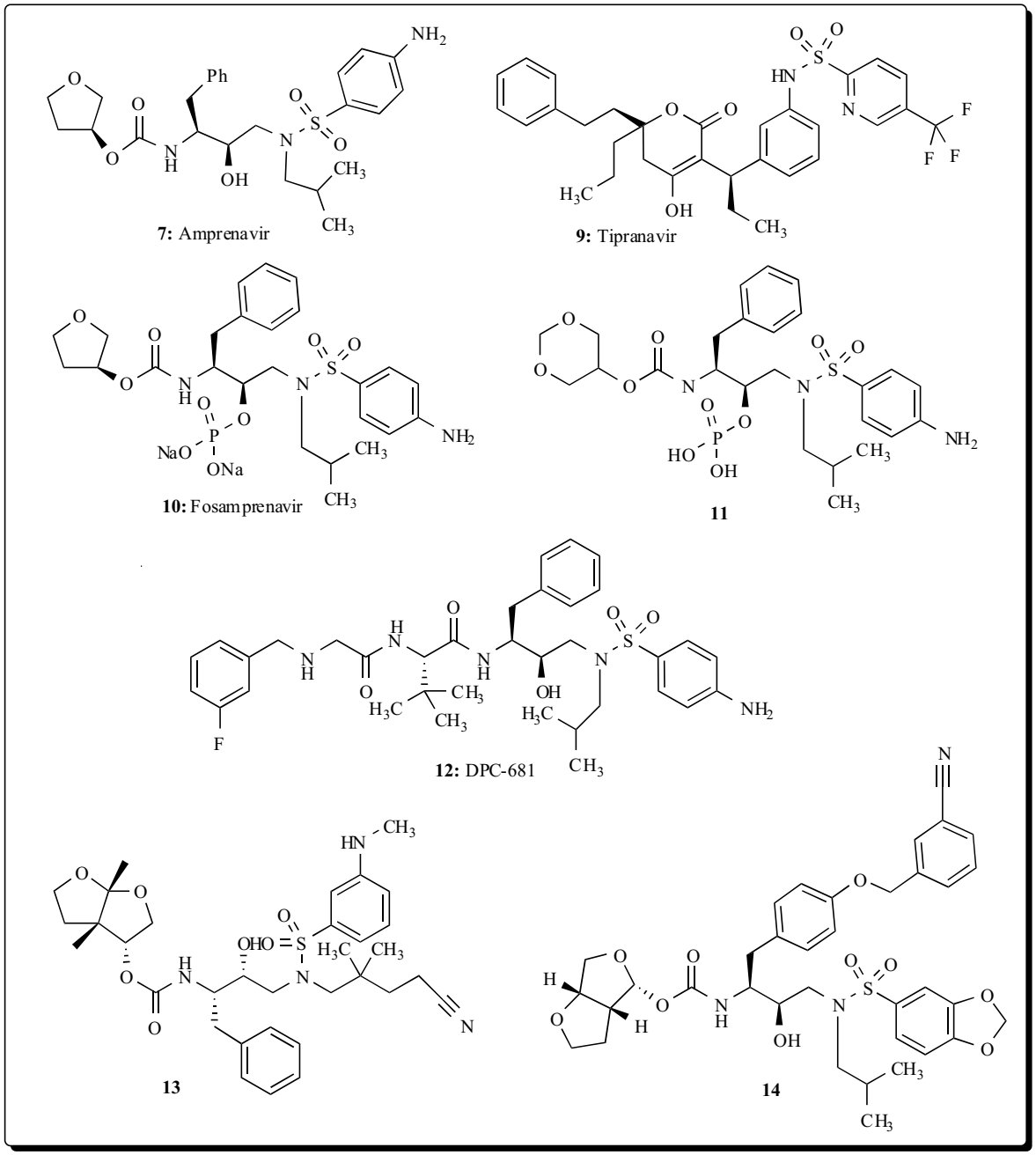
2. HIV PROTEASE INHIBITORS

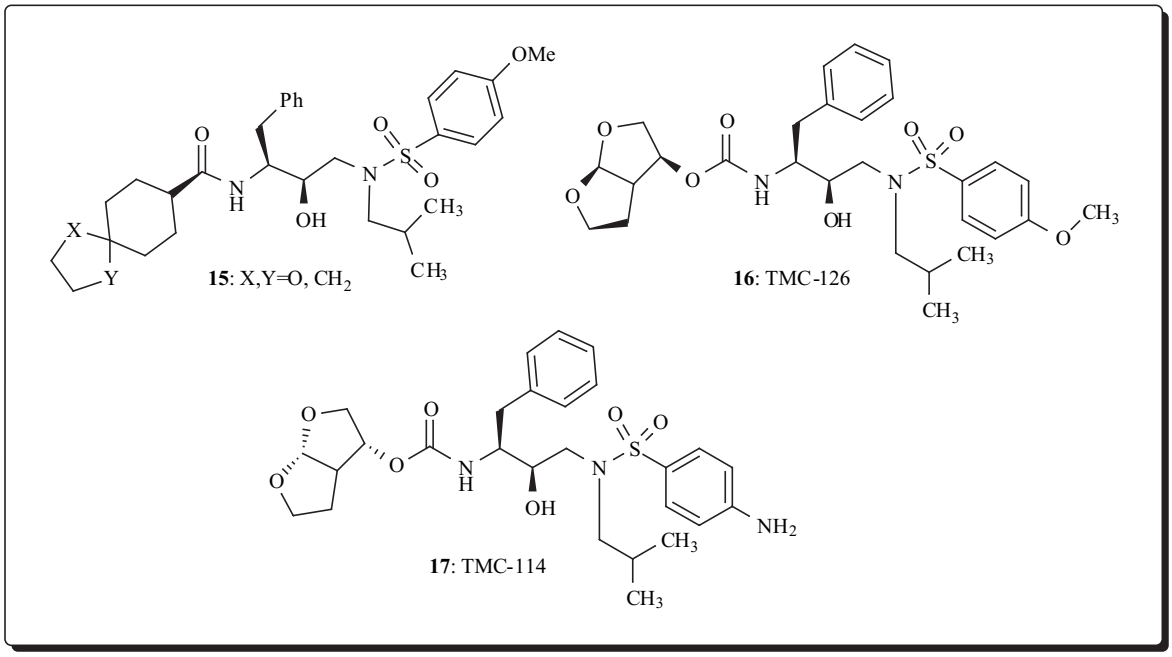
The HIV genome consists of two single-stranded, positive-sense RNA molecules organized in three major coding elements: *gag*, *pol* and *env* genes [12]. The *gag* and *pol* gene products are translated from a single unspliced polycistronic mRNA that encodes both genes. The 55 kDa Gag polyprotein (Pr55gag) contains sequences of the structural proteins of the virion, i.e., matrix, capsid and nucleocapsid proteins, along with several peptides involved in the assembly and morphogenesis of mature capsids [18]. The *pol* gene encodes the three enzymes of the virus, the protease (PR), the reverse transcriptase (RT) and the integrase (IN) [12,18]. These proteins are translated as part of larger polyprotein precursor, Pr160gag-pol, which results from ribosomal frameshift and readthrough during translation of the *gag* gene. The PR, which belongs to the class of aspartic proteases, cleaves the Pr55gag and Pr160gag-pol precursor polyproteins to structural and functional proteins, and its inhibition arrests maturation and blocks the infectivity of the nascent virions [12,18]. Ever since introduced in clinics in 1996, PR inhibitors (PIs) changed the course of the HIV infection, mainly in combination with other antiviral agents, such as RT inhibitors (of the nucleoside type, NRTI, or the non-nucleoside type, NNRTIs) [4,12]. Presently, six drugs containing PR inhibitors (PIs) are clinically available, i.e., saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, and lopinavir, and several other compounds (such as tipranavir **9**) are in advanced clinical evaluation [12]. Two of these drugs, amprenavir [19] **7** and tipranavir [20] **9** are sulfonamide derivatives.

HIV PR is a dimeric aspartic protease consisting of two identical, non-covalently associated subunits of 99 amino acid residues, associated in a twofold, C-2 symmetric fashion [18,21]. The active site is formed at the dimer interface and contains two conserved catalytic aspartic acid

residues, one from each monomer, Asp 25 and Asp 125, respectively [17,21]. A water molecule situated in the neighbourhood of the two aspartic acid residues mentioned above acts as the nucleophile in catalysis [17,21]. The substrate binding cleft is composed of equivalent residues from each subunit, and is lined on one side by the two aspartic acid residues mentioned above, and on the other by a pair of twofold related, antiparallel β-hairpin structures denominated "flaps" [17,21]. These flaps are highly flexible and undergo significant structural changes by inhibitor binding [17,21]. This protease cleaves some otherwise difficultly hydrolysable peptide bonds such as for example Tyr – Pro and Phe – Pro, as well as other peptide bonds incorporating Leu, Met, Asn, or Phe as P1 residues, and Ala, Met, Phe, Leu or Tyr as P1' residues (the nomenclature of Schechter and Berger [100] is used) [17,21]. Just this rather unusual catalytic activity, i.e., cleavage of Tyr(Phe) – Pro bonds was very much used in the design of PIs, and led in fact to the discovery of the clinically used compounds mentioned above.

All the clinically used compounds, including **7** and **9**, possess a hydroxyl moiety which mimics the transition state hydrated amide (diol) structure presumed to represent the intermediate of the peptide bond hydrolysis [17,21]. Amprenavir was clearly designed in such a way as to possess this moiety incorporated in a hydroxyethylamine isostere of the transition state, as well as the Phe peptidomimetic group in P1 [19]. The main innovation of this type of inhibitors is the presence of the sulfonamide moiety at the P2' site. This sulfonamide moiety is important for at least three reasons: (i) one of its oxygen atoms interacts with a water molecule (the so-called "flap" water) present in all HIV PR complexes investigated up to now [17,21]; (ii) the aromatic group

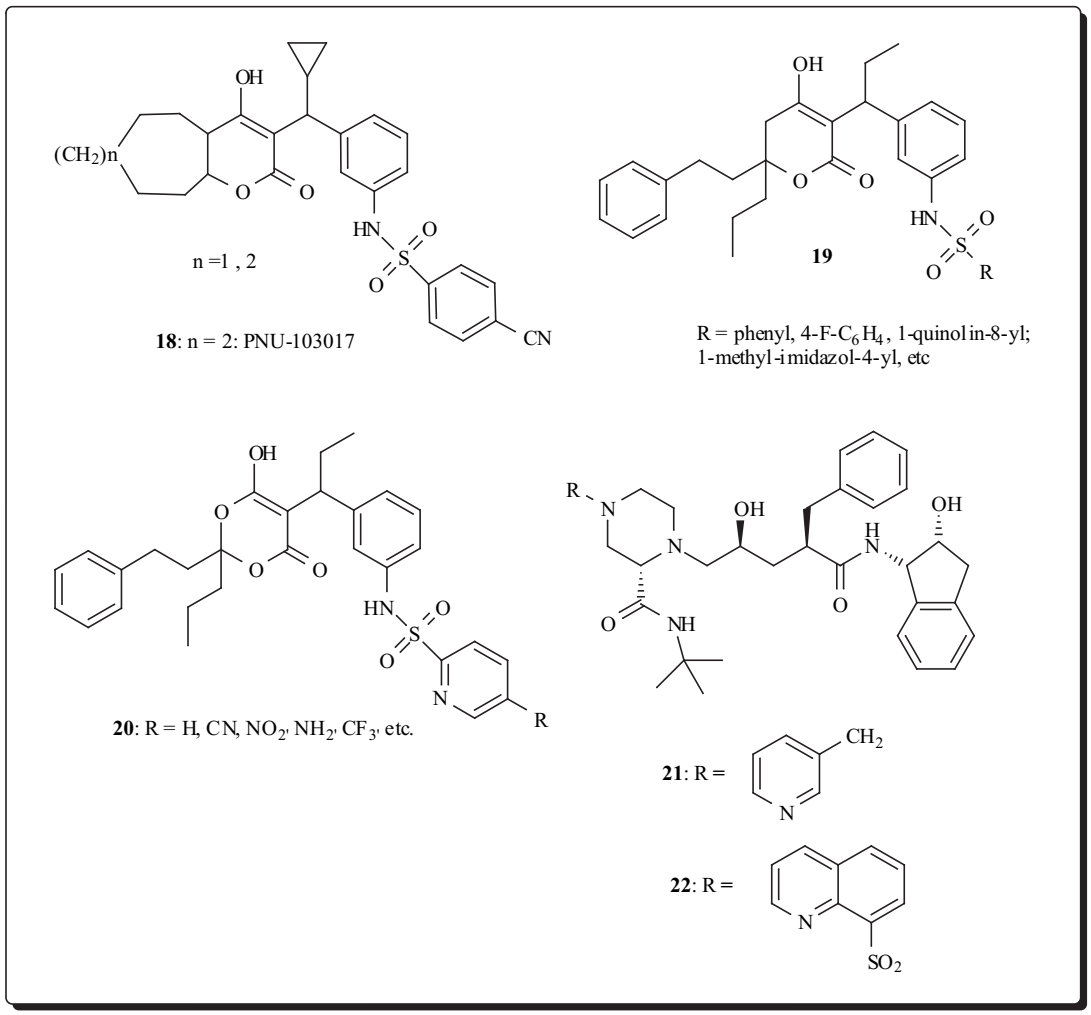




connected to the sulfonamide moiety interacts with the S2' sub-site of the enzyme, (iii) this moiety assures an increased water solubility to such PIs. In fact amprenavir has a rather high water solubility (of 190 μg/mL) as compared to other PIs, as well as a good lipophilicity. In this way, this compound possesses a very high oral bioavailability. Obviously, **7** is a very potent inhibitor of HIV PR, with a K_I of 0.6 nM against the wild type enzyme. It also appreciably inhibits mutated HIV PRs [19]. Recently, fosamprenavir **10** has been reported as an interesting prodrug of amprenavir [20,21]. Either the disodium salt **10** or the corresponding calcium salt GW433908 have shown bioequivalence to amprenavir and comparable safety features in phase I and II clinical trials [20,21]. In contrast to amprenavir, the prodrug(s) may show conveniently low pill count and the potential for single daily dosing, since amprenavir offers the longest half-life among the currently available PIs [98,99]. Some amprenavir-like derivatives of types **11-17** were also recently reported [21,22]. DPC-681 **12** is very active against wild type and highly mutated HIV proteases, and phase I clinical trials of this drug are ongoing [21,22]. These compounds possess many of the structural features already present in amprenavir, but generally incorporate different P2' binding moieties, and this probably explains their good activity against mutated viruses that lost susceptibility to the first generation PIs. An extension of the amprenavir-like structures has also been achieved in compounds of type **15, 16**, which incorporate a spiro S3 binding moiety and a slightly modified arylsulfonamide group (i.e., *p*-methoxybenzenesulfonamide instead of *p*-aminobenzenesulfonamide present in amprenavir) [22,23]. Of particular interest are the derivatives in clinical development by Tibotec, TMC-114 **17** [24] (TMC-126 **16** was the prototypic version of this type of PI), which although quite similar structurally to amprenavir, show very good activity against multi-drug resistant viral isolates at extremely low (sub-nanomolar) concentrations. The first clinical trials in humans of these derivatives are currently ongoing [24]. TMC-114 showed a lack of induction of high levels of phenotypic resistance after repeated *in vitro* passage with different HIV clinical and laboratory isolates [24]. The passaged isolates exhibited only a 10-fold resistance when exposed to a concentration of 100 nM TMC-114 and cross-resistance was observed only to saquinavir [24].

Tipranavir **9** has been discovered through a broad screening program that evidenced that some 4-hydroxy-benzopyran-2-one or the corresponding monocyclic derivatives possess affinity in the micromolar range for HIV PR [25]. X-ray crystallography showed that the 4-OH group of tipranavir-like PIs is nearly symmetrically bound to the two catalytic aspartic acid residues, whereas the carbonyl oxygen of C-2 replaces the flap water molecule and makes two hydrogen bonds with the NH groups of the flap residues Ile 50 and Ile 150 [25]. Furthermore, the sulfonamide moiety makes several strong hydrogen bonds with several active site residues, whereas the phenethyl and propyl groups of C-6 extend into the S1' and S2' subsites, respectively. The ethyl and phenyl groups at C-3α occupy the S1 and S2 subsites, whereas the trifluoromethyl-2-pyridyl moiety the S3 subsite [25]. These multiple interactions explain the very high inhibitory power of tipranavir (K_I < 1 nM) against wild type and mutant PRs [25]. Many tipranavir-like derivatives were also reported possessing different substitution patterns at the arylsulfonamide, C-3α and C-6 positions of the heterocyclic ring, or incorporating a 6-hydroxy-1,3-dioxin-4-one moiety instead of the coumarone one, of types **18-20** [26-30]. Many such derivatives exhibited low nanomolar affinity for the wild type and mutated HIV PRs [26-30].

Indinavir **21** is one of the most widely clinically used PIs. It possesses an IC_{50} value of 0.41 nM against HIV PR and a good water solubility of 70 μg/ mL, being thus orally bioavailable [31]. An even better inhibitory activity showed the 8-quinoline-sulfonamide derivative structurally related with indinavir, **22**, which with an IC_{50} of 0.013 nM is one of the most potent PI ever reported, but its rather low water solubility (in the range of < 1 μg/mL) precluded with its clinical development [31].

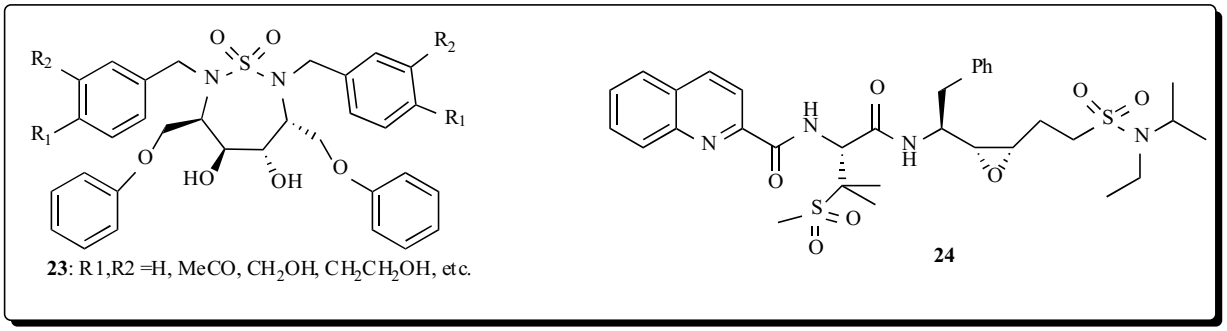


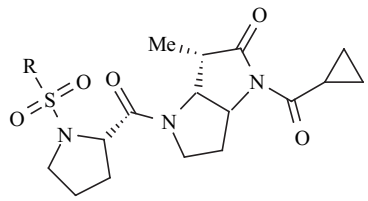
Another class of potent HIV-1 PIs incorporating sulfonamide-related moieties is represented by the symmetric cyclic sulfamide derivatives **23**, reported by Hallberg's group [32-34]. In contrast to the structurally related cyclic ureas, sulfamides **23** bind to the PR nonsymmetrically, i.e., the left hand phenoxymethyl moiety is accommodated within the S1 subsite, the right-hand similar moiety of the inhibitor to the S2' subsite; the left-hand (substituted)benzyl group binds within the S2 subsite, and the right-hand similar moiety to the S1' subsite of the enzyme, respectively, whereas one of the oxygen atoms of the SO_2 group mimics the tetrahedrally coordinated flap water molecule of the PR [32-34]. Some of these cyclic sulfamides showed affinity in the range of 3 – 43 nM against the wild type HIV PR [32-34].

Irreversible PIs containing sulfonamide and sulfone bond isosteres, of type **24** were reported by Choy *et al.* [35]. Derivative **24** showed a rapid, time-dependent inactivation of the HIV-1 protease, and high antiviral potency in cell cultures, with an IC_{50} value of 6.6 nM [35].

3. HERPES VIRUS PROTEASES (HCMV, HSV) INHIBITORS

The herpes viruses are enveloped, linear double-strand DNA viruses with a distinctive morphology, containing an icosadeltahedral capsid surrounded by an amorphous tegument [36,37]. The viral genomes range in size from 125 to 230 kilobases, encoding for 70 – 200 gene products [37].





25: R = 4-Me-C_6H_4; 3-i-PrO-C_6H_4;dansyl, etc.

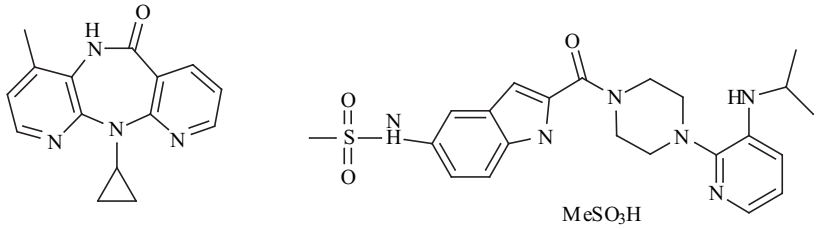
Eight human herpesviruses have been identified so far, divided in three sub-families as follows: (i) α-herpesviruses, which include HSV-1, HSV-2 and varicella-zoster virus (VZV). They cause widespread disease in both immunocompetent and immunocompromized patients, such as mucosal-labial herpes (cold sores) – HSV-1, or genital herpes – HSV-2, respectively. VZV infection is also widespread in the human population, causing chickenpox following primary infection in children and shingles (herpes zoster) upon reactivation in adults [37]; (ii) β-herpesviruses include HCMV, human herpesviruses 6 and 7 (HHV-6 and HHV-7). HCMV provokes serious pulmonary (pneumonitis) or ocular (retinitis) diseases, mainly in immunocompromised patients or transplant recipients [36,37]. In addition, congenital CMV infection provokes morbidity in approximately 15 % of babies asymptomatic at birth [37]. HHV-6 (and to a lesser extent HHV-7) is the primary cause of roseola, infecting greater than 95 % of babies, whereas its reactivation is associated with complications in transplant recipients [37]; (iii) γ-herpesviruses include Epstein-Barr virus (EBV) and the newly identified human herpesvirus 8 (HHV-8) [37]. Primary EBV infection is the leading cause of infectious mononucleosis in adolescence and early adulthood, but this virus is also associated with Burkitt's lymphoma, nasopharyngeal carcinoma and Hodgkin's disease [37]. HHV-8 is associated with Kaposi's sarcoma in AIDS and solid organ transplant populations [37], and may be a significant factor in the development of multiple myeloma [36,37].

Sulfonamide-based antivirals were also recently designed against the HCMV protease [36,37]. In contrast to the HIV PR discussed earlier, this enzyme belongs to the serine PR family, and together with similar proteases isolated from other herpes viruses, it is characterized by a particular catalytic triad, of the type His, His, Ser within the active site [38].

It was observed that α-methylpyrrolidine-5,5-trans-lactam derivatives of type **25** act as mechanism-based inhibitors of the HCMV δAla protease, acylating reversibly and in a time-dependent manner the active site nucleophile Ser 132. This serine PR hydrolyzes peptide bonds of the type Ala-Ser [36-38]. Compounds of type **25** bind to this PR with the 6-Me group within the S1 subsite, the *N*-4-cyclopropylcarbonyl moiety within the S1' subsite and the bulky, hydrophobic arylsulfonyl-pyrrolidine-2-carbonyl moiety within the S3 subsite [36]. Derivatives incorporating the dansyl-(*S*)-proline moiety in this position showed the best activity (IC_{50} of 0.34 μM) and also specificity over related serine proteases (such as elastase, thrombin or acetyl cholinesterase), which were inhibited with IC_{50} values greater than 10 – 200 μM [36].

4. NON NUCLEOSIDE HIV REVERSE TRANSCRIPTASE INHIBITORS

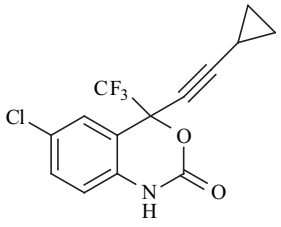
Among the drugs used for the treatment of HIV infection are also the NNRTIs [17]. Three such compounds are presently used clinically, nevirapine **26**, delavirdine **27** and efavirenz **28**, whereas two other agents, emivirine **29** and



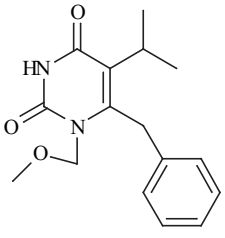
26: Nevirapine

$MeSO_3H$

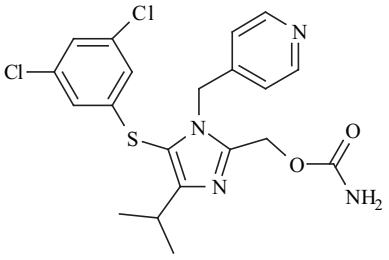
27: Delavirdine (mesylate)



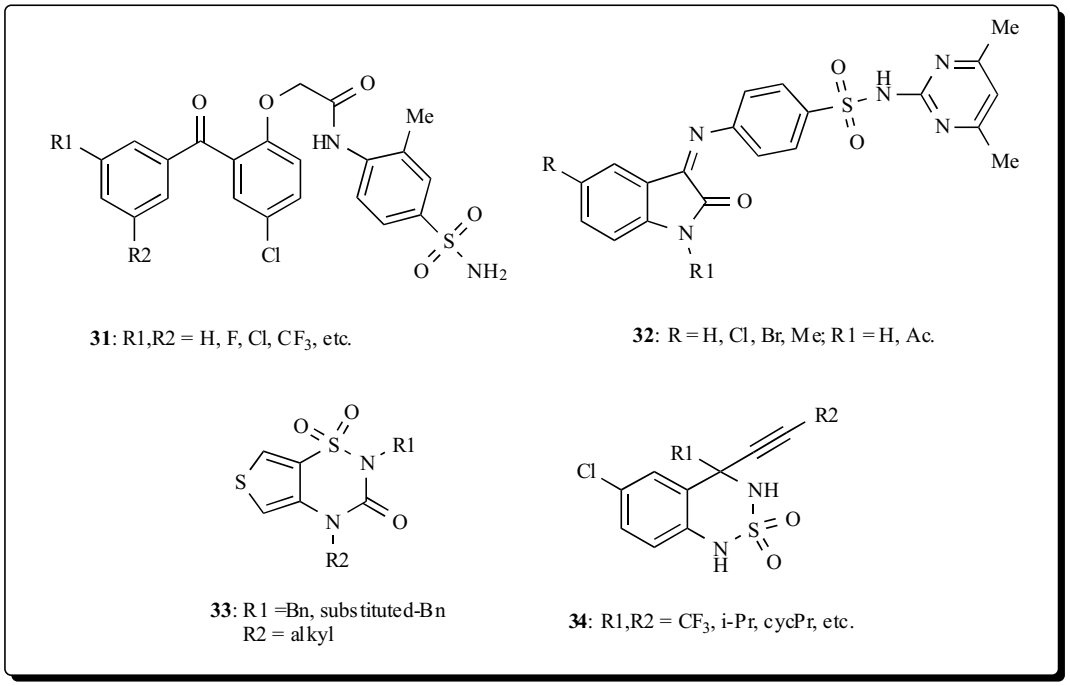
28: Efavirenz



29: Emivirine



30:Capravirine



capravirine **30** are in advanced clinical trials and may soon be released for clinical use [17,39,40].

NNRTIs block HIV-1 infection by binding to the enzyme RT, at an allosterically located, non-substrate binding site [39,40]. This binding site is situated at about 10 Å from the substrate binding site (where the NRTIs bind for example) being both spatially and functionally associated with it [39,40].

From the chemical point of view, NNRTIs possess a large variety of structures, being a heterogeneous class of compounds (as exemplified by the very diverse structures **26-30** above). The rapid emergence of NNRTI-resistant viruses (due to mutation of RT, and more specifically, of amino acid residues surrounding the NNRTI-binding site, such as L101I, K103N, V106A, E138K, V179D, Y181C, Y188H, G190A, P225H, F227L and P236L among others) [39,40] led to much synthetic effort for identifying potent NNRTIs belonging to other classes of compounds, as well as derivatives effective for resistant strains to the clinically used inhibitors mentioned above [17, 39,40].

As seen from the structures above, delavirdine **27** incorporates a methylsulfamoyl moiety in its molecule, but recently some other sulfonamide derivatives incorporating such functionalities have been reported.

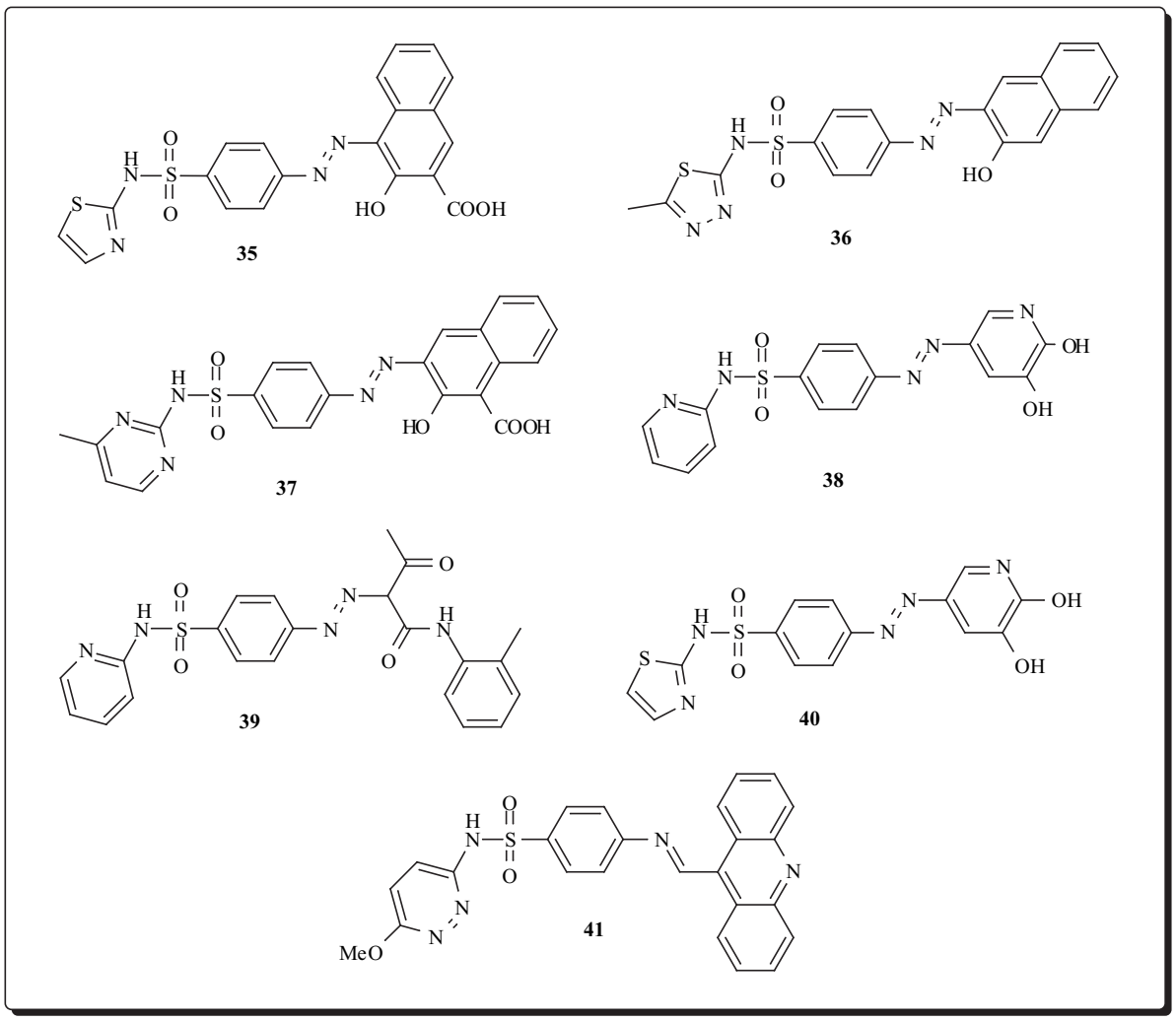
Recently, benzophenones of type **31** [41] incorporating primary sulfonamide moieties and 4-[(1,2-dihydro-2-oxo-3*H*-indol-3-ylidene)amino]-*N*-(4,6-dimethyl-2-pyrimidinyl) benzenesulfonamides of type **32** [42] were reported to possess strong anti-HIV properties, their mechanism of action being that of the NNRTIs. Probably by using efavirenz **28** as lead molecule, the thienothiadiazines **33** and the benzothiadiazines **34** were recently reported as new classes of NNRTIs [43,44] Both these types of derivatives incorporate the sulfamoyl functionalities in a fused bicyclic ring system, and possess strong anti-HIV-1 properties, with IC_{50} in the range of 0.18 – 60 μM [43,44].

5. HIV INTEGRASE INHIBITORS

Integration of viral DNA into host cell chromosomal DNA to form provirus is an essential step in the virus life cycle [45,46]. This process is mediated by integrase (IN), a 32 kDa enzyme, that catalyses two separate but chemically similar reactions, known as 3'-processing and DNA strand transfer [45,46]. In the first reaction, IN removes the terminal dinucleotide (GT) next to a conserved cytosine-adenine sequence from each 3'-end of the viral DNA. The enzyme then attaches the processed 3'-ends of the viral DNA to the host cell DNA in the second, strand transfer reaction [45,46]. Since there is no human counterpart of HIV IN, this enzyme is a good target for developing novel antiviral drugs [45,46].

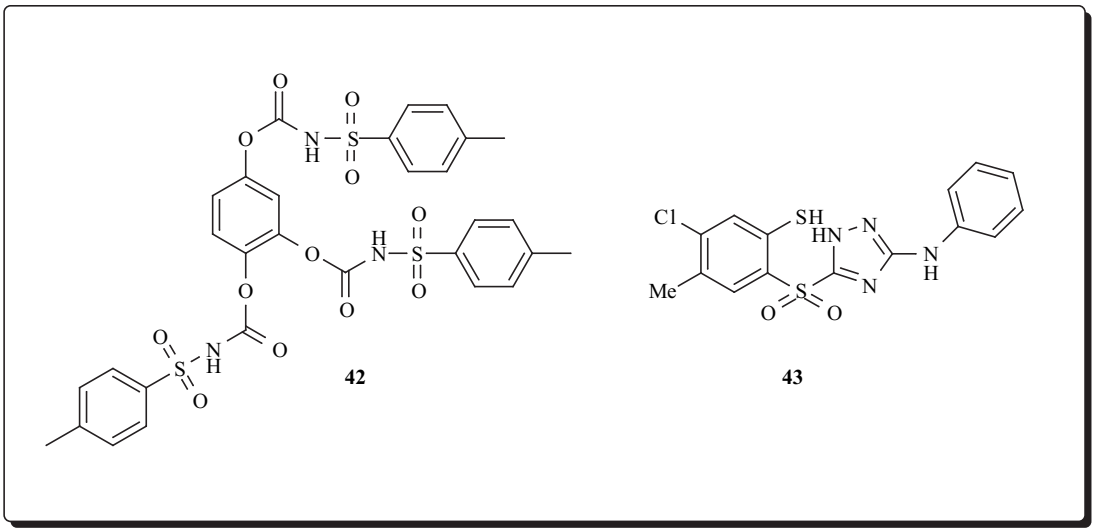
Pommier's and Neamati's groups [45-48] identified many classes of IN inhibitors through database search and extensive assay of large libraries/collections of compounds. Many such derivatives proved to belong to different classes of sulfonamides. Thus, investigation of the antibacterial sulfanilamide derivatives used clinically (such as sulfamethoxazole, sulfadiazine, sulfathiazole, sulfadimethoxine, sulfisoxazole, etc.) for their interaction with IN, lead to the observation that sulfisoxazole and sulfasalazine were the most potent IN inhibitors, with IC_{50} values in the range of 50 – 100 μg/mL [47]. Much better inhibitory properties proved to possess derivatives with bulkier groups in their molecules, of types **35 – 41**, which possess IC_{50} values in the range of 21 – 60 μM for the 3'-processing reaction and 20 – 50 μM for the integration reaction, respectively [48].

Neamati [46] showed that most of these compounds participate in networks of hydrogen bonds with the active site residue Gln 148. For example, in the case of compound **39**, the NH group of the sulfonamide moiety donates a H-bond to the oxygen of the $CONH_2$ moiety of Gln 148, one of the sulfonamide oxygen atoms is an acceptor of a H-bond from Gln 62, whereas the other oxygen of the SO_2 group



accepts a H-bond from His 114 (these two residues belong to the inhibitors binding site of IN, as shown by X-ray crystallography) [49]. Recently, very potent IN inhibitory properties were detected for the tris-sulfonamide derivative **42**, which shows IC_{50} values of 5.5 μM for the integration reaction and is inactive against the 3'-processing reaction, respectively (Johnston, A.; Supuran, C.T.; Pommier, Y., unpublished results). This compound is an interesting lead for developing better IN inhibitors, since for the moment no derivative belonging to this class arrived to clinical use.

Neamati *et al.* [50] have investigated a large series of 2-mercaptobenzenesulfonamides possessing bulky aromatic/heterocyclic moieties substituting the sulfonamide functionality, as HIV IN inhibitors. One of the best



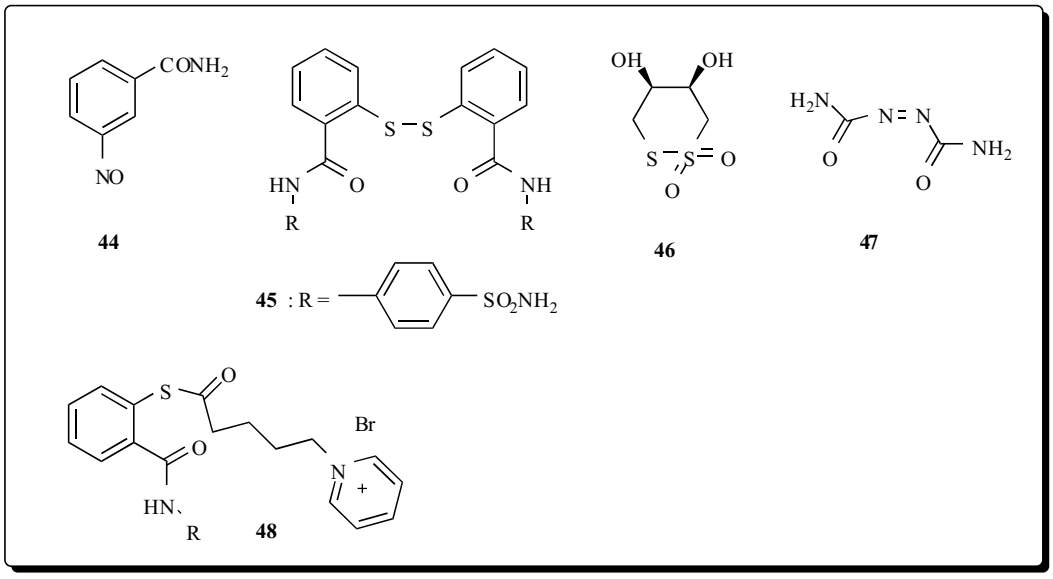
derivatives in this series was **43** and its disulfide derivative which showed IC_{50} values of 16 μM for the 3'-processing reaction and 12 μM for the strand transfer reaction, respectively (for **43**). For the disulfide, the corresponding values were of 4 μM and 8 μM, respectively. These derivatives also showed moderately good activity as antivirals in a cellular anti-HIV assay, with a therapeutic index of 2.7 for **43**, and 16.5 for its disulfide [50].

6. COMPOUNDS INTERACTING WITH VIRAL ZINC FINGER PROTEINS

A new target for the development of anti-HIV therapies has been reported by Rice *et al.* to be the HIV-1 nucleocapsid zinc finger protein NCp7 [51,52]. All retroviruses (except spumaretroviruses) contain in their nucleocapsid (NC) proteins one or two copies of a conserved sequence motif termed the cysteine array or the Cys-His box, comprising the C-$(X)_2$-C-$(X)_4$-H-$(X)_4$-C sequence, where C represents cysteine, H histidine, X any amino acid, and the number after the parenthesis the number of amino acid residues between the two elements mentioned above [53,54]. This motif is also known as the NC zinc finger, since a Zn(II) ion is tetrahedrally coordinated by the thiolate of the three Cys residues and the imidazole nitrogen belonging to the histidine residue [53,54]. The NC proteins of all retroviruses are indeed essential for the viral life cycle since they select viral RNA from cellular RNA for dimerization and packaging, promote binding of the essential *t*-RNA primer to the primer site, stimulate reverse transcription and protect the viral RNA from nucleases, and their mutation/modification are not tolerated [54-58]. Rice *et al.* [51,52] showed that it is possible to target NCp7 by chemically modifying the nucleophilic sulfur atoms coordinating the Zn(II) ions in the above-mentioned zinc fingers, with 3-nitrosobenzamide derivatives **44**, disulfide benzamides (DIBAs) **45**, dithianes of type **46** or azodicarbonamide **47** among others, the process being followed by zinc extrusion from the zinc finger and production of non-infective virions. Furthermore, no resistance to such agents has been observed, since, as stated above, no mutations in the NC proteins are tolerated by retroviruses, HIV included [52-58].

The mechanism of action of such zinc finger targeting agents of types **44-47** has been investigated in details [55], being shown that the zinc is ejected in a stoichiometric, oxidative manner. Initially, the antiviral agent (i.e., a dithiobisamide of type **45**) forms an intermolecular disulfide bond (by means of a disulfide exchange reaction) due to an electrophilic attack on one of the sulfur atoms coordinating the Zn(II) of the zinc finger. This process leads to the destabilization of the Zn(II) ion coordination sphere, and is followed by ejection of the metal ion from the zinc finger. The initial process may then be followed by the formation of intramolecular disulfides within the protein as well as disulfide bond rearrangements [55], but anyhow, the final result is the denaturation of the zinc finger protein, which is lethal for the virus, leading to the formation of non-infective virions [52-58].

A large number of isomeric 2,2'-, 3,3'- and 4,4'-disulfide benzamides (DIBAs) have been reported [56], but only the 2,2'-isomers of type **45** showed good zinc ejecting and antiviral properties. A second generation type of compounds is constituted by the pyridinioalkanoyl thiolesters (PATEs) **48** [57]. Some of the most effective antiviral derivatives belonging to the DIBAs are aromatic sulfonamides (derivatives of sulfanilamide, homosulfanilamide or orthanilamide) [56]. The presence of the free sulfonamide moiety on the other hand is known to be associated with powerful inhibition of a zinc enzyme possessing an important physiological function in bacteria, plants and vertebrates, i.e., carbonic anhydrase [7,8]. In that case, the ionized sulfonamide moiety is directly coordinated to the Zn(II) ion, which may be not the case of the derivatives **45** and **48** mentioned above, in their interaction with the Zn(II) ion of the zinc finger. Still, it would be important to verify whether the presence of the sulfonamide moiety may in a way or another directly influence the zinc-binding of the antiviral derivatives to the NCp7 zinc finger, considering the affinity of Zn(II) to coordinate sulfonamides in their deprotonated form [7,8].



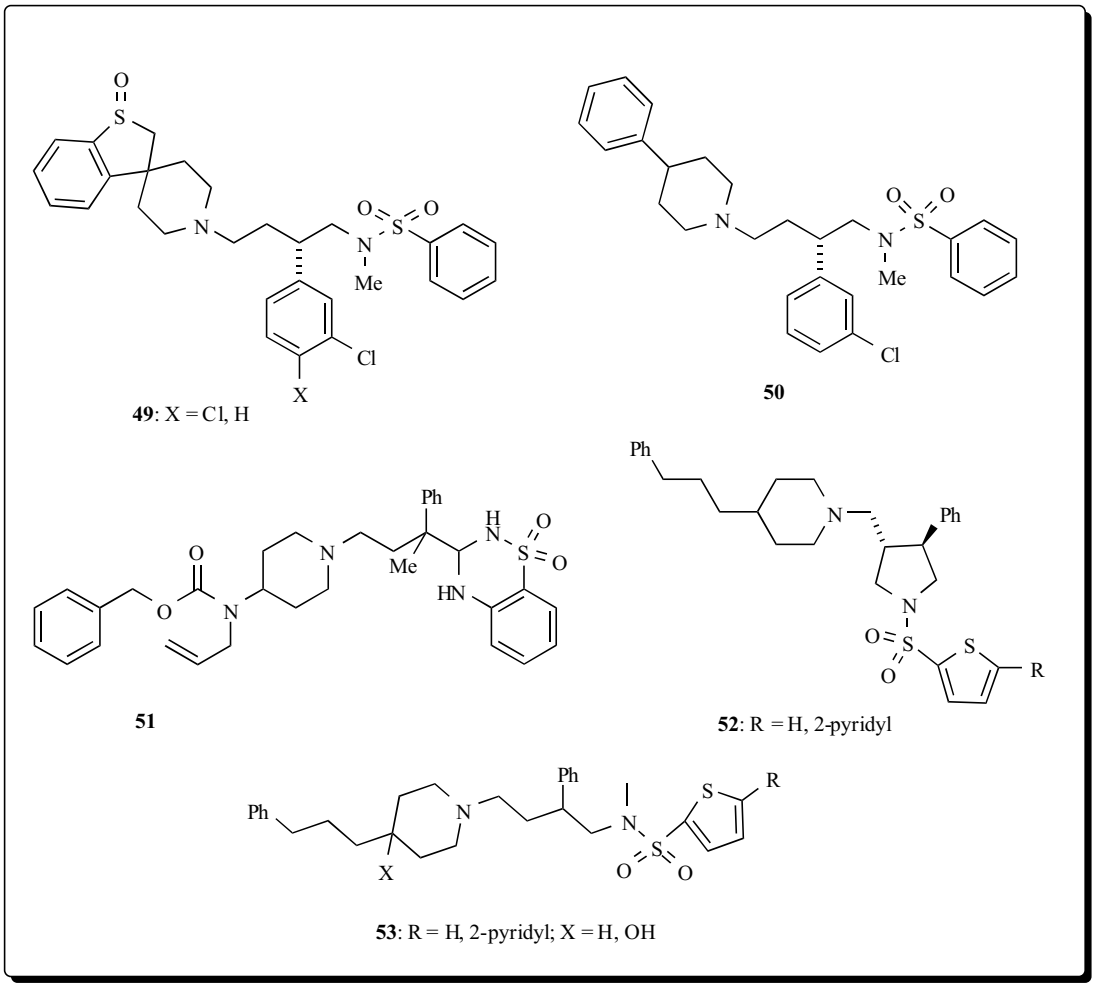
It has also been proven that some of these agents of types **45** and **48** possess selectivity for the viral zinc finger protein (NCp7), without appreciably reacting with several cellular zinc fingers, such as the poly(ADP-ribose) polymerase (PARP) and the Sp1 and GATA-1 transcription factors, nor did they inhibit HeLa nuclear extract mediated transcription [59]. This finding is of great importance in validating these compounds as valuable leads for the development of new antiviral drugs.

7. HIV ENTRY INHIBITORS

HIV entry within the target cells involves a series of molecular events that started to be understood in detail in the last period. The T-lymphocyte cell surface protein CD4 is the primary receptor involved in the interaction with the viral glycoprotein gp120, but a cellular coreceptor is also needed for the successful entry of the virus within the cell [4, 60-62]. At least two types of such coreceptors have been identified so far, both belonging to the chemokine family of seven-transmembrane-spanning receptors coupled to a G-protein signalling pathway: [4, 63-65] the CC chemokine receptor 5 (CCR5) (which binds the chemotactic chemokines, the monocyte inflammatory protein (MIP)-1α, and MIP-1β, and RANTES [**r**egulated upon **a**ctivation **n**ormal **T**-cell **e**xpress and **s**ecreted]) and the CXC chemokine receptor 4 (CXCR4) (which binds the stromal derived factor (SDF)-1 as ligand) [60-65]. These receptors therefore are the gateways for HIV entry, determinants of viral tropism and sensitivity. CCR5 receptor is used by macrophage (M)-tropic viruses and CXCR4 is used by T-lymphocyte (T)-tropic virus [4, 60-64].

The three main steps of virus entry within the cell can be detailed as follows: (i) Attachment of the virus to the host cells. This process involves the formation of a complex between the trimeric gp120 – gp41 viral glycoproteins, the CD4 receptor and the chemokine coreceptor (CCR5 or CXCR4) [60-65]; (ii) Interaction of the virus with the coreceptors. The amino-terminal fusion peptide of gp41 is inaccessible in the native state, but following interaction of gp120 with the CD4 protein, a conformational change occurs, leading to exposure of the gp120 third hypervariable (V3) domain loop, with insertion of the aminoterminal peptide into the target cell membranes, *via* a "prehairpin" intermediate [60-64]; (iii) Fusion of the virus and host cell membranes. Intramolecular interactions between the C- and N-terminal peptide regions of gp41 lead to the formation of a hairpin configuration (actually a trimer of hairpins), which is followed by juxtaposition of the host cell and viral membranes, i.e., membrane fusion [60-64].

Considering the complexity of the molecular events briefly outlined above, it is envisageable that all these three steps have been considered for the drug design of HIV entry inhibitors [60-64]. Indeed, several approaches have been reported ultimately of agents that interact with one of the



steps mentioned above, such as for example: fusogenic particle peptide antagonists (agents of peptidic nature that interact with the gp120/gp41 – CD4 – chemokine receptor interaction); chemokine and chemokine derivatives as fusion inhibitors; peptide-based antagonists of the CXCR4 receptors as well as small molecule chemokine antagonists of either CXCR4 or CCR5 receptors [60-65]. Some recently reported small molecule antagonists of the chemokine receptors involved in HIV pathogenesis are of the sulfonamide type and will be reviewed here.

Finke's group showed that the substituted 2-aryl-1-[*N*-(methyl)-*N*-(phenylsulfonyl)amino]-4-(piperidin-1-yl)butanes **49** and the related derivative **50** possess strong CCR5 antagonistic activity [65,66]. These compounds have been identified as CCR5 antagonists through an extensive screening programme of Merck collections of derivatives, using a HTS binding assay of labelled-MIP-1α to stably expressed human CCR5 receptors in CHO cells [65,66]. A number of sulfonamides possessing 1-(*N*-alkyl-*N*-phenylsulfonylamino)-2-(3,4-dichlorophenyl)-4-(piperidin-1-yl)butane moieties were found active in this way, whereas the corresponding carboxamides were devoid of CCR5 antagonistic activity [144]. Thus, the synthetic efforts were then concentrated on such lead molecules, which eventually led to the spiro-2,3-dihydrobenzthiophene-3,4'-piperidin-1'-yl derivative **49** (X = Cl) – as sulfoxide – showing a nanomolar affinity for the CCR5 receptor (IC_{50} = 35 nM), without appreciable binding to the related CCR1, CCR2 and CCR3 receptors (IC_{50} values over 1000 nM) [65]. Subsequent modification of the arylsulfonamide moiety by a large variety of alkyl-, substituted aryl or hetaryl moieties were detrimental to the binding affinity, and the phenylsulfonamido group of **49** has been preserved as so. The halogenophenyl moiety of the lead **49** has also been extensively modified, but generally the obtained derivatives were less active then the dichloroderivative (except for the 3-chlorophenyl-derivative, **49** (X = H) [66]). In the same paper, the spiro-2,3-dihydrobenzthiophene-3,4'-piperidin-1'-yl moiety of **49** has been replaced by the 4-phenyl-piperidine moiety present in **50**, leading to a compound with slightly improved binding affinity for the CCR5 receptor (IC_{50} = 30 nM for **50**) [66].

Further synthetic work of the same group led to some more potent CCR5 antagonists, such as **51** (its R_f isomer has an IC_{50} of 18 nM) [67] and **52, 53** (discovered by a combinatorial chemistry approach) [67]. Some of these last derivatives are indeed excellent and selective CCR5 antagonists, possessing IC_{50} values in the range of 2 - 20 nM [68]. Although detailed *ex vivo*/*in vivo* data are not yet available, these compounds represent promising leads for the development of new antivirals.

8. CONCLUSIONS

The sulfonamides constitute an important class of drugs, with several types of pharmacological agents possessing a host of biological activities, of the antibacterial, anti-carbonic anhydrase, diuretic, hypoglycemic, antithyroid, antiprotease and antiviral type among others. A large number of structurally novel sulfonamide derivatives have recently been reported to show substantial antiviral activity, both *in vitro* and/or *in vivo*. Although they have a common chemical motif of aromatic/heterocyclic sulfonamide, there are a variety of mechanisms of their antiviral action. Striking progress has been achieved in the design of sulfonamide-based antivirals. Some of the clinically used HIV protease inhibitors belong to this family of compounds, and the sulfamoyl functionalities present in their molecules are essential for strong binding to the enzyme and good oral bioavailability of the drug. Furthermore, many of the new types of NNRTIs, integrase inhibitors, the compounds interacting with viral zinc finger proteins or several small molecule HIV entry inhibitors also belong to varied classes of sulfonamides, proving indeed that these versatile moieties may be of great help for the modern drug design.

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REFERENCES

[1] De Clercq, E. *Nature Drug Discov*. **2002**, *1*, 13-25.
[2] Richman, D.D. *Nature* **2001**, *410*, 995-1001.
[3] Nabel, G.J. *Nature* **2001**, *410*, 1002-1007.
[4] Scozzafava, A.; Mastrolorenzo, A.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem*. **2002**, *17*, 69-76.
[5] Drew, J. *Science* **2000**, *287*, 1960-1964.
[6] Supuran, C.T. *Exper Opin. Invest. Drugs* **2003**, *12*, 283-287.
[7] Supuran, C.T.; Scozzafava, A. *Exp. Opin. Ther. Patents* **2000**, *10*, 575-600.
[8] Supuran, C.T.; Scozzafava, A. *Exp. Opin. Ther. Patents* **2002**, *12*, 217-242.
[9] Supuran, C.T.; Scozzafava, A. *Curr. Med. Chem.- Imm., Endoc. Metab. Agents,* **2001**, *1*, 61-97.
[10] Boyd, A.E. *Diabetes* **1988**, *37*, 847-850.
[11] Thornber, C.W. *Chem. Soc. Rev*. **1979**, *8*, 563-580.
[12] Ogden, R.C.; Flexner, C.W., Eds. *Protease Inhibitors in AIDS Therapy*. Marcel Dekker: New York, Basel, **2001**.
[13] Supuran, C.T.; Scozzafava, A.; Mastrolorenzo, A. *Exp. Opin. Ther. Patents* **2001**, *11*, 221-259.
[14] Supuran, C.T.; Scozzafava, A.; Clare, B.W. *Med. Res. Rev*. **2002**, *22,* 329-372.
[15] Scozzafava, A.; Supuran, C.T. *J. Med. Chem*. **2000**, *43*, 3677-3687.
[16] Wouters, J.; Michaux, C.; Durant, F.; Dogné, J.M.; Delarge, J.; Masereel, B. *Eur. J. Med. Chem*. **2000**, *35*, 923-929.
[17] De Clercq, E. *Curr. Med. Chem*. **2001**, *8*, 1543-1572.
[18] Erickson, J.W. HIV-1 protease as a target for AIDS therapy. In *Protease inhibitors in AIDS therapy*, Ogden, R.C.; Flexner, C.W. Eds., Marcel Dekker, New York, **2001**, pp. 1-25.
[19] Tung, R.D.; Livingston, D.J.; Rao, B.G.; Kim, E.E.; Baker, C.T., Boger, J.S.; Chambers, S.P.; Deininger, D.D.; Dwyer, M.; Elsayed, L.; Fulghum, J.; Li, M.; Murcko, M.A.; Navia, M.A.; Novak, P.; Pazhanisamy, S.; Stuver, C.; Thomson, J.A. Design and synthesis of amprenavir, a novel HIV protease inhibitor. In *Protease inhibitors in AIDS therapy*, Ogden, R.C.; Flexner, C.W. Eds., Marcel Dekker, New York, **2001**, pp. 101 – 118.
[20] Gatell, J.M. *J. HIV Ther*. **2001**, *6*, 95-99.
[21] Casini, A.; Scozzafava, A.; Supuran, C.T. *Exp. Opin. Ther. Patents* **2002**, *12*, 1307-1327.
[22] Rusconi, S.; La Seta Catamancio, S. *Expert Opin. Investig. Drugs* **2002**, *11*, 387-395.
[23] Ghosh, A.K.; Shin, D.; Swanson L. *Farmaco* **2001**, *56*, 29-32.
[24] De Meyer, S.; Azijn, H.; van Ginderen, M.; de Baere, I.; Pauwels, S.; De Bethune, M.P. *Antiviral Ther*. **2002**, *7*, S5.
[25] Chrusciel, R.A.; Thaisrivongs, S.; Nicholas, J.A. HIV protease inhibitors in early development. *Protease inhibitors in AIDS therapy*, Ogden, R.C.; Flexner, C.W. Eds., Marcel Dekker, New York, **2001**, pp. 119 – 137.

[26] Wlodawer, A.; Gustchina, A. *Biochim. Biophys. Acta* **2000**, *1477*, 16-34.
[27] Schechter, I.; Berger, A. *Biochem. Biophys. Res. Commun*. **1967**, *27*, 157-162.
[28] Turner, S.R.; Strohbach, J.W.; Tommasi, R.A.; Aristoff, P.A.; Johnson, P.D.; Skulnick, H.I.; Dolak, L.A.; Seest, E.P.; Tomich, P.K.; Bohanon, M.J.; Horng, M.M.; Lynn, J.C.; Chong, K.T.; Hinshaw, R.R.; Watenpaugh, K.D.; Janakiraman, M.N.; Thaisrivongs, S. *J. Med. Chem*. **1998**, *41*, 3467-3476.
[29] Skulnick, H.I.; Johnson, P.D.; Aristoff, P.A.; Morris, J.K.; Lovasz, K.D.; Howe, W.J.; Watenpaugh, K.D.; Janakiraman, M.N.; Anderson, D.J.; Reischer, R.J.; Schwartz, T.M., Banitt, L.S.; Tomich, P.K.; Lynn, J.C.; Horng, M.M.; Chong, K.T.; Hinshaw, R.R.; Dolak, L.A.; Seest, E.P.; Schwende, F.J.; Rush, B.D.; Howard, G.M.; Toth, L.N.; Wilkinson, K.R.; Kakuk, T.J.; Johnson, C.W.; Cole, S.L.; Zaya, R.M.; Zipp, G.L.; Possert, P.L.; Dalga, R.J.; Zhong, W.Z.; Williams, M.G.; Romines, K.R. *J. Med. Chem*. **1997**, *40*, 1149-1164.
[30] Lee, Y.S.; Lee, Y.S.; Lee, J.Y.; Kim, S.N.; Lee, C.K.; Park, H. *Bioorg. Med. Chem. Lett*. **2000**, *10*, 2625-2627.
[31] Dorsey, B.D.; Vacca, J.P. Discovery and early development of indinavir. In *Protease inhibitors in AIDS therapy*, Ogden, R.C.; Flexner, C.W. Eds., Marcel Dekker, New York, **2001**, pp. 65-83.
[32] Hulten, J.; Bonham, N.M.; Nillroth, U.; Hansson, T.; Zuccarello, G.; Bouzide, A.; Aqvist, J.; Classon, B.; Danielson, U.H.; Karlen, A.; Kvarnstrom, I.; Samuelsson, B.; Hallberg, A. *J. Med. Chem.* **1997**, *40*, 885-897.
[33] Backbro, K.; Lowgren, S.; Osterlund, K.; Atepo, J.; Unge, T.; Hulten, J.; Bonham, N.M.; Schaal, W.; Karlen, A.; Hallberg, A. *J. Med. Chem.* **1997**, *40*, 898-902.
[34] Hulten, J.; Andersson, H.O.; Schaal, W.; Danielson, U.H.; Classon, B.; Kvarnstrom, I.; Karlen, A.; Unge, T.; Samuelsson, B.; Hallberg, A. *J. Med. Chem.* **1999**, *42*, 4054-4061.
[35] Choy, N.; Choi, H.; Jung, W.H.; Kim, C.R.; Yoon, H.; Kim, S.C.; Lee, T.G.; Koh, J.S. *Bioorg. Med. Chem. Lett*. **1997**, *7*, 2635-2638.
[36] Borthwick, A.D.; Crame, A.J.; Ertl, P.F.; Exall, A.M.; Haley, T.M.; Hart, G.J.; Mason, A.M.; Pennell, A.M.K.; Singh, O.M.P.; Weingarten, G.G.; Woolven, J.M. *J. Med. Chem*. **2002**, *45*, 1-18.
[37] Ilies, M.A.; Scozzafava, A.; Supuran, C.T. *Exp. Opin. Ther. Patents* **2002**, *12*, 1181-1214.
[38] Shieh, H.S.; Kurumbail, R.G.; Stevens, A.M.; Stegeman, R.A.; Sturman, E.J.; Pak, J.Y.; Witwer, A.J.; Palmier, M.O.; Wiegand, R.C.; Holwerda, B.C.; Stallings, W.C. *Nature* **1996**, *383*, 279-282.
[39] De Clercq, E. *J. Pharmacol. Exptl. Ther.* **2001**, *297*, 1-10.
[40] Condra, J.H.; Miller, M.D.; Hazuda, D.J.; Emini, E.A. *Annu. Rev. Med*. **2002**, *53*, 541-555.
[41] Andrews, C.W.; Chan, J.H.; Freeman, G.A.; Romines, K.R.; Tidwell, J.H. *WO Patent* **2001**, 17982.
[42] Selvam, P.; Chandramohan, M.; De Clercq, E.; Witvrouw, M.; Pannecouque, C. *Eur. J. Pharm. Sci*. **2001**, *14*, 313-316.
[43] Arranz, E.; Dìaz, J.A.; Ingate, S.T.; Witvrouw, M.; Pannecouque, C.; Balzarini, J.; De Clercq, E.; Vega, S. *Bioorg. Med. Chem.* **1999**, *7*, 2811-2822.
[44] Corbett, J.W.; Gearhart, L.A.; Ko, S.S.; Rodgers, J.D.; Cordova, B.C.; Klabe, R.M.; Erickson-Viitanen, S.K. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 193-195.
[45] Pommier, Y.; Neamati, N. *Adv. Virus Res*. **1999**, *52*, 427-458.
[46] Neamati, N. *Exp. Opin. Invest. Drugs* **2001**, 10, 281-296.
[47] Neamati, N.; Mazumder, A.; Zhao, H.; Sunder, S.; Burke, T.R.; Schultz, R.J.; Pommier, Y. *Antivir. Chem. Chemother*. **1997**, *8*, 385-393.
[48] Chen, I.J.; Neamati, N.; Nicklaus, M.C.; Orr, A.; Anderson, L.; Barchi, J.J.; Kelley, J.A.; Pommier, Y.; MacKerell, A.D. *Bioorg. Med. Chem.* **2000**, *8*, 2385-2398.
[49] Goldkur, Y.; Craigie, R.; Cohen, G.H.; Fujiwara, T.; Yoshinaga, T.; Fujishita, T.; Sugimoto, H.; Endo, T.; Murai, H.; Davies, D.R. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 13040-13043.
[50] Neamati, N.; Mazumder, A.; Sunder, S.; Owen, J.M.; Schultz, R.J.; Pommier, Y. *Antivir. Chem. Chemother*. **1997**, *8*, 485-495.
[51] Rice, W.G.; Schaeffer, C.A.; Harten, B.; Villinger, F.; South, T.L.; Summers, M.F.; Henderson, L.E.; Bess, J.W. Jr; Arthur, L.O.; McDougal, J.S. *Nature* **1993**, *361*, 473-475.
[52] Rice, W.G.; Supko, J.G.; Malspeis, L.; Buckheit, R.W. Jr; Clanton, D.; Bu, M.; Graham, L.; Schaeffer, C.A.; Turpin, J.A.; Domagala, J. *Science* **1995**, *270*, 1194-1197.
[53] Mely, Y.; Cornille, F.; Fournie-Zaluski, M.C.; Darlix, J.L.; Roques, B.P.; Gerard, D. *Biopolymers* **1991**, *31*, 899-906.
[54] Ramboarina, S.; Moreller, N.; Fournie-Zaluski, M.C.; Roques, B.P. *Biochemistry* **1999**, *38*, 9600-9607.
[55] Loo, J.A.; Holler, T.P.; Sanchez, J.; Gogliotti, R.; Maloney, L.; Reily, M.D. *J. Med. Chem.* **1996**, *39*, 4313-4320.
[56] Domagala, J.M.; Bader, J.P.; Gogliotti, R.D.; Sanchez, J.P.; Stier, M.A.; Song, Y.; Prasad, J.V.; Tummino, P.J.; Scholten, J.; Harvey, P.; Holler, T.; Gracheck, S.; Hupe, D.; Rice, W.G.; Schultz, R. *Bioorg. Med. Chem.* **1997**, *5*, 569-579.
[57] Huang, M.; Maynard, A.; Turpin, J.A.; Graham, L.; Janini, G.M.; Covell, D.G.; Rice, W.G. *J. Med. Chem.* **1998**, *41*, 1371-1381.
[58] Turpin, J.A.; Song, Y.; Inman, J.K.; Huang, M.; Wallqvist, A.; Maynard, A.; Covell, D.G.; Rice, W.G.; Appella, E. *J. Med. Chem.* **1999**, *42*, 67-86.
[59] Basrur, V.; Song, Y.; Mazur, S.J.; Higashimoto, Y.; Turpin, J.A.; Rice, W.G.; Inman, J.K.; Appella, E. *J. Biol. Chem.* **2000**, *275*, 14890-14897.
[60] Tomaras, G.D.; Greenberg, M.L. *Curr. Infect. Dis. Rep*. **2001**, *3*, 93-99.
[61] Mastrolorenzo, A.; Scozzafava, A.; Supuran, C.T. *Exp. Opin. Ther. Patents*, **2001**, *11*, 1245-1252.
[62] Kwong, P.D.; Wyatt, R.; Robinson, J.; Sweets, R.W.; Sodroski, J.; Hendrickson W.A. *Nature* **1998**, *393*, 648-659.
[63] Stantchev, T.S.; Broder, C.C. *Cytokine Growth Factors Rev.* **2001**, *12*, 219-243.
[64] Root, M.J.; Kay, M.S.; Kim, P.S. *Science* **2001**, *291*, 884-888.
[65] Dorn, C.; Finke, P.E.; Oates, B.; Budhu, R.J.; Mills, S.G.; MacCoss, M.; Malkowitz, L.; Springer, M.S.; Daugherty, B.L.; Gould, S.L.; DeMartino, J.A.; Siciliano, S.J.; Carella, A.; Carver, G.; Holmes, K.; Danzeisen, R.; Hazuda, D.; Kessler, J.; Lineberger, J.; Miller, M.; Schleif, W.A.; Emini, E.A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 259-264.
[66] Finke, P.E.; Meurer, L.C.; Oates, B.; Mills, S.G.; MacCoss, M.; Malkowitz, L.; Springer, M.S.; Daugherty, B.L.; Gould, S.L.; DeMartino, J.A.; Siciliano, S.J.; Carella, A.; Carver, G.; Holmes, K.; Danzeisen, R.; Hazuda, D.; Kessler, J.; Lineberger, J.; Miller, M.; Schleif, W.A.; Emini, E.A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 265-270.
[67] Kim, D.; Wang, L.; Caldwell, C.G.; Chen, P.; Finke, P.E.; Oates, B.; MacCoss, M.; Mills, S.G.; Malkowitz, L.; Gould, S.L.; DeMartino, J.A.; Springer, M.S.; Hazuda, D.; Miller, M.; Kessler, J.; Danzeisen, R.; Carver, G.; Carella, A.; Holmes, K.; Lineberger, J.; Schleif, W.A.; Emini, E.A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 3103-3106.
[68] Willoughby, C.A.; Berk, S.C.; Rosauer, K.G.; Degrado, S.; Chapman, K.T.; Gould, S.L.; Springer, M.S.; Malkowitz, L.; Schleif, W.A.; Hazuda, D.; Miller, M.; Kessler, J.; Danzeisen, R.; Holmes, K.; Lineberger, J.; Carella, A.; Carver, G.; Emini, E.A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 3137-3141.