Control of HIV Through the Inhibition of HIV-1 Integrase: A Medicinal Chemistry Perspective

C.P. Gordon[1], R. Griffith[2,*] and P.A. Keller[1,*]

[1]*Department of Chemistry, University of Wollongong, Wollongong, NSW 2522, Australia;* [2]*School of Environmental and Life Sciences, University of Newcastle, Callaghan, NSW 2308, Australia*

Abstract: This article reviews the current status of classes of HIV-1 integrase enzyme inhibitors. These classes include peptide-based inhibitors, natural products, polyhydroxylated aromatics, diketo acids, naphthyridines, and sulfonated compounds including sulfonic acids. Discussions of structure activity relationships are presented and include the current overview of the structure-based model, suitable for the further design and development. To date, the advances in the medicinal chemistry of HIV-1 integrase inhibitors have relied mostly on ligand-based designs leading to most displaying similar binding interactions within the active site or at the dimer interface. This paves the way for single enzyme mutations rendering entire compound classes inactive and thus, the requirement for second and third generation inhibitors with novel modes of binding is apparent. To facilitate future structure-based drug design efforts, a model of the biologically relevant structure of the HIV-1 integrase enzyme, a dimer of dimers has also been discussed.

Key Words: HIV-1 Integrase, Inhibitor, Inhibitor classes, Structure-activity relationship.

INTRODUCTION

The human immunodeficiency virus (HIV), first identified in 1983, is commonly accepted as the causative agent of acquired immunodeficiency syndrome (AIDS) [1]. The prognosis of AIDS patients who have full access to current therapies has dramatically changed since the first cases of AIDS were reported. Prior to the inception of the first anti-HIV drug, AZT [2,3], in 1987 the life expectancy for AIDS patients was less than 1 year, whereas today the median survival for HIV-positive patients receiving treatment exceeds 8 years [4]. Moreover, the median survival for patients prescribed current combination therapies has not been determined, as these were only introduced 8 years ago. This dramatic change is due to the development of effective therapies, to early detection of HIV-positive individuals, and a sustained effort to analyse and understand viral resistance mechanisms, which can be overcome by rational drug development and combination therapy [4].

At present, triple therapy, or highly active antiretroviral therapy (HAART), is the standard treatment for infection. HAART typically consists of a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor (NNRTI) in combination with two nucleoside reverse transcriptase inhibitors (NRTI) [5-10]. These multi-drug combination regimens have made it possible to suppress the replication of HIV to such an extent that the virus becomes undetectable in infected individuals. Unfortunately, HAART requires patients to adhere to long-term complicated dosing regimens resulting in many patients becoming non-compliant.

*Address correspondence to these authors at the Department of Chemistry, University of Wollongong, Wollongong, NSW 2522, Australia; Tel: +62 2 4221 4692; Fax: +61 2 4221 4287; keller@uow.edu.au

School of Environmental and Life Sciences, University of Newcastle, Callaghan, NSW 2308, Australia; Tel: +61 2 4921 6990; Fax: +61 2 4921 6923; E-mail: Renate.Griffith@newcastle.edu.au

Despite rapid advances in the treatment of HIV infection, eradication of the virus has failed due to the persistence of latent HIV-1 in resting memory $CD4^+$ T cells [11-16]. Furthermore, the emergence of HIV strains resistant to antiretroviral drugs aimed at any of the aforementioned viral targets is an inevitable phenomenon. Thus, there is a clear and present need to develop new therapies, which target alternative steps in the viral cycle.

HIV-1 INTEGRASE AS A POTENTIAL TARGET FOR ANTIVIRAL THERAPY

An important, yet unexploited potential therapeutic target is the HIV-1 integrase enzyme (IN). IN is a particularly attractive antiviral target as it is indispensable in the HIV lifecycle and perhaps more importantly it has no cellular counterparts indicating that specific and non-toxic inhibitors could be developed [17].

The genome of this retrovirus is encoded as RNA and during the viral lifecycle, after translation into DNA (by reverse transcriptase), it needs to be integrated into the host genome – this is facilitated by IN. Specifically, it catalyses two distinct reactions, these being 3′-processing and strand transfer. During 3′-processing, which occurs in the cytoplasm of the infected cell, the enzyme catalyses the resection of a 5′-GT dinucleotide from each end of the viral DNA thereby generating the nucleophilic 3′-hydroxyl ends required for strand transfer [18-20]. This water-mediated endonucleolytic cleavage of the 5′-GT dinucleotides occurs immediately 3′ to a highly conserved CA dinucleotide, any alteration of this sequence prevents IN from catalysing 3′-processing [21-23].

Following 3′-processing, integrase undergoes a structural change in preparation for the binding of the acceptor (chromosomal) DNA [24,25]. IN, still bound to the 3'-processed viral DNA, translocates to the nucleus of the infected cell as

1573-4064/07 $50.00+.00 © 2007 Bentham Science Publishers Ltd.

a part of a pre-integration complex (PIC), wherein the terminal 3'-OH of the viral DNA attacks the host DNA [26-29]. Strand transfer is coordinated in such a way that each of the 3′-hydroxyl ends attack a DNA phosphodiester bond on each strand of the chromosomal DNA with a five-base-pair stagger across the DNA major groove [4,30,31]. This leaves a five-base single-stranded gap at each junction between the integrated viral DNA and chromosomal DNA, and a two-base flap at the 5′-ends of the viral DNA, which are repaired by cellular DNA repair enzymes [4].

HIV IN STRUCTURE AND FUNCTIONAL DOMAINS

HIV-1 IN is a 32-kDa protein, composed of 288 amino acids. When dissecting the molecular structure of the HIV-1 IN, three discrete domains can be distinguished, the amino-terminal domain (NTD), the catalytic core domain (CCD) and the carboxyl terminal domain (CTD).

The NTD encompasses residues 1–50 and has been shown to interact with DNA as part of the IN complex but does not exhibit DNA binding properties when isolated in solution [32-34]. In keeping with integrases of retroviruses, retrotransposons, and even many prokaryotic transposable elements, the NTD contains a histidine-histidine-cysteine-cysteine motif (HHCC) to which Zn^{2+} binds in a 1:1 stoichiometry [35-37]. The domain comprises a four α-helix bundle which forms a dimer in solution. The domains are stabilised by a hydrophobic region and the zinc binding motif, consequently the mutation of a single residue within the HHCC results in the domain losing structure and DNA binding ability [32,37].

The catalytic core is structurally remarkably similar to other retroviral integrases and retrotransposons [38]. This family of DNA-processing enzymes typically contain a canonical Asp, Asp, Glu motif, which in HIV-1 IN is formed by Asp-64, Asp-116 and Glu-152, with the two aspartic acid residues forming a coordination complex with a divalent metal [39-42]. Although it is well accepted that a metal cofactor is required for catalysis, to date no definitive conclusions have been reached regarding the type, Mg^{2+} of Mn^{2+}, or number of metal ions required [43,44]. However, it is generally accepted that Mg^{2+} is a more likely cofactor given its one million-fold abundance over Mn^{2+} in cells [45,46]. Further although the X-ray structures show one metal ion within the active site, it is believed that the second metal ion comes with DNA potentially coordinating with the carboxylate oxygen atoms of Glu-152 and possibly Asp-64 [43].

The C-terminal domain is primarily composed of β-strands and is the least conserved domain among the retroviral integrases [40,47-49]. The isolated C-terminal domain has been shown to bind DNA in a nonspecific manner and also dimerises when isolated in solution [40]. In addition, this domain has been implicated in protein–protein interactions [50] as it has been shown to interact with RT, an interaction which appears to be crucial for RT catalytic activity [51,52].

At present, crystal structures of the NTD [35,36], CCD [53,39,40,38,54-58], CTD [48,49], as well as the CCD with the NTD [59], and the CCD with the CTD [39], have been reported. However, crystallisation of the entire enzyme has proven to be difficult and to date no complete X-ray crystal structure has been reported. Consequently, the relative spatial arrangement of these structural domains, and their interactions with DNA substrates has remained largely unknown. However, as illustrated in (Fig. **1**), it appears as if the enzyme adopts at least a dimeric conformation in which the active sites of the opposing units are on opposite sides of the complex. As shown in (Fig. **1**), this dimerisation is mediated by a number of hydrophobic interactions between various secondary structures found at the dimeric interface, such as alpha helices 1, 3, 5, 6 and beta-strand 3 [60].

In a recently published model (Fig. **1**, (**c**) and (**d**)), it has been proposed that a dimer of dimers is required for activity [61]. In this model only two complete IN units, one from each dimer, are directly involved in catalysis, while the other two units provide structural support to the complex and participate in the binding of the viral DNA [61]. As illustrated in Fig. **1** (**c**) and (**d**), the CCDs (dark-blue) of the two units directly involved in catalysis encapsulate the chromosomal DNA (light-blue) while their NTDs (red) appear to play a crucial role in the binding and positioning of the chromosomal DNA. Further, the CTDs (yellow) of two catalytically involved units appear to play a dual role binding the viral and chromosomal DNA, while the NTDs (green) and CTDs (black) of the two units not directly involved in catalysis appear to assist in the binding and positioning of the viral DNA (yellow) [61].

HIV-1 INTEGRASE INHIBITORS

In contrast to RT and PR, the paucity of structural information has hampered structure-based discovery of selective inhibitors targeted to IN. As a consequence, many groups have turned to substrate-based drug design and high-through screening of chemical or natural product libraries to search for novel lead molecules. Such studies have unearthed a plethora of inhibitors based on diverse scaffolds including substrate-based inhibitors, numerous natural products, polyhydroxylated aromatics, aryl-β-diketoacids, naphthyridines, styrylquinolines, a number of peptides, and sulfur based analogues.

SUBSTRATE-BASED IN INHIBITORS

Rational design commonly begins with the investigation of compounds that are based on, or mimic the natural enzyme substrate. Amongst the first substrate-based HIV-1 IN inhibitors reported were the dinucleotides 5'-pGT-3' (**1**, Table **1**), which is excised from the 3′-end of the viral DNA during 3′-processing, and 5'pCA-3' (**2**, Table **1**), which is immediately upstream [62]. Upon further investigation three other dinucleotides, 5'pCT-3', 5'-pAC-3', and 5'-pAT-3' (**3**-**5**, respectively, Table **1**) were also identified as potent inhibitors of IN [62].

Unfortunately, small oligonucleotides are rapidly cleaved by cellular nucleases. Consequently a number of studies were initiated to investigate the inhibitory effects of a number of non-natural nucleotides. The initial non-natural derivatives, which included a number isodeoxycytosine [27] and cyclic analogues [63] were essentially inactive. However, further investigations lead to the development of a

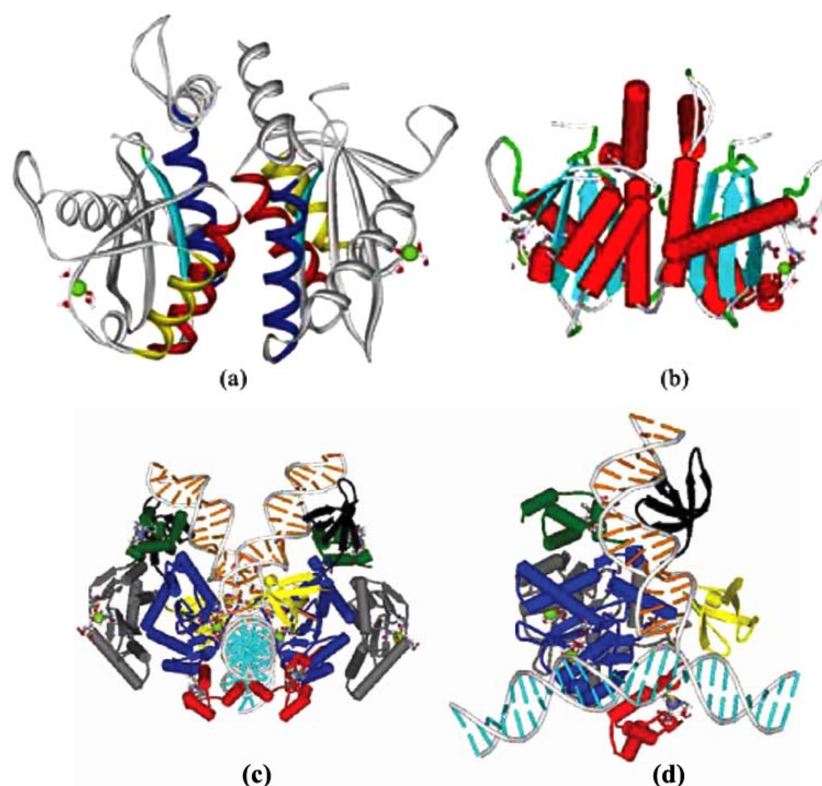


Fig. (1). (a) and **(b) – ribbon and cartoon representations**: Dimerisation of the core domains is mediated by a number of hydrophobic interactions. The primary interactions are between alpha helices 1, 3, 6 and beta-strand 3 which are coloured red, yellow, dark blue, and light blue respectively. The active-site Mg^{2+} ion and co-crystallised water molecules are coloured by atom type (PBD code 1QS4). The residues of the catalytic triad are represented as sticks and coloured by atom type. The core domain adopts a dimeric formation in which the active-sites of the opposing domains are on opposite faces of the complex. **(c)** and **(d)**: The CCDs (dark-blue) of the two units directly involved in catalysis encapsulate the chromosomal DNA (light-blue) while their NTDs (red) appear to play a crucial role in the binding and positioning of the chromosomal DNA [61]. Further the CTDs (yellow) of two catalytically involved units appear to play a dual role binding the viral and chromosomal DNA, while the NTDs (green) and CTDs (black) of the two units not directly involved in catalysis appear to assist in the binding and positioning of the viral DNA (yellow). (**d**) Additional view: 'Sliced in half' and rotated 90 °C to the right.

number of potent inhibitors which included dinucleotide **6** [64] and the isodeoxyuracil derivative **7** [65] (Fig. **2**).

A number of oligonucleotides have also been identified as potent IN inhibitors [66], however to date the most potent oligonucleotides reported were a series of analogues composed entirely from deoxyguanosine and thymidine, known as guanosine quartets [67]. However, these quartets are not only IN inhibitors, as their primary viral target is gp120. Nevertheless, compound **8** (Zintevir) [67] is currently in preclinical trials.

SMALL-MOLECULE NATURAL PRODUCT IN INHIBITORS

Over the past several years, a plethora of compounds isolated from natural sources have been identified as HIV-1 IN inhibitors. As illustrated in (Fig. **4**), amongst the first identified were aurintricarboxylic acid (**9**) [68], the *Fusarium heterosporum* isolate equisetin (**10**) [69], integric acid (**11**) [70] from *Xylaria* sp., several lichen acids of the depside and depsidone families, such as chloroparellic acid (**12**) [71] and stictic acid (**13**) [71], and a number of lignanolides, ex-

Table 1. Sequence and IC50 Values of a Number of Dinucleotide HIV-1 IN Inhibitors

Nucleotide	Sequence	3'-Processing Inhibition IC_{50} (μM)	Strand Transfer Inhibition IC_{50} (μM)
1	5'-pGT-3'	22.0	7.0
2	5'-pCA-3'	105.0	13.0
3	5'-pCT-3'	8.0	6.0
4	5'-pAC-3'	6.0	3.0
5	5'-pAT-3'	7.0	7.0

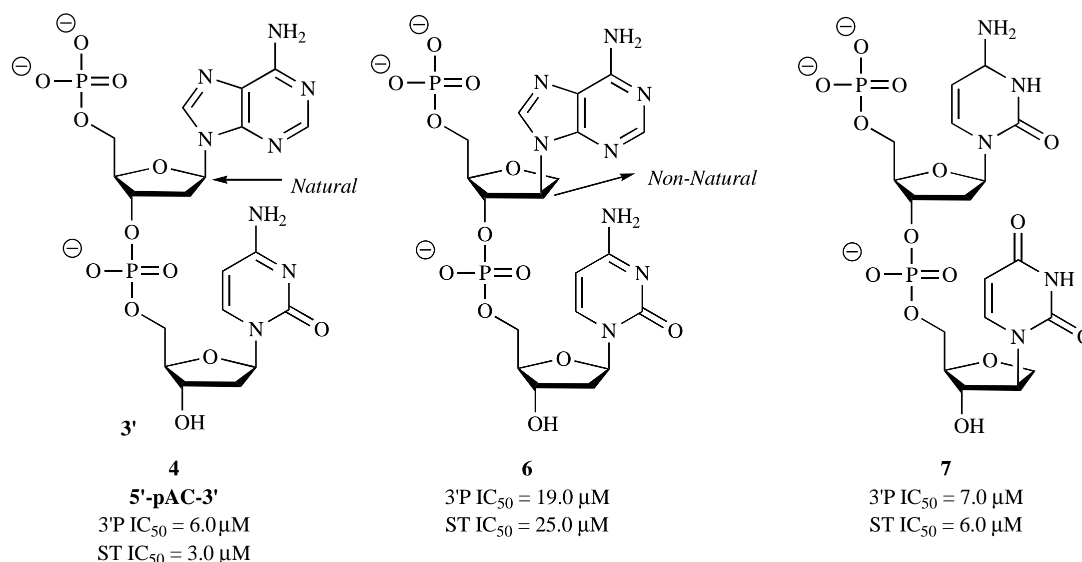


Fig. (2). Structure and activity of the natural substrate-based inhibitor **4**, the non-natural derivative **6**, and the isodeoxyuracil derivative **7** against 3′-processing (3′P) and strand transfer (ST).

emplified by β-conidendrol (**14**) [72]. Others include many tetracyclins, such as the DNA binder doxorubicin (**15**) [73], antioxidants such as flavones, and a number of compounds isolated from food stuffs such as curcumin (**16**) [74], which was isolated from the widely used spice cumin.

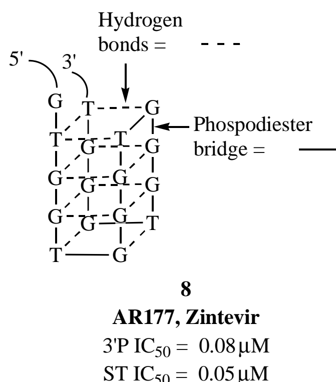


Fig. (3). The structure and activity of the guanosine quartet Zintevir.

More recently, a number of biaryl fungal extracts have also been identified as HIV-1 IN inhibitors (Fig. **5**). Examples include xanthoviridicatin E (**17**) [75], which was isolated from *Penicillium chrysogenum* as well as isochaetochromin B_1 and D_1 (**18** & **19**), extracted from *Fusarium* species [76]. Also isolated from *Fusarium* species were a series of oxygenated tetracyclic triterpenoids typified by integracide A and integracide B (**20** & **21**) [77]. Other fungal isolates include xerocomic acid (**22**) [78], extracted from a strain of *Xeromphalina junipericola*, hydroxyterphenyllin (**23**) [78] from *Aspergillus candidus*, the polyketide australifunginol (**24**) [79] from a *Cytospora* species and a series of sesterterpenoids such as ophiobolin C (**25**) [80].

Clearly, numerous naturally occurring compounds based on extremely diverse scaffolds display inhibitory effects against HIV-1 IN. Thus, elucidation of the common essential structural and chemical features required for activity is an extremely onerous task. As a result, many groups have adopted molecular modelling approaches, such as pharmocophore generation, to determine important three-dimensional arrangements and essential functional groups needed to effectively interact with the enzyme [81-89]. However, a consistent theme in a significant number of natural product IN inhibitors is the presence of polyhydroxylated aromatic moieties. Consequently, a significant amount of research has focused on identifying and subsequently modifying polyphenolic IN inhibitors.

POLYHYDROXYLATED AROMATIC HIV-1 INHIBITORS

The group of polyhydroxylated aromatics (PHAs) embodies the most numerous and diverse class of HIV IN inhibitors. As illustrated in (Fig. **6**), the majority of the PHAs share a common architecture consisting of two aryl units, one of which contains the 1,2-catechol pattern, separated by a linker segment. Examples of the class include flavones such as quercetagetin (**26**) [90], caffeic acid phenethyl ester analogues (CAPE) (**27**) [90], and chicoric acids (**28**) [91,92].

To date no definitive conclusions regarding the inhibitory action of the PHAs have been reported. It is unclear whether the hydroxyl moieties act by chelating the active-site magnesium ions or are simply involved in hydrogen bonding within the catalytic core. However, a co-crystallised structure of the enzyme core with the catechol-containing **30** (Fig. **7**) [57], suggests that the PHAs may elicit their inhibitory effects by causing steric obstruction at the dimer interface, possibly leading to an arrest in the formation of an enzymatically functional multimeric complex. Unfortunately the precise binding mode of **30** is unclear as the ligand in this structure is only partially resolved.

Although a plethora of PHAs have been identified as potent *in vitro* IN inhibitors [93-95], the *in vivo* antiviral

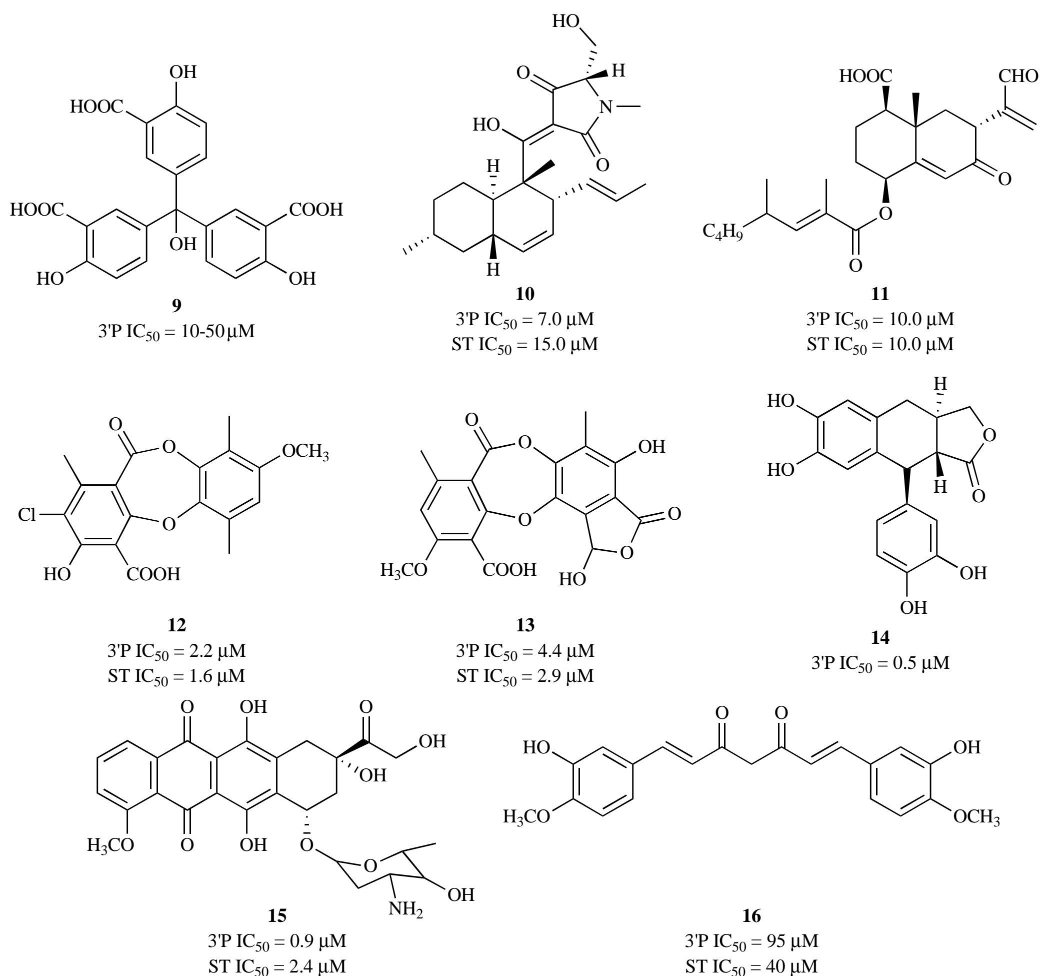
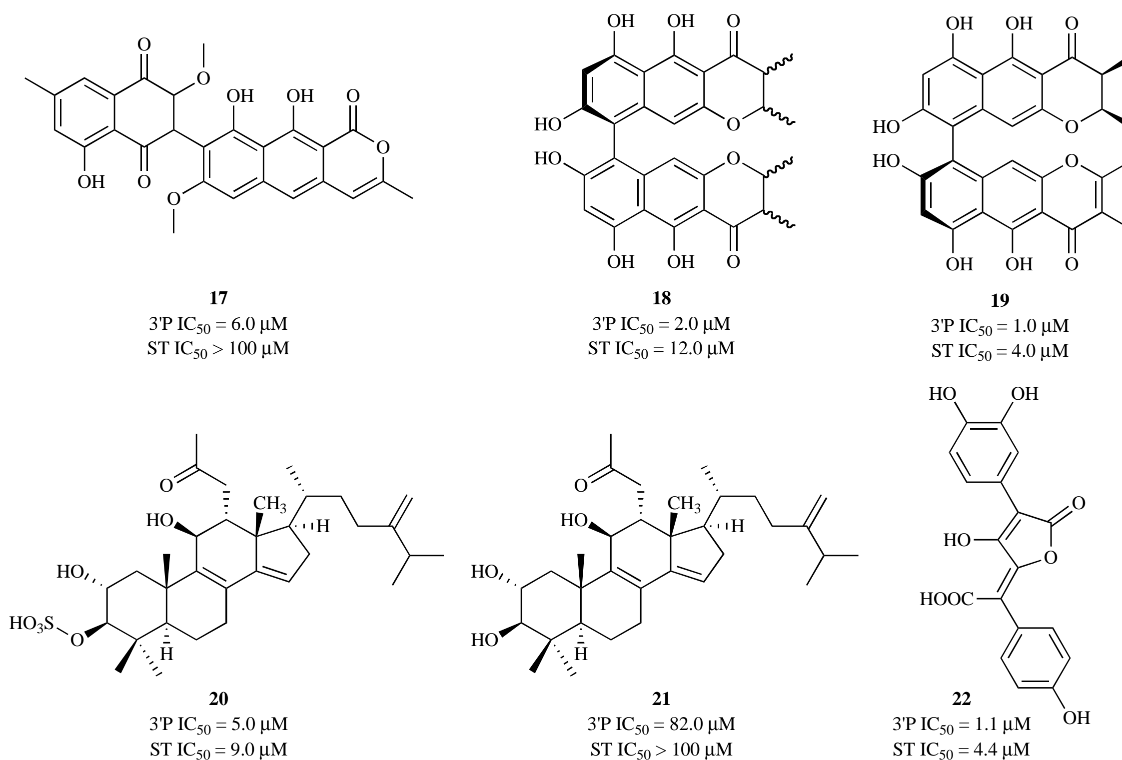


Fig. (4) The structure and activity of a number of natural products identified as HIV-1 IN inhibitors.



(Fig. (5). Contd….)

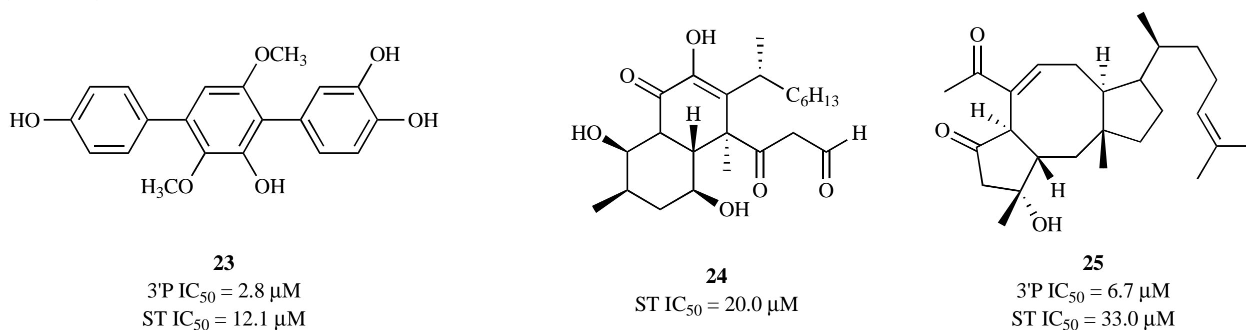


Fig. (5). The structure and activity of a number of natural products recently identified as HIV-1 IN inhibitors.

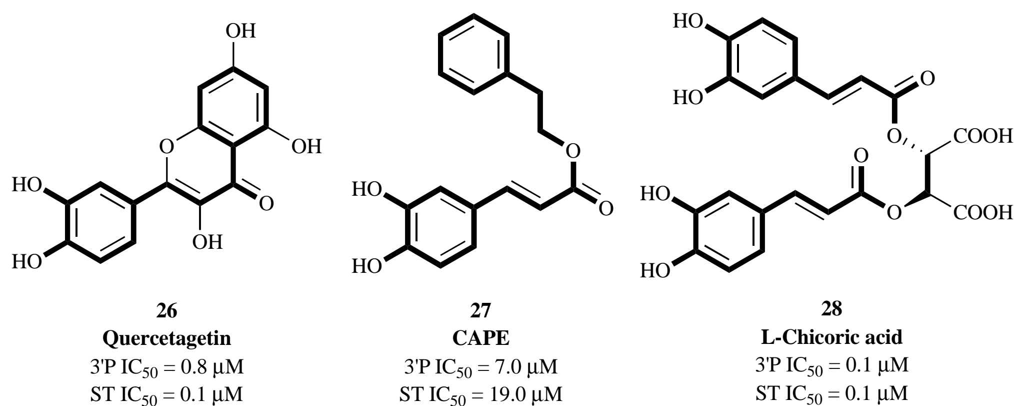


Fig. (6). The structure and activity of quercetagetin (**26**), CAPE (**27**), and L-chicoric acid (**28**). The majority of the PHAs share a common architecture consisting of two aryl units, one of which contains the 1,2-catechol pattern, separated by a linker segment.

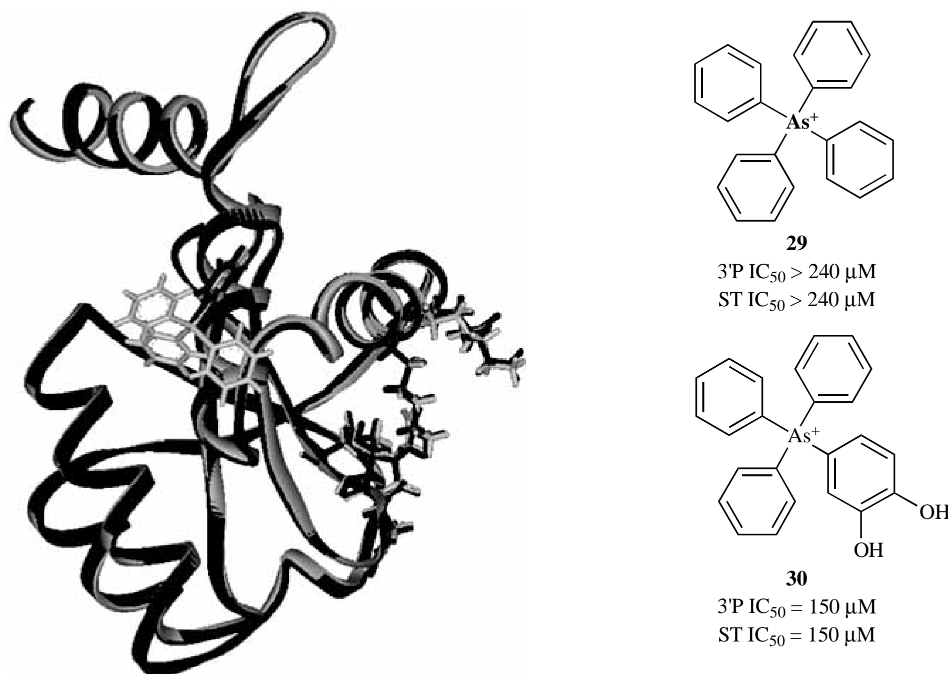


Fig. (7). Superimposition of the HIV-1 core backbone co-crystallised with the inactive derivative **29** (PBD code 1HYV[57]; core domain, grey) on the core domain backbone co-crystallised with **30** (PBD code 1HYZ[57]; black). It has been proposed that the binding of **30** induces a number of conformational changes to a number of key active-site residues while binding of **29** does not induce any conformational changes [57]. However, superimposition of the two structures reveals no significant conformational changes to any key active-site residues (active-site residues of both 1HYV and 1HYZ are represented as sticks).

effects of a significant number of PHAs can be attributed to non-integrase-dependent phenomena. For example, the primary antiviral target of the L-chicoric acid analogues is the viral envelope glycoprotein gp120 [96]. Furthermore, a large portion of the PHAs are cytotoxic which is believed to result from non-selective binding and, perhaps more importantly, from the formation of oxidised species, such as semiquinones or orthoquinones, which form protein or possibly DNA adducts [97,98].

In an attempt to separate the mechanism of cytotoxicity and IN inhibition a number of monohydroxylated [95] derivatives as well as methoxy (**31**, Fig. **8**) [95] and acetyl protected catechol analogues [99] were produced, none of which exhibited a significant inhibitory response. Further, as illustrated by **33** and **34** [93], which were designed to mimic the two distinct rotational isomers of CAPE, it appears that subtle conformational factors play a significant role in inhibitor binding. This indicates the Achilles' heal of the PHAs is the absolute requirement of a catechol moiety.

In an order to identify novel PHA inhibitors devoid of the critical catechol functionality a ligand based molecular modelling approach was adopted from which a number of novel salicylhydrazides were identified as IN inhibitors (**35** & **36**, Fig. **9**) [100]. A structure-activity relationship (SAR) study of the lead compounds indicated that the two 2-hydroxy moieties may mimic a catechol as the removal of one or both moieties [100], or separation of the hydroxyls *via* chain extension of the hydrazine linker yielded inactive compounds [100]. To investigate the potential binding mode of these compounds three mercaptosalicylhydrazide compounds (MSHs), **37**, **38**, and **39** were designed and synthesised. The MSHs were found to be 300-fold less cytotoxic and exhib-

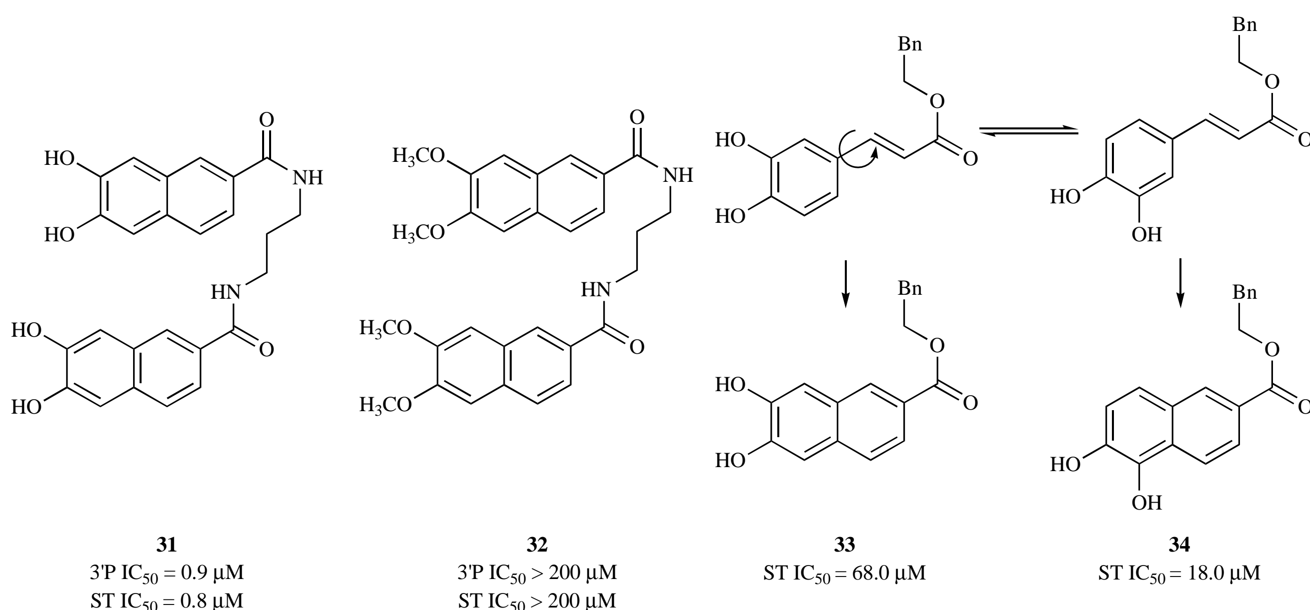


Fig. (8). The structure and activity of **31** and **32** illustrating the dramatic reduction in activities resulting from the protection of the catachol moieties. Further the structure and activity of **33** and **34**, which were designed to mimic the two distinct rotational isomers of CAPE indicating that subtle conformational factors play a significant role in inhibitor binding.

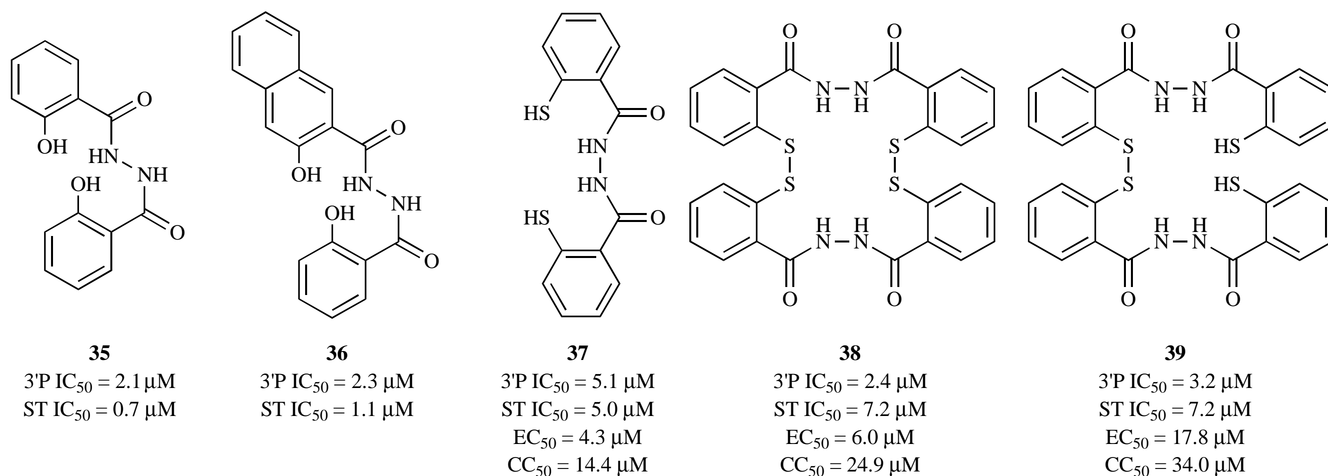


Fig. (9). The structure and activity of the salicylhydrazides **35** and **36** identified during a pharmacophore study [100] and the mercaptosalicylhydrazide compounds (MSHs) **37**-**39** synthesised in an ensuing SAR study [100].

ited antiviral activity. Further site-directed mutagensis and molecular modelling studies indicated that the MSHs bind to Cys-65, located in the active-site, and chelate Mg^{2+} [100].

Coumarins, exemplified by **40** [101] (Fig. **10**), are also a novel class of non-catechol-containing PHA IN inhibitors. An SAR study of over 30 novel coumarins indicated that a coumarin dimer (**41**) [101] was sufficient for activity and in general, a hydrophobic linker was required for retention of potency while hydroxylation of the coumarin ring at positions C4 and C7 increased potency (**42** & **43**) [101]. Further, a photoaffinity study, which utilised a number of coumarins possessing a photo-activatable benzophenone moiety, indicated that binding occurred at the core dimer interface [102]. suggesting the coumarins exhibit their inhibitory effects by causing a steric obstruction of the dimer complex [102].

Despite the enormous number of PHA IN inhibitors identified, only a handful of patents have been filed. To date, the only patented PHAs are the chicoric acids [96], 3,5-dicaffeoylquinic acids [103], and caffeoyl naphthalenesulfonamides [99]. Two other patented compounds are an amino acid derivative [95] and the non-viral entry inhibitor lithospermic acid [95], isolated from *Salvia miltiorrhiza*. However, all of these compounds have failed to reach clinical trials, presumably due to poor cellular uptake and non-specific inhibition. Nevertheless, the development of a therapeutically viable PHA remains an attractive proposition as it is envisaged that a diet rich in natural PHAs such as chicoric acids and lithospermic acids, commonly found in vegetables such as lettuce, may reduce therapeutic doses [104].

ARYL-β-DIKETOACID IN INHIBITORS

Of the currently reported classes of HIV-1 IN inhibitors, the aryl-β-diketoacid containing compounds (DKAs), exemplified by L-731988 (**44**) [105], are the most developed biological validated inhibitors. Indeed a member of this class S-1360 (**45**) [106] was one of the first IN inhibitors to enter into clinical trials. The DKAs or 4-aryl-3-oxo-2-hydroxy-butenoic acids as they adopt this tautomeric conformation in solution [107], were independently identified by both the Merck and Shionogi companies [108-110].

Although the diketo functionality, which is believed to chelate two magnesium ions [111], is an intrinsic feature of the DKAs it is not sufficient for activity. As typified by compound **46** [112] (Fig. **12b**) which appears to be the minimum scaffold required for activity, DKAs require an aryl portion and an acid portion.

A member of the DKA family, 5CITEP (**47**, Fig. **12a**) [54], was subsequently cocrystallised with the enzyme catalytic core providing the first X-ray crystal structure of an inhibitor in complex with the active-site [54]. The co-crystal

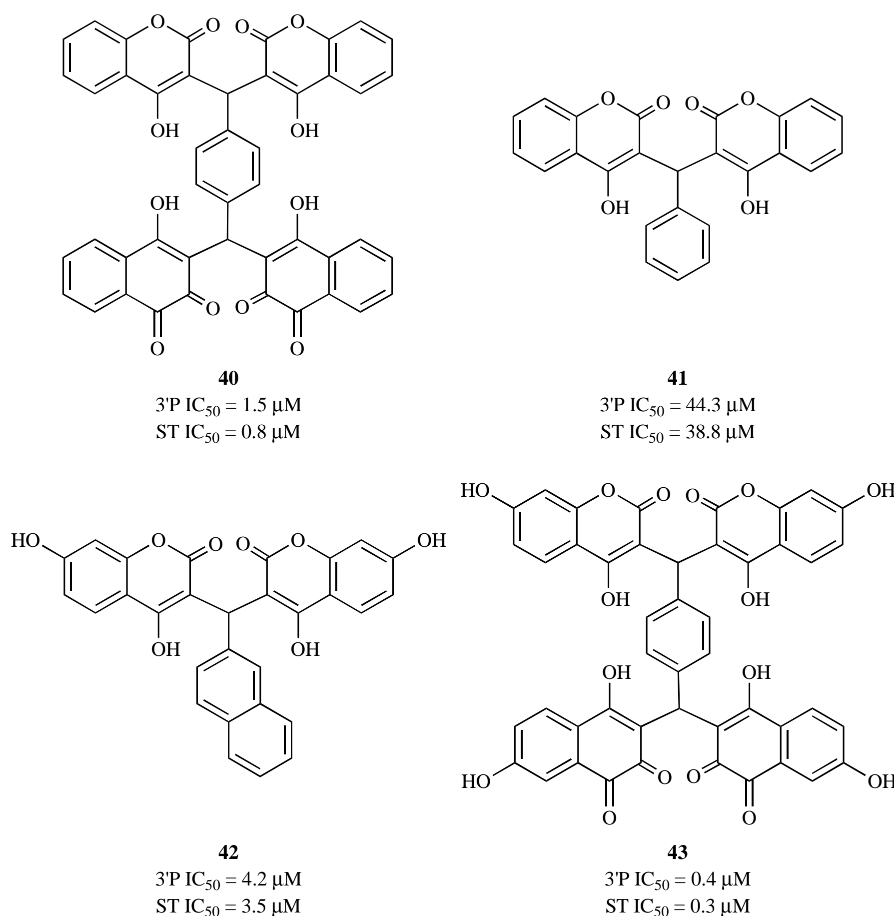


Fig. (10). Structure and activity of a number of coumarin-based HIV-1 integrase inhibitors.

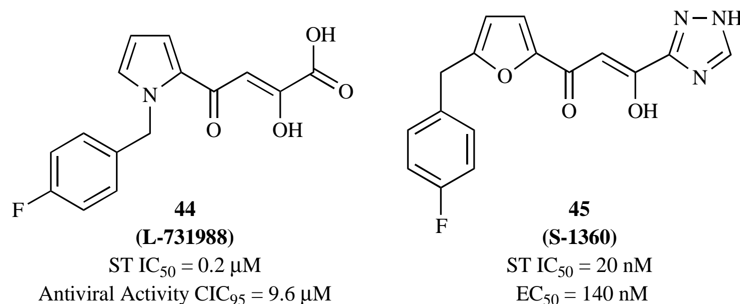


Fig. (11). The structure and activity of two members of the DKA family of HIV-1 IN inhibitors.

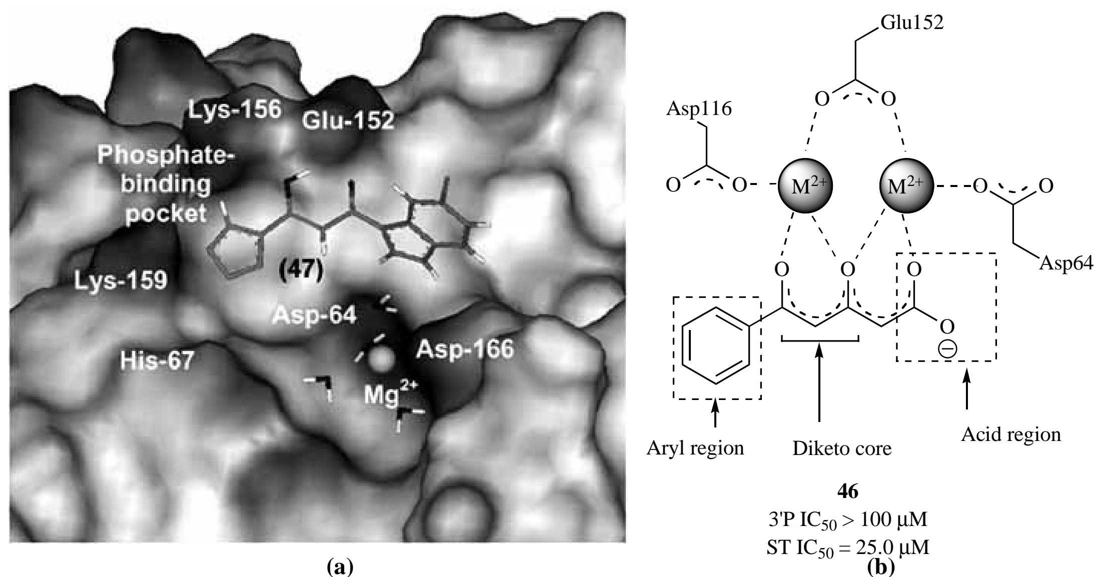


Fig. 12: (**a**) Co-crystallised structure of the HIV-1 IN catalytic core (a solvent exposed surface is shown with charged residues highlighted) with 5CITEP (**47**). The tetrazole moiety occupies the phosphate binding pocket. The co-crystallised crystal structure discloses minimal interactions between the ligand and the resolved Mg^{2+}. (**b**) The structure, activity, and proposed di-metal binding interactions of **46**.

structure of the core with 5CITEP [54] indicates the carboxylic acid, or in the case of 5CITEP the carboxylic mimic tetrazole, occupies the phosphate bind-pocket forming interactions with Lys-156 and Lys-159, whilst the aryl portion occupies a hydrophobic region of the active-site. However, the 5CITEP co-crystallised crystal structure disclosed minimal interactions between the ligand and the resolved Mg^{2+}.

In general, the DKAs are characterised by an ability to afford preferential inhibition of strand transfer (ST) versus 3-processing (3'-P). The high selectivity for the strand-transfer reaction indicates that the DKAs bind to the enzyme following 3'-processing [4]. This inhibitory response is generally believed to be dependent on the presence of a divalent metal ion(s), which are chelated by the diketo moiety subsequently blocking DNA substrate binding [113,43,407,114]. Support for such a hypothesis is provided by the lack of inhibitory action elicited by the DKAs in the absence of Mn^{2+} or Mg^{2+} [115]. However, the 5CITEP cocrystallised crystal structure discloses minimal interactions between the ligand and the resolved Mg^{2+}. Thus, there are growing and popular notions in support of a two-metal ion theory, which is a typical feature of various DNA binding enzymes such as Hepatitis C virus (HCV) polymerase [43,111].

A number of structure-activity-relationship (SAR) studies have revealed that the aryl portion of the DKAs can accommodate a diverse range of substituents. It appears as if the most significant increases in whole cell activity and reduction of cytotoxicity can be gained with the introduction of hydrophobic substituents on the aryl moiety such as benzyloxy, benzyl, or phenyloxy at the 3, and 3,5-positions (**48** [112] & **49** [105], Fig. **13**). However, as illustrated by **50** [116], the size of these substituents appears to be limited and if hydrophilic substituents are introduced selective inhibition of strand-transfer is removed **51** [117] suggesting such analogues may elicit there inhibitory response *via* a slightly different mechanism.

Slight improvements in the antiviral activity of the mono-substituted variants, exemplified by **52** [118] (Fig. **14**), can be gained with the introduction of a halogen atom at the 2'-position on the distal benzene ring, while halogen introduction at the 3'-position and the 4'-position leads to no increase or a significant loss in activity respectively [118]. Further, slight increases in whole cell activity can be achieved with the introduction of small substituents such as methoxy (**53**, Fig. **14**) [118], ethoxy, and isopropoxy at the 2-position [117,118].

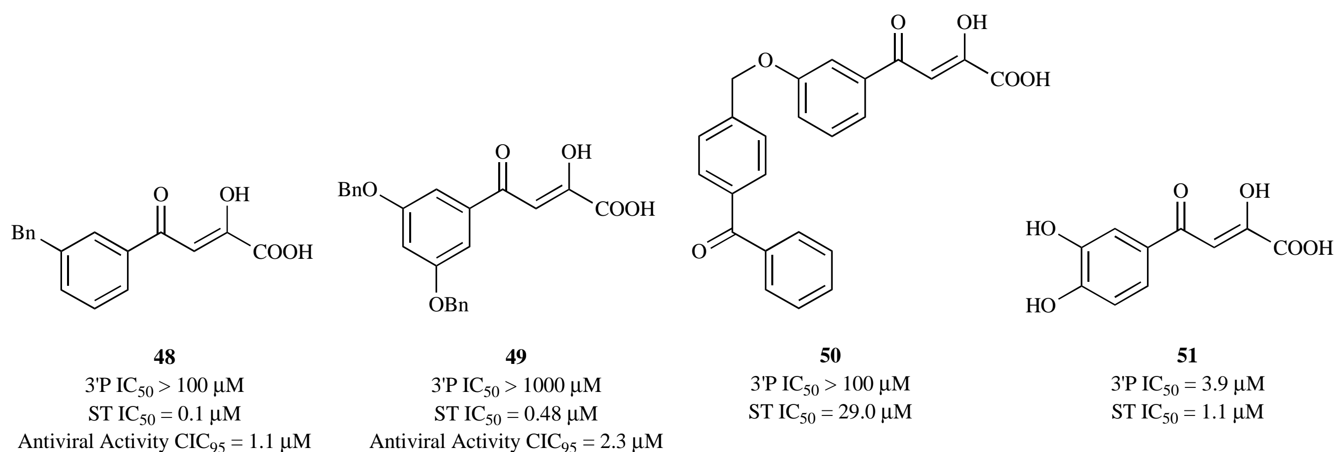


Fig. (13). Structure and activity of a number of DKA inhibitors.

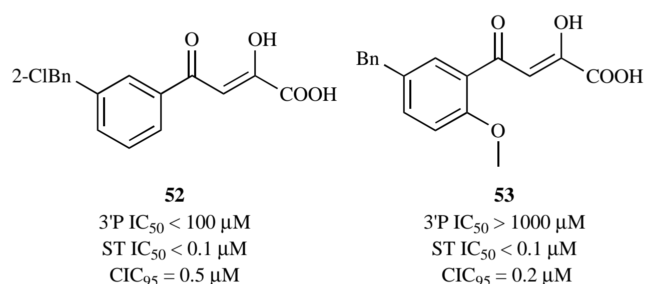


Fig. (14). Structure and activity of a number of DKA inhibitors with **53** illustrating whole cell activity can be increased with the addition at C2.

Although it is established that substituents at the 3, 4, or 5 positions on the aryl portion play a crucial role in the inhibitory process, it is unclear what electronic and chemical properties of such subsitituents are required to elicit potent antiviral effects. Replacement of the benzyloxy group on **49** [118] (Fig. **13**) with a benzoylamino group [118] reduces potency by approximately 100-fold. However, substituting the benzyloxy substituent with an azidomethyl group [119] reduces potency only by approximately 4-fold. Further quinoline and ferrocenyl groups were poorly tolerated leading to inactive or cytotoxic inhibitors (**54** & **55**, Fig. **15**) [112]. Moreover, while a number of the indole derivatives exhibited potent *in vitro* activity, the majority displayed limited antiviral activity and the select few analogues displaying *in vivo* inhibition (e.g. **56** Fig. **15**) [112,120] were cytotoxic.

In keeping with the indole DKAs, purine (**57** & **58**, Fig. **16**) [121] and pyrimidine (**59** & **60**) [122] analogues displayed potent *in vitro* activity, reduced *in vivo* activity and reduced strand-transfer selectivity. However, the reduction of selectivity of these analogues is not expected as they mimic the natural substrate.

It is apparent that the subsitituent orientation relative to the diketo acid functionality is significant. As illustrated by compounds **61** to **65** [118], (Fig. **17**) potency increases as the angle between the two lines extending from the benzyl and diketoacid moiety increases from 60° to 120° and decreases as the angle exceeds 120°.

In contrast to the aryl portion of the DKAs, the central diketo moiety has received considerably less attention with the only major study investigating the effect of elongating the diketo group from a 2,4-dioxobutanoic acid to a 2,4-dioxo-5-hexenoic acid [123]. This extension was conceived as a result of partial superimposition between the cinnamoyl group of various natural and synthetic IN inhibitors (Fig. **18**). One of the highly potent compounds from this series, **66** [123] displayed moderate *in vitro* activity against strand-transfer and 3'-processing, however, surprisingly **66**, showed remarkable antiviral activity, each with EC_{50} values of 1.5 µM, similar to the most potent IN inhibitors reported.

An additional variation of the DKAs includes a number of dimeric acids, these inhibitors were designed on the hypothesis that two diketo moieties could simultaneously bind

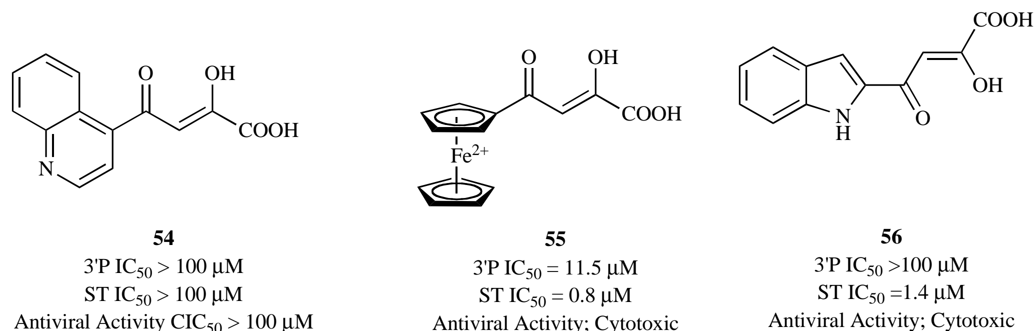


Fig. (15). Structure and activity of a number of DKA variants.

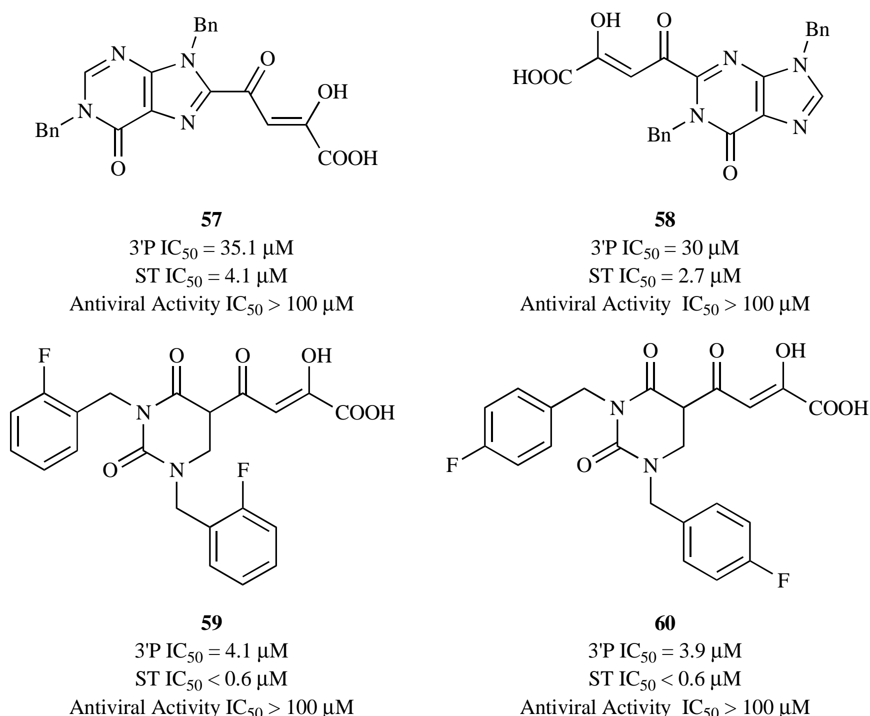


Fig. (16). The structure and activity of the purine **57** & **58**, [121] and pyrimidine **59** & **60**, [122] DKA analogues.

to the two divalent metal ions within the IN active-site. As a whole, the dimeric derivatives displayed reduced strand-transfer selectivity and the initial dimeric acids, **67** [112], **68** [112], and **69** [112], displayed limited antiviral activity (Fig. **19**).

Second generation dimeric DKAs include a number of amide (**70**, Fig. **20**) [43] or benzyl **71** [43] linked analogues. Unfortunately, the majority of the amide-linked derivatives were found to be cytotoxic and it is unclear what effects linker geometry has on activity [43]. In contrast, the benzyl-linked diketoacids were considerably less toxic, however, the majority displayed minimal anti-viral activity.

An additional series of dimeric based DKA analogues were developed based on the premise that alkylation at the 1-

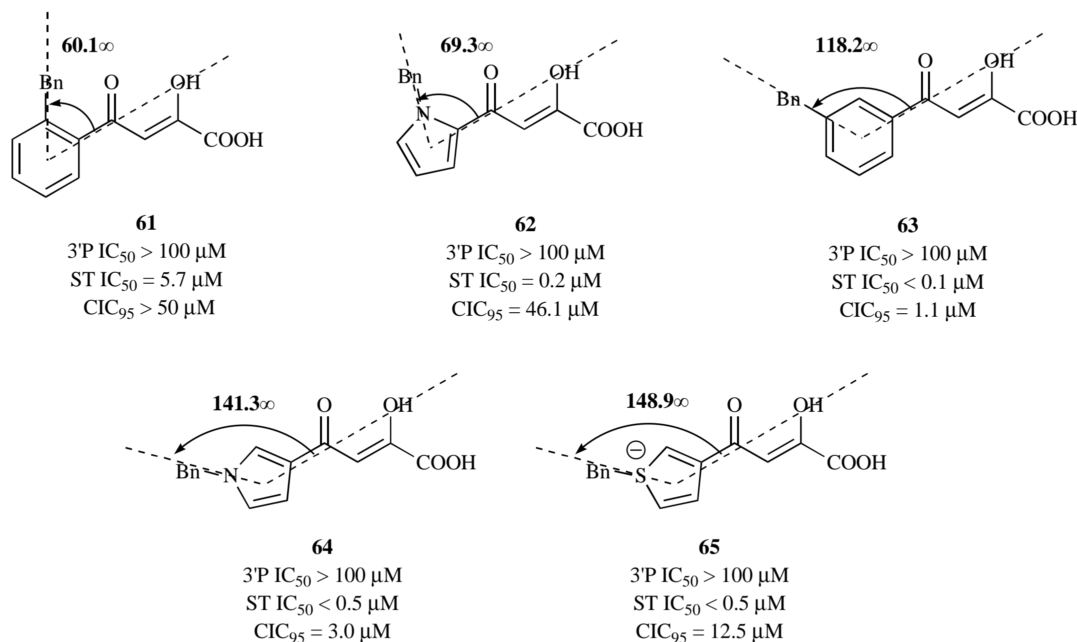


Fig. (17). A number of DKA derivatives illustrating potency increases as the angle between the two lines extending from the benzyl and diketoacid moiety increases from 60° to 120° and decreases as the angle exceeds 120°.

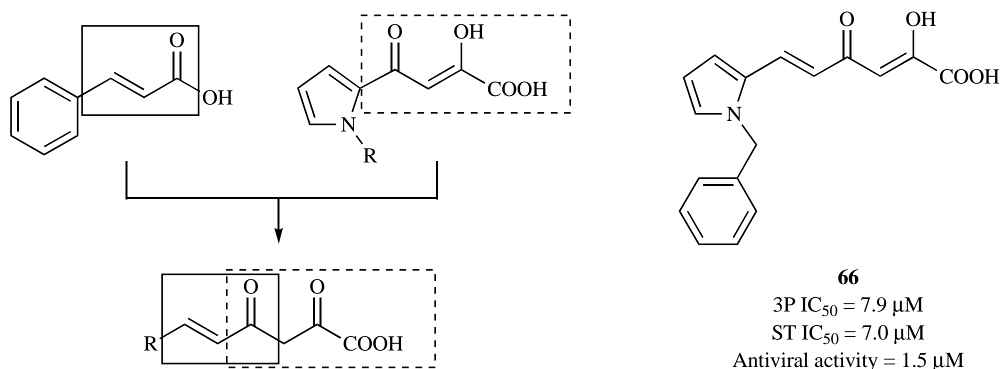


Fig. (18). The design principle behind the dioxohexenoic acids and the structure and activity the most active member of the series **66**.

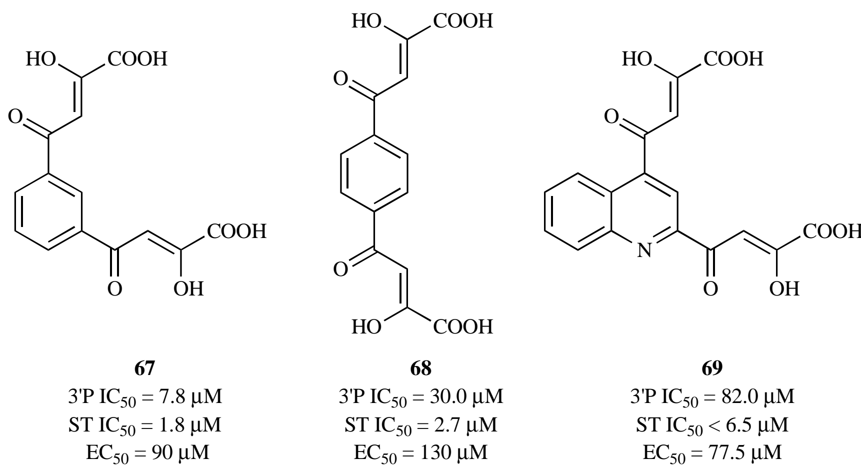


Fig. (19). The structure and activity of the first dimeric DKA analogues.

position with a benzyl group to obtain 1,3-disubstituted compounds[115] would afford the geometric requirements for optimal IN inhibitory activity. This approach proved valid with the two 1-*p*-fluorobenzyl-derivatives **74** & **75** (Fig. **21**) displaying considerably lower effective concentrations than the unsubstituted counterparts (**72** & **73**) [115]. Furthermore **75** is the most potent bifunctional diketoacid derivative reported to date.

Unfortunately, the 1,3-diketoacid functionality is biologically labile, giving rise to the derivatives containing diketoacid bioisosteres. A number of diketoacid bioisosteres have been shown to be tolerated on the acid side of the scaffold, these include diketotriazole **45** [106], diketotetrazole **47** [54], keto-iminoacid **76** [124] and diketopyridine [125].

However, it appears that the size of the heteroaryl ring is limited as various substituted tetrazole analogues (**77**, **78**, and **79**, Fig. **23**) [112] were devoid of activity. A possible explanation for this loss of activity arises from inspection of the 5CITEP co-crystallised structure (Fig. **12**), which infers that the tetrazole ring occupies the relatively polar and con-

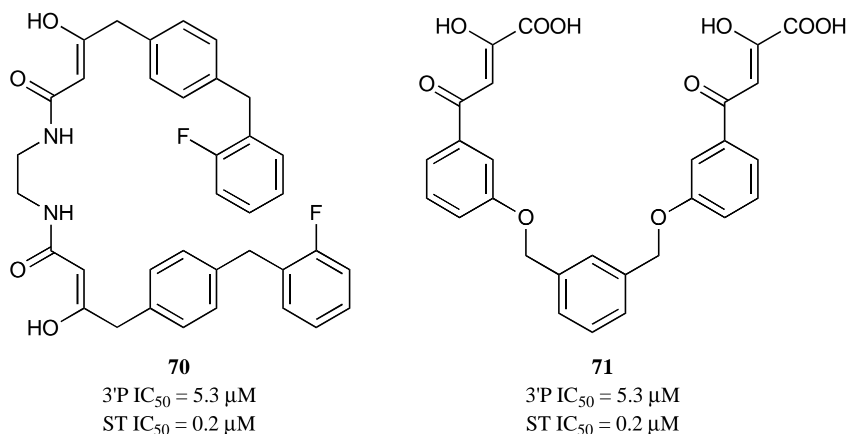


Fig. (20). Structure and activity of the second generation dimeric DKAs **70** and **71**.

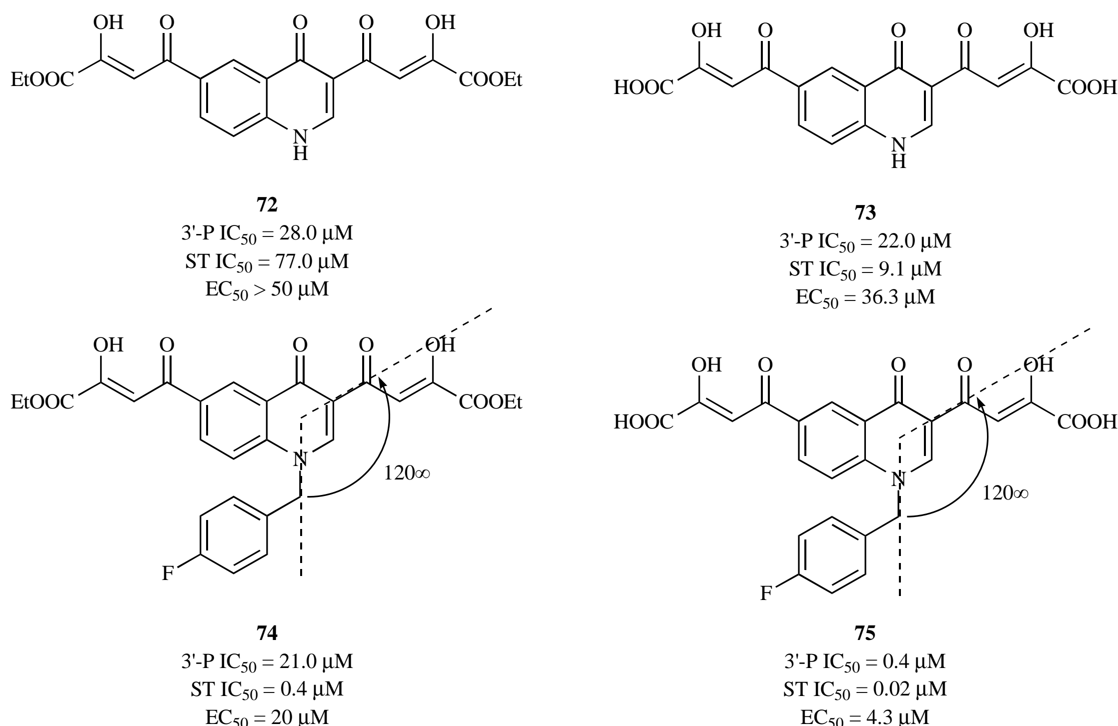


Fig. (21). Structure and activity of the bifunctional quinolonyl DKA derivatives **72**-**75**. Further these compounds highlight the need for a free carboxylic acid moiety.

fined phosphate-binding pocket [54]. Further, as illustrated by compound **80** (Fig. **23**) [124], it appears that an optimum distance between diketomimetic atoms must be maintained.

NAPHTHYRIDINE IN INHIBITORS

In order to mimic the diketoacid motif three functional regions are required, a ketone mimic, an enolisable ketone, and carboxyl oxygen, all of which adopt a coplanar conformation with the aryl portion of the DKA scaffold. Based on this rational, it was hypothesised that a similar arrangement of key hydroxyl groups could be achieved by a 1,2-catechol and by incorporating an oxygen bridge, coplanarity between to two phenyl rings could be achieved (**81**, Fig. **24**) [126]. Unfortunately, as outlined previously 1,2-catechols are biologically labile producing toxic metabolites, while coplanar polyaromatic ring systems typically exhibit poor physical properties [127]. However, based on previous SAR studies, it was proposed these limitations could be alleviated with the removal of the bridgehead carbon and substitution of one of the catechol hydroxyl groups with an aromatic nitrogen [126]. Furthermore, it was anticipated that resulting loss of coplanarity could be re-established with the incorporation of a heteroatom at an appropriate position on the aryl ring. These considerations led to the synthesis of a number of potent [1,6]naphthyridine analogues (**82** & **83**, Fig. **24**) [126].

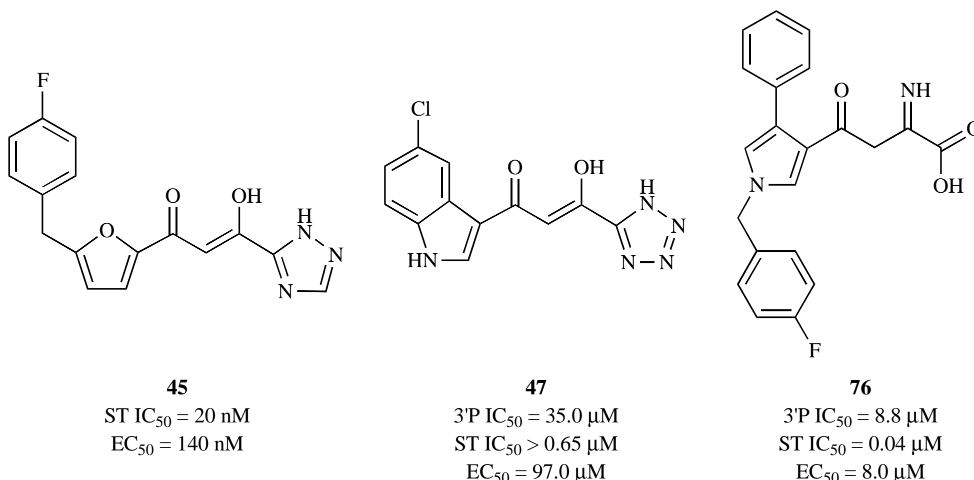


Fig. (22). Structure and activities of a number of HIV-1 IN inhibitors possessing diketoacid bioisosteres.

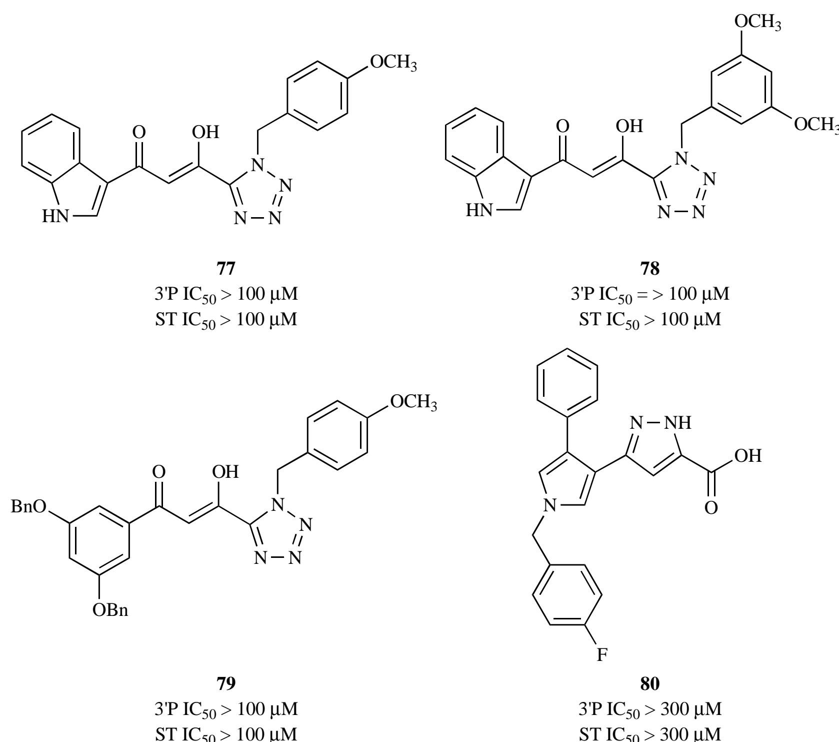


Fig. (23). Structures and activities of compounds containing substituted tetrazole moieties and the structure of the pyrazole containing derivative **80**.

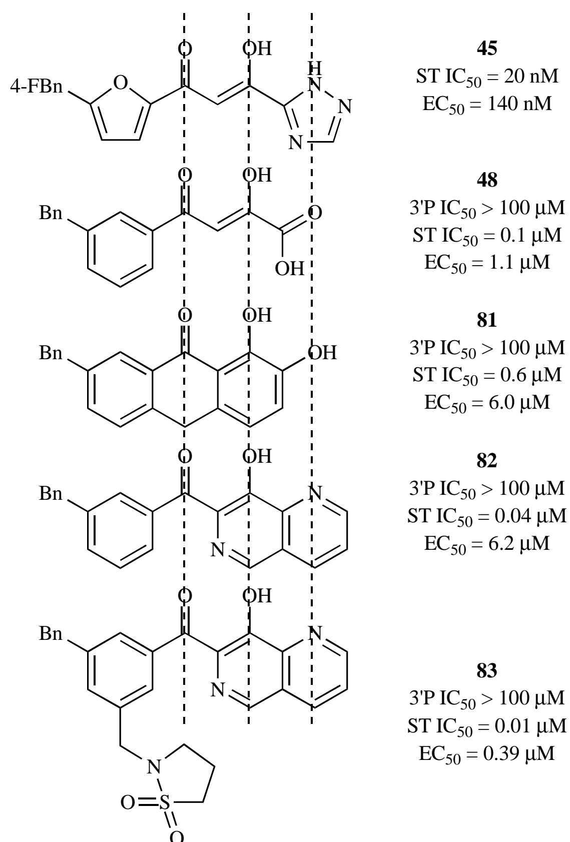


Fig. (24). Structures and activities of a number of HIV-1 IN inhibitors possessing diketoacid bioisosteres. The atoms of the diketoacid bioisosteres are with the dotted lines.

Extensions of the 8-hydroxy-[1,6]naphthyridine series lead to the 8-hydroxy-[1,6]naphthyridine-7-carboxamide derivatives (**84**, Fig. **25**) [128]. Significant improvements in both intrinsic potency and pharmacokinetic properties were obtained with the addition of polar functionalities, such as uracils, amides, and heterocyles, at the 5-position on the naphthyridine backbone [128]. A member of this series, L-870810 **85** [129,28] entered phase I/II clinical trails and although phase II trials were halted due to liver and kidney toxicity another member of this series L-870812 **86** [128] is currently still in trials.

STYRYLQUINOLINES IN INHIBITORS

A number of heterocyclic IN inhibitors which overlay the 8-hydroxy-[1,6]naphthyridine-7-carb oxamide pharmacophore have been identified. Amongst the first reported were the styrylquinolines (SQs), exemplified by compound **87** [130] (Fig. **26**) In contrast to the 8-hydroxy-[1,6]naphthyridine-7-carboxamides the SQs display non-integrase dependant antiviral activity and reduced selective inhibition of strand-transfer [131]. It has been demonstrated that the SQs specifically and efficiently inhibit *in vitro* nuclear import of IN suggesting that they inhibit the interaction between the enzyme and the cellular factor required for its nuclear import [131].

Unlike the PHAs, the catechol moiety of **87** does not induce toxicity nor is its crucial for activity [132]. A substantial amount of SAR data relating to the SQs has been complied [133-136,130] and it is evident that numerous aryl moieties such as furan, thiophene, 4-nitrobenzene, and pyridine are well tolerated on the catechol side of the SQ scaffold [132]. In contrast to the catechol portion, variations to

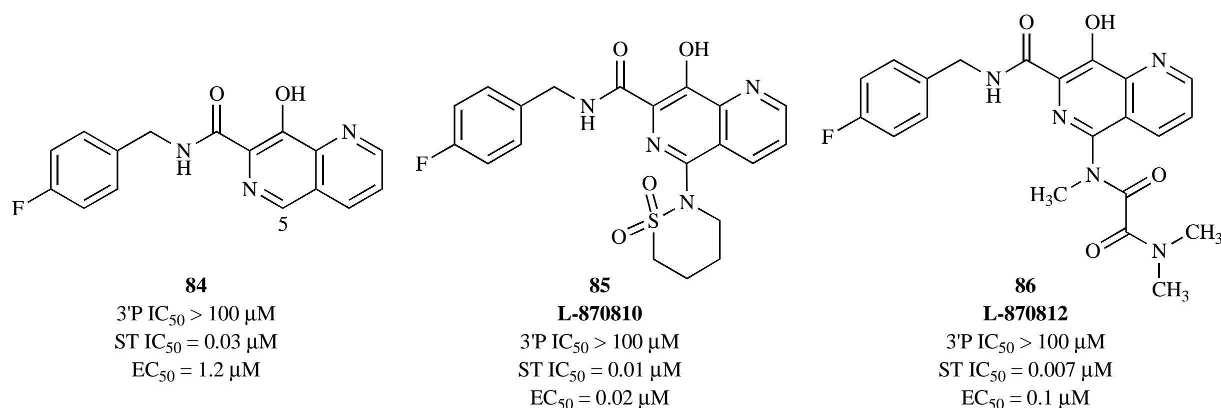


Fig. (25). The structure and activity of the the 8-hydroxy-[1,6]naphthyridine-7-carboxamide derivatives **84**-**86**.

the linker functionality are poorly tolerated [133]. Further, as expected, removal [134], protection [130] or extension [136] of the carboxylic acid functionality was detrimental to activity. An additional variation to the SQ scaffold theme was a series of styrylbenzofuran derivatives [135], however, these derivatives were considerably less active than their quinoline counterparts. However, a significant increase in activity, and unfortunately cytotoxicity, can be achieved with the introduction of the 3,4,5-trihydroxybenzene moiety [132], yet, methyl protection of one of these hydroxyls results in the dramatic reduction of cytotoxicity, so much so that compound **88** [132] (FZ-41, Fig. **26**) is poised to enter clinical trials.

More recently, 4-quinolone-3-carboxylic acid was identified as a promising masked diketoacid scaffold. Significant improvements in the antiviral activity of lead compound **89** [127] (Fig. **27**) were achieved with the introduction of 2-fluoro and 3-chloro substituents into the distal benzene ring and alkylation of the quinolone nitrogen with a hydroxyethyl moiety **90** [127]. Further, the introduction of a methoxy group at the 7-position of the quinolone ring of and an isopropyl group at the 1*S*-position of the hydroxyethyl **91**, JTK-303 [127] moiety led to a far more potent inhibitor which is currently under phase I/II clinical trials.

PEPTIDE INHIBITORS OF HIV-1 INTEGRASE

Recent advances in peptide synthesis and combinatorial chemistry have resulted in the generation and screening of peptide libraries becoming an essential tool in the drug dis-

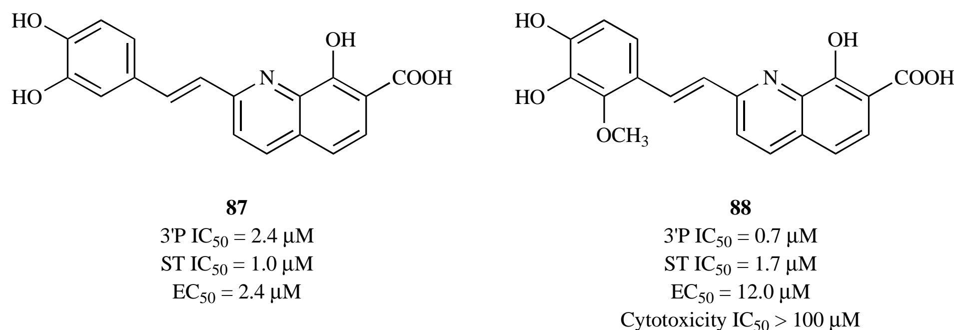


Fig. (26). Structure and activity of the styrylquinoline derivatives **87** and **88** (FZ-41) which is poised to enter clinical trials.

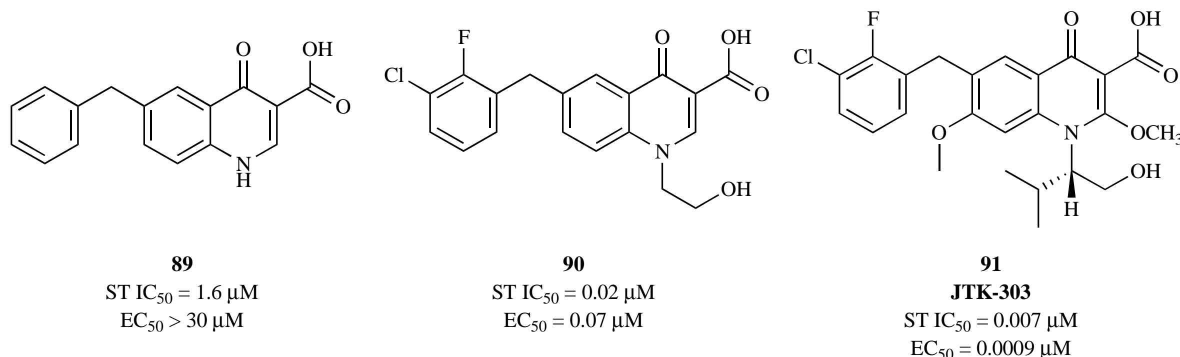


Fig. (27). Structure and activity of the 4-quinolone-3-carboxylic acid derivatives **89**, **90** and **91** (FZ-41) which is poised to enter clinical trials.

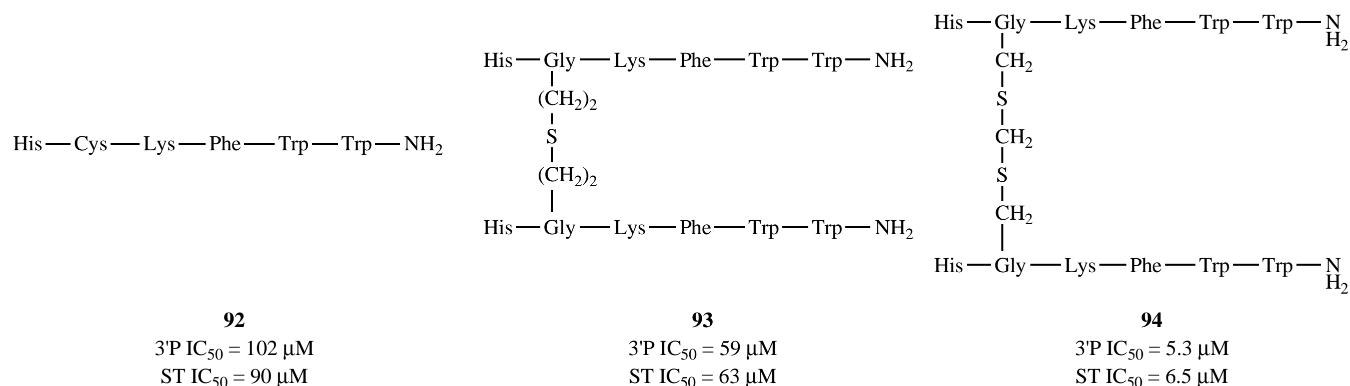


Fig. (28). Structure and activity of the peptide base inhibitors **92**-**94**.

covery process. However, surprisingly, only a handful of peptides or peptidomimetics have been identified as IN inhibitors.

The first peptide IN inhibitor isolated from a combinatorial library was the hexapeptide **92** [137] (Fig. **28**) During an ensuing SAR study of **92**, an number of dimer analogues possessing variable linkers, such as L-homolanthionine **93** [138] or L-djenkolic acid **94** [138], were synthesised. Of these compounds the dithiomethylene linked dimer **94** showed an approximately 20-fold higher potency compared to the monomer indicating that the dimeric peptide may act as a bivalent inhibitor, simultaneously occupying neighbouring catalytic sites.

Additional IN peptides include Indolicidin (**95**, Table **2**) a tryptophan rich 13-mer natural antibacterial peptide [139,140], a 33-mer known as I_{33} [141], and a number of 7-mer derivatives exemplified by **96** and **97** (Table **2**) which were identified from the random screening of a heptapeptide phage-display library [142].

Recent studies have shown that the catalytic activity of IN is inhibited *in vitro* by HIV-1 reverse transcriptase (RT) [143]. In a random screen of HIV-1 RT derived peptides two 20-residue-long peptides **98** [143] and **99** [143] were identified as possessing anti-IN activity (Table **3**).

As outlined previously the catalytic core dimerises through mutual interactions between a number of secondary structures, such as alpha helices 1, 3, 5, 6 and beta-strand 3 [60]. Consequently, two synthetic peptides, INH1 [144] which corresponds to α1-helix residues 93-107 and compound INH5 [144], corresponding to α5-helix residues 167-187, were synthesised. Both of these compounds exhibited inhibitory effects with INH5 displaying IC_{50} values of 85 nM and 60 nM for 3'-processing and strand transfer activity respectively [144]. More recently, the mimics of all the interfacial secondary structures were synthesised. Although shorter than the initial mimics, the α1 (**100** Table **4**) [60], and α5 **101** [60] derivatives along with α-6 mimic **102** [60] displayed potency activity against 3'-processing. Further, protein crosslinking experiments conducted in the presence of the α1, α5, and, α5 indicated the reduced amounts of dimer-core domains were formed indicating that these peptides prevent dimerisation of the IN core domains [60].

SULFONATED HIV-1 INTEGRASE INHIBITORS

Diverse arrays of sulfur containing compounds such as sulfonates, sulfones, and sulfides, have been identified as HIV-1 IN inhibitors, however with respect to quantity, the most important class is the sulfonamides. Sulfonamides are well known antimicrobial drugs with a well established

Table 2. Sequence and Activity of the Peptide Based IN Inhibitors 95-97

Peptide	Sequence	3'-Processing Inhibition IC_{50} (μM)	Strand Transfer Inhibition IC_{50} (μM)
95	ILPWKWPWWPWRR-NH_2	60	57
96	HLEHLLF	0.1	13
97	FHNHGKQ	1.0	4.9

Table 3. Sequence and Activity of RT Derived IN Inhibitors 98 and 99

Peptide	RT-Derived Sequence	Position in RT (residue no.)	3'-Processing Inhibition IC_{50} (μM)	Strand Transfer Inhibition IC_{50} (μM)
98	KILEPFRKQNPDIVIYQYMD	Palm (133-185)	4.8	4.5
99	ELVNQIIEQLIKKEKVYLAW	RNase H (516-535)	6.9	5.0

Table 4. Sequence and activity of RT derived IN inhibitors 100 and 102

Peptide	Secondary Structure	Sequence	3'-Processing Inhibition IC_{50} (μM)
100	α1	$Q^{95}ETAYFLLKLAGRWP^{109}$-$CONH_2$	3.5
101	α5	$H^{171}LKTAVQMAVFIHNFKR^{187}$-$CONH_2$	3.0
102	α6	$A^{196}GERIVDIIATDIQ^{210}$-$CONH_2$	2.0

safety profile and are extensively used in the treatment of *Pneumocystis carinii* pneumonia, a leading cause of morbidity and mortality in AIDS patients. Consequently, a number of studies have focused on identifying sulfonamide based IN inhibitors, with a number of structurally diverse derivatives identified (**103-105** Fig. **29**) [145].

Amongst the more potent derivatives were a series of 2-mercaptobenzenesulfonamides (MBSAs), typified by **106** [146] (Fig. **30**). The majority of the MBSAs share a common architecture consisting of aryl units, one of which possesses a 2-mercapto moiety, separated by a sulfonamide-heteroaryl linker. SAR studies of the MBSAs indicated that various hydrophobic functionalities such as butane, were tolerated on the heteroaryl side of the scaffold [146]. Moreover, the introduction of a 4-chlorophenyl amide substituent on the mercaptophenol ring increased activity, however, crucial to activity was the presence of a free mecapto moiety [146].

Although number of MBSAs displayed potent *in vitro* activity, they were found to be metabolically unstable. In an attempt to improve stability, a number of cyclic derivatives were produced (**107** & **108** Fig. **30**) [147]. Unfortunately, none of the 3-aroyl-1,1-dioxo-1,4,2-benzodithiazines displayed potent activity, however, a number of these derivatives displayed significant differential potencies against the IN mutant C65S, versus the wild type enzyme, suggesting that these compounds potentially interact with C65, which in close proximity to the highly conserved catalytic triad [147].

The lack of potency displayed by the cyclic sulfonamide derivatives is believed to be primarily due to the lack of a

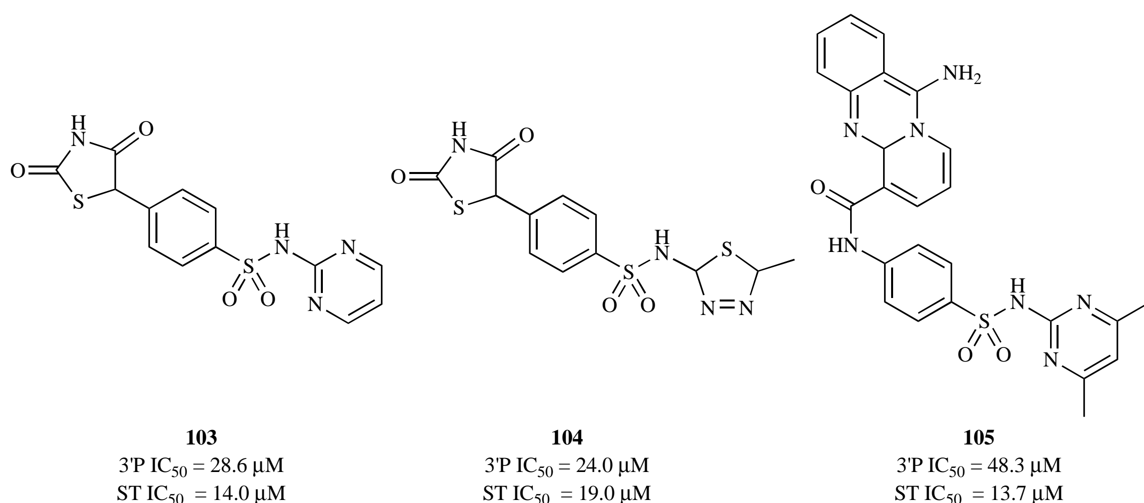


Fig. (29). Structure and activity of a number of sulfonamide based HIV-1 IN inhibitors.

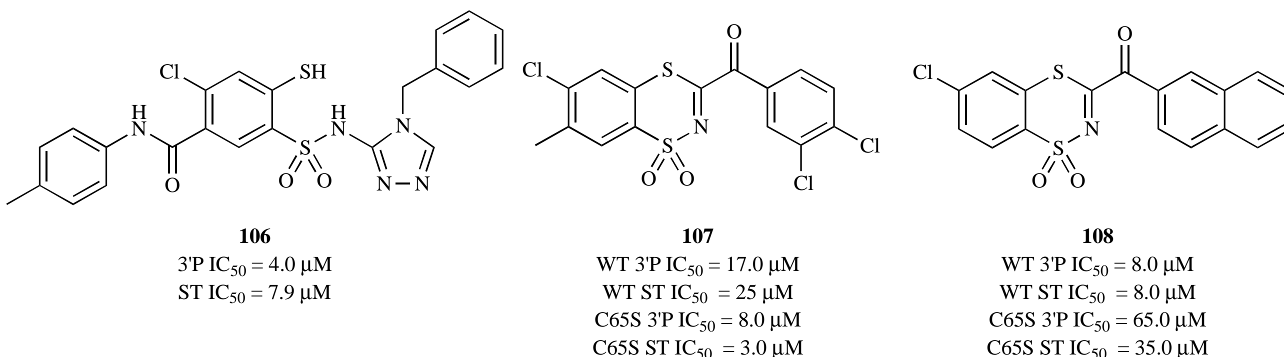


Fig. (30). Structure and activity of the 2-mercaptobenzenesulfonamide **106** and the 3-aroyl-1,1-dioxo-1,4,2-benzodithiazines derivatives **107** and **108**.

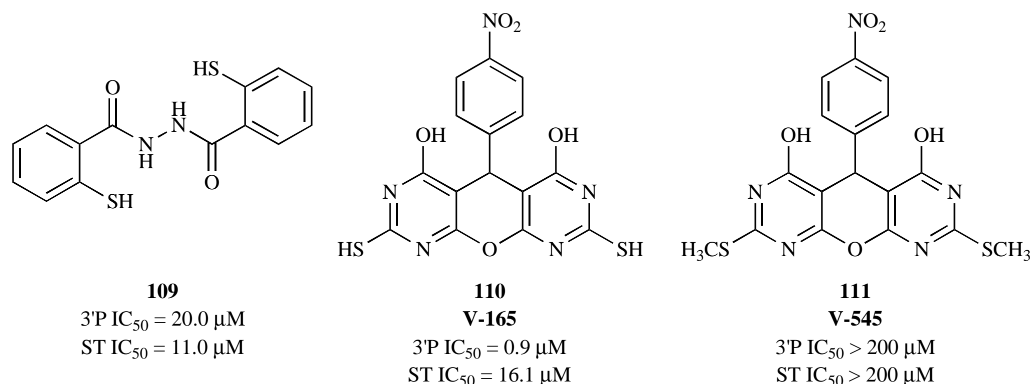


Fig. (31). Structure and activity of the mercapto containing derivative **109** and **110** in addition the protected analogue **111**.

free mercaptoaryl group. The mercapto moiety is a well know hydroxy isostere and is present in a number of IN inhibitor such as **109** (Fig. **31**) [100] and the phenyldipyrimidine analogue **110** V-165 [148] which is currently in preclinical trials. As with the cyclic sulfonamides, protection of the free mercapto moieties of **110** yielded an inactive compound (**111**, Fig. **31**) [148].

In keeping with sulfonamides, sulfone based compounds have a well established safety profile with a number of sulfone derivatives used in the treatment of malaria. A handful of sulfone-based compounds have been identified as IN inhibitors, the most potent being the geminal disulfonates, exemplified by **112** [148] (Fig. **32**) Further, a number of relatively simple diarylsulfones, exemplified by compound **113** [149] were identified in the screen of a National Cancer Institute (NCI) sulfone drug repository. However, a number of diarylsulfones have previously been reported as RT inhibitors, thus giving a possible explanation to their antiviral activity [150].

NAPHTHALENESULFONIC ACIDS AS POTENTIAL HIV-1 INTEGRASE INHIBITORS

Amongst the very first IN inhibitors reported was the polyanionic sulfonate suramin (**114**) [151-155] (Fig. **33**). A substantial number of *bis*-naphthalenesulfonic acids derivatives such as Suramin, have previously reported as HIV-1, and HIV-2 RT inhibitors [154,156-159,155].

Despite the potent anti-IN activity of suramin, and the *in vivo* activity displayed a number of naphthalenesulfonic acid analogues, to date, only a handful of derivatives, such as the moderately active monomers **115** [160], **116** [160], (Fig. **34**) and the active derivative **117** (**Y3**) have been identified as HIV-1 inhibitors. In addition to **Y3**, two other naphthalenesulfonic acids, **118** (**Y1**) [161] and **119** (**Y2**)[161], were

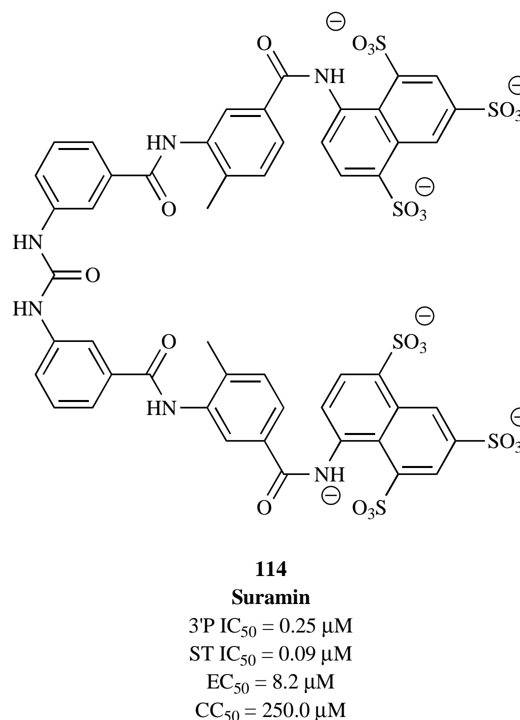


Fig. (33). The structure and activity of the polyanionic sulfonate suramin.

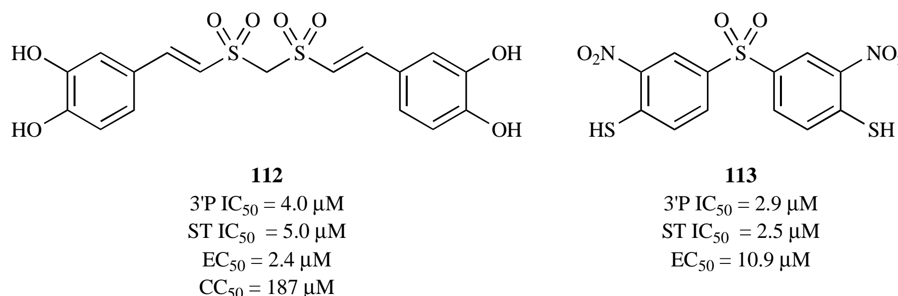


Fig. (32). The structure and activity of the geminal disulfonate **112** and the disulfone **113**.

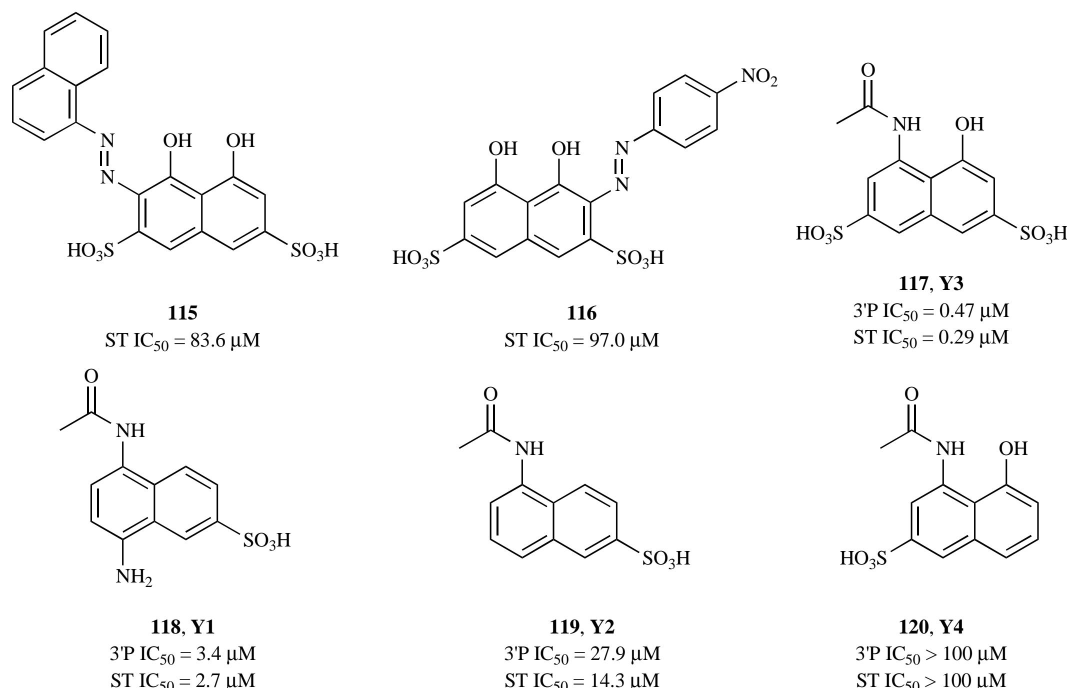


Fig. (34). Structure and activity of a number of naphthalene sulfonate HIV-1 integrase inhibitors.

identified using a three-point pharmacophore to screen the NCI three-dimensional database while an additional derivative **120** (**Y4**) [161] was synthesised in a insuring SAR study of the Y3 series.

No definitive conclusions regarding the mechanisms by which the naphthalenesulfonic acids inhibit IN have been reported, yet there is a consensus that they may reside within a third and as yet unexploited binding-site. In a co-crystallised structure of the avian sarcoma virus (ASV) core domain, which shares a 24% sequence homology with HIV-1 IN and 3-D structural alignment RMS deviation of 1.4 Å [162,54] **Y3** resided within a pocket removed from the active-site [161]. However, at present, there is no structural information of any HIV-1 IN-inhibitor complex that binds in this manner.

THE REQUIREMENT FOR SECOND AND THIRD GENERATION IN INHIBITORS

After a decade of research the first-generation IN inhibitors have been patented [164] and are entering clinical trials. However, continual cell-based viral exposure of a number of the most promising inhibitors in cells such as L-870812 has resulted in the emergence of a number of resistant strains [163]. Further, the current generation of IN inhibitors all appear to display similar binding interactions residing within the active-site or dimer interface. Thus, a single mutation within either of these regions may render a whole class of compounds inactive. This observation emphasises the need for the discovery of second and third generation IN inhibitors of novel structure and inhibition mechanisms.

ABBREVIATIONS

AIDS = Acquired immunodeficiency syndrome

CAPE = Caffeic acid phenethyl ester

CCD = Catalytic core domain

CTD = Carboxyl terminal domain

DKA = Aryl-β-diketoacid

HAART = Highly active antiretroviral therapy

HCV = Hepatitis C virus

HHCC = Histidine-histidine-cysteine-cysteine

HIV = Human immunodeficiency virus

IN = HIV-1 integrase enzyme

MBSA = 2-mercaptobenzenesulfonamides

MSH = Mercaptosalicylhydrazide

NCI = National Cancer Institute

NNRTI = Non-nucleoside reverse transcriptase inhibitor

NRTI = Nucleoside reverse transcriptase inhibitor

NTD = Amino-terminal domain

PHA = Polyhydroxylated aromatics

RT = Reverse transcriptase

SAR = Structure-activity relationship

SQ = Styrylquinolines

REFERENCES

[1] Broder, S.; Gallo, R. C. *N. Engl. J. Med.,* **1984**, *311* (20), 1292-1297.

[2] Lambert, D. M.; Bartus, H.; Fernandez, A. V.; Bratby-Anders, C.; Leary, J. J.; Dreyer, G. B.; Metcalf, B. W.; Petteway, S. R., Jr. *Antiviral Res.,* **1993**, *21*(4), 327-342.

[3] McCune, J. M.; Namikawa, R.; Shih, C. C.; Rabin, L.; Kaneshima, H. *Science,* **1990**, *247*(4942), 564-566.

[4] Pommier, Y.; Johnson, A. A.; Marchand, C. *Nat. Rev. Drug Discov.,* **2005**, *4*(3), 236-248.

[5] Berns, J. S.; Kasbekar, N. *Clin. J. Am. Soc. Nephrol.,* **2006**, *1*(1), 117-129.

[6] Burton, C. T.; Mela, C. M.; Rosignoli, G.; Westrop, S. J.; Gotch, F. M.; Imami, N. *Curr. Med. Chem.,* **2006**, *13*(26), 3203-3211.

[7] Grabar, S.; Weiss, L.; Costagliola, D. *J. Antimicrob. Chemother.,* **2006**, *57*(1), 4-7.

[8] Haugaard, S. B. *Expert Opin. Drug Metabol. Toxicol.,* **2006**, *2*(3), 429-445.

[9] Langmann, P.; Zilly, M.; Winzer, R.; Klinker, H. *Curr. Pharm. Anal.,* **2006**, *2*(3), 205-217.

[10] Pomerantz, R. J.; Nunnari, G. *Cell Death during HIV Infection,* **2006**, CRC Press LLC, Boca Raton, Fla: 457-474.

[11] Bailey, J.; Blankson, J. N.; Wind-Rotolo, M.; Siliciano, R. F. *Curr. Opin. Immunol.,* **2004**, *16*(4), 470-476.

[12] Lassen, K.; Han, Y.; Zhou, Y.; Siliciano, J.; Siliciano, R. F. *Trends Mol. Med.,* **2004**, *10*(11), 525-531.

[13] Lassen, K. G.; Bailey, J. R.; Siliciano, R. F. *J. Virol.,* **2004**, *78*(17), 9105-9114.

[14] Re, M. C.; Vitone, F.; Bon, I.; Schiavone, P.; Gibellini, D. *New Microbiol.,* **2006**, *29*(2), 81-88.

[15] Shen, A.; Zink, M. C.; Mankowski, J. L.; Chadwick, K.; Margolick, J. B.; Carruth, L. M.; Li, M.; Clements, J. E.; Siliciano, R. F. *J. Virol.,* **2003**, *77*(8), 4938-4949.

[16] Siliciano, J. D.; Kajdas, J.; Finzi, D.; Quinn, T. C.; Chadwick, K.; Margolick, J. B.; Kovacs, C.; Gange, S. J.; Siliciano, R. F. *Nat. Med.,* **2003**, *9*(6), 727-728.

[17] Zhu, K. Dissertation, University of California, Los Angeles, CA, USA, **2002**.

[18] Lee, S. P.; Kim, H. G.; Censullo, M. L.; Han, M. K. *Biochem.,* **1995**, *34*(32), 10205-10214.

[19] Tramontano, E.; La Colla, P.; Cheng, Y.-C. *Biochem.,* **1998**, *37*(20), 7237-7243.

[20] Wang, Y.; Klock, H.; Yin, H.; Wolff, K.; Bieza, K.; Niswonger, K.; Matzen, J.; Gunderson, D.; Hale, J.; Lesley, S.; Kuhen, K.; Caldwell, J.; Brinker, A. *J. Biomol. Screen.,* **2005**, *10*(5), 456-462.

[21] Mazumder, A.; Gupta, M.; Pommier, Y. *Nucleic Acids Res.,* **1994**, *22*(21), 4441-4448.

[22] Semenova, E. A.; Pokrovskii, A. G. 2002-126506, WO2234535, **2004**.

[23] Snasel, J.; Rejman, D.; Liboska, R.; Tocik, Z.; Ruml, T.; Rosenberg, I.; Pichova, I. *Eur. J. Biochem.,* **2001**, *268*(4), 980-986.

[24] Marchand, C.; Zhang, X.; Pais, G. C. G.; Cowansage, K.; Neamati, N.; Burke, T. R., Jr.; Pommier, Y. *J. Biol. Chem.,* **2002**, *277*(15), 12596-12603.

[25] Neamati, N.; Marchand, C.; Pommier, Y. *Advances in Pharmacology (San Diego, CA, United States)* **2000**, *49*(HIV-1: Molecular Biology and Pathogenesis: Clinical Applications), 147-165, 141 plate.

[26] Barreca, M. L.; De Luca, L.; Iraci, N.; Chimirri, A. *J. Med. Chem.,* **2006**, *49*(13), 3994-3997.

[27] Chi, G.; Neamati, N.; Nair, V. *Bioorg. Med. Chem. Lett.,* **2004**, *14*(19), 4815-4817.

[28] Espeseth, A. S.; Felock, P.; Wolfe, A.; Witmer, M.; Grobler, J.; Anthony, N.; Egbertson, M.; Melamed, J. Y.; Young, S.; Hamill, T.; Cole, J. L.; Hazuda, D. J. *Proc. Natl. Acad. Sci. USA,* **2000**, *97*(21), 11244-11249.

[29] Mazumder, A.; Engelman, A.; Craigie, R.; Fesen, M.; Pommier, Y. *Nucleic Acids Res.,* **1994**, *22*(6), 1037-1043.

[30] Pommier, Y.; Marchand, C.; Neamati, N. *Antiviral Res.,* **2000**, *47*(3), 139-148.

[31] Yoder, K. E.; Bushman, F. D. *J. Virol.,* **2000**, *74*(23), 11191-11200.

[32] Schauer, M.; Billich, A. *Biochem. Biophys. Res. Commun.,* **1992**, *185*(3), 874-880.

[33] Van den Ent, F. M. I.; Vos, A.; Plasterk, R. H. A. *J. Virol.,* **1999**, *73*(4), 3176-3183.

[34] Yi, J.; Arthur, J. W.; Dunbrack, R. L., Jr.; Skalka, A. M. *J. Biol. Chem.,* **2000**, *275*(49), 38739-38748.

[35] Cai, M.; Huang, Y.; Caffrey, M.; Zheng, R.; Craigie, R.; Clore, G. M.; Gronenborn, A. M. *Protein Sci.,* **1998**, *7*(12), 2669-2674.

[36] Cai, M.; Zheng, R.; Caffrey, M.; Craigie, R.; Clore, G. M.; Gronenborn, A. M. *Nat. Struct. Biol.,* **1997**, *4*(7), 567-577.

[37] Zheng, R.; Jenkins, T. M.; Craigie, R. *Proc. Natl. Acad. Sci. USA,* **1996**, *93*(24), 13659-13664.

[38] Dyda, F.; Hickman, A. B.; Jenkins, T. M.; Engelman, A.; Craigie, R.; Davies, D. R. *Science,***1994**, *266*(5193), 1981-1986.

[39] Chen, J. C.; Krucinski, J.; Miercke, L. J.; Finer-Moore, J. S.; Tang, A. H.; Leavitt, A. D.; Stroud, R. M. *Proc. Natl. Acad. Sci. USA,* **2000**, *97*(15), 8233-8238.

[40] Chen, J. C. H.; Krucinski, J.; Miercke, L. J. W.; Finer-Moore, J. S.; Tang, A. H.; Leavitt, A. D.; Stroud, R. M. *Proc. Natl. Acad. Sci. USA,* **2000**, *97*(15), 8233-8238.

[41] Maignan, S.; Guilloteau, J.-P.; Zhou-Liu, Q.; Clement-Mella, C.; Mikol, V. *J. Mol. Biol.,* **1998**, *282*(2), 359-368.

[42] Maignan, S.; Guilloteau, J. P.; Zhou-Liu, Q.; Clement-Mella, C.; Mikol, V. *J. Mol. Biol.,* **1998**, *282*(2), 359-368.

[43] Long, Y.-Q.; Jiang, X.-H.; Dayam, R.; Sanchez, T.; Shoemaker, R.; Sei, S.; Neamati, N. *J. Med. Chem.,* **2004**, *47*(10), 2561-2573.

[44] Steitz, T. A. DNA *J. Biol. Chem.,* **1999**, *274*(25), 17395-17398.

[45] Cowan, J. A. Introduction to the biological chemistry of magnesium ion. *Biological Chemistry of Magnesium* VCH, New York NY **1995**, 1-23.

[46] Cowan, J. A. *J. Biol. Inorg. Chem.,* **1997**, *2*(2), 168-176.

[47] Chen, Z.; Yan, Y.; Munshi, S.; Li, Y.; Zugay-Murphy, J.; Xu, B.; Witmer, M.; Felock, P.; Wolfe, A.; Sardana, V.; Emini, E. A.; Hazuda, D.; Kuo, L. C. *J. Mol. Biol.,* **2000**, *296*(2), 521-533.

[48] Eijkelenboom, A. P. A. M.; Sprangers, R.; Hard, K.; Lutzke, R. A. P.; Plasterk, R. H. A.; Boelens, R.; Kaptein, R. *Proteins: Struct., Funct., Genet.,* **1999**, *36*(4), 556-564.

[49] Lutzke, R. A. P.; Plasterk, R. H. A. *J. Virol.,* **1998**, *72*(6), 4841-4848.

[50] Ramcharan, J.; Colleluori, D. M.; Merkel, G.; Andrake, M. D.; Skalka, A. M. *Retrovirology,* **2006**, *3*, 34-44.

[51] Nymark-McMahon, M. H.; Beliakova-Bethell, N. S.; Darlix, J.-L.; Le Grice, S. F. J.; Sandmeyer, S. B. *J. Virol.,* **2002**, *76*(6), 2804-2816.

[52] Woerner, A. M.; Klutch, M.; Levin, J. G.; Marcus-Sekura, C. J. *AIDS Res. Hum. Retroviruses,* **1992**, *8*(2), 297-304.

[53] Bujacz, G.; Alexandratos, J.; Zhou-Liu, Q.; Clement-Mella, C.; Wlodawer, A. *FEBS Lett.,* **1996**, *398*(2,3), 175-178.

[54] Goldgur, 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*(23), 13040-13043.

[55] Goldgur, Y.; Dyda, F.; Hickman, A. B.; Jenkins, T. M.; Craigie, R.; Davies, D. R. *Proc. Natl. Acad. Sci. USA,* **1998**, *95*(16), 9150-9154.

[56] Marchand, C.; Johnson, A. A.; Karki, R. G.; Pais, G. C. G.; Zhang, X.; Cowansage, K.; Patel, T. A.; Nicklaus, M. C.; Burke, T. R., Jr.; Pommier, Y. *Mol. Pharmacol.,* **2003**, *64*(3), 600-609.

[57] Molteni, V.; Greenwald, J.; Rhodes, D.; Hwang, Y.; Kwiatkowski, W.; Bushman, F. D.; Siegel, J. S.; Choe, S. *Acta Crystallogr. D,* **2001**, *57*(Pt 4), 536-544.

[58] Wlodawer, A. *Adv. Virus Res.,* **1999**, *52*, 335-350.

[59] Wang, J.-Y.; Ling, H.; Yang, W.; Craigie, R. *EMBO J.,* **2001**, *20*(24), 7333-7343.

[60] Zhao, L.; O'Reilly, M. K.; Shultz, M. D.; Chmielewski, J. *Bioorg. Med. Chem. Lett.,* **2003**, *13*(6), 1175-1177.

[61] Wielens, J.; Crosby, I. T.; Chalmers, D. K. *J. Comput. Aided Mol. Des.,* **2005**, *19*(5), 301-317.

[62] Mazumder, A.; Uchida, H.; Neamati, N.; Sunder, S.; Jaworska-Maslanka, M.; Wickstrom, E.; Zeng, F.; Jones, R. A.; Mandes, R. F.; Chenault, H. K.; Pommier, Y. *Mol. Pharmacol.,* **1997**, *51*(4), 567-575.

[63] Chi, G.; Seo, B. I.; Nair, V. *Nucleos. Nucleot. Nucl.,* **2005**, *24*(5-7), 481-484.

[64] Taktakishvili, M.; Neamati, N.; Pommier, Y.; Pal, S.; Nair, V. *J. Am. Chem. Soc.,* **2000**, *122*(24), 5671-5677.

[65] Taktakishvili, M.; Neamati, N.; Pommier, Y.; Nair, V. *Bioorg. Med. Chem. Lett.,* **2001**, *11*(11), 1433-1435.

[66] Brodin, P.; Pinskaya, M.; Buckle, M.; Parsch, U.; Romanova, E.; Engels, J.; Gottikh, M.; Mouscadet, J.-F. *Biochemistry,* **2002**, *41*(5), 1529-1538.

[67] Mazumder, A.; Neamati, N.; Ojwang, J. O.; Sunder, S.; Rando, R. F.; Pommier, Y. *Biochemistry,* **1996**, *35*(43), 13762-13771.

[68] Cushman, M.; Sherman, P. *Biochem. Biophys. Res. Commun.,* **1992**, *185*(1), 85-90.

[69] Singh, S. B.; Zink, D. L.; Goetz, M. A.; Dombrowski, A. W.; Polishook, J. D.; Hazuda, D. J. *Tetrahedron Lett.* **1998**, *39*(16), 2243-2246.
[70] Singh, S. B.; Felock, P.; Hazuda, D. J. *Bioorg. Med. Chem. Lett.,* **2000**, *10*(3), 235-238.
[71] Neamati, N.; Hong, H.; Mazumder, A.; Wang, S.; Sunder, S.; Nicklaus, M. C.; Milne, G. W. A.; Proksa, B.; Pommier, Y. *J. Med. Chem.,* **1997**, *40*(6), 942-951.
[72] Eich, E.; Pertz, H.; Kaloga, M.; Schulz, J.; Fesen, M. R.; Mazumder, A.; Pommier, Y. *J. Med. Chem.,* **1996**, *39*(1), 86-95.
[73] Fesen, M. R.; Kohn, K. W.; Leteurtre, F.; Pommier, Y. *Proc. Natl. Acad. Sci. USA,* **1993**, *90*(6), 2399-2403.
[74] Mazumder, A.; Raghavan, K.; Weinstein, J.; Kohn, K. W.; Pommier, Y. *Biochem. Pharmacol.,* **1995**, *49*(8), 1165-1170.
[75] Singh, S. B.; Zink, D. L.; Guan, Z.; Collado, J.; Pelaez, F.; Felock, P. J.; Hazuda, D. J. *Helv. Chim. Acta,* **2003**, *86*(10), 3380-3385.
[76] Singh, S. B.; Zink, D. L.; Bills, G. F.; Teran, A.; Silverman, K. C.; Lingham, R. B.; Felock, P.; Hazuda, D. J. *Bioorg. Med. Chem. Lett.,* **2003**, *13*(4), 713-717.
[77] Singh, S. B.; Ondeyka, J. G.; Schleif, W. A.; Felock, P.; Hazuda, D. J. *J. Nat. Prod.,* **2003**, *66*(10), 1338-1344.
[78] Singh, S. B.; Jayasuriya, H.; Dewey, R.; Polishook, J. D.; Dombrowski, A. W.; Zink, D. L.; Guan, Z.; Collado, J.; Platas, G.; Pelaez, F.; Felock, P. J.; Hazuda, D. J. *J. Ind. Microbiol. Biotechnol.,* **2003**, *30*(12), 721-731.
[79] Jayasuriya, H.; Guan, Z.; Polishook, J. D.; Dombrowski, A. W.; Felock, P. J.; Hazuda, D. J.; Singh, S. B. *J. Nat. Prod.,* **2003**, *66*(4), 551-553.
[80] Tomoda, H.; Tabata, N.; Ohyama, Y.; Omura, S. *J. Antibiot.,* **2003**, *56*(1), 24-29.
[81] Carlson, H. A.; Masukawa, K. M.; Lins, R. D.; Briggs, J. M.; Jorgensen, W. L.; McCammon, J. A. A dynamic pharmacophore model for HIV-1 integrase. *Book of Abstracts, 217th ACS National Meeting, Anaheim, Calif., March 21-25* **1999**, CINF-064.
[82] Deng, J.; Lee, K. W.; Briggs, J. M. Prediction of new HIV-1 integrase lead inhibitors using dynamic pharmacophore models based on MD snapshots. *Abstracts of Papers, 225th ACS National Meeting, New Orleans, LA, United States, March 23-27,* **2003**, COMP-203.
[83] Deng, J.; Lee, K. W.; Sanchez, T.; Cui, M.; Neamati, N.; Briggs, J. M. *J. Med. Chem.,* **2005**, *48*(5), 1496-1505.
[84] Deng, J.; Sanchez, T.; Neamati, N.; Briggs, J. M. *J. Med. Chem.,* **2006**, *49*(5), 1684-1692.
[85] Hoffmann, R. D.; Meddeb, S.; Langer, T. Use of 3D pharmacophore models in 3D database searching. *Computational Medicinal Chemistry for Drug Discovery,* Marcel Dekker Inc, New York, NY, **2004**, 461-482.
[86] Hong, H.; Neamati, N.; Wang, S.; Nicklaus, M. C.; Mazumder, A.; Zhao, H.; Burke, T. R., Jr.; Pommier, Y.; Milne, G. W. A. *J. Med. Chem.,* **1997**, *40*(6), 930-936.
[87] Neamati, N.; Hong, H.; Sunder, S.; Milne, G. W.; Pommier, Y. *Mol. Pharmacol.,* **1997**, *52*(6), 1041-1055.
[88] Neamati, N.; Hong, H.; Sunder, S.; Milne, G. W. A.; Pommier, Y. *Mol. Pharmacol.,* **1997**, *52*(6), 1041-1055.
[89] Nicklaus, M. C.; Neamati, N.; Hong, H.; Mazumder, A.; Sunder, S.; Chen, J.; Milne, G. W. A.; Pommier, Y. *J. Med. Chem.,* **1997**, *40*(6), 920-929.
[90] Fesen, M. R.; Pommier, Y.; Leteurtre, F.; Hiroguchi, S.; Yung, J.; Kohn, K. W. *Biochem. Pharmacol.,* **1994**, *48*(3), 595-608.
[91] Robinson, W. E., Jr.; Cordeiro, M.; Abdel-Malek, S.; Jia, Q.; Cho, S. A.; Reinecke, M. G.; Mitchell, W. M. *Mol. Pharmacol.,* **1996**, *50*(4), 846-855.
[92] Robinson, W. E., Jr.; Reinecke, M. G.; Abdel-Malek, S.; Jia, Q.; Chow, S. A. *Proc. Natl. Acad. Sci. USA,* **1996**, *93*(13), 6326-6331.
[93] Burke, T. R., Jr.; Fesen, M.; Mazumder, A.; Yung, J.; Wang, J.; Carothers, A. M.; Grunberger, D.; Driscoll, J.; Pommier, Y.; Kohn, K. *J. Med. Chem.,* **1995**, *38*(21), 4171-4178.
[94] Zhang, X.; Neamati, N.; Lee, Y. K.; Orr, A.; Brown, R. D.; Whitaker, N.; Pommier, Y.; Burke, T. R. *Bioorg. Med. Chem.,* **2001**, *9*(7), 1649-1657.
[95] Zhao, H.; Neamati, N.; Mazumder, A.; Sunder, S.; Pommier, Y.; Burke, T. R., Jr. *J. Med. Chem.,* **1997**, *40*(8), 1186-1194.
[96] Pluymers, W.; Neamati, N.; Pannecouque, C.; Fikkert, V.; Marchand, C.; Burke, T. R., Jr.; Pommier, Y.; Schols, D.; De Clercq, E.; Debyser, Z.; Witvrouw, M. *Mol. Pharmacol.,* **2000**, *58*(3), 641-648.
[97] Liehr, J. G.; Roy, D. *Free Radic. Biol. Med.,* **1990**, *8*(4), 415-423.
[98] Stanwell, C.; Ye, B.; Yuspa, S. H.; Burke, T. R. Jr. *Biochem. Pharmacol.,* **1996**, *52*(3), 475-480.
[99] Xu, Y.-W.; Zhao, G.-S.; Shin, C.-G.; Zang, H.-C.; Lee, C.-K.; Lee, Y. S. *Bioorg. Med. Chem.,* **2003**, *11*(17), 3589-3593.
[100] Neamati, N.; Pommier, Y.; Lin, Z.; Burke, T. Preparation of thiosalicylhydrazides as inhibitors of HIV-1 integrase. 2000-US6361, WO2000053577, **2000**.
[101] Zhao, H.; Neamati, N.; Hong, H.; Mazumder, A.; Wang, S.; Sunder, S.; Milne, G. W. A.; Pommier, Y.; Burke, T. R., Jr. *J. Med. Chem.,* **1997**, *40*(2), 242-249.
[102] Al-Mawsawi, L. Q.; Fikkert, V.; Dayam, R.; Witvrouw, M.; Burke, T. R., Jr.; Borchers, C. H.; Neamati, N. *Proc. Natl. Acad. Sci. USA,* **2006**, *103*(26), 10080-10085.
[103] Mazumder, A.; Neamati, N.; Sunder, S.; Schulz, J.; Pertz, H.; Eich, E.; Pommier, Y. Curcumin *J. Med. Chem.,* **1997**, *40*(19), 3057-3063.
[104] Bailly, F.; Cotelle, P. *Curr. Med. Chem.,* **2005**, *12*(15), 1811-1818.
[105] Hazuda, D. J.; Felock, P.; Witmer, M.; Wolfe, A.; Stillmock, K.; Grobler, J. A.; Espeseth, A.; Gabryelski, L.; Schleif, W.; Blau, C.; Miller, M. D. *Science,* **2000**, *287*(5453), 646-650.
[106] Billich, A. *Curr. Opin. Invest. Drugs,* **2003**, *4*(2), 206-209.
[107] Maurin, C.; Bailly, F.; Buisine, E.; Vezin, H.; Mbemba, G.; Mouscadet, J. F.; Cotelle, P. *J. Med. Chem.,* **2004**, *47*(22), 5583-5586.
[108] Selnick, H. G.; Hazuda, D. J.; Egbertson, M.; Guare, J. P., Jr.; Wai, J. S.; Young, S. D.; Clark, D. L.; Medina, J. C. Preparation of nitrogen-containing 4-heteroaryl-2,4-dioxobutyric acids useful as HIV integrase inhibitors. 99-US12095, WO9962513, **1999**.
[109] Young, S. D.; Egbertson, M.; Payne, L. S.; Wai, J. S.; Fisher, T. E.; Guare, J. P., Jr.; Embrey, M. W.; Tran, L.; Zhuang, L.; Vacca, J. P.; Langford, M.; Melamed, J.; Clark, D. L.; Medina, J. C.; Jaen, J. Preparation of aromatic and heteroaromatic 4-aryl-2,4-dioxobutyric acid derivatives useful as HIV integrase inhibitors. 99-US12093, WO9962520, **1999**.
[110] Young, S. D.; Wai, J. S.; Embrey, M. W.; Fisher, T. E. Heterocyclyldioxobutanoates as HIV integrase inhibitors. 99-US12094, WO9962897, **1999**.
[111] Summa, V.; Petrocchi, A.; Pace, P.; Matassa, V. G.; De Francesco, R.; Altamura, S.; Tomei, L.; Koch, U.; Neuner, P. *J. Med. Chem.,* **2004**, *47*(1), 14-17.
[112] Pais, G. C. G.; Zhang, X.; Marchand, C.; Neamati, N.; Cowansage, K.; Svarovskaia, E. S.; Pathak, V. K.; Tang, Y.; Nicklaus, M.; Pommier, Y.; Burke, T. R. Jr. *J. Med. Chem.,* **2002**, *45*(15), 3184-3194.
[113] Dayam, R.; Neamati, N. *Bioorg. Med. Chem.,* **2004**, *12*(24), 6371-6381.
[114] Summa, V.; Petrocchi, A.; Matassa, V. G.; Taliani, M.; Laufer, R.; De Francesco, R.; Altamura, S.; Pace, P. *J. Med. Chem.* **2004**, *47*(22), 5336-5339.
[115] Di Santo, R.; Costi, R.; Roux, A.; Artico, M.; Lavecchia, A.; Marinelli, L.; Novellino, E.; Palmisano, L.; Andreotti, M.; Amici, R.; Galluzzo, C. M.; Nencioni, L.; Palamara, A. T.; Marchand, C.; Pommier, Y. *J. Med. Chem.,* **2006**, *49*(6), 1939-1945.
[116] Zhang, X.; Marchand, C.; Pommier, Y.; Burke, T. R. *Bioorg. Med. Chem. Lett.,* **2004**, *14*(5), 1205-1207.
[117] Maurin, C.; Bailly, F.; Mbemba, G.; Mouscadet, J. F.; Cotelle, P. *Bioorg. Med. Chem.,* **2006**, *14*(9), 2978-2984.
[118] Wai, J. S.; Egbertson, M. S.; Payne, L. S.; Fisher, T. E.; Embrey, M. W.; Tran, L. O.; Melamed, J. Y.; Langford, H. M.; Guare, J. P., Jr.; Zhuang, L.; Grey, V. E.; Vacca, J. P.; Holloway, M. K.; Naylor-Olsen, A. M.; Hazuda, D. J.; Felock, P. J.; Wolfe, A. L.; Stillmock, K. A.; Schleif, W. A.; Gabryelski, L. J.; Young, S. D. *J. Med. Chem.,* **2000**, *43*(26), 4923-4926.
[119] Zhang, X.; Pais, G. C. G.; Svarovskaia, E. S.; Marchand, C.; Johnson, A. A.; Karki, R. G.; Nicklaus, M. C.; Pathak, V. K.; Pommier, Y.; Burke, T. R. *Bioorg. Med. Chem. Lett.,* **2003**, *13*(6), 1215-1219.
[120] Sechi, M.; Derudas, M.; Dallocchio, R.; Dessi, A.; Bacchi, A.; Sannia, L.; Carta, F.; Palomba, M.; Ragab, O.; Chan, C.; Shoemaker, R.; Sei, S.; Dayam, R.; Neamati, N. *J. Med. Chem.,* **2004**, *47*(21), 5298-5310.
[121] Nair, V.; Uchil, V.; Neamati, N. *Bioorg. Med. Chem. Lett.,* **2006**, *16*(7), 1920-1923.

[122] Nair, V.; Chi, G.; Ptak, R.; Neamati, N. *J. Med. Chem.,* **2006**, *49*(2), 445-447.

[123] Costi, R.; Di Santo, R.; Artico, M.; Roux, A.; Ragno, R.; Massa, S.; Tramontano, E.; La Colla, M.; Loddo, R.; Marongiu, M. E.; Pani, A.; La Colla, P. *Bioorg. Med. Chem. Lett.,* **2004**, *14*(7), 1745-1749.

[124] Di Santo, R.; Costi, R.; Artico, M.; Ragno, R.; Greco, G.; Novellino, E.; Marchand, C.; Pommier, Y. *Farmaco,* **2005**, *60*(5), 409-417.

[125] Hazuda, D. J.; Anthony, N. J.; Gomez, R. P.; Jolly, S. M.; Wai, J. S.; Zhuang, L.; Fisher, T. E.; Embrey, M.; Guare, J. P., Jr.; Egbertson, M. S.; Vacca, J. P.; Huff, J. R.; Felock, P. J.; Witmer, M. V.; Stillmock, K. A.; Danovich, R.; Grobler, J.; Miller, M. D.; Espeseth, A. S.; Jin, L.; Chen, I. W.; Lin, J. H.; Kassahun, K.; Ellis, J. D.; Wong, B. K.; Xu, W.; Pearson, P. G.; Schleif, W. A.; Cortese, R.; Emini, E.; Summa, V.; Holloway, M. K.; Young, S. D. *Proc. Natl. Acad. Sci. USA,* **2004**, *101*(31), 11233-11238.

[126] Zhuang, L.; Wai, J. S.; Embrey, M. W.; Fisher, T. E.; Egbertson, M. S.; Payne, L. S.; Guare, J. P., Jr.; Vacca, J. P.; Hazuda, D. J.; Felock, P. J.; Wolfe, A. L.; Stillmock, K. A.; Witmer, M. V.; Moyer, G.; Schleif, W. A.; Gabryelski, L. J.; Leonard, Y. M.; Lynch, J. J., Jr.; Michelson, S. R.; Young, S. D. *J. Med. Chem.,* **2003**, *46*(4), 453-456.

[127] Sato, M.; Motomura, T.; Aramaki, H.; Matsuda, T.; Yamashita, M.; Ito, Y.; Kawakami, H.; Matsuzaki, Y.; Watanabe, W.; Yamataka, K.; Ikeda, S.; Kodama, E.; Matsuoka, M.; Shinkai, H. *J. Med. Chem.,* **2006**, *49*(5), 1506-1508.

[128] Embrey, M. W.; Wai, J. S.; Funk, T. W.; Homnick, C. F.; Perlow, D. S.; Young, S. D.; Vacca, J. P.; Hazuda, D. J.; Felock, P. J.; Stillmock, K. A.; Witmer, M. V.; Moyer, G.; Schleif, W. A.; Gabryelski, L. J.; Jin, L.; Chen, I. W.; Ellis, J. D.; Wong, B. K.; Lin, J. H.; Leonard, Y. M.; Tsou, N. N.; Zhuang, L. *Bioorg. Med. Chem. Lett.,* **2005**, *15*(20), 4550-4554.

[129] Dayam, R.; Sanchez, T.; Neamati, N. *ChemMedChem,* **2006**, *1*(2), 238-244.

[130] Bonnenfant, S.; Thomas, C. M.; Vita, C.; Subra, F.; Deprez, E.; Zouhiri, F.; Desmaele, D.; d'Angelo, J.; Mouscadet, J. F.; Leh, H. *J. Virol.,* **2004**, *78*(11), 5728-5736.

[131] Mousnier, A.; Leh, H.; Mouscadet, J.-F.; Dargemont, C. *Mol. Pharmacol.,* **2004**, *66*(4), 783-788.

[132] Zouhiri, F.; Mouscadet, J. F.; Mekouar, K.; Desmaele, D.; Savoure, D.; Leh, H.; Subra, F.; Le Bret, M.; Auclair, C.; d'Angelo, J. *J. Med. Chem.,* **2000**, *43*(8), 1533-1540.

[133] Benard, C.; Zouhiri, F.; Normand-Bayle, M.; Danet, M.; Desmaele, D.; Leh, H.; Mouscadet, J.-F.; Mbemba, G.; Thomas, C.-M.; Bonnenfant, S.; Le Bret, M.; d'Angelo, J. *Bioorg. Med. Chem. Lett.,* **2004**, *14*(10), 2473-2476.

[134] Mekouar, K.; Mouscadet, J.-F.; Desmaeele, D.; Subra, F.; Leh, H.; Savoure, D.; Auclair, C.; d'Angelo, J. *J. Med. Chem.,* **1998**, *41*(15), 2846-2857.

[135] Yoo, H.; Lee, J. Y.; Park, J. H.; Chung, B. Y.; Lee, Y. S. *Farmaco,* **2003**, *58*(12), 1243-1250.

[136] Zouhiri, F.; Desmaele, D.; d'Angelo, J.; Ourevitch, M.; Mouscadet, J.-F.; Leh, H.; Le Bret, M. *Tetrahedron Lett.,* **2001**, *42*(46), 8189-8192.

[137] Puras, R. A.; Vink, C.; Plasterk, R. H. A. *Nucleic Acids Res.,* **1994**, *22*(20), 4125-4131.

[138] Krajewski, K.; Long, Y.-Q.; Marchand, C.; Pommier, Y.; Roller, P. P. *Bioorg. Med. Chem. Lett.,* **2003**, *13*(19), 3203-3205.

[139] Krajewski, K.; Marchand, C.; Long, Y.-Q.; Pommier, Y.; Roller, P. P. *Bioorg. Med. Chem. Lett.,* **2004**, *14*(22), 5595-5598.

[140] Robinson, W. E., Jr.; McDougall, B.; Tran, D.; Selsted, M. E. *J. Leukocyte Biol.,* **1998**, *63*(1), 94-100.

[141] de Soultrait, V. R.; Caumont, A.; Durrens, P.; Calmels, C.; Parissi, V.; Recordon, P.; Bon, E.; Desjobert, C.; Tarrago-Litvak, L.; Fournier, M. *Biochim. Biophys. Acta-Gene Struct. Expression,* **2002**, *1575*(1-3), 40-48.

[142] Desjobert, C.; De Soultrait, V. R.; Faure, A.; Parissi, V.; Litvak, S.; Tarrago-Litvak, L.; Fournier, M. *Biochemistry,* **2004**, *43*(41), 13097-13105.

[143] Oz Gleenberg, I.; Avidan, O.; Goldgur, Y.; Herschhorn, A.; Hizi, A. *J. Biol. Chem.,* **2005**, *280*(23), 21987-21996.

[144] Maroun, R. G.; Gayet, S.; Benleulmi, M. S.; Porumb, H.; Zargarian, L.; Merad, H.; Leh, H.; Mouscadet, J.-F.; Troalen, F.; Fermandjian, S. *Biochemistry,* **2001**, *40*(46), 13840-13848.

[145] Nicklaus, M. C.; Pommier, Y.; Mazumder, A. Hiv-1 integrase inhibitors. 3D searching and active site docking. *Book of Abstracts, 210th ACS National Meeting, Chicago, IL, August 20-24* **1995**(Pt. 1), CINF-022.

[146] Kuo, C.-L.; Assefa, H.; Kamath, S.; Brzozowski, Z.; Slawinski, J.; Saczewski, F.; Buolamwini, J. K.; Neamati, N. *J. Med. Chem.,* **2004**, *47*(2), 385-399.

[147] Brzozowski, Z.; Saczewski, F.; Sanchez, T.; Kuo, C.-L.; Gdaniec, M.; Neamati, N. *Bioorg. Med. Chem.,* **2004**, *12*(13), 3663-3672.

[148] Pannecouque, C.; Pluymers, W.; Van Maele, B.; Tetz, V.; Cherepanov, P.; De Clercq, E.; Witvrouw, M.; Debyser, Z. *Curr. Biol.,* **2002**, *12*(14), 1169-1177.

[149] Neamati, N.; Mazumder, A.; Zhao, H.; Sunder, S.; Burke, T. R., Jr.; Schultz, R. J.; Pommier, Y. *Antimicrob. Agents Chemother.,* **1997**, *41*(2), 385-393.

[150] Meadows, D. C.; Mathews, T. B.; North, T. W.; Hadd, M. J.; Kuo, C. L.; Neamati, N.; Gervay-Hague, J. *J. Med. Chem.,* **2005**, *48*(14), 4526-4534.

[151] Carteau, S.; Mousacadet, J. F.; Goulaouic, H.; Subra, F.; Auclair, C. *Arch. Biochem. Biophys.,* **1993**, *305*(2), 606-610.

[152] Carteau, S.; Mouscadet, J. F.; Goulaouic, H.; Subra, F.; Auclair, C. *Arch. Biochem. Biophys.,* **1993**, *305*(2), 606-610.

[153] Farnet, C. M.; Wang, B.; Lipford, J. R.; Bushman, F. D. *Proc. Natl. Acad. Sci. USA,* **1996**, *93*(18), 9742-9747.

[154] Mohan, P.; Hopfinger, A. J.; Baba, M. *Antivir. Chem. Chemother.,* **1991**, *2*(4), 215-222.

[155] Tan, G. T.; Wickramasinghe, A.; Verma, S.; Singh, R.; Hughes, S. H.; Pezzuto, J. M.; Baba, M.; Mohan, P. *J. Med. Chem.,* **1992**, *35*(26), 4846-4853.

[156] Mohan, P.; Loya, S.; Avidan, O.; Verma, S.; Dhindsa, G. S.; Wong, M. F.; Huang, P. P.; Yashiro, M.; Baba, M.; Hizi, A. *J. Med. Chem.,* **1994**, *37*(16), 2513-2519.

[157] Mohan, P.; Singh, R.; Baba, M. *J. Med. Chem.,* **1991**, *34*(1), 212-217.

[158] Mohan, P.; Singh, R.; Wepsiec, J.; Gonzalez, I.; Sun, D. K.; Sarin, P. S. *Life Sci.,* **1990**, *47*(12), 993-999.

[159] Mohan, P.; Wong, M. F.; Verma, S.; Huang, P. P.; Wickramasinghe, A.; Baba, M. *J. Med. Chem.,* **1993**, *36*(14), 1996-2003.

[160] Barreca, M. L.; Rao, A.; De Luca, L.; Zappala, M.; Gurnari, C.; Monforte, P.; De Clercq, E.; Van Maele, B.; Debyser, Z.; Witvrouw, M.; Briggs, J. M.; Chimirri, A. *J. Chem. Inf. Comput. Sci.,* **2004**, *44*(4), 1450-1455.

[161] Lubkowski, J.; Yang, F.; Alexandratos, J.; Wlodawer, A.; Zhao, H.; Burke, T. R., Jr.; Neamati, N.; Pommier, Y.; Merkel, G.; Skalka, A. M. *Proc. Natl. Acad. Sci. USA,* **1998**, *95*(9), 4831-4836.

[162] Bujacz, G.; Jaskolski, M.; Alexandratos, J.; Wlodawer, A.; Merkel, G.; Katz, R. A.; Skalka, A. M. *Structure,* **1996**, *4*(1), 89-96.

[163] Fikkert, V.; Van Maele, B.; Vercammen, J.; Hantson, A.; Van Remoortel, B.; Michiels, M.; Gurnari, C.; Pannecouque, C.; De Maeyer, M.; Engelborghs, Y.; De Clercq, E.; Debyser, Z.; Witvrouw, M. *J. Virol.* **2003**, *77*(21), 11459-11470.

[164] Cotelle, P., *Recent Pat. Anti-Infecti. Drug Discov.,* **2006**, *1*(1), 1-15.

Received: 01 November, 2006 Revised: 15 November, 2006 Accepted: 16 November, 2006