# New Developments in Diketo-Containing Inhibitors of HIV-1 Integrase

Guisen Zhao*, Chao Wang, Chuan Liu and Hongxiang Lou*

*College of Pharmacy, Shandong University, Jinan, Shandong 250012, PR China*

**Abstract:** HIV-1 integrase is one of the three enzymes, which are critical for viral replication. It catalyzes the integration of the HIV genome into the cellular chromosome. Since there is no known human homolog to integrase, its inhibition is one of the most promising novel drug targets for anti-retroviral therapy with potential advantage over existing therapies. To date, numerous compounds with diverse structural features have been reported as integrase inhibitors, among which the diketo-containing inhibitors of HIV-1 integrase represent a major lead for anti-HIV drug development. The discovery of diketo acids plays an important role in validating integrase as a legitimate target for treatment of AIDS. In this review, we summarize several drug candidates in clinical trials and new diketo-containing inhibitors of HIV-1 integrase discovered recently.

**Key Words:** Human immunodeficiency virus, integrase, inhibitors, diketo acids.

## 1. INTRODUCTION

Human immunodeficiency virus type-1 (HIV-1) is the etiological agent of the acquired immunodeficiency syndrome (AIDS). Reverse transcriptase (RT) and protease (PR) play the fundamental role among retroviral targets useful for chemotherapeutic intervention, [1]. The highly active antiretroviral therapy (HAART), which is based on the combination use of reverse transcriptase inhibitors and protease inhibitors, effectively suppresses the HIV-1 replication to such an extent that the virus becomes undetectable in the blood of the infected persons. However, HAART fails to eradicate viral replication and the emergence of multidrug resistant viral strains in infected patients can complicate the response to the treatment [2, 3]. Therefore, it is demanded for the development of novel drugs, which target other steps in the viral replication cycle.

The integration of double stranded viral DNA into the cellular chromosome, a process catalyzed by the HIV-1 integrase (IN), is essential for viral replication. Inhibition of this process provides an attractive strategy for antiretroviral drug design. Moreover, there is no known human homologue to IN and the reactions catalyzed by IN are unique. This allows the design of selective inhibitors with little or no side effects and makes IN as an attractive target for therapeutic intervention [4, 5].

## 2. THE STRUCTURE AND FUNCTION OF IN

HIV-1 integrase, a 32kDa protein encoded by pol gene, belongs to the superfamily of polynucleotidyl transferases. It consists of 288 amino acids in three structurally and functionally distinct domains Fig. (**1**). The N-terminal domain includes residues 1-50 and is characterized by a conserved and essential HHCC (H12 and H16, C40 and C43) motif that binds one zinc atom. It consists of a bundle of three α-helices and its function is protein multimerization [6, 7]. The catalytic core domain comprises residues 51-212 and is responsible for catalysis as well as specific contacts with viral DNA. This domain contains a triad of acidic residues, the D, D-35-E (D64, D116, and E152) motif, which plays an important role in binding divalent magnesium ion cofactors and has also been found in other retroviral integrases and bacterial transposases. Mutation of any of these three residues abolishes or severely diminishes all catalytic activities of the protein. The triad coordinates a divalent metal ion, usually a $Mg^{2+}$ *in vivo*, which is required for catalytic activity [8, 9]. The C-terminal domain features residues 213-288 and has a SH3-like fold. It is the least conserved of the three domains and thought to be involved in nonspecific DNA binding [10].

The IN-catalyzed insertion of retrotranscribed viral DNA into the host cell's genomic DNA takes place through a complex process, which consists of three biochemical steps: (i) cleavage of a dinucleotide pair from the 3'-end of the viral DNA (termed "3'-processing", 3'-P), (ii) insertion of the resulting shortened strands into the host-cell chromosome (termed "strand transfer", ST) and (iii) removal of the two unpaired nucleotides at the 5'-end of the viral DNA and gap-filling process [11, 12]. Due to the absence of any known human homolog, IN is considered as an attractive and validated target for the development of novel ant-HIV drugs for its key role in HIV-1 replication.

## 3. STRUCTURE-ACTIVITY RELATIONSHIPS AND MECHANISM OF ACTION OF DIKETO-CONTAINING IN INHIBITORS

The first IN inhibitors were reported approximately 10 years ago [13, 14]. In the past few years, numerous compounds with diverse structural features have been discovered and reported as IN inhibitors, including oligonucleotides, peptides, polyhydroxylated compounds, quinoline derivatives, hydrazides and quinolones [15-22]. Most of these compounds inhibit IN function in extracellular enzyme assays, but often lack inhibitory potency or fail to show antiviral activity against HIV-infected cells [23-25]. Beside above mentioned inhibitors, diketo-containing compounds, mainly diketo acids (DKAs) and their derivatives represent the most

*Address correspondence to these authors at College of Pharmacy, Shandong University, Jinan, Shandong 250012, PR China;
E-mail: guisenzhao@sdu.edu.cn; louhongxiang@sdu.edu.cn

1389-5575/07 $50.00+.00 

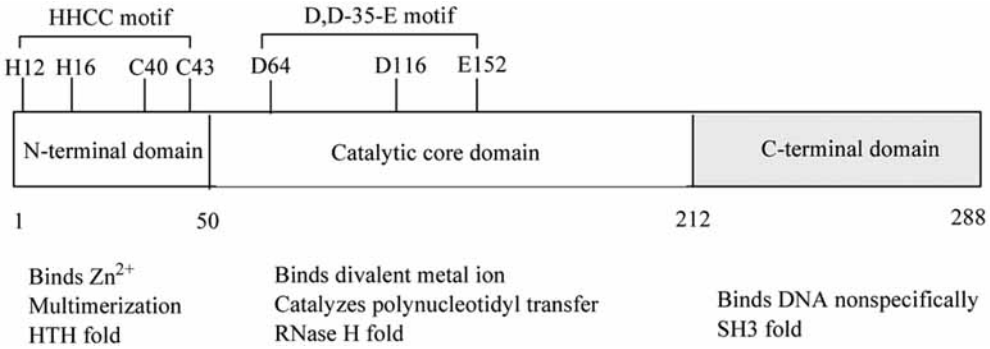



**Fig. (1).** General structure of HIV-1 integrase.

promising class of compounds that are both selective HIV-1 IN inhibitors and antiviral agents [26, 27].

The structure of DKAs and their derivatives can be outlined in a general formula Fig. (**2**). DKAs comprise of three structural components, which are a common β-diketopropyl

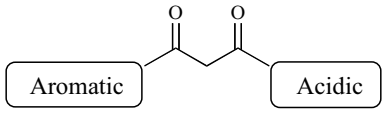



**Fig. (2).** General formula of diketo acids.

linker, different left aromatic and right acidic portions. It is believed that the diketo acid moiety is the key pharmacophore for enzyme inhibition and that the aromatic group adjacent to the diketo acid moiety improves potency and selectivity of compounds. Many SAR studies on DKAs have been conducted to search for a clinical candidate. Most of them focus on the aromatic ring and acidic group. The left aromatic portion has been replaced with benzene and various nitrogen-, sulfur-, and oxygen-containing heterocycles, maintaining good ST inhibitory potency. Introduction of acidic functionality or another diketo acid side chain into the left side aromatic ring results in the enhancement of 3'-P inhibitory potency and a reduction of selectivity towards ST [28, 29]. The right acid functionality has been substituted by a variety of groups including carboxylate, triazoles, tetrazoles, thiazoles, etc. It is observed that both tetrazole and carboxyl moieties provide potent inhibition in biochemical ST assays, while only carboxylate-bearing agents are antiviral within the tested concentration range [28].

The relative orientation of aryl substituents and diketo acid side chain is important for intrinsic potency of DKAs Fig. (**3**). It is found that IN and viral replication inhibitory activities increase as the angle between the aryl substituent and the diketo acid side chain increases from 60° to 118°, e.g. compounds (**1**-**3**). As the angle of bisection increases further, (e.g. in compound (**4**)), the inhibitory potency decreases. Electronic effects, size and position of substituents on the distal aryl ring also have profound effects on inhibitory activities. Introduction of a fluorine substituent at the 2'-position of the distal benzene furnishes compound (**5**) with strong inhibitory potency. Isopropoxy or methoxy group introduced at 2-position on the central benzene ring, i.e., compound (**6**), leads to significant enhancement of antiviral activity, whereas a methoxy group at the 3-position or 4-position, i.e., compounds (**7** and **8**), decreases potency Fig. (**3**) [30]. Moreover, ST and HIV-1 infectivity inhibitory potency of DKAs decreases as the distance between central and distal aryl ring increases [31].

Most of the DKAs selectively inhibit ST and exhibit potent antiviral effects against HIV-infected cells. Further studies have indicated that, unlike numerous other IN inhibitors whose antiviral effects can be attributable to non-IN-dependent phenomena [32, 33], members of the DKAs family disrupt viral infectivity in a manner consistent with inhibition of integration [31, 33, 34]. DKAs have been shown to inhibit viral replication by competing with the substrate DNA in binding to the IN active site and selectively blocking ST, while being inhibitory to ST function by sequestering the divalent cations bound in the active site of IN [35, 36].

DKAs' binding to IN is expected to be mediated by the interaction of the carboxylic acid function with $Mg^{2+}$ at site I, i.e., metal coordinated by D64 and D116 [28, 35]. A direct interaction of diketo acid with divalent cofactor in the IN active site has been shown using functional and binding assays [35]. Pharmacomodulations have shown that the free acidic function is essential for the activity. Due to its high affinity to $Mg^{2+}$, diketo acid may bind to metal at site I, which is expected to be the site of 3'-P [37]. This binding may not alter the catalysis of the 3'-P but allows to position the "diketo tweezers" in the vicinity of the other $Mg^{2+}$, i.e., site II, between D64 and E152. The second $Mg^{2+}$, probably carried into the IN active site by host DNA may be sequestrated by the tweezers, thus inhibiting ST [24].

During HIV-1 infection, selective inhibition of ST allows the viral DNA to become accessible to metabolism by cellular recombination and repair enzymes, which leads to the irreversible blocking of viral replication due to incompetent and unstable integration [38].

## 4. DIKETO ACIDS

The first reported diketo-containing molecule is curcumin (**9**). Its IN inhibitory activity has been reported with $IC_{50}$ values equal to 150 μM for 3'-P and 140 μM for ST respectively [39]. Two synthetic analogs of curcumin with no methoxy groups, discaffeolymethane (**10**) and rosmarinic acid (**11**) are found to be very potent, which are found to bind in the enzyme catalytic core. The presence of any methoxy

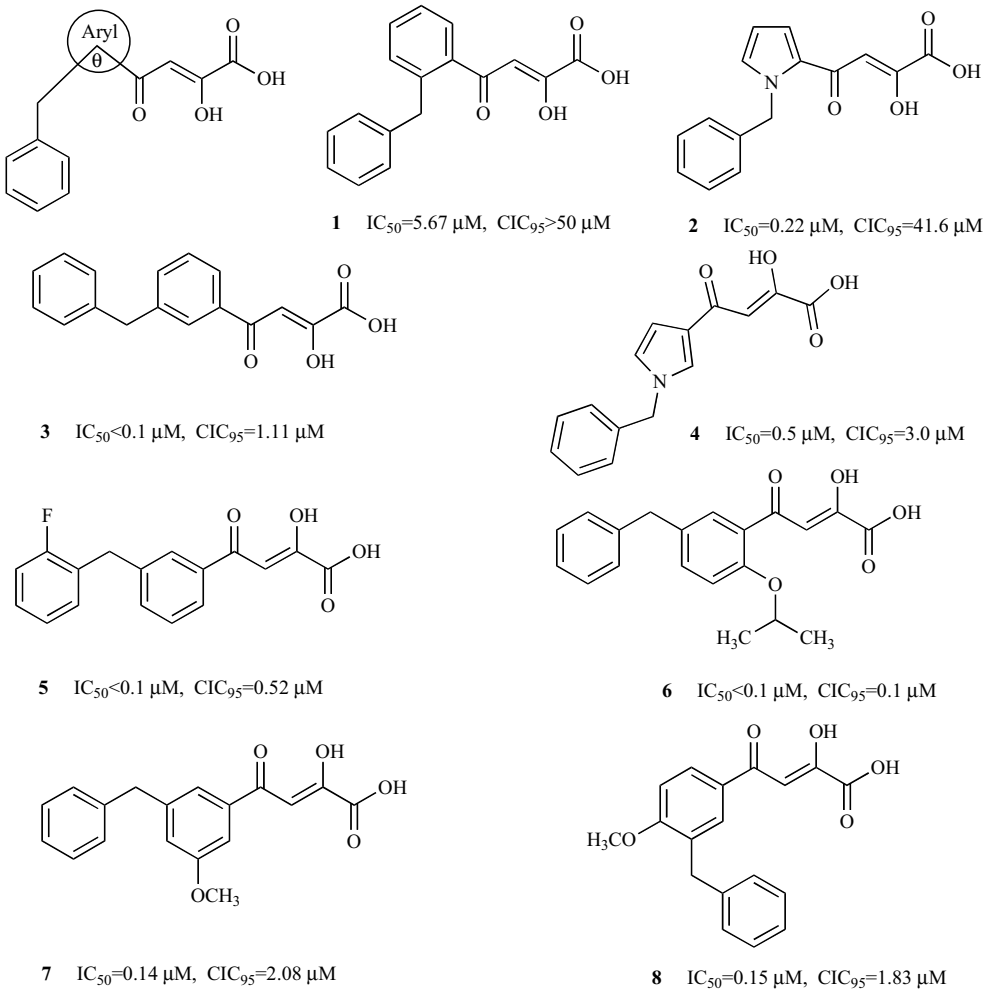



**Fig. (3).** Schematic drawing of the angle of bisection and structures of compound **1-8**.

group is shown to decrease the potency (**12**), suggesting that the methoxy group is not preferred Fig. (**4**) [40].

The DKAs are the first class validated as IN inhibitors and have been characterized in considerable detail. The Shionogi compound 5CITEP (**13**) belongs to the diketo acid-containing compound (**14**), because the tetrazole group is a well-known bioisostere of a carboxylic acid Fig. (**5**) [41].

It is co-crystallized in close association with the catalytic D, D-35-E triad, which provides the first and crystal structure of the core domain of IN bound to an inhibitor located

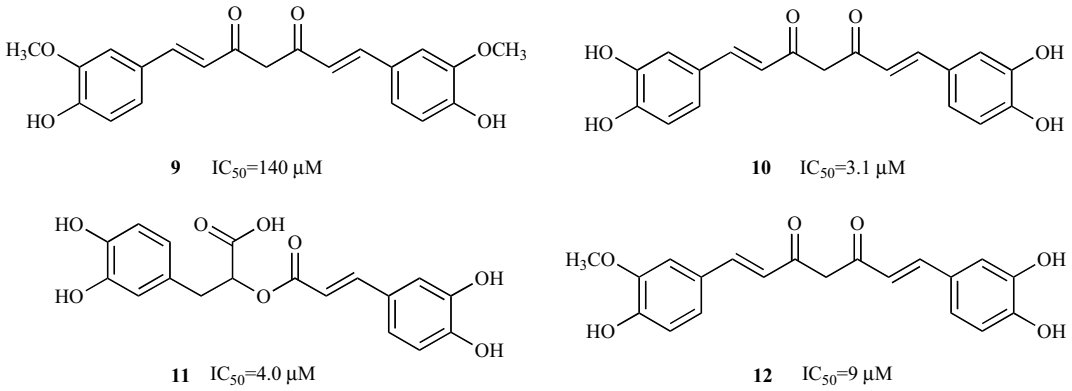



**Fig. (4).** Structures of compound **9-12**.

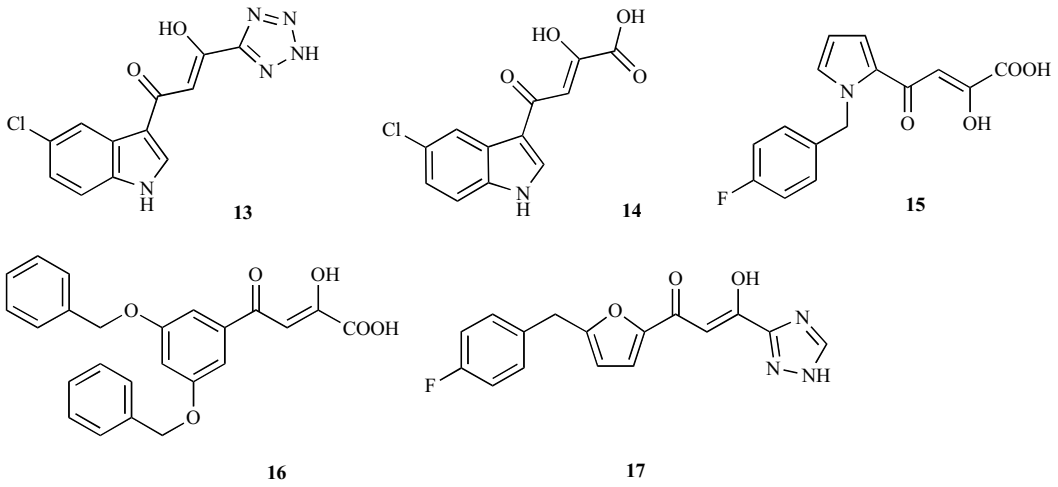



**Fig. (5).** Structures of compound **13-17**.

centrally within the active site. In the 5CITEP-IN crystal structure, the inhibitor forms a variety of hydrogen bonds with amino acids in the active site [42]. This crystal structure offers a platform for antiviral drug design, docking and molecular dynamics studies. However, the information provided by this 5CITEP-IN crystal structure is questionable. Subsequent docking of 5CITEP onto snapshots of IN active site region indicated the potential for the existence of two conformations of this flexible inhibitor in the docked structure and two possible ligand-binding regions adjacent to the IN active site [43].

L-731988 (**15**) and L-708906 (**16**) are two potent inhibitors disclosed through a random screen of more than 250000 samples by Merck. The two early compounds selectively inhibit ST, with $IC_{50}$ values of 0.05 and 0.1 μM respectively against recombinant IN. They also inhibit HIV-1 replication in cell cultures with $EC_{50}$ values below 2 μM and exhibit a $CC_{50}$>50 μM [31, 44]. In biochemical assays, the concentration of L-731988 which required inhibiting 3'-P was 70 times higher than the concentration required inhibiting ST [31]. It also inhibited preincubation complexes in cell based assays with an $IC_{50}$ value of 0.08 μM. Furthermore, IN mutations confer resistance to the inhibitory effects of L-731988 on both ST and HIV-1 infectivity [31]. L-708906 (**16**) displays activity against various HIV-1, HIV-2 and SIV strains at micromolar concentration [34]. Compared to 5CITEP, L-708906 shows comparable ST inhibitory potency and is at least 28-fold more selective for ST than 5CITEP [29, 34, 36].

S-1360 (**17**), developed by Shionogi and GlaxoSmith-Kline, is the first IN inhibitor to enter into clinical trials. Compared to 5CITEP (**13**), it retains the diketo functionality and contains a furan ring substituted at the 5-position with a 4-fluorobenzyl group. S-1360 inhibits IN catalytic activity in the nanomolar range ($IC_{50}$=20 nM). The compound is a potent inhibitor of viral replication, with $EC_{50}$, $EC_{90}$, and $CC_{50}$ values of 0.2 μM, 0.74 μM and 12 μM, respectively in MTT assay. Moreover, it is also active against a variety of clinical isolates (including multidrug-resistant strains), displaying mean $EC_{50}$ and $CC_{50}$ values of 0.14 μM and 110 μM respectively. Viruses resistant to S-1360 have been identified *in vitro*, which contains Q148K, I151L and N155S mutations proximal to the putative active site of IN. Synergistic effects are observed when S-1360 is dosed *in vitro* in combination with various NRTIs, NNRTIs and PIs. It is also active in a mouse MT-4 *in vivo* assay and shows efficacy ($ED_{50}$=7.1 mg/kg) similar to capravirine ($ED_{50}$=10 mg/kg) and zidovudine ($ED_{50}$=2 mg/kg) [45, 46]. S-1360 reached phase II, but was halted in 2003. Data from clinical trail suggest the involvement of a non-cytochrome P450 clearance pathway. Reduction of S-1360 at the carbon linked to the triazole heterocycle generates a key metabolite in humans. This metabolic instability is probably the reason of the abandonment of its development [47].

### 4.1. Nucleobase Scaffold Based Diketo Acids

Recently, some conceptually novel DKAs with nucleobase scaffolds have been discovered as powerful IN inhibitors. These compounds involve examples of both pyrimidine and purine scaffolds Fig. (**6**). It is found that the nucleobase scaffold, the substituents and the specific spatial relationship of substituents in the scaffold are critical for potent IN inhibitory activity. Unlike other reported DKAs which are commonly inhibitors of only ST, pyrimidine-based DKAs can effectively inhibit both 3'-P and ST. Compound (**18**) with a pyrimidine nucleobase scaffold bearing a diketo acid and two hydrophobic benzyl groups shows strong inhibition of 3'-P and ST, with $IC_{50}$ values of 3.7 and 0.2 μM respectively. Its analogs (**19**) and (**20**) also exhibit potent inhibitory activity for both key steps of IN enzymology. However, compounds (**21**) and (**22**) with purine nucleobase scaffolds show much lower 3'-P inhibitory potency. Both compounds (**21**) and (**22**) are strong inhibitors of ST. It is however, not entirely clear how the difference generates. Molecular modeling data disclose that the regiochemical arrangement and preferred conformation of DKAs with pyrimidine nucleobase scaffolds allow for more effective overlap of these DKAs with both the 3'-P and ST pockets within the catalytic site. Docking experiments indicate that the uracil amide carbonyl (4-position) of compound (**18**) participates in the binding of this inhibitor to the active site [48, 49].

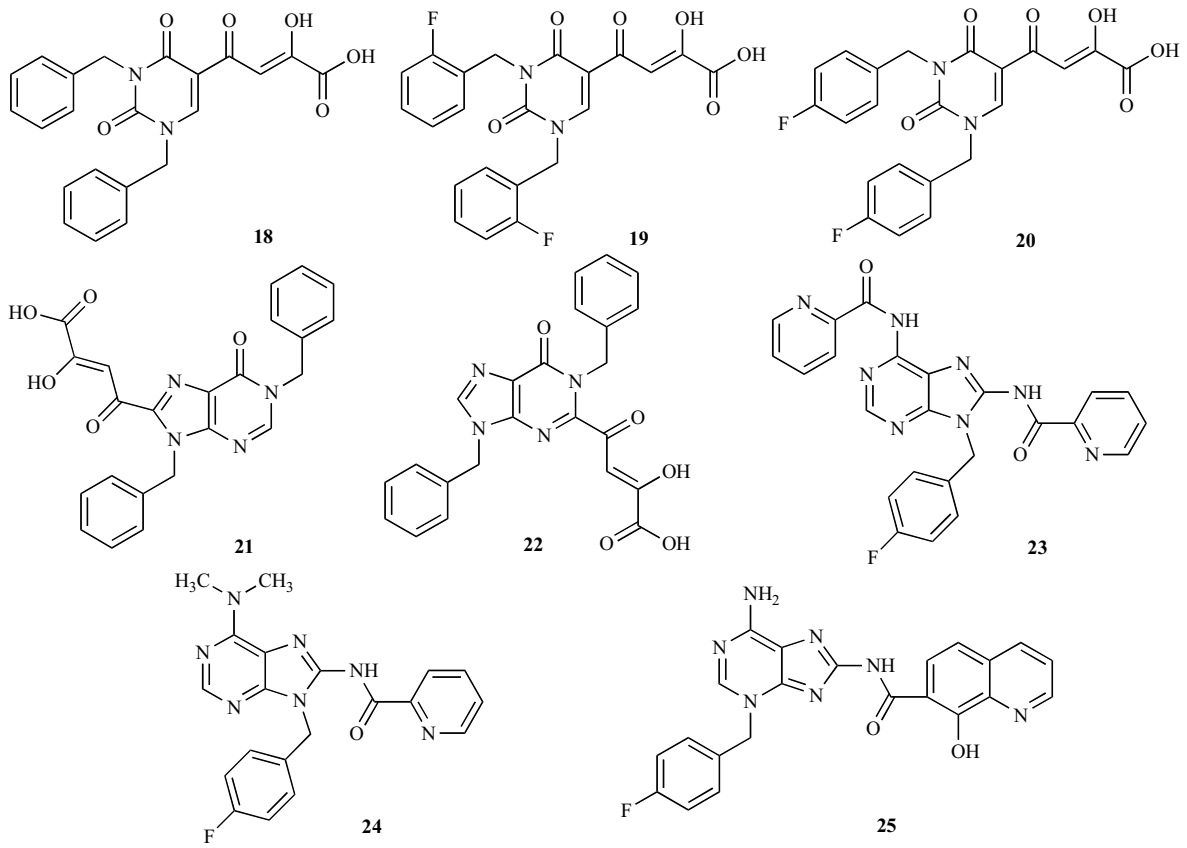


**Fig. (6).** Structures of compound **18-25**.

Compound (**18**) exhibits conspicuously potent antiviral efficacy in the inhibition of viral replication in peripheral blood mononuclear cell (PBMC). It is extremely active with $IC_{50}$ values in the nanomolar range. The $IC_{90}$ values for compound (**18**), being 3.91 μM (HIV-1TEKI) and 1.54 μM (HIV-1 NL4-3) respectively, are also compelling. Its antiviral efficacy data are equivalent to or greater than zidovudine and have proved to be well over 1 order of magnitude greater than some other diketo-containing IN inhibitors in PBMC. Furthermore, compound (**18**) shows no cytotoxicity at test concentration in PBMC and HeLa-CD4-LTR-β-gal cells. The results of biological assays make compound (**18**) the most active inhibitor of HIV replication of DKAs [48].

In addition, another series of diketo acid analogs with purine scaffolds has been synthesized by incorporating a purine ring in the aryl moiety and replacing the labile diketo acid moiety with other divalent metal chelating ligands including the two-point ligand picolinamide and the three-point ligand 8-hydroxy-quinoline-7-carboxamide. Data of the anti-IN activities assays indicate that these purine derivatives inhibit recombinant HIV-IN at low micromolar range, but are less potent than L-731988 (**15**). No significant difference in potency is observed between the two types of metal chelating ligands used in this study, which affect inhibitory potency depending on the substitution position of the 4-fluorobenzyl group. The C6-, C8-dipicolinamide substituted purine (**23**) exhibits greater potency against IN than compound (**24**) and with mono-picolinamide and compound (**25**), it is suggested that an additional metal chelating ligand contribute to the increase in potency and the spatial arrangement of the ligand can be crucial [50].

### 4.2. Carbazolone-Containing Diketo Acids

Taking compound (**14**) and L-731988 (**15**) as reference structures, carbazol-4-one and carbazol-1-one containing DKAs have been designed and synthesized by connecting the β-carbon of diketo acid to the indole ring *via* a methylene chain bridge and adding conformational restraint onto the open-chain form of the diketo acid Fig. (**7**). The results of biological assays indicate that compounds (**26-31**) show anti-IN activity in the low micromolar range, but are less than the lead DKAs [51]. This suggests that the open-chain form of diketo acid adopts a better conformation for binding to IN. This reduction in activity may also be attributed to the steric hindrance introduced by the methylene bridge or may result from a twisted coplanarity of $Mg^{2+}$ and α,γ-diketo complex with bulky β-substituents leading to a decrease in chelating strength. The geometry of diketo acid moiety may be crucial to anti-IN potency. Compound (**29**) shows a 2- to 3-fold increase in anti-IN activity compared with compound (**26**). With 6-fluorine substitution, compounds (**27** and **30**) exhibit better activity than corresponding compounds (**26** and **29**). Alkylation of the nitrogen of compound (**26**) with a 4-fluorobenzyl group (**28**) leads to significant decrease in activity, suggesting that the free nitrogen atom may directly

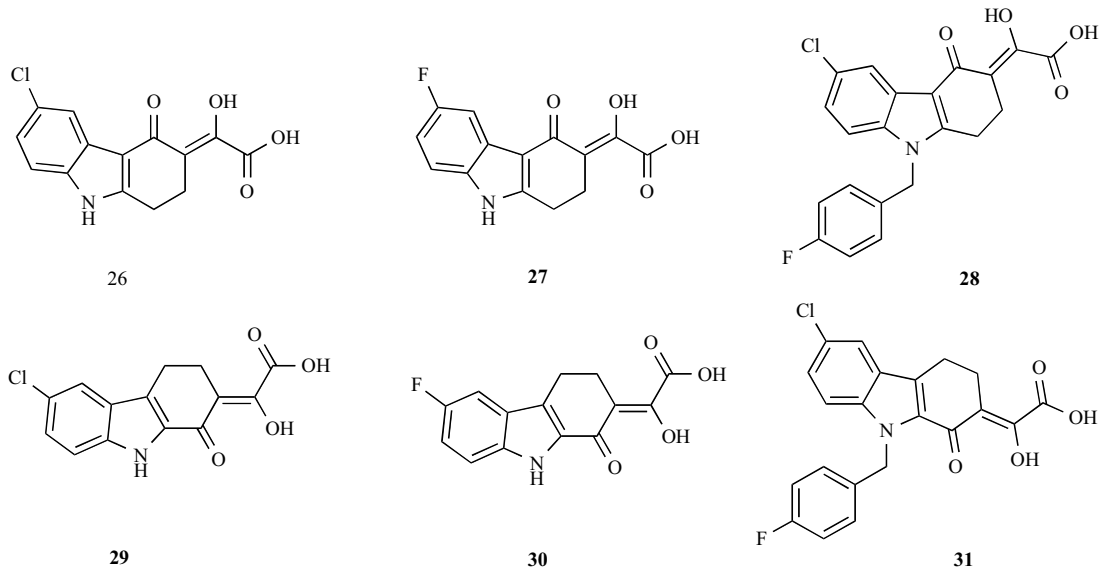



**Fig. (7).** Structures of compound **26-31**.

interact with the active site of IN. However, compound (**31**) adding a 4-fluorobenzyl group to carbazol-1-one (**31**) has little effect on activity [51].

### 4.3. Indole Diketo Acids

Several patents from Shionogi report indole derivatives with IN inhibitory activity [52, 53]. These compounds contain an indole-3-(1,3-diketo) skeleton, among which compound (**32**) shows high potency against IN with an $IC_{50}$ value of 0.13 μg/ml. Further studies on indole-β-diketo acid analogs suggest that compounds (**33** and **34**) with a free carboxylate group exhibit stronger IN inhibitory potency and better selectivity for ST than the indole-β-diketoesters (**35** and **36**), but only compound **35f** shows an interesting selectivity for ST (SI=7) Fig. (**8**). This indicates that the free carboxylic function is necessary for a strong interaction with the catalytic residues on the IN active site. The dioxole ring as well as the position of the diketo acid group result in no sig-

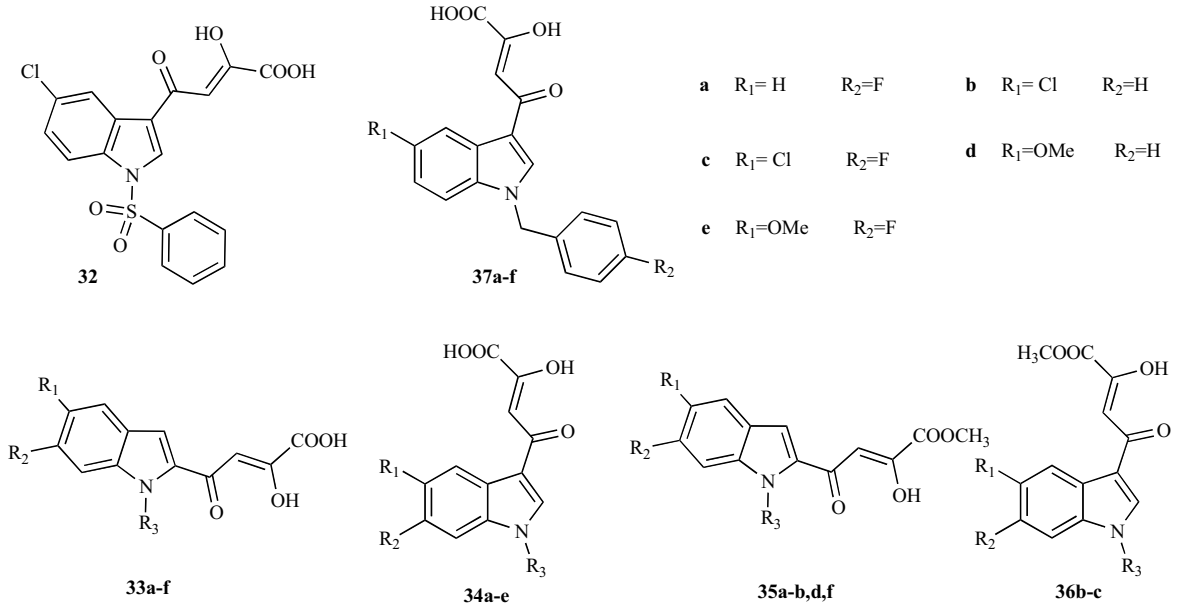



**Fig. (8).** Structures of compound **32-37**.

nificant variation of IN inhibitory activity. Moreover, the presence of the alkyl substituents on the indolic nitrogen seems to somewhat influence the potency. Interestingly, the high ST inhibitory potency of compounds **33e** and **34e** suggests that the large substituent on the N1 position of indole provides higher activity irrespective of the position (2 or 3) of the diketo acid group on the core indole. However, only the indole-3-β-diketo compounds (**34a**, **34c**, and **36c**) show antiviral activity much lower than the cytotoxic concentrations, with $EC_{50}$ values of 37, 9, and 19 μM respectively [54]. Compounds (**37a-e**) show potent inhibitory activity toward ST in comparison to 3'-P. Compared to unsubstituted parent compound (**34e**), the introduction of a chlorine atom on the indolic ring (**37b**) leads to a significant decrease of anti-IN activity, while the presence of a methoxy group in the same position (**37d**) does not influence the ST inhibitory potency. With a 4-fluorine atom on the benzyl moiety, compounds (**37a** and **37c**) are 2-fold more potent than compound (**34e**) approximately. Furthermore, compound (**37e**) with a 4-fluo-robenzyl moiety and a methoxy group on the indole system, shows IN inhibitory activity with an $IC_{50}$ value of 0.004 μM [55].

### 4.4. Catechol–Diketo Acid Hybrids

The catechol–DKA hybrids are composed of two groups, the catechol and 2-hydroxy-4- oxobutenoic acid Fig. (**9**). The IN inhibitory potency demonstrates that these compounds are active against IN with slight decrease in the 3'-P/ST selectivity. Compound (**38**) with free carboxylic and phenolic hydroxyl group shows strong inhibitory potency of 3'-P and ST, with $IC_{50}$ values of 3.9 and 1.1 μM respectively. In contrast, diketoester (**39**) is inactive. Compound (**40**) bearing two methoxy groups on the phenyl ring is only modestly active on ST. Therefore, it is the multi-active site that leads to the differences. Replacing the 3, 4-dihydroxyphenyl group by a 6, 7-dihydroxynaphthyl moiety, compound (**41**) shows similar IN inhibitory potency with analog (**38**). But compound (**42**), the ester of (**41**), is only about 4-fold less active than compound (**41**). Since diketoesters are known to be generally inactive, it is considered that the IN inhibitory activity of compound (**41**) is probably due to the presence of the 6, 7-dihydroxynaphthyl moiety. Compounds (**43** and **44**) are tested in order to evaluate the influence of the catechol function on the activity. Unfortunately, they are both inactive in anti-IN activity assay; being similar to compounds (**45** and **46**). Concerning the antiviral activity, compound (**38**) is active at non-cytotoxicity concentrations ($EC_{50}$=46μM, $CC_{50}$= 160μM) and exhibits modest therapeutic index (TI=3.5). Compounds (**41** and **42**) display similar antiviral properties, but the acid (**41**) is twice less toxic than the ester (**42**). Both of them inhibit the HIV-1 cytopathic effect in cell-based assays at cytotoxic concentrations. Summarizing the result, the presence of an acidic function on the left side of the diketo acid significantly influences the selectivity toward ST and the antiviral properties [56].

### 4.5. Azido-Containing Diketo Acids

The azido-containing DKAs IN inhibitors have been discovered during the process of preparing variants of compound (**16**). It appears that the azidomethyl group is a suitable replacement for benzyloxy group in compound (**16**). However, the two groups have quite different electronic and geometric properties. Compound (**47**) with 3,5-di-azidomethyl substitution, selectively inhibits the ST of IN with an $IC_{50}$ value of 2 μM, but shows no activity in the 3'-P assay at a tested concentration of 100 μM. It also shows moderate antiviral activity ($EC_{50}$=5μM) in an assay using HIV-1 infected cells with low cytotoxicity ($CC_{50}$>50 μM). Compounds (**48**, **49** and **50**) show better ST inhibitory activity, but exhibit less antiviral activity than the parent compound (**47**) in HIV-1 infected cells. Mono-azidomethyl analog (**51**) is approximately equivalent to or greater than compound (**47**) in both IN and antiviral assays, while the analog (**52**) shows less activity against ST and exhibits no antiviral activity at a concentration of 25 μM. Introduction of methoxyl or isopropoxy groups into compound (**51**) results in a reduction in both the ST and antiviral assays. The nitrile-containing compound (**53**) shows equivalent IN inhibitory activity as compound (**51**), but exhibits significantly loss of antiviral activity ($EC_{50}$>25 mM) in HIV-1 infected cells Fig. (**10**). Both azido and nitrilomethano groups are found to be sterically and electronically similar and to exhibit similar metal dependencies [57].

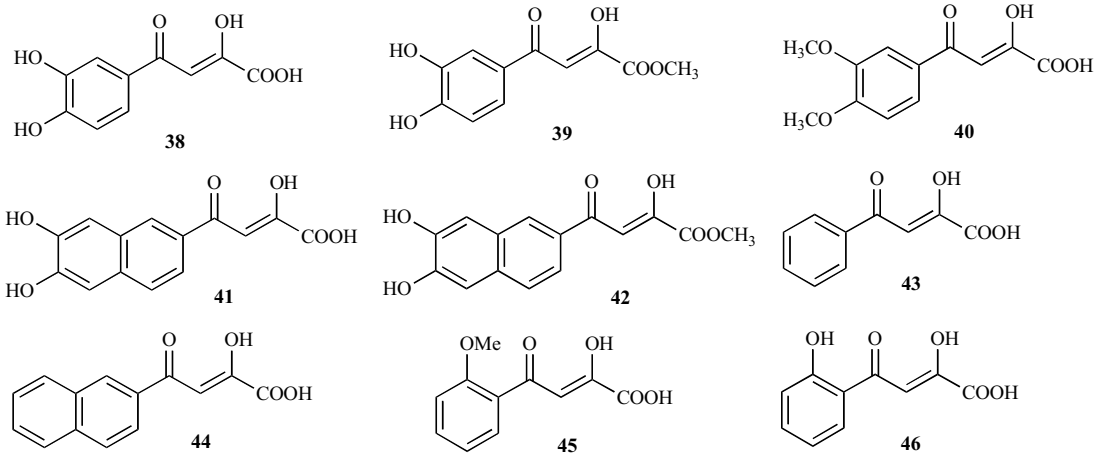



**Fig. (9).** Structures of compound **38-46**.

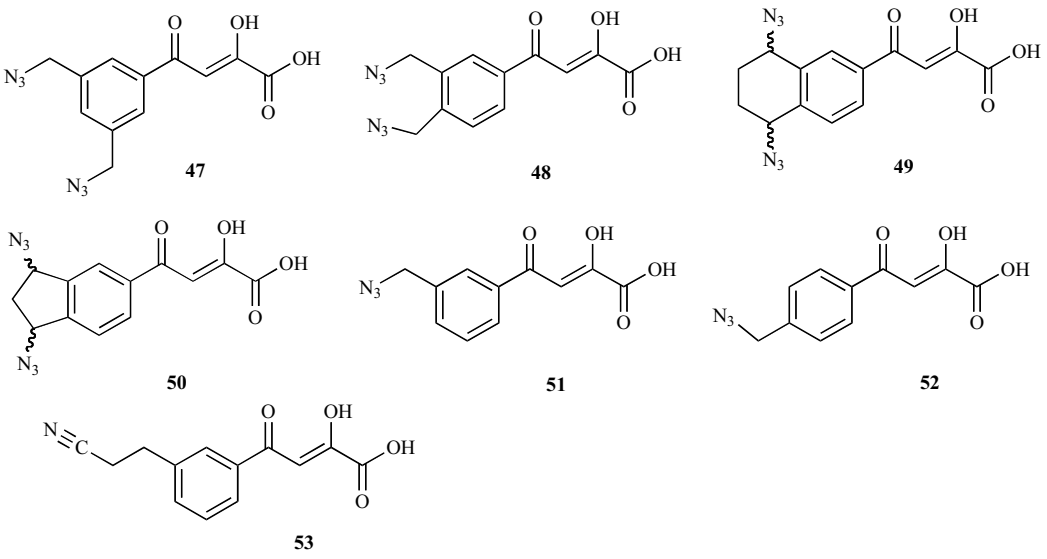


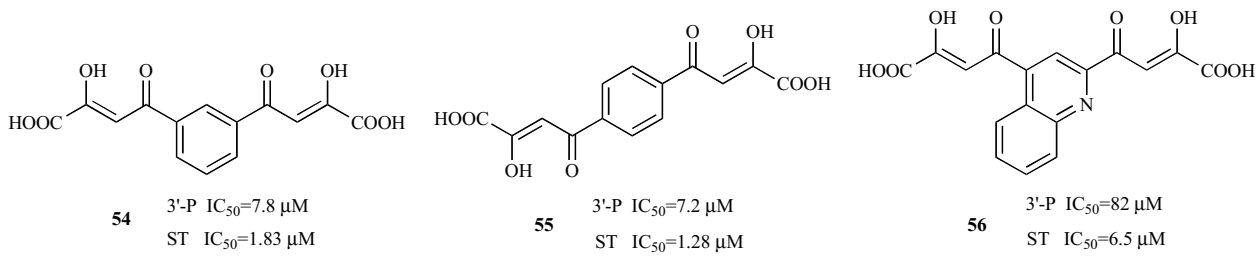
**Fig. (10).** Structures of compound **47-53**.

### 4.6. Bifunctional Diketo Acids

Some bifunctional DKAs (BDKAs) have been reported as IN inhibitors, which are characterized by the presence of two diketo acid chains in the skeleton of molecular Fig. (**11**). Compound (**54**) displays effective ST inhibitory potency and significantly enhanced 3'-P inhibitory potency, which is due to its ability of competing with the target DNA and blocking access of the viral DNA substrate to the enzymes active site [28]. Compound (**55**) with the second diketo acid chain attached to the 4-position displays similar IN inhibitory potency. Compared with compounds (**54** and **55**), compound (**56**) inhibits 3'-P and ST at higher concentration [28]. Based on these findings, a new binding mode for DKAs is proposed. Since HIV-1 IN catalyzes the insertion of a donor DNA substrate into an acceptor DNA template, both DNA duplexes are required to bind two adjacent sites. In this model, it is suggested that BDKAs can interact with both donor and acceptor sites, being inhibitors of both 3'-P and ST. In contrast, monofunctional DKAs bind only to the acceptor site, selectively inhibiting ST [29, 36]. Compound (**54**) exhibits little or no antiviral activity in whole cell assays, which may be due to its highly charged nature that prevents it from crossing the cell membrane.

Compound (**57**) is a potent IN inhibitor for both 3'-P ($IC_{50}$=12nM) and ST ($IC_{50}$=0.20μM). It shows high antiviral activity against HIV-1 infected H9/HTLVIIIB cells ($EC_{50}$= 4.29μM, $EC_{90}$=40μM) and low cytotoxicity ($CC_{50}$>200μM, TI>46.6). In contrast, compounds (**58** and **59**) are 8 and 5 times less potent than the parent compound **57** respectively, while compound (**60**) is inactive below 50 μM concentration Fig. (**12**) [58].

Two different sets of diketo acid dimers have also been designed and synthesized as novel bifunctional analogs Fig. (**13**). It is hypothesized that such dimeric compounds may simultaneously bind to two divalent metal ions on the active site of IN. The IN inhibitory data demonstrates that all diketo acid dimers exhibit potent inhibitory activity against IN and the amide-linked dimers (**61a–e**) show a much better selectivity for ST versus 3'-P and a stronger antiviral efficacy in HIV-infected cells than the benzyloxy-linked dimers (**62a-c**) and compound (**62d**). The intrinsic selectivity for ST of the amide-linked dimeric inhibitors decreases as the length of the linear linker increases from two carbons to six carbons chain. The highly rigid compound (**61a**) shows somewhat decreased selectivity for ST, but exhibits the most potent antiviral activity. Similarly, compound (**61e**) with the highly flexible linker also shows moderate antiviral activity. Compounds (**61b-d**) show high potency and selectivity for ST, but exhibit no significant antiviral activity. Among the benzyloxy-linked diketo acid dimers, the ortho-substituted diketo acid (**62a**) shows the most potent antiviral activity, while other two analogs (**62b-c**) present lack of antiviral activity.



**54** 3'-P $IC_{50}$=7.8 μM; ST $IC_{50}$=1.83 μM

**55** 3'-P $IC_{50}$=7.2 μM; ST $IC_{50}$=1.28 μM

**56** 3'-P $IC_{50}$=82 μM; ST $IC_{50}$=6.5 μM

**Fig. (11).** Structures of compound **54-56**.

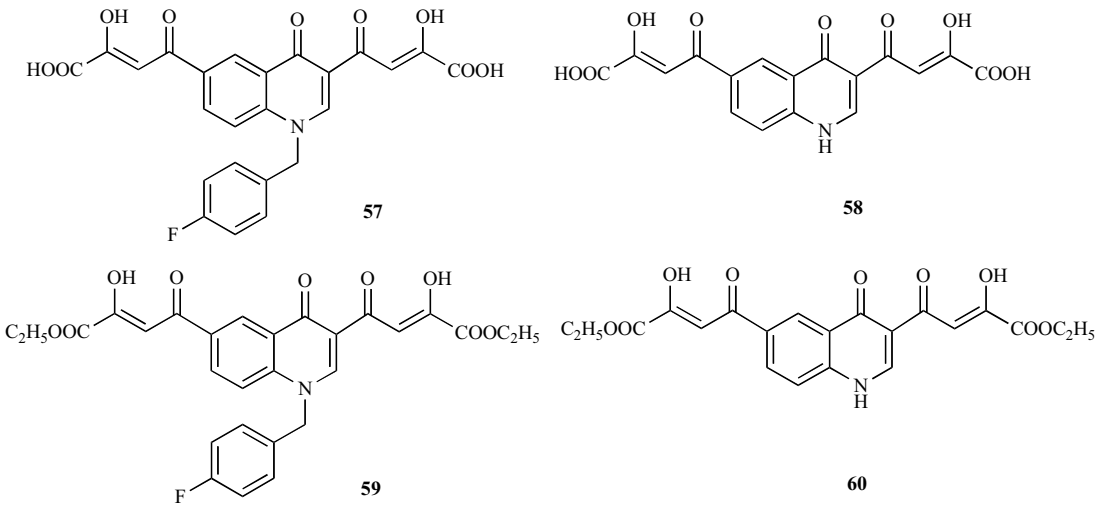



**Fig. (12).** Structures of compounds **57-60**.

Compound (**62d**) exhibits moderate selectivity against ST, but displays potent antiviral activity in HIV-1 infected CEM cells. It seems that the size and shape of the linker of the dimers have remarkable influence on the ST selectivity, the antiviral activity as well as the toxicity [59].

### 4.7. Diketo Acid-Containing Ferrocene

Diketo acid-containing ferrocene has been discovered as an IN inhibitor in early SAR studies of DKAs Fig. (**14**). Compound (**63**) shows strong ST inhibitory potency in an extracellular HIV-1 IN assay, with an $IC_{50}$ value of 0.81 μM

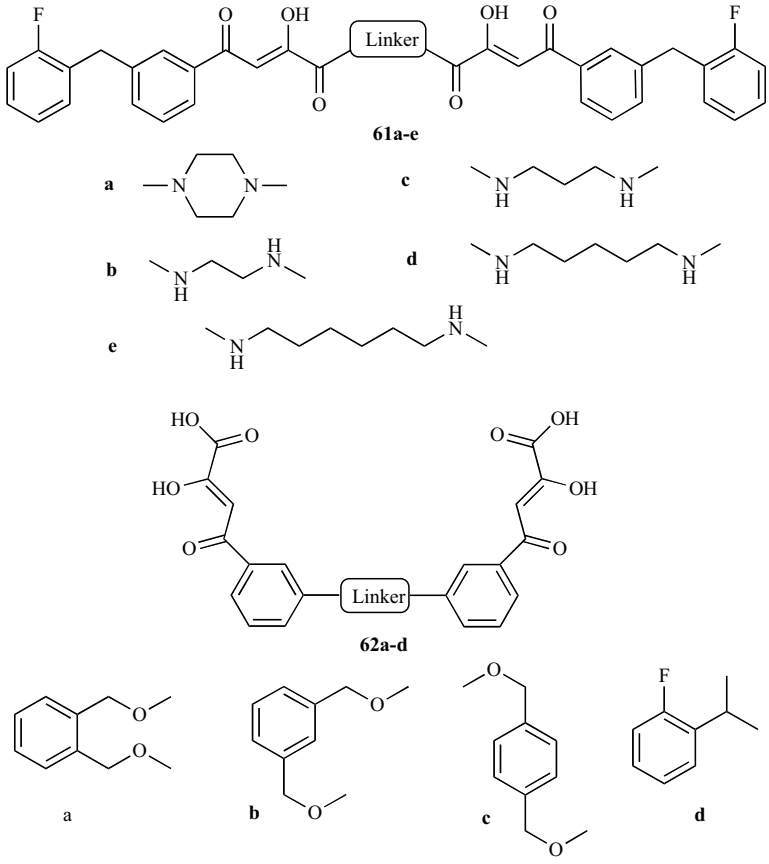



**Fig. (13).** Structures of compound **61-62**.

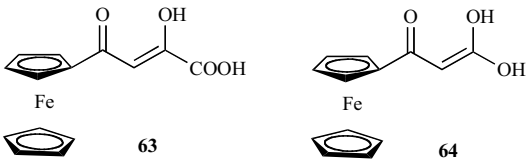



**Fig. (14).** Structures of compound **63-64**.

[28]. Some other diketo acid-containing ferrocene inhibitors are also reported in the following molecular interaction field, density functional and docking studies [60]. Results of docking studies show that these compounds exhibit high docking scores and compound (**64**) yields the best GOLD score (40.5). The results also confirm the existence of a novel binding trench in HIV-1 IN [43], which can bind both the 5CITEP as well as the ferrocene potential inhibitors. The introduction of the transition metals rotatable rings, and flexible dihedrals accommodates the ferrocene derivatives at the two binding trenches of the active site.

### 4.8. Heteroaryl Diketohexenoic Acids

A series of heteroaryl diketohexenoic acids has been designed as a promising tool to investigate how elongation of the diketobutanoic group will affect anti-IN activity. The diketohexenoic acids are characterized by a side chain, which is conceived as the result of a partial superimposition between the cinnamoyl group and the diketobutanoic acid moiety Fig. (**15**). Compounds (**65**) and (**66**) show strong inhibitory potentcy of IN ($IC_{50}$ for 3'-P: 7.9 and 8.9 μM; $IC_{50}$ for ST: 7 and 7.5 μM, respectively) in enzyme assays. Compared to the compound (**15**), compound (**65**) shows decreased IN inhibitory activity and loss of selectivity for ST, which is due to the different steric and electronic arrangement of the phenylmethyl moieties of compound (**65**) and compound (**15**) in the enzyme binding site. Other members of this series inhibit IN at higher concentrations ranging from 22 to 95 μM, which results from the steric hindrance along with differences in the electronic effects exerted by the substituents in the 4-position of the phenyl ring. Surprisingly, data of antiviral assay indicate that compounds (**65** and **66**) turn out to be potent and selective in cell-based assays, both with $EC_{50}$ values of 1.5 μM, being similar to the antiviral activity exhibited by compound (**15**). Not an improvement of antiviral activity but a seven-fold loss of potency is observed when a fluorine atom is introduced at the 4-position of the phenyl ring. The introduction of chlorine, methyl, methoxy or nitro substituents leads to totally inactive products. Moreover, acid derivatives are less cytotoxic than the corresponding esters, whereas no difference in cell-based antiviral activities is observed by comparing the active acids with the related esters. In the optimization of compound (**65**), efforts are focused on derivatives bearing one or two substituents at ortho and/or meta position of the phenyl ring. Compared with the lead (**65**), most of the synthesized derivatives show no significant improvement in the potency. Only the 2, 6-difluoro derivative (**67**) exhibits significant potency, with $EC_{50}$ and $CC_{50}$ values of 0.3 and 37 μM, respectively (TI=123). Anti-IN activity of compound (**68**) is 2-fold greater than compound (**15**), but it exhibits less antiviral activity. With a pyrazole masking the diketobutanoic acid chain, compound (**69**) is found to be a completely inactive derivative. Replac-

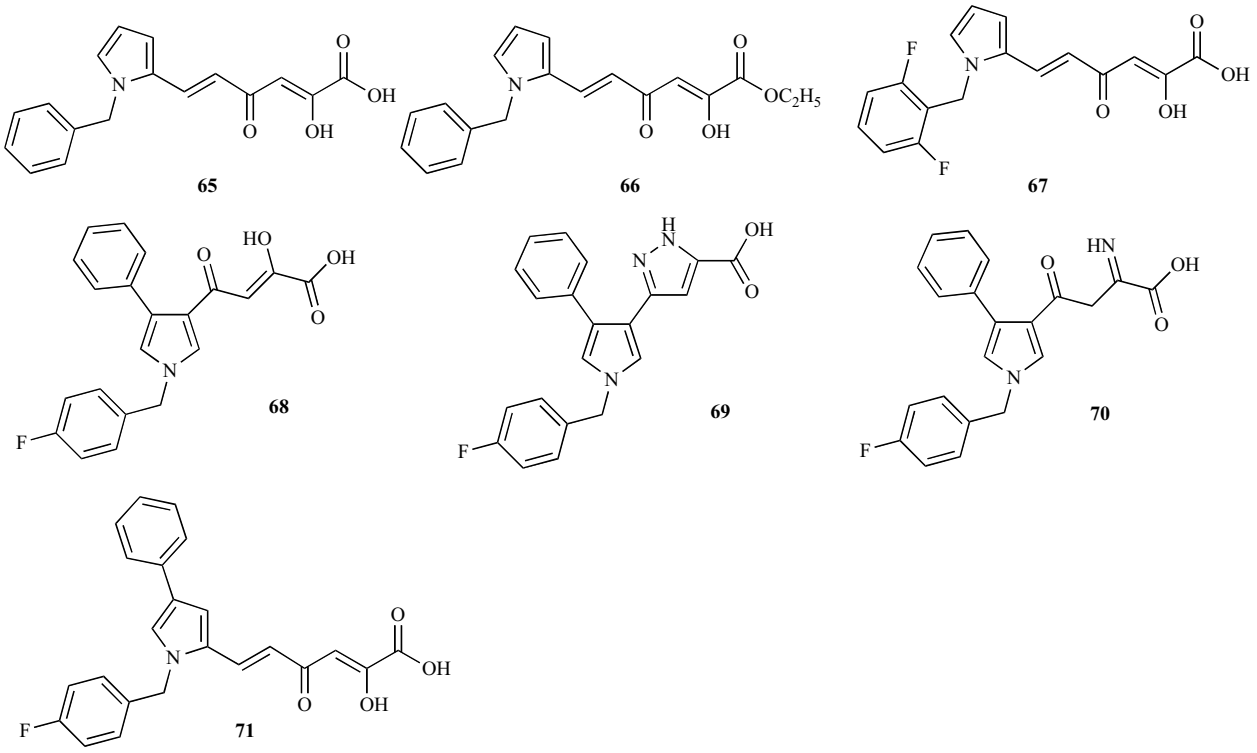



**Fig. (15).** Structures of compound **65-71**.

ing the diketobutanoic acid chain with imino-keto or diketohexenoic acid moieties leads to compounds (**70** and **71**), which are 2 and 10 times less potent than compound (**68**) in enzyme assays. In addition, all the indolyldiketohexenoic acids designed in the further development are unable to block the HIV-1 replication in cell-based assays at concentrations lower than 100 μM [61, 62].

### 4.9. Triketoacids

A new triketoacid-based chemotype has been discovered to inhibit ST selectively. These compounds are characterized by inserting a carbonyl group between the aryl and 2, 4-dioxobutanoic acid moiety Fig. (**16**). Compound (**72**) is a moderately potent inhibitor of the isolated IN and of HIV-1 in cell culture, with $IC_{50}$ and $EC_{50}$ values of 1 and 120 μM, respectively. It can inhibit IN in the context of the pre-integration complex (PIC) ($IC_{50}$=0.6μM) isolated from infected cells as well. However, it does not inhibit other viral or DNA-processing enzymes and is inactive against other viruses. After a series of passages in cell culture, two resistant strains that maintained in the presence of compound (**72**) are isolated. Similar to compound (**15**), two unique sets of mutations for each resistant isolates (T66I and L172F, V151A and V165I), are located in the core domain of IN being close to the active site catalytic D,D-35-E triad. Introduction of the T66I mutation into the HIV-NL4-3 strain results in resistance to compound (**72**) in cell culture. The result confirms that the triketoacid inhibitor (**72**) targets IN. Moreover, a brief SAR investigation is carried out to determine the binding mode to $Mg^{2+}$ of triketoacid inhibitors. Replacing the carboxylic acid with a tetrazole, compound (**73**) is essentially inactive. In addition, compound (**74**) is only somewhat less potent than compound (**72**). Therefore, triketoacids may bind to IN in a manner similar to the DKAs. Since the saturated derivative (**74**) is an effective inhibitor of IN, compound (**75**) is synthesized by removing the carbonyl group adjacent to the aryl group. It is disclosed that compound (**75**) exhibits strong IN inhibitory potency, with an $IC_{50}$ value of 0.01 μM [63, 64].

Further SAR survey related to the phenyl ring indicates that introduction of an electron-withdrawing group at the ortho- or meta-position enhances *in vitro* activity. The phenyl ring can be replaced by pyridine, but its activity is sensitive to regiochemistry with the more exposed 3-isomer weaker than the 2-isomer. With a naphthyl instead of phenyl group, compound (**76**) shows a noticeable improvement in inhibitory activity, which is in accordance with the hypothesis that a hydrophobic binding site exists in the region between the ortho- and meta-sites of phenyl ring. In contrast, compounds (**77** and **78**), which also have an additional phenyl ring, do not demonstrate increased potency, indicating that the improved activity seen with compound (**76**) is not simply due to non-specific, hydrophobic binding. This suggests that a specific aryl-binding domain exists within IN, which can distinguish subtle differences in structure. Several other triketo analogs have been synthesized and tested for anti-IN potency. The results show that compound (**79a**) is active against ST of purified HIV-IN in soluble mutant, with an $IC_{50}$ value of 80 μM. Compounds (**79b-e**) are less effective against IN (65).

Comparison of the triketoacids with two different DKAs, compound (**14** and **15**), provide some insight into the SAR and helps to establish a model for the binding conformation. It is assumed that two different aryl-binding domains exist in the active site of IN. Triketoacid is able to access either one with a simple change of enol configuration.

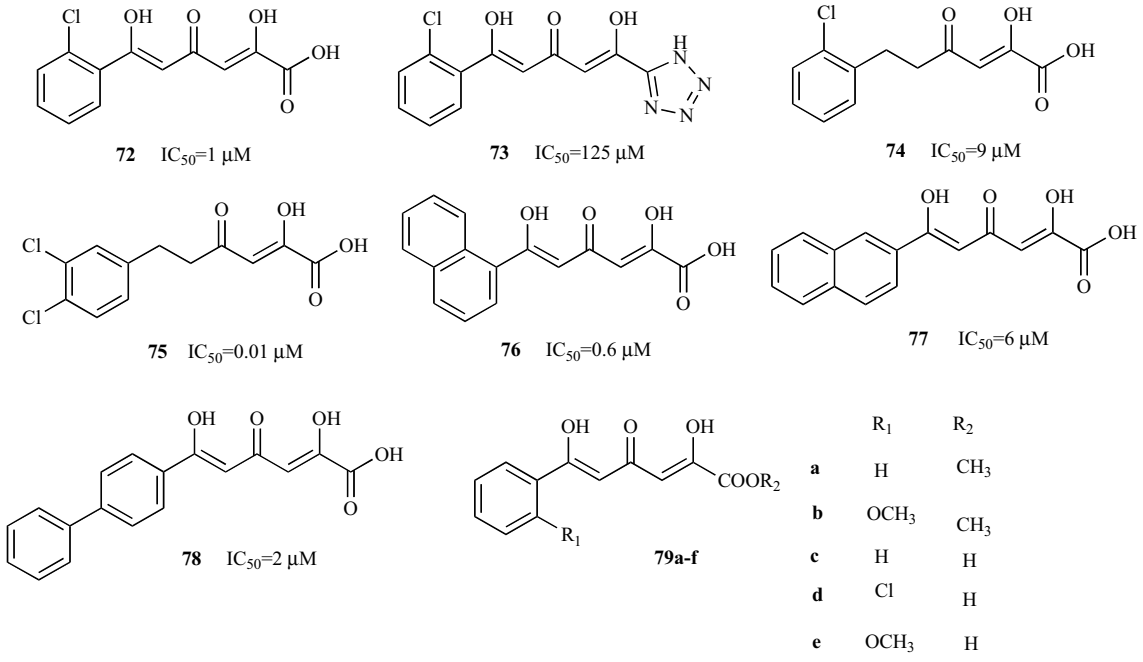



**Fig. (16).** Structures of compound **72-79**.

## 5. 8-HYDROXY-1,6-NAPHTHYRIDINES

The 1,3-diketo acid functionality is essential for IN inhibitory activity of DKAs. However, the 1,3-diketo acid moiety is biologically labile. 8-hydroxy-[1,6]-naphthyridine is discovered as a suitable replacement which still fit onto the critical pharmacophore and maintains antiviral activity Fig. (**17**). In the novel derivatives one of the naphthyridine nitrogen atoms is thought to function as a bioequivalent of the carboxylate anion of the diketo acids, while the enol tautomer of one of the ketones is represented by an isosteric phenolic hydroxyl group.

Compound (**80**) maintains inhibitory potency against ST with an $IC_{50}$ value of 0.04 μM. It also potently inhibits viral replication at a $CIC_{95}$ value of 6.2 μM. The presence of the nitrogen atom at the 6-position of naphthyridine allows the molecule to attain a conformation in which the central phenyl ring and the naphthyridine ring are coplanar. Further elaboration of compound (**80**) leads to compound (**81**), which contains a sultam substitution at the 3 position of the phenyl ring. Compound (**81**) shows strong ST inhibitory potency with an $IC_{50}$ value of 0.01 μM and also exhibits antiviral activity at a $CIC_{95}$ value of 0.39 μM in cell-based assays. However, it shows significant loss in potency against drug resistant IN with T66I and S153Y double mutations. This indicates that the mechanism of action of compound (**81**) is similar to that of the DKAs [66].

A related series of compounds, the 8-hydroxy-1,6-naphthyridin-7-carboxamide derivatives are synthesized by replacing the aryl ketone in above naphthyridine ketones with a carboxamide group. With a 4-fluorobenzyl carboxamide, compound (**82**) potently inhibits ST of HIV-1 integration with an $IC_{50}$ value of 33.4 nM. It inhibits viral replication with a $CIC_{95}$ of 1.25 μM in cell culture, which decreases 4-fold in the presence of 50% normal human serum (NHS), due to its higher binding to human plasma protein (99.2%) [67]. Introduction of a six-membered cyclic sulfonamide substituent at the 5-position of the 8-hydroxy-1,6-naphthyridine core, provides the drug candidate L-870810 (**83**). The inhibitor selectively blocked ST with an $IC_{50}$ value of 8 or 15 nM when assayed using 0.5 nM and 5 nM target DNA respectively, while being less effective at inhibiting 3'-P ($IC_{50}$ value of 85 and 250 nM in 0.5 and 5 nM target DNA). The preferential inhibition of ST and the sensitivity of L-870,810 to the concentration of target substrate suggest that this inhibitor binds to IN at a site similar to the target DNA. In a competition-binding experiment, L-870810 displaces a ra-

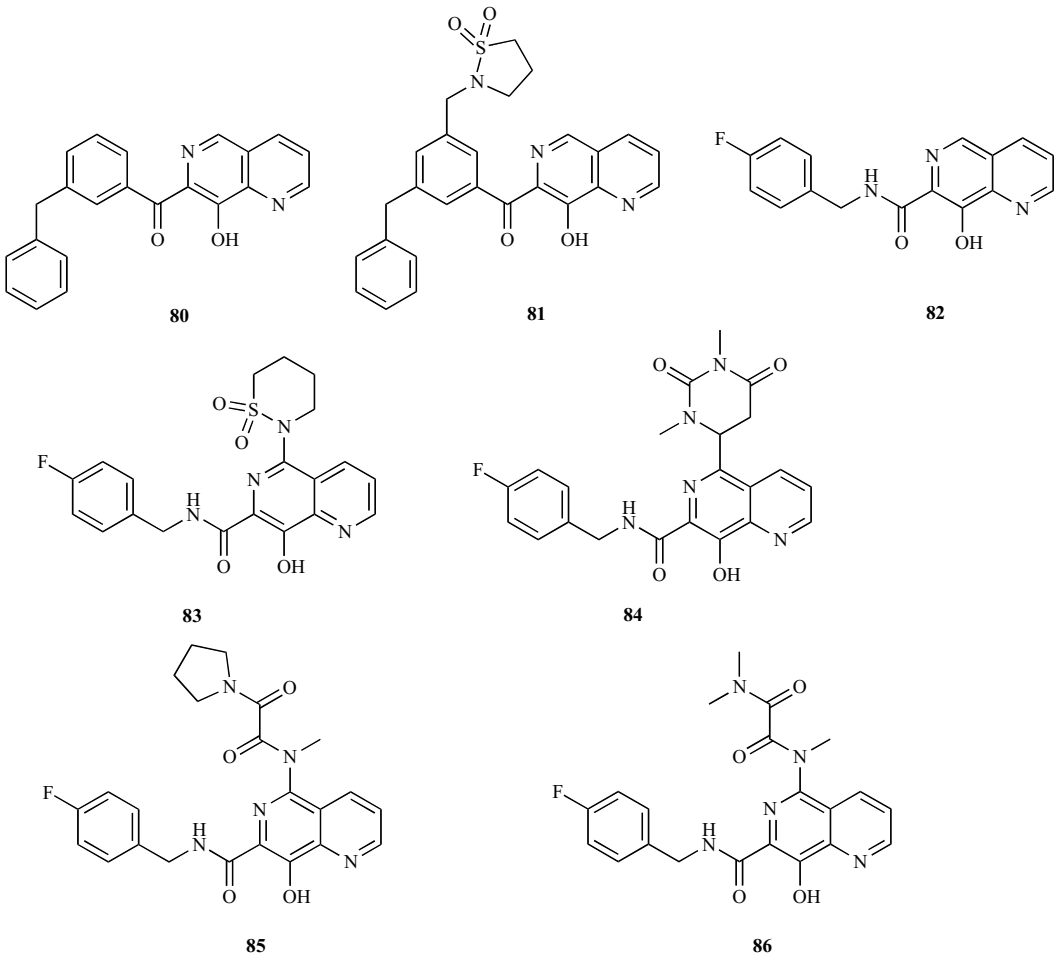



**Fig. (17).** Structures of compound **80-86**.

diolabeled diketo acid containing inhibitor from the IN donor complex with a $K_i$ of 3 nM, indicating that the inhibitor binds to the same or overlapping region on the IN active site as DKAs. It exhibits antiviral activity and is active against wild-type and multi-drug resistant strains of HIV-1, HIV-2 and SIV in a manner consistent with its effect on IN, with an $IC_{50}$ value of 4 nM. L-870810 is a potent IN inhibitor with improved bioavailability compared to previously reported DKAs, with an oral bioavailability of 41%, 24%, and 51% and low plasma clearance of 2.8, 2.0, and 6.6 $ml \cdot min^{-1} \cdot kg^{-1}$ in rats, dogs, and rhesus, respectively [68]. However, clinical research on L-870,810 was stopped after unacceptable liver and kidney cell toxicity found in dogs [69].

Some highly active HIV-1 IN inhibitors have been synthesized by the introduction of a 5,6-dihydrouracil functionality in the 5-position of compound (**82**). Compound (**84**), the (-) enantiomer of the dimethylated dihydrouracil, is a 150-fold more potent antiviral agent than compound (**82**). It displays antiviral activity with $CIC_{95}$ value of 20.1 nM in 10% fetal bovine serum (FBS) and 40.2 nM in 50% NHS. Good pharmacokinetics had been observed when it was dosed in rats and dogs [67]. Another potent series of 5-amino derivatives of 8-hydroxy-1,6-naphthyridine-7-carboxamide has also been synthesized. Compound (**85**) blocks viral growth with a $CIC_{95}$ value of 63 nM in the presence of 10% FBS. Compound (**86**) also known as L-870812 is demonstrated to be efficacious against replication of simian-human immunodeficiency virus (SIV) 89.6P in infected rhesus macaques. It inhibits the ST activity of recombinant HIV and SIV IN *in vitro* with an $IC_{50}$ value of 40 nM. It displays balanced biological and physical properties, with an antiviral $CIC_{95}$ of 103 nM and a moderate affinity for human serum protein (93.2%) that lead to a modest 2.5-fold shift of the $CIC_{95}$ to 250 nM in the presence of 50% NHS. L-870812 also exhibits excellent pharmacokinetic profiles in rats and monkeys [70].

## 6. TRICYCLIC ANALOGUES

A novel class of tricyclic phthalimide analogs has been designed and synthesized as IN inhibitors by Verschueren *et al*. Data from the biochemical and biological evaluation of these compounds suggest that all the tricyclic phthalimide analogs demonstrate a similar activity on the enzyme when compared with compound (**83**) but are less selective. Compound (**87**) Fig. (**18**) is the enzymatically most active compound in this series with an $IC_{50}$ value of 112 nM on IN.

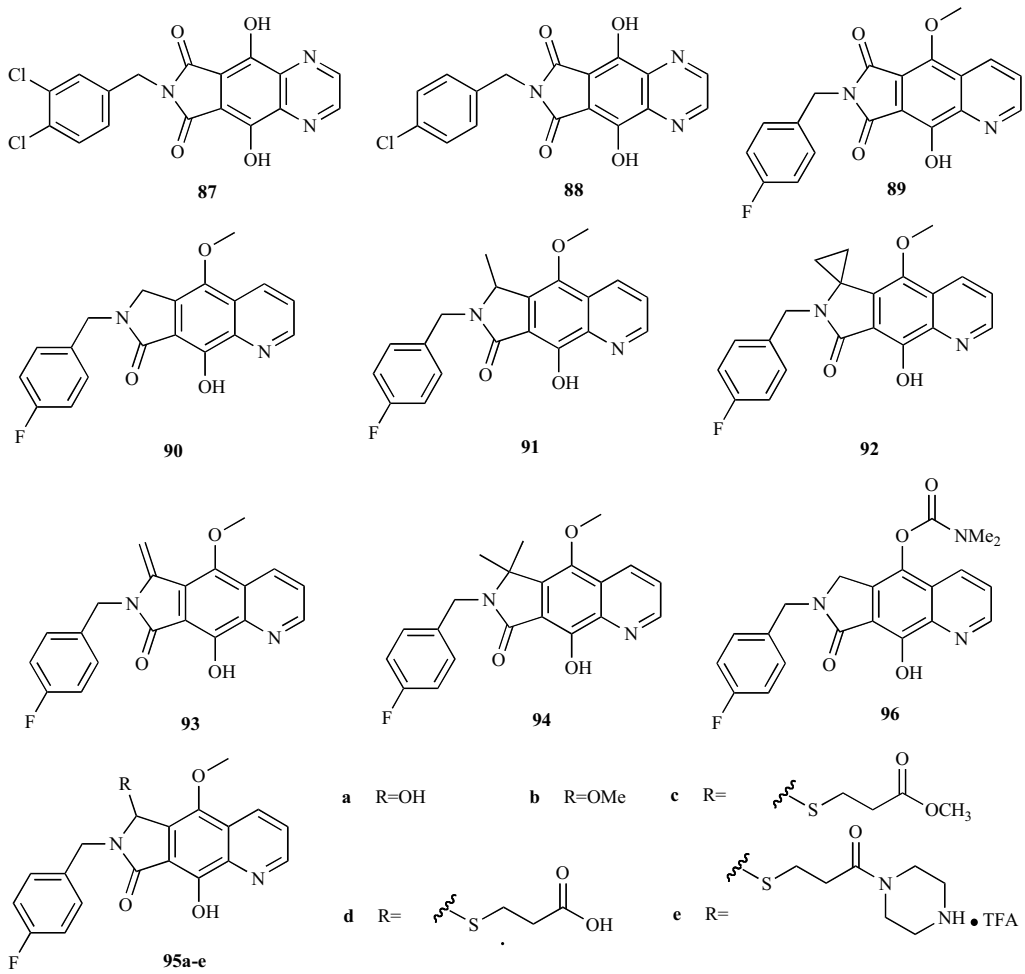



**Fig. (18).** Structures of compound **87-96**.

Compound (**88**) exhibits an $EC_{50}$ of 270 nM against viral replication in a cell-based assay. Molecular docking studies indicate that the carbonyl and hydroxy oxygens chelate the $Mg^{2+}$ ion in the active site. The D64 and D116 acid side chains and two water molecules complete the octahedral coordination sphere of the ion. Important hydrogen bonds between inhibitors and the enzyme are observed. SAR investigation demonstrates that the presence of a single carbonyl-hydroxy-aromatic nitrogen motif is essential for the enzymatic activity. However, only one such motif is necessary. Hydrophobic substituent on the phthalimide nitrogen is also important, which interacts with the flexible loop formed by residues 140-148 [71].

Another series of tricyclic IN inhibitors has been designed based on conformational analysis of compound (**83**) and docking the designed inhibitor into the active site of an IN/DNA complex model [72]. Regarding the tricyclic scaffold, the pyridyl nitrogen, the C-9 phenol and the C-8 carbonyl form the pharmacophore of the compounds in binding to the enzyme [73]. Similar to DKAs, the phenol and the C-8 carbonyl group *via* a bidentate coordination, chelate to the available $Mg^{2+}$ ion in the catalytic core of IN [74]. Among the synthesized compounds, tricyclic phthalimide analog (**89**) shows good IN inhibitory activity ($IC_{50}$=0.08 μM), while lack of significant anti-HIV potency in the cell-based assay. Removing the C-6 carbonyl, compound (**90**) exhibits substantially improved potency in the cell-based assay, with an $EC_{50}$ value of 0.089 μM. The improved potency of compound (**90**) over compound (**89**) can be due to the increased solubility and permeation through the cell membrane. Further investigation on the effect of substitution at the C-6 suggests that compounds substituted with small alkyl groups show higher activity against ST. Among the substitutions placed at the C-6, methyl analog (**91**) and the spirocyclopropyl compound (**92**) have the most attractive *in vitro* profiles. Compound (**93**) maintaining the planar conformation shows weaker activity against IN and viral replication. Compound (**94**) decreases by an order of magnitude compared to the spirocyclopropyl analog (**92**). With polar substitutions, compounds (**95a-e**) have poor potency in the antiviral assay. For the remarkably enhanced activity of C-5 carbamate (**96**) Fig. (**19**) in cell-based assay, the effects of other functional groups at C-5 and the relationship between substitutions at the C-5 and the C-6 positions are explored. All the three

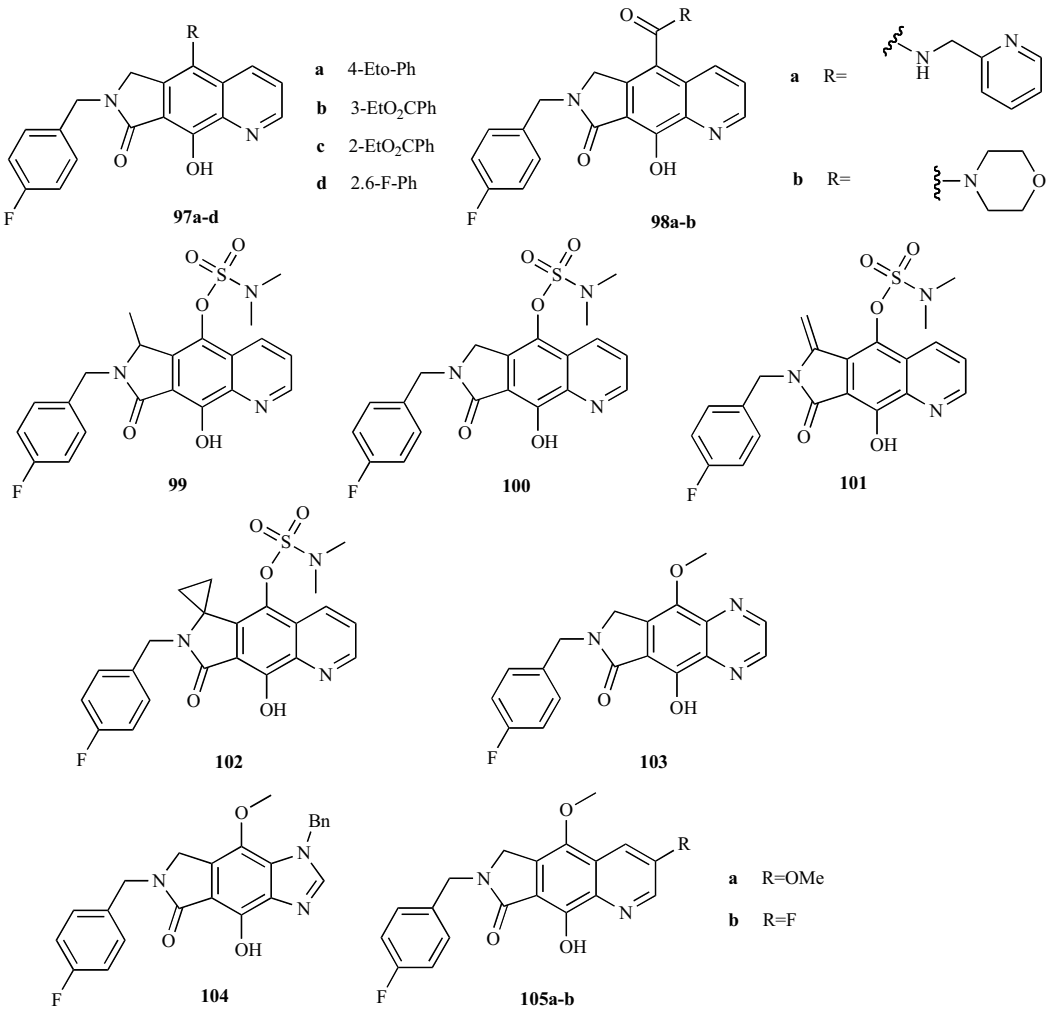



**Fig. (19).** Structures of compound **97-105**.

simple mono-substituted bi-aryl analogs (**97a**–**c**) show moderate potency against IN and HIV. Compound (**97d**) displays further improved activity by the introduction of a 2, 6-difluorophenyl group. Compounds (**98a** and **98b**) exhibit $IC_{50}$ in sub-micromolar and $EC_{50}$ in low nanomolar ranges. The methyl sulfamate (**99**) as well as the dihydrosulfamate (**100**) both have impressive antiviral activities. In contrast, compounds (**101** and **102**) bearing a sulfamate exhibit poorer enzymatic activity. Compound (**103**) with a pyrazine instead of the pyridine shows a clear reduction in antiviral activity compared to compound (**90**). Moreover, the imidazole analog (**104**) completely loses inhibition activity toward the enzyme along with its antiviral potency. With a methoxy substitution at C-3 in pyridine, compound (**105a**) largely preserves the enzymatic activity while improving anti-HIV potency in the cell assay when compared to compound (**94**). Furthermore, replacing hydrogen with fluorine at C-3, compound (**105b**) shows significant enhancement of enzymatic activity, but at the same time, loses anti-HIV activity in the cell assay significantly.

SAR studies indicate that the pyridine ring is optimal in the novel tricyclic-based scaffold. Both the shape of heterocycles and the electronic properties of nitrogen in the ring are important for biological activity.

## 7. SALICYLIC ACID AND RHODANINE CONTAINING COMPOUNDS

A novel class of IN inhibitors containing salicylic acid and rhodanine group has been discovered by using a common feature pharmacophore model derived from DKAs. Results of the IN inhibitory activities suggest that compounds containing both salicylic acid and rhodanine group show significant inhibitory potency against IN, while the presence of either a salicylic acid or a rhodanine group alone does not. Amongst the analogs with both of the two components, compounds (**106a-c**) show high potency against ST, with $IC_{50}$ values of 11, 17 and 11 μM respectively Fig. (**20**). With 5-nitro- or 5-chlorophenol, compounds (**106e** and **106f**) effectively inhibit ST with $IC_{50}$ values of 18 and 17 μM, while compounds with 3- or 4-benzoic acid exhibit moderate activity. Some of the compounds containing only a salicylic acid show inhibitory potency against IN. The most potent compounds (**107** and **108**) inhibit 3'-P and ST activities of IN with $IC_{50}$ values of 13, 16 μM and 12, 8 μM, respectively. In addition, most of the obtained compounds do not show a significant antiviral activity. Compound (**106d**) shows the best antiviral activity, with an $EC_{50}$ value of 21 μM. SAR studies concluded that electron withdrawing groups on the 3- and 4- or 2- and 5-positions on the left aromatic ring along with certain substituents on the 4-position of the rhodanine ring have considerable impact on inhibitory potency against IN. The rhodanine ring is an important structural unit and its presence would lead to increased IN inhibitory potency [75].

## 8. OTHER DIKETO-CONTAINING COMPOUNDS

Several pharmacophoric fragments and their incorporation on various aromatic or heteroaromatic rings have been identified through virtual screening of the National Cancer Institute database and structure-based drug design strategies. In addition, a series of 5-aryl(heteroaryl)-isoxazole- 3-carboxylic acids has been designed and synthesized as biological isosteric analogs of DKA. Result of IN inhibitory assays show that compound (**110d**) exhibits the most potent activity with an $IC_{50}$ value of 10 μM. Comparison of compound (**110d**) with other cyanoketo acids indicates that substitution of the ester carbonyl with carboxylate functionality leads to a significant increase in activity. Most of the other compounds (**109a-e**, **110a-c**, **111**, **112**, **114a-e**) inhibit IN at a high micromolar concentration, ranging from 100 to 660 μM, while compounds (**113, 115a**, **115c**–**115e**) are essentially inactive ($IC_{50}$>1000 μM) Fig. (**21**) [65].

Many other diketo-containing compounds or bioisosteres of DKAs have also been reported in patents since IN was identified as a potential target for the therapy of HIV infec-

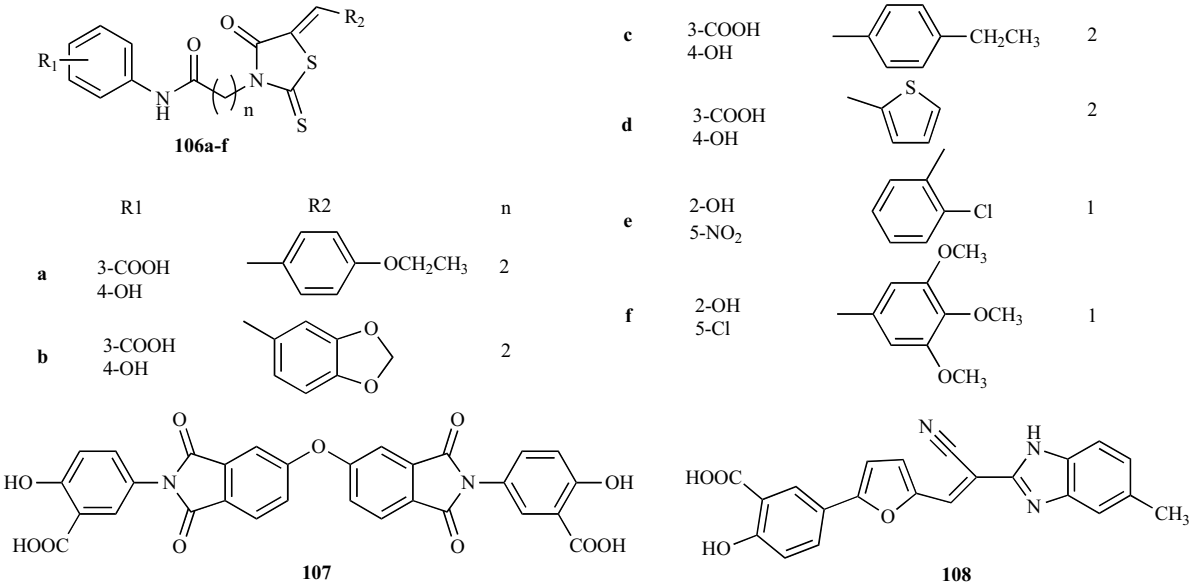



**Fig. (20).** Structures of compound **106-108**.

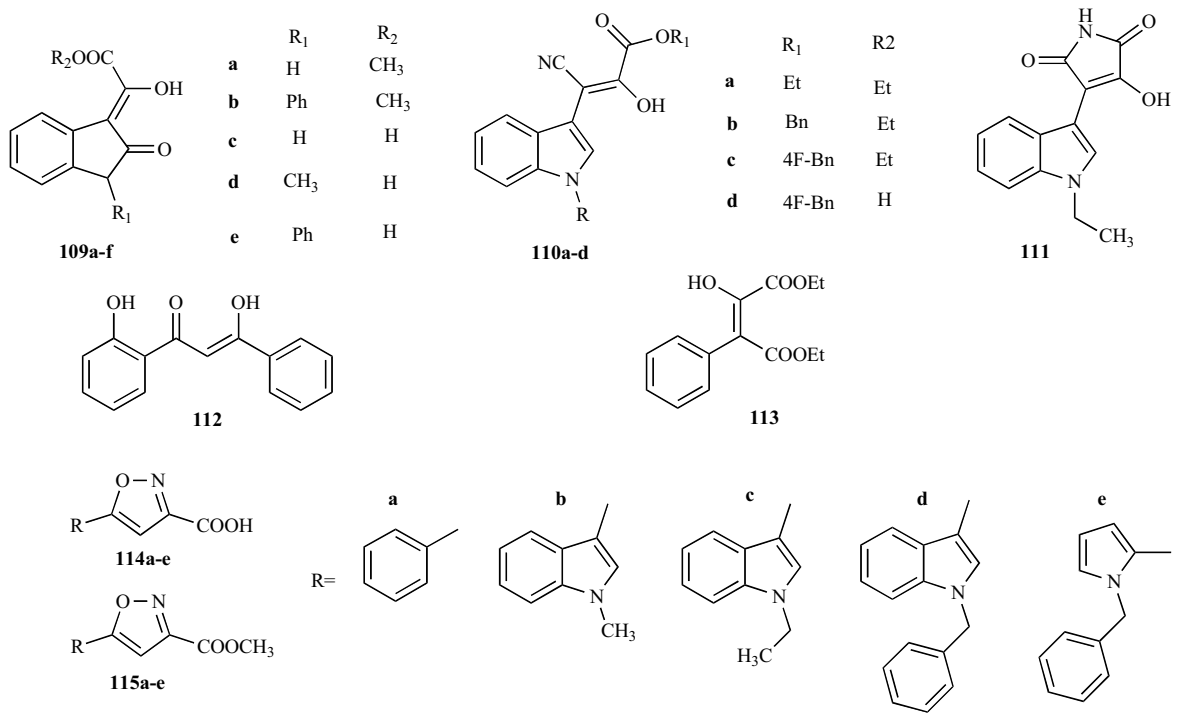
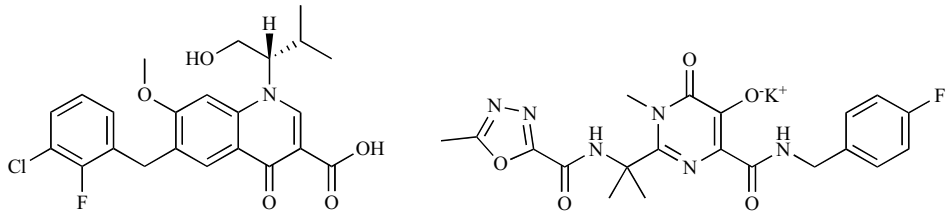



**Fig. (21).** Structures of compound **109-115**.

tion. Merck has conducted an exhaustive study on these compounds (**47**, **76-80**). However, the IN inhibitory potencies of most compounds are not provided; representative compounds show strong potency in IN assays, with $IC_{50}$ values less than 10 μM. In further assays for the inhibition of HIV replication, some compounds exhibit high antiviral activity, with $IC_{95}$ values often less than 10 μM. Regrettably, little understanding of the SAR requirements for high-affinity interaction with IN has been investigated on the diverse range of patented diketo-containing compounds.

GS-9137 (**116**) also named JTK-303 Fig. (**22**), is a novel IN inhibitor derived from quinolone antibiotics. Similar to reported DKAs, the compound is much more potent at inhibiting ST than 3'-P, with an $IC_{50}$ value of 7.2 nM. GS-9137 shows potent antiviral activity against the laboratory strains tested, with $EC_{50}$ ranging from 0.1 to 0.7 nM. Its antiviral activity is moderately reduced by the addition of 50% human serum. The compound shows potent antiviral activity against all clinical isolates including both subtype B and non-B subtypes of HIV-1. The average $EC_{50}$ of GS-9137 is 0.62 nM for the 8 subtypes of HIV-1 (11 viruses) and is 0.53 nM for the single HIV-2 clinical isolate. The compound exhibits comparable or greater potency than zidovudine, efavirenz and nelfinavir against all the isolates tested. GS-9137 retains antiviral activity against drug-resistant HIV-1 carrying resistance mutations to multiple drug classes, with average $EC_{50}$ of 0.46 nM. Although two of the isolates (MDR 1385 and MDR 3761) were completely resistant to zidovudine, efavirenz, and nelfinavir ($EC_{50}$s>1 μM), they were highly sensitive to GS-9137 ($EC_{50}$s<1 nM). GS-9137 displays additivity to highly synergistic antiviral activity *in vitro* with the following antiretroviral medications: lamivudine, lamivudine/zidovudine, zidovudine, tenofovir, tenofovir/lamivudine, efavirenz, indinavir and nelfinavir. When administered with food, it shows a half-life of approximately three hours in a Phase I pharmacokinetics study using single oral doses of GS-9137. Moreover, GS-9137 has good oral bioavailability in preclinical animal studies, with 34.1% and 29.6% for rats and dogs, respectively. Gilead has developed this candidate into phase II clinical trials [81-83].



**116** **117**

**Fig. (22).** Structures of compound **116** and **117**.

MK-0518 (**117**) known as hydroxypyrimidinone carboxamide derivative is a leading candidate developed by Merck. It is a novel IN inhibitor with potent *in vitro* activity against HIV-1 ($IC_{95}$=33nM in 50% human serum) and good bioavailability in uninfected subjects. Preclinical evaluation of MK-0518 indicates that the compound is not a potent inhibitor or inducer of CYP3A4 and it is predominantly metabolized by glucuronidation, specifically by the enzyme UGT1A1. MK-0518 has been investigated in Phase II trials for the treatment of HIV in patients, who are treatment-naive and in those who have multi-drug-resistant infection. After 10 days of monotherapy in treatment-naive patients, the compound shows effective activity with HIV RNA decreases of 1.7–2.2 log10 copies/mL for 100-600 mg treatment groups and is generally well-tolerated at all doses. In a second Phase II, randomized, double-blinded study, all triple-class experienced patients are placed on an optimized background ART therapy and receive either a placebo or 200 mg, 400 mg and 600 mg twice daily dosages of MK-0518. At the 16-week interim analysis, the percentage of patients achieving viral load levels less than 400 copies/mL ranges from 64% to 84% across all doses studied, compared with 22% for placebo. The percentage of patients achieving viral load levels less than 50 copies/mL ranges from 56% to 72%, compared with 19% for placebo. In completed trials, MK-0518 displays no significant food effect and appears compatible with all currently available antiretroviral medications. Phase III trials are underway to further evaluate efficacy, safety, and tolerability of MK-0518 in patients failing HAART on optimized background therapy [84-86].

## CONCLUSION

Unlike RT and PR, the paucity of structural information and no X-ray structure of full-length IN with or without the DNA substrate available have hampered structure-based discovery of selective inhibitors targeted to IN. To date, no IN inhibitor is approved by FDA for clinical use. Despite the lack of full-length structural information of IN, numerous compounds of a variety of chemical classes have been discovered using IN-specific assays. Diketo-containing compounds have emerged as a most promising class of IN inhibitors, some of which exhibited antiviral activity in cell-based assays, consistent with their inhibitory effect on IN. This provides the proof-of-concept for IN as a legitimate retroviral drug target. Furthermore, it is exciting that MK-0518 is currently in Phase III clinical trials in human volunteers. This promising candidate may be validated as the first IN inhibitor to treat HIV infection. The combination use of new agents targeting different steps in the viral cycle with current antiretrovirals might potently suppress HIV replication and limit the emergence of viral resistance.

## ACKNOWLEDGEMENTS

This work was supported by National Natural Science Foundation of China (20472045 and 20672069) and Shandong Natural Science Foundation (Y2003C19).

## ABBREVIATIONS

HIV = Human immunodeficiency virus

SIV = Simian-human immunodeficiency virus

AIDS = Acquired immunedeficiency syndrome

RT = Reverse transcription

PR = Protease

IN = Integrarse

NRTIs = Nucleoside reverse transcriptase inhibitors

NNRTIs = Non- nucleoside reverse transcriptase inhibitors

PIs = Protease inhibitors

SH3 = Src homology 3

3'-P = 3'-processing

ST = Strand transfer

DKAs = Diketo acids

SAR = Structure-activity relationship

SI = Selectivity index

TI = Therapeutic index

PBMC = Peripheral blood mononuclear cell

NHS = Normal human serum

FBS = Fetal bovine serum

PIC = Pre-integration complex

CYP3A4 = Cytochrome P3A4

UGT1A1 = UDP-glucuronosyltransferase 1A1

## REFERENCES

[1] Mustata, G.I.; Brigo, A.; Briggs, J.M. *Bioorg. Med. Chem. Lett.*, **2004**, *14*, 1447.
[2] Stephenson, J. *JAMA*, **1997**, *277*, 614.
[3] Cohen, J. *Science*, **2002**, *296*, 2320.
[4] Richman, D. D. *Nature*, **2001**, *410*, 995.
[5] Pommier, Y.; Johnson, A. A.; Marchand, C. *Nat. Rev. Drug Discov*., **2005**, *4*, 236-248.
[6] Craigie, R. *J. Biol. Chem*., **2001**, *276*, 23213.
[7] Zheng, R.; Jenkins, T.M.; Craigie, R. *Proc. Natl. Acad. Sci. USA*, **1996**, *93*, 13659.
[8] Kulkosky, J.; Jones, K.S.; Katz, R.A.; Mack, J.P.; Skalka, A.M. *Mol. Cell. Biol*., **1992,** *12*, 2331.
[9] Engelman, A.; Craigie, R. *J. Virol.*, **1992**, *66*, 6361.
[10] Engelman, A.; Hickman, A.B.; Craigie, R. *J. Virol*., **1994**, *68*, 5911.
[11] Chiu, T.K.; Davies, D.R. *Curr. Top. Med. Chem*., **2004**, *4*, 965.
[12] Pommier, Y.; Johnson, A.A.; Marchand, C. *Nat. Rev. Drug Discov*., **2005**, *4*, 236.
[13] Cushman, M.; Sherman, P. *Biochem. Biophys. Res. Commun*., **1992**, *185*, 85.
[14] Fesen, M.R.; Kohn, K.W.; Leteurtre, F.; Pommier, Y. *Proc. Natl. Acad. Sci. USA*, **1993**, *90*, 2399.
[15] Jing, N.; Marchand, C.; Liu, J.; Mitra, R.; Hogan, M.E.; Pommier, Y. *J. Biol. Chem*., **2000**, *275*, 21460.
[16] Krajewski, K.; Long, Y.Q.; Marchand, C.; Pommier, Y.; Roller, P.P. *Bioorg. Med. Chem. Lett.,* **2003**, *13*, 3203.
[17] de Soultrait, V.R.; Desjobert. C.; Tarrago-Litvak, L. *Curr. Med. Chem*., **2003**, *10*, 1765.
[18] Xu, Y.W.; Zhao, G.S.; Shin, C.G.; Zang, H.C.; Lee, C.K.; Lee, Y.S. *Bioorg. Med. Chem.*, **2003**, *11*, 3589.
[19] Costi, R.; Santo, R.D.; Artico, M.; Massa, S.; Ragno, R.; Loddo, R.; La Colla. M.; Tramontano, E.; La Colla, P.; Pani, A. *Bioorg. Med. Chem,,* **2004**, *12*, 199.
[20] Benard, C.; Zouhiri, F.; Normand-Bayle, M.; Danet, M.; Desmaele, D.; Leh, H.; Mouscadet, J.F.; Mbemba, G.; Thomas, C.M.; Bon-

nenfant, S.; Le Bret, M.; d'Angelo, J. *Bioorg. Med. Chem. Lett*., **2004**, *14*, 2473.
[21] Zhao, H.; Neamati, N.; Sunder, S.; Hong, H.; Wang, S.; Milne, G.W.; Pommier, Y.; Burke, T.R. Jr. *J. Med. Chem*., **1997**, *40*, 937.
[22] 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,* 1506.
[23] Dayam, R.; Neamati, N. *Curr. Pharm. Des*., **2003**, *9*, 1789.
[24] Maurin, C.; Bailly, F.; Cotelle, P. *Curr. Med. Chem.*, **2003**, *10*, 1795.
[25] Dayam, R.; Deng, J.; Neamati, N. *Med. Res. Rev*., **2006**, *26*,271.
[26] Huang, M.; Richards, W. G.; Grant, G. H. *J. Phys. Chem. A*, **2005**, *109*, 5198.
[27] Sechi, M.; Bacchi, A.; Carcelli, M.; Compari, C.; Duce, E.; Fisicaro, E.; Rogolino, D.; Gates, P.; Derudas, M.; Al-Mawsawi, L.Q.; Neamati, N. *J. Med. Chem*., **2006,** *49*, 4248.
[28] Pais, G.C.; Zhang, X.; Marchand, C.; Neamati, N.; Cowansage, K.; Svarovskaia, S.; Pathak, V.K.; Tang, Y.; Nicklaus, M.; Pommier, Y.; Burke, T.R. Jr. *J. Med. Chem.*, **2002** , *45*, 3184.
[29] Marchand, C.; Zhang, X.; Pais, G.C.; Cowansage, K.; Neamati, N.; Burke, T.R. Jr.; Pommier, Y. *J. Biol. Chem*., **2002**, *277*, 12596.
[30] 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*, 4923.
[31] 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*, 646.
[32] Pluymers, W.; Neamati, N.; Pannecouque, C.; Fikkert, V.; Marchand, C.; Burke, T. R.; Pommier, Y.; Schols, D.; DeClercq, E.; Debyser, Z.; Witvrouw, M. *Mol. Pharmacol*., **2000**, *58*, 641.
[33] Vandegraaff, N.; Kumar, R.; Hocking, H.; Burke, T. R., Jr.; Mills, J.; Rhodes, D.; Burrell, C. J.; Li, P. *Antimicrob. Agents Chemother*., **2001**, *45*, 2510.
[34] Pluymers, W.; Pais, G.; Van Maele, B.; Pannecouque, C.; Fikkert, V.; Burke, T.R. Jr.; De Clercq, E. Witvrouw, M.; Neamati, N.; Debyser, Z. *Antimicrob. Agents Chemother.*, **2002**, *46*, 3292.
[35] Grobler, J.A.; Stillmock, K.; Hu, B.; Witmer, M.; Felock, P.; Espeseth, A.S.; Wolfe, A.; Egbertson, M.; Bourgeois, M.; Melamed, J.; Wai, J.S.; Young, S.; Vacca, J.; Hazuda, D.J. *Proc. Natl. Acad. Sci. USA*, **2002**, *99*, 6661.
[36] 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*, 11244.
[37] Bernardi, F.; Bottoni, A.; De Vivo, M.; Garavelli, M.; Keseru, G.; Naray-Szabo, G. *Chem. Phys. Lett*., **2002**, *362*, 1.
[38] Condra, J.H.; Miller, M.D.; Hazuda, D.J.; Emini, E.A. *Annu. Rev. Med*., **2002**, *53*, 541.
[39] Mazumder, A.; Raghavan, K.; Weinstein, J.N.; Kohn, K.W.; Pommier, Y. *Biochem. Pharmacol*., **1995**, *49*, 1165.
[40] Mazumder, A.; Neamati, N.; Sunder, S.; Schutz, J.; Pertz, H.; Eich, E.; Pommier, Y. *J. Med. Chem*., **1997**, *40*, 3057.
[41] Herr, R.J. *Bioorg. Med. Chem.*, **2002**, *10*, 3379.
[42] 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*, 13040.
[43] Schames, J.R.; Henchman, R.H.; Siegel, J.S.; Sotriffer, C.A.; Ni, H.; McCammon, J.A. *J. Med. Chem.*, **2004**, *47*, 1879.
[44] Svarovskaia, E.S.; Barr, R.; Zhang, X.; Pais, G.C.; Marchand, C.; Pommier, Y.; Burke, T.R. Jr.; Pathak, V.K. *J. Virol*., **2004**, *78*, 3210.
[45] Yoshinaga, T.; Sato, A.; Fujishita, T.; Fujiwara, T. 9thConference on Retroviruses and Opportunistic Infections. Seattle, USA. 2002.
[46] Billich, A. *Curr. Opin. Invest. Drugs*, **2003**, *4*, 206.
[47] Cotelle, P. *Recent Pat. Anti Infec. Drug Discov*., **2006**, *1*, 1.
[48] Nair, V.; Chi, G.; Ptak, R.; Neamati, N. *J. Med. Chem.*, **2006**, *49*, 445.
[49] Nair, V.; Uchil, V.; Neamati, N. *Bioorg. Med. Chem. Lett.*, **2006**, *16,* 1920.
[50] Li, X.; Vince, R. *Bioorg. Med. Chem*., **2006**, *14*, 5742.
[51] Li, X.; Vince, R. *Bioorg. Med. Chem*., **2006**, *14*, 2942.
[52] Fujishita, T.; Yoshinaga, T. U.S. Patent 6,716,605, **2004**.
[53] Fujishita, T.; Yoshinaga, T. U.S. Patent 6,333,323, **2001**.
[54] 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*, 5298.
[55] Barreca, M.L.; Ferro, S.; Rao, A.; De Luca, L.; Zappala, M.; Monforte, A.M.; Debyser, Z.; Witvrouw, M.; Chimirri, A. *J. Med. Chem*., **2005**, *48*, 7084.
[56] Maurin. C,l.; Bailly, F.; Mbemba, G.; Mouscadet, J.F.; Cotelle, P. *Bioorg. Med. Chem*., **2006**, *14*, 2978.
[57] Zhang, X.; Pais, G.C.; 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*, 1215.
[58] 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.; Pommier, Y.; Marchand, C. *J. Med. Chem*., **2006**, *49*, 1939.
[59] Long, Y.Q.; Jiang, X.H.; Dayam, R.; Sanchez, T.; Shoemaker, R.; Sei, S.; Neamati, N. *J. Med. Chem*., **2004**, *47*, 2561.
[60] da Silva, C.H.; Del Ponte, G.; Neto, A.F.; Taft, C.A. *Bioorg. Chem*., **2005,** *33*, 274.
[61] 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*, 1745.
[62] Di Santo, R.; Costi, R.; Artico, M.; Ragno, R.; Greco, G.; Novellino, E.; Marchand, C.; Pommier, Y. *Farmaco*., **2005**, *60*, 409.
[63] Walker, M.A.; Johnson, T.; Ma, Z.; Banville, J.; Remillard, R.; Kim, O.; Zhang, Y.; Staab, A.; Wong, H.; Torri, A.; Samanta, H.; Lin, Z.; Deminie, C.; Terry, B.; Krystal, M.; Meanwell, N. *Bioorg. Med. Chem. Lett.*, **2006**, *16*, 2920.
[64] Walker, M.A.; Johnson, T.D.; Kim, O.A. *PCT Int. Appl*. WO 01/98248 A2, **2001**.
[65] Sechi, M.; Sannia, L.; Carta, F.; Palomba, M.; Dallocchio, R.; Dessi, A.; Derudas, M.; Zawahir, Z.; Neamati, N. *Antivir. Chem. Chemother*., **2005**, *16*, 41.
[66] 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*, 453.
[67] 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*, 4550.
[68] 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*, 11233.
[69] http://www.hivandhepatitis.com/recent/experimental_drugs/032805_a.html.
[70] Guare, J.P.; Wai, J.S.; Gomez, R.P.; Anthony, N.J.; Jolly, S.M.; Cortes, A.R.; Vacca, J.P.; Felock, P.J.; Stillmock, K.A.; Schleif, W.A.; Moyer, G.; Gabryelski, L.J.; Jin, L.; Chen, I.W.; Hazuda, D.J.; Young, S.D. *Bioorg. Med. Chem. Lett*., **2006**, *16*, 2900.
[71] Verschueren, W.G.; Dierynck, I.; Amssoms, K.I.; Hu, L.; Boonants, P.M.; Pille, G.M.; Daeyaert, F.F.; Hertogs, K.; Surleraux, D.L.; Wigerinck, P.B. *J. Med. Chem.*, **2005**, *48*, 1930.
[72] Metobo, S.E.; Jin, H.; Tsiang, M.; Kim, C.U. *Bioorg. Med. Chem. Lett*., **2006**, *16*, 3985.
[73] Jin, H.; Cai, R.Z.; Schacherer, L.; Jabri, S.; Tsiang, M.; Fardis, M.; Chen, X.; Chen, J.M.; Kim, C.U. *Bioorg. Med. Chem. Lett.*, **2006**, *16*, 3989.
[74] Fardis, M.; Jin, H.; Jabri, S.; Cai, R.Z.; Mish, M.; Tsiang, M.; Kim, C.U. *Bioorg. Med. Chem. Lett*., **2006**, *16*, 4031.
[75] Dayam, R.; Sanchez, T.; Clement, O.; Shoemaker, R.; Sei, S.; Neamati, N. *J. Med. Chem*., **2005**, *48*, 111.
[76] Wai, J.S.; Williams, P.D.; Langford, H.M. *PCT Int. Appl*. WO2005/120516 A2, **2005**.

[77] Wai, J.S.; Vacca, J.P.; Zhuang, L.; Kim, B.; Lyle, T.A.; Wiscount, C.M.; Egbertson, M.S.; Neilson, L.A.; Embrey, M.W.; Fisher, T.E.; Staas, D.D. *PCT Int. Appl*. WO 2005/110415 A1, **2006**.
[78] Williams, P.D.; Wai, J.S.; Embrey, M.W.; Staas, D.D.; Zhuang, L.; Langford, H.M. *PCT Int. Appl*. WO 2005/092099 A1, **2005**.
[79] Han, W.; Egbertson, M.; Wai, J.S.; Zhuang, L.; Ruzek, R.D.; Perlow, D.S.; Isaacs, R.C., A.; Cameron, M.; Foster, B.S.; Dolling, U.H.; Hoerrner, R.S.; Obligado, V.E.; Neilson, L.A.; Kim, B.; Payne, L.S.; Morrisette, M.M.; Williams, P.D.; Pye, P.J.; Angelaud, R.; Mancheno, D.E.; Askin, D. *PCT Int. Appl*. WO 2005/087768 A1, **2005**.
[80] Wai, J.S.; Fisher, T.E.; Zhuang, L.; Staas, D.D.; Lyle, T.A.; Kim, B.; Embrey, M.W.; Wiscount, C.M.; Tran, L.O.; Egbertson, M.; Savage, K.L. *PCT Int. App*l. WO 2005/041664 A1, **2005**.
[81] http://www.natap.org/2006/CROI/CROI_13.htm.
[82] http://www.retroconference.org/2006/Abstracts/26798.HTM.
[83] 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*, 1506.
[84] Lataillade, M.; Kozal, M.J. *AIDS Patient Care STDS*., **2006**, *20*, 489.
[85] http://www.aidsinfo.nih.gov/DrugsNew/DrugDetailT.aspx?MenuItem=Drugs&int_id=420& Search=Off& ClassID=&TypeID=.
[86] http://www.retroconference.org/2006/Abstracts/27911.HTM.

**Received: 13 September, 2006 Revised: 03 October, 2006 Accepted: 04 October, 2006**