Design and discovery of HIV-1 integrase inhibitors

Nouri Neamati, Sanjay Sunder and Yves Pommier

The rapid emergence of human immunodeficiency virus (HIV) strains resistant to available drugs implies that effective treatment modalities will require the use of a combination of drugs targeting different sites of the HIV life cycle. Integrase is emerging as a novel target for intervention by chemotherapeutics. As part of a program to develop novel antiviral agents, we have studied a large number of compounds with activity against HIV-1 replication in the National Cancer Institute (NCI) Antiviral Drug Program. In this review, we present a comprehensive review of all such inhibitors reported to date.

Retroviruses contain three large open reading frames *gag*, *pol* and *env*. The *pol* gene encodes for reverse transcriptase, protease and integrase (IN). Of the three *pol* gene products, IN is the only protein responsible for inserting viral DNA into host chromosomal targets and is known to be essential for effective viral replication[1–3]. Furthermore, no cellular counterpart has been identified. Thus, the virus-encoded protein IN is an attractive target for selective inhibition. Based on X-ray crystal structures, it has recently been suggested that retroviral integrases belong to a superfamily of polynucleotidyl transferases that includes the Mu transposase and the nucleases RNase H and RuvC (Ref. 2). During viral infection, IN catalyzes two consecutive steps. Initially, IN prepares linear viral DNA for integration by removing two nucleotides from each 3′-end, leaving the recessed 3′-OH termini. After transfer to the nucleus in the preintegration complex, IN catalyzes the integration of the viral DNA into the host chromosome.

Integration occurs via a transesterification of phosphodiester bonds in which a host DNA strand is cut and joined to the processed viral 3′ terminus. These two steps, known as 3′-processing and DNA strand transfer, respectively, can be easily measured in an *in vitro* assay employing purified recombinant IN and oligonucleotides corresponding to the U5 or U3 ends of the HIV long terminal repeat (LTR) sequence in the presence of a divalent metal ion (Mn^{2+} or Mg^{2+}). The *in vitro* assays have recently been reviewed[4]. Completion of HIV DNA integration requires processing of the 5′-ends of the viral DNA and ligation to the host DNA. This step is referred to as gap-filling or 5′-processing; its molecular mechanisms are unknown but probably require host cellular components.

Although the X-ray crystal structures of the HIV-1 IN core domain (amino acids 50–212)[5] and ASV (Ref. 6) integrase have been solved, no X-ray crystal structure of an IN–drug complex is yet available. Therefore, rational drug design for inhibitors has been limited. We have explored several alternative strategies for identifying novel inhibitors of IN. For example, we have used molecular modeling techniques to generate pharmacophores from a series of active compounds against IN (Refs 7–10) and used such pharmacophores to identify new classes of inhibitors. We have also synthesized derivatives from lead compounds[11–18] and used chemically modified inhibitors as photoaffinity probes. We have recently identified a unique binding site on IN that is responsible for the inhibitory mechanism of nucleotides[18] such as dideoxyuridine and AZT monophosphates[19]. In parallel, we have screened large numbers of compounds that have been shown to possess activity in the NCI Antiviral

Nouri Neamati, Sanjay Sunder and **Yves Pommier***, Laboratory of Molecular Pharmacology, Division of Basic Sciences, National Cancer Institute, Building 37, Room 5C25, Bethesda, MD 20892-4255, USA. tel: +1 301 496 5944, fax: +1 301 402 0752, e-mail: pommier@nih.gov

  1359-6446/96/$17.00. PII: S1359-6446(97)01105-7

Drug Screening cellular assays against HIV-1. From these endeavors, several distinct classes of IN inhibitors have been identified.

We have observed that different enzyme or drug preparations and/or assay conditions sometimes resulted in 2–3 fold differences in potency for several inhibitors. In general, the results reported by others have been reproduced in our laboratory. However, it is becoming more attractive to compare and contrast the results obtained against purified IN with those utilizing preintegration complex assays[20,21]. In screening compounds for IN, we have mainly confided in compounds with known antiviral activity.

In this review, we have attempted to summarize all of the IN inhibitors reported in the literature. We have selected lead compounds and pertinent derivatives from each inhibitor class and classified them into several conveniently arranged groups (Tables 1–15 and Figures 1–9). The corresponding 50% inhibitory concentrations (IC_{50}) for the inhibition of 3′-processing and strand transfer are indicated in each table.

From nucleotides to guanosine quartets

In our original study of IN inhibition by 3′-azido-3′-deoxythymidine (**1**, AZT) and its metabolites, we observed that AZT mono-, di- and triphosphate (**2–4**) exhibited an IC_{50} for strand transfer in the range of 100–150 μM (Ref. 22). By contrast, AZT was inactive up to 400 μM. This original observation indicated that a phosphate group is required for activity, perhaps through interaction with a positively charged IN residue. In addition, when we examined other nucleotide analogues (**5–9**) including those that inhibit HIV-1 replication, differences in sugar conformations and substitutions also appeared important (Table 1) and suggested the existence of a specific nucleotide binding site on IN (Ref. 12). Interestingly, biologically relevant nucleotides such as ATP are active only at high but near physiological concentrations (1–3 mM). Selective binding of mononucleotides to IN is consistent with the existence of a polynucleotide binding region on the enzyme[23]. In addition, high-affinity RNA ligands of IN have also been identified[24].

During viral infection, IN catalyzes the excision of the last two nucleotides from each 3′-end of the viral LTRs. The dinucleotide released (5′-pGT) is conserved in all HIV-1 strains and the dinucleotide immediately upstream (5′-pCA) is conserved among all retroviruses[3]. Testing of such dinucleotides showed that the 5′-pCA (**11**) and 5′-pGT (**22**) dinucleotides exhibited IC_{50} values in the low micromolar range.

Table 1. Nucleotides and guanosine quartets[a]

No.	Compound	IC_{50} (μM) 3′-Processing	Strand transfer
1	AZT	>400	>400
2	AZTMP	200	20
3	AZTDP	150	120
4	AZTTP	300	150
5	D4TMP	110 ± 20	95 ± 15
6	FdTMP	95 ± 15	70 ± 12
7	L-ddCMP	50 ± 8	45 ± 10
8	L-5FddCMP	46 ± 6	39 ± 4
9	L-5FddCTP	68 ± 2	48 ± 10
10	5′-CA	>132	60
11	5′-pCA-3′	105 ± 20	13 ± 5
12	5′-pCC-3′	15 ± 0.5	12 ± 5
13	5′-pCG-3′	71 ± 11	12 ± 2
14	5′-pCT-3′	8 ± 1	6 ± 1
15	5′-pAA-3′	>100	100 ± 30
16	5′-pAC-3′	6 ± 2	3 ± 1
17	5′-pAG-3′	15 ± 7	9 ± 4
18	5′-pAT-3′	7 ± 2	7 ± 3
19	5′-pGA-3′	>100	100 ± 40
20	5′-pGC-3′	>100	100 ± 40
21	5′-pGG-3′	54 ± 34	12 ± 4
22	5′-pGT-3′	22 ± 1	7 ± 1
23	5′-pTA-3′	85 ± 21	18 ± 10
24	5′-pTC-3′	35 ± 17	37 ± 9
25	5′-pTG-3′	65 ± 35	31 ± 4
26	5′-pTT-3′	73 ± 27	53 ± 4
27	5′-CAG-3′	57 ± 12	53 ± 11
28	5′-CTA-3′	65 ± 15	25 ± 5
29	5′-pCTA-3′	27 ± 6	6 ± 2
30	5′-pCTT-3′	>100	13 ± 4
31	5′-pCAT-3′	>100	20 ± 4
32	5′-pCAC-3′	93 ± 19	27 ± 6
33	5′-pCAA-3′	42 ± 9	22 ± 5
34	5′-pCAG-3′	57 ± 11	15 ± 4
35	5′-pGTC-3′	40 ± 5	9 ± 1
36	5′-pGTCA-3′	32 ± 4	5 ± 1
37	5′-GTGGTGGGT-GGGTGGGT-3′	0.08 ± 0.02	0.05 ± 0.01
38	5′-GTGGTGGGT-GGGTGGGT-3′[b]	0.11 ± 0.01	0.08 ± 0.07
39	5′-GTGGTGGGT-GTGGTGGGT-3′	0.15 ± 0.03	0.13 ± 0.02
40	5′-GTGGTTGGT-GTGGTTGGT-3′	0.76	0.61

[a]Refs 22 (compounds **1–4**), 12 (compounds **5–9**), 18 (compounds **10–36**) and 11 (compounds **37–40**).
[b]This oligonucleotide is the RNA version of **37**.

Interestingly, in contrast to mononucleotides, the 5′-phosphate was not required for activity. The dinucleotides were in general more potent than mononucleotides. Upon testing all possible dinucleotide combinations, a base sequence selectivity for the inhibition of IN was observed. For example, the 5′-pCT (**14**), 5′-pAC (**16**), and 5′-pAT (**18**), with IC_{50} values below 10 μM for both 3′-processing and strand transfer were the most potent inhibitors. On the other hand, the dinucleotides 5′-pAA (**15**), 5′-pGA (**19**), and 5′-pGC (**20**) showed low activity at 100 μM (Table 1)[18]. Increasing the number of bases to three and four (**27–36**) did not markedly increase potency. However, when we examined a series of oligonucleotides composed entirely of deoxyguanosine and thymidine, known as guanosine quartets, their IC_{50} values plummeted to 2–4 orders of magnitude lower than the mononucleotides (compounds **37–40**, Table 1)[11]. Our results indicated that the sequence of the loops between the quartets are important for optimal potency against purified IN. In addition, the most potent IN inhibitors are also the most potent inhibitors of HIV-1 replication in cell-based assays. At present the guanosine quartet, T30177 (compound **37**) is undergoing Phase I clinical studies as the first IN inhibitor.

DNA binders as inhibitors of IN

It is logical to target DNA substrates required for IN binding. However, a limitation to such an approach is the difficulty in obtaining selective LTR binders. Several classes of DNA binders and well known inhibitors of other DNA-binding proteins such as topoisomerases have been evaluated against purified IN (Refs 25–27). The topoisomerase II inhibitors, doxorubicin and mitoxantrone (compounds **41** and **43**, Table 2 and Figure 1), inhibited IN catalytic activity at low micromolar concentrations. In general, the topoisomerase II inhibitors that were not effective DNA binders were not effective inhibitors of IN. In addition, the topoisomerase I poison camptothecin was inactive against purified IN (Refs 25,26). However, DNA binding did not correlate closely with integrase inhibition. A non-DNA binder, dihydroxynaphtaquinone (**44**), a moiety present in both compounds **41** and **43**, inhibited IN at low micromolar concentrations[25]. This finding led to the exploration of hydroxylated aromatic compounds (see below), including flavones (**45**) and caffeic acid phenethyl ester (CAPE; **46**). The weak DNA binders primaquine (**47**) and chloroquine (**48**) were active against purified IN (Table 2). Moreover, the phenanthroline–cuprous complexes that are known to bind to the minor groove of DNA only at concentrations of ≥50 μM (Ref. 28) were effective inhibitors of IN at concentrations of 1–25 μM (Ref. 28). By contrast, the physiological DNA groove binders spermine (**49**) and spermidine (**50**) had no effect against IN (Table 3)[25,29].

Table 2. Topoisomerase poisons and other natural product inhibitors of HIV-1 integrase[25]

No.	IC_{50} (μM) 3′-Processing	Strand transfer
41	0.9 ± 0.7	2.4
42	>100	>100
43	3.8 ± 0.6	8.0
44	5.7 ± 2.7	2.5
45	19.4 ± 9.9	11.0 ± 5.9
46	7	19
47	15.3 ± 3.6	3.6
48	13.1 ± 10.0	5.7

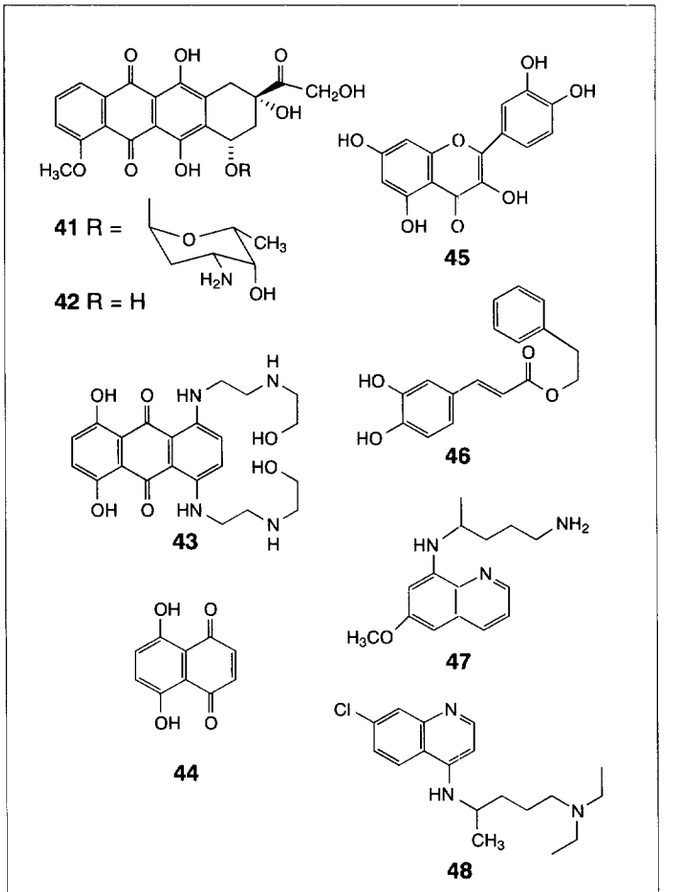


Figure 1. *Topoisomerase poisons and other natural product inhibitors of HIV-1 integrase. See also Table 2.*

Table 3. DNA groove binders[29]

No.	Compound	IC_{50} (μM) 3′-Processing	Strand transfer
49	Spermine	>100	>100
50	Spermidine	>100	>100
51	Net-CO-Net	42.8	29.0
52	Net-CO$(CH_2)_2$CO-Net	5.8 ± 1.6	7.5 ± 2.5
53	Net-CO$(CH_2)_9$CO-Net	37.6	10.0
54	Net-CO$(CH_2)_{10}$CO-Net	33.8	8.7
55	Dist-CO$(CH_2)_2$CO-Dist	21 ± 8.5	9.5 ± 1.2
56	Dist-CO$(CH_2)_6$CO-Dist	0.02	0.01
57	Dist-CO$(CH_2)_8$CO-Dist	0.03	0.01
58	Dist-CO$(CH_2)_{22}$CO-Dist	12.2 ± 0.3	8.0
59	Distamycin	57	50
60	Hoechst 33258	>100	>100
61	DAPI	>100	>100
62	Pentamidine	>100	>100
63	Berenil	>100	>100

Abbreviations: Net, netropsin; Dist, distamycin.

IN is known to bind to specific sequences located on both extremities of the DNA on the HIV LTR. These sites are highly conserved in all HIV genomes (for alignment of HIV-1 LTRs, see Ref. 3) and could provide potential targets for the selective inhibition of integration. The retroviral LTR that recognizes a DNA binding site on IN contains an AT-rich sequence. The presence of this sequence has been exploited as a possible target for the DNA minor groove binder netropsin[30] and triple helix-forming oligonucleotides[31].

In an effort to elucidate the role of AT-binding agents in inhibition of IN function, we have recently examined a series of novel lexitropsins and compared their potency with those of classical DNA minor groove binders (Table 3)[29]. Lexitropsin (not shown) and several bis-distamycins (**55–58**) were identified as potent inhibitors of IN catalytic activity at low nanomolar concentrations. Interestingly, many of these lexitropsins were effective inhibitors of HIV-1 replication in CEM cells[32]. However, the monomeric minor groove binders such as distamycin (**59**), Hoechst 33258 (**60**), DAPI (**61**), pentamidine (**62**) and berenil (**63**) exhibited poor activity against purified IN. Thus, the antiviral activity of some of the IN inhibitors targeting the HIV LTRs provides a rationale for their further development as anti-HIV drugs.

Hydroxylated aromatics (catechol-containing inhibitors)

In our original study we identified CAPE (**46**) as a novel, non-DNA binding inhibitor of IN (Ref. 25). When we expanded this early observation by testing several naturally occurring hydroxylated aromatics, such as flavones[14], it became clear that a prerequisite for potency was the presence of a catechol moiety. As expected, flavones with additional hydroxyl groups exhibited enhanced potency. Quercetagetin (**77**) with IC_{50} values of 0.8 and 0.1 μM for 3′-processing and strand transfer, respectively, was the most potent inhibitor[14]. Subsequent comparative molecular field analysis (CoMFA) of flavones revealed a correlation between the inhibitory activity of the flavones and the steric and electrostatic field around them[10]. Flavones are known to inhibit a wide variety of enzymes *in vitro*, yet, in cell-based assays, not all flavones are cytotoxic. In fact, flavones naturally occurring in fruits and beverages can act as antioxidants and cancer-preventive agents[33], and some have been reported with moderate antiviral activity[34,35]. For example, curcumin (**78**), which is isolated from the widely used spice curry, is an antiviral agent with moderate potency against IN (Ref. 36). Therefore, identification of a novel IN inhibitor based on natural products is attractive.

Other natural products such as lignans and lignaloids[37,38] and the bis-catechols, α- and β-conidendrol, were reported to inhibit IN at submicromolar concentrations (Table 4 and Figure 2)[39]. However, all of the methylated derivatives were considerably less potent. A consistent theme in the active inhibitors depicted in Figure 2 is the presence of at least one catechol center. Compounds with two catechol units separated by a linker were, in general, more potent

Table 4. Lignanolides and related bis-catechols[a]

No.	IC_{50} (μM) 3′-Processing	Strand transfer[b]
64	>100	>100
65	100	100
66	21.4 ± 15.0	5.4 ± 4.0
67	0.5	ND
68	1.7	ND
69	7	ND
70	>35	ND
71	0.5	ND
72	35	ND
73	>35	ND

[a]Refs 38 (compounds **64–66**) and 39 (compounds **67–73**).
[b]ND, not determined.

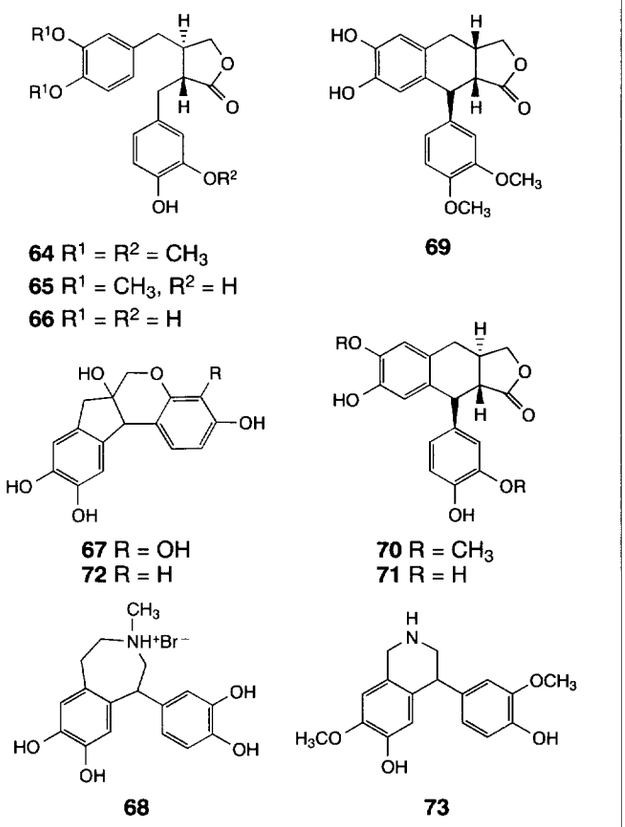


Figure 2. *Lignanolides and related bis-catechols. See also Table 4.*

Table 5. CAPE, CAPE-amides and other hydroxylated aromatics[a]

No.	IC_{50} (μM) 3′-Processing	Strand transfer
46	7	19
74	> 100	> 100
75	33	33
76		4
77	0.8 ± 0.3	0.1 ± 0.1
78	95	40
79	6.0 ± 1.0	3.1 ± 0.1

[a]Refs 14 (compounds **46** and **77**), 13 (compounds **74–76**), 36 (compound **78**) and 43 (compound **79**).

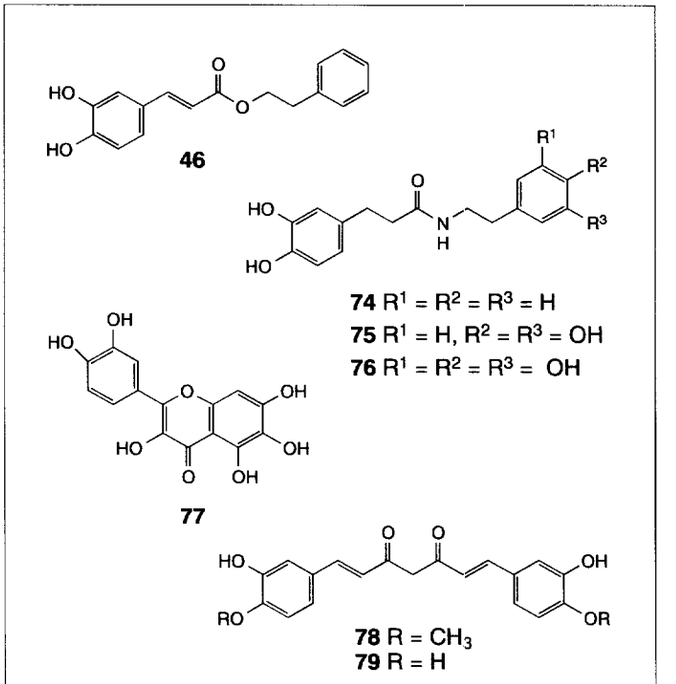


Figure 3. *CAPE, CAPE-amides and other hydroxylated aromatics. See also Table 5.*

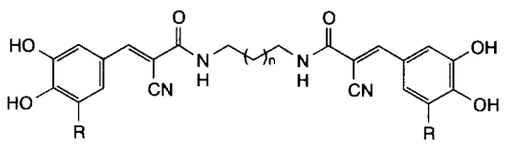
inhibitors. This is true for other classes of inhibitors discussed below.

In a separate study, designed to examine specific features of the CAPE (**46**) structure for anti-IN activity, 30 derivatives with various modifications of the parent compound were synthesized and tested in an assay specific for IN. Although the CAPE amide (**74**) was less potent than CAPE (Table 5 and Figure 3), increased hydroxylation of the phenyl ring increased potency against IN (Ref. 13).

The hydroxylated aromatic inhibitors of tyrosine kinases were also shown to have significant potency against purified IN. For example, several tyrphostins (**80–86**), the synthetic analogs of erbstatin, inhibited IN at low micromolar concentrations (Table 6). We showed that the catechol moieties are required for activity and that the length of the linker and aromatic ring substitutions were less critical for activity[40]. Although tyrphostins did not inhibit topoisomerase I at concentrations active against IN, the lack of target selectivity is perhaps responsible for their cytotoxicity and therefore lack of protection against HIV-1 infection in cell culture.

More recently, the hydroxylated aromatics containing catechol moieties, caffeoylquinic acids[8,41,42] and chicoric acid (**91**)[41,42] (Figure 4) have been reported to have antiviral activity and inhibit purified IN. As shown in Table 7, the dicaffeoylquinic acids **90**, **92–96**, rosmarinic acid (**89**)[43] and the chicoric acid **91** are potent inhibitors of IN, whereas the monomeric precursors, **87** and **88** are inactive[8,41,42].

Table 6. The tyrphostins[40]



No.	n	R	IC_{50} (μM) 3′-Processing	Strand transfer
80	0	H	1.9	0.8
81	0	OH	0.4 ± 0.1	0.2
82	0	Br	0.8 ± 0.4	0.4 ± 0.3
83	0	NO_2	3.3	1.9
84	2	OH	0.7 ± 0.5	0.6 ± 0.1
85	3	OH	0.5 ± 0.1	0.2
86	4	H	1.4 ± 0.6	1.1 ± 0.5

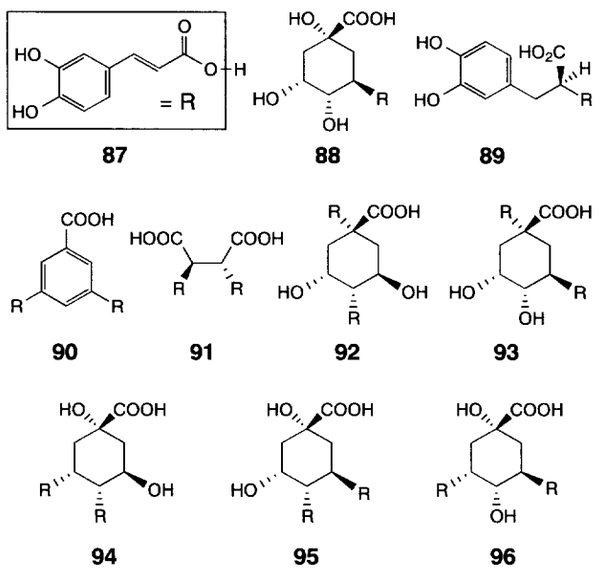


Figure 4. *Caffeoylquinic acids and related compounds. See also Table 7.*

In an independent study, we identified several naturally occurring lichen acids (see below) as inhibitors of IN and identified two distinct pharmacophores based on these compounds[8]. Subsequent searching of the NCI 3D database revealed some 800 compounds containing one or the other pharmacophore. Among these compounds we identified several dicaffeoylquinic acids as potent inhibitors of IN. However, these compounds did not show any protection against HIV-1 infected CEM cells in the NCI Antiviral Screen Program protocol[8].

Table 7. Caffeoylquinic acids and related compounds[a]

No.	IC_{50} (μM) 3′-Processing	Strand transfer
87	>200	>200
88	87.8	45.8
89	9 ± 7	4.0 ± 1.5
90	1.38 ± 0.21	4.71 ± 0.96
91	0.15 ± 0.02	0.13 ± 0.08
92	9.5 ± 2.0	7.8 ± 1.4
93	0.68 ± 0.08	1.08 ± 0.27
94	0.79 ± 0.17	0.54 ± 0.17
95	0.25 ± 0.04	0.46 ± 0.15
96	0.64 ± 0.2	0.66 ± 0.4

[a]Refs 41 (compounds **87, 90, 91, 93–96**), 8 (compounds **88** and **92**) and 43 (compound **89**).

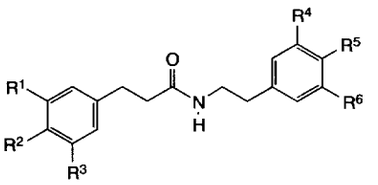
In conclusion, it seems that, even though the reported hydroxylated aromatic inhibitors are from completely unrelated structures, most share common structural features composed of two aryl units separated by a central linker. Frequently, a catechol moiety is required for increased potency and, as discussed above, catechol-containing inhibitors were proven toxic perhaps as a result of nonspecific binding to other targets. The mechanism of toxicity may stem from the formation of oxidized species, such as semiquinones or orthoquinones, which form protein or possibly DNA adducts[44,45]. Thus, the requirement of a catechol moiety is the Achilles' heel in the design of IN inhibitors based on hydroxylated aromatics.

Non-catechol containing aromatics

In an attempt to separate the mechanism of cytotoxicity and IN inhibition we have designed and synthesized a series of monohydroxylated arylamides and determined their inhibitory activities against IN (Table 8)[15]. Unfortunately, none of the monohydroxyl derivatives (**98–101**) or ortho-substituted analogs (**102–104**) exhibited significant inhibitory potency against IN up to 350 μM. In addition, protection of the hydroxyl groups also abolished activity (compounds **106** and **108**; Table 9).

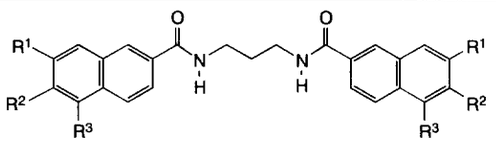
We thus sought a unique way of circumventing formation of reactive quinone species while preserving the required pharmacophore for activity. Using a molecular modeling approach, we identified unique pharmacophores among the reported IN inhibitors, most of which contained a catechol center[7–9]. Subsequent searching of the NCI 3D database revealed many structures devoid of the catechol moiety. A series of novel inhibitors generated from the

Table 8. Arylamides[15]



No.	R^1	R^2	R^3	R^4	R^5	R^6	IC_{50} (μM) 3′ Processing	Strand transfer
97	OH	OH	H	H	OH	OH	33.0	33.0
98	H	OH	H	H	OH	H	>350	>350
99	OH	H	H	H	OH	H	>350	>350
100	H	OH	H	H	H	OH	>350	>350
101	OH	H	H	H	H	OH	>350	>350
102	OH	H	OH	H	OH	OH	>350	>350
103	OH	H	OH	OH	H	OH	>350	>350
104	OH	OH	H	OH	H	OH	>350	>350

Table 9. Bis-arylamides[15]



No.	R^1	R^2	R^3	IC_{50} (μM) 3′-Processing	Strand transfer
105	OH	OH	H	0.98 ± 0.5	0.81 ± 0.1
106	OCH_3	OCH_3	H	>200	>200
107	H	OH	OH	0.23 ± 0.05	0.11 ± 0.07
108	H	OCH_3	OCH_3	>200	>200

Table 10. Hydrazides[9,17]

No.	IC_{50} (μM) 3′-Processing	Strand transfer
109	2.07 ± 0.75	0.73 ± 0.13
110	9.1 ± 3.7	5.8 ± 1.3
111	2.3 ± 0.3	1.1 ± 0.15
112	>200	>200
113	>100	>100
114	>100	>100
115	6.7 ± 0.8	5.2 ± 1.5
116	>100	>100

pharmacophore search were tested against IN. As a result, novel hydrazides were identified as effective inhibitors of IN (Refs 9,17). The *N,N′*-bis-salicylhydrazine (**109**) was used as a lead compound, and subsequent synthesis of several analogs revealed an interesting structure–activity relationship amongst these inhibitors (Table 10 and Figure 5)[17]. It was shown that the length of the linker and the presence of the 2-hydroxyl group were critical for potency against purified IN. Although the 2-hydroxyl group could be replaced by a mercapto group (compound **110**), resulting in decreased potency, the removal of the 2-hydroxyls (compounds **113** and **114**) or their replacement with amino groups, carboxyl groups or halogens (N. Neamati *et al.*, unpublished) yielded inactive compounds.

Coumarins are also a novel class of non-catechol-containing inhibitors of IN (Ref. 46). For example, coumermycin A1 (Ref. 12) inhibited IN at low micromolar ranges, whereas its monomeric derivatives were inactive. In contrast to coumermycin A1, several coumarins exhibit antiviral activity and also inhibit HIV-1 protease[46]. The tetrameric 4-hydroxycoumarin (**117**), with IC_{50} values of 1.5 and 0.8 against 3′-processing and strand transfer, respectively (Table 11 and Figure 6), was identified as a lead compound. In a subsequent study designed to dissect and simplify the lead structure we synthesized over 30 novel coumarins and established a structure–activity relationship among this class of inhibitors (Table 11). We

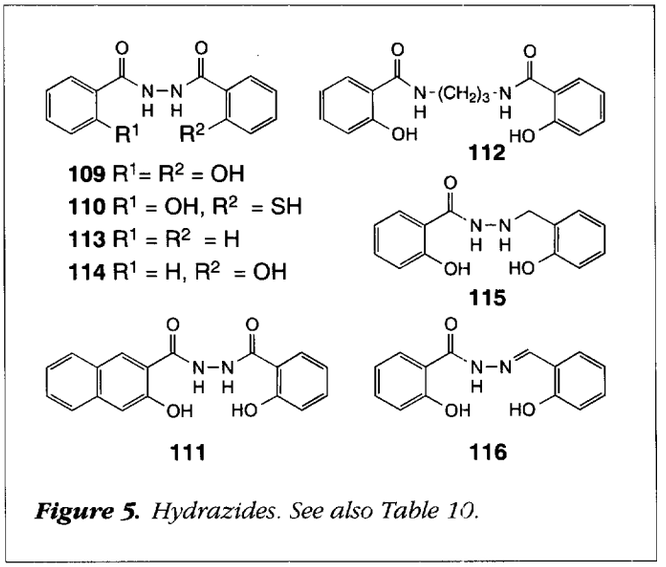


Figure 5. *Hydrazides. See also Table 10.*

Table 11. Coumarins[a]

No.	IC_{50} (μM) 3′-Processing	Strand transfer
117	1.5 ± 0.5	0.8 ± 0.3
118	0.4 ± 0.1	0.3 ± 0.3
119	7.5 ± 2.0	2.7 ± 0.9
120	10.5	12.0
121	4.2 ± 0.7	3.5 ± 1.2
122	7.0 ± 0.1	1.8 ± 0.5
123	50	24

[a]Refs 46 (compounds **117**, **119** and **120**) and **16** (compounds **118** and **121–123**).

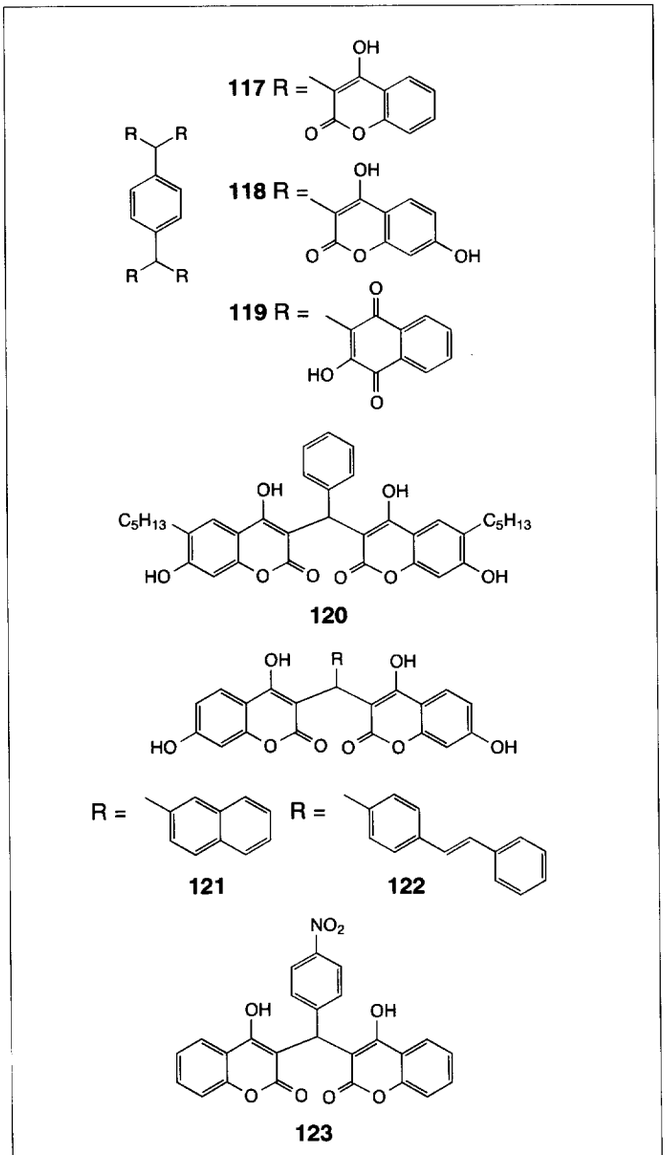


Figure 6. *Coumarins. See also Table 11.*

found that a coumarin dimer was sufficient for activity and that in general a hydrophobic linker was required for retention of potency and hydroxylation of the aromatic ring increased potency[16].

Other non-catechol-containing inhibitors are also known. In fact, polyanionic inhibitors of gp120 are, in general, active

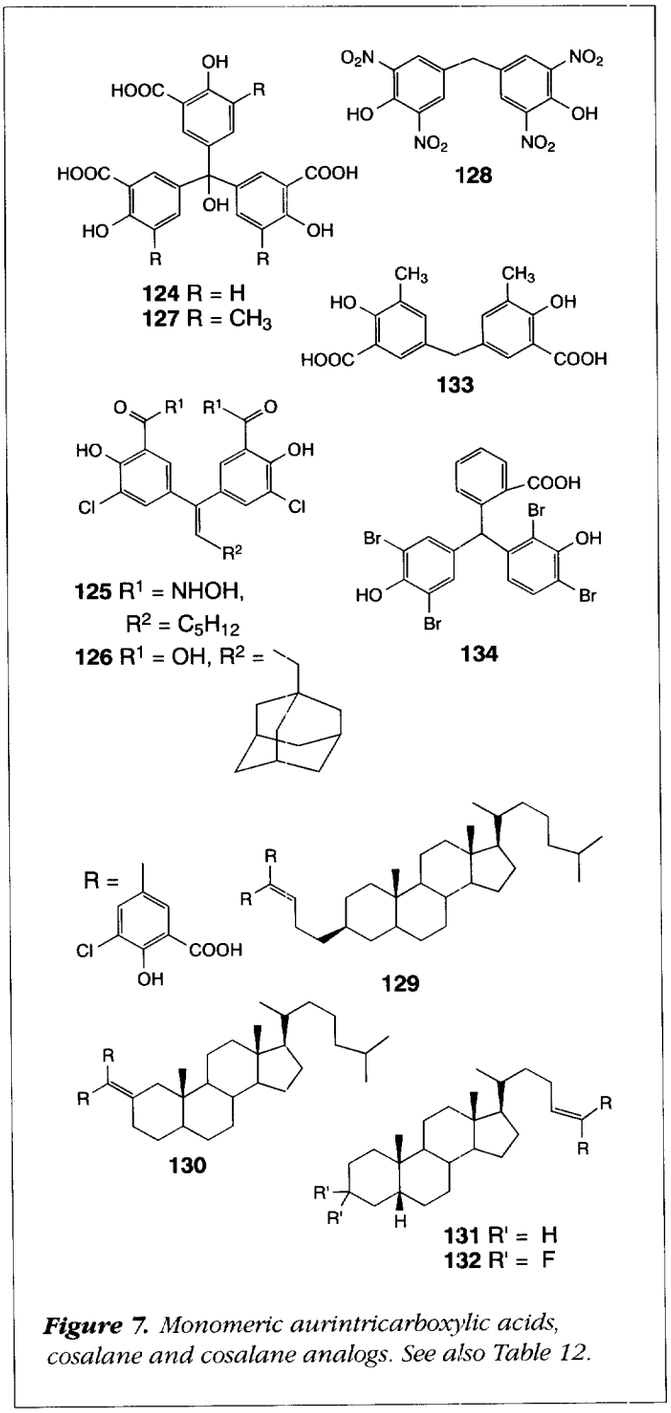


Figure 7. *Monomeric aurintricarboxylic acids, cosalane and cosalane analogs. See also Table 12.*

Table 12. Monomeric aurintricarboxylic acids, cosalane and cosalane analogs[a]

No.	IC_{50} (μM) 3'-Processing	Strand transfer
124	10–50	
125	4	
126	7	
127	32.8	23.0
128	49.7	43.9
129	25	
130	>100	
131	2.2	
132	2.2	
133	61.8 ± 8.6	68.5 ± 13.5
134	25.6	27.2

[a]Refs 47 (compound **124**), 48 (compounds **125**, **126** and **129–132**) and 8 (compounds **127**, **128**, **133** and **134**).

Table 13. Depsides depsidones, and related compounds[8]

No.	IC_{50} (μM) 3'-Processing	Strand transfer
135	4.6 ± 1.6	6.5 ± 3.9
136	2.2	1.6
137	4.4	2.9
138	42.2	28.1
139	6.1	6.1
140	8.3	4.0
141	11.6	7.9
142	1.1	0.9

against recombinant IN, and monomeric aurintricarboxylic acid (**124**; Figure 7) was among the first compounds identified to inhibit IN (Ref. 47). In a later study, novel lipophilic analogs of aurintricarboxylic acids and cosalane (**129**) were prepared and tested against IN and protease[48]. Several such analogs inhibited IN at low micromolar concentrations (Table 12). Interestingly, when the disalicylmethane moiety was attached directly to the steroid, no anti-IN potency was observed (compound **130**; Table 12), however, a greater potency against HIV-1 protease[48] was exhibited. In a recent study, several monomeric aurintricarboxylic acid analogs were found to exhibit moderate potency against IN (Ref. 8). In this study, it was demonstrated that the carboxylic group could be replaced by electron-withdrawing bromo or nitro groups (compounds **128** and **134**) without loss in potency. Additionally, it was shown that compounds containing only two phenyl rings are equally active against IN (compounds **128** and **133**).

Among many non-catechol-containing natural products that we have tested, several lichen acids of the depside and depsidone families exhibited remarkable potency against IN and moderate activity against HIV-1 replication[8]. For example, virensic acid (**135**), chloroparellic acid (**136**), and stictic acid (**137**) exhibited IC_{50} values of 2–6 mM against IN (Table 13 and Figure 8). The monohydroxylated derivative **138** exhibited moderate potency against IN. Subsequently, we used molecular modeling studies and discovered two distinct three-point pharmacophores among

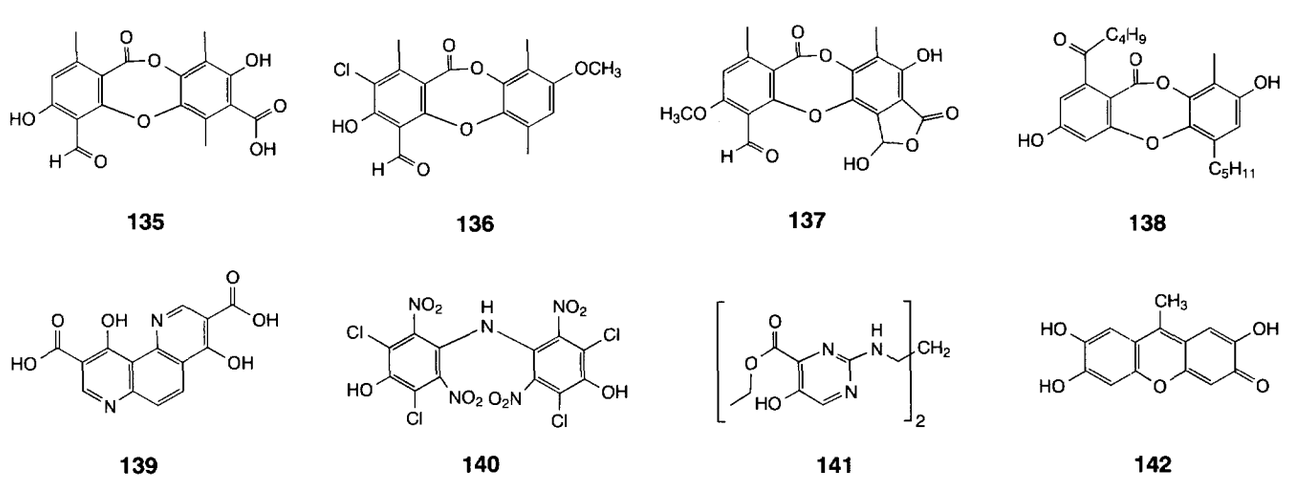


Figure 8. *Depsides, depsidones and related compounds. See also Table 13.*

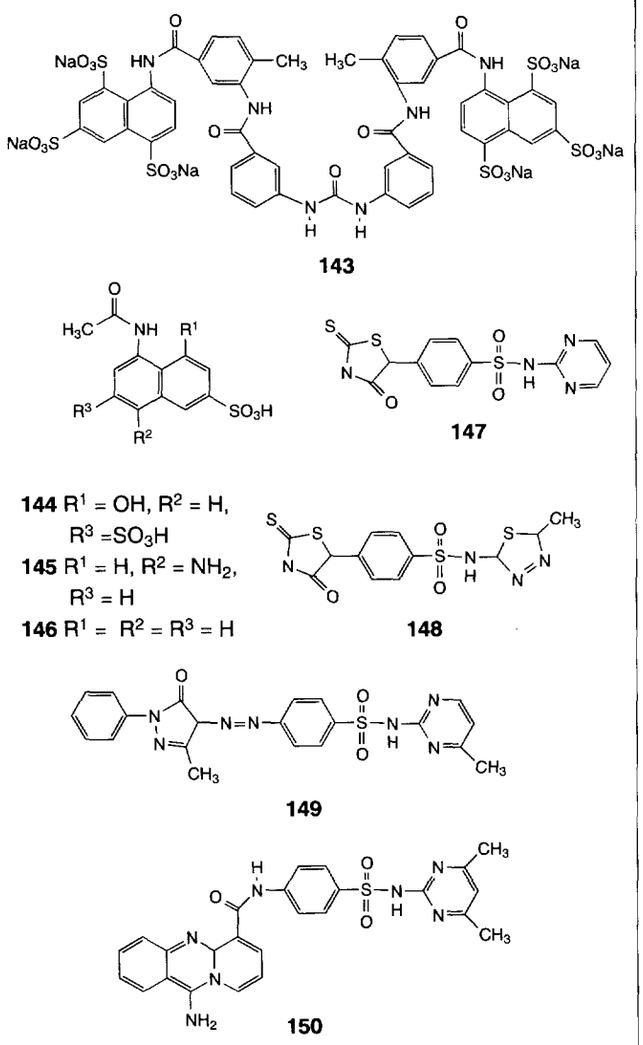


Figure 9. *Sulfonic acids and sulfonilamides. See also Table 14.*

Table 14. Sulfonic acids and sulfonilamides[a]

No.	IC_{50} (μM) 3′-Processing	Strand transfer
143	0.25	0.09
144	0.47 ± 0.02	0.29 ± 0.04
145	3.4 ± 0.8	2.7 ± 0.4
146	27.9 ± 8.6	14.3 ± 0.6
147	28.6 ± 11.6	14.0 ± 9.7
148	24.0 ± 8.2	19.0 ± 8.3
149	49.0 ± 9.5	23.6 ± 1.9
150	48.3 ± 25.8	13.7 ± 8.9

[a]Refs 49 (compound **143**) and 7 (compounds **144–150**).

the lichen acids[8]. A search of the NCI 3D database yielded a variety of novel inhibitors, such as compounds **139–142** (Table 13). These inhibitors, with IC_{50} values below 10 μM, could be further explored as lead compounds. Studies are under way to determine the mechanisms of action of these inhibitors *in vivo*.

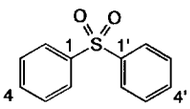
Sulfonates, sulfones, sulfonamides and sulfides

The polyanionic sulfonate suramin (**143**; Figure 9) and dextran sulphate, known to bind to gp120, were among the first inhibitors of IN reported[26,49]. We have also examined several sulfonates, which generally appear to be potent inhibitors of purified IN (N. Neamati, A. Mazumder and Y. Pommier, unpublished). Although intracellular uptake of sulfonic acids is not likely, and the specificity of such compounds for IN remains to be elucidated, we have recently identified other lipophilic and uncharged sulfur-containing compounds as potent inhibitors of IN. For example, several sulfonamides (compounds **147–150**; Table 14 and Figure 9), diarylsulfones[50] (compounds **151–162**; Table 15), and aromatic disulfides (N. Neamati *et al.*, submitted for publication) were found to inhibit IN function at low micromolar concentrations. Sulfonamides and sulfones are interesting classes of compounds because sulfa drugs are well known antimicrobial agents for which great efficacy against *pneumocystis carinii* pneumonia, a leading cause of morbidity and mortality in AIDS patients, has been shown. It is appealing to design IN inhibitors based on these classes of compounds, thus taking advantage of a structural class with a well-established safety profile. For example, we have already established that compounds containing a mercapto group adjacent to an amino, hydroxyl or carboxyl group have anti-IN activity. In a recent communication, we identified novel 2-mercaptobenzenesulfonamides as potent inhibitors of IN with activity against HIV-1 infected CEM cells (N. Neamati *et al.*, submitted for publication). Studies are in progress to generate HIV-1 mutants using novel sulfonamides.

Peptides and peptidomimetics

Application of a combinatorial peptide library in designing protease inhibitors has been remarkably successful. Unfortunately, potent peptide-based inhibitors of IN have not emerged. R.A.P. Lutzke *et al.* screened a synthetic peptide combinatorial library and identified a hexapeptide HCKFWW as an inhibitor of IN with IC_{50} values in the

Table 15. Diarylsulfones[50]



No.	Substituent	IC_{50} (μg/ml) 3′-Processing	Strand transfer
151	3,3′-$(NO_2)_2$, 4,4′-$(F)_2$	51.0 ± 8.8	59.0 ± 8.9
152	2,2′,4,4′-$(NO_2)_4$	60.0	76.5
153	4,4′-$(N{=}NOH)_2$. 2Na	6.5 ± 3.0	6.1 ± 0.9
154	3,3′-$(NH2)_2$, 4,4′-$(OH)_2$	4.5 ± 3.0	4.9 ± 3.0
155	4,4′-$[(NHN{=}C(CN)_2]_2$	25.9 ± 6.7	21.9 ± 1.9
156	3,3′-$(CHO)_2$, 4,4′-$(OH)_2$	0.6 ± 0.2	1.3 ± 0.2
157	3,4-$(CO)_2O$, 3′,4′-$(CO)_2O$	20.9 ± 1.7	18.6 ± 2.0
158	3,4-$(COOH)_2$, 3′,4′-$(COOH)_2$	29.8 ± 8.0	29.5 ± 5.0
159	3,3′-$(NO_2)_2$, 4,4′-$(COOH)_2$	55	83
160	3,3′,5-$(NO_2)_3$, 4,4′-$(Cl)_2$	67.5 ± 25.0	65.6 ± 19.6
161	3,3′-$(NO_2)_2$, 4,4′-$(SH)_2$	2.9 ± 0.7	2.5 ± 0.5
162	3,3′-$(NO_2)_2$, 4,4′-$(OCH_2COOH)_2$	70.0 ± 3.2	64.2 ± 14.5

micromolar range[51]. However, selectivity of the inhibition and the drug-binding site of such hexapeptides remain to be elucidated. More recently, a 30-mer peptide encompassing amino acids 147–175 of IN was found to dimerize with IN and to reduce IN activity, albeit at very high concentrations (IC_{50} in mM range)[52].

In our efforts of studying compounds with known antiviral activity from the NCI Antiviral Drug Screening Program, we have identified mastoparan and other peptidomimetics as inhibitors of IN in our *in vitro* assay (N. Neamati and Y. Pommier, unpublished). Mastoparan, which is a 14-mer peptide isolated from wasp venom and now commercially available, inhibits IN at low micromolar concentrations. However, mastoparan and the hexapeptide HCKFWW are effective DNA binders at high concentrations (N. Neamati and Y. Pommier, unpublished).

Inhibition of IN by the anti-HIV plant proteins MAP30 and GAP31 has also been reported[53]. These proteins exhibit anticancer activity and appear to be nontoxic because they are unable to enter healthy cells[53]. Monoclonal antibodies (mAb) directed against various segments of IN have recently been shown to exhibit remarkable potency[54–56]. The preliminary observations indicate that a single-chain variable antibody fragment (SFv33) directed against the C-terminus of IN protects human PBMC against infection by HIV-1 (Ref. 57). The gene therapy approach of mAb in AIDS patients will await further development.

Conclusions

Our efforts of combining rational drug selection from compounds exhibiting known activity against HIV-1 with molecular modeling and pharmacophore searching of the NCI 3D database have enabled us to select several sets of structurally diverse IN inhibitors from a large collection. The information gleaned from the reported inhibitors has thus paved the way for further rational drug design. For example, several of the potent inhibitors identified are water-soluble and could serve as candidates for co-crystallization studies with IN. Studies are under way to determine the X-ray crystal structure of the IN–drug complex. We have also 'docked' several of the inhibitors into the active site of IN. Our preliminary investigations attest a linear correlation between the IC_{50} values and the free energy of binding. Additionally, several inhibitors have been selected for development of IN mutants *in vitro*.

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In short ...

Allergan claim that retinoic acid receptor (RAR)-specific synthetic retinoid tazarotene (Zorac®) offers new prospects in the treatment of mild-to-moderate psoriasis. The company sponsored a satellite symposium at the 6th Congress of the European Academy of Dermatology and Venereology, held in Dublin in September, to highlight the drug.Tazarotene was recently licensed for use in the UK and is also approved in some other countries, including the USA and Germany; approval for use in several other European countries is expected shortly. "Tazarotene is as or more effective than all existing treatments" said Professor Ronald Marks (University of Wales College of Wales, Cardiff, UK), "we believe it will be a very effective therapy". Tazarotene is applied once daily as a topical preparation. Trials have so far shown tazarotene to be more effective and easier to use than many existing therapies (psoriasis has been treated with a range of preparations, including emollients, keratolytic agents, coal tar preparations, anthralin, corticosteroids and dithranol). According to Dr Alan Menter (Baylor University Medical Center, Dallas, TX, USA) "It is a spectacular drug that is better than any other topical treatment", but he went on to explain that toxic effects associated with oral retinoids are well established, and although adverse effects seen with topical administration of tazarotene are much less severe, it has been found to cause erythema and burning/itching sensations. He went on to explain how these effects could be effectively minimized by co-administration of mid- and high-potency topical steroids in mild-to-moderate plaque psoriasis (corticosteroids are already widely used in treatment of the condition).
Good results in the treatment of psoriasis have also been observed with the vitamin D analogue calcipotriol (Devonex®), and the results of trials comparing the performance of these two agents are eagerly awaited.

SIBIA Neurosciences, Inc. has presented favorable Phase I clinical data on its Parkinson's drug, SIB1508Y, at the 11th Annual Symposium on Etiology, Pathogenesis and Treatment of Parkinson's Disease, which preceded the Annual Meeting of the American Neurological Association. In a single-dose Phase I trial evaluating the safety and pharmacokinetics in a single rising-dose tolerance, placebo-controlled and blinded study in 48 healthy male volunteers (under both fasted and fed conditions), SIB1508Y appeared to be well tolerated and rapidly absorbed following oral administration. Phase II studies are expected to commence early in 1998. According to Dr David E. McClure, Vice President of Clinical Development and Regulatory Affairs, "[SIBIA Neurosciences] are pleased by the safety profile exhibited by SIB1508Y in this single-dose study and in the recently completed multiple-dose Phase I study. We look forward to initiating studies in Parkinson's disease patients shortly, with the intent of demonstrating a positive effect on both cognitive and motor deficits."