Progress of Bis(heteroaryl)piperazines (BHAPs) as Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs) against Human Immunodeficiency Virus Type 1 (HIV-1)

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Abstract: Since the first case of acquired immunodeficiency syndrome (AIDS) was reported in 1981, AIDS, as the global disease affecting 33.2 million people in 2007, has always been an unsolved problem worldwide. Reverse transcriptase (RT) is a crucial enzyme in the life cycle of human immunodeficiency virus type 1 (HIV-1), and thereby has been the prime drugs target for antiretroviral (ARV) therapy against AIDS. To date, two classes of RT inhibitors (RTIs), *e.g.*, nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs), and a lot of compounds tested as RTIs have been described. To our knowledge, bis(heteroaryl)piperazines (BHAPs) have been considered as one class of promising NNRTIs, such as structurally and chemically related NNRTI delavirdine, which was approved by the U. S. Food and Drug Administration (FDA) for the treatment of HIV-1 infection in 1997. In this mini-review, we make attempts to report the progress of synthesis and structure–activity relationship (SAR) of BHAPs, in the meantime, the synergistic inhibition of HIV-1 replication by combining delavirdine with other HIV-1 inhibitors is also discussed. It will pave the way for the design and development of BHAPs as anti-HIV-1 agents in AIDS chemotherapy in the future.

Key Words: Bis(heteroaryl)piperazine, synthesis, acquired immunodeficiency syndrome, human immunodeficiency virus type 1, non-nucleoside reverse transcriptase inhibitor, structure–activity relationship.

INTRODUCTION

The human immunodeficiency virus type-1 (HIV-1) is a member of a class of viruses known as retroviruses, which are the primary causative agents of acquired immunodeficiency syndrome (AIDS), and cause life-threatening opportunistic infections associated with the progressive failure of the immune system [1].

HIV infection in humans is now pandemic. HIV/AIDS still remains a leading cause of mortality and has always been an unsolved problem worldwide since the first case of AIDS was reported in 1981. According to World Health Organization (WHO) / Joint United Nations Programme on HIV/AIDS (UNAIDS) estimates published in December 2007 with a notable downward number of people living with HIV from 39.5 million in 2006 to 33.2 million [2, 3], 2.5 million people have been newly infected in 2007, and 2.1 million died from AIDS in 2007 [2].

In recent years, therefore, there has been an extensive search for drugs selectively blocking HIV-1 replication, and several celluar targets for AIDS chemotherapy have been found such as HIV-1 reverse transcriptase (RT), protease, integrase (IN) [4], and CCR5 or CXCR4 receptor [5]. Especially RT, which catalyzes the conversion of the viral genomic RNA into the proviral DNA as an essential and absolutely required step in the life cycle of HIV-1 for viral rep1ication, is a key enzyme in the life cycle of HIV-1, and thereby has been an important drugs target for highly active antiretroviral therapy (HAART) against AIDS [6, 7]. To date, two classes of RT inhibitors (RTIs), such as nucleoside reverse transcriptase inhibitors (NRTIs), *e.g.*, zidovudine (AZT, **1**), stavudine (d4T, **2**), zalcitabine (ddc, **3**), and abacavir (ABC, **4**), and non-nucleoside reverse transcriptase inhibitors (NNRTIs), *e.g.*, nevirapine (**5**), delavirdine (**6**), and efavirenz (**7**), for the treatment of HIV infections, were approved by the U. S. Food and Drug Administration (FDA) for clinical use in the world [8].

However, the rapid emergence of viral variants resistant to HIV-1 inhibitors and severe side effects limit the efficacy of existing anti-AIDS therapies [9-11]. Meanwhile, as compared with HAART, the development of an HIV/AIDS vaccine may represent an alternative route to prevent the spread of this virus. One of the greatest challenges to developing a preventative HIV vaccine is the diversity of HIV-1 isolates [12]. Recently, HIV/AIDS vaccine in the clinical trial has been stopped because it failed to protect against HIV infection, and may even have made some people more susceptible [13, 14].

Consequently, although the development of a new, selective, efficacy and safe chemotherapeutical drug for the prevention of the spread of HIV-1 infection is a complex, lengthy and expensive process, it still remains a high priority for medical research worldwide. Some excellent reviews on anti-HIV drugs design [15-17], anti-HIV therapy [18-21], HIV-1 reverse transcriptase inhibitors [22, 23], HIV-1

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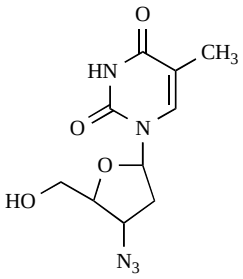
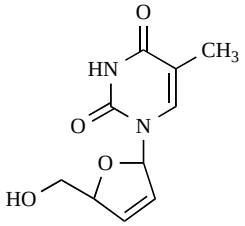
integrase inhibitors [24, 25], and HIV-1 RNase H inhibitors are available in recent years [26].

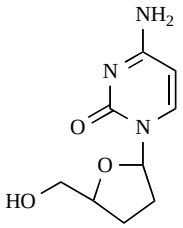
To our knowledge, bis(heteroaryl)piperazines (BHAPs) have been considered as one class of promising NNRTIs, such as structurally and chemically related NNRTI delavirdine (**6**), which was approved by FDA for the treatment of HIV-1 infection in 1997. In the present mini-review, we make attempts to report the progress of synthesis and structure–activity relationship (SAR) of BHAPs as NNRTIs, in the meantime, the synergistic inhibition of HIV-1 replication by combining delavirdine with other HIV-1 inhibitors is also surveyed.



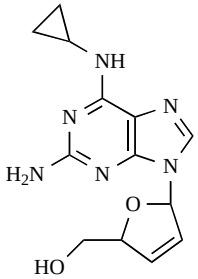
AZT (**1**), trade name: Combivir



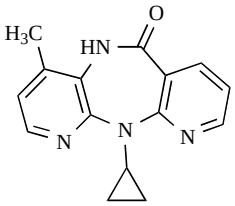
d4T (**2**), trade name: Zerit



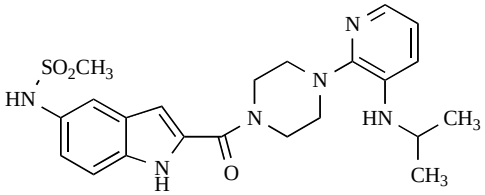
ddc (**3**), trade name: HIVID



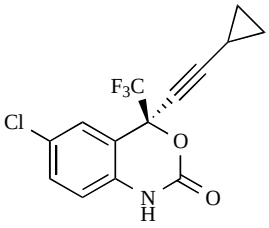
ABC (**4**), trade name: Ziagen



nevirapine (**5**), trade name: Viramune



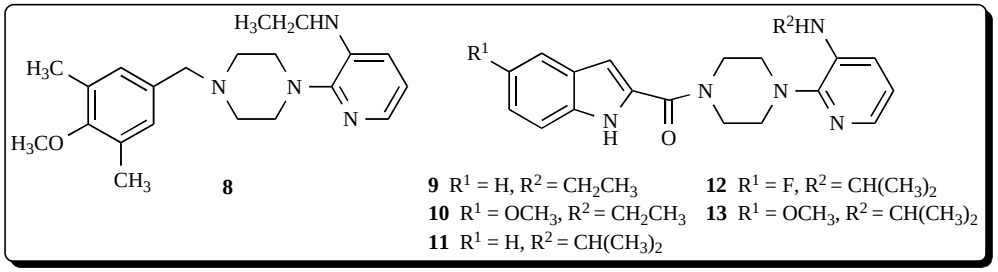
delavirdine (**6**), trade name: Rescriptor



efavirenz (**7**), trade name: Sustiva

Fig. (1). Structures of zidovudine (**1**), stavudine (**2**), zalcitabine (**3**), and abacavir (**4**), nevirapine (**5**), delavirdine (**6**), and efavirenz (**7**).

Table 1. Potently Blocking HIV-1 Replication in Human Lymphocytes and Inhibition of HIV-1 RT of BHAPs 9-13 (μM)[a]



8

9 R^1 = H, R^2 = CH_2CH_3
10 R^1 = OCH_3, R^2 = CH_2CH_3
11 R^1 = H, R^2 = $CH(CH_3)_2$
12 R^1 = F, R^2 = $CH(CH_3)_2$
13 R^1 = OCH_3, R^2 = $CH(CH_3)_2$

Compd.	MT-2/IIIb		PBMC/D34		H9/IIIb		RT inhibition
	ED_{50}	CC_{50}	ED_{50}	CC_{50}	ED_{50}	CC_{50}	IC_{50}
8	2	15	1-10	>10	1	10	20
9	0.3	>30	0.01	>10	0.06	>10	0.85
10	<0.2	>20	0.001	>10	0.04	>10	1.3
11	0.3	>27	0.001	>10	0.04	>10	0.25
12	0.3	>26	0.003	>10	0.05	>10	0.21
13	0.3	>25	0.001	>10	0.06	63	0.25
1	0.07	123	0.001	10	0.03	63	0.15

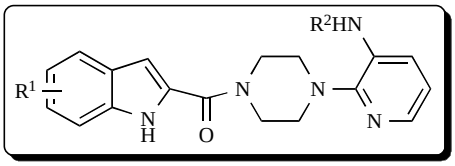
[a]HIV-1 infectivity and drug cytotoxicity studies were conducted in MT-2 and H9 cells with the HIV-1 IIIb isolate and in PBMC with the HIV-1 D34 isolate as described. CC_{50} is defined as the concentration of drug required to decrease cell viability 50% compared to uninfected controls. ED_{50} is 50% effective antiviral dose. All data represent the median values from at least two or three independent determinations with standard deviations ≈10% or less. The IC_{50} values of BHAPs were determined by using recombinant HIV-1 RT and synthetic poly(rA)-oligo(dT) template-primer as described.

SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIP (SAR)

The present review covers developments of synthesis and structure–activity relationship (SAR) of BHAPs as NNRTIs, which are presented in chronological order to demonstrate sequential progress in this area.

In previous paper, Busso and McQuade *et al.* found that *N*-ethyl-2-{4-[(4-methoxy-3,5-dimethylphenyl)methyl]-1-piperazinyl}-3-pyridinamine **8** exhibited anti-HIV activity [27, 28]. Then Romero *et al.* used compound **8** as a lead compound for chemical modifications. As shown in Table **1**, replacing the benzyl ring of **8** with indole-2-carbonyl group gave bis(heteroaryl)piperazines (BHAPs) **9**-**13**, which were evaluated with greatly enhanced inhibitory activity based on the formation of HIV-1 (IIIb or D34 isolate)-induced syncytia in MT-2, H9 and PBMC cells. For example, the ED_{50} values of **9**-**13** were 0.001-0.3 μM, while the ED_{50} value of **8** was 1-10 μM. In the meantime, the concentrations for the inhibition of HIV-1 RT could be lowered by 10- to 100-fold as compared with **8**, for example, the IC_{50} values of **9**-**13** were 0.21-1.3 μM, while the IC_{50} value of **8** was 20 μM. Moreover, the inhibition of RT by **11**-**13** was similar to that determined for **1** (0.21-0.25 *vs* 0.15 μM) [29].

Table 2. Inhibition of HIV-1 RT of BHAP Analogs 6, and 14-36



Compd.	R^1	R^2	RT inhibition[a]	
			% at 100 μM	IC_{50} (μM)
14	H	Et	96	4.0
15	5-OCH_3	Et	92	5.2
16	5-OH	Et	89	2
17	H	*i*-Pr	96	1.2
18	5-OH	*i*-Pr	98	1.3
19	5-OCH_2Ph	*i*-Pr	74	4.3
20	5-CH_3	*i*-Pr	68	34
21	5-NH_2	*i*-Pr	94	1
22	6-OCH_3	*i*-Pr	97	1.3
23	6-F	*i*-Pr	96	4.4
24	6-OCH_2Ph	*i*-Pr	65	37
25	6-OH	*i*-Pr	97	1.2
26	6-CN	*i*-Pr	74	3.9
27	6-CHO	*i*-Pr	94	1.4
28	5-OSO_2CH_3	*i*-Pr	97	5.5
29	5-$NHCOCH_3$	*i*-Pr	94	1.8
30	5-$NHCO_2CH_3$	*i*-Pr	96	2.5
31	5-$NHCOCF_3$	*i*-Pr	85	27
32	5-$NHSO_2CF_3$	*i*-Pr	94	8.3
33	6-$NHSO_2CH_3$	*i*-Pr	98	0.5

(Table 2) Contd…..

Compd.	R^1	R^2	RT inhibition[a]	
			% at 100 μM	IC_{50} (μM)
34	6-OSO_2CH_3	*i*-Pr	97	2.2
35	6-CH_2OH	*i*-Pr	98	0.76
36	5-$N(CH_3)SO_2CH_3$	*i*-Pr	95	5.6
5	/	/	93	5.5
6	5-$NHSO_2CH_3$	*i*-Pr	98	1.1

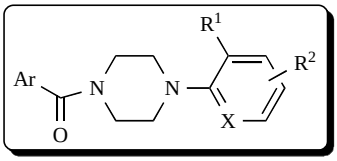
[a]The HIV-1 RT *in vitro* assay was carried out with recombinant enzyme using the template-primer poly(rA)-$(dT)_{10}$ and dTTP as the mononucleotide substrate as described. IC_{50} was determined by assaying at four drug concentrations.

In order to further explore optimum substitution patterns, a variety of BHAP analogs, including substitution of the indole moiety and the aminopyridine portion, were prepared and evaluated *in vitro* by recombinant HIV-1 RT screening assay. As shown in Table **2**, preliminary SAR showed the following interesting characteristics: (1) The effect of substituents on the indole on RT inhibitory activity was obvious. For example, when 5-methyl (34 μM for **20**), 6-benzyloxy (37 μM for **24**), or 5-(trifluoromethanecarbonyl) amino (27 μM for **31**) group was introduced on the indole ring, the RT inhibitory activity of the corresponding compounds were reduced sharply as compared to nevirapine (5.5 μM for **5**). On the contrary, when some substituents (*e.g.*, 5-methylsulfonamide (1.1 μM for **6**), 5-hydroxy (1.3 μM for **18**), 5-amino (1 μM for **21**), 6-hydroxy (1.2 μM for **25**), and 6-formyl groups (1.4 μM for **27**), *etc*.), were introduced on the indole ring, the corresponding compounds displayed more potent activity than **5**. Especially **33** and **35** showed the most potent RT inhibitory activity (0.5 μM for **33**, and 0.76 μM for **35**), *i.e.*, slight structural modifications on the 5- or 6-position of indole nucleus in the BHAP template could cause dramatic increases in potency. (2) The 3-(isopropylamino)pyridyl series exhibited more potent RT inhibitory activity than the corresponding 3-(ethylamino) pyridyl series (**17** *vs.* **14**, and **18** *vs.* **16**). (3) Through in-depth RT inhibition assay, **6** had an IC_{50} value comparable to that of AZT (**1**)-triphosphate (0.26 *vs.* 0.15 μM), and **6** was much more effective than **1** against HIV-1_{IIIB} in PBMC (0.1-1 *vs.* 1 nM) [30].

BHAPs **37**-**94** were synthesized by the chemical modifications of the left- and right-hand aryl portion of **8** and evaluated for the inhibition of HIV-1 RT. Preliminary SAR showed the following interesting characteristics (Table **3**): (1) Replacement of the substituted aryl moiety with 2-indolyl group provided BHAPs that were more potent than **8**, most noteworthy, compound **38** exhibited the most potent inhibition of RT with 96% at 100 μM. However, many other heterocycles, such as thienyl, furyl, benzofuryl, benzimidazolyl, benzothiazolyl, benzoxazolyl, quinolyl, and pyrazinyl groups, *etc*., were not the suitable replacements for the left-hand aryl moiety of **8** (**44**-**54** *vs.* **8**). (2) The 2-ethylamino or 2-isopropylamino substituent on the right-hand phenyl or pyridyl ring was very important for BHAPs having the RT inhibitory activity. For example, in the phenyl series, replacing the 2-ethylamino (**60**, 78%) or 2-isopropylamino substituent (**61**, 87%) with 2-ethyl (**56**, 0%), 2-cyano (**57**, 0%), or 2-methoxy group (**58**, 33%) usually caused the

activity to be lost except 2-ethoxy (**59**, 76%); in the pyridine series, some substituents, such as 3-nitro (**65**, 5%), 3-amino (**66**, 20%), 3-cyano (**67**, 5%), 3-acetamido (**68**, 0%), 3-(methylamino) (**71**, 85%), 3-(benzylamino) (**74**, 14%), and 3-(1-ethylpropy1)amino groups (**76**, 82%), *etc*., were not as favorable as 3-(ethylamino) (**38**, 96%), 3-(isopropylamino) (**73**, 96%), and *sec*-butylamino (**75**, 95%) ones. Meanwhile, when the fluoro atom or methoxy group was introduced on the 5-position of indole portion of **38**, **73**, and **87**, respectively, the corresponding compounds were also more potent for the inhibition of RT than **8** (**89**-**94** *vs*. **8**). (3) The 2-(indolylcarbonyl) moiety and the 3-(ethylamino)- or 3-(isopropy1amino)pyridine substituent were therefore necessary for BHAPs possessing good anti-HIV-1 RT activity [31].

Table 3. Inhibition of HIV-1 RT of BHAP Analogs 37-94[a]



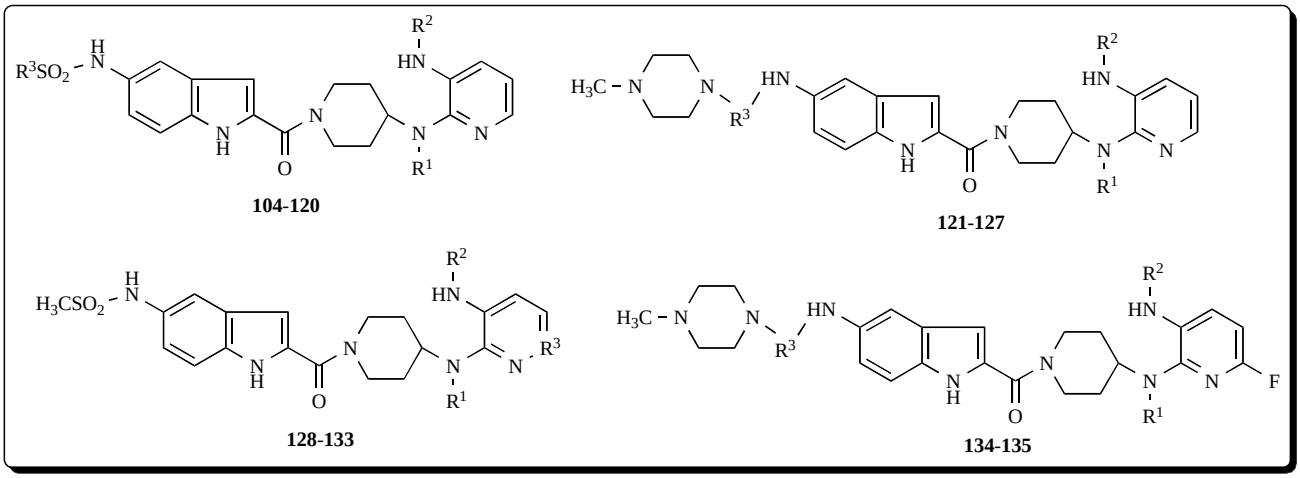
Compd.	Ar	X	R^1	R^2	% inhibition of RT[b]
8	/	/	/	/	73
37	5-methoxy-4,6,7-trimethyl-2-indolyl	N	NHEt	H	63
38	2-indolyl	N	NHEt	H	96
39	3-indolyl	N	NHEt	H	22
40	4-indolyl	N	NHEt	H	28
41	5-indolyl	N	NHEt	H	45
42	7-indolyl	N	NHEt	H	87
43	2-pyrryl	N	NHEt	H	87
44	2-thienyl	N	NHEt	H	60
45	2-furyl	N	NHEt	H	39
46	2-benzofuryl	N	NHEt	H	37-57
47	2-benzimidazolyl	N	NHEt	H	69
48	2-benzothienyl	N	NHEt	H	50
49	2-benzothiazolyl	N	NHEt	H	0
50	2-benzoxazolyl	N	NHEt	H	0
51	2-quinolyl	N	NHEt	H	0
52	3-quinolyl	N	NHEt	H	14
53	2-pyrazinyl	N	NHEt	H	0
54	3-(1,2-benzopyronyl)	N	NHEt	H	16
55	2-naphthyl	N	NHEt	H	74
56	2-indolyl	CH	Et	H	0
57	2-indolyl	CH	CN	H	0
58	2-indolyl	CH	OMe	H	33
59	2-indolyl	CH	OEt	H	76
60	2-indolyl	CH	NHEt	H	78
61	2-indolyl	CH	NH-*i*-Pr	H	87
62	2-indolyl	CH	NH-*i*-Pr	4-CF_3	22
63	2-indolyl	CH	NH-*i*-Pr	4-F	83
64	2-indolyl	CH	NH-*i*-Pr	5-F	77
65	2-indolyl	N	NO_2	H	5
66	2-indolyl	N	NH_2	H	20
67	2-indolyl	N	CN	H	5

(Table 3) Contd…..

Compd.	Ar	X	R^1	R^2	% inhibition of RT[b]
68	2-indolyl	N	NHCOMe	H	0
69	2-indolyl	N	N(Et)Ac	H	8
70	2-indolyl	N	NEt_2	H	69
71	2-indolyl	N	NHMe	H	85
72	2-indolyl	N	NHPr	H	64
73	2-indolyl	N	NH-*i*-Pr	H	96
74	2-indolyl	N	$NHCH_2Ph$	H	14
75	2-indolyl	N	NH-*s*-Bu	H	95
76	2-indolyl	N	$NHCHEt_2$	H	82
77	2-indolyl	N	CONHMe	H	0
78	2-indolyl	N	CONH-*t*-Bu	H	50
79	2-indolyl	N	COMe	H	30
80	2-indolyl	N	COEt	H	40
81	2-indolyl	N	CO-*i*-Pr	H	26
82	2-indolyl	N	CO-*t*-Bu	H	58
83	2-indolyl	N	CO_2Me	H	82
84	2-indolyl	N	CO_2Et	H	68
85	2-indolyl	N	CH_2NH-*i*-Pr	H	0
86	2-indolyl	N	$NHCH_2$-*c*-Pr	H	71
87	2-indolyl	N	NH-*t*-Bu	H	89
88	2-indolyl	N	$NHCH_2CF_3$	H	40
89	5-fluoro-2-indolyl	N	NHEt	H	93
90	5-methoxy-2-indolyl	N	NHEt	H	92
91	5-fluoro-2-indolyl	N	NH-*i*-Pr	H	96
92	5-methoxy-2-indolyl	N	NH-*i*-Pr	H	97
93	5-fluoro-2-indolyl	N	NH-*t*-Bu	H	89
94	5-methoxy-2-indolyl	N	NH-*t*-Bu	H	86

[a]The HIV-1 RT *in vitro* assay was carried out with recombinant enzyme using the template-primer poly(rA)-$(dT)_{10}$ and dTTP as the mononucleotide substrate. [b]% inhibition at 100 μM.

Table 4. Inhibitory Activities of AAP- BHAPs 104-135 on HIV-1 WT and Mutant RT Enzyme[a]



Compd.	R^1	R^2	R^3	IC_{50} (μM)		
				WT	P236L	Y181C
104	Me	*i*-Pr	Me	0.5	1.5	1.1
105	Me	*t*-Bu	Me	0.39	1.46	0.51
106	Me	*t*-Amyl	Me	0.35	3.33	0.53

(Table 4) Contd…..

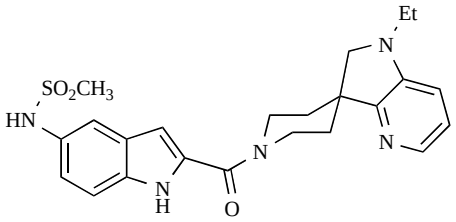
Compd.	R^1	R^2	R^3	IC_{50} (μM)		
				WT	P236L	Y181C
107	Me	CH_2-*c*-Pr	Me	0.52	14.6	2.91
108	Me	*c*-Pr	Me	0.09	0.21	0.28
109	Me	1-Me-*c*-Pr	Me	0.16	1.45	0.37
110	Et	*i*-Pr	Me	0.25	0.2	0.40
111	*n*-Pr	*i*-Pr	Me	0.24	0.24	0.61
112	*c*-Pr	*i*-Pr	Me	0.23	0.7	0.94
113	Et	*t*-Bu	Me	0.32	0.46	0.66
114	*n*-Pr	*t*-Bu	Me	0.48	1.45	2.48
115	Et	Et	Me	0.46	0.54	0.88
116	Et	Et	Et	0.40	0.26	0.48
117	Et	Et	*i*-Pr	0.45	0.33	0.66
118	*n*-Pr	Et	Me	0.15	0.52	0.95
119	*i*-Pr	Et	Me	0.38	2.6	3.6
120	Et	Pr	Me	0.13	0.28	0.45
121	Et	Et	-CO-	0.14	0.24	1.23
122	Et	Et	$-SO_2-$	0.24	0.44	1.11
123	Et	*i*-Pr	-CO-	0.14	0.18	0.41
124	Et	*i*-Pr	$-SO_2-$	0.38	0.57	0.77
125	Et	*t*-Bu	-CO-	0.22	0.37	0.61
126	Et	*t*-Bu	$-SO_2-$	0.79	1.25	1.83
127	*n*-Pr	*i*-Pr	-CO-	0.19	0.40	0.86
128	Me	*i*-Pr	N	0.74	4.56	1.31
129	Me	*i*-Pr	C-Cl	0.36	1.7	2.13
130	Me	*t*-Bu	C-F	0.53	1.9	0.72
131	Et	*i*-Pr	C-F	0.46	0.54	0.88
132	Et	*t*-Bu	C-Cl	1.02	1.52	3.0
133	Et	*t*-Bu	C-F	0.73	1.05	1.06
134	Et	*t*-Bu	-CO-	0.21	0.43	0.79
135	Et	*t*-Bu	$-SO_2-$	0.83	1.09	2.07

[a]The HIV-1 RT *in vitro* assays were carried out at 200 nM template-primer poly$(rA)_{600}$-oligo$(dT)_{10}$ and the RT enzymes (WT, P236L, and Y181C) were used as the homodimers.

Based on molecular modeling and X-ray crystallography of atevirdine (**15**), a novel spiro-BHAP analog **95** was designed and synthesized (Fig. **2**). By RT inhibition assay at 100 μM, although **95** was much less active than **15** (38% *vs.* 92%), the fact that it retained some RT inhibitory activity was encouraging [32].

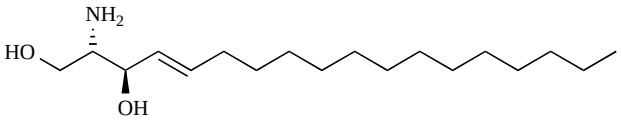
In order to impart the advantages (including liposomal delivery to infected macrophages and improved blood-brain penetration) of some lipid-drug conjugates, compounds **98**-**103** were prepared from **18** and **21** *via* a fatty acid-like linker to sphingosine **96** and psychosine **97** (Fig. **2**). At the test concentration of 100 nM, the antiviral activities of RT inhibition of the amide-linked ceramide cognates **98**-**100** were 59%, 59% and 66%, respectively, *i.e.*, the influence of varying the length of the alkyl spacer between two carbonyl groups to RT inhibitory activity was not obvious (**98** *vs.* **99** *vs.* **100**); the antiviral activities of RT inhibition of the ether-linked ceramide cognates **101**-**103** were 76%, 44% and 66%, respectively, that is, spacer groups between the carbonyl and the phenoxy exhibited influence on the inhibition of RT (**101** *vs.* **102**). But no activity was seen for all compounds at the concentration of 10 nM [33].

Based upon compound **104** demonstrating enhanced antiviral activity against both resistant viruses as compared with **6**, Romero *et al.* then focused on the synthesis of a variety of (alkylamino)piperidine BHAP analogs (AAP-BHAPs). The influences of different substituents of the pyridine ring, aminopiperidine linker, and indole ring on the



38% RT inhibition at 100 |*M

95



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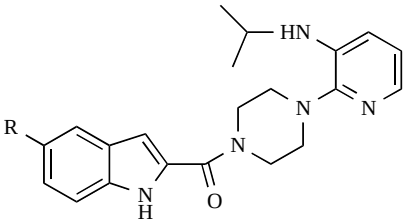
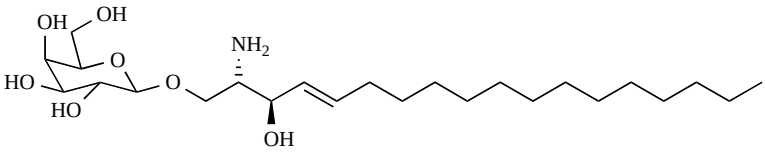


18: R = OH; **21:** R = NH_2



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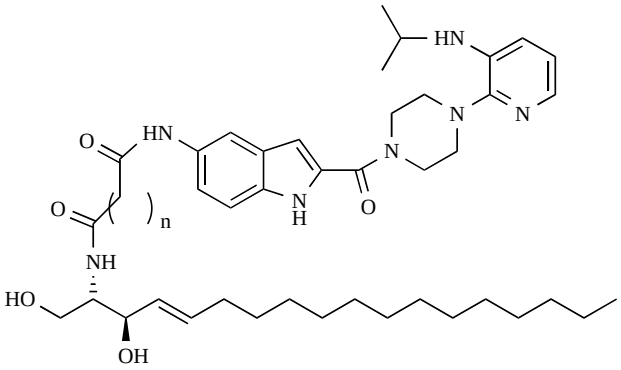
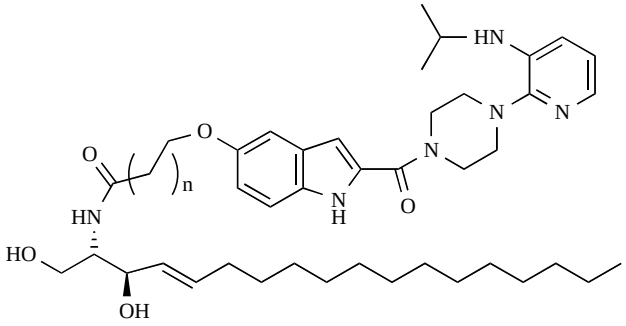
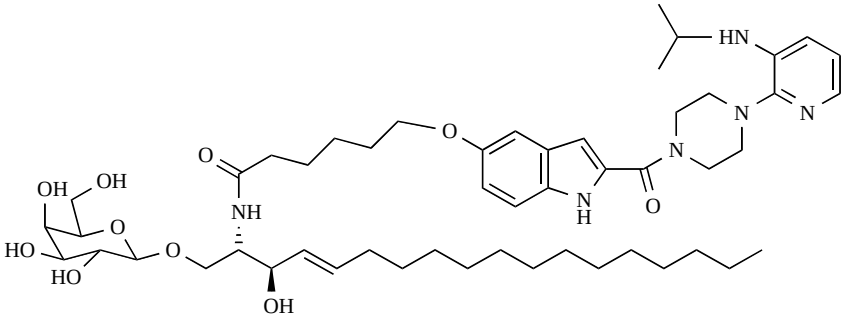


98: n =2; **99:** n =4; **100**: n=8



101: n =2; **102:** n =4



103

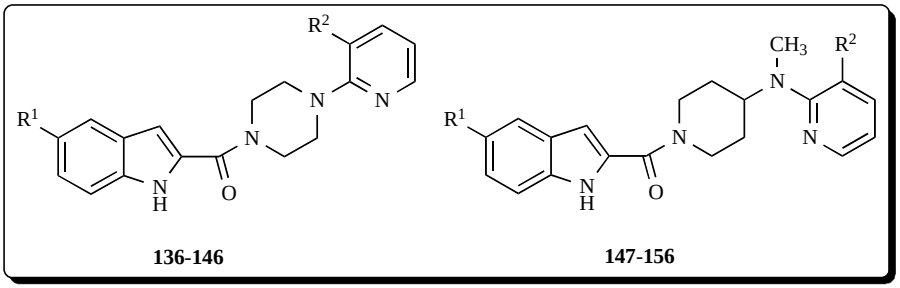
Fig. (2). Structures of spiro-BHAP analog **95**, and lipid-BHAP conjugates **98**-**103**.

inhibitory activities of AAP-BHAPs **104**-**135** were investigated. As shown in Table **4**, preliminary SAR showed the following interesting characteristics: (1) When the bulky substituent was introduced on the 3-position of the pyridine portion, the corresponding compound was usually obtained in good activity. For example, the *tert*-butyl analog **105** demonstrated inhibitory activity superior to the isopropyl one **104**, particularly with respect to activity against the Y181C mutant enzyme (0.51 *vs.* 1.1 μM). Especially, the cyclopropyl analog **108** exhibited the potency enzyme inhibitory activities, and the IC_{50} values of **108** against WT, P236L, and Y181C were 0.09, 0.21, and 0.28 μM, respectively. Meanwhile, in all cases introduction of the halogen atom (fluorine or chlorine) on the 6-position of the

pyridine ring caused decreases in the IC_{50} values against the three RT enzymes as compared with the deshalo parents (**129** *vs.* **104**, **130** *vs.* **105**, **131** *vs.* **110**, and **132** and **133** *vs.* **113**). (2) When a (methylamino)piperidine linker was changed to an (ethylamino)- or (*n*-propylamino)piperidine one, the corresponding compound was usually obtained in good activity. For example, the IC_{50} values of **104**, **110**, and **111** against WT, P236L, and Y181C were 0.5/0.25/0.24 μM, 1.5/0.2/0.24 μM, and 1.1/0.40/0.61 μM, respectively. The same results were also found in the 3-(*tert*-butylamino) pyridine series (**105** *vs.* **113** and **114**). (3) In all cases, the compounds containing the water-solubilizing urea substituents on the indole ring were more potent than those containing the sulfamoyl substituents against the panel of RTs (**121** *vs.* **122**, **123** *vs.* **124**, **125** *vs.* **126**, and **134** *vs.* **135**). In the meantime, usually introduction of the methylsulfonamide group on the 5-position of the indole ring of AAP-BHAPs caused increases in the IC_{50} values against the three RT enzymes as compared with the (4-methyl-1-piperazinyl) sulfonylamino ones (**110** *vs.* **124**, and **113** *vs.* **126**) [34].

Some novel BHAPs and AAP-BHAPs were synthesized and initially evaluated *in vitro* for their ability to inhibit recombinant HIV-1 RT. As shown in Table **5**, the isopropyl and ethyl ethers **140**-**143** showed the inhibitory activity comparable to **6** (1.1-2.1 *vs* 1.1 μM), demonstrating that the alkoxy group was a good alternative for the alkylamine moiety. The ethylamine and *tert*-butylamine analogs **138** and **139** were also quite active against the RT, and the corresponding IC_{50} values were 2.7 and 4.8 μM, respectively. However, both benzyl ethers **144** and **145** showed no activity in the initial assay. The AAP-BHAPs **147**-**150** containing 3-

Table 5. Inhibition of HIV-1 RT of BHAPs 136-146 and AAP-BHAPs 147-156



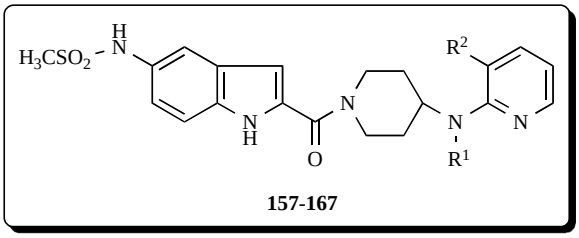
136-146 **147-156**

Compd.	R^1	R^2	RT inhibition[a]	
			% at 100 μM	IC_{50} (μM)
6	$NHSO_2CH_3$	NH-*i*-Pr	98	1.1
136	OCH_3	NH_2	0	ND[b]
137	$NHSO_2CH_3$	NH_2	0	ND
138	$NHSO_2CH_3$	NHEt	96	2.7
139	$NHSO_2CH_3$	NH-*t*-Bu	97	4.8
140	H	O-*i*-Pr	98	1.8
141	$NHSO_2CH_3$	O-*i*-Pr	99	1.1
142	H	OEt	98	2.1
143	$NHSO_2CH_3$	OEt	98	1.9
144	H	OCH_2Ph	0	ND
145	$NHSO_2CH_3$	OCH_2Ph	0	ND
146	$NHSO_2CH_3$	O-*t*-Bu	92	ND
147	H	NHEt	96	2.2
148	H	NH-*i*-Pr	96	2.0
149	$NHSO_2CH_3$	NH-*i*-Pr	98	2.2
150	$NHSO_2CH_3$	NH-*t*-Bu	97	3.1
151	H	OEt	94	3.2
152	H	O-*i*-Pr	96	2.0
153	$NHSO_2CH_3$	OEt	96	3.4
154	$NHSO_2CH_3$	O-*i*-Pr	91	1.4
155	$NHSO_2CH_3$	$NHCD(CH_3)_2$	98	ND[a]
156	$NHSO_2CH_3$	$NHCH(CD_3)_2$	98	ND

[a]The HIV-1 RT *in vitro* assay was carried out with recombinant enzyme using the template-primer poly(rA)-$(dT)_{10}$ and dTTP as the mononucleotide substrate. [b]Not determined.

alkylamino substituents exhibited very good potency against the RT. The 3-alkoxypyridine series **151**-**154** also possessed sufficient potency. Moreover, within the alkoxy series, the piperazine-linked compounds appeared to possess activities comparable to the piperidine-linked ones (**142** *vs.* **151**, and **143** *vs.* **153**) [35].

Table 6. Inhibition of HIV-1 RT of AAP-BHAPs 157-167



157-167

Compd.	R^1	R^2	IC_{50} (μM)[a]		
			WT	P236L	Y181C
6	/	/	0.26	18.0	8.32
104	Me	NH-*i*-Pr	0.50	1.5	1.1
115	Et	NHEt	0.43	0.36	0.39
157	Me	Et	0.061	1.1	2.0
158	Me	*n*-Pr	84%[b]	40%[b]	25%[b]
159	Me	*i*-Bu	0.51	1.1	2.6
160	Et	Et	0.098	0.21	0.43
161	Et	*n*-Pr	0.27	0.37	0.45
162	Et	*i*-Bu	0.41	0.43	0.88
163	*n*-Pr	Et	0.13	0.54	0.91
164	Me	CH_2OMe	0.14	6.8	11
165	Me	C(O)OMe	0.25	13	21
166	Et	CH_2OMe	0.14	0.30	0.84
167	Me	CH_2OH	50%[c]	7%[c]	15%[c]

[a]The HIV-1 RT *in vitro* assay was carried out with recombinant enzyme using the template-primer poly(rA)$_{600}$-oligo(dT)$_{10}$. [b]At 1 μM. [c]At 50 μM.

Some AAP-BHAPs **157**-**167** containing a 3-alkylpyridine were prepared and evaluated *in vitro* against a panel of recombinant RTs, which included wild-type (WT) RT, and the P236L and Y181C mutant RTs. As shown in Table **6**, preliminary SAR showed the following interesting characteristics: (1) When the 3-alkylamino substituent on the pyridine ring was replaced by a 3-alkyl substituent, the corresponding compound usually retained activity against recombinant P236L and WT RT (**115** *vs.* **160**). (2) The (ethylamino)piperidine-linked analogs were more potent against P236L and Y181C mutant RTs than the corresponding (methylamino)-piperidine-linked ones (**160** *vs.* **157**, and **162** *vs.* **159**), while the (propylamino)piperidine-linked analog **163** was 2-fold less potent than the (ethylamino)piperidine-linked one **160** against P236L and Y181C RTs. (3) When the ethyl group was introduced on the 3-position of the pyridine ring, the corresponding compound showed the better activities against all three enzymes than the one containing *n*-propyl or *i*-butyl group on the 3-position of the pyridine ring (**157** *vs* **158** and **159**). (4) (Methylamino)piperidine **164** containing a methyl ether in the 3-pyridine ring lost considerable potency against P236L and Y181C RTs as compared with its WT potency, while the (ethylamino)piperidine-linked analog **166** retained good potency against all three enzymes. (5) Introduction of an ester group on the 3-position of the pyridine ring of **165** caused decreases in the IC_{50} values against the P236L and Y181C RT enzymes. Especially, a 3-hydroxymethyl-substituted pyridine **167** exhibited decreased activities for all three enzymes [36].

Guillaumel *et al*. synthesized a series of vinylogous BHAP analogs **168**-**174** (Fig. **3**), and evaluated for their protective effect against the cytopathogenic activity of WT HIV-1. However, all compounds were inactive at the concentrations up to 10^{-6} μM [37].

SYNERGISTIC INHIBITION OF HIV-1 REPLICATION BY DELAVIRDINE AND OTHER HIV-1 INHIBITORS

The emergence of drug-resistant strains of HIV-1 and the toxicity of several antiviral nucleoside analogs led to the use of combination antiretroviral regimens for the treatment of HIV-1 infection [38]. As with all other RT inhibitors described to date, the viral variants resistant to delavirdine (**6**) also emerged. Interestingly, Dueweke *et al.* found that the combination of **6** with AZT (**1**), each at 0.5 μM, could totally prevent viral spread against HIV-1_{IIIB}-infected MT-4 cells, while **1** at 3 μM prolonged the interval to the rapid burst of viral replication about 7 days, and **6** at 1 μM delayed viral spread until 42 days [39]. Synergistic inhibition of HIV-1 replication by **6** and **175** (Fig. **3**) has also been observed in HIV-1-infected CEM cells. For example, when **6** (0.09 μM) was combined with **175** (0.11 μM) against HIV-1_{IIIB}-infected CEM cells, virus breakthrough could be prevented for more than 77 days as compared to 8 days with **6** (0.09 μM) as a single agent [40]. Consequently, breakthrough of virus was markedly delayed or even suppressed with the use of drug combinations, while the concentrations of the individual drugs could be lowered by 10- to 25-fold compared with the individual use of the compounds. However, **6** should be further evaluated in preclinical and clinical studies in the treatment of acquired immune deficiency syndrome as potential new agent to be used in combination chemotherapy with other HIV-1 inhibitors. Vasudevachari *et al.* considered that the combination therapy approaches may benefit from potential synergism in the antiviral effects of mechanistically distinct inhibitors of reverse transcriptase while minimizing the selection of drug-resistant strains [41].

CONCLUSION

Nowadays, HIV/AIDS continues to remain a leading cause of mortality and has always been an unsolved problem worldwide. In order to diminish the undesirable toxicity and the emergence of resistant strains, the development of new, selective, efficacy and safe chemotherapeutical drugs for the prevention of the spread of HIV-1 infection still remains a high priority for medical research. In this mini-review, the progress of synthesis and SAR of BHAPs as one class of NNRTIs is reported, meanwhile, the synergistic inhibition of HIV-1 replication by combining delavirdine with other HIV-1 inhibitors is also described, which will make the BHAPs as

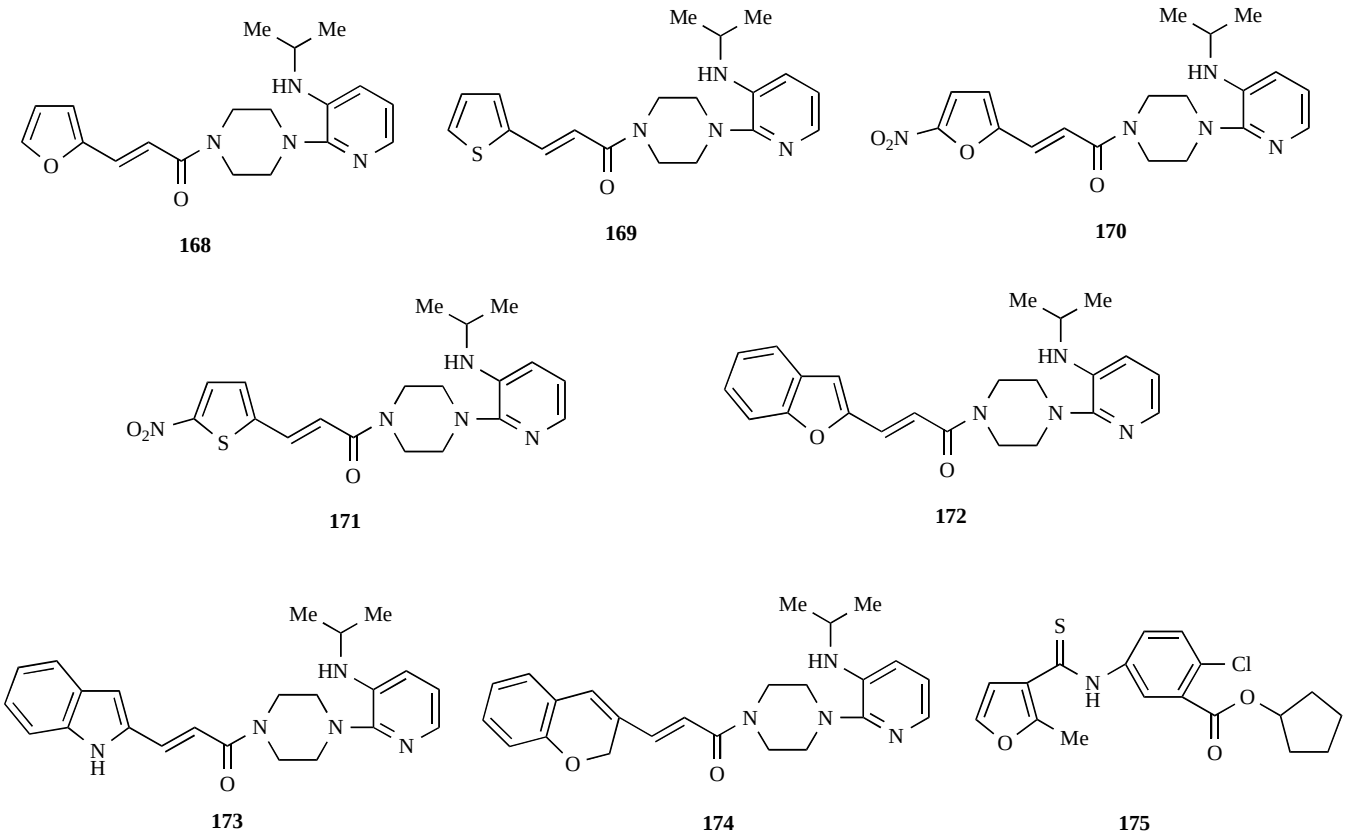


Fig. (3). Structures of vinylogous BHAP analogs **168**-**174**, and thiocarboxanilide derivative **175**.

the excellent candidates to be further pursued for the combination chemotherapy of HIV-1 infection.

ACKNOWLEDGEMENTS

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ABBREVIATIONS

AAP	=	Alkylaminopiperidine
AAP-BHAP	=	(Alkylamino)piperidine-bis(heteroaryl) piperazine
Ac	=	Acetyl
AIDS	=	Acquired immune deficiency syndrome
ARV	=	Antiretroviral
AZT	=	3'-Azido-3'-deoxythymidine
BHAP	=	Bis(heteroaryl)piperazine
t-Bu	=	*tert*-Butyl
Et	=	Ethyl
FDA	=	Food and Drug Administration
FIs	=	Fusion inhibitors
HAART	=	Highly active antiretroviral therapy
HIV-1	=	Human immunodeficiency virus type 1
IN	=	Integrase
PBMC	=	Peripheral blood mononuclear cells
i-Pr	=	*iso*- Propyl
RT	=	Reverse transcriptase
RTIs	=	RT inhibitors
NRTIs	=	Nucleoside reverse transcriptase inhibitors
NNRTIs	=	Non-nucleoside reverse transcriptase inhibitors
SAR	=	Structure–activity relationship
WHO	=	World Health Organization
WT	=	Wilde type
UNAIDS	=	Joint United Nations Programme on HIV/AIDS

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