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Benzophenone Derivatives: a Novel Scaffold as Non-Nucleoside HIV-1 Reverse Transcriptase Inhibitors

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Abstract

Developing non-nucleoside HIV-1 reverse transcriptase inhibitors (NNRTIs) with improved drug resistance profiles is still a great challenge owing to the rapid emergence of resistant virus. For the potential activity against both wild-type and clinically relevant NNRTI-resistant mutant HIV-1 strains, the novel NNRTI benzophenone derivatives (BPs) have drawn great attention. Considerable efforts on modifying this template have led to the identification of the most active BP analogue, GW678248, which has been progressed to the phase II clinical studies owing to its high anti-HIV-1 activity, low clearance and stable metabolic property. Therefore, this review focused on the benzophenone deravitives provided valuable SAR information for further modifying this scaffold.

Keywords: HIV; NNRTI; SAR; Benzophenone; Inhibitor

Introduction

Since the identification of the human immunodeficiency virus (HIV) as the causative agent of AIDS, searching for more safe and effective drugs for HIV therapy continues to be a hot topic [1,2]. Till now, 27 approved anti-HIV drugs have been marketed for the treatment of HIV infection, which have dramatically alleviated the AIDS issues particularly after the application of highly active antiretroviral therapy (HAART) [3-6]. Being the most important components of HAART, the marketed non-nucleoside inhibitors of reverse transcriptase (NNRTIs), nevirapine (**1**, NVP) [7], delavirdine (**2**, DLV) [8], efavirenz (**3**, EFE) [9-11], etravirine (**4**, ETV) [12,13] and rilpivirine (**5**, RPV) [14-17], which exhibited great resistance to the presence of drug resistance mutations, have been proved effective for the AIDS therapy (Figure 1). Nevertheless, due to the rapid emergence of drug resistance, more effective HIV inhibitors are still required to allow continued suppression of the viral infection [18-20].

Recently, benzophenone derivatives, originated in a high-throughput screening in 1995 [21], attracted more attention for their greatly improved profiles of activity against both wild-type and clinically relevant NNRTI-resistant mutant strains of HIV-1. Structural modifications on this backbone have resulted in producing several novel BP analogues, such as **7** (GW4511) [22] and **8** (GW678248) [23,24], which possess high anti-HIV activity, low clearance and stable metabolic property (Figure 2). In particular, GW678248 also has been advanced to the clinical exploration for the excellent potency in inhibiting the mutant HIV strains (IC_{50}<1 nM) [25]. As our great interest, we have designed and synthesized a series of new potential BP inhibitors, such as PAFAs, NPEs, and so on [26-29]. Based on these studies, the structure and activity relationships (SARs) of BPs were summarized and provided for further structure optimizations in this manuscript.

A-Ring SARs

Apparently, the shape of A-ring has a significant impact on the potency of the BP analogues. As shown in table 1, phenyl-substituted analogue **9a** not only showed nanomolar concentration level of anti-HIV activity assayed in MT-4 cell, but also exhibited equivalent potency against syncytia (SYN) and p24 antigen (p24) in C8166 cells infected with HIV-1. Replacement of the A-ring with a more polar pyridyl ring (**9b**) or the saturated cyclophenyl ring (**9c**) resulted in a great loss of anti-HIV activity [21].

By moving a small substituent around the A-ring, compounds **10a-d** were obtained and evaluated for the activity against both the wild-type and the mutant HIV viruses (Table 2) [23]. Clearly, placement of a small group at *meta* position (**10c**) is far more favorable than the substitution of the same group at *ortho* (**10b**) or *para* position (**10c**). Furthermore, substitution at the *meta* position (**10c**) offers an advantage over the unsubstituted analogues (**10a**), particularly against the critical mutant viruses K103N and Y181C.

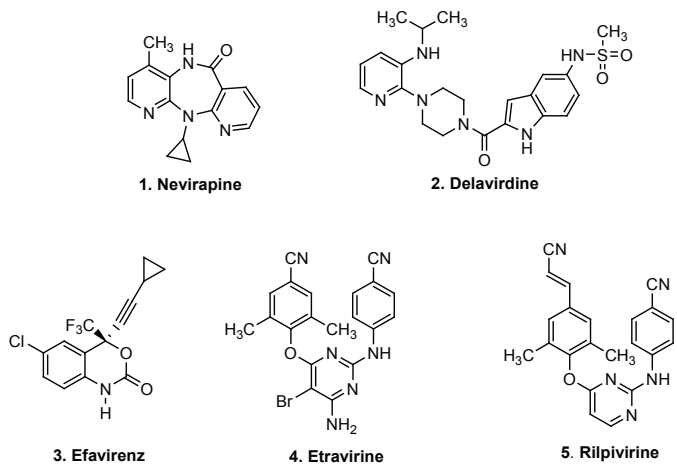

Figure 1: Structures of currently approved NNRTIs.

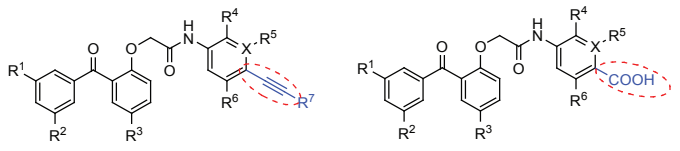

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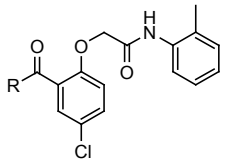
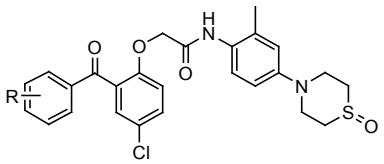
Figure 2: Novel benzophenone NNRTI structures.



Compound	R	RT[a]	MTM[b]	TOX[c]	SYN[d]	p24[e]
9a	Phenyl	0.01	0.0004	>0.005	0.0002	0.0012
9b	Pyridyl	0.16	<0.01	10	--[f]	--
9c	Cyclophentyl	1.8	0.22	>100	--	--

Table 1: BP analogues with various A-ring and their anti-HIV activity.
[a]IC_{50} *versus* RT of HIV-1 (μg/mL), AZT triphosphate control=0.02 ± 0.005 μM;
[b]IC_{50} *versus* HIV-1 (strain RF)-infected MT-4 cells (μg/mL), AZT=0.002–0.02 μM;
[c]IC_{50} *versus* uninfected MT-4 cells (mg/mL);
[d] IC_{50} *versus* syncytia formation in infected (C8166-cells μg/mL), AZT=0.002–0.02 μM;
[e]IC_{50} *versus* p24 antigen formation in infected (C8166-cells μg/mL), AZT=0.001–0.01 μM;
[f]Undetectable.



Compound	R	potency [IC_{50}, nM][a]			
		WT	K103N	Y181C	V106A
10a	H	17	100	150	1400
10b	*o*-CN	1500	155	700	2000
10c	*m*-CN	4	5	6	--[b]
10d	*p*-CN	26	44	355	1400

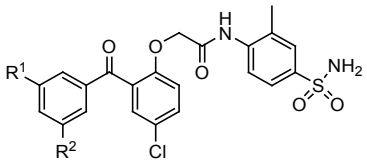
Table 2: SARs of various substituents on the A-ring.
[a]IC_{50} values determined using a HeLa-MAGI assay. Wild-type virus (WT) was the HxB2 strain. K103N, Y181C, and V106A mutants were isogenic with the HxB2 virus backbone;
[b]Undetectable.

Romines et al. [23] further explored the *meta* substitution patterns on the A-ring (Table 3). Addition of a methyl group (**11h**) or a chloro group (**11i**) at the second *meta* position on the A-ring of the mono-*meta* substituted analogue (**10c**), had remarkable impact on the ability of the analogue to inhibit the V106A strain, without sacrificing the potency in the wild-type, K103N, and Y181C viruses. The disubstituted analogues shown in table 3 have very good profiles against the wild-type, K103N, Y181C, and V106A viruses, including the fluoro, trifluoromethyl (**11b**), the chloro, bromo (**11f**), and the chloro, methyl (**11g**). Although all of these were more potent against the V106A strain than the mono-*meta* substituted analogue, they were not quite as potent as the methyl, cyano analogue (**11h**). Use of electron-donating substituents (**11c**) was clearly less beneficial than the electron withdrawing substituents, although potency against the Y181C mutant virus was less affected than potency against the other viruses in the panel. Use of neutral substituents (methyl groups), however, was very well tolerated. In fact, the dimethyl-substituted analogue (**11d**) was as potent as the methyl, cyano-substituted analogue (**11h**).

B-Ring SRAs

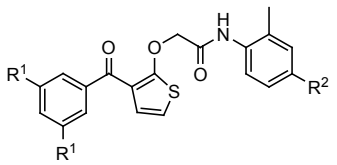
Three versions of the thiophene B-ring analogues **12a-c** were prepared and evaluated for the activity against wild-type HIV in MT4 cells (Table 4) [23]. Compared with the chlorophenyl B-ring (**9a,** IC_{50}=10 nM), the thiophenyl compound **12a** showed approximately 30-fold decrease of activity. Although the activities of compounds **12b** and **12c** were improved, their potency was low relative to the analogues with the chlorophenyl B-ring (**9a**).

In 2011, Ma et al. [27] obtained a series of benzophenone derivatives (**13a-h**) with B-ring substituted by a naphthyl ring to increase the π–π interactions with the mutant type of HIV viruses (Table 5). The results showed that most of these compounds were highly effective, and the most active analogue **13d** was able to inhibit HIV-1 replication within 4.9 nM concentrations. However, these compounds are still less sensitive to the A_{17} mutant HIV viruses (micromolar level). Although the naphthyl B-substituted analogues were not as potent as the lead compound, they provided a new scaffold worthy of further optimizations.



Compound	R^1	R^2	potency [IC_{50}, nM]a			
			WT	K103N	Y181C	V106A
11a	H	CN	0.7	1.5	1.5	30
11b	F	CF_3	1.4	2.0	2.9	10.5
11c	OMe	OMe	16	370	13	500
11d	Me	Me	0.4	3.3	3.0	4.
11e	Br	CF_3	30	93	290	18
11f	Cl	Br	2.2	6.1	6.9	13
11g	Cl	Me	1.2	3.6	3.3	9.4
11h	Me	CN	0.5	0.9	1.9	1.4
11i	Cl	CN	0.5	1.0	0.7	3.4
11j	CF_3	CN	1.0	1.1	2.0	5.6
EFV[b]			0.8	25	1.6	1.8
NVP[b]			88	5800	10300	8200

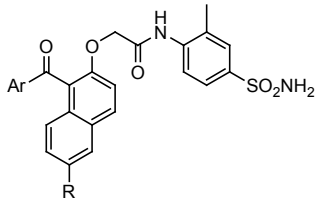
Table 3: SARs of 3,5-disubstituents on the A-ring.
[a]IC_{50} values determined using a HeLa MAGI assay. Wild-type virus (WT) was the HxB2 strain. K103N, Y181 C and V106A mutants wereisogenic with the HxB2 virus backbone;
[b]EFV is efavirenz; NVP is nevirapine.



Compound	X	R	WT [IC_{50}, nM][a]
12a	H	H	920
12b	F	H	247
12c	F	SO_2NH_2	88

Table 4: BP analogues bearing a thiophenyl B-ring and their anti-HIV activity

[a]IC_{50} values were determined using HIV-1 III_B wild-type virus in an acute infection assay in MT4 cells.



Compound	Ar	R	EC_{50}[a]		CC_{50} [μM][c]	SI[d]
			III_B [nM]	A_{17} [μM][b]		
13a	Ph	H	52.7	9.6	33.8	641.8
13b	3-Me-Ph	H	13.5	2.4	40.4	2992.0
13c	3,5-Me_2-Ph	H	7.8	8.4	39.1	5038.4
13d	3,5-Cl,CN-Ph	H	4.9	2.1	50.8	10347.9
13e	3,5-Me_2-Ph	Br	7573.8	10.2	28.5	3.8
13f	3-Me-Ph	Br	2299.8	3.4	6.8	2.9
13g	3-Me-Ph	CN	790.6	10.4	15.2	19.2
13h	3,5-Me_2-Ph	CN	1777.9	16.3	128.1	72.0
GW678248			0.7	0.00138	>385.8	>567382.0
AZT			10.6	0.00812	5601.7	528975.3

Table 5: BP analogues bearing a naphthyl B-ring and their anti-HIV activity.

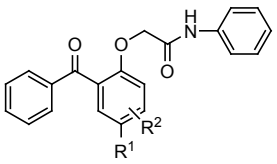
[a]EC_{50}: effective concentration of compound required to protect the cell against viral cytopathogenicity by 50%in C8166 cells;

[b]A_{17}: HIV-1 mutated strain bearing both K103N andY181C mutations;

[c]CC_{50}: cytotoxic concentration of compound that reduces the normal uninfected C8166 cell viability by 50%;

[d]SI: selectivity index: ratio CC_{50}/EC_{50} (HIV-1 III_B).

As illustrated in table 6, compounds **14a-f** were designed and synthesized to investigate the SARs of substitutions on the B ring [21]. Obviously, 5-chloro substituted **14a** was as potent as 5-fluorine substituted **14b** in inhibiting HIV viruses assayed in both RT and cell level. Replacement of chlorine with a polar imidazole group (**14c**) resulted in a significant loss of activity. The dichloro-substituted compounds **14d-f** also showed significant SAR information that the 4,5-dichloro-substituted



Compound	R1	R^2	RT[a]	MTM[b]	TOX[c]	SYN[d]	p24[e]
14a	Cl	H	0.01	0.0004	>0005	0.0002	0.0012
14b	F	H	0.003	0.09	10	0.014	0.006
14c	*N*-imidazolyl	H	>10	>10	10	--[f]	--
14d	Cl	3-Cl	>100	--	--	--	--
14e	Cl	5-Cl	0.14	0.23	100	--	--
14f	Cl	6-Cl	>100	--	--	--	--

Table 6: SARs of various substituents on the B-ring

[a]IC_{50} *versus* RT of HIV-1 (μg/mL), AZT triphosphate control=0.02 ± 0.005 μM;

[b]IC_{50} *versus* HIV-1 (strain RF)-infected MT-4 cells (μg/mL), AZT=0.002–0.02 μM;

[c]IC_{50} *versus* uninfected MT-4 cells (mg/mL);

[d] IC_{50} *versus* syncytia formation in infected (C8166-cells μg/mL), AZT=0.002–0.02 μM;

[e]IC_{50} *versus* p24 antigen formation in infected (C8166-cells μg/mL), AZT=0.001–0.01 μM;

[f]Undetectable.

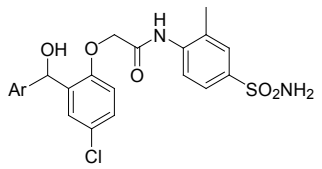
14e retained the activity of the mono substituted **14a**, whereas the 3,3- and 5,6-dichloro derivatives **14d**,**f** were devoid of activity.

Ma et al. [28] further explored the importance of the carbonyl group between A and B-rings in 2011 (Table 7). By reducing the carbonyl group, they synthesized a series of benzhydrol derivatives **15a-h** and evaluated their anti-HIV activity in C8166 cells. Among these molecules, most were able to inhibit wild-type HIV-1 at lower than 1 μM. In particular, compound **15d** was identified as the highest active inhibitor against the wild-type HIV-1, with an EC_{50} value of 0.12 μM and a selectivity index value of 312.73. Although some of them exhibited moderate activity against the double mutant strain A_{17} (K103N+Y181C), these inhibitors were still not quite sensitive to the resistant profiles. Taken together, this exploration induced a valuable conclusion that the benzophenone carbonyl is essential for BPs to maintain their high anti-HIV potency.

Also based on GW678248, Tucker et al. [30] changed the keto group between A and B-rings into an ether linker, to synthesize a series of diaryl ether derivatives **16a-d** which possess broad antiviral activity against a number of key clinical mutations. As shown in table 8, adding the 3-chloro substituent to the central aryl ring (**16a**) increased the activity versus WT and the Y181C mutant RT enzyme. Addition of a *meta* cyano substituent to the A-ring resulted in a large enhancement of potency (**16c**) versus WT and both the K103N and Y181C enzymes, and the further addition of a second *meta* chloro substituent provided a more moderate enhancement in potency versus the K103N mutant (**16d**). This di *meta* substitution on the A-ring appears to provide excellent potency versus WT and the key mutant enzymes, which is similarly to the SAR seen with GW678248.

Inspired by the active diaryl ether HIV inhibitors, Gu et al. [29] designed a series of naphthyl phenyl ether analogues (**NPEs**, **17a-i**, Table 9), in which the bulky naphthalene ring was introduced to replace the benzene ring (A ring). The evaluation for their activities against HIV-1 in C8166 cells showed that most of the compounds moderately repress the replication of HIV-1 virus. In particular, compound **17f**

showed the highest activity against the wild-type HIV-1 with an EC_{50} value of 4.60 nM, along with the moderate activity against the double mutant strain HIV-1 A_{17} (K103N+Y181C) and HIV-2 strain ROD, with EC_{50} values of 0.82 and 4.40 μM, respectively. Unfortunately, this structural modification led to about 5-fold decrease of anti-HIV activity compared with GW678248.



Compound	Ar	EC_{50}[nM][a]		CC_{50} [μM][c]	SI[d]
		III_B	A_{17}[b]		
15a	Ph	1.12	16.46	33.40	29.92
15b	3-Me-Ph	0.48	9.70	33.15	69.05
15c	3-Cl-Ph	0.72	12.98	33.25	46.40
15d	3-NO_2-Ph	0.12	4.34	36.85	312.73
15e	3,5-Me_2-Ph	0.18	2.08	29.36	165.97
15f	3,5-Cl_2-Ph	1.06	9.45	13.125	12.40
15g	3,5-Cl,Br-Ph	0.54	7.79	22.92	42.39
15h	3,5-Cl,CN-Ph	0.39	6.84	31.58	80.75

Table 7: Benzhydrol derivatives **15a-h** and their anti-HIV activity

[a]EC_{50}: effective concentration of compound required to protect the cell against viral cytopathogenicity by 50% in C8166 cells;

[b]A_{17}: HIV-1 mutated strain bearing both K103N andY181C mutations;

[c]CC_{50}: cytotoxic concentration of compound that reduces the normal uninfected C8166 cell viability by 50%;

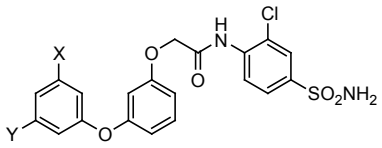
[d]SI: selectivity index: ratio CC_{50}/EC_{50} (HIV-1 III_B).

In 2011, Ma et al. [26] synthesized a class of *N*-phenylaryl formamide derivatives (**PAFAs**, **18a-i**, Table 10), considering that the flexible amido linker between A and B rings might not only improve the adaptation of the inhibitor to RT, but also promote the H-bond bindings with key mutant amino acids Y188 or Y181. Within these inhibitors, more than half possess anti-wild-type HIV-1 EC_{50} values ranging from 0.3 nM to 5.1 nM, and the selectivity index values ranging from 10616 to 271000. In particular, compound **18c** (EC_{50}=0.30 nM, SI=184578), **18f** (EC_{50}=0.37 nM, SI=212819), **18g** (EC_{50}=0.32 nM, SI=260617) and **18h** (EC_{50}=0.27 nM, SI=271000) displayed the highest activity against this type virus as potent as GW678248. Moreover, the four compounds also were effective to inhibit the double mutant strain A_{17} (K103N+Y181C) with EC_{50} values of 0.29 μM, 0.14 μM, 0.10 μM and 0.27 μM, respectively. Interestingly, compound **18g**, the broad-spectrum anti-HIV inhibitor, also showed potent activity against HIV-2 ROD with an EC_{50} value of 4.37 μM.

C-Ring SARs

Wyatt et al. [21] first explored the preliminary SARs of the C-ring in 1995. As shown in table 11, the *N*-cyclohexylamide **19a** lost the total activity against several mutant HIV viruses compared with the phenyl-substituted **19b**. Although the 2-methylbenzyl amide derivative **19c** was less active than **19b** in enzyme and cellular assays, it was significantly more metabolically stable. The bicyclic amide **19f** synthesized as a constrained version of **19c** proved to be a potent inhibitor of the enzyme and exhibited excellent activity in antiviral assays, but was less metabolically stable than **19c.** Also, compound **19e** with (*N*,*N*-diethylamino)-propoxy substituent on the C-ring have the significant metabolic stability problem. All these results indicated that the anilino aromatic ring was essential for their potential anti-HIV activity, while the 2-sustituted methyl of C-ring increased the metabolic stability of BPs.

By replacing the phenyl ring (C-ring) with a pyridine ring, Romines et al. [23] synthesized a family of pyridine-substituted BP derivatives (**20a-g**) and evaluated their anti-HIV activity (Table 12). Apparently, the sulfonamide substitution at the pyridine ring was quite well tolerated (**20c-g**, IC_{50}<10 nM). Placement of the pyridine nitrogen at the X^3 position (**20g**) attenuated potency against both wild-type (IC_{50}=18 nM) and K103N mutant viruses (IC_{50}=93 nM), while installing the pyridine



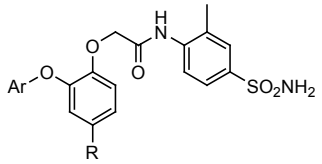
Compound	Ar	Inhibition of RT polymerase, IC_{50} [nM][a]			Antiviral activity in cell culture, IC_{50} [nM][b]			
		WT	K103N	Y181C	WT [10% FBS]	K103N [10% FBS]	Y181C [10% FBS]	WT [50% FBS]
16a	Ph	51.7	192.4	711	--[c]	--	--	--
16b	3-Cl-Ph	10.50	218	25	--	--	--	--
16c	3-CN-Ph	0.96	14.4	1.2	8	--	--	--
16d	3,5-Cl,CN-Ph	2.11	3.48	2.66	7.56	--	--	125

Table 8: Diaryl ether derivatives **16a-d** and their anti-HIV activity

[a]Compounds were evaluated in a standard SPA assay. Values are the geometric mean of at least two determinations;

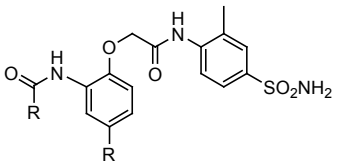
[b]CIC_{95} (Cell culture inhibitory concentration) is defined as the concentration at which the spread of virus is inhibited by >95% in MT-4 human T-lymphoid cells maintained in RPMI 1640 medium containing either 10% FBS or 50% NHS. Values are the geometric mean of at least two determinations. No cytotoxicity was observed for any of the compounds up to the upper limit of the assay (8.3 μM);

[c]Undetectable.



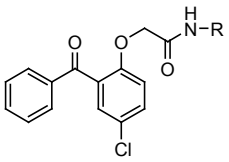
Compd	Ar	R	EC_{50} [nM][a]			CC_{50} [μM][c]	SI[d]
			III_B [nM]	A_{17} [μM]b	HIV-2 ROD[μM]		
17a	2-Naphthyl	Cl	79.10	1.79	27.26	118.98	1504.17
17b	6-Br-2-Naphthyl	Cl	244.30	2.49	20.03	31.33	128.24
17c	1-Cl-2-Naphthyl	Cl	169.04	1.73	13.27	40.72	240.89
17d	6-Br-2-Naphthyl	F	181.28	1.77	96.01	>357.53	>1972.25
17e	1- Cl-3-Me-2-Naphthyl	Cl	73.12	1.07	3.00	42.61	582.74
17f	1- Br-6-CN-2-Naphthyl	F	4.60	0.82	4.40	35.28	7669.56
17g	6-CN-2-Naphthyl	Cl	116.50	2.33	>383.16	>383.16	>3288.92
17h	6-CN-2-Naphthyl	F	38.77	1.02	165.97	>395.63	>10201.53
17i	3-Me-2-Naphthyl	Cl	58.44	0.76	20.74	76.71	1312.63

Table 9: Naphthyl phenyl ether analogues **17a-l** and their anti-HIV activity
[a]EC_{50}: effective concentration of compound required to protect the cell against viral cytopathogenicity by 50%in C8166 cells;
[b]A_{17}: HIV-1 mutated strain bearing both K103N andY181C mutations;
[c]CC_{50}: cytotoxic concentration of compound that reduces the normal uninfected C8166 cell viability by 50%;
[d]SI: selectivity index: ratio CC_{50}/EC_{50} (HIV-1 III_B).



Compound	R	EC_{50} [nM][a]			CC_{50} [μM][c]	SI[d]
		III_B [nM]	A_{17} [μM][b]	HIV-2 ROD [μM]		
18a	Ph	3.67	1.59	72.10	207.37	56548
18b	3-Me-Ph	1.46	0.46	29.60	52.90	36155
18c	2-NO_2-Ph	0.30	0.29	11.85	54.78	184578
18d	3-NO_2-Ph	0.66	0.46	36.10	58.84	89425
18e	2,4-Me_2-Ph	0.56	0.09	41.36	47.60	85339
18f	2,5-Me_2-Ph	0.37	0.14	27.68	79.70	212819
18g	3,4-Me_2-Ph	0.32	0.10	4.37	84.11	260617
18h	3,5-Cl_2-Ph	0.27	0.27	18.01	72.39	271000
18i	3,5-Cl,CN-Ph	0.96	0.29	23.16	131.08	136287

Table 10: *N*-Phenylaryl formamide derivatives **18a-i** and their anti-HIV activity
[a]EC_{50}: effective concentration of compound required to protect the cell against viral cytopathogenicity by 50% in C8166 cells;
[b]A_{17}: HIV-1 mutated strain bearing both K103N and Y181C mutations;
[c]CC_{50}: cytotoxic concentration of compound that reduces the normal uninfected C8166 cell viability by 50%;
[d]SI: selectivity index: ratio CC_{50}/EC_{50} (HIV-1 III_B).



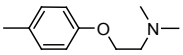
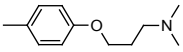
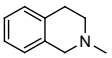
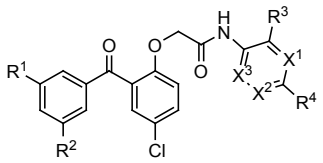
Compound	R	RT[a]	MTM[b]	TOX[c]	SYN[d]	p24[e]	half-life [h]	
							mouse serum[f]	mouse S9 live[g]
19a		14.5	--[h]	--	--	--	--	--
19b		0.01	0.0004	>0.005	0.0002	0.0012	<0.25	<0.25
19c		2.5	0.028	10	0.05	0.032	2.8	>6
19d		0.45	>10	10	--	--	--	--
19e		0.097	<0.01	>0.5	0.00009	<0.0005	<0.25	>2
19f		0.006	0.014	>0.5	0.011	0.016	1.2	2.8

Table 11: SARs of C-ring.
[a]IC_{50} *versus* RT of HIV-1 (μg/mL), AZT triphosphate control=0.02 ± 0.005 μM;
[b]IC_{50} *versus* HIV-1 (strain RF)-infected MT-4 cells (μg/mL), AZT=0.002–0.02 μM;
[c]IC_{50} *versus* uninfected MT-4 cells (mg/mL);
[d]IC_{50} *versus* syncytia formation in infected (C8166-cells μg/mL), AZT=0.002–0.02 μM;
[e]IC_{50} *versus* p24 antigen formation in infected C8166-cells μg/mL L), AZT=0.001–0.01 μM;
[f]Stability of compound in mouse serum preparation;
[g]Stability of compounds in mouse S9 liver preparation;
[h]Undetectable.



Compound	R^1	R^2	R^3	R^4	X^1	X^2	X^3	potency [IC_{50}, nM][a]			
								WT	K103N	Y181C	V106A
20a	F	F	H	Cl	CH	N	CH	--[b]	67	--	--
20b	F	F	H	OMe	CH	N	CH	--	72	--	--
20c	Me	CN	Me	SO_2NH_2	N	CH	CH	2	6	4	3
20d	Cl	CN	Me	SO_2NH_2	N	CH	CH	1	3	3	7
20e	Me	CN	Me	SO_2NH_2	CH	N	CH	1	7	4	2
20f	Cl	CN	Me	SO_2NH_2	CH	N	CH	2	5	2	10
20g	Me	CN	Me	SO_2NH_2	CH	CH	N	18	93	--	--

Table 12: SARs of various substituents on the C-ring.
[a]IC_{50} values were determined using a HeLa MAGI assay. Wild-type virus (WT) was the HxB2 strain. K103N, Y181C, and V106A mutants were isogenic with the HxB2 virus backbone;
[b]Undetectable.

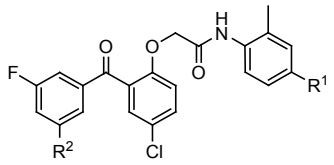
nitrogen at either the X^1 (**20c**,**d**) or X^2 (**20e**,**f**) positions gave analogues with excellent potency against both wild-type and mutant viruses with IC_{50} values ranging from 1 to 10 nM.

In 2006, a family of potent HIV inhibitors **21**-**22** featuring a *para*-substituted alkynyl or carboxyl group on the C-ring were reported [31,32]. All these compounds were able to inhibit the wild-type and the single or the double mutant HIV strains within nanomolar concentrations. Aquino et al. [33] further explored the SARs of the *para* substituent on the C-ring, and obtained three chiral benzophenone analogues **23**-**25**. All the three chiral molecules exhibited less than 10 nM potency against the wild-type and a panel of mutant HIV strains. Excitingly, their concentrations for 50% of maximal effect on the key mutant strains, such as K103, Y181 and Y188, were even lower than 1 nM (Figure 3).

As shown in table 13, various *para*-substituents on the C-ring were employed to further investigate the SARs of BPs [23]. Conversion of the ether linkage to secondary amines or amides (**26b**-**e**) was little benefit to these changes relative to the unsubstituted analogue **26a**. Yet, compound **26e** is quite potent against both wild-type (IC_{50}=5 nM) and K103N mutant viruses (IC_{50}=9 nM). Changing the terminal amine with other groups proved to be more consistently beneficial. The sulfonamide (**26f**), urea (**26g**), and ether (**26h**) substituents were all favorable in the antiviral assays. The best results were achieved when the *para* position was substituted with a sulfonamide (**26i**). This analogue had excellent potency against both wild-type virus and the two key mutant viruses associated with NNRTI resistance (K103N and Y181C) with an EC_{50} value of lower than 3 nM concentrations. Analogues with the sulfonamide substitution (**26j**,**k**) were also active in the antiviral assays, but were not quite as potent as **26i**. In general, substitution at the *para* position was beneficial. These explorations also indicated that most of the compounds did not show notable difference in potency ($p<0.05$), even in cases with fairly significant changes (e.g., **26b** vs **26i**).

SARs of the Linker between B- and C-Ring

In the case of the linker, a very few modifications were performed. In the original paper in 1995, Wyatt et al. [21] reported that reducing the amide linker chain length significantly lost the anti-HIV activity (Table 14). Therefore, more work on modifying this linker is necessary to further explore its SARs.



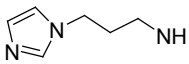
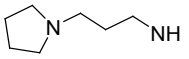
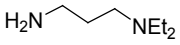
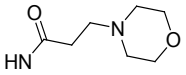
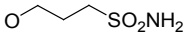
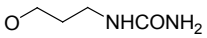
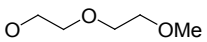
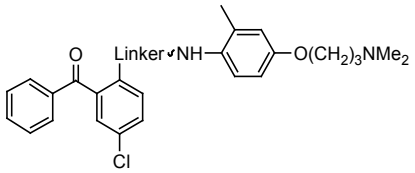
Compound	R^1	R^2	potency [IC_{50}, nM]			
			WT[a]	WT[b]	K103N[b]	Y181C[b]
26a	H	F	25	8	38	25
26b		F	37	--	158	--
26c		F	18	--	109	--
26d		F	41	--	96	--
26e		F	5	--	9	--
26f		CF_3	7	3	4	6
26g		CF_3	6	4	4	10
26h		CF_3	6	6	16	12
26i	SO_2NH_2	CF_3	1	2	2	3
26j	SO_2NHMe	CF_3	8	3	2	--
26k	SO_2NMe_2	CF_3	10	8	6	10

Table 13: SARs of various substituents at the *C*-4 position of C-ring

[a]IC_{50} values were determined using HIV-1 III_B wild-type virus in an acute infection assay in MT4 cells; [b]IC_{50} values were determined using a HeLa MAGI assay system. The wild-type virus was the HxB2 strain, and the K103N and Y181C mutants were isogenic with the HxB2 virus backbone;
[c]Undetectable.



Compound	R[1]	RT[a]	MTM[b]	TOX[c]	SYN[d]	p24[f]
27a	-CH_2CO-	0.35	0.0044	>0.5	0.016	0.0029
27b	-CO-	>l00	--[g]	--	--	--

Table 14: SARs of the linker between B- and C-ring.
[a]IC_{50} *versus* RT of HIV-1 (μg/mL), AZT triphosphate control=0.02 ± 0.005 μM;
[b]IC_{50} *versus* HIV-1 (strain RF)-infected MT-4 cells (μg/mL), AZT=0.002–0.02 μM;
[c]IC_{50} *versus* uninfected MT-4 cells (mg/mL);
[d]IC_{50} *versus* syncytia formation in infected (C8166-cells μg/mL), AZT=0.002–0.02 μM;
[e]IC_{50} *versus* p24 antigen formation in infected (C8166-cells μg/mL), AZT=0.001–0.01 μM;
[g]Undetectable.

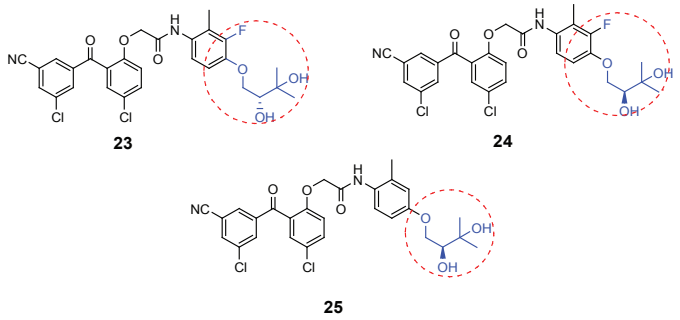


Figure 3: Structures of potent benzophenones with various substituents at *C*-4 position of C-ring.

Summary

The use of NNRTIs as an important part of successful combination therapy in HIV/AIDS patients have been well established and widely accepted. In recent years, vast progress has been achieved in the development of NNRTI, especially to improve their antiviral potency. Furthermore, the NNRTI clinical pipeline also seems not to be that impressive as hoped. Nevertheless, the major cause of treatment failure, fast emergence of clinical resistance is still the serious issue for NNRTIs. During the past decade, benzophenone derivatives, have been transformed from a lead with moderate activity against wild-type HIV and little or no activity against clinically relevant mutants to very promising series with excellent in vitro potency against wild-type and key mutant viruses with the unremitting efforts. To provide valuable SAR information for producing more effective benzophenone HIV inhibitors, the SARs of BPs were provided in this manuscript, which were overviewed as below: (1) the carbonyl between the A- and B-ring was necessary for maintaining the pharmacodynamics conformation of this template. (2) *Meta* substitution on the A-ring would require a polar aprotic and sterically small substituent, such as cyano, methyl, which would reach into the hydrophobic region adjacent to the side chains of Y181 and Y188. (3) The methyl substituent *ortho* to the amide on the C-ring gave the molecule a measure of metabolic stability, and the *para* substitution counter the lipophilic nature was much tolerated. In spite of this valuable SAR information, there are still some unclear SARs, for instance, the impact on the activity by changing the linker between B- and C-ring, the impact of the replacement of both A- and B-rings with a bulky aromatic ring, and so on. Therefore, it is essential to extensively optimize this promising template to generate more active anti-HIV agents.

Acknowledgements

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