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Original article

Synthesis, structure–activity relationships, and docking studies of *N*-phenylarylformamide derivatives (PAFAs) as non-nucleoside HIV reverse transcriptase inhibitors

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ABSTRACT

A series of *N*-phenylarylformamide derivatives (PAFAs) with anti-wild-type HIV-1 activity (EC_{50} values) ranging from 0.3 nM to 5.1 nM and therapeutic index (TI) ranging from 10 616 to 271 000 were identified as novel non-nucleoside reverse transcriptase inhibitors. Among them, compound **13g** (EC_{50} = 0.30 nM, TI = 184 578), **13l** (EC_{50} = 0.37 nM, TI = 212 819), **13m** (EC_{50} = 0.32 nM, TI = 260 617) and **13r** (EC_{50} = 0.27 nM, TI = 271 000) displayed the highest activity against this type virus nearly as potent as lead compound GW678248. Moreover, all of them were also active 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. In particular, compound **13m**, which showed broad-spectrum anti-HIV activity, was also effective to inhibit the HIV-2 ROD replication within 4.37 μM concentration.

1. Introduction

Since the identification of the human immunodeficiency virus (HIV) as the causative agent of AIDS [1–4], the search for safe and effective agents for HIV treatments has become a major focus for drug discovery groups worldwide. Although the highly active antiretroviral therapy (HAART) combination regimens such as nevirapine (NVP, **1**, Fig. 1) [5], delavirdine (DLV, **2**, Fig. 1) [6], efavirenz (EFV, **3**, Fig. 1) [7], etravirine (ETV, **4**, Fig. 1) [8] and rilpivirine (RPV, **5**, Fig. 1) [9,10], which have been approved by US FDA as novel HIV-1 NNRTIs, are proving to be effective for AIDS therapy. Unfortunately, the genetic barrier of current NNRTIs is relatively low, and single mutations begin to reduce the susceptibility of virus to drug. Furthermore, cross resistance between the approved NNRTIs is quite common, and patients are generally forced to abandon the approved NNRTIs altogether once they have developed resistance to one of its members [11,12]. Therefore, an urgent need has arisen for more potent NNRTIs that possess both a broad spectrum of antiviral activity against key mutant strains and a high genetic barrier to the selection of new mutant strains.

Benzophenone derivatives (BPs, Fig. 2), originated in a high-throughput screening in 1995 [13], are typical NNRTIs and very efficacious against both wild-type and clinically relevant NNRTI-resistant mutant HIV-1 strains [13–19]. For searching more active BPs as NNRTIs, considerable efforts on the modifications of BPs have been made and led to identify several highly potent inhibitors, such as GW564511 (**7**, Fig. 2) [13] and GW678248 (**8**, Fig. 2) [15,16]. The initial SARs of BPs showed that the keto group between A- and B-rings was important for maintaining their high anti-HIV activity [19,20]. Therefore, few modifications on the keto template were performed in the following structure optimizations. A flexible amido linker between A- and B-rings might not only improve the

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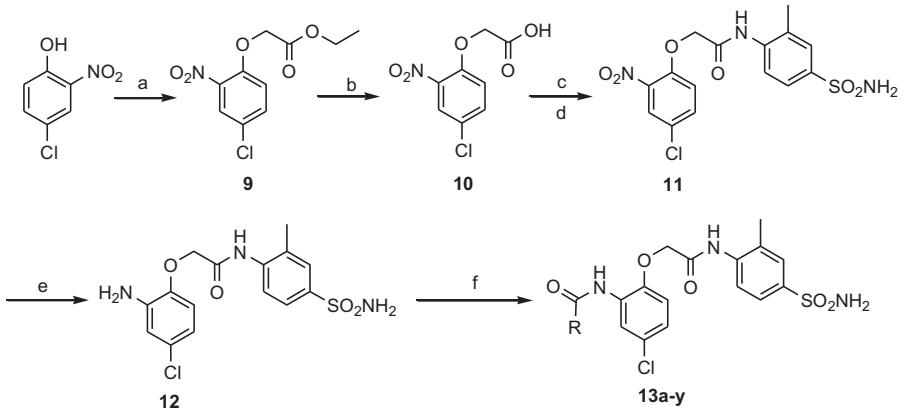

Scheme 1. Synthesis of *N*-phenylarylformamide analogues **13a**–**y**. Reagents and conditions: (a) $BrCH_2COOEt$, K_2CO_3, *n*-Bu_4NBr, acetone, 2 h, 50 °C, 91%; (b) $LiOH{\cdot}H_2O$, THF–H_2O–EtOH, r.t, 2 h, 89%; (c) $SOCl_2$, 80 °C, 1 h; (d) 4-amino-3-methylbenzenesulfonamide, $NaHCO_3$, acetone, 80 °C, 2 h, 85%; (e) Fe, NH_4Cl, acetone-H_2O, 80 °C, 12 h, 82%; (f) RCOCl, $NaHCO_3$, acetone, r.t, 30 min, 70–89%.

adaptation of the inhibitor to RT, but also promote the H-bond bindings with key mutant amino acids Tyr188 or Tyr181. Herein, a series of *N*-phenylarylformamide derivatives (PAFAs) were synthesized and evaluated for their anti-HIV activity. Moreover, their preliminary structure–activity relationships (SARs) and the possible binding modes with RT were also explored in this manuscript.

2. Results and discussion

2.1. Chemistry

As depicted in Scheme 1, alkylation of the staring material 4-chloro-2-nitrophenol with ethyl bromoacetate [21], and subsequent hydrolysis gave the acid derivative **10** [22]. Treatment acid chloride of **10** with 4-amino-3-methylbenzenesulfonamide in basic condition provided the compound **11** [8]. After reducing the nitro-compound **11** in the presence of Fe–NH_4Cl, amino compound **12** was obtained in 82% yield. Finally, coupling **12** with the appropriately substituted of benzoyl chloride was accomplished by mixing with $NaHCO_3$ in the solvent of acetone at room temperature to provide the corresponding target compounds **13a**–**y** in 70–89% yield.

2.2. Biological activity

All title molecules were evaluated for the activity against wild-type HIV-1 strain III_B, the double mutant HIV-1 strain A_{17} (K103N + Y181C) and HIV-2 strain ROD in C8166 cells [23,24]. For comparation, lead compound GW678248 and the FDA-approved drug, zidovudine (AZT) were also tested as reference compounds, and the activity data is interpreted in CC_{50} (cytotoxicity), EC_{50} (anti-HIV activity) and TI (therapeutic index, given by the CC_{50}/EC_{50} ratio).

As illustrated in Tables 1and 2, more than half of the newly synthesized compounds displayed strong potency against the wild-type HIV-1 strain III_B with EC_{50} values ranging from 0.30 nM to 5.10 nM, and therapeutic index values from 10 616 to 271 000. In particular, analogues **13g** (EC_{50} = 0.30 nM, TI = 184 578), **13l** (EC_{50} = 0.37 nM, TI = 212 819), **13m** (EC_{50} = 0.32 nM, TI = 260 617) and **13r** (EC_{50} = 0.27 nM, TI = 271 000) were identified as the maximum inhibitors nearly as potent as GW678248 against this type of virus, indicating that the flexibility of amide linkage might improve the adaptation of inhibitor to RT.

To explore the potential SARs, great efforts on the modifications of the A-ring, which placed in the top hydrophobic pocket lined by the important aromatic residues Tyr181, Tyr188 and Trp229 was carried out [20]. It was found that relatively small changes in structure did prove to have a significant impact on the anti-wild-type potency. In general, placement of a small group at the *meta* position (**13c**) was more favourable than at the *ortho* (analogue **13b**) or at the *para* (analogue **13d**) position, but for the nitro group, the *ortho*-substituted analogue **13g** was 2-fold higher than the *meta*-substituted analogue **13h**. In the case of *meta*-substituted analogues **13b**–**j**, the order of the potency against wild-type virus was NO_2 > Me > Cl > F > CF_3, but the trifluoro-substituted analogue **13i** almost lost 1000-fold activity in particular.

Additionally, several analogues with di-*meta* substitutions at different positions (analogue **13k**–**u**) were also synthesized and

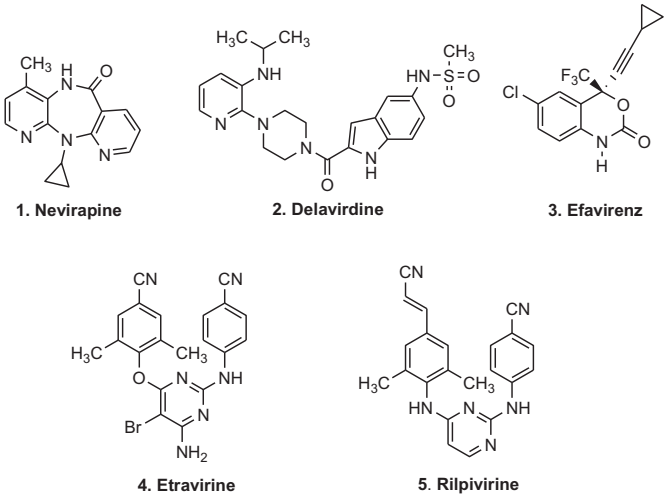

Fig. 1. Structures of currently marketed NNRTIs.

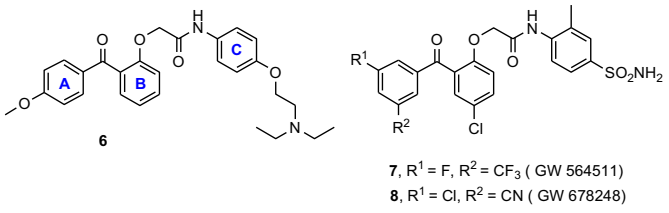

Fig. 2. Structures of potent benzophenones.

Table 1
Biological activity of compounds **13a**–**y** against wild-type HIV-1 and HIV-2 ROD in C8166 cells.[a]

Compd	R	EC_{50}[b]		CC_{50} (μM)[c]	TI[d]
		HIV-1 III_B (nM)	HIV-2 (μM)		
13a	Ph	3.67	72.10	207.37 ± 16.98	56 548
13b	2-Me–Ph	17.93	55.51	67.27 ± 9.40	3751
13c	3-Me–Ph	1.46	29.60	52.90 ± 6.48	36 155
13d	4-Me–Ph	5.10	57.33	54.17 ± 7.72	10 616
13e	3-Cl–Ph	3.19	52.47	59.66 ± 12.85	18 722
13f	3-F–Ph	3.53	185.87	242.98 ± 25.37	68 811
13g	2-NO_2–Ph	0.30	11.85	54.78 ± 8.87	184 578
13h	3-NO_2–Ph	0.66	36.10	58.84 ± 10.86	89 425
13i	3-CF_3–Ph	1505.73 ± 1002.08	81.31	123.63 ± 9.92	82
13j	4-MeO–Ph	204.38 ± 8.42	58.76	69.53 ± 7.83	340
13k	2,4-DiMe–Ph	0.56	41.36	47.60 ± 3.93	85 339
13l	2,5-DiMe–Ph	0.37	27.68	79.70 ± 2.48	212 819
13m	3,4-DiMe–Ph	0.32	4.37	84.11 ± 8.48	260 617
13n	3,5-DiMe–Ph	1.16	25.87	36.45 ± 4.04	31 327
13o	2,4-DiCl–Ph	2.97	14.42	61.14 ± 12.40	20 615
13p	2,5-DiCl–Ph	2.34	21.57	42.12 ± 4.55	18 004
13q	3,4-DiCl–Ph	51.40	55.52	157.50 ± 21.95	3064
13r	3,5-DiCl–Ph	0.27	18.01	72.39 ± 1.11	271 000
13s	3,5-Cl,Br–Ph	3088.87 ± 1820.53	61.49	113.92 ± 2.55	37
13t	3,5-DiBr–Ph	45.89	56.49	89.66 ± 13.36	1954
13u	3,5-Cl,CN–Ph	0.96	23.16	131.08 ± 10.96	136 287
13v	PhCH=CH	95395.72 ± 3663.03	77.45	163.39 ± 6.42	2
13w	2,6-DiCl–$PhCH_2$	248504.98 ± 97472.42	160.17	>359.16	>2
13x	1-Naphthyl	109.16 ± 51.82	97.68	>381.69	>3497
13y	2-Naphthyl	100.77 ± 21.05	31.59	37.60 ± 10.82	373
GW678248		0.103 ± 0.052	2.1	>386.8	>37 773 585
AZT		12.80 ± 3.12	39.0	5401.4 ± 345.5	422 067

[a] Data represent the mean of at least two separate experiments.
[b] Compound concentration required to protect C8166 cells against viral cytopathogenicity by 50%.
[c] Compound concentration that decreases the uninfected C8166 cell viability by 50%.
[d] Therapeutic index: CC_{50}/EC_{50} ratio (wild–type).

evaluated for anti-HIV activity to further explore the SARs of A-ring. In fact, most of the di-substituted analogues showed strong anti-HIV-1 activity with EC_{50} values less than 3.00 nM except for 3,4-dichlo analogue (**13q**, EC_{50} = 51.40 nM), 3,5-Cl,Br analogue (**13s**, EC_{50} = 3088.87 nM) and 3,5-dibromo analogue (**13t**, EC_{50} = 45.89 nM), and notably, 3,5-dichloro analogue **13r** was

Table 2
Biological activity of compounds **13a**–**y** against mutant HIV-1 virus A_{17} (K103N + Y181C) in C8166 cells.[a]

Compd	R	EC_{50}(μM)[b]	CC_{50}(μM)[c]	TI[d]
13a	Ph	1.59 ± 0.13	207.37 ± 16.98	130.17
13b	2-Me–Ph	2.29 ± 0.40	67.27 ± 9.40	29.37
13c	3-Me–Ph	0.46 ± 0.02	52.90 ± 6.48	114.73
13d	4-Me–Ph	0.93 ± 0.08	54.17 ± 7.72	58.16
13e	3-Cl–Ph	1.49 ± 0.70	59.66 ± 12.85	39.93
13f	3-F–Ph	1.02 ± 0.31	242.98 ± 25.37	239.29
13g	2-NO_2–Ph	0.29 ± 0.21	54.78 ± 8.87	188.25
13h	3-NO_2_Ph	0.46 ± 0.32	58.84 ± 10.86	128.07
13i	3-CF_3–Ph	52.94	123.63 ± 9.92	2.34
13j	4-MeO–Ph	18.07	69.53 ± 7.83	3.85
13k	2,4-DiMe–Ph	0.09 ± 0.03	47.60 ± 3.93	543.07
13l	2,5-DiMe–Ph	0.14 ± 0.01	79.70 ± 2.48	559.58
13m	3,4-DiMe–Ph	0.10 ± 0.05	84.11 ± 8.48	836.04
13n	3,5-DiMe–Ph	0.33 ± 0.04	36.45 ± 4.04	109.55
13o	2,4-DiCl–Ph	0.33 ± 0.09	61.14 ± 12.40	186.99
13p	2,5-DiCl–Ph	0.42 ± 0.13	42.12 ± 4.55	100.29
13q	3,4-DiCl–Ph	1.14 ± 0.37	157.50 ± 21.95	137.67
13r	3,5-DiCl–Ph	0.27 ± 0.02	72.39 ± 1.11	268.23
13s	3,5-Cl,Br–Ph	27.75	113.92 ± 2.55	4.11
13t	3,5-DiBr–Ph	1.39 ± 0.38	89.66 ± 13.36	64.66
13u	3,5-Cl,CN–Ph	0.29 ± 0.03	131.08 ± 10.96	453.99
13v	PhCH=CH	153.62	163.39 ± 6.42	1.06
13w	2,6-DiCl–$PhCH_2$	240.55	>359.16	>1.49
13x	1-Naphthyl	21.54	>381.69	>17.72
13y	2-Naphthyl	8.91	37.60 ± 10.82	4.22
GW678248		0.00028 ± 0.000038	>386.8	>1 369 863
AZT		0.0228	4913.8	215 627

[a] Data represent the mean of at least two separate experiments.
[b] Compound concentration required to protect C8166 cells against viral cytopathogenicity by 50%.
[c] Compound concentration that decreases the uninfected C8166 cell viability by 50%.
[d] Therapeutic index: CC_{50}/EC_{50} ratio.

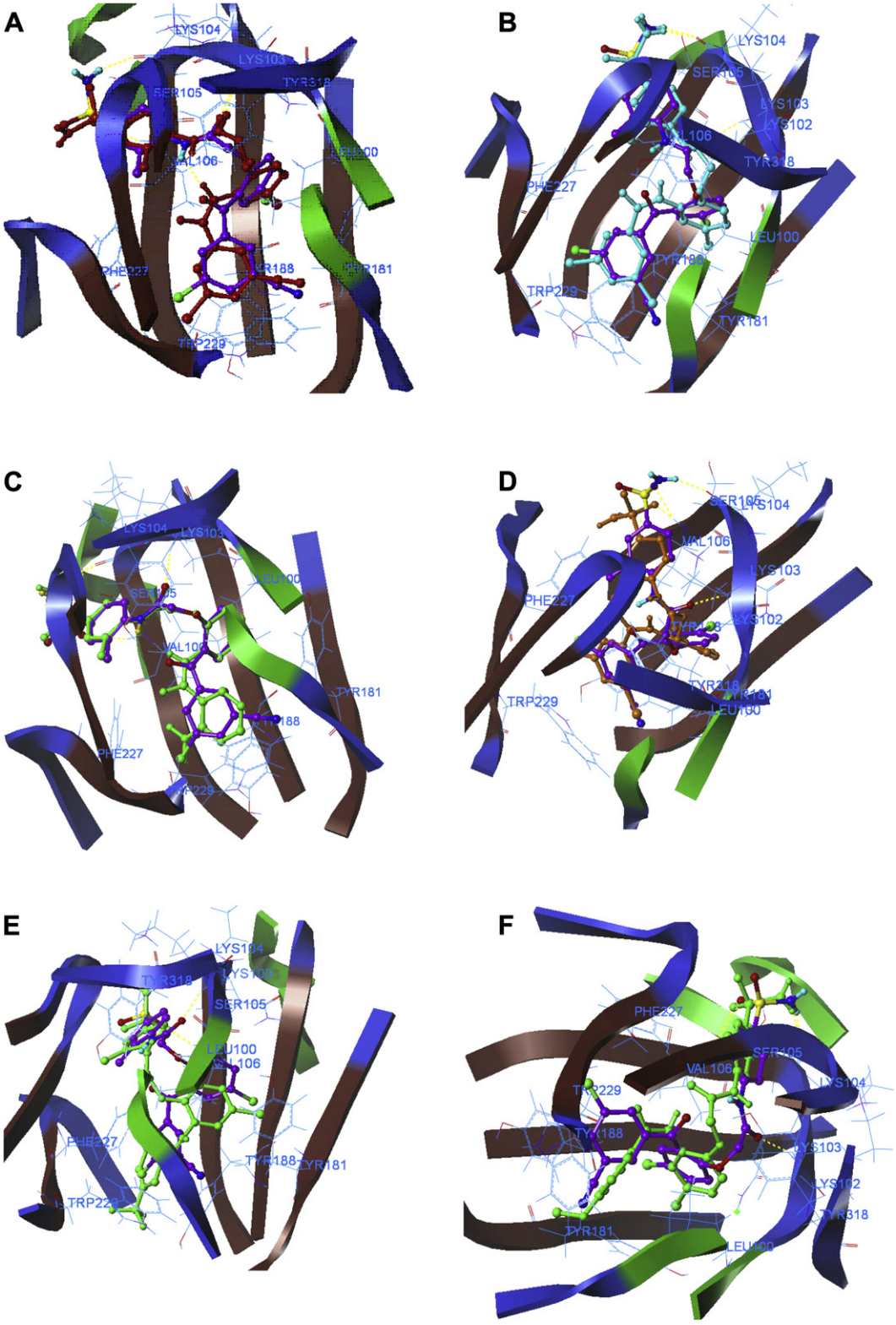

Fig. 3. A: Superimposition of the docked conformations of GW678248 (purple) and **13u** (red). B: Superimposition of the docked conformations of GW678248 (purple) and **13r** (cyan). C: Superimposition of the docked conformations of GW678248 (purple) and **13g** (green). D: Superimposition of the docked conformations of GW678248 (purple) and **13s** (red). E: Superimposition of the docked conformations of GW678248 (purple) and **13i** (green). F: Superimposition of the docked conformations of GW678248 (purple) and **13j** (green). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Selected compounds assayed against HIV-1 RT (wild-type).

Compd	IC_{50} (nM)[a]
13m	1.14
13u	2.05
GW678248	2.69

[a] Compound concentration required to inhibit HIV-1 RT_{wt} activity by 50%.

identified as the most active inhibitor with an EC_{50} value of 0.27 nM. As seen from the result, it was found that introducing another group on the A-ring of the *mono*-substituted analogues strengthen the interaction with RT.

In addition, four peculiar structure molecules **13v**–**y** were prepared with the aim to enhance the π–π interactions with amino acid resides Tyr188 and Tyr181 [25], Unfortunately, the test result indicated that compound **13v** and **13w** completely lost the ability to inhibit HIV-1 within more than 25 μM concentration. While interestingly, both 1-naphthyl analogues **13x** and 2-naphthyl analogues **13y** displayed moderate inhibitory activity with EC_{50} values of 109.16 nM and 100.77 nM, respectively.

Furthermore, all of the compounds were also evaluated for activity against the double mutant HIV-1 strain A_{17} (K103N + Y181C) in parallel with the HIV-2 strain ROD. In these assays, most of the derivatives exhibited moderate potency against the drug-resistant viral strain within less than 1 μM concentration. Notably, analogues **13g**, **13l**, **13m** and **13r**, the most active inhibitors against wild-type HIV-1 virus, also showed the strongest potency to inhibit the mutant type virus A_{17} (K103N + Y181C) with EC_{50} values of 0.29 μM, 0.14 μM, 0.10 μM and 0.27 μM, respectively. Nevertheless, almost all of them lost the ability to inhibit the HIV-2 strain ROD ($EC_{50} > 4.37$ μM) except for **13m** ($EC_{50} = 4.37$ μM), indicating that *N*-phenylarylformamide derivatives might be HIV-1 non-nucleoside reverse transcriptase inhibitors only.

To directly prove the target of *N*-phenylarylformamide derivatives was HIV-1 RT, compound **13m** and **13u**, the most active inhibitors tested in C8166 cell cultures, were also chosen to evaluate the potency against HIV-1 RT_{wt} at molecular level in parallel with the reference compound GW678248. A poly (rA)/oligo $(dT)_{15}$ homopolymer template with the HIV antigen detection ELISA was used for quantifying expression of HIV-1 RT_{wt} in culture medium [26]. As shown in Table 3, apparently, both **13m** and **13u** exhibited strong capacity to inhibit the HIV-1 RT_{wt} with IC_{50} values of 1.14 nM and 2.05 nM, respectively.

2.3. Molecular simulation

To investigate the binding modes of these derivatives with HIV-1 RT (wild-type), several representative analogues **13g**, **13i**, **13j**, **13r**, **13s** and **13u** (**13g**, **13r** and **13u** were the most active inhibitors, while **13i**, **13j** and **13s** showed the least potency) were docked into the non-nucleoside binding site (NNBS) of HIV-1 RT_{wt} (PDB code: 3DLG) [20] according to the basis of the protocols, using Base-builder program and Surflex-Dock program interfaced with SYBYL-1.2 [27,28]. The default SYBYL-X 1.2 parameters were used. For comparation, the lead compound GW678248 was also docked to the NNBS and superimposed onto the reference compound according to the same the protocols [29–31].

As seen in Fig. 3, a panel of tightening interactions were formed by GW678248 with RT, such as: a) the van der Waals interactions between the cyano group at *meta*-position of the A-ring and the amino acid residue Trp229; b) the H-bond interactions formed by the NH group that linking the B- and C-rings with Lys103, the NH of the sulfonamide group with Lys104 and the oxygen atom of the sulfonamide group with Val106; c) the π–π interactions formed by the A-ring with Tyr188, and B-ring with Tyr181. Apparently, all of the docked conformations of **13g**, **13r** and **13u** were superimposed well onto that of GW678248, indicating that their interactions with RT were comparable to GW678248. However, some of the important H-bonds with the key mutant amino acid residues of RT were disappeared (**13g** lost the H-bond with Val106, **13r** lost the H-bond with Lys103, and **13u** lost the H-bond with Lys104), which might lead to the falling activity against mutant HIV virus A_{17} (K103N + Y181C). While for the analogue **13i**, **13j** and **13s**, which showed less potent against HIV, were superimposed badly onto GW678248 for their docked conformations. Moreover, all of them lost the H-bond with Lys103, which was a key mutant amino acid for producing drug resistance. In summary, all these analyses were in accordance with the test result.

3. Conclusion

In summary, we report a novel series of *N*-phenylarylformamide derivatives (PAFAs) endowed with high anti-HIV activity as potent NNRTIs. Among these strong inhibitors, more than half showed relative low concentration activity against wild-type HIV-1 virus within a range of 0.3 nM–5.1 nM. In particular, compound **13g** (EC_{50} = 0.30 nM, TI = 184 578), **13l** (EC_{50} = 0.37 nM, TI = 212819), **13m** (EC_{50} = 0.32 nM, TI = 260617) and **13r** (EC_{50} = 0.27 nM, TI = 271000) displayed the most activity against this type virus nearly as potent as GW678248. Furthermore, all of them were also effective to inhibit the double mutant HIV-1 strain A_{17} (K103N + Y181C) with EC_{50} values of 0.29 μM, 0.14 μM, 0.10 μM and 0.27 μM, respectively. In addition, both the preliminary SARs and the binding modes with RT were also discussed in this manuscript. Overall, the designed PAFAs exhibited promising activity against the anti-wild-type HIV-1 virus, although were not effective to inhibit the A_{17} (K103N + Y181C) virus.

4. Experimental section

4.1. Chemistry

All chemicals used were purchased from commercial sources, were of analytical grade and were used without further purification. Melting points were measured on a SGW X-1 microscopic melting-point apparatus and were uncorrected. Mass spectra were obtained on a Waters Quattro Micromass instrument using electrospray ionization (ESI) techniques. ^{1}H NMR and ^{13}C NMR spectra on a Brucker AV 400 MHz spectrometer were recorded in [D]DMSO. Chemical shifts are reported in δ (ppm) units relative to the internal standard tetramethylsilane (TMS). Elemental analyses were performed on a Carlo Erba 1106 instrument and the results of elemental analyses for C, H and N were within ±0.4% of the theoretical values. TLC analyses were run on silica gel 60 F254 plates (Merck) using a variety of solvent systems and a fluorescent indicator for visualization. Spots were visualized under 254 nm UV illumination. Flash chromatography separations were obtained on Silica Gel (300–400 mesh) using EtOAc/hexane as eluents.

4.2. General procedure for the synthesis of *N*-[4-(aminosulfonyl)-2-methylphenyl]-2-(4-chloro-2-nitrophenoxy)acetamide (*11*)

Thionyl chloride (21.8 mL, 0.30 mol) was added dropwise to **10** (23.19 g, 0.10 mol), the mixture was stirred at 80 °C for 2 h, and then concentrated in vacuo. The crude product was used immediately without further purification or characterization.

A solution of the crude product (0.10 mol) in acetone (50 mL) was added dropwise to the mixture of 4-amino-3-methylbenzenes

ulfonamide (18.62 g, 0.10 mol), acetone (100 mL), sodium bicarbonate (25.20 g, 0.30 mol) and water (1 mL) over 20 min, and the resulting mixture was stirred at room temperature for 2 h. The reaction mixture was then poured into water (150 mL) and extracted with CH_2Cl_2 (2 × 100 mL). The aqueous layer was then filtered to give crude product as a beige solid. The crude material was suspended in 1 N HCl and then filtered and washed with CH_2Cl_2, MeOH, and hexane to give **11** as an off-white solid (33.92 g, 85%). mp 253.7–254.8 °C; ^{1}H NMR ([D]DMSO) δ_{ppm} = 2.31 (s, 3H, C*H*$_3$), 5.04 (s, 2H, C*H*$_2$), 7.41 (d, *J* = 8.8 Hz, 1H, Ar*H*), 7.64 (d, *J* = 8.4 Hz, 1H, Ar*H*), 7.69 (s, 1H, Ar*H*), 7.81 (dd, *J* = 8.4 Hz, *J* = 8.8 Hz, 2H, Ar*H*), 8.09 (s, 1H, Ar*H*); ^{13}C NMR ([D]DMSO) δ = 17.7, 68.0, 117.3, 123.5, 123.8, 124.7, 124.8, 124.9, 127.7, 131.0, 134.1, 138.4, 140.3, 149.6, 165.7, MS (ESI$^+$) *m*/*z*: 400 [M + H]$^+$. Calcd for $C_{15}H_{14}ClN_3O_6S$ 399.

4.3. General procedure for the synthesis of N-[4-(aminosulfonyl)-2-methylphenyl]-2-(4-chloro-2-aminophenoxy)acetamide (12)

A mixture of compound **11** (39.98 g, 0.10 mol) and ammonium chloride (13.37 g, 0.25 mol) in acetone–water (330 mL, acetone: H_2O = 2: 1) solution was heated to 80 °C, then iron powder (22.30 g, 0.40 mol) was added slowly, and the resulting mixture was stirred at this temperature for 12 h. After completion, filtrated, the blank reside was washed with DMSO (3 × 50 mL), and the combined filtration was poured into water (1000 mL), filtered and washed with H_2O (3 × 200 mL) and CH_2Cl_2 (100 mL), then dried to give crude product as a white solid **12** (30.26 g, 82%). mp 251.1–252.9 °C; ^{1}H NMR ([D]DMSO) δ_{ppm} = 2.32 (s, 3H, C*H*$_3$), 5.04 (s, 2H, C*H*$_2$), 7.30 (s, 2H, SO_2NH_2), 7.41–8.09 (m, 6H, Ar*H*), 9.53 (1H, N*H*); ^{13}C NMR ([D]DMSO) δ = 17.7, 68.0, 117.3, 123.5, 123.9, 124.7, 124.9, 127.8, 131.0, 134.2, 138.5, 139.6, 140.3, 149.6, 165.7. MS (ESI$^+$) *m*/*z*: 370 [M + H]$^+$. Calcd for $C_{15}H_{16}ClN_3O_4S$ 369.

4.4. General procedure for the synthesis of N-phenylarylformamide derivatives (**13a**–**y**)

A mixture of **12** (3.70 g, 0.01 mol), acetone (50 mL) and sodium bicarbonate (3.36 g, 0.04 mol) was cooled to 0 °C in an ice bath, then acyl chloride (0.02 mol) was added dropwise over 10 min, and the resulting mixture was allowed to warm to room temperature and stirred for another 30 min. The reaction mixture was then poured into water (150 mL), filtered and washed with CH_2Cl_2, MeOH, and hexane to give crude product as a beige solid, which was purified by crystallization from acetonitrile to prepare the target compounds **13a**–**y** as a white solid.

4.4.1. N-[4-(Aminosulfonyl)-2-methylphenyl]-2-[4-chloro-2-(benzoyl)aminophenoxy]acetamide (**13a**)

Yield 83%; mp 237.8–238.5 °C; ^{1}H NMR ([D]DMSO) δ_{ppm} = 2.14 (s, 3H, C*H*$_3$), 4.93 (s, 2H, C*H*$_2$), 7.29 (m, 2H, SO_2NH_2), 7.19–7.99 (m, 11H, Ar*H*), 9.81(s, 1H, N*H*), 10.03 (s, 1H, N*H*); ^{13}C NMR ([D]DMSO) δ = 17.5, 68.3, 114.9, 123.4, 123.7, 125.0, 125.1, 125.2, 127.6 (2C), 127.8, 128.6 (2C), 128.9, 132.0, 132.4, 134.0, 138.3, 140.8, 148.3, 166.5, 167.0; MS (ESI$^-$) *m*/*z*: 472 [M–H]$^-$. Anal. calcd for $C_{22}H_{20}ClN_3O_5S$: C 55.75, H 4.25, N 8.87, Cl 7.48, S 6.77, found: C 55.74, H 4.26, N 8.85, Cl 7.51, S 6.75.

4.4.2. N-[4-(Aminosulfonyl)-2-methylphenyl]-2-[4-chloro-2-(2-methylbenzoyl)aminophenoxy]acetamide (**13b**)

Yield 85%; mp 239.6–240.5 °C; ^{1}H NMR ([D]DMSO) δ_{ppm} = 2.11 (s, 3H, C*H*$_3$), 2.44 (s, 3H, C*H*$_3$), 4.88 (s, 2H, C*H*$_2$),7.28 (m, 2H, SO_2NH_2), 7.20–8.05 (m, 10H, Ar*H*), 9.76 (s, 1H, N*H*), 9.89 (s, 1H, N*H*); ^{13}C NMR ([D]DMSO) δ = 17.5, 19.4, 68.0, 114.4, 123.0, 123.7, 124.8, 125.0, 125.2, 125.8, 127.3, 127.6, 128.6, 130.0, 130.7, 132.5, 135.6, 136.3, 138.2, 140.9, 147.8, 166.6, 168.0; MS (ESI$^-$) *m*/*z*: 486 [M–H]$^-$. Anal. calcd for $C_{23}H_{22}ClN_3O_5S$: C 56.61, H 4.54, N 8.61, Cl 7.27, S 6.57, found: C 56.60, H 4.52, N 8.60, Cl 7.29, S 6.56.

4.4.3. N-[4-(Aminosulfonyl)-2-methylphenyl]-2-[4-chloro-2-(3-methylbenzoyl)aminophenoxy]acetamide (**13c**)

Yield 87%; mp 240.2–241.8 °C; ^{1}H NMR ([D]DMSO) δ_{ppm} = 2.13 (s, 3H, C*H*$_3$), 2.36 (s, 3H, C*H*$_3$), 4.93 (s, 2H, C*H*$_2$), 7.31 (m, 2H, SO_2NH_2), 7.19–7.98 (m, 10H, Ar*H*), 9.83 (s, 1H, N*H*), 10.02 (s, 1H, N*H*); ^{13}C NMR ([D]DMSO) δ = 17.6, 20.9, 68.4, 115.1, 123.3, 123.7, 124.8, 125.0, 125.1, 125.2, 127.7, 128.1, 128.5, 128.9, 132.4, 132.6, 134.0, 138.0, 138.3, 140.9, 148.3, 165.6, 167.0; MS (ESI$^-$) *m*/*z*: 486 [M–H]$^-$. Anal. calcd for $C_{23}H_{22}ClN_3O_5S$: C 56.61, H 4.54, N 8.61, Cl 7.27, S 6.57, found: C 56.58, H 4.52, N 8.60, Cl 7.29, S 6.58.

4.4.4. N-[4-(Aminosulfonyl)-2-methylphenyl]-2-[4-chloro-2-(4-methylbenzoyl)aminophenoxy]acetamide (**13d**)

Yield 85%; mp 239.9–241.0 °C; ^{1}H NMR ([D]DMSO) δ_{ppm} = 2.15 (s, 3H, C*H*$_3$), 2.39 (s, 3H, C*H*$_3$), 4.93 (s, 2H, C*H*$_2$), 7.28 (m, 2H, SO_2NH_2), 7.19–7.99 (m, 10H, Ar*H*), 9.81 (s, 1H, N*H*), 9.94 (s, 1H, N*H*); ^{13}C NMR ([D]DMSO) δ = 17.5, 21.0, 68.3, 114.9, 123.2, 123.7, 124.9, 125.1, 125.2, 127.6 (2C), 127.7, 129.0, 129.1 (2C), 131.2, 132.3, 138.3, 140.8, 142.2, 148.2, 165.2, 167.0; MS (ESI$^-$) *m*/*z*: 486 [M–H]$^-$. Anal. calcd for $C_{23}H_{22}ClN_3O_5S$: C 56.61, H 4.54, N 8.61, Cl 7.27, S 6.57, found: C 56.63, H 4.52, N 8.59, Cl 7.28, S 6.58.

4.4.5. N-[4-(Aminosulfonyl)-2-methylphenyl]-2-[4-chloro-2-(3-chlorobenzoyl)aminophenoxy]acetamide (**13e**)

Yield 79%; mp 244.3–245.4 °C; ^{1}H NMR ([D]DMSO) δ_{ppm} = 2.13 (s, 3H, C*H*$_3$), 4.92 (s, 2H, C*H*$_2$), 7.29 (m, 2H, SO_2NH_2), 7.20–8.03 (m,10H, Ar*H*), 9.76 (s, 1H, N*H*), 10.18 (s, 1H, N*H*); ^{13}C NMR ([D] DMSO) δ = 17.5, 68.4, 115.2, 123.7, 123.8, 125.0, 125.2, 125.4, 126.5, 127.5, 127.7, 128.6, 130.6, 131.8, 132.3, 133.4, 136.1, 138.3, 140.8, 148.6, 164.2, 167.0; MS (ESI$^-$) *m*/*z*: 507 [M–H]$^-$. Anal. calcd for $C_{22}H_{19}Cl_2N_3O_5S$: C 51.98, H 3.77, N 8.27, Cl 13.95, S 6.31, found: C 51.99, H 3.75, N 8.25, Cl 13.97, S 6.32.

4.4.6. N-[4-(Aminosulfonyl)-2-methylphenyl]-2-[4-chloro-2-(3-fluorobenzoyl)aminophenoxy]acetamide (**13f**)

Yield 79%; mp 242.2–243.1 °C; ^{1}H NMR ([D]DMSO) δ_{ppm} = 2.12 (s, 3H, C*H*$_3$), 4.97 (s, 2H, C*H*$_2$), 7.62 (m, 2H, SO_2NH_2), 7.19–7.93 (m, 10H, Ar*H*); MS (ESI$^-$) *m*/*z*: 490 [M–H]$^-$. Anal. calcd for $C_{22}H_{19}ClFN_3O_5S$: C 53.72, H 3.89, N, Cl 7.21, F 3.86, S 6.52, found: C 53.72, H 3.89, N, Cl 7.20, F 3.88, S 6.51.

4.4.7. N-[4-(aminosulfonyl)-2-methylphenyl]-2-[4-chloro-2-(2-nitrobenzoyl)aminophenoxy]acetamide (**13g**)

Yield 77%; mp 245.7–246.9 °C; ^{1}H NMR ([D]DMSO) δ_{ppm} = 2.10 (s, 3H, C*H*$_3$), 4.86 (s, 2H, C*H*$_2$), 7.28 (m, 2H, SO_2NH_2), 7.19–8.19 (m, 10H, Ar*H*), 9.60 (s, 1H, N*H*), 10.36 (s, 1H, N*H*); ^{13}C NMR ([D]DMSO) δ = 17.5, 68.0, 114.6, 122.5, 123.7, 124.3, 124.8, 124.9, 125.0, 127.6, 128.2, 129.3, 131.1, 132.2, 132.3, 134.2, 138.2, 140.8, 146.2, 147.4, 164.8, 166.4; MS (ESI$^-$) *m*/*z*: 516 [M–H]$^-$. Anal. calcd for $C_{22}H_{19}ClN_4O_7S$: C 50.92, H 3.69, N 10.80, Cl 6.83, S 6.18, found: C 50.90, H 3.71, N 10.78, Cl 6.85, S 6.17.

4.4.8. N-[4-(Aminosulfonyl)-2-methylphenyl]-2-[4-chloro-2-(3-nitrobenzoyl)aminophenoxy]acetamide (**13h**)

Yield 74%; mp 236.5–237.7 °C; ^{1}H NMR ([D]DMSO) δ_{ppm} = 2.11 (s, 3H, C*H*$_3$), 4.92 (s, 2H, C*H*$_2$), 7.62 (m, 2H, SO_2NH_2), 7.22–8.79 (m, 10H, Ar*H*), 9.74 (brs, 1H, N*H*), 10.43 (brs, 1H, N*H*); ^{13}C NMR ([D] DMSO) δ = 17.4, 68.3, 115.2, 122.5, 123.7, 124.3, 124.8, 125.1, 125.8, 126.5, 127.6, 128.3, 130.3, 132.0, 134.1, 135.6, 138.2, 140.7, 147.8, 148.9, 163.6, 166.9; MS (ESI$^-$) *m*/*z*: 516 [M–H]$^-$. Anal. calcd for $C_{22}H_{19}ClN_4O_7S$: C 50.92, H 3.69, N 10.80, Cl 6.83, S 6.18, found: C 50.91, H 3.70, N 10.82, Cl 6.81, S 6.16.

*4.4.9. N-[4-(Aminosulfonyl)-2-methylphenyl]-2-[4-chloro-2-(3-trifluoromethylbenzoyl)aminophenoxy]acetamide (**13i**)*

Yield 72%; mp 257.2–258.1 °C; ^{1}H NMR ([D]DMSO) δ_{ppm} = 2.11 (s, 3H, C*H*$_3$), 4.92 (s, 2H, C*H*$_2$), 7.66 (m, 2H, SO$_2$N*H*$_2$), 7.22–8.32 (m, 10H, Ar*H*), 9.73 (s, 1H, N*H*), 10.34 (s, 1H, N*H*); ^{13}C NMR ([D]DMSO) δ = 17.4, 68.4, 115.3, 122.5–129.5 (q, 1C, 122.5, 125.3, 129.2, 129.5), 123.7, 124.1, 124.3 (q, 1C), 124.8, 125.2, 125.3, 125.6, 127.9, 128.5 (2C), 129.9, 131.8, 132.1, 135.1, 138.3, 140.8, 148.8, 164.3, 167.0; MS (ESI$^-$) *m*/*z*: 539 [M–H]$^-$. Anal. calcd for $C_{23}H_{19}ClF_3N_3O_5S$: C 50.97, H 3.53, N 7.75, Cl 6.54, F 10.52, S 5.92, found: C 50.98, H 3.52, N 7.77, Cl 6.51, F 10.50, S 5.90.

*4.4.10. N-[4-(Aminosulfonyl)-2-methylphenyl]-2-[4-chloro-2-(4-methoxybenzoyl)aminophenoxy]acetamide (**13j**)*

Yield 82%; mp 241.1–242.2 °C; ^{1}H NMR ([D]DMSO) δ_{ppm} = 2.15 (s, 3H, C*H*$_3$), 3.83 (s, 3H, C*H*$_3$O), 4.93 (s, 2H, C*H*$_2$), 7.30 (s, 2H, SO$_2$N*H*$_2$), 7.05–7.98 (m, 10H, Ar*H*), 9.84 (s, 1H, N*H*), 9.90 (s, 1H, N*H*); ^{13}C NMR ([D]DMSO) δ = 17.6, 55.5, 68.3, 113.8 (2C), 114.9, 123.4, 123.7, 124.8, 125.1, 125.2, 126.1, 127.7, 129.1, 129.6 (2C), 132.4, 138.1, 140.8, 148.3, 162.3, 164.9, 167.0; MS (ESI$^-$) *m*/*z*: 502 [M–H]$^-$. Anal. calcd for $C_{23}H_{22}ClN_3O_6S$: C 54.82, H 4.40, N 8.34, Cl 7.03, S 6.36, found: C 54.80, H 4.41, N 8.32, Cl 7.05, S 6.33.

*4.4.11. N-[4-(Aminosulfonyl)-2-methylphenyl]-2-[4-chloro-2-(2,4-dimethylbenzoyl)aminophenoxy]acetamide (**13k**)*

Yield 84%; mp: 248.5–249.6 °C; ^{1}H NMR ([D]DMSO) δ_{ppm} = 2.12 (s, 3H, C*H*$_3$), 2.32 (s, 3H, C*H*$_3$), 2.41 (s, 3H, C*H*$_3$), 4.88 (s, 2H, C*H*$_2$), 7.28 (s, 2H, SO$_2$N*H*$_2$), 7.09–8.05 (m, 9H, Ar*H*), 9.78 (s, 2H, 2N*H*); ^{13}C NMR ([D]DMSO) δ = 17.5, 19.5, 20.8, 68.1, 114.4, 122.8, 123.7, 124.6, 125.0, 126.1, 127.6, 127.7, 128.7, 131.3, 131.4, 132.5, 133.3, 135.8, 138.2, 139.8, 140.9, 147.7, 166.7, 167.9; MS (ESI$^-$) *m*/*z*: 501 [M–H]$^-$. Anal. calcd for $C_{24}H_{24}ClN_3O_5S$: C 57.42, H 4.82, N 8.37, Cl 7.06, S 6.39, found: C 57.41, H 4.81, N 8.36, Cl 7.05, S 6.40.

*4.4.12. N-[4-(Aminosulfonyl)-2-methylphenyl]-2-[4-chloro-2-(2,5-dimethylbenzoyl)aminophenoxy]acetamide (**13l**)*

Yield 85%; mp 244.7–245.8 °C; ^{1}H NMR ([D]DMSO) δ_{ppm} = 2.10 (s, 3H, C*H*$_3$), 2.29 (s, 3H, C*H*$_3$), 2.38 (s, 3H, C*H*$_3$), 4.88 (s, 2H, C*H*$_2$), 7.30 (s, 2H, SO$_2$N*H*$_2$), 7.17–8.03 (m, 9H, Ar*H*), 9.79 (s, 1H, N*H*), 9.90 (s, 1H, N*H*); ^{13}C NMR ([D]DMSO) δ = 17.5, 19.0, 20.4, 68.1, 114.5, 123.0, 123.7, 124.8, 125.0, 125.2, 127.7, 127.8, 128.6, 130.6, 130.7, 132.5, 132.6, 134.8, 136.2, 138.3, 140.9, 147.8, 166.7, 168.1; MS (ESI$^-$) *m*/*z*: 501 [M–H]$^-$. Anal. calcd for $C_{24}H_{24}ClN_3O_5S$: C 57.42, H 4.82, N 8.37, Cl 7.06, S 6.39, found: C 57.40, H 4.81, N 8.38, Cl 7.07, S 6.40.

*4.4.13. N-[4-(Aminosulfonyl)-2-methylphenyl]-2-[4-chloro-2-(3,4-dimethylbenzoyl)aminophenoxy]acetamide (**13m**)*

Yield 87%; mp 248.8–249.7 °C; ^{1}H NMR ([D]DMSO) δ_{ppm} = 2.10 (s, 3H, C*H*$_3$), 2.29 (s, 6H, 2C*H*$_3$), 4.87 (s, 2H, C*H*$_2$), 7.28 (s, 2H, SO$_2$N*H*$_2$), 7.19–8.06 (m, 9H, Ar*H*), 9.76 (s, 1H, N*H*), 9.88 (s, 1H, N*H*); ^{13}C NMR ([D]DMSO) δ = 16.0, 17.5, 19.8, 68.0, 114.4, 123.0, 123.7, 124.7 (2C), 124.9, 125.3, 125.2, 127.6, 128.5, 131.0, 132.5, 133.6, 137.3 (2C), 138.2, 140.9, 147.7, 166.6, 168.7; MS (ESI$^-$) *m*/*z*: 500 [M–H]$^-$. Anal. calcd for $C_{24}H_{24}ClN_3O_5S$: C 57.42, H 4.82, N 8.37, Cl 7.06, S 6.39, found: C 57.41, H 4.80, N 8.38, Cl 7.10, S 6.36.

*4.4.14. N-[4-(aminosulfonyl)-2-methylphenyl]-2-[4-chloro-2-(3,5-dimethylbenzoyl)aminophenoxy]acetamide (**13n**)*

Yield 89%; mp: 252.4–253.7 °C; ^{1}H NMR ([D]DMSO) δ_{ppm} = 2.15 (s, 3H, C*H*$_3$), 2.32 (s, 6H, 2C*H*$_3$), 4.93 (s, 2H, C*H*$_2$), 7.31 (s, 2H, SO$_2$N*H*$_2$), 7.19–7.99 (m, 9H, Ar*H*), 9.84 (s, H, N*H*), 9.98 (s, H, N*H*); ^{13}C NMR ([D]DMSO) δ = 17.6, 20.8 (2C), 68.6, 115.2, 123.2, 123.7, 124.9, 125.0, 125.4 (3C), 127.8, 129.1, 132.4, 133.4, 134.1, 137.9 (2C), 138.4, 140.9, 148.3, 165.7, 167.1; MS (ESI$^-$) *m*/*z*: 500 [M–H]$^-$. Anal. calcd for $C_{24}H_{24}ClN_3O_5S$: C 57.42, H 4.82, N 8.37, Cl 7.06, S 6.39, found: C 57.41, H 4.83, N 8.38, Cl 7.07, S 6.40.

*4.4.15. N-[4-(Aminosulfonyl)-2-methylphenyl]-2-[4-chloro-2-(2,4-dichlorobenzoyl)aminophenoxy]acetamide (**13o**)*

Yield 73%; mp 253.1–254.3 °C; ^{1}H NMR ([D]DMSO) δ_{ppm} = 2.14 (s, 3H, C*H*$_3$), 4.86 (s, 2H, C*H*$_2$), 7.65 (s, 2H, SO$_2$N*H*$_2$), 7.19–8.09 (m, 9H, Ar*H*), 9.68 (s, 1H, N*H*), 10.22 (s, 1H, N*H*); ^{13}C NMR ([D]DMSO) δ = 17.6, 68.0, 114.6, 122.7, 123.7, 124.9, 125.0, 125.1, 127.4, 127.7, 128.0, 129.3, 130.6, 131.4, 132.4, 135.1, 135.2, 136.2, 140.9, 147.5, 164.4, 166.4; MS (ESI$^-$) *m*/*z*: 540 [M–H]$^-$. Anal. calcd for $C_{22}H_{18}Cl_3N_3O_5S$: C 48.68, H 3.34, Cl 19.59, N 7.74, S 5.91, found: C 48.70, H 3.35, Cl 19.60, N 7.75, S 5.90.

*4.4.16. N-[4-(Aminosulfonyl)-2-methylphenyl]-2-[4-chloro-2-(2,5-dichlorobenzoyl)aminophenoxy]acetamide (**13p**)*

Yield 71%; mp 253.3–254.1 °C; δ_{ppm}: ^{1}H NMR ([D]DMSO) δ_{ppm} = 2.15 (s, 3H, C*H*$_3$), 4.85 (s, 2H, C*H*$_2$), 7.29 (m, 2H, SO$_2$N*H*$_2$), 7.20–8.08 (m, 9H, Ar*H*), 9.67 (s, 1H, NH), 10.28 (s, 1H, NH); ^{13}C NMR ([D]DMSO) δ = 17.6, 68.1, 114.7, 122.9, 123.7, 125.0, 125.1 (2C), 127.7, 127.9, 128.8, 128.9, 131.1, 131.5, 131.9, 132.4, 137.8, 138.2, 140.9, 147.6, 163.9, 166.4; MS (ESI$^-$) *m*/*z*: 540 [M–H]$^-$. Anal. calcd for $C_{22}H_{18}Cl_3N_3O_5S$: C 48.68, H 3.34, Cl 19.59, N 7.74, S 5.91, found: C 48.69, H 3.35, Cl 19.58, N 7.72, S 5.90.

*4.4.17. N-[4-(Aminosulfonyl)-2-methylphenyl]-2-[4-chloro-2-(3,4-dichlorobenzoyl)aminophenoxy]acetamide (**13q**)*

Yield 74%; mp 249.8–251.7 °C; ^{1}H NMR ([D]DMSO) δ_{ppm} = 2.13 (s, 3H, C*H*$_3$), 4.91 (s, 2H, C*H*$_2$), 7.28 (m, 2H, SO$_2$N*H*$_2$), 7.21–8.23 (m, 9H, Ar*H*), 9.74 (s, 1H, NH), 10.24 (s, 1H, NH); ^{13}C NMR ([D]DMSO) δ = 17.5, 68.4, 123.7, 123.9, 124.9, 125.1, 127.6, 127.7, 128.0, 128.4, 129.6 (2C, 129.59, 129.65), 130.8, 131.4, 132.2, 134.5, 134.8, 138.2, 140.8, 148.7, 163.4, 166.9; MS (ESI$^-$) *m*/*z*: 541 [M–H]$^-$. Anal. calcd for $C_{22}H_{18}Cl_3N_3O_5S$: C 48.68, H 3.34, Cl 19.59, N 7.74, S 5.91, found: C 48.71, H 3.30, Cl 19.57, N 7.75, S 5.89.

*4.4.18. N-[4-(Aminosulfonyl)-2-methylphenyl]-2-[4-chloro-2-(3,5-dichlorobenzoyl)aminophenoxy]acetamide (**13r**)*

Yield 82%; mp 254.7–255.8 °C; ^{1}H NMR ([D]DMSO) δ_{ppm} = 2.15 (s, 3H, C*H*$_3$), 4.90 (s, 2H, C*H*$_2$), 7.28 (m, 2H, SO$_2$N*H*$_2$), 7.21–8.14 (m, 9H, Ar*H*), 9.73(s, 1H, NH), 10.30 (s, 1H, NH); ^{13}C NMR ([D]DMSO) δ = 17.6, 68.6, 115.5, 122.7 (2C), 123.7, 124.0, 124.9, 125.2, 125.7, 127.7, 128.3, 129.7 (2C), 132.2, 136.6, 137.7, 138.3, 140.8, 143.8, 162.8, 167.0; MS (ESI$^-$) *m*/*z*: 541 [M–H]$^-$. Anal. calcd for $C_{22}H_{18}Cl_3N_3O_5S$: C 48.68, H 3.34, Cl 19.59, N 7.74, S 5.91, found: C 48.69, H 3.36, Cl 19.60, N 7.75, S 5.90.

*4.4.19. N-[4-(Aminosulfonyl)-2-methylphenyl]-2-[4-chloro-2-(3-chloro-5-bromobenzoyl)aminophenoxy]acetamide (**13s**)*

Yield 72%; mp 265.4–266.3 °C; ^{1}H NMR ([D]DMSO) δ_{ppm} = 2.14 (s, 3H, C*H*$_3$), 4.90 (s, 2H, C*H*$_2$), 7.67 (m, 2H, SO$_2$N*H*$_2$), 7.20–8.10 (m, 9H, Ar*H*); ^{13}C NMR ([D]DMSO) δ = 17.6, 68.6, 115.5, 122.5, 123.7, 124.0, 125.0, 125.2, 125.5, 126.9, 127.7, 128.9, 129.3, 132.2, 133.8, 134.5, 137.8, 138.3, 140.8, 148.8, 162.9, 167.0; MS (ESI$^-$) *m*/*z*: 586 [M–H]$^-$. Anal. calcd for $C_{22}H_{18}Cl_2BrN_3O_5S$: C 44.99, H, 3.09 Br 13.61, Cl 12.07, N 7.16, S 5.46, found: C 45.00, H, 3.10, Br 13.60, Cl 12.05, N 7.18, S 5.48.

*4.4.20. N-[4-(Aminosulfonyl)-2-methylphenyl]-2-[4-chloro-2-(3,5-bromobenzoyl)aminophenoxy]acetamide (**13t**)*

Yield 75%; mp 297.6–298.5 °C; ^{1}H NMR ([D]DMSO) δ_{ppm} = 2.16 (s, 3H, C*H*$_3$), 4.92 (s, 2H, C*H*$_2$), 7.29 (m, 2H, SO$_2$N*H*$_2$), 7.21–8.13 (m, 9H, Ar*H*), 9.74 (s, 1H, NH), 10.29 (s, 1H, NH); ^{13}C NMR ([D]DMSO) δ = 17.6, 68.7, 115.6, 122.8 (2C), 123.8, 124.0, 124.9, 125.2, 125.7, 127.7, 128.4, 129.7 (2C), 132.2, 136.6, 137.7, 138.3, 140.8, 148.7, 162.9, 167.3; MS (ESI$^-$) *m*/*z*: 631 [M–H]$^-$. Anal. calcd for $C_{22}H_{18}ClBr_2N_3O_5S$: C 41.83, H 2.87, Br 25.30, Cl 5.61, N 6.65, S 5.08, found: C 41.81, H 2.86, Br 25.32, Cl 5.60, N 6.63, S 5.07.

*4.4.21. N-[4-(Aminosulfonyl)-2-methylphenyl]-2-[4-chloro-2-(3-chloro-5-cyanobenzoyl)aminophenoxy]acetamide (**13u**)*

Yield 65%; mp 255.7–256.8 °C; ^{1}H NMR ([D]DMSO) δ_{ppm} = 2.14 (s, 3H, C*H*$_3$), 4.91 (s, 2H, C*H*$_2$), 7.28 (m, 2H, SO$_2$N*H*$_2$), 7.22–8.37 (m,

9H, Ar*H*), 9.73 (s, 1H, NH), 10.37 (s, 1H, NH); ^{13}C NMR ([D]DMSO) δ = 17.5, 68.5, 113.5, 115.4, 117.0, 123.7, 123.9, 124.8, 125.2, 125.7, 127.7, 128.2, 130.3, 132.1, 132.4, 134.4, 134.8, 137.1, 138.2, 140.8, 148.6, 162.7, 166.9; MS (ESI$^-$) *m*/*z*: 532 [M−H]$^-$. Anal. calcd for $C_{23}H_{18}Cl_2N_4O_5S$: C 51.79, H 3.40, Cl 13.29, N 10.50, S 6.01, found: C 51.80, H 3.41, Cl 13.30, N 10.52, S 6.00.

4.4.22. N-[4-(Aminosulfonyl)-2-methylphenyl]-2-{[4-chloro-2-[(E)-cinnamoyl]aminophenoxy}acetamide (**13v**)

Yield 68%; mp 263.2–264.3 °C; ^{1}H NMR ([D]DMSO) δ_{ppm} = 1.99 (s, 3H, C*H*$_3$), 4.89 (s, 2H, C*H*$_2$), 7.21 (d, 1H, *J* = 8.8 Hz, C*H*), 7.54 (d, 1H, *J* = 8.8 Hz, C*H*), 7.60 (m, 2H, SO$_2$N*H*$_2$), 7.29–8.30 (m, 11H, Ar*H*); ^{13}C NMR ([D]DMSO) δ = 17.4, 68.1, 114.6, 123.4, 123.6, 125.1 (2C), 125.7, 126.4, 127.1, 127.6(2C), 128.3(2C), 129.7, 130.5, 132.4, 133.2, 134.2, 138.3, 140.8, 148.0, 166.6, 167.6; MS (ESI$^-$) *m*/*z*: 499 [M−H]$^-$. Anal. calcd for $C_{24}H_{22}CN_3O_5S$: C 57.66, H 4.44, Cl 7.09, N 8.40, S 6.41, found: C 57.68, H 4.45, Cl 7.10, N 8.41, S 6.40.

4.4.23. N-[4-(Aminosulfonyl)-2-methylphenyl]-2-[4-chloro-2-(2,6-dichlorophenylacetyl)aminophenoxy]acetamide (**13w**)

Yield 77%; mp 297.5–298.3 °C; ^{1}H NMR ([D]DMSO) δ_{ppm} = 2.28 (s, 3H, C*H*$_3$), 4.18 (s, 2H, C*H*$_2$), 4.90 (s, 2H, C*H*$_2$), 7.30 (s, 2H, SO$_2$N*H*$_2$), 7.16–8.01 (m, 9H, Ar*H*), 9.73 (s, 1H, NH), 9.96 (s, 1H, NH); ^{13}C NMR ([D]DMSO) δ = 17.7, 38.5, 68.0, 114.4, 121.5, 123.7, 123.9, 124.8, 125.0, 127.7, 128.1(2C), 128.7, 129.5, 131.9, 131.1, 135.6 (2C), 138.4, 140.7, 146.8, 166.6, 167.2; MS (ESI$^-$) *m*/*z*: 555 [M−H]$^-$. Anal. calcd for $C_{23}H_{20}Cl_3N_3O_5S$: C 49.61, H 3.62, Cl 19.10, N 7.55, S 5.76, found: C 49.60, H 3.60, Cl 19.13, N 7.54, S 5.75.

4.4.24. N-[4-(Aminosulfonyl)-2-methylphenyl]-2-[4-chloro-2-(1-naphthoyl)aminophenoxy]acetamide (**13x**)

Yield 74%; mp 262.4–263.1 °C; ^{1}H NMR ([D]DMSO) δ_{ppm} = 1.99 (s, 3H, C*H*$_3$), 4.90 (s, 2H, C*H*$_2$), 7.61 (s, 2H, SO$_2$N*H*$_2$), 7.22–8.31 (m, 13H, Ar*H*); ^{13}C NMR ([D]DMSO) δ = 17.4, 68.2, 114.6, 123.3, 123.6, 125.0 (3C), 125.1 (2C), 125.7, 126.4, 127.1, 127.6, 128.3, 128.7, 129.7, 130.5, 132.4, 133.1, 134.1, 138.4, 140.8, 148.0, 166.6, 167.6; MS (ESI$^-$) *m*/*z*: 522 [M−H]$^-$. Anal. calcd for $C_{26}H_{22}ClN_3O_5S$: C 59.60, H 4.23, Cl 6.77, N 8.02, S 6.12, found: C 59.62, H 4.25, Cl 6.75, N 8.00, S 6.14.

4.4.25. N-[4-(Aminosulfonyl)-2-methylphenyl]-2-[4-chloro-2-(2-naphthoyl)aminophenoxy]acetamide (**13y**)

Yield 71%; mp 281.1–282.3 °C; ^{1}H NMR ([D]DMSO) δ_{ppm} = 2.13 (s, 3H, C*H*$_3$), 4.96 (s, 2H, C*H*$_2$), 7.67 (s, 2H, SO$_2$N*H*$_2$), 7.22–8.63 (m, 13H, Ar*H*), 9.86 (s, 1H, NH), 10.24 (s, 1H, NH); ^{13}C NMR ([D]DMSO) δ = 17.6, 68.4, 115.1, 123.3, 123.7, 124.2, 125.1 (2C), 125.2, 127.0, 127.7 (2C), 128.1, 128.2 (2C), 128.9, 129.0, 131.4, 132.1, 132.4, 134.4, 138.3, 140.9, 148.4, 165.6, 167.0; MS (ESI$^-$) *m*/*z*: 522 [M−H]$^-$. Anal. calcd for $C_{26}H_{22}ClN_3O_5S$: C 59.60, H 4.23, Cl 6.77, N 8.02, S 6.12, found: C 59.61, H 4.25, Cl 6.79, N 8.02, S 6.09.

4.5. Anti-HIV assays

4.5.1. Cytotoxicity assay

The cytotoxicity of compounds on C8166 cells were assessed by MTT colorimetric assay as described previously [23]. The absorbance at 570 nm/630 nm ($A_{570/630}$) was read in an ELISA reader (Elx800, Bio-Tek Instrument Inc., USA). The minimum cytotoxic concentration that caused the reduction of viable cells by 50% (CC_{50}) was determined from dose response curve.

4.5.2. Syncytium reduction assay

In the presence of 100 μL various concentrations of compounds, C8166 cells (4×10^5/mL) were infected with viruses (HIV-1$_{IIIB}$, HIV-1$_{A17}$, and HIV-2$_{ROD}$) at a multiplicity of infection (M.O.I) of 0.06. The final volume per well was 200 μL. AZT and GW678248 were used for drug control. After 3 days of culture, the number of syncytia (multinucleated giant cells) was scored under an inverted microscope; 50% effective concentration to blocking syncytia formation (EC_{50}) was calculated [24].

4.6. RT inhibition assay

According to the method reported by Liu [26], assays against HIV-1 RT$_{wt}$ were performed using a poly(rA)/oligo (dT)$_{15}$ homopolymer template with HIV antigen detection ELISA for quantifying expression of HIV-1 RT in culture medium. Oligo (dT) was immobilized via its 50-terminal phosphate to Covalink-NH microtiter plates. The biotin-dUTP was incorporated by reverse transcriptase. The reaction mixture contained 50 mM Tris–HCl (pH 8.3), 3 mM $MgCl_2$, 75 mM KCl, 5 mM DTT (d,l-dithiothreitol), 0.13 mg/mL BSA, 10 mg/mL poly (A), 0.75 mM biotin-11-dUTP, and 1.5 mM dTTP. After incubation at 37 °C for 1 h, the plate was washed three times with a wash buffer containing 50 mM Tris–HCl (pH 7.5), 0.15 M NaCl, 0.05 mM $MgCl_2$, and 0.02% Tween-20. After 100 mL of 1% BSA was added to each well and incubated for 30 min at room temperature, the plate was washed with the same buffer. Subsequently 50 mL of SA-ALP (alkaline phosphatase streptavidin) solution (100 ng/mL) was added per well and then incubated for 1 h at 37 °C. The plate was washed as above and then 50 mL of PNPP (p-nitrophenyl phosphate, disodium) (1 mg/mL, pH 9.5) was added, after 30 min at 37 °C the reaction was stopped by addition of 0.5 M NaOH. The products were detected and quantified using a colorimetric streptavidin alkaline phosphatase reporter system.

4.7. Molecular simulation

Molecular modelling was carried out with the Tripos molecular modelling packages Sybyl-1.2. All the molecules for docking were built by using the standard bond lengths and angles from the Sybyl-X 1.2/base Builder, and then were optimized for 2000-generations two until the maximum derivative of energy became 0.1kcal·mol^{-1} Å^{-1}, using the Tripos force field. The flexible docking method, called Surflex-Dock program docks ligand automatically into a receptor's ligand binding site using a protomol based approach and an empirically derived scoring function [27,28]. The protomol is a computational representation of a putative ligand that binds to the intended binding site and is a unique and essential element of the docking algorithm. Surflex-Dock's scoring function, which contains hydrophobic, polar, repulsive, entropic, and salvation terms, was trained to estimate the dissociation constant (K_d) expressed in $-\log(K_d)^2$ unit. Prior to docking, the protein was prepared by removing water molecules, ligand and other useless small molecules from the complex of HIV-1 RT with GW564511 (PDB code: 3DLG) [20], in parallel with adding polar hydrogen atoms to protein. Other parameters were set as defaults for Surflex-Dock, such as the starting conformations per molecule of 0, the angstroms to expand search grid of 8, the max number of rotatable bonds per molecule of 100, and the maximum number of poses per ligand of 20. During the docking procedure, all of the single bonds of residue side chains inside the defined RT binding pocket were regarded as rotatable or flexible bonds, and the ligand was allowed to rotate on all single bonds and move flexibly within the tentative binding pocket. The atomic charges were recalculated by using the Kollman all-atom approach for the protein and the Gasteiger–Hückel approach for the ligand. The binding interaction energy was calculated to include van der Waals, electrostatic, and torsional energy terms defined in the Tripos force field. The structure optimization was performed for 20000-generations using a genetic.

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Appendix A. Supplementary data

Supplementary data related to this article can be found online at doi:10.1016/j.ejmech.2012.03.032.

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