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Novel diarylpyridinones, diarylpyridazinones and diarylphthalazinones as potential HIV-1 nonnucleoside reverse transcriptase inhibitors (NNRTIs)

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ABSTRACT

In this Letter, we report on diarylpyridinone, diarylpyridazinone and diarylphthalazinone analogs as potential inhibitors of HIV-1 nonnucleoside reverse transcriptase (NNRTIs). The most promising compounds in these series are three diarylpyridazinones **25a**, **25l** and **25n** which demonstrated submicromolar activity against wild-type HIV-1 and moderate activity against the single mutant strain Ba-L V106A.

1. Introduction

According to the most recent UNAIDS report, worldwide 60 million people have been infected by the human immunodeficiency virus (HIV) and about 25 million patients died of AIDS.[1] HIV-1 reverse transcriptase (RT) is one of the most important viral enzymes and plays a vital role in the HIV-1 life cycle. There are two known drug-target sites, the substrate catalytic site and an allosteric site that is distinct from but located closely to the substrate site.[2–4] Nonnucleoside reverse transcriptase inhibitors (NNRTIs) interact with the allosteric site in a noncompetitive manner to distort the enzyme's active conformation and hence disrupt the function of the enzyme. The first-generation NNRTIs such as nevirapine (**1**),[5] delavirdine (**2**)[6] and efavirenz (**3**)[7] exhibit very potent anti-HIV-1 activity and low toxicity. However, rapid drug-resistance emergence due to single point mutations[8] in the NNRTI binding site, compromises their clinical utility. Etravirine (TMC125, **4**),[9] a diarylpyrimidine (DAPY) was recently approved as a next-generation NNRTI for the treatment of HIV-1 infection in treatment-experienced patients who show evidence of HIV-1 resistant strains. It exhibits high potency against wild-type and a number of mutated viral strains with nanomolar EC_{50} values and has demonstrated a higher genetic barrier[10] to the emergence of drug-resistance. The most advanced NNRTI (approved recently by FDA) is another DAPY derivative rilpivirine (TMC278, **5**)[11], which shows a better potency and safety profile than **4**. It allows once daily administration and will be prescribed for treatment of HIV-1 infection in treatment-naive patients.[12] Dapivirine, also known as TMC120 (**6**)[13a] is currently under development for HIV-microbicidal applications (Fig. 1).[13b]

The excellent pharmacological profiles of the DAPYs **4–6** have encouraged several research groups to explore next-generation NNRTI agents.[14] In this Letter, we report on the synthesis and antiviral activity of diarylpyridinone, diarylpyridazinone and diarylphthalazinone analogs (Series A–C).

2. Results and discussion

2.1. Compound design and molecular modeling studies

On the basis of SAR data from the DAPY series, our strategy was to synthesize novel series of NNRTIs by replacing the central pyrimidine ring. The new compounds designed in this study have

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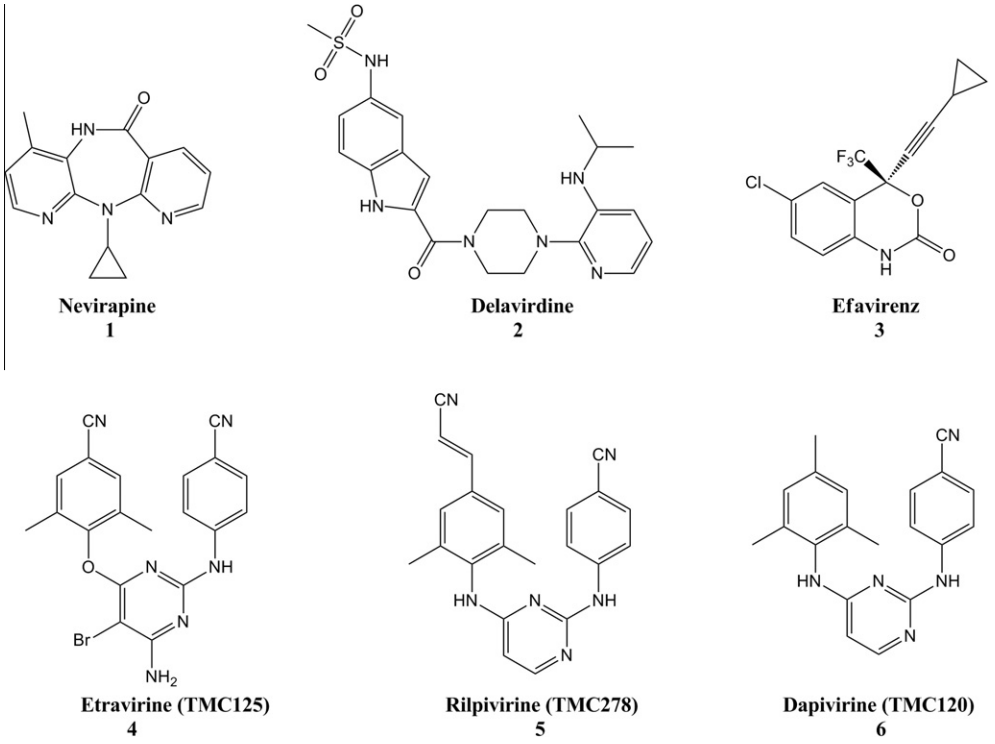


Figure 1. Structures of reported HIV-1 NNRTI compounds.

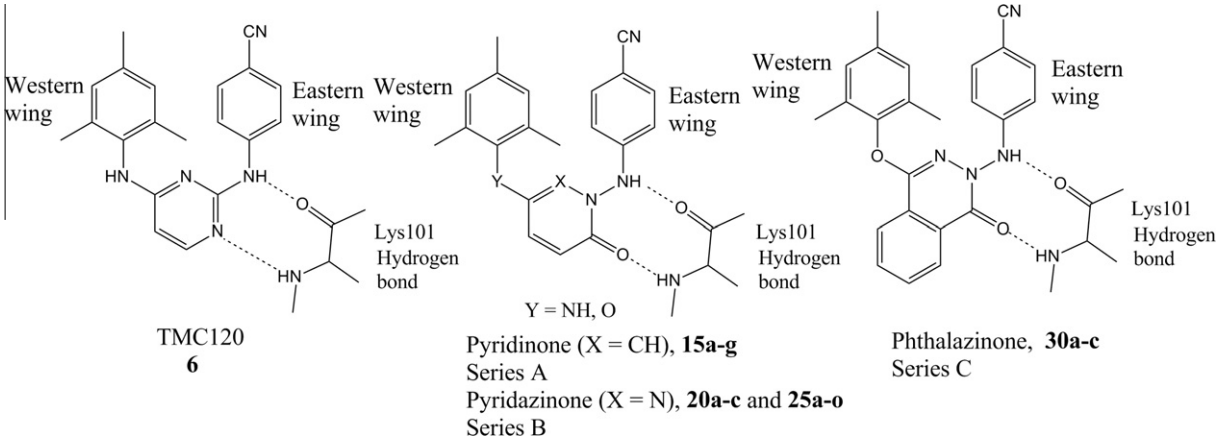


Figure 2. Design of diarylpyridinones, diarylpyridazinones and diarylphthalazinones based on the interactions of TMC 120 (**6**) with its binding site on reverse transcriptase.

a central pyridinone, pyridazinone and phthalazinone which retain the two phenyl rings in the Western and Eastern wing. In TMC120 (**6**) the NH linker connecting the central pyrimidine ring and the eastern wing serves as hydrogen donor with the backbone carbonyl of Lys101 and the pyrimidine nitrogen at position 1 serves as hydrogen bond acceptor with the backbone α-amino group of Lys101. In our newly designed compounds the NH linker attached to a nitrogen of the central ring is maintained whereas the carbonyl group of the central ring serves as hydrogen bond acceptor. The presence of the central ring will assure a similar molecular topology and flexibility, and hence we expected the new compounds would have binding orientations and conformations similar to those of the DAPYs (Fig. 2).

Figure 3 shows the superposition of low-energy docking conformations of our compounds **15a**, **25a** and **30a** as the representatives of the pyridinone, pyridazinone and phthalazinone series, respectively, along with TMC125 (**4**) and TMC120 (**6**). The model reveals that the designed compounds have the potential to bind to HIV-1 reverse transcriptase in a similar horseshoe conformation.[9b]

2.2. Chemistry

The synthesis of compounds belonging to Series A–C is outlined in Schemes 1–3. Scheme 1 shows the synthesis of diarylpyridinones **15a–g** (Series A). Selective *O*-benzylation of bromopyridin-2-one **7** provided bromopyridine **8**.[15] Palladium-catalyzed amination of bromopyridine **8** with amines **9a–c** gave the respective amino-substituted pyridines **10a–c**.[15] Catalytic debenzylation of aminopyridines **10a–c** was easily achieved to afford the corresponding pyridin-2-ones **11a–c**. Phosphine-based amino transfer reagent *O*-(diphenylphosphinyl)hydroxyl amine (**12**) was synthesized according to a reported procedure.[16] *N*-amination of the pyridin-2-ones **11a–c** using **12** yielded the *N*-aminopyridin-2-ones

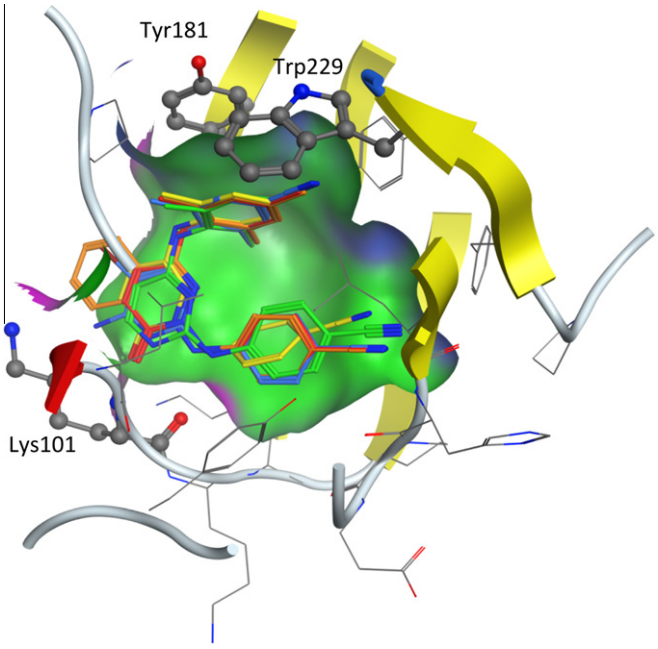


Figure 3. Superposition of low-energy docking binding conformations of newly designed compounds **15a** (yellow), **25a** (red), **30a** (orange) and DAPYs **4** (blue) and **6** (green).

13a–c.[17] Coupling[18] of arylboronic acids **14a–d** with *N*-aminopyridin-2-ones **13a–c** furnished the desired compounds **15a–g** (Series A).

Scheme 2 shows the synthesis of diarylpyridazinones **20a–c** and **25a–o** (Series B). Fusion of 3,6-dichloropyridazine (**16**) with 2,4,6-trimethylaniline (**9a**) yielded 6-chloro-*N*-mesitylpyridazin-3-amine (**17**). Reaction of **16** with sodium phenoxide derivatives **21a–e** afforded the corresponding 3-aryloxy-6-chloropyridazines **22a–e**. Hydrolysis of **17** and **22a–e** in glacial acetic acid furnished the corresponding 6-(mesitylamino)pyridazin-3(2*H*)-one (**18**)[14h] and 6-aryloxypyridazin-3(2*H*)-ones **23a–e**, respectively. *N*-amination of the pyridazin-3-one **18** and **23a–e** with amine **12** yielded *N*-aminopyridazin-3-one **19**[19] and **24a–e**, respectively. Coupling of **19** and **24a–e** with arylboronic acids **14a–f** delivered the desired compounds **20a–c** and **25a–o** (Scheme 2).

Scheme 3 shows the synthesis of diarylphthalazinones **30a–c** (Series C). Reaction of **26** with phenols **21a** and **21d** afforded 1-chloro-4-aryloxyphthalazines **27a** and **27b** which were hydrolyzed to furnish 4-aryloxyphthalazin-1(2*H*)-ones **28a** and **28b**, respectively. *N*-amination of **28a–b** gave **29a–b** which were coupled with arylboronic acids **14a** and **14e** to yield the desired compounds **30a–c**.

2.3. Antiviral activity against wild-type HIV-1

The compounds **15a–g**, **20a–c**, **25a–o** and **30a–c** were evaluated for their anti-HIV-1 activity and cytotoxicity in TZM-bl cells in comparison with TMC120 (**6**). The results, expressed as EC_{50} (50% effective concentration), CC_{50} (50% cytotoxic concentration) and SI (selectivity index given by the CC_{50}/EC_{50} ratio) values are summarized in Tables 1–3. The compounds **15a–g** showed no activity (Table 1).

Table 2 shows the anti-HIV-1 activity of pyridazinones **20a–c** and **25a–o**. Compound **20a** (R_1=H and R_2=CN) showed micromolar activity (EC_{50} = 1.37 μM). This is about 700 times higher than the EC_{50} value of the corresponding pyrimidine compound **6** (TMC120). Compound **25a**, the oxygen analog of **20a**, was slightly more potent. Replacement of the para-methyl group with chlorine (**25l**) and bromine (**25n**) resulted in similar anti-HIV-1 activity. Moreover, compound **25n** demonstrated less cytotoxicity, resulting in a more favorable selectivity index. Replacement of the para-cyano or its shift to the meta position was detrimental for potency.

Similar conclusions can be formulated for the phthalazinones **30a–c** (Table 3). In summary, replacement of the pyrimidine core of the DAPYs with pyridinone, pyridazinone and phthalazinone resulted in compounds with lower anti-HIV-1 activity in comparison with TMC120, but with similar structure–activity relationship around the two aryl substituents.

2.4. Antiviral activity against mutant strains of HIV-1

Three submicromolar compounds **25a**, **25l** and **25n** were tested against single mutant strains, Ba-L V106A and VI829 Y181C in comparison with **6** (TMC120). These three compounds exhibited moderate activity against the Ba-L V106A with EC_{50} values lower than 5 μM but lost the potency against VI829 Y181C (Table 4).

2.5. Anti-reverse transcriptase activity

The compounds **15a**, **20a**, **25a** and **30a** were further evaluated for anti-reverse transcriptase activity in comparison with **6** (TMC120) and efavirenz in an enzymatic recombinant HIV-1 RT-activity assay and the results are compared with anti-HIV-1 activity (Table 5). All compounds are consistently much more active against the whole virus compared to their inhibitory activity against the reverse transcriptase (150–1250 times). The reasons for this discrepancy remain unclear.

2.6. Crystal structures

In order to confirm the position of amination at nitrogen instead of oxygen with the aminating reagent **12**, we determined the structures of one of the intermediates **24c** and one of the target compounds **25o** by X-ray analysis. Their structures are shown in Figures 4 and 5, respectively.[20]

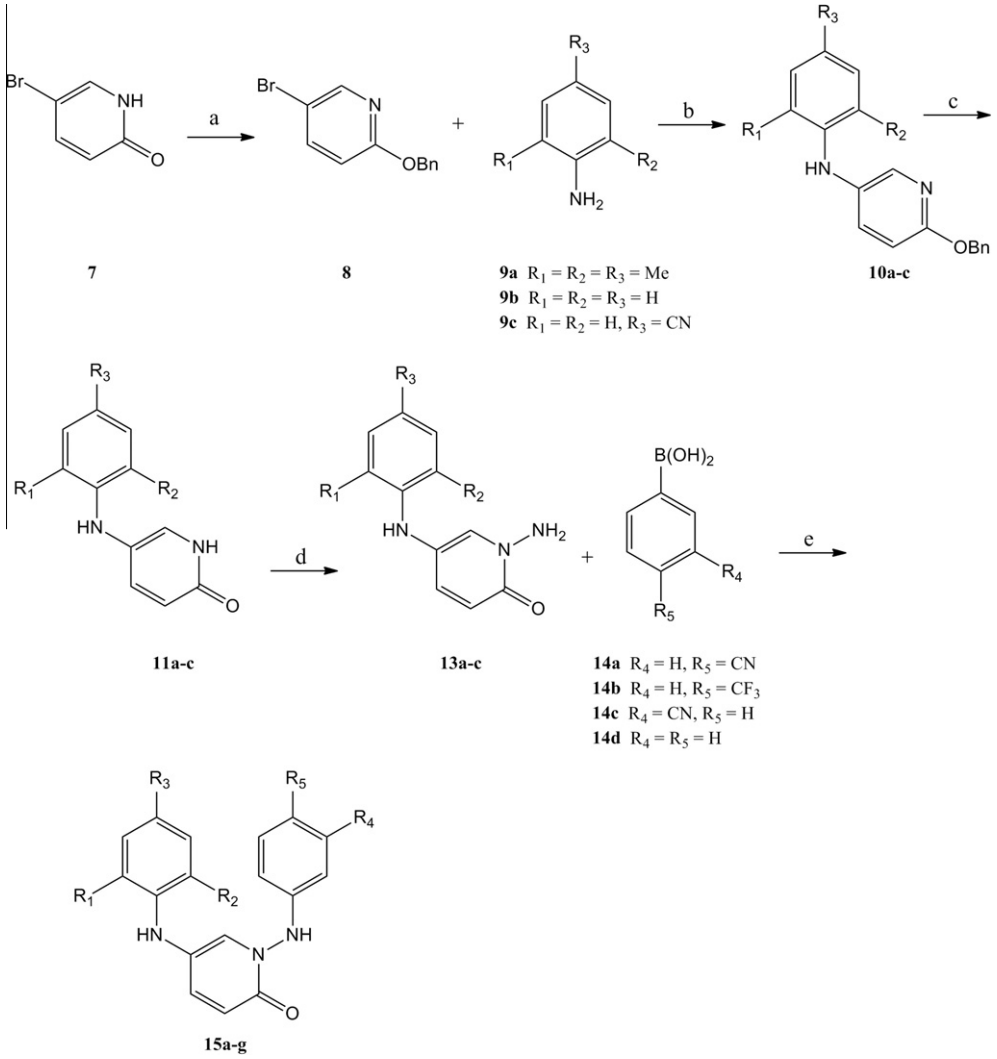
3. Conclusion

In summary, we designed and synthesized three novel series of pyridinones **15a–g** (Series A), pyridazinones **20a–c** and **25a–o** (Series B) and phthalazinones **30a–c** (Series C) and evaluated their activity against wild-type HIV-1 in TZM-bl cells. The most promising compounds in these series are three diarylpyridazinones **25a**, **25l** and **25n** which showed submicromolar activity. Furthermore, **25a**, **25l** and **25n** were tested against single mutant strains, Ba-L V106A and VI829 Y181C. The activities against mutated strains of HIV-1 indicated that the pyridazinones **25a**, **25l** and **25n** showed moderate activity against Ba-L V106A with EC_{50} values lower than 5 μM and no activity against VI829 Y181C. In addition, enzyme inhibitory assays were performed with selected compounds against HIV-1 wtRT.

4. Experimental section

4.1. Chemistry

Reagents were supplied by Sigma–Aldrich and Acros. Characterization of all compounds was done with ^{1}H NMR and mass spectrometry. ^{1}H NMR spectra were recorded on a Bruker Avance DRX-400 spectrometer (400 MHz) and coupling constants (*J*) are reported in Hz. Single crystal X-ray diffraction data were recorded



Scheme 1. Synthetic route to target compounds **15a–g**. Reagents and conditions: (a) $C_6H_5CH_2Br$, Ag_2CO_3, dry THF, reflux; (b) $Pd_2(dba)_3$, 2-dicyclohexylphosphino-2′-(*N*,*N*-dimethylamino)biphenyl, NaO*t*Bu, dry toluene, 85 °C; (c) H_2, Pd/C, MeOH/EtOAc; (d) (diphenylphosphinyl)hydroxyl amine, Cs_2CO_3, dry DMF, 50 °C; (e) $Cu(OAc)_2$, CH_2Cl_2, Et_3N, rt.

at 100 K on a Bruker Smart 1000 system with Mo Kα radiation. Column chromatography was performed on a Flashmaster II instrument (Jones Chromatography) with Isolute columns pre-packed with silica gel. Electrospray ionization (ESI) mass spectra were acquired on an ion trap mass spectrometer (Bruker Daltonics Esquire™ 3000 plus). LC–MS spectra were recorded on an Agilent 1100 Series LC system equipped with a C18 column (2.1 × 50 mm, 5 mm, Supelco, Sigma–Aldrich) coupled to the mass spectrometer; solvent A: H_2O + 0.1% formic acid, solvent B: CH_3CN + 0.1% formic acid; gradient: 5% B → 100% B over 23 min at 0.2 mL min^{-1}. HPLC was performed on a Gilson instrument equipped with a C18 column (4.6 mm × 25 cm, 5 mm, Ultrasphere™ ODS), with standard UV detection at λ 214 nm; solvent A: H_2O + 0.1% trifluoroacetic acid (TFA), solvent B: CH_3CN + 0.1% TFA; gradient: 10% B → 100% B over 36 min at 1 mL min^{-1}. The synthesis of compounds **8**, **17**, **18**, **22a** and **23a** has been reported elsewhere[14h,15] and *O*-(diphenylphosphinyl)hydroxyl amine (**12**) was synthesized according to a reported procedure.[16]

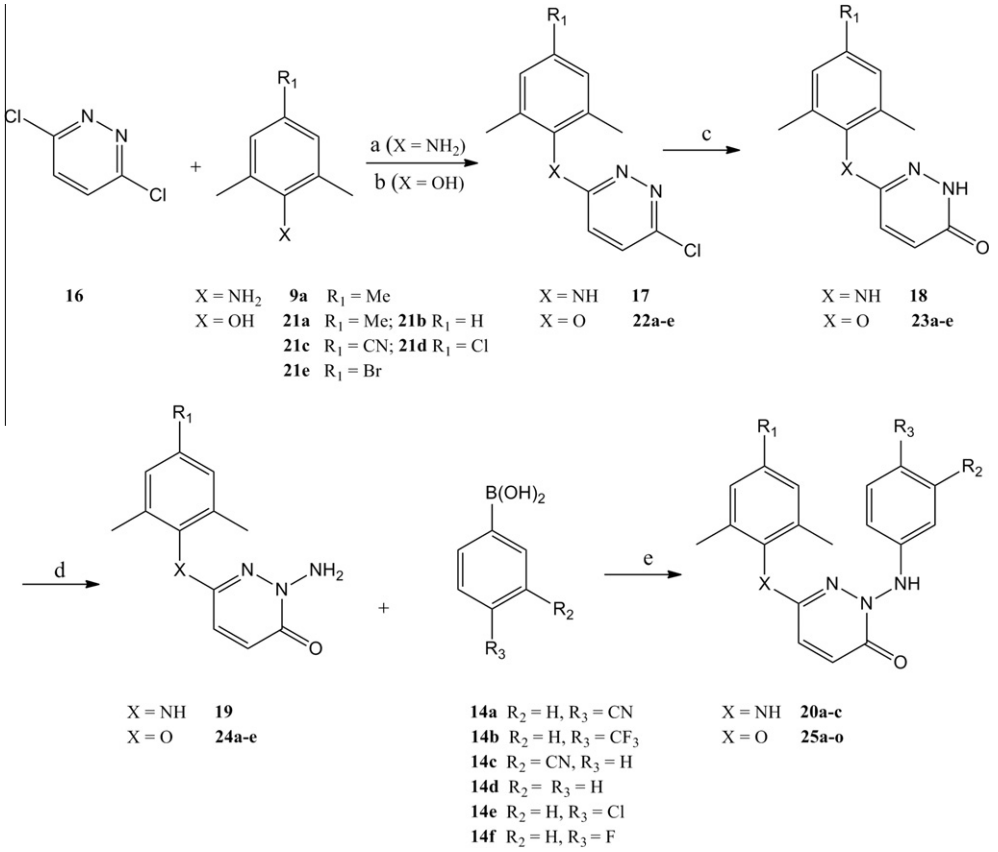
4.2. 2-(benzyloxy)-5-bromopyridine (8)

To a solution of **1** (1.4 g, 8 mmol) in dry THF (80 mL) was added benzyl bromide (1.14 mL, 9.6 mmol) and Ag_2CO_3 (1.32 g, 4.8 mmol) and subsequently refluxed in the dark overnight. The reaction mixture was filtered through celite and concentrated to provide brown solid which was flash chromatographed on silica gel eluting with 10% EtOAc in hexanes to provide 1.8 g (85%) of a white solid;^{1}H NMR ($CDCl_3$) δ (ppm) 5.34 (s, 2H), 6.71 (d, 1H, *J* = 8.7 Hz), 7.25–7.44 (m, 5H), 7.64 (dd, 1H, *J* = 8.8 Hz), 8.20 (d, 1H, *J* = 2.5 Hz); MS (ESI) *m/z* 289 $(M+Na)^+$.

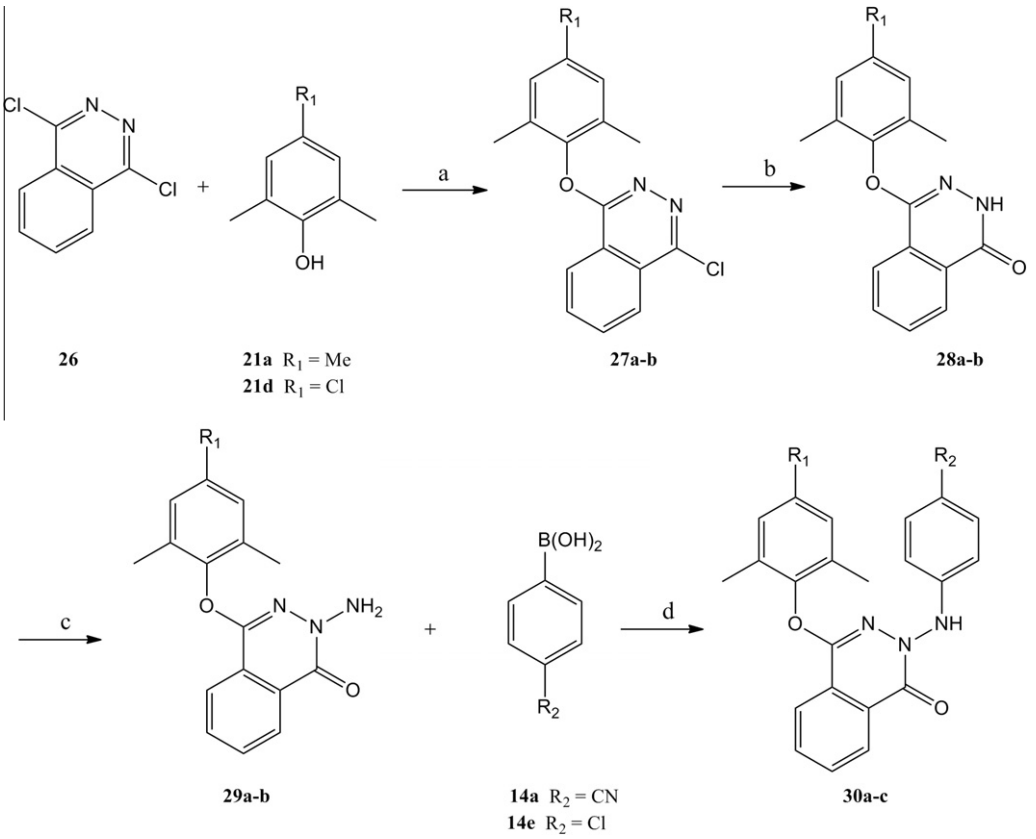
4.2.1. General procedure for the synthesis of 10a–c

Compound **8** (7.5 mmol), **9a–c** (10.5 mmol), Pd_2dba_3 (0.19 mmol), 2′-(dicyclohexyl phosphino)-*N*,*N*-dimethylbiphenyl-2-amine (0.75 mmol) and NaOtBu (10.5 mmol) were weighed in a 50 mL RB flask and dry toluene (20 mL) was added and flushed with argon for 15 min. Subsequently the reaction mixture was stirred at 85 °C overnight, cooled to room temperature, diluted with EtOAc and filtered through a pad of celite and concentrated to obtain a dark brown solid which was flash chromatographed on silica gel eluting with 30% EtOAc in hexanes.

4.2.1.1. 6-(Benzyloxy)-*N*-mesitylpyridin-3-amine (10a). Yield 50%; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.17 (s, 6H), 2.30 (s, 3H), 5.3 (s, 2H), 6.65 (d, 1H, *J* = 8.8 Hz), 6.82 (dd, 1H, *J* = 8.8 Hz), 6.93 (s, 2H),

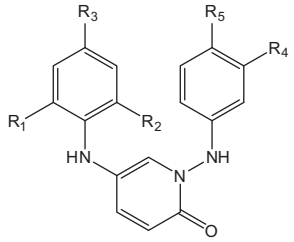


Scheme 2. Synthetic route to target compounds **20a–c** and **25a–o**. Reagents and conditions: (a) Fusion, 150 °C; (b) NaH, DMF, rt; (c) glacial acetic acid, reflux; (d) (diphenylphosphinyl)hydroxyl amine, Cs_2CO_3, dry dioxane, 60 °C; (e) $Cu(OAc)_2$, CH_2Cl_2, Et_3N, rt.



Scheme 3. Synthetic route to target compounds **30a–c**. Reagents and conditions: (a) NaH, DMF, rt; (b) glacial acetic acid, reflux; (c) (diphenylphosphinyl)hydroxyl amine, NaH, dry dioxane, 100 °C; (d) $Cu(OAc)_2$, CH_2Cl_2, Et_3N, rt.

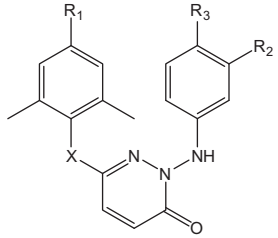
Table 1
Anti-HIV-1 activity of **15a–g** in TZM-bl cells



Compd	R_1	R_2	R_3	R_4	R_5	EC_{50} (μM)	CC_{50} (μM)	SI
15a	Me	Me	Me	H	CN	>10	nd[a]	—
15b	Me	Me	Me	H	CF_3	>10	nd[a]	—
15c	Me	Me	Me	CN	H	>10	nd[a]	—
15d	Me	Me	Me	H	H	>10	nd[a]	—
15e	H	H	H	H	CN	>10	nd[a]	—
15f	H	H	H	H	H	>10	nd[a]	—
15g	H	H	CN	H	CN	>10	nd[a]	—
6						0.002	2.88	1440

[a] nd, not determined when EC_{50} >10 μM.

Table 2
Anti-HIV-1 activity of **20a–c** and **25a–o** in TZM-bl cells



Compd	X	R_1	R_2	R_3	EC_{50} (μM)	CC_{50} (μM)	SI
20a	NH	Me	H	CN	1.37	>100	>69
20b	NH	Me	H	CF_3	>10	nd[a]	—
20c	NH	Me	CN	H	>10	5.04	< 1
25a	O	Me	H	CN	0.69	19.95	29
25b	O	Me	H	CF_3	10	21.15	2.1
25c	O	Me	H	F	9.64	23.53	2.4
25d	O	Me	H	Cl	3.95	22.27	5.6
25e	O	Me	CN	H	>10	nd[a]	—
25f	O	H	H	CN	7.63	>100	>13
25g	O	H	H	F	>10	nd[a]	—
25h	O	H	CN	H	>10	nd[a]	—
25i	O	H	H	H	>10	nd[a]	—
25j	O	CN	H	CN	1.75	24.74	14
25k	O	CN	CN	H	>10	nd[a]	—
25l	O	Cl	H	CN	0.60	14.10	23
25m	O	Cl	CN	H	>5	nd[a]	—
25n	O	Br	H	CN	0.64	>100	>193
25o	O	Br	H	Cl	3.19	63.46	20
6					0.002	2.88	1440

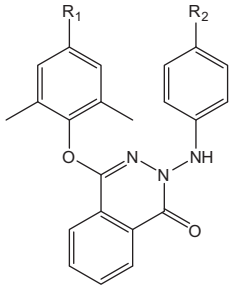
[a] nd, not determined.

7.30–7.39 (m, 3H), 7.44 (d, 2H, *J* = 8.2 Hz), 7.52 (d, 1H, *J* = 3.0 Hz); MS (ESI) *m/z* 320 $(M+H)^+$.

4.2.1.2. 6-(Benzyloxy)-*N*-phenylpyridin-3-amine (10b). Yield 56%; ^1H NMR ($CDCl_3$) δ (ppm) 5.36 (s, 2H), 6.78 (d, 1H, *J* = 8.2 Hz), 6.84–6.88 (m, 3H), 7.20–7.48 (m, 8H), 8.00 (d, 1H, *J* = 2.8 Hz); MS (ESI) *m/z* 278 $(M+H)^+$.

4.2.1.3. 4-(6-(Benzyloxy)pyridin-3-ylamino)benzonitrile (10c). Yield 42%; ^1H NMR ($CDCl_3$) δ (ppm) 5.40 (s, 2H), 6.78 (d, 2H, *J* = 8.4 Hz), 6.86 (d, 1H, *J* = 8.8 Hz), 7.27–7.51 (m, 8H), 8.07 (d, 1H, *J* = 2.7 Hz); MS (ESI) *m/z* 324 $(M+Na)^+$.

Table 3
Anti-HIV-1 activity of **30a–c** in TZM-bl cells



Compd	R_1	R_2	EC_{50} (μM)	CC_{50} (μM)	SI
30a	Me	CN	1.39	60.40	43.3
30b	Cl	CN	3.60	51.23	14.2
30c	Cl	Cl	>10	nd[a]	—
6			0.002	2.88	1440

[a] nd, not determined.

Table 4
Activity of **25a**, **25l** and **25n** against mutated strains of HIV-1

Compd	WT Ba-L	WT VI829	Ba-L V106A		VI829 Y181C	
	EC_{50} (μM)	EC_{50} (μM)	EC_{50} (μM)	FC	EC_{50} (μM)	FC
25a	0.69	0.16	1.12	2	>10	>64
25l	0.60	0.50	4.55	8	>10	>20
25n	0.64	0.34	2.97	5	>10	>29
6	0.002	0.002	0.0025	1.3	0.0108	5.4

Table 5
HIV-1 RT inhibitory activity in comparison with anti HIV-1 activity

Compd	WT Ba-L EC_{50} (μM)	WT RT IC_{50} (μM)
15a	>10	760
20a	1.37	547
25a	0.69	100
30a	1.39	928
6	0.002	2.5
Efavirenz	0.002	1.52

4.2.2. General procedure for synthesis of 11a–c

To a solution of **10a–c** (3 mmol) in MeOH (20 mL) and EtOAc (10 mL) was added 10% Pd/C (1.5 mmol). The reaction mixture was stirred for 3 h under hydrogen atmosphere and filtered through a celite pad. After removal of solvent the crude mixture was chromatographed on silica using 10% MeOH in DCM.

4.2.2.1. 5-(Mesitylamino)pyridin-2(1*H*)-one (11a). Yield 79%; ^1H NMR (MeOD) δ (ppm) 2.15 (s, 6H), 2.25 (s, 3H), 6.17 (dd, 1H, *J* = 3.0 Hz), 6.53 (dd, 1H, *J* = 9.6 Hz), 6.91 (s, 2H), 7.36 (dd, 1H, *J* = 9.6 Hz); MS (ESI) *m/z* 229 $(M+H)^+$.

4.2.2.2. 5-(Phenylamino)pyridin-2(1*H*)-one (11b). Yield 73%; ^1H NMR (MeOD) δ (ppm) 6.57 (dd, 1H, *J* = 9.6 Hz), 6.75–6.81 (m, 3H), 7.14–7.19 (m, 2H), 7.23 (d, 1H, *J* = 2.6 Hz), 7.55 (dd, 1H, *J* = 9.6 Hz); MS (ESI) *m/z* 187 $(M+H)^+$.

4.2.2.3. 4-(6-Oxo-1,6-dihydropyridin-3-ylamino)benzonitrile (11c). Yield 76%; ^1H NMR (MeOD) δ (ppm) 6.60 (dd, 1H, *J* = 9.6 Hz), 6.78–6.80 (m, 2H), 7.33 (d, 1H, *J* = 2.9 Hz), 7.45–7.48 (m, 2H), 7.54 (dd, 1H, *J* = 9.6 Hz); MS (ESI) *m/z* 212 $(M+H)^+$.

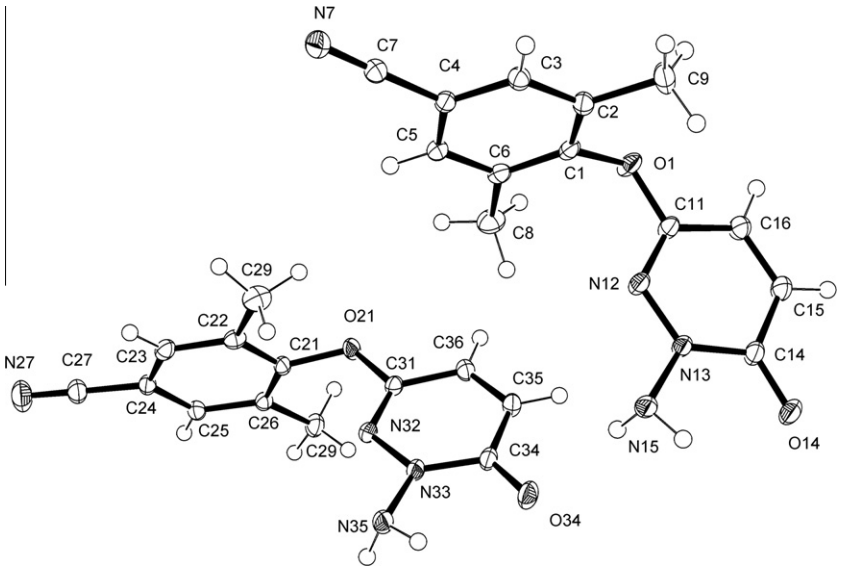


Figure 4. Crystal structure of compound **24c**.

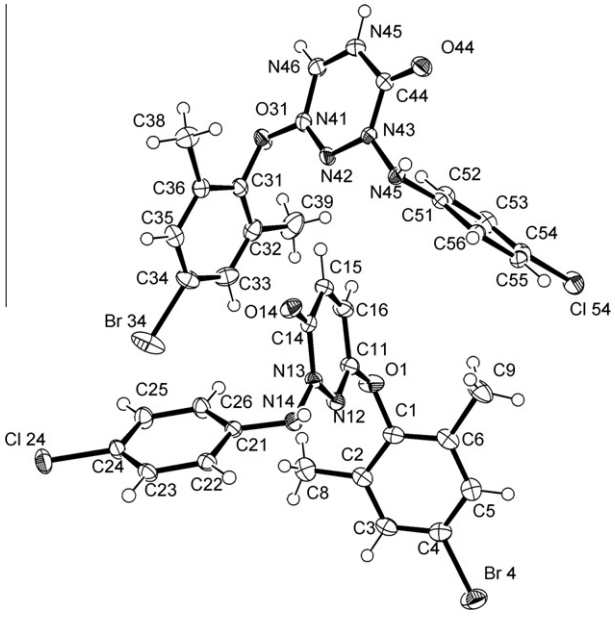


Figure 5. Crystal structure of compound **25o**.

4.2.3. General procedure for the synthesis of 13a–c

To a solution of **11a–c** (1.8 mmol) in DMF (20 mL) was added cesium carbonate (2.16 mmol) and allowed to stir for 15 min. Then **12** (2.16 mmol) was added and the reaction mixture was stirred at room temperature for 36 h and subsequently at 50 °C for 12 h. Water was added to the reaction mixture, extracted with EtOAc (3 × 100 mL) and the combined organic layers were washed with water and evaporated. The crude mixture was chromatographed on silica using 10% MeOH in DCM.

4.2.3.1. 1-Amino-5-(mesitylamino)pyridin-2(1*H*)-one (13a). Yield 41%; ^{1}H NMR (MeOD) δ (ppm) 2.16 (s, 6H), 2.26 (s, 3H), 6.49 (d, 1H, J = 3.0 Hz), 6.55 (d, 1H, J = 9.6 Hz), 6.92 (s, 2H), 7.22 (dd, 1H, J = 9.5 Hz); MS (ESI) m/z 244 $(M+H)^+$.

4.2.3.2. 1-Amino-5-(phenylamino)pyridin-2(1*H*)-one (13b). Yield 58%; ^{1}H NMR (MeOD) δ (ppm) 6.60 (d, 1H, J = 9.6 Hz), 6.75–6.82 (m, 3H), 7.15–7.19 (m, 2H), 7.42 (dd, 1H, J = 9.4 Hz), 7.57 (d, 1H, J = 2.8 Hz); MS (ESI) m/z 202 $(M+H)^+$.

4.2.3.3. 4-(1-Amino-6-oxo-1,6-dihydropyridin-3-ylamino)benzonitrile (13c). Yield 64%; ^{1}H NMR (MeOD) δ (ppm) 6.65 (d, 1H, J = 9.4 Hz), 6.81 (d, 2H, J = 8.3 Hz), 7.43 (br s, 1H), 7.47 (d, 2H, J = 8.1 Hz), 7.69 (br s, 1H); MS (ESI) m/z 227 $(M+H)^+$.

4.2.4. General procedure for the synthesis of 15a–g

To a solution of **13a–c** (0.38 mmol) in dichloromethane (7 mL) was added diacetoxycopper hydrate (0.38 mmol), Et_3N (0.45 mmol) and **14a–d** (0.45 mmol) in dichloromethane (0.5 mL) was added dropwise, allowed to stir at room temperature for 2 h. Then the reaction mixture was concentrated and chromatographed on silica using 10% MeOH in DCM. The resulting fine sticky powders/oily materials were lyophilized to powders.

4.2.4.1. 4-(5-(Mesitylamino)-2-oxopyridin-1(2*H*)-ylamino)-benzonitrile (15a). Yield 23%; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.19 (s, 6H), 2.26 (s, 3H), 6.41 (br s, 1H), 6.63 (d, 2H, J = 8.4 Hz), 6.74 (br s, 1H), 6.90 (br s, 2H), 7.20–7.30 (m, 2H), 7.45 (d, 2H, J = 8.4 Hz); MS (ESI) m/z 345 $(M+H)^+$; HPLC (214 nm) t_r 23.8 min, 98%; LC–MS (214 nm) t_r 17.1 min, 100%.

4.2.4.2. 5-(Mesitylamino)-1-(4-(trifluoromethyl)phenylamino)-pyridin-2(1*H*)-one (15b). Yield 26%; ^{1}H NMR (MeOD) δ (ppm) 2.19 (s, 6H), 2.24 (s, 3H), 6.67 (d, 2H, J = 7.8 Hz), 6.89 (d, 2H, J = 8.7 Hz), 7.46 (br s, 2H), 7.61 (br s, 2H), 7.77 (br s, 1H); MS (ESI) m/z 388 $(M+H)^+$; HPLC (214 nm) t_r 27.4 min, 85%.

4.2.4.3. 3-(5-(Mesitylamino)-2-oxopyridin-1(2*H*)-ylamino)-benzonitrile (15c). Yield 22%; ^{1}H NMR (MeOD) δ (ppm) 2.20 (s, 6H), 2.23 (s, 3H), 6.20 (br s, 1H), 6.69 (d, 1H, J = 9.6 Hz), 6.80 (br s, 1H), 6.86 (d, 1H, J = 7.8 Hz), 6.91 (br s, 2H), 7.20 (d, 1H, J = 7.3 Hz), 7.36 (t, 1H, J = 7.9 Hz), 7.45 (d, 1H, J = 8.9 Hz); MS 346 $(M+H)^+$; HPLC (214 nm) t_r 23.5 min, 95%.

4.2.4.4. 5-(Mesitylamino)-1-(phenylamino)pyridin-2(1*H*)-one (15d). Yield 25%; ^{1}H NMR (MeOD) δ (ppm) 2.15 (s, 6H), 2.84 (s, 3H), 6.30 (br s, 1H), 6.58 (d, 2H, J = 7.0 Hz), 6.66 (d, 1H,

J = 9.9 Hz), 6.90 (br s, 1H), 7.18 (t, 2H, *J* = 7.6 Hz), 7.34 (d, 2H, *J* = 8.7 Hz), 7.59 (d, 1H, *J* = 7.0 Hz); MS (ESI) *m/z* 342 $(M+Na)^+$; HPLC (214 nm) t_r 24.8 min, 77%.

4.2.4.5. 4-(2-Oxo-5-(phenylamino)pyridin-1(2*H*)-ylamino)benzonitrile (15e). Yield 39%; ^{1}H NMR (MeOD) δ (ppm) 6.71 (d, 1H, *J* = 8.70 Hz), 6.73–6.78 (m, 1H), 6.80–6.82 (m, 1H), 6.85–6.87 (m, 2H), 7.17–7.21 (m, 2H), 7.43 (d, 1H, *J* = 2.8 Hz), 7.54–7.57 (m, 2H), 7.59 (d, 1H, *J* = 3.0 Hz), 7.61 (d, 1H, *J* = 3.0 Hz); MS (ESI) *m/z* 303 $(M+H)^+$; HPLC (214 nm) t_r 19.4 min, 100%; LC–MS (214 nm) t_r 14.8 min, 100%.

4.2.4.6. 1,5-Bis(phenylamino)pyridin-2(1*H*)-one (15f). Yield 26%; ^{1}H NMR (MeOD) δ (ppm) 6.70–6.73 (m, 2H), 6.77–6.81 (m, 1H), 6.84 (dd, 2H, *J* = 8.6 Hz) 6.90–6.94 (m, 1H), 7.14–7.25 (m, 4H), 7.30–7.38 (m, 1H), 7.50 (d, 1H, *J* = 2.7 Hz), 7.60 (dd, 1H, *J* = 7.0.0 Hz); MS (ESI) *m/z* 300 $(M+Na)^+$; HPLC (214 nm) t_r 20.9 min, 77%.

4.2.4.7. 4-((5-((4-Cyanophenyl)amino)-2-oxopyridin-1(2*H*)-yl)-amino)benzonitrile (15g). Yield 37%; ^{1}H NMR (MeOD) δ (ppm) 6.75–6.80 (m, 3H), 6.85–6.87 (m, 2H), 7.48–7.50 (m, 2H), 7.56–7.59 (m, 2H), 7.62 (dd, 2H, *J* = 7.3 Hz); MS (ESI) *m/z* 328 $(M+H)^+$; HPLC (214 nm) t_r 17.6 min, 100%; LC–MS (214 nm) t_r 14.2 min, 100%.

4.2.4.8. 6-Chloro-*N*-mesitylpyridazin-3-amine (17). Compound **16** (7.45 g, 50 mmol) and **9a** (7.75 mL, 55 mmol) were mixed together and stirred at 150 °C for 3 h. The crude mixture was chromatographed on silica using 10% MeOH in DCM to provide 1.8 g (40%) of a white solid. ^{1}H NMR ($CDCl_3$) δ (ppm) 2.17 (br s, 6H), 2.30 (s, 3H), 6.28 (d, 1H, *J* = 9.1 Hz), 6.94 (s, 2H), 7.13 (d, 1H, *J* = 9.4 Hz); MS (ESI) *m/z* 248 $(M+H)^+$.

4.2.4.9. 6-(Mesitylamino)pyridazin-3(2*H*)-one (18). Compound **17** (1.98 g, 8 mmol) was refluxed in glacial acetic acid (25 mL) at 120 °C for 6 h. The solvent was removed under reduced pressure, water (50 mL) was added and the solid product obtained was collected by filtration. Purification of the crude solid by column chromatography using 8% MeOH in DCM afforded 0.14 g (51%) white solid. ^{1}H NMR ($CDCl_3$) δ (ppm) 2.16 (s, 6H), 2.25 (s, 3H), 6.85 (d, 1H, *J* = 9.9 Hz), 6.88 (s, 2H), 7.12 (d, 1H, *J* = 9.9 Hz); MS (ESI) *m/z* 228 $(M-H)^-$.

4.2.4.10. 2-Amino-6-(mesitylamino)pyridazin-3(2*H*)-one (19). To a solution of **18** (0.46 g, 2 mmol) in dry dioxane (12 mL) was added NaH (90 mg, 2.2 mmol). The mixture was stirred at 60 °C for 1 h and then cooled to room temperature, **12** (0.52 g, 2.2 mmol) was added and allowed to stir at 60° for 24 h. Water was added to the reaction mixture, extracted with EtOAc (3 × 75 mL) and the combined organic layers were washed with water. Then the evaporation of solvent afforded a light brown solid which was used in next step without further purification. MS (ESI) *m/z* 267 $(M+Na)^+$.

4.2.5. General procedure for the synthesis of 20a–c

To a solution of **19** (0.6 mmol) in dichloromethane (9 mL) was added diacetoxycopper hydrate (0.6 mmol), Et_3N (0.72 mmol) and **14a–c** (0.72 mmol) in dichloromethane (1 mL) was added dropwise and allowed to stir at room temperature for 2 h. The reaction mixture was then concentrated and chromatographed on silica using 10% MeOH in DCM. The resulting oily materials were lyophilized to powders.

4.2.5.1. 4-(3-(Mesitylamino)-6-oxopyridazin-1(6*H*)-ylamino)-benzonitrile (20a). Yield 17%; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.15 (s, 6H), 2.21 (s, 3H), 6.67 (d, 2H, *J* = 7.6 Hz), 6.84 (br s, 2H), 7.03 (d, 1H, *J* = 9.6 Hz), 7.22 (d, 1H, *J* = 9.0 Hz), 7.50 (d, 2H, *J* = 8.0 Hz); MS (ESI) *m/z* 346 $(M+H)^+$; HPLC (214 nm) t_r 20.4 min, 100%.

4.2.5.2. 6-(Mesitylamino)-2-(4-(trifluoromethyl)phenylamino)-pyridazin-3(2*H*)-one (20b). Yield 26%; ^{1}H NMR (MeOD) δ (ppm) 2.15 (s, 6H), 2.20 (s, 3H), 6.70 (br s, 2H), 6.84 (br s, 2H), 7.04 (d, 1H, *J* = 9.7 Hz), 7.21 (br s, 1H), 7.46 (d, 2H, *J* = 7.5 Hz); MS (ESI) *m/z* 389 $(M+H)^+$; HPLC (214 nm) t_r 24.1 min, 97%.

4.2.5.3. 3-(3-(Mesitylamino)-6-oxopyridazin-1(6*H*)-ylamino)-benzonitrile (20c). Yield 16%; ^{1}H NMR (MeOD) δ (ppm) 2.15 (s, 6H), 2.19 (s, 3H), 6.83 (br s, 4H), 7.03 (d, 1H, *J* = 9.7 Hz), 7.24–7.33 (m, 3H); MS (ESI) *m/z* 346 $(M+H)^+$; HPLC (214 nm) t_r 20.5 min, 100%.

4.2.6. General procedure for the synthesis of 22a–e

To a solution of **16** (10 mmol) and **21a–e** (10 mmol) in dry DMF (20 mL) was added NaH (11 mmol) portionwise (1 h) and the reaction mixture was stirred at room temperature for 3 h, poured on ice-cold water (40 mL), the precipitated solid was filtered and dried under high vacuum.

4.2.6.1. 3-Chloro-6-(mesityloxy)pyridazine (22a). Yield 80%; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.10 (s, 6H), 2.31 (s, 3H), 6.93 (s, 2H), 7.12 (d, 1H, *J* = 9.1 Hz), 7.47 (d, 1H, *J* = 9.1 Hz); MS (ESI) *m/z* 273 $(M+Na)^+$.

4.2.6.2. 3-Chloro-6-(2,6-dimethylphenoxy)pyridazine (22b). Yield 78%; ^{1}H NMR (DMSO-d_6) δ (ppm) 2.05 (s, 6H), 7.10–7.18 (m, 3H), 7.62 (d, 1H, *J* = 9.2 Hz), 7.96 (d, 1H, *J* = 9.1 Hz); MS (ESI) *m/z* 259 $(M+Na)^+$.

4.2.6.3. 4-(6-Chloropyridazin-3-yloxy)-3,5-dimethylbenzonitrile (22c). Yield 80%; ^{1}H NMR (DMSO-d_6) δ (ppm) 2.10 (s, 6H), 7.20 (s, 2H), 7.42 (d, 1H, *J* = 9.1 Hz), 7.60 (d, 1H, *J* = 9.1 Hz); MS (ESI) *m/z* 261 $(M+Na)^+$.

4.2.6.4. 3-Chloro-6-(4-chloro-2,6-dimethylphenoxy)pyridazine (22d). Yield 71%; ^{1}H NMR (DMSO-d_6) δ (ppm) 2.05 (s, 6H), 7.28 (s, 2H), 7.67 (d, 1H, *J* = 9.1 Hz), 7.98 (d, 1H, *J* = 9.2 Hz); MS (ESI) *m/z* 293 $(M+Na)^+$.

4.2.6.5. 3-(4-Bromo-2,6-dimethylphenoxy)-6-chloropyridazine (22e). Yield 87%; ^{1}H NMR (DMSO-d_6) δ (ppm) 2.05 (s, 6H), 7.40 (s, 2H), 7.67 (d, 1H, *J* = 9.2 Hz), 7.99 (d, 1H, *J* = 9.2 Hz); MS (ESI) *m/z* 337 $(M+Na)^+$.

4.2.7. General procedure for the synthesis of 23a–e

Compounds **22a–e** (4 mmol) were refluxed in glacial acetic acid (25 mL) at 120 °C for 6 h. The solvent was removed under reduced pressure, water (50 mL) was added and the solid products formed were collected by filtration.

4.2.7.1. 6-(Mesityloxy)pyridazin-3(2*H*)-one (23a). Yield 79%; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.13 (s, 6H), 2.31 (s, 3H), 6.92 (s, 2H), 7.04 (d, 1H, *J* = 9.9 Hz), 7.28 (d, 1H, *J* = 9.9 Hz), 9.94 (s, 1H); MS (ESI) *m/z* 231 $(M+H)^+$.

4.2.7.2. 6-(2,6-Dimethylphenoxy)pyridazin-3(2*H*)-one (23b). Yield 88%; ^{1}H NMR (DMSO-d_6) δ (ppm) 2.10 (s, 6H), 7.01–7.08 (m, 2H), 7.11 (br s, 1H), 7.13 (br s, 1H), 7.48 (d, 1H, *J* = 9.9 Hz), 12.1 (s, 1H); MS (ESI) *m/z* 239 $(M+Na)^+$.

4.2.7.3. 3,5-Dimethyl-4-(6-oxo-1,6-dihydropyridazin-3-yloxy)-benzonitrile (23c). Yield 54%; ^{1}H NMR (DMSO-d_6) δ (ppm) 2.13 (s, 6H), 7.06 (d, 1H, *J* = 9.9 Hz), 7.53 (d, 1H, *J* = 9.9 Hz), 7.67 (s, 2H), 12.17 (s, 1H); MS (ESI) *m/z* 264 $(M+Na)^+$.

4.2.7.4. 6-(4-Chloro-2,6-dimethylphenoxy)pyridazin-3(2*H*)-one (23d). Yield 97%; ^{1}H NMR (DMSO-d_6) δ (ppm) 2.08 (s, 6H), 7.03 (d, 1H, J = 9.9 Hz), 7.20 (s, 2H), 7.49 (d, 1H, J = 9.9 Hz), 12.12 (s, 1H); MS (ESI) m/z 275 (M+Na)$^+$.

4.2.7.5. 6-(4-Bromo-2,6-dimethylphenoxy)pyridazin-3(2*H*)-one (23e). Yield 91%; ^{1}H NMR (DMSO-d_6) δ (ppm) 2.08 (s, 6H), 7.03 (d, 1H, J = 9.9 Hz), 7.36 (s, 2H), 7.49 (d, 1H, J = 9.9 Hz), 12.12 (s, 1H); MS (ESI) m/z 319 (M+Na)$^+$.

4.2.8. General procedure for the synthesis of 24a–e

To a solution of **23a–e** (2 mmol) in dry dioxane (12 mL) was added NaH (2.2 mmol). The mixture was stirred at 60 °C for 1 h and then cooled to room temperature, **12** (2.2 mmol) was added and allowed to stir at 60 °C for 24 h. Water was added to the reaction mixture and extracted with EtOAc (3 × 75 mL). The combined organic layers were washed with water and evaporation of solvent afforded light brown solids which were used in next step without further purification.

4.2.9. General procedure for the synthesis of 25a–o

To a solution of **24a–e** (0.8 mmol) in dichloromethane (7 mL) was added diacetoxycopper hydrate (0.8 mmol), Et_3N (0.96 mmol) and **14a–f** (0.96 mmol) in dichloromethane (1.5 mL) was added dropwise and allowed to stir at room temperature for 2 h. The reaction mixture was concentrated and chromatographed on silica using 10% MeOH in DCM. The resulting fine sticky powders/oily materials were lyophilized to powders.

4.2.9.1. 4-(3-(Mesityloxy)-6-oxopyridazin-1(6*H*)-ylamino)benzonitrile (25a). Yield 11%; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.13 (s, 6H), 2.23 (s, 3H), 6.60 (br s, 2H), 6.85 (s, 2H), 7.35 (br s, 1H), 7.45 (br s, 2H), 7.78 (br s, 1H); MS (ESI) m/z 347 (M+H)$^+$; HPLC (214 nm) t_r 22.3 min, 100%.

4.2.9.2. 6-(Mesityloxy)-2-(4-(trifluoromethyl)phenylamino)pyridazin-3(2*H*)-one (25b). Yield 20%; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.13 (s, 6H), 2.24 (s, 3H), 6.70 (d, 2H, J = 8.4 Hz), 6.85 (br s, 2H), 7.20 (d, 1H, J = 9.8 Hz), 7.31 (d, 1H, J = 9.2 Hz), 7.42 (d, 1H, J = 8.4 Hz), 7.55 (br s, 1H); MS (ESI) m/z 390 (M+H)$^+$; LC–MS (214 nm) t_r 16.8 min, 100%.

4.2.9.3. 2-(4-Fluorophenylamino)-6-(mesityloxy)pyridazin-3(2*H*)-one (25c). Yield 15%; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.12 (s, 6H), 2.29 (s, 3H), 6.77 (br s, 2H), 6.89 (s, 2H), 6.94 (br s, 2H), 7.20 (d, 1H, J = 9.4 Hz), 7.55 (br s, 1H); MS (ESI) m/z 362 (M+Na)$^+$; LC–MS (214 nm) t_r 14.6 min, 100%.

4.2.9.4. 2-(4-Chlorophenylamino)-6-(mesityloxy)pyridazin-3(2*H*)-one (25d). Yield 17%; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.13 (s, 6H), 2.29 (s, 3H), 6.88 (br s, 2H), 6.89 (d, 2H, J = 9.4 Hz), 7.15–7.22 (m, 2H), 7.28–7.33 (m, 1H), 7.47–7.54 (m, 1H); MS (ESI) m/z 380 (M+Na)$^+$; LC–MS (214 nm) t_r 16.8 min, 100%.

4.2.9.5. 3-(3-(Mesityloxy)-6-oxopyridazin-1(6*H*)-ylamino)benzonitrile (25e). Yield 11%; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.13 (s, 6H), 2.23 (s, 3H), 6.79 (s, 1H), 6.85 (s, 3H), 7.19–7.28 (m, 2H), 7.34 (d, 1H, J = 9.8 Hz), 7.97 (br s, 1H); MS (ESI) m/z 369 (M+Na)$^+$; HPLC (214 nm) t_r 22.9 min, 100%.

4.2.9.6. 4-(3-(2,6-Dimethylphenoxy)-6-oxopyridazin-1(6*H*)-ylamino)benzonitrile (25f). Yield 15%; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.17 (s, 6H), 6.68 (d, 1H, J = 8.8 Hz), 7.05 (s, 3H), 7.21 (d, 1H, J = 9.8 Hz), 7.34 (d, 1H, J = 9.8 Hz), 7.45–7.49 (m, 3H); MS (ESI) m/z 355 (M+Na)$^+$; HPLC (214 nm) t_r 20.5 min, 99%.

4.2.9.7. 6-(2,6-Dimethylphenoxy)-2-(4-fluorophenylamino)pyridazin-3(2*H*)-one (25g). Yield 14%; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.18 (s, 6H), 6.79–6.82 (m, 2H), 6.89–6.93 (m, 2H), 7.10 (s, 2H), 7.18 (d, 1H, J = 9.8 Hz), 7.28 (d, 1H, J = 4.0 Hz), 7.57 (br s, 1H); MS (ESI) m/z 348 (M+Na)$^+$; HPLC (214 nm) t_r 22.1 min, 98%.

4.2.9.8. 3-(3-(2,6-Dimethylphenoxy)-6-oxopyridazin-1(6*H*)-ylamino)benzonitrile (25h). Yield 15%; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.18 (s, 6H), 6.86 (s, 1H), 6.92 (d, 1H, J = 9.8 Hz), 7.00–7.11 (m, 3H), 7.18–7.39 (m, 3H), 7.81 (br s, 1H); MS (ESI) m/z 355 (M+Na)$^+$; HPLC (214 nm) t_r 20.8 min, 99%.

4.2.9.9. 6-(2,6-Dimethylphenoxy)-2-(phenylamino)pyridazin-3(2*H*)-one (25i). Yield 12%; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.19 (s, 6H), 6.77–6.81 (m, 2H), 6.89–7.03 (m, 1H), 7.07 (br s, 2H), 7.19–7.24 (m, 3H), 7.29 (d, 1H, J = 9.8 Hz), 7.53 (br s, 1H); MS (ESI) m/z 330 (M+Na)$^+$; HPLC (214 nm) t_r 21.7 min, 97%.

4.2.9.10. 4-(1-(4-Cyanophenylamino)-6-oxo-1,6-dihydropyridazin-3-yloxy)-3,5-dimethylbenzonitrile (25j). Yield 22%; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.21 (s, 6H), 6.65 (d, 2H, J = 8.6 Hz), 7.27–7.30 (m, 1H), 7.38 (t, 3H, J = 9.2 Hz), 7.49 (d, 1H, J = 8.6 Hz), 7.59 (br s, 1H); MS (ESI) m/z 380 (M+Na)$^+$; LC–MS (214 nm) t_r 15.8 min, 92%.

4.2.9.11. 4-(1-(3-Cyanophenylamino)-6-oxo-1,6-dihydropyridazin-3-yloxy)-3,5-dimethylbenzonitrile (25k). Yield 20%; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.23 (s, 6H), 6.86 (br s, 1H), 6.97 (br s, 1H), 7.28–7.37 (m, 2H), 7.40–7.49 (m, 3H), 7.67 (br s, 1H); MS (ESI) m/z 380 (M+Na)$^+$; LC–MS (214 nm) t_r 16.1 min, 100%

4.2.9.12. 4-(3-(4-Chloro-2,6-dimethylphenoxy)-6-oxopyridazin-1(6*H*)-ylamino)benzonitrile (25l). Yield 14%; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.13 (s, 6H), 6.61 (d, 1H, J = 9.3 Hz), 7.04 (s, 2H), 7.22–7.26 (m, 1H), 7.56–7.64 (m, 4H); MS (ESI) m/z 389 (M+Na)$^+$; HPLC (214 nm) t_r 22.69 min, 98%

4.2.9.13. 3-(3-(4-Chloro-2,6-dimethylphenoxy)-6-oxopyridazin-1(6*H*)-ylamino)benzonitrile (25m). Yield 21%; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.16 (s, 6H), 6.87 (s, 1H), 6.95 (d, 1H, J = 9.8 Hz), 7.07 (br s, 2H), 7.22–7.37 (m, 4H), 7.66 (br s, 1H); MS (ESI) m/z 389 (M+Na)$^+$; HPLC (214 nm) t_r 22.9 min, 90%.

4.2.9.14. 4-(3-(4-Bromo-2,6-dimethylphenoxy)-6-oxopyridazin-1(6*H*)-ylamino)benzonitrile (25n). Yield 17%; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.14 (s, 6H), 6.65 (d, 2H, J = 7.0 Hz), 7.20 (d, 2H, J = 9.2 Hz), 7.24 (s, 1H), 7.35 (d, 1H, J = 9.8 Hz), 7.47 (d, 2H, J = 7.0 Hz), 7.60 (s, 1H); MS (ESI) m/z 413 (M+H)$^+$; LC–MS (214 nm) t_r 17.2 min, 100%.

4.2.9.15. 6-(4-Bromo-2,6-dimethylphenoxy)-2-(4-chlorophenylamino)pyridazin-3(2*H*)-one (25o). Yield 16%; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.12 (s, 6H), 6.65 (d, 2H, J = 6.8 Hz), 7.12–7.16 (m, 3H), 7.27 (d, 2H, J = 9.8 Hz), 7.56 (s, 1H); MS (ESI) m/z 422 (M+H)$^+$; LC–MS (214 nm) t_r 19.1 min, 100%.

4.2.10. General procedure for the synthesis of 27a–b

To a solution of **26** (1 mmol) and **21a** and **21d** (1 mmol) in dry DMF (5 mL) was added NaH (1.1 mmol) portionwise and the reaction mixture was allowed to stir at room temperature for 3 h, poured on ice-cold water (20 mL) and the solid precipitated out was filtered and dried under high vacuum.

4.2.10.1. 1-Chloro-4-(mesityloxy)phthalazine (27a). Yield 97%; ^{1}H NMR (DMSO-d_6) δ (ppm) 2.04 (s, 6H), 2.31 (s, 3H), 7.0 (s, 2H), 8.22–8.26 (m, 2H), 8.29 (br s, 1H), 8.54–8.56 (m, 1H); MS (ESI) m/z 299 (M+H)$^+$.

4.2.10.2. 1-Chloro-4-(4-chloro-2,6-dimethylphenoxy)phthalazine (27b). Yield 98%; ^{1}H NMR (DMSO-d_6) δ (ppm) 2.13 (s, 6H), 7.3 (s, 2H), 8.21–8.29 (m, 2H), 8.30–8.33 (m, 1H), 8.53–8.57 (m, 1H); MS (ESI) *m/z* 320 $(M+H)^+$.

4.2.11. General procedure for the synthesis of 28a–b

Compounds **27a–b** (4 mmol) were refluxed in glacial acetic acid (25 mL) at 120 °C for 6 h. The solvent was removed under reduced pressure, water (50 mL) was added and the solid products obtained were collected by filtration.

4.2.11.1. 4-(Mesityloxy)phthalazin-1(2*H*)-one (28a). Yield 87%; ^{1}H NMR (DMSO-d_6) δ (ppm) 2.07 (s, 6H), 2.26 (s, 3H), 6.95 (s, 2H), 7.96–8.07 (m, 2H), 8.24–8.30 (m, 2H), 11.8 (s, 1H); MS (ESI) *m/z* 303 $(M+Na)^+$.

4.2.11.2. 4-(4-Chloro-2,6-dimethylphenoxy)phthalazin-1(2*H*)-one (28b). Yield 90%; ^{1}H NMR (DMSO-d_6) δ (ppm) 2.11 (s, 6H), 7.26 (s, 2H), 7.97–8.07 (m, 2H), 8.24–8.30 (m, 2H), 11.8 (s, 1H); MS (ESI) *m/z* 324 $(M+Na)^+$

4.2.12. General procedure for the synthesis of 29a–b

To a solution of **28a–b** (2.2 mmol) in dry dioxane (12 mL) was added NaH (3.3 mmol). The mixture was stirred at 100 °C for 1 h and then cooled to room temperature, **12** (2.86 mmol) was added and allowed to stir at 100 °C for 24 h. Water was added to the reaction mixture and extracted with EtOAc (3 × 75 mL). The combined organic layers were washed with water and evaporation of solvent afforded light brown solids which were used in the next step without further purification.

4.2.13. General procedure for the synthesis of 30a–c

To a solution of **29a–b** (0.75 mmol) in dichloromethane (7 mL) was added diacetoxycopper hydrate (0.75 mmol), Et_3N (0.9 mmol) and **14a/14e** (0.9 mmol) in dichloromethane (1.5 mL) was added dropwise and allowed to stir at room temperature for 2 h. The reaction mixture was concentrated and chromatographed on silica using 5% MeOH in DCM. The resulting oily materials were lyophilized to powders.

4.2.13.1. 4-(4-(Mesityloxy)-1-oxophthalazin-2(1*H*)-ylamino)-benzonitrile (30a). Yield 40%; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.16 (s, 6H), 2.25 (s, 3H), 6.55 (d, 2H, *J* = 8.80 Hz), 6.83 (s, 2H), 7.35 (d, 2H, *J* = 7.0 Hz), 7.69 (s, 1H), 7.88–7.99 (m, 2H), 8.31 (d, 1H, *J* = 8.0 Hz), 8.49 (d, 1H, *J* = 8.0 Hz); MS (ESI) *m/z* 397 $(M+H)^+$; LC–MS (214 nm) t_r 20.1 min, 100%.

4.2.13.2. 4-(4-(4-Chloro-2,6-dimethylphenoxy)-1-oxophthalazin-2(1*H*)-ylamino)benzonitrile (30b). Yield 18%; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.15 (s, 6H), 6.65 (d, 2H, *J* = 7.0 Hz), 7.05 (s, 2H), 7.43 (d, 2H, *J* = 7.4 Hz), 7.55 (s, 1H), 7.9–8.01 (m, 2H), 8.30 (d, 1H, *J* = 7.4 Hz), 8.48 (d, 1H, *J* = 7.4 Hz); MS (ESI) *m/z* 418 $(M+H)^+$; LC–MS (214 nm) t_r 19.2 min, 100%.

4.2.13.3. 4-(4-Chloro-2,6-dimethylphenoxy)-2-(4-chlorophenylamino)phthalazin-1(2*H*)-one (30c). Yield 24%; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.16 (s, 6H), 6.68 (d, 2H, *J* = 7.0 Hz), 7.07 (s, 2H), 7.13–7.21 (m, 3H), 7.9–8.0 (m, 2H), 8.3 (d, 1H, *J* = 7.1 Hz), 8.48 (d, 1H, *J* = 7.0 Hz); MS (ESI) *m/z* 427 $(M+H)^+$; LC–MS (214 nm) t_r 21.6 min, 100%.

4.3. Modeling

The molecular modeling study was performed using MOE 2009.10 software (Chemical Computing Group) to investigate the binding mode of our compounds with NNBS of HIV-1 RT. The coordinates of the NNBS were taken from the crystal structure of the RT/4-(4-(mesitylamino)pyrimidin-2-ylamino)benzonitrile (TMC120) complex (pdb code: 1S6Q).[9b] Throughout the docking process, the program standard settings were employed, unless stated otherwise. Prior to the docking, the X-ray structure was protonated with the Protonate 3D algorithm. First, selected target compounds were drawn in MOE, protonated, minimized and merged in a database. Then, this database was used to generate all possible conformations of these compounds using the Conf-search algorithm (Low mode). All these low energy conformations were withheld for docking. Finally, an automated docking protocol was employed to dock all conformations in the active site of HIV-1-RT, using TMC-120 as a template ligand. The first pose, sorted on docking score, of each docked compound was used as such in Figure 3.

4.4. Antiviral assay

4.4.1. Cells

The JC53-BL cell line, also known as the TZM-bl cell line (NIH AIDS Research and Reference Reagent Program, Germantown, USA), was used for the evaluation of anti-HIV-1 activity of the diarylpyridinones, diarylpyridazinones and diarylphthalazinones. TZM-bl cells were cultured in Dulbecco's Minimum Essential Medium (DMEM) (Lonza) containing 10% heat-inactivated FBS and 50 μg gentamycin/mL at 37 °C in a humidified 5% CO_2, 95% air environment. Twice a week the cells were treated with 0.25% trypsin – 1 mM EDTA (Lonza) for 10 minutes. The resulting cell suspension was washed with an equivalent amount of TZM-bl medium and subsequently seeded in a T75 culture flask (Greiner Bio-One, Germany) at 10^6 cells in 20 mL medium.

4.4.2. TZM-bl virus inhibition assay

The antiviral activity of the newly designed compounds was measured by pre-incubating ten thousand TZM-bl cells (at 10^5 cells/mL in culture medium supplemented with 30 μg/mL DEAE dextran) in a 96-well plates for 30 min at 37 °C, 5% CO_2 in the presence or absence of serial dilutions of the respective compound. Subsequently, 200 $TCID_{50}$ of HIV-1 BaL or mutant virus (V106A or Y181C) was added to each well and cultures were incubated for 48 h before quantifying luciferase activity, using a TriStar LB941 luminometer (Berthold Technologies GmbH & Co., KG, Bad Wildbad, Germany). Each condition was evaluated in triplicate wells and in at least two independent experiments. The antiviral activity of the compound was expressed as the percentage of viral inhibition compared to the untreated controls and subsequently plotted against the compound concentration. Nonlinear regression analysis was used to calculate the 50% effective concentration (EC_{50}) based on at least two independent measurements and using GraphPad Prism version 5.03 for Windows (GraphPad Software, San Diego, CA, USA).

4.4.3. Reverse transcriptase activity assay

In order to asses the activity of a selected number of compounds on recombinant HIV-1 reverse transcriptase, the Lenti RT activity kit from CAVIDI (Uppsala, Sweden) was used according to the manufacturer's protocol. In brief, a serial dilution series of each compound was prepared starting at 10^3 μM down to 10^{-4} μM. TMC-120 and efavirenz, two NNRTIs with low nM anti-HIV activity, were used as controls. Each compound was tested against 25 and 2.5 pg of recombinant HIV-1 RT, in three independent experiments.

The anti-RT activity of each compound was expressed as the percentage of residual RT activity compared to 'no-compound' controls and subsequently plotted against the compound concentration. IC_{50} values were calculated by nonlinear regression

analysis using GraphPad Prism version 5.03 for Windows (GraphPad Software, San Diego, CA, USA).

4.4.4. WST-1 cytotoxicity assay

The Water Soluble Tetrazolium-1 (WST-1) Cell Proliferation Assay is a colorimetric assay for the measurement of cell proliferation and viability. The assay is based on the cleavage of the tetrazolium salt WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate) to a formazan dye by a complex cellular mechanism. This bioreduction is largely dependent on the glycolytic production of NAD(P)H in viable cells. Therefore, the amount of formazan dye formed correlates directly to the number of viable cells in the culture, and can be quantified by measuring the absorbance at 450 nm in a multiwell plate reader. The greater the number of viable cells, the greater the amount of formazan dye produced following the addition of WST-1. Cytotoxicity of each compound was evaluated using this WST-1 viability assay, according to the manufacturer's instructions (Roche, Vilvoorde, Belgium).

Briefly, ten thousand TZM-bl cells were seeded in a 96-well plates and cultured for 2 days in the presence of a serial dilution of compound. After this 48 h exposure, Cell Proliferation Reagent, WST-1, was added and absorbance at 450 nm was quantified after 90 min using a microplate reader (BioRad, Tokio, Japan). Each compound was tested in three replicate wells and in at least two independent experiments. The percentage cell viability, compared to untreated controls, was plotted against the compound concentration and nonlinear regression analysis was performed using GraphPad Prism version 5.02 for Windows (GraphPad Software, San Diego, CA, USA) to calculate the 50% cytotoxic concentration (CC_{50}).

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