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Design, synthesis, and structure–activity relationships of 1,3-dihydrobenzimidazol-2-one analogues as anti-HIV agents

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ABSTRACT

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) have become very important components in the antiretroviral combination therapies used to treat HIV. Recently, our group identified some 1,3-dihydrobenzimidazol-2-one derivatives and their sulfones as a potent and novel class of NNRTIs. We herein report the synthesis and biological evaluation of the new compounds in which different structural modifications have been introduced in order to investigate their effects on RT inhibition.

1. Introduction

Since the identification of HIV-1 as the cause of acquired immune deficiency syndrome (AIDS), considerable medical advances have been made and nowadays a variety of drugs are available for the clinical treatment of HIV-1 infection.[1,2] These drugs mainly inhibit the following key enzymes in the HIV-1 life cycle: reverse transcriptase (RT), protease (PR), and more recently integrase (IN) as well as viral entry.[3–5]

In highly active antiretroviral therapy (HAART), potent combinations of three or more reverse transcriptase and protease inhibitors are used to suppress replication of HIV.[6] HAART combination regiments have proven to be effective in controlling disease progression and prolonging the survival of HIV-infected patients. However, the therapeutic situation is challenged by the rapid mutation of the virus to yield resistant strains and by the emergence of unpleasant side effects.[7]

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are a variety of non-competitive inhibitors that bind specifically to a hydrophobic pocket in proximity to the DNA polymerase active site of the RT enzyme; most of them are highly specific against HIV-1 RT and are characterized by low toxicity and favorable pharmacokinetic properties.[8]

However, only four NNRTIs (nevirapine, delavirdine, efavirenz, and recently etravirine) have been approved for clinical use by the FDA. Unfortunately, treatment with these drugs results in the rapid emergence of RT mutants.[8,9] Therefore, development of novel NNRTIs with enhanced therapeutic spectra and different resistance mutation profiles is urgently required to successfully employ this class of drugs in combination therapy.

In recent papers, aimed at the discovery of new NNRTIs, we reported a 3D-pharmacophore model for a second generation of NNRTIs. We used this model for molecular modeling studies which led to the rational discovery of N1-substituted 1,3-dihydro-2*H*-benzimidazol-2-ones and their sulfones and some of them have proven to be potent HIV-1 RT inhibitors (Fig. 1).[10–12]

SAR studies highlighted that compounds containing a sulfonyl moiety were more potent than the analogues with a methylene linker. Moreover, 3,5-phenylsubstituted derivatives with a chlorine atom at 6 position of the benzimidazolone system proved to be less

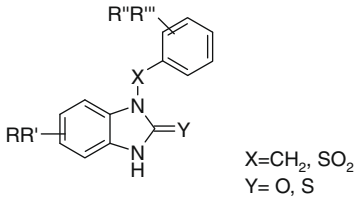

Figure 1. Structure of N1-substituted 1,3-dihydro-2*H*-benzimidazol-2-ones.

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toxic and more active than nevirapine and in some cases than efavirenz, against both wild-type and mutant strains of HIV-1.

Supported by these promising results, we planned the synthesis of new benzimidazolone analogues and their upper homologue quinoxalinones with the aim of establishing further SARs on this class of NNRTIs.

2. Results and discussion

As part of a project devoted to structural optimization and to provide further explanation of the structure–activity relationships of 1,3-dihydrobenzimidazol-2-one derivatives, different structural modifications were introduced on both the benzimidazol-2-one system as well as on the aromatic portion at the N-1 atom.

Using the skeleton of the above molecules as scaffold, new potential NNRTIs were designed in which the imidazol-2-one nucleus was converted into a six-membered ring homologue, like piperazine, keeping the functional groups that are able to act as hydrogen bond acceptor and donor.

The chlorine atom on the bicycle system and the sulfonyl group were also retained as they are of paramount importance in increasing antiviral activity.

Furthermore, the substitution on the arylsulfonyl portion was explored in order to investigate its effects on antiretroviral activity and on RT inhibition (Fig. 2).

The new molecules were synthesized following the reaction sequence reported in Scheme 1.

The 5-chloro-2-nitroaniline was N-substituted by treatment with the appropriate arylsulfonylchloride using sodium hydride as base and the *N*-(5-chloro,2-nitrophenyl)-benzenesulfonamides thereby obtained (**10**–**18**) were reduced with Zn dust in acidic medium to give derivatives **19–27**.

The cyclization of the aminoderivatives (**19–27**) with phosgene gave compounds (**1a–9a**), while the synthesis of N_1-substituted-3,4-dihydroquinoxalin-2(1*H*)-ones (**1c–9c**) was carried out by reacting the suitable aminoderivative with chloroacetyl chloride by microwave-irradiation and subsequent cyclization of the intermediate obtained (**1b–9b**), with a catalytic amount of ethyldiisopropylamine (EDIPA) as base.

Both analytical and spectral data (^{1}H NMR) of all synthesized compounds are in full agreement with the proposed structures.

All the designed compounds were evaluated for their inhibition of the cytopathic effects of HIV-1 (III_B) in MT-4 cells. Compound-induced cytotoxcity was also measured in MT-4 cells in parallel with antiviral activity. The obtained compounds were also tested for their ability to inhibit the enzymatic activity of HIV-1 RT.

Some intermediates of the reaction (**1b–9b)** were tested for anti-HIV assays, considering that they could be 'open models' of the designed molecules (**1c–9c**) which maintain all of the key structural requirements for RT enzyme inhibition analogous to the corresponding closed form.

The results from the cell-based and RT assays are summarized in Table 1 and are compared with nevirapine and efavirenz as reference drugs.

As shown in Table 1, several of the new compounds prevented the cytopathic effects of HIV-1 IIIB at micromolar or nanomolar concentrations and, in some cases, were minimally toxic to MT-4 cells, thus resulting in high selectivity indices.

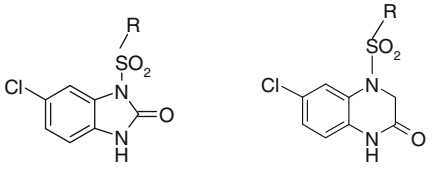
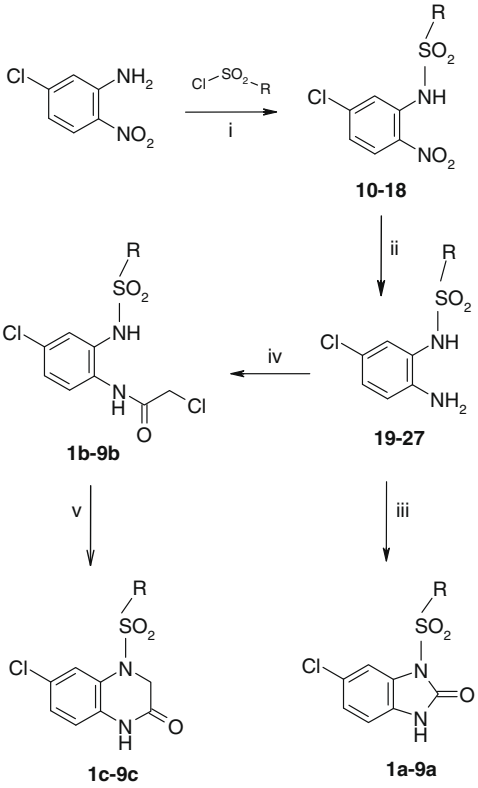


Figure 2. Structure of benzimidazolone and quinoxalinone derivatives.



Compd	R
1a*, 1b, 1c,	2,6-difluorophenyl
2a*, 2b, 2c	3,5-difluorophenyl
3a, 3b, 3c	2,6-dichlorophenyl
4a, 4b, 4c	3,5-dichlorophenyl
5a#, 5b, 5c	3,5-dimethylphenyl
6a, 6b, 6c	2-fluorophenyl
7a, 7b, 7c	3-fluorophenyl
8a, 8b, 8c	4-fluorophenyl
9a, 9b, 9c	3-cyanophenyl

Scheme 1. Reagents and conditions: (i) Dioxane, NaH, 0–5 °C, 30 min; (ii) Zn/HCl, EtOH, 80 °C, 1 h; (iii) 20% toluene solution of $COCl_2$, HCl 2 N, Δ, 4 h; (iv) $ClCH_2COCl$, mw: 250 W, 25 °C, 10 min; (v) THF anhydrous, EDIPA, rt, 45 min. *, #See Refs. 11,12.

It can be observed that anti-HIV activity is dependent on the nature and position of the substituents on the arylsulfonyl portion. In particular, the more potent compounds turned out to be derivatives **5a**, **5b**, and **5c** which were active at 0.002, 0.047, and 0.1 μM concentration, respectively, and which presented a 3,5-dimethylphenylsulfonyl substituent on the nitrogen atom of the bicycle system.

Also the presence of two chlorine atoms in 2,6 or 3,5 positions of the phenyl ring provided compounds active against cell replication at good micromolar concentrations (compd **3a**, **3b**, **3c,** and **4a**). The mono substitution with a fluorine atom in 2 or 3 position seems to influence anti-HIV activity positively (compd **6a** and **7a**), whereas the 4-substituted derivative **8a** is inactive.

The anti-HIV-1 activity in MT-4 cells reported in Table 1 shows that the benzimidazolone nucleus provided the most active compounds whereas the substitution of the imidazol-2-one nucleus with a six-member ring homologue seems to have a negative influence on anti-HIV activity. In fact, compounds **1a–9a** were more

Table 1
Anti-RT and anti-HIV-1 activities, cytotoxicity and selectivity index in MT-4 cells

Compd	IC_{50}[a] (μM)	EC_{50}[b] (μM)	CC_{50}[c] (μM)	SI[d]
1a	30 ± 1.5	1.566 ± 0.174	194.12 ± 9.90	124
1b	30.36 ± 12.65	6.96 ± 2.38	174.59 ± 20.11	25
1c	>40	20.68 ± 5.52	179.98 ± 12.96	9
2a	0.1 ± 0.01	0.069 ± 0.055	202.47 ± 83.45	2934
2b	27.83 ± 5.57	11.21 ± 0.30	179.40 ± 16.17	16
2c	>40	13.99 ± 4.40	187.03 ± 7.44	13
3a	>100	1.515 ± 0.16	>331	218
3b	32.70 ± 2.33	1.50 ± 0.85	159.95 ± 9.27	107
3c	1.67 ± 0.75	1.09 ± 0.58	>319.15	>293
4a	0.003 ± 0.001	0.048 ± 0.005	179.27 ± 16.07	3735
4b	15.88 ± 6.77	8.99 ± 3.83	>291.97	>32
4c	>40	26.66 ± 18.43	⩾205.80	8
5a	0.005 ± 0.001	0.002 ± 0.0003	39 ± 8.2	17,846
5b	3.36 ± 0.52	0.047 ± 0.0073	35.11 ± 5.65	747
5c	>40	0.1 ± 0.059	80.58 ± 50.45	782
6a	0.85	0.23 ± 0.02	>382.57	>1663
6b	NT[e]	NT[e]	NT[e]	NT[e]
6c	>40	6.10 ± 2.34	196.70 ± 20.63	32
7a	0.132	0.043 ± 0.009	127.50 ± 87.07	2965
7b	NT[e]	NT[e]	NT[e]	NT[e]
7c	>40	7.04 ± 0.73	189.19 ± 4.19	27
8a	>40	35.99 ± 12.67	⩾355.02	⩾10
8b	NT[e]	NT[e]	NT[e]	NT[e]
8c	>40	>172.50	172.49 ± 36.68	<1
9a	0.36	0.149 ± 0.089	205.41 ± 23.70	1357
9b	283.68 ± 21	>155.89	>155.89	<1
9c	>40	⩾47.73	186.27 ± 12.02	⩽4
Nevirapine	0.18 ± 0.02	0.073 ± 0.015	>15	>205
Efavirenz	0.004 ± 0.001	0.0009 ± 0.0002	>6	>6666

[a] Concentration required to inhibit by 50% the in vitro RNA-dependent DNA polymerase activity of recombinant rt.
[b] Effective concentration required to reduce HIV-1-induced cytopathic effect by 50% in MT-4 cells.
[c] Cytotoxic concentration required to reduce MT-4 cell viability by 50%.
[d] Selectivity index:ratio CC_{50}/EC_{50}.
[e] Not tested.

active than the corresponding quinoxalinone derivatives **1c–9c** as well as **1b–9b**.

Furthermore, most of the compounds **1b–9b** showed only weak inhibitory activity against RT whereas derivatives **1c-9c** were inactive, with the exception of 2,6-dichloro substituted derivative (**3c**) which was active at 1.67 μM concentration.

Interestingly, the 3,5-dichlorophenyl substituted compound (**4a**) presented inhibitory activity at 0.003 μM thus confirming a previous hypothesis that the 3,5-phenylsubstituted moiety interacted with the hydrophobic roof of the non-nucleoside inhibitors binding site.[11]

In summary, new potent anti-HIV agents have been obtained active at nanomolar concentration and with very low toxicity. The biological results suggest that the benzimidazolone nucleus is the best scaffold of these series of derivatives as it provides the most active compounds. The results obtained will be used as basis strategy point for the design of new molecules with improved anti-HIV properties.

3. Experimental

3.1. Chemistry

All microwave-assisted reactions were carried out in a CEM Focused Microwave Synthesis System, Model Discover working at the power necessary for refluxing under atmospheric conditions. Melting points were determined on a Kofler hot stage apparatus and are uncorrected. Elemental analyses (C, H, N) were carried out on a C. Erba Model 1106 Elemental Analyzer and the results were within ±0.4% of the theoretical values. Merck Silica Gel 60 F_{254} plates were used for TLC. ^{1}H NMR spectra were measured with a Varian Gemini 300 spectrometer in $CDCl_3$ with TMS as internal standard or in DMSO-d_6. Coupling constants (J) are reported in hertz, and chemical shifts are expressed in δ (ppm).

3.1.1. General procedures for the synthesis of *N*-(5-chloro-2-nitrophenyl)-benzenesulfonamides (12–13, 15–18)

The appropriate aryl sulfonyl chloride (3 mmol) was added dropwise to a solution of 5-chloro,2-nitroaniline (2 mmol) in dioxane (6 ml) at 0 °C, using dry sodium hydride (10 mmol) as catalyst. The mixture was stirred for 30 min. The reaction mixture was then quenched with a saturated $NaHCO_3$ solution, extracted with chloroform, and dried over Na_2SO_4. After removal of the solvent under reduced pressure, the residue was powdered by treatment with diethyl ether.

3.1.1.1. *N*-(5-Chloro-2-nitrophenyl)-2,6-dichlorobenzenesulfonamide (12). Mp: 176–178 °C, yield 46%. ^{1}H NMR ($CDCl_3$): 7.10–8.16 (m, 6H, ArH), 10.77 (br s, 1H, NH). Anal. Calcd for $C_{12}H_7Cl_3N_2O_4S$: C, 37.77; H, 1.85; N, 7.34. Found: C, 37.56; H, 1.98; N, 7.57.

3.1.1.2. *N*-(5-Chloro-2-nitrophenyl)-3,5-dichlorobenzenesulfonamide (13). Mp: 111–115 °C, yield 69%. ^{1}H NMR ($CDCl_3$): 7.17–8.17 (m, 6H, ArH), 10.07 (br s, 1H, NH). Anal. Calcd for $C_{12}H_7Cl_3N_2O_4S$: C, 37.77; H, 1.85; N, 7.34. Found: C, 37.55; H, 2.03; N, 7.08.

3.1.1.3. *N*-(5-Chloro-2-nitrophenyl)-3-cyanobenzenesulfonamide (15). Mp: 160 °C dec, yield 65%. ^{1}H NMR ($CDCl_3$): 7.18–8.17 (m, 7H, ArH), 10.06 (br s, 1H, NH). Anal. Calcd for $C_{13}H_8ClN_3O_4S$: C, 46.23; H, 2.39; N, 12.44. Found: C, 46.31; H, 2.49; N, 12.17.

3.1.1.4. *N*-(5-Chloro-2-nitrophenyl)-2-fluorobenzenesulfonamide (16). Mp: 106–108 °C, yield 100%. ^{1}H NMR ($CDCl_3$): 7.13–8.13 (m, 7H, ArH), 10.02 (br s, 1H, NH). Anal. Calcd for $C_{12}H_8ClFN_2O_4S$: C, 43.58; H, 2.44; N, 8.47. Found: C, 43.74; H, 2.31; N, 8.79.

3.1.1.5. *N*-(5-Chloro-2-nitrophenyl)-3-fluorobenzenesulfonamide (17). Mp: 108–110 °C, yield 82%. ^{1}H NMR ($CDCl_3$): 7.10–8.15 (m, 7H, ArH), 10.03 (br s, 1H, NH). Anal. Calcd for $C_{12}H_8ClFN_2O_4S$: C, 43.58; H, 2.44; N, 8.47. Found: C, 43.22; H, 2.65; N, 8.13.

3.1.1.6. *N*-(5-Chloro-2-nitrophenyl)-4-fluorobenzenesulfonamide (18). Mp: 95–98 °C, yield 65%. ^{1}H NMR ($CDCl_3$): 7.15–8.12 (m, 7H, ArH), 10.05 (sa, 1H, NH). Anal. Calcd for $C_{12}H_8ClFN_2O_4S$: C, 43.58; H, 2.44; N, 8.47. Found: C, 43.15; H, 2.79; N, 8.68.

3.1.2. General procedures for the synthesis of *N*-(2-aminophenyl-5-chloro)-benzenesulfonamides (21–22, 24–27)

The mixture of appropriate *N*-(5-chloro-2-nitrophenyl)-benzenesulfonamide (0.6 mmol) in 3 ml HCl and 4 ml EtOH anhydrous was stirred in an ice bath, then zinc dust (20 mmol) was gradually added in several portions. Once this addition was complete, the reaction mixture was heated in a water bath for 1 h; it was then cooled, made alkaline with NaOH 2 N and extracted with ethyl acetate. The extract was washed with water, dried over Na_2SO_4, and evaporated. The residue was crystallized from ethanol or diethyl ether.

3.1.2.1. *N*-(2-Aminophenyl-5-chloro)-2,6-dichlorobenzenesulfonamide (21). Mp: 256–258 °C, yield 92%. ^{1}H NMR (DMSO-d_6): 5.36 (br s, 2H, NH_2), 6.60–7.87 (m, 6H, ArH). Anal. Calcd for $C_{12}H_9Cl_3N_2O_2S$: C, 40.99; H, 2.58; N, 7.97. Found: C, 40.77; H, 2.70; N, 7.80.

3.1.2.2. *N*-(2-Aminophenyl-5-chloro)-3,5-dichlorobenzenesulfonamide (22). Mp: 206–208 °C, yield 100%. ^{1}H NMR (DMSO-d_6): 5.39 (br s, 2H, NH_2), 6.69–7.74 (m, 6H, ArH). Anal. Calcd for $C_{12}H_9Cl_3N_2O_2S$: C, 40.99; H, 2.58; N, 7.97. Found: C, 40.77; H, 2.70; N, 7.80.

3.1.2.3. *N*-(2-Aminophenyl-5-chloro)-3-cyanobenzenesulfonamide (24). Mp: 225–227 °C, yield 98%. ^{1}H NMR (DMSO-d_6): 5.15 (br s, 2H, NH_2), 6.53–8.14 (m, 7H, ArH). Anal. Calcd for $C_{13}H_{10}ClN_3O_2S$: C, 50.74; H, 3.28; N, 13.65. Found: C, 50.97; H, 3.48; N, 13.80.

3.1.2.4. *N*-(2-Aminophenyl-5-chloro)-2-fluorobenzenesulfonamide (25). Mp: 157–159 °C, yield 100%. ^{1}H NMR (DMSO-d_6): 5.15 (br s, 2H, NH_2), 6.59–7.71 (m, 7H, ArH). Anal. Calcd for $C_{12}H_{10}ClFN_2O_2S$: C, 47.93; H, 3.35; N, 9.31. Found: C,48.14; H, 3.52; N, 9.78.

3.1.2.5. *N*-(2-Aminophenyl-5-chloro)-3-fluorobenzenesulfonamide (26). Mp: 139–141 °C, yield 93%. ^{1}H NMR (DMSO-d_6): 5.09 (br s, 2H, NH_2), 6.59–7.65 (m, 7H, ArH), 9.53 (sa, 1H, NH). Anal. Calcd for $C_{12}H_{10}ClFN_2O_2S$: C, 47.93; H, 3.35; N, 9.31. Found: C, 47.59; H, 3.07; N, 9.63.

3.1.2.6. *N*-(2-Aminophenyl-5-chloro)-4-fluorobenzenesulfonamide (27). Mp: 231–234 °C, yield 100%. ^{1}H NMR (DMSO-d_6): 4.62 (br s, 2H, NH_2), 6.31–6.49 (m, 7H, ArH). Anal. Calcd for $C_{12}H_{10}ClFN_2O_2S$: C, 47.93; H, 3.35; N, 9.31. Found: C, 48.22; H, 3.71; N, 9.75.

3.1.3. General procedure for the synthesis of 1-arylsulfonyl-1,3-dihydro-2*H*-benzimidazol-2-ones (3a–4a, 6a–9a)

An excess of a 20% toluene solution of phosgene (1 ml) was added to a solution of the appropriate *N*-(2-aminophenyl-5-chloro)-benzenesulfonamide (0.25 mmol) in HCl 2 N (4 ml), and the resulting mixture was heated for 4 h. After cooling, the reaction mixture was neutralized with NaOH 2 N, extracted with ethyl acetate, washed with water, and evaporated under reduced pressure. The residue was crystallized from ethyl acetate.

3.1.3.1. 6-Chloro-1-(2,6-dichlorophenylsulfonyl)-1,3-dihydro-2*H*-benzimidazol-2-one (3a). Mp: 187 °C dec., yield 40%. ^{1}H NMR ($CDCl_3$): 6.96 (d, J = 8.42, 1H, H-4), 7.19 (dd, J = 8.42, 1H, H-5), 7.40–7.52 (m, 3H, ArH), 7.91 (s, 1H, H-7). Anal. Calcd for $C_{13}H_7Cl_3N_2O_3S$: C, 41.35; H, 1.87; N, 7.42. Found: C, 41.15; H, 1.48; N, 7.76.

3.1.3.2. 6-Chloro-1-(3,5-dichlorophenylsulfonyl)-1,3-dihydro-2*H*-benzimidazol-2-one (4a). Mp: 239–240 °C, yield 62%. ^{1}H NMR (DMSO-d_6): 6.85 (d, J = 8.24, 1H, H-4), 7.04 (dd, J = 8.24, 1H, H-5), 7.53 (s, 1H, H-7), 8.01 (s, 2H, H-2′,6′), 8.05 (s, 1H, H-4′). Anal. Calcd for $C_{13}H_7Cl_3N_2O_3S$: C, 41.35; H, 1.87; N, 7.42. Found: C, 41.51; H, 1.73; N, 7.38.

3.1.3.3. 6-Chloro-1-(3-cyanophenylsulfonyl)-1,3-dihydro-2*H*-benzimidazol-2-one (6a). Mp: 220–223 °C, yield 28%. ^{1}H NMR (DMSO-d_6): 7.03 (d, J = 8.51, 1H, H-4), 7.24 (d, J = 7.69, 1H, H-5), 7.74 (s, 1H, H-7), 7.87–7.90 (m, 1H, H-5′), 8.26–8.39 (m, 2H, ArH), 8.59 (s, 1H, H-2′), 11.64 (br s, 1H, NH). Anal. Calcd for $C_{14}H_8ClN_3O_3S$: C, 50.38; H, 2.42; N, 12.59. Found: C, 50.71; H, 2.64; N, 12.27.

3.1.3.4. 6-Cloro-1-(2-fluorophenylsulfonyl)-1,3-dihydro-2*H*-benzimidazol-2-one (7a). Mp: 192–197 °C, yield 72%. ^{1}H NMR (DMSO-d_6): 7.03 (d, J = 8.51, 1H, H-4), 7.22 (d, J = 8.79, 1H, H-5), 7.44–7.53 (m, 2H, ArH), 7.60 (s, 1H, H-7), 7.81–8.09 (m, 2H, ArH). Anal. Calcd for $C_{13}H_8ClFN_2O_3S$: C, 47.79; H, 2.47; N, 8.57. Found: C, 47.33; H, 2.69; N, 8.78.

3.1.3.5. 6-Chloro-1-(3-fluorophenylsulfonyl)-1,3-dihydro-2*H*-benzimidazol-2-one (8a). Mp: 222–224 °C yield 70%. ^{1}H NMR (DMSO-d_6): 7.52 (s, 1H, H-7), 7.63–7.81 (m, 6H, ArH). Anal. Calcd for $C_{13}H_8ClFN_2O_3S$: C, 47.79; H, 2.47; N, 8.57. Found: C, 48.05; H, 2.18; N, 8.21.

3.1.3.6. 6-Chloro-1-(4-fluorophenylsulfonyl)-1,3-dihydro-2*H*-benzimidazol-2-one (9a). Mp: 313–315 °C yield 47%. ^{1}H NMR (DMSO-d_6): 6.88–6.96 (m, 7H, ArH), 10.75 (br s, 1H, NH). Anal. Calcd for $C_{13}H_8ClFN_2O_3S$: C, 47.79; H, 2.47; N, 8.57. Found: C, 47.41; H, 2.84; N, 8.96.

3.1.4. General procedure for the synthesis of 2-chloro-*N*-[4-chloro-2-(phenylsulfonamido)-phenyl]acetamides (1b–9b)

The appropriate *N*-(2-aminophenyl-5-chloro)-benzenesulfonamide (1 mmol) was added to an excess of 2-chloroacetyl chloride and the resulting mixture was stirred and irradiated in a microwave oven (W 100, 10 min, 50 °C) without solvent. After cooling, the reaction mixture was evaporated under reduced pressure and the obtained residue was crystallized from diethyl ether.

3.1.4.1. 2-Chloro-*N*-[4-chloro-2-(2,6-difluoro-phenylsulfonamido)-phenyl]acetamide (1b). Mp: 130–133 °C, yield 100%. ^{1}H NMR (DMSO-d_6): 4.26 (s, 2H, CH_2), 7.16 (s, 1H, H-6), 7.24–7.32 (m, 3H, ArH), 7.58 (d, J = 8.79, 1H, H-3), 7.71–7.78 (m, 1H, H-4′), 10.31 and 10.65 (br s, 2H, NH and NH). Anal. Calcd for $C_{14}H_{10}Cl_2F_2N_2O_3S$: C, 42.55; H, 2.55; N, 7.09. Found: C, 42.73; H, 2.28; N, 7.40.

3.1.4.2. 2-Chloro-*N*-[4-chloro-2-(3,5-difluoro-phenylsulfonamido)-phenyl]acetamide (2b). Mp: 148–150 °C, yield 100%. ^{1}H NMR (DMSO-d_6): 4.23 (s, 2H, CH_2), 7.11 (s, 1H, H-6), 7.31 (dd, J = 8.79, 1H, H-3), 7.35–7.38 (m, 2H, ArH), 7.58 (d, J = 8.79, 1H, H-4), 7.61–7.69 (m, 1H, H-4′), 10.02 and 10.30 (br s, 2H, NH and NH). Anal. Calcd for $C_{14}H_{10}Cl_2F_2N_2O_3S$: C, 42.55; H, 2.55; N, 7.09. Found: C, 42.70; H, 2.33; N, 7.46.

3.1.4.3. 2-Chloro-*N*-[4-chloro-2-(2,6-dichloro-phenylsulfonamido)-phenyl]acetamide (3b). Mp: 154–157 °C, yield 50%. ^{1}H NMR (DMSO-d_6): 4.32 (s, 2H, CH_2), 7.08 (s, 1H, H-6), 7.25 (dd, J = 8.54, 1H, H-3), 7.50–7.90 (m, 4H, ArH), 9.72 and 9.92 (br s, 2H, NH and NH). Anal. Calcd for $C_{14}H_{10}Cl_4N_2O_3S$: C, 39.28; H, 2.35; N, 6.54. Found C, 39.49; H, 2.62; N, 6.28.

3.1.4.4. 2-Chloro-*N*-[4-chloro-2-(3,5-dichloro-benzenesulfonylamido)-phenyl]acetamide (4b). Mp: 149–151 °C, yield 51%. ^{1}H NMR (DMSO-d_6): 4.23 (s, 2H, CH_2), 7.08(s, 1H, H-6), 7.33 (d, J = 8.54, 1H, H-3), 7.55–7.63 (m, 3H, ArH), 7.99 (s, 1H, H-4′), 9.59 and 9.98 (br s, 2H, NH and NH). Anal. Calcd for $C_{14}H_{10}Cl_4N_2O_3S$: C, 39.28; H, 2.35; N, 6.54. Found: C, 39.59; H, 2.52; N, 6.21.

3.1.4.5. 2-Chloro-*N*-[4-chloro-2-(3,5-dimethyl-phenylsulfonamido)-phenyl]acetamide (5b). Mp: 149–150 °C, yield 61%. ^{1}H NMR (DMSO-d_6): 3.54 (s, 6H, CH_3), 4.41 (s, 2H, CH_2), 7.22 (s, 1H, H-6), 7.45–7.48 (m, 4H, ArH), 7.83 (d, J = 8.79, 1H, H-4), 9.73 and 9.87 (br s, 2H, NH and NH). Anal. Calcd for $C_{16}H_{16}Cl_2N_2O_3S$: C, 49.62; H, 4.16; N, 7.23. Found: C, 49.37; H, 4.43; N, 7.08.

3.1.4.6. 2-Chloro-*N*-[4-chloro-2-(3-cyano-phenylsulfonamido)-phenyl]acetamide (6b). Mp: 176–178 °C, yield 82%. ^{1}H NMR ($CDCl_3$): 4.18 (s, 2H, CH_2), 7.02 (s, 1H, H-6), 7.29 (dd, J = 8.51, 1H, H-4), 7.54 (d, J = 8.51, 1H, H-3), 7.65 (t, 1H, H-5′), 7.89–7.93 (m, 2H, H-4′,6′), 8.03 (s, 1H, H-2′), 8.58 (br s, 2H, NH and NH). Anal. Calcd for $C_{15}H_{11}Cl_2N_3O_3S$: C, 46.89; H, 2.89; N, 10.94. Found: C, 46.51; H, 2.96; N, 10.75.

3.1.4.7. 2-Chloro-*N*-(4-chloro-2-(2-fluoro-phenylsulfonamido)-phenyl)acetamide (7b). Mp: 124–129 °C, yield 71%. ^{1}H NMR (DMSO-d_6): 4.26 (s, 2H, CH_2), 7.10 (s, 1H, H-6), 7.26–7.73 (m, 6H, ArH), 9.73 and 10.05 (br s, 2H, NH and NH). Anal. Calcd for $C_{14}H_{11}Cl_2FN_2O_3S$: C, 44.58; H, 2.94; N, 7.43. Found: C, 44.83; H, 3.12; N, 7.71.

3.1.4.8. 2-Chloro-*N*-[4-chloro-2-(3-fluoro-phenylsulfonamido)-phenyl]acetamide (8b). Mp: 137–140 °C, yield 94%. ^{1}H NMR (DMSO-d_6): 4.25 (s, 2H, CH_2), 7.03 (s, 1H, H-6), 7.28 (dd, J = 8.51, 1H, H-3), 7.46–7.66 (m, 5H, ArH), 9.56 and 9.91 (br s, 2H, NH and NH). Anal. Calcd for $C_{14}H_{11}Cl_2FN_2O_3S$: C, 44.58; H, 2.94; N, 7.43. Found: C, 44.26; H, 2.45; N, 7.86.

3.1.4.9. 2-Chloro-*N*-[4-chloro-2-(4-fluoro-phenylsulfonamido)-phenyl]acetamide (9b). Mp: 167–169 °C, yield 59%. ^{1}H NMR (DMSO-d_6): 4.26 (s, 2H, CH_2), 7.03 (s, 1H, H-6), 7.26 (d, 1H, H-3), 7.40–7.98 (m, 5H, ArH), 10.01 and 10.04 (br s, 2H, NH and NH). Anal. Calcd for $C_{14}H_{11}Cl_2FN_2O_3S$: C, 44.58; H, 2.94; N, 7.43. Found: C, 44.70; H, 2.69; N, 7.25.

3.1.5. General procedure for the synthesis of 4-arylsulfonyl-3,4-dihydro-1*H*-quinoxalin-2-ones (1c–9c)

An excess of ethyldiisopropylamine (EDIPA) was added dropwise to a solution of the appropriate 2-chloro-*N*-(4-chloro-phenyl)-acetamide (1 mmol) in anhydrous THF (2 ml). The mixture was stirred for 1 h at room temperature.

The reaction mixture was washed with a saturated $NaHCO_3$ solution and then dried over Na_2SO_4. After removal of the solvent under reduced pressure, the residue was crystallized from diethyl ether.

3.1.5.1. 6-Chloro-4-(2,6-difluorophenylsulfonyl)-3,4-dihydro-1*H*-quinoxalin-2-one (1c). Mp: 214–217 °C, yield 50%. ^{1}H NMR (DMSO-d_6): 4.42 (s, 2H, CH_2), 6.85 (d, J = 8.51, 1H, H-2), 7.23–7.52 (m, 4H, ArH), 7.71–7.80 (m, 1H, H-4′), 10.65 (br s, 1H, NH). Anal. Calcd for $C_{14}H_9ClF_2N_2O_3S$: C, 46.87; H, 2.53; N, 7.81. Found: C, 46.30; H, 2.67; N, 7.53.

3.1.5.2. 6-Chloro-4-(2,6-dichlorophenylsulfonyl)-3,4-dihydro-1*H*-quinoxalin-2-one (2c). Mp: 204–207 °C, yield 86%. ^{1}H NMR (DMSO-d_6): 4.37 (s, 2H, CH_2), 6.86 (d, J = 8.51, 1H, H-2), 7.26 (dd, J = 8.51, 1H, H-3), 7.42 (s, 1H, H-5), 7.50–7.95 (m, 3H, ArH), 10.68 (br s, 1H, NH). Anal. Calcd for $C_{14}H_9Cl_3N_2O_3S$: C, 42.93; H, 2.32; N, 7.15. Found: C, 42.75; H, 2.56; N, 7.38.

3.1.5.3. 6-Chloro-4-(3,5-difluorophenylsulfonyl)-3,4-dihydro-1*H*-quinoxalin-2-one (3c). Mp: 191–194 °C, yield 44%. ^{1}H NMR (DMSO-d_6): 4.35 (s, 2H, CH_2), 6.83 (d, J = 8.79, 1H, H-2), 7.15 (s, 2H, H-2′,6′), 7.38 (dd, J = 8.51, 1H, H-3), 7.53 (s, 1H, H-5), 7.73–7.79 (m, 1H, H-4′), 10.51 (br s, 1H, NH). Anal. Calcd for $C_{14}H_9ClF_2N_2O_3S$: C, 46.87; H, 2.53; N, 7.81. Found: C, 46.49; H, 2.20; N, 7.63.

3.1.5.4. 6-Chloro-4-(3,5-dichlorophenylsulfonyl)-3,4-dihydro-1*H*-quinoxalin-2-one (4c). Mp: 167–170 °C, yield 55%. ^{1}H NMR (DMSO-d_6): 4.35 (s, 2H, CH_2), 6.83 (d, J = 8.79, 1H, H-2), 7.33 (s, 2H, H-2′,6′), 7.41(d, J = 8.79, 1H, H-3), 7.54 (s, 1H, H-5), 8.08 (s, 1H, H-4′), 10.19 (br s, 1H, NH). Anal. Calcd for $C_{14}H_9Cl_3N_2O_3S$: C, 42.93; H, 2.32; N, 7.15. Found: C, 42.65; H, 2.58; N, 7.36.

3.1.5.5. 6-Chloro-4-(3,5-dimethylphenylsulfonyl)-3,4-dihydro-quinoxalin-2(1*H*)-one (5c). Mp: 181–184 °C, yield 30%. ^{1}H NMR (DMSO-d_6): 2.21 (s, 6H, CH_3), 4.26 (s, 2H, CH_2), 6.78 (d, J = 8.51, 1H, H-2), 6.99–7.50 (m, 5H, ArH), 10.35 (br s, 1H, NH). Anal. Calcd for $C_{16}H_{15}ClN_2O_3S$: C, 54.78; H, 4.31; N, 7.98. Found: C, 54.56; H, 4.79; N, 7.47.

3.1.5.6. 6-Chloro-4-(3-cyanodimethylphenylsulfonyl)-3,4-dihydro-1*H*-quinoxalin-2-one (6c). Mp: 217–220 °C, yield 17%. ^{1}H NMR (DMSO-d_6): 4.30 (s, 2H, CH_2), 6.79 (d, J = 8.79, 1H, H-2), 7.33–7.53 (m, 6H, ArH), 10.40 (br s, 1H, NH). Anal. Calcd for $C_{15}H_{10}ClN_3O_3S$: C, 51.80; H, 2.90; N, 12.08. Found: C, 51.46; H, 3.20; N, 12.35.

3.1.5.7. 6-Chloro-4-(2-fluorophenylsulfonyl)-3,4-dihydro-1*H*-quinoxalin-2-one (7c). Mp: 183–186 °C, yield 100%. ^{1}H NMR (DMSO-d_6): 4.37 (s, 2H, CH_2), 6.83 (d, J = 8.51, 1H, H-2), 7.28–7.44 (m, 3H, ArH), 7.49 (s, 1H, H-5), 7.61–7.77 (m, 2H, ArH), 10.57 (br s, 1H, NH). Anal. Calcd for $C_{14}H_{10}ClFN_2O_3S$: C, 49.35; H, 2.96; N, 8.22. Found: C, 49.67; H, 3.34; N, 8.56.

3.1.5.8. 6-Chloro-4-(3-fluorophenylsulfonyl)-3,4-dihydro-1*H*-quinoxalin-2-one (8c). Mp: 172–175 °C, yield 35%. ^{1}H NMR (DMSO-d_6): 4.32 (s, 2H, CH_2), 6.79 (d, J = 8.51, 1H, H-2), 7.24–7.26 (m, 2H, ArH), 7.35 (dd, J = 8.51, 1H, H-3), 7.53 (s, 1H, H-5), 7.56–7.61 (m, 2H, ArH), 10.43 (br s, 1H, NH). Anal. Calcd for $C_{14}H_{10}ClFN_2O_3S$: C, 49.35; H, 2.96; N, 8.22. Found: C, 49.70; H, 2.64; N, 8.53.

3.1.5.9. 6-Chloro-4-(4-fluorophenylsulfonyl)-3,4-dihydro-1*H*-quinoxalin-2-one (9c). Mp: 189–191 °C, yield 86%. ^{1}H NMR (DMSO-d_6): 4.34 (s, 2H, CH_2), 6.79 (d, J = 8.51, 1H, H-2), 7.38 (dd, J = 8.51, 1H, H-3), 7.54 (s, 1H, H-5), 7.64–8.22 (m, 4H, ArH), 10.41 (br s, 1H, NH). Anal. Calcd for $C_{14}H_{10}ClFN_2O_3S$: C, 49.35; H, 2.96; N, 8.22. Found: C, 49.51; H, 2.66; N, 8.38.

3.2. Anti-HIV activity assays

3.2.1. HIV-1 RT RNA-dependent DNA polymerase activity assay

Poly(rA)/oligo(dT) was used as a template for the RNA-dependent DNA polymerase reaction by HIV-1 RT. For the activity assay, a 25 μl final reaction volume contained: TDB buffer (50 mM Tris–HCl (pH 8.0), 1 mM dithiothreitol (DTT), 0.2 mg/ml bovine serum albumine (BSA), 2% glycerol), 10 mM $MgCl_2$, 0.5 mg of poly(rA):oligo(dT)$_{10:1}$ (0.3 mM 3′-OH ends), 10 mM ^{3}[H]dTTP 1 Ci/mmol and was finally introduced into tubes containing aliquots of different enzyme concentrations (5–10 nM rt). After incubation at 37 °C for the indicated time, 20 μl from each reaction tube were spiked on glass fiber filters GF/C and immediately immersed in 5% ice-cold trichloroacetic acid (TCA) (AppliChem GmbH, Darmstadt). Filters were washed three times with 5% TCA and once with ethanol for 5 min, then dried and, lastly, EcoLume® Scintillation cocktail (ICN, Research Products Division, Costa Mesa, CA USA) was added to detect the acid-precipitable radioactivity using a PerkinElmer® Trilux MicroBeta 1450 Counter.

3.2.2. RT inhibition assays

Reactions were performed under the conditions described for the HIV-1 RT RNA-dependent DNA polymerase activity assay.[13] The incorporation of radioactive dTTP into poly(rA)/oligo(dT) was monitored in the presence of increasing amounts of the inhibitors to be tested. Data were then plotted according to Lineweaver-Burke and Dixon. For K_i determinations an interval of inhibitor concentrations of between 0.2 K_i and 5 *Ki* was used. Experiments were done in triplicate. Experimental errors (±SD) were ⩽10%.

3.2.3. In vitro anti-HIV assay

The methodology of the anti-HIV assays has been previously described.[14] Briefly, MT-4 cells were infected with HIV-1 (III_B) at ~100-times the $CCID_{50}$ (50% cell culture infective dose) per ml of cell suspension. Hundred microliters of the infected cell suspension were then transferred to microtiter plate wells, mixed with

100 μl of the appropriate dilutions of the test compounds, and further incubated at 37 °C. After 5 days (MT-4) of incubation, the number of viable MT-4 cells was determined. The 50% effective concentration (EC_{50}) was defined as the concentration of compound required to reduce the virus-induced cytopathicity by 50%.

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