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Novel 1,3-dihydro-benzimidazol-2-ones and their analogues as potent non-nucleoside HIV-1 reverse transcriptase inhibitors

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ABSTRACT

A series of novel benzimidazolones and their analogues, characterized by the presence of one or more methyl groups or other bioisosteric moieties at different positions of the phenyl ring at N-1, were synthesized and evaluated as inhibitors of human immunodeficiency virus type-1 (HIV-1). Most of the new compounds proved to be highly effective in inhibiting both HIV-1 replication in MT4 cells with minimal cytotoxicity and RT enzyme at nanomolar concentrations. Some derivatives were also tested against RTs containing single amino acid mutations responsible for resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs). The different potencies displayed by the new compounds were studied using molecular modeling.

1. Introduction

Standard therapy for human immunodeficiency virus type 1 (HIV-1) infection was based, for many years, on potent cocktails of drugs targeting the viral protease (PR) and reverse transcriptase (RT).[1] More recently, two viral entry inhibitors, *enfuvirtide*[2] and *maraviroc*,[3] and a viral integrase inhibitor (IN), *raltegravir*[4] have been added to these regimens.

Although this treatment has curtailed the number of AIDS deaths, it is often associated with severe and long term side effects. Moreover, the incomplete suppression of HIV replication is responsible for the emergence of drug-resistant HIV strains. Therefore, a continued research effort is required to develop more potent and less toxic antiviral compounds to cope with the rapidly emerging resistant strains.[5]

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) have become very important components in antiretroviral combination therapies due to their unique mechanism of action, low toxicity and favorable pharmacokinetics properties.[6,7]

Consequently, many efforts have been directed in recent years to the development of novel broad spectrum NNRTIs that inhibit both wild-type and drug-resistant variants of RT and four NNRTIs (nevirapine, delavirdine, efavirenz, and etravirine) have been approved for clinical use to date.[8]

In recent papers, we reported a 3D-pharmacophore model which led to the rational discovery of N1-substituted 1-3-dihydro-2*H*-benzimidazol-2-ones and their sulfones as potent HIV-1 RT inhibitors.[9–11]

The most active derivative (**1**) (Fig. 1) of this class of NNRTIs, characterized by the presence of a 3,5-dimethylphenylsulfonyl moiety at N1 of the benzimidazolone system and a chlorine atom

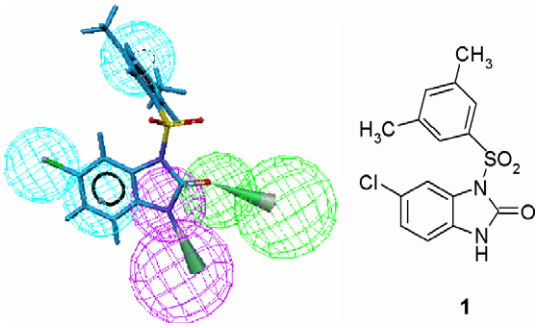

Figure 1. 3D-pharmacophore model for second-generation NNRTIs aligned to compound **1** (cyan: hydrophobic groups; green: hydrogen bond acceptor; magenta: hydrogen bond donor).

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at C-6, showed very low toxicity and potent antiretroviral activity similar to that of efavirenz and higher than nevirapine against both wild-type and in some mutant strains of HIV-1.[10]

SAR studies highlighted that the nature and the position of the substituents at N1 and on the benzene ring of the benzimidazolone moiety significantly influenced the anti-HIV activity.[11] Moreover, the opening or the replacement of the imidazol-2-one nucleus with a homologous six-membered ring decreased the potency.[12]

Our molecular modeling studies performed on this class of compounds highlighted the positive contribution to antiviral activity of both a chlorine atom on the benzimidazole system (Fig. 1) and, in particular, the electron-rich sulfonyl group, which is able to form intermolecular interactions with important residues of the non-nucleoside inhibitor binding pocket (NNIBP). We also reported that 3,5-dimethylphenyl-substituted benzimidazolones showed the greatest activity levels, and docking studies suggested that this might be due to the ability of the methyl groups to create additional intermolecular interactions with hydrophobic residues present within the NNIBP space.[10,11]

Supported by these promising results and with the aim of obtaining more potent compounds and to establish further SAR on this class of NNRTIs, we designed and synthesized new benzimidazolones and their analogues in which the chlorine atom and the sulfonyl group were maintained in view of their paramount importance.

More specifically, we decided to prepare other 3,5-dimethylphenyl-substituted derivatives in which the benzene ring of the benzimidazolone system was replaced by a suitably heteroaromatic nucleus (as pyridine, **2**) or where the carbonyl group was converted into the bioisosteric thiocarbonyl moiety (**3**).

We also designed new derivatives in which other structural modifications had been made to the aromatic portion at the N1 atom, such as the introduction of one or more methyl groups or other bioisosteric substituents in different positions of the phenyl ring (Fig. 2).

The influence of these structural changes on the antiretroviral activity of this class of compounds is reported and discussed herein. The different degrees of potency displayed by the new molecules against wild-type and mutants of RTs were studied by molecular modeling approaches.

2. Results and discussion

2.1. Chemistry

The synthesis of new benzimidazolone derivatives (**4–16**) and analogues (**2–3**) is reported in Scheme 1. The 5-chloro-2-nitroaniline or 2-amino-6-chloro-3-nitropyridine was N-substituted by treatment with the appropriate arylsulfonylchloride in the presence of sodium hydride at 0–5 °C in dioxane .

The obtained *N*-sulfoxide derivatives **32–46** were reduced with Zn dust in HCl and ethanol at 80 °C.

The subsequent cyclization of the aminoderivatives **17–31** with phosgene at room temperature afforded compounds **1–2** and **4–16**, while compound **3** was achieved when using thiophosgene as reagent.

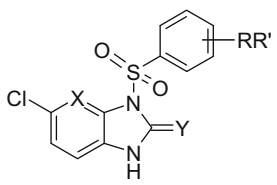


Figure 2. Structural modifications of new N_1-substituted 1,3-dihydro-2*H*-benzimidazol-2-ones.

The desired products were obtained in good yield and high purity (more than 95%). Both analytical and spectral data (^{1}H NMR) of all synthesized compounds were in full agreement with the proposed structures.

2.2. Biological activity

The new synthesized compounds **1–16** were evaluated by enzymatic tests for their ability to inhibit RT activity as well as HIV-1 (III_B) replication in MT-4 cell cultures in parallel with cytotoxic activity, and compared with nevirapine and efavirenz which were used as reference drugs (Table 1).

Selected compounds were also tested against RTs containing the K103N, Y181I, and L100I single amino acid mutations responsible for resistance to NNRTIs (Table 2).

The biological activity data show that most of the new compounds inhibit RT and prevent the cytopathic effect of HIV-1 IIIB at nanomolar concentrations and with minimal toxicity to MT-4 cells, resulting in remarkably high selectivity indices (SI).

Among all the tested compounds, derivatives **3**, **5**, **8**, **9** and **16**, proved to be highly potent and showed inhibitory potencies superior to that of nevirapine and in some cases were comparable to that of efavirenz and our lead compound **1**.

As shown in Table 1, the results obtained highlighted that modifications on the bicycle system influenced the activity profile of the molecules. In particular, the replacement of the benzene ring of the benzimidazolone system with a pyridine resulted in a reduction in the anti-HIV activity, whereas the conversion of the carbonyl group into the bioisosteric thiocarbonyl moiety caused a substantial decrease of the selectivity index, even if both compounds retained potent RT inhibitory effects.

Furthermore, the anti-HIV-1 activity was influenced by the nature and the position of the substituents introduced on the aromatic portion at N1.

Considering first of all the effect of the monomethyl substitution, the best results were obtained by the presence of a methyl function at 3 position of the phenyl ring at the N1. Moreover, the insertion of two or more methyl groups on the same moiety also provided potent compounds (**8**, **9**, **10**) as suggested by molecular modeling studies (see Section 2.3).

In contrast, the replacement of the methyl with a trifluoromethyl moiety (**13** and **14)** or 3-OCF_3 (**15)** led to a decrease in antiretroviral activity whereas its bioisostere (**16)** 3-OCH_3 substituted exhibited high potency and selectivity.

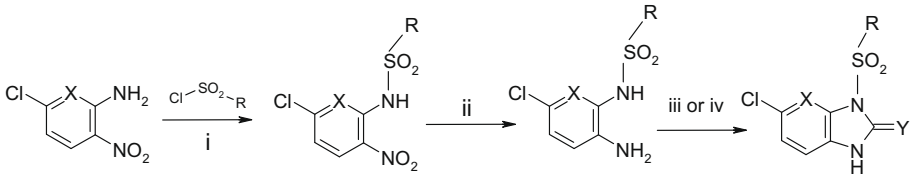
Compound **9**, characterized by a 3,4-dimethyl-phenyl substitution, showed the best activity profile both in enzymatic tests on wt RT (IC_{50} = 0.004 μM) and in cellular assays (EC_{50} = 0.006 μM) as well as the best selectivity index (>61857). Overall, **9** showed an anti-HIV profile superior to that of nevirapine and comparable to that of efavirenz.

All of the ten selected derivatives were potent inhibitors of the L100I RT mutant at submicromolar concentration; some inhibited the K103N RT mutant in the micromolar range, whereas they were generally inactive against Y181I.

The most active compounds (**2**, **3** and **9)** were more active than nevirapine. However, they were less active than reference **1** and efavirenz both against K103N and L100I RT HIV-1.

2.3. Computational results

In order to explore the possible binding conformation of the designed compounds and their interaction mode with RT, a molecular modeling study was performed using the program AUTODOCK[13] and the docking protocol that we successfully applied in our previous papers on RT.[9,10,14]



X= CH, N **32-46** **17-31** **1-16**

Compd	X	Y	R	Compd.	X	Y	R
1*	CH	O	3,5-$(CH_3)_2$-C_6H_3	**9**	CH	O	3,4-$(CH_3)_2$-C_6H_3
2	N	O	3,5-$(CH_3)_2$-C_6H_3	**10**	CH	O	2,4,6-$(CH_3)_3$-C_6H_2
3	CH	S	3,5-$(CH_3)_2$-C_6H_3	**11**	CH	O	2,3,5,6-$(CH_3)_4$-C_6H
4	CH	O	2-CH_3-C_6H_4	**12**	CH	O	2,3,4,5.6-$(CH_3)_5$-C_6
5	CH	O	3-CH_3-C_6H_4	**13**	CH	O	3,5-$(CF_3)_2$-C_6H_3
6	CH	O	4-CH_3-C_6H_4	**14**	CH	O	3-CF_3-C_6H_4
7	CH	O	2,4-$(CH_3)_2$-C_6H_3	**15**	CH	O	3-OCF_3-C_6H_4
8	CH	O	2,5-$(CH_3)_2$-C_6H_3	**16**	CH	O	3-OCH_3-C_6H_4

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) acetone, $CSCl_2$, 1 h. *See Ref. 10.

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

Compd	IC_{50} (μM)[a]	EC_{50} (μM)[b]	CC_{50} (μM)[c]	SI[d]
1[e]	0.005 ± 0.001	0.0022 ± 0.0003	39 ± 8.2	17846
2	0.020 ± 0.002	0.026 ± 0.0015	>272.35	>10475
3	0.006 ± 0.001	0.0082 ± 0.002	34.85 ± 4.42	4250
4	0.269 ± 0.027	1.92 ± 2.04	>387.27	>201
5	0.021 ± 0.002	0.0151 ± 0.004	>307.92	>20528
6	0.149 ± 0.015	0.046 ± 0.015	133.53 ± 109.73	2902
7	0.63 ± 0.060	0.12 ± 0.026	39.28 ± 4.27	327
8	0.049 ± 0.005	0.0097 ± 0.002	>371.14	>37114
9	0.004 ± 0.0004	0.006 ± 0.002	>371.14	>61857
10	0.0012 ± 0.0001	1.002 ± 0.645	>356	>355
11	0.040 ± 0.004	0.063 ± 0.016	⩾342.60	>5412
12	0.047 ± 0.005	0.129 ± 0.006	235.6 ± 26.81	1826
13	0.31 ± 0.030	0.31 ± 0.15	32.31 ± 11.93	104
14	1.72 ± 0.170	0.50 ± 0.02	>331.79	>664
15	0.305 ± 0.031	3.66 ± 1.93	>127.56	>35
16	0.052 ± 0.005	0.035 ± 0.014	>368.99	>10543
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] See Ref. 10.

Docking of compounds **1**–**16** into the RT allosteric pocket, extracted from the structure of the complex RT/efavirenz (PDB code 1FK9)[15] generated a number of possible binding conformations with the corresponding AUTODOCK estimated free binding energies.

Table 2
HIV-1 RT inhibitory activity of compounds **1–3**, **5**, **8–10** and **16** against mutant enzymes carrying single amino acid substitutions

Compd	IC_{50}[a] (μM)		
	L100I	Y181I	K103N
1	0.009 ± 0.001	>40	0.4 ± 0.05
2	0.07 ± 0.006	27 ± 2	2.8 ± 0.2
3	0.05 ± 0.006	18 ± 1	0.9 ± 0.1
5	0.07 ± 0.005	>40	10 ± 1
8	0.38 ± 0.04	>40	>40
9	0.03 ± 0.003	>40	2.61 ± 0.26
10	0.01 ± 0.001	>40	>40
11	0.18 ± 0.02	>40	>40
12	0.08 ± 0.01	>40	6.7 ± 0.7
16	0.33 ± 0.03	>40	10.92 ± 1.10
Nevirapine	7 ± 1	30 ± 3	4 ± 0.5
Efavirenz	0.05 ± 0.006	0.5 ± 0.05	0.2 ± 0.03

[a] Compound concentration IC_{50} (μM) required to inhibit by 50% the RT activity of the indicated strain.

The cluster analysis revealed a predominant ligand orientation within the binding pocket and the most energetically favorable conformation for each molecule was chosen for further analysis. Figure 3A reports the docking results of **1**–**16** derivatives compared to the crystallized position of efavirenz: compounds **1**–**16** were bound in the NNIBP with an analogous binding mode and interacted with RT residues of the allosteric pocket in a similar fashion to efavirenz. The potent inhibitory effects of several synthesized derivatives might also be due to the ability of the methyl groups to occupy the hydrophobic space near the 'roof' of the NNRTI binding pocket (consisting of P95, Y181, Y188, and W229),[10,16] thus creating additional intermolecular interactions as reported in Figure 4 for derivative **9**, the most interesting of our compounds.

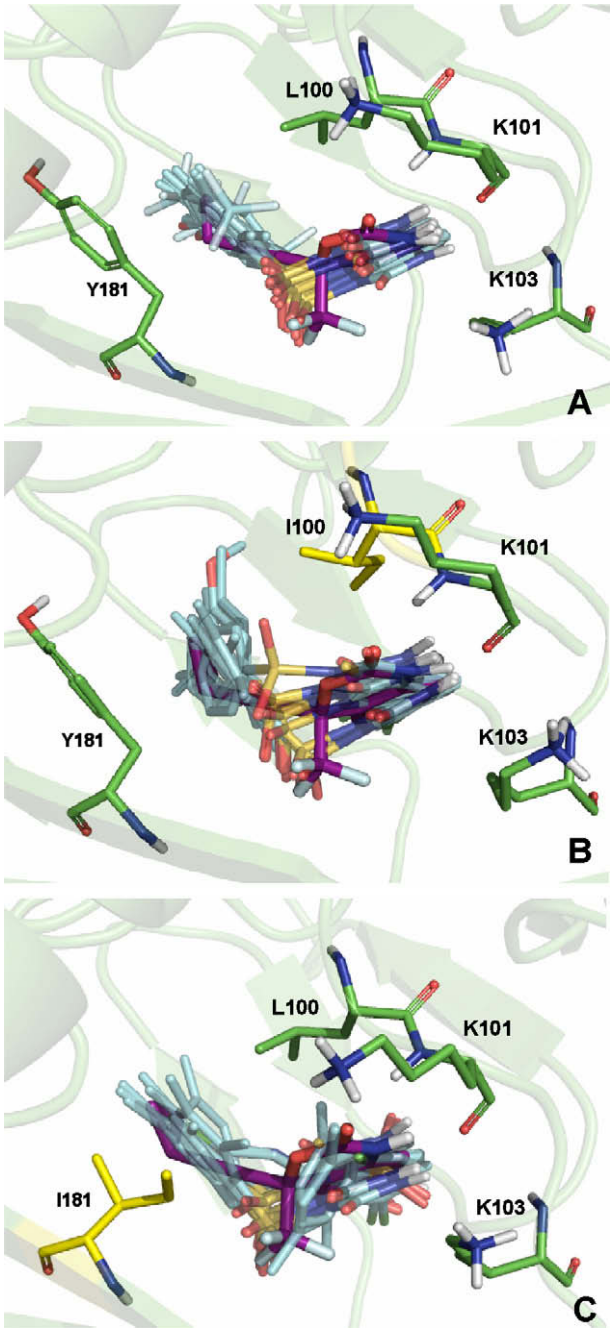


Figure 3. (A) Binding mode of compounds **1**–**16** (cyan) compared to the crystallized position of efavirenz (violet) in wild-type RT. (B) Binding mode of compounds **1**–**3**, **5**, **8**–**12**, **16** (cyan) efavirenz (violet) in mutant L100I (yellow) RT. (C) Binding mode of compounds **1**–**3**, **5**, **8**–**12**, **16** (cyan) efavirenz (violet) in mutant Y181I (yellow) RT. This figure was prepared using the program PYMOL.

We carried out further docking studies in order to understand the behavior of the most active compounds against different mutant enzymes.

For the mutation L100I the crystal Structure of L100I mutant HIV-1 reverse transcriptase in complex with GW420867X (PDB code 2OPQ) was used[17] for AUTODOCK docking experiments.

A docking study of GW420867X was performed to validate the ability of the method. The program perfectly reproduced the experimental position of the ligand with root-mean square deviation of 0.28 Å (1 cluster in 250 runs out of 250).

We docked derivatives **1–3**, **5**, **8–12**, **16** and efavirenz, as reference compound.

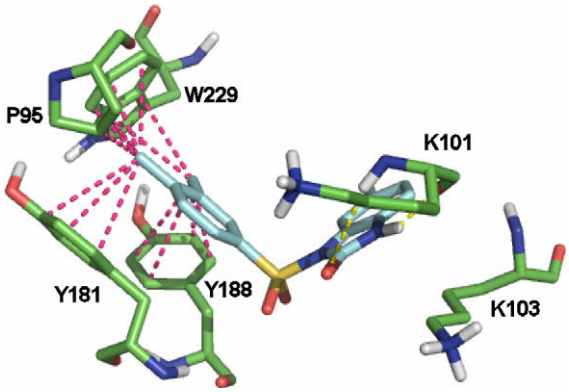


Figure 4. Intermolecular interactions (shown as dashed magenta lines) between the 3,4-dimethyl groups of compound **9** and NNIBP residues P95, Y181, Y188 and W229. Hydrogen bonds (shown as dashed yellow lines) are formed between the ligand and RT. This figure was prepared using the program PYMOL. PYMOL Molecular Graphics System; v 0.99; DeLano Scientific: San Carlos, CA, USA, 2002.

Both efavirenz and our compounds showed a binding mode similar to that assumed in wild-type; in fact the benzimidazol-2-one moiety is oriented in the same position near the K103 and the phenyl ring close to Y181 thus making hydrogen bonds possible between the amide group and K101 (Fig. 3B).

In fact, the activity of these compounds in the presence of the L100I mutation remains comparable to that against wild-type RT, probably due to the hydrophobic nature of both leucine and isoleucine, as well as to the similar size of the two residues.

For the mutation Y181I, since the structure of RT with this mutation is not available in the Protein Data Bank, we used the crystal structure of Y181C mutant HIV-1 RT in complex with efavirenz[18] (PDB code 1JKH), in which we changed the cysteine 181 in isoleucine in order to obtain a plausible model (see Section 4).

Once again, the AUTODOCK program was run for derivatives **1–3**, **5**, **8–12**, **16** and efavirenz obtaining a different binding mode for our compounds whereas the efavirenz showed an orientation similar to that observed with wild-type enzyme.

In fact, in the benzimidazolones, although the phenyl ring is oriented near the I181, the remaining part of the structure is pointed in a different direction thus losing the interaction with K101 in the active site (Fig. 3C).

The Y181 ring is important to create π–π interactions with phenyl ring at N-1, stabilizing the orientation of the remaining part of the molecule. Thus, the mutation of Y181I causes the loss of this π–π interaction thereby creating new hydrophobic contacts between I181 and lipophilic groups such as methyl substituents or chlorine atom. However, these new hydrophobic interactions are not sufficient to stabilize a suitable orientation of our compounds in the binding pocket.

Nevertheless, there is also a decrease in the activity in the case of efavirenz, but it is not so relevant because it maintains better its orientation due to the presence of hydrophobic cyclopropyl nucleus which is less influenced by the mutation of aromatic tyrosine in the lipophilic isoleucine residue.

Molecular docking simulations on the K103N RT were not performed as it has clearly been established that this mutation has generally a minimal influence on the bound conformation of NNRTIs, while this mutation greatly affects the kinetics of the inhibitor-binding process by stabilizing the unbound state of RT.[19–23]

3. Conclusion

In the context of our ongoing efforts to prepare novel potent NNRTIs, a number of new derivatives have been synthesized. The

results obtained, in both enzymatic tests on wt RT and in cellular assays, showed high potency and very low cytotoxicity for the majority of compounds.

An excellent HIV-1 inhibitory profile is shown by compound **9** with a potency superior to that of nevirapine and comparable to that of compound **1** and efavirenz but with a higher selectivity index (>61857).

Furthermore, compounds **2**, **3** and **9** also proved to be potent inhibitors of RTs carrying the K103N and L100I mutations.

The molecular modeling studies helped us to rationalize the relationships between the molecular structure of the inhibitors and their activity toward RTs.

4. Experimental section

4.1. Chemistry

Melting points were determined on a BUCHI Melting Point B-545 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 and purity of tested compounds was >95%. Merck Silica Gel 60 F_{254} plates were used for TLC; column chromatography was performed on Merck Silica Gel 60 (230–400 mesh) and flash chromatography (FC) on Biotage SP1 EXP. ^{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).

4.1.1. General procedures for the synthesis of *N*-(5-chloro-2-nitrophenyl)-benzenesulfonamides (32, 34–46)

The mixture of the appropriate arylsulfonyl chloride (3 mmol) and the 5-chloro-2-nitroaniline or 2-amino-6-chloro-3-nitropyridine (2 mmol) in dioxane (6 mL), was stirred for 30 min at 0 °C using dry sodium hydride (10 mmol) as catalyst. 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 crystallized from diethyl ether.

4.1.1.1. *N*-(5-Chloro-2-nitrophenyl)-3,5-dimethylbenzenesulfonamide (32). Compound **32** was prepared as previously reported.[10]

4.1.1.2. *N*-(5-Chloro-2-nitrophenyl)-2-methylbenzenesulfonamide (34). Mp: 142–144 °C, yield 100%. ^{1}H NMR (DMSO-d_6): δ 2.56 (s, 3H, CH_3), 7.30 (s, 1H, H-6), 7.35–7.59 (m, 4H, H-4, 3′,4′,5′), 7.77 (d, J = 7.96, 1H, H-6′), 7.96 (d, J = 8.79, 1H, H-3), 10.59 (br s, 1H, NH). Anal. Calcd for $C_{13}H_{11}ClN_2O_4S$: C, 47.79; H, 3.39; N, 8.57. Found: C, 48.03; H, 3.21; N, 8,69.

4.1.1.3. *N*-(5-Chloro-2-nitrophenyl)-3-methylbenzenesulfonamide (35). Mp: 127–130 °C, yield 99%. ^{1}H NMR ($CDCl_3$): δ 2.41 (s, 3H, CH_3), 7.10 (d, J = 9.06, 1H, H-4), 7.39–7.71 (m, 4H, H-6, 4′,5′,6′), 7.87 (s, 1H, H-2′), 8.09 (d, J = 9.06, 1H, H-3), 10.01 (br s, 1H, NH). Anal. Calcd for $C_{13}H_{11}ClN_2O_4S$: C, 47.79; H, 3.39; N, 8.57. Found: C, 47.43; H, 3.45; N, 8.84.

4.1.1.4. *N*-(5-Chloro-2-nitrophenyl)-4-methylbenzenesulfonamide (36). Mp: 133–135 °C, yield 100%. ^{1}H NMR (DMSO-d_6): δ 2.35 (s, 3H, CH_3), 7.27 (s, 1H, H-6), 7.38 (d, J = 7.96, 2H, H-3′,5′), 7.44 (d, J = 8.79, 1H, H-4), 7.65 (d, J = 7.69, 2H, H-2′,6′), 7.96 (d, J = 8.79, 1H, H-3), 10.47 (br s, 1H, NH). Anal. Calcd for $C_{13}H_{11}ClN_2O_4S$: C, 47.79; H, 3.39; N, 8.57. Found: C, 47.35; H, 3.72; N, 8.26.

4.1.1.5. *N*-(5-Chloro-2-nitrophenyl)-2,4-dimethylbenzenesulfonamide (37). Mp: 125–127 °C, yield 100%. ^{1}H NMR (DMSO-d_6): δ 2.31 (s, 3H, CH_3, C-4′), 2.51 (s, 3H, CH_3, C-2′), 7.17 (d, J = 7.14, 1H, H-5′), 7.23 (s, 1H, H-6), 7.32 (s, 1H, H-3′), 7.38 (d, J = 8.51, 1H, H-4), 7.66 (d, J = 7.69, 1H, H-5′), 7.96 (d, J = 8.51, 1H, H-3), 10.46 (br s, 1H, NH). Anal. Calcd for $C_{14}H_{13}ClN_2O_4S$: C, 49.34; H, 3.85; N, 8.22. Found: C, 49.78; H, 3.44; N, 8.57.

4.1.1.6. *N*-(5-Chloro-2-nitrophenyl)-2,5-dimethylbenzenesulfonamide (38). Mp: 259–261 °C, yield 100%. ^{1}H NMR (DMSO-d_6): δ 2.27 (s, 3H, CH_3, C-4′), 3.55 (s, 3H, CH_3, C-2′), 6.44 (d, J = 7.42, 1H, H-4′), 6.98–7.08 (m, 2H, H-3′,4), 7.27 (s, 1H, H-6), 7.35 (d, J = 8.79, 1H, H-3), 7.68 (s, 1H, H-5′). Anal. Calcd for $C_{14}H_{13}ClN_2O_4S$: C, 49.34; H, 3.85; N, 8.22. Found: C, 48.94; H, 3.52; N, 8.07.

4.1.1.7. *N*-(5-Chloro-2-nitrophenyl)-3,4-dimethylbenzenesulfonamide (39). Mp: 154–155 °C, yield 98%. ^{1}H NMR (DMSO-d_6): δ 2.25 (s, 3H, CH_3, C-4′), 2.27 (s, 3H, CH_3, C-3′), 7.29 (s, 1H, H-6), 7.34 (d, J = 7.96, 1H, H-5′), 7.41 (d, J = 9.06, 1H, H-6′), 7.49 (d, J = 8.79, 1H, H-4), 7.56 (s, 1H, H-2′), 7.96 (d, J = 8.79, 1H, H-3), 10.39 (br s, 1H, NH). Anal. Calcd for $C_{14}H_{13}ClN_2O_4S$: C, 49.34; H, 3.85; N, 8.22. Found: C, 49.65; H, 4.21; N, 8.34.

4.1.1.8. *N*-(5-Chloro-2-nitrophenyl)-2,4,6-trimethylbenzenesulfonamide (40). Mp: 117–119 °C, yield 82%. ^{1}H NMR ($CDCl_3$): δ 2.22 (s, 3H, CH_3), 2.48 (s, 6H, $2CH_3$), 6.46 (d, J = 9.06, 1H, H-4), 6.76 (s, 2H, H-3′,5′), 6.92 (s, 1H, H-6), 7.63 (d, J = 9.06, 1H, H-3), 10.19 (br s, 1H, NH). Anal. Calcd for $C_{15}H_{15}ClN_2O_4S$: C, 50.78; H, 4.26; N, 7.90. Found: C, 50.39; H, 4.51; N, 7.73.

4.1.1.9. *N*-(5-Chloro-2-nitrophenyl)-2,3,5,6-tetramethylbenzenesulfonamide (41). Mp: 184–186 °C, yield 71%. ^{1}H NMR (DMSO-d_6): δ 2.21 (s, 6H, CH_3, C-3′,5′), 2.39 (s, 6H, CH_3, C-2′,6′), 7.21 (s, 1H, H-6), 7.29 (s, 1H, H-4′), 7.42 (d, J = 8.79, 1H, H-4), 8.01 (d, J = 8.79, 1H, H-3), 10.41 (br s, 1H NH). Anal. Calcd for $C_{16}H_{17}ClN_2O_4S$: C, 52.10; H, 4.65; N, 7.59. Found: C, 51.86; H, 4.23; N, 7.14.

4.1.1.10. *N*-(5-Chloro-2-nitrophenyl)-2,3,4,5,6-pentamethylbenzenesulfonamide (42). Mp: 182–184 °C, yield 100%. ^{1}H NMR (DMSO-d_6): δ 2.17 (s, 6H, CH_3, C-3′,5′), 2.23 (s, 3H, CH_3, C-4′), 2.43 (s, 6H, CH_3, C-2′,6′), 7.21 (s, 1H, H-6), 7.39 (d, J = 8.79, 1H, H-4), 8.01 (d, J = 8.79, 1H, H-3), 10.36 (br s, 1H, NH). Anal. Calcd for $C_{17}H_{19}ClN_2O_4S$: C, 53.34; H, 5.00; N, 7.32. Found: C, 53.61; H, 5.33; N, 7.68.

4.1.1.11. *N*-(5-Chloro-2-nitrophenyl)-3,5-bistrifluoromethylbenzenesulfonamide (43). Mp: 121–124 °C, yield 95%. ^{1}H NMR ($CDCl_3$): δ 7.17 (d, J = 9.06, 1H, H-4), 7.80 (s, 1H, H-6), 8.09–8.37 (m, 4H, H-3, 2′,4′,6′), 10.07 (br s, 1H, NH). Anal. Calcd for $C_{14}H_7ClF_6N_2O_4S$: C, 37.47; H, 1.57; N, 6.24. Found: C, 37.15; H, 1.79; N, 6.61.

4.1.1.12. *N*-(5-Chloro-2-nitrophenyl)-3-trifluoromethylbenzenesulfonamide (44). Mp: 120–123 °C, yield 100%. ^{1}H NMR ($CDCl_3$): δ 7.13 (d, J = 8.51, 1H, H-4), 7.67–7.88 (m, 4H, H-6, 4′,5′,6′), 8.08 (d, J = 8.24, 1H, H-3), 8.14 (s, 1H, H-2′), 10.04 (br s, 1H, NH). Anal. Calcd for $C_{13}H_8ClF_3N_2O_4S$: C, 41.01; H, 2.12; N, 7.36. Found: C, 41.44; H, 2.50; N, 7.64.

4.1.1.13. *N*-(5-Chloro-2-nitrophenyl)-3-trifluoromethoxybenzenesulfonamide (45). Mp: 95–97 °C, yield 94%. ^{1}H NMR ($CDCl_3$): δ 7.16 (d, J = 8.99, 1H, H-4), 7.47 (d, J = 8.51, 1H, H-4′), 7.54–7.59 (m, 1H, H-5′), 7.73 (s, 1H, H-6), 7.83 (d, J = 7.69, 1H, H-6′), 7.87 (s, 1H, H-2′), 8.09 (d, J = 9.06, 1H, H-3), 9.99 (br s, 1H, NH). Anal.

Calcd for $C_{13}H_8ClF_3N_2O_5S$: C, 39.36; H, 2.03; N, 7.06. Found: C, 39.62; H, 2.31; N, 6.95.

4.1.1.14. *N*-(5-Chloro-2-nitrophenyl)-3-methoxybenzenesulfonamide (46). Mp: 111–113 °C, yield 73%. ^{1}H NMR ($CDCl_3$): δ 3.84 (s, 3H, CH_3), 7.12 (d, J = 8.79, 1H, H-4), 7.27 (s, 1H, H-6), 7.37–7.48 (m, 3H, H-4′,5′,6′), 7.89 (s, 1H, H-2′), 8.09 (d, J = 8.79, 1H, H-3), 9.99 (br s, 1H, NH). Anal. Calcd for $C_{13}H_{11}ClN_2O_5S$: C, 45.56; H, 3.23; N, 8.17. Found: C, 45.29; H, 3.37; N, 8.40.

4.1.2. General procedure for the synthesis of *N*-(6-chloro-3-nitropyridin-2-yl)-3,5-dimethylbenzenesulfonamide (33)

Using a similar procedure for **32**, **34–46**, compound **33** was prepared from 2-amino-6-chloro-3-nitropyridine (2 mmol) and the appropriate aryl sulfonyl chloride (3 mmol).

Mp: 215–219 °C, yield 77%. ^{1}H NMR (DMSO-d_6): δ 2.34 (s, 6H, CH_3), 7.25–7.35 (m, 2H, H-4, H-4′), 7.70 (s, 2H, H2′,6′), 8.42 (d, J = 8.24, 1H, H-3). Anal. Calcd for $C_{13}H_{12}ClN_3O_4S$: C, 45.69; H, 3.54; N, 12.29. Found: C, 45.48; H, 3.81; N, 12.57.

4.1.3. General procedures for the synthesis of *N*-(2-aminophenyl-5-chloro)-benzenesulfonamides (17, 19–31)

Zinc dust (20 mmol) was added in several portions to a solution of the appropriate *N*-(5-chloro-2-nitrophenyl)-benzenesulfonamide (0.6 mmol) in 3 mL HCl 2 N and 4 mL EtOH anhydrous under stirring in an ice bath. The reaction mixture was heated in a water bath for 1 h. After cooling, it was made alkaline with NaOH 2 N and extracted with ethyl acetate. The extract was washed with water, dried over Na_2SO_4 and evaporated under reduced pressure. The residue was powdered by treatment with ethanol or diethyl ether.

4.1.3.1. *N*-(2-Aminophenyl-5-chloro)-3,5-dimethylbenzenesulfonamide (17). Compound **17** was prepared as previously reported.[10]

4.1.3.2. *N*-(2-Aminophenyl-5-chloro)-2-methylbenzenesulfonamide (19). Mp: 163–165 °C, yield 100%. ^{1}H NMR (DMSO-d_6): δ 2.55 (s, 3H, CH_3), 5.10 (br s, 2H, NH_2), 6.57 (d, J = 8.79, 1H, H-3), 6.62 (s, 1H, H-6), 6.79 (d, J = 8.51, 1H, H-4), 7.27–7.36 (m, 2H, H-3′,4′), 7.44–7.49 (m, 1H, H-5′), 7.71 (d, J = 7.69, 1H, H-6′). Anal. Calcd for $C_{13}H_{13}ClN_2O_2S$: C, 52.61; H, 4.44; N, 9.44. Found: C, 52,33; H, 4.87; N, 9.14.

4.1.3.3. *N*-(2-Aminophenyl-5-chloro)-3-methylbenzenesulfonamide (20). Mp: 116–118 °C, yield 44%. ^{1}H NMR (DMSO-d_6): δ 2.66 (s, 3H, CH_3), 5.25 (br s, 2H, NH_2), 6.76 (d, J = 8.51, 1H, H-3), 6.82 (s, 1H, H-6), 7.06 (d, J = 8.51, 1H, H-4), 7.54–7.68 (m, 4H, H-2′,4′,5′,6′), 9.52 (br s, 1H, NH). Anal. Calcd for $C_{13}H_{13}ClN_2O_2S$: C, 52.61; H, 4.44; N, 9.44. Found: C, 52.97; H, 4.23; N, 9.79.

4.1.3.4. *N*-(2-Aminophenyl-5-chloro)-4-methylbenzenesulfonamide (21). Mp: 158–160 °C, yield 87%. ^{1}H NMR (DMSO-d_6): δ 2.34 (s, 3H, CH_3), 5.07 (br s, 2H, NH_2), 6.60 (d, J = 8.51, 1H, H-3), 6.72 (s, 1H, H-6), 6.87 (d, J = 8.51, 1H, H-4), 7.33–7.57 (m, 4H, H-2′,3′,5′,6′), 9.37 (br s, 1H, NH). Anal. Calcd for $C_{13}H_{13}ClN_2O_2S$: C, 52.61; H, 4.44; N, 9.44. Found: C, 52.25; H, 4.18; N, 9.82.

4.1.3.5. *N*-(2-Aminophenyl-5-chloro)-2,4-dimethylbenzenesulfonamide (22). Mp: 115–117 °C, yield 34%. ^{1}H NMR (DMSO-d_6): δ 2.28 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 5.12 (br s, 2H, NH_2), 6.59 (d, J = 8.51, 1H, H-3), 6.65 (s, 1H, H-6), 6.85 (d, J = 8.51, 1H, H-4), 7.11 (d, J = 8.24, 1H, H-5′), 7.18 (s, 1H, H-3′), 7.58 (d, J = 7.96, 1H, H-6′), 9.38 (br s, 1H, NH). Anal. Calcd for $C_{14}H_{15}ClN_2O_2S$: C, 54.10; H, 4.86; N, 9.01. Found: C, 54.46; H, 4.57; N, 9.29.

4.1.3.6. *N*-(2-Aminophenyl-5-chloro)-2,5-dimethylbenzenesulfonamide (23). Mp: 150–152 °C, yield 48%. ^{1}H NMR (DMSO-d_6): δ 2.27 (s, 3H, CH_3, C-4′), 2.51 (s, 3H, CH_3, C-2′), 5.15 (br s, 2H, NH_2), 6.61 (s, 1H, H-6), 6.64 (d, J = 9.06, 1H, H-3), 6.91 (d, J = 8.51, 1H, H-4), 7.22–7.35 (m, 2H, H-3′,4′), 7.56 (s, 1H, H-6′), 9.36 (br s, 1H, NH). Anal. Calcd for $C_{14}H_{15}ClN_2O_2S$: C, 54.10; H, 4.86; N, 9.01. Found: C, 54.34; H, 4.99; N, 9.41.

4.1.3.7. *N*-(2-Aminophenyl-5-chloro)-3,4-dimethylbenzenesulfonamide (24). Mp: 95–97 °C, yield 100%. ^{1}H NMR (DMSO-d_6): δ 2.21 (s, 3H, CH_3, C-4′), 2.25 (s, 3H, CH_3, C-3′), 4.70 (br s, 1H, NH_2), 6.41–6.76 (m, 3H, H-3, 4, 6), 7.16 (d, J = 7.96, 1H, H-5′), 7.37 (d, J = 7.96, 1H, H-2′), 7.44 (s, 1H, H-6′). Anal. Calcd for $C_{14}H_{15}ClN_2O_2S$: C, 54.10; H, 4.86; N, 9.01. Found: C, 53.83; H, 5.25; N, 8.77.

4.1.3.8. *N*-(2-Aminophenyl-5-chloro)-2,4,6-trimethylbenzenesulfonamide (25). Mp: 146–148 °C, yield 45%. ^{1}H NMR (DMSO-d_6): δ 2.68 (s, 3H, CH_3), 2.74 (s, 6H, CH_3), 5.37 (br s, 2H, NH_2), 6.76 (s, 1H, H-6), 6.88 (d, J = 8.54, 1H, H-3), 7.16 (d, J = 8.54, 1H, H-4), 7.25 (s, 2H, H-3′,5′). Anal. Calcd for $C_{15}H_{17}ClN_2O_2S$: C, 55.46; H, 5.28; N, 8.62. Found: C, 55,14; H, 5.67; N, 8.23.

4.1.3.9. *N*-(2-Aminophenyl-5-chloro)-2,3,5,6-tetramethylbenzenesulfonamide (26). Mp: 161–163 °C, yield 63%. ^{1}H NMR (DMSO-d_6): δ 2.19 (s, 6H, CH_3, C-3′,5′), 2.34 (s, 6H, CH_3, C-2′,6′), 5.14 (br s, 2H, NH_2), 6.54 (s, 1H, H-6), 6.64 (d, J = 8.51, 1H, H-3), 6.92 (d, J = 8.51, 1H, H-4), 7.23 (s, 1H, H-4′), 9.34 (br s, 1H, NH). Anal. Calcd for $C_{16}H_{19}ClN_2O_2S$: C, 56.71; H, 5.65; N, 8.27. Found: C, 56,38; H, 5.14; N, 8.45.

4.1.3.10. *N*-(2-Aminophenyl-5-chloro)-2,3,4,5,6-pentamethylbenzenesulfonamide (27). Mp: 171–173 °C, yield 89%. ^{1}H NMR (DMSO-d_6): δ 2.15 (s, 6H, CH_3, C-3′,5′), 2.22 (s, 3H, CH_3, C-4′), 2.36 (s, 6H, CH_3, C-2′,6′), 5.13 (br s, 2H, NH_2), 6.63–6.66 (m, 2H, H-3,6), 6.92 (d, J = 8.51, 1H, H-4), 9.47 (br s, 1H, NH). Anal. Calcd for $C_{17}H_{21}ClN_2O_2S$: C, 57.86; H, 6.00; N, 7.94. Found: C, 57.43; H, 6.25; N, 7.59.

4.1.3.11. *N*-(2-Aminophenyl-5-chloro)-3,5-bistrifluoromethylbenzenesulfonamide (28). Mp: 182–185 °C, yield 44%. ^{1}H NMR (DMSO-d_6): δ 5.39 (br s, 2H, NH_2), 6.70 (d, J = 8.24, 1H, H-3), 6.84 (d, J = 8.24, 1H, H-4), 6.93 (s, 1H, H-6), 8.26 (s, 1H, H-4′), 8.35 (s, 2H, H-2′,6′). Anal. Calcd for $C_{14}H_9ClF_6N_2O_2S$: C, 40.16; H, 2.17; N, 6.69. Found: C, 39.82; H, 2.44; N, 6.24.

4.1.3.12. *N*-(2-Aminophenyl-5-chloro)-3-trifluoromethylbenzenesulfonamide (29). Mp: 116–119 °C, yield 59%. ^{1}H NMR (DMSO-d_6): δ 5.12 (br s, 2H, NH_2), 6.65 (d, J = 8.79, 1H, H-3), 6.70 (s, 1H, H-6), 6.93 (d, J = 8.79, 1H, H-4), 7.77–7.97 (m, 3H, H-6, 4′,5′), 8.03 (d, J = 7.69, 1H, H-6′), 9.55 (br s, 1H, NH), 7.85 (d, J = 7.41, 1H, H-6′). Anal. Calcd for $C_{13}H_{10}ClF_3N_2O_2S$: C, 44.52; H, 2.87; N, 7.99. Found: C, 44.91; H, 2.56; N, 8.24.

4.1.3.13. *N*-(2-Aminophenyl-5-chloro)-3-trifluoromethoxybenzenesulfonamide (30). Mp: 121–124 °C, yield 87%. ^{1}H NMR (DMSO-d_6): δ 5.51 (br s, 2H, NH_2), 6.68 (d, J = 7.96, 1H, H-3), 6.91 (s, 1H, H-6), 6.96 (d, J = 8.51, 1H, H-4), 7.46–7.55 (m, 3H, H-4′,5′), 7.75 (s, 1H, H-2′), 7.85 (d, J = 7.41, 1H, H-6′). Anal. Calcd for $C_{13}H_{10}ClF_3N_2O_3S$: C, 42.58; H, 2.75; N, 7.64. Found: C, 42.29; H, 3.11; N, 8.06.

4.1.3.14. *N*-(2-Aminophenyl-5-chloro)-3-methoxybenzenesulfonamide (31). Mp: 117–120 °C, yield 66%. ^{1}H NMR (DMSO-d_6): δ 5.11 (br s, 2H, NH_2), 6.79 (d, J = 8.51, 1H, H-3), 6.71 (s, 1H, H-6), 6.91 (d, J = 8.79, 1H, H-4), 7.18–7.48 (m, 4H, H-2′,4′,5′,6′), 9.39 (br s, 1H, NH), 7.85 (d, J = 7.41, 1H, H-6′). Anal. Calcd for

$C_{13}H_{13}ClN_2O_3S$: C, 49.92; H, 4.19; N, 8.96. Found: C, 49.54; H, 4.53; N, 9.25.

4.1.4. General procedure for the synthesis of *N*-(3-amino-6-chloropyridin-2-yl)-3,5-dimethylbenzenesulfonamide (18)

Using a similar procedure for **17**, **19–31**, compound **18** was prepared from *N*-(6-chloro-3-nitropyridin-2-yl)-3,5-dimethylbenzenesulfonamide **33** (0.6 mmol).

Mp: 202–205 °C, yield 78%. ^{1}H NMR (DMSO-d_6): δ 2.17 (s, 6H, CH_3), 5.38 (br s, 2H, NH_2), 6.63 (d, J = 7.69, 1H, H-3), 7.01 (s, 1H, H-4′), 7.21 (d, J = 8.51, 1H, H-4), 7.39 (s, 2H, H-2′,6′). Anal. Calcd for $C_{13}H_{14}ClN_3O_2S$: C, 50.08; H, 4.53; N, 13.48. Found: C, 50.51; H, 4.18; N, 13.81.

4.1.5. General procedure for the synthesis of 1-arylsulfonyl-1,3-dihydro-2*H*-benzimidazol-2-ones (1, 4–16)

An excess of phosgene 20% toluene (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 stirred and heated for 4 h. The reaction mixture was then cooled, neutralized with NaOH 2 N, extracted with ethyl acetate, washed with water and dried over Na_2SO_4. After evaporation under reduced pressure, the residue was crystallized by ethyl acetate or diethyl ether.

4.1.5.1. 6-Chloro-1-(3,5-dimethylphenylsulfonyl)-1,3-dihydro-2*H*-benzimidazol-2-one (1). Compound 1 was prepared as previously reported.[10]

4.1.5.2. 6-Chloro-1-(2-methylphenylsulfonyl)-1,3-dihydro-2*H*-benzimidazol-2-one (4). Mp: 272–274 °C, yield 81%. ^{1}H NMR (DMSO-d_6): δ 2.40 (s, 3H, CH_3), 7.09 (d, J = 8.24, 1H, H-4), 7.27 (d, J = 8.24, 1H, H-5), 7.45–7.70 (m, 4H, H-7, H-3′,4′,5′), 8.09 (d, J = 7.96, 1H, H-6′), 11.73 (br s, 1H, NH). Anal. Calcd for $C_{14}H_{11}ClN_2O_3S$: C, 52.10; H, 3.44; N, 8.68. Found: C, 52.57; H, 3.73; N, 8.24.

4.1.5.3. 6-Chloro-1-(3-methylphenylsulfonyl)-1,3-dihydro-2*H*-benzimidazol-2-one (5). Mp: 213–216 °C, yield 43%. ^{1}H NMR (DMSO-d_6): δ 2.36 (s, 3H, CH_3), 6.80 (d, J = 8.51, 1H, H-4), 6.98 (dd, J = 2.19, J = 8.51, 1H, H-5), 7.44–7.52 (m, 3H, H-4′,5′,6′), 7.74 (s, 1H, H-7), 7.77 (s, 1H, H-2′). Anal. Calcd for $C_{14}H_{11}ClN_2O_3S$: C, 52.10; H, 3.44; N, 8.68. Found: C, 51.76; H, 3.80; N, 8.92.

4.1.5.4. 6-Chloro-1-(4-methylphenylsulfonyl)-1,3-dihydro-2*H*-benzimidazol-2-one (6). Mp: 274–277 °C, yield 87%. ^{1}H NMR (DMSO-d_6): δ 2.38 (s, 3H, CH_3), 7.02 (d, J = 8.51, 1H, H-4), 7.24 (d, J = 8.51, 1H, H-5), 7.46 (d, J = 8.24, 2H, H-3′,5′), 7.72 (s, 1H, H-7), 7.92 (d, J = 8.24, 2H, H-2′,6′), 11.66 (br s, 1H, NH). Anal. Calcd for $C_{14}H_{11}ClN_2O_3S$: C, 52.10; H, 3.44; N, 8.68. Found: C, 52.34; H, 3.15; N, 8.53.

4.1.5.5. 6-Chloro-1-(2,4-dimethylphenylsulfonyl)-1,3-dihydro-2*H*-benzimidazol-2-one (7). Mp: 206–208 °C, yield 40%. ^{1}H NMR (DMSO-d_6): δ 2.34 (s, 3H, CH_3, C-4′), 2.47 (s, 3H, CH_3, C-2′), 7.06 (d, J = 8.24, 1H, H-4), 7.23 (s, 1H, H-3′), 7.24 (d, J = 8.51, 1H, H-2′), 7.29 (d, J = 8.24, 1H, H-5), 7.66 (s, 1H, H-7), 7.96 (d, J = 7.96, 1H, H-6′), 11.58 (br s, 1H, NH). Anal. Calcd for $C_{15}H_{13}ClN_2O_3S$: C, 53.49; H, 3.89; N, 8.32. Found: C, 53.10; H, 3.61; N, 8.03.

4.1.5.6. 6-Chloro-1-(2,5-dimethylphenylsulfonyl)-1,3-dihydro-2*H*-benzimidazol-2-one (8). Mp: 272–274 °C, yield 41%. ^{1}H NMR (DMSO-d_6): δ 2.33 (s, 3H, CH_3, C-5′), 2.37 (s, 3H, CH_3, C-2′), 7.08 (d, J = 8.51, 1H, H-4), 7.26 (dd, J = 1.92, J = 8.51, 1H, H-5), 7.34 (d, J = 7.96, 1H, H-4′), 7.49 (d, J = 7.69, 1H, H-3′), 7.68 (s, 1H, H-7), 7.90 (s, 1H, H-6′), 11.71 (br s, 1H, NH). Anal. Calcd for $C_{15}H_{13}ClN_2O_3S$: C, 53.49; H, 3.89; N, 8.32. Found: C, 53.85; H, 3.46; N, 8.61.

4.1.5.7. 6-Chloro-1-(3,4-dimethylphenylsulfonyl)-1,3-dihydro-2*H*-benzimidazol-2-one (9). Mp: 260–262 °C, yield 59%. ^{1}H NMR (DMSO-d_6): δ 2.29 (s, 6H, CH_3), 7.02 (d, J = 8.51, 1H, H-4), 7.23 (dd, J = 1.92, J = 8.51, 1H, H-5), 7.42 (d, J = 7.96, 1H, H-5′), 7.72–7.80 (m, 3H, H-7, H-2′,6′). Anal. Calcd for $C_{15}H_{13}ClN_2O_3S$: C, 53.49; H, 3.89; N, 8.32. Found: C, 53.11; H, 3.97; N, 8.74.

4.1.5.8. 6-Chloro-1-(2,4,6-trimethylphenylsulfonyl)-1,3-dihydro-2*H*-benzimidazol-2-one (10). Mp: 240–242 °C, yield 62%. ^{1}H NMR ($CDCl_3$): δ 2.31 (s, 3H, CH_3), 2.62 (s, 6H, $2CH_3$), 6.92 (d, J = 8.24, 1H, H-4), 6.99 (s, 2H, H-3′,5′), 7.17 (d, J = 8.24, H-5), 7.96 (s, 1H, H-7). Anal. Calcd for $C_{16}H_{15}ClN_2O_3S$: C, 54.78; H, 4.31; N, 7.98. Found: C, 54.31; H, 4.74; N, 7.53.

4.1.5.9. 6-Chloro-1-(2,3,5,6-tetramethylphenylsulfonyl)-1,3-dihydro-2*H*-benzimidazol-2-one (11). Mp: 241–243 °C, yield 86%. ^{1}H NMR (DMSO-d_6): δ 2.24 (s, 6H, CH_3, C-3′,5′), 2.39 (s, 6H, CH_3, C-2′,6′), 7.10 (d, J = 8.51, 1H, H-4), 7.28 (d, J = 8.51, 1H, H-5), 7.40 (s, 1H, H-4′), 7.66 (s, 1H, H-7), 11.70 (br s, 1H, NH). Anal. Calcd for $C_{17}H_{17}ClN_2O_3S$: C, 55.96; H, 4.70; N, 7.68. Found: C, 55.58; H, 4.36; N, 7.88.

4.1.5.10. 6-Chloro-1-(2,3,4,5,6-pentamethylphenylsulfonyl)-1,3-dihydro-2*H*-benzimidazol-2-one (12). Mp: 243–245 °C, yield 85%. ^{1}H NMR (DMSO-d_6): δ 2.20 (s, 6H, CH_3, C-3′,5′), 2.27 (s, 3H, CH_3, C-4′), 2.42 (s, 6H, CH_3, C-2′,6′), 7.10 (d, J = 8.51, 1H, H-4), 7.27 (d, J = 8.51, 1H, H-5), 7.65 (s, 1H, H-7), 11.68 (br s, 1H, NH). Anal. Calcd for $C_{18}H_{19}ClN_2O_3S$: C, 57.06; H, 5.05; N, 7.39. Found: C, 57.45; H, 5.27; N, 7.69.

4.1.5.11. 6-Chloro-1-(3,5-bistrifluoromethylphenylsulfonyl)-1,3-dihydro-2*H*-benzimidazol-2-one (13). Mp: 238–241 °C, yield 16%. ^{1}H NMR (DMSO-d_6): δ 7.04 (d, J = 8.51, 1H, H-4), 7.24 (d, J = 8.24, 1H, H-5), 7.78 (s, 1H, H-7), 8.66 (s, 1H, H-4′), 8.69 (s, 2H, H-2′,6′), 11.76 (br s, 1H, NH). Anal. Calcd for $C_{15}H_7ClF_6N_2O_3S$: C, 40.51; H, 1.59; N, 6.30. Found: C, 40.17; H, 1.88; N, 5.92.

4.1.5.12. 6-Chloro-1-(3-trifluoromethylphenylsulfonyl)-1,3-dihydro-2*H*-benzimidazol-2-one (14). Mp: 208–211 °C, yield 15%. ^{1}H NMR (DMSO-d_6): δ 6.96 (d, J = 8.24, 1H, H-4), 7.17 (d, J = 8.51, 1H, H-5), 7.67 (s, 1H, H-7), 7.87–7.92 (m, 1H, H-5′), 8.18 (d, J = 7.96, 1H, H-4′), 8.33–8.35 (m, 2H, H-2′,6′). Anal. Calcd for $C_{14}H_8ClF_3N_2O_3S$: C, 44.63; H, 2.14; N, 7.44. Found: C, 44.79; H, 1.88; N, 7.63.

4.1.5.13. 6-Chloro-1-(3trifluoromethoxyphenylsulfonyl)-1,3-dihydro-2*H*-benzimidazol-2-one (15). Mp: 207–209 °C, yield 24%. ^{1}H NMR (DMSO-d_6): δ 6.81 (d, J = 8.24, 1H, H-4), 7.02 (d, J = 8.51, 1H, H-5), 7.51 (s, 1H, H-7), 7.72–7.99 (m, 4H, H-2′,4′,5′,6′). Anal. Calcd for $C_{14}H_8ClF_3N_2O_4S$: C, 42.82; H, 2.05; N, 7.13. Found: C, 42.57; H, 2.41; N, 7.78.

4.1.5.14. 6-Chloro-1-(3-methoxyphenylsulfonyl)-1,3-dihydro-2*H*-benzimidazol-2-one (16). Mp: 270–273 °C, yield 17%. ^{1}H NMR (DMSO-d_6): δ 3.81 (s, 3H, CH_3), 7.03 (d, J = 8.24, 1H, H-4), 7.24 (d, J = 8.24, 1H, H-5), 7.36–7.72 (m, 5H, H-7, H-2′,4′,5′,6′), 11.7 (br s, 1H, NH). Anal. Calcd for $C_{14}H_{11}ClN_2O_4S$: C, 49.64; H, 3.27; N, 8.27. Found: C, 50.06; H, 2.92; N, 8.55.

4.1.6. General procedure for the synthesis of 6-chloro-1-(3,5-dimethylphenylsulfonyl)-1*H*-imidazo[4,5-*b*]pyridin-2(3*H*)-one (2)

Using a similar procedure for **1**, **4–16**, compound **2** was prepared from *N*-(3-amino-6-chloropyridin-2-yl)-3,5-dimethylbenzenesulfonamide **18** (0.25 mmol).

Mp: 160–163 °C, yield 52%. ^{1}H NMR (DMSO-d_6): δ 2.35 (s, 6H, CH_3), 7.27 (d, J = 8.24, 1H, H-4), 7.40–7.43 (m, 2H, H-5, H-4′), 7.69 (s, 2H, H-2′,6′), 11.81 (br s, 1H, NH). Anal. Calcd for $C_{14}H_{12}ClN_3O_3S$: C, 49.78; H, 3.58; N, 12.44. Found: C, 49.33; H, 3.95; N, 12.24.

4.1.7. General procedure for the synthesis of 6-chloro-1-(3,5-dimethylphenylsulfonyl)-1,3-dihydro-2*H*-benzimidazol-2-thione (3)

Thiophosgene (0.25 mmol) was added to a solution of *N*-(2-aminophenyl-5-chloro)-3,5-dimethylbenzenesulfonamide **17** (0.25 mmol) in acetone and the resulting mixture was stirred for 1 h at room temperature. The reaction solvent was evaporated under reduced pressure and the residue was crystallized from ethanol.

Mp: 234–237 °C, yield 14%. ^{1}H NMR (DMSO-d_6): δ 2.35 (s, 6H, CH_3), 7.04 (d, J = 8.51, 1H, H-4), 7.24 (dd, J = 1.92, J = 8.51, 1H, H-5), 7.43 (s, 1H, H-4′), 7.68 (s, 2H, H-2′,6′), 7.73 (s, 1H, H-7), 11.67 (br s, 1H, NH). Anal. Calcd for $C_{15}H_{13}ClN_2O_2S$: C, 51.06; H, 3.71; N, 7.94. Found: C, 51.47; H, 3.30; N, 7.58.

4.2. Anti-HIV activity assays

4.2.1. 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 albumin (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 lastly, was 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 was 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 acid-precipitable radioactivity using a PerkinElmer® Trilux MicroBeta 1450 Counter.

4.2.2. RT inhibition assays

Reactions were performed under the conditions described for the HIV-1 RT RNA-dependent DNA polymerase activity assay.[24] 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 K_i was used. Experiments were done in triplicate. Experimental errors (±SD) were $\leqslant$10%.

4.2.3. In vitro anti-HIV assay

The methodology of the anti-HIV assays has been previously described.[25] 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 five days (MT-4) 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%.

4.3. Computational procedures

The structures of ligands were constructed using the Schrodinger Maestro[26] and were then submitted to Polak–Ribiere conjugate gradient minimization (0.0005 kJ/(Å mol) convergence). The target macromolecules from 1FK9, 2OPS and 1JKH PDB complexes were prepared using the protein preparation workflow in Maestro.[26] 1JKH was used as a template to build a Y181I RT model; prior to the last step of the protein preparation workflow, cysteine 181 was mutated to isoleucine, and this sidechain was submitted to an exhaustive conformational search, the lowest-energy conformer was finally selected for the last step of the protein preparation workflow (i.e. relaxation of the complex using the OPLS force-field.[27]

All docking calculations were performed using AUTODOCK version 4.0.[13]

For each enzyme, a grid box of 60–60–60 points (0.375 spacing) was centered on the allosteric site, using the crystallographic positions of the ligands as reference, and used to define the docking space.

The GA-LS method was used with the default settings and 250 docking runs were performed for each ligand/enzyme docking. The docking results were clustered using an rmsd tolerance of 1 Å and the lowest-energy conformer was selected as binding conformation. Autodock input files were prepared using AUTODOCKTools.[28]

The resulting complexes were then minimized using 200 steps of steepest descent minimization followed by 200 steps of Polak–Ribiere conjugate gradient minimization using the MACROMODEL program[29] (OPLS force-field[27]).

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