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Novel N_1-substituted 1,3-dihydro-2*H*-benzimidazol-2-ones as potent non-nucleoside reverse transcriptase inhibitors

Anna-Maria Monforte [a,*], Angela Rao [a], Patrizia Logoteta [a], Stefania Ferro [a], Laura De Luca [a], Maria Letizia Barreca [b], Nunzio Iraci [a], Giovanni Maga [c], Erik De Clercq [d], Christophe Pannecouque [d], Alba Chimirri [a]

[a] *Dipartimento Farmaco-Chimico, Università di Messina, Viale Annunziata, 98168 Messina, Italy*
[b] *Dipartimento di Chimica e Tecnologia del Farmaco, Via del Liceo 1, 06123 Perugia, Italy*
[c] *Istituto di Genetica Molecolare IGM-CNR, via Abbiategrasso 207, 27100 Pavia, Italy*
[d] *Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium*

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ABSTRACT

Several N_1-substituted 1,3-dihydro-2*H*-benzimidazol-2-ones were synthesized and evaluated as anti-HIV agents. Some of them proved to be highly effective in inhibiting HIV-1 replication at nanomolar concentration as potent non-nucleoside HIV-1 RT inhibitors (NNRTIs) with low cytotoxicity. SAR studies highlighted that the nature of the substituents at N_1 and on the benzene ring of benzimidazolone moiety significantly influenced the anti-HIV activity of this class of potent antiretroviral agents.

1. Introduction

Human immunodeficiency virus (HIV) is the primary cause of acquired immunodeficiency syndrome (AIDS). The replication of HIV-1 in infected patients can be reduced considerably by HAART, a highly active combination of drugs with multiple viral targets.

Officially approved drugs for anti-HIV treatment belong to the class of nucleoside/nucleotide and non-nucleoside reverse transcriptase inhibitors (NRTIs and NNRTIs), to protease inhibitors (PIs) and more recently to viral entry (Enfuvirtide)[1,2] and integrase inhibitors (Isentress).[3]

Despite the successes with such treatments, the permanent use of anti-AIDS drugs induces drug-resistant viral variants and the emergence of unwanted metabolic side effects.[4]

NNRTIs are a structurally diverse group of compounds, which inhibit the enzyme in an allosteric mode by binding at about 10 Å from the polymerase active site causing a distortion of the catalytic aspartate triad in a non-competitive fashion.[5]

First-generation NNRTIs, such as nevirapine and delavirdine, easily lose their inhibitory potential against mutant virus strains that contain single amino acid mutations in their RT. Also the antiviral potency of second-generation NNRTIs, such as efavirenz, significantly decreases although this occurs after two or more mutations in the HIV-1 RT.[5]

As a consequence, there is a great need for additional drugs to further optimize and improve the efficacy of long term HIV treatment.

In previous papers, we combined different computational methods in order to obtain information for rational drug design[6] and in connection with these investigations, additional molecular modeling and synthetic approaches were applied to discover potent and selective HIV-1 RT inhibitors.

In particular, we reported a 3D pharmacophore model for a second generation of NNRTIs, built by using a combined ligand- and structure-based molecular modeling approach, consisting of five features: three hydrophobic groups, one hydrogen bond acceptor, and one hydrogen bond donor (Fig. 1).[7]

We used this model for molecular modeling studies which led to the discovery of N_1-substituted 1,3-dihydro-2H-benzimidazol-2-ones and some derivatives of this series proved to be potent HIV-1 RT inhibitors.[7]

Considering 6-chloro-1-(2,6-difluorobenzyl)-substituted derivative (**1a**) (Scheme 1) as a starting point for lead optimization strategy we designed and synthesized other new benzimidazolone analogues such as derivative **2a**, characterized by the presence of a 3,5-dimethylbenzyl moiety and its sulfonyl derivative **2b**, which showed lower toxicity and antiretroviral activity similar to that of

* Corresponding author. Tel.: +39 90 6766477; fax: +39 90 6766402.
E-mail address: ammonforte@pharma.unime.it (A.-M. Monforte).

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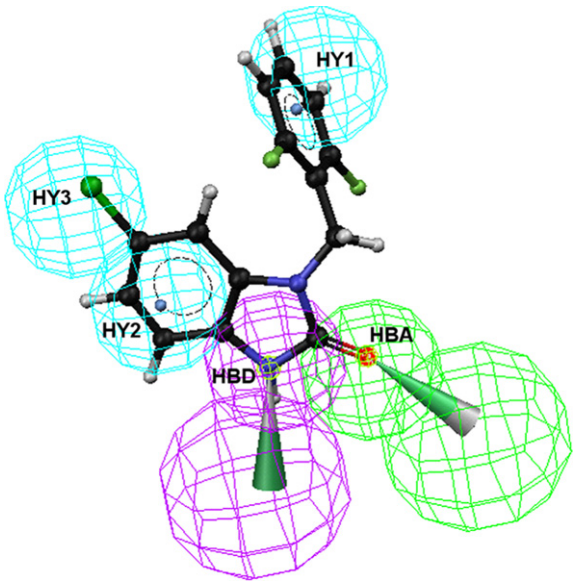


Figure 1. 3D pharmacophore model for second-generation NNRTIs aligned to compound **1a**. HY1–HY3: hydrophobic groups; HBA: hydrogen bond acceptor; HBD: hydrogen bond donor.

efavirenz and greater than that of nevirapine, two of the four NNRTIs currently available in antiretroviral therapy.[8]

Starting from these promising results, on which we have recently published some preliminary findings,[8] we report here the synthesis of new N_1-substituted 1,3-dihydro-2*H*-benzimidazol-2-ones in which different structural modifications have been introduced (Fig. 2). Docking and SAR studies have also been performed.

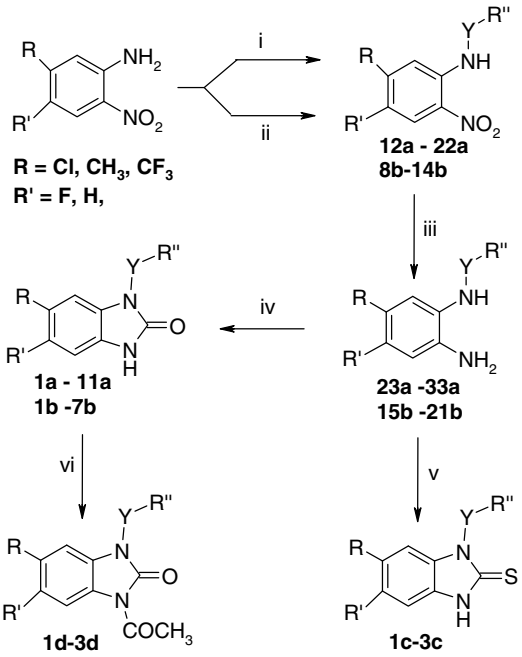
2. Results and discussion

In order to better determine the structural characteristics that were able to improve the anti-HIV-1 activity of this class of NNRTIs and to investigate the effects of different chemical modifications on the RT inhibition, an extensive SAR was examined by varying the nature and the position of the substituents both on the benzene ring of the benzimidazolone moiety and on the aromatic portion at the N-1 atom.

In addition, we changed the linker connecting the benzimidazole system with N-1 substituents in order to investigate the effects on RT inhibition induced by the modified conformational disposition of the two butterfly wings, an important characteristic of HIV-1 RT inhibitors.[8]

Moreover, we have planned further structural modifications such as the conversion of the carbonyl group of the benzimidazolone nucleus into the bioisostere thiocarbonyl one thus increasing the lipophilicity of the molecules and the introduction of an acetyl group on NH of some synthesized compounds in order to confirm the importance of the NH residue of the benzimidazolone ring in the interaction with Lys101 of the RT enzyme.[7]

The synthesis of the new molecules was achieved by following the reaction sequence reported in Scheme 1. The 2-nitroanilines were N-substituted by treatment with the appropriate substituted-benzylbromides in the presence of potassium carbonate; in this step the microwave-irradiation shortened the reaction times, giving the desired products (**12a**–**22a**) in high yields. The *N*-sulfoxide derivatives were obtained by reacting with arylsulfonylchloride using sodium hydride as base. The intermediates (**12a**–**22a**, **8b**–**14b**) were reduced with Zn dust in acidic medium. The cyclization of the aminoderivatives (**23a**–**33a**, **15b**–**21b**) with phosgene afforded compounds (**1a**–**11a**, **1b**–**7b**), while using thiophosgene



Compd	R	R'	Y	R"
1a*, 1c, 1d	Cl	H	CH_2	2,6- difluorophenyl
1b	Cl	H	SO_2	2,6- difluorophenyl
2a#, 2c, 2d	Cl	H	CH_2	3,5-dimethylphenyl
2b#	Cl	H	SO_2	3,5-dimethylphenyl
3a, 3c, 3d	Cl	H	CH_2	3,5-difluorophenyl
3b	Cl	H	SO_2	3,5-difluorophenyl
4a,	CH_3	H	CH_2	2,6- difluorophenyl
4b	CH_3	H	SO_2	2,6- difluorophenyl
5a,	CH_3	H	CH_2	3,5-dimethylphenyl
5b	CH_3	H	SO_2	3,5-dimethylphenyl
6a	CH_3	H	CH_2	3,5-difluorophenyl
6b	CH_3	H	SO_2	3,5-difluorophenyl
7a	Cl	F	CH_2	3,5-dimethylphenyl
7b	Cl	F	SO_2	3,5-dimethylphenyl
8a	Cl	F	CH_2	3,5-difluorophenyl
9a	Cl	F	CH_2	2,6- difluorophenyl
10a	CF_3	H	CH_2	3,5-dimethylphenyl
11a	CF_3	H	CH_2	2,6- difluorophenyl

Scheme 1. Reagents and conditions: (i) DMF, K_2CO_3 , M_W, 2 steps 250 W, 120 °C, 6 min; 250 W, 130 °C, 6 min; (ii) dioxane, NaH, 0–5 °C, 30 min; (iii) Zn/HCl, EtOH, 80 °C, 1 h; (iv) 20% toluene solution of $COCl_2$, HCl 2 N, Δ, 4 h; (v) acetone, $CSCl_2$, 1 h; (vi) CH_2Cl_2, $ClCOCH_3$/TEA, 30 min. See Refs. 6,7.

as reagent, compounds (**1c**–**3c**) were obtained. The N-acetylated derivatives (**1d**–**3d**) were synthesized using acetyl chloride with a catalytic amount of TEA.

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

All compounds were evaluated in enzymatic tests for their ability to inhibit RT activity as well as HIV-1 (III_B) replication in MT-4 cell cultures and also cytotoxic activity, and compared with nevirapine and efavirenz, which were used as reference drugs.

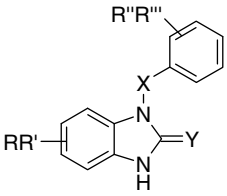


Figure 2. Structural modifications of new N_1-substituted 1,3-dihydro-2*H*-benzimidazol-2-ones.

As shown in Table 1, most of the new compounds inhibited RT and in particular prevented the cytopathic effect of HIV-1 IIIB at nanomolar concentrations with low toxicity to MT-4 cells resulting in high selectivity indices. Several derivatives were more potent and less toxic than nevirapine and, in some cases, than efavirenz.

Considering first of all the effect of the substituents on the phenyl ring at N-1, it is interesting to note that the 3,5-difluorophenyl derivatives were generally more active than the corresponding 2,6-substituted compounds. This underlines the importance of hydrophobic contacts with the surrounding lipophilic HY1 region of the pharmacophore model, more favorable by 3,5-substitution.

In particular the greatest activity levels of the 3,5-dimethyl derivatives might be due to the ability of the 3,5-dimethyl moiety to occupy the hydrophobic space near the 'roof' of the NNRTI binding pocket (consisting of P95, Y181, Y188, and W229),[8,9] thus creating additional intermolecular interactions (Fig. 3).

Furthermore, the biological effects also confirm that compounds containing a sulfonyl moiety are more potent and less toxic than the analogues with a methylene linker, with the exception of the 2,6-difluoro substituted derivative **1a**. The greatest potency of the arylsulfonyl derivatives might also be due to the electronic characteristics of the sulfonyl groups that are able to make closer intermolecular contacts with the NNIBP residues (V106, V179, Y181, and Y188) with respect to the methylene linker, as we have suggested in a previous paper (Fig. 4).[8]

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

Compound	IC_{50}[a] (μM)	EC_{50}[b] (μM)	CC_{50}[c] (μM)	SI[d]
1a	0.70 ± 0.1	0.24 ± 0.05	>424	>1891
1b	30 ± 1.5	1.566 ± 0.174	194.12 ± 9.90	124
1c	—	0.74 ±0.345	5.95 ± 0.4	8
1d	>100	0.534 ± 0.39	101.80 ± 11.31	190
2a	0.15 ± 0.02	0.032 ± 0.007	8.9 ± 0.8	281
2b	0.005 ± 0.001	0.002 ± 0.0003	39 ± 8.2	17,846
2c	0.046 ± 0.006	0.076 ± 0.030	0.305 ± 0.018	4
2d	>100	0.0117 ± 0.015	7.18 ± 0.7	614
3a	0.1 ± 0.01	0.289 ± 0.1	>424	1467
3b	0.1 ± 0.01	0.069 ± 0.055	202.47 ± 83.45	2934
3c	0.160 ± 0.02	>0.737	1.204 ± 0.508	2
3d	38.5 ± 2.5	1.663 ± 0.148	40.388 ± 1.217	24
4a	>100	18.56 ± 5.58	236.41 ± 23.12	13
4b	1,3 ± 0.1	0.195 ± 0.143	2.22 ± 0.185	11
5a	76.92 ± 7.5	0.075 ± 0.034	0.856 ± 0.4	11
5b	0.1 ± 0.01	0.0063 ± 0.0037	>308	48,889
6a	>400	>72.95	72.95 ± 60.37	<1
6b	0.2 ± 0.02	0.065 ± 0.014	23.37 ± 11.16	360
7a	>100	0.938 ± 0.45	>410	438
7b	0.158 ± 0.02	0.45 ± 0.11	139.60 ± 50.76	310
8a	0.003 ± 0.0004	>33.90	>400	12
9a	0.14 ± 0.02	28.975 ± 17.59	266.88 ± 58.942	9
10a	0.28 ± 0.03	0.425 ± 0.202	37.418 ± 7.28	88
11a	0.017 ± 0.002	5.497 ± 1.425	>424	77
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}.

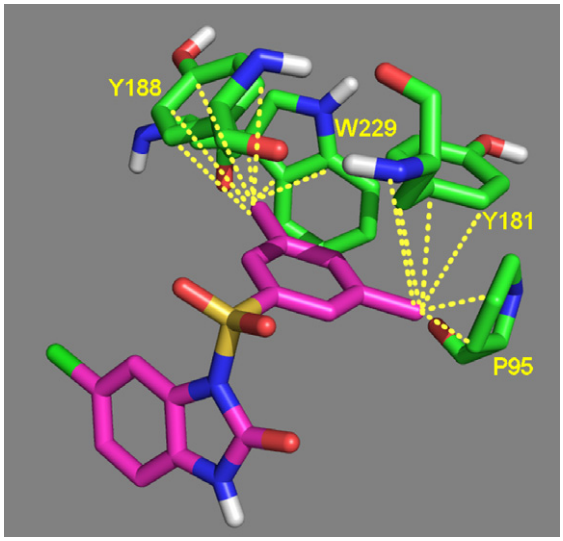


Figure 3. Intermolecular interactions between the 3,5-dimethyl groups of compound **2b** and NNIBP residues P95, Y181, Y188, and W229.

Moreover, the biological data of the synthesized compounds highlighted that the nature and position of the substituent on the benzene ring of benzimidazolone moiety greatly influence both the anti-HIV activity and the enzyme inhibition. In particular, the presence of a chlorine atom or a methyl group at the 6 position of the benzimidazolone system provided the most active compounds. The introduction of the CF_3 group in position 6 and the disubstitution in 5 and 6 positions of the benzene fused ring generally led to a reduction of the anti-HIV activity.

The replacement of the carbonyl moiety with the isosteric thiocarbonyl functionality does not lead to a substantial variation in activity highlighting that a further increase in the lipophilicity resulting from this structural modification has no influence on the antiviral activity of the molecules but causes a substantial reduction of the selectivity index.

Furthermore, the introduction of the acetyl group on NH was detrimental to the RT inhibition, confirming the importance of the NH residue of the benzimidazolone ring in the interaction with the enzyme. In contrast the antiviral activity in cell assays was preserved thus suggesting that this discrepancy could be due to a hydrolytic consequence in cell tests (compounds **1d**–**3d**).

In conclusion, novel anti-HIV agents acting as RT inhibitors have been obtained. Some derivatives are more potent and less toxic than nevirapine, one of the only four NNRTIs approved by the FDA.

The SAR data collected will be useful to design and synthesize new NNRT inhibitors with improved anti-HIV properties.

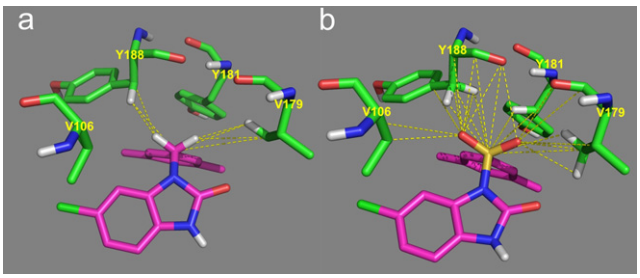


Figure 4. Intermolecular interactions between the methylene linker of compound **2a** and the sulfonyl group of compound **2b** and NNIBP residues V106, V179, Y181, and Y188.

3. Experimental

3.1. Chemistry

All microwave-assisted reactions were carried out in a CEM Focused Microwave Synthesis System, Model Discover working at the potency 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 procedure for the synthesis of N-substituted-2-nitroanilines (14a–22a)

The appropriate benzyl bromide (3 mmol) and anhydrous potassium carbonate (10 mmol) were added to a solution of 5(4) substituted 2-nitroaniline (2 mmol) in DMF (5 ml) in a cylindrical quartz tube (2 cm). The reaction mixture was then stirred and irradiated in a microwave oven for two steps (I step: W 250, 6 min, 120 °C; II step: W 250, 6 min, 130 °C), cooled, filtered and, after addition of water (60 ml), extracted with chloroform (2× 50 ml). After removal of the solvent under reduced pressure, the residue was powdered by treatment with diethyl ether and recrystallized from ethanol.

3.1.1.1. 5-Chloro-1-(3,5-difluorobenzyl)-2-nitroaniline (14a). Mp: 122–124 °C, yield 68%. ^{1}H NMR ($CDCl_3$): 4.53 (d, 2H, CH_2), 6.67–8.19 (m, 6H, ArH), 8.47 (br s, 1H, NH). Anal. Calcd for $C_{13}H_9ClF_2N_2O_2$: C, 52.28; H, 3.04; N, 9.38. Found: C, 51.95; H, 3.19; N, 9.13.

3.1.1.2. 5-Methyl-1-(2,6-difluorobenzyl)-2-nitroaniline (15a). Mp: 96–98 °C, yield 72%. ^{1}H NMR ($CDCl_3$): 2.35 (s, 3H, CH_3), 4.58 (d, 2H, CH_2), 6.46–8.07 (m, 6H, ArH), 8.35 (br s, 1H, NH). Anal. Calcd for $C_{14}H_{12}F_2N_2O_2$: C, 60.43; H, 4.35; N, 10.07. Found: C, 60.33; H, 4.51; N, 10.11.

3.1.1.3. 5-Methyl-1-(3,5-dimethylbenzyl)-2-nitroaniline (16a). Mp: 115–116 °C, yield 81%. ^{1}H NMR ($CDCl_3$): 2.30 and 2.31 (s, 9H, CH_3), 4.44 (d, 2H, CH_2), 6.45–8.11 (m, 6H, ArH), 8.40 (br s, 1H, NH). Anal. Calcd for $C_{16}H_{18}N_2O_2$: C, 71.09; H, 6.71; N, 10.36. Found: C, 71.25; H, 6.88; N, 10.27.

3.1.1.4. 5-Methyl-1-(3,5-difluorobenzyl)-2-nitroaniline (17a). Mp: 134 °C, yield 40%. ^{1}H NMR ($CDCl_3$): 2.28 (s, 3H, CH_3), 4.54 (d, 2H,CH_2), 6.47–8.09 (m, 6H, ArH); 9.07 (br s, 1H, NH). Anal. Calcd for $C_{14}H_{12}F_2N_2O_2$: C, 60.43; H, 4.35; N, 10.07. Found: C, 60.83; H, 4.75; N, 9.87.

3.1.1.5. 5-Chloro,4-fluoro-(3,5-dimethylbenzyl)-2-nitroaniline (18a). Mp: 98–100 °C, yield 89%. ^{1}H NMR ($CDCl_3$): 2.35 (s, 6H, CH_3), 4.41 (d, 2H, CH_2), 6.88–8.02 (m, 5H, ArH), 8.26 (br s, 1H, NH). Anal. Calcd for $C_{15}H_{14}ClFN_2O_2$: C, 58.35; H, 4.57; N, 9.02. Found: C, 58.49; H, 4.41; N, 9.22.

3.1.1.6. 5-Chloro,4-fluoro-(3,5-difluorobenzyl)-2-nitroaniline (19a). Mp: 125–126 °C, yield 42%. ^{1}H NMR ($CDCl_3$): 4.52 (d, 2H, CH_2); 6.74–8.05 (m, 5H, ArH); 8.92 (br s, 1H, NH). Anal. Calcd for $C_{13}H_8ClFN_2O_2$: C, 49.31; H, 2.55; N, 8.85. Found: C, 48.97, H, 2.25: N, 8.76.

3.1.1.7. 5-Chloro,4-fluoro-(2,6-difluorobenzyl)-2-nitroaniline (20a). Mp: 102–104 °C, yield 58%. ^{1}H NMR: 4.56 (d, 2H, CH_2), 6.89–8.00 (m, 5H, ArH), 8.23 (br s, 1H, NH). Anal. Calcd for $C_{13}H_8ClF_3N_2O_2$: C, 49.31; H, 2.55; N, 8.85. Found: C, 49.64; H, 2.33; N, 9.05.

3.1.1.8. 5-Trifluoromethyl-1-(3,5-dimethylbenzyl)-2-nitroaniline (21a). Mp: 102–105 °C, yield 91%. ^{1}H NMR ($CDCl_3$): 2.32 (s, 6H, CH_3), 4.46 (d, 2H, CH_2), 6.86–8.31 (m, 6H, ArH), 8.37 (br s, 1H, NH). Anal. Calcd for $C_{16}H_{15}F_3N_2O_2$: C, 71.09; H, 6.71; N, 10.36. Found: C, 71.25; H, 6.88; N, 10.27.

3.1.1.9. 5-Trifluoromethyl-1-(2,6-difluorobenzyl)-2-nitroaniline (22a). Mp: 105–106 °C, yield 25%. ^{1}H NMR ($CDCl_3$): 4.64 (d, 2H, CH_2); 6.87–8.29 (m, 6H, ArH); 8.40 (br s, 1H, NH). Anal. Calcd for $C_{14}H_9F_5N_2O_2$: C, 50.61; H, 2.73: N, 8.43. Found: C, 51.03; H, 3.11; N, 8.78.

3.1.2. General procedure for the synthesis of *N*-(2-nitrophenyl)-benzenesulfonamides (8b, 10b–14b)

Dry sodium hydride (10 mmol) was added to a stirred solution of 5(4) substituted 2-nitroaniline (2 mmol) in dioxane (6 ml) at 0 °C. The mixture was stirred for 10 min. The appropriate aryl sulfonyl chloride (3 mmol) was added dropwise and a saturated $NaHCO_3$ solution was added after 30 min to quench the reaction. The reaction mixture was 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.

3.1.2.1. *N*-(5-Chloro-2-nitrophenyl)-2,6-difluorobenzenesulfonamide (8b). Mp: 103–105 °C, yield 55%. ^{1}H NMR (DMSO-d_6): 7.01–8.19 (m, 6H, ArH), 10.49 (br s, 1H, NH). Anal. Calcd for $C_{12}H_7ClF_2N_2O_4S$: C, 41.33; H, 2.02; N, 8.03. Found: C, 41.48; H, 1.91; N, 8.23.

3.1.2.2. *N*-(5-Chloro-2-nitrophenyl)-3,5-difluorobenzenesulfonamide (10b). Mp: 105–107 °C, yield 60%. ^{1}H NMR: 7.03–8.17 (m, 6H, ArH), 10.06 (br s, 1H, NH). Anal. Calcd for $C_{12}H_7ClF_2N_2O_4S$: C, 41.33; H, 2.02; N, 8.03. Found: C, 41.52; H, 2.00; N, 7.90.

3.1.2.3. *N*-(5-Methyl-2-nitrophenyl)-2,6-difluorobenzenesulfonamide (11b). Mp: 122–124 °C, yield 51%. ^{1}H NMR: 2.39 (s, 3H, CH_3), 6.94–8.11 (m, 6H, ArH), 10.48 (br s, 1H, NH). Anal. Calcd for $C_{13}H_{10}F_2N_2O_4S$: C, 47.56; H, 3.07; N, 8.53. Found: C, 47.47; H, 2.98; N, 8.71.

3.1.2.4. *N*-(5-Methyl-2-nitrophenyl)-3,5-dimethylbenzenesulfonamide (12b). Mp: 120–122 °C, yield 74%. ^{1}H NMR: 2.33 (s, 6H, 3′,5′-CH_3), 2.41 (s, 3H, CH_3), 6.92–8.04 (m, 6H, ArH), 9.93 (br s, 1H, NH). Anal. Calcd for $C_{15}H_{16}N_2O_4S$: C, 56.24; H, 5.03; N, 8.74; Found: C, 56.37; H, 5.30; N, 8.60.

3.1.2.5. *N*-(5-Methyl-2-nitrophenyl)-3,5-difluorobenzenesulfonamide (13b). Mp: 113–114 °C, yield 57%. ^{1}H NMR: 2.45 (s, 3H, CH_3), 7.01–8.07 (m, 6H, ArH), 9.96 (br s, 1H, NH). Anal. Calcd for $C_{13}H_{10}F_2N_2O_4S$: C, 47.56; H, 3.07; N, 8.53. Found: C, 47.65; H, 3.19; N, 8.31.

3.1.2.6. *N*-(5-Chloro-4-fluoro-2-nitrophenyl)-3,5-dimethylbenzenesolfonamide (14b). Mp: 164–165 °C, yield 88%. ^{1}H NMR ($CDCl_3$): 2.35 (s, 6H, CH_3), 7.21–7.99 (m, 5H, ArH), 9.69 (br s, 1H, NH). Anal. Calcd for $C_{14}H_{12}ClFN_2O_4S$: C, 46.87; H, 3.37; N, 7.81. Found: C, 46.70; H, 3.52; N, 7.90.

3.1.3. General procedure for the synthesis of N_1-(substituted-benzyl)-2-amino-anilines (25a–33a)

The mixture of appropriate N-substituted-2-nitroanilines (0.6 mmol) in 3 ml HCl and 4 ml EtOH was stirred vigorously, then zinc dust (20 mmol) was added in several portions at room temperature. After this addition was completed, the reaction mixture was heated in a water bath for 1 h, cooled, made alkaline with NaOH 2 N, and then extracted with ethyl acetate. The extract was washed with water, dried over Na_2SO_4 and evaporated. The residue was crystallized from ethanol.

3.1.3.1. 2-Amino-5-chloro-1-(3,5-difluorobenzyl)-aniline (25a). Mp: 115–116 °C, yield 94%. ^{1}H NMR (DMSO-d_6): 3.50 (br s, 2H, NH_2), 3.90 (br s, 1H, NH), 4.31 (s, 2H, CH_2), 6.49–6.94 (m, 6H, ArH). Anal. Calcd for $C_{13}H_{11}ClF_2N_2$: C, 58.11; H, 4.13; N, 10.43. Found: C, 58.36; H, 3.90; N, 10.59.

3.1.3.2. 2-Amino-5-methyl-1-(2,6-difluorobenzyl)-aniline (26a). Mp: 70–72 °C, yield 98%. ^{1}H NMR ($CDCl_3$): 2.26 (s, 3H, CH_3), 3.28 (br s, 3H, NH and NH_2), 4.20 (s, 2H, CH_2), 6.47–7.02 (m, 6H, ArH). Anal. Calcd for $C_{14}H_{14}F_2N_2$: C, 67.73; H, 5.68; N, 11.28. Found: C, 67.86; H, 5.77; N, 11.09.

3.1.3.3. 2-Amino-5-methyl-1-(3,5-dimethylbenzyl)-aniline (27a). Mp: 60–62 °C, yield 99%. ^{1}H NMR ($CDCl_3$): 2.25, 2.32 (s, 9H, CH_3), 3.38 (br s, 3H, NH and NH_2), 4.37 (s, 2H, CH_2), 6.49–7.29 (m, 6H, ArH). Anal. Calcd for $C_{16}H_{20}ClN_2$: C, 79.96; H, 8.39; N, 11.66. Found: C, 79.84; H, 8.51; N, 11.49.

3.1.3.4. 2-Amino-5-methyl-1-(3,5-difluorolbenzyl)-aniline (28a). Mp: 80–82 °C, yield 55%. ^{1}H NMR ($CDCl_3$): 2.21 (s, 3H, CH_3); 3.40 (br s, 3H, NH and NH_2); 4.32 (s, 2H, CH_2); 6.36–6.94 (m, 6H, ArH). Anal. Calcd for $C_{14}H_{14}F_2N_2$: C, 67.73; H, 5.68; N, 11.28. Found: C, 67.65; H, 5.54; N, 10.98.

3.1.3.5. 2-Amino-5-chloro,4-fluoro-1-(3,5-dimethylbenzyl)-aniline (29a). Mp: 102–105 °C, yield 98%. ^{1}H NMR ($CDCl_3$): 2.29 (s, 6H, CH_3), 3.47 (br s, 3H, NH and NH_2), 4.13 (s, 2H, CH_2), 6.52–7.00 (m, 6H, ArH). Anal. Calcd for $C_{15}H_{16}CFlN_2$: C, 64.63; H, 5.79; N, 10.05. Found: C, 64.51; H, 5.96; N, 9.88.

3.1.3.6. 2-Amino-5-chloro,4-fluoro-1-(3,5-difluorolbenzyl)-aniline (30a). Mp: 75–77 °C, yield 90%. ^{1}H NMR ($CDCl_3$): 3.52 (br s, 3H, NH and NH_2), 4.26 (s, 2H, CH_2); 6.50–6.98 (m, 5H, ArH). Anal. Calcd for $C_{13}H_{10}ClF_3N_2$: C, 54.47; H, 3.52; N, 9.77. Found: C, 54.30; H, 3.34, N, 9.67.

3.1.3.7. 2-Amino-5-chloro,4-fluoro-1-(2,6-difluorolbenzyl)-aniline (31a). Mp: 71–73 °C, yield 95%. ^{1}H NMR ($CDCl_3$): 3.38 (br s, 1H, NH), 3.56 (br s, 2H, NH_2), 4.30 (s, 2H, CH_2), 6.50–7.29 (m, 5H, ArH). Anal. Calcd for $C_{13}H_{10}ClF_3N_2$: C, 79.96; H, 8.39; N, 11.66. Found: C, 65.48; H, 5.64; N, 9.76.

3.1.3.8. 2-Amino-5-trifluoromethyl-1-(3,5-dimethylbenzyl)-aniline (32a). Mp: 106–109 °C, yield 91%. ^{1}H NMR ($CDCl_3$): 2.33 (s, 6H, CH_3), 3.60 (br s, 3H, NH and NH_2), 4.21 (s, 2H, CH_2), 6.70–7.02 (m, 6H, ArH). Anal. Calcd for $C_{16}H_{17}F_3N_2$: C, 65.29; H, 5.82; N, 9.52. Found: C, 79.84; H, 8.51; N, 11.49.

3.1.3.9. 2-Amino-5-trifluoromethyl-1-(2,6-difluorolbenzyl)-aniline (33a). Mp: 77–79 °C, yield 95%. ^{1}H NMR ($CDCl_3$): 3.59 (br s, 3H, NH and NH_2), 4.40 (s, 2H, CH_2), 6.70–7.31 (m, 6H, ArH). Anal. Calcd for $C_{14}H_{11}F_5N_2$: C, 55.63; H, 3.67: N, 9.27. Found: C, 55.40; H, 3, 80; N, 9.30.

3.1.4. General procedure for the synthesis of *N*-(2-aminophenyl)-benzenesulfonamides (15b, 17b–21b)

With a similar procedure for compounds **23a**–**33a**, the *N*-(2-aminophenyl)-benzenesulfonamides (**15b**–**21b**) were prepared starting from the appropriate *N*-(2-nitrophenyl)-benzenesulfonamide (0.6 mmol).

3.1.4.1. *N*-(2-Aminophenyl-5-chloro)-2,6-difluorobenzenesulfonamide (15b). Mp: 133–135 °C, yield 99%. ^{1}H NMR ($CDCl_3$): 3.47 (br s, 2H, NH_2), 4.20 (br s, 1H, NH), 7.01–8.19 (m, 6H, ArH). Anal. Calcd for $C_{12}H_9ClF_2N_2O_2S$: C, 45.22; H, 2.85; N, 8.79; Found: C, 45.36; H, 2.99; N, 8.62.

3.1.4.2. *N*-(2-Aminophenyl-5-chloro)-3,5-difluorobenzenesulfonamide (17b). Mp: 136–138 °C, yield 99%. ^{1}H NMR ($CDCl_3$): 5.12 (br s, 3H, NH and NH_2), 6.45–7.41 (m, 6H, ArH). Anal. Calcd for $C_{12}H_9ClF_2N_2O_2S$: C, 45.22; H, 2.85; N, 8.79. Found: C, 45.11; H, 2.88; N, 8.95.

3.1.4.3. *N*-(2-Aminophenyl-5-methyl)-2,6-difluorobenzenesulfonamide (18b). Mp: 176–178 °C, yield 86%. ^{1}H NMR ($CDCl_3$): 2.01 (s, 3H, CH_3), 3.77 (br s, 3H, NH and NH_2), 6.47–7.37 (m, 6H, ArH). Anal. Calcd for $C_{13}H_{12}F_2N_2O_2S$: C, 52.34; H, 4.05; N, 9.39. Found: C, 52.28; H, 4.19; N, 9.30.

3.1.4.4. *N*-(2-Aminophenyl-5-methyl)-3,5-dimethylbenzensolfonamide (19b). Mp: 125–127 °C, yield 77%. ^{1}H NMR ($CDCl_3$): 2.08 (s, 3H, CH_3), 2.34 (s, 6H, 3′,5′-CH_3), 3.82 (br s, 2H, NH_2), 5.88 (br s, 1H, NH), 6.35–7.36 (m, 6H, ArH). Anal. Calcd for $C_{15}H_{18}N_2O_2S$: C, 62.04; H, 6.25; N, 9.65. Found: C, 62.21; H, 6.33; N, 9.44.

3.1.4.5. *N*-(2-Aminophenyl-5-methyl)-3,5-difluorobenzensulfonamide (20b). Mp: 120–122 °C, yield 97%. ^{1}H NMR ($CDCl_3$): 2.06 (s, 3H, CH_3), 2.42 (br s, 3H, NH and NH_2), 6.44–7.28 (m, 6H, ArH). Anal. Calcd for $C_{13}H_{12}F_2N_2O_2S$: C, 52.34; H, 4.05; N, 9.39. Found: C, 52.56; H, 4.08; N, 9.32.

3.1.4.6. *N*-(2-Aminophenyl-5-chloro-4-fluoro)-3,5-dimethylbenzensulfonamide (21b). Mp: 142–143 °C, yield 96%. ^{1}H NMR ($CDCl_3$): 2.24 (s, 6H, CH_3), 3.50 (br s, 1H, NH), 4.13 (br s, 2H, NH_2), 6.38–7.31 (m, 5H, ArH). Anal. Calcd for $C_{14}H_{14}ClFN_2O_2S$: C, 51.14; H, 4.29; N, 8.52. Found: C, 51.48; H, 4.13; N, 8.61.

3.1.5. General procedure for the synthesis of N_1-substituted 1,3-dihydro-2*H*-benzimidazol-2-ones (3a–11a)

An excess of a 20% toluene solution of phosgene (1 ml) was added to a solution of the appropriate N_1-(substituted-benzyl)-2-amino-anilines (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.

3.1.5.1. 6-Chloro-1-(3,5-difluorobenzyl)-1,3-dihydro-2*H*-benzimidazol-2-one (3a). Mp: 195 °C, yield 96%. ^{1}H NMR ($CDCl_3$): 5.01 (s, 2H, CH_2), 6.70–6.69 (m, 1H, H-4′), 6.81–6.82(m, 3H, H-7, H-2′, H-6′), 7.00 (d, J = 8.24, 1H, H-5), 7.07 (d, J = 8.24, 1H, H-4), 8.73 (br s, 1H, NH). Anal. Calcd for $C_{14}H_9ClF_2N_2O$: C, 57.06; H, 3.08; N, 9.51. Found: C, 56.91; H, 3.32; N, 9.62.

3.1.5.2. 6-Methyl-1-(2,6-difluorobenzyl)-1,3-dihydro-2*H*-benzimidazol-2-one (4a). Mp: 250–253 °C, yield 32%. ^{1}H NMR ($CDCl_3$): 2.33 (s, 3H, CH_3), 5.14 (s, 2H, CH_2), 6.61–6.95 (m, 5H, H-4, H-5, H-7, H-3′, H-5′), 7.21–7.31 (m, 1H, H-4′), 8.34 (br s, 1H, NH). Anal. Calcd for $C_{15}H_{12}F_2N_2O$: C, 65.69; H, 4.41; N, 10.21. Found: C, 65.73; H,4.57; N, 10.40.

3.1.5.3. 6-Methyl-1-(3,5-dimethylbenzyl)-1,3-dihydro-2*H*-benzimidazol-2-one (5a). Mp: 142 °C dec, yield 55%.^{1}H NMR ($CDCl_3$): 2.28 (s, 6H, CH_3), 2.33 (s, 3H, CH_3), 4.97 (s, 2H, CH_2), 6.70 (s, 1H, H-7), 6.83–6.95 (m, 5H, H-4, H-5, H-2′, H-4′, H-6′), 8.29 (br s, 1H, NH). Anal. Calcd for $C_{16}H_{15}ClN_2O$: C, 76.66; H, 6.81; N, 10.52. Found: C, 76.70; H, 6.68; N, 10.44.

3.1.5.4. 6-Methyl-1-(3,5-difluorobenzyl)-1,3-dihydro-2*H*-benzimidazol-2-one (6a). Mp: 191–193 °C, yield 50%. ^{1}H NMR ($CDCl_3$): 2.34 (s, 3H, CH_3); 5.01 (s, 2H, CH_2); 6.64 (s, 1H, H-7); 6.69–6.75 (m, 1H, H-4′), 6.82–6.90 (m, 3H, H-5, H-2′, H-6′), 6.97 (d, J = 7.69, 1H, H-4), 8.29 (br s, 1H, NH). Anal. Calcd for $C_{15}H_{12}F_2N_2O$: C, 65.69; H, 4.41; N, 10.21. Found: C, 65.71; H, 4.60; N, 10.35.

3.1.5.5. 6-Chloro,5-fluoro-(3,5-dimethylbenzyl)-1,3-dihydro-2*H*-benzimidazol-2-one (7a). Mp: 221–223 °C, yield 72%. ^{1}H NMR ($CDCl_3$): 2.29 (s, 6H, CH_3), 4.95 (s, 2H, CH_2), 6.84–6.94 (m, 5H, H-4, H-7, H-2′, H.4′, H-6′), 8.87 (br s, 1H, NH). Anal. Calcd for $C_{14}H_8ClF_3N_2O$: C, 53.78; H, 2.58; N, 8.96. Found: C, 53.96; H, 2.37; N, 10.59.

3.1.5.6. 6-Chloro,5-fluoro-(3,5-difluorobenzyl)-1,3-dihydro-2*H*-benzimidazol-2-one (8a). Mp: 216–218 °C, yield 44%. ^{1}H NMR ($CDCl_3$): 5.01 (s, 2H, CH_2), 6.73–6.83 (m, 4H, H-7, H-2′, H-4′, H-6′), 6.98 (d, J = 8.51, 1H, H-4), 9.69 (br s, 1H, NH). Anal. Calcd for $C_{14}H_8ClF_3N_2O$: C, 53.; H, 4.63; N, 9.19. Found: C, 63.55; H, 4.60; N, 9.54.

3.1.5.7. 6-Chloro,5-fluoro-(2,6-difluorobenzyl)-1,3-dihydro-2*H*-benzimidazol-2-one (9a). Mp: 221–223 °C, yield 75%. ^{1}H NMR ($CDCl_3$): 5.12 (s, 2H, CH_2), 6.88–6.97 (m, 4H, H-4, H-7, H,3′, H-5′), 7.26–7.31 (m, 1H, H-4′), 8.87 (br s, 1H, NH). Anal. Calcd for $C_{14}H_8ClF_3N_2O$: C, 63.06; H, 4.63; N, 9.19. Found: C, 63.44; H, 4.45; N, 9.43.

3.1.5.8. 6-Trifluoromethyl-1-(3,5-dimethylbenzyl)-1,3-dihydro-2*H*-benzimidazol-2-one (10a). Mp: 198–201 °C, yield 58%. ^{1}H NMR ($CDCl_3$): 2.28 (s, 6H, CH_3), 5.02 (s, 2H, CH_2), 6.93 (s, 3H, H-2′, H-4′, H-6′), 7.13–7.16 (m, 2H, H-4, H-7), 7.34 (d, J = 7.41, 1H, H-5), 8.91 (br s, 1H, NH). Anal. Calcd for $C_{17}H_{15}F_3N_2O$: C, 63.75; H, 4.72; N, 8.75. Found: C, 63.48; H, 5.08; N, 8.91.

3.1.5.9. 6-Trifluoromethyl-1-(2,6-difluorobenzyl)-1,3-dihydro-2*H*-benzimidazol-2-one (11a). Mp: 243–245 °C, yield 60%. ^{1}H NMR ($CDCl_3$): 5.19 (s, 2H, CH_2); 6.91–6.97 (m, 2H, H-4, H-7); 7.12 (d, J = 8.24, 1H, H-5), 7.25–7.61 (m, 3H, H-3′, H-4′, H-5′), 8.66 (br s, 1H, NH). Anal. Calcd for $C_{15}H_9F_5N_2O$: C, 54.89; H, 2.76; N, 28.94. Found: C, 54.48; H, 2.47; N, 29.02.

3.1.6. General procedure for the synthesis of 1-arylsulfonyl-1,3-dihydro-2*H*-benzimidazol-2-ones (1b, 3b–7b)

With a similar procedure for compounds **1a**–**11a** the 1-arylsulfonyl-1,3-dihydro-2*H*-benzimidazol-2-ones (**1b**–**7b**) were prepared from the appropriate *N*-(2-aminophenyl)-benzenesulfonamides (0.25 mmol).

3.1.6.1. 6-Chloro-1-(2,6-difluorophenylsulfonyl)-1,3-dihydro-2*H*-benzimidazol-2-one (1b). Mp: 163–165 °C, yield 54%. ^{1}H NMR (DMSO-d_6): 7.24 (d, J = 8.51, 1H, H-5), 7.34–7.40 (m, 3H, H-4, H-3′, H-5′), 7.58 (s, 1H, H-7), 7.69–7.92 (m, 1H, ArH), 10.78 (br s, 1H, NH). Anal. Calcd for $C_{13}H_7ClF_2N_2O_3S$: C, 45.30; H, 2.05; N, 8.13. Found: C, 45.44; H, 2.19; N, 7.98.

3.1.6.2. 6-Chloro-1-(3,5-difluorophenylsulfonyl)-1,3-dihydro-2*H*-benzimidazol-2-one (3b). Mp: 250 °C dec, yield 75%. ^{1}H NMR (DMSO-d_6): 7.05 (d, J = 8.51, 1H, H-4), 7.26 (d, J = 8.51, 1H, H-5), 7.75 (s, 1H, H-7), 7.80–7.89 (m, 3H, H-2′, H-4′, H-6′), 11.79 (br s, 1H, NH). Anal. Calcd for $C_{13}H_7ClF_2N_2O_3S$: C, 45.30; H, 2.05; N, 8.13. Found: C, 45.52; H, 2.01; N, 7.97.

3.1.6.3. 6-Methyl 1-(2,6-difluorophenylsulfonyl)-1,3-dihydro-2*H*-benzimidazol-2-one (4b). Mp: 198–200 °C, yield 39%. ^{1}H NMR ($CDCl_3$): 2.42 (s, 3H, CH_3), 6.91 (d, J = 7.96, 1H, H-5), 6.94–7.07 (m, 3H, H-4, H-3′, H-5′), 7.56–7.63 (m, 1H, H-4′), 7.70 (s, 1H, H-7), 7.97 (br s, 1H, NH). Anal. Calcd for $C_{14}H_{10}F_2N_2O_3S$: C, 51.85; H, 3.11; N, 8.64. Found: C, 51.95; H, 2.98; N, 8.73.

3.1.6.4. 6-Methyl 1-(3,5-dimethylphenylsulfonyl)-1,3-dihydro-2*H*-benzimidazol-2-one (5b). Mp: 280 °C dec, yield 43%. ^{1}H NMR ($CDCl_3$): 2.37 (s, 6H, CH_3), 2.44 (s, 3H, CH_3), 6.89 (d, J = 7.96, 1H, H-5), 6.99 (d, J = 7.96, 1H, H-4), 7.27–7.76 (m, 4H, H-7, H-2′, H-4′, H-6′), 8.01 (br s, 1H, NH). Anal. Calcd for $C_{16}H_{16}N_2O_3S$: C, 60.74; H, 5.10; N, 8.85. Found: C, 60.97; H, 5.00; N, 8.71.

3.1.6.5. 6-Methyl 1-(3,5-difluorophenylsulfonyl)-1,3-dihydro-2*H*-benzimidazol-2-one (6b). Mp: 230–232 °C dec, yield 45%. ^{1}H NMR (DMSO-d_6): 2.32 (s, 3H, CH_3), 6.82 (d, J = 7.69, 1H, H-5), 6.91 (d, J = 7.69, 1H, H-4), 7.49 (s, 1H, H-7), 7.73–7.79 (m, 3H, H-2′, H-4′, H-6′), 11.51 (br s, 1H, NH). Anal. Calcd for $C_{14}H_{10}F_2N_2O_3S$: C, 51.85; H, 3.11; N, 8.64. Found: C, 51.80; H, 2.96; N, 8.77.

3.1.6.6. 6-Chloro-5-fluoro-1-(3,5-dimethylphenylsulfonyl)-1,3-dihydro-2*H*-benzimidazol-2-one (7b). Mp: 245 °C dec, yield 47%. ^{1}H NMR (DMSO-d_6): 2.30 (s, 6H, CH_3), 6.65 (d, J = 10.44, 1H, H-4), 7.29 (s, 1H, H-4′), 7.38 (d, J = 7.69, 1H, H-7), 7.52 (s, 2H, H-2′, H-6′). Anal. Calcd for $C_{13}H_6ClF_3N_2O_3S$: C, 43.05; H, 1.67; N, 7.72. Found: C, 43.30; H, 1.52; N, 7.84.

3.1.7. General procedure for the synthesis of N_1-substituted 1,3-dihydro-2*H*-benzimidazol-2-thiones (1c–3c)

Thiophosgene (0.25 mmol) was added to a solution of N_1-substituted-2-amino-5-chloroaniline (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.

3.1.7.1. 6-Chloro-1-(2,6-difluorobenzyl)-1,3-dihydro-2*H*-benzimidazol-2-thione (1c). Mp: 210–212 °C, yield 88%. ^{1}H NMR ($CDCl_3$): 5.49 (s, 2H, CH_2), 7.04–7.10 (m, 2H, H-3′, H-5′), 7.15–7.22 (m, 2H, H-4, H-5), 7.39 (m, 1H, H-4′), 7.49 (s, 1H, H-7), 12.95 (br s, 1H, NH). Anal. Calcd for $C_{14}H_9ClF_2N_2S$: C, 54.11; H, 2.92; N, 9.01. Found: C, 53.67; H, 3.04; N, 9.58.

3.1.7.2. 6-Chloro-1-(3,5-dimethylbenzyl)-1,3-dihydro-2*H*-benzimidazol-2-thione (2c). Mp: 263–265 °C, yield 45%. ^{1}H NMR ($CDCl_3$): 2.28 (s, 6H, CH_3), 5.40 (s, 2H, CH_2), 7.02 (s, 3H, H-2′, H-4′, H-6′), 7.10 (s, 1H, H-7), 7.13–7.18 (m, 2H, H-4, H-5), 9.69 (br s, 1H,NH). Anal. Calcd for $C_{16}H_{15}ClN_2S$: C, 63.46; H, 4.99; N, 9.25. Found: C, 63.58; H, 5.13; N, 9.52.

3.1.7.3. 6-Chloro-1-(3,5-difluorobenzyl)-1,3-dihydro-2*H*-benzimidazol-2-thione (3c). Mp: 223–225 °C, yield 56%. ^{1}H NMR ($CDCl_3$): 5.46 (s, 2H, CH_2), 6.73–6.86 (m, 3H, H-2′, H-4′, H-6′), 6.98 (s, 1H, H-7), 7.16–7.23 (m, 2H, H-4, H-5), 10.34 (br s, 1H, NH). Anal. Calcd for $C_{14}H_9ClF_2N_2S$: C, 54.11; H, 2.92; N, 9.01. Found: C, 54.37; H, 2.53; N, 9.54.

3.1.8. General procedure for the synthesis of N_1-substituted 1-*H*-2*H*-3-acetyl-benzimidazol-2-one (1d–3d)

Triethylamine and then acetyl chloride (0.3 mmoli) were added dropwise to a solution of 1*H*-2*H*-benzimidazolone (**1a**, **2a** or **3a**)

(0.2 mmol) in dichloromethane (3 ml); the mixture was stirred for 30 min at room temperature. Successively, the reaction mixture was diluted with chloroform, washed with water and evaporated under reduced pressure. The residue was crystallized from ethanol.

3.1.8.1. 3-Acetyl-6-chloro-1-(2,6-difluorobenzyl)-1,3-dihydro-2*H*-benzimidazol-2-one (1d). Mp: 155–157 °C, yield 84%. ^{1}H NMR ($CDCl_3$): 2.77 (s, 3H, CH_3), 5.11 (s, 2H, CH_2), 6.93–7.33 (m, 5H, H-5, H-7, H-3′, H-4′, H-5′), 8.12 (d, J = 8.51, 1H, H-4). Anal. Calcd for $C_{16}H_{11}ClF_2N_2O_2$: C, 57.07; H, 3.29; N, 8.32. Found: C, 57.12; H, 3.18; N, 8.46.

3.1.8.2. 3-Acetyl-6-chloro-1-(3,5-dimethylbenzyl)-1,3-dihydro-2*H*-benzimidazol-2-one (2d). Mp: 152–154 °C, yield 99%. ^{1}H NMR ($CDCl_3$): 2.30 (s, 6H, CH_3), 2.80 (s, 3H, $COCH_3$), 4.94 (s, 2H, CH_2), 6.88–6.94 (m, 4H, H-7, H-2′, HP-4′, H-6′), 7.10 (d, J = 8.51, 1H, H-5), 8.13 (d, J = 8.51, 1H, H-4). Anal. Calcd for $C_{18}H_{17}ClN_2O_2$: C, 65.75; H, 5.21; N, 8.52. Found: C, 65.66; H, 5.46; N, 8.34.

3.1.8.3. 3-Acetyl-6-chloro-1-(3,5-difluorolbenzyl)-1,3-dihydro-2*H*-benzimidazol-2-one (3d). Mp: 121–123 °C, yield 23%. ^{1}H NMR ($CDCl_3$): 2.79 (s, 3H, CH_3), 4.99 (s, 2H, CH_2), 6.75–6.92 (m, 4H, H-2′, H-4′, H-6′), 7.15 (d, J = 8.51, 1H, H-5), 8.17 (d, J = 8.51, 1H, H-4). Anal. Calcd for $C_{16}H_{11}ClF_2N_2O_2$: C, 57.07; H, 3.29; N, 8.32. Found: C, 57.24; H, 3.40; N, 8.52.

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 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, finally, introduced into tubes containing aliquots of different enzyme concentrations (5–10 nM RT). After incubation at 37 °C for 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, finally, added with EcoLume® Scintillation cocktail (ICN, Research Products Division, Costa Mesa, CA, USA) to detect the acid-precipitable radioactivity by 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.[11] 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 Ki determinations an interval of inhibitor concentrations between 0.2 Ki and 5 Ki was used. Experiments have been done in triplicate. Experimental errors (±SD) were $\leqslant$10%.

3.2.3. In vitro anti-HIV assay

The methodology of the anti-HIV assays has been previously described.[10] Briefly, MT-4 cells were infected with HIV-1 (III_B) at ~100-times the $CCID_{50}$ (50% cell culture infective dose) per milliliter of cell suspension. One-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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References and notes

1. De Clercq, E. *J. Clin. Virol.* **2004**, *30*, 115.
2. Imamichi, T. *Curr. Pharm.* **2004**, *10*, 4039.
3. www.fda.gov/bbs/topics/NEWS/2007/NEW01726.html.
4. Vandamme, A. M.; Van Vaerenbrgh, K.; De Clercq, E. *Antiviral Chem. Chemother.* **1998**, *9*, 187.
5. Das, K.; Lewi, P. J.; Hughes, S. H.; Arnold, E. *Prog. Biophys. Mol. Biol.* **2005**, *88*, 209.
6. Barreca, M. L.; Balzarini, J.; Chimirri, A.; De Clercq, E.; De Luca, L.; Höltje, H. D.; Höltje, M.; Monforte, A. M.; Monforte, P.; Pannecouque, C.; Rao, A.; Zappalà, M. *J. Med. Chem.* **2002**, *45*, 5410.
7. Barreca, M. L.; Rao, A.; Balzarini, J.; De Luca, L.; Zappalà, M.; Monforte, A. M.; Maga, G.; Pannecouque, C.; Balzarini, J.; De Clercq, E.; Chimirri, A.; Monforte, P. *J. Med. Chem.* **2005**, *48*, 3433.
8. Barreca, M. L.; Rao, A.; De Luca, L.; Iraci, N.; Monforte, A. M.; Maga, G.; De Clercq, E.; Pannecouque, C.; Balzarini, J.; Chimirri, A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1956.
9. Hopkins, A. L.; Ren, J.; Tanaka, H.; Baba, M.; Okamato, M., et al *J. Med. Chem.* **1999**, *42*, 4500.
10. Balzarini, J.; Karlsson, A.; Perez-Perez, M. J.; Vrang, L.; Walbers, J., et al *Virology* **1993**, *192*, 246.
11. Maga, G.; Amacker, M.; Ruel, N.; Hubscher, U.; Spadari, S. *J. Mol. Biol.* **1997**, *274*, 738.