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Discovery of novel benzimidazolones as potent non-nucleoside reverse transcriptase inhibitors active against wild-type and mutant HIV-1 strains

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Abstract—Molecular modeling studies led to the rational discovery of N_1-arylsulfonyl-1,3-dihydro-2*H*-benzimidazol-2-one as a novel template for the design of new non-nucleoside reverse transcriptase inhibitors (NNRTIs) that are active against wild-type and mutant strains of HIV-1. It is worth noting that compound **3** proved to have antiretroviral activity similar to that of efavirenz and greater than that of nevirapine, two of the three NNRTIs currently available in antiretroviral therapy.

Current anti-AIDS therapy is based on drugs that belong either to the class of nucleoside/nucleotide (NRTIs/NtRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease or entry inhibitors. NNRTIs are a structurally diverse group of compounds which interact with a specific allosteric non-substrate binding pocket site of HIV-1 RT (non-nucleoside inhibitor binding pocket—NNIBP), leading to a non-competitive inhibition of the enzyme.[1] Three NNRTIs are currently licensed for use in antiretroviral therapy: nevirapine, delavirdine, and efavirenz.

Studies carried out on crystal structures of different RT/NNRTI complexes show that NNRTIs share a common binding mode even if they have different shapes and sizes.[2] The success of anti-HIV treatment with inhibitors that target RT, analogously to other drugs, has been limited by the rapid development of drug resistance and emergence of unwanted side-effects.

These problems have been the impetus for the design and development of new anti-AIDS agents that are highly active against drug-resistant mutant strains and have low levels of toxicity.

In a previous paper, we reported a 3D pharmacophore model for second generation NNRTIs consisting of five features: three hydrophobic groups, one hydrogen bond acceptor group, and one hydrogen bond donor group, and these both bind with two important RT Lysines (K101 and/or K103).[3] We used this model for the rational design of 1-benzyl-1,3-dihydro-2*H*-benzimidazol-2-ones as potential NNRTIs.[3]

In particular, biological tests showed that the 6-chloro-1-(2,6-difluorobenzyl)-substituted derivative (**1**, Fig. 1 and Scheme 1) was associated with significant activity against HIV-1 in cell culture and HIV-1 RT in cell-free assays, with a high selectivity index (SI > 1766) (Table 1). However, compound **1** was a poor inhibitor of viruses containing NNRTI-characteristic mutations. In fact when tested in CEM cell cultures against an extensive panel of mutant virus strains, the activity of compound **1** was generally maintained against L100I and E138K RT HIV-1, but it lost antiviral activity against K103N, Y181C, and Y188H RT HIV-1 strains (Table 2).

Keywords: NNRTI; Anti-HIV; Molecular modeling; Pharmacophore.
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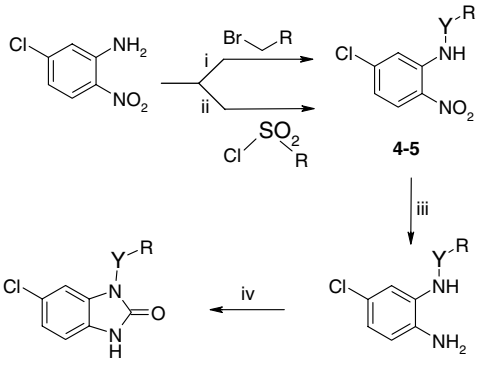

Compound	Y	R
1	CH_2	2,6- difluorophenyl[a]
2, 4, 6	CH_2	3,5-dimethylphenyl
3, 5, 7	SO_2	3,5-dimethylphenyl

Scheme 1. Reagents and conditions: (i) DMF, K_2CO_3, Δ, 2 h; (ii) appropriate halide, NaH, DMF, 0 °C, 30 min; (iii) Zn/HCl, EtOH, 80°C, 1 h; (iv) 20% toluene solution of $COCl_2$, HCl 2 N, Δ, 4 h. [a]See Ref. 3.

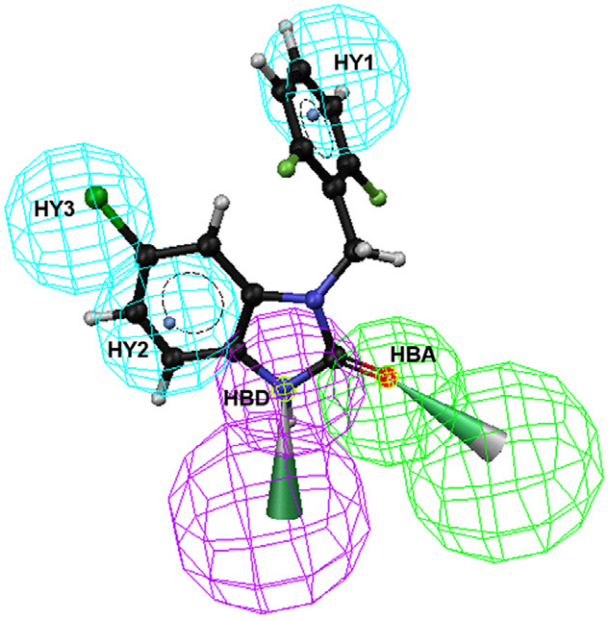

Figure 1. 3D pharmacophore model for second generation NNRTIs aligned to compound **1**. (HY1–HY3: hydrophobic groups; HBA: hydrogen bond acceptor; HBD: hydrogen bond donor).

Considering compound **1** as a starting point for lead optimization strategy, the aim of this paper was to design and synthesize derivatives that are more potent and less toxic than compound **1**, and are especially more active against the commonly found NNRTI-resistant HIV-1 mutants.

It has previously been demonstrated that hydrophobic substituents attached to the 3 and 5 positions of the phenyl group of NNRTIs invariably resulted in an enhancement of antiviral activity.[4–6] In particular Hopkins et al. showed that the introduction of 3,5-dimethyl groups onto the phenyl ring of MKC-442 resulted in a compound (GCA-186—Fig. 2) which demonstrated a greater inhibitory effect against the Y181C mutant virus as well as against the clinically important mutant K103N RT virus strain.[4]

The superimposition of GCA-186 on our pharmacophore model (Fig. 3) showed that the 3,5-dimethylphenyl group of this ligand occupied the hydrophobic feature HY1 corresponding to the difluorophenyl group of compound **1** (Fig. 1), thus suggesting an interesting chemical modification on this part of our derivative **1**.

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]
1	0.70 ± 0.1	0.24 ± 0.05	>424	>1766
2	0.15 ± 0.02	0.032 ± 0.007	8.9 ± 0.8	281
3	0.005 ± 0.001	0.002 ± 0.0003	39 ± 8.2	17846
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}.

Table 2. Anti-HIV-1 activity against wild-type and mutant HIV-1 strains in CEM cells

	EC_{50}[a] (μM)					
Compound	HIV-1IIIB	L100I	K103N	E138K	Y181C	Y188H
1	0.374 ± 0.204	3.1 ± 0.47	>50	2.5 ± 2.3	>50	>50
2	0.081 ± 0.042	0.049 ± 0.004	⩾3.0	0.665 ± 0.315	>3.0	>3.0
3	0.003 ± 0.0003	0.0060 ± 0.0	0.029 ± 0.0277	0.015 ± 0.017	0.16 ± 0.01	1.5 ± 0.42
Nevirapine	0.03 ± 0.00	0.199 ± 0.086	1.3 ± 0.26	0.207 ± 0.026	2.5 ± 0.0	>75
Efavirenz	0.0027 ± 0.0004	0.0573 ± 0.0191	0.218 ± 0.046	0.0096 ± 0.0026	0.0061 ± 0.0034	0.049 ± 0.0611

[a] Effective concentration (μM) to protect CEM cells against the HIV-induced cytopathicity (giant cell formation) by 50%.

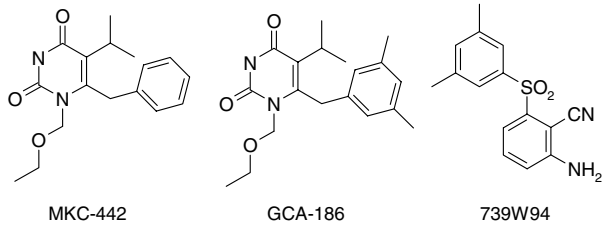

Figure 2. Structures of MKC-442, its 3,5-dimethyl derivatives GCA-186 and 739W94.

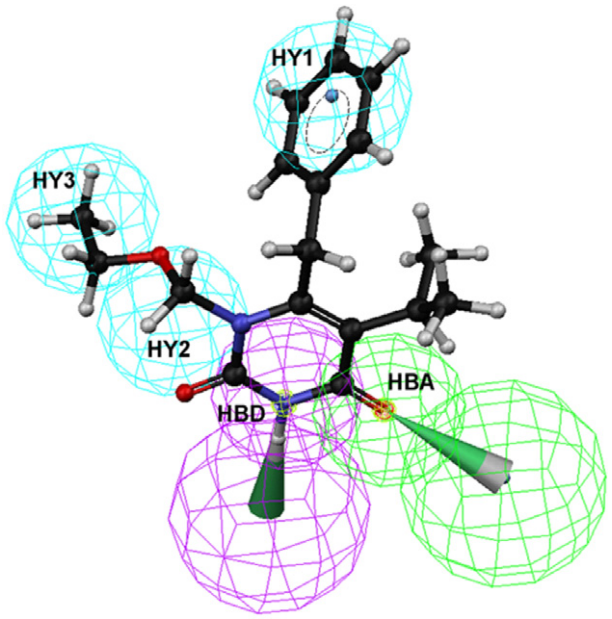

Figure 3. GCA-186 aligned on the 3D pharmacophore model (HY1–HY3: hydrophobic groups; HBA: hydrogen bond acceptor; HBD: hydrogen bond donor). Fit value for X-ray conformer = 3.88.

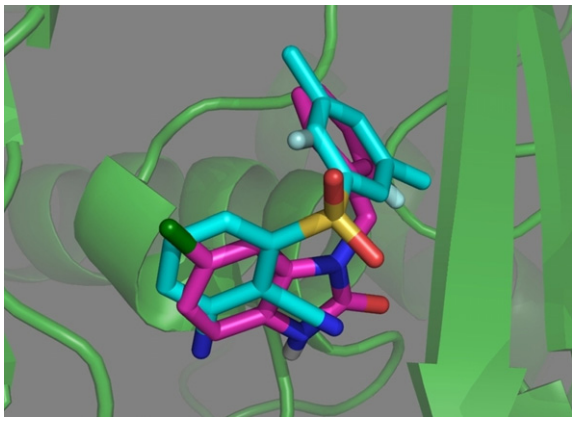

Figure 4. Autodock-predicted binding mode of **1** (magenta) compared to the crystallized position of 739W94 (cyan). The RT protein structure is shown in cartoon format (green). This figure was prepared using the program PyMOL.[8]

Furthermore, some arylsulfonylbenzonitriles and phenylsulfonylindol-2-carboxamides were reported as potent and selective anti-HIV agents against both wild-type and NNRTI-resistant mutant strains.[5–7] In particular the importance of the sulfone oxygens of the 2-amino-6-arylsulfonylbenzonitriles has been described and the crystal structure of one of the most potent analogues in the series (739W94, Fig. 2) in complex with RT has been elucidated.[5] The best docked conformation of compound **1** within NNIBP,[3] compared to the experimental position of 739W94, showed that the sulfonyl group of 739W94 overlapped with the methylene spacer between the difluorophenyl and the benzimidazol-2-one scaffold of **1** (Fig. 4).

On these bases, we designed analogues of compound **1** in order to investigate the influence of the suggested chemical modifications both on anti-HIV-1 activity and HIV-1 RT inhibition: derivative **2** was characterized by the presence of a 3,5-dimethylbenzyl moiety at N-1 while compound **3** was its sulfonyl derivative (Scheme 1).

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 3.0.5[9] and the docking protocol that we successfully applied in our previous papers on RT.[3,10] Docking of compounds **1**–**3** into the RT allosteric pocket, extracted from the structure of the complex RT/efavirenz (PDB code 1FK9),[11] generated a number of possible binding conformations with the corresponding Autodock energy values. The cluster analysis revealed a predominant ligand orientation within the binding pocket (65, 66, and 23 conformations in the first ranked cluster for **1**, **2**, and **3**, respectively) and the most energetically favorable conformation for each ligand was chosen for further analysis. Figure 5 reports docking results of **1**–**3** derivatives compared to the crystallized position of efavirenz: compounds **1**–**3** were bound in the NNIBP with an analogous binding mode and interacted in a fashion similar to efavirenz with RT residues of the allosteric pocket. The amide group of the imidazolone skeleton interacted with the main chain of K101 by hydrogen bond interactions, whereas *p*-chloroaniline and 2,6-difluorophenyl or 3,5- dimethylphenyl moieties created interactions with the hydrophobic binding pocket. The synthesis of the designed 1-substituted-1,3-dihydro-2*H*-benzimidazol-2-ones **2**–**3** was set up and carried out according to the reaction sequence reported in Scheme 1.

The 5-chloro-2-nitroaniline was N-substituted by treatment with the appropriate arylbromide in the presence of potassium carbonate to give intermediate **4** by microwave irradiation or with arylsulfonylchloride and a catalytic amount of sodium hydride to give intermediate **5**. Compounds **4**–**5** were reduced with Zn dust in acidic medium. Finally, the target compounds **2**–**3** were obtained by cyclization of the amino derivatives **6**–**7** with phosgene. The new compounds were evaluated in enzymatic tests for their ability to inhibit RT activity as well as HIV-1 replication in MT-4 cell cultures and also cytotoxic activity, and compared with derivative **1** (Table 1). Nevirapine and efavirenz were used as reference drugs.

All compounds were also tested in CEM cell culture against an extensive panel of mutant virus strains that

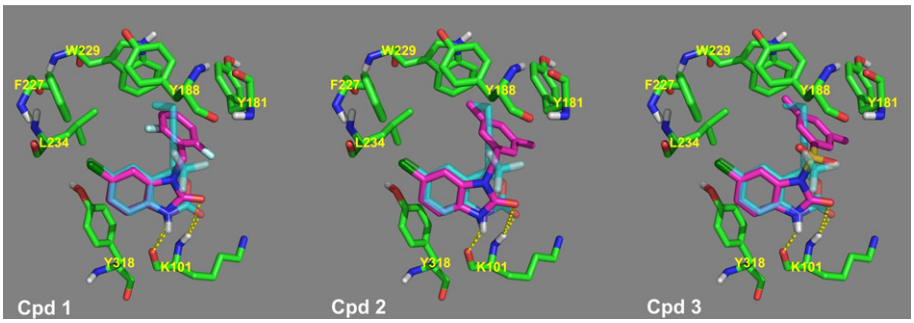

Figure 5. AutoDock-predicted binding mode of compound **1**–**3** (magenta) compared to the experimental position of efavirenz (cyan). Important residues of RT binding site are shown in green. Hydrogen bonds (shown as dashed yellow lines) are formed between the ligands and RT. This figure was prepared using the program PyMOL.[8]

contain a single mutation in their RT that is characteristic of the HIV-1 NNRTI-resistance profile (Table 2). As expected, the biological data highlighted the importance of a 3,5-dimethylaryl moiety for optimal activity and showed that compounds **2** and **3** were more active than derivative **1** both in RT and in HIV-1 tests. Moreover, N_1-arylsulfonyl derivative **3** was endowed with remarkable activity in the nanomolar range against both wild-type and NNRTI-resistant mutant virus strains (Tables 1 and 2).

Interestingly, compound **3** showed an anti-HIV-1 profile superior to that of nevirapine and comparable to that of efavirenz, with a higher selectivity index (SI = 17,846). When tested against HIV-1 strains carrying clinically relevant NNRTI-resistance mutations, this analogue was found to be: (i) generally at least 10 times more active than nevirapine against all tested strains; (ii) 10 times more potent than efavirenz against viruses with the L100I or K103N mutation, (iii) potent as efavirenz against E138K mutant, and (iv) less potent than efavirenz against the NNRTI-resistant HIV-1 strain with a Y181C or Y188H mutation. Moreover, it is worth noting that derivative **3** demonstrated antiviral activity against HIV-1_{IIIB} that was comparable to or better than that of molecules used for inspiration (MKC-442: EC_{50} = 0.004 μM; CGA-186: EC_{50} = 0.001 μM; 739W94: EC_{50} = 0.01 μM).[4,5] These results confirmed our idea that the introduction of a 3,5-dimethylbenzyl moiety and a sulfonyl group could be a valid strategy to increase the potency of our NNRTIs against both wild-type and drug-resistant mutant virus strains. As suggested by Hopkins et al., the greater potency against Y181C- and K103Asn-resistant HIV-1 strains of compounds bearing a 3,5-dimethylbenzyl group (e.g., **3**) might be due to the ability of this moiety: (i) to occupy a hydrophobic space near the 'roof' of the NNRTI binding pocket thus creating additional intermolecular interactions with the neighboring residues and (ii) to increase the van der Waals contact with the highly conserved W229 at the top of RT NNIBP, thereby reducing the dependence on binding to Y181.[4] In order to analyze the protein/ligand interactions for each docked compound, we used Ligplot 4.22[12] and observed a direct correlation between the contact numbers of the tested derivatives with the three highly conserved RT aminoacids (W229, L234, and Y318) and their different potency against mutant HIV-1 strains (see Supplementary data). The greatest activity of compound **3** might also be due to the electronic characteristics of the sulfonyl group: this linker was able to make close contacts with residues V106, V179, Y181, and Y188, while the methylene linker of compound **2** makes contacts only with residues V179 and Y188 (Fig. 6).

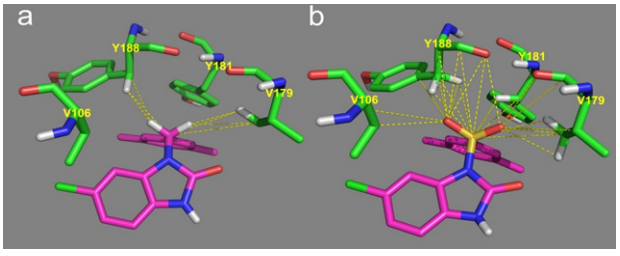

Figure 6. Intermolecular interactions (yellow lines) between the methylene linker of compound **2** (a) and the sulfonyl group of compound **3** (b) and NNIBP residues V106, V179, Y181, and Y188.

It is interesting to note that the SO_2 group of compound **3**, in its selected docking position, overlies the trifluoromethyl moiety of efavirenz (Fig. 5), thus suggesting that an electron-rich group in this region of NNIBP might positively influence the biological profile of NNRTIs. Moreover, non-bonded contacts of compound **2** with Y188 involve only the side chain of the residue, while in the case of compound **3** the same contacts involve both the side and main chains (Fig. 6). In summary, N_1-arylsulfonyl-1,3-dihydro-2*H*-benzimidazol-2-one was shown to be a novel template for the design of new NNRTIs active against wild-type and mutant strains of HIV-1. The most important goal of this study resulted in the discovery of compound **3**, a new NNRTI with anti-HIV potency similar to that of efavirenz. The skeleton of this molecule will now be used to introduce diverse chemical modifications to optimize its antiviral potential.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.01.025.

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