1-BENZOYL-2-(2-NITROPHENYL)-1*H*-BENZIMIDAZOLE DERIVATIVES: A NOVEL APPROACH TO THE DEVELOPMENT OF NEW HIV-1 REVERSE TRANSCRIPTASE INHIBITORS.

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ABSTRACT

A novel approach to the development of a new class of HIV-1 RT inhibitors is reported. The 1-benzoyl-2-aryl-1*H*-benzimidazole series was designed as a combination of two previously reported active scaffolds, the benzimidazole and benzoyl moieties. The active compounds of the series effectively blocked the reverse transcription in the micromolar range in an *in vitro* assay containing the wild-type enzyme. We have demonstrated that the 2-nitrophenyl C-2 substituent is an important structural feature for the desired biological activity in this series. Molecular docking experiments suggest that the active compounds adopt a butterfly-like conformation within the binding pocket of the enzyme, with the benzoyl moiety located in an extended hydrophobic region defined mainly by Tyr181, Tyr188, and Trp229.

Keywords: NNRTIs; HIV-1 RT; molecular modeling; benzoylbenzimidazole derivatives

INTRODUCTION

The discovery and development of new antiviral drugs in the search for better treatments has been a main goal for scientists. During the last two decades, an important number of organic compounds exhibiting antiviral activity, including products isolated from natural sources, have been published.[1]

The non-nucleoside reverse transcriptase inhibitors (NNRTIs) are vital components of multi-drug regimens for HIV therapy.[2,3] This class of anti-AIDS drugs is a structurally diverse group of compounds that specifically inhibit HIV-1 reverse transcriptase (HIV-1 RT) by interacting with a specific allosteric nonsubstrate binding site,[4] causing short and long-range conformational changes that disrupt the activity of DNA polymerase.[5] However, in spite of the positive impact of highly active antiretroviral therapy, there are significant problems with drug-resistant strains of HIV-RT,[6] and with drug toxicity.[7] Therefore, the development of more potent and less aggressive drugs for the treatment of AIDS is continually needed.[8] Of the approved drugs for the treatment of HIV infection, at present only three molecules belong to the NNRTI class: nevirapine, delavirdine and efavirenz (Figure 1).[9]

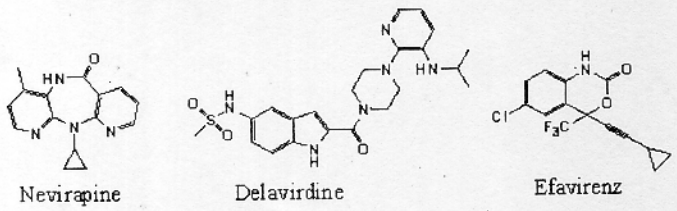


Figure 1. Current clinically used NNRTIs.

The first benzimidazole derivatives reported as potential anti-HIV-1 agents were the 1-aryl-1*H*,3*H*-thiazolo[3,4-*a*]benzimidazoles (TBZs), which proved to be highly active as NNRTIs.[10] Specifically, TBZ 1-(2,6-difluorophenyl)-3*H*-[1,3]thiazolo[3,4-*a*]benzimidazole has been shown clearly to be the most potent inhibitor, and is considered as a lead compound for 2-aryl-substituted benzimidazole derivatives. The extensive oxidation of the sulfur atom, responsible for TBZ degradation to less potent sulfoxide and sulfone metabolites, led to its isosteric replacement or the removal of the thiazole ring. These modifications led in turn to the discovery of a new series of 1*H*,3*H*-oxazolo[3,4-*a*]benzimidazoles, OBZs,[11] and 1-benzyl-2-arylbenzimidazoles, known generically as bis-phenylbenzimidazoles, BPBIs,[12] both families structurally related to TBZs (Figure 2).

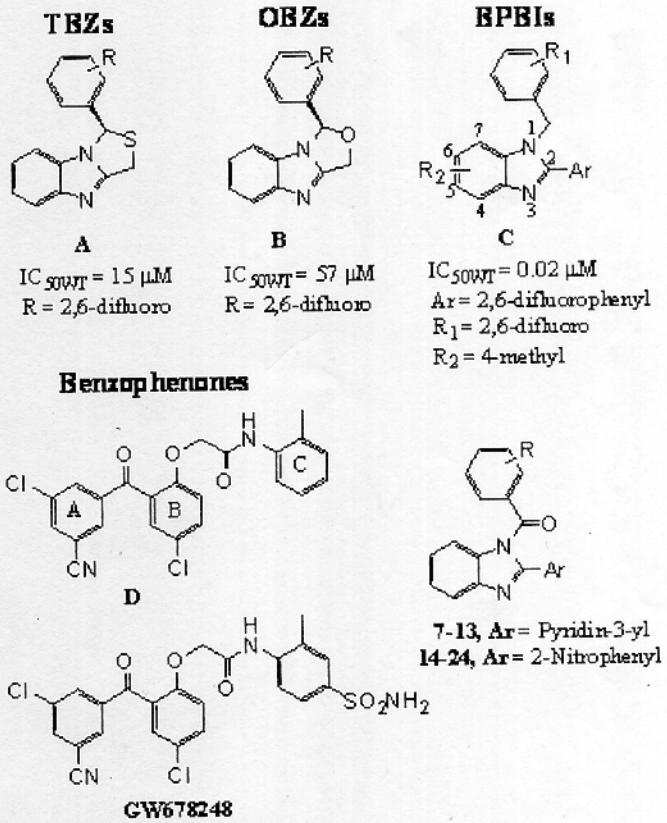


Figure 2. Active NNRTI benzimidazole and benzophenone derivatives, and general structure of the designed series.

The biological activity of TBZ, OBZ and BPBI derivatives is linked to their ability to assume a "butterfly-like" conformation, associated with the biological activity of other NNRTIs,[13] and to a suitable spatial location of lipophilic and electron-rich groups in the hydrophobic pocket of the enzyme.[14] This conformation has also been evidenced by comparing the crystallographic structures of several inhibitors, leading to a common NNRTI pharmacophore description.[15] Structure-activity relationship studies on the BPBI system have shown that aromatic substituents at position 2 play a crucial role in the

stabilization of the interaction of these compounds with the viral enzyme.[16] Replacement of the benzene ring at C2 by bulkier systems (e.g. naphthyl) had a negative influence on the inhibiting capacity of the ligands.[16] In particular, it has been reported that the high binding affinity of compound **C** is related to π-stacking interactions between the electron-deficient difluorobenzoyl ring system and electron-rich Tyr181, Tyr188 and Trp299 residues in the binding site that allow electronic and hydrophobic interactions between the enzyme and the ligand.[17]

The present work is aimed at extending these studies to the synthesis and anti-RT evaluation of two novel series of 2-aryl-1*H*-benzimidazoles containing a benzoyl system instead of the reported benzyl group as an *N*1 substituent (Figure 2). Our interest in attaching a substituted benzoyl moiety to the active 2-aryl-benzimidazole framework arises from the high anti-HIV-1 activity reported for this scaffold.[18] Moreover, research on benzophenones as NNRTIs performed by GlaxoSmithKline and the University of Oxford led to the synthesis and evaluation of several series of derivatives like GW678248, a potent inhibitor currently in phase 2 clinical studies (Figure 2).[19] Analysis of the crystal structure of compound **D** complexed with wild-type HIV-RT indicates that the benzoyl moiety (A-ring) lies within the hydrophobic region adjacent to the side chains of Tyr181 and Tyr188, in a manner similar to the benzyl system on BPBIs.[20] Our designed series can also be considered as analogues of OBZs in which the oxazole ring has been removed. Since the electronic properties of the 2,6 fluorine atoms in the most active compounds TBZs and OBZs (figure 2) and the carbonyl group of the benzoyl derivatives proposed are qualitatively similar, we might expect that their effect in the electron density of the phenyl ring is also comparable. This hypothesis was supported by quantum chemical calculations (see supplementary information), that showed a highly similar electronic density distribution in the phenyl ring for the previously outlined compounds.

Our preliminary docking experiments carried out on 2-aryl-1-benzoyl benzimidazoles showed that the 2-nitrophenyl ring, which has not been studied as a C-2 substituent in BPBIs, can enhance the butterfly-like conformation of these compounds within the RT binding pocket. Moreover, despite the high prevalence of the pyridine ring among NNRTIs, just one benzyl BPBI bearing a pyridine at C-2 has been reported, displaying activity in the micromolar range.[12] Considering the goal of this study, this paper details the synthesis and biological assays of a series of *N*1-benzoyl-2-pyridyn-3-yl- and 2-(2-nitrophenyl)-1*H*-benzimidazoles, **7-24,** exploring the effect of some benzoyl ring substitution patterns on RT inhibition (Figure 2).

EXPERIMENTAL

Chemistry

All organic solvents used for the synthesis were of analytical grade. Melting points were determined on a Stuart Scientific SMP3 apparatus and are uncorrected. IR spectra were recorded on a Bruker Vector 22 spectrophotometer using KBr discs. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker APC-200. The chemical shifts are expressed in ppm (δ scale) downfield from TMS, *J* values are given in Hertz for solutions in $CDCl_3$ unless otherwise indicated. Column chromatography was performed on Merck silica gel 60 (70-230 mesh). Thin layer chromatographic separations were performed on Merck silica gel 60 (70-230 mesh) chromatofoils. Elemental analyses were carried out on a FISONS EA 1108 CHNS-O analyzer.

2-Pyridin-3-yl-1*H*-benzimidazole (3): A solution of 3-pyridine-carboxaldehyde (500 mg, 4.67 mmole) and *o*-phenylenediamine (505 mg, 4.67 mmole) in ethanol (100 mL) was stirred at room temperature for 48 hours. The reaction mixture was then poured into water (220 mL) and a precipitate was separated by filtration. The precipitate was purified by recrystallization from water, isolating compound **3** as a pale yellow solid. Yield: 95%; mp: 255–256 °C; IR (KBr) cm^{-1}: 1446 (NH). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 12.58-12.70 (b.s., 1H, NH), 9.43 (dd, 1H, J_1= 2.3, J_2=0.8, H-2'), 8.66 (dd, 1H, J_1= 4.8, J_2= 1.5, H-4'), 8.54-8.51 (m, 1H, H-6'), 7.82-7.76 (m, 1H, H-4), 7.48-7.56 (m, 1H, H-7), 7.44 (dd, 1H, J_1= 8.1, J_2= 4.9, H-5'), 7.21-7.31 (m, 2H, H-5,6). ^{13}C NMR ($CDCl_3$) δ: 150.8, 149.8, 138.2, 134.4, 131.3, 126.7, 125.8, 125.5, 124.0, 123.1, 120.8, 113.5. *Anal.* Calcd. for $C_{12}H_9N_3$ (MW: 195.22): C, 73.83%; H, 4.65%; N, 21.52%. Found: C, 73.51%; H, 4.60%; N, 21.90%.

General synthetic procedure for 1-benzoyl-2-pyridin-3-yl-1*H*-benzimidazole derivatives 7-13.

A solution of 2-pyridin-3-yl-1*H*-benzimidazole **3** and the corresponding benzoyl chloride in anhydrous THF, was stirred for 24 hours under reflux. Triethylamine was added to trap the hydrogen chloride. The reaction mixture was filtered and the crude product was purified by silica gel column chromatography (ethyl acetate:dichlorometane 2:1) and recrystallized, yielding the aroyl benzimidazole as a solid.

1-Benzoyl-2-pyridin-3-yl-1*H*-benzimidazole (7). Prepared from **3** (500 mg, 2.56 mmole) and benzoyl chloride (180 mg, 1.28 mmole). Yield = 46%; mp: 138–139 °C (ethanol); IR (KBr) cm^{-1}: 1701 (C=O). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.85 (1H, dd, J_1= 2.0, J_2= 0.5, H-2'), 8.56 (1H, dd, J_1= 4.9, J_2=1.5, H-4'), 7.94 (1H, m, H-6'), 7.90 (1H, d, *J*= 7.8, H-6"), 7.75 (2H, dd, J_1= 8.3, J_2= 1.0, H-4, 2"), 7.58 (1H, m, H-7), 7.44-7.39 (4H, m, H-3", 4", 5",5'), 7.33 (1H, m, H-5), 7.25 (1H, dd, J_1= 7.8, J_2= 4.9, H-6). ^{13}C NMR ($CDCl_3$) δ: 167.8, 150.1, 149.2, 142.9, 135.6, 133.9, 133.5, 130.7, 130.1, 2x128.8, 2x127.1, 125.6, 125.2, 124.5, 124.2, 122.1, 114.2. *Anal.* Calcd. for $C_{19}H_{13}N_3O$ (MW: 299.33): C,76.24%; H, 4.38%; N, 14.04%. Found: C, 76.11%; H, 4.28%; N, 14.10%.

1-(4-Nitrobenzoyl)-2-pyridin-3-yl-1*H*-benzimidazole (8). Prepared from **3** (500 mg, 2.56 mmole) and *p*-nitrobenzoyl chloride (238 mg, 1.28 mmole). Yield = 96%; mp:155 – 156 °C (ethanol); IR (KBr) cm^{-1}: 1701 (C=O), 1529,1292 (NO_2). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.79 (dd, 1H, J_1=2.0, J_2= 0.4, H-2'), 8.58 (dd, 1H, J_1= 4.9, J_2=1.0, H-4'), 8.24-8.22 (m, 2H, H-3", 5"), 7.98-7.93 (m, 2H, H-2",6"), 7.90-7.87 (m, 2H, H-6',4), 7.54-7.47 (m, 2H, H-7,5'), 7.41 (m, 1H, H-5), 7.30 (m, 1H, H-6). ^{13}C NMR ($CDCl_3$) δ: 165.1, 150.6, 149.8, 148.2, 142.9, 136.2, 134.8, 133.8, 131.9, 131.5, 2x128.1, 125.7, 125.3, 2x124.9, 123.1, 122.3, 113.2. *Anal.* Calcd. for $C_{19}H_{12}N_4O_3$ (MW: 344,32): C, 66.28%; H, 3.51%; N, 16.27%. Found: C, 66.55%; H, 3.38%; N, 16.32%.

1-(4-Chlorobenzoyl)-2-pyridin-3-yl-1*H*-benzimidazole (9). Prepared from **3** (500 mg, 2.56 mmole) and *p*-chlorobenzoyl chloride (224 mg, 1.28 mmole). Yield = 52%; mp: 154 –155 °C (ethanol); IR (KBr)cm^{-1}: 1701 (C=O). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.83 (dd, 1H, J_1= 2.0, J_2= 0.5, H-2'), 8.59 (dd, 1H, J_1= 5.1, J_2=1.8, H-4'), 7.95-7.92 (m, 1H, H-6'), 7.91 (d, 1H, *J*= 8.1 Hz, H-6"), 7.70-7.67 (m, 2H, H-4, 2"), 7.44-7.37 (m, 4H, H-3",5",5',7), 7.40-7.32 (m, 1H, H-5), 7.28 (m, 1H, H-6). ^{13}C NMR ($CDCl_3$) δ: 166.4, 150.48, 149.8, 142.9, 136.3, 134.4, 132.8, 2x130.6, 2x129.5, 125.3, 124.8, 123.7, 123.1, 2x122.4, 120.5, 113.3. *Anal.* Calcd. for $C_{19}H_{12}ClN_3O$ (MW: 333.77): C, 68.37%; H, 3.62%, N, 12.59%. Found: C, 67.93%; H, 3.54%; N, 12.62%.

1-(4-Fluorobenzoyl)-2-pyridin-3-yl-1*H*-benzimidazole (10). Prepared from **3** (500 mg, 2.56 mmole) and *p*-fluorobenzoyl chloride (203 mg, 1.28 mmole). Yield = 51%; mp: 192 – 193 °C (ethanol); IR (KBr) cm^{-1}: 1703 (C=O); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.84 (dd, 1H, J_1= 2.0, J_2= 0.5, H-2'), 8.58 (dd, 1H, J_1= 4.9, J_2=1.5, H-4'), 7.96-7.93 (m, 1H, H-6'), 7.90 (d, 1H, *J*= 8.3 Hz, H-4), 7.81-7.76 (m, 2H, H-2", 6"), 7.44-7.39 (m, 2H, H-5',7), 7.36-7.32 (m, 1H, H-5), 7.28 (m, 1H, H-6), 7.12-7.07 (m, 2H, H-3",5"). ^{13}C NMR ($CDCl_3$) δ: 167.6, 166.1 (d, 2C, J_{C-F}= 175 Hz), 150.9, 150.7, 149.8, 143.0, 141.2, 136.3, 134.3, 131.9, 2x129.5, 125.3, 124.9, 123.1, 120.6, 2x116.6, 113.2. *Anal.* Calcd. for $C_{19}H_{12}FN_3O$ (MW: 317.32): C, 71.92%; H, 3.81%, N, 13.24%. Found: C, 71.30%; H, 3.75%; N, 13.21%.

4-[(2-Pyridin-3-yl-1*H*-benzimidazol-1-yl)carbonyl]aniline (11). A stirred suspension of 1-(4-nitrobenzoyl)-2-pyridin-3-yl-1*H*-benzimidazole **8** (300 mg, 0.95 mmole), iron powder (1000 mg, 17.86 mmole) and acetic acid-ethanol-water (80 mL, 1:1:1 v/v) was heated at 50-60 °C for 1h. The mixture was then diluted with water (30 mL), neutralized with sodium hydrogencarbonate and extracted with ethyl acetate (3x70 mL). The organic layer was dried over Na_2SO_4 and the solvent evaporated in vacuo to give a whitish solid. Further purification of the crude by column chromatography (chloroform:ethyl acetate = 1:1) followed by recrystallization afforded **11**. Total yield = 43%; mp: 171-172°C (ethanol); IR (KBr) cm^{-1}: 3423 (NH_2), 1603 (CO); ^{1}H NMR (acetone-d_6) δ: 9.34 (d, 1H, *J*= 1.6, H-2'), 8.57 (dd, 1H, J_1= 4.2, J_2= 1.5, H-4'), 8.49 (dd, 1H, J_1= 4.6, J_2= 1.4, H-5'), 7.96-7.93 (m, 1H, H-6'), 7.59-7.56 (m, 2H, H-4,7), 7.46-7.43 (m, 2H, H-2",6"), 7.41-7.15 (m, 4H, H-5,6, 5",3"), 5.20-3.80 (b.s., 2H, NH_2). ^{13}C NMR (acetone-d_6) δ: 167.8, 151.2, 149.2, 148.0, 141.5, 138.9, 134.1, 133.0, 2x130.8, 130.7, 125.6, 124.0, 123.0, 120.6, 116.8, 2x115.3, 113.3. *Anal.* Calcd. for $C_{19}H_{14}N_4O$ (MW: 314.34): C, 72.60%; H, 4.49%; N, 17.82%; Found: C, 72.32%; H, 4.69%; N, 17.83%.

***N,N*-Dimethyl-4-[(2-pyridin-3-yl-1*H*-benzimidazol-1-yl)carbonyl]aniline (12).** Prepared from **3** (500 mg, 2.56 mmole) and *p*-dimethylaminobenzoyl chloride (244 mg, 1.28 mmole). Yield = 53%; mp: 153–154 °C (ethanol); IR (KBr) cm^{-1}: 1686 (C=O). ^{1}H NMR (200 MHz, $CDCl_3$)

δ: 8.99 (dd, 1H, J_1= 2.0, J_2= 0.5, H-2'), 8.59 (dd, 1H, J_1= 7.8, J_2= 4.9, H-4'), 8.06-8.03 (m, 1H, H-6'), 7.88 (d, 1H, *J*=7.8, H-4), 7.72-7.68 (m, 3H, H-2", 6", 7), 7.38-7.34 (m, 1H, H-5'), 7.32-7.24 (m, 2H, H-5, 6), 6.58-6.62 (m, 2H, H-3", 5"), 3.37 (s, 6H, 2 x CH_3). ^{13}C NMR ($CDCl_3$) δ: 167.4, 154.6, 150.9, 150.3, 149.6, 142.9, 138.8, 134.5, 132.9, 2x130.8, 2x124.9, 123.1, 120.2, 115.6, 112.8, 2x111.0, 2x40.0. *Anal.* Calcd. for $C_{21}H_{18}N_4O$ (MW: 342.39): C, 73.67%; H, 5.30%; N, 16.36%. Found: C, 73.84%; H, 5.63%; N, 15.86%.

1-(3,4,5-Trimethoxybenzoyl)-2-pyridin-3-yl-1*H*-benzimidazole (13). Prepared from **3** (500 mg, 2.56 mmole) and 3,4,5-trimethoxybenzoyl chloride (295 mg, 1.28 mmole). Yield = 44%; mp: 151–152 °C (ethanol); IR (KBr) cm^{-1}: 2835 (CO-CH_3), 1683 (C=O). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.89 (d, 1H, *J*= 1.5, H-2'), 8.60 (dd, 1H, J_1= 4.8, J_2=1.3, H-4'), 8.00-7.97 (m, 1H, H-6'), 7,58 (m, 1H, H-5'), 7.45-7.40 (m, 2H, H-4,7), 7.40-7.37 (m, 1H, H-5), 7.30 (m, 1H, H-6), 7.01(s, 2H, H-2", 6"), 3.91(s, 3H, OCH_3), 3.79 (s, 6H, 2 x OCH_3). ^{13}C NMR ($CDCl_3$) δ: 167.9, 153.3, 151.1, 150.5, 149.5, 142.9, 136.1, 135.0, 131.9, 2x127.0, 125.1, 124.8, 2x123.1, 120.5, 113.2, 2x108.4, 61.1, 2x56.4. *Anal.* Calcd. for $C_{22}H_{19}N_3O_4$ (MW: 389,40): C, 67.86%; H, 4.92%, N, 10.79%. Found: C, 67.64%; H, 4.96%; N, 10.86%.

2-(2-Nitrophenyl)-1*H*-benzimidazole (4). A solution of 2-nitrobenzaldehyde (150 mg, 0.99 mmole) and *o*-phenylenediamine (107 mg, 0.99 mmole) in ethanol (20 mL) was stirred at room temperature for 48 hours and extracted with chloroform (3 x 30 mL). The extract was washed with water, dried over magnesium sulfate and concentrated under vacuum. The crude product was recrystallized from ethanol to give pure benzimidazole (4). Yield: 85%; mp: 277–279 °C; IR (KBr) cm^{-1}: 3440 (NH). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 12.38 (br s, 1H, NH), 8.00-7.25 (m, 8H, Ar-H). ^{13}C NMR ($CDCl_3$) δ: 178.5, 148.9, 148.8, 142.6, 139.2, 132.8, 132.2, 130.5, 124.6, 123.3, 122.2, 119.8, 111.8. *Anal.* Calcd. for $C_{13}H_9N_3O_2$ (MW: 239.23): C, 65.26%; H, 3.79%; N, 17.57%. Found: C, 64.90%; H, 4.09%; N, 17.34%.

General synthetic procedure for 1-benzoyl-2-(2'-nitrophenyl)-1*H*-benzimidazole derivatives 14-24.

A solution of 2-(2'-nitrophenyl)-1*H*-benzimidazole **4** and the corresponding benzoyl chloride in anhydrous THF was stirred at room temperature for 24 hours. Triethylamine was added to trap the hydrogen chloride. The reaction mixture was filtered, and the crude product was purified by silica gel column chromatography (ethyl acetate:dichlorometane 2:1) and recrystallized, yielding the target compound as a solid.

1-Benzoyl-2-(2-nitrophenyl)-1*H*-benzimidazole (14). Prepared from **4** (500 mg, 2.56 mmole) and benzoyl chloride (180 mg, 1.28 mmole). Yield = 46%; mp: 138–139 °C (ethanol); IR (KBr) cm^{-1}: 1697 (C=O), 1524 (NO_2); ^{1}H NMR(200 MHz, $CDCl_3$) δ: 8.17 (d, 1H, *J*= 7.8, H-3'), 7.87-7.43 (m, 7H, H-4, 7, 4', 5', 6', 2", 6"), 7.42-7.18 (m, 4H, H-4', 3", 4", 5"), 6.93 (d, 1H, *J*= 7.6 Hz, H-5 or 6). ^{13}C NMR ($CDCl_3$) δ: 167.9, 150.5, 147.3, 142.9, 133.9, 133.6, 133.0, 132.5, 130.8, 130.1, 2x128.8, 2x126.3, 127.6, 124.9, 124.7, 124.5, 120.7, 114.1. *Anal.* Calcd. for $C_{20}H_{13}N_3O_3$ (MW: 343.34): C, 69.96%; H, 3.82%; N, 12.24%. Found: C, 69.32 %; H, 3.44 %; N, 12.18 %.

1-(2-Nitrobenzoyl)-2-(2-nitrophenyl)-1*H*-benzimidazole (15). Prepared from **4** (527 mg, 2.5 mmole) and *p*-nitrobenzoyl chloride (588 mg, 2.5mmole). Yield = 48%; mp: 140-141 °C (ethanol); IR (KBr) cm^{-1}: 1720 (C=O), 1529 (NO_2). ^{1}H-NMR (DMSO-d_6) δ: 8.20 (t, 2H, *J*=7.5, H-3', 3"), 7.84-7.70 (m, 7H, H-4, 7, 5', 6', 4", 5", 6"), 7.26-7.47 (m, 3H, H-5, 6, 4'). ^{13}C-NMR ($CDCl_3$) δ: 168.2, 149.9, 146.9, 145.6, 140.7, 135.3, 134.1, 132.8, 132.2, 131.6, 129.0, 127.6, 2x125.8, 125.2, 124.9, 124.2, 120.2, 116.8, 114.2. *Anal.* Calcd. for $C_{20}H_{12}N_4O_5$ (MW: 388.33): C, 61.86%; H, 3.11%; N, 14.43%. Found: C, 61.90%; H, 3.17%; N, 14.43%.

1-(2,6-Difluorobenzoyl)-2-(2-nitrophenyl)-1*H*-benzimidazole (16). Prepared from **4** (478 mg, 2.0 mmole) and 2,6-difluorobenzoyl chloride (353 mg, 2.0 mmole). Yield = 51%; mp: 178–179 °C (ethanol); IR (KBr) cm^{-1}: 1698 (C=O), 1529 (NO_2), 1007 (C-F); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.10 (d, 1H, *J*=7.8 , H-3'), 7.80-7.27 (m, 8H, 4, 5, 6, 7, 4', 5', 6', 4"), 6.78 (t, 2H, *J*= 8.1, H-3", 5"). ^{13}C-NMR ($CDCl_3$) δ: 166.7, 159.2 (d, 2C, J_{C-F}= 388 Hz), 158.8, 156.5, 149.6, 146.9, 142.9, 134.0 (t, 3C, J_{1-3}=14 Hz), 132.5, 131.2, 127.1, 126.2, 125.6, 124.5, 120.7, 114.5, 112.3 (d, 2C, J_{1-2}= 28 Hz), 109.3, 108.5, 107.7. *Anal.* Calcd. for $C_{20}H_{11}F_2N_3O_3$ (MW: 379.32): C, 66.33%; H, 2.92%, N, 11.08%. Found: C, 66.41%; H, 2.56%; N, 11.21%.

1-(2,6-Dichlorobenzoyl)-2-(2-nitrophenyl)-1*H*-benzimidazole (17). Prepared from **4** (527 mg, 2.5 mmole) and 2,6-difluorobenzoyl chloride (461 mg, 2.5 mmole). The reaction mixture was stirred for 48 hours under reflux. Yield = 53 %, mp: 177–178°C (ethanol); IR (KBr) cm^{-1}: 1716 (C=O), 1522 (NO_2), C-Cl (933); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.25 (d, 1H, *J*= 7,9, H-3'), 7.98-7.62 (m, 4H, H-4,7,5',6'), 7.62-7.26 (m, 6H, H-5, 6, 4', 3", 4", 5"). ^{13}C NMR ($CDCl_3$) δ: 166.0, 147.4, 142.9, 134.6, 2x134.0, 132.5, 131.7, 130.7, 2x129.2, 128.6, 127.5, 126.6, 126.1, 125.5, 124.5, 120.9, 115.5, 111.3. *Anal.* Calcd. for $C_{20}H_{11}Cl_2N_3O_3$ (MW: 412.23): C, 58.27%; H, 2.69%, N, 10.19%. Found: C, 57.63%; H, 3.15 %; N, 9.78%.

1-(4-Nitrobenzoyl)-2-(2-nitrophenyl)-1*H*-benzimidazole (18). Prepared from **4** (547 mg, 2.3 mmole) and *p*-nitrobenzoyl chloride (425 mg, 2.3 mmole). Yield = 90%; mp: 189-190 °C (ethanol); IR (KBr) cm^{-1}: 1702 (C=O), 1577 (NO_2); ^{1}H NMR (DMSO-d_6) δ: 8.19 (d, 2H, *J*= 6.9, H-3", 5"), 8.15 (d, 1H, *J*=7.5, H-3'), 7.88 (d, 2H, *J*=8.1, H-2", 6"), 7.84-7.58 (m, 4H, H-4, 7, 5', 6'), 7.41-7.19 (m, 3H, H-5, 6, 4'). ^{13}C NMR ($CDCl_3$) δ: 165.6, 149.5, 149.4, 146.9, 142.3, 137.7, 133.5, 132.8, 130.9, 2x130.5, 128.1, 126.3, 125.0, 2x124.6, 124.1, 123.3, 119.9, 114.1. *Anal.* Calcd. for $C_{20}H_{12}N_4O_3$ (MW: 388.33): C, 61.86%; H, 3.11%; N, 14.43%. Found: C, 61.89%; H, 3.21%; N, 14.62%.

1-(4-Chlorobenzoyl)-2-(2-nitrophenyl)-1*H*-benzimidazole (19). Prepared from **4** (519 mg, 2.2 mmole) and *p*-chlorobenzoyl chloride (380 mg, 2.2 mmole). Yield = 38%; mp: 167–168 °C (ethanol); IR (KBr) cm^{-1}: 1700 (C=O), C-Cl (757); ^{1}H NMR (DMSO-d_6) δ: 8.10 (d, 1H, *J*=7.8, H-3'), 7.80-7.46 (m, 6H, H-4, 7, 5', 6', 2", 6"), 7.41-7.23 (m, 5H, H-5, 6, 4', 3", 5"). ^{13}C NMR (DMSO-d_6) δ: 166.5, 149.5, 147.1, 142.3, 138.6, 133.9, 133,.2, 132.9, 131.4, 131.3, 2x130.9, 2x128.7, 126.2, 125.1, 124.6, 124.3, 120.0, 114.4. *Anal.* Calcd for $C_{20}H_{12}ClN_3O_3$ (MW: 377.78): C, 63.59%; H, 3.20%; N, 11.12%; Found: C, 64.00%; H, 3.52%; N, 10.86%.

1-(4-Fluorobenzoyl)-2-(2-nitrophenyl)-1*H*-benzimidazole (20). Prepared from **4** (547 mg, 2.3 mmole) and *p*-fluorobenzoyl chloride (365 mg, 2.3 mmole). Yield = 50%, mp: 167–168 °C (ethanol); IR (KBr) cm^{-1}: 1704 (C=O), 1534 (NO_2), 936 (C-F); ^{1}H NMR (DMSO-d_6) δ: 8.14 (d, 1H, *J*=7.5, H-3'), 7.85-7.42 (m, 6H, H-4, 7, 5', 6', 2", 6"), 7.41-7.23 (m, 5H, H-5, 6, 3", 5"). ^{13}C NMR (DMSO-d_6) δ: 166.6, 166.1 (d, 2C, J_{C-F}= 175), 150.4, 147.4, 143.0, 133.6, 133.0, 2x130.8, 128.6, 127.5, 124.7 (d, 3C, J_{1-3}= 7), 120.8, 118.1, 117.4, 116.2 (d, 2C, J_{1-2}=14), 113.8. *Anal.* Calcd. for $C_{20}H_{12}FN_3O_3$ (MW: 361.33): C, 66.48%; H, 3.35%, N, 11.63%. Found: C, 66.48 %; H,3.69 %; N,11.73%.

***N,N*-Dimethyl-4-{[2-(2-nitrophenyl)-1*H*-benzimidazol-1-yl)carbonyl]carbonyl} aniline (21).** Prepared from **4** (500 mg, 2.1 mmole) and *p*-dimethylaminobenzoyl chloride (384 mg, 2.0 mmole). The reaction mixture was stirred for 48 hours under reflux. Yield = 40%; mp: 264–265 °C (ethanol); IR (KBr) cm^{-1}: 1526 (C=O), 1348 (N-C); ^{1}H NMR (DMSO-d_6) δ: 8.11 (d, 1H, *J*=7.1, H-3'), 7.86-7.66 (m, 7H, H-4, 7, 4', 5', 6', 2", 6"), 7.46-7.44 (m, 2H, H-5,6), 6.91 (s, 2H, 3",5"), 3.38 (s, 6H, 2xCH_3). ^{13}C NMR (DMSO-d_6) δ: 167.7, 153.5, 152.9, 150.2, 147.2, 143.0, 141.9, 134.3, 134.2, 133.7, 131.8, 127.8, 127.1, 125.7, 125.1, 124.8, 120.5, 115.1, 108.2, 107.9, 60.5, 56.6. *Anal.* Calcd. for $C_{22}H_{18}N_4O_3$ (MW: 386.40): C, 68.38%; H, 4.70%, N, 14.50%. Found: C, 68.01%; H, 4.28%; N, 14.75 %.

1-(2-Methoxybenzoyl)-2-(2-nitrophenyl)-1*H*-benzimidazole (22). Prepared from **4** (540 mg, 2.3 mmole) and 2-methoxybenzoyl chloride (427 mg, 2.3 mmole). Yield = 47%; mp: 130-131 °C (ethanol); IR (KBr) cm^{-1}: 1702 (C=O), 1316, 1342 (OCH_3); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.05 (d, 1H, *J*= 8, H-3'), 7.84-7.56 (m, 5H, H-4, 7, 5', 6', 6"), 7.45-7.26 (m, 4H, H-5, 6, 4', 4"), 6.94-6.76 (m, 2H, 3",5"), 4.01 (s, 3H, OCH_3). ^{13}C-NMR ($CDCl_3$) δ: 165.9, 155.8, 149.7, 146.5, 142.3, 133.9, 133.8, 132.7, 132.5, 131.3, 128.9, 126.3, 125.5, 124.8, 124.0, 122.3, 120.5, 119.9, 114.3, 111.3, 55.6. *Anal.* Calcd. for $C_{21}H_{15}N_3O_4$ (MW: 373.36) : C, 67.56%; H, 4.05%; N, 11.25%. Found: C, 67.65 %; H, 4.55; %; N, 10.87 %.

1-(3,4,5-Trimethoxybenzoyl)-2-(2-nitrophenyl)-1*H* benzimidazole (23): Prepared from **4** (555 mg, 2.3 mmole) and *p*-3,4,5-trimethoxybenzoyl chloride (535 mg, 2.3 mmole). Yield = 52%; mp: 163–164 °C (ethanol); IR (KBr) cm^{-1}: 1701 (C=O), 1527 (NO_2), 1125 (O-CH_3); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.17 (d, 1H, *J*=7.2, H-3'), 7.86-7.62 (m, 4H, H-4, 7, 5', 6'), 7.39-7.14 (m, 3H, H-5, 6, 4'), 6.95 (s, 2H, H-2", 6"), 3.91 (s, 3H, OCH_3), 3.76 (s, 6H, 2 x OCH_3). ^{13}C NMR ($CDCl_3$) δ: 167.8, 151.3, 146.9, 145.0, 141.5, 138.9, 134.6, 133.3, 132.2, 130.5, 126.2, 126.1, 125.3, 124.9, 123.6, 119.5, 118.1, 110.6, 2x105.1, 60.5, 2x56.2. *Anal.* Calcd. for $C_{23}H_{19}N_3O_6$ (MW: 433.41): C, 63.74%; H, 4.42%, N, 9.70%. Found: C, 63.67 %; H, 4.00%; N, 9.78%.

1-(Biphen-4-yl-carbonyl)-2-(2-nitrophenyl)-1*H*-benzimidazole (24): Prepared from **4** (511mg, 2.1 mmole) and *p*-phenylbenzoyl chloride (464 mg, 2.1 mmole). Yield = 59%; mp: 162-163 °C (ethanol); IR (KBr) cm^{-1}: 1702 (C=O), 1526 (NO_2); ^1H NMR (DMSO-d_6) δ: 8.11 (d, 1H, *J*=7.8, H-3'), 7.86-7.68 (m, 9H, H-2",3",5",6", 5',6',4',4, 7), 7.65-7.29 (m, 7H, H-5,6, *p*-C_6H_5). ^{13}C NMR (DMSO-d_6) δ: 167.2, 149.6, 147.3, 145.0, 142.2, 138.4, 133.8, 133.4, 2x132.9, 131.1, 130.9, 2x130.3, 2x129.0, 128.5, 126.9, 126.7, 126.4, 125.0, 124.5, 124.3, 120.0, 118.3, 114.1. *Anal.* Calcd. for $C_{26}H_{17}N_3O_3$ (MW: 419.43): C, 74.45%; H, 4.09%; N, 10.02%; Found: C, 74.18%; H; 4.36%; N, 10.03%.

Reverse transcriptase assay. Biological activity of the synthesized compounds was determined by examining their ability to inhibit HIV-1 RT (wild type) at 10 μM drug concentration (Table 1). The HIV-1 reverse transcriptase used in this study was a recombinant enzyme constituting part of a commercially available enzyme kit (Retrotech-GmbH, Germany).

Enzymatic assays. Briefly, inhibition of recombinant reverse transcriptase enzyme was measured by the incorporation of biotin-dUTP into oligo(dT) homopolymer template primers. All tested compounds were dissolved in dimethylsulfoxide (DMSO). In all inhibition experiments the enzyme and either lysis buffer or compound solution was co-incubated for 60 min at 37 °C in a shaking incubator. All the tests were performed including nevirapine (10 μM) as the positive control. Residual enzymatic activity was calculated relative to the initial linear reaction rates found when no drug was added. The data were represented as percent inhibition relative to basal conditions, which were calculated from mean OD values as follows: $100 - (OD_{max} - OD_{sample} \times 100/OD_{max})$.

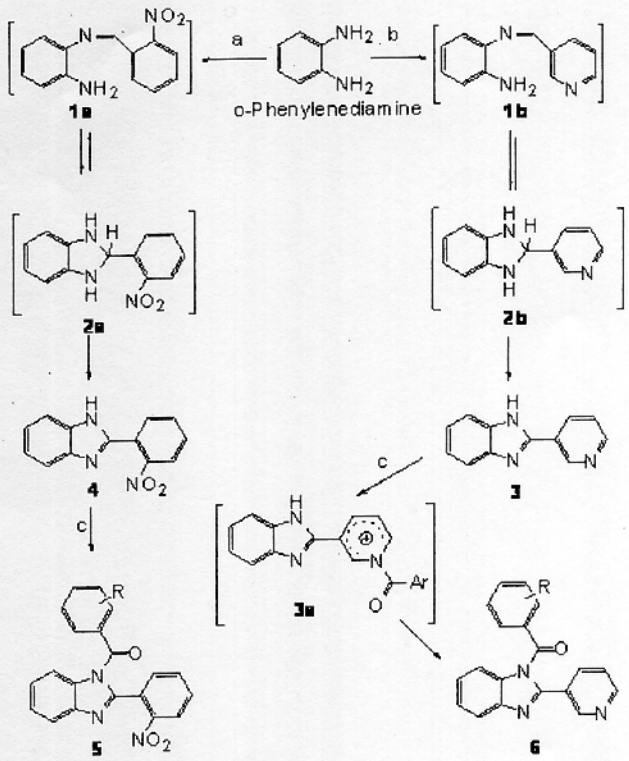
Data analysis. Experiments were performed in triplicate and repeated at least three times. Values quoted are given as means ± S.E.M. for the number of independent experiments indicated.

Molecular modeling

Docking studies to RT were carried out using AutoDock 3.0.5.[21] The X-ray structure of wild-type HIV-RT complexed with the inhibitor 8-Cl-TIBO,[13] already reported as a suitable template for activity prediction of novel BPBIs 16 and TIBO derivatives,[22] was selected as target for our modeling studies. The structure was retrieved from the Protein Data Bank[23] with entry code 1HNV. Compounds 8-Cl-TIBO, 4Me-BPBI, and derivatives 12, 17, 19, 20 and 21 were optimized by the AM1 semi-empirical procedure using the InsightII/Mopac module.[24] The population in the genetic algorithm was 50, the energy evaluations were 250,000 and the maximum number of iterations was 27,000. After docking, the 100 solutions were clustered into groups with RMS deviations less than 1.0 Å. Solutions were then minimized until convergence, considering residues at 6 Å from the mass center of each ligand using the CVFF force field as implemented in the InsightII/Discover module, and then ranked according to the Energy_Estimate 2 Ludi scoring function as implemented in the InsightII/Ludi module.[25] The lowest energy conformation was selected for analysis. All figures were displayed using DS Visualizer 1.7.[26]

RESULTS AND DISCUSSION

A synthetic route was sought that would provide a rapid possibility of modifying the substitution pattern on the benzene ring of the benzoyl moiety. The target benzimidazoles were synthesized according to the synthetic strategy displayed in Scheme 1. *o*-Phenylenediamine was the starting material. Benzimidazole **3** was efficiently obtained by condensation of *o*-phenylenediamine and 3-pyridinecarboxaldehyde in ethanol, with continuous stirring at room temperature. In a similar strategy, benzimidazole **4** was prepared by coupling *o*-phenylenediamine with 2-nitrobenzaldehyde in ethanol under reflux.[27]



Scheme 1. Synthesis of *N*1-benzoyl-2-arylbenzimidazoles. Reaction conditions: a) 2-nitrobenzaldehyde, ethanol, room temp. reflux, 48h, yield 95%; b) 3-pyridinecarboxaldehyde, ethanol, room temp., 48 h., yield 85%; c) benzoyl halide, THF, reflux, NEt_3, yields 38-96%.

According to the literature, the generation of benzimidazoles **3** and **4** involves the imine intermediates **1a** and **1b**, followed by a second nucleophilic attack to give the benzimidazolidines **2a** and **2b**, which are finally converted into the benzimidazoles by aerobic oxidation, as depicted in Scheme 2. Benzimidazoles **3** and **4** were subsequently reacted with a series of benzoyl chlorides under reflux to yield the corresponding series of *N*1-benzoyl-2-pyridyn-3-yl- and *N*1-benzoyl-2-(2-nitrophenyl)-1*H*-benzimidazoles **5** and **6**. On the basis of the well known catalytic effect of pyridine in acylation reactions, production of the N1-benzoylbenzimidazole derivative **6** probably occurs *via* the formation of the more reactive intermediate **3a**, which would generate the target compounds by a nucleophilic attack of the benzimidazole, as shown in Scheme 2. As expected, these reactions had moderate yields due to the low nucleophilicity of the benzimidazoles.

Biological assay

Compounds **7-24** were tested at 10 μM concentration in an *in vitro* wild-type HIV-1 RT inhibition assay including nevirapine (10 μM) as the positive control. The range of inhibition by the active compounds was from 11.5% to 53.3%. As seen in Table 1, compounds **17** and **21** showed the highest activity (53.5% and 50.7%, respectively), while the 2-(3-pyridyl) derivatives **7-11** and **13** did not significantly inhibit HIV-1 RT. Comparison of the active derivatives **19**, **20** and **21** with their 2-pyridin-3-yl analogues **9**, **10** and **12** reveals that the 2-(2-nitrophenyl) ring was consistently better at conferring inhibitory activity to the compounds.

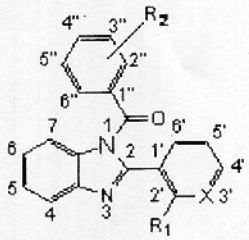


Table 1. HIV-1 reverse transcriptase inhibition assay results for compounds **7–24**.

Entry	X	R_1	R_2	Inhib. (%) 10μM[a]
7	N	H	H	na
8	N	H	4-NO_2	na
9	N	H	4-Cl	na
10	N	H	4-F	na
11	N	H	4-NH_2	na
12	N	H	4-$N(CH_3)_2$	11.5±1.7
13	N	H	3,4,5-OCH_3	na
14	C	NO_2	H	na
15	C	NO_2	2-NO_2	na
16	C	NO_2	2, 6-F	na
17	C	NO_2	2, 6-Cl	53.3±1.9
18	C	NO_2	4-NO_2	na
19	C	NO_2	4-Cl	36.0±1.5
20	C	NO_2	4-F	11.3±1.4
21	C	NO_2	4-$N(CH_3)_2$	50.7±2.3
22	C	NO_2	2-OCH_3	na
23	C	NO_2	3,4,5-OCH_3	na
24	C	NO_2	4-Phe	na
Nevirapine	-	-	-	85.5±1.0

[a] Values are the means±S.E.M. of at least three separate experiments (na = not active).

A detailed look at the data in Table 1 shows the following: (a) Substitution of the benzoyl ring with chlorine at the 2 and 6 positions yielded the best inhibitor, **17**. (b) As shown by the inhibition data of compounds **17**, **19** and **20**, halogen substitution on the benzoyl ring enhances the anti-RT activity. This suggests that the electron-withdrawing effect of the substituents has a positive influence on the activity. This result was also consistent with our hypothesis and is in good agreement with previous studies reported for *N*1-benzyl benzimidazoles, as discussed above. Nevertheless, it is very clear that the 2-(2-nitrophenyl) derivatives have the best activity in the RT assay, since compounds **7-11** and **13** bearing similar electron withdrawing substituents, were inactive. (c) Notably, compound **21**, bearing a *p*-dimethylamine group, showed inhibitory potency in the micromolar range in spite of the electron-donating character of the substituent.

In order to investigate the binding mode of the designed ligands, a molecular docking study was carried out with 8-Cl-TIBO, 4Me-BPBI, and derivatives **12**, **17**, **19**, **20** and **21**, by means of the AutoDock v3.0.5 software. Selected solutions were further minimized and scored using the LUDI scoring function.

Visual inspection of the minimized complexes for the active compounds of our series shows the molecules adopting a butterfly-like conformation similar to that of other NNRTIs reported in the literature. For all the derivatives, the benzoyl moiety is located within an extended hydrophobic region known as wing I, defined by the aromatic side chains of Tyr181, Tyr188, and Trp229 as well as by Pro95 and Leu100 in the non-nucleoside binding pocket (NNBP), while the benzimidazole core is positioned in the opposite region, defined mainly by Lys101, Lys103, Pro236 and Tyr318, known as wing II.

Active compounds **12** and **21**, bearing a *p*-dimethylaminophenyl group as **R2** substitution, showed a similar spatial orientation as the inhibitors 8-Cl-TIBO and BPBIs. The common features in the binding mode of 8-Cl-TIBO and derivative **21** are shown in Figure 3.

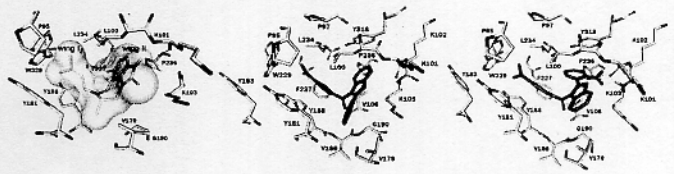


Figure 3. From left to right. Comparison of the crystallographically determined binding mode of 8-Cl-TIBO in complex with wild-type HIV-RT (PDB entry code 1HNV), and predicted binding modes for compounds **12** and **21,** showing the similar spatial location of the dimethyl groups. Hydrogen bonds are displayed as green dashes. Hydrogen atoms and some residues are omitted for clarity.

Results showed **12** assuming an orientation similar to that reported for BPBIs. Moreover, there is no formation of H–bonds between the ligand and residues on the NNBP, the *p*-dimethylaminophenyl group is accommodated into an extended hydrophobic region defined by the aromatic side chains of Tyr181, Tyr188, and Trp229 as well as by Pro95 and Leu100 on wing I in the NNBP, with the carbonyl group facing the floor of the NNBP, between the Val106 side chain and Val189 main chain atoms. This orientation allows the ligand to establish a parallel π-stacking interaction between the phenyl ring and Tyr188 (centroid distance 4,4 Å), while the benzimidazole core is placed on wing II, with its phenyl ring between Pro236 and Tyr318, establishing a π-stacking interaction with this latter (centroid distance 4,8 Å), and hydrophobic interactions with residues Leu100 at the top of the NNBP, and Val106 at the bottom. The imidazole core could establish aliphatic contacts with Gly190 and the Lys103 side-chain. This conformation suggests that substitution at positions 6 and 7 on the benzimidazole ring with small hydrophobic groups could improve activity and that the core is sensitive to mutations in the Val106 region. Nevertheless, substitutions at position 3 and 5 in the *N*1-benzoyl ring could enhance activity against Y181 mutants. The 2-aryl substituent (3-pyridyl) points toward the solvent-exposed region, and makes hydrophobic contacts with Tyr181, Val179, and Gly190.

The predicted **21**/HIV-RT complex reveals a different binding mode. The 2-aryl substituent (2-nitrophenyl) is placed toward the inside of the pocket, and is able to establish a double H-bond between the nitro group and the Lys103 backbone, while a π-stacking interaction with Tyr318 may contribute to stabilization of the complex. Another interesting result in this study is that the nitro group of the C-2 substituent seems to induce a butterfly-like conformation due to steric repulsion. This spatial arrangement permits a conformational arrangement of **21** in the binding pocket so as to optimize π-stacking interactions between the benzoyl moiety and Tyr181 and Tyr188.

In regard to the orientation of the *p*-dimethylaminophenyl group in **21**, our molecular modeling results showed the methyl groups in close contact with Pro95, Leu100 Tyr181, Tyr188 and Trp229 residues, resulting in a more efficient use of lipophilic subsite contacts compared to **12** and the inactive derivatives of the series. Furthermore, examination of the **21-RT** complex reveals the loss of coplanarity between the dimethylamino group and the aromatic ring, not allowing resonance of the unpaired electrons of the nitrogen orbital with the aromatic ring. This conformation is probably induced by interaction of the methyl groups with the surrounding aminoacids mentioned above. Moreover, when a *p*-amino group replaced the *p*-dimethylamino group in compound **11**, the weak activity of **12** disappeared. This suggests that the activity of **12** and **21** is not due to the electron-donor properties of nitrogen but to improved hydrophobic contacts with the surrounding residues in the binding site due to the methyl groups.

The carbonyl group in **21** appeared oriented to the bottom of the non-nucleotide binding pocket, allowing dipolar interactions with the side chain of Val106 and the backbone of Val 189 and Gly190. The benzimidazole core is located toward Lys101 and Lys103. Moreover, the phenyl ring of the benzoyl system is not long enough to interact extensively with residues V179 and G190. This spatial distribution probably enhances a conformational arrangement positioning the benzoyl moiety in the NNBP so as to optimize a π-stacking interaction with Y181 and Y188. This binding mode is very similar to that reported for some indolyl aryl sulfones (IASs) and arylsulfonylbenzonitrile derivatives.[30,31]

Similarly, the model of the **17**/HIV-RT complex suggests a binding mode dominated by the same features as for 4Me-BPBI and its analogue *N*1(2,6-dichlorobenzyl)-4Me-BPBI (not shown), with the C2 phenyl ring occupying a pocket formed by the side chains of Leu100, Lys101, Lys103 and Val179 (Figure 4). The nitro group is oriented to the bottom of the pocket, between the side chain of Val106 and the backbone of Gly190. The phenyl moiety of the benzimidazole core is surrounded by the Val106 and Leu234 side chains, and an aromatic π-stacking interaction with Tyr318 is suggested.

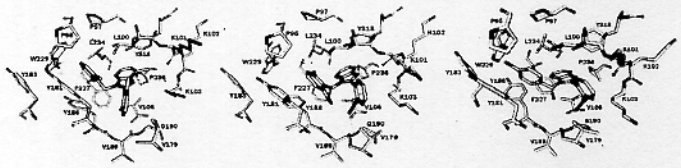


Figure 4. From left to right. Comparison of the predicted binding modes of 4-MeBPBI, and compounds **17** and **19**. Hydrogen bonds are displayed as green dashes. Hydrogen atoms and some residues are omitted for clarity.

Regarding the 2,6-dichlorobenzoyl moiety of **17** (Figure 4) as for its analogue *N*1(2,6-dichlorobenzyl)-4Me-BPBI, the chlorine atom at position 2 makes hydrophobic contacts with Tyr188, Trp229, and Leu234, whereas the phenyl ring makes hydrophobic contacts with Pro95 and Leu100, as well as parallel π-stacking with Tyr181. The two halogen atoms should have an electronic-withdrawing effect that can enhance the π-stacking interaction.

In general, examination of the HIV-RT complexes with all the active compounds of the series shows that the phenyl groups of the π-deficient benzoyl system and Tyr181/Tyr188 are associated in a coplanar arrangement of their aromatic rings, allowing favorable π-stacking interactions which contribute to the stabilization of the complexes. This spatial arrangement may also lead to a partial charge transfer between the electron-rich aromatic ring of Tyr181/Tyr188 and the phenyl ring of the benzoyl system. Nevertheless, the presence of inactive compounds in this series clearly suggests that the electron-withdrawing effect of the carbonyl, which could enhance the abovementioned π-stacking interactions through the phenyl ring of the benzoyl system, is not enough for strong inhibition, while the substitution pattern of this ring as well as substitution at C-2 are very important for the inhibitory activity.

CONCLUSIONS

In the search for novel structures with anti-RT activity, we have reported the discovery of a novel class of inhibitors combining the active moieties benzoyl and *N*1-substituted 2-arylbenzimidazole. The *N*1-benzoyl-2-pyridin-3-yl- and 2-(2-nitrophenyl)benzimidazole derivatives were obtained in two steps in moderate to good yields. All the final products were characterized by ^{1}H and ^{13}CNMR spectra, IR spectra, and by CHN elemental analysis. The series was evaluated by *in vitro* assays of their inhibitory activity against the wild-type reverse transcriptase of HIV-1.

Molecular modeling studies suggested that the active compounds could bind to RT in two different modes according to the C-2 substitution, allowing a binding mode similar to that of other reported NNRTIs such as 8-Cl-TIBO, BPBIs and Nevirapine. The π-deficient aromatic ring of the benzoyl system contributes to the good overall binding properties of these compounds, probably through hydrophobic, π-stacking and charge transfer interactions. The 2-nitro phenylbenzimidazoles **17** and **21**, which showed the highest inhibitory activity against RT-HIV, represent a valuable advance in the search for novel NNRTIs and can be considered a starting point for lead optimization, which is currently in progress.

ACKNOWLEDGMENS

This project was supported by FONDECYT (Grant 1050950). The assistance of Dr. Otto Faff (Retrotech, GmbH) with the anti-RT assays, and support from Fundación Ciencia para la Vida and Fundación Chilena para Biología Celular is gratefully acknowledged.

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