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Il Farmaco 57 (2002) 819–823

IL FARMACO

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Synthesis and anti-HIV activity of 1-(2,6-difluorophenyl)-1H,3H-thiazolo[3,4-a]benzimidazole structurally-related 1,2-substituted benzimidazoles

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Received 15 April 2002; accepted 1 July 2002

Abstract

The synthesis of 1,2-substituted benzimidazoles is reported. These novel derivatives share chemical similarities with 1-aryl-1H,3H-thiazolo[3,4-a]benzimidazoles, a class of HIV-1 NNRTIs studied widely. All compounds prepared were tested in MT-4 cells to explore their potential anti-HIV activity and were found to be less potent than 1-(2,6-difluorophenyl)-1H,3H-thiazolo[3,4-a]benzimidazole (TBZ).
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Keywords: 1,2-Substituted benzimidazoles; Anti-HIV agents; NNRTI

1. Introduction

Since the beginning of anti-AIDS chemotherapy, HIV-1 reverse transcriptase (RT) has been the main target in the attack against this virus and the majority of drugs under clinical use are inhibitors of such enzyme [1]. These inhibitors can be classified into two groups: nucleosides (NRTIs), that as chain terminators block the growth of the DNA strand, and the non-nucleosides (NNRTIs), which are a structurally diverse group of compounds that inhibit the enzyme in an allosteric mode by binding to a pocket near the polymerase active site. In contrast to the former group, NNRTIs present low toxicity and high selectivity. In recent years, three NNRTIs namely nevirapine, delavirdine and efavirenz have been approved by FDA for clinical use in combined therapy [2].

In previous papers [3,4] we reported a series of 1-aryl-1H,3H-thiazolo[3,4-a]benzimidazoles which proved to be highly active as HIV-1 NNRTIs. Extensive structure–activity relationship (SAR) studies have been performed within this family of compounds and it has been observed that specific requirements exist with regard to the structural determinants for optimum anti-HIV activity. The C-1 substituent plays a decisive and crucial role in the interaction of these compounds with the HIV-1 RT: a 2,6-dihalo substituted phenyl ring at C-1 furnished rewarding results and gave a large improvement in potency. In particular, 1-(2,6-difluorophenyl)-1H,3H-thiazolo[3,4-a]benzimidazole (TBZ, Fig. 1) proved to be the most potent inhibitor and has been selected as lead compound of this series.

We have also demonstrated previously [5] that the biological activity of TBZ is associated with the ability to assume a 'butterfly-like' conformation, which allows a binding mode similar to other NNRTIs by means of a suitable spatial location of lipophilic and electron-rich groups. From this study, it resulted that the plausible pharmacophoric elements for TBZ were: the benzene fused ring, the aryl group at C-1 and the nitrogen atom of the thiazole nucleus.

Furthermore, the potential therapeutic utility of TBZ is hampered by the metabolic oxidation of the thiazole

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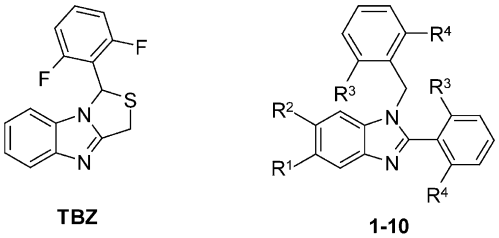
PII: S0014-827X(02)01300-9



R^1 = H, Me, MeO, benzyl
R^2 = H, Me, MeO, COOH, benzyl
R^3 = F, Cl
R^4 = H, F, Cl

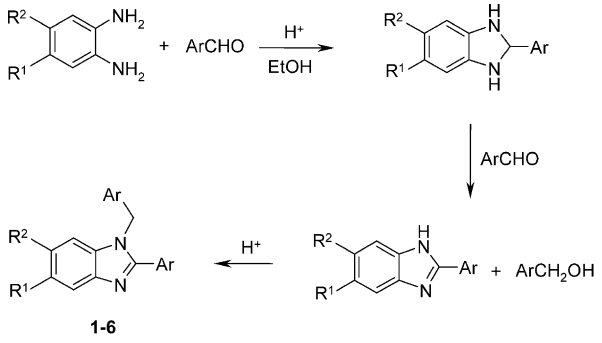
Fig. 1.

ring of TBZ leading to the formation of less potent sulfoxide and sulfone metabolites [6].

On these bases, we have removed the thiazolidine nucleus and synthesized a series of 1-benzyl-2-arylbenzimidazoles (Fig. 1) as new NNRTIs. The elimination of the thiazole nucleus allowed keeping all key structural requirements, that is two π-systems and a nitrogen atom, for enzyme inhibition.

2. Results and discussion

The synthesis of 2-aryl-1-benzylbenzimidazoles was accomplished by reacting a 1,2-phenylenediamine with an excess of an aromatic aldehyde in the presence of a catalytic amount of *p*-toluenesulfonic acid, as shown in Scheme 1.



Comp	R^1	R^2	Ar
1	H	H	2-FC_6H_4
2	H	H	2,6-$F_2C_6H_3$
3	H	COOH	2,6-$F_2C_6H_3$
4	CH_3	CH_3	2,6-$F_2C_6H_3$
5	CH_3	H	2,6-$F_2C_6H_3$
6	H	CH_3	2,6-$F_2C_6H_3$

Scheme 1.

The reaction proceeds through the formation of 2-arylbenzimidazoline, generated in situ from 1,2-phenylenediamine and the appropriate aromatic aldehyde. Thanks to the well-known reducing ability exhibited by this compound [7], the aromatic aldehyde is converted into the corresponding alcohol which in turn alkylates the 2-arylbenzimidazole formed in this redox process.

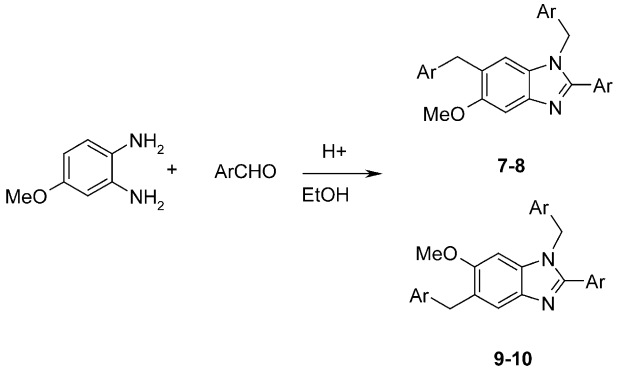
When the aromatic diamine employed is strongly activated by the presence of a methoxy substituent at position 4, a Friedel–Craft alkylation also occurs and the 2-aryl-1-benzylbenzimidazoles 5 or 6-benzyl substituted were isolated as the main products of the reaction (Scheme 2).

The structural assignment of compounds **3** and **7**–**10** has been carried out on the basis of long-range heteronuclear chemical shift correlation (LR-HETCOR) experiments.

The key entry point is the identification of the C-7a resonance which is assigned on the basis of its correlation with the *N*-benzyl methylene protons. In compound **3** this carbon is long-range correlated only to H-4 resonance, so indicating that the carboxylic group is linked to C-6.

In derivatives **7**–**10**, the relative position of the two substituents on the benzene fused ring has been established on the basis of the presence, in the spectra of **7**–**8**, of cross-peaks between the CH_2-6 and C-5 and C-7, whereas in derivatives **9**–**10** the same methylene protons are long-range coupled with C-4 and C-6.

The antiviral activities of the synthesized compounds **1**–**10** were evaluated in MT-4 cells acutely infected with HIV-1 (III_B) or HIV-2 (ROD) and compared with TBZ.



Comp	Ar
7	2,6-$F_2C_6H_3$
8	2-Cl,6-FC_6H_3
9	2,6-$F_2C_6H_3$
10	2-Cl,6-FC_6H_3

Scheme 2.

Table 1
Anti-HIV activity and cytotoxicity for compounds **1**–**10**

Comp.	EC_{50} [a] (μM)		CC_{50} [b] (μM)
	HIV-1_{IIIb}	HIV-2_{ROD}	
1	2.62±0.25	> 23.33	23.33±2.81
2	2.01±0.47	> 33.63	33.63±7.02
3	> 116.66	> 116.66	116.66±12.0
4	> 6.15	> 6.15	6.15±0.39
5	4.89±1.32	> 31.95	31.95±4.01
6	7.35±2.16	> 30.45	30.45±3.42
7	31.22±2.83	> 31.35	31.35±2.75
8	> 18.75	> 18.75	18.75±2.85
9	> 5.40	> 5.40	5.40±2.05
10	> 126.67	> 126.67	126.67±33.81
TBZ	0.60±0.5	> 48.60	48.60±5.5

[a] Concentration required to reduce HIV-induced cytopathic effect by 50%.
[b] Concentration required to reduce MT-4 cell viability by 50%.

The ability of the structural changes introduced in the benzimidazole system to influence the anti-HIV activity is shown in Table 1.

Derivatives **1**, **2**, **5** and **6** were found to selectively inhibit HIV-1 (III_B) replication whereas, like other NNRTIs, none of the compounds inhibited the replication of HIV-2 (ROD) in MT-4 cells.

The biological results obtained suggest that in this series of compounds the anti-HIV activity is maintained when the benzene-fused ring is not substituted, whereas the substitution in position 5 and/or 6 with bulky group negatively influences the biological activity. However, new tested compounds were found to have low efficacy. They were much less potent than their structural congener TBZ here used as reference compound (Table 1).

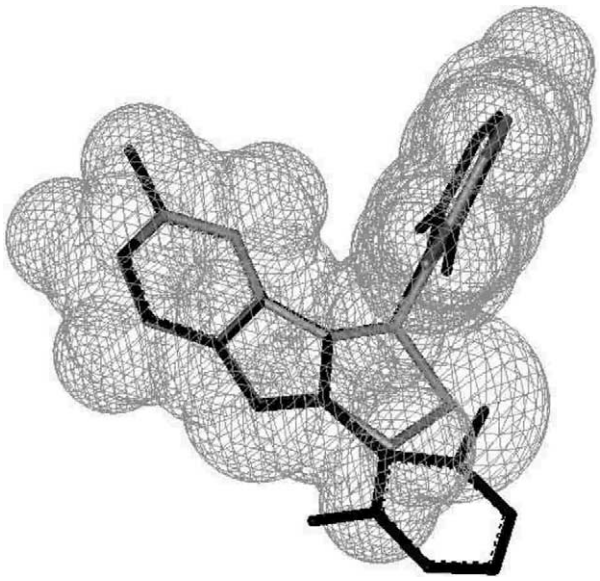


Fig. 2. Superimposition of TBZ (grey) and **6** (black) resulting from QXP fitting.

On the basis of our previous modeling studies on TBZ derivatives and other NNRTIs [5], the 3D structure of new benzimidazole derivatives was compared with that of TBZ aiming at the maximum overlap of the most plausible pharmacophoric elements, with the purpose to rationalize the lack of activity observed for benzimidazole derivatives **1**–**10**.

The molecular model of compound **6** was constructed using the SYBYL 6.7 molecular modeling software [8] and fitted with TBZ. As seen in Fig. 2, considerable similarity exists between the energy minimized butterfly-like shape of TBZ and **6**.

The obtained results suggest that also the new compounds could bind to RT assuming a butterfly-like conformation essential for enzyme inhibition, but the extra phenyl ring at C-2 probably enhance too much the steric hindrance to well accommodate into the apolar pocket of RT.

3. Experimental

3.1. Chemistry

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. Elemental analyses (C, H, N) were carried out on a Carlo Erba Model 1106 elemental analyzer and the results are within ±0.4% of the theoretical values. Merck silica gel 60 F_{254} plates were used for analytical TLC; column chromatography was performed on Merck silica gel 60 (230–400 mesh). ^{1}H NMR spectra were recorded in $CDCl_3$ on a Varian Gemini-300 spectrometer. Chemical shifts were expressed in δ (ppm) relative to TMS as internal standard and coupling constants (J) in Hz. HETCOR and LR-HETCOR experiments were carried out by using the standard software package.

3.1.1. Synthesis of 1-benzyl-2-arylbenzimidazoles (*1*–*10*)

To a stirred solution of 1,2-phenylenediamine (8 mmol) in ethanol (80 ml), the appropriate aromatic aldehyde (16 mmol) and *p*-toluenesulfonic acid (16 mmol) were added. The reaction mixture was refluxed for 2 h, then diluted with water and extracted with chloroform. The collected organic phases were dried over sodium sulfate and the solvent evaporated under reduced pressure to give an oily residue which was powdered by treatment with a mixture of ethanol and diethyl ether to afford compounds **4**, **5** and **7**, or separated by silica gel column chromatography eluting with cyclohexane/ethyl acetate 4:6. All compounds were recrystallized from ethanol.

*3.1.2. 1-(2′-Fluorobenzyl)-2-(2′-fluorophenyl)-benzimidazole (**1**)*

Yield 34%, m.p. 86–88 °C. ^{1}H NMR: 5.38 (s, 2H, CH_2), 6.73–7.90 (m, 12H, ArH). ^{13}C NMR: 42.05 (CH_2), 110.38 (C-7), 115.23 and 115.91 (C-3′, C-3″), 118.26 and 122.79 (C-1′, C-1″), 119.99 (C-4), 122.50 and 123.13 (C-5, C-6), 124.20, 124.56, 128.20, 129.38, 132.04, 132.17 (C-4′, C-4″, C-5′, C-5″, C-6′, C-6″), 135.06 (C-7a), 143.13 (C-2), 149.15 (C-3a), 158.29 and 161.58 (C-2′, C-2″).

*3.1.3. 1-(2′,6′-Difluorobenzyl)-2-(2′,6′-difluorophenyl)-benzimidazole (**2**)*

Yield 20%, m.p. 103–105 °C. ^{1}H NMR: 5.38 (s, 2H, CH_2), 6.79–7.87 (m, 10H, ArH).

*3.1.4. 6-Carboxy-1-(2′,6′-difluorobenzyl)-2-(2′,6′-difluorophenyl)-benzimidazole (**3**)*

Yield 10%, m.p. 223 °C dec. ^{1}H NMR: 5.45 (s, 2H, CH_2), 6.80–8.39 (m, 9H, ArH). ^{13}C NMR: 36.69 (CH_2), 108.05 and 110.90 (C-1′ and C-1″), 111.51 and 111.91 (C-3′, C-5′, C-3″, C-5″), 113.19 (C-7), 120.10 (C-4), 124.56 (C-5), 124.66 (C-2), 130.92 and 132.82 (C-4′, C-4″), 134.48 (C-7a), 146.18 (C-2), 146.81 (C-3a), 160.97, 161.05, 161.18, 161.27 (C-2′, C-2″, C-6′, C-6″), 171.73 (C=O).

*3.1.5. 1-(2′,6′-Difluorobenzyl)-2-(2′,6′-difluorophenyl)-5,6-dimethyl-benzimidazole (**4**)*

Yield 70%, m.p. 189–191 °C. ^{1}H NMR: 2.36 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 5.31 (s, 2H, CH_2), 6.76–7.58 (m, 8H, ArH).

*3.1.6. 1-(2′,6′-Difluorobenzyl)-2-(2′,6′-difluorophenyl)-5-methyl-benzimidazole (**5**)*

Yield 27%, m.p. 136–138 °C. ^{1}H NMR: 2.47 (s, 3H, CH_3), 5.33 (s, 2H, CH_2), 6.76–7.62 (m, 9H, ArH).

*3.1.7. 1-(2′,6′-difluorobenzyl)-2-(2′,6′-difluorophenyl)-6-methyl-benzimidazole (**6**)*

Yield 34%, m.p. 143–145 °C. ^{1}H NMR: 2.48 (s, 3H, CH_3), 5.33 (s, 2H, CH_2), 6.77–7.72 (m, 9H, ArH).

*3.1.8. 1,6-(2′,6′-Difluorobenzyl)- 2-(2′,6′-difluorophenyl)-5-methoxybenzimidazole (**7**)*

Yield 14%, m.p. 192–196 °C. ^{1}H NMR: 3.94 (s, 3H, OCH_3), 4.13 (s, 2H, CH_2), 5.24 (s, 2H, CH_2), 6.65–7.56 (m, 11H, ArH). ^{13}C NMR: 22.78 (CH_2-6), 36.37 (CH_2-N), 52.72 (OCH_3), 91.42 (C-7), 111.01, 111.24, 111.40, 111.56, 111.75, 111.84 (C-3′. C-5′, C-3″, C-5″, C-3‴, C-5‴), 116.08 (C-1′, C-1″, C-1‴), 120.03 (C-4), 123.70 (C-6), 127.84, 130.67, 132.00 (C-4′, C-4″, C-4‴), 133.79 (C-7a), 137.23 (C-3a), 141.51 (C-2), 155.03 (C-5), 159.96, 160.54, 160.85, 161.40, 161.58, 161.86 (C-2′, C-2″, C-2‴, C-6′, C-6″, C-6‴).

*3.1.9. 1,6-(2′-Chloro-6′-fluorobenzyl)-2-(2′-chloro-6′-fluorophenyl)-5-methoxybenzimidazole (**8**)*

Yield 23%, m.p. 223–225 °C. ^{1}H NMR: 3.94 (s, 3H, OCH_3), 4.19 (s, 2H, CH_2), 5.19 (d, 1H, H_2), 5.29 (d, 1H, H_2), 6.59–7.44 (m, 11H, ArH).

*3.1.10. 1,5-(2′,6′-Difluorobenzyl)- 2-(2′,6′-difluorophenyl)-6-methoxybenzimidazole (**9**)*

Yield 15%, m.p. 169–171 °C. ^{1}H NMR: 3.88 (s, 3H, OCH_3), 4.08 (s, 2H, CH_2), 5.29 (s, 2H, CH_2), 6.77–7.45 (m, 11H, ArH). ^{13}C NMR: 22.69 (CH_2–5), 36.29 (CH_2–N), 55.61 (OCH_3), 91.27 (C-7), 110.88, 111.12, 111.31, 111.52, 111.67, 111.83 (C-3′, C-3″, C-3‴, C-5′, C-5″, C-5‴), 116.10 (C-1′), 119.87 (C-4), 123.53 (C-5), 127.84, 130.68, 132.00 (C-4′, C-4″, C-4‴), 133.64 (C-7a), 137.74 (C-3a), 141.80 (C-2), 154.84 (C-6), 160.78, 160.86,161.02, 161.15, 161.20, 161.22 (C-2′, C-2″, C-2‴, C-6′, C-6″, C-6‴).

*3.1.11. 1,5-(2′-Chloro-6′-fluorobenzyl)-2-(2′-chloro-6′-fluorophenyl)-6-methoxybenzimidazole **10***

Yield 7%, m.p. 195–199 °C. ^{1}H NMR: 3.88 (s, 3H, OCH_3), 4.14 (s, 2H, CH_2), 5.22 (d, 1H, H_2), 5.31 (d, 1H, H_2), 6.63–7.49 (m, 11H, ArH).

3.2. Pharmacology

The antiviral experiments using MT-4 cells and HIV-1 (III_B) and HIV-2 (ROD) strains were performed following procedures that have already been described [9,10].

Acknowledgements

These investigations were supported in part by the Ministero dell'Istruzione, dell'Università e della Ricerca (COFIN 2000).

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