Potent Antifungal and Antistaphylococcal 2-Anilinobenzimidazoles

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A series of 21 anilinobenzimidazoles with a 2,4-difluorophenyl group were synthesized and their minimum inhibitory concentrations (MIC) determined by the tube dilution method. Most of the compounds exhibited excellent MIC values against *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA). Among them, compound **27** having dibromo substitution at the 4- and 6-position of the benzimidazole ring showed the best antifungal activity against *Candida krusei* with a MIC value of 3.12 μg/mL that surpassed that of the reference drug fluconazole.

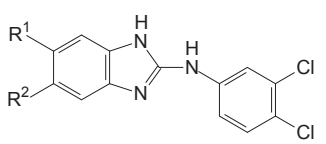
Key words: Anilinobenzimidazoles, Antistaphylococcal Activity, Antifungal Activity

Introduction

Previously, we have reported the synthesis and very potent antistaphylococcal activities of a series of 2-anilinobenzimidazole derivatives (Özden *et al.*, 2008). Compounds **I** and **II** (Fig. 1) were identified as the most active agents against *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA), with MIC values of 0.095 and 0.19 μg/mL, respectively. According to our preliminary results, these compounds exhibited good *in vitro* antifungal activity as well, particularly against *Candida krusei*.

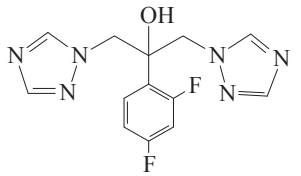
C. krusei is a well known fungal pathogen in patients with hematologic malignancies as well as in transplant recipients. It is naturally resistant against some of the antifungal azoles such as fluconazole. The mortality rate due to *C. krusei* fungemia is much higher than that due to more common *C. albicans* infections. Therefore it is urgently needed to discover new agents that will be effective against *C. krusei*.

The use of classical antifungal therapies has been limited due to toxicity, low efficacy, and resistance. New formulations and structural modifications have been developed which increase the absorption and efficacy. These new agents may provide additional options for the treatment of superficial fungal infections, and they may help to overcome the limitations of current treatments (Gupta and Tomas, 2003). Among them, three new triazole drugs, *viz.* voriconazole, ravuconazole, and posaconazole (Fig. 2), with broad-spectrum activity, have been developed and may be of use in both systemic and superficial fungal infections. Voriconazole has a high bioavailability and has been successfully used with immunocompromised patients, including those infected with *C. krusei*. Ravuconazole is active against *Candida* spp., *Cryptococcus neoformans*, *Aspergillus fumigates* and dermatophytes. Posaconazole shows excellent activity against *Candida* spp., including *C. krusei*, *C. glabrata*, *C. guilliermondii*, and *C. dubliniensis*, all of which are inherently less susceptible to fluconazole. All of these triazole derivatives have a 2,4-difluorophenyl ring, which is a very effective pharmacophore group for good antifungal activity. In studies aimed at the development of fluconazole derivatives, it has been reported that compounds with a 2,4-difluorophenyl group showed the best *in vivo* antifungal activity (Jiang *et al.*, 2011; Kagoshima and Konosu, 2006).

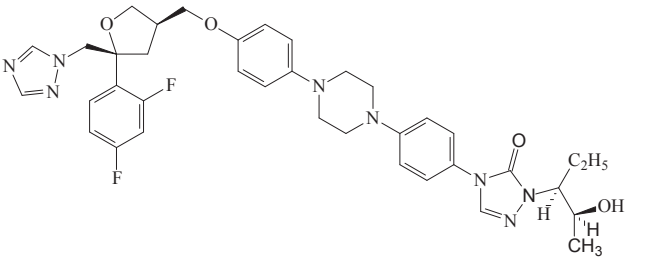


I : R^1 = H R^2 = Br **II** : R^1 = R^2 = Cl

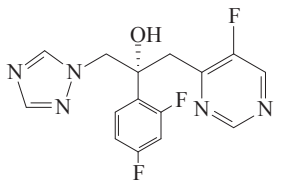
Fig. 1. Potent antistaphylococcal anilinobenzimidazoles.



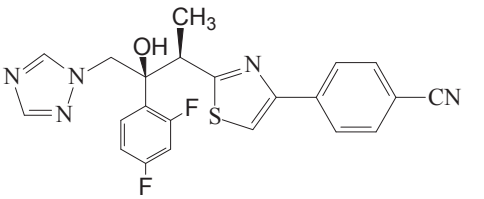
Fluconazole



Posaconazole



Voriconazole



Ravuconazole

Fig. 2. Fluconazole and other new antifungal triazoles used in therapy.

In the present study, we have designed a series of novel anilinobenzimidazoles, containing a difluorophenyl group, with the aim to find an antifungal spectrum against *Candida* species.

Results and Discussion

Chemical syntheses

Syntheses of non-commercial starting materials were carried out according to methods described in the literature (Scheme 1). 4,5-Dibromo-*o*-phenylenediamine (**2**) was prepared via the acylation of *o*-phenylenediamine with *p*-tolylsulfonyl chloride, followed by addition of bromine at 0–5 °C. Finally, hydrolyzation of **1** with concentrated H_2SO_4 afforded the desired compound **2** (Cheeseman, 1962). 3,6-Dichloro-*o*-phenylenediamine (**3**) was prepared by nitration of 1,4-dichlorobenzene followed by reduction with $Sn/SnCl_2$ (Baudy *et al.*, 1993). Reduction of 2,4-dibromo-6-nitroaniline by Zn/acetic acid gave 3,5-dibromo-*o*-phenylenediamine (**4**) under moderate conditions (Scott and Tomlinson, 1959).

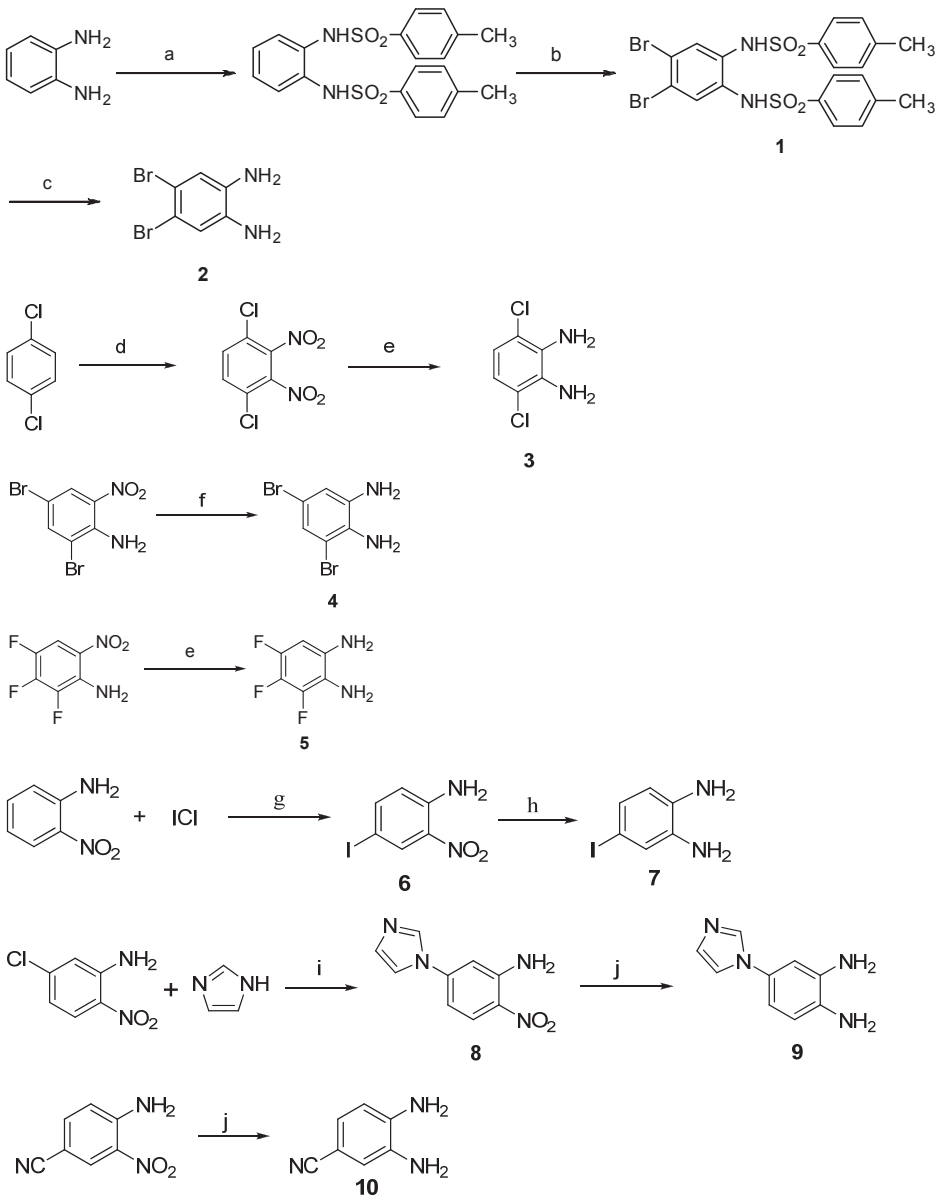
Similarly, 3,4,5-trifluoro-*o*-phenylenediamine (**5**) was prepared from its nitro derivative (Keana *et al.*, 1995). Heating of 2-nitroaniline with iodine monochloride in anhydrous sodium acetate/glacial acetic acid gave compound **6** (Price *et al.*, 2003), which was reduced to 4-iodo-*o*-phenylenediamine (**7**) (Wilson and Hunt, 1983). 5-Imidazol-1-yl-2-nitroaniline (**8**) was prepared by the reaction of 5-chloro-2-nitroaniline with imidazole in DMSO/KOH. Reduction of this compound with H_2/Pd-C afforded 4-imidazol-1-yl-*o*-phenylenediamine (**9**) (Güngör *et al.*, 1992). Using similar catalytic hydrogenation conditions, 4-amino-3-nitrobenzonitrile was converted to 3,4-diaminobenzonitrile (**10**) (Fairley *et al.*, 1993).

The target anilinobenzimidazoles were synthesized using the three different methods given in Scheme 2:

Method A: Synthesis of 2-*N*-(substituted) aminobenzimidazoles utilizing CuCl-promoted intramolecular cyclization of *N*-(2-aminoaryl)thioureas (Wang *et al.*, 2004).

Method B: Cyclization using an ethyl carbodiimide polymer (EDC) polymer (Cee and Downing, 2006).

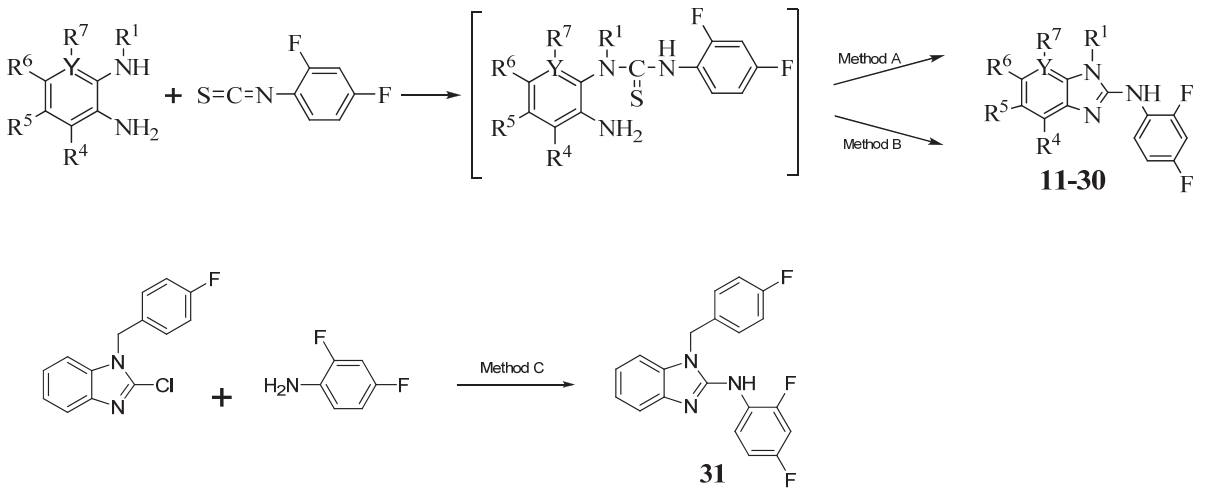
Method C: Heating of 1-(4-fluorobenzyl)-2-chlorobenzimidazole with 2,4-difluoroaniline at high temperature (Tunçbilek *et al.*, 1997).



Scheme 1. Syntheses of non-commercial starting materials **1**–**10**. Reagents: a) *p*-Tolylsulfonyl chloride; b) Br_2; c) concentrated H_2SO_4; d) HNO_3/H_2SO_4; e) $Sn/SnCl_2$; f) Zn/CH_3COOH; g) CH_3COOH/CH_3COONa; h) $SnCl_2$; i) DMSO/KOH; j) H_2/Pd-C.

Antimicrobial activities

The *in vitro* antifungal and antistaphylococcal activities of the synthesized compounds were evaluated against two pathogenic fungi, *viz. C. albicans* ATCC 10231 and *C. krusei* ATCC 6258, and against two bacteria, *viz. S. aureus* ATCC 25923 and methicillin-resistant *S. aureus* ATCC 43300 (MRSA). Sultamicillin, ampicillin, fluconazole, and miconazole were used as reference compounds. The *in vitro* minimal inhibitory concentrations (MIC_{100}) of the compounds were determined using the microbroth dilution method (Jorgensen *et al.*, 1999; Shadomy and Pfaller, 1991). Compounds **1**–**31** and the reference drugs



Scheme 2. Syntheses of anilinobenzimidazoles **11**–**31**. Reagents: Method A, CuCl, iPr_2NEt, toluene, CH_3CN; Method B, EDC/THF; Method C, DMF.

were dissolved in DMSO/H_2O (50:50, v/v), at a concentration of 200 μg/mL. The concentration was adjusted to 50 μg/mL by fourfold dilution with growth medium and bacterial solution in the first tube. Data were not recorded for the initial solution, because of the high DMSO content (12.5%). Tubes containing bacteria or fungi were incubated at 36 °C for 18 h and 48 h, respectively. MIC_{100} values are summarized in Table I.

As mentioned earlier, these compounds were prepared for their promising antifungal activities. However, they exhibited better antistaphylococcal rather than antifungal activities. Among compounds **11**–**31**, compound **27** with bromine atoms at the 4- and 6-position of the benzimidazole ring showed the best antifungal activity against *C. krusei* with a MIC value of 3.12 μg/mL, surpassing that of fluconazole. It is well known that fluconazole has no inhibitory effect against *C. krusei* at 25 μg/mL. Additionally, none of the synthesized compounds had significant inhibitory activity against *C. albicans*. Thus, in contrast to our expectations, substitution of the benzimidazoles with 2,4-difluoroaniline at C-2 is not beneficial for the antifungal activity.

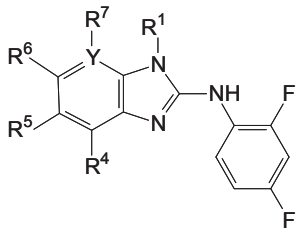
Most of the compounds were active against both *S. aureus* and MRSA. The activities of **22** and **29** were equal to that of ampicillin against *S. aureus* (0.39 μg/mL). Compounds **19**, **24**–**28** had very potent activity against both *S. aureus* and MRSA, with MIC values of 0.78–1.56 μg/mL. Halogen and CF_3 substitution of the benzimidazole ring increased the antibacterial activity. Additionally, while ampicillin and sultamicillin were practically inactive against MRSA (25 μg/mL), the anilinobenzimidazoles which were effective against *S. aureus* were also active against MRSA. This is a significant advantage over the clinically used drugs, since vancomycin is the only antibiotic that is effective against MRSA infections. Antibacterial activity completely disappeared with the substitution of the N^1-imidazole ring with the *p*-fluorobenzyl group, as exemplified for **31**. This finding suggests that the tautomeric NH group of the imidazole ring could be important for a potent antistaphylococcal effect.

Experimental

General

Melting points were measured with a capillary melting point apparatus (Büchi Labortechnik, Flawil, Switzerland) and were uncorrected. The ^{1}H NMR (400 MHz) and ^{19}F NMR (376.5 MHz) spectra were recorded on Varian Mercury 400 MHz FT-NMR spectrophotometers (Varian Inc., Palo Alto, CA, USA). Coupling constants (*J*) are given in Hertz (Hz). LC/MS analyses were performed with Waters Alliance and Micromass ZQ instruments (Waters Corporation, Milford, MA, USA), using the ESI(+) mode. Elemental analyses were taken on a Leco 932 CHNS-O analyzer (Leco, St. Joseph, MI, USA). For the HCl salts of the synthesized compounds, the free bases were dissolved in

Table I. MIC_{100} values of the references and compounds **11**–**31**.



Compound	Y	R^1	R^4	R^5	R^6	R^7	Method	MIC_{100} [μg/mL]			
								S. aureus	MRSA	*C. albicans*	*C. krusei*
11	C	H	H	H	H	H	A	>50	>50	25	25
12	C	H	H	F	H	H	A	25	12.5	25	25
13	C	H	H	Cl	H	H	A	12.5	6.25	25	25
14	C	H	H	Br	H	H	A	6.25	6.25	25	25
15	N	H	H	Br	H	H	B	>50	>50	25	25
16	C	H	H	I	H	H	A	3.12	3.12	12.5	25
17	C	H	H	NO_2	H	H	A	6.25	3.12	25	25
18	C	H	H	CN	H	H	A	50	50	25	25
19	C	H	H	CF_3	H	H	A	1.56	1.56	25	25
20	C	H	H	$COOCH_3$	H	H	B	50	50	25	25
21	C	H	H	imidazol-1-yl	H	H	B	50	50	25	25
22	C	H	H	Cl	Cl	H	A	0.39	0.39	25	25
23	C	H	Cl	H	H	Cl	B	>50	>50	25	25
24	C	H	Cl	H	Cl	H	B	0.78	0.78	12.5	6.25
25	C	H	H	H	Cl	Cl	B	1.56	0.78	12.5	6.25
26	C	H	H	Br	Br	H	A	0.78	3.12	25	25
27	C	H	Br	H	Br	H	B	0.78	0.78	6.25	3.12
28	C	H	CH_3	CH_3	Br	H	A	1.56	1.56	12.5	25
29	C	H	H	CF_3	H	Br	A	0.39	0.39	6.25	12.5
30	C	H	F	F	F	H	B	>50	>50	25	25
31	C	*p*-fluorobenzyl	H	H	H	H	C	>50	>50	25	25
Ampicillin								0.39	25	-	-
Sultamicillin								0.39	25	-	-
Miconazole								-	-	0.19	0.78
Fluconazole								-	-	1.56	25

ethanol and dry HCl gas was passed through the solution. The salts frequently contained fractional moles of solvation water and/or ethanol; for compounds **14**, **16**, **17**, **19**, and **22**, ^{1}H NMR spectroscopy confirmed the presence of solvent.

Method A

A mixture of the corresponding *o*-phenylenediamine (4 mmol) and substituted phenylisothiocyanate (4 mmol) was heated under reflux in acetonitrile (2.7 mL) and toluene (1 mL) for 15 min. To this mixture cellite (0.4 g), *N*-ethyldiisopropylamine (2 mL), and CuCl (0.9 g) were added. The resulting mixture was heated to 80 °C and kept at this temperature for 1 h. The reaction mixture was then cooled to 40 °C and filtered at this temperature. The filtrate was extracted with 7% ammonium hydroxide and dried over anhydrous Na_2SO_4; then the solvent was evaporated. The residue was purified by column chromatography on silica gel with a mixture of ethyl acetate/*n*-hexane in the appropriate ratio.

Method B

A mixture of the corresponding *o*-phenylenediamine (4 mmol), substituted phenylisothiocyanate (4 mmol) and 1.590 g of 1-(3-dimetylaminopropyl)-3-ethyl carbodiimide polymer (EDC) in THF

(15 mL) was heated in a sealed tube at 70 °C, for 3 h. The reaction mixture was then cooled, filtered, and the solvent evaporated.

Method C

A mixture of 1-(*p*-flourobenzyl)-2-chloro-1*H*-benzimidazole (1 mmol) and 2,4-difluoroaniline (1 mmol) in DMF (0.8 mL) was heated under reflux for 18 h at 140 °C. The reaction mixture was cooled, then sodium hydroxide solution (20%) was added, and extracted with ethyl acetate.

2-(2,4-Difluoroanilino)-1H-benzimidazole hydrochloride (**11**), Method A: The crude product was purified by chromatography on silica gel (EtOAc/*n*-hexane 1:3, v/v). – Yield 11.3%. – M.p. 216 – 218 °C. – ^{1}H NMR (DMSO-d_6): δ = 7.23–7.28 (3H, m, H-3‘, 4, 7), 7.39–7.43 (2H, m, H-5, 6), 7.52–7.57 (1H, m, H-5‘), 7.65–7.71 (1H, m, H-6‘). – ^{19}F NMR (CD_3OD): δ = –110.77, –119.04. – MS (ESI): *m/z* = 246 [M+H]$^+$ (100). – $C_{13}H_9F_2N_3 \cdot HCl \cdot 0.75H_2O$: calcd. C 52.8, H 3.90, N 14.2; found C 52.71, H 4.04, N 13.9.

5(6)-Fluoro-2-(2,4-difluoroanilino)-1H-benzimidazole (**12**), Method A: The crude product was purified by chromatography on silica gel (EtOAc/*n*-hexane 1:3). – Yield 26.2%. – M.p. 220–224 °C. – ^{1}H NMR (DMSO-d_6): δ = 7.07–7.12 (1H, m, H-3‘), 7.22–7.23 (1H, m, H-4), 7.24 – 7.27 (1H, dd, J_o = 8.8 Hz, J_m = 2.4 Hz, H-6), 7.38–7.42 (1H, m, H-7), 7.50–7.56 (1H, m, H-5‘), 7.69–7.755 (1H, m, H-6‘). – MS (ESI): *m/z* = 246 [M+H]$^+$ (100). – $C_{13}H_8F_3N_3$: calcd. C 59.32, H 3.06, N 15.96; found C 59.37, H 3.05, N 15.50.

5(6)-Chloro-2-(2,4-difluoroanilino)-1H-benzimidazole (**13**) (Özden *et al.*, 2008), Method A: The crude product was purified by chromatography on silica gel (EtOAc/*n*-hexane 1:3). – Yield 19.1%. – M.p. 195–199 °C. – ^{1}H NMR (CD_3OD): δ = 7.04–7.09 (1H, m, H-3‘), 7.14–7.19 (2H, m, H-6, 5‘), 7.30–7.35 (2H, m, H-4, 7), 7.76–7.82 (1H, m, H-6‘). – ^{19}F NMR (CD_3OD): δ = –118.44, –125.06. – MS (ESI): *m/z* = 280 [M+H]$^+$ (100), 282 [M+H+2]$^+$ (32). – $C_{13}H_8ClF_2N_3$: calcd. C 55.83, H 2.88, N 15.02; found C 56.00, H 2.71, N 14.93.

5(6)-Bromo-2-(2,4-difluoroanilino)-1H-benzimidazole hydrochloride (**14**), Method A: The crude product was purified by chromatography on silica gel (EtOAc/*n*-hexane 1:3). – Yield 12.5%. – M.p. 234–237 °C. – ^{1}H NMR (CD_3OD): δ = 7.05–7.11 (1H, m, H-3‘), 7.15–7.21 (1H, m, H-5‘), 7.26–7.33 (2H, m, H-6, 7), 7.51 (1H, s, H-4), 7.73–7.79 (1H, m, H-6‘). – MS (ESI): *m/z* = 324 [M+H]$^+$ (100), 326 [M+H+2]$^+$ (98). – $C_{13}H_8BrF_2N_3 \cdot HCl \cdot 0.2C_2H_5OH$: calcd. C 43.52, H 2.78, N 11.36; found C 43.03, H 3.05, N 10.97.

6-Bromo-2-(2,4-difluoroanilino)-3H-imidazo-[4,5-b]pyridine (**15**), Method B: The crude product was extracted with ethylacetate. – Yield 26.7%. – M.p. 352–356 °C. – ^{1}H NMR (DMSO-d_6): δ = 7.11–7.16 (1H, m, H-3‘), 7.29–7.36 (1H, m, H-5‘), 7.82 (1H, d, J_m = 2.4 Hz, H-5), 8.11 (1H, d, J_m = 2 Hz, H-7), 8.28–8.30 (1H, m, H-6‘). – MS (ESI): *m/z* = 325 [M+H]$^+$ (100), 327 [M+H+2]$^+$ (98). – $C_{12}H_7BrF_2N_4$: calcd. C 44.33, H 2.17, N 17.23; found C 44.17, H 2.23, N 16.88.

5(6)-Iodo-2-(2,4-difluoroanilino)-1H-benzimidazole hydrochloride (**16**), Method A: The crude product was purified by chromatography on silica gel (EtOAc/*n*-hexane 1:3). – Yield 11.77%. – M.p. 233–236 °C. – ^{1}H NMR (DMSO-d_6): δ = 7.23–7.26 (2H, m, H-3‘, 7), 7.49–7.56 (2H, m, H-5‘, 6), 7.67–7.73 (2H, m, H-6‘, 4). – MS (ESI): *m/z* = 372 [M+H]$^+$ (100). – $C_{13}H_8F_2IN_3 \cdot HCl \cdot 0.4C_2H_5OH \cdot 0.2H_2O$: calcd. C 38.58, H 2.76, N 9.78; found C 38.30, H 2.43, N 9.96.

5(6)-Nitro-2-(2,4-difluoroanilino)-1H-benzimidazole hydrochloride (**17**), Method A: The crude product was purified by chromatography on silica gel (EtOAc/*n*-hexane 1:3). – Yield 15.96%. – M.p. 233–236 °C. – ^{1}H NMR (DMSO-d_6): δ = 7.26–7.29 (1H, m, H-3‘), 7.52–7.55 (1H, m, H-5‘), 7.59 (1H, d, J_o = 8.8 Hz, H-7), 7.88–7.94 (1H, m, H-6‘), 8.14–8.17 (1H, dd, J_o = 8.4 Hz, J_m = 2 Hz, H-6), 8.23 (1H, d, J_m = 2 Hz, H-4). – ^{19}F-NMR (CD_3OD): δ = –109.94, –118.80. – MS (ESI): *m/z* = 291 [M+H]$^+$ (100). – $C_{13}H_8F_2N_4O_2 \cdot 1.5HCl \cdot 0.2C_2H_5OH$: calcd. C 45.44, H 3.04, N 15.82; found C 45.68, H 2.83, N 15.50.

2-(2,4-Difluoroanilino)-1H-benzimidazol-5-carbonitrile hydrochloride (**18**), Method A: The crude product was purified by chromatography on silica gel (EtOAc/*n*-hexane 1:3). – Yield 14.5%. – M.p. 231–233 °C. – ^{1}H NMR (DMSO-d_6): δ = 7.25–7.29 (1H, m, H-3‘), 7.52–7.58 (2H, m, H-6, 5‘), 7.68 (1H, d, J_o = 8.4 Hz, H-7), 7.84–7.90 (2H, m, H-4, 6‘).– ^{19}F NMR (CD_3OD): δ = –110.10, –118.91. – MS (ESI): *m/z* = 271 [M+H]$^+$ (100). – $C_{14}H_8F_2N_4 \cdot 1.5HCl \cdot 0.2C_2H_5OH$: calcd. C 51.76, H 3.22, N 16.76; found C 51.71, H 3.23, N 16.31.

5(6)-Trifluoromethyl-2-(2,4-difluoroanilino)-1H-benzimidazole hydrochloride (**19**), Method A: The crude product was purified by chromatography on silica gel (EtOAc/*n*-hexane 1:3). – Yield 17.2%. – M.p. 211–215 °C. – ^{1}H NMR (DMSO-d_6): δ = 7.22–7.27 (1H, m, H-3‘), 7.49–7.55 (1H, m, H-5‘), 7.58 (2H, s, H-4, 6), 7.69 (1H, s, H-7), 7.73–7.79 (1H, m, H-6‘). – ^{19}F NMR (CD_3OD): δ = –62.96, –110.77, –119.06. – MS (ESI): *m/z* = 314 $[M+H]^+$ (100). – $C_{14}H_8F_5N_3 \cdot HCl \cdot 0.5C_2H_5OH$: calcd. C 48.33, H 3.24, N 11.27; found C 47.96, H 3.16, N 11.52.

2-(2,4-Difluoroanilino)-1H-benzimidazole-5(6)-methyl carboxylate (**20**), Method B: The crude product was recrystallized from cold dichloromethane. – Yield 38.5%. – M.p. 272–276 °C. – ^{1}H NMR (CD_3OD): δ = 3.89 (3H, s, -CH_3), 6.98–7.04 (1H, m, H-3‘), 7.06–7.12 (1H, m, H-5‘), 7.33 (1H, d, J_o = 8.4 Hz, H-7), 7.78–7.80 (1H, dd, J_o = 8.4 Hz, J_m = 1.6 Hz, H-6), 7.89–7.94 (1H, m, H-6‘), 7.96 (1H, s, H-4). – MS (ESI): *m/z* = 304 $[M+H]^+$ (100). – $C_{15}H_{11}O_2F_2N_3 \cdot 0.75H_2O$: calcd. C 56.87, H 3.97, N 13.26; found C 57.08, H 3.38, N 12.67.

2-(2,4-Difluoroanilino)-5(6)-(imidazol-1-yl)-1H-benzimidazole (**21**), Method B: The crude product was purified by chromatography on silica gel (EtOAc/*n*-hexane 1:5). – Yield 13.8%. – M.p. 241–245 °C. – ^{1}H NMR (DMSO-d_6): δ = 6.79–6.81 (1H, dd, J_o = 8.4 Hz, J_m = 2.4 Hz, H-6), 6.95–6.98 (1H, m, H-3‘), 7.01 (1H, s) 7.11–7.17 (2H, m), 7.23 (1H, d, J_m = 2 Hz, H-4), 7.49 (1H, s), 7.96 (1H, s, H-7), 8.63–8.68 (1H, m, H-6‘).– MS (ESI): *m/z* = 312 $[M+H]^+$ (100). – $C_{16}H_{11}F_2N_5 \cdot 0.25H_2O$: calcd. C 60.85, H 3.67, N 22.18; found C 61.34, H 3.97, N 21.59.

5,6-Dichloro-2-(2,4-difluoroanilino)-1H-benzimidazole hydrochloride (**22**), Method A: The crude product was purified by chromatography on silica gel (EtOAc/*n*-hexane 1:3), then dissolved in propanol, and recrystallized from diethyl ether. – Yield 12.2%. – M.p. 250–253 °C. – ^{1}H NMR (DMSO-d_6): δ = 7.24–7.28 (1H, m, H-3‘), 7.52–7.57 (1H, m, H-5‘), 7.64 (2H, s, H-4, 7), 7.81–7.87 (1H, m, H-6‘). – ^{19}F NMR (CD_3OD): δ = –110.27, –118.93. – MS (ESI): *m/z* = 314 $[M+H]^+$ (100), 316 $[M+H+2]^+$ (60), 318 $[M+H+4]^+$ (10). – $C_{13}H_7Cl_2F_2N_3 \cdot 1.5\ HCl \cdot 0.5\ H_2O$: calcd. C 41.29, H 2.51, N 11.11; found C 41.32, H 2.15, N 11.09.

4,7-Dichloro-2-(2,4-difluoroanilino)-1H-benzimidazole hydrochloride (**23**), Method B: The crude product was recrystallized from dichloromethane. – Yield 11.5%. – M.p. 260–263 °C. – ^{1}H NMR (acetone-d_6): δ = 7.07–7.18 (4H, m, H-5, 6, 3‘, 5‘), 8.47 (1H, s, exchangeable D_2O), 8.79–8.85 (1H, m, H-6‘), 10.8 (1H, s, exchangeable D_2O). – MS (ESI): *m/z* = 314 $[M+H]^+$ (100), 316 $[M+H+2]^+$ (70), 318 $[M+4]^+$ (12). – $C_{13}H_7Cl_2F_2N_3$: calcd. C 49.70, H 2.25, N 13.38; found C 49.22, H 2.34, N 13.70.

4,6-Dichloro-2-(2,4-difluoroanilino)-1H-benzimidazole (**24**), Method B: The crude product was purified by chromatography on silica gel (EtOAc/*n*-hexane 1:4). – Yield 23.3%. – M.p. 335–339 °C. – ^{1}H NMR (CD_3OD): δ = 7.16–7.21 (1H, m, H-3‘), 7.27–7.33 (1H, m, H-5‘), 7.38 (1H, d, J_m = 1.6 Hz, H-5), 7.43 (1H, d, J_m = 1.6 Hz, H-7), 7.63–7.69 (1H, m, H-6‘). – ^{19}F NMR (CD_3OD): *d* = –125.43, –118.49. – MS (ESI): *m/z* = 314 $[M+H]^+$ (100), 316 $[M+H+2]^+$ (75), 318 $[M+H+4]^+$ (15). – $C_{13}H_7Cl_2F_2N_3$: calcd. C 49.71, H 2.25, N 13.38; found C 49.97, H 2.51, N 13.19.

4,5-Dichloro-2-(2,4-difluoroanilino)-1H-benzimidazole (**25**), Method B: The crude product was purified by chromatography on silica gel (EtOAc/*n*-hexane 1:4). – Yield 12.4%. – M.p. 245–249 °C. – ^{1}H NMR (DMSO-d_6): δ = 7.12–7.18 (1H, m, H-3‘), 7.17 (1H, d, J_o = 8.8 Hz, H-6), 7.29 (1H, d, J_o = 8.8 Hz, H-7), 7.32–7.38 (1H, m, H-5‘), 8.39 – 8.46 (1H, m, H-6‘). – MS (ESI): *m/z* = 314 $[M+H]^+$ (100), 316 $[M+H+2]^+$ (60), 318 $[M+H+4]^+$ (10). – $C_{13}H_7Cl_2F_2N_3 \cdot 0.3\ H_2O$: calcd. C 48.86, H 2.39, N 13.15; found C 48.45, H 2.33, N 13.12.

5,6-Dibromo-2-(2,4-difluoroanilino)-1H-benzimidazole hydrochloride (**26**), Method A: The crude product was purified by chromatography on silica gel (EtOAc/*n*-hexane 1:3). – Yield 25%. – M.p. 257–260 °C. – ^{1}H NMR (DMSO-d_6): δ = 7.24–7.29 (1H, m, H-3‘), 7.51–7.57 (1H, m, H-5‘), 7.52 (2H, s, H-4, 7), 7.84–7.90 (1H, m, H-6‘). – MS (ESI): *m/z* = 402 $[M+H]^+$ (52), 404 $[M+H+2]^+$ (100), 406 $[M+H+4]^+$ (53). – $C_{13}H_7Br_2F_2N_3 \cdot HCl \cdot 0.5H_2O$: calcd. C 34.81, H 2.02, N 9.37; found C 34.69, H 1.89, N 9.50.

4,6-Dibromo-2-(2,4-difluoroanilino)-1H-benzimidazole (**27**), Method B: The crude product was purified by chromatography on silica gel (EtOAc/*n*-hexane 1:3). – Yield 13.5%. – M.p. 192–196 °C. – ^{1}H NMR (CD_3OD): δ = 7.17–7.21 (1H, m, H-3‘), 7.28–7.33 (1H, m, H-5‘), 7.50 (1H,

d, J_m = 1.6 Hz, H-5), 7.62–7.67 (1H, m, H-6‘), 7.70 (1H, d, J_m = 1.6 Hz, H-7). – MS (ESI): *m/z* = 402 $[M+H]^+$ (58), 404 $[M+H+2]^+$ (100), 406 $[M+H+4]^+$ (57). – $C_{13}H_7Br_2F_2N_3 \cdot 1.25H_2O$: calcd. C 36.69, H 2.25, N 9.87; found C 36.96, H 2.15, N 9.62.

6-Bromo-4,5-dimethyl-2-(2,4-difluoroanilino)-1H-benzimidazole hydrochloride (**28**), Method A: The crude product was purified by chromatography on silica gel (EtOAc/*n*-hexane 1:3). – Yield 10.3%. – M.p. 292–295 °C. – ^{1}H NMR (DMSO-d_6): δ = 2.23 (3H, s, -CH_3), 2.32 (3H, s, -CH_3), 7.12–7.15 (1H, m, H-3‘), 7.33 (1H, s, H-7), 7.38–7.44 (1H, m, H-5‘), 7.56–7.62 (1H, m, H-6‘). – MS (ESI): *m/z* = 352 $[M+H]^+$ (100), 354 $[M+H+2]^+$ (51). – $C_{15}H_{12}BrF_2N_3 \cdot HCl \cdot 0.5H_2O$: calcd. C 45.30, H 3.54, N 10.56; found C 45.83, H 3.89, N 10.71.

4(7)-Bromo-5(6)-trifluoromethyl-2-(2,4-difluoroanilino)-1H-benzimidazole hydrochloride (**29**), Method A: The crude product was purified by chromatography on silica gel (EtOAc/*n*-hexane 1:3). – Yield 12.3%. – M.p. 229–233 °C. – ^{1}H NMR (CD_3OD): δ = 7.17–7.22 (1H, m, H-3‘), 7.29–7.35 (1H, m, H-5‘), 7.66–7.72 (2H, m, H-5, 6‘), 7.84 (1H, s, H-7). – ^{19}F NMR (CD_3OD): δ = –63.26, –110.39, –119.07. – MS (ESI): *m/z* = 392 $[M+H]^+$ (100), 394 $[M+H+2]^+$ (98). – $C_{14}H_7BrF_5N_3 \cdot HCl$: calcd. C 39.23, H 1.88, N 9.80; found C 39.78, H 2.13, N 9.93.

4,5,6(5,6,7)-Trifluoro-2-(2,4-difluoroanilino)-1H-benzimidazole (**30**), Method B: The crude product was recrystallized from dicholoromethane. – Yield 45.5%. – M.p. 143–147 °C. – ^{1}H NMR (DMSO-d_6): δ = 7.10–7.15 (1H, m, H-3‘), 7.18–7.24 (1H, m, H-7), 7.27–7.33 (1H, m, H-5‘), 8.20–8.26 (1H, m, H-6‘). – ^{19}F NMR (CD_3OD): δ = –123.23, –130.00, –153.32, –159.57, –179.67. – MS (ESI): *m/z* = 300 $[M+H]^+$ (100). – $C_{13}H_6F_5N_3 \cdot H_2O$: calcd. C 49.22, H 2.54, N 13.25; found C 49.49, H 2.68, N 13.24.

1-(p-Fluorobenzyl)-2-(2,4-difluoroanilino)-1H-benzimidazole (**31**), Method C: The crude product was purified by chromatography on silica gel (EtOAc/*n*-hexane 1:3). – Yield 31.4%. – M.p. 165–168 °C. – ^{1}H NMR ($CDCl_3$): δ = 5.28 (2H, s, -CH_2-), 6.68 (1H, m, H-3‘), 6.86 (1H, m, H-5‘), 7.05 (2H, m, H-3‘‘, 5‘‘), 7.17–7.26 (5H, m,), 7.54 (1H, d, J_o = 7.6 Hz), 8.23–8.29 (1H, m, H-6‘). – ^{19}F NMR (CD_3OD): δ = –78.50, –112.44, –120.06. – MS (ESI): *m/z* = 354 $[M+H]^+$ (100). – $C_{20}H_{14}F_3N_3 \cdot 0.1H_2O$: calcd. C 67.63, H 4.03, N 11.83; found C 67.62, H 4.07, N 12.04.

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