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Synthesis of some new benzimidazolecarboxamides and evaluation of their antimicrobial activity

Hakan Göker [a,*], Meral Tunçbilek [a], Gülgün Ayhan [a], Nurten Altanlar [b]

[a] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ankara University, 06100 Tandogan, Ankara, Turkey*

[b] *Department of Microbiology, Faculty of Pharmacy, Ankara University, 06100 Tandogan, Ankara, Turkey*

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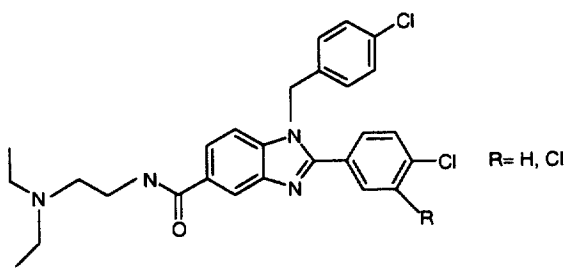
Abstract

A series of 1,2-disubstituted benzimidazole-5(6)-carboxamides was prepared and evaluated in vitro for antimicrobial activity against *Staphyloccus aureus*, *Escherichia coli* and *Candida albicans*. The precursor benzimidazolecarboxylic acids **4a–c** and **9a–c** were prepared via oxidative condensation of diaminobenzoic acids with aldehydes and via several steps over the 2(1*H*)-benzimidazolones, respectively. All acids were converted to their acyl chlorides with $SOCl_2$, then amidified with several *N,N'*-dialkylaminoethyl derivatives. Compounds **8a–c**, **20** and **22** exhibited the best activity.

Keywords: Benzimidazolecarboxamides; Antimicrobial activity

1. Introduction

We have already reported the synthesis and antimicrobial evaluation of a series of *N'*-(*N,N*-dialkylaminoethyl)-benzimidazole-5(6)-carboxamides [1]. The study revealed that compounds **I** exhibited potent antimicrobial activity. We planned to modify the structure of compounds **I** in order to find new antimicrobial agents.



I

2. Chemistry

In the present work, benzimidazole-5(6)-carboxylic acids **4a–c** were prepared by the method of Scheme 1 via oxidative condensation of **3a–c** and aldehydes with cupric ion [2]. Nucleophilic substitution of the methyl 4-chloro-3-nitrobenzoate with halogenated benzylamines gave **1a–c**. Their reduction with $NiCl_2$/Zn [3] produced **2a–c**. Alkaline hydrolysis of these compounds afforded **3a–c**. Compound **5** was prepared as previously reported by us [4] (Scheme 1). For the synthesis of 2-aminosubstituted benzimidazolecarboxylates (Scheme 2), the 2(1*H*)-benzimidazolones **6a,b** were prepared by the reaction of the corresponding *o*-phenylenediamines **2a,c**, with 1,1'-carbonyldiimidazole. These compounds were converted to 2-chlorinated derivatives **7a,b** with $POCl_3$/HCl.

The nucleophilic substitution of **7a,b** with several amines gave **8a–c**. Alkaline hydrolysis of these compounds afforded **9a–c**. All the benzimidazolecarboxylic acids were converted to acyl chlorides with $SOCl_2$, and dehydrohalogenation between the corresponding acyl chlorides and piperazine or ethylenediamines gave the required benzimidazolecarboxamides **10–25** (Table 1). In order to obtain water-soluble compounds, the HCl salts of the bases were prepared by bubbling dry hydrogen chloride in an isopropanol/ether solution of the base; the precipitated hydrochlorides were washed with ether and dried in vacuum to give a pure solid.

3. Experimental

Melting points were determined with a Buchi SMP-20 melting point apparatus and are uncorrected. All the instru-

* Corresponding author. Tel.: (90)-312-2220471; fax: (90)-312-213 10 81; e-mail: goker@pharmacy.ankara.edu.tr

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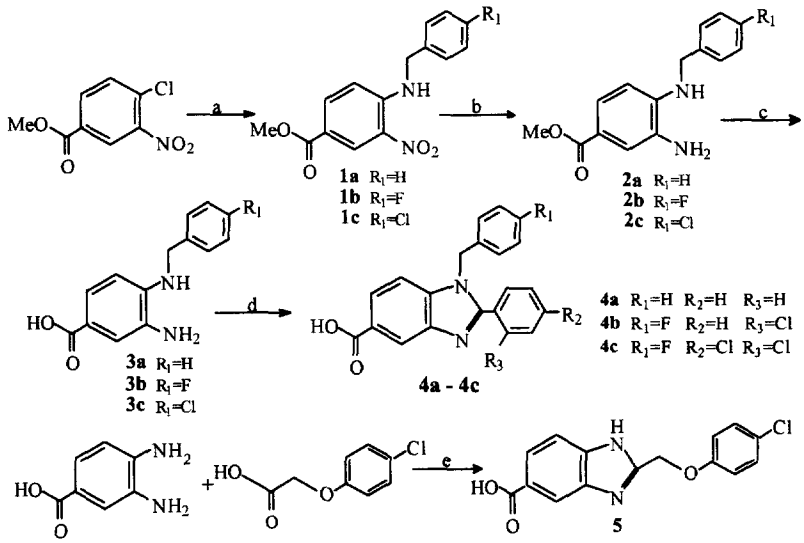
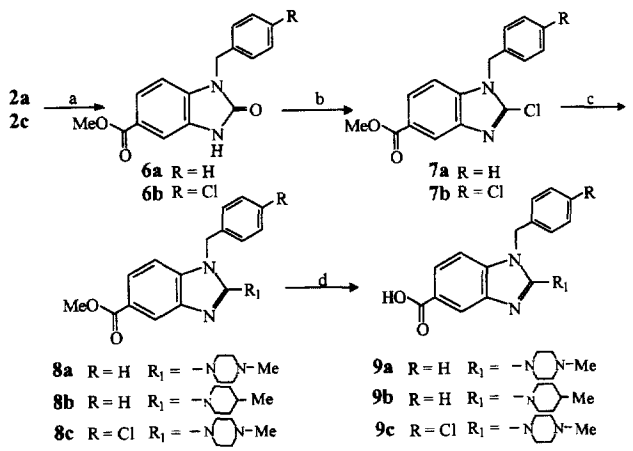


Scheme 1. Preparation of compounds **4a–c** and **5**. Reagents: (a) benzylamine or *p*-fluorobenzylamine or *p*-chlorobenzylamine; (b) $NiCl_2/Zn$; (c) NaOH/HOH; (d) Cu(II) acetate/H_2S, corresponding benzaldehydes; (e) 4NHCl.



Scheme 2. Synthesis of 2-aminosubstituted benzimidazolecarboxylates. Reagents: (a) 1,1′-carbonyldiimidazole; (b) $POCl_3$/HCl; (c) 1-methyl piperazine or 4-methyl piperidine; (d) NaOH/HOH.

mental analyses were performed by TUBITAK (Ankara) using the Bruker AC 400 NMR spectrophotometer, the VG Platform II mass spectrometer and the Leco CHNS 932 elemental analyzer. For the chromatographic analyses, Merck silica gel 60 (230–400 mesh ASTM) was used. Known intermediates were prepared according to the method cited for each of them: 2-(phenoxy)methyl-5-[1*H*]benzimidazolecarboxylic acid [4], methyl 3-nitro-4-(phenylmethyl)aminobenzoate **1a** [1], methyl 4-(*p*-chlorophenylmethyl)amino-3-nitrobenzoate m.p. 141°C (recrystallization from EtOH) **1c** [1], methyl 3-amino-4-phenylmethylaminobenzoate **2a** [1], methyl 3-amino-4-(*p*-chlorophenylmethyl)aminobenzoate m.p. 221° (recrystallization from EtOH) **2c** [1], 3-amino-4-phenylmethyl-amino benzoic acid **3a** [1], 3-amino-4-(*p*-chlorophenylmethyl)amino benzoic acid **3c** [1].

3.1. Methyl 4-(p-fluorophenylmethyl)amino-3-nitro benzoate ***1b***

p-Fluoro-benzylamine (9.6 mmol, 1.2 g) was added to a solution of methyl 4-chloro-3-nitrobenzoate (4.65 mmol, 1 g) in *N,N*-dimethylformamide (DMF) (2 ml) and heated for 5 h at 80°C. The mixture was allowed to cool and water was added. The resultant yellow precipitate was filtered and washed with water. M.p. 121–122°C, yellow in colour (1.15 g, yield 81.32%). *Anal.* (C, H, N) for $C_{15}H_{13}FN_2O_4$.

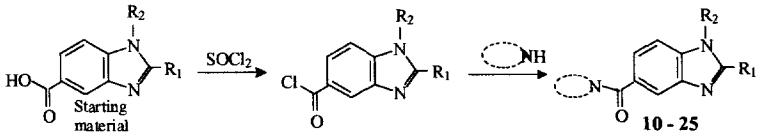
3.2. Methyl 3-amino-4-(p-fluorophenylmethyl)amino benzoate ***2b***

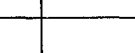
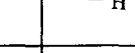
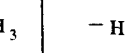
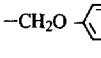
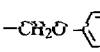
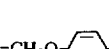
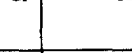
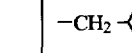
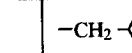
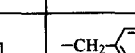
Compound **1b** (2.5 mmol, 0.76 g) and $NiCl_2 \cdot 6HOH$ (5 mmol, 1.2 g) were suspended in methanol (13 ml) and Zn (20 mmol, 1.3 g) was added in portions with stirring. The solution was then refluxed for 1 h. The precipitate was separated by filtration while hot, and washed with methanol. The filtrate and the washing were combined and the solvent was partially evaporated off and the product crystallized from EtOH, m.p. 151°C, light grey in colour (0.45 g, yield 65.6%). ^{1}H NMR($CDCl_3$): 3.31 (br s, 2H, $-NH_2$), 3.86(s, 3H, $COOCH_3$), 4.31 (s, 1H, $NHCH_2$), 4.38 (s, 2H, $NHCH_2$), 6.61(d, $J_o = 8.33$ Hz, 1H, H-5), 7.1–7.4 (4H, phenyl aromat.), 7.45 (d, 1H, $J_m = 1.7$ Hz, H-2), 7.57 (dd, 1H, $J_o = 8.3$ Hz, $J_m = 1.65$ Hz, H-6). *Anal.* (C, H, N) for $C_{15}H_{15}FN_2O_2$.

3.3. 3-Amino-4-(p-chlorophenylmethyl)aminobenzoic acid ***3b***

The solution of **2b** (5 mmol, 1.37 g) in EtOH (10 ml) was treated with 10% NaOH (10 ml) and refluxed for 1h over a water bath. The reaction mixture was cooled, diluted with water and neutralized with glacial acetic acid. The crude

Table 1
Physical and spectral data of compounds **10–25**



No	R_1	R_2	N	mp[a], (°C)	Formula[b]	NMR[c] (δ ppm) (DMSO-d_6)	Mass (EI, 70ev)	Starting Material
10		– H		270-5	$C_{18}H_{20}N_4OS$ 3HCl . H_2O	2.65(s,3H,CH_3) 6.9-7.83(aromat,4H)	340.16(M^+,5.3), 283.1(9.2), 241.1(16.2), 213.1, 99(32.1), 83.02(82.6), 70.08(100)	Lit .1
11		– H		298	$C_{19}H_{19}ClN_4O$ 2HCl . $0.5H_2O$	7-7.6(aromat,6H) 8.05(d,1H,J_o=8.06 Hz,H-6)	354.2(M^+,3.8), 356(1.3), 297(7.2), 299(2.8), 255(11.6) 257.12(4.14), 227 (4.1), 229 (1.3), 83.09(81.8), 70.2(100)	Lit. 1
12		– H		(a) 160-5	$C_{19}H_{18}Cl_2N_4O$ H_2O	7.2-7.8(aromat,4H) 7.72(s,1H,H-4) 7.92(d,1H, J_o=8.4 Hz, H-6)	388(M^+,1.8), 390(1.1), 392 (0.2), 331(3.6), 333(2.4), 335 (0.4), 289(5.2), 291(3.7), 293(0.6), 83(76.8), 70(100)	Lit .1
13		– H		>300	$C_{20}H_{22}N_4O_2$ 2HCl . $0.5H_2O$	3.63(s,OMe,3H) 7-7.6(aromat,6H) 8.05(d,1H,H-6)	350.34(M^+,6.08), 293.29 (7.94), 267.29(10.9), 98.98(42.23), 83(79.05), 70(100)	Lit .1
14		– H		(a) 90-5	$C_{26}H_{26}N_4O_2$ 2HCl . H_2O	4.72(s,2H,OCH_2) 6.6-7.3(aromat,10H), 7.37(s,1H,H-4), 7.82 (d,1H, J_o=8.77Hz,H-6)	426.32(M^+,1.76), 369.3(3.58), 343.31 (8.04), 327.29(8.07), 91(100), 70 (63.24), 83(63.24) 69.92(83.24)	Lit .1
15	$-CH_2O$ 	– H		(a) 90	$C_{20}H_{22}N_4O_2$ HCl . H_2O	5.35(s,2H,CH_2O) 6.9-7.8(8H,aromat)	350.35(M^+,6.2), 293.34 (12.1) 251.27(7.52), 200.2(6), 158.1 (18.1), 130.03(37.42), 99.02 (46.6), 83(85.3), 70(100)	Lit .1
16	$-CH_2O$ 	– H	$HOCH_2CH_2N$ 	240-45	$C_{21}H_{24}N_4O_3$ 2HCl . $2.5H_2O$	2.5(2H,CH_2N), 3.8 (2H,$HOCH_2$), 5.65 (s,2H,CH_2O), 7.0-8.2 (aromat, 8H)	380.32(M^+,0.7), 364.3(0.5), 251.26(55.4), 158.06(52.27), 130(86.36), 99.98(100), 93.96 (66.84)	Lit .1
17	$-CH_2O$ 	– H		272-75	$C_{20}H_{21}ClN_4O_2$ 2HCl . $0.5H_2O$	5.64(s,2H,CH_2O), 7.2(d 2H,J_o=8.96Hz,H-2',6'), 7.4(d,2H, J_o=8.9Hz, H-3',5'), 7.55(dd,1H, J_o=8.4Hz,H-7), 7.85 (d,1H, J_o=8.4Hz,H-6), 7.9(s,1H,H-4)	384.22(M^+,3.02), 386.21 (1.14), 327.21(4.54), 329.2(1.72), 285.16(2.91), 287.17(0.99), 200.12 (5.25), 158(17.28), 82.86 (81.07), 70(100).	Lit 1
18		$-CH_2$ 		(a) 120	$C_{26}H_{26}N_4O$ 2HCl . $1.5H_2O$	5.59(s,2H,CH_2) 7.05-7.95(13H, aromat)	410.24(M^+,3.4), 353.25(5.4) 328.3(7.7), 311.3(11.4), 91 (100), 82.86(86), 70(50.7)	4a
19		$-CH_2$ 		(a) 110	$C_{26}H_{25}ClN_4O$ 2HCl . $0.5H_2O$	5.49(s,2H,CH_2) 7.0-7.95(12H, aromat)	444.23(M^+,5.9), 387.2(6.9), 362.19(9.7), 345.2(13.1), 99.1(35.76), 91.1(83.7), 83.04(100), 70.1(77.91)	Lit.1
20		$-CH_2$ 		(a) 120	$C_{26}H_{24}Cl_2N_4O$ 2HCl . $3H_2O$	5.69(s,2H,CH_2) 7.1-8.0(11H, aromat)	478.27(M^+,3.8), 480.3(2.7), 421.2(5.5), 423(4.3), 379 (7.4), 381(5), 383(0.97), 125 62.6), 127(21.5), 70(100).	Lit.1
21		$-CH_2$ 		235-40	$C_{26}H_{23}Cl_2FN_4O$ 2HCl . $1.5H_2O$	5.69(s,2H,CH_2) 7.1-8.0(10H, aromat)	496.1(M^+,2.2), 498(1.5), 439.2(4.29), 441(3), 443(0.5 397(5.6), 399(3.7), 109(98) 82.99(100), 70(91.34).	4c
22		$-CH_2$ 	$(Me)_2NCH_2CH_2NH$	268-70	$C_{25}H_{24}ClFN_4O$ 2HCl . $2H_2O$	5.43(s,2H,CH_2) 7.1-8.0(11H, aromat)	451(M^+,1.5), 453(0.48), 406 (2), 408(0.74), 380(38.8), 382(15.5), 362.9(15.7), 109 (99.5), 82.9(78), 70.6(100)	4b
23		$-CH_2$ 	$(Me)_2NCH_2CH_2NH$	(a) 110	$C_{24}H_{32}N_6O$ 3HCl . $3.5H_2O$	5.55(s,2H,CH_2) 7.27-7.38 (aromat., 6H), 8.05(d,1H,H-6), 8.3 (1H,H-4)	421.4(M^+,15), 350.4(25.7), 337(22.9), 305.4(47.5), 279 (32.7), 235(23.5), 91(18.58), 71.1(14.66), 58.2(100).	9a
24		$-CH_2$ 	$(Me)_2NCH_2CH_2NH$	(a) 118	$C_{25}H_{33}N_5O$ 3HCl . $3H_2O$	5.49(s,2H,CH_2), 7.3-7.5 (aromat.,6H), 7.94(d,1H,H-6), 8.1(1H,H-4)	420.4(M+1), 348.4(46.7), 332.4, 305.4, 257.4(17), 213.3(15.3), 91.1(50.2), 71(33.5), 58.3(100).	9b
25		$-CH_2$ 	$(Me)_2NCH_2CH_2NH$	(a) 105	$C_{24}H_{31}ClN_6O$ 2HCl . $2H_2O$	5.45(s,2H,CH_2), 7.25-7.5(aromat.,5H), 7.8(d, 1H H-6), 8.15(1H,H-4)	454.1(M^+,2.3), 456(0.97), 384(6.86), 386(2.4), 371(3.3 300(18.1), 302(6.8), 125(43) 127(16), 70.7(97.3), 58(100)	9c

[a] Because of bubbling at this point, there is no sharp melting point.
[b] All compounds gave satisfactory C, H, N analysis.
[c] Common protons of **10–21**: 2.2–2.4 (CH_3 protons of piperazine), 2.6–2.9 (methylene protons of piperazine neighbours to the N atoms having group Me), 3.5–4 (other methylene protons of piperazine); **22–25**: 2.6–2.8 (s, 6H, N–$(Me)_2$), 2.89–3.2 (t, 2H, CH_2NMe_2), 3.5–3.7 (q or t, 2H, $CONHCH_2$), 9.1–9.3 (t, N*H*CO).

product was collected and then crystallized with EtOH. M.p. 185°C (1.05 g, yield 80.7%). *Anal.* (C, H, N) for $C_{14}H_{13}FN_2O_2$.

3.4. 2-Phenyl-1-phenylmethyl-1H-benzimidazole-5-carboxylic acid **4a**

3a (5 mmol, 1.21 g) was suspended in water (20 ml) and MeOH (20 ml). A solution of the benzaldehyde (7 mmol, 0.742 g) in MeOH (20 ml) was added and stirred for 5 min, followed by addition of a solution of cupric acetate (7.4 mmol, 1.5 g) in water (20 ml). The resulting mixture was stirred vigorously and heated briefly to boiling and then filtered while hot. The precipitate was washed with a mixture of water and MeOH (1:1) and dried. 1 g of this precipitate was dissolved in EtOH (20 ml) containing concentrated HCl (1 ml). A solution of Na_2S (2 ml, 50%) in water was added and the mixture was filtered while hot to remove the copper sulfide. The filtrate was concentrated to half volume, diluted with water twice, cooled, and the mixture made alkaline with NaOH solution (10%), stirred and filtered. The filtrate was acidified with acetic acid solution (10%) and the precipitate was collected. Crystallization of the crude product from EtOH gave **4a**. M.p. 225°C, (0.9 g, yield 55.2%). MS (70 eV): 328.31 ($M^{+\bullet}$, 18.81), 237, 193, 119, 91 (100). *Anal.* (C, H, N) for $C_{21}H_{16}N_2O_2$.

3.5. 2-(o-Chlorophenyl)-1-(p-fluorophenylmethyl)-1H-benzimidazole-5-carboxylic acid **4b**

Compound **4b** was prepared in analogy to **4a**, starting from **3b** and *o*-chlorobenzaldehyde. M.p. 215°C, (0.96 g, yield 50.4%). ^{1}H NMR($CDCl_3$): 5.28 (s, 2H, CH_2), 6.9–7.71 (aromat., 9H), 8.09 (dd, J_o = 8.5 Hz, 1H, H-6), 8.69 (s, 1H, H-4); MS (70 eV): 380.22 ($M^{+\bullet}$, 12.77), 382.22 (M+2, 4.37), 364, 345, 108.9 (100). *Anal.* (C, H, N) for $C_{21}H_{14}ClFN_2O_2$.

3.6. 2-(2,4-Dichlorophenyl)-1-(p-fluorophenylmethyl)-1H-benzimidazole-5-carboxylic acid **4c**

Compound **4c** was prepared in analogy to **4a**, starting from **3b** and 2,4-dichlorobenzaldehyde. M.p. 258–260°C, (0.85 g, yield 40.94%). ^{1}H NMR($CDCl_3$): 5.26 (s, 2H, CH_2), 6.9–7.5 (aromat., 8H), 8.01 (dd, J_o = 8.8 Hz, 1H, H-6), 8.69 (s, 1H, H-4); MS (70 eV): 414.09 ($M^{+\bullet}$, 10.04), 416.13 (M+2, 6.5), 418 (M+4, 1.38), 364, 108.94 (100). *Anal.* (C, H, N) for $C_{21}H_{13}Cl_2FN_2O_2$.

3.7. 2-(p-Chlorophenoxy)methyl-1H-benzimidazole-5(6)-carboxylic acid **5**

3,4-Diaminobenzoic acid (5 mmol, 0.76 g), *p*-chlorophenoxyacetic acid (5 mmol, 0.93 g) and 4N HCl (25 ml) were heated under reflux for 4 h. After cooling, the mixture was adjusted to pH 7 with 10% NaOH. The precipitated solid was collected, and washed with water. After drying, crystallization of the crude product from EtOH gave **5**. M.p. 236°C, (0.53 g, yield 35%). ^{1}H NMR(DMSO-d_6): 5.37 (s, 2H, CH_2), 7.12 (d, 2H), 7.36 (d, 2H), 7.6–8.2 (3H, aromat.); MS (70 eV): 302($M^{+\bullet}$, 7.97), 304 (M+2, 2.91), 175 (100). *Anal.* (C, H, N) for $C_{15}H_{11}ClN_2O_3$.

3.8. Methyl 1-(phenylmethyl)-2(1H)-benzimidazolone-5-carboxylate **6a**

A solution of **2a** (1.28 g, 5 mmol) in 20 ml of dry tetrahydrofuran (THF) was cooled and a solution of *N,N'*-carbonyldimidazole (1.63 g, 10 mol) in 5 ml of dry THF was added rapidly with stirring. The cooling bath was removed and stirring was continued overnight. The reaction mixture was diluted with water, the precipitate was filtered, washed with water and recrystallized from EtOH. M.p. 200°C (1.2 g, yield 85.5%). MS (70 eV): 282($M^{+\bullet}$, 11.62), 251 (6.3), 91 (100). *Anal.* (C, H, N) for $C_{16}H_{14}N_2O_3$.

3.9. Methyl 1-(p-chlorophenylmethyl)-2(1H)-benzimidazolone-5-carboxylate **6b**

Compound **6b** was prepared in analogy to **6a**, starting from **2c**; white-coloured product, m.p. 248°C (1.37 g, yield 86.7%). MS (70 eV): 316 ($M^{+\bullet}$, 6.4), 318 (M+2, 2.12), 125 (100), 127 (32.8). *Anal.* (C, H, N) for $C_{16}H_{13}ClN_2O_3$.

3.10. Methyl 2-chloro-1-(phenylmethyl)-1H-benzimidazole-5-carboxylate **7a**

A mixture of **6a** (1 g, 3.54 mmol) and $POCl_3$ (15 ml) was refluxed with stirring for 4 h and dry hydrogen chloride was passed through the refluxing liquid. Then $POCl_3$ was evaporated, the reaction mixture was poured into ice-cold water, 4N NaOH was added and the precipitate was collected. Crystallization of the residue from EtOH gave **7a** as colourless crystals. M.p. 160–162°C (0.73 g, yield 68.6%). MS (70 eV): 300 ($M^{+\bullet}$, 6.9), 302 (M+2, 2.09), 269, 178, 91 (100). *Anal.* (C, H, N) for $C_{16}H_{13}ClN_2O_2$.

3.11. Methyl 2-chloro-1-(p-chlorophenylmethyl)-1H-benzimidazole-5-carboxylate **7b**

Compound **7b** was prepared in analogy to **7a** starting from **6b**; white-coloured product, m.p. 157–159°C (0.98 g, yield 58.6%). MS (70 eV): 334.08 ($M^{+\bullet}$, 18.88), 336.1 (12.88), 338.13 (3.08), 178.05 (4.91), 180.07 (1.82), 149.05 (58.16), 125 (100), 126.83 (43.37). *Anal.* (C, H, N) for $C_{16}H_{12}Cl_2N_2O_2$.

3.12. General synthesis method for **8a–c**

The mixture of **7a,b** (5 mmol) and corresponding amine derivatives (7.5 mmol) in a few ml of DMF was stirred and heated (at 110–120°C) until all the starting material benzim-

Table 2
Physical and mass data of compounds **8a–9c**

Comp.	Yield (%)	M.p. (°C)	MS (70 eV)/EI	Formula [a]	Purification
8a	53.5	144–145	364 ($M^{+\cdot}$, 1.07), 333, 294.5 (10.1), 91 (100)	$C_{21}H_{24}N_4O_2$	flash-chromatography, $CHCl_3$/isopropanol (10:2)
8b	52.7	127	363 ($M^{+\cdot}$, 11.42), 272 (37.19), 244 (12.19), 91 (100)	$C_{22}H_{25}N_3O_2$	flash-chromatography, CH_2Cl_2
8c	46.3	115	398.14 ($M^{+\cdot}$, 1.91), 400 (1.03), 328.1 (9.49), 330 (6.53), 315 (9.7), 317 (3.9), 125.07 (100), 127 (33)	$C_{21}H_{23}ClN_4O_2$	flash-chromatography, $CHCl_3$/isopropanol (10:2)
9a	76.5	very hygroscopic	350.5 ($M^{+\cdot}$, 2.7), 280.5 (16.08), 267.4 (16.86), 91 (100)	$C_{20}H_{22}N_4O_2$	
9b	72.3	237	349.5 ($M^{+\cdot}$, 17.1), 258.5 (26.37), 230.4 (10.53), 91 (100)	$C_{21}H_{23}N_3O_2$	
9c	62.7	294	384.1 ($M^{+\cdot}$, 4.8), 386 (2), 314 (14.7), 316 (5.28), 301 (10.63), 303.1 (4.32), 125.03 (100), 127 (33.4)	$C_{20}H_{21}ClN_4O_2$	

[a] All compounds gave satisfactory C, H, N analysis.

Table 3
The in vitro antibacterial and antifungal activity of **10–25**

Comp. [a]	Growth inhibition zone diameter (mm)		
	E. coli	*S. aureus*	*C. albicans*
8a	18	17	16
8b	12	21	10
8c	14	20	11
11	14	–	–
20	–	15	14
21	10	14	–
22	11	16	11
24	–	10	10
25	–	–	12
Fluconazole			16.5
Miconazole			18
Ampicillin	22	29	

[a] Other tested compounds have no activity.

idazoles were used up. The reaction mixture was made alkaline with dilute Na_2CO_3 solution, extracted with AcOEt, washed with water, dried over Na_2SO_4 and evaporated. Purification conditions are given in Table 2.

*3.13. General synthesis method for **9a–c***

Compounds **8a–c** (5 mmol) in EtOH (10 ml) were refluxed in 10% NaOH (10 ml) for 1 h over a water bath. The reaction mixture was cooled, diluted with water and neutralized with acetic acid. The crude product was precipitated and then crystallized with EtOH (Table 2).

*3.14. General synthesis method for **10–25***

Related benzimidazolecarboxylic acids (0.3–0.5 g) were refluxed in benzene (5 ml) with $SOCl_2$ (10 ml) for 2 h at 80°C. Then the solvent and excess $SOCl_2$ were evaporated completely and the residue was dissolved in chloroform (20 ml). Excess corresponding amine derivatives were added and the mixture was stirred and heated for 30 min at 50°C. Chloroform was evaporated and the residue was dissolved in AcOEt (20 ml). This mixture was washed with Na_2CO_3 (5%), then with saturated NaCl solution and water, dried over anhydrous $CaCl_2$ and evaporated. Some physicochemical properties and spectral findings of **10–25** are given in Table 1.

4. Antimicrobial activity technique

A paper disc (8 mm in diameter) was soaked in a 2000 μg/ml solution of the test compound in propylene glycol (**8a–c**, **9a–c**, **10**, **12**, **13**, **15**, **18**, **20**, **21**, **22**) or in water and placed on an agar plate containing fungi or bacteria cells, which was incubated at 37°C for 24 h. Propylene glycol as a blank has no inhibition zone. The diameter of the growth inhibition zone around the paper disc was measured.

5. Results and discussion

Compounds **8a–25** were evaluated for antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* in vitro. Table 3 shows the results of in vitro activity determination by a paper disc assay (measuring the diameter of the inhibition zone around a paper disc soaked in a solution of test compounds). Among the investigated compounds, **8a–c**, **20**, **22** showed the best activity. Methyl benzimidazole-5-carboxylate derivatives **8a** had potent activity comparable to that of fluconazole against *C. albicans*. Derivatives **8a–c** showed the largest growth inhibition zone diam-

eter against the bacteria, with **8b** giving the best result against *S. aureus*. On the other hand the amides **23–25**, corresponding to the ester derivatives **8a–c**, had no good inhibitory activity. Compound **20** also showed strong inhibitory activity against *C. albicans*, while **20** and **22** had moderate activity against *S. aureus*. None of the benzimidazolecarboxamides **10–25** exhibited activity towards *E. Coli*. On the whole, the results of compounds **10–25** indicate that the introduction of a 4-halogenated benzyl residue on position 1 of the benzimidazole moiety led to compounds which exhibited a higher activity than that of the corresponding unsubstituted compounds.

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