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Synthesis and antimicrobial activity of some new benzimidazole carboxylates and carboxamides

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Abstract

Some benzimidazole carboxylates and carboxamides were synthesized and evaluated for their antimicrobial activities against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. Among the investigated compounds **2d** exhibited best activity against *C. albicans*. © 1999 Elsevier Science S.A. All rights reserved.

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1. Introduction

In our previous papers [1,2] we described the synthesis and antimicrobial activities of some benzimidazole carboxylates and carboxamides. Since the carboxylate derivatives showed more activity than the carboxamide, we planned to synthesize some new benzimidazole methylcarboxylates and some other carboxamides substituted at C-2 with several aromatic or heteroaromatic groups.

2. Chemistry

Benzimidazole carboxylates **2a–f** were prepared by the reaction of **1a–f** with methanolic hydrogen chloride. Compounds **1a** and **1f** were converted to acyl chlorides with $SOCl_2$ then dehydrohalogenation between these acyl chlorides and 2-aminomethylpyridine and 4-methylpiperidine gave **3** and **4**, respectively (Scheme 1). The intermediate **5** was prepared as previously reported by us [1]. Compounds **6** and **7** were prepared via oxidative condensation of **5** and appropriate aldehydes with sodium hydrosulphite [3].

3. Experimental

Compounds **1a–f** were prepared by the method already reported by us in Ref. [1]. Melting points were determined with a Büchi SMP-20 melting point apparatus and are uncorrected. All the instrumental analyses were performed by TUBITAK (Instrumental Analysis Laboratory, Ankara) with a Bruker AC 400 NMR spectrophotometer using TMS internal standard and $CDCl_3$, a VG Platform II mass spectrometer and a Leco CHNS 932 elemental analyzer. For the chromatographic analyses Merck Silica Gel 60 (230–400 mesh ASTM) was used.

3.1. General procedure for the synthesis of compounds 2a–f

Benzimidazole carboxylic acids **1a–f** (0.3 g, 0.925 mmol) were added to 10 ml of 10% hydrogen chloride methanolic solution and refluxed until the starting material was completely exhausted. The methanol was removed and the residue was made alkaline with dilute Na_2CO_3 solution. The precipitate was collected and crystallized from methanol–water (if necessary active carbon was used) giving compounds **2a–f**. Some physico-chemical properties and spectral findings of **2a–f** are given in Table 1.

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3.2. Synthesis of compounds **3** and **4**

Compounds **1a** and **1f** (0.3 g) were refluxed in benzene (4 ml) with $SOCl_2$ (6 ml) for 2 h. Then the solvent and the excess of $SOCl_2$ were completely evaporated and the residue was dissolved in chloroform (10 ml). An excess of corresponding amine derivatives was added and the mixture was stirred and heated for 2 h at 50°C. Chloroform was evaporated and the residue was dissolved in AcOEt (20 ml). This mixture was washed with 5% Na_2CO_3, then with saturated NaCl solution and water, dried over anhydrous $CaCl_2$ and evaporated. Compounds **3** and **4** were purified by column chromatography using AcOEt:isopropanol (15:2) and $CHCl_3$:acetone (5:2) as eluent, respectively. Some physico-chemical properties and spectral data of **3** and **4** are given in Table 2.

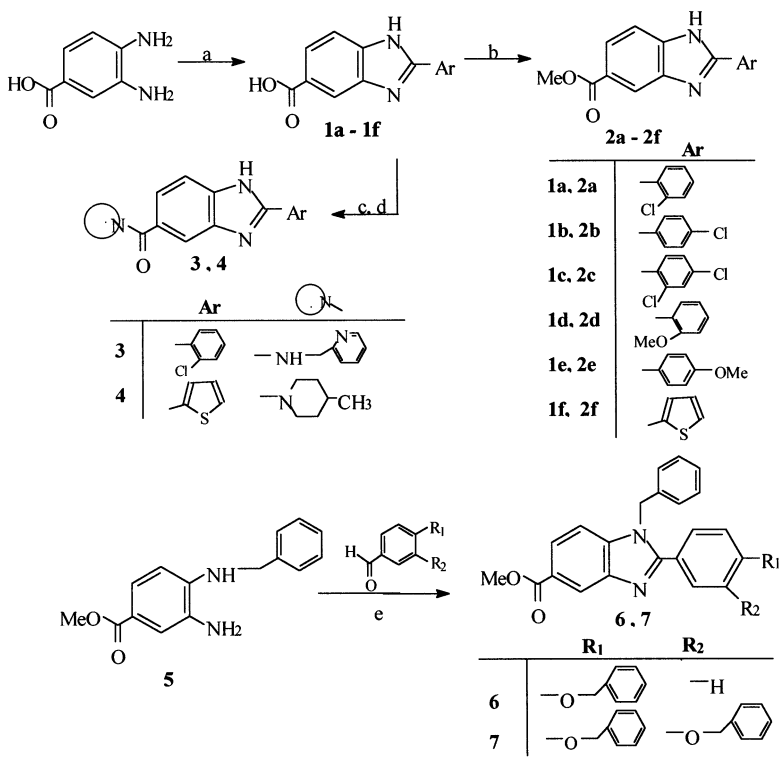
3.3. Synthesis of compounds **6** and **7**

The appropriate aldehyde derivative (1.5 mmol) was dissolved in 5 ml EtOH. 0.160 g of $NaHSO_3$ in 5 ml water was added in portions to the cooled ethanolic solution. The formed precipitate was filtered off and dried. A total of 1.2 mmol of this precipitate and 1.2 mmol of **5** in 6 ml of DMF were heated at 110°C for 4.5 h. At the end of this period the reaction mixture was cooled, poured into water and extracted with $CHCl_3$, washed with water, dried over anhydrous Na_2SO_4, and then crystallized from acetone–*n*-hexane. Some physico-chemical properties and spectral data of **6** and **7** are given in Table 2.

4. Antimicrobial activity

The in vitro antimicrobial activity of the compounds was tested by the tube dilution technique [4]. Test and reference compounds (ampicillin trihydrate, fluconazole and ketoconazole) were dissolved in 12.5% DMSO, at concentrations of 200 μg/ml, further dilutions of the compounds and standards in the test medium were prepared at the required concentrations of 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 μg/ml. The final inoculum size was 10^5 CFU/ml. The minimum inhibitory concentrations (MIC) were defined as the lowest concentrations of the compounds that prevented visible growth. It was determined that the solvent had no antimicrobial activity against any of the test microorganism.

All the compounds were tested for their in vitro growth inhibitory activity against *Staphylococcus aureus* ATCC 250 as Gram positive and *Escherichia coli*



Scheme 1. Synthesis of the compounds **1a–7**. Reagents: (a) Cu(II)acetate/H_2S, corresponding benzaldehydes; (b) CH_3OH/HCl; (c) $SOCl_2$; (d) 2-amino-methylpyridine and 4-methylpiperidine; (e) $NaHSO_3$.

Table 1
Some physico-chemical properties and spectral findings of compounds **2a–f**

Comp.	Yield (%)	M.p. (°C)	Formula	NMR (δ ppm)	MS (70 eV, EI)
2a	75	207	$C_{15}H_{11}N_2O_2Cl{\cdot}0.6H_2O$	3.89 (s, 3H, $COOCH_3$), 7.53–7.93 (aromat, 7H)	286 ($M^{\bullet+}$) (38.19), 288 ($M+2$) (14.29), 255 (33.52), 227 (11.26), 137 (6.25), 111 (8.17), 63 (100)
2b	70	207	$C_{15}H_{11}N_2O_2Cl$	3.94 (s, 3H, $COOCH_3$), 7.50 (d, 2H, $J_o = 8.51$ Hz, H-3′,5′), 7.62 (d, 1H, $J_o = 8.42$ Hz, H-7), 7.92 (dd, 1H, $J_o = 8.42$ Hz, $J_m = 1.4$ Hz, H-6), 8.20 (d, 2H, $J_o = 8.54$ Hz, H-2′,6′), 8.30 (s, 1H, H-4)	286 ($M^{\bullet+}$) (44.74), 288 ($M+2$) (20.00), 255 (55.26), 227 (17.89), 137 (16.58), 111 (18.95), 63 (100)
2c	78	242	$C_{15}H_{10}N_2O_2Cl_2{\cdot}1.8H_2O$	3.94 (s, 3H, $COOCH_3$), 7.48 (dd, 1H, $J_o = 8.39$ Hz, $J_m = 1.30$ Hz, H-5′), 7.62 (d, 1H, $J_m = 1.48$ Hz, H-3′), 7.69 (d, 1H, $J_o = 8.50$ Hz, H-7), 7.94 (d, 1H, $J_o = 8.48$ Hz, H-6), 8.00 (d, 1H, $J_o = 8.37$ Hz, H-6′), 8.36 (s, 1H, H-4)	320 ($M^{\bullet+}$) (2.10), 289 (2.01), 171 (14.74), 145 (7.73), 63 (100)
2d	70	103	$C_{16}H_{14}N_2O_3{\cdot}1.9H_2O$	3.89 (s, 3H, $COOCH_3$), 4.05 (s, 3H, OCH_3), 7.15 (ddd, 1H, $J_o = 7.53$ Hz, H-5′), 7.27 (d, 1H, $J_o = 8.37$ Hz, H-3′), 7.52 (ddd, 1H, $J_o = 8.52$ Hz, $J_m = 1.46$ Hz, H-4′), 7.71 (d, 1H, $J_o = 8.46$ Hz, H-7), 7.85 (d, 1H, $J_o = 8.31$ Hz, H-6), 8.29 (s, 1H, H-4), 8.36 (d, 1H, $J_o = 7.00$ Hz, H-6′)	282 ($M^{\bullet+}$) (100), 251 (5.17), 223 (2.82)
2e	73	226	$C_{16}H_{14}N_2O_3{\cdot}0.35H_2O$	3.86 (s, 3H, $COOCH_3$), 3.88 (s, 3H, OCH_3), 7.14 (d, 2H, $J_o = 8.75$ Hz, H-3′,5′), 7.64 (d, 1H, $J_o = 8.40$ Hz, H-7), 7.83 (d, 1H, $J_o = 8.41$ Hz, $J_m = 1.1$ Hz, H-6), 8.15 (d, 2H, $J_o = 8.79$ Hz, H-2′,6′), 8.16 (s, 1H, H-4)	281 $(M-1)^+$ (1.63), 149 (100)
2f	65	231	$C_{13}H_{10}N_2O_2S$	3.94 (s, 3H, $COOCH_3$), 7.59–7.63 (m, 2H, H-7,4′), 7.86 (d, 1H, $J = 5$ Hz, H-3′), 7.90 (d, 1H, $J = 8.54$ Hz, H-6), 8.26 (m, 2H, H-4′,5′)	258 (M^+) (3.90), 227 (6.56), 199 (4.33), 109 (45.25), 83 (22.17)

RSKK 313 as Gram negative bacteria and a fungus *Candida albicans* RSKK 628. MIC values of new and reference compounds are presented in Table 3.

4.1. Antibacterial activity assay

The cultures were obtained in Mueller–Hinton Broth (Difco) for all the bacteria after 18–24 h of incubation at 37 ± 1°C. Testing was carried out in Mueller–Hinton Broth at pH 7.4 and two-fold dilution technique was applied. A set of tubes containing only inoculated broth was kept as controls. After incubation for 18–24 h at 37 ± 1°C, the last tube with no growth of microorganism was recorded to represent MIC expressed in μg/ml.

4.2. Antifungal activity assay

The yeast *C. albicans* was maintained in Sabouraud Dextrose Broth (Difco) after incubation for 48 h at 25 ± 1°C. Testing was performed in Sabouraud Dextrose Broth at pH 7.4 and the two-fold dilution technique was applied. A set of tubes containing only inoculated broth was kept as controls. After incubation for 48 h at 25 ± 1°C, the last tube with no growth of yeast was recorded to represent MIC expressed in μg/ml.

5. Results and discussion

Compounds **2a–f**, **3**, **4**, **6** and **7** were evaluated for their antimicrobial activity against *S. aureus*, *E. coli* and *C. albicans* by tube dilution technique. Their antibacterial and antifungal activities were determined as MIC values. Table 3 shows the results of in vitro activity determination by a tube dilution method. Among the investigated compounds **2d** showed better activity than fluconazole and compounds **2a**, **2c**, **2e**, **2f**, **3** and **4** exhibited comparable activities to that of

Table 2
Some physicochemical properties and spectral data of compounds **3–7**

Comp.	Yield (%)	M.p. (°C)	Formula	NMR (δ ppm)	Mass (70 eV, EI)
3	75	127	$C_{20}H_{15}N_4OCl{\cdot}3HCl{\cdot}2H_2O$	4.91 (d, 2H, CH_2), 7.65 (ddd, 1H, $J = 7.56$ Hz, H-4′), 7.72 (ddd, 1H, $J = 7.38$ Hz, H-5′), 7.78 (d, 1H, $J = 8.02$ Hz, H-7), 7.90–7.92 (m, 2H, H-3′,6′), 8.00 (ddd, 2H, $J = 9.21$ Hz, H-4″,5″), 8.10 (d, 1H, $J = 8.60$ Hz, H-3″), 8.48–8.52 (m, 2H, H-6,4), 8.84 (d, 1H, $J = 5.53$ Hz, H-6″), 9.79 (t, 1H, NH)	362 ($M^{+\bullet}$) (18.02), 255 (10.96), 227 (3.81), 137 (1.70), 107 (100), 92 (33.44)
4	60	135	$C_{18}H_{19}N_3OS$	7.52 (dd, 1H, $J_o = 8.51$ Hz, $J_p = 0.91$ Hz, H-7), 7.78 (m, 1H, $J_{4',5'} = 5.1$ Hz, $J_{4',3'} = 2.88$ Hz, H-4′), 7.82 (s, 1H, H-3′), 7.85 (d, 1H, $J_o = 8.5$ Hz, H-6), 8.16 (dd, 1H, $J_{5',4'} = 5.1$ Hz, $J_{5',3'} = 0.82$ Hz, H-5′), 9.02 (dd, 1H, $J_m = 2.7$ Hz, $J_p = 0.95$ Hz, H-4)	325 ($M^{+\bullet}$) (2.29), 227 (29.10), 199 (23.51), 109 (11.29), 98 (7.56), 83 (9.00), 69 (33.77)
6	50	164	$C_{29}H_{24}N_2O_3$	4.04 (s, 3H, $COOCH_3$), 5.21 (s, 2H, O–CH_2), 5.56 (s, 2H, N–NH_2), 7.13–7.53 (aromat, 13H), 7.72 (dd, 2H, $J_{2',3'} = J_{6',5'} = 8.73$ Hz, $J_{2',6'} = J_{6',2'} = 1.79$ Hz, H-2′,6′), 8.05 (dd, 1H, $J_{6,7} = 8.49$ Hz, $J_{6,4} = 1.41$ Hz, H-6), 8.64 (d, 1H, $J_{4,6} = 1.10$ Hz, H-4)	342 ($M-3'$, 4′-$OCH_2H_5C_6$), 311, 283, 251, 221, 192 (1.07), 91 (100).
7	55	145	$C_{36}H_{30}N_2O_4$	3.94 (s, 3H, $COOCH_3$), 4.98 (s, 2H, 3′-O–CH_2), 5.20 (s, 2H, 4′-OCH_2), 5.39 (s, 2H, N–CH_2), 6.98–7.44 (aromat, 19H), 7.95 (d, 1H, $J_{6,7} = 8.55$ Hz, H-6), 8.54 (s, 1H, H-4)	448 ($M^{+\bullet}$), 449 ($M+1$), 450 ($M+2$), 417, 358, 327, 267, 121 (2.80), 93 (100)

Table 3
Antimicrobial activities [a] of the synthesized compounds

Comp.	*C. albicans*	*S. aureus*	*E. coli*
2a	50	50	>100
2b	100	50	>100
2c	50	100	>100
2d	25	50	>100
2e	50	50	>100
2f	50	100	100
3	50	50	>100
4	50	50	>100
6	>100	100	>100
7	100	100	>100
Fluconazole	50	n.t. [b]	n.t.
Ketoconazole	12.5	n.t.	n.t.
Ampicillin	n.t.	6.25	3.125

[a] Minimum inhibitory concentration.
[b] n.t., not tested.

fluconazole against *C. albicans*. The other compounds had no valuable inhibitory activity.

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