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Synthesis and potent antimicrobial activity of some novel methyl or ethyl 1*H*-benzimidazole-5-carboxylates derivatives carrying amide or amidine groups

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Abstract—A series of benzimidazole-5-carboxylic acid alkyl ester derivatives carrying amide or amidine substituted methyl or phenyl groups at the position C-2 were synthesised and evaluated for antibacterial and antifungal activities against *S. aureus*, methicillin resistant *S. aureus* (MRSA), *S. faecalis*, methicillin resistant *S. epidermidis* (MRSE), *E. coli* and *C. albicans*. The results showed that while all simple acetamides are essentially inactive, aromatic amides and amidines have potent antibacterial activities. Aromatic amidine derivatives **13f–h** exhibited the best inhibitory activity with 1.56–0.39 μg/mL MIC values against MRSA and MRSE.

1. Introduction

Numerous benzimidazole derivatives containing ester groups on the benzene ring have been synthesised for their antifungal, insecticidal, herbicidal,[1,2] anti-inflammatory[3] and potential anthelmintic[4] activities. It is also well known that amides,[5–7] amidines[8–10] and combinations of both[11,12] are present in a variety of antimicrobial, antiparasitic, anthelmintic, antiviral and antitumoural agents. Furthermore, our previous work and that of others[1,12–14] showed that benzimidazolecarboxamides display good antibacterial and antimycotic activity. Taking into consideration these structural features and the expectation of low toxicity with ester function present, we planned to prepare a series of benzimidazoles carrying the ester group at the benzene ring of the benzimidazole core and with additional substitution at the position C-2 by alkyl or aryl groups possessing amides or amidines. Their antimicrobial activity against bacteria and fungi will be described.

Keywords: Methicillin-resistant *S. aureus*; Methicillin-resistant *S. epidermidis*; Ethyl 1*H*-benzimidazole-5-carboxylate; *N*,*N*′-Dialkylamidine.
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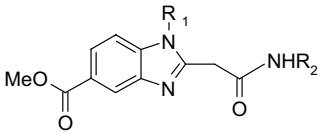
2. Result and discussion

2.1. Chemistry

The synthetic pathway for preparation of the targeted benzimidazoles **6a–h** (Table 1)and **8a–f** (Table 2) is shown in Scheme 1. 4-Chloro-3-nitrobenzoic acid **1** was converted to its methyl or ethyl ester **2a** and **b**, then the chlorine atom of **2** was replaced with amines by aromatic nucleophilic substitution. Reduction of the nitro group of **3a–h** (Table 5) afforded **4b–f**, **4h–j** (Table 6). Compounds **4a** and **g** were prepared according to our previous methods.[15a,b] The reaction of **4a–f** with ethyl β-amino-β-ethoxy acrylate HCl[16] gave ethyl 5-methoxycarbonyl-1-alkylsubstituted-1*H*-benzimidazole-2-acetate derivatives **5a–f** (Table 7). The acetamides **6a–h** were prepared by the aminolysis of **5a–f** in moderate yield without reaction with the aromatic ester (Table 1). Compounds **7a–d** (Table 8) and **12a–d** (Scheme 2, Table 9) were obtained in good yield by condensation of **4g–j** with the $Na_2S_2O_5$ adduct of 4-carboxybenzaldehyde or 4-cyanobenzaldehyde, respectively, in DMF.[17] The carboxyl groups of **7a–d** were converted to the amides **8a–f**, first by acid chloride formation using $SOCl_2$, then by reaction with the several amines. Compound **9** was prepared by heating **4a** with ethyl cyanoacetate according to a previously published method.[18a,b,19] However, treatment of the other *N*-substituted

Table 1. Yields, physicochemical and spectral properties of **6a**–**h**



Compd	R_1	R_2	Yield	Mp	Formulas	NMR ($CDCl_3$) (δ ppm)	MS ESI (*m*/*z*) (M+H) (rel int 100%)	Eluant for c.c.
6a	H	Isopropyl	54	168–173	$C_{14}H_{17}N_3O_3$	1.09 (d, 6H, J = 6.6), 3.85 (s, 3H), 3.94 (s, 2H), 4.01 (m, 1H, J = 6.7), 7.3 (d, 1H, J = 6), 7.5 (d, 1H, J_o = 8.5), 7.9 (d, 1H, J_o = 8.5, J_m = 1.3), 8.25 (d, 1H, J_m = 1.3)	276	EtOAc–EtOH (90:10)
6b	Ethyl	*n*-Butyl	66	142	$C_{17}H_{23}N_3O_3$	0.82 (t, 3H, J = 7.3), 1.24 (m, 2H, J = 7.2), 1.34 (t, 3H, J = 7.3), 1.42 (m, 2H), 3.19 (q, 2H, J = 6), 3.86 (s, 2H), 3.87 (s, 3H), 4.21 (q, 2H, J = 7.3), 7.33 (d, 1H, J = 8.6), 7.8 (t, 1H), 7.96 (d, 1H, J_o = 8.5, J_m = 1.2), 8.36 (d, 1H, J_m = 1.1)	318	EtOAc
6c	Methyl	3-Diethyl aminopropyl	21	[a]	$C_{19}H_{28}N_4O_3{\cdot}2HCl{\cdot}3H_2O$	0.95 (t, 6H, J = 7.1), 1.64–1.68 (m, 2H), 2.40–2.5 (m, 6H), 3.38 (q, 2H), 3.85 (s, 3H), 3.9 (s, 2H), 3.97 (s, 3H), 7.36 (d, 1H, J_o = 8.4), 8.05 (dd, 1H, J_o = 8.5, J_m = 1.5), 8.2 (br s, 1H), 8.44 (d, 1H, J_m = 1)	361	Chloroform–isopropanol (10:2)
6d	Cyclopropyl	3-Pyridilmethyl	25	183–185	$C_{20}H_{20}N_4O_3$	1.01 (2H), 1.27 (2H), 3.20 (m, 1H), 3.88 (s, 3H), 4.01 (s, 2H), 4.5 (d, 2H, J = 6), 7.2 (d, 1H), 7.5 (d, 1H, J_o = 8.6), 7.6 (d, 1H), 7.95 (dd, 1H, J_o = 8.5, J_m = 1.6), 8.3 (d, 1H, J_m = 1.3), 8.45 (dd, 1H, J_o = 8.5, J_m = 1.6), 8.51 (d, 1H, J = 1.9), 8.77 (br s, 1H)	365	Chloroform–isopropanol (10:2)
6e	Benzyl	3-Dimethyl aminopropyl	19	139–142	$C_{23}H_{28}N_4O_3{\cdot}2HCl{\cdot}2H_2O$	1.63 (m, 2H), 2.02 (s, 6H), 2.30 (t, 2H, J = 6.4), 3.33 (q, 2H, J = 6.2), 3.86 (s, 2H), 3.98 (s, 3H), 5.46 (s, 2H), 7.09 (dd, 2H, J_o = 7.7, J_m = 2.4), 7.3–7.4 (m, 4H), 8.0 (dd, 1H, J_o = 8.5, J_m = 1.5), 8.2 (br s, 1H), 8.49 (d, 1H, J_m = 1.2)	409	Chloroform–isopropanol (10:2)

6f	*p*-Chlorobenzyl	Isobutyl	64	202–204	$C_{22}H_{24}ClN_3O_3$	0.83 (d, 6H, J = 6.6), 1.72 (m, 1H, J = 6.7), 3.02 (t, 2H, J = 6.4), 3.84 (s, 2H), 3.88 (s. 3H), 5.42 (s, 2H), 6.93 (d, 1H, J = 8.4), 7.25 (m, 4H), 7.94 (t, 1H), 7.96 (dd, 1H, J_o = 8.5, J_m = 1.4), 8.41 (d, 1H, J_m = 1.2)	414	EtOAc–*n*-hexane (8:2)
6g	*p*-Chlorobenzyl	Methyl aminoethyl	29	148–150	$C_{21}H_{23}ClN_4O_3{\cdot}2HCl{\cdot}H_2O$	2.45 (s, 3H), 2.76 (t, 2H), 3.38 (q, 2H), 3.84 (s, 2H), 3.97 (s, 3H), 5.47 (s, 2H), 7.01 (d, 2H, J_o = 8.4), 7.30–7.32 (m, 3H), 7.73 (br s, 1H), 8.1 (dd, 1H, J_o = 8.6, J_m = 1.4), 8.48 (d, 1H, J_m = 1)	415	Chloroform–isopropanol (10:2)
6h	*p*-Chlorobenzyl	Isopropyl aminoethyl	64	149–153	$C_{23}H_{27}ClN_4O_3{\cdot}2HCl{\cdot}0.75H_2O$	1.09 (d, 6H), 2.82 (t, 2H), 2.88 (m, 1H), 3.4 (q, 2H), 3.86 (s, 2H), 3.97 (s, 3H), 5.47 (s, 2H), 7.02 (d, 2H, J_o = 8.3), 7.30–7.32 (m, 3H), 7.91 (br s, 1H), 8.01 (dd, 1H, J_o = 8.3), 8.47 (d, 1H)	443	Chloroform–isopropanol (10:2)

[a] Very hygroscopic, no sharp mp.

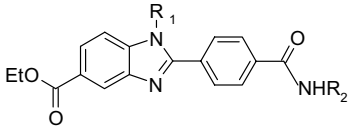
o-phenylendiamines **4h–j** with ethyl cyanoacetate led to unidentified mixtures. When we have attempted to convert the cyano group of **9**, to the *N*-butylacetamidine by the well-known Pinner reaction,[20] **10** and amide compound **11** were obtained instead of the expected amidine. It was reported that ester has been obtained in similar this kind of reactions.[18a,b] To our knowledge, there is only one example of a benzimidazole-2-acetamidine in the literature and it was not prepared by Pinner reaction (obtained by using $AlCl_3/NH_3$).[21] Meanwhile, conversion of the aromatic methyl ester to ethyl ester was performed in a straightforward manner. By treating the aromatic nitriles **12a–d**, with dry HCl gas in ethanol which formed the imidate esters and was followed by the reaction with various amines in ethanol gave the amidines **13a–i** (Table 3) in moderate yields.

Unexpectedly, the *N*,*N*′-disubstituted amidine **13d** was obtained from the reaction at room temperature, normally higher temperatures (50–55 or 80 °C) and 4-fold excess of amine are required to lead to the formation of *N*,*N*′-disubstituted amidines.[11,22,23] In our case we had to use 3- to 4-fold excess of amine, in order to observe significant reaction. In contrast, **13f** and **g** were formed with only small amounts of *N*,*N*′-dialkylated amidines (5% and 6%) according to the LC/MS chromatogram. These mixtures exhibited very close retention times of 5.29 min (*m*/*e* 647, 100%): 5.86 min (*m*/*e* 523, 100%) and 6.07 min (*m*/*e* 717, 100%): 6.85 min (*m*/*e* 591, 100%), respectively. After column chromatography, the mono-alkylated amidines **13f** and **g** were obtained by crystallisation from isopropanol, however, the pure diamidines could not be isolated. The formation ratio for the di- and monoalkylated amidines of **13h** is 21% (4.05 min, *m*/*e* 783, 100%) and 79% (6.99 min, *m*/*e* 625, 100%) respectively, according to the LC/MS chromatogram. In contrast to the previous example the retention times are sufficiently different that the mixture was readily separated by column chromatography. The dialkylated amidine derivative has been identified as **13i**. The other derivatives of series **13** do not form detectable amounts of dialklylated amidines. To fully understand the factors controlling the monoalkyl/dialkylamidine ratio further studies are necessary.

2.2. Microbiology

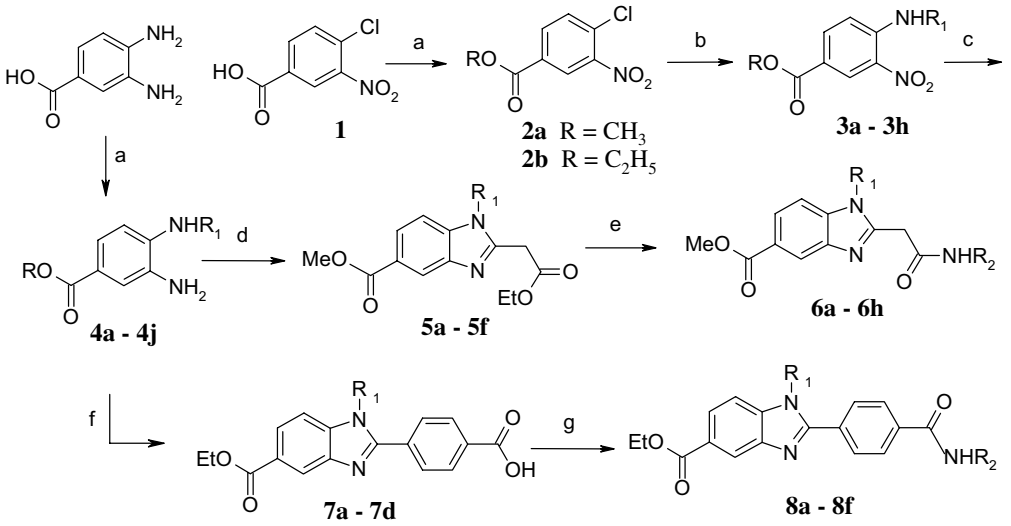
All of the benzimidazoles series **6**, **8**, **11** and **13** were tested for in vitro antibacterial activity against Gram-negative *Escherichia coli*, Gram-positive *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA, clinical isolate), *Streptococcus faecalis*, methicillin-resistant *Staphylococcus epidermidis* (MRSE, clinical isolate) bacteria and antifungal activity against *Candida albicans* by the agar diffusion method,[24] which has been extensively used by us.[24] The compounds showing antimicrobial activity by this method were further tested by the macro-broth dilution assay[25a,b] to determine the MICs which are listed in Table 4. The synthesised compounds and the reference drugs were dissolved in DMSO–H_2O (50%), at a concentration of 400 μg/mL. The concentration was adjusted to 100 μg/mL by 4-fold dilution with media culture and bacteria

Table 2. Yields, physicochemical and spectral properties of **8a–f**

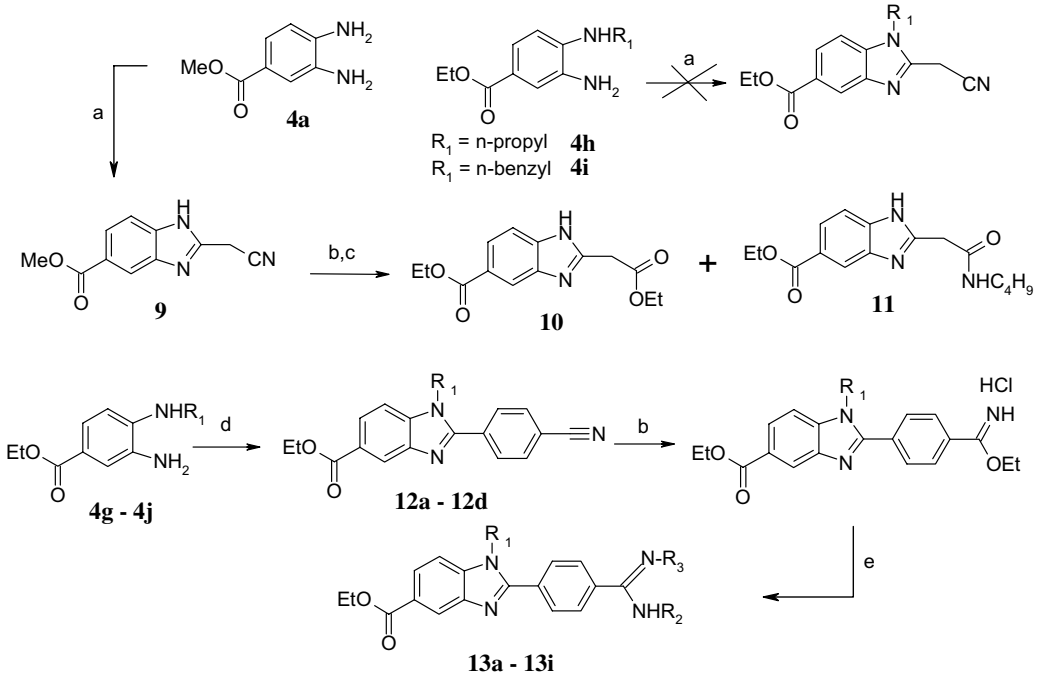


Compd	R_1	R_2	Formulas	Mp (°C)	Yield (%)	NMR ($CDCl_3$) (δ ppm)	MS (ESI+) *m/z* (rel intensity: 100%)
8a	H	Isopropyl	$C_{20}H_{21}N_3O_3$	250–252	71	1.11 (d, 6H, J = 6.6), 1.26 (t, 3H, J = 7), 4.08 (m, 1H, J = 6.7), 4.2 (q, 2H, J = 7), 7.5 (d, 1H, J_o = 8.5), 7.79 (dd, 1H), 7.89 (m, 3H), 8.14 (m, 3H)	352
8b	Propyl	Isopropyl	$C_{23}H_{27}N_3O_3{\cdot}0.5H_2O$	145–147	45	0.78 (t, 3H, J = 7.4), 1.24 (d, 6H, J = 6.6), 1.35 (t, 3H, J = 7.2), 1.74 (m, 2H, J = 7.5), 4.15 (t, 2H, J = 7.6), 4.25 (m, 1H, J = 6.6), 4.35 (q, 2H, J = 7.2), 6.1 (d, 1H, J = 7.5), 7.38 (d, 1H, J = 8.6), 7.72 (d, 2H), 7.86 (d, 2H), 7.95 (dd, 1H, J_o = 8.5, J_m = 1.3), 8.49 (d, 1H, J = 1.1)	394
8c	Benzyl	Isopropyl	$C_{27}H_{27}N_3O_3{\cdot}0.75H_2O$	153	44	1.18 (d, 6H, J = 6.6), 1.34 (t, 3H, J = 7.2), 4.25 (m, 1H, J = 6.6), 4.34 (q, 2H, J = 7.2), 5.39 (s, 2H), 6.03 (d, 1H, J = 7.6), 6.96 (m, 2H), 7.3 (m, 4H), 7.68 (d, 2H), 7.76 (d, 2H), 7.92 (dd, 1H, J_o = 8.5, J_m = 1.3), 8.53 (d, 1H, J = 1.1)	442
8d	Benzyl	4-Chlorobenzyl	$C_{31}H_{26}ClN_3O_3{\cdot}0.1H_2O$	175	54	1.34 (t, 3H, J = 7.1), 4.33 (q, 2H, J = 7.1), 4.54 (d, 2H, J = 5.7), 5.4 (s, 2H), 6.9 (1H), 6.95 (m, 2H), 7.22 (overlapped, 8H), 7.66 (d, 2H), 7.79 (d, 2H), 7.96 (dd, 1H, J_o = 8.5, J_m = 1.2), 8.52 (d, 1H, J = 1)	524
8e	Benzyl	Ethyl aminoethyl	$C_{28}H_{30}N_4O_3{\cdot}2.5H_2O$	188–192	43	1.3 (6H, overlapped), 2.94 (q, 2H), 2.97 (2H), 3.73 (q, 2H), 5.34 (s, 2H), 6.93 (2H), 7.11 (d, 1H), 7.14 (m, 3H), 7.63 (d, 2H), 7.87 (dd, 1H, J_o = 8.5, J_m = 1.2), 8.0 (d, 2H), 8.43 (d, 1H), 8.52 (t, 1H)	471
8f	2,4-Dichlorobenzyl	Ethyl aminoethyl	$C_{28}H_{28}Cl_2N_4O_3{\cdot}H_2O$	100[a]	35	1.08 (t, 3H), 1.12 (t, 3H), 2.7 (q, 2H), 2.85 (2H), 3.52 (q, 2H), 4.33 (q, 2H), 5.38 (s, 2H), 6.54 (d, 1H), 7.04 (m, 2H), 7.3 (t, 1H), 7.4 (d, 1H), 7.6 (d, 2H), 7.84 (d, 2H), 7.92 (dd, 1H, J_o = 8.4, J_m = 1.1), 8.49 (d, 1H, J_m = 1)	539

[a] Bubbling, no sharp mp.



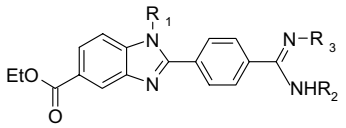
Scheme 1. Reagents: (a) methanolic or ethanolic HCl; (b) R_1NH_2; (c) H_2·Pd/C, for **4f** and **j** $NiCl_2$/Zn; (d) ethyl β-amino-β-ethoxyacrylate·HCl; (e) corresponding amines; (f) $Na_2S_2O_5$ adduct of 4-carboxybenzaldehyde; (g) $SOCl_2$/various amines.



Scheme 2. Reagents: (a) $NCCH_2CO_2C_2H_5$; (b) EtOH/dry HCl gas; (c) $C_4H_9NH_2$; (d) $Na_2S_2O_5$ adduct of 4-cyano-benzaldehyde; (e) corresponding amines.

solution. Data were not taken for the initial solution because of the high DMSO concentration (12.5%). Since the compound **13i** is insoluble in DMSO–H_2O (50%), its antimicrobial activity has only been determined by the diffusion method and it has some growth inhibition zone, however, not as well as the others. As shown in Table 4, the acetamide derivatives **6g** and **h** exhibit only a modest inhibitory effect with a MIC values of 50 μg/mL against *S. aureus*. All of the acetamide–benzimidazoles **6** series, are essentially inactive. Among the benzamide series, compound **8e** and **f** show good activity, in particular against *S. aureus*, MRSA and MRSE. All the aromatic amidine analogues **13** exhibited strong ability to inhibit *S. aureus* with most of the MICs in the low micromolar range. The most active compounds are **13f–h** and exhibit MIC values of 0.78–1.56 μg/mL against *S. aureus*. While ampicillin and sultamicillin is nearly inactive against MRSA (50 and 25 μg/mL and no inhibition zone with the diffusion method), compounds **13f–h** exhibited the greatest activity with MICs between 0.78–0.39 μg/mL. Activity against drug-resistant microorganisms, which are clinically difficult to treat, is very important for the next generation of antibacterial agents. Consequently the results for **13f–h** are quite encouraging. Since compounds **13f–h** had superior activity against MRSA and MRSE, when compared to

Table 3. Yields, physicochemical and spectral properties of **13a–i**



Compd	R_1	R_2	R_3	Formulas	Mp (°C)	Yield (%)	NMR (DMSO-d_6) (δ ppm)	LC/MS ESI(+), m/z M+H, 100% (Retention time)
13a	H	Isopropyl	H	$C_{20}H_{22}N_4O_2 \cdot 0.5C_3H_8O \cdot 0.75HOH$	95[a] 224–225	35	1.1 (d, 6H, J = 6.3), 1.26 (t, 3H, J = 7.1), 3.77 (m, 1H, J = 6.3), 4.23 (q, 2H, J = 7.1), 7.53 (d, 1H, J = 8.4), 7.67 (dd, 1H, J_o = 8.4, J_m = 1.4), 7.78 (d, 2H, J = 8.4), 8.09 (d, 1H, J = 1.1), 8.2 (d, 2H, J = 8.4)	351 (5.81)
13b	H	Phenylethyl	H	$C_{25}H_{24}N_4O_2 \cdot 0.5C_3H_8O \cdot 0.5HOH$	95[a] 205	33	1.55 (t, 3H, J = 7), 3.13 (t, 2H, J = 6.5), 3.67 (t, 2H, J = 6.1), 4.52 (q, 2H, J = 6.9), 7.4 (m, 7H), 7.77 (d, 1H, J = 8.3), 7.96 (d, 2H), 8.1 (d, 1H, J = 8.2), 8.49 (s, 1H)	413 (6.35)
13c[b]	Propyl	Isopropyl	H	$C_{23}H_{28}N_4O_2 \cdot 2HCl \cdot 0.5EtOH \cdot HOH$	100–135[a] 253–255	41	0.65 (t, 3H, J = 7.3), 1.21 (d, 6H, J = 5.5), 1.29 (t, 3H, J = 7.1), 1.62 (m, 2H, J = 7.4), 4.07 (m, 1H, J = 7.6), 4.29 (m, 4H, J = 6.9), 7.9 (d, 3H), 7.93 (dd, 1H, J_o = 8.6, J_m = 1.3), 8.01 (d, 2H, J = 8.5), 8.27 (d, 1H, J_o = 1), 9.25 (s, 1H), 9.57 (s, 1H), 9.74 (d, 1H)	393 (5.8)
13d[b]	Propyl	Propyl	Propyl	$C_{26}H_{34}N_4O_2 \cdot 2HCl \cdot 0.1HOH$	Very hygroscopic	40	0.67 (tt, 6H, partly overlapped), 0.9 (t, 3H, J = 7.2), 1.28 (t, 3H, J = 7.2), 1.45 (m, 2H, J = 7.3), 1.62 (m, 4H), 3.03 (q, 2H, J = 6.3), 3.37 (q, 2H, J = 6.3), 4.29 (m, 4H), 7.76 (d, 2H, J = 8.2), 7.86 (d, 1H, J = 8.3), 7.91 (dd, 1H, J_o = 8.2, J_m = 1.4), 8.00 (d, 2H, J = 8.2), 8.26 (s, 1H), 9.53 (t, 1H), 9.76 (t, 1H)	435 (6.2)
13e	Benzyl	Isopropyl	H	$C_{27}H_{28}N_4O_2 \cdot 0.75HOH$	100–110[a]	35	1.49 (d, 6H, J = 6.4), 1.61 (t, 3H, J = 7.1), 4.22 (t, 3H, J = 7.1), 4.22 (m, 1H, J = 6.2), 4.61 (q, 2H, J = 7.12), 5.67 (s, 2H), 7.26 (m, 2H), 7.51 (m, 4H), 7.89 (d, 2H, J = 8.2), 7.95 (d, 2H, J = 8.3), 8.2 (dd, 1H, J_o = 8.5, J_m = 1.4), 8.76 (d, 1H, J_m = 1.1)	441 (5.89)
13f	Benzyl	4-Chlorobenzyl	H	$C_{31}H_{27}ClN_4O_2 \cdot 0.25C_3H_8O \cdot 0.5HOH$	93–95[a] 138–140	30	(With 1 drop D_2O): 1.31 (t, 3H, J = 7.1), 4.31 (4H, overlapped), 5.63 (s, 2H), 6.96 (d, 2H, J = 6.8), 7.25 (m, 3H), 7.36 (d, 2H, J = 8.4), 7.45 (d, 2H, J = 8.7), 7.62 (d, 1H, J = 8.8), 7.76 (d, 2H, J = 8.4), 7.88 (dd, 1H, J_o = 8.2, J_m = 1.1), 7.97 (d, 2H, J = 8.3), 8.32 (d, 1H, J = 1.2)	523 (5.86)

13g	2,4-Dichloro benzyl	4-Chlorobenzyl	H	$C_{31}H_{25}Cl_3N_4O_2{\cdot}2C_3H_8O$	93–95[a] 157–159	37	(With 1 drop D_2O): 1.41 (t, 3H, $J = 7.1$), 4.42 (q, 2H, $J = 7.2$), 4.49 (s, 2H), 5.77 (s, 2H), 6.86 (d, 1H, $J = 8$), 7.4 (m, 2H), 7.55 (d, 2H, $J = 8.3$), 7.68 (m, 2H), 7.83 (d, 2H), 7.98 (m, 2H), 8.12 (d, 2H, $J = 8.4$), 8.48 (s, 1H)	591 (6.85)
13h	2,4-Dichloro benzyl	3,4-Dichloro benzyl	H	$C_{31}H_{24}Cl_4N_4O_2{\cdot}0.25C_3H_8O$	187–191	39	(With 1 drop D_2O): 1.33 (t, 3H, $J = 7.1$), 4.33 (4H, overlapped), 5.61 (s, 2H), 6.65 (d, 1H, $J = 8.4$), 7.27 (dd, 1H, $J_o = 8.4$, $J_m = 1.8$), 7.38 (d, 1H, $J = 8.3$), 7.55 (m, 2H), 7.64 (m, 4H), 7.85 (dd, 1H, $J_o = 8.1$, $J_m = 1.2$), 7.94 (d, 2H, $J = 8.4$), 8.33 (s, 1H)	625 (6.99)
13i	2,4-Dichloro benzyl	3,4-Dichloro benzyl	3,4-Dichloro benzyl	$C_{38}H_{28}Cl_6N_4O_2{\cdot}HOH$	153–157	11	1.33 (t, 3H, $J = 7.1$), 4.13 (s, 2H), 4.33 (q, 2H, $J = 7.2$), 4.43 (s, 2H), 5.64 (s, 2H), 6.63 (d, 1H, $J = 8$), 6.95 (d, 1H, $J = 7.8$), 7.1 (s, 1H), 7.25 (d, 1H), 7.4 (m, 4H), 7.61 (m, 4H), 7.73 (d, 2H, $J = 7.7$), 7.88 (d, 1H, $J = 7.6$), 8.33 (s, 1H)	783 (4.05)

[a] HCl salt of **13c** and **d** were crystallised from EtOH.
[b] Because of the strong hydrogen bonding properties of amidine groups, these compounds absorb crystallisation solvents which has been caused bubbling at this point. Elemental analysis and NMR results also confirm this finding.

the clinically important agents oxacillin (diffusion method), ampicillin and sultamicillin, further studies are needed to clarify their mechanism of action.

It was reported that natural product distamycin A, a DNA minor-groove binding ligand, has weak antibacterial activity, with moderate or high DNA binding affinity.[26] Nevertheless, distamycin has served as a prototype for the development of minor-groove binding ligands with subnanomolar binding affinity. Dimeric analogous of distamycin A have recently been reported with strong DNA binding properties which is thought to be necessary for good antibacterial activity. Furthermore, some researchers[27] are focused on the optimisation of distamycin A analogues for therapeutic application in the treatment of severe infections caused by drug-resistant Gram-positive bacteria. Some of the analogues exhibited very potent antibacterial activity and were shown to bind A/T rich target sequences that are commonly found in bacterial promoters and replication origins. These results that they inhibit DNA replication and RNA transcription and consequently kill bacteria. Recently, internal and C-terminal benzimidazole[28,29] modifications of distamycin A were published by the same group. In addition, it has been reported that benzimidazole ring is an important structural motif of various types of DNA minor groove binding ligands.[27,28,30–32] Hence, a similar mechanism of action may exist for our benzimidazoles, since they have also very potent inhibitory activity against drug resistance Gram-positive bacteria. At the same time, low cytotoxicity should be expected, since the unmodified distamycin A has only limited cytotoxicity[33] and it has also monocationic amidine moiety like the compounds **13a**–**i**. It appears that benzyl or halogenated benzyl substitution at the position N^1 enhance the antibacterial activities against *S. aureus*, for both series **8** and **13**. The best result was obtained against *S. faecalis* with compound **13g**. All the synthesised compounds were inactive (MICs $\geqslant 50$ μg/mL) against Gram-negative bacteria *E. Coli* possibly due to poor permeability of the additional outer membrane. To study the selectivity for inhibition of bacterial growth, all these compounds were also tested against the fungi *C. albicans*. Generally all of the compounds were less effective against fungi compared to bacteria.

3. Conclusion

Introduction of aromatic amidine groups into the benzimidazole system gives a good profile of Gram-positive antibacterial activity. In particular, compounds **13f**–**h** having chlorinated benzyl groups at the position N^1 and 2-(4-*N*-benzylcarboxamidinophenyl) benzimidazoles exhibited the greatest activity with MIC values ranging from 1.56 to 0.39 μg/mL against *S. aureus*, methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant *S. epidermidis* (MRSE), respectively. Detailed mechanistic studies are required to understand the potent activity of these compounds. In vivo studies of compounds such as **13f**–**h** are necessary to fully evaluate the potential of these compounds.

Table 4. Antibacterial and antifungal activity of compounds **6g–13h** (MIC minimum inhibitory concentration μg/mL)

Compound	*S. aureus* ATCC 25923	MRSA[a]	*S. faecalis* ATCC 29212	MRSE[b]	*E. coli* ATCC 25922	*C. albicans* ATCC 10231
6g	50	>50	>50	50	> 50	>50
6h	50	>50	>50	25	>50	>50
8e	12.5	50	50	12.5	>50	50
8f	6.25	6.25	12.5	6.25	50	25
13a	12.5	>50	>50	>50	>50	50
13b	3.12	6.25	12.5	3.12	>50	50
13c	6.25	>50	>50	50	>50	50
13d	3.12	>50	>50	25	>50	50
13e	3.12	50	>50	12.5	>50	50
13f	0.78	0.78	6.25	1.56	>50	12.5
13g	1.56	0.78	3.12	0.78	>50	12.5
13h	1.56	0.39	3.12	1.56	>50	6.25
Ampicillin	0.39	50	0.78			
Sultamicillin	0.78	25	1.56	3.12		
Gentamisin					0.78	
Fluconazole						1.56

[a] MRSA: methicillin-resistant *S. aureus* (clinical isolate).
[b] MRSE: methicillin-resistant *S. epidermidis* (clinical isolate).

4. Experimental

Melting points were measured with a capillary melting point apparatus (Buchi SMP 20 and Electrotermal 9100) and are uncorrected. The IR spectra were recorded on a Jasco FT/IR-420 spectrometer as KBr discs. Compounds **3a–g**: 1709–1730 cm^{-1} (C=O ester); **4a–h**: 1692–1730 cm^{-1} (C=O ester). The ^1H NMR spectra were recorded with Bruker DPX-400 and VARIAN Mercury 400 FT-NMR spectrophotometers, δ scale (ppm) from TMS. Mass spectra were taken on Waters Micromass ZQ by using ESI(+) method. LC/MS analyses for **13** series were performed with Waters Alliance and Micromass ZQ by using MeOH and C-18 column (15 cm) with a flow rate of 0.5 mL/min. LC equipped with a diode array UV detection monitoring at 254 nm. The mixtures ratio were calculated from the peak area. Elemental analyses were taken on a Leco 932 CHNS-O analyzer (TUBlTAK Instrumental Analyze Lab., Ankara). For the HCl salts of the synthesised compounds, the free bases were dissolved in isopropanol and dry HCl gas was passed through the solution, some salts were highly hygroscopic and no sharp melting points could be observed.

4.1. General procedure for synthesis of 3a–h

To a solution of **2a** or **b** (4.6 mmol) in DMF (1.5 mL), the corresponding amines (9.3 mmol) were added and the mixture was heated for 3–8 h at 80 °C until starting material was consumed. The mixture was allowed to cool, H_2O and EtOH were added for **3a–c** and for **3d–h**, respectively. The resultant yellow precipitate was filtered and washed with H_2O, crystallised from EtOH or EtOH–H_2O (Table 5).

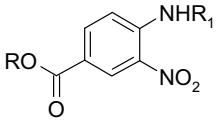
4.2. General procedure for synthesis of 4b–e and 4h–j

Compounds **3a–d**, **f–h** (3.4 mmol) in EtOH (75 mL) was reduced by hydrogenation using 40 psi of H_2 and 10% Pd–C (40 mg) until cessation of H_2 uptake. The catalyst was filtered on a bed of Celite, washed with EtOH and the filtrate was concentrated in vacuo. The crude amines were used for the further steps without crystallisation, grey-purple in colour (Table 6).

4.3. General procedure for synthesis of 5a–f

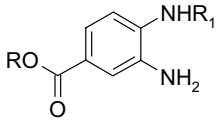
To a solution of **4a–f** (2 mmol) in DMF (1 mL), ethyl-β-amino-β-ethoxyacrylate HCl (4.7 mmol, 0.74 g) was

Table 5. Yields, formulas and physical properties of **3a–h**



Compd	R	R_1	Formulas	Mp (°C)	Yield (%)
3a	Methyl	Methyl	$C_9H_{10}N_2O_4$	142–143 lit.[34] 145	79
3b	Methyl	Ethyl	$C_{10}H_{12}N_2O_4$	95 lit.[35]	67.5
3c	Methyl	Cyclopropyl	$C_{11}H_{12}N_2O_4$	80–82	78
3d	Methyl	Benzyl	$C_{15}H_{14}N_2O_4$	101 lit.[13] 98	
3e	Methyl	*p*-Chlorobenzyl	$C_{15}H_{13}ClN_2O_4$	141–142 lit.[1] 141	
3f	Ethyl	*n*-Propyl	$C_{12}H_{16}N_2O_4$	78	84
3g	Ethyl	Benzyl	$C_{16}H_{16}N_2O_4$	93	71
3h	Ethyl	2,4-Dichlorobenzyl	$C_{16}H_{14}Cl_2N_2O_4$	126	70

Table 6. Yields, physicochemical and spectral properties of **4a–j**



Compd	R	R_1	Formulas	Mp (°C)	Yield (%)
4a	Methyl	H	$C_8H_{10}N_2O_2$	Lit.[15a]	
4b	Methyl	Methyl	$C_9H_{12}N_2O_2$	99–100 lit.[36] 102	86
4c	Methyl	Ethyl	$C_{10}H_{14}N_2O_2$	136–137	94
4d	Methyl	Cyclopropyl	$C_{11}H_{14}N_2O_2$	108–110	93
4e	Methyl	Benzyl	$C_{15}H_{16}N_2O_2$	126–127 lit.[13] 125	
4f	Methyl	*p*-Chlorobenzyl	$C_{15}H_{15}ClN_2O_2$	221–223 lit.[1] 221	
4g	Ethyl	H	$C_9H_{12}N_2O_2$	Lit.[15b]	
4h	Ethyl	*n*-Propyl	$C_{12}H_{18}N_2O_2$	Oily-semisolid	94
4i	Ethyl	Benzyl	$C_{16}H_{18}N_2O_2$	103–104	93
4j	Ethyl	2,4-Dichlorobenzyl	$C_{16}H_{16}Cl_2N_2O_2$	119	55

added and the mixture was stirred for 8 h at 40 °C. The mixture was cooled, water was added and then extracted with chloroform. The extract was washed H_2O and evaporated. The residue was purified by column chromatography (EtOAc) to give **5a–f**. All of them were obtained as white solids (Table 7).

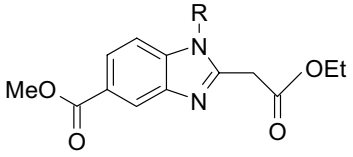
4.4. General procedure for synthesis of 6a–h

A mixture of **5a–f** (3 mmol), and corresponding amines (11 mmol) was heated for 3–8 h under reflux (for **5a**; 4 days). After cooling, water was and the mixture was extracted with chloroform. The extract was washed with Na_2CO_3 solution (5%) and H_2O, dried (anhydrous Na_2SO_4) and evaporated. The residue was purified by column chromatography to give **6a–h** as white solids (Table 1).

4.5. General procedure for synthesis of 7a–d and 12a–d

4-Carboxy or 4-cyanobenzaldehyde (15 mmol) were dissolved in 50 mL EtOH and sodium metabisulfite (1.6 g) in 10 mL H_2O was added in portions. The reaction mixture was stirred vigorously and more EtOH was added. The mixture was kept in a refrigerator for a several hours. The precipitate was filtered and dried

Table 7. Yields, physicochemical and spectral properties of **5a–f**



Compd	R	Mp (°C)	Formulas	Yield (%)	NMR ($CDCl_3$) (δ ppm)	IR ν cm^{-1} (C=O)
5a	H	113–114	$C_{13}H_{14}N_2O_4$	27	1.23 (t, 3H, J = 7.1), 3.86 (s, 3H), 4.06 (s, 2H), 4.19 (q, 2H, J = 7.1), 7.53 (d, 1H, J_o = 8.5), 7.91 (d, 1H, J_o = 8.5), 8.25 (s, 1H)	1717 and 1721
5b	Methyl	120–122	$C_{14}H_{16}N_2O_4$	51.5	1.14 (t, 3H, J = 7.13), 3.66 (s, 3H), 3.79 (s, 3H), 3.90 (s, 2H), 4.08 (q, 2H, J = 7.2), 7.21 (d, 1H, J_o = 8.5), 7.87 (dd, 1H, J_o = 8.5, J_m = 1.5), 8.28 (d, 1H, J_m = 1)	1715
5c	Ethyl	107–109	$C_{15}H_{18}N_2O_4$	65	1.17 (t, 3H, J = 7.1), 1.35 (t, 3H), 3.84 (s, 3H), 3.92 (s, 2H), 4.09–4.17 (4H overlapped), 7.26 (d, 1H, J_o = 8.5), 7.91 (dd, 1H, J_o = 8.5, J_m = 1.5), 8.34 (d, 1H, J_m = 1.3)	1715
5d	Cyclopropyl	105–108	$C_{16}H_{18}N_2O_4$	29	1.12–1.15 (2H), 1.22–1.33 (5H), 3.3 (m, 1H), 3.96 (s, 3H), 4.16 (s, 2H), 4.26 (q, 2H, J = 7.2), 7.58 (d, 1H, J_o = 8.5), 8.03 (dd, 1H, J_o = 8.5, J_m = 1.5), 8.44 (d, 1H, J_m = 1.4)	1713 and 1723
5e	Benzyl	113–115	$C_{20}H_{20}N_2O_4$	64	1.11 (t, 3H, J = 7.13), 3.83 (s, 3H), 3.86 (s, 2H), 4.02 (q, 2H), 5.33 (s, 2H), 6.95 ve 7.2 (m, 6H), 7.87 (dd, 1H, J_o = 8.5, J_m = 1.4), 8.38 (d, 1H, J_m = 1.3)	1719
5f	*p*-Chlorobenzyl	128–129	$C_{20}H_{19}ClN_2O_4$	59	1.25 (t, 3H, J = 7.14), 3.97 (s, 3H), 3.99 (s, 2H), 4.16 (q, 2H), 5.43 (s, 2H), 7.02 (d, 2H, J_o = 8.4), 7.23 (d, 1H, J_o = 8.6), 7.32 (d, 2H, J = 8.4), 8.0 (dd, 1H, J_o = 8.5, J_m = 1.5), 8.51 (d, 1H)	1717

(yield over 93%). The mixture of these salts (2 mmol) and **4h–j** in DMF (5 mL) were heated at 130 °C for 4 h. The reaction mixture was cooled, poured into H_2O, and the solid was filtered, crystallisation of crude product was from EtOH to give **7a–d** and **12a–d** as white solids (Tables 8 and 9).

4.6. General procedure for synthesis of 8a–f

Compounds **7a–d** (1 mmol) were heated at reflux in benzene (4 mL) with $SOCl_2$ (3 mL) for 2 h at 80 °C. Then solvent and excess of $SOCl_2$ were evaporated and the residue was dissolved in chloroform (10 mL). Excess of the corresponding amine derivatives (1 mL) was added and the mixture was stirred and heated for 30 min at 50 °C. Chloroform was added, washed with Na_2CO_3 (5%), H_2O and evaporated (Table 2). Compounds **8a** and **d** recrystallised from EtOH, **8b–c** and **8e–f** were purified by column chromatography using EtOAc and chloroform–isopropanol–isopropylamine (100:50:1) as eluant, respectively.

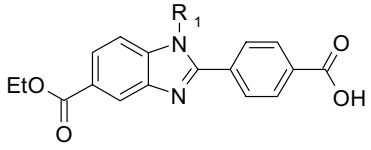
4.7. Methyl 2-cyanomethyl-5-benzimidazolecarboxylate 9

The mixture of **4a** (0.7 g, 4.2 mmol) and 0.8 g of ethyl cyanoacetate were heated for 2 h at 200 °C. After cooling, the residue was extracted with chloroform and purified by column chromatography using EtOAc–*n*-hexane (50%) as eluant and decolourised with activated carbon. Yield 0.31 g, 34%, mp 218–220 °C, ^{1}H NMR (DMSO-d_6) δ 3.78 (s, 3H), 4.38 (s, 2H), 7.53 (1H), 7.45 (d, 1H), 8.1 (s, 1H), 12.8 (s, 1H). MS ESI *m*/*z* (rel intensity): 216 (M+H, 100%), $C_{11}H_9N_3O_2$, Anal. (C, H, N).

4.8. General procedure for synthesis of 10, 11 and 13a–i

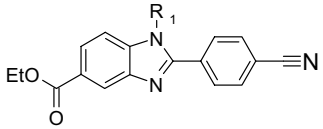
Compounds **9**, **12a–d** (2.5 mmol) were suspended in absolute EtOH, cooled in a ice–salt bath and dry HCl gas was then passed through the solution for 40 min. The stoppered flask was stirred at room temperature for 3 days. The solution was diluted with dry ether. The imidate ester hydrochloride separated as an oil. Excess solvent was decanted, and dried under vacuum at room temperature. The imidate esters of **13e–h** were precipitated as white solids, washed with ether, then dried under vacuum at room temperature. All imidate esters were used directly without characterisation. A suspension of imidate ester HCl in absolute EtOH was stirred with corresponding amines (4-fold excess) for overnight at 25–30 °C. The reaction mixture was evaporated, stirred with dilute Na_2CO_3 solution then extracted with dichloromethane. The solvent was evaporated and the residue purified by column chromatography using dichloromethane–isopropanol–isopropylamine (100:50:1.5) mixture as eluant for **13a–h**. In the **13h** case, **13i** was obtained from column chromatography by eluting with dichloromethane–isopropanol–isopropylamine (100:50:0.5). The solid of **9** treated with butylamine as mentioned above, gave compound **10** by column chromatography using EtOAc–*n*-hexane (1:1), yield 42%, mp 110 °C, ^{1}H NMR ($CDCl_3$) δ 1.23 (t, 3H, $J = 7.1$), 1.33 (t, 3H, $J = 7$), 3.86 (s, 3H), 4.04 (s, 2H), 4.30 (q, 2H, $J = 7.1$), 4.33 (q, 2H, $J = 7.1$), 7.53 (d, 1H, $J_o = 8.5$), 7.91 (dd, 1H, $J_o = 8.5$, $J_m = 1.2$), 8.25 (d, 1H, $J_m = 1.1$), MS (ESI) *m*/*z* (rel intensity): 277 (M+H, 100%), $C_{14}H_{16}N_2O_4$ Anal. (C, H, N). Then the column was eluted a second time with EtOAc–EtOH (95:5), **11**

Table 8. Yields, formulas and mp of **7a–d**



Compd	R_1	Formulas	Mp (°C)	Yield (%)	IR ν cm^{-1} COOEt COOH overlapped
7a	H	$C_{17}H_{14}N_2O_4$	>300	69	1683
7b	Propyl	$C_{20}H_{20}N_2O_4$	268–270	71	1703
7c	Benzyl	$C_{24}H_{20}N_2O_4$	265	79	1702
7d	2,4-Dichlorobenzyl	$C_{24}H_{18}Cl_2N_2O_4$	>300	65	1708

Table 9. Yields, formulas and mp of **12a–d**



Compd	R_1	Formulas	Mp (°C)	Yield (%)	IR ν cm^{-1}	
					COOEt	CN
12a	H	$C_{17}H_{13}N_3O_2$	>300	73	1685	2226
12b	Propyl	$C_{20}H_{19}N_3O_2$	130–131	68	1704	2230
12c	Benzyl	$C_{24}H_{19}N_3O_2$	161	63	1717	2225
12d	2,4-Dichlorobenzyl	$C_{24}H_{17}Cl_2N_3O_2$	213–215	61	1711	2229

was obtained, mp 194–195 °C, yield 11%. ^{1}H NMR (DMSO-d_6) δ 0.85 (t, 3H, $J = 7.3$), 1.32 (m, 7H, $J = 7.1$, overlapped), 3.07 (q, 2H, $J = 6.8$), 3.76 (s, 2H), 4.3 (q, 2H, $J = 7.1$), 7.56 (d, 1H, $J = 8.3$), 7.78 (d, 1H, $J = 8.1$), 8.09 (s, 1H), 8.27 (t, 1H), 12.5 (s, 1H), MS (ESI) *m/z* (rel intensity): 304 (M+H, 100%), $C_{16}H_{21}N_3O_3$, Anal. (C, H, N).

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