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ORIGINAL ARTICLE

Synthesis and potent antistaphylococcal activity of some new 2-[4-(3,4-dimethoxyphenoxy)phenyl]-1,*N*-*di*substituted-1*H*-benzimidazole-5-carboxamidines

M. Orhan Püsküllü[1,4], Sulhiye Yıldız[2], Yalçın Duydu[3], Aylin Üstündağ[3], and Hakan Göker[1]

[1]*Department of Pharmaceutical Chemistry,* [2]*Department of Microbiology, and* [3]*Department of Toxicology, Faculty of Pharmacy, Ankara University, Tandogan, Ankara, Turkey, and* [4]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Erciyes University, Kayseri, Turkey*

Abstract

A series of new 2-[4-(3,4-dimethoxyphenoxy)phenyl]-1,*N*-*di*substituted-1*H*-benzimidazole-5-carboxamidines (**23**–**33**) have been synthesized and evaluated for their potential antistaphylococcal activity. Cytotoxic effects of the compounds were investigated by the neutral red uptake (NRU) cytotoxicity test. Most of the compounds exhibited good MICs values against *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA). Compound **28** with *N*-cyclohexylcarboxamidine group at the 5-position was found to be the most potent agent, with the MIC value of 3.12 μg/mL.

Keywords

1*H*-benzimidazole-5-carboxamidines, 1*H*-benzimidazole-5-carboxylic acid, 1*H*-benzimidazole-5-sulfonamide, antistaphylococcal activity, MRSA

History

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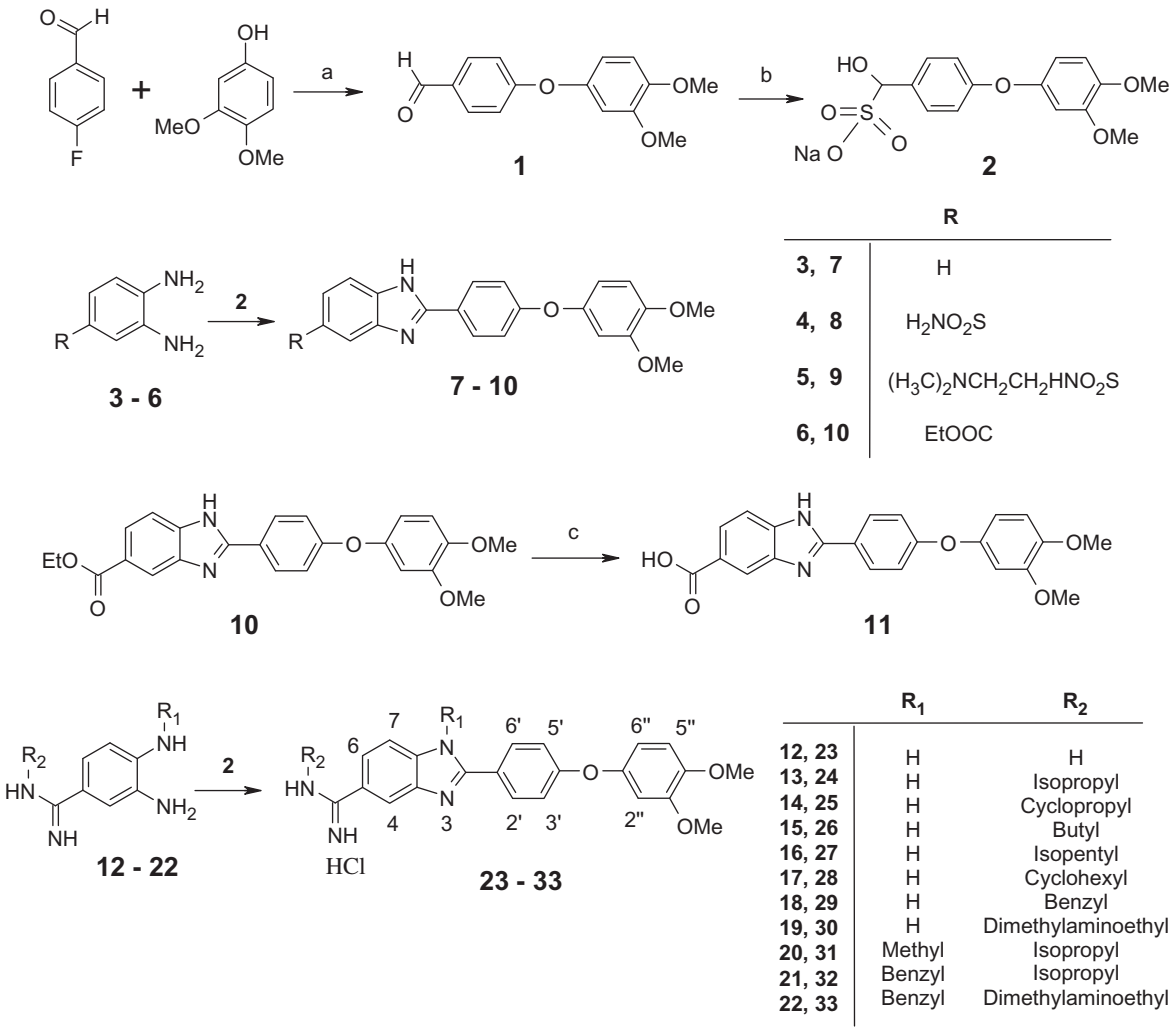
Introduction

Numerous benzimidazole derivatives containing amidine groups on the benzene ring have been synthesized for their antifungal, insecticidal, herbicidal, anti-inflammatory, and potential anthelmintic activities[1]. It is also well known that amides, amidines, and combinations of both are present in a variety of antimicrobial, antiparasitic, anthelmintic, antiviral, and antitumoral agents. Furthermore, our previous work[2–4] and that of others showed that benzimidazolecarboxamidines display good antibacterial and antimycotic activity. The literature survey reveals that one of the benzimidazolecarboxamidine having 3,4-(dimethoxy-phenoxy)-phenyl moiety (**23**, unpublished structural data)[5] was reported as an inhibitor of histidine protein kinase (HPK) from the bacterial two-component system (TCS) which are composed of a HPK and a response regulator (RR) for signal transduction. TCS are pervasive among bacteria, and this signal transduction pathway used bacteria to sense and response environmental changes[5]. In pathogenic bacteria, the TCS often regulates the expression of virulence factors. Since TCS is not found in animal cells, inhibiting this system is very attractive targets for developing novel antimicrobial agents, in particular against multidrug-resistant (MDR) bacterial infections such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecium* (VRE). Taking into consideration these structural features and the expectation of low toxicity without halogen atoms [e.g. (a) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is one of the most toxic agents which is known to be a human carcinogen[6], (b) polychlorinated dibenzofurans and polyhalogenated biphenyls[6] are a group of halogenated organic compounds which are highly toxic environmental pollutants, (c) chloramphenicol is a naturally occurring antibiotic derivative which is reasonably anticipated to be a human carcinogen[6], (d) *p*-chloroamphetamine (PCA)[7] and 3,4-dichloro-amphetamine (DCA) have higher neurotoxicity than amphetamine, (e) 1H-β-D-ribofuranoside-2-bromo-5,6-dichlorobenzimidazole (BDCRB)[8] was discovered as an antiviral drug, however, that compound has not found clinical application, because of its toxic aglycone, (f) similarly, some 4,5,6,7-tetrabromobenzimidazoles[9] having a trifluoromethyl, pentafluoroethyl, heptafluoropropyl side chain attached to position-2 of the benzimidazole ring exhibit strong cytotoxicity against the host cell lines in the low micromolar range (0.5–2.0 μM), (g) 2-benzylthio-5,6-dichloro-3-(β-D-ribofuranosyl)indole was developed as antiviral agents against HCMV, but it also was somewhat cytotoxic[10]]. In the present work, we have planned to prepare a series of benzimidazoles carrying 4-(3,4-dimethoxyphenoxy) phenyl at the C-2 position, with *N*-substituted amidines and evaluate their antistaphylococcal activity against MRSA.

Chemistry

The reaction sequences employed for the synthesis of the target compounds are outlined in Scheme 1. Compound **1**[11] was prepared by a reaction between the p-fluorobenzaldehyde and

Address for correspondence: Prof. Dr. Hakan Göker, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ankara University, 06100 Tandogan, Ankara, Turkey. Tel/Fax: +90 312 2230562. E-mail: goker@ankara.edu.tr



	R
3, 7	H
4, 8	H_2NO_2S
5, 9	$(H_3C)_2NCH_2CH_2HNO_2S$
6, 10	EtOOC

	R_1	R_2
12, 23	H	H
13, 24	H	Isopropyl
14, 25	H	Cyclopropyl
15, 26	H	Butyl
16, 27	H	Isopentyl
17, 28	H	Cyclohexyl
18, 29	H	Benzyl
19, 30	H	Dimethylaminoethyl
20, 31	Methyl	Isopropyl
21, 32	Benzyl	Isopropyl
22, 33	Benzyl	Dimethylaminoethyl

Scheme 1. Synthesis of the 1,N-*di*substituted-1*H*-benzimidazolcarboxamidines **7**–**11** and **23**–**33**.

the 3,4-dimethoxyphenol in good yield, then sodium metabisulfite adduct (**2**) of this aldehyde was prepared. Cyclization of **2** with several N-(substituted)-1,2-phenylendiamines **3**–**6** and **12**–**22** gave the targeted benzimidazoles **7**–**10** and **23**–**33**. Compound **11** was prepared from alkaline hydrolysis of ester **10**.

Experimental

Chemistry

Melting points were determined with Buchi SMP-20 (Büchi. Labortechnik, Flawil, Switzerland) and Electrotermal 9100 (Varian, Palo Alto, CA) capillary melting point apparatus and were uncorrected. The ^{1}H NMR spectra were recorded with a Varian Mercury-400 FT-NMR spectrometer (Palo Alto, CA), in DMSO-d_6 or $CDCl_3$. The mass spectra were taken on a Waters ZQ micromass LC-MS spectrometer (Waters Corporation, Milford, MA) by using the ESI(+) method and elemental analysis was performed on a LECO 932 CHNS analyzer (Leco Corporation, St. Joseph, MI) and satisfactory results $\pm$ 0.4% of calculated values were obtained. The compounds reported as salts were frequently analyzed correctly for fractional moles of water and/or crystalization solvent of solvation; proton NMR confirmed the presence of solvent. Column chromatography was accomplished on silica gel 60 (40–63 µm particle size) (Merck, Darmstadt, Germany). All starting materials and reagents were purchased from Aldrich, Merck or Fluka. The compounds are reported as salts were frequently analyzed correctly for fractional moles of water and/or EtOH of solvation; proton NMR confirmed the presence of solvent. Because of the tautomeric effect of imidazole ring, some NMR data, particularly 2D-NMR experiments, did not give satisfactory results. In the C^{13} spectra and HSQC experiments of compound **7**, two carbon peaks and the cross peak between the C-4,7 and H-4,7 were not observable, respectively. In order to prevent the tautomeric effects, the compounds were dissolved in DMSO-d_6, followed by a tiny amount of dry NaH, and 2–3 drops of D_2O were added to the NMR tube and stirred well. As it is reported in the experiment section, this time very satisfactory NMR results have been observed for compound **7**, since the tautomeric effect was totally disappeared. Intermediates **1**[11], **4**–**5**[12], **12**–**13**, **15**[3], **14**, **20**[3,4], **16**[3], **17** and **18**[3,4], **19**[4], **21**[3], and **22**[13] were prepared according to our previously published method. HCl salts of compounds were prepared by using dry HCl gas in EtOH or isopropanol (Scheme 1).

Sodium bisulphite adduct of 4-(3,4-dimethoxy)phenoxy-benzaldehyde (2)

Compound **1** (1.94 g, 7.5 mmol) was dissolved in EtOH (25 mL) and sodium metabisulfite (0.8 g) (5 mL of water) 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 white precipitate was filtered and dried, and used for the further steps without purification and characterization.

General synthesis of compounds 7–10 and 23–33

The mixture of **2** (0.181 g, 0.5 mmol) and compounds **3–6** and **12–22** in DMF (2 mL) was heated at 120 °C for 4 h. The reaction mixture was cooled, poured into water, and made alkaline with dilute K_2CO_3 solution. The resulting precipitate was collected by filtration and dried.

2-[4-(3,4-Dimethoxyphenoxy)phenyl]-1*H*-benzimidazole (7)

The residue was purified by crystallization from methanol; yield 65.5%; mp: 232–233 °C; **^{1}H NMR** δ ppm (DMSO-d_6): 3.76 and 3.78 (s,s, 6H, OCH_3), 6.65 (dd, 1H, $J_o = 8.4$, $J_m = 2.8$, H-6″), 6.83 (d, 1H, $J_m = 2.8$, H-2″), 7.00 (d, 1H, $J_o = 8.8$, H-5″), 7.1 (d, 2H, $J_o = 8.8$, H-3′, 5′), 7.19 (m, 2H, H-5,6), 7.57 (br. s, 2H, H-4,7), 8.15 (d, 2H, $J_o = 8.8$, H-2′,6′); **^{13}C-NMR** and **HSQC** δ ppm (DMSO-d_6): 55.6 and 55.8 (OCH_3), 105.2 (CH-2″), 111.2 (CH-6″), 112.4 (CH-5″), 117.1 (C-3′,5′), 121.8 (CH-5,6), 124.3, 128.2 (CH-2′,6′), 145.7, 148.7, 149.8, 150.8, 159.5; **^{1}H-NMR** δ ppm (DMSO-d_6 + NaH + D_2O): 3.70 and 3.73 (s,s, 6H, OCH_3), 6.56 (dd, 1H, $J_o = 8.4$, $J_m = 2.8$, H-6″), 6.72 (d, 1H, $J_m = 2.8$, H-2″), 6.82 (m, 2H, H-5,6), 6.93 (d, 2H, $J_o = 8.8$, H-3′,5′), 6.96 (d, 1H, $J_o = 8.8$, H-5″), 7.4 (m, 2H, H-4,7), 8.16 (d, 2H, $J_o = 8.8$, H-2′,6′). **^{13}C-NMR** and **HSQC** and **HMBC** δ ppm (DMSO-d_6 + NaH + D_2O): 159.5 (C-2), 157.3 (C-4′), 150.4 (C-4″), 149.9 (C-3″), 147.0 (C-3a, 7a), 145.4 (C-1″), 131.9 (C-1′), 128.5 (CH-2′,6′), 118.4 (CH-5,6), 117.7 (CH-3′,5′), 116.2 (CH-4,7), 112.8 (CH-5″), 110.9 (CH-6″), 104.8 (CH-2″), 56.0 and 56.2 (OCH_3); **MS** (ESI+) *m/z*: 347 (M + H, 100%); Anal. calcd. for $C_{21}H_{18}N_2O_3$ (C, H, N).

2-[4-(3,4-Dimethoxyphenoxy)phenyl]-1*H*-benzimidazole-5(6)-sulphonamide (8)

The residue was purified by column chromatography using dichloromethane:isopropanol:ammonium hydroxide (25%) (20:2:0.05) as an eluent, white color product; yield 15.5%; mp: 280–281 °C; **^{1}H-NMR** δ ppm (DMSO-d_6 + one drop of D_2O): 3.71 and 3.74 (s,s, 6H, OCH_3), 6.63 (dd, 1H, $J_o = 8.8$, $J_m = 2.8$, H-6″), 6.78 (d, 1H, $J_m = 2.8$, H-2″), 6.97 (d, 1H, $J_o = 8.8$, H-5″), 7.07 (d, 2H, $J_o = 8.8$, H-3′,5′), 7.66 (br.d, 2H, H-6,7), 7.99 (br.s, 1H, H-4), 8.12 (d, 2H, $J_o = 8.8$, H-2′,6′); **MS** (ESI+) *m/z*: 426 (M + H, 100%); Anal. calcd. for $C_{21}H_{19}N_3O_5S$ (C, H, N, S).

N-[2-(Dimethylamino)ethyl]-2-[4-(3,4-dimethoxyphenoxy)-phenyl]-1*H*-benzimidazole-5 (6)-sulphonamide HCl (9)

The residue was purified by column chromatography using dichloromethane:isopropanol:ammonium hydroxide (25%) (12:2:0.1) as an eluent. Oily residue was solidified by ethanolic HCl, white color product; yield 27.7%; mp: 273–275 °C; **^{1}H-NMR** δ ppm (DMSO-d_6 + one drop of D_2O): 2.75 [s, 6H, $N(CH_3)_2$], 3.08 (t, 2H, $J = 5.6$, CH_2NHSO_2), 3.14 (t, 2H, $J = 5.6$, NCH_2), 3.71 and 3.75 (s,s, 6H, OCH_3), 6.66 (dd, 1H, $J_o = 8.4$, $J_m = 2.4$, H-6″), 6.8 (d, 1H, $J_m = 2.4$, H-2″), 7.01 (d, 1H, $J_o = 8.4$, H-5″), 7.15 (d, 2H, $J_o = 8.4$, H-3′,5′), 7.85 (dd, 1H, $J_o = 8.4$, $J_m = 1.2$, H-6), 7.92 (d, 1H, $J_o = 8.4$, H-7), 8.16 (d, 1H, $J_m = 1.2$, H-4), 8.21 (d, 2H, $J_o = 8.4$, H-2′,6′); **^{13}C-NMR** δ ppm (DMSO-d_6): 162.8, 152.5, 150.7, 148.6, 146.9, 137.1 (br.s), 136.3, 134.4 (br.s), 131.1, 123.7, 118.9, 117.9, 115.6, 114.2, 113.3, 112.4, 106.2, 56.6, 56.5, 56.3, 43.1, 38.3; **MS** (ESI+) *m/z*: 497 M + H, 100%); Anal. calcd. for $C_{25}H_{28}N_4O_5S$ 2HCl.2.5 HOH (C, H, N, S).

Ethyl 2-[4-(3,4-dimethoxyphenoxy)phenyl]-1*H*-benzimidazole-5-carboxylate (10)

The residue was purified by crystallization from methanol; yield 55%; mp: 177–179 °C; **^{1}H-NMR** δ ppm ($CDCl_3$): 1.39 (t, 3H, CH_3CH_2), 3.81 & 3.87 (s,s, 6H, OCH_3), 4.4 (q, 2H, CH_3CH_2), 6.59 (dd, 1H, $J_o = 8.4$, $J_m = 2.4$, H-6″), 6.65 (d, 1H, $J_m = 2.4$, H-2″), 6.86 (d, 1H, $J_o = 8.4$, H-5″), 6.98 (d, 2H, $J_o = 8.4$, H-3′,5′), 7.59 (br.s, 1H, H-7), 7.95 (d, 1H, $J_o = 8.8$, H-6), 8.06 (d, 2H, $J_o = 8.4$, H-2′,6′), 8.32 (br.s, 1H, H-4); **^{13}C-NMR** δ ppm ($CDCl_3$): 167.4, 160.7, 153.9, 150.0, 149.1, 146.1, 128.6, 124.9, 124.3, 123.3, 117.6, 111.8, 111.6, 104.8, 61.1, 56.3, 56.0, 14.4; **MS** (ESI+) *m/z*: 419 (M + H, 100%); Anal. calcd. for $C_{24}H_{22}N_2O_5$ (C, H, N).

2-[4-(3,4-Dimethoxyphenoxy)phenyl]-1*H*-benzimidazole-5-carboxylic acid (11)

Compound **10** (1 mmol) was dissolved in 3% NaOH in 50% ethanol–water (3 mL) and heated under reflux for 1 h and cooled water and acetic acid were added to obtain the carboxylic acid, filtered, and recrystallized from methanol; yield 95%; mp: 244–246 °C; **^{1}H-NMR** δ ppm (CD_3OD): 3.78 and 3.81 (s,s, 6H, OCH_3), 6.59 (dd, 1H, $J_o = 8.4$, $J_m = 2.4$, H-6″), 6.73 (d, 1H, $J_m = 2.4$, H-2″), 6.93 (d, 1H, $J_o = 8.4$, H-5″), 7.03 (d, 2H, $J_o = 8.4$, H-3′,5′), 7.57 (d, 1H, $J_o = 8.4$, H-7), 7.92 (dd, 1H, $J_o = 8.4$, $J_m = 1.2$, H-6), 8.02 (d, 2H, $J_o = 8.4$, H-2′6 ′), 8.26 (s, 1H, H-4); **^{13}C-NMR** δ ppm (CD_3OD): 170.4, 161.0, 154.0, 150.5, 149.7, 146.4, 142.1 (br.s), 138.7 (br.s), 128.6, 126.8, 124.3, 123.4, 117.4, 116.7, 113.9, 112.6, 111.7, 105.2, 55.7, 55.4; **MS** (ESI+) *m/z*: 391 (M + H, 100%); Anal. calcd. for $C_{22}H_{18}N_2O_5$ (C, H, N).

2-[4-(3,4-Dimethoxyphenoxy)phenyl]-1*H*-benzimidazole-5(6)-carboxamidine (23)

The residue was purified by column chromatography using chloroform:methanol:ammonium hydroxide (25%) (110:20:5) as an eluant; yield 27%; mp: 180 °C bubb.; **^{1}H-NMR** δ ppm (DMSO-d_6): 3.72 and 3.74 (s,s, 6H, OCH_3), 6.59 (dd, 1H, $J_o = 8.4$, $J_m = 2.4$, H-6″), 6.79 (d, 1H, $J = 2.4$, H-2″), 6.96 (d, 1H, $J_o = 8.4$, H-5″), 7.01 (d, 2H, $J_o = 8.4$, H-3′,5′), 7.41 (dd, 1H, $J_o = 8.4$, $J_m = 2$, H-6), 7.5 (d, 1H, $J_o = 8.4$, H-7), 8.00 (d, 1H, $J_m = 1.6$, H-4), 8.22 (d, 2H, $J_o = 8.4$, H-2′,6′); **^{13}C-NMR** and **HSQC** δ ppm (DMSO-d_6): 166.5, 159.9, 159.2, 150.5, 150.1, 148.5, 146.2, 144.6, 129.1 (CH-2′,6′), 120.3, 119.6 (CH-6), 117.7 (CH-3′,5′), 116.1 (CH-4), 115.5 (CH-7), 113.3 (CH-5″), 111.5 (CH-6″), 105.6 (CH-2″), 56.6 and 56.3 (OCH_3). (Because of the tautomeric effect one carbon atom is not observable); **MS** (ESI+) *m/z*: 389 (M + H, 100%); Anal. calcd. for $C_{22}H_{20}N_4O_3 \cdot 1.5HOH$ (C, H, N).

N-Isopropyl-2-[4-(3,4-dimethoxyphenoxy)phenyl]-1*H*-benzimidazole-5(6)-carboxamidine HCl (24)

The residue was purified by column chromatography using dichloromethane:isopropanol:ammonium hydroxide (25%) (60:30:2) as an eluant; yield 28.5%; mp: 295–298 °C; **^{1}H-NMR** δ ppm (DMSO-d_6)**:** 1.28 [d, 6H, $CH(CH_3)_2$], 3.73 and 3.75 (s,s, 6H, OCH_3), 4.12 [m, 1H, $CH(CH_3)_2$], 6.68 (dd, 1H, $J_o = 8.4$, $J_m = 2.8$, H-6″), 6.83 (d, 1H, $J_m = 2.8$, H-2″), 7.0 (d, 1H, $J_o = 8.4$, H-5″), 7.16 (d, 2H, $J_o = 8.8$, H-3′,5′), 7.74 (d, 1H, $J_o = 8.4$, H-6), 7.89 (d, 1H, $J_o = 8.4$, H-7), 8.1 (s, 1H, H-4), 8.52 (d, 2H, $J_o = 8.6$, H-2′,6′), 9.25 (s, 1H), 9.62 (s, 1H), 9.77 and 9.8 (1H); **^{13}C-NMR** δ ppm (DMSO-d_6): 162.8, 162.3, 152.1, 150.7, 148.6, 146.9, 137.1, 133.9, 131.3, 126.0, 125.5, 118.8, 117.9, 115.6, 114.8, 113.3, 112.3, 106.2, 56.6, 56.4, 45.9, 21.9; **MS** (ESI+) *m/z*: 431 (M + H, 100%); Anal. calcd. for $C_{25}H_{26}N_4O_3 \cdot 3HCl$ (C, H, N).

N-Cyclopropyl-2-[4-(3,4-dimethoxyphenoxy)phenyl]-1*H*-benzimidazole-5 (6)-carboxamidine HCl (25)

The residue was purified by column chromatography using dichloromethane:isopropanol:ammonium hydroxide (25%) (60:30:2) as an eluant; yield 27%; mp: 294–296 °C; **^{1}H-NMR** δ ppm (DMSO-d_6): 0.87 (m, 2H, cylopropyl methylene H), 0.97 (m, 2H, cylopropyl methylene H), 2.82 (m, 1H, cylopropyl methine H), 3.76&3.79 (s,s, 6H, OCH_3), 6.70 (dd, 1H, $J_o = 8.4$, $J_m = 2.8$, H-6″), 6.85 (d, 1H, $J_m = 2.8$, H-2″), 7.03 (d, 1H, $J_o = 8.2$, H-5″), 7.18 (d, 2H, $J = 8.8$, H-3′,5′), 7.73 (dd, 1H, $J_o = 8.4$, $J_m = 2.0$, H-6), 7.89 (d, 1H, $J_o = 8.4$, H-7), 8.14 (d, 1H, $J_m = 1.8$, H-4), 8.43 (d, 2H, $J = 8.4$, H-2′,6), 9.23 (s, 1H), 9.81 (s, 1H), 10.13 (s, 1H); **MS** (ESI+) *m/z*: 429 (M + H, 100%); Anal. calcd. for $C_{25}H_{24}N_4O_3 \cdot 2HCl$ (C, H, N).

N-Butyl-2-[4-(3,4-dimethoxyphenoxy)phenyl]-1*H*-benzimidazole-5(6)-carboxamidine HCl (26)

The residue was purified by column chromatography using dichloromethane:isopropanol:ammonium hydroxide (25%) (60:30:2) as an eluant; yield 34%; mp: 270 °C bubb.; **^{1}H-NMR** δ ppm (DMSO-d_6): 0.95 (t, 3H, $CH_2CH_2CH_2C\underline{H}_3$), 1.42 (m, 2H, $CH_2CH_2C\underline{H}_2CH_3$), 1.66 (m, 2H, $CH_2C\underline{H}_2CH_2CH_3$), 3.44 (q, 2H, $C\underline{H}_2CH_2CH_2CH_3$), 3.75 and 3.78 (s,s, 6H, OCH_3), 6.68 (dd, 1H, $J_o = 8.4$, $J_m = 2.8$, H-6″), 6.84 (d, 1H, $J_m = 2.8$, H-2″), 7.01 (d, 1H, $J_o = 8.4$, H-5″), 7.12 (d, 2H, $J_o = 8.8$, H-3′,5′), 7.6 (dd, 1H, $J_o = 8.4$, $J_m = 1.8$, H-6), 7.77 (d, 1H, $J_o = 8.4$, H-7), 8.05 (s, 1H, H-4), 8.32 (d, 2H, $J_o = 8.6$, H-2′,6′), 9.07 (s, 1H), 9.49 (s, 1H), 9.79 (1H); **^{13}C-NMR** δ ppm (DMSO-d_6): 164.1, 161.1, 154.3, 150.7, 149.3, 146.6, 129.8, 123.6, 123.3, 122.9, 117.9, 113.3, 112.1, 106.0, 56.6, 56.4, 43.2, 30.1, 26.2, 20.2; **MS** (ESI+) *m/z*: 445 (M + H, 100%); Anal. calcd. for $C_{26}H_{28}N_4O_3 \cdot 1.5HCl \cdot 0.25C_3H_8O$ (C, H, N).

N-Isopentyl-2-[4-(3,4-dimethoxyphenoxy)phenyl]-1*H*-benzimidazole-5 (6)-carboxamidine HCl (27)

The residue was purified by column chromatography using dichloromethane:isopropanol:ammonium hydroxide (25%) (60:30:2) as an eluant; yield 32%; mp: >300 °C; **^{1}H-NMR** δ ppm (DMSO-d_6): 0.95 (d, 6H, $CH_2CH_2CH(C\underline{H}_3)_2$), 1.59 (q, 2H, $CH_2C\underline{H}_2CH(CH_3)_2$), 1.71 (m, 1H, $CH_2CH_2C\underline{H}(CH_3)_2$), 3.44 (q, 2H, $C\underline{H}_2CH_2CH(CH_3)_2$), 3.75 & 3.78 (s,s, 6H, OCH_3), 6.68 (dd, 1H, $J_o = 8.5$, $J_m = 2.4$, H-6″), 6.85 (d, 1H, $J_m = 2.4$, H-2″), 7.03 (d, 1H, $J_o = 8.4$, H-5″), 7.17 (d, 2H, $J_o = 8.8$, H-3′,5′), 7.69 (d, 1H, $J_o = 8.4$, $J_m = 1.3$, H-6), 7.87 (d, 1H, $J_o = 8.4$, H-7), 8.09 (d, 1H, $J_m = 1.2$, H-4), 8.37 (d, 2H, $J_o = 8.4$, H-2′,6′), 9.09 (s, 1H), 9.56 (s, 1H), 9.83 (1H); **MS** (ESI+) *m/z*: 459 (M + H, 100%); Anal. Calcd. for $C_{27}H_{30}N_4O_3 \cdot 2HCl$ (C, H, N).

N-Cyclohexyl-2-[4-(3,4-dimethoxyphenoxy)phenyl]-1*H*-benzimidazole-5 (6)-carboxamidine HCl (28)

The residue was purified by column chromatography using dichloromethane:isopropanol:ammonium hydroxide (25%) (60:30:2) as an eluant; yield 33%, mp: 298 °C; **^{1}H-NMR** δ ppm (DMSO-d_6): 1.1–2 (m, 10H, methylene protons of cyclohexyl), 3.77 (m, 1H, methine proton of cyclohexyl, overlapped), 3.75 and 3.78 (s,s, 6H, OCH_3), 6.68 (dd, 1H, $J_o = 8.4$, $J_m = 2.8$, H-6″), 6.83 (d, 1H, $J_m = 2.8$, H-2″), 7.01 (d, 1H, $J_o = 8.4$, H-5″), 7.16 (d, 2H, $J_o = 8.8$, H-3′,5′), 7.69 (dd, 1H, $J_o = 8.4$, $J_m = 1.2$, H-6), 7.86 (d, 1H, $J_o = 8.4$, H-7), 8.07 (d, 1H, $J_m = 1.2$, H-4), 8.45 (d, 2H, $J_o = 8.4$, H-2′,6′), 9.25 (s, 1H), 9.56 (s, 1H), 9.68 & 9.71 (1H); **^{13}C-NMR** δ ppm (DMSO-d_6): 162.6, 162.5, 152.5, 150.7, 148.7, 146.9, 131.0, 125.6, 125.1, 119.7, 117.9, 115.8, 114.9, 113.3, 112.3, 106.15, 56.7, 56.5, 52.8, 31.8, 26.3, 25.5, 25.0; **MS** (ESI+) *m/z*: 471 (M + H, 100%); Anal. calcd. for $C_{28}H_{30}N_4O_3 \cdot 2HCl \cdot 0.1HOH$ (C, H, N).

N-Benzyl-2-[4-(3,4-dimethoxyphenoxy)phenyl]-1*H*-benzimidazole-5 (6)-carboxamidine HCl (29)

The residue was purified by column chromatography using dichloromethane:isopropanol:ammonium hydroxide (25%) (60: 30: 1) as an eluant; yield 42.5%; mp: 297 °C; **^{1}H-NMR** δ ppm (DMSO-d_6): 3.73 and 3.75 (s,s, 6H, OCH_3), 4.74 (d, 2H, Ph-C$\underline{H}_2$-NH), 6.68 (dd, 1H, $J_o = 8.4$, $J_m = 2.8$, H-6″), 6.83 (d, 1H, $J_m = 2.8$, H-2″), 7.01 (d, 1H, $J_o = 8.4$, H-5″), 7.16 (d, 2H, $J_o = 8.8$, H-3′,5′), 7.32 (m, 1H, Ph-H), 7.38 (m, 2H, Ph-H), 7.48 (d, 2H, Ph-H), 7.79 (d, 1H, $J_o = 8.4$, $J_m = 1.1$, H-6), 7.82 (d, 1H, $J_o = 8.4$, H-7), 8.19 (s, 1H, H-4), 8.47 (d, 2H, $J_o = 8.8$, H-2′,6′), 9.44 (s, 1H), 9.8 (s, 1H), 10.5 (br.t, 1H); **^{13}C-NMR** δ ppm (DMSO-d_6): 163.6, 162.6, 152.5, 150.7, 148.7, 146.9, 137.9 (br.s), 136.3, 134.7 (br.s), 131.1, 129.3, 128.5, 125.3, 125.2, 119.4 (br.s), 117.9, 115.8, 115.0, 113.3, 112.3, 106.2, 56.6, 56.4, 46.4; **MS** (ESI+) *m/z*: 479 (M + H, 100%)]; Anal. calcd. for $C_{29}H_{26}N_4O_3 \cdot 2HCl$ (C, H, N).

N-[2-(Dimethylamino)ethyl]-2-[4-(3,4-dimethoxyphenoxy)-phenyl]-1*H*-benzimidazole-5 (6)-carboxamidine HCl (30)

The residue was purified by column chromatography using chloroform:methanol:ammonium hydroxide (25%) (110:40:5) as an eluant; yield 34%; mp: 260–270 °C bubb.; **^{1}H-NMR** δ ppm (DMSO-d_6): 2.88[d, 6H, $NH^+(CH_3)_2$], 3.49[q, 2H,$(CH_3)_2NHCH_2C\underline{H}_2$], 3.76 & 3.79 (s,s, 6H, OCH_3), 3.99[q, 2H,$(CH_3)_2NHC\underline{H}_2CH_2$], 6.70 (dd, 1H, $J_o = 8.4$, $J_m = 2.8$, H-6″), 6.87 (d, 1H, $J_m = 2.8$, H-2″), 7.03 (d, 1H, $J_o = 8.8$, H-5″), 7.20 (d, 1H, $J_o = 9.2$, H-3′,5′), 7.94 (m, 2H, H-6,7), 8.3 (s, 1H, H-4), 8.53 (d, 2H, $J_o = 8.8$, H-2′,6′), 9.78 (s, 1H), 10.03 (s, 1H), 10.3 (br.t, 1H), 11 (br.m, 1H); **^{13}C-NMR** δ ppm (DMSO-d_6): 163.9, 162.8, 152.2, 150.7, 148.6, 146.9, 137.4 (br.s), 134.1 (br.s), 131.3, 125.7, 125.6, 118.9, 117.9, 115.9, 114.8, 113.3, 112.4, 106.2, 56.6, 56.5, 54.6, 42.9, 38.8; **MS** (ESI+) *m/z*: 460 (M + H, 100%); Anal. calcd. for $C_{26}H_{29}N_5O_3 \cdot 4HCl \cdot 0.5HOH$ (C, H, N).

N-Isopropyl-1-methyl-2-[4-(3,4-dimethoxyphenoxy)-phenyl]-1*H*-benzimidazole-5-carboxamidine HCl (31)

The residue was purified by column chromatography using dichloromethane:isopropanol:ammonium hydroxide (25%) (60:30:1) as an eluant; yield 29.5%; mp: >295 °C; **^{1}H-NMR** δ ppm (DMSO-d_6): 1.31[d, 6H, $CH(C\underline{H}_3)_2$], 3.76 and 3.78 (s,s, 6H, OCH_3), 3.97 (s, 3H, N^1-CH_3), 4.12[m, 1H, $C\underline{H}(CH_3)_2$], 6.68 (dd, 1H, $J_o = 8.4$, $J_m = 2.8$, H-6″), 6.86 (d, 1H, $J_m = 2.8$, H-2″), 7.01 (d, 1H, $J_o = 8.4$, H-5″), 7.13 (d, 2H, $J_o = 8.8$, H-3′,5′), 7.69 (d, 1H, $J_o = 8.4$, H-6), 7.91 (m, 3H, H-7,2′,6′ overlapped), 8.14 (s, 1H, H-4), 9.09 (s, 1H), 9.46 (s, 1H), 9.57 & 9.59 (1H); **MS** (ESI+) *m/z*: 445 (M + H, 100%); Anal. Calcd. for $C_{26}H_{28}N_4O_3 \cdot 1.5$ HCl (C, H, N).

N-Isopropyl-1-benzyl-2-[4-(3,4-dimethoxyphenoxy)-phenyl]-1*H*-benzimidazole-5-carboxamidine HCl (32)

The residue was purified by column chromatography using dichloromethane:isopropanol:ammonium hydroxide (25%) (100:50:2) as an eluant; yield 31.7%; mp: 105–110 °C bubb.; **^{1}H-NMR** δ ppm (DMSO-d_6): 1.27 [d, 6H, $CH(C\underline{H}_3)_2$], 3.71 and 3.74 (s,s, 6H, OCH_3), 4.15 [m, 1H, $C\underline{H}(CH_3)_2$], 5.76 (s, 2H, N^1-CH_2-Ph), 6.65 (dd, 1H, $J_o = 8.4$, $J_m = 2.8$, H-6″), 6.81 (d, 1H, $J_m = 2.8$, H-2″), 6.9 (d, 1H, $J_o = 8.4$, H-5″), 7.07–7.12 (m, 4H, H-3′,5′ & Ph-H), 7.24–7.23 (m, 4H, H-6 & Ph-H), 7.74 (d, 1H, $J_o = 8.8$, H-7), 7.84 (d, 2H, $J_o = 8.4$, H-2′,6′), 8.23 (s, 1H, H-4), 9.34 (s, 1H), 9.6 (s, 1H), 9.75 & 9.77 (1H); **MS** (ESI+) *m/z*: 521

Table 1. *In vitro* antistaphylococcal activity and cytotoxicity results of compound **7–11**, **23–33** and their calculated physico-chemical parameters by Lipinski's rule.

	Antistaphylococcal activity			Parameter*				
No.	*S. aureus* (ATTC 25923)	MRSA (ATTC 43300)	Cytotoxicity IC_{50}	*a-don*	*a-acc*	Log *P*	M.Wt.	TPSA
7	>25	>25	NT	1	3	4.03 ± *0.46*	346.38	52.08
8	>25	>25	NT	2	5	2.13 ± *0.84*	425.46	112.24
9	>25	>25	NT	2	6	2.93 ± *0.91*	496.57	101.49
10	>25	>25	NT	1	4	4.54 ± *0.84*	418.44	78.38
11	>25	25	NT	2	5	3.70 ± *0.84*	390.39	89.38
23	12.5†	12.5	44.4	3	5	2.46 ± *0.84*	388.42	101.95
24	12.5	6.25	55.3	3	5	3.48 ± *0.97*	430.49	87.96
25	>25	25	15.6	3	5	2.96 ± *0.97*	428.48	87.96
26	12.5	6.25	7.3	3	5	4.19 ± *0.97*	444.53	87.96
27	12.5	6.25	28.6	3	5	4.54 ± *0.97*	458.55	87.96
28	6.25	3.12	35.5	3	5	4.66 ± *0.97*	470.56	87.96
29	12.5	6.25	38.8	3	5	4.37 ± *0.97*	478.54	87.96
30	>25	>25	NT	3	6	2.75 ± *0.99*	459.54	91.20
31	>25	>25	NT	2	5	4.11 ± *1.02*	444.52	79.17
32	6.25	6.25	NT	2	5	5.89 ± *1.02*	520.62	79.17
33	25	25	NT	2	6	5.17 ± *1.04*	549.66	82.41
Ampicillin	0.78	>25	–					
Sultamicillin	0.78	25	–					
Vancomycin	0.78	0.78	–					
Na dodecyl sulfate	–	–	43.8					

NT, not tested; MRSA, methicillin resistant *Staphylococcus aureus*; *a-don*, the number of H-bond donor; *a-acc*, the number of H-bond acceptor (Marvin Sketch 6.2.0). Log *P* calculated lipophilicity (ACD Chemsketch 2012).TPSA, topological polar surface area (Perkin Elmer Chem Draw Pro).
*All calculations were made over the bases of formulas.
†This value was reported as 8 μg/mL in Ref. [5].

(M + H, 100%); Anal. Calcd. for $C_{32}H_{32}N_4O_3 \cdot 3HCl \cdot 0.5C_3H_8O$ (C, H, N).

N-(2-Dimethylaminoethyl)-1-benzyl-2-[4-(3,4-dimethoxyphenoxy)phenyl]-1*H*-benzimidazole-5-carboxamidine HCl (33)

The residue was purified by column chromatography using dichloromethane:isopropanol:ammonium hydroxide (25%) (100:50:2) as an eluant; yield 35.5%; mp: 100–105 °C bubb.; **^{1}H-NMR** δ ppm (DMSO-d_6): 2.86 [d, 6H, $NH^+(C\underline{H}_3)_2$], 3.47 [q, 2H,$(CH_3)_2NHCH_2C\underline{H}_2$], 3.72 and 3.75 (s,s, 6H, OCH_3), 3.98[q, 2H,$(CH_3)_2NHC\underline{H}_2CH_2$], 5.77 (s, 2H, N^1-CH_2-Ph), 6.46 (dd, 1H, $J_o = 8.4$, $J_m = 1.8$, H-6″), 6.82 (d, 1H, $J_m = 1.8$, H-2″), 6.99 (d, 1H, $J_o = 8.4$, H-5″), 7.11 (m, 4H, H-3′,5′ & Ph-H), 7.29 (m, 3H, Ph-H), 7.86 (m, 3H, H-2′,6′,6), 7.93 (d, 1H, $J_o = 8.6$, H-7), 8.4 (s, 1H, H-4), 9.81 (s, 1H), 9.94 (s, 1H), 10.3 (br.t, 1H), 11.1 (s, 1H); **^{13}C-NMR** δ ppm (DMSO-d_6): 163.9, 161.8, 154.6, 150.7, 148.8, 146.9, 137.9, 136.9, 136.1, 132.5, 129.6, 128.7, 127.1, 125.4, 125.3, 120.1, 118.7, 117.8, 113.3, 112.3, 106.2, 62.7, 56.6, 56.5, 54.6, 43.0, 38.8; **MS** (ESI+) *m/z*: 550 (M + H, 100%); Anal. calcd. for $C_{33}H_{35}N_5O_3 \cdot 4HCl \cdot 0.5C_3H_8O$ (C, H, N).

Biological activities

(a) Microbiological studies[14]

Activity tests were performed in Mueller–Hinton broth (MHB) (Difco, Difco Laboratories, Detroit, MI). Four or five *S. aureus* colonies from overnight growth on Tryptic Soy Agar (Merck, Darmstadt, Germany) were suspended in 5 mL saline and the turbidity was adjusted to match that of a 0.5 McFarland Standard. Then, a portion of the standardized suspension was diluted 1:100 (106 CFU/mL) with MHB. One mL of this dilution was added to each tube containing 1 mL of the compound diluted in MHB. All tubes were incubated at 37 °C for 20 h and MIC's were determined.

(b) NRU cytotoxicity test[15]

The HeLa cell line was cultured as a monolayer in an appropriate tissue culture flask at 37 °C with 5% CO_2 in F12 HAM (Biol. Ind.) containing 10% heat inactivated fetal bovine serum (Sigma, St. Gallen, Switzerland), 50 μg/mL penicillin and 50 μg/mL streptomycin (Biol. Ind.). When cells approach confluence they were removed from the flask by trypsinization. After determination of the cell number, the culture is seeded into a 96-well micro titer plate (1×10^4 cells/well) and incubated at 37 °C with 5% CO_2 for 24 h. Afterwards the culture medium were removed and the cells were treated with 100 μl treatment medium containing either eight concentrations of test chemical or the positive control (sodium dodecyl sulfate, Sigma) or the vehicle (medium). After 24 h of treatment period, the medium was removed and the cells were washed with 150 μl PBS. Thereafter, PBS was aspirated and the cells were incubated in 100 μl of neutral red medium for additional 3 h. After this final incubation period, neutral red medium were discarded and washed with 150 μl PBS. Finally 150 μl EtOH/acetic acid (1% glacial acetic acid, 50% EtOH, %49 H_2O) solution was added to all wells and the 96-well plate was shaken for 10 min in a micro-plate shaker. The absorption of the colored solution was measured at 540 nm by a micro-plate reader and the related IC_{50} values were computed.

Results and discussion

The synthesized benzimidazoles **7–11** and **23–33** were evaluated for antibacterial activity against Gram-positive *S. aureus* and methicillin-resistant *S. aureus* (MRSA) as *in vitro* by the macro-broth dilution[9] assay and the MICs values are listed in Table 1. The synthesized compounds and reference drugs were dissolved in water or DMSO–water (40%) at a concentration of 400 μg/mL. The concentration was adjusted to 100 μg/mL by four-fold dilution with media culture and bacteria solution at the first tube. Data were not taken for the initial solution because of the high DMSO concentration (10%). MIC values were determined as

the lowest concentration at which no growth was observed upon visual inspection after incubating for 20 h at 37 °C. From the results of the antistaphylococcal activity of the synthesized compounds **7**–**11** and **23**–**33**, the following structure activity relationship can be presented: the antistaphyloccoccal activity was considerably affected by substitution pattern at the C-5(6) position of benzimidazole ring. Unsubstitution **7**, substitution with sulfonamide **8**, N-alkylsulfonamide **9**, ester **10**, and carboxylic acid **11** of this position resulted in decrease activity which is almost inactive against both bacteria. However, on the same position, substitution with carboxamidine **23**–**33** showed good antistaphylococcal activity profile with the MIC values of between the 25 and 3.12 μg/mL (Table 1).

It is apparent that carboxyamidine moiety at this position is critical for optimal antistaphylococcal activity. The mechanism of action should be explained by the inhibition of histidine protein kinases from bacterial two-component systems as it was previously reported[5]. Among them, compound **28** with the cyclohexyl group at the nitrogen atom of carboxamidine exhibited best potent activity with a MIC value of 3.12 μg/mL, which is only two times less active than Vancomycin and Ampicillin. The presence of lipophilic substituent such as cyclohexyl on the amidine moiety provided a positive influence on antibacterial activity. While ampicillin and sultamicillin are practically inactive against MRSA (25 μg/mL), most of the benzimidazole-carboxamidines **13**–**23** are effective against MRSA, this is highly important advantage. Although there are no enough samples, it may be concluded that, N^1-substitution of benzimidazole moiety results in loss of activity as in compounds **31**–**33**. In contrast, in last decade, several new physico-chemical parameters have been introduced for absorption prediction, including molecular size and shape descriptors, hydrogen-bonding capabilities, and surface properties[16]. A set of rules imposing limitations on log *P*, molecular weight, and the number of hydrogen bond donors and acceptors (known as the ''rule of 5'') introduced by Lipinski[17] (not more than 5 hydrogen bond donors, not more than 10 hydrogen bond acceptors, a molecular weight under 500 Da, and log *P* of less than 5) has become particularly popular. Another very useful parameter for prediction of drug candidate properties is the topological polar surface area (TPSA)[18] that is defined as a sum of surfaces of polar atoms (usually oxygens, nitrogens, and attached hydrogens) in a molecule. This parameter has been shown to correlate very well with ADME properties, including blood–brain barrier crossing tendency. For this purpose, we have also calculated these parameters by using some computer program (ACD Chemsketch 2012, Marvin Sketch 6.2.0 and Perkin Elmer Chem Draw Pro, PerkinElmer, Inc., Waltham, MA) and are reported in Table 1. As seen here, all of our active compounds obey these rules, it means that, these compounds should present good profile of ADME.

For further pharmacological study, cytotoxic properties of the microbiologically active benzimidazolecarboxamidines against HeLa cells were determined using neutral red uptake (NRU) cytotoxicity test. The results are summarized in Table 1. It was observed that with the exception of **26** none of the tested compounds exhibited significant cytotoxic effect on HeLa cells.

Conclusion

In summary, a new series of benzimidazolecarboxamidines have been successfully synthesized starting from uncommercial *o*-phenylenediamines and sodium bisulphite adduct of 4-(3,4-dimethoxy)phenoxybenzaldehyde. All new compounds were confirmed by ^{1}H-NMR, ^{13}C-NMR, COSY, HSQC, HMBC, LC-MS, and elemental analyze data. The *in vitro* antistaphylococcal activity revealed that all the carboxamidines showed moderate to potent inhibitory activity, against *S. aureus* and methicillin-resistant *S. aureus* (MRSA) strains and compound **28** displayed close activity to the reference clinical drugs. In our previous work[2–4], we had published more potent amidine type of benzimidazoles, however, their cytotoxic properties were not cleared. This result manifested that compound **28** should be worthy to be further investigation.

Declaration of interest

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