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Preliminary communication

Synthesis and potent antibacterial activity against MRSA of some novel 1,2-disubstituted-1*H*-benzimidazole-*N*-alkylated-5-carboxamidines

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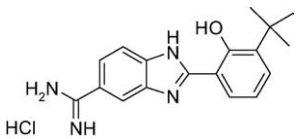
Abstract

A series of 28 novel 1,2-disubstituted-1*H*-benzimidazole-*N*-alkylated-5-carboxamidine derivatives were synthesized and evaluated for in vitro antibacterial activities against *Staphylococcus aureus* and methicillin resistant *S. aureus* (MRSA) by the tube dilution method. The results showed that compounds **45**–**46** and **55**–**57**, having 3,4-dichloro substituted phenyl at the position C-2, of *N*-bulky alkyl substituted benzimidazolecarboxamidines exhibited the greatest activity with MIC values of 1.56–0.39 μg/ml.

Keywords: 1*H*-benzimidazole-carboxamidines; Methicilline resistant *S. aureus*; Antibacterial activity

1. Introduction

There has been an alarming increase in the incidence of Gram-positive infections. Among them *Staphylococcus aureus* is one of the most common human pathogens. It is the primary bacteria in many skin and mucous membrane infections and easily transmitted to uninfected areas of the body. Moreover, methicillin resistant *S. aureus* (MRSA) represents a therapeutic problem of increasing importance, especially in hospital patients [1,2]. MRSA attracted attention in the late 1960s in Europe and the first report on its importance was published in USA. Thereafter, MRSA infection became a large issue in the 1980s [3]. At the moment most of the known commercial antibiotics are inactive against the MRSA. Vancomycin, the most effective antibiotic against MRSA infection, was not available for use in Japan until 1992 and therefore it was difficult to treat MRSA due to the lack of effective drugs [3]. In addition, with the recent increased use of vancomycin in methicilline resistance *S. aureus* infections, resistance to vancomycin has been spreading. Recent reports from Japan [4] and the USA [5] of the isolation of an intermediate vancomycin-resistant strain of *S. aureus* have appeared which increases the urgency for development of new classes of antibacterial agents. Recently, some novel amidinobenzimidazoles [6] (Formula 1) have been identified as inhibitors of the bacterial KinA/SpoOF two-component system TCS (which include a histidine protein kinase HPK and a response regulator RR) which are responsible for adaption of bacteria to the environmental changes and survival within the host. These amidino inhibitors display good in vitro antibacterial activity in particular, against Gram-positive bacteria (i.e. *S. aureus*, MRSA, VRE vancomycin-resistant *Enterococcus*). Based on these considerations, we have done some modifications on the benzimidazole ring and a series of *N*-alkylated carboxamidines were prepared which show promising activity against MRSA.



Formula 1.

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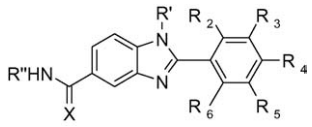
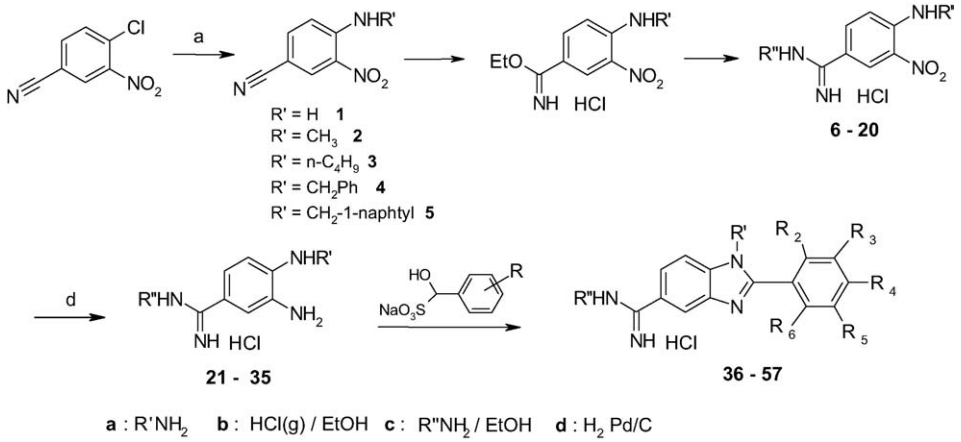
2. Chemistry

The synthetic pathways for preparation of the benzimidazoles listed in Tables 1 and 2 is shown in Scheme 1. Nucleophilic displacement of the chloro group of 4-chloro-3-nitrobenzonitrile by the reaction with several amines in DMF gave **1**–**5**. The nitrile group of these compounds were converted into the imidate ester, using Pinner method [7], and the imidate esters was used directly without characterization to make the amidines **6**–**20**. Their reduction with H_2, Pd/C

Table 1
Formulas and in vitro antibacterial activities as MIC (μg/ml) for **36**–**64**



Numbers	X	R′	R″	R_2	R_3	R_4	R_5	R_6	*S. aureus* ATTC 25923	MRSA ATCC 43300	MRSA clinical isolate from blood	MRSA clinical isolate from wound
36	NH	H	H	H	Cl	Cl	H	H	6.25	6.25	6.25	3.12
37	NH	H	isopropyl	H	H	Cl	H	H	12.5	100	12.5	6.25
38	NH	H	isopropyl	H	Cl	H	H	H	12.5	50	12.5	12.5
39	NH	H	isopropyl	H	Cl	Cl	H	H	12.5	12.5	1.56	1.56
40	NH	H	cyclopropyl	H	Cl	Cl	H	H	6.25	12.5	3.12	6.25
41	NH	H	n-butyl	H	Cl	Cl	H	H	3.12	12.5	1.56	1.56
42	NH	H	isopentyl	H	Cl	Cl	H	H	1.56	6.25	0.78	0.78
43	NH	H	cyclohexyl	H	Cl	Cl	H	H	3.12	6.25	3.12	3.12
44	NH	H	CH_2Ph	H	Cl	Cl	H	H	1.56	1.56	1.56	0.78
45	NH	H	2.4-dichlorobenzyl	H	Cl	Cl	H	H	0.78	0.78	0.39	1.56
46	NH	H	CH_2-1-naphtyl	H	Cl	Cl	H	H	0.39	0.78	0.39	0.78
47	NH	H	isopropyl	H	Cl	H	Cl	H	12.5	12.5	12.5	12.5
48	NH	H	isopropyl	Cl	H	H	H	Cl	> 100	NT	NT	NT
49	NH	H	isopropyl	H	F	F	H	H	50	NT	NT	NT
50	NH	H	isopropyl	H	OCH_3	OCH_3	H	H	> 100	NT	NT	NT
51	NH	H	isopropyl	H	H	CN	H	H	100	NT	NT	NT
52	NH	methyl	isopropyl	H	Cl	Cl	H	H	> 100	NT	NT	NT
53	NH	n-butyl	isopropyl	H	Cl	Cl	H	H	6.25	12.5	6.25	6.25
54	NH	CH_2Ph	isopropyl	H	Cl	Cl	H	H	6.25	12.5	3.12	3.12
55	NH	CH_2-1-naphtyl	isopropyl	H	Cl	Cl	H	H	0.78	3.12	0.39	3.12
56	NH	CH_2-1-naphtyl	isopentyl	H	Cl	Cl	H	H	0.78	1.56	0.39	3.12
57	NH	CH_2-1-naphtyl	CH_2Ph	H	Cl	Cl	H	H	0.78	1.56	0.78	3.12
63	O	H	isopropyl	H	F	F	H	H	> 100	NT	NT	NT
64	O	H	n-butyl	H	Cl	Cl	H	H	> 100	NT	NT	NT
Formula 1									0.78	0.78	0.39	0.78
Ampicillin									0.78	50	50	50
Sultamicillin									0.39	25	25	25



Scheme 1.

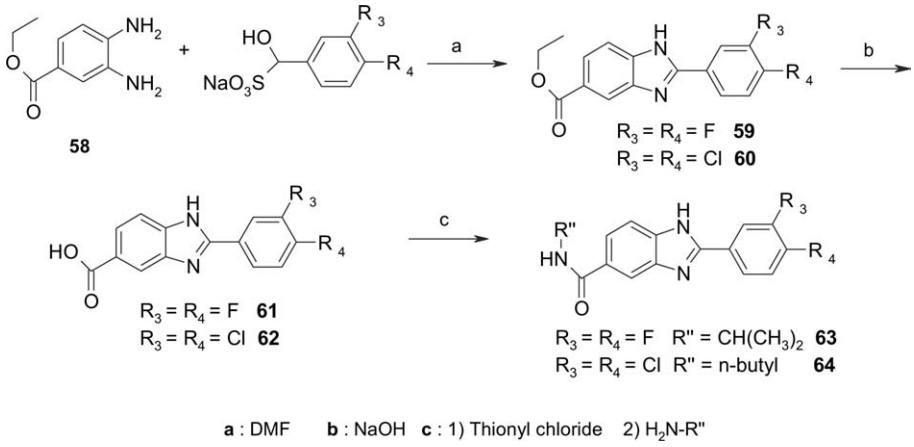
Table 2
Physical and spectral data for compounds **36–64**

Numbers	M.p. (°C)	Yield (%)	Formula Anal. (C,H,N)	^{1}H-NMR (δ ppm) (DMSO-d_6)	Mass (ESI+) M + 1	Isolation
36	305–309	57.3	$C_{14}H_{10}Cl_2N_4$ 2HCl·1.5HOH	7.84(m,3H), 8.28(m,2H), 8.6(m,1H), 9.18 and 9.47(s,2H)	305(100), 307(70), 309(12)	Cryst. 2 N HCl
37	340–345	62	$C_{17}H_{17}ClN_4$ 2HCl	1.29(d,6H), 4.14(m,1H), 7.66(d, *J* = 8.8,1H), 7.73 (d, *J* = 8.4,2H), 7.87(d, *J* = 8.4,1H), 8.1(s,1H), 8.41 (d, *J* = 8.4,2H), 9.13(s,1H), 9.52(s,1H), 9.67(d,1H)	313(100), 315(31)	Cryst. Ethanolic HCl
38	316–320	65	$C_{17}H_{17}ClN_4$ 2HCl·0.75HOH	1.29(d,6H), 4.1(m,1H), 7.64–8.45(aromat.7H), 9.1(s,1H), 9.49(s,1H), 9.63(d,1H)	313(100), 315(31)	Cryst. Ethanolic HCl
39	317–318	49	$C_{17}H_{16}Cl_2N_4$ 2HCl·0.25HOH	1.31(d,6H), 4.11(m), 7.61–8.61(aromat. 6H), 9.08(s,1H), 9.46(s,1H), 9.61(d,1H)	347(100), 349(65), 351(11)	$CHCl_3$/isopropanol/NH_3 (100:50:2.5) cc
40	324–327	29	$C_{17}H_{14}Cl_2N_4$ 2HCl·HOH	0.86 & 0.95(m,4H), 2.8(m,1H), 7.62–8.6(aromat. 6H), 9.16(s,1H), 9.71(s,1H), 10.2(d,1H)	345(100), 347(67), 349(12),	$CHCl_3$/isopropanol/isopropylamine (100:50:2.5) cc
41	326–329	66	$C_{18}H_{18}Cl_2N_4$ 2HCl	0.93(t,3H), 1.39(m,2H), 1.65(m,2H), 3.44(q,2H), 7.64–8.6(aromat. 6H), 9.09(s,1H), 9.53(s,1H), 9.85(d,1H)	361(100), 363(70), 365(14)	$CHCl_3$/isopropanol/NH_3 (100:50:2.5) cc
42	322–328	51	$C_{19}H_{20}Cl_2N_4$ 2HCl	0.95(d,6H), 1.59(m,2H), 1.69(m,1H), 3.45 (q,2H), 7.63–8.61(aromat. 6H), 9.08(s,1H), 9.52(s,1H), 9.78(d,1H)	375(100), 377(65), 379(12)	$CHCl_3$/isopropanol/isopropylamine (100:50:2.5) cc
43	310–320 bubb.	81	$C_{20}H_{20}Cl_2N_4$ 2HCl·5HOH	1.01–1.97(m,9H), 3.4(m,1H), 7.55–8.56 (aromat. 6H), 9.18(s,1H), 9.44(s,1H), 9.53(d,1H)	387(100), 389(66), 391(13)	Cryst. Ethanolic HCl
44	110–120 bubb.	39	$C_{21}H_{16}Cl_2N_4$ 0.5EtOH·0.75HOH	4.6(s,2H), 7.28–8.41(aromat. 11H)	395(100), 397(66), 399(13)	$CHCl_3$/isopropanol/NH_3 (100:50:2) cc
45	140–150 bubb.	54.5	$C_{21}H_{14}Cl_4N_4$ 0.5EtOH·0.5HOH	4.53(s,2H), 7.28–8.41(aromat. 9H)	463(70), 465(100), 467(50), 469(10)	$CHCl_3$/isopropanol/NH_3 (100:50:1) cc
46	125 bub 218–220	25	$C_{25}H_{18}Cl_2N_4$ 0.5C_3H_8O·HOH	4.98(s,2H), 7.52–8.41(aromat. 13H)	445(100), 447(70), 449(12)	$CHCl_3$/isopropanol/NH_3 (100:50:1) cc
47	306–308	53	$C_{17}H_{16}Cl_2N_4$ 2HCl	1.3(d,6H), 4.13(m,1H), 7.63–8.44(m,6H), 9.15(s,1H), 9.51(s,1H), 9.66(d,1H)	347(100), 349(63), 351(11)	Cryst. Ethanolic HCl
48	285–290	22	$C_{17}H_{16}Cl_2N_4$ 2HCl·0.5EtOH·HOH	1.29(d,6H), 4.16(m,1H), 7.64–8.15(m,6H), 9.26(s,1H), 9.52(s,1H), 9.63(d,1H)	347(100), 349(61), 351(12)	$CHCl_3$/isopropanol/NH_3 (100:50:4) cc
49	323–326	32	$C_{17}H_{16}F_2N_4$ 2HCl·0.5EtOH·HOH	1.3(d,6H), 4.11(m,1H), 7.63–8.53(m,6H), 9.11(s,1H), 9.5(s,1H), 9.64(d,1H)	315(100)	$CHCl_3$/isopropanol/NH_3 (100:50:2) cc
50	304–309	40.5	$C_{19}H_{22}N_4O_2$ 2HCl·3HOH	1.312(d,6H), 3.88(s,3H), 3.92(s,3H), 4.11(m,1H), 7.27(d,1H), 7.69(d,1H), 7.87(d,1H), 8.11(m, 2H), 8.18(s,1H), 9.14(s,1H), 9.56(s,1H), 9.71(d,1H)	339(100)	$CHCl_3$/isopropanol/NH_3 (100:50:3) cc
51	325–327	71	$C_{18}H_{17}N_5$ 2HCl·HOH	1.32(d,6H), 4.11 (m,1H), 7.66(d,1H), 7.69(d,1H), 8.11(m,3H), 8.57(m,2H), 9.16(s,1H), 9.53(s,1H), 9.66(d,1H)	304(100)	Cryst. Ethanolic HCl

(continued on next page)

Table 2
(continued)

Numbers	M.p. (°C)	Yield (%)	Formula Anal. (C,H,N)	^{1}H-NMR (δ ppm) (DMSO-d_6)	Mass (ESI+) M + 1	Isolation
52	298–300	44	$C_{18}H_{18}Cl_2N_4$ 2HCl·0.25C_3H_8O·0.5HOH	1.29(d,6H), 3.99(s,3H), 4.16(m,1H), 7.75–8.19 (aromat.6H), 9.21(s,1H), 9.56(s,1H), 9.68(d,1H)	361(100), 363(66), 365(12)	$CHCl_3$/isopropanol/isopropylamine (100:50:3) cc
53	125 bub 300–303	42	$C_{21}H_{24}Cl_2N_4$ 2HCl·0.1EtOH·HOH	0.75(t,3H), 1.1(m,2H), 1.31(d,6H), 1.63(m,2H), 4.15(m,1H), 4.42(t,2H), 7.72–8.18(m,6H), 9.21(s,1H), 9.54(s,1H), 9.64(d,1H)	403(100), 405(70), 407(14)	$CHCl_3$/isopropanol/isopropylamine (100:50:3) cc
54	100 bub 290–293	30	$C_{24}H_{22}Cl_2N_4$ 2HCl·0.25EtOH·1.5HOH	1.28(d,6H), 4.12(m,1H), 5.72(s,2H), 6.97–8.2 (m,11H), 9.17(s,1H), 9.49(s,1H), 9.6(d,1H)	437(100), 439(62), 441(11)	$CHCl_3$/isopropanol/isopropylamine (100:50:2) cc
55	150 bub 290–293	25.5	$C_{28}H_{24}Cl_2N_4$ 2HCl·HOH	1.28(d,6H), 4.1(m,1H), 6.2(s,2H), 6.54 (d,1H), 7.3–8.25(other aromat. 12H), 9.11(s,1H), 9.46(s,1H), 9.57(d,1H)	487(100), 490(71), 492(13)	$CHCl_3$/isopropanol/NH_3 (100:50:2) cc
56	180 bub 293–298	17	$C_{30}H_{28}Cl_2N_4$ 2HCl·HOH	0.9(d,6H), 1.56(q,2H), 1.66(m,1H), 3.42(q,2H), 6.18(s,2H), 6.5(d,1H), 7.29–8.23(aromat. 12H), 9.02(s,1H), 9.44(s,1H), 9.68(s,2H)	515(100), 517(55), 519(10)	$CHCl_3$/isopropanol/NH_3 (100:50:1) cc
57	125 bub 250–255	22.5	$C_{32}H_{24}Cl_2N_4$ 1.5HCl·0.25C_3H_8O	4.74(s,2H), 6.2(s,2H), 6.54(d,1H), 7.3–8.35 (m,17H), 9.3(s,1H), 9.63(s,1H), 10.36(s,1H)	535(100), 537(65), 539(12)	$CHCl_3$/isopropanol/NH_3 (100: 50: 1) cc
59	233–235	88	$C_{16}H_{12}F_2N_2O_2$	1.34(d,6H), 4.33(q,2H), 7.66–8.2(aromat. 6H)	303(100)	Crys. DMF/EtOH (5:95)
60	246–248	85	$C_{16}H_{12}Cl_2N_2O_2$	1.34(d,6H), 4.33(q,2H), 7.68–8.39(aromat. 6H), 13.4(s,1H)	335(100), 337(62), 338(11)	Crys. DMF/EtOH (15:85)
61	> 300	90	$C_{14}H_8F_2N_2O_2$	7.63–8.25(6H)	275(100)	Crys. DMF/EtOH (5:95)
62	> 300	91	$C_{14}H_8Cl_2N_2O_2$	7.68(d, J = 8,1H), 7.85(d,2H), 8.16(m,2H), 8.41(d, J = 2,1H)	307(100), 309(69), 311(12)	Crys. DMF/EtOH (15:85)
63	243–244	56	$C_{17}H_{15}F_2N_3O$·0.3HOH	1.19(d,6H), 4.12(m,1H), 7.60–8.23 (aromat. 6H), 13(br.s,1H)	316(100)	EtOAc: n-Hexane 50% cc
64	253–256	51	$C_{18}H_{17}Cl_2N_3O$	0.91(t,3H), 1.35(m,2H), 1.52(m,2H), 3.33 (t,2H), 7.62–8.47 (m,6H), 13.3(br.s,1H)	362(100), 364(65), 366(13)	EtOAc: n-Hexane 50% cc



Scheme 2.

produced **21**–**35**. Condensation of these derivatives with the $Na_2S_2O_5$ adduct of appropriate benzaldehydes [8] gave the targeted benzimidazoles **36**–**57**. Since these amidines exhibited very potent antibacterial activity against *S. aureus*, it was deemed important to prepare the corresponding amide derivatives (Scheme 2) to study the role of the amidino center. So, compound **59** and **60** were obtained by the condensation of **58** with the $Na_2S_2O_5$ adduct of 3,4-di-(fluoro/chloro)-benzaldehydes [8] in DMF. Saponification of ester gave **61**–**62**. The carboxyl group was converted to the acid chloride using $SOCl_2$, then reaction with isopropyl or butylamine gave desired amide derivatives **63**, **64**.

3. Results and discussion

The benzimidazoles **36**–**64** were tested in vitro for antibacterial activity against Gram-positive *S. aureus* (ATCC 25923), MRSA (ATCC 43300 and *clinical isolates from wound and blood*), for antibacterial activity by the macrobroth 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 fourfold 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%). The parent amidine (**36**) and compounds with relatively small alkyl groups on the amidino group (**37**–**40**) are only moderately effective against the bacteria studied. Introduction of more lipophilic substituents on the amidine group (**41**–**46**) increases the antibacterial activity significantly yielding very promising compounds such as **45** and **46**. It seems that 3,4-dichloro substitution on the *2*-phenyl yields the most active compounds since the MIC value of **39** is superior to that of **48**–**51**, the latter include other chloro isomers and fluoro, methoxy and cyano substituted analogues. The combination of moderate sized *N*-substitution on the benzimidazole N-atom with the *N*-isopropylamidino group does not enhance the antibacterial effect (**52**–**54**). However, more lipophilic substitution on the benzimidazole N-atom does lead to quite active compounds (**55**–**57**). Replacement of the cationic amidine unit by the neutral amide group results in the loss of activity (**63**, **64**).

4. Conclusion

This work demonstrates that monoamidines in the benzimidazole series can show very good activity profiles versus Gram-positive bacteria *S. aureus*. Compounds **45**–**46** and **55–57**, having *N*-bulky alkyl substituted amidino groups at position C-5 and 3,4-dichloro substituted phenyl at the position C-2 of benzimidazole exhibited the greatest activity against *S. aureus*, MRSA with MIC values of 1.56–0.39 μg/ml. More extensive study is needed to confirm these preliminary results and in vivo and mode of action studies are required to be able to optimize the effectiveness of this series of compounds.

5. Experimental

Uncorrected melting points were measured on a Electrothermal 9100 capillary melting point apparatus. ^{1}H-NMR spectra were recorded employing VARIAN Mercury 400 FT spectrometer, chemical shifts (δ) are in ppm relative to TMS, and coupling constants (J) are reported in hertz. Mass spectra were taken on Waters Micromass ZQ by using ESI(+) method. Microanalyses were performed by Leco CHNS-932 (TUBITAK Ins. Anal. Lab., Ankara) and were within ± 0.4% of calculated values. All chemicals and solvents were purchased from Aldrich Chemical Co. or Fischer Scientific. Compounds **2** [10], **3** [11], **4** [11] and Formula 1 [6] were prepared according to the literature method.

*5.1. 4-[(Naphtalen-1-ylmethyl)-amino]-3-nitrobenzonitrile (**5**)*

To a solution of 4-chloro-3-nitrobenzonitrile (7 mmol, 1.28 g) in DMF (3 ml), 1-naphthalenemethylamine (10 mmol,

1.57 g) was added and the mixture was heated for 5 h at 110 °C. The hot reaction mixture was allowed to recrystallization from EtOH. A second recrystallization of the product from EtOH gave pure **5**, yield 77.8%, 1.65 g, m.p. 147–8 °C, yellow in color, ^{1}H-NMR ($CDCl_3$) δ: 5.01(d,2H), 6.94(d,1H), 7.43–7.6(m,5H), 7.87(dd,1H), 7.92(d,2H), 8.54(s,1H), 8.75(s,1H), ^{13}C-NMR ($CDCl_3$) δ: 45.37, 98.8, 115.2, 117.8, 122.4, 125.4, 125.5, 126.3, 126.9, 129.1, 129.2, 130.6, 130.9, 131.8, 132.1, 134.03, 137.8, 146.9 Ref. [12]: ^{1}H-NMR ($CDCl_3$) δ: 5.01(d,2H), 6.92(d,1H), 7.4–7.6(m,6H), 7.87(dd,1H), 8.55(s,1H), 8.74(s,1H), ^{13}C-NMR ($CDCl_3$) δ: 45.5, 98.3, 115.4, 118.0, 122.5, 125.62, 125.64, 126.5, 127.1, 129.3, 129.4, 130.8, 131.0, 132.0, 132.3, 134.2, 138.0, 147.1, $C_{18}H_{13}N_3O_2$. ^{13}C-NMR is in agreement with the data given in Ref. [12]. However in their NMR spectra, the peak at 7.92 ppm was not reported.

5.2. General procedure for synthesis of (*6–20*)

1–5 (4.5 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 esters were precipitated as yellow solids, washed with ether, then dried under vacuum at room temperature. All imidate esters were used directly without characterization. A suspension of imidate ester HCl in absolute EtOH was stirred with corresponding amines (1.5–2-fold excess) overnight at 25–30 °C. The reaction mixture was evaporated, and diluted with ether, the precipitate filtered, washed with ether, then dried (Table 3). For compound **13** and **14**, the dark orange colored precipitate which first appeared was removed by filtration, and the mother liquor yielded a second crop of pure yellow colored product.

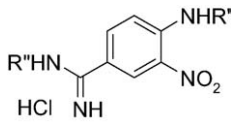
5.3. General procedure for synthesis of (*21–35*)

Compounds **6–20** (3.5 mmol) in EtOH (75 ml) was subjected to hydrogenation using 40 psi of H_2 and 10% Pd–C (40 mg) until of H_2 uptake ceased. The catalyst was filtered on a bed of Celite, washed with EtOH, and the filtrate was concentrated in vacuo. The crude *o*-phenylenediamines (gray–purple–black in color) were used for the further steps without crystallization (Table 4). In order to prevent halogen reduction of compound **28**, 15 psi of H_2 pressure was employed.

5.4. General procedure for synthesis of (*36–57*) and (*59–60*)

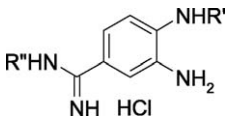
The corresponding benzaldehydes (15 mmol) were dissolved in 50 ml EtOH and sodium metabisulfite (1.6 g) in 10 ml H_2O was added in portions. The reaction mixture was stirred vigorously and more EtOH was added. The mixture was kept in a refrigerator for a several hours. The precipitate was filtered and dried (yield over 93%). The mixture of these salts (2 mmol) and **21–35** and **58** in DMF (5 ml) were heated at 120 °C for 4 h. The reaction mixture was cooled, poured into H_2O, made alkaline with dilute Na_2CO_3 solution and the solid was filtered. Purification methods are given in Table 2.

Table 3
Formulas, m.p., yields and mass spectra of **6–20**



Numbers	R′	R″	Formula	M.p. (°C)	Yield (%)	MS ESI(+) M + H (100%)	Ref.
6	–H	–H	$C_7H_8N_4O_2$	> 300 Ref. [13] > 300	71	181	[13]
7	–H	Isopropyl	$C_{10}H_{14}N_4O_2$	274–276 Ref. [14] > 300	68	223	[14]
8	–H	cyclopropyl	$C_{10}H_{12}N_4O_2$	259–262	51	221	[15]
9	–H	n-butyl	$C_{11}H_{16}N_4O_2$	265–268 Ref. [16] 265	69	237	[16,17]
10	–H	Isopentyl	$C_{12}H_{18}N_4O_2$	270–275	67	251	
11	–H	cyclohexyl	$C_{13}H_{18}N_4O_2$	> 300	60	263	[15,16]
12	–H	$–CH_2Ph$	$C_{14}H_{14}N_4O_2$	285–288	61	271	[16]
13	–H	2,4-dichlorobenzyl	$C_{14}H_{12}Cl_2N_4O_2$	155 bubb. 280–282	44	339(100), 341(63), 343(11)	
14	–H	$–CH_2$-1-naphtyl	$C_{18}H_{16}N_4O_2$	235–240	32	321	
15	–methyl	Isopropyl	$C_{11}H_{16}N_4O_2$	260–261	76	237	
16	–n-butyl	Isopropyl	$C_{14}H_{22}N_4O_2$	202–206	59	279	
17	$–CH_2Ph$	Isopropyl	$C_{17}H_{20}N_4O_2$	239–241	51	313	
18	$–CH_2$-1-naphtyl	Isopropyl	$C_{21}H_{22}N_4O_2$	225–231	64	363	
19	$–CH_2$-1-naphtyl	Isopentyl	$C_{23}H_{26}N_4O_2$	288–290	63	391	
20	$–CH_2$-1-naphtyl	$–CH_2Ph$	$C_{25}H_{22}N_4O_2$	250–255	66	411	

Table 4
Formulas, m.p., yields and mass spectra of **21–35**



Numbers	R′	R″	Formula	M.p. (°C)	Yield (%)	MS ESI(+) M + H (100%)	Ref.
21	–H	–H	$C_7H_{10}N_4$	238–239 Ref. [13] 237–9	94	151	[13]
22	–H	isopropyl	$C_{10}H_{16}N_4$	228 Ref. [14] 227–31	93	193	[14]
23	–H	cyclopropyl	$C_{10}H_{14}N_4$	105–110	91	191	[15]
24	–H	n-butyl	$C_{11}H_{18}N_4$	167–170 Ref. [16] 170 Ref. [17] 150.4	95	207	[16,17]
25	–H	isopentyl	$C_{12}H_{20}N_4$	115–120	93	221	
26	–H	cyclohexyl	$C_{13}H_{20}N_4$	110(Bubb.)	92	233	[15,16]
27	–H	$–CH_2Ph$	$C_{14}H_{16}N_4$	80(Bubb) 125–127	91	241	[16]
28	–H	2,4-dichlorobenzyl	$C_{14}H_{14}Cl_2N_4$	[a]	76	309(100) 311(68) 313(11)	
29	–H	$–CH_2$-1-naphtyl	$C_{18}H_{18}N_4$	[a]	94	291	
30	–methyl	isopropyl	$C_{11}H_{18}N_4$	117–120	91	207	
31	–n-butyl	isopropyl	$C_{14}H_{24}N_4$	128–130	90	249	
32	$–CH_2Ph$	isopropyl	$C_{17}H_{22}N_4$	106–110	89	283	
33	$–CH_2$-1-naphtyl	isopropyl	$C_{21}H_{24}N_4$	waxy	95	333	
34	$–CH_2$-1-naphtyl	isopentyl	$C_{23}H_{28}N_4$	waxy	94	361	
35	$–CH_2$-1-naphtyl	$–CH_2Ph$	$C_{25}H_{24}N_4$	semi-solid	92	381	

[a] No sharp melting points.

5.5. General procedure for synthesis of (*61–62*)

Compounds **59** and **60** (1 mmol) were dissolved in 3% NaOH in % 50 EtOH–HOH (10 ml) and heated under reflux for 1 h, cooled, water was added and acetic acid was added to obtain the carboxylic acids. Purification methods are given in Table 2.

5.6. General procedure for synthesis of (*63–64*)

61 and **62** (0.75 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 (0.5 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. Purification methods are given in Table 2.

5.7. Microbiological studies

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, Merck KGaA, Darmstadt) 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 (10^6 CFU/ml) with MHB. One milliliter of this dilution was added to each tube containing 1 ml of the compound diluted in MHB.

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