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Synthesis and Potent Antimicrobial Activity of Some Novel N-(Alkyl)-2-Phenyl-1*H*-Benzimidazole-5-Carboxamidines

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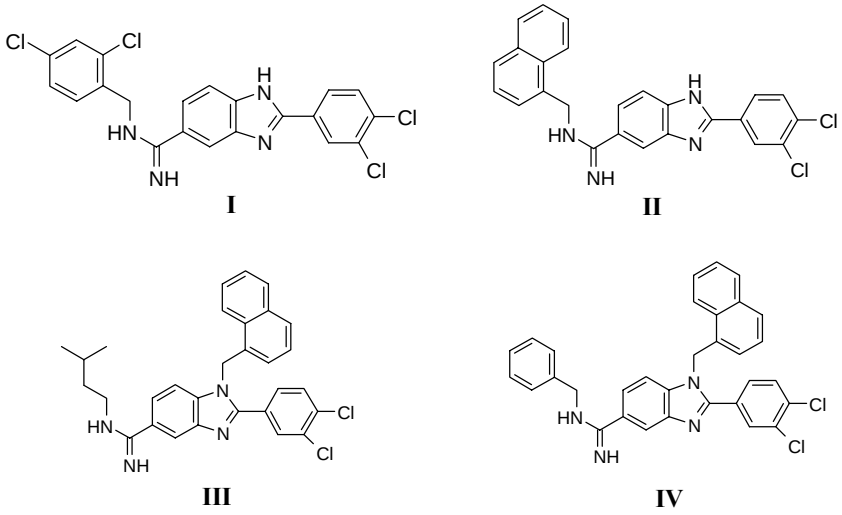
Abstract: A series of 22 novel 1,2-disubstituted-1*H*-benzimidazole-*N*-alkylated-5-carboxamidine derivatives were synthesized and evaluated for *in vitro* antibacterial activity against *S. aureus* and methicillin resistant *S. aureus* (MRSA), *E. coli*, *E. faecalis* and for antifungal activity against *C. albicans*. Compound **59** [1-(2,4-dichlorobenzyl)-N-(2-diethylaminoethyl)-1*H*-benzimidazole-5-carboxamidine], with a 3,4-dichlorophenyl group at the C-2 position, displayed the greatest activity (MIC = 3.12 µg/mL against both some bacteria and the fungus *C. albicans*).

Keywords: 1*H*-Benzimidazole carboxamidines, methicillin-resistant *S. aureus* (MRSA), antibacterial activity and antifungal activity.

Introduction

We have already reported the synthesis of a series of 1,2-disubstituted-1*H*-benzimidazole-*N*-alkylated-5-carboxamidine derivatives and their very potent antibacterial activities against *S. aureus* and methicillin resistant *S. aureus* [1]. The study revealed that compounds **I**–**IV** (Figure 1) exhibited the best activity, with MIC values of 0.78 - 0.39 µg/mL against these species. As part of a continuing program focused on development of new antimicrobial benzimidazole carboxamidines, we planned to modify the structure of compounds **I**–**IV**.

Figure 1

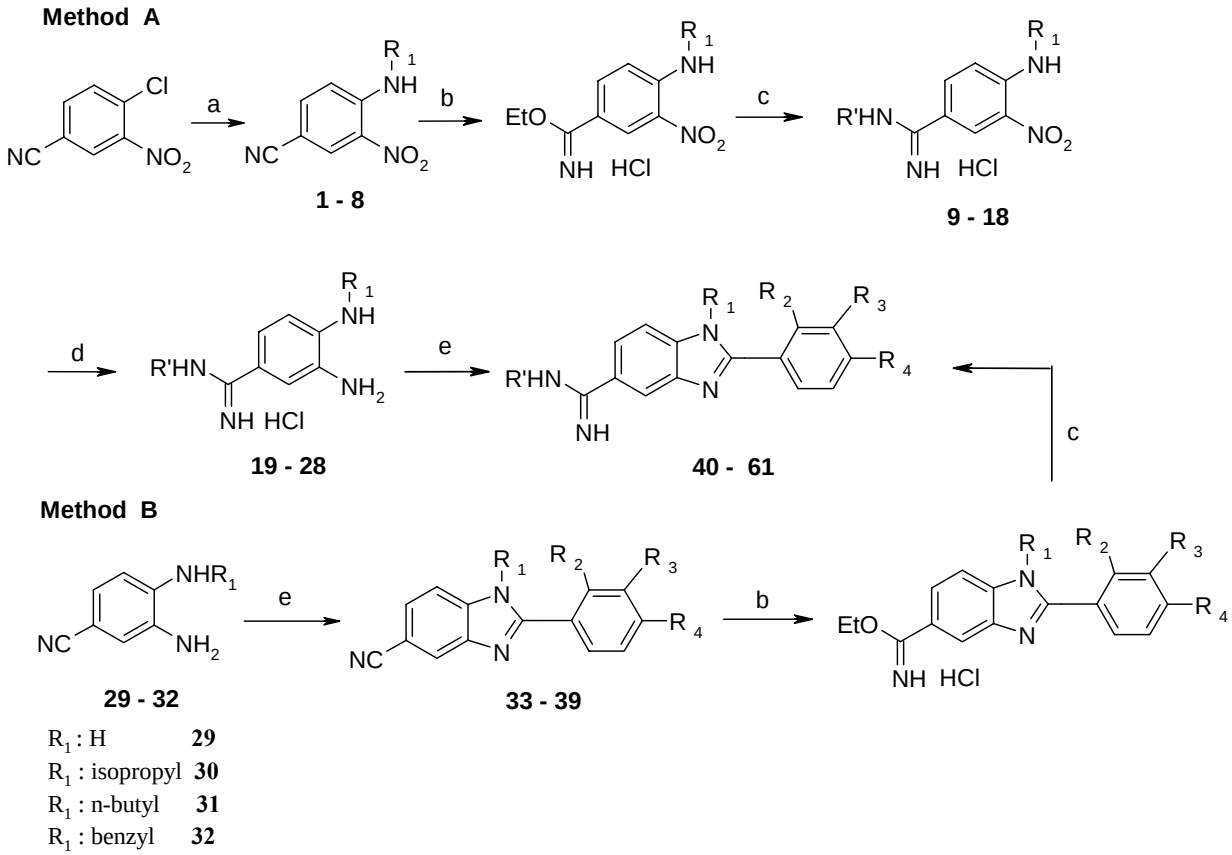


Results and Discussion

Chemistry

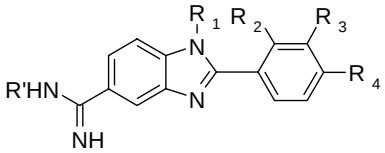
Syntheses of the target benzimidazoles (Tables 1, 2) were achieved by two different methods, as shown in Scheme 1.

Scheme 1



Method A involved nucleophilic displacement in DMF of the chloro group of 4-chloro-3-nitro-benzonitrile by reaction with several amines to give **1**–**8** (Table 3). The cyano group was then converted into the imidate ester, using a modified Pinner method [1], and the imidate esters were used directly to make the corresponding benzamidines **9**–**18** (Table 4). Their reduction with H_2, Pd/C produced **19**–**28** (Table 5). Condensation of these derivatives with the $Na_2S_2O_5$ adducts of several benzaldehydes afforded the corresponding benzimidazoles **45**–**52, 55** and **59**–**61** [2]. *Method B* involved cyclization of **29**–**32** with the $Na_2S_2O_5$ adducts of various benzaldehydes to afford 5-cyano-benzimidazoles **33**–**39** (Table 6), following this, the cyano groups were converted into the imidate esters, as in *method A,* and these were used to prepared the corresponding amidine compounds **40**–**44**, **53**, **54** and **56**–**58**. For its practical advantages this method was used in particular for cyano-benzimidazoles, which have better solubility in EtOH, although the yields were low.

Table 1. Formulas and *in vitro* antibacterial and antifungal activities of **40** - **61**.



No.	Substituents					Minimal inhibitory concentration, μg/mL					
	R'	R_1	R_2	R_3	R_4	*S .a.*	MRSA	MRSA*	*E. c.*	*E. f.*	*C. a.*
40			Cl			>50	>50	>50	>50	>50	>50
41	$CH(CH_3)_2$		Cl		Cl	>50	>50	>50	>50	>50	>50
42	$CH(CH_3)_2$				F	>50	>50	>50	>50	>50	>50
43	$(Et)_2NCH_2CH_2$				F	>50	>50	>50	>50	>50	>50
44	$(Me)_2NCH_2CH_2$		Cl			>50	>50	>50	>50	>50	>50
45	$(Me)_2NCH_2CH_2$			Cl	Cl	12.5	12.5	12.5	>50	50	12.5
46	$CH(CH_3)_2$	CH_3			CN	>50	>50	>50	>50	>50	>50
47	$CH(CH_3)_2$	CH_3		OCH_3	OCH_3	>50	>50	>50	>50	>50	>50
48	Cyclopropyl				$COOCH_3$	>50	>50	>50	>50	>50	>50
49	$PhCH_2$				COOH	>50	>50	>50	>50	>50	>50
50	$PhCH_2$				$COOCH_3$	12.5	25	3.12	>50	50	25
51	Cyclohexyl				COOH	>50	>50	>50	>50	>50	>50
52		n-butyl				>50	>50	>50	>50	>50	>50
53	$(Me)_2NCH_2CH_2$	n-butyl			F	>50	>50	>50	>50	>50	>50
54	$CH(CH_3)_2$	$CH(CH_3)_2$			F	>50	>50	>50	>50	>50	>50
55	$(Me)_2NCH_2CH_2$	Ph		Cl	Cl	12.5	3.12	12.5	>50	25	12.5
56	$(Me)_2NCH_2CH_2$	$PhCH_2$		Cl	Cl	12.5	12.5	12.5	>50	25	12.5
57	$(Et)_2NCH_2CH_2$	$PhCH_2$		Cl	Cl	12.5	12.5	6.25	50	12.5	12.5
58	$(Me)_2NCH_2CH_2$	$PhCH_2$	Cl		Cl	50	12.5	12.5	>50	50	25
59	$(Et)_2NCH_2CH_2$	2,4-*di*-Cl-benzyl		Cl	Cl	3.12	3.12	3.12	12.5	6.25	3.12
60	$(Et)_2NCH_2CH_2$	$PhCH_2CH_2$		OCH_3	OCH_3	>50	>50	>50	>50	>50	>50
61	Isobutyl	$PhCH_2CH_2$		Cl	Cl	6.25	6.25	6.25	>50	12.5	12.5
Ref			OH		t-butyl	0.78	0.78	0.78			
Sult						0.39	25	25		1.56	

Table 1. Cont.

No.	R'	R_1	R_2	R_3	R_4	***S .a.***	**MRSA**	**MRSA***	***E. c.***	***E. f.***	***C. a.***
Amp						0.78		50		0.78	
Cip									0.39		
Flu											1.56

S.a.*: Staphylococcus aureus* (ATCC 25923); **MRSA** (methicillin resistant *Staphylococcus aureus*, ATCC 43300); **MRSA*** (Methicillin resistant *Staphylococcus aureus*, clinical isolate); ***E. c.***: *Escherichia coli* (ATCC 25922); ***E. f.***: *E. faecalis* (ATCC 29212); ***C. a.****: Candida albicans* (ATCC 10231); **Ref**: this compound was found to be the most active compound against *S. aureus* by Weidner-Wells *et al* [3]; **Sult**: Sultamicillin; **Amp**: Ampicillin; **Cip**: Ciprofloxacin; **Flu**: Fluconazole

Antimicrobial Activity

The benzimidazoles **40**–**61** were tested by the macro-broth dilution [4] assay for *in vitro* antibacterial activity against Gram positive *Staphylococcus aureus*, methicillin resistant *Staphylococcus aureus* (MRSA, a clinical isolate from a wound), *Enterococcus faecalis* and Gram negative *Escherichia coli* and for antifungal activity against *Candida albicans.* The MIC 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 was not taken for the initial solution because of the high DMSO concentration (10 %). We have already reported that 3,4-dichloro substitution on the 2-phenyl group of amidinobenzimidazoles plays an important role in their antibacterial activity [1]. Thus, the most active compound, **59**, and less active compounds **45**, and **55**–**57** all have a 3,4-dichlorophenyl group at the C-2 position. Replacement of 3,4-dichloro substitution with other functions such as fluoro, cyano, methoxy, carboxyl or methyl ester caused a reduction in inhibitory activities, and only compound **50**, having a methyl ester group, exhibited moderate activity against MRSA with a MIC of 3.12 μg/mL. More lipophilic substituents on the benzimidazole *N*-atom such as phenyl, benzyl and 2,4-dichlorobenzyl do lead to quite active compounds (**55**–**59**), however substitution with methyl, butyl and isopropyl (cf. **46**, **47**, **52**–**54**) gave no significant activity. Introduction of *N,N*-diethylaminoethyl substitution on the cationic amidine **59** led to inhibitory activity against *E. coli* and *C. albicans.* This is a very important result, as it represents the first example to date of inhibitory activity against *E. coli* with these amidinobenzimidazoles. Except for compound **59**, none of the compounds showed important inhibitory activity against *E. faecalis* and *C. albicans*.

Conclusions

Introduction of aromatic amidine groups into the benzimidazole system gives a good profile of Gram-positive antibacterial activity. In particular, 1-(2,4-dichlorobenzyl)-N-(2-diethylaminoethyl)-1*H*-benzimidazole-5-carboxamidine (**59**), having a 3,4-dichlorophenyl at the C-2 position, exhibited the greatest activity, with a MIC value of 3.12 μg/mL against *S. aureus* and MRSA. Detailed mechanistic studies are required to understand the potent activity of this compound.

Acknowledgments

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Experimental

General

Uncorrected melting points were measured on an Electrothermal 9100 capillary melting point apparatus. ^{1}H-NMR spectra were recorded employing a Varian Mercury 400 MHz FT spectrometer, chemical shifts (δ) are in ppm relative to TMS, and coupling constants (*J*) are reported in Hertz. Mass spectra were taken on a Waters Micromass ZQ using the ESI(+) method. Microanalyses were performed by Leco CHNS-932. Some HCl salts of compounds **40–61** were prepared by using dry HCl gas in EtOH or isopropanol. All chemicals and solvents were purchased from Aldrich Chemical Co. or Fischer Scientific. Compounds **29–32** were synthesized as described in our previous study [2]

Table 2. Physical and spectral data for compounds **40 – 61**.

No	Mp (^{O}C)	Yield (%)	Formula Calculated Found	^{1}H-NMR δ ppm (DMSO-d6) (if not stated otherwise)	MS (ESI+) m/z	Synthesis Method and Isolation Column Chromatography if not stated otherwise
40	>300	35	**$C_{14}H_{11}ClN_4$** · 2HCl · H_2O C: 46.50 H: 4.18 N: 15.5 C: 46.24 H: 3.99 N: 15.3	7.59 - 7.97 (arom. 7H), 8.31 (s), 9.30 (s), 9.53 (s)	271 (100) 273 (33)	(B) Crys. Ethanolic HCl
41	>290	40	**$C_{17}H_{16}Cl_2N_4$** · 2HCl · 1.5H_2O C: 45.66 H: 4.73 N: 12.5 C: 45.67 H: 4.45 N: 12.4	1.21 (d, 6H), 4.03 (m, 1H), 7.6 (m, 2H), 7.8(d, J=8.5, 1H), 7.83 (d, J=2, 1H), 7.92 (d, J=8.4, 1H), 8.05 (d, J=1.5, 1H), 9.06 (s), 9.42 (s), 9.54 (d)	347 (100) 349 (65) 351 (11)	(B) CH_2Cl_2 : Isopropanol : NH_3 (100 : 60 : 4)
42	>300	28	**$C_{17}H_{17}FN_4$** · 2HCl · 1.5H_2O C: 51.52 H: 5.59 N: 14.1 C: 51.90 H: 5.31 N: 14.1	1.29 (d, 6H), 4.08 (m, 1H), 7.51 (t, 2H), 7.67 (d, J=8.3, 1H), 7.87 (d, J=8.4, 1H), 8.08 (s, 1H), 8.44 (m, 2H), 9.08 (s), 9.48 (s), 9.62 (d)	297 (100)	(B) CH_2Cl_2 : Isopropanol : NH_3 (100 : 60 : 3)
43	>300	41	**$C_{20}H_{24}FN_5$** · 3HCl · 2H_2O C: 48.15 H: 6.26 N: 14.1 C: 48.01 H: 6.02 N: 13.99	1.29 (t, 6H), 3.26 (4H), 3.46 (2H), 4.00 (2H), 7.56 (t, 2H), 7.84 (d, J=8.4, 1H), 7.92 (d, J=8.4, 1H), 8.28 (s, 1H), 8.52 (m, 2H), 9.67 (s), 9.93 (s), 10.21 (1H), 10.96 (s)	354 (100)	(B) CH_2Cl_2 : Isopropanol : NH_3 (100 : 60 : 3)
44	100-110 bubb	37	***$C_{18}H_{20}ClN_5$** · 4HCl · 1.5H_2O · 0.5C_2H_6O C: 42.27 H: 5.97 N: 12.97 C: 42.54 H: 6.04 N: 13.01	(CD_3OD): 3.05 (6H), 3.66 (t, 2H), 4.03 (t, 2H), 7.69 (m, 1H), 7.82 (m, 2H), 7.99 (d, 1H), 8.102 (s, 2H), 8.54 (s, 1H)	342 (100) 344 (33)	(B) CH_2Cl_2 : Isopropanol : NH_3 (100 : 60 : 3)
45	95-100 bubb	18	**$C_{18}H_{19}Cl_2N_5$** · 1.5HCl 0.25H_2O · 0.25C_3H_8O C: 49.99 H: 5.14 N: 15.54 C: 49.97 H: 5.14 N: 15.23	2.23 (s, 6H), 2.58 (t, 2H), 3.53 (t, 2H), 7.56 (dd, J=8.4, 1.6, 1H), 7.80 (d, J=8.8, 1H), 7.86 (d, J=8.4, 1H), 8.06 (s, 1H), 8.25 (dd, J= 8.4 1.8, 1H), 8.50 (d, J=1.8, 1H)	376 (100) 378 (69) 380 (11)	(A) CH_2Cl_2 : Isopropanol : Ethylamine (100 : 50 : 3)
46	200-210 bubb	30	**$C_{19}H_{19}N_5$** · 1.5H_2O · 0.1C_3H_8O C: 66.15 H: 6.55 N: 19.98 C: 66.19 H: 5.97 N: 19.82	1.23 (d, 6H), 3.95 (m, 1H), 3.97 (s, 3H), 7.76 (m, 2H), 8.09 (m, 5H)	318 (100)	(A) CH_2Cl_2 : Isopropanol : Propylamine (100 : 50 : 4)
47	100-110 bubb	26	**$C_{20}H_{24}N_4O_2$** · 1.8H_2O C: 62.42 H: 7.22 N: 14.55 C: 62.62 H: 6.94 N: 14.21	(DMSO-d_6 + 1 drop D_2O): 1.17 (d, 6H), 3.85-3.90 (10H), 7.15 (d, J=8.4, 1H), 7.4 (s, 2H), 7.62 (m, 2H), 7.97 (s, 1H)	353 (100)	(A) CH_2Cl_2 : Isopropanol : Propylamine (100 : 50 : 3)
48	285-290 bubb	60	**$C_{19}H_{18}N_4O_2$** · 3H_2O · 0.3C_3H_8O C: 58.80 H: 6.54 N: 13.78 C: 58.72 H: 6.20 N: 13.36	0.77 (2H), 0.91 (d, 2H), 2.79 (1H), 3.89 (s, 3H), 7.53 (d, J=8, 1H), 7.67 (d, J=8.4, 1H), 8.04 (s, 1H), 8.09 (d, J=7.8, 2H), 8.38 (d, J=8, 2H)	335 (100)	(A) Cryst. Isopropanol

Table 2. Cont.

49	260-270 bubb	34	$C_{22}H_{18}N_4O_2 \cdot 2H_2O$ C: 65.01 H: 5.45 N: 13.77 C: 64.95 H: 5.23 N: 13.78	4.73 (s, 2H), 7.35-8.30 (aromat. 12H)	371 (100)	(A) Cryst. EtOH
50	265-275 bubb	52	$C_{23}H_{20}N_4O_2 \cdot 0.25H_2O$ C: 71.03 H: 5.31 N: 14.40 C: 71.07 H: 5.14 N: 14.32	3.88 (s, 3H), 4.53 (s, 2H), 7.27-7.56 (m, 7H), 8.03-8.06 (m, 3H), 8.37 (d, J=8.4, 2H),	385 (100)	(A) CH_2Cl_2 : Isopropanol : Propylamine (100 : 100 : 6)
51	>300	64	$C_{21}H_{22}N_4O_2 \cdot 2HCl \cdot 1.5H_2O$ C: 54.55 H: 5.88 N: 12.12 C: 54.26 H: 5.89 N: 12.62	1.21-1.42-1.63-1.77-1.98 (m, 10H), 3.78 (1H), 7.65 (d, J=8.4, 1H), 7.87 (d, J=8.4, 1H), 8.1 (s, 1H), 8.15 (d, J=8, 2H), 8.47 (d, J=7.6, 2H), 9.19 (s), 9.50 (s), 9.63 (d)	363 (100)	(A) Cryst. Ethanolic HCl
52	150-155 bubb	22	$C_{18}H_{20}N_4 \cdot 2HCl \cdot 2.2H_2O$ $0.5C_3H_8O$ C: 53.84 H: 7.04 N: 12.88 C: 53.98 H: *** N: 12.81	(Base) : 0.64 (t, 3H), 1.01 (m, 2H), 1.56 (m, 2H), 4.33 (t, 2H), 7.56 (m, 3H), 7.76 (m, 3H), 7.95 (d, J=8.6, 1H), 8.23 (d, J=1.4, 1H), 9.18 (s), 9.41 (s)	293 (100)	(A) CH_2Cl_2 : Isopropanol : NH_3 (100 : 50 : 4)
53	205-210 bubb	32	$C_{22}H_{28}FN_5 \cdot 3HCl \cdot 2H_2O$ C: 50.14 H: 6.69 N: 13.29 C: 50.34 H: 6.52 N: 12.92	0.78 (t, 3H), 1.20 (m, 2H), 1.70 (m, 2H), 2.90 (s, 6H), 3.51 (t, 2H), 4.03 (t, 2H), 4.45 (t, 2H), 7.55 (m, 2H), 8.01 (m, 3H), 8.10 (d, J=8.6, 1H), 8.42 (d, J=1.3, 1H), 9.77 (s), 9.97 (s), 10.28 (s)	382 (100)	(B) CH_2Cl_2 : Isopropanol : Ethylamine (100 : 50 : 3)
54	115-120 bubb	20	$C_{20}H_{23}FN_4 \cdot 3HCl \cdot 1.25H_2O$ C: 51.07 H: 6.11 N: 11.91 C: 51.05 H: 6.31 N: 11.55	1.34 (d, 6H), 1.66 (d, 6H), 4.2 (m, 1H), 4.8 (m, 1H), 7.54 (m, 2H), 7.74 (d, J=8.7, 1H), 7.86 (m, 2H), 8.22 (2H), 9.21 (s), 9.58 (s), 9.68 (d)	339 (100)	(B) CH_2Cl_2 : Isopropanol : Ethylamine (100 : 50 : 3)
55	295-300 bubb	30	$C_{24}H_{23}Cl_2N_5 \cdot 3HCl \cdot 1.25H_2O$ C: 49.34 H: 4.91 N: 11.98 C: 49.45 H: *** N: 11.87	2.85 (s, 6H), 3.44 (t, 2H), 3.92 (t, 2H), 7.37-7.80 (m, 10H), 8.41 (d, J=1.2, 1H), 9.52 (s), 9.83 (s), 10.07 (s), 10.81 (s)	452 (100) 454 (68) 456 (12)	(A) CH_2Cl_2 : Isopropanol : NH_3 (100 : 50 : 15)
56	130-135 bubb	49	$C_{25}H_{25}Cl_2N_5 \cdot 3HCl \cdot 1.25H_2O$ C: 50.18 H: 5.14 N: 11.7 C: 50.06 H: 5.22 N: 11.49	2.90 (6H), 3.50 (t, 2H), 4.03 (q, 2H), 5.74 (s, 2H), 7.04 (m, 2H),7.34 (m, 3H), 7.82 (m, 4H), 8.02 (d, J=1.9, 1H), 8.42 (d, J=1.4, 1H), 9.71 (s), 9.86 (s), 10.15 (d), 11.14 (s)	466 (100) 468 (68) 470 (11)	(B) CH_2Cl_2 : Isopropanol : Ethylamine (100 : 50 : 5)
57	120-130 bubb	46	$C_{27}H_{29}Cl_2N_5 \cdot 3HCl \cdot 2H_2O$ C: 50.68 H: 5.67 N: 10.94 C: 50.66 H: 5.62 N: 10.92	1.27 (t, 6H), 3.24 (4H), 3.45 (2H), 3.99 (2H), 5.72 (s, 2H), 6.98 (m, 2H), 7.27 (m, 3H), 7.81 (m, 4H), 8.01 (d, J=2, 1H), 8.35 (s, 1H), 9.61 (s), 9.81 (s), 10.11 (s), 10.97 (s)	494 (100) 496 (68) 498 (12)	(B) CH_2Cl_2 : Isopropanol : Ethylamine (100 : 50 : 3)
58	**	33	** $C_{25}H_{25}Cl_2N_5 \cdot HCl$	(CD_3OD): 3.29 (6H), 3.63 (t, 2H), 3.99 (t, 2H), 5.57 (s, 2H), 7.03 (m, 2H), 7.26 (m, 3H), 7.61 (dd, J=8.4 , 2, 1H), 7.68 (d, J=8.1, 1H), 7.82 (d, J=2, 1H), 7.95 (m, 2H), 8.42 (s, 1H)	466 (100) 468 (71) 470 (13)	(B) CH_2Cl_2 : Isopropanol : NH_3 (100 : 50 : 0.5)
59	120-125 bubb	35	$C_{27}H_{27}Cl_4N_5 \cdot 3HCl \cdot H_2O$ C: 46.64 H: 4.71 N: 10.07 C: 46.80 H: 4.82 N: 9.86	1.25 (t, 6H), 3.23 (m, 4H), 3.41 (t, 2H), 3.84 (2H), 5.71 (s, 2H), 6.73 (d, J=8.8, 1H), 7.30 (dd, J=8.4 2, 1H), 7.62-7.81 (m, 5H), 7.93 (d, J=2, 1H), 8.35 (s, 1H), 9.64 (s), 9.83 (s), 10.13 (s), 10.96 (s)	562 (80) 564 (100) 566 (51) 568 (14)	(A) CH_2Cl_2 : Isopropanol : NH_3 (100 : 50 : 0.5)
60	127-130 bubb.	30	$C_{30}H_{37}N_5O_2 \cdot 3HCl \cdot H_2O$ C: 54.34 H: 6.99 N: 10.56 C: 54.40 H: 6.80 N: 10.45	1.29 (t, 6H), 3.08 (t, 2H), 3.25 (4H), 3.48 (d, 2H), 3.84 (s,3H), 3.89 (s,3H), 4.04 (q, 2H), 4.83 (t, 2H), 6.9 (m, 2H), 7.12-7.31 (m, 6H), 8.03 (d, J=8.8, 1H), 8.27 (d, J=8.8, 1H), 8.36 (s, 1H), 9.87 (s), 10.08 (s), 10.44 (s), 11.1 (s)	500 (100)	(A) CH_2Cl_2 : Isopropanol : iso-propylamine (90 : 30 : 2)
61	288-290	19	$C_{26}H_{26}Cl_2N_4 \cdot 1.5HCl$ C: 60.04 H: 5.33 N: 10.77 C: 60.05 H: 5.50 N: 10.66	0.96 (d,6H), 2.06 (m, 1H), 2.93 (t, 2H), 3.16 (t, 2H), 4.63 (t, 2H), 6.67 (d, J=7.2, 2H), 7.03 (t, J=7.2, 2H), 7.11 (m, 1H), 7.45 (2H), 7.70 (m, 2H), 8.01 (d, J=8.8, 1H), 8.12 (s, 1H), 9.02 (s), 9.44 (s), 9.75 (s)	465 (100) 467 (67) 469 (11)	(A) CH_2Cl_2 : Isopropanol : ethylamine (100 : 50 : 0.5)

* Hygroscopic; ** Very hygroscopic, not measurable; *** No satisfactory result.

4-(2,4-Dichlorobenzyl)amino-3-nitrobenzonitrile (**7**)

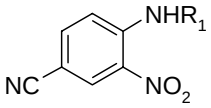
A mixture of 2,4-dichlorobenzylamine (3.52 g, 20 mmol) and 4-chloro-3-nitrobenzonitrile (2 g, 10.95 mmol) in DMF (3 mL) was heated under reflux for 4h at 120°C. The mixture was allowed to cool and EtOH was added. The resultant yellow precipitate was filtered, washed with water and crystallised from EtOAc-*n*-hexane; yield 57 %; mp: 192°C; ^{1}H-NMR ($CDCl_3$): 4.66 (d, J=5.6, 2H), 6.8

(d, J=9.2, 1H), 7.24 (m, 2H), 7.47 (d, J=2, 1H), 7.58 (dd, J_o=9.2, J_m=2, 1H), 8.55 (d, J=2, 1H), 8.78 (br.t, 1H).

4-(2-Phenylethyl)amino-3-nitrobenzonitrile (**8**)

2-Phenylethylamine (1.3 g, 10.8 mmol) and 4-chloro-3-nitrobenzonitrile (1g, 5.4 mmol) were allowed to react for 2 h, at 100°C and the product wasisolated as described for **7**; yield 79 %; mp: 111°C; ^{1}H-NMR ($CDCl_3$): 3.04 (t, J=7.1, 2H), 3.61 (q, J=6.8, 2H), 6.89 (d, J=8.8, 1H), 7.25-7.37 (m, 4H), 7.58 (dd, J_o=8.8, J_m=2, 1H), 8.44 (br.t, 1H), 8.47 (d, J=2, 1H).

Table 3. Formulas and melting points of **1–8**.



Comp	R_1	Formula	Ref.
1	H	$C_7H_5N_3O_2$	Commercial
2	methyl	$C_8H_7N_3O_2$	Lit [1]
3	*iso*-propyl	$C_{10}H_{11}N_3O_2$	Lit [2]
4	n-butyl	$C_{11}H_{13}N_3O_2$	Lit [2]
5	phenyl	$C_{13}H_9N_3O_2$	lit [5] 126°C, 126 °C
6	benzyl	$C_{14}H_{11}N_3O_2$	Lit [2]
7	2,4-dichlorobenzyl	$C_{14}H_9Cl_2N_3O_2$	---
8	$PhCH_2CH_2$	$C_{15}H_{13}N_3O_2$	---

General Procedure for Synthesis of **9–18, 40–44, 53, 54, 56–58**

1–8 (4.5 mmol) and **33–39** (Table 6, 1 mmol) were suspended in absolute EtOH, cooled in a ice-salt bath, and dry HCl gas was passed through the solution for 40 min. The solution was stirred in a stoppered flask at room temperature for 3 days and then diluted with dry ether. The imidate esters precipitated as yellow solids, which were 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 the amines (1.5 - 2 fold excess) overnight at 25-30°C. The reaction mixture was evaporated and diluted with ether, the precipitate was filtered, washed with ether, then dried. Compounds **9–18** (Table 4) were used without purification as HCl salts for the next steps since they were prepared completely pure. In contrast, crude products **40–44**, **53**, **54**, **56–58** were treated with dilute Na_2CO_3 solution, then water. Further purification methods are given in Table 2.

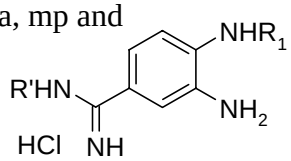
Table 4. Formulas, spectroscopic data, m.p. and yields of **9 - 18**.

Comp	R'	R_1	Formula	NMR δ ppm (DMSO-d_6)	Mass (ESI+)	mp (°C)	Yield (%)
9		butyl	$C_{11}H_{16}N_4O_2$	0.895 (t, 3H), 1.34 (m, 2H), 1.56 (m, 2H), 3.41 (q, 2H), 7.22 (d, J=9.2, 1H), 7.95 (dd, J=9.2, 2.4, 1H), 8.6 (t, 1H), 8.67 (d, J=2.4, 1H), 9.2 (br.s)	237 (100)	200-4	62
10	*iso*-propyl	methyl	$C_{11}H_{16}N_4O_2$			Lit [1]	

Table 4. Cont.

11	$(CH_3)_2N(CH_2)_2$		$C_{11}H_{17}N_5O_2$	2.24 (s, 6H), 2.58 (t, 2H), 3.5 (t, 2H), 7.14 (d, J=8.8,1H), 7.5 (dd, J=8.8, 2, 1H), 8.09 (br.s, 2H), 8.48 (d, J=2, 1H).	252 (100)	220-230 (bubb) Lit [6]	
12	*cyclo*-propyl		$C_{10}H_{12}N_4O_2$			Lit [1]	
13	*cyclo*-hexyl		$C_{13}H_{18}N_4O_2$			Lit [1]	
14	Benzyl		$C_{14}H_{14}N_4O_2$			Lit [1]	
15	$(CH_3)_2N(CH_2)_2$	phenyl	$C_{17}H_{21}N_5O_2$	2.32 (s, 6H), 2.69 (2H), 3.4 (br.s), 3.56 (t, 2H), 7.12 (d, J=9..3, 1H), 7.28-7.49 (m, 5H), 7.85 (dd, J=9.2, 2.3, 1H), 8.61 (d, J=2.3, 1H), 9.85 (s, 1H)	328 (100)	Hygros. 110 (bubb)	87
16	*iso*-butyl	$PhCH_2CH_2$	$C_{19}H_{24}N_4O_2$	(CD_3OD): 1.05 (d, 6H), 2.07 (m,1H), 3.05 (t, 2H), 3.25 (d, 2H), 3.72 (m, 2H), 7.21 (m, 2H), 7.3 (m, 4H), 7.79 (dd, J=9.2 2.4, 1H), 8.53 (t, 1H), 8.59 (d, J=2.4, 1H)	341 (100)	247-9	75
17	$(C_2H_5)_2N(CH_2)_2$	$PhCH_2CH_2$	$C_{21}H_{29}N_5O_2$	(CD_3OD): 1.11 (t, 6H), 2.68 (q, 4H), 2.78 (t, 2H), 3.03 (t, 2H), 3.57 (t, 2H), 4.7 (s, 2H), 7.21 (m, 2H), 7.3 (m, 4H), 7.81 (dd, J=8.8 ,2.4, 1H), 8.62 (d, J=2.4, 1H).	384 (100)	236-9	85
18	$(C_2H_5)_2N(CH_2)_2$	2,4-dichloro benzyl	$C_{20}H_{25}Cl_2N_5O_2$	(CD_3OD): 1.11 (t, 6H), 2.68 (q, 4H), 2.79 (t, 2H), 3.57 (t, 2H), 4.78 (t, 2H), 6.98 (d, J=9.2, 1H), 7.30 (dd, J=8.6 2.4, 1H), 7.37 (d, J=8.8, 1H), 7.53 (d, J=2, 1H), 7.78 (dd, J=9.2, 2.4, 1H), 8.68 (d, J=2.4, 1H)	438 (100) 440 (65) 442 (12)	203-5	89

Table 5. Formulas, spectroscopic data, mp and yields of **19 - 28**.



Comp	**R'**	**R_1**	**Formula**	**NMR** δ ppm (DMSO-d_6)	**MS** (ESI+)	**mp** (^oC)	**Yield** (%)
19		butyl	$C_{11}H_{18}N_4$	0.88 (t, 3H), 1.36 (m, 2H), 1.56 (m, 2H), 3.1 (t, 2H), 5.01 (br.s, 2H), 5.57 (br.s, 2H), 6.46 (d, J=8, 1H), 6.9 (s, 1H), 7.07 (d, J=8.4, 1H), 8.58 (s, 2H), 8.74 (s, 2H).	207(100)	285	94
20	*iso*-propyl	methyl	$C_{11}H_{18}N_4$			Lit [1]	
21	$(CH_3)_2N(CH_2)_2$		$C_{11}H_{19}N_5$	(+ one drop D_2O): 2.2 (s, 6H), 2.54 (t, 2H), 3.4 (t, 2H), 6.57 (d, J=8, 1H), 6.85 (m, 2H)	222(100)	Lit [6]	
22	*cyclo*-propyl		$C_{10}H_{14}N_4$			Lit [1]	
23	*cyclo*-hexyl		$C_{13}H_{20}N_4$			Lit [1]	
24	benzyl		$C_{14}H_{16}N_4$			Lit [1]	
25	$(CH_3)_2N(CH_2)_2$	phenyl	$C_{17}H_{23}N_5$	(+ one drop D_2O) : 2.27 (s, 6H), 2.63 (t, 2H), 3.5 (t, 2H), 6.85-7.26 (8H)	298(100)	*	90

Table 5. Cont.

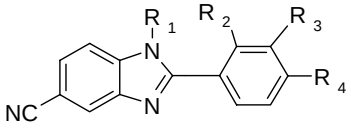
26	*iso*-butyl	-CH_2CH_2 Ph	$C_{19}H_{26}N_4$	0.92 (d, 6H), 1.96 (m, 1H), 2.9 (t, 2H), 3.17 (t, 2H), 3.31(2H), 4.39 (t, 1H), 5.03 (2H), 5.66 (t,1H), 6.57 (d, J=8.4, 1H), 6.87 (d, J=1.6, 1H), 6.98 (dd, J=8.4 1.6, 1H), 7.2-7.3 (m, 5H), 8.57 (s, 1H), 8.9 (s, 1H)	311(100)	98-100	94
27	$(C_2H_5)_2N(CH_2)_2$	-CH_2CH_2 Ph	$C_{21}H_{31}N_5$	(CD_3OD): 1.09 (t, 6H), 2.65 (q, 4H), 2.77 (t, 2H), 2.96 (t, 2H), 3.47 (t, 2H), 3.52 (t, 2H), 6.68 (d, J=8, 1H), 7.01 (d, J=2, 1H), 7.14-7.3 (m, 6H)	354(100)	*	95
28	$(C_2H_5)_2N(CH_2)_2$	2,4-dichloro-benzyl	$C_{20}H_{27}Cl_2N_5$	(CD_3OD): 1.14 (t, 6H), 2.76 (4H), 2.89 (2H), 3.57 (t, 2H), 4.54 (s, 2H), 6.37 (d, J=8, 1H), 7.06 (d, J=2, 2H), 7.25 (dd, J=8.2 2, 1H), 7.31 (d, J=8.4, 1H), 7.49 (d, J=2, 1H)	408(100) 410(65) 412(11)	*	92

***** No sharp melting point.

General Procedure for Synthesis of **19** – **28**

Compound **9–18** (3.5 mmol) in EtOH (75 mL) was subjected to hydrogenation using 40 psi of H_2 and 10 % Pd-C (40mg) until uptake of H_2 ceased. The catalyst was filtered on a bed of Celite, washed with EtOH, and the filtrate was concentrated *in vacuo*. The crude o-phenylenediamines (grey–purple–black in colour) were used for the subsequent steps without crystallisation (Table 5). In order to prevent halogen reduction of compound **28**, 15 psi of H_2 pressure was employed.

Table 6. Formulas, spectroscopic data, mp and yields of **33 - 39**.



Comp	R_1	R_2	R_3	R_4	Formula	NMR δ ppm ($CDCl_3$)	Mass (ESI+)	mp (^oC)	Yield (%)	İsolation
33		Cl			$C_{14}H_8ClN_3$			Lit [2]		
34		Cl		Cl	$C_{14}H_7Cl_2N_3$			Lit [2]		
35				F	$C_{14}H_8FN_3$			Lit [2]		
36	*iso*-propyl			F	$C_{17}H_{14}FN_3$			Lit [2]		
37	*n*-butyl			F	$C_{18}H_{16}FN_3$			Lit [2]		
38	benzyl		Cl	Cl	$C_{21}H_{13}Cl_2N_3$	5.47 (s, 2H), 7.05 (m, 2H), 7.3-7.55 (m,7H), 7.82 (d, J=2, 1H), 8.16 (d, J=1.5, 1H)	91(100) 378(50) 380(33) 382(6)	192	75	Cryst. EtOH-water
39	benzyl	Cl		Cl	$C_{21}H_{13}Cl_2N_3$	5.27 (s, 2H), 6.91(m, 2H), 7.26-7.57 (m, 8H), 8.16 (1H)	91(100) 378(54) 380(31) 382(5)	195-6	73	Cryst. EtOH-water

General Procedure for Synthesis of **33–39, 45–52, 55, 59–61**

The corresponding benzaldehydes (15 mmol) were dissolved in EtOH (50 mL) and sodium metabisulfite (1.6 g) in H_2O (10 mL) 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 (yields over 93 %). The mixture of these salts (1 mmol) and **19–28** and **29–32** (1 mmol) in DMF (1-2 mL) was heated at 120^{o}C for 4h. The reaction mixture was cooled, poured into H_2O, and the solid was filtered. Purification methods are given in Tables 2 and 6.

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Sample availability : Available from the authors.