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Original article

Synthesis, potent anti-staphylococcal activity and QSARs of some novel 2-anilinobenzazoles

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Abstract

Synthesis and anti-staphylococcal activity of a number of substituted 2-anilinobenzimidazoles, benzothiazoles and benzoxazoles are reported. The anti-staphylococcal activities were evaluated in standard in vitro MIC assay method. While anilinobenzimidazole derivatives **11**–**45** showed very potent anti-staphylococcal activities (greatest activity with an MIC value of 0.095 μg/mL), none of the 2-anilinobenzothiazoles and benzoxazole derivatives exhibited inhibitory activity. QSAR analysis of the anilinobenzimidazoles was studied on the relationship between the anti-staphylococcal activity (MIC in μg/ml) and extrapolated log k_w values.

Keywords: Anilinobenzimidazole; Anilinobenzothiazole; Anilinobenzoxazole; Anti-staphylococcal activity; k_w Capacity factor; QSAR

1. Introduction

Multiple drug-resistant organisms such as MRSA (methicillin-resistant *Staphylococcus aureus*), VRE (vancomycin resistant enterococci) and MRSE (methicillin-resistant *Staphylococcus epidermidis)* are becoming common causes of infections in the acute and long-term care units in hospitals. Infections caused by staphylococci can become persistent and recurring and can be difficult to treat with available antibacterial agents [1]. The emergence of these resistant bacteria has created a major concern and an urgent need for antibacterial agents in structural classes distinct from known antibacterial agents. The intravenous injection of vancomycin, the most effective antibiotic against MRSA infection, had not been permitted in Japan until 1992; it was therefore difficult to treat MRSA due to the lack of effective drugs [2]. Newly designed agents with different chemical structures against MRSA have been urgently expected. Continuing our interest in this field, we found a patent [3] concerning the synthesis of the anilinobenzimidazoles, which have very potent anti-staphylococci activity (Fig. 1). Earlier, we have also reported some anilinobenzimidazoles that showed significant antibacterial activities in MIC assay [4]. In addition, a series of anilinobenzimidazoles carrying different heterocycles such as 1,2,3-thiadiazole, 1,3,4-thiadiazole, thiazolidine, 2,3-dihydrothiazole, 1,3,4-oxadiazole, semicarbazone and substituted thiosemicarbazones have been found to possess in vitro growth inhibition activity against various bacteria and fungi [5]. Very recently, two new aminobenzimidazoles (Fig. 2), a novel class of dual-targeting compounds (dual inhibition of GyrB and ParE) with very potent antibacterial activity, were reported [6].

These results prompted us to prepare a series of new *N*-(substituted)anilinobenzazoles and establish their QSAR analysis, as well.

2. Chemistry

Non-commercial starting material *o*-phenylenediamines **1** [7], **2** [8], **3** [9], **4** [8], **5** [10], **6** [11], 2,5(6)-dichlorobenzimidazole **7** [12], 2-chloro-5(6)-trifluorobenzimidazole **9** [13], and aniline derivative **10** [9] were prepared according to the

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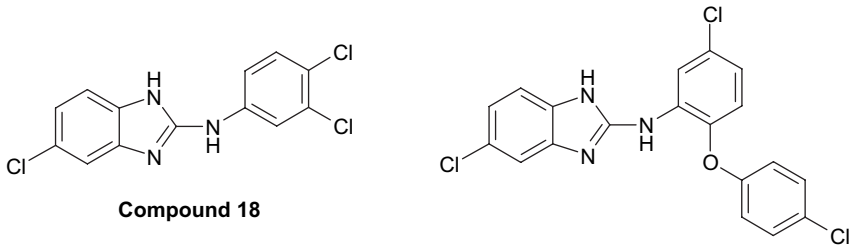

Compound 18

Fig. 1. Chemical structures of known anilinobenzimidazoles.

literature methods, which are given in Scheme 1. Earlier, there was limited number of methods for the preparation of 2-(*N*-substituted)-aminobenzimidazole derivatives. In one of the old method [3,4] corresponding 2-chlorobenzimidazole derivatives have been heated with the appropriate anilines, at elevated temperature, which is also given here as Method C, for a few samples (Scheme 2). Another approach involves the cyclodesulfurization of pre-formed thioureas between the appropriate *o*-phenylenediamines and isothiocyanates. For this purpose, in Method B, dicyclohexylcarbodiimide (DCCI) was used as desulfurization agents [14]; however, isolation of the desired compound was highly tedious. In Method A, copper (I) chloride [15] was used as desulfurization agents. This modification has the advantage that a large variety of compounds with diverse substitution patterns can be easily synthesized and, therefore, the SAR could be rapidly delineated. Furthermore, very recently polymer-supported carbodiimide [16] was suggested by simplified product isolation, one-pot method and in high yield for the same reaction. Novel 2-anilinobenzothiazoles **47**, **48** and benzoxazole **51** derivatives were prepared as outlined in Scheme 3, by the previously reported literature methods.

3. Results and discussion

All of the 2-anilinobenzazoles **11**–**45**, **47**, **48** and **51** were tested for in vitro antibacterial activity against Gram-positive *S. aureus*, methicillin-resistant *S. aureus* (MRSA) for antibacterial activity by the agar diffusion method, which has been extensively used by us [17]. Among them compounds **12**, **13**, **32**, **47**, **48** and **51** did not give any growth inhibition zone. The others exhibiting good antibacterial activity by this method were further tested by the macro-broth dilution assay [18] to determine the MIC values which are listed in Table 1. The synthesized compounds and reference drugs were dissolved in DMSO–water (50%) mixture at a concentration of 400 μg/ml.

The final concentration was adjusted to 100 μg/mL by four-fold dilution with media culture and bacterial solution in the first tube. Data were not taken for the initial solution because of the high DMSO concentration (12.5%). Antibacterial activity disappeared totally by the substitution of N^1-imidazole ring with ethyl **12**, *p*-chlorophenyl **13**, butyl **32**. In addition, with the replacement of imidazole ring with thiazoles **47**, **48** and oxazole **51** moities inhibitory activity was again totally lost. This means that tautomeric NH of imidazole ring is very important for potent anti-staphylococci effect, so imidazole moiety was the best bioisoster of the benzazoles. Halogen substitution, particularly at the 3,4-position of the benzene ring of aniline, increases antibacterial activity, in contrast with electron-donor group such as methoxy compound **31** have no good inhibitory activity. Similarly, the substitution of C-5(6) position, with electron-withdrawing group such as halogens, NO_2, CF_3, CN and COOMe always increases the antibacterial activity, in contrast with amino group **41**, at the same position activity decreased too much. Among the anilinobenzimidazoles compound **33**, **35** and **38** are the most active with an MIC value of 0.095–0.19 μg/mL against both *S. aureus*. While ampicillin and sultamicillin are practically inactive against MRSA (25 μg/mL), all the compounds belonging to the anilinobenzimidazoles are effective against MRSA, this is highly important advantage.

3.1. Lipophilicity (by determination of k_w parameter) and QSAR analysis

OSAR analysis was performed for 32 active anilinobenzimidazoles **11**, **14**–**31**, **33**–**45**, by multiple regression analysis. As well-known, the lipophilicity of the chemical agents is an important parameter for its distribution in biological systems. For defining lipophilicity, the logarithm of the octanol–water partition coefficient (log *P*) has become the most widely used

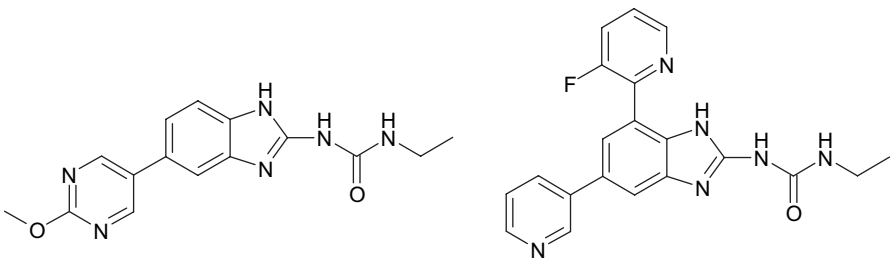

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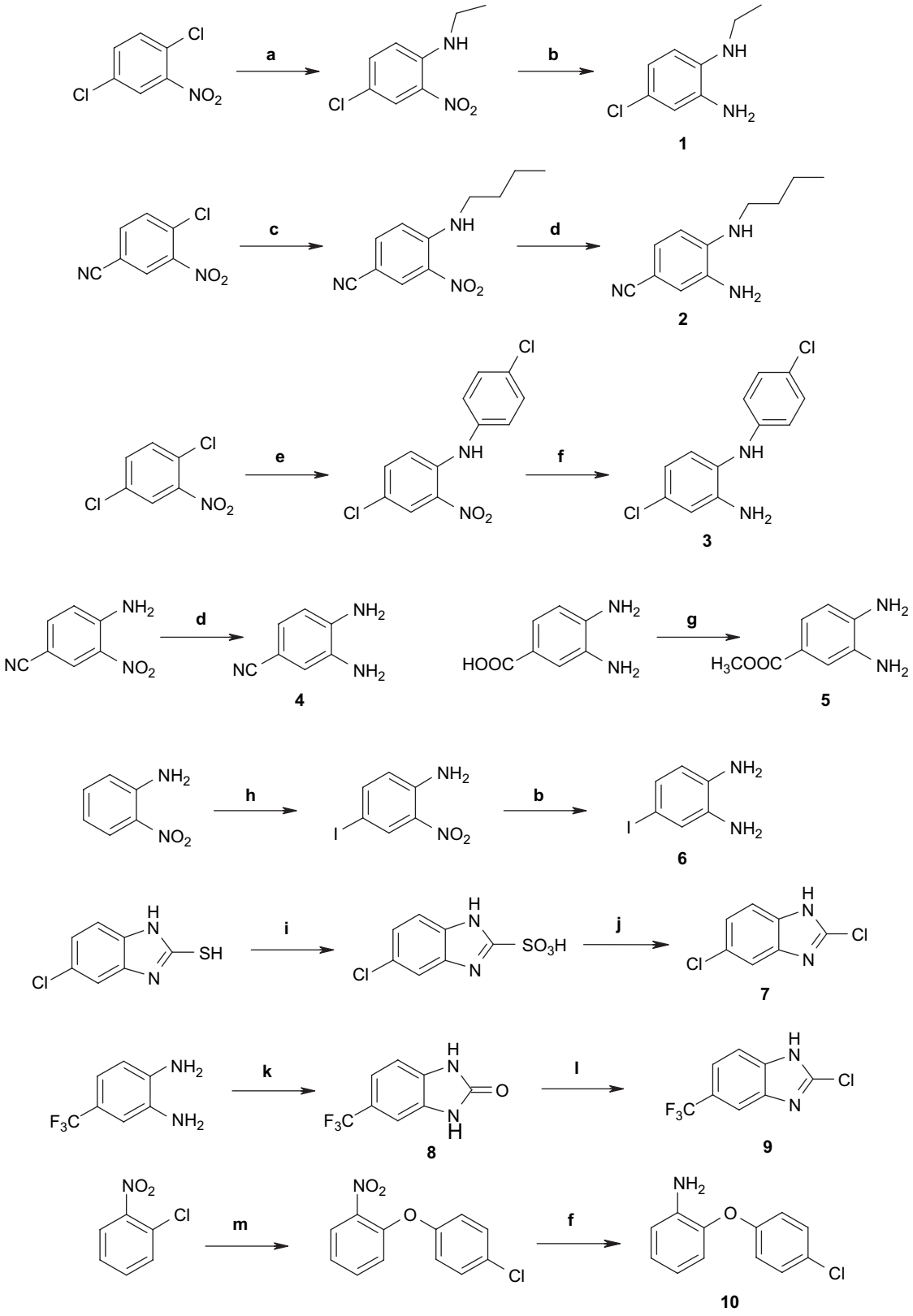
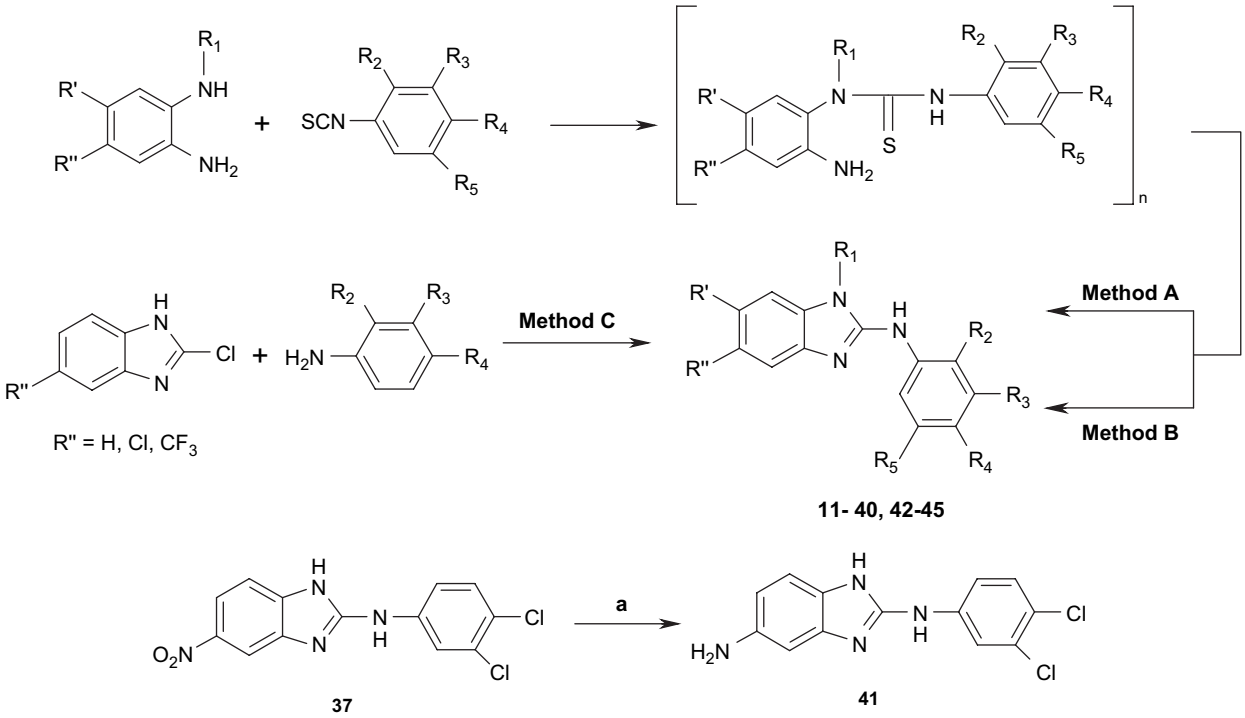
Fig. 2. Chemical structures of dual-targeting aminobenzimidazoles.



Reagents : **a)** Et-NH_2 **b)** Sn/HCl **c)** Bu-NH_2 **d)** H_2.Pd/C **e)** p-Chloroaniline **f)** Zn/CH_3COOH **g)** abs. CH_3OH / dry HCl (gas) **h)** ICl/CH_3COOH **i)** H_2O_2/NaOH **j)** PCl_5/$POCl_3$ **k)** CDI **l)** $POCl_3$ **m)** p-Chlorophenol

Scheme 1. Synthesis of *o*-phenylenediamines **1**–**6**, 2-chlorobenzimidazoles **7**, **9** and aniline derivative **10**.



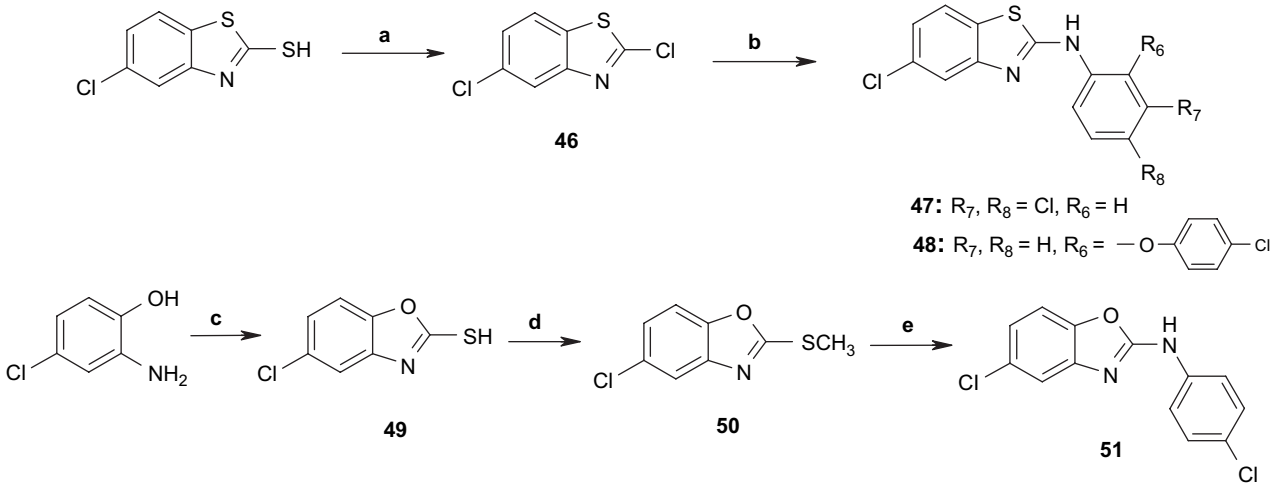
Reagents : **Method A)** CuCl/i-Pr_2NEt **Method B)** DCCI **Method C)** High temp in DMF **a)** Sn/HCl

Scheme 2. Synthesis of anilinobenzimidazoles **11**–**45**.

parameter. However, this method is rather laborious, time-consuming and has several problems, such as poor solubility of the compounds in one of the phases and instability in aqueous media. Owing to this kind of difficulties in making log *P* measurements by the traditional shake-flask method, several chromatographic approaches have been developed. Among them, the determination of log k' data from isocratic reversed-phase high performance liquid chromatography (RP-HPLC) and their extrapolation to elution with 100% water (log k_w) has become a well-accepted method for the determination of lipophilicity [19–21]. Since, the log k_w values correlate well with calculated log *P* octanol values, it is an available index of lipophilicity, for QSAR analysis [22–25]. This method provides an easy, reliable and accurate way to determine the concentration of a compound in solvents used for the measurement of partition coefficients. The chromatographic retention time directly relates to the compound's distribution between the mobile and stationary phases. The chromatographic capacity factors, k', were calculated according to Eq. (1).

$$k' = (t_R - t_0)/t_0 \quad (1)$$

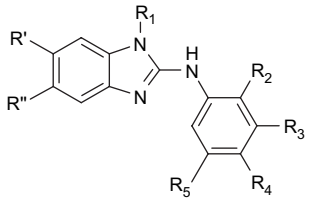
t_R, the retention time of the solute; t_0, column dead time.

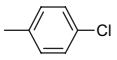
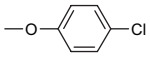
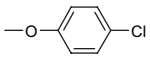


Reagents: **a)** SO_2Cl_2 **b)** Corresponding anilines **c)** CS_2/KOH/CH_3OH **d)** $(CH_3)_2SO_4$ **e)** p-Chloroaniline

Scheme 3. Synthesis of anilinobenzothiazoles **47**, **48** and anilinobenzoxazoles **51**.

Table 1
Formulas and anti-staphylococci activity of compounds **11–45**



No	R″	R′	R_1	R_2	R_3	R_4	R_5	*S. aureus* ATCC 25923 MIC (μg/mL)	log 1/*C*	*S. aureus* ATCC 43300 MIC (μg/mL)	log 1/C
11					Cl	Cl		3.12	4.95	6.25	4.65
12	Cl		C_2H_5		Cl	Cl		NT		NT	
13	Cl				Cl	Cl		NT		NT	
14	Cl			Cl		Cl		0.39	5.90	0.39	5.90
15	Cl			Cl			CF_3	0.78	5.65	0.78	5.65
16	Cl							1.56	5.38	1.56	5.38
17	Cl			F		F		12.5	4.35	6.25	4.65
18	Cl				Cl	Cl		0.39	5.90	0.39	5.90
19	Cl				Cl	F		1.56	5.28	1.56	5.28
20	Cl				CF_3	Cl		0.39	5.95	0.39	5.95
21	Cl				CF_3			0.39	5.90	1.56	5.30
22	Cl				Cl		Cl	0.39	5.90	0.39	5.90
23	Cl				F		F	1.56	5.25	1.56	5.25
24	Cl				F			6.25	4.62	6.25	4.62
25	Cl					F		3.12	4.92	12.5	4.32
26	Cl					Cl		3.12	4.95	1.56	5.25
27	Cl					N_3		3.12	4.96	6.25	4.66
28	Cl					Br		1.56	5.31	0.78	5.62
29	Cl					OCF_3		0.78	5.62	0.78	5.62
30	Cl					CF_3		0.39	5.90	0.39	5.90
31	Cl					OCH_3		50	3.74	50	3.74
32	CN		C_4H_9			F		NT		NT	
33	CF_3							0.19	6.33	0.19	6.33
34	F				Cl	Cl		1.56	5.28	1.56	5.28
35	Br				Cl	Cl		0.095	6.55	0.19	6.28
36	I				Cl	Cl		0.78	5.71	0.78	5.71
37	NO_2				Cl	Cl		0.78	5.62	1.56	5.32
38	CF_3				Cl	Cl		0.19	6.26	0.19	6.26
39	CN				Cl	Cl		1.56	5.28	1.56	5.28
40	COOMe				Cl	Cl		1.56	5.34	0.78	5.64
41	NH_2				Cl	Cl		25	4.07	50	3.77
42	NO_2					Cl		1.56	5.27	1.56	5.27
43	Cl	Cl			Cl	Cl		0.39	5.95	0.39	5.95
44	Cl	Cl				Cl		0.39	5.90	0.39	5.90
45	Cl	CH_3			Cl	Cl		0.39	5.92	0.39	5.92
Ampicillin								0.78		25	
Sultamicillin								0.78		25	

NT: not tested, since they have no clear growth inhibition zone by the disc diffusion method.

It was reported that log k_w is more closely related to log P_{OCT} than isocratic capacity factors. However, log k_w values are usually too high to obtain experimentally, and therefore have to be calculated using extrapolation techniques. For this purpose, the mixture of methanol–water has been used as mobile phase. Methanol appears to be the preferred organic modifier for the determination of lipophilicity by reversed-phase HPLC [26]. The log k_w values were determined from intercepts of linear plots between log k' and φ_M, expressed by Eq. (2) and signifies the capacity factor of the solute in pure water.

$$\log k' = \log k_w + S\varphi_M \quad (2)$$

φ_M, volume fraction of methanol in isocratic elution HPLC; k_w, extrapolated capacity factor for 100% water in isocratic HPLC; S, the slope of the regression curve, should be related to the solvent strength of the pure organic solvent.

Table 2 shows the retention data of the synthesized anilinobenzimidazoles at different volume fractions of methanol in water. Table 3 collects the data from regression analysis of the relation between log k' and φ_M, and includes the partition coefficients, log P. QSAR analysis was carried out by the existence of correlation between log $1/C$ (the MIC value expressed in molar concentration units in Table 1) and log k_w as shown in Eqs. (3) and (4) (parabolic relation).

For *S. aureus* ATCC 25923:

$$\log 1/C = -0.328 <\pm 0.22> [\log k_w]^2 + 3.808 <\pm 1.96> \log k_w - 4.998 <\pm 4.38>$$
$$\log k_w \text{ optimum} = 5.81 <5.27/8.33>$$
$$(N = 32; r = 0.905; s = 0.281; F = 65.529; R^2 = 0.81902) \quad (3)$$

For *S. aureus* ATCC 43300 (MRSA):

$$\log 1/C = -0.350 <\pm 0.21> [\log k_w]^2 + 4.060 <\pm 1.91> \log k_w - 5.713 <\pm 4.28>$$
$$\log k_w \text{ optimum} = 5.80 <5.29/7.76>$$
$$(N = 32; r = 0.917; s = 0.275; F = 76.604; R^2 = 0.8408) \quad (4)$$

In the above equations, R^2 is the square of correlation coefficient; S is the standard error; F is the mean square radio; N is the number of compounds; r is the correlation coefficient.

Eqs. (3) and (4) enable us to conclude that the anti-staphylococci activity of the anilinobenzimidazoles was related to capacity factors log k_w. According to the equation, the anti-staphylococci activity of 2-anilinobenzimidazole derivatives can be predicted and more compounds with different substituents can be designed on the basis of QSAR studies.

In addition, log P values were calculated by using several computer programs which are different from each others (Table 3). In order to find out the most closer log P values from three different programs to log k_w, the linearity equations between log P values and measured log k_w were established. Best correlations were found by ALOGPS's values. The corresponding regression equation is as follows:

Table 2
Isocratic log k' values of **11**, **14–31**, **33–45** for different volume fractions of methanol, φ_M, in water

Compd.	Volume fractions, φ_M		
	0.85	0.80	0.70
11	−0.226	0.020	0.507
14	0.057	0.324	0.839
15	0.020	0.320	0.912
16	0.121	0.424	1.030
17	−0.391	−0.143	0.323
18	0.023	0.321	0.898
19	−0.175	0.118	0.657
20	0.0364	0.347	0.975
21	−0.183	0.112	0.683
22	0.158	0.439	1.024
23	−0.196	0.075	0.597
24	−0.368	−0.100	0.382
25	−0.476	−0.175	0.289
26	−0.233	0.065	0.564
27	−0.173	0.080	0.562
28	−0.145	0.121	0.644
29	−0.130	0.172	0.769
30	−0.153	0.150	0.735
31	−0.527	−0.277	0.160
33	0.133	0.468	1.138
34	−0.149	0.117	0.641
35	0.094	0.379	0.945
36	0.157	0.445	1.040
37	0.026	0.279	0.810
38	0.075	0.389	1.010
39	−0.182	0.073	0.607
40	−0.160	0.127	0.681
41	−0.765	−0.514	−0.056
42	−0.257	−0.015	0.453
43	0.283	0.591	1.233
44	0.039	0.325	0.895
45	0.178	0.475	1.070

$$\log k_w = 0.906 <\pm 0.13> \log P_{calc(ALOGPS)} + 0.302 <\pm 0.60>$$
$$(N = 32; r = 0.937; s = 0.234; F = 216.47; R^2 = 0.8779) \quad (5)$$

To obtain the quantitative effects of the log P parameters of anilinobenzimidazoles, on their anti-staphylococci activity, OSAR analysis with log P (ALOGPS) parameter was operated against both bacteria as shown in Eqs. (6) and (7).

For *S. aureus* ATCC 25923:

$$\log 1/C = -0.345 <\pm 0.25> [\log P]^2 + 4.02 <\pm 2.44> \log P - 5.708 <\pm 5.78>$$
$$\log P \text{ optimum} = 5.83 <5.33/8.85>$$
$$(N = 32; r = 0.824; s = 0.374; F = 30.78; R^2 = 0.678) \quad (6)$$

For *S. aureus* ATCC 43300 (MRSA):

$$\log 1/C = -0.386 <\pm 0.25> [\log P]^2 + 4.464 <\pm 2.38> \log P - 6.911 <\pm 5.63>$$
$$\log P \text{ optimum} = 5.78 <5.34/7.62>$$
$$(N = 32; r = 0.849; s = 0.364; F = 37.57; R^2 = 0.7208) \quad (7)$$

It is significant that the results obtained from Eqs. (6) and (7) did not give satisfactory correlation than Eqs. (3) and (4).

Table 3
Regression analysis of the relationship between the volume fraction of methanol, φ_M, and $\log k'$; partition coefficients, $\log P$ values of compounds **11**, **14**–**31**, **33**–**45**

Compd.	$\log k_w$	$-S$	R^2	ALOGPS $\log P$ (average)	ACD $\log P$	ChemOffice Clog P
11	3.926	4.884	1.0000	4.33	5.04	5.46
14	4.483	5.204	0.9999	5.07	5.25	6.33
15	5.073	5.943	1.0000	5.18	5.68	6.58
16	5.272	6.060	1.0000	6.00	6.32	7.64
17	3.647	4.746	0.9998	4.24	4.19	5.19
18	4.976	5.824	0.9999	5.09	5.64	6.21
19	4.527	5.524	0.9996	4.70	5.33	5.76
20	5.357	6.261	1.0000	5.19	6.22	6.38
21	4.719	5.764	0.9999	4.70	5.09	5.85
22	5.071	5.784	0.9999	5.21	5.82	6.33
23	4.292	5.277	0.9999	4.35	4.75	5.19
24	3.868	4.974	0.9993	4.14	4.23	5.03
25	3.823	5.034	0.9954	4.00	4.19	5.03
26	4.258	5.267	0.9979	4.25	4.73	5.60
27	3.986	4.889	0.9999	4.06	4.70	5.27
28	4.324	5.256	1.0000	4.47	4.91	5.75
29	4.963	5.990	1.0000	4.85	4.73	5.88
30	4.874	5.910	0.9999	4.77	4.75	5.85
31	3.350	4.550	0.9988	3.68	3.69	4.77
33	5.828	6.700	1.0000	6.17	6.29	7.89
34	4.326	5.263	1.0000	4.50	5.09	5.64
35	4.915	5.671	1.0000	5.19	5.81	6.36
36	5.166	5.896	0.9999	5.60	6.07	6.62
37	4.475	5.239	0.9999	4.22	4.77	5.33
38	5.372	6.230	1.0000	5.30	5.61	6.43
39	4.295	5.271	0.9999	4.25	4.48	5.04
40	4.600	5.597	0.9999	4.36	5.02	5.53
41	3.241	4.706	0.9995	3.41	3.76	4.91
42	3.762	4.726	0.9999	3.63	3.87	4.73
43	5.673	6.346	0.9999	5.74	6.11	6.81
44	4.889	5.706	1.0000	5.10	5.20	6.21
45	5.233	5.947	1.0000	5.41	6.10	6.71

4. Conclusion

The synthesis of the 34 new anilinobenzimidazoles (**11**–**17**, **19**–**45**), two anilinobenzothiazoles (**47**–**48**) and one anilinobenzoxazole (**51**) was performed by moderate yield, in multiple steps, using three different methods. The mostly used method was CuCl-promoted intramolecular cyclization of *N*-(2-aminoaryl)thioureas for the synthesis of anilinobenzimidazoles (Method A). According to obtained antimicrobial results, these anilinobenzimidazole series can show very good activity profiles versus Gram-positive bacteria *S. aureus* and MRSA. Compounds **35** and **38** having bromo or CF_3 groups at position C-5(6) and 3,4-dichloro substituted phenyl at position C-2 of benzimidazole exhibited the greatest activity against *S. aureus* and MRSA with MIC values of 0.095–0.19 μg/mL. QSAR analysis performed between the log 1/*C* (the MIC value expressed in molar concentration units against bacteria) and extrapolated $\log k_w$ values of the anilinobenzimidazoles can provide hydrophobicity parameters which are strongly related to $\log P$ in the standard octanol–water system and good correlation was observed. More extensive study is needed to confirm these in vitro results and in vivo and mode of action studies are required.

5. Experimental section

5.1. Lipophilicity HPLC determination (capacity factor, k)

The HPLC separation module Waters Alliance 2695 and Waters Photodiode Array Detector 2487 (Waters Corp., Milford, MA, USA) were used. XTerra® MS C_{18} 5 μm, 4.6 × 250 mm was used as chromatographic column. HPLC grade methanol and double glass distilled water were used in all determination as mobile phase. The total flow of the column was 0.9 mL/min. The detection wavelength of 254 nm was chosen. The column dead time, t_0, of the system was measured by acetone (2 μl). Retention times and dead times for all the synthesized compounds were measured at three different methanol concentrations in the mobile phase in Table 2. Retention times (t_R) were measured in minutes, and capacity factors were calculated (k'). k_w Value was calculated using regression analysis program GraphPad Instat ver. 3.0.5 by extrapolation technique.

5.1.1. Lipophilicity calculations

The logarithm of the partition coefficient for *n*-octanol–water, $\log P$ was calculated using the programs ACD/ChemSketch ver. 10.02 (Advanced Chemistry Development Inc., Toronto, Canada) and ALOGPS (average $\log P$) ver 2.1 (Virtual Computational Chemistry Laboratory, Munich, Germany), Clog P values (the logarithm of *n*-octanol–water partition coefficient based on established chemical interactions) were generated by means of CS ChemOffice Ultra ver. 8.0.3 software.

5.2. QSAR analysis

QSAR models were derived by multiple regression analyses that were performed using the BILIN program to determine the coefficients of the correlation equations. BILIN Program, version 1994, graciously provided by Prof. Dr. H. Kubinyi, BASF AG, Ludwigshafen, Germany.

Melting points were measured with a capillary melting point apparatus (Buchi SMP 20) and are uncorrected. The IR spectra were recorded on a Jasco FT/IR-420 spectrometer as KBr discs. The ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded using VARIAN Mercury 400 FT-NMR spectrophotometers, δ scale (ppm) from TMS. Coupling constants (J) are given in hertz (Hz). LC/MS analyses were performed with Waters Alliance and Micromass ZQ by using ESI (+). Elemental analyses were taken on a Leco 932 CHNS-O analyzer. For the HCl salts of the synthesized compounds, the free bases were dissolved in ethanol and dry HCl gas was passed through the solution. Compounds **46** [27], **49** [28] and **50** [29] were prepared according to the literature method.

*5.2.1. 2-Chloro-5(6)-trifluoromethyl-1H-benzimidazole (**9**)*

5(6)-Trifluoromethyl-1,3-dihydro-benzimidazole-2-one **8** (0.295 g, 1.46 mmol) was heated under reflux in $POCl_3$ (5 ml) for 13 h. Excess of $POCl_3$ was evaporated in vacuo, then neutralized with saturated $NaHCO_3$, extracted with EtOAc and purified by flash chromatography (EtOAc–hexane 1:2), 0.2 g, 62%, mp 186–188 °C, ^{1}H NMR (CD_3OD) δ 7.44–7.46 (dd, 1H, $J_o = 8.4$ Hz, $J_m = 1.2$ Hz), 7.55 (d, 1H, $J_o = 8.8$ Hz), 7.72 (s, 1H). MS *m/z* (ESI+): 221 (%100) (M + H), 223 (%34.4) (M + H + 2).

5.2.1.1. Method A. A mixture of corresponding *o*-phenylenediamines (4 mmol) and substituted phenylisothiocyanates (4 mmol) was heated under reflux in CH_3CN (2.7 ml) and toluene (11 ml) for 15 min. To this mixture Celite (0.4 g), *N*-ethyldiisopropylamine (2 ml) and CuCl (0.9 g) were added. The resulting mixture was heated to 80 °C and kept at this temperature for 1 h. The reaction mixture was then cooled to 40 °C and filtered. The filtrate was washed with a 4:1 mixture of toluene and acetonitrile. The combined filtrates were washed with 7% ammonium hydroxide and evaporated. The solvents were removed and the residue purified by column chromatography on silica gel with mixture of EtOAc–hexane in the appropriate ratio.

5.2.1.2. Method B. A mixture of corresponding *o*-phenylenediamines (4 mmol) and substituted phenylisothiocyanates (4 mmol) was heated under reflux in benzene (60 ml) for 15 min. To this mixture DCCI (1.05 g, 5.1 mmol) was added and heated for 10 h. The reaction mixture was extracted with dilute Na_2CO_3 solution and water several times. When precipitate occurred between two phases, precipitate was combined with benzene and evaporated. Purification was done by column chromatography on silica gel with mixture of EtOAc–hexane in the appropriate ratio as an eluent, after preparing HCl salt, pure product was crystallized at least two times from ethanol.

5.2.1.3. Method C. A mixture of corresponding 2-chloro-1*H*-benzimidazoles (1 mmol) and substituted anilines (1 mmol) in DMF (2 mL) was heated under reflux for 30 h, at 160–170 °C. The cooled crude product mixture was boiled with diluted HCl and filtered. The filtered solid was then washed with dilute sodium hydroxide solution, then with water and dried. Purification was done by column chromatography on silica gel using different volume ratio of EtOAc–hexane as an eluent.

*5.2.2. 2-(3,4-Dichloroanilino)-1H-benzimidazole HCl (**11**). Method C*

Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:3) to obtain **11**; yield 62%; mp 304–307 °C, ^{1}H NMR (DMSO-d_6): δ 7.26–7.28 (m, 2H), 7.46–7.50 (m, 3H), 7.70 (d, 1H, $J_o = 8.8$ Hz), 7.81 (d, 1H, $J_m = 2.8$ Hz), ^{13}C NMR (DMSO-d_6): δ 147.8, 137.51, 132.56, 132.06, 130.57, 128.24, 124.74, 124.23, 123.31, 112.76. MS ESI (+) *m/z*: 278 (100%) (M + H), 280 (65.4%) (M + H + 2), 282 (11.3%) (M + H + 4). Anal. calcd. for $C_{13}H_9Cl_2N_3 \cdot HCl$: C 49.63, H 3.20, N 13.36, found: C 49.43, H 3.34, N 13.15.

*5.2.3. 1-Ethyl-5-chloro-2-(3,4-dichloroanilino)-1H-benzimidazole (**12**). Method A*

Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:3) to obtain **12**; yield 56%; mp 217–220 °C, ^{1}H NMR (DMSO-d_6): δ 1.27 (t, 3H), 4.25–4.30 (q, 2H), 7.09–7.12 (dd, 1H, $J_o = 8.4$ Hz, $J_m = 1.2$ Hz), 7.39 (d, 1H, $J_o = 8.4$ Hz), 7.48 (d, 1H, $J_m = 1.2$ Hz), 7.58 (d, 1H, $J_o = 9.2$ Hz), 7.86–7.89 (dd, 1H, $J_o = 9.2$ Hz, $J_m = 2$ Hz), 8.31 (d, 1H, $J_m = 2$ Hz), ^{13}C NMR (DMSO-d_6): δ 150.62, 143.35, 141.40, 132.61, 131.55, 131.12, 126.12, 123.19, 120.65, 119.85, 118.93, 116.76, 110.24, 37.61, 14.95. MS ESI (+) *m/z*: 340 (%100) (M + H), 342 (%98.1) (M + H + 2), 344 (%32.5) (M + H + 4), 346 (%4.4) (M + H + 6). Anal. calcd. for $C_{15}H_{12}Cl_3N_3 \cdot 0.1\ H_2O$: C 52.89, H 3.55, N 12.34, found: C 52.61, H 3.59, N 12.27.

*5.2.4. 1-(4-Chlorophenyl)-5-chloro-2-(3,4-dichloroanilino)-1H-benzimidazole (**13**). Method A*

Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:6) to obtain **13**; yield 17%; mp 243–247 °C, ^{1}H NMR ($CDCl_3$): δ 6.87 (d, 1H, $J_o = 8$ Hz), 7.05–7.08 (dd, 1H, $J_o = 8.4$ Hz, $J_m = 2$ Hz), 7.36–7.66 (m, 7H), 7.86 (d, 1H, $J_m = 2.4$ Hz), ^{13}C NMR ($CDCl_3$): δ 148.93, 143.02, 138.42, 136.23, 133.14, 132.32, 131.50, 130.85, 128.89, 128.33, 126.09, 121.86, 120.05, 118.02, 117.83, 109.10. MS ESI (+) *m/z*: 422 (%78.1) (M + H), 424 (%100) (M + H + 2), 426 (%51.3) (M + H + 4), 428 (%11.9) (M + H + 6), 430 (%1.3) (M + H + 8). Anal. calcd. for $C_{19}H_{11}Cl_4N_3 \cdot 0.1\ H_2O$: C 53.70, H 2.66, N 9.89, found: C 53.65, H 2.62, N 9.83.

*5.2.5. 5(6)-Chloro-2-(2,4-dichloroanilino)-1H-benzimidazole HCl (**14**). Method A*

Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:3) to obtain **14**; yield 17%; mp 301–306 °C, ^{1}H NMR (DMSO-d_6): δ 7.25–7.28 (dd, 1H, $J_o = 8.4$ Hz, $J_m = 2$ Hz), 7.41 (d, 1H, $J_o = 8.8$ Hz), 7.46 (d, 1H, $J_m = 2$ Hz), 7.56–7.59 (dd, 1H, $J_o = 8.8$ Hz, $J_m = 2.4$ Hz), 7.79 (d, 1H, $J_o = 8.8$ Hz), 7.85 (d, 1H, $J_m = 2.4$ Hz), ^{13}C NMR (DMSO-d_6): δ 149.14, 132.38, 132.12, 131.61, 130.40, 129.86, 129.49, 128.94, 128.77, 127.26, 123.19, 113.26, 111.86. MS ESI (+) *m/z*: 312 (%100) (M + H), 314 (%95) (M + H + 2), 316 (%32.5) (M + H + 4), 318 (%3.8) (M + H + 6). Anal. calcd. for $C_{13}H_8Cl_3N_3 \cdot HCl$: C 44.73, H 2.60, N 12.04, found: C 44.65, H 2.66, N 11.91.

*5.2.6. 5(6)-Chloro-2-(2-chloro-5-trifluoromethylanilino)-1H-benzimidazole HCl (**15**). Method A*

Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:3) to obtain **15**; yield 17%; mp 271–276 °C, ^{1}H NMR (DMSO-d_6): δ 7.28–7.31 (dd, 1H, $J_o = 8.8$ Hz, $J_m = 2$ Hz), 7.46–7.48 (d, 1H, $J_o = 8.8$ Hz),

7.52–7.53 (d, 1H, $J_m = 1.6$ Hz), 7.75–7.77 (d, 1H, $J_o = 8.8$ Hz), 7.90–7.93 (d, 1H, $J_o = 8.8$ Hz), 8.34 (s, 1H). MS ESI (+) *m/z*: 346 (%100) (M + H), 348 (%73.8) (M + H + 2), 350 (%12.5) (M + H + 4). Anal. calcd. for $C_{14}H_8Cl_2F_3N_3$. HCl: C 43.95, H 2.37, N 10.98, found: C 44.01, H 2.40, N 10.91.

5.2.7. 5(6)-Chloro-2-[2-(4′-chlorophenoxy)anilino]-1H-benzimidazole HCl (**16**). Method C

Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:4) to obtain **16**; yield 46%; mp 116–119 °C, ^{1}H NMR (CD_3OD): δ 6.80–6.83 (d, 2H), 7.07–7.09 (dd, 1H), ve 7.18–7.39 (m, 7H), 7.53–7.55 (dd, 1H, $J_o = 8$ Hz, $J_m = 1.6$ Hz), ^{13}C NMR (CD_3OD): δ 155.48, 151.13, 149.82, 131.21, 129.86, 129.55, 129.37, 129.05, 128.84, 127.34, 126.73, 125.46, 124.11, 120.65, 119.33, 112.72, 111.81. MS ESI (+) *m/z*: 370 (%100) (M + H), 372 (%67.5) (M + H + 2), 374 (%11.9) (M + H + 4). Anal. calcd. for $C_{19}H_{13}Cl_2N_3O \cdot 1.4$ HCl: C 54.17, H 3.45, N 9.98, found: C 54.06, H 4.06, N 9.63.

5.2.8. 5(6)-Chloro-2-(2,4-difluoroanilino)-1H-benzimidazole HCl (**17**). Method A

Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:3) to obtain **17**; yield 33.5%; mp 97–101 °C, ^{1}H NMR (DMSO-d_6): δ 7.01–7.39 (m, 5H), 8.52 (s, 1H). MS ESI (+) *m/z*: 280 (%100) (M + H), 282 (%32.5) (M + H + 2). Anal. calcd. for $C_{13}H_8ClF_2N_3 \cdot 0.5$ HCl: C 52.41, H 2.88, N 14.11, found: C 52.63, H 3.14, N 13.89.

5.2.9. 5(6)-Chloro-2-(3,4-dichloroanilino)-1H-benzimidazole HCl (**18**) [3]. Method A

Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:3) to obtain **18**; yield 29%; mp 306–309 °C, ^{1}H NMR (DMSO-d_6): δ 7.24–7.27 (dd, 1H, $J_o = 8.8$ Hz, $J_m = 2.4$ Hz), 7.40–7.47 (m, 2H), 7.49 (d, 1H, $J_m = 2.8$ Hz), 7.69 (d, 1H, $J_o = 8.8$ Hz), 7.87 (d, 1H, $J_m = 2.8$ Hz), ^{13}C NMR (DMSO-d_6): δ 149.05, 137.73, 132.78, 132.51, 132.03, 130.6, 127.92, 124.37, 123.83, 123.01, 114.09, 112.7. MS ESI (+) *m/z*: 310 (%100) (M − H), 312 (%97.5) (M + H), 314 (%31.9) (M + H + 2), 316 (%3.8) (M + H + 4). Anal. calcd. for $C_{13}H_8Cl_3N_3 \cdot$HCl: C 44.73, H 2.60, N 12.04, found: C 44.67, H 2.65, N 11.97.

5.2.10. 5(6)-Chloro-2-(3-chloro-4-fluoroanilino)-1H-benzimidazole HCl (**19**). Method C

Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:3) to obtain **19**; yield 13%; mp >300 °C, ^{1}H NMR (DMSO-d_6): δ 7.28–7.30 (dd, 1H, $J_o = 8.8$ Hz, $J_m = 2$ Hz), 7.43 (d, 1H, $J_o = 8.8$ Hz), 7.45 (d, 1H, $J_m = 2$ Hz), 7.51–7.60 (m, 2H), 7.85–7.87 (dd, 1H, $J_{HH} = 2.8$ Hz, $J_{HF} = 6.4$ Hz), ^{13}C NMR (DMSO-d_6): δ 156.89, 154.97, 154.46, 149.76, 134.65, 132.91, 130.70, 127.78, 125.43, 124.26, 123.68, 120.95, 120.76, 118.53, 118.31, 113.93, 112.58. MS ESI (+) *m/z*: 296 (%100) (M + H), 298 (%40) (M + H + 2), 300 (%6.25) (M + H + 4). Anal. calcd. for $C_{13}H_8Cl_2FN_3 \cdot$HCl: C 46.95, H 2.73, N 12.63, found: C 46.74, H 2.84, N 12.29.

5.2.11. 5(6)-Chloro-2-(4-chloro-3-trifluoromethylanilino)-1H-benzimidazole HCl (**20**). Method A

Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:3) to obtain **20**; yield 11.5%; mp 119–224 °C, ^{1}H NMR (DMSO-d_6): δ 7.02 (d, 1H, $J_o = 8$ Hz), 7.27–7.39 (m, 2H), 7.61–7.63 (d, 1H, $J_o = 8.8$ Hz), 8.07–8.09 (d, 1H, $J_o = 9.2$ Hz), 8.28 (s, 1H). MS ESI (+) *m/z*: 346 (%100) (M + H), 348 (%63.8) (M + H + 2), 350 (%10.6) (M + H + 4). Anal. calcd. for $C_{14}H_8Cl_2F_3N_3 \cdot$HCl: C 43.95, H 2.37, N 10.98, found: C 43.91, H 2.50, N 10.73.

5.2.12. 5(6)-Chloro-2-(3-trifluoromethylanilino)-1H-benzimidazole HCl (**21**). Method C

Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:3) to obtain **21**; yield 33.5%; mp 244–249 °C, ^{1}H NMR (CD_3OD): δ 7.31–7.73 (m, 7H), ^{13}C NMR (CD_3OD): δ 149.19, 136.63, 132.5 (q, $J = 33$ Hz), 131.29, 130.81, 129.7, 128.73, 127.27, 125.23, 124.46, 123.91 (q, $J = 3.9$ Hz), 120.56 (q, $J = 3.9$ Hz), 112.95, 111.95. MS ESI (+) *m/z*: 312 (%100) (M + H), 314 (%41.3) (M + H + 2). Anal. calcd. for $C_{14}H_9ClF_3N_3 \cdot 1.3$ HCl: C 46.83, H 2.89, N 11.70, found: C 47.06, H 2.97, N 11.78.

5.2.13. 5(6)-Chloro-2-(3,5-dichloroanilino)-1H-benzimidazole HCl (**22**). Method A

Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:3) to obtain **22**; yield 17%; mp >300 °C, ^{1}H NMR (DMSO-d_6): δ 7.25–7.28 (dd, 1H, $J_o = 8.4$ Hz, $J_m = 2$ Hz), 7.41–7.42 (m, 1H), 7.45 (d, 1H, $J_o = 8.8$ Hz), 7.48 (d, 1H, $J_m = 1.6$ Hz), 7.60 (d, 2H, $J_m = 1.6$ Hz), ^{13}C NMR (DMSO-d_6): δ 148.81, 140.37, 135.36, 133.07, 130.87, 127.88, 124.82, 123.79, 120.57, 114.22, 112.84. MS ESI (+) *m/z*: 312 (%100) (M + H), 314 (%96.3) (M + H + 2), 316 (%31.3) (M + H + 4), 318 (%3.8) (M + H + 6). Anal. calcd. for $C_{13}H_8Cl_3N_3 \cdot$HCl: C 44.73, H 2.60, N 12.04, found: C 44.80, H 2.66, N 11.91.

5.2.14. 5(6)-Chloro-2-(3,5-difluoroanilino)-1H-benzimidazole HCl (**23**). Method B

Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:3) to obtain **23**; yield 11%; mp >300 °C, ^{1}H NMR (DMSO-d_6): δ 7.03–7.07 (m, 1H), 7.25 (d, 1H, $J_o = 8.8$ Hz), 7.30 (d, 2H, $J_o = 8.8$ Hz), 7.41–7.44 (dd, 1H, $J_o = 8.4$ Hz, $J_m = 1.6$ Hz), 7.47 (s, 1H), ^{13}C NMR (DMSO-d_6): δ 164.75 (d, $J = 15$ Hz), 162.32 (d, $J = 15.4$ Hz), 149.24, 140.92 (t, $J = 13.2$ Hz), 134.05, 131.69, 127.62, 123.5, 114.3, 112.99, 104.8 (d, $J = 27$ Hz), 100.26 (t, $J = 27$ Hz). MS ESI (+) *m/z*: 280 (%100) (M + H), 282 (%34.4) (M + H + 2). Anal. calcd. for $C_{13}H_8ClF_2N_3 \cdot 0.9$ HCl: C 49.97, H 2.87, N 13.45, found: C 49.71, H 2.91, N 13.15.

*5.2.15. 5(6)-Chloro-2-(3-fluoroanilino)-1H-benzimidazole HCl (**24**). Method B*

Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:1) to obtain **24**; yield 11%; mp 285–290 °C, ^{1}H NMR (DMSO-d_6): δ 7.08–7.13 (m, 1H), 7.28–7.34 (m, 2H), 7.43–7.55 (m, 4H). MS ESI (+) *m/z*: 262 (%100) (M + H), 264 (%40.6) (M + H + 2). Anal. calcd. for $C_{13}H_9ClFN_3 \cdot HCl$: C 52.37, H 3.38, N 14.09, found: C 52.19, H 3.43, N 13.96.

*5.2.16. 5(6)-Chloro-2-(4-fluoroanilino)-1H-benzimidazole HCl (**25**). Method C*

Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:3) to obtain **25**; yield 16%; mp 275–278 °C, ^{1}H NMR (DMSO-d_6): δ 7.27–7.29 (dd, 1H, $J_o = 8.4$ Hz, $J_m = 1.2$ Hz), 7.34–7.38 (m, 2H), 7.40 (d, 1H, $J_o = 8.4$ Hz), 7.44 (d, 1H, $J_m = 1.2$ Hz), 7.54–7.57 (m, 2H). MS ESI (+) *m/z*: 262 (%100) (M + H), 264 (%31.9) (M + H + 2). Anal. calcd. for $C_{13}H_9ClFN_3 \cdot HCl$: C 52.37, H 3.38, N 14.09, found: C 52.10, H 3.42, N 13.79.

*5.2.17. 5(6)-Chloro-2-(4-chloroanilino)-1H-benzimidazole HCl (**26**). Method A*

Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:3) to obtain **26**; yield 10.3%; mp 280–290 °C, ^{1}H NMR (DMSO-d_6): δ 7.28–7.30 (dd, 1H, $J_o = 8.4$ Hz, $J_m = 2$ Hz), 7.42 (d, 1H, $J_o = 8.8$ Hz), 7.46 (d, 1H, $J_m = 2$ Hz), 7.56 (s, 4H), ^{13}C NMR (DMSO-d_6): 149.23, 136.15, 132.36, 130.48, 130.32, 130.21, 127.96, 124.96, 123.89, 113.93, 112.52. MS ESI (+) *m/z*: 278 (%100) (M + H), 280 (%66.2) (M + H + 2), 282 (%11.8) (M + H + 4). Anal. calcd. for $C_{13}H_9Cl_2N_3 \cdot HCl$: C 49.63, H 3.20, N 13.36, found: C 49.57, H 3.37, N 13.31.

*5.2.18. 5(6)-Chloro-2-(4-azidoanilino)-1H-benzimidazole HCl (**27**). Method A*

Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:3) to obtain **27**; yield 11.1%; mp 304–307 °C, ^{1}H NMR (CD_3OD): δ 7.13–7.16 (d, 2H), 7.20–7.23 (dd, 1H, $J_o = 8.8$ Hz, $J_m = 2$ Hz), 7.29 (d, 1H, $J_o = 8.8$ Hz), 7.33 (d, 1H, $J_m = 2$ Hz), 7.38–7.40 (d, 2H), ^{13}C NMR (CD_3OD): δ 149.68, 139.86, 132.24, 130.81, 129.52, 128.73, 125.93, 124.27, 120.64, 112.74, 111.76. MS ESI (+) *m/z*: 285 (%100) (M + H), 287 (%31.9) (M + H + 2). Anal. calcd. for $C_{13}H_9ClN_6 \cdot 2\ HCl \cdot 1.5\ H_2O$: C 40.59, H 3.67, N 21.85, found: C 40.21, H 3.17, N 24.29.

*5.2.19. 5(6)-Chloro-2-(4-bromoanilino)-1H-benzimidazole HCl (**28**). Method B*

Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:3) to obtain **28**; yield 10%; mp 295–298 °C, ^{1}H NMR (DMSO-d_6): δ 7.28–7.30 (dd, 1H, $J_o = 8.4$ Hz, $J_m = 2$ Hz), 7.43 (d, 1H, $J_o = 8.4$), 7.47–7.50 (m, 3H), 7.68 (d, 2H, $J_o = 8.4$ Hz), ^{13}C NMR (DMSO-d_6): δ 149.3, 136.71, 133.22, 132.62, 130.44, 127.89, 125.12, 123.81, 118.49, 113.93, 112.54. MS ESI (+) *m/z*: 322 (%76.3) (M + H), 324 (%100) (M + H + 2), 326 (%24.4) (M + H + 4). Anal. calcd. for $C_{13}H_9BrClN_3 \cdot HCl$: C 43.49, H 2.81, N 11.70, found: C 43.26, H 3.10, N 11.61.

*5.2.20. 5(6)-Chloro-2-(4-trifluoromethoxyanilino)-1H-benzimidazole HCl (**29**). Method A*

Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:3) to obtain **29**; yield 15%; mp 265–269 °C, ^{1}H NMR (DMSO-d_6): δ 7.25–7.28 (dd, 1H, $J_o = 8.4$ Hz, $J_m = 2$ Hz), 7.41–7.43 (d, 1H, $J_o = 8.8$ Hz), 7.45–7.48 (m, 3H), 7.60–7.62 (d, 2H). MS ESI (+) *m/z*: 328 (%100) (M + H), 330 (%34.4) (M + H + 2). Anal. calcd. for $C_{14}H_9ClF_3N_3O \cdot HCl$: C 46.18, H 2.77, N 11.54, found: C 46.38, H 2.71, N 11.46.

*5.2.21. 5(6)-Chloro-2-(4-trifluoromethylanilino)-1H-benzimidazole HCl (**30**). Method C*

Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:3) to obtain **30**; yield 13%; mp 269–272 °C, ^{1}H NMR (CD_3OD): δ 7.28–7.30 (dd, 1H, $J_o = 8.4$ Hz, $J_m = 1.6$ Hz), 7.35–7.41 (m, 2H), 7.57 (d, 2H, $J_o = 8.4$ Hz), 7.77 (d, 2H, $J_o = 8.4$ Hz). MS ESI (+) *m/z*: 312 (%100) (M + H), 314 (%34.4) (M + H + 2). Anal. calcd. for $C_{14}H_9ClF_3N_3 \cdot HCl \cdot 1.7\ H_2O$: C 44.39, H 3.57, N 11.09, found: C 44.97, H 3.59, N 10.50.

*5.2.22. 5(6)-Chloro-2-(4-methoxyanilino)-1H-benzimidazole HCl (**31**). Method A*

Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:3) to obtain **31**; yield 19%; mp 230–235 °C, ^{1}H NMR (DMSO-d_6): δ 3.80 (s, 3H), 7.06–7.08 (d, 2H, $J_o = 7.2$), 7.26–7.28 (dd, 1H, $J_o = 8.4$ Hz, $J_m = 1.6$ Hz), 7.37 (d, 2H, $J_o = 8.8$ Hz), 7.39–7.41 (m, 2H), ^{13}C NMR (DMSO-d_6): δ 158.72, 150.17, 131.98, 129.89, 129.16, 127.92, 126.43, 123.86, 115.8, 113.67, 112.24, 56.19. MS ESI (+) *m/z*: 274 (%100) (M + H), 276 (%40) (M + H + 2). Anal. calcd. for $C_{14}H_{12}ClN_3O \cdot 1.5\ HCl$: C 51.20, H 4.14, N 12.80, found: C 51.37, H 4.73, N 12.68.

*5.2.23. 1-Butyl-5-cyano-2-(4-fluoroanilino)-1H-benzimidazole (**32**). Method A*

Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:3) to obtain **32**; yield 48.7%; mp 218–223 °C, ^{1}H NMR ($CDCl_3$): δ 0.98 (t, 3H), 1.40–1.46 (m, 2H), 1.77–1.82 (m, 2H), 4.03 (t, 2H), 7.06–7.10 (m, 2H), 6.27 (s, 1H), 7.18 (d, 1H, $J_o = 8$ Hz), 7.39–7.41 (dd, 1H, $J_o = 8$ Hz, $J_m = 1.6$ Hz), 7.56–7.60 (m, 2H), 7.80 (s, 1H). MS ESI (+) *m/z*: 309 (%100) (M + H). Anal. calcd. for $C_{18}H_{17}FN_4$: C 70.11, H 5.56, N 18.17, found: C 70.30, H 5.66, N 17.97.

*5.2.24. 5(6)-Trifluoromethyl-2-[2-(4′-chlorophenoxy)anilino]-1H-benzimidazole (**33**). Method C*

Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:3) to obtain **33**; yield 48%; mp 67–70 °C, ^{1}H NMR ($CDCl_3$): δ 6.58 (d, 2H), 6.77 (d, 1H), 6.89–7.49 (m, 8H), 8.12 (d, 1H). MS ESI (+) *m/z*: 404

(%100) (M + H), 406 (%31.9) (M + H + 2). Anal. calcd. for $C_{20}H_{13}ClF_3N_3O \cdot 4H_2O \cdot 0.1\ C_4H_8O_2$: C 50.56, H 4.53, N 8.67, found: C 50.56, H 3.64, N 8.93.

5.2.25. 5(6)-Fluoro-2-(3,4-dichloroanilino)-1H-benzimidazole HCl (**34**). Method A

Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:3) to obtain **34**; yield 27%; mp >310 °C, ^{1}H NMR (DMSO-d_6): δ 7.10–7.15 (m, 1H), 7.30–7.33 (dd, 1H, $J_o = 8.4$ Hz, $J_m = 2$ Hz), 7.45–7.53 (m, 2H), 7.72 (d, 1H, $J_o = 8.4$ Hz), 7.87 (d, 1H, $J_m = 2$ Hz), ^{13}C NMR (DMSO-d_6): δ 159.42 (d, $J = 236$ Hz), 149.15, 137.78, 132.5, 132.3, 132.15, 132.02, 127.9 (d, $J = 11$ Hz), 124.33, 122.97, 113.7 (d, $J = 9$ Hz), 111.1 (d, $J = 25$ Hz), 100.26 (d, $J = 27$ Hz). MS ESI (+) *m/z*: 296 (%100) (M + H), 298 (%68.1) (M + H + 2), 300 (%11.3) (M + H + 4). Anal. calcd. for $C_{13}H_8Cl_2FN_3 \cdot 1.1$ HCl: C 46.44, H 2.73, N 12.50, found: C 46.66, H 2.72, N 12.52.

5.2.26. 5(6)-Bromo-2-(3,4-dichloroanilino)-1H-benzimidazole HCl (**35**). Method A

Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:3) to obtain **35**; yield 58%; mp 306–310 °C, ^{1}H NMR (DMSO-d_6): δ 7.38 (s, 2H), 7.48–7.50 (dd, 1H, $J_o = 9.2$ Hz, $J_m = 2.4$ Hz), 7.59 (d, 1H, $J_m = 1.2$ Hz), 7.68 (d, 1H, $J_o = 8.4$ Hz), 7.86 (d, 1H, $J_m = 2.4$ Hz), ^{13}C NMR (DMSO-d_6): δ 148.86, 137.81, 133.33, 132.48, 131.98, 131.12, 127.73, 126.46, 124.14, 122.80, 115.49, 115.44, 114.55. MS ESI (+) *m/z*: 356 (%85) (M + H), 358 (%100) (M + H + 2), 360 (%60) (M + H + 4). Anal. calcd. for $C_{13}H_8BrCl_2N_3 \cdot 1.4$ HCl: C 38.26, H 2.32, N 10.30, found: C 38.30, H 2.46, N 10.44.

5.2.27. 5(6)-Iodo-2-(3,4-dichloroanilino)-1H-benzimidazole HCl (**36**). Method A

Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:3) to obtain **36**; yield 38%; mp 284–286 °C, ^{1}H NMR (DMSO-d_6): δ 7.27 (d, 1H, $J_o = 8.4$ Hz), 7.47–7.50 (dd, 1H, $J_o = 8.4$ Hz, $J_m = 2.8$ Hz), 7.55–7.57 (dd, 1H, $J_o = 8$ Hz, $J_m = 1.6$ Hz), 7.71 (d, 1H, $J_o = 8.4$ Hz), 7.76 (d, 1H, $J_m = 1.2$ Hz), 7.83 (d, 1H, $J_m = 2.8$ Hz), ^{13}C NMR (DMSO-d_6): δ 148.27, 137.56, 133.04, 132.57, 132.45, 132.08, 131.06, 128.17, 124.62, 123.22, 121.00, 114.92. MS ESI (+) *m/z*: 404 (%100) (M + H), 406 (%65.6) (M + H + 2), 408 (%11.3) (M + H + 4), 277 (M + H − I). Anal. calcd. for $C_{13}H_8Cl_2IN_3 \cdot 0.9$ HCl: C 35.74, H 2.05, N 9.62, found: C 35.81, H 2.43, N 9.17.

5.2.28. 5(6)-Nitro-2-(3,4-dichloroanilino)-1H-benzimidazole HCl (**37**). Method B

Crude product was purified by chromatography on silica gel (EtOAc–EtOH 95:5) to obtain **37**; yield 46%; mp 292–294 °C, ^{1}H NMR (DMSO-d_6): δ 7.55 (d, 1H, $J_o = 8.4$ Hz), 7.57–7.60 (dd, 1H, $J_o = 9.2$ Hz, $J_m = 2$ Hz), 7.66 (d, 1H, $J_o = 8.8$ Hz), 8.03 (d, 1H, $J_m = 2.8$ Hz), 8.07–8.09 (dd, 1H, $J_o = 8.8$ Hz, $J_m = 2.4$ Hz), 8.20 (d, 1H, $J_m = 2.4$ Hz), 10.15 (s, 1H), 11.6 (br s, 1H). MS ESI (+) *m/z*: 323 (%100) (M + H), 325 (%65.6) (M + H + 2), 327 (%11.9) (M + H + 4). Anal. calcd. for $C_{13}H_8Cl_2N_4O_2 \cdot 2$ HCl: C 39.42, H 2.55, N 14.15, found: C 39.28, H 2.55, N 14.33.

5.2.29. 5(6)-Trifluoromethyl-2-(3,4-dichloroanilino)-1H-benzimidazole HCl (**38**). Method A

Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:3) to obtain **38**; yield 12%; mp 270–272 °C, ^{1}H NMR (CD_3OD): δ 7.43–7.47 (dd, 1H, $J_o = 8.4$ Hz, $J_m = 2$ Hz), 7.63–7.76 (m, 5H). MS ESI (+) *m/z*: 346 (%100) (M + H), 348 (%65.6) (M + H + 2), 350 (%13.1) (M + H + 4). Anal. calcd. for $C_{14}H_8Cl_2F_3N_3 \cdot$HCl: C 43.94, H 2.37, N 10.98, found: C 43.72, H 2.45, N 10.95.

5.2.30. 5(6)-Cyano-2-(3,4-dichloroanilino)-1H-benzimidazole (**39**). Method A

Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:2) to obtain **39**; yield 17%; mp 357–358 °C, ^{1}H NMR (DMSO-d_6): δ 7.43–7.58 (m, 3H), 7.66 (dd, 1H, $J_o = 8.8$ Hz, $J_m = 2.4$ Hz), 7.76 (br s, 1H), 8.23 (d, 1H, $J_m = 2.4$ Hz). MS ESI (+) *m/z*: 303 (%100) (M + H), 305 (%65.6) (M + H + 2), 307 (%13.1) (M + H + 4). Anal. calcd. for $C_{14}H_8Cl_2N_4 \cdot 0.1\ C_4H_8O_2$: C 55.44, H 2.84, N 17.96, found: C 55.17, H 2.84, N 17.94.

5.2.31. 5(6)-Carboxymethyl-2-(3,4-dichloroanilino)-1H-benzimidazole HCl (**40**). Method A

Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:3) to obtain **40**; yield 38%; mp 254–256 °C, ^{1}H NMR (DMSO-d_6): δ 3.81 (s, 3H), 7.36–7.45 (dd, 1H), 7.52–7.55 (d, 1H), 7.62–7.65 (dd, 1H), 7.68–7.70 (dd, 1H), 7.93 (d, 1H), 8.23 (d, 1H, $J_m = 2.8$ Hz), 10.1 (d, 1H) and 11.5 (d, 1H). MS ESI (+) *m/z*: 336 (%100) (M + H), 338 (%65.6) (M + H + 2), 340 (%13.1) (M + H + 4). Anal. calcd. for $C_{15}H_{11}Cl_2N_3O_2$: C 53.59, H 3.30, N 12.50, found: C 53.39, H 3.48, N 12.59.

5.2.32. 5(6)-Amino-2-(3,4-dichloroanilino)-1H-benzimidazole HCl (**41**)

The mixture of 37 (0.6 g, 1.52 mmol), $SnCl_2$ (1 g) and Sn (1 g) in conc. HCl acid (15 mL) and 15 mL ethanol was heated under reflux. The reaction mixture was cooled and basified with NaOH solution and extracted with EtOAc. Crude product was purified by chromatography on silica gel (EtOAc–EtOH 50:10) to obtain **41**; yield 57%; mp >300 °C, ^{1}H NMR (DMSO-d_6): δ 7.23–7.25 (dd, 1H, $J_o = 8.8$ Hz, $J_m = 2$ Hz), 7.48–7.57 (m, 3H), 7.69 (d, 1H, $J_o = 8.8$ Hz), 7.88 (d, 1H, $J_m = 2.8$ Hz). MS ESI (+) *m/z*: 293 (%100) (M + H), 295 (%62.5) (M + H + 2), 297 (%10.6) (M + H + 4). Anal. calcd. for $C_{13}H_{10}Cl_2N_4 \cdot 2.15$ HCl: C 42.02, H 3.30, N 15.08, found: C 42.46, H 3.54, N 15.12.

5.2.33. 5(6)-Nitro-2-(4-chloroanilino)-1H-benzimidazole (**42**). Method A

Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:3) to obtain **42**; yield 20%; mp 304–307 °C, ^{1}H NMR (DMSO-d_6): δ 7.38 (d, 2H, $J_o = 8.8$ Hz),

7.43 (d, 1H, $J_o = 7.2$ Hz), 7.79 (d, 2H, $J_o = 9.2$ Hz), 7.95–7.98 (dd, 1H, $J_o = 8.4$ Hz, $J_m = 2$ Hz), 8.14 (d, 1H, $J_m = 2$ Hz), 10.1 (d, 1H) and 11.5 (d, 1H). MS ESI (+) *m/z*: 289 (%100) (M + H), 291 (%31.9) (M + H + 2). Anal. calcd. for $C_{13}H_9ClN_4O_2 \cdot 0.6\ H_2O$: C 52.13, H 3.43, N 18.71, found: C 52.08, H 3.26, N 18.36.

5.2.34. 5,6-Dichloro-2-(3,4-dichloroanilino)-1H-benzimidazole HCl (**43**). Method B

Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:3) to obtain **43**; yield 21%; mp >300 °C, ^{1}H NMR (DMSO-d_6): δ 7.51–7.54 (dd, 1H, $J_o = 8.8$ Hz, $J_m = 2.4$ Hz), 7.63 (s, 2H), 7.68 (d, 1H), 7.97 (d, 1H), ^{13}C NMR (DMSO-d_6): δ 150.27, 138.23, 133.06, 132.40, 131.90, 127.12, 125.39, 123.44, 122.19, 114.30. MS ESI (+) *m/z*: 346 (%76.3) (M + H), 348 (%100) (M + H + 2), 350 (%49.4) (M + H + 4), 352 (%10.6) (M + H + 6), 354 (%0.9) (M + H + 8). Anal. calcd. for $C_{13}H_7Cl_4N_3 \cdot HCl$: C 40.72, H 2.10, N 10.96, found: C 40.64, H 2.30, N 10.90.

5.2.35. 5,6-Dichloro-2-(4-chloroanilino)-1H-benzimidazole HCl (**44**). Method A

Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:3) to obtain **44**; yield 16%; mp >300 °C, ^{1}H NMR (CD_3OD): δ 7.36–7.39 (d, 2H), 7.47 (s, 2H), 7.46–7.48 (d, 2H), ^{13}C NMR (CD_3OD): δ 150.07, 134.19, 133.24, 130.31, 129.68, 127.75, 125.71, 113.25. MS ESI (+) *m/z*: 312 (%100) (M + H), 314 (%93.8) (M + H + 2), 316 (%31.3) (M + H + 4), 318 (%3.8) (M + H + 6). Anal. calcd. for $C_{13}H_8Cl_3N_3 \cdot HCl$: C 44.73, H 2.60, N 12.04, found: C 44.38, H 3.05, N 12.04.

5.2.36. 5(6)-Chloro-6(5)-methyl-2-(3,4-dichloroanilino)-1H-benzimidazole HCl (**45**). Method A

Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:3) to obtain **45**; yield 36.4%; mp >310 °C, ^{1}H NMR (DMSO-d_6): δ 2.38 (s, 3H), 7.42 (s, 1H), 7.49–7.51 (m, 2H), 7.73 (d, 1H, $J_o = 8.4$ Hz), 7.87 (d, 1H, $J_m = 2.4$ Hz), ^{13}C NMR (DMSO-d_6): δ 148.83, 137.73, 132.51, 132.03, 130.99, 130.80, 130.70, 128.40, 127.89, 124.36, 122.99, 114.45, 112.87. MS ESI (+) *m/z*: 326 (%100) (M + H), 328 (%95.6) (M + H + 2), 330 (%30) (M + H + 4), 332 (%3.8) (M + H + 6). Anal. calcd. for $C_{14}H_{10}Cl_3N_3 \cdot HCl$: C 46.31, H 3.05, N 11.57, found: C 45.93, H 3.21, N 11.45.

5.2.37. 5-Chloro-2-(3,4-dichloroanilino)-1,3-benzothiazole (**47**)

The mixture of **46** (0.250 g, 1.23 mmol) and 3,4-dichloroaniline (0.213 g, 1.32 mmol) in DMF (0.5 mL) was heated at 140 °C for 15 min. Water was added, white precipitate was crystallized from EtOH; yield 72%; mp 244–246 °C, ^{1}H NMR (DMSO-d_6) δ 7.18–7.21 (dd, 1H, $J_o = 8.4$ Hz, $J_m = 2$ Hz), 7.54–7.58 (m, 2H), 7.66 (d, 1H, $J_m = 1.2$ Hz), 7.82 (d, 1H, $J_o = 8.8$ Hz), 8.16 (d, 1H, $J_m = 2$ Hz). ^{13}C NMR (DMSO-d_6) δ 163.47, 153.52, 140.89, 131.95, 131.45, 131.44, 129.50, 124.22, 123.26, 123.21, 119.81, 119.62, 118.66. MS ESI (+) *m/z*: 329 (%100) (M + H), 331 (%100) (M + H + 2), 333 (%50.6) (M + H + 4), 335 (%7.5) (M + H + 6). Anal. calcd. for $C_{13}H_7Cl_3N_2S \cdot 0.1\ H_2O$: C 47.11 H 2.19, N 8.45, found: C 46.95, H 2.14, N 8.6.

5.2.38. 5-Chloro-2-[2-(4′-chlorophenoxy)anilino]-1,3-benzothiazole (**48**)

The mixture of **46** (0.12 g, 0.59 mmol) and 2-(4′-chlorophenoxy)aniline (0.140 g, 0.62 mmol) in DMF (0.5 mL) was heated under reflux at 190 °C for 1 h. Dilute NH_4OH solution was added, and extracted with EtOAc. Crude product was purified by chromatography on silica gel (EtOAc–hexane 1:6) to obtain **48**; yield 39%; mp 127–129 °C, ^{1}H NMR ($CDCl_3$) δ 6.86–7.28 (m, 8H), 7.52 (d, 1H, $J_o = 8.4$ Hz), 7.65 (d, 1H, $J_m = 2.4$ Hz), 8.02 (s, 1H), 8.35 (dd, 1H, $J_o = 8.4$ Hz, $J_m = 1.6$ Hz), ^{13}C NMR ($CDCl_3$) δ 163.68, 155.19, 153.09, 145.78, 132.32, 131.39, 130.19, 129.33, 128.84, 124.79, 124.01, 123.22, 121.60, 120.28, 120.13, 119.83, 118.30. MS ESI (+) *m/z*: 387 (%100) (M + H), 389 (%71.3) (M + H + 2), 391 (%15.6) (M + H + 4). Anal. calcd. for $C_{19}H_{12}Cl_2N_2OS \cdot 0.1\ H_2O \cdot 0.1\ C_4H_8O_2$: C 58.56 H 3.29, N 7.04, found: C 58.42, H 3.09, N 7.37.

5.2.39. 5-Chloro-2-(4-chloroanilino)-1,3-benzoxazole (**51**)

The mixture of **50** (0.3 g, 1.5 mmol) and 4-chloroaniline (0.577 g, 4.53 mmol) was heated at 150 °C for 2 h. Crude product was crystallized from EtOH to obtain **50**; yield 56%; mp 244–248 °C, ^{1}H NMR ($CDCl_3$) δ 7.10–7.13 (dd, 1H, $J_o = 8.8$ Hz, $J_m = 1.6$ Hz), 7.24 (s, 1H), 7.36 (d, 2H, $J_o = 8.4$ Hz), 7.46 (d, 1H, $J_m = 2$ Hz), 7.57 (d, 2H, $J_o = 8.4$ Hz). MS ESI (+) *m/z*: 279 (%100) (M + H), 281 (%71.3) (M + H + 2), 283 (%11.9) (M + H + 4). Anal. calcd. for $C_{13}H_8Cl_2N_2O$: C 55.94 H 2.89, N 10.04, found: C 56.04, H 3.02, N 9.90.

5.3. Microbiological studies

Activity tests were performed in Mueller–Hinton broth (MHB) (Difco, Difco Laboratories, Detroit, MI, USA). Four or five *S. aureus* colonies from overnight growth on Tryptic Soy Agar (Merck, 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 millilitre of this dilution was added to each tube containing 1 mL of the compound diluted in MHB. All tubes were incubated at 35 °C for 18 h and MIC's were determined.

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