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Design, synthesis, antibacterial evaluation and molecular docking studies of some newer benzothiazole containing aryl and alkaryl hydrazides

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Abstract

The alarming rise of bacterial resistance is occurring worldwide and endangering the efficacy of antibiotics. Therefore, development of new and efficient antibacterial agents remains paramount. In the present work we designed and synthesized a series of N'-(1,3-benzothiazol-2-yl)-substituted aryl/aralkyl hydrazides **C1-C27** and evaluated them *in vitro* for their antibacterial activity. Among all tested compounds, **C10**, **C15**, and **C24** showed potent activity against *Staphylococcus aureus* ATCC 43300 (MRSA). Minimum bactericidal concentration studies of synthesized compounds are performed against selected bacterial strains. Time kill kinetics showed that the compounds **C10** and **C15** possess bactericidal activity against MRSA ATCC 43300, while compound **C24** possess bactericidal activity against *S. aureus* NCIM 5022. In the extra-precision docking compounds **C1-C27** exhibited interactions mainly with the N-terminal and central domains of *S. aureus* GyrB catalytic pocket. Binding free energy (ΔG_{bind}) of compounds **C1-C27**/3U2K complexes were computed by MM-GBSA approach. Free energy components indicated Coulomb energy term as favourable for binding, while van der Waals and electrostatic solvation energy terms strongly disfavoured the binding. ADMET properties of synthesized compounds **C1-C27** are also computed.

Key words Benzothiazole; arylhydrazides; *Staphylococcus aureus*, MIC; binding free energy

Introduction

The rapid rise of bacterial resistance to antibiotics is a worldwide problem and increases healthcare costs and mortality.[1] Specifically, infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA), carbapenem-resistant *Enterobacteriaceae* (CRE), multi-drug-resistant *Mycobacterium tuberculosis* (MDR-TB), vancomycin-resistant *Enterococcus* (VRE), and multidrug-resistance *Streptococcus pneumonia* are hard to treat.[2] This has necessitated an interest in the development of new and effective antibacterial agents to fight antibiotic resistant bacterial strains**.**

Benzothiazoles (BT) are sulphur-containing heterocyclic constituted from a benzene ring fused to a thiazole ring. Benzothiazole derivatives have been synthesized by different synthetic paths.[3-5] Due to associated biological and pharmacological activities, BTs played significant role in synthetic and medicinal chemistry. BT is present as core nucleus in various therapeutic agents like ethoxazolamide, riluzole, viozan, zopolrestat, Tiaramide, Halethazole, Frentizole, Thioflavin-T etc.[6]. Many patents have been published on benzothiazoles highlighting its importance and few derivatives are in different phases of clinical trials.[7-10] Compounds possessing 1,3-benzothiazole nucleus[11-13] gained attention due to their diverse biological activities like amyotrophic lateral sclerosis[11], anti-inflammatory,[12] antifungal,[13] hypoglycemic,[14] anthelmintics,[15] anticancer,[16] PPARα antagonists[17] and Jun N-terminal kinase inhibitors.[18]

In addition, 2-hydrazinobenzothiazole linked to the arylidene moieties (**1**)[19,20] exhibited promising antibacterial activity. Functionalized 2-hydrazinobenzothizole (**2**)[21] linked to the isatin and some carbohydrates is also reported to possess antibacterial activity. In another investigation a series of 2-hydrazinobenzothiazoles have been designed and synthesized using the molecular hybridization of 2-hydrazinyl benzothiazole and 4-(aryloxy)benzaldehyde.[22]Specifically, compound **3** exhibited promising activity against *Mycobacterium tuberculosis* H37Rv (MIC, 1.5 μg/mL).

On the same line, aryl/alkyl hydrazides are considered as an important scaffold in the drug discovery and development processes because of their wide range pharmacological activities. In search of the potent antibacterial agents synthesis and antimicrobial activity of 2-bromo-4-methoxy benzohydrazide linked to the substituted thiazole moieties has been described by Raj et al.[23]; in particular, compound **4** showed promising activity against *Pseudomonas aeruginosa* with MIC of 6.25 μg/mL. In another study Peng et al.[24] reported the antibacterial activity of substituted benzohydrazide derivatives linked to the substituted benzylidene derivatives. Among all the tested compounds, derivative **5** exhibited significant activity against *Bacilus subtilis* (MIC, 3.1 μg/mL). In another research, Kumar et al.[25] synthesized 3-ethoxy-4-hydroxybenzylidene hydrazides linked to the alkyl groups and evaluated their antibacterial activity. Compound **6** showed higher antibacterial activity against *Staphylococcus aureus*, *Bacilus subtilis* and *Eschericia coli* than that of ciprofloxacin. Further, 2-(2,3-dihydro-1-benzofuran-6-yl)acetohydrazide linked to the benzylidene moiety in compound **7** showed significant activity against both Gram-positive and Gram-negative bacteria.[26]

Molecular hybridization is shown to be valuable medicinal chemistry strategy that aims to combine two molecules in a new and single chemical entity. It is recognized as an effective approach to design ligands to modulate the affinity against targets of interest.[27,28] Based on these reported good performance of molecular hybridization, combining benzothiazole ring with alkyl/aryl hydrazide moieties in a single molecular scaffold was presumed to be a reasonable and promising approach. In view of this data, we aimed the development of new antibacterial agents using molecular hybridization approach. In this context, various molecules were designed by incorporating substituted alkyl/substituted aryl/aralkyl carbonyl parmacophore into the 2-hydrazinobenzothiazole to combine the synergistic activity of both moieties. In the present study we carried out 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCl) and N-hydroxybenzotriazole (HOBt) mediated synthesis of novel N'-(1,3-benzothiazol-2-yl)-substituted aryl/aralkyl hydrazides **C1-C27** and subsequent evaluated for their activity against selected strain of bacteria.

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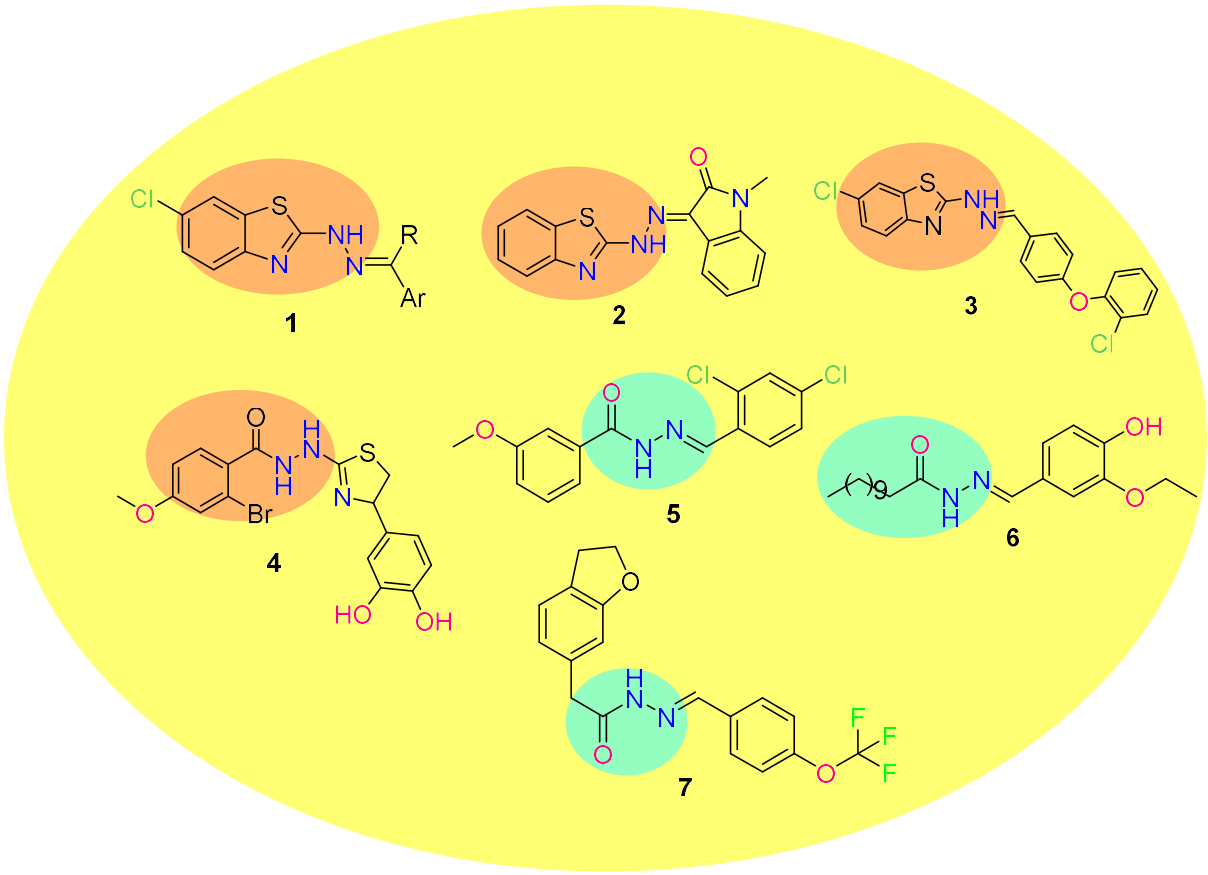
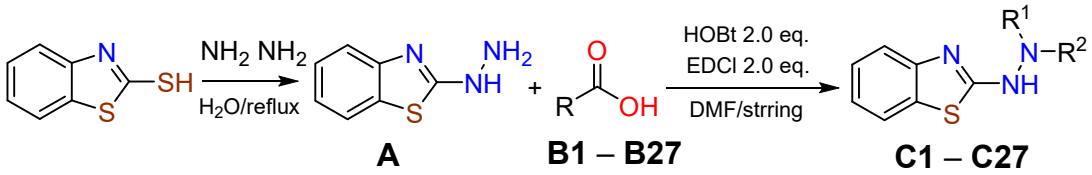


Figure 1. Designing of pharmacophoric groups into benzothiazole containing aryl and alkaryl hydrazides.

RESULTS AND DISCUSSION

Coupling of carboxylic acids with amines have been successfully carried out under the catalysis of EDCl/HOBt in moderate to high yield.[29-31] Amidation reaction was performed using EDCl/HOBt under ultrasound irradiation with 65-78% yield.[32] Amides have also been synthesized in high yields (81-93%) with EDCl/HOBt using different solvents like dichloromethane, acetone and tetrahydrofuran under reflux.[33,34] Keeping in view of this versatile uses, we carried out the coupling reaction of 2-hydrazino-1,3-benzothiazole with different aromatic carboxylic acids using EDCl/HOBt in N,N-dimethyl formamide (DMF) at room temperature. We synthesized new N'-(1,3-benzothiazol-2-yl)-substituted aryl/aralkylhydrazides **C1-C27** as outlined in *Scheme 1*. 2-Hydrazino-1,3-benzothiazole (**A**) was obtained by the reaction of 2-mercaptobenzothiazole with hydrazine hydrate (98% w/w) in water.[35] Stirring of 2-hydrazino-1,3-benzothiazole with appropriate substituted aromatic acids **B1-B27** in the presence of HOBt and EDCl[36] in DMF afforded title compounds **C1-C27** in 78.2-94.2% yield. Optimization of the reaction conditions for the synthesis of N'-(1,3-benzothiazol-2-yl)-substituted benzohydrazides derivatives **C1-C27** started by reacting 2-hydrazino-1,3-benzothiazole (**A**) and 4-methyl benzoic acid as model substrates. Increase in the reaction time did

not improve the reaction yield remarkably. To further improve the reaction yield other solvents including chloroform and dichloromethane were also tested and but did not improve the yield.



	R		R^1	R^2
B1	4-CH_3-C_6H_4	**C1**	4-CH_3-C_6H_4-C(=O)	H
B2	C_6H_5	**C2**	C_6H_5-C(=O)	C_6H_5–C(=O)
B3	C_6H_5-$(CH_2)_2$	**C3**	C_6H_5-$(CH_2)_2$-C(=O)	H
B4	4-NO_2-C_6H_4	**C4**	4-NO_2-C_6H_4-C(=O)	H
B5	3-Cl-4-NO_2-C_6H_3	**C5**	3-Cl-4-NO_2-C_6H_3-C(=O)	H
B6	4-OH-C_6H_4	**C6**	4-OH-C_6H_4-C(=O)	H
B7	3,5-di-OH-C_6H_3	**C7**	3,5-di-OH-C_6H_3-C(=O)	H
B8	3,5-di-Cl-C_6H_3	**C8**	3,5-di-Cl-C_6H_3-C(=O)	H
B9	4-pyridyl	**C9**	pyridine-3-yl-C(=O)	H
B10	4-F-C_6H_4	**C10**	4-F-C_6H_4-C(=O)	H
B11	2-quinolinyl	**C11**	quinoline-2-yl-C(=O)	H
B12	2-I-C_6H_4	**C12**	2-I-C_6H_4-C-(=O)	H
B13	3-CH_3O-2-NO_2-C_6H_3	**C13**	3-CH_3O-2-NO_2-C_6H_3-C(=O)	H
B14	3-CH_3-2-NO_2-C_6H_3	**C14**	3-CH_3-2-NO_2-C_6H_3-C(=O)	H
B15	3,5-di-NO_2-C_6H_3	**C15**	3,5-di-NO_2-C_6H_3-C(=O)	H
B16	C_6H_5-CH(OH)-CH_2	**C16**	C_5H_5-CH(OH)-CH_2-C(=O)	H
B17	2-F-C_6H_4	**C17**	2-F-C_6H_4-C(=O)	H
B18	2-Br-4-pyridyl	**C18**	3-Br-pyridine-4-yl-C(=O)	H
B19	2,4,6-tri-Cl-C_6H_2	**C19**	2,4,6-tri-Cl-C_6H_2-C(=O)	H
B20	2,4-di-Cl-C_6H_3	**C20**	2,4-di-Cl-C_6H_3-C(=O)	H
B21	2-thienyl	**C21**	thiophene-2-yl-C(=O)	H
B22	C_6H_5O-CH_2	**C22**	thiophene-2-yl-C(=O)	H
B23	2-CH_3-C_6H_4	**C23**	2-CH_3-C_6H_4-C(=O)	H
B24	4-C_4H_9O-C_6H_4	**C24**	4-C_4H_9O-C_6H_4-C(=O)	H
B25	4-CH_3O-C_6H_4	**C25**	4-CH_3O-C_6H_4-C(=O)	H
B26	C_6H_5-$(CH_2)_2$	**C26**	C_6H_5-$(CH_2)_2$-C(=O)	C_6H_5-$(CH_2)_2$-C(=O)
B27	2-thienyl	**C27**	thiophene-2-yl-C(=O)	thiophene-2-yl-C(=O)

Scheme 1. The synthetic route of the target compounds **C1-C27**.

The structures of newly synthesized compounds **C1-C27** is characterized by ^{1}H NMR, ^{13}C NMR and HRMS spectral data (see Supplementary *Figure S1-S27*). In the Fourier-Transform Infrared (FT-IR) spectra of compounds **C1-C27** characteristics absorption bands of >C=O, >C=N and C-S-C were observed, respectively in the range 1655 to 1678, 1611 to 1637 and 667 to 696 cm^{-1}. Characteristic NH and NHNH absorption bands were observed in the range between 3253 to 3369 cm^{-1}. In the ^{1}H NMR spectra of synthesized compounds absorption bands due to NHNH/NH appeared in the range at δ 4.77-12.28 ppm. Moreover, in compound **C24** NHNH signal has not appeared may be due to the replacement of exchangeable protons with deuterium of residual DCl. The ^{1}H NMR spectrum of representative compound **C1** (*Figure S1a*) showed a sharp singlet at δ 4.99 ppm indicating the presence of two -NHNH- protons. A singlet signal integrating for three protons is observed at δ 2.43 ppm and is assigned to -CH_3 group. Eight aromatic protons of this compound appeared in the region at δ 7.28 to 7.84 ppm. Further, in the ^{1}H NMR spectra of compounds **C1-C27** aromatic protons showed splitting patterns which agree with the substitution

pattern of respective compounds. The chemical shift and coupling constant (*J*) values of aromatic protons were assigned accordingly with their position. In compound **C1**, two protons present on position five and six of benzothiazole ring appeared as double doublet at δ 7.84 ppm (J = 8.0, 2.3 Hz), while two protons present on position four and seven of the same ring showed a doublet signal at δ 7.78 ppm (J = 8.1 Hz). A multiplet signal appeared at δ 7.49-7.32 ppm is assigned to two protons present at position two and six of the phenyl ring of 4-methylphenylhydrazino moiety. Two protons present at position three and five of this phenyl ring appeared as doublet signal at δ 7.28 ppm (J = 9.4 Hz). In the ^{13}C NMR spectrum of compound **C1** (*Figure S1b*) the carbonyl carbon of hydrazide exhibited a singlet signal at δ 170.55 ppm, while the azomethine carbon of the benzothiazole ring appeared as a singlet at δ 161.44 ppm. A singlet signal appeared at δ 29.38 ppm is ascribed to the -CH_3 group. Remaining ten SP^2 hybridized aromatic carbons appeared in their expected region between δ 7.28 to 7.84 ppm. Mass spectrum of **C1** (*Figure S1c*) showed $[M + H]^+$ peak at m/z 284.0845 (100%), which corresponds to its molecular formula $C_{15}H_{13}N_3OS$.

Minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC and MBC of synthesized compounds **C1-C27** were determined by broth microdilution method (CLSI 2007)[37] and results are presented, respectively in *Table 1* and *Table 2*. It can be seen from *Table 1* that compounds **C14** exhibited maximum activity against *S. aureus* NCIM 5021 with MIC of 20.75 μM, whereas compounds **C4**, **C5**, **C11**, **C14**, **C18-C20** showed moderate activity (MICs, 24.02 to 48.04 μM). High activity of **C14** against this strain may be attributed to the presence of bulky NO_2 and CH_3 groups, respectively on position two and three of the phenyl ring attached to the hydrazide function. Against *S. aureus* NCIM 5022 compounds **C4** and **C24** showed high activity with MICs of 24.81 and 20.75 μM, respectively. Whereas compounds **C5**, **C8**, **C12-C15** and **C18-C20** displayed moderate activity (MICs, 39.21 to 46.12 μM) against this strain when compared to the standard drugs ciprofloxacin (MIC, 6.33 μM) and gentamicin (MIC, 16.95 μM). Presence of bulky NO_2 and n-butoxy groups on position four of the phenyl rings, respectively in **C4** and **C24** may be attributed for their high activity. Five compounds i.e. **C10**, **C12**, **C15**, **C16** and **C24** exhibited potent activity (MICs, 19.73 to 27.14 μM) against *S. aureus* ATCC 43300 (MRSA) in comparison to the standard drugs ciprofloxacin (MIC, 95.97 μM) and gentamycin (MIC, 41.24 μM). Compounds **C8**, **C11**, **C13**, **C14**, **C14** and **C19** also displayed promising activity (MICs, 40.52 to 47.13 μM) against this strain. It is evident that presence of electronegative atoms either on positions two or three of the phenyl ring (**C10**, **C12** and **C24**) or presence of electron withdrawing goups on position three and five of the phenyl ring (**C15**) is optimum for activity against *S. aureus* ATCC 43300. In general presence of alkyl substituted phenyl ring (**C1**, **C23**), unsubstitued phenyl ring (**C2**) or when phenyl ring is attached to the hydrazide moiety via alkyl groups (**C3**, **C26**) markedly decreased activity against *S. aureus* ATCC 43300. Against *Staphylococcus epidermidis* NCIM 2493 compounds **C13** and **C21** possessing, respectively 3-methoxy-2-nitrophenyl and thiophene rings linked to the hydrazide moiety showed maximum activity (MICs, 22.65 and 28.32 μM, respectively), while all other tested compounds showed moderate to low activity. However, activity of compounds **C13** and **C21** is far low against this strain in comparision to the tested standard drugs ciprofloxacin (MIC, 6.33 μM) and gentamycin (MIC, 17.16 μM). Against *Mycobacterium sp* NCIM 2984 only one compound i.e. **C24** displayed promising activity (MIC, 21.81 μM),

while all other tested compounds were observed to less active against this strain. However, activity of **C24** against this strain was comparatively less compared to the standard drugs ciprofloxacin (MIC, 3.62 μM) and gentamycin (MIC, 16.95 μM). When tested against *K. pneumoniae* NCIM 2706 two compounds i.e. **C18** and **C19** possessing, respectively 3-bromopyridine and 2,4,6-trichlorobenzene rings linked to the hydrazide moiety displayed promising activity (MICs, 20.24 and 21.19 μM). However, activity of these two compounds against this strain was observed to be significantly less compared to the standard drugs ciprofloxacin (MIC, 6.63 μM) and gentamycin (MIC, 2.30 μM). When tested against *E. coli* NCIM 2065 only three compounds **C11**, **C18** and **C20** showed promising activity with MICs of 24.65, 20.61 and 22.17 μM, respectively. But activity of these three compounds is low compared to the tested standard drugs ciprofloxacin (MIC, 6.94 μM) and gentamycin (MIC, 2.09 μM). It is evident that the presence of electron rich heterocyclic ring linked to the hydrazide moiety is optimum for activity against Gram-negative *K. pneumoniae* NCIM 2706 and *E. coli* NCIM 2065. Against another Gram-negative bacteria *P. aeruginosa* NCIM 2036 tested compounds exhibited moderate to less activity (MICs, 38.20 to 220.14 μM) compared to the standard drugs. It is evident that most of the compounds studied here have shown broad spectrum antibacterial activity which is agreement with earlier study.[38,39]

Table 1. Minimum inhibitory concentration in μM of the synthesized compounds **C1-C27**

Comp. No	Minimum inhibitory concentration (μM)*							
	[a]*S.a*	[b]*S. a*	[c]*MRSA*	[d]*S.e*	[e]*M. t*	[f]*K. p*	[g]*P. a*	[h]*E. c*
C1.	112.72±0.74	55.12±0.68	220.84±0.39	112.36±0.68	112.36±0.89	112.36±0.77	220.84±0.68	220.14±0.47
C2.	168.43±0.81	83.54±0.69	166.02±0.68	83.54±0.98	41.23±0.98	84.08±0.49	167.36±0.89	83.81±0.86
C3.	>420	210.84±0.56	104.91±0.49	210.84±0.84	105.92±0.74	210.16±0.47	107.27±0.76	104.24±0.89
C4.	48.04±0.25	24.81±0.76	50.58±0.71	101.17±0.75	199.79±0.49	48.04±0.49	48.04±0.68	98.62±0.68
C5.	45.01±0.89	43.00±0.69	91.18±0.49	45.11±0.82	90.89±0.64	45.30±0.69	44.15±0.23	89.17±0.49
C6.	218.00±0.76	109.70±0.72	219.75±1.06	217.29±1.19	109.35±0.41	219.75±0.23	111.80±0.74	111.80±0.86
C7.	207.42±0.49	105.53±0.21	207.42±0.89	104.545±0.91	104.87±0.76	105.86±0.93	103. ±0.63	208.41±0.68
C8.	92.84±0.09	46.12±0.76	44.64± 0.09	45.82±0.89	93.43±0.45	91.65±0.89	45.82±0.60	91.65±0.59
C9.	56.97±0.87	56.60±0.59	56.97±0.98	115.42±0.80	28.85±0.43	57.71±0.80	58.45±0.69	115.79±0.39
C10.	52.55±0.76	54.29±0.89	27.14±1.34	54.29 ±0.67	27.49±0.69	108.94±0.85	53.25±0.86	53.94±0.57
C11.	49.00±0.79	49.31±0.76	47.13±0.88	47.13±0.59	47.13±0.80	98.94±0.69	49.31±0.49	24.65±0.89
C12.	78.43±0.49	39.21±0.96	19.74±0.69	80.460.68	39.21±0.47	40.23±0.89	38.20±0.88	39.72±0.76
C13.	92.34±0.67	43.85±0.85	43.56±0.77	22.65±0.99	90.89±0.49	43.85±0.49	45.88±0.47	90.89±0.79
C14.	24.02±0.49	44.37±0.47	48.420.44	47.51±0.76	46.29±0.85	47.51±0.09	97.12±0.89	45.98±0.78
C15.	87.38±0.87	43.69±0.97	21.70±0.09	41.74±0.58	44.25±0.68	86.54±0.39	43.96±0.47	86.54±1.19
C16.	101.79±0.79	98.92±0.85	25.52±0.88	101.15±1.21	48.18±0.39	99.87±0.89	101.47±0.34	198.8±0.79
C17.	53.25±0.49	108.59±0.28	53.94±0.71	109.98±0.89	53.94±0.09	53.25±0.79	53.25±0.09	218.57±0.89
C18.	44.38±0.85	43.24±0.85	90.20±0.57	90.20±0.86	44.38±0.61	20.24±0.04	90.20±0.64	20.61±0.79
C19.	42.66±0.49	40.25±0.74	40.52±0.49	85.06±0.75	40.52±0.49	21.19±0.74	41.59±0.90	85.06±0.44
C20.	46.12 ±0.74	44.64±0.94	92.25±1.14	92.25±0.47	45.82±0.95	93.13±0.43	44.64±0.74	22.17±0.88
C21.	57.38±1.21	114.04±0.86	114.04±0.74	28.32±0.56	115.49±0.64	56.29±0.89	57.74±0.48	55.56±0.70
C22.	210.12±0.32	104.44±1.32	208.78±0.71	209.78±0.93	53.11±0.89	104.55±0.89	207.44±0.73	105.22±0.54
C23.	111.52±0.49	110.81±0.92	221.98±1.12	112.22±0.81	56.11±0.49	55.76±1.31	110.11±0.49	220.92±0.95

C24.	167.34±0.85	20.75±0.74	20.75±0.89	84.86±0.74	21.81±0.82	84.86±0.74	42.30±0.85	41.23±0.75
C25.	106.23±0.59	53.11±0.12	106.56±0.74	207.78±0.68	51.44±0.52	106.56±0.42	106.56±0.09	208.45±0.89
C26.	73.56±0.49	37.01±0.92	144.57±0.85	36.55±0.88	145.03±0.69	73.56±0.88	72.86±0.90	144.80±0.62
C27.	163.17±0.85	82.49±1.14	81.19±9.37	161.36±0.94	162.65±1.02	161.36±0.49	82.49±0.62	160.84±0.76
Cipro floxacin	6.18±0.82	6.33±0.52	95.97±0.78	6.33±0.52	3.62±0.52	6.63±0.76	1.62±0.94	6.94±0.52
Genta micin	17.16±0.99	16.95±0.92	41.240.78	17.16±0.98	16.95±0.62	2.30±0.88	4.60±0.83	2.09±0.52

*Values are mean ± SEM (n = 3).

Ciprofloxacin and Gentamicin used as positive control

[a]*S. a: Staphylococcus aureus* NCIM 5021; [b]*S. a: Staphylococcus aureus* NCIM 5022;[c]*S. a: Methicillin resistant Staphylococcus aureus* ATCC 43300 (MRSA); [d]*S. e: Staphylococcus epidermidis NCIM* 2493; [e]M.*t. a :Mycobacterium sp* NCIM 2984; [f]*K. p: Klebsiella pneumoniae* NCIM 2706; [g]*P. a: Pseudomonas aeruginosa* NCIM 2036;[h]*E. c: Escherichia coli* NCIM 2065.

MIC: Minimum inhibitory concentration.

MBC of tested compounds is reported in *Table 2*. It is evident that all ten tested compounds (**C4**, **C5**, **C9-C11**, **C14**, **C17**, **C18**, **C21** and **C26)** are bactericidal (MBC/MIC ≥4) against *S. aureus* NCIM 5021. Moreover, all twelve compounds tested against *S. aureus* NCIM 5022 showed bactericidal activity (MBC/MIC ≥4). On the other hand, eight tested compounds (**C4**, **C10**, **C12**, **C14**, **C15, C17**, **C19** and **C24**) displayed bactericidal activity (MBC/MIC ≥4) against *S. aureus* ATCC 43300 (MRSA), while compounds **C9** exhibited bacteriostatic activity (MBC/MIC, 8) against this strain. All six compounds (**C5**, **C12**, **C13**, **C17**, **C18** and **C21)** tested against *S. epidermidis* NCIM 2493 were observed to be bactericidal (MBC/MIC ≥4). The results of MBC screening of eight tested compounds (**C9-C11**, **C14**, **C18**, **C19**, **C23** and **C24)** against *Mycobacterium sp* NCIM 2984 indicated the bactericidal activity (MBC/MIC ≥4). Except compound **C5** (MBC/MIC, 8), all other eleven tested compounds **C1**, **C2**, **C4**, **C12-15** and **C18-C21** showed bactericidal activity against *K. pneumoniae* NCIM 2706. Against *P. aeruginosa* NCIM 2036 two tested compounds **C5** and **C21** showed bactericidal activity, while six compounds **C8**, **C10**, **C12**, **C17**, **C19** and **C24** exhibited bacteriostatic activity (MBC/MIC, 8). When tested against *E. coli* NCIM 2931, all six tested compounds **C10-C12**, **C14**, **C20** and **C24** displayed bactericidal activity (MBC/MIC ≥4).

Time-kill assay

As expected from the MIC values, tested compounds **C10** and **C15** displayed significant bactericidal activity towards *S. aureus* ATCC 43300 (MRSA) in comparison to the untreated bacteria and bacteria treated with standard drug ciprofloxacin. The time kill kinetic profiles of **C10** and **C15** (*Figure 2a* and *2b*) displayed bactericidal activity with a ≥3log10 reduction in viable cell count at tested concentrations of 13.75 and 27.14 μM in case of **C10** and 10.85 and 21.70 μM in case of **C15** after 24 h exposure. The killing rate of **C24** (*Figure 2c*) towards *S. aureus* NCIM 5022 was similar to that of **C10** and **C15** observed against *S. aureus* ATCC 43300. This compound showed bactericidal activity at concentrations of 10.37 μM and 20.75 μM after 24 h exposure. However, all three tested compounds exhibited lower bactericidal activity compared to the standard drug ciprofloxacin tested at 47.98 μM and

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95.97 μM concentrations against *S. aureus* ATCC 43300 and also against *S. aureus* NCIM 5022 at 3.16 μM and 6.33 μM concentrations. It is important to note that time kill kinetics of compounds **C10**, **C15** and **C24** are consistent with their respective MIC values.

Table 2. Minimum bactericidal concentrations (MBC) and MBC/MIC ratio of some selected compounds.

Comp.	MBC[S*] & MBC/MIC	[a]*S.a*	[b]*S. a*	[c]*MRSA*	[d]*S.e*	[e]*M. t*	[f]*K. p*	[g]*P. a*	[h]*E. c*
C1	MBC	--	111.1±0.87	--	--	--	220.5±0.85	--	--
	MBC/MIC	--	2	--	--	--	2	--	--
C2	MBC	--	--	--	--	--	334.7±0.99	--	--
	MBC/MIC	--	--	--	--	--	4	--	--
C4	MBC	100.2±0.47	100.2±0.88	198.8±0.97	--	--	198.8±0.93	--	--
	MBC/MIC	2	4	4	--	--	4	--	--
C5	MBC	90.3±0.47	90.3±0.59	--	89.4±0.73	--	357.8±0.91	179.9±0.80	--
	MBC/MIC	2	2	--	2	--	8	4	--
C8	MBC	--	93.2±0.94	--	--	--	--	367.1±1.04	--
	MBC/MIC	--	2	--	--	--	--	8	--
C9	MBC	115.6±1.02	116.3±0.92	461.8±0.97	--	115.9±0.91	--	--	--
	MBC/MIC	2	2	8	--	4	--	--	--
C10	MBC	107.6±0.80	--	108.8±0.94	--	107.5±0.91	--	433.3±0.68	107.4±0.87
	MBC/MIC	2	--	4	--	4	--	8	2
C11	MBC	98.4±0.44	95.9±0.82	--	--	98.1±0.99	--	--	99.9±0.57
	MBC/MIC	2	2	--	--	2	--	--	4
C12	MBC	--	77.9±0.81	78.4±0.79	80.9±0.90	--	79.9±0.91	313.6±0.85	78.2±0.49
	MBC/MIC	--	2	4	2	--	2	8	2
C13	MBC	--	--	--	92.9±1.04	--	268.0±0.93	--	--
	MBC/MIC	--	--	--	4	--	4	--	--
C14	MBC	94.0±0.94	96.7±0.67	97.1±0.81	--	94.1±0.49	188.4±0.80	--	93.4±0.47
	MBC/MIC	4	2	2	--	2	4	--	2
C15	MBC	--	--	88.3±0.91	--	--	347.6±0.89	--	--
	MBC/MIC	--	--	4	--	--	4	--	--
C17	MBC	109.7±0.7	--	108.5±0.88	215.2±0.79	--	--	434.7±0.76	--
	MBC/MIC	2	--	2	2	--	--	8	--
C18	MBC	90.4±0.94	89.4±1.21	--	177.4±0.89	89.2±0.90	89.8±0.86	357.7±0.94	--
	MBC/MIC	2	2	--	2	2	4	4	--
C19	MBC	--	--	84.6±0.69	--	83.9±0.90	85.2±0.74	335.1±0.97	--
	MBC/MIC	--	--	2	--	2	4	8	--
C20	MBC	--	91.3±0.79	--	--	--	368.9±0.94	--	94.6±0.79
	MBC/MIC	--	2	--	--	--	4	--	4
C21	MBC	112.5±0.58	--	--	115.3±0.75	--	227.2±0.88	224.7±0.78	--
	MBC/MIC	2	--	--	4	--	4	4	--
C23	MBC	--	--	--	--	221.9±0.79	--	--	--
	MBC/MIC	--	--	--	--	4	--	--	--

C24	MBC	--	82.3±0.77	81.6±0.94	--	84.6±0.78	--	331.9±0.82	82.1±0.67
	MBC/MIC	--	4	4	--	4	--	8	2
C25	MBC	--	106.5±0.99	--	--	--	--	--	--
	MBC/MIC	--	2	--	--	--	--	--	--
C26	MBC	144.0±0.98	--	--	--	--	--	--	--
	MBC/MIC	2	--	--	--	--	--	--	--
Ciprofloxacin	MBC	2.1±0.45	1.9±0.78	2.1±0.89	2.0±0.68	2.2±0.83	1.9±0.88	2.0±0.96	2.0±0.86
	MBC/MIC	1	<1	<1	1	<1	<1	<1	<1

[$]minimum bactericidal concentration (μM).

*Values are mean ± SEM (n = 3); --: not determined.

Ciprofloxacin used as positive control

[a]*S. a: Staphylococcus aureus* NCIM 5021; [b]*S. a: Staphylococcus aureus* NCIM 5022;[c]*S. a: Methicillin resistant Staphylococcus aureus* ATCC 43300 (MRSA); [d]*S. e: Staphylococcus epidermidis* NCIM 2493; [e]*M.t. a :Mycobacterium sp* NCIM 2984; [f]*K. p: Klebsiella pneumoniae* NCIM 2706; [g]*P. a: Pseudomonas aeruginosa* NCIM 2036;[h]*E. c: Escherichia coli* NCIM 2065.

MIC: Minimum inhibitory concentration (μM).

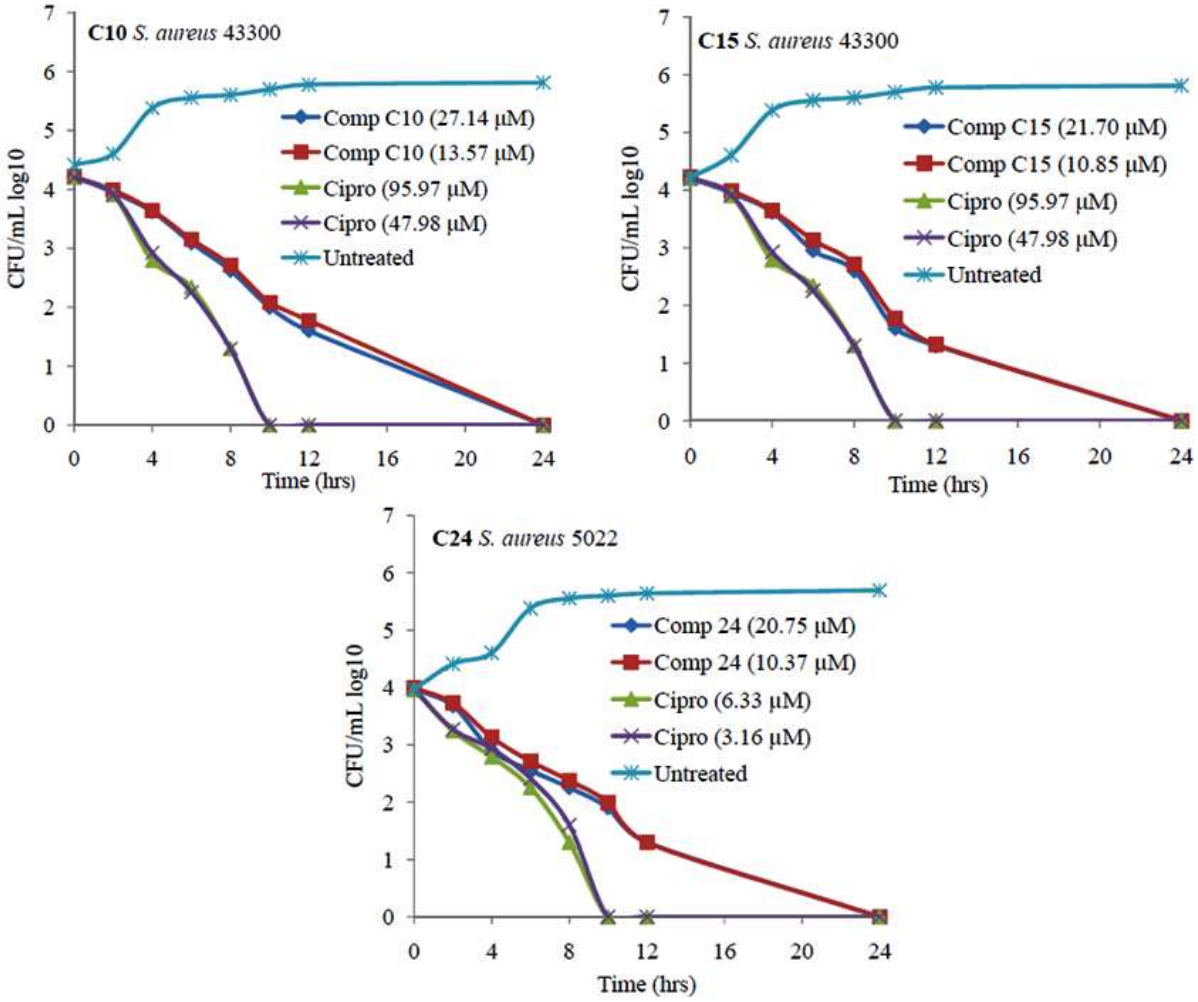


Figure 2. Time kill curves of **C10** and **C15** against *S. aureus* ATCC 43300 and **C24** against *S. aureus* NCIM 5022.

Computational study

The ATP dependent bacterial DNA GryB, a type II topoisomerase is actively involved in introducing negative supercoils into DNA.[40] It is vital for the biosynthesis of peptidoglyan, an essential component of bacterial cells. This enzyme is ubiquitous in bacteria but has not been found in humans, making it an attractive target for the investigation of new antibacterial agents.[41] Coumarins like novobiocin and naturally occurring cyclothialidines binds to GyrB and competitively inhibit the binding of ATP.[42,43] Based on these observations and activity of synthesized compounds against *S. aureus* we investigated the binding modes of these compounds in the catalytic pocket of *S. aureus* GyrB by extra-precision docking.

Docking studies revealed that compounds **C1-27** occupied the same site of catalytic pocket of 3U2K as co-crystal structure and exhibited hydrogen bonding, π-π stacking, π-cation and salt bridge interactions with the key binging residues of catalytic pocket (*Table 3*, *Figure 2a-c* also Supplementary *Figure S28* and *Table S1*). The high active compounds **C10** (G_{score} -5.72 kcal/mol), **C15** (G_{score} -5.82 kcal/mol) and **C24** (G_{score} -5.89 kcal/mol) well occupied the catalytic pocket and exhibited interactions mainly with key binding residues of the N-terminal and

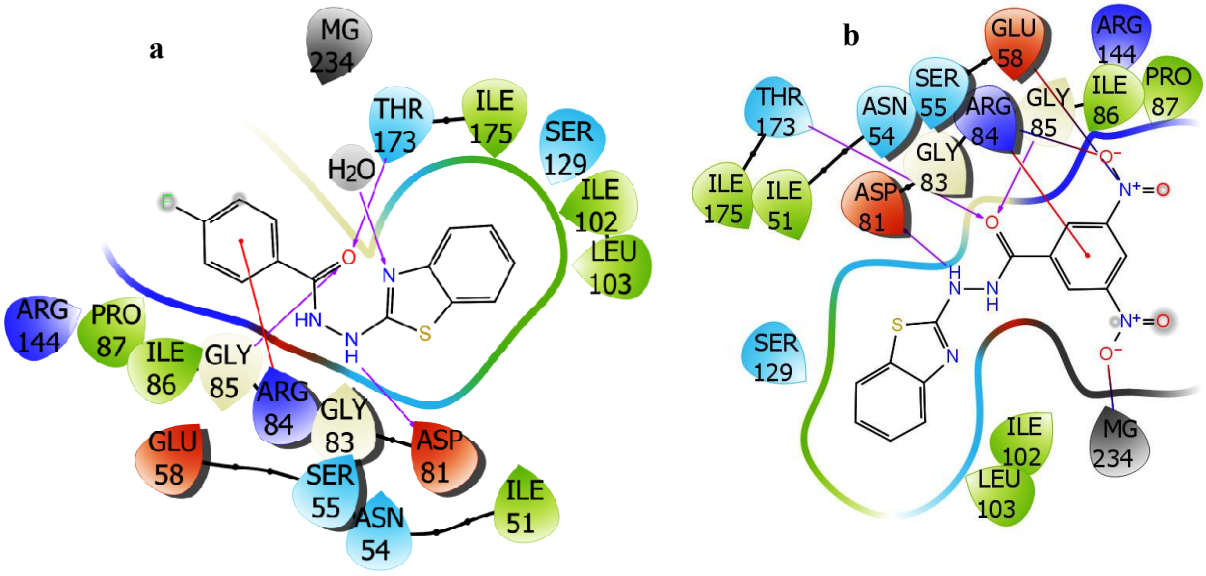
central domains (*Figure 2a-c*). Precisely, carbonyl oxygen of the hydrazide function in compound **C10** accepted two hydrogen bonds one each from Gly85 and Thr173. One of nitrogen atom of NH-NH fragment established hydrogen bonding interaction with charged residue Asp81. This compound is further stabilized in the catalytic pocket by π-cation interaction of thiazole part of the benzothiazole ring with protonated NH_2 of Arg84. In case of compound **C15** carbonyl oxygen of the hydrazide function exhibited similar hydrogen bonding interaction with Gly85 and Thr173 as observed in compound **C10**. Nitrogen atom of one the nitro group established salt bridge interaction with charged residue Glu58, while oxygen atom another nitro group exhibited coordination bond with Mg234. Like **C10**, this compound is also stabilized in the catalytic pocket by π-cation interaction of phenyl ring of 3,5-dinitropheyl moiety with Glu84. Like **C10** and **C15**, compound **C15** displayed similar hydrogen bonding interactions with Gly85 and Thr173 and π-cation interaction with Arg84. Additionally, in this compound oxygen atom of butoxy group present on the phenyl ring displayed hydrogen-bonding interaction with Asn54. In general other compounds also displayed hydrogen bonding interactions with Asp81, Gly85 and Thr173; π-cation interactions with Gly 85 and Arg84; salt bridge interactions with Gly85. It is evident from above results that apart from hydrogen bonding, π-cation and salt bridge interactions also play crucial role for the stabilization of compounds **C1-C27** within the catalytic pocket.

Table 3. Extra-precision docking result and contribution of binding free energy (MM-GBSA) (kcal/mol) between synthesized compounds **C1-C27** in the catalytic pocket of *S. aureus* GyrB enzyme (pdb.3U2K).

Comp.	[a]g_{score}	[b]ΔG_{Bind}	[c]ΔG_{Coul}	[d]ΔG_{Cov}	[e]ΔG_{HB}	[f]ΔG_{Lipo}	[g]ΔG_{Solv}	[h]ΔG_{vdW}	[i]$\Delta G_{packing}$
C1	-5.75	-57.26	-34.01	-1.72	-1.75	-11.93	33.86	33.86	-2.43
C2	-4.35	-46.51	-25.59	-12.45	1.76	-14.33	41.25	41.25	-3.29
C3	-5.09	-67.30	-33.88	-2.22	-0.73	-13.7	19.82	19.82	-1.84
C4	-5.91	-50.37	-41.61	0.13	-2.55	-11.96	47.61	47.61	-4.34
C5	-5.10	-54.02	-17.75	-8.64	-1.49	-10.48	28.71	28.71	-4.58
C6	-4.86	-36.81	-63.55	-3.14	-0.56	-5.46	77.49	77.49	-3.38
C7	-5.87	-41.28	-72.8	-7.45	-2.01	-4.10	84.86	84.86	-2.96
C8	-6.09	-57.03	-33.45	-11.16	-0.84	-12.85	34.51	34.51	-3.50
C9	-4.73	-48.23	-32.06	-7.71	-1.09	-9.91	38.84	38.84	-5.35
C10	-5.72	-71.99	-29.43	-2.80	-1.27	-11.98	33.34	33.34	-4.02
C11	-5.15	-54.56	-17.96	-5.16	-0.27	-7.56	26.7	26.70	-6.41
C12	-5.45	-55.74	-22.92	-5.22	-1.30	-11.61	24.11	24.11	-3.52
C13	-5.72	-62.14	-31.03	0.75	0.04	-13.59	24.98	24.98	-2.89
C14	-5.29	-42.85	-25.91	-4.08	-0.12	-12.34	44.89	44.89	-4.71
C15	-5.82	-67.87	-45.31	0.42	-2.16	-13.73	43.8	43.80	-2.10
C16	-5.66	-59.42	-36.8	-4.85	1.55	-12.72	26.56	26.56	-1.32
C17	-4.78	-36.12	-37.62	-5.06	-0.91	-9.00	47.4	47.40	-5.68
C18	-5.06	-48.63	-7.26	-5.54	-0.02	-12.03	23.05	23.05	-4.90
C19	-5.40	-54.43	-44.64	-0.81	0.48	-17.23	31.82	31.82	-3.70
C20	-4.85	-48.71	-30.07	-1.33	-2.05	-12.99	39.66	39.66	-4.04
C21	-4.26	-51.18	-22.42	-3.17	-1.8	-10.37	26.88	26.88	-4.70
C22	-6.30	-59.19	-27.1	-4.5	-2.33	-10.97	29.52	29.52	-4.48
C23	-5.12	-44.19	-3.55	-13.77	1.88	-9.77	21.54	21.54	-3.26
C24	-5.89	-71.28	-22.64	6.91	-2.54	-19.21	21.07	21.07	-6.56
C25	-4.44	-54.51	-20.85	-5.09	-1.16	-12.36	27.13	27.13	-4.88
C26	-5.72	-45.91	-29.51	-1.53	2.64	-17.38	43.50	43.50	-1.83
C27	-4.72	-42.3	-11.43	-0.70	1.29	-15.55	26.88	26.88	-5.18

[a]glide score; [b]free energy of binding; [c]Coulomb energy; [d]covalent energy (internal energy); [e]hydrogen bonding energy; [f]hydrophobic energy (non-polar contribution estimated by solvent accessible surface area); [g]electrostatic solvation energy; [h]van der Waals energy; [i]packing energy.

Binding free energy (ΔG_{bind}) of compounds **C1-27**/3U2K ranges between -36.12 to -71.99 kcal/mol showing high binding affinity for most compounds with *S. aureus* GyrB (*Table 3*). Among all compounds **C10**, **C15** and **C24** exhibited high negative value of ΔG_{bind} (-71.99, -67.87 and -71.28 kcal/mol, respectively) which is observed to be in correlation with MIC values against *S. aureus* ATCC 43300. It is evident from the computed energy components that Coulomb (ΔG_{Coul}) and hydrophobic (ΔG_{Lipo}) energy terms are favourable for the binding of ligands to *S. aureus* GyrB, whereas electrostatic solvation (ΔG_{Solv}, 19.82 to 84.86 kcal/mol) and van der Waals (ΔG_{vdW}, 19.82 to 77.49 kcal/mol) energy terms strongly disfavours the binding. Except for compounds **C5**, **C11**, **C18**, **C23-C25** and **C27** (ΔG_{Coul}, -3.55 to -22.64 kcal/mol), Coulomb energy (ΔG_{Coul}, -3.55 to -17.96 kcal/mol) term is observed to be strongly favourable for the binding of all other compounds. The high negative value in these compounds indicated it as the driving force for binding. In most of the compounds covalent energy (-0.70 to -13.77 kcal/mol) term is observed to either weakly unfavourable or moderately unfavourable for the binding.



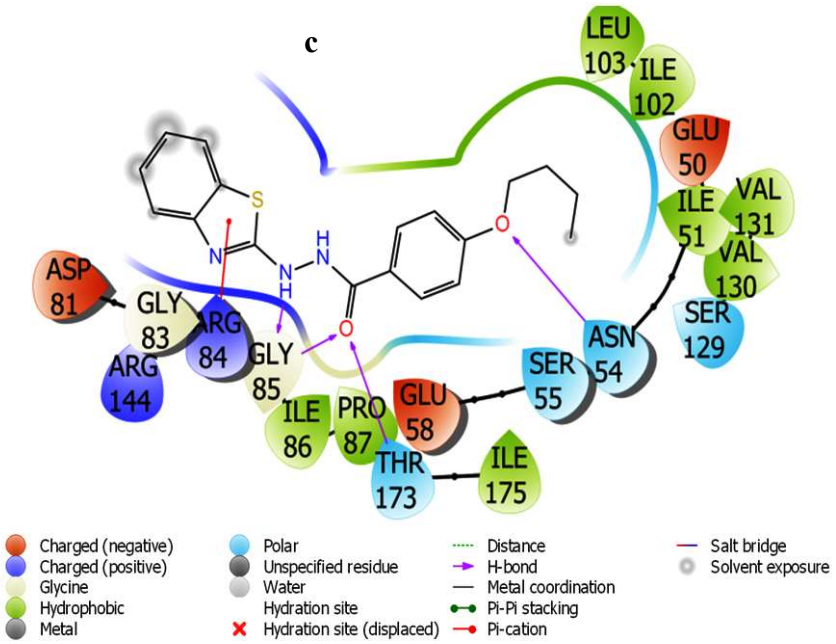


Figure 3 Plot represent interaction of compounds (a) **C10** (b) **C15** and (c) **C24** with the catalytic pocket residues of *S. aureus* GyrB (pdb.3U2K).

Absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties prediction

ADMET properties were computed to assess the pharmacokinetic and safety profiles of synthesized compounds **C1-C27**. It is evident from supplementary *Table S2* that none of the synthesized molecules exhibited blockage of human ether-a-go-go (HERG) K^+ channels and obeyed Lipinski's rule of five (0 to 1) indicating drug-like property of these molecules. These compounds are observed to be non toxic as predicted by the AMES test. Predicted Caco-2 cell permeability (a model for the gut-blood barrier) for **C1-C27** is within the range 0.22 to 1.43 cm/sec showing the non-active transport of these compounds. Total polar surface area (TPSA) of compounds ranges between 82.2 to 173.9 $Å^2$ and are well within the recommended range (7 to 200 $Å^2$). The predicted central nervous system (CNS) permeability (-1.34 to -2.07) is well within the recommended range of -2 to +2. The human intestinal absorption ranges between 88.54 to 97.88% indicating the favourable kinetic profile of these compounds. All compounds are observed to be inhibitors of some of the Cytochrome P450s. In addition, all synthesized compounds exhibit zero Pains alert (PA), indicating absence of PAN assay interference structure.

Conclusions

In the present work we synthesized new benzothiazole containing aryl and alkaryl hydrazides **C1-C27**. All compounds were characterized by spectral data and screened for their antibacterial activity. Compounds **C10**, **C15** and **C24** showed high activity against *S. aureus* ATCC 43300 (MRSA) and also exhibited bactericidal activity against this strain in MBC determination. In time kill kinetics **C10** and **C15** displayed bactericidal activity towards *S. aureus* ATCC 43300 (MRSA), respectively at concentrations of 13.75 and 10.85 µM after 24 h exposure. Compound **C24** exhibited bactericidal activity towards *S. aureus* NCIM 5022 at concentrations of 10.37 µM after 24

h exposure. Among all tested compounds, **C20** and **C24** showed maximum activity, respectively against *Mycobacterium sp* NCIM 2984 (MICs, 21.81 μM) and *E. coli* NCIM 2065 (MIC, 22.17 μM). On the other hand, tested compounds were observed to less active against *P. aeruginosa* NCIM 2036. In extra-precision docking, **C1-C27** showed interactions mainly with the N-terminal and central domains of the catalytic pocket. Components of binding free energy calculation revealed Coulomb energy term strongly favourable for binding of most of the compounds with *S. aureus* GyrB. In ADMET prediction all compounds obeyed Lipinski's rule of five. Results show that compound **C24** exhibited promising activity against *S. aureus* NCIM 5022 (MIC, 20.75 μM), *S. aureus* ATCC 43300 (MIC, 20.75 μM) and *Mycobacterium sp* NCIM 2984 (MIC, 21.81 μM). This compound also exhibited bactericidal activity against *S. aureus* NCIM 5022 (MBC/MIC, 4). In addition, the binding free energy of compound **C24** as computed by the MM-GBSA approach is -71.28 kcal/mol, indicating high binding affinity of this compound towards *S. aureus* GyrB protein. Further, we can see from *Figure 3c* that *n*-butyl fragment of *n*-butoxy group is located near to the hydrophobic pocket. This *n*-butyl fragment may further be exchanged with other hydrophobic groups to increase the hydrophobic interactions and binding affinity towards *S. aureus* GyrB. Hence chemical structure of his compound may further be optimized to increase the antibacterial activity of this compound against *S. aureus* and also against *Mycobacterium sp*.

Experimental

Material and Methods

All the glass wares were oven dried prior to use. The purification and drying of solvents were carried wherever required. All reactions were examined by thin layer chromatography (TLC) using pre-coated aluminum back thin layer chromatography (TLC) Silica Gel F254 plates (Merck, Ltd., Germany). All the chemicals and solvents were purchased from Sigma Aldrich, Merck, Finar, Alfa aesar and LOBA Chemie. The melting point of the synthesized compounds was determined using Veego VMP-1 melting point apparatus expressed in °C and are uncorrected. Fourier transform infrared (FT-IR) spectroscopy was performed either by Shimadzu or Perkin Elmer-Spectrum Two spectrophotometers. ^{1}H and ^{13}C-NMR spectra were recorded either in deuterated chloroform ($CDCl_3$) or dimethyl sulfoxide ((D_6)DMSO) at 300, 400 or 500 and 101 or 125 MHz, respectively using Bruker AV-III 400 spectrometer (Germany). Chemical shifts were recorded in ppm using the solvent as internal standard. High resolution mass spectra (HRMS) were recorded using Xevo G2-XS QTof Quadrupole Time-of-Flight Mass Spectrometer (USA) with positive electrospray ionization (ESI) mode at 70 ev.

2-Hydrazino-1,3-benzothiazole (**A**) was synthesized from 2-mercaptobenzothizole (**A**) following the procedure described in the literature (m.p. 192 ^{0}C, lit. 189-193 ^{0}C).[35]

General procedure for the synthesis of *N*'-(1,3-benzothiazol-2-yl)-substituted aryl/aralkyl hydrazides (C1-C27)

2-Hydrazino-1,3-benzothiazole (**A**) (0.242 g, 0.0014 mol) and hydroxybenzotriazole (HOBt) (0.450 g, 0.0029 mol) was successively added to the corresponding substituted benzoic acid (0.2 g, 0.0014 mol) in N,N-dimethylformamide (DMF) (15 mL). The mixture was cooled to 0 °C in an ice bath with stirring and then 1-ethyl-3-(3-dimethylaminopropyl)carbodiimde hydrochloride (EDCl) (0.563 gm, 0.00294 mol) was added. The reaction

mixture was then slowly allowed to reach the room temperature over 1 h and then stirring was further continued at this temperature till completion of reaction. Progress of reaction was monitored with TLC using n-hexane:ethylacetate (1:4) as eluent. The reaction was quenched by saturated $NaHCO_3$ solution and then extracted with EtOAc (20 mL x 3). The organic layer was dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue thus obtained was purified by column chromatography on silica gel Merck (100-200 mesh) in glass columns (2 or 3 cm diameter) using 25-30 grams of silica gel per one gram of the residue. The elution of column was started with n-hexane and then eluent polarity was gradually increased with ethyl acetate Compounds $\mathbf{C_{1\text{-}27}}$ thus obtained in yield of 78-95%.

Synthetic route for compounds **C1-C27** is presented in *Scheme 1*. Characterization data of compounds **C1-C27** is given in supplementary.

Antibacterial activity assay

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The newly synthesized compounds **C1-C27** were screened against seven bacterial strains, *Staphyloccocus aureus* NCIM 5021, *Staphyloccocus aureus* NCIM 5022, methicillin resistant *Staphyloccocus aureus* ATCC 43300 (MRSA strain), *Staphylococcus epidermidis* NCIM 2493, *Mycobacterium tuberculosis* NCIM 2984, *Klebsiella pneumoniae* NCIM 2706, *Pseudomonas aeruginosa* NCIM 2036 and *Escherichia coli* NCIM 2065 following the guidelines of Clinical Laboratories Standard Institute (CLSI 2007)[37]. The tests were performed in Mueller Hinton medium (Hi-media) by broth microdilution method, in 96-well microtiter plates. Compounds **C1-C27** was dissolved in sterile dimethyl sulfoxide (DMSO) to screen their antibacterial activity. Ciprofloxacin and gentamicin were dissolved in sterile DMSO and used as positive control, while sterile DMSO served as a negative control. The final volume for MIC protocols was 100 µL, whereas DMSO concentration in assays well was less than 1%. Synthesized compounds were tested in the concentration range of 19.74 to 221.98 µM. The bacterial suspensions at 10^5 Colony Forming Unit/mL (CFU/mL) concentrations were inoculated to the corresponding wells. Following inoculation 96-well microtitre plates was incubation at 37 °C for 24 h. Plates were then agitated and read for the absorbance at a wavelength of 600 nm. All tests were performed in triplicate and the results were taken as a mean. After MIC determination, 50 µL aliquot from each well was sub-cultured on Mueller-Hinton agar plates and were further incubated at 37 °C for 24 h. The MBC was defined as the lowest concentration of the tested compound responsible for 99.9% reduction in bacterial viable count. All experiments were repeated in triplicate for each strain. The ratio MBC/MIC ≤2 indicates bactericidal activity, while MBC/MIC ratio ≥4 shows bacteriostatic activity of test compound. MIC and MBC values (µM) of the tested compounds are presented in *Table 1* and *Table 2*, respectively.

Time-kill assay

Compounds **C10**, **C15** and **C24** exhibited promising MIC values against *S. aureus* ATCC 43300. Compound **C24** also exhibited high activity against *S. aureus* NCIM 5022. These three compounds are selected for further antibacterial evaluation by the broth macro-dilution method following the guidelines of Clinical Laboratories Standard Institute (CLSI 2007).[37] Inoculum suspension of *S. aureus* NCIM 5022 *S. aureus* ATCC 43300 and was

serially diluted to achieve a concentration of 10^5 CFU/mL and treated with respective test compounds (**C10**, **C15** and **C24**) respectively at 13.57, 10.85 and 10.37 μM, and also at 27.14, 21.70 and 20.75 μM concentrations. Ciprofloxacin was used as positive control against *S. aureus* ATCC 43300 at 47.98 μM and 95.97 μM concentrations and against *S. aureus* NCIM 5022 at 3.16 μM and 6.33 μM concentrations. The inoculum cultures were incubated at 37 °C and 50 μL from the corresponding cultures were collected at timed intervals (0, 2, 4, 6, 8, 12, and 24 h) and sub-cultured on nutrient agar medium. After incubation at 37 °C for 24 h CFUs were determined. Data were analyzed by plotting the $\log_{10}$ CFU per millilitre versus time (h). A reduction of ≥3 $\log_{10}$ cfu/mL compared to the initial inoculums is defined as the bactericidal activity, whereas <3 $\log_{10}$ cfu/mL decrease compared to the initial inoculum corresponds to bacteriostatic activity.

Computational Studies

Molecular docking and binding free energy calculation

It was of particular interest to investigate the mechanism by which title compounds exerted their antibacterial activities. Grid-based extra-precision glide docking was performed for compounds **C1-C12** in the catalytic pocket of *S. aureus* GyrB. For this purpose 3D-crystal structure of *S. aureus* GyrB 24-kDa N-terminal domain (pdb.3U2K, resolution 1.64 Å)[44] was retrieved from the protein data bank and prepared by the Protein Preparation Wizard[45] using prime[46] and OPLS3e force field.[47] Around centre of the co-crystal ligand a 10 Å glide grid was generated. Synthesized compounds **C1-C27** structures were sketched with the builder panel of Maestro version 12.0 and optimized with LigPrep module using OPLS3e force field. Low energy conformations of all compounds were docked into the prepared catalytic pocket of 3U2K in extra-precision mode using Glide[48] without applying any constraint. Further, binding free energy (ΔG_{bind}) of docked complexes was computed by the MM-GBSA approach using VSGB 2.0 energy model[49] and OPLS3e force field.

Absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties prediction

ADMET properties of compounds play vital roles in design and development of drug. ADMET properties of the synthesized compounds were computed by SwissADME and (http://www.swissadme.ch/) and pkCSM (http://biosig.unimelb.edu.au/pkcsm/prediction) online tools. ADMET properties, principle descriptors of compounds **C1-C27** including hERG inhibition potential, AMES toxicity, Lipinski number of violations and PAINS number of alerts were taken into consideration (Supplementary *Table S2*) to assess the acceptability for rational drug design.

Conflict of interest

The authors declare that they have no conflict of interest.

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Authors Contribution

Swarupa Rani gurram was the author, who synthesized the literature and drafted the paper. Dr. Md. Afzal Azam provided conceptual inputs and revised the manuscript. All authors read and approved the final paper.

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Graphical Abstract

N'-(1,3-benzothiazol-2-yl)-substituted benzohydrazides were synthesized by direct coupling of 2-hydrazino-1,3-benzothiazole with different aromatic carboxylic acids under catalysis of HOBt/EDCl in DMF.