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Original research paper

Synthesis and pharmacophore modeling of novel quinazolines bearing a biologically active sulfonamide moiety

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In the present work, interaction of the strategic starting material, methyl 2-isothiocyanatobenzoate (**1**), with sulfa drugs resulted in the formation of methyl 2-[3-(4-(*N*-substituted sulfamoyl)phenyl)thioureido] benzoates **2–5**, which upon reaction with hydrazine hydrate afforded *N*-amino derivatives **6–9.** Triazoloquinazoline derivatives **10–18** were obtained *via* reaction of compounds **6–8** with aromatic aldehydes. Also, the reaction of compound **8** with formic acid gave the corresponding triazoloquinazoline derivative **19**. Triazinoquinazoline derivatives **22**, **23** were obtained *via* reaction of *N*-amino derivatives **6** or **8** with ethyl chloroacetate. Interaction of **6** with diethyloxalate yielded triazoloquinazoline **26**. The synthesized compounds were screened for their *in vitro* antimicrobial activities and some of them exhibited promising antibacterial activity compared to ampicillin as positive control. Compounds that revealed significant activity are able to satisfy effectively the proposed pharmacophore geometry.

Keywords: quinazolines, fused quinazolines, sulfonamide, antimicrobial, pharmacophore

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Condensed heterocyclo-quinazolines are a large group of polyheterocycles with diverse interesting biological activities (1). Many synthetic methods have been developed to prepare the titled compounds, but few used the versatile and widely synthesized intermediate methyl 2-isothiocyanatobenzoate (2–6). Also, several quinazoline-(3*H*)-one derivatives containing hydrazone, thiosemicarbazide, pyrazole moieties and 1,2,4-triazolo[4,3-*a*]quinazolin-5-(4*H*)-one derivatives are reported as potential growth inhibitors of *Staphylococcus aureus* (7). In addition, a series of 2-oxo-azetidinylquinazolin-4(3*H*)-ones were reported as good antimicrobial agents (8). On the other hand, 4(3*H*)-quinazolinones with 3-substitution have been reported to be associated with antimicrobial properties. The 3-substitutions were reported in substituted phenyl ring moieties (9), bridged phenyl rings (10), heterocyclic rings (11) and aliphatic systems (12). Also, 2,3-disubstituted-4(3*H*)-quinazolinones were reported to possess antimicrobial properties (13). From the literature survey, it was found that the corresponding triazoloquinazolinones seemed to be more potent than tetrazinoquinazolinones and indolotriazinoquinazolinones. The presence of triazole nucleus at N-1 and C-2 of quinazolinone ring works better for antimicrobial activity of this series of compounds than the others (14) which showed moderate to good antifungal activity (15). Moreover, sulfonamides constitute an important class of drugs, with several types of pharmacological activities, specially antimicrobial activities (6–19).

In the light of these facts, and in continuation of our work aimed at developing new approaches for the synthesis of polyfunctionally substituted heterocyclic compounds of expected biological activity (20, 21), the current work aims to synthesize some novel derivatives including hybrid molecules of substituted quinazolines, triazoloquinazoline or triazinoquinazoline scaffold conjugated to sulfonamide moieties in order to get a new lead structure with enhanced antimicrobial activity. In this study, we used the LigandScout program to establish the microbial growth inhibitor pharmacophore sites by analyzing a training set of the synthesized compounds. The generated pharmacophore model was validated using a test set of synthesized compounds.

EXPERIMENTAL

Melting points were determined on a Gallenkamp melting point apparatus (UK) and are uncorrected. Infrared (IR) spectra were recorded on a Shimadzu MR 470 infrared spectrophotometer (Shimadzu, Japan) using KBr pellets. ^{1}H NMR spectra were recorded on a Varian EM 360 (240 MHz) instrument (Varian, UK) using TMS as an internal standard (chemical shift in δ ppm). Elemental analyses (C, H, N) were performed on a Perkin-Elmer 2400 Instrument (USA). All compounds were within ±0.4 % of the theoretical values. Mass spectra were run using a HP Model MS-5988 (Hewlett Packard).

General syntheses

*Methyl 2-(3-(4-(*N*-substituted sulfamoyl)phenyl)thioureido)benzoate (***2***–***5***).* – A mixture of methyl 2-isothiocyanatobenzoate **1** (1.93 g, 0.01 mol) and sulfa drug (0.01 mol) in dimethylformamide (20 mL) was stirred at room temperature for 4 h. The reaction mixture

was poured onto ice water and the obtained product was recrystallized from ethanol to give compounds **2–5**, respectively.

4-(3-Amino-4-oxo-3,4-dihydroquinazolin-2-ylamino)-N-*substituted benzenesulfonamides (**6–9**).* – A mixture of **2–5** (0.01 mol) and hydrazine hydrate (1.0 g, 0.02 mol) in butanol (30 mL) was refluxed for 5 h. After cooling the reaction mixture was poured onto ice water and the solid obtained was recrystallized from dioxane to give **6–9**, respectively.

N-*substituted-4-(9–oxo–2–phenyl[1,2,4]triazolo[5,1-*b*]quinazolin-3(9H)-yl)benzenesulfonamides (**10–18**).* – A mixture of **6–8** (0.01 mol) and aromatic aldehyde (0.01 mol) in glacial acetic acid (20 mL) containing fused sodium acetate (0.5 g) was heated under reflux for 4 h, the solvent was concentrated and the residue was recrystallized from dioxane to give **10–18**, respectively.

N-*(4,6-dimethylpyrimidin-2-yl)-4-(9-oxo-[1,2,4]triazolo[5,1-*b*]quinazolin-3(9H)-yl)benzen esulfonamide (**19**).* – A solution of compound **8** (4.37 g, 0.01 mol) in formic acid (20 mL) was heated under reflux for 8 h. The solvent was evaporated under vacuum and the residue was recrystallized from ethanol to give **19**.

N-*carbamidoyl-4-(2,10-dioxo-2,3-dihydro-1*H*[1,2,4]triazino[3,2-*b*]quinazolin-4(10*H*)-yl)benzenesulfonamide (***22***)* and N-*(4,6-dimethylpyrimidin-2-yl)-4-(2,10-dioxo-2,3-dihydro-1*H*[1,2,4]triazino[3,2-*b*]quinazolin-4(10*H*)-yl)benzenesulfonamide (***23***).* – A mixture of compound **6** or **8** (0.01 mol) and ethyl chloroacetate (1.22 g, 0.01 mol) in methanol (20 mL) containing sodium methoxide (0.54 g, 0.01 mol) was refluxed for 10 h. After cooling, the reaction mixture was poured onto ice water and the solid obtained was recrystallized from dioxane to give (**22**, **23)**, respectively.

*Ethyl 3-(4-(*N*-carbamidoylsulfamoyl)phenyl)-9-oxo-3,9-dihydro[1,2,4]triazolo[5,1-*b*]quinazoline-2-carboxylate (**26**)* – A mixture of compound **6** (3.73 g, 0.01 mol) and diethyl oxalate (1.46 g, 0.01 mol) in methanol (20 mL) containing sodium methoxide (0.54 g, 0.01 mol) was refluxed for 8 h. After cooling, the reaction mixture was poured onto ice water, acidified by diluted HCl and the solid obtained was recrystallized from dioxane to give **26**.

N-*carbamidoyl-4-(-4-oxo-3-(3-phenylthioureido)-3,4-dihydroquinazolin-2-ylamino)benzene sulfonamide (**29**)* and N-*(2,6-dimethoxypyrimidin-4-yl)-4-[4-oxo-3-(3-phenylthioureido)-3,4-dihydroquinazolin-2-ylamino)benzenesulfonamide (**30**).* – A mixture of compound **6** or **9** (0.01 mol) and phenyl isothiocyanate (1.35 g, 0.01 mol) in ethanol (50 mL) was refluxed for 8 h. The solvent was then concentrated and the residue was recrystallized from ethanol to give **29, 30,** respectively.

In vitro antimicrobial activity

Antimicrobial screening of the newly synthesized compounds was undertaken using the agar diffusion assay (22). DMSO solvent was used as a negative control, and ampicillin and miconazole were used as reference drugs for tested samples (23). The synthesized compounds were tested against two Gram-negative (*Serratia marcesens* IMRU-70, *Proteus mirab.* NTC-289), two Gram-positive (*Staphylococcus aureus* NCTC-7447, *Bacillus cereus* ATCC-14579) strains and one fungal (*Aspergillus ochraceus* AUCC-230) strain.

Ligand based pharmacophore modeling

The study was carried out using the software LigandScout (version 3.0). LigandScout program was used to derive the 3D chemical feature-based pharmacophores from the structural data of the synthesized compounds (Schemes 1–4) using default settings (24). Compounds **2–7**, **9–13**, **15–19**, **22–23**, **26**, **29** and **30** are included in the modeling method. Prior to the generation of pharmacophore hypotheses, the training set of compounds which were converted to 3D structure, were used to generate diverse conformations. Diverse Conformation Generation protocol implemented in the LigandScout program was used to generate conformations using the best conformation model generation method. Other parameters like maximum number of 500 conformers, and an energy threshold value of 20 kcal mol^{-1} above the global energy minimum were chosen during conformation generation. During generation of pharmacophore hypothesis, four pharmacophoric features such as hydrogen, bond acceptor (HBA), hydrogen bond donor (HBD), ring aromatic (RA) and hydrophobic (HY) features were selected based on the feature mapping results. All parameters were set to their default values.

Pharmacophore validation

The generated pharmacophore hypothesis was validated using the test set and leave-one-out methods.

Test set method. – Compounds **2, 9, 11, 22** and **30** were selected as test set compounds. This method is used to elucidate whether the generated pharmacophore hypothesis is able to predict the activities of compounds other than the training set and classify them correctly on their activity scale. Conformation generation for test set compounds was carried out in a similar way as for training set compounds using a conformation analysis algorithm. The compounds associated with their conformations were subsequently used for pharmacophore mapping using the ligand pharmacophore mapping protocol with the best/flexible search option.

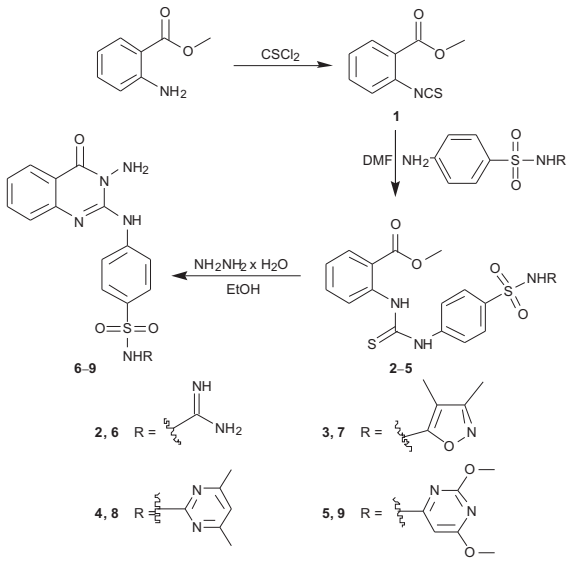
Leave-one-out method. – The pharmacophore hypothesis was cross validated by the leave-one-out method. In this method, one compound is left out when generating a new pharmacophore model and its affinity is predicted using that new model. The model building and estimation cycle is repeated until each compound was left out once (25). This test is performed to verify whether the correlation coefficient of the training set compounds is strongly dependent on one particular compound or not (26).

RESULTS AND DISCUSSION

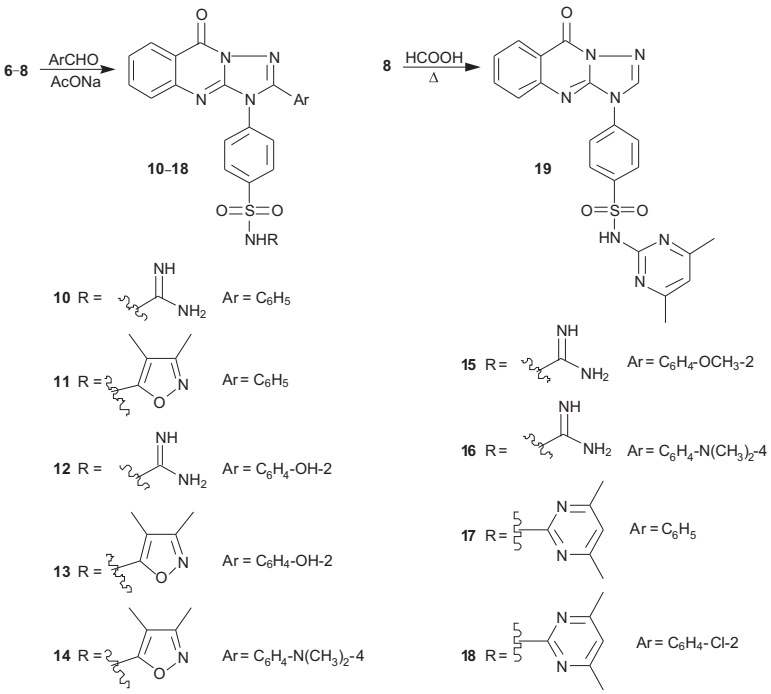
Chemistry

The sequence of reactions followed in the synthesis of new compounds is followed in Schemes 1-4. Tables I and II show the physicochemical and the spectral data, respectively.

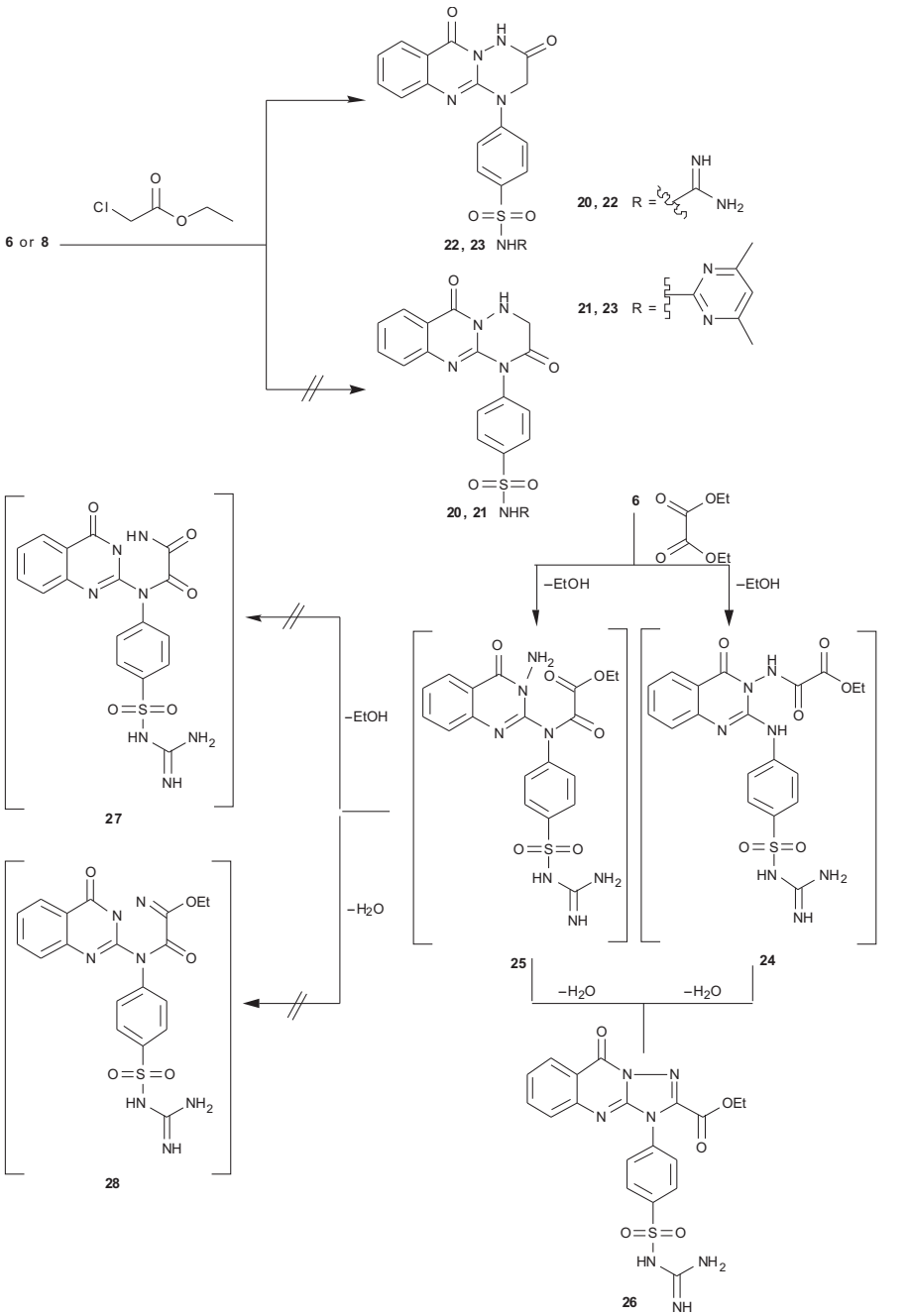
In Scheme 1, the treatment of methyl anthranilate with thiophosgene gave the corresponding methyl 2-isothiocyanatobenzoate (27, 28). The reactivity of isothiocyanato **1** towards nitrogen nucleophile was investigated. Thus, interaction of **1** with sulfa drugs



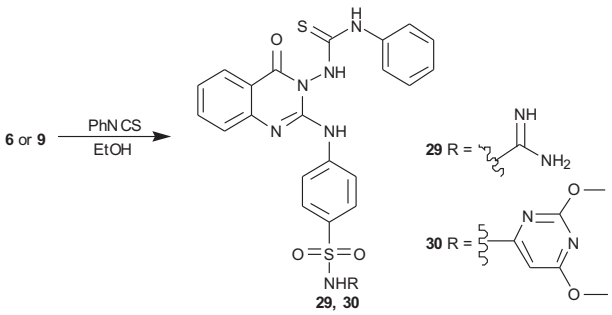
Scheme 1.



Scheme 2.


6 or 8
22, 23 NHR
20, 22 R =
21, 23 R =
20, 21 NHR
6
–EtOH
–EtOH
–EtOH
–H2O
–H2O
–H2O
27
28
25
24
26

Scheme 3.



Scheme 4.

in dimethylformamide at room temperature yielded the corresponding methyl-2-(3-(4-(*N*-substituted-sulfamoyl) phenylthioureidobenzoate derivatives **2–5**, respectively. The IR spectra of compounds **2–5** revealed the absence of the NCS band and the presence of characteristic bands attributed to the NH, C=O, C=S and SO_2 groups. ^{1}H NMR spectra of compounds **2–5** indicated the presence of a singlet at 3.7-3.9 ppm that could be assigned to OCH_3 and a singlet at 12.1-13.1 ppm due to SO_2NH.

Treatment of compounds **2–5** with hydrazine hydrate in refluxing ethanol afforded the cyclic *N*-amino compounds **6–9**. The formation of *N*-amino derivatives **6–9** proceeded *via* loss of 1 mol of H_2S (lead acetate paper), followed by intramolecular cyclization to give **6–9,** respectively. IR spectra of compounds **6–9** revealed the presence of characteristic bands of NH_2, C=O and SO_2 groups. ^{1}H-NMR spectra of compounds **6–9** showed a characteristic signal at 5.5-5.8 ppm corresponding to the *N*-amino group.

Triazoloquinazoline derivatives **10–18** were obtained *via* reaction of **6–8** with aromatic aldehydes in acetic acid containing fused sodium acetate (Scheme 2). IR and ^{1}HNMR spectra of compounds **10-18** revealed disappearance of the *N*-amino group.

Table I. Physical and analytical properties of the newly synthesized compounds

Compd.	R	Ar	Formula (M_r)	M.p. (°C)	Yield (%)	Analysis (calcd./found) (%)		
						C	H	N
2		–	$C_{16}H_{17}N_5O_4S_2$ (407.47)	> 300	82	47.16/47.31	4.21/4.40	17.19/17.57
3		–	$C_{20}H_{20}N_4O_5S_2$ (460.53)	> 300	76	52.16/52.35	4.38/4.52	12.17/12.34
4		–	$C_{21}H_{21}N_5O_4S_2$ (471.55)	> 300	69	53.49/53.72	4.49/4.23	14.85/14.63
5		–	$C_{21}H_{21}N_5O_6S_2$ (503.55)	279–281	80	50.09/50.21	4.20/4.43	13.91/13.72

6		–	$C_{15}H_{15}N_7O_3S$ (373.39)	252–254	77	48.25/48.11	4.05/4.19	26.26/26.47
7		–	$C_{19}H_{18}N_6O_4S$ (426.45)	128–130	81	53.51/53.70	4.25/4.12	19.71/19.46
8		–	$C_{20}H_{19}N_7O_3S$ (437.48)	115–117	70	54.91/54.77	4.38/4.54	22.41/22.20
9		–	$C_{20}H_{19}N_7O_5S$ (469.47)	136–138	79	51.17/51.38	4.08/4.22	20.88/20.69
10		Ph	$C_{22}H_{17}N_7O_3S$ (459.48)	> 300	62	57.51/57.34	3.73/3.51	21.34/21.62
11		Ph	$C_{26}H_{20}N_6O_4S$ (512.54)	> 300	66	60.93/60.76	3.93/3.74	16.40/16.26
12		Ph-OH-*o*	$C_{22}H_{17}N_7O_4S$ (475.48)	> 300	71	55.57/55.66	3.60/3.43	20.62/20.81
13		Ph-OH-*o*	$C_{26}H_{20}N_6O_5S$ (528.54)	> 300	68	59.08/59.31	3.81/3.64	15.90/15.56
14		Ph-$N(CH_3)_2$-*p*	$C_{28}H_{25}N_7O_4S$ (555.61)	> 300	73	60.53/60.42	4.54/4.71	17.65/17.39
15		C_6H_4-OCH_3-*o*	$C_{23}H_{19}N_7O_4S$ (489.51)	> 300	68	56.43/56.25	3.91/3.79	20.03/20.23
16		Ph-$N(CH_3)_2$-*p*	$C_{24}H_{22}N_8O_3S$ (502.55)	> 300	68	57.36/57.60	4.41/4.72	22.30/22.49
17		Ph	$C_{27}H_{21}N_7O_3S$ (523.57)	> 300	76	61.94/61.72	4.04/4.22	18.73/18.60
18		C_6H_4-Cl-*o*	$C_{27}H_{20}ClN_7O_3S$ (558.01)	220–222	68	58.12/58.35	3.61/3.40	17.57/17.69
19	–	–	$C_{21}H_{17}N_7O_3S$ (447.47)	174–177	62	56.37/56.10	3.83/3.70	21.91/21.80
22		–	$C_{17}H_{15}N_7O_4S$ (413.41)	225–227	74	49.39/49.14	3.66/3.42	23.72/23.56
23		–	$C_{22}H_{19}N_7O_4S$ (477.50)	260–262	81	55.34/55.10	4.01/4.22	20.53/20.32
26	–	–	$C_{19}H_{17}N_7O_5S$ (455.45)	92–94	66	50.11/50.38	3.76/3. 91	21.53/21.77
29		–	$C_{22}H_{20}N_8O_3S_2$ (508.58)	116–118	82	51.96/51.80	3.96/3.73	22.03/22.17
30		–	$C_{27}H_{24}N_8O_5S_2$ (604.66)	131–133	70	53.63/53.51	4.00/4.23	18.53/18.25

Table II. Spectral characterization of the newly synthesized compounds

Compd.	IR (ν_{max}, cm^{-1})	^1H NMR (DMSO-d_6) (d, ppm)	Mass (m/z, %)
2	3250, 3125 (NH, NH_2), 3050 (CH-arom.), 2970, 2850 (CH-aliph.), 1695 (C=O), 1604 (C=N), 1265 (C=S), 1350, 1113 (SO_2)	3.8 (s, 3H, OCH_3), 6.8 (s, 2H, NH_2, exchangeable with D_2O), 7.3–7.9 (m,9H, Ar-H + NH), 11.3 (s, 2H, 2NH thiourea, exchangeable with D_2O), 13.1 (s, 1H, SO_2NH, exchangeable with D_2O)	
3	3220, 3150 (NH), 3090 (CH-arom.), 2980, 2870 (CH-aliph.), 1685 (C=O), 1610 (C=N), 1260 (C=S), 1345, 1115 (SO_2)	2.4 (s, 6H, $2CH_3$), 3.7 (s, 3H, OCH_3), 6.9–7.9 (m, 8H, Ar-H), 11.2 (s, 2H, 2NH, exchangeable with D_2O), 12.9 (s, 1H, SO_2NH, exchangeable with D_2O)	460 [M^+] (2.25), 176 (100)
4	3390, 3251 (NH), 3035 (CH-arom.), 2950, 2865 (CH-aliph.), 1710 (C=O), 1600 (C=N), 1265 (C=S), 1345, 1161 (SO_2)	2.3 (s, 6H, $2CH_3$), 3.9 (s, 3H, OCH_3), 6.8–7.9 (m, 9H, Ar-H+CH pyrimidine), 11.4 (s, 2H, 2 NH, exchangeable with D_2O), 12.1 (s, 1H, SO_2NH, exchangeable with D_2O)	471 [M^+] (31.6), 65 (100)
5	3398, 3274, 3178 (NH), 3070 (CH-arom.), 2970, 2860 (CH-aliph.), 1732 (C=O), 1620 (C=N), 1265 (C=S), 1388, 1168 (SO_2)	3.8, 3.9 (2s, 9H, $3OCH_3$), 6.8–7.9 (m, 8H, Ar-H), 8.6 (s, 1H, CH pyrimidine), 11.1 (s, 2H, 2NH, exchangeable with D_2O), 12.4 (s, 1H, SO_2NH, exchangeable with D_2O)	
6	3350, 3251 (NH, NH_2), 3035 (CH-arom.), 1720 (C=O), 1610 (C=N), 1315, 1158 (SO_2)	5.8 (s, 2H, N-NH_2, exchangeable with D_2O), 6.7 (s, 2H, NH_2, exchangeable with D_2O), 6.9–8.0 (m, 9H, Ar-H + NH), 9.1 (s, 1H, NH-ph, exchangeable with D_2O), 11.6 (s, 1H, SO_2NH, exchangeable with D_2O)	373 [M^+] (4.52), 108 (100)
7	3350, 3201 (NH, NH_2), 2989, 2850 (CH-aliph.), 1698 (C=O), 1612 (C=N), 1315, 1153 (SO_2)	2.2 (s, 6H, $2CH_3$), 5.6 (s, 2H, N-NH_2, exchangeable with D_2O), 6.8–8.1 (m, 8H, Ar-H), 9.0 (s, 1H, NH-Ph, exchangeable with D_2O), 10.9 (s, 1H, SO_2NH, exchangeable with D_2O)	426 [M^+] (0.88), 331 (100)
8	3321, 3250 (NH, NH_2), 3058 (CH-arom.), 2923, 2865 (CH-aliph.), 1685 (C=O), 1600 (C=N), 1311, 1188 (SO_2)	2.3 (s, 6H, $2CH_3$), 5.5 (s, 2H, N-NH_2, exchangeable with D_2O), 6.6 (s, 1H, CH pyrimidine), 6.9–7.9 (m, 8H, Ar-H), 8.9 (s, 1H, NH-Ph, exchangeable with D_2O), 10.9 (s, SO_2NH, exchangeable with D_2O)	437 [M^+] (2.27), 278 (100)
9	3301, 3205 (NH, NH_2), 2993, 2889 (CH-aliph.), 1685 (C=O), 1627 (C=N), 1315, 1157 (SO_2)	3.8 (s, 6H, $2OCH_3$), 5.5 (s, 2H, N-NH_2, exchangeable with D_2O), 5.9 (s, 1H, CH pyrimidine), 7.6–7.9 (m, 8H, Ar-H), 8.8 (s, 1H, NH-Ph, exchangeable with D_2O), 10.8 (s, 1H, SO_2NH, exchangeable with D_2O)	469 [M^+] (0.1), 92 (100)
10	3425, 3301 (NH, NH_2), 1680(C=O), 1604(C=N), 1315, 1141 (SO_2)	6.8 (s, 2H, NH_2, exchangeable with D_2O), 7.0–7.9 (m, 14H, Ar-H + NH), 11.2 (s, 1H, SO_2NH, exchangeable with D_2O)	459 [M^+] (7.61), 91 (100)
11	3448 (NH), 3100 (CH-arom.), 2990, 2850 (CH-aliph.), 1697 (C=O), 1617 (C=N), 1350, 1157 (SO_2)	2.4 (s, 6H, $2CH_3$), 7.1–8.1 (m, 13H, Ar-H), 11.1 (s, 1H, SO_2NH, exchangeable with D_2O)	
12	3440 (OH), 3400, 3301 (NH, NH_2), 1705 (C=O), 1610 (C=N), 1310, 1157 (SO_2)	6.7 (s, 2H, NH_2, exchangeable with D_2O), 6.9–7.9 (m, 13H, Ar-H + NH, exchangeable with D_2O), 10.8 (s, 1H, SO_2NH, exchangeable with D_2O), 11.0 (s, 1H, OH, exchangeable with D_2O)	475 [M^+] (8.50), 207 (100)

13	3433 (OH), 3310 (NH), 1695 (C=O), 1604 (C=N), 1334, 1157 (SO_2)	2.3 (s, 6H, $2CH_3$), 6.9–7.9 (m, 12H, Ar-H), 10.8 (s, 1H, SO_2NH, exchangeable with D_2O), 11.6 (s, 1H, OH, exchangeable with D_2O)	528 [M^+] (2.56) 287 (100)
14	3425 (NH), 2970, 2865 (CH-aliph.), 1705 (C=O), 1620 (C=N), 1320, 1164 (SO_2)	2.2 (s, 6H, $2CH_3$), 2.8 (s, 6H, $N(CH_3)_2$), 6.9-8.1 (m, 12H, Ar-H), 10.7 (s, 1H, SO_2NH, exchangeable with D_2O).	555 [M^+] (0.62), 148 (100)
15	3456, 3310 (NH, NH_2), 3050 (CH-arom.), 2950, 2839 (CH-aliph.), 1697 (C=O), 1628 (C=N), 1330, 1164 (SO_2)	3.8 (s, 3H, OCH_3), 5.8 (s, 2H, NH_2, exchangeable with D_2O), 6.9–8.2 (m, 13H, Ar-H + NH), 11.4 (s, 1H, SO_2NH, exchangeable with D_2O)	
16	3448, 3305 (NH, NH_2), 2950, 2823 (CH-aliph.), 1698 (C=O), 1630 (C=N), 1315, 1164 (SO_2)	2.9 (s, 6H, $2CH_3$), 6.4 (s, 2H, NH_2, exchangeable with D_2O), 6.9–8.1 (m, 13H, ArH + NH), 11.0 (s, 1H, SO_2NH, exchangeable with D_2O)	502 [M^+] (1.6), 207 (100)
17	3424 (NH), 3058 (CH-arom.), 2932, 2855 (CH-aliph.), 1696 (C=O), 1625 (C=N), 1316, 1154 (SO_2)	2.4 (s, 6H, $2CH_3$), 6.5 (s, 1H, CH pyrimidine), 6.9–8.2 (m, 13H, Ar-H), 10.9 (s, 1H, SO_2NH, exchangeable)	523 [M^+] (5.1), 105 (100)
18	3446 (NH), 2926, 2862 (CH-aliph.), 1688 (C=O), 1592 (C=N), 1312, 1160 (SO_2), 767 (C-Cl)	2.3 (s, 6H, $2CH_3$), 6.8–8.1 (m, 13H, Ar-H + CH pyrimidine), 11.2 (s, 1H, SO_2NH, exchangeable with D_2O)	558 [M^+] (2.2), 139 (100)
19	174–177 °C; IR, (KBr, cm^{-1}): 3430 (NH), 2926, 2850 (CH-aliph.), 1684 (C=O), 1598 (C=N), 1318, 1164 (SO_2)	2.4 (s, 6H, $2CH_3$), 6.5 (s, 1H, CH pyrimidine), 6.9–8.0 (m, 9H, Ar-H + CH triazole), 10.9 (s, 1H, SO_2NH, exchangeable with D_2O)	447 [M^+] (8.3), 273 (100)
22	3444, 3310 (NH, NH_2), 3080 (CH-arom.), 1708, 1690 (2C=O), 1596 (C=N), 1318, 1164 (SO_2)	4.3 (s, 2H, CH_2CO), 6.4 (s, 2H, NH_2, exchangeable with D_2O), 6.9–8.1 (m, 9H, Ar-H, + NH), 8.3 (s, 1H, NHCO, exchangeable with D_2O), 10.9 (s, 1H, SO_2NH, exchangeable with D_2O)	413 [M^+] (5.2), 314 (100)
23	3324 (NH), 2950, 2863 (CH-aliph.), 1708, 1686 (2C=O), 1596 (C=N), 1324, 1154 (SO_2)	2.2 (s, 6H, $2CH_3$), 4.3 (s, 2H, CH_2CO), 6.6 (s, 1H, CH pyrimidine), 6.9–8.1 (m, 8H, Ar-H), 8.4 (s, 1H, NHCO, exchangeable with D_2O), 10.7 (s, 1H, SO_2NH, exchangeable with D_2O)	477 [M^+] (4.6), 175 (100)
26	3421, 3271 (NH, NH_2), 3100 (CH-arom.), 1685, 1650 (2C=O), 1604 (C=N), 1314, 1172 (SO_2)	1.3 (t, 3H, CH_3), 4.3 (q, 2H, CH_2), 6.6 (s, 2H, NH_2, exchangeable with D_2O), 6.9–8.1 (m, 9H, Ar-H+ NH), 10.6 (s, 1H, SO_2NH, exchangeable with D_2O)	455 [M^+] (1.33), 92 (100)
29	3425, 3215 (NH, NH_2), 1698 (C=O), 1610 (C=N), 1284 (C=S), 1315, 1168 (SO_2)	6.4 (s, 2H, NH_2, exchangeable with D_2O), 6.9–8.2 (m, 14H, Ar-H + NH), 8.4, 8.6 (2s, 2H, 2NH thiourea, exchangeable with D_2O), 9.4 (s, 1H, NH-Ph, exchangeable with D_2O), 10.9 (s, 1H, SO_2NH, exchangeable with D_2O)	508 [M^+] (2.6), 193 (100)
30	3205 (NH), 3035 (CH-arom.), 2927, 2865 (CH-aliph.), 1668 (C=O), 1550 (C=N), 1242 (C=S), 1315, 1168 (SO_2)	3.8 (s, 6H, 2 OCH_3), 6.9–8.2 (m, 13H, Ar-H), 8.5, 8.6 (2s, 2H, 2NH, exchangeable with D_2O), 9.7 (s, 1H, NH-Ph, exchangeable with D_2O), 10.7 (s, 1H, SO_2NH, exchangeable with D_2O)	604 [M^+) (3.10), 186 (100)

Also, reaction of compound **8** with formic acid as one carbon cyclizing agent gave the triazoloquinazoline derivative **19.** ^{1}H NMR spectrum of compound **19** indicated the presence of two methyl groups at δ 2.4 ppm and CH-pyrimidine at δ 6.5 ppm.

Interaction of compound **6** or **8** with ethyl chloroacetate in methanol containing sodium methoxide yielded the triazinoquinazoline derivatives **22** and **23**, respectively (Scheme 3). The structure of compounds **22** and **23** was suggested rather than structures **20** and **21**, based on the assumption that the reaction basic condition allowed it to proceed through formation of sodium salt on the less basic NH nitrogen atom, and elimination of sodium chloride followed by cyclization (25). In addition, the IR spectrum of compound **22** showed bands at 1708 and 1690 cm^{-1} (2C=O); also, the IR spectrum of compound **23** revealed bands at 1708 and 1686 cm^{-1} (2C=O), which were at lower frequency than it was expected for structures **20** and **21**. Further evidence was the ^{1}H NMR spectrum that showed a singlet at 4.3 ppm for methylene protons. On the other hand, when compound **6** was reacted with diethyl oxalate, the corresponding triazoloquinazoline derivative **26** was obtained (Scheme 3). This was confirmed by its elemental analysis, ^{1}H NMR and mass spectral data. These results are in agreement with the method previously reported (29). ^{1}H NMR spectrum of compound **26** revealed a triplet at 1.3 ppm and a quartet at 4.3 ppm attributed to the ethyl ester group.

Finally, thioureido derivatives **29** and **30** were obtained *via* reaction of compounds **6** or **9** with phenyl isothiocyanate (Scheme 4). IR spectra of compounds **29** and **30** showed the presence of bands for NH, C=O, C=S and SO_2 groups. ^{1}H NMR spectrum of compound **29** indicated the presence of NH_2 of guanidine moiety at 6.4 ppm. ^{1}H NMR spectrum of compound **30** revealed a signal at 3.8 ppm attributed to two methoxy groups.

In vitro *antimicrobial activity*

Table III lists the results of screening the tested compounds against the Gram-negative bacteria *S. marcescens* and *P. mirabilis*, and Gram-positive bacteria *S. aureus* and *B. cereus*, in addition to the pathogenic fungi *A. ochraceus Wilhelm*.

Compounds **2, 15, 17** and **30** (*MIC* 0.192–0.239 μmol mL^{-1}) were more active than ampicillin (*MIC* 0.248 μmol mL^{-1}) against *S. marcescens*, while compounds **5** and **11** (*MIC* 0.244–0.249 μmol mL^{-1}) were found equipotent to ampicillin. Against *P. mirabilis*, compounds **7** and **30** (*MIC* 0.207 and 0.220 μmol mL^{-1}) were found to be more active than ampicillin, compound **16** (*MIC* 0.249 μmol mL^{-1}) was nearly as active as ampicillin. Compounds **5**, **10**, **13** and **17** (*MIC* 0.179–0.237 μmol mL^{-1}) showed higher activity against *S. aureus* than ampicillin. In addition, compounds **11**, **14**, and **30** (*MIC* 0.183–0.225 μmol mL^{-1}) were more active against *B. cereus* while compound **29** (*MIC* 0.246 μmol mL^{-1}) exhibited equipotent activity as ampicillin. Finally, compounds **2**, **5**, **11**, **15**, **17** and **30** were of comparable activity to the reference drug against bacteria, but of low activity against *A. ochraceus*.

Pharmacophore modeling

Bioisosterism between the pharmacophoric group of ampicillin and the functional group of sulfonamide compounds has been reported (Fig. 1) (30). Also, it was hypothesized that the difference in charges between two heteroatoms of the same dipolar phar-

Table III. Minimal inhibitory concentration (MIC, μmol mL^{-1}) of the newly synthesized compounds

Compd.	*Serratia marcesens*	*Proteus mirabilis*	*Staphylococcus aureus*	*Bacillus cereus*	*Aspergillus ochraceus Wilhelm*
2	0.230	0.307	0.614	0.614	0.307
3	NA	0.544	0.408	0.272	0.204
4	0.531	0.796	0.372	0.796	NA
5	0.249	0.348	0.186	0.373	0.249
6	0.503	1.341	0.670	1.005	0.469
7	0.646	0.220	NA	NA	NA
8	NA	NA	0.286	0.429	0.215
9	0.267	0.267	0.400	0.533	0.200
10	0.545	0.272	0.204	0.272	0.381
11	0.244	0.488	0.342	0.183	0.488
12	0.790	0.395	0.526	0.368	NA
13	0.355	0.331	0.237	0.355	0.237
14	NA	NA	NA	0.225	0.451
15	0.192	0.256	0.511	0.358	0.256
16	0.498	0.249	0.498	0.747	0.187
17	0.239	0.478	0.179	0.335	0.359
18	0.336	0.672	0.896	0.448	0.224
19	NA	0.839	0.559	0.420	0.210
22	0.303	0.454	0.908	1.211	0.605
23	0.524	0.786	0.262	0.524	0.393
26	0.824	0.385	1.099	0.275	0.550
29	0.492	0.738	0.738	0.246	0.984
30	0.207	0.207	0.621	0.207	0.620
Ampicillin	0.248	0.248	0.248	0.248	0.248
Miconazole	–	–	–	–	0.050
DMSO	–	–	–	–	–

NA – *MIC* > 1.5 μmol mL^{-1}.

macophore site (Xδ^-…..Yδ^+) may facilitate the inhibition of bacteria more than viruses. Hence, the antifungal activity is related to the possible secondary electronic interaction with the positively charged target sites (30).

In view of these findings and *in vitro* results against the Gram-negative bacterium *S. marcescens*, it was thought worthwhile to search for supportive coordination between *in silico* studies with *in vitro* results. Elucidation of the binding approaches for the synthesized compounds is based on finding the active structures. Schemes 1–4 show the structure of the training set compounds (**4–7**, **10**, **13**, **15–18**, **23**, **26**, **29**) and test set compounds (**2**, **9**, **11**, **22**, **30**). The test set compounds were selected to represent structural variations in the training set. On the assumption that active compounds bind in a similar fashion at the active site, we employed the LigandScout program (24) to evaluate the common fea-

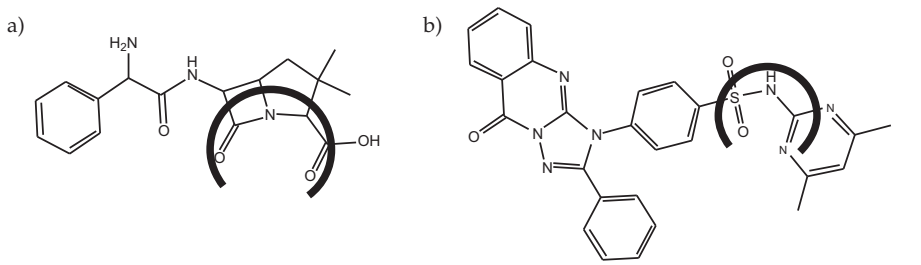


Fig. 1. Structure of: a) reference standard (ampicillin), b) representative compound **17** of the synthesized analogues. Common potential antibacterial pharmacophores are delineated with a bold line.

tures essential for activity and the hypothetical geometries adopted by these ligands in their most active forms. Thus, these compounds were submitted to pharmacophore model generation based on shared chemical features. Diverse conformations within the 20 kcal mol^{-1} energy range were generated and submitted to the alignment procedure.

The successful pharmacophore run resulted in the generation of 10 hypotheses (Hypo1-10) (Table IV). With the exception of Hypo 2, 3, 5, 7 and 10, all other hypotheses were composed of two hydrophobes, one aromatic ring, three hydrogen bond acceptor, one hydrogen bond donor. Based on its highest rank score and its mapping into all training set molecules, Hypo1 was considered to be the best hypothesis statistically. This hypothesis was selected for further investigation and analysis. The top-ranked chemical feature-based pharmacophore model identified in this study is shown in Fig. 2. This pharmacophore model contains seven chemical features: two hydrophobes, one aromatic ring, three hydrogen bond acceptors and one hydrogen bond donor.

All synthesized compounds were mapped onto Hypo1 with scoring the orientation of the mapped compound within the hypothesis features using a »fit value« score. As

Table IV. Summary of the generated pharmacophores with antibacterial activity against S. marcesens *(IMRU-70)*

Hypothesis	Feature	Rank score
Hypo1	HHRAAAD	0.8613
Hypo2	HHRRAAAD	0.8594
Hypo3	HHAAAD	0.8523
Hypo4	HHRAAAD	0.8518
Hypo5	HHAAAAD	0.8500
Hypo6	HHRAAAD	0.8495
Hypo7	HHAAAD	0.8478
Hypo8	HHRAAAD	0.8419
Hypo9	HHRAAAD	0.8398
Hypo10	HHAAAAD	0.8391

H – hydrophobic, R – aromatic ring, A – hydrogen bond acceptor, D – hydrogen bond donor

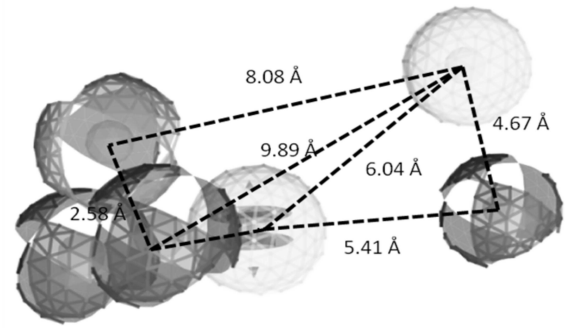


Fig. 2. Proposed pharmacophore model of antibacterial activity.

quick and primary validation of Hypo1, mapping of compounds was found to show good agreement between the fit value and the biological activity (Table V). Initial investigation of the results shown in Table V reveal moderate correlation between the fit value and the biological activity of each of the tested compounds. Highly active

Table V. Output for Hypo1 mapping and predictive model of S. marcesens

Compd.	*MIC* (μmol mL^{-1})	p*MIC* [a]	Fit value	pMIC_{pred} [b]
2	0.230	0.638	75.28	0.601
4	0.531	0.275	56.15	0.262
5	0.249	0.605	75.29	0.601
6	0.503	0.299	57.14	0.280
7	0.646	0.190	57.12	0.279
9	0.267	0.574	67.09	0.456
10	0.545	0.264	60.19	0.334
11	0.244	0.612	74.94	0.595
12	0.790	0.103	46.33	0.088
13	0.355	0.450	60.44	0.338
15	0.192	0.717	73.58	0.571
16	0.498	0.303	62.19	0.369
17	0.239	0.622	73.23	0.565
18	0.336	0.474	65.94	0.435
22	0.303	0.519	74.49	0.587
23	0.524	0.281	57.19	0.280
26	0.824	0.084	46.97	0.099
29	0.492	0.308	73.94	0.577
30	0.207	0.684	73.09	0.601

[a,b] Negative log of minimal inhibitory concentration in experiment and predicted

p*MIC* = 43.534 fit value + 46.612, n = 19, SE = 0.097, R^2 = 0.7712 *(1)* where n is the number of compounds, SE is the standard error, R^2 is the squared correlation coefficient.

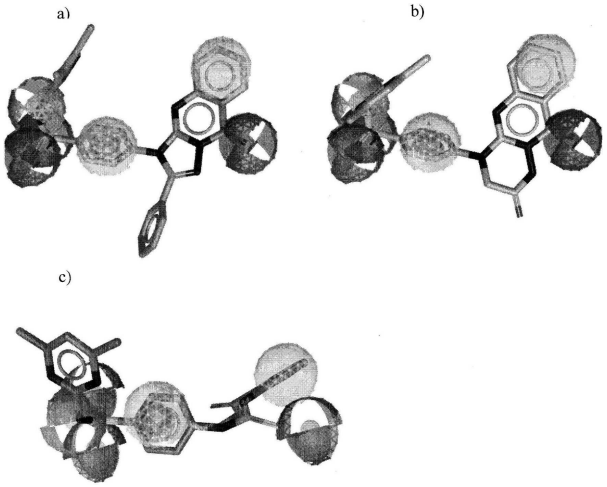


Fig. 3. a) Best aligned pose of compound **11** (*MIC* 0.244 μmol mL^{-1}) superposed with the query (Hypo1), b) Best aligned pose of compound **23** (*MIC* 0.524 μmol mL^{-1}) fitted inadequately with the query (Hypo1), c) Best aligned pose of weak **4** (*MIC* 0.531 μmol mL^{-1}) overlaid onto the pharmacophore model (Hypo1).

compounds showed a range of fit values of 75.29–67.09 where moderately active derivatives had a lower fit value average of 65.0. This initial correlation encouraged us to generate a linear model based on »fit value« to predict the biological activity of the compounds under study. The generated model (*Eq. 1*) showed very good statistics and was used successfully to calculate the activity of the tested compounds (Table V).

Figs. 3a-c show as an example the alignment of the hypothesis model with compounds **4**, **11** and **23**. A closer look at the mapped structures reveals the importance of certain structural features for activity. The quinazolinone scaffold is thought to be critical for activity, where the slight displacement of its fused benzene ring away from the hydrophobic pharmacophore center (Fig. 3b) or displacement of its carbonyl oxygen away from the hydrogen bond acceptor pharmacophore center (Fig. 3c) can partially explain the lack of activity. Also, the slight displacement of the benzene ring of sulfanilamide moiety away from the hydrophobic pharmacophore center (Fig. 3c) can partially explain the lack of activity. Another feature that is common to all compounds is the two sulfonyl hydrogen bond acceptor feature and the sulfonamide NH hydrogen bond donor of the sulfanilamide moiety.

Further structural modifications need to be tried at the sulfanilamide moiety before we can further discuss the importance of this feature for activity. Aromatic ring was found to be oriented to the hydrophobic feature and its plane was planar to that of the quinazoline ring. However, there is no evidence that the size of such a group has a determinant effect on the antibacterial activity. The two hydrogen bond acceptor features were found to lie on the opposite side of the hydrogen bond acceptor feature at a distance of 2.58 Å.

CONCLUSIONS

We report here the synthesis of some new quinazolines, triazoloquinazolines and triazinoquinazolines bearing a biologically active sulfonamide moiety. Compounds methyl 2-(3-(4-(*N*-carbamimidoylsulfamoyl)phenyl)thioureido)benzoate (**2**), methyl 2-(3-(4-(N-(2,6-dimethoxypyrimidin-4-yl)sulfamoyl)phenyl)thioureido)benzoate (**5**), *N*-(3,4-dimethylisoxazol-5-yl)-4-(9-oxo-2-phenyl[1,2,4]triazolo[5,1-*b*]quinazolin-3(9*H*)-yl)benzenesulfonamide (**11**), *N*-carbamimidoyl-4-(2-(2-methoxyphenyl)-9-oxo[1,2,4]triazolo[5,1-*b*]quinazolin-3(9*H*)-yl)benzenesulfonamide (**15**), *N*-(4,6-dimethylpyrimidin-2-yl)-4-(9-oxo-2-phenyl[1,2,4]triazolo[5,1-*b*]quinazolin-3(9*H*)-yl)benzenesulfonamide (**17**) and *N*-(2,6-dimethoxypyrimidin-4-yl)-4-[4-oxo-3-(3-phenylthioureido)-3,4-dihydroquinazolin-2-ylamino)benzenesulfonamide (**30**) exhibited significant antibacterial activity compared to ampicillin as positive control. Pharmacophore modelling study revealed that these compounds are able to effectively satisfy the proposed common feature sites using energy accessible conformers (E_{conf} < 20 kcal mol^{-1}).

Further studies which are in progress in our laboratory are focused on the study of the modification effect of the benzene ring of the sulfanilamide group by its replacement with a substituted or fused benzene ring or even its replacement with aliphatic hydrocarbons of different lengths in addition to modification of the sulfonamide group by the replacement with other carbonyl moieties.

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