Arch. Pharm. Res.
DOI 10.1007/s12272-013-0094-6

Archives of Pharmacal Research
www.springer.com/12272

RESEARCH ARTICLE

Synthesis, antimicrobial evaluation and molecular modelling of novel sulfonamides carrying a biologically active quinazoline nucleus

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Abstract A novel series of quinazolines **5**–**10**, triazoloquinazolines **11**–**17** and triazinoquinazoline **19** bearing a biologically active sulfonamide moiety were synthesized, utilizing methyl 2-isothiocyanato benzoate **2**. Some of the newly synthesized compounds revealed promising bacterial growth inhibition, compared with the ampicillin, as the reference drug. A LigandScout approach-generated pharmacophore model for the *Staph aureus* bacteria growth inhibition was done. The degree of fitting of the test set compounds (**3**, **4**, **6**, **8**, **11**, **17**) to the generated hypothetical model revealed a qualitative measure of the more or less microbial inhibition of *Staphylococcus aureus*. Compounds (**7**, **8**, **10**, **12**, **15**, **17** and **22**), which revealed significant activity, are able to effectively satisfy the proposed pharmacophore geometry, using the energy accessible conformers ($E_{conf} < 20$ kcal/mol).

Keywords Sulfonamides · Quinazolines · Fused quinazolines · Antimicrobial · Pharmacophore modelling

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Introduction

In the previous papers, we have described the preparation of heterocyclic compounds with condensed nuclei containing the quinazoline system (Ghorab et al. 1998, 1999, 2000, 2006a, b; Barakat et al. 2007; Abdel-Gawad et al. 2000). Quinazoline derivatives have been reported to possess a significant activity as antihypertensive (Ram et al. 1990), antifibrillatory, choleretic, antiphlogistic (Bekhit et al. 2001), antimitotic (Jiang et al. 1990), antifungal (Lopez et al. 2001) and anticonvulsant agents (Farghaly and Moharram 1999). Triazoles were reported to possess diverse pharmacological activities, such as antimicrobial (Ghorab et al. 2001), antitumor (Heiba et al. 2006) and anti-inflammatory activities (Hardtmann and Kathawala 1977). In addition 1,2,4-triazine derivatives were found to posses antimicrobial activity (Zhang et al. 2002). On the other hand, several nitrogen and sulfur-containing heterocyclic compounds, incorporating sulfonamide moiety, were found to possess a wide range of biological activities (Abou El-Ella et al. 2008; Ismail et al. 2006; Ghorab et al. 2004, 2006a, b; El-Sharief et al. 2002). In continuation of our interest in the synthesis of heterocycles containing sulfonamide moiety (Ghorab 2000), we report herein, a facile route for the synthesis of some new quinazolines having a biologically active triazole or triazine and sulfonamide moieties in one molecule to explore

Published online: 26 March 2013

their antimicrobial activities. 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 (**5**, **7**, **10**, **12**–**15**, **19**, **22**). The generated pharmacophore model was validated using a test set of synthesized compounds (**3**, **4**, **6**, **8**, **11**, **17**).

Materials and methods

Chemistry

Melting points were determined on Gallen-kamp melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded on shimadzu MR 470 infrared spectrophotometer, using the KBr pellets. ^{1}HNMR spectra were recorded on a Varian EM 360 (240 MHz) instrument, using TMS as an internal standard (chemical shift in δ ppm). Microanalytical data (C, H, N) were determined at the Microanalytical centre, Cairo University, Egypt. Mass spectra were run using HP Model MS-5988.

*Methyl 2-(3-(4-(N-substituted sulfamoy)phenyl)thioureidobenzoates (**3**, **4**)*

To a solution of methyl 2-isothiocyanato benzoate **2** (1.93 g, 0.01 mol) in dimethylformamide (20 mL), sulfadiazine or sulfamerazine (0.01 mol) were added. The reaction mixture was stirred at room temperature for 4 h. The reaction mixture was poured onto ice water and the obtained precipitate was filtered and recrystallized from ethanol to give **3**, **4**, respectively.

*Methyl 2-(3-(4-(N-pyrimidin-2-ylsulfamoy)phenyl)thioureido) benzoate (**3**)* Yield, 81 %; m.p. > 300 °C; IR (KBr, cm^{-1}): 3255, 3220, 3150(3NH), 3035 (CH arom.), 1662 (C=O), 1620(C=N), 1265 (C=S), 1346, 1161 (SO_2). ^{1}H-NMR in (DMSO-d_6) δ: 3.9 [s, 3H, OCH_3], 6.7–7.8 [m, 9H, Ar–H + CH pyrimidine], 8.5 [s, 2H, 2CH pyrimidine], 10.9 [s, 1H, SO_2NH, D_2O exchangeable], 12.1 [s, 2H, 2NH, D_2O exchangeable]. MS (m/z): 443 [M^+] (48), 175 (100). Anal. Calcd. For $C_{19}H_{17}N_5O_4S_2$: C, 51.46; H, 3.86; N, 15.79. Found: C, 51.22; H, 3.53; N, 16.10.

*Methyl 2-(3-(4-(N(4-methylpyrimidin-2-yl)sulfamoy)phenyl) thioureido)benzoate (**4**)* Yield, 74 %; m.p. 279–281 °C; IR (KBr, cm^{-1}): 3420, 3380, 3150(3NH), 1660(C=O), 1610 (C=N), 1350, 1160 (SO_2). ^{1}H-NMR in (DMSO-d_6) δ: 2.4(s, 3H, CH_3), 3.9(s, 3H, OCH_3), 6.7–8.1(m, 10H, Ar–H + 2CH-pyrimidine), 11.0(s, H, SO_2NH, D_2O exchangeable), 12.3(s, 2H, 2NH, D_2O exchangeable). Anal. Calcd. For $C_{20}H_{19}N_5O_4S_2$: C, 52.50; H, 4.19; N, 15.31. Found: C, 52.35; H, 4.41; N, 15.11.

*4-(3-Amino-4-oxo-3,4-dihydroquinazolin-2-ylamino)-N-(substitutedpyrimidin-2-yl)benzenesulfonamides (**5**, **6**)*

A mixture of **3** or **4** (0.01 mol) and hydrazine hydrate (1.0 g, 0.02 mol) in n-butanol (30 mL) was refluxed for 5 h. The reaction mixture was cooled, then poured onto ice water and the obtained solid was recrystallized from dioxane to give **5**, **6**, respectively.

*4-(3-Amino-4-oxo-3,4-dihydroquinazolin-2-ylamino)-N-(pyrimidin-2-yl)benzenesulfonamide (**5**)* Yield, 68 %; m.p. 228–239 °C; IR (KBr, cm^{-1}): 3390, 3209(NH, NH_2), 1700(C=O), 1315, 1153 (SO_2). ^{1}H-NMR in (DMSO-d_6) δ: 5.8 [s, 2H, NH_2, D_2O exchangeable], 6.7–8.1 [m, 9H, Ar–H + CH pyrimidine], 8.6 [s, 2H, 2CH pyrimidine], 9.2 [s, 1H, NH, D_2O exchangeable], 11.1 [s, 1H, SO_2NH, D_2O exchangeable]. MS (m/z): 409 [M^+] (3.2), 228 (100). Anal. Calcd. For $C_{18}H_{15}N_7O_3S$: C, 52.80; H, 3.69; N, 23.95. Found: C, 52.68; H, 3.53; N, 23.86.

*4-(3-Amino-4-oxo-3,4-dihydroquinazolin-2-ylamino)-N-(4-methylpyrimidin-2-yl)benzenesulfonamide (**6**)* Yield, 77 %; m.p. 205–207 °C; IR (KBr, cm^{-1}): 3365, 3210 (NH, NH_2), 1680 (C=O), 1390, 1150 (SO_2). ^{1}H-NMR in (DMSO-d_6) δ: 2.2 [s, 3H, CH_3], 5.6 [s, 2H, NH_2, D_2O exchangeable], 6.7–7.9 [m, 9H, Ar–H + CH-pyrimidine), 8.2 (s, 1H, N=CH pyrimidine), 9.1 [s, 1H, NH, D_2O exchangeable], 11.0 [s, 1H, SO_2NH, D_2O exchangeable]. Anal. Calcd. For $C_{19}H_{17}N_7O_3S$: C, 53.89; H, 4.05; N, 23.15. Found: C, 53.67; H, 4.35; N, 23.46.

*(E)-4-(3-(Substituted benzylideneamino-4-oxo-3,4-dihydroquinazolin-2-ylamino)-N-substituted benzenesulfonamides (**7**–**10**)*

A mixture of compound **5** (4.09 g, 0.01 mol) and required aromatic aldehydes in ethanol (20 mL) was refluxed for 4 h. The solvent was concentrated, and the residue was recrystallized from ethanol to give **7**–**10**, respectively.

*(E)-4-(3-(Benzylideneamino)4-oxo-3,4-dihydroquinazolin-2-ylamino)-N-(pyrimidin-2-yl) benzenesulfonamide (**7**)* Yield, 69 %; m.p. 98–100 °C; IR (KBr, cm^{-1}): 3390, 3270 (2NH), 2930, 2840(CH-aliph.), 1690 (C=O), 1376, 1150 (SO_2). ^{1}H-NMR in (DMSO-d_6) δ: 6.7–8.1 [m, 14H, Ar–H + CH pyrimidine], 9.1 [s, 1H, NH, D_2O exchangeable], 9.9 [s, 1H, N=CH], 11.6 [s, 1H, SO_2NH, D_2O exchangeable]. MS (m/z): 497 [M^+] (3.70), 131(100). Anal. Calcd. For $C_{25}H_{19}N_7O_3S$: C, 60.35; H, 3.85; N, 19.71. Found: C, 60.05; H, 3.51; N, 19.41.

*(E)-4-(3-(2-Hydroxybenzylideneamino)-4-oxo-3,4-dihydroquinazolin-2-ylamino)-N-(pyrimidin-2-yl)benzenesulfonamide (**8**)* Yield, 71 %; m.p. 205–207 °C; IR (KBr, cm^{-1}): 3500 (OH), 3210, 3180(2NH), 1708(C=O), 1315, 1130 (SO_2). ^{1}H-NMR in (DMSO-d_6) δ: 6.9–8.1 [m, 13H, Ar–H + CH pyrimidine], 8.6 [s, 2H, 2N=CH pyrimidine], 8.8 [s, 1H, NH, D_2O exchangeable], 9.3 [s, 1H, N=CH], 9.9 [s, 1H, OH, D_2O exchangeable], 11.4 [s, 1H, SO_2NH, D_2O exchangeable]. MS (m/z): 513 [M^+] (1.67), 240 (100). Anal. Calcd. For $C_{25}H_{19}N_7O_4S$: C, 58.47; H, 3.73; N, 19.09. Found: C, 58.19; H, 3.42; N, 19.25.

*(E)-4-(3-(2-Methoxybenzylideneamino)-4-oxo-3,4-dihydroquinazolin-2-ylamino)-N-(pyrimidin-2-yl) benzenesulfonamide (**9**)* Yield, 82 %; m.p. 158–160 °C; IR (KBr, cm^{-1}): 3444, 3336 (2NH), 2927, 2839 (CH-aliph.) 1681 (C=O), 1604 (C=N), 1303, 1164 (SO_2). ^{1}H-NMR in (DMSO-d_6) δ: 3.7 [s, 3H, OCH_3], 6.7–8.0 [m, 13H, Ar–H + CH pyrimidine], 8.7 [s, 2H, 2N=CH pyrimidine], 9.0 [s, 1H, NH, D_2O exchangeable], 9.8 [s, 1H, N=CH], 11.7 [s, 1H, SO_2NH, D_2O exchangeable]. Anal. Calcd. For $C_{26}H_{21}N_7O_4S$: C, 59.19; H, 4.01; N, 18.59. Found: C, 59.40; H, 4.22; N, 18.25.

*(E)-4-(3-(4-(Dimethylamino)benzylideneamino)-4-oxo-3,4-dihydroquinazolin-2-ylamino)-N-(pyrimidin-2-yl) benzenesulfonamide (**10**)* Yield, 66 %; m.p. 250–252 °C; IR (KBr, cm^{-1}): 3225, 3175 (2NH), 2980, 2860 (CH-aliph.), 1685(C=O), 1620(C=N), 1310, 1150 (SO_2). ^{1}H-NMR in (DMSO-d_6) δ: 2.8 [s, 6H, $2CH_3$], 6.7–8.1 [m, 13H, Ar–H + CH pyrimidine], 8.6 [s, 2H, 2N=CH pyrimidine], 9.1 [s, 1H, NH, D_2O exchangeable], 9.6 [s, 1H, N=CH], 11.0 [s, 1H, SO_2NH, D_2O exchangeable]. Anal. Calcd. For $C_{27}H_{24}N_8O_3S$: C, 59.99; H, 4.47; N, 20.73. Found: C, 59.63; H, 4.21; N, 20.85.

*N-(4-Methylpyrimidin-2-yl)-4-(9-oxo-2-(substitutedphenyl)-[1,2,4]triazolo[5,1-b]quinazolin-3(9H)-yl)benzenesulfonamides (**11**–**13**)*

A mixture of **6** (4.23 g, 0.01 mol) and the corresponding aromatic aldehydes (0.01 mol) in glacial acetic acid (30 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 ethanol to give **11**–**13**, respectively.

*N-(4-Methylpyrimidin-2-yl)-4-(9-oxo-2-phenyl-[1,2,4]triazolo[5,1-b]quinazolin-3(9H)-yl)benzenesulfonamide (**11**)* Yield, 69 %; m.p. 150–152 °C; IR (KBr, cm^{-1}): 3432(NH), 1685(C=O), 1592(C=N), 1318, 1148 (SO_2). ^{1}H-NMR in (DMSO-d_6) δ: 2.4 [s, 3H, CH_3], 6.7–8.2 [m, 15H, Ar–H + 2CH pyrimidine], 11.1 [s, 1H, SO_2NH, D_2O exchangeable]. MS (m/z): 509 [M^+] (1.67), 60 (100). Anal. Calcd. For $C_{26}H_{19}N_7O_3S$: C, 61.29; H, 3.76; N, 19.24. Found: C, 61.06; H, 3.52; N, 19.11.

*4-(2-(2-Hydroxyphenyl)-9-oxo-[1,2,4]triazolo[5,1-b]quinazolin-3(9H)-yl)-N-(4-methylpyrimidin-2-yl)benzenesulfonamide (**12**)* Yield, 80 %; m.p. > 300 °C; IR (KBr, cm^{-1}): 3402 (OH), 3232 (NH), 1685 (C=O), 1604 (C=N), 1326, 1126 (SO_2). ^{1}H-NMR in (DMSO-d_6) δ: 2.3 [s, 3H, CH_3], 6.8–8.1 [m, 14H, Ar–H + 2CH pyrimidine], 10.2 [s, 1H, OH, D_2O exchangeable], 11.6 [s, 1H, SO_2NH, D_2O exchangeable]. Ms (m/z): 525 [M^+] (0.1), 175(100). Anal. Calcd. For $C_{26}H_{19}N_7O_4S$: C, 59.42; H, 3.64; N, 18.66. Found: C, 59.13; H, 3.48; N, 18.39.

*4-(2-(4-(Dimethylamino)phenyl)-9-oxo-[1,2,4]triazolo[5,1-b]quinazolin-3(9H)-yl)-N-(4-methylpyrimidin-2-yl)benzenesulfonamide (**13**)* Yield, 84 %; m.p. 139–141 °C; IR (KBr, cm^{-1}): 3402 (NH), 2920, 2850 (CH-aliph.), 1688 (C=O), 1311, 1176(SO_2). ^{1}H-NMR in (DMSO-d_6) δ: 2.2 [s, 3H, CH_3], 2.9 [s, 6H, $N(CH_3)_2$], 6.7–8.2 [m, 14H, Ar–H + 2CH pyrimidine], 11.4 [s, 1H, SO_2NH, D_2O exchangeable]. Anal. Calcd. For $C_{28}H_{24}N_8O_3S$: C, 60.86; H, 4.38; N, 20.28. Found: C, 60.59; H, 4.16; N, 20.01.

*4-(9-Oxo-[1,2,4]triazolo[5,1-b]quinazolin-3(9H)-yl)-N-(pyrimidin-2-yl)benzenesulfonamide (**14**)*

A solution of **5** (4.09 g, 0.01 mol) in formic acid (50 mL) was heated, under reflux for 8 h.; the solvent was concentrated and the residue was recrystallized from ethanol to give **14**. Yield, 66 %; m.p. 188–190 °C; IR (KBr, cm^{-1}): 3340 (NH), 3100 (CH-arom.), 1685 (C=O), 1319, 1145(SO_2). ^{1}H-NMR in (DMSO-d_6) δ: 6.7–8.0 [m, 10H, Ar–H + CH triazole + CH pyrimidine], 8.4 [s, 2H, 2N=CH pyrimidine], 11.6 [s, 1H, SO_2NH, D_2O exchangeable]. Ms (m/z): 419 [M^+] (13.43), 315(100). Anal. Calcd. For $C_{19}H_{13}N_7O_3S$: C, 54.41; H, 3.12; N, 23.38. Found: C, 54.15; H, 3.02; N, 23.19.

*4-(2-Methyl-9-oxo-[1,2,4]triazolo[5,1-b]quinazolin-3(9H)-yl)-N-(4-methylpyrimidin-2-yl)benzenesulfonamide (**15**)*

A solution of **6** (4.23 g, 0.01 mol) in acetic anhydride (50 mL) was heated, under reflux for 8 h.; the solvent was concentrated and the residue was recrystallized from ethanol to give **15**. Yield, 64 %; m.p. 194–196 °C; IR (KBr, cm^{-1}): 3382 (NH), 3016(CH-arom.), 2932, 2781 (CH-aliph.), 1676 (C=O), 1315, 1176 (SO_2). ^{1}H-NMR in (DMSO-d_6) δ: 1.3 [s, 3H, CH_3 triazole], 2.4 [s, 3H, CH_3], 6.7–8.2 [m, 10H, Ar–H + 2N=CH pyrimidine], 11.0 [s, 1H, SO_2NH, D_2O exchangeable]. MS (m/z): 447 [M^+] (0.2), 257(100). Anal. Calcd. For $C_{21}H_{17}N_7O_3S$: C, 56.37; H, 3.83; N, 21.91. Found: C, 56.15; H, 3.63; N, 21.75.

*4-(2-(2-Chlorophenyl)-9-oxo-[1,2,4]triazolo[5,1-b]quinazolin-3(9H)-yl)-N-(substitutedpyrimidin-2-yl)benzenesulfonamides (**16**, **17**)*

A mixture of **5** or **6** (0.01 mol) and 2-chlorobenzoylchloride (1.75 g, 0.01 mol) in benzene (20 mL) was refluxed for 10 h. The reaction mixture was cooled, then was concentrated and the obtained solid was recrystallized from ethanol to give **16**, **17**, respectively.

*4-(2-(2-Chlorophenyl)-9-oxo-[1,2,4]triazolo[5,1-b]quinazolin-3(9H)-yl)-N-(pyrimidin-2-yl)benzenesulfonamide (**16**)* Yield, 83 %; m.p. 111–113 °C; IR (KBr, cm^{-1}): 3150 (NH), 3050 (CH-arom.), 1680 (C=O), 1604 (C=N), 1350, 1156 (SO_2), 768 (C–Cl). ^{1}H-NMR in (DMSO-d_6) δ: 6.7–8.0 [m, 13H, Ar–H + CH pyrimidine], 8.7 [s, 2H, 2N=CH pyrimidine], 11.4 [s, 1H, SO_2NH, D_2O exchangeable]. MS (m/z): 529 [M^+] (0.3), 139 (100). Anal. Calcd. For $C_{25}H_{16}ClN_7O_3S$: C, 56.66; H, 3.04; N, 18.50. Found: C, 56.42; H, 3.18; N, 18.36.

*4-(2-(2-Chlorophenyl)-9-oxo-[1,2,4]triazolo[5,1-b]quinazolin-3(9H)-yl)-N-(4-methylpyrimidin-2-yl) benzenesulfonamide (**17**)* Yield, 75 %; m.p. 121–123 °C; IR (KBr, cm^{-1}): 3424(NH), 3076 (CH-arom.), 2950, 2858 (CH-aliph.), 1690 (C=O), 1312, 1105 (SO_2), 750 (C–Cl). ^{1}H-NMR in (DMSO-d_6) δ: 2.4 [s, 3H, CH_3], 6.7–8.1 [m, 14H, Ar–H + 2CH pyrimidine], 10.9 [s, 1H, SO_2NH, D_2O exchangeable]. Anal. Calcd. For $C_{26}H_{18}ClN_7O_3S$: C, 57.41; H, 3.34; N, 18.02. Found: C, 57.21; H, 3.13; N, 18.31.

*4-(2,10-Dioxo-2,3-dihydro-1H-[1,2,4]triazino[3,2-b]quinazolin-4(10H)-yl)-N-(pyrimidin-2-yl)benzenesulfonamide (**19**)*

A mixture of **5** (4.09 g, 0.01 mol) and ethyl chloroacetate (1.23 g, 0.01 mol) in methanol, containing sodium methoxide (0.054 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 **19**. Yield, 68 %; m.p. 201–203 °C; IR (KBr, cm^{-1}): 3410(NH)), 3039 (CH-arom.), 1681 (C=O), 1310, 1156 (SO_2). ^{1}H-NMR in (DMSO-d_6) δ: 4.3 [s, 2H, CH_2], 6.7–8.2 [m, 9H, Ar–H + CH pyrimidine], 8.6 [s, 2H, 2N=CH pyrimidine], 8.9 [s, 1H, NH, D_2O exchangeable], 11.6 [s, 1H, SO_2NH, D_2O exchangeable]. MS (m/z): 449 [M^+] (5.10), 200 (100). Anal. Calcd. For $C_{20}H_{15}N_7O_4S$: C, 53.45; H, 3.36; N, 21.82. Found: C, 53.31; H, 3.14; N, 21.60.

*Ethyl 9-oxo-3-(4-(N-pyrimidin-2-ylsulfamoyl)phenyl)-3,9-dihydro-[1,2,4]triazolo[5,1-b]quinazoline-2-carboxylate (**22**)*

A mixture of **5** (4.09 g, 0.01 mol) and diethyloxalate (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 and the solid obtained was recrystallized from dioxane to give **22**. Yield, 81 %; m.p. 80–82 °C; IR (KBr, cm^{-1}): 3433 (NH), 2940, 2862 (CH aliph.), 1710, 1677 (2 C=O), 1558 (C=N), 1342, 1157(SO_2). ^{1}H-NMR in (DMSO-d_6) δ: 1.3 [t, 3H, CH_3], 4.3 [q, 2H, CH_2], 6.7–8.1 [m, 9H, Ar–H + CH pyrimidine], 8.5 [s, 2H, 2N=CH pyrimidine], 11.2 [s, 1H, SO_2NH, D_2O exchangeable]. MS (m/z): 491 [M^+] (2.3), 341(100). Anal. Calcd. For $C_{22}H_{17}N_7O_5S$: C, 53.76; H, 3.49; N, 19.95. Found C, 53.52; H, 3.32; N, 19.75.

Antimicrobial screening

The antimicrobial activity screening of the newly synthesized compounds was undertaken using the agar cup diffusion (8 mm diameter) assay against the microbial organisms, listed in Table 1 (Singh and Wahi 2011). The agar media were inoculated with the test organism, and a solution of test compound 2 and 1 μmol/mL in DMSO. Ampicillin (2 μmol/mL and 1 μmol/mL), in addition to fluconazole (2 μmol/mL and 1 μmol/mL), were used as a reference for antibacterial and antifungal activity, respectively. The zones of inhibition were measured after 24 h incubation.

For determination of MIC value (Singh and Wahi 2011), by a serial plate dilution method, five milligrams of each test compounds were dissolved in 1 mL of dimethylsulfoxide (DMSO), separately, to prepare the stock solution. From the stock solution, serial dilutions were prepared. Thus, proper amounts of the different concentrations of compounds were pipetted on the blank disks, which were placed on the plates. The plates were incubated at 37 °C for 24 h. The minimum inhibitory concentrations (MICs), the lowest concentration (μmol/mL) of the test compound that resulted in no visible growth on the plates were recorded. DMSO was used as a solvent control to ensure that the solvent had no effect on the bacterial growth. The results of the antimicrobial activities are summarized in Table 1.

Ligand based pharmacophore modeling

LigandScout program (version 3.0) was used to derive the 3D chemical feature-based pharmacophores from the structural data of the synthesized compounds (Schemes 1, 2, 3,4), using the default settings (Friederike et al. 2002). Compounds (**5**, **7**, **10**, **12**, **13**–**15**, **19**, **22**) as training set were included in the modeling method. Prior to the generation of pharmacophore hypotheses, the training set compounds, which were converted to 3D structure, were used to generate diverse conformations. LigandScout program was used to generate the

Table 1 Minimal inhibitory concentration (MIC μM/mL) of the newly synthesized compounds

Compounds	*S. marcescens* (IMRU-70)	*P. mirabilis* (NTC-289)	*S. aureus* (NCTC-7447)	*B. cereus* (ATCC-14579)	*A. ochraceus* Wilhelm (AUCC-230)
3	0.50	0.25	0.75	0.19	0.50
4	0.82	0.27	0.55	0.21	0.27
5	0.31	0.23	0.92	0.31	0.43
6	0.73	0.37	0.97	0.97	0.97
7	0.42	0.56	0.28	0.85	0.56
8	NA	0.89	0.30	0.59	0.30
9	0.47	NA	NA	0.95	0.47
10	0.23	0.23	0.35	0.46	0.17
11	0.49	0.37	0.49	0.34	0.25
12	0.33	0.48	0.24	0.24	NA
13	0.34	0.91	0.45	NA	0.45
14	0.30	0.60	0.60	NA	0.22
15	0.84	1.12	0.39	0.56	1.12
16	0.95	0.71	NA	0.24	0.24
17	0.46	0.35	0.23	0.17	0.46
19	0.39	1.11	0.84	0.42	0.21
22	0.25	0.19	0.36	0.51	NA
Ampicillin	0.25	0.25	0.25	0.25	NA
Fluconazole	–	–	–	–	0.050

NA compounds having MIC value >1.5 μM

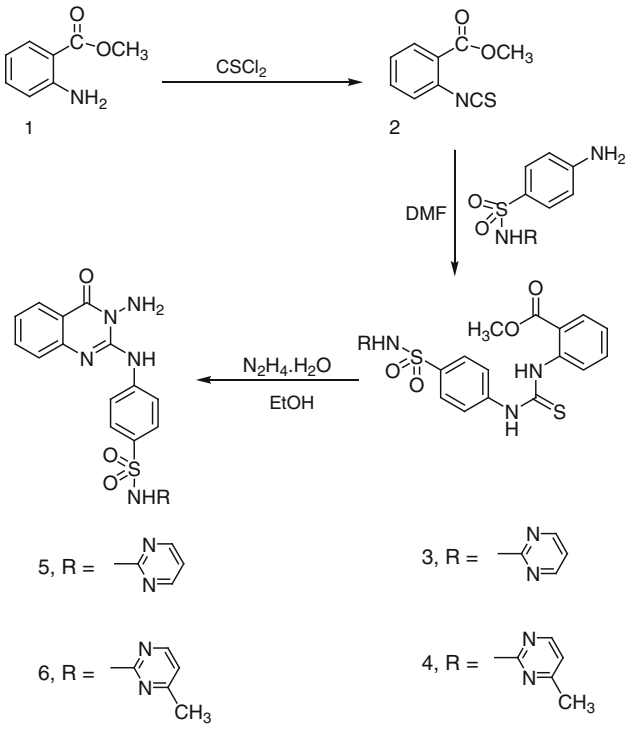
conformations, using the BEST conformation model generation method. Other parameters, like the maximum number of 250 conformers and an energy threshold value of 20 kcal/mol above the global energy minimum, were chosen during conformation generation. During the pharmacophore hypothesis generation, three pharmacophoric features, like hydrogen bond acceptor (HBA), hydrogen bond donor (HBD) and hydrophobic feature (HY) were selected to produce reliable pharmacophore model for our experimental results.

Pharmacophore validation

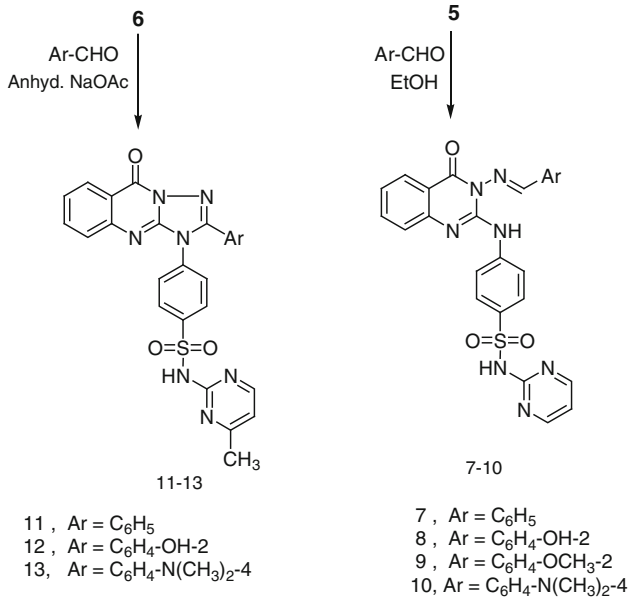
The generated pharmacophore hypothesis was validated using a test set, and leave-one-out methods.

Pharmacophore validation using test set

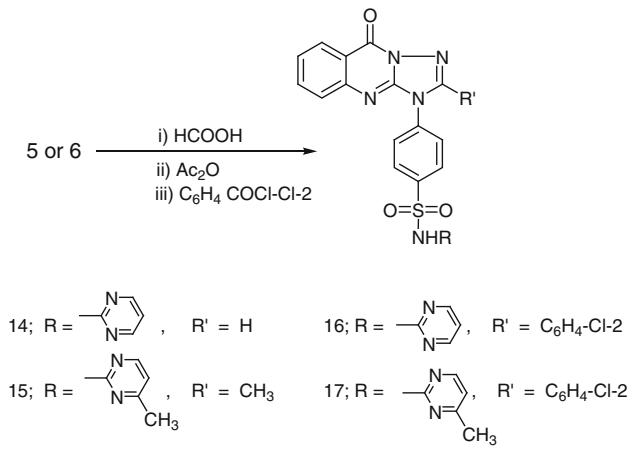
Compounds (**3**, **4**, **6**, **8**, **11**, **17**) were selected as a test set. This method is used to elucidate whether the generated pharmacophore hypothesis is proficient to predict the activities of compounds other than the training set and classify them correctly in their activity scale. The conformation generation for the test set compounds was carried out in a similar way, like the training set compounds using BEST conformation analysis algorithm, implemented within the LigandScout program with setting values, as same as those used with the training set. The compounds associated with their conformations were subsequently carried out for pharmacophore mapping (Friederike et al. 2002).



Scheme 1 Synthetic pathway of 3-aminoquinazolines bearing sulfonamide moiety



Scheme 2 Synthetic pathway of 3-benzylidinylaminoquinazolines and triazoloquinazolines having sulfonamide moiety



Scheme 3 Synthetic pathway of triazoloquinazolines containing sulfonamide moiety

Pharmacophore validation using leave-one-out

The pharmacophore hypothesis is cross validated by the leave-one-out method. In this method, one compound is left in the generation of 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 (Inte:ligand GmbH, Vienna, Austria) (Wolber and Langer 2005). This test is performed to verify whether the correlation coefficient of the training set compounds is strongly dependent on one particular compound or not (John et al. 2011).

Results

Chemistry

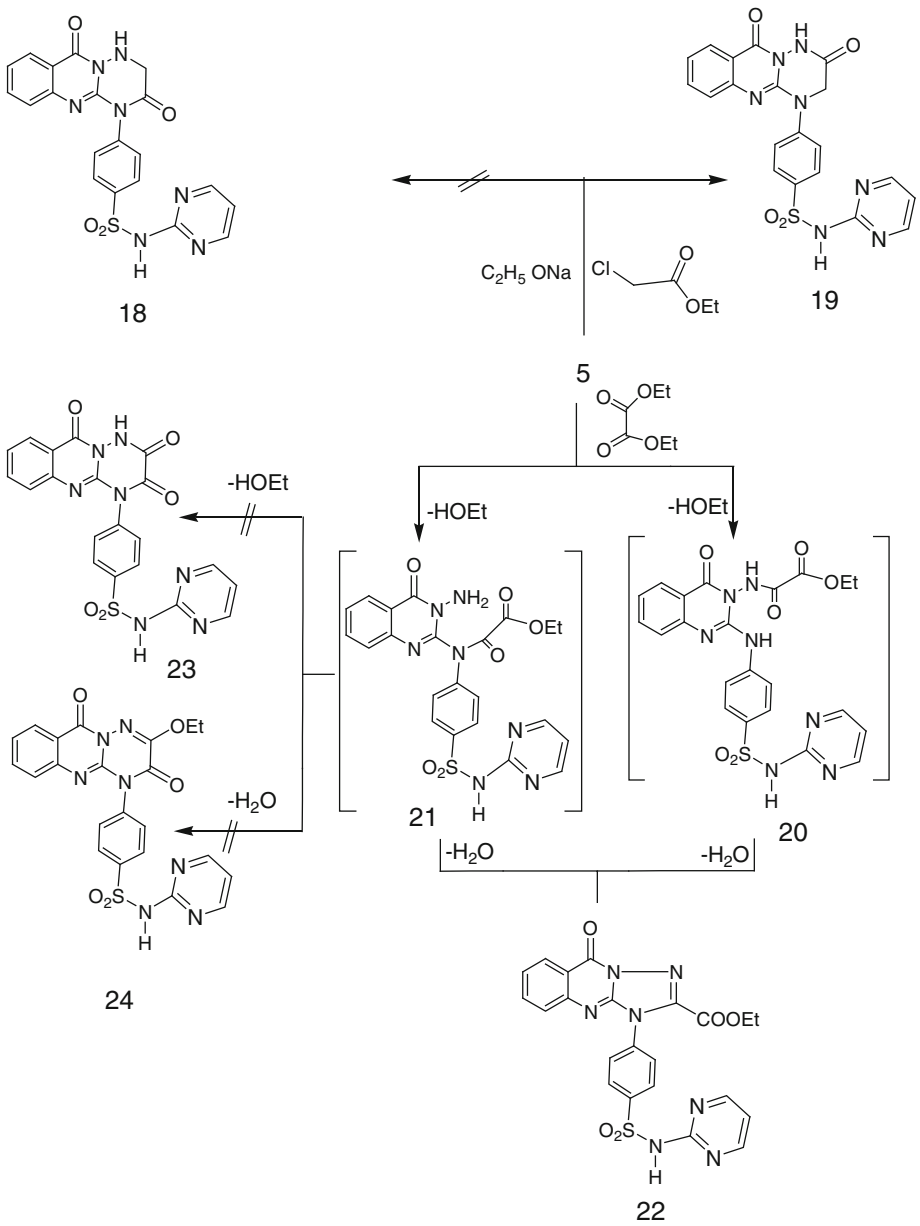
As a part of a program aimed at the synthesis of novel quinazolines, triazoloquinazolines and triazinoquinazoline, bearing a biologically active sulfonamide moiety, which could be useful for biological screening, we have investigated the possible utility of methyl 2-isothiocyanatobenzoate **2** (Guccion et al. 1995) to react with sulfadiazine or sulfamerazine in dimethylformamide, at room temperature, to give thioureido derivatives **3**, **4**, respectively, in high yield Scheme 1. Structure of compounds **3**, **4** was established based on the elemental and spectral data.

Compounds **3**, **4** allowed a fruitful, one step synthesis of several heterocyclic rings via their reaction with hydrazine hydrate. The formation of N-amino derivatives **5** and **6** proceeded via the elimination of one molecule of H_2S, followed by intramolecular cyclization. IR spectra exhibited the presence of characteristic bands for N-amino group. ^{1}H NMR spectra revealed the presence of a singlet at 5.8 and 5.6 ppm, due to N-amino group. Mass spectrum of compound **5** revealed a molecular ion peak m/z at 409, with a base peak m/z at 228. Schiff's bases **7–10** were obtained by the reaction of **5** with the corresponding aromatic aldehydes, Scheme 2. Upon a reaction of compound **6** with aromatic aldehydes in acetic acid containing fused sodium acetate yielded the corresponding triazoloquinazoline derivatives **11–13**, respectively (Scheme 2). Triazoloquinazolines **14–17** were prepared by a reaction of N-amino derivatives **5**, **6** with one carbon cyclizing reagent, namely formic acid, acetic anhydride and/or 2-chlorobenzoylchloride (Scheme 3). Reaction of compound **5**, with ethyl chloroacetate in presence of sodium methoxide, furnished the triazinoquinazoline **19**, rather than its isomeric structure **18** (Scheme 4). Interaction of compound **5** with diethyloxalate gave a triazoloquinazoline derivative **22**, which was confirmed by its elemental analysis, ^{1}H-NMR and mass spectral data. These results are in agreement with the method previously reported (Fahmi et al. 1994). ^{1}H-NMR spectrum indicated the presence of a triplet at 1.3 ppm and a quartet at 4.3 ppm assigned to ester moiety. Mass spectrum revealed a molecular ion peak m/z at 491, with a base peak at 172.

Antimicrobial screening

Table 1 lists the screening results of the tested compounds against the Gram-negative bacteria, *Serratia marcescens*, *Proteus mirabilis*, and the Gram-positive bacteria, *Staphylococcus aureus* and *Bacillus cereus*, in addition to the pathogenic fungi, *Aspergillus ochraceus* Wilhelm.

Scheme 4 Synthetic pathway of triazinoquinazoline carrying sulfonamide moiety



Discussion

Chemistry

IR spectra of compounds **3** and **4** revealed the absence of NCS band and presence of characteristic bands for NH, CH-aliphatic, SO_2, C=S. ^{1}H NMR spectra of 3 and 4 indicated the presence of a singlet at 3.9 due to the methoxy group and a singlet at 10.9 and 11.0 corresponding to SO_2NH. Mass spectrum of compound **3** showed a molecular ion peak m/z at 443 with a base peak m/z at 175.

The structure of compounds **7**–**10** was elucidated based on the elemental analysis and spectral data. The IR spectra of **7**–**10** revealed the absence of NH_2 bands. ^{1}H NMR spectra exhibited the disappearance of signals NH_2 and appearance of signals of benzylidine N=CH protons at 9.3–9.9 ppm. Their mass spectra were in agreement with their molecular weights. The structure of **11**–**13** was established on the bases of elemental analysis and spectral data. IR spectra showed the absence of NH_2 bands. ^{1}H NMR spectra revealed the disappearance of NH_2 signals and presence of signals at 10.2 ppm, corresponding to the phenolic OH group in compound **12** and 2.9 ppm due to dimethylamino group in compound **13**. Their mass spectra are in agreement with their molecular weights. IR spectra showed the absence of NH_2 bands. ^{1}H NMR spectrum of compound **15** revealed the disappearance of NH_2 signal and presence of a singlet at 1.3 ppm, due to a methyl group at triazole ring. Mass spectra were in accordance with their molecular weights. Structure **19** was suggested rather than

structure **18**, based on the assumption that the reaction basic condition allowed it to proceed through the formation of sodium salt on the less basic NH, and elimination of sodium chloride, followed by cyclization (Liu et al. 1992). In addition, the IR spectrum of isolated product showed a (C=O) band at 1681 cm^{-1}, which was at less frequency than that expected for structure **18**. Further evidence was the ^{1}H-NMR spectrum, which showed a singlet at 4.3 ppm for the methylene protons. Its mass spectrum showed a molecular ion peak m/z at 449, with a base peak at 200.

^{1}H-NMR spectrum of compound **22** indicated the presence of a triplet at 1.3 ppm and a quartet at 4.3 ppm assigned to ester moiety. Mass spectrum revealed a molecular ion peak m/z at 491, with a base peak at 172. These results are in agreement with the method previously reported (Fahmi et al. 1994).

Antimicrobial screening

Antimicrobial screening revealed that compounds **5**, **10**, **22** were found slightly more potent or equipotent to ampicillin against *S. marcescens.* Also, compounds **3**, **4**, **5**, **10**, **22** were found slightly more potent, or equipotent to ampicillin against *P. mirabilis*. In addition, compounds **12**, **17** were found slightly more potent or equipotent to ampicillin against *S. aureus*.

Compared with an antimicrobial activity of ampicillin against *B. cereus*, compounds **3**, **4**, **12**, **17** were more potent, while compounds **5**–**11**, **15**–**22** were less potent, and compounds **13**, **14** were inactive. All the compounds were found weak active against *A. ochraceus* Wilhelm, compared with that of the fluconazole.

Pharmacophore modeling

It was reported that there are bioisosterism between the pharmacophoric group of ampicillin and the functional group of the sulfonamide compounds (Fig. 1) (Chohan et al. 2010). Also, it was hypothesized that the difference in charges between the two heteroatoms of the same dipolar pharmacophore site ($X\delta^-\ldots Y\delta^+$) may facilitate the inhibition of bacteria, more than viruses.

In view of these findings and well obtained in vitro results, it was thought worthy to search for supportive coordination, between in silico studies with in vitro antibacterial results. The antibacterial results of the synthesized compounds (Table 1), against the Gram-positive bacteria *S. Aureus*, were selected for pharmacophore modelling study. The 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 (**5**, **7**, **10**, **12**–**15**, **19**, **22**) and test set compounds (**3**, **4**, **6**, **8**, **11**, **17**). On the assumption that the active compounds bind in a similar fashion at the active site, we employed the LigandScout program (Wolber and Langer 2005) to evaluate the common features essential for the activity, and the hypothetical geometries adopted by these ligands in their most active forms. Thus, these compounds were submitted for pharmacophore model generation based on the shared chemical features. Conformation generation within 20 kcal/mol energy range were generated and submitted to the alignment procedure.

The successful pharmacophore run resulted in the generation of 10 models (model-1: model-10, Table 2). Based on its highest rank score and its mapping into all training set molecules, model-1 was considered to be the best

Table 2 Summary of the generated pharmacophores of the antibacterial activity against *S. aureus*

Hypothesis	Features	Rank score
Model-1	HHHHAAAD	0.9121
Model-2	HHHHAAAD	0.9053
Model-3	HHHHAAAD	0.9029
Model-4	HHHHAAAD	0.9009
Model-5	HHHHHAAAD	0.8902
Model-6	HHHHHAAAD	0.8872
Model-7	HHHHHAAAD	0.8862
Model-8	HHHHHAAAD	0.8680
Model-9	HHHHHAAAD	0.8589
Model-10	HHHHHAAAD	0.8484

H hydrophobic, *A* hydrogen bond acceptor, *D* hydrogen bond donor

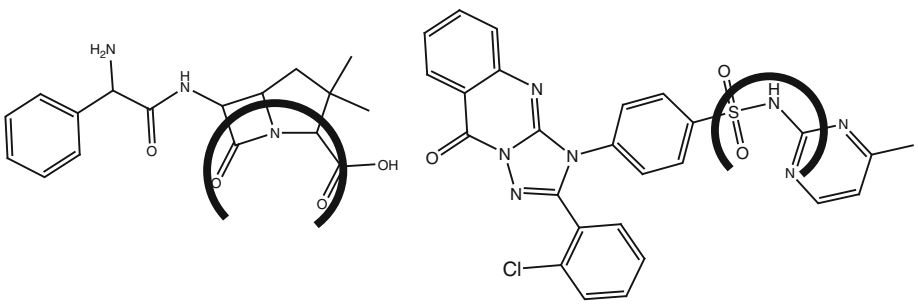


Fig. 1 Common potential antibacterial pharmacophores (delineated with *bold black line*) **a** ampicillin and **b** representative compound (**17**) of the synthesized analogues

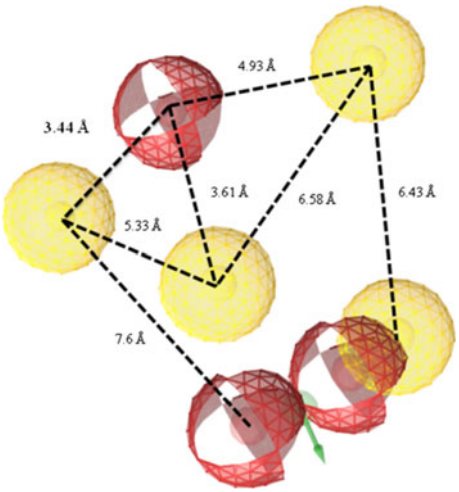


Fig. 2 Proposed pharmacophore model of growth inhibition activity against *S. aureus* (*red* HBA; *yellow* hydrophobic; *green* HBD)

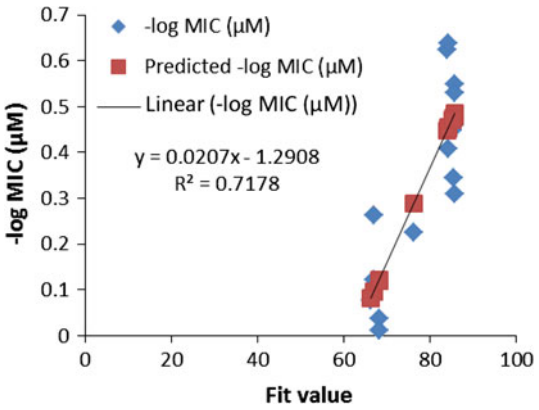


Fig. 3 A chart representing the negative logarithm of the minimal inhibitor concentration in μmol (dependent value) against LigandScout program output fit value (independent value)

Table 3 Output for model-1 mapping and predictive model *S. aureus*

Compounds	MIC (μM)	−log MIC (μM)	Fit value	Pred. −log MIC (μM) −log	Residual
3	0.75	0.122	66.96	0.096	0.026
4	0.55	0.262	66.98	0.096	0.166
5	0.92	0.038	68.2	0.121	−0.083
6	0.97	0.011	68.15	0.120	−0.109
7	0.28	0.549	85.7	0.484	0.065
8	0.30	0.529	85.76	0.485	0.044
10	0.35	0.459	85.62	0.482	−0.023
11	0.49	0.309	85.68	0.483	−0.174
12	0.24	0.623	83.98	0.448	0.175
13	0.45	0.344	85.36	0.477	−0.133
14	0.60	0.224	76.22	0.287	−0.063
15	0.39	0.407	84.32	0.455	−0.048
17	0.23	0.638	84.24	0.453	0.185
19	0.84	0.078	66.27	0.081	−0.003
22	0.36	0.448	85.16	0.472	−0.024

hypothesis, statistically. Such model was selected for further investigation and analysis. The top-ranked chemical feature-based pharmacophore model (model-1), identified in this study, is shown in Fig. 2. This pharmacophore model contains eight chemical features: four hydrophobes, three HBA and one HBD.

All synthesized compounds were mapped onto model-1, with scoring the orientation of a mapped compound within the hypothesis features, using a "fit value" score. As a quick and primary validation of model-1, mapping of the compounds found to show a good agreement between the fit value and the biological activity (Table 3). Initial investigation of the results shown in Table 3 reveals a good correlation between the fit value and the biological activity of the screened compounds. The highly active compounds show a range of fit value of (85.76–83.98), while the rest compounds varied from moderate to weak activity showed a range of fit value of 76.22–66.27. This initial correlation encouraged us to generate a linear model, based on "fit value" to predict the biological activity of the compounds understudy. The generated model (Eq. 1) showed very good statistics and was used successfully to calculate the activity of the tested compounds (Fig. 3; Table 3).

$$-\log\ \mathrm{MIC}\ (\mu\mathrm{M}) = 0.0207\ \text{fit value} - 1.2908$$
$$n = 15,\ \text{st error} = 0.115,\ R = 0.847,\ R^2 = 0.718,$$

where n is the number of compounds and R is the multiple correlation coefficients.

Figure 4a, b shows as an example of the alignment of the hypothesis model with the most active compounds **12** and **17**, respectively, and Fig. 5a–c shows an example as the alignment of the hypothesis model with the weakly active compounds **3**, **4** and **19** respectively. A closer look at the mapped structures (Fig. 5a, b) reveals the importance of certain structural features for activity. The aryl-substituted triazoloquinazolinone scaffold is thought to be critical for activity where the opening of the triazine ring or unsubstituted triazine affect badly on the fit value of the compounds to the pharmacophore model, which is also reflected on its experimental weak activity. Also, the slight displacement of the benzene ring of sulfanilamide moiety away from the hydrophobic pharmacophore center, in addition to the absence of hydrophobic substituent on the fused triazine ring (Fig. 5c) can partially explain their weak activity. The rest feature that is common for all compounds is the two sulfonyl HBA feature and the NH HBD of sulfonamide moiety.

Fig. 4 **a** Best aligned pose of compound **12** (MIC 0.24 μmol) superposed with the query (model-1). **b** Best aligned pose of compound **17** (MIC 0.23 μmol) fitted inadequately with the query (model-1)

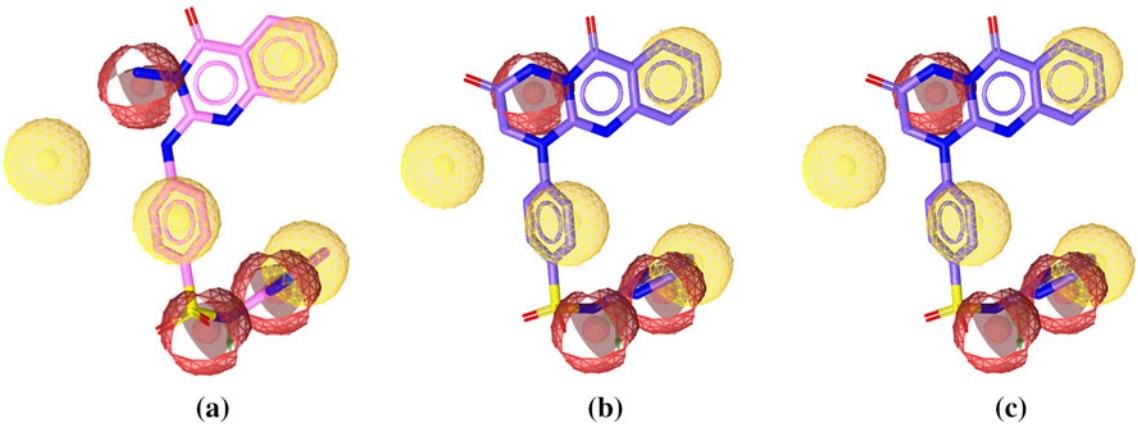


Fig. 5 **a** Best aligned pose of the less active compound **5** (MIC 0.92 μmol) overlaid onto the pharmacophore model (model-1). **b** Best aligned pose of the less active compound **6** (MIC 0.97 μmol) overlaid onto the pharmacophore model (model-1). **c** Best aligned pose of the less active compound **19** (MIC 0.84 μmol) overlaid onto the pharmacophore model (model-1)

Further structural modifications need to be tried at the sulfadiazine moiety before we can discuss the importance of such feature for activity. Aromatic ring found to be oriented into the HY, and its plane is perpendicular to that of the triazoloquinazoline ring. However, there is no evidence that the size of such group has a determinant effect on the antibacterial activity. Further studies are in progress in our laboratory, and are focused on the study of the modification effect of the benzene ring of the sulfadiazine group by replacement with substituted or fused benzene ring or even its replacement with heterocyclic ring or aliphatic chain of different lengths, in addition to the modification of the sulfonamide group by replacement by other carbonyl moieties.

Conclusion

A novel series of triazoloquinazolines and triazinoquinazoline bearing sulfonamide moiety were synthesized and biologically evaluated. Compounds **4**, **7**, **8**, **10**–**13**, **15**, **17**, **19** and **22** showed promising antibacterial activities, compared with the reference drug. Pharmacophore modelling study of the antibacterial effect of the synthesized compounds against *S. aureus* strains revealed that compounds **12** and **17**, slightly more potent than ampicillin, were able to effectively satisfy the proposed common feature sites using the energy accessible conformers ($E_{conf} < 20$ kcal/mol).

Acknowledgments The authors are grateful to the sponsorship of the Research Centre, College of Pharmacy and the Deanship of Scientific Research, King Saud University, Riyadh 11451, Saudi Arabia.

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