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NOVEL PYRROLOPYRIMIDINES AND TRIAZOLOPYRROLOPYRIMIDINES CARRYING A BIOLOGICALLY ACTIVE SULFONAMIDE MOIETIES AS ANTICANCER AGENTS

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Abstract: A new series of pyrroles **5**, **6**, pyrrolopyrimidines **8**, **11**–**14**, **16**–**29**, triazolo-pyrrolopyrimidines **9**, **10** and **15** carrying a biologically active sulfonamide moities were synthesized using 2-amino-3-cyano-4-(4-bromophenyl)pyrrole **5** as a strategic starting material. The structures of the prepared compounds were confirmed by elemental analyses, IR, ^{1}H-NMR and ^{13}C-NMR data. All of the synthesized compounds showed promising anticancer activity against breast cancer cell line (MCF7) compared to doxorubicin as reference drug, especially compounds **5**–**17**, **21**–**24** and **28** with better IC_{50} than that of doxorubicin. In order to suggest the mechanism of action of their cytotoxic activities, molecular docking on the active site of c-Src was done and good results were obtained.

Keywords: anticancer, pyrrolopyrimidines, triazolopyrrolopyrimidines, sulfonamides

The design as well as identification of some new molecules for the treatment of diseases such as cancer is an important undertaking in medicinal chemistry research. The pyrrole, pyrrolopyrimidine and triazolopyrrolopyrimidine derivatives have been known to posses wide spectrum of biological properties specially anticancer activity (1–10). Pyrrolo[2,3-d]pyrimidines have aroused recent attention from chemical and biological points of view, since they have useful properties as antimetabolites in purine biochemical reactions (11). Several mechanisms of action explaining their anticancer activity include protein kinases inhibition such as c-Src, Akt and EGFR (12–14). On the other hand, sulfonamides have recently been reported to show potent anticancer activity (15–17). To explore the synergistic effect resulting from combining pyrrole, pyrrolopyrimidine and triazolopyrrolopyrimidines with sulfonamide moiety, the present work describes the synthesis and molecular docking of these novel derivatives on the active site of c-Src enzyme hoping to discover novel anticancer agents and suggest a mechanism of action for their cytotoxic activities.

EXPERIMENTAL

Chemistry

Melting points (^{o}C) were determined in open capillaries on a Gallenkamp melting point apparatus (Sanyo Gallenkamp, Southborough, UK) and were uncorrected. Precoated silica gel plates (silica gel 0.25 mm, 60 GF254, Merck, Germany) were used for thin layer chromatography, dichloromethane/methanol (9.5 : 0.5, v/v) mixture was used as a developing solvent system and the spots were visualized by ultraviolet light and/or iodine. Infrared spectra were recorded in KBr discs using IR-470 Shimadzu spectrometer (Shimadzu, Tokyo, Japan). NMR spectra in DMSO-d_6 were recorded on Bruker Ac-500 UltraShield NMR spectrometer (Bruker, Flawil, Switzerland; δ, ppm) at 500 MHz, using TMS as internal standard and peak multiplicities are designed as follows: s, singlet; d, doublet; t, triplet; m, multiplet. Elemental analyses were performed on Carlo Erba 1108 Elemental Analyzer (Heraeus, Hanau, Germany). All compounds gave results within ± 0.4% of the theoretical values.

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4-(2-(4-Bromophenyl)-2- oxoethylamino)benzenesulfonamide (3)

A mixture of sulfanilamide **1** (1.72 g, 0.01 mol) and 4-bromophenacylbromide **2** (2.77 g, 0.01 mol) was refluxed in *N*,*N*'-dimethylformamide (20 mL) in the presence of catalytic amount of triethylamine for 6 h. The solid obtained was filtered off and recrystallized from ethanol to give **3**. Yield 89%, m.p. 232.6°C. IR (KBr, cm^{-1}): 3358, 3255 (NH, NH_2), 3100 (CH arom.), 2970, 2863 (CH aliph.), 1685 (C=O), 1381, 1157 (SO_2).^{1}H-NMR (DMSO-d_6, δ, ppm): 4.7 (s, 2H, CH_2), 6.6 (s, 1H, NH, D_2O exchangeable), 6.7, 7.5 (2d, 4H, Ar-H, AB system, *J* = 7.1 Hz), 7.8, 8.0 (2d, 4H, Ar-H, AB system, *J* = 6.9 Hz).^{13}C-NMR (DMSO-d_6, δ, ppm): 49.4, 111.4 (2), 127.1, 127.7, 129.9 (2), 130.7 (2), 131.8 (2), 133.9, 150.8, 195.3. Analysis: calcd. for $C_{14}H_{13}BrN_2O_3S$ (369.23): C, 45.54; H, 3.55; N, 7.59%; found: C, 45.54; H, 3.31; N, 7.24%.

4-(2-Amino-4-(4-bromophenyl)-3-cyano-1H-pyrrol-1-yl)benzenesulfonamide (5)

A mixture of compound **3** (3.69 g, 0.01 mol) and malononitrile (0.66 g, 0.01 mol) in ethanol (20 mL) containing sodium ethoxide (0.5 g) was refluxed for 8 h. The reaction mixture was cooled and acidified with dil. HCl. The solid obtained was filtered off and recrystallized from dioxane to give **5**. Yield 78%, m.p. 221.2°C. IR (KBr, cm^{-1}): 3419, 3344, 3238 (NH_2), 3095 (CH arom.), 2187 (C=N), 1635 (C=N), 1342, 1176 (SO_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 6.1 (s, 2H, NH_2, D_2O exchangeable), 7.0 (s, 1H, CH pyrrole), 7.5–7.9 (m, 10H, Ar-H + SO_2NH_2).^{13}C-NMR (DMSO-d_6, δ, ppm): 70.5, 113.5, 117.5, 119.5, 121.3 (2), 125.2, 127.3, 131.2 (2), 131.6 (2), 132.3 (2), 139.5, 142.9, 148.5. Analysis: calcd. for $C_{17}H_{13}BrN_4O_2S$ (417.28): C, 48.93; H, 3.14; N, 13.43%; found: C, 48.71; H, 3.50; N, 13.16%.

Ethyl N-4-(4-bromophenyl)-3-cyano-1-(4-sulfamoylphenyl)-1H-pyrrol-2-ylformimidate (6)

A mixture of compound **5** (4.17 g, 0.01 mol) and triethylorthoformate (20 mL) was refluxed for 6 h. The reaction mixture was cooled and then poured onto ice/water. The formed residue was recrystallized from ethanol to give **6**. Yield 78%, m.p. 160.2°C. IR (KBr, cm^{-1}): 3151, 3136 (NH_2), 2987, 2865 (CH aliph.), 2206 (C≡N), 1629 (C=N), 1354, 1155 (SO_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.0 (t, 3H, CH_3), 4.3 (q, 2H, CH_2), 7.6–7.9 (m, 8H, Ar-H), 8.1 (s, 1H, CH pyrrole), 8.5 (s, 2H, SO_2NH_2, D_2O exchangeable), 8.7 (s, 1H, N=CH). ^{13}C-NMR (DMSO-d_6, δ, ppm): 13.6, 63.6, 79.1, 116.8, 117.4, 120.3, 122.8 (2), 125.4, 127.7, 127.8 (2), 131.5 (2), 131.8 (2), 138.5, 140.6, 145.3, 161.9. Analysis: calcd. for $C_{20}H_{17}BrN_4O_3S$ (473.34): C, 50.75; H, 3.62; N, 11.84%; found: C, 50.48; H, 3.91; N, 11.54%.

4-(3-Amino-5-(4-bromophenyl)-4-imino-3,4-dihydropyrrolo[2,3-d]pyrimidin-7-yl)benzenesulfonamide (8)

A mixture of **6** (4.73 g, 0.01 mol) and hydrazine hydrate (1.0 g, 0.02 mol) was stirred in ethanol (20 mL) at room temperature for 1 h, the solid formed was filtered and recrystallized from ethanol to give **8**. Yield 91%, m.p. 184.5°C. IR (KBr, cm^{-1}): 3294, 3230, 3150 (NH, NH_2), 1639 (C=N), 1375, 1163 (SO_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 5.6 (s, 2H, NH_2, D_2O exchangeable), 6.9 (s, 1H, CH pyrrole), 7.4–7.9 (m, 10H, Ar-H + SO_2NH_2), 8.0 (s, 1H, CH pyrimidine), 8.1 (s, 1H, NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6, δ, ppm): 103.7, 119.8, 120.1, 121.1 (2), 124.0, 126.7, 130.8 (2), 131.1 (2), 132.9 (2), 139.5, 141.9, 142.8, 147.6, 152.9. Analysis: calcd. for $C_{18}H_{15}BrN_6O_2S$ (459.32): C, 47.07; H, 3.29; N, 18.30%; found: C, 47.39; H, 3.51; N, 18.64%.

4-(9-(4-Bromophenyl)-7H-pyrrolo[3,2-e][1,2,4]triazolo[1.5-c]pyrimidin-7-yl)benzenesulfonamide (9)

A solution of **8** (4.59 g, 0.01 mol) in formic acid (20 mL) was refluxed for 6 h, and the reactoin mixture was then concentrated. The separated crystals were recrystallized from ethanol to give **9**. Yield 82%, m.p. 347.2°C. IR (KBr, cm^{-1}): 3402, 3267 (NH_2), 3082 (CH arom.), 1620 (C=N), 1370, 1165 (SO_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.5 (s, 1H, CH pyrrole), 7.6–8.2 (m, 10H, Ar-H + SO_2NH_2), 8.6 (s, 1H, CH triazole), 9.6 (s, 1H, CH pyrimidine). ^{13}C-NMR (DMSO-d_6, δ, ppm): 104.1, 117.1, 120.0 (2), 124.7, 124.9 (2), 126.8, 129.2 (2), 131.3 (2), 131.7, 136.5, 139.2, 141.3, 142.7, 148.4, 154.3. Analysis: calcd. for $C_{19}H_{13}BrN_6O_2S$ (469.31): C, 48.62; H, 2.79; N, 17.91%; found: C, 48.96; H, 2.49; N, 17.59%.

4-(9-(4-Bromophenyl)-2-(cyanomethyl)-7H-pyrrolo[3,2-e][1,2,4]triazolo[1,5-c]pyrimidin-7-yl)benzenesulfonamide (10)

A mixture of **8** (4.59 g, 0.01 mol) and ethylcyanoacetate (1.13 g, 0.01 mol) was refluxed for 10 h, in ethanol (20 mL) containing sodium ethoxide (0.23 g, 0.01 mol), the reaction mixture was then acidified with dil. HCl. The obtained solid was recrystallized from dioxane to give **10**. Yield 79%,

m.p. 301.2°C. IR (KBr, cm^{-1}): 3344, 3251 (NH_2), 3062 (CH arom.), 2922, 2861 (CH aliph.), 2270 (C≡N), 1625 (C=N), 1365, 1161 (SO_2).^{1}H-NMR (DMSO-d_6, δ, ppm): 4.5 (s, 2H, CH_2), 7.5 (s, 1H, CH pyrrole), 7.6–8.3 (m, 10H, Ar-H + SO_2NH_2), 9.6 (s, 1H, CH pyrimidine). ^{13}C-NMR (DMSO-d_6, δ, ppm): 18.0, 109.1, 117.1, 120.1, 124.6 (2), 125.0, 126.8 (2), 129.3, 131.3 (2), 131.6 (2), 136.3, 139.1, 141.5, 142.7, 149.4, 158.4, 164.2. Analysis: calcd. for $C_{21}H_{14}BrN_7O_2S$ (508.35): C, 49.62; H, 2.78; N, 19.29%; found: C, 49.38; H, 2.46; N, 19.58%.

N-(5-(4-bromophenyl)-4-imino-7-(4-sulfamoylphenyl)-4H-pyrrolo[2,3-d]pyrimidin-3-(7H)-yl)acetamide (11)

A solution of **8** (4.59 g, 0.01 mol) in acetic anhydride (20 mL) was refluxed for 2 h. The solid obtained was recrystallized from acetic acid to give **11**. Yield 68%, m.p. 337.5°C. IR (KBr, cm^{-1}): 3420, 3280 (NH, NH_2), 3082 (CH arom.), 2940, 2860 (CH aliph.), 1710 (C=O), 1625 (C=N), 1348, 1161 (SO_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.1 (s, 3H, $COCH_3$), 7.4 (s, 1H, CH pyrrole), 7.6–8.4 (m, 10H, Ar-H + SO_2NH_2), 8.5 (s, 1H, CH pyrimidine), 9.6 (s, 1H, NH imino, D_2O exchangable), 12.2 (s, 1H, NHCO, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6, δ, ppm): 23.2, 103.6, 117.2, 120.0, 124.2 (2), 124.3, 128.8, 129.2 (2), 131.5 (2), 137.3 (2), 140.7, 141.2, 148.8, 163.6, 168.9, 172.0. Analysis: calcd. for $C_{20}H_{17}BrN_6O_3S$ (501.36): C, 47.91; H, 3.42; N, 16.76%; found: C, 47.66; H, 3.68; N, 16.50%.

General procedure for the synthesis of compounds (12–14)

A mixture of compound **8** (4.59 g, 0.01 mol) and benzoyl chloride derivatives (0.01 mol) in pyridine (20 mL) was refluxed for 6 h. The reaction mixture was poured onto ice water. The formed residue was recrystallized from dioxane to give **12–14**, respectively.

N-(5-(4-bromophenyl)-4-imino-7-(4-sulfamoylphenyl)-4H-pyrrolo[2,3-d]pyrimidin-3-(7H)-yl)benzamide (12)

Yield 68%, m.p. 247.8°C. IR (KBr, cm^{-1}): 3217, 3186, 3130 (NH, NH_2), 3093 (CH arom.), 1683 (C=O), 1591 (C=N), 1393, 1174 (SO_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.4 (s, 1H, CH pyrrole), 7.5–8.4 (m, 15H, Ar-H + SO_2NH_2), 9.5 (s, 1H, CH pyrimidine), 9.6 (s, 1H, NH imino, D_2O exchangeable), 12.8 (s, 1H, NHCO, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6, δ, ppm): 104.8, 117.6, 120.6, 123.7 (2), 124.5, 126.5, 127.1 (2), 128.6 (2), 129.7 (2), 131.4 (2), 132.8 (2), 133.2, 133.4, 136.1, 136.4, 143.7, 162.9, 165.5, 167.2. Analysis: calcd. for $C_{25}H_{19}BrN_6O_3S$ (563.43): C, 53.29; H, 3.40; N, 14.92% found: C, 53.50; H, 3. 68; N, 14.66%.

N-(5-(4-bromophenyl)-4-imino-7-(4-sulfamoylphenyl)-4H-pyrrolo[2,3-d]pyrimidin-3-(7H)-yl)-4-chlorobenzamide (13)

Yield 71%, m.p. 258.6°C. IR (KBr, cm^{-1}): 3220, 3165 (NH, NH_2), 3056 (CH arom.), 1683 (C=O), 1591 (C=N), 1391, 1176 (SO_2), 761 (C-Cl). ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.5 (s, 1H, CH pyrrole), 7.6–7.9 (m, 14H, Ar-H + SO_2NH_2), 8.0 (s, 1H, CH pyrimidine), 9.7 (s, 1H, NH imino, D_2O exchangeable), 12.9 (s, 1H, NHCO, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6, δ, ppm): 109.8, 119.3, 121.4, 123.3 (2), 125.2, 128.8, 129.5 (2), 130.3 (2), 130.8 (2) 131.0 (2), 134.0 (2), 134.9, 136.1, 136.9, 137.7, 146.1, 161.7, 165.1, 166.4. Analysis: calcd. for $C_{25}H_{18}BrClN_6O_3S$ (596.00): C, 50.22; H, 3.03; N, 14.06%; found: C, 50.01; H, 3.28; N, 14.36%.

N-(5-(4-bromophenyl)-4-imino-7-(4-sulfamoylphenyl)-4H-pyrrolo[2,3-d]pyrimidin-3-(7H)-yl)-3,4,5-trimethoxybenzamide (14)

Yield 73%, m.p. 260.8°C. IR (KBr, cm^{-1}): 3323, 3300, 3277 (NH, NH_2), 3081 (CH arom.), 2946, 2871 (CH aliph.), 1656 (C=O), 1593 (C=N), 1391, 1163 (SO_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.7, 3.9 (2s, 9H, $3OCH_3$), 7.0 (s, 1H, CH pyrrole), 7.5–8.4 (m, 12H, Ar-H + SO_2NH_2), 8.5 (s, 1H, CH pyrimidine), 9.5 (s, 1H, NH imino, D_2O exchangeable), 12.9 (s, 1H, NHCO, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6, δ, ppm): 55.9 (2), 60.5, 103.7 (2), 108.3, 117.2, 119.3, 120.0 (2), 124.5, 125.6, 126.8 (2),129.3, 131.8 (2), 136.2 (2), 139.3, 141.3, 142.5, 142.6, 148.6 (2), 152.5, 155.1, 162.0. Analysis: calcd. for $C_{28}H_{25}BrN_6O_6S$ (652.07): C, 51.46; H, 3.86; N, 12.86%; found: C, 51.81; H, 3.57; N, 12.61%.

4-(9-(4-Bromophenyl)-2-thioxo-2H-pyrrolo[3,2-e][1,2,4]triazolo[1,5-c]pyrimidin-7(3H)-yl)benzenesulfonamide (15)

A mixture of **8** (4.59 g, 0.01 mol), carbon disulfide (2 mL) in pyridine (20 mL) was refluxed for 12 h, the reaction mixture was cooled and poured onto ice water. The separated solid was filtered off and recrystallized from acetic acid to give **15**. Yield 82%, m.p. 315.1°C. IR (KBr, cm^{-1}): 3346, 3311, 3248 (NH, NH_2), 3095 (CH arom.), 1581 (C=N), 1359, 1159 (SO_2), 1242 (C=S). ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.4 (s, 1H, CH pyrrole), 7.5–8.0 (m, 10H, Ar-H + SO_2NH_2), 9.6 (s, 1H, NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6, δ, ppm): 110.2, 117.0, 119.2, 121.9

(2), 124.6, 127.0, 129.9 (2), 130.3 (2), 132.2 (2), 138.0, 138.1, 142.8, 143.4, 153.3, 178.9. Analysis: calcd. for $C_{19}H_{13}BrN_6O_2S_2$ (501.38): C, 45.52; H, 2.61; N, 16.76%; found: C, 45.19; H, 2.38; N, 16.98%.

4-(5-(4-Bromophenyl)-4-imino-3-(propan-2-ylideneamino)-3H-pyrrolo[2,3-d]pyrimidin-7(4H)-yl)benzenesulfonamide (16)

A mixture of **8** (4.59 g, 0.01 mol) and dry acetone (30 mL) was refluxed for 18 h; on cooling the reaction mixture was concentrated and the product obtained was filtered and recrystallized from a mixture of acetic acid and ethanol (1 : 2, v/v) to give **16**. Yield 78%, m.p. 228.2°C. IR (KBr, cm^{-1}): 3365, 3246, 3176 (NH, NH_2), 3100 (CH arom.), 2972, 2927 (CH aliph.), 1595 (C=N), 1381, 1161 (SO_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.1 (s, 6H, $2CH_3$), 6.8 (s, 1H, CH pyrrole), 7.1–8.0 (m, 10H, Ar-H + SO_2NH_2), 8.1 (s, 1H, CH pyrimidine), 8.2 (s,1H, NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6, δ, ppm): 19.8, 27.1, 100.0, 118.8, 119.6, 124.0 (2), 124.4, 126.6, 129.7 (2), 131.2 (2), 132.2 (2), 140.2, 142.2, 147.3, 154.6, 166.2, 208.3. Analysis: calcd. for $C_{21}H_{19}BrN_6O_2S$ (499.38): C, 50.51; H, 3.83; N, 16.83%; found: C, 50.27; H, 3.59; N, 16.55%.

General procedure for the synthesis of compounds (17–19)

A mixture of **8** (4.59 g, 0.01 mol) and sulfonyl chloride derivatives (0.01 mol) in dry benzene (30 mL) containing pyridine (1 mL) was refluxed for 8 h. The solid obtained was recrystallized from ethanol to give **17–19**, respectively.

4-(5-(4-Bromophenyl)-4-imino-3-(sulfamoylphenyl)-3,4-dihydropyrrolo[2,3-d]pyrimidin-7-yl)benzenesulfonamide (17)

Yield 74%, m.p. 188.8°C. IR (KBr, cm^{-1}): 3340, 3219, 3219 (NH, NH_2), 3066 (CH arom.), 1618 (C=N), 1326, 1161 (SO_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.3 (s, 1H, CH pyrrole), 7.4–8.3 (m, 15H, Ar-H + SO_2NH_2), 8.5 (s, 1H, CH pyrimidine), 8.9 (s, 1H, NH imino, D_2O exchangeable), 12.7 (s, 1H, $NHSO_2$, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6, δ, ppm): 111.4, 119.2, 124.8, 125.4 (2), 125.6, 126.9, 127.6 (2), 128.3 (2), 128.9 (2), 130.3 (2), 130.6 (2), 131.6, 132.0, 142.6, 145.4, 148.2, 151.9, 152.4. Analysis: calcd. for $C_{24}H_{19}BrN_6O_4S_2$ (598.00): C, 48.08; H, 3.19; N, 14.02%; found: C, 47.79; H, 3.52; N, 14.32%.

4-(5-(4-Bromophenyl)-4-imino-3-(-sulfamoylphenyl)-3,4-dihydropyrrolo[2,3-d]pyrimi-din-7-yl)-4-methylbenzenesulfonamide (18)

Yield 69%, m.p. 324.6°C. IR (KBr, cm^{-1}): 3331, 3263, 3171 (NH, NH_2), 3084 (CH arom.), 2932, 2867 (CH aliph.), 1595 (C=N), 1332, 1172 (SO_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.2 (s, 3H, CH_3), 6.7 (s, 1H, CH pyrrole), 7.0–7.9 (m, 14H, Ar-H + SO_2NH_2), 8.0 (s, 1H, CH pyrimidine), 8.1 (s, 1H, NH imino, D_2O exchangeable), 8.6 (s, 1H, $NHSO_2$, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6, δ, ppm): 20.7, 100.0, 118.6, 121.4, 124.8 (2), 125.4, 126.8, 126.9 (2), 128.0 (2), 130.7 (2), 130.8 (2), 132.0 (2), 138.2, 142.9, 143.2, 145.1, 145.3, 147.5, 152.3. Analysis: calcd. for $C_{25}H_{21}BrN_6O_4S_2$ (613.51): C, 48.94; H, 3.45; N, 13.70%; found: C, 48.60; H, 3.71; N, 13.47%.

4-Bromo-N-(5-(4-bromophenyl)-4-imino-7-(4-sulfamoylphenyl)-4H- pyrrolo[2,3-d]pyrimidin-3-(7H)-yl)benzenesulfonamide (19)

Yield 62%, m.p. 296.6°C. IR (KBr, cm^{-1}): 3405, 3332, 3261 (NH, NH_2), 3084 (CH arom.), 1595 (C=N), 1384, 1174 (SO_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 6.6 (s, 1H, CH pyrrole), 7.4–8.0 (m, 14H, Ar-H + SO_2NH_2), 8.1 (s, 1H, CH pyrimidine), 8.6 (s, 1H, NH imino, D_2O exchangeable), 8.8 (s, 1H, $NHSO_2$, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6, δ, ppm): 100.0, 118.6, 121.4, 121.6 (2), 124.8, 126.2, 126.9, 127.0 (2), 127.6 (2), 130.7 (2), 130.8 (2), 132.0 (2), 138.1, 143.3, 144.2, 145.1, 147.5, 152.4. Analysis: calcd. for $C_{24}H_{18}Br_2N_6O_4S_2$ (678.38): C, 42.49; H, 2.67; N, 12.39%; found: C, 42.76; H, 2.48; N, 12.08%.

General procedure for the synthesis of compounds (20 and 21)

A mixture of **8** (4.59 g, 0.01 mol) and isothiocyanate derivatives (0.01 mol) in absolute ethanol (20 mL) was refluxed for 6 h, the reaction mixture was concentrated and the separated crystals were recrystallized from dioxane to give **20** and **21**, respectively.

4-(5-(4-Bromophenyl)-3-(3-butylthioureido)-4-imino-3,4-dihydropyrrolo[2,3-d]pyrimidin-7-yl)benzenesulfonamide (20)

Yield 59%, m.p. 245.6°C. IR (KBr, cm^{-1}): 3415, 3390 (NH, NH_2), 3084 (CH arom.), 2958, 2872 (CH aliph.), 1637 (C=N), 1340, 1182 (SO_2), 1265 (C=S). ^{1}H-NMR (DMSO-d_6, δ, ppm): 0.9 (s, 3H, CH_3), 1.3 (m, 2H, $\underline{CH_2}CH_3$), 1.4–1.5 (m, 2H, $\underline{CH_2}CH_2NH$), 3.2 (t, 2H, $\underline{CH_2}NH$), 6.9 (s, 1H, CH pyrrole), 7.4–8.0 (m, 10H, Ar-H + SO_2NH_2), 8.1 (s, 1H, CH pyrimidine), 8.3 (s, 1H, NH imino, D_2O exchangeable), 9.1 (s, 2H, 2NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6, δ, ppm): 13.4, 19.6,

31.1, 44.1, 102.3, 119.7, 122.5, 123.7 (2), 124.5, 126.7, 129.4 (2), 131.7 (2), 132.9 (2), 134.8, 139.5, 148.6, 157.6, 166.2, 189.2. Analysis: calcd. for $C_{23}H_{24}BrN_7O_4S_2$ (574.52): C, 48.08; H, 4.21; N, 17.07%; found: C, 48.33; H, 4.49; N, 17.36%.

4-(5-(4-Bromophenyl)-4-imino-3-(3-phenylthioureido)-3,4-dihydropyrrolo[2,3-d]pyrimi-din-7-yl)benzenesulfonamide (21)

Yield 74%, m.p. 83.6°C. IR (KBr, cm^{-1}): 3215, 3196, 3116 (NH, NH_2), 3049 (CH arom.), 1597 (C=N), 1375, 1157 (SO_2), 1292 (C=S). ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.1 (s, 1H, CH pyrrole), 7.3–7.9 (m, 15H, Ar-H + SO_2NH_2), 8.1 (s, 1H, CH pyrimidine), 8.4 (s, 1H, NH imino, D_2O exchangeable), 11.0 (s, 2H, 2NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6, δ, ppm): 102.7, 118.1, 121.5, 122.5 (2), 123.5, 124.9, 126.8, 127.6 (2), 128.7 (2), 130.5 (2), 131.3 (2), 135.3 (2), 139.9, 140.8, 142.1, 147.9, 157.6, 162.2, 187.1. Analysis: calcd. for $C_{25}H_{20}BrN_7O_2S_2$ (594.51): C, 50.51; H, 3.39; N, 16.49%; found: C, 50.21; H, 3.10; N, 16.87%.

General procedure for the synthesis of compounds (22–29)

A mixture of **8** (4.59 g, 0.01 mol) and the corresponding aromatic aldehydes (0.01 mol) was refluxed in acetic acid (30 mL) for 5 h, the reaction mixture was then concentrated and the separated solid crystals were recrystallized from dioxane to give **22**–**29**, respectively.

4-(5-(4-Bromophenyl)-4-imino-3-(4-methylbenzylideneamino)-3,4-dihydropyrrolo[2,3-d]pyrimidin-7-yl)benzenesulfonamide (22)

Yield 88%, m.p. 314.1°C. IR (KBr, cm^{-1}): 3321, 3191, 3136 (NH, NH_2), 1624 (C=N), 1330, 1161 (SO_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.2 (s, 3H, CH_3), 7.2 (s, 1H, CH pyrrole), 7.3–8.2 (m, 14H, Ar-H + SO_2NH_2), 8.4 (s, 1H, CH pyrimidine), 9.5 (s, 1H, NH imino, D_2O exchangeable), 9.9 (s, 1H, N=CH). ^{13}C-NMR (DMSO-d_6, δ, ppm): 21.0, 103.8, 117.2, 122.6, 123.2 (2), 124.4, 126.9, 128.8 (2), 129.3 (2), 129.5 (2), 130.5 (2), 131.3, 133.9 (2), 136.1, 139.2, 141.3, 142.5, 145.1, 163.2, 192.5. Analysis: calcd. for $C_{26}H_{21}BrN_6O_2S$ (561.45): C, 55.62; H, 3.77; N, 14.97%; found: C, 55.96; H, 3.44; N, 14.66%.

4-(5-(4-Bromophenyl)-3-(4-hydroxybenzylideneamino)-4-imino-3,4-dihydropyrrolo[2,3-d]pyrimidin-7-yl)benzenesulfonamide (23)

Yield 82%, m.p. >350°C. IR (KBr, cm^{-1}): 3470 (OH), 3385, 3321, 3196 (NH, NH_2), 1593 (C=N), 1381, 1159 (SO_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 6.9 (s, 1H, CH pyrrole), 7.5–8.1 (m, 14H, Ar-H + SO_2NH_2), 8.5 (s, 1H, NH imino, D_2O exchangeable), 8.6 (s,1H, CH pyrimidine), 9.5 (s, 1H, N=CH), 10.0 (s, 1H, OH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6, δ, ppm): 103.7, 115.7 (2), 117.6, 120.0, 120.6 (2), 123.2, 124.5, 126.8, 128.8 (2), 129.4 (2), 130.5 (2), 131.4 (2), 131.8, 139.3, 142.6, 149.3, 159.7, 161.0, 162.9. Analysis: calcd. for $C_{25}H_{19}BrN_6O_3S$ (563.43): C, 53.29; H, 3.40; N, 14.92%; found: C, 53.55; H, 3.71; N, 14.60%.

4-(5-(4-Bromophenyl)-4-imino-3-(4-methoxybenzylideneamino)-3,4-dihydropyrrolo[2,3-d]pyrimidin-7-yl)benzenesulfonamide (24)

Yield 79%, m.p. 253.0°C. IR (KBr, cm^{-1}): 3292, 3230, 3101 (NH, NH_2), 2970, 2841 (CH aliph.), 1591 (C=N), 1340, 1157 (SO_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.7 (s, 3H, OCH_3), 6.8 (s, 1H, CH pyrrole), 6.9–8.1 (m, 14H, Ar-H + SO_2NH_2), 8.2 (s, 1H, CH pyrimidine), 9.6 (s, 1H, NH, D_2O exchangeable), 9.9 (s, 1H, N=CH). ^{13}C-NMR (DMSO-d_6, δ, ppm): 55.0, 113.6, 114.4 (2), 118.9, 119.6, 121.0 (2), 124.4, 126.6, 127.9, 129.6 (2), 131.7 (2), 132.3 (2), 133.7 (2), 139.6, 140.0, 142.1, 145.3, 148.7, 158.9, 160.1. Analysis: calcd. for $C_{26}H_{21}BrN_6O_3S$ (577.45): C, 54.08; H, 3.67; N, 14.55%; found: C, 54.39; H, 3.29; N, 14.26%.

4-(3-Benzo[d][1,3]dioxol-5-ylmethyleneamino)-5-(4-bromophenyl)-4-imino-3,4-dihydropyrrolo[2,3-d]pyrimidin-7-yl)benzenesulfonamide (25)

Yield 66%, m.p. 310.4°C. IR (KB, cm^{-1}): 3385, 3261, 3141 (NH, NH_2), 2976, 2895 (CH aliph.), 1595 (C=N), 1363, 1161 (SO_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 6.1 (s, 2H, CH_2), 6.9 (s, 1H, CH pyrrole), 7.0–8.1 (m, 13H, Ar-H + SO_2NH_2), 8.4 (s, 1H, CH pyrimidine), 9.5 (s, 1H, NH imino, D_2O exchangeable), 9.8 (s, 1H, N=CH). ^{13}C-NMR (DMSO-d_6, δ, ppm): 101.6, 108.5, 117.3, 118.9, 119.6, 121.8, 123.7 (2), 124.4, 124.5, 126.8, 128.5, 129.4 (2), 131.7 (2), 132.3 (2), 136.1, 139.9, 145.3, 147.7, 148.7, 149.3, 163.0, 190.9. Analysis: calcd. for $C_{26}H_{19}BrN_6O_4S$ (591.44): C, 52.80; H, 3.24; N, 14.21%; found: C, 52.48; H, 3.56; N, 14.50%.

4-(5-(4-Bromophenyl)-3-(4-chlorobenzylideneamino)-4-imino-3,4-dihydropyrrolo[2,3-d]pyrimidin-7-yl)benzenesulfonamide (26)

Yield 83%, m.p. 375.0°C. IR (KBr, cm^{-1}): 3421, 3271 (NH, NH_2), 3051 (CH arom.), 1595 (C=N), 1334, 1161 (SO_2), 727 (C-Cl). ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.4 (s, 1H, CH pyrrole), 7.5–8.3 (m, 14H, Ar-H + SO_2NH_2), 8.4 (s, 1H, CH

pyrimidine), 8.5 (s, 1H, NH imino, D_2O exchangeable), 9.6 (s, 1H, N=CH). ^{13}C-NMR (DMSO-d_6, δ, ppm): 104.6, 118.6, 121.2, 122.0 (2), 123.9, 124.6, 126.8 (2), 128.8 (2), 129.4 (2), 131.4 (2), 133.5, 134.6 (2), 136.0, 136.4, 139.3, 143.1, 162.2, 167.2. Analysis: calcd. for $C_{25}H_{18}BrClN_6O_2S$ (581.87): C, 51.60; H, 3.12; N, 14.44%; found: C, 51.35; H, 3.49; N, 14.12%.

4-(5-(4-Bromophenyl)-4-imino-3-(4-nitrobenzylideneamino)-3,4-dihydropyrrolo[2,3-d]pyrimidin-7-yl)benzenesulfonamide (27)

Yield 75%, m.p. 375.5°C. IR (KBr, cm^{-1}): 3350, 3271 (NH, NH_2), 3072 (CH arom.), 1625 (C=N), 1346, 1163 (SO_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.4 (s, 1H, CH pyrrole), 7.5– 8.2 (m, 14H, Ar-H + SO_2NH_2), 8.4 (s, 1H, CH pyrimidine), 9.6 (s, 1H, NH imino, D_2O exchangeable), 10.1 (s, 1H, N=CH). ^{13}C-NMR (DMSO-d_6, δ, ppm): 103.9, 117.7, 122.6, 123.4 (2), 124.2, 124.6 (2), 126.8, 129.3 (2), 130.2 (2), 130.6 (2), 131.7 (2), 136.7, 137.0, 138.1, 143.0, 144.3, 151.2, 162.8, 192.3. Analysis: calcd. for $C_{25}H_{18}BrN_7O_2S$ (592.42): C, 50.68; H, 3.06; N, 16.55%; found: C, 50.37; H, 3.30; N, 16.82%.

4-(5-(4-Bromophenyl)-4-imino-3-(2,3,4-trimethoxybenzylideneamino)-3,4-dihydropyrrolo[2,3-d]pyrimidin-7-yl)benzenesulfonamide (28)

Yield 71%, m.p. 275.5°C. IR (KB, cm^{-1}): 3371, 3298, 3221 (NH, NH_2), 2939, 2839 (CH aliph.), 1624 (C=N), 1338, 1165 (SO_2). ^{1}H-NMR (DMSO-d_6, D_2O, δ, ppm): 3.7, 3.8 (2s, 9H, $3OCH_3$), 6.7 (s, 1H, CH pyrrole), 7.3–8.1 (m, 12H, Ar-H + SO_2NH_2), 8.4 (s, 1H, CH pyrimidine), 8.6 (s, 1H, NH imino, D_2O exchangeable), 9.5 (s, 1H, N=CH). ^{13}C-NMR (DMSO-d_6, δ, ppm): 55.6 (2), 59.2, 104.6, 106.8, 107.8, 119.6, 122.1, 124.3 (2), 124.4, 124.8, 126.7, 130.2 (2), 130.8 (2), 133.1 (2), 136.1, 137.2, 139.8, 142.6, 147.3, 154.0, 157.3, 163.4, 163.8. Analysis: calcd. for $C_{28}H_{25}BrN_6O_5S$ (637.50): C, 52.75; H, 3.95; N, 13.18%; found: C, 52.40; H, 3.64; N, 13.55%.

4-(5-(4-Bromophenyl)-4-imino-3-(thiophen-2-ylmethyleneamino)-3,4-dihydropyrrolo[2,3-d]pyrimidin-7-yl)benzenesulfonamide (29)

Yield 56%, m.p. 334.2°C. IR (KBr, cm^{-1}): 3321, 3195 (NH, NH_2), 3055 (CH arom.), 1627 (C=N), 1327, 1159 (SO_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.0 (s, 1H, CH pyrrole), 7.1– 8.1 (m, 13H, Ar-H + SO_2NH_2), 8.4 (s, 1H, CH pyrimidine), 9.5 (s, 1H, NH imino, D_2O exchangeable), 11.6 (s, 1H, N=CH). ^{13}C-NMR (DMSO-d_6, δ, ppm): 103.6, 119.6, 120.0, 123.6 (2), 124.4, 124.6, 126.6, 126.8, 128.3, 128.9, 129.3 (2), 130.7 (2), 136.1 (2), 139.2, 141.5, 142.6, 149.2, 159.4, 184.2. Analysis: calcd. for $C_{23}H_{17}BrN_6O_2S_2$ (553.45): C, 49.91; H, 3.10; N, 15.18%; found: C, 49.66; H, 3.43; N, 15.55%.

Molecular docking

All the molecular modeling studies were carried out on an Intel Pentium 1.6 GHz processor, 512 MB memory with Windows XP operating system using Molecular Operating Environment (MOE, 10.2008) software. All the minimizations were performed with MOE until a RMSD gradient of 0.05 kcal mol^{-1}Å^{-1} with MMFF94X force field and the partial charges were automatically calculated. The X-ray crystallographic structure of c-Src complex with its ligand (PDB ID: 1YOL) was obtained from the protein data bank. The enzyme was prepared for docking studies where: (i) Ligand molecule was removed from the enzyme active site. (ii) Hydrogen atoms were added to the structure with their standard geometry. (iii) MOE Alpha Site Finder was used for the active sites search in the enzyme structure and dummy atoms were created from the obtained alpha spheres. (iv) The obtained model was then used in predicting the ligand enzymes interactions at the active site (Table 1).

***In vitro* antitumor activity**

Human tumor breast cell line (MCF7) was used in this study. The cytotoxic activity was measured *in vitro* for the newly synthesized compounds using the sulforhodamine-B stain (SRB) assay using the method of Skehan et al. (18). The *in vitro* anticancer screening was done by the pharmacology unit at the National Cancer Institute, Cairo University. Cells were plated in 96-multiwell plate (104 cells/well) for 24 h before treatment with the compound(s) to allow attachment of cell to the wall of the plate. The tested compounds were dissolved in dimethyl sulfoxide. Different concentrations of the compound under test (10, 25, 50, and 100 µM) were added to the cell monolayer. Triplicate wells were prepared for each individual concentration. Monolayer cells were incubated with the compound(s) for 48 h at 37°C and in atmosphere of 5% CO_2. After 48 h, cells were fixed, washed and stained for 30 min with 0.4% (w/v) SRB dissolved in 1% acetic acid. Excess unbound dye was removed by four washes with 1% acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug con-

Table 1. Binding scores and amino acid interactions of the docked compounds on the active site of c-Src.

Compd. No.	S Kcal/mol	Amino acid interactions	Interacting groups	H bond length [Å]
5	–13.6201	Asp 350, Met 343 Ser 347	NH_2, N pyrimidine	3.22, 2.22 3.09
6	–12.7631	Asp 350	SO_2NH_2	1.62
8	–10.6603	Met 343, Gln 277	NH_2, SO_2NH_2	2.9, 2.51
9	–12.3317	Gln 277	N imidazole	2.78
10	–13.1892	Thr 340	SO_2NH_2	2.27
11	–16.1225	Gln 277, Lys 274	C=NH, C=O	3.03, 2.94
12	–17.7816	Leu 275	NH, NH	1.59, 1.69
13	–17.7263	Met 343, Gln 277	SO_2NH_2 NH, C=O	1.94, 3.18, 2.50, 2.53
14	–14.4151	Thr 340, Gln 277	SO_2NH_2,C=O	1.94, 2.79
15	–8.0453	Leu 275	SO_2NH_2	2.83
16	–16.6572	Asp 350, Asp 406	SO_2NH_2, C=NH	1.63, 1.53
17	–8.8088	Asp 350	SO_2NH	2.73
18	–13.2570	Gln 277	C=NH	2.55, 2.57
19	–14.0169	Gln 277	C=NH	2.58, 2.57
20	–15.3704	Leu 275	C=NH	1.81
21	–14.2411	Met 343, Gln 277	SO_2NH_2, C=NH	2.1, 1.4
22	–12.7915	Gln 277, Asp 350	C=NH, C=NH	2.91, 2.71
23	–13.9751	Thr 340	OH	2.47
24	–14.5953	Leu 275	C=NH	1.87
25	–19.0510	Gln 277	C=NH	2.93, 3.21
26	–18.3491	Met 343	SO_2NH_2, SO_2NH_2	1.73, 2.75
27	–14.1116	Leu 275	C=NH	2.02
28	–18.5521	Thr 340	SO_2NH_2	2.19
29	–18.3311	Asp 406, Asp 350	C=NH, SO_2NH_2	1.78, 207

centration is plotted to get the survival curve for breast tumor cell line after the specified time. The molar concentration required for 50% inhibition of cell viability (IC_{50}) was calculated and compared to the reference drug doxorubicin (CAS, 25316-40-9). The surviving fractions were expressed as the means ± standard error and the results are given in Table 2.

RESULTS AND DISCUSSION

Chemistry

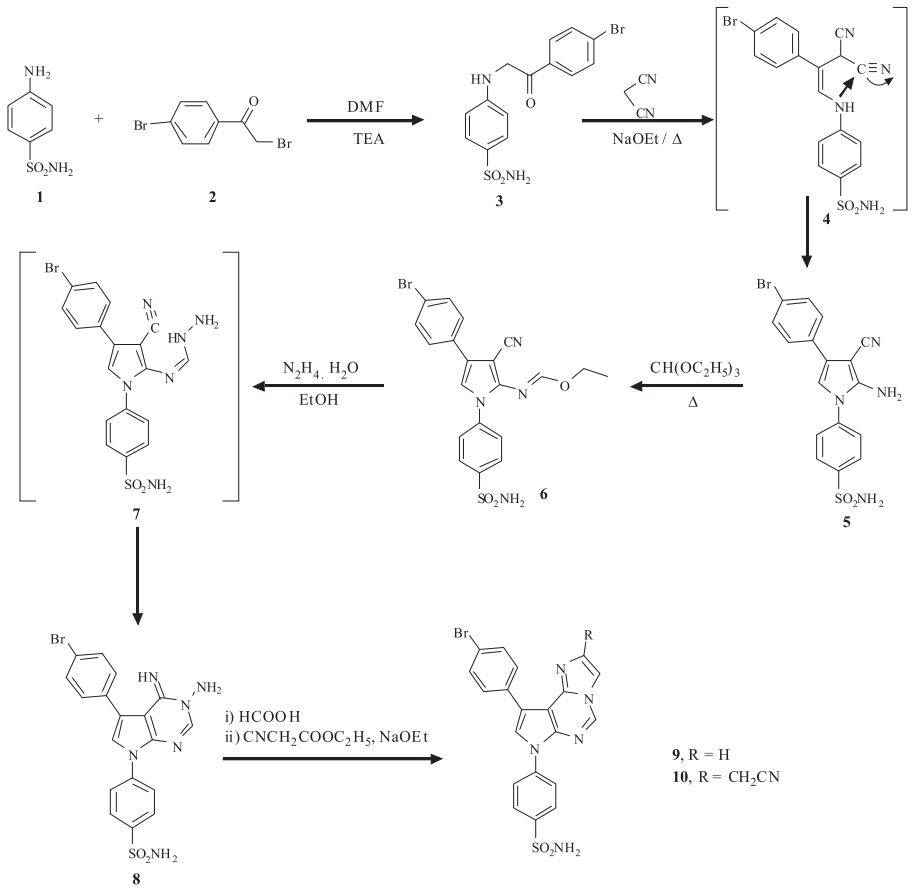
The synthetic procedures adopted to obtain the target compounds are depicted in Schemes 1 and 2. In this work, the reactivity of sulfanilamide **1** towards phenacyl bromide was studied. Thus, interaction of sulfanilamide **1** with 2-bromo-1-(4-bromophenyl)ethanone **2** furnished the corresponding 4-(2-(4-bromophenyl)-2-oxoethylamino)benzenesulfonamide **3**, which upon reaction with malononitrile in refluxing ethanol containing sodium ethoxide yielded the strategic starting material, pyrrole-2-amino-3-carbonitrile **5** (Scheme 1). The formation of compound **5** was assumed to proceed *via* condensation of compound **3** with malononitrile to give the intermediate **4** followed by intramolecular cyclization to give the pyrrole derivative **5**. The structure of compound **3** was proved *via* elemental analysis and spectral data. The IR spectrum of compound **3** revealed the presence of characteristic bands for NH, NH_2, C=O and SO_2. Also ^{1}H-NMR spectrum indicated the presence of a singlet at 4.7 ppm, which could be assigned to CH_2 group. The IR spectrum of compound **5** exhibited bands for NH_2, CN and SO_2 groups. The ^{1}H-NMR spectrum of **5** showed signals

at 6.1 ppm due to NH_2 group and 7.9 ppm for SO_2NH_2 group. Compound **5** was reacted with triethylorthoformate to give the corresponding ethoxymethylene amino derivative **6**. The IR spectrum of compound **6** revealed the presence of characteristic bands for NH_2, CN and SO_2 groups. The ^{1}H-NMR spectrum of **6** showed a triplet for CH_3 group and a quartet for CH_2 group. When compound **6** was reacted with hydrazine hydrate in ethanol at room temperature, the *N*-amino-imino derivative **8** was obtained in good yield. The structure of compound **8** was supported on the basis of elemental analyses and spectral data. The IR spectrum exhibited the disappearance of the C=N band and the presence of bands at 3294, 3230, 3150 cm^{-1} for NH, NH_2, 1639 cm^{-1} for C=N and 1375, 1163 cm^{-1} for SO_2. Moreover, the ^{1}H-NMR spectrum of compound **8** exhibited singlet at 5.6 ppm due to NH_2 group. Compound **8** was reacted with different one carbon cyclizing agents to prepare some new pyrrolopyrimidine derivatives carrying 1,2,4-triazole moiety. Thus, the corresponding 1,2,4-triazolopyrrolopyrimidine derivatives **9** and **10** were obtained *via* reaction of compound **8** with formic acid or ethyl cyanoacetate in the presence of sodium ethoxide. The IR spectrum of compound **9** revealed a band at 1620 cm^{-1} for C=N. The ^{1}H-NMR spectrum of **9** showed singlet at 8.6 ppm attributed to CH of triazole. Also the IR spectrum of **10** revealed band at 2270 cm^{-1} C=N. The ^{1}H-NMR spectrum of **10** in DMSO-d_6

Table 2. *In vitro* anticancer screening of the synthesized compounds against human breast cancer cell line (MCF7).

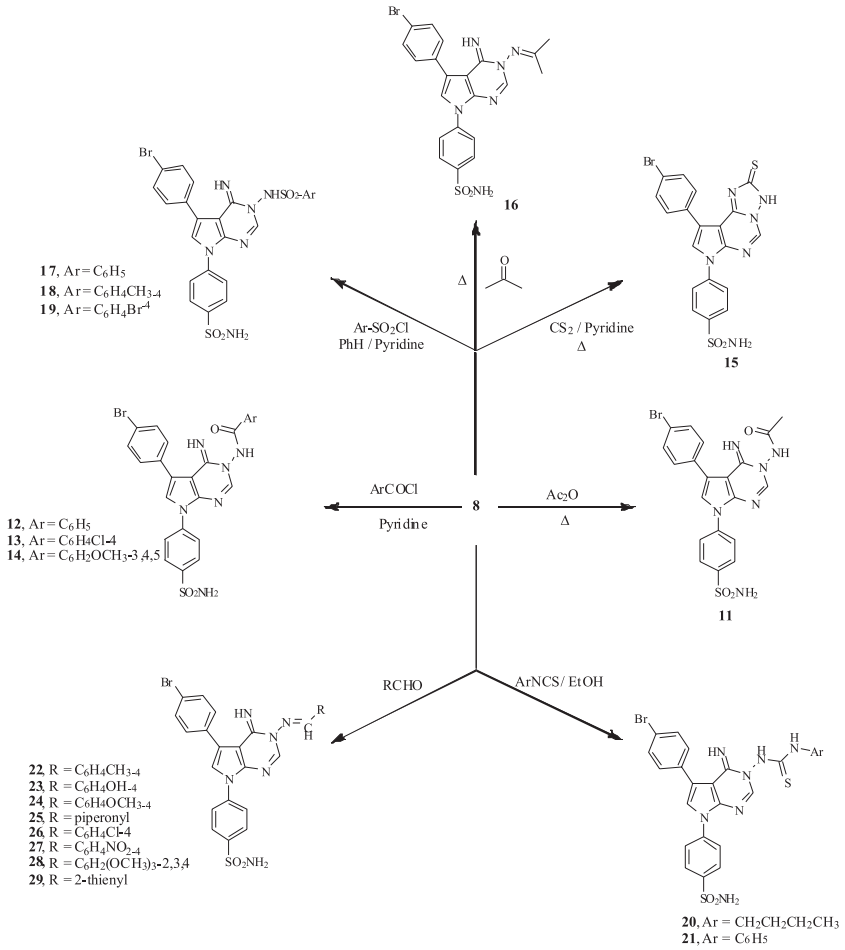
Compound	Compound concentration (μM)				IC_{50} (μM)
	10 μM	25 μM	50 μM	100 μM	
	Surviving fraction (Mean ± S.E.)*				
Doxorubicin	0.314 ± 0.032	0.309 ± 0.016	0.251 ± 0.023	0.266 ± 0.032	8.02
5	0.327 ± 0.121	0.273 ± 0.043	0.233 ± 0.011	0.255 ± 0.020	7.56
6	0.340 ± 0.090	0.294 ± 0.021	0.246 ± 0.110	0.256 ± 0.002	7.56
8	0.330 ± 0.211	0.309 ± 0.016	0.227 ± 0.110	0.268 ± 0.132	7.29
9	0.310 ± 0.101	0.318 ± 0.104	0.298 ± 0.100	0.322 ± 0.018	7.29
10	0.344 ± 0.004	0.312 ± 0.111	0.276 ± 0.040	0.343 ± 0.011	7.56
11	0.340 ± 0.149	0.284 ± 0.101	0.212 ± 0.146	0.266 ± 0.155	7.56
12	0.377 ± 0.021	0.356 ± 0.100	0.260 ± 0.001	0.294 ± 0.088	7.84
13	0.361 ± 0.021	0.324 ± 0.048	0.260 ± 0.099	0.245 ± 0.011	7.84
14	0.256 ± 0.044	0.211 ± 0.001	0.187 ± 0.032	0.270 ± 0.001	6.46
15	0.316 ± 0.010	0.323 ± 0.001	0.370 ± 0.124	0.311 ± 0.100	7.29
16	0.305 ± 0.007	0.263 ± 0.071	0.218 ± 0.100	0.244 ± 0.029	7.01
17	0.316 ± 0.011	0.264 ± 0.001	0.195 ± 0.021	0.177 ± 0.049	7.56
18	0.321 ± 0.001	0.311 ± 0.111	0.299 ± 0.029	0.299 ± 0.056	8.19
19	0.344 ± 0.092	0.310 ± 0.044	0.299 ± 0.008	0.280 ± 0.019	8.20
20	0.401 ± 0.001	0.367 ± 0.066	0.281 ± 0.047	0.300 ± 0.100	8.21
21	0.266 ± 0.011	0.269 ± 0.009	0.187 ± 0.006	0.266 ± 0.018	6.74
22	0.307 ± 0.001	0.256 ± 0.011	0.249 ± 0.096	0.259 ± 0.077	7.29
23	0.285 ± 0.001	0.246 ± 0.042	0.212 ± 0.033	0.218 ± 0.011	7.29
24	0.358 ± 0.010	0.300 ± 0.085	0.289 ± 0.011	0.355 ± 0.021	7.84
25	0.367 ± 0.093	0.345 ± 0.088	0.299 ± 0.024	0.299 ± 0.001	8.10
26	0.399 ± 0.001	0.387 ± 0.001	0.299 ± 0.077	0.310 ± 0.019	8.16
27	0.393 ± 0.012	0.289 ± 0.001	0.290 ± 0.044	0.300 ± 0.078	8.16
28	0.303 ± 0.011	0.280 ± 0.067	0.249 ± 0.067	0.352 ± 0.011	7.01
29	0.389 ± 0.001	0.389 ± 0.001	0.284 ± 0.022	0.300 ± 0.019	8.16

* Each value is the mean of three values ± standard error.



Scheme 1. Synthetic pathways for compounds **3–10**

exhibited singlet at 4.5 ppm due to CH_2CN group. The monoacetyl derivative **11** was obtained *via* reaction of **8** with acetic anhydride (Scheme 2). The IR spectrum of **11** revealed the presence of a characteristic band at 1710 cm^{-1} for C=O group, while its ^{1}H-NMR showed singlet at 2.1 ppm attributed to the presence of acetyl group. Interaction of compound **8** with benzoyl chloride derivatives in pyridine afforded the corresponding benzamide derivatives **12–14**, respectively. The IR spectrum of **12** showed band at 1683 cm^{-1} (C=O). The ^{1}H-NMR spectrum of **12** revealed the presence of singlet at 9.6 ppm for imino group. The IR spectrum of **13** showed band at 761 cm^{-1} (C-Cl). The ^{1}H-NMR spectrum of **14** exhibited signals at 3.7, 3.9 ppm attributed to three methoxy groups. When compound **8** was allowed to react with CS_2, the triazolopyrimide-2-thione **15** was obtained. The IR spectrum of **15** revealed bands at 1242 cm^{-1} (C=S), 1359, 1159 cm^{-1} (SO_2). In addition, condensation of the key compound **8** with dry acetone gave the condensed product **16**, its ^{1}H-NMR revealed singlet at 1.1 ppm attributed to two methyl groups. On the other hand, interaction of compound **8** with sulfonyl derivatives in dry benzene containing few drops of pyridine yielded the corresponding sulfonamide derivatives **17–19**, respectively. The ^{1}H-NMR spectrum of **18** revealed the presence of singlet at 2.2 ppm attributed to tolyl group. Nucleophilic reaction of compound **8** on the highly positive carbon of the isothiocyanate yielded the corresponding thiourea derivatives **20**, **21** (Scheme 2). Their structures were confirmed based on their spectral data, where the IR spectra of compounds **20** and **21** showed bands at 1292, 1265 cm^{-1} (C=S). The ^{1}H-NMR spectrum of compound **20** showed a triplet at 0.9 ppm due to the methyl group. The benzylidene derivatives **22–29** were obtained in a good yield *via* the reaction of compound **8** with aromatic aldehydes. The IR spectra showed the presence of bands of imino groups. Also ^{1}H-NMR spectra of compounds **22–29** showed singlets at 9.5–11.6 ppm for N=CH groups.



Scheme 2. Synthetic pathways for compounds **11**–**29**

Molecular docking

Several classes of inhibitors are currently used to inhibit the activity of c-Src in a number of cell types. However, they often show poor selectivity within the c-Src family, which in mammals comprises at least eight members involved in many key functions of the cell (19). Recently, we have shown that c-Src inhibitors of the pyrrolopyrimidine class exhibit a powerful inhibitory activity and a several-fold greater selectivity for c-Src against most tyrosine kinases (20–22). This suggests that c-Src can be activated downstream of receptor activator of NF-κB (RANK) (23), a member of the tumor necrosis factor (TNF) receptor superfamily that is involved in cell differentiation, function, and survival (24–26). In order to perform the aim of the present investigations, the authors have performed molecular docking of the synthesized compounds on the active sites of c-Src, which may lead to an understanding of their effect as antitumor agents. The protein data bank file (PDB ID:1YOL) was selected for this purpose. The file contains c-Src enzyme co-crystallized with a pyrrolopyrimidine ligand. All docking procedures were achieved by MOE (Molecular Operating Environment) software 10.2008 provided by chemical computing group, Canada. Docking on the active site of c-Src enzyme was performed for all synthesized compounds. Docking protocol was verified by redocking of the co-crystallized ligand in the vicinity of the active site of the enzyme with energy score (S) = –22.6799 Kcal/mol and root mean standard deviation (RMSD) = 0.8205 (Fig. 1). The ligand interacts with the active site amino acids by three interactions: with Met 343 with hydrogen bond of 3.09 Å, with Glu 341 with hydrogen bond of 2.22 Å and with Thr 340 with hydrogen bond of 3.22 Å. All the synthesized compounds were docked on the active site of the enzyme showing good fitting. The

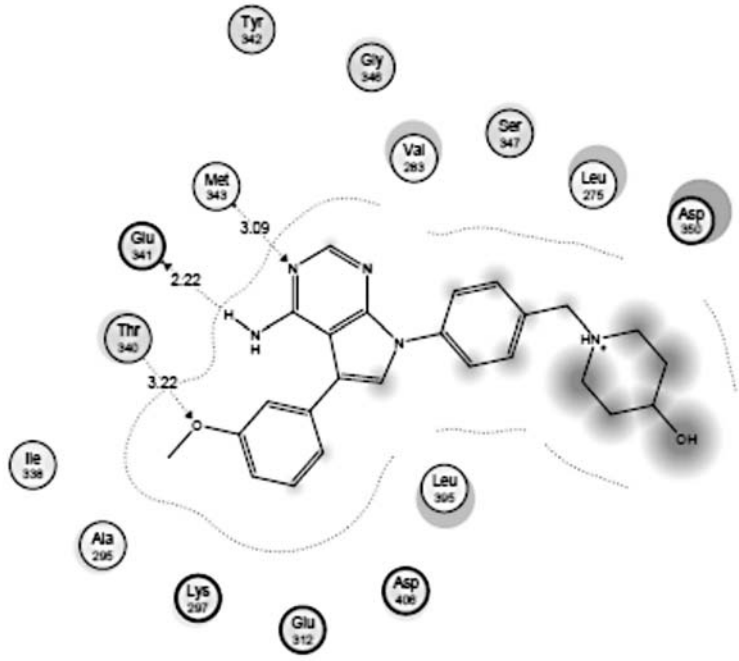


Figure 1. Pyrrolopyrimidine ligand on the active site c-Src enzyme

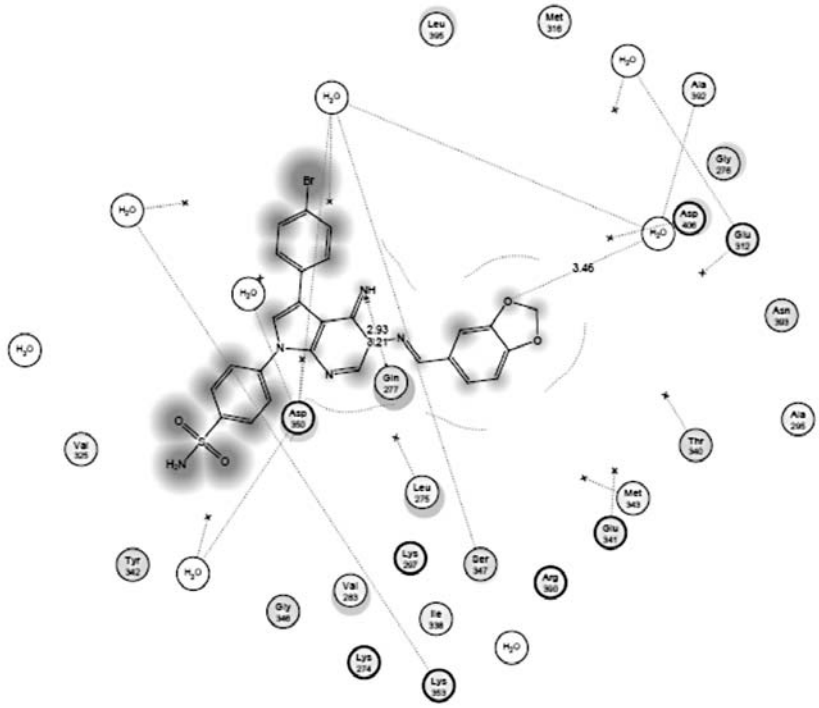


Figure 2. Compound **25** on the active site c-Src enzyme

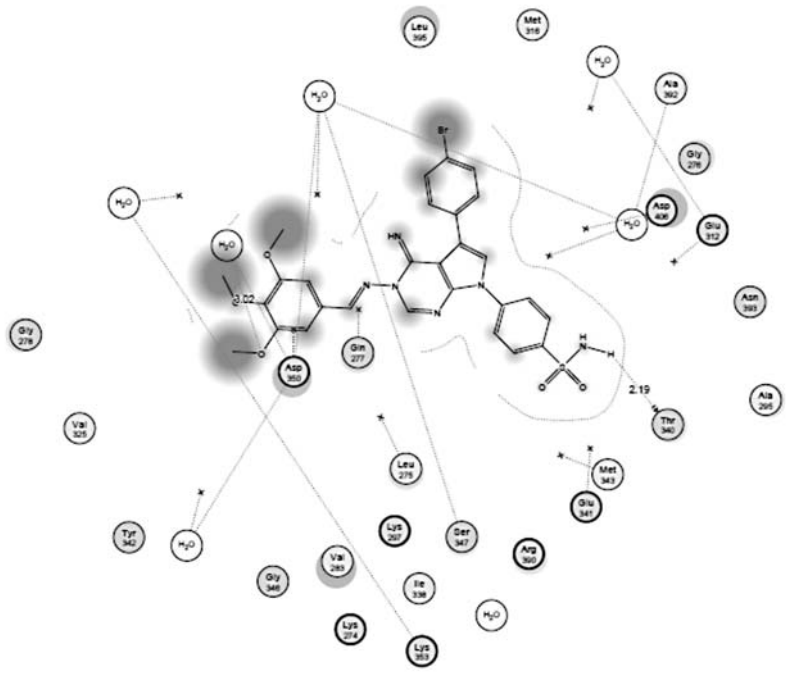


Figure 3. Compound **28** on the active site c-Src enzyme

energy score (S) as well as amino acid interactions of the synthesized compounds were listed in (Table 1). The best energy scores were exhibited by compounds **25** and **28** with S = –19.0510 and –18.5521, respectively. Figures 2 and 3 show the amino acid interactions with compounds **25** and **28**, respectively. On the other hand, one or more of the amino acids that were interacting with the co-crystalized ligand has/have interacted with compounds **5**, **8**, **13**, **14**, **21**, **23**, **26** and **28**. This was not the case in the rest of compounds as they interacted with different amino acids of the active site other than the ones interacted with the co-crystalized ligand.

***In vitro* antitumor activity**

The newly synthesized compounds were evaluated for their *in vitro* cytotoxic activity against human breast cancer cell line (MCF7). Doxorubicin, which is one of the most effective anticancer agents, was used as the reference drug in this study. The relationship between surviving fraction and drug concentration was plotted to obtain the survival curve of breast cancer cell line (MCF7). The response parameter calculated was the IC_{50} value, which corresponds to the concentration required for 50% inhibition of cell viability. Table 2 shows the *in vitro* cytotoxic activity of the synthesized compounds where all compounds exhibited significant activity compared to the reference drug. All the synthesized compounds showed promising cytotoxic activities especially compounds **5**–**17**, **21**–**24** and **28** with IC_{50} values in the range of 6.46–7.84 μM, which is better than that of doxorubicin with IC_{50} value of 8.02 μM. On the other hand, compounds **18**–**20**, **25**–**27** and **29** showed IC_{50} values in the range of 8.16–8.21 μM, which indicated lower activity but comparable to that of doxorubicin.

The strategic synthone – compound **5**, showed IC_{50} value of 7.56 μM and upon reaction with ethyl orthoformate to give compound **6** did not lead to a more active derivative, as its IC_{50} value retains the same, while cyclization to the iminoamino pyrrolopyrimidine derivative **8** improved the activity, as its IC_{50} yielded 7.29 μM. Annelation to imidazo derivatives **9** did not improve the activity with the same IC_{50} value but substitution with CH_2CN as in compound **10** again returned the IC_{50} to that of the staring compound **5**. This was also the case in the acetyl derivative **11** with the same IC_{50} value of 7.56 μM. The activity decreased upon treatment of compound **8** with benzoyl chloride and/or 4-chlorobenzoyl chloride to reach 7.84 μM for compounds **12** and **13**. A marked increase in activity was observed with the trimethoxy derivative **14**

having IC_{50} = 6.46 μM. The triazolo derivative **15** showed IC_{50} value of 7.29 μM which was improved in case of the iminopyrrolopyrimidine derivative **16** to reach 7.01 μM. Treatment of compound **8** with several sulfonylchloride derivatives lead to the formation of compounds **17**–**19** in which the unsubstituted aromatic derivative **17** was the best one among them with IC_{50} value of 7.56 μM, while compounds **18** and **19** showed IC_{50} values of 8.19 and 8.20 μM, respectively. The aliphatic thiourea derivative **20** had the worst IC_{50} of 8.21 μM, while its aromatic analogue **21** showed very good IC_{50} value of 6.74 μM. Finally, the Schiff's base derivatives **22**–**29** showed IC_{50} values in the range of 7.01–8.16 μM. Again the trimethoxy derivative **28** was the most active among this series with IC_{50} value of 7.01 μM, while compounds **26**, **27** and **29** with a substitution with electron withdrawing groups chloro, nitro and 2-thienyl showed IC_{50} value of 8.16 μM. On the other hand, compounds **22**, **23** and **24** with electron donating groups CH_3, OH and OCH_3 showed IC_{50} value of 7.29, 7.29 and 7.84 μM, respectively.

Relationship between molecular docking and cytotoxic activity

All of the synthesized compounds were fit in the active site of c-Src enzyme. The docking scores range between –19.0510 and –8.0453 kcal/mol. However, only compounds **5**, **8**, **13**, **14**, **21**, **23**, **26** and **28** interacted with either Met 343 or Thr 340, which are two of the amino acids that interacted with the co-crystallized ligand. The above mentioned compounds have shown better IC_{50} values than that of doxorubicin except for compound **26** with IC_{50} value of 8.16 μM. Compound **28** case was interesting as its docking score was one of the best docking score of –18.5521 kcal/mol and its interaction with one of the amino acids that interacted with the co-crystallized ligand (Thr 340) with a hydrogen bond of 2.19 Å. In spite of these findings, no linear relationship could be postulated between docking results and biological activities and this could be a trial to suggest a mechanism of action for the cytotoxic activities of the synthesized compounds that still needs further investigations.

CONCLUSION

A novel series of pyrrolopyrimidines and triazolopyrimidines carrying a biologically active sulfonamide moieties were synthesized and evaluated for their anti-breast cancer activity. Most of the synthesized compounds showed promising anticancer activity against breast cancer cell line (MCF7) compared to doxorubicin as positive control especially compounds **5**–**17**, **21**–**24** and **28** with better IC_{50} than that of doxorubicin.

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