Anti-breast Cancer of Some Novel Pyrrole and Pyrrolopyrimidine Derivatives Bearing A Biologically Active Sulfonamide Moiety

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Abstract: Several novel pyrroles **5**, **6**, **11-15**, **20** and pyrrolopyrimidines **7- 10**, **16- 19**, **21-26** having a biologically active sulfonamide moieties were synthesized in order to study their anticancer activity. The prepared compounds were characterized by elemental analyses, IR, ^{1}H-NMR and ^{13}C-NMR spectra, then screened as anticancer agents against human breast cancer cell line (MCF7). All the tested compounds showed good cytotoxic activity better than the reference drug Doxorubicin as reference drug with IC_{50} values ranging between 6.46-7.86 µM except compounds **19** and **31** which showed comparable activity with IC_{50} values of 8.30, 8.39 µM, respectively. In order to predict the mechanism of action for their activity, molecular docking on the active site of c-Src kinase enzyme was performed and good results were obtained.
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1. Introduction

The formation of new fused heterocyclic compounds is an important task for medicinal chemists from various points of views. Pyrrole and pyrrolopyrimidine for example, have been evaluated pharmacologically as antimicrobial [Dang and Gomez-Galeno, 2002], analgesic [Danchev et al., 2005], anti-inflammatory [Jarvis et al., 2002], antiviral [Gangjee et al., 2005] and anticancer activity [De Clereq et al., 1987; Finch et al., 1997; Krawczyk et al., 1995]. Pyrrolo[2,3-d]pyrimidines have aroused recent attention from chemical and biological view points since they have useful properties as antimetabolites in purine biochemical reactions [Hildik and Shaw, 1971]. Several mechanisms are also involved in their cytotoxic activities as being antifolate inhibitors of dihydrofolate reductases [Germ et al., 1990; Schisky and Perry, 1990], tyrosine Kinase c-Src or Lck inhibitors [Calderwood et al., 2002; Almann et al., 2002], Cyclin dependent kinase (CDK) inhibitors [Morgan et al., 1998; Kitagawa et al., 1993] or adenosine receptor antagonists [Esteve et al., 2006; Merighi et al., 2003]. In addition, Sulfonamides are known to posses good antitumor activity in vivo and in vitro through several mechanisms of action [Owa et al., 1999; Ghorab et al., 2006; Rostom, 2006; Casini et al., 2002]. Due to our interest in the development of novel anticancer agents, in this study we report the synthesis and biological evaluation of novel pyrrolo and pyrrolopyrimidine derivatives carrying biologically active sulfonamide moiety hoping that the newly synthesized compounds might show significant anticancer activity. Also, molecular docking of the newly synthesized compounds was achieved on the active site of c-Src kinase in a trial to suggest the mechanism of action for their cytotoxic activity.

2. Experimental

2.1. Chemistry

General methods

All chemicals used in this study were purchased from Aldrich and Fluka. Melting points (^{o}C, uncorrected) were determined in open capillaries on a Gallenkemp melting point apparatus (Sanyo Gallenkemp, Southborough, UK) and were uncorrected. Precoated silica gel plates (silica gel 0.25 mm, 60 G F254; Merck, Germany) were used for thin layer chromatography, dichloromethane/methanol (9.5:0.5) mixture was used as a developing solvent system and the spots were visualized by ultraviolet light and/or iodine. Infra red spectra were recorded in KBr discs using IR-470 Shimadzu spectrometer (Shimadzu, Tokyo, Japan). NMR spectra (in DMSO-d6) were recorded on Bruker AC-500 Ultra Shield 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).

2.1.14-(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 3 h. The solid obtained was filtered off and recrystallized from ethanol to give **3**. Yield % 89, m.p. 232.6 ^{o}C, IR:$\upsilon_{max.}$/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, D_2O) δ: 4.7 (s, 2H, CH_2), 6.6 (s, 1H, NH, D_2O exchangeable), 6.7, 8.0 (2d, 4H, Ar-H, AB system, *J*=6.9 Hz). ^{13}C-NMR (DMSO-d_6): 49.4, 111.4 (2), 127.1, 127.7, 129.9 (2), 130.7 (2), 131.8 (2), 133.9, 150.8, 195.3. Anal. Calcd. for $C_{14}H_{13}BrN_2O_3S$ (369.23): C, 45.54; H, 3.55; N, 7.59. Found: C, 45.86; H, 3.31; N, 7.24.

2.1.2. 4-(2-Amino-4-(4-bromophenyl)-3-cyano-1*H*-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 ^{o}C, IR:$\upsilon_{max.}$/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, D_2O) δ: 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): 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. Anal. 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.

2.1.3. Ethyl *N*-4-(4-bromophenyl)-3-cyano-1-(4-sulfamoylphenyl)-*1H*-pyrrol-2-ylfor-mimi-date (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 ^{o}C, IR:$\upsilon_{max.}$/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, D_2O) δ: 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): 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. Anal. 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.

2.1.4. 4-(5-(4-Bromophenyl)-4-imino-3-substituted-3,4-dihydropyrrolo[2,3-d]pyrimidine-7-yl)benzenesulfonamides (7-10).

General procedure

A mixture of **6** (4.73 g, 0.01 mol.) and the appropriate aliphatic amine (0.01 mol.) was stirred in ethanol (20 mL) at room temperature for 2 h. The solid formed was filtered and recrystallized from dioxane to give **7-10**, respectively.

2.1.5. 4-(5-(4-Bromophenyl)-4-imino-3-methyl-3,4-dihydropyrrolo[2,3-d]pyrimidine-7-yl)benzenesulfonamide (7).

Yield % 91, m.p. 286.2 ^{o}C, IR:$\upsilon_{max.}$/cm^{-1} 3410, 3380, 3211 (NH, NH_2), 3075 (CH arom.), 2970, 2836 (CH aliph.), 1610 (C=N), 1398, 1157 (SO_2). ^{1}H-NMR (DMSO-d_6, D_2O) δ: 2.4 (s, 3H, CH_3), 6.4 (s, 1H, CH pyrrole), 7.4-7.9 (m, 11H, Ar-H + SO_2NH_2 + NH), 8.0 (s, 1H, CH pyrimidine). ^{13}C-NMR (DMSO-d_6): 34.7, 103.4, 119.5, 120.4, 120.7 (2), 123.8, 126.7, 130.6 (2), 131.5 (2), 132.9 (2), 139.6, 141.8, 143.0, 147.8, 154.7. Anal. Calcd. for $C_{19}H_{16}BrN_5O_2S$ (458.33): C, 49.79; H, 3.52; N, 15.28;. Found: C, 49.48; H, 3.19; N, 15.56.

2.1.6. 4-(5-(4-Bromophenyl) -3-ethyl-4-imino- 3,4-dihydropyrrolo[2,3-d]pyrimidine-7-yl)benzenesulfonamide (8).

Yield % 65, m.p. 244.2 ^{o}C, IR:$\upsilon_{max.}$/cm^{-1} 3361, 3312, 3270 (NH, NH_2) 3065 (CH arom.), 2946, 2851 (CH aliph.), 1612 (C=N), 1378, 1161 (SO_2). ^{1}H-NMR (DMSO-d_6, D_2O) δ: 1.0 (t, 3H, CH_3), 4.0 (q, 2H, CH_2), 6.3 (s, 1H, CH pyrrole), 7.4- 7.9 (m, 11H, Ar-H + SO_2NH_2 + NH), 8.1 (s, 1H, CH pyrimidine). ^{13}C-NMR (DMSO-d_6): 14.1, 38.9, 103.5, 119.6, 120.5, 120.9 (2), 123.9, 124.0, 126.7 (2), 127.0 (2), 132.7 (2), 139.5, 141.9, 142.9, 147.4, 153.4. Anal. Calcd. for $C_{20}H_{18}BrN_5O_2S$ (472.36): C, 50.85; H, 3.84; N, 14.83;. Found: C, 50.61; H, 3.58; N, 14.60.

2.1.7. 4-(5-(4-Bromophenyl)-4-imino-3-propyl-3,4-dihydropyrrolo[2,3-d]pyrimidine-7-yl)benzenesulfonamide (9).

Yield % 69, m.p. 174.1 ^{o}C, IR:$\upsilon_{max.}$/cm^{-1} 3310, 3290, 3150 (NH, NH_2) 3095 (CH arom.), 2964, 2861 (CH aliph.), 1627 (C=N), 1378, 1159 (SO_2). ^{1}H-NMR (DMSO-d_6, D_2O) δ: 0.9 (t, 3H, CH_3), 1.7 (m, 2H, $\underline{CH_2}CH_3$), 3.9 (t, 2H, $\underline{CH_2}CH_2CH_3$), 6.6 (s, 1H, CH pyrrole), 7.4- 7.9 (m, 11H, Ar-H + SO_2NH_2 + NH), 8.0 (s, 1H, CH pyrimidine). ^{13}C-NMR (DMSO-d_6): 10.8, 21.1, 47.8, 103.6, 119.6, 120.5, 120.7 (2), 123.9, 126.7, 130.7 (2), 131.5 (2), 132.9 (2), 139.6, 141.9, 142.7, 147.8, 152.4. Anal. Calcd. for $C_{21}H_{20}BrN_5O_2S$ (486.38): C, 51.86; H, 4.14; N, 14.40;. Found: C, 51.50; H, 4.44; N, 14.75.

2.1.8. 4-(3-Benzyl-5-(4-Bromophenyl)- 4-imino-3,4-dihydropyrrolo[2,3-d]pyrimidine-7-yl)benzenesulfonamide (10).

Yield % 72, m.p. 157.8 °C, IR:$\upsilon_{max.}$/cm^{-1} 3380, 3329, 3290 (NH, NH_2), 3059 (CH arom.), 2960, 2920 (CH aliph.), 1625 (C=N), 1328, 1159 (SO_2).^{1}H-NMR (DMSO-d_6, D_2O) δ: 5.5 (s, 2H, CH_2), 6.7 (s, 1H, CH pyrrole), 7.2- 8.2 (m, 16H, Ar-H + SO_2NH_2 + NH), 8.5 (s, 1H, CH pyrimidine).^{13}C-NMR (DMSO-d_6): 48.6, 103.7, 119.8, 120.5, 120.9 (2), 124.0, 124.9, 126.3, 126.7 (2), 127.0 (2), 127.4 (2), 128.3 (2), 131.5 (2), 132.8, 137.5, 139.5, 142.0, 142.6, 147.9. Anal. Calcd. for $C_{25}H_{20}BrN_5O_2S$ (534.43): C, 56.18; H, 3.77; N, 13.10. Found: C, 56.48; H, 3.46; N, 13.42.

2.1.9. 4-(4-(4-bromophenyl)-3-cyano-2-((2-methylhydrazinyl)methyleneamino)-1*H*-pyrr-ol-1-yl)benzenesulfonamide (11).

A mixture of compound **6** (4.73 g, 0.01 mol.) and methyl hydrazine (0.46 g, 0.01 mol.) in ethanol (20 mL) was stirred at room temperature for 2 h, the solid formed was filtered and recrystallized from ethanol to give **11**. Yield 77%, m.p. 149.3 °C, IR:$\upsilon_{max.}$/cm^{-1} 3294, 3210, 3170 (NH, NH_2), 3100 (CH arom.), 2955, 2862 (CH aliph.), 2196 (C≡N), 1627 (C=N), 1379, 1161 (SO_2). ^{1}H-NMR (DMSO-d_6, D_2O) δ: 2.4(s, 3H, CH_3), 3.14, 3.19 (2s, 2H, 2NH, D_2O exchangeable), 5.2 (s, 1H, CH pyrrole), 7.4- 7.9 (m, 10H, Ar-H + SO_2NH_2), 8.3 (s, 1H, N=CH). ^{13}C-NMR (DMSO-d_6): 39.1, 75.1, 115.2, 115.3, 118.5, 119.8 (2), 122.1, 125.0, 126.4 (2), 127.6 (2), 131.6 (2), 140.2, 142.0, 150.3, 156.8. Anal. Calcd. for $C_{19}H_{17}BrN_6O_2S$ (473.35): C, 48.21; H, 3.62; N, 17.75. Found: C, 48.50; H, 3.31; N, 17.48.

2.1.10. *N*`-(4-(4- Bromophenyl)-3- cyano-1- (4-sulfamoylphenyl)-1*H*-pyrrol-2-yl)-*N*-(4-substitutedsulfamoylphenyl)formimidamides (12-15).

General procedure

A mixture of compound **6** (4.73 g, 0.01 mol.) and sulfa drugs namely sulfanilamide, sulfaisoxazole, sulfathiazole and/or sulfapyridine (0.01 mol.) in ethanol (20 mL) was refluxed for 1 h. The obtained solid was recrystallized from dioxane to give **12-15**, respectively.

2.1.11. *N*`-(4-(4- Bromophenyl)-3- cyano-1- (4-sulfamoylphenyl)-1*H*-pyrrol-2-yl)-*N*-(4-sulfamoylphenyl)formimidamide (12).

Yield 76%, m.p. 161.1 °C, IR:$\upsilon_{max.}$/cm^{-1} 3376, 3255 (NH, NH_2), 3095 (CH arom.), 2946, 2866 (CH aliph.), 2208 (C≡N), 1635 (C=N), 1328, 1163 (SO_2). ^{1}H-NMR (DMSO-d_6, D_2O) δ: 6.8 (s, 1H, CH pyrrole), 7.2- 8.0 (m, 16H, Ar-H + $2SO_2NH_2$), 8.5 (s, 1H, N=CH), 10.4 (s, 1H, NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6): 112.4, 114.9 (2), 116.9, 117.5, 119.3, 120.3 (2), 122.7, 123.1, 125.2 (2), 126.5 (2), 127.4 (2), 127.8, 131.8 (2), 132.8, 139.4, 143.7, 145.3, 160.8. Anal. Calcd. for $C_{24}H_{19}BrN_6O_4S_2$ (599.48): C, 48.08; H, 3.19; N, 14.02. Found: C, 48.36; H, 3.51; N, 14.31.

2.1.12. *N*`-(4-(4- Bromophenyl)-3- cyano-1- (4-sulfamoylphenyl)-1*H*-pyrrol-2-yl)-*N*-(4-(3,4-dimethylisoxazol-5-yl) sulfamoylphenyl)formimidamide (13).

Yield % 68, m.p. 193.4 °C, IR:$\upsilon_{max.}$/cm^{-1} 3485, 3381, 3236 (NH, NH_2), 3076 (CH arom.), 2946, 2814 (CH aliph.), 2202 (C≡N), 1629 (C=N), 1346, 1163 (SO_2). ^{1}H-NMR (DMSO-d_6, D_2O) δ: 1.6, 2.0 (2s, 6H, $2CH_3$), 6.2 (s, 1H, CH pyrrole), 6.6-7.9 (m, 14H, Ar-H + SO_2NH_2), 8.0 (s, 1H, N=CH), 10.3 (s, 1H, SO_2NH), 10.4 (s, 1H, NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6): 5.8, 10.2, 104.2, 112.6, 117.6 (2), 118.3, 119.6, 122.0, 123.7 (2), 124.3, 128.6, 128.9 (2), 129.4 (2), 130.6, 130.8 (2), 133.7 (2), 136.7, 139.8, 146.3, 149.2, 153.2, 156.2, 161.1. Anal. Calcd. for $C_{29}H_{24}BrN_7O_5S_2$ (694.58): C, 50.15; H, 3.48; N, 14.12. Found: C, 50.52; H, 3.79; N, 14.46.

2.1.13. *N*`-(4-(4- Bromophenyl)-3- cyano-1- (4-sulfamoylphenyl)-1*H*-pyrrol-2-yl)-*N*-(4-(*N*-thiazol-2-ylsulfamoylphenyl)formimidamide (14).

Yield % 76, m.p. 201.1 °C, IR:$\upsilon_{max.}$/cm^{-1} 3354, 3319, 3277 (NH, NH_2), 3093 (CH arom.), 2900, 2802 (CH aliph.), 2202 (C≡N), 1595 (C=N), 1323, 1138 (SO_2). ^{1}H-NMR (DMSO-d_6, D_2O) δ: 6.5 (s, 1H, CH pyrrole), 6.7- 7.9 (m, 14H, Ar-H + SO_2NH_2), 8.0 (s, 1H, N=CH), 10.5 (s, 1H, SO_2NH, D_2O exchangeable), 12.4 (s, 1H, NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6): 107.4, 112.1 (2), 112.4, 113.5, 117.5, 119.3, 123.1 (2), 124.1, 125.2, 125.3 (2), 126.1 (2), 127.0 (2), 127.3, 127.7 (2), 131.6, 131.9, 145.1, 148.5, 152.2, 161.5, 167.9. Anal. Calcd. for $C_{27}H_{20}BrN_7O_4S_3$ (682.59): C, 47.51; H, 2.95; N, 14.36. Found: C, 47.21; H, 2.63; N, 14.09.

2.1.14. *N*`-(4-(4- Bromophenyl)-3- cyano-1- (4-sulfamoylphenyl)-1*H*-pyrrol-2-yl)-*N*-(4-(*N*-pyridin-2-ylsulfamoylphenyl)formimidamide (15).

Yield % 59, m.p. 176.6 °C, IR:$\upsilon_{max.}$/cm^{-1} 3415, 3309, 3244 (NH, NH_2), 3068 (CH arom.), 2930, 2867 (CH aliph.), 2202 (C≡N), 1635 (C=N), 1382, 1151 (SO_2). ^{1}H-NMR (DMSO-d_6, D_2O) δ: 6.6 (s, 1H, CH pyrrole), 6.8-7.9 (m, 18H, Ar-H + SO_2NH_2), 8.1 (s, 1H, N=CH), 10.3 (s, 1H, SO_2NH, D_2O exchangeable), 11.0 (s, 1H, NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6): 112.1, 112.4, 113.5, 114.9 (2), 117.0, 117.6, 119.3, 119.5 (2), 120.5, 121.4, 123.2 (2), 125.3 (2), 127.1 (2), 127.3, 128.8 (2), 131.6, 138.7, 139.5, 142.9, 146.2, 148.5, 152.7, 164.0. Anal. Calcd. for $C_{29}H_{22}BrN_7O_4S_2$ (676.56): C, 51.48; H, 3.28; N, 14.49. Found: C, 51.79; H, 3.56; N, 14.18.

2.1.15. 4-(5-(4-Bromophenyl)-4-imino-7-(4-sulfamoylphenyl)-4*H*-pyrrolo[2,3-d]pyrimidin-3(7*H*)-yl)-*N*-(1-phenyl-1*H*-pyrazol-5-yl)benzenesulfonamide (16).

A mixture of compound **6** (4.73 g, 0.01 mol.) and sulfaphenazole (3.94 g, 0.01 mol.) in DMF (20 mL) containing 3 drops of triethylamine was refluxed for 6 h. The obtained solid was recrystallized from dioxane to give **16**. Yield % 69, m.p. 152.5 ^{o}C, IR:$\upsilon_{max.}$/cm^{-1} 3430, 3371, 3210 (NH, NH_2), 3055 (CH arom.), 1618 (C=N), 1378, 1161 (SO_2).^{1}H-NMR (DMSO-d_6, D_2O) δ: 6.9 (s, 1H, CH pyrrole), 7.0- 7.9 (m, 21H, Ar-H + SO_2NH_2 + 2CH pyrazole), 8.2 (s, 1H, NH imino, D_2O exchangeable), 8.3 (s, 1H, CH pyrimidine), 10.4 (s, 1H, SO_2NH, D_2O exchangeable).^{13}C-NMR (DMSO-d_6): 89.6, 112.5, 113.5 (2), 115.4, 117.5, 119.4 (2), 121.3 (2), 123.8, 124.9, 125.2, 126.8 (2), 127.1 (2), 128.4 (2), 128.7 (2), 128.9, 130.6 (2), 131.6, 132.2, 139.5, 141.0, 141.8, 142.9, 148.5, 159.9, 162.2. Anal. Calcd. for $C_{33}H_{25}BrN_8O_4S_2$ (741.64): C, 53.44; H, 3.40; N, 15.11. Found: C, 53.71; H, 3.12; N, 15.39.

2.1.16. 4-(5-(4-Bromophenyl)-3-(substituted)-4-imino-3,4-dihydropyrrolo[2,3-d]pyri-midin 7-yl)benzenesulfonamide (17-19).

General procedure

A mixture of compound **6** (4.73 g, 0.01 mol.) and aromatic amines namely 4-aminophenazone or 3,5-dimethoxyaniline and/or 2,4-dibromoaniline (0.01 mol.) in DMF (20 mL) containing 3 drops of triethylamine was refluxed for 24 h. The obtained solid was recrystallized from dioxane to give **17-19**, respectively.

2.1.17. 4-(5-(4-Bromophenyl)-3-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)- 4-imino-3,4-dihydropyrrolo[2,3-d]pyrimidin-7-yl)benzenesulfonamide (17).

Yield % 81, m.p. 127.4 ^{o}C, IR:$\upsilon_{max.}$/cm^{-1} 3412, 3391, 3262 (NH, NH_2), 3075 (CH arom.), 2961, 2836 (CH aliph.), 1688 (C=O), 1618 (C=N), 1378, 1151 (SO_2).^{1}H-NMR (DMSO-d_6, D_2O) δ: 2.3 (s, 3H, CH_3), 3.2 (s, 3H, NCH_3), 7.0 (s, 1H, CH pyrrole), 7.1- 8.0 (m, 15H, Ar-H + SO_2NH_2), 8.1 (s, 1H, CH pyrimidine), 8.3 (s, 1H, NH imino, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6): 14.2, 38.9, 109.1, 112.9 (2), 120.5, 123.7, 124.4, 125.3, 126.3 (2), 127.3, 128.7, 129.0 (2), 130.6 (2), 131.9 (2), 132.4, 133.1 (2), 134.2, 137.8, 139.0, 141.8, 158.9, 161.5, 162.3. Anal. Calcd. for $C_{29}H_{24}BrN_7O_3S$ (630.52): C, 55.24; H, 3.84; N, 15.55. Found: C, 55.56; H, 3.61; N, 15.17.

2.1.18. 4-(5-(4-Bromophenyl)-3-(3,5-dimethoxyphenyl) -4-imino -3,4-dihydropyrrolo-[2,3-d]pyrimidin- 7-yl)benzenesulfonamide (18).

Yield % 83, m.p. 118.2 ^{o}C, IR:$\upsilon_{max.}$/cm^{-1} 3314, 3300, 3233 (NH, NH_2), 3077 (CH arom.), 2926, 2848 (CH aliph.), 1600 (C=N), 1378, 1161 (SO_2).^{1}H-NMR (DMSO-d_6, D_2O) δ: 3.8 (s, 6H, $2OCH_3$), 6.9 (s, 1H, CH pyrrole), 7.0- 8.0 (m, 13H, Ar-H + SO_2NH_2), 8.4 (s, 1H, CH pyrimidine), 10.3 (s, 1H, SO_2NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6): 55.4 (2), 94.6 (2), 95.7 (2), 117.5, 119.4, 121.3 (2), 123.5, 124.1, 125.2 (2), 127.8 (2), 130.7 (2), 132.3, 139.5, 142.9, 148.5, 160.4, 161.1 (2), 162.2. Anal. Calcd. for $C_{26}H_{22}BrN_5O_4S$ (580.45): C, 53.80; H, 3.82; N, 12.07. Found: C, 53.50; H, 3.61; N, 12.37.

2.1.19. 4-(5-(4-Bromophenyl)-3-(2,4-dibromophenyl) -4-imino -3,4-dihydropyrrolo[2,3-d]pyrimidin- 7-yl)benzenesulfonamide (19).

Yield % 68, m.p. 161.1 ^{o}C, IR:$\upsilon_{max.}$/cm^{-1} 3389, 3311, 3218 (NH, NH_2), 3066 (CH arom.), 1613 (C=N), 1378, 1156 (SO_2).^{1}H-NMR (DMSO-d_6, D_2O) δ: 6.6 (s, 1H, CH pyrrole), 7.0- 8.0 (m, 13H, Ar-H + SO_2NH_2), 8.2 (s, 1H, CH pyrimidine), 10.3 (s, 1H, NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6):106.1, 113.5, 116.6, 117.5, 119.4, 121.3, 125.2 (2), 125.3, 127.1, 127.4 (2), 127.8 (2), 130.9, 131.5 (2), 132.3, 133.4, 139.5,141.8, 145.3, 148.5, 162.2. Anal. Calcd. for $C_{24}H_{16}BrN_5O_2S$ (678.19): C, 42.50; H, 2.38; N, 10.33. Found: C, 42.82; H, 2.71; N, 10.68.

2.1.20. *N*'-(4-(4-Bromophenyl)-3-cyano-1-(4-sulfamoylphenyl)-1*H*-pyrrol-2-yl)-*N*-(4-ethoxyphenyl)formimidamide (20) and 4-(5-(4-bromophenyl)-3-(4-ethoxyphenyl)-4-imino-3,4-dihydropyrrolo[2,3-d]pyrimidin-7-yl)benzenesulfonamide (21).

A mixture of compound **6** (4.73 g, 0.01 mol.) and p-phentidene (1.37 g, 0.01 mol.) in ethanol (20 mL) was refluxed for 1 h. The obtained solid was recrystallized from ethanol to give **20**, while compound **21** was obtained in a good yield via reaction of compound **6** (4.73 g, 0.01 mol.) with p-phenetidene (1.37 g, 0.01 mol.) in DMF (20 mL) in presence of TEA (3 drops) was refluxed for 5 h. The obtained solid was recrystallized from dioxane to give **21**.

20: Yield % 76, m.p. 186.5 ^{o}C, IR:$\upsilon_{max.}$/cm^{-1} 3390, 3334, 3290 (NH, NH_2), 3080 (CH arom.), 2978, 2870 (CH aliph.), 2200 (C≡N), 1593 (C=N), 1388, 1163 (SO_2). ^{1}H-NMR (DMSO-d_6, D_2O) δ: 1.3 (t, 3H, CH_3), 4.0 (q, 2H, CH_2), 6.6 (s, 1H, CH pyrrole), 6.8-8.0 (m, 14H, Ar-H + SO_2NH_2), 8.9 (s, 1H, N=CH), 10.5 (s, 1H, NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6): 14.5, 63.3, 113.5, 114.4 (2), 115.2 (2), 115.3, 117.5, 119.5, 120.5 (2), 121.3, 122.5, 125.4 (2), 126.8 (2), 127.6 (2), 131.7, 132.5, 139.5, 142.9, 148.5, 154.8. Anal. Calcd. for $C_{26}H_{22}BrN_5O_3S$ (564.45): C, 55.32; H, 3.93; N, 12.41. Found: C, 55.67; H, 3.67; N, 12.12.

21: Yield 89 %, m.p. 129.9 ^{o}C, IR:$\upsilon_{max.}$/cm^{-1} 3412, 3378, 3190 (NH, NH_2), 3071 (CH arom.), 2930, 2871 (CH aliph.), 1597 (C=N), 1378, 1161 (SO_2).^{1}H-

NMR (DMSO-d_6, D_2O) δ: 1.3 (t, 3H, CH_3), 4.1 (q, 2H, CH_2), 6.8 (s, 1H, CH pyrrole), 6.9- 8.0 (m, 14H, Ar-H + SO_2NH_2), 8.4 (s, 1H, CH pyrimidine), 10.0 (s, 1H, NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6): 14.6, 63.1, 114.3, 155.1 (2), 115.2 (2), 117.5, 119.6, 120.5 (2), 122.3, 123.2, 125.2 (2), 127.3 (2), 130.6 (2), 132.3, 139.5, 139.9, 142.9, 148.5, 150.3, 162.2. Anal. Calcd. for $C_{26}H_{22}BrN_5O_3S$ (564.45): C, 55.32; H, 3.93; N, 12.41. Found: C, 54.94; H, 3.71; N, 12.12.

General procedure for the synthesis of compounds (22-26).

A mixture of compound **6** (4.73 g, 0.01 mol.) and aromatic amines amely 4-aminopyridine or 2-amino-5-choro pyridine or 2-amino pyrimidine or 2-amino-4,6-dimethyl pyrimidine and/or 5-aminouracil (0.01 mol.) in DMF (20 mL) in presence of TEA (3 drops) was refluxed for 8 h. The obtained solid was recrystallized from dioxane to give **22-26**, respectively.

2.1.21. 4-(5-(4-Bromophenyl)-4-imino-3-(pyridine-4-yl)-3,4-dihydropyrrolo[2,3-d]pyrim-idin-7-yl)benzenesulfonamide (22).

Yield % 62, m.p. 134.0 °C, IR:$\upsilon_{max.}$/cm^{-1} 3421, 3386, 3261 (NH, NH_2), 3061 (CH arom.), 1612 (C=N), 1378, 1151 (SO_2).^{1}H-NMR (DMSO-d_6, D_2O) δ: 6.4 (s, 1H, CH pyrrole), 6.9- 8.0 (m, 14H, Ar-H + SO_2NH_2), 8.4 (s, 1H, CH pyrimidine), 10.0 (s, 1H, NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6): 100.7, 108.9 (2), 120.5, 123.1, 123.6 (2), 124.3, 125.2, 126.3 (2), 127.3(2), 130.5 (2), 132.3, 132.6, 139.4, 141.3, 149.8 (2), 153.0, 162.2. Anal. Calcd. for $C_{23}H_{17}BrN_6O_2S$ (521.39): C, 52.98; H, 3.29; N, 16.12. Found: C, 52.71; H, 3.52; N, 16.46.

2.1.22. 4-(5-(4-Bromophenyl)-3-(5-chloropyridin-2yl)--4-imino-3,4-dihydropyrrolo[2,3-d]pyrimidin-7-yl)benzenesulfonamide (23).

Yield % 66, m.p. 275.2 °C, IR:$\upsilon_{max.}$/cm^{-1} 3391, 3312, 3276 (NH, NH_2), 3056 (CH arom.), 1610 (C=N), 1376, 1151 (SO_2), 751 (C-Cl).^{1}H-NMR (DMSO-d_6, D_2O) δ: 7.3 (s, 1H, CH pyrrole), 7.4- 8.0 (m, 13H, Ar-H + SO_2NH_2), 8.3 (s, 1H, CH pyrimidine), 8.5 (s, 1H, NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6): 103.3, 114.4, 116.2, 120.9, 123.5, 123.6 (2), 124.7, 126.8, 127.0 (2), 127.1 (2), 130.7 (2), 131.8, 132.6, 137.5, 139.4, 146.0, 150.6, 151.5, 162.2. Anal. Calcd. for $C_{23}H_{16}BrClN_6O_2S$ (555.83): C, 49.70; H, 2.90; N, 15.12. Found: C, 49.43; H, 2.61; N, 15.39.

2.1.23. 4-(5-(4-Bromophenyl)- 4-imino- 3-(pyrimidin-2-yl)) -3,4-dihydropyrrolo[2,3-d]pyramidin-7-yl)benzenesulfonamide (24).

Yield 59 %, m.p. 132.2 °C, IR:$\upsilon_{max.}$/cm^{-1} 3361, 3346, 3265 (NH, NH_2), 3067 (CH arom.), 1621 (C=N), 1378, 1161 (SO_2).^{1}H-NMR (DMSO-d_6, D_2O) δ: 6.9 (s, 1H, CH pyrrole), 7.0- 8.1 (m, 13H, Ar-H + SO_2NH_2), 8.2 (s, 1H, CH pyrimidine), 10.6 (s, 1H, NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6): 108.3, 110.0, 119.4, 123.8, 125.2, 125.3 (2), 126.8, 127.0 (2), 127.1 (2), 127.4 (2), 131.9, 132.3, 148.5, 152.4, 159.6, 159.9, 161.0, 162.2. Anal. Calcd. for $C_{22}H_{16}BrN_7O_2S$ (522.38): C, 50.58; H, 3.09; N, 18.77. Found: C, 50.18; H, 3.31; N, 18.99.

2.1.24. 4-(5-(4-Bromophenyl)-3-(4,5-dimethylpyrimidin-2-yl)- 4-imino-3,4-dihydropyrrolo-[2,3-d]pyrimidin-7-yl)benzenesulfonamide (25).

Yield % 64, m.p. 118.9 °C, IR:$\upsilon_{max.}$/cm^{-1} 3417, 3361, 3312 (NH, NH_2), 3076 (CH arom.), 2981, 2861 (CH aliph.), 1596 (C=N), 1378, 1161 (SO_2). ^{1}H-NMR (DMSO-d_6, D_2O) δ: 2.3 (s, 6H, $2CH_3$), 6.4 (s, 1H, CH pyrimidine-dimethyl), 7.2 (s, 1H, CH pyrrole), 7.3-8.0 (m, 10H, Ar-H + SO_2NH_2), 8.6 (s, 1H, CH pyrimidine), 10.5 (s, 1H, NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6): 23.2 (2), 108.0, 112.7, 117.5, 118.5, 119.2 (2), 120.5, 123.2, 125.6 (2), 129.2 (2), 131.1 (2), 132.3, 133.5, 139.5, 151.0, 161.5, 162.3, 166.8, 167.9. Anal. Calcd. for $C_{24}H_{20}BrN_7O_2S$ (549.05): C, 52.37; H, 3.66; N, 17.81. Found: C, 52.71; H, 3.37; N, 17.50.

2.1.25. 4-(5-(4-Bromophenyl)-3-(2,4-dihydroxypyrimidin-5-yl)- 4-imino-3,4-dihydro-py-rrolo [2,3-d]pyrimidin-7-yl)benzenesulfonamide (26).

Yield % 78, m.p. >350 °C, IR:$\upsilon_{max.}$/cm^{-1} 3470 (OH), 3376, 3333, 3189 (NH, NH_2), 3077 (CH arom.), 1598 (C=N), 1379, 1151 (SO_2).^{1}H-NMR (DMSO-d_6, D_2O) δ: 6.7 (s, 1H, CH pyrrole), 7.0- 7.9 (m, 10H, Ar-H + SO_2NH_2 + CH uracil), 8.8 (s, 1H, CH pyrimidine), 9.1(s, 1H, CH pyrimidine), 10.0 (s, 1H, NH, D_2O exchangeable), 13.3 (s, 2H, 2OH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6): 114.2, 119.8, 121.9, 122.7 (2), 123.6, 125.4, 127.6, 129.3 (2), 130.1 (2), 130.8 (2), 136.0, 136.7, 138.6, 141.9, 149.3, 152.7, 160.6, 162.2. Anal. Calcd. for $C_{22}H_{16}BrN_7O_4S$ (554.38): C, 47.66; H, 2.91; N, 17.69. Found: C, 47.28; H, 2.68; N, 17.46.

2.1.26. 4-(2-Amino-9-(4-bromophenyl)-7*H*-pyrrolo[3,2-e][1,2,4]triazolo[1,5-c]pyrimi-din-7-yl)benzenesulfonamide (28).

A mixture of compound **6** (4.73 g, 0.01 mol.) and thiosemicarbazide (0.91 g, 0.01 mol.) in DMF (20 mL) containing 3 drops of triethylamine was refluxed for 24 h. The obtained solid was recrystallized from dioxane to give **28**. Yield 74 %, m.p. 112.0 °C, IR:$\upsilon_{max.}$/cm^{-1} 3391, 3376, 3210 (NH_2), 3066 (CH arom.), 1621 (C=N), 1378, 1161 (SO_2).^{1}H-NMR (DMSO-d_6, D_2O) δ: 6.3 (s, 1H, CH pyrrole), 7.0 (s, 2H, NH_2, D_2O exchangeable), 7.4- 8.2 (m, 10H, Ar-H + SO_2NH_2), 9.2 (s, 1H, CH pyrimidine. ^{13}C-NMR (DMSO-d_6): 104.1, 119.4, 123.8 (2), 124.6, 127.1 (2), 127.8, 128.7 (2), 131.3 (2), 132.6, 136.4, 139.3, 142.6, 149.4, 155.6, 162.2. Anal. Calcd. for

$C_{19}H_{14}BrN_7O_2S_2$ (484.33): C, 47.12; H, 2.91; N, 20.24. Found: C, 47.19; H, 3.00; N, 19.98.

2.1.27. 4-(9-(4-Bromophenyl)-2-phenyl-7*H*-pyrrolo[3,2-e][1,2,4]triazolo[1,5-c]pyrim-idin-7-yl)benzenesulfonamide (30).

A mixture of compound **6** (4.73 g, 0.01 mol.) and benzoylhydrazine (1.36 g, 0.01 mol.) in DMF (20 mL) containing 3 drops of triethylamine was refluxed for 24 h. The obtained solid was recrystallized from acetic acid to give **30**. Yield 69 %, m.p. 213.6 ^oC, IR:$\upsilon_{max.}/cm^{-1}$ 3410, 3386 (NH_2), 3091 (CH arom.), 1618 (C=N), 1378, 1151 (SO_2).^{1}H-NMR (DMSO-d_6, D_2O) δ: 7.5 (s, 1H, CH pyrrole), 7.6- 8.3 (m, 15H, Ar-H + SO_2NH_2), 9.2 (s, 1H, CH pyrimidine). ^{13}C-NMR (DMSO-d_6): 103.8, 117.2, 119.9 (2), 124.0, 124.6 (2), 126.3 (2), 126.7, 126.9, 128.7 (2), 129.2 (2), 129.6, 130.4 (2), 131.1, 131.5, 135.9, 139.2, 141.2, 149.1, 162.2. Anal. Calcd. for $C_{25}H_{17}BrN_6O_2S$ (545.41): C, 55.05; H, 3.14; N, 15.41. Found: C, 55.13; H, 3.01; N, 14.99.

2.1.28. 4-(5-(4-Bromophenyl)-3-(5,6-dimethyl-1,2,4-triazin-3-yl)-4-imino-3,4-dihydro-pyrrolo[2,3-d]pyrimidin-7-yl)benzenesulfonamide (31).

A mixture of compound **6** (4.73 g, 0.01 mol.) and 3-amino-5,6-dimethyl-1,2,4-triazine (1.22 g, 0.01 mol.) in DMF (20 mL) in presence of of triethylamine (3 drops) was refluxed for 6 h. The obtained solid was recrystallized from dioxane to give **31.** Yield 58 %, m.p. 145.5 ^oC, IR:$\upsilon_{max.}/cm^{-1}$ 3341, 3311, 3256 (NH, NH_2), 3045 (CH arom.), 2962, 2831 (CH aliph.), 1611 (C=N), 1387, 1151 (SO_2).^{1}H-NMR (DMSO-d_6, D_2O) δ: , 2.7 (s, 6H, $2CH_3$), 6.9 (s, 1H, CH pyrrole), 7.1- 7.9 (m, 10H, Ar-H + SO_2NH_2), 9.8 (s, 1H, CH pyrimidine), 10.8 (s, 1H, NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6): 21.2 (2), 113.5, 119.5, 121.3, 125.6 (2), 126.1, 127.3, 128.2 (2), 130.5 (2), 132.3 (2), 132.8, 139.5, 141.8, 142.9, 148.5, 159.9, 161.5, 162.2. Anal. Calcd. for $C_{23}H_{19}BrN_8O_2S$ (551.42): C, 50.10; H, 3.47; N, 20.32. Found: C, 50.43; H, 3.19; N, 20.66.

2.2. 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 $mol^{-1}A^{o-1}$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.

2.3. Biological screening

2.3.1. 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 Sulfo-Rhodamine-B stain (SRB) assay using the reported method [Skehan et al., 1990]. 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. Test compounds were dissolved in dimethylsulfoxide. 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 ^oC and in atmosphere of 5% CO_2. After 48 h, cells were fixed, washed and stained for 30 min with 0.4% (wt/vol) SRB dissolved in 1% acetic acid. Excess unbound dye was removed by four washes with 1% acetic acid and attached stain was recovered with Trise-EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration 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 means ± standard error .

2.3.2. Molecular Docking

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 (**Figure 1**). The ligand interacts with the active site amino acids by three interactions: with Met 343 with hydrogen bond of 3.09 A^o, with Glu

341 with hydrogen bond of 2.22 A^o and with Thr 340 with hydrogen bond of 3.22 A^o as shown in **Figure 1**. All the synthesized compounds were docked on the active site of the enzyme showing good fitting. The 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 **15** and **31** with S = -20.1891and -20.3610 Kcal/mol, respectively. **Figure 2** and **3** describes the amino acid interactions with compounds **15** and **31**, respectively.

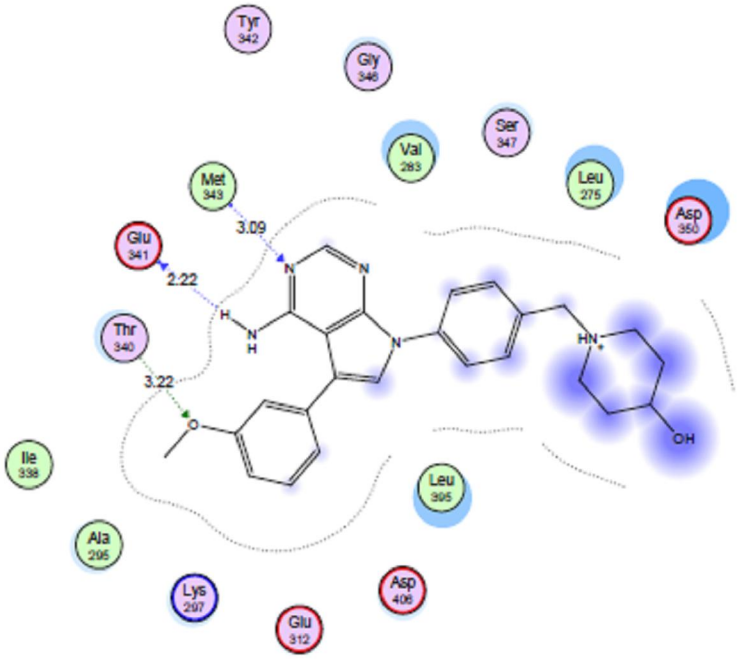

Figure 1. pyrrolpyrimidine ligand on the active site c-Src enzyme

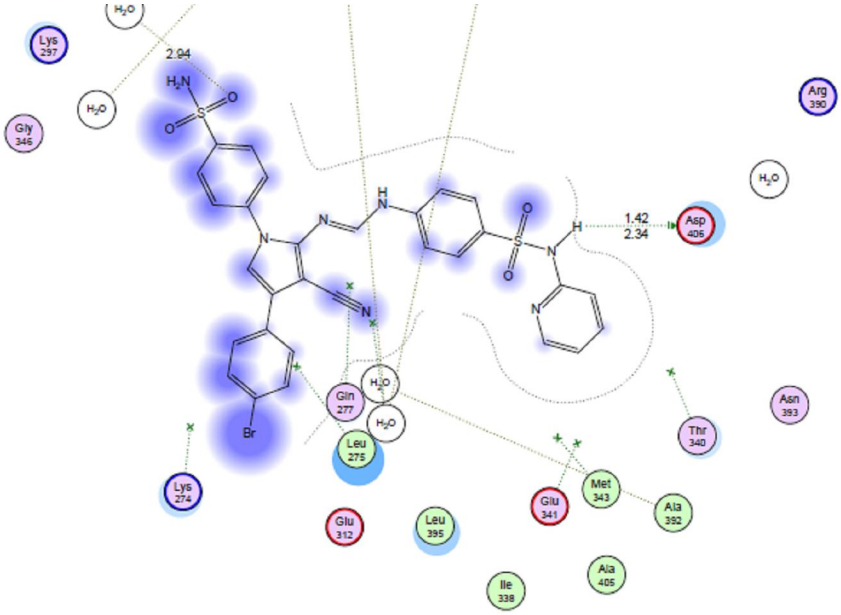

Figure 2. Compound **15** on the active site c-Src enzyme

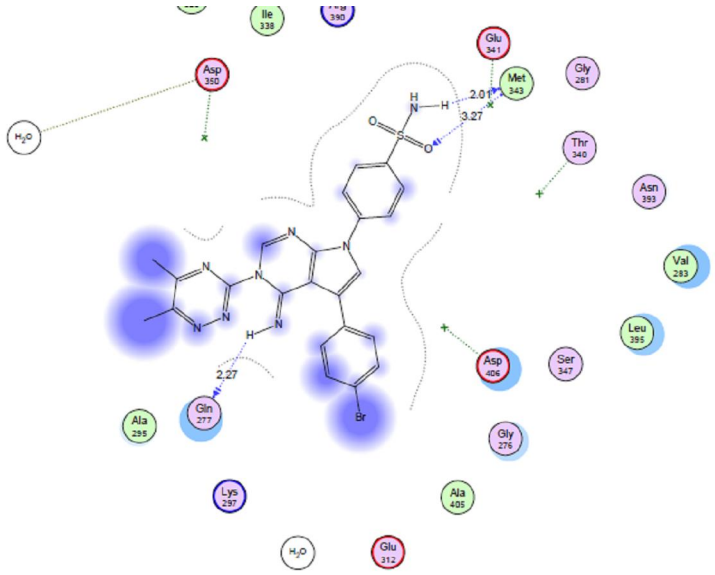

Figure 3. Compound **31** on the active site c-Src enzyme

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

Compound No.	S Kcal/Mol	Amino acid interactions	Interacting groups	H bond length A°
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
7	-7.3581	Asp 350	SO_2NH_2	1.31
8	-7.9267	Asp 406, Leu 275 Ser 347	C=NH, SO_2NH_2 SO_2	1.37, 1.54 2.95
9	-11.6258	Asp 406, Leu 275	C=NH, SO_2NH_2	1.39, 1.74
10	-16.2385	Asp 406, Leu 275	C=NH, SO_2NH_2	1.36, 1.85
11	-14.2511	Met 343, Glu 312 Asp 406	NH, SO_2NH_2 SO_2NH_2	2.45, 1.30 2.26
12	-10.3500	Gln 277, Arg 390 Asp 388	NH, SO_2 SO_2NH_2	1.64, 2.61 1.50
13	-17.5183	Lys 274	CN	3.31
14	-15.3214	Asp 350, Leu 274 Gln 277	SO_2NH_2,CN NH	1.49, 2.77 2.28
15	-20.1891	Asp 406	NH	1.42, 2.34
16	-17.3110	Asp 406, Gln 277	SO_2NH, C=NH	1.29, 2.54
17	-14.9782	Gln 277	C=NH	2.60, 2.49
18	-19.2743	Asp 406	OCH_3, C=NH	3.47, 1.40
19	-17.1698	Asp 350	SO_2NH_2	2.02, 2.23
20	-13.6863	Asp 406, Leu 275	NH, SO_2NH_2	1.09, 1.69
21	-15.7630	Met 343	SO_2NH_2 SO_2NH_2	1.91, 3.69
22	-15.6582	Asp 406, Asp 350	C=NH, SO_2NH_2	1.31, 2.28
23	-17.8734	Gln 277	C=NH	2.53, 2.81
24	-17.1883	Asp 350	SO_2NH_2	1.51
25	-17.6001	Asp 406, Leu 275	C=NH, SO_2NH_2	2.60, 1.59
26	-11.1602	Met 343 Asp 350	SO_2NH_2, SO_2NH_2 OH	1.63, 2.90 3.04
28	-14.7554	Asn 303, Met 343	SO_2NH_2, NH_2	1.80, 2.16
30	-12.1645	Asp 406 Asp 350	NH SO_2NH_2	1.43 1.57, 2.3
31	-20.3610	Gln 277 Met 343	C=NH SO_2NH_2	2.27 2.01, 3.27

2.3.3. Biological screening

2.3.3.1. 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.

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

Compound	Compound concentration (µM)				IC_{50} (µM)
	10µM	25µM	50µM	100µM	
	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	0.300±0.001	0.266±0.011	0.251±0.114	0.275±0.112	7.01
7	0.271±0.011	0.247±0.008	0.251±0.003	0.263±0.012	6.74
8	0.274±0.100	0.259±0.002	0.246±0.021	0.284±0.031	6.74
9	0.279±0.007	0.300±0.044	0.250±0.022	0.258±0.021	6.74
10	0.301±0.100	0.274±0.181	0.300±0.024	0.311±0.051	7.01
11	0.311±0.041	0.222±0.162	0.231±0.011	0.288±0.042	6.88
12	0.264±0.001	0.230±0.033	0.250±0.051	0.272±0.064	6.74
13	0.301±0.077	0.282±0.021	0.251±0.111	0.288±0.133	7.29
14	0.329±0.041	0.288±0.022	0.282±0.001	0.317±0.035	7.29
15	0.309±0.011	0.281±0.052	0.277±0.011	0.280±0.044	7.90
16	0.294±0.101	0.232±0.005	0.265±0.041	0.321±0.009	7.01
17	0.320±0.002	0.333±0.004	0.291±0.015	0.361±0.072	7.29
18	0.281±0.055	0.344±0.014	0.358±0.020	0.363±0.030	7.01
19	0.300±0.011	0.295±0.013	0.296±0.012	0.311±0.009	8.30
20	0.325±0.019	0.289±0.011	0.300±0.019	0.356±0.018	7.29
21	0.365±0.021	0.280±0.049	0.255±0.031	0.349±0.011	7.86
22	0.367±0.052	0.365±0.014	0.317±0.022	0.323±0.019	7.84
23	0.208±0.070	0.208±0.081	0.215±0.062	0.276±0.077	6.46
24	0.313±0.052	0.311±0.031	0.296±0.068	0.310±0.002	7.32
25	0.330±0.044	0.292±0.001	0.290±0.069	0.294±0.022	7.01
26	0.254±0.061	0.290±0.032	0.229±0.045	0.257±0.004	6.46
28	0.247±0.001	0.228±0.014	0.231±0.008	0.239±0.011	6.46
30	0.338±0.022	0.302±0.017	0.324±0.052	0.267±0.034	7.84
31	0.389±0.011	0.344±0.083	0.267±0.039	0.256±0.001	8.39

* Each value is the mean of three values ± Standard Error.

2.4. Chemistry

The synthetic procedures adopted to obtain the target compounds are depicted in **Figures (4-7)**. In this work the reactivity of Sulfanilamide **1** towards phenacyl bromide was studied. Thus, interaction of Sulfanilamide **1** with phenacyl bromide **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-*0*-aminocarbonitrile **5** (**Fig. 4**). 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. 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. IR spectrum of compound **5** exhibited bands for (NH_2), (CN) and (SO_2) groups. ^{1}H-NMR spectrum of **5** (in DMSO-d_6) 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**. IR spectrum of compound **6** revealed the presence of charectrestic bands for (NH_2), (CN) and (SO_2) groups. ^{1}H-NMR spectrum of **6** in (DMSO-d_6) showed a triplet for CH_3 group and a quartet for CH_2 group. This later compound was used as intermediate for the synthesis of the key imino-amino derivatives **7-10** by cyclization with different amines as depicted in (**Fig. 4**). The structure of compounds **7-10** was proved on the basis of elemental analyses, IR, ^{1}H-NMR and ^{13}C-NMR spectra. IR spectra of compounds **7-10** revealed the absence of (CN) band and the presence of bands corresponding to (NH) and (CH aliphatic). ^{1}H-NMR spectrum for compound **7** showed singlet at 2.4 ppm for CH_3 group. ^{1}H-NMR spectrum for compound **8** revealed a triplet at 1.0 ppm for CH_3 group and a quartet at 4.0 ppm for CH_2 group. ^{1}H-NMR spectrum for compound **9** exhibited a triplet at 0.9 ppm due to CH_3 group and . ^{1}H-NMR spectrum of compound **10** showed singlet at 5.2 ppm for CH_2 group. Interaction of compound **6** with methyl hydrazine in ethanol at room temperature gave the 2-methyl hydrazinyl derivative **11**. IR spectrum of **11** revealed bands at 3294, 3210, 3170 (NH, NH_2), 2196 (CN). ^{1}H-NMR spectrum of compound **11** (in DMSO-d_6) showed singlet at 2.4 ppm assigned to CH_3 group. Reaction of compound **6** with sulfonamide derivatives afforded the corresponding formimidamide derivatives **12-15**, respectively (**Fig. 5**). IR spectra of compounds **12-15** revealed the presence of (CN) band. ^{1}H-NMR spectra for compounds **12-15** showed singlet for N=CH group. On the other hand, interaction of **6** with sulfaphenazole in DMF in presence of triethylamine as catalyst yielded the corresponding pyrrolopyrimidine derivative **16**. IR spectrum of **16** showed the absence of (CN) band. ^{1}H-NMR spectrum of **16** (in DMSO-d_6) exhibited singlet at 8.2 ppm due to the imino group. Compound **17** was obtained via reaction of compound **6** with 4-aminophenazole (**Fig. 6**). IR spectrum of compound **17** revealed the absence of (CN) band. ^{1}H-NMR showed signals at 2.3 ppm for CH_3 group and 3.2 ppm attributed to NCH_3. Interaction of compound **6** with 3,4-dimthoxyaniline and/or 2,4-dibromoaniline afforded the pyrrolopyrimidine derivatives **18** and **19**, respectively. IR spectra of compounds **18** and **19** revealed the absence of (CN) band. ^{1}H-NMR spectrum of compound **18** showed singlet at 3.8 ppm attributed to two methoxy groups. ^{1}H-NMR of compound **19** exhibited singlet at 8.2 ppm attributed to CH of pyrimidine. When compound **6** was reacted with *p*-phentidene in ethanol gave the corresponding pyrrole derivative **20**, while in refluxing DMF containing triethylamine as a catalyst afforded cyclic system pyrrolopyrimidine **21**. The structures of compounds **20** and **21** were confirmed via elemental analyses and spectral data. IR spectrum of compound **20** revealed the presence of (CN) band. ^{1}H-NMR spectrum in (DMSO-d_6) showed triplet at 1.3 ppm and a quartet at 4.0 ppm attributed to OC_2H_5 group. IR spectrum of compound **21** revealed the absence of (CN) band. ^{1}H-NMR spectrum of **21** (in DMSO-d_6) exhibited singlet at 8.4 ppm attributed to CH of pyrimidine. Interaction of compound **6** with 4-aminopyridine and/or 2-amino-3-chloropyridine in DMF containing catalytic amount of triethylamine furnished pyrrolopyrimidine derivatives **22** and **23**, respectively. IR spectra of compounds **22** and **23** revealed the absence of (CN) band. ^{1}H-NMR spectra of compounds **22** and **23** (in DMSO-d_6) exhibited signals at 8.5 and 8.6 ppm attributed to imino groups. Pyrrolopyrimidines **24- 26** were obtained in good yields via reaction of compound **6** with 2-aminopyrimidine, or 2-amino-4,6-dimethylpyrimidine and/or 5-aminouracil in DMF containing triethylamine. IR spectra of compounds **24- 26** showed the absence of (CN) group. ^{1}H-NMR spectra of **24- 26** revealed the presence of a singlet attributed to CH of pyrimidine. The pyrrolotriazolopyrimidines **28** and **30** were obtained via reaction of compound **6** with thiosemicarbazide and/or benzoylhydrazide via the formation of intermediates **27** and **29**, respectively (**Fig 7**). IR spectra of compounds **28** and **30** revealed the absence of (CN) band. ^{1}H-NMR spectrum of compound **28** exhibited a singlet at 7.0 ppm attributed to (NH_2) group, while ^{1}H-NMR spectrum of compound **30** revealed a singlet at 9.2 ppm attributed to CH of pyrimidine. Finally, the reaction of compound **6** with 3-amino-5,6-dimethyl-1,2,4-triazine afforded the triazino derivative **31**. IR spectrum of compound **31** revealed the absence of (CN) band . ^{1}H-NMR of compound **31** exhibited singlet at 2.7 ppm attributed to two CH_3 groups.

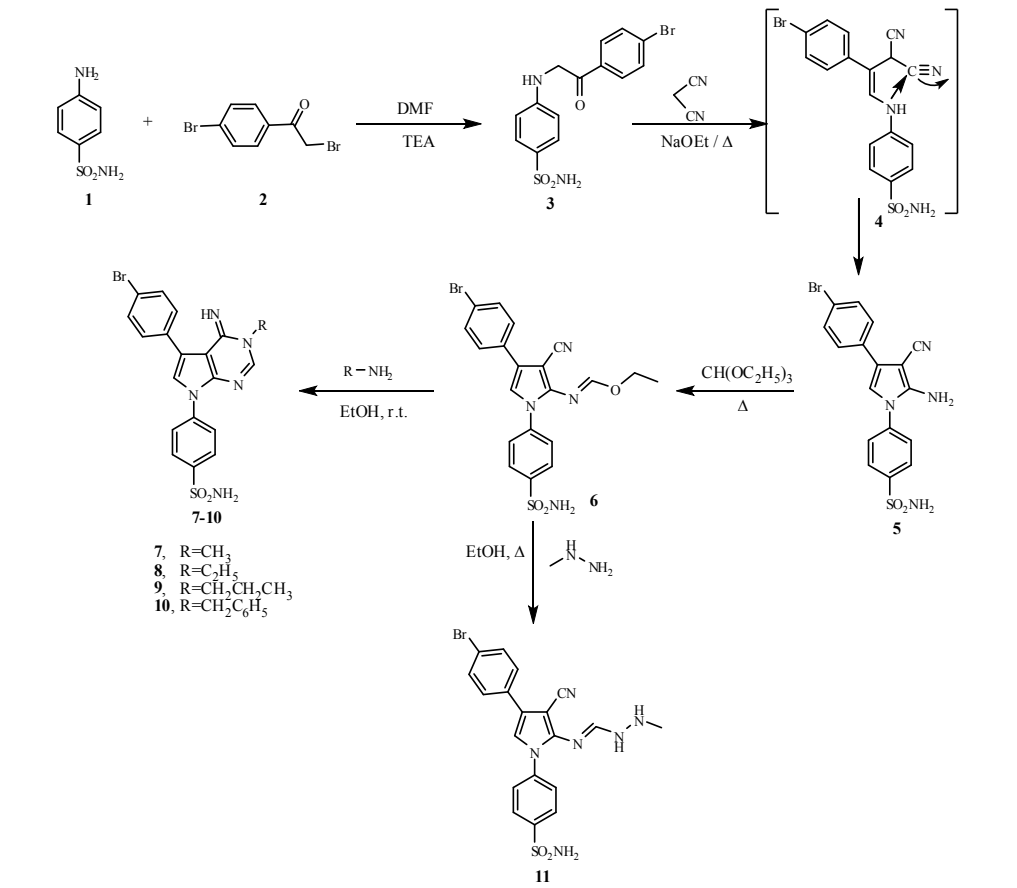

Figure 4. Synthetic pathways for compounds (**5-11**)

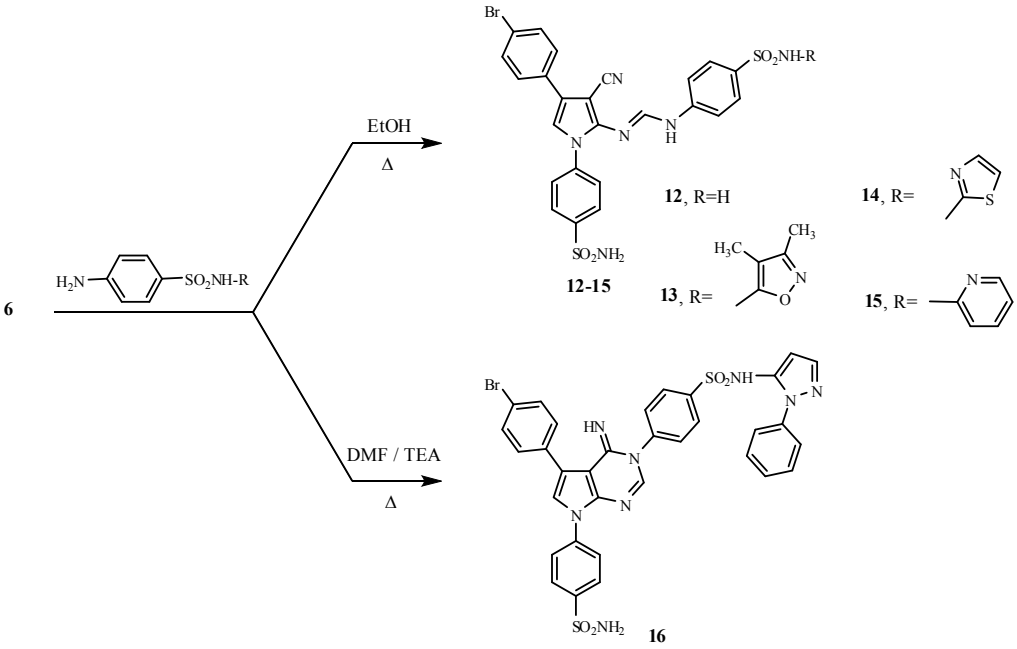

Figure 5. Synthetic pathways for compounds (**12-16**)

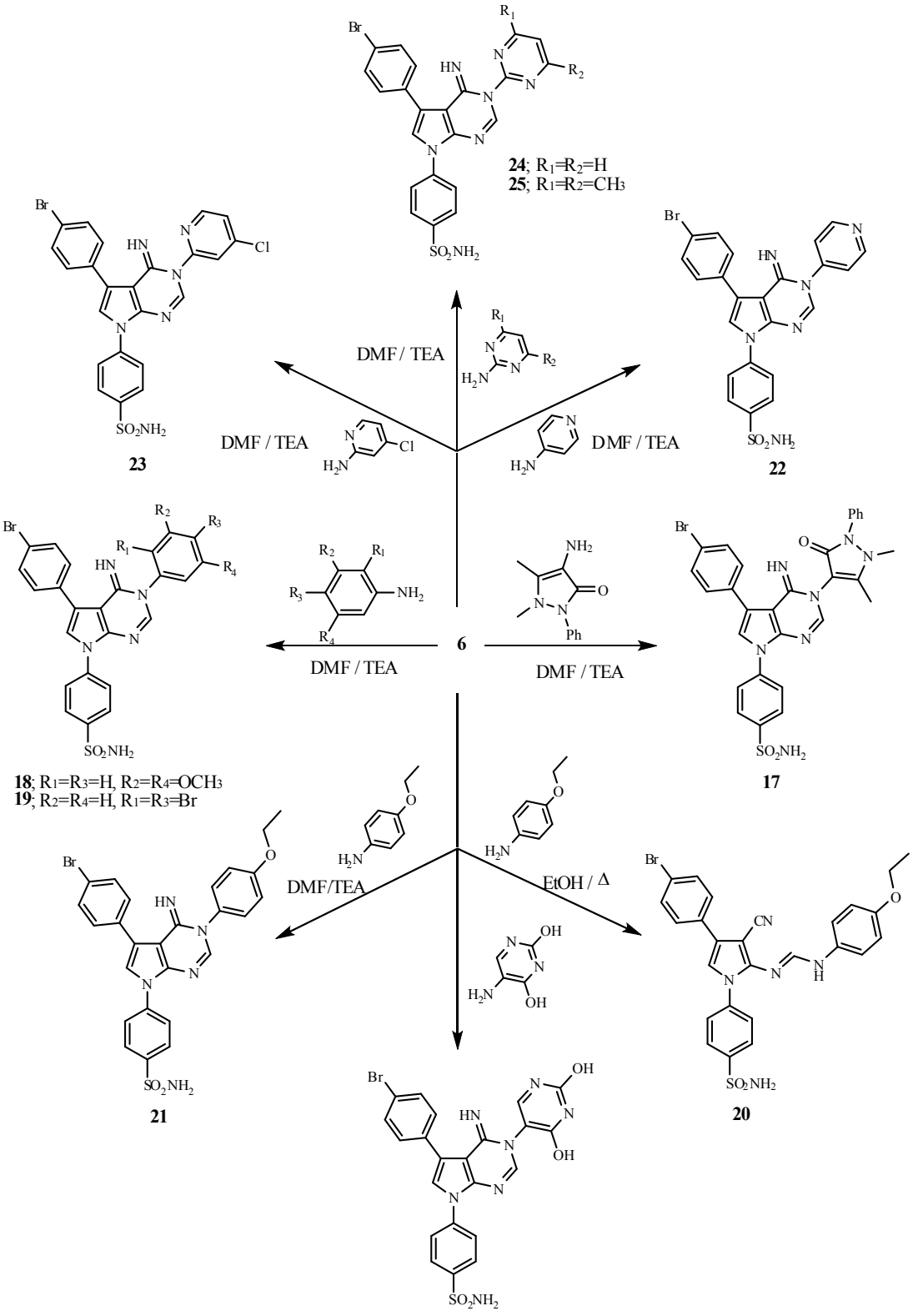

Figure 6. Synthetic pathways for compounds (**17-26**)

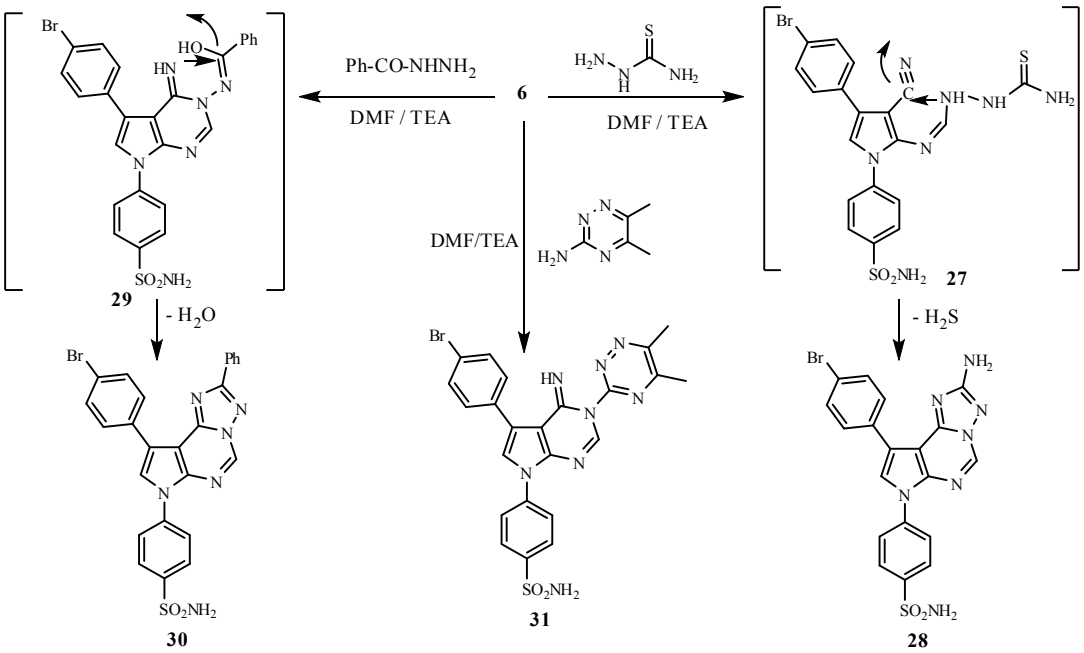

Figure 7. Synthetic pathways for compounds (**28**, **30**, **31**)

3.1.1. Molecular Docking

Protein kinases play a crucial role in signal transduction as well as in cellular proliferation, differentiation, and various regulatory mechanisms. The inhibition of growth related kinases, especially tyrosine kinases, might provide new therapies for diseases such as cancer [Traxler et al., 2001]. The progress made in the crystallization of protein kinases has confirmed that the ATP-binding domain of tyrosine kinases is an attractive target for drug design. Over the past years, numerous reviews have covered the design, synthesis and biological evaluation of ATP site-directed inhibitors of PTKs [MacMahon et al., 1998; Traxler, 1998; Showalter and Kraker, 1997; Strawn and Shawver, 1998; Bridges, 1998], among them was pyrrolo[d]pyrimidines [Traxler et al., 1996]. PKI166, a pyrrolopyrimidine derivative inhibits c-Src kinase with an IC_{50} of 0.13 µM [Traxler et al., 2001] which was encouraging in this investigation to perform molecular docking study on the active site of c-Src kinase enzyme to explain the cytotoxic activity of the synthesized compounds. All the synthesized compounds were fit into the active site of the enzyme with good energy scores comparable to the co-crystallized ligand and most of the compounds showed good amino acids interactions with the active sites amino acids. Compounds **5**, **8**, **11**, **12** and **14** interacted with three amino acids of the active site of the enzyme while compounds **9**, **10**, **16**, **22**, **25**, **26**, **28**, **30** and **31** interacted with two amino acids of the active site of the compounds. On the other hand, compounds **6**, **7**, **9**, **13**, **15**, **17**, **18**, **19**, **20**, **21**, **23** and **24** interacted with one amino acid of the active site of the enzyme. Docking score energies ranged between -7.3581 and -20.3610 Kcal/mol. Compounds **15** and **31** showed the best scoring energy of -20.1891and -20.3610 Kcal/mol, respectively. Upon the previously mentioned results we can assume that c-Src inhibition could be a suggested mechanism of action for the cytotoxic activity of the newly synthesized compounds.

3.1.2. Biological screening

3.1.2.1. In vitro antitumor activity

All the synthesized compounds showed better cytotoxic activity than Doxorubicin except compounds **19** and **31** with IC_{50} values of 8.30 and 8.39 µM, respectively. The pyrrolo derivatives (**5-11**) showed good cytotoxic activity better than that of Doxorubicin with IC_{50} values ranging between 6.47-7.56 µM. The o-aminocarbonitrile derivative **5** was the least active compound in this series while the formation of formimidate derivative **6** tends to increase the activity to 7.01 µM. On the other hand, cyclization to pyrrolopyrimidine system (**7-10**) increased the activity with methyl, ethyl and propyl substitution on N number 3 with IC_{50} values of 6.74 µM for compounds **7**, **8** and **9** while, the activity remains the same with benzyl derivative **10** with IC_{50} value 7.01 µM. Treatment of the formimidate derivative **6** with methyl hydrazine revealed compound **11** with better activity and IC_{50} value of 6.88 µM.

In the second series representing compounds (**12-16**) the tested compounds showed cytotoxic activity ranging between 6.74- 7.90 µM . Compound **12** was the most active compound in this series with IC_{50} value

of 6.74 µM with unsubstituted sulfa derivative. Upon conducting the same reaction with substituted sulfa drugs the formed compounds showed less cytotoxic activity with IC_{50} values of 7.29, 7.29 and 7.90 µM for compounds **13**, **14** and **15**, respectively. In addition to, the formation of the pyrrolopyrimidine compound with substituted sulfa derivative **17** still show less activity than compound **12** with IC_{50} value of 7.01 µM.

Compounds (**17- 19**, **21- 26** and **31**) all represent pyrrolopyrimidine derivatives which showed better cytotoxic activity than Doxorubicin except compound **19** and **31** with IC_{50} values of 8.30 and 8.39 µM. Compound **23** was the most active compound with IC_{50} value of 6.46 µM with a 4-chloropyridine substituent at N number 3 of pyrimidine ring also, compound **26** showed the same IC_{50} value with a 2,6-dihydroxypyrimidine derivative at N number 3 on the pyrimidine ring. The rest of compound in this series were less active than compound **23** and **26** but still more active than Doxorubicin except compound **19** and **31** with IC_{50} of 8.30 and 8.39 µM, respectively. The pyrrolo derivative **20** showed good activity with IC_{50} value of 7.29 µM, while the tiazolopyrrolpyrimidine derivatives **28** and **30** showed IC_{50} values of 6.46 and 7.84 µM, respectively. This could be attributed to the amino substitution on compound **28** that was replaced by a larger phenyl substitution in compound **30**.

The promising results of cytotoxic activity of the synthesized compounds especially compounds **7- 9**, **11**, **23**, **26** and **28** urge more investigations for their mechanism of action. The trial in the present investigation to predict an assumption on the mechanism of action of the synthesized compounds was conducted through molecular docking on the active site of c-Src based on the similarities between the synthesized compounds and the enzyme inhibitors of this enzymes. Also, the good energy scores for these compounds as well as their interactions with the active site amino acids could aid in understanding the mechansim of action for their cytotoxic activity.

3. Conclusion

The objective of the present study was to synthesize and investigate the anti-breast cancer activity of some novel pyrrole and pyrrolopyrimidine derivatives bearing a biologically active sulfonamide moieties. All the tested compounds exhibited anti-breast cancer activity higher than that the positive control Doxorubicin as reference drug.

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