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Design, synthesis and molecular docking studies of new Quinoxaline-linked-1,2,4-triazole-Sulfonamide hybrids as Anti-proliferative agents

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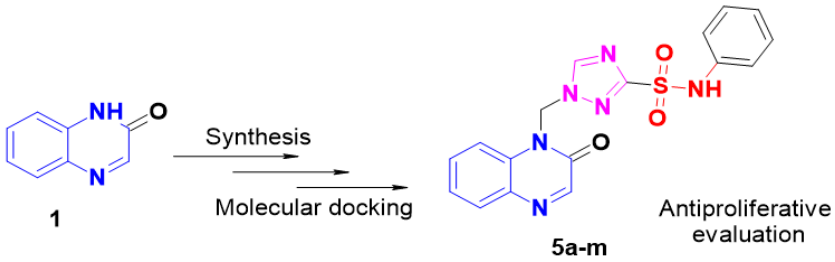
Article

ABSTRACT

A new series of quinoxaline linked 1,2,4-triazole sulfonamide derivatives were designed and efficiently synthesized. All compounds were characterized by their IR, ^{1}HNMR, ^{13}CNMR, and Mass spectral data, and elemental analysis. The final compounds (**5a-m)** were screened for *in vitro* anti-proliferative activity against cancer cell lines HeLa (lung), A549 (carcinoma), MCF-7 (breast) and HCT116 (colon).



The results revealed that the compounds **5k, 5l** and **5d** have shown promising activity as compared to etoposide. Predominantly, the compound **5k** displayed greater activity on HeLa, A549, MCF-7and HCT116 with IC_{50} values of 1.97±0.09, 1.84±0.07, 3.10±0.04and 4.10±0.07 than the standard drug etoposide. Moreover, molecular docking studies of **5k, 5l** and **5d** on EGFR receptor suggested that the most potent compound **5k** strongly binds to protein EGFR (pdbid: 4HJO). Furthermore, the compounds **5k** and **5l** displayed promising inhibitory activity over tyrosine kinase EGFR when compared with the standard erlotinib.

Keywords: Quinoxaline, 1,2,4-triazole, Sulfonamide, anti-cancer drugs, Anti-proliferative, Tyrosine kinase, EGFR inhibitory activity

INTRODUCTION

As a major worldwide health problem, Cancer is regarded as the most common cause of death after cardiovascular diseases. It accounts for one in each seven deaths in the world.[1] Despite the outstanding development and advances in cancer management, the high rate of occurrence and mortality related to cancer leads to constant research for new anticancer agents.[2] Furthermore, the multi-drug resistance (MDR) in cancer chemotherapy is a big challenge for researchers in this field. In this regard for the past few decades, there has been a keen attention on the development of heterocyclic compounds-based drugs towards development of efficient cure for the different cancers. The quinoxaline-based derivatives have emerged among the lead heterocyclic molecules as effective drugs that possess anticancer activity.[3,4] Interestingly, two anticancer drugs namely tirapazamine **(1)** and chloroquinoxaline sulfonamide (CQS) **(2)** (figure 1) containing quinoxaline moiety are already present in the market. The CQS acts as a poison for topoisomerase II alpha and topoisomerase II beta, thereby causing double-stranded breaks in DNA, accumulation of unrepaired DNA, and apoptosis.[5,6] Similarly, the AG1295 has shown selective blockade of EGFR kinase resulting into controlling normal cell growth, apoptosis and other cellular functions.[7–10] On the other hand, 1,2,4-triazoles act as important pharmacophores by interacting with the biological receptors with high affinity owing to their dipole character, hydrogen bonding capacity, rigidity and solubility.[11–14] In addition, there are some commercially available anticancer drugs such as anastrozole, letrozole which contain 1,2,4-triazole moiety.[15,16] Besides, the sulfonamide moiety has evoked high favor in medicinal chemistry, and a variety of sulfonamide derivatives have been developed with a wide assortment of biological activities. Some sulfonamide derivatives have been approved by FDA for cancer therapy. For example, Belinostat, a histone deacetylase (HDAC) inhibitor, is the third approved drug to treat T-cell lymphoma after Vorinostat and Romidepsin.[17]

The literature and marketed drugs indicate that potential anti-cancer drug molecules have quinoxaline, 1,2,4-triazole and sulfonamide as active pharmacophores in them. Based on the significant anticancer applications of quinoxaline, 1,2,4-triazole and sulfonamide pharmacophores, herein, we combined quinoxaline, 1,2,4-triazole and sulfonamide pharmacophores as single framework i.e quinoxaline 1,2,4-triazole-sulfonamide derivatives by hybridization approach (Figure 1).

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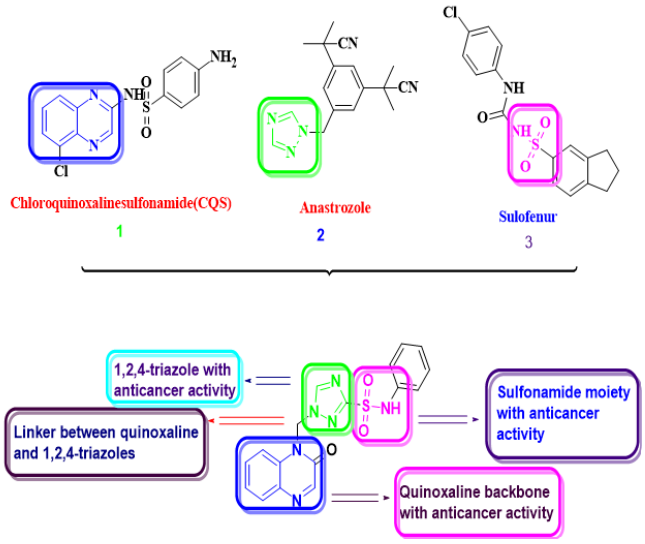
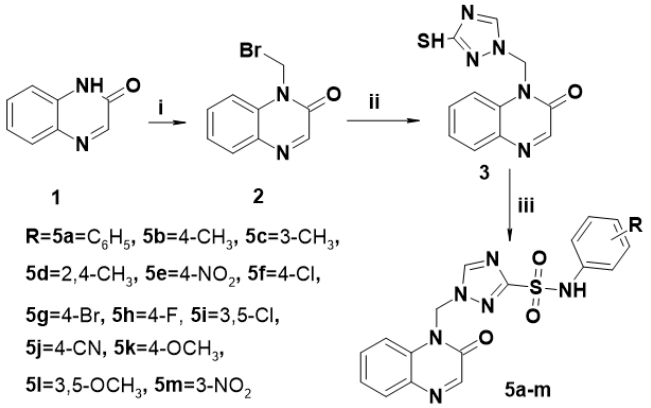


Figure 1. Pharmacophore Hybridization Strategy



Scheme 1. Synthesis of compounds. Reaction conditions: (i). dibromomethane, Acetonitrile, K_2CO_3, reflux, 10h, 80℃; (ii) EtOH, 1-formyl-3-thiosemicarbazide, NaOH, 80min, HCl, 0℃, 30 min.; (iii) H_2O_2, $POCl_3$, CH_3CN, Amberlite IRA-400 (Cl-), Aromatic amines(4a-m), r.t. 90min.

EXPERIMENTAL

All reagents and solvents were purchased from SD Fine Chemicals limited and used without further purification. TLC was performed using Merck silica gel 60 F254 pre-coated plates (0.25 mm), and silica gel (particle size 60–120 mesh) was used for column chromatography. Melting points were determined on a Cintex apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were measured on a Bruker (400 MHz) and Mercury (75 MHz) NMR spectrometers using DMSO-*d*6 as a solvent and TMS as the internal standard. Mass spectra were measured on a Jeol JMC-300 spectrometer (ESI, 70 eV). Microanalyses were performed on a Perkin-Elmer 2400 analyzer. The compound 1-(bromomethyl)quinoxalin-2(1*H*)-one **(2)** were prepared according to reported procedure (Scheme 2).[18,19]

General procedure for the synthesis of 1-((3-mercapto-1*H*-1,2,4-triazol-1-yl)methyl) quinoxalin-2(1*H*)-one (3):

A mixture of 1-(bromomethyl)quinoxalin-2(1*H*)-one **(2)** (0.03moles) and 1-formyl-3-thiosemicarbazide (0.03moles) is dissolved in ethanol (20mL) and sodium hydroxide (0.10moles) in 30 ml. of water in a round-bottomed flask and is heated on a steam bath for 80 minutes. The solution is cooled for 30 minutes in an ice bath and then treated with 20 ml. of concentrated hydrochloric acid. The reaction mixture is again cooled in an ice bath for 2 hours, and the 1-((3-mercapto-1*H*-1,2,4-triazol-1-yl)methyl)quinoxalin-2(1*H*)-one that precipitates out is collected by suction filtration. The thiol is dissolved in 50 ml of boiling water and the solution is filtered through a fluted filter paper to obtain a white color solid.

General procedure for the synthesis of 1-((2-oxoquinoxalin-1(2*H*)-yl)methyl)-*N*-phenyl-1*H*-1,2,4-triazole-3-sulfonamide (5a-m):

A mixture of thiols (0.05mol) and aromatic amines **(4a-m)** (0.05mol), H_2O_2 (30%, 0.03 mol, 4mL), and $POCl_3$ (10 mol, 0.25 g) was stirred in CH_3CN (10 mL) at 25°C for 5 minutes.

The completion of the reaction was shown by the TLC, then Amberlite IRA-400 (Cl^-) (0.05 g, 0.001 mol of OH^-) in acetonitrile (10mL) was added. The resulting mixture was stirred at room temperature for 85 minutes. After completion of the reaction, the catalyst was filtered off and the filtrate was concentrated in *vaccum*. The reaction mixture was then diluted with water (20mL) and extracted with EtOAc(3×10mL). The combined ethyl acetate extracts were dried with anhydrous Na_2SO_4, and then concentrated under reduced pressure and then recrystallized from EtOH to afford the pure product *N*-phenyl-[1,2,4]triazolo [4,3-*a*]quinoxaline-1-sulfonamide **(5a-m)**.

1-((2-oxoquinoxalin-1(2*H*)-yl)methyl)-*N*-phenyl-1*H*-1,2,4-triazole-3-sulfonamide (5a): Yield 79%, mp 238-240°C**.** IR spectrum, ν, cm^{-1}: 1343 (-SO_2), 1513 (–C=N), 1626 (–N=N), 3134 (=CH), 3224 (-NH). ^{1}HNMR spectrum, δ, ppm: 6.12 s (2H, -CH_2-), 7.32-7.41 m (5H, Ar-H), 7.57-7.69 m (4H, quin-H), 8.10 s (1H, triazole-H), 8.17 bs (1H,-NH), 9.12 s (1H, quin-H). ^{13}C NMR spectrum, δC, ppm: 67.5, 120.3, 123.2, 126.8, 127.5, 129.1, 130.2, 131.4, 135.4, 138.1, 139.3, 147.3, 153.2, 157.4, 163.7. MS: *m/z* 383 $(M+H)^+$; Anal. Cal. for $C_{17}H_{14}N_6O_3S$: C, 53.40; H, 3.69; N, 21.98. Found, %: C, 53.40; H, 3.69; N, 21.97%.

1-((2-oxoquinoxalin-1(2*H*)-yl)methyl)-*N*-(p-tolyl)-1*H*-1,2,4-triazole-3-sulfonamide(5b): Yield 83%, mp 252-254°C**.** IR spectrum, ν, cm^{-1}: 1332 (-SO_2), 1619 (–N=N), 1510 (–C=N), 3124 (=CH), 3213 (-NH). ^{1}HNMR spectrum, δ, ppm: 2.21 s (3H, -CH_3), 6.04 s (2H, -CH_2-), 7.31 d (2H, *J*=4.2Hz, Ar-H), 7.36 d (2H, *J*=4.2Hz, Ar-H), 7.44 t (1H, *J*=4.5Hz, quin-H), 7.49 t (1H, *J*=4.6Hz, quin-H), 7.56 d (1H, *J*=4.8Hz, quin-H), 7.61 d (1H, *J*=4.9Hz, quin-H), 8.02 s (1H, triazole-H), 8.13 bs (1H, -NH), 9.10 s (1H, quin-H). ^{13}C NMR spectrum, δC, ppm: 21.5, 66.5, 119.8, 122.1, 126.2, 127.0, 128.7, 129.2, 131.0, 134.7, 137.6, 138.7, 146.5, 152.9, 157.1, 162.9. MS: *m/z* 397 $(M+H)^+$; Anal. Cal. for $C_{18}H_{16}N_6O_3S$: C, 54.54; H, 4.07; N, 21.20. Found, %: C, 54.53; H, 4.06; N, 21.19%.

1-((2-oxoquinoxalin-1(2*H*)-yl)methyl)-*N*-(m-tolyl)-1*H*-1,2,4-triazole-3-sulfonamide (5c): Yield 72%, mp 259-261°C**.**

IR spectrum, ν, cm^{-1}: 1336 (-SO_2), 1514 (–C=N), 1621 (–N=N), 3126 (=CH), 3217 (-NH). ^{1}H NMR spectrum, δ, ppm: 2.24 s (3H, -CH_3), 6.05 s (2H, -CH_2-), 7.27 s (1H, Ar-H), 7.33-7.39 m (3H, Ar-H), 7.47 t (1H, *J*=4.2Hz, quin-H), 7.51 t (1H, *J*=4.3Hz, quin-H), 7.57 d (1H, *J*=4.5Hz, quin-H), 7.64 d (1H, *J*=4.7Hz, quin-H), 8.04 s (1H, triazole-H),8.17 bs (1H, -NH), 9.13 s (1H, quin-H). ^{13}C NMR spectrum, δC, ppm: 21.8, 66.7, 119.9, 120.3, 122.6, 126.5, 127.2, 128.8, 129.3, 130.1, 131.2, 134.9, 137.6, 138.9, 146.8, 153.1, 157.4, 162.5. MS: *m/z* 397 $(M+H)^+$; Anal. Cal. for $C_{18}H_{16}N_6O_3S$: C, 54.54; H, 4.07; N, 21.20. Found, %: C, 54.52; H, 4.05; N, 21.18%.

***N*-(2,4-dimethylphenyl)-1-((2-oxoquinoxalin-1(2*H*)-yl)methyl)-1*H*-1,2,4-triazole-3-sulfonamide(5d):** Yield 69%, mp 279-281°C**.** IR spectrum, ν, cm^{-1}: 1308 (-SO_2), 1503 (–C=N), 1614 (–N=N), 3120 (=CH), 3206 (NH). ^{1}H NMR spectrum, δ, ppm: 2.21 s (3H, -CH_3), 2.25 (3H, -CH_3), 5.97 s (2H, -CH_2-), 7.26 s (1H, Ar-H), 7.31 d (1H, *J*=4.0Hz, Ar-H), 7.36 d (1H, *J*=4.1Hz, Ar-H), 7.41 t (1H, *J*=3.9Hz, quin-H), 7.47 t (1H, *J*=4.0Hz, quin-H), 7.54 d (1H, *J*=4.3Hz, quin-H), 7.59 d (1H, *J*=4.4Hz, quin-H), 7.98 s (1H, triazole-H), 8.06 bs (1H, -NH), 9.07 s (1H, quin-H). ^{13}C NMR spectrum, δC, ppm: 19.5, 21.3, 66.4, 118.3, 122.6, 126.3, 127.5, 128.7, 129.2, 130.4, 132.7, 133.4, 134.4, 135.2, 138.5, 146.3, 151.4, 156.3, 162.1. MS: *m/z* 411 $(M+H)^+$; Anal. Cal. for $C_{19}H_{18}N_6O_3S$: C, 55.60; H, 4.42; N, 20.48. Found, %: C, 55.59; H, 4.41; N, 20.46%.

***N*-(4-nitrophenyl)-1-((2-oxoquinoxalin-1(2*H*)-yl)methyl)-1*H*-1,2,4-triazole-3-sulfonamide (5e):** Yield 65%, mp 267-269°C**.** IR spectrum, ν, cm^{-1}: 1354 (-SO_2), 1527 (–C=N), 1630 (–N=N), 3142 (=CH), 3230 (NH). ^{1}H NMR spectrum, δ, ppm: 6.13 s (2H, -CH_2-), 7.52 d (2H, *J*=5.3Hz, Ar-H), 7.59 d (2H, *J*=5.2Hz, Ar-H), 7.65 t (1H, *J*=4.9Hz, quin-H), 7.71 t (1H, *J*=4.8Hz, quin-H), 7.78 d (1H, *J*=4.8Hz, quin-H), 7.86 d (1H, *J*=4.7Hz, quin-H), 8.17 s (1H, triazole-H), 8.26 bs (1H, -NH), 9.42 s (1H, quin-H). ^{13}C NMR spectrum, δC, ppm: 67.4, 121.5, 124.2, 125.6, 127.4, 130.1, 131.4, 135.7, 138.4, 143.2, 146.3, 147.1, 152.4, 158.2, 163.9. MS: *m/z* 428 $(M+H)^+$; Anal. Cal. for $C_{17}H_{13}N_7O_5S$: C, 47.77; H, 3.07; N, 22.94. Found, %: C, 47.76; H, 3.06; N, 22.93%.

***N*-(4-chlorophenyl)-1-((2-oxoquinoxalin-1(2*H*)-yl)methyl)-1*H*-1,2,4-triazole-3-sulfonamide (5f):** Yield 68%, mp 246-248°C. IR spectrum, ν, cm^{-1}: 1325 (-SO_2), 1518 (–C=N), 1621 (–N=N), 3123 (=CH), 3224 (-NH). ^{1}H NMR spectrum, δ, ppm: 6.10 s (2H, -CH_2-), 7.49 d (2H, *J*=5.4 Hz, Ar-H), 7.55 d (2H, *J*=5.5Hz, Ar-H), 7.61 t (1H, *J*=5.0Hz, quin-H), 7.66 t (1H, *J*=5.1Hz, quin-H), 7.72 d (1H, *J*=5.2Hz, quin-H), 7.78 d (1H, *J*=5.3Hz, quin-H), 8.14 s (1H, triazole-H), 8.16 bs (1H, -NH), 9.21 s (1H, quin-H). ^{13}C NMR spectrum, δC, ppm: 66.5, 120.2, 123.1, 124.2, 126.7, 129.2, 130.4, 134.2, 137.1, 142.4, 145.4, 146.6, 151.6, 157.1, 163.8. MS: *m/z* 416 $(M+H)^+$, 418 (M+2); Anal. Cal. for $C_{17}H_{13}ClN_6O_3S$: C, 48.98; H, 3.14; N, 20.16. Found, %: C, 48.97; H, 3.13; N, 20.15%.

***N*-(4-bromophenyl)-1-((2-oxoquinoxalin-1(2*H*)-yl)methyl)-1*H*-1,2,4-triazole-3-sulfonamide (5g):**

Yield 61%, mp 274-276°C**.** IR spectrum, ν, cm^{-1}: 1322 (-SO_2), 1516 (–C=N), 1618 (–N=N), 3120 (=CH), 3221 (NH). ^{1}H NMR spectrum, δ, ppm: 6.09 s (2H, -CH_2-), 7.45 d (2H, *J*=5.0 Hz, Ar-H), 7.53 d (2H, *J*=5.1Hz, Ar-H), 7.59 t (1H, *J*=4.8Hz, quin-H), 7.64 t (1H, *J*=4.8Hz, quin-H), 7.69 d (1H, *J*=4.6Hz, quin-H), 7.76 d (1H, *J*=4.5Hz, quin-H), 8.09 s (1H, triazole-H), 8.14 bs (1H, -NH), 9.14 s (1H, quin-H). ^{13}C NMR spectrum, δC, ppm: 66.1, 119.3, 122.6, 123.9, 126.3, 129.0, 130.1, 134.0, 136.5, 142.0, 145.1, 146.3, 151.4, 156.8, 163.6. MS: *m/z* 460 $(M+H)^+$, 462 (M+2); Anal. Cal. for $C_{17}H_{13}BrN_6O_3S$: C, 44.26; H, 2.84; N, 18.22. Found, %: C, 44.25; H, 2.83; N, 18.21%.

***N*-(4-fluorophenyl)-1-((2-oxoquinoxalin-1(2*H*)-yl)methyl)-1*H*-1,2,4-triazole-3-sulfonamide (5h):** Yield 64%, mp 281-283°C**.** IR spectrum, ν, cm^{-1}: 1347 (-SO_2), 1523 (–C=N), 1630 (–N=N), 3134 (=CH), 3245 (-NH). ^{1}H NMR spectrum, δ, ppm: 6.11 s (2H, -CH_2-), 7.52 d (2H, *J*=5.6Hz, Ar-H), 7.58 d (2H, *J*=5.9Hz, Ar-H), 7.63 t (1H, *J*=5.2Hz, quin-H), 7.69 t (1H, *J*=5.3 Hz, quin-H), 7.76 d (1H, *J*=5.5Hz, quin-H), 7.84 d (1H, *J*=5.6Hz, quin-H), 8.12 s (1H, triazole-H), 8.22 bs (1H, -NH), 9.29 s (1H, quin-H). ^{13}C NMR spectrum, δC, ppm: 67.5, 121.5, 123.7, 125.2, 129.5, 131.2, 132.5, 136.3, 138.2, 144.3, 146.5, 147.9, 153.1, 158.2, 164.2. MS: *m/z* 401 $(M+H)^+$; Anal. Cal. for $C_{17}H_{13}FN_6O_3S$: C, 51.00; H, 3.27; N, 20.99. Found, %: C, 51.00; H, 3.26; N, 20.98%.

***N*-(3,5-dichlorophenyl)-1-((2-oxoquinoxalin-1(2*H*)-yl)methyl)-1*H*-1,2,4-triazole-3-sulfonamide(5i):** Yield 66%, mp 277-279°C**.** IR spectrum, ν, cm^{-1}: 1356 (-SO_2), 1534 (–C=N), 1642 (–N=N), 3143 (=CH), 3251 (NH). ^{1}H NMR spectrum, δ, ppm: 6.14 s (2H, -CH_2-), 7.51 s (2H, Ar-H), 7.58 s (1H, Ar-H),7.63 t (1H, *J*=6.3Hz, quin-H), 7.68 t (1H, *J*=6.2Hz, quin-H), 7.76 d (1H, *J*=6.0Hz, quin-H), 7.81 d (1H, *J*=5.9Hz, quin-H), 8.15 s (1H, triazole-H), 8.21 bs (1H, -NH), 9.27 s (1H,q uin-H). ^{13}C NMR spectrum, δC, ppm: 66.9, 120.8, 124.3, 125.2, 127.3, 129.9, 130.8, 134.9, 138.3, 143.1, 145.8, 147.2, 152.3, 157.8, 164.4. MS: *m/z* 451 $(M+H)^+$, 453(M+2); Anal. Cal. for $C_{17}H_{12}Cl_2N_6O_3S$: C, 45.25; H, 2.68; N, 18.62. Found, %: C, 45.24; H, 2.68; N, 18.61%.

***N*-(4-cyanophenyl)-1-((2-oxoquinoxalin-1(2*H*)-yl)methyl)-1*H*-1,2,4-triazole-3-sulfonamide (5j):** Yield 68%, mp 259-261°C**.** IR spectrum, ν, cm^{-1}: 1354 (-SO_2), 1527 (–C=N), 1630 (–N=N), 3142 (=CH), 3230 (-NH). ^{1}H NMR spectrum, δ, ppm: 6.15 s (2H, -CH_2-), 7.51 d (2H, *J*=6.6Hz, Ar-H), 7.57 d (2H, *J*=6.5Hz, Ar-H), 7.64 t (1H, *J*=6.3Hz, quin-H), 7.69 t (1H, *J*=6.2Hz, quin-H), 7.76 d (1H, *J*=6.1Hz, quin-H), 7.84 d (1H, *J*=6.0Hz, quin-H), 8.17 s (1H, triazole-H), 8.24 bs (1H, -NH), 9.39 s (1H, quin-H). ^{13}C NMR spectrum, δC, ppm: 67.0, 119.7, 121.1, 123.7, 125.2, 127.1, 129.3, 131.0, 135.6, 137.8, 142.9, 146.2, 147.1, 152.3, 158.3, 164.9. MS: *m/z* 408 $(M+H)^+$; Anal. Cal. for $C_{18}H_{13}N_7O_3S$: C, 53.07; H, 3.22; N, 24.07. Found, %: C, 53.06; H, 3.21; N, 24.06%.

***N*-(4-methoxyphenyl)-1-((2-oxoquinoxalin-1(2*H*)-yl)methyl)-1*H*-1,2,4-triazole-3-sulfonamide (5k):** Yield 74%, mp 269-271°C**.** IR spectrum, ν, cm^{-1}: 1340 (-SO_2), 1523 (–C=N), 1621 (–N=N), 3122 (=CH), 3210 (NH). ^{1}H NMR spectrum, δ, ppm: 3.89 s (3H, -CH_3), 6.11 s (2H, -CH_2-), 7.39 d (2H, *J*=6.2Hz, Ar-H), 7.45 d (2H, *J*=6.5Hz, Ar-H), 7.51 t (1H, *J*=6.2Hz, quin-H), 7.58 t (1H, *J*=6.3Hz, quin-H), 7.64 d (1H, *J*=6.0Hz, quin-H), 7.72 d (1H, *J*=5.9Hz, quin-H), 8.15 s (1H, triazole-H), 8.17 bs (1H, -NH), 9.26 s (1H, quin-H). ^{13}C NMR spectrum, δC, ppm:

57.5, 67.1, 116.3, 120.3, 123.1, 126.2, 128.7, 130.4, 131.5, 135.3, 138.2, 146.3, 152.1, 157.3, 158.1, 162.8. MS: *m/z* 413 $(M+H)^+$; Anal. Cal. for $C_{18}H_{16}N_6O_4S$: C, 52.42; H, 3.91; N, 20.38. Found, %: C, 52.41; H, 3.90; N, 20.37%.

***N*-(3,5-dimethoxyphenyl)-1-((2-oxoquinoxalin-1(2*H*)-yl)methyl)-1*H*-1,2,4-triazole-3-sulfonamide(5l):** Yield 79%, mp 271-273°C**.** IR spectrum, ν, cm^{-1}: 1332 ($-SO_2$), 1514 (–C=N), 1618 (–N=N), 3116 (=CH), 3202 (NH). ^{1}H NMR spectrum, δ, ppm: 3.92 s (6H, $-CH_3$), 6.13 s (2H, $-CH_2$-), 7.37 s (1H, Ar-H), 7.44 s (2H, Ar-H), 7.50 t (1H, *J*=5.8Hz, quin-H), 7.57 t (1H, *J*=5.9Hz, quin-H), 7.63 d (1H, *J*=6.4Hz, quin-H), 7.69 d (1H, *J*=6.3Hz, quin-H), 8.14 s (1H, triazole-H), 8.14b s (1H, -NH), 9.19 s (1H, quin-H). ^{13}C NMR spectrum, δC, ppm: 56.8, 66.5, 115.7, 119.8, 122.6, 125.7, 127.4, 129.5, 130.2, 134.5, 137.8, 145.7, 151.8, 156.2, 157.6, 162.1. MS: *m/z* 443 $(M+H)^+$; Anal. Cal. for $C_{19}H_{18}N_6O_5S$: C, 51.58; H, 4.10; N, 8.99. Found, %: C, 51.57; H, 4.09; N, 18.98%.

***N*-(3-nitrophenyl)-1-((2-oxoquinoxalin-1(2*H*)-yl)methyl)-1*H*-1,2,4-triazole-3-sulfonamide (5m):** Yield 59%, mp 278-280°C**.** IR spectrum, ν, cm^{-1}: 1362 ($-SO_2$), 1532 (–C=N), 1634 (–N=N), 3146 (=CH), 3235 (NH). ^{1}H NMR spectrum, δ, ppm: 6.16 s (2H, $-CH_2$-), 7.62-7.56 m (3H, Ar-H), 7.64 s (1H, Ar-H), 7.69 t (1H, *J*=6.8Hz, quin-H), 7.76 t (1H, *J*=6.9Hz, quin-H), 7.82 d (1H, *J*=7.2 Hz, quin-H), 7.89 d (1H, *J*=7.4Hz, quin-H), 8.18 s (1H, triazole-H), 8.29 bs (1H,-NH), 9.45 s (1H, quin-H). ^{13}C NMR spectrum, δC, ppm: 67.8, 121.7, 124.7, 126.3, 127.6, 128.4, 129.1, 130.4, 131.4, 136.1, 139.3, 141.5, 146.3, 148.1, 152.3, 158.5, 163.4. MS: *m/z* 428 $(M+H)^+$; Anal. Cal. for $C_{17}H_{13}N_7O_5S$: C, 47.77; H, 3.07; N, 22.94. Found, %: C, 47.77; H, 3.05; N, 22.92%.

MTT assay

For this assay we have taken 1×10^4 Cells/well and loaded in 200 mL of DMEM. 10% FBS was dropped in every well of 96-well micro culture plates which are incubated for 24 hours at 37°C temperature in a CO_2 incubator. All the derivative compounds were diluted to the preferred concentrations in culture medium and added to the wells with corresponding vehicle control. After 2 days of incubation, 10 mL of MTT (5 mg/mL) was introduce into each well and the plates were incubated for additional 4 h. The supernatant from each well was carefully removed; formed crystals were dissolved in 100 mL of DMSO. Absorbance was recorded at 540 nm wavelength.

Tyrosine kinase EGFR inhibitory activity

The study was carried out by EGFR Kinase Assay Kit (PBS Bioscience, catalog # 40321). Erlotinib was used as standard. The results of all the three compounds were in triplicates. The IC_{50} value of the compounds and reference is calculated by taking average of the three experiments and standard deviation of three experiments was taken into consideration.

RESULTS AND DISCUSSION

Chemistry

The designed final target quinoxaline-1,2,4-triazole sulfonamide derivatives (**5a-m)** were synthesized efficiently by following the simple synthetic approach as detailed in **Scheme 1.** The starting compound quinoxalin-2(1*H*)-one **(1)** is prepared according to reported procedure.[19] Then, the compound (**1)** is reacted with dibromomethane using K_2CO_3 as base in acetonitrile under reflux condition for 10hrs which gave the corresponding intermediate 1-(bromomethyl) quinoxalin-2(1*H*)-one **(2)**. Then the compound 1-(bromomethyl)quinoxalin-2(1*H*)-one **(2)** is treated with hydrazinecarbothioamide in ethanol followed by formic acid to afforded the corresponding 1-((3-mercapto-1*H*-1,2,4-triazol-1-yl)methyl)quinoxalin-2(1*H*)-one**(3).**[20] Finally the 1-((3-merca pto-1*H*-1,2,4-triazol-1-yl)methyl)quinoxalin-2(1*H*)-one **(3)** was treated with several aromatic amines using phosphorous oxy chloride and hydrogen peroxide in the presence of *N*-benzyl tri-methyl ammonium chloride (Amberlite IRA-400(Cl^-)) in dry acetonitrile at room temperature for 90 minutes to produce the designed 1-((2-oxoquinoxalin-1(2*H*)-yl)methyl)-*N*-phenyl-1*H*-1,2,4-triazole-3-sulfonamide (**5a-m)** in good to high yields.

Anti-Proliferative Activity:

All the newly synthesized quinoxaline linked 1,2,4-triazole-sulfonamide derivatives **(5a-5m)** were evaluated for their *in vitro* anti-proliferative activity against a panel of four cancer cell lines such as HeLa, A549, MCF-7 and HCT116 employing MTT method[19] and the Etoposide was used as positive control. The IC_{50} values for anti-proliferative potency of the compounds are shown in Table 1. The compounds **5k**, **5l**, and **5d** showed good anti-proliferative affectivity on all the tested cancer cell lines.

Table 1: *In vitro* anti-cancer activity of synthesized compounds (5a-m) with IC_{50} in μM[a]

Compd	[b]HeLa	[c] A549	[d] MCF-7	[e] HCT116
5a	10.05±0.12	12.10±0.06	10.09±0.09	12.05±0.07
5b	11.23±0.04	11.01±0.08	12.06±0.07	13.03±0.09
5c	12.20±0.13	12.08±0.53	13.10±0.10	12.06±0.57
5d	4.06±0.09	5.20± 0.02	6.30±0.09	6.30± 0.10
5e	13.20 ±0.05	13.07±0.09	14.30±0.10	11.04±0.07
5f	12.04±0.10	11.30±0.10	13.04±0.09	12.06±0.01
5g	13.16±0.05	12.10 ± 0.05	11.10±0.06	12.02±0.09
5h	13.01± 0.02	10.05 ± 0.03	12.06± 0.05	12.03± 0.10
5i	13.02± 0.05	12.60±0.07	13.04±0.06	12.05±0.07
5j	12.03±0.03	11.90± 0.02	13.07±0.04	13.20±0.03
5k	1.97±0.09	1.84 ± 0.07	3.10±0.04	4.10±0.07
5l	2.36 ±0.08	3.02±0.10	4.02±0.09	5.05±0.13
5m	10.03±0.12	12.08±0.02	10.50±0.19	12.02±0.13
Etoposide	2.17 ± 0.02	2.10±0.02	3.12±0.02	4.18±0.10

ND = Not determined. [a] Each data represents as mean ±S.D values from three different experiments performed in triplicates. [b] HeLa: human cervical cancer cell line. [c] A549: human lung adenocarcinoma cancer cell line. [d]: MCF-7: breast adenocarcinoma cell line. [e] HCT116: human colon carcinoma

Among the compound **5k** with 4-methoxy substituent on phenyl ring showed potent activity against all the cell lines than the standard drug Etoposide with IC_{50} ((µM) values 1.97±0.09 (HeLa), 1.84 ±0.07 (A549), 3.10±0.04 (MCF-7) and 4.10±0.07 (HCT116) respectively. Moderate activity is shown by the compound with 3,5-dimethoxy group **5l** with IC_{50} ((µM) values 2.36±0.08 (HeLa), 3.02±0.10(A549), 4.02±0.09 (MCF-7) and 5.05±0.13 (HCT116). The introduction of weak electron donating methyl groups on phenyl rings at 2nd and 4th positions **5d** exhibited little lower activity with IC_{50} (µM) values 4.06±0.09 (HeLa), 5.20± 0.02 (A549), 6.30±0.09 (MCF-7) and 6.30± 0.10 (HCT116) as compared to compounds **5k** and **5l**. Finally, the activity order against four cancer cell lines for the top three potent compounds **5k, 5l**, and **5d** as follows **5k>5l>5d.**

Molecular Docking Studies

The epidermal growth factor receptor (EGFR) was taken as the target for molecular docking studies as it was one of the leading targets in developing cancer drugs.[21] EGFR is a cell-surface receptor which plays an important role in the ductal development of the mammary glands.[22] When the EGFR is over expressed it leads to a number of cancers.[23–27] The EGFR is downloaded in pdb format (pdb.id-4HJO) from protein data bank.[28] Molecular docking studies of 1,2,4-triazole hybrids (**5d**, **5k** and **5l**) with epidermal growth factor receptor as the target revealed that they have strong interaction with the protein (**Table 2**). The compound **5k** has exhibited highest binding energy (-9.52 Kcal/mol) and inhibition constant (104.30 nM). It has formed two hydrogen bonds with MET769 and CYS773 amino acid with bond lengths 2.03 A° and 1.97 A° respectively. The compound **5d** has exhibited -8.34 Kcal/mol binding energy and 811.13 nanomolar inhibition constant. It has formed one hydrogen bond with MET769 amino acid with bond length 1.82 A°. It has formed π–cation with LYS721 residue. Similarly the ligand **5l** has interacted with the protein with the binding energy -8.84Kcal/mol and inhibition constant 329.06 in nanomolar concentration. The compound **5l** has formed two hydrogen bonds with LYS851 and ARG817 residues with bond lengths 1.99 A° and 2.11 A° respectively and exhibited π–π stacking with PHE699.

Table 2. Molecular docking interaction parameters of compounds (5d, 5k and 5l) with the epidermal growth factor receptor (PDB ID-4HJO)

Comp	Binding Energy (Kcal/mol)	Inhibition Constant (Nano Molar)	No. of hydrogen bonds	Residues involved in hydrogen bonding (bond length in Å°)
5d	-8.31	811.13	1	MET769(1.82)
5k	-9.52	104.30	2	MET769(2.03), CYS773(1.97)
5l	-8.84	329.06	2	LYS851(1.99), ARG817(2.11)

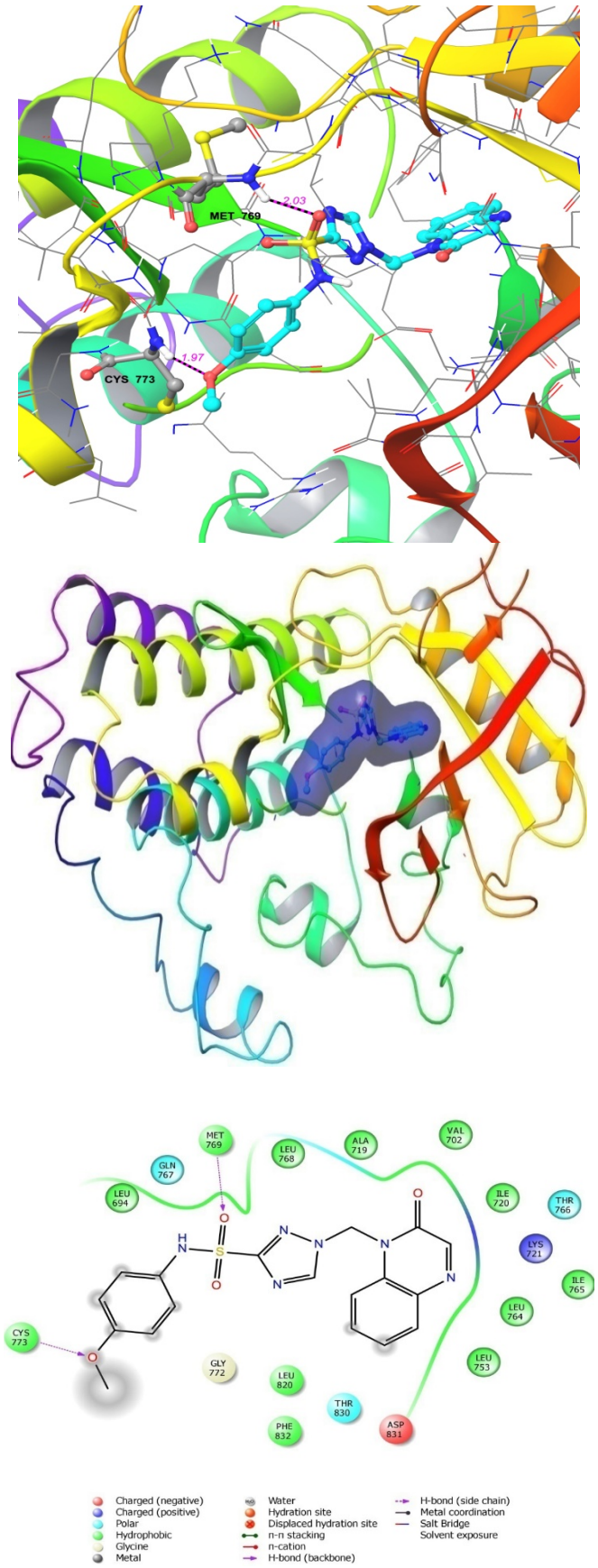


Figure 2. 2D and 3D interactions of compound 5k

IN VITRO TYROSINE KINASE EGFR INHIBITORY ACTIVITY

The inhibitory activity against tyrosine kinase EGFR was carried out for three potent compounds (**5k**, **5l**, and **5d**). According to the results (Table 3) the compounds **5k** and **5l** have shown greater inhibitory activity compared to reference Erlotinib drug (IC_{50}; 0.39µM) with IC_{50} 0.39µM and 0.44µM respectively. However, the compounds **5d** have exhibited almost similar inhibitory activity (IC_{50}; 0.58µM respectively) compared to that of the Erlotinib. These results strongly support the *in vitro* anticancer activity also.

Table 3. Kinase inhibitory acitivity

Compound	IC_{50}(µM)*
5d	0.58±0.06
5k	0.39±0.07
5l	0.44±0.02
Erlotinib	0.46±0.01

*Average of triplicates± standard deviation

CONCLUSION

We herein described the synthesis of some new 1-((2-oxoquinoxalin-1(2*H*)-yl)methyl)-*N*-phenyl-1*H*-1,2,4-triazole-3-sulfonamide derivatives **(5a-m).** The target molecules investigated for their *in vitro* cytotoxicity by MTT assay using etoposide as standard drug. The *in vitro* anti-cancer activity of the compounds **(5a-m)** over four human cancer cell lines i. e. MCF-7 (breast), HeLa (cervical), A549 (lung) and HCT116 (colon carcinoma). Among them, compound **5k** showed higher activity against all the cancer cell lines than standard drug. The other compounds **5l** showed nearer activity against all the cancer cell lines compared to standard drug. The other compound **5d** exhibited good activity as compared to standard. The remaining compounds showed moderate to less activity against all the cancer cell lines tested in this study.

CONFLICT OF INTEREST

Authors declare no conflict of interest of any kind – academic or financial is there for publication of this piece of work.

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