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Quinazoline and tetrahydropyridothieno[2,3-d]pyrimidine derivatives as irreversible EGFR tyrosine kinase inhibitors: influence of the position 4 substituent

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Experimental

Chemistry

Solvents and reagents were obtained from commercial suppliers and used as received. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX 500 spectrometer. Chemical shifts are referenced to the residual protonated solvent signals. The purities of the tested compounds 4a-4p and 10a-10e were determined by HPLC coupled with mass spectrometry and were higher than 95% in all cases. Mass spectrometric analysis (HPLC-ESI-MS) was performed on a TSQ quantum (Thermo Electron Corporation) instrument equipped with an ESI source and a triple quadrupole mass detector (Thermo Finnigan). The MS detection was carried out at a spray voltage of 4.2 kV, a nitrogen sheath gas pressure of 4.0 x 10^5 Pa, an auxiliary gas pressure of 1.0 x 10^5 Pa, a capillary temperature of 400 ºC, a capillary voltage of 35 V, and a source CID of 10 V. All samples were injected by an autosampler (Surveyor, Thermo Finnigan) with an injection volume of 10 μL. An RP C18 NUCLEODUR 100-3 (125 x 3 mm) column (Macherey-Nagel) was used as the stationary phase. The solvent system consisted of water containing 0.1% TFA (A) and 0.1% TFA in acetonitrile (B). HPLC-Method: flow rate 400 μL/min. The percentage of B started at an initial of 5%, was increased up to 100% during 16 min, kept at 100% for 2 min, and flushed back to 5% in 2 min. Melting points are uncorrected and were determined on Buchi melting point apparatus (B-540). The IR spectra were measured on Nicolet 380 FT-IR spectrometer.

Ethyl *N*-(2-cyano-4-nitrophenyl)formimidate (1). 5g (30.6 mmol) of 2-amino-5-nitrobenzonitrile was refluxed in 50ml of triethyl orthoformate for 24 hours in the

presence of 10 drops of acetic anhydride. The reaction was then concentrated under vaccum and the remaining residue was poured on ice water where a precipitate has been formed. The ppt. was filtered under vacuum and left to dry and purified by column chromatography using (Dichloromethane/Hexane 4:1) as eluent to give compound **1**. Yield 82% (5.5 g, solid); IR: 2228.6 cm^{-1} (C≡N); ^{1}H NMR (500 MHz, DMSO- d_6): δ 8.67 (d, *J* = 2.6 Hz, 1H), 8.43 (dd, *J* = 8.9, 2.7 Hz, 1H), 8.22 (s, 1H), 7.46 (d, *J* = 8.9 Hz, 1H), 4.36 (q, *J* = 7.0 Hz, 2H), 1.35 (t, *J* = 7.1 Hz, 3H).

General procedure for the synthesis of *N*-(substituted)-6-nitroquinazolin-4-amine (2a-2o). Compound **1** (5 mmol) was refluxed for 1 hour with the respective amine derivative (5 mmol) in 8ml glacial acetic acid. A precipitate is formed during the reaction which is filtered on hot and the precipitate is then washed with diethyl ether to give the corresponding nitroquinazoline derivatives **2a-2o**. If a precipitate is not formed, the solution is poured on ice water and the formed precipitate is filtered followed by washing with diethyl ether to give the corresponding nitroquinazoline derivative.

***N*-(2-bromo-6-fluorophenyl)-6-nitroquinazolin-4-amine (2a)**. Yield 67% (1.21 g, solid); ^{1}H NMR (500 MHz, DMSO- d_6): δ 10.70 (s, 1H), 9.49 (s, 1H), 8.56 (dd, *J* = 8.9, 1.7 Hz, 2H), 7.90 (s, 1H), 7.78 (dd, *J* = 8.2, 6.1 Hz, 1H), 7.46 (s, 1H), 7.16 (s, 1H). LC/MS (+ESI): m/z = 362.75 (M + H).

***N*-(4-bromo-2-fluorophenyl)-6-nitroquinazolin-4-amine (2b).** Yield 71% (1.28 g, solid); ^{1}H NMR (500 MHz, Acetone): δ 9.71 (s, 1H), 9.38 (d, *J* = 1.6 Hz, 1H), 8.70 (s, 1H), 8.60 (dd, *J* = 9.2, 2.1 Hz, 1H), 8.01 (d, *J* = 9.1 Hz, 1H), 7.83 (t, *J* = 8.3 Hz, 1H), 7.54 (d, *J* = 10.0 Hz, 1H), 7.48 (d, *J* = 8.5 Hz, 1H). ^{13}C NMR (126 MHz, Acetone) δ 160.60, 158.60, 157.48 (d, $^{1}J_{C\text{-}F}$ = 254.2 Hz), 154.40, 146.12, 131.02, 129.90, 128.47 (d,

$^{4}J_{C\text{-}F}$ = 3.4 Hz), 127.45, 120.90, 120.28 (d, $^{2}J_{C\text{-}F}$ = 23.3 Hz), 119.47, 118.37 (d, $^{3}J_{C\text{-}F}$ = 9.2 Hz). 115.26. LC/MS (+ESI): m/z = 362.99 (M + H).

***N*-(4-bromo-2-methylphenyl)-6-nitroquinazolin-4-amine (2c).** Yield 62% (1.11 g, solid); ^{1}H NMR (500 MHz, Acetone) δ 9.61 (s, 1H), 9.35 (d, *J* = 1.7 Hz, 1H), 8.58 (dd, *J* = 9.2, 2.4 Hz, 2H), 7.97 (d, *J* = 9.2 Hz, 1H), 7.54 (s, 1H), 7.48 – 7.32 (m, 2H), 2.32 (s, 3H). ^{13}C NMR (126 MHz, Acetone): δ 161.08, 158.93, 154.46, 153.48, 145.91, 138.83, 138.82, 134.16, 130.83, 130.82, 130.21, 127.24, 120.95, 115.20, 18.22. LC/MS (+ESI): m/z = 359.02 (M + H).

***N*-(4-bromo-3-methylphenyl)-6-nitroquinazolin-4-amine (2d)**. Yield 65% (1.16 g, solid); ^{1}H NMR (500 MHz, DMSO- d_6) δ 10.41 (s, 1H), 9.61 (d, *J* = 2.4 Hz, 1H), 8.70 (s, 1H), 8.52 (dd, *J* = 9.2, 2.4 Hz, 1H), 7.90 (d, *J* = 9.2 Hz, 1H), 7.81 (d, *J* = 2.4 Hz, 1H), 7.69 (dd, *J* = 8.6, 2.5 Hz, 1H), 7.58 (d, *J* = 8.7 Hz, 1H), 2.37 (s, 3H). ^{13}C NMR (126 MHz, DMSO- d_6) δ 158.53, 157.48, 152.96, 144.50, 138.02, 137.21, 131.95, 129.34, 126.56, 124.87, 122.02, 120.74, 118.81, 114.39, 22.59. LC/MS (+ESI): m/z = 358.86 (M + H).

***N*-(2-fluoro-3-methylphenyl)-6-nitroquinazolin-4-amine (2e).** Yield 67% (0.99 g, solid); ^{1}H NMR (500 MHz, DMSO- d_6) δ 10.49 (s, 1H), 9.58 (s, 1H), 8.61 (s, 1H), 8.55 (dd, *J* = 9.2, 2.4 Hz, 1H), 7.93 (d, *J* = 9.1 Hz, 1H), 7.35 (t, *J* = 6.4 Hz, 1H), 7.24 (t, *J* = 6.9 Hz, 1H), 7.16 (t, *J* = 7.7 Hz, 1H), 2.30 (s, 3H). ^{13}C NMR (75 MHz, DMSO- d_6) δ 159.85, 157.94, 155.39 (d, $^{1}J_{C\text{-}F}$ = 245.9 Hz), 152.95, 144.50, 129.48, 129.40 (d, $^{4}J_{C\text{-}F}$ = 4.8 Hz), 126.68, 125.87, 125.24 (d, $^{3}J_{C\text{-}F}$ = 7.8 Hz), 125.05 (d, $^{2}J_{C\text{-}F}$ = 11.2 Hz), 123.80 (d, $^{4}J_{C\text{-}F}$ = 4.5 Hz), 120.94, 113.94, 14.22 (d, $^{4}J_{C\text{-}F}$ = 4.0 Hz). LC/MS (+ESI): m/z = 298.95 (M + H).

***N*-(4-bromo-3-methoxyphenyl)-6-nitroquinazolin-4-amine (2f)**. Yield 75% (1.4 g, solid); ^{1}H NMR (500 MHz, DMSO- d_6) δ 10.39 (s, 1H), 9.62 (d, *J* = 2.4 Hz, 1H), 8.74 (s, 1H), 8.54 (dd, *J* = 9.2, 2.4 Hz, 1H), 7.93 (d, *J* = 9.2 Hz, 1H), 7.67 (d, *J* = 2.0 Hz, 1H), 7.58 (d, *J* = 8.6 Hz, 1H), 7.55 (dd, *J* = 8.6, 2.1 Hz, 1H), 3.89 (s, 3H). ^{13}C NMR (126 MHz, DMSO- d_6) δ 158.59, 157.46, 155.19, 152.99, 144.55, 139.35, 132.37, 130.59, 129.57, 129.36, 126.62, 120.67, 115.88, 107.08, 56.14. LC/MS (+ESI): m/z = 374.73 (M + H).

***N*-(2,4-dimethoxyphenyl)-6-nitroquinazolin-4-amine (2g)**. Yield 70% (1.14 g, solid); ^{1}H NMR (500 MHz, DMSO- d_6): δ 10.12 (s, 1H), 9.57 (d, *J* = 2.4 Hz, 1H), 8.52 (dd, *J* = 9.1, 2.6 Hz, 2H), 7.88 (d, *J* = 9.2 Hz, 1H), 7.29 (d, *J* = 8.6 Hz, 1H), 6.70 (d, *J* = 2.6 Hz, 1H), 6.59 (dd, *J* = 8.6, 2.7 Hz, 1H), 3.81 (s, 3H), 3.76 (s, 3H). ^{13}C NMR (126 MHz, DMSO- d_6): δ 160.40, 159.11, 158.16, 155.24, 153.04, 144.24, 129.24, 128.88, 126.40, 120.90, 119.02, 114.01, 104.62, 99.24, 55.60, 55.40. LC/MS (+ESI): m/z = 327.15 (M + H).

***N*-(2-ethylphenyl)-6-nitroquinazolin-4-amine (2h).** Yield 66% (0.97 g, solid); ^{1}H NMR (500 MHz, DMSO- d_6) δ 10.38 (s, 1H), 9.58 (s, 1H), 8.54 (dd, *J* = 9.2, 2.4 Hz, 1H), 8.50 (s, 1H), 7.90 (d, *J* = 9.2 Hz, 1H), 7.37 (d, *J* = 6.7 Hz, 1H), 7.31 (dd, *J* = 8.2, 3.5 Hz, 1H), 7.28 (d, *J* = 3.7 Hz, 2H), 2.56 (q, *J* = 7.6 Hz, 2H), 1.09 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (126 MHz, DMSO- d_6): δ 160.51, 158.20, 153.07, 144.49, 140.91, 135.94, 129.40, 128.90, 128.29, 127.43, 126.70, 126.55, 120.98, 114.02, 24.11, 14.35.

***N*-(3-ethylphenyl)-6-nitroquinazolin-4-amine (2i)**. Yield 69% (1.01 g, solid); ^{1}H NMR (500 MHz, Acetone) δ 9.70 (s, 1H), 9.37 (d, *J* = 2.3 Hz, 1H), 8.74 (s, 1H), 8.56 (dd, *J* = 9.2, 2.4 Hz, 1H), 7.96 (d, *J* = 9.2 Hz, 1H), 7.80 (dd, *J* = 8.1, 1.2 Hz, 1H), 7.75 (t, *J* = 1.6

Hz, 1H), 7.32 (t, *J* = 7.8 Hz, 1H), 7.05 (dd, *J* = 7.6, 0.6 Hz, 1H), 2.68 (q, *J* = 7.6 Hz, 2H), 1.25 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (126 MHz, Acetone): δ 159.95, 158.71, 154.53, 145.94, 145.60, 139.63, 130.90, 129.40, 127.13, 125.00, 122.80, 120.90, 120.67, 115.60, 29.48, 15.97.

***N*-(4-ethylphenyl)-6-nitroquinazolin-4-amine (2j).** Yield 67% (0.98 g, solid); ^{1}H NMR (500 MHz, DMSO- d_6): δ 10.35 (s, 1H), 9.58 (d, *J* = 2.4 Hz, 1H), 8.62 (s, 1H), 8.49 (dd, *J* = 9.2, 2.4 Hz, 1H), 7.86 (d, *J* = 9.2 Hz, 1H), 7.67 (d, *J* = 8.4 Hz, 2H), 7.23 (d, *J* = 8.5 Hz, 2H), 2.60 (q, *J* = 7.6 Hz, 2H), 1.19 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (126 MHz, DMSO- d_6): δ 158.88, 157.83, 153.12, 144.48, 140.30, 136.00, 129.44, 127.85, 126.57, 123.15, 120.86, 114.40, 27.81, 15.74.

4-((6-nitroquinazolin-4-yl)amino)benzenesulfonamide (2k). Yield 78% (1.34 g, solid); ^{1}H NMR (500 MHz, DMSO- d_6): δ 10.61 (s, 1H), 9.67 (d, *J* = 2.4 Hz, 1H), 8.78 (s, 1H), 8.57 (dd, *J* = 9.2, 2.4 Hz, 1H), 8.07 (d, *J* = 8.8 Hz, 2H), 7.96 (d, *J* = 9.2 Hz, 1H), 7.88 (d, *J* = 8.8 Hz, 2H), 7.32 (s, 2H). ^{13}C NMR (126 MHz, DMSO- d_6): δ 158.72, 157.40, 153.03, 144.68, 141.53, 139.28, 129.63, 126.76, 126.30, 122.17, 120.83, 114.44. LC/MS (+ESI): m/z = 346.09 (M + H).

***N*-carbamimidoyl-4-((6-nitroquinazolin-4-yl)amino)benzenesulfonamide (2l).** Yield 75% (1.45 g, solid); ^{1}H NMR (500 MHz, DMSO- d_6) δ 10.58 (s, 1H), 9.66 (d, *J* = 1.9 Hz, 1H), 8.77 (s, 1H), 8.56 (dd, *J* = 9.2, 2.5 Hz, 1H), 8.00 (d, *J* = 8.6 Hz, 2H), 7.95 (d, *J* = 9.2 Hz, 1H), 7.83 – 7.81 (m, 1H), 7.81 – 7.79 (m, 1H), 6.72 (s, 4H). ^{13}C NMR (126 MHz, DMSO- d_6) δ 158.71, 158.13, 157.47, 153.04, 144.66, 141.00, 139.86, 129.62, 126.77, 126.23, 122.07, 120.87, 114.46. LC/MS (+ESI): m/z = 387.87 (M + H).

4-((6-nitroquinazolin-4-yl)amino)-*N*-(thiazol-2-yl)benzenesulfonamide (2m). Yield 73% (1.56 g, solid); ^{1}H NMR (500 MHz, DMSO- d_6) δ 12.72 (s, 1H), 10.60 (s, 1H), 9.66 (d, *J* = 2.2 Hz, 1H), 8.77 (s, 1H), 8.55 (dd, *J* = 9.2, 2.4 Hz, 1H), 8.06 (d, *J* = 8.7 Hz, 2H), 7.95 (d, *J* = 9.2 Hz, 1H), 7.88 – 7.86 (m, 1H), 7.86 – 7.84 (m, 1H), 7.26 (d, *J* = 4.6 Hz, 1H), 6.84 (d, *J* = 4.6 Hz, 1H). ^{13}C NMR (126 MHz, DMSO- d_6) δ 168.80, 158.66, 157.40, 153.04, 144.69, 141.82, 137.32, 129.66, 126.80, 126.55, 124.51, 122.06, 120.87, 114.47, 108.19. LC/MS (+ESI): m/z = 428.79 (M + H).

4-((6-nitroquinazolin-4-yl)amino)-*N*-(pyridin-2-yl)benzenesulfonamide (2n). Yield 75% (1.58 g, solid); ^{1}H NMR (500 MHz, DMSO- d_6): δ 11.90 (s, 1H), 10.59 (s, 1H), 9.66 (d, *J* = 2.3 Hz, 1H), 8.78 (s, 1H), 8.56 (dd, *J* = 9.2, 2.4 Hz, 1H), 8.07 (d, *J* = 8.8 Hz, 2H), 8.03 (dd, *J* = 5.5, 1.1 Hz, 1H), 7.96 (d, *J* = 9.2 Hz, 1H), 7.95 – 7.91 (m, 2H), 7.73 (ddd, *J* = 8.9, 7.2, 1.9 Hz, 1H), 7.19 (d, *J* = 8.7 Hz, 1H), 6.88 (ddd, *J* = 7.0, 5.5, 0.9 Hz, 1H). ^{13}C NMR (126 MHz, DMSO- d_6): δ 158.64, 157.35, 153.03, 144.70, 141.98, 140.23, 140.21, 136.69, 136.67, 129.65, 127.35, 126.79, 121.94, 120.84, 115.72, 114.48, 113.65. LC/MS (+ESI): m/z = 423.09 (M + H).

***N*-cyclohexyl-6-nitroquinazolin-4-amine (2o)**. Yield 55% (0.74 g, solid); ^{1}H NMR (500 MHz, Acetone): δ 9.10 (d, *J* = 2.5 Hz, 1H), 8.60 (s, 1H), 8.47 (dd, *J* = 9.2, 2.5 Hz, 1H), 7.84 (d, *J* = 9.2 Hz, 1H), 4.51 – 4.15 (m, 1H), 2.16 – 2.10 (m, 2H), 2.09 (s, 1H), 1.86 – 1.79 (m, 2H), 1.73 – 1.67 (m, 1H), 1.50 – 1.41 (m, 4H), 1.29 – 1.18 (m, 1H). ^{13}C NMR (126 MHz, Acetone) δ 160.76, 159.34, 154.42, 145.33, 130.43, 126.73, 120.63, 115.20, 51.30, 33.05, 26.39, 26.04. LC/MS (+ESI): m/z = 273.17 (M + H).

General procedure for the synthesis of compunds (3a-3o). According to the reported procedure,[1] a mixture of the respective nitroquinazoline derivative **2a-2o** (3 mmol) and

stannous chloride (15 mmol) in MeOH (20 ml) was stirred at reflux for 1 h under nitrogen atmosphere. The excess MeOH was removed under reduced pressure; the remaining residue was dissolved in ethyl acetate (200 ml) and basified with aqueous $NaHCO_3$ solution. The resulting mixture was filtrated under vacuum followed by separation of the organic phase from the aqueous phase. The aqueous phase was extracted with ethyl acetate (2 x 20 ml), these organic fractions were combined, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to obtain the corresponding aminoquinazoline derivatives **3a-3o.**

***N*^{4}-(2-bromo-6-fluorophenyl)quinazoline-4,6-diamine (3a)**. Yield 75% (0.75 g, solid); ^{1}H NMR (500 MHz, DMSO- d_6): δ 9.19 (s, 1H), 8.18 (s, 1H), 7.72 (dd, J = 8.8, 6.0 Hz, 1H), 7.65 (s, 1H), 7.50 (d, J = 7.8 Hz, 1H), 7.23 (d, J = 15.8 Hz, 2H), 7.04 (d, J = 6.7 Hz, 1H), 5.66 (s, 2H). LC/MS (+ESI): m/z = 332.85 (M + H).

***N*^{4}-(4-bromo-2-fluorophenyl)quinazoline-4,6-diamine (3b).** Yield 78% (0.78 g, solid); ^{1}H NMR (300 MHz, DMSO- d_6) δ 9.29 (s, 1H), 8.22 (s, 1H), 7.61 (dd, J = 9.9, 2.2 Hz, 1H), 7.55 (dd, J = 8.7, 6.4 Hz, 2H), 7.43 (dd, J = 8.6, 1.3 Hz, 1H), 7.27 (d, J = 2.2 Hz, 1H), 7.24 (s, 1H), 5.63 (s, 2H). ^{13}C NMR (75 MHz, DMSO- d_6) δ 156.47, 156.39 (d, $^1J_{C-F}$ = 251.5 Hz), 149.85, 147.34, 142.55, 128.89 (d, $^5J_{C-F}$ = 2.4 Hz), 128.61, 127.35 (d, $^4J_{C-F}$ = 3.5 Hz), 126.97 (d, $^3J_{C-F}$ = 11.8 Hz), 123.87, 119.12 (d, $^2J_{C-F}$ = 23.7 Hz), 116.95 (d, $^3J_{C-F}$ = 9.2 Hz). 116.35, 100.82. LC/MS (+ESI): m/z = 332.84 (M + H).

***N*^{4}-(4-bromo-2-methylphenyl)quinazoline-4,6-diamine (3c).** Yield 78% (0.77 g, solid); ^{1}H NMR (500 MHz, Acetone): δ 8.28 (s, 1H), 8.26 (s, 1H), 7.60 (t, J = 8.4 Hz, 2H), 7.47 (d, J = 1.8 Hz, 1H), 7.39 (dd, J = 8.5, 2.1 Hz, 1H), 7.34 – 7.29 (m, 2H), 5.12 (s, 2H), 2.31 (s, 3H). ^{13}C NMR (126 MHz, Acetone): δ 157.84, 151.60, 148.04, 144.58, 138.37,

137.05, 133.76, 130.29, 129.87, 128.81, 124.41, 118.54, 117.41, 101.75, 18.21. LC/MS (+ESI): m/z = 329.0 (M + H).

***N*^{4}-(4-bromo-3-methylphenyl)quinazoline-4,6-diamine (3d).** Yield 80% (0.79 g, solid); ^{1}H NMR (500 MHz, DMSO- d_6) δ 9.34 (s, 1H), 8.35 (s, 1H), 7.86 (d, J = 2.4 Hz, 1H), 7.71 (dd, J = 8.7, 2.6 Hz, 1H), 7.54 (d, J = 6.7 Hz, 1H), 7.52 (d, J = 6.5 Hz, 1H), 7.35 (d, J = 2.3 Hz, 1H), 7.25 (dd, J = 8.9, 2.4 Hz, 1H), 5.57 (s, 2H), 2.36 (s, 3H). ^{13}C NMR (126 MHz, DMSO- d_6): δ 155.80, 149.68, 147.26, 142.65, 139.54, 136.84, 131.74, 128.67, 123.79, 123.68, 120.98, 116.91, 116.68, 100.96, 22.68.

***N*^{4}-(2-fluoro-3-methylphenyl)quinazoline-4,6-diamine (3e).** Yield 82% (0.66 g, solid); ^{1}H NMR (500 MHz, DMSO- d_6) δ 9.17 (s, 1H), 8.21 (s, 1H), 7.52 (d, J = 8.8 Hz, 1H), 7.40 (td, J = 7.5, 2.1 Hz, 1H), 7.27 (d, J = 2.2 Hz, 1H), 7.24 (dd, J = 8.8, 2.4 Hz, 1H), 7.15 – 7.07 (m, 2H), 5.59 (s, 2H), 2.27 (d, J = 1.9 Hz, 3H). ^{13}C NMR (126 MHz, DMSO- d_6) δ 156.88, 155.13 (d, $^1J_{C-F}$ = 245.4 Hz), 150.11, 147.20, 142.48, 128.57, 127.85 (d, $^4J_{C-F}$ = 4.6 Hz), 126.92 (d, $^3J_{C-F}$ = 12.7 Hz), 125.26, 124.61 (d, $^2J_{C-F}$ = 16.2 Hz), 123.66, 123.49 (d, $^4J_{C-F}$ = 4.2 Hz), 116.34, 101.00, 14.28 (d, $^4J_{C-F}$ = 4.0 Hz). LC/MS (+ESI): m/z = 268.97 (M + H).

***N*^{4}-(4-bromo-3-methoxyphenyl)quinazoline-4,6-diamine (3f).** Yield 83% (0.86 g, solid); ^{1}H NMR (500 MHz, DMSO- d_6) δ 9.97 (s, 2H), 8.48 (s, 1H), 7.95 (s, 1H), 7.70 (d, J = 2.1 Hz, 1H), 7.61 (d, J = 8.9 Hz, 1H), 7.55 (d, J = 8.6 Hz, 1H), 7.52 (dd, J = 8.6, 2.1 Hz, 1H), 7.46 (d, J = 2.3 Hz, 1H), 7.33 (dd, J = 8.9, 2.4 Hz, 1H), 3.86 (s, 3H). ^{13}C NMR (126 MHz, DMSO- d_6) δ 156.48, 155.17, 148.32, 148.12, 140.05, 138.45, 132.28, 126.07, 124.36, 116.33, 115.63, 106.92, 104.50, 101.23, 56.09. LC/MS (+ESI): m/z = 344.88 (M + H).

***N*^{4}-(2,4-dimethoxyphenyl)quinazoline-4,6-diamine (3g).** Yield 80% (0.70 g, solid); ^{1}H NMR (500 MHz, DMSO- d_6): δ 8.53 (s, 1H), 8.18 (s, 1H), 7.66 (d, *J* = 8.7 Hz, 1H), 7.49 (d, *J* = 9.3 Hz, 1H), 7.26 – 7.15 (m, 2H), 6.68 (d, *J* = 2.6 Hz, 1H), 6.56 (dd, *J* = 8.7, 2.6 Hz, 1H), 5.53 (s, 2H), 3.80 (s, 3H), 3.79 (s, 3H). ^{13}C NMR (126 MHz, DMSO- d_6): δ 157.45, 156.80, 153.54, 150.29, 147.10, 142.16, 128.58, 126.49, 123.24, 121.01, 116.26, 104.29, 100.56, 99.01, 55.72, 55.33. LC/MS (+ESI): m/z = 297.19 (M + H).

***N*^{4}-(2-ethylphenyl)quinazoline-4,6-diamine (3h).** Yield 79% (0.62 g, solid); ^{1}H NMR (500 MHz, DMSO- d_6): δ 9.10 (s, 1H), 8.09 (s, 1H), 7.49 (d, *J* = 8.8 Hz, 1H), 7.33 – 7.26 (m, 3H), 7.26 – 7.19 (m, 3H), 5.52 (s, 2H), 2.55 (q, *J* = 7.5 Hz, 2H), 1.08 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (126 MHz, DMSO- d_6): δ 157.69, 150.40, 147.01, 142.17, 140.61, 137.17, 128.47, 128.39, 128.18, 126.27, 126.11, 123.36, 116.07, 101.16, 24.08, 14.09.

***N*^{4}-(3-ethylphenyl)quinazoline-4,6-diamine (3i).** Yield 77% (0.61 g, solid); ^{1}H NMR (300 MHz, DMSO- d_6) δ 9.27 (s, 1H), 8.32 (s, 1H), 7.73 (d, *J* = 8.1 Hz, 1H), 7.66 (s, 1H), 7.52 (d, *J* = 8.9 Hz, 1H), 7.37 (d, *J* = 2.3 Hz, 1H), 7.27 (d, *J* = 7.5 Hz, 1H), 7.22 (d, *J* = 2.4 Hz, 1H), 6.91 (d, *J* = 7.5 Hz, 1H), 5.57 (s, 2H), 2.62 (q, *J* = 7.6 Hz, 2H), 1.21 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (75 MHz, DMSO- d_6) δ 156.08, 149.93, 147.22, 143.85, 142.55, 139.93, 128.64, 128.24, 123.54, 122.42, 121.06, 119.27, 116.72, 101.16, 28.34, 15.63. LC/MS (+ESI): m/z = 265.02 (M + H).

***N*^{4}-(4-ethylphenyl)quinazoline-4,6-diamine (3j)**. Yield 78% (0.62 g, solid); ^{1}H NMR (500 MHz, DMSO- d_6) δ 9.25 (s), 8.29 (s), 7.73 (d, *J* = 8.5 Hz), 7.51 (d, *J* = 8.8 Hz), 7.36 (d, *J* = 2.3 Hz), 7.23 (dd, *J* = 8.9, 2.4 Hz), 7.18 (d, *J* = 8.5 Hz), 5.53 (s), 2.59 (q, *J* = 7.6 Hz), 1.19 (t, *J* = 7.6 Hz). ^{13}C NMR (126 MHz, DMSO- d_6): δ 156.09, 149.95, 147.10, 142.51, 138.35, 137.51, 128.57, 127.53, 123.43, 121.95, 116.61, 101.16, 27.66, 15.78.

4-((6-aminoquinazolin-4-yl)amino)benzenesulfonamide (3k). Yield 82% (0.77 g, solid); ^{1}H NMR (500 MHz, DMSO- d_6): δ 9.63 (s, 1H), 8.40 (s, 1H), 8.07 (d, *J* = 8.7 Hz, 2H), 7.80 (d, *J* = 8.8 Hz, 2H), 7.57 (d, *J* = 8.9 Hz, 1H), 7.37 (d, *J* = 2.2 Hz, 1H), 7.28 (dd, *J* = 8.9, 2.2 Hz, 1H), 7.23 (s, 2H), 5.64 (s, 2H). ^{13}C NMR (126 MHz, DMSO- d_6): δ 155.64, 149.45, 147.46, 143.17, 142.82, 137.43, 128.73, 126.24, 123.98, 120.52, 116.82, 100.81. LC/MS (+ESI): m/z = 316.15 (M + H).

4-((6-aminoquinazolin-4-yl)amino)-N-carbamimidoylbenzenesulfonamide (3l). Yield 85% (0.91 g, solid); ^{1}H NMR (500 MHz, DMSO- d_6): δ 9.56 (s, 1H), 8.39 (s, 1H), 8.00 (d, *J* = 8.8 Hz, 2H), 7.73 (d, *J* = 8.9 Hz, 2H), 7.56 (d, *J* = 8.9 Hz, 1H), 7.36 (d, *J* = 2.3 Hz, 1H), 7.27 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.68 (s, 4H), 5.62 (s, 2H). ^{13}C NMR (126 MHz, DMSO- d_6): δ 158.04, 155.66, 149.52, 147.40, 142.82, 142.60, 138.03, 128.73, 126.12, 123.90, 120.41, 116.81, 100.86.

4-((6-aminoquinazolin-4-yl)amino)-N-(thiazol-2-yl)benzenesulfonamide (3m). Yield 79% (0.94 g, solid); ^{1}H NMR (500 MHz, DMSO- d_6): δ 9.67 (d, *J* = 4.9 Hz, 1H), 8.39 (s, 1H), 8.04 (d, *J* = 8.8 Hz, 2H), 7.85 (s, 1H), 7.80 – 7.75 (m, 2H), 7.56 (d, *J* = 8.9 Hz, 1H), 7.35 (d, *J* = 2.3 Hz, 1H), 7.24 (d, *J* = 4.6 Hz, 1H), 6.85 – 6.82 (m, 1H), 6.81 (d, *J* = 4.6 Hz, 1H), 5.64 (s, 2H). ^{13}C NMR (126 MHz, DMSO- d_6): δ 155.60, 149.36, 147.48, 144.70, 135.47, 128.61, 126.49, 124.41, 124.00, 122.07, 121.54, 120.47, 116.83, 108.00, 100.82.

4-((6-aminoquinazolin-4-yl)amino)-N-(pyridin-2-yl)benzenesulfonamide (3n). Yield 83% (0.97 g, solid); ^{1}H NMR (500 MHz, DMSO- d_6) δ 11.73 (s, 1H), 9.66 (s, 1H), 8.40 (s, 1H), 8.06 (d, *J* = 1.8 Hz, 1H), 8.05 (d, *J* = 5.2 Hz, 2H), 7.88 – 7.83 (m, 2H), 7.71 (ddd, *J* = 8.7, 7.2, 1.9 Hz, 1H), 7.56 (d, *J* = 8.9 Hz, 1H), 7.34 (d, *J* = 2.3 Hz, 1H), 7.28 (dd, *J* =

8.9, 2.4 Hz, 1H), 7.17 (dt, *J* = 8.6, 0.9 Hz, 1H), 6.88 (ddd, *J* = 7.1, 5.4, 0.9 Hz, 1H), 5.68 (s, 2H). ^{13}C NMR (126 MHz, DMSO- d_6) δ 155.56, 152.86, 149.34, 147.54, 143.71, 142.72, 139.89, 134.51, 128.66, 127.42, 124.07, 122.00, 120.39, 116.88, 116.06, 113.31, 100.79. LC/MS (+ESI): m/z = 392.92 (M + H).

***N*^{4}-cyclohexylquinazoline-4,6-diamine (3o).** Yield 80% (0.58 g, solid); ^{1}H NMR (500 MHz, Acetone): δ 8.23 (d, *J* = 42.6 Hz, 1H), 7.44 (dd, *J* = 42.8, 8.8 Hz, 1H), 7.13 (td, *J* = 33.1, 16.5 Hz, 2H), 6.49 (d, *J* = 32.5 Hz, 1H), 4.84 (d, *J* = 36.6 Hz, 2H), 4.21 (s, 1H), 2.02 – 1.94 (m, 2H), 1.82 – 1.57 (m, 3H), 1.48 – 1.27 (m, 4H), 1.25 – 1.08 (m, 1H). ^{13}C NMR (126 MHz, Acetone): δ 158.45, 152.40, 147.24, 143.95, 129.83, 123.51, 102.34, 84.10, 50.28, 33.49, 26.52, 26.09. LC/MS (+ESI): m/z = 243.21 (M + H).

General procedure for the synthesis of compounds (4a-4j, 4o). A mixture of the corresponding aminoquinazoline derivative **3a-3j, 3o** (1 mmol) and $NaHCO_3$ (1.3 mmol) was stirred at 0°C in acetone (10 ml) under nitrogen atmosphere. This is then followed by dropwise addition of acryloyl chloride (1.3 mmol) and then was stirred for 30 min. at 0°C. Excess solvent was then removed under reduced pressure and the remaining residue was neutralized using $NaHCO_3$ solution. The formed solid was then filtered and the purified using column chromatography with ethylacetate as eluent.

General procedure for the synthesis of compounds (4k-4n).

Same above procedure except that the solvent used in the reaction was DMF instead of acetone and the eluent in column chromatography was Dichloromethane:Methanol 100:5.

***N*-(4-((2-bromo-6-fluorophenyl)amino)quinazolin-6-yl)acrylamide (4a).** Yield 56% (0.21 g, solid); m.p. 303-304°C; ^{1}H NMR (500 MHz, DMSO- d_6): δ 10.52 (s, 1H), 9.78 (s, 1H), 8.81 (s, 1H), 8.46 (s, 1H), 7.92 (d, *J* = 8.3 Hz, 1H), 7.85 – 7.72 (m, 2H), 7.63 (s,

1H), 7.14 (s, 1H), 6.53 (dd, *J* = 16.5, 10.4 Hz, 1H), 6.34 (d, *J* = 16.9 Hz, 1H), 5.83 (d, *J* = 10.1 Hz, 1H). LC/MS (+ESI): m/z = 386.99 (M + H).

***N*-(4-((4-bromo-2-fluorophenyl)amino)quinazolin-6-yl)acrylamide (4b).** Yield 58% (0.22 g, solid); m.p. 234-236°C; ^{1}H NMR (500 MHz, DMSO- d_6): δ 10.50 (s, 1H), 9.91 (s, 1H), 8.81 (s, 1H), 8.40 (s, 1H), 7.89 (dd, *J* = 8.9, 2.1 Hz, 1H), 7.77 (d, *J* = 8.5 Hz, 1H), 7.67 – 7.57 (m, 1H), 7.49 (s, 1H), 7.45 (dd, *J* = 8.3, 1.6 Hz, 1H), 6.52 (dd, *J* = 17.0, 10.1 Hz, 1H), 6.34 (dd, *J* = 17.0, 1.9 Hz, 1H), 5.83 (dd, *J* = 10.1, 1.9 Hz, 1H). LC/MS (+ESI): m/z = 386.99 (M + H).

***N*-(4-((4-bromo-2-methylphenyl)amino)quinazolin-6-yl)acrylamide (4c).** Yield 59% (0.22 g, solid); m.p. 261-262°C; ^{1}H NMR (500 MHz, DMSO- d_6): δ 10.46 (s, 1H), 9.66 (s, 1H), 8.78 (d, *J* = 2.1 Hz, 1H), 8.36 (s, 1H), 7.87 (dd, *J* = 9.0, 2.2 Hz, 1H), 7.76 (d, *J* = 8.9 Hz, 1H), 7.53 (d, *J* = 1.9 Hz, 1H), 7.42 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.29 (d, *J* = 8.4 Hz, 1H), 6.53 (dd, *J* = 17.0, 10.2 Hz, 1H), 6.34 (dd, *J* = 17.0, 1.9 Hz, 1H), 5.83 (dd, *J* = 10.1, 1.9 Hz, 1H), 2.17 (s, 3H). ^{13}C NMR (126 MHz, DMSO- d_6): δ 163.29, 158.44, 153.60, 146.55, 137.62, 137.14, 136.40, 132.77, 131.59, 129.38, 128.96, 128.33, 127.29, 126.93, 118.41, 115.00, 112.28, 17.77. LC/MS (+ESI): m/z = 383.03 (M + H).

***N*-(4-((4-bromo-3-methylphenyl)amino)quinazolin-6-yl)acrylamide (4d)**. Yield 63% (0.24 g, solid); m.p. 296-297°C; ^{1}H NMR (500 MHz, DMSO- d_6): δ 10.47 (s, 1H), 9.83 (s, 1H), 8.80 (d, *J* = 2.0 Hz, 1H), 8.54 (s, 1H), 7.89 (dd, *J* = 9.0, 2.2 Hz, 1H), 7.82 (d, *J* = 2.4 Hz, 1H), 7.79 (d, *J* = 8.9 Hz, 1H), 7.66 (dd, *J* = 8.6, 2.5 Hz, 1H), 7.56 (d, *J* = 8.7 Hz, 1H), 6.53 (dd, *J* = 17.0, 10.2 Hz, 1H), 6.35 (dd, *J* = 17.0, 1.9 Hz, 1H), 5.84 (dd, *J* = 10.1, 1.9 Hz, 1H), 2.37 (s, 3H). ^{13}C NMR (126 MHz, DMSO- d_6): δ 163.30, 157.37, 153.23,

146.73, 138.97, 136.96, 136.51, 131.81, 131.55, 128.43, 127.39, 127.15, 124.57, 121.71, 117.73, 115.44, 112.38, 22.65. LC/MS (+ESI): m/z = 383.05 (M + H).

***N*-(4-((2-fluoro-3-methylphenyl)amino)quinazolin-6-yl)acrylamide (4e).** Yield 65% (0.21 g, solid); m.p. 229-231°C; ^{1}H NMR (500 MHz, DMSO- d_6): δ 10.48 (s, 1H), 9.75 (s, 1H), 8.81 (d, J = 1.8 Hz, 1H), 8.42 (s, 1H), 7.88 (dd, J = 9.0, 2.1 Hz, 1H), 7.78 (d, J = 8.9 Hz, 1H), 7.37 (t, J = 7.0 Hz, 1H), 7.17 (t, J = 6.7 Hz, 1H), 7.12 (t, J = 7.7 Hz, 1H), 6.53 (dd, J = 17.0, 10.1 Hz, 1H), 6.34 (dd, J = 17.0, 1.8 Hz, 1H), 5.83 (dd, J = 10.2, 1.8 Hz, 1H), 2.29 (s, 3H). ^{13}C NMR (126 MHz, DMSO- d_6) δ 163.31, 158.37, 155.30 (d, $^{1}J_{C-F}$ = 246.1 Hz), 153.57, 146.55, 136.47, 131.58, 128.41, 128.36, 127.32, 126.96, 126.49 (d, $^{3}J_{C-F}$ = 12.7 Hz), 125.46, 124.72 (d, $^{2}J_{C-F}$ = 16.1 Hz), 123.56 (d, $^{4}J_{C-F}$ = 4.1 Hz). 115.06, 112.22, 14.25 (d, $^{4}J_{C-F}$ = 3.9 Hz). LC/MS (+ESI): m/z = 323.18 (M + H).

***N*-(4-((4-bromo-3-methoxyphenyl)amino)quinazolin-6-yl)acrylamide (4f).** Yield 62% (0.25 g, solid); m.p. 268-269°C; ^{1}H NMR (500 MHz, DMSO- d_6): δ 10.49 (s, 1H), 9.85 (s, 1H), 8.81 (d, J = 2.1 Hz, 1H), 8.58 (s, 1H), 7.91 (dd, J = 9.0, 2.2 Hz, 1H), 7.80 (d, J = 8.9 Hz, 1H), 7.69 (d, J = 2.0 Hz, 1H), 7.56 (dd, J = 8.7, 2.1 Hz, 1H), 7.54 (d, J = 8.6 Hz, 1H), 6.53 (dd, J = 17.0, 10.2 Hz, 1H), 6.35 (dd, J = 17.0, 1.9 Hz, 1H), 5.84 (dd, J = 10.1, 1.9 Hz, 1H), 3.87 (s, 3H). ^{13}C NMR (126 MHz, DMSO- d_6): δ 163.33, 157.28, 155.13, 153.16, 146.74, 140.38, 136.58, 132.22, 131.53, 128.49, 127.45, 127.18, 115.50, 115.35, 112.27, 106.60, 103.98, 56.04. LC/MS (+ESI): m/z = 399.02 (M + H).

***N*-(4-((2,4-dimethoxyphenyl)amino)quinazolin-6-yl)acrylamide (4g)**. Yield 68% (0.24 g, solid); m.p. 178-180°C; ^{1}H NMR (500 MHz, DMSO- d_6): δ 10.45 (s, 1H), 9.13 (s, 1H), 8.68 (d, J = 2.0 Hz, 1H), 8.36 (s, 1H), 7.89 (dd, J = 9.0, 2.2 Hz, 1H), 7.73 (d, J = 8.9 Hz, 1H), 7.49 (d, J = 8.6 Hz, 1H), 6.69 (d, J = 2.6 Hz, 1H), 6.57 (dd, J = 8.7, 2.7 Hz, 1H),

6.52 (dd, *J* = 17.0, 10.2 Hz, 1H), 6.33 (dd, *J* = 17.0, 1.9 Hz, 1H), 5.82 (dd, *J* = 10.1, 1.9 Hz, 1H), 3.80 (s, 3H), 3.77 (s, 3H). ^{13}C NMR (126 MHz, DMSO- d_6): δ 163.32, 158.46, 158.10, 154.31, 153.77, 146.38, 136.29, 131.62, 128.28, 127.40, 127.25, 126.68, 120.47, 115.04, 111.94, 104.41, 99.13, 55.68, 55.35. LC/MS (+ESI): m/z = 351.18 (M + H).

***N*-(4-((2-ethylphenyl)amino)quinazolin-6-yl)acrylamide (4h)**. Yield 61% (0.19 g, solid); m.p. 148-150°C; ^{1}H NMR (500 MHz, DMSO- d_6) δ 10.49 (s, 1H), 9.66 (s, 1H), 8.73 (s, 1H), 8.31 (s, 1H), 7.90 (dd, *J* = 8.9, 1.8 Hz, 1H), 7.74 (d, *J* = 8.9 Hz, 1H), 7.33 (d, *J* = 4.3 Hz, 1H), 7.26 (d, *J* = 4.1 Hz, 3H), 6.52 (dd, *J* = 17.0, 10.2 Hz, 1H), 6.33 (dd, *J* = 17.0, 1.5 Hz, 1H), 5.82 (dd, *J* = 10.2, 1.4 Hz, 1H), 2.55 (q, *J* = 7.5 Hz, 2H), 1.08 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (126 MHz, DMSO- d_6): δ 163.84, 159.56, 154.32, 146.90, 141.22, 137.33, 136.77, 132.01, 129.05, 128.68, 127.89, 127.42, 127.11, 126.70, 115.38, 112.90, 112.87, 24.54, 14.60. LC/MS (+ESI): m/z = 319.21 (M + H).

***N*-(4-((3-ethylphenyl)amino)quinazolin-6-yl)acrylamide (4i)**. Yield 65% (0.21 g, solid); m.p. 216-217°C; ^{1}H NMR (500 MHz, DMSO- d_6): δ 10.45 (s, 1H), 9.74 (s, 1H), 8.79 (d, *J* = 2.1 Hz, 1H), 8.52 (s, 1H), 7.90 (dd, *J* = 8.9, 2.2 Hz, 1H), 7.77 (d, *J* = 8.9 Hz, 1H), 7.69 (dd, *J* = 8.1, 1.1 Hz, 1H), 7.63 (t, *J* = 1.6 Hz, 1H), 7.28 (t, *J* = 7.8 Hz, 1H), 6.97 (dd, *J* = 7.6, 0.5 Hz, 1H), 6.53 (dd, *J* = 17.0, 10.2 Hz, 1H), 6.35 (dd, *J* = 17.0, 1.9 Hz, 1H), 5.83 (dd, *J* = 10.1, 1.9 Hz, 1H), 2.63 (q, *J* = 7.6 Hz, 2H), 1.22 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (126 MHz, DMSO- d_6): δ 163.28, 157.56, 153.41, 146.71, 143.88, 139.34, 136.36, 131.58, 128.34, 128.22, 127.32, 127.05, 123.03, 121.68, 119.88, 115.45, 112.58, 28.23, 15.50. LC/MS (+ESI): m/z = 319.19 (M + H).

***N*-(4-((4-ethylphenyl)amino)quinazolin-6-yl)acrylamide (4j)**. Yield 63% (0.20 g, solid); m.p. 229-230°C; ^{1}H NMR (500 MHz, DMSO- d_6) δ 10.45 (s, 1H), 9.74 (s, 1H),

8.77 (d, *J* = 1.3 Hz, 1H), 8.48 (s, 1H), 7.89 (dd, *J* = 8.9, 1.8 Hz, 1H), 7.76 (d, *J* = 8.9 Hz, 1H), 7.70 (d, *J* = 8.3 Hz, 2H), 7.21 (d, *J* = 8.3 Hz, 2H), 6.53 (dd, *J* = 17.0, 10.2 Hz, 1H), 6.34 (dd, *J* = 17.0, 1.5 Hz, 1H), 5.83 (dd, *J* = 10.2, 1.5 Hz, 1H), 2.61 (q, *J* = 7.5 Hz, 2H), 1.20 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (126 MHz, DMSO- d_6): δ 163.28, 157.59, 153.44, 146.68, 139.05, 136.95, 136.32, 131.58, 128.31, 127.58, 127.31, 127.00, 122.60, 115.40, 112.56, 27.67, 15.71. MS (+ESI): m/z = 319.2 (M + H).

***N*-(4-((4-sulfamoylphenyl)amino)quinazolin-6-yl)acrylamide (4k).** Yield 59% (0.22 g, solid); m.p. 269-271°C; ^{1}H NMR (500 MHz, DMSO- d_6): δ 10.52 (s, 1H), 10.09 (s, 1H), 8.84 (d, *J* = 1.9 Hz, 1H), 8.61 (s, 1H), 8.04 (d, *J* = 8.8 Hz, 2H), 7.93 (dd, *J* = 9.0, 2.1 Hz, 1H), 7.83 (d, *J* = 8.7 Hz, 3H), 7.27 (s, 2H), 6.53 (dd, *J* = 17.0, 10.1 Hz, 1H), 6.35 (dd, *J* = 17.0, 1.7 Hz, 1H), 5.85 (dd, *J* = 10.1, 1.7 Hz, 1H). ^{13}C NMR (126 MHz, DMSO- d_6): δ 163.38, 157.32, 153.06, 146.86, 142.61, 138.19, 136.72, 131.50, 128.54, 127.52, 127.32, 126.23, 121.40, 115.57, 112.24. LC/MS (+ESI): m/z = 370.09 (M + H).

***N*-(4-((4-(*N*-carbamimidoylsulfamoyl)phenyl)amino)quinazolin-6-yl)acrylamide (4l).** Yield, 55% (0.23 g, solid); m.p. 282-284°C; ^{1}H NMR (500 MHz, DMSO- d_6): δ 10.51 (s, 1H), 10.03 (s, 1H), 8.82 (d, *J* = 1.8 Hz, 1H), 8.59 (s, 1H), 7.97 (d, *J* = 8.7 Hz, 2H), 7.94 (dd, *J* = 9.0, 2.0 Hz, 1H), 7.82 (d, *J* = 8.9 Hz, 1H), 7.75 (d, *J* = 8.7 Hz, 2H), 6.69 (s, 4H), 6.54 (dd, *J* = 17.0, 10.1 Hz, 1H), 6.35 (dd, *J* = 17.0, 1.7 Hz, 1H), 5.84 (dd, *J* = 10.2, 1.7 Hz, 1H). ^{13}C NMR (126 MHz, DMSO- d_6): δ 163.35, 158.07, 157.32, 153.11, 146.84, 142.02, 138.80, 136.68, 131.53, 128.50, 127.46, 127.28, 126.11, 121.28, 115.55, 112.30. LC/MS (+ESI): m/z = 412.10 (M + H).

***N*-(4-((4-(*N*-(thiazol-2-yl)sulfamoyl)phenyl)amino)quinazolin-6-yl)acrylamide (4m).** Yield 60% (0.27 g, solid); m.p. 279-280°C; ^{1}H NMR (500 MHz, DMSO- d_6): δ 10.29 (s,

2H), 9.86 (s, 2H), 8.62 (s, 2H), 8.38 (s, 2H), 7.81 (d, *J* = 8.3 Hz, 4H), 7.71 (dd, *J* = 9.2, 2.2 Hz, 3H), 7.60 (d, *J* = 8.8 Hz, 6H), 7.03 (s, 4H), 6.30 (dd, *J* = 17.0, 10.1 Hz, 2H), 6.12 (dd, *J* = 17.0, 1.7 Hz, 2H), 5.62 (dd, *J* = 10.2, 1.7 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 163.36, 162.27, 157.31, 153.04, 146.86, 142.64, 138.18, 136.74, 131.52, 128.54, 127.48, 127.30, 126.23, 121.39, 116.20, 116.17, 115.59, 112.28. LC/MS (+ESI): m/z = 453.13 (M + H).

***N*-(4-((4-(*N*-(pyridin-2-yl)sulfamoyl)phenyl)amino) quinazolin-6-yl)acrylamide (4n)**. Yield 63% (0.28 g, solid); m.p. 210-212°C; ^{1}H NMR (500 MHz, DMSO- d_6): δ 8.78 (s, 1H), 8.57 (s, 1H), 8.04 (d, *J* = 8.7 Hz, 2H), 8.01 (d, *J* = 5.4 Hz, 1H), 7.95 (d, *J* = 8.8 Hz, 2H), 7.82 – 7.76 (m, 2H), 7.75 – 7.69 (m, 1H), 7.28 (d, *J* = 8.7 Hz, 1H), 6.90 (t, *J* = 6.3 Hz, 1H), 6.50 (s, 1H), 6.49 – 6.47 (m, 1H), 5.85 (dd, *J* = 8.4, 3.4 Hz, 1H). ^{13}C NMR (126 MHz, DMSO- d_6): δ 166.25, 159.27, 154.50, 147.75, 144.40, 144.22, 141.75, 141.69, 138.32, 137.38, 132.07, 129.07, 128.90, 128.79, 128.44, 122.63, 117.20, 117.03, 115.83, 112.73. LC/MS (+ESI): m/z = 447.14 (M + H).

***N*-(4-(cyclohexylamino)quinazolin-6-yl)acrylamide (4o)**. Yield 68% (0.20 g, solid); m.p. 182-184°C; ^{1}H NMR (500 MHz, DMSO- d_6) δ 10.34 (s, 1H), 8.49 (d, *J* = 2.1 Hz, 1H), 8.37 (s, 1H), 7.83 – 7.78 (m, 2H), 7.64 (d, *J* = 8.9 Hz, 1H), 6.50 (dd, *J* = 17.0, 10.1 Hz, 1H), 6.31 (dd, *J* = 17.0, 1.9 Hz, 1H), 5.80 (dd, *J* = 10.1, 1.9 Hz, 1H), 4.24 – 4.13 (m, 1H), 1.92 (d, *J* = 12.2 Hz, 2H), 1.77 (d, *J* = 12.9 Hz, 2H), 1.65 (d, *J* = 12.8 Hz, 1H), 1.46 – 1.32 (m, 4H), 1.21 – 1.14 (m, 1H). ^{13}C NMR (126 MHz, DMSO- d_6): δ 163.13, 158.25, 154.10, 146.21, 135.58, 131.62, 127.94, 127.06, 126.52, 114.99, 112.82, 49.35, 31.89, 25.37, 25.07. LC/MS (+ESI): m/z = 297.21 (M + H).

6-*tert*-butyl 3-ethyl 2-amino-4,5-dihydrothieno[2,3-*c*]pyridine-3,6(7*H*)-dicarboxylate (5). According to the reported procedure.[2]

***tert*-butyl 4-oxo-3,4,5,6-tetrahydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidine-7(8*H*)-carboxylate (6).** According to the reported procedure.[2]

***tert*-butyl 4-chloro-5,6-dihydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidine-7(8*H*)-carboxylate (7).** According to the reported procedure.[2]

General procedure for the synthesis of compounds (8a-8f).

A mixture of **7** (3 mmol) and the corresponding amine (3.2 mmol) in 1ml ethanol was refluxed for 8 h. The reaction mixture was concentrated, and the residue was partitioned between water and dichloromethane; the organic layer separated, dried over anhydrous $MgSO_4$, and concentrated. The crude product was purified by silica gel column chromatography using a mixture of Dichloromethane:Methanol (100:3) to give compounds **8a-8e**.

***tert*-butyl 4-((2-fluoro-3-methylphenyl)amino)-5,6-dihydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidine-7(8*H*)-carboxylate (8a).** Yield 53% (0.66 g, solid); LC/MS (+ESI): m/z = 414.65 (M + H).

***tert*-butyl 4-((4-bromo-2-fluorophenyl)amino)-5,6-dihydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidine-7(8*H*)-carboxylate (8b).** Yield 50% (0.72 g, solid); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.61 (t, *J* = 8.7 Hz, 1H), 8.57 (s, 1H), 7.35 (s, 1H), 7.34 – 7.27 (m, 2H), 4.72 (s, 2H), 3.87 (t, *J* = 5.7 Hz, 2H), 3.15 (t, *J* = 5.4 Hz, 2H), 1.51 (s, 9H). LC/MS (+ESI): m/z = 478.62 (M + H).

***tert*-butyl 4-((4-bromo-3-methylphenyl)amino)-5,6-dihydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidine-7(8*H*)-carboxylate (8c).** Yield 46%

(0.65 g, solid); ^{1}H NMR (300 MHz, DMSO- d_6) δ 8.42 (s, 1H), 8.20 (s, 1H), 7.61 (s, 1H), 7.51 (d, *J* = 1.2 Hz, 2H), 4.67 (s, 2H), 3.69 (t, *J* = 5.4 Hz, 2H), 3.20 (s, 2H), 2.34 (s, 3H), 1.45 (s, 9H). LC/MS (+ESI): m/z = 474.61 (M + H).

***tert*-butyl 4-((3-ethylphenyl)amino)-5,6-dihydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidine-7(8*H*)-carboxylate (8d).** Yield 48% (0.59 g, solid); ^{1}H NMR (500 MHz, DMSO- d_6) δ 8.40 (s, 1H), 8.15 (s, 1H), 7.53 (dd, J = 8.1, 1.2 Hz, 1H), 7.25 (t, *J* = 7.8 Hz, 1H), 6.95 (dd, *J* = 7.6, 0.5 Hz, 1H), 4.67 (s, 2H), 3.69 (s, 2H), 3.21 (t, *J* = 5.6 Hz, 2H), 2.61 (q, *J* = 7.6 Hz, 2H), 1.45 (s, 9H), 1.20 (t, *J* = 7.6 Hz, 3H). LC/MS (+ESI): m/z = 410.67 (M + H).

***tert*-butyl 4-((4-ethylphenyl)amino)-5,6-dihydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidine-7(8*H*)-carboxylate (8e).** Yield 47% (0.57 g, solid); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.49 (s, 1H), 7.50 (d, *J* = 8.4 Hz, 2H), 7.23 (d, *J* = 8.5 Hz, 2H), 6.92 (s, 1H), 4.71 (s, 2H), 3.85 (t, *J* = 5.6 Hz, 2H), 3.14 (s, 2H), 2.66 (q, *J* = 7.6 Hz, 2H), 1.51 (s, 9H), 1.25 (t, *J* = 7.6 Hz, 3H). LC/MS (+ESI): m/z = 410.72 (M + H).

***tert*-butyl 4-(cyclohexylamino)-5,6-dihydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidine-7(8*H*)-carboxylate (8f).** Yield 42% (0.49 g, solid); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.39 (s, 1H), 5.07 (d, *J* = 7.0 Hz, 1H), 4.65 (s, 2H), 4.28 – 4.08 (m, 1H), 3.80 (t, *J* = 5.7 Hz, 2H), 2.99 (s, 2H), 2.15 – 1.97 (m, 2H), 1.83 – 1.60 (m, 4H), 1.50 (s, 9H), 1.35 – 1.17 (m, 4H). LC/MS (+ESI): m/z = 388.66 (M + H).

General procedure for the synthesis of compounds (9a-9f).

To a mixture of the corresponding intermediate **8a-8f** (1.5 mmol) in dichloromethane (2mL) at 0°C was added trifluoroacetic acid (TFA) (1mL) and then warmed to room temperature. The reaction mixture was stirred for 2 h, removed the solvent under vacuum,

and neutralized the residue by slow addition of sodium bicarbonate solution and then extracted with ethyl acetate. The organic layer separated, dried over anhydrous $MgSO_4$, and concentrated to give **9a-9f** and they were used directly for the next step without further purification.

Compound	**9a**	**9b**	**9c**	**9d**	**9e**	**9f**
% Yield	89	82	86	94	83	85
Amount (g)	0.42	0.46	0.48	0.43	0.38	0.36
Physical State	solid	solid	solid	solid	solid	solid
LC/MS(+ESI): m/z (M + H)=	314.80	378.48	374.59	310.80	310.89	288.97

General procedure for the synthesis of compounds (10a-10f).

A mixture of the corresponding intermediate **9a-9f** (1 mmol) and $NaHCO_3$ (1.3 mmol) was stirred at 0°C in acetone (10 ml) under nitrogen atmosphere. This is then followed by dropwise addition of acryloyl chloride (1.3 mmol) and then was stirred for 30 min. at 0°C. Excess solvent was then removed under reduced pressure and the remaining residue was neutralized using $NaHCO_3$ solution. The formed solid was then filtered and the purified using column chromatography using a mixture of dichloromethane:methanol (100:1) as eluent.

1-(4-((2-fluoro-3-methylphenyl)amino)-5,6-dihydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidin-7(8*H*)-yl)prop-2-en-1-one (10a). Yield 25% (92 mg, solid); m.p. 189-190°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.56 (s, 1H), 8.45 (d, *J* = 7.6 Hz, 1H), 7.31 (d, *J* = 23.4 Hz, 1H), 7.08 (t, *J* = 7.8 Hz, 1H), 6.92 (t, *J* = 7.3 Hz, 1H), 6.74 – 6.53 (m, 1H), 6.45 – 6.29 (m, 1H), 5.81 (d, *J* = 9.6 Hz, 1H), 4.90 (d, *J* = 44.8 Hz, 2H), 4.04 (d, *J* = 49.4 Hz, 2H), 3.23 (s, 2H), 2.32 (d, *J* = 2.0 Hz, 3H). LC/MS (+ESI): m/z = 368.73 (M + H).

1-(4-((4-bromo-2-fluorophenyl)amino)-5,6-dihydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidin-7(8*H*)-yl)prop-2-en-1-one (10b). Yield 28% (121 mg, solid); m.p. 231-233°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.60 (s, 1H), 8.57 (s, 1H), 7.36 – 7.30 (m, 2H), 7.22 (s, 1H), 6.78 – 6.52 (m, 1H), 6.38 (t, *J* = 14.4 Hz, 1H), 5.82 (d, *J* = 9.5 Hz, 1H), 4.91 (d, *J* = 44.8 Hz, 2H), 4.05 (d, *J* = 44.3 Hz, 2H), 3.22 (s, 2H). LC/MS (+ESI): m/z = 432.46 (M + H).

1-(4-((4-bromo-3-methylphenyl)amino)-5,6-dihydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidin-7(8*H*)-yl)prop-2-en-1-one (10c). Yield 30% (128 mg, solid); m.p. 216-218°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.52 (s, 1H), 7.51 (d, *J* = 8.6 Hz, 1H), 7.48 (d, *J* = 2.4 Hz, 1H), 7.37 (s, 1H), 6.87 (d, *J* = 38.4 Hz, 1H), 6.73 – 6.53 (m, 1H), 6.37 (t, *J* = 14.2 Hz, 1H), 5.81 (d, *J* = 10.4 Hz, 1H), 4.90 (d, *J* = 45.1 Hz, 2H), 4.03 (d, *J* = 43.8 Hz, 2H), 3.20 (s, 2H), 2.42 (s, 3H). LC/MS (+ESI): m/z = 428.63 (M + H).

1-(4-((3-ethylphenyl)amino)-5,6-dihydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidin-7(8*H*)-yl)prop-2-en-1-one (10d). Yield 22% (80 mg, solid); m.p. 105-107°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.51 (s, 1H), 7.49 (s, 1H), 7.38 (s, 1H), 7.31 (t, *J* = 7.8 Hz, 1H), 7.01 (dd, *J* = 7.6, 0.6 Hz, 1H), 6.92 (d, *J* = 37.2 Hz, 1H), 6.75 – 6.53 (m, 1H), 6.44 – 6.27 (m, 1H), 5.80 (d, *J* = 10.6 Hz, 1H), 4.90 (d, *J* = 45.0 Hz, 2H), 4.03 (d, *J* = 46.7 Hz, 2H), 3.21 (s, 2H), 2.68 (q, *J* = 7.6 Hz, 2H), 1.27 (t, *J* = 7.6 Hz, 3H). LC/MS (+ESI): m/z = 364.70 (M + H).

1-(4-((4-ethylphenyl)amino)-5,6-dihydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidin-7(8*H*)-yl)prop-2-en-1-one (10e). Yield 26% (94 mg, solid); m.p. 201-202°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.49 (s, 1H), 7.49 (d, *J* = 7.9 Hz, 2H), 7.22 (d, *J* = 8.3 Hz, 2H), 6.89 (d, *J* = 36.7 Hz, 1H), 6.74 – 6.52 (m, 1H), 6.44 – 6.27 (m, 1H), 5.80 (d, *J* = 10.8 Hz, 1H),

4.89 (d, *J* = 44.7 Hz, 2H), 4.02 (d, *J* = 45.9 Hz, 2H), 3.19 (s, 2H), 2.65 (q, *J* = 7.6 Hz, 2H), 1.25 (t, *J* = 7.6 Hz, 3H). LC/MS (+ESI): m/z = 364.75 (M + H).

1-(4-(cyclohexylamino)-5,6-dihydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidin-7(8*H*)-yl)prop-2-en-1-one (10f). Yield 23% (78 mg, solid); m.p. 150-152°C; ^{1}H NMR (500 MHz, MeOD) δ 8.24 (s, 1H), 6.86 (ddd, *J* = 38.8, 16.8, 10.6 Hz, 1H), 6.28 (dd, *J* = 16.6, 9.3 Hz, 1H), 5.83 (t, *J* = 11.9 Hz, 1H), 4.95 – 4.67 (m, 2H), 4.16 – 4.07 (m, 1H), 4.01 (t, *J* = 5.6 Hz, 2H), 3.15 (d, *J* = 25.2 Hz, 2H), 2.04 (d, *J* = 9.6 Hz, 2H), 1.80 (dd, *J* = 9.4, 3.3 Hz, 2H), 1.68 (d, *J* = 12.6 Hz, 1H), 1.47 – 1.40 (m, 4H), 1.32 – 1.27 (m, 2H). LC/MS (+ESI): m/z = 342.95 (M + H).

Biological screening

Cell Culture and Plating

Cancer cell lines cultured included cell lines with wild type EGFR (SKBR-3 mammary carcinoma) and with mutant EGFR (H1975). Both cell lines were maintained in RPMI-1640 media supplemented with 10% fetal bovine serum in a 37°C humidified incubator with 5% CO_2 and subcultured twice weekly. Only cultures exhibiting greater than 95% viability were used in any experiment (determined by trypan blue exclusion). Cells were seeded in 96-well standard assay plates at a density of 5,000 cells/well for growth assays and 10,000 cells/well in optical quality PerkinElmer ViewPlate for immunofluorescence, then allowed to acclimate overnight before compound addition or stimulation with EGF.

Cytoblot Assay[3]

Serial dilutions of each compound were added to at least 3 replicate wells each 30 min prior to EGF stimulation (200 ng/mL). Each plate included a positive control (Iressa, 20μm) and negative control (DMSO). Cytoblot assays were conducted in H1975 (EGF

mutant) cell line. Phosphorylated EGFR was specifically detected (Cell Signaling Technology anti-PY1068 rabbit monoclonal antibody) to quantify the level of receptor autophosphorylation in response to EGF stimulation. Secondary goat anti-rabbit conjugate labeled with horseradish peroxidase enzyme was added, followed by addition of enhanced chemiluminescence reagent (ECL; Pierce Pico West). The resulting luminescence was quantitated using a Molecular Devices Paradigm multilabel microplate reader. Raw luminescence data were plotted to generate dose response curves and IC_{50} values.

Growth Assay

SKBR3 and H1975 cells were treated with 8 concentrations of inhibitors ranging from 50 µM to 8 nM (specifially, the doses tested were 50uM, 25uM, 10uM, 5uM, 1uM, 0.2uM, 0.04uM, and 0.008uM) followed by EGF stimulation (100 ng/mL) 1 h later. Cells were incubated for an additional 72 h at 37°C. Relative cell growth was determined by addition of Promega CellTiter Glo luciferase-based measure of ATP content, and the resulting luminescence was measured using a Molecular Devices Spectramax Paradigm microplate reader in luminescence mode. Growth inhibition data were analyzed using DMSO as a baseline (negative control equal to 0% growth inhibition) with GraphPad Prism curve fitting software. IC50 values are representative of the results at least two independent concentration-response experiments with three replicates per concentration.

EGFR kinase phosphorylation assay

Phosphorylation assays were performed in a final volume of 20 µl containing 8 mM MOPS (pH 7.0), 0.2 mM EDTA, 10 mM MnCl2, 200 µM substrate peptide, 0.25 mM DTT, 0.1 mg/ml BSA, 10 ng EGFR-Kinase (Cat. No. 40187, BPS Bioscience), 10 mM

magnesium acetate, 100 μM γ–[32P]ATP, and inhibitors at different concentrations or DMSO control (1.25% v/v). Reactions were started by the addition of the magnesium acetate/ATP mixture. After 30 min incubation at 30°C, 5 μl of each reaction was spotted on phosphocellulose P81 paper (Whatman). The P81 paper was then washed 5 times with 50 mM phosphoric acid for 15 min, dried and exposed to a phosphorimager screen, which was scanned and densitometrically analyzed the next day. The sequence of the substrate peptide was derived from phospholipase C-γ1 and had the sequence "KHKKLAEGSAYEEV", according to Fry *et al.* [4]

Molecular modeling

The proteins used for the docking was downloaded from the protein data bank (PDB 2J5F, 3W2P). The proteins were first prepared for docking using MOE software where the proteins were protonated and saved for docking. The ligands were drawn on MOE and energy minimized and then saved as "mol2" file. Docking was done using GOLD software, where the proteins are first prepared by removing the water molecules and extracting the co-crystallized ligands. The docking of the compounds included a covalent interaction which was done by specifying the atoms in the ligand and the protein that will covalently bind together and then docking was done using CHEMPLP as the scoring function and Goldscore as a rescoring function. The viewing of the results was done using PyMOL software and the side chains from the docked molecules were hidden to facilitate the viewing process.

References

1. S. Madapa, Z. Tusi, A. Mishra, K. Srivastava, S. K. Pandey, R. Tripathi, S. K. Puri and S. Batra, *Bioorg Med Chem*, 2009, **17**, 222-234.

2. C. H. Wu, M. S. Coumar, C. Y. Chu, W. H. Lin, Y. R. Chen, C. T. Chen, H. Y. Shiao, S. Rafi, S. Y. Wang, H. Hsu, C. H. Chen, C. Y. Chang, T. Y. Chang, T. W. Lien, M. Y. Fang, K. C. Yeh, C. P. Chen, T. K. Yeh, S. H. Hsieh, J. T. Hsu, C. C. Liao, Y. S. Chao and H. P. Hsieh, *J Med Chem*, 2010, **53**, 7316-7326.
3. G. Chiosis and A. B. Keeton, *Methods Mol Biol*, 2009, **486**, 139-149.
4. D. W. Fry, A. J. Kraker, A. McMichael, L. A. Ambroso, J. M. Nelson, W. R. Leopold, R. W. Connors and A. J. Bridges, *Science*, 1994, **265**, 1093-1095.