

Supporting Information

Synthesis and biological evaluation of 4-anilinoquinolines as potent inhibitors of epidermal growth factor receptor

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Table of contents

Experimental	S2
4-Chloro-6-nitroquinoline (7).	S2
<i>N</i>-(3-Bromophenyl)-6-nitroquinolin-4-amine (8a).	S3
<i>N</i>-4-(3-Bromophenyl)quinoline-4,6-diamine (11a).	S3
4-Chloro-6,7-bis(2-methoxyethoxy)quinoline (15a).	S4
Figure S1	S6
References	S6

Experimental

4-Chloro-6-nitroquinoline (7). A mixture of concentrated nitric acid (6 mL) and concentrated sulfuric acid (6 mL) was added slowly to a solution of 4-hydroxyquinoline 5 (5 g, 34.5 mmol) in concentrated sulfuric acid (6 mL). The temperature of the reaction mixture was kept at -15 to 0 °C. The mixture was stirred for 2 h at the same temperature, added to ice-water (1:1) and the precipitated crude product 6-nitroquinolin-4-ol (6) (5.4 g, 88.5%) was collected by filtration and dried under high vacuum. The crude product was used as such for the following step. A two neck flask was flushed with argon and charged with 6-nitroquinolin-4-ol (6) (2 g, 10.5 mmol) and POCl₃ (10 mL). A catalytic amount of DMF was added and the reaction mixture was refluxed for three hours. Excess of POCl₃ was removed under vacuum followed by neutralization with aq. ammonium hydroxide. The resulting solid was filtered off and dried affording 4-chloro-6-nitroquinoline (7) as an off-white solid in 95% yield (2.1 g). ¹H NMR (400 MHz, Acetone-*d*₆) δ 9.11 (d, *J* = 2.5 Hz, 1H, H5), 9.07 (d, *J* = 4.7 Hz, 1H, H2), 8.60 (dd, *J* = 2.5, 9.2 Hz, 1H, H7), 8.35 (d, *J* = 9.2 Hz, 1H, H8), 7.92 (d, *J* = 4.7 Hz, 1H, H3). ¹³C NMR (100 MHz, Acetone-*d*₆) δ 154.34, 151.32, 146.79, 143.94, 132.46, 125.84, 124.18, 123.66, 121.01. HRMS (ESI-MS) Calc.: 209.0112 for C₉H₆ClN₂O₂ [M+H⁺], Found: 209.0112.

***N*-(3-Bromophenyl)-6-nitroquinolin-4-amine (8a).** A mixture of 7 (2 g, 5.6 mmol), *m*-bromoaniline (1.15 mL, 10.6 mmol) and a catalytic amount of conc. HCl was refluxed in isopropanol (5 mL) at 90°C for 16 h. After cooling the reaction mixture, 10% aqueous NaHCO₃ was added to the aqueous layer and extracted three times with ethyl acetate. The combined organic layers were dried over Na₂SO₄ and the volatiles were removed *in vacuo* yielding an orange solid. The crude was purified on silica gel (DCM/MeOH, 100:1 to 100:10) afford 2.8 g (85%) of yellow solid product 8a. ¹H NMR (400 MHz, CD₃CN) δ 9.51 (d, *J* = 2.4 Hz, 1H), 9.51 (d, *J* = 2.4 Hz, 1H), 8.60 (d, *J* = 5.5 Hz, 1H), 8.41 (dd, *J* = 2.4, 9.3 Hz, 1H), 8.05 (d, *J* = 9.3 Hz, 1H), 7.62 (s, br, 1H), 7.43 (d, *J* = 2.6 Hz, 1H), 7.40 – 7.31 (m, 2H), 7.10 (d, *J* = 5.5 Hz, 1H). ¹³C NMR (100 MHz, CD₃CN) δ 153.86, 151.57, 150.47, 144.71, 142.23, 131.61, 130.77, 127.74, 125.93, 123.46, 122.77, 121.99, 120.78, 117.92, 103.61. HRMS (ESI-MS) Calc.: 344.0029 for C₁₅H₁₁BrN₃O₂ [M+H⁺], Found: 344.0033.

***N*-4-(3-Bromophenyl)quinoline-4,6-diamine (11a).** A mixture of 8a (2 g, 5.8 mmol) and iron (3.2 g, 58 mmol) was suspended in aqueous ethanol (500 mL, 70% v/v) containing acetic acid (3.3 mL, 58 mmol) and heated under reflux for 3 h. The reaction mixture was cooled to room temperature (RT) and basified with ammonium hydroxide (25% ammonia in water). The resulting slurry was then filtered through celite and the filtrate was extracted with ethyl acetate. The organic phase was dried over Na₂SO₄ and evaporated to dryness. The obtained solid was thoroughly dried under high vacuum to produce a 1.1 g (60%) of greenish solid 11a. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.71 (s, br, 1H), 8.24 (d, *J* = 5.0 Hz, 1H), 7.65 (d, *J* = 8.8 Hz, 1H), 7.43 (s, 1H), 7.30-7.26 (m, 2H), 7.22 – 7.08 (m, 3H), 6.99 (d, *J* = 5.0 Hz, 1H), 5.54 (s, br, 2H). ¹³C NMR (100 MHz, DMSO) δ 147.17, 145.88, 145.09, 144.89, 143.12, 131.94, 130.40, 124.99, 123.37, 122.92, 122.77, 122.25, 119.15, 105.37, 101.10. MS (ESI): *m/z* 314.3 (M + H)⁺.

4-Chloro-6,7-bis(2-methoxyethoxy)quinoline (15a). Step 1: A mixture of 4-nitrobenzene-1,2-diol (3.0 g, 19.3 mmol), 1-bromo-2-methoxyethane (5.9 g, 42.5 mmol) and potassium carbonate (8.0 g, 58 mmol) was heated at 100 °C in dry DMF (10 mL) for 16 h. Upon completion of the reaction, the mixture was acidified with 2N hydrochloric acid followed by extraction with ethyl acetate (3 x 10 mL) and the combined organic layers were washed with water and dried over Na₂SO₄. The volatiles were removed *in vacuo* leading to 4.65 g (88%) of a reddish oil of 1,2-bis(2-methoxyethoxy)-4-nitrobenzene. Step 2: The crude 1,2-bis(2-methoxyethoxy)-4-nitrobenzene (4.65 g) was dissolved in 80 mL of EtOH and hydrogenated using 10% Pd/C (125 mg) under H₂ at atmospheric pressure for 8 h. The catalyst was filtered off and the filtrate was evaporated *in vacuo* leading to a crude reddish-yellow oil of 3,4-bis(2-methoxyethoxy)aniline (3.4 g, 82%). Step 3: The crude compound 3,4-bis(2-methoxyethoxy)aniline (2.0 g, 8.3 mmol) was heated with diethyl 2-(ethoxymethylene)malonate (2.3 g, 10 mmol) at 100°C for 3 h, then cooled to room temperature. Volatiles of the reaction mixture were evaporated *in vacuo* and the resulting crude compound was purified using silica gel column chromatography (cyclohexane/ethyl acetate; 2:3) to obtain diethyl 2-((3,4-bis(2-methoxyethoxy)phenylamino)methylene)malonate (2.4 g, 79%). ¹H NMR (400 MHz, CDCl₃) δ 10.94 (d, *J* = 13.7 Hz, 1H), 8.40 (d, *J* = 13.7 Hz, 1H), 6.91 (d, *J* = 8.5 Hz, 1H), 6.71 (d, *J* = 2.6 Hz, 1H), 6.68 (dd, *J* = 2.6, 8.5 Hz, 1H), 4.32 – 4.18 (m, 4H), 4.18 – 4.08 (m, 4H), 3.78 – 3.67 (m, 4H), 3.44 (s, 3H), 3.43 (s, 3H), 1.36 (t, *J* = 7.1 Hz, 3H), 1.30 (t, *J* = 7.1 Hz, 3H). Step 4: 2-((3,4-bis(2-methoxyethoxy)phenylamino)methylene)malonate (1.0 g, 2.4 mmol) was heated in diphenyl ether at 280 °C for 2 h. Upon cooling the reaction mixture was diluted with petroleum ether. The resulting solid was collected by filtration affording a crude solid of ethyl 4-hydroxy-6,7-bis(2-methoxyethoxy)quinoline-3-carboxylate (610 mg, 68%). This crude intermediate was

treated with 10% aq. NaOH (10 mL) in MeOH (10 mL) and the mixture was heated under reflux for 3 h. Upon completion of the reaction, the reaction mixture was acidified with 10% aqueous HCl. The resulting solid was collected by filtration and washed with CHCl₃/MeOH (100 mL/20 mL) to obtain 380 mg (77%) of crude 6,7-bis(2-methoxyethoxy)quinolin-4-ol (14a) which was thoroughly dried under high vacuum. The 6,7-bis(2-methoxyethoxy)quinolin-4-ol (14a) was then heated under reflux with excess of POCl₃ (1 mL) for 1 h. The reaction mixture was concentrated *in vacuo* and resulting residue was dissolved in CHCl₃/MeOH (4:1) followed by the addition of ammonium hydroxide (25% ammonia in water, 1 mL). The mixture was extracted with ethyl acetate and the organic layer was dried over Na₂SO₄ and concentrated *in vacuo*. The resulting residue was purified by column chromatography using CH₂Cl₂/MeOH (100:1 to 100:7) as eluent to obtain a 321 mg (79%) of 4-chloro-6,7-bis(2-methoxyethoxy)quinoline 15a. MS (ESI): *m/z* 312 (M + H)⁺.

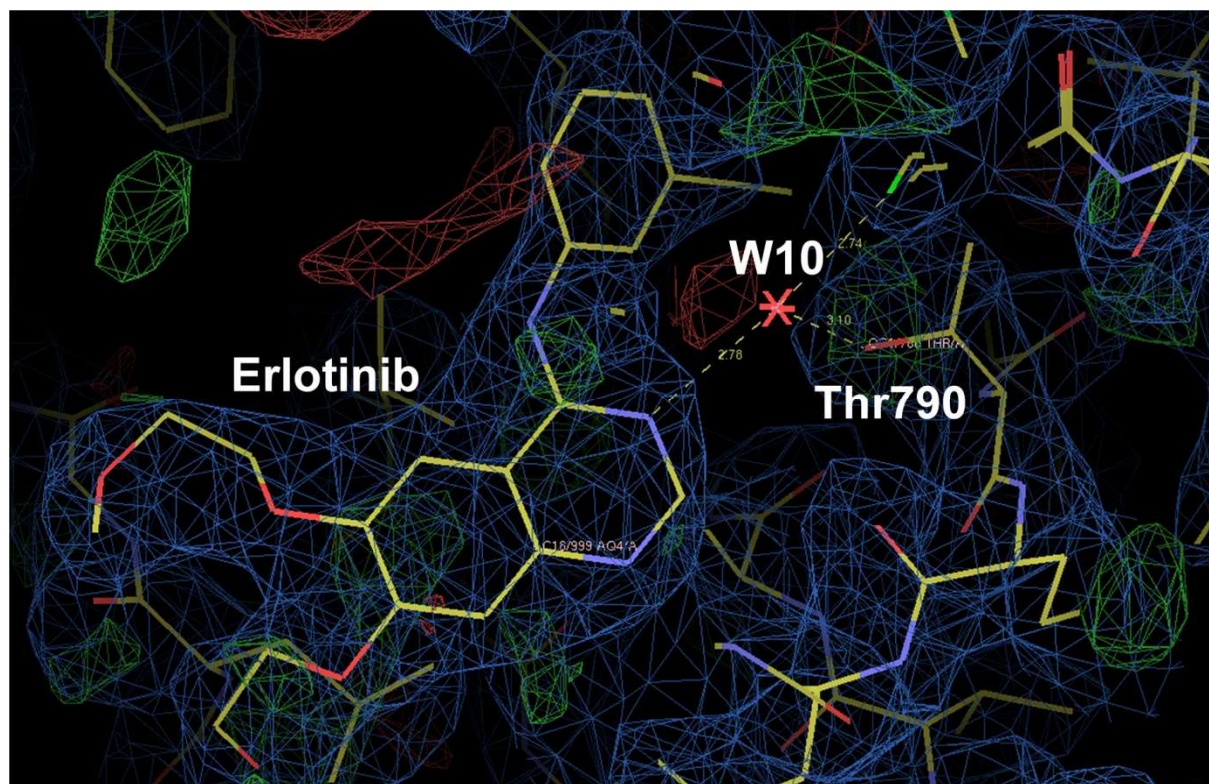


Figure S1: Electron density maps of Erlotinib in complex with the catalytic domain of EGFR (PDB-code: 1M17).¹ Electron density maps ($2Fo - Fc$ (blue) contoured at 1σ ; positive (green) and negative (red) difference densities ($Fo - Fc$) contoured at 2.5σ) were calculated from available structure factors and reveal no electron density for the discussed water molecule (W10).

References.

1. Stamos, J.; Sliwkowski, M. X.; Eigenbrot, C. Structure of the epidermal growth factor receptor kinase domain alone and in complex with a 4-anilinoquinazoline inhibitor. *Journal of Biological Chemistry* 2002, 277, 46265-46272.