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Scaffold Morphing Led to Evolution of 2,4-Diaminoquinoline and Aminopyrazolopyrimidine As Inhibitors of ATP Synthesis Pathway

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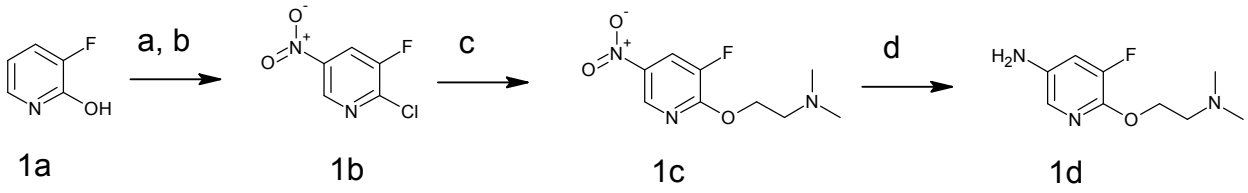
1. Materials and methods

All commercial reagents and solvents were used without further purification. Analytical thin-layer chromatography (TLC) was performed on SiO_2 plates on alumina. Visualization was accomplished by UV irradiation at 254 and 220 nm. Flash column chromatography was performed using the Biotage Isolera flash purification system with SiO_2 60 (particle size 0.040−0.055 mm, 230−400 mesh). Purity of all final derivatives for biological testing was confirmed to be >95% as determined using the following conditions: a Shimadzu HPLC instrument with a Hamilton reverse phase column [HxSil, C18, 3µm, 2.1 mm × 50 mm (H2)]. Eluent A: 5% CH_3CN in H_2O, eluent B: 90% CH_3CN in H_2O. A flow rate of 0.2 ml/min was used with UV detection at 254 and 214 nm. The structure of the intermediates and end products was confirmed by ^1H NMR and mass spectroscopy. Proton magnetic resonance spectra were determined in DMSO- d_6 unless otherwise stated, using Bruker DRX-300 or Bruker DRX-400 spectrometers, operating at 300 MHz, or 400 MHz, respectively. Splitting patterns are indicated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad peak. LCMS data was acquired using Agilent LCMS VL series. Source: ES ionization, coupled with an Agilent 1100 series HPLC system and an Agilent 1100 series PDA as the front end. HRMS data was acquired using an Agilent 6520, Quadrupole-Time of flight tandem mass spectrometer (Q-Tof MS/MS) coupled with an Agilent 1200 series HPLC system.

1.1. Schemes and experimental procedure for the synthesis of intermediate (1d)

Scheme and reagents:



Scheme 1A. 6-(2-(Dimethylamino) ethoxy-5-fluoropyridin-3-amine

Reagents and conditions: **a**) H_2SO_4, HNO_3, 0 ^oC, 3 h. (**b**) $POCl_3$, PCl_5, 60 ^oC, 1 h. (**c**) 2-(dimethylamino)ethanol, NaH, THF, 0-25 ^oC, 2 h. (**d**) Pd-C, EtOH, H_2(bubbled), RT, 2 h.

Experimental procedures:

Step-1. 2-Chloro-3-fluoro-5-nitropyridine (1b)

To a solution of 3-fluoropyridin-2-ol (25 g, 0.353 mole) in H_2SO_4 (100 ml), HNO_3 (75 ml) was added drop wise at 0 °C and the resulting solution was stirred at 0 °C for 3 h. After completion of the reaction, ice was added onto the reaction mass and stirred for 0.5 h. The solid formed was filtered and dried well to give 3-fluoro-5-nitropyridin-2-ol. Yield: 13.0 g (37%). ESMS calculated. 158.1 found: 157.0 (M-H). The crude 3-fluoro-5-nitropyridin-2-ol (13.0 g, 0.0822 mole) dissolved in $POCl_3$ (130 ml) was heated to 60 °C. At that temperature, PCl_5 (25.6 g, 0.1233 mole) was added

portion wise. After completion of the reaction, the reaction mixture was evaporated completely and quenched with ice water. The residue was dissolved in EtOAc and the organic layer was washed with saturated $NaHCO_3$ solution. Evaporation of the organic layer afforded 2-chloro-3-fluoro-5-nitropyridine. Yield: 10.0 g (70%). ESMS calculated. 176.5 found: 177.4 (M+H).

Step-2. 2-((3-Fluoro-5-nitropyridin-2-yl)oxy)-N,N-dimethylethan -1-amine (1c)

To a solution of N,N-dimethylethanol amine (6.05 g, 0.067 mol) in THF (250 ml), 60 % Sodium hydride (3.39 g, 0.084 mol) was added in portion wise manner at 0 °C. After 15 min, a solution of 2-chloro-3-fluoro-5-nitropyridine (10 g, 0.056 mol) in THF (100 ml) was added drop wise and the resulting solution was stirred at RT for 1 h. After completion of the reaction, the mixture was quenched with crushed ice and the resulting mixture was evaporated under reduced pressure. The residue was partitioned between water and EtOAc. The organic layer was dried over anhydrous sodium sulphate and concentrated. The crude product was purified by column chromatography using EtOAc- pet ether. The required product eluted at 80% EtOAc-pet ether. Purification resulted in **2-((3-Fluoro-5-nitropyridin-2-yl)oxy)-N,N-dimethylethan-1-amine (III)** as a yellow colored solid. Yield: 9.0 g (69%). ESMS calculated: 229.2; found: 230.2 (M+H).

Step-3. 6-(2-(Dimethylamino) ethoxy-5-fluoropyridin-3-amine (1d)

To a solution of 2-((3-fluoro-5-nitropyridin-2-yl)-N,N-dimethylethan-1-amine (9.0 g, 0.0392 mol) in ethanol (450 ml), Pd/C (4.0 g) was added in portion wise manner at RT and the resulting solution was stirred at RT for 2 h under H_2 gas atmosphere. After the completion of the reaction, the reaction mixture was evaporated to get title compound as brown solid. Yield: 7.0 g (90%). ESMS calculated: 199.2; found: 200.4 (M+H).

1.2. Experimental procedures for the synthesis of compounds 3 to compound 26

1.2.1. General procedure for the synthesis of 2,4-diaminoquinazolines (3c-i) demonstrated through the synthesis of N4-(6-(2-(Dimethyl amino)ethoxy)-5-fluoropyridin-3-yl)-6,7-dimethoxy-N2-(2-(tetrahydro-2H-pyran-4-yl)ethyl)quinazoline-2,4-diamine (3i)

Step-1. 2-Chloro-N-(6-(2-(dimethylamino) ethoxy) -5-fluoropyridin-3-yl)-6,7-dimethoxy quinazolin-4-amine (2i)

In a thermal vial, 6-(2-(dimethylamino)ethoxy)-5-fluoropyridin-3-amine (77 mg, 0.39 mmol) dissolved in dry DMF (2 ml) was placed at 0 ^oC. To this, sodium hydride (20.38 mg, 0.42 mmol) was added. The resulting reaction mixture was stirred for 10 min at room temperature. A solution of 2,4-dichloro-6,7-dimethoxyquinazoline (100 mg, 0.39 mmol) dissolved in DMF (2 ml) was added drop-wise and the reaction mixture was heated at 80 ^oC for 2 h. Completion of reaction was monitored by LCMS. Then the solvent was evaporated and the crude product was purified through combiflash chromatography using DCM-MeOH gradient. The required product elutes at 20% DCM-MeOH. Yield: 75 mg (46%). ESMS calculated: 421.8; found: 422.3 (M+).

Step-2. N4-(6-(2-(Dimethylamino)ethoxy)-5-fluoropyridin-3-yl)-6,7-dimethoxy-N2-(2-(tetrahydro-2H-pyran-4-yl)ethyl)quinazoline-2,4-diamine (3i)

In a microwave vial, a suspension of 2-chloro-N-(6-(2-(dimethylamino)ethoxy)-5-fluoropyridin-3-yl)-6,7-dimethoxyquinazolin-4-amine (50 mg, 0.12 mmol), 2-(tetrahydro-2H-pyran-4-yl)ethanamine (77 mg, 0.59 mmol) and sodium carbonate (75 mg, 0.71 mmol) in *n*-BuOH (2 ml) was subjected to Microwave at 170 ^{o}C for 90 min. Completion of the reaction was monitored by LCMS. After completion of the reaction, *n*-BuOH was evaporated and the residue was re-dissolved in ethyl acetate (25 ml). The organic layer was washed with water, brine, dried over Na_2SO_4 and evaporated. The crude residue was purified through reverse phase chromatography using acetonitrile-water gradient. Yield: 27 mg (44.3%), dissolved in DMSO and purified through reverse phase chromatography. ^{1}H NMR: (400 MHz, DMSO- d_6): δ ppm 1.03 - 1.27 (m, 2 H), 1.39 - 1.54 (m, 2 H), 1.59 (d, J = 10.3 Hz, 3 H), 2.22 (s, 6 H), 2.65 (t, J = 5.8 Hz, 2 H), 3.18 - 3.32 (m, 4 H), 3.75 - 3.84 (m, 2 H), 3.85 (s, 6 H), 4.43 (t, J = 5.8 Hz, 2 H), 6.59 (br. s., 1 H), 6.75 (s, 1 H), 7.60 (s, 1 H), 8.26 (d, J = 12.4 Hz, 1 H), 8.40 (br. s., 1 H), 9.23 (br. s., 1 H); ESMS calculated: 514.2; found: 515.2 (M+H); HRMS calculated for $C_{26}H_{35}FN_6O_4$, 515.2776; found: 515.2766.

N4-(5-tert-butyl-1-methyl-1H-pyrazol-3-yl)-N2-(2-(tetrahydro-2H-pyran-4-yl) ethyl)quinazoline -2,4-diamine (3c)

Yield: 28.9%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.16 (d, J = 7.9 Hz, 3 H), 1.38 (s, 9 H), 1.46 - 1.56 (m, 2 H), 1.57 - 1.70 (m, 2 H), 3.24 (t, J = 11.3Hz, 2 H), 3.36 - 3.46 (m, 2 H), 3.74 - 3.92 (m, 5 H), 6.74 (s, 2 H), 7.01 (t, J = 7.25 Hz, 1 H), 7.23 (d, J = 8.1 Hz, 1 H), 7.41 - 7.56 (m, 1 H), 8.31 (d, J = 8.10 Hz, 1 H), 9.89 (br. s., 1 H); ESMS calculated: 408.5; found: 409.2 (M+H); HRMS calculated for $C_{23}H_{32}N_6O$: 409.2710; found: 409.2709.

N4-(5-(tert-butyl)-1-methyl-1H-pyrazol-3-yl)-6,7-dimethoxy-N2-(2-(tetrahydro-2H-pyran-4-yl)ethyl)quinazoline-2,4-diamine (3d)

Yield: 30.1%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.01 - 1.30 (m, 3 H), 1.38 (s, 9 H), 1.46 - 1.56 (m, 2 H), 1.56 - 1.68 (m, 2 H), 3.24 (t, J = 11.7 Hz, 2 H), 3.35 - 3.48 (m, 2 H), 3.83 (d, J = 9.98 Hz, 11 H), 6.38 (t, J = 5.65 Hz, 1 H), 6.68 (s, 1 H), 6.75 (s, 1 H), 7.76 (s, 1 H), 9.78 (br. s., 1 H); ESMS calculated: 468.6: found; 469.0 (M+); HRMS calculated for $C_{25}H_{36}N_6O_3$: 469.2921; found: 469.2921.

N4-(5-Isopropylthiazol-2-yl)-6,7-dimethoxy-N2-(2-(tetrahydro-2H-pyran-4-yl)ethyl) quinazoline-2,4-diamine (3e)

Yield: 15.9%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.32 (d, J = 6.8 Hz, 6 H), 1.49 - 1.72 (m, 4 H), 3.06 - 3.19 (m, 1 H), 3.21 - 3.29 (m, 1 H), 3.29 - 3.38 (m, 6 H), 3.49 (br. s, 2 H), 3.77 - 3.91

(m, 6 H), 6.65 (br. s., 1 H), 6.75 (s, 1 H), 7.21 (s, 1 H), 7.88 (s, 1 H), 11.31 - 11.76 (m, 1 H); ESMS calculated: 457.6; found: 458.0 (M+); HRMS calculated for $C_{23}H_{31}N_5O_3S$: 458.2220; found: 458.2234.

6,7-Dimethoxy-N4-(6-methoxy-5-methylpyridin-3-yl)-N2-(2-(tetrahydro-2H-pyran-4-yl)ethyl) quinazoline-2,4-diamine (3f)

Yield 29.4%.^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.04 - 1.21 (m, 3 H), 1.38 - 1.51 (m, 2 H), 1.57 (d, J = 9.42 Hz, 2 H), 2.18 (s, 3 H), 3.15 - 3.32 (m, 4 H), 3.74 - 3.95 (m, 11 H), 6.45 (t, J = 5.6 Hz, 1 H), 6.72 (s, 1 H), 7.61 (s, 1 H), 7.82 - 7.94 (m, 1 H), 8.48 (br. s., 1 H), 9.06 (br. s., 1 H); ESMS calculated: 453.5; found: 454.5 (M+H); HRMS calculated for $C_{24}H_{31}N_5O_4$: 454.2448; found: 454.2440.

6,7-Dimethoxy-N4-(6-methoxy-5-methylpyridin-3-yl)-N2-(2-(pyridin-3-yl)ethyl)quinazoline-2,4-diamine (3g)

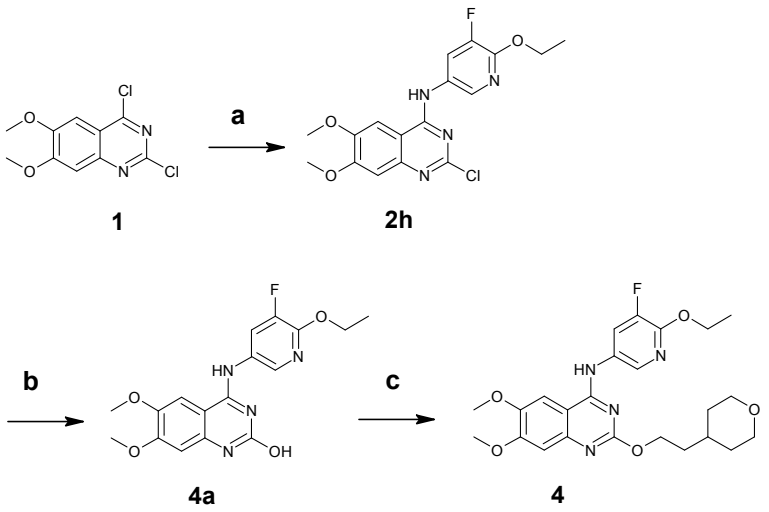
Yield: 18.6%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.15 (s, 3 H), 2.85 (m, 2 H), 3.42 - 3.57 (m, 2 H), 3.86 (s, 6H), 3.99 (s, 3 H), 6.66 (br. s., 1 H), 6.78 (s, 1 H), 7.21 - 7.35 (m, 1 H), 7.50 - 7.72 (m, 2 H), 7.78 - 7.95 (m, 1 H), 8.32 - 8.54 (m, 3 H), 9.12 (br. s., 1 H); ESMS calculated: 446.5; found: 447.5 (M+H); HRMS calculated for $C_{24}H_{26}N_6O_3$: 447.2138; found: 447.2142.

N4-(6-Ethoxy-5-fluoropyridin-3-yl)-6,7-dimethoxy-N2-(2-(tetrahydro-2H-pyran-4-yl)ethyl) quinazoline-2,4-diamine (3h)

Yield: 33.4%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.94 - 1.31 (m, 3 H), 1.36 (t, J = 6.9 Hz, 3 H), 1.47 (d, J = 6.0 Hz, 2 H), 1.59 (d, J = 10.7 Hz, 2 H), 3.14 - 3.33 (m, 4 H), 3.75 - 3.92 (m, 8 H), 4.39 (q, J = 7.0 Hz, 2 H), 6.61 (br. s., 1 H), 6.75 (s, 1 H), 7.60 (s, 1 H), 8.26 (d, J = 13.0Hz, 1 H), 8.40 (br. s., 1 H), 9.24 (br. s., 1 H); ESMS calculated: 471.5; found: 472.0 (M+); HRMS calculated for $C_{24}H_{30}FN_5O_4$: 472.2354; found: 472.2350.

1.2.2. Schemes and experimental procedure for the synthesis of N-(6-Ethoxy-5-fluoropyridin-3-yl)-6,7-dimethoxy-2-(2-(tetrahydro-2H-pyran-4-yl)ethoxy)-quinazolin-4-amine (compound 4)

Scheme and reagents:



Scheme 1B. Synthesis of compound-4

Reagents and conditions: **a**) 6-ethoxy-5-fluoro-pyridin-2-amine, NaH, DMF, 0-25 ^{o}C, 5 h. (**b**) AcOH, NaOAc, DMF, 80 ^{o}C, 16 h. (**c**) DMSO, $CsCO_3$, 4-(2-bromoethyl)tetrahydro-2*H*-pyran, 100 ^{o}C, 16 h

Experimental procedures:

Step-1. 2-Chloro-N-(6-ethoxy-5-fluoropyridin-3-yl)-6,7-dimethoxyquinazolin-4-amine (2h)

To a stirred solution of 2,4-dichloro-6,7-dimethoxyquinazoline (1.5 g, 0.0057 mol) in DMF (25 ml), sodium hydride (0.577 g, 0.01445 mol) was added portion wise at 0 °C. This was followed by drop wise addition of a solution of 6-ethoxy-5-fluoro-pyridin-2-amine (1.08 g, 0.00694 mol) in DMF (10 ml) and the resulting solution was stirred at RT for 5 h. Then the reaction mixture was quenched by pouring into crushed ice. The precipitate formed was collected by filtration, washed with water and dried to afford 2-chloro-N-(6-ethoxy-5-fluoropyridin-3-yl)-6,7-dimethoxyquinazolin-4-amine as a gummy solid. Yield: 1.6 g (73.05%). ESMS calculated: 378.8; found: 379.5 (M+H).

Step-2. 4-((6-Ethoxy-5-fluoropyridin-3-yl)amino)-6,7-dimethoxyquinazolin-2-ol (4a)

To a stirred solution of 2-chloro-N-(6-ethoxy-5-fluoropyridin-3-yl)-6,7-dimethoxyquinazolin-4-amine (1.6 g, 0.0042 mol) in acetic acid (20 ml), sodium acetate (0.7 g, 0.0084 mol) was added. The reaction mixture was heated to 80 °C for 16 h and then poured into ice water slowly and stirred vigorously for 1 h. The resulting precipitate was collected by filtration, washed with water, followed by hexane and then dried to afford 4-((6-ethoxy-5-fluoropyridin-3-yl)amino)-6,7-dimethoxyquinazolin-2-ol as a colourless solid. Yield: 0.98 g (69.0%). ESMS calculated: 360.3; found: 361.5 (M+H).

Step-3. N-(6-Ethoxy-5-fluoropyridin-3-yl)-6,7-dimethoxy-2-(2-(tetrahydro-2H-pyran-4-yl) ethoxy)-quinazolin-4-amine (4)

To a stirred solution of 4-((6-ethoxy-5-fluoropyridin-3-yl) amino)-6,7-dimethoxyquinazolin-2-ol (0.1 g, 0.000275 mol) in DMSO (3 ml), cesium carbonate (0.271 g, 0.000825 mol) was added. This was followed by dropwise addition of a solution of 4-(2-bromoethyl)tetrahydro-2H-pyran (0.05 ml, 0.00330 mol) in THF (5 ml) and the resulting mixture was heated to 100 °C for 16 h. Then the reaction mixture was poured into ice water and extracted with ethyl acetate twice. The combined organic layer was washed with water and brine, dried over sodium sulphate and concentrated. The crude product obtained was purified by preparative HPLC to result in N-(6-ethoxy-5-fluoropyridin-3-yl)-6,7-dimethoxy-2-(2-(tetrahydro-2H-pyran-4-yl)ethoxy)quinaz-olin-4-amine as a viscous liquid. Yield: 14 mg (10.4%). ^{1}H NMR: (400 MHz, DMSO-d_6): δ 1.16-1.19 (m, 3H), 1.38 (t, J = 7.0 Hz, 3H), 1.56 (d, J = 13.8 Hz, 2H), 1.62-1.65 (m, 2H), 3.28 (t, J = 20.0 Hz, 2H), 3.86 (m, 2H), 3.93 (s, 6H), 4.40 (m, 4H), 6.75 (s, 1H), 7.01 (s, 1H), 7.86 (m, 1H), 8.12 (d, J = 11.9 Hz, 1H), 8.25 (s, 1H); ESMS calculated: 472.5; found: 473.2 (M+1); HRMS calculated for $C_{24}H_{29}FN_4O_5$: 473.2194; found: 473.2192.

1.2.3. General procedure for the synthesis of 2, 4-diaminoquinolines (8a-g) demonstrated through synthesis of N4-(6-Ethoxy-5-fluoropyridin-3-yl)-6,7-dimethoxy-*N*2-(2-(tetrahydro-2*H*-pyran-4-yl)ethyl)quinoline-2,4-diamine (8a)

Step 1. 2,4-Dihydroxy-6,7-dimethoxyquinoline (6a)

To a solution of malonic acid (3.96 g, 0.0380 mol) in $POCl_3$ (5.30 g, 0.0345 mol) 3,4-difluoroaniline (50 g, 0.0345 mol) was added drop wise at 0 °C. The resulting solution was stirred at 120 °C for 3 h. After completion of the reaction, the reaction mixture was cooled to 0 °C and 10% NaOH solution was added to get a clear solution. Addition of 2N HCl afforded to get green colored solid. The solid was filtered, washed with petroleum ether and dried under vacuum. Yield: 4.6 g (60.28%). ESMS calculated: 221.2; found: 223.2 (M+1).

Step 2. 2, 4-Dichloro-6,7-dimethoxyquinoline (6)

A mixture of 2,4-dihydroxy-6,7-dimethoxy quinoline (4.6 g, 0.0207 mol) and $POCl_3$ (140 ml) was heated to 120 °C for 6 h. Then the reaction mixture was quenched onto ice-water. The solid formed was filtered, washed with ether, petroleum ether and dried under vacuum. Yield 3.3 g (61.79%). ESMS calculated. 258.1: found: 259.0 (M+1).

Step 3. 4-Chloro-6, 7-dimethoxy-N-(2-(tetrahydro-2H-pyran-4-yl)ethyl)quinolin-2-amine (7)

A solution of 2,4-dichloro-6,7-dimethoxy quinoline (0.5 g, 2.04 mmol) in dry toluene (10 ml) was added to 2-(tetrahydro-2H-pyran-4-yl)ethan-1-amine (0.317 g, 6.54 mmol), sodium *tert*-butoxide (0.310 g, 3.07 mmol) and purged with N_2 for 15 min. This was followed by addition of

XantPhos (0.023 g, 0.04 mmol) and Pd_2dba_3 (0.023 g, 0.02 mmol) the resulting mixture was heated to 120 °C under microwave for 1 h. Then the reaction mixture was evaporated completely under reduced pressure. The crude product showed the mixture of two products. 2-substituted quinoline was isolated, purified through column chromatography using 2% DCM in MeOH as an eluent to give compound **7** as yellow solid. The isolated compound was confirmed by 2D NMR. The purification Yield: 0.32 g (37%). ESMS calculated: 350.8; found: 351.5 (M+1).

N4-(6-Ethoxy-5-fluoropyridin-3-yl)-6,7-dimethoxy-N2-(2-(tetrahydro-2H-pyran-4-yl)ethyl)quinoline-2,4-diamine (8a)

To a solution of 4-chloro-6,7-dimethoxy-N-(2-(tetrahydro-2H-pyran-4-yl)ethyl) quinolin-2-amine (0.32 g, 2.04 mmol) in dry toluene (5 ml), 6-ethoxy-5-fluoropyridin-3-amine (0.17 g, 1.09 mmol) and sodium *tert*-butoxide (0.3 g, 3 mmol) were added and purged with N_2 for 15 min. This was followed by addition of XantPhos (0.012 g, 0.02 mmol) and Pd_2dba_3 (0.012 g, 0.01 mmol) the resulting mixture was heated to 120 °C under microwave for 1 h. After the completion of the reaction, the reaction mixture was evaporated completely. The resulting crude product was purified by reverse phase preparative HPLC to give the title compound **8a** as an off-white solid. Yield: 0.08 g (19%). ^{1}H NMR: (400 MHz, DMSO-d_6): δ 1.10-1.28 (m, 2H), 1.39 (t, J = 12.0 Hz, 3H), 1.59-1.63 (m, 4H), 3.29-3.40 (m, 3H), 3.86 (q, J = 32.0 Hz, 3H), 3.87 (s, 6H), 4.45 (q, J = 20.0 Hz, 2H), 5.82 (s, 1H), 7.24 (s, 1H), 7.64 (s, 1H), 7.91 (d, J = 11.12 Hz, 1H), 8.06 (s, 1H), 8.25-8.32 (m, 1H), 9.91 (s, 1H), 11.94 (s, 1H). ESMS calculated: 470.5; found: 471.2 (M+H); HRMS calculated for $C_{25}H_{31}FN_4O_4$: 471.5443; found: 471.2399.

N4-(6-(2-(Dimethylamino)ethoxy)-5-fluoropyridin-3-yl)-6,7-dimethoxy-N2-(2-(tetrahydro-2H-pyran-4-yl)ethyl)quinoline-2,4-diamine (8b)

Yield: 21%. ^{1}H NMR: (400 MHz, DMSO-d_6): δ 1.19-1.22 (m, 2H), 1.60-1.53 (m, 6H), 2.89 (s, 6H), 3.16 (s, 1H), 3.59 (m, 3H), 3.83-3.87 (m, 8H), 4.69-4.71 (m, 2H), 5.84 (s, 1H), 7.64 (s, 1H), 7.98 (dd, J = 10.4, Hz, 1H), 8.10 (s, 1H), 8.29 (s, 1H), 9.83 (s, 1H), 10.00 (s, 1H), 12.08 (s, 1H). ESMS calculated: 513.6; found: 514.4 (M+H); HRMS calculated for $C_{27}H_{36}FN_5O_4$: 514.2823; found: 514.2812.

N2-(2-Chlorophenethyl)-N4-(6-(2-(dimethylamino)ethoxy)-5-fluoropyridin-3-yl)-6,7-di methoxyquinoline-2,4-diamine (8c)

Yield: 7%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.89 (s, 6H), 3.04-3.05 (m, 2H), 3.88 (s, 6H), 3.53 (m, 3H), 4.73 (d, J = 4.0 Hz, 2H), 5.92 (s, 1H), 7.23-7.31 (m, 2H), 7.31 (m, 1H), 7.38-7.39 (m, 1H), 7.42-7.43 (m, 1H), 7.65 (s, 1H), 7.97 (m, 1H), 8.11 (s, 1H), 8.50 (s, 1H), 9.95 (s, 1H), 10.06 (s, 1H), 12.33 (s, 1H). ESMS calculated: 540.0; found: 541.4 (M+H); HRMS calculated for $C_{28}H_{31}ClFN_5O_3$: 540.2171; found: 540.2171.

2-(3-(2-Chlorophenyl)pyrrolidin-1-yl)-N-(6-(2-(dimethylamino)ethoxy)-5-fluoropyridin-3-yl)-6,7-dimethoxyquinolin-4-amine (8d)

Yield: 33.3%. ^{1}H NMR: (400 MHz, DMSO-d_6): δ 2.30 (m, 1H), 2.40 (s, 6H). 2.83 (m, 2H), 3.45-3.58 (m, 3H), 3.86 (s, 6H), 4.32-4.35 (m, 1H), 4.47-4.49 (t, 2H), 5.94 (s, 1H), 6.91 (m, 1H), 7.06 (s, 1H), 7.26-7.48 (m, 5H), 7.58 (s, 1H), 7.81 (d, 1H), 8.02 (s, 1H). ESMS calculated. 566.1: found: 567.2 (M+H); HRMS calculated for $C_{30}H_{33}ClFN_5O_3$: 566.2328; found: 566.2318.

2-(3-(2-chlorophenyl)pyrrolidin-1-yl)-N-(5-fluoro-6-((1-methylpyrrolidin-2-yl)methoxy)pyridin-3-yl)-6,7-dimethoxyquinolin-4-amine (8e)

Yield: 32%. ^{1}H NMR: (400 MHz, DMSO-d_6): δ 1.85-1.96 (m, 4H), 2.10-2.32 (m, 6H), 2.97-2.98 (m, 3H), 3.61-3.63 (m, 3H), 3.96 (s, 6H), 4.51-4.56 (m, 1H), 4.69-4.73 (m, 1H), 5.77 (s, 1H), 7.35-7.39 (m, 2H), 7.48-7.52 (m, 3H), 7.81 (s, 1H), 8.00-8.13 (m, 1H), 8.13 (s, 1H), 9.66 (s, 1H), 9.99 (s, 1H), 11.59 (s, 1H). ESMS calculated: 592.1; found: 593.4 (M+H); HRMS calculated for $C_{32}H_{35}ClFN_5O_3$: 592.2484; found: 592.2496.

N5-(2-(3-(2-Chlorophenyl)pyrrolidin-1-yl)-6,7-dimethoxyquinolin-4-yl)-N2-(2-(dimethylamino) ethyl)-3-fluoropyridine-2,5-diamine (8f)

Yield: 14.5%. (400 MHz, DMSO-d_6): δ 2.15-2.30 (m, 1H), 2.50 (m, 2H), 2.83 (m, 6H), 3.31 (m, 2H), 3.7 (m, 2H), 3.71 (m, 5H), 3.91-3.96 (m, 6H), 5.63 (s, 1H), 7.10 (s, 1H), 7.31-7.34 (m, 2H), 7.50 (t, J = 7.0 Hz, 3H), 7.66 (d, J = 11.92 Hz, 1H), 7.83 (s, 1H), 7.96 (s, 1H), 9.58 (s, 1H), 11.56 (s, 1H). ESMS calculated: 565.1; found: 566.2 (M+H); HRMS calculated for $C_{30}H_{34}ClFN_6O_2$: 565.2488; found: 565.2477.

2-(3-(2-Chlorophenyl)pyrrolidin-1-yl)-N-(6-(2-(dimethylamino)ethoxy)-5-fluoropyridin-3-yl)-6,7-difluoroquinolin-4-amine (8g)

Yield: 72%. ^{1}H NMR: (400 MHz, DMSO-d_6): δ ppm 2.10 (t, J = 12.0 Hz, 1H), 2.23 (s, 6H), 2.36 (d, J = 0.8 Hz, 1H), 2.67 (d, J = 10.8 Hz, 2H), 3.46-3.52 (m, 2H), 3.59 (s, 1H), 3.82 (t, J = 14.4 Hz, 2H), 4.43 (t, J = 11.2 Hz, 2H), 6.08 (s, 1H), 7.27-7.38 (m, 3H), 7.41-7.48 (m, 2H), 7.81 (d, J = 12.0 Hz, 1H), 8.10-8.16 (m, 2H), 8.59 (s, 1H). ESMS calculated: 542.0; found: 543.2 (M+H); HRMS calculated for $C_{28}H_{27}ClF_2N_5O_{1:}$ 542.1928; found: 542.1932.

1.2.4. Synthesis of N7-(6-Ethoxy-5-fluoro pyridin-3-yl)-N5-(2-(tetrahydro-2H-pyran-4-yl) ethyl) pyrazolo[1,5-a] pyrimidine-5,7-diamine (12)

Step 1. Pyrazolo[1,5-a]pyrimidine-5,7(4H,6H)-dione (9)

To a stirred solution of sodium (138 mg, 6.02 mmol) in ethanol (15 ml), 1H-pyrazol-3-amine (500 mg, 6.02 mmol) was added. This was followed by addition of diethyl malonate (1.010 ml, 6.62 mmol) at room temperature. The reaction mixture was refluxed at 85 °C for 18 h. Completion of reaction was followed by LCMS. After completion of reaction, the reaction mixture was cooled to room temperature and evaporated. Acidification of residue with 1N HCl to pH 3.0 resulted in white solid. The solid was filtered and dried to result in 570 mg of pure pyrazolo[1,5-a]pyrimidine-5,7(4H,6H)-dione **9** with 62% yield. ESMS calculated: 151.1; found: 152.0 (M+H).

Step 2. 5,7-Dichloropyrazolo[1,5-a]pyrimidine (10)

A solution of pyrazolo[1,5-a]pyrimidine-5,7(4H,6H)-dione **9** (400 mg, 2.65 mmol) in $POCl_3$ (6 ml, 64.37 mmol) was refluxed for 3 h. Completion of the reaction was monitored by LCMS. After completion of reaction, the reaction mixture was poured onto ice and the residue was extracted into dichloromethane (30 ml). The organic layer was washed with water, dried over Na_2SO_4 and evaporated. Purification of crude residue through combiflash chromatography using EtOAc-Hexane gradient resulted in 150 mg of pure 5,7-dichloropyrazolo[1,5-a] pyrimidine **10** with 30.1% yield. ESMS calculated: 188.0; found: 188.0 (M+).

Step 3. 5-Chloro-N-(4-ethoxy-3-fluorophenyl) pyrazolo[1,5-a]pyrimidin-7-amine (11)

To a cooled solution of 6-ethoxy-5-fluoropyridin-3-amine (108 mg, 0.69 mmol) dissolved in DMF (3 ml), sodium hydride (60 mg, 1.25 mmol) was added. After stirring the reaction mixture for 15min at room temperature, 5,7-dichloropyrazolo[1,5-a]pyrimidine **10** (130 mg, 0.69 mmol) was added and the reaction mixture was heated for 2 h at 75 ^oC. Completion of reaction was monitored by LCMS. Then the reaction mixture was evaporated and the residue was diluted with water. The separated solid was filtered and dried. This resulted in 200 mg of pure 5-chloro-N-(4-ethoxy-3-fluorophenyl)pyrazolo[1,5-a]pyrimidin-7-amine **11** with 94% yield. ESMS calculated: 306.7; found: 307.7 (M+H).

Step 4. N7-(6-Ethoxy-5-fluoropyridin-3-yl)-N5-(2-(tetrahydro-2H-pyran-4-yl)ethyl) pyrazolo [1,5-a]pyrimidine-5,7-diamine (12)

In a microwave vial, a solution of 5-chloro-N-(6-ethoxy-5-fluoropyridin-3-yl)pyrazolo[1,5-a]pyrimidin-7-amine **11** (200 mg, 0.65 mmol) dissolved in *n*-BuOH (2 ml) was placed. 2-(tetrahydro-2H-pyran-4-yl)ethanamine (336 mg, 2.60 mmol), sodium carbonate (413 mg, 3.90 mmol) and DIEA (0.681 ml, 3.90 mmol) were added onto this reaction mixture and subjected to microwave irradiation for 75 min at 170 °C. Completion of reaction was monitored by LCMS. Then the solvent was evaporated and the residue was redissolved in ethyl acetate (25 ml). The organic layer was washed with water, dried over Na_2SO_4 and evaporated. Purification of crude product by reverse phase chromatography using ACN-H_2O gradient resulted in 90 mg of pure form of N7-(6-ethoxy-5-fluoropyridin-3-yl)-N5-(2-(tetrahydro-2H-pyran-4-yl)ethyl) pyrazolo [1,5-a]pyrimidine-5,7-diamine **12** with 34.6% yield. ^1H NMR (300 MHz, DMSO-d_6) δ ppm 1.05 - 1.24 (m, 2 H) 1.33 - 1.38 (m, 2 H) 1.38 - 1.48 (m, 3 H) 1.57 (d, J = 10.1 Hz, 3 H) 3.18 - 3.32 (m, 4 H) 3.81 (dd, J = 10.9, 3.39 Hz, 2 H) 4.42 (q, J = 7.0 Hz, 2 H) 5.48 (s, 1 H) 5.93 (d, J = 2.07 Hz, 1 H) 6.76 (t, J = 5.2 Hz, 1 H) 7.76 - 7.91 (m, 2 H) 8.05 (d, J = 2.0 Hz, 1 H) 9.24 (s, 1 H). ESMS calculated: 400.4; found: 401.2 (M+H); HRMS calculated for $C_{20}H_{25}FN_6O_2$: 401.2095; found: 401.2095.

1.2.5. General procedure for the synthesis of pyrazolopyrmidines demonstrated through the synthesis of N-(6-(2-(Dimethylamino)ethoxy)-5-fluoropyridin-3-yl)-2-(4-fluorophenyl)-5-phenyl-pyrazolo[1,5-a]pyrimidin-7-amine (17a)

Step 1. 3-(4-Fluorophenyl)-1H-pyrazol-5-amine (14)

To a solution of 3-(4-fluorophenyl)-3-oxopropanenitrile (7 g, 4.29 mmol) in ethanol (700 ml), hydrazine hydrate (2.57 g, 51.49 mmol) was added and heated to 80 °C for 4 h. Evaporation of the reaction mixture afforded a thick solid which was dissolved in ethyl acetate (200 ml), washed with water (200 ml). The organic layer separated was washed with brine solution, dried over sodium sulphate and evaporated under reduced pressure to yield the product as an off-white solid. Yield 2.5 g (33%). ESMS calculated: 177.2; found: 178.4 (M+H).

Step 2. 2-(4-Fluorophenyl)-5-phenylpyrazolo [1,5-a]pyrimidin-7(4H)-one (15)

A mixture of 3-(4-fluorophenyl)-1H-pyrazol-5-amine **14** (11 g, 62.08 mmol), ethyl 3-oxo-3-phenyl-propanoate (14.3 g, 74.5 mmol) and acetic acid (165 ml) was heated to 120 °C in a sealed tube for 12 h. Evaporation of the resulting mixture under reduced pressure afforded the product as off-white solid. Yield 9 g (48%). ESMS calculated. 305.3: found: 304.0 (M-H).

Step 3. 7-Chloro-2-(4-fluorophenyl)-5-phenyl pyrazolo[1,5-a]pyrimidine (16)

A suspension of 2-(4-fluorophenyl)-5-phenyl pyrazolo[1,5-a]pyrimidin-7(4H)-one **15** (9 g, 29.47 mmol) and phosphrous oxychloride (180 ml) was heated to 135 °C for 3 h. The reaction mass was concentrated under reduced pressure, neutralized with sodium bicarbonate, extracted with ethyl acetate (2 x 300 ml). The combined organic layer was washed with brine solution, dried over Na_2SO_4, and concentrated under reduced pressure to get the crude product as a brown colored liquid. The crude product was purified by column chromatography (silica gel 230-400 mesh eluted with 15% EtOAc petroleum ether) to give the title product **16** as a light yellow solid. Yield 6.5 g (68%). ESMS calculated: 323.7; found: 324.2 (M-H).

Step 4. N-(6-(2-(Dimethylamino)ethoxy)-5-fluoropyridin-3-yl)-2-(4-fluorophenyl)-5-phenylpyrazolo[1,5-a]pyrimidin-7-amine (17a)

To a stirred solution of 7-chloro-2-(4-fluorophenyl)-5-phenylpyrazolo[1,5-a]pyrimidine **16** (4 g, 12.35 mmol) in dry DMF (40 ml), 6-(2-(dimethylamino)ethoxy)-5-fluoropyridin-3-amine (2.44 g, 12.35 mmol) and K_2CO_3 (4.2 g, 30.8 mmol) were added and heated to 75 °C for 2 h. Then the reaction mixture was taken in water (150 ml), extracted with EtOAc (2 x 150 ml). The combined organic layer was washed with saturated ammonium chloride solution, followed by brine solution and dried over anhydrous sodium sulphate. Evaporation of the organic layer afforded the crude product as a green colored liquid. It was then purified by column chromatography (silica gel 230-400 mesh eluted with 90% DCM in MeOH (containing 1% of AcOH) to afford the product **17a** as a green colored solid. Yield 2 g (33%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.26 (s, 6H), 2.71-2.72 (m, 2H), 4.50 (t, J = 8.0 Hz, 2H), 6.64 (s, 1H), 7.09 (s, 1H), 7.35-7.37 (m, J=12Hz, 2H), 7.47-7.49 (m, 3H), 8.04-8.17 (m, 3H), 8.19-8.22 (m, 3H), 9.87 (s, 1H). ESMS calculated: 486.5; found: 487.2 (M+H); HRMS calculated for $C_{27}H_{24}F_2N_6O$: 487.2052; found: 487.2051.

N-(6-(2-(dimethylamino)ethoxy)-5-fluoropyridin-3-yl)-2-(4-fluorophenyl)-5-(trifluoromethyl) pyrazolo[1,5-a]pyrimidin-7-amine (17b)

Yield 22.7%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.29 (s, 6H), 2.76 (m, 2H), 4.42-4.44 (m, 2H), 6.68 (s, 1H), 7.05 (s, 1H), 7.25-7.29 (m, 2H), 7.45-7.48 (m, 2H), 8.30-8.34 (m, 2H), 10.03 (s, 1H). ESMS calculated: 478.4; found: 479.2 (M+H); HRMS calculated for $C_{22}H_{19}F_5N_6O$: 479.1612; found: 479.1609.

1.2.6. General procedure for the synthesis of N-(6-(2-(Dimethylamino)ethoxy)-5-fluoropyridin-3-yl)-2-(4-fluorophenyl)-5-phenyl-1H-pyrrolo[3,2-b]pyridin-7-amine (26)

Step 1. 3-Nitropyridine-2,4-diol (19)

To a stirred solution of pyridine-2,4-diol (20 g, 180.01 mmol) in concentrated sulfuric acid (89 ml), 70% nitric acid (89 ml) was added drop wise at 0 °C over a period of 30 min. After stirring at 0 °C for 0.5 h, the reaction mixture was poured onto ice-water and stirred vigorously for 1 h. The resulting yellow precipitate was filtered, washed with water and then dried to afford 3-nitropyridine-2,4-diol **19** as a yellow solid. Yield 24 g, (85.4%). ESMS calculated: 156.1; found: 157.6 (M+H).

Step 2. 2, 4-Dichloro-3-nitropyridine (20)

A solution of 3-nitropyridine-2,4-diol **19** (23 g, 147.34 mmol) in $POCl_3$ (300 ml) was placed in a sealed tube and heated at 135 °C. After 12 h, it was concentrated completely and the resulting crude was poured onto ice-water. The reaction mixture was extracted with ethyl acetate twice. The combined organic layer was washed with water and brine, dried over sodium sulphate and concentrated under reduced pressure. The resulting crude residue was triturated with hexane and the solid obtained was collected by filtration and dried to get 2, 4-dichloro-3-nitropyridine **20** as a pale yellow solid. Yield 20.5 g, (72.18%). ESMS calculated: 193.0; found: 195.5 (M+2H).

Step 3. 2, 4-Dichloropyridin-3-amine (21)

A stirred solution of 2,4-dichloro-3-nitropyridine **20** (20 g, 106.22 mmol) in ethanol (200 ml) was added to ammonium chloride (34.2 g, 637.32 mmol), iron powder (29.2 g, 531.14 mmol) and water (50 ml). Then the reaction mixture was heated at 80 °C for 5 h. It was later filtered celite pad, washed with ethanol and the filtrate was concentrated. The residue was partitioned between dichloromethane and water. The organic layer was washed with brine, dried over sodium sulphate and concentrated to get 2,4-dichloropyridin-3-amine **21** as a dark brown solid. Yield 14.2 g (82.08%). ESMS calculated: 163.0; found: 165.2 (M+2H).

Step 4. 6-Bromo-2, 4-dichloropyridin-3-amine (22)

To a stirred solution of 2,4-dichloropyridin-3-amine **21** (14.2 g, 87.116 mmol) in DMF (455 ml), a solution of NBS (18.6 g, 105.31 mmol) dissolved in DMF (200 ml) was added drop wise at

0 °C over a period of 20 min. After stirring at 0 °C for 1 h, the reaction mixture was poured onto ice-water and stirred vigorously and the resulting solid was collected by filtration, washed with water and dried under vacuum to afford 6-bromo-2,4-dichloropyridin-3-amine **22** as an off-white solid. Yield 10.0 g (47.61%). ESMS calculated: 241.9; found: 243.4 (M+2H).

Step 5. 2,4-Dichloro-6-phenylpyridin-3-amine (23)

To a stirred solution of 6-bromo-2,4-dichloropyridin-3-amine **22** (5 g, 20.669 mmol) and phenyl boronic acid (2.5 g, 20.660 mmol) in 1,4-dioxane (750 ml), dry potassium carbonate (10.9 g, 102.83 mmol) was added followed by water (100 ml). The reaction mixture was purged with N_2 for 20 min, tetrakis(triphenylphosphine) palladium (1.19 g, 1.033 mmol) was added and degassed with N_2 for another 10 min. The reaction mixture was heated to 85 °C for 12 h. The reaction mixture was concentrated completely and the resulting crude mixture was partitioned between ethyl acetate and water. The compound in organic layer was washed with brine dried over sodium sulphate and concentrated. The crude product obtained was purified by silica gel column chromatography using 10-90% ethyl acetate in petroleum ether to get 2,4-dichloro-6-phenyl pyridin-3-amine **23** as an off white solid. Yield 4.0 g (80.97%). ESMS calculated: 239.1; found: 240.2 (M+1).

Step 6. 4-Chloro-2-((4-fluorophenyl)ethynyl)-6-phenylpyridin-3-amine (24)

A stirred solution of 2,4-dichloro-6-phenylpyridin-3-amine **23** (2 g, 8.36 mmol) in triethylamine (50 ml) was added to $Pd(PPh_3)_2Cl_2$ (0.29 g, 0.41 mmol) followed by copper iodide (0.07 g, 0.41 mmol) at RT. The reaction mixture was purged with N_2 for 20 min and then cooled to 0 °C followed by drop wise addition of 4-fluorophenyl acetylene (1.5 g, 15.54 mmol). The resulting reaction mixture was heated to 80 °C for 5 h. It was then concentrated completely and the resulting crude was partitioned between ethyl acetate and water. The organic layer was separated, washed with saturated brine solution, dried over anhydrous sodium sulphate and concentrated. The crude product obtained after concentration was purified by silica gel column chromatography using 5% ethyl acetate in petroleum ether to get 4-chloro-2-((4-fluorophenyl)-ethynyl)-6-phenylpyridin-3-amine **24** as a yellow solid. Yield 1.2 g (45%). ESMS calculated: 322.7; found: 323.2 (M+1).

Step 7. 7-Chloro-2-(4-fluorophenyl)-5-phenyl-1H-pyrrolo[3,2-b]pyridine (25)

To a stirred solution of 4-chloro-2-((4-fluorophenyl)ethynyl)-6-phenylpyridin-3-amine **24** (1.5 g, 4.655 mmol) in THF (15 ml), potassium tert-butoxide (0.52 g, 4.65 mmol) was added at RT. The reaction was subjected to microwave irradiation at 100 °C for 1 h. The reaction mixture was concentrated completely and the resulting residue was partitioned between ethyl acetate and water. The organic layer separated was washed with saturated brine solution, dried over anhydrous sodium sulphate and concentrated to get 7-chloro-2-(4-fluorophenyl)-5-phenyl-1H-pyrrolo[3,2-b]pyridine **25** as an off-white solid. Yield 1.17 g (78%). ESMS calculated: 322.7; found: 323.2 (M+1).

Step 8. N-(6-(2-(Dimethylamino)ethoxy)-5-fluoro pyridin-3-yl)-2-(4-fluorophenyl)-5-phenyl-1*H*-pyrrolo[3,2-b]pyridin-7-amine (26)

To a stirred solution of 7-chloro-2-(4-fluorophenyl)-5-phenyl-1H-pyrrolo[3,2-b]pyridine **26** (0.6 g, 1.86 mmol) in dry THF (17 ml), 6-(2-(dimethylamino)ethoxy)-5-fluoropyridin-3-amine (0.36 g, 1.86 mmol), sodium *tert*-butoxide (0.44 g, 4.57 mmol) and t-Butyl Xphos (63 mg, 0.09 mol) were added in a microwave vial. The reaction mixture was purged with N_2 for 15 min and subjected to microwave irradiation at 100 °C for 2 h. The reaction mixture was concentrated under reduced pressure and the residue extracted with EtOAc (100 ml). The combined organic layer was washed with saturated ammonium chloride solution, followed by brine solution and dried over sodium sulphate. The crude product was purified by silica gel column chromatography using 15% MeOH in DCM to give the title product **26** as a light brown solid. Yield 57 mg (6.3%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.64 (s, 6H), 3.24 (s, 2H), 4.63 (t, J = 8.0 Hz, 2H), 7.04 (s, 1H), 7.10 (s, 1H), 7.36-7.39 (m, 3H), 7.45-7.47 (m, 2H), 7.93-7.95 (m, 3H), 8.06-8.07 (m, 2H), 8.14 (dd, J = 4.0, 23.80 Hz, 1H), 9.50 (s, 1H), 12.26 (s, 1H), ESMS calculated: 485.5; found: 486.2 (M+H); HRMS calculated for $C_{28}H_{25}F_2N_5O$: 485.2027; found: 485.2027.

1.3. Molecular Modeling Studies

1.3.1. Homology modeling of Fo unit of *M. tuberculosis* ATP synthase

The knowledge about the 3D structure of a target may significantly add to the understanding of essential structural requirements as well as biological activity variation among the different ligands acting on the same target. The BLAST analysis of the “a” and “c” subunit of the *Mycobacterium tuberculosis* ATP synthase (Swissprot entry code: P9WPV6 and P9WPS0 respectively) showed the maximum sequence identity (38% and 52% respectively) with “a” and “c” subunit of the NMR-based *Escherichia coli* ATP synthase protein (PDB ID: 1C17). This suggested that the latter may serve as a good candidate for the selection of a template for this subunit. In view of the above, a 3D structural model of Fo unit of the *M. tuberculosis* ATP synthase was generated using NMR-based structural model of *E. coli* ATP synthase (PDB ID: 1C17) as a template and the previously reported alignment.

Highly conserved residues were anchored and the plausible models were generated in Prime module of Schrodinger software package.[1-3] Homology model was inspected to ensure that the side chains of the conserved residues were aligned to the template. The outlier residues were examined using the Ramachandran plot and refined using the "refine loops" tool with "serial-loop sampling" procedure of Prime. The resulting plausible model for *M. tuberculosis* ATP synthase was backbone-constrained, energy minimized using OPLS 2005 force field using MacroModel.[20] Protein was prepared using protein preparation wizard implemented in Schrödinger software package.

1.3.2. Ligand and Protein Preparation

Protein was prepared using Protein preparation wizard implemented in Schrodinger package using default options: bond orders were assigned, hydrogens were added and water molecules 5 Å beyond hetero groups were deleted. Hydrogens were then optimized using the exhaustive sampling option and the protein was minimized to an rmsd limit from the starting structure of 0.3 Å using the Impref module of Impact with the OPLS_2005 force field. The ligands were prepared using LigPrep module of Schrodinger package. Conformational search for each ligand was performed using MacroModel with OPLS_2005 force field, water as solvent and remaining as default parameters. All the calculations were performed on a Linux workstation equipped with four parallel Intel Xeon X5460 processors (2.8 GHZ) with 12 GB total RAM.

1.3.3. Molecular Docking

Three dimensional resolved crystal structure of bedaquiline was downloaded from Cambridge Structural Database and used for the docking studies. The binding conformation of bedaquiline [3] showed an excellent overlap with its crystal structure.

The bound conformation of modeled bedaquiline with ATP synthase protein[3] was used to define the active site grid for docking. The optimized 3D-structures of new ligands were docked within 15 Å radius. All the docking outputs of ligands occupied the interface between the two c-subunits (chain B and chain C) and the a-subunit of ATP synthase. Post docking analysis was performed to select the best binding pose of synthesized ligands where the ligands were ranked based on their docking score and rmsd with respect to protein bound conformation of bedaquiline. Interestingly, among all the docking outputs for each ligand, the conformation with the highest docking score showed least rmsd with respect to protein bound conformation of bedaquiline. Hence, they were selected as the best binding pose for these ligands.

1.4. Biological Assays

1.4.1. Materials

Mycobacterial membrane vesicles from *Mycobacterium smegmatis* were prepared in house. Sub mitochondrial particles (SMP) was provided by Prof. Harvey Rubin, University of Pennsylvania. Beetle Luciferin, potassium salt (CAS: E1605) and Quantilum recombinant luciferase (CAS: E1702) were procured from Promega. ATP Bioluminescence kit CLSII (CAS: 11699695001) was purchased from Roche. Lysosensor Green DND-153 (CAS: L7354) was purchased from Life technologies. NADH (CAS: N8129) and ADP (CAS: A2754) were purchased from SIGMA. 384 well black flat bottom plates were procured from Corning (CAS: 3573). Reference inhibitors: Dicyclohexylcarbodimide (DCCD, Catalog no: D80002), Thioridazine (CAS: T-9025) and carbonylchloride 3-chlorophenyl hydrazone (CCCP, CAS: C2759) were procured from SIGMA. Bedaquiline was synthesized in house.

1.4.2. NADH driven ATP synthesis assay (Myc_ATPS)

The ATP synthesis activity was determined in isolated membrane vesicles from *M. smegmatis*. The 384 well plate assay with an assay volume of 30 µl used 8 µg/ml *M. smegmatis* inverted membrane vesicles (IMVs). Briefly, 15 µl of enzyme mix containing IMVs and 5 mM $MgCl_2$ in HEPES-NaOH buffer, pH 7.6 was added to 384 well plates containing 1 µl of 30X compound dilutions. Reaction was initiated by adding 15 µl of substrate mix containing 0.3 mM NADH, 15 µM ADP and 0.1 mM KH_2PO_4. Plates were incubated for 60 min at room temperature. The amount of ATP synthesized was measured by adding 30 µl of Lucifierin/luciferase reagent and the luminescence measured using TECAN Infinite F500 immediately. IC_{50} values were calculated using GraphPad Prism software.

1.4.3. Mitochondrial ATP synthesis assay (SMP_ATPS)

ATP synthesized by mitochondrial ATP synthase using ADP and inorganic phosphate, Pi was measured using ATP Bioluminescence Assay Kit CLS II and purified bovine SMP. Briefly, 20 µl of reagent mix-1 (purified bovine SMP along with ADP, potassium phosphate and CLSII detection reagent in Tris acetate buffer, pH 7.5) was added to 384 well black plates (corning 3573) containing 30X compound dilutions. Reaction was initiated by the addition of 10 µl of reagent mix-2 containing 0.2 mM NADH. Luminescence was read kinetically in Tecan Infinite F500 over 20 minutes. IC_{50} values were calculated using GraphPad Prism software.

1.4.4. Membrane damage assay in BCG

Mycobacterium bovis BCG cells were grown in 7H9 media containing 10% ADC. At A_{600} 0.5-0.6, cells were harvested and resuspended in 7H9 media such that A_{600} was about 3.0. Cells were then loaded with the fluorescence probe, $DiOC_2$ at a final concentration of 1µM and incubated for 30 minutes in dark. After 30 minutes, 10 µl of cell suspension (~10^7 cells) was dispensed using multidrop into 384 well plates containing 1µl of 30X compound dilutions and 39 µl 7H9 media bringing the total assay volume to 50 µl. Plates were incubated in the dark for 60 minutes and fluorescence was read in Tecan Safire with excitation at 485 nm and emission at 600 nm. $DiOC_2$ can delocalize the charge on the membrane, penetrate and accumulate on the inner negative surface. Stacked $DiOC_2$ molecules having excitation wavelength of 488nm & emission at 600nm show higher fluorescence with high membrane potential. CCCP, a proton ionophore was used as positive control for membrane damage and chloramphenicol was used as negative control. IC_{50} values were calculated using GraphPad Prism software.

1.4.5. Intracellular ATP pool measurement

3 ml of *M. bovis* BCG cells grown till A_{600} ~0.1 in 7H9 broth was exposed to 5X MIC concentration of a set of test compounds and two reference inhibitors Isoniazid (INH) and bedaquiline. At the end of 20 hours of compound exposure, cells were harvested, resuspended in 300 µl of 1X PBS pH 7.4, added a pinch of Zirconia beads (Biospec products), lysed by bead beating in a mini bead beater (Biospec products) with two pulses of 20 seconds each. 30 µl of the cell lysates were immediately used to measure the ATP levels by adding 30 µl of

Lucifierin/luciferase reagent followed by measuring luminescence in TECAN Infinite F500. Percent depletion in ATP levels for each compound was calculated by taking the ATP levels measured in control BCG cells not exposed to any compound as 100%.

1.4.6. Microbiology methods

Determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), *in vitro* survival kinetics were performed as reported earlier.[4] For an initial MIC_{90} estimate, a small set of *M. tuberculosis* clinical strains which included a set of sensitive strains and a set of single drug resistant strains were used to test the MIC. Concentration of the compound at which ≥ 80% growth inhibition in a turbidometric read method was considered as MIC. In-house generated bedaquiline (Bedaquiline^R) resistant strains having both I66M and A63P mutations were also used to test cross resistance with the compounds. Bedaquiline^R strains were generated following the general mutant generation and characterization procedures as reported earlier.[5]

1.4.7. Pharmacokinetics

AstraZeneca Animal Ethics Committee, registered with the Government of India (registration no. CPCSEA 99/5) approved all animal experimental protocols and usage. Pharmacokinetics of **17a** and **8g** were analyzed in healthy as well as infected mice in the efficacy study. Blood samples from infected animals were collected and processed in a biosafety level 3 (BSL3) laboratory. The compounds were orally administered in a suspension containing 0.5% hydroxypropyl methylcellulose (HPMC) & Tween 80 at a 10 ml/kg dose volume. Blood samples were collected from groups of 3 mice per time point by puncturing the saphenous vein at 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 6 h or 8 h and 24 h after dosing. The separated plasma samples were precipitated by adding chilled acetonitrile (1:10 v/v) containing carbamazepine as internal standard (100 ng/ml). Samples were vortexed, and centrifuged at 4000 rpm for 30 min at 10 °C. The resulting supernatant was mixed with mobile phase (50% acetonitrile in water containing 0.1% formic acid). 10 µl of sample was injected onto a liquid chromatographic system (Prominence UPLC *XR,* Shimadzu Corporation, Kyoto, Japan) coupled to triple quadrupole mass spectrometer (API 3000, AB Sciex, Thornhill, ON, Canada). Samples were separated on LC column (Phenomenex Synergy Hydro-RP, 50x4.6mm, 80A, 4.6 µm) by isocratic elution with 40 parts of 5 mM ammonium formate containing 0.1% v/v formic acid and 60 parts of acetonitrile at a flow rate of 0.6 ml/min. Mass spectrometer was operated in positive ion mode and analytes were detected by multiple reaction monitoring (MRM). Concentrations of analytes were determined from a standard curve obtained by plotting known concentrations of the analyte against peak area ratios (analyte/internal standard peak response). Area under the concentration versus time PK profile (AUC) was calculated by non-compartmental analysis (WinNonLin 5.2.1; Pharsight Inc.).

1.4.8. Pharmacodynamics

AstraZeneca Animal Ethics Committee, registered with the Government of India (registration no. CPCSEA 99/5) approved all animal experimental protocols and usage. BALB/c mice were infected in an aerosol chamber (10,000 CFU of *M. tuberculosis* H37Rv per mouse). Infected mice were housed in individually ventilated cages (Allentown Technologies, USA) in a BSL3 facility. Treatment was initiated 3 days post infection. Vehicle- and compound-treated groups contained 3 mice each. An additional 3 mice were sacrificed just after infection to serve as early controls. **17a**, **8g** and isoniazid were formulated in 0.5% HPMC/Tween. Infected mice were treated with an oral dose of 100 and 200 mg/kg of **17a** and **8g** and also10 mg/kg of isoniazid, once daily, 6 days per week, for 2 weeks. Mice were sacrificed after completion of treatment and suitable dilutions of their lung homogenates were plated on 7H11 agar to determine viable CFU in mouse lung tissue. One-way analysis of variance (ANOVA) followed by the Bonferroni's multiple-comparison test was used for analyzing efficacy data.

2. Computational analysis

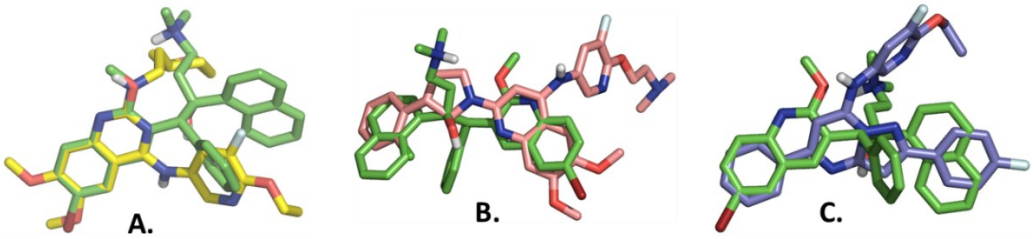


Fig. S1. 3-Dimensional (3D) flexible superimposition of compounds with bedaquiline (green color carbon) **A.** compound **3h**; **B.** compound **8d**; **C.** compound **17a**.

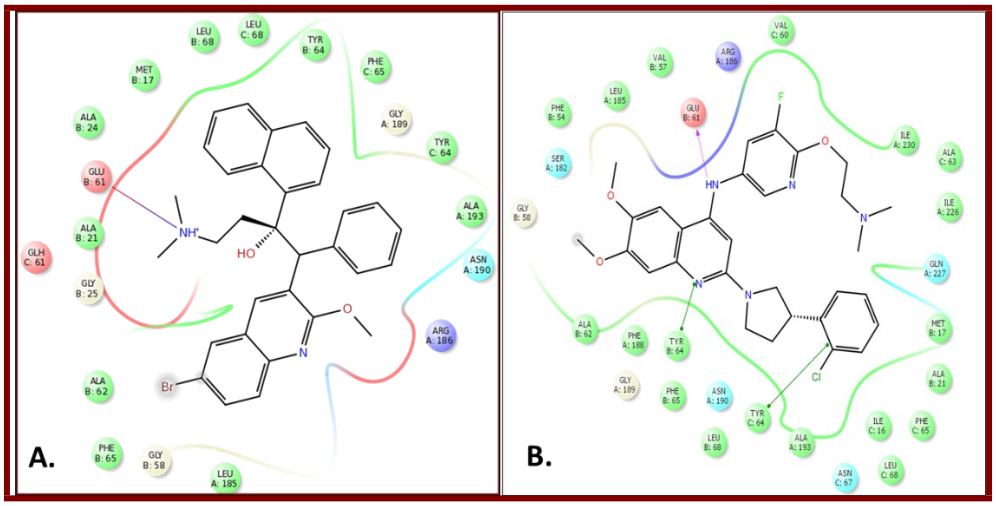


Fig. S2. 2-Dimensional (2D) binding site interaction of inhibitors at the ATPase binding site **A.** bedaquiline; **B.** compound **8d**.

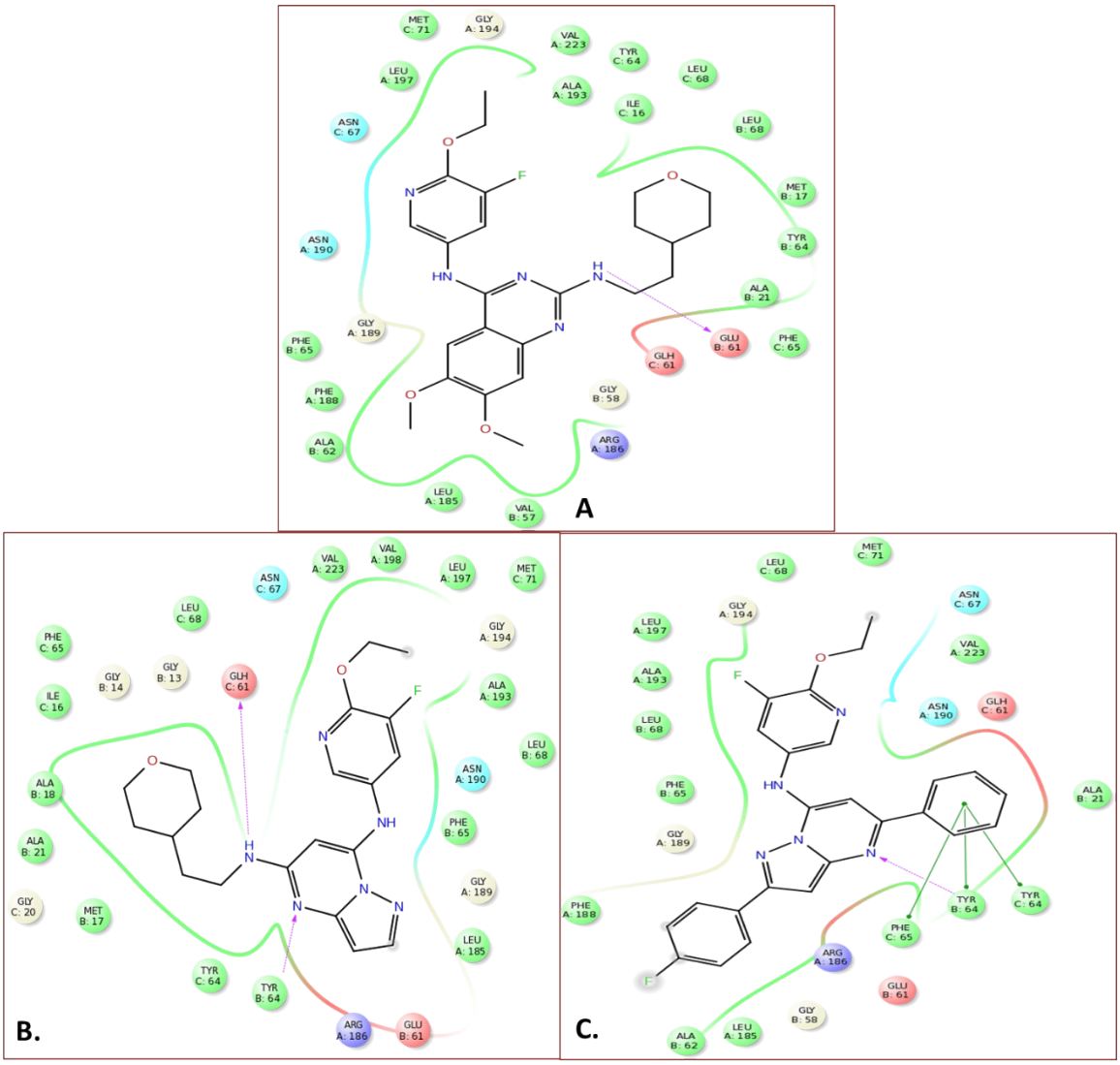


Fig. S3. 2D binding site interaction of inhibitors at the ATPase binding site; **A.** compound **3h**; **B.** compound **12**; **C.** compound **17a**.

3. Compound identity: NMR and HPLC profiles

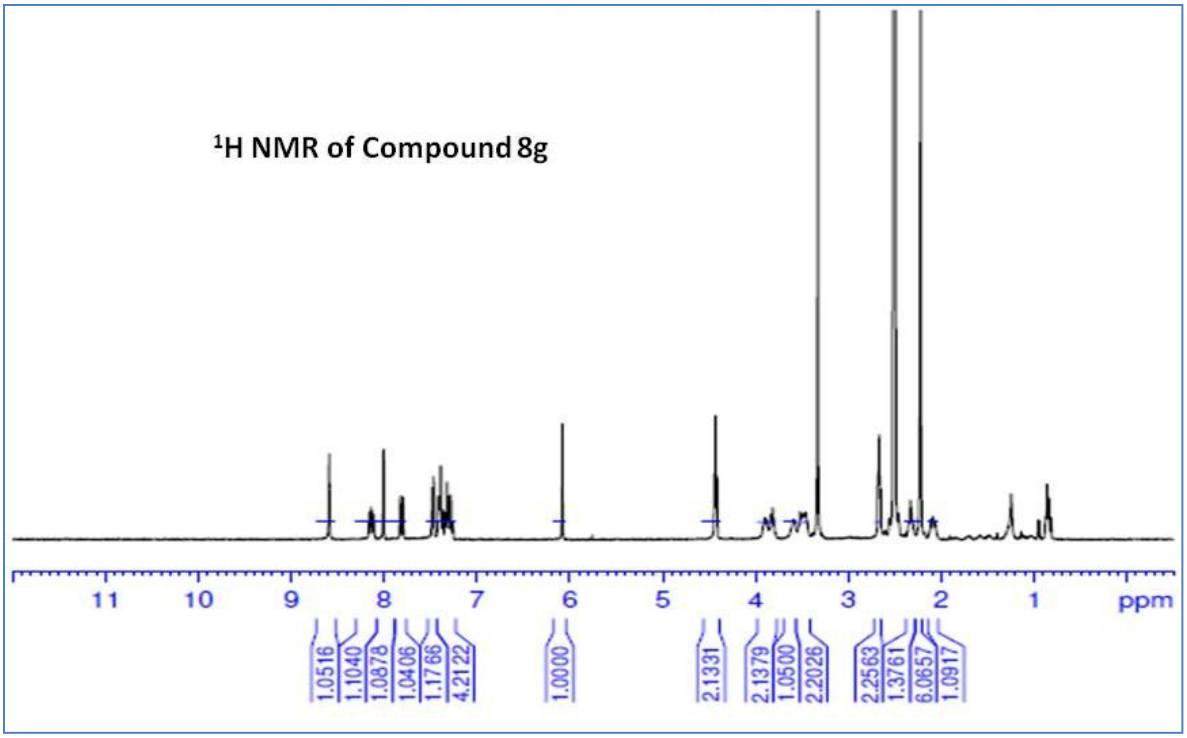


Fig. S4. ^{1}H NMR: (400 MHz, DMSO-d_6) of compound **8g**

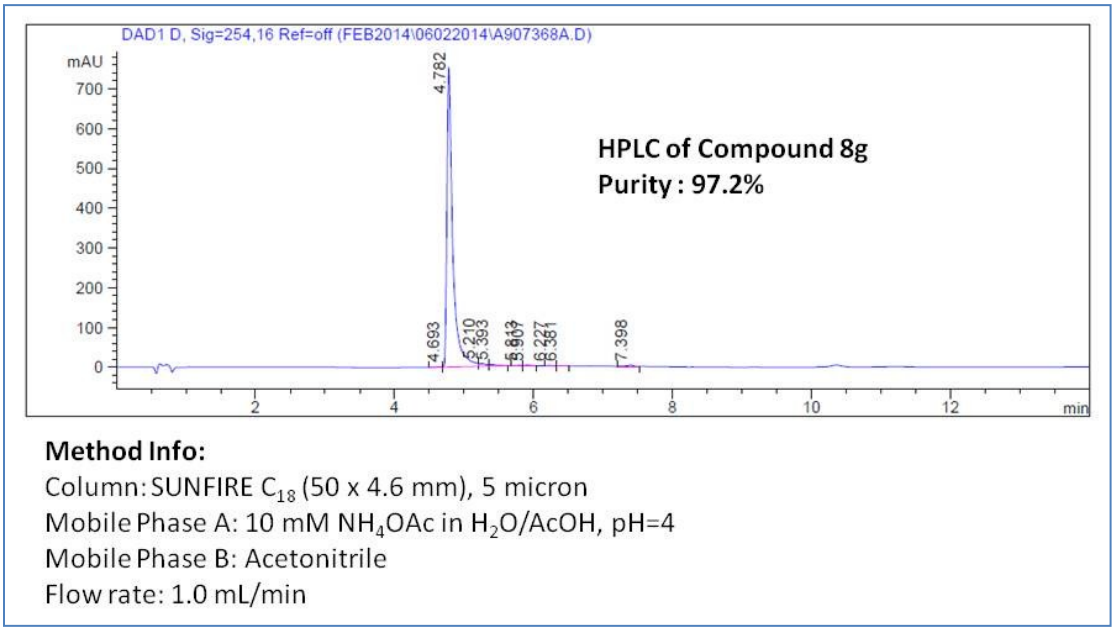


Fig. S5. HPLC of compound **8g**

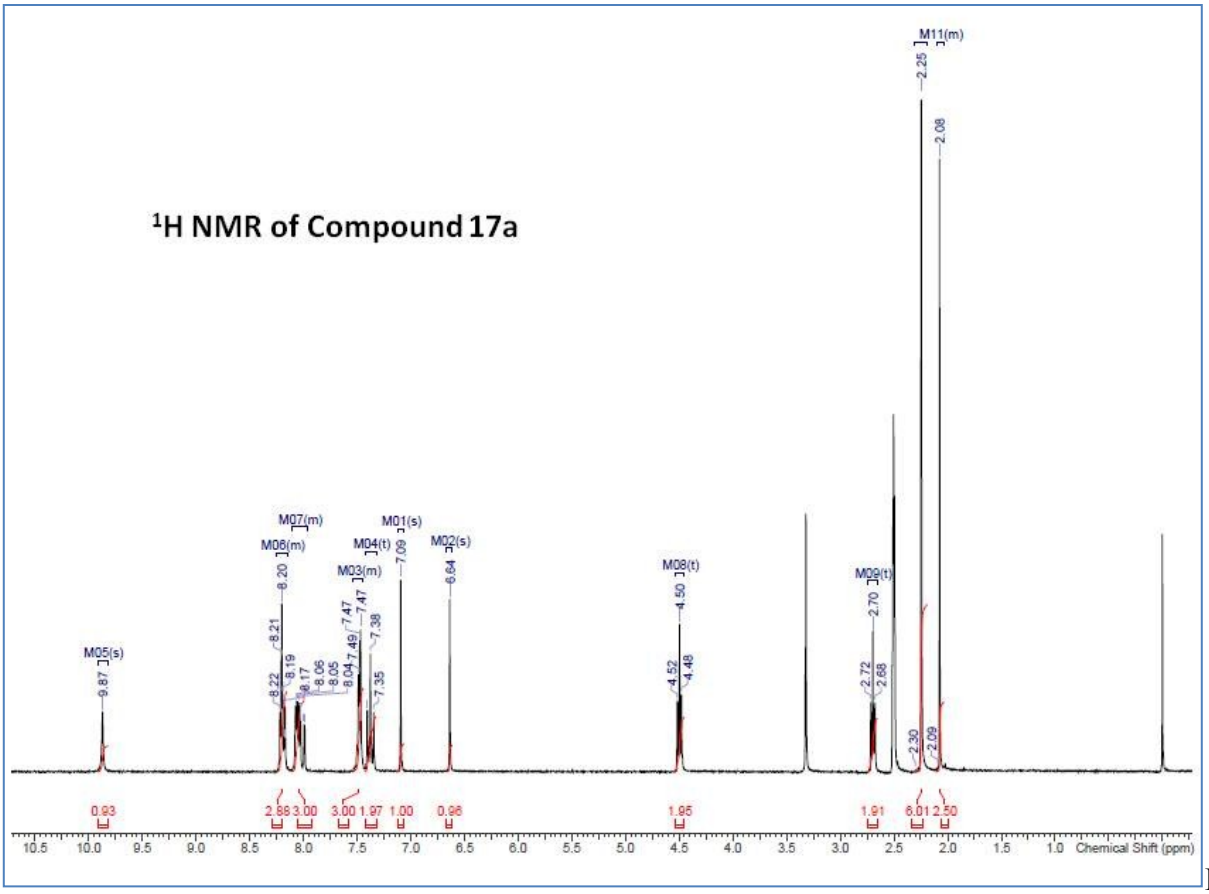


Fig. S6. ^{1}H NMR: (400 MHz, DMSO-d_6) of compound **17a**

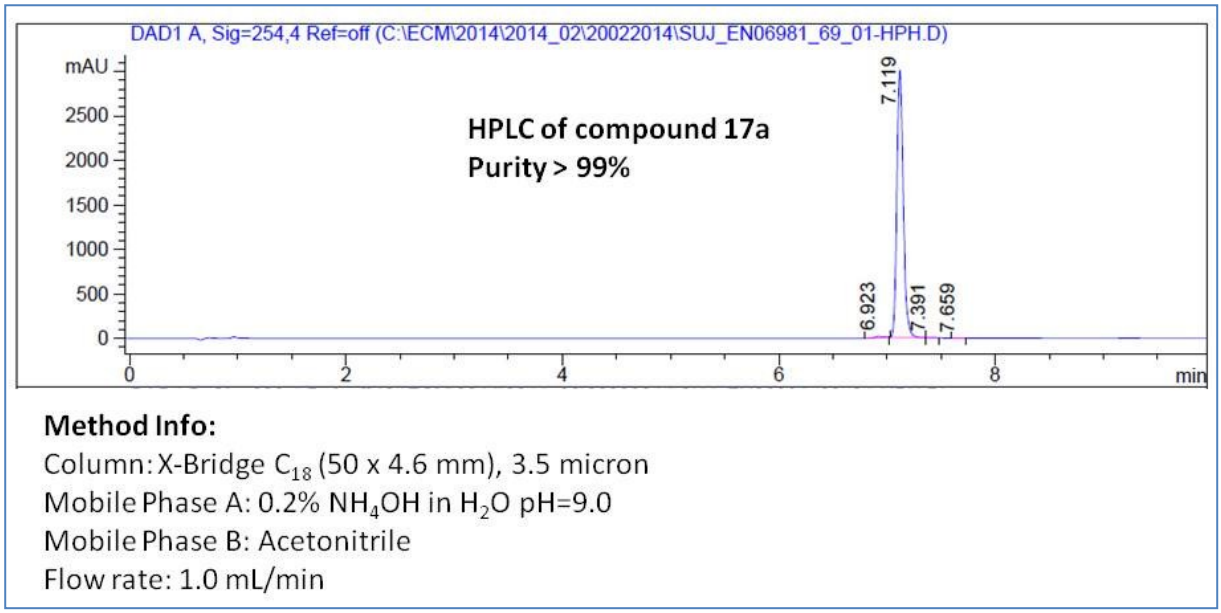


Fig. S7. HPLC of compound **17a**

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