

Design, Synthesis and Biological Evaluation of a Series of Novel Axl Kinase Inhibitors

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Supporting Information - 19 pages

I. Homology Modeling and Analysis of Inhibitor-Axl Complexes

The input sequence of human Axl kinase (NP_06871) and its kinase domain (518-843 AA) were obtained from RCSB (protein data bank) and were evaluated using Blast sequence search. Three protein kinases, c-Met (PDB: 2RFN), MER (PDB: 3BPR) and IGF-1R (PDB: 1P4O) were found to exhibit high E-value to that of the target sequence and these homologues structures have been solved crystallographically. The structural model of Axl kinase was built using these three template structure. The multiple sequence alignment performed using the Prime and Clustal programs identified the identical, conserved and active site residues (**Figure 1**). The final Axl model (**Figure 2**) derived from the Prime homology modeling workflow was examined using Profile-3D. The Profile-3D and 3D-1D score plots of the model are positive over the entire length of the protein in a moving-window scan to the template structure. Additionally, PROCHECK was used to verify the correct geometry of the dihedral angles and the handedness of the model-built structure. The Axl homology model complex structure was energy minimized and the lowest global energy structure were used to calculate intermolecular binding energies in performing the virtual screening/docking for lead identification and optimization.

CLUSTAL 2.0.11 multiple sequence alignment

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AXL      -----ISEELKEKLRDVMVDRH-----KVALGKTLGEGEFGAVMEGQLN 556
MER      -----NKLEDVVIDRN-----LLILGKILGEGEFGSVMEGNLK 607
c-Met    -----ALNPELVQAVQHVVIGPSSL---IVHFNEVIGRGHFGCVYHGTL 1098
IGF-1R   -----EVAREKITMSRELQGGSFQMVYEGVAK 1019
          :. * . : : : : * . * * * . *

AXL      --QDD-SILKVAVKTMKIAICTRSELEDFLSEAVCMKEFDHPNVMRLIGVCFQGSERESF 613
MER      --QEDGTSLKVAVKTMKLDNSSHREIEEFLSEAACMKDFSHPNVIRLLGVCIE MSS-QGI 664
c-Met    --DNDGKKIHCAVKSLNR-ITDIGEVSQFLTEGIIMKDFSHPNVLSLLGICLRSEG---- 1151
IGF-1R   GVVKDEPETRVAIKTVNEAASMR-ERIEFLNEASVMKEFNCHHVRLG VVVSQGGP---- 1074
          . * : * : : : * : * * . * * : * : * : . .

AXL      PAVVILPFMKHGDLSHFLLYSR--LGDQPVYLP--TQMLVKFMADIASGMEYLS TKRFI 669
MER      PKPMVILPFMKYGDLSHTYLLYSR--LETGPKHIP--LQTLKFMVDIALGMEYLSNRNFL 720
c-Met    -SPLVVLPYMKHGDLRNFIRN-----ETHNPT--VKDLIGFGLQVAKGMKYLASKKFV 1201
IGF-1R   --TLVIMELMTRGDLKSYLRLSRPEMENN PVLAPPSLSKMIQMAGEIADGMAYLNANKFV 1132
          . : : : * . * * : : : . . : : : : * * * * . * :

AXL      HRDLAARN CMLNENMSVCVA DFLSKKIYNGDYRQG--RIAKMPVKWIAIESLADR VYT 727
MER      HRDLAARN CMLRDDMTVCVA DFLSKKIYSGDYRQG--RIAKMPVKWIAIESLADR VYT 778
c-Met    HRDLAARN CMLDEKFTVKVA DFLGARDMYDKEYYSVHNKTGAKLPVKWMALES LQTQKFT 1261
IGF-1R   HRDLAARN CMVAEDFTVKIGDFGMTRDIYETDYRKG--GKGLLPVRWMSPE SLKDG VFT 1190
          * * * * * : : : * : * * : : : * . * * : * * : * * : *

AXL      SKSDVWSFGVTMWEIATRGTPTYPGVENSEIYDYL RQGNRLKQPADCLDGLYALMSRCWE 787
MER      SKSDVWAFGVTMWEIIRTRGMTYPGVQNHMYDYLLHGHR LKQPEDCLDELYEIMYSCWR 838
c-Met    TKSDVWSFGVLLWELMTRGAPPYDVNTFDITVYLLQGRRLQPEYCFDPLYEVMLK CWH 1321
IGF-1R   TYSDVWSFGVLLWEIATLAEQPYQGLSNEQVLR FVMEGGLLDKPDNCPDMLFELMRMCWQ 1250
          : * * * : * * : * * . * * . . : : : * * * * * : * * *

AXL      LNPQDRPSFTELREDLNTL KALP-PAQEPDEILYVN--MDEGGGYPEPPGAAGGADPP 843
MER      TDPLDRPTFSVLR LQLEKLLLESLP-DV----- 864
c-Met    PKAEMRPSFSELVSRISAIFSTFIGEHYVH----- 1351
IGF-1R   YNPKMRPSFLEI ISSIKEEMEPGF----REVSFYSEENK----- 1283
          . . * * : : : . . . . . * : :

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Figure 1. Multiple sequence alignment of the target Axl kinase and its homologous template sequences was performed using Prime. Mer kinase exhibits the highest sequence identity and sequence similarity of 69 and 83% with 1% gaps. c-Met and IGF-1R had 46 and 41% sequence identity with Axl kinase.

* identical residues, : highly conserved residues, . similar residues. The amino acid residues highlighted in magenta color are the critical active site residues and the residues in red are the kinase gatekeeper.

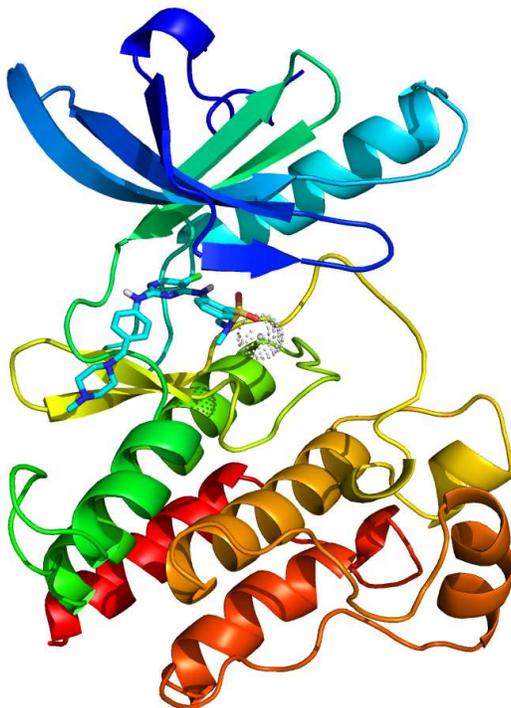


Figure 2. Homology model of Axl kinase derived from c-Met and IGF-1R kinases, shown in color by secondary structure type. The model was further refined by utilizing the recently published Mer kinase crystal structure which exhibited 69% sequence identity with the target sequence. The initial lead compound **13** bound to Axl kinase is shown in color-by-atom type.

II. General Chemical Procedures

All routine reagents and solvents were purchased from Sigma Aldrich and used as received. They were of reagent grade, purity $\geq 99\%$. Specialty chemicals and building blocks obtained from several suppliers were of the highest offered purity (always $\geq 95\%$).

Reactions were monitored by TLC on Baker flexible-backed plates coated with 200 μm of silica gel containing a fluorescent indicator. Preparative TLC was performed on 20cm x 20cm Analtech Uniplates coated with a 1000 or 2000 μm silica gel layer containing a fluorescent (UV 254) indicator. Elution mixtures are reported as v:v. Spot visualization was achieved using UV light.

Microwave-assisted reactions were performed on a Biotage Initiator 2.5 at various powers.

Hydrogenation reactions were performed on a standard Parr hydrogenation apparatus.

Flash chromatography was performed on a Teledyne Isco CombiFlash RF 200 using appropriately sized Rediseq Rf Gold or Standard normal-phase silica or reversed-phase C-18 columns. Crude compounds were adsorbed on silica gel, 70-230 mesh 40 \AA (for normal phase) or celite (for reversed-phase) and loaded into solid cartridges. Elution mixtures are reported as v:v

Mass spectrometry was performed on a Waters Quattro-II triple quadrupole mass spectrometer. All samples were analyzed by positive ESI-MS and the mass-to-charge ratio (m/z) of the protonated molecular ion is reported.

NMR spectroscopy was performed on a Mercury 400 MHz equipped with a 5mm broadband probe using standard pulse sequences and operating at 400 MHz. Chemical shifts (δ) are reported in parts-per-million (ppm) relative to the residual solvent signals. Coupling constants (J-values) are expressed in Hz.

General Suzuki cross-coupling procedure (procedure A):

The desired aryl chloride (1.0 eq), arylboronic acid (1.05 eq) and triphenylphosphine (0.04 eq) were dissolved in a mixture of THF (final concentration of limiting reagent 0.1 mM) and 1 M aqueous sodium carbonate (2.0 eq) after which palladium(II) acetate (0.02 eq) was added and the solution was refluxed for 12 h. Following cooling, the solvent was removed in vacuo and the residue was dissolved in EtOAc and poured in water. The organic phase was separated, the aqueous phase was extracted with EtOAc, the combined organic phases were washed with water and brine then dried over Na₂SO₄ and concentrated. The crude material was purified by flash chromatography.

General Buchwald-Hartwig amination procedure (procedure B):

The desired aryl chloride (1.0 eq), aniline derivative (1.05 eq) and potassium carbonate (1.5 eq) were dissolved in *t*BuOH (concentration of limiting reagent 0.05 - 0.1 mM). The resulting mixture was thoroughly degassed after which Pd₂(dba)₃ (0.05 eq) and XantPhos (0.05 eq) were added and the solution was refluxed for 12 h. Following cooling, the reaction mixture was diluted with EtOAc and poured in water. The organic phase was separated, the aqueous phase was extracted with EtOAc, the combined organic phases were washed with water and brine then dried over Na₂SO₄ and concentrated. The crude material was purified by flash chromatography.

General procedure for the amination of 4-chloropyrimidines (procedure C):

The desired 4-chloropyrimidine (1.00 eq) and DIPEA (1.20 eq) were dissolved in IPA (concentration of limiting reagent 0.05 - 0.1 mM). The desired aniline (1.05 eq) was added and the resulting mixture was refluxed for 12h. Following cooling, the solvent was removed in vacuo, the residue was dissolved in EtOAc and poured in water. The organic phase was separated, the aqueous phase was extracted with EtOAc, the combined organic phases were washed with water and brine then dried over Na₂SO₄ and concentrated. The crude material was purified by flash chromatography.

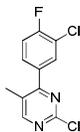
General chlorosulfonation procedure (procedure D):

The desired aryl compound (1.0 eq) was dissolved in chlorosulfonic acid (at least 10.0 eq. or the smallest volume necessary to ensure complete dissolution) and the resulting mixture was stirred for 4 h (room temperature or 90 °C) after which it was carefully poured on ice. The resulting solution was extracted twice with EtOAc, the combined organic phases were washed with water and brine then dried over Na₂SO₄ and concentrated. The crude material was purified by flash chromatography.

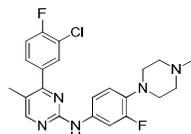
General sulfonamide synthesis (procedure E):

The desired sulfonyl chloride (1.00 eq) and DIPEA (2.50 eq) were dissolved in THF (concentration of limiting reagent 0.05 - 0.1 mM). The desired amine (1.05 eq) was added and the resulting mixture was stirred at room temperature for 2 h. Following removal of the solvent, the crude material was purified by flash chromatography.

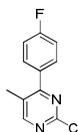
III. Experimental details and characterization for selected compounds



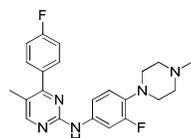
2-chloro-4-(3-chloro-4-fluorophenyl)-5-methylpyrimidine: prepared according to procedure A from 2,4-dichloro-5-methylpyrimidine and (3-chloro-4-fluorophenyl)boronic acid in 70% yield (white solid). Flash chromatography eluent: Hex/EtOAc 95/5 gradually increasing to 80/20. ¹H NMR (400 MHz, CDCl₃): δ 8.50 (s, 1H), 7.71 (dd, 1H, J = 2.2, 7.0 Hz), 7.52 (m, 1H), 7.26 (t, 2H), 2.38 (s, 3H). MS (ESI): 257.0 [M+H]⁺



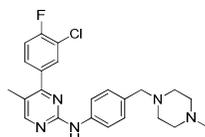
3: prepared according to procedure B from 2-chloro-4-(3-chloro-4-fluorophenyl)-5-methylpyrimidine and 3-fluoro-4-(4-methylpiperazin-1-yl)aniline in 73% yield (yellow solid). Flash chromatography eluent $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 99/1 gradually increasing to 95/5. ^1H NMR (400 MHz, CDCl_3): δ 8.30 (s, 1H), 7.69 (dd, 1H, $J = 6.8, 2.2$ Hz), 7.57 (dd, 1H, $J = 14.5$ Hz, 2.2 Hz), 7.50 (m, 1H), 7.13 (d, 1H, $J = 8.5$ Hz), 6.98 (s, 1H), 6.91 (t, 1H, $J = 9.1$ Hz), 3.06 (bs, 4H), 2.59 (bs, 4H), 2.34 (s, 3H), 2.24 (s, 3H). MS (ESI): 430.3 $[\text{M}+\text{H}]^+$, 215.7 $[\text{M}+2\text{H}]^{2+}$



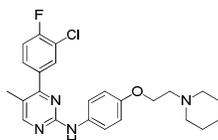
2-chloro-4-(4-fluorophenyl)-5-methylpyrimidine: prepared according to procedure A from 2,4-dichloro-5-methylpyrimidine and (4-fluorophenyl)boronic acid in 76% yield (white solid). Flash chromatography eluent: Hex/EtOAc 95/5 gradually increasing to 80/20. ^1H NMR (400 MHz, CDCl_3): δ 8.48 (s, 1H), 7.64 (m, 2H), 7.17 (m, 1H), 2.37 (s, 3H)



4: prepared according to procedure B from 2-chloro-4-(4-fluorophenyl)-5-methylpyrimidine and 3-fluoro-4-(4-methylpiperazin-1-yl)aniline in 73% yield (yellow solid). Flash chromatography eluent $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 99/1 gradually increasing to 95/5. ^1H NMR (400 MHz, CDCl_3): δ 8.28 (s, 1H), 7.60 (m, 3H), 7.15 (t, 3H, $J = 8.8$ Hz), 6.91 (t, 1H, $J = 9.1$ Hz), 3.06 (bs, 4H), 2.59 (bs, 4H), 2.34 (s, 3H), 2.24 (s, 3H). MS (ESI): 396.3 $[\text{M}+\text{H}]^+$, 198.7 $[\text{M}+2\text{H}]^{2+}$

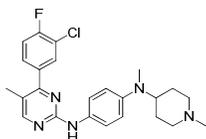


5: prepared according to procedure B from 2-chloro-4-(3-chloro-4-fluorophenyl)-5-methylpyrimidine and 4-((4-methylpiperazin-1-yl)methyl)aniline in 84% yield (yellow solid). Flash chromatography eluent $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 99/1 gradually increasing to 95/5. ^1H NMR (400 MHz, CDCl_3): δ 8.30 (s, 1H), 7.70 (m, 1H), 7.56 (d, 2H, $J = 8.6$ Hz), 7.51 (m, 1H), 7.25 (d, 2H), 7.05 (s, 1H), 3.46 (s, 2H), 2.46 (bs, 8H), 2.28 (s, 3H), 2.25 (s, 3H). MS (ESI): 426.3 $[\text{M}+\text{H}]^+$, 213.7 $[\text{M}+2\text{H}]^{2+}$

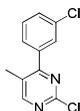


6: prepared according to procedure B from 2-chloro-4-(3-chloro-4-fluorophenyl)-5-methylpyrimidine and 4-(2-(piperidin-1-yl)ethoxy)aniline in 47% yield (yellow solid). Flash chromatography eluent $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 99/1 gradually increasing to 95/5. ^1H NMR (400 MHz, CDCl_3): δ 8.26 (s, 1H), 7.19 (dd, 1H, $J = 1.9, 7$ Hz),

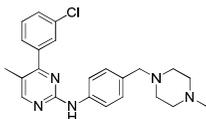
7.49 (m, 1H), 7.47 (d, 2H, J = 8.9 Hz), 7.21 (d, 1H, J = 8.5 Hz), 6.87 (d, 2H, 8.9 Hz), 4.13 (s, 2H), 2.83 (s, 2H), 2.57 (bs, 4H), 2.22 (s, 3H), 1.64 (bs, 4H), 1.45 (s, 2H). MS (ESI): 441.3 [M+H]⁺, 221.2 [M+2H]²⁺



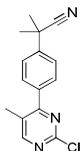
*N*¹-(4-(3-chloro-4-fluorophenyl)-5-methylpyrimidin-2-yl)-*N*⁴-methyl-*N*⁴-(1-methylpiperidin-4-yl)benzene-1,4-diamine **7**: prepared according to procedure B from 2-chloro-4-(3-chloro-4-fluorophenyl)-5-methylpyrimidine and *N*¹-methyl-*N*¹-(1-methylpiperidin-4-yl)benzene-1,4-diamine in 25% yield (yellow solid). Flash chromatography eluent CH₂Cl₂/MeOH 99/1 gradually increasing to 95/5. ¹H NMR (400 MHz, CDCl₃): δ 8.24 (s, 1H), 7.69 (dd, 1H, J = 7.2, 1.7 Hz), 7.49 (m, 1H), 7.41 (d, 2H, J = 8.9 Hz), 7.20 (d, 1H, 8.5 Hz), 6.79 (d, 2H, J = 8.5 Hz), 3.49 (m, 1H), 3.00 (d, 2H, J = 10.6 Hz), 2.74 (s, 3H), 2.34 (s, 3H), 2.21 (s, 3H), 2.11 (m, 2H), 1.89 (m, 2H), 1.72 (d, 2H, J = 11.6 Hz). MS (ESI): 440.3 [M+H]⁺, 220.8 [M+2H]²⁺



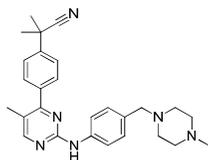
2-chloro-4-(3-chlorophenyl)-5-methylpyrimidine: prepared according to procedure A from 2,4-dichloro-5-methylpyrimidine and (3-chlorophenyl)boronic acid in 68% yield (white solid). Flash chromatography eluent: Hex/EtOAc 95/5 gradually increasing to 80/20. ¹H NMR (400 MHz, CDCl₃): δ 8.50 (s, 1H), 7.61 (m, 1H), 7.45 (m, 3H), 2.37 (s, 3H). MS (ESI): 239.0 [M+H]⁺



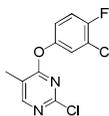
4-(3-chlorophenyl)-5-methyl-*N*-(4-((4-methylpiperazin-1-yl)methyl)phenyl)pyrimidin-2-amine **8**: prepared according to procedure B from 2-chloro-4-(3-chlorophenyl)-5-methylpyrimidine and 4-((4-methylpiperazin-1-yl)methyl)aniline in 77% yield (yellow solid). Flash chromatography eluent CH₂Cl₂/MeOH 99/1 gradually increasing to 95/5. ¹H NMR (400 MHz, CDCl₃): δ 8.31 (s, 1H), 7.61 (s, 1H), 7.56 (d, 2H, J = 8.2 Hz), 7.48 (m, 1H), 7.40 (m, 2H), 7.25 (d, 2H), 3.45 (s, 2H), 2.45 (bs, 8H), 2.27 (s, 3H), 2.24 (s, 3H). MS (ESI): 408.4 [M+H]⁺, 204.7 [M+2H]²⁺



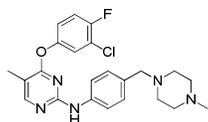
2-(4-(2-chloro-5-methylpyrimidin-4-yl)phenyl)-2-methylpropanenitrile: prepared according to procedure A from 2,4-dichloro-5-methylpyrimidine and (4-(2-cyanopropan-2-yl)phenyl)boronic acid in 80% yield (white solid). Flash chromatography eluent: Hex/EtOAc 95/5 gradually increasing to 80/20. ¹H NMR (400 MHz, CDCl₃): δ 8.49 (s, 1H), 7.65 (d, 2H, J = 8.8 Hz), 7.59 (d, 2H, J = 8.4 Hz), 2.38 (s, 3H), 1.75 (s, 6H). MS (ESI): 272.2 [M+H]⁺



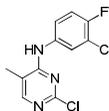
2-methyl-2-(4-(5-methyl-2-((4-((4-methylpiperazin-1-yl)methyl)phenyl)amino)pyrimidin-4-yl)phenyl)propanenitrile 9: prepared according to procedure B from 2-(4-(2-chloro-5-methylpyrimidin-4-yl)phenyl)-2-methylpropanenitrile and 4-((4-methylpiperazin-1-yl)methyl)aniline in 39% yield (yellow solid). Flash chromatography eluent $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 99/1 gradually increasing to 95/5. $^1\text{H NMR}$ (400 MHz, CDCl_3): δ 8.30 (s, 1H), 7.66 (d, 2H, $J = 8.6$ Hz), 7.58 (d, 2H, $J = 4.7$ Hz), 7.56 (d, 2H, $J = 4.7$ Hz), 7.25 (d, 2H), 3.44 (s, 2H), 2.42 (bs, 8H), 2.26 (s, 6H), 1.76 (s, 6H). MS (ESI): 441.4 $[\text{M}+\text{H}]^+$, 221.4 $[\text{M}+2\text{H}]^{2+}$



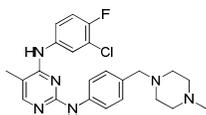
2-chloro-4-(3-chloro-4-fluorophenoxy)-5-methylpyrimidine potassium carbonate (424 mg, 3.07 mmol) was added to a solution of 2,4-dichloro-5-methylpyrimidine (250 mg, 1.534 mmol) and 3-chloro-4-fluorophenol (247 mg, 1.687 mmol) in acetone (Volume: 10 ml) and the reaction mixture was refluxed for 12 h. Following cooling, the solvent was removed in vacuo and the residue was dissolved in EtOAc (20 mL) and poured in water (20 mL). The organic phase was separated, the aqueous phase was extracted with EtOAc (20 ml), the combined organic phases were washed with water (40 mL) and brine (40 mL) then dried over Na_2SO_4 and concentrated. The crude material was purified by flash chromatography eluting with Hex/EtOAc 98/2 gradually increasing to 90/10 to yield 2-chloro-4-(3-chloro-4-fluorophenoxy)-5-methylpyrimidine as a white solid (472 mg, 1.728 mmol, 100% yield). $^1\text{H NMR}$ (400 MHz, CDCl_3): δ 8.30 (s, 1H), 7.45 (t, 1H, $J = 8.4$ Hz), 7.03 (dd, 1H, $J = 9.4, 2.6$ Hz), 6.94 (d, 1H, $J = 8.8$ Hz), 2.29 (s, 3H). MS (ESI): 272.9 $[\text{M}+\text{H}]^+$



4-(3-chloro-4-fluorophenoxy)-5-methyl-N-(4-((4-methylpiperazin-1-yl)methyl)phenyl)pyrimidin-2-amine 10: prepared according to procedure B from 2-chloro-4-(3-chloro-4-fluorophenoxy)-5-methylpyrimidine and 4-((4-methylpiperazin-1-yl)methyl)aniline in 59% yield (yellow solid). Flash chromatography eluent $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 99/1 gradually increasing to 95/5. $^1\text{H NMR}$ (400 MHz, CDCl_3): δ H 8.10 (s, 1H), 7.43 (t, 1H, $J = 8.4$ Hz), 7.23 (d, 2H), 7.09 (d, 2H, $J = 8.2$ Hz), 7.04 (dd, 1H, $J = 9.6, 2.4$ Hz), 6.94 (m, 1H), 3.42 (s, 2H), 2.46 (bs, 8H) 2.27 (s, 3H), 2.18 (s, 3H). MS (ESI): 442.3 $[\text{M}+\text{H}]^+$, 221.7 $[\text{M}+2\text{H}]^{2+}$



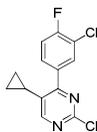
2-chloro-N-(3-chloro-4-fluorophenyl)-5-methylpyrimidin-4-amine: concentrated HCl (0.05 ml, 1.646 mmol) was added to mixture of 2,4-dichloro-5-methylpyrimidine (100 mg, 0.613 mmol), 3-chloro-4-fluoroaniline (94 mg, 0.644 mmol), DIPEA (2 ml, 11.45 mmol) and DMAP (8 mg, 0.065 mmol) in DMA (Volume: 5 ml). The resulting mixture was heated to 140 °C overnight, after which it was allowed to cool to room temperature and partitioned between water (30 mL) and EtOAc (30 mL). The organic phase was separated, the aqueous phase acidified to pH 6 with dilute HCl and extracted with EtOAc (30 mL). The combined organic phases were washed with water (60 mL) and brine (60 mL), dried over Na_2SO_4 then filtered and evaporated. The resulting crude yellow oil was purified by flash chromatography eluting with Hex/EtOAc 90/10 gradually increasing to 70/30 to yield 2-chloro-N-(3-chloro-4-fluorophenyl)-5-methylpyrimidin-4-amine as a white oil that solidified on standing (73 mg, 0.268 mmol, 44% yield). MS (ESI): 272.0 $[\text{M}+\text{H}]^+$



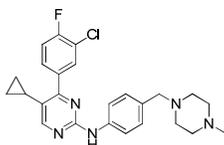
*N*⁴-(3-chloro-4-fluorophenyl)-5-methyl-*N*²-(4-((4-methylpiperazin-1-yl)methyl)phenyl)pyrimidine-2,4-diamine **11**: prepared according to procedure B from 2-chloro-*N*-(3-chloro-4-fluorophenyl)-5-methylpyrimidin-4-amine and 4-((4-methylpiperazin-1-yl)methyl)aniline in 78% yield (yellow solid). Flash chromatography eluent CH₂Cl₂/MeOH 99/1 gradually increasing to 95/5. MS (ESI): 440.4 [M+H]⁺



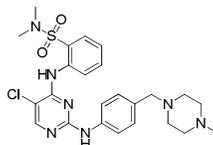
2,4-dichloro-5-cyclopropylpyrimidine: DMF (0.575 ml, 7.43 mmol) was added dropwise to a solution of oxalyl dichloride (0.638 ml, 7.43 mmol) in 1,2-dichloroethane (Volume: 20 ml) and the resulting white suspension was stirred for 10 min at room temperature. After all gas evolution had stopped 5-cyclopropyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one (250 mg, 1.486 mmol) was added and the reaction mixture was refluxed for 12 h after which it was allowed to cool to room temperature, poured in chloroform (20 mL), washed with water (2 x 40 mL) then brine (40 mL), dried over Na₂SO₄ and concentrated. The crude material was purified by flash chromatography eluting with Hex/EtOAc 100/0 gradually increasing to 90/10 to yield 2,4-dichloro-5-cyclopropylpyrimidine (258 mg, 1.365 mmol, 92% yield) as a pale yellow oil. ¹H NMR (400 MHz, CDCl₃): δ 8.16 (s, 1H), 2.03 (m, 1H), 1.14 (m, 2H), 0.78 (m, 2H). MS (ESI): 189.0 [M+H]⁺



2-chloro-4-(3-chloro-4-fluorophenyl)-5-cyclopropylpyrimidine: prepared according to procedure A from 2,4-dichloro-5-cyclopropylpyrimidine and (3-chloro-4-fluorophenyl)boronic acid in 37% yield (pale yellow oil). Flash chromatography eluent: Hex/EtOAc 95/5 gradually increasing to 80/20. No characterization was performed.

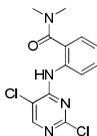


4-(3-chloro-4-fluorophenyl)-5-cyclopropyl-*N*-(4-((4-methylpiperazin-1-yl)methyl)phenyl)pyrimidin-2-amine **12**: prepared according to procedure B from 2-chloro-4-(3-chloro-4-fluorophenyl)-5-cyclopropylpyrimidine and 4-((4-methylpiperazin-1-yl)methyl)aniline in 51% yield (yellow solid). Flash chromatography eluent CH₂Cl₂/MeOH 99/1 gradually increasing to 95/5. ¹H NMR (400 MHz, CDCl₃): δ 8.22 (s, 1H), 7.90 (dd, 1H, J = 7.0, 2.0 Hz), 7.72 (m, 1H), 7.55 (d, 2H, J = 8.6 Hz), 7.22 (d, 1H, J = 8.6 Hz), 7.09 (d, 2H, J = 8.6 Hz), 3.45 (s, 2H), 2.43 (bs, 8H), 2.26 (s, 3H), 1.86 (m, 1H), 0.92 (m, 2H), 0.63 (m, 2H). MS (ESI): 452.3 [M+H]⁺, 226.9 [M+2H]²⁺

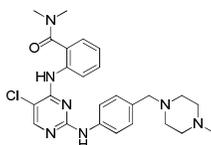


2-((5-chloro-2-((4-((4-methylpiperazin-1-yl)methyl)phenyl)amino)pyrimidin-4-yl)amino)-*N,N*-dimethylbenzenesulfonamide **13**: prepared according to procedure B from 2-((2,5-dichloropyrimidin-4-yl)amino)-*N,N*-dimethylbenzenesulfonamide and 4-((4-methylpiperazin-1-yl)methyl)aniline in 62% yield

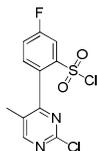
(yellow solid). Flash chromatography eluent CH₂Cl₂/MeOH 99/1 gradually increasing to 95/5. ¹H NMR (400 MHz, CDCl₃): δ 8.53 (d, 1H, J = 8.2 Hz), 8.11 (s, 1H), 7.84 (dd, 1H, J = 8.2, 1.3 Hz), 7.54 (m, 1H), 7.45 (d, 2H, J = 8.2 Hz), 7.22 (m, 3H), 3.47 (s, 2H), 2.72 (s, 6H), 2.48 (bs, 8H), 2.29 (s, 3H). ¹³C NMR (100 MHz, DMSO-d₆): 157.7, 155.2, 154.9, 138.9, 136.7, 133.9, 131.6, 130.1, 129.0, 129.0, 124.4, 124.0, 123.7, 119.5, 119.5, 104.4, 61.7, 54.7, 54.7, 52.4, 52.4, 45.7, 37.2, 37.2. HRMS (ESI⁺) calculated for [M+H]⁺: 516.1942982; found m/z: 516.19471.



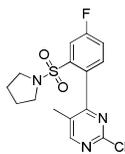
2-((2,5-dichloropyrimidin-4-yl)amino)-N,N-dimethylbenzamide: prepared according to procedure C from 2,4,5-trichloropyrimidine and 2-amino-N,N-dimethylbenzamide in 82% yield (white solid). Flash chromatography eluent CH₂Cl₂/MeOH 100/0 gradually increasing to 96/4. ¹H NMR (400 MHz, CDCl₃): δ 9.62 (s, 1H), 8.30 (d, 1H, J = 8.2 Hz), 8.14 (s, 1H), 7.43 (dt, 1H, J = 7.9, 1.0 Hz), 7.28 (dd, 1H, J = 7.7, 1.2 Hz), 7.11 (t, 1H, J = 7.5 Hz), 3.08 (s, 3H), 3.04 (s, 3H). MS (ESI): 311.0 [M+H]⁺



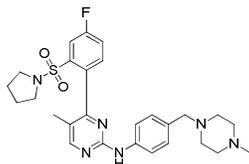
2-((5-chloro-2-((4-((4-methylpiperazin-1-yl)methyl)phenyl)amino)pyrimidin-4-yl)amino)-N,N-dimethylbenzamide 14: prepared according to procedure B from 2-((2,5-dichloropyrimidin-4-yl)amino)-N,N-dimethylbenzamide and 4-((4-methylpiperazin-1-yl)methyl)aniline in 32% yield (yellow solid). Flash chromatography eluent CH₂Cl₂/MeOH 99/1 gradually increasing to 95/5. ¹H NMR (400 MHz, CDCl₃): δ 9.07 (s, 1H), 8.35 (d, 1H, J = 8.2 Hz), 8.05 (s, 1H), 7.46 (s, 2H, J = 8.2 Hz), 7.38 (t, 1H, J = 7.8 Hz), 7.29 (d, 1H, J = 7.5 Hz), 7.21 (d, 2H, J = 8.2 Hz), 7.10 (t, 1H, J = 7.5 Hz), 7.01 (s, 1H), 3.45 (s, 2H), 3.11 (s, 3H), 3.04 (s, 3H), 2.43 (bs, 8H), 2.27 (s, 3H). MS (ESI): 480.1 [M+H]⁺



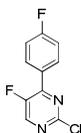
2-(2-chloro-5-methylpyrimidin-4-yl)-5-fluorobenzene-1-sulfonyl chloride: prepared according to procedure D from 2-chloro-4-(4-fluorophenyl)-5-methylpyrimidine in 32% yield (white solid). Flash chromatography eluent Hex/EtOAc 98/2 gradually increasing to 80/20. ¹H NMR (400 MHz, CDCl₃): δ 8.57 (s, 1H), 8.26 (dd, 1H, J = 6.4, 2.4 Hz), 8.07 (m, 1H), 7.48 (t, 1H, J = 9.0 Hz), 2.42 (s, 3H). MS (ESI): m/z 321.0 [M+H]⁺



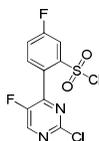
2-chloro-4-(4-fluoro-2-(pyrrolidin-1-ylsulfonyl)phenyl)-5-methylpyrimidine: prepared according to procedure E from 2-(2-chloro-5-methylpyrimidin-4-yl)-5-fluorobenzene-1-sulfonyl chloride and pyrrolidine in 48% yield (white solid). Flash chromatography eluent Hex/EtOAc 95/5 gradually increasing to 70/30. ¹H NMR (400 MHz, CDCl₃): δ 8.52 (s, 1H), 8.15 (dd, 1H, J = 6.2, 2.2 Hz), 7.88 (m, 1H), 7.34 (t, 1H, J = 9.2 Hz), 3.37 (m, 4H), 2.38 (s, 3H), 1.85 (m, 4H). MS (ESI): m/z 356.0 [M+H]⁺



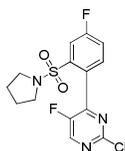
4-(4-fluoro-2-(pyrrolidin-1-ylsulfonyl)phenyl)-5-methyl-N-(4-((4-methylpiperazin-1-yl)methyl)phenyl)pyrimidin-2-amine 15: prepared according to procedure B from 2-chloro-4-(4-fluoro-2-(pyrrolidin-1-ylsulfonyl)phenyl)-5-methylpyrimidine and 4-((4-methylpiperazin-1-yl)methyl)aniline in 60% yield (yellow solid). Flash chromatography eluent CH₂Cl₂/MeOH 99/1 gradually increasing to 95/5. ¹H NMR (400 MHz, CDCl₃): δ 8.32 (s, 1H), 8.21 (dd, 1H, J = 6.6, 2.2 Hz), 7.87 (m, 1H), 7.55 (d, 2H, J = 8.5 Hz), 7.32 (t, 1H, J = 9.0 Hz), 7.25 (d, 2H), 7.10 (bs, 1H), 3.45 (s, 2H), 3.39 (m, 4H), 2.46 (bs, 8H), 2.27 (s, 3H), 1.86 (m, 4H). MS (ESI): m/z 525.2 [M+H]⁺



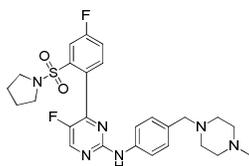
2-chloro-5-fluoro-4-(4-fluorophenyl)pyrimidine: prepared according to procedure A from 2,4-dichloro-5-fluoropyrimidine and (4-fluorophenyl)boronic acid in 100% yield (white solid). Flash chromatography eluent: Hex/EtOAc 100/0 gradually increasing to 80/20. ¹H NMR (400 MHz, CDCl₃): δ 8.50 (d, 1H, J = 2.9 Hz), 8.17 (m, 2H), 7.20 (t, 2H, J = 8.4 Hz). MS (ESI): m/z 227.0 [M+H]⁺



2-(2-chloro-5-fluoropyrimidin-4-yl)-5-fluorobenzene-1-sulfonyl chloride: prepared according to procedure D from 2-chloro-5-fluoro-4-(4-fluorophenyl)pyrimidine in 72% yield (yellow oil). Flash chromatography eluent Hex/EtOAc 98/2 gradually increasing to 80/20. ¹H NMR (400 MHz, CDCl₃): δ 8.78 (dd, 1H, J = 6.6, 2.2 Hz), 8.62 (d, 1H, J = 2.9 Hz), 8.58 (m, 1H), 7.51 (t, 1H, J = 9.0 Hz). MS (ESI): m/z 325.0 [M+H]⁺

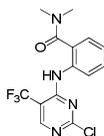


2-chloro-5-fluoro-4-(4-fluoro-2-(pyrrolidin-1-ylsulfonyl)phenyl)pyrimidine: prepared according to procedure E from 2-(2-chloro-5-fluoropyrimidin-4-yl)-5-fluorobenzene-1-sulfonyl chloride and pyrrolidine in 95% yield (pale yellow solid). Flash chromatography eluent Hex/EtOAc 95/5 gradually increasing to 70/30. ¹H NMR (400 MHz, CDCl₃): δ 8.66 (dd, 1H, J = 6.6, 2.2 Hz), 8.55 (d, 1H, J = 2.9 Hz), 8.35 (m, 1H), 7.36 (t, 1H, J = 9.2 Hz), 3.38 (m, 4H), 1.86 (m, 4H). MS (ESI): m/z 360.0 [M+H]⁺

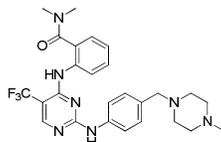


5-fluoro-4-(4-fluoro-2-(pyrrolidin-1-ylsulfonyl)phenyl)-N-(4-((4-methylpiperazin-1-yl)methyl)phenyl)pyrimidin-2-amine 16: prepared according to procedure B from 2-chloro-5-fluoro-4-(4-fluoro-2-(pyrrolidin-1-ylsulfonyl)phenyl)pyrimidine and 4-((4-methylpiperazin-1-yl)methyl)aniline in 60% yield (yellow solid). Flash chromatography eluent CH₂Cl₂/MeOH 99/1 gradually increasing to 95/5. ¹H NMR (400 MHz, CDCl₃): δ 8.65 (dd, 1H, J = 6.8, 2.0 Hz), 8.30 (d, 1H, J = 3.3 Hz), 8.24 (m, 1H), 7.50 (d,

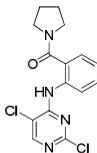
2H, J = 8.4 Hz), 7.30-7.19 (m, 4H), 3.43 (s, 2H), 3.34 (m, 4H), 2.46 (bs, 8H), 2.26 (s, 1H), 1.79 (m, 4H). MS (ESI): m/z 529.2 [M+H]⁺



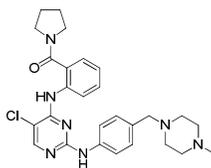
2-((2-chloro-5-(trifluoromethyl)pyrimidin-4-yl)amino)-N,N-dimethylbenzamide: prepared according to procedure C from 2,4-dichloro-5-(trifluoromethyl)pyrimidine and 2-amino-N,N-dimethylbenzamide in 84% yield (white solid). Flash chromatography eluent CH₂Cl₂/MeOH 100/0 gradually increasing to 96/4. ¹H NMR (400 MHz, CDCl₃): δ 9.52 (s, 1H), 8.40 (s, 1H), 8.20 (d, 1H, J = 8.2 Hz), 7.45 (dt, 1H, J = 8.0, 1.0 Hz), 7.30 (dd, 1H, J = 7.5, 1.0 Hz), 7.17 (t, 1H, J = 7.5 Hz), 3.07 (s, 6H). MS (ESI): 345.1 [M+H]⁺



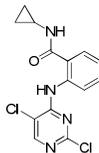
N,N-dimethyl-2-((2-((4-(4-methylpiperazin-1-yl)methyl)phenyl)amino)-5-(trifluoromethyl)pyrimidin-4-yl)amino)benzamide 17: prepared according to procedure B from 2-((2-chloro-5-(trifluoromethyl)pyrimidin-4-yl)amino)-N,N-dimethylbenzamide and 4-((4-methylpiperazin-1-yl)methyl)aniline in 12% yield (pale yellow oil that solidifies on standing). Flash chromatography eluent CH₂Cl₂/MeOH 99/1 gradually increasing to 95/5. ¹H NMR (400 MHz, CDCl₃): δ 8.46 (s, 1H), 8.28 (s, 1H), 8.15 (d, 1H, J = 8.2 Hz), 7.44 (d, 2H, J = 8.5 Hz), 7.30 (d, 2H, J = 8.2 Hz), 7.21 (t, 1H, J = 7.5 Hz), 7.02 (t, 1H, J = 7.4 Hz), 6.81 (s, 1H), 3.50 (s, 2H), 3.09 (s, 3H), 2.96 (s, 3H), 2.49 (bs, 8H), 2.30 (s, 3H). MS (ESI): 514.2 [M+H]⁺



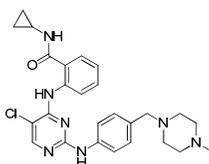
(2-((2,5-dichloropyrimidin-4-yl)amino)phenyl)(pyrrolidin-1-yl)methanone: prepared according to procedure C from 2,4,5-trichloropyrimidine and (2-aminophenyl)(pyrrolidin-1-yl)methanone in 47% yield (white solid). Flash chromatography eluent CH₂Cl₂/MeOH 100/0 gradually increasing to 96/4. ¹H NMR (400 MHz, CDCl₃): δ 8.35 (d, 1H, J = 8.5 Hz), 8.12 (s, 1H), 7.41 (m, 2H), 7.09 (dt, 1H, J = 7.5, 1.0 Hz), 3.60 (t, 2H, J = 7.0 Hz), 3.47 (t, 2H, J 6.5 Hz), 1.96-1.77 (m, 4H) MS (ESI): m/z 337.0 [M+H]⁺



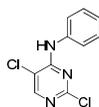
(2-((5-chloro-2-((4-(4-methylpiperazin-1-yl)methyl)phenyl)amino)pyrimidin-4-yl)amino)phenyl)(pyrrolidin-1-yl)methanone 18: prepared according to procedure B from (2-((2,5-dichloropyrimidin-4-yl)amino)phenyl)(pyrrolidin-1-yl)methanone and 4-((4-methylpiperazin-1-yl)methyl)aniline in 73% yield (yellow solid). Flash chromatography eluent CH₂Cl₂/MeOH 99/1 gradually increasing to 95/5. ¹H NMR (400 MHz, CDCl₃): δ 9.68 (s, 1H), 8.40 (d, 1H, J = 8.2 Hz), 8.04 (s, 1H), 7.46 (d, 2H, J = 8.2 Hz), 7.39-7.34 (m, 2H), 7.21 (d, 2H, J = 8.2 Hz), 7.06 (t, 1H, J = 7.5 Hz), 3.65 (t, 2H, J = 7.0 Hz), 3.48 (t, 2H, J = 6.3 Hz), 3.44 (s, 2H), 2.44 (bs, 8H), 2.26 (s, 3H), 1.95 (m, 2H), 1.83 (m, 2H). MS (ESI): 506.1 [M+H]⁺



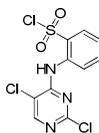
N-cyclopropyl-2-((2,5-dichloropyrimidin-4-yl)amino)benzamide: prepared according to procedure C from 2,4,5-trichloropyrimidine and 2-amino-N-cyclopropylbenzamide in 66% yield (white solid). Flash chromatography eluent CH₂Cl₂/MeOH 100/0 gradually increasing to 96/4. ¹H NMR (400 MHz, CDCl₃): δ 8.68 (d, 1H, J = 8.6 Hz), 8.20 (s, 1H), 7.53 (t, 1H, J = 7.9 Hz), 7.42 (d, 1H, J = 7.9 Hz), 7.09 (t, 1H, J = 7.5 Hz), 6.39 (bs, 1H), 2.89 (m, 1H), 0.90 (m, 2H), 0.63 (m, 2H). MS (ESI): m/z 323.0 [M+H]⁺



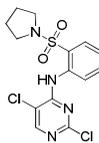
(2-((5-chloro-2-((4-((4-methylpiperazin-1-yl)methyl)phenyl)amino)pyrimidin-4-yl)amino)-N-cyclopropylbenzamide 19: prepared according to procedure B from *N-cyclopropyl-2-((2,5-dichloropyrimidin-4-yl)amino)benzamide* and 4-((4-methylpiperazin-1-yl)methyl)aniline in 38% yield (yellow solid). Flash chromatography eluent CH₂Cl₂/MeOH 99/1 gradually increasing to 95/5. ¹H NMR (400 MHz, CDCl₃): δ 8.64 (d, 1H, J = 8.5 Hz), 8.11 (s, 1H), 7.50 (d, 2H, J = 8.2 Hz), 7.44 (m, 2H), 7.25 (d, 2H, J = 8.2 Hz), 7.07 (t, 1H, J = 7.5 Hz), 6.38 (bs, 1H), 3.50 (s, 2H), 2.93 (qu, 1H, J = 3.4 Hz), 2.55 (bs, 8H), 2.35 (s, 3H), 0.92 (m, 2H), 0.65 (m, 2H). MS (ESI): m/z 492.1 [M+H]⁺



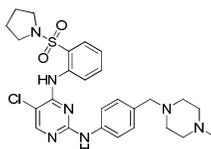
2,5-dichloro-N-phenylpyrimidin-4-amine: prepared according to procedure C from 2,4,5-trichloropyrimidine and aniline in 92% yield (white solid). Flash chromatography eluent Hex/EtOAc 98/2 gradually increasing to 80/20. ¹H NMR (400 MHz, CDCl₃): δ 8.18 (s, 1H), 7.61 (d, 2H, J = 7.9 Hz), 7.39 (t, 2H, J = 8.0 Hz), 7.23 (bs, 1H), 7.18 (t, 1H, J = 7.4 Hz). MS (ESI): m/z 239.9 [M+H]⁺



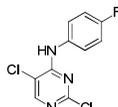
2-((2,5-dichloropyrimidin-4-yl)amino)benzene-1-sulfonyl chloride: prepared according to procedure D from 2,5-dichloro-N-phenylpyrimidin-4-amine in 6% yield (white solid). Flash chromatography eluent Hex/EtOAc 98/2 gradually increasing to 80/20. ¹H NMR (400 MHz, CDCl₃): δ 9.27 (s, 1H), 8.68 (d, 1H, J = 8.5 Hz), 8.35 (s, 1H), 8.06 (d, 1H, J = 8.2 Hz), 7.81 (t, 1H, J = 7.7 Hz), 7.34 (t, 1H, J = 7.7 Hz). MS (ESI): m/z 337.9 [M+H]⁺



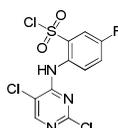
2,5-dichloro-N-(2-(pyrrolidin-1-ylsulfonyl)phenyl)pyrimidin-4-amine: prepared according to procedure E from 2-((2,5-dichloropyrimidin-4-yl)amino)benzene-1-sulfonyl chloride and pyrrolidine in 85% yield (white solid). Flash chromatography eluent Hex/EtOAc 95/5 gradually increasing to 70/30. ¹H NMR (400 MHz, CDCl₃): δ 9.96 (s, 1H), 8.59 (d, 1H, J = 8.6 Hz), 8.25 (s, 1H), 7.89 (dd, 1H, J = 8.0, 1.5 Hz), 7.63 (td, 1H, J = 8.0, 1.7 Hz), 7.25 (td, 1H, J = 7.2, 1.0 Hz), 3.21 (m, 4H), 1.79 (m, 4H). MS (ESI): m/z 373.1 [M+H]⁺



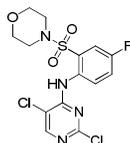
5-chloro-N2-(4-((4-methylpiperazin-1-yl)methyl)phenyl)-N4-(2-(pyrrolidin-1-ylsulfonyl)phenyl)pyrimidine-2,4-diamine 20: prepared according to procedure B from 2,5-dichloro-N-(2-(pyrrolidin-1-ylsulfonyl)phenyl)pyrimidin-4-amine and 4-((4-methylpiperazin-1-yl)methyl)aniline in 50% yield (yellow solid). Flash chromatography eluent CH₂Cl₂/MeOH 99/1 gradually increasing to 95/5. ¹H NMR (400 MHz, CDCl₃): δ 9.58 (s, 1H), 8.61 (d, 1H, J = 8.2 Hz), 8.18 (s, 1H), 7.96 (d, 1H, J = 7.9 Hz), 7.58 (t, 1H, J = 7.7 Hz), 7.51 (d, 2H, J = 8.2 Hz), 7.30 (d, 2H, J = 8.2 Hz), 7.26 (m, 1H), 3.53 (s, 2H), 3.30 (m, 4H), 2.53 (bs, 8H), 2.34 (s, 3H), 1.84 (m, 4H). MS (ESI): m/z 542.20 [M+H]⁺



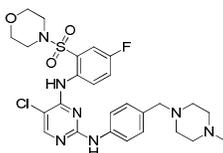
2,5-dichloro-N-(4-fluorophenyl)pyrimidin-4-amine: prepared according to procedure C from 2,4,5-trichloropyrimidine and 4-fluoroaniline in 100% yield (white solid). Flash chromatography eluent Hex/EtOAc 98/2 gradually increasing to 80/20. ¹H NMR (400 MHz, CDCl₃): δ 8.18 (s, 1H), 7.55 (m, 2H), 7.17 (bs, 1H), 7.09 (t, 2H, J = 8.5 Hz). MS (ESI): m/z 258.0 [M+H]⁺



2-((2,5-dichloropyrimidin-4-yl)amino)-5-fluorobenzene-1-sulfonyl chloride: prepared according to procedure D from 2,5-dichloro-N-(4-fluorophenyl)pyrimidin-4-amine in 64% yield (pale yellow solid). Flash chromatography eluent Hex/EtOAc 98/2 gradually increasing to 80/20. ¹H NMR (400 MHz, CDCl₃): δ 8.61 (qu, 1H, J = 4.6 Hz), 8.32 (s, 1H), 7.76 (dd, 1H, J = 7.2, 3.1 Hz), 7.52 (td, 1H, J = 8.3, 3.1 Hz). MS (ESI): m/z 358.0 [M+H]⁺

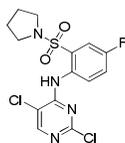


2,5-dichloro-N-(4-fluoro-2-(morpholinosulfonyl)phenyl)pyrimidin-4-amine: prepared according to procedure E from 2-((2,5-dichloropyrimidin-4-yl)amino)-5-fluorobenzene-1-sulfonyl chloride and morpholine in 93% yield (pale yellow solid). Flash chromatography eluent Hex/EtOAc 95/5 gradually increasing to 70/30. ¹H NMR (400 MHz, CDCl₃): δ 9.54 (s, 1H), 8.57 (dd, 1H, J = 9.2, 4.8 Hz), 8.27 (s, 1H), 7.54 (dd, 1H, J = 7.9, 3.1 Hz), 7.39 (m, 1H), 3.67 (m, 4H), 3.05 (m, 4H). MS (ESI): m/z 407.0 [M+H]⁺

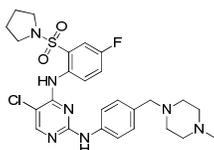


5-chloro-N4-(4-fluoro-2-(morpholinosulfonyl)phenyl)-N2-(4-((4-methylpiperazin-1-yl)methyl)phenyl)pyrimidine-2,4-diamine 21: prepared according to procedure B from 2,5-dichloro-N-(4-fluoro-2-(morpholinosulfonyl)phenyl)pyrimidin-4-amine and 4-((4-methylpiperazin-1-yl)methyl)aniline in 62% yield (yellow solid). Flash chromatography eluent CH₂Cl₂/MeOH 99/1 gradually increasing to 95/5. ¹H NMR (400 MHz, CDCl₃): δ 9.12 (s, 1H), 8.49 (dd, 1H, J = 4.8, 9.2 Hz), 8.10 (s, 1H), 7.54 (dd, 1H, J =

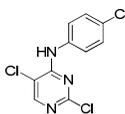
3.1, 7.9 Hz), 7.40 (d, 2H, J = 8.2 Hz), 7.27-7.20 (m, 3H), 3.64 (m, 4H), 3.47 (s, 2H), 3.06 (m, 4H), 2.47 (bs, 8H), 2.28 (s, 3H). MS (ESI): m/z 576.2 [M+H]⁺



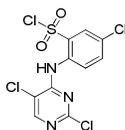
2,5-dichloro-N-(4-fluoro-2-(pyrrolidin-1-ylsulfonyl)phenyl)pyrimidin-4-amine: prepared according to procedure E from 2-((2,5-dichloropyrimidin-4-yl)amino)-5-fluorobenzene-1-sulfonyl chloride and pyrrolidine in 72% yield (white solid). Flash chromatography eluent Hex/EtOAc 95/5 gradually increasing to 70/30. ¹H NMR (400 MHz, CDCl₃): δ 9.74 (s, 1H), 8.54 (dd, 1H, J = 9.2, 4.8 Hz), 8.25 (s, 1H), 7.61 (dd, 1H, J = 8.2, 3.1 Hz), 7.34 (m, 1H), 3.22 (m, 4H), 1.81 (m, 4H).



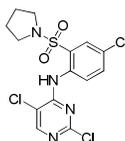
5-chloro-N4-(4-fluoro-2-(pyrrolidin-1-ylsulfonyl)phenyl)-N2-(4-((4-methylpiperazin-1-yl)methyl)phenyl)pyrimidine-2,4-diamine 22: prepared according to procedure B from 2,5-dichloro-N-(4-fluoro-2-(pyrrolidin-1-ylsulfonyl)phenyl)pyrimidin-4-amine and 4-((4-methylpiperazin-1-yl)methyl)aniline in 78% yield (yellow solid). Flash chromatography eluent CH₂Cl₂/MeOH 99/1 gradually increasing to 95/5. ¹H NMR (400 MHz, CDCl₃): δ 9.30 (s, 1H), 8.49 (dd, 1H, J = 9.2, 4.8 Hz), 7.60 (dd, 1H, J = 8.0, 2.9 Hz), 7.41 (d, 2H, J = 8.2 Hz), 7.23 (d, 2H, J = 8.2 Hz), 7.19 (m, 1H), 3.48 (s, 2H), 3.23 (m, 4H), 2.50 (bs, 8H), 2.31 (s, 3H), 1.78 (m, 4H). MS (ESI): m/z 560.3 [M+H]⁺



2,5-dichloro-N-(4-chlorophenyl)pyrimidin-4-amine: prepared according to procedure C from 2,4,5-trichloropyrimidine and 4-chloroaniline in 99% yield (white solid). Flash chromatography eluent Hex/EtOAc 98/2 gradually increasing to 80/20. ¹H NMR (400 MHz, CDCl₃): δ 8.20 (s, 1H), 7.56 (d, 2H, J = 8.5 Hz), 7.35 (d, 2H, J = 8.5 Hz), 7.20 (bs, 1H). MS (ESI): m/z 274.0 [M+H]⁺

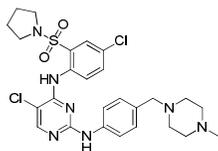


5-chloro-2-((2,5-dichloropyrimidin-4-yl)amino)benzene-1-sulfonyl chloride: prepared according to procedure D from 2,5-dichloro-N-(4-chlorophenyl)pyrimidin-4-amine in 77% yield (pale yellow solid). Flash chromatography eluent Hex/EtOAc 98/2 gradually increasing to 80/20. ¹H NMR (400 MHz, CDCl₃): δ 9.29 (s, 1H), 8.67 (d, 1H, J = 9.2 Hz), 8.36 (s, 1H), 8.03 (d, 1H, J = 2.7 Hz), 7.55 (dd, 1H, J = 9.1, 2.6 Hz). MS (ESI): m/z 373.8 [M+H]⁺

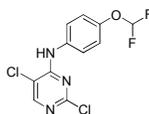


2,5-dichloro-N-(4-chloro-2-(pyrrolidin-1-ylsulfonyl)phenyl)pyrimidin-4-amine: prepared according to procedure E from 5-chloro-2-((2,5-dichloropyrimidin-4-yl)amino)benzene-1-sulfonyl chloride and pyrrolidine in 60% yield (pale yellow solid). Flash chromatography eluent Hex/EtOAc 95/5 gradually

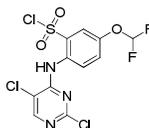
increasing to 70/30. $^1\text{H NMR}$ (400 MHz, CDCl_3): δ 9.87 (s, 1H), 8.56 (d, 1H, $J = 8.9$ Hz), 8.26 (s, 1H), 7.85 (d, 1H, $J = 2.4$ Hz), 7.57 (dd, 1H, $J = 8.9, 2.7$ Hz), 3.22 (m, 4H), 1.81 (m, 4H). MS (ESI): m/z 407.0 $[\text{M}+\text{H}]^+$



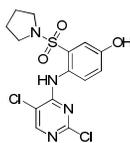
5-chloro-N4-(4-chloro-2-(pyrrolidin-1-ylsulfonyl)phenyl)phenyl)-N2-((4-methylpiperazin-1-yl)methyl)phenyl)pyrimidine-2,4-diamine 23: prepared according to procedure B from 2,5-dichloro-N-(4-chloro-2-(pyrrolidin-1-ylsulfonyl)phenyl)pyrimidin-4-amine and 4-((4-methylpiperazin-1-yl)methyl)aniline in 26% yield (yellow solid). Flash chromatography eluent $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 99/1 gradually increasing to 95/5. $^1\text{H NMR}$ (400 MHz, CDCl_3): δ 8.46 (dd, 1H, $J = 6.5, 2.1$ Hz), 8.42 (s, 1H), 8.10 (m, 1H), 7.52 (d, 2H, $J = 8.2$ Hz), 7.31 (t, 1H, $J = 9.2$ Hz), 7.26 (d, 2H, $J = 8.2$ Hz), 3.45 (s, 2H), 3.39 (m, 4H), 2.46 (bs, 8H), 2.27 (s, 3H), 1.85 (m, 4H). MS (ESI): m/z 576.2 $[\text{M}+\text{H}]^+$



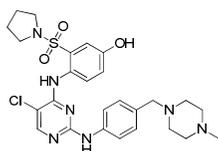
2,5-dichloro-N-(4-(difluoromethoxy)phenyl)pyrimidin-4-amine: prepared according to procedure C from 2,4,5-trichloropyrimidine and 4-(difluoromethoxy)aniline in 100% yield (pale yellow solid). Flash chromatography eluent Hex/EtOAc 98/2 gradually increasing to 80/20. $^1\text{H NMR}$ (400 MHz, CDCl_3): δ 8.18 (s, 1H), 7.59 (d, 2H, $J = 8.9$ Hz), 7.24 (s, 1H), 7.14 (d, 2H, $J = 8.9$ Hz). MS (ESI): m/z 306.0 $[\text{M}+\text{H}]^+$



2-((2,5-dichloropyrimidin-4-yl)amino)-5-(difluoromethoxy)benzene-1-sulfonyl chloride: prepared according to procedure D from 2,5-dichloro-N-(4-(difluoromethoxy)phenyl)pyrimidin-4-amine in 36% yield (pale yellow solid). Flash chromatography eluent Hex/EtOAc 98/2 gradually increasing to 80/20. $^1\text{H NMR}$ (400 MHz, CDCl_3): δ 8.27 (s, 1H), 8.23 (d, 1H, $J = 2.6$ Hz), 8.25 (dd, 1H, $J = 8.8$ Hz, 2.6 Hz), 7.47 (bs, 1H), 7.36 (d, 1H, $J = 8.8$ Hz). MS (ESI): m/z 396.0 $[\text{M}+\text{H}]^+$

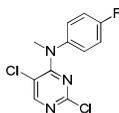


4-((2,5-dichloropyrimidin-4-yl)amino)-3-(pyrrolidin-1-ylsulfonyl)phenol: prepared according to procedure E (via unexpected cleavage of the CHF_2 group) from 2-((2,5-dichloropyrimidin-4-yl)amino)-5-(difluoromethoxy)benzene-1-sulfonyl chloride and pyrrolidine in 60% yield (pale yellow solid). Flash chromatography eluent Hex/EtOAc 95/5 gradually increasing to 70/30. $^1\text{H NMR}$ (400 MHz, CDCl_3): δ 8.16 (m, 1H), 7.97 (m, 1H), 7.44 (dd, 1H, $J = 8.8, 2.6$ Hz), 7.40 (bs, 1H), 7.03 (m, 1H), 3.35 (m, 4H), 1.84 (m, 4H). MS (ESI): m/z 389.1 $[\text{M}+\text{H}]^+$

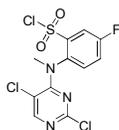


4-((5-chloro-2-((4-methylpiperazin-1-yl)methyl)phenyl)amino)pyrimidin-4-yl)amino)-3-(pyrrolidin-1-ylsulfonyl)phenol 24: prepared according to procedure B from 4-((2,5-dichloropyrimidin-4-yl)amino)-3-

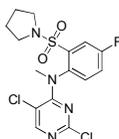
(pyrrolidin-1-ylsulfonyl)phenol and 4-((4-methylpiperazin-1-yl)methyl)aniline in 27% yield (yellow solid). Flash chromatography eluent CH₂Cl₂/MeOH 99/1 gradually increasing to 95/5. ¹H NMR (400 MHz, CDCl₃): δ 8.08 (s, 1H), 7.87 (d, 1H, J = 8.4 Hz), 7.67 (s, 1H), 7.44 (d, 2H, J = 8.1 Hz), 7.26 (1H), 7.23 (d, 2H, J = 8.4 Hz), 7.10 (d, 1H), 3.53 (s, 2H), 3.33 (m, 4H), 2.63 (bs, 8H), 2.43 (s, 3H), 1.83 (m, 4H). MS (ESI): m/z 558.1 [M+H]⁺



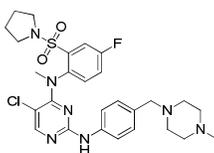
2,5-dichloro-N-(4-fluorophenyl)-N-methylpyrimidin-4-amine: prepared according to procedure C from 2,4,5-trichloropyrimidine and 4-fluoro-N-methylaniline in 100% yield (pale yellow solid). Flash chromatography eluent Hex/EtOAc 99/1 gradually increasing to 80/20. ¹H NMR (400 MHz, CDCl₃): δ 8.02 (s, 1H), 7.12-7.02 (m, 4H), 3.46 (s, 3H). MS (ESI): m/z 272.0 [M+H]⁺



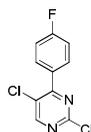
2-((2,5-dichloropyrimidin-4-yl)(methyl)amino)-5-fluorobenzene-1-sulfonyl chloride: prepared according to procedure D from 2,5-dichloro-N-(4-fluorophenyl)-N-methylpyrimidin-4-amine in 77% yield (white solid). Flash chromatography eluent Hex/EtOAc 98/2 gradually increasing to 80/20. ¹H NMR (400 MHz, CDCl₃): δ 8.16 (s, 1H), 7.69 (dd, 1H, J = 5.7, 2.8 Hz), 7.48 (m, 1H), 7.35 (t, 1H, J = 9.0 Hz), 3.54 (s, 3H).



2,5-dichloro-N-(4-fluoro-2-(pyrrolidin-1-ylsulfonyl)phenyl)-N-methylpyrimidin-4-amine: prepared according to procedure E from 2-((2,5-dichloropyrimidin-4-yl)(methyl)amino)-5-fluorobenzene-1-sulfonyl chloride and pyrrolidine in 100% yield (pale yellow solid). Flash chromatography eluent Hex/EtOAc 95/5 gradually increasing to 70/30. ¹H NMR (400 MHz, CDCl₃): δ 8.06 (s, 1H), 7.63 (dd, 1H, J = 5.69, 2.9 Hz), 7.31 (m, 1H), 7.21 (t, 1H, J = 9.0 Hz), 3.49 (s, 3H), 3.35 (m, 4H), 1.84 (m, 4H). MS (ESI): m/z 405.0 [M+H]⁺

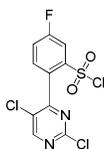


5-chloro-N4-(4-fluoro-2-(pyrrolidin-1-ylsulfonyl)phenyl)-N4-methyl-N2-(4-((4-methylpiperazin-1-yl)methyl)phenyl)pyrimidine-2,4-diamine 25: prepared according to procedure B from 2,5-dichloro-N-(4-fluoro-2-(pyrrolidin-1-ylsulfonyl)phenyl)-N-methylpyrimidin-4-amine and 4-((4-methylpiperazin-1-yl)methyl)aniline in 39% yield (yellow solid). Flash chromatography eluent CH₂Cl₂/MeOH 99/1 gradually increasing to 95/5. ¹H NMR (400 MHz, CDCl₃): δ 7.92 (s, 1H), 7.58 (m, 1H), 7.45 (d, 2H, J = 8.5 Hz), 7.25 (m, 1H), 7.21 (d, 2H, J = 8.4 Hz), 7.13 (t, 1H, J = 9.0 Hz), 7.10 (s, 1H), 3.43 (s, 3H), 3.41 (s, 2H), 3.31 (m, 4H), 2.38 (bs, 8H), 2.22 (s, .79 (m, 4H). MS (ESI): m/z 574.2 [M+H]⁺

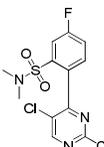


2,5-dichloro-4-(4-fluorophenyl)pyrimidine: prepared according to procedure A from 2,4,5-trichloroethylpyrimidine and (4-fluorophenyl)boronic acid in 100% yield (white solid). Flash

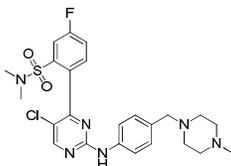
chromatography eluent: Hex/EtOAc 98/2 gradually increasing to 80/20. ^1H NMR (400 MHz, CDCl_3): δ 8.63 (s, 1H), 7.94 (dd, 2H, $J = 8.9, 5.5$ Hz), 7.19 (t, 1H, $J = 8.8$ Hz). MS (ESI): m/z 243.0 $[\text{M}+\text{H}]^+$



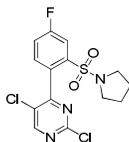
2-(2,5-dichloropyrimidin-4-yl)-5-fluorobenzene-1-sulfonyl chloride: prepared according to procedure D from 2,5-dichloro-4-(4-fluorophenyl)pyrimidine in 76% yield (white solid). Flash chromatography eluent Hex/EtOAc 98/2 gradually increasing to 80/20. ^1H NMR (400 MHz, CDCl_3): δ 8.72 (s, 1H), 8.59 (dd, 1H, $J = 6.3, 2.2$ Hz), 8.34 (m, 1H), 7.49 (t, 1H, $J = 9.1$ Hz). MS (ESI): m/z 337.0 $[\text{M}-\text{Cl}+\text{MeOH}]^+$



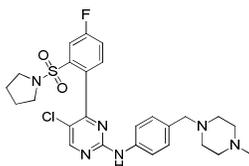
2-(2,5-dichloropyrimidin-4-yl)-5-fluoro-N,N-dimethylbenzenesulfonamide: prepared according to procedure E from 2-(2,5-dichloropyrimidin-4-yl)-5-fluorobenzene-1-sulfonyl chloride and dimethylamine (as a 2 M solution in THF) in 47% yield (white solid). Flash chromatography eluent Hex/EtOAc 95/5 gradually increasing to 70/30. ^1H NMR (400 MHz, CDCl_3): δ 8.36 (dd, 1H, $J = 6.6, 1.5$ Hz), 8.28 (s, 1H), 8.06 (m, 1H), 7.26 (t, 1H, $J = 9.0$ Hz), 2.83 (s, 6H). MS (ESI): m/z 350.0 $[\text{M}+\text{H}]^+$



2-(5-chloro-2-((4-((4-methylpiperazin-1-yl)methyl)phenyl)amino)pyrimidin-4-yl)-5-fluoro-N,N-dimethylbenzenesulfonamide 26: prepared according to procedure B from 2-(2,5-dichloropyrimidin-4-yl)-5-fluoro-N,N-dimethylbenzenesulfonamide and 4-((4-methylpiperazin-1-yl)methyl)aniline in 44% yield (yellow solid). Flash chromatography eluent $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 99/1 gradually increasing to 95/5. ^1H NMR (400 MHz, CDCl_3): δ 8.39 (m, 2H), 8.08 (m, 1H), 7.48 (d, 2H, $J = 8.4$ Hz), 7.30-7.20 (m, 4H), 3.41 (s, 2H), 2.82 (s, 6H), 2.40 (bs, 8H), 2.22 (s, 3H). MS (ESI): m/z 519.2 $[\text{M}+\text{H}]^+$

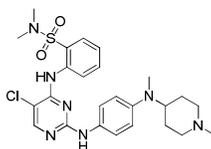


2,5-dichloro-4-(4-fluoro-2-(pyrrolidin-1-ylsulfonyl)phenyl)pyrimidine: prepared according to procedure E from 2-(2,5-dichloropyrimidin-4-yl)-5-fluorobenzene-1-sulfonyl chloride and pyrrolidine in 88% yield (white solid). Flash chromatography eluent Hex/EtOAc 95/5 gradually increasing to 70/30. ^1H NMR (400 MHz, CDCl_3): δ 8.67 (s, 1H), 8.49 (dd, 1H, $J = 6.2, 2.2$ Hz), 8.11 (m, 1H), 7.35 (t, 1H, $J = 9.1$ Hz), 3.39 (m, 4H), 1.86 (m, 4H). MS (ESI): m/z 375.94 $[\text{M}+\text{H}]^+$

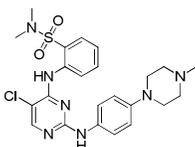


5-chloro-4-(4-fluoro-2-(pyrrolidin-1-ylsulfonyl)phenyl)-N-(4-((4-methylpiperazin-1-yl)methyl)phenyl)pyrimidin-2-amine 27: prepared according to procedure B from 2,5-dichloro-4-(4-fluoro-

2-(pyrrolidin-1-ylsulfonyl)phenyl)pyrimidine and 4-((4-methylpiperazin-1-yl)methyl)aniline in 96% yield (yellow solid). Flash chromatography eluent CH₂Cl₂/MeOH 99/1 gradually increasing to 95/5. ¹H NMR (400 MHz, CDCl₃): δ 9.48 (s, 1H), 8.55 (d, 1H, J = 8.9 Hz), 8.11 (s, 1H), 7.85 (d, 1H, J = 2.4 Hz), 7.42 (1H), 7.41 (d, 2H, J = 8.2 Hz), 7.25 (d, 2H, J = 8.2 Hz), 3.48 (s, 2H), 3.24 (m, 4H), 2.49 (bs, 8H), 2.29 (s, 3H), 1.79 (m, 4H). MS (ESI): m/z 545.2 [M+H]⁺



2-((5-chloro-2-((4-(methyl(1-methylpiperidin-4-yl)amino)phenyl)amino)pyrimidin-4-yl)amino)-N,N-dimethylbenzenesulfonamide **28**: prepared according to procedure B from 2-((2,5-dichloropyrimidin-4-yl)amino)-N,N-dimethylbenzenesulfonamide and N¹-methyl-N¹-(1-methylpiperidin-4-yl)benzene-1,4-diamine in 34% yield (yellow solid). Flash chromatography eluent CH₂Cl₂/MeOH 99/1 gradually increasing to 95/5. ¹H NMR (400 MHz, CDCl₃): δ 9.45 (s, 1H), 8.60 (d, 1H, J = 8.2 Hz), 8.06 (s, 1H), 8.18 (d, 1H, J = 8.0 Hz), 7.47 (t, 1H, J = 7.7 Hz), 7.31 (d, 2H, J = 8.9 Hz), 7.16 (t, 1H, J = 7.5 Hz), 6.77 (d, 2H, J = 8.9 Hz), 3.51 (tt, 1H, J = 11.3, 3.8 Hz), 3.00 (d, 2H, J = 10.6 Hz), 2.76 (s, 3H), 2.72 (s, 3H), 2.34 (s, 3H), 2.11 (t, 2H, J = 10.2 Hz), 1.90 (q, 2H, J = 11.3 Hz), 1.73 (d, 2H, J = 12.0 Hz). MS (ESI): 530.3 [M+H]⁺



5-fluoro-4-(4-fluoro-2-(pyrrolidin-1-ylsulfonyl)phenyl)-N-(4-((4-methylpiperazin-1-yl)methyl)phenyl)pyrimidin-2-amine **29**: prepared according to procedure B from 2-((2,5-dichloropyrimidin-4-yl)amino)-N,N-dimethylbenzenesulfonamide and 4-((4-methylpiperazin-1-yl)methyl)aniline in 24% yield (yellow solid). Flash chromatography eluent CH₂Cl₂/MeOH 99/1 gradually increasing to 95/5. ¹H NMR (400 MHz, CDCl₃): δ 8.56 (d, 1H, J = 8.4 Hz), 8.07 (s, 1H), 7.82 (d, 1H, J = 8.1 Hz), 7.49 (t, 1H, J = 7.7 Hz), 7.36 (d, 2H, J = 8.8 Hz), 7.17 (t, 1H, J = 7.5 Hz), 7.01 (bs, 1H), 6.89 (d, 2H, J = 8.8 Hz), 3.16 (m, 4H), 2.71 (s, 6H), 2.58 (m, 4H), 2.35 (s, 3H). MS (ESI): m/z 502.2 [M+H]⁺

IV. Cell culture

PSN-1 cells were acquired ACACC and cultured in RPMI 1620 media supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (100 IU/ml penicillin and 100 µg/ml streptomycin) at 37 °C and 5% CO₂.

V. Axl Kinase activity assay

Test compounds were diluted to desired concentrations in kinase reaction buffer (50 mM HEPES pH 7.5, 10 mM MgCl₂, 1mM EGTA, 2 mM DTT, and 0.01% v/v Tween-20) and were briefly incubated with Axl kinase (Invitrogen Corporation, Carlsbad, California). The Axl kinase used was recombinant human Axl kinase (catalytic domain, amino acids 473-894) with a histidine tag. The reaction was initiated by the addition of ATP and fluorescein-labeled poly-GT substrate (poly Glu:Tyr, 4:1 polymer; Invitrogen). Concentration of the various components in the assay (10 µl reaction volume) were: 1% DMSO, 93 ng/ml Axl kinase, 20 µM ATP, and 200 nM fluorescein poly-GT substrate. Following addition of ATP and fluorescein poly-GT substrate, incubation was for 60 min at room temperature, the enzyme reaction is stopped by addition of 10 µl terbium-labeled anti-phosphotyrosine PY20 antibody in EDTA-containing buffer. Final concentration of EDTA and antibody after addition to the reaction is 10 mM and 2 nM, respectively. The terbium conjugated antibody generates a time-resolved FRET signal with the fluorescein molecule (bound to the poly-GT substrate) when the substrate is phosphorylated. After one

hour incubation at room temperature, fluorescence was measured with excitation of 320 nm and dual emission of 495 and 520 nm on an EnVision microplate reader (PerkinElmer). Signal is expressed in terms of a TR-FRET ratio (fluorescence intensity at 520 nm to 495nm).

VI. Cell Proliferation Assay

For cell proliferation assays, 45 μ l containing 1000 cells per well were seeded into solid white 384-well plates in appropriate cell growth media containing 10% FBS and incubated overnight at 37 °C and 5% CO₂. The following day, test compounds were diluted in serum free growth media to 10x desired concentrations and 5 μ l was added to each well. Combined compound and cells were incubated for 96 hours. Following incubation, 40 μ l of ATP-Lite solution (PerkinElmer, Inc., Waltham, Massachusetts) was added to each well, incubated for an additional 10 minutes at room temperature and luminescence was measured on an EnVision microplate reader. Percent cell viability for test compounds was calculated by comparing treated wells to appropriate controls (e.g. vehicle treated) included on each plate.

VII. GAS6 – Axl – Akt Signaling Assays

Pancreatic cancer cells (PSN-1 cell line) were seeded in 1 ml of the appropriate growth media with 10% FBS into 6-well plates (8 x 10⁵ cells/well) and incubated overnight at 37°C and 5% CO₂. The following day, serum-containing growth media was replaced by serum-free media and incubated for 4 hr, and then test compounds were added to the cells at desired concentrations and incubated for an additional 2 hr. To stimulate Axl signaling, Gas6 was added to each well to a concentration of 3 μ g/ml and incubated for 10 min. The cells were lysed immediately and the lysates were used in a multiplex ELISA kit (Meso Scale Discovery, Gaithersburg, Maryland) to quantitate the total AKT and phospho-AKT (Ser473) according to the manufacturer's protocol. The same lysates were analyzed in a phospho-Axl ELISA (R&D Systems) using the protocol provided with the kit from the manufacturer.

VIII. IC50 calculation

IC₅₀ values are determined using GraphPad Prism 5 software. The data were entered as an X-Y plot into the software as percent inhibition for each concentration of the drug. The concentration values of the drug were log transformed and the nonlinear regression was carried out using the "sigmoidal dose-response (variable slope)" option within the GraphPad software to model the data and calculate IC₅₀ values. The IC₅₀ values reported are the concentration of drug at which 50% inhibition was reached.

IX. Kinase Panel Profiling

SelectScreen® (Invitrogen) was utilized for the kinase selectivity screening. The panel comprised 75 distinct protein kinases. The activity profiling as performed at 200 nM of test compound with ATP at Km apparent for each kinase, and the percent inhibition at that concentration was determined for each. Assay protocols and conditions are available at <http://www.invitrogen.com/site/us/en/home/Products-and-Services/Services/Screening-and-Profiling-Services/SelectScreen-Profiling-Service/SelectScreen-Kinase-Profiling-Service.html?CID=fl-kinaseprofiling>.

X. Cell Cycle Analysis by Flow Cytometry

PSN-1 cells were treated as indicated for 24 hours, harvested with trypsin and washed in PBS. Cell pellets (1 million cells) were resuspended in 1 mL of Krishan's buffer (0.1% sodium citrate, 0.02 mg/mL RNase A, 0.3% NP40) containing 0.05 mg/mL propidium iodide and incubated for 4 hours in the dark. DNA content analysis was performed using a Becton Dickinson FACScan (San Jose, CA), modeling 10,000 events per sample.