Supporting Information

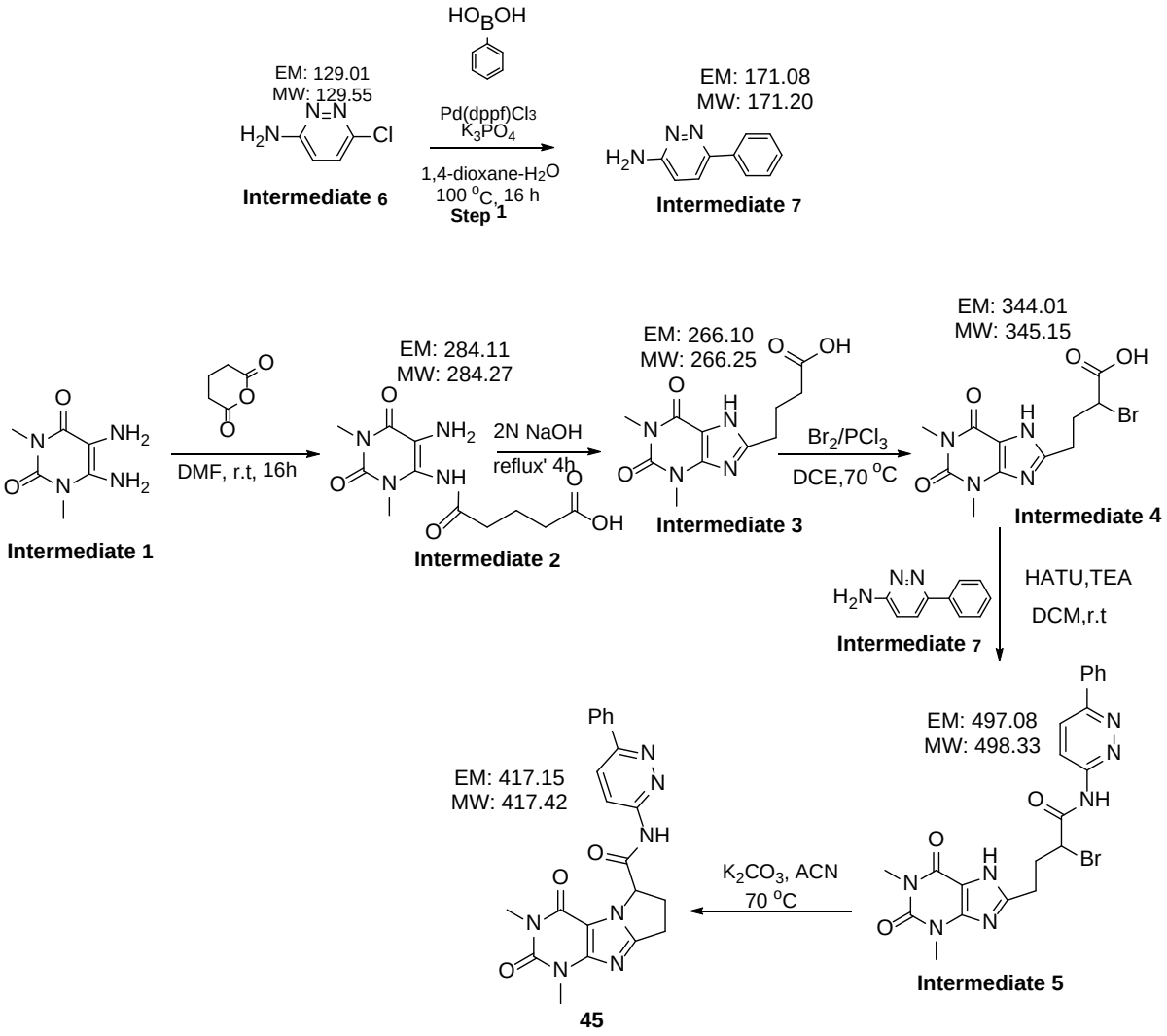
Pharmacophore Model for Wnt/Porcupine Inhibitors and Its Use in Drug Design

Anders Poulsen[*]*, Soo Yei Ho, Weiling Wang, Jenefer Alam, Duraiswamy A. Jeyaraj, Shi Hua Ang, Eldwin Sum Wai Tan, Grace Ruiting Lin, Vivien Wei Wen Cheong, Zhiyuan Ke, May Ann Lee and Thomas H. Keller*

Experimental Therapeutics Centre, Agency for Science, Technology and Research, 31 Biopolis Way, Nanos Level 3, Singapore 138669

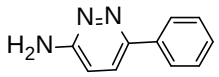
* Experimental Therapeutics Centre, Agency for Science, Technology and Research, 31 BiopolisWay, Nanos Level 3, Singapore 138669. Tel.: +65 6478 8767, Fax: +65 6478 8768, e-mail: apoulsen@etc.a-star.edu.sg.

Synthetic scheme of 45



Step 1: 6-phenylpyridazin-3-amine.

EM: 171.08
MW: 171.20



Intermediate 7

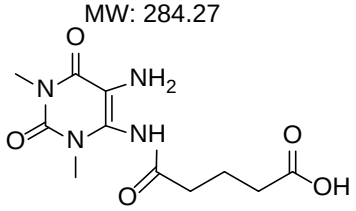
The stirred solution of **6-chloropyridazin-3-amine** (2 g, 15.44 mmol), **phenyl boronic acid** (2.82 g, 23.16 mmol) and potassium phosphate (6.5 g, 30.88 mmol) in 1,4 dioxane (40 mL) and water (10 mL) was degassed for 15 min with argon. Pd(dppf)Cl_2.DCM (630 mg, 0.77 mmol) was added to reaction mixture and heated to reflux for 16 hours. After completion, the reaction mixture was concentrated and water (50 mL) was added to reaction mixture and extracted with EtOAc (2x50 mL). The combined EtOAc layers were washed with brine (50 mL), dried (Na_2SO_4) and concentrated under vacuum. The crude compound was purified by column chromatography to afford 1.4 g (54%) of intermediate **7** as a off white solid.

^{1}H NMR (400 MHz, DMSO-d_6): 7.96-7.94 (d, *J* =7.6 Hz, 2H), 7.82-7.80 (d, *J* = 9.2 Hz, 1H), 7.48-7.44 (t, *J* = 9.2 Hz, 2H), 7.40-7.38 (t, *J* = 6.8 Hz, 1H), 6.86-6.83 (d, *J* = 9.2 Hz, 1H), 6.50 (brs, 2H).

LC-MS: m/z 172 (M+H) with a purity of 98.7 %.

Step 2: 5-(5-amino-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-ylamino)-5-oxopentanoic acid.

EM: 284.11
MW: 284.27

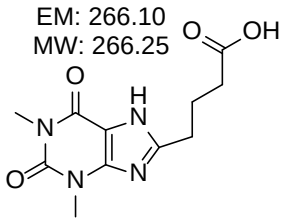


Intermediate 2

Intermediate **1** (3.5 g, 0.205 mmol) and glutamic anhydride (3.52 g, 30.882 mmol) in DMF (35 mL) was stirred at r.t for 16 hours. After completion, the reaction mixture was concentrated, washed with diethyl ether to give 4 g of crude intermediate **2** as brown solid. The crude compound was taken to next step without purification.

LC-MS: m/z 282.9 (M-H) with a purity of 33%.

Step 3: 4-(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)butanoic acid.



Intermediate 3

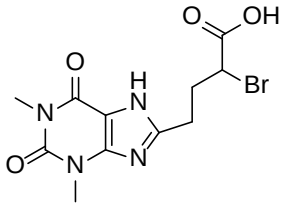
Intermediate **2** (3 g, 10.563 mmol) in 2M NaOH (60 mL) was heated to reflux for 4 hours. After completion, the reaction mixture was concentrated and neutralized with 6N HCl and precipitated solid was collected by filtration and washed with minimum amount of water and dried to give 1.3 g of intermediate **3** (46%) as light brown solid.

^{1}H NMR (400 MHz, DMSO-d_6): 13.20 (brs, 1H), 12.09 (brs, 1H), 3.32 (s, 3H), 3.22 (s, 3H), 2.73-2.70 (t, *J* = 7.2 Hz, 2H), 2.29-2.24 (t, *J* = 7.2Hz, 2H), 1.95-1.87 (q, *J* =7.2 Hz, 2H).

LC-MS: m/z 267.1 (M+H) with a purity of 98.85 %.

Step 4: 2-bromo-4-(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)butanoic acid.

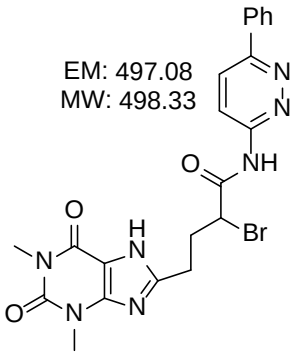
EM: 344.01
MW: 345.15



Intermediate 4

Intermediate **3** (200 mg, 0.751 mmol) in 1,2 dichloroethane (5 mL) was added bromine (180 mg,1.12 mmol) and PCl_3 (0.05 mL) at r.t and heated at 65 ^{o}C for 6 hours and 100 ^{o}C for 1 hour. The reaction mixture was concentrated and basified with aq. $NaHCO_3$ solution (20 mL) and washed with EtOAc (20 mL). The aqueous layer was acidified with aq. $KHSO_4$ solution and product was extracted with EtOAc (2x30 mL). The combined organic layers were dried over Na_2SO_4, concentrated under vacuum to give 50 mg (crude) (LC-MS 19%) of intermediate **4** and it was taken to next step without purification.

Step 5: 2-bromo-4-(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)-N-(6-phenylpyridazin-3-yl)butanamide.

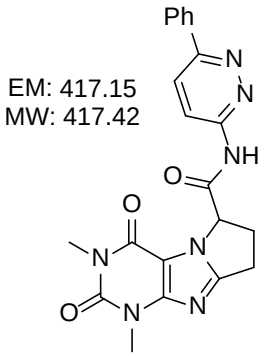


Intermediate 5

A stirred solution of intermediate **4** (50 mg, 0.145 mmol) in DCM (20 mL) was treated with HATU (66 mg, 0.174 mmol), TEA (0.03 mL, 0.217 mmol) and intermediate **7** (25 mg, 0.145 mmol) and stirred at r.t for 16 h. After completion, water (10 mL) was added to the reaction mixture and extracted with DCM (2x20 mL). The combined DCM layers were washed with brine solution (10 mL) and dried over Na_2SO_4 and concentrated under vacuum. The crude compound was purified by column chromatography to afford 20 mg (28%) of intermediate **5.**

MS: m/z 496 [M-2], 498 [M].

Step 6: 1,3-dimethyl-2,4-dioxo-N-(6-phenylpyridazin-3-yl)-2,3,4,6,7,8-hexahydro-1H-pyrrolo[1,2-f]purine-6-carboxamide.



EM: 417.15
MW: 417.42

45

Compound **5** (20 mg, 0.04 mmol) in acetonitrile (5 mL) was added K_2CO_3 (11 mg, 0.080 mmol) and reaction mixture was heated at 70 ^{o}C for 16 hours. After completion, the reaction mixture was concentrated and water (20 mL) was added to reaction mixture and extracted with $CHCl_3$ (2x20 mL). The combined $CHCl_3$ layers were washed with brine (10 mL), dried (Na_2SO_4) and concentrated under vacuum. The crude compound was purified by column chromatography to afford 8 mg (47%) of **45** as off white solid.

^{1}H NMR (400 MHz, DMSO-d_6): 11.42 (brs, 1H), 8.51-8.49 (d, *J* = 9.2 Hz, 1H), 8.01-7.99 (m,2H), 7.90-7.87 (d, *J* = 9.6 Hz,1H), 7.52-7.47 (m, 3H), 5.60-5.58 (t, *J* = 7.6 Hz, 1H), 3.58 (s, 3H) 3.45 (s, 3H), 3.38-3.16 (m, 2H), 3.05-2.95 (m, 2H).

LC-MS: m/z 421.1 (M-H) with a purity of 98.81 %.

Screening Strategy

No Porcupine biochemical assay suitable for high throughput screening or lead optimization has been published. We
employed a cellular pathway screening for hit finding and LO. A HEK293-STF cell line expressing Wnt3A that contains
a luciferase reporter for β-catenin mediated transcriptional activation was used [1-5]. Hits that act downstream of
surface receptor activation by Wnt (e.g. tankyrase inhibitors) was identified with a second assay and eliminated. A
HEK293-STF cell line without Wnt3A expression was used and screening was done in the presence of Wntconditioned
medium16 (HEK293-STF/Wnt) [1-5]. Hits were also assayed for general cytotoxicity in a CellTiter-Glo

Luminescent Cell Viability Assay assay and cytotoxic compounds eliminated [1-5]. Advanced compounds were tested
in secondary assays as referenced below.

Secondary assays

Cytoplasmic and nuclear β-catenin assay [1,8]
Phospho-LRP6 assay [1]
Palmitoylation-dependent Wnt-WLS interaction assay [6,8]
Wnt3A palmitoylation assay [6-8]
PORCN overexpression assay [6-8]
β-catenin target genes assay [6,8]
Wnt/β-catenin signaling assay [6,8]

References

[1] Ho, S. Y.; Blanchard, S. E.; Duraiswamy, A. J.; Alam, J.; Adsool, V. A. Purine Diones as WNT Pathway
Modulators. PCT/SG2014/000217, **2014**.
[2] Duraiswamy, A. J.; Alam, J. WNT Pathway Modulator. PCT/SG2014/000183, **2014**.
[3] Ho, S. Y.; Alam, J.; Duraiswamy, A. J.; Wang, W. L. WNT Pathway Inhibitors. PCT/SG2014/000602, **2014**.
[4] Wang, W. L.; Ho, S. Y.; Alam, J.; Poulsen, A.; Duraiswamy, J. Phthalimide Derivatives as Modulators of WNT
Pathway. GB1409783.6, **2014**.
[5] Alam, J.; Poulsen, A.; Ho, S. Y.; Wang, W. L.; Duraiswamy, A. J. Maleimide Derivatives as Modulators of WNT
Pathway. PCT/SG2014/000601, **2014**.
[6] Madan, B.; Ke, Z.; Lim, S. Q. Y.; Wang, S.; Alam, J.; Ho, S. Y.; Jeyaraj, D. A.; Ghosh, K.; Aliyev, J.; Chew, Y. S.;
Ding, L. J.; Pendharkar, V.; Sangthongpitag, K.; Keller, T.; Lee, M. A.; Virshup, D. M. Novel PORCN inhibitors
are Safe and Effective in the Treatment of Wnt-Dependent Cancers. In: Proceedings of the AACR-NCI-EORTC
International Conference: Molecular Targets and Cancer Therapeutics; **2013** Oct 19-23; Boston, MA. Philadelphia (PA): AACR; Mol. Cancer Ther. **2013**; 12(11 Suppl.): Abstract nr C248.
[7] Duraiswamy, A. J.; Lee, M. A.; Madan, B.; Ang, S. H.; Tan, E.; Cheong, V.; Ke, Z.; Pendharkar, V.; Ding, L.;
Chew, Y. C.; Sangthongpitag, K.; Poulsen, A.; Ho, S. Y.; Virshup, D. M.; Keller, T. H. Discovery and Optimization
Of A WntAcyltransferase Porcupine Inhibitor. Under review by J. Med. Chem.
[8] Madan, B.; Ke, Z.; Harmston. N.; Ho, S. Y.; Oliver, F. A.; Alam, J.; Jeyaraj, D. A.; Pendharkar, V.; Ghosh, K.;
Virshup, I. H.; Manoharan, V.; Ong, E. HQ; Sangthongpitag, K.; Hill, J.; Petretto, E.; Keller, T.; Lee, M. A.;
Matter, A.; Virshup, D. M. Wnt addiction treated by PORCN inhibition. Under review by Oncogene.