Bioorganic & Medicinal Chemistry Letters 22 (2012) 3198–3202

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and structure–activity relationships of 1,2,4-triazolo[1,5-*a*]pyrimidin-7(3*H*)-ones as novel series of potent β isoform selective phosphatidylinositol 3-kinase inhibitors

Robert M. Sanchez [a,*], Karl Erhard [a], Mary Ann Hardwicke [b], Hong Lin [a], Jeanelle McSurdy-Freed [c], Ramona Plant [b], Kaushik Raha [d], Cynthia M. Rominger [b], Michael D. Schaber [e], Michael D. Spengler [c], Michael L. Moore [a], Hongyi Yu [a], Juan I. Luengo [a], Rosanna Tedesco [a], Ralph A. Rivero [a]

[a] *Cancer Metabolism Chemistry, GlaxoSmithKline, 1250 South Collegeville Road, Collegeville, PA 19426, USA*
[b] *Cancer Metabolism Biology, GlaxoSmithKline, 1250 South Collegeville Road, Collegeville, PA 19426, USA*
[c] *Cancer Metabolism DMPK, GlaxoSmithKline, 1250 South Collegeville Road, Collegeville, PA 19426, USA*
[d] *Platform Technology Sciences, Computational Chemistry, GlaxoSmithKline, 1250 South Collegeville Road, Collegeville, PA 19426, USA*
[e] *Platform Technology Sciences, Screening and Compound Profiling, GlaxoSmithKline, 1250 South Collegeville Road, Collegeville, PA 19426, USA*

ARTICLE INFO

Article history:
Received 8 February 2012
Accepted 8 March 2012
Available online 16 March 2012

Keywords:
PI3K-β inhibitor
PTEN-deficient
Phosphatidylinositol 3-kinase
Triazolopyrimidinone
Structure–activity relationship

ABSTRACT

A series of 1,2,4-triazolo[1,5-*a*]pyrimidin-7(3*H*)-ones with excellent enzyme inhibition, improved isoform selectivity, and excellent inhibition of downstream phosphorylation of AKT has been identified. Several compounds in the series demonstrated potent (~ 0.100 μM IC_{50}) growth inhibition in a PTEN deficient cancer cell line.

The phosphatidylinositol 3-kinases (PI3K) are a family of enzymes involved in the regulation of cell survival, metabolism and proliferation.[1] Disruption of the PI3K signaling pathway plays a key role in the formation of cancerous cells from normal cells, and inactivation of the tumor suppressor protein PTEN is often seen through aberrant pathway activation.[2] This lipid-phosphatase is one of the most commonly mutated proteins in human cancers and loss of PTEN protein expression as measured by immunohistochemical staining of PTEN has been observed in approximately 40% of glioblastoma, 50% of prostate, 57% of endometrial cancers, in addition to a number of other tumor types, including melanoma and breast cancers.[3] Preclinical studies have shown that tissue-specific deletion of the PI3K-β isoform in the prostate specifically reduces PI3K signaling and blocks the formation of aggressive prostate tumors.[4] Based on the premise that the PI3K-β isoform is necessary for cancer proliferation, a program was initiated to identify PI3K-β selective inhibitors for the treatment of cancers driven by loss of PTEN.

A series of novel imidazopyrimidinones, exemplified by compound **1,** were previously designed in our laboratories. Although potent and selective, the high clearance observed in rodent PK analysis precluded further in vivo studies.[5] In an effort to improve on the overall profile of the imidazo[1,2-*a*]-pyrimidin-5(1*H*)-ones, the isosteric 1,2,4-triazolo[1,5-*a*]pyrimidin-7(3*H*)-ones illustrated in Figure 1 were explored. This modest change allowed for the opportunity to leverage our knowledge of the structure–activity relationships to quickly identify potent and selective analogs.

The synthetic route used to prepare the 1,2,4-triazolo[1,5-*a*]pyrimidin-7(3*H*)-ones is shown in Scheme 1. The triazole I[6] was treated with diethyl malonate and sodium ethoxide in ethanol

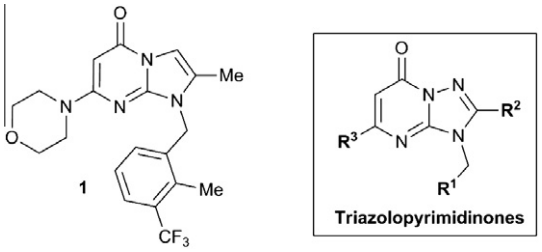
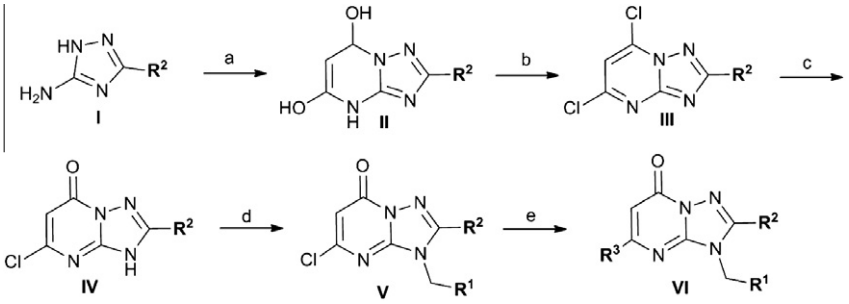


Figure 1. Structures of imadazopyrimidines and triazolopyrimidinones.

* Corresponding author.
E-mail address: robert.m.sanchez@gsk.com (R.M. Sanchez).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.03.039



Scheme 1. Synthesis of 1,2,4-triazolo[1,5-*a*]pyrimidin-7(3*H*)-ones. (a) Diethyl malonate, NaOEt, EtOH, reflux, 18 h (49–84%); (b) $POCl_3$, 80 °C, 2 h, (7–73%); (c) NaOH, 100 °C, 1 h, (20–50%); (d) R^1–Br, K_2CO_3, DMF, microwave, 120 °C, 10 min, (25–54%); (e) morpholine, EtOH, microwave, 150 °C, 1 h (25–88%).

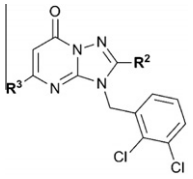
at reflux to yield dihydroxy cyclized product **II**. This was chlorinated with phosphorus oxychloride to yield di-chlorinated product **III**. The dichlorinated compound was hydrolyzed to **IV** with sodium hydroxide at 100 °C. Selective alkylation of the triazole ring was accomplished by treatment with substituted benzyl halides and potassium carbonate under microwave irradiation at 120 °C to provide **V**, followed by treatment with morpholine under microwave irradiation at 150 °C to give the desired product **VI.**

The structure–activity relationships previously established within the imidazopyrimidinone series allowed for the focus of efforts to be centered on 2,3 disubstituted phenyl groups at R^1. Specifically, Table 1 contains the isoform enzyme data for representative compounds where R^1 is 2,3-dichloro phenyl. These compounds exhibited excellent PI3K-β activities ranging from IC_{50} values of 0.025–0.0013 μM. The addition of a 2-methyl group to the morpholine was well tolerated. Chiral separation of the enantiomers (compounds **6** and **7**) resulted in a more active enantiomer **7**, but in this case provided no real advantage over the unsubstituted analog **2**.[7] Furthermore, the series displayed improved selectivity versus the delta enzyme (6 to 50-fold).

As can be seen in Table 2, a modest increase in activity was observed when R^1 was the 2-methyl, 3-trifluoromethyl phenyl compared with the 2,3-dichlorophenyl moiety. In this instance, several analogs (**9**, **10**, **14**, **15**) were very potent inhibitors of the PI3K-β enzyme with IC_{50} values of 0.001 μM or less. A preference for one stereoisomer of 2-methyl morpholine (**14**) was observed, but provided no real advantage over the unsubstituted analog **9**. Importantly, excellent selectivity versus all the other enzyme isoforms was maintained.

An important step in the progression of intracellular enzyme inhibitors is the ability to illustrate activity in a cellular context. In this case, mechanistic cell-based activity was demonstrated by measuring the inhibition of phosphorylation of AKT at serine 473 in a PTEN deficient breast cancer cell line, MDA-MB-468.[10] The plot of PI3K-β-enzyme pIC_{50} values of compounds **2–15** versus the pIC_{50} values of pAKT inhibition seen in Chart 1 shows a 1.5-fold log shift but is in acceptable agreement between PI3K-β enzyme and the cellular inhibition of AKT phosphorylation.

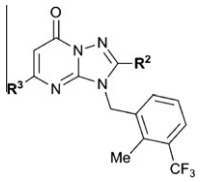
Table 1
Biochemical activity[b] in PI3K isoforms[a]



No.	R^2	R^3	PI3K enzyme IC_{50} (μM)			
			α	β	δ	γ
2	Me	4-Morpholinyl	0.63	0.0013	0.050	0.50
3	SMe	4-Morpholinyl	1.60	0.0016	0.031	0.50
4	*c*Pr	4-Morpholinyl	2.50	0.0025	0.130	2.50
5	Me	(2-Me)-4-Morph (±)	0.32	0.0020	0.025	0.25
6	Me	(2-Me)-4-Morph (E1)	2.50	0.0250	0.500	7.90
7	Me	(2-Me)-4-Morph (E2)	1.00	0.0013	0.040	0.50
8	SMe	4-Pyridyl[c]	0.40	0.0130	0.079	2.00

[a] IC_{50} values of β and δ activities are means of at least 2 experiments.
[b] Ref.8.
[c] Ref.9.

Table 2
Biochemical activity[8] in PI3K isoforms[a]



No.	R^2	R^3	PI3K enzyme IC_{50} (μM)			
			α	β	δ	γ
9	Me	4-Morpholinyl	0.63	0.0006	0.020	0.79
10	SMe	4-Morpholinyl	0.32	0.0004	0.025	1.30
11	cPr	4-Morpholinyl	2.50	0.0013	0.040	1.60
12	Me	(2-Me)-4-Morph (±)	0.40	0.0013	0.010	0.16
13	Me	(2-Me)-4-Morph (E1)	1.60	0.0031	0.063	0.79
14	Me	(2-Me)-4-Morph (E2)	0.32	0.0003	0.004	0.06
15	SMe	(2-Me)-4-Morph	1.30	0.0010	0.025	0.20

[a] IC_{50} values of β and δ activities are means of at least 2 experiments.

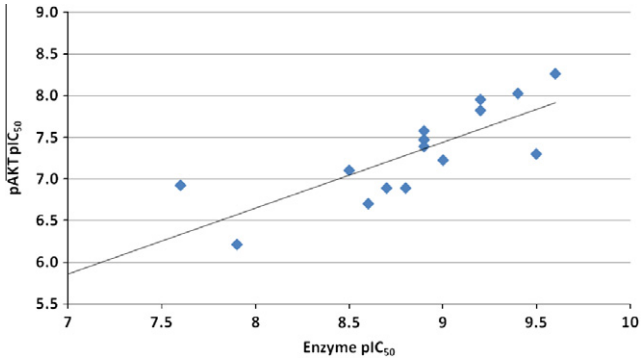
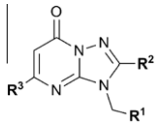


Chart 1. Enzyme versus cell activity compounds **2–15.**

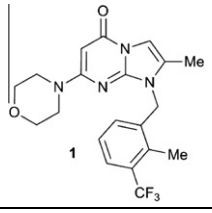
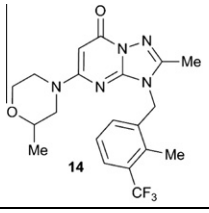
Table 3
Cellular and proliferation activity in MDA-MB-468 cell line[a]



No.	R^1	R^2	R^3	MDA-MB-468 IC_{50} (μM) EC_{50}	
				pAKT	Growth
2	(2,3-diCl)Ph	Me	4-Morpholinyl	0.0340	0.170
3	(2,3-diCl)Ph	SMe	4-Morpholinyl	0.0130	0.240
4	(2,3-diCl)Ph	*c*Pr	4-Morpholinyl	0.0200	0.260
7	(2,3-diCl)Ph	Me	(2-Me)-4-Morph(E2)	0.0034	0.110
9	(2-Me,3-CF_3)Ph	Me	4-Morpholinyl	0.0015	0.110
10	(2-Me,3-CF_3)Ph	SMe	4-Morpholinyl	0.0051	NA
11	(2-Me,3-CF_3)Ph	cPr	4-Morpholinyl	0.0041	0.040
12	(2-Me,3-CF_3)Ph	Me	(2-Me)-4-Morph(±)	0.0027	0.055
13	(2-Me,3-CF_3)Ph	Me	(2-Me)-4-Morph(E1)	0.0079	0.310
14	(2-Me,3-CF_3)Ph	Me	(2-Me)-4-Morph(E2)	0.0005	0.013

[a] IC_{50} values given are means of at least 2 experiments.

Table 4
Comparison of compounds **1** and **14**[a]

Selective PI3K-β inhibitor comparison (μM)	 **1**	 **14**
PI3K-β IC_{50}	0.0013	0.0003
PI3K-δ IC_{50}	0.0079	0.0040
mTOR[b] IC_{50}	32	6.3
DNA-PK[b] IC_{50}	0.80	0.16
MDA-MB-468 pAKT IC_{50}	0.078	0.005
HCC1954 pAKT IC_{50}	> 29	2.5
MDA-MB-468 Growth EC_{50}	0.14	0.013
HCC1954 Growth EC_{50}	9.7	2.2

[a] IC_{50} values given are means of at least 2 experiments.
[b] Member of the phosphatidylinositol 3-kinase related kinase family.

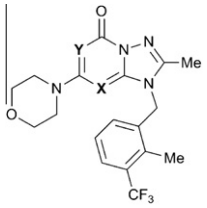
In an effort to understand the downstream consequences of inhibiting the phosphorylation of AKT, the PTEN deficient breast cancer cell line, MDA-MB-468, was evaluated to determine the effect of the compounds on tumor cell growth. A relevant cell line would be one that is PTEN deficient, as our hypothesis is that growth of PTEN deficient cell lines will be sensitive to PI3K-β inhibitors, while growth of wild-type PTEN cell-lines should be unaffected by selective PI3K-β inhibitors. As can be seen in Table 3, most of the more potent enzyme and cellular inhibitors also inhibited proliferation in MDA-MB-468 cell lines at sub-micromolar concentrations.[11] Compound **14** was a particularly potent inhibitor of phosphorylation of AKT and cell proliferation with IC_{50} values of 0.0005 and 0.0133 μM, respectively, whereas the less preferred isomer **13** demonstrated reduced IC_{50}'s of 0.079 and 0.031 μM, respectively.

As shown in Table 4, triazolopyrimidinone **14** compared very favorably with previously reported selective PI3K-β inhibitor compound **1**. The selectivity versus the delta isoform improved to greater than 10-fold, and the selectivity versus two other members of the phophatidylinositol 3-kinase related family of proteins mTOR and DNA-PK was 21,000 and 500-fold, respectively. Importantly, compound **14** was significantly more potent at inhibiting the phosphorylation of AKT in MDA-MB-468 cells as well as inhibiting proliferation in the same cell line. Both compounds **1** and **14** were significantly less active in the cellular pAKT and proliferation assays in a PTEN wild-type cell line (HCC1954), supporting our hypothesis of the selectivity of PI3K-β inhibitors for PTEN deficient tumor cell lines compared to PTEN wild-type.

While a second novel PI3K-β inhibitor chemotype with good *in vitro* activity had been identified, the compounds in this series suffered from very high rat clearance rates, similar to the isosteric imidazopyrimidinone series,[12] and thus were not appropriate for in vivo studies. A hypothesis that the high clearance might be related to the potential reactivity of the carbonyl, led to the preparation of the isomeric compound **16**[13] where the carbonyl might be less electrophilic. This compound, shown in Table 5, had slightly diminished enzyme and cell activity with rat clearance rates that were still too high for the molecules to be considered for in vivo analysis.

In summary, a novel series of triazolopyrimidinones as potent and selective inhibitors of PI3K-β has been described. Compounds

Table 5
Comparison of compounds **9** and **16**[a]



No.	X	Y	PI3K-β IC_{50}(μM)	pAKT IC_{50} (μM)	Growth Inhib. EC_{50} (μM)	Rat CL [b,c] (mL/min/kg)
9	N	C	0.0006	0.015	0.11	130
16	C	N	0.0020	0.092	0.21	83

[a] IC_{50} and clearance values given are means of at least 2 experiments.
[b] Ref. 14.
[c] Ref. 15.

in this series are the most potent and selective PI3K-β inhibitors reported to date with excellent anti-proliferation activity in a PTEN deficient cell line. While the overall profile of these new inhibitors represents an improvement over previously reported PI3K-β selective inhibitors, the observed high rodent clearance rates precluded their utility for in vivo analysis.

Acknowledgment

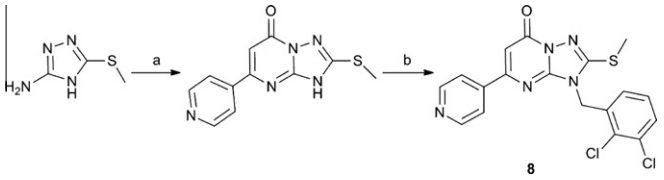
The authors thank Dr. Minghui Wang for NMR structural confirmation of compounds **2** and **8.**

Supplementary data

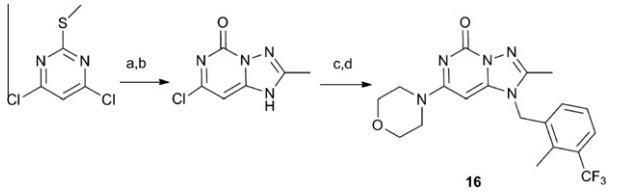
Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.03.039.

References and notes

1. Engleman, J. A.; Luo, J.; Cantley, L. C. *Nat. Rev. Genet.* **2006**, *7*, 60.
2. Wong, K.-K.; Engelman, J. A.; Cantley, L. C. *Curr. Opin. Genet. Dev.* **2010**, *20*, 87; (b) Cantley, L. C. *Science* **2002**, *296*, 1655.
3. Example references for the following cancers: (a) Glioblastoma, A.; Waldron, J. S.; Yang, I.; Han, S.; Tihan, T.; Sughrue, M. E.; Mills, S. A.; Pieper, R. Q.; Parsa, A. T. *J. Clin. Neurosci.* **2010**, *17*, 1543; Prostate cancer: (b) Carver, B. S.; Tran, J.; Gopalan, A.; Chen, Z.; Shaikh, S.; Carracedo, A.; Alimonti, A.; Nardella, C.; Varmeh, S.; Scardino, P. T.; Cordon-Cardo, C.; Gerald, W.; Pandolfi, P. P. *Nat. Genet.* **2009**, *41*, 619; Melanoma: (c) Abdel-Rahman, M. H.; Yang, Y.; Zhou, X.-P.; Craig, E. L.; Davidorf, F. H.; Eng, C. *J. Clin. Oncol.* **2006**, *24*, 288; Breast cancer: (d) Stefansson, O. A.; Jonasson, J. G.; Olafsdottir, G.; Esteller, M.; Johannsson, O. T.; Eyfjord, E. *Epigenetics* **2011**, *6*, 638; Endometrial cancer: (e) Cheung, L. W. T.; Hennessy, B.; Li, J.; Yu, S.; Myers, A. P.; Djordjevic, B.; Lu, Y.; Stemke-Hale, A.; Dyer, M. D.; Zhang, F.; Ju, Z.; Cantley, L. C.; Scherer, S. E.; Liang, H.; Lu, K. H.; Broaddus, R. R.; Mills, G. B. *Cancer Discov.* **2011**, *1*, 170.
4. (a) Wee, S.; Wiederschain, D.; Maira, S.-M.; Loo, A.; Miller, C.; DeBeaumont, R.; Stegmeier, F.; Yao, Y. M.; Lengauer, C. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 13057; (b) Jia, S.; Liu, Z.; Zhang, S.; Liu, P.; Zhang, L.; Lee, S. H.; Zhang, J.; Signoretti, S.; Loda, M.; Roberts, T. M.; Zhao, J. J. *Nature* **2008**, *454*, 776; Lee, S. H.; Poulogiannis, G.; Pyne, S.; Jia, S.; Zou, L.; Signoretti, S.; Loda, M.; Cantley, L. C.; Roberts, T. M. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *105*, 11002; (d) Hill, K. M.; Kalifa, S.; Das, J. R.; Bhatti, T.; Gay, M.; Williams, D.; Taliferro-Smith, L.; De Marzo, A. M. *The Prostate* **2010**, *70*, 755.
5. Lin, H.; Erhard, K.; Harwicke, M. A.; Luengo, J. I.; Mack, J.; McSurdy-Freed, J.; Plant, R.; Raha, K.; Rominger, C.; Sanchez, R. M.; Schaber, M. D.; Schulz, M. J.; Spengler, M. D.; Tedesco, R.; Xie, R.; Zheng, J.; Rivero, R. A. *Bioorg. Med. Chem. Lett.* **2012**. http://dx.doi.org/10.1016/ j.bmcl.2012.01.092.
6. Huang, Y.; Hu, X.-Q.; Shen, D.-P.; Chen, Y.-F.; Xu, P.-F. *Molecular Diversity* **2007**, *11*, 73.
7. Enantiomers were separated by chiral HPLC, absolute configuration unknown. E1 and E2 are denoted as the compounds relative elution off the column. Details can be found in Supplementary data.
8. Inhibition of PI3K-isoforms was measured using a continuous read time-resolved fluorescence resonance energy transfer displacement assay Gray, A.; Olsson, H.; Batty, I. H.; Priganica, L.; Downes, C. P. *Anal. Biochem.* **2003**, *313*, 234.
9. Compound **8** was prepared as shown in the following scheme.



Reagents and conditions: (a) ethyl 3-oxo-3-(4-pyridinyl)propanoate, AcOH, 120 °C, overnight, 18%; (b) 1,2-dichlorobenzyl bromide, K_2CO_3, DMF, 125 °C, microwave, 30 min, 18%.
10. Breast cancer cells were plated, incubated for approximately 16-20 hours and then treated with compound for 30 minutes. Final DMSO concentration on all cells was 0.15%. The cells were washed with Tris buffer and lysed in MesoScale Discovery (MSD) lysis buffer containing protease and phosphatase inhibitors (included in MSD kit). MSD Ser473-AKT duplex plates (Cat # MS6000) were used according to the manufacturer's instructions and plates were read on a SECTOR™ Imager 6000 using MSD Workbench software. For analysis of the Ser473-pAKT concentration response curves, the data was normalized using the corresponding total AKT value (sum of pAKT and AKT signal) and plotted as the percent of the DMSO-treated control values. The data was fit in Graphpad Prism version 4 for Windows (Graphpad Software, San Diego, California).
11. For the tumor cell growth assay, breast cancer cell lines were plated under anchorage independent conditions. To generate anchorage independent growth conditions, a 0.6% agar/media + 10% fetal bovine serum (FBS) solution was made to generate a bottom agar layer in the plates to prevent cell attachment. After solidification, a cell solution in 0.3% agar/media + 10% FBS was added to the plates. After the cell layer solidified, media + 10% FBS was added to the top of the cells. A 0.3% Brij 35 (Sigma B4184) solution in media + 10% FBS was added as a background control. The cells were incubated for approximately 16–20 h, treated with compound and the plates incubated for 6 days. One plate of cells was processed at the time of compound addition to quantify the starting number of cells. The final concentration of DMSO in all wells was 0.15%. Following the 6-day incubation, Alamar Blue (Invitrogen #DAL1100) was added to the cells, incubated for 6 h and the plates read on a Spectramax (Gemini EM)) at 530 nm (excitation) and 590 nm (emission). For analysis of cell growth inhibition dose response curves, the data was plotted as the percent of the DMSO-treated control samples. Values from wells containing 0.3% Brij 35 were subtracted from all samples for background correction. The data was fit in Excel with a 4 parameter logistical fit using XLfit from ID Business solutions (IDBS, model 205).
12. Nine analogs were evaluated and rat clearance averaged >3 times rat hepatic blood flow.
13. Compound **16** was prepared as shown in the following scheme.



Reagents and conditions: (a) NH_2NH_2, THF, rt, overnight, 91%; (b) triethylorthoformate, THF, AcOH, 135 °C, microwave, 30 min; KOH, MeOH

70 °C, 2 h, 60%; (Siegel, S.; Int. Patent WO113469, **2008**); (c) (3-trifluoromethyl-2-methyl)benzyl bromide, K_2CO_3, DMF, rt, overnight; (d) morpholine, DIEA, MeOH, RT, overnight, 3% (combined steps c and d).

14. Clearance is calculated following IV administration. The formulation for an IV dose is 1% DMSO, 20% Encapsin in saline at a drug concentration of 0.25 mg/kg.
15. All studies were conducted after review by the GSK Institutional Animal Care and Use Committee and in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals.