Bioorganic & Medicinal Chemistry Letters 18 (2008) 5299–5302

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Optimization of a series of multi-isoform PI3 kinase inhibitors

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ARTICLE INFO

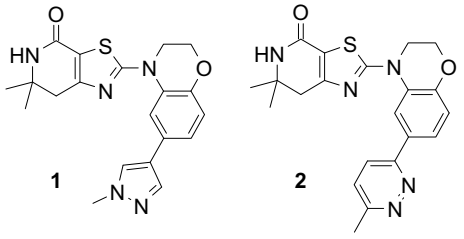
Article history:
Received 2 July 2008
Revised 3 August 2008
Accepted 14 August 2008
Available online 20 August 2008

Keywords:
PI3
PI3 kinase
Kinase
Benzoxazine
Inflammation

ABSTRACT

Optimization of the cellular and pharmacological activity of a novel series of PI3 kinase inhibitors targeting multiple isoforms is described.

Previous communications from our group have disclosed the discovery of novel morpholino- and benzoxazino-dihydrothiazolopyridinones as multi-isoform inhibitors of class 1 phosphoinositide-3-kinases (PI3K).[1,2] Of particular interest are compounds demonstrating inhibitory activity against both the δ and γ isoforms of PI3K, which have been shown to play crucial roles in inflammatory responses.[3] It is hoped that such compounds will prove useful as therapeutic agents for the treatment of chronic inflammatory diseases including rheumatoid arthritis and multiple sclerosis.[3c] Lead pyrazole-benzoxazine compound **1** demonstrated good in vitro and in vivo pharmacokinetic properties, moderate activity in a PI3Kδ/γ driven in vitro cellular assay and significant activity in a PI3K-dependent primary pharmacological model of inflammation. Similarly, pyridazine analogue **2** demonstrated excellent in vivo PK profile and a moderate cellular activity against PI3K. However, both compounds **1** and **2** suffered from low solubility and moderate selectivity issues against other kinases.[4]



Herein we discuss attempts to optimize this series with emphasis on increasing cellular and pharmacological activity and improving compound solubility and selectivity whilst retaining the desirable pharmacokinetic profile of these lead compounds. Solubility was tackled through increasing polarity of the compounds and through attempts to disrupt the highly planar conjugated scaffold through steric disruption. Improving compound activity in the PI3Kδ/γ-driven fMLP assay was addressed through attempting to increase activity against the target proteins, optimizing cellular permeability through modulation of log*D* and polarity, or a combination of both.

Decoration of the pyrazole ring in compound **1** and pyridazine ring in compound **2** with polar and non-polar functionalities was achieved as shown in Scheme 1. Suzuki coupling of previously described bromide **5** with pyrazole boronate esters **3a–k**, synthesized as shown in Scheme 2 from **3a** and **3b** or sourced commercially, gave compounds **8a–k**.[1] Likewise, Suzuki coupling of boronate ester **6** with commercially available pyrazole bromides **7a–d** gave compounds **8l–o**. Chiral preparative chromatography of **8g** gave enantiomers **8p** and **8q**.[5] Conversion of **5** to amino-benzoxazine intermediate **9** was effected through Buchwald coupling with benzophenone imine followed by acid hydrolysis.[6] Palladium-catalyzed coupling or direct nucleophilic substitution of **9** with 2-chloropyridazines **4b–i** (synthesized as in Scheme 2 or sourced commercially) gave *N*-linked pyridazines **10a–g**. Direct-linked pyridazines **11a–e** were synthesized via Suzuki coupling of boronate ester **6** with pyridazine chlorides**4b, c, g, h** and **j**, respectively.

Compounds **8a–f** and **8i** show that 3,5-dimethylation of the pyrazole ring results in a drop in activity against the PI3Kγ isoform but a good improvement in in vitro microsomal and hepatocytic

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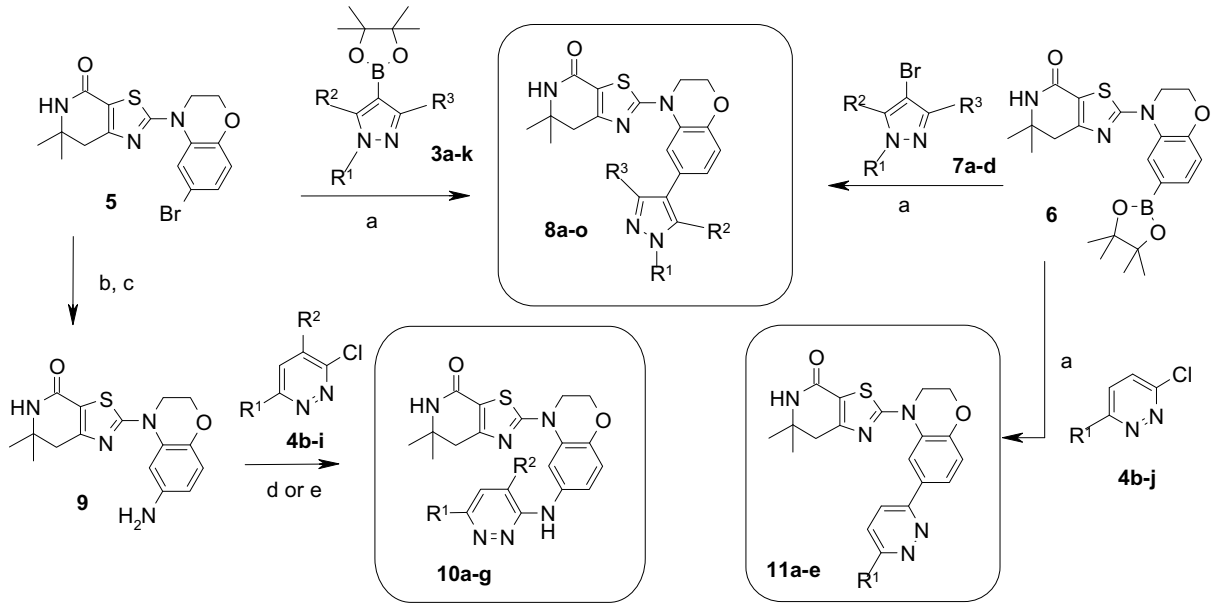
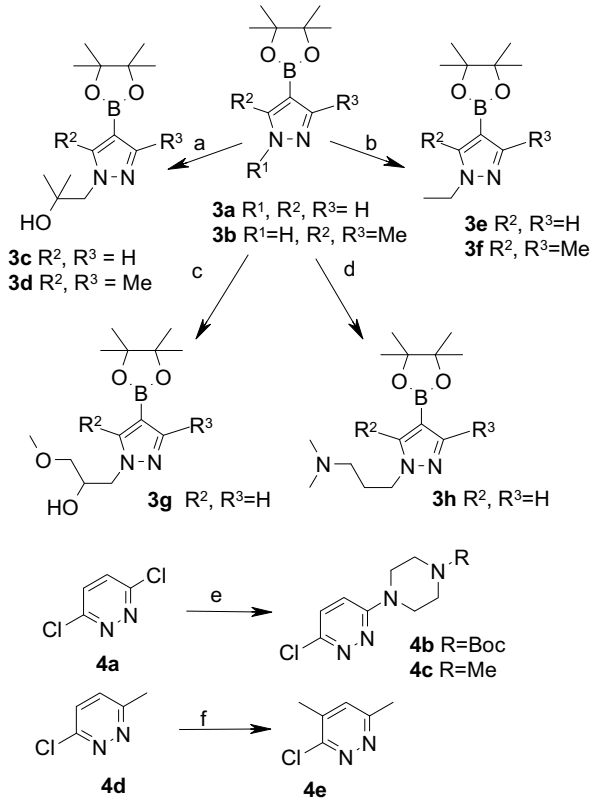
doi:10.1016/j.bmcl.2008.08.042



Scheme 1. Reagents and conditions: (a) $Pd(PPh_3)_4$, K_3PO_4, DME/H_2O, $^nBu_4NBr$, 120 °C, 1 h (8–71% yield); (b) Pd_2dba_3, (+/−)-BINAP, THF, benzophenoneimine, NaO^{t}Bu, 120 °C, 20 min; (c) HCl, THF, rt, 18 h (87% yield over 2 steps); (d) 1,1-bis(di-*tert*-butylphosphino)ferrocene palladium dichloride, NaO^{t}Bu, PhMe, 120 °C, 2 h (10–51% yield); (e) ^{i}PrOH, 150 °C, 3 h (9–10% yield).



Scheme 2. Reagents and conditions: (a) isobutylene oxide, Cs_2CO_3, 110 °C, 1 h (81–86%); (b) NaH, EtI, THF, rt, 18 h (58–98%); (c) NaHMDS, THF, $ClCH_2CH(OH)CH_2OCH_3$, 80 °C, 19 h (57%); (d) NaHMDS, THF, $ClCH_2CH_2CH_2N(CH_3)_2$, 80 °C, 19 h (25%); (e) 1-boc-piperazine or 1-Me-piperazine, THF, $^iPr_2NEt$, 180 °C, 2 h (47–52%); (f) AcOH, $AgNO_3$, H_2SO_4, H_2O, 75 °C, 30 min (50%).

turnover. The drop in activity against the γ isoform translated into a significant drop in the cellular activity of these compounds.[7] Compound **8i** gave significantly improved solubility over parent **1**, suggesting that attempted disruption of the planarity of the benzoxazine-pyrazole bond through steric hindrance of the aryl–aryl bond significantly affects solubility as intended. Substitution of the pyrazole 1-position with a *tert*-butyl alcohol moiety (**8c**) gave a significant improvement in solubility along with a reduction of in vitro clearance. Combination of reduced planarity through 3,5-dimethylation and the presence of the *tert*-butyl alcohol moiety resulted in a cumulative increase in solubility (**8d**). Simple mono-methylation at the 3-position of the pyrazole ring conferred no improvement in solubility (**8l**). Replacement of the *N*-methyl group in **1** with an ethyl group (**8e**) resulted in a drop in PI3Kγ and cellular activity, and combination of *N*-ethyl pyrazole with planarity-disrupting 3,5-dimethylation gave a decent increase in solubility and cellular potency whilst compromising in vitro DMPK stability (**8f**). *N*-Substitution of the pyrazole ring with a 2-hydroxy-3-methoxypropyl chain (**8g**) resulted in good activity in both enzyme assays, translating to high cellular potency. This compound also demonstrated good in vitro clearance, including improved stability in human microsomes over lead compound **1**, and also demonstrated superior solubility, presumably due to the increased polarity of the compound. The isolated enantiomers of **8g** (**8p** and **8q**) showed that although the (*R*)-enantiomer **8p** demonstrated slightly better pharmacokinetic stability in vitro, no major advantage was presented by the chirally pure compounds over parent racemate. *N*-Benzyl analogue **8j** and *N*-methyl-(3-pyridyl) analogue **8k** demonstrated significant activity against the PI3Kδ isoform, good activity against the PI3Kγ isoform, and in the case of **8j**, very good cellular potency. *N*-substitution of the pyrazole with alkylamine chains gave enhanced solubility (**8m, 8n**). Exceptional reduction of in vitro clearance in both rat and human microsomes was observed for these compounds; however, they proved to be highly susceptible to hepatocytic clearance. Interestingly, the tertiary propylamine **8h** had significantly reduced PI3Kγ activity relative to the corresponding primary propylamine **8n**, as did the shortened primary ethylamine **8m**. Substitution at the 5-position of the pyrazole with a primary amine substituent (**8o**) improved solubility and rat in vitro clearance, but gave no advantage over parent **1** in terms of cellular and enzyme potency (Table 1).

Table 1
IC_{50} values[a] and PK properties of substituted 4-thiazolyl-[2,3]-dihydrobenzoxazine-6-pyrazole analogues against PI3Kδ and γ isoforms

Compound	Synthetic precursor[b]	R^1	R^2	R^3	PI3K IC_{50}		Cl_{Mic}[c] rat (human)	Cl_{Hep}[d] rat	FMLP[e] IC_{50}	log*D*[f]	Solubility at pH 7.4[g]
					δ	γ					
1	—	Me	H	H	32	78	13 (13)	0	111	2.63	48
2	—	Me	H	—	139	107	8 (10)	3	220	2.32	13
8a	**3a**	H	H	H	16	22	84 (53)	13	nd	2.68	37
8b	**3b**[h]	H	Me	Me	32	201	29 (14)	6	76	3.04	19
8c	**3c**	$CH_2C(CH_3)_2OH$	H	H	54	50	15 (5)	2	37	2.74	143
8d	**3d**	$CH_2C(CH_3)_2OH$	Me	Me	65	257	9 (6)	nd	65	2.30	>500
8e	**3e**	Et	H	H	17	70	17 (22)	2	212	2.73	17
8f	**3f**	Et	Me	Me	43	180	31 (25)	5	84	3.57	215
8g	**3g**	$CH_2CH(OH)CH_2OMe$	H	H	14	52	14 (8)	3	38	2.19	>500
8h	**3h**	$(CH_2)_3NMe_2$	H	H	29	767	21 (13)	15	167	nd	nd
8i	**3i**	Me	Me	Me	45	327	44 (23)	7	75	3.23	349
8j	**3j**	Bn	H	H	4	48	45 (25)	7	21	3.91	4
8k	**3k**	CH_2(3-pyridyl)	H	H	4	35	84 (53)	13	nd	2.94	37
8l	**7a**	Me	H	Me	8	18	23 (20)	nd	nd	3.00	7
8m	**7b**	$CH_2CH_2NH_2$	H	H	39	131	1 (0)	11	274	1.16	>500
8n	**7c**	$(CH_2)_3NH_2$	H	Me	4	51	1 (2)	18	67	0.38	>500
8o	**7d**	Me	NH_2	H	36	70	1 (13)	3	102	1.98	>500
8p	**8g**[i]	(*S*)-$CH_2CH(OH)CH_2OMe$	H	H	14	46	21 (17)	nd	37	nd	nd
8q	**8g**[i]	(*R*)-$CH_2CH(OH)CH_2OMe$	H	H	9	51	13 (13)	4	25	nd	>500
10a	**4b** (d)[j]	1-Piperazine	H	—	21	34	0 (1)	nd	86	0.60	77
10b	**4c** (d)	1-(4-Me)-piperazine	H	—	30	35	18 (15)	10	19	1.76	234
10c	**4e** (e)	Me	Me	—	245	203	23 (20)	Nd	nd	1.97	>500
10d	**4f** (e)	Me	H	—	4	20	21 (23)	0	8	2.39	94
10e	**4g** (d)	NMe_2	H	—	12	24	81 (83)	36	nd	nd	142
10f	**4h** (d)	OMe	H	—	7	20	35 (23)	5	nd	2.74	6
10g	**4i** (d)	Ph	H	—	6	34	53 (34)	7	nd	3.72	11
11a	**4b**[j]	1-Pipeazine	—	—	103	767	0 (0)	nd	288	2.45	>500
11b	**4c**	1-(4-Me)-Piperazine	—	—	231	1288	8 (26)	nd	209	1.75	102
11c	**4g**	NMe_2	—	—	103	138	40 (63)	nd	nd	2.61	9
11d	**4h**	OMe	—	—	89	222	9 (7)	3	493	0.22	2
11e	**4j**	NH_2	—	—	72	174	9 (9)	18	125	1.94	226

[a] Values are quoted in nM, and are means of three experiments.
[b] Synthetic precursor (coupling conditions used).
[c] Compound concentration 0.5 μM, values quoted in μL/min/mg protein.
[d] Compound concentration 2.0 μM, values quoted in μL/min/mg protein
[e] Values quoted in nM.
[f] Experimentally determined.
[g] Values in μM (500 μM limit of detection).
[h] SEM-protected pyrazole boronic ester used, deprotected during work-up.
[i] Isolated from **8g** via chiral preparative HPLC.
[j] *N*-Boc protecting group removed during work-up (nd, not determined).

In general, the *N*-linked pyridazines **10a–g** demonstrated significantly improved activity against both PI3Kδ and γ isoforms relative to the direct-linked pyridazine **2**. Unfortunately, most of these compounds also demonstrated very high in vitro clearances in both rat and human microsomes. Piperazine-substituted analogue **10a** was an exception to this, although despite good activity against both PI3K isoforms the cellular activity and solubility of this compound were compromised. Interestingly, the *N*-methylated piperazine analogue **10b** showed similar levels of activity in the enzyme assay to **10a**, which in this case did translate into very good activity in the cellular assay. Closer investigation into this result demonstrated a correlation between low log*D* (<1.5) and higher drop-off from both PI3Kδ and PI3Kγ enzyme activity into cellular activity (Fig. 1a). Substitution *ortho* to the anime linker in **10c** resulted in loss of activity but improved solubility. Of greatest interest for this series was analogue **10d**, which displayed exceptional activity in both the enzyme and cellular assays whilst retaining acceptable in vitro PK properties, including noticeable stability in rat hepatocytes. Despite good activity against PI3Kδγ, electron-rich pyridazines **10e** and **10f**, and phenyl-substituted analogue **10g** suffered from very high in vitro clearance and poor solubility.

Pyridazine analogues **11a–e** displayed similar or slightly improved activity against PI3Kδ relative to parent **2**; however, only **11e** gave an improvement in cellular activity, and all analogues showed reduced activity against the PI3K γ isoform. Only piperazine analogue **11a** showed a significant improvement in microsomal stability and solubility.

Profiling of compounds **8g** and **10d** against a panel of 50 different kinases revealed that although compound **8g** retained a degree of off-target activity (>50% inhibition of both Pim-1 and CK-2 at 10 μM), compound **10d** demonstrated no off-target activity. Activity of both **8g** and **10d** against other class 1 PI3K isoforms was measured. The PI3K α and β activity (IC_{50}) of **8g** was 243 nM and 222 nM, respectively, approximately a 5- to 10-fold selectivity bias towards the δ and γ isoforms. For 10d the activity (IC_{50}) was 24 nM and 23 nM, respectively, suggesting this compound to have a greater 'pan' class 1 PI3K isoform profile than **8g**.

Further evaluation of this series through in vivo pharmacokinetic profiling of some of these compounds can be seen in Table 2. Rat plasma protein binding of these compounds varied significantly. A correlation between measured log*D* and plasma protein binding was observed, suggesting that compounds with lower log*D* demonstrate an increased likelihood of having greater free-fraction in plasma (Fig. 1b). Compound **8c** achieved high exposure when dosed orally to Han-Wistar rats (3 mg/kg), whilst other pyrazole compounds **8f, 8g** and **8j** demonstrated moderate exposures. No significant difference between chirally pure **8q** and racemate parent **8g** was observed. *N*-Linked pyridazine **10d** demonstrated

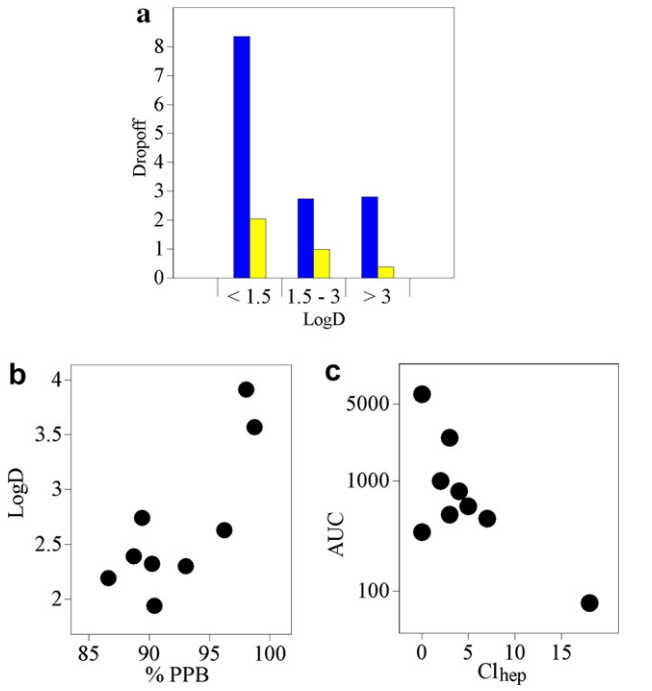


Figure 1. Relationship between (a) average fold drop-off in cellular activity (fMLP IC_{50}) versus PI3Kδ enzyme IC_{50} (blue) and PI3Kγ enzyme IC_{50} (yellow) relative log*D* for compounds within the whole UCB series; (b) measured log*D* at pH 7.4 and plasma protein binding (rat) for all compounds in Table 2; (c) in vitro hepatocytic clearance and in vivo exposure (log scale) for compounds in Table 2.

Table 2
In vivo PK analysis of key compounds orally dosed in Han-Wistar rats[a]

Compound	C_{max} (ng/mL)	AUC (ng h/mL)	% PPB[b]
1	1216	6162	96.2
2	185	2471	90.2
8c	195	1005	89.4
8f	139	591	98.7
8g	159	495	86.6
8j	103	455	98.0
8q	187	807	86.6
10d	105	345	88.7
11e	14	78	90.4

[a] Dosed at 3 mg/kg po.
[b] % Plasma protein bound in blood (male Han-Wistar rat).

slightly lower C_{max} and AUC than the pyrazole series, and direct-linked amino-pyridazine **11e** had very low exposure. Despite most of these compounds having relatively similar profiles in the in vitro rat and human microsomal clearance model, none demonstrated levels of exposure in vivo comparable to parent compounds **1** and **2**. Analysis of the link between in vitro hepatocytic clearance and in vivo exposure suggests a logarithmic correlation between these two factors, indicating that for this series of compounds hepatocytic clearance may be key to achieving good in vivo exposure (Fig. 1c).

Despite demonstrating significantly lower oral exposures relative to lead compounds **1** and **2**, the increased free-fraction and improved solubility and cellular activity of **8g** and **10d** led us to investigate the activity of these compounds in vivo. Acute activation of rat T-cells by anti-CD3 antibody treatment causes release of IL2, both in vitro and in vivo. As shown in Figure 2a, **8g** and **10d** inhibited CD3-induced IL2 release in male Lewis rats with ED_{50}s of 5 mg/kg and 20 mg/kg, respectively, compared with an ED_{50} of 25 mg/kg for compound **1**. When efficacy in vivo is represented using free compound exposure in plasma (EC_{50} free) for this series, a good correlation is seen to the in vitro potency (Fig. 2b).[8] This demonstrates the clear overall improvement in compound properties of **8g** and **10d**, compared to **1**.

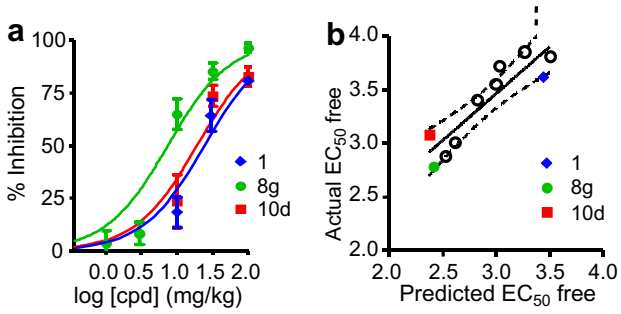


Figure 2. Efficacy of PI3K inhibitors in in vivo models. (a) Dose–response curves for compounds **1**, **8g** and **10d** in CD3-induced IL2 release in male Lewis rats. (b) Correlation between in vitro and in vivo efficacy, expressed as EC_{50} (plasma free-fraction for in vivo). The regression line and 95% confidence intervals are represented as solid and dotted lines, respectively.[8]

In conclusion, we have demonstrated optimization of a series of multi-isoform PI3K inhibitors. General cellular and enzyme activity has been improved, and compounds with good solubility and kinase selectivity have been identified, whilst retaining or improving on the good in vivo pharmacological activity demonstrated by the parent leads.

Acknowledgments

The authors acknowledge the significant contributions of the DMPK, molecular biology and CADD groups at UCB Cambridge and the medicinal chemistry group at UCB Slough in helping to carry out this research.

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4. Compound **1** (10 μM) demonstrated >50% inhibition against 2 kinases from a panel of 50 (Pim-1 and SAPK2a).
5. Separation performed on chiralpack IA column using EtOH/heptane (60:40) as eluant. Absolute configuration or **8p** and **8q** assigned through discreet synthesis of **8q** from reaction of **8a** with (*S*)-4-methoxymethyl-1,2-dioxolan-2-one (NaOH, DMF, 155 °C, 4 h, 49%, >98% ee).
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8. All data points in Figure 2b represent compounds from the morpholino- or benzoxazino-dihydro-thiazolopyridinone series of PI3K inhibitors described in Ref. 2b.