Bioorganic & Medicinal Chemistry Letters 18 (2008) 4700–4704

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Achieving multi-isoform PI3K inhibition in a series of substituted 3,4-dihydro-2H-benzo[1,4]oxazines

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ARTICLE INFO

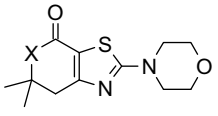
Article history:
Received 10 June 2008
Revised 27 June 2008
Accepted 30 June 2008
Available online 5 July 2008

Keywords:
Kinase
PI3K
Inflammation
Small molecule
Isoform

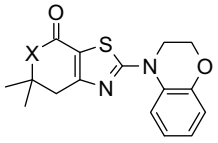
ABSTRACT

The SAR and pharmacokinetic profiles of a series of multi-isoform PI3K inhibitors based on a 3,4-dihydro-2H-benzo[1,4]oxazine scaffold are disclosed.

Production of the second messenger phosphatidylinositol 3,4,5-triphosphate by the Class 1 phospoinositide-3-kinases is well known to be a key event in a wide number of cellular processes, including metabolic control, cell survival and growth and cytokine-induced inflammatory responses.[1] As such inhibition of one or more of the four isoforms of the Class 1 PI3Ks presents opportunities for the treatment of conditions such as cancer, chronic inflammatory disorders and allergy.[2] The PI3Kδ and PI3Kγ isoforms are largely expressed in hematopoietic cells and have been shown to play crucial roles in inflammatory responses.[3] Furthermore genetic targeting of both the δ and γ isoforms of PI3K has revealed them to be suitable drug targets for chronic disease therapy, as mice lacking a catalytically functional form of either of these two enzymes are healthy and viable.[4] In this letter, we present the discovery and optimization of inhibitors of Class 1 PI3 Kinases with an emphasis on the δ and γ isoforms, with view to validating such compounds as a treatment for inflammatory disorder.



1 X=CH_2
2 X=NH



3 X=CH_2
4 X=NH

Efforts to identify inhibitors of PI3Kδ/γ isoforms via a pharmacophore-directed medium-throughput screen yielded 5,5-dimethyl-2-morpholin-4-yl-5,6-dihydro-4H-benzothiazol-7-one **1**, originating from a commercially available screening library.[5,6] Interestingly, structurally related compounds in which the morpholine group is replaced by a 4-pyridyl moiety have recently been reported as MK-2 and Cdc7 inhibitors.[7] The use of morpholine as a backbone binder in lipid kinases such as PI3K is well documented.[8] Compound **1** was considered a promising chemical starting point due to its low molecular weight and resultant high ligand efficiency (0.4164 kcal · mol^{-1} against PI3Kγ).[9] It was postulated that replacement of the cyclic ketone moiety with a more synthetically tractable lactam (**2**) would lower log *D* of the scaffold whilst retaining activity, thus improving physicochemical aspects of the scaffold.

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doi:10.1016/j.bmcl.2008.06.104

A crystal structure of **1** binding to PI3Kγ (Fig. 1)[10] led to the hypothesis of increasing binding activity through fusion of a phenyl ring across the [2,3] C–C bond of the backbone-binding morpholine unit (**3**, **4**). It was envisaged that this would rigidify the morpholine unit resulting in a tighter binding into the cavity whilst retaining the overall size and distribution of binding elements within the ligand. Furthermore, it was expected that the presence of an aromatic unit in this position would allow for a favourable edge-face interaction with Trp812, thus gaining an overall increase in potency.

A variety of substituted [2,3]benzo- and [2,3]pryridyl-fused derivatives of **1** and **2** were prepared via two equally expedient routes (Scheme 1). Benzoxazines **6**, sourced commercially or synthesized from the corresponding aminophenols **5** via cyclization with chloroacetyl chloride followed by borane reduction of the cyclic amide, were reacted with thiocarbonyldiimidazole under microwave irradiation followed by ammonium hydroxide quench to yield benzoxazinethioureas **7**. Thiazole formation via urea-bromoketone condensation with brominated cyclic di-ketone **8** or keto-lactam **9**, synthesized via bromination of 5,5-dimethyl-cyclohexane-1,3-dione or 6,6-dimethyl-piperidine-2,4-dione, respectively, yielded the desired compounds **10** (Route A). Alternatively, formation of 2-bromo-thiazoloketone **11** or 2-bromothiazololactam **12** via condensation of thiourea with **8** or **9** and subsequent Sandmeyer transformation, followed by nucleophilic displacement with benzoxazines **6** yielded the desired compounds **10** (Route B). Friedel–Crafts acetylation of **4** with acetic anhydride gave **13**. Suzuki reaction of 6- and 7-bromobenzoxazines **10e** and **10h** with phenyl pinacolatoboronate ester gave **14** and **15**, respectively. Suzuki coupling of 10 h with cyclopropyl-pinacolato boronate ester yielded **16**. Lithium-halogen exchange of 10 h followed by DMF quench and reductive amination with dimethylamine gave **17**. 7-Pyrido[1,4]oxazine **19** was synthesized through nucleophilic displacement of **12** with 4-amino-3-hydroxypyridine **18**, followed by cyclization with 1,2-dibromoethane (Scheme 2). 8-Pyrido[1,4]oxazine **21** was synthesized from 3-amino-2-hydroxypyridine **20** in a manner analogous to Route B used for benzo[1,4]oxazines **10**. These compounds were tested against a number of different isoforms of PI3K (Table 1).

Unsubstituted benzoxazine analogue **3** showed a 2-fold increase in γ isoform activity whilst retaining δ isoform activity. A similar effect was observed in the lactam analogue **4**, although in this case a slight drop in δ isoform activity was observed. Pyridyl-fused analogues (**19**, **21**) were not tolerated, presumably due to an unfavourable electronic effect of the pyridine ring on the backbone-binding morpholine oxygen. As expected, simple substitution at the 8-position of the benzoxazine ring (**10a**, **10b**) severely compromised activity due to the steric constraints of the PI3K pocket. Substitution at the 6- and 7-positions was generally well

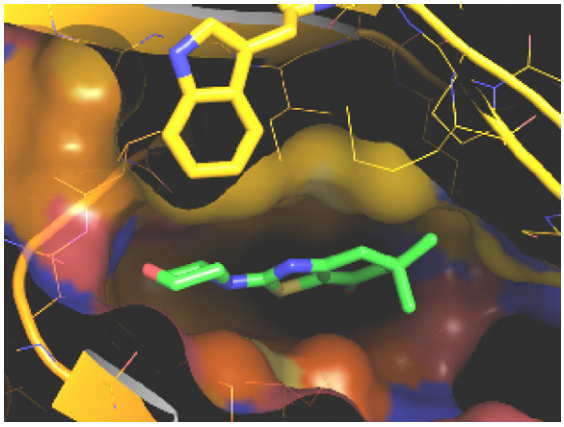
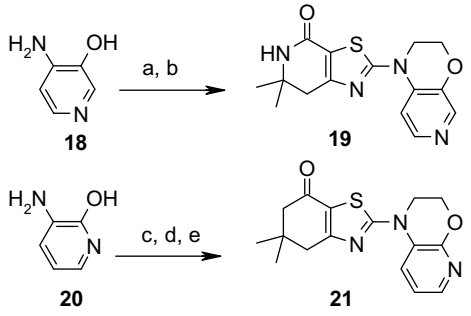
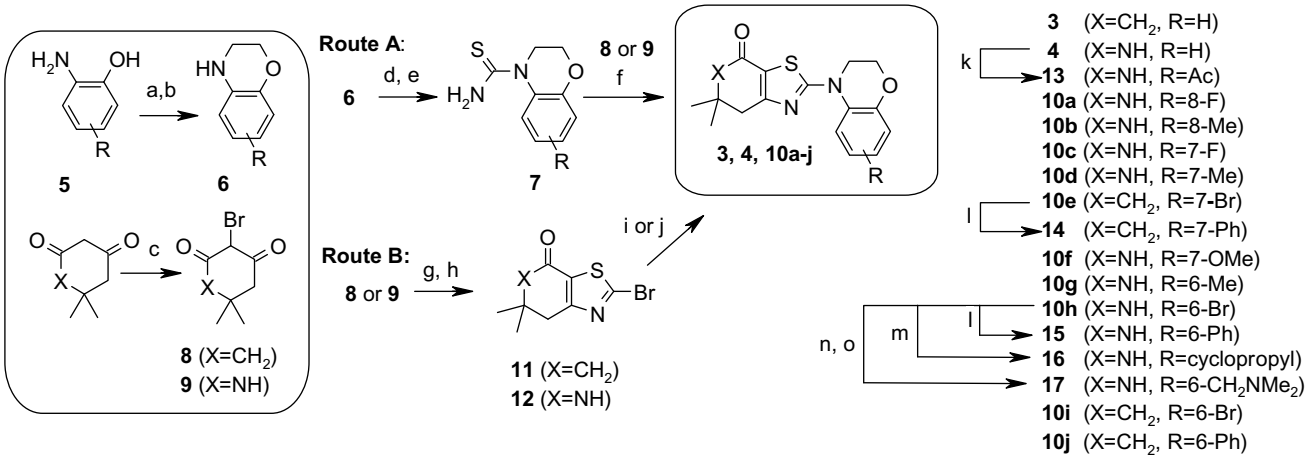


Figure 1. Crystal structure of 1 (green) bound to PI3Kγ (human) demonstrating the availability of TRP812 residue (yellow) for a possible edge-face interaction.



Scheme 2. Reagents and conditions: (a) **12**, $^{i}Pr_2NEt$, MeOH, 80 °C, 38%; (b) 1,2-dibromoethane, K_2CO_3, DMSO, 100 °C, 35%; (c) ClC(=O)CH_2Cl; DCM, $^{i}Pr_2Net$; (d) BH_3, THF, 66% over 2 steps; (e) **11**, NaO^{t}Bu, Pd($^{t}Bu_3P)_2$, 140 °C, 10%.



Scheme 1. Reagents and conditions: (a) ClC(=O)CH_2Cl, 1,2-DME, $^{i}Pr_2NEt$, 80 °C; (b) BH_3, THF, 80 °C, 21–90% over 2 steps; (c) NBS, MeCN, 70 °C, 88–95%; (d) thiocarbonyl-1,1-diimidazole, THF, 120 °C; (e) NH_4OH, H_2O/THF, rt, 30–82% over 2 steps; (f) THF, $^{i}Pr_2NEt$, rt, 51–92%; (g) $H_2NC(=S)NH_2$, $^{i}Pr_2NEt$, THF, 80 °C; (h) $CuBr_2$, ^{t}BuONO, MeCN, rt, 69% over 2 steps; (i) **6** $^{i}Pr_2NEt$, ^{i}PrOH, 80 °C, 5%; (j) **6**, Pd($^{t}Bu_3P)_2$, PhMe, NaO^{t}Bu, 140 °C, >95%; (k) $AlCl_3$, DMF, Ac_2O, 70 °C, 86%; (l) $Pd(PPh_3)_4$, Na_2CO_3, THF/H_2O, $PhB(OH)_2$, 120 °C; (m) $Pd(PPh_3)_4$, K_3PO_4, THF/H_2O, cyclopropylboronic acid pinacolato ester, 120 °C, 26%; (n) ^{n}BuLi, THF, DMF −78 °C, 45%; (o) $HNMe_2$, $PhSiH_3$, Bu_2SnCl_2, THF, 120 °C, 7%.

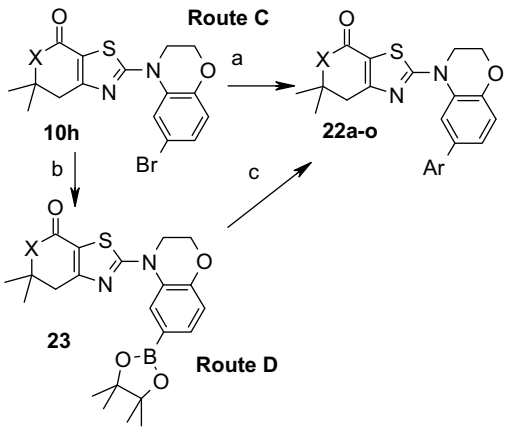
Table 1
IC_{50} values of substituted 1-thiazolyl-[2,3]-dihydrobenzoxazine analogues against various PI3K isoforms[a]

Compound	X	R	Route	PI3K (IC_{50})			
				δ	γ	α	β
1	CH_2	—	—	701	1660	1333	693
2	NH	—	—	722	5285	2372	450
3	CH_2	—	B	614	829	676	511
4	NH	—	A	1631	959	nd	1226
10a	NH	8-F	A	11,240	16,840	nd	11,690
10b	NH	8-Me	A	15,280	nd	nd	nd
10c	NH	7-F	A	783	357	nd	1245
10d	NH	7-Me	B	605	531	nd	1621
10e	CH_2	7-Br	A	549	3130	2847	5344
10f	NH	7-OMe	A	230	440	1131	1593
10g	NH	6-Me	A	357	617	1812	964
10h	NH	6-Br	A	153	321	1108	1625
10i	CH_2	6-Br	A	249	829	1972	1919
10j	CH_2	6–Ph	B	80	854	2666	7991
13	NH	6-Ac	—	366	318	nd	1448
14	CH_2	7-Ph	—	1050	>20,000	>20,000	nd
15	NH	6-Ph	—	50	80	nd	3656
16	NH	6-cyclopropyl	—	155	210	nd	834
17	NH	6–CH_2NMe_2	—	7574	>20,000	>20,000	10,320
19	NH	—	—	>20,000	nd	nd	nd
21	CH_2	—	—	5731	6654	11,330	5309

[a] Values are quoted in nM, and are means of three experiments (nd, not determined).

tolerated, and a significant increase in activity against the δ and γ isoforms was observed for compounds featuring 6-alkylation (**16**) and 6-arylation (**10j, 15**). Interestingly, 7-arylation resulted in a significant loss in activity, particularly against the PI3Kγ isoform (**14**). Substitution at the 5-position of the benzoxazine ring was not explored as it was expected that the resulting steric clash between a 5-substituent and the bicylic thiazole moiety at the 4-position would result in disruption of planarity of the core scaffold with subsequent loss of activity. In general, activity against the α and β isoforms of PI3K remained constant across the series (between 1 and 2 μM), with compound **3** having the most balanced "pan"-isoform activity and 6-aryl analogue **15** demonstrating best selectivity for δ and γ isoforms over the β isoform.

The significant increase in activity against the δ and γ isoforms afforded by aromatic substitution at the 6-position of the benzoxazine scaffold prompted further investigation through synthesis of a series of 7-heteroaryl analogues. 6-heteroaryl-benzo[1,4]oxazine analogues were synthesized via microwave-promoted Suzuki coupling as shown in Scheme 3 (**22a–o**). These compounds were tested for their activity against PI3Kδ and γ isoforms (Table 2).

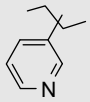
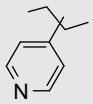
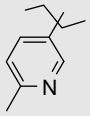
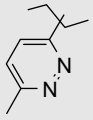
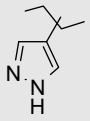
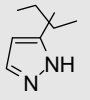
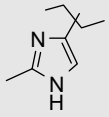
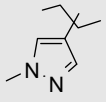


Scheme 3. Reagents and conditions: (a) $ArB(OR)_2$, $Pd(PPh_3)_4$, K_3PO_4, DME:H_2O, 140 °C; (b) $Pd(dppf)Cl_2$, bis-pinacolatodiborane, THF, KOAc, 125 °C, 64%; (c) ArBr, $Pd(PPh_3)_4$, K_3PO_4, DME:H_2O, 140 °C.

Most heteroaromatic groups were well tolerated, and in certain cases such as pyrazoles **22i** and **22n**, a modest increase in activity was observed over the parent 6-phenyl analogue **15**. Simple methylation of the groups was also tolerated, although in the case of **22m** a large loss in activity was observed, presumably due to sterically induced twisting out of plane of the aryl–aryl bond. In general activity against the α and β isoforms remained between 5- and 10-fold lower than the corresponding δ and γ isoform activity. In a selectivity screen of 240 kinases, compound **22d** demonstrated >80% inhibition at 10 μM for 2.5% of the panel. A further 8% of the panel was inhibited at between 50% and 80%. Compound **22n** demonstrated similar levels of selectivity in a screen against 50 kinases.[11] The only kinase against which both **22d** and **22n** demonstrated off-target activity was Pim-1.

Several compounds from this series were further profiled in a PI3Kδ/γ-driven cellular assay monitoring inhibition of superoxide production by fMLP-stimulation of TNFα-primed human neutrophils,[12] and various in vitro PK properties were determined (Table 3). The increase in potency observed in the enzyme assays for simple benzo[1,4]oxazine **3** and 6-phenyl substituted analogues **10j** and **15** over hit compounds **1** and **2** translated into the cellular assay, confirming that this series of compounds was capable of cell penetration. 6-Heteroaryl substituted compounds **22d**, **22n** and **22o** displayed a 5-fold increase in cellular potency relative to the parent 6-phenyl derivatives **10j** and **15**. The difference in cellular potency exhibited by compounds **22n** and **22o** suggests that the dihydrothiazolopyridone core scaffold is less cell permeable than the corresponding dihydrobenzothiazolone scaffold. In vitro pharmacokinetic profiling indicated that the benzoxazine compound **3** is considerably more prone to both microsomal and hepatocytic clearance than the parent morpholine hits (**1, 2**). Fortunately, substitution at the 6-position with aryl and hetroaryl groups significantly reduced the in vitro clearance of the compounds. The thiazololactam-analogues **22d** and **22n** had approximately 2-fold lower microsomal and hepatocytic clearance in vitro than the thiazoloketo counterparts (**22e, 22o**). This may be associated to a difference in log *D* or the decreased metabolic liability of an amide moiety relative to the ketone moiety. The low-moderate in vitro microsomal and hepatocytic clearance exhibited by compounds **22d, 22g** and **22n** led us to investigate their in vivo pharmacokinet-

Table 2
IC_{50} values of 6-heteroaryl 4-thiazolyl-[2,3]-dihydrobenzoxazine analogues against various PI3K isoforms[a]

Compound	X	Ar	Route (Yield)	PI3K (IC_{50})			
				δ	γ	α	β
22a	CH_2		C[b] (32%)	74	258	677	2001
22b	CH_2		C (63%)	20	113	172	376
22c	CH_2		C (24%)	94	159	825	1048
22d	NH		C (30%)	21	28	116	452
22e	CH_2		C (45%)	26	73	164	308
22f	NH		D (68%)	123	189	791	623
22g	NH		D (37%)	139	107	738	1325
22h	NH		C[c] (29%)	81	163	736	727
22i	NH		C[d] (74%)	16	22	89	156
22j	CH_2		C (34%)	17	77	188	298
22k	NH		C (60%)	90	145	917	1470
22l	NH		D (14%)	51	107	636	325
22m	NH		D (4%)	285	819	2900	3521
22n	NH		C (70%)	32	78	295	540
22o	CH_2		C (16%)	49	52	294	336

[a] Values are quoted in nM, and are means of three experiments.
[b] 6-Chloropyridyl-2-boronic acid used, 6-Cl removed via 10% Pd on C, cyclohexene, EtOH, 120 °C.
[c] *N*-SEM-imidazole-5-boronic acid used in crosscoupling, SEM removed in workup.
[d] *N*-Boc-pyrazole-4-boronic acid used in cross coupling, Boc removed in workup.

Table 3
Cellular potency and in vitro PK properties of key compounds

Compound	fMLP[a] (IC_{50})	ClMic[b] (rat)	ClMic[b] (human)	ClHep[c] (rat)
1	1660	6	0	7
2	2965	nd	1	1
3	759	220	47	162
10j	267	15	18	nd
15	573	39	21	nd
22d	57	17	25	7
22e	nd	25	48	nd
22g	220	8	10	3
22n	111	13	13	0
22o	44	39	36	8

[a] Assay monitoring inhibition of cellular superoxide production in human neutrophils,[9] values are quoted in nM.
[b] Compound concentration 0.5 μM, values quoted in μL/min/mg protein.
[c] Compound concentration 2.0 μM, values quoted in μL/min/mg protein; nd, not done.

Table 4
In vivo PK analysis and pharmacological efficacy of lead compounds in Han–Wistar rats[a]

Compound	C_{max} (ng/mL)	AUC (ng h/mL)	Cl_{iv}[b] (mL/min/kg)	*F*%	ED_{50}[c] (mg/kg)
22d	649	2884	—	—	15
22g	185	2471	13	66	—
22n	1216	6162	7	97	25

[a] Dosed at 3 mg/kg po.
[b] Dosed at 1 mg/kg iv.
[c] Anti-CD3 induced IL2 release in male Lewis rat; nd, not done.

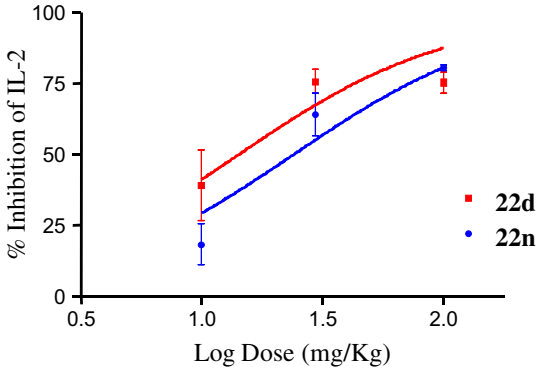


Figure 2. Dose–response for **22d** and **22n** in an anti-CD3 antibody induced IL2 release in male Lewis rats (values: mean ± SEM; $n = 8$).

ics and efficacy. Intravenous and oral dosing of **22g** and **22n** identified that the low in vitro clearances of these compounds translated well in vivo affording high bioavailabilities for both compounds (66% and 97%, respectively). All compounds exhibited high oral exposure (Table 4). Compounds were tested in a model of acute T-cell activation by anti-CD3 antibody treatment in male Lewis rats. Activation of T-cells can be measured by release of IL2 into blood, which was inhibited by both compounds **22d** and **22n** (Fig. 2).[13] Oral dosing of **22d** and **22n** inhibited IL2 release with ED_{50}'s of 15 and 25 mg/kg, respectively.

In conclusion, a novel series of multi-isoform PI3K inhibitors with an activity bias towards the δ and γ isoforms have been developed. Significant enzyme and cellular potency has been achieved across the series, and compounds with acceptable in vitro DMPK properties, good oral exposure and efficacy in a primary pharmacological model of inflammation have been identified. Further optimization of the selectivity and pharmacological profile of this compound series is ongoing.

Acknowledgments

The authors acknowledge the significant contributions of the DMPK, molecular biology and CADD groups at UCB Cambridge in helping carrying out this research.

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10. PDB ID code 3DPD.
11. Kinases inhibited by **22d** (10 μM) at >80% were TSSK1, Pim-1, CaMKII (3 isoforms) and CK1. Interestingly **22d** showed no activity against mTOR which is a frequent co-target of PI3K inhibitors (mTOR IC_{50} > 15 μM). Compound **22n** (10 μM) demonstrated >50% inhibition against 2 kinases from a panel of 50 (Pim-1 and SAPK2a).
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