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Cis-Amide isosteric replacement in thienobenzoxepin inhibitors of PI3-kinase

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ABSTRACT

Substructural class effects surrounding replacement of a 'cis' *N*-methyl aniline amide within potent and selective thienobenzoxepin PI3-kinase inhibitors are disclosed. While a simple aryl to alkyl switch was not tolerated due to differences in preferred amide conformation, heterocyclic amide isosteres with maintained aryl substitution improved potency and metabolic stability at the cost of physical properties. These gains in potency allowed lipophilic deconstruction of the arene to simple branched alkyl substituents. As such, overall lipophilicity-neutral, MW decreases were realized relative to the aniline amide series. The improved properties for lead compound **21** resulted in high permeability, solubility and bioavailability.

As a result of our effort toward discovery of novel PI3-kinase inhibitors for oncology applications,[1] we recently disclosed an HTS derived thienobenzoxepin series (compounds **1**, Fig. 1A).[2] As part of this disclosed work, we described aniline amide substituents (R1) that improved clearance and potency. Herein, work directed toward replacement of the aniline amide is presented. The rationale for this replacement was three fold: (1) to improve clearance as *N*-dealkylation and amide hydrolysis were major identified metabolites in vitro; (2) to reduce risk of reactive metabolite generation through above-said metabolism;[3] and (3) to improve binding efficiency through conformational restriction and targeted interactions in the affinity pocket of PI3Kα.

The metabolic instability and potential for aniline release in this series is evidenced by experiments in human liver microsomes (HLM). Strikingly, 93% of the 132 compounds synthesized were labile in HLM (predicted Cl_{hep} > 14.2 mL/min/kg) when oxidative mechanisms of metabolism were enabled (+NADPH, Fig. 1A).[4] Although several potential metabolic hot spots exist for oxidation (for example: *N*-methyl group, aliphatic methylenes), we believed this instability was largely a result of amidase-mediated hydrolysis of the aniline amide. In support of this hypothesis, 59% of the same group of compounds (n = 132) had moderate or worse metabolic stability in HLM even in the absence of NADPH which presumably limited oxidative metabolism (Fig. 1A, -NADPH).

As expected based on the known binding mode of these inhibitors (Fig. 1C), simple replacement of the 2-chlorophenyl ring with cycloaliphatic (**4**) or branched alkyl substituents (**3**) was ineffective (Fig. 1B, >125-fold and 55-fold loss in biochemical potency, respectively). Interestingly, compounds such as **5** bind to PI3Kγ with the amide in an '*anti–cis*' orientation (Fig. 1B, conformation A; Fig. 1C, cyan color): the carbonyl and methyl group pointing toward the polar affinity pocket while the 2-chlorophenyl ring is directed toward a lipophilic pocket formed by residues in the *p*-loop. The favorable binding energy of **2** relative to **3** and **4** can be partially explained through small-molecule conformational analysis (Fig. 1B). *Trans* rotamers of **2** (R = 2–Cl–C_6H_4) are calculated to be 1.6–3.5 kcal/mol higher in energy relative to *cis* rotamers (DFT/B3LYP(6-31G**), vacuum). This somewhat surprising preference for *cis*-orientation of *N*-methyl aniline amides in the solid state has been well-noted in the literature.[5] These observations are in contrast to the typically expected *trans* rotamer that is favored for typical *N,N*-dialkyl amides (i.e., **3** and **4**, lowest energy rotamer calculated to be rotamer B). Also of note is the preference for alignment of the thiophene sulfur with the carbonyl oxygen (i.e., rotamer D is calculated to be 0.7 kcal/mol lower energy than A for compound **2**).[6] Because aliphatic replacements of the 2-haloaryl group were not tolerated, we sought to examine isosteric replace-

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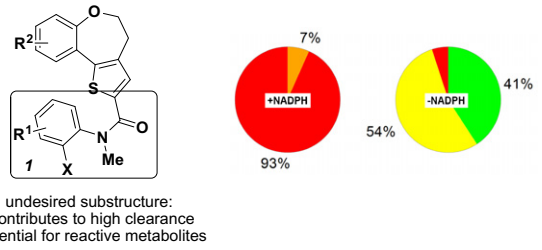
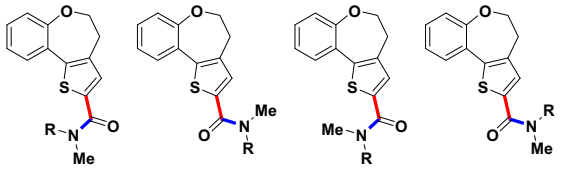
A. Binned HLM Cl_{hep} (+/- NADPH)



undesired substructure:
-contributes to high clearance
-potential for reactive metabolites

B. Calculated relative energies for select rotamers (kcal/mol)



	relative rotamer energies (kcal/mol):				
compound	A	B	C	D	PI3Kα IC_{50}
R= 2-Cl-C_6H_4 (**2**)	0.72	2.30	3.47	0	16 nM
R= ^{i}Pr (**3**)	1.15	0	1.09	0.66	> 2 μM
R= cyclohexyl (**4**)	1.56	0	1.07	0.66	885 nM

C. X-ray structure of compound *5* in PI3Kγ aligned with GDC-0980.

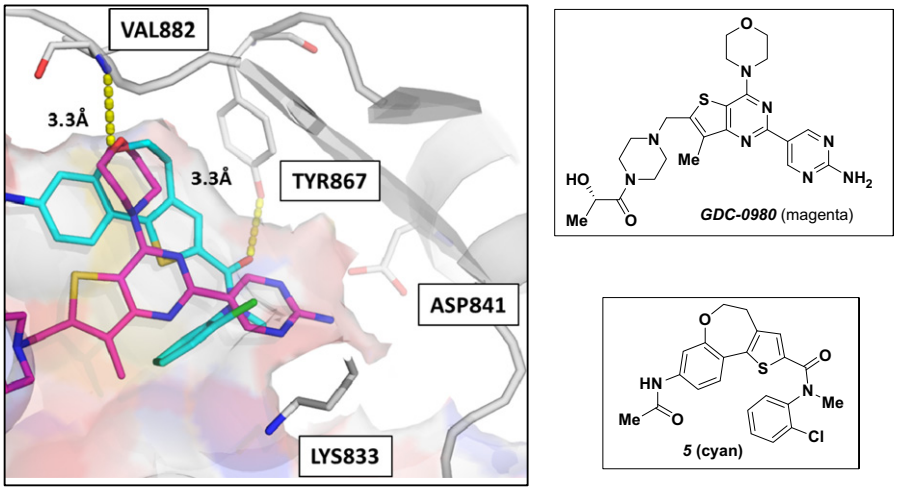


Figure 1. (A) Thienobenzoxepin PI3K-inhibitors with *N*-methyl aniline amide substructure are metabolized in human liver microsomes in the presence and absence of NADPH (132 compounds, bin description: Cl_{hep} 0–6.2 (green); 6.3–14.2 (orange); >14.2 mL/min/kg (red)). (B) Relative energy calculations for compounds **2**, **3**, and **4** (DFT/B3LYP-6-31G**, vacuum). (C). Crystal structure of PI3Kγ with **5** (cyan, PDB-ID-3R7R), thienopyrimidine GDC-0980 (magenta, PDB-ID-3TL5) aligned.[8] Solvent accessible surface is shown along with key PI3Kγ residues. Compound **5** binds with a *cis*-orientation of the *N*-methyl aniline amide and overlay with GDC-0980 indicates room for heterocyclic replacement. Yellow dashed lines indicate potential polar interactions; distances are indicated with black text.

ments of this *cis*-bound amide.[7] More specifically, we envisaged conformational restriction through cyclization of the *N*-methyl amide to 5-membered heterocycles. Although a potential detriment to physicochemical properties (increased lipophilicity and aromatic ring count), we hoped that an increase in potency would result from conformational restriction or through targeted interactions with the affinity pocket. Unoccupied volume and potential targetable interactions were evident based on overlay with alternative classes of PI3K inhibitors such as clinical PI3K inhibitor GDC-0980 (Fig. 1C, magenta, note proximity of TYR867, ASP841, and LYS833).[8] In turn, it was hoped that significant potency enhancement would allow eventual removal/deconstruction of the lipophilic 2-halo aromatic ring that was not possible in the amide series.

Heterocylic amide isosteres were designed to maintain an H-bond accepting atom adjacent to the thiophene–heterocycle biaryl linkage (postulated water mediated interaction with TYR867) and to accept an H-bond from the sidechain of LYS833 and/or donate an H-bond to the sidechain of ASP841. A selection of analogs prepared with either 2-chlorophenyl or 2,4-diflurophenyl substitution is shown in Table 1 (*N*-methyl aniline amide analogs **2**, and **6–8** included for comparison). Meeting our initial desire for maintenance/improvement in biochemical potency, direct isosteric replacement of the N-Me-amide with various 5-membered heterocycles was successful. In fact, many compounds prepared were more potent than we could accurately determine under our PI3Kα IC_{50} assay conditions ([PI3Kα] = 0.5 nM). Because of this, we commonly examined the biochemical potency of analogs without carboxamide substitution at the 8-position to gauge the effect of isosteric replacements on intrinsic affinity for PI3Kα. 4-Aryl-1,2,4-triazoles (**9**/**10**) were designed to only maintain the water-mediated H-bond interaction with TYR867 without adding additional polar interactions. However, compound **10** had improved biochemical and cell potency (measured phosphorylated pAKT-S473 in PC3 cells) relative to its closest aniline amide comparator **7**. 1-Aryltetrazole (**11**), 5-aryloxazole (**12**), and 1-aryl-1,2,4-triazoles (**13**/**14**)[9] were designed to accept an H-bond from LYS833 and all were well-tolerated relative to their closest *N*-methyl ani-