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Discovery of a Potent Class of PI3Kα Inhibitors with Unique Binding Mode via Encoded Library Technology (ELT)

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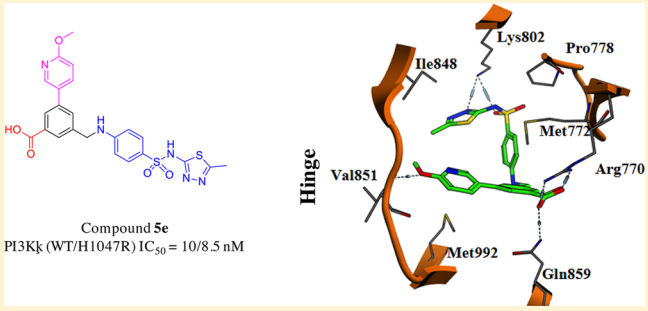
Supporting Information

ABSTRACT: In the search of PI3K p110α wild type and H1047R mutant selective small molecule leads, an encoded library technology (ELT) campaign against the desired target proteins was performed which led to the discovery of a selective chemotype for PI3K isoforms from a three-cycle DNA encoded library. An X-ray crystal structure of a representative inhibitor from this chemotype demonstrated a unique binding mode in the p110α protein.



KEYWORDS: *Encoded Library technology, ELT, PI3Kα, p110α, PI3K p110α (H1047R)*

Despite advances in research and development, discovery of novel, safe, and efficacious small-molecule chemical entities as therapeutic agents remains a major challenge in the pharmaceutical industry. It was speculated that this might have led to a decrease in the number of small molecule drugs launched over the past decade.[1,2] One major contributor to low output in the drug discovery process is limitation of suitable chemotypes or scaffolds for medicinal chemistry program initiation.[3] DNA-encoded chemical libraries as a new hit identification platform have been explored for over a decade now.[4,5] Our group has recently reported on the application of encoded library technology (ELT) as a novel hit and lead discovery platform complementary to existing methods.[6−13] In pursuit of an isoform and/or mutant selective class of phosphoinositide 3-kinase α (PI3Kα) inhibitors, ELT was utilized to discover additional chemotypes to our in-house existing scaffolds. In this publication, we report one class of potent and selective PI3Kα inhibitors discovered through an ELT endeavor.

A few classes of small molecule pan-PI3K inhibitors are reported in clinical development for oncology applications. Some of these pan-inhibitors include ZSTK-474,[14] GDC-0941,[15] XL-147,[16] BKM-120,[17] and CH-5132799.[18] Selective inhibitors such a INK-1117[16] and NVP-BYL719[19] have been reported that target PI3Kα, the most frequently mutated kinase in human cancer,[20] making it a promising target in cancer therapy. A frequent mutation in the p110α kinase domain is H1047R.[21] Recently we described the discovery a pan-PI3K inhibitor for clinical evaluation.[22] In an effort to identify a novel and potentially isoform and/or mutant selective class of PI3K p110α inhibitors, we performed an ELT selection against a set of libraries. The process of affinity selection was performed against both His-tagged PI3Kα wild type and the mutant H1047R. The His affinity tags allowed for the target to be isolated by immobilization on the solid matrix, PhyNexus IMAC (immobilized metal affinity chromatography) resin tip. Once the target was immobilized, it was exposed to the library and nonbinding library members were removed through a simple resin wash. This was repeated twice (three rounds total) after which the binders were eluted by heat denaturation of the resin bound target, followed by PCR and DNA sequencing. For the PI3Kα wild type we obtained 76 457 unique sequences, and for the PI3Kα mutant (H1047R) we obtained 47 060 unique

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sequences. The outcome was analyzed to determine the binding library members that were specific to the proteins.

Selection of a preferred scaffold was found from one of our well established libraries that was designed around three cycles of chemistry to provide a library (DEL-A) with a complexity of 3.5 million compounds. As described in Figure 1, the library is

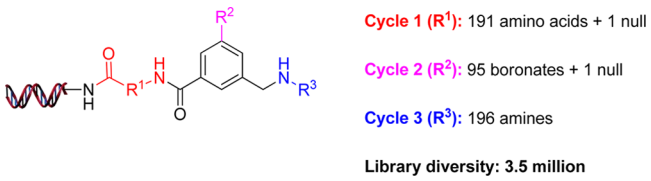


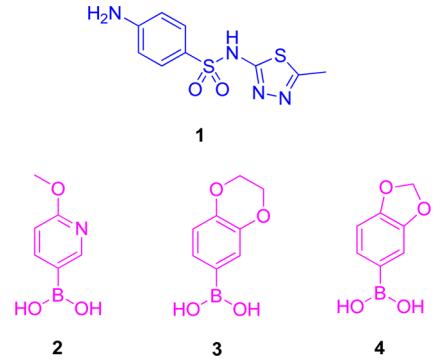
Figure 1. Design of DEL-A: "null" indicates that the reaction was carried out without addition of the desired BB amino acid (R^1) or boronate (R^2).

composed of 191 amino acids at cycle 1 (R^1), 95 boronates at cycle 2 (R^2), and 196 amines at cycle 3 (R^3). The R^1 residues were utilized as the attachment point to the ELT headpiece DNA through their carboxylate group. The details of the library synthesis will be the subject of a different publication in the near future.

A cubic scatter plot in which each axis represents a cycle of diversity in the library was used to analyze and visualize the selected library members for His-tagged PI3Kα wild type and the mutant (H1047R). After removal of the low copy-number molecules from the analysis, the most selected and highly enriched families were observed to be of the same scaffolds and chemotypes with copy counts greater than 20-fold above the background (Figure 2), indicating potential for lack of mutant selective inhibitors.[6] The feature was confirmed by repeating the PI3Kα mutant (H1047R) selection against the same library in the presence of ZSTK474,[14] a known and potent ATP competitive inhibitor. The cube analysis of the data demonstrated that the previously selected feature (family) was competed away in the presence of a known inhibitor, leading us to conclude that the selected feature was interacting with PI3Kα at the ATP binding site. We then initiated off-DNA feature confirmation of the original PI3Kα mutant (H1047R) selection.

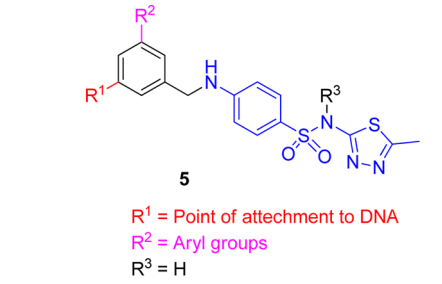
The visualizations in Figure 2 show the population after removal of the sequences that occurred fewer than 2 times to simplify data analysis. This analysis revealed the preference for one main family of compounds over the plane within the cube represented by a 4-amino-*N*-(5-methyl-1,3,4-thiadiazol-2-yl)-benzenesulfonamide building block (**1**, Scheme 1), a cycle 3 based monosynthon. Within the plane, one observes three

Scheme 1. Selected Building Blocks at Cycle 3 (1) and Cycle 2 (2, 3, and 4)



major lines based on cycle 2 boronate building blocks (**2**–**4**, Scheme 1). There is no preference for cycle 1 in the selection. The possible scaffold obtained from the selection is defined by the library backbone, building blocks **1** at cycle 3 and **2**, **3**, and **4** at cycle 2. On the basis of the feature enrichment over no target (NT) background noise, building block **2** at cycle 3 was potentially a stronger outcome than **3** or **4**. A representation of the active pharmacophore is illustrated in Scheme 2.

Scheme 2. Inhibitor Pharmacophore Defined by Selection Analysis



To confirm the feature activity, a handful of compounds needed to be synthesized off-DNA after feature analysis. In off-DNA small molecule synthesis, a choice was made of where to truncate the linker to the encoding DNA. Since the selection analysis indicated that cycle 1 was not selected for, two representative amines were chosen in the first round of off-

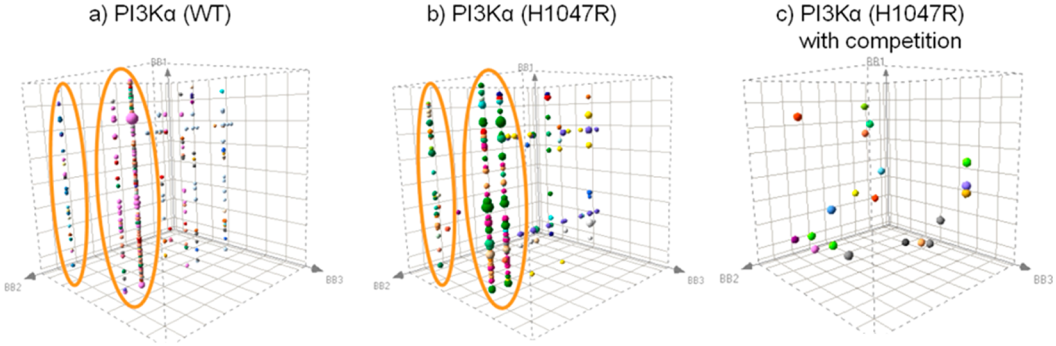


Figure 2. PI3Kα wild type selection (left), mutant (H1047R) selection (middle), and mutant selection with ZSTK474competitor (right). Library members with a single copy were removed to simplify visualization.

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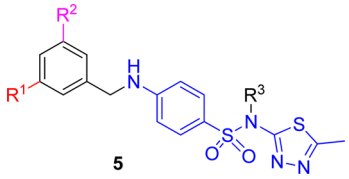
DNA synthesis in place of the corresponding amino acids. In addition, analogs were planned in which cycle 1 was eliminated (**5a**–**f**, Table 1). The representative set of the selected

Table 1. Proposed Target Compounds for Off-DNA Synthesis and Structure–Activity Relationship Exploration



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Compound #	R^1	R^2	R^3
5a			H
5b			H
5c	$-CO_2Me$	-I	H
5d	$-CO_2Me$		H
5e	$-CO_2H$		H
5f	-H		H
5g	$-CO_2H$		H
5h	$-CO_2H$		H
5i			Me
5j			H

molecules was synthesized off-DNA following the synthetic strategy in Scheme 3. The synthetic strategy was planned as such to establish the selected and conserved moiety in the scaffold earlier in the synthesis to allow for testing truncated analogs and intermediates and fully enumerated compounds. Reductive amination of the selected aniline with aldehyde **6** followed by Suzuki cross-coupling of the resultant aryl iodide product with an arylboronic acid afforded **7**. Saponification of an ester in **7** gave the acid **8**, which after coupling with the desired amine afforded the final compounds **5**.

The off-DNA synthesized compounds were assayed for their activity against PI3Kα to confirm the activity of the selected feature. The data obtained for all compounds are reported in Table 2. Fully enumerated compounds **5a** and **5b** showed excellent activity against PI3Kα wild type and mutant H1047R.

Table 2. Feature Activity (IC_{50}, nM) Confirmation versus the PI3K Isoforms[a]

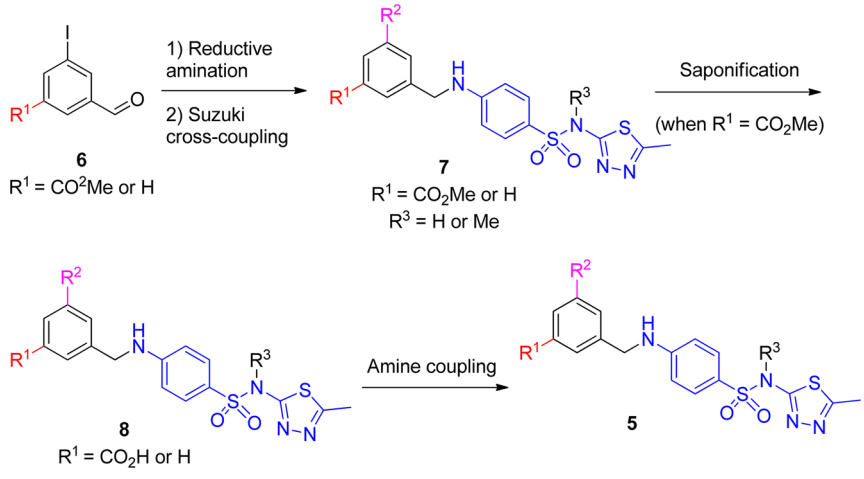
compd	α (WT)	α (H1047R)	β	δ	γ
5a	6.5	13.0	700	10	4.4
5b	9.6	11.0	398	4.0	10
5c	>25 000	13 000	–	–	–
5d	46.0	30.0	–	–	–
5e	10	8.5	149	59	2.6
5f	102	–	765	44	52
5g	12.6	–	125.9	63.1	2.0
5h	39.8	–	501	199	4.0
5i	2000	>10 000	>10 000	2000	>10 000
5j	16	16	790	20	25

[a]"–" indicates that compounds were not tested in biochemical assay.

The methyl ester **5d** obtained similar activity as **5a** and **5b**, confirming the selection result that cycle 1 is not necessary for scaffold activity. The intermediate lacking R2 (**5c**) showed no activity against PI3Kα, a strong indication that the methoxypyridine moiety is critical for inhibitor activity. Elimination of cycle 1 and replacement of the carboxamide with a free carboxylic acid moiety afforded **5e**, with similar or better PI3Kα activity than the corresponding **5a** and **5b**. Removal of the carboxylic acid in **5e** afforded **5f** with a 10-fold decrease in PI3Kα activity, indicating a potential interaction between the carboxylic acid moiety and the enzyme active site. As mentioned earlier, our selection data showed two additional building blocks selected at cycle 2. These two building blocks are boronates **3** and **4**. The corresponding final carboxylic acids for these two boronates were synthesized analogous to the synthesis of **5e** (**5g** and **5h**). Both **5g** and **5h** showed similar PI3Kα activity as **5e**. PI3K isoform assay data for the final carboxylic acids indicate 5- to 10-fold selectivity for α over β and δ. In general, the selected compounds showed no selectivity against mutant H1047R but they appear to be more selective for the γ isofom.

To further investigate the mode of the scaffold binding to the enzyme active site, the crystal structure of **5e** bound to the ATP

Scheme 3. Synthesis of the Target Off-DNA Compounds



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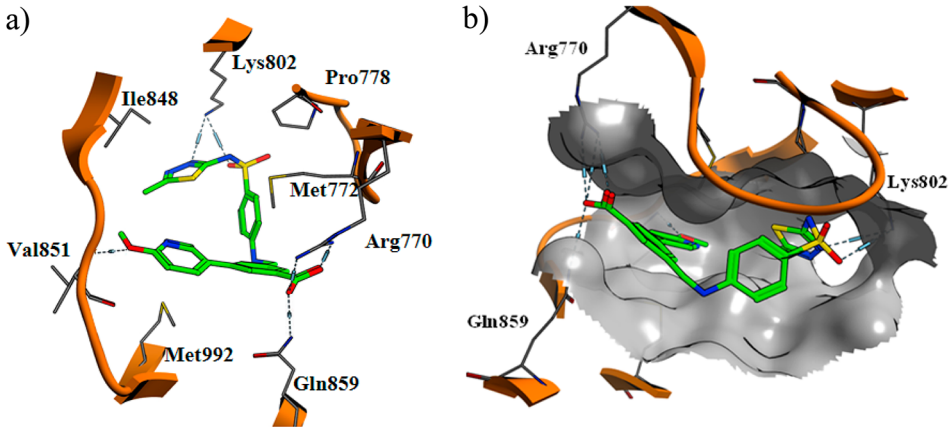

Figure 3. X-ray structure of **5e** bound to PI3Kα: (a) key interactions of the inhibitor in the active site depicted; (b) surface representation of the active site with the bound inhibitor demonstrates steric packing and interactions with the residues of the P-loop.

site of PI3Kα was obtained (Figure 3). As anticipated from the selection preference for the methoxypyridine group, the methoxy group is a hinge binder with a classic hydrogen bond interaction between the methoxy oxygen with −NH of Val-851. The thiadiazole and phenyl sulfonamide moieties bypass the Ile-848 "gatekeeper" and Tyr-836 to access the "affinity pocket" of the active site. This mode of binding is different from a canonical type I kinase inhibitor binding mode, which tends to maximize interactions further into the back pocket by sterically packing against and making interactions with the residues in the vicinity of the gatekeeper Ile-848.[23] The unusual binding mode of **5e** allows extensive interactions with residues in the front of the pocket such as Arg-770 and Met-772 from the P-loop and Gln-859 from the C-terminal lobe of the catalytic domain. Since the residue composition of the P-loop or the glycine-rich loop of protein kinases is markedly different from lipid kinases, the ELT scaffold is likely to be selective for PI3K if these differential interactions are maximized. The inward turn of the phenyl sulfonamide moiety, facilitated by the methylamine linker, places it in proximity to the catalytic component of the enzyme, specifically the catalytic Lys-802 residue. The sulfonamide and thiadiazole groups establish two major hydrogen bonds with $-NH_3^+$ of Lys-802. One of these interactions is through the thiadiazole nitrogen, and the second interaction is through the oxygen atom of the sulfonamide group (Figure 2a). Additionally, pK_a calculations on **5e** suggest that at physiological pH of 7.4, the major species (83%) carries a negative charge on the deprotonated nitrogen atom of the sulfonamide group.[24,25] The presence of a negative charge on this moiety in the major species is likely to result in favorable charge complementarity between the inhibitor and the catalytic Lys-802, which could contribute to the low nanomolar potency of **5e** in PI3Kα. Indeed, methylation of the sulfonamide nitrogen as exemplified in **5i** resulted in significantly attenuated potency across PI3K isoforms (Table 1). Methylation of the nitrogen is also likely to result in steric clashes with residues in this region; therefore, this result is explained as a net unfavorable steric and electrostatic effect. The X-ray structure reveals that the carboxylic acid group at the para-position of the central phenyl ring is involved in a network of an ionic salt-bridge interaction with Arg-770 and a hydrogen bond with Gln-859 (Figure 2b). These strong electrostatic interactions of the carbonyl group indicate its importance in the activity of the amide and carboxylate analogs, although being solvent exposed the effect might be attenuated. Lastly, an indication that the benzylamine group picks up a hydrogen bonding interaction by acting as a H-bond donor to the backbone carbonyl oxygen of Ser-919 residue through its amine moiety and the methyl group on the thiadiazole establishes close van der Waal interactions with Tyr-836 in the back pocket of the active site.

The selection data showed no preference for the cycle 1 synthon, and as the crystal structure reveals, there are no major interactions between this region of the inhibitor and the protein. Therefore, cycle 1 likely only functions as a linker to attach the inhibitor to the DNA in the library. The DNA attachment point on the scaffold provides a handle that could be used to alter the physicochemical properties of the inhibitors.

In summary, we report here the discovery of a novel and potent class of inhibitors of the p110α catalytic subunit of PI3Kα using ELT as the lead identification technology. We have demonstrated ELT selection for disynthons (cycles 2 and 3), allowing for truncation of the selection based on a variable cycle 1. This demonstrates the power of the technology to establish preliminary internal SAR before obtaining off-DNA activity of the compounds and the utility of the selection-based internal data to guide medicinal chemistry lead optimization efforts. The ELT selection output generated a scaffold exhibiting modest isoform selectivity for PI3Kα over PI3Kβ and PI3Kδ. The X-ray crystal structure of inhibitor **5e** in PI3Kα demonstrated a unique binding mode in the ATP binding pocket with major interactions with the hinge point and the "affinity pocket" that are consistent with the selection results and off-DNA compound activity.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details for the synthesis of all the compounds and in vitro ADME and in vivo PK/PD data. This material is available free of charge via the Internet at http://pubs.acs.org.

Accession Codes

The coordinates of PI3Kα p110a in complex with **5e** were deposited under PDB code 4YKN.

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Notes

The authors declare no competing financial interest.

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