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Diversity-oriented synthesis as a strategy for fragment evolution against GSK3β.

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Diversity Oriented Synthesis; Fragment-based drug discovery; Fragment growing; GSK3β

ABSTRACT: Traditional fragment-based drug discovery (FBDD) relies heavily on structural analysis of the hits bound to their targets. Herein, we presented a complimentary approach based on diversity-oriented synthesis (DOS). This DOS-based fragment collection was able to produce initial hit compounds against the target GSK3β, allow the systematic synthesis of related fragment analogues to explore fragment-level SAR, and finally lead to the synthesis of a more potent hit compound.

Fragment-based drug discovery (FBDD) is an established approach for generating therapeutic lead molecules.¹⁻⁴ Key to the optimization process is the application of structure-based methods to guide the synthetic decision-making of growing fragment hits into more potent compounds.⁵⁻⁸ However, the strong reliance on highly resolved fragment-target structures can be limiting. Targets that do not readily lend themselves to structural guided approaches provide little recourse to FBDD.^{9,10} A strategy for evolving more potent compounds from fragments that does not completely depend on structure-based methods would be a useful tool within the FBDD arena, making the approach more broadly applicable.

There have been recent discussions related to evolving fragment scaffolds without structural guidance. For example, Konrat and coworkers described a strategy for using protein meta-analysis and ligand-based NMR techniques to generate leads against two targets without 3D structures.¹¹ Similarly, Viola and coworkers reported a systematic chemistry approach to explore fragment-level structure-activity relationships.¹²

The development of other methods to evolve and optimize fragments¹³ without structural guidance would certainly expand the generality of the FBDD approach. Toward this goal, we herein describe the application of diversity-oriented synthesis (DOS) to discover and optimize fragments to the kinase GSK3 β .



Figure 1. A logical chemical synthesis strategy related to the fragments would impact optimization and modification of fragment hits. Fragments constructed using highly modular syntheses would enable rapid substitutions around a fragment core. Therefore, the generation of fragment-SAR using modular fragment syntheses would aid the fragment evolution process as a complementary approach to structure-guided approaches.

DOS involves the generation of skeletal, stereochemical, regiochemical and appendage variation using a common synthesis scheme.¹⁴⁻¹⁵ The build/couple/pair (B/C/P) strategy in DOS was developed to guide, in a modular fashion, the synthetic planning processes to efficiently generate diverse compounds.¹⁶⁻¹⁸ This produces a broader degree of variation in molecular structure in a set of compounds compared to other approaches. Importantly, this molecular diversity is produced under the aegis of one general synthesis pathway, which significantly simplifies the synthetic planning and development

Supporting information for this article is given via a link at the encor Plus Environment the document.

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throughout the discovery and lead generation phases. The merits of the B/C/P approach have been demonstrated in various high-throughput screening (HTS) applications.¹⁹⁻²¹ Here, we extend the concept of DOS towards the application of fragment-baseddrug discovery (Figure 1).

A set of 86 fragments was prepared and screened against glycogen synthase kinase 3β (GSK3 β), a serine threonine kinase which is aberrantly overexpressed in cancer and Alzheimer's disease.²²⁻²⁵ These fragments were derived from three different pathways²⁶⁻²⁸ using the DOS B/C/P approach (Figure **2a**). Accordingly, these modular routes could be exploited to generate fragment-level SAR from an identified hit.



Figure 2. a. Three different build/couple/pair pathways leading to a library of skeletally and stereochemically diverse fragments. **b**. Fragment hits from a DSF screening at 2.5 mM against GSK3 β . Compounds displaying a thermal shift ≥ 0.5 °C are shown.

As shown in Figure 2b, differential scanning fluorimetry (DSF) was used as the primary screening method for detecting fragment binding against GSK3 β . Interestingly, three compounds from the same synthetic pathway (pathway III) displayed weak thermal stabilization (Δ T) of 0.5 °C or greater (Figure **2b**).

The detailed synthesis of **1S** is shown in Figure **3a**.²⁶ Here, the build phase consisted of procuring commercially available arylnitrofluorides and α -amino esters. The appropriate arylnitrofluorides were then coupled with amino esters using S_NAr reactions to yield **1S'**. Subsequent reduction of the nitro group under mild conditions afforded the aniline, which underwent concomitant cyclization (the pairing phase) to the desired product **1S**. The enantiomeric excess of compounds produced by this synthetic pathway was verified by chiral HPLC analysis to be greater than 95% in all cases, allowing for activity comparison using compounds with equivalent enantiopurity.

Based on the B/C/P pathway described above, a series of structurally similar compounds to parent fragment **1S** was synthesized and evaluated in DSF experiments. In parallel, using ADP-GloTM assays, single point biochemical inhibitions were also performed to rank compounds based on their inhibition against GSK3β (at 1 mM). As shown in Figure **3b**, fragment **1R** (antipode of the initial hit **1S**) showed a larger Δ T and inhibition than the original fragment hit **1S** (1.7 °C and 46% inhibition at 1 mM, Figure **3b**). The variation in thermal stabilizations and inhibitions of both fragment enantiomers against GSK3β provided early validation of fragment binding.

Some preliminary SAR could also be observed from the set of piperazinone compounds synthesized. Fused pyridyl rings to the piperazinone scaffolds were found to stabilize GSK3 β more favorably than their phenyl counterparts. As shown in Figure **3b**, both enantiomeric compounds **4S** and **4R** had no measurable effect in the DSF. It was also found that the position of the nitrogen in the pyridyl rings affected the thermal stabilization of GSK3 β . Importantly, depending on the position of the pyridine-N, the extent of variation in activity and thermal stabilizations of the corresponding enantiomers was different. We hypothesize that these regioisomers bind to GSK3 β through non-identical binding conformations, accounting for differences in stabilization between antipodes. Based on the qualitative DSF and point inhibition results, fragment **1R** was prioritized for further optimization.



Figure 3. a. Synthesis of **1S** as a representation of the B/C/P pathway. **b.** DSF and biochemical assay results of analogues of **1S. c.** Chemical modifications on **1R** to identify key binding interactions and probe potential pocket for fragment growing. DSF assays were performed at 2.5 mM unless otherwise indicated; biochemical assays were performed at 1 mM.

Pyridinyl amides are known hinge binding motifs against kinases.²⁹ We postulated that this interaction is also present for fragment **1R** (Figure **3c**). This hypothesis was further corroborated with the synthesis of compounds **8R** and **9R**. The deletion of the amide carbonyl in **1R** abolished thermal stabilization of fragment **8R**. Additionally, removal of the hydrogen bond donor capacity by *N*-methylation of the amide nitrogen (compound **9R**) resulted in significantly lower stabilization of GSK3 β .



Figure 4 a. Saturation transfer differential (STD) NMR spectrum of **1R** against GSK3 β **b.** WaterLOGSY NMR spectrum of **1R** against GSK3 β . Both NMR techniques further validated binding of fragment **1R** against GSK3 β . **c.** Using ITC, **1R** was determined to bind against GSK3 β with $K_d = 610 \mu$ M (LE = 0.37).

At this stage, Saturation Transfer Differential (STD) and WaterLOGSY NMR experiments³⁰ were also performed to validate binding of **1R**. As shown in Figure **4**, both ligand-based NMR experiments show compound **1R** binding unambiguously towards GSK3 β , corroborating thermal shifts results. With the results in hand, Isothermal Titration Calorimetry (ITC) was then used to quantitatively evaluate the equilibrium dissociation constant (K_d) of fragment **1R**. Compound 1R was found to bind to GSK3 β with $K_d = 0.6$ mM with a desirable ligand efficiency (LE) of 0.37 (Figure **4c**).

In an effort to evaluate the importance of the chiral center residing in 1R, three analogues were prepared (Figure 3c). Oxidation of 1R afforded compound 10, therefore removing the chirality of 1R. This planar fragment resulted in diminished activity and low thermal stability as compared to 1R. Installation of a gem-dimethyl group produced compound 11, which did not provide thermal shifts equivalent to 1R. This indicated that the binding pocket cannot easily accommodate two hydrophobic groups on both faces of the fragment. Finally, to probe the size of the binding pocket, a larger isopropylcontaining analogue 12R was synthesized which showed a ΔT of 2.3 °C (0.6 °C more stable than 1R). Given this last observation, we concluded that the chiral center, in particular with the *R* configuration, was a potential growth vector to increase potency. Therefore, from the fragment SAR generated at this point, we were equipped with a rational plan to evolve the fragment into a more potent compound.

The B/C/P pathway was once again utilized to generate analogues of **1R** with larger groups from the corresponding amino ester starting materials (optimization phase). Both enantiomers were synthesized to provide side-by-side comparisons of the two stereogenic growth vectors. DSF and single point inhibition assays were used to evaluate their binding interaction and inhibitory activity, respectively.



Figure 5. A small set of compounds were synthesized, extending from a growth vector identified from previous SAR. For ranking purposes, DSF assays were performed at 1.25 mM for comparison and point inhibition biochemical assays were performed at 1 mM.

As shown in Figure 5, the binding of larger aromatic groups was accommodated in GSK3 β similarly to aliphatic groups in compounds 1R and 12R. Interestingly, an additional hydroxyl group on the phenyl ring (compound 14R) led to a significant increase in potency, leading us to surmise that a larger, electron-rich aromatic group could be tolerated. Satisfyingly, compound 15R with an indolyl substitution resulted in a 4.5 °C thermal stabilization when tested at 1.25 mM. Results from the biochemical assay conducted at a single concentration (1 mM) correlated well with the DSF results.

Finally, ITC was used to quantify the binding of **15R**, the compound which displayed the greatest thermal stabilization against GSK3 β . As shown in Figure **6a** compound **15R** bound to GSK3 β with $K_d = 9 \ \mu$ M, a greater than 60-fold improvement in binding over **1R**. Additionally, **15R** was found to inhibit GSK3 β with an IC₅₀ = 18 μ M. The enantiomer **15S** was also tested and was ~18-fold less active than **15R** (IC₅₀ = 322 μ M, Figure **6b**), a result that is reflected by the low thermal shifts observed by DSF,

As shown in Figure 6c, 15R demonstrates 50-fold selectivity against GSK3 β over the closely related kinase Cyclindependent Kinase 5 (CDK5). The selectivity profile of the chiral compound 15R against GSK3 β over CDK5 is an attractive starting point for the preparation of additional ligands with more enhanced selectivity toward GSK3 β . It is also noteworthy that compound 15R has a low molecular weight of 278 g/mol (LE of 0.33) and this provides further growth opportunities towards achieving greater potency.

In most FBDD efforts, X-ray analysis is performed at the earliest stage to guide optimization chemistry. However, it was our strategy to demonstrate that the modular B/C/P chemistry would be advantageous towards optimizing a fragment

hit. To corroborate the fragment growing approach, X-ray crystal structures of GSK3 β in complex with **1R** and **15R** were determined.³¹



Figure 6. (a) ITC K_d determination of **15R** against GSK3 $\beta K_d =$ 9.1 μ M (b) Biochemical inhibition measurements of **15R** (IC₅₀ = 18 μ M) and **15S** (IC₅₀ = 322 μ M) against GSK3 β (c) IC₅₀ measurement of **15R** and **15S** against CDK.

Consistent with our SAR analysis, 1R and 15R were found to occupy the ATP-binding site, which is at the interface between the N-terminal β-strand domain and C-terminal α-helix domain³² (see Supporting Information Figure S4). Most interactions with 15R are attributed to the hinge region (D133-T138) of GSK3ß (Figure 7). The amide NH of 15R makes a hydrogen bond with the backbone oxygen of D133. The pyridyl nitrogen forms an additional hydrogen bond with the backbone NH of V135. The structural results were consistent with our synthetically obtained SAR studies. More interestingly, the indole substituent is found to be off plane to the hinge binding region. As a result, multiple hydrophobic interactions with residues F67 and V70 are formed, contributing to the ~60-fold improvement in binding potency over the initial fragment. There is also possibility of hydrogen bond or salt bridge formation between the indole nitrogen on 15R and the side chain of D200. However, the distance between these two atoms is 3.4 Å, suggesting the interaction to be weak. In contast, the S enantiomer failed to provide these interactions in a stereochemically matched manner, leading to diminished affinity.



Figure 7. X-ray crystal structure of 1R and 15R against GSK3 β . The 2Fo-Fc (1 σ) and Fo-Fc maps (3 σ) around **15R** are shown as blue and orange meshes, respectively. Polar interactions are displayed as red dashed lines. PDB ID for **1R**: 4J71, for **15R**: 4J1R

The X-ray crystal structure of the fragment hit **1R** occupied the same site as **15R** (Figure 7). However, due to modest resolution, an unambiguous placement of **1R** in the electron density was not possible. Multiple binding modes are commonly encountered in the X-ray analysis of fragments to targets given that they may contain other energetically similar weak binding poses. Guided by SAR, our strategy enabled us to generate analogs of **1R** in a systematic fashion to produce a more potent compound when a highly resolved structure could not be achieved with the initial fragment hit. Although X-ray did not guide our chemical optimizations, we envision that our approach will also be useful with an X-ray structure of the fragment-ligand complex since the modular B/C/P chemistry can be used to prepare specific analogs suggested from the structure.

In conclusion, a set of novel fragments was generated using a DOS-driven B/C/P approach. This set of compounds was screened against GSK3 β , and from the screen the pyridyl piperazinone fragment hit **1R** ($K_d = 0.6$ mM) was identified. It is noteworthy that the stereoselective binding preference of a particular antipode of the piperazinone ring lends towards validation of fragment binding. More importantly, the modular synthesis allows access to different analogs of the fragment hit, enabling quick SAR determination around the fragment. Consequently, fragment growing was exploited through the chiral center of **1R** leading to a more potent binder **15R** ($K_d =$ 9.1 µM). Finally, both **1R** and **15R** were soaked against GSK3 β and the X-ray crystal structure revealed a successful fragment growing approach.

Based on the work described here, we contend that a modular diversity-oriented synthetic approach towards designing fragments is warranted. The ability to efficiently control the modification of a fragment core would greatly assist fragmentlevel SAR. This in turn expedites structure-guided FBDD and more crucially provides a way of fragment evolution when no structure information is available.

Supporting Information

Chemical structures and PMI analysis of DOS fragments, detailed synthesis and characterization of compounds 1 to 15, DSF, biochemical assays, NMR Spectroscopy, isothermal titration calorimetry and crystallography studies, are included in Supporting Information.

The Supporting Information is available free of charge on the ACS Publications website.

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REFERENCES

- Chessari,G.; Woodhead, A. J. Recent developments in fragmentbased drug discovery. *Drug. Discov. Today* 2009, 14, 668-675.
- (2) Congreve, M.; Chessari, G.; Tisi, D.; Woodhead, A. J. Recent developments in fragment-based drug discovery. J. Med. Chem. 2008, 51, 3661-3680.
- (3) Murray, C. W.; Rees, D. C. The rise of fragment-based drug discovery. Nat. Chem. 2009, 1, 187-192.
- van Montfort, R. L. M.; Collins, I. Fragment-Based Methods in Drug Discovery: It's the Small Things that Matter. *Curr. Top. Med. Chem.* 2009, 9, 1676-1677.
- (5) Blundell, T. L.; Jhoti, H.; Abell, C. High-throughput crystallography for lead discovery in drug design. *Nat. Rev. Drug Discov.* 2002, *1*, 45-54.
- (6) Ciulli, A.; Abell, C. Fragment-based approaches to enzyme inhibition. *Curr. Opin. Biotech.* 2007, *18*, 489-496.
- (7) Hennig,M.; Ruf, A.; Huber, W. Combining biophysical screening and X-ray crystallography for fragment-based drug discovery. *Top. Curr. Chem.* 2012, 317, 115-143.
- (8) Jhoti, H.; Cleasby, A.; Verdonk, M.; Williams, G. Fragment-based screening using X-ray crystallography and NMR spectroscopy. *Curr. Opin. Chem. Biol.* **2007**, *11*, 485-493.
- (9) Granseth,E.; Seppala, S.; Rapp, M.; Daley, D. O.; Von Heijne, G. Membrane protein structural biology - How far can the bugs take us? *Mol. Membr. Biol.* 2007, 24, 329-U323.
- (10) Torres, J.; Stevens, T. J.; Samso, M. Membrane proteins: the 'Wild West' of structural biology. *Trends Biochem. Sci.* 2003, 28, 137-144.
- (11) Henen, M. A.; Coudevylle, N.; Geist, L.; Konrat, R. Toward Rational Fragment-Based Lead Design without 3D Structures. J. Med.Chem. 2012, 55, 7909-7919.
- (12) Thangavelu, B.; Bhansali, P.; Viola, R. E. Elaboration of a Fragment Library Hit Produces Potent and Selective Aspartate semialdehyde Dehydrogenase Inhibitors. *Bioorg. Med. Chem.* **2015**, *23*, 6622-6631.
- (13) Hung, A. W.; Silvestre, H. L.; Wen, S.; George, G. P.; Boland, J.; Blundell, T. L.; Ciulli, A.; Abell, C. Optimization of inhibitors of Mycobacterium tuberculosis Pantothenate Synthetase based on group efficiency analysis. *Chem. Med. Chem.* **2016**, 5, 38-42.
- (14) Burke, M. D.; Schreiber, S. L. A planning strategy for diversity-oriented synthesis. Angew. Chem. Int. Edit. 2004, 43, 46-58.
- (15) Schreiber, S. L. Target-oriented and diversity-oriented organic synthesis in drug discovery. *Science* **2000**, *287*, 1964-1969.
- (16) Nielsen, T. E.; Schreiber, S. L. Diversity-oriented synthesis Towards the optimal screening collection: A synthesis strategy. *Angew. Chem. Int. Edit.* 2008, 47, 48-56.

- (17) Spiegel, D. A.; Schroeder, F. C.; Duvall, J. R.; Schreiber, S. L. An oligomer-based approach to skeletal diversity in small-molecule synthesis. *J. Am. Chem. Soc.* **2006**, *128*, 14766-14767.
- (18) Dandapani, S.; Marcaurelle, L. A. Current strategies for diversityoriented synthesis. *Curr. Opin. Chem. Biol.* 2010, *14*, 362-370.
- (19) Marcaurelle, L. A.; Comer, E.; Dandapani, S.; Duvall, J. R.; Gerard, B.; Kesavan, S.; Lee, M. D.; Liu, H. B.; Lowe, J. T.; Marie, J. C.; Mulrooney, C. A.; Pandya, B. A.; Rowley, A.; Ryba, T. D.; Suh, B. C.; Wei, J. Q.; Young, D. W.; Akella, L. B.; Ross, N. T.; Zhang, Y. L.; Fass, D. M.; Reis, S. A.; Zhao, W. N.; Haggarty, S. J.; Palmer, M.; Foley, M. A. An Aldol-Based Build/Couple/Pair Strategy for the Synthesis of Medium- and Large-Sized Rings: Discovery of Macrocyclic Histone Deacetylase Inhibitors. J. Am. Chem. Soc. 2010, 132, 16962-16976.
- (20) Peng, L. F.; Stanton, B. Z.; Maloof, N.; Wang, X.; Schreiber, S. L. Syntheses of aminoalcohol-derived macrocycles leading to a smallmolecule binder to and inhibitor of Sonic Hedgehog. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6319-6325.
- (21) Stanton, B. Z.; Peng, L. F.; Maloof, N.; Nakai, K.; Wang, X.; Duffner, J. L.; Taveras, K. M.; Hyman, J. M.; Lee, S. W.; Koehler, A. N.; Chen, J. K.; Fox, J. L.; Mandinova, A.; Schreiber, S. L. A small molecule that binds Hedgehog and blocks its signaling in human cells. *Nat. Chem. Biol.* **2009**, *5*, 154-156.
- (22) Hanger, D. P.; Anderton, B. H.; Noble, W. Tau phosphorylation: the therapeutic challenge for neurodegenerative disease. *Trends Mol. Med.* 2009, 15, 112-119.
- (23) Hernandez, F.; Lucas, J. J.; Avila, J. GSK3 and Tau: Two Convergence Points in Alzheimer's Disease. J. Alzheimers Dis. 2013, 33, S141-144.
- (24) Hoeflich, K. P.; Luo, J.; Rubie, E. A.; Tsao, M. S.; Jin, O.; Woodgett, J. R. Requirement for glycogen synthase kinase-3 beta in cell survival and NF-kappa B activation. *Nature* **2000**, *406*, 86-90.
- (25) Luo, J. Glycogen synthase kinase 3 beta (GSK3 beta) in tumorigenesis and cancer chemotherapy. *Cancer Lett.* 2009, 273, 194-200.
- (26) Chen, J. J.; Qian, W. Y.; Biswas, K.; Viswanadhan, V. N.; Askew, B. C.; Hitchcock, S.; Hungate, R. W.; Arik, L.; Johnson, E. Discovery of dihydroquinoxalinone acetamides containing bicyclic amines as potent Bradykinin B1 receptor antagonists. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4477-4481.
- (27) Hung, A. W.; Ramek, A.; Wang, Y.; Kaya, T.; Wilson, J. A.; Clemons, P. A.; Young, D. W. Route to three-dimensional fragments using diversity-oriented synthesis. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 6799-6804.
- (28) Zhong, C.; Wang, Y.; Hung, A. W.; Schreiber, S. L.; Young, D. W. Diastereoselective control of intramolecular aza-Michael reactions using achiral catalysts. *Org. Lett.* **2011**, *13*, 5556-5559.
- (29) Gould, A. E.; Adams, R.; Adhikari, S.; Aertgeerts, K.; Afroze, R.; Blackburn, C.; Calderwood, E. F.; Chau, R.; Chouitar, J.; Duffey, M. O.; England, D. B.; Farrer, C.; Forsyth, N.; Garcia, K.; Gaulin, J.; Greenspan, P. D.; Guo, R. B.; Harrison, S. J.; Huan, S. C.; Iartchouk, N.; Janowick, D.; Kim, M. S.; Kulkarni, B.; Langston, S. P.; Liu, J. X.; Ma, L. T.; Menon, S.; Mizutani, H.; Paske, E.; Renou, C. C.; Rezaei, M.; Rowland, R. S.; Sintchak, M. D.; Smith, M. D.; Stroud, S. G.; Tregay, M.; Tian, Y. A.; Veiby, O. P.; Vos, T. J.; Vyskocil, S.; Williams, J.; Xu, T. L.; Yang, J. J.; Yano, J.; Zeng, H. B.; Zhang, D. M.; Zhang, Q.; Galvin, K. M. Design and Optimization of Potent and Orally Bioavailable Tetrahydronaphthalene Raf Inhibitors. *J. Med. Chem.* 2011, *54*, 1836-1846.
- (30) Ludwig, C.; Guenther, U. L. Ligand based NMR methods for drug discovery. Front. Biosci. (Landmark Ed.), 2009, 14, 4565-4574.
- (31) Bax, B.; Carter, P. S.; Lewis, C.; Guy, A. R.; Bridges, A.; Tanner, R.; Pettman, G.; Mannix, C.; Culbert, A. A.; Brown, M. J. B.; Smith, D. G.; Reith, A. D. The structure of phosphorylated GSK-3 beta complexed with a peptide, FRATtide, that inhibits beta-catenin phosphorylation. *Structure* 2001, *9*, 1143-1152.
- (32) ter Haar, E.; Coll, J. T.; Austen, D. A.; Hsiao, H. M.; Swenson, L.; Jain, J. Structure of GSK3 beta reveals a primed phosphorylation mechanism. *Nat. Struct. Biol.* **2001**, *8*, 593-596.

Table of Content

Chemistry that is suitably modular and efficient using the build/couple/pair pathway can provide a complementary driving force for optimization than structure-guided approaches in the area of FBDD. Systematic alterations around the entire fragment core would enable determination of fragment-level SAR, which could identify critical binding features of the hit. Moreover, it would reveal specific growth vectors on the scaffold that could be exploited to generate more potent compounds.

