

JUSWINDER SINGH
LEONA E. LING
J. SCOTT SAWYER
WEN-CHERNG LEE
FAMING ZHANG
JONATHAN M. YINGLING

Successful discovery of T β RI (ALK5) kinase inhibitors using HTS, target-hopping and virtual screening

The protein kinase family is emerging as an exciting class of targets for drug discovery¹. Protein kinases play a pivotal role in control of cellular signaling and are involved in proliferation, differentiation and metabolism². Aberrant signaling of protein kinases has been identified in a wide range of diseases including cancer³ and inflammation⁴. The protein kinases exemplify a family where the potential exists to accelerate lead discovery and optimization by inferring between the massive amount of chemical and biological information between gene family members.

A critical hurdle facing all new drug discovery projects is the identification of useful lead molecules. Here we will describe distinct strategies used to identify inhibitors against a novel kinase drug target. It provides an excellent example of highlighting distinct lead discovery strategies and how existing information on a gene family can be used to accelerate the lead discovery process.

T β RI AS A TARGET FOR THE DEVELOPMENT OF ANTI-FIBROTIC AGENTS

The TGF β pathway plays a pivotal role in progressive fibrotic diseases of the lung, liver and kidney that are major causes of morbidity and mortality, and represents an exciting target for the development of novel anti-fibrotic agents⁵. There are several potential points for therapeutic intervention. Potential therapeutic agents which inhibit TGF β ligand include anti-TGF β antibodies, TGF β antisense oligonucleotides or various TGF β binding proteins⁶. Therapeutic intervention in TGF β 's intracellular signal transduction pathway is also possible⁷. The intracellular pathway is particularly attractive given that the type I (T β RI) and type II are kinases that are amenable to small molecule inhibition⁷. Of these kinases, T β RI is the best characterized with regards to its domain structure, biochemical activity and biological function⁷.

TARGET HOPPING

Target hopping capitalizes on knowledge of a given compound's activity against other kinase family members as the starting point for lead discovery. Evers *et al.*⁷ discovered a weak T β RI inhibitor using a structure-guided target hopping strategy in which structural insights into the interaction of an inhibitor for one kinase family member was used to infer binding to another kinase domain. The fluorophenyl substituent of **1** (Figure 1; SB203580), a potent p38 α kinase inhibitor, is able to access the hydrophobic pocket of p38 α due to the small size of the gatekeeper residue. The mutation of this amino acid position to a larger residue abolished binding to p38 α .

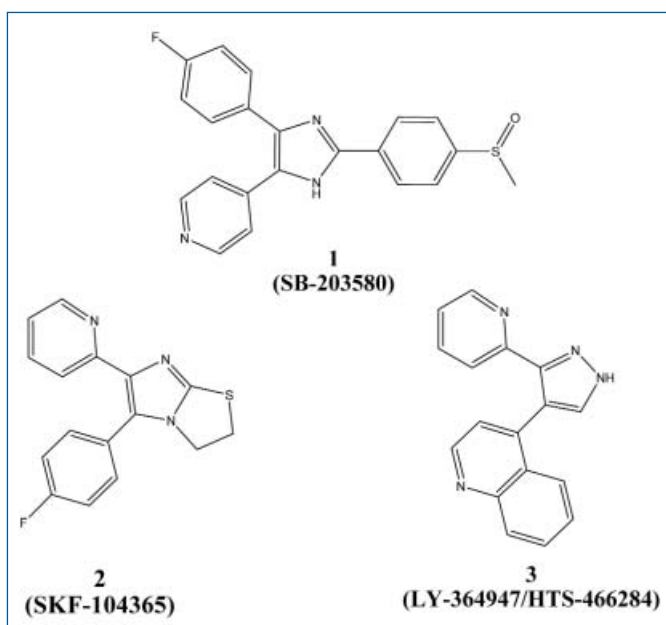


Figure 1: Chemical structures of current T β RI inhibitors from GSK, Lilly Research Laboratories and Biogen Idec.

kinase^{8,9}. Noting that T β RI had a similarly small gatekeeper residue, Evers *et al.* postulated that a p38 α inhibitor such as **1** may show T β RI kinase inhibitor activity. Their hypothesis was confirmed when **1** was found to inhibit T β RI kinase autophosphorylation with an IC₅₀ of 20 micromolar⁸.

HTS

High-Throughput screening (HTS) still remains the best-validated approach to discovering useful inhibitors against drug targets¹⁰. The process involves rapidly testing large, ideally diverse, compound collections against the drug target and does not require any a priori information into structure or compound preferences of the target. Both GSK and Lilly Research Laboratories employed a HTS screen to identify useful potent inhibitors against T β RI.

GlaxoSmithKline screened their internal compound collection for inhibitors of T β RI kinase's ability to phosphorylate Smad3¹¹. The screen identified **2** (SKF-104365), a cyco-fused imidazole compound that had a 2-pyridyl group. This compound, unlike the 4-pyridyl containing imidazoles **1**, inhibited T β RI more potently (IC₅₀ = 1.6 μ M) than p38 α (IC₅₀ > 50 μ M)¹¹.

Independently, the Lilly Research Laboratories discovered a highly potent, 2-pyridyl-containing disubstituted pyrazole inhibitor¹². TGFβ-dependent cell-based HTS of their large internal compound collection identified several active compounds¹². Subsequent testing of these hits against a constitutively active form of the TβRI kinase domain (T204D) identified a very potent inhibitor, **3** (LY364947)¹². Compound **3** had an IC₅₀ of 51 nM for inhibition of TβRI autophosphorylation and a cellular IC₅₀ of 47 nM in a TGFβ-induced p3TP-Lux reporter assay¹². This compound, like **2**, contained a 2-pyridyl group. It also exhibited weaker inhibition of p38α (IC₅₀ = 740 nM) compared to its significant activity against TβRI¹³.

VIRTUAL SCREENING

Virtual screening holds great promise as an inexpensive and fast alternative to high-throughput screening (HTS) to discover useful starting points for drug discovery projects^{14,15}. Ligand-based virtual screening approaches develop a pharmacophore query which represents the 3D arrangement of a set of chemical features/functional groups from an inhibitor that are critical to interacting with the receptor which can be used to search large chemical databases¹⁴.

The starting point for the Biogen Idec TβRI virtual screen was SB203580 **1**, which was used to construct a pharmacophoric query¹⁶. Our objective was to search for molecules with alternative chemistries to SB203580 in the commercially available database that satisfied five pharmacophore features and also had a similar shape (Fig. 2). The choice of pharmacophore features was based upon a derived alignment of p38-SB203580 into the ATP site of TβRI. Two hydrogen-bond acceptor features were predicted to interact with the side chain amino group of Lys232 and the backbone NH of His283¹⁶. This lysine position is strictly conserved across the kinase family and is involved in coordination of the triphosphate moiety of ATP¹⁷, while the backbone NH is involved in binding of the adenine moiety of ATP¹⁷. The majority of kinase inhibitors that have been deposited in the protein databank form interactions with these positions¹⁸. Three of the four aromatic centers in SB203580 were selected as being required. Eighty-seven hits that satisfied the computational filter were purchased

for testing in a TβRI autophosphorylation assay. Compound **3** was identified as a hit, shown to be ATP competitive and demonstrated to have an IC₅₀ of 27 nM¹⁶. This compound inhibited a TGFβ-induced PAI-luciferase reporter cellular assay with an IC₅₀ of 60 nM¹⁶. The Biogen Idec team also confirmed that **3** exhibited weaker p38α inhibition compared to TβRI (Ling, unpublished results).

X-RAY ANALYSIS OF SMALL MOLECULE BINDING TO TβRI

Crystal structures of **3** have been published by the Lilly and Biogen Idec groups (Figure 3^{12,16}). As expected, the

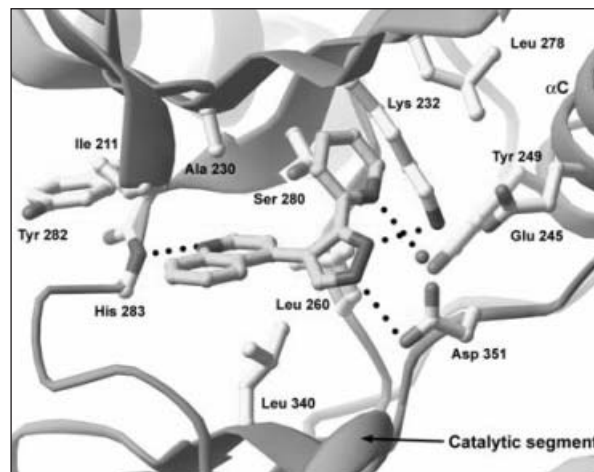


Figure 3: X-ray structures of TβRI in complex with **3**. Residues contacting **3** are shown yellow. Hydrogen bond interactions made by the inhibitors are shown as dashed lines. The water molecule is indicated as a red sphere.

compound binds to the ATP site of the kinase, and is consistent in terms of its binding interactions with the virtual screening hypothesis. The structures also provided an explanation for the binding of the 2-pyridyl group which had not been predicted by the modeling studies. It binds to the hydrophobic pocket while its pyridyl nitrogen participates in a tetrahedral water-mediated network of hydrogen bonds with the enzyme^{12,16}.

CONCLUSION

We have highlighted distinct lead generation strategies converging on a common pharmacophore for TβRI inhibition. The successful identification of useful inhibitors using target hopping, HTS and virtual screening provides further validation that all three approaches are powerful and complementary approaches to lead discovery. The discovery of two potent TβRI inhibitor series by Lilly Research Laboratories and GlaxoSmithKline confirms the power of HTS to discover useful inhibitors against a novel drug target. Alternatively, the value of target-hopping is illustrated by the discovery of **1** as a TβRI inhibitor using the knowledge of structural similarities between the inhibitor binding site of the TβRI and p38α. Finally, the discovery of **3** by Biogen Idec using an *in silico* virtual screen illustrates its role as a significant new drug discovery tool to enable highly focused screening strategies to complement HTS¹⁹. The fact that **3** was identified using HTS and virtual screening suggests that focused library design and screening may be a valuable addition to the lead discovery approaches in the future and lead to improved hit rates against gene families.

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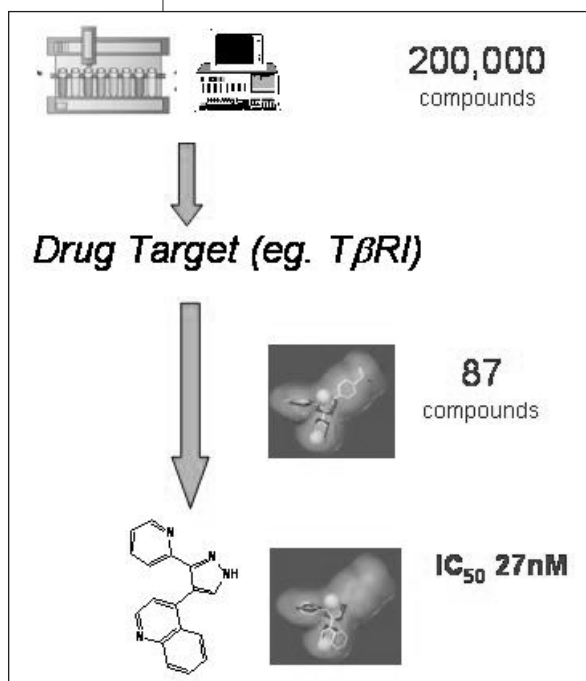


Figure 2: Lead discovery by virtual screening. An electronic database of 200,000 compounds was constructed from commercial vendors. These were screened against the TβRI using a shape-based query based upon X-ray bound conformation of **1** in p38α. The shape component of the query is shown in blue, while the aromatic features are shown as red planes, and the hydrogen-bond acceptors as green spheres. A virtual screen of the 200,000 compounds using the shape-based query identified 87 hits which were purchased and tested. Compound **3** inhibited TGFBR autophosphorylation with an IC₅₀ of 27 nM. The fit of **3** with the shape-based query is shown.

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JUSWINDER SINGH¹, LEONA E. LING¹, J. SCOTT SAWYER², WEN-CHERNG LEE¹, FAMING ZHANG², JONATHAN M. YINGLING²

1. Biogen Idec, 12 Cambridge Center, Cambridge, MA02142

2. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285