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The discovery of potent and selective pyridopyrimidin-7-one based inhibitors of B-Raf^{V600E} kinase

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ABSTRACT

Herein we describe the discovery of a novel series of ATP competitive B-Raf inhibitors via structure based drug design (SBDD). These pyridopyrimidin-7-one based inhibitors exhibit both excellent cellular potency and striking B-Raf selectivity. Optimization led to the identification of compound **17**, a potent, selective and orally available agent with excellent pharmacokinetic properties and robust tumor growth inhibition in xenograft studies.

The Raf family protein kinases, consisting of A-Raf, B-Raf, and C-Raf, are central components of the MAPK pathway that regulates cellular proliferation, differentiation, and survival.[1] Raf kinases act downstream of RAS and are responsible for MEK and ERK activation. BRAF gene mutations may lead to MAPK pathway amplification via constitutive activation of B-Raf, and are present in ~7% of all cancers,[2] with high frequency in malignant melanoma (30–70%).[3,4] The most common (>90%) mutation in B-Raf is a glutamic acid for valine substitution at residue 600 (V600E),[2] which leads to constitutive kinase activity 500-fold greater than wild-type B-Raf,[5] and correlates with increased malignancy and decreased response to chemotherapy.[6] A number of drug candidates targeting the B-Raf^{V600E} mutation have entered clinical trials in recent years. Several of these, such as vemurafenib[7] and dabrafenib[8] have shown clinical efficacy, thus validating B-Raf^{V600E} as a cancer target.

Our lab recently reported the discovery of several novel series of potent and selective inhibitors of B-Raf^{V600E}.[9] A common feature for these inhibitors (Fig. 1) is an amide linker connecting various hinge-binding templates to an aryl sulfonamide, with the amide –NH making a weak hydrogen bond to the hydroxyl of the gatekeeper residue Thr529.[9a] The initial series of amide-linked B-Raf^{V600E} inhibitors utilized a pyridine as a hydrogen bond acceptor, which was postulated to bind to the –NH of hinge residue Cys532. The micromolar enzymatic and cellular activity of pyridine amide **1** was improved 30-fold by introducing an amino group at the 6-position and a bromine atom at the 5-position.[9a]

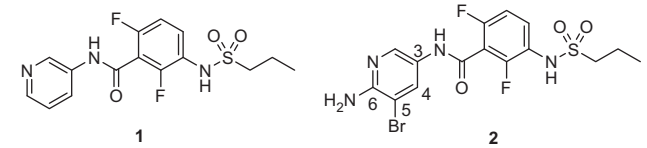

Figure 1. B-Raf^{V660E} inhibitors **1** and **2**.

An X-ray crystal structure[9a] of B-Raf^{WT} in complex with amino pyridine amide **2** revealed that the newly installed 6-amino group is hydrogen-bonded to the carbonyl of Cys532, the sulfonamide moiety forms several hydrogen bonds with the backbone of the DFG sequence, and the propyl chain occupies a small lipophilic pocket that is enlarged by an outward shift of the αC-helix (Fig. 2).

The bromine atom makes key lipophilic contacts to the side chains of Ile463, Val471, Trp531, and Phe583.

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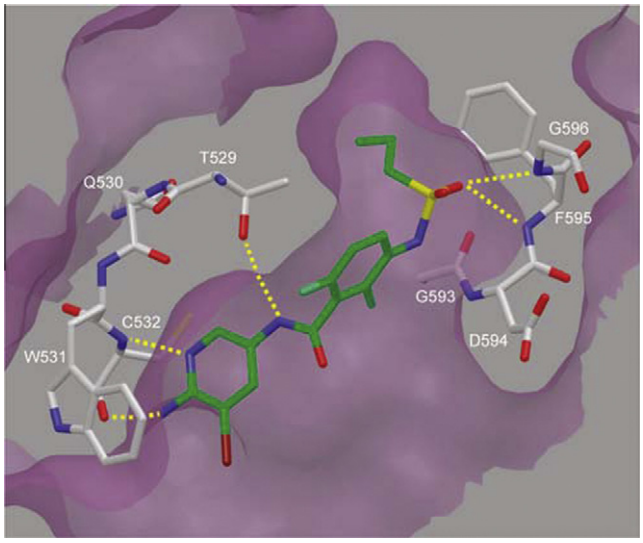
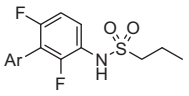
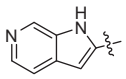
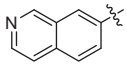
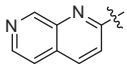
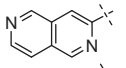
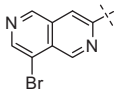
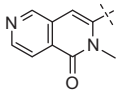
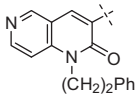


Figure 2. X-ray crystal structure of **2** in complex with B-Raf^{WT}. The cleft surface is rendered in violet, select residues are depicted in white, and the inhibitor is green. Hydrogen-bonding interactions are illustrated with yellow dashed lines. Several residues that are involved in hydrophobic interactions with **2** are omitted for clarity and are described in the text. The propyl group resides in a pocket that is enlarged by an outward shift of the αC-helix. The DFG sequence (D594-G596) resides in its active (DFG-in) conformation.

Table 1
B-Raf activity of compounds **3–9**



Compd	Ar	B-Raf^{V600E} IC_{50}[a] (nM)	pERK IC_{50}[a] (nM)
3		>10,000	—[b]
4		340	—[b]
5		3606	—[b]
6		241	703
7		72	469
8		71	445
9		13	1163

[a] IC_{50} values reflect the average from at least two separate experiments.
[b] Not determined.

The crystal structure also revealed two characteristic conformational features of the amide series: a near co-planar arrangement of the amide group with the hinge-binding scaffold and a near 90° torsion angle of the amide relative to the 2,6-di-halo substituted central phenyl ring. This resulted in a near orthogonal spatial arrangement between the propylsulfonamide bearing phenyl ring and the hinge-binding pyridine ring, and positions them to interact optimally with residues in the ATP pocket. Although the amide linker functioned efficiently as a spacer and a conformational control, its chemical and metabolic stability were perceived as liabilities. Indeed, potentially toxic aniline metabolites generated from in vivo cleavage of the amide bond were observed. To address these concerns, we initiated a program to identify alternative linkers.

Our strategy was based on the observation that the amide linker adopted a near co-planar conformation in relationship to the pyridine ring. Wishing to retain the propyl sulfonamide moiety, a key feature for the DFG-in/αC-helix shifted binding mode, we explored the approach of connecting the amide to the 4-position of the pyridine to form a fused heterocycle (Fig. 3). The resulting inhibitor with the appropriate torsion angle between the newly introduced bicycle and the propylsulfonamide bearing phenyl ring should maintain all the critical interactions with the enzyme.

Due to synthetic accessibility, initial hinge-binding template evaluation focused on fused heterocycles with a single hinge contact to the main chain –NH of Cys532. It was anticipated that once an optimal scaffold was identified, activity could be further improved 4- to 5-fold by installing an amino group at the appropriate position to interact with the carbonyl of Cys532.[9a] Selected examples are shown in Table 1.[10]

Pyrrolopyridine **3** was prepared in an attempt to retain the amide –NH interaction with the gatekeeper residue Thr529, but was inactive. Modeling studies revealed that the 6,5-fused ring system repositioned the central di-halo aryl ring and the propylsulfonamide tail, rendering **3** unable to maintain key contacts with the DFG motif. In contrast, molecular modeling suggested that the propyl sulfonamide on the 6,6-fused template should occupy a similar space when compared to the amide series. As predicted, isoquinoline **4**, showed ca. 10-fold improvement in enzyme inhibition over **1**. The new core also made an improved hydrophobic contact with Phe583 on the floor of the ATP pocket. Incorporating a nitrogen atom into the second ring made a striking difference to activity depending on the substitution pattern. Compound **5** was less active most likely owing to a desolvation penalty upon burial of a strong hydrogen bond acceptor. Binding activity was restored for 2,6-naphthyridine **6**, where N2 is more accessible to solvent, with concomitant submicromolar cellular activity. Consistent with the observation made in the amide series, 8-bromo naphthyridine **7** showed improved potency presumably due to added hydrophobic contacts with the protein. Further improvement over **6** could also be achieved by introducing a carbonyl group into the second ring. The resulting 2,6-naphthyridin-1-one **8** showed similar activity to **7**. Finally, 1,6-naphthyridin-2-one **9**, an inhibitor with reversed amide connectivity, showed the most promising enzyme activity. Results from modeling of compound **9** suggested that the potency gain could be attributed to improved hydrophobic contact between the phenethyl substituent on the amide nitrogen and residues of the P-loop (Fig. 4).

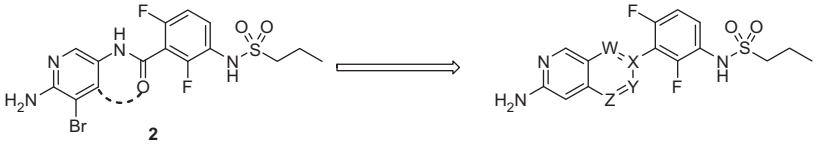


Figure 3. Schematic illustration of the amide linker replacement strategy.