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Identification of pyrazolo[1,5-*a*]pyrimidine-3-carboxylates as B-Raf kinase inhibitors

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ABSTRACT

B-Raf kinase plays a critical role in the Raf-MEK-ERK signaling pathway and inhibitors of B-Raf could be used in the treatment of melanomas, colorectal cancer, and other Ras related human cancers. We have identified novel small molecule pyrazolo[1,5-*a*]pyrimidine derivatives as B-Raf kinase inhibitors. Structure–activity relationship was generated for various regions of the scaffold to improve the biochemical profile.

Raf kinases are serine/threonine protein kinases. They play central role in cell growth and survival and are components of Raf-MEK-ERK signaling pathway.[1] This pathway normally mediates cellular responses to growth signals, however, Ras mutations that are associated with 30% of all cancers constitutively activate this pathway. Of the three Raf human isoforms (A-, B- and C-), activating B-Raf mutations have been found in 66% of malignant melanomas[2] and in a smaller fraction of other cancers including those of the colorectum. The most common B-Raf mutation is valine substitution by glutamic acid at amino acid position (V600E). B-Raf mutations in these cancers were found in a systematic genome-wide screening effort to detect alterations in genes that control cell proliferation, differentiation, and death. Inhibitors of B-Raf could be used in the treatment of melanomas, colorectal cancer, and other Ras related human cancers thus making B-Raf kinase a compelling drug discovery target.

A number of small molecule inhibitors of B-Raf have emerged in the recent past[3] including the most intensively studied Sorafenib,[4] triarylimidazole SB-590885[5] and azaindole PLX-4720[6] (Fig. 1). As part of our on-going effort to identify B-Raf inhibitors, we established an HTS assay to monitor the kinase activity of both wt and mutant (V600E) forms of this protein. This assay was an adaptation of our previously described assay system to detect inhibitors of both C-Raf and MEK1 kinases.[7] Briefly, B-Raf was used to phosphorylate GST-tagged inactive MEK1. MEK1 phosphorylation was measured by a phospho-specific antibody that detected B-Raf phosphorylation of the two serine residues at positions 217 and 221 on MEK1.[8] From this effort pyrazolo[1,5-*a*]pyrimidine-3-carboxylate **1** (Fig. 2) was identified as a promising B-Raf kinase inhibitor.

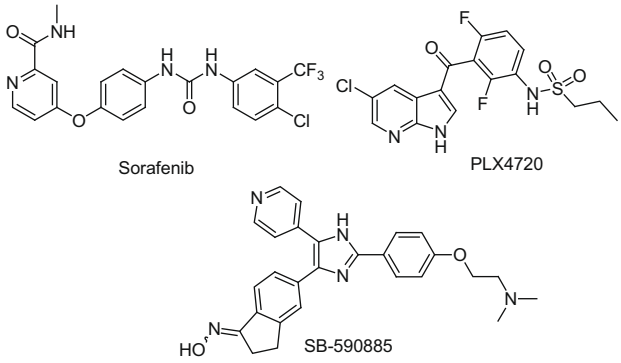

Figure 1. Examples of B-Raf kinase inhibitors.

Compound **1** showed an IC50 of 1.5 μM in the B-Raf kinase assay. It inhibited the growth of a variety of tumor cell lines including BXPC-3 (IC_{50}: 3.25 μM), HT29 (IC_{50}: 7.0 μM), A375 (IC_{50}: 3.8 μM), SW620 (IC_{50}: 8.3 μM), LOVO (IC_{50}: 3.87 μM), WM266-4 (IC_{50}: 6.2 μM) and CaCo-2 (IC_{50}: 6.6 μM). It is a novel scaffold for B-Raf kinase inhibition. This compound was found to be selective when tested against a panel of kinases including PDK-1, m-TOR, Tpl2,

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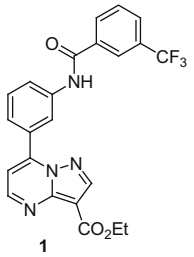


Figure 2. B-Raf inhibitor hit **1** (pyrazolo[1,5-*a*]pyrimidine-3-carboxylate) identified from high through-put screen.

BTK, AKT, CDK-4, LCK, LYN, KDR, IGFR-1, SRC and PI3 Kα (IC_{50} > 10 μM). The key structural features of the compound had high degree of resemblance to the reported B-Raf kinase inhibitor Sorafenib. A docking study was performed with the standard Glide2.0 SP procedure, using the crystal structure of Sorafenib_B-Raf complex.[9] Illustrated in Figure 3 is a binding model of compound **1** in complex with B-Raf. The model indicated that the amide of compound **1** makes two hydrogen bonds to the enzyme: one to the side chain of Glu500 and another to the backbone NH of Asp593. No specific polar interaction was found between the ester moiety and B-Raf, but the ester does make hydrophobic interactions with Ile462, Trp530 and Phe582 with the ethyl group pointing toward a solvent accessible region. On the other end of the molecule, the aromatic ring of the amide region sits at a hydrophobic pocket which consists of Ile 512, His 573 and Ile 571. The ring is partially exposed to solvent at the C4 position. Based on this information, the initial structure–activity relationship for the ester moiety, amide linker and the aromatic ring of the amide region were explored.

The analogs designed to obtain structure–activity relationship were synthesized by following the synthetic sequence employed for compound **1** as shown in Scheme 1.[10] Reaction of 3-nitroacetophenone **2** with *N*,*N*-dimethylformamidedimethylacetal followed by condensation with 5-aminopyrazole-4-carboxylate **4** afforded ester **5**. Selective reduction of the nitro group of compound **5** afforded the key intermediate aniline **6** which was further derivatized to the required amides by reacting with appropriate acid chlorides. Reacting the common intermediate amine **6** with various isocyanates or triphosgene and appropriate amines generated urea analogs. Pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid **8** and carboxamides **9** were prepared starting from the ester **1**, which was hydrolyzed to the acid and coupled with different amines (Scheme 2).

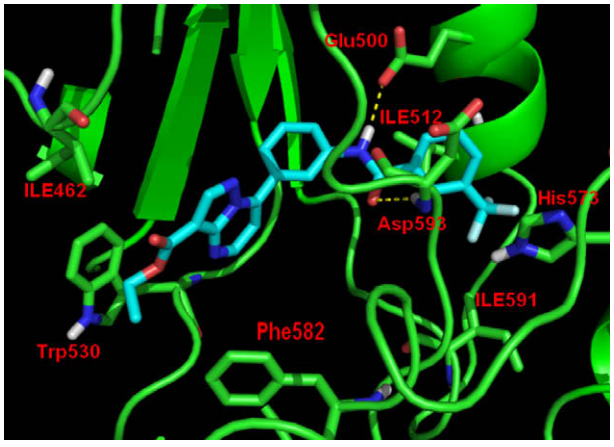
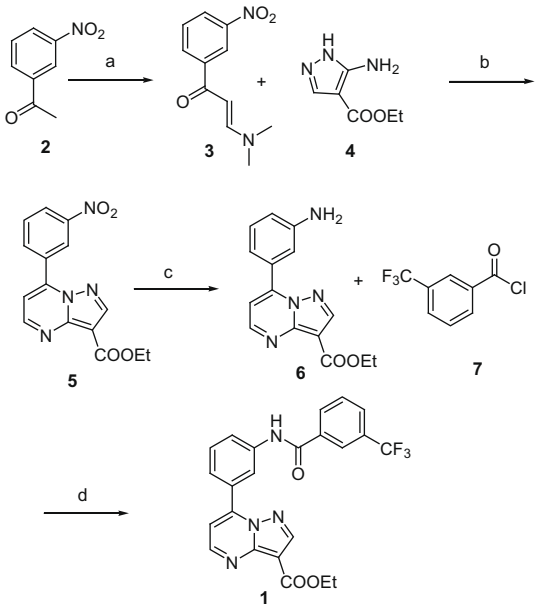
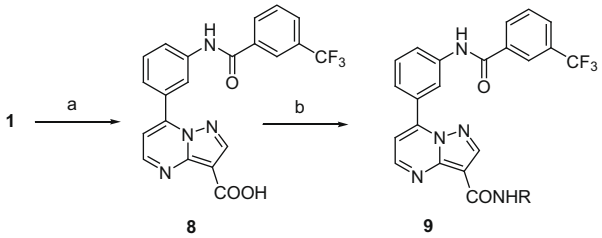


Figure 3. Docked conformation of compound **1** in the active site of B-Raf.



Scheme 1. Reagents and conditions: (a) DMF–DMA, reflux, 18 h, 79%; (b) acetic acid, 80 °C, 12 h, 72%; (c) Fe, acetic acid, 80 °C, 3 h, 60%; (d) pyridine, rt, 70%.



Scheme 2. Reagents and conditions: (a) 2 M LiOH, THF–MeOH, 40 °C, 6 h, 90%; (b) RNH_2, PYBOP, DIEA, DMF, rt, 12 h.

Initial understanding of the SAR was directed towards determining the importance of the trifluoromethyl group and its position on the aromatic ring (Table 1) while maintaining the rest of the molecule intact. Elimination of the group resulted in an unsubstituted phenyl analog **10** with significantly reduced activity. Replacing the CF_3 group with other substituents was also attempted. Both electron withdrawing groups and electron releasing groups were introduced to probe the electronic effects. Most of the aromatic substituents including halogens were not favorable. However, introduction of trifluoromethoxy group (compound **16**) maintained the activity indicating that the lipophilicity of the group is probably more important than the electronics for binding interactions as indicated by our docking studies. Moving the CF_3 group from *meta* position of the aromatic ring (R^2) to the *para* position (R^3) reduced the activity as shown by analog **17**.

Having established the requirement of a lipophilic group like CF_3 in the R^2 position of the aromatic ring, we explored the tolerance of an additional substituent in that ring as shown by analogs **18–22** in Table 1. Introduction of a small group like methyl, methoxy and chloro in the R^3 position along with CF_3 group in the R^2 position of the aromatic ring was very well tolerated (compounds 18–20). However, moving these substituents to the R_5 position of the aromatic ring in the presence of the CF_3 group in the R^2 position (compound **21** and **22**) was not favorable. This is probably due to the steric hindrance introduced by these groups around the amide linker which forms two key hydrogen bond interactions