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Discovery of 3(*S*)-thiomethyl pyrrolidine ERK inhibitors for oncology ☆

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ABSTRACT

Compound **5** (SCH772984) was identified as a potent inhibitor of ERK1/2 with excellent selectivity against a panel of kinases (0/231 kinases tested @ 100 nM) and good cell proliferation activity, but suffered from poor PK (rat AUC PK @10 mpk = 0 μM h; F% = 0) which precluded further development. In an effort to identify novel ERK inhibitors with improved PK properties with respect to **5**, a systematic exploration of sterics and composition at the 3-position of the pyrrolidine led to the discovery of a novel 3(*S*)-thiomethyl pyrrolidine analog **28** with vastly improved PK (rat AUC PK @10 mpk = 26 μM h; F% = 70).

Aberrant and hyper activation of the RAS/RAF/MEK/ERK signaling pathway, a member of the MAPK kinase pathway, plays a central role in the underlying proliferation mechanisms of several human tumor subtypes. ERK is the downstream target of the MAPK kinase pathway and is constitutively activated in many tumor cells (Melanoma: 60% BRAF mutant; 15–20% NRAS, Colon: 50% KRAS mutant, 15% BRAF, Pancreatic: 90% KRAS, NSCLC: 30% KRAS).[1] ERK inhibition selectively induces many cellular events including cell differentiation, cell proliferation and apoptosis.[2] Of late, several highly optimized ERK inhibitors such as the pyrrole **1** by Vertex/Biomed Valley Discoveries,[3] the pyridone **2** by Genentech,[4] fused pyrrolo-diazepanone **3** by Novartis[5] and most recently, the irreversible acrylate inhibitor **4** by Astrazeneca[6] have been reported (Fig. 1).

An excellent review[7] by Samatar and Poulikakos describes the discovery and development of various RAS-ERK inhibitors that are being studied at various phases in both clinical and pre-clinical development. The seminal work by these groups has led to the development of more effective therapies for patients with tumors dependent on the ERK signaling pathway. The goal of our research effort was to overcome RAS-ERK resistance believed to be tied to down field effectors in the MAPK pathway. Designing potent and selective RAS-ERK inhibitors that could mediate drug resistance is a daunting challenge that has been a major focus for both academia and the pharmaceutical industry.[8] We reported[9] the discovery of a highly potent, kinase selective ERK1/2 inhibitor **5** (SCH772984) that displayed unique dual mechanism of action (inhibits both phosphorylation of ERK and RSK) and demonstrated *anti*-tumor efficacy in both the BRAF/RAS mutant cell line xenograft models (Fig. 1).

Compound **5** is a potent ERK inhibitor (ERK2 IC_{50} = 1 nM), exhibiting high kinase selectivity (0/231 kinases tested @ 100 nM), which was derived from an initial high throughput screening hit **6** (ERK2 IC_{50}=18.6 μM) (Fig. 2). Compound **6** was obtained through an in-house neomorph small molecule library which was screened against the unphosphorylated (in-active) form of the target protein ERK2 utilizing an automated ligand identification system (ALIS). Subsequent SAR following hit validation from **6** afforded compound **7** (ERK2 IC_{50} = 2.7 μM).[10] Introduction of the

☆ Dedicated to Professor Dale L. Boger, Professor The Scripps Research Institute, San Diego, CA, USA on the occasion of his 65th birthday.

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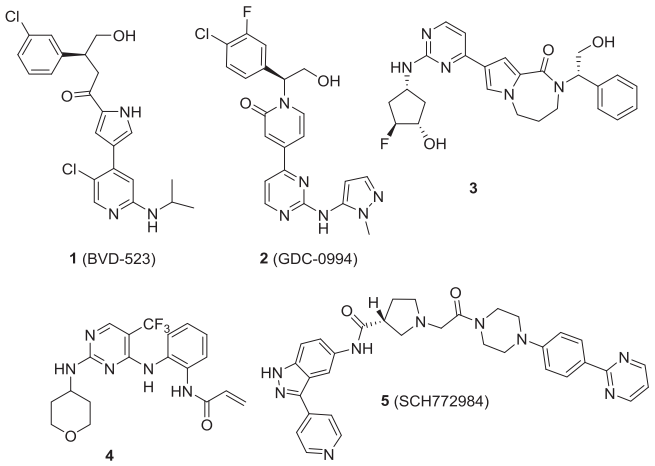

Fig. 1. Representative structures of ERK inhibitors **1**–**5**.

indazole pharmacophore provided enhanced potency and selectivity giving rise to compound **5**; however, compound **5** suffered from both poor absorption and bioavailability in rat, as seen in the plasma drug concentration measured by area under the concentration-time curve (AUC) (Rat AUC @ 10 mpk = 0 nM h; F% = 0), that precluded this target for further development. Based upon our interest in developing an oral ERK compound for *in vivo* biological studies, we chose to further explore the SAR of this novel class of ERK inhibitors. Herein we report our research efforts toward this aim (Fig. 2).

To enable our structure based drug design (SBDD) effort, we obtained the X-ray crystal structure of **5** bound to ERK2, shown in Fig. 4. The binding mode is similar to other inhibitors in the series and has been extensively discussed previously.[10] Briefly, the two indazole nitrogen atoms of **5** form hydrogen bonds with Asp104 and Met106 at the hinge region of the ERK2 ATP binding site. The pyridine N atom forms an H-bond with Lys112, while the two amide carbonyl O atoms, along with the protonated pyrrolidine N atom, are involved in an H-bond network with gatekeeper Gln103 and catalytic Lys52. In addition, upon binding of **5** to ERK2, the G-loop undergoes a large conformation change which flips the Try34 side-chain and generates a new side pocket for **5** to extend into, thus produces a novel binding conformation where aromatic pi-pi stacking interactions between the pyrrolidine and Tyr34, and the distal phenyl pyrimidine and Tyr62, were observed. This unique binding conformation leads to excellent kinase selectivity for **5** and its analogs.

Based on the X-ray studies, the pyrrolidine amide linkage is involved in pivotal hydrogen bonding interactions in the active site with the gate keeper residues (Asp165, Gln103 Glu69) of the ERK protein vis-à-vis a water hydrogen bond network (depicted in Fig. 3). We began our SAR studies by replacing the unsubstituted pyridine (potentially susceptible to *N*-oxide formation mediated by cytochrome P450 enzymes) at the 3-position of the indazole, which has a key interaction with the lysine 112 residue of ERK, with a *p*-fluoro phenyl group to afford compound **8**. Compound **8** maintained ERK potency ERK2 IC_{50} = 5.5 nM, but did not show any sign of improving pharmacokinetics (PK) in the rat (Rat AUC @ 10 mpk = 0 nM h) Table 1.

In order to better understand the underlying mechanism for poor bioavailability for this class of compounds, metabolic identification studies with compound **8** were undertaken. Incubation of compound ^{3}H-**8** in cryo-preserved rat and human hepatocytes at 10 μM and 1 μCi/mL for 0, 2 and 4 h at 37 °C followed by metabolite characterization identified the indazole amide linkage as the major metabolic pathway giving rise to metabolites **9** and **10** (Fig. 4).

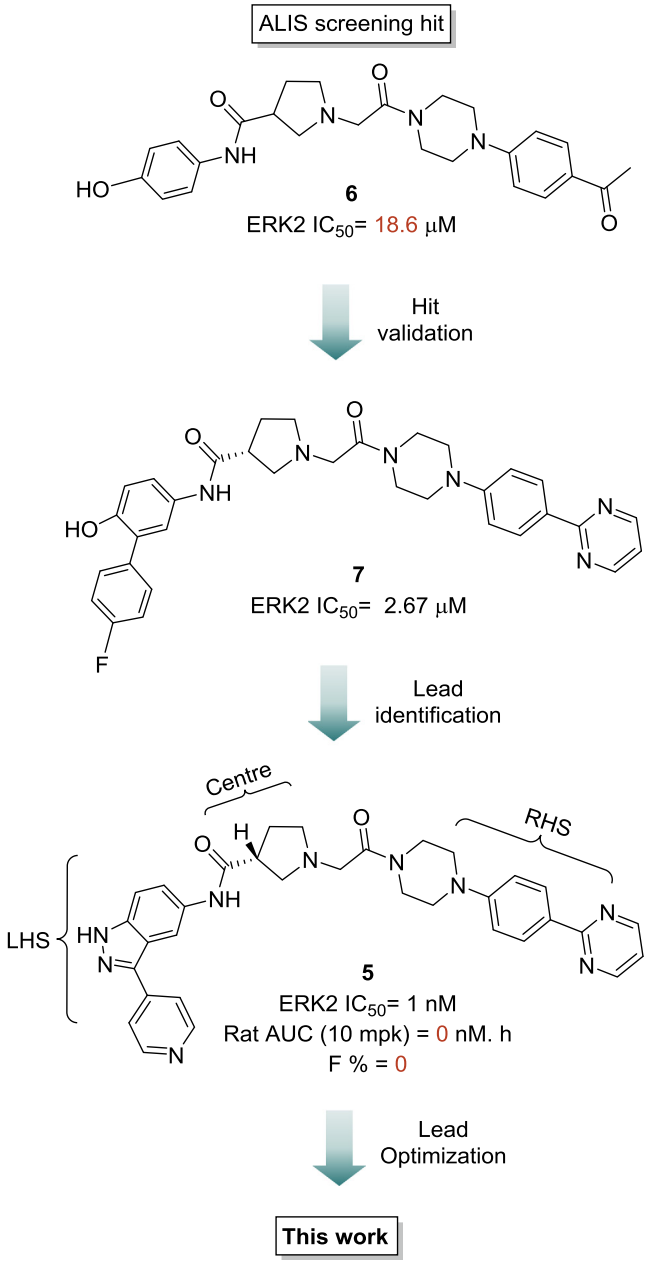

Fig. 2. Hit to lead identification of ERK inhibitor **5** (SCH772984).

Guided by *in silico* modeling, an initial chemistry strategy was aimed at structural modifications (*viz.*, elimination, disposition, isostere and steric hindrance) around the pyrrolidine amide region

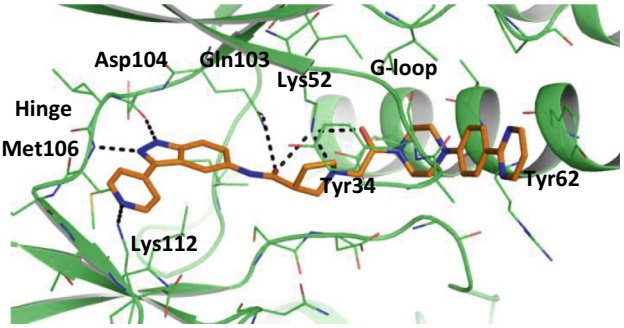

Fig. 3. X-ray crystal structure of **5** in the active site of ERK2 with hydrogen bond interactions to the key residues highlighted in dashed lines.