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Discovery and in vitro evaluation of potent kinase inhibitors: Pyrido[1′,2′:1,5]pyrazolo[3,4-*d*]pyrimidines

Michael J. Alberti, Elizabeth P. Auten, Karen E. Lackey, Octerloney B. McDonald, Edgar R. Wood, Frank Preugschat, Geoffrey J. Cutler, Laurie Kane-Carson, Wei Liu and David K. Jung*

GlaxoSmithKline, 5 Moore Drive, Research Triangle Park, NC 27709, USA

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Abstract—The discovery, synthesis, potential binding mode, and in vitro kinase profile of several pyrido[1′,2′:1,5]pyrazolo[3,4-*d*]-pyrimidines as potent kinase inhibitors are discussed.

Protein kinases catalyze the phosphorylation of tyrosine and serine/threonine residues in various proteins involved in the regulation of all functions.[1] Protein kinases can be broadly classified as receptor (e.g., EGFr, c-erbB2, PDGFr, and VEGFR2) or non-receptor (e.g., c-src, b-raf, and ZAP70) kinases. Inappropriate or uncontrolled activation of many of these kinases, by over-expression, constitutive activation, or mutation, has been shown to result in uncontrolled cell growth.[2] Drug discovery efforts have targeted this aberrant kinase activity in cancer, asthma, psoriasis, and inflammation, to name a few.[3]

Recent advances in the identification of erbB family kinase inhibitors have created hope for the modulation of uncontrolled cell growth in cancer therapy for solid tumors.[4] For example, the compounds shown in Figure 1, Iressa™ and GW572016, continue to show promising results in clinical trials on cancer patients.[5] Gleevec™'s activity in bcl-abl or c-kit-mediated malignancies is well-documented and CEP1347 looks promising for Parkinson's disease.[6,7] Despite these tremendous results in the development of signaling inhibitors, there remains a gap in the understanding of the selectivity and required inhibition profile of kinase inhibitors to achieve efficacy without introducing toxicity.[8]

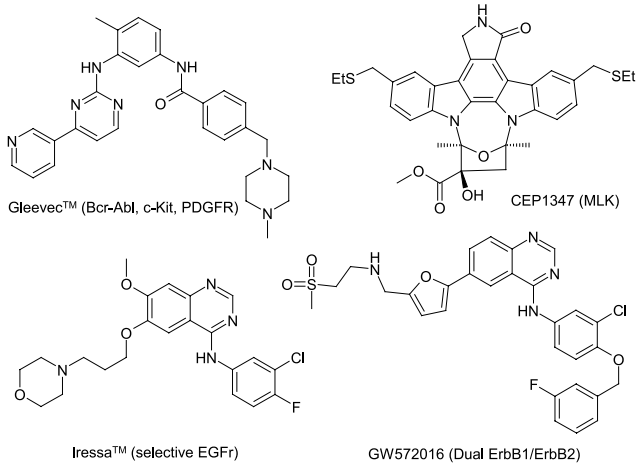

Figure 1. Successful examples of kinase inhibitors that have progressed to clinical trials and patient care.

Herein, we report the generation of a novel scaffold where the substitution pattern targets different regions of the ATP-binding site of the protein kinase domain to create differentially selective molecules. Based on literature reports, linearly fused tricyclic core systems have been used as scaffolds for kinase inhibitors.[9] Our goal was to develop a novel tricyclic core ring system that could be decorated with a variety of diverse substituents to examine their structure–activity relationship (SAR) against a panel of kinase inhibition assays. Herein, we report the results of the utility of this approach to generate useful, selective tool compounds.

Keyword: Kinase inhibitor; VEGFR; GSK; Erb; EGFR.
* Corresponding author. Tel.: +1 919 483 8391; fax: +1 919 483 6053; e-mail: david.k.jung@gsk.com

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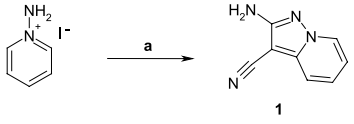
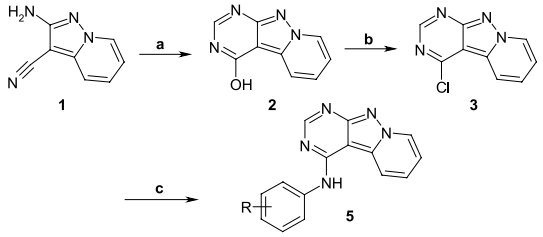
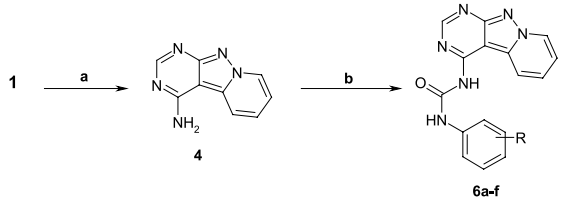


Scheme 1. Synthesis of key intermediate 2-amino-3-cyano-pyrazolo[1,5-*a*]pyridine **1**. Reagents: (a) malononitrile, ethanol, potassium carbonate (25%).



Scheme 2. Synthesis of *N*-phenylpyrido[1′,2′:1,5]pyrazolo[3,4-*d*]pyrimidin-4-amine derivatives **5a**–**d**. Reagents and conditions: (a) formic acid, cat. sulfuric acid, 90 °C; (b) $POCl_3$ (61%, two steps); (c) anilines, *i*-PrOH, reflux.



Scheme 3. Synthesis of *N*-phenyl-*N*′-pyrido[1′,2′:1:5]pyrazolo[3,4-*d*]pyrimidin-4-yl-urea derivatives **6a**–**f**. Reagents and conditions: (a) formamide, MW 240 °C (79%); (b) isocyanates, MeCN, 25 °C, 16 h.

The syntheses of two novel classes of kinase inhibitors, *N*-phenyl-pyrido[1′,2′:1,5]pyrazolo[3,4-*d*]-pyrimidin-amine and *N*-phenyl-*N*′-pyrido[1′,2′:1,5]pyrazolo [3,4-*d*]-pyrimidin-4-yl-urea derivatives, are shown in Schemes 1–3.

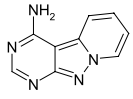
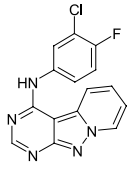
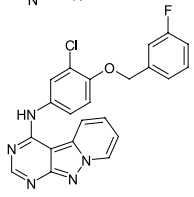
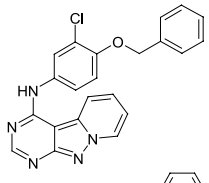
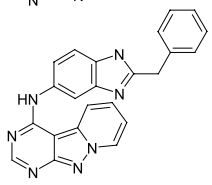
The key aminonitrile intermediate **1** was synthesized by combining the commercially available *N*-aminopyridinium iodide and malononitrile in ethanol. Heating this mixture with microwave irradiation in the presence of 2 equiv of potassium carbonate generates the desired product. This intermediate has been used to prepare two classes of kinase inhibitors. Preparation of those derivatives with aniline substituents at the 4-position is shown in Scheme 2, and a synthetic approach used to prepare inhibitors with urea substitution at the 4-position is shown in Scheme 3.

The synthesis of anilino-substituted analogs was started by heating the aminonitrile **1** with formic acid in the presence of catalytic sulfuric acid (Scheme 2). The resulting pyrimidinone derivative **2** was converted to the corresponding chloroimidate **3** with phosphorous oxychloride. The final displacement of the chloride with an appropriate aniline was accomplished by heating in isopropyl alcohol to give the desired *N*-phenylpyrido[1′,2′:1,5]pyrazolo[3,4-*d*]pyrimidin-4-amine compounds (**5a**–**d**).

Scheme 3 depicts the sequence of reactions used to prepare urea-substituted derivatives **6a**–**f**. Subjecting the aminonitrile intermediate **1** to microwave irradiation in the presence of formamide results in the formation of tricyclic amine **4**. Stirring this product overnight with an isocyanate in acetonitrile gave the desired *N*-phenyl-*N*′-pyrido[1′,2′:1,5]-pyrazolo[3,4-*d*]pyrimidin-4-yl-urea compounds. The synthetic sequences shown above are amenable to the production of large number of compounds by scaling up key intermediates **3** and **4**, and adding the final diversity in a parallel array format.[10,11]

Compounds **4**, **5a**–**d**, and **6a**–**f** are representative examples of derivatives that can be synthesized by the methods described above. The compounds were evaluated in a panel of kinase enzyme assays and the data for erbB2, EGFR, GSK3, and VEGFR2 are summarized in Tables 1 and 2. The free amino-substituted derivative **4** showed no activity against EGFR, erbB2, GSK3, or VEGFR. However, replacement of the amino substituent with aniline derivatives resulted in potent inhibitors of erbB2 and/or EGFR, with selectivity over GSK3 and VEGFR. The SAR that confers potency and selectivity to the quinazoline series for the erbB family. TK inhibition was evaluated in *N*-phenylpyrido[1′,2′:1,5]pyrazolo[3,4-*d*]pyrimidin-4-amines.[12] For example, **5a** has a 'small' anilino group, and like Iressa™, is selective for EGFR

Table 1. *N*-Phenylpyrido[1′,2′:1,5]pyrazolo[3,4-*d*]pyrimidin-4-amines

Compound	Structure	ErbB2[14]	EGFR[14]
4		>10	>10
5a		>10	0.200
5b		0.063	0.032
5c		0.200	0.050
5d		1.58	5.01

Kinase enzyme inhibition expressed as IC_{50} values in micromolar.[14] The IC_{50} values are >10 μM for GSK3 and VEGFR2 for compounds **4** and **5a**–**d**.