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Design and synthesis of a pyrido[2,3-*d*]pyrimidin-5-one class of anti-inflammatory FMS inhibitors

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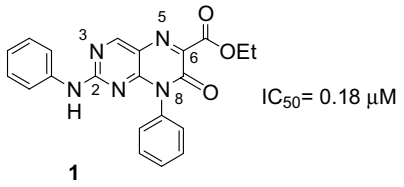
Abstract—A series of pyrimidinopyridones has been designed, synthesized and shown to be potent and selective inhibitors of the FMS tyrosine kinase. Introduction of an amide substituent at the 6-position of the pyridone core resulted in a significant potency increase. Compound **24** effectively inhibited in vivo LPS-induced TNF in mice greater than 80%.

Increased tumor-associated macrophage numbers have been associated with tumor progression.[1] In addition, macrophage numbers present within target tissues have been strongly correlated with disease severity in rheumatoid arthritis,[2] and immune nephritis.[3] In some solid tumors, such as breast cancer, elevated macrophage numbers are thought to contribute to disease progression and poor survivability.[4–6] The proliferation and survival of macrophages, monocytes, and their progenitors are driven by macrophage colony-stimulating factor (M-CSF or CSF-1).[7] Binding of CSF-1 to its exclusive receptor, colony-stimulating factor-1 receptor (FMS), induces receptor dimerization and autophosphorylation which leads to the phosphorylation of downstream signaling proteins, and the subsequent differentiation and activation of cells in the macrophage lineage.[8] Animal studies with CSF-1 deficient mice suggest that CSF-1/FMS is a crucial component of a positive cycle that drives chronic inflammation.[9–11] Thus, the inhibition of FMS has great potential in treating human diseases such as rheumatoid arthritis as well as certain cancers where macrophages are pathogenic.

By screening a focused kinase library using Thermofluor® technology,[12] pteridinone **1** was identified as a confirmed hit with an IC_{50} of 0.18 μM for FMS (Fig. 1). A small set of these pteridinones was synthesized and it was observed that replacement of the N-8 phenyl of **1** with an indan ring resulted in a 10-fold potency increase (data not shown).



1

Figure 1. High-throughput screening hit **1**.

Compound **1** was modeled in FMS (PDB ID: 2i0y)[13] in order to evaluate the interactions required for binding (Fig. 2). Related pyrido[2,3-*d*]pyrimidin-7-one kinase inhibitors have previously been shown to interact with the hinge region via hydrogen bonds to the amino NH and N-3 of the pyrimidine ring.[14] The model was built assuming similar interactions for the amino NH and the pteridinone N-3 of hit **1** with, respectively, the backbone carbonyl and backbone NH of the hinge residue Cys666. In addition, the following interactions were predicted by this model: a hydrogen bond between the side-chain hydroxyl of Thr663 and the pteridinone N-5, a hydrogen bond between either the pteridinone carbonyl or the ester carbonyl and the terminal amine of Lys616 (depending on side chain conformation), and hydrophobic interactions for the N-8 phenyl.

Keywords: FMS; cFMS; Colony-stimulating factor-1 receptor; CSF-1; M-CSF; Macrophage; Pyrimidinopyridone.
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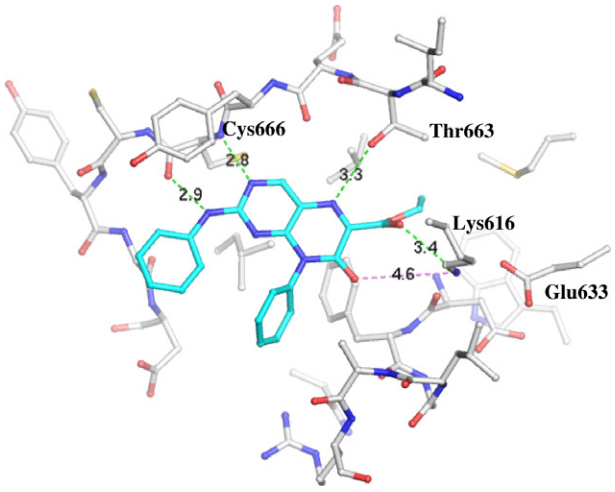

Figure 2. Model of **1** (cyan carbons) in FMS kinase (white carbons). Hydrogen bonds and potential hydrogen bonds are shown as green and magenta dotted lines, respectively.

Based on this model of pteridinone binding, novel scaffolds were explored in an attempt to improve potency. One of the most promising designs was constructed from a 5,8-dihydro-pyrido[2,3-*d*]pyrimidin-5-one scaffold. Represented by **2** (Fig. 3), this scaffold was predicted to bind with a minimal shift of the ring system away from Thr663. With an IC_{50} of 0.25 μM for FMS, **2** showed a 10-fold potency loss compared to its pteridinone analogue (IC_{50} = 0.02 μM). A detailed SAR study was then conducted to improve its in vitro potency. Herein, the structure–activity relationships at three areas of the new scaffold are reported: the C-6 carboxylate ester, N-8 hydrophobic substitution and C-2 anilino substitution.

A linear synthetic method was developed to quickly synthesize analogues of **2** (Scheme 1). An amine, for example, indan-5-ylamine, was reacted with ethyl 3-chloropropionate at elevated temperature in the presence of an inorganic base and a catalytic amount of tetrabutylammonium bromide to afford the aminopropionate ester **3**, which was treated with ethyl 4-chloro-2-methylthio-5-pyrimidinecarboxylate to produce the 4-substituted aminopyrimidine **4**. Cyclization of this diester under Dieckmann conditions afforded bicyclic compound **5**. Subsequent halogenation with bromine followed by dehydrohalogenation gave **6**.[15] The thiomethyl moiety was oxidized to the sulfone **7**, which was subsequently displaced with an amine or aniline by nucleophilic substitution. The resulting carboxylate ester **2** was converted to the carboxylic acid **8** by hydrolysis, or to the amides **9** and **11**–**34** by reaction with the corresponding amines in methanol in a pressure bottle.

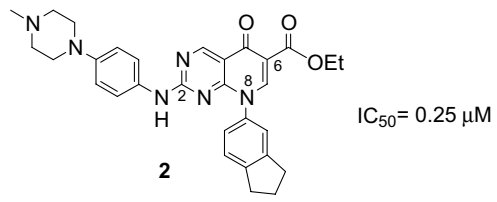

Figure 3. A novel 5,8-dihydro-pyrido[2,3-*d*]pyrimidin-5-one scaffold represented by **2**.

Compound **10**, where N-1 was replaced with CH, was synthesized by the methods shown in Scheme 2. 4,6-Dihydroxynicotinic acid ethyl ester **35** was obtained in two steps from diethyl 1,3-acetonedicarboxylate.[16] Treatment of **35** with phosphorous oxychloride gave 4,6-dichloronicotinic acid ethyl ester **36**. The subsequent nucleophilic displacement yielding **37** and Dieckmann cyclization followed by halogenation/dehydrohalogenation which yielded **38** were similar to the methods outlined in Scheme 1. Substitution of the 2-position chloride of **38** with the piperazinyl aniline was carried out under microwave conditions to afford ethyl ester **39**, which was then converted to the amide **10** by treatment with methanolic ammonia.

All compounds were tested in an in vitro enzyme assay for their abilities to inhibit ATP-induced auto-phosphorylation of FMS (Tables 1 and 2).[17] The most potent compounds were also tested in a functional assay, which was based upon inhibition of CSF-1 driven proliferation of bone marrow-derived macrophages (BMDM).[18] In this cell-based assay, compound **2** was moderately active with an IC_{50} value of 0.47 μM. According to the model, the C-2 anilino substituents are largely solvent-exposed. It seemed likely that the C-6 carboxylate ester and N-8 hydrophobic substitutions would be more important in terms of binding at the active site due to their more intimate contact with the protein. Thus, the C-2 substitution was initially fixed as 4-(4-methyl-piperazin-1-yl)-phenylamine, while the C-6 and N-8 positions were explored (Table 1). The C-6 carboxylic acid **8** was about fourfold less active, whereas transformation of the ester to primary amide **9** resulted in a significant increase in potency. The model of **9** in FMS suggested that the amide could make an intramolecular hydrogen bond to the C-5 carbonyl stabilizing a conformation of the molecule that optimizes hydrogen bonding between the amide carbonyl and the terminal amine of Lys616. The model does not predict that N-1 of the pyrimidine ring is making a direct interaction with FMS. However, removal of N-1 as in **10** decreased potency about fourfold. The greater potency of **9** is likely due to the lower basicity of the aminopyrimidine system, which favors the neutral form of the molecule necessary for interaction with Cys666.

Small alkyl substitution on the C-6 amide was well tolerated, though activity diminished with increasing size of the alkyl group (**11**, **12**). The N-8 substituents occupy a hydrophobic pocket of the active site. Whereas aliphatic rings (**13**–**15**) maintained activity, the unsubstituted phenyl compound **16** significantly attenuated potency. This trend also had been observed within the pteridinone high-throughput screening hit series. Benzylic substitution (**17**) also resulted in a potency decrease, although to a lesser extent than phenyl substitution.