Bioorganic & Medicinal Chemistry Letters 23 (2013) 2787–2792

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Structure-based design, SAR analysis and antitumor activity of PI3K/mTOR dual inhibitors from 4-methylpyridopyrimidinone series

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ARTICLE INFO

Article history:
Received 15 December 2012
Revised 23 January 2013
Accepted 1 February 2013
Available online 13 February 2013

Keywords:
SBDD
Kinase inhibitor
PI3K/mTOR dual inhibitor
Cancer
Antitumor
Effective polar surface area
ePSA
Metabolite

ABSTRACT

PI3K, AKT and mTOR, key kinases from a frequently dysregulated PI3K signaling pathway, have been extensively pursued to treat a variety of cancers in oncology. Clinical trials of PF-04691502, a highly potent and selective ATP competitive kinase inhibitor of class 1 PI3Ks and mTOR, from 4-methylpyridopyrimidinone series, led to the discovery of a metabolite with a terminal carboxylic acid, PF-06465603. This paper discusses structure-based drug design, SAR and antitumor activity of the MPP derivatives with a terminal alcohol, a carboxylic acid or a carboxyl amide.

The phosphatidylinositol 3-kinase (PI3K) signaling pathway plays crucial roles in cell growth, proliferation and survival, and is a frequently dysregulated pathway in human cancers.[1,2] Inhibitors of key kinases in the pathway, including PI3K, AKT and mTOR, have been extensively pursued in oncology in recent years.[3] In order to effectively block the PI3K pathway, overcome feedback loops,[4] and block PI3K independent mTOR activation, our strategy to target PI3K signaling pathway is focused on pursuing PI3K/mTOR dual inhibitors. PF-04691502 is a highly potent and selective ATP competitive kinase inhibitor of class 1 PI3Ks and mTOR, and has progressed to phase I/II clinical trials for the treatment of solid tumors (see Fig. 1).[5,6]

PF-04691502, derived from 4-methylpyridopyrimidinone (MPP) series, exhibited potent in vitro activity against class I PI3K isoforms and mTOR, with mPI3Kα K_i of 0.57 nM (mouse PI3Kα was used as a surrogate of human PI3Kα in the primary screening), and mTOR K_i of 16 nM. In a BT20 cell assay, measuring inhibition of AKT phosphorylation at S473, PF-04691502 exhibited excellent cellular potency with an IC_{50} of 13 nM. Co crystal structure of PF-04691502 bound in PI3Kγ was determined (Fig. 2).[5] The aminopyrimidine formed key hydrogen bonds with the hinge residue, Val 882. The methyl at the 4 position on the MPP core fit tightly in a small hydrophobic pocket unique to PI3K and mTOR, conferring excellent kinase selectivity for the MPP derivatives. The ring nitrogen on the methoxypyridine formed a key hydrogen bond with a conserved water molecule in the selectivity pocket. The terminal alcohol of the hydroxyethyl formed an intramolecular H bond with the ether oxygen atom off the cyclohexyl ring, reducing the effective number of hydrogen bond donors, and helping the compound to achieve excellent permeability and cellular potency.

Structural analysis also revealed that the terminal alcohol was located in a solvent exposed region surrounded by polar residues, and there was space between the terminal alcohol and the polar residues. Modeling studies, exemplified by docking **29** in PI3Kγ as illustrated in Figure 3, indicated a primary carboxyl amide would fit in the space, with the amide carbonyl pointing towards the solvent, and an intramolecular H bond between the amide N–H and the ether oxygen could be formed. In PF-04691502 co crystal structure with PI3Kγ, the Lys 833 side chain was pointing down as shown in Figure 2, and there was no H bond interaction between MeO-pyridine and Lys 833. However, the F atom on the 4-MeO-5-F-3-pyridine in **29** pushed Lys 833 up and inward as illustrated in Figure 3, the terminal amino group from Lys 833 side

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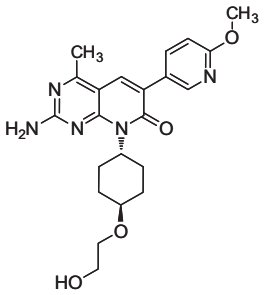
http://dx.doi.org/10.1016/j.bmcl.2013.02.020



PF-04691502

Figure 1. Chemical structure of PF-04691502.

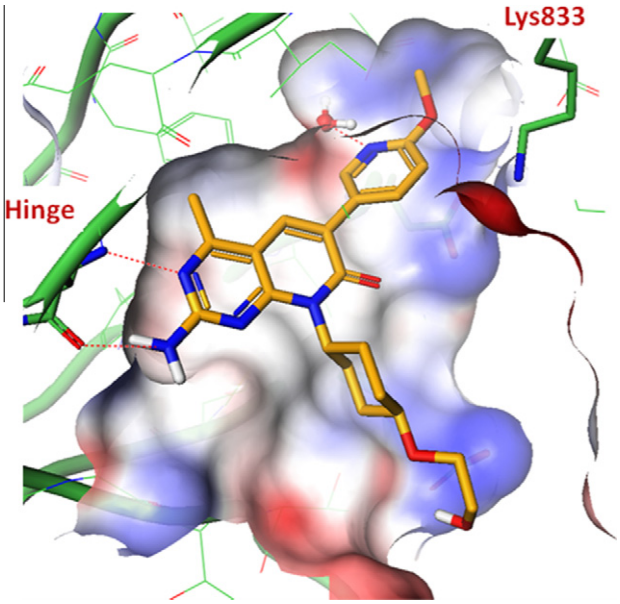


Figure 2. Co crystal structure of PF-04691502 bound in PI3Kγ.

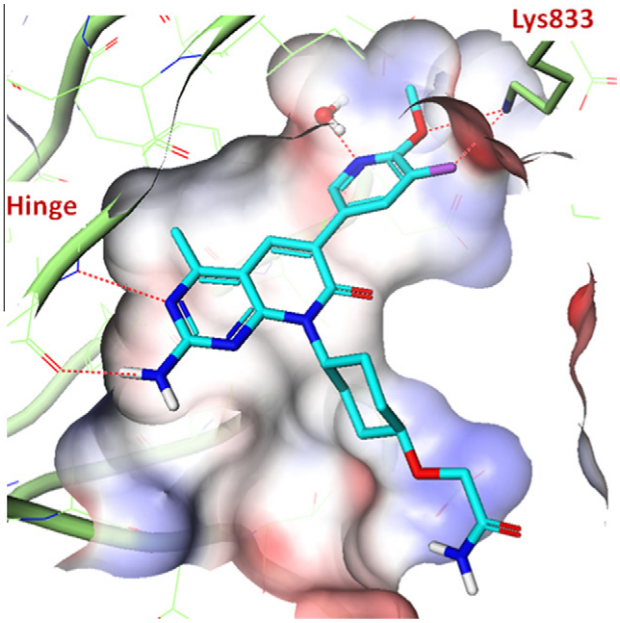


Figure 3. Amide derivative **29** modeled in PI3Kγ.

chain was in close distance to form H bonds with both the oxygen atom and the F atom. The H bond interaction between the conserved water molecule and the pyridine ring N was retained. The interaction between 4-MeO-5-F-3-pyridine and Lys 833 and the conserved water molecule was recently reported both for a modified MPP derivative[7] and for AMG 511, in which 4-MeO-5-F-3-pyridine was attached to a different scaffold.[8] Based on the modeling studies, we decided to investigate the SAR by replacing 4-MeO-3-pyridine in PF-04691502 with different heteroaryl groups including 4-MeO-5-F-3-pyridine. In addition, the terminal amide derivatives had much higher Topological Polar Surface Area (PSA) than the terminal alcohol derivatives such as PF-04691502, so we were interested in comparing the SAR between the amides and the alcohols, for example, binding affinities with mPI3Kα and mTOR, correlation between PSA, LogD, permeability and cellular potency.

Illustrated in Scheme 1 is the synthetic route for preparing MPP derivatives with cis-cyclohexyl moiety substituted at the 8 position; the *trans*-cyclohexyl derivatives were prepared by using the corresponding *trans*-4-aminocyclohexanol at step 1.[9] Compound **1** was reacted with *cis*-4-aminocyclohexanol to produce compound **2**, which was reacted with *tert*-butyl bromoacetate, followed by quenching with MeOH to give the isolated methyl ester **3**. The ester in **3** was reacted with ammonia to yield compound **4**, which was reacted with hydroxylamine in ethanol to generate compound **5**. Palladium-catalyzed coupling of **5** with ethyl acrylate yielded compound **6**, which was subjected to intramolecular cyclization to generate compound **7**. Bromination of **7** with NBS, followed by Suzuki coupling reactions with an appropriate bronic acid or bronic ester gave the desired product **9**.

To assess the metabolic profile of PF-04691502 in humans in vivo, the plasma obtained from patients dosed with PF-04691502 at 8 mg QD in a clinical pharmacology dose escalation study was analyzed.[10] The high dose was selected for metabolite profiling to ensure that all metabolites were detected in the human plasma. The total ion chromatogram of plasma showed 4 peaks. The retention times and the molecular ions of the peaks at 24.81 and 26.80 min were identical to the PF-04447949[11] and unchanged PF-04691502, respectively (Fig. 4). The additional peaks at 25.24 min (M2) and 29.07 (M6) showed molecular ions at *m*/*z* 602.2 and 440.19. The fragmentation pattern of the two molecular ions suggested the M2 and M6 were the glucuronide conjugate of PF-04691502 and the carboxylic acid metabolite, respectively (Fig. 5). Although the site of conjugation was unknown, the formation of M2 involved catalysis by uridine glucuronyl transferase (UGT). On the other hand, the formation of M6 was mediated by oxidation of the alcohol either by CYP450 or via alcohol dehydrogenase to the aldehyde that was oxidized to the corresponding acid, ({*trans*-4-[2-amino-6-(6-methoxypyridin-3-yl)-4-methyl-7-oxopyrido[2,3-*d*]pyrimidin-8(7*H*)-yl]cyclohexyl}oxy)acetic acid (M6, PF-06465603) (Scheme 2). The identity of M6 was further confirmed by comparison of its mass spectrum and retention time with an authentic standard, which was prepared from PF-04691502 by oxidizing the primary alcohol to the acid (Scheme 3)

MPP derivatives with different heteroaryl groups off the 6 position, with *cis* or *trans*-cyclohexyl off the 8 position, and with terminal alcohol or terminal amide were prepared following the synthetic route described in Scheme 1. The in vitro potency, calculated SFLogD, HLM in vitro clearance, permeability (RRCK), and PSA for the alcohol and amide derivatives, and the metabolite **10** are summarized in Table 1.

In the mPI3Kα assay, compounds with six-membered heterocycle are in general more potent than those with 5 or 10-membered heterocycles as shown in Figure 6, suggesting six-membered heterocycle fits the best in PI3Kα binding site. Modeling studies with **29** indicated that the F atom could help position Lys 833 side chain to form H bonds with both the oxygen atom and the F atom of the