

Discovery of a Highly Potent and Selective MEK Inhibitor: GSK1120212 (JTP-74057 DMSO Solvate)

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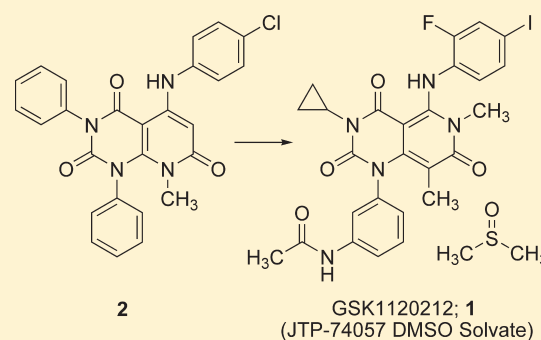
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S Supporting Information

ABSTRACT: Inhibition of mitogen-activated protein kinase/extracellular signal-regulated kinase (MEK) represents a promising strategy for the discovery of a new generation of anticancer chemotherapeutics. Our synthetic efforts, beginning from the lead compound **2**, were directed at improving antiproliferative activity against cancer cells as well as various drug properties. These efforts led to the discovery of *N*-{3-[3-cyclopropyl-5-(2-fluoro-4-iodophenylamino)-6,8-dimethyl-2,4,7-trioxo-3,4,6,7-tetrahydro-2*H*-pyrido[4,3-*d*]pyrimidin-1-yl]phenyl}acetamide dimethylsulfoxide solvate (GSK1120212, JTP-74057 DMSO solvate; **1**), a selective and highly potent MEK inhibitor with improved drug properties. We further confirmed that the antiproliferative activity correlates with cellular MEK inhibition and observed significant antitumor activity with daily oral dosing of **1** in a tumor xenograft model. These qualities led to the selection of **1** for clinical development.

KEYWORDS: GSK1120212, JTP-74057, antiproliferative, MEK inhibitor



Activation of the p42/44 MAPK signaling pathway comprising mitogen-activated protein kinase/extracellular signal-regulated kinase (ERK) kinase (MEK)–ERK has been implicated in the pathogenesis and progression of various human malignant tumors.^{1,2} The MEK–ERK pathway is often activated by mutation of upstream factors, BRAF or Ras, or by the signals of constitutively activated cell-surface receptors.^{1–6} Therefore, inhibition of the p42/44 MapK pathway is an attractive therapeutic strategy for multiple cancers. Indeed, several small molecules that target the pathway, including MEK and Raf inhibitors, are being tested in human clinical studies.^{1,2,6}

During a high-throughput screening for compounds that can induce expression of the cyclin-dependent kinase (CDK) 4/6 inhibitor p15^{INK4b}, we identified **2** (Figure 1).⁷ Subsequent experiments confirmed that **2** has an antiproliferative activity against human cancer cell lines ACHN (renal adenocarcinoma) and HT-29 (colorectal adenocarcinoma) with IC₅₀ values of 4800 and 990 nM, respectively. We conducted a medicinal chemistry campaign, chemically modifying **2** to optimize for these antiproliferative effects. Our synthetic efforts led to the discovery of orally bioavailable GSK1120212 (JTP-74057 DMSO solvate) **1** (Figure 1), demonstrating selective inhibition of proliferation in various BRAF mutant cancer cell lines. This

compound was confirmed through molecular target analyses^{7,8} to be a highly potent and selective inhibitor of MEK1/2. We describe herein the structure–activity relationship (SAR) studies on **2** guided by ACHN and HT-29 cancer cell lines growth inhibitory activity and the detailed characteristics of **1**.⁹

Compound **2** has three aromatic rings on the pyridopyrimidine core, making it an unattractive starting point for further optimization due to the high hydrophobicity (ClogP 6.3). With the aim to reduce hydrophobicity of **2**, we replaced each aromatic ring with small alkyl groups by turns. Replacement of the benzene ring at the 3-position (upper left) of the pyridopyrimidine core with a cyclopropyl ring and a chlorine atom on the aniline ring with bromine resulted in **3** (Figure 1), which exhibited reduced hydrophobicity (ClogP 5.0) and improved potency by 4-fold. Replacement of benzene rings at position 1 (lower left) or 5 (upper right) with lower alkyl groups resulted in a loss of potency (data not shown). Encouragingly, **3** showed the first sign of antitumor effect in an in vivo model, the nude mouse HT-29 xenograft model (data not shown). We next introduced a

Received: January 5, 2011

Accepted: February 20, 2011

Published: February 28, 2011

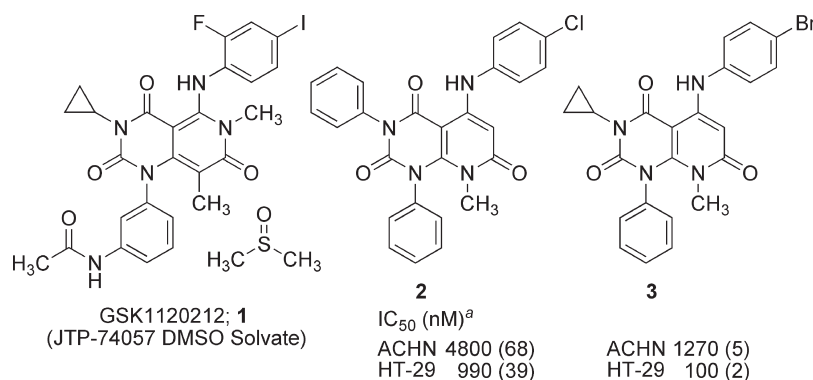
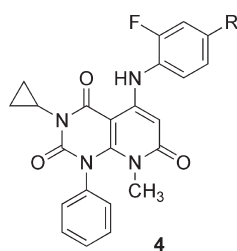


Figure 1. Structures (1–3) and cellular activities (2 and 3). ^aNumbers in parentheses represent numbers of determinations.

Table 1. Substitution at the 4-Position of Aniline SAR^a



compd	R	IC_{50} (nM)	
		ACHN	HT-29
4a	H	10000 (1)	1300 (1)
4b	Me	1300 (2)	110 (2)
4c	Et	185 (3)	27 (3)
4d	Pr	2100 (2)	230 (2)
4e	cPr	290 (2)	39 (2)
4f	vinyl	104 (3)	15 (3)
4g	C≡CH (ethynyl)	42 (2)	5.9 (2)
4h	C≡C–Me	>3000 (1)	>1000 (1)
4i	CN	>10000 (1)	1600 (1)
4j	F	>3000 (1)	>1000 (1)
4k	Cl	4600 (2)	102 (2)
4l	Br	325 (4)	15 (4)
4m	I	13 (2)	1.5 (2)

^aNumbers in parentheses represent numbers of determinations.

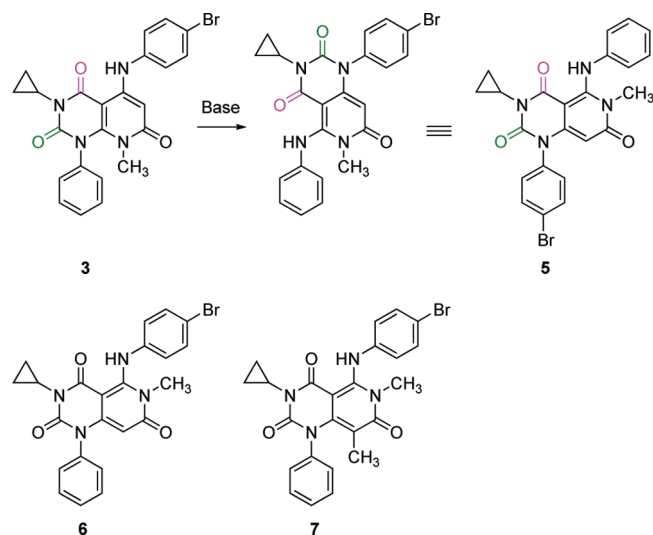
small substituent (e.g., methyl, halogen, methoxy, etc.) at the 2- or 3-position of the aniline ring of **3**. Introduction of fluorine atom at the 2-position of the aniline ring (**4l**, Table 1) improved the potency by ca. 4-fold as compared to **3** with a small increase in ClogP (5.2).

To further increase the potency, we proceeded to explore the substitution at the 4-position on the aniline ring (Table 1). To investigate the size limit of the substituent at the 4-position, we first replaced the bromine atom of **4l** with alkyl groups (**4b–e**). As the size increased from hydrogen (**4a**) to ethyl (**4c**), the compounds became more potent, while removal of the bromine atom of **4l** resulted in loss of activity (**4a**). Propyl (**4d**) showed less potency, but cyclopropyl (**4e**) restored potency to a similar level of **4c**. Introduction of the unsaturated alkyls vinyl (**4f**) and

ethynyl (**4g**) further increased the potency, although one-carbon extension deprived **4g** of activity (**4h**). These results suggested the existence of a sterically constrained region of the target protein of these compounds in the vicinity of the para-substituents. The compound with cyano (**4i**) showed weak potency in spite of having similar shape and size as the ethynyl group of potent compound **4g**. We next introduced halogen atoms (**4j–m**) and found that iodine, synthetic intermediate for **4g** etc., significantly increased the potency to IC_{50} values of 13 and 1.5 nM for ACHN and HT-29, respectively (**4m**). Thus, we succeeded in improving potency by 500-fold from the lead **2** to **4m**.

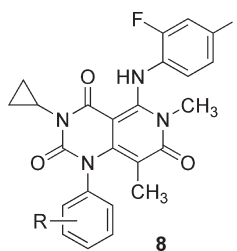
Unfortunately, the compounds detailed above, possessing a pyrido[2,3-*d*]pyrimidine core including **4m**, were proven to be unstable under weak basic conditions and not appropriate for further development as drug candidates. For example, treatment of **3** with potassium carbonate in methanol/chloroform at ambient temperature provided **5** having a pyrido[4,3-*d*]pyrimidine core in 97% yield. We assumed that **5** was formed through the opening of the pyrimidinedione ring by the attack of nucleophile (in this case methanol) at the C-2 carbonyl and subsequent ring closure to another pyrimidinedione involving the neighboring nitrogen atom of the other aniline (upper right). Compound **5** was totally inactive (Table 2). However, we hypothesized that placement of appropriate substituents in the same alignment as **4m** to the postarrangement stable scaffold might afford chemically stable and highly active compounds, because the spatial arrangement of two benzene and cyclopropane rings and three carbonyls on two heterocyclic cores of **3** and **5** was almost identical. Although the inhibitory activity was not restored by just a transposition of bromine atom from lower left phenyl (**5**) to the aniline ring at the 5-position (**6**), incorporation of the methyl to **6** at the 8-position of the heterocyclic core to yield **7** raised the potency up to the level of prerearranged **3**. We reasoned that this was due to a potential effect on the orientation of the neighboring phenyl ring at the 1-position of the heterocyclic core. Finally, introduction of 2-F,4-I aniline moiety in the same substitution fashion as **4m** to give **8a** provided a substantial boost in potency as we anticipated (Table 3).

Although **8a** exhibited IC_{50} values of 25 and 1.7 nM for ACHN and HT-29, respectively, this compound displayed very low aqueous solubility due to the high ClogP of 6.0. We investigated polar group substitution of the phenyl ring at the 1-position of the heterocyclic core as a means to optimize physicochemical properties while further improving potency. An acetamide and a methanesulfonamide analogues were found to be the most active among a variety of polar groups explored. Representative

Table 2. Heteroaromatic Core Transformation SAR^a

compd	IC ₅₀ (nM)	
	ACHN	HT-29
3	1270 (2)	100 (2)
5	>3000 (1)	>1000 (1)
6	>3000 (1)	>1000 (1)
7	830 (2)	135 (2)

^a Numbers in parentheses represent numbers of determinations.

Table 3. Polar Group Substitution SAR^a

compd	R	IC ₅₀ (nM)	
		ACHN	HT-29
8a	H	25 (2)	1.7 (2)
8b	3-NHCOMe	9.8 (5)	0.57 (10)
8c	4-NHCOMe	195 (2)	14 (2)
8d	3,5-diNHCMe	>1000 (1)	>100 (1)
8e	3-NHSO ₂ Me	6.4 (5)	0.52 (5)
8f	4-NHSO ₂ Me	22 (5)	1.4 (5)

^a Numbers in parentheses represent numbers of determinations.

examples are shown in Table 3. Compounds **8b** and **8e** substituted at the meta-position improved potency by 3-fold and were coupled with the reduced hydrophobicity (ClogP **8b**, 5.0; **8e**, 4.8). Disubstituted analogue **8d** exhibited no activity up to 100 nM. Compounds **8c** and **8f** each had a para-substituent and

Table 4. PK Related Parameters^a

compd	solubility (PBS)	Caco2 permeability	AUC ^b	% F ^b
4l	<0.4	<0.1	7.7	2.1
4m	<0.3	<0.1		
8a	<0.2	14.0		
8b	5.3	26.0	88.3	30
8e	3.3	17.1	59.2	59

^a Units: solubility, μM ; Caco2 permeability, $\text{cm/s} \times 10^{-6}$; and AUC, $\mu\text{M h}$. ^b AUC and % F were determined after dosing orally to mice at 30 mg/kg in 0.5% methyl cellulose suspension.

showed moderately less or similar potency as compared to **8a**. Consistent with their reduced hydrophobicity, **8b** and **8e** showed improved solubility and permeability as compared to **8a** (Table 4) as well as better bioavailability than **4l**. Compound **8b** (JTP-74057) represented the best combination of desired features, with IC₅₀ values of 9.8 and 0.57 nM for ACHN and HT-29, respectively, and was subsequently determined to be a candidate for further development based on the results of in vivo xenograft model and toxicity studies.

Initial batches of **8b** having a mp of 180 °C showed acceptable oral bioavailability as shown in Table 4. However, through crystalline polymorphism studies, the most stable form of mp 300 °C appeared and was found to show poor oral exposure. Compound **8b** was weakly basic compound and not suitable for stable salt formation. In hope of restoring oral bioavailability, we prepared various solvates. Fortunately, we found that two solvates with class 3 solvent acetic acid and DMSO showed similar oral bioavailability to **8b** with an mp of 180 °C. DMSO solvate **1** showed desirable solid state properties and was selected for development.

Because the molecular target of this compound series was still unknown at that time, we carried out a compound-immobilized affinity chromatography in which the analogues of **8b** were immobilized to resin. As a result, we found that **8b** binds to MEK1/2 and inhibits its kinase activity with high specificity.^{7,8,10,11}

To confirm that cellular antiproliferative activity is due to cellular inhibition of MEK1/2 kinase activity, the DMSO solvate **1** was tested in both a cellular phospho-ERK1/2 assay and cell proliferation assays. In BRAF mutant SK-MEL-28 cells and KRAS mutant HCT116 cells, **1** caused dose-dependent inhibition of ERK1/2 phosphorylation as well as dose-dependent growth inhibition. We then considered the question of the relationship between the extent of ERK1/2 phosphorylation (p-ERK1/2) inhibition and the extent of growth inhibition in similar time frames. To address that relationship, we compared the respective curves of phosphoinhibition and growth inhibition after a 72 h drug treatment (Figure 2). In both SK-MEL-28 and HCT116 cells, **1** inhibits 50% p-ERK1/2 at nearly equivalent concentrations (0.8 and 1.8 nM, respectively). However, as the slopes of the curves reflect, in SK-MEL-28 cells, **1** inhibits 90% p-ERK1/2 at a lower concentration (3.4 nM) than in HCT116 (33.3 nM). Furthermore, in both cell lines, 50% growth inhibition was only achieved at concentrations **1** that produced near complete ERK1/2 inhibition (85 and 90%, respectively). These data suggest that near complete inhibition of ERK1/2 phosphorylation is required to achieve significant cell growth inhibition and that cells inherently differ in the slope of their MEK inhibition dose–response curve.

In addition, **1** was then evaluated in vivo in an A549 (KRAS mutant cell line) xenograft model, orally dosing daily for 21 days

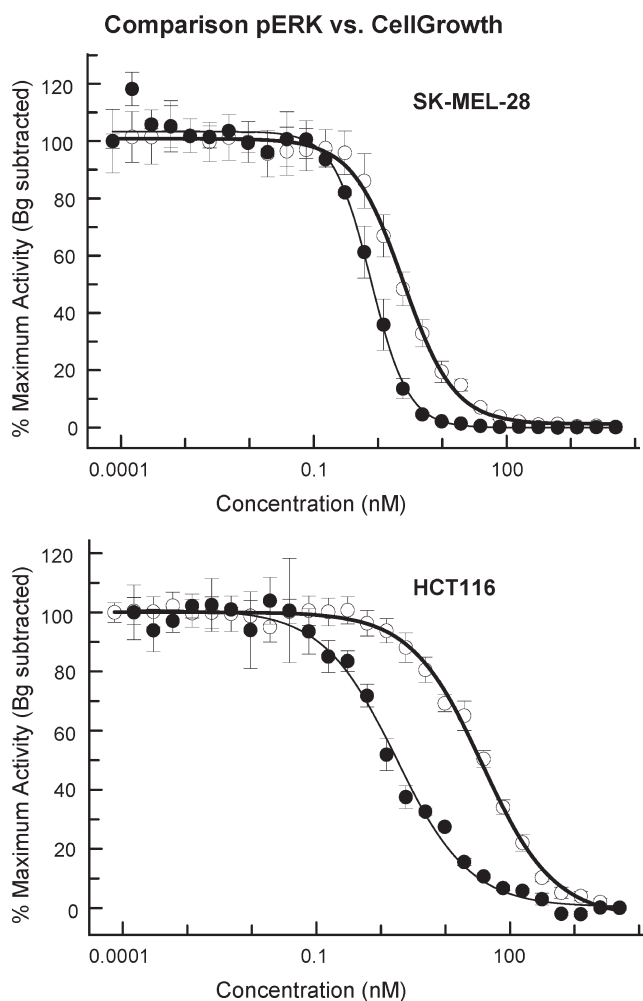


Figure 2. In both BRAF^{V600E} SK-MEL-28 cells and KRAS^{G13D} HCT116 cells, compound **1** (GSK1120212) potently inhibits both phosphorylation of ERK1/2 (filled circles) and cell growth (open circles) over 72 h. Differences in growth assay IC₅₀ values between cell lines appear to correlate with the concentrations required for ca. 90% inhibition of ERK1/2 phosphorylation.

(qd × 21). In this study (Figure 3), near complete tumor growth inhibition was observed at 5.0 and 2.5 mg/kg [92 and 87% tumor growth inhibition (TGI), respectively] and to a lesser degree at 0.5 and 0.1 mg/kg (62 and 58% TGI). [Notably, although 5 mg/kg was the maximally tolerated dose (MTD) in this study, 3 mg/kg is the typically observed MTD.] Dose-dependent antitumor activity with **1** treatment has been similarly reported for several other KRAS and BRAF mutant tumor models.¹⁰

Although SAR studies from the lead compound to JTP-74057 were guided by cancer cell lines growth inhibitory activity rather than MEK inhibitory activity, we realized after confirmation of the enzymatic target that the resultant compounds shared SAR features to those of known MEK inhibitors.^{12,13} In particular, the best substitution fashion of the aniline ring, 2-F,4-I, of **1** is also employed in the second-generation MEK inhibitor PD0325901¹² (Pfizer). The methyl group of **1**, introduced at the 8-position of the heterocyclic core to boost the potency, is found in the corresponding position of pyridone type MEK inhibitors.^{14–16} A marked difference of **1** from the previously identified MEK inhibitors is its unique pyrido[4,3-*d*]pyrimidine core structure. This unique feature likely contributes to **1**'s good pharmacokinetic profile, with

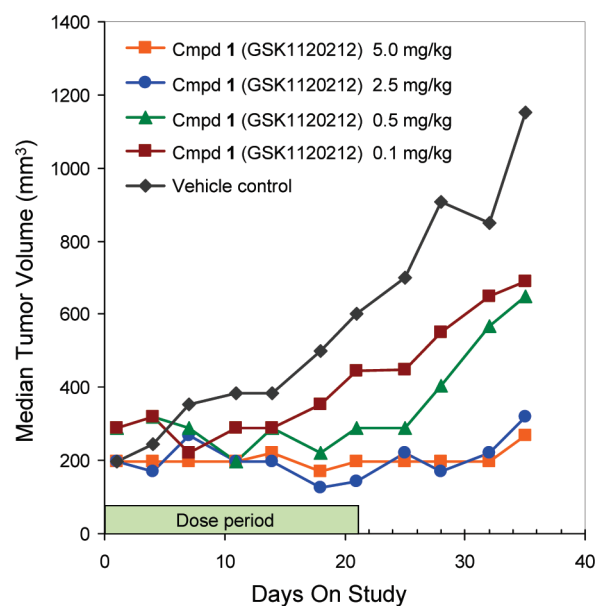


Figure 3. Compound **1** causes significant antitumor activity in KRAS^{G12S} A549 tumor xenograft model. Daily oral administration for 21 days resulted in significant and dose-dependent tumor growth inhibition.

sustained plasma drug concentrations and narrow “peak/trough” ratio,¹⁰ resulting in significant anticancer activity with once-daily dosing.

In conclusion, starting from an initial lead **2**, SAR studies to improve antiproliferative activity against cancer cells culminated in the discovery of **1**, GSK1120212 (JTP-74057 DMSO Solvate), a highly potent, selective, and orally active MEK inhibitor. Compound **1** displays remarkable potency in cellular assays for ERK1/2 phosphorylation and growth inhibition and excellent in vivo activity. Inhibition of the MEK–ERK pathway offers a very promising therapeutic strategy for cancers with activating mutant Ras and Raf, and **1** has been advanced into phase III trials for advanced or metastatic BRAF mutant melanoma as well as phase I and II studies in subjects with solid tumors or leukemia.

■ ASSOCIATED CONTENT

Supporting Information. Biological assays and experimental procedures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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■ ACKNOWLEDGMENT

We thank our Analytical Research and Development Laboratories for collecting analytical data and Dr. Jun-ichi Haruta for helpful discussions.

■ ABBREVIATIONS

MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase; ERK, mitogen-activated protein kinase/extracellular signal-regulated kinase

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- (16) In ref 14, it was pointed out that N-Me pyridones are active in the kinase and cellular assays, but N-H pyridones are not potent. They speculated that the drop of activity of N-H pyridones may be attributed to the loss of the key hydrogen bond to Ser212 due to partial tautomerization of the pyridone system. However, in our case, introduction of a methyl group in the corresponding position, that is, 6 to 7, does not change the tautomerization state of the pyrido[4,3-d]pyrimidine core, so we speculated that methyl group affected the orientation of neighboring phenyl ring.