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Letters

Potent and Selective Mitogen-Activated Protein Kinase Kinase (MEK) 1,2 Inhibitors. 1. 4-(4-Bromo-2-fluorophenylamino)-1-methylpyridin-2(1*H*)-ones

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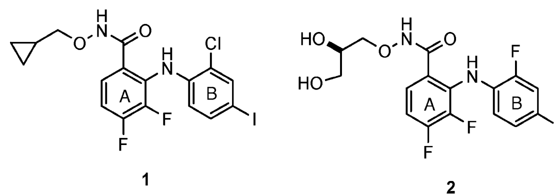
Abstract: The role of MEK 1,2 in cancer tumorgenesis has been clearly demonstrated preclinically, and two selective inhibitors are currently undergoing clinical evaluation to determine their role in the human disease. We have discovered 4-(4-bromo-2-fluorophenylamino)-1-methylpyridin-2(1*H*)-ones as a new class of ATP noncompetitive MEK inhibitors. These inhibitors exhibit excellent cellular potency and good pharmacokinetic properties and have demonstrated the ability to inhibit ERK phosphorylation in HT-29 tumors from mouse xenograft studies.

One of the most important and well understood signal transduction pathways involved in human cancer is the Ras/Raf/MEK/ERK signal transduction pathway. Examination of cell lines and primary tumor samples have shown constitutive activation of the ERK pathway in cancers of the lung, colon, pancreas, kidney, and ovary.[1] Furthermore, oncogenic mutations reside in this pathway. Oncogenic forms of Ras are found in almost one-third of human cancers, including 50% of colon, 30% lung, and greater than 9 out of 10 pancreatic cancers.[2] A majority of malignant melanoma (60%) and papillary thyroid cancers (40−70%) harbor B-Raf mutations.[3] The functional result of these mutations is a constitutively active ERK kinase cascade. Overexpression of receptor tyrsosine kinases also contributes to activation of the Ras/Raf/MEK/ERK pathway. For example, overexpression of the HER2 receptors has been shown to increase signaling through this pathway.[4] MEK 1,2 (mitogen-activated protein kinase kinase) is a pivotal player in this pathway. It is downstream of both Ras proteins and Raf kinases. Its only known substrates are ERK 1,2.

Chart 1. Structures of MEK 1,2 Inhibitors[5,6]



Three selective MEK inhibitors have entered clinical trials for cancer: **1** (CI-1040)[5] and **2** (PD 0325901)[6] both from Pfizer (Chart 1) and ARRY-142886[7] (also known as AZD6244) from Array BioPharma in collaboration with AstraZeneca.[8] Anthranilic hydroxamate **1** was subsequently withdrawn from the clinic because of lack of efficacy, probably due to poor pharmaceutical characteristics.[9] Both ARRY-142886 and **2** are superior to **1** in both pharmaceutical properties and preclinical efficacy.[6,7] Reports from the clinic on these promising inhibitors are just starting to emerge.[10,11] All three inhibitors are highly selective for MEK 1,2 and have been shown to be noncompetitive inhibitors with respect to ATP and ERK.[5−7] X-ray cocrystal structures of MEK with anthranilic acid type MEK inhibitors (the same structural class as **1** and **2**) have been disclosed.[12] The structure shows that these inhibitors bind in a pocket adjacent to the nucleotide-binding site in the presence of ATP.

MEK is a very attractive anticancer target because of its integral role in cancer tumorgenesis and the fact that noncompetitive, exquisitely selective inhibitors can be designed. Our goal was to design novel, ATP noncompetitive MEK inhibitors that capitalize on the key binding features present in the published crystal structures. In the disclosure of the cocrystal structures of MEK with inhibitors, some key binding interactions were highlighted (Figure 1).[6b,12] These include the B-ring occupying a hydrophobic pocket formed by residues Leu118, Ile126, Val127, Ile141, Met143, Phe129, Phe209, and Val211 and forming an edge-to-face interaction with Phe209. The diol of the hydroxamate also interacts with the terminal phosphate of ATP and Lys97. We hypothesized that the hydroxamate and the hydrophobic B-ring are the two key pharmacophore elements of these inhibitors. Additionally, it has been reported that the aryl fluoride of the anthranilic acid MEK inhibitors (such as **1**

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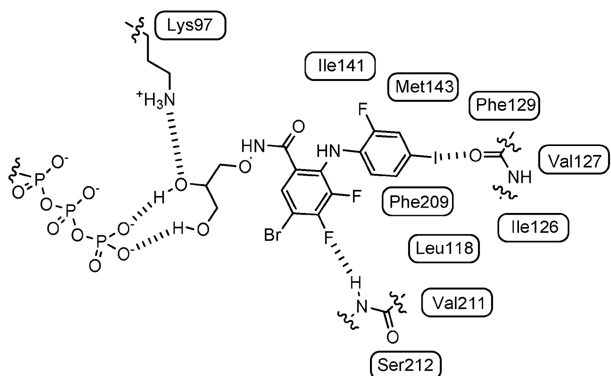
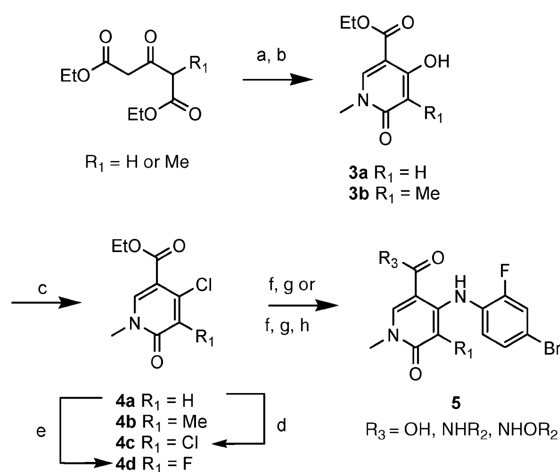


Figure 1. Key binding interactions of anthranilic acid type inhibitor and MEK 1.[6b,12]

Scheme 1[a]



[a] Reagents: (a) $HC(OEt)_3$, Ac_2O; (b) $MeNH_2$, H_2O; (c) $POCl_3$, Δ; (d) NCS; (e) Selectfluor; (f) LiOH; (g) 4-bromo-2-fluoroaniline, LiHMDS, −78 °C; (h) EDCI, HOBt and NHR_2 or $NHOR_2$.

and **2**) is both within the proper distance and direction to make a hydrogen bond to the backbone NH of Ser212.[12] While this latter interaction may contribute to the binding affinity of these inhibitors, further inspection of the X-ray structure suggests an appropriate H-bond acceptor, in place of the fluorine, would lead to compounds with increased potency. Strengthening this interaction may also allow for greater flexibility in the design of inhibitors with improved physicochemical properties (i.e., solubility, permeability, and metabolic stability). We decided to examine 4-(4-bromo-2-fluorophenylamino)-1-methylpyridin-2(1*H*)-ones for their potential to inhibit MEK.[13] Docking studies indicated that these inhibitors should form strong hydrogen bonds to the backbone NHs of both Ser212 and Val211.[14]

The desired 4-(4-bromo-2-fluorophenylamino)-1-methylpyridin-2(1*H*)-ones can be prepared by condensation of either 3-oxopentanedioic acid diethyl ester or diethyl 2-methyl-3-oxopentanedioate with triethyl orthoformate followed by cyclization with methylamine in a two-step, one-pot procedure to give hydroxyl pyridone ester **3** (Scheme 1). Pyridone ester **3** is then treated with $POCl_3$ to give the corresponding chloride **4** which can be further functionalized with either NCS or Selectfluor for derivatives where R_1 = Cl or F. Saponification followed by S_NAr coupling with 4-bromo-2-fluoroaniline in the presence of LiHMDS at low temperature with the inclusion of standard coupling with EDCI for amide and hydroxamate derivatives furnished the desired analogues **5**.

The enzymatic activity of these inhibitors was determined as previously described.[15] Inhibition of basal ERK phosphorylation in Malme-3M cells was used as the mechanistic cellular assay. Inhibition of cellular viability of Malme-3M cells was used as the functional cellular assay. It is interesting to note that within this series of inhibitors, the enzyme and cell data did not correspond well. In several instances, the compounds under evaluation were found to be more potent in the mechanistic cellular experiments than in enzymatic studies. Two possible explanations for this observation are (1) the use of a constitutively active mutant protein for the enzymatic screen and (2) the presence of auxiliary proteins that are not present in the cell-free system which can influence the conformation and/or accessibility of cellular MEK. Regardless, the enzyme data is included for completeness although the decisions to progress into further studies were solely based on the cellular assays.

Table 1. Activity of 5-Substituted 4-(4-Bromo-2-fluorophenylamino)-1-methylpyridin-2(1*H*)-ones

Compd	R_2	R_1	IC_{50} (nM)[a]	pERK (nM)[b]	Cell Viability (nM)[b]
6	$cPrCH_2$	H	39	26 ± 1	165 ± 25
7		Cl	124	27 ± 7	195 ± 15
8		F	6.8	3.4 ± 0.9	22 ± 9
9		Me	40	7.5 ± 0.6	46 ± 8
10	$HOCH_2CH_2$	H	30	73 ± 5	460 ± 20
11		Cl	45	26 ± 1	175 ± 35
12		F	9.3	22 ± 3	115 ± 15
13		Me	18	2.0 ± 0.1	11 ± 2
14			6786	>10,000	>5,000
15			4496	>10,000	>5,000

[a] Estimated error is 35% based on historical variability of the control compound. [b] Cell assay results expressed as mean ± SEM (n = 2).

The first series of 4-(4-bromo-2-fluorophenylamino)-1-methylpyridin-2(1*H*)-ones prepared showed excellent potency as evidenced by analogue **6** displaying 26 and 165 nM activity in the mechanistic (pERK) and functional (cell viability) cellular assays, respectively (Table 1). Examination of the five position of the pyridone showed that potency could be improved. In the cyclopropyl methyl hydroxamate series, the 5-F compound **8** and 5-Me compound **9** were more potent than the unsubstituted analogue **6** with single digit nanomolar activities in the mechanistic cellular assay. The hydroxyl ethyl hydroxamate analogues showed the same trend. However, the 5-Me **13** analogue was ca. 10-fold more potent than the 5-F **12** in both cellular assays. As predicted, both the methoxy pyridine **14** and the chloro pyridine **15** were inactive, suggesting the important role of the putative pyridone carbonyl−backbone NH Ser212/Val211 hydrogen bond for MEK inhibition in this series.

Evaluation of the carboxylate region revealed some important results (Table 2). As also shown in Table 1, the cyclopropyl methyl hydroxamate **8** was found to be more potent than the hydroxyethyl hydroxamate **12**. Interestingly, the primary amide **16** was shown to exhibit near equal potency to the hydroxamate **8**. The excellent activity of the amide **16** was somewhat a surprise, as it is significantly smaller than the hydroxamates

Table 2. Activity of 4-(4-Bromo-2-fluorophenylamino)-1-methylpyridin-2(1*H*)-one Hydroxamates, Amides, and Acid

compd	R_1	R_3	IC_{50} (nM)[a]	pERK (nM)[b]	cell viability (nM)[b]
8	F	$cPrCH_2ONH$	6.8	3.4 ± 0.9	22 ± 9
12		$HO(CH_2)_2ONH$	9.3	22 ± 3	115 ± 15
16		NH_2	14	7.1 ± 0.3	48 ± 3
17		OH	30	> 10,000	> 5,000
18		MeNH	118	68 ± 22	405 ± 5
19		$cPrCH_2NH$	2200	1206 ± 76	> 5000
20		HONH	15	18 ± 2	860 ± 240
21		MeONH	43	22 ± 1	145 ± 25
22		EtONH	33	8.8 ± 0.8	52 ± 1
23	Me	MeONH	105	39 ± 0.3	265 ± 25
24		EtONH	82	10.6 ± 0.2	118 ± 23

[a] Estimated error is 35% based on historical variability of the control compound. [b] Cell assay results expressed as mean ± SEM ($n = 2$).

and lacks the N−O moiety which may act as a hydrogen bond acceptor. Importantly, enzymatic profiling of compound **16** showed that this series binds noncompetitively with respect to ATP. The carboxylic acid **17** was active in the enzyme assay but void of cellular activity. The inactivity of the carboxylic acid **17** in cells is likely a result of poor permeability. Secondary amides were not nearly as potent as the primary amide **16,** with the methyl amide **18** ca. 10-fold weaker in all the assays and the cyclopropyl methyl amide **19** in the micromolar range. The hydroxamic acid **20** showed excellent activity in the mechanism based cellular assay (1 h compound exposure) but lost significant activity in the functional cellular assay (3-day compound exposure). Similar results with hydroxamic acid MEK inhibitors have been reported.[16] It has been postulated that the hydroxamic acids are metabolically unstable upon extended cellular exposure and thus lose activity over time. Finally, simple methyl **21** and **23** and ethyl **22** and **24** hydroxamates retained good activity in both assays.

Most of these pyridone analogues show excellent cellular activity even though they appear to be unable to interact with the phosphate of ATP as proposed for the anthranilic acid MEK inhibitors. The potency of these inhibitors may be driven more by their interaction with the backbone NHs of both Ser212 and Val211 than by an interaction with the phosphate of ATP. The hydroxyethyl hydroxamate analogue **12** may interact with the phosphate. However, it is a weaker inhibitor than other analogues in this series. In fact, several compounds, including amide **16** and ethyl hydroxamate **22**, are more potent. It may be that the increase in binding affinity obtained by interaction of the terminal hydroxyl of **12** with the phosphate is offset by a desolvation penalty. It is unclear if the oxygen of the hydroxamate is interacting with the protein as the amide **16** shows excellent activity. It appears a more subtle effect is in play with the amides in contrast to the hydroxamates. Increasing the lipophilic nature of the hydroxamates increases potency (i.e. **8** > **22** > **21**). The opposite is true of the amides examined (**16** > **18** > **19**). This trend in the amide series could reflect interaction with the protein through a water network which may be disrupted by larger substituents. Alternatively, the active conformation of the secondary amides may be *s*-trans, in this case, the larger the substituent, the larger the strain energy encountered in the active conformation.

Table 3. In Vitro Stability, Permeability, and Solubility and Rat PK (iv, 1 mg/kg; po, 10 mg/kg)

		compd **13**	compd **16**	compd **22**	compd **23**	compd **24**
hepat. Cl[a]		6	9	2	5	5
Caco-2		low	high	high	high	high
sol. @ pH 6.5[b]		450	26	26	200	60
CL[c]		8.0	5.7	0.7	1.5	1.9
Vd[d]		1.1	0.8	0.4	0.4	0.4
AUC[e]	iv	2.26	2.5	24.1	11.5	8.9
	po	12.0	23	138	88.3	75
$T_{1/2}$ (h)	iv	1.6	1.8	6	3.5	2.5
	po	3.0	11	12	4.7	4.7
%*F*		53	100	58	73	80

[a] Human hepatocyte clearance (mL/min/kg). [b] Aqueous solubility on crystalline solid (μg/mL). [c] mL/min/kg. [d] L/kg. [e] μg/h/kg.

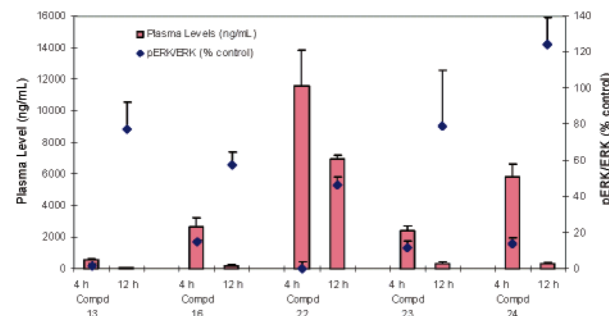


Figure 2. PK/PD evaluation of 4-(phenylamino)pyridine-2-(1*H*)-ones after 10 mg/kg po dose in HT-29 tumor-bearing mice.

The selectivity of a subset of these inhibitors was examined. Compounds **13**, **22**, **23**, and **24** were screened against a panel of over 20 protein kinases at 10 μM. None of the compounds tested showed greater than 50% inhibition against any of the enzymes. This excellent selectivity may be a result of noncompetitive binding nature of these inhibitors.

We next turned our attention to physiochemical and in vivo properties of these inhibitors. Compounds **13**, **16**, **22**, **23**, and **24** were selected for further evaluation. With the exception of the primary amide **16** (human hepatocyte CL = 9 mL/min/kg), the compounds showed good human hepatic stability and acceptable solubility at pH 6.5 (Table 3). Compounds **16**, **22**, **23**, and **24** were found to be highly permeable in the Caco-2 assay. The more polar analogue **13** with the hydroxylethyl hydroxamate demonstrated low permeability in this assay. The rat pharmacokinetic profiles after a 1 mg/kg iv dose and 10 mg/kg po dose demonstrated good to excellent half-lives, extraction ratios below 10%, and bioavailabilities from 53 to 100%.

These five analogues were evaluated in vivo in HT-29 tumor-bearing mice for their ability to inhibit pERK in tumors that are then excised 4 and 12 h after a single 10 mg/kg oral dose (Figure 2). All of the compounds showed greater than 80% inhibition of pERK at the 4 h time point while only compounds **16** and **22** demonstrated ~50% inhibition at the 12 h time point. Plasma exposure of the compounds varied widely. Compound **22** had extremely high plasma levels at 4 h with complete inhibition of pERK. However, despite plasma levels of **22** greater than 6000 ng/mL, inhibition of pERK at 12 h was only 50%. In contrast, at 4 h, compound **13** was able to completely inhibit pERK levels in the excised tumor with plasma levels below 600 ng/mL.

In conclusion, we have discovered new 4-(4-bromo-2-fluorophenylamino)-1-methyl-pyridin-2(1*H*)-one MEK inhibitors with excellent potency and physiochemical properties. Several very potent inhibitors have been designed that do not have the B-ring iodide nor hydroxyl containing hydroxamates. In fact,

potent inhibitors have been prepared that do not contain the hydroxamate at all (compound **16**). The ability to replace the iodide with bromide results in a reduction in both lipophilicity and molecular weight. We hypothesize that these inhibitors bind in a manner similar to those depicted by the X-ray cocrystal studies of earlier inhibitors with one important difference: a strong interaction between the carbonyl of the pyridone and the backbone NHs of Ser212 and Val211. Establishing this strong binding interaction allowed us to generate potent MEK inhibitors void of the B-ring iodide and, in some cases, to replace the hydroxamate moiety. Several of these pyridone MEK inhibitors were examined in vivo for their ability to inhibit pERK in HT-29 tumor-bearing mice. Compounds **13**, **16**, **22**, **23**, and **24** showed greater than 80% inhibition of pERK at 4 h after a single 10 mg/kg oral dose in this model. Further progress on these inhibitors will be reported in due course.

Supporting Information Available: Experimental details on the preparation of compounds and characterization as well as details on biological, in vitro ADME, and in vivo assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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