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2-Alkylamino- and alkoxy-substituted 2-amino-1,3,4-oxadiazoles—O-Alkyl benzohydroxamate esters replacements retain the desired inhibition and selectivity against MEK (MAP ERK kinase)

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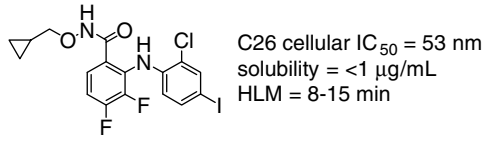
ABSTRACT

This paper reports a second generation MEK inhibitor. The previously reported potent and efficacious MEK inhibitor, PD-184352 (CI-1040), contains an integral hydroxamate moiety. This compound suffered from less than ideal solubility and metabolic stability. An oxadiazole moiety behaves as a bioisostere for the hydroxamate group, leading to a more metabolically stable and efficacious MEK inhibitor.

The mitogen-activated protein kinase pathway is thought to be essential in cellular growth and differentiation. The family of mitogen-activated protein (MAP) kinases can be activated in response to a wide variety of extracellular stimuli.[1,2] In particular, MEK (MAP kinase kinase) is an attractive target.[3] This kinase is activated by a cascade of phosphorylation events downstream from ras. Its only known substrates are the MAP kinases, ERK-1 and ERK-2. These kinases phosphorylate a number of transcription factors that control cell growth (e.g., ELK-1). Because of its substrate selectivity in the ras pathway, MEK is an ideal target for intervention of proliferative diseases.[3]



C26 cellular IC_{50} = 53 nm
solubility = <1 μg/mL
HLM = 8-15 min

CI-1040

An orally active, potent inhibitor of MEK (CI-1040) was reported by this group.[4] This compound inhibits MEK in a colon 26 cell based assay with an IC_{50} of 53 nM. CI-1040 was shown to not be competitive with ATP and is exquisitely selective for MEK (IC_{50} > 10 μM against 27 other kinases).[4a] However, this compound suffers from poor solubility and also shows a metabolic liability (human liver microsomes). It was believed that these problems were due in a large part to the key hydroxamic ester moiety. In order to circumvent this liability, a survey of five-membered heterocycles was undertaken. After investigating a number of heterocyclic systems, a 5-amino-1,3,4-oxadiazole was found to have moderate activity against MEK (Table 1).

The synthesis of this oxadiazole[11] is shown in Scheme 1. The anion of 4-iodo-2-methyl aniline was prepared using LiHMDS. This anion was added to the Li salt of 2,3,4-trifluorobenzoic acid in the presence of excess LiHMDS to afford the anthranilic acid **4**. This substitution occurs exclusively at the 2-position of the benzoic acid due to coordination of the aniline anion with the carboxylate. Formation of the hydrazide followed by condensation with cyanogen bromide affords the amino-oxadiazole **1**. Attempts to reductively aminate the oxadiazole **1** proved to be difficult. A number of aldehydes and conditions were tried, but no generally applicable set of conditions were found.

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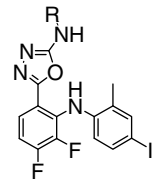
Table 1
Initial 1,3,4-oxadiazole data



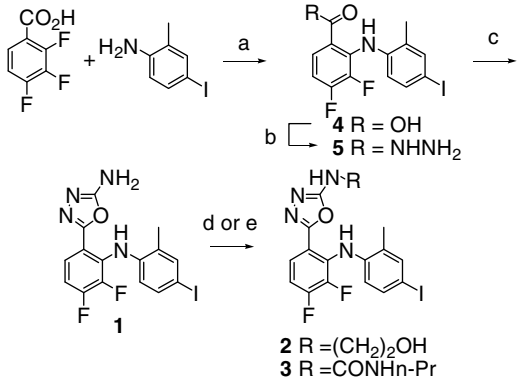
Compound	R	C26 IC_{50}[a] (M)
1	H	0.21
2	CH_2CH_2OH	1.50
3	$CONHCH_2CH_2CH_3$	10

[a] Western blot analysis was used to measure MEK inhibitory activity in cultured C26 colon carcinoma cells.

Two derivatives were made, compounds **2** and **3**, in rather low yields after extended reaction times. Neither of these substitutions was found to be advantageous. During this investigation, it was noted that a fluorine in the 2′-position of the anthranilic acid greatly increased potency in the hydroxamate series.[4b,4c] We investigated this substitution and found that a similar effect occurred in the oxadiazole series (Table 2). Simple replacement of the 2′ methyl with a fluorine, compound **6**, provided a 5-fold increase in activity, and provided a compound of similar potency to CI-1040. Addition of a chlorine **7** increased potency slightly. The importance of the 4′-iodo is exemplified by **8**, a simple bromo substitution gives only moderate activity.

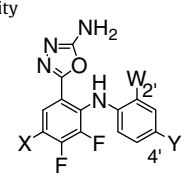
In order to circumvent the poor reactivity of the 5-amino-1,3,4-oxadiazole, we required a new synthesis. The hydrazide **9** was treated with CDI to provide the intermediate oxazolidinone **10**.[5] This was then treated with various amines to give the semicarbazides **11**,[6] which could be cyclized with either triphenylphosphine or polymer bound triphenylphosphine and carbon tetrachloride[7] to provide the desired 5-amino-oxadiazoles **12**. In the case of **12b–d** (Table 3), the ω-amino BOC was removed using HCl in methanol. In this case, no opening of the oxadiazole was observed (Scheme 2).

The results of the SAR of the substituted amino oxadiazoles is shown in Table 3. The goal of this series of compounds was to maintain or increase potency with a concomitant increase in solubility and metabolic stability. As can be seen, this series proved to be metabolically stable in a human liver microsome assay. Affect on solubility for the most part was disappointing, with the majority of analogs having a solubility on par with CI-1040. Compound **12a** proved to be the most soluble, and retained an activity 2-fold better than CI-1040.



Scheme 1. Reagents and conditions: (a) LiHMDS, THF, −78 °C to rt, 90%; (b) hydrazine, PyBop, CH_2Cl_2, THF, rt, 94%; (c) CNBr, $NaHCO_3$, water, dioxane, rt, 86%; (d) hydroxyacetaldehyde, $NaB(OAc)_3H$, DCE, rt, 64 h, 35%; (e) propyl isocyanate, toluene, 130 °C, 52%.

Table 2
Effect of 2′ F on cellular activity



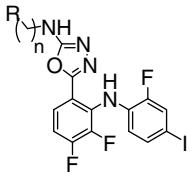
Compound	W	X	Y	C26 IC_{50}[a] (M)
1	CH_3	H	I	0.210
6	F	H	I	0.040
7	F	Cl	I	0.018
8	F	H	Br	0.140

[a] Western blot analysis was used to measure MEK inhibitory activity in cultured C26 colon carcinoma cells.

Compound **12a** was further evaluated in an ex vivo pharmacodynamic assay. This assay analyzed p-ERK suppression in tumors following an oral dose of inhibitor. Mice bearing subcutaneously implanted colon 26 tumors were treated with one dose of the inhibitor and after each time point the tumors were excised and Western blots were used to measure p-ERK levels versus vehicle treated controls. As can be seen in Figure 1, **12a** showed superior inhibition of p-ERK levels relative to CI-1040. Most noticeably, at 10 h, **12a** completely suppresses phosphorylation of ERK by MEK at 100 mg/kg dose, and even at 12.5 mg/kg dose, outperforms CI-1040 (100 mg/kg). A high level of inhibition was retained at the highest dose even at 24 h post-dose.

We next investigated the effect of **12a** in vivo. Colon C26 tumors were grown to 200 mg over 8 days, then drug (or vehicle) was administered PO from days 9 to 15 (see Table 4). Compared to CI-1040 (BID, 100 mg/kg), **12a** not only provided 3/3 complete re-

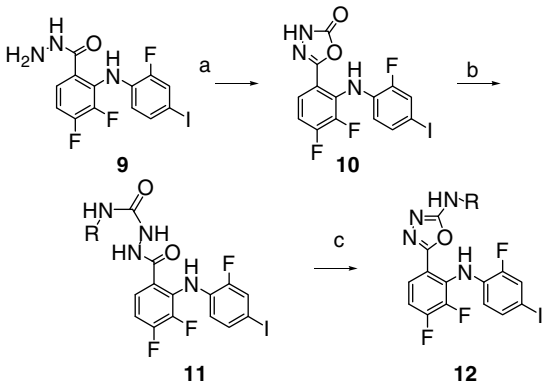
Table 3
SAR for a series a substituted oxadiazoles



Compound	R	*n*	C26 IC_{50}[a] (M)	Solubility[a] (μg/mL)	HLM[b] $t_{1/2}$ (min)
6	H	0	0.040	<3	40
12a	$NHCH_3$	2	0.027	6.7	40
12b	NH_2	2	0.015	<3	40
12c	NH_2	3	0.076	5.4	40
12d	NH_2	4	0.045	ND	ND
12e	$NH(CH_3)_2$	2	0.140	ND	ND
12f	OH	2	0.015	<3	40
12g	OH	3	0.028	<3	40
12h	OH	4	0.012	<3	40
12i	OH	5	0.120	<3	40
12j	Morpholine	2	0.032	4.7	40
12k	OMe	3	0.080	<3	35
12l	$NH(CH_2CH_3)_2$	3	0.153	<3	40
12m	Morpholine	3	0.311	<3	40
12n	$(rac)CH_2CHOHCH_2CHOH$	0	0.067	<3	40
12o	$(S)CH_2CHOHCH_2CHOH$	0	0.091	3.5	40
12p	$CH(CH_2CHOH)_2$	0	0.770	8.8	40

[a] Solubility determined by measuring the concentration of the compound in a saturated solution over time. In this procedure, increasing aliquots of a 10 mM solution of a compound in DMSO are added to a series of 15 vials containing 1.8 mL of 50 mM sodium phosphate buffer at pH 6.5. The solutions are prepared over the range of 1–60 mg/mL and are allowed to stand overnight at ambient temperature. The samples are vortexed, then HPLC analysis is performed.

[b] Human liver microsomal stability, maximum $t_{1/2}$ is 40 min for this assay.



Scheme 2. Reagents and conditions: (a) CDI, DMF, 93%; (b) RNH_2, ethanol, 100 °C, 67–99%; (c) PPh_3, Et_3N, CCl_4, CH_2Cl_2, 45 °C, 31–98%.

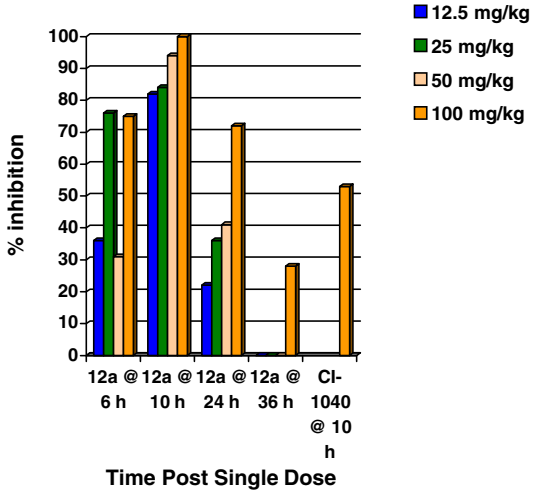


Figure 1. Ex vivo assay of **12a** compared to CI-1040 in colon 26 tumors.

Table 4
In vivo test results

Drug	Dose[a]	Schedule	% Weight change[b]	CR[c]	PR[d]	T–C[e]	Net log_{10} Kill[f]
Vehicle	0	Days 9–15	−3.6 (14)	0/3	0/3	—	−0.6
Vehicle	0	BID;Days 9–15	−4.0 (14)	0/3	0/3	—	−0.6
12a	300	Days 9–15	−4.5 (14)	3/3	—	9.1	0.3
12a	200	Days 9–15	−0.1 (16)	2/3	1/3	8.7	0.3
12a	100	Days 9–15	+	0/3	1/3	6.0	0.0
12a	150	BID;Days 9–15	−4.0 (14)	3/3	—	10.2	0.4
12a	100	BID;Days 9–15	+	3/3	—	7.9	0.2
12a	50	BID;Days 9–15	+	0/3	0/3	7.4	0.1
CI-1040[g]	100	BID;Days 9–15	−4.8 (18)	0/3	0/3		0.1

[a] Dose is in mg/kg, oral gavage. Vehicle for 0331257-2B was 5% EtOH + 5% Tween 80 in saline.

[b] Maximum treatment related weight loss, expressed as a percent of mean group weight at initial treatment. Value in parentheses indicates the day the maximum weight loss was recorded. A net weight gain is represented by a "+".

[c] A complete response represents a tumor that decreased in mass to less than 62 mg during the study.

[d] A partial response represents a tumor that decreased in mass by at least 50% compared to the tumor mass at initial treatment.

[e] T–C is the difference, in days, for the median treated and control tumors to reach 750 mg.

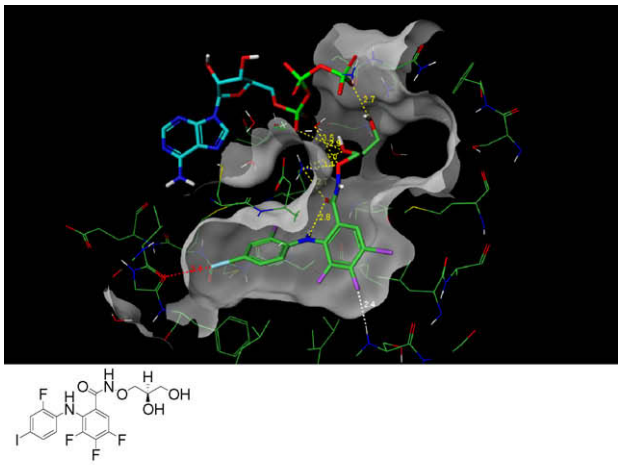
[f] Net log10 tumor cell kill represents the change in tumor burden during therapy. A negative value indicates a net increase in tumor mass during therapy, while a positive value indicates a net tumor burden reduction during therapy. Values near zero indicate tumor stasis during therapy.

[g] CI-1040 data from separate test, included for comparison. Median control tumor size at treatment was 245 mg. Vehicle for CI-1040 was 0.5% hydroxymethylcellulose + 0.2% Tween 80 in water.

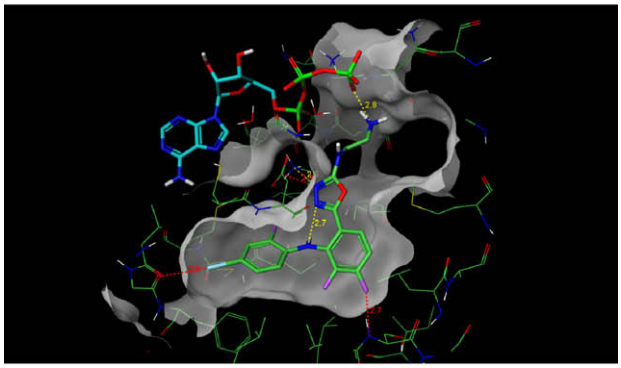
sponses, but also showed a net weight gain for the animals in the study. The Net log10 Kill is also improved relative to CI-1040.

We have obtained a co-crystal structure[8] of the ternary complex of a related inhibitor PD-318088 (**13**), MgATP, and MEK1 (62–393) (Fig. 2a). Selectivity for these inhibitors can be explained via binding of CI-1040 to a novel inhibitor binding site located in an interior hydrophobic pocket adjacent to but distinct from the MgATP binding site. This binding region has low sequence homology to other known protein kinases.

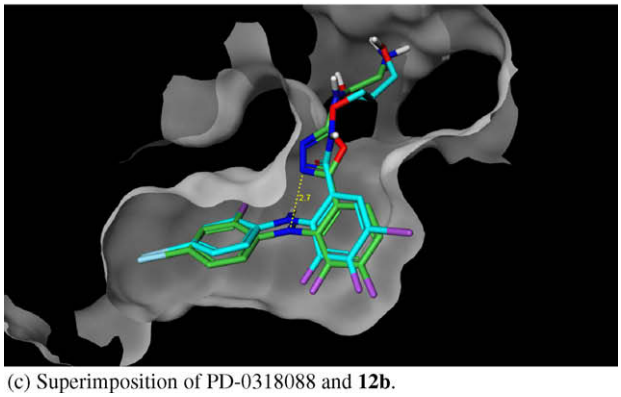
One key interaction with the enzyme is a dipolar interaction of the 4-fluoro atom of PD-318088 (**13**) and the backbone amide of Ser 212.[9] The hydroxyl groups of the sidechain are also positioned to allow hydrogen bonding directly to ATP and to Lys 97. The structure suggests that the PD-318088-like inhibitors act by preventing the autophosphorylation of MEK and the phosphorylation of ERK substrate by trapping MEK in a "closed" but catalytically inactive conformation.



(a) X-ray of PD-0318088 (**13**) bound to MEK active site and ATP



(b) X-ray of **12b**.



(c) Superimposition of PD-0318088 and **12b**.

Figure 2. X-ray co-crystal structures of PD-0318088 and **12b**.

A crystal structure of **12b**[10] shows that the interaction of the enzyme with the fluorine is preserved. Importantly, an internal hydrogen bond from the aniline NH to the nitrogen of the oxadiazole positions the second nitrogen to efficiently bond to Lys 97, mimicking the hydroxamate binding mode (Fig. 2b).

In conclusion, we have shown that an oxadiazole moiety is a useful replacement for a hydroxamic ester in a series of MEK inhibitors. While solubility was not greatly improved, the compounds were metabolically stabile. In addition, these inhibitors proved superior to CI-1040 in both ex vivo pharmacodynamic assays and in vivo experiments in C26 tumors.

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10. PDB ID Code 3EQB.
11. All compounds were fully characterized by ^{1}H NMR and MS.