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The discovery of the benzhydroxamate MEK inhibitors CI-1040 and PD 0325901

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ABSTRACT

A novel series of benzhydroxamate esters derived from their precursor anthranilic acids have been prepared and have been identified as potent MEK inhibitors. 2-(2-Chloro-4-iodo-phenylamino)-*N*-cyclopropylmethoxy-3,4-difluoro-benzamide, **CI-1040**, was the first MEK inhibitor to demonstrate in vivo activity in preclinical animal models and subsequently became the first MEK inhibitor to enter clinical trial. **CI-1040** suffered however from poor exposure due to its poor solubility and rapid clearance, and as a result, development of the compound was terminated. Optimization of the diphenylamine core and modification of the hydroxamate side chain for cell potency, solubility, and exposure with oral delivery resulted in the discovery of the clinical candidate *N*-(2,3-dihydroxy-propoxy)-3,4-difluoro-2-(2-fluoro-4-iodo-phenylamino)-benzamide **PD 0325901**.

The mitogen-activated protein (MAP) kinase intracellular signaling pathways are involved in the regulation of various cellular functions.[1,2] Levels of phosphorylated MAP kinases are increased by a multitude of mitogenic stimuli, including growth factors coupled through tyrosine kinase receptors, hormones that bind to G protein coupled receptors, cytokines, and phorbol esters. This suggests signaling through MAP kinases is essential to multiple cellular functions.[3] One of these pathways, the Raf/MEK/ERK pathway, plays a major role in the regulation of cellular growth, differentiation, and proliferation, therefore, modulation of the cascade could be a useful approach for treating proliferative disorders such as cancer.[4] Raf-activated MAP/ERK kinase (MEK) activates the MAP kinase known as extracellular signal-regulated kinase (ERK) through a highly specific double phosphorylation.[5] The pharmacological role of MEK has been widely investigated using the weak in vitro MEK inhibitor **PD 0098059**, Fig. 1 a compound discovered in our laboratories.[6]

In the past, the role of this compound was limited to in vitro study in that exposure levels required for such a weak inhibitor to modulate target in vivo were unattainable due to its poor solubility. In this article we describe the discovery of the new series of benzhydroxamate esters from which emerged **CI-1040** (compound **24**), the first MEK inhibitor to enter clinical trials.[7,8] We furthermore summarize the optimization of the benzhydroxamate series that culminated in the identification of the second-generation clinical candidate **PD 0325901** (compound **34**).[9]

Scheme 1 describes the general procedure for preparing the benzhydroxamate esters.[13] The appropriate 2-fluorobenzoic acid (**1**) and aniline derivatives are treated with excess base in a solvent (e.g., LiHMDS in THF) to give a substituted *N*-phenylanthranilic acid (**2**). Coupling of the appropriate hydroxylamine to an *N*-phenylanthranilic acid core using PyBOP, BOP, or Ph_2POCl afforded the desired products (**3**).

In cases where a free hydroxyl or diol was protected as an allyl ether or acetal, respectively, deprotection was accomplished using pTsOH in $MeOH/H_2O$.

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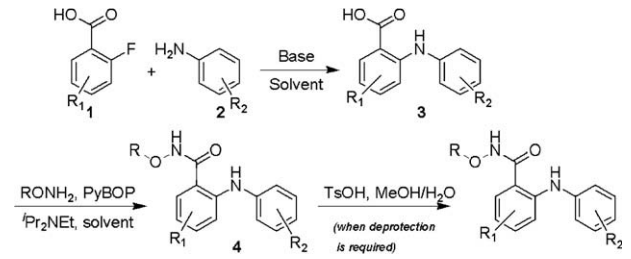


Scheme 1. General procedure for the preparation of the benzhydroxamates.

The goal of the medicinal chemistry program was to identify lead matter that possesses in vivo activity when administered orally in an animal model. We believed that it would be necessary to identify novel compounds with activity in the low nanomolar range for both a kinase cascade assay and the whole cell assay. A kinase cascade assay was employed as the primary screen for potential MEK inhibitors through the discovery of **CI-1040**.

This assay evaluates ^{32}P phosphate incorporation into myelin basic protein (MBP) by MAPK in the presence of MEK. MAPK is only weakly active in the basal state, and is activated upon phosphorylation by ATP-activated MEK. Order of addition experiments were required to determine whether inhibition of MBP phosphorylation in the assay is due to MEK or MAPK inhibition.[6] X-ray crystallography data previously reported has confirmed the benzhydroxamates bind as ternary MEK-ATP-inhibitor complexes.[10]

The secondary screen was a whole cell (K-Balb or murine colon 26) assay that measured levels of phosphorylated ERK (pERK) after treatment with compound and stimulation of the kinase pathway with platelet-derived growth factor (PDGF) versus DMSO vehicle-treated controls. The K-Balb cell line is a K-ras transformed murine fibroblast line that is fast growing, easy to handle, and gives a strong response to growth factor stimulation, thus making it quite suitable for measuring the suppression of pERK levels with inhibitors.

High-throughput screening identified a novel iodoanthranilic acid (**5**) as a potent inhibitor of MEK (Table 1). These acids generally showed weak activity in cells at 10 μM regardless of their kinase inhibition potencies. The *N*-phenylanthranilic acid template was used to systematically study substituent effects on the diphenylamine core. It was evident that the iodo substituent was favored at the 4′ position, as evidenced by compounds (**7**–**9**). This series also indicated that a substituent, in this case a methyl, was desired at the 2′ position, as evidenced by compounds **6** and **9**. Additionally, a halo substituent at the 4 position of ring A, in particular a fluorine (compound **10**), was optimal. X-ray data[10] published after this work had been completed revealed that the fluorine has a dipolar interaction with the Ser212 amide backbone hydrogen in the MEK1 structure.[11]

Later synthetic efforts in our laboratories that are yet unpublished have sought to exploit this key interaction. Another favorable substitution on the A-ring is fluorine at position R_1. Analog pair (**10**/**13**) demonstrate the fluorine's moderate potency-enhancing effect.

Carboxylic acid isosteres were investigated in order to obtain cellular potency with this class of inhibitors. Benzhydroxamic acid (R=H) analogs (**15**–**16**) are potent inhibitors in both the kinase and cellular assays (Table 2).

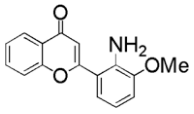
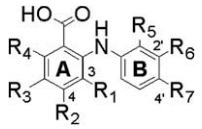


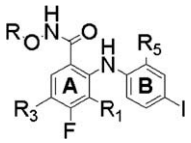
Figure 1. Structure of MEK inhibitor **PD 0098059**.

Table 1
Activity of the 2-phenylamino-benzoic acids.



Compound	R_1	R_2	R_3	R_4	R_5	R_6	R_7	Kinase cascade IC_{50} (nM)
5	H	Cl	H	H	Me	H	I	554
6	H	H	H	H	Me	H	I	390
7	H	H	H	H	I	H	H	>10,000
8	H	H	H	H	H	I	H	>10,000
9	H	H	H	H	H	H	I	3050
10	H	F	H	H	Me	H	I	15
11	H	H	F	H	Me	H	I	166
12	H	H	H	F	Me	H	I	518
13	F	F	H	H	Me	H	I	5.8
14	F	F	F	H	Me	H	I	6.8

Table 2
Activity of the 4-fluoro-*N*-substituted-2-(4-iodo-phenylamino)-benzamides.



Compound	R	R_3	R_1	R_5	Kinase cascade IC_{50} (nM)	K-Balb cell IC_{50} (nM)	
						1 h	24 h
15	H	H	H	Me	7.96	3.55	624
16	H	H	H	Cl	2.8	1.59	645
17	Me	H	H	Me	26	11.8	50
18	Et	H	F	Me	20	4.18	47
19	^{n}Pr	H	F	Me	18	4.42	136
20	^{i}Pr	H	F	Me	35	8.18	232
21	$-CH_2{}^{c}Pr$	H	H	Me	27	23.5	257
22	$-CH_2{}^{c}Pr$	H	H	Cl	13	54.9	84.6
23	$-CH_2{}^{c}Pr$	H	F	Me	7.0	6.48	172
24	$-CH_2{}^{c}Pr$	H	F	Cl	2.3	2.45	35.4
25	$-CH_2{}^{c}Pr$	Br	F	Me	7.8	26.4	113
26	$-CH_2{}^{c}Bu$	Br	F	Me	10	75.8	326
27	$-CH_2{}^{c}C_5H_9$	Br	F	Me	23	119	661
28	$-CH_2{}^{c}C_6H_{11}$	Br	F	Me	48	1140	1780

A variety of small alkyl and cycloalkylmethyl esters (**17**–**28**) are likewise potent in both assays. With the cycloalkylmethyl esters, potencies are diminished with increasing ring size from cyclopropyl to cyclohexyl. The ester series distinguishes itself from the acid series when compounds are incubated with the K-Balb cells for 24 h before stimulation with growth factor. Loss in cell potency over time is in general more pronounced with the benzhydroxamic acids than with the O-substituted ester analogs. We speculate the esters are more resistant to metabolic processes within the cells such as hydrolysis and O-glucuronidation leading to a lower rate of metabolic degradation of parent compound concentrations and thus less receding of cell activity over time. Analog (**24**), **CI-1040**, emerged from the field to demonstrate in vivo activity with oral administration in a mouse tumor model.[12] This compound advanced into Phase 2 but failed for inadequate efficacy caused by insufficient systemic exposure. The clinical trials of **CI-1040** thus failed to validate MEK as a viable anticancer drug target, and it was clear that a backup medicinal chemistry program should more aggressively address the physical and pharmacokinetic property issues connected with the termination of the drug's development.

The solubility of (**24**) is less than 1 μg/mL in, pH 6.5, phosphate buffer, making it clear that low in vivo exposure is at least partly due to solubility limitations. In animals and humans, a high-dose formulation of **CI-1040** was administered up to three times daily in order to achieve sustained plasma levels required for the target modulation required to observe expected efficacy. The goal of the backup program was the optimization of both in vitro potency and aqueous solubility that would translate to a lower dose administered once daily to achieve sustained plasma concentrations required to validate MEK as an anticancer target.

In order to increase solubility in the second-generation MEK inhibitor series, a number of polar hydroxylamine side chains were prepared and were coupled with some of the most potent *N*-phenylanthranilic acids to produce the series of hydroxylated and dihydroxylated benzhydroxamate ester MEK inhibitors described in Table 3. We made two significant SAR observations with this work. First, a comparison between the colon 26 cell potencies of CI-1040 and its hydroxyethyl hydroxamate analog (**30**) reveal a 10-fold improvement due to the hydroxyethyl over the cyclopropylmethyl side chain. The improvement in cell potency of the racemic dihydroxypropyl hydroxamate ester analog (**32**) over CI-1040 is not as dramatic. The second critical finding was the discovery that substitution of fluorine for chlorine at R_5 in CI-1040 gives the cyclopropylmethyl hydroxamate ester analog (**29**) a 35-fold increase in colon 26 cell MEK inhibition. The same substitution in the hydroxyethyl hydroxamate ester pair (analogs **30** and **31**) provides a 50-fold improvement in cell potency and for the first time gave a subnanomolar cellular MEK inhibitor. The two SAR advances also provided the benefit of improved aqueous solubility (Table 3). Neither the fluorine at R_5 nor the hydroxyethyl side chain provided significant increases in solubility in and of themselves as seen in comparisons between the analog pairs (**24**/**29**) and (**24**/**30**). A comparison of analogs (**29**), (**30**), and (**31**) show that the two substitutions together provide a modest improvement in solubility in the pH 6.5 buffer. Synergy between the R_5 fluorine and the dihyroxypropyl side chain clearly give the most dramatic increases in aqueous solubility as observed for the racemate-enantiomeric trio (**33**), (**34**), and (**35**).

A comparison between key physical properties and in vitro metabolism of **CI-1040** (**24**) and **PD 0325901** (**34**) is presented in Table 4.

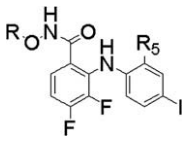
The second-generation MEK inhibitor demonstrates significantly greater stability over CI-1040 (**24**) when assayed with either human liver microsomes or with human hepatocytes. **PF-0325901** (**34**) also shows higher permeability than (**24**). The in vitro profiles suggest **PD 0325901** (**34**) should be able to achieve higher systemic exposures than **CI-1040** (**24**) in vivo.

Both **CI-1040** and **PD 0325901** were tested for the ability to inhibit pERK in subcutaneously implanted murine colon 26 tumors in mice 24 h after a single oral administration of compound at various doses. Consistent with the in vitro potency and ADME profiles, **PD 0325901** (**34**) clearly differentiates itself from **CI-1040** (**24**) ex vivo.

The compound significantly suppresses pERK levels in the tumors at doses lower than those for which effect can be seen for **CI-1040**. At 100 mpk, **CI-1040** provides only 14% inhibition of pERK. In contrast, **PD 0325901** inhibits tumor pERK levels 84% at 50 mpk. Even at 12.5 mpk, **PD 0325901** inhibits pERK 59% one day after the dose is administered. These data translate to the corresponding colon 26 mouse in vivo model (Table 5). All mice show some response at the MTD (QD, 25 mg/kg, 14 days) of **PD 0325901**, whereas to achieve a similar result, mice must be dosed TID with 300 mg of **CI-1040** for 14 days. The MTD for **CI-1040** cannot be achieved in this model.

Both the foundation established by the study of the ERK signaling pathway using the in vitro pharmacological probe **PD 0098509**[6] and the identification of Parke–Davis collection screen hit, the *N*-phenylanthranilic acid (**2**), provided the medicinal chemistry program a suitable small molecule template to begin pursuing an orally administered in vivo active MEK inhibitor for the treatment of cancer. Optimization of the substitutions around the diphenylamine core for in vitro potency and selection of a benzhydroxamate ester that retained robust MEK inhibition activity in cells over time produced the first orally active MEK inhibitor

Table 3
Activity and solubility of the 3,4-difluoro-2-(2-halo-4-iodo-phenylamino)-*N*-(2-hydroxy-ethoxy)-benzamides.



Compound	R	R_5	C26 IC_{50} (nM)	Sol (pH 6.5) (μg/mL)
24 (**CI-1040**)	$-CH_2{}^cPr$	Cl	35	<1
29	$-CH_2{}^cPr$	F	1.0	<1
30	$-CH_2CH_2OH$	Cl	3.5	<1
31	$-CH_2CH_2OH$	F	0.07	5
32	(±)-$CH_2CHOH(CH_2OH)$	Cl	19	—
33	(±)-$CH_2CHOH(CH_2OH)$	F	0.48	147
34 (**PD 0325901**)	*R*-(−)-$CH_2CHOH(CH_2OH)$	F	0.33	190
35	*S*-(+)-$CH_2CHOH(CH_2OH)$	F	0.82	255

Table 4
Comparison of the physical and metabolic properties of **CI-1040** and **PD 032590**.

Compound	HLM $t_{1/2}$ (min)	HuHep $t_{1/2}$ (min)	Log*D*	Caco-2 (cm/s)	C26 IC_{50} (nM)	Sol (pH 6.5) (μg/mL)
24 (**CI-1040**)	11.5	100	>5	16.2×10^{-6}	3.5	<1
34 (**PD 0325901**)	>100	370	4.6	72.7×10^{-6}	0.33	190

Table 5
In vivo comparison of antitumor activity by orally dosed **CI-1040** and **PD 0325901** in advanced stage C26 mouse colon carcinoma.

Compound	% pERK Inhibition in subcutaneous-implanted murine C26 tumors in mice 24 hours post-dose (PO)					
	Dose[a] (mg/kg/treatment)	Treatment schedule	Total daily dose (mg/kg)	% Complete response[b]	% Partial response[b]	T-C[c] (days)
24 (**CI-1040**)	75	TID × 14 days	225	10	20	10.8
	300		900	70	30	12.7
34 (**PD 0325901**)	1.6	QD × 14 days	1.6	20	0	7.7
	25		25	70	30	15.4

[a] A maximum tolerated dose (MTD) is defined as the highest non-lethal dose that does not produce a weight loss greater than 15%. A minimal effective dose (MED) is defined as the dose that produces at least 20% response rate (CR + PR), or stasis in tumor growth. CI-1040 doses are the MED and the highest dose given. Sufficient exposure cannot be obtained to establish the MTD for **CI-1040**. The doses of **PD 0325901** are the MED and the MTD. The vehicle for both compounds is 0.5% hydroxypropylmethylcellulose + 0.2% Tween 80 in water.

[b] A complete response (CR) is defined as a 100% reduction of initial tumor mass. Partial response (PR) is defined as at least 50% reduction in tumor mass.

[c] Tumor growth delay (T-C) is the difference in days for the control and treated tumors to reach a mass of 750 mg.

in an animal model.[12] **CI-1040** (**24**) suffered from low systemic exposure because of its insolubility and rapid metabolism, and these liabilities prevented the drug from achieving sufficient efficacy in the clinic. The medicinal chemistry program addressed the pharmacokinetic issues in the discovery of **PD 0325901** (**34**) by altering the structure to improve aqueous solubility. In so doing, new synergies were realized by combining the optimized diphenylamine core substitutions with small mono- and dihydroxylated hydroxamate ester side chains. This lead to both improved physical properties, as well as a greatly improved pharmacological profile and clinical candidate.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.10.054.

References and notes

1. Cowan, K. J.; Storey, K. B. *J. Exp. Biol.* **2003**, *206*, 1107.
2. (a) Herra, R.; Sebolt-Leopold, J. S. *Trends. Mol. Med.* **2002**, *8*, S27; (b) Kohno, M.; Pouyssegur, J. *Ann. Med.* **2006**, *38*, 200; (c) Thompson, N.; Lyons, J. *Curr. Opin. Pharmacol.* **2005**, *5*, 350; (d) Wallace, E. M.; Lyssikatos, J.; Blake, J. F.; Seo, J.; Yang, H. W.; Yeh, T. C.; Perrier, M.; Jarski, H.; Marsh, V.; Poch, G.; Livingston, M. G.; Otten, J.; Hingorani, G.; Woessner, R.; Lee, P.; Winkler, J.; Koch, K. *J. Med. Chem.* **2006**, *49*, 441; (e) Wallace, E. M.; Lyssikatos, J. P.; Yeh, T.; Winkler, J. D.; Koch, K. *Curr. Topics Med. Chem.* **2005**, *5*, 215.
3. Guan, K. L. *Cell. Signal.* **1994**, *6*(6), 581.
4. Alessandrini, A. *Cancer Metastasis—Biol. Treat.* **2002**, *3*, 35.
5. Payne, D. M.; Rossomando, A. J.; Martino, P.; Erickson, A. K.; Her, J. H.; Shabanowitz, J.; Hunt, D. F.; Weber, M. J.; Sturgill, T. W. *EMBO J.* **1991**, *10*(4), 885.
6. Dudley, D. T.; Pang, L.; Decker, S. J.; Bridges, A. J.; Saltiel, A. R. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 7686.
7. LoRusso, P. M.; Adjei, A. A.; Varterasian, M.; Gadgeel, S.; Reid, J.; Mitchell, D. Y.; Hanson, L.; DeLuca, P.; Bruzek, L.; Piens, J.; Asbury, P.; Van Becelaere, K.; Herrera, R.; Sebolt-Leopold, J.; Meyer, M. B. *J. Clin. Oncol.* **2005**, *23*, 5281.
8. Rinehart, J.; Adjei, A. A.; LoRusso, P. M.; Waterhouse, D.; Hecht, J. R.; Natale, R. B.; Hamid, O.; Varterasian, M.; Asbury, P. E.; Kaldjian, P.; Gulyas, S.; Mitchell, D. Y.; Herrera, R.; Sebolt-Leopold, J.; Meyer, M. B. *J. Clin. Oncol.* **2004**, *22*, 4456.
9. Sebolt-Leopold, J. Abstract of Papers, 229th ACS National Meeting, San Diego, CA, United States, March 13–17, 2005, MEDI-310.
10. Ohren, J. F.; Chen, H.; Pavlovsky, A.; Whitehead, C.; Zhang, E.; Kuffa, P.; Yan, C.; McConnel, P.; Spressard, C.; Banotai, C.; Mueller, W. T.; Delaney, A.; Omer, C.; Sebolt-Leopold, J.; Dudley, D.; Leung, I.; Flamme, C.; Warmus, J. S.; Kaufman, M.; Barrett, S.; Tecle, H.; Hasemann, C. *Nat. Struct. Mol. Biol.* **2004**, *11*, 1192.
11. (a) Razgulin, A. V.; Mecozzi, S. *J. Med. Chem.* **2006**, *49*, 7902; (b) Olsen, J. A.; Banner, D. W.; Seiler, P.; Wagner, B.; Tschopp, T.; Obst-Sander, U.; Kansy, M.; Müller, K.; Diederich, F. *ChemBiochem* **2004**, *5*, 666; (c) Dunitz, J. D.; Taylor, R. *Eur. Chem. J.* **1997**, *3*, 89.
12. Sebolt-Leopold, J. S.; Dudley, D. T.; Herrera, R.; Van Becelaere, K.; Wiland, A.; Gowan, R. C.; Tecle, H.; Barrett, S. D.; Bridges, A.; Przybranowski, S.; Leopold, W. R.; Saltiel, A. R. *Nat. Med.* **1999**, *5*, 810.
13. All final compounds were fully characterized, see supplemental material.