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Discovery of TAK-733, a potent and selective MEK allosteric site inhibitor for the treatment of cancer

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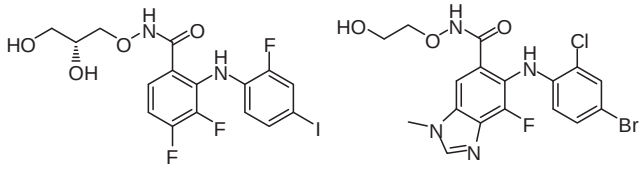
ABSTRACT

A novel 5-phenylamino-8-methylpyrido[2,3-*d*]pyrimidine-4,7(3*H*,8*H*)-dione series of MEK inhibitors has been developed using structure-based drug design. Lead optimization of this series led to the discovery of TAK-733. This was advanced to Phase I clinical studies for cancer treatment.

The RAF–MEK–ERK pathway mediates proliferative and anti-apoptotic signaling from growth factors and oncogenic factors such as RAS and RAF in mutant phenotypes that promote tumor growth, progression, and metastasis.[1] The RAF–MEK–ERK pathway therefore provides molecular targets with potentially broad therapeutic applications in cancerous and non-cancerous hyperproliferative disorders, immunomodulation and inflammation.[2] This pathway is inappropriately activated in 30% of all human cancers, resulting in the activation of ERK via phosphorylation. Point mutations in BRAF that are primarily responsible for MEK activation have been identified in a majority of melanomas as well as a significant portion of thyroid and colorectal cancers.[3] Selecting BRAF mutant patients for treatment regiments with MEK inhibitors may therefore lead to increased efficacy in cancer treatment. Early work in the field has led to the advancement of the compounds PD0325901[4] and AZD-6244,[5] which has recently completed Phase II clinical trials (Fig. 1). Several other compounds have subsequently entered Phase I clinical evaluation for cancer.[6]

The crystal structure of a close analog of PD0325901 (PDB code 1S9J) in the MEK1 allosteric site has been described and analyzed for important interactions.[7] Our analysis of this structure led to the design of compound **1** (Fig. 2) by molecular modeling.[8] It was our intention to replace the metabolically liable hydroxamate functionality by incorporating the amide portion into a fused bicyclic ring system, as precedented in literature.[9] This bicyclic design had the potential for efficient binding due to its low degree of conformational freedom. The pyridopyrimidinedione ring system was found to be a highly complementary fit to the allosteric pocket. The energy-minimized model indicated that the carbonyl oxygen at the 7-position would make hydrogen bonding interactions with the backbone NHs of Val211 and Ser212, similar to pyridone-based inhibitors described previously.[10] The carbonyl oxygen at the 4-position would make a hydrogen bond to the side-chain NH_3 of Lys97. The 2-fluoro-4-iodoaniline moiety has been previously reported as a recognition motif for the hydrophobic pocket of the MEK allosteric site.[11] As in the 1S9J structure, the 2-fluoro-4-iodoaniline would bind in the lipophilic pocket formed by Ile141, Met143, Val127, Phe129, Leu118, and Phe209.



PD0325901 AZD-6244

Figure 1. Early MEK inhibitors in clinical studies.

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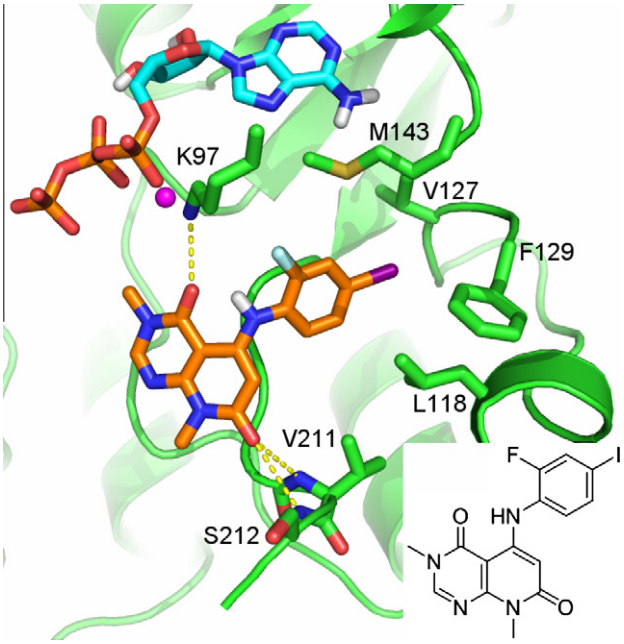
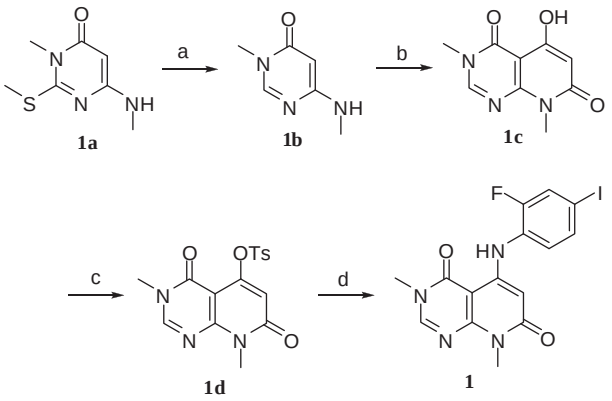


Figure 2. The minimized model of compound **1** in the MEK1 protein–ATP complex. The yellow dashed lines indicate hydrogen bonds between the ligand and the protein–ATP complex.

Compound **1** was prepared as outlined in Scheme 1. Reductive cleavage of the thiomethyl group of commercially available compound **1a** gave aminopyrimidinone **1b** which, upon treatment with ethyl malonate, provided the ring-cyclized product **1c**. Tosylation of compound **1c** followed by heat mediated displacement with 2-fluoro-4-iodoaniline gave target molecule **1**.[12]

Encouraged by the enzymatic activity[13] (IC_{50} = 26 nM) of compound **1**, we designed additional analogs of this lead utilizing molecular modeling. Target molecules **9–23** were accessed using two converging routes 2 and 3 (Scheme 2). The key steps include: (a) cyclization of 6-(methylamino)pyrimidin-4(3*H*)-one with a malonate to form the pyridopyrimidinedione ring system, (b)



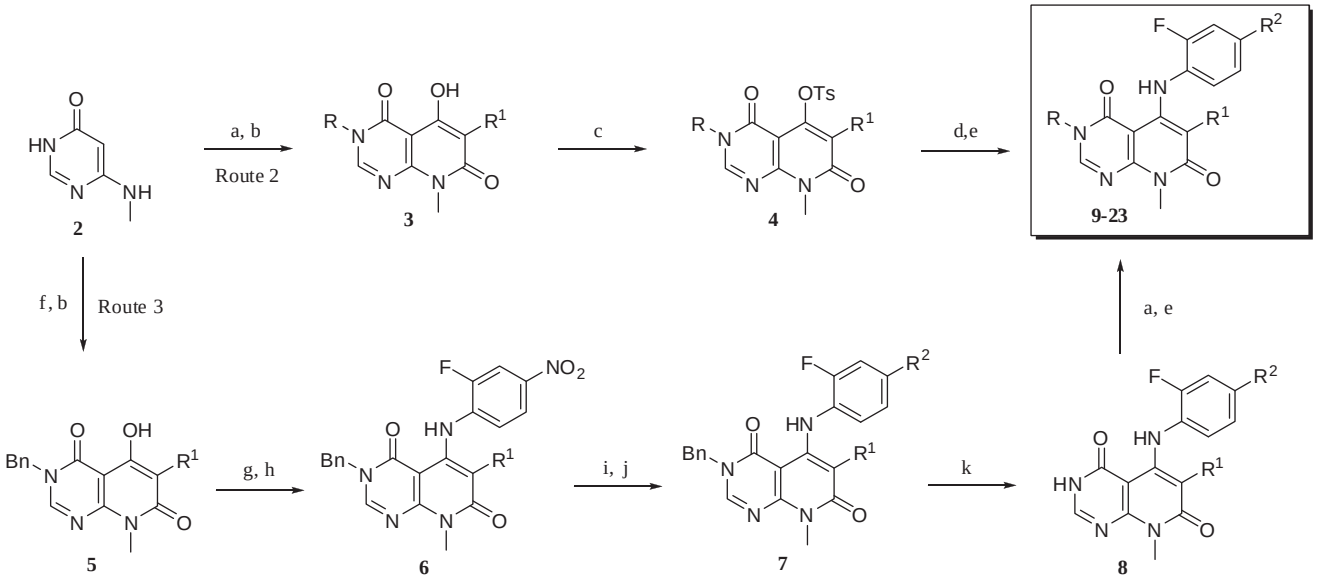
Scheme 1. Synthesis of compound **1** (Route 1). Reagents and conditions: (a) Raney Ni, MeOH, rt; (b) $CH_2(CO_2Et)_2$, Ph_2O, NMP, 240 °C; (c) TsCl, Et_3N, ACN, rt; (d) 2-fluoro-4-iodoaniline, 125 °C.

conversion of the C5 hydroxyl group to a leaving group and its displacement with an aniline, and (c) N-alkylation at N3 to attach an R group substituent.

Our SAR strategy involved exploring three variable positions on the bicyclic scaffold. We hypothesized that analogs with polar R substituents would have the potential to interact with Lys97 and the ATP phosphate and thus improve enzyme potency. A series of polar R groups based around alcohols of varying chain length were designed and synthesized. As seen in Table 1, alcohols and diols with two and three carbon chain lengths showed 3–6 fold increased potency. As compound **11** demonstrated promising in vitro activity[14] (IC_{50} = 4.6 nM, Colo205 EC_{50} = 27 nM) and pharmaceutical properties, it was chosen for further lead optimization.

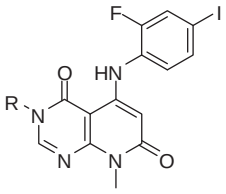
With the R group of compound **11** fixed, we began to examine substitutions at R^1 and R^2 (Table 2). Of the three R^2 variants examined, iodine was the most potent in our enzyme assay, and neither bromine nor acetylene at R^2 improved microsomal stability.

With favorable R^1 and R^2 groups in place, we sought to expand our search for novel R groups to optimize potency, pharmacokinetics and physical properties of the series. Compounds **26** and **27** required the development of a new synthetic route (Scheme 3).



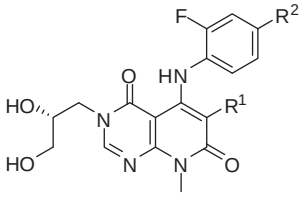
Scheme 2. Synthesis of compounds **9–23** (Routes 2–3). Reagents and conditions: (a) R–X, K_2CO_3, DMF; (b) $CH_2(CO_2Et)_2$ or $CCH_3(CO_2Et)_2$, Ph_2O, NMP, 240 °C; (c) TsCl, Et_3N, ACN; (d) substituted aniline, LDA, THF, −78 °C; (e) when R^1 is H: NCS, DMF or Selectfluor, ACN; (f) benzyl bromide, K_2CO_3, EtOH; (g) $POCl_3$; (h) 2-fluoro-4-nitroaniline, $Pd_2(dba)_3$, Xantphos, NaO*t*Bu, dioxane; (i) Zn, AcOH; (j) $NaNO_2$, HCl, KI *or* $NaNO_2$, CuBr, HBr, ACN, H_2O; (k) $Pd(OH)_2$, HCO_2NH_4, dioxane.

Table 1
Initial SAR of various R groups



Compd	Route	R	MEK1 IC_{50} (nM)	A375 EC_{50} (nM)	Colo205 EC_{50} (nM)	HLM/RLM $t½$ (min)
1	1	Me	26	86	49	>200/13
9	2	CH_2CH_2OH	9.8	60	38	37/11
10	2	$CH_2CH_2CH_2OH$	4.9	23	16	>200/20
11	3	(*R*)-$CH_2CHOHCH_2OH$	4.6	26	27	48/25
12	3	(*S*)-$CH_2CHOHCH_2OH$	8.3	40	38	>200/>200
13	2	(*S*)-$CH_2CHOHCH_2CH_2OH$	53	479	142	>200/40
14	3	$CH_2CH_2N(CH_3)_2$	740	NA	NA	29/8

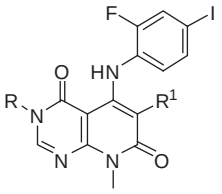
Table 2
Selective SAR of substitutions at R^1 and R^2



Compd	Route	R^1	R^2	MEK1 IC_{50} (nM)	A375 EC_{50} (nM)	Colo205 EC_{50} (nM)	HLM/RLM $t½$ (min)
11	3	H	I	4.6	26	27	48/25
15	3	Cl	I	5.9	25	10	22/>200
16	3[15]	Me	I	3.8	7.4	7.9	117/118
17*	3	F	I	3.2	3.1	2.1	143/135
18	3	F	Br	16	50	38	188/49
19	3[16]	F	CCH	4.8	11	10	49/33

* TAK-733.

Table 3
Further SAR of polar R groups, with optimal R^1 and R^2 groups in place



Compd	Route	R	R^1	MEK1 IC_{50} (nM)	A375 EC_{50} (nM)	Colo205 EC_{50} (nM)	HLM/RLM $t½$ (min)
17	3	(*R*)-$CH_2CHOHCH_2OH$	F	3.2	3.1	2.1	143/135
20	3	(*S*)-$CH_2CHOHCH_2OH$	F	3.2	3.0	2.6	183/91
21	3	(*S*)-$CH_2CHOHCH_2OH$	Me	5.0	8.0	13	>200/>200
22	3[18]	CH_2CH_2OH	F	8.6	6.3	3.7	51/29
23	3	CH_2CH_2OH	Me	4.0	18	9.0	>200/>200
26	4	$CH(CH_2OH)_2$	F	3.3	8.0	3.5	31/48
27	4	(*R*)-$OCH_2CHOHCH_2OH$	F	3.1	3.0	1.0	35/28

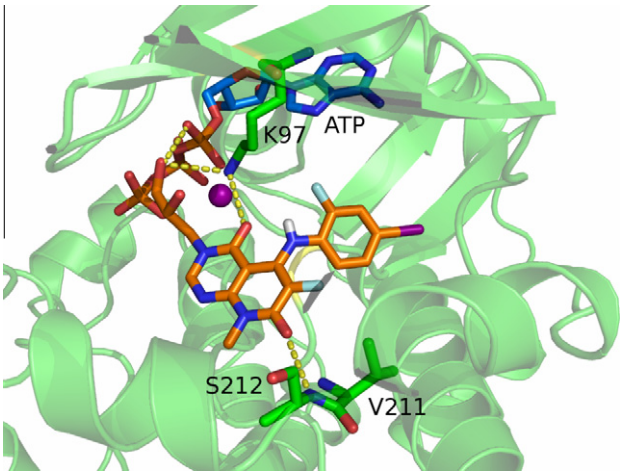


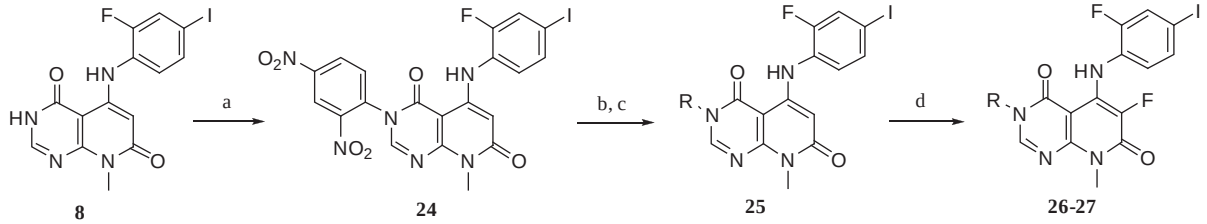
Figure 3. The X-ray co-crystal structure of TAK-733 in the MEK1 allosteric site.

Reaction of compound **8** with 1-chloro-2,4-dinitrobenzene gave compound **24**. The treatment of **24** with an amine led to a ring opening and re-cyclization accompanied by the displacement of 2,4-dinitroaniline.[17] Deprotection under acidic conditions followed by fluorination with Selectfluor provided the final compounds **26** and **27**.

As analysis of the data in Table 3 shows, the diol compounds with R^1 as fluorine exhibited the most potent enzyme and cell activities. Compounds **17** and **20** showed increased microsomal stability compared to analogs **26** and **27**.

TAK-733 exhibited potent enzymatic and cell activity with an IC_{50} of 3.2 nM against constitutively active MEK enzyme and an EC_{50} of 1.9 nM against ERK phosphorylation in cells. TAK-733 did not inhibit any other kinases, receptors or ion channels that were tested with inhibitor concentrations up to 10 μM.[19] TAK-733 was found to bind plasma protein moderately (ca. 97% for human and 96% for mouse), and exhibit high permeability and high microsomal stability across species. It did not inhibit P450s up to 30 μM.

The co-crystal structure of TAK-733 in the MEK1 allosteric site has been solved (Fig. 3).[20] As predicted, the pyridone oxygen makes a hydrogen bond with the backbone NH of Ser212. The 2-fluoro-4-iodoaniline moeity sits in the deep lipophilic pocket. The pyrimidinone oxygen makes a hydrogen bond with Lys97, and the propanediol terminal hydroxyl interacts with both Lys97 and the ADP phosphate.



Scheme 3. Synthesis of compounds **26** and **27** (Route 4). Reagents and conditions: (a) 1-chloro-2,4-dinitrobenzene, K_2CO_3, DMF; (b) (*R*)-*O*-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)hydroxylamine or 2,2-dimethyl-1,3-dioxan-5-amine, K_2CO_3 or Cs_2CO_3, DMF; (c) HCl, THF; (d) Selectfluor, CH_3CN,DMF.

Table 4
Selected PK parameters for compound **17** (TAK-733)[21]

Species	CL (mL/min/kg)	Vdss (mL/kg)	MRT_{iv} (h)	MRT_{po} (h)	*F* (%)
Mouse	17.2	1955	1.9	4.6	67
Rat	13.7	1643	2.0	11.5	71
Dog	5.9	3070	8.9	12.9	76
Monkey	11.1	3745	5.8	23.8	>38[22]

The pharmacokinetics of TAK-733 was evaluated in nude mouse, rat, dog and monkey (Table 4). Low clearance and high oral bioavailability were observed in all species. TAK-733 demonstrated broad antitumor activity in mouse xenograft models of human cancer including models of melanoma, colorectal, NSCLC, pancreatic and breast cancer. Further detailed in vivo data will be presented in future publications.

In summary, we have discovered a series of potent, selective, ATP-noncompetitive MEK inhibitors. TAK-733 has demonstrated potent anticancer activity in several mouse xenograft models. It is well tolerated with pharmacokinetics and pharmacodynamics that support once-daily oral dosing in humans. Based on its potent antitumor activities and its safety profiles, TAK-733 was advanced to Phase I clinical studies for cancer treatment.

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12. Full experimental procedures for compounds **1**–**27** are contained within the following patent application: Dong, Q.; Gong, X.; Kaldor, S. W.; Kanouni, T.; Scorah, N.; Wallace, M. B.; Zhou, F. PCT Int. Patent Appl. WO 08/079814.
13. *MEK1 enzyme assay*: Inhibition of compounds relative to MEK1 were determined using a cascade assay method in 384 well format under the following reaction conditions: test compounds serial diluted in DMSO were diluted into assay buffer (50 mM HEPES pH 7.3, 10 mM NaCl, 10 mM $MgCl_2$, 0.01% Brij35, 1 mM DTT) and added into ERK1, fluorescent labeled ERK1 substrate: IPTTPITTYFFFK-5FAM-COOH, and the reaction was initiated with 1 nM MEK1 and 400 μM ATP or 10 μM ATP. Reaction product was determined quantitatively by fluorescent polarization using progressive IMAP beads from Molecular Devices. Inhibition constants (IC_{50}) were calculated using standard mathematical models. An ERK1 assay was also conducted to rule out that inhibition was due to ERK1 in the cascade assay. Since all compounds tested showed almost identical IC_{50} when assayed at 400 μM or 10 μM ATP, only IC_{50} results assayed at 400 μM ATP were listed. Based on K_{mATP} for MEK1 at 20 μM determined using direct assay method (not shown), no potency shift when compounds were assayed at $10 \times K_m$ and $0.5 \times K_m$ ATP concentration indicated compounds were not ATP competitive inhibitors.
14. A375 and Colo205 EC_{50}s were generated using a cellular colorimetric MTS assay which measures newly produced NADH. Briefly, human cancer cell lines were seeded between 3000 and 10,000 cells per 96 well and incubated for 16 h in a humidified 5% CO_2 atmosphere incubator at 37 °C. Cells were then incubated with an eleven point dilution of test compound in duplicate for 72 h and subsequently assayed for NADH levels via the CellTiter 96-AQueous® kit (Promega) which utilizes a MTS tetrazolium salt conversion. The resulting colorimetric reaction was read on a spectrophotometer (Molecular Devices) at OD 490 nm and EC_{50} values of compound concentration vs. total NADH levels were calculated in Activity Base (IDBS). It is important to note the A375 and Colo205 cell lines both posses the BRAF(V600E) mutation making them reliant upon MEK signaling for survival. All compounds listed were also tested against the PC3 cell line whose survival is independent of MEK signaling and served as a control for MEK inhibitory selectivity. The EC_{50}s generated for all compounds listed were at a minimum 50 fold higher in the PC3 cell line.
15. A *para*-methoxybenzyl (PMB) protecting group was used in place of the benzyl protecting group.
16. Compound **19** was prepared from compound **17** via a Sonigashira reaction with TMS-acetylene, followed by deprotection with TBAF.

17. (a) Fukuoka, M.; Shuto, S.; Minakawa, N.; Ueno, Y.; Matsuda, A. *J. Org. Chem.* **2000**, *65*, 5238; (b) Fukuoka, M.; Shuto, S.; Minakawa, N.; Ueno, Y.; Matsuda, A. *Tetrahedron Lett.* **1999**, *40*, 5361; (c) Oliviero, G.; Amato, J.; Borbone, N.; D'Errico, S.; Piccialli, G.; Bucci, E.; Piccialli, V.; Mayol, L. *Tetrahedron* **2008**, *64*, 6475.
18. The fluorination step (e) was carried out on intermediate **6** prior to nitro group reduction rather than as a final step.
19. *Kinase Panel*: Abl1, AKT3, c-RAF, CamK1Δ, CDK2/cyclinA, cMet, cSRC, EGFR, GSK3β, IR, JAK3, P38α, PDGFRβ, PDK1, PKCα, PLK3, Syk, Tie2.
20. Protein Data Bank code is 3PP1. Purified recombinant Mek1 was prepared in a buffer containing 20 mM HEPES (pH 7.5), 150 mM ammonium acetate, 20 mM DTT, 0.25 mM TCEP, 0.1 mM EDTA, 5 mM ATP, and 10 mM $MgCl_2$. The protein–inhibitor complex was co-purified using 200 nM of the inhibitor with recombinant MEK1 and concentrated to 16 mg/mL. Crystals were grown at 4 °C using the sitting-drop, vapor-diffusion technique. 50 nL of the protein–inhibitor complex was mixed with an equal volume of reservoir solution containing 8–12% MPD and 0.1 M MES (pH 6.0). Crystals were mounted into nylon loops, transferred into a reservoir solution supplemented with 22% ethylene glycol, and flash frozen in liquid nitrogen.
21. Compounds were administered intravenously (IV vehicle: 30% b-cyclodextrin in 0.05 M MSA) and orally (oral vehicle: 0.5% MC) at 1.4/7.8, 1.0/4.2, 0.6/2.3, and 0.5/2.0 mg/kg for mouse, rat, dog, and monkey, respectively.
22. Longer blood collection is needed to better assess bioavailability of this compound.