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Design and synthesis of orally available MEK inhibitors with potent in vivo antitumor efficacy

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ABSTRACT

The structure-based design, synthesis, and biological evaluation of two novel series of potent and selective MEK kinase inhibitors are described herein. The elaboration of a lead pyrrole derivative to a bicyclic dihydroindolone core provided compounds with high potency and good drug-like pharmaceutical properties. Further scaffold modification afforded a series of dihydroindolizinone inhibitors, including an orally available advanced preclinical MEK inhibitor with potent in vivo antitumor efficacy.

The RAS/RAF/MEK/ERK cascade plays a central role in the signaling required for cellular transformation and proliferation.[1] Downstream in the signaling pathway, the MEK kinases catalyze the phosphorylation of the MAPK substrates ERK1 and ERK2, and thus the inhibition of MEK kinases have the potential to control cell growth. MEK inhibitors could conceivably be utilized as therapeutic agents in hyperproliferative disorders.[2] In a previous Letter, we discussed the structure-based design and synthesis of pyrrole derived MEK inhibitors.[3] In continuation of this research, we elaborated on the pyrrole moiety to develop bicyclic compounds which are orally bioavailable and efficacious in tumor xenograft models.

The key binding features of a representative compound (**1**) from the initial pyrrole series[3] in the MEK allosteric site are illustrated in Figure 1.[4] Hydrogen bonding interactions are observed between the hydroxyamate oxygens and Lys97. The exocyclic acetate carbonyl forms a hydrogen bond with the backbone NH's of both Val211 and Ser212. The iodoaniline moiety[5] binds in a lipophilic pocket, where the iodo group makes an electrostatic interaction with the backbone carbonyl of Val127.

It was our goal to improve the in vitro potency and microsomal stability of compound **1** (IC_{50} = 18 nM, Colo205 EC_{50} = 12 nM, RLM $t_{1/2}$ = 14 min) by exploring modification at the 4- and 5-positions of the pyrrole. We reasoned that rotationally locking the carbonyl moiety at the 5-position of pyrrole **1** by the formation of a bicyclic core (Fig. 1, dashed line) would increase potency by making favorable hydrophobic interactions with Ile216 while limiting molecular conformations. Previous work determined that substitution at the 4-position of the pyrrole was tolerated[3] without any unfavorable conformational perturbation of the neighboring hydroxamate group.

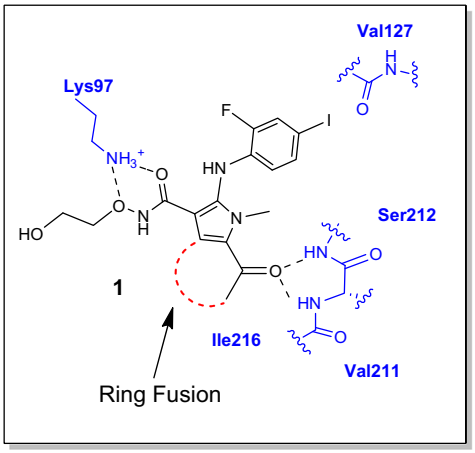

Figure 1. Key interactions of pyrrole compound **1** in the MEK allosteric site.

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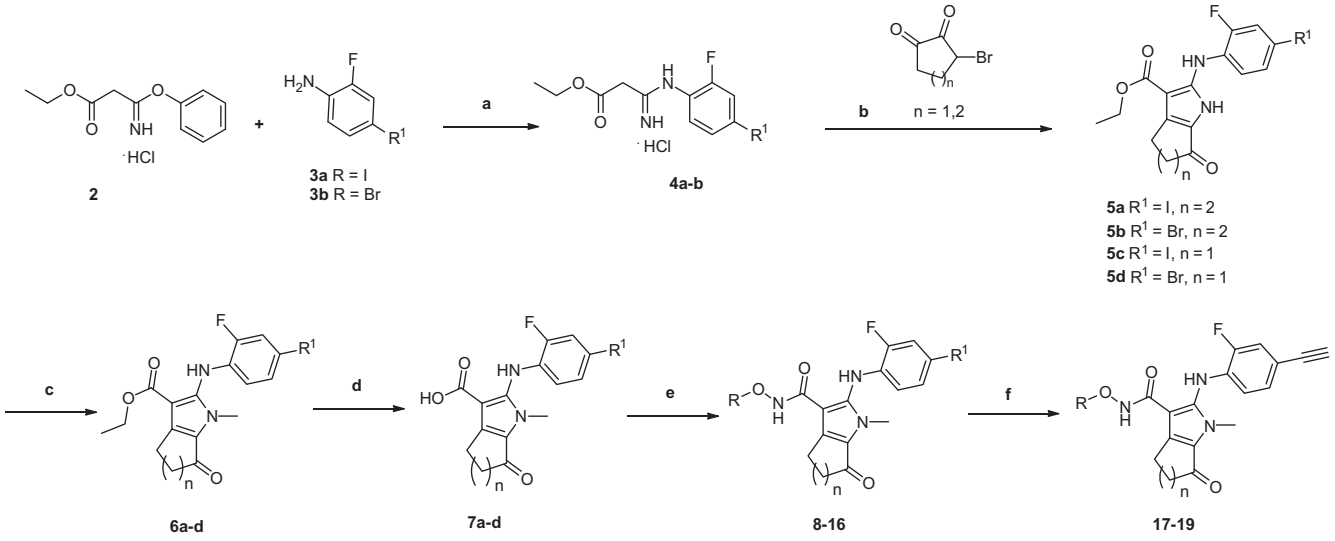
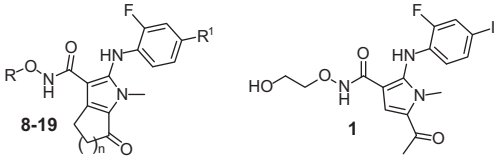


Scheme 1. Synthesis of compounds **8**–**19**. Reagents and conditions: (a) EtOAc, reflux, (60–96%); (b) 1-$NaHCO_3$ wash; 2-THF, reflux, (17–24%); (c) Me_2SO_4, Cs_2CO_3, DMF, (65–90%); (d) 1 N NaOH, THF, MeOH, (57–80%); (e) R-ONH_2, EDCI/HOBt, Et_3N, DMF, (16–50%); (f) 1-TMS-acetylene, $PdCl_2$(dppf), Et_3N, CuI, THF; 2-TBAF, THF, (19–80%).

Table 1
Selected data for bicyclic *N*-methyl pyrrole analogs



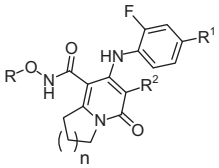
Compd	R	R^1	*n*	MEK1 IC_{50}[10] (nM)	Colo205 EC_{50}[11] (nM)	A375 EC_{50}[11] (nM)	HLM/RLM $t_{1/2}$, (min)
1	—	—	—	18	12	14	36/14
8	–$(CH_2)_2OH$	I	2	14	1.0	1.0	3.7/5.5
9	(*S*)-$CH_2CHOHCH_2OH$	I	2	17	2.0	2.5	22/36
10	(*R*)-$CH_2CHOHCH_2OH$	I	2	20	2.5	3.0	36/26
11	–$(CH_2)_2OH$	I	1	8.9	2.0	4.0	10/14
12	(*S*)-$CH_2CHOHCH_2OH$	I	1	11	5.0	7.0	64/54
13	(*R*)-$CH_2CHOHCH_2OH$	I	1	54	9.0	13	86/54
14	–$(CH_2)_2OH$	Br	2	18	34	31	25/20
15	(*S*)-$CH_2CHOHCH_2OH$	Br	2	76	53	76	76/70
16	(*R*)-$CH_2CHOHCH_2OH$	Br	2	52	58	72	94/85
17	–$(CH_2)_2OH$	CCH	2	32	10	15	34/38
18	(*R*)-$CH_2CHOHCH_2OH$	CCH	2	41	62	72	>200/>200
19	–$(CH_2)_2OH$	CCH	1	36	17	34	107/150

The bicyclic pyrroles **8**–**19** were synthesized according to the general route shown in Scheme 1.[6] The phenol displacement of ethyl 3-imino-3-phenoxypropanoate hydrochloride **2** with a halo-substituted aniline (**3a**–**b**) in refluxing ethyl acetate gave the imidate HCl salts **4a**–**b**.[7] The free base of intermediates **4a**–**b** underwent a cyclization with either 3-bromocyclohexane-1,2-dione[8] or 3-bromocyclopentane-1,2-dione[9] in refluxing THF to furnish the bicyclic core compounds **5a**–**d**. The resulting bicyclic pyrroles were then subjected to a selective N-methylation with dimethyl sulfate to give **6a**–**d**, followed by the saponification with aqueous sodium hydroxide to give the carboxylic acids **7a**–**d**. The subsequent hydroxamides **8**–**16** were made via the EDC/HOBt mediated coupling of **7a**–**d** with selected amines in the presence of triethylamine. Compounds **17**–**19** were prepared from their respective iodides via a Sonogashira reaction with trimethylsilyl-acetylene, followed by TMS-deprotection with tetrabutylammonium fluoride.

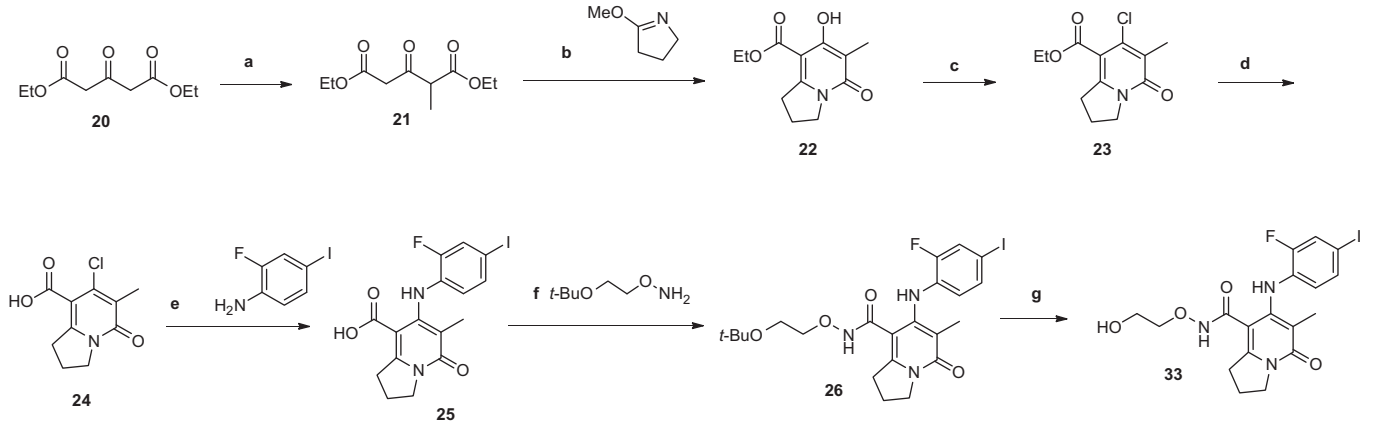
The in vitro properties of compounds **8**–**19** are presented in Table 1. Both the 5,6 and 5,5-bicyclic analogs of the pyrrole compound **1** (compounds **8** and **11**) improved cellular potency (Colo205 EC_{50} = 1.0 nM and 2.0 nM, respectively); however, microsomal stability was reduced (HLM $t_{1/2}$ < 10 min). To improve the microsomal stability of **8** and **11** we designed less lipophilic analogs. The diol compounds (**9**, **10**, **12**, and **13**) showed improved microsomal stability, albeit with no improvement in potency. As expected, compounds from the less lipophilic 5,5 series displayed higher microsomal stability than analogous 5,6 compounds. We also examined replacements for the iodine on the phenyl ring, due to its high molecular weight, lipophilic nature, and potential for metabolic instability. However, both the bromo and acetylene analogs **14**–**19** showed no potency advantages in enzymatic assays, and a 5- to 15-fold loss of activity in the cell assays. The in vitro potency of compound **17**, however, is comparable to the initial pyrrole **1**, and has the advantage of being devoid of the iodine moiety.

After successful demonstration of the advantages of the additional ring fusion in the pyrrole series, a similar strategy was employed in the context of known pyridone-based inhibitors[12] to develop a series of dihydroindolizinone compounds **27**–**34** (Table 2). The additional fused semi-saturated ring would occupy similar

Table 2
Selected data for dihydroindolizinone analogs



Compd	R	R^1	R^2	*n*	MEK1 IC_{50}[10] (nM)	Colo205 EC_{50}[11] (nM)	A375 EC_{50}[11] (nM)	HLM/RLM $t_{1/2}$, (min)
27	$-(CH_2)_2OH$	I	F	1	25	1.0	3.0	>200/124
28	$-(CH_2)_2OH$	I	F	2	57	12	30	111/41
29	(*S*)-$CH_2CHOHCH_2OH$	I	F	1	94	44	139	>200/>200
30	(*R*)-$CH_2CHOHCH_2OH$	I	F	1	39	27	69	>200/>200
31	$-(CH_2)_2OH$	CCH	F	1	69	12	33	128/>200
32	$-(CH_2)_2OH$	I	H	1	29	5.0	13	134/62
33	$-(CH_2)_2OH$	I	Me	1	38	1.0	2.0	99/40
34	$-(CH_2)_2OH$	I	Cl	1	109	3.0	5.0	47/54



Scheme 2. Synthesis of compound **34**. Reagents and conditions: (a) 1-Et_3N, $MgCl_2$, 2-NaH, MeI, Benzene, (80%); (b) Et_3N, rt, (22%); (c) $POCl_3$, DMAP, reflux, (63%); (d) 1 N NaOH, THF, MeOH, (95%); (e) LiHMDS, THF, 0 °C, (22%); (f) EDCI, HOBt, Et_3N, DMF, (84%); (g) TFA, (92%).

space as the fused ring of the bicyclic pyrrole series, and it could potentially make favorable hydrophobic interactions with Ile216 and Met219.

The dihydroindolizinones **27**–**34** were made according to the general route for the synthesis of compound **33** shown in Scheme 2.[13,14] Diethyl 3-oxopentandioate **20** formed a bidentate complex with magnesium chloride in the presence of triethylamine, which allowed a mono-methylation with sodium hydride and iodomethane to furnish **21**.[15] The subsequent cyclization was accomplished by stirring **21** in triethylamine at room temperature for 5 days to give the bicyclic intermediate **22** in modest yields. Chlorination with $POCl_3$ gave **23**, which was then hydrolyzed to the acid **24** with 1 N NaOH. Next, displacement of the chlorine on **24** with 2-fluoro-4-iodoaniline added the hydrophobic pocket substituent to produce **25**. The protected sidechain *O*-(2-(*tert*-butoxy)ethyl)-hydroxylamine[16] was coupled with **25** using typical EDCI/HOBt conditions to give the intermediate **26**, which was deprotected with neat TFA to give the final compound **33**.

The in vitro data of compounds **27**–**34** are presented in Table 2. Compound **27** showed excellent cellular potency (Colo205 EC_{50} = 1.0 nM) and microsomal stability, but expanding the five-membered fused ring to six-membered ring (**28**) or changing the alcohol tail to a diol (**29**, **30**) decreased enzymatic and cellular activity. At R^2, the analogs with F and Me (**27**, **33**) were more potent in cellular assays than the compounds with H or Cl (**32**, **34**).

The pharmacokinetic properties in rats were determined for selected compounds as shown in Table 3. The 5,6-bicyclic pyrrole compound **10** showed moderate clearance with 12% oral bioavailability. This is consistent with poor membrane permeability which we attributed to the increased hydrogen bond donor count due to the diol group. In support of this, the bicyclic pyrrole compounds **17** and **19** with one less hydrogen bond donor showed reduced clearance and concomitant increased oral exposure of 61% and 51%, respectively. The dihydroindolizinone compounds **27** and **33** exhibited moderate exposure, with % F of 21% and 29%, respectively. None of these bicyclic compounds were found to inhibit a broader panel of kinases or CYP-450s at inhibitor concentrations up to 10 μM.[18]

Table 3
Rat PK parameters for select compounds[17]

Compd	CL (mL/min/kg)	V_{dss} (mL/kg)	AUC/dose (ng h/mL)(mg/kg)	F (%)
1	52	2896	144	46
10	23.7	1158	88	12
17	5.9	596	1670	61
19	15.6	1186	512	51
27	12.9	745	262	21
33	21.4	971	204	29

The pharmacodynamic properties of compounds **17** and **33** in HT-29 tumors are shown in Figure 2. Compound **17** showed nearly complete inhibition of phosphorylation of the downstream substrates ERK-1/2 at 30 mpk at 4 h. In comparison, compound **33**

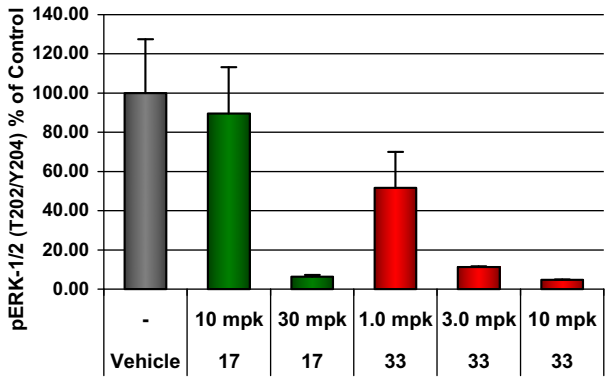


Figure 2. Pharmacodynamic data of compounds **17** and **33** in human colon adenocarcinoma HT-29 tumors at 4 h.

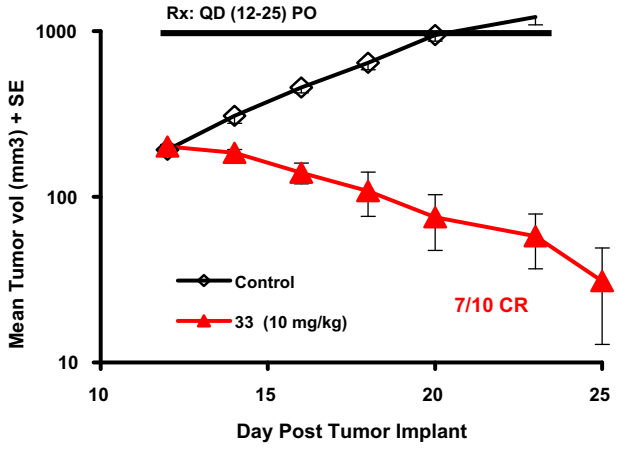


Figure 3. HL-60 (NRAS) xenograft response at MTD for compound **33**.

gave a p-ERK-1/2 inhibition dose response with nearly complete 4-h biomarker suppression at 3.0 mpk that correlated with compound plasma concentration. An HL-60 xenograft study was conducted with compound **33** at the maximum tolerable dose (Fig. 3). Daily oral administration of 10 mpk of **33** induced significant tumor regression in the promyelocytic leukemia xenograft model.

In summary, we designed and synthesized dihydroindolone and dihydroindolizinone analogs as potent and selective MEK inhibitors. Optimization of the series led to compounds that are orally bioavailable and efficacious in tumor xenograft models. Further evaluation of lead compounds as pre-clinical candidates is ongoing.

Acknowledgments

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10. *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 × K_m and 0.5 × K_m ATP concentration indicated compounds were not ATP competitive inhibitors.
11. *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 versus 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.
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17. Compounds were administered intravenously and orally at 1 and 5 mg/kg, respectively.
18. *Kinase panel*: Abl1, AKT3, c-RAF, CamK1Δ, CDK2/cyclinA, cMet, cSRC, EGFR, GSK3β, IR, JAK3, P38α, PDGFRβ, PDK1, PKCα, PLK3, Syk, Tie2.