ACS Medicinal Chemistry Letters

Letter

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ACS Med. Chem. Lett., **Just Accepted Manuscript** • DOI: 10.1021/acsmedchemlett.8b00220 • Publication Date (Web): 14 Jun 2018

Just Accepted

ACS Publications

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

	Windsor, William; Merck, Bishop, Robert; Merck and Co Inc Samatar, Ahmed; Merck and Co Inc

MK-8353: Discovery of an Orally Bioavailable Dual Mechanism ERK Inhibitor for Oncology

Sobhana Babu Boga,*‡ Yongqi Deng,† Liang Zhu,† Yang Nan,† Alan B. Cooper,‡ Gerald W. Shipps Jr.,† Ronald Doll,‡, Neng-Yang Shih,‡, Hugh Zhu,‡ Robert Sun,‡ Tong Wang,† Sunil Paliwal,‡ Hon-Chung Tsui,‡ Xiaolei Gao,‡ Xin Yao,‡ Jagdish Desai,‡ James Wang,‡ Abdul Basit Alhassan,‡ Joseph Kelly,‡ Mehul Patel,† Kiran Muppalla,† Subrahmanyam Gudipati,‡ Li-Kang Zhang,‡ Alexei Buevich,‡ David Hesk,‡ Donna Carr,‡ Priya Dayananth,‡ Stuart Black,‡ Hong Mei,‡ Kathleen Cox,‡ Bradley Sherborne,‡ Alan W. Hruza,‡ Li Xiao,‡ Weihong Jin,‡ Brian Long, ‡ Gongjie Liu, ‡ Stacey A. Taylor, ‡ Paul Kirschmeier,‡ William T. Windsor,‡ Robert Bishop,‡ Ahmed A. Samatar‡

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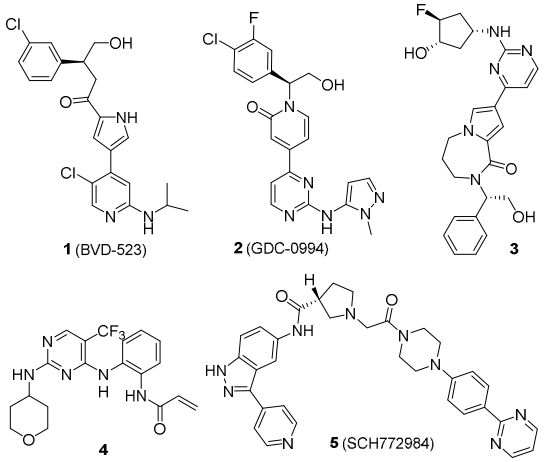
KEYWORDS: *MK-8353, MAPK pathway, ERK inhibitor, kinase selectivity, lead optimization, oncology*

ABSTRACT: The emergence and evolution of new immunological cancer therapies has sparked a rapidly growing interest in discovering novel pathways to treat cancer. Toward this aim, a novel series of pyrrolidine derivatives (compound **5)** were identified as potent inhibitors of ERK1/2 with excellent kinase selectivity and dual mechanism of action but suffered from poor pharmacokinetics (PK). The challenge of PK was overcome by the discovery of a novel 3(*S*)-thiomethyl pyrrolidine analog **7**. Lead optimization through focused SAR led to the discovery of a clinical candidate **MK-8353** suitable for twice daily oral dosing as a potential new cancer therapeutic.

The MAPK pathway plays a central role in regulating mammalian cell growth, differentiation, survival and migration by relaying extracellular signals. Activation of the MAPK pathway occurs via a cascade of protein phosphorylation events. One of the critical components in this pathway is the small GTPase RAS which recruits and activates the serine/threonine kinase RAF. Activated RAF phosphorylates and activates MEK1/2 which in turn phosphorylates and activates ERK1/2. Once activated, ERK1/2 promotes the transcription of genes that are involved in cell cycle regulation, differentiation and survival[1]. Aberrant activation of the MAPK pathway has been demonstrated to be an important feature common to several human tumor types and several components of the pathway have become attractive drug targets. Gain of function mutations of the RAS oncogene are found in several human cancers including pancreatic (90%), colorectal (50%) and lung (30%). Gain of function B-RAF mutations, which lead to constitutive activation of the MEK-ERK pathway, are found in melanoma(~ 70%); colorectal(15%) and thyroid tumors(50%).[2] In addition, non-small cell lung cancer and pancreatic tumors have been shown to have high incidence of RAS mutation. Tumors with mutations in BRAF or RAS are usually non-overlapping in occurrence. The transforming activity of the RAS and BRAF mutations suggests that inhibition of the downstream targets such as ERK would be a good strategy for the development of therapeutic agents. Inhibition of ERK should effectively inhibit all signal transduction downstream from MEK since ERK is the only known substrate for MEK. To date, several signal transduction inhibitors e.g., Vemurafenib(BRAF), Dabrafenib(BRAF), and Trametinib(MEK)) targeting this pathway have been approved and various RAS-ERK inhibitors are being studied at various phases of development.[3] Reactivation of the ERK1/2 phosphorylation and signaling has been frequently associated with secondary resistance to these upstream inhibitors.[4] Recently several highly optimized ERK inhibitors such as the pyrrole **1** by Vertex/Biomed Valley Discoveries,[5] the pyridone **2** by Genentech,[6] fused pyrrolo-diazepanone **3** by Novartis[7] and irreversible acrylate inhibitor **4** by Astrazeneca[8] have been reported (Figure 1) highlighting the importance of exploring targeted inhibition of more downstream components of MAPK signaling.

We previously reported the discovery of a pyrrolidine based ERK inhibitor **5** (SCH-772984)[9] which was derived from an initial high throughput screening hit **6** utilizing an automated ligand identification system (ALIS)[10] and validation[11] (Figure 2). Compound **5** showed excellent kinase selectivity against a panel of kinases while displaying nanomolar ERK1/2 potency in both biochemical and cellular assays. Interestingly, compound **5** displayed dual mechanism of action effecting both the inhibition of MEK phosphorylation of ERK1/2 and inhibition of its intrinsic kinase function with nanomolar potency. This pyrrolidine compound **5** served as a Proof-of-Concept (PoC) lead, that demonstrated single agent efficacy in several xenograft mouse models with either KRAS or BRAF mutant cell lines.

Figure 1. Representative structures of ERK inhibitors **1**-**5**

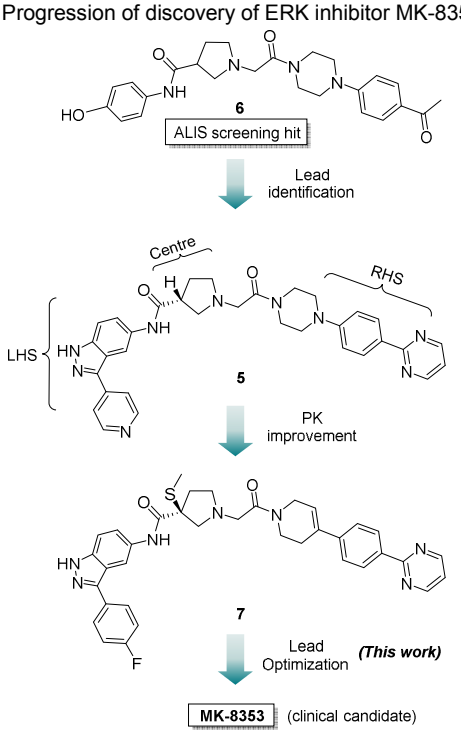


Compound **5** suffered from poor pharmacokinetics (PK) which precluded further development due to high clearance and low permeability leading to poor absorption and bioavailability in rat, as seen in the plasma drug concentration measured by area under the concentration-time curve (AUC) (rat AUC @ 10 mpk = 0 μM.h; F % = 0; Figure 2). The conventional approaches of lowering the clearance by reducing hydrophobicity and lowering logD did not improve oral exposure as lowering logD further deteriorated the permeability. Although compound **5** provided an excellent starting point for our medicinal chemistry program, our goal was to develop a potent, selective and orally bioavailable ERK1/2 inhibitor for oncology. Cold metabolite identification studies revealed pyrrolidine amide hydrolysis between centre core and Left Hand Side (LHS) due to proteolytic enzymes. We suspected that this hydrolysis was responsible for poor PK. Systematic introduction of steric hindrance groups at the 3-position of the pyrrolidine led to the discovery of 3(*S*)-thiomethyl pyrrolidine analog **7**(Figure 2). This group vastly improved the PK (rat/dog AUC PK @10 mpk = 26/17 μM.h; rat/dog F % = 70/75, see reference[12] for detailed SAR analysis) but with the loss of 2-3 fold cell potency when compared to PoC compound **5**. Herein we report further optimization of the lead **7** to which led to the discovery of a clinical candidate **MK-8353**, a highly potent and selective ERK1/2 inhibitor with good oral bioavailability across multiple pre-clinical species. MK-8353 displayed *anti*-tumor efficacy in several BRAF-mutant models.

Although thiomethyl compound **7** improved PK properties from the PoC lead compound **5**, it required further optimization to improve potency, reduce hERG and improve the overall profile in selecting a single clinical candidate for progression. Crystal structure of compound **5** and other related molecules in this series revealed that the

pyrimidine group in the RHS forms a key π-π interaction with Tyr-62 of the ERK protein.[12,13] The nature of the linker connecting the pyrimidine to the centre core is critical for optimal binding as it orients the pyrimidine to pick up the π-π interaction with Tyr-62. We began SAR scrutinizing the piperidine-ene (double bond oxidation–a potential liability) on the RHS of the molecule **7** while holding the *p*-fluorophenyl indazole on the LHS constant and the results are summarized in Table 1. Replacing piperidine-ene in **7** with piperazine to obtain compound **8** maintained ERK1/2 potency but showed a decreased rat AUC by 7-fold. Substitution of fluorine on the *o*-position of phenyl ring to obtain compound **9** resulted in 16 fold loss in ERK2 potency, potentially changing the trajectory angle of pyrimidine to a less optimal position. Reducing the double bond of piperidine-ene **7** to a tetrahydropyridine group to obtain compound **10** and substitution of fluorine on the *o*-position of phenyl ring to obtain compound **11** both resulted in 2-3 fold loss in ERK2 potency.

Figure 2 Progression of discovery of ERK inhibitor MK-8353



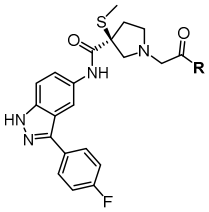
Compound	5	7
Enzymatic ERK1/2 IC_{50} (nM)	8/1	41/7
Cell Proliferation HT-29/Colo-205 IC_{50} (nM)	59/16	118/45
CYP 3A4 (co/pre) (mM)	7.8/5.7	>30/2
hERG Rb (%I) @1.5/5 mg/ml	12/3	8/21
Hu & Dog hep. CL (mL/min/M)	ND	18/7
10^6Caco II (P_{app}/cm.S^{-1})	4	60
Rat AUC @ 10 mpk (μM.hr.)	0	26
Clp (mL/min/kg)	122	8.4
t½ (h)	1.5	2.5
Mean residence time (hr.)	1.6	2.5
Bioavailability F %	0	70
Dog AUC @ 10 mpk (nM.hr.)	ND	17
Clp (mL/min/kg)	ND	7.6
t½ (h)	ND	2.0
Mean residence time (hr.)	ND	5.1
F %	ND	75

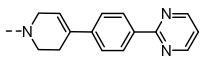
ND, Not Determined.

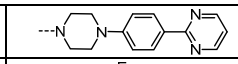
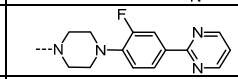
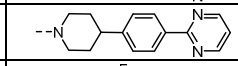
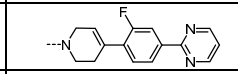
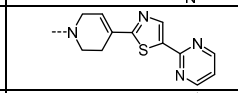
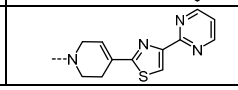
Attempted replacement of the phenyl group between the piperidine-ene ring and the pyrimidine ring in **7** with a 5-membered thiazole ring resulted in two regio-isomers **12** and **13** with much decreased ERK1/2 potency. This limited SAR illustrated that the piperidine-ene phenyl pyrimidine construction of the RHS of the molecule **7** was optimal in maintaining the ERK potency with improved PK.

Concurrently, we rapidly investigated 3(*S*)-thiomethoxy pyrrolidine compound **7** that provided the balanced enzymatic ERK potency and PK properties while we continued medicinal chemistry efforts to further improve physicochemical properties such as cell potency (HT-29 and colo-205) and other off-targets *viz*., hERG by combining various LHS and RHS modifications and the results are summarized in **Table 2**. Change of the *p*-flouro phenyl group attached to the indazole on the LHS of compound **7** to a 4,5-methylenedioxy phenyl compound **14** gave an excellent 4-fold boost in rat AUC (106 μM at 10 mpk) and 1.5 fold improvement in cellular potency but suffered from strong CYP 3A4 inhibition (pre-incubation) with an in vitro IC_{50} = 0.3 μM. In general, this series of ERK compounds are not potent inhibitors of human CYPs 1A2, 2C9, 2C19 or 2D6 but are time dependent inhibitors of CYP3A4, and thus potentially raise the concerns for drug-drug interactions when co-administered with drugs that are primarily metabolized by CYP 3A4. The 5-methoxy pyridyl, compound **15** showed a similar profile but with only marginal improvement in hERG activity and rat AUC. Various heterocyclic rings were explored on the RHS and LHS. For example, 2-ethylamino-1,3,4-oxadiazole compound **16** (with *p*-flouro phenyl indazole in LHS) and 2-ethylamino-1,3-thiazole compound **17** (with 2-methylpyridine indazole in LHS) showed only a modest rat AUC 4.9 μM and 4.8 μM at 10 mpk respectively. However, a more electron deficient 1-methyl-1,2,4-triazole compound **18** improved both ERK1/2 enzymatic and cellular (HT-29) potency by 3 to 4-fold and at the same time restored the rat oral AUC exposure. To remove the potential metabolic liability of the methoxy group in 5-methoxy pyridyl compound **18**, introduction of a 5-isopropoxy pyridyl group to obtain compound **19** led to a 10-fold loss of ERK1/2 potency potentially due to steric hindrance and non-alignment of favorable Lys112 interaction of ERK. Enabled by structure based drug design (SBDD), an effort to re-orient 5-isopropoxy O atom which forms a key H-bond with Lys112 of ERK when changed to 4-isopropoxy pyridyl indazole on the LHS (see X-ray, Figure-**4**) and in combination with the potent, optimal 1-methyl-1,2,4-triazole RHS resulted in the discovery of compound **20**. Compound **20** displayed overall improved ERK1/2 enzymatic, cell potency, hERG selectivity and good rat oral AUC. Further replacement of 1-methyl-1,2,4-triazole with pyrimidine, compound **21** led to a loss of ERK1/2 and cell potency(2 fold) with no advantage in hERG selectivity. At this point, compound **20** was selected as a pre-clinical candidate (**MK-8353**) for further development and evaluated *in vivo* studies based on its *in vitro* potency (ERK1/2 IC_{50} = 20/7 nM), cellular activity (HT29/Colo-205 IC_{50} = 51/23 nM) and in conjunction with its favorable off-target hERG activity and pharmacokinetic profile. Interestingly, the sulfone methyl pyrrolidine analog of compound **20** gave compound **22** and the opposite enantiomer 3(*R*)-thiomethoxy of compound **20** gave compound **23.** Both compounds were shown to be weaker in ERK1/2 potency, illustrating the importance of stereospecific structural requirement for this class of inhibitors for optimal potency.[12]

Table 1. Targeted modifications on the right hand side portion of compound **7**



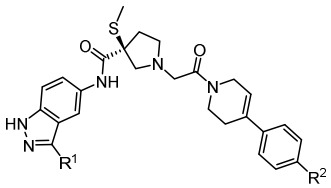
Compound #	R	aERK1/2 IC_{50} (nM)	HT29 IC_{50} (nM)	Colo-205 IC_{50} (nM)	hERG Rb %I @ 1.5/5 μg/ml	Rat AUC (μM.hr)[a]
7		41/7	118	45	8/21	25.9

8		25/5.6	87	47	3/47	3.6
9		663/114	ND	ND	ND	ND
10		43/23	245	76	22/12	ND
11		135/25	209	83	11/32	ND
12		207/36	543	>111	-5/10	ND
13		952/291	ND	ND	6/19	ND

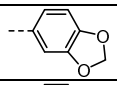
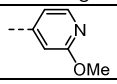
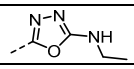
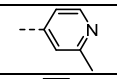
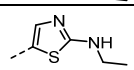
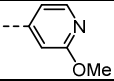
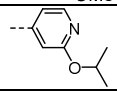
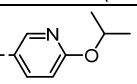
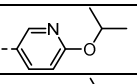
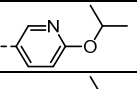
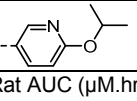
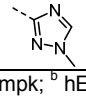
[a] Rat AUC (μM.hr) @ 10 mpk

The full profile of the clinical compound MK-8353 is depicted in Figure 3. MK-8353 is a potent and selective inhibitor of both active and inactive ERK1 and ERK2 kinases (IC_{50}=20 and 7 nM, respectively). The overall kinase selectivity profile of MK-8353 was characterized against a panel of 233 mammalian serine/threonine and tyrosine kinases. Only three kinases showed greater than 50% inhibition at the 1 μM test concentration and none of the 231 off-target kinases were inhibited at 100 nM.

Table 2. Combination of LHS and RHS modifications of the 3(*S*)-thiomethoxy pyrrolidine core.

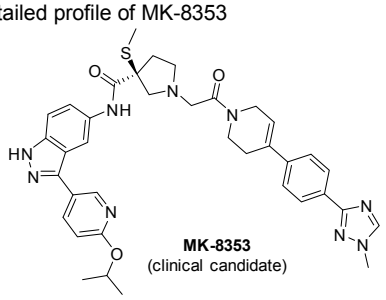


The excellent selectivity of MK-8353 for ERK1/2 can be attributed to a large compound induced conformational change in the polyglycine loop of ERK (see crystal structure, Figure-4). A2058 melanoma cells were used to characterize the in-cell target engagement profile of MK-8353. A typical western analysis profile of MK-8353 (see supporting info.) caused a dose proportional decrease in pERK1/2 and pRSK levels with complete suppression of pERK1/2 at 30 nM in A2058 cells, illustrating the dual mechanism of action. MK-8353 was not a potent inhibitor of human CYPs 1A2, 2C9, 2C19 or 2D6 but inhibits CYP 3A4 (pre-incubation) *in vitro* and showed inhibition of CYP 3A4 and 2C8 (IC_{50} = 1.7 & 3.5 μM), which can cause drug-drug interactions when co administered with drugs that are primarily metabolized by CYP 2C8 or 3A4. MK-8353 is a weak inhibitor of hERG current, producing 16% inhibition at 0.6 μM. There were no test article-related changes in PR, QRS, and QT/QTc intervals in telemetered guinea-pigs exposed at 30 & 100 mg/kg with exposure multiples of 2-3 fold (based on total C_{max}). Based on the metabolite characterization in rat bile, urine and plasma, the primary metabolic pathways appeared to be oxidative dealkylation of the parent compound leading to a loss of the isopropyl group as well as the amide cleavage of the parent with subsequent oxidative and conjugation reactions. No glutathione conjugate metabolites were found.[18]

Compound #	R^1	R^2	aERK1/2 IC_{50} (nM)	HT29 IC_{50} (nM)	Colo-205 IC_{50} (nM)	hERG Rb %I @ 1.5/5 μg/ml	Rat AUC (μM.hr)[a]
7			41/7	118	45	8/21	25.9
14			45/13	77	29	19/16	106
15			19/5	60	34	2/15 7/19[b]	30.8
16			17/5	49	22	-3/6	4.9
17			34/6	131	30	-7/-3	4.8
18			7.9/1.7	80	10	0.8/7.2[b]	16.9
19			74/27	99	41	3/10[a]	ND
20 (**MK-8353**)			20/7	51	23	-3.5/6 -1.7/-0.2[b]	34.5
21			93/13	79	57	25/37	ND
22[14]			228/29	243	86	4/5[b]	ND
23[15]			ND/729	ND	>1000	6/10[b]	ND

[ND] Not Determined; [a] Rat AUC (μM.hr) @ 10 mpk; [b] hERG (IW) %I@ 1/10 μM.

Figure 3. Detailed profile of MK-8353



Compound	**MK-8353**
Molecular weight	691.8
Melting point (°C)	133
ERK 1 & 2 IC_{50} (nM)	20 & 7
Cell proliferation IC_{50} (nM) A2058, HT-29, Colo-205	371, 51, 23
Caspase (nM)	185
CYP Co/Pre-incubation IC_{50} (μM) 3A4; 2C8; 2C9	>5/1.7; >5/3.5; >5/>5
hERG voltage clamp	16% I @ 600 nM
Caco II AP>BL/ BL>AP(nM/sec)	134 / 400
Plasma protein binding: rat/dog/monkey/mice/human	>99% bound

The *in vivo* pharmacokinetics and metabolism of MK-8353 were evaluated in male CD1 mice, Sprague Dawley (SD) rats, guinea pigs, beagle dogs, and cynomologus monkeys. With the exception of monkeys, MK-8353 showed moderate clearance after IV administration in all species, with a half-life range of 1.3-2.8 hr and a mean residence time range of 1.5-4.0 hr (Table 3 and 4). Acceptable oral bioavailability was seen in mice, rats and dogs (23-80%) but low oral bioavailability in monkeys (2%). The permeability observed in Caco-2 cells was high (135 nm/sec), suggesting that intestinal absorption and permeability in humans should also be high. The steady-state volume of distribution in mice, dogs and monkeys was in the range of 0.9-3.3 L/kg, while in rats it was 0.1 L/kg.

The crystal structure of ERK with the ATP competitive inhibitor MK-8353 has been determined at 1.45 Å resolutions. An overall view of this structure is shown below (Figure 4). The indazole ring of the inhibitor inserts into the ATP binding cavity and mimics the hydrogen bonding of adenine to the hinge region of ERK and H-bonds to the backbone Asp104 CO and Met106 NH. Another strong interaction is the H-bond from the catalytic Lys52 to the pyrrolidine nitrogen which requires the deprotonated form of the amino nitrogen; a pKa near neutral pH is essential. Tyr34 folds under the Gly-rich loop (conformational shift) and stacks with the pyrrolidine ring. This rearrangement opens a cavity that is occupied by the pendant methyl-triazole where it stacks with Tyr62. 3(*S*)-thiomethoxy group that allows to retain strong binding to ERK point towards the binding region defined by residues Asn152 and Cys164. The numerous interactions which MK-8353 makes with ERK contribute to its potency and selectivity versus other kinases.

Figure 4. X-ray crystal structure of MK-8353 (PDB:6DCG) in the active site of ERK2 with hydrogen bond interactions to the key residues highlighted in dashed lines.

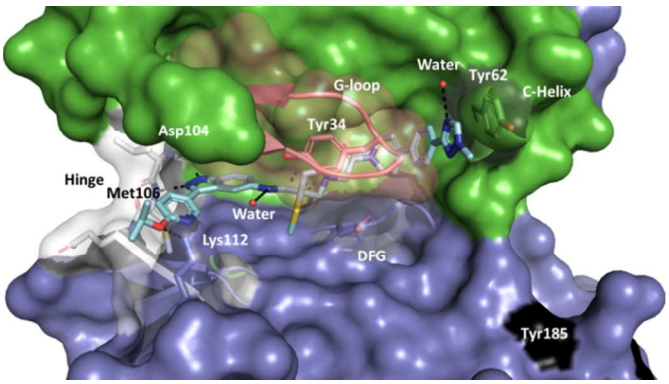


Table 3. Mean Pharmacokinetic Parameters of MK-8353 following intravenous administration[b]

Species	Dose (mg/kg)	n	AUC (0-∞) (μM.hr)	Half-life (hr)	MRT (hr)	Clearance (mL/min/kg)	$V_{d,ss}$ (L/kg)
Mouse	10	30	19.4	1.8	1.5	38	3.3
SD Rat	3	3	85	1.6	2.3	1.0	0.1
Dog	3	3	20	2.8	4.0	3.9	0.9
Monkey	1	3	1.3	1.3	1.9	17.9	2.0

[b] aqueous solution of 20% hydroxypropyl-β-cyclodextrin (HPβCD)

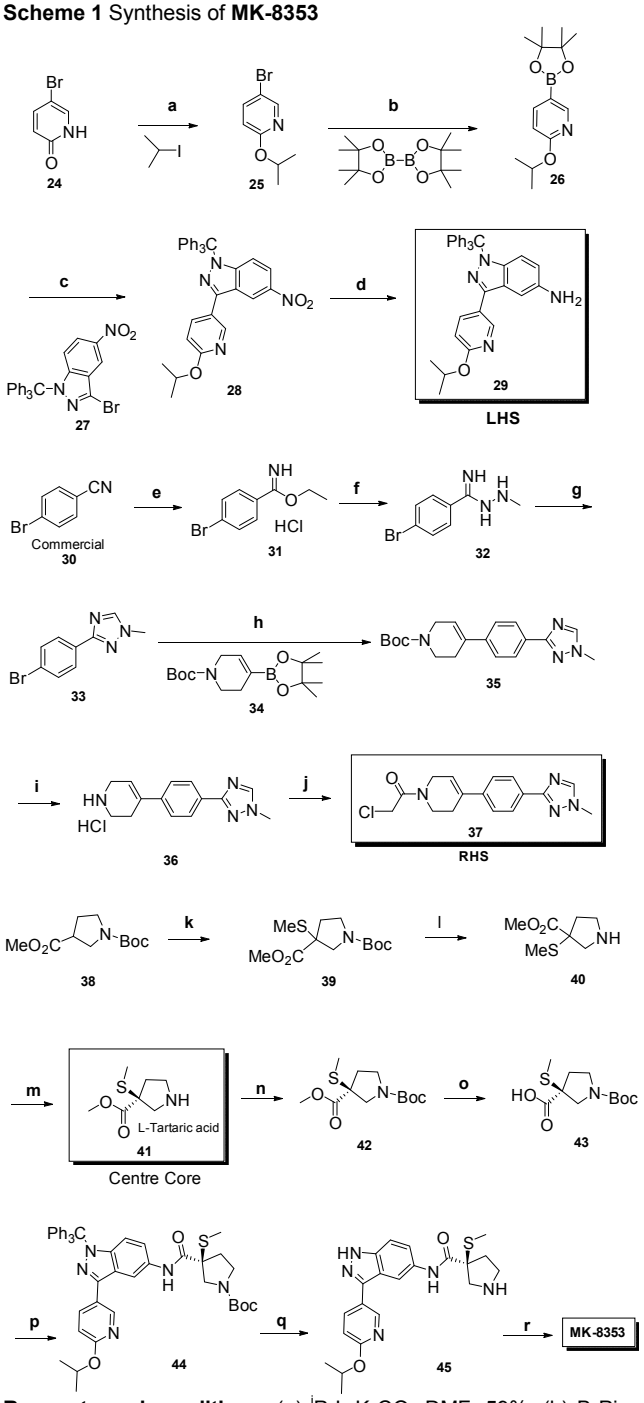
Table 4. Mean Pharmacokinetic Parameters of MK-8353 following oral administration[c]

Species	Dose (mg/kg)	n	C_{max} (μM)	T_{max} (hr)	AUC (0-∞) (μM.hr)	Bioavailability (%)
Mouse	30	27	4.6	1.0	15.3	80
SD rat	10	3	24	1.7	143	50
Dog	30	3	6.5	4.0	45	23
Monkey	10	3	0.06	1.4	0.2	2

[c] 0.4% hydroxypropyl methylcellulose (0.4% MC)

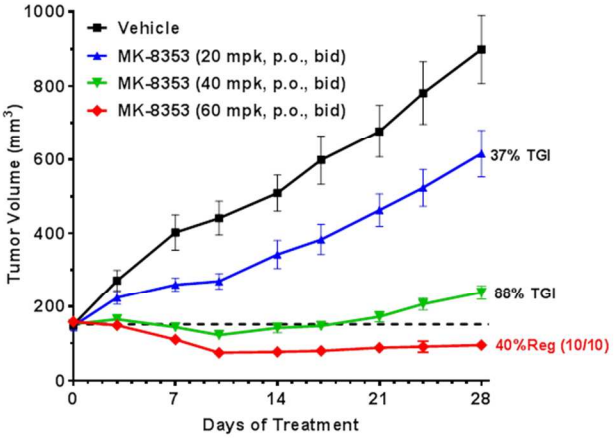
In addition to inhibiting the kinase activity of ERK, MK-8353 prevents the phosphorylation of ERK by MEK.[18] By contrast, a published Vertex ERK inhibitor,[4] which maintains the original apo-ERK conformation of Tyr34, inhibits the enzymatic activity of ERK, but not its phosphorylation by MEK. Apparently, phosphorylation of ERK requires not only the full length ERK protein, but also a precise conformation of ERK. Successful ERK phosphorylation by MEK appears to critically depend on retaining the native conformation within Tyr34 and the Gly-rich loop. Efficacy of *anti*-tumor activity of MK-8353 was established in the Colo-205 human colon xenograft model. Female nude mice bearing Colo-205 human colon tumor xenografts were randomized for treatment with MK-8353 at the doses shown Figure 5. After 28 days of treatment, MK-8353 at all the doses tested significantly inhibited Colo-205 tumor growth compared to vehicle control ($P < 0.05$ or $P < 0.0001$). At the 20 and 40 mg/kg, p.o., bid dose MK-8353 caused 37% and 88% TGI respectively. At the 60 mg/kg, p.o., bid doses MK-8353 caused mean tumor regressions of 40%. Projection of the human dose regimen was carried out based on an effective average plasma exposure of 31 uM.hr, corresponding to a 72% tumor growth inhibition in the colo-205 mouse model. The projected human dose regimen is bid with a dose range of 400 mg (based on dogs) to 1800 mg (based on monkeys).[18]

Scheme 1 Synthesis of **MK-8353**



Reagents and conditions: (a) ^{i}PrI, K_2CO_3 DMF, 59%; (b) B_2Pin_2, $Pd(dppf)_2Cl_2$, KOAc, DMSO; (c) **27**, $Pd(Ph_3P)_4$, Na_2CO_3 Tol-EtOH-H_2O; Pd/C, 100 °C, 80%; (d) Pd/C, H_2 ^{i}PrOH-Toluene, 100%; e) HCl, EtOH, 56%; (f) $MeNHNH_2$, pyridine, 85%; (g) Formic acid, 84%; (h) **34**, $Pd(dppf)_2Cl_2$, K_2CO_3, DME/water, 73%; (i) HCl, Dioxane, 100%; (j) Chloro acetyl chloride, Et_3N, CH_2Cl_2, 67%; (k) LDA, $(SMe)_2$, THF, -78°C, 67%; (l) TFA, CH_2Cl_2, 90%; (m) L-tartaric acid, MeOH, 78%; (n) $(Boc)_2O$, Et_3N, DMF, 100%; (o) *aq*. LiOH (2.0M), THF/MeOH, 92%; (p) LHS **29**, HATU, iPr_2EtN, DMF, 60%; (q) TFA, CH_2Cl_2, 73%; (r) RHS **37**, $^{i}Pr_2EtN$, DMF, 50 °C, 70%.

Figure 5. *Anti*-tumor activity of MK-8353 on the Colo-205 human colon tumor xenograft model



The discovery synthesis of ERK inhibitor MK-8353 relied on a convergent approach utilizing three fragments *viz.*, LHS substituted *iso*-propoxy indazole (LHS **29)**, RHS piperidine-ene phenyl triazole (RHS **37)**, 3(*S*)-thiomethyl pyrrolidine centre core **41** and final coupling as depicted in Scheme 1. Chemo-selective alkylation of 5-bromopyridin-2(1*H*)-one **24** with isopropyl iodide using K_2CO_3 followed by borylation gave 2-isopropoxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine **26**. Suzuki reaction of the commercially available 3-bromo-5-nitro-1-trityl-1*H*-indazole **27**[17] with **26** gave the nitro indazole **28** in good yield after chromatography. Reduction of **28** gave LHS **29** as an oil in good yield without chromatography, with an overall yield 20%. RHS **37** was prepared by reaction of the commercially available bromo-4-cyanobenzene **30** with ethanol under acidic conditions to give **31** followed by methyl hydrazine to form the hydrazinoimidate **32** in modest yield. After reaction with formic acid the bromophenyl-*N*-methyl triazole intermediate **33** was obtained. The tetrahydropyridine ring was introduced by a Suzuki reaction of the commercially available Boc protected tetrahydropyridine-boronate **35** to obtain the tricyclic ring system **36**. RHS fragment, chloroacetamide **37,** was obtained in excellent yield by reaction of the deprotected **36** with chloroacetyl chloride. The centre core, 3(*S*)-thiomethyl pyrrolidine **41** was obtained in good yield in 3 steps starting from commercially available pyrrolidine intermediate **38**. Deprotonation with LDA at -78 °C and quenching with dimethyl disulfide gave the centre core 3-thiomethyl Pyrrolidine **39** in moderate yield.[16] Subsequent Boc deprotection gave **40** and chiral resolution by crystallization with L-tartaric acid and filtration from methanol gave the pure enantiomer 3(*S*)-thiomethyl pyrrolidine **41** in good yield with >99% purity. Boc protection followed by hydrolysis of the methyl ester gave **43** in good yield. The final coupling of the intermediates is proceeded by coupling LHS **29** with centre core derivative **43** using HATU to give **44** in good yield. After deprotection of both trityl and Boc groups with TFA gave **44** in 73% after purification. Final coupling between **45** and RHS **37** using Hünig's base at 50 °C resulted **MK-8353** in good yield after chromatography. The convergent discovery synthesis of MK-8353 is 19 steps with the longest linear synthesis of 9 steps proceeding in an unoptimized 3 % overall yield (Scheme1). Optimization of this synthesis to provide kg quantities will be discussed in future publications.

In conclusion, structure-based drug design afforded a novel conformationally restricted potent pyrrolidine series of ERK inhibitors. 3(*S*)-thiomethyl substitution of the pyrrolidine core retarded amide metabolism and ameliorated the challenge of PK in this chemotype. A systematic SAR exploration of LHS and RHS led to the discovery of orally bioavailable ERK inhibitor MK-8353. MK-8353 displayed excellent potency, high kinase selectivity and dual mechanism of action inhibition. MK-8353 also demonstrated significant *in vivo* efficacy, demonstrated by tumor growth inhibition & regressions in BRAF/KRAS tumor models. The detailed pharmacological and clinical evaluation of MK-8353 as a potential treatment of cancer showed that it was well tolerated up to 400 mg twice daily and exhibited antitumor activity in patients with BRAFV600-mutant melanoma.[19]

ASSOCIATED CONTENT\

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.
Kinase Selectivity, PK Profiles, X-ray Data-collection, Experimental procedures, NMR, MS data

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

ACKNOWLEDGMENT

We thank Drs. John Piwinski, Cathy Strader, Cecil Pickett, Malcolm MacCoss at Merck & Co., Inc., Kenilworth, NJ USA for their support and encouragement. Drs. William Greenlee and Robert Aslanian for support of the program.

ABBREVIATIONS

ERK, extracellular signal-regulated kinases, MAPK, mitogen-activated protein kinases; SBDD, structure based drug design; SAR, structure-activity relationship; PK, pharmacokinetics; AUC, area under curve; TGI, tumor growth inhibiition; PoC, Proof of Concept

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MK-8353: Discovery of an Orally Bioavailable Dual Mechanism ERK Inhibitor for Oncology

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