Bioorganic & Medicinal Chemistry 16 (2008) 9202–9211

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

4-Anilino-7-alkenylquinoline-3-carbonitriles as potent MEK1 kinase inhibitors

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ARTICLE INFO

Article history:
Received 4 August 2008
Revised 27 August 2008
Accepted 5 September 2008
Available online 9 September 2008

Keywords:
Anticancer
MEK1
Kinase inhibitor
3-Quinolinecarbonitriles

ABSTRACT

A series of substituted 7-alkenyl 4[3-chloro-4-(1-methyl-1*H*-imidazol-2-ylsulfanyl)]anilino-3-quinolinecarbonitrile analogs were synthesized and evaluated as MEK1 kinase inhibitors. The synthetic details, structure–activity relationships, biological activity, and selected oral exposure studies of these analogs are described. From these studies, compound **5m** was chosen as a strong candidate for further evaluation. The selectivity of **5m** was ascertained against a panel of 17 kinases, where activity was observed against EGFR, Src, Lyn, and IR kinases. Western blot studies in WM-266 cells demonstrated that **5m** inhibited phosphorylation of ERK, while additional kinase pathways tested showed no inhibition at up to 10 μM of **5m**. PK studies, as well as a xenograft and in vivo biomarker studies are described for **5m**.

1. Introduction

The Ras-MAPK pathway is an essential signaling cascade that controls cellular growth, proliferation, and survival. Activation of this pathway under the influence of growth factor receptors or hormones results in Ras being induced into an active GTP-bound state, which in turn stimulates the serine/threonine kinase Raf. Raf kinase phosphorylates and activates mitogen-activated protein kinase kinases (MEK) 1 and 2, which phosphorylates mitogen-activated protein kinase (MAPK). The activated MAPK proteins translocate to the nucleus, where they phosphorylate downstream targets that result in growth and proliferation.[1] Inappropriate Ras activation is associated with ~30% of all human cancers, making both Ras and its downstream effectors attractive targets for pharmaceutical intervention.[2a,b] Additionally, B-Raf mutations have been associated with certain tumor types, particularly melanoma and colorectal cancer.[3] It has been observed that tumor formation can be associated with either Ras or Raf mutations, but not both.[4] As signaling by either mutation proceeds via the downstream target MEK kinase, several groups have focused their attention on discovering MEK inhibitors. Thus, researchers at Pfizer identified CI-1040 as a potent MEK1 kinase inhibitor with oral activity in vivo against selected tumor lines.[5] This compound entered human clinical trials, but was subsequently replaced by analog PD0325901, which demonstrated significantly improved bioavailability over CI-1040.[6a,b] A MEK1 inhibitor discovered at Array Biopharma (AZD6244) is also currently being evaluated in clinical trials as a potential anticancer agent.[6a,7]

High-throughput screening identified 6,7-dimethoxyquinoline-3-carbonitriles as potent MEK1 kinase inhibitors.[8,9] Of particular interest was a series of 4-[3-chloro-4-(1-methyl-1*H*-imidazol-2-ylsulfanyl)anilinoquinoline-3-carbonitriles **I** (Chart 1) which were highly potent inhibitors in cellular proliferation assays. These compounds were shown to be ATP competitive inhibitors of MEK1 kinase,[9] in contrast to the allosteric, non-ATP competitive MEK inhibitors CI-1040, PD0325901, and AZD6244. Of these compounds, **II** was identified as a potent MEK1 inhibitor (IC_{50}: 3 nM), with exceptional activity (IC_{50}: 7 nM) against LoVo cells (a K-Ras mutant human colon tumor line), as well as other selected cell lines. Western blot studies revealed that this compound strongly inhibited phosphorylation of ERK in LoVo cells, although additional off-target activity was observed to cause inhibition of MEK phosphorylation as well. While **II** displayed potent in vitro activity as well as in vivo activity following ip dosing, it was poorly soluble in aqueous media and undetectable in plasma following oral dosing. As a result, it was not orally active in vivo. Similarly, additional C-6, C-7 dialkoxy substituted analogs[10] were found to have poor oral bioavailability. Other quinoline-3-carbonitrile analogs investigated include the C-7 aryl[11] analogs and C-7 amino[12] substituted series. A C-7 (4-diethylamino)piperidine substituted analog was found to have oral activity against BXPC3 xenografts when dosed orally at 25 and 50 mg/kg twice daily.[12] However, further evaluation of this compound revealed low oral bioavailability in rats (2%), as well as highly variable blood levels following oral dosing. Thus, further efforts were undertaken to find MEK1 kinase inhibitors

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doi:10.1016/j.bmc.2008.09.009

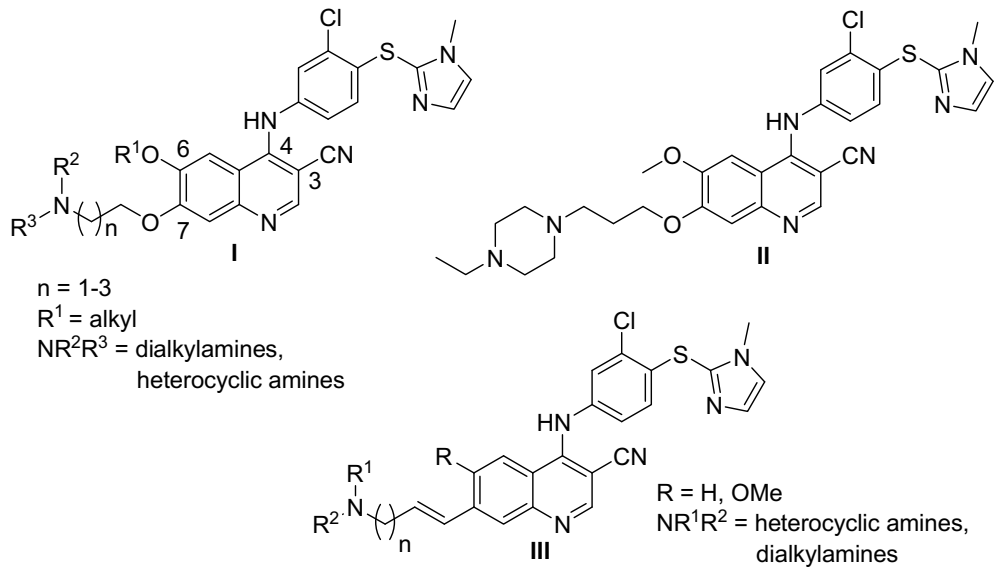


Chart 1.

with improved oral bioavailability. The present work describes the synthesis and detailed SAR of the C-7 alkenyl substituted quinoline-3-carbonitriles. Structurally, they are described by the general formula **III** shown in Chart 1.

2. Chemistry

The target compounds all possessed the 3-chloro-4-(1-methyl-1*H*-imidazol-2-ylsulfanyl)aniline headpiece, as this provided the optimal mixture of MEK1 enzyme and cellular activity for the 3-quinolinecarbonitriles.[9] Additionally, the final products were synthesized with water-solubilizing amines attached via the alkene linker at C-7, based on the SAR provided by the previously synthesized MEK1 inhibitors.[9–12] Scheme 1 outlines the method by which we initially synthesized the desired target compounds, with installation of the different amines being the last step of the reaction sequence. Thus, the reaction of propyn-1-ol, butyn-1-ol, and pentyn-1ol with tributyltin hydride respectively provided **2a**[13] and **3a**,[14] **2b**, and **3b**, as separable mixtures. Following chromatographic separation of the isomers on silica gel, alcohols **2a and b** were reacted with quinoline-3-carbonitrile intermediates **4a and b**[15] under Stille coupling conditions[16] to provide **5a–c**. Allylic alcohol **5a** was converted to target compounds **5d–f** via reaction with acetic anhydride, followed by a palladium (II) acetate-mediated coupling with the requisite amines. The alcohol residues of **5b** and **c** were tosylated, then displaced by the corresponding amines to provide **5g–h, k, and p**. While this reaction sequence provided several analogs, one issue was that the intermediate alcohols **5a–c** were highly insoluble in a variety of solvents, with the C-6 methoxy substituted **5c** being particularly insoluble. In addition, the target compounds in some cases required more than one chromatographic separation to provide $\geqslant$98:2 *trans*/*cis* product ratios.

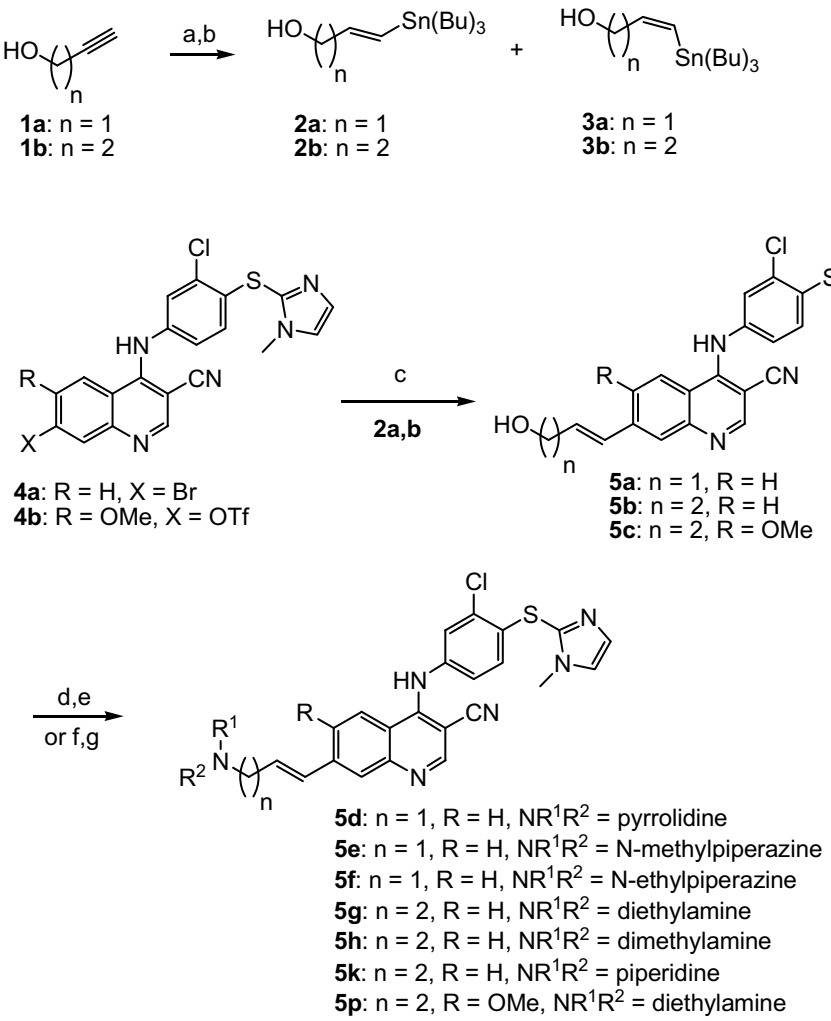
In order to circumvent the solubility issues, another set of analogs was prepared by the sequence outlined in Scheme 2. Intermediates **2b** and **c** were first tosylated, and then reacted with amines to provide amino intermediates **6a–e**. Following the purification by silica gel chromatography, the amino-substituted vinyl tin intermediates **6a–e** were reacted with **4a and b** by Stille coupling to provide target compounds **5i, j, n, o, q, and r**. This methodology proved useful in readily providing the target compounds, although some were again found to require careful chromatography to provide $\geqslant$98:2 *trans*/*cis* product ratios.

Pure *trans*-substituted compounds **5l** and **m** were obtained by the methodology shown in Scheme 3. Hydroboration of alkynes **7a** and **b** in the presence of Schwartz reagent provided the intermediate boronates **8a** and **b** with excellent *trans*-selectivity.[17] Suzuki coupling of **8a** and **b** with **4a** in a mixture of toluene/EtOH/H_2O provided **5l** and **m** in high yield and purity following silica gel chromatography (the *cis*-isomer being $\leqslant$0.5% as determined by analytical HPLC).

3. Biology

As previously determined for the 6,7-dialkoxy substituted 4-[3-chloro-4-(1-methyl-1*H*-imidazol-2-ylsulfanyl)anilinoquinoline-3-carbonitriles,[9] the C-7 alkenyl substituted quinoline-3-carbonitriles described here were confirmed to be MEK1 inhibitors. The in vitro inhibitory activity of compounds **II** and **5a–r** is detailed in Table 1. Despite having low solubility (aqueous solubility: 1 μg/mL at pH 7.4), alcohols **5a–c** had potent activity in both the enzyme and cellular assays, demonstrating that the water-solubilizing amines were not necessary for in vitro activity. Overall, the in vitro potency of **5a** and **b** was similar to the C-6 hydrogen substituted analogs **5d–o**, which possess amine water-solubilizing groups. While the C-7 substituted allylic amine analogs **5d–f** appeared to be slightly less potent than the comparably substituted homoallylic amines, there was little variability in the activity of the different amine substituted compounds. The importance of the *trans*-geometry for the C-7 alkene was demonstrated by determining and comparing the IC_{50}s of selected compounds for which sufficient quantities of the *cis*-isomers were isolated. For example, while **5j** had a Raf/MEK IC_{50} of 20 nM, the *cis*-isomer was far less potent with a Raf/MEK IC_{50} of 435 nM.

The most obvious improvement in activity was provided by the C-6 methoxy substituent. Thus, homoallylic alcohol **5c**, and amino-substituted analogs **5p–r** were the most potent MEK1 kinase inhibitors, with comparable activity to **II**. In cellular assays, however, mixed results were observed for the C-6 methoxy substituted analogs. While all of these analogs showed good potency against LoVo cells, it proved to be challenging to obtain consistent antiproliferative IC_{50}s when using the BXPC3 cells. Thus, for example, while compounds **5p** and **r** appeared to have some activity at low nanomolar concentrations in several runs, the dose–response curves did not reach 50% inhibition, and are therefore reported as having



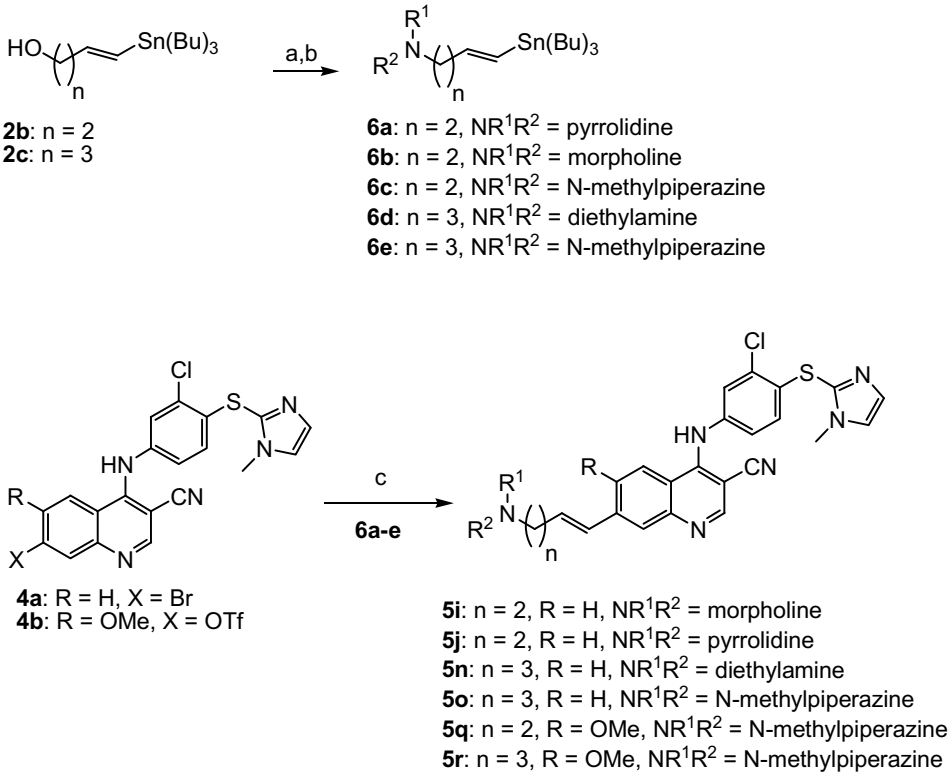
Scheme 1. Preparation of **5a**–**h**, **k**, and **p**. Reagents and conditions: (a) Bu_3SnH, AIBN, 100 °C; (b) separation of isomers by silica gel chromatography (hexane/EtOAc); (c) $Pd(PPh_3)_4$, PPh_3, NMP, 100 °C; (d) $(CH_3CO)_2O$, HOAc, 50 °C; (e) R^1R^2NH, $Pd(PPh_3)_4$, NMP, 25 °C; (f) TosCl, 2,6-lutidine, 45 °C; (g) R^1R^2NH, THF, 60 °C.

>1000 nM activity. The same phenomenon was observed for analog **5n**, which has a water-solubilizing diethylamine group attached at C-7 via a *trans*-pent-1-enyl linker. It is possible that the low aqueous solubility of these analogs may be a contributing factor to these results. The aqueous solubilities of **5n**, **p**, and **r** were measured to be 3, 3, and 2 μg/mL, respectively, at pH 7.4, in contrast to lower molecular weight analogs such as **5d** and **g** (aqueous solubilities 20 and 28 μg/mL, respectively, at pH 7.4).

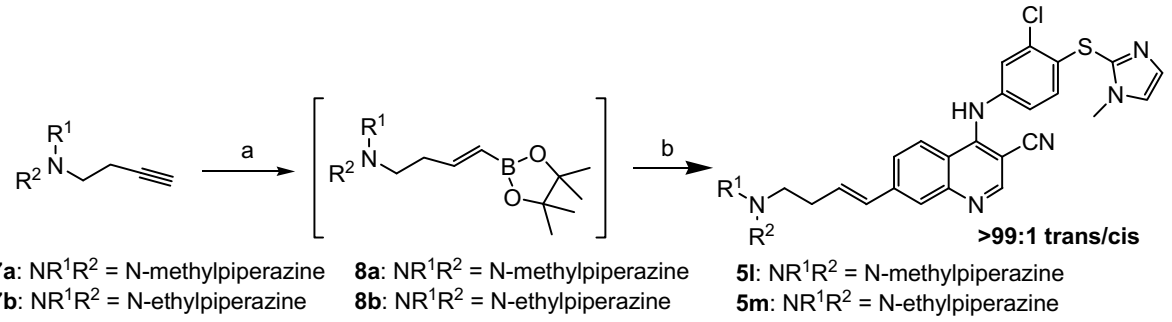
As it had been demonstrated that the majority of the synthesized analogs had similar in vitro potency, our main focus was to find a compound that would have the optimal physical characteristics to provide improved oral bioavailability. Thus, a number of analogs were tested in preliminary exposure studies, for which blood levels were measured at 2 and 4 h following a single oral dose of 50 mg/kg in a nude mouse model. Representative data are shown in Table 2. Overall, the allylic amine substituted analogs such as **5d** and **i** had poor blood levels following a single oral dose of 50 mg/kg. The homoallylic amines **5j**, **l**, and **m** had significantly higher blood levels, with **5m** providing the best exposure at both time points. Despite its poor solubility in aqueous methocel/Tween, C-6 methoxy substituted **5q** had measurable plasma levels following oral dosing, although these were lower than the correspondingly substituted 6-*H* substituted analog, **5l**.

With a potent in vitro activity profile, and good exposure levels following oral administration to nude mice, compound **5m** was chosen for more extensive evaluation. As shown in Table 3, **5m** showed good selectivity versus a number of kinases, but it did have significant activity against IR, EGFR, Src, and Lyn. These results indicate that the biological effects of **5m** on tumor cells may be augmented by the inhibition of kinases in addition to MEK1. Of these results, the potent activity against IR kinase was of most concern, given the role it plays in the modulation of glucose.[20]

The cellular activity profile of compound **5m** was determined against a panel of additional cell lines. In addition to the LoVo and BXPC3 activity as shown in Table 1, **5m** was found to inhibit H358 (lung), WM266-4, A375 (melanoma) cell lines with IC_{50}s of 50, 25, and 26 nM, respectively. In contrast, **5m** was a weak inhibitor of CaCo-2 cells (IC_{50}: 1400 nM). The cellular profile was therefore as anticipated for a MEK1 kinase inhibitor, having significantly more potent activity against K-ras mutant (LoVo, H358) and B-Raf mutant (WM266-4, A375) cell lines than ras wild-type cells (Caco-2). The cellular inhibition of MEK1 by **5m** was measured in the WM266-4 cell line (Fig. 1). With an IC_{50} of 0.225 μM for the inhibition of phospho-ERK, **5m** did inhibit MEK1 in these cells, albeit at concentrations higher than that required to inhibit cellular proliferation. Since **5m** had shown good activity against additional kinases, further studies were performed to determine whether it would inhibit the associated pathways in the WM266-4 cells. Thus, cell lysates have been tested for inhibition of phosphorylation of EGFR, IR, IGFR, Src, SAP, JNK, P38, and overall phospho-tyrosine



Scheme 2. Preparation of **5i**, **j**, **n**, **o**, **q**, and **r**. Reagents and conditions: (a) TosCl, 2,6-lutidine, 25 °C; (b) R^1R^2NH, THF, 25 °C, then 45 °C; (c) $Pd(PPh_3)_4$, PPh_3, NMP, 100 °C.



Scheme 3. Preparation of **5l** and **m**. Reagents and conditions: (a) pinacolborane, Cp_2ZrHCl, 25 °C; (b) **4a**, $Pd(PPh_3)_4$, toluene/EtOH/aq K_2CO_3, reflux.

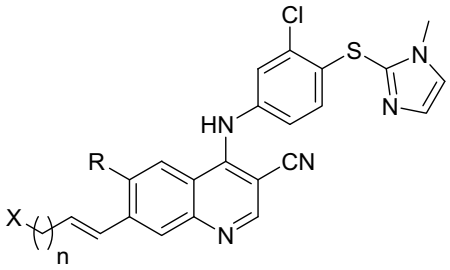
kinase inhibition. These studies showed that **5m** did not inhibit these pathways in WM266-4 cells at up to 10 μM (data not shown), indicating that its activity against these additional kinases was not contributing to the observed cellular antiproliferative activity.

In pharmaceutical profiling assays, **5m** was found to be moderately stable when exposed to male CD-1 mouse and rat microsomes, with half-lives of 16.5 and 21 min, respectively. Permeability, as measured in a PAMPA assay, was 0.45×10^{-6} cm/s. Since compound **5m** had shown the highest blood levels of the analogs evaluated in the exposure studies following oral dosing, a full PK study was carried out to determine its suitability for in vivo studies (Table 4).

Thus, **5m** was dosed iv at 2 mg/kg in a pH 3 aqueous solution (it was necessary to solubilize **5m** at low pH, since the aqueous solubility for this analog was a modest 4 μg/mL at pH 7.4) and at 50 mg/kg orally in female nude mice. Upon oral dosing, the compound achieved a C_{max} of 510 ng/mL, with the T_{max} being achieved at 1 h post-administration. The volume of distribution (V_{ss}) was moderate at 2.7 L/kg, and the half-life favorable at 4.7 h. As a secondary peak was observed with orally dosed **5m**, this suggested possible entero-hepatic recycling. Following an iv dose of **5m**, the half-life was 0.9 h, with moderate/high clearance (66 mL/min/kg). The oral bioavailability was 17%, which was modest, but among the highest observed for any 3-chloro-4-(1-methyl-1*H*-imidazol-2-ylsulfanyl)aniline substituted quinoline-3-carbonitriles. A subsequent rat PK study provided similar results, with iv half-lives of 2.5 and 1.8 h, and somewhat improved volume of distribution of 5.7 and 7.6 L/kg in female and male rats, respectively. The oral bioavailability of **5m** was 23% and 13% in female and male rats, respectively.

Given that **5m** had modest plasma levels following oral dosing, it was administered twice daily (bid) to nude mice implanted with H358 xenografts at 25, 50, and 100 mg/kg orally (Fig. 2). At the highest dose of 100 mg/kg bid, complete suppression of tumor growth was observed. Statistically significant tumor growth inhibition was achieved at the 50 mg/kg bid dose, while the 25 mg/kg dose had a marginal effect on inhibiting tumor growth. On removal of the tumors at 2 h after the 100 mg/kg final dose was given, the resulting lysates showed almost complete inhibition of phospho-ERK (Fig. 3), consistent with the proposed mechanism of action for **5m**.

Table 1
Inhibition in the Raf/MEK1 coupled assay, and cellular activity for compounds **II** and **5a–r**



Compound	R	*n*	X	Raf/MEK IC_{50}[a] (nM)	LoVo IC_{50}[a] (nm)	BXPC3 IC_{50}[a] (nm)
II	—	—	—	3	7	26
5a	H	1	OH	35	21	44
5b	H	2	OH	36	21	ND
5c	OMe	2	OH	5	9	25
5d	H	1	Pyrrolidine	59	25	40
5e	H	1	*N*-Methylpiperazine	55	41	40
5f	H	1	*N*-Ethylpiperazine	52	44	42
5g	H	2	Diethylamine	26	25	73
5h	H	2	Dimethylamine	21	33	32
5i	H	2	Morpholine	11	27	39
5j	H	2	Pyrrolidine	20	31	51
5k	H	2	Piperidine	10	18	43
5l	H	2	*N*-Methylpiperazine	19	14[b]	48[b]
5m	H	2	*N*-Ethylpiperazine	12	34	48
5n	H	3	Diethylamine	25	37	>1000
5o	H	3	*N*-Methylpiperazine	33	20	41
5p	OMe	2	Diethylamine	3	8	>1000[c]
5q	OMe	2	*N*-Methylpiperazine	4	7	>1000[c]
5r	OMe	3	*N*-Methylpiperazine	6	8	>1000[c]

[a] IC_{50} values reported represent the means of at least two separate determinations.
[b] IC_{50} values are from a single determination.
[c] Compounds highly insoluble, <50% inhibition was observed over a broad concentration range for this assay.

Table 2
Plasma concentration of selected compounds following an oral dose of 50 mg/kg in nude mice (average of three mice/measurement)

Compound	Plasma concentration (2 h) ng/mL	Plasma concentration (4 h) ng/mL	% CV[a] (2 h, 4 h)
5d	72[b]	11	43[b], 69
5i	95	22	55, 41
5j	319	82	20, 31
5l	368	85	33, 18
5q	267	76	33, 17
5m	494	132	53, 48

[a] Coefficient of variation (CV) = standard deviation/mean × 100.
[b] Plasma concentration of **5d** was measured at 1 h post-dosing.

Table 3
Kinase selectivity profile of **5m**

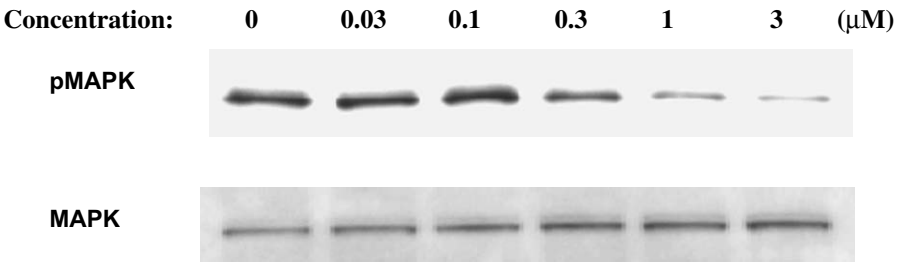
Kinase (ATP concentration)	IC_{50} (μM)
MEK1 (100 μM)	0.012
EGFR (10 μM)	0.010
IR (100 μM)	0.032
Src (100 μM)	0.055
Lyn (20 μM)	0.058
IGFR (100 μM)	0.220
KDR (1 μM)	1.0
PDK1 (100 μM)	3.10
B-raf (100 μM)	8.80
IKK beta (2 μM)	9.17
P70S6 (2 μM)	20
PKC theta (6 μM)	27.4
PI3K alpha (25 μM)	>10
AKT (20 μM)	>30
mTOR (100 μM)	>30
Tpl2 (50 μM)	>40
MK2 (1 μM)	>100

4. Summary and conclusion

A series of C-7 alkenylquinoline-3-carbonitriles was evaluated as MEK1 kinase inhibitors. For the initial synthetic pathway for this series, our plan was to introduce the diverse amine water-solubilizing groups in the last step. However, alternative pathways were pursued due to the insolubility of several intermediates, as well as significant quantities of the unwanted *cis*-isomer being present in the product mixtures. Compounds of interest were subsequently efficiently synthesized by utilizing vinylboronic acids and Suzuki coupling chemistry under appropriate conditions to provide essentially only the desired C-7 *trans-alkenyl* products.

A number of compounds were found to be potent MEK1 kinase inhibitors in vitro, as was true for the previously explored series of quinoline-3-carbonitriles possessing the C-4 [3-chloro-4-(1-methyl-1*H*-imidazol-2-ylsulfanyl)]aniline substituent. However, in contrast to the earlier series of analogs, these C-7 alkenyl substituted compounds showed significant plasma levels following oral dosing. Of these, **5m** was a clear lead candidate due to its good in vitro potency profile, and highest plasma exposure levels observed for the tested compounds.

Further biological evaluation of **5m** revealed that it had additional activity against kinases other than MEK1. Western blot analyses carried out with **5m** have so far revealed evidence of phospho-ERK suppression, but no evidence of inhibition of other pathways that we have evaluated. Despite this observation, it is certainly possible that additional kinase activities are contributing to the potent cellular antiproliferative activity of **5m**. Further studies are underway to determine the activity of **5m** against an expanded set of kinases, and the biological relevance of any additional kinase activities will be evaluated. At this time, we have established **5m** as a lead compound MEK1 inhibitor with oral activity against H358 xenografts.



Dose (μM)	% Inhibition
0	0
0.03	22
0.1	18
0.3	69
1	88
3	95

Figure 1. Inhibition of p-MAPK in WM266-4 cells treated with varying concentrations of **5m**. Cells were incubated with compound for 2.5 h. Cell lysates were prepared and analyzed as described in Section 5. Inhibition of p-MAPK was determined by densitometric measurement of autoradiograms, followed by normalizing these values by calculating a p-MAPK/MAPK ratio. Ratios of treated samples were divided by the ratio determined for the control to derive the percent inhibition at that dose. By plotting these values, the IC_{50} of p-MAPK inhibition was calculated be 0.225 μM.

Table 4
Pharmacokinetic properties of **5m** in nude mouse and rat

Species	Female nude mouse	Female rat	Male rat
Route	Intravenous		
Dose (mg/kg)	2	1	1
Cl (mL/min/kg)	66.0	35.7	70.3
V_{ss} (L/kg)	2.7	5.7	7.6
$T_{1/2}$ (h)	0.9	2.5	1.8
Route	Oral		
Dose (mg/kg)	50	10	10
C_{max} (ng/mL)	510	298	61
AUCINF (ng h/mL)	2170	988	204
Oral bioavailability (% F)	17	23	13

Note. Cl, clearance; V_{ss}, steady state volume of distribution; $T_{1/2}$, half-life of compound in plasma; C_{max}, maximum observed plasma concentration; AUCINF, area under the concentration curve extrapolated to infinity.

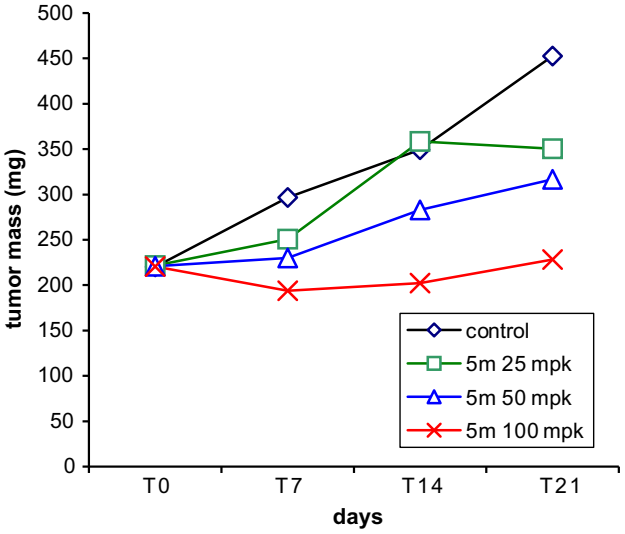


Figure 2. Dose–response of **5m** dosed orally twice daily (bid) versus H358 (lung) xenografts implanted in nude mice. Each group ($n = 15$) was dosed twice daily with compound in vehicle or vehicle alone (0.05% methocel/2% Tween). Tumor size at all time points in treatment arms of 50 mg and 100 mg/kg were statistically different than control tumors ($p \leqslant 0.05$, Student's *t*-test).

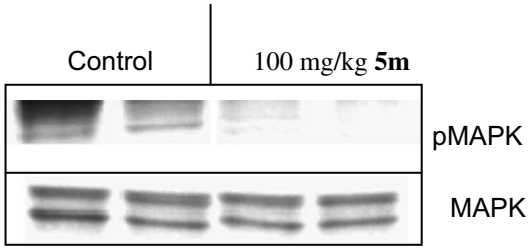


Figure 3. p-MAPK suppression in H358 tumors following 21 days oral dosing with **5m** at 100 mg/kg twice daily. Tumors excised from euthanized animals were stored at −80 °C until assayed. Tumor lysates were prepared and analyzed as outlined in Section 5.

5. Experimental

5.1. General methods

Melting points were determined in open capillary tubes on a Meltemp melting point apparatus and are uncorrected. ^{1}H NMR spectra were determined at 400 MHz on a DRX-400 spectrometer, with chemical shifts (δ) reported in parts per million referenced to Me_4Si. Electrospray (ES) mass spectra were recorded in positive mode on a Micromass Platform or an LCT spectrometer. Electron Impact (EI) mass spectra were obtained on a Finnigan MAT-90 spectrometer. All reagents and solvents obtained from commercial suppliers were used without further purification. Reactions were carried out under nitrogen as an inert atmosphere. Flash chromatography was carried out with Baker 40 μM silica gel.

5.2. Specific procedures

5.2.1. (*E*)-1-(tri-*n*-Butylstannyl)-1-buten-4-ol (2b)

A stirred mixture of 3-butyn-1-ol (3.50 g, 50 mmol) and AIBN (0.25 g, 1.5 mmol), contained in large round-bottomed flask equipped with a reflux condenser, was outgassed with N_2 at 25 °C and treated with tri-*n*-butyltin hydride (20.2 mL, 75 mmol). The mixture was slowly heated to 90 °C and the resulting vigorous exothermic reaction was moderated by removal of the heating bath and reflux of the butynol. After the initial reaction, the

solution was stirred at 100 °C for 18 h. The solution was cooled and the crude product was subjected to chromatography on silica gel with a 3–20% gradient of EtOAc in hexane. After elution of a small amount of *Z*-isomer, the *E*-isomer was obtained as a colorless oil (10.3 g, 57%). The NMR spectrum was identical to that reported.[14]

5.2.2. 4-({3-Chloro-4-[(1-methyl-1*H*-imidazol-2-yl)thio]phenyl}-amino)-7-[(1*E*)-3-hydroxyprop-1-enyl]quinoline-3-carbonitrile (5a)

To 10 mL of N_2-purged NMP was added Ph_3P (280 mg, 1.08 mmol) and $Pd(OAc)_2$ (61 mg, 0.27 mmol). The mixture was warmed to 50 °C and stirred for 1 h. The resulting red solution of palladium catalyst[21] was cooled to 0 °C and treated successively with **4a** (1.26 g, 2.67 mmol) and a solution of stannane **2a**[13] (1.40 g, 4.0 mmol) in 3.4 mL of NMP. The mixture was warmed to 100 °C during 20 min, stirred for 1 h, cooled to 25 °C, and stirred with H_2O and 2:1 hexane/Et_2O. The resulting tan solid was filtered off, washed with H_2O and 2:1 hexane/Et_2O, and dried to give 1.18 g (98% yield) of **5a**. A portion of this material was digested in hot acetone to provide a light tan solid, mp 220–240 °C (dec.); MS (ESI) *m*/*z* 448.0 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 9.87 (s, 1H), 8.62 (s, 1H), 7.87 (s, 1H), 7.84 (s, 1H), 7.55 (s, 1H), 7.46 (d, *J* = 2.5 Hz, 1H), 7.19 (d, *J* = 2.5 Hz), 7.17 (s, 2H), 6.77 (m, 1H), 6.54 (d, *J* = 8.7 Hz, 1H), 5.02 (t, *J* = 5.5 Hz, 1H), 4.20 (m, 2H), 3.61 (s, 3H). Elemental Anal. Calcd for $C_{23}H_{18}ClN_5OS{\cdot}0.8H_2O$: C, 59.75; H, 4.27; N, 15.15. Found: C, 60.01; H, 4.06; N, 14.05.[22]

5.2.3. 4-({3-Chloro-4-[(1-methyl-1*H*-imidazol-2-yl)thio]phenyl}-amino)-7-[(1*E*)-4-hydroxybut-1-enyl]quinoline-3-carbonitrile (5b)

Compound **5b** was prepared by the procedure used for the preparation of compound **5a** using stannane **2b**. Flash chromatography (silica gel, 10:1 CH_2Cl_2/MeOH) gave a 77% yield of **5b** as an amber solid, mp 205–210 °C; MS (ESI) *m*/*z* 462.2 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 9.86 (s, 1H), 8.60 (s, 1H), 8.31 (s, 1H), 7.82 (s, 1H), 7.80 (s, 1H), 7.55 (d, *J* = 1.3 Hz, 1H), 7.44 (s, 1H), 7.17 (d, *J* = 1.3 Hz, 1H), 6.66 (m, 1H), 6.53 (d, *J* = 8.5 Hz, 1H), 4.66 (t, *J* = 5.3 Hz, 1H), 3.61 (s, 3H), 3.58 (m, 2H), 2.42 (m, 2H). Elemental Anal. Calcd for $C_{24}H_{20}ClN_5OS{\cdot}0.9CH_3OH$: C, 60.93; H, 4.85; N, 14.27. Found: C, 61.09; H, 4.46; N, 14.05.

5.2.4. 4-({3-Chloro-4-[(1-methyl-1*H*-imidazol-2-yl)thio]phenyl}-amino)-7-[(1*E*)-4-hydroxybut-1-enyl]-6-methoxyquinoline-3-carbonitrile (5c)

Compound **5c** was prepared from triflate **4b** and stannane **2b** by the procedure used for the preparation of **5b**. Digestion of the crude product with 10:1 CH_2Cl_2/MeOH gave a 35% yield of **5c** as a white solid, mp 273–278 °C; MS (ESI) *m*/*z* 492.0 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 9.67 (s, 1H), 8.50 (s, 1H), 7.98 (s, 1H), 7.70 (s, 1H), 7.55 (d, *J* = 1.2 Hz, 1H), 7.44 (m, 1H), 7.16 (d, *J* = 1.2 Hz, 1H), 6.79 (d, *J* = 16.2 Hz, 1H), 6.62 (m, 2H), 6.56 (d, *J* = 8.6 Hz, 1H), 4.65 (t, *J* = 5.3 Hz), 1H), 3.94 (s, 3H), 3.60 (s, 3H), 3.94 (s, 3H), 3.56 (m, 2H), 2.42 (m, 2H). Elemental Anal. Calcd for $C_{25}H_{22}ClN_5O_2S{\cdot}0.5CH_3OH$: C, 60.29; H, 4.76; N, 13.79. Found: C, 60.55; H, 4.44; N, 13.84.

5.2.5. 4-({3-Chloro-4-[(1-methyl-1*H*-imidazol-2-yl)thio]phenyl}-amino)-7-[(1*E*)-3-pyrrolidin-1-ylprop-1-enyl]quinoline-3-carbonitrile (5d)

Step 1: A solution of **5a** (1.43 g, 3.2 mmol) and acetic anhydride (24 mL, 254 mmol) in 24 mL of HOAc was stirred at 50 °C for 19 h and concentrated to dryness. The residue was stirred in dilute $NaHCO_3$ and 1:1 hexane/Et_2O. The resulting solid was filtered, washed with water and 1:1 hexane/Et_2O, and dried to give 1.64 g (100%) of (2*E*)-3-[4-({3-chloro-4-[(1-methyl-1*H*-imidazol-2-yl)thio]phenyl}amino)-3-cyanoquinolin-7-yl]prop-2-enyl acetate as an amber solid, sufficiently pure for the next step. A portion was subjected to flash chromatography (silica gel, CH_2Cl_2/EtOAc/HOAc) to give (2*E*)-3-[4-({3-chloro-4-[(1-methyl-1*H*-imidazol-2-yl)thio]phenyl}amino)-3-cyanoquinolin-7-yl]prop-2-enyl acetate as a light yellow solid, mp 181–193 °C (dec.) MS (ESI) *m*/*z* 490.1 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 9.89 (s, 1H), 8.62 (s, 1H), 8.35 (d, *J* = 9.3 Hz, 1H), 7.89 (br s, 2H), 7.55 (s, 1H), 7.46 (s, 1H), 7.18 (m, 1H), 7.17 (s, 1H), 6.89 (d, *J* = 6.9 Hz, 1H), 6.70 (m, 1H), 6.53 (d, *J* = 6.9 Hz, 1H), 4.78 (d, *J* = 5.3 Hz, 2H), 3.61 (s, 3H), 2.10 (s, 3H).

Step 2: A stirred mixture of the crude acetate (2*E*)-3-[4-({3-chloro-4-[(1-methyl-1*H*-imidazol-2-yl)thio]phenyl}amino)-3-cyanoquinolin-7-yl]prop-2-enyl acetate (196 mg, 0.40 mmol), pyrrolidine (133 μL, 1.6 mmol), and 0.80 mL of NMP was treated with $Pd(Ph_3P)_4$ (46 mg, 0.04 mmol) under N_2 at 25 °C. After 1 h, the mixture was stirred with 4:1 hexane/EtOAc and dilute $NaHCO_3$. The crude product was filtered off, washed with water and 4:1 hexane/EtOAc, and dried. Purification by flash chromatography (silica gel, 25:25:2:1 CH_2Cl_2/EtOAc/MeOH/TEA) gave **5d** as 106 mg (53%) of a yellow solid, mp 182–190 °C; MS (ESI) *m*/*z* 501.1 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) d 9.86 (s, 1H), 8.60 (s, 1H), 8.32 (d, *J* = 9.1 Hz, 1H), 7.88 (s, 1H), 7.86 (d, *J* = 9.1 Hz, 1H), 7.52 (d, *J* = 1.2 Hz, 1H), 7.44 (s, 1H), 7.16 (d, *J* = 1.2 Hz, 1H), 7.14 (m, 1H), 6.73 (m, 2H), 6.54 (d, *J* = 8.5 Hz, 1H), 3.61 (s, 3H), 3.37 (m, 2H), 2.61 (m, 2H), 1.74 (m, 4H). Elemental Anal. Calcd for $C_{27}H_{25}ClN_6S{\cdot}0.3H_2O$: C, 64.03; H, 5.09; N, 16.59. Found: C, 64.06; H, 5.02; N, 16.54.

5.2.6. 4-({3-Chloro-4-[(1-methyl-1*H*-imidazol-2-yl)thio]phenyl}-amino)-7-[(1*E*)-3-(4-methylpiperazin-1-yl)prop-1-enyl]quinoline-3-carbonitrile (5e)

Following the procedure described for **5d**, allylic alcohol **5a** was acetylated and reacted with *N*-methylpiperazine to provide compound **5e** as a yellow solid in 27% yield, mp 209–212 (dec.); MS (ESI) *m*/*z* 530.1 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 9.89 (s, 1H), 8.60 (s, 1H), 7.54 (s, 1H), 7.44 (s, 1H), 7.16 (br s, 2H), 6.76 (d, 1H, *J* = 8.0 Hz), 6.58–6.67 (br m, 3H), 6.55 (d, 1H, *J* = 10.0 Hz), 3.61 (s, 3H), 3.15 (d, *J* = 6.7 Hz, 2H), 2.50 (s, 3H), 2.44 (br s, 4H), 2.34 (br s, 4H), 2.17 (s, 3H). Elemental Anal. Calcd for $C_{28}H_{28}ClN_7S{\cdot}0.45CH_3OH$: C, 62.76; H, 5.35; N, 18.00. Found: C, 62.61; H, 5.57; N, 17.64.

5.2.7. 4-({3-Chloro-4-[(1-methyl-1*H*-imidazol-2-yl)thio]phenyl}-amino)-7-[(1*E*)-3-(4-ethylpiperazin-1-yl)prop-1-enyl]quinoline-3-carbonitrile (5f)

Following the procedure described for **5d**, allylic alcohol **5a** was acetylated and reacted with *N*-ethylpiperazine to provide compound **5f** as a yellow solid in 36% yield, mp 224–225; MS (ESI) *m*/*z* 544.1 $[M+H]^+$; ^1H NMR spectrum (400 MHz, DMSO-d_6) δ 9.89 (s, 1H), 8.60 (s, 1H), 7.54 (s, 1H), 7.44 (s, 1H), 7.16 (br s, 2H), 6.76 (d, 1H, *J* = 8.0 Hz), 6.58–6.67 (br m, 3 H), 6.55 (d, 1H, *J* = 10.0 Hz), 3.61 (s, 3H), 3.15 (d, *J* = 6.7 Hz, 2H), 2.50 (br s, 2H), 2.44 (br s, 4H), 2.34 (d, *J* = 6.0 Hz, 4H), 0.99 (t, 3H). Elemental Anal. Calcd for $C_{29}H_{30}ClN_7S{\cdot}\ 0.75H_2O$: C, 62.46; H, 5.69; N, 17.58. Found: C, 62.14; H, 5.62; N, 17.40.

5.2.8. 4-({3-Chloro-4-[(1-methyl-1*H*-imidazol-2-yl)thio]phenyl}-amino)-7-[(1*E*)-4-(diethylamino)but-1-enyl]quinoline-3-carbonitrile (5g)

Step 1: A mixture of **5b** (0.77 g, 1.67 mmol), tosyl chloride (1.59 g, 8.35 mmol), and 16.7 mL of 2,6-lutidine was stirred at 45 °C for 18 h and stirred at 25 °C with water (0.75 mL; 42 mmol) for 1 h. The resulting suspension was partitioned with CH_2Cl_2 and dilute $NaHCO_3$. The organic layer was washed with water, dried, and concentrated. The residue was stirred in 10:1 Et_2O/acetone. The tan solid was filtered off, washed with Et_2O, and dried at 25 °C to give 0.84 g, mp 135–150 °C (dec.). The crude (2*E*)-3-[4-({3-chloro-4-[(1-methyl-1*H*-imidazol-2-yl)thio]phenyl}amino)-3-cyanoquinolin-7-yl]but-3-enyl 4-methylphenylsulfonate was used without further purification.

Step 2: To a stirred suspension of crude (2*E*)-3-[4-({3-chloro-4-[(1-methyl-1*H*-imidazol-2-yl)thio]phenyl}amino)-3-cyanoquinolin-7-yl]but-3-enyl 4-methylphenylsulfonate (185 mg, 0.30 mmol) in 5.0 mL of THF was added diethylamine (2.5 mL, 24 mmol). The resulting solution was stirred at 60 °C for 20 h. The mixture was concentrated to remove THF, and the residue was partitioned between 20:1 CH_2Cl_2/MeOH and dilute $NaHCO_3$. The organic layer was washed with water, dried, and concentrated. Flash chromatography (silica gel, EtOAc/MeOH/TEA) gave a 30% yield of **5g** as an off-white solid, mp 230–235 °C; MS (ESI) *m*/*z* 517.1 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 9.85 s, 1H, 8.59 s, 1H), 8.30 (d, *J* = 8.8 Hz, 1H), 7.81 (s, 1H), 7.78 (d, *J* = 8.8 Hz, 1H), 7.54 (d, *J* = 1.1 Hz, 1H), 7.43 (s, 1H), 7.16 (d, *J* = 1.1 Hz, 1H), 7.15 (m, 1H), 6.67 (m, 2H), 6.54 (d, *J* = 8.6 Hz, 1H), 3.61 (s, 3H), 2.56 (m, 2H), 2.37 (q, *J* = 6.8 Hz, 4H), 0.98 (t, *J* = 6.8 Hz, 6H). Elemental Anal. Calcd for $C_{28}H_{29}ClN_6S$: C, 65.04; H, 5.65; N, 16.25. Found: C, 64.74; H, 5.42; N, 16.06.

5.2.9. 4-({3-Chloro-4-[(1-methyl-1*H*-imidazol-2-yl)thio]phenyl}-amino)-7-[(1*E*)-4-(dimethylamino)but-1-enyl]quinoline-3-carbonitrile (5h)

Following the procedure described for **5g**, allylic alcohol **5b** was tosylated and reacted with dimethylamine to provide compound **5h** as an off-white solid in 32% yield, mp 191–198 °C; MS (ESI) *m*/*z* 489.1 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 9.87 s, 1H), 8.59 (s, 1H), 8.30 (d, *J* = 9.3 Hz, 1H), 7.81 (s, 1H), 7.80 (d, *J* = 9.3 Hz, 1H), 7.55 (d, *J* = 1.1 Hz, 1H), 7.44 (s, 1H), 7.17 (d, *J* = 1.1 Hz, 1H), 7.16 (m, 1H), 6.66 (m, 2H), 6.53 (d, *J* = 8.4 Hz, 1H), 3.61 (s, 3H), 2.40 (m, 4H), 2.18 (s, 6H). Elemental Anal. Calcd for $C_{26}H_{25}ClN_6S{\cdot}0.25CH_3CO_2C_2H_5$: C, 63.45; H, 5.32; N, 16.44. Found: C, 63.68; H, 5.09; N, 16.28.

5.2.10. Preparation of 6a–e. Representative procedure: 1-methyl-4-[(3*E*)-4-(tri-*n*-butylstannyl)but-3-enyl]piperazine (6c)

Step 1: To a stirred solution of (*E*)-1-(tri-*n*-butylstannyl)-1-buten-4-ol (5.42 g, 15 mmol) in 30 mL of 2,6-lutidine was added tosyl chloride (8.58 g, 45 mmol) while maintaining at 25 °C. After 20 h, the excess tosyl chloride was decomposed by the addition of 30 mL of water and 5 mL of pyridine while cooling to maintain at 25 °C. The resulting mixture was portioned with CH_2Cl_2 and dilute $NaHCO_3$. The organic layer was washed with water and dried over $MgSO_4$. Following concentration at <35 °C, finally at 0.5 mmHg, crude (3*E*)-4-(tri-*n*-butylstannyl)but-3-enyl 4-methylbenzenesulfonate was obtained as an oil.

Step 2: A solution of the crude (3*E*)-4-(tri-*n*-butylstannyl)but-3-enyl 4-methylbenzenesulfonate (1.55 g, 3.0 mmol), 1-methylpiperazine (1.33 mL, 12 mmol), and 3.0 mL of THF was stirred for 24 h at 25 °C and subsequently heated to 45 °C for 2 h. The solution was concentrated to dryness under vacuum, and the residue was partitioned with 1:1 hexane/Et_2O and dilute $NaHCO_3$. The organic layer was washed with water, dried, and concentrated at <30 °C to give **6c** as an amber oil which was used in the next step without purification.

5.2.11. 4-({3-Chloro-4-[(1-methyl-1*H*-imidazol-2-yl)thio]phenyl} amino)-7-[(1*E*)-4-morpholin-4-ylbut-1-enyl]quinoline-3-carbonitrile (5i)

To a N_2-purged mixture of **4a** (188 mg, 0.40 mmol), **6b** (0.24 g, 0.56 mmol), and 2.0 mL of NMP was added $Pd(Ph_3P)_4$ (46 mg, 0.04 mmol). The mixture was warmed to 100 °C, and the resulting solution was stirred for 1 h, cooled, and partitioned with CH_2Cl_2 and water. The organic layer was dried and concentrated to give a residue that was stirred in 10:1 Et_2O/hexane. The resulting solid was filtered off and subjected to flash chromatography (silica gel, 5:1 EtOAc/MeOH) to give 134 mg (63%) of **5i** as a light yellow solid, mp 232–238 °C; MS (ESI) *m*/*z* 531.1 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 9.86 (s, 1H), 8.60 (s, 1H), 8.31 (d, *J* = 8.6 Hz, 1H), 7.81 (s, 1H), 7.80 (d, *J* = 8.6 Hz, 1H), 7.55 (d, *J* = 1.1 Hz, 1H), 7.44 (s, 1H), 7.17 (d, *J* = 1.1 z, 1H), 7.16 (m, 1H), 6.66 (m, 2H), 6.53 (d, *J* = 8.6 Hz, 1H), 3.61 (s, 3H), 3.58 (m, 4H), 2.43 (m, 8H). Elemental Anal. Calcd for $C_{28}H_{27}ClN_6OS$: C, 63.33; H, 5.12; N, 15.82. Found: C, 63.13; H, 4.89; N, 15.47.

5.2.12. 4-({3-Chloro-4-[(1-methyl-1*H*-imidazol-2-yl)thio]phenyl}-amino)-7-[(1*E*)-4-pyrrolidin-1-ylbut-1-enyl]quinoline-3-carbonitrile (5j)

Following the procedure described for **5i**, **6a** was reacted with **4a** to provide compound **5j** as an off-white solid in 40% yield, mp 185–191 °C; MS (ESI) *m*/*z* 515.1 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 9.87 (s, 1H), 8.31 (d, *J* = 8.6 Hz, 1H), 7.81 (d, *J* = 8.6 Hz, 1H), 7.55 (d, *J* = 1.1 Hz, 1H), 7.44 (s, 1H), 7.17 (d, *J* = 1.1 Hz, 1H), 7.16 (m, 1H), 6.66 (m, 2H), 6.53 (d, *J* = 8.6 Hz, 1H), 3.61 (s, 3H), 2.55 (m, 4H), 2.45 (m, 4H), 1.71 (m, 4H). Elemental Anal. Calcd for $C_{28}H_{27}ClN_6S{\cdot}0.25H_2O$: C, 64.73; H, 5.33; N, 16.17. Found: C, 64.67; H, 5.14; N, 16.07.

5.2.13. 4-({3-Chloro-4-[(1-methyl-1*H*-imidazol-2-yl)thio]phenyl}-amino)-7-[(1*E*)-4-piperidin-1-ylbut-1-enyl]quinoline-3-carbonitrile (5k)

Following the procedure described for **5g**, allylic alcohol **5b** was tosylated and reacted with piperidine to provide compound **5k** as an off-white solid in 48% yield, mp 205–210 °C; MS (ESI) *m*/*z* 529.1 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 9.86 (s, 1H), 8.60 (s, 1H), 8.30 (d, *J* = 9.1 Hz, 1H), 7.81 (s, 1H), 7.80 (d, *J* = 9.1 Hz, 1H), 7.55 d, *J* = 1.1 Hz, 1H), 7.44 (s, 1H), 7.17 (d, *J* = 1.1 Hz, 1H), 7.16 (m, 1H), 6.66 (m, 2H), 6.53 (d, *J* = 8.6 Hz, 1H), 3.61 (s, 3H), 2.43 (m, 2H), 2.38 (m, 4H), 1.50 (m, 4H), 1.38 (m, 4H). Elemental Anal. Calcd for $C_{29}H_{29}ClN_6S$: C, 65.83; H, 5.52; N, 15.88. Found: C, 65.59; H, 5.13; N, 15.48.

5.2.14. 4-({3-Chloro-4-[(1-methyl-1*H*-imidazol-2-yl)thio]phenyl}-amino)-7-[(1*E*)-4-(4-methylpiperazin-1-yl)but-1-enyl]quinoline-3-carbonitrile (5l)

To a mixture of **7a** (1.52 g, 10.0 mmol) and 4,4,5,5-tetramethyl-[1,3,2]dioxaborolane (2.98 g, 17.2 mmol) was added bis(cyclopentadienyl)-zirconium chloride hydride (0.13 g, 0.05 mmol). The resulting mixture was stirred at room temperature for 24 h to provide (*E*)-1-methyl-4-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)but-3-enyl)piperazine (**8a**), which was subsequently dissolved in toluene/ethanol/water (100 mL/10 mL/10 mL). Compound **4a** (2.36 g, 5.0 mmol), K_2CO_3 (2.1 g, 15.2 mmol), and $Pd(PPh_3)_4$ (0.46 g, 0.4 mmol) were added. The reaction mixture was heated at 85 °C for 5 h and allowed to cool to room temperature. To this mixture was added 100 mL of a saturated $NaHCO_3$ solution. The resulting solid was collected by filtration, washed with water, EtOAc, and dried under vacuum. The crude solid was purified by silica gel flash column chromatography (gradient 95:5–4:1 CH_2Cl_2/MeOH) to give 1.70 g (63%) of **5l** as an off-white solid, mp 142–143 °C; MS (ESl) *m*/*z* 544.2 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 9.86 (s, 1H), 8.59 (s, 1H), 8.30 (d, *J* = 8.7 Hz, 1H), 7.80 (s, 1H), 7.79 (d, *J* = 8.7 Hz, 1H), 7.54 (d, *J* = 1.1 Hz, 1H), 7.44 (s, 1H), 7.16 (d, *J* = 1.1 Hz, 1H), 7.15 (m, 1H), 6.64 (m, 2H), 6.54 (d, *J* = 8.6 Hz, 1H), 3.61 (s, 3H), 2.37 (m, 12H), 2.15 (s, 3H). HPLC analysis (CH_3CN/H_2O/TFA reverse phase) showed the *trans*/*cis* ratio to be 98.5:0.5. Elemental Anal. Calcd for $C_{29}H_{30}ClN_7S{\cdot}0.2H_2O$: C, 63.59; H, 5.59; N, 17.90. Found: C, 63.46; H, 5.57; N, 17.84.

5.2.15. 4-({3-Chloro-4-[(1-methyl-1*H*-imidazol-2-yl)thio]phenyl}-amino)-7-[(1*E*)-4-(4-ethylpiperazin-1-yl)but-1-enyl]quinoline-3-carbonitrile (5m)

Following the procedure described for **5l**, **7b** was converted **8b**, and reacted with **4a** to provide compound **5m** as a yellow solid in 73% yield, mp 194–204 °C; MS (ESI) *m*/*z* 558.1 $[M+H]^+$; ^1H NMR

(400 MHz, DMSO-d_6) δ 9.84 (s, 1H), 8.60 (s, 1H), 8.30 (d, J = 8.8 Hz, 1H), 7.79 (m, 2H), 7.54 (s, 1H), 7.43 (s, 1H), 7.16 (s, 2H), 6.65 (m, 2H), 6.54 (d, J = 8.8 Hz, 1H), 3.61 (s, 3H), 2.46–2.29 (m, 14H), 0.98 (t, J = 6.8 and 7.2 Hz, 3H). Elemental Anal. Calcd for $C_{30}H_{32}ClN_7S{\cdot}0.3H_2O$: C, 63.94; H, 5.83; N, 17.40. Found: C, 64.01; H, 5.67; N, 17.14.

5.2.16. 4-({3-Chloro-4-[(1-methyl-1*H*-imidazol-2-yl)thio]phenyl}-amino)-7-[(1*E*)-5-(diethylamino)pent-1-enyl]quinoline-3-carbonitrile (5n)

Following the procedure described for **5i**, **6d** was reacted with **4a** to provide compound **5n** as an off-white solid in 49% yield, mp 220–225 °C; MS (ESI) m/z 531.1 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.86 (s, 1H), 8.59 (s, 1H), 8.30 (d, J = 8.8 Hz, 1H), 7.81 (s, 1H), 7.80 (d, J = 8.8 Hz, 1H), 7.54 (d, J = 1.1 Hz, 1H), 7.42 (d, J = 1.8 Hz, 1H), 7.16 (d, J = 1.1 Hz, 1H), 7.14 (d, J = 1.8 Hz, 1H), 6.66 (m, 2H), 6.54 (d, J = 8.6 Hz, 1H), 3.61 (s, 3H), 2.47 (q, J = 7.3 Hz, 4H), 2.43 (m, 2H), 2.26 (m, 2H), 1.59 (m, 2H), 0.95 (t, J = 7.3 Hz, 6H). Elemental Anal. Calcd for $C_{29}H_{31}ClN_6S$: C, 65.58; H, 5.88; N, 15.82. Found: C, 65.59; H, 5.91; N, 15.79.

5.2.17. 4-({3-Chloro-4-[(1-methyl-1*H*-imidazol-2-yl)thio]phenyl}-amino)-7-[(1*E*)-5-(4-methylpiperazin-1-yl)pent-1-enyl]quinoline-3-carbonitrile (5o)

Following the procedure described for **5i**, **6e** was reacted with **4a** to provide compound **5o** as an off-white solid in 64% yield, mp 219–227 (dec.) °C; MS (ESI) m/z 558.1 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.85 (s, 1H), 8.58 (s, 1H), 8.30 (d, J = 8.8 Hz, 1H), 7.81 (s, 1H), 7.80 (d, J = 8.8 Hz, 1H), 7.54 (d, J = 1.1 Hz, 1H), 7.42 (m, 1H), 7.16 (d, J = 1.1 Hz, 1H), 7.14 (m, 1H), 6.65 (m, 2H), 6.54 (d, J = 8.8 Hz, 1H), 3.61 (s, 3H), 2.51 (m, 6H), 2.31 (m, 6H), 2.14 (s, 3H), 1.62 (m, 2H). Elemental Anal. Calcd for $C_{30}H_{32}ClN_7S$: C, 64.56; H, 5.78; N, 17.57. Found: C, 64.27; H, 5.67; N, 17.54.

5.2.18. 4-({3-Chloro-4-[(1-methyl-1*H*-imidazol-2-yl)thio]phenyl}-amino)-7-[(1*E*)-4-(diethylamino)but-1-enyl]-6-methoxyquinoline-3-carbonitrile (5p)

Following the procedure described for **5g**, allylic alcohol **5c** was tosylated and reacted with diethylamine to provide compound **5p** as a yellow solid in 54% yield, mp 194–202 °C; MS (ESI) m/z 547.2 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.70 (s, 1H), 8.50 (s, 1H), 7.96 (s, 1H), 7.71 (s, 1H), 7.54 (d, J = 1.2 Hz, 1H), 7.44 (s, 1H), 7.16 (d, J = 1.2 Hz, 1H), 7.15 (m, 1H), 6.79 (d, J = 15.9 Hz, 1H), 6.61 (dt, J = 15.9 and 7.0 Hz, 1H), 6.56 (d, J = 8.8 Hz), 1H), 5.30 (s, 1H), 3.94 (s, 3H), 3.60 (s, 3H), 3.36 (q, J = 7.0 Hz, 4H), 2.57 (m 3H), 0.98 (t, J = 7.0 Hz, 6H). Elemental Anal. Calcd for $C_{29}H_{31}ClN_6OS{\cdot}1.25H_2O$: C, 61.15; H, 5.93; N, 14.75. Found: C, 61.44; H, 6.02; N, 14.41.

5.2.19.4-({3-Chloro-4-[(1-methyl-1*H*-imidazol-2-yl)thio]phenyl}-amino)-6-methoxy-7-[(1*E*)-4-(4-methylpiperazin-1-yl)but-1-enyl]quinoline-3-carbonitrile (5q)

Following the procedure described for **5i**, **6c** was reacted with **4b** to provide compound **5q** as an off-white solid in 50% yield, mp 223–230 (dec.) °C; MS (ESI) m/z 574.2 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.67 (s, 1H), 8.50 (s, 1H), 7.96 (s, 1H), 7.70 (s, 1H), 7.54 (d, J = 1.2 Hz, 1H), 7.44 (s, 1H), 7.17 (m, 1H), 7.16 (d, J = 1.2 Hz, 1H), 6.79 (d, J = 15.4 Hz, 1H), 6.59 (dt, J = 15.4 and 6.0 Hz, 1H), 6.56 (d, J = 8.9 Hz, 1H), 3.94 (s, 3H), 3.60 (s, 3H), 2.38 (m, 12H), 2.16 (s, 3H). Elemental Anal. Calcd for $C_{30}H_{32}ClN_7OS$: C, 62.76; H, 5.62; N, 17.08. Found: C, 62.44; H, 5.54; N, 16.97.

5.2.20.4-({3-Chloro-4-[(1-methyl-1*H*-imidazol-2-yl)thio]phenyl}-amino)-6-methoxy-7-[(1*E*)-5-(4-methylpiperazin-1-yl)pent-1-enyl]quinoline-3-carbonitrile (5r)

Following the procedure described for **5i**, **6e** was reacted with **4b** to provide compound **5r** as a yellow solid in 50% yield, mp 215–221 (dec.) °C; MS (ESI) m/z 588.1 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.66 (s, 1H), 8.50 (s, 1H), 7.98 (s, 1H), 7.69 (s, 1H), 7.54 (d, J = 1.2 Hz, 1H), 7.44 (s, 1H), 7.17 (m, 1H), 7.16 (d, J = 1.2 Hz, 1H), 6.75 (d, J = 15.9 Hz, 1H), 6.63 (dt, J = 15.9 and 6.7 Hz, 1H), 6.57 (d, J = 8.6 Hz, 1H), 3.94 (s, 3H), 3.60 (s, 3H), 2.29 (m, 12H), 2.16 (s, 3H), 1.62 (m, 2H). Elemental Anal. Calcd for $C_{31}H_{34}ClN_7OS{\cdot}0.5CH_3OH$: C, 62.62; H, 6.01; N, 16.23. Found: C, 62.64; H, 5.71; N, 16.14.

5.3. Biological evaluation

5.3.1. Enzyme studies

MEK1 enzyme activities were determined by the previously described high throughput, non-radioactive Raf/MEK1/MAPK ELISA assay.[18] Additional in-house protein kinase assays were performed by immunologically based ELISA or DELFIA protocols using the appropriate phospho-specific antibodies.

5.3.2. Cellular studies

Cellular proliferation assays[19] (Sulfo-rhodamine B staining) were carried out using LoVo, CaCo-2 (colon), BXPC3 (pancreatic) and WM266-4 and A375 (melanoma) human tumor cell lines.

5.3.3. Phosphorylation studies

WM266-4 human melanoma cells were grown in DMEM with 10% FBS. For phosphoblot analysis, cells were grown in 6-well plates and when semi-confluent, exposed to compound for 2.5 h. Cells were solubilized at 4 °C in cell lysis buffer (150 mM NaCl, 10 mM Tris, pH 7.4, 1% (w/v) Triton X-100, 0.5% (w/v) sodium deoxycholate, 0.1% (w/v) SDS with protease inhibitors: 1 mM EDTA, 7.5 TIU/mL aprotinin, 10 μg/mL pepstatin A, 30 μM leupeptin and a 1:100 dilution of phosphatase inhibitor cocktails I (P-2850) and II (P-5726) (Sigma Chemical Co., St. Louis, MO)). Lysate was clarified by centrifugation at 17,000g for 10 min. Total lysate protein was determined by the BCA protein assay (Pierce Biochemical). Equal amounts of protein lysate (30 μg) were separated on SDS–PAGE gels (4–20% gradient) and transferred to nitrocellulose. After blocking non-specific binding sites on the nitrocellulose blot with 3% (w/v) BSA in TBS-T (Tris-buffered saline with 0.1% Tween 20), blots were incubated with anti-MAPK (diluted 1:1000) or anti-phosphoMAPK (diluted 1:2000) (Sigma Chemical Co., St. Louis, MO) for 1.5 h followed by washing in TBS-T. The blots were then incubated with appropriate secondary antibody conjugated to horse radish peroxidase (Sigma Chemical Co, St. Louis, MO) The blots were again washed with TBS-T followed by exposure to ECL and exposure to autoradiographic film.

5.3.4. In vivo studies

All animal studies were performed in accordance with internal IACUC standards. Human non-small cells lung cancer cells (American Type Culture Collection, Rockville, Maryland # CRL-5807) were grown in RPMI (Gibco/BRL, Gaithersburg, MD) supplemented with 10% FBS (Gemini Bio-Products Inc., Calabasas, CA). Athymic nu/nu female mice (Charles River, Wilmington, MA) were injected SC in the flank area with 10×10^6 cells plus Matrigel (BD Biosciences, Billerica, MA). When tumors attained a mass of between 200 and 250 mg, the mice were randomized into treatment groups (day zero), 10 animals per group. Animals were administered compound orally BID on days 1 through 20 post-staging (day zero) with either 25, 50, or 100 mg/kg/dose of compound prepared in 0.5% methocel/2% Tween 80 as the vehicle control. Tumor mass was determined every 7 days [(length $\times$ width^2)/2] for 21 days post-staging. Relative tumor growth (mean tumor mass on days 7, 14, and 21 divided by the mean tumor mass on day zero) was determined for each treatment group. Statistical analysis of the vehicle control tumor group versus the treated groups at each time point

were determined by using the Student *t*-test. A *p*-value ($p \leqslant 0.05$) indicates a statistically significant reduction in relative tumor growth of treated group compared to the vehicle control.

Phosphoblotting studies on tumor samples: after animals were sacrificed, tumor samples were harvested and tumor lysates prepared as described in Section 5.3.3.

5.3.5. Exposure studies and pharmacokinetics

Compounds for oral (po) administration were made as a suspension in 0.5% methylcellulose and 1% Tween 80. For iv administration, the compounds were dissolved in 0.5% methylcellulose and 1% Tween 80, with the pH lowered to 3 by aqueous HCl. Mice were manually randomized and treated with a single dose of 50 mg/kg by po gavage. After drug dosing, three mice per treatment group were sacrificed at 0.25, 0.5, 1, 4, 8, and 24 h by cardiac stick exsanguinations under isoflurane anesthesia, and plasma samples were collected. Analysis of plasma was carried out using liquid chromatography/mass spectrometry analysis. In brief, 50-μL plasma samples were extracted with 0.2 mL acetonitrile. The organic layer was collected following extraction, evaporated to dryness, and reconstituted in 0.05 mL of acetonitrile/water (1:1, v/v). Samples were analyzed with an API-3000 triple quadrupole mass spectrometer (PE Sciex, Foster City, CA). Analysis of data for the calculation of pharmacokinetic parameters was carried out using noncompartmental analysis with WinNonlin v. 4.1 (Pharsight Corporation, Mountain View, CA).

Acknowledgments

The authors acknowledge the Wyeth Analytical Chemistry Department for providing spectral data and elemental analyses, and the Chemical Technology group for the pharmaceutical profiling data. Additionally, we thank Dr. Tarek Mansour for his support.

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