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Bioorganic & Medicinal Chemistry Letters 16 (2006) 5561–5566
Bioorganic & Medicinal Chemistry Letters

Identification of isothiazole-4-carboxamidines derivatives as a novel class of allosteric MEK1 inhibitors

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Received 2 June 2006; revised 4 August 2006; accepted 7 August 2006
Available online 24 August 2006

Abstract—The development of potent, orally bioavailable, and selective series of 5-amino-3-hydroxy-*N*(1-hydroxypropane-2-yl)isothiazole-4-carboxamidine inhibitors of MEK1 and MEK-2 kinase is described. Optimization of the carboxamidine and the phenoxyaniline group led to the identification of **55** which gave good potency as in vitro MEK1 inhibitors, and good oral exposure in rat.

There are three mitogen-activated protein (MAP) kinase pathways that control a variety of cellular regulation. The c-jun kinase pathway (JNK) is important in the regulation of many transcription factors particularly in response to cellular stress. The p-38 pathway plays a critical role in the activation of inflammatory responses. The third and, by far, the most important and well-understood signal transduction pathway is the Ras/Raf/MEK/ERK pathway that controls fundamental cellular processes such as proliferation, differentiation, survival, and apoptosis.[1,2] The MAPK pathway represents a cascade of phosphorylation events including three pivotal kinases: Raf, MEK (MAP kinase/ERK kinase), and ERK (extracellular signal-regulated kinase). The Ras–Raf–MEK–ERK pathway is activated by a range of growth factor receptors. When Raf is activated, it phosphorylates MEK at two serine residues. The activated MEK, in turn, phosphorylates the threonine and tyrosine residues of MAPK[3] or ERK. ERK regulates downstream signaling complexes of transcription factors that affect gene expression.[4] Constitutive activation of the Ras–Raf–MEK–ERK pathway has been demonstrated in several cancer types, including pancreatic, colon, lung, and melanoma. Inhibition of this pathway via MEK1/2 is an attractive strategy for therapeutic intervention in cancer because it has the potential to block inappropriate signal transduction regardless of the upstream position of the oncogenic aberration. Furthermore, ERK1/2 are the only known substrates for MEK1/2.[5–13] These kinases present new opportunities for the development of novel anti-cancer drugs designed to be target-specific and probably less toxic than conventional chemotherapeutic agents. A number of drugs inhibiting Ras, Raf or MEK are currently under clinical investigation.[14,15] Several highly specific MEK1/2 inhibitors such as PD98059, U0126, and CI-1040 have been identified in recent years. These compounds inhibit the activation of MEK1/2 non-competitively with respect to ATP. The crystal structure of human MEK-1/2 complexed with an analog of PD184352 demonstrated that these biaryl amine-based compounds are bound to an allosteric site adjacent to ATP binding pocket.[14,16,17] Previously, we reported the initial results of SAR study of the cyanoisothiazole scaffold as a MEK-1/2 inhibitor.[18] We disclosed the discovery of the potent and selective MEK-1 and MEK-2 inhibitor (**1**), which exhibits an IC_{50} value of 38 nM and EC_{50} value of 375 nM. The optimization of **1** led to a series of potent MEK-1/2 kinase inhibitors, and culminated in the idenfication of the compound 5-(4-(2,5-dichlorophenoxy)phenylamino)-3-

Keywords: MEK inhibitor; MEK-1; MEK-2; Isothiazole carboxamidine; Allosteric inhibitors.
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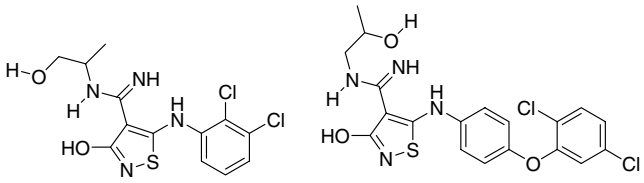
0960-894X/$ - see front matter
doi:10.1016/j.bmcl.2006.08.048

hydroxy-*N*-(2- hydroxypropyl)isothiazole-4-carboximidamide (**55**), that showed excellent oral exposure in the rat. Several derivatives were synthesized in order to optimize their drug-like properties, that is, their biological, physicochemical, and pharmacokinetics profile (PK). This paper describes the structure-guided design and structure–activity relationships (SAR) of this series.



1 **55**

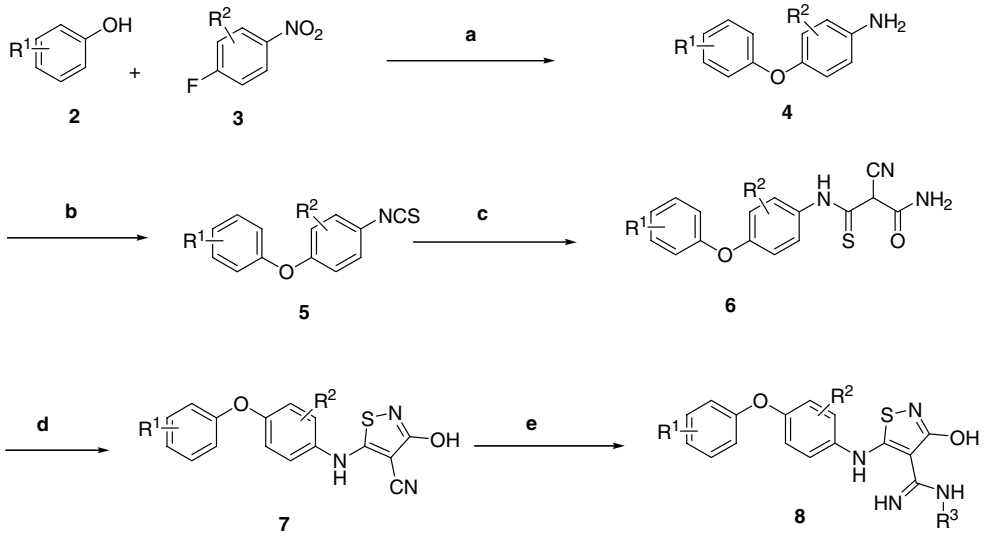
A series of compounds were generated to investigate the SAR of the 5-aminoaryl-3-hydroxy-*N*-alkylisothiazole-4-carboximidamide scaffold. Their inhibitory activities against both MEK1 and MEK2 were evaluated and IC_{50} values were determined using an enzymatic assay as described elsewhere.[18,19] In most cases, the IC_{50} values for MEK1 and MEK2 are similar. For simplicity, we only report the IC_{50} values against MEK1 here. A straightforward five-step synthetic approach is detailed in Scheme 1. Displacement of the fluorine atom in 4-fluoronitrobenzenes **3** by a variety of phenols **2** provided a series of 4-phenoxynitrobenzene intermediates that were reduced by Fe in the presence of ammonium chloride to afford anilines **4**. Isothiazoles **7** were prepared from the corresponding isothiocyanates **5**, which were either commercially available or freshly prepared from the corresponding anilines **4**. The isothiocyanates **5** were reacted with cyanoacetamide in the presence of powdered KOH in DMF to provide the corresponding 2-cyano-3-(4-phenoxyphenylamino)-3-thioxopropanamides **6**, which were cyclized in the presence of bromine to give the corresponding isothiazoles **7**. The carboxamidines **8** were prepared by refluxing **7** in the presence of excess of amines.[18]

Optimization of **8** led to a number of potent, orally active MEK-1/2 kinase inhibitors. The SAR was focused on changes at three positions: substitutions at either ring of the phenoxyaniline (R^1 and R^2) and substitutions at the carboxamidine (R^3).

With regard to SAR of **8** we first examined the optimization of the linker connecting rings B and C in the para position of phenylamino isothiazoe carboxamidine series. We explored a variety of the linkers as we have highlighted in Table 1. Except for the *gem*-dimethyl-linked **15**, which showed a reduction in both MEK enzymatic activity and cell proliferation, activity was retained for a variety of linkers.[19] Interestingly, this series showed poor to modest rat PK properties, characterized by high clearance and low oral bioavailability (see Table 1).

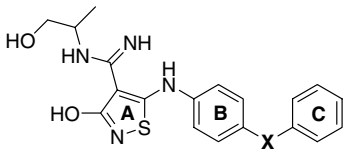
The selectivity profiles of MEK-1 inhibitors **9** and **10** were examined. They were assessed against a panel of 85 protein kinases.[19] The data confirmed the generally good selectivity profile of **9** for MEK-1. The excellent activity of the compound **10** against CHK-2 was somewhat a surprise (see Table 2). This excellent selectivity may be a result of non-competitive binding nature of isothiazole cabroxamidine scaffold.[19]

To circumvent the low oral bioavailability of the isothiazole cabroxamidine series, HPLC/MS/MS analysis of **9** incubated with rat liver microsomes indicated more extensive oxidation of the ring C over ring B. Indeed, any metabolism in the molecule was suppressed by halogen substitutions in the rings B and C, and improved the activities as well as rat PK properties (see Table 3).



Scheme 1. General synthesis of 3-hydroxy-5-(4-phenylamino)isothiazole-4-carboxyamidines. Reagents and conditions: (a) K_2CO_3, DMF, reflux, 16 h; Fe, MeOH, NH_4Cl, reflux, 16 h; (b) $CSCl_2$, aq K_2CO_3, $CHCl_3$, rt, 2 h; (c) cyanoacetamide, KOH, DMF, rt, 16 h; (d) Br_2, EtOAc, rt, 1–2 h; (e) R^3-NH_2, EtOH, reflux, 16 h.

Table 1. The linker between the rings B and C: MEK-1 activity and oral-IV AUC of 3-hydroxy-*N*-(2-hydroxy-1-methyl-ethyl)-5-phenylamino-isothiazole-4-carboxamidine



Compound	X	IC_{50} (nM)	EC_{50} (nM)	IV AUC (ng h/ml)	Oral AUC (ng h/ml)
9	O	33	42	8321	1135
10	NH	240	45	859	1236
11	CH_2	58	130	7254	220
12	C(O)	120	100	2897	60
13	S	45	650	6905	265
14	SO_2	150	278	8540	4251
15	$C(CH_3)_2$	7900	1500	9250	2350

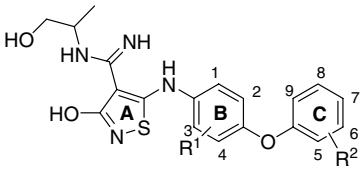
Table 2. Selectivity profile of **9** and **10** (IC_{50}, nM)

Compound	MEK1	Aurora	Axl	CHK2	Flt3	P70S6
9	26	260	455	142	795	2620
10	266	265	389	13	448	1000

Several analogs of **9** containing halogen substituents on the ring C were investigated (see Table 3). Initial evaluation of mono-substitution in the para position of the ring C with chlorine, bromine, methylene, trifluoromethyl or any group larger than the methyl group revealed no improvement in potency, but demonstrated large gains in oral PK. In contrast, any combination of di-halogen substitutions in the ring C retains potency, and improves rat PK properties. However, the substitution by different halogens in ring B did not contribute to either the potency or rat PK properties. When substitution of both phenyl rings B and C was simultaneously attempted, the PK profile improved considerably, but there was no improvement in potency. In this series, the compounds **16**, **17**, **18**, **20**, **27**, **38**, and **40** exhibited good oral AUC exposure in rat and retained most of the activities. The best compound in this series is the compound **25** that showed an IC_{50} value of 30 nM, EC_{50} value of 130 nM, and good oral AUC exposure in rat of 47,400 ng h/ml. Importantly, enzymatic profiling of the compound **25** shows that it binds also non-competitively with respect to ATP.[19]

Additionally, we turned our attention to optimization of the alkyl substituent at the carboxamidine moiety. Using (2,5-dichlorophenoxyamino)-3-hydroxyisothiazole as the starting scaffold, we evaluated the influence of carboxamidine substituents containing a water-soluble moiety. Most of the compounds in this series displayed double digit nanomolar IC_{50} values in the MEK-1 enzymatic assay and double digit nanomolar EC_{50} values against EGF-stimulated pERK MDA-MB-231 cancer cells.[19] However, there was strong variation in the oral PK values with only compounds **51**, **52**, and **55** exhibiting good oral AUC exposure in rat and retaining most of the activities (see Table 4).

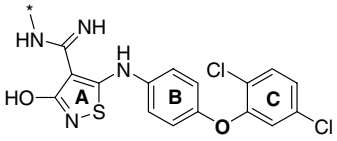
Table 3. MEK-1 activity and oral AUC of 3-hydroxy-*N*-(2-hydroxy-1-methyl-ethyl)-5-(4-phenoxy-phenylamino)-isothiazole-4-carboxamidine

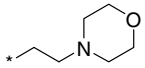
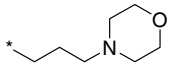
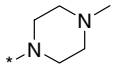
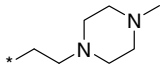
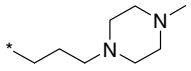
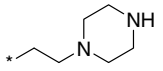
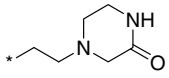
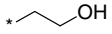
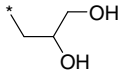
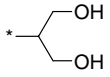
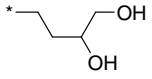
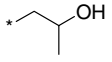
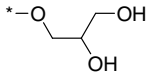
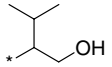
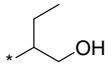
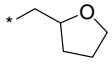


Compound	Ring-C R^2	Ring-B R^1	IC_{50} (nM)	EC_{50} (nM)	Oral AUC (ng h/ml)
16	6-CF_3	H	44	140	28,654
17	6-CF_3–5-F	H	58	123	13,003
18	6-CF_3–5-Cl	H	120	95	20,395
19	5-Cl–8-CF_3	H	540	350	21,867
20	6-F–8-CF_3	H	140	30	15,391
21	6-NMe_2	H	160	130	900
22	5-Cl–6-CF_3	2-Cl	67	70	5711
23	5-Cl–6-CF_3	2-F	52	88	4500
24	5-F–8-CF_3	H	60	330	31,270
25	5-Cl–8-Cl	H	30	130	47,400
26	5-Cl–8-F	H	80	260	900
27	5-Cl–8-Cl	2-F	26	220	22,226
28	5-Cl–8-F	2-Me	280	350	nd
29	5-Cl–8-F	2-Cl	30	>10	nd
30	5-Cl–6-Cl	H	75	100	5260
31	5-F–6-F	H	20	42	9427
32	5-Cl–6-Cl	2-Cl	27	>10	4275
33	5-F–6-Cl	H	145	200	12,567
34	6-Cl–8-Cl	H	20	1450	11,700
35	6-F–8-F	H	207	128	5135
36	5-Cl–7F	H	85	250	6256
37	5-Cl–7F	2Cl	107	37	5687
38	5-Cl–7-Cl	H	64	140	26,221
39	5-F–7-F	H	207	150	16,500
40	5Cl–6F–8F	H	37	78	20,330
41	5Cl–6F–8F	2F	27	332	14,925
42	5-Cl–7-F	1-Me	57	250	12,696

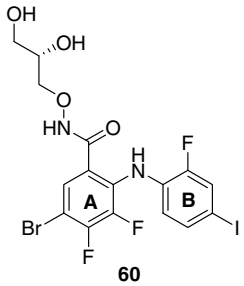
The disclosure of the first MEK X-ray crystallographic structure with (*S*)-5-bromo-*N*-(2,3-dihydroxypropoxy)-3,4-difluoro-2-(2-fluoro-4-iodophenylamino)benzamide (**60**)[14] (PDB code: 1S9J) revealed an allosteric binding

Table 4. MEK-1 activity and oral AUC of 5-[4-(2,5-dichloro-phenoxy)-phenylamino]-3-hydroxy-isothiazole-4-carboxamidine



Compound	X	IC_{50} (nM)	EC_{50} (2 M)	Oral AUC (ng h/ml)
43		32	47	2876
44		40	35	617
45		147	284	6862
46		34	113	1979
47		137	118	262
48		298	605	2422
49		58	43	nd
50		44	49	87
51		46	47	14,557
52		46	54	12,468
53		35	120	5105
54		34	22	2020
55		28	75	23,908
56		56	444	nd
57		744	564	22,370
58		40	694	14,568
59		34	65	425

pocket adjacent to the ATP-binding site, providing a possible platform for the design of ATP non-competitive, selective inhibitors.



60

On the assumption that the isothiazoles bind in the same pocket as hydroxamate **60**, an initial working hypothesis for the equivalency of the hydroxamate and the isothiazole series was envisaged. Thus, ring A of **60** was considered the isosteric equivalent of the isothiazole ring and ring B of **60** that of the attached aminoaryl moiety. In the crystallographic structure, the B ring of **60** is found ensconced in a largely hydrophobic environment, with a face-to-edge interaction with Phe209 of the DFG motif. Hydrogen bonds form a critical feature of the interaction of **60** with the protein. One of the F atoms in the A ring forms a hydrogen bond with the backbone NH of Ser212, the hydroxamate oxygens form a hydrogen-bonding network with Lys97 and a structural water, while the diol oxygens interact with Lys97 and one of the terminal phosphate oxygens.

The docked structure of **61**, derived using GOLD v. 3.0 software, shows the isothiazole ring to be somewhat offset compared to ring A of **60** (Fig. 1). In this orientation, the nitrogen atom in the isothiazole captures the important hydrogen-bonding interaction with Ser212. This mode of overlay disposes the amidinoalkyl sidechain in a completely different trajectory compared to the alkylhydroxamate sidechain, obviating any need to explain the overlap of non-isoelectronic groups, that is, the amidine and the hydroxamate groups. However, no evidence of significant interactions for the amidino group was discovered in this orientation that would compensate for the hydrogen-bond network associated with the hydroxamate sidechain of **60**. This overlay suggests that **61** may share the same allosteric binding pocket as **60** with an overlap of certain pharmacophoric features only.

Availability of SAR data for a series of 2-anilinobenzoates,[14] a precursor series to the hydroxamates represented by **60**, provided an opportunity to test the credibility of our working hypothesis. In the 2-anilinobenzoate series, activity was observed after introducing an iodine atom in the para position to the nitrogen in ring B. In a slightly modified series of 2-(4-substituted anilino)-5-nitrobenzoates, the iodo substitution was associated with a 15-fold better enzymatic activity than the bromo substitution. Phenyl, alkyl, alkoxy and thioalkyl substitutions at that position rendered the compounds inactive. In addition, placing a chloro or methyl

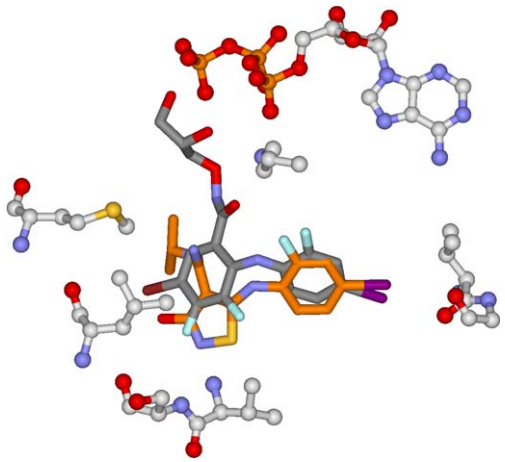


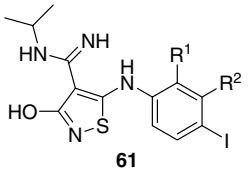
Figure 1. Overlay of the docked model of **61** (gold carbons) with **60** (gray carbons).

substitution meta to the iodo group enhanced activity 10-fold. Encouragingly, very similar SAR patterns were observed for the corresponding isothiazole series (see Table 5). The iodo analog **61** showed submicromolar activity, while over 10-fold increase in activity was observed when the R^1 position was occupied by either a methyl **62** or a chloro **63** group. Moving the chlorine in **61** to the R^2 position **64** resulted in a 5-fold abrogation of activity.

When the iodo group of **61** is replaced with an aryloxy moiety, the docking model fails to provide an equivalent binding pose. It could be speculated that either the aryloxy group is accommodated through induced fit, or that these compounds are binding at an alternate binding site. The poor EC_{50} values for **61**–**64** were difficult to explain, but may have been caused by differences in the cellular uptake of this series of the compounds compared to the aryloxy analogs.

In order to obtain more concrete evidence, X-ray crystallographic experiments were attempted on several isothiazoles. A combination of instability and insolubility of the isothiazoles in the crystallization buffer conditions prevented further work in this area. These problems may also help explain the poor cellular activity obtained for these compounds. Nevertheless, this computer

Table 5. MEK-1 activity of 3-hydroxy-5-(4-iodophenylamino)-*N*-isopropylisothiazole-4-carboximidamide



61

Compound	R^1	R^2	IC_{50} (nM)	EC_{50} (nM)
61	H	H	510	8321
62	Me	H	62	1480
63	Cl	H	42	>10,000
64	H	Cl	200	6190

modeling and docking study suggests that these isothiazole compounds could bind to the same allosteric pocket that a PD184352 analog binds and act as an allosteric inhibitor for MEK1 and MEK2. The ideas obtained from this study did, however, provide an excellent platform for the design of alternate, non-labile scaffolds to be discussed in future publications.

In conclusion, we have identified a series of 3-hydroxy-4-carboxyalkylamidino-5-arylamino isothiazole carboxamidines as a novel class of allosteric MEK-1/2 inhibitors. The present systematic SAR studies of 5-phenylamino-4-cyano-3-hydroxy-isothiazole led to improvement in both enzymatic and cellular assays, and showed promising oral bioavailability.

Acknowledgments

The authors thank colleagues from the Drug Development Department & PK Group of Dr. Chin-Chung Lin, the Toxicology Group of Dr. Domenico Vitarella, the Analytical Group of Dr. Jingfan Huang, and the HTS Group of Dr. Paul Diaz.

Supplementary data

Experimental details on biological data. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.08.048.

References and notes

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19. For IC_{50} assay, EC_{50} assay, kinase profiling, and kinetics, please see Supplementary data.