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Discovery of 3-hydroxy-4-carboxyalkylamidino-5-arylamino-isothiazoles as potent MEK1 inhibitors

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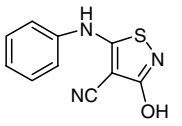
Abstract—3-Hydroxy-4-carboxyalkylamidino-5-arylamino-isothiazoles were discovered as potent in vitro MEK1 inhibitors.

Chemotherapy of cancer by traditional cytotoxic agents has limited efficacy and lacks specificity. This has led to the development of therapies aimed at aberrant molecular pathways of tumor growth in the hope of greater efficacy and fewer side effects. Recent studies validated protein kinases as key regulators of all pathways of cancer and many kinase inhibitors are now being developed. Imatinib,[1] a tyrosine kinase inhibitor, is the first molecular targeted agent to be approved in US and other countries for the treatment of chronic myelogenous leukemia (CML). Other drugs, recently approved, for the treatment of cancer based on target approach include, Gefitinib,[2a] Erlotinib,[2b] Trastuzumab,[2c] Cetuximab,[2d] and Bevacizumab.[2e] While drugs such as Sorafenib, Sutent, Zactima, and AG-013736, in clinical development for different kinds of cancers, rely on intervention of multiple target pathways.[3] The aforementioned drugs have proven that therapies targeting specific enzymes in signal transduction pathways are successful.

Mitogen activated protein kinase (MEK[4]), a dual specificity kinase, has central role in extracellular signal regulated kinase (ERK) pathway in regulating cell growth and survival, differentiation, and angiogenesis. MEK phosphorylates threonine and tyrosine (T183 and Y185) residues of its substrates ERK 1 and 2 (MAP kinase) resulting in their activation.[5] MEK and ERK are frequently dysregulated in human cancers.[6]

Further it was found overexpression and activation of these enzymes were associated with various human cancers including kidney, breast, colon, and oral carcinomas, leukemia, and glial neoplasmas.[7] MEK1/2 are the only enzymes known to activate ERK1/2 and it is believed their inhibition might be useful in controlling the growth of cancer cells. At present, several pharmaceutical companies are pursuing intervention of this pathway in the treatment of various cancers. PD-0325901 and ARRY-142886, the lead compounds in clinical development, represent such examples wherein MEK[8] is inhibited to suppress the tumor growth.

During a high-throughput screening of Valeant library of compounds several 4-cyano-3-hydroxy-5-arylamino-isothiazoles, such as **1** (IC_{50}: 7 μM), were found as lead in vitro inhibitors of MEK.[1] Isothiazole[9] scaffold is present in a wide range of compounds possessing a wide range of activities. Fused isothiazole moiety has been present in drugs such as antipsychotics Geodon[10] and Perispirone[11] (Sumitomo Chemical, Japan). Further, many isothiazole derivatives show antiviral,[12] histone acetyltransferase inhibitor,[13] cholinergic channel activa-



1, IC_{50}: 7 μM

Figure 1.

Keywords: Isothiazoles; 3-Hydroxy-isothiazoles; Cancer; MEK1; MAPK; Protein kinases.
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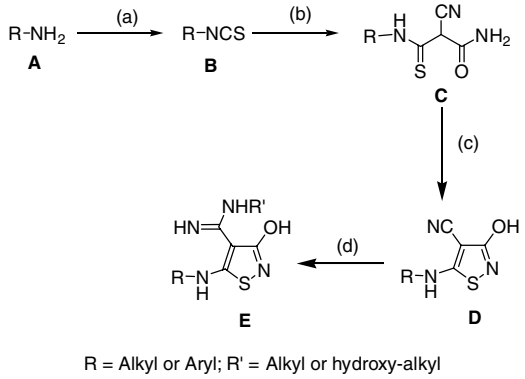
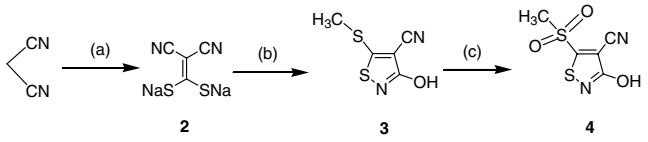
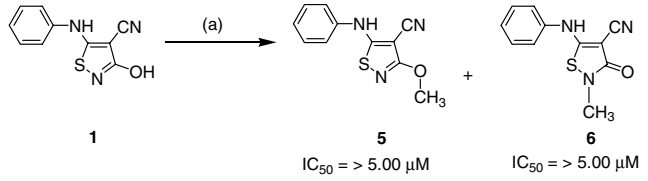
tor,[14] and herbicide and fungicide[15] activities. In light of few studies involving isothiazoles as kinase inhibitors,[16] we initiated SAR to find novel and potent MEK inhibitors. In this communication, we present preliminary results of this study.



Scheme 1. Reagents and conditions: (a) $CSCl_2$, aq K_2CO_3, $CHCl_3$, rt, 2 h; (b) cyanoacetamide, KOH, DMF, rt, 16 h; (c) Br_2, EtOAc, rt, 1–2 h; (d) $R'NH_2$, EtOH, 90 °C, 16 h.



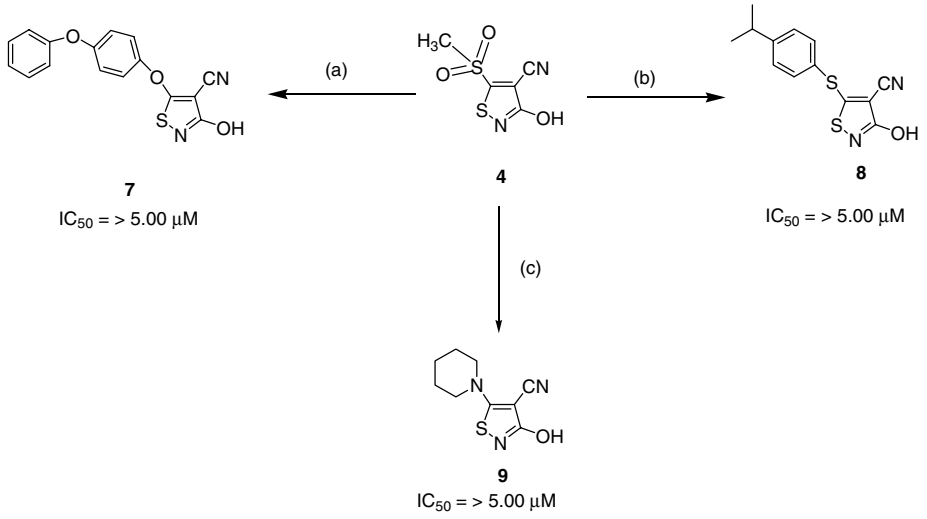
Scheme 2. Reagents: (a) CS_2, NaOH, EtOH; (b) i—H_2O_2, MeOH; ii—MeI, MeOH; (c) H_2O_2, Ac_2O, AcOH.



Scheme 3. Reagents and conditions: (a) NaH, MeI, DMF, rt, 4 h.

The SAR studies of lead isothiazole **1** (Fig. 1) to improve the potency involved modifications at four positions, viz. 3-hydroxy group, linker between aryl group and isothiazole moiety, 5-arylamino, and 4-cyano groups. The required 3-hydroxy-4-nitrile-5-arylamino-isothiazoles[17] (**D**) have been synthesized from the corresponding isothiocyanates (**B**) which were either commercially available or readily prepared from the respective amines (**A**) (Scheme 1). Isothiocyanates (**B**) were treated with cyanoacetamide in presence of powdered KOH in DMF to give substituted cyanoacetamides (**C**) which were cyclised by Br_2 to give the target 5-arylamino-isothiazole derivatives (**D**). However, some 5-alkyl or cycloakylamino-isothiazoles, prepared in the course of SAR studies, were obtained by the reaction of corresponding amines with sulfone **4** (Scheme 2) which was synthesized from malononitrile following the literature procedure.[18] Thus, smalononitrile was treated with CS_2 in presence of NaOH to obtain di-sodium salt **2** which was subjected to oxidative cyclization followed by its methylation leading to sulfide **3**. The sulfide **3** was then oxidized to desired sulfone **4**.

With regard to SAR of **1** we first examined the relevance of 3-hydroxy group. Accordingly, 3-methoxy-4-cyano-5-phenylamino isothiazole **5** and 4-cyano-5-phenylamino-*N*-methyl-3-isothiazolone **6** were synthesized from 5-phenylamino-4-cyano-3-hydroxy-isothiazole **1** (Scheme 3). Both modifications (**5** and **6**) resulted in reducing the activity.[19] Further replacement of hydroxyl with an halogen or a proton also resulted in the loss of activity (data not shown). These results indicate that a free hydroxyl at position 3 is essential for activity. The preferred tautomer of hydroxyl group is expected to be determined by X-ray crystallographic studies of MEK1 enzyme in presence of inhibitor.



Scheme 4. Reagents and conditions: (a) 30% aq NaOH, PhOPhOH, 80 °C, 16 h; (b) 30% aq NaOH, *p*-^{i}Pr–PhSH, 80 °C, 16 h; (c) piperidine, EtOH, 80 °C, 16 h.

These results would be discussed in future communications.

Next, we focused our attention on the linker of isothiazole core to aryl group at position 5. To this extent, we prepared the corresponding phenoxy **7** and phenyl sulfide **8** derivatives as shown in Scheme 4 from sulfone **4**. These modifications resulted in further loss of activity.

The intermediates sulfide **3** and sulfone **4** used in syntheses of the above modifications were also inactive. Additionally, 5-cycloalkylamino-isothiazole **9**, representing N,N-substituted amino group at 5-position, also showed loss of activity. The loss of activity from all of the above modifications indicated that a hydrogen bond donor, such as an amino group (NH), is also essential to retain the activity apart from 3-hydroxyl group (vide supra).

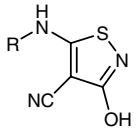
In order to explore the suitable substitution on amino group at 5 position of isothiazole **1**, a variety of alkyl and arylamino groups (Table 1) were examined. In general, alkyl groups (**10**–**13**) resulted in increased IC_{50} values indicating loss of activity. Notably, aliphatic rings (**14**) or aromatic groups (**15**–**18**) attached to 5-amino-isothiazole core by a spacer (1–2 carbons) also lost activity. This result suggests that inclusion of a spacer (1–2 carbons) between 5-alkyl or aromatic-amino group and isothiazole core is not tenable. Heterocycles on 5-amino group (**19**) did not improve the potency either.

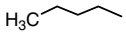
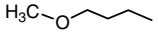
After establishing the essential requirements, viz. presence of 3-hydroxy group, a free NH group at position 5 and exclusion of a spacer between aryl and amino groups at this position; to retain the activity, we prepared several substituted 5-arylamino derivatives (Table 1). A variety of groups were examined to improve potency further involving substitutions such as methyl (**20**, *o*, *p*, or *m* –Me), electron donating (**21**: *o*, *p*, or *m* –OCH3) and withdrawing groups (**22**: *o*, *p*, or *m* $-NO_2$, **23**–**25**) on the aryl group. None of the single modifications had improved activity except for halogen (**26**–**27**) derivatives. However, introduction of two halogen substitutions (**28**–**31**) on phenyl ring has substantially improved (28-fold over lead **1**) activity as in the case of **31**.

Meanwhile 4-nitrile group on isothiazole was not affected in any of the above modifications thus far. Hence, we wanted to explore whether its modifications would further improve the activity. We prepared the corresponding amide, hydrazide, hydroxyamidine, tetrazole, and amidine (**32**–**36**, Table 2) derivatives following standard literature procedures involving nitriles. Amongst these modifications only *N*-alkylcarboxyamidines (**E**, Scheme 1; **36**, Table 2) had retained the potency gained previously (**26**–**31**, Table 1).

This prompted us to find whether the activity of carboxyalkylamidines can be improved further by modifying 5-phenylamino group. Hence, a variety of cyclohexylm-

Table 1. SAR of 5-alkyl/arylamino-4-cyano-3-hydroxy-isothiazole



Compound	R	IC_{50} (μM)
10		>5.00
11		>5.00
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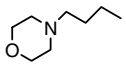
13		>5.00
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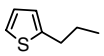
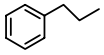
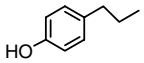
15		>5.00
16		>5.00
17		>5.00
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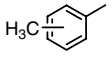
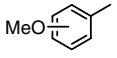
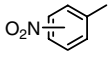
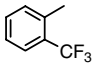
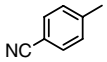
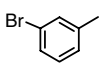
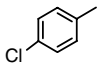
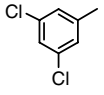
19		>5.00
20		>5.00
21		>5.00
22		>5.00
23		>5.00
24		>5.00
25		>5.00
26		0.50
27		0.41
28		0.80

(*continued on next page*)

Table 1 (*continued*)

Compound	R	IC_{50} (μM)
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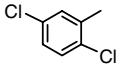
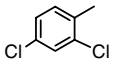
30		0.62
31		0.25

Table 2. SAR of 5-arylamino-4-cyano-3-hydroxy-isothiazole

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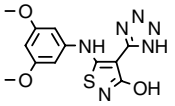
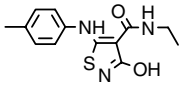
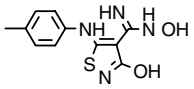
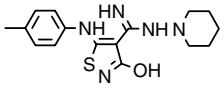
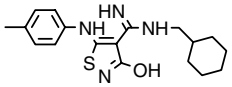
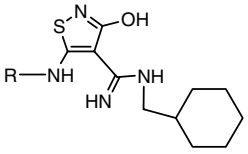
32		>2.00
33		>2.00
34		>2.00
35		>2.00
36		0.74

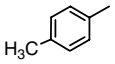
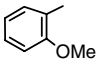
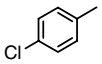
ethylene amidines[20] were prepared with various substitutions on 5-arylamino group. However, as in the case of 4-nitrile SAR studies (**26**–**31**, Table 1), only mono- and di-halogen derivatives (**39**–**50**) had displayed low micromolar inhibition concentrations except for *p*-ester derivative (**44**) unlike its related nitrile (**25**, Table 1 and Table 3).

Among other substitutions studied on amidines small alkyl groups and alkyl groups with hydrophilic groups (OH) are favored and had improved the activity substantially. Further, it was also found that *ortho* substitution on phenyl ring has improved the potency (**51** vs **53**; **52** vs **54**, Table 4) by 8- to 20-fold. *ortho* Substitution on 5-phenylamino group coupled with hydroxyalkyl-carboxyamidino substitution has further enhanced potency by 6-fold (**59** vs **60**, Table 4).

In summary, we designed and synthesized and have shown that a variety of 3-hydroxy-4-carboxyalkylamidino-5-arylamino-isothiazoles are potent in vitro MEK[1] inhibitors. The present systematic structure–activity relationship studies of 5-phenylamino-4-cyano-3-hydroxy-isothiazole led to more than 180-fold (**1** vs **60**) improvement in vitro inhibition of MEK1. Future reports will examine other modifications of 5-arylamino-4-carboxyalkylamidino-3-hydroxy-isothiazole scaffold and its cellular assays and bioavailability studies.

Table 3. SAR of 5-arylamino-4-carboxyalkylamidino-3-hydroxy-isothiazole



Compound	R	IC_{50} (μM)
37		1.20
38		1.44
39		0.42
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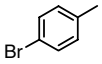
41		0.43
42		0.50
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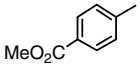
44		0.35
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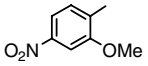
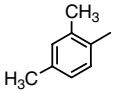
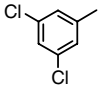
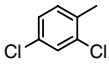
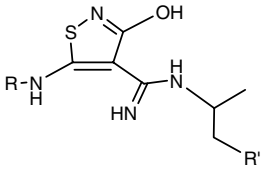
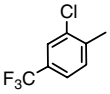
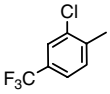
47		0.67
48		0.69
49		0.79
50		0.41

Table 4. SAR of 5-arylamino-4-carboxyalkylamidino-3-hydroxy-isothiazole



Compound	R′ = H R	IC_{50} (μM)	Compound	R′ = OH R	IC_{50} (μM)
51		0.51	**52**		1.44
53		0.062	**54**		0.073
55		0.042	**56**		0.044
57		0.068	**58**		0.086
59		0.22	**60**		0.038

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.05.019.

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19. For IC_{50} assay procedure and conditions, please see the supporting data.
20. ^{1}H and ES-MS data of select compounds are given in the supporting data.