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Beyond U0126. Dianion chemistry leading to the rapid synthesis of a series of potent MEK inhibitors

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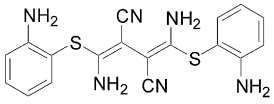
Abstract—Employing phenylmalonitrile dianion chemistry, a large number of analogues of MEK inhibitor lead SH053 ($IC_{50} = 140$ nM) were rapidly synthesized leading to single digit nM inhibitors, displaying submicromolar AP-1 transcription inhibition in COS-7 cells. Compound **41**, exhibiting a MEK $IC_{50} = 12$ nM showed ip activity in a TPA-induced ear edema model with an $ED_{50} = 5$ mg/kg.

1. Introduction

Steroids exert their anti-inflammatory effect via inhibition of the transcription factors AP-1 and NF-κB, both of which are regulators of the immune response genes.[1] Unfortunately, steroids also interact with the glucocorticoid response elements (GREs) in gene promoters resulting in the enhancement of transcription,[2] which in turn leads to undesirable side effects.[3] Inhibition of AP-1 and/or NF-κB without interaction with the GREs would constitute an ideal anti-inflammatory drug.

In a previous letter,[4] we disclosed the discovery of U0126, a compound which functionally antagonizes AP-1 transcriptional activity via upstream inhibition of MEK[5] (MAP kinase kinase or MAPKK), a dual specificity kinase in the mitogen-activated protein kinase (MAPK) cascade.[5] MEK inhibition thus appears to be an attractive anti-inflammatory[6] mechanism to pursue. Recently, MEK inhibitors have been shown to display anticancer properties as well.[7–9]



U0126
MEK IC_{50} = 0.07 ± 0.02 μM
AP-1 IC_{50} = 1.0 ± 0.2 μM

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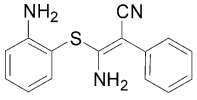
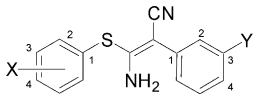
U0126 readily undergoes cyclization and therefore is not a stable entity.[4] We believed that keeping the molecule symmetric was not important and that most likely, both vinylogous cyanamides were not required for good binding affinity. Since the vinylogous cyanamide moiety is flat, we thought that we could replace it with a simple benzene ring, leading to molecule SH053 which exhibited good binding affinity.[10,11]



SH053
MEK IC_{50} = 0.14± 0.05 μM
AP-1 IC_{50} = 9.2± 4.0 μM

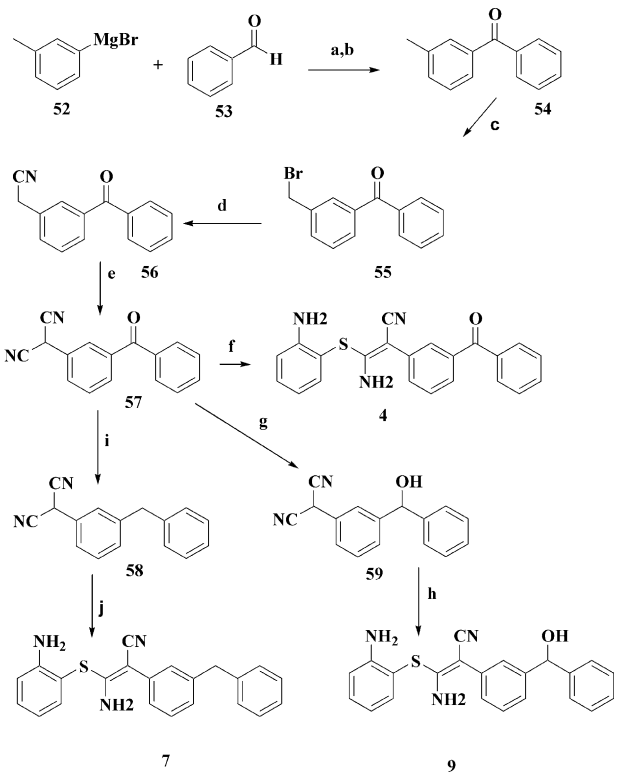
We further hypothesized that SH053 is less potent than U0126 because it lacks U0126s right-most phenyl ring which most likely binds in a hydrophobic pocket. One of the first set of compounds to be made to test this hypothesis involved the addition of a phenoxy group to SH053 (compounds **1**, **2**, and **3** in Table 1). Compound **2**, to our delight, exhibited an increased affinity for MEK ($IC_{50} = 0.04 \pm 0.01$ μM; AP-1 $IC_{50} = 3.03 \pm 0.9$ μM). Replacement of the oxygen linker of **2** with a carbonyl group leads to decreased MEK inhibition (**4**). However, the methylene and CH(OH) linkers yield compounds of equivalent MEK inhibitory potency (**7** and **9** versus **2**). *N*-Methyl amides **10** and **11** were weak inhibitors. Moving the amino group from the *ortho* to the *para* position weakens potency (**4** versus **5**; **7** versus **8**).

Table 1. SH053 analogues



No.	X	Y	MEK IC_{50} (uM)[a]	AP-1 IC_{50} (uM)
SH053	2-NH_2	H	0.14±0.05	9.20±4.0
1	2-NH_2	2-*O*-Ph	0.51±0.01	—
2	2-NH_2	3-*O*-Ph	0.04±0.01	3.03±0.9
3	2-NH_2	4-*O*-Ph	0.93 ($n=1$)	—
4	2-NH_2	3-CO-Ph	0.41±0.06	6.17
5	4-NH_2	3-CO-Ph	1.81 ($n=1$)	—
6	2-NH_2	4-CO-Ph	0.54±0.20	3.13
7	2-NH_2	3-CH_2-Ph	0.04±0.01	3.2
8	4-NH_2	3-CH_2-Ph	0.45 ($n=1$)	38.8
9	2-NH_2	3-CH(OH)-Ph	0.03±0.01	2.07±0.8
10	2-NH_2	3-CO-N(CH_3)-Ph	4.01 ($n=1$)	—
11	4-NH_2	3-CO-N(CH_3)-Ph	45% @ 10 uM	—

Synthesis of the CO and CH(OH) linked diaryl intermediates was rather lengthy, especially when the appropriate benzophenone was not commercially available (Scheme 1). Fortunately, we discovered a novel synthetic sequence involving dianion chemistry (see Section 2), thus permitting the rapid synthesis of the benzhydrol analogues summarized in Table 2.



Scheme 1. (a) THF, 10 °C, 97%; (b) MnO_2, CH_2Cl_2, (99% when using 2-pyridinecarboxaldehyde, for example); (c) NBS, benzoyl peroxide, CCl_4, reflux, 70%; (d) Et_4NCN, CH_2Cl_2, reflux, 65%; (e) LDA, 2-ClBnSCN, 42%; (f) 2-aminothiophenol, Et_3N, 25 °C, 37%; (g) $NaBH_4$, MeOH, 0 °C, 57%; (h) 2-aminothiophenol, Et_3N, 25 °C, 59%; (i) TFA, $NaBH_4$ pellets, 25 °C, 51% (CAUTION: do not use powdered $NaBH_4$—explosion hazard); (j) 2-aminothiophenol, Et_3N, 25 °C, 57%.

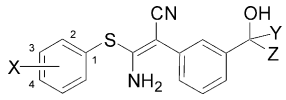
In Table 2 we find that placement of a polar and/or H-bonding functionality at the *para*-position of the Z = Ph group leads to greater MEK and AP-1 inhibition. Thus, *para*-NO_2 (**30**) and *para*-CN (**33**) yield molecules that exhibit MEK IC_{50}'s of 8 and 7 nM, respectively, and AP-1 IC_{50}'s of 0.3 μM each, the latter being almost 7-fold better than that of **9**, and 30-fold better than that of SH053. The carbomethoxy group (**38**) leads to lower potency compared to the NO_2 and CN groups. Of the pyridine analogues (**39**–**41**), the 4-isomer again shows the greatest affinity. The furans appear to be less potent, but the thiophenes appear to be equipotent to pyridine **41** (**44**–**47**). Enlargement of the Z = Ph group of **9** by replacement with Z = naphthyl (**12**, **13**) and benzodioxanyl (**14**) leads to loss in potency most likely due to steric hindrance. Addition of a simple methyl group to Z = Ph also has the same effect (**16**, **18**, **20**). The same is true for a CF_3 group (**48**, **49**). Extension of the Z = Ph by the insertion of a methylene group decreases potency (**25**). Removal of the phenyl group or replacement by cyclohexyl leads to decreased potency (**23** and **22**, respectively). When Y≠H, decreased binding is observed (**26** and **27**). Compound **50** was an intermediate to **51**, which along with **42**, contain groups which are charged at physiological pH. Both show extremely weak AP-1 IC_{50}'s most likely due to poor cell penetration. On the other hand, polar, but neutral groups such as NO_2, CN, CO_2Me (**30**, **33**, and **38**) all show good cell penetration with the most potent AP-1 IC_{50} values in Table 2.

Modifications on the left aromatic ring of the molecule were investigated as well. The 2-OH group was about as potent as the 2-NH_2 group (compare **36** and **33**; **29** and **28**) with respect to MEK IC_{50}'s, but 2-fold less potent for AP-1 inhibition. The other groups, 4-NH_2 (**34**), 2-OMe (**37**), and H (**35**), are all less potent in the 4-CN series. The same is true for the 4-NH_2 group in other series as well (**4** versus **5**; **9** versus **15**; **16** versus **17**; **18** versus **19**; **20** versus **21**).

Overall, the SAR closely followed that established in the U0126 series,[4] suggesting that these two chemotypes might bind at the same site on MEK. In binding kinetics studies with U0126, it was found that the binding to MEK was noncompetitive with either ERK or ATP.[6] To determine the mechanism of MEK inhibition by our new series of inhibitors, the effects of varying concentrations of **41** on the enzyme velocity at varying substrate concentrations was measured, and the data analyzed using classical Michaelis–Menten kinetics.[12] Noncompetitive inhibition with respect to ERK and uncompetitive inhibition with respect to ATP was demonstrated for **41**. Hence, **41** displayed significant affinity for MEK only when ATP was bound to the enzyme.

The ability of **41** to displace ^{3}H-labeled U0126 from MEK in the presence and absence of ATP was also investigated. In the presence of ATP, a dose-dependent displacement of ^{3}H-U0126 by **41** was observed. However, when ATP was excluded from the buffer, **41** failed to displace the radioligand, consistent with binding selectively to the ATP-replete enzyme. The data suggest

Table 2. Benzhydrol MEK inhibitors and analogues



No.	X	Y	Z	MEK IC_{50} (uM)	AP-1 IC_{50} (uM)
9	2-NH_2	H	Ph	0.034±0.006	2.07±0.8
12	2-NH_2	H	2-Napthyl	0.076±0.007	3.25±0.0
13	2-NH_2	H	1-Napthyl	0.047±0.008	2.59±0.6
14	2-NH_2	H	1,4-Benzo-dioxan-6-yl	0.025±0.004	2.10±0.1
15	4-NH_2	H	Ph	0.570 ($n=1$)	10.7
16	2-NH_2	H	2-CH_3–Ph	0.024±0.008	2.69±1.5
17	4-NH_2	H	2-CH_3–Ph	0.659±0.008	3.67±1.5
18	2-NH_2	H	3-CH_3–Ph	0.057±0.036	2.4
19	4-NH_2	H	3-CH_3–Ph	0.503 ($n=1$)	N/A
20	2-NH_2	H	4-CH_3–Ph	0.050±0.033	3.0
21	4-NH_2	H	4-CH_3–Ph	0.390±0.039	N/A
22	2-NH_2	H	Cyclohexyl	0.056±0.030	4.51±1.1
23	2-NH_2	H	CH_3	0.183±0.000	66.7
24	2-NH_2	CH_3	CH_3	0.340 ($n=1$)	40
25	2-NH_2	H	Bn	0.170±0.019	19.9
26	2-NH_2	Ph	Ph	2.220 ($n=1$)	N/A
27	2-NH_2	CH_3	Ph	0.682±0.200	N/A
28	2-NH_2	H	3-NO_2–Ph	0.017 ($n=1$)	1.6
29	2-OH	H	3-NO_2–Ph	0.025±0.008	3.22±0.9
30	2-NH_2	H	4-NO_2–Ph	0.008±0.000	0.30±0.0
31	2-NH_2	H	2,4-di-NO_2–Ph	0.008±0.002	0.30±0.0
32	2-NH_2	H	3-CN–Ph	0.013±0.006	1.08±0.6
33	2-NH_2	H	4-CN–Ph	0.007±0.002	0.32±0.1
34	4-NH_2	H	4-CN–Ph	0.053±0.006	1.13±0.2
35	H	H	4-CN–Ph	0.053 ($n=1$)	1.2
36	2-OH	H	4-CN–Ph	0.008±0.004	0.64
37	2-OMe	H	4-CN–Ph	2.081±0.000	N/A
38	2-NH_2	H	4-(COOMe)–Ph	0.014±0.003	0.29±0.2
39	2-NH_2	H	2-Pyridyl	0.099±0.030	11.8±2.0
40	2-NH_2	H	3-Pyridyl	0.025±0.012	2.72±0.2
41	2-NH_2	H	4-Pyridyl	0.012±0.003	1.40±0.5
42	2-NH_2	H	4-(N-Me)Pyridinium I^-	0.330 ($n=1$)	50
43	2-NH_2	H	Ph-F_5	0.044±0.005	0.87±0.2
44	2-NH_2	H	2-Furyl	0.323±0.110	12.2
45	2-NH_2	H	3-Furyl	0.055±0.009	7.02±1.4
46	2-NH_2	H	Thiophen-2-yl	0.017±0.003	2.22±0.2
47	2-NH_2	H	Thiophen-3-yl	0.017 ($n=1$)	2.4
48	2-NH_2	H	3-CF_3–Ph	0.053±0.029	5.7±0.0
49	2-NH_2	H	4-CF_3–Ph	0.057±0.027	3.97±0.3
50	2-NH_2	H	4-[N^2-Ph_3C-Tetrazol-5-yl]Ph	0.255±0.000	6.88
51	2-NH_2	H	4-[Tetrazol-5-yl]Ph $N(CH_2CH_2OH)_3$	0.035±0.001	25

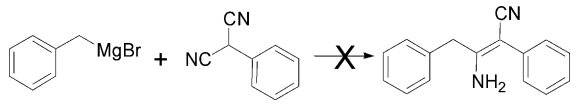
that there is a common binding site for U0126 and **41**, however, the accessibility of this binding site appears to be modulated by the binding of ATP. We may infer therefore that the vinylogous cyanamide portions of **41** and U0126 most likely overlap and bind in the same site. The pyridyl portion of **41**, however, probably binds in a different site from U0126 which is accessible only in the presence of ATP.

For U0126, ATP binding had a minimal effect on its affinity for MEK. In contrast, **41** had a significantly greater affinity for MEK when ATP was bound to the enzyme. Conformational changes are thought to mediate the phosphotransfer activity of MEK and other members of the MAP kinase family. The present data suggest that ATP mediated conformational changes of the protein may also play a significant role in the binding of certain inhibitors as well, such as **41**. Compound **41** is selective for MEK, displaying IC_{50} values of >1 μM for the related MAP kinases MKK3 and MKK4.[13]

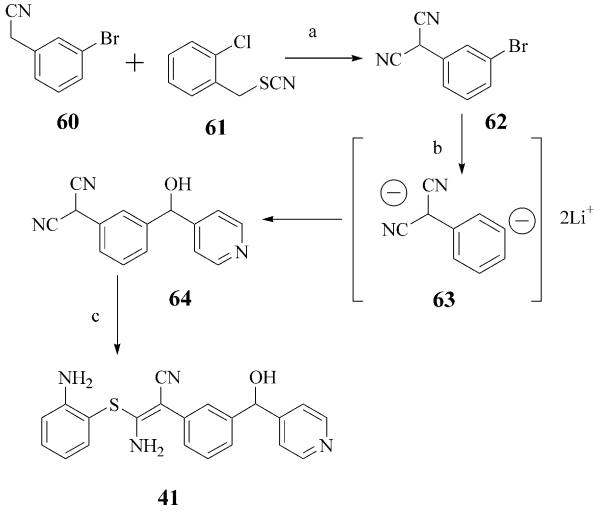
Due to its superior solubility properties, pyridine **41** was tested for in vivo anti-inflammatory activity against phorbol ester (TPA) mediated ear edema in the mouse.[14] When administered intraperitoneally as a solution in 1:1 PEG:EtOH, the ED_{50} was 5 mg/kg.

2. Chemistry

Initial lead compounds were synthesized by the methods outlined in Scheme 1. The key cyanation step (e) was done by the method of Cava.[15] Ethers **1**–**3** were synthesized in a analogous fashion from the commercially available phenoxybenzyl cyanides. Amides **10** and **11** were made similarly from the corresponding *N*-methyl-*N*-phenyltoluamides.



Scheme 2.



Scheme 3. Dianion chemistry. (a) LDA, 0 °C to 25 °C, benzene, 80%; (b) 2 equiv *n*-BuLi, −70 °C, THF, 4-pyridinecarboxaldehyde, 54%; (c) 2-aminothiophenol, Et_3N, THF, 25 °C, 62%.

In an attempt to replace the sulfur atom of SH053 with a carbon, we initially tried to add benzyl Grignard to phenylmalononitrile (Scheme 2). Even with 7 equiv of Grignard reagent, starting material phenylmalononitrile was obtained unchanged. This meant that the malononitrile anion protects both nitriles from nucleophilic attack.

Using this 'anionic protection' we found that we could generate a dianion of phenylmalononitrile (Scheme 3) and quench it with a wide variety of aldehydes and ketones. Subsequent reaction with aryl thiols led to a library of CH(OH) and CR(OH) linker analogues, which are summarized in Table 2. Thus, Cava cyanation leads to malononitrile **62** (Scheme 3). Deprotonation of the malononitrile proton followed by halogen–metal exchange at −70 °C with two equivalents of *n*-BuLi yields dianion **63** as an orange slurry. Immediate quenching with, for example, 4-pyridine-carboxaldehyde yields **64**. Vinylogous cyanamide formation yields compound **41**. The dianion forming reaction was scaled up to 0.15 moles for the synthesis of **41**.

3. Conclusion

We found that one of the vinylogous cyanamides of U0126 could be effectively replaced by a benzene ring, leading to the discovery of SH053. Attachment of an additional phenyl ring with a *para*-H-bonding substituent increases the potency by 1 order of magnitude over that of SH053. The binding pocket for this additional aromatic ring is limited in size, since naphthalene and benzodioxanyl replacements (**13** and **14**) lowered affinity. The *N*-methyl amides (**10** and **11**) being 2 atom linkers could also be less potent due to size limitations imposed by this terminal aromatic pocket. Cell penetration is required for MEK inhibition and thus charged compounds display poor AP-1 transcription inhibition. With the replacement of a vinylogous cyanamide group of U0126 with a benzene ring, we have increased both chemical stability and potency. Some of the compounds described in Tables 1 and 2 have been found to be stable in aqueous buffer at pH = 1 whereas U0126 is not. Further work will be aimed at replacing the remaining cyanamide moiety. The in vitro results translated into in vivo anti-inflammatory activity seen in the TPA mediated ear edema mouse model.

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