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Pyrrolopyridazine MEK inhibitors

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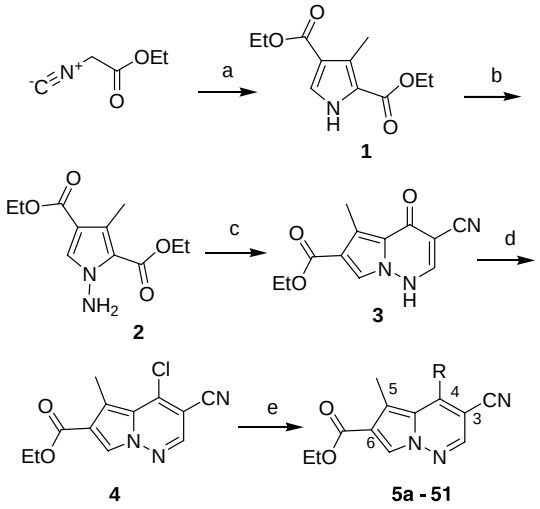
Abstract—The synthesis and SAR of a series of pyrrolopyridazine MEK inhibitors are reported. Optimal activity was achieved by incorporation of a 4-phenoxyaniline substituent at C4 and an acylated amine at C6.

Cell growth and differentiation in response to extracellular stimuli and certain intracellular oncogenes are mediated through various intracellular signal transduction pathways. A key player in this kinase-signaling cascade from growth factors to the cell nucleus is the mitogen-activated protein kinase (MAPK) pathway.[1,2] This pathway involves a series of kinases at multiple levels. Members of the Raf family of kinases are downstream of Ras. When Raf is activated, it phosphorylates MEK (MAPK/ERK kinase, or MAP kinase) on two serine residues. The activated MEK in turn phosphorylates the threonine and tyrosine residues of MAPK (or ERK, extracellular signal-regulated kinase). Activated MAPK then translocates and accumulates in the nucleus, where it effects cell growth and proliferation through phosphorylation of various substrates.[3]

Overexpression and overactivation of MEK or ERK have been found to be associated with various human cancers.[4] It has been demonstrated that inhibition of MEK prevents activation of ERK and subsequent activation of ERK substrates in cells, resulting in inhibition of cell growth stimulation and reversal of the phenotype of Ras-transformed cells.[5] Therefore, inhibition of the MAPK pathway through inhibition of Raf, MEK, or ERK presents a unique opportunity to block uncontrolled cell growth and, thus, has potential therapeutic utility in cancer treatment.

Multiple isoforms exist within the MEK family.[2,3] Among them, MEK-1 has been extensively studied and various types of MEK-1 inhibitors have been reported.[6–13] Here we report the synthesis and SAR of pyrrolopyridazine MEK inhibitors.

The synthesis[14] of the pyrrolopyridazine core, as shown in Scheme 1, started with the formation of the diethyl



Scheme 1. Reagents and conditions: (a) DBU, acetaldehyde, THF, 55 °C, 17 h, 42%; (b) NaH, DMF, then *O*-(2,4-dinitrophenyl)hydroxylamine, 10–25 °C, 12 h, 75%; (c) 1,1-diethoxypropionitrile, TsOH, toluene, reflux, 12 h, then DBU, 80 °C, 1 h, 40%; (d) $POCl_3$, 75 °C, 2 h, 77%; (e) RH, Et_3N or K_2CO_3, THF or DMF, rt to reflux, 1–72 h, 10–100% depending on R.

Keywords: MEK inhibitor; MEK1; Pyrrolopyridazine.
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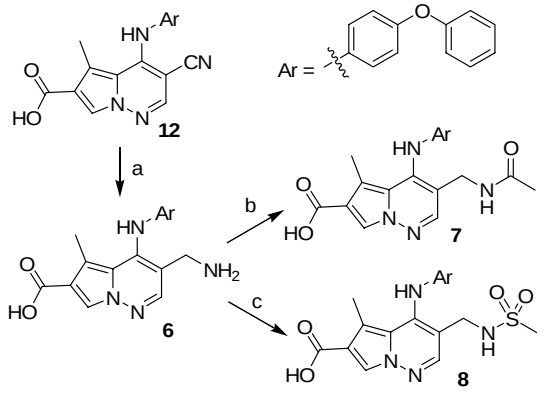
pyrrole-2,4-dicarboxylate **1** by condensation of ethyl isocyanoacetate with acetaldehyde in the presence of DBU. N-Amination of **1** gave **2**, which was condensed with diethoxypropionitrile and the resulting intermediate was cyclized under basic condition to give the 3-cyanopyrrolopyridazine core **3**. Compound **3** was treated with $POCl_3$ to give the 4-chloro compound **4**, which readily reacted with various nucleophiles to give compounds **5a**–**l**.

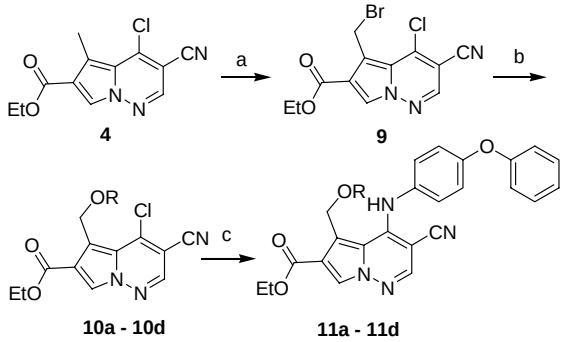
Synthetic modification at C3 is shown in Scheme 2. Hydrogenation of the C3 cyano group of **12** provided the corresponding benzyl amine **6**, which may be acetylated or sulfonylated to give compounds such as **7** or **8**.

Modification at C5 is shown in Scheme 3. α-Bromination of the C5 methyl group of **4** afforded **9**, which underwent substitution reactions to give the alcohol or ether compounds **10a**–**d**. Subsequent nucleophilic aromatic substitution with 4-phenoxyaniline yielded compounds **11a**–**d**.

The C6 position was subjected to the most intense modifications (Scheme 4). Hydrolysis of the ethyl ester **5b** led to the carboxylic acid **12**, which was a good starting point for further modifications. Amide coupling with various primary or secondary amines, or anilines, gave



Scheme 2. Reagents and conditions: (a) H_2 (1 atm), Pd/C, MeOH, THF, TFA, rt, 17 h, 100%; (b) Ac_2O, Et_3N, THF, rt, 10 min, then NaOH, rt, 2 h, 81%; (c) $MeSO_2Cl$, Et_3N, THF, rt, 10 min, 27%.



Scheme 3. Reagents and conditions: (a) NBS, benzoyl peroxide, CCl_4, 77 °C, 3 h, 99%; (b) ROH, $NaHCO_3$, 25–70 °C, 3 h to 3 days, 80–100%; (c) 4-phenoxyaniline, Et_3N, THF, 70 °C, 1 h, 92%.

the corresponding amides **13a**–**c**. Certain amides were cyclized to form heterocycles, as illustrated by benzimidazole **25**. Curtius rearrangement of the carboxylic acid **12** with DPPA, proceeding through the acyl azide, and then the isocyanate intermediates, afforded either the carbamate (**15a**–**d**) by trapping with an alcohol, or the urea (**14a**–**e**) by trapping with TMS–azide to form the carbamoyl azide[15–17] first, which then reacted readily with a primary amine to give the urea product. Hydrogenolysis of the benzyl carbamate **15e** gave the C6 amino compound **16**, which upon acylation yielded the C6 N-linked amides such as **17a**–**c**. Sulfonylation of **16** gave the sulfonamide **18**, reductive amination gave the benzyl amine **19**, and copper-mediated boronic acid coupling gave the aniline **20**.[18] The acid **12** can be decarboxylated to give **21**. The ester **5b** can react with methyl Grignard reagent to give **22**, which yielded the C6 hydroxyl compound **23** upon benzylic hydroperoxide rearrangement.[19] Alkylation of **23** with alkyl halides or Mitsunobu reaction with alcohols afforded the ether compounds such as **24a** and **b**.

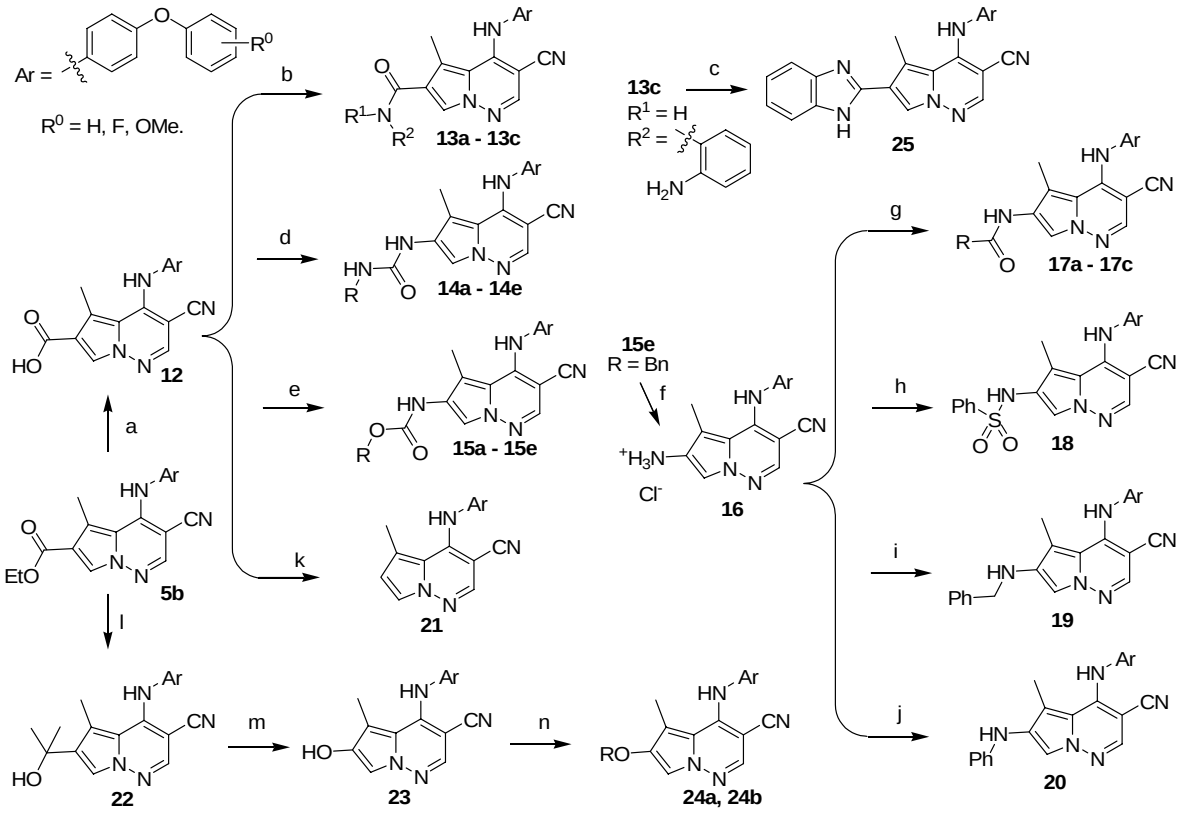
The compounds were tested in an in vitro 96-well plate assay measuring their inhibitory activity against MEK-1.[20,21] The IC_{50} values of select compounds are shown in Tables 1–5.

The SAR of the C3 and C4 positions of the pyrrolopyridazine is generally very tight. Selected modifications to the C3 cyano group, shown in Scheme 2, led to complete loss of MEK activity (Table 1). The pyrrolotriazine compound **26**[22–24] also showed significantly weaker MEK activity than the corresponding C–cyano compound **5b**, suggesting that the C3 cyano group plays a critical role for MEK activity.

The 4-phenoxyaniline is a key group at the C4 position for MEK activity. Modifications to this group, such as one atom extension/contraction or O/N exchange, generally lead to a dramatic loss of activity (Table 2). Some exceptions include: (1) a $–CH_2–$ in place of the oxygen between the two phenyl rings (**5f**); and (2) certain substituents on the *ortho* (**5k**) or *meta* position of the terminal phenyl ring, which led to a slight improvement of MEK activity.

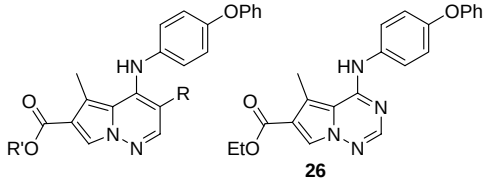
Although modifications at the C5 methyl are generally tolerated, no improvement of MEK activity was observed (Table 3).

Extensive modifications at C6 yielded diverse SAR (Table 4). A wide variety of substituents are tolerated, although a carbonyl one atom away from the C6 carbon of the pyrrolopyridazine core is preferred, as is illustrated by the activity of the ureas (**14a**,**b**), carbamates (**15a**,**b**), and N-linked amides (**17a**–**c**) being better than the C-linked amides (**13a**,**b**) and ethers (**24a**,**b**). Removal of this amide carbonyl (**19**) or conversion to a sulfonyl (**18**) resulted in a dramatic loss of activity. Both hydrophobic groups (**17b**,**c**) and hydrophilic ones (**14b** and **15b**) at the other side of the carbonyl can lead to potent activity. Small groups, such as H (**21**), OH (**23**), and NH_2 (**16**), but not certain bulky ones such as $–CMe_2OH$



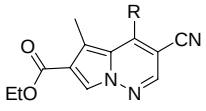
Scheme 4. Reagents and conditions: (a) NaOH, H_2O, EtOH, THF, 80 °C, 6 h, 95%; (b) NR_1R_2, EDC, HOAt, THF, 60 °C, 15 h, 30–99%; (c) CSA, toluene, 110 °C, 5 h, 33%; (d) $(PhO)_2PON_3$, Et_3N, 1,4-dioxane, 25 °C, 15 h, then $TMSN_3$, 80 °C, 2 h, then RNH_2, 25 °C, 1 h, 50–80%; (e) $(PhO)_2PON_3$, Et_3N, 1,4-dioxane, 25 °C, 15 h, then ROH, 75 °C, 4 h, 30–80%; (f) H_2 (1 atm), Pd/C, MeOH, rt, 30 min, then HCl (4 M in dioxane, to form HCl salt), 100%; (g) RCOOH, EDC, HOAt, THF, 60 °C, 15 h, 35–95%; (h) $PhSO_2Cl$, DIEA, 1,2-dichloroethane, rt, 16 h, 27%; (i) PhCHO, $NaBH(OAc)_3$, 1,2-dichloroethane/AcOH, rt, 16 h, 11%; (j) $PhB(OH)_2$, $Cu(OAc)_2$, Et_3N, CH_2Cl_2, rt, 16 h, 15%; (k) Cu_2O, diethyleneglycol, 155 °C, 21 h, 6%; (l) MeMgBr, THF, 0–50 °C, 1 h, 64%; (m) $BF_3{\cdot}OEt_2$, H_2O_2, CH_2Cl_2, −5 °C, 1 h, 65%; (n) ROH, Ph_3P, DEAD, THF, 0–25 °C, 2 h, 60–90%.

Table 1. MEK inhibitory activity of pyrrolopyridazines with C3 variations



Compound	R	R′	MEK IC_{50} (μM)
5b	–CN	–Et	0.094
12	–CN	–H	0.147
6	$–CH_2NH_2$	–H	>25
7	$–CH_2NHAc$	–H	>25
8	$–CH_2NHSO_2Me$	–H	>25
26	—	—	3.47

Table 2. MEK inhibitory activity of pyrrolopyridazines with C4 variations



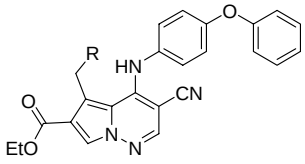
Compound	R	MEK IC_{50} (μM)
5a	–NHPh	>25
5b	–NHPh-4-OPh	0.094
5c	–NHPh-3-OPh	2.65
5d	–NHPh-4-Ph	>25
5e	–NHPh-4-NHPh	2.32
5f	$–NHPh-4-CH_2Ph$	0.068
5g	–NHPh-4-COPh	1.11
5h	–OPh-4-OPh	5.42
5i	$–NHCH_2Ph-4-OPh$	>25
5j	$–NHPh-4-OCH_2Ph$	>25
5k	–NHPh-4-OPh-2-OMe	0.023
5l	–NHPh-4-OPh-4-Cl	0.642

(**22**), at C6 are tolerated. Certain heterocycles such as benzimidazole (**25**) at the C6 position also demonstrated potent inhibition of MEK.

Some of our most active compounds were obtained by combining the best substituents at C4 and C6. Several such examples are shown in Table 5.

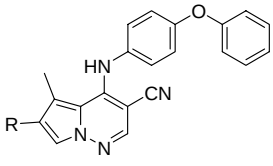
The 3-cyanopyrrolopyridazines are highly selective against other kinases such as VEGFR-2.[24] For example, two of the most active MEK inhibitors **14b** and **15b** both had less than 5% inhibition against VEGFR-2 at concentrations up to 50 μM.

Table 3. MEK Inhibitory activity of pyrrolopyridazines with C5 variations



Compound	R	MEK IC_{50} (μM)
5b	–H	0.094
11a	–OH	0.203
11b	–OMe	0.191
11c	–OEt	0.331
11d	$–OCH_2CH_2OMe$	0.566

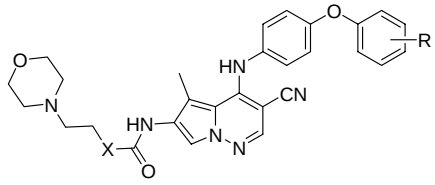
Table 4. MEK inhibitory activity of pyrrolopyridazines with C6 variations



Compound	R	MEK IC_{50} (μM)
21	–H	0.241
23	–OH	0.181
24a	–OMe	0.805
16	$–NH_2$	0.190
22	$–CMe_2OH$	>25
5b	–COOEt	0.094
12	–COOH	0.147
13a	–CONHMe	0.397
13b	$–CONH(CH_2)_2$-*N*-morpholine	0.386
14a	–NHCONHMe	0.018
14b	$–NHCONH(CH_2)_2$-*N*-morpholine	0.036
15a	–NHCOOMe	0.023
15b	$–NHCOO(CH_2)_2$-*N*-morpholine	0.058
17a	–NHCOMe	0.087
17b	–NHCOPh	0.042
17c	–NHCOPh-3-NHAc	0.014
18	$–NHSO_2Ph$	14.6
19	$–NHCH_2Ph$	6.13
20	–NHPh	0.361
24b	$–O(CH_2)_2$-*N*-morpholine	0.521
25	–2-Benzimidazolyl	0.168

Compounds active in the MEK enzyme assay were tested in cell assays. The MEK2 cells are Rat1 cells transformed by human MEK-1 cDNA with two activating mutations (S218E and S222E).[25] Compounds that inhibit the growth of such cells indicate a strong probability of MEK inhibition. The compounds were also tested against two human tumor cell lines: HT-29 and Colo205. The cellular inhibitory activities of the pyrrolopyridazines are generally 10- to 100- fold less potent than their enzyme activities. It is shown as a general trend that more active MEK inhibitors are more potent against tumor cell growth (Table 6).

Table 5. MEK Inhibitory activity of pyrrolopyridazines with optimal C4 and C6 substituents



Compound	X	R	MEK IC_{50} (μM)
14c	NH	–2-OMe	0.024
14d	NH	–2-F	0.033
14e	NH	–3-OMe	0.029
15c	O	–2-OMe	0.048
15d	O	–3-OMe	0.076

Table 6. Cell growth inhibitory activity of select pyrrolopyridazines

Compound	MEK enzyme IC_{50} (μM)	MEK2 cell (μM)	HT-29 (μM)	Colo205 (μM)
5b	0.094	>20	—	—
14a	0.018	9.78	7.38	—
14b	0.036	2.45	0.86	3.90
15a	0.023	7.65	7.64	—
15b	0.058	0.50	3.76	2.88
15c	0.048	3.67	3.78	2.06
15d	0.076	—	2.35	0.72
17b	0.042	12.1	2.13	2.42
17c	0.014	2.50	1.34	1.86
21	0.241	—	>10	>10

In conclusion, a series of pyrrolopyridazines have been prepared as MEK inhibitors. The SAR at the 3–6 positions have been explored. The most active compounds have a cyano group at C3 position, 4-phenoxyaniline with optional *ortho* or *meta* substitution at the terminal phenyl at C4, methyl or alkoxymethyl at C5, and carbamate, urea or N-linked amide at C6. These compounds have potent MEK inhibitory activity and inhibit the growth of several human tumor cell lines.

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