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MEK (MAPKK) Inhibitors. Part 2: Structure–Activity Relationships of 4-Anilino-3-cyano-6,7-dialkoxyquinolines

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Abstract—A series of 4-anilino-3-cyano-6,7-dialkoxyquinolines with different substituents attached to the 4-anilino group has been prepared that are potent MEK (MAP kinase kinase) inhibitors. The best activity is obtained when a phenyl or a thienyl group is attached to the *para*-position of the aniline through a hydrophobic linker, such as an oxygen, a sulfur, or a methylene group. The most active compounds show low nanomolar IC_{50}'s against MEK (MAP kinase kinase), and have potent growth inhibitory activity in LoVo cells (human colon tumor line).

Mammalian cells use diverse signaling pathways to instruct the nucleus about genes to turn on and off in the regulation of cell growth and cell cycling. The mitogen-activated protein kinase (MAPK) pathway (Raf-MEK-ERK phosphorylation cascade) is a key signaling pathway linking signals from growth factors and hormones acting at cell surface receptors to transcription factors in the nucleus which control gene expression and function.[1] However, this pathway also serves oncogenic signals in cancer cells by linking signals from membrane receptor oncogenes and intracellular oncogenic kinases to the nucleus. Ras is an upstream activator of the MAPK pathway and oncogenic forms of Ras are known. Ras is mutated in roughly 30% of all human solid tumors, including 50% of all colon cancers and 90% of all pancreatic cancers.[2] Although mutations of Raf[3] have not yet been shown in human tumors, overexpression, hyperactivity, or dysregulation of c-Raf[3] has been shown to be associated with various human cancers and tumor cells.[3] Cells transformed with v-Raf are tumorigenic when injected into mice[4] and v-Raf has been shown to transform human tissue and cells.[4] MEK (MAP kinase kinase) and ERK (MAP kinase) are dysregulated in human cancers[5] and over-activation of c-Raf is associated with dysregulated ERK and MEK in human tumors and tumor cell lines.[5g] Thus, inhibition of the MAPK pathway through inhibition of Raf, MEK, or ERK presents a unique opportunity to block uncontrolled cell growth and, therefore, has potential therapeutic utility in developing cancer agents.[6] Raf inhibitors,[7] MEK inhibitors,[8] and receptor and non-receptor tyrosine kinase inhibitors[9] have been reported in the literature.

We have previously reported a series of 3-cyano-4-(phenoxyanilino) quinolines that are novel and potent inhibitors of MEK (MAP kinase kinase) activity.[10] The most active compounds have alkoxy groups at both the 6- and 7-positions. Our thesis is that cyanoquinoline molecules represent new, unique inhibitors of MEK, which is an important new target for cancer therapeutics. We now report the structure–activity relationships of a series of 4-anilino-3-cyano-6,7-dialkoxyquinolines with different substitutions on the 4-anilino group. The substitution patterns at the 6- and 7-positions were those which have been shown to provide the best activity: 6,7-dimethoxy- or 6-methoxy-7-(3-morpholino-4-yl-propoxy).[10]

Compounds were prepared using methods described in the previous paper.[10a] Compounds were tested for MEK kinase inhibition using two related fluorescence based (DELFIA) ELISA assays: a coupled MEK assay (which uses activated Raf to activate an inactive

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GST-MEK)[10a] and a direct MEK assay (which by-passes the need for activated Raf by using an activated GST-MEK).[10a] Cells were obtained from ATCC (Rockville, MD) and inhibition of cell proliferation was monitored as described before.[10a]

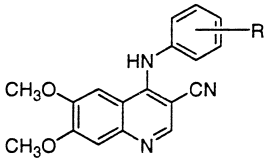
We have extended our initial findings on 3-cyano-4-(phenoxyanilino)-6,7-dialkoxyquinolines by evaluating the SARs surrounding the substitutions on the 4-anilino group. The structures of such 4-anilino-3-cyano-6,7-dimethoxyquinoline compounds (**1**–**10**) and their IC_{50} values are shown in Table 1.

As shown in Table 1, compound **1**, with a *para*-phenoxy group on the aniline at the 4-position, shows low nanomolar IC_{50}'s in both assays. Replacement of the oxygen with an amino group (**2**) greatly decreases activity, while replacement of the same oxygen with a methylene group (**3**) retains the activity. This result suggests that a hydrogen bond donor is detrimental to activity. A flexible linkage between the two phenyl rings is crucial as the activity decreases by more than two orders of magnitude when a phenyl group is attached directly to the aniline at the *para*-position (**4**). The size of the phenoxy group is also important for activity. Replacement with a methoxy (**5**), a hydroxy (**6**), or a methylsulfanyl (**7**) group invariably leads to a decrease in activity. The activity is reduced by 6- to 7-fold when a 4-chloro is introduced to the phenoxy group (**8**). The position of the phenoxy group on the aniline is also very important. When it is moved from the *para*-position (**1**) to the *meta*-position (**9**), the activity is greatly reduced. Moving the phenoxy to the *ortho*-position (**10**) further reduces activity. The activity in the direct MEK assay is consistent with that in the coupled MEK assay, although the IC_{50} values in the coupled assay are 4- to 10-fold better. This may reflect the fact that coupled kinase systems enhance the signal amplification and sensitivity of an assay.[11] Furthermore, the kinase components of the MAPK cascade have been shown to change from graded to all or none responses as the phosphorylation cascade proceeds;[11] thus, different steps in the cascade may be more or less sensitive to inhibitors.

A key finding in our earlier paper[10a] was the fact that introducing a morpholinoalkoxy group at the 7-position, while maintaining a 6-alkoxy group, contributed to greater potency in the cell growth assay. We have extended these studies and focused our synthetic and biological efforts on a study of the SARs of analogues with different substituents at the *para*-position of the 4-anilino group of 4-anilino-3-cyano-6-methoxy-7-(3-morpholino-4-yl-propoxy) quinolines (**11**–**26**). The structures of these compounds and the IC_{50} values for kinase inhibition are shown in Table 2.

Several conclusions can be reached from these results. We confirmed our earlier findings (shown in Table 1) that a benzyl group is comparable to a phenoxy group on the 4-aniline (**11** vs **12**) while a methoxy group greatly reduces activity (**13**). In addition, we found a phenylsulfanyl group (**14**) is at least as potent as a phenoxy or a benzyl group. An unsaturated ring system is required for this position since replacement of the phenyl ring with a saturated cyclohexyl group reduces activity by 2–3 orders of magnitude (**11**, **12**, and **14** vs **15**, **16**, and **17**). We then studied the effects of attaching different heteroaryl groups through a methylene or an oxygen linkage. Pyridinyl groups were first introduced in place of the phenyl group owing to the geometric similarity of these groups. As shown in Table 2, inhibitory activity

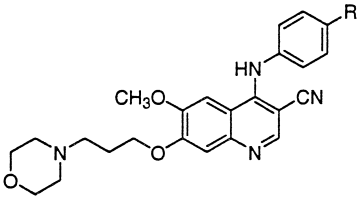
Table 1. Kinase inhibitory activity of compounds **1**–**10**[a]



Compound	R	IC_{50} (μM) Coupled MEK[4]	IC_{50} (μM) Direct MEK[4]
1	*para*-Phenoxy	0.0062	0.034
2	*para*-Phenylamino	0.16	1.2
3	*para*-Benzyl	0.0036	0.036
4	*para*-Phenyl	1.1	9.5
5	*para*-Methoxy	0.52	3.0
6	*para*-Hydroxy	0.55	2.2
7	*para*-Methylsulfanyl	0.28	1.3
8	*para*-(4-Chlorophenoxy)	0.043	0.21
9	*meta*-Phenoxy	0.060	0.16
10	*ortho*-Phenoxy	>10	>10

[a]Values are means of two experiments, each experiment with $n=3$ replicates. The variability is within 10% of the mean.

Table 2. Kinase inhibitory activity of compounds **11**–**26**[a]



Compound	R	IC_{50} (μM) Coupled MEK[4]	IC_{50} (μM) Direct MEK[4]
11	Phenoxy	0.0024	0.0070
12	Benzyl	0.0027	0.014
13	Methoxy	0.099	0.25
14	Phenylsulfanyl	0.0011	0.0057
15	Cyclohexyloxy	0.11	0.32
16	Cyclohexylmethyl	0.20	3.3
17	Cyclohexylsulfanyl	0.021	0.30
18	4-Pyridinylmethyl	0.19	0.65
19	3-Pyridinylmethyl	0.029	0.10
20	2-Pyridinylmethyl	0.018	0.072
21	4-Pyridinyloxy	0.026	0.081
22	3-Furylmethyl	0.014	0.043
23	2-Furylmethyl	0.020	0.066
24	3-Thienylmethyl	0.0042	0.023
25	2-Thienylmethyl	0.0044	0.020
26	2-Phenylethyl	0.033	0.15

[a]Values are means of two experiments, each experiment with $n=3$ replicates. The variability is within 10% of the mean.

increases in order from 4-pyridinyl to 3-pyridinyl and then to 2-pyridinyl (**18**, **19**, and **20**). However, even the most active of the three, compound **20**, is about 5-fold less active than the corresponding phenyl analogue, compound **12**. Similarly, the 4-pyridinyloxy compound **21** is 10-fold less active than the corresponding phenoxy analogue **11**. Both 2- and 3-furyl groups are also 5- to 10-fold less active than the corresponding phenyl group (**22** and **23** vs **12**), while 2- and 3-thienyl groups are only 2-fold less active (**24** and **25** vs **12**). To study the space requirement of the linker between the two aryl groups, we also extended the methylene linkage in **12** to its ethyl homologue (**26**). This resulted in a 10-fold loss of activity.

The active compounds in Tables 1 and 2 appear to be relatively selective for MEK. These compounds showed less activity (with IC_{50}'s greater than 10 μM) on other serine/threonine kinases (cdk2 and cdk4, AKT, ERK) and tyrosine kinases (EGFR, HER2/NEU, ECK, and VEGF related KDR). Other related MAPK family members are being tested.

In addition to enzyme inhibitory activity, several of these compounds exhibit cell growth inhibitory activity. Cell proliferation was monitored by harvesting monolayer cell cultures with trypsin after exposing the cells to 10% serum containing compound for 3 days. The cells were counted by an electronic particle counter. In some cases, cells were counted on a hemocytometer under light microscopy with trypan blue staining. Compounds **1**, **11**, **12**, and **14** were found to be inhibitors of growth in a LoVo human tumor cell line. The IC_{50} values of these compounds are shown in Table 3. These compounds were not cytotoxic at concentrations near the IC_{50} values for growth inhibition as observed by light microscopy of the cells and by trypan blue dye exclusion. At concentrations on the order of 5–10 μM, some compounds show morphological evidence of cytotoxicity. Western blot studies have shown that these compounds also inhibit phosphorylation of cellular ERK1,2 at concentrations of 200–900 nM (data not shown), which are equivalent to, or slightly higher than, the IC_{50} values required for growth inhibition.

We have recently reported on the SARs for a series of 3-cyano-4-(phenoxyanilino)-6,7-dialkoxyquinolines that are potent MEK inhibitors and represent novel structures with relatively selective MEK activity.[10] We now have extended these SAR studies by preparing a series of 4-anilino-3-cyano-6,7-dialkoxyquinolines with different substituents on the 4-anilino group. A study of these SARs shows that inhibitory activity is best when the *para*-position of the 4-anilino group is connected to a phenyl or a thienyl group through a hydrophobic linker, such as an oxygen, a sulfur or a methylene group. The most active compounds show low IC_{50} values (2–50 nM) against MEK (MAP kinase kinase), and have potent (around 200 nM) growth inhibitory activity in LoVo cells (human colon tumor). Introduction of a morpholinoalkoxy group at the 7-position yields compounds that are more active on cells than the corresponding 6,7-dimethoxy compounds. We have established that these compounds are competitive with ATP (data to be published separately) and thus, they have a different mode of action than PD 98059 or U 0126, other MEK inhibitors, which are noncompetitive with ATP and appear to bind to MEK.

Table 3. Tumor cell growth inhibition by compounds **1**, **11**, **12**, and **14** in LoVo human colon tumor line[a]

Compound	IC_{50} (μM)
1	0.63
11	0.19
12	0.21
14	0.22

[a]Values are means of three experiments, each experiment with $n=3$ replicates.

Our colleagues at Wyeth-Ayerst Research have recently published on two different series of cyanoquinolines[9e–g] that inhibit EGFR kinase and Src kinase. These compounds are different from, but related to, structures presented in this manuscript. Although the core structures of the compounds are similar, the anilino groups at the 4-position are different. Quinazolines have been shown to be good tyrosine kinase inhibitors. Although quinazolines[9a–d] are close analogues of the cyanoquinolines, we have clearly shown that quinazolines do not inhibit MEK.[10a] Several other quinazolines have been tested on MEK and IC_{50} values were found to be greater than 10 μM.

References and Notes

1. (a) Seeger, R.; Krebs, E. G. *FASEB J.* **1995**, *9*, 726. (b) Cobb, M. H.; Goldsmith, E. J. *J. Biol. Chem.* **1995**, *270*, 14843. (c) Schaeffer, H. J.; Weber, M. J. *Mol. Cell. Biol.* **1999**, *19*, 2435. (d) Garrington, T. P.; Johnson, G. L. *Curr. Opin. Cell. Biol.* **1999**, *11*, 211.
2. (a) Bos, J. L. *Cancer Res.* **1989**, *49*, 4682. (b) Kiaris, H.; Spandidoss, D. A. *Int. J. Oncol.* **1995**, *7*, 413.
3. (a) Callans, L. S.; Naama, H.; Khandelwal, M.; Plotkin, R.; Jardines, L. *Annu. Surg. Oncol.* **1995**, *2*, 38. (b) Berger, D. H.; Jardines, L. A.; Chung, H.; Ruggeri, B. *J. Surg. Res.* **1997**, *69*, 199. (c) El-Ashry, D.; Miller, D. L.; Kharbanda, S.; Lippman, M. E.; Kern, F. G. *Oncogene* **1997**, *15*, 423.
4. (a) Fang, X. J.; Keating, A.; Flowers, M.; Liew, C. C.; Gupta, H.; Mills, G. B.; Sherman, M. *Carcinogenesis* **1993**, *14*, 669. (b) Bisgaard, H. C.; Mackay, A. R.; Gomez, D. E.; Ton, P. T.; Thorgeirsson, S. S.; Thorgeirsson, U. P. *Invasion Metastasis* **1997**, *17*, 240. (c) Scully, J. L.; Edwards, P. A. W. *Int. J. Cancer* **1991**, *48*, 128. (d) Hampton, L. L.; Worland, P. J.; Yu, B.; Thorgeirsson, S. S.; Huggett, A. C. *Cancer Res.* **1990**, *50*, 7460.
5. (a) Oka, H.; Chatani, Y.; Hoshino, R.; Ogawa, O.; Kakehi, Y.; Terachi, T.; Okada, Y.; Kawaichi, M.; Kohno, M.; Yoshida, O. *Cancer Res.* **1995**, *55*, 4182. (b) Sivaraman, V. S.; Wang, H.; Nuovo, G. J.; Malbon, C. C. *J. Clin. Invest.* **1997**, *99*, 1478. (c) Kono, Y.; Kondo, K.; Iwata, H.; Senga, T.; Akiyama, S.; Ito, K.; Takagi, H.; Hamaguchi, M. *Jpn. J. Cancer Res.* **1998**, *89*, 903. (d) Towatari, M.; Iida, H.; Tanimoto, M.; Iwata, H.; Hamaguchi, M.; Saito, H. *Leukemia* **1997**, *11*, 479. (e) Mandell, J. W.; Hussaini, I. M.; Zecevic, M.; Weber, M. J.; VadenBerg, S. R. *Am. J. Pathol.* **1998**, *153*, 1411. (f) Mishima, K.; Yamada, E.; Masui, K.; Shimokawara, T.;

Takayama, K.; Sugimura, M.; Ichijima, K. *Modern Pathol.* **1998**, *11*, 886. (g) Hoshino, R.; Chatani, Y.; Yamori, T.; Tsuruo, T.; Oka, H.; Yoshida, O.; Shimada, Y.; Ari-i, S.; Wada, H.; Fujimoto, J.; Kohno, M. *Oncogene* **1999**, *18*, 813.
6. (a) Stein, B.; Anderson, D. *Annu. Rep. Med. Chem.* **1996**, *31*, 289. (b) Sedlacek, H. H. *Drugs* **2000**, *59*, 435. (c) Levitt, M. L.; Koty, P. P. *Invest. New Drugs* **1999**, *17*, 213. (d) Cohen, P. *Curr. Opin. Chem. Biol.* **1999**, *3*, 459. (e) Levitzki, A. *Pharmacol. Ther.* **1999**, *82*, 231.
7. (a) Monia, B. P. *Anti-Cancer Drug Des.* **1997**, *12*, 327. (b) Geiger, T.; Muller, M.; Monia, B. P.; Fabbro, D. *Clin. Cancer Res.* **1997**, *3*, 1179. (c) Lau, Q. C.; Brusselbach, S.; Muller, R. *Oncogene* **1998**, *16*, 1899.
8. (a) Simon, C.; Hicks, M. J.; Nemechek, A. J.; Mehta, R.; O'Malley, B. W.; Goepfert, H.; Flaitz, C. M.; Boyd, D. *Br. J. Cancer* **1999**, *80*, 1412. (b) Dudley, D. T.; Pang, L.; Decker, S. J.; Bridges, A. J.; Saltiel, A. R. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 7686. (c) Alessi, D. R.; Cuenda, A.; Cohen, P.; Dudley, D. T.; Saltiel, A. R. *J. Biol. Chem.* **1995**, *270*, 13585. (d) Sebolt-Leopold, J. S.; Dudley, D. T.; Herrera, R.; Van Becelaere, K.; Wiland, A.; Gowan, R. C.; Tecle, H.; Barrett, S. D.; Bridges, A.; Przybranowski, S.; Leopold, W. R.; Saltiel, A. R. *Nat. Med.* **1999**, *5*, 810. (e) Nishio, K.; Fukuoka, K.; Fukumoto, H.; Sunami, T.; Iwamoto, Y.; Suzuki, T.; Usuda, J.; Saijo, N. *Int. J. Oncol.* **1999**, *14*, 461. (f) Favata, M. F.; Horiuchi, K. Y.; Manos, E. J.; Daulerio, A. J.; Stradley, D. A.; Feeser, W. S.; Van Dyk, D. E.; Pitts, W. J.; Earl, R. A.; Hobbs, F.; Copeland, R. A.; Magolda, R. L.; Scherle, P. A.; Trzaskos, J. M. *J. Biol. Chem.* **1998**, *273*, 18623.
9. (a) Denny, W. A.; Rewcastle, G. W.; Bridges, A. J.; Fry, D. W.; Kraker, A. J. *Clin. Exp. Pharmacol. Physiol.* **1996**, *23*, 424. (b) Fry, D. W. *Pharmacol. Ther.* **1999**, *82*, 207. (c) Smaill, J. B.; Palmer, B. D.; Rewcastle, G. W.; Denny, W. A.; McNamara, D. J.; Dobrusin, E. M.; Bridges, A. J.; Zhou, H.; Showalter, H. D. H.; Winters, R. T.; Leopold, W. R.; Fry, D. W.; Nelson, J. M.; Slintak, V.; Elliot, W. L.; Roberts, B. J.; Vincent, P. W.; Patmore, S. J. *J. Med. Chem.* **1999**, *42*, 1803. (d) Discafani, C. M.; Carroll, M. L.; Floyd, M. B.; Hollander, I. J.; Husain, Z.; Johnson, B. D.; Kitchen, D.; May, M. K.; Malo, M. S.; Minnick, A. A.; Nilakantan, R.; Shen, R.; Wang, Y.-F.; Wissner, A.; Greenberger, L. M. *Biochem. Pharmacol.* **1999**, *57*, 917. (e) Wissner, A.; Berger, D. M.; Boschelli, D. H.; Floyd, M. B., Jr.; Greenberger, L. M.; Gruber, B. C.; Johnson, B. D.; Mamuya, N.; Nilakantan, R.; Reich, M. F.; Shen, R.; Tsou, H.-R.; Upeslacis, E.; Wang, Y. F.; Wu, B.; Ye, F.; Zhang, N. *J. Med. Chem.* **2000**, *43*, 3244. (f) Wang, Y. D.; Miller, K.; Boschelli, D. H.; Ye, F.; Wu, B.; Floyd, M. B.; Powell, D. W.; Wissner, A.; Weber, J. M.; Boschelli, F. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2477. (g) Boschelli, D. H.; Wang, Y. D.; Ye, F.; Wu, B.; Zhang, N.; Dutia, M.; Powell, D. W.; Wissner, A.; Arndt, K.; Weber, J. M.; Boschelli, F. *J. Med. Chem.* **2001**, *44*, 822.
10. (a) Zhang, N.; Wu, B.; Powell, D.; Wissner, A.; Floyd, M. B.; Kovacs, E. D.; Toral-Barza, L.; Kohler, C. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2825. (b) Zhang, N.; Wu, B.; Powell, D.; Wissner, A.; Kovacs, E. D.; Toral-Barza, L.; Kohler, C. In *Frontiers of Biotechnology & Pharmaceuticals*; Science: New York, 2000; Vol. 1, pp 305–313.
11. (a) Ferrell, J. E., Jr. *Trends Biochem. Sci.* **1997**, *22*, 288. (b) Ferrell, J. E., Jr. *Trends Biochem. Sci.* **1998**, *23*, 461.