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Synthesis and Biological Evaluation of 4-[(3-Methyl-3*H*-imidazol-4-yl)-(2-phenylethynyl-benzyloxy)-methyl]-benzonitrile as Novel Farnesyltransferase Inhibitor

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Abstract—Farnesyltransferase inhibitors (FTIs) have emerged as a novel class of anticancer agents. Analogues of the potent FTI, 4-[3-biphenyl-1-hydroxy-1-(3-methyl-3*H*-imidazol-4-yl)-prop-2-ynyl]-1-yl-benzonitrile, were synthesized and tested in vitro for their inhibitory activities. The most promising compound identified from this series is analogue **11** that possesses potent enzymatic and cellular activities.

GTP-bound Ras proteins those are responsible for initiating an intracellular phosphorylation cascade, and consequently play an important role in normal cellular physiology and pathophysiology.[1] Oncogenic Ras proteins commonly found in human tumors[1,2] are locked in the activated GTP-bound state, which leads to a continuously activated phosphorylation cascade. An essential prerequisite for the function of the Ras protein is its association with the plasma membrane. Ras proteins are initially synthesized in the cytoplasm where they undergo posttranslational farnesylation of the cysteine unit of the so-called CAAX box (C, cysteine; A, any aliphatic amino acid; X, serine or methionine) in the pre-Ras protein by the enzyme protein farnesyltransferase (FT).[3] Once the protein substrate is farnesylated, the AAX tripeptide is cleaved and the new C-terminal cysteine carboxylate is methylated. The processed proteins become relocated to the cell membrane, a step that is essential for their function. This association transmits extracellular signals to the nucleus and leads to cell proliferation[4,5]

The antitransforming properties of farnesyltransferase inhibitors (FTIs), a novel class of cancer therapeutics, have been widely investigated in the past decade.[6–9] FTIs were originally developed with the aim of inhibiting the posttranslational prenylation and oncogenic activity of Ras. It has become apparent that inhibition of Ras prenylation is not necessary for these compounds to exhibit antitumor activity. Instead, inhibition of Rho-B and possibly other cellular proteins might also account for the efficacy against malignant tumors.[10–13] Although, the mechanism of action of these agents is still debated, FTIs have shown impressive efficacy in preclinical models of human cancers.

Our initial discovery of compound **1** as a potent, non-peptidic, non-sulhydryl, selective inhibitor of FT prompted an investigation of SAR centered around this compound. The goal of our research is to identify additional molecules within this class of FTIs that not only have good in vitro potency but may also have different physical properties (i.e., solubility and crystallinity). We have previously discovered that modification of the phenyl ring of the biaryl skeleton in **1** affected the inhibitory potency of analogues.[14] However, we have not thoroughly examined the effect of the linker on the potency. Since it has been demonstrated previously that the *ortho* phenyl ring could accommodate a large group, we replaced the acetylenic moiety with a methyl ether linkage in **1**, and introduced an acetylenic likage between the two *ortho* phenyl rings as shown in the

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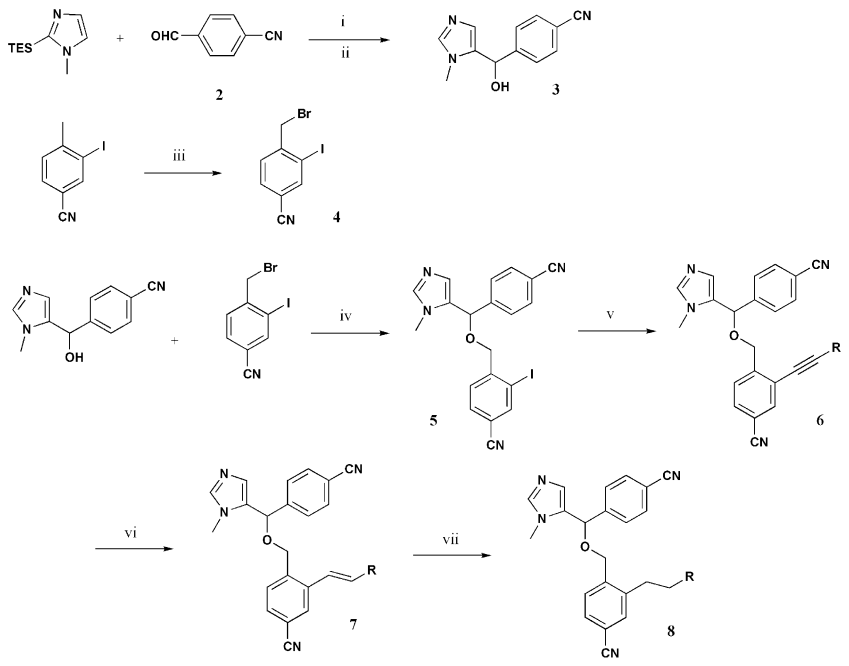
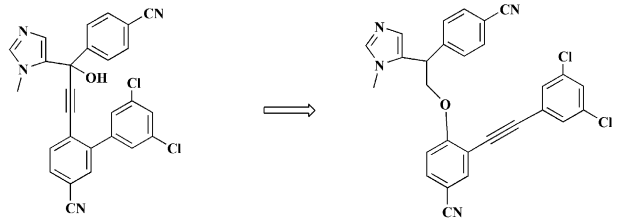


Scheme 1. (i) *n*-BuLi, −78 °C; (ii) TBAF, THF; (iii) NBS, AIBN, CCl_4; (iv) NaH, THF; (v) $Pd(PPh_3)_4$, CuI, THF; (vi) Pt/C; H_2; (vii) Pd/C; H_2.

structure **A**. It was anticipitated that these modifications would increase the solubility of FTIs and maintain the potency.



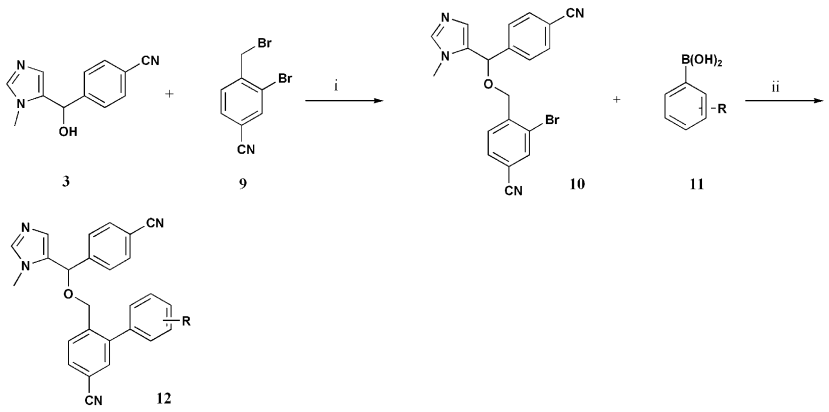
1 **A**

Acetylenic analogues were prepared using the reaction sequences shown in Scheme 1. Formation of the secondary alcohol **3** was accomplished by formation of an imidazole anion with *n*-butyl lithium followed by addition of the anion to the aldehyde **2**. The TES group was then removed with TBAF. Bromide **4** was prepared from the corresponding toluene by bromination with *N*-bromosuccinimide in carbon tetrachloride. Alkylation of alcohol **3** with bromide **4** gave the core intermediate **5**, which was coupled with various acetylenes utilizing Sonogashira reaction conditions to give the acetylene analogues **6**. The acetylene analogues were hydrogenated to olefinic compounds **7** with hydrogen in the presence of platinum on charcoal. Further reduction to saturated analogues **8** was then accomplished by hydrogenation of the olefins with palladium on carbon in ethyl acetate.

Biphenyl analogues were synthesized using a similar route (Scheme 2). Thus, coupling of a substituted benzyl bromide **9** (prepared by bromination of the corresponding toluene with NBS) with alcohol **3** provided the bromide **10** as a common intermediate. Suzuki reaction of bromide **10** with various substituted boronic acids **11** gave the biphenyl analogues **12** in reasonable yields.

Acetylenic, vinyl and alkyl analogues of methyl ether FTIs were tested for their inhibitory activity against both FTase and GGTase-1 enzymes. The effects of variation of the phenyl group in the phenyl acetylene series have been studied in detail (Table 1). The 4-NO_2 analogue (**21**) was found to be the most potent FTase inhibitor in this series. Both electron-donating and withdrawing groups are tolerated at the C3- and C4-position. Interestingly, introduction of the ethylene group between the phenyl group and acetylene had little effect on the potency (**17** vs **11**). To probe the steric volume of acetylene pocket we replaced the phenyl ring with a long aliphatic chain, but this had little impact on the potency (**14** vs **11**). The same phenomenon was also observed when the phenyl moeity was replaced with a bulkyl *t*-butyl group (**12** vs **11**). Only a 2-fold decrease in potency was observed when a polar hydroxyl group was introduced to the aliphatic chain (**14** vs **19**). Replacement of the phenyl group with a saturated cyclohexyl moiety (**20**) gave similar potency as well. In contrast, the inhibitory activity appeared to be affected by steric effects at the C4 position. Analogue **16** that possesses a large phenyl group at the C4-position is less potent than either the unsubstituted phenyl analogue **11** or cyano substituted phenyl analogue **18**. Finally, introduction of a nitrogen atom into the acetylenic phenyl ring resulted in decreased potency in both the FTase and GGTase-1 assays (**15** vs **11**).

With regard to activity against the GGTase, introduction of electron withdrawing groups such as nitro or cyano resulted in an increase in potency. In contrast, a large group at the C4 position caused a huge reduction in potency. In general, the acetylene series showed good selectivity with the exception of compounds **14**, **18**, and **21**, which have approximately only 150-fold selectivity in favor of FTase inhibition.



Scheme 2. (i) NaH, THF; (ii) $Pd(PPh_3)_4$, CsF, DME.

The introduction of a NO_2 or CN group at the C4-position of the phenyl ring in the acetylene series was found to result in a 116- to 360-fold decrease in Ras processing potency compared with analogue **11**. Introduction of a hydroxyl group at the C3 position caused a similar reduction in potency, and potency was further lowered by replacement of the phenyl moiety with aliphatic groups (cf. **12** and **17**). The only acceptable substituent in the Ras processing was found to be the cyclohexyl moiety (**20**).

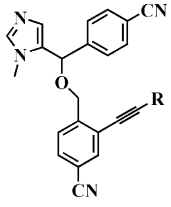
The effects of changing the acetylene to an olefin on the potency of inhibiting FTase (Table 2) have been studied with only a few analogues. The 4-NO_2 analogue **23** was found to be the most potent compound to inhibit FTase in this series. However, only a minor change in potency was observed when the 4-nitrophenyl group was replaced with either pyridine or a long aliphatic moiety.

The selectivity of olefinic analogues against FTase was also evaluated. It was found that replacement of the acetylene in the phenyl acetylene moiety with an olefinic group caused a 5-fold decrease in selectivity (**22** vs **15**). In contrast to this result, no change in selectivity was observed when the acetylene was replaced with an olefin in the 4-nitrophenyl analogues (**23** vs **21**).

Replacement of acetylene with an olefin in the acetylene series results in a 16-fold increase in Ras processing potency (**23** vs **21**). The nitro analogue **23** was identified as the most potent compound in the olefin series synthesized so far.

We then turned our attention to the effect of the acetylenic linker on the inhibitory potency (Table 3). Removal of the acetylene moiety of the phenyl acetylene analogue resulted in only minor change in potency in the inhibition of both the FTase and GGTase-1

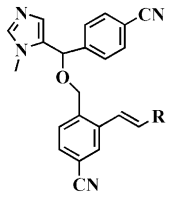
Table 1. FTase inhibition, GGTase-1 inhibition, and Ras processing data for acetylenic analogues **11**–**21**



Compd	R	FTase IC_{50} (nM)[a]	GGTase IC_{50} (nM)[b]	Selectivity (GGT/FT)	Ras EC_{50} (nM)[c]
11	Ph	0.95	1800	1900	0.1
12	*t*-Bu	0.62	490	790	76.9
13	3-OH–Ph	1	1300	1300	>0.1
14	*n*-C_6H_{12}	0.9	160	178	>0.1
15	2-Pyridine	2.9	16,000	5517	>0.1
16	4-Ph–Ph	10	>10,000	>1000	>0.1
17	–CH_2–CH_2–Ph	1.4	540	386	130
18	4-CN–Ph	0.63	124	196	11.6
19	–$(CH_2)_4$–OH	1.7	1600	941	20.9
20	1-OH-cyclohexyl	0.87	845	971	1.93
21	4-NO_2–Ph	0.55	150	273	36

[a]Concentration of compound required to reduce the human FTase-catalyzed incorporation of [^{3}H] FPP into recombinant Ras CVIM by 50%.
[b]Concentration of compound required to reduce the human GGTase-catalyzed incorporation of [^{3}H] GGPP into biotinylated peptide corresponding to the C-terminal of human K-Ras by 50%.
[c]Compound concentration needed to reduce 50% of farnesylation in NIH-3T3H-ras cell line.

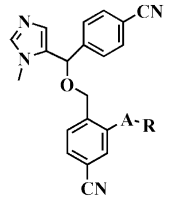
Table 2. FTase inhibition, GGTase-1 inhibition, and Ras processing data for olefinic analogues **22**–**24**



Compd	R_1	FTase IC_{50} (nM)[a]	GGTase IC_{50} (nM)[b]	Selectivity (GGT/FT)	Ras EC_{50} (nM)[c]
22	2-Pyridine	1.6	1800	1125	23.1
23	4-NO_2–Ph	0.61	110	180	2.24
24	–CH–CH–*n*-Pr	1.3	220	169	16.7

[a]Concentration of compound required to reduce the human FTase-catalyzed incorporation of [^{3}H] FPP into recombinant Ras CVIM by 50%.
[b]Concentration of compound required to reduce the human GGTase-catalyzed incorporation of [^{3}H] GGPP into biotinylated peptide corresponding to the C-terminal of human K-Ras by 50%.
[c]Compound concentration needed to reduce 50% of farnesylation in NIH-3T3H-ras cell line.

Table 3. FTase inhibition, GGTase-1 inhibition, and Ras processing data for biphenyl analogues **25**–**28** and **22**



Compd	R	A	FTase IC_{50} (nM)[a]	GGTase IC_{50} (nM)[b]	Selectivity (GGT/FT)	Ras EC_{50} (nM)[c]
25	Ph	Bond	1.3	3500	2690	140
11	Ph	C≡C	0.95	1800	1900	0.1
26	4-CN–Ph	Bond	1.3	9000	6900	<0.1
18	4-CN–Ph	C≡C	0.63	124	196	11.6
27	4-Ph–Ph	Bond	1.1	150	136	<0.1
16	4-Ph–Ph	C≡C	10	>10,000	>1000	>0.1
21	4-NO_2–Ph	C≡C	0.55	150	273	36
23	4-NO_2Ph	CH=CH	0.61	110	180	2.24
12	*t*-Bu	C≡C	0.62	490	790	76.9
28	*t*-Bu	CH_2CH_2	0.95	310	326	0.54

[a]Concentration of compound required to reduce the human FTase-catalyzed incorporation of [^{3}H] FPP into recombinant Ras CVIM by 50%.
[b]Concentration of compound required to reduce the human GGTase-catalyzed incorporation of [^{3}H] GGPP into biotinylated peptide corresponding to the C-terminal of human K-Ras by 50%.
[c]Compound concentration needed to reduce 50% of farnesylation in NIH-3T3H-ras cell line.

enzymes (**25** vs **11**). In contrast to this result, a 77-fold decrease in potency was observed in the inhibition of GGTase-1 when the acetylene was removed from the 4-CN phenyl analogue (**26** vs **18**). However, little change in the FTase potency was observed (Table 3). Reduction of the acetylene to an olefin had little effect on the inhibition of both enzymes (**23** vs **21**). The same trend was observed when the triple bond was reduced to a single bond (**12** vs **28**). In general, acetylenic analogues were less potent inhibitors of Ras processing activity than their corresponding alkyl, alkenyl or biphenyl analogues.

In summary, we have varied the substituent pattern at two phenyl rings of the imidazole acetylenic alcohol such as compound **1** and examined the resultant effects on inhibitory activity. The inhibitory activity (Tables 1–3) indicates that large substituents are not well tolerated at the C4-position of the phenyl ring. In general, compounds having either the phenyl or alkyl structure as shown in Table 1 possess acceptable inhibitory activities against the GGTase-1 enzyme, while the 2-pyridyl analogues possess the weakest activity. The selectivity for FTase inhibition over GGTase-1 is highest in the 2-pyridyl series as shown in Table 1. This study has also shown that the effect of the acetylene moiety on potency is substrate dependent. However, only a small effect is observed when the acetylene is reduced to either a double or single bond. Compound **11** is the most potent FTI identified from this study.

References and Notes

1. (a) Clarke, S. *Annu. Rev. Biochem.* **1992**, *61*, 355. (b) Sawyer, C. L.; Denny, C. T. *Cell* **1994**, *77*, 171.

2. Barbacid, M. *Annu. Rev. Biochem.* **1987**, *56*, 779.
3. Lowy, D. R.; Willumsen, B. M. *Annu. Rev. Biochem.* **1993**, *62*, 851.
4. Zhang, F. L.; Casey, P. J. *Annu. Rev. Biochem.* **1996**, *65*, 241.
5. Clarke, S.; Vogel, J. P.; Deschenes, R. J.; Stock, J. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4643.
6. Gutierrez, L.; Magee, A. I.; Marshall, C. J.; Hancock, J. F. *EMBO J.* **1989**, *8*, 1093.
7. Kohl, N. E.; Mosser, S. D.; deSolms, S. J.; Giuliani, E. A.; Pompliano, D. L.; Graham, S. L.; Smith, R. L.; Scolnick, E. M.; Oliff, A. J.; Gibbs, J. B. *Science* **1993**, *260*, 1934.
8. James, G. L.; Goldstein, J. L.; Brown, M. S.; Rawson, T. E.; Somers, T. C.; McDowell, R. S.; Crowley, C.; Lucas, B. K.; Levinson, A. D.; Marster, J. C., Jr. *Science* **1993**, *260*, 1937.
9. Nigam, M.; Seong, C.-M.; Qian, Y.; Hamilton, A. D.; Sebti, S. M. *J. Biol. Chem.* **1993**, *268*, 20695.
10. Wallace, A.; Koblan, K. S.; Hamilton, K.; Marqui-Omar, D. J.; Miller, P. J.; Mosser, S. D.; Omer, C. A.; Schaber, M. D.; Cortese, R.; Oliff, A. I.; Gibbs, J. B.; Pessi, A. *J. Biol. Chem.* **1996**, *271*, 31306.
11. Prendergast, G. *Curr. Opin. Cell Biol.* **2000**, 166.
12. Du, W.; Lebowitz, P. F.; Prendergast, G. C. *Mol. Cell. Biol.* **1999**, *19*, 183.
13. Prendergast, G. C.; Du, W. *Drug Resist. Update* **1999**, *2*, 81.
14. Lin, N.-H.; Wang, L.; Cohen, J.; Gu, W.-Z.; Frost, D.; Zhang, H.; Rosenberg, S.; Sham, H. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1293.