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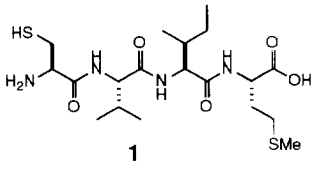
DIARYLETHER INHIBITORS OF FARNESYL-PROTEIN TRANSFERASE

Christopher J. Dinsmore,* Theresa M. Williams, Kelly Hamilton,[†] Timothy J. O'Neill,[†] Elaine Rands,[†] Kenneth S. Koblan,[†] Nancy E. Kohl,[†] Jackson B. Gibbs,[†] Samuel L. Graham, George D. Hartman, and Allen I. Oliff[†]

Department of Medicinal Chemistry and [†]Department of Cancer Research, Merck Research Laboratories, West Point, PA 19486

Abstract: The design and synthesis of simple nonpeptide inhibitors of farnesyl-protein transferase (FTase) are described. Cysteine-derived diarylether frameworks are appropriate structural replacements for the *C*-terminal tetrapeptide portion of the Ras protein, and possess in vitro potency against FTase. Inhibitory activity is dependent on the ring-substitution pattern, and does not require the presence of a *C*-terminal carboxylate group.

The important role of the *ras* oncogene product Ras p21 in growth regulatory signal transduction pathways, leading to either normal or abnormal cellular proliferation, is dependent on its post-translational modification which results in its ability to associate with the inner surface of the cell membrane.[1] Mutated Ras proteins have been implicated in cell transformation, and are frequently found in human cancers.[2] A critical modification of Ras is the *S*-farnesylation of a cysteine residue at the carboxy terminal tetrapeptide sequence, often referred to as the CaaX motif (e.g., CVIM **1**).[3] As this prenylation event is catalyzed by the farnesyl-protein transferase enzyme (FTase), inhibition of FTase is thought to be an attractive approach for the development of safe and selective anticancer drugs, and this has stimulated aggressive research activity.[4] Thus far, FTase inhibitors (FTIs) have been shown to prevent protein farnesylation in cell culture and to selectively suppress *ras*-transformed cell growth.[4,5] Further validation has been achieved by the inhibition of *ras*-dependent tumor growth in nude mice as well as the regression of tumors in Ha-*ras*-transgenic mice.[6]



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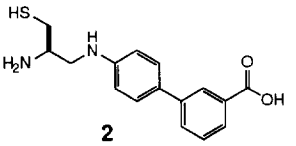

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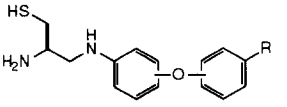

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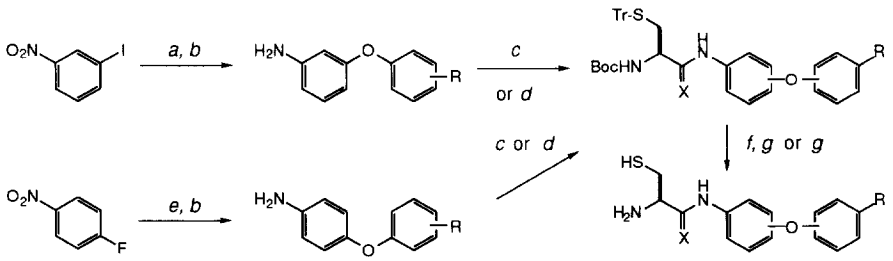
Many classes of FTase inhibitors that mimic the CaaX motif of Ras have been described.[7] Recent reports have revealed that the *C*-terminal tripeptide aaX portion can be dramatically simplified to such peptidomimetic fragments as carboxybiphenyls (e.g. **2**)[7b,c] as well as noncarboxylate pseudopeptides[7d] and acylpiperazines[7e] to furnish FTIs with significant in vitro and in vivo activity against the enzyme. As a part of our program to design conformationally constrained mimics of the CaaX motif, we investigated the diarylether fragment as a hydrophobic surrogate for aaX which contains elements of rigidity and limited flexibility in the form of a central hinge (e.g., **3**). In this paper, we describe the syntheses and activity profiles of a number of these FTIs, including the impact of regiochemistry and substituent effects on inhibitory activity.

A flexible synthetic approach to the desired substrates (Scheme 1) relied on the Ullmann coupling of 3-iodonitrobenzene or the S_NAr substitution of 4-fluoronitrobenzene to produce aminodiarylether templates with the

Scheme 1.



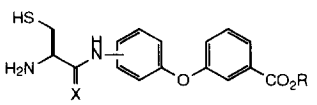
(a) R-C_6H_4-OH, NaH, CuBr•DMS, Py, 80 °C. (b) Fe, HOAc, MeOH, reflux. (c) for X = H_2; *N*-Boc-*S*-Tr-cysteinal, $Na(AcO)_3BH$, 4 Å MS, AcOH, $ClCH_2CH_2Cl$. (d) for X = O; *N*-Boc-*S*-Tr-cysteine, EDC, HOBt, DMF. (e) R-C_6H_4-OH, K_2CO_3, DMF, 80 °C. (f) for R = CO_2H; NaOH, THF, H_2O, 0 °C. (g) TFA, Et_3SiH, CH_2Cl_2, 0 °C-rt.

meta or *para* substitution pattern, respectively. Amide coupling or reductive coupling was followed by either ester hydrolysis and acid-promoted deprotection of the cysteinyl moiety or by deprotection alone to afford the carboxylate and non-carboxylate diarylether FTIs.

Peptide-derived FTIs such as **1** (IC_{50} 165 nM)[3b] have generally exhibited potency ~100-fold greater than that of the corresponding *C*-terminal methyl esters, indicating the important role of the carboxylate group in binding to the enzyme.[3-5] Recently, the biological activity of the biaryl FTI **2** (IC_{50} 150 nM) was attributed in part to an interaction of the negatively charged carboxylate with a positively charged residue in the enzyme active site.[7b,c] As such, the initial design of diarylether derivatives in this study incorporated the carboxylate group as a putative binding element (*e.g.* **3**, R = CO_2H).

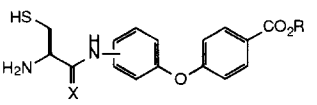
A systematic comparison of the relative activities of regioisomeric diarylether inhibitors against FTase was conducted in an effort to discern the optimal substitution for this array (Tables 1 and 2). Unexpectedly, the potencies of the diarylether acids were only ~3-to 6-fold greater than those of the corresponding methyl esters (e.g. **3b** vs. **3a**), suggestive of only weak participation by the acid functionality at best. As had been defined in other FTI structural classes,[7a] reduction of the cysteine amide to an amine provided a significant enhancement of

Table 1



compd	isomer	X	R'	IC_{50} (nM)[a]
3a	*meta*	O	Me	45,000
3b	*meta*	O	H	6,900
3c	*meta*	H_2	H	250
4a	*para*	O	Me	45,000
4b	*para*	O	H	16,000
4c	*para*	H_2	H	403

Table 2



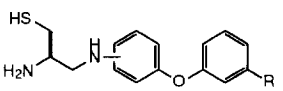
compd	isomer	X	R	IC_{50} (nM)[a]
5a	*meta*	O	Me	>100,000
5b	*meta*	O	H	>100,000
5c	*meta*	H_2	H	3,800
6a	*para*	O	Me	>100,000
6b	*para*	O	H	39,000
6c	*para*	H_2	H	3,150

(a) Concentration of compound required to reduce the FTase-catalyzed incorporation of [^{3}H]FPP into recombinant Ha-Ras by 50%. The assay used enzyme purified from bovine brain at a concentration of ca. 1 nM, as described in ref 5a.

potency (e.g. **3c** vs. **3b**), perhaps owing to the relaxation of an unfavorable conformational restriction. An analysis of the four possible variations in *meta* and *para* regiochemistry revealed that although the aminoaryl ring tolerated either pattern quite well with only a slight preference for *meta*-substitution, the *C*-terminal benzoate ring was much more sensitive to positional changes. Thus, *meta-meta* isomer **3c** (IC_{50} 250 nM) was only two-fold more potent than the corresponding *para-meta* isomer **4c**, but >10-fold more potent than either the *meta-para* (**5c**) or *para-para* (**6c**) isomers.

Additional structure-activity relationships confirmed the notion that in this partially flexible diarylether series the carboxylic acid functional group is not required for binding. In a panel of *para-meta*-substituted diarylethers (Table 3), the stepwise decrease in oxidation state of the *C*-terminal substituent, and ultimately its removal, resulted in little overall change in activity. Although the potency of benzoic acid analog **4c** (IC_{50} 403 nM) is slightly enhanced relative to the methyl ester **4d** (650 nM), the neutral benzyl alcohol derivative **7** (335 nM) is just as active. In fact, nonpolar substituents such as methyl (**8**, 615 nM) and hydrogen (**9**, 460 nM) are effective replacements for the carboxylate group, suggesting that subtle steric interactions around the terminal ring environment may contribute as significantly to potency in this series as an ionic component. Interestingly, the *meta*-series exhibited enhanced sensitivity to substituent effects. While the methyl-substituted (**10**, 330 nM) and carboxyl-substituted (**3c**, 250 nM) derivatives were very similar, the unsubstituted diarylether was 10-fold less potent (**11**, 2,300 nM).

Table 3



			IC_{50} (nM)[a]		soft agar IC_{90} (μM)[c]	
compd	isomer	R	FTase	GGTase-I[b]	Ha-*ras*	*raf*
4c	*para*	CO_2H	403	nd	≥50	≥50
4d	*para*	CO_2Me	650	2,300	10-30	10-30
7	*para*	CH_2OH	335	nd	~30	30-50
8	*para*	CH_3	615	nd	10-30	10-30
9	*para*	H	460	5,000	10-30	10-30
3c	*meta*	CO_2H	250	5,800	>50	>50
10	*meta*	CH_3	330	nd	nd	nd
11	*meta*	H	2,300	nd	nd	nd

(a) See corresponding footnote in Table 1. (b) See ref 5a for assay conditions. (c) Concentration required to achieve a 90% reduction in size and number of colonies of RAT1 v-Ha-*ras* (Ras2B) or RAT1 v-*raf*-(Raf1a1) transformed cells in soft agar relative to vehicle-treated control. Assay conditions are described in ref 5b.

Several of the diarylethers selectively inhibited FTase over the closely related geranylgeranyltransferase-I (GGTase-I, Table 3), an enzyme that recognizes CaaX sequences containing leucine as the terminal residue.[5a] Despite significant modifications of the substituent at the *C*-terminal aryl ring, selectivity was maintained in the 3- to 20-fold range. In an assay that measured inhibition of cell growth in soft agar, compounds that lack a carboxyl

substituent (**4d**, **8**, **9**) reduced the size and number of colonies of Ha-*ras* transformed Rat1 cells by 90% at 10-30 μM concentrations. Derivatives with more polar substituents required higher concentrations (**7**, IC_{90} ~30 μM; **4c**, ≥50 μM). Notably, this growth inhibition was not selective, as inhibition of *ras*-independent *raf*-transformed cells occurred at similar IC_{90} values. Thus, although the compounds possess inhibitory activity against FTase in vitro, no conclusion can be drawn with regard to the mechanism of inhibition of cell growth in soft agar; the observed activity in cell culture may be derived from a mechanism other than the inhibition of Ha-Ras farnesylation.

The results demonstrate that structural simplification of the carboxy-tetrapeptidic CaaX box using a diarylether core is a viable strategy toward noncarboxylate FTase inhibitors with in vitro activity. The optimization of this new structural class is ongoing.

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