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Diaryl Ether Inhibitors of Farnesyl-Protein Transferase

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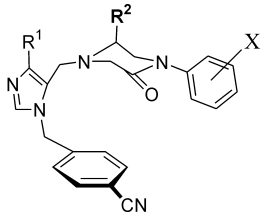
Abstract—Imidazolemethyl diaryl ethers are potent inhibitors of farnesyl-protein transferase. The S_NAr displacement reaction used to prepare these diaryl ethers was amenable to rapid parallel synthesis of FPTase inhibitors. The use of a broad range of commercially available phenols quickly identified compounds which proved active in cells.

The protein encoded by *ras* is an important part of the signal transduction pathway in the life cycle of the cell. Mutations in the *ras* gene can lead to uncontrolled cell growth and have been reported in human tumors.[1] A crucial post-translational modification required for activation of the Ras protein is farnesylation of the C-terminus. Blockade of this activation step through inhibition of farnesyl-protein transferase (FPTase) has been accomplished by an assortment of synthetic compounds as possible therapeutics in the treatment of human cancer.[2–4] In particular, piperazinones of the general formula **1a** have been shown to be potent inhibitors of FPTase.[5]

In order to generate structural information about the bound conformation of these inhibitors, transferred NOE (trNOE) studies using the FPTase enzyme and piperazinone **1b** ($IC_{50} = 475$ nM) were performed.[6] The results support the folded conformation shown for these compounds in the active site with the *p*-cyanophenyl moiety positioned underneath the piperazinone ring. Examination of a model based on the trNOE data indicated that the lipophilic binding site occupied by the X-phenyl group in **1b** could be accessed from the cyanophenyl group rather than from the imidazole group.[7,8] In support of this proposal, Figure 1 shows an overlay of **1b** (in a conformation consistent with trNOE data) with a low energy conformer of the biphenyl ether **2**.[9] This indicates that, when the imidazole and cyanophenyl groups are closely aligned, the biphenyl group of **2** is indeed able to reach the lipophilic pocket occupied by the X-phenyl group of **1b**.



1a $R^1 = R^2 = H$
1b $R^1 = CH_3$,
$R^2 = CH_2CH_2SO_2CH_3$,
X = *m*-Cl

To test this hypothesis, compound **2** was prepared as outlined in Scheme 1. It was tested against both FPTase and the closely related enzyme geranylgeranyl-protein transferase-I (GGPTase-I). **2** inhibited the in vitro farnesylation of Ras by FPTase (Table 1, $IC_{50} = 86 \pm 43$ nM) and was more selective versus GGPTase-I ($IC_{50} = 12{,}000$ nM). Related analogues (Table 1) were synthesized using the same methodology with the exception of compound **3**, which was prepared as shown in Scheme 2. Removal of the phenyl group to give **5** led to a 10-fold loss in potency. The effect of altering the spacing between the aryl groups was explored. Extending the chain length by one or two carbon atoms (**6** and **7**) improved potency against FPTase 2- to 4-fold. Truncation to a two-atom linker (**4**) resulted in slightly reduced activity. Further contraction to the diaryl ether provided compound **3** with

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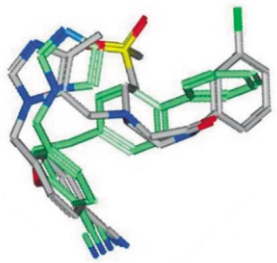
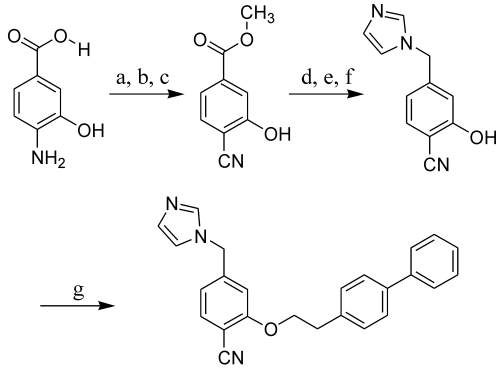


Figure 1. Stick overlay (**2** in green).



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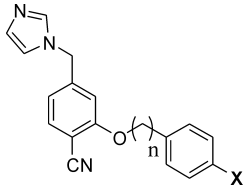
Scheme 1. Reagents and conditions: (a) CH_3OH, HCl, rt, 5 h, 96%; (b) HCl, $NaNO_2$, THF, 0 °C, 20 min; KI, 45 min, 14%; (c) $Zn(CN)_2$, $Pd(Ph_3P)_4$, DMF, 100 °C, 1 h, 83%; (d) $LiBH_4$, THF, reflux 22 h, 48%; (e) CBr_4, Ph_3P, DMF, CH_2Cl_2, rt, 1.5 h, 78%; (f) imidazole, DMF, rt, 16 h, 64%; (g) 2-(1,1′-biphenyl-4-yl)ethyl methanesulfonate, Cs_2CO_3, DMF, 80 °C, 1.5 h, 63%.

Table 1. Activity of unsubstituted arylalkyl ethers



Compounds	X	*n*	FPTase IC_{50} (nM)[a]	GGPTase-I IC_{50} (nM)[b]
2	Ph	2	86±43	12,000±1000
3	H	0	230	3700±900 (2)
4	H	1	1500	6800±900 (2)
5	H	2	860	7000±3100 (2)
6	H	3	200	3100±900 (2)
7	H	4	440	380±50 (3)

[a]Concentration of compound required to inhibit by 50% the rate of incorporation of [^{3}H]FPP into recombinant Ras-CVIM by human FPTase.

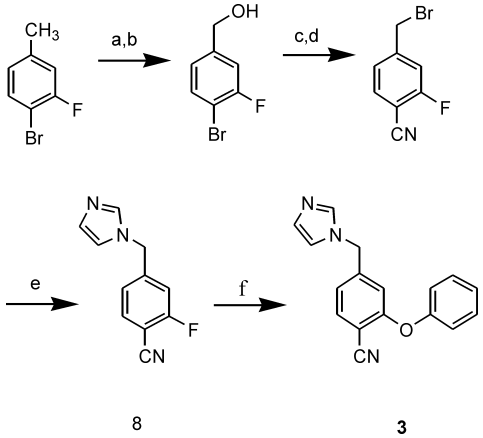
[b]Concentration of compound required to inhibit by 50% the rate of incorporation of [^{3}H]GGPP into a biotinylated peptide corresponding to the C-terminus of human Ki-Ras by human GGPTase-I. In both FPTase and GGPTase-I, results from a single experiment are reported unless noted otherwise in parentheses; values are estimated to be reliable within 2-fold. In the FPTase assay, two sets of dilution plates were prepared and tested in all experiments.

potency comparable to **6** and only 3-fold less potent than the initial lead compound **2**. A somewhat different pattern was observed for GGPTase-I activity, which was minimally affected as the length of the linker increased from one to four atoms but improved 10-fold with the five-atom linker in **7**.

The amenability of the synthetic route outlined in Scheme 2 to rapid analogue synthesis led to the identification of compound **3** as a promising structure for further exploration. An S_NAr displacement reaction was used to couple *N*-(4-cyano-3-fluorobenzyl) imidazole **8** with a wide variety of commercially available phenols. Representative examples of compounds prepared in this manner are shown in Table 2. Single chlorine substitution in any of the positions gave compounds (**9**, **11**, and **13**) that were 4-fold more potent than the unsubstituted parent **3**. Methoxy (**14**, **15**, and **16**) and phenyl (**17**, **18**, and **19**) substitution exhibited a marked preference for the *meta*-position. This finding was reinforced by the loss in potency seen in going from 2-naphthyl (**20**) to 1-naphthyl (**21**).

Other *meta*-substituents in Table 2 show an increase in their ability to inhibit the FPTase enzyme with an increase in size, such as the 4- to 6-fold increase of the halogens from F to Cl to Br (**10**, **11**, and **12**). The alkyne (**26**) and its analogous, less potent nitrile (**27**), in conjunction with the acetyl (**28**) and *t*-butyl (**29**), suggest that there is a large space that can accommodate these structures.

Disubstituted phenoxy compounds (**20**–**25**) were among the most potent. Interestingly, the 2,4-dichloro compound **22** was 5-fold more potent against FPTase than the mono-substituted analogues with concomitant improvement in GGPTase-I activity. 2,3-Dimethoxy analogue **24** was a potent FPTase inhibitor (IC_{50} 1 nM) but a very weak inhibitor of GGPTase-I (IC_{50} 15 μM).

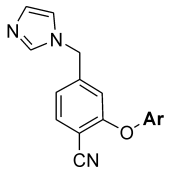


8 **3**

Scheme 2. Synthesis of *N*-(4-cyano-3-fluorobenzyl)imidazole **8** and S_NAr displacement by phenol. Reactions and conditions: (a) $KMnO_4$, pyridine, H_2O, reflux, 40 h, 94%; (b) BH_3·THF, 5 °C to rt, 24 h, 92%; (c) $Zn(CN)_2$, $Pd(Ph_3P)_4$, DMF, 95 °C, 18 h, 66%; (d) NBS, DMS, CH_2Cl_2, −20 to 0 °C to rt, 18 h, 65%; (e) imidazole, DMF, rt, 18 h, 51%; (f) phenol, Cs_2CO_3, DMF, 50 °C, 3 h, 88%.

The 3-phenyl substituted compound **18** is among the more potent FPTase inhibitors. The biphenyl unit was extended by insertion of several heteroatom linkers (Table 3). Inhibitory potency against FPTase was generally independent of the electronic nature of the linker as the amides **34** and **35**, ketone **31**, ether **32**, and aniline **33** were equipotent. However, the methylene-linked compound **30** was 16-fold less active. In the GGPTase-I assay, the amides were the least well-tolerated among the linking elements.

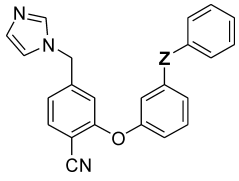
Table 2. Mono- and disubstituted phenyl ethers



Compounds	Ar	FPTase IC_{50} (nM)[a]	GGPTase-I IC_{50} (nM)[a]
9	2-Cl-Ph	41±12 (2)	1100±200 (2)
10	3-F-Ph	180	4400
11	3-Cl-Ph	53±20 (2)	3200±600 (2)
12	3-Br-Ph	38	2900
13	4-Cl-Ph	38±18 (2)	920±90 (3)
14	2-OCH_3-Ph	340	28,000
15	3-OCH_3-Ph	40	3500
16	4-OCH_3-Ph	290	3500
17	2-Ph-Ph	110	740±80 (2)
18	3-Ph-Ph	9.6	520
19	4-Ph-Ph	3400	1300
20	2-Naphthyl	1.1±0.1 (2)	520
21	1-Naphthyl	150	3200
22	2,4-diCl-Ph	8	280
23	3,4-diCl-Ph	28	1200
24	2,3-diOCH_3-Ph	1	15,000
25	2,5-diOCH_3-Ph	100	18,000
26	3-Ethynyl-Ph	27	2600
27	3-Cyano-Ph	130±10 (2)	16,000
28	3-Acetyl-Ph	45	6100
29	3-*t*-Butyl-Ph	27±13 (2)	520

[a]See corresponding notes in Table 1.

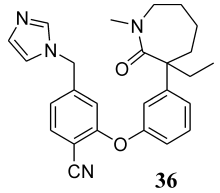
Table 3. Heteroatom aryl linkers



Compounds	Z	FPTase IC_{50} (nM)[a]	GGPTase-I IC_{50} (nM)[a]
30	CH_2	230	600
31	C=O	13	840
32	O	14±1.0 (2)	280
33	N	17	630
34	C(O)NH	38±21 (2)	3300±1000 (3)
35	HNC(O)	38	2000±30 (2)

[a]See corresponding notes in Table 1.

The attempt to discover novel structures by screening a wide variety of commercially available phenols in this library led to compound **36**. The inclusion of the amide group into the otherwise lipophilic seven-membered ring of the large, racemic caprolactam **36** yielded one of the most potent compounds, both in the FPTase and GGPTase-I assays.



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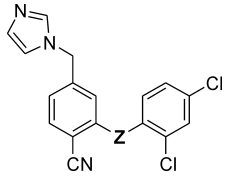
FPTase IC_{50} 2.9 nM
GGPTase-I IC50 7.1 nM

The nucleophilic aromatic substitution reaction also provided a facile synthesis of diaryl thioethers (Table 4). The thioether **37** was significantly more potent than the ether analogue **22**. The sulfoxide **38** and sulfone **39** of thioether **37** were also prepared and tested. As the oxidation state of the sulfur increased, potency drastically decreased in both the FPTase and GGPTase-I assays.

The most potent of the library compounds were tested further in a cell-based assay which measured displacement of a radiolabeled inhibitor from FPTase in cells.[10] Of the compounds tested, the racemic caprolactam **36** had the highest FPTase occupancy level in cells with an IC_{50} of 4.9 nM (Table 5).

From the analysis of information provided by trNOE experiments, a novel series of aryl ether inhibitors was elucidated. Identification of a core structure (**3**) that could be readily prepared by derivatization of the

Table 4. Effect of oxidation on thioethers



Compounds	Z	FPTase IC_{50} (nM)[a]	GGPTase-I IC_{50} (nM)[a]
22	O	8	280
37	S	<1	23±12 (3)
38	S=O	390	2000±800 (2)
39	O=S=O	5400	8800±2000 (2)

[a]See corresponding notes in Table 1.

Table 5. Cell-based assay

Compound	FPTase cell assay IC_{50} (nM)[a]
22	260
24	14
36	4.9±0.1 (2)
37	96±53 (3)

[a]Concentration of sample required to displace 50% of a radiolabeled farnesyltransferase inhibitor (FTI) from FPTase in cultured v-Ha-*ras*-transformed RAT1 cells.[10]

fluoro-cyano intermediate **8** via a one-step synthesis expedited the discovery process. Overall, the *meta*-position was recognized as the best site for optimization. Commercial availability of a wide variety of substrates broadened the number and scope of substrates that could be investigated. Inhibitors were discovered that were potent and selective against farnesyl-protein transferase and showed a wide range of activity against geranylgeranyl-protein transferase-I. Selected compounds exhibited activity in cell culture. Further elaboration of these diaryl ethers will be reported in subsequent publications.

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9. One hundred conformations of **2** were generated using JG. The subsequent conformations were energy minimized with Macromodel using the procedure outlined above. The resulting energy minimized conformations were superimposed using SQ. Miller, M. D.; Sheridan, R. P.; Kearsley, S. K. *J. Med. Chem.* **1999**, *42*, 1505.
10. Lobell, R. B.; Gibson, R., manuscript in preparation. To assess the potency of the FTIs in cells, a competitive binding assay was utilized to determine the concentration of the FTI required to displace 50% of a radiolabeled FTI from FPTase in cultured v-Ha-*ras* transformed RAT1 cells. This radiotracer, [^{125}I] 4-{[5-({(2*S*)-4-(3-iodophenyl)-2-[2-(methylsulfonyl)-ethyl]-5-oxopiperazin-1-yl}methyl)-1*H*-imidazol-1-yl]methyl}-benzonitrile, has $\sim$50,000 high affinity binding sites (apparent K_d of $\sim$1 nM) in the Rat1 cell line. The nonspecific binding signal, determined by the addition of 1000-fold excess unlabeled competitor FTI, is typically 5-fold lower than the specific binding signal. The assay provides comparable results using a variety of cell lines, and is performed as follows. Cells are seeded at 200,000 cells per well in 24-well tissue culture plates and cultured for 16 h. The radiotracer ($\sim$300–1000 Ci/mmol) is diluted into culture media to a concentration of 1 nM, along with the desired concentration of test FTI, and then added to the cell monolayers. After a 4 h incubation at 37 °C, the cells are briefly rinsed with phosphate-buffered saline, removed from the culture plate by trypsinization, and then subjected to gamma counting using a CobraII® gamma counter (Packard Instrument Company). Dose-inhibition curves and IC_{50} values are derived from a four-parameter curve-fitting equation using SigmaPlot® Software.