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POTENT AND ORALLY BIOAVAILABLE NONCYSTEINE-CONTAINING INHIBITORS OF PROTEIN FARNESYLTRANSFERASE

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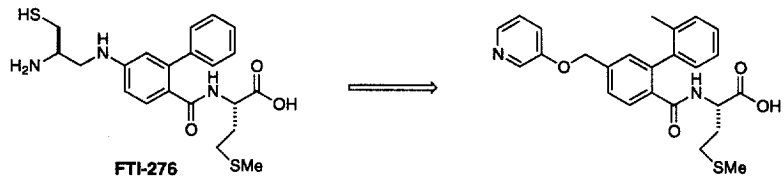
Abstract: Potent and orally bioavailable nonthiol-containing inhibitors of protein farnesyltransferase are described. Oral bioavailability was achieved by replacement of the pyridyl ether moiety of **1** with a 2-substituted furan ether to give **4**. Potency was regained with 2,5-disubstituted furan ethers while maintaining the bioavailability inherent in **4**. *p*-Chlorophenylfuran ether **24** is 0.7 nM in vitro (FTase) and is 32% bioavailable in the mouse, 30% bioavailable in rats, and 21% bioavailable in dogs.

Oncogenic Ras proteins are found in 20–30% of all human tumors including those of the lung (30%), colon (50%), and pancreas (90%).[1] Mutated Ras proteins are constitutively active and promote uncontrolled cell division.[2] The Ras protein is functional only after undergoing a series of posttranslational modifications. Farnesylation of a cysteine residue near the C-terminus by the enzyme farnesyltransferase (FTase) is required for membrane association and signal transduction. Inhibition of FTase will render Ras inactive and block the uncontrolled mitogenic signaling pathway.[3–7]

Several structurally distinct classes of farnesyltransferase inhibitors are known.[8] The first reported inhibitors were designed to mimic the Ras C-terminal tetrapeptide (CVFM), which is the minimum recognition sequence. FTI-276 is an example of this class of inhibitors where the central two amino acids have been successfully replaced by a hydrophobic biphenyl core and are flanked by cysteine and methionine.[9,10]

Figure 1



FTI-276
IC_{50} FTase = 0.5 nM

IC_{50} FTase = 0.4 nM

We recently reported the transformation of FTI-276 into the potent noncysteine-containing FTase inhibitor **1** (Figure 1).[11] Although **1** proved to be potent in vitro and active in whole cells as the methionine acid, it displayed poor bioavailability. Earlier, we observed that phenyl ether **2** displayed significantly better absorption characteristics than the corresponding pyridyl ether, albeit at a great cost in potency (IC_{50} pyridyl ether = 4.0 nM, **2** = >1 μM, Figure 2). However, it answered an important question concerning bioavailability. It was clear that the pyridyl ether moiety posed a pharmacokinetic liability.

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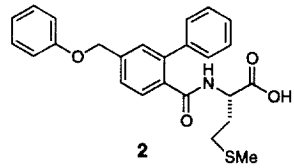
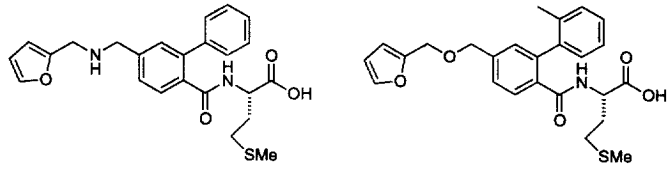
Figure 2



2

The *ortho*-methyl biphenyl core has shown a dramatic uniform increase in potency of every noncysteine-containing farnesyltransferase inhibitor that we have synthesized.[11] This has given us great latitude when designing cysteine replacements. A combinatorial chemistry library of various benzylic amines furnished us with furfurylamine derivative **3** (IC_{50} = 560 nM). We desired a potent compound devoid of a basic nitrogen. Accordingly, **4** was synthesized to improve the pharmacokinetic profile (Figure 3).

Figure 3



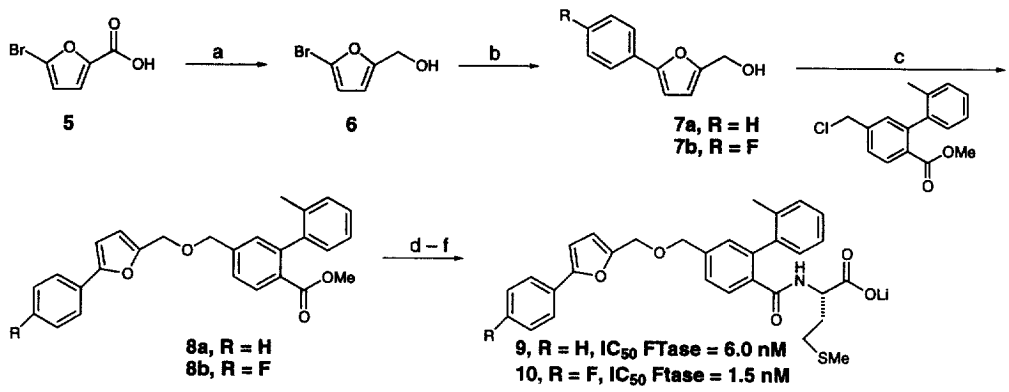
3 **4**

In vitro assay against FTase showed **4** to be 43 nM in potency. Bioavailability of **4** in rats was measured to be 30%. This result stimulated a research effort to build potency into furan ether **4** without sacrificing bioavailability. To that end, it was decided to substitute the furan at the 5-position to investigate the size of the binding site. Aryl groups were chosen simply because of the ease of chemistry and commercially available starting materials.

Commercially available 5-bromo-2-furoic acid **5** was transformed to alcohol **6** and coupled to phenylboronic acid and 4-fluorophenylboronic acid using Suzuki conditions to give **7a** and **7b**. These furan adducts were coupled to the benzyl chloride activated *o*-methyl substituted biphenyl core using a crown ether mediated SN2 displacement to give **8a** and **8b**. Intermediates **8a** and **8b** contain aryl methyl esters that were hydrolyzed, coupled to L-methionine methyl ester, hydrolyzed and then lyophilized to give their respective lithium salts **9** and **10** as white amorphous solids (Scheme 1).

In vitro assay against FTase showed **9** and **10** to be 6.0 nM and 1.5 nM in potency, respectively. The EC_{50} is a measure of inhibition of ras processing in a whole cell assay. Compound **9** showed 70% inhibition of ras processing at 10 μM while **10** showed an EC_{50} = 0.45 μM. In addition, preliminary IV and ID pharmacokinetic data indicated that **9** and **10** maintained good absorption profiles. It was clear that a substituted phenyl ring appended to the 5-position of the furan looked promising. These results prompted further investigation.

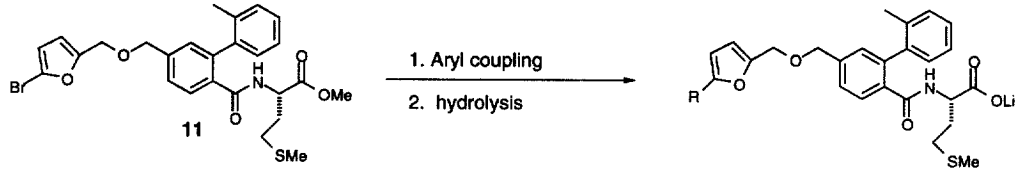
Scheme 1



a. IBCF, NMM, THF, 0 °C ; $NaBH_4$ 92% b. $RPhB(OH)_2$, $PdCl_2(PPh_3)_2$, Cs_2CO_3, DMF, 80 °C, 60-72% c. NaH, 15-crown-5, DMF, 25 °C R = H, 82%; R = F, 18% d. LiOH, MeOH, H_2O, reflux, 85-90%. e. L-methionine methyl ester HCl, EDAC, HOOBT, TEA, DMF, 81-94% f. LiOH, 1.05 equiv., THF, H_2O, quant.

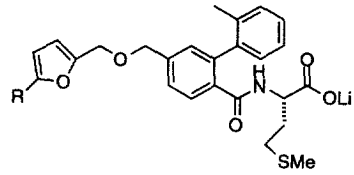
It was decided to prepare a library of various aryl-substituted furan ethers that could lead us to the most potent and bioavailable compound in this series. We decided to perform the aryl coupling as late as possible in the synthesis with commercially available boronic acids. Accordingly, multigram quantities of late intermediate **11** (IC_{50} = 3.0 nM) was prepared using the same chemistry outlined in Scheme 1 to give us access to any substituted system in two steps. A Suzuki coupling followed by hydrolysis of the methionine methyl ester gave us a focused library of various aryl-substituted furan ethers (Table 1). Pyridyl-substituted furans were synthesized using Stille couplings followed by hydrolysis of the methionine methyl ester.

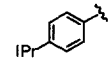
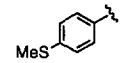
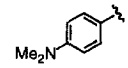
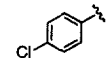
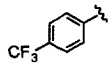
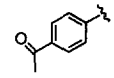
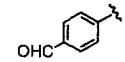
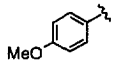
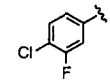
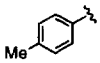
Scheme 2



The fluoro-(**10**, **12**, **13**), trifluoromethyl-(**14**, **15**, **16**) and methoxy-(**17**, **18**, **19**) substituted phenyl series were prepared in ortho, meta, and para substituted fashion. It was evident from these compounds that the para position of the aryl ring at the 5-position of the furan ring is optimal for potency. The nature of the substituent is somewhat important as the most potent compounds contain an electron-withdrawing substituent at the para position (**10** and **24**). Strongly electron donating groups rendered those compounds (**17** and **22**) less potent.

Table 1



Compd	R group	IC_{50} (nM)	EC_{50} (μM)	Compd	R group	IC_{50} (nM)	EC_{50} (μM)
9		6.0	>1	**21**		2.9	0.78
10		1.5	0.44	**22**		6.2	10
12		7.6	10	**23**		2.6	0.90
13		6.3	>1	**24**		0.7	0.10
14		3.0	1.2	**25**		6.0	10
15		8.8	---	**26**		6.6	>1
16		9.9	---	**27**		1.2	>1
17		8.4	>1	**28**		4.9	---
18		12	>1	**29**		2.4	>0.3
19		20	>1	**30**		6.5	0.3
20		2.9	1.8	**31**		19	>0.3

The *p*-chlorophenylfuran **24** was the most potent of the series with an IC_{50} of 0.7 nM and an EC_{50} of 0.10 μM. Importantly, **24** retained the pharmacokinetic profile inherent in **4**, the lead compound in this series. As detailed in Table 2 and Table 3, **24** is 30% orally bioavailable in rats and 21% orally bioavailable in dogs. The compounds contained herein are greater than 10,000-fold selective for FTase over GGTase I.

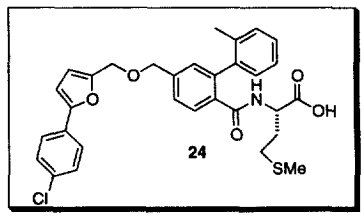


Table 2. PK Profile in Conscious Rats

$T_{1/2}$ (h)	Vc (l/kg)	$AUC_{0-\infty}$ (μg•h/mL)	C_{max} (mg/mL)	T_{max} (h)	$AUC_{0-\infty}$ (μg.h/mL)	F (%)
1.3	0.21 ± 0.07	8.1 ± 0.83	1.25 ± 0.38	0.7 ± 0.2	2.39 ± 0.11	30 ± 1.4

IV dose **24**, 10 mg/kg (n = 4); Oral dose **24**, 10 mg/kg (n = 3).

Table 3. PK Profile in Conscious Dogs

$T_{1/2}$ (h)	Vc (l/kg)	$AUC_{0-\infty}$ (μg•h/mL)	C_{max} (mg/mL)	T_{max} (h)	$AUC_{0-\infty}$ (μg.h/mL)	F (%)
1.24	0.08 ± 0.01	4.937 ± 0.407	0.713 ± 0.529	0.4 ± 0.25	1.040 ± 0.293	21.1 ± 5.93

IV dose **24**, 10 mg/kg (n = 4); Oral dose **24**, 10 mg/kg (n = 3).

In conclusion, we were successful in identifying **4**, a 43 nM noncysteine-containing CAAX box inhibitor of farnesyltransferase devoid of a basic nitrogen that proved to be orally bioavailable. We built 0.7 nM in vitro potency and 100 nM cellular potency into this compound through substitution of the furan ring to give **24**, which retains the PK profile of the lead compound **4**. Future effort will be dedicated toward improving cellular potency.

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