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POTENT INHIBITORS OF PROTEIN FARNESYLTRANSFERASE: HETEROARENES AS CYSTEINE REPLACEMENTS

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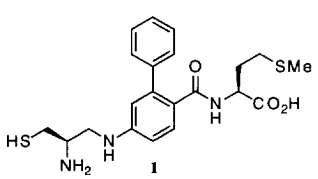
Abstract: Synthesis and biological evaluation of heteroarenes as reduced cysteine replacements are described. Of the heteroaryl groups examined with respect to FT inhibitor FTI-276 (**1**), pyridyl was the replacement found to be most effective. Substitutions at C4 of the pyridyl moiety did not affect the *in vitro* activity. Compound **9a** was found to have moderate *in vivo* bioavailability.

Introduction The *ras* oncogene product, Ras p21, is a key player in regulation of cell proliferation.[1] In a significant percentage of human cancers, genetic mutations lead to the production of mutant Ras proteins which are constitutively activated.[2] Both the wild-type and the mutant Ras proteins must undergo a series of post-translational modifications involving the C-terminus CAAX box, where C is cysteine, A is an aliphatic amino acid, and X is serine or methionine. Farnesyltransferase (FT) catalyzed *S*–farnesylation of the cysteine residue of CAAX box is the essential step of the process.[3] Animal models of cancers with *ras* mutations have demonstrated that FT inhibitors are efficacious and yet surprisingly nontoxic,[4a,4b] though their exact mechanism of action appears to be more complicated than initially thought.[4] Furthermore, FT inhibitors were found to inhibit the growth of cultured cancer cells with either mutated or wild type *ras*.[4b] Synergistic effects between FT inhibitors and either cytotoxic agents (taxol and epothiolones) [5a] or radiation[5b] have been observed on human cancer cell lines. Intensive research efforts have been focused on identifying and developing small molecule inhibitors of FT as potential anticancer agents,[6] and clinical trials of at least two FT inhibitors have started.[7] Recently, this laboratory reported the modification of FTI-276 (**1**)[8] to a potent inhibitor **2**, where a pyridyl ether function has replaced the reduced cysteine moiety.[9] We wish to report further structure-activity relationship (SAR) investigations of heteroarenes aimed at improving both *in vitro* and *in vivo* potencies.



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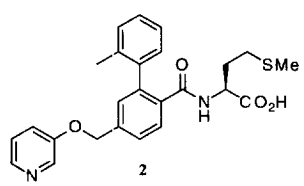

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Chemistry To better meet the need for large quantities of advanced intermediates, the Suzuki coupling of aryl chlorides was developed during the course of this investigation.[10] As shown in Scheme 1, chloride **3**, derived

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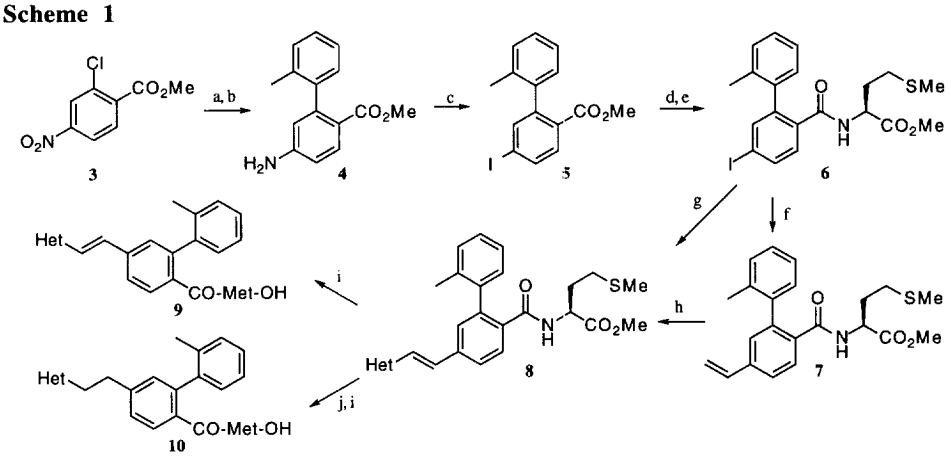
from commercially inexpensive 2-chloro-4-nitrobenzoic acid, was coupled with *o*-tolylboronic acid, followed by hydrogenation, to give **4** in 91% yield. The amine was converted to iodide **5** by a Sandmeyer reaction, followed by elaboration of the ester functionality to provide methionine amide **6**. A Stille reaction with tributyl(vinyl)tin gave vinylbiphenyl **7** in 92% yield. A series of *trans* alkenes (**8**) was synthesized utilizing Heck chemistry by either reacting vinylheteroarenes with **6** or reacting heteroaryl halides with **7**. Saponification gave acid **9**. Alternatively, **8** could be first hydrogenated and then converted to acid **10**. The hydrogenation step required 1.2 molar equivalents of palladium, presumably due to catalyst poisoning by the methionine sulfide.

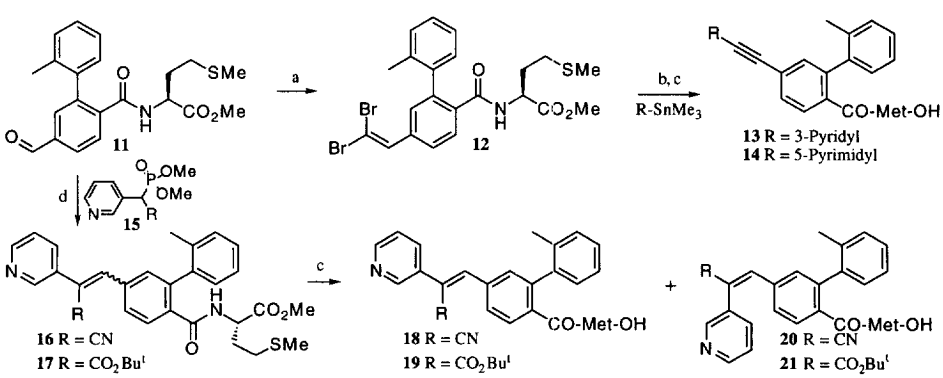
Scheme 1



(a) *o*-TolylB(OH)$_2$, Pd(PPh$_3$)$_2$Cl$_2$ (3 mol%), aqueous Na$_2$CO$_3$, toluene, reflux; (b) H$_2$ (1 atm), 10% Pd/C, MeOH, 91%, 2 steps; (c) NaNO$_2$, KI, 6.0 N HCl/acetone (1/1 vol.), −5 to 0 °C, 77%; (d) LiOH (saturated, aqueous), MeOH, reflux; (e) *L*-methionine methyl ester hydrochloride, EDAC, HOBt, Et$_3$N, THF, 89%; (f) tributyl(vinyl)tin, Pd(PPh$_3$)$_2$Cl$_2$, toluene, reflux, 92%; (g) vinylheteroarene, Pd(dppf)Cl$_2$, Et$_3$N, DMF, 100 °C, 55-79%; (h) Heteroaryl–X (X = I, Br, OTf), Pd(dppf)Cl$_2$, Et$_3$N, DMF, 100 °C, 41-85%; (i) 1.0 N LiOH, MeOH, 100%; (j) H$_2$ (1 atm), 10% Pd/C (120 mol% Pd), MeOH, 45-100%.

Unsymmetrical alkynes **13** and **14** were synthesized from a novel Stille reaction between vinyldibromide **12** and a corresponding heteroaryl(trimethyl)tin.[11] Trisubstituted alkenes **18–21** were synthesized from aldehyde **11**[9] and known phosphonates **15**.[12] The olefin stereochemistry was determined by 2-D NMR.

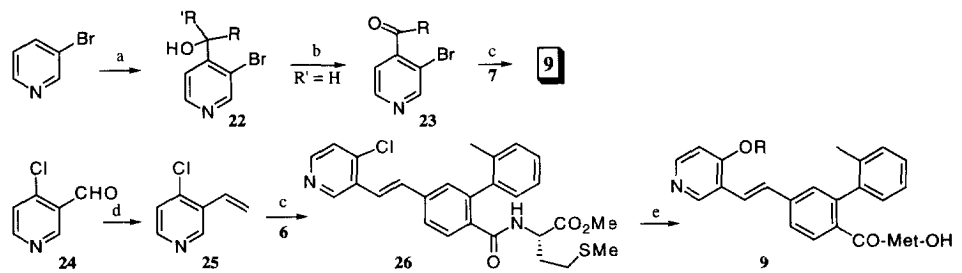
Scheme 2



(a) CBr$_4$, PPh$_3$, 0 °C, 95%; (b) 3-pyridyl(trimethyl)tin [or 5-pyrimidyl(trimethyl)tin], Pd(PPh$_3$)Cl$_2$, EtN*i*Pr$_2$, DMF, 80 °C, 70-75%; (c) 1.0 N LiOH, MeOH, 100%; (d) KO*t*Bu, THF, 75-80%.

Because the initial biological results indicated that *trans* alkenyl pyridine (**9a**) was preferred (Table 1), we next explored substitutions on the pyridyl ring of **9**. Substitutions at C5 and C6 (Table 1) resulted in less potent compounds than parent **9a**, however, no loss in *in vitro* potency was observed with C4 substitutions. For the preparation of the later derivatives, 3-bromopyridine was deprotonated and then reacted with aldehydes or ketones to give **22** in good yields.[13] Oxidation gave pyridyl ketones **23**. Both alcohols **22** and ketones **23** were subjected to Heck reactions as illustrated in Scheme 1. Attempts to deoxygenate alcohols **22** failed, due to difficulty in cation generation (Et_3SiH, TFA) or interference of bromine in palladium catalyzed hydrogenolysis. Ketopyridines (**9o, 9r**) derived from **23** are less basic than parent **9a**. 4-Hydroxymethylpyridines (**9l, 9m, 9n, 9p, 9q**) derived from **22** are of similar basicity. On the other hand, 4-alkoxypyridines (**9t** to **9w**) derived from known 4-chloro-3-formylpyridine (**24**)[14] are more basic than pyridine. Large appendant alkyl groups render some of these compounds considerably more lipophilic than the parent **9a**.

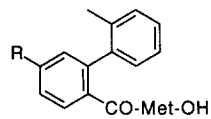
Scheme 3



(a) LDA, THF, -78 °C, R(CO)R', 50-75%; (b) TPAP, NMO, DCM, 81-95%; (c) **7** (or **6**), $Pd(dppf)Cl_2$, Et_3N, DMF, 100 °C 67%; (d) $MePPh_3Br$, LDA, THF, 100%; (e) RONa, THF, then (one-pot), LiOH, 1.0 N, 85-100%.

Biological Results and Discussion The *in vitro* FT inhibitory activities (IC_{50})[9] were determined against FT using the SPA assay. The substrates used were ^{3}H-farnesyl pyrophosphate and a biotin-linked K-ras(B) decapeptide (KKSKTKCVIM). The cellular H-ras processing inhibitory activities (EC_{50})[9] were determined with subconfluent NIH3T3 Ras transformed cells. The results are summarized in Table 1. It was previously determined by combinatorial and traditional medicinal chemistry efforts[9] that 3-pyridine linked by a 2-atom spacer to the biphenyl core is important in replacing the reduced cysteine moiety in FTI-276 (**1**). As such, nitrogen-containing heteroarenes were chosen. It was found that pyridine and imidazole were superior to other heteroarenes (entries 1–11). The reduced cysteine thiol in FTI-276 (**1**) was assumed to bind to zinc(II) ion in the active site of FT similar to the cysteine residue in the CAAX box, thus the more basic nitrogens in pyridine and imidazole might provide a better replacement for cysteine thiol for Zn(II) coordination. Pyridine is better than imidazole in the cellular assay for inhibition of Ras processing (entries 2–8), possibly because the more basic imidazole might prevent the inhibitors from penetrating cell membranes. The spacer is also important for activity. Where the pyridyl is *cis* to the biphenyl core, compounds **20** and **21** are less active than the *trans* examples **18** and **19** respectively (entries 14–17). Alkynes **13** and **14** are 5-fold less potent than the corresponding *trans* alkenes **9a** and **9e**. When the *trans* alkenyl and alkyl pyridines **9a** and **10a** and imidazoles **9b** and **10b** were subjected to pharmacokinetic (pk) studies (iv and po) in rats, the alkenes (**9a, 9b**) were found to be more orally bioavailable.

Table 1. FT inhibitory and Ras processing activities.



Entry	R (compound)	IC_{50} (nM)	EC_{50} (μM)	Entry	R (compound)	IC_{50} (nM)	EC_{50} (μM)
1	FTI-276 (**1**)	0.51	1.0[a]	18	**9g**	16	>1
2	**2**	0.40	0.35	19	**9h**	0.80	64% inh. @ 1
3	**9a**	0.61	79% inh. @ 0.3 (0.13)	20	**9i**	26	nd
4	**10a**	2.1	nd[b]	21	**9j**	4.7	>1
5	**9b**	0.20	>1	22	**9k**	12	nd
6	**10b**	0.21	>1	23[c]	**9l**	0.54	63% inh. @ 0.3
7	**9c**	0.63	>1	24	**9m**	0.81	74% inh. @ 1
8	**10c**	0.15	>1	25	**9n**	0.58	67% inh. @ 0.3
9	**9d**	2.4	>1	26	**9o**	0.92	1.0
10	**9e**	25	nd	27	**9p**	0.66	63% inh. @ 1
11	**9f**	57	nd	28[d]	**9q**	0.37	61% inh. @ 1
12	**13**	2.7	1.0	29[d]	**9r**	0.12	74% inh. @ 1
13	**14**	140	nd	30	**9s**	0.29	60% inh. @ 0.3
14	**18**	0.59	57% inh. @ 0.3	31	**9t**	0.29	75% inh. @ 0.3
15	**20**	3.6	60% inh. @ 0.3	32	**9u**	2.9	66% inh. @ 0.3
16	**19**	0.91	80% inh. @ 0.3	33	**9v**	1.4	49% inh. @ 0.3
17	**21**	15	35% inh. @ 0.3	34	**9w**	0.30	nd

[a] See Reference 9. [b] nd: not determined. [c] Chex: cyclohexyl. [d] Adam: adamantyl.

These results led to examination of substituted *trans* pyridyl alkenes. Substitutions at pyridyl C6 (**9g**, **9i**) gave 100 fold decrease in biological activities. Substitutions at C5 (**9j**, **9k**) also caused a drop in IC_{50} and EC_{50}

values. Isoquinolinyl (**9h**) showed similar activity to pyridyl (**9a**), possibly due to the relatively small substitution at C5. Substitutions at C4 are well tolerated, and the resulting compounds (**9l** to **9w**, entries 23 to 34) are also of similar *in vitro* potency. Large lipophilic groups, such as diphenylmethyl and 1-adamantyl (entries 27, 28), do not affect the inhibitory activities both against the enzyme and in the cellular Ras processing assays.

The pharmacokinetics of the compounds with good *in vitro* activities were evaluated in rats. Imidazoles (**9b**, **10b**) showed very poor bioavailability compared to pyridines, and compounds with alkoxy substituents at pyridyl C4 (**9t** to **9w**) also showed poor values, possibly due to higher basicity. However, compounds with lipophilic substitution (**9l** to **9r**) also showed reduced bioavailability. Compound **9a** was found to be the most favored pharmacokinetically among all the pyridines[15] examined. Pharmacokinectic data for studies of compound **9a** in the rat and the dog are listed in Table 2.

Table 2. Pharmacokinetic Data of Compound **9a**.

Species	Dosage (mg/kg)[b]	IV Dose			Oral Dose			
		t1/2 (h)	Vc (l/kg)	AUC (0-∞) (μg•h/mL)	Cmax (μg/mL)	Tmax (h)	Auc (0-∞) (μg•h/mL)	F[c] (%)
Rat[a]	10	2.15	0.35	6.66	0.321	0.33	0.87	23
Dog[a]	5	0.55	0.06	10.5	2.83	0.38	2.56	24.5

[a] Blood plasma samples were analyzed by HPLC. [b] N = 3 for both the rat and the dog. [c] Bioavailability (F) = Oral AUC / IV AUC

In conclusion, we have determined that *trans* pyridyl alkene **9a** represents the best heteroarene in replacing the reduced cysteine moiety in FTI-276. This compound not only retains both FT inhibition and whole cell Ras processing inhibition activities, but also demonstrates an improved pharmacokinetic profile in the rat and the dog. Further work on this series and related ones continues as we search for FT inhibitors with better pharmacokinetic profiles and *in vivo* efficacious in nude mice implanted with human tumors.

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