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Novel and Selective Imidazole-Containing Biphenyl Inhibitors of Protein Farnesyltransferase

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Abstract—A series of imidazole-containing biphenyls was prepared and evaluated in vitro for inhibition of FTase and cellular Ras processing. Several of these analogues, such as **21**, are potent inhibitors of FTase (< 1 nM), FTase/GGTase selective (> 300-fold) and cellularly active (≤ 80 nM). An X-ray crystal structure of inhibitor **21** bound to rat farnesyltransferase is also presented.
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Ras proteins play an integral role in the transmission of signals from extracellular stimuli to the nucleus and the ras gene has been identified as one of the most frequently mutated genes in human cancers.[1,2] Ras proteins must undergo a series of posttranslational modifications to provide the active species, the first occurring through the transfer of a farnesyl group from farnesylpyrophosphate (FPP) to the cysteine near the C-terminus (CAAX box) of the protein.[3] This transfer is catalyzed by the enzyme protein farnesyltransferase (FTase). Thus, the inhibition of FTase represents a potential target for the development of small molecule anticancer agents.[4]

A number of structurally distinct families of farnesyltransferase inhibitors (FTIs) have been disclosed in the literature.[5] Diverse examples from two of these classes include imidazole-containing R115777 (**1**, FTase IC_{50} 0.57 nM)[6] and tricyclic inhibitor SCH66336 (**2**, FTase IC_{50} 7.8 nM),[7] both of which are reported to be in Phase III clinical trials for cancer.[8] Another structural class encompasses the RAS C-terminal tetrapeptide mimics in which the central two amino acids have been replaced with a biphenyl core which is flanked by cysteine and methionine.[9] Our laboratories have published extensively on a modification of this motif in which the cysteine is replaced with aryl, alkyl or heterocyclic side chains;[10] this work culminated in the discovery of the clinical candidate ABT-839 (**3**, FTase IC_{50} 1.0 nM).

1, R115777

2, SCH66336

3, ABT-839

4

Because many of these amino acid-containing biphenyl compounds possessed modest bioavailability and a short half-life, potent analogues lacking the methionine moiety were desired. Despite the observation that the methionine residue is crucial for efficient binding of these compounds with FTase, a series of potent inhibitors arising from the lead structure **4** (FTase IC_{50}

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65 nM) has recently been discovered.[11] While that work was on-going, a separate series of compounds was examined in which the biphenyl/imidazole hydroxymethylene-tether was replaced by an aminomethylene linker with alkyl and alkylaryl substituents on the tether nitrogen (e.g., **9**, Scheme 1). Previous work on amino acid-containing biphenyl inhibitors with this imidazole tether[12] suggested that this modification would be permitted although its effect on the enzyme selectivity or cellular activity of these compounds was not clear. The SAR of this tether-modified series against FTase and cellular RAS processing is described here.

The preparation of the tether-modified compounds (Scheme 1) began with methyl 3-chloro-4-cyanobenzoate, prepared in two steps from 4-amino-2-chlorobenzonitrile,[13] which was coupled with 2-methylphenyl boronic acid in good yield;[14] saponification of the biphenyl ester with LiOH at room temperature gave carboxylic acid **5**. This compound was subjected to Curtius reaction conditions in *t*-BuOH and the resulting *N*-(*tert*-butoxycarbonyl)aniline was isolated and deprotected to afford aniline **6**. Using reductive amination conditions,[15] aniline **6** was coupled with aldehyde **7**, prepared by regioselective formylation of 1-methyl-2-triethylsilylimidazole[16] with *N*-formylmorpholine and *tert*-butyllithium, to give imidazole **8** after in situ silyl deprotection. This compound was then *N*-alkylated with a variety of alkyl halides using KO*t*Bu to afford inhibitors such as **9**.[17] Substitution of 2-methylboronic acid with 1-naphthylboronic acid in the first step of the synthesis gave naphthylbiphenyl analogues such as **21** in comparable yields.

Synthesis of the imidazole *N*-desmethyl analogues were prepared as shown in Scheme 2. Aniline **6** was coupled with 1-(triphenylmethyl)imidazole-4-carboxaldehyde, prepared in two steps from 4-(hydroxymethyl)-imidazole,[18] under reductive amination conditions to give compound **10**; deprotection of the imidazole provided inhibitor **11**.

Scheme 1. Reagents and conditions: (a) 2-methylphenylboronic acid, CsF, cat. $Pd(OAc)_2$, cat. 2-(dimethylamino)-2′-dicyclohexylphosphinobiphenyl, dioxane, 80%; (b) LiOH, 1:1 THF/MeOH, 91%; (c) DPPA, Et_3N, *t*-BuOH; (d) TFA, CH_2Cl_2, 56% two steps; (e) **7** (2 equiv), $NaBH(OAc)_3$ (2.7 equiv), AcOH (5 equiv), DCE, 68%; (f) benzyl chloride, KO*t*Bu, THF, 0 °C, 70% (g) *t*-BuLi, 4-formylmorpholine, THF, −78 °C, >95%.

The ability of these compounds to inhibit FTase in vitro was examined along with their selectivity against the related prenyltransferase, geranylgeranyltransferase I (GGTase), which is believed to be essential for normal cellular processes (Table 1).[19] While the parent imidazolylmethyl compound **8** was only weakly active against FTase (IC_{50} 1 μM), substitution of the biphenyl/imidazole-tether nitrogen improved potency significantly. As it had been reported that addition of 4-substituted benzyl groups to other non-peptidic, imidazole-containing FTIs enhanced potency,[20] initial work focused on benzyl and cyanobenzyl substitution. As expected, *N*-benzyl derivative **9** (FTase IC_{50} 11 nM) was much more potent than **8**, but equipotent with the 4-cyanobenzyl analogue **12**. Interestingly, the 3-cyano compound **13** (FTase IC_{50} 0.97 nM) was 10-fold more potent than the 4-cyano inhibitor. Both **12** and **13** were also quite selective for inhibition of FTase versus GGTase (100- to 1000-fold).

The presence and substitution of the imidazole was important for enzymatic activity. *N*-Desmethyl analogue **11** (FTase IC_{50} 84 nM) was a weaker inhibitor than **9**, while replacement of the imidazole of **9** with a 3-pyr-

Scheme 2. Reagents and conditions: (a) 1-(triphenylmethyl)imidazole-4-carboxaldehyde (2 equiv), $NaBH(OAc)_3$ (2.7 equiv), AcOH (5 equiv), DCE, 74%; (b) benzyl chloride, KO*t*Bu, THF, 0 °C, 92%; (c) Et_3SiH, TFA, CH_2Cl_2, 87%.

Table 1. FTase and GGTase inhibition of *o*-tolyl analogues

Compd	R	FTase IC_{50} (nM)[19]	GGTase IC_{50} (nM)[19]
8	H	1000	ND
9	$PhCH_2$	11	ND
12	(4-CNPh)CH_2	10	1000
13	(3-CNPh)CH_2	0.97	1200

Table 2. FTase and GGTase inhibition of naphthyl analogues

Compd	R	FTase IC_{50} (nM)[19]	GGTase IC_{50} (nM)[19]
14	H	31	ND
15	Methyl	38	ND
16	*n*-Hexyl	1.9	180
17	$PhCH_2$	1.5	ND
18	PhCO	14	310
19	$PhSO_2$	18	1900
20	$(4\text{-}CNPh)CH_2$	1.5	1000
21	$(3\text{-}CNPh)CH_2$	0.39	150
22	$(3\text{-}ClPh)CH_2$	0.45	230
23		0.37	650

idyl gave a significant loss of potency (FTase IC_{50} 450 nM, compound not shown).

In light of the observation that replacement of the *o*-tolyl of **4** with 1-naphthyl gave a > 20-fold enhancement of FTase inhibitory activity,[11] most of the tether substitution work was completed on this biphenyl core (e.g., **14** in Table 2). Regardless of tether substitution, the naphthyl analogues were consistently more potent against FTase than the corresponding *o*-tolyl compounds by 3- to 30-fold. The unsubstituted and tether *N*-methylated analogues (**14** and **15**, respectively) were the weakest in this series while nitrogen substitution with *n*-hexyl or benzyl gave very potent inhibitors (FTase IC_{50} 1.9 nM and 1.5 nM, respectively). The *N*-benzoyl (**18**) and *N*-benzenesulfonyl (**19**) compounds had intermediate enzymatic potency against FTase. Consistent with the *o*-tolyl series SAR, the 4-cyanobenzyl analogue **20** was equipotent with the benzyl-substituted inhibitor **17** while the 3-cyano derivative **21** exhibited the best potency of the series (FTase IC_{50} 0.39 nM). The use of 3-chlorobenzyl (**22**) or (3-cyanopyrid-4-yl)methyl (**23**) gave inhibitors equipotent with **21**. The imidazole *N*-desmethyl analogue of **17** was somewhat less potent (FTase IC_{50} 11 nM, compound not shown).

Table 3. Enzymatic and cellular data for selected inhibitors

Compd	FTase IC_{50} (nM)[19]	RAS EC_{50} (μM)[21]
1	0.57	0.002
2	7.8	0.16
12	10	29% inh. @ 1
13	0.97	0.07
20	1.5	64% inh. @ 1
21	0.39	0.05
22	0.45	45% inh. @ 1
23	0.37	0.08

Concerning GGTase inhibitory activity, all compounds in the naphthyl series had significant FTase/GGTase

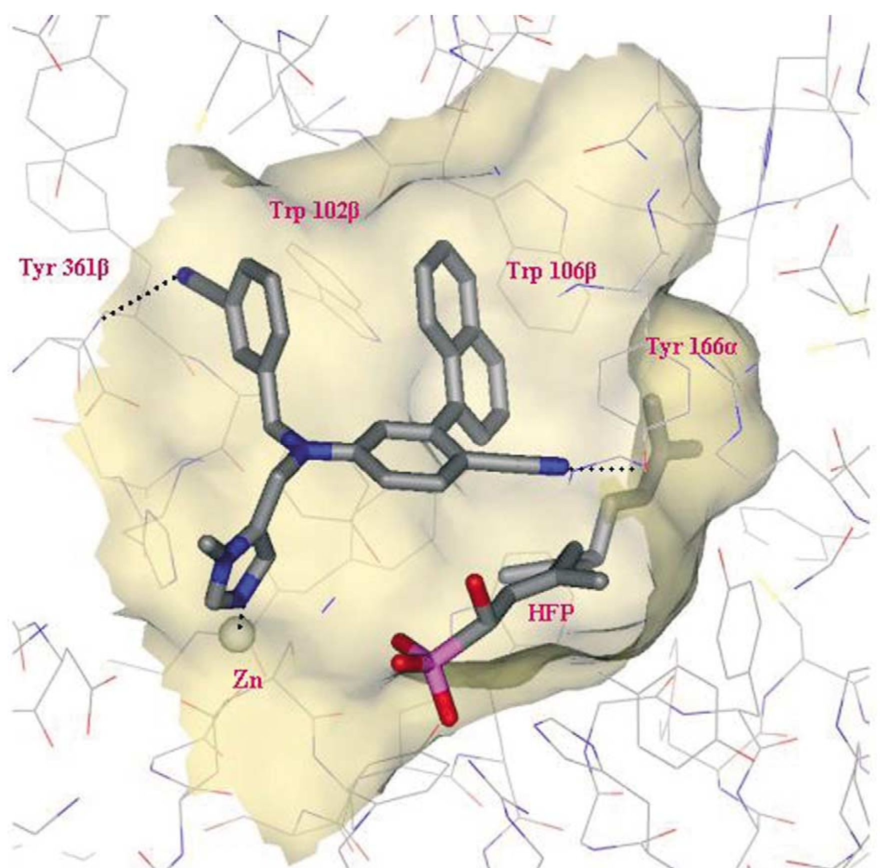

Figure 1. Inhibitor **21** bound to rat farnesyltransferase and α-hydroxyfarnesylphosphonic acid (HFP).

selectivity ($\geq$90-fold) with inhibitor **23** being the most selective (1600-fold).

The cellular potency of selected compounds was evaluated in a RAS processing assay and a portion of that data, including the reference inhibitors **1** and **2**, is shown in Table 3.[21] While there was a general correlation between FTase enzymatic activity and inhibition of RAS processing (e.g., **12** vs **13**), there were a number of examples of equipotent FTase inhibitors with significantly different cellular activity (e.g., **21** or **23** vs **22**). The most potent inhibitor in this assay (**21**) compared favorably to clinical candidate **2** in both enzymatic and cellular potency.

However, pharmacokinetic studies in rat of several of these inhibitors, including compound **21**, revealed short half-lives ($\leq$1 h) and low oral bioavailability (<10%).

An X-ray crystal structure was acquired for inhibitor **21** bound to rat farnesyltransferase along with α-hydroxyfarnesylphosphonic acid (HFP), an FPP analogue (Fig. 1).[22,23] It was suspected that the imidazole-containing biphenyl inhibitors (e.g., **4** and **9**) maintain binding affinity to the FTase active site by replacing the important methionine-enzyme interaction of inhibitor **3** with an interaction between the imidazole and the putative active site Zn^{2+} ion. The data from the X-ray structure supported this hypothesis and also revealed several other key inhibitor/enzyme interations which are consistent with the SAR of this series. In possibly the most important interaction, the distal imidazole nitrogen of **21** coordinates with the active site zinc (2.36 Å) through displacement of a water molecule, an interaction not observed with the methionine-containing biphenyl inhibitors such as **3** but similar to the zinc-imidazole interaction of FTase-bound **1**.[22b] The naphthyl moiety sits in a hydrophobic pocket formed by Trp102β and Trp106β, with a naphthyl/phenyl dihedral angle of 60.1°. The tether *N*-benzyl ring fits on a shelf created by Tyr361β with the *m*-cyano substituent resting in a niche formed by the displacement of a water molecule and making a hydrogen bond with the enzyme backbone (3.22 Å). The crystal structure also revealed several interactions between **21** and the bound HFP as well as a hydrogen bond between the biphenyl cyano group and the side-chain of Tyr166β (3.47 Å).

In summary, a series of potent and novel imidazole-containing biphenyl inhibitors of FTase has been discovered which has significant FTase/GGTase selectivity and cellular Ras processing inhibitory activity. An X-ray crystal structure of the most potent member of the series with rat farnesyltransferase revealed several key inhibitor/enzyme interactions including an imidazole/zinc coordination and two cyano/enzyme hydrogen bonds. Analysis of several of these compounds in rat indicated an unacceptable pharmacokinetic profile. Further modification of the imidazole-containing biphenyl FTase inhibitors will be reported in due course.

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