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Aryloxy Substituted *N*-Arylpiperazinones as Dual Inhibitors of Farnesyltransferase and Geranylgeranyltransferase-I

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Abstract—A series of aryloxy substituted piperazinones with dual farnesyltransferase/geranylgeranyltransferase-I inhibitory activity was prepared. These compounds were found to have potent inhibitory activity in vitro and are promising agents for the inhibition of Ki-Ras signaling.

Introduction

Oncogenically activated Ras protein has been implicated in the growth of 20–30% of all human tumors.[1] Transforming mutations eliminate the intrinsic GTPase activity of Ras, resulting in constitutively activated Ras and growth signaling independent of extracellular growth factors, leading to uncontrolled proliferation. Among the *ras* genes, Ki-*ras* is the most relevant target for an anticancer agent since this accounts for approximately 90% of the altered Ras found in human cancers. Strategies for controlling Ras mediated oncogenic cellular proliferation have focused on preventing the prenylation of Ras by inhibition of farnesyl-protein transferase (FPTase), an enzyme which catalyzes the *S*-alkylation of a cysteine residue in the C-terminal tetrapeptide sequence of Ras.[2] This post translational modification is required for Ras activation and its inhibition in altered Ras should control proliferation. FPTase inhibitors (FTIs) have been shown to selectively inhibit *ras*-transformed cell growth in cell culture, to inhibit the growth of *ras*-dependent tumors in mice, and are currently undergoing human clinical trials both as single agents and in combination with other anti-cancer agents.[3] However, Ki-Ras prenylation in FTI treated cells has been reported.[4] When farnesylation of Ki-Ras is inhibited by an FTI, geranylgeranyltransferase-I (GGPTase-I), an analogous prenyltransferase, is able to activate Ki-Ras through geranylgeranylation. Thus, dual FPTase/GGPTase-I inhibitors should prevent prenylation of Ki-Ras, and have significant potential as cancer chemotherapeutic agents.

Inhibitor Design

A wide array of FPTase inhibitors that mimic the Ca_1a_2X tetrapeptide C-terminus of Ras have been described.[3d,e] Improvements in the biological properties of FTIs have been achieved through the use of non-peptide structural replacements for the central a_1a_2 portion, the deletion of the carboxyl-containing terminus, and substitution of the cysteine moiety with alternative non-thiol groups. Extensive work in this area resulted in the identification of piperazinone FTIs,[5] such as **1** (Fig. 1), which underwent clinical evaluation.[3c,e] This compound is a dual prenyl-protein transferase inhibitor (FPTase $IC_{50}=2$ nM, GGPTase-I $IC_{50}=98$ nM).[6] Our desire to improve GGPTase-I inhibitory activity prompted investigation of piperazinone derivatives with substitution on the cyanobenzyl ring. Through this work, it was discovered that aryloxy substitution (e.g. **2**) can have an enhancing effect on GGPTase-I activity. Herein, we describe the optimization of these compounds as dual prenyl-transferase inhibitors.

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Synthesis of Inhibitors

The cyanofluorobenzyl imidazole aldehydes **6** were prepared from the corresponding bromofluorotoluenes as outlined in Scheme 1. For example, 1-(4-cyano-3-fluorobenzyl)-5-imidazolecarboxaldehyde **6a** was prepared in five steps starting from 4-bromo-3-fluorotoluene. Cyanation with zinc cyanide was followed by NBS bromination to give **4a**. A protected imidazole was alkylated with this benzyl bromide giving **5a**. Deprotection of the hydroxyl group and subsequent oxidation gave the aldehyde **6a**.

Reductive amination of aldehydes **6a** and **6b** with the piperazinone amine **7**[7] was followed by treatment of the product with an aryloxide and cesium carbonate in DMF to give the desired aryloxypiperazinones **8** and **9** (Scheme 2). Compounds without the nitrile (e.g., **10**) were prepared by reductive alkylation of the appropriate aryl piperazinone **7** with aldehyde **6c**, followed by Ullmann coupling[8] with phenol.

Structure–Activity Relationships

Aryloxy substitution on the cyanophenyl ring has a profound effect on GGPTase-I activity while leaving FPTase activity relatively unaffected (Table 1). Addition of phenoxide to the 3-position of **1** gave **8a** (FPTase IC_{50} = 7 nM, GGPTase-I IC_{50} = 22 nM). This represented a 5-fold increase in affinity for GGPTase-I while maintaining FPTase potency similar to **1**. Substituting thiophenoxy in this position (**8b**) reduced affinity for FPTase. In some cases, substitution on the aromatic ring had a significant effect on activities. The *ortho*-chlorophenoxy derivative **8c** had the most profound effect, maintaining the high intrinsic FPTase potency of **1** while increasing GGPTase-I activity over 100-fold (GGPTase-I IC_{50} = 0.7 nM). The *meta* and *para* chloro analogues were equipotent to unsubstituted **8a** in GGPTase-I inhibition. Increasing the size of the phenoxy substituent had a deleterious effect on GGPTase-I activity. Compounds **8g** and **8h** had reduced GGPTase-I inhibitory activity relative to **1** but maintained high potency versus FPTase.

It was found that some compounds behave as slow tight binders to GGPTase-I.[6a,b] In these cases, in vitro studies in which IC_{50} values are measured without prior incubation of the inhibitor and enzyme gave higher IC_{50} values then those with a 30 min incubation period. For example, **8c** and **8i** exhibited ca. 30-fold differences in IC_{50} (Table 1). Furthermore, inhibition of GGPTase-I by **8c** was determined to be competitive with geranylgeranylpyrophosphate (GGPP) and dependent on the presence of inorganic or organic phosphate ions. Many different phosphates could satisfy this requirement, and ATP was chosen because it is effective at physiological concentrations.[6a,b,10]

Previous studies have documented the importance the cyano group plays in FPTase inhibition activity.[11] It was anticipated that reorienting or removing this group could reduce FPTase activity while maintaining GGPTase-I activity. In an attempt to produce a GGPTase-I selective inhibitor, such modifications were explored (Table 2). In compound **9a** the orientation of the nitrile and phenoxy groups was reversed. Unexpectedly, FPTase potency was maintained while GGPTase-I potency suffered. In compounds **10a–c** the cyano group

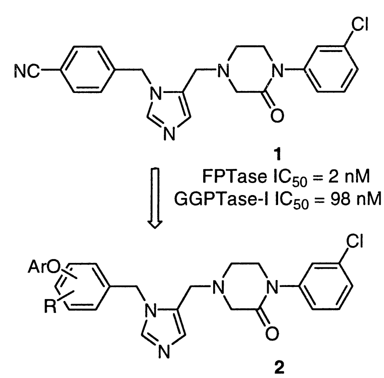
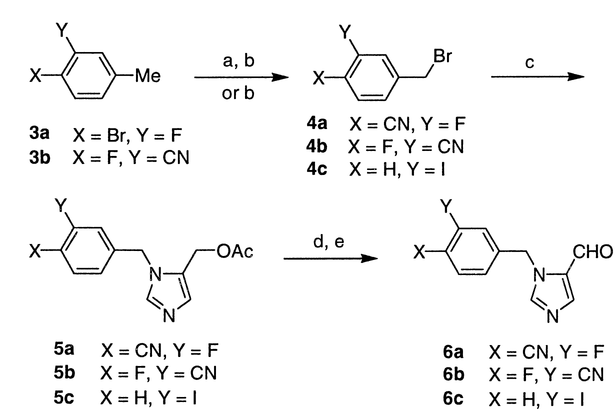
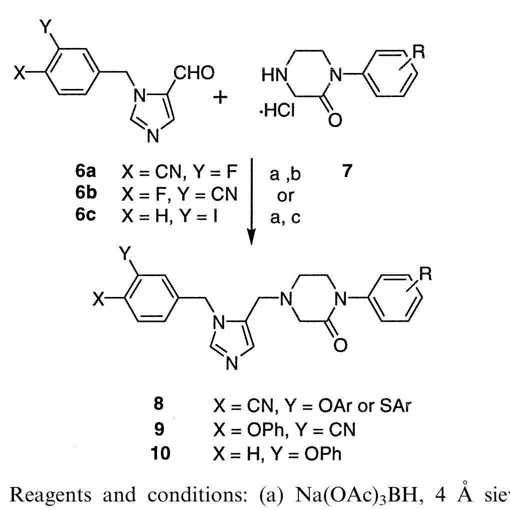


Figure 1. Design of improved FTI-GGTIs.



Scheme 1. Reagents and conditions: (a) $Pd(PPh_3)_4$, $Zn(CN)_2$, DMF, 80 °C, 96%; (b) NBS, CCl_4, benzoyl peroxide, reflux, 43–50%; (c) 1-trityl-4-(acetoxymethyl)imidazole, EtOAc, 60 °C; MeOH, 79–93%; (d) LiOH, THF, H_2O, 68–83%; (e) pyridine–SO_3 complex, DMSO, Et_3N, 86–90%.



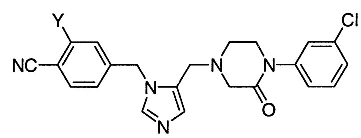
Scheme 2. Reagents and conditions: (a) $Na(OAc)_3BH$, 4 Å sieves, DCE, 34–67%; (b) for **8** and **9**; ArOH or ArSH, Cs_2CO_3, DMF, 31–60%; (c) for **10**; PhOH, CuBr·DMS, NaH, pyridine, reflux, 9–21%.

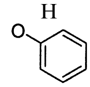
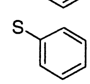
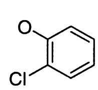
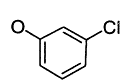
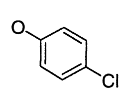
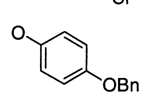
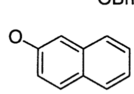
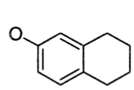
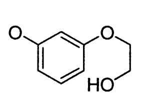
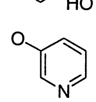
is deleted. In these cases, FPTase inhibitory potency has been drastically reduced yet these compounds remain moderately potent and selective GGPTase-I inhibitors. This supports the idea that the phenoxy group is important to GGPTase-I binding, and that selective inhibitors can be prepared by manipulating substituents.

In addition to the in vitro FPTase inhibition assay, FPTase binding in cells was also determined (Table 1). An unfortunate consequence of the added lipophilicity of these compounds was their poor performance in the cell based assay. For many analogues, cellular FPTase values were off by 10-fold or more as compared to in vitro FPTase IC_{50}s. It is postulated that the increased lipophilicity of the aryloxy compounds impedes cell penetration. When polar substituents were introduced onto the aryloxy groups, cellular FPTase values responded positively. Substitution with *O*-(2-hydroxyethyl)resorcinol gave **8i** (FPTase $IC_{50}=2.5$ nM, GGPTase-I $IC_{50}=1.3$ nM, $FPTase_{cell}$ $IC_{50}=8$ nM) which was more than 10-fold more active in the cell based assay than the unsubstituted phenyl compound **8a**. Replacement of the phenol in **8a** with 3-hydroxypyridine gave **8j**, the latter being 6-fold more potent in cells.

Inhibition of the geranylgeranylation of Rap1a is used as a measure of the ability of a GGTI to prevent prenylation

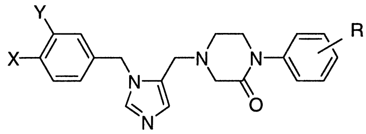
Table 1. FPTase and GGPTase-I inhibition data for aryloxy derivatives **8a–j**



Compd	Y	In vitro (IC_{50}, nM)		In cell culture (nM)	
		FPTase[a]	GGPTase-I[b]	FPTase (IC_{50}) binding[d]	Rap1a (MIC) prenylation[e]
1	H	2	98	3	1000
8a		7	18	110	300–1000
8b		18	12	255	nd
8c		7	0.7 (20)[c]	41	300
8d		4	20	93	nd
8e		19	15	350	nd
8f		32	62	311	nd
8g		1	199	48	nd
8h		4	(290)[c]	nd	nd
8i		2.5	1.3 (35)[c]	8	3000
8j		11	52	17	1000

[a]Concentration of compound required to reduce the human FPTase-catalyzed incorporation of [^{3}H]FPP into recombinant Ras-CVIM by 50%.[9a]
[b]Concentration of compound required to reduce the human GGPTase-I-catalyzed incorporation of [^{3}H]GGPP into biotinylated peptide corresponding to the C-terminus of human Ki-Ras by 50%. Assay run with 30 min preincubation of enzyme and inhibitor in the presence of 5 mM ATP.[6]
[c]Same as footnote b without prior incubation of inhibitor and enzyme.
[d]Concentration of sample required to displace 50% of a radiolabeled farnesyltransferase inhibitor (FTI) from FPTase in cultured Ha-*ras* transformed RAT1 cells.[9b]
[e]Minimal concentration of compound required to inhibit Rap1a processing in PSN-1 cells.[6]

Table 2. FPTase and GGPTase-I inhibition for piperazinones **9a** and **10a–c**



Compd	X	Y	R	FPTase IC_{50} (nM)[a]	GGPTase-I IC_{50} (nM)[b]
8a	CN	OPh	3-Cl	7	18
9a	OPh	CN	3-Cl	9	809
10a	H	OPh	3-Cl	1230	65
10b	H	OPh	4-Cl	670	136
10c	H	OPh	4-CF_3	5770	155

[a]See footnote a in Table 1.
[b]See footnote b in Table 1.

in cells.[6a,b] The compounds in this series that were tested in the Rap1a processing assay were active with MICs in the 0.3–3 μM range, confirming GGPTase-I inhibition in cells. Interestingly, Rap1a inhibition was not significantly greater than that for **1** in spite of these compounds having greater in vitro potency towards GGPTase-I. This is most likely due to their reduced cell penetration in comparison to **1**.

In anchorage-independent growth inhibition assays in soft agar, **8a** was less effective than **1** at blocking colony formation of *v*-H-*ras* transformed RAT1 cells (IC_{90} **8a** = 0.3 μM, IC_{90} **1** = 0.1 μM), consistent with its reduced FTase binding in cell culture (vide supra). General cell cytotoxicity elicited by **8a** is only observed at ~30-fold higher concentrations (~80% RAT1 cell survival up to 10 μM as assessed by viability staining with MTT). Interestingly, inhibition by **8a** of K-*ras* transformed cell colonies required only slightly higher concentration (IC_{90} = 0.3–1 μM) than was required for H-*ras*, resulting in a ratio of K-*ras*/H-*ras* IC_{90} which is lower than for previously characterized selective *N*-arylpiperazinone FTIs[5] (ratio ca. 1–3 vs 10–20). A determination of whether this is the result of dual versus selective prenyltransferase inhibition will require further studies.

Conclusion

The inclusion of aryloxy substituents on the cyanobenzyl portions of certain dual FPTase/GGPTase-I inhibitors can substantially improve GGPTase-I inhibitory potency while leaving the relatively high intrinsic FPTase inhibitory potency unaffected. The poor cell penetration seen in this series can be positively addressed by the inclusion of polar functionality within the aryloxy substituent. Further modifications, leading to the deletion of the nitrile, yield GGPTase-I selective compounds. The ability to modulate the relative degree of FPTase and GGPTase-I inhibition could prove important in the design of antineoplasic drugs of the prenyl-protein transferase inhibitor class.

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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.

10. For **8c**, the slope of a plot of log(IC_{50}) versus log[GGPP] is 0.93 in the presence of 5 mM ATP, suggestive of a GGPP-competitive inhibitor. In the absence of 5 mM ATP in the assay, GGPTase-I IC_{50} = 140 nM.

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