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Macrocyclic piperazinones as potent dual inhibitors of farnesyltransferase and geranylgeranyltransferase-I

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Abstract—A series of macrocyclic piperazinone compounds with dual farnesyltransferase/geranylgeranyltransferase-I inhibitory activity was prepared. These compounds were found to be potent inhibitors of protein prenylation in cell culture. A hypothesis for the binding mode of compound **3o** in FPTase is proposed.

There has been considerable interest in protein prenyltransferase inhibitors over recent years, due to the importance of Ras proteins in cell growth and oncogenesis,[1] coupled with the observation that Ki-Ras requires prenylation for biological function.[2] Selective farnesyltransferase (FPTase) inhibitors show promising activity in preclinical and clinical studies,[3] but they are incapable of blocking the function of Ki-Ras, since they fail to inhibit its alternative geranylgeranylation by geranylgeranyl-transferase-I (GGPTase-I). While non-Ras proteins may therefore be important for the biological effects of FPTase inhibitors,[4] blocking Ki-Ras function remains an important strategy in cancer research. Dual FPTase-GGPTase-I inhibitors (FTI-GGTIs) have been shown to inhibit the prenylation of Ki-Ras in cell culture and in animals, albeit with a narrow in vivo therapeutic index.[5]

We recently discovered that *N*-arylpiperazinone FTI-GGTIs such as compound **1** (L-778,123) bind to FPTase in a folded conformation, and that potency and selectivity enhancements are achieved by enforcing a macrocyclic constraint (Fig. 1).[6,7] Macrocycle **2** emerged as a highly potent FTI with only moderate activity versus GGPTase-I. Unexpectedly, whereas **1** inhibits GGPTase-I in a GGPP-competitive manner,[8] constrained **2** was found to be protein substrate-competitive, suggesting different GGPTase-I binding modes for **1** and **2**.[6] By replacing the naphthyl ring in **2** with a substituted benzyl group, we have discovered a strategy for modulating the relative prenyltransferase inhibitory activities in this series. Here, we describe the synthesis and biological activities of macrocyclic compounds (**3**) with dramatically increased GGPTase-I inhibition, leading to highly potent macrocyclic dual FTI-GGTIs.[9]

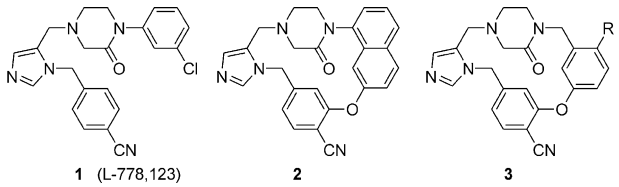

Figure 1. Piperazinone prenyltransferase inhibitors.

Compounds **3a–s** (Table 1) in this study were prepared in analogy to recently reported syntheses.[6,10] The phenol **4** was converted in four steps to piperazinone **6**

Keywords: Inhibitor; Farnesyltransferase; Geranylgernyltransferase.
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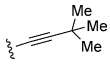
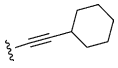
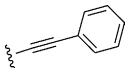
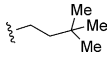
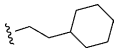
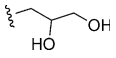
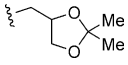
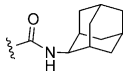
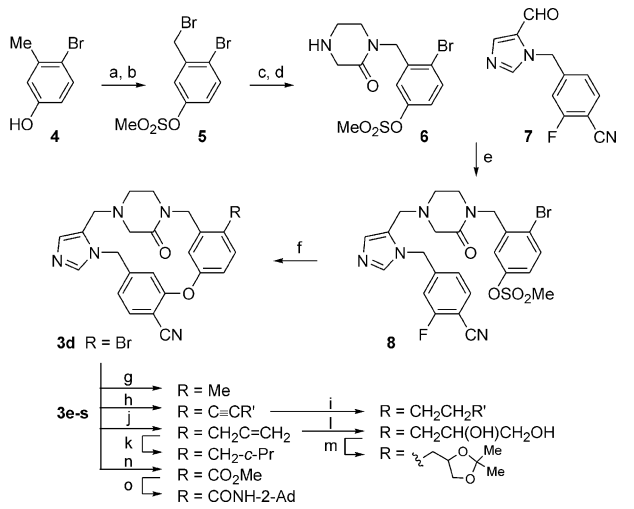
Table 1. FPTase and GGPTase-I inhibition data and cell culture activity for compounds **1**, **2**, and **3a–s**

Compds	R (in **3**)	FPTase in vitro IC_{50} (nM)[a]	GGPTase-I in vitro IC_{50} (nM)[b]	GGTase-I Slope log(IC_{50}) vs log[GGPP][d]	FPTase binding in cell culture IC_{50} (nM)[e]	Rap1a processing in cell culture MIC (nM)[f]
1	—	2	98	1.42	3.6	1000
(+)-**2**	—	0.1	301	−0.44	0.29	10,000
3a	H	2.2	3780	0.08	3.3	30,000
3b	F	<1	2750	nd	1	nd
3c	Cl	<1	450	nd	0.30	nd
3d	Br	0.45	100	nd	1.1	10,000
3e	Methyl	0.26	502	nd	0.33	nd
3f	Ethyl	0.66	124	−0.16	1.2	1000
3g	Allyl	0.1	107	nd	0.59	1000
3h	*n*-Propyl	0.31	97	−0.12	0.44	1000
3i		0.5	212	nd	1.4	3000
3j		38	55 (135)[c]	0.32	10	1000
3k		364	2.4 (26)[c]	0.68	170	300
3l		645	534	nd	nd	nd
3m		0.66	238	nd	2.2	10,000
3n		19	2.1 (18)[c]	0.48	28	100
3o		1.6	1.7	−0.25	5.7	30
(±)-**3p**		6.8	1050	nd	40	nd
(±)-**3q**		0.5	46 (36)[c]	0.25	0.86	<300
3r		24	420	nd	20	3000
3s		20	43	0.28	30	100

[a] Concentration required to reduce the human FPTase-catalyzed incorporation of [^{3}H]FPP into recombinant Ras-CVIM by 50%.[11a]
[b] Concentration 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.[8a]
[c] In parentheses: same as footnote b without prior incubation of enzyme and inhibitor.
[d] Qualitative measure of GGPP competition, with slopes of 0 and 1 indicating noncompetitive and competitive binding, respectively. IC_{50} values were determined at various GGPP concentrations in the presence of 5 mM ATP.[8a]
[e] Concentration required to displace 50% of a radiolabeled FTI from FPTase in cultured Ha-*ras* transformed RAT1 cells.[11b]
[f] Minimum concentration of compound required to inhibit Rap1a processing in PSN-1 cells.[8a]

(Scheme 1), which was reductively alkylated with aldehyde **7** to give the cyclization precursor **8**. Intramolecular S_NAr reaction provided **3d**. Compounds **3a–c** were prepared in an analogous fashion. The bromide **3d** was used in palladium-mediated cross-coupling reactions to afford, with additional straightforward transformations, **3e–s**.

Assessment of the in vitro inhibitory activities for compounds (Table 1) revealed a clear trend relating increased substituent size (R in **3**) to enhanced potency versus GGPTase-I, without significant effects on FPTase inhibition. This was true of both halogen substituents (**3a**, **3b–d**) and simple alkyl groups (**3a**, **3e–h**). The addition of bulky aliphatic substituents to a series



Scheme 1. Reagents and conditions: (a) MsCl, Et_3N, DCM, 0 °C; (b) NBS, AIBN, CCl_4, reflux, 61% for 2 steps; (c) *N*-Boc-piperazinone, NaH, DMF, 0 °C, 92%; (d) HCl, EtOAc, 0 °C, 98%; (e) **7**, $Na(AcO)_3BH$, 4 Å sieves, DCE, 69% (f) Cs_2CO_3, 0.05 M DMSO, 80 °C, 74%; (g) Me_4Sn, $PdBn(PPh_3)_2Cl$, HMPA, 70 °C, 72%; (h) R′C≡CH, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, DMF, 90 °C; (i) H_2, Pd/C, EtOH; (j) allyl-$SnBu_3$, $Pd(PPh_3)_4$, DMF, 110 °C, 99%; (k) CH_2N_2, $Pd(OAc)_2$, $CHCl_3$, 9%; (l) $K_2OsO_4{\cdot}2H_2O$, $K_3Fe(CN)_6$, K_2CO_3, quinuclidine, 1:1 *t*-BuOH:H_2O, 0 °C, 68%; (m) Acetone, CSA, reflux, 100%; (n) CO, $Pd(OAc)_2$, dppp, Et_3N, DMSO, 110 °C; $(Me_3Si)CHN_2$, 35%; (o) 2-adamantyl-NH_2, EDC·HCl, HOBt, DMF, 81%.

of alkynes (**3i**–**3k**) enhanced GGPTase-I inhibition and decreased potency versus FPTase, providing selective GGTI **3k** (IC_{50} 2.4 nM), which displayed slow tight binding to GGPTase-I. These results suggest that the GGPTase-I active site is better able to accommodate bulky hydrophobic groups in this region of the inhibitor than is FPTase. The phenylacetylide **3l** was poorly tolerated by both enzymes. Potent dual FPTase-GGPTase-I inhibition could be attained by removing the acetylene conformational constraint (**3m**–**o**), with the 2-(cyclohexyl)ethyl FTI-GGTI **3o** being the most potent overall (IC_{50} FPTase 1.6 nM, GGPTase-I 1.7 nM). The incorporation of polar functionality caused decreases in inhibitory activity for both FPTase and GGPTase-I (**3p**, **3r**), but this effect was attenuated by the addition of aliphatic functionality (**3q** versus **3p**, **3s** versus **3r**).

A preliminary kinetic analysis of GGPTase-I inhibition by compounds was undertaken. A plot of log (IC_{50}) versus log [GGPP] provided a qualitative assessment of inhibitory mechanism[8a] (Table 1), and indicated that compounds of structure **3** spanned the range from not competitive with respect to GGPP (presumably competitive with respect to protein substrate) to mixed type. Interestingly, while the selective GGTI **3k** displayed mixed type inhibition (slope 0.68), the most potent FTI-GGTI **3o** was not competitive with GGPP

Table 2. Inhibition of protein prenylation in PSN-1 cells by **1**,**2**, and **3o**,**q**

Entry	Compds	Protein processing EC_{50} (nM)[a]			EC_{50} ratio Ki-Ras/HDJ2
		HDJ2	Rap1a	Ki-Ras	
1	**1**	92	6760	6300	68
2	(+)-**2**	2	>10,000	>10,000	>5000
3	(+)-**2** + GGTI	2[b]	nd	90[b]	47[b]
4	**3o**	12	140	1065	89
5	**3q**	10	690	1515	150

[a] Dose of compound that caused half-maximal inhibition of prenylation in PSN-1 cells, determined by scanning immunoblots of whole cell extracts.[5]
[b] Inhibition by (+)-**2**, adding 1 μM of a selective GGTI (EC_{50} HDJ2 = 23 μM; Rap1a = 2 nM, Ki-Ras >100 μM).[5] The resulting Ki-Ras/HDJ2 ratio reflects the optimal balance of activities for inhibition of Ki-Ras.

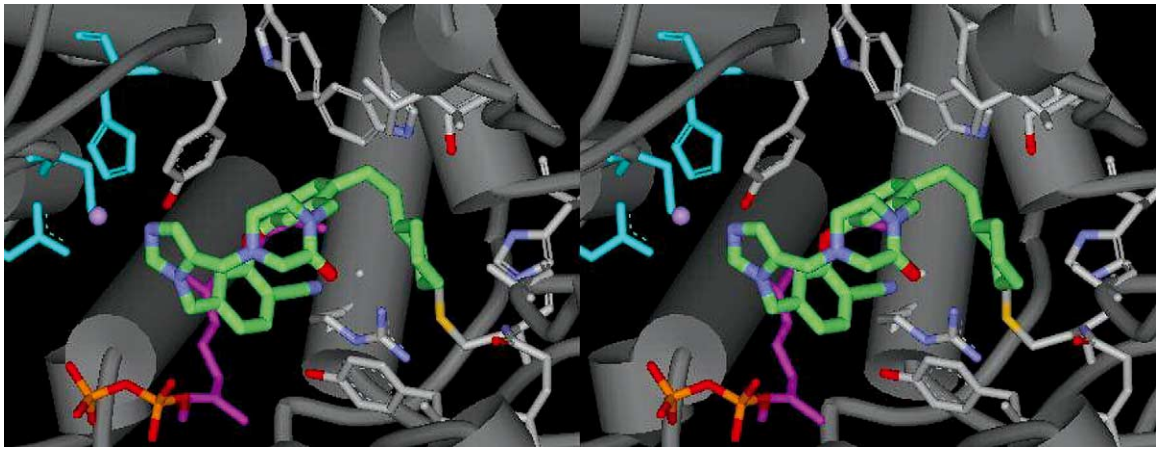


Figure 2. Proposed FPTase·**3o**·FPP ternary complex structure in cross-eyed stereoview, based on molecular modeling.[12] Inhibitor **3o** is colored green, the farnesyl group of FPP is magenta, the Zn ion is purple, Zn-ligating residues are cyan, and several key FTase residues are colored gray.

(slope = −0.25), despite close structural similarity between the two compounds.

The activities of compounds in cell culture were consistent with their in vitro potencies (Table 1). IC_{50} values determined in an FPTase in-cell enzyme occupancy assay were generally within 2–4-fold of the corresponding in vitro inhibition IC_{50}s, indicating high cell permeability. The minimal concentrations required to detect the unprocessed form of the specifically geranylgeranylated protein Rap1a roughly correlated with the in vitro GGPTase-I IC_{50}s. In a more detailed study, the relative FPTase and GGPTase-I inhibition activities of FTI-GGTIs **3o** and **3q** were assessed in a uniform cell background, and compared to the activities of **1** and **2** (Table 2). As detailed elsewhere,[5] **1** inhibited the processing of farnesylated and geranylgeranylated proteins (HDJ2 and Rap1a, respectively), and was therefore able to suppress the prenylation of Ki-Ras (entry 1). The more potent FTI **2** was unable to block Ki-Ras prenylation due to insufficient GGTase-I inhibition (entry 2). However, the addition of high concentration of a potent GGTI provided the necessary inhibition to observe unprenylated Ki-Ras (entry 3). This experiment indicated the maximum achievable level of Ki-Ras inhibition by a dual FTI-GGTI with a given level of FPTase potency, and thus the optimal balance of these enzyme activities for Ki-Ras inhibition (Ki-Ras/HDJ2 EC_{50} ratio ~47).[5] Compound **3o** (entry 4) was shown to be more potent at inhibiting the prenylation of all three proteins relative to **1**, and is a closely balanced FTI-GGTI (ratio ~89). Compound **3q** is also more potent than **1**, but is slightly excessive in its FTI activity (entry 5).

A hypothesis for the binding mode of the macrocyclic dual FTI-GGTI **3o** in FPTase (Fig. 2) was derived from molecular modeling studies based on published X-ray crystal structures of related macrocyclic inhibitor·FPTase·FPP ternary complexes.[12] As in related complexes,[12b] the imidazole moiety is ligated to zinc, the cyanophenyl group is stacked against the isoprenoid chain of FPP, and the benzylpiperazinone portion is in contact with key hydrophobic residues. Notably, the cyclohexylethyl group is angled toward a large cavity in the active site. Given that the mechanism of GGPTase-I inhibition by **3o** is not competitive with respect to GGPP, it is possible that the inhibitory binding mode of **3o** in GGPTase-I is qualitatively similar to the FPTase·**3o**·FPP complex.

In summary, several of the macrocyclic prenyltransferase inhibitors prepared in this study demonstrate dual FPTase and GGPTase-I inhibition. Compound **3o** potently inhibits both enzymes in cell culture with relative activities that are near-optimally balanced for the inhibition of Ki-Ras prenylation.

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12. (a) A model of the FPTase active site containing FPP was derived from the 1LD8 pdb entry (ref 12b). Random conformations of **3o** were generated using the distance geometry method JG (S. Kearsley, Merck & Co., Inc., unpublished). These conformations were energy minimized in the static active site using Batchmin and the MMFFs force field, while restraining the Zn-ligating nitrogen to its crystallographic position. The lowest energy structure is shown. (b) Bell, I. M.; Gallicchio, S. N.; Abrams, M.; Beese, L. S.; Beshore, D. C.; Bhimnathwala, H.; Bogusky, M. J.; Buser, C. A.; Culberson, J. C.; Davide, J.; Ellis-Hutchings, M.; Fernandes, C.; Gibbs, J. B.; Graham, S. L.; Hamilton, K. A.; Hartman, G. D.; Heimbrook, D. C.; Homnick, C. F.; Huber, H. E.; Huff, J. R.; Kassahun, K.; Koblan, K. S.; Kohl, N. E.; Lobell, R. B.; Lynch, J. J., Jr.; Robinson, R.; Rodrigues, A. D.; Taylor, J. S.; Walsh, E. S.; Williams, T. M.; Zartman, C. B. *J. Med. Chem.* **2002**, *45*, 2388.