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Oxo-piperazine Derivatives of *N*-Arylpiperazinones as Inhibitors of Farnesyltransferase

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Abstract—The evaluation of SAR associated with the insertion of carbonyl groups at various positions of *N*-arylpiperazinone farnesyltransferase inhibitors is described herein. 1-Aryl-2,3-diketopiperazine derivatives exhibited the best balance of potency and pharmacokinetic profile relative to the parent 1-aryl-2-piperazinones.

Introduction

Farnesyltransferase (FTase) is a zinc metalloenzyme that catalyzes the *S*-alkylation of a cysteine residue in the C-terminal tetrapeptide sequence of proteins using farnesylpyrophosphate (FPP), thus enabling their participation in signal transduction during cell proliferation.[1] One of the protein substrates for FTase, K-Ras, has been implicated in the growth of 20–30% of all human tumors.[2] In these tumors, mutated Ras loses its normal GTPase function, becomes constitutively bound to GTP, and transmits growth signals independent of extracellular growth factors to downstream mitogenic effectors. Strategies for regulating unimpeded oncogenic *ras* signalling have focused on FTase, inhibitors of which have significant potential as cancer chemotherapeutic agents.[3] FTase 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.[4]

A wide array of FTIs that mimic the C-terminal Ca_1a_2X tetrapeptide of Ras (e.g., CVIM) has been described.[4a,e] In a previous approach to pharmacologically improved FTIs, the use of conformational constraints in conjunction with carboxyl deletion and cysteinyl group replacement provided low molecular weight piperazinone FTIs with potent in vitro and in vivo activity.[5] Thus, 1-aryl-2-piperazinone FTIs (e.g., **1**) exhibit high levels of potency and antiproliferative activity, and have good oral bioavailability (Fig. 1).[5,6]

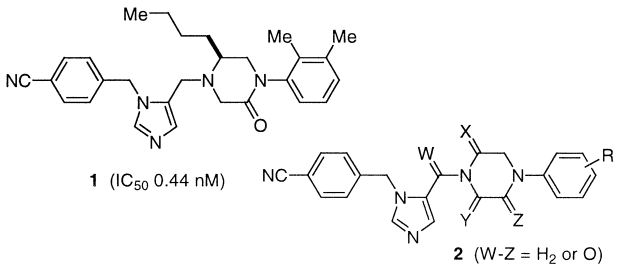

Figure 1. 1-Aryl-2-piperazinone FTI **1** and oxo-piperazine derivatives **2**.

We chose to investigate SAR of compounds closely related to **1**. The in vivo duration of **1** is limited by rapid metabolic transformation via *N*-dealkylations and *C*5-side-chain substituent hydroxylations.[6] In attempts to improve the overall pharmacokinetic profile of compounds related to **1**, we investigated oxo-piperazines **2** with the hope of blocking or attenuating metabolism, and altering compound polarity. Herein, we describe features that are consistent with maintaining

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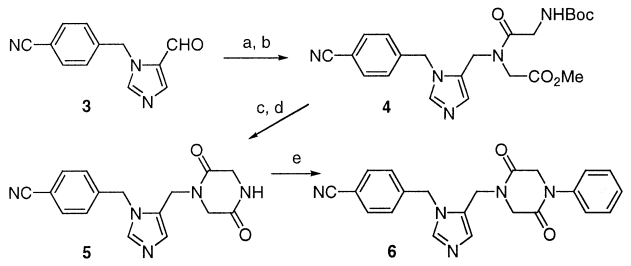


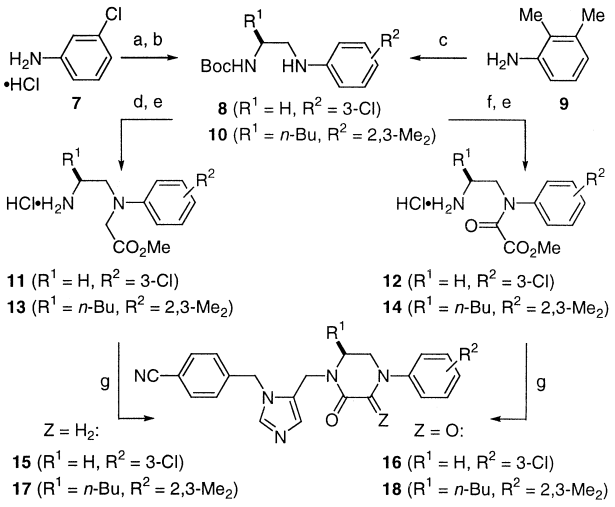
Scheme 1. Reagents and conditions: (a) Gly-OMe, $Na(AcO)_3BH$, 4 Å MS, DCE, 56%; (b) Boc-Gly-OH, EDC, HOBt, Et_3N, DMF, 81%; (c) HCl, EtOAc, 0 °C, 100%; (d) Et_3N, DCM, 62%; (e) Ph_3Bi, $Cu(OAc)_2$, Et_3N, DCM, 34%.

high enzyme activity and cell-potency, and that provide improvements in pharmacokinetic half-life.[7]

Chemistry

The 1-aryl-2-piperazinones **19**, **22**, and **25** (cf **2**; Z = O) were synthesized by analogy to previously described methods.[5,6,8] The 1-aryl-2,5-diketopiperazine **6** (cf. **2**; X, Z = O) was prepared as described in Scheme 1. Aldehyde **3**[5] was reductively alkylated with glycine methyl ester, then acylated with *N*-Boc-glycine to provide **4**. Deprotection and cyclization gave diketopiperazine **5**, which was subjected to $Cu(OAc)_2$-mediated *N*-arylation[9] to give **6**. The piperazine carboxamides **23** and **24** (cf **2**; W = O and W, Z = O, respectively) were prepared by sodium chlorite oxidation[10] of aldehyde **3**, followed by EDC-mediated amide coupling to 1-(3-chlorophenyl)piperazine and 1-(3-chlorophenyl)-2-piperazinone,[8] respectively.

The 1-aryl-3-piperazinones **15** and **17** (cf. **2**; Y = O) and the 1-aryl-2,3-diketopiperazines **16**, **18**, **20**, and **26** (cf. **2**; Y, Z = O) were prepared using a tandem reductive alkylation and cyclization process recently developed for convergent syntheses of unsymmetrical 2,3-diketopiperazines (Scheme 2).[11] For example, intermediate **8**, prepared by aminoethylation[12] and protection of 3-chloroaniline **7**, was alkylated with methyl bromoacetate to give the salt **11** after treatment with HCl. This was subjected to a tandem reductive alkylation and cyclization reaction with aldehyde **3** to afford the desired piperazinone **15**. Likewise, **8** was acylated with methyl oxalyl chloride to provide the salt **12** after deprotection, and this was converted to 2,3-diketopiperazine **16**. The substituted analogues **17** and **18** were constructed by reductive alkylation of 2,3-dimethylaniline **9** with *N*-Boc-norleucinal to give **10**, followed by the same reaction sequences described above. The reactions of substrates **11** and **12** with aldehyde **3** are less efficient than similar reactions using nonbasic aldehydes (cf. 45% yield using **3** vs 61–74% using substituted benzaldehydes).[11] Greater proportions of unalkylated piperazinone or diketopiprazine byproducts were formed. This may be due, in part, to the basicity of the imidazole moiety, which may accelerate premature cyclization relative to reductive alkylation. The reactions of the substituted analogues **13** and **14** were less efficient still (18% yield), probably because



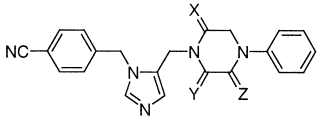
Scheme 2. Reagents and conditions: (a) 2-oxazolidinone, $MeO(CH_2)_2$-$O(CH_2)_2OH$, 160 °C; (b) Boc_2O, $NaHCO_3$, THF–H_2O, 80% for 2 steps; (c) *N*-Boc-norleucinal, $Na(AcO)_3BH$, AcOH, 4 Å MS, DCE, 63%; (d) $BrCH_2CO_2Me$, K_2CO_3, DMF, 60 °C, 45% from **8**, 58% from **10**; (e) HCl, EtOAc, 0 °C, 100%; (f) $ClCOCO_2Me$, $NaHCO_3$, EtOAc–H_2O, 0 °C, 90% from **8**, 58% from **10**; (g) $Na(AcO)_3BH$, 4 Å MS, DCE, 0 °C to rt, 45% from **11**, 48% from **12**, 18% from **13**, 18% from **14**.

premature cyclization was exacerbated by the Thorpe–Ingold effect.[13]

Structure–Activity Relationships

An initial survey of *N*-[1-(4-cyanobenzyl)imidazolyl-5-methyl]-2-piperazinone analogues (Table 1) revealed that the position of oxo-groups within the framework significantly impacts the enzyme activities. Relative to the parent piperazinone **19** (FTase IC_{50} 5 nM), the more polar 2,3-diketopiperazine **20** was only 3-fold less active versus FTase, and at least several fold less active versus the related enzyme geranylgeranyltransferase-I (GGTase-I). The isomeric 2,5-diketopiperazine **6**, with similar physical properties, was substantially less active (FTase IC_{50} 950 nM). Taken with the known increase in

Table 1. FTase inhibition, GGTase-I inhibition, and physical property data for parent piperazinone **19** and diketopiperazines **20** and **6**



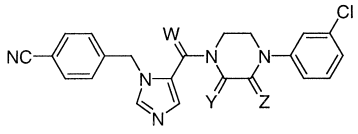
Compd	X	Y	Z	FTase IC_{50} (nM)[a]	GGTase-I IC_{50} (μM)[b]	Log P[c]
19	H_2	H_2	O	5	8.7	1.32
20	H_2	O	O	16	>20	0.46
6	O	H_2	O	950	nd	0.55

[a]Concentration of compound required to reduce the human FTase-catalyzed incorporation of [^{3}H]FPP into recombinant Ras-CVIM by 50% (ref 14a).
[b]Concentration of compound required to reduce the human GGTase-I-catalyzed incorporation of [^{3}H]GGPP into biotinylated peptide corresponding to the C-terminus of human Ki-Ras by 50% (ref 14b).
[c]Log of the octanol–water partition coefficient.

potency induced by the hydrophobic *n*-butyl group in **1** and related compounds,[5,6] this indicates the presence of a hydrophobic binding pocket in the FTase active site that is intolerant to the presence of a carbonyl group at the piperazine *C*5 position.

Additional studies (Table 2) focused on compounds lacking the offending *C*5 carbonyl group (X = O in **2**). The unoxygenated piperazine **21** (IC_{50} = 250 nM), was 100-fold less potent than the parent 1-aryl-2-piperazinone **22** (IC_{50} = 2 nM), and was the least active in the series. This reduction in potency was accompanied by ~10-fold increase in the concentration required to inhibit the anchorage-independent growth of Harvey-*ras* transformed cells in soft agar. The 1-aryl-3-piperazinone **15** was 50-fold less active than **22**, while another isomeric compound **23** was 10-fold less active. However, the 2,3-diketopiperazine **16** (IC_{50} = 11 nM) exhibited the best in vitro activity relative to **22**, and also exhibited the lowest log P in the series. That the activities of these compounds in vitro and in cell culture roughly correlate, and that general cell cytotoxicity is only observed at much higher concentrations (>25 μM) than are required for growth inhibition, suggest that the intracellular mechanism is due to inhibition of FTase. Taken together, the results suggest that converting the amino-group in 1-aryl-2-piperazinones to an sp^2-hybridized amide decreases potency by an amount dependent on the placement of the carbonyl group.

Table 2. FTase inhibition, cell growth inhibition, cytotoxicity, and physical property data for compounds **15**–**16** and **21**–**24**



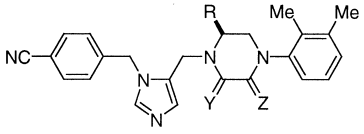
Compd	W	Y	Z	FTase IC_{50} (nM)[a]	H-*ras* soft agar IC_{90} (μM)[b]	Cytotoxic endpoint IC_{20} (μM)[c]	Log P[d]
21	H_2	H_2	H_2	250	1.0	25	>3.4
22	H_2	H_2	O	2	0.1	>100	2.11
15	H_2	O	H_2	100	1	>100	2.51
23	O	H_2	H_2	21	0.1–1	>100	2.79
24	O	H_2	O	33	1	>100	nd
16	H_2	O	O	11	1	>100	1.24

[a]See footnote a in Table 1.
[b]Concentration required to achieve 90% reduction of anchorage-independent growth of RAT1 v-Ha-*ras* transformed cells in soft agar relative to vehicle-treated control (ref 15).
[c]Highest nontoxic concentration (~80% cell survival) for cultured RAT1 cells as assessed by viability staining with MTT (ref 16).
[d]See footnote c in Table 1.

Closer derivatives of 1-aryl-2-piperazinone compound **1** were investigated with the expectation that the biological activity of oxo-piperazine analogues could be improved by the addition of the lipophilic alkyl chain to the piperazine ring (Table 3). Although 20-fold improvement in activity was observed in the 1-aryl-2-piperazinone series upon *C*5-alkylation (**25**, IC_{50} = 11 nM vs **1**, IC_{50} = 0.44 nM), only 7-fold benefit was afforded in the 2,3-diketopiperazines (**26**, IC_{50} = 20 nM vs **18**, IC_{50} = 3 nM). Surprisingly, the 1-aryl-3-piperazinone analogue **17** was as potent as **18** in vitro, an unexpected result in light of the 10-fold difference between non-alkylated analogues **15** (IC_{50} = 100 nM) and **16** (IC_{50} = 11 nM). Interestingly, despite their equivalent activity in vitro, a >100-fold difference in cell potency between **17** and **18** was observed both in soft agar and on poly(HEMA) coated microtiter plates, with **18** achieving the best activity overall (H-*ras* poly-HEMA IC_{50} = 15 nM).

In pharmacokinetic analyses, the compounds were administered orally to two dogs as a mixture with up to eleven other compounds, each at 1 mg/kg, with compound **22** included as a reference in each combination dose (Table 4).[18] To monitor the data for potential artifacts due to drug–drug interactions using this protocol, pharmacokinetic profiles relative to the internal standard **22** were assessed and were considered meaningful since the data for **22** were not significantly altered from single administration data. Relative to the corresponding 1-aryl-2-piperazinones, the 1-aryl-3-piper-

Table 3. FTase inhibition, cell growth inhibition, cytotoxicity, and physical property data for compounds **1**, **17**–**18**, and **25**–**26**



Compd	R	Y	Z	FTase IC_{50} (nM)[a]	H-*ras* soft agar IC_{90} (μM)[c]	H-*ras* poly(HEMA) IC_{50} (nM)[d]	Cytotoxic endpoint IC_{20} (μM)[e]	Log P[f]
25	H	H_2	O	11	1	83	10	2.00
26	H	O	O	20	>1	nd	10	1.11
1	*n*-Bu	H_2	O	0.44[b]	0.1–1	28	25-50	>3.7
17	*n*-Bu	O	H_2	1.3[b]	1–10	2,510	>100	>3.5
18	*n*-Bu	O	O	3	0.1–1	15	10	3.30

[a]See footnote a in Table 1.
[b]Enzyme concentration = 10 pM instead of 1 nM in assay.
[c]See footnote b in Table 2.
[d]Concentration required to inhibit by 50% the anchorage-independent proliferation of RAT1 v-Ha-ras transformed cells on poly-(HEMA)-coated plates relative to vehicle-treated control (ref 17).
[e]See footnote c in Table 2.
[f]See footnote c in Table 1.

Table 4. Pharmacokinetic data in dogs following combination dosing in dogs at 1 mpk po[a]

Compd	C_{max} (μM)	$t_{1/2}$ (h)	AUC (μM h)	AUC rel **22**[c]
		1-Aryl-2-piperazinones		
22[b]	2.98±1.27	0.79±0.16	5.30±2.19	1.0
25	1.06	0.5	1.40	0.4
1	0.14	0.6	0.15	0.06
		1-Aryl-3-piperazinones		
15	3.14	1.2	8.58	2.3
17	0.96	2.3	3.46	0.4
		1-Aryl-2,3-diketopiperazines		
16	3.11	2.2	12.50	3.3
26	2.29	1.0	4.43	1.2
18	1.95	0.9	4.25	0.5

[a]Compounds were administered orally to two dogs as mixtures with 11 other compounds, each at 1 mg/kg, with compound **22** included in each experiment as an internal reference. Plasma extracts were analyzed by LC/MS/MS, and unless otherwise indicated reported data are the average of two dogs (see ref 18).
[b]Mean data from 19 experiments. These are in good agreement with single compound administration data.
[c]The ratio of the compound's average AUC to that of the internal standard **22** from the same experiment.

azinones achieved greater $t_{1/2}$ and AUC relative to the 1-aryl-2-piperazinones, consistent with the possibility that altered metabolism is responsible (cf. **15** vs **22** and **17** vs **1**). Likewise, the 1-aryl-2,3-diketopiperazines consistently displayed improved pharmacokinetic properties relative to the 1-aryl-2-piperazinones (cf. **16** vs **22**, **26** vs **25** and **18** vs **1**).

Conclusion

The manipulation of the oxo group position within the 1-aryl-2-piperazinone framework results in the modulation of FTase enzyme activity, polarity, and pharmacokinetic profiles in dogs following oral administration. In the case of 1-aryl-3-piperazinones, a *C*5-alkyl substituent is beneficial to in vitro FTase inhibition activity, but activity in cell culture (e.g., for **17**) is poor. In comparison, the 2,3-diketopiperazines are in some cases as potent as the corresponding 1-aryl-2-piperazinones in cell culture (e.g., **18** vs **1**). The 2,3-diketopiperazine template offers a promising avenue toward potent FTIs with improved pharmacokinetic properties.

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