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Synthesis and Evaluation of a Novel Series of Farnesyl Protein Transferase Inhibitors as Non-Peptidic CAAX Tetrapeptide Analogues

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Abstract—A novel series of compounds, derived from 4-amino-phenyl piperazine, has been designed to selectively inhibit farnesyl protein transferase (FPTase) as CAAX tetrapeptide analogues. Certain of these compounds were shown to possess low nanomolar inhibitory activity both against the isolated enzyme and in cultured cells.

FPTase catalyzes a key step in the post-translational modification of different kind of proteins implicated in cell proliferation. This reaction transfers a farnesyl group from farnesyl pyrophosphate onto the cysteine residue present in the carboxy terminal tetrapeptide sequence of these proteins, called CAAX box with the C referring to the prenylated cysteine, A referring to aliphatic amino acids and X referring to methionine, serine or glutamine.[1,2] Among the proteins farnesylated, Ras proteins were identified as an important target since they are key components in cellular signal transduction pathway, controlling cell proliferation and differentiation, and since mutations of the *ras* gene have been observed in about 30% of human cancers.[3] In order to function, both mutated and wild type Ras must move to the inner face of the plasma membrane. This ability is only acquired after several post-translational modifications that create a membrane-binding domain on the C-terminal portion of the protein. In these chemical processes, because farnesylation alone can support Ras transforming activity, inhibition of FPTase was envisioned as a way to down regulate *ras* function.[4,5] However, it is now generally acknowledged that other proteins may also implicated in the anti-tumor activity of farnesyl transferase inhibitors (FTIs). Indeed, about 20 different proteins have been identified as substrates for FPTase in mammalian cells[6] and some of them like RhoB or centromer binding proteins represent complementary or alternative targets to elaborate a new possible mechanism of the antiproliferative activity.[7]

One of the milestones in the development of small FTIs was the finding that all the major recognition elements responsible for interaction with the enzyme were carried by the isolated CAAX tetrapeptide.[8] Since then, rational design has been able to produce an important number of non-peptidic CAAX analogues as potent inhibitors of FPTase.[9,10] The originality of this target is that most of the FTIs discovered possess very diverse chemical structures relative to each other. This diversity is probably due to the very wide catalytic site of the enzyme and to the different chemical approaches used to identify these inhibitors (screening of natural products or libraries of synthetic compounds). However, most of the FTIs described to date display relatively poor cellular activity compared to their inhibitory potencies on the isolated enzyme. Our own research in this field has led to potent inhibitors of FPTase both on the isolated enzyme and in cellular models.

Our strategy was also focused on CAAX box analogues. To mimic the central core of the CVIM-tetrapeptide we decided to use the amino-phenyl piperazine scaffold that we previously worked in a CNS project. This scaffold

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displays some interesting structural analogies with existing compounds of the literature.[16] To interact with the zinc atom we added an imidazole ring, a well-known alternative to the cysteine thiol group, on the amino function. From this substituted scaffold we synthesized and screened a first combinatorial library from which we identified a hit (compound **1**, Fig. 1) that displayed an IC_{50} of 600 nM on the inhibition of FPTase. Even modest, this activity was sufficient to decide to optimize this compound.

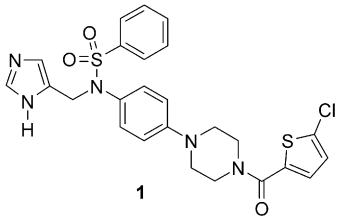
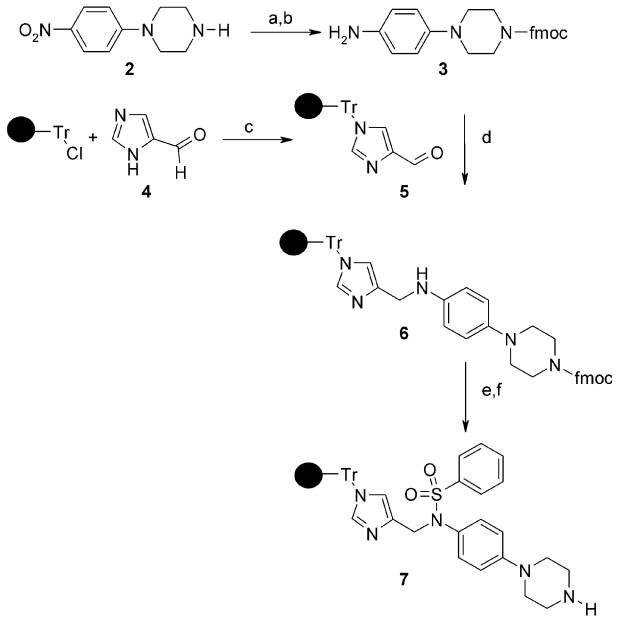


Figure 1. Structure of lead compound **1**.

Optimization of the right hand part of compound **1** has been realized using solid phase parallel synthesis, since anchorage on a solid support was possible using one of the imidazole nitrogens. Synthesis of the first intermediate resin is described in Scheme 1. 4-Nitro-phenyl piperazine **2** is first protected with a fmoc group and then reduced by catalytic hydrogenation to give compound **3**. Imidazole carboxaldehyde was regio-selectively anchored to a PS-chlorotrityl chloride resin and then reductively aminated with compound **3** to provide intermediate **6**. This resin was treated with phenyl sulfonyl chloride in pyridine followed by removal of the fmoc protecting group to give resin **7**.

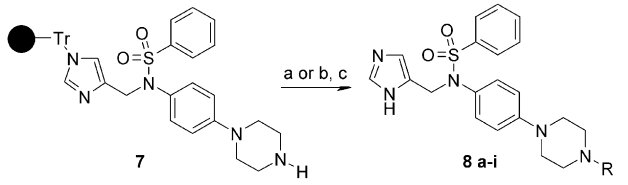


Scheme 1. Reagents and conditions: (a) Fmoc-OSu, DMF, Na_2CO_3, rt, 30 min; (b) H_2, Pd/C, MeOH/THF; (c) DIEA, DMF; (d) $NaBH(OAc)_3$, AcOH, DCE, rt, 7 h; (e) $PhSO_2Cl$, Pyridine, rt, 4 h (f) piperidine, DMF.

This intermediate can then undergo a diverse set of reactions with either acid chlorides, sulfonyl chlorides, isocyanates or isothiocyanates followed by cleavage of the resin in acidic conditions to afford small libraries of compounds (Scheme 2). Among these compounds, the highest inhibitory activities were obtained with thiourea derivatives, which surprisingly and inexplicably, had lower IC_{50} values than the corresponding ureas (Table 1, compare **8b** and **8d** to **8i** and **8g**). However, the most potent compounds in this series were poorly active in a cellular Ras-processing assay[13] (**8a** and **8b**).

We then focused our attention on modification of the left hand part of compound **1**. We first decided to substitute the imidazole by a 4-cyanobenzyl group[11] (Scheme 3). 4-Nitro-phenyl piperazine **2** was first treated with 5-chloro-thiophene-2-carboxylic acid to give compound **9**, which was then reduced with tin chloride to give amine **10**. Reductive coupling with 4-cyanobenzyl imidazole carboxaldehyde afforded compound **11**. From this intermediate we were able to prepare compound **12c** which corresponds to the imidazole-substituted equivalent of compound **1**. This modification led to a major reduction in the IC_{50} value of **12c** as compared to **1** (2 vs 600 nM respectively). However, this compound also demonstrated only poor cellular activity in the Ras-processing assay ($IC_{50} = 200$ nM).

To optimize the cellular activity we decided to focus our efforts on a modification or replacement of the phenyl sulfonyl group. We used solution phase parallel synthesis with polymer-supported reagents and resin scavengers in order to rapidly optimize this series of compounds (Scheme 4). Reactions with sulfonyl chlorides or acid chlorides were performed using PS-DIEA as

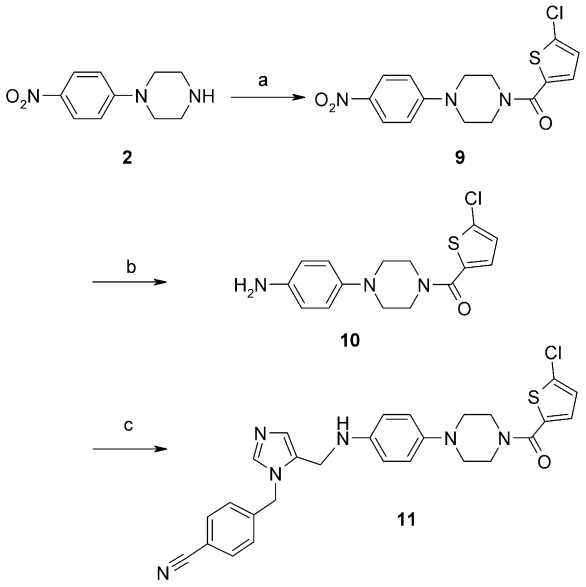


Scheme 2. Reagents and conditions: (a) RNCO or RNCS, Toluene, 70 °C, 10 h; (b) RCOCl or RSO_2Cl, DIEA, DCM, rt, 4 h; (c) TFA, DCM, Et_3SiH, 1 h.

Table 1.

Compd	R	FPTase[a] IC_{50} (nM)	Processing[b] IC_{50} (μM)	GGPTase[a] IC_{50} (nM)
8a	CS–NH–iBu	6	0.8	>1000
8b	CS–NH–cHexyl	20	1	>1000
8c	CS–NH–CH_2–cHexyl	30	nd	>1000
8d	CS–NH–CH_2–Ph	60	nd	>1000
8e	CS–NH–$(CH_2)_2$–Ph	100	nd	>1000
8f	CS–NH–Ph	600	nd	>1000
8g	CO–NH–CH_2–Ph	600	nd	nd
8h	CONHPh	1000	nd	nd
8i	CO–NH–cHexyl	1000	nd	nd

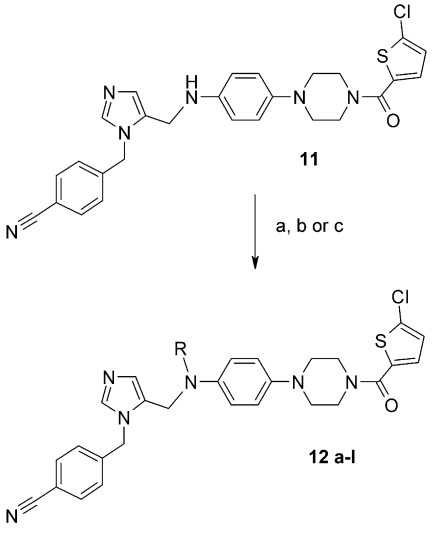
[a]FPTase and GGPTase I inhibitory assays were performed as described in ref 12 ($n = 2$).
[b]Farnesylation of H-Ras in DLD-1 cells was monitored.[13]



Scheme 3. Reagents and conditions: (a) 5-Cl-thiophene-2-carboxylic acid, HOOBT, EDC, DIEA, DCM, rt, 4 h, 84%; (b) $SnCl_2.2H_2O$, EtOH, reflux, 24 h, 94%; (c) 4-CN-benzyl imidazole carboxaldehyde, DCE, $NaBH(OAc)_3$, AcOH, rt, 24 h, 84%.

base and PS-trisamine as scavenger to remove excess reagent. Reductive aminations were also performed using MP-BH_3CN as reducing agent and PS-trisamine as scavenger to remove excess aldehyde. About 80 compounds were synthesized using these methodologies and data on a selection of the most interesting compounds is summarized in Table 2.

From these results, it appears that replacement of the sulfonyl substituent by a benzoyl group has no influence on FPTase inhibitory activity but led to a real improvement of the IC_{50} value on the cellular Ras-processing assay (compare **12b** and **12c**). Substitution of the benzoyl group gave very surprising results in terms of Ras-processing with important variability, in the case of



Scheme 4. Reagents and conditions: (a) (i) RCOCl, DCM, PS-DIEA, rt, 1 h; (ii) PS-Trisamine, 5 h; (b) (i) RSO_2Cl, DCM, PS-DIEA, rt, 1 h; (ii) PS-Trisamine, 5 h; (c) (i) RCHO, MeOH, AcOH, rt, 3 h then MP-BH_3CN, rt, overnight, 48 h; (ii) PS-Trisamine, rt, overnight.

Table 2.

Compd	R	FPTase[a] IC_{50} (nM)	Processing[b] IC_{50} (μM)	GGPTase[a] IC_{50} (nM)
12a	H	70	nd	nd
12b	COPh	2	0.003	> 1000
12c	SO_2Ph	2	0.2	nd
12d	CO–3-(Cl)Ph	2	0.002	> 1000
12e	CO–2-(F)Ph	2	0.02	nd
12f	CO–3-(F)Ph	2	0.006	> 1000
12g	CO–4-(F)Ph	2	0.01	400
12h	CO–2-(OMe)Ph	2	> 10	nd
12i	CO–3-(OMe)Ph	2	0.007	700
12j	CO–4-(OMe)Ph	2	0.08	nd
12k	CH_2–3-(F)Ph	20	nd	nd
12l	CH_2–4-(F)Ph	70	nd	nd

[a]FPTase and GGPTase I inhibitory assays were performed as described in ref 12 ($n = 2$).
[b]Farnesylation of H-Ras in DLD-1 cells was monitored.[13]

the methoxy substituent, depending on its position on the phenyl group (compare **12h**, **12i** and **12j**). The presence of a carbonyl or sulfonyl group seems necessary to obtain good IC_{50} values, as demonstrated by the compounds prepared by reductive amination (compare **12k** and **12l** to **12f** and **12g**). For the most interesting compounds, GGPTase inhibition was measured and the results obtained demonstrated a very high selectivity of these compounds for FPTase over GGPTase (Table 2). Overall, the results reported here show a very interesting in vitro profile for 4 compounds (**12b**, **12d**, **12f** and **12i**). Among these, **12d** (Fig. 2) demonstrated equal inhibitory activity on both the isolated enzyme and the Ras-processing cellular assay, associated with a high selectivity for FPTase over GGPTase.

Using the coordinates of a recently published X-ray crystal structure of human FPTase,[14,15] compound **12d** was docked into the active site using the flexible docking

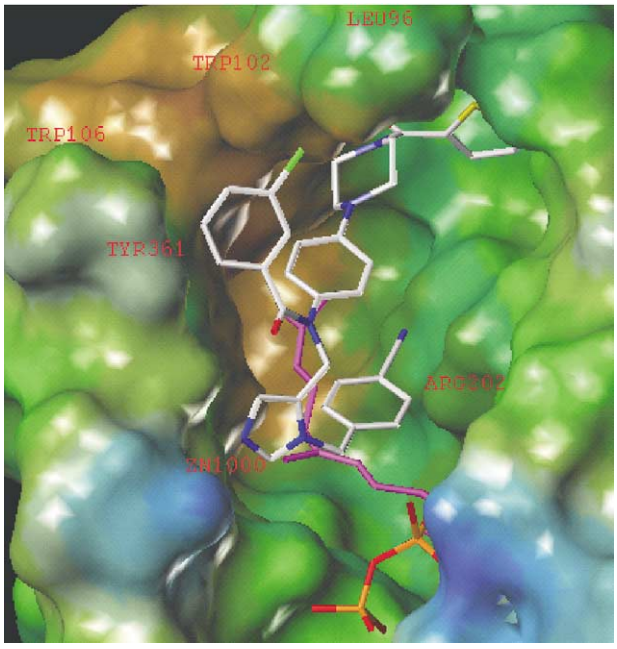


Figure 2. Proposed binding mode of compound **12d** in human FPTase active site. Connolly surface and lipophilic potential are represented. For clarity, isoprenoid chain of FPP was colored in purple.

program FlexX (Tripos Inc.) (Fig. 2). On the Connolly surface of the active site the lipophilic potential, calculated using the *MOLCAD* program (Tripos Inc.), has been represented. The cyanobenzyl group was used as starting fragment and placed in the same position as the cyanobenzyl in the crystallized complex, making stacking with FPP isoprenoid and pointing towards Arg202β and Tyr166α. Then, FlexX has positioned the rest of the molecule, fragment by fragment, into the active site searching for favourable interactions between the ligand and the amino acid residues. The docking program calculated an interaction between the imidazole and the zinc cation as anticipated by analysis of the crystal structure. The aminophenyl piperazine group fits into a hydrophobic pocket defined by Trp102β, Trp106β and Tyr361β, which, in the case of CA_1A_2X peptide substrates, hosts the aliphatic amino acid A_2. The chlorobenzoyl group interacts with an additional pocket constituted by Tyr361β, Trp106β and Leu96β. The chlorothiophene moiety is placed in the hydrophobic pocket constituted by Tyr131α and His149β interacting usually with the methionine residue of CAAM peptide substrates, while the carbonyl oxygen forms a hydrogen bond with the Trp 102β.

In summary, 4-amino-phenyl piperazine derivatives were found to be effective templates that give rise to a series of potent inhibitors of FPTase displaying nanomolar activities on cellular Ras-processing assay. Molecular modelling demonstrates that these compounds act as CAAX analogues and interact efficiently with the different parts of the active site. The most interesting molecules are in the process of being evaluated in-vivo and these results will be reported in due course.

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