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Farnesyltransferase inhibitors: *CAAX* mimetics based on different biaryl scaffolds

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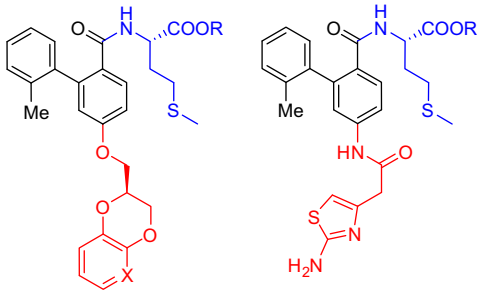
ABSTRACT

Mimetics of the C-terminal *CAAX* tetrapeptide of Ras protein were designed as farnesyltransferase (FTase) inhibitors (FTIs) by replacing *AA* with *o*-aryl or *o*-heteroaryl substituted *p*-hydroxy- or *p*-aminobenzoic acid, while maintaining the replacement of *C* with 1,4-benzodioxan-2-ylmethyl or 2-amino-4-thiazolylacetyl residue as in previous *CAAX* mimetics. Both FTase inhibition and antiproliferative effect were showed by two thiazole derivatives, namely those with 1-naphthyl (**10** and **10a**) or 3-furanyl (**15** and **15a**) in the central spacer, and by the benzodioxane derivative with 2-thienyl (**6** and **6a**) in the same position. Accumulation of unprenylated RAS was demonstrated in cells incubated with **15a**. Consistently with FTIs literature, such results delineate the biaryl scaffold not only as a spacer but also as a sensible area of these mimetic molecules, where modifications at the branching aromatic ring are not indifferent and should be matter of further investigation.

The G-protein Ras plays an important role in mediating cellular responses to growth signals and the appearance of its oncogenic mutants is associated to a high percentage of human cancers.[1,2] Activation of Ras protein requires its localization to the cell membrane, which is conditioned by farnesylation of the cysteine residue of the C-terminal *CAAX* tetrapeptidic sequence by the enzyme farnesyltransferase (FTase).[3] Inhibition of FTase has proved to exert antiproliferative effect and a number of FTase inhibitors (FTIs), whose development began in the early nineties, have been submitted to clinical trials as anticancer drugs.[4–8] Although the exact biological mechanism is still under debate, the efficient antitumor activity with low toxicity of FTIs and their synergistic effects with other antineoplastic drugs offer interesting perspectives for cancer therapy.[9–13,8,14]

Design of *CAAX* mimetics is a currently applied approach to the development of FTIs.[8,15] Common feature of such mimetics, which have gradually evolved into non-peptidic and non-thiol molecules, is the presence of a relatively rigid spacer bearing a fragment mimicking the cysteine residue *C* and its zinc chelating ability and a *X* residue, such as methionine, or an alternative fragment interacting with a hydrophobic pocket in the enzyme active site. Based on this strategy, we have recently developed the FTIs **1**–**3** all having 2-*o*-tolylbenzoyl as a rigid spacer and methionine as a *X* residue while differing in the cysteine replacing fragment, namely pyridodioxan-2-ylmethyloxy in **1**, benzodioxan-2-ylmethyloxy in **2** and 2-amino-4-thiazolylacetamido in **3** (Chart 1).[16–18]

The esters **1a**–**3a** exert inhibition of Ras prenylation and antiproliferative effect on rat aortic smooth muscle cells in the low micromolar range, while inhibition of FTase activity in the low nanomolar range has been determined for the free acids **1** and **3**.



X = N; R = H (**1**), Me (**1a**)
X = CH; R = H (**2**), Me (**2a**)

R = H (**3**), $CHMe_2$ (**3a**)

Chart 1.

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In a continuation of our effort to identify new FTIs, we considered modifying compounds **2** and **3**. The portion mimicking cysteine, containing the benzodioxane or the 2-aminothiazole substructure, was retained unaltered as well as the *X* residue methionine, whereas the central spacer 2-*o*-tolylbenzoyl, which is responsible for correctly positioning the two side fragments, was replaced with other rigid systems. Docking studies on the *CAAX* mimetic FTI-2148, a potent non-thiol FTI,[19] have shown that the 2-*o*-tolylbenzoyl is not only a mere spacer replacing *AA*, but also a substructure interacting with a hydrophobic pocket of FTase through its tolyl residue and that such a pocket can accommodate larger groups than tolyl.[20] Therefore, we planned the replacement of tolyl with a heteroaromatic ring, such as thiophene and furan, or with a larger aromatic system, such as naphthalene and isoquinoline, in order to verify if the extra interaction potential due to the additional heteroatom or ring would improve the enzyme inhibition. In particular, we designed the six analogues **4**–**9** of **2** and the six analogues **10**–**15** of **3**, where 2-*o*-tolyl is replaced by 1-naphthyl, 4-isoquinolinyl, 2- or 3-thienyl, and 2- or 3-furanyl, and the corresponding isopropyl esters **4a**–**15a** (Chart 2).

The isopropyl esters **4a**–**15a** were prepared as prodrugs of the acids **4**–**15** in order to facilitate cell membrane penetration and to evaluate the cell activity of the acids exhibiting FTase inhibition.

To synthesize compounds **4a**–**9a**, the mesylate group of racemic 2-mesyloxymethyl-1,4-benzodioxane[21,22] was displaced by methyl 2-bromo-4-hydroxybenzoate.[23–25] Subsequent Suzuki coupling of the resultant bromophenyl ether with the suitable aryl or heteroaryl boronic acid gave the intermediate methyl esters **16**–**21**, which were converted into the corresponding carboxylic acids **22**–**27** and then condensed with the isopropyl ester of L-methionine to yield the desired esters **4a**–**9a**. Hydrolysis of these esters afforded the carboxylic acids **4**–**9** (Scheme 1).

A different synthetic strategy was adopted to prepare compounds **10**–**15**. Firstly, we synthesized the methyl 2-aryl- or 2-heteroaryl-4-nitrobenzoates **28**–**33** by Suzuki coupling of methyl 2-bromo-4-nitrobenzoate with the suitable aryl or heteroaryl boronic acid. The methyl benzoates **28**–**33** were then hydrolyzed and condensed with the isopropyl ester of L-methionine. The NO_2 group was reduced to NH_2 and the resulting anilines **46**–**51** converted into the anilides **52**–**57** by reaction with 2-tritylamino-4-thiazolyl acetic acid.[18] Subsequent N-detritylation provided the esters **10a**–**15a**, which were hydrolyzed to the acids **10**–**15** (Scheme 2) (See Supplementary data for reactions conditions and work-up, yields and spectral data).

The inhibition of FTase activity was first determined using a FTase fluorescent assay[25] and FTI-276 (IC_{50} 9 nM)[18] and **3** (IC_{50} 49 nM)[18] as reference compounds. In the initial screening, the free acids **4**–**15** were tested at a 20 μM and the percentual inhibition at such concentration was determined. Results are summarized in Table 1. Two compounds, namely **7** and **15**, showed a near 100% inhibition with an IC_{50} equal to 5.0 and 2.8 μM, while the others exhibited lower inhibitory activities or were not active.

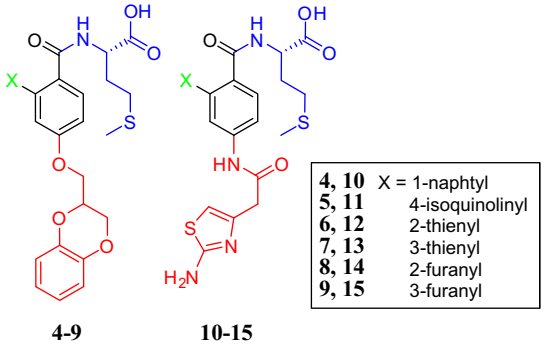
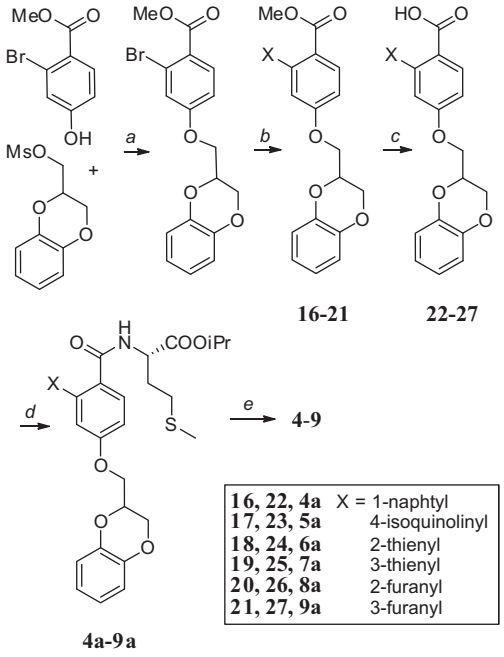


Chart 2.

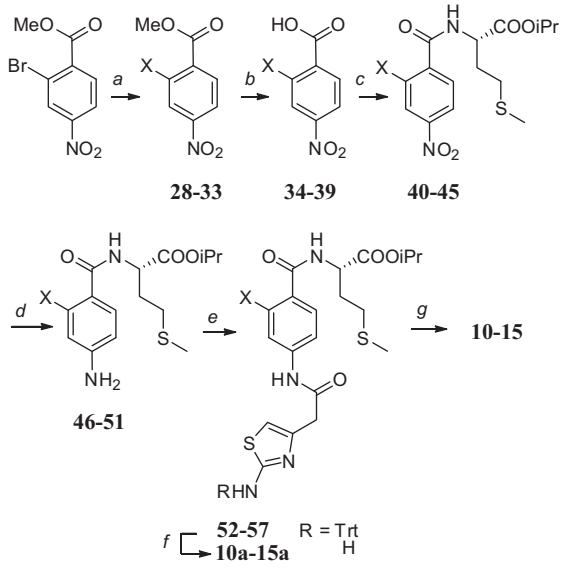


Scheme 1. Synthesis of compounds **4**–**9** and of the corresponding isopropyl esters **4a**–**9a**. Reagents and conditions: (a) K_2CO_3, DMF, 30 min, room temperature and, after addiction of the mesylate, 60 °C, 18 h, 76%; (b) $Pd(PPh_3)_4$, $XB(OH)_2$, DMF or THF or toluene/methanol, $Na_3PO_4{\cdot}12H_2O$ or Na_2CO_3/H_2O or K_2CO_3/H_2O, room temperature or 100 °C, 18 h, 58–95%; (c) NaOH 1 M at 60 °C or NaOH 4 M at room temperature, methanol, 18 h, 60–100%; (d) L-methionine isopropyl ester, HOBt, EDAC·HCl, DIPEA, DMF, 18 h, room temperature, 67–91%; (e) NaOH 1 M, MeOH, 18 h, room temperature, 100%.

The isopropyl esters **4a**–**15a**, prepared to ameliorate membrane permeability and to study the effects on cell growth, were tested by a cellular assay measuring inhibition of rat aortic SMC proliferation. As shown in Table 2, most of them proved to inhibit rat SMC proliferation with IC_{50} values in the micromolar range. In particular, compound **15a**, the ester of the most potent FTase inhibitor of the series, reduced rat SMC growth in a concentration dependent manner with an IC_{50} value of 250 μM, whereas compound **7a**, whose free acid **7** is only little less potent than **15** as a FTI, showed no antiproliferative activity.

To study whether the ability of compound **15** to interfere with FTase activity in vitro was maintained also in cultured cells by its ester **15a**, we investigated the Ras prenylation by western blot analysis from total cell lysates of rat SMCs. Cells were incubated for 72 h in the presence or absence of **15a** at concentrations ranging from 25 to 400 μM and in the presence of the 3-methyl-3-glutaryl-coenzyme A (HMG-CoA) reductase inhibitor simvastatin (2 μM) as positive control. The compound **15a** interfered with Ras farnesylation in a concentration-dependent manner with approximately 100 μM IC_{50}, as demonstrated by the appearance of a slower migrating band corresponding to the unprenylated form of the protein, also detected after incubation with simvastatin (Fig. 1) (See Supplementary data for FTase inhibition tests and for cell proliferation and Ras prenylation assays).

In terms of FTase inhibition, two of the six designed replacements of *o*-tolyl with new aryl or heteroaryl systems are invariably detrimental, namely those with 4-isoquinolinyl and 2-furanyl. On the contrary, the other four tolyl replacements result in a moderate FTase inhibition but depending on the compounds series: 1-naphthyl and 3-furanyl in the 2-aminothiazoles (compounds **10** and **15**), 2- and 3-thienyl in the benzodioxanes (compounds **6** and **7**). However, of the two thienyl substituted esters, one has a



28, 34, 40, 46, 52, 10a	X = 1-naphtyl
29, 35, 41, 47, 53, 11a	4-isoquinolinyl
30, 36, 42, 48, 54, 12a	2-thienyl
31, 37, 43, 49, 55, 13a	3-thienyl
32, 38, 44, 50, 56, 14a	2-furanyl
33, 39, 45, 51, 57, 15a	3-furanyl

Scheme 2. Synthesis of compounds **10**–**15** and of the corresponding esters **10a**–**15a**. Reagents and conditions: (a) $Pd(PPh_3)_4$, $XB(OH)_2$, DMF or THF or toluene/methanol, $Na_3PO_4{\cdot}12H_2O$ or Na_2CO_3/H_2O or K_2CO_3/H_2O, room temperature or 110 °C, 18 h, 56–97%; (b) NaOH 1 or 2.5 M, methanol, room temperature 18 h or 60 °C 2 h, 78–100%; (c) L-methionine isopropyl ester, HOBt, EDAC·HCl, DIPEA, DMF, room temperature, 18 h, 67–91%; (d) $SnCl_2$, EtOAc, reflux, 3 h, 86–100%; (e) 2-(2-tritylamino-4-thiazolyl)acetic acid,, HOBt, EDAC·HCl, DIPEA, DMF, room temperature, 18 h, 46–92%; (f) HCOOH, DCM, room temperature, 18 h, 40–100%; (g) NaOH 1 M, MeOH, 18 h, room temperature, 78–100%.

Table 1
Inhibitory activities of compounds **4**–**15** (20 μM) on FTase expressed as inhibition ratio (%)

Compounds	Inhibition ratio (%)	Compounds	Inhibition ratio (%)
4	41.7	**10**	90.4
5	14.5	**11**	0
6	90.0	**12**	53.8
7	96.6	**13**	20.9
8	51.5	**14**	15.4
9	48.7	**15**	98.7

Table 2
Inhibition of rat SMC proliferation by compounds **4a**–**15a**

Cell proliferation		Cell proliferation	
Compounds	Cell proliferation IC_{50} (μM)	Compounds	IC_{50} (μM)
4a	155	**10a**	81
5a	n.a.	**11a**	252
6a	81	**12a**	200
7a	n.a.	**13a**	123
8a	99	**14a**	110
9a	61	**15a**	259

n.a.: not active.

significantly lower antiproliferative activity than the lead compound (cf. 81 μM IC_{50} of **6a** and 6.6 μM IC_{50} of **2a**[17]) and the other, **7a**, is devoid of antiproliferative effect. On the other hand, in the 2-aminothiazole series, FTase inhibition by **10** and **15** is in the low

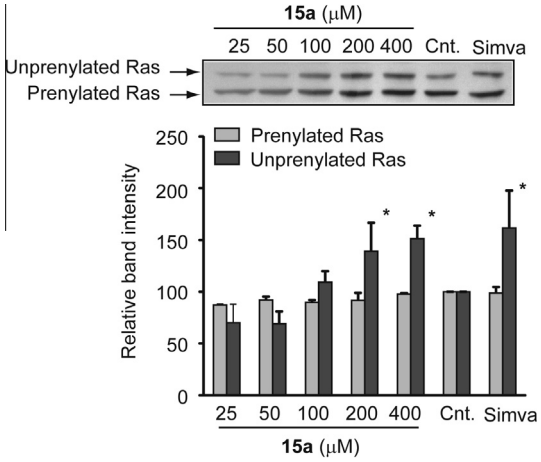


Figure 1. After 72 h incubation of the cells with indicated concentrations of compound **15a** and 2 μM simvastatin (Simva), total cell lysates were prepared and Ras prenylation evaluated by Western blotting analysis with a specific antibody anti Ras (clone RAS10, Millipore). The slower migrating band represents the unprenylated form of Ras, while the faster migrating band is prenylated Ras.

micromolar range, while **3** has a 49 nM IC_{50}[17] and also the tests on cultured cells indicate a slight superiority of **3a** to **10a** and **15a**.

Overall, such results indicate that none of the aryl systems selected to replace the *o*-tolyl residue has improved the activity profile of the lead FTIs **2** and **3**. Nevertheless, **6**, **10** and **15** can be considered new FTIs with moderate activity in vitro and, when esterified, in whole cells. Furthermore, the antiproliferative effect of **15a** has been proved to be associated with unprenylated Ras accumulation in the cell, as previously demonstrated for **2a** and **3a**.[17,18]

The non univocal effects of the same structural modifications of the central 2-*o*-tolylbenzoyl substructure in the two series of compounds confirm that the role of such scaffold is more complex than that of a mere spacer supporting the two pharmacophoric side portions. Reasonably, the *o*-tolyl residue is determinant for their correct orientation and is itself responsible for favorable contacts within the active site, as suggested by the advantages from the *ortho* methyl substitution on the branching aromatic ring, which have been imputed both to the increased hydrophobic surface area and conformational constraint.[26] As the 2-*o*-tolylbenzoyl replacements we have made do not improve FTase inhibitory activity, but neither prejudice it, compounds **6**, **10** and **15** are candidates for being optimized and subjected to a wider SAR study with reference to the biaryl scaffold. In perspective, finer modifications, such as the introduction of an *ortho*-substituent at the branching aromatic ring of the spacer, would have to be considered by analogy with the transformation of biphenyl core into *o*-tolylphenyl, previously reported as highly beneficial.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.04.078.

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