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Phenothiazine-based CaaX competitive inhibitors of human farnesyltransferase bearing a cysteine, methionine, serine or valine moiety as a new family of antitumoral compounds

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ABSTRACT

A new family of CaaX competitive inhibitors of human farnesyltransferase based on phenothiazine and carbazole skeleton bearing a L-cysteine, L-methionine, L-serine or L-valine moiety was designed, synthesized and biologically evaluated. Phenothiazine derivatives proved to be more active than carbazole-based compounds. Phenothiazine **1b** with cysteine residue was the most promising inhibitor of human farnesyltransferase in the current study.

The interest in protein farnesyltransferase (FTase) as a potential cancer target is maintained in recent years.[1–6] FTase is a heterodimeric zinc metalloenzyme, responsible for posttranslational modification and activation of Ras proteins. Ras proteins undergo three sequential enzymatic posttranslational modifications. The first step of isoprenylation is catalyzed by FTase and consists of a covalent attachment of the farnesyl group of farnesyldiphosphate (FPP) on the cysteine residue from the C-terminus of tetrapeptidic (CaaX) sequence of Ras proteins.[7] In the CaaX motif, the letter C represents a cysteine and the letter a denotes an aliphatic amino acid. FTase recognizes proteins carrying in X a serine, methionine, glutamine or alanine.[8,9] The isoprenylation process plays a key role in the signaling pathway that allows cell division. Thus, preventing the farnesylation process by inhibiting FTase can represent an approach in cancer chemotherapy.

The main part of molecules targeting FTase are competitive inhibitors of CaaX box. CaaX competitive inhibitors of FTase (FTIs) will thus compete with the terminal cysteine of Ras proteins (replacement of aliphatic amino acids by aromatic amino acids and modification of the methylation reaction of the C-terminus). Cell permeability problems have been reported in the past for some CaaX competitive FTIs, due to their peptide structure,[10] sensitive to peptidases (e.g., compound **VII**, CVIM, Fig. 1). For this reason, currently developed FTIs are nonpeptide. These compounds are often capable of chelating the zinc cation of FTase and have a methionine as X residue[6,11] (e.g., compound **VIII**,[11] Fig. 1).

Based on our previous efforts in identifying new FTIs (compounds **I**–**VI**, Fig. 1)[2,12–15] and in order to enrich the existing SAR on this family of antitumoral agents, we were then interested in the design and synthesis of CaaX competitive inhibitors of FTase with phenothiazine or carbazole skeleton bearing a cysteine, methionine, serine or valine residue as potential zinc chelating unit (compounds **1a**–**i** and **2a**–**i**, Fig. 1).

The title compounds **1a**–**i**, **2a**–**i** were synthesized as outlined in Scheme 1. Starting acids **6**–**8** were obtained by Michael addition reaction of carbazole **12**, phenothiazine **13** or 2-chlorophenothiazine **14** with acrylonitrile in the presence of Triton B,[16,17]

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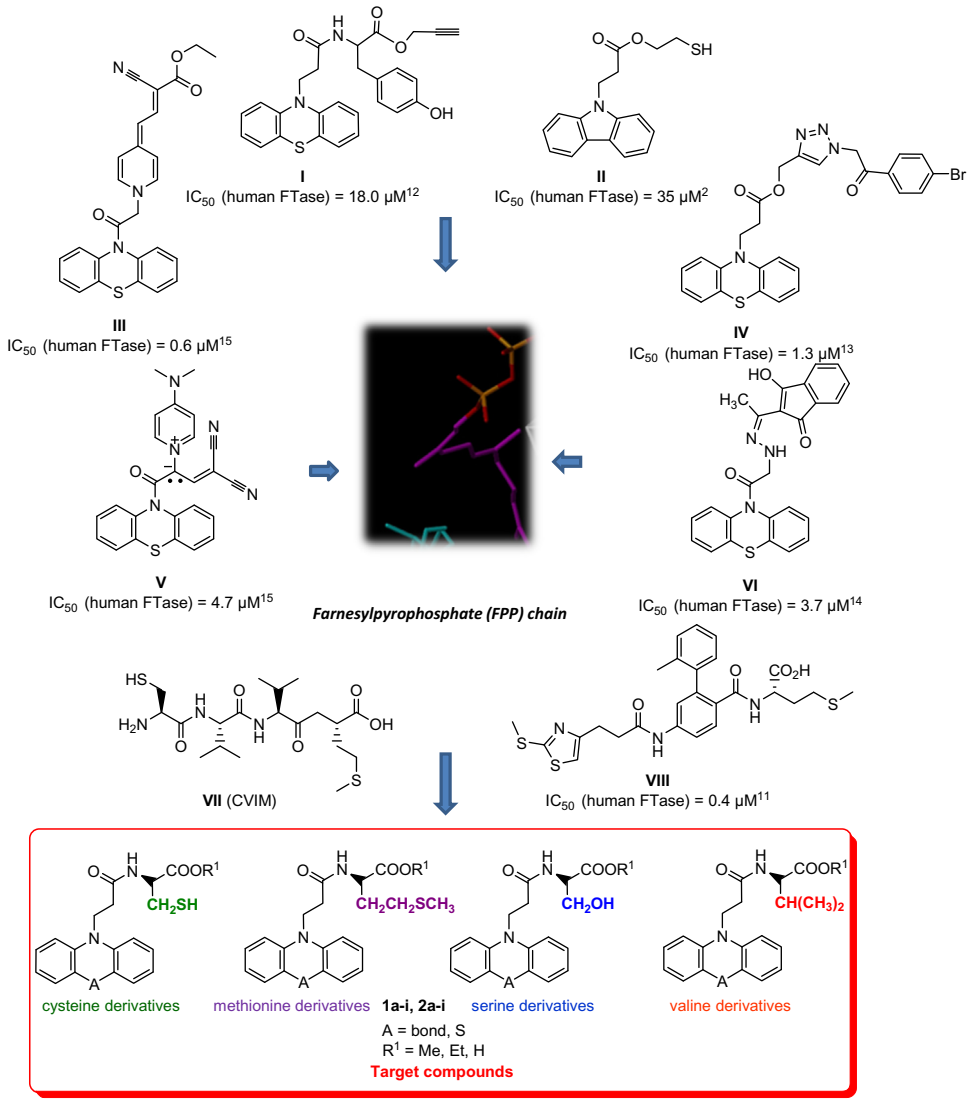

Figure 1. Structure of FTase inhibitors discovered in previous research work (compounds **I**–**VIII**) and of target compounds (**1a**–**i**, **2a**–**i**).

followed by hydrolysis of nitrile **9**–**11** with aqueous sodium hydroxide in methanol.[16,17] The key intermediates, activated esters **3**, **4**,[2,13] **5**, were then prepared by reaction between carboxylic acids **6**, **7** and **8** with *N*-hydroxysuccinimide in the presence of EDCI [1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride].[18] Furthermore, coupling reactions of activated esters **3** and **4** with L-cysteinyl, methionyl, serinyl or valinyl esters provided the corresponding esters **2a**–**h** (Scheme 1). In these reactions, the target cysteinyl ester derivative **2b** bearing a phenothiazine unit was obtained in 53% yield by reacting *N*-hydroxysuccinimide activated ester **7** with L-cysteine ethyl ester hydrochloride, but the dimer **2b$_d$** was also isolated in 43% yield (Scheme 2). Finally, the saponification of esters **2a**–**h** furnished carboxylic derivatives **1a**–**h** (Scheme 1). In the 2-chlorophenothiazine series, only the coupling reaction of activated ester **5** with L-methionine methyl ester hydrochloride was realized in order to obtain the corresponding ester **2i**, which was then saponified to the corresponding acid **1i** (Scheme 1).

In the interest of exploring the importance on the biological efficiency of three carbon atoms chain between the phenothiazinic nitrogen atom and the methionyl residue, the introduction of a triazolyl unit as a different tensor was then envisaged. To reach the target triazole derivative **20** (Scheme 3), phenothiazine **13** was first reacted with 1-bromo-3-chloropropane using sodium hydride as base and provided chloro derivative **15**.[19] Azide **16** was next obtained in good yield by action of sodium azide in water/chloroform in the presence of TBAB phase-transfer catalysis medium at room temperature.[20] The construction of 1,2,3-triazole ring in compound **17** was then achieved by *click chemistry*.[21,22] The saponification of ethyl ester **17** straightforwardly provided carboxylic acid **18** which was further coupled, after in situ activation in the presence of 1-hydroxybenzotriazole and EDCI, with L-methionine methyl ester hydrochloride to obtain intermediate **19** in 69% yield. Synthesis of the target triazole derivative **20** was finally achieved in very good yield by simple saponification of methyl ester **19** (Scheme 3).

The activity of all synthesized phenothiazine and carbazole derivatives was evaluated on human FTase.[23] Results are reported in Table 1 and Figures 2 and 3. Examination of the inhibitory profile of these two series emphasizes, without exception, greater biological potential for derivatives bearing a phenothiazine unit (e.g., phenothiazine **1b** (IC_{50} (FTase) = 4.7 ± 0.5 μM) vs carbazole **1a** (IC_{50} (FTase) = 65.4 ± 5.1 μM); phenothiazine **1d** (IC_{50} (FTase) = 11.7 ± 0.9 μM) vs carbazole **1c** (IC_{50} (FTase) = 40.2 ± 2.2 μM),