Bioorganic & Medicinal Chemistry Letters 25 (2015) 4447–4452

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

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

Gina-Mirabela Dumitriu [a], Elena Bîcu [a], Dalila Belei [a], Benoît Rigo [b,c], Joëlle Dubois [d], Amaury Farce [b,e], Alina Ghinet [a,b,c,*]

[a] Department of Organic Chemistry, Faculty of Chemistry, 'Al. I. Cuza' University of Iasi, B-dul Carol I, Nr. 11, Corp A, 700506 Iasi, Romania
[b] Inserm, LIRIC-U995, Université de Lille 2, CHRU de Lille, Faculté de Médecine—Pôle Recherche, Place Verdun, F-59045 Lille Cedex, France
[c] Hautes Etudes d'Ingénieur (HEI), UCLille, Laboratoire de pharmacochimie, 13 rue de Toul, BP 41290, F-59014 Lille Cedex, France
[d] Institut de Chimie des Substances Naturelles, UPR2301 CNRS, Centre de Recherche de Gif, Avenue de la Terrasse, F-91198 Gif-sur-Yvette Cedex, France
[e] Université de Lille 2, Faculté des Sciences Pharmaceutiques et Biologiques, Laboratoire de Chimie Thérapeutique, 3 rue du Professeur Laguesse, BP 83, F-59006 Lille Cedex, France

ARTICLE INFO

Article history:
Received 20 July 2015
Revised 3 September 2015
Accepted 4 September 2015
Available online 7 September 2015

Keywords:
Farnesyltransferase
Phenothiazine
Cysteine
Methionine
Serine
Valine
CAAX inhibitor

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.

© 2015 Elsevier Ltd. All rights reserved.

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]

* Corresponding author.
E-mail address: alina.ghinet@hei.fr (A. Ghinet).

http://dx.doi.org/10.1016/j.bmcl.2015.09.008
0960-894X/© 2015 Elsevier Ltd. All rights reserved.

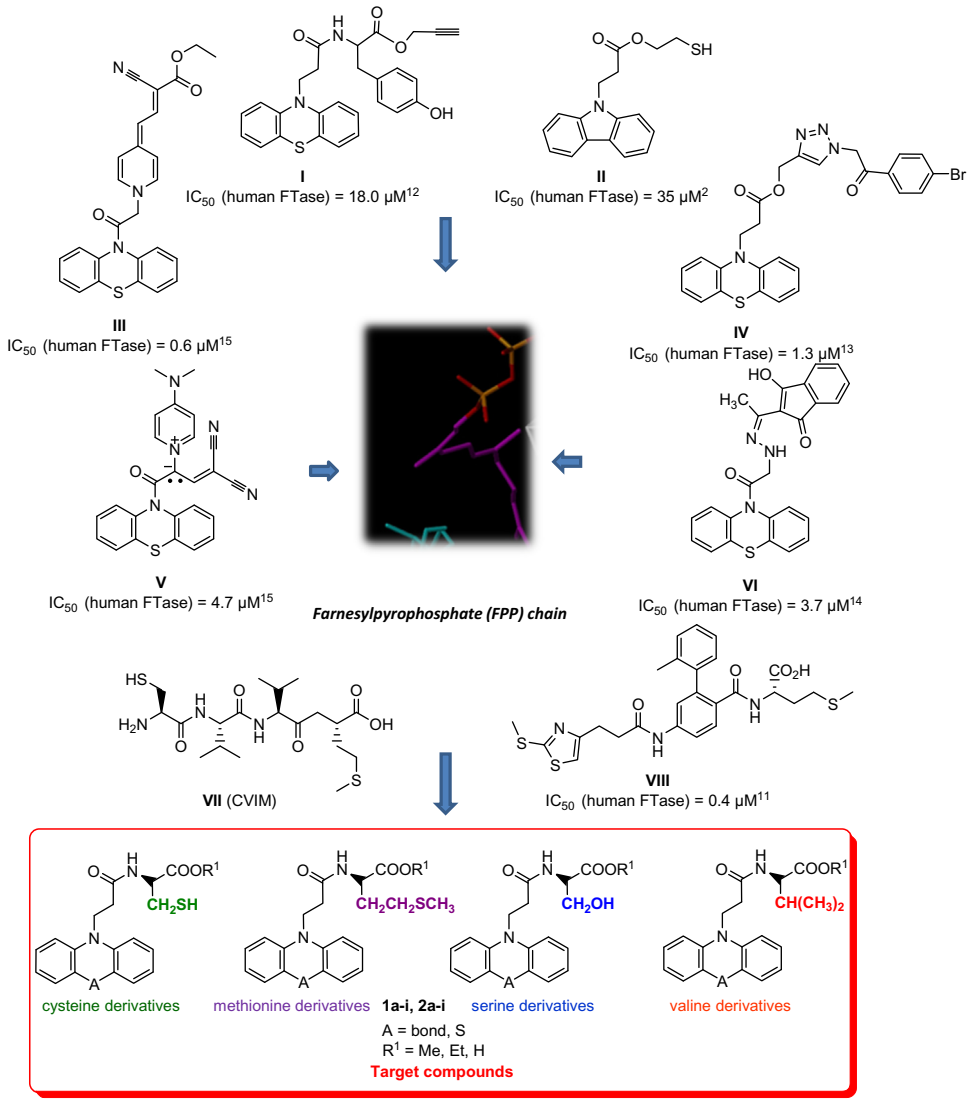
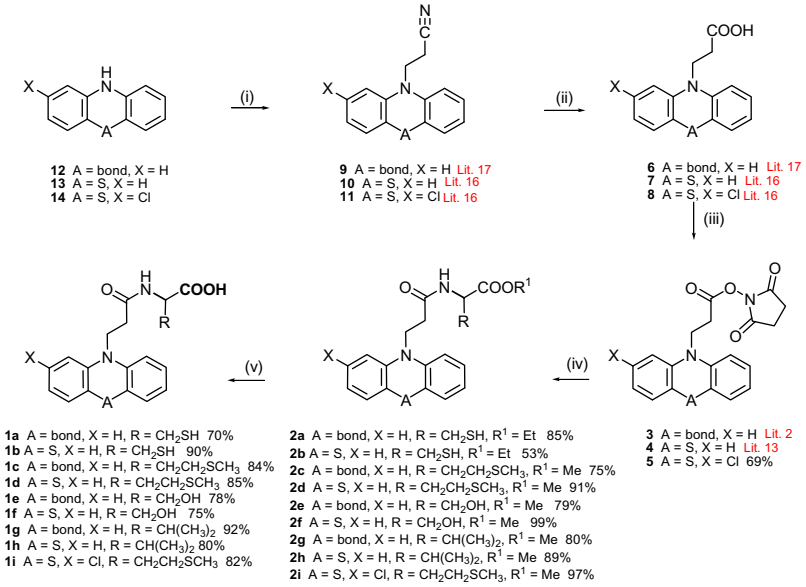


Figure 1. Structure of FTase inhibitors discovered in previous research work (compounds **I**–**VIII**) and of target compounds (**1a**–**i**, **2a**–**i**).

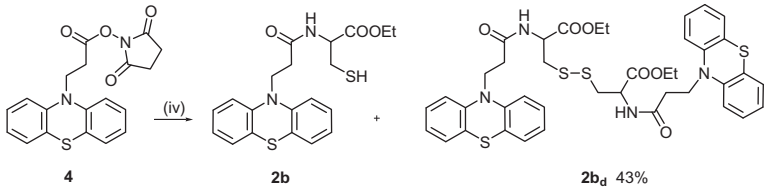
followed by hydrolysis of nitrile **9**–**11** with aqueous sodium hydroxide in methanol.[16,17] The key intermediates, activated esters **3**, **4**,[2,13] **5**, were then prepared by reaction between carboxylic acids **6**, **7** and **8** with *N*-hydroxysuccinimide in the presence of EDCI [1-ethyl-3-(3-dimethylaminopropyl)carbodiimide 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),



Scheme 1. Reagents and conditions: (i) acrylonitrile, Triton B, 0 °C to reflux, 2 h; (ii) aqueous NaOH, MeOH, reflux, 15 h; (iii) 1.2 equiv *N*-hydroxysuccinimide, 1.2 equiv EDCI, CH_2Cl_2, rt, 24 h; (iv) 1.2 equiv L-cysteine ethyl ester hydrochloride, L-methionine methyl ester hydrochloride, L-serine methyl ester hydrochloride or L-valine methyl ester hydrochloride, 1.2 equiv triethylamine, CH_2Cl_2, rt, 24 h; (v) 5 equiv NaOH 2 N, 80 °C, 2–5 h.



Scheme 2. Reagents and conditions: (iv) 1.2 equiv L-cysteine ethyl ester hydrochloride, 1.2 equiv triethylamine, CH_2Cl_2, rt, 24 h.

Table 1). This result is in accordance with previous reported studies which highlight the phenothiazine nucleus as well tolerated bulky unit for the A_2 binding site of human farnesyltransferase.[12,13,15] The 2-chloro substitution of the phenothiazine unit is tolerated, but does not improve the activity. Thus, methionine compound **1i** has similar FTase affinity as unsubstituted derivative **1d** (e.g., compound **1i**: IC_{50} (FTase) = 18.9 ± 3.2 μM vs compound **1d**: IC_{50} (FTase) = 11.7 ± 0.9 μM, Table 1). Therefore, this chemical modulation was not envisaged in the cysteine, serine or valine series. Moreover, carboxylic acids **1a–i** showed superior inhibitory potency compared to their ester analogues **2a–i** (Table 1 and Fig. 2). This highlights a better chelating power of the zinc cation of the enzyme for carboxylic acids versus ethyl or methyl esters. Similar tendency was previously observed in the FTase inhibitors domain.[12]

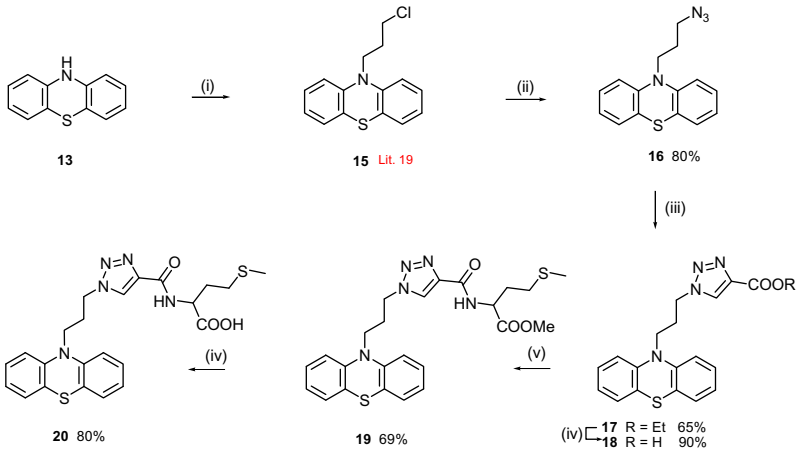
The study of the nature of the aminoacid residue on the biological properties revealed that in the carbazole series, the cysteine and methionine analogues (e.g., carbazoles **1a** and **1c**, Table 1) were more active than derivatives bearing a serine or a valine unit (e.g., carbazoles **1e** and **1g**, Table 1). In the phenothiazine series, cysteine derivative **1b** was the best FTase inhibitor. Methionine and serine analogues (compounds **1c** and **1f**, respectively) showed slightly decreased activities and valine derivative **1h** presented a modest activity (IC_{50} (FTase) = 44.7 μM) (Table 1).

In order to gain supplementary structure–activity relationships in the current family of CaaX competitive inhibitors of human farnesyltransferase, modulations have been envisaged on the spacer between the nitrogen atom from the phenothiazine unit and the amide function. The insertion of a 1,2,3-triazole ring as a spacer was thus realized (Scheme 3 and Fig. 3). Since the 2-chloro substitution of the phenothiazine unit in compound **1i** did not result in improved inhibitory properties (Fig. 3), the spacer variation was realized only on compound **1d**. However, the insertion of the triazole unit in compound **20** and consequently, the increase of the spacer length, was not tolerated and resulted in diminished biological potency (e.g., compound **1d** vs **20**, Fig. 3). The three-carbon atoms chain between the phenothiazine nitrogen and the amino group from the aminoacid residue proved to be important for the FTase inhibition.

Farnesyltransferase structure[24] was taken from the 1LD7 entry of the RCSB Protein Data Bank.[25] The crystallized inhibitor and water molecules were removed to permit docking of the studied compounds, built from the standard fragments library of Sybyl 6.9.1[26] with GOLD 5.1.[27] Thirty solutions were generated and classed through an in-house scoring function based on GoldScore[27] and X-Score functions.[28] The consistency of the results was assessed by visually examining the conformation cluster.

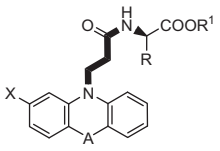
Molecular docking was realized on the best candidates issued from this study **1b**, **1d**, **1f** and **1i** (Fig. 4) in order to validate the zinc-chelating potential of the free carboxylic acids and understand the positioning and interactions of these molecules in the active site of FTase.

Phenothiazine derivative bearing a cysteine moiety **1b** (Fig. 4 (a)) has a set of conformations placed on the same side and oriented in the same direction in the active site of the protein and with a score superior to serine derivative **1f**. The carboxylic acid is in a favorable position and interacts with the zinc atom of the protein, a fact significant for FTase inhibitory properties.



Scheme 3. Reagents and conditions: (i) 1.2 equiv 1-bromo-3-chloropropane, 1.2 equiv NaH, dimethylformamide;[19] (ii) 3 equiv sodium azide, 0.45 equiv tetrabutylammoniumbromide (TBAB), $CHCl_3/H_2O$ 2:1, rt, 24 h; (iii) 1 equiv ethyl propiolate, 0.1 equiv $CuSO_4{\cdot}5H_2O$, 0.2 equiv sodium ascorbate, *t*-BuOH/H_2O 10:2, rt, 24 h; (iv) 5 equiv NaOH 2 N, 80 °C, 2 h; (v) 1 equiv L-methionine methyl ester hydrochloride, 1.05 equiv 1-hydroxybenzotriazole, 1 equiv EDCI, 1 equiv triethylamine, CH_2Cl_2, rt, 24 h.

Table 1
Inhibitory activities of compounds **1a–i**, **2a–i**, **2b$_d$** on human FTase



Compound	A	R	R^1	X	% Inh (FTase)[a,b]	IC_{50}[b] (μM ± SD[c])	R^2[d]
1a	Bond	CH_2SH	H	H	67	65.4 ± 5.1	0.923
1b	S	CH_2SH	H	H	92	4.7 ± 0.5	0.964
1c	Bond	$CH_2CH_2SCH_3$	H	H	73	40.2 ± 2.2	0.759
1d	S	$CH_2CH_2SCH_3$	H	H	92	11.7 ± 0.9	0.959
1e	Bond	CH_2OH	H	H	33	n.d.[e]	—
1f	S	CH_2OH	H	H	94	12.0 ± 2.6	0.845
1g	Bond	$CH(CH_3)_2$	H	H	22	n.d.	—
1h	S	$CH(CH_3)_2$	H	H	76	44.7 ± 3.5	0.938
1i	S	$CH_2CH_2SCH_3$	H	Cl	96	18.9 ± 3.2	0.947
2a	Bond	CH_2SH	CH_2CH_3	H	15	n.d.	—
2b	S	CH_2SH	CH_2CH_3	H	10	n.d.	—
2b$_d$	S	$CH_2S)_2$	CH_2CH_3	H	0	n.d.	—
2c	Bond	$CH_2CH_2SCH_3$	CH_3	H	51	n.d.	—
2d	S	$CH_2CH_2SCH_3$	CH_3	H	5	n.d.	—
2e	Bond	CH_2OH	CH_3	H	23	n.d.	—
2f	S	CH_2OH	CH_3	H	63	n.d.	—
2g	Bond	$CH(CH_3)_2$	CH_3	H	12	n.d.	—
2h	S	$CH(CH_3)_2$	CH_3	H	52	n.d.	—
2i	S	$CH_2CH_2SCH_3$	CH_3	Cl	0	n.d.	—

[a] Inhibition ratio of protein farnesyltransferase at a 100 μM concentration.
[b] Values represent mean of two experiments.
[c] SD: standard deviation.
[d] R^2: regression factor.
[e] Not determined.

The methionine derivative **1d** (Fig. 4(b)) occupies globally the same place in the active site as compound **1b**. Again, carboxylic acid function is placed next to the zinc cation to insure complexation and thus, inactivation of the enzyme.

Serine derivative **1f** has two possible conformations. One conformation seems significantly larger and is depicted in Figure 4 (c). The carboxylic acid interacts with the zinc atom of farnesyltransferase. The tricycle lodges globally the same place as derivatives **1b** and **1d**, explaining the similar biological activity.

2-Chlorophenothiazine derivative **1i** is the least active of these four compounds. It can adopt four different conformations suitably oriented. The conformation with the best score is represented in Figure 4(d). The tricycle location is not stable, adopting at least three different positions in the active site.

The comparison of the various compounds seems to indicate a two point interaction mode. The first and most evident is the interaction with the zinc atom of the enzyme, which is chelated by at least the acid moiety of all the molecules. Small substituents on the neighboring branch of the side chain are able to form a second interaction, which may count for a part of the activity of compounds **1b** and **1f**. The ethyl linker in the side chain of the amino acid is too long to permit a correct placement of the terminal sulfur of **1d** and **1i**.

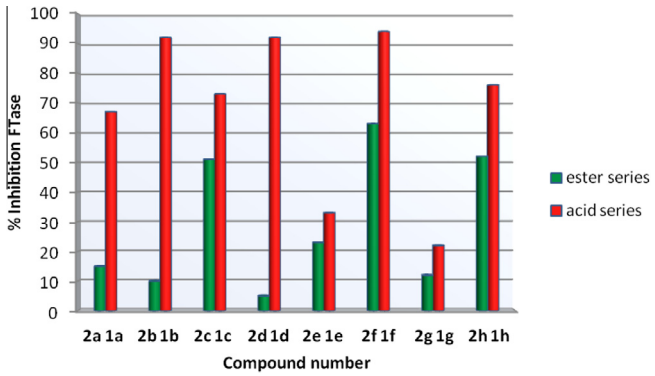


Figure 2. Comparative inhibitory activity on human FTase in the carbazole and phenothiazine series at a 100 μM concentration: esters **2a**–**h** (in green) versus corresponding acids **1a**–**h** (in red).

The second is less apparent when looking only at the docking results but is evident when checking the biological results. The tricyclic base of the compound has a privileged position for a high activity. In fact, none of the compounds with a bond in position A appears in the best compounds. Interestingly, the difference between **1b** and **1f** is the presence of a second conformation for the last, with the tricycle off to the side of the pocket, along the FPP. This may explain the diminished activity of compound **1f** compared to compound **1b**. This conclusion is further proved by compound **1i**. Substitution at R^1 hinders rather seriously the positioning of the tricycle and therefore has a degrading effect on the stability of the best interaction conformation of the tricycle, with compound **1i** having three possible placements in the binding site.

A new series of phenothiazine and carbazole derivatives bearing a L-cysteine, L-methionine, L-serine or L-valine moiety was designed, synthesized and evaluated as human farnesyltransferase inhibitors. The preferred synthetic strategy appealed primarily to an activated ester coupling reaction with esters of the four different aminoacids mentioned above and provided target esters **2a–i** and **19** in very good yields. A final straightforward saponification reaction provided corresponding carboxylic acids **1a–i** and **20**. A biological screening was assessed on all final compounds in order to evaluate the ability to inhibit human farnesyltransferase. Phenothiazine derivative bearing a cysteine residue **1b** was the

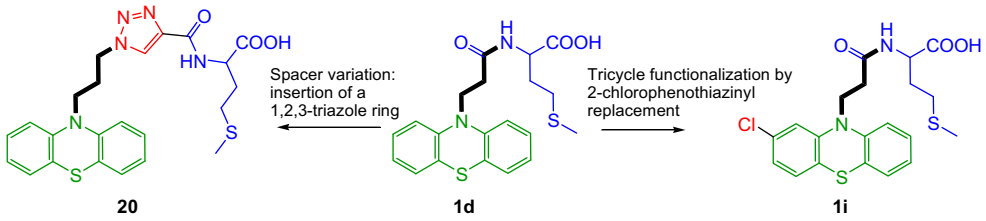


Figure 3. Inhibitory activity of 1,2,3-triazole-4-methionine derivative **20** on human FTase compared to phenothiazines **1d** and **1i**.

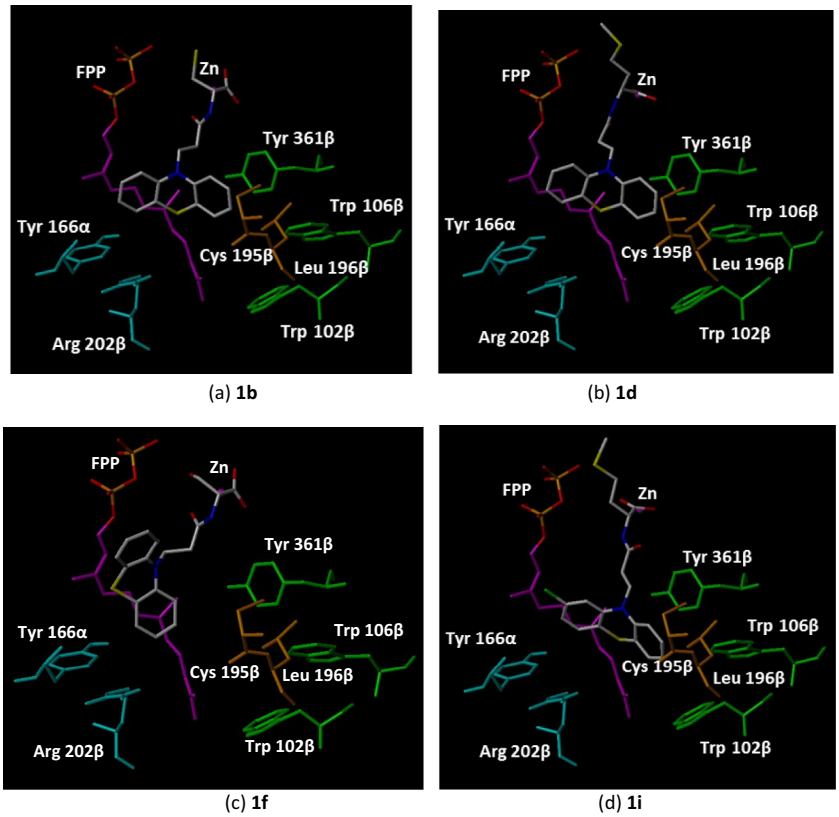


Figure 4. Docking of compounds **1b**, **1d**, **1f** and **1i** in the active site of FTase: (a) compound **1b**, (b) compound **1d**, (c) compound **1f**, (d) compound **1i**.

most interesting inhibitor of human farnesyltransferase in the current study with an IC_{50} value in the micromolar domain. Some important QSAR have been established on the basis of the development and biological evaluation of this new family of CaaX competitive inhibitors of human farnesyltransferase: (1) the phenothiazine skeleton has great importance for the biological activity on human FTase; the replacement by a carbazole unit resulted in diminished inhibitory potential; (2) the substitution of the phenothiazine unit in position 2 by a chloro group is tolerated and consequently conserves the biological properties; (3) carboxylic acid derivatives **1a**–**i** are more active in vitro than corresponding esters **2a**–**i**, thus suggesting a greater zinc chelating potential for the free carboxylic group compared to an ester moiety; (4) cysteine derivatives have slightly improved inhibitory properties compared to methionine and serine analogues; the valinyl-substituted compounds presenting the most modest activity in the current study; (5) finally, the spacer length between the phenothiazinic nitrogen and the amide group is also important for the affinity toward FTase; the insertion of a triazole ring, and thus increase of the spacer length, diminishes the biological potency on the protein.

The best candidates issued from this study could constitute strategic hits for developing anticancer agents with improved characteristics.

Acknowledgements

This work was supported by the strategic grant POSDRU/159/1.5/S/137750, Project "Doctoral and Postdoctoral programs support for increased competitiveness in Exact Sciences research" cofinanced by the European Social Found within the Sectorial Operational Program Human Resources Development 2007–2013 (PhD scholarship G.-M.D.).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.09.008.

References and notes

1. Yang, L.; Liu, W.; Mei, H.; Zhang, Y.; Yu, X.; Xu, Y.; Li, H.; Huang, J.; Zhao, Z. *MedChemComm* **2015**, *6*, 671.
2. Dumitriu, G.-M.; Ghinet, A.; Belei, D.; Rigo, B.; Gautret, P.; Dubois, J.; Bîcu, E. *Lett. Drug Des. Discovery* **2015**, *12*, 85.
3. Shen, M.; Pan, P.; Li, Y.; Li, D.; Yu, H.; Hou, T. *Drug Discovery Today* **2015**, *20*, 267.
4. Holland, A. J.; Cleveland, D. W.; Desai, A.; Reis, R. M.; Pereira, C.; Gassmann, R.; Niessen, S.; Andres, D. A.; Spielmann, H. P. *Mol. Biol. Cell* **1845**, *2015*, 26.
5. Moorthy, N. S. H. N.; Sousa, S. F.; Ramos, M. J.; Fernandes, P. A. *Curr. Med. Chem.* **2013**, *20*, 4888.
6. Straniero, V.; Pallavicini, M.; Chiodini, G.; Ruggeri, P.; Fumagalli, L.; Bolchi, C.; Corsini, A.; Ferri, N.; Ricci, C.; Valoti, E. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 2924.
7. Russo, P.; Loprevite, M.; Cesario, A.; Ardizzoni, A. *Curr. Med. Chem. Anticancer Agents* **2004**, *4*, 123.
8. Zhang, F. L.; Casey, P. J. *Annu. Rev. Biochem.* **1996**, *65*, 241.
9. Zhang, H. In *Handbook of Metalloproteins*; Messerschmidt, Albrecht, Ed.; John Wiley & Sons Ltd: Chichester, UK, 2004; Vol. 3, p 37.
10. Kral, A. M.; Diekl, R. E.; deSolms, S. J.; Williams, T. M.; Kohl, N. E.; Omer, C. A. *J. Biol. Chem.* **1997**, *272*, 27319.
11. Bolchia, C.; Pallavicinia, M.; Berninib, S. K.; Chiodinia, G.; Corsinib, A.; Ferrib, N.; Fumagallia, L.; Straniero, V.; Valoti, E. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5408.
12. Dumitriu, G. M.; Ghinet, A.; Bîcu, E.; Rigo, B.; Dubois, J.; Farce, A.; Belei, D. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 3180.
13. Belei, D.; Dumea, C.; Samson, A.; Farce, A.; Dubois, J.; Bîcu, E.; Ghinet, A. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4517.
14. Baciu-Atudosie, L.; Ghinet, A.; Farce, A.; Dubois, J.; Belei, D.; Bîcu, E. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 6896.
15. Abuhaie, C.-M.; Ghinet, A.; Farce, A.; Dubois, J.; Gautret, P.; Rigo, B.; Belei, D.; Bîcu, E. *Eur. J. Med. Chem.* **2013**, *59*, 101.
16. Godefroi, E. F.; Wittle, E. L. *J. Org. Chem.* **1956**, *21*, 1163.
17. Tierney, M. T.; Grinstaff, M. W. *Org. Lett.* **2000**, *2*, 3413.
18. Nakajima, S.; Ikada, Y. *Bioconjugate Chem.* **1995**, *6*, 123.
19. Harrold, M. W.; Chang, Y. A.; Wallace, R. A.; Farooqui, T.; Wallace, L. J.; Uretsky, N.; Miller, D. D. *J. Med. Chem.* **1987**, *30*, 1631.
20. Belei, D.; Bicu, E.; Jones, P. G.; Birsa, M. L. *J. Heterocycl. Chem.* **2011**, *48*, 129.
21. Rostovtsev, V.; Green, L.; Fokin, V.; Sharpless, B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596.
22. Li, Z.; Seo, T.; Ju, J. *Tetrahedron Lett.* **2004**, *45*, 3143.
23. *Farnesyltransferase assay:* Assays were carried out in 96-well plates, prepared with a Biomek NKMC and a Biomek 3000 from Beckman Coulter and read on a Wallac Victor fluorimeter from Perkin–Elmer. per well, 20 μL of farnesylpyrophosphate (10 μM) was added to 180 μL of a solution containing 2 μL of varied concentrations of potential inhibitors (dissolved in DMSO) and 178 μL of a solution composed by 10 μL of partially purified recombinant human FTase (1.5 mg/mL) and 1.0 mL of dansyl-GCVLS peptide (in the following buffer: 5.6 mM DTT, 5.6 mM $MgCl_2$, 12 μM $ZnCl_2$ and 0.2% (w/v) octyl-b-D-glucopyranoside, 52 mM Tris/HCl, pH 7.5). Fluorescence development was recorded for 15 min (0.7 s per well, 20 repeats) at 30 °C with an excitation filter to 340 nm and an emission filter of 486 nm. Each measurement was realized twice, in duplicate or in triplicate. The kinetic experiments were realized under the same conditions, either with FPP as varied substrate with a constant concentration of Dns-GCVLS of 2.5 μM, or with Dns-GCVLS as varied substrate with a constant concentration of FPP of 10 μM. Nonlinear regressions were performed by KaleidaGraph 4.03 software. Coudray, L.; de Figueiredo, R. M.; Duez, S.; Cortial, S.; Dubois, J. *J. Enzyme Inhib. Med. Chem.* **2009**, *24*, 972.
24. 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.
25. Berman, H. M.; Westbrook, J.; Feng, Z.; Gary, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E. *Nucleic Acids Res.* **2000**, *28*, 235.
26. SYBYL 6.9.1, Tripos Associates, 1699 South Hanley Road, St. Louis, MO 63144.
27. Jones, G.; Willett, P.; Glen, R. C.; Leach, A. R.; Taylor, R. *J. Mol. Biol.* **1997**, *267*, 727.
28. Wang, R.; Lai, L.; Wang, S. *J. Comput. Aided Mol. Des.* **2002**, *16*, 11.