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Original article

Synthesis and biological evaluation of a new series of phenothiazine-containing protein farnesyltransferase inhibitors

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ABSTRACT

Two new families of human farnesyltransferase inhibitors **13a**–**m** and **14a**–**d**, based on a phenothiazine scaffold, were synthesized. Compounds **14a** and **14b** were the most promising inhibitors of human farnesyltransferase with IC_{50} values of 0.7 and 0.6 μM, respectively.

1. Introduction

Since the discovery of protein farnesyltransferase (FTase) in the late 1980s, its inhibition has generated much attention as an important target for the conception of new anticancer agents with reduced toxicity [1].

This heterodimeric metalloenzyme belongs to the prenyltransferase protein family. It catalyses the transfer of a farnesyl moiety (C_{15}) from farnesyl pyrophosphate (FPP) to the free thiol group of the C cysteine found in the terminal CaaX motif (where a are aliphatic amino acids and X a serine, a methionine, an alanine or a glutamine) [2] of a group of membrane small G-proteins such as lamin A and lamin B, RhoB or RhoE. This G-protein superfamily is actively involved in many important cellular signalling pathways and plays an important role in carcinogenesis. Indeed, protein farnesylation is an important post-translational modification occurring on several cell signalling proteins *e.g.* oncogenic Ras proteins [3].

Activation of Ras proteins requires association with the plasma membrane which is mainly dependent on farnesylation [4]. Inhibition of farnesyltransferase prevents membrane localization of Ras, and thus constitutes a valid target for the conception of new cytostatic anticancer drugs [5]. Hence, farnesyltransferase inhibitors (FTIs) have been developed as promising drugs for cancer treatment, and diverse compounds (R-115777 [6,7], SCH-66336 [8], BMS-214662 [9]) have emerged from these studies [10] before undergoing clinical trials (Fig. 1). The approaches leading to new FTIs included random screening of chemical libraries [11] or natural as well as rational design of the CaaX peptidomimetics products [12], have also been described.

We have recently discovered inhibitors, heretofore unencountered [13], such as compound **1** (Fig. 1), bearing a phenothiazine unit. The investigation of the steric tolerance of the A_2 binding site of farnesyltransferase revealed that the ferrocene unit was a well-tolerated bulky group (compound **2**, Fig. 1) [14]. We then investigated the possibility of obtaining phenothiazine-containing FTIs able to coordinate with the zinc atom of the protein *via* chelating groups and to interact with the A_2 binding site *via* the

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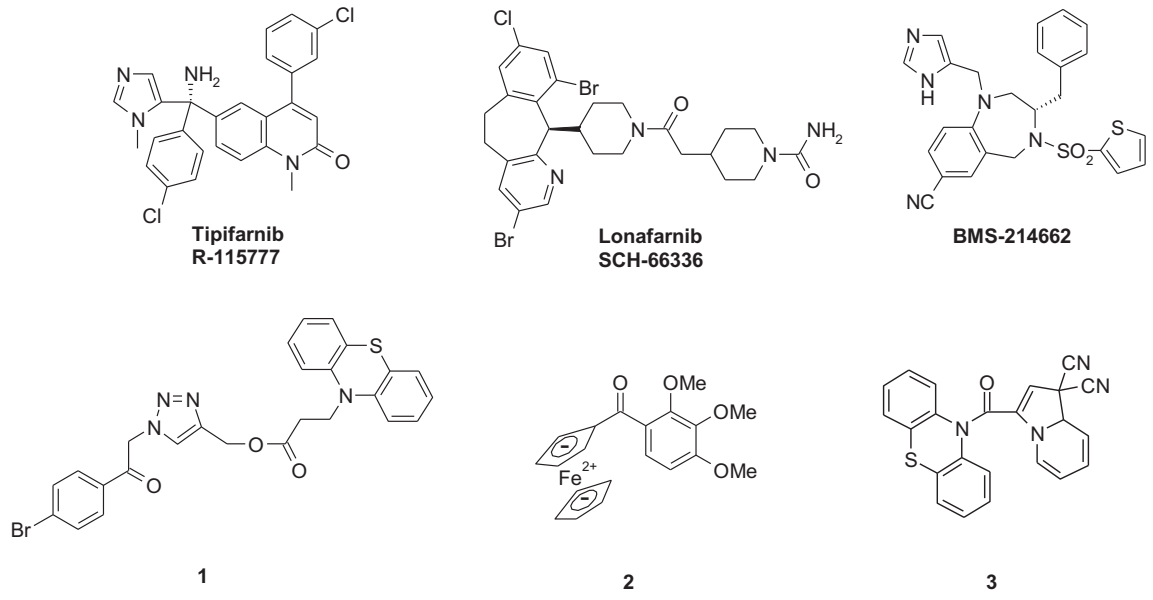


Fig. 1. Structure of some known FTIs and of compounds **1**–**3**.

phenothiazine unit. Then, we decided to perform a preliminary SAR study on the phenothiazine derivatives bearing an indolizine unit with some structural modifications of the scaffold of compound **3** (Fig. 1).

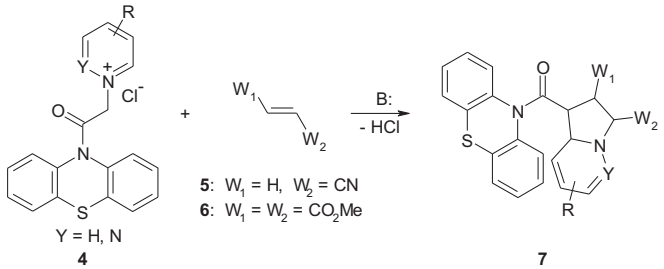
2. Results and discussion

2.1. Chemistry

3-Acylsubstituted tetrahydroindolizine derivatives **7** or dihydrobenzoindolizine can be obtained *via* a 1,3-dipole cycloaddition reaction between *N*-ylides, generated *in situ* from pyridazinium or benzopyridinium salts **4** in the presence of different bases, and different substituted alkenes **5** or **6** (Scheme 1) [15], and we thought that by using ethoxymethylene malononitrile instead of alkenes **5** and **6**, a double bond could be introduced in the pyrrolidine ring of compound **3**.

We were interested in the synthesis of compounds bearing complexing groups (CN, CO_2R) that can bind the zinc atom of protein farnesyltransferase. Different pyridinium salts **9a**–**f** were reacted with trisubstituted alkenes (2-cyano-3-ethoxyacrylonitrile **10**, ethyl 2-cyano-3-ethoxyacrylate **11** or (3*E*)-4-ethoxy-1,1,1-trifluorobut-3-en-2-one **12**). After the reactions were performed, we found that the target compounds **3a**–**m**, analogues to **7**, were not obtained. In their stead, carbanion disubstituted *N*-ylides **13a**–**m** were isolated (Scheme 2 and Table 1).

To the best of our knowledge, cycloimmonium ylides have not been encountered in the FTIs field and no biological properties have been described in the literature for the phenothiazin–pyridinium ylides. Therefore, we decided to synthesize a series of compounds with a ylide skeleton and to investigate the affinity of these new compounds towards protein farnesyltransferase.



Scheme 1. Synthesis of tetrahydroindolizines **7** [15].

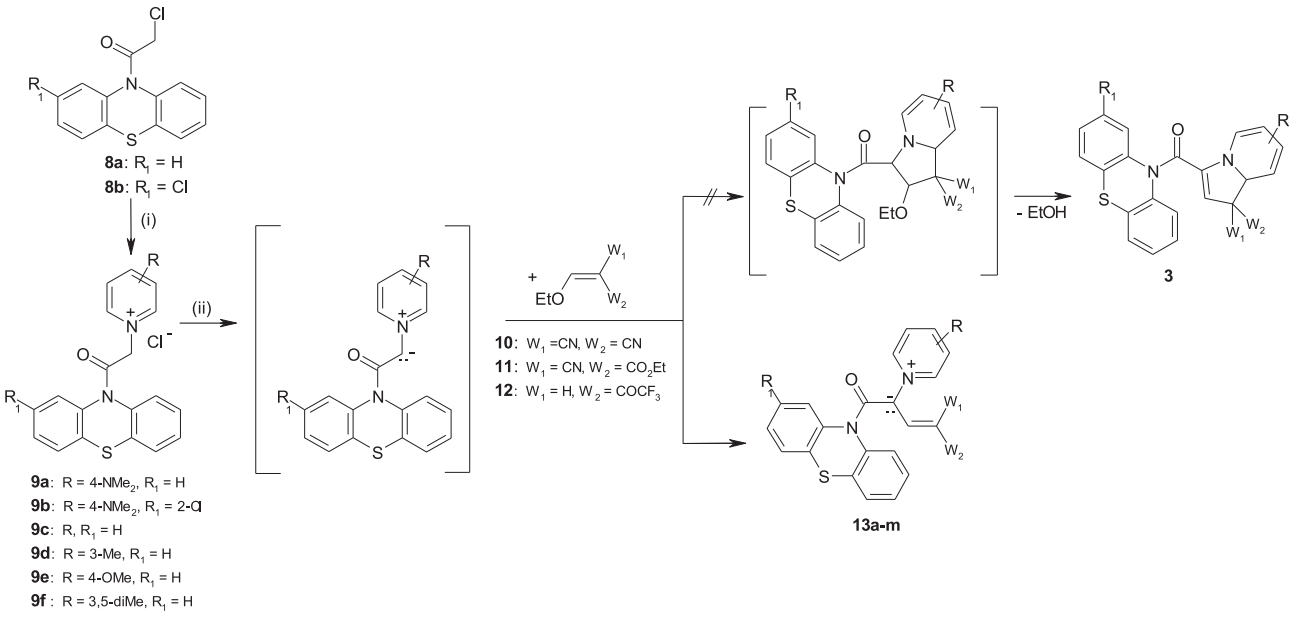
The obtention of carbanion disubstituted *N*-ylides could be due to the two cyano and ester groups, linked to the carbanion of the Michael addition intermediary, that prevent positioning near the carbocation in the α-pyridinium position and/or at the existing extended conjugation.

Note also that hydrolysis of trifluoromethyl group occurred when the reaction was realized with salt **9a** in presence of alkene **12** to afford ylide **13c** (Table 1) in 57% yield. Such hydrolyses have been reported in the literature [16].

As can be observed from Table 1, the reaction is quite general; however, when the same sequence was realized with picoline salts **9g** and **9h**, unexpected red compounds **14a**–**d** were obtained as unique reaction products, in place of ylides **13n**–**q** (Scheme 3). Note that very few references have described the reaction of the methyl group of 2- and 4-picolinium salts with dipolarophiles. Only five allylidenedihydropyridine derivatives were isolated when ketenethioacetal (CMAN) was reacted with picolinium salts [17]; however, no investigation on the generalization of the synthetic method has been performed.

With this in mind, we furtherered the generalization of the synthesis of such pyridineylidene derivatives. When the phenothiazine unit was replaced by a phenyl moiety, the reaction provided the corresponding ylide **15** in 78% yield (Table 2, entry 1). The replacement of the phenothiazine by a morpholine unit or by a diethylamino group resulted in the synthesis of pyridin-(1*H*)-ylidene derivatives **16** and **17** in 41% and 56% yields, respectively; also, with morpholine salt **9j**, a by-product **19** [18] was isolated in 16% yield, which resulted from the cleavage of the amide function, followed by the reaction of the morpholine with alkene **11** (Table 2, entries 6 and 7). Finally, the same sequence realized with pyridinium salt containing a carbazole unit (**9l**) yielded only the cleavage product 9*H*-carbazole **18** (Table 2, entry 8).

The study of the generalization of the synthesis of pyridin-(1*H*)-ylidene derivatives starting from 2- and 4-picolinium salts showed that an amide function was essential for reaction effectiveness; this could perhaps result from stabilization of intermediates charged species.



Scheme 2. Synthesis of ylides **13a**–**m**. Reagents and conditions: (i) pyridine derivative (2–5 equiv), EtOAc, reflux, 24 h; (ii) DBU (1 equiv), DMF or acetonitrile, alkene (2-cyano-3-ethoxyacrylonitrile (**10**), ethyl 2-cyano-3-ethoxyacrylate (**11**) or (3*E*)-4-ethoxy-1,1,1-trifluorobut-3-en-2-one (**12**)) (2 equiv), 70 °C, 24 h.

2.2. Biological evaluation

The activity of the two new families of synthesized compounds was evaluated on human FTase. Results are summarized in Tables 3 and 4. In the ylide series of compounds **13** (structures given in Scheme 2 and Table 1), the 4-dimethylamino substitution of the pyridinium ring proved to be favourable to bioactivity. Except the 3,5-dimethylpyridinium ylides **13l** and **13m**, that showed comparable biological potency, all other structural modifications of the pyridinium ring (H, 3-Me or 4-OMe) caused a slight reduction in biological potential (compounds **13f**, **13h** or **13j** *vs* **13a**, Table 3). Structural modifications of the chelating groups were also realized: the replacement of one cyano group by an ethyl ester generally conserved affinity towards FTase (compound **13a** *vs* **13b**, Table 3). In addition, when only a carboxyl unit was kept to interact with the zinc atom of the protein, a significant decrease in biological potential was observed (**13a**: $IC_{50} = 4.67 \pm 0.36$ μM; **13c**: $IC_{50} = 86.49 \pm 16.96$ μM). Finally, the substitution of the phenothiazine unit by a 2-chloro group conserved biological potency (compound **13b** *vs* **13e**, Table 3).

Table 1
Structure of starting pyridinium salts **9a**–**f** and obtained ylides **13a**–**m**.

Salt N°	Starting salt		Product N°	Ylide			
	R	R_1		R	R_1	W_1	W_2
9a	4-NMe_2	H	**13a**	4-NMe_2	H	CN	CN
			13b	4-NMe_2	H	CN	CO_2Et
			13c	4-NMe_2	H	H	CO_2H
9b	4-NMe_2	2-Cl	**13d**	4-NMe_2	2-Cl	CN	CN
			13e	4-NMe_2	2-Cl	CN	CO_2Et
9c	H	H	**13f**	H	H	CN	CN
			13g	H	H	CN	CO_2Et
9d	3-Me	H	**13h**	3-Me	H	CN	CN
			13i	3-Me	H	CN	CO_2Et
9e	4-OMe	H	**13j**	4-OMe	H	CN	CN
			13k	4-OMe	H	CN	CO_2Et
9f	3,5-diMe	H	**13l**	3,5-diMe	H	CN	CN
			13m	3,5-diMe	H	CN	CO_2Et

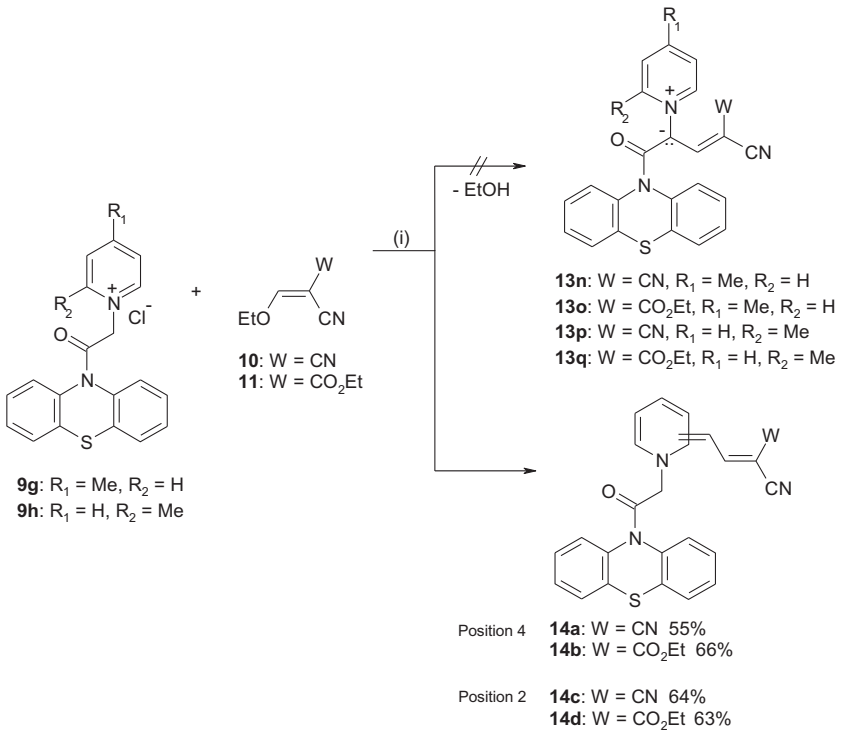
In the pyridinylidene series of products **14** (structures given in Scheme 4 and Table 2), the *p*-substituted compounds showed significant biological potential with protein farnesyltransferase inhibition potencies in the submicromolar range (e.g. **14a**: $IC_{50} = 0.65 \pm 0.1$ μM, Table 4). As seen in the previous ylide series, the replacement of a cyano chelating unit with an ethyl ester, had no effect on biological potential (compound **14a** *vs* **14b**, Table 4). The *o*-substituted derivatives **14c** and **14d**, showed a slight reduction in inhibitory activity, compared to the *p*-substituted derivatives **14a** and **14b**. Finally, the replacement of the phenothiazine unit by a diethylamino group caused a significant decrease of the inhibitory potential (compound **14b** *vs* **17**, Table 4), highlighting the importance of the phenothiazine group in these series.

2.3. Molecular modelling

Farnesyltransferase structure was taken from the 1LD7 [19] entry of the RCSB Protein Data Bank [20]. 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 [21] with GOLD 5.1 [22]. Thirty solutions were generated and classed through an in-house scoring function based on GoldScore [22] and X-Score functions [23]. The consistency of the results was assessed by visually examining the conformation cluster.

Compound **14b** adopts a single conformation described in Fig. 2(a), where the cyano and the ethyl ester groups are in front of the zinc atom and the carbonyl moiety next to the nitrogen atom of the phenothiazine forms a hydrogen bond with Arg 202β. The dinitrile **14a** binds in a similar way to ester **14b** and adopts a single conformation, without forming hydrogen bonds (Fig. 2(b)).

The affinity of *o*-substituted compounds **14c** and **14d** is weaker than that of *p*-substituted compounds **14a** and **14b**. They display different conformations. However, one conformation of compound **14c** fits into the binding site with the cyano units in front of the zinc atom of the enzyme, while the phenothiazine ring establishes stacking interactions with Tyr361β. On other conformations, possible hydrogen bonding could further anchor the compound **14c** with the hydroxyl Tyr 166α at the level of the cyano group



Scheme 3. Products obtained in case of reaction between dipolarophiles and 2- and 4-picoline salts **9g** and **9h**. Conditions: (i) DBU (1 equiv), DMF, 70 °C, 24 h.

(Fig. 2(d)). Compound **14d** displays a similar behaviour, with the cyano group in interaction with the zinc atom and stacking interactions between the phenothiazine group and Tyr361β (Fig. 2(c)).

3. Conclusions

The biological evaluation of the effect of synthesized products on human farnesyltransferase, allowed us to discover that new ylides with a phenothiazine scaffold possess protein farnesyltransferase inhibition potencies in the low micromolar range.

The nature of the substituent on the pyridine unit proved to be important in determining inhibitory activity and replacement of the cyanoacrylonitrile chelating function by a cyanoethylacrylate group generally conserved biological activity.

Unexpected products were isolated from 2- and 4-picolinium salts. Compounds **14a** and **14b** were the most promising inhibitors of human farnesyltransferase in the present study, with IC_{50} values in the submicromolar range. The obtained results indicate that phenothiazine derivatives deserve further investigation as new cytotoxic agents of improved characteristics.

4. Experimental section

4.1. Materials and methods-general remarks

Starting materials are commercially available and were used without further purification. Melting points were measured on a Buchi 510 apparatus and are raw values. NMR spectra were acquired at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR on a Bruker DRX 400 spectrometer or on a Varian 400 MHz Premium Shielded® spectrometer with tetramethylsilane as internal standard, at room temperature. Chemical shifts (δ) are expressed in ppm relative to TMS as internal standard. Thin layer chromatography (TLC) was realized on Macherey Nagel silica gel plates with fluorescent indicator and was visualized under a UV-lamp at 254 nm and 366 nm. Column chromatographies were performed using a Combi*Flash* Rf Companion (Teledyne-Isco System) and Redi*Sep* packed columns. IR spectra were recorded on a Bruker Tensor 27. Elemental analysis (C, H, N, S) of new compounds was determined on a Thermo Electron apparatus by 'Pôle Chimie Moléculaire', Faculté de Sciences Mirande, Université de Bourgogne, Dijon, France.

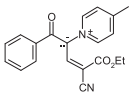
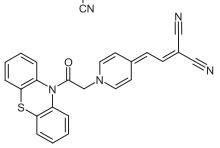
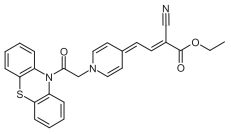
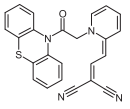
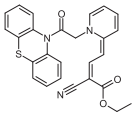
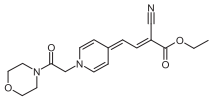
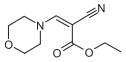
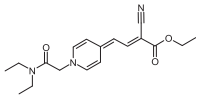
4.1.1. General procedure for the synthesis of pyridinium salts (**9a**–**l**)

The pyridine derivative (2–5 equiv) was added to a solution of halogenated compound (1 equiv) in ethyl acetate. The resulting mixture was stirred at reflux for 24 h. After cooling the reaction media to room temperature, the obtained precipitate was filtered, washed with ethyl acetate or acetone and recrystallized from ethanol to give the corresponding pure salt.

*4.1.1.1. 1-[2-Oxo-2-(10H-phenothiazin-10-yl)ethyl]-4-dimethylaminopyridinium chloride (**9a**).* White solid, 93% yield; mp 228–230 °C. IR ν_{max}/cm^{-1}: 1692, 1651, 1578, 1456, 1216, 1177, 775. ^{1}H NMR (DMSO-d_6/D_2O 1:1, 400 MHz) δ ppm: 3.20 (s, 6H, N(CH_3)$_2$), 5.55 (br s, 2H, COCH_2N), 7.07 (d, J = 7.8 Hz, 2H, Py*H*), 7.32–7.94 (m, 8H, Ar*H*), 8.30 (d, J = 7.8 Hz, 2H, Py*H*). ^{13}C NMR (DMSO-d_6/D_2O 1:1, 100 MHz) δ 40.0 ($2CH_3$), 57.7 (CH_2), 106.7 (2CH), 126.7–138.0 (8CH + 4C), 143.4 (2CH), 155.9 (C), 165.4 (C). Anal. calcd for $C_{21}H_{20}ClN_3OS$: C, 63.39; H, 5.07; N, 10.56; S, 8.06. Found: C, 63.04; H, 4.97; N, 10.11%.

*4.1.1.2. 1-[2-Oxo-2-(10H-2-chlorophenothiazin-10-yl)ethyl]-4-dimethylaminopyridinium chloride (**9b**).* White solid, 92% yield; mp 225 °C. IR ν_{max}/cm^{-1}: 1681, 1648, 1572, 1459, 1210, 1175, 775. ^{1}H NMR (DMSO-d_6/D_2O 1:1, 400 MHz) δ ppm: 3.21 (s, 6H, N(CH_3)$_2$), 5.74 (br s, 2H, COCH_2N), 7.10 (d, J = 7.4 Hz, 2H, Py*H*), 7.36–7.55 (m, 4H, Ar*H*), 7.66 (d, J = 8.0 Hz, 2H, Ar*H*), 7.97 (br. s, 1H, Ar*H*), 8.39 (d, J = 7.4 Hz, 2H, Py*H*). ^{13}C NMR (DMSO-d_6/D_2O 1:1, 100 MHz) δ 39.4 ($2CH_3$), 57.6 (CH_2), 106.9 (2CH), 126.0–138.0 (8CH + 4C), 143.4 (2CH), 155.9 (C),

Table 2
Study of the generalization of the synthesis of pyridin-(1*H*)-ylidene derivatives starting from 2- and 4-picolinium salts.

Entry	Salt no	Starting salt		Product no	Structure final product
		Y	R		
1	**9i**	C_6H_5	4-Me	**15**	
2	**9g**	10*H*-phenothiazin-10-yl	4-Me	**14a**	
3				**14b**	
4	**9h**		2-Me	**14c**	
5				**14d**	
6	**9j**	Morpholin-4-yl	4-Me	**16**	
				19	
7	**9k**	NEt_2	4-Me	**17**	
8	**9l**	9*H*-carbazol-9-yl	4-Me	**18**	

165.4 (C). Anal. calcd for $C_{21}H_{19}Cl_2N_3OS$: C, 58.34; H, 4.43; N, 9.72; S, 7.42. Found: C, 58.74; H, 4.57; N, 9.91%.

*4.1.1.3. 1-[2-Oxo-2-(10H-phenothiazin-10-yl)ethyl]pyridinium chloride (**9c**).* White solid, 90% yield; mp 252–253 °C. IR ν_{max}/cm^{-1}: 1628, 1479, 1460, 1372, 1259, 1177, 760, 570. ^1H NMR (DMSO-d_6/D_2O 1:1, 400 MHz) δ ppm: 5.35 (br s, 1H, COCH_2N), 6.11 (br s, 1H, COCH_2N), 7.26–7.48 (m, 4H, Ar*H*), 7.50–7.84 (m, 4H, Ar*H*), 8.04 (t, $J = 7.0$ Hz, 2H, Py*H*), 8.54 (t, $J = 7.8$ Hz, 1H, Py*H*), 8.77 (d, $J = 6.0$ Hz, 2H, Py*H*). ^{13}C NMR (DMSO-d_6/D_2O 1:1, 100 MHz) δ 62.0 (CH_2), 124.0–139.0 (10CH + 4C), 146.3 (2CH), 146.9 (CH), 164.5 (C). Anal. calcd for $C_{19}H_{15}ClN_2OS$: C, 64.31; H, 4.26; N, 7.89; S, 9.04. Found: C, 64.38; H, 4.18; N, 7.90%.

Table 3
Inhibitory activities of ylides **13a**–**m** on human FTase.

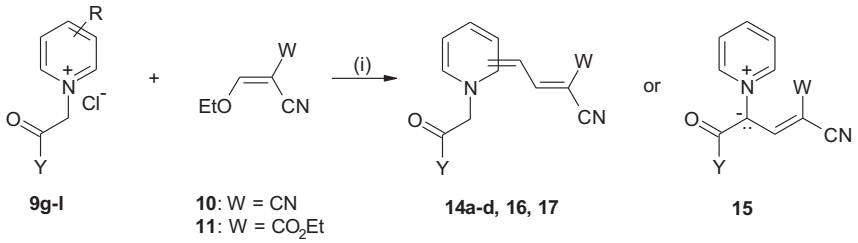
Compound no.	IC_{50} (μM ± SD)[a]
13a	4.67 ± 0.36
13b	5.33 ± 0.55
13c	86.49 ± 16.96
13d	3.18 ± 0.23
13e	4.92 ± 0.45
13f	13.10 ± 1.02
13g	14.98 ± 2.31
13h	10.98 ± 1.65
13i	7.01 ± 0.93
13j	10.29 ± 1.48
13k	13.74 ± 0.99
13l	6.36 ± 0.74
13m	7.27 ± 0.73

[a] Values represent mean of two experiments.

Table 4
Inhibitory activities of compounds **14a**–**d** and **17** on human FTase.

Compound no.	IC_{50} (μM ± SD)[a]
14a	0.65 ± 0.10
14b	0.63 ± 0.06
14c	1.20 ± 0.29
14d	1.07 ± 0.17
15	n.t.[b]
16	n.t.
17	43.09 ± 9.81

[a] Values represent mean of two experiments.
[b] not tested.



Scheme 4. Study of the generalization of the synthesis of new unexpected compounds. Conditions: (i) DBU (1 equiv), DMF, 70 °C, 24 h.

*4.1.1.4. 1-[2-Oxo-2-(10H-phenothiazin-10-yl)ethyl]-3-methylpyridinium chloride (**9d**).* White solid, 82% yield; mp 160–163 °C. IR ν_{max}/cm^{-1}: 1677, 1461, 1372, 1303, 1262, 1166, 780, 748, 574. ^1H NMR (DMSO-d_6/D_2O 1:1, 400 MHz) δ ppm: 2.55 (s, 3H, CH_3), 5.53 (br s, 1H, $COCH_2N$), 6.54 (br s, 1H, $COCH_2N$), 7.38–7.59 (m, 5H, Ar*H*), 7.64–7.80 (m, 3H, Ar*H*), 8.17 (dd, J = 8.0, 6.0 Hz, 1H, pyridine Ar*H*), 8.58 (d, J = 8.0 Hz, 1H, pyridine Ar*H*), 9.04 (d, J = 6.0 Hz, 1H, Py*H*), 9.12 (s, 1H, Py*H*). ^{13}C NMR (DMSO-d_6/D_2O 1:1, 100 MHz) δ 18.2 (CH_3), 62.0 (CH_2), 124.8–138.0 (8CH + 5C), 138.4 (CH), 144.4 (CH), 146.3 (CH), 147.2 (CH), 164.7 (C). Anal. calcd for $C_{20}H_{17}ClN_2OS$: C, 65.12; H, 4.65; N, 7.59; S, 8.69. Found: C, 64.90; H, 5.06; N, 8.26%.

*4.1.1.5. 1-[2-Oxo-2-(10H-phenothiazin-10-yl)ethyl]-4-methoxypyridinium chloride (**9e**).* White solid, 85% yield; mp 192–194 °C. IR ν_{max}/cm^{-1}: 1677, 1640, 1520, 1480, 1300, 1261, 1198, 999, 748, 536. ^1H NMR (DMSO-d_6/D_2O 1:1, 400 MHz) δ ppm: 4.02 (s, 3H, OCH_3), 5.14 (br s, 1H, $COCH_2N$), 5.93 (br s, 1H, $COCH_2N$), 7.27–7.80 (m, 10H, 8H phenoth. + 2H pyridine Ar*H*), 8.54 (d, J = 7.4 Hz, 2H, Py*H*). ^{13}C NMR (DMSO-d_6/D_2O 1:1, 100 MHz) δ 58.4 (CH_3), 60.1 (CH_2), 113.2 (2CH), 124.0–139.0 (8CH + 4C), 147.6 (2CH), 165.0 (C), 171.7 (C). Anal. calcd for $C_{20}H_{17}ClN_2O_2S$: C, 62.41; H, 4.45; N, 7.28; S, 8.33. Found: C, 62.48; H, 5.15; N, 6.98%.

*4.1.1.6. 1-[2-Oxo-2-(10H-phenothiazin-10-yl)ethyl]-3,5-dimethylpyridinium chloride (**9f**).* White solid, 88% yield; mp 262–264 °C. IR ν_{max}/cm^{-1}: 1682, 1462, 1367, 1310, 1263, 1182, 755, 599. ^1H NMR (DMSO-d_6/D_2O 1:1, 400 MHz) δ ppm: 2.37 (s, 6H, $N(CH_3)_2$), 5.25 (br s, 1H, $COCH_2N$), 5.97 (br s, 1H, $COCH_2N$), 7.27–7.50 (m, 4H, Ar*H*), 7.53–7.83 (m, 4H, Ar*H*), 8.20 (s, 1H, Py*H*), 8.43 (s, 2H, Py*H*). ^{13}C NMR (DMSO-d_6/D_2O 1:1, 100 MHz) δ 18.6 ($2CH_3$), 62.4 (CH_2), 123.0–131.0 (10CH + 2C), 139.2 (2C), 143.6 (2C), 148.5 (CH), 165.3 (C). Anal. calcd for $C_{21}H_{19}ClN_2OS$: C, 65.87; H, 5.00; N, 7.32; S, 8.37. Found: C, 65.86; H, 4.91; N, 7.76%.

*4.1.1.7. 1-[2-Oxo-2-(10H-phenothiazin-10-yl)ethyl]-4-methylpyridinium chloride (**9g**).* White solid, 87% yield; mp 265–266 °C. IR ν_{max}/cm^{-1}: 1677, 1635, 1462, 1374, 1261, 1132, 975, 785, 508.

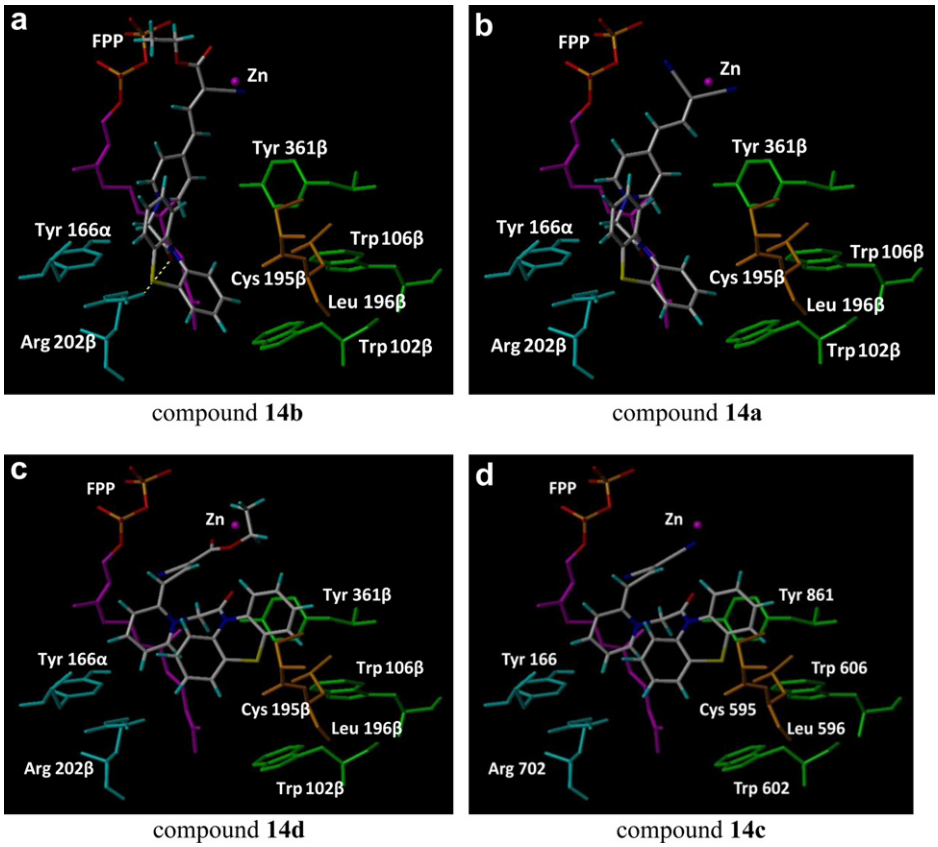


Fig. 2. Docking of compounds **14a**–**d** in the active site of FTase: (a) compound **14b**, (b) compound **14a**, (c) compound **14d**, (d) compound **14c**.

^{1}H NMR (DMSO-d_6/D_2O 1:1, 400 MHz) δ ppm: 2.56 (s, 3H, CH_3), 5.31 (br s, 1H, COCH_2N), 6.11 (br s, 1H, COCH_2N), 7.27–7.64 (m, 8H, Ar*H*), 7.88 (d, J = 6.8 Hz, 2H, Py*H*), 8.66 (d, J = 6.8 Hz, 2H, Py*H*). ^{13}C NMR (DMSO-d_6/D_2O 1:1, 100 MHz) δ 22.0 (CH_3), 61.2 (CH_2), 124.0–140.0 (8CH + 4C phenoth. + 2CH pyridine), 145.4 (2CH pyridine), 160.9 (C), 164.7 (C). Anal. calcd for $C_{20}H_{17}ClN_2OS$: C, 65.12; H, 4.65; N, 7.59; S, 8.69. Found: C, 64.90; H, 5.06; N, 8.26%.

*4.1.1.8. 1-[2-Oxo-2-(10H-phenothiazin-10-yl)ethyl]-2-methylpyridinium chloride (**9h**).* White solid, 81% yield; mp 256–258 °C. IR ν_{max}/cm^{-1}: 2918, 1675, 1629, 1462, 1346, 1263, 1181, 1033, 759, 547. ^{1}H NMR (DMSO-d_6/D_2O 1:1, 400 MHz) δ ppm: 2.55 (s, 3H, CH_3), 5.31 (br s, 1H, COCH_2N), 6.05 (br s, 1H, COCH_2N), 7.25–7.75 (m, 8H, Ar*H*), 7.89–7.97 (m, 2H, Py*H*), 8.44 (dt, J = 7.8, 1.1 Hz, 1H, Py*H*), 8.82 (d, J = 6.0 Hz, 1H, Py*H*). ^{13}C NMR (DMSO-d_6/D_2O 1:1, 100 MHz) δ 19.7 (CH_3), 59.5 (CH_2), 124.0–140.0 (10CH + 4C), 147.1 (CH), 146.9 (CH), 156.2 (C), 164.0 (C). Anal. calcd for $C_{20}H_{17}ClN_2OS$: C, 65.12; H, 4.65; N, 7.59; S, 8.69. Found: C, 64.92; H, 4.98; N, 7.96%.

*4.1.1.9. 1-[2-Oxo-2(phenyl)ethyl]-4-methylpyridinium chloride (**9i**).* White solid with the same physico-chemical properties as described in the literature [24]; 85% yield.

*4.1.1.10. 1-(2-Morpholin-4-yl-2-oxoethyl)-4-methylpyridinium chloride (**9j**).* Off-white solid; 48% yield. ^{1}H NMR (D_2O, 400 MHz) δ ppm: 2.56 (s, 3H, CH_3), 3.31 (t, J = 4.6 Hz, 2H, NCH_2CH$_2$O), 3.46 (t, J = 4.7 Hz, 2H, NCH$_2$CH_2O), 3.66 (t, J = 4.7 Hz, 2H, NCH$_2$CH_2O), 3.72 (sym m, J = 4.6 Hz, 2H, NCH_2CH$_2$O), 5.56 (s, 2H, COCH_2N), 7.80 (d, J = 6.9 Hz, 2H, Ar*H*), 8.40 (d, J = 6.9 Hz, 2H, Ar*H*). ^{13}C NMR (D_2O, 100 MHz) δ 21.5 (CH_3), 44.9 (CH_2), 59.5 (CH_2), 63.5 (CH_2), 65.9 (CH_2), 66.1 (CH_2), 128.3 (2CH), 144.6 (2CH), 161.5 (C), 164.7 (C). Anal. calcd for $C_{12}H_{17}ClN_2O_2$: C, 56.14; H, 6.67; N, 10.91. Found: C, 56.37; H, 6.46; N, 11.07%.

*4.1.1.11. 1-[2-(Diethylamino)-2-oxoethyl]-4-methylpyridinium chloride (**9k**).* Very hygroscopic white solid; 95% yield. IR ν_{max}/cm^{-1}: 3379 (hydrated form), 1641, 1521, 1472, 1433, 1385, 1308, 1273, 1203, 1147, 951. ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.06 (t, J = 7.1 Hz, 3H, NCH$_2$CH_3), 1.25 (t, J = 7.1 Hz, 3H, NCH$_2$CH_3), 2.64 (s, 3H, CH_3), 3.33 (q, J = 7.1 Hz, 2H, NCH_2CH$_3$), 3.39 (q, J = 7.1 Hz, 2H, NCH_2CH$_3$), 5.77 (s, 2H, COCH_2N), 8.01 (d, J = 6.6 Hz, 2H, Ar*H*), 8.86 (d, J = 6.6 Hz, 2H, Ar*H*). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 12.7 (CH_3), 13.6 (CH_3), 21.4 (CH_3), 40.1 (CH_2), 40.7 (CH_2), 60.0 (CH_2), 127.6 (2CH), 145.3 (2CH), 159.4 (C), 163.6 (C). Anal. calcd for $C_{12}H_{19}ClN_2O$ $5H_2O$: C, 43.31; H, 8.78; N, 8.42. Found: C, 44.04; H, 9.16; N, 8.87%.

*4.1.1.12. 1-[2-Oxo-2-(9H-carbazol-9-yl)ethyl]-4-methylpyridinium chloride (**9l**).* White solid, 78% yield; mp 294–296 °C ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.72 (s, 3H, ArCH_3), 6.67 (s, 2H, COCH_2N), 7.53 (t, J = 7.6 Hz, 2H, Ar*H*), 7.61 (t, J = 7.6 Hz, 2H, Ar*H*), 8.15 (d, J = 6.4 Hz, 2H, Py*H*), 8.26–8.31 (m, 4H, Ar*H*), 9.02 (d, J = 6.4 Hz, 2H, Py*H*). ^{13}C NMR (DMSO-d_6/D_2O 1:1, 100 MHz) δ 21.8 (CH_3), 64.3 (CH_2), 116.5 (2CH), 120.6 (2CH), 124.7 (2CH), 126.1 (2C), 127.9 (2CH), 128.2 (2CH), 137.5 (2C), 145.5 (2CH), 160.5 (C), 166.5 (C).

*4.1.2. General procedure for the preparation of ylides (**13a**–**m**)*

DBU (1 equiv) was added to a solution of pyridinium salt (1 equiv) in a minimum volume of solvent (DMF or acetonitrile). The reaction media became yellow. This corresponded to the *in situ* formation of the ylure. The trisubstituted alkene (2-cyano-3-ethoxyacrylonitrile (**10**), ethyl 2-cyano-3-ethoxyacrylate (**11**) or (3*E*)-4-ethoxy-1,1,1-trifluorobut-3-en-2-one (**12**)) (2 equiv) was then added. The reaction mixture became red-brown and was heated at 70 °C for 24 h. After cooling to room temperature, cold distilled water was added to the reaction media. The obtained precipitate was filtered, washed with cold distilled water and recrystallized from absolute ethanol. Some ylures necessitated supplementary chromatographic purification using ethanol as eluent.

*4.1.2.1. 3-(2-Cyanoacrylonitrile)-10H-phenothiazin-10-yl-carbonyl-4-dimethylaminopyridinium methylide (**13a**).* Yellow solid; 51% yield; mp 281–283 °C. IR ν_{max}/cm^{-1}: 2189, 2168, 1634,1567, 1540, 1279, 1259, 1169, 751, 595. ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.19 (s, 6H, N(CH_3)$_2$), 6.71 (s, 1H, C*H*═), 6.86 (d, J = 7.2 Hz, 2H, Py*H*), 7.21 (t, J = 7.6 Hz, 2H, Ar*H*), 7.34 (t, J = 7.6 Hz, 2H, Ar*H*), 7.43 (d, J = 7.6 Hz, 2H, Ar*H*), 7.80 (d, J = 7.6 Hz, 2H, Ar*H*), 8.00 (d, J = 7.2 Hz, 2H, Py*H*). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 39.7 (2CH$_3$), 40.1 (C), 106.9 (2CH), 112.5 (C), 118.0 (C), 121.2 (C), 124.9 (2CH), 126.0 (2CH), 127.6 (2CH), 127.7 (2CH), 130.0 (2C) 140.6 (2C), 143.3 (CH), 146.0 (2CH), 155.8 (C), 163.6 (C). Anal. calcd for $C_{25}H_{19}N_5OS$: C, 68.56; H, 4.34; N, 15.99. Found: C, 68.58; H, 4.26; N, 15.29%.

*4.1.2.2. 3-(2-Cyanoethylacrylate)-10H-phenothiazin-10-yl-carbonyl-4-dimethylaminopyridinium methylide (**13b**).* Yellow-greenish solid; 57% yield; mp 287–289 °C. IR ν_{max}/cm^{-1}: 3539, 3456, 2193, 1645, 1618, 1541, 1197, 1097, 757. ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.10 (t, J = 7.2 Hz, 3H, $CO_2CH_2CH_3$), 3.19 (s, 6H, N(CH_3)$_2$), 3.91 (q, J = 7.2 Hz, 2H, $CO_2CH_2CH_3$), 6.85 (d, J = 7.2 Hz, 2H, Py*H*), 7.16 (t, J = 7.6 Hz, 2H, Ar*H*), 7.28 (t, J = 7.6 Hz, 2H, Ar*H*), 7.38 (d, J = 7.6 Hz, 2H, Ar*H*), 7.61 (s, 1H, C*H*═), 7.75 (d, J = 7.6 Hz, 2H, Ar*H*), 7.98 (d, J = 7.2 Hz, 2H, Py*H*). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 14.6 (CH_3), 39.7 (2CH$_3$), 58.6 (CH_2), 65.4 (C), 106.8 (2CH), 110.9 (C), 119.4 (C), 124.1 (2CH), 125.5 (2CH), 127.5 (4CH), 129.4 (2C), 141.2 (2C), 142.5 (CH), 146.2 (2CH), 155.7 (C), 165.0 (C), 167.0 (C). Anal. calcd for $C_{27}H_{24}N_4O_3S$: C, 66.86; H, 4.95; N, 11.55. Found: C, 66.74; H, 5.15; N, 11.41%.

*4.1.2.3. 3-(Prop-2-enoic acid)-10H-phenothiazin-10-yl-carbonyl-4-dimethylaminopyridinium methylide (**13c**).* Yellow solid; 57% yield; mp 207–210 °C. IR ν_{max}/cm^{-1}: 1630, 1564, 1516, 1440, 1182, 1150, 754, 714. ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.19 (s, 6H, N(CH_3)$_2$), 4.55 (d, J = 12.8 Hz, 1H, C*H*═), 6.84 (d, J = 7.2 Hz, 2H, pyridine Ar*H*), 7.16 (t, J = 7.6 Hz, 2H, phenoth. Ar*H*), 7.29 (t, J = 7.6 Hz, 2H, phenoth. Ar*H*), 7.37 (d, J = 7.6 Hz, 2H, phenoth.), 7.72–7.78 (m, 3H, 2H phenoth. Ar*H* + C*H*═), 7.93 (d, J = 7.2 Hz, 2H, pyridine Ar*H*). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 39.7 (2CH$_3$), 85.8 (CH), 107.3 (2CH), 115.5 (C), 124.5 (2CH), 126.0 (2CH), 127.5 (4CH), 129.7 (2C), 140.5 (2C), 140.7 (CH), 144.6 (2CH), 155.3 (C), 164.0 (C), 168.9 (C). Anal. calcd for $C_{24}H_{21}N_3O_3S$: C, 66.58; H, 4.85; N, 9.71; S, 7.39. Found: C, 66.31; H, 4.86; N, 9.61%.

*4.1.2.4. 3-(2-Cyanoacrylonitrile)-10H-(2-chlorophenothiazin)-10-yl-carbonyl-4-dimethylaminopyridinium methylide (**13d**).* Yellow solid; 41% yield; mp 271–273 °C. IR ν_{max}/cm^{-1}: 2193, 2173, 1630, 1537, 1278, 1171, 743, 599. ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 3.22 (s, 6H, N(CH_3)$_2$), 6.63 (d, J = 7.6 Hz, 2H, Py*H*), 7.02 (s, 1H, C*H*═), 7.10–7.17 (m, 2H, Ar*H*), 7.21 (d, J = 7.6 Hz, 1H, Ar*H*), 7.25–7.30 (m, 2H, Ar*H*), 7.49 (d, J = 7.6 Hz, 1H, Ar*H*), 7.64 (d, J = 7.6 Hz, 2H, Py*H*), 7.88 (s, 1H, Ar*H*). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 40.3 (2CH$_3$), 43.3 (C), 107.0 (2CH), 111.6 (C), 118.3 (C), 120.6 (C), 124.4 (CH), 123.7 (CH), 125.8 (CH), 126.3 (CH), 127.8 (CH), 128.0 (CH), 128.2 (CH), 129.1 (C), 130.6 (C), 133.1 (C), 140.9 (C), 141.7 (C), 145.6 (CH), 146.3 (2CH), 156.1 (C), 164.3 (C). Anal. calcd for $C_{25}H_{18}ClN_5OS$: C, 63.56; H, 3.81; N, 14.83; S, 6.78. Found: C, 63.18; H, 4.16; N, 15.02%.

*4.1.2.5. 3-(2-Cyanoethylacrylate)-10H-(2-chlorophenothiazin)-10-yl-carbonyl-4-dimethylaminopyridinium methylide (**13e**).* Yellow solid; 38% yield; mp 250–253 °C. IR ν_{max}/cm^{-1}: 2178, 1644, 1630, 1557, 1365, 1191, 1098, 746. ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.21 (t,

J = 7.2 Hz, 3H, $CO_2CH_2CH_3$), 3.21 (s, 6H, $N(CH_3)_2$), 4.08 (q, J = 7.2 Hz, 2H, $CO_2CH_2CH_3$), 6.66 (d, J = 7.2 Hz, 2H, Py*H*) 7.07–7.13 (m, 2H, Ar*H*), 7.21 (d, J = 8.0 Hz, 2H, Ar*H*), 7.31 (d, J = 7.2 Hz, 1H, Ar*H*), 7.50 (d, J = 8.0 Hz, 1H, Ar*H*), 7.71 (d, J = 7.2 Hz, 2H, Py*H*), 7.80 (s, 1H, C*H*═), 7.87 (s, 1H, Ar*H*). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.6 (CH_3), 40.2 ($2CH_3$), 59.8 (CH_2), 68.1 (C), 106.9 (2CH), 110.3 (C), 120.2 (C), 124.0 (CH), 124.3 (CH), 125.4 (CH), 125.8 (CH), 127.6 (CH), 127.9 (CH), 128.1 (CH), 129.0 (C), 130.3 (C), 133.0 (C), 141.6 (C), 142.3 (C), 144.2 (CH), 146.5 (2CH), 156.2 (C), 165.7 (C), 167.1 (C). Anal. calcd for $C_{27}H_{23}ClN_4O_3S$: C, 62.41; H, 4.43; N, 10.78; S, 6.16. Found: C, 62.08; H, 4.26; N, 10.39%.

*4.1.2.6. 3-(2-Cyanoacrylonitrile)-10H-phenothiazin-10-yl-carbonyl-pyridinium methylide (**13f**).* Orange solid; 36% yield; mp 292–294 °C. IR ν_{max}/cm^{-1}: 2193, 2172, 1631, 1542, 1458, 1358, 1284, 1256, 1174, 746, 589. ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 6.84 (s, 1H, C*H*═), 7.22 (t, J = 7.6 Hz, 2H, Ar*H*), 7.35 (t, J = 7.6 Hz, 2H, Ar*H*), 7.40 (d, J = 7.6 Hz, 2H, Ar*H*), 7.86 (d, J = 8.0 Hz, 2H, Ar*H*), 8.01 (t, J = 6.8 Hz, 2H, Py*H*), 8.50 (t, J = 7.6 Hz, 1H, Py*H*), 8.93 (d, J = 6.0 Hz, 2H, Py*H*). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 40.2 (C), 114.0 (C), 116.9 (C) 120.4 (C), 125.1 (2CH), 126.3 (2CH), 127.5 (2CH), 127.7 (2CH), 127.8 (2CH), 130.3 (2C), 140.0 (2C), 142.5 (CH), 145.6 (CH), 149.1 (2CH), 162.6 (C). Anal. calcd for $C_{23}H_{14}N_4OS$: C, 69.96; H, 3.54; N, 14.19. Found: C, 69.58; H, 3.97; N, 13.90%.

*4.1.2.7. 3-(2-Cyanoethylacrylate)-10H-phenothiazin-10-yl-carbonyl-pyridinium methylide (**13g**).* Orange solid; 42% yield; mp 259–261 °C. IR ν_{max}/cm^{-1}: 2181, 1644, 1615, 1560, 1334, 1232, 1195, 1098, 754, 596. ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.23 (t, J = 7.0 Hz, 3H, $CO_2CH_2CH_3$), 4.11 (q, J = 7.0 Hz, 2H, $CO_2CH_2CH_3$), 7.12 (t, J = 7.6 Hz, 2H, Py*H*) 7.20–7.35 (m, 4H, Ar*H*), 7.70–7.80 (m, 4H, Ar*H*), 7.92 (s, 1H, C*H*═), 8.20 (t, J = 7.6 Hz, 1H, Py*H*), 8.32 (d, J = 6.0 Hz, 2H, Py*H*). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.6 (CH_3), 60.0 (CH_2), 68.1 (C), 112.3 (C), 119.4 (C), 124.8 (2CH), 126.0 (2CH), 126.9 (2CH), 127.5 (2CH), 127.8 (2CH), 131.3 (2C), 140.7 (2C), 142.7 (CH), 143.5 (CH), 148.5 (2CH), 164.2 (C), 166.9 (C). Anal. calcd for $C_{25}H_{19}N_3O_3S$: C, 67.94; H, 4.30; N, 9.51; S, 7.24. Found: C, 67.6; H, 4.54; N, 9.48%.

*4.1.2.8. 3-(2-Cyanoacrylonitrile)-10H-phenothiazin-10-yl-carbonyl-3-methylpyridinium methylide (**13h**).* Orange solid; 49% yield; mp 170–174 °C. IR ν_{max}/cm^{-1}: 2193, 2171, 1632, 1537, 1455, 1353, 1280, 1250, 1170, 754, 589. ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.39 (s, 3H, CH_3), 6.92 (s, 1H, C*H*═), 7.20 (dt, J = 7.6, 0.8 Hz, 2H, phenoth. Ar*H*), 7.31–7.38 (m, 4H, phenoth. Ar*H*), 7.78–7.87 (m, 3H, 1H pyridine and 2H phenoth. Ar*H*), 8.27 (d, J = 8.0 Hz, 1H, H-4 pyridine Ar*H*), 8.69 (d, J = 6.0 Hz, 1H, H-6 pyridine Ar*H*), 8.75 (s, 1H, H-2 pyridine Ar*H*). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 17.6 (CH_3), 40.5 (C), 114.2 (C), 117.0 (C), 120.5 (C), 125.1 (2CH), 126.2 (2CH), 126.7 (CH), 127.6 (2CH), 127.8 (2CH), 130.4 (2C), 138.0 (C), 139.8 (2C), 142.4 (CH), 145.6 (CH), 146.2 (CH), 147.9 (CH), 162.5 (C). Anal. calcd for $C_{24}H_{16}N_4OS$: C, 70.50; H, 3.91; N, 13.70; S, 7.83. Found: C, 70.40; H, 4.39; N, 13.28%.

*4.1.2.9. 3-(2-Cyanoethylacrylate)-10H-phenothiazin-10-yl-carbonyl-3-methylpyridinium methylide (**13i**).* Red solid; 43% yield; mp 220–224 °C. IR ν_{max}/cm^{-1}: 2181, 1658, 1635, 1527, 1475, 1231, 1196, 764, 597. ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.23 (t, J = 7.2 Hz, 3H, $CO_2CH_2CH_3$), 2.46 (s, 3H, ArCH_3), 4.09 (q, J = 7.2 Hz, 2H, $CO_2CH_2CH_3$), 7.11 (t, J = 7.6 Hz, 2H, phenoth. Ar*H*), 7.20–7.30 (m, 4H, phenoth. Ar*H*), 7.59 (t, J = 6.8 Hz, 1H, pyridine Ar*H*), 7.78 (d, J = 7.6 Hz, 2H, phenoth. Ar*H*), 7.94 (d, J = 6.8 Hz, 1H, pyridine Ar*H*), 8.00 (s, 1H, C*H*═), 8.07 (s, 1H, pyridine Ar*H*), 8.12 (d, J = 6.8 Hz, 1H, pyridine Ar*H*). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.6 (CH_3), 18.7 (CH_3), 60.0 (CH_2), 68.2 (C), 112.3 (C), 119.4 (C), 124.8 (2CH), 125.9 (2CH), 126.2 (CH), 127.5 (2CH), 127.7 (2CH), 131.2 (2C), 138.1 (C), 140.7 (2C), 142.7 (CH), 144.0 (CH), 145.6 (CH), 147.9 (CH), 164.2 (C), 167.0 (C). Anal. calcd for $C_{26}H_{21}N_3O_3S$: C, 68.49; H, 4.60; N, 9.21; S, 7.02. Found: C, 68.55; H, 4.82; N, 9.20%.

*4.1.2.10. 3-(2-Cyanoacrylonitrile)-10H-phenothiazin-10-yl-carbonyl-4-methoxypyridinium methylide (**13j**).* Yellow solid; 50% yield; mp 242–245 °C. IR ν_{max}/cm^{-1}: 2181, 2170, 1625, 1511, 1238, 1167, 1124, 938, 746, 595. ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 4.06 (s, 3H, OCH_3), 7.07 (d, J = 7.2 Hz, 2H, pyridine Ar*H*), 7.08 (s, 1H, CH═), 7.15 (t, J = 7.6 Hz, 2H, phenoth. Ar*H*), 7.27–7.32 (m, 4H, phenoth. Ar*H*), 7.71 (d, J = 7.6 Hz, 2H, phenoth. Ar*H*), 8.03 (d, J = 7.2 Hz, 2H, pyridine Ar*H*). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 43.0 (C), 57.7 (CH_3), 112.4 (2CH), 118.0 (2C), 120.2 (C), 125.0 (2CH), 126.3 (2CH), 127.6 (2CH), 127.9 (2CH), 131.4 (2C), 140.4 (2C), 144.7 (CH), 149.9 (2CH), 163.5 (C), 170.7 (C). Anal. calcd for $C_{24}H_{16}N_4O_2S$: C, 67.84; H, 3.76; N, 13.19; S, 7.53; Found: C, 67.41; H, 3.66; N, 12.90%.

*4.1.2.11. 3-(2-Cyanoethylacrylate)-10H-phenothiazin-10-yl-carbonyl-4-methoxypyridinium methylide (**13k**).* Yellow solid; 41% yield; mp 211–213 °C. IR ν_{max}/cm^{-1}: 2181, 1674, 1624, 1512, 1455, 1312, 1185, 1103, 748, 593. ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.22 (t, J = 7.2 Hz, 3H, $CO_2CH_2CH_3$), 4.02 (s, 3H, OCH_3), 4.09 (q, J = 7.2 Hz, 2H, $CO_2CH_2CH_3$), 7.05–7.19 (m, 4H, 2H pyridine and 2H phenoth. Ar*H*), 7.24 (t, J = 7.6 Hz, 2H, phenoth. Ar*H*), 7.30 (d, J = 7.6 Hz, 2H, phenoth. Ar*H*), 7.71 (d, J = 7.6 Hz, 2H, phenoth. Ar*H*), 7.82 (s, 1H, C*H*═), 8.07 (d, J = 6.8 Hz, 2H, pyridine Ar*H*). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.6 (CH_3), 57.5 (CH_2), 59.8 (CH_3), 67.4 (C), 111.0 (C), 112.3 (2CH), 120.0 (C), 124.7 (2CH), 125.8 (2CH), 127.4 (2CH), 127.7 (2CH), 131.2 (2C), 140.9 (2C), 143.1 (CH), 149.9 (2CH), 164.7 (C), 167.0 (C), 170.4 (C). Anal. calcd for $C_{26}H_{21}N_3O_4S$: C, 66.16; H, 4.45; N, 8.90. Found: C, 66.41; H, 4.28; N, 8.91%.

*4.1.2.12. 3-(2-Cyanoacrylonitrile)-10H-phenothiazin-10-yl-carbonyl-3,5-dimethylpyridinium methylide (**13l**).* Yellow solid; 57% yield; mp 266–269 °C. IR ν_{max}/cm^{-1}: 2194, 2171, 1639, 1536, 1456, 1255, 1029, 759, 594. ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.30 (s, 6H, $2CH_3$), 7.13 (s, 1H, C*H*═), 7.20 (t, J = 7.6 Hz, 2H, phenoth. Ar*H*), 7.32–7.36 (m, 4H, phenoth. Ar*H*), 7.85 (d, J = 7.6 Hz, 2H, phenoth. Ar*H*), 8.06 (s, 1H, pyridine Ar*H*), 8.53 (s, 2H, pyridine Ar*H*). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 17.5 ($2CH_3$), 40.7 (C), 114.4 (C), 117.1 (C), 120.7 (C), 125.1 (2CH), 126.2 (2CH), 127.5 (2CH), 127.7 (2CH), 130.5 (2C), 137.1 (2C), 139.6 (2C), 142.2 (CH), 145.2 (2CH), 145.8 (CH), 162.4 (C). Anal. calcd for $C_{25}H_{18}N_4OS$: C, 71.00; H, 4.26; N, 13.25; S, 7.57. Found: C, 70.77; H, 4.31; N, 13.25%.

*4.1.2.13. 3-(2-Cyanoethylacrylate)-10H-phenothiazin-10-yl-carbonyl-3,5-dimethylpyridinium methylide (**13m**).* Orange solid; 61% yield; mp 245–246 °C. IR ν_{max}/cm^{-1}: 2190, 1672, 1628, 1522, 1457, 1188, 1021, 755, 598. ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.25 (t, J = 6.8 Hz, 3H, $CO_2CH_2CH_3$), 2.38 (s, 6H, $2CH_3$), 4.14 (q, J = 6.8 Hz, 2H, $CO_2CH_2CH_3$), 7.10 (t, J = 7.6 Hz, 2H, phenoth. Ar*H*), 7.21 (d, J = 7.6 Hz, 2H, phenoth. Ar*H*), 7.26 (t, J = 7.6 Hz, 2H, phenoth. Ar*H*), 7.69 (s, 1H, C*H*═), 7.81 (d, J = 7.6 Hz, 2H, phenoth. Ar*H*), 7.87 (s, 2H, pyridine Ar*H*), 8.09 (s, 1H, pyridine Ar*H*). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.6 (CH_3), 18.5 ($2CH_3$), 59.9 (CH_2), 68.0 (C), 112.3 (C), 119.4 (C), 124.9 (2CH), 125.8 (2CH), 127.4 (2CH), 127.5 (2CH), 131.0 (2C), 137.2 (2C), 140.5 (2C), 142.4 (CH), 144.7 (CH), 144.9 (2CH), 164.1 (C), 167.2 (C). Anal. calcd for $C_{27}H_{23}N_3O_3S$: C, 69.00; H, 4.89; N, 8.94. Found: C, 68.87; H, 4.64; N, 8.75%.

*4.1.3. General procedure for the synthesis of compounds **14a–d**, **15**, **16** and **17***

DBU (1 equiv) was added to a solution of cycloimmonium salt (1 equiv) in a minimum volume of DMF. The reaction media become yellow. This corresponded to the formation *in situ* of the

intermediate ylure. The trisubstituted alchene (2-cyano-3-ethoxyacrylonitrile **10** or ethyl 2-cyano-3-ethoxyacrylate **11**) (2 equiv) was then added. The reaction mixture become red and was heated at 70 °C for 24 h. A precipitate was formed during the reaction. After cooling to room temperature, the obtained precipitate was filtered, washed with absolute ethanol and recrystallized from absolute ethanol. Compound **16** necessitated a supplementary chromatographic purification using AcOEt/*n*-heptane 8/2 as eluent.

*4.1.3.1. {2-[1-[2-Oxo-2-(10H-phenothiazin-10-yl)ethyl]pyridin-4(1H)-ylidene]ethylidene}malononitrile (**14a**).* Orange red solid; 55% yield; mp 265–267 °C. IR ν_{max}/cm^{-1}: 2187, 1680, 1652, 1543, 1461, 1261, 1176, 754. ^1H NMR ($(CD_3)_2CO$, 400 MHz) δ ppm: 5.20 (br s, 2H, NCH_2CO), 5.61 (d, J = 14.0 Hz, 1H, CH=), 6.70 (d, J = 6.4 Hz, 1H, pyridine ArH), 7.25–7.32 (m, 3H, 2H phenoth. and 1H pyridine ArH), 7.35 (dt, J = 8.0, 1.2 Hz, 2H, phenoth. ArH), 7.50 (dd, J = 8.0, 1.2 Hz, 2H, phenoth. ArH), 7.57 (d, J = 7.9 Hz, 2H, phenoth. ArH), 7.59 (d, J = 14.0 Hz, 1H, CH=), 7.66 (d, J = 6.4 Hz, 2H, pyridine ArH). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 53.1 (C), 59.5 (CH_2), 102.2 (CH), 112.8 (CH), 117.4 (C), 117.7 (CH), 119.6 (C), 127.1 (2CH), 127.5 (2CH), 127.7 (2CH), 128.1 (2CH), 132.8 (2C), 137.6 (2C), 140.2 (CH), 141.2 (CH), 149.3 (CH), 152.5 (C), 165.2 (C). Anal. calcd for $C_{24}H_{16}N_4OS$: C, 70.57; H, 3.95; N, 13.72. Found: C, 69.99; H, 3.62; N, 12.35%.

*4.1.3.2. Ethyl (2Z)-2-cyano-4-[1-[2-oxo-2-(10H-phenothiazin-10-yl) ethyl]pyridin-4(1H)-ylidene]but-2-enoate (**14b**).* Red solid; 66% yield; mp 268–271 °C. IR ν_{max}/cm^{-1}: 2183, 1675, 1652, 1553, 1256, 1197, 1093, 852, 749. ^1H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.20 (t, J = 7.2 Hz, 3H, $CO_2CH_2CH_3$), 4.08 (q, J = 7.2 Hz, 2H, $CO_2CH_2CH_3$), 5.20 (br s, 2H, NCH_2CO), 5.65 (d, J = 14.0 Hz, 1H, CH=), 6.85 (br s, 1H, pyridine ArH), 7.25–7.50 (m, 5H, 4H phenoth. and 1H pyridine ArH), 7.64 (d, J = 7.6 Hz, 2H, phenoth. ArH), 7.70 (d, J = 7.6 Hz, 2H, phenoth. ArH), 7.75 (br s, 2H, pyridine ArH), 8.05 (d, J = 14.0 Hz, 1H, CH=). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 14.6 (CH_3), 57.7 (CH_2), 59.0 (CH_2), 75.7 (C), 101.8 (CH), 112.8 (CH), 118.0 (CH), 120.0 (C), 126.9 (2CH), 127.6 (4CH), 128.2 (2CH), 132.0 (2C), 137.0 (C), 140.7 (CH), 141.4 (CH), 146.3 (CH), 152.7 (C), 165.5 (C), 166.0 (C). Anal. calcd for $C_{26}H_{21}N_3O_3S$: C, 68.55; H, 4.65; N, 9.22. Found: C, 68.04; H, 4.59; N, 9.35%.

*4.1.3.3. {(2E)-2-[1-[2-Oxo-2-(10H-phenothiazin-10-yl)ethyl]pyridin-2(1H)-ylidene]ethylidene}malononitrile (**14c**).* Yellow solid; 64% yield; mp 270–271 °C. IR ν_{max}/cm^{-1}: 2196, 2188, 1680, 1545, 1283, 1176, 772, 576. ^1H NMR (DMSO-d_6, 400 MHz) δ ppm: 4.96 (br s, 1H, NCH_2CO), 5.16 (d, J = 13.6 Hz, 1H, CH=), 5.50 (br s, 1H, NCH_2CO), 6.85 (t, J = 6.8 Hz, 1H, pyridine ArH), 7.38–7.58 (m, 4H, phenoth. ArH), 7.63–7.80 (m, 5H, 4H phenoth. + 1H pyridine ArH), 7.83 (d, J = 13.6 Hz, 1H, CH=), 7.87 (d, J = 6.8 Hz, 1H, pyridine ArH), 7.91 (d, J = 6.8 Hz, 1H, pyridine ArH). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 50.2 (CH_2), 56.2 (C), 92.7 (CH), 114.3 (CH), 118.1 (C), 120.3 (C), 120.6 (CH), 121.0–138.0 (8CH phenoth. + 4C), 138.3 (CH), 142.3 (CH), 149.6 (CH), 153.1 (C), 164.2 (C). Anal. calcd for $C_{24}H_{16}N_4OS$: C, 70.57; H, 3.95; N, 13.72; S, 7.85. Found: C, 70.35; H, 4.42; N, 12.72%.

*4.1.3.4. Ethyl (2Z,4E)-2-cyano-4-[1-[2-oxo-2-(10H-phenothiazin-10-yl)ethyl]pyridin-2(1H)-ylidene]but-2-enoate (**14d**).* Red solid; 63% yield; mp 135–137 °C. IR ν_{max}/cm^{-1}: 2184, 1684, 1521, 1412, 1224, 1156, 1082, 748. ^1H NMR ($(CD_3)_2CO$, 400 MHz) δ ppm: 1,24 (t, J = 7.2 Hz, 3H, $CO_2CH_2CH_3$), 4.14 (q, J = 7.2 Hz, 2H, $COCH_2CH_3$), 5.19 (br s, 2H, NCH_2CO), 5.42 (d, J = 13.6 Hz, 1H, CH=), 6.62 (t, J = 6.8 Hz, 1H, H pyridine, ArH), 7.33–7.50 (m, 5H, 4H phenoth. + 1H pyridine ArH), 7.55–7.70 (m, 3H, 2H phenoth. + 1H pyridine ArH), 7.71–7.85 (m, 3H, 2H phenoth. + 1H pyridine ArH), 8.17 (d, J = 13.6 Hz, 1H, CH=). ^{13}C NMR ($(CD_3)_2CO$, 100 MHz) δ 14.1 (CH_3), 56.1 (CH_2), 59.2 (CH_2), 79.7 (C), 91.5 (CH), 112.1 (CH), 119.0 (C), 120.1 (CH), 123.0–129.0 (8CH), 134.0 (2C), 136.7 (CH), 141.8 (CH), 143.0 (2C), 148.2 (CH), 153.9 (C), 164.2 (C), 165.7 (C). Anal. calcd for $C_{26}H_{21}N_3O_3S$: C, 68.55; H, 4.65; N, 9.22; S, 7.04. Found: C, 68.44; H, 4.71; N, 9.22%.

*4.1.3.5. 3-(2-Cyanoethylacrylate)-benzoyl-4-methylpyridinium methylide (**15**).* Yellow solid, 78% yield; mp 252–254 °C. IR ν_{max}/cm^{-1}: 2185, 1697, 1640, 1516, 1233, 1172, 1115, 736, 662. ^1H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.22 (t, J = 7.2 Hz, 3H, $CO_2CH_2CH_3$), 2.70 (s, 3H, ArCH_3), 4.14 (q, J = 7.2 Hz, 2H, $CO_2CH_2CH_3$), 7.41–7.52 (m, 3H, phenyl ArH), 7.62 (d, J = 6.8 Hz, 2H, phenyl ArH), 7.74 (d, J = 5.6 Hz, 2H, phenyl ArH), 8.17 (s, 1H, CH=), 8.31 (d, J = 5.6 Hz, 2H, pyridine ArH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.5 (CH_3), 22.5 (CH_3), 60.3 (CH_2), 70.2 (C), 118.8 (C), 121.1 (C), 127.9 (2CH), 128.4 (2CH), 128.5 (2CH), 130.7 (CH), 138.6 (C), 145.2 (CH), 147.7 (2CH), 158.7 (C), 166.8 (C), 185.4 (C). Anal. calcd for $C_{20}H_{17}N_2O_3$: C, 71.99; H, 5.09; N, 8.39. Found: C, 71.57; H, 4.88; N, 8.31%.

*4.1.3.6. Ethyl (2Z)-2-cyano-4-[1-(2-morpholin-4-yl-2-oxoethyl)pyridin-4(1H)-ylidene]but-2-enoate (**16**).* Red oil; 41% yield. ^1H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.27 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 3.32 (t, J = 4.6 Hz, 2H, NCH_2CH_2O), 3.49 (t, J = 4.7 Hz, 2H, NCH_2CH_2O), 3.69 (t, J = 4.7 Hz, 2H, NCH_2CH_2O), 3.76 (sym m, J = 4.6 Hz, 2H, NCH_2CH_2O), 4.21 (q, J = 7.2 Hz, 2H, $CO_2CH_2CH_3$), 4.55 (s, 2H, $COCH_2N$), 5.76 (d, J = 13.9 Hz, 1H, CH=), 6.44 (sym m, 1H, ArH), 6.93–6.99 (m, 3H, ArH), 8.08 (d, J = 13.9 Hz, 1H, CH=). Anal. calcd for $C_{18}H_{21}N_3O_4$: C, 62.96; H, 6.16; N, 12.24. Found: C, 62.79; H, 6.43; N, 12.51%.

*4.1.3.7. Ethyl 2-cyano-3-morpholin-4-yl acrylate (**19**) [18]. By-product from the synthesis of compound **16**.* White solid; 16% yield. IR ν_{max}/cm^{-1}: 2206, 1682, 1608, 1439, 1368, 1285, 1260, 1211, 1163, 1099, 1028, 1004, 855, 762. ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.31 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 3.50 (large s, 2H, CH_2), 3.79 (t, J = 4.8 Hz, 4H, $2CH_2$), 4.05 (large s, 2H, CH_2), 4.24 (q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 7.70 (s, 1H, CH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 14.4 (CH_3), 46.7 (CH_2), 55.2 (CH_2), 61.1 (CH_2), 66.0 (CH_2), 66.9 (CH_2), 70.6 (C), 117.8 (C), 155.6 (CH), 166.5 (C).

*4.1.3.8. Ethyl (2Z)-2-cyano-4-[1-[2-(diethylamino)-2-oxoethyl]pyridin-4(1H)-ylidene]but-2-enoate (**17**).* Red solid; 56% yield. IR ν_{max}/cm^{-1}: 2972, 2183, 1648, 1519, 1480, 1183, 1135, 1072, 1019, 948, 860, 815. ^1H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.16 (t, J = 7.1 Hz, 3H, NCH_2CH_3), 1.28 (t, J = 7.2 Hz, 3H, $CO_2CH_2CH_3$), 1.31 (t, J = 7.1 Hz, 3H, NCH_2CH_3), 3.33 (q, J = 7.1 Hz, 2H, NCH_2CH_3), 3.42 (q, J = 7.1 Hz, 2H, NCH_2CH_3), 4.23 (q, J = 7.2 Hz, 2H, $CO_2CH_2CH_3$), 4.55 (s, 2H, $COCH_2N$), 5.77 (d, J = 13.9 Hz, 1H, CH=), 6.44 (sm, 1H, ArH), 6.93–6.99 (m, 3H, ArH), 8.08 (d, J = 13.9 Hz, 1H, CH=). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.9 (CH_3), 14.4 (CH_3), 14.6 (CH_3), 41.1 (CH_2), 56.8 (CH_2), 60.3 (CH_2), 81.8 (C), 103.7 (CH), 112.5 (CH), 117.9 (CH), 119.8 (C), 137.9 (CH), 138.6 (CH), 148.2 (CH), 151.3 (C), 163.9 (C), 166.8 (C). Anal. calcd for $C_{18}H_{23}N_3O_3$: C, 65.63; H, 7.04; N, 12.76. Found: C, 65.29; H, 7.33; N, 12.75%.

4.2. Molecular modelling

Based on previous studies [25], 1LD7 was chosen due to the structural similarity of the cocrystallized ligand with the compounds related in this work. The reference ligand and water molecules were removed, then the cocrystallized inhibitor was repositioned into the binding site defined as a 10Å sphere around its position in the crystal, needing a distance constraint to maintain proximity to the zinc. This constraint was later withdrawn as metal atoms were directly taken into account by newer versions docking software and the studied compounds became more remote from the cocrystallized inhibitor. The protonation state of the protein and ligands was found to be compatible with a pH of 7.5 and the

geometry of the ligands optimized using Gasteiger–Hückel partial charges, with a formal charge of +2 applied to iron atoms and a dielectric constant set to 4. A careful inspection of the geometry and charges of the ferrocene moiety was done to verify the distance and superposition of the aromatic-ion-aromatic stack. Due to limitations in the stability of an unbound complex in these molecular mechanics settings, it was necessary to tether the stack with bonds between the iron and the surrounding carbons, of 2Å length. After applying a formal charge of +2 on the farnesyltransferase zinc, metal atoms were handled by GOLD with the default geometry corresponding to their coordination. The 30 docking solutions were ranked based on a consensus scoring by GoldScore and X Score, then, visually inspected to assess the consistency of the best ranking conformation and select the most inhibitory clusters or conformation, when several were found.

4.3. Farnesyltransferase assay [26]

Assays were realized 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 farnesyl pyrophosphate (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-ß-D-glucopyranoside, 52 mM Tris/HCl, pH 7.5). Fluorescence was recorded for 15 min (0.7 s per well, 20 repeats) at 30 °C with an excitation filter at 340 nm and an emission filter of 486 nm. Each measurement was reproduced 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. Non linear regressions were performed with KaleidaGraph 4.03 software.

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