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Synthesis and biological evaluation of a new series of *N*-ylides as protein farnesyltransferase inhibitors



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ABSTRACT

A new family of 30 benzoylated *N*-ylides **4** and **5** was synthesized and evaluated for the inhibitory activity on human protein farnesyltransferase. Most of these novel compounds possessed in vitro inhibition potencies in the micromolar range. The nature of the substituents on the pyridine and phenyl units proved to be important in determining inhibitory activity and generally, the replacement of the cyanoacrylonitrile function by a cyanoethylacrylate group decreased the biological potential on farnesyltransferase. These results completed our SAR study on this original class of *N*-ylides.

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The maturation of many proteins requires one or more posttranslational modifications including prenylation, proteolysis and carboxymethylation.¹ The prenyltransferases are a family of zinc metalloenzymes that catalyze the addition of a prenyl unit to a cysteine thiol group of a set of proteins, causing their localization on the plasma membrane.²⁻⁴ Many G-proteins, such as Ras, Rho, Rac and CDC42, are located on the plasma membrane or endomembranes. This superfamily is actively involved in many important cellular signaling pathways, and plays an important role in carcinogenesis. The G-protein superfamily is the most important category of human CAAX proteins (A: aliphatic amino acids; X: methionine, glutamine or serine for farnesyltransferase, leucine or isoleucine for type I geranylgeranyltransferase). 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.⁵ As one of the most important G-proteins. Ras protein has a wellestablished role in oncogenesis. Ras proteins function as switches that control growth signals from cell surface receptors to nuclear transcription factors. Human cancer studies show that gene mutational activation of the Ras subfamily (K-Ras, N-Ras and H-Ras)

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occurs in many type of cancers, such as adenocarcinomes,^{6a–e} melanomas, hepatocellular cancer, myelodysplastics syndrome and acute myelogenous leukemia,^{6f–h} thyroid follicular and papillary carcinoma, bladder and renal cell cancer.^{6i,j}

Inhibition of protein farnesyltransferase (FTase) prevents membrane localization of Ras, and so constitutes a valid target for the conception of new cytostatic anticancer drugs.⁷ Farnesyltransferase inhibitors (FTIs) have thus been developed as a new class of promising drugs for cancer treatment.⁸ The main FTase inhibitors that have undergone clinical development⁹ are non peptidic, heterocyclic compounds such as Tipifarnib (R-115777),¹⁰ L-778123,¹¹ BMS-214662,¹² Lonafarnib (SCH-66336)¹³ and SCH-226374.¹⁴

We recently described that bulky lipophilic groups such ferrocene¹⁵ (Compounds 1) or phenothiazine¹⁶ (Compounds 2) can be placed in the A_2 binding site of farnesyltransferase. During the synthesis of other phenothiazine inhibitors,¹⁷ some *N*-ylides products such as **3** were isolated which were observed to display farnesyltransferase inhibition properties. In this light, we were intrigued by the fact that for the first time *N*-ylides compounds were encountered in the farnesyltransferase field (Ferrocenyl compounds are generally not considered as ylides). Thus we decided to perform a preliminary SAR study and some structural modifications of the scaffold of **3**, based mainly on replacement of the phenothiazine group by conventional aromatic nucleus, and we described here the results of this preliminary investigation (Fig. 1).

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Starting from the structure of compound 3, we were interested in the synthesis of *N*-ylides **4** and **5** (Scheme 2) bearing complexing groups (CN, CO₂Et) that could potentially bind the zinc atom of protein farnesyltransferase (Scheme 2). The initial reagents were commercial bromo or chloroacetyl benzenes 6A-J (Scheme 2). As for bromoacetyl-3,4,5-trimethoxybenzene 6K, it was obtained in 43% yield from the reaction of 3,4,5-trimethoxyacetophenone with bromine in acetic acid.¹⁸ In this reaction, bromoacetyl-2-bromo-3,4,5-trimethoxybenzene 6L was also isolated (Scheme 1). Now, bromoacetyl or chloroacetyl substituted benzenes 6 were reacted with pyridines **7** to give pyridinium salts $\mathbf{8}^{19}$ which were reacted with 2-cyano-3-ethoxyacrylonitrile 9 or ethyl 2-cyano-3ethoxyacrylate **10** in the presence of DBU,^{17,20} leading to the new ylides 4 and 5 (Table 1 and Scheme 2). In this reaction, indolizines **11** which could be generated from cyclization of salts 12^{21} were not detected. The obtention of carbanion disubstituted *N*-vlides **4** and **5** could be due to the two cyano or cyanoester groups, linked to the carbanion of the Michael addition intermediary 12, that prevent positioning near the carbocation in the α -pyridinium position of **12**,¹⁷ or to the rapid elimination of ethoxy anion leading to the new intermediate 13 (Scheme 2).

As can be observed from Scheme 2, the reaction is quite general. It is interesting to note that picolinium salt **8Ab** only furnished corresponding ylides **4Ab** and **5Ab** and that pyridinylidene compounds **14** and **15** were not observed. In contrast, under the same conditions, the methyl group of phenothiazine derivative **16** reacted with acrylates **9** or **10** to give products **17** and **18**¹⁷ as it was reported for some other picolinium salts (Scheme 3).²² The study of the generalization of the synthesis of pyridine-(1*H*)-ylidene derivatives starting from 2- and 4-picolinium salts has been realized and showed that an amide function was essential for reaction effectiveness;²² this could perhaps result from stabil-ization of intermediate charged species.

The activity of the new series of synthesized *N*-ylides was evaluated on human FTase.²³ Results are summarized in Table 2.

The nature of the substitution of the pyridine ring, of the phenyl group and the potential chelating bicyano or cyano ethylacetate unit proved to be important for the biological potential. The best results were obtained with 4-dimethylaminopyridinium derivatives 4Ba, 4Da, 4Ea and 4Ka (Tables 1 and 2) exhibiting IC₅₀ values from 12.5 to 19.7 μ M (Table 2). These ylides have also in common a dicyano group. The replacement of one cyano unit by an ethyl ester in compounds 5Ba, 5Da, 5Ea and 5Ka decreased the inhibitory activity (IC50 values from 31.9 to 48.0 µM). Next, the study of the influence of the pyridinium substitution on the FTase activity revealed that, except 3,5-dimethylpyridinium ylides **4Gd** and **4Kd**, that showed comparable potential to that of corresponding 4-dimethylamino ylides 4Ga and 4Ka, all other structural modifications (H, 4-OMe, 4-Me or 3,4-diOMe) of the pyridinium ring caused a slight reduction of the inhibitory activity. Finally, the *p*-chloro and the *p*-bromo substitutions of the phenyl ring proved to be favorable to bioactivity. We then investigated the influence of different substituents (F, CN, OMe, NO₂ or Me) at the para-position of the phenyl ring. Except the p-methyl compound 4Ba which conserved similar activity (IC₅₀ = $14.47 \pm 1.28 \mu$ M), all other structural modifications caused a significant reduction in biological activity (e.g. compound 4Da vs 4Ha, Table 2). A 3,4,5-triOMe substitution of the same phenyl unit was also favorable to bioactivity (ylide **4Ka**: $IC_{50} = 15.56 \pm 1.04 \mu M$) while its replacement by an unsubstituted phenyl moiety in ylide 4Aa caused an important reduction of the biological potential ($IC_{50} = 46.19$ ± 3.36 μM).

To further expand the understanding of the experimental results, molecular modeling studies for compound **4Aa** and the phenothiazine derivative **3** were carried out in the active site of protein FTase. Farnesyltransferase structure was taken from the 1LD7²⁴ entry of the RCSB Protein Data Bank.²⁵ The cocrystallized inhibitor and water molecules were removed to permit docking of the studied compounds, built from the standard fragments library of Sybyl



Figure 1. Structure of FTIs.



Scheme 1. Reagents and conditions: (i) bromine (1.2 equiv), AcOH, rt, 2 h.



Scheme 2. Reagents and conditions: (i) pyridine derivative 7a-d (2-5 equiv), EtOAc or acetone, reflux, 24 h; (ii) DBU (1 equiv), DMF or acetonitrile, alkene (2-cyano-3-ethoxyacrylonitrile (9) or ethyl 2-cyano-3-ethoxyacrylate (10)) (2 equiv), 70 °C, 24 h.

6.9.1²⁶ with GOLD 5.1.²⁷ Thirty solutions were generated and classed through an in-house scoring function based on GoldScore²⁷ and X-Score functions.²⁸

The phenothiazine derivative **3** adopts a single conformation described in Fig. 2(a), where the cyano groups form two hydrogen bonds with tyrosines 361β and 365β . The pyridine ring and the phenothiazine unit of the same compound **3** are in a favorable position to establish stacking interactions with Tyr 361β and Tyr

166α, respectively. The *p*-chlorophenyl analogue **4Da** displays different conformations. However, one conformation of ylide **4Da** fits into the binding site with the cyano units forming hydrogen bonds with tyrosines 361β and 365β (Fig. 2(b)) in the same way as the phenothiazine derivative **3**. On the other hand, the chlorophenyl ring of ylide **4Da** is too small to occupy the hydrophobic cavity compared to phenothiazine tricycle in compound **3** (Fig. 2(b)). This highlights the importance of the phenothiazine moiety and

Table 2

Table 1
Structure of synthesized N-ylides 4 and 5

Starting	Product	Product Ylide							Yield
salt no	no	х	Y	Ζ	R_1	R ₂	R ₃	W	(%)
8Aa	4Aa	Н	NMe_2	Н	Н	Н	Н	CN	82
	5Aa	Н	NMe_2	Н	Н	Н	Н	CO ₂ Et	86
8Ba	4Ba	Н	NMe_2	Н	Н	Me	Н	CN	82
	5Ba	Н	NMe_2	Н	Н	Me	Н	CO ₂ Et	54
8Ca	4Ca	Н	NMe_2	Н	Н	F	Н	CN	72
	5Ca	Н	NMe_2	Н	Н	F	Н	CO_2Et	76
8Da	4Da	Н	NMe_2	Н	Н	Cl	Н	CN	63
	5Da	Н	NMe_2	Н	Н	Cl	Н	CO_2Et	42
8Ea	4Ea	Н	NMe_2	Н	Н	Br	Н	CN	51
	5Ea	Н	NMe_2	Н	Н	Br	Н	CO_2Et	77
8Fa	4Fa	Н	NMe_2	Н	Н	CN	Н	CN	65
	5Fa	Н	NMe_2	Н	Н	CN	Н	CO_2Et	56
8Ga	4Ga	Н	NMe_2	Н	Н	OMe	Н	CN	68
	5Ga	Н	NMe_2	Н	Н	OMe	Н	CO_2Et	54
8Ha	4Ha	Н	NMe_2	Н	Н	NO_2	Н	CN	89
	5Ha	Н	NMe_2	Н	Н	NO_2	Н	CO_2Et	56
8Ka	4Ka	Н	NMe_2	Н	OMe	OMe	OMe	CN	74
	5Ka	Н	NMe_2	Н	OMe	OMe	OMe	CO_2Et	69
8Ia	4Ia	Н	NMe_2	Н	-		Н	CN	60
					NHCO	CH_2O-			
	5Ia	Н	NMe_2	Н	-		Н	CO_2Et	50
					NHCO	CH_2O-			
8Ab	4Ab	Н	Me	Н	Н	Н	Н	CN	78
	5Ab	Н	Me	Н	Н	Н	Н	CO_2Et	76
8Gc	4Gc	Н	OMe	Н	Н	OMe	Н	CN	47
	5Gc	Н	OMe	Н	Н	OMe	Н	CO_2Et	68
8Jc	4Jc	Н	OMe	Н	OMe	OMe	Н	CN	78
	5Jc	Н	OMe	Н	OMe	OMe	Н	CO_2Et	75
8Gd	4Gd	Me	Н	Me	Н	OMe	Н	CN	75
	5Gd	Me	Н	Me	Н	OMe	Н	CO_2Et	68
8Kd	4Kd	Me	Н	Me	OMe	OMe	OMe	CN	78
	5Kd	Me	Н	Me	OMe	OMe	OMe	CO_2Et	79

Compd No.	% ^{a,b}	$IC_{50} (\mu M \pm SD^c)^b$	R ^{2d}
4Aa	79	46.19 ± 3.36	0.972
5Aa	68	30.94 ± 1.77	0.970
4Ba	89	14.47 ± 1.28	0.980
5Ba	65	43.64 ± 2.63	0.952
4Ca	81	23.57 ± 0.91	0.991
5Ca	64	62.3 ± 3.50	0.976
4Da	95	12.49 ± 0.79	0.992
5Da	100	31.89 ± 8.94	0.806
4Ea	91	19.69 ± 0.88	0.995
5Ea	100	36.37 ± 10.39	0.830
4Fa	77	23.85 ± 2.24	0.976
5Fa	69	46.19 ± 3.36	0.977
4Ga	99	22.19 ± 2.58	0.965
5Ga	64	62.67 ± 4.11	0.972
4Ha	88	28.23 ± 2.21	0.985
5Ha	N.D. ^e	N.D.	-
4Ka	93	15.56 ± 1.04	0.990
5Ka	73	48.00 ± 2.03	0.988
4Ia	92	25.85 ± 3.18	0.962
5Ia	62	55.22 ± 3.14	0.967
4Ab	29	N.D.	-
5Ab	45	N.D.	-
4Gc	83	27.34 ± 2.02	0.973
5Gc	51	N.D.	-
4Jc	77	31.36 ± 0.87	0.997
5Jc	88	20.84 ± 2.37	0.897
4Gd	75	27.39 ± 0.48	0.998
5Gd	49	N.D.	-
4Kd	82	20.13 ± 0.59	0.994
5Kd	90	43.48 ± 5.00	0.938

Inhibitory activities of ylides 4 and 5 on human farnesyltransferase

^a Inhibition 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.

explains the higher affinity towards human FTase obtained with phenothiazine-containing ylides¹⁷ compared to benzoylated ylides presented in this article (e.g. phenothiazine **3**: (IC₅₀ (FTase) = $4.67 \pm 0.36 \,\mu$ M) vs compound **4Da**: (IC₅₀ (FTase) = $12.49 \pm 0.79 \,\mu$ M)).

In summary, 30 new benzoylated *N*-ylides **4** and **5** have been synthesized and evaluated for their inhibitory activity on human farnesyltransferase. Most of these novel compounds were found to possess in vitro inhibition potencies in the micromolar range. However, given the modest inhibitory activity values obtained, non-specific interactions are not excluded.

The nature of the substituent on the pyridine group proved to be important in determining inhibitory activity and generally, the replacement of the cyanoacrylonitrile function by a cyanoethylacrylate group decreased the biological potential on FTase.

The study performed in the present work, based mainly on the replacement of the phenothiazine group of a previously described series of *N*-ylides¹⁷ by conventional aromatic nucleus, emphasizes the importance of the phenothiazine unit in the structure of FTIs and completes our structure–activity relationships on this original class of *N*-ylides.



Scheme 3. Reagents and conditions: (i) DBU (1 equiv), DMF, 70 °C, 24 h.



Figure 2. Docking of phenothiazine derivative 3 (a) and *N*-ylide 4Da (b) in the active site of protein FTase.

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Supplementary data

Supplementary data (synthesis details and physico-chemical characterization for all new compounds) associated with this article can be found, in the online version, at http://dx.doi.org/ 10.1016/j.bmcl.2013.08.088.

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