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FULL PAPER

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Exploring isoxazoles and pyrrolidinones decorated with the 4,6-dimethoxy-1,3,5-triazine unit as human farnesyltransferase inhibitors

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Abstract

Unprecedented triazinyl-isoxazoles were afforded via an effective cycloaddition reaction between nitrile oxides and the scarcely described 2-ethynyl-4,6-dimethoxy-1,3,5-triazine as dipolarophile. The biological evaluation of the newly synthesized compounds showed that the inhibition of human farnesyltransferase by zinc complexation could be improved with triazine-isoxazole moieties. The replacement of the isoxazole unit by a pyrrolidin-2-one was detrimental to the inhibitory activity while the pyrrolidin-2-thione derivatives conserved the biological potential. The potential of selected compounds to disrupt protein farnesylation in Chinese hamster ovary (CHO) cells transfected with pEGFP-CAAX was also evaluated.

KEYWORDS
antitumor compound, cycloaddition, farnesyltransferase, inhibitor, isoxazole, pyrrolidine, triazine

1 | INTRODUCTION

Human farnesyltransferase (FTase) inhibitors are appealing compounds, largely studied in oncology to fight cancer and currently investigated for their potentialities to treat other pathologies such as sepsis,[1] Hutchinson–Gilford progeria syndrome, chronic hepatitis D, cardiovascular diseases, or neurodegenerative disorders.[2] Indeed farnesylation of proteins plays a vital role in cell progression.[3] Mainly, regulation of cyclin and cyclin-dependent kinase was obtained in part from farnesylation of Ras and Rho protein, and from protein Rheb which also influenced the Ras signaling pathway and is involved in the cell cycle. There are three human Ras genes: H-Ras, K-Ras, and N-Ras, and Ras oncogenes are known to be mutated in 30% of cancers.[4] The H-Ras localization is successfully inhibited by FTase inhibitors. Clinical trials are ongoing with known inhibitors of FTase such as lonafarnib and tipifarnib.[5] Lonafarnib (Sarasar®, Merck) is currently being investigated in six different clinical trials in combination with: (a) everolimus in a Phase I/II trial in progeria treatment; (b) ritonavir in a Phase II trial on patients with chronic delta hepatitis; (c) pravastain (a statin) and zoledronic acid (a bisphosphonate) in an open label Phase II efficacy trial in children with progeria; (d)

temozolomide in Phase I study in treating patients with recurrent glioblastoma; (e) ritonavir and lambda interferon in a Phase II trial to verify if the three-drug combination is safe and effective to treat chronic hepatitis D infection; and (f) ritonavir with and without pegylated (PEG) IFN-alfa-2a in a Phase III, matrix design, partially double-blind, randomized study in patients chronically infected with hepatitis delta virus.[5] Tipifarnib (Zarnestra®, Johnson & Johnson, NYSE: JNJ) is also studied in seven different Phase II clinical trials as the unique main ingredient: (a) in advanced squamous non-small-cell lung cancer with oncogene HRAS mutations; (b) in subjects with chronic myelomonocytic leukemia; (c) in patients with myelodysplastic syndromes; (d) in subjects with relapsed or refractory peripheral T-cell lymphoma; (e) for the treatment of head and neck cancer with HRAS mutations and impact of HRAS on response to therapy; (f) in patients with squamous head and neck cancer with H-Ras mutations, for which there is no curative therapy available; and (g) in treating patients with relapsed or refractory non-Hodgkin's lymphoma.[5] However, the problem with FTase inhibitors has been the impossibility of blocking localization of K-Ras, due to its ability to bypass FTase and to undergo alternative prenylation via structurally close geranylgeranyltransferase. Interestingly, neo-substrates for FTase have been recently developed which prevent the alternative prenylation by geranylgeranyltransferase, mislocalizing oncogenic K-Ras in cells.[6] New therapeutic area opens for molecules inactivating FTase and original powerful inhibitors are expected by the medicinal chemistry community. In the fight against tumor cell proliferation, we have already developed new families of human FTase.[7]

Because this heterodimeric metalloenzyme (45 and 48 kDa)[8] utilizes a zinc and a magnesium atom for its activation, scaffolds bearing complexing groups able to bind the metallic cations of the protein can lead to FTase inhibitors.

The *s*-triazine moiety is a pharmacophore with considerable therapeutic interest,[9] and many derivatives of this structure have been synthesized for their herbicidal[10] and antifungal[11] activities. Some 2,4-dimethoxytriazines display anticancer activity,[12] and our group described a new synthesis of this scaffold[13] that we have valued in the development of antitumoral agents targeting mitotic events.[14] Of particular interest is that starting from propionyl chloride, 2-ethynyl-4,6-dimethoxy-1,3,5-triazine **1** was obtained in 55% yield, whose condensation with azides furnished dimethoxytriazinyl-triazoles **2**. These new compounds have the potential to complex the zinc atom of FTase, but presented only a modest activity as FTase inhibitors and as anticancer agents.[15] It is known that 3,5-diarylisoxazoles display pharmaceutical properties as interleukin-8 receptor antagonists[16] or hypolipidemic agents,[17] and weak anticancer activity.[18] We are now interested in the synthesis of arylisoxazoles substituted by a dimethoxytriazine group, with the general structure **3**. Indeed the possibility for zinc complexation of the isoxazole moiety[19] could be improved by the triazinyl substituent. Therefore, target compounds **3** should exhibit inhibitory properties on human FTase and thus antitumoral activity. Next, the replacement of the isoxazole ring was envisaged in target pyrrolidin-2-(thi)ones **4** and **14** to conclude on its importance on the biological activity. The latter compounds conserve a heteroatom next to the triazine unit, and the (thio)carbonyl moiety should furthermore be able to form possible hydrogen bonds in the active site of the protein (Figure 1).

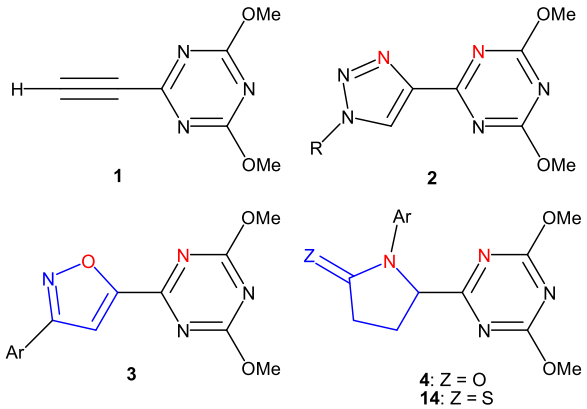


FIGURE 1 Structure of triazinic dipolarophile **1**, previously identified farnesyltransferase inhibitors dimethoxytriazinyl-triazoles **2**, and target dimethoxytriazinyl isoxazoles **3** and pyrrolidin-2-(thi)ones **4** and **14**

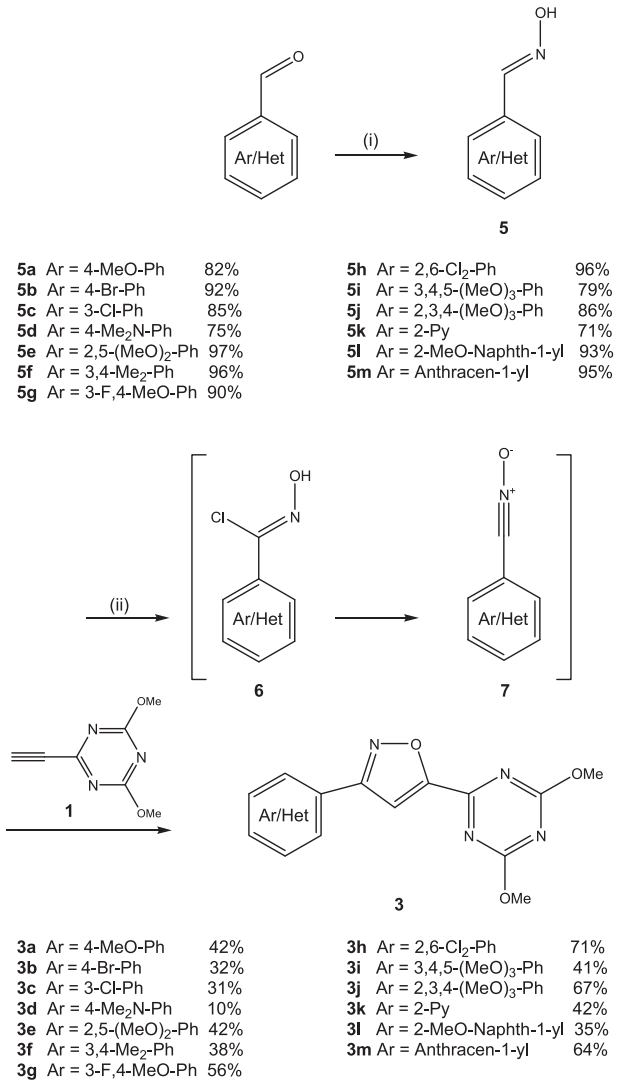
2 | RESULTS AND DISCUSSION

2.1 | Chemistry

The literature devoted to 2-ethynyl-4,6-dimethoxy-1,3,5-triazine **1** is extremely scarce.[1][20] We have recently proved that this dipolarophile constitutes a valuable intermediate in the synthesis of molecules with biological potential.[14] From a synthetic point of view, alkyne **1** is also interesting since the dimethoxytriazine unit has a very pronounced electro-withdrawing effect and should behave in the same way as classical methyl or ethyl propiolates in cycloaddition reactions. The synthetic strategy chosen for target isoxazoles **3** used ethynyldimethoxytriazine **1** as key reagent.

The most important methods described so far for the synthesis of 3,5-isoxazoles are the condensation of 1,3-diketones with hydroxylamine[21] and the [3+2] dipolar cycloaddition between alkynes and nitrile oxides.[22] In this context, oxidation of oxime was promoted by Magtrieve™ (Sigma-Aldrich, France) (CrO_2),[23] alkyl nitrites,[24] IBX,[25] or more often by *N*-chlorosuccinimide.[26] This latter procedure was privileged for our synthetic strategy to obtain target isoxazolyl-dimethoxytriazines **3** starting from oximes **5**. These compounds were obtained by using hydroxylamine hydrochloride in basic conditions,[27] but it is possible to work without base in refluxing alcohol,[28] and we obtained even easier very good yields of known oximes **5a–m**,[29] by stirring a mixture of hydroxylamine hydrochloride and aldehyde in a water/ethanol at room temperature (Scheme 1). Reaction of *N*-chlorosuccinimide with aldoximes **5** in the presence of dipolarophile **1** and potassium hydrogen carbonate in refluxing ethyl acetate furnished the cycloadduct **3** directly, without isolation of intermediate imidoyl chlorides **6** or nitrile oxides **7** (Scheme 1).

[1]Only five references deal with this dipolarophile: references 14, 15, and 20 from our group.



SCHEME 1 Synthesis of compounds **3a–m**. Reagents and conditions: (i) Hydroxylamine hydrochloride (1.2 equiv), EtOH, H_2O, rt, 4–24 hr; (ii) *N*-chlorosuccinimide (1.2 equiv), $KHCO_3$ (5 equiv), ethyl acetate, reflux, 24–72 hr

Cycloaddition of a nitrile oxide to a substituted alkyne can lead to two regioisomers, the 4- and/or 5-substituted isoxazoles. The same reaction was largely studied on substituted olefins. Reactions of mono-substituted alkenes gave the 5-substituted analogues with almost complete regioselectivity.[22] In contrast, alkynes substituted by strong electro-withdrawing groups can furnish two isomers.[30] For these reasons, we have investigated the X-ray diffraction spectra of isoxazoles **3g** and **3h** to prove their structure, and confirmed the formation of 5-substituted isoxazoles as unique regioisomers in our synthetic procedure (Figures S1 and S2).

The low to middle yields of some of the isolated isoxazoles **3** are in accordance with the literature which described that electro-donating groups are not very favorable for dipolar cycloadditions,[31] and this was confirmed by the condensation of 2,5-dimethoxybenzaldoxime **5e** with ethyl propiolate in the same experimental conditions, which leads to isoxazole **8**,[2] in only 33% yield (Scheme 2). On the other side, we did not observe the synthesis of furoxan *via* nitrile oxide **7** dimerization. Compound **8** has also been studied by single crystal X-ray diffraction method and proved the structure (Figures S1 and S2).

To justify the importance of the isoxazole ring on the activity against FTase, we have considered the replacement with a pyrrolidin-2-one then with a pyrrolidin-2-thione unit. Syntheses started from methyl pyroglutamate **9** which was first *N*-arylated according to a described procedure[32] providing methyl esters **10** and **11** in good yields (Scheme 3). Esters were further saponified, and the resulting carboxylic acids **12** and **13** were first activated with thionyl chloride in refluxing dichloromethane, then reacted with zinc dimethyl imidodicarbonimidate in the presence of pyridine and 4 Å powdered molecular sieves affording new pyrrolidinone-triazines **4a** and **4b**, by adapting a reported procedure.[13] The investigation of compounds **4** by chiral supercritical fluid chromatography indicated the presence of two enantiomers (see the chromatogram in the Supporting Information). Variation on the lactam carbonyl of pyrrolidin-2-ones **4** was also obtained by their reaction with phosphorus pentasulfide in toluene, in the presence of hexamethyldisiloxane (HMDSO). Thiolactams **14a,b** were thus obtained in very good yields of 91% and 89% respectively.

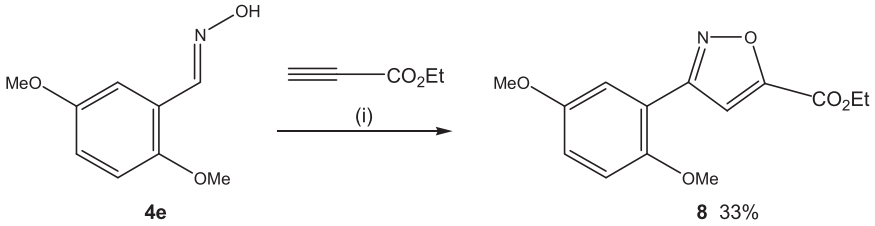
2.2 | Biological evaluation

Isoxazoles **3a–m** and **8**, pyrrolidinones **4a,b** and pyrrolidine-2-thiones **14a,b** were tested on human FTase protein. The results showed that isoxazole **3d**, substituted by *p*-dimethylaminophenyl unit was the best inhibitor in the studied triazine-isoxazoles series (Table 1). The 2,6-dichlorophenyl substituted isoxazole **3h** showed moderate activity against FTase, whereas the other chemical modulations realized on target isoxazoles abolished the biological efficiency (Table 1). Interestingly, isoxazole **8** presented comparable potency (IC_{50} value in the micromolar range) to the best triazine-isoxazole **3d** (Table 1). The replacement of the isoxazole unit by a pyrrolidin-2-one moiety in compounds **4a** and **4b** resulted in complete loss of inhibitory activity. The importance of the lactam carbonyl unit was also evaluated by synthesizing corresponding pyrrolidin-2-thiones **14a,b**. It is noteworthy that this modulation was beneficial for biological potential suggesting a conformational space available in the protein binding site in that position, and could serve as valuable tool for preclinical research on inhibitors with improved activity on human FTase. Moreover, among the studied non-sulfur triazine derivatives **3** and **4**, triazine-isoxazoles were more active than the pyrrolidin-2-one analogues (Table 1).

2.3 | Molecular modeling

Representatives of the best compounds in the current study (compounds **3d** and racemate **14a**) were selected for docking in the

[2]Isoxazole **8** is a commercial compound; however, the synthesis is not reported in SciFinder.



SCHEME 2 Synthesis of compound **8**. Reagents and conditions: (i) Ethyl propiolate (1.0 equiv), *N*-chlorosuccinimide (1.2 equiv), $KHCO_3$ (5 equiv), ethyl acetate, reflux, 24 hr

FTase binding site to understand their mode of linkage (Figure 2). Starting from the crystallographic data of FTase in the presence of an inhibitor (1LD7), the docking of the compounds was realized with GOLD suit 5.1. Thus, 30 solutions were generated for each compound and the consistency of their superposition assessed visually. The most representative conformations of each cluster were kept as the final solution of the docking run.

Compound **3d** presents a large group of 18 well-superimposed poses. The general position is quite close to the farnesyldiphosphate and roughly parallel, with the dimethylamine in front of the zinc atom (Figure 2a). This conformation is stabilized by two well-conserved hydrogen bond groups, one from the triazine to Arg 702, and depending on the generosity of the angle tolerance, the isoxazole is also able to form a hydrogen bond with this residue; the other from the terminal methoxy to Gln 167. No other discernible cluster can be seen in the 12 other poses, each independently placed more or less perpendicular to the cluster.

Compound **14a** in (*S*)-configuration displays a large majority of its poses in a single, well-defined cluster of 22 poses (Figure 2 b). It binds rather low in the site of the enzyme, at the end of the farnesyldiphosphate far from the zinc. While well encased in an aromatic cage, the most notable stacking being with Tyr 166 and 861, there is no hydrogen bond. The eight other poses are not so well correlated, although they are either a simple translation of the solution toward the zinc atom or a rotation in the same position to fit in the same aromatic cage with another set of stacking.

On the contrary, in *R* configuration, there are two clusters holding a total of 27 poses. The more numerous comprises 18 poses and is very similar to that of its enantiomer (Figure 2c). The difference of chirality imposes a very minor tilting of the whole molecule, permitting a hydrogen bond between one the methoxy and Arg 702.

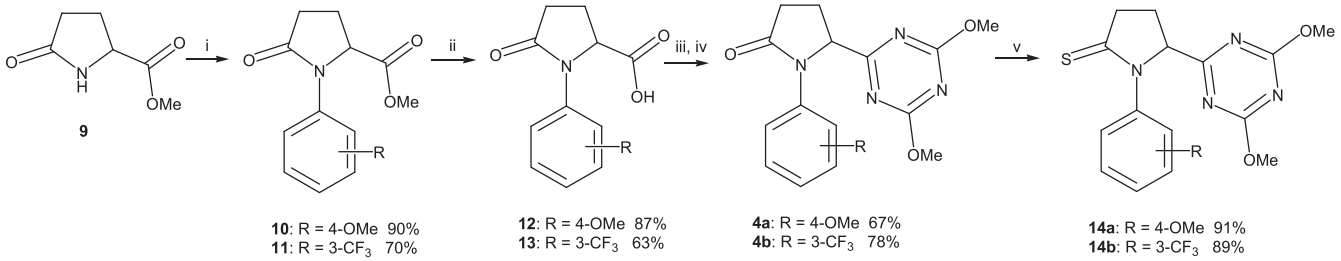
The second cluster contains nine poses which are placed in a different way (Figure 2d). The pyrrolidinethione is oriented toward the farnesyldiphosphate rather than away from it and the whole molecule is much closer to the zinc. The stacking with Tyr 861 is kept and a hydrogen bond appears between the single methoxy and Tyr 800.

The molecular modeling studies thus show that our initial hypothesis concerning the chelation of the zinc atom of the protein by the triazinyl-isoxazole or pyrrolidin-2-(thi)one unit is not validated, different mode of action of these molecules as being privileged.

To evaluate the potential of compounds **3d**, **8**, and **14a** to disrupt protein farnesylation in cells, Chinese hamster ovary (CHO) cells were transfected with pEGFP-CAAX (Addgene), a mammalian expression plasmid encoding farnesylation motif of H-Ras with *N*-terminus EGFP, and resulting cells were treated with selected compounds at a 30-μM concentration and with dimethyl sulfoxide (DMSO) as negative control. All three compounds did not affect the membrane localization (Figure 3).

3 | CONCLUSIONS

In summary, our previous discovery of triazinyl-triazoles as potential chelating molecules of the zinc of human FTase has encouraged us to perform herein a study of triazine-isoxazoles **3a–m** to try to optimize the inhibitory activity on human FTase. For the synthesis of target isoxazoles, an unequivocal and effective methodology was privileged starting from the scarcely described 2-ethynyl-4,6-dimethoxy-1,3,5-triazine **1**. This dipolarophile can be used in the same way as largely described alkyl propiolates and could provide original scaffolds of biological interest. Biological studies demonstrated that compounds of general structure **3** were potent against human FTase. Among them, *p*-dimethylaminophenyl isoxazole **3d** showed the strongest inhibition activity. Isoxazole **8**, obtained from ethyl propiolate, used as comparative cycloaddition dipolarophile, had equivalent FTase inhibitory activity. Compounds **3** and **8** can serve not only as valuable hits for research of potent FTase



SCHEME 3 Synthesis of compounds **14a,b**. Reagents and conditions: (i) Aryl bromide (1.1 equiv), CuI (0.5 equiv), *N,N′*-dimethylethylenediamine (1.0 equiv), Cs_2CO_3 (2.0 equiv), dioxane, inert atm, 60°C, 12 hr; (ii) $NaOH_{aq}$ 2 N, 80°C, 1–16 hr, then conc. HCl; (iii) $SOCl_2$ (1.5 equiv), CH_2Cl_2, reflux, 2–3 hr; (iv) zinc derivative (0.7 equiv), pyridine, CH_2Cl_2, molecular sieves 4 Å, rt, 12 hr; (v) P_4S_{10} (0.33 equiv), HMDSO (2.0 equiv), toluene, reflux, 3–5 hr

TABLE 1 Results of the human farnesyltransferase assay

Cpd nos	% Inh (FTase)[a,b]	IC_{50} (μM) ± SD	Cpd no.	% Inh (FTase)[a,b]	IC_{50} (μM) ± SD
3a	0	–	**3h**	53	–
3b	31	–	**3i**	28	–
3c	35	–	**3j**	17	–
3d	86	37.31 ± 4.18	**3k**	25	–
3e	0	–	**3l**	14	–
3f	0	–	**3m**	0	–
3g	33	–	**8**	87	37.22 ± 12.75
4a	17	–	**14a**	86	3.82 ± 0.59
4b	18	_	**14b**	96	41.06 ± 6.63

Note. SD: standard deviation.
[a]Inhibition ratio of protein farnesyltransferase at a 100 μM concentration.
[b]Values represent the mean of two experiments.

inhibitors but also as functionalized scaffolds for additional chemical modulations on the 3,5-dimethoxytriazine ring. In addition, the replacement of the isoxazole unit by a pyrrolidin-2-thione in compounds **14** was effective in terms of inhibitory potential. Compounds **3d**, **8**, and **14b** showed, however, limitation to disrupt protein farnesylation in pEGFP-CAAX transfected CHO cells. In conclusion, in the current context of lack of emergence of innovative human FTase inhibitors series and given all new therapeutic perspectives that open up for such molecules in rare diseases (e.g., Hutchinson–Gilford progeria syndrome), Delta hepatitis, cardiovascular, or neuroinflammatory diseases, we have just discovered new chemical fragments that open the way for the next generation of FTase inhibitors.

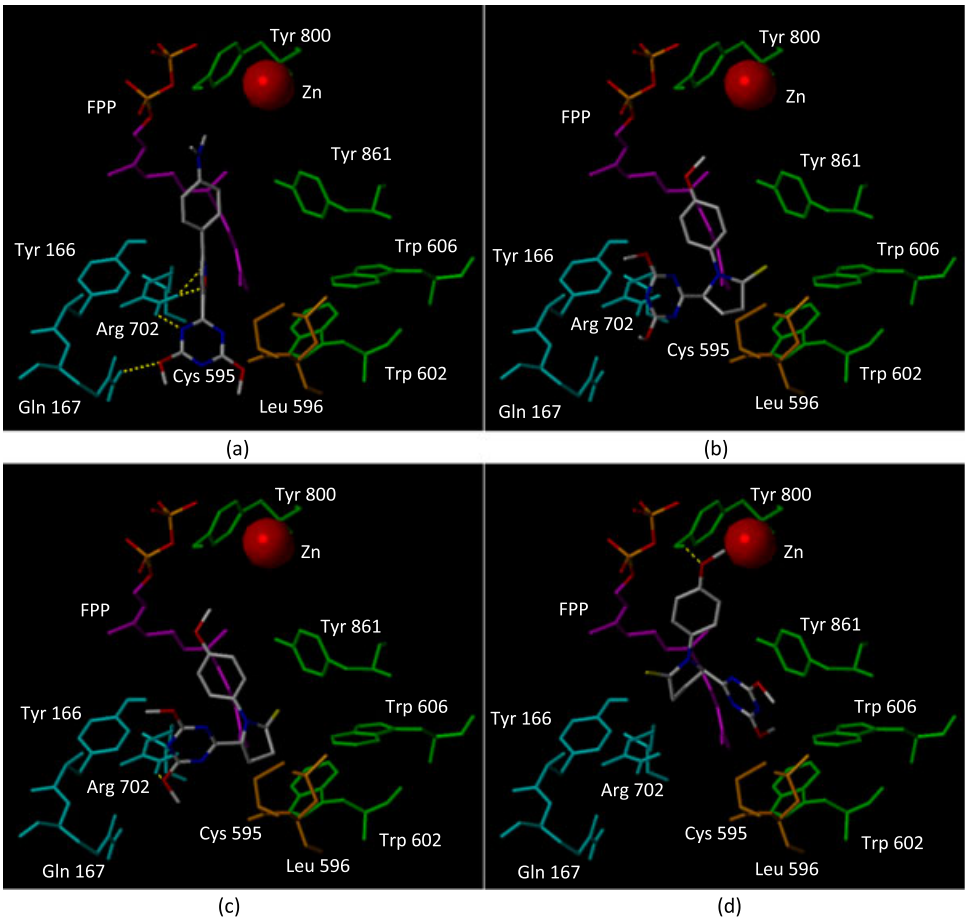


FIGURE 2 Docking of compounds **3d** and **14a** in the farnesyltransferase-binding site: (a) **3d**, (b) (*S*)-**14a**, (c) (*R*)-**14a**, first cluster, (d) (*R*)-**14a**, second cluster

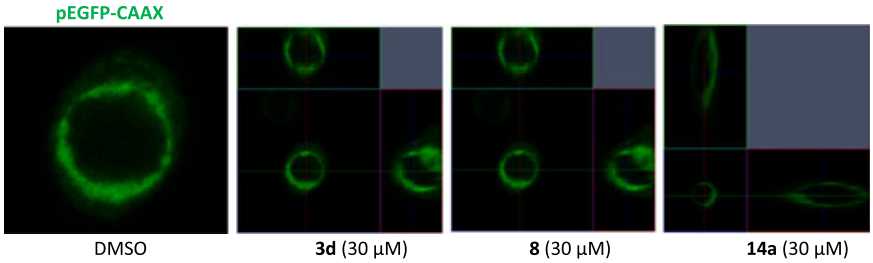


FIGURE 3 Lack of effect of **3d**/**8**/**14b** on membrane localization of a GFP construct. CHO cells were transfected with pEGFP-CAAX plasmid followed by treatment as shown for 16 hr

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

Starting materials were commercially available and were used without further purification. Melting points were measured on a MPA 100 OptiMelt® (Stanford Research Systems, Sunnyvale, CA) apparatus and are uncorrected. NMR spectra were acquired at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR on a Varian 400-MR spectrometer (Agilent Technologies France SAS, Les Ulis) or on a Bruker Avance DRX 400 spectrometer (Bruker BioSpin, Wissembourg, France) or at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR on a Bruker Avance III spectrometer. Chemical shifts (δ) are given in ppm relative to TMS (0 ppm). Splitting patterns are designed as: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; quint, quintuplet; m, multiplet and sym m, symmetric multiplet. Coupling constants *J* are reported in Hertz (Hz). Column chromatographies were performed with a CombiFlash Rf Companion (Teledyne-Isco System, Lincoln, NE) using RediSep (Serlabo Technologies, Vedene, France) packed columns. IR spectra were recorded on a Varian 640-IR FT-IR spectrometer (Agilent Technologies France SAS, Les Ulis, neat). Mass spectra were acquired on a Finnigan MAT 90X spectrometer (Thermo Scientific, MA), electron impact (EI). Elemental analyses (C, H, N) of new compounds were determined on a Thermo Electron apparatus by "Pôle Chimie Moléculaire-Welience," Faculté de Sciences Mirande, Université de Bourgogne (Dijon, France).

The InChI codes of the investigated compounds together with some biological activity data are provided as the Supporting Information.

4.1.2 | General procedure for the sysnthesis of isoxazoles 3a–m and 8

2-Ethynyl-4,6-dimethoxy-1,3,5-triazine **1** (1 equiv), $KHCO_3$ (5 equiv), *N*-chlorosuccinimide (1.2 equiv) and few drops of distilled water were added to a solution of oxime **5a–m** (1.1 equiv) in ethyl acetate. The resulting mixture is refluxed till the complete consumption of the oxime (monitored by TLC). After cooling to room temperature, the crude was filtered to remove insoluble salts. The organic filtrate was washed with distilled water, dried over anhydrous Na_2SO_4 and evaporated to dryness. The resulting solid was recrystallized from absolute EtOH to provide pure isoxazole **3a–m** or **8**.

2,4-Dimethoxy-6-[3-(4-methoxyphenyl)isoxazol-5-yl]-1,3,5-triazine (3a)

The general procedure was followed using 2-ethynyl-4,6-dimethoxy-1,3,5-triazine **1** (1.0 g, 6.06 mmol, 1 equiv), 4-methoxybenzaldoxime **5a** (1.0 g, 6.66 mmol, 1.1 equiv), *N*-chlorosuccinimide (0.98 g, 7.26 mmol, 1.2 equiv), $KHCO_3$ (3.04 g, 30.4 mmol, 5 equiv), and ethyl acetate (20 ml). Yield: 0.81 g (42%); beige solid, mp (EtOH) 175–176°C; R_f = 0.57 (EtOAc/*n*-hexane, 1:1). IR (neat): 1610, 1532, 1505, 1458, 1428, 1406, 1389, 1351, 1309, 1253, 1187, 1076, 976, 844, 795 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.86 (s, 3 H, OCH_3), 4.14 (s, 6 H, $2OCH_3$), 6.99 (d, *J* = 8.8 Hz, 2 H, Ar*H*), 7.48 (s, 1 H, Ar*H*), 7.81 (d, *J* = 8.8 Hz, 2 H, Ar*H*). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 55.4 (CH_3), 55.7 (2 CH_3), 106.3 (CH), 115.5 (2 CH), 120.8 (C), 128.3 (2 CH), 161.3 (C), 162.8 (C), 165.3 (C), 165.9 (C), 173.0 (2 C). Anal. calcd. for $C_{15}H_{14}N_4O_4$: C, 57.32; H, 4.49; N, 17.83. Found: C, 57.50; H, 4.62; N, 18.05%.

2-[3-(4-Bromophenyl)isoxazol-5-yl]-4,6-dimethoxy-1,3,5-triazine (3b)

The general procedure was followed using 2-ethynyl-4,6-dimethoxy-1,3,5-triazine **1** (0.6 g, 3.64 mmol, 1 equiv), 4-bromobenzaldoxime **5b** (0.8 g, 4.0 mmol, 1.1 equiv), *N*-chlorosuccinimide (0.58 g, 4.36 mmol, 1.2 equiv), $KHCO_3$ (1.82 g, 18.18 mmol, 5 equiv), and ethyl acetate (40 ml). Yield: 0.42 g (32%); beige solid, mp (EtOH) 217–218°C; R_f = 0.45 (EtOAc/*n*-hexane, 1:1). IR (neat): 1593, 1545, 1509, 1499, 1463, 1425, 1384, 1354, 1296, 1258, 1149, 1064, 939, 850, 715 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.15 (s, 6 H, $2OCH_3$), 7.50 (s, 1 H, Ar*H*), 7.62 (d, *J* = 8.4 Hz, 2 H, Ar*H*), 7.74 (d, *J* = 8.8 Hz, 2 H, Ar*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 55.8 (2 CH_3), 106.2 (CH), 124.8 (C), 127.2 (C), 128.3 (2 CH), 132.3 (2 CH), 162.3 (C), 165.1 (C), 166.5 (C), 173.0 (2 C). MS (EI) *m/z* (%): 362 (78) [M$^+$], 224 (97), 222 (100), 194 (25), 141 (15), 76 (14). Anal. calcd. for $C_{14}H_{11}BrN_4O_3$: C, 46.30; H, 3.05; N, 15.43. Found: C, 46.49; H, 3.22; N, 15.81%.

2-[3-(3-Chlorophenyl)isoxazol-5-yl]-4,6-dimethoxy-1,3,5-triazine (3c)

The general procedure was followed using 2-ethynyl-4,6-dimethoxy-1,3,5-triazine **1** (0.78 g, 4.66 mmol, 1 equiv), 3-chlorobenzaldoxime **5c** (0.8 g, 5.14 mmol, 1.1 equiv), *N*-chlorosuccinimide (0.78 g, 5.82 mmol, 1.2 equiv), $KHCO_3$ (2.42 g, 24.24 mmol, 5 equiv) and ethyl acetate (40 ml). Yield: 0.50 g (31%); yellow solid, mp (EtOH) 201–203°C; R_f = 0.69 (EtOAc/*n*-hexane, 1:1). IR (neat): 1538, 1509, 1469, 1447, 1433, 1397, 1384, 1347, 1255, 1200, 1151, 1074, 940, 851, 758 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.16 (s, 6 H, 2 OCH_3),

7.41–7.47 (m, 1 H, Ar*H*), 7.52 (s, 1 H, Ar*H*), 7.76 (d, *J* = 8.0, 1.6 Hz, 1 H, Ar*H*), 8.16 (d, *J* = 7.2 Hz, 1 H, Ar*H*), 7.89 (s, 1 H, Ar*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 55.8 (2 CH_3), 106.3 (CH), 125.0 (CH), 127.0 (CH), 130.1 (C), 130.4 (CH), 130.5 (CH), 135.2 (C), 162.1 (C), 165.1 (C), 166.6 (C), 173.0 (2 C). Anal. calcd. for $C_{14}H_{11}ClN_4O_3$: C, 52.76; H, 3.48; N, 17.58. Found: C, 53.01; H, 3.62; N, 17.77%.

N-{4-[5-(4,6-Dimethoxy-1,3,5-triazine-2-yl)isoxazol-3-yl]-phenyl}-*N*,*N*-dimethylamine (3d)

The general procedure was followed using 2-ethynyl-4,6-dimethoxy-1,3,5-triazine **1** (0.9 g, 5.46 mmol, 1 equiv), 4-dimethylaminobenzaldoxime **5d** (1.0 g, 6.06 mmol, 1.1 equiv), *N*-chlorosuccinimide (0.86 g, 6.44 mmol, 1.2 equiv), $KHCO_3$ (2.72 g, 27.2 mmol, 5 equiv), and ethyl acetate (40 ml). Yield: 0.12 g (10%); yellow solid, mp (EtOH) 180–184°C; R_f = 0.75 (EtOAc/*n*-hexane, 1:1). IR (neat): 1611, 1539, 1504, 1457, 1434, 1387, 1357, 1228, 1196, 1174, 1036, 981, 815, 796, 757 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.05 (s, 6 H, 2CH_3), 4.15 (s, 6 H, 2 OCH_3), 6.88 (s, 2 H, Ar*H*), 7.47 (s, 1 H, Ar*H*), 7.77 (d, *J* = 8.4 Hz, 2 H, Ar*H*). ^{13}C NMR (100 MHz, $CDCl_3$): 40.3 (2 CH_3), 55.7 (2 CH_3), 98.4 (CH), 114.8 (2 CH), 122.6 (C), 128.2 (2 CH), 149.6 (C), 165.3 (C), 165.9 (C), 173.0 (2 C), 173.1 (C). Anal. calcd. for $C_{16}H_{17}N_5O_3$: C, 58.71; H, 5.23; N, 21.39. Found: C, 59.04; H, 5.50; N, 21.62%.

2-[3-(2,5-Dimethoxyphenyl)isoxazol-5-yl]-4,6-dimethoxy-1,3,5-triazine (3e)

The general procedure was followed using 2-ethynyl-4,6-dimethoxy-1,3,5-triazine **1** (0.30 g, 1.82 mmol, 1 equiv), 2,5-dimethoxybenzaldoxime **5e** (0.36 g, 4.02 mmol, 1.1 equiv), *N*-chlorosuccinimide (0.29 g, 2.18 mmol, 1.2 equiv), $KHCO_3$ (0.9 g, 9.08 mmol, 5 equiv), and ethyl acetate (30 ml). Yield: 0.26 g (42%); beige solid, mp (EtOH) 177–179°C; R_f = 0.45 (EtOAc/*n*-hexane, 1:1). IR (neat): 1541, 1512, 1460, 1421, 1376, 1301, 1263, 1116, 1029, 983, 807, 739 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.84 (s, 3 H, OCH_3), 3.88 (s, 3 H, OCH_3), 4.16 (s, 6 H, 2 OCH_3), 6.97 (s, 1 H, Ar*H*), 6.99 (d, *J* = 3.2 Hz, 1 H, Ar*H*), 7.55 (d, *J* = 2.8 Hz, 1 H, Ar*H*), 7.99 (s, 1 H, Ar*H*). ^{13}C NMR (100 MHz, $CDCl_3$): 55.7 (2 CH_3), 55.9 (CH_3), 56.1 (CH_3), 110.2 (CH), 112.9 (CH), 113.5 (CH), 117.5 (C), 117.8 (CH), 151.7 (C), 153.6 (C), 160.7 (C), 165.1 (C), 165.5 (C), 173.0 (2 C). Anal. calcd. for $C_{16}H_{16}N_4O_5$: C, 55.81; H, 4.68; N, 16.27. Found: C, 56.11; H, 4.90; N, 16.54%.

2-[3-(3,4-Dimethylphenyl)isoxazol-5-yl]-4,6-dimethoxy-1,3,5-triazine (3f)

The general procedure was followed using 2-ethynyl-4,6-dimethoxy-1,3,5-triazine **1** (0.60 g, 3.64 mmol, 1 equiv), 3,4-dimethylbenzaldoxime **5f** (0.6 g, 4.02 mmol, 1.1 equiv), *N*-chlorosuccinimide (0.58 g, 4.38 mmol, 1.2 equiv), $KHCO_3$ (1.82 g, 18.18 mmol, 5 equiv), and ethyl acetate (40 ml). Yield: 0.43 g (38%); beige solid, mp (EtOH) 171–174°C; R_f = 0.74 (EtOAc/*n*-hexane, 1:1). IR (neat): 1615, 1541, 1503, 1467, 1446, 1396, 1377, 1343, 1228, 1197, 1148, 1074, 943, 865, 718 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.36 (s, 3 H, CH_3), 2.37 (s, 3 H, CH_3), 4.19 (s, 6 H, 2 OCH_3), 7.29 (t, *J* = 6.8 Hz, 1 H, Ar*H*), 7.55 (s, 1 H, Ar*H*), 7.62 (d, *J* = 7.6 Hz, 1 H, Ar*H*), 7.72 (s, 1 H, Ar*H*). ^{13}C NMR (100 MHz, $CDCl_3$): 19.8 (2 CH_3), 55.7 (2 CH_3), 106.5 (CH), 124.3 (CH), 125.8 (C), 127.9 (CH), 130.3 (CH), 137.4 (C), 139.3 (C), 163.2 (C), 165.3 (C), 165.9 (C), 173.0 (2 C). Anal. calcd. for $C_{16}H_{16}N_4O_3$: C, 61.53; H, 5.16; N, 17.94. Found: C, 61.82; H, 5.50; N, 18.11%.

2-[3-(3-Fluoro-4-methoxyphenyl)isoxazol-5-yl]-4,6-dimethoxy-1,3,5-triazine (3g)

The general procedure was followed using 2-ethynyl-4,6-dimethoxy-1,3,5-triazine **1** (0.7 g, 4.24 mmol, 1 equiv), 3-fluoro-4-methoxybenzaldoxime **5g** (0.8 g, 4.72 mmol, 1.1 equiv), *N*-chlorosuccinimide (0.68 g, 5.1 mmol, 1.2 equiv), $KHCO_3$ (2.12 g, 20.12 mmol, 5 equiv), and ethyl acetate (50 ml). Yield: 0.75 g (56%); white solid, mp (EtOH) 160–163°C; R_f = 0.6 (EtOAc/*n*-hexane, 1:1). IR (neat): 1619, 1549, 1509, 1460, 1389, 1355, 1317, 1280, 1130, 1104, 1017, 934, 870, 762 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.96 (s, 3 H, OCH_3), 4.16 (s, 6 H, 2 OCH_3), 7.06 (t, *J* = 8.6 Hz, 1 H, Ar*H*), 7.47 (s, 1 H, Ar*H*), 7.60 (dt, *J* = 8.6, 1.2 Hz, 1 H, Ar*H*), 7.64 (dd, *J* = 11.8 Hz, 1 H, Ar*H*). ^{13}C NMR (100 MHz, $CDCl_3$): 55.8 (2 CH_3), 56.3 (CH_3), 106.2 (CH), 113.5 (d, *J* = 2.3 Hz, CH), 114.7 (d, *J* = 20.2 Hz, CH), 121.2 (d, *J* = 7.2 Hz, C), 123.2 (d, *J* = 3.1 Hz, CH), 149.5 (d, *J* = 10.3 Hz, C), 152.5 (d, *J* = 246.7 Hz, CF), 162.0 (d, *J* = 2.6 Hz, C), 165.1 (C), 166.3 (C), 173.0 (2 C). Anal. calcd. for $C_{15}H_{13}FN_4O_4$: C, 54.22; H, 3.94; N, 16.86. Found: C, 54.46; H, 4.14; N, 17.07%.

2-[3-(2,6-Dichlorophenyl)isoxazol-5-yl]-4,6-dimethoxy-1,3,5-triazine (3h)

The general procedure was followed using 2-ethynyl-4,6-dimethoxy-1,3,5-triazine **1** (0.3 g, 1.82 mmol, 1 equiv), 2,6-dichlorobenzaldoxime **5h** (0.38 g, 2.0 mmol, 1.1 equiv), *N*-chlorosuccinimide (0.3 g, 2.18 mmol, 1.2 equiv), $KHCO_3$ (0.9 g, 8.9 mmol, 5 equiv), and ethyl acetate (30 ml). Yield: 0.46 g (71%); beige solid, mp (EtOH) 150–152°C; R_f = 0.66 (EtOAc/*n*-hexane, 1:1). IR (neat): 1546, 1514, 1493, 1453, 1386, 1233, 1105, 1046, 940, 851, 731 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.16 (s, 6 H, 2 OCH_3), 7.33 (s, 1 H, Ar*H*), 7.35–7.39 (m, 1 H, Ar*H*), 7.45 (d, *J* = 8.0 Hz, 2 H, Ar*H*). ^{13}C NMR (125 MHz, $CDCl_3$): 55.9 (2 CH_3), 110.3 (CH), 127.7 (C), 128.4 (2 CH), 131.6 (CH), 135.7 (2 C), 159.7 (C), 165.2 (C), 166.1 (C), 173.1 (2 C). MS (EI) *m/z* (%): 352 (44) [M^+], 317 (36), 214 (62), 212 (100), 184 (25), 157 (10). Anal. calcd. for $C_{14}H_{10}Cl_2N_4O_3$: C, 47.61; H, 2.85; N, 15.86. Found: C, 47.80; H, 3.20; N, 16.00%.

2,4-Dimethoxy-6-[3-(3,4,5-trimethoxyphenyl)-isoxazol-5-yl]-1,3,5-triazine (3i)

The general procedure was followed using 2-ethynyl-4,6-dimethoxy-1,3,5-triazine **1** (0.44 g, 2.66 mmol, 1 equiv), 3,4,5-trimethoxybenzaldoxime **5i** (0.62 g, 2.94 mmol, 1.1 equiv), *N*-chlorosuccinimide (0.42 g, 3.2 mmol, 1.2 equiv), $KHCO_3$ (1.34 g, 13.34 mmol, 5 equiv), and ethyl acetate (20 ml). Yield: 0.40 g (41%); white solid, mp (EtOH) 176–179°C; R_f = 0.72 (EtOAc/*n*-hexane, 1:1). IR (neat): 1587, 1502, 1461, 1418, 1388, 1351, 1309, 1236, 1204, 1125, 1041, 1032, 944, 854, 766 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.91 (s, 3 H, OCH_3), 3.95 (s, 6 H, 2 OCH_3), 4.16 (s, 6 H, 2 OCH_3), 7.10 (s, 2 H, Ar*H*), 7.51 (s, 1 H, Ar*H*). ^{13}C NMR (125 MHz, $CDCl_3$): 55.8 (2 CH_3), 56.3 (2 CH_3), 61.0 (CH_3), 104.1 (2 CH), 106.6 (CH), 123.6 (C), 140.0 (C), 153.7 (2 C), 163.0 (C), 165.2 (C),

166.2 (C), 173.0 (2 C). MS (EI) *m/z* (%): 375 (18) [M^+], 374 (100), 359 (52), 331 (18), 234 (12), 72 (9). Anal. calcd. for $C_{17}H_{18}N_4O_6$: C, 54.54; H, 4.85; N, 14.97. Found: C, 54.61; H, 5.09; N, 15.15%.

2,4-Dimethoxy-6-[3-(2,3,4-trimethoxyphenyl)isoxazol-5-yl]-1,3,5-triazine (3j)

The general procedure was followed using 2-ethynyl-4,6-dimethoxy-1,3,5-triazine **1** (0.3 g, 1.82 mmol, 1 equiv), 2,3,4-trimethoxybenzaldoxime **5j** (0.42 g, 2.0 mmol, 1.1 equiv), *N*-chlorosuccinimide (0.29 g, 2.18 mmol, 1.2 equiv), $KHCO_3$ (0.9 g, 9.08 mmol, 5 equiv), and ethyl acetate (30 ml). Yield: 0.46 g (67%); white solid, mp (EtOH) 150–152°C; R_f = 0.48 (EtOAc/*n*-hexane, 1:1). IR (neat): 1544, 1498, 1466, 1414, 1382, 1346, 1285, 1227, 1190, 1076, 1041, 966, 936, 853, 796 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 3.91 (s, 3 H, O*CH*$_3$), 3.92 (s, 3 H, O*CH*$_3$), 3.93 (s, 3 H, O*CH*$_3$), 4.16 (s, 6 H, 2 O*CH*$_3$), 6.79 (d, *J* = 9.0 Hz, 1 H, Ar*H*), 7.66 (d, *J* = 8.5 Hz, 1 H, Ar*H*), 7.66 (s, 1 H, Ar*H*). ^{13}C NMR (125 MHz, $CDCl_3$): 55.8 (2 CH_3), 56.3 (CH_3), 61.1 (CH_3), 61.4 (CH_3), 108.0 (CH), 110.0 (CH), 115.3 (C), 124.0 (CH), 142.7 (C), 152.5 (C), 155.7 (C), 160.7 (C), 165.5 (C), 165.7 (C), 173.1 (2 C). Anal. calcd. for $C_{17}H_{18}N_4O_6$: C, 54.54; H, 4.85; N, 14.97. Found: C, 54.73; H, 5.13; N, 15.22%.

2,4-Dimethoxy-6-(3-pyridine-2-ylisoxazol-5-yl)-1,3,5-triazine (3k)

The general procedure was followed using 2-ethynyl-4,6-dimethoxy-1,3,5-triazine **1** (1.0 g, 6.06 mmol, 1 equiv), 2-pyridinaldoxime **5k** (1.0 g, 6.66 mmol, 1.1 equiv), *N*-chlorosuccinimide (0.98 g, 7.26 mmol, 1.2 equiv), $KHCO_3$ (3.04 g, 30.4 mmol, 5 equiv) and ethyl acetate (40 ml). Yield: 0.96 g (42%); beige solid, mp (EtOH) 192–194°C; R_f = 0.53 (EtOAc/*n*-hexane, 1:1). IR (neat): 1542, 1511, 1493, 1469, 1401, 1370, 1346, 1256, 1223, 1107, 1020, 937, 849, 746 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 4.14 (s, 6 H, 2 O*CH*$_3$), 7.39 (t, *J* = 6.4 Hz, 1 H, Ar*H*), 7.84 (dt, *J* = 8.0, 1.6 Hz, 1 H, Ar*H*), 7.88 (s, 1 H, Ar*H*), 8.16 (d, *J* = 8.0 Hz, 1 H, Ar*H*), 8.72 (d, *J* = 4.4 Hz, 1 H, Ar*H*). ^{13}C NMR (100 MHz, $CDCl_3$): 55.7 (2 CH_3), 107.6 (CH), 122.0 (CH), 125.0 (CH), 137.5 (CH), 147.5 (C), 149.5 (CH), 163.6 (C), 165.2 (C), 166.5 (C), 173.0 (2 C). Anal. calcd. for $C_{13}H_{11}N_5O_3$: C, 54.74; H, 3.89; N, 24.55. Found: C, 55.11; H, 4.08; N, 24.91%.

2,4-Dimethoxy-6-[3-(2-methoxy-1-naphthyl)isoxazol-5-yl]-1,3,5-triazine (3l)

The general procedure was followed using 2-ethynyl-4,6-dimethoxy-1,3,5-triazine **1** (0.3 g, 1.82 mmol, 1 equiv), 2-methoxy-1-naphthaldoxime **5l** (0.4 g, 2.0 mmol, 1.1 equiv), *N*-chlorosuccinimide (0.29 g, 2.18 mmol, 1.2 equiv), $KHCO_3$ (0.9 g, 9.08 mmol, 5 equiv), and ethyl acetate (30 ml). Yield: 0.23 g (35%); beige solid, mp (EtOH) 212–215°C; R_f = 0.67 (EtOAc/*n*-hexane, 1:1). IR (neat): 1546, 1516, 1504, 1484, 1469, 1394, 1371, 1348, 1274, 1226, 1101, 1038, 924, 852, 734 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 3.97 (s, 3 H, O*CH*$_3$), 4.17 (s, 6 H, 2 O*CH*$_3$), 7.36–7.41 (m, 2 H, Ar*H*), 7.45–7.48 (m, 2 H, Ar*H*), 7.84 (d, *J* = 8.4 Hz, 1 H, Ar*H*), 7.95 (d, *J* = 8.4 Hz, 1 H, Ar*H*), 7.99 (d, *J* = 9.2 Hz, 1 H, Ar*H*). ^{13}C NMR (100 MHz, $CDCl_3$): 55.7 (2 CH_3), 56.6 (CH_3), 111.3 (C), 112.0 (CH), 124.1 (CH), 124.6 (2 CH), 127.6 (CH), 128.1 (CH), 128.9 (C), 132.0 (CH), 132.9 (C), 155.5 (C), 159.8 (C), 165.1 (C), 165.5 (C), 173.0 (2 C). Anal. calcd. for $C_{19}H_{16}N_4O_4$: C, 62.63; H, 4.43; N, 15.38. Found: C, 62.88; H, 4.79 N, 15.62%.

2-[3-(9-Anthryl)isoxazol-5-yl]-4,6-dimethoxy-1,3,5-triazine (3m)

The general procedure was followed using 2-ethynyl-4,6-dimethoxy-1,3,5-triazine **1** (0.68 g, 4.12 mmol, 1 equiv), anthracene-9-carbaldehyde oxime **5m** (1.0 g, 4.52 mmol, 1.1 equiv), *N*-chlorosuccinimide (0.66 g, 4.94 mmol, 1.2 equiv), $KHCO_3$ (2.12 g, 21.12 mmol, 5 equiv), and ethyl acetate (50 ml). Yield: 0.76 g (64%); yellow solid, mp (EtOH) 178–181°C; R_f = 0.78 (EtOAc/*n*-hexane, 1:1). IR (neat): 1543, 1495, 1435, 1348, 1318, 1225, 1202, 1105, 1041, 940, 884, 786 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 4.18 (s, 6 H, 2 O*CH*$_3$), 7.44–7.54 (m, 5 H, Ar*H*), 7.87 (d, *J* = 8.7 Hz, 2 H, Ar*H*), 8.07 (d, *J* = 8.5 Hz, 2 H, Ar*H*), 8.62 (s, 1 H, Ar*H*). ^{13}C NMR (100 MHz, $CDCl_3$): 55.8 (2 CH_3), 112.0 (CH), 122.0 (C), 125.4 (2 CH), 125.5 (2 CH), 126.8 (2 CH), 128.6 (2 CH), 129.4 (CH), 130.6 (2 C), 131.2 (2 C), 161.8 (C), 165.4 (C), 166.1 (C), 173.1 (2 C). Anal. calcd. for $C_{22}H_{16}N_4O_3$: C, 68.74; H, 4.20; N, 14.58. Found: C, 69.01; H, 4.52 N, 14.90%.

Ethyl 3-(2,5-dimethoxyphenyl)isoxazole-5-carboxylate (8)

The general procedure was followed using ethyl propiolate (1.0 ml, 3.0 mmol, 1 equiv), 2,5-dimethoxybenzaldoxime **5e** (0.6 g, 3.32 mmol, 1.1 equiv), *N*-chlorosuccinimide (0.48 g, 2.18 mmol, 1.2 equiv), $KHCO_3$ (1.5 g, 15.06 mmol, 5 equiv), and ethyl acetate (30 ml). Yield: 0.30 g (33%); beige solid, mp (EtOH) 55–56°C; R_f = 0.52 (EtOAc/*n*-hexane, 1:1). IR (neat): 1730, 1562, 1575, 1503, 1470, 1417, 1377, 1301, 1260, 1119, 1048, 964, 882, 768 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 1.44 (t, *J* = 7.5 Hz, 3 H, C*H*$_3$), 3.83 (s, 3 H, O*CH*$_3$), 3.88 (s, 3 H, O*CH*$_3$), 4.46 (q, 2 H, C*H*$_2$), 6.95 (d, *J* = 9.0 Hz, 1 H, Ar*H*), 7.00 (dd, *J* = 9.0, 3.0 Hz, 1 H, Ar*H*), 7.45 (s, 1 H, Ar*H*), 7.51 (d, *J* = 3.0 Hz, 1 H, Ar*H*). ^{13}C NMR (125 MHz, $CDCl_3$): 14.3 (CH_3), 56.0 (CH_3), 56.3 (CH_3), 62.3 (CH_2), 111.2 (CH), 113.1 (CH), 113.6 (CH), 117.4 (C), 118.1 (CH), 151.7 (C), 153.5 (C), 157.3 (C), 160.1 (C), 160.6 (C). Anal. calcd. for $C_{14}H_{15}NO_5$: C, 60.64; H, 5.45; N, 5.05. Found: C, 60.99; H, 5.73; N, 5.82%.

4.1.3 | General procedure for the synthesis of pyrrolidin-dimethoxytriazines 4a and 4b (Scheme 3)

A mixture of carboxylic acid (1 equiv) and thionyl chloride (1.5 equiv) in dichloromethane was refluxed, under inert atmosphere, for 2–34 hr. The pale yellow solution was concentrated in vacuo to give the crude acid chloride as a yellow pale solid in a quantitative yield and used without further purification.

Under inert atmosphere, a solution of acid chloride (1 equiv) in anhydrous CH_2Cl_2 was added dropwise (30 min) to a stirred mixture of zinc salt (0.7 equiv) and powdered 4 Å molecular sieves in distilled pyridine. After the addition was complete, the mixture was stirred at rt for 12 hr. The mixture was filtered upon completion of the reaction, the solid was washed with CH_2Cl_2 and the filtrate was concentrated in vacuo. The residue was coevaporated with toluene (3 × 5 ml) to remove pyridine. The remaining slurry was dissolved in

CH_2Cl_2 (10 ml), washed with 1 N HCl (3 × 10 ml), and then with aqueous saturated $NaHCO_3$ solution (10 ml). The residue obtained upon evaporation was crystallized or purified by column chromatography, then recrystallized from appropriate solvent to give pure dimethoxytriazine **4a** or **4b** (Scheme 3).

5-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-1-(4-methoxyphenyl)pyrrolidin-2-one (4a)

The general procedure was followed using carboxylic acid **12** (1.5 g, 6.4 mmol), thionyl chloride (0.7 ml) in 10 ml dichloromethane, then zinc derivative (1.4 g, 4.4 mmol), pyridine (20 ml), and dichloromethane (14 ml) in presence of powdered 4 Å molecular sieves (5.0 g). The residue obtained upon evaporation was purified by column chromatography (EtOAc/*n*-heptane 8:2) to provide pure triazine **4a**. Yield: 1.42 g (67%); white solid; mp (EtOAc/*n*-heptane) 141–142°C; R_f (DCM/MeOH 95:5) = 0.36. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.04–2.20 (m, 1 H, CH_2CH_2CH), 2.48–2.96 (m, 3 H, CH_2CH_2CH), 3.76 (s, 3 H, OCH_3), 4.00 (s, 6 H, $2OCH_3$), 5.13 (dd, *J* = 7.8, 2.8 Hz, 1 H, CH_2CH_2CH), 6.82 (d, *J* = 9.0 Hz, 2 H, Ar*H*), 7.36 (d, *J* = 9.0 Hz, 2 H, Ar*H*). Anal. calcd. for $C_{16}H_{18}N_4O_4$: C, 58.17; H, 5.49; N, 16.96. Found: C, 58.57; H, 5.50; N, 16.46.

5-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-1-[3-(trifluoromethyl)phenyl]pyrrolidin-2-one (4b)

The general procedure was followed using carboxylic acid **13** (0.3 g, 1.1 mmol), thionyl chloride (0.1 ml) in 8 ml dichloromethane, then zinc derivative (0.2 g, 0.6 mmol), pyridine (8 ml), and dichloromethane (9.5 ml) in presence of powdered 4 Å molecular sieves (1.0 g). The residue obtained upon evaporation was purified by column chromatography (EtOAc/*n*-heptane 6:4) to provide pure triazine **4b**. Yield: 0.31 g (78%); white solid; R_f (EtOAc/*n*-heptane 75:25) = 0.43. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.09–2.28 (m, 1 H, CH_2CH_2CH), 2.55–2.98 (m, 3 H, CH_2CH_2CH), 3.99 (s, 6 H, $2OCH_3$), 5.19–5.26 (m, 1 H, CH_2CH_2CH), 7.39 (dd, *J* = 15.3, 8.0 Hz, 2 H, Ar*H*), 7.64 (d, *J* = 8.0 Hz, 1 H, Ar*H*), 7.92 (s, 1 H, Ar*H*). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 25.0 (CH_2), 31.0 (CH_2), 55.5 ($2CH_3$), 64.2 (CH), 116.4 (C), 118.4 (q, *J* = 7.8, 3.9 Hz, CH), 121.5 (q, *J* = 7.0, 3.1 Hz, CH), 124.4 (t, *J* = 1.6 Hz, CH), 129.4 (CH), 139.0 (C), 150.3 (d, *J* = 5.5 Hz, C), 172.9 (C), 174.9 (2 C), 181.4 (C). ^{19}F ($CDCl_3$, 376 MHz): δ = −62.7 (s, 3 F, ArCF_3). Anal. calcd. for $C_{16}H_{15}N_4O_3F_3$: C, 52.18; H, 4.11; N, 15.21. Found: C, 52.57; H, 4.41; N, 14.96.

4.1.4 | General procedure for the synthesis of thiolactams 14a and 14b (Scheme 3)

A mixture of lactam **4a** or **4b** (1 equiv), phosphorus pentasulfide (0.33 equiv) and HMDSO (2.0 equiv) in toluene was stirred at reflux for 3–5 hr. The volatiles were removed in vacuo. The crude was dissolved in dichloromethane, washed with water and dried on $MgSO_4$. Volatiles were removed in vacuo and the crude product was purified by flash chromatography on silica with EtOAc/*n*-heptane 3:7 to give the pure thiolactam **14a** or **14b** as yellow solid.

5-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-1-(4-methoxyphenyl)pyrrolidin-2-thione (14a)

The general procedure was followed using pyrrolidinone **4a** (0.2 g, 0.45 mmol), phosphorus pentasulfide (0.03 g, 0.14 mmol), HMDSO (0.2 ml, 0.9 mmol) in toluene (15 ml). The mixture was stirred at reflux for 3 hr. Yield: 0.19 g (91%); yellow solid; mp (EtOAc/*n*-heptane) 169–170°C; R_f (DCM/MeOH 95:5) = 0.70. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.05–2.19 (m, 1 H, CH_2CH_2CH), 2.57–2.89 (m, 3 H, CH_2CH_2CH), 3.79 (s, 3 H, OCH_3), 4.01 (s, 6 H, $2OCH_3$), 5.33 (m, 1 H, CH_2CH_2CH), 6.94 (d, *J* = 8.1 Hz, 2 H, Ar*H*), 7.41 (d, *J* = 8.1 Hz, 2 H, Ar*H*). Anal. calcd. for $C_{16}H_{18}N_4O_3S$: C, 55.48; H, 5.24; N, 16.17. Found: C, 55.91; H, 5.37; N, 16.60.

5-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-1-[3-(trifluoromethyl)phenyl]pyrrolidine-2-thione (14b)

The general procedure was followed using pyrrolidinone **4b** (0.2 g, 0.54 mmol), phosphorus pentasulfide (0.04 g, 0.16 mmol), HMDSO (0.23 ml, 1.1 mmol) in toluene (10 ml). The mixture was stirred at reflux for 5 hr. Yield: 0.18 g (89%); yellow solid; R_f (DCM/MeOH 95:5) = 0.72. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.14–2.34 (m, 1 H, CH_2CH_2CH), 2.51–2.82 (m, 1 H, CH_2CH_2CH), 3.12–3.46 (m, 2 H, CH_2CH_2CH), 3.94 (s, 6 H, $2OCH_3$), 5.32 (ddd, *J* = 9.0, 4.0, 1.6 Hz, 1 H, CH_2CH_2CH), 7.46 (t, *J* = 5.9 Hz, 1 H, Ar*H*), 7.54 (d, *J* = 6.2 Hz, 1 H, Ar*H*), 7.57 (d, *J* = 7.2 Hz, 1 H, Ar*H*), 7.70 (s, 1 H, Ar*H*). ^{19}F (DMSO-d_6, 376 MHz): δ = −62.18 (s, 3 F, ArCF_3). Anal. calcd. for $C_{16}H_{15}N_4O_2SF_3$: C, 50.00; H, 3.93; N, 14.58. Found: C, 49.77; H, 4.09; N, 14.18.

4.2 | Biological assays

4.2.1 | Human FTase studies

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 fluorometer from Perkin–Elmer (Waltham, MA). 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 of 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% (wt/vol) octyl-β-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 (Synergy Software, PA).[33]

4.2.2 | Live-cell imaging assays

CHO cells were plated into 35-mm culture plates 24 hr before transfection with a plasmid pEGFP-CAAX (Addgene) by using Amaxa

SF Cell Line 4D-Nucleofector X Kit L (Lonza). Four hours after transfection, fresh media were added along with vehicle or drug at the appropriate concentrations as stated in the figure legends. At the end of treatment, confocal live-cell imaging on a LSM-710 microscope (Carl Zeiss, Inc., Thornwood, NY) at ×40 magnification was performed.

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