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Synthesis and biological evaluation of a new class of triazin–triazoles as potential inhibitors of human farnesyltransferase

Liliana Lucescu[1] · Elena Bîcu[1] · Dalila Belei[1] · Sergiu Shova[2] · Benoît Rigo[3,4] · Philippe Gautret[3,4] · Joëlle Dubois[5] · Alina Ghinet[1,3,4]

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Abstract A new synthesis of ethynyldimethoxytriazine **1**, an important platform-compound for developing new chemical entities for anticancer research and for other biological applications, is described. Compound **1** was further reacted with azides **5a–i** to provide triazin–triazoles **2a–i**, which were tested on human farnesyltransferase and on the NCI-60 human tumor cell lines. Synthesis of other dimethoxytriazine derivatives **15** and **16**, linked to a sp^2 or a sp^3 carbon atom were also studied.

Electronic supplementary material The online version of this article (doi:10.1007/s11164-015-2131-1) contains supplementary material, which is available to authorized users.

✉ Alina Ghinet
alina.ghinet@hei.fr

[1] Department of Organic Chemistry, Faculty of Chemistry, 'Al. I. Cuza' University of Iasi, B-dul Carol I, Nr. 11, Corp A, 700506 Iasi, Romania

[2] 'Petru Poni' Institute of Macromolecular Chemistry, 41A Grigore Ghica Voda Alley, 700487 Iasi, Romania

[3] Inserm, U995-LIRIC, Faculté de Médecine - Pôle Recherche, Université Lille 2, CHRU de Lille, Place Verdun, 59045 Lille Cedex, France

[4] UCLille, Laboratoire de Pharmacochimie, Hautes Etudes d'Ingénieur (HEI), 13 rue de Toul, 59046 Lille, France

[5] Centre de Recherche de Gif, Institut de Chimie des Substances Naturelles, UPR2301 CNRS, Avenue de la Terrasse, 91198 Gif-sur-Yvette Cedex, France

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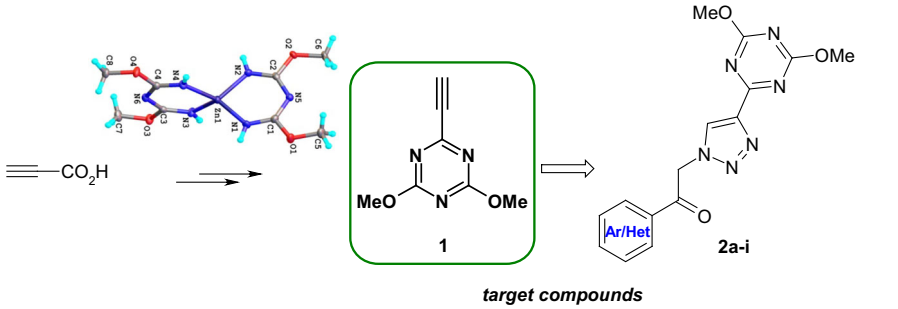
Graphical Abstract



Keywords Dimethoxytriazine · Triazole · Triazin–triazole · Farnesyltransferase inhibitor · Ethynyldimethoxytriazine

Introduction

Many *s*-triazine derivatives have been synthesized, particularly for their considerable therapeutic potential [1–13] and their antifungal [14–20] or herbicidal [21–23] properties. In this context, very few dimethoxytriazines have been described [24–34], and some of their 2-(alk-1′-ynyl) derivatives such as **I–III** [35] present interesting cytotoxic activities toward different tumor cell lines (Fig. 1). The method utilized for the synthesis of these acetylenic compounds is the palladium catalyzed cross-coupling between alk-1-ynes and 2-chloro-4,6-dimethoxy-1,3,5-triazine [36–38]. As for their 2-alkyl analogs, they were often obtained by reacting zinc bis(imino-bismethyl carbamate) **3** with acid chloride or anhydride [25, 26, 33, 34], and we have reported that 4 Å molecular sieves and pyridine can be advantageously utilized in this reaction [39, 40]. The molecular structure of zinc salt was initially described as **3a** [24], but we recently proved from an RX study (see 'Supporting information' section) that the real structure was **3b**,[1] as suggested in a patent [25, 26], but not confirmed (Fig. 2).

The farnesylated proteins play an important role in cell cycle progression [41]. Ras and Rho proteins are involved in the regulation of cyclin and CDK (cyclin-dependent kinase) inhibitors. In addition, studies have shown that Rheb was a protein involved in the cell cycle that also affected the Ras signaling pathway. Therefore, it is necessary to design new farnesyltransferase inhibitors (FTIs) that specifically inhibit the farnesylation of target proteins involved in cell proliferation. In this field, our team already developed new families of human farnesyltransferase (FTase) inhibitors [42–45]. FTase is a heterodimeric metalloenzyme with two subunits: α (48 kDa) and β (45 kDa) [46]. To be activated, farnesyltransferase needs

[1] CCDC-1045377 (for zinc derivative **3b**) and CCDC-1045376 (for phenothiazine derivative **16**) contain the crystallographic data for this contribution. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

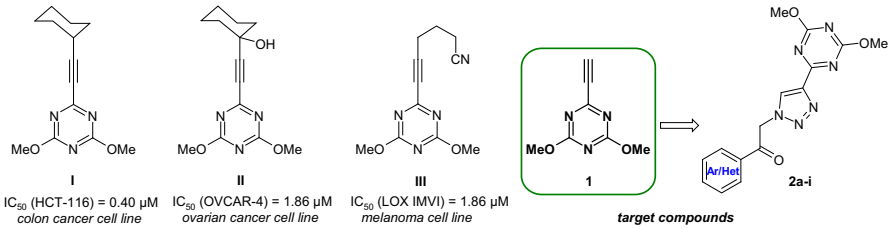


Fig. 1 Structures of some biologically active dimethoxytriazines **I–III** [35] and target compounds **1** and **2a–i**

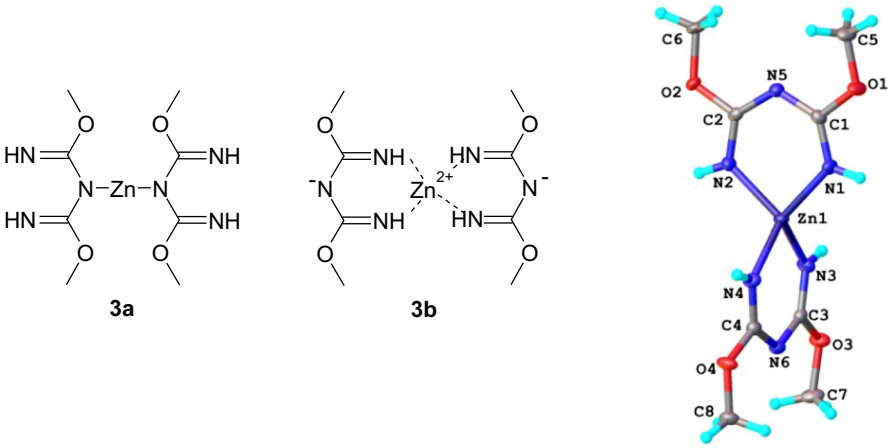


Fig. 2 Structure of zinc salt **3**: initially described structure **3a** and X-ray proved structure **3b**

a zinc and a magnesium atom. In order to block the activity of farnesyltransferase, we became interested in the design and the synthesis of FTIs bearing chelating units. Thus, after the successful investigation of compounds bearing complexing groups (CN, CO_2R) that bind the zinc atom of protein farnesyltransferase [42], we describe here the study of compounds with a triazin–triazole unit as a potential zinc chelating moiety.

Experimental

Materials and methods

Starting materials are commercially available. Melting points were measured on an MPA 100 OptiMelt® apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were acquired at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR on a Varian 400-MR spectrometer. Chemical shifts (δ) are given in parts per million

relative to $CDCl_3$ (7.26; 77.1 ppm), DMSO-d_6 (2.47; 40.0 ppm) or TMS. Splitting patterns are designed: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; m, multiplet, and sym m, symmetric multiplet. Coupling constants J are reported in Hertz (Hz). Thin layer chromatographies (TLC) were realized on Macherey–Nagel silica gel plates with fluorescent indicator and were visualized under a UV-lamp at 254 and 366 nm. Column chromatographies were performed with a CombiFlash Rf Companion (Teledyne-Isco System) using RediSep packed columns. IR spectra were recorded on a Varian 640-IR FT-IR Spectrometer. Elemental analyses (C, H, N) of new compounds were determined on a Thermo Electron apparatus by 'Pôle Chimie Moléculaire', Faculté de Sciences Mirande, Université de Bourgogne, Dijon, France.

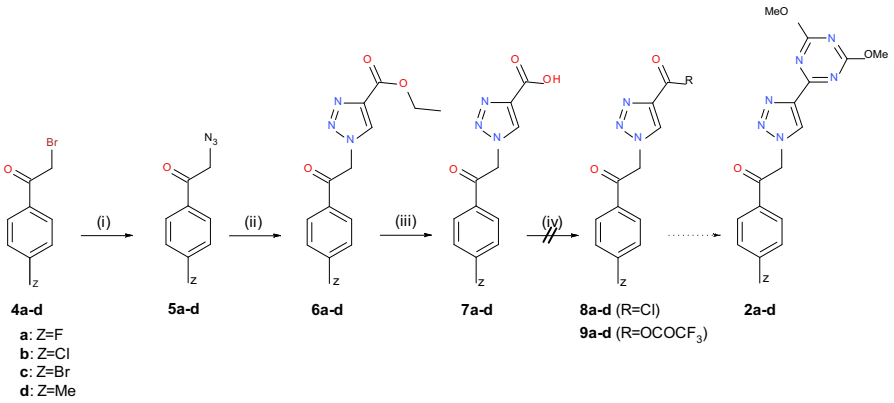
Chemistry

We were recently involved in the synthesis of protein FTIs [43–45], and we decided to synthesize products **2a–i** (Fig. 1), in order to investigate the possibility for their dimethoxytriazine linked to the triazole heterocycle to bind to the zinc atom of this protein. However, we did not succeed in the activation of acids **7a–d** [47, 48],[2] whatever the conditions tested ($SOCl_2$/DMF [49], oxalyl chloride/CH_2Cl_2/DMF [50], TFAA/TFA [51]) (Scheme 1; Table 1). Thus, we considered obtaining the target compounds **2a–i** starting from acetylenic derivative **1** (Scheme 2). The synthetic pathway started from propiolic acid **10**, which after activation as acid chloride, then treatment with salt **3b**, furnished the target acetylenic derivative. The instability and hazardous potential of the propioloyl chloride is described in the literature [52]. Moreover, spontaneous inflammation sometimes occurring during the isolation of propiolic acid chloride, was attributed to trace amounts of monochloroacetylene formed during distillation [52, 53]. For this reason, we did not try to isolate the pure propioloyl chloride **11** and we used it further without any purification.

The reaction of 90 % pure propioloyl chloride **11** containing 10 % of addition by-products **12** and **13**, with salt **3b** in our general conditions [39, 40] was performed, leading to 55 % of acetylenic product **1** and 30 % of 2-[(*E*)-2-chlorovinyl]-4,6-dimethoxy-1,3,5-triazine **14** (Scheme 2). Compound **1** was reacted with azides **5a–i** to provide the target compounds **2a–i** in good yields (Scheme 2; Table 2).

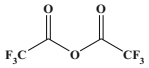
Because of the formation of secondary products (**12–14**) occurring during the synthesis of propioloyl chloride **11** and the reaction with salt **3b** in our general conditions [39, 40] (Scheme 2), condensation of hydroxysuccinyl esters **20** and **22** with precursor **3b** was studied. While starting from acids **10**, **19** and **21**, pyridinyl or phenothiazinyl derivatives **15**, and **16** were easily formed in medium to good yields, and activation of propiolic acid **10** (CDI and *N*-hydroxysuccinimide) led only to the addition of imidazole to the triple bound, providing compound **17** (Scheme 3); this reaction is reminiscent of the formation of (*Z*) and (*E*)-3-chloroacryloyl chlorides **12**

[2] Acids **7a–d** were obtained by saponification of the corresponding known esters.



Scheme 1 Initial envisaged chemical strategy for the isolation of triazin–triazoles **2a**–**d**. *Reagents and conditions*: *i* NaN_3 (1.1–1.2 equiv), TBAB (0.11–0.15 equiv), $CHCl_3$, H_2O, room temperature (RT), 24 h, 58–90 % yield; *ii* ethyl propiolate (1 equiv), $CuSO_4{\cdot}5H_2O$ (0.1 equiv), sodium ascorbate (0.3 equiv), *t*-BuOH, MeCN, H_2O, RT, 24 h, 33–71 % yield; *iii* 30 % KOH aq sln, EtOH, reflux, 15 min, 84–97 % yield; *iv* conditions described below in Table 1

Table 1 Tested conditions for the activation of carboxylic acids **7a**–**d**

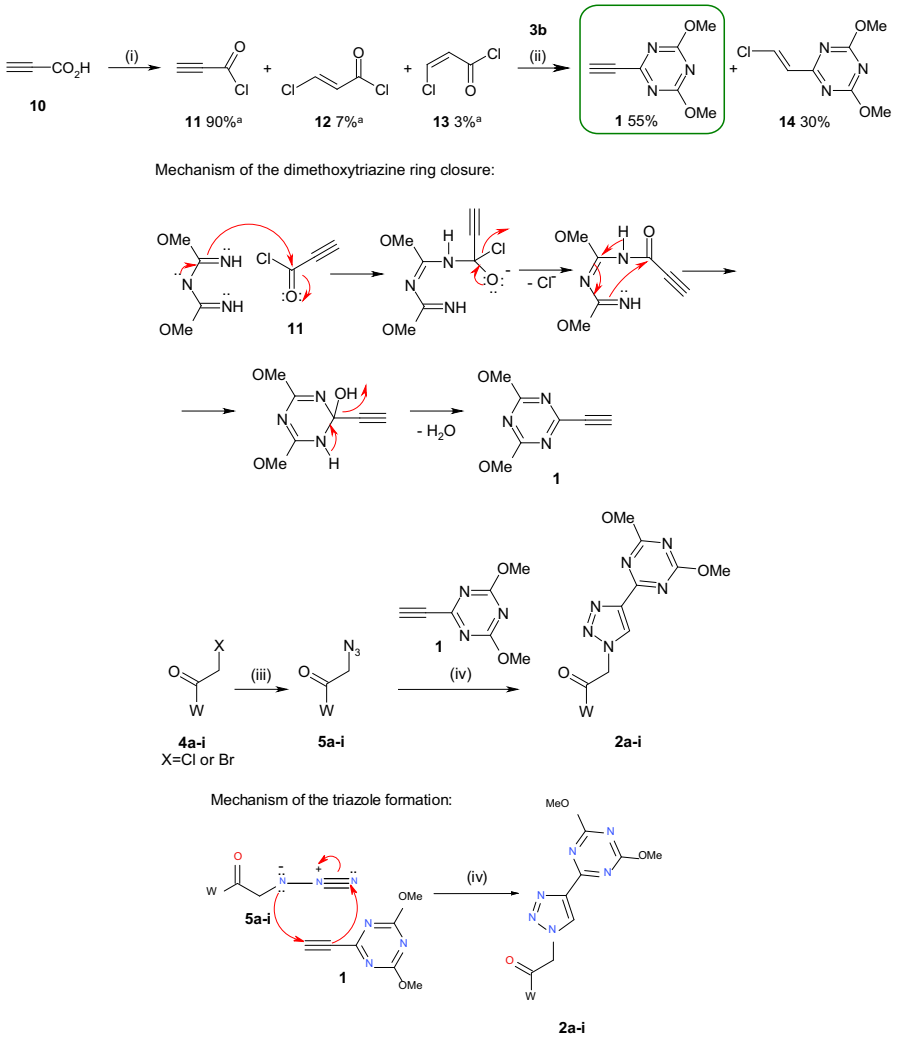
Entry	Reagent (no. equiv.)	Solvent	Time (h)	Temperature	Yield[a] (%)
1	$SOCl_2$ (2)	DMF	4	reflux	0
2	 (2)	CH_2Cl_2/DMF	2	reflux	0
3	$SOCl_2$ (10)	-	2	reflux	0
4	$SOCl_2$ (36)	DMF	2	reflux	0
5	 (4)	F_3C-COOH	26	reflux	0

Entries 1 and 5: Conditions described in reference [39] for the activation of a carboxylic acid group. Entry 2: Conditions described in reference [50]. Entry 3: Conditions described in reference [49]. Entry 4: Conditions described in reference [51]

[a] Tested conditions resulted in formation of insoluble material

and **13** from the addition of chloride ion to propioloyl chloride **11** (Scheme 2), and the addition of the same ion to acetylenic compound **1** as described in Scheme 2.

In order to attest the withdrawing effect of the dimethoxytriazine unit, acetylenic derivative **1** was reacted with diethylamine in dichloromethane at RT and provided enamine **18** in a good 70 % yield (Scheme 3).

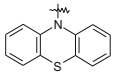
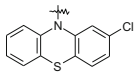


Scheme 2 Synthesis and formation mechanism of 2-ethynyl-4,6-dimethoxy-1,3,5-triazine **1** and of target triazin–triazoles **2a–i.** [a] ^{1}H NMR yields. *Reagents and conditions*: *i* PCl_5 (1 equiv), CH_2Cl_2, RT; *ii* zinc dimethylimidodicarbonimidate **3b** (0.7 equiv), powdered 4 Å molecular sieves, RT, 24 h; *iii* NaN_3 (1.1–1.2 equiv), TBAB (0.11–0.15 equiv), $CHCl_3$, H_2O, RT, 24 h, 58–90 % yield; *iv* $CuSO_4 5H_2O$ (0.1 equiv), sodium ascorbate (0.2–0.3 equiv), *t*-BuOH, MeCN, H_2O, RT, 24 h, 26–83 % yield

The crystal structure of triazine **16** is shown in Fig. 3. It comprises two crystallographic independent but chemically identical molecules, denoted as **A** and **B** (See 'Supporting information' section for full crystallographic data).

In both **A** and **B** asymmetric molecules, the phenothiazine units feature a butterfly structure with a boat conformation for the central six-membered ring and *syn–anti conformation* for the two dimethoxy-1,3,5-triazine fragments. The folding dihedral angle between the benzene rings of the phenothiazine system is 140.65(6)°

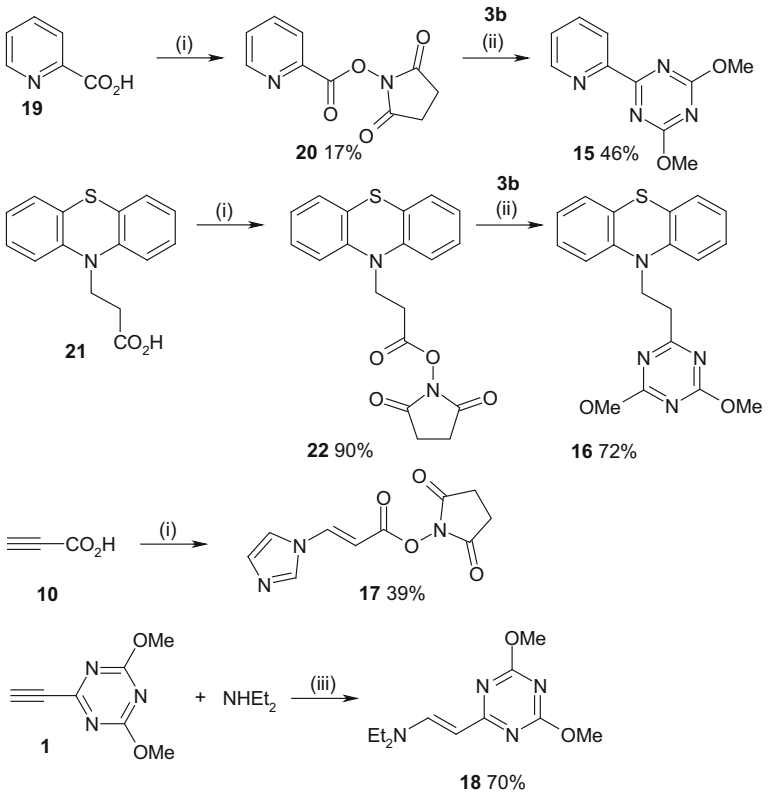
Table 2 Structure and isolated yields of triazin–triazoles **2a–i**

Compound	W	Yield	Compound	W	Yield
2a		52%	**2f**		79%
2b		65%	**2g**		61%
2c		58%	**2h**		83%
2d		38%	**2i**		26%
2e		43%			

and 140.83(5)° for the two crystallographic independent molecules **A** and **B**, respectively. In the crystal packing, two asymmetric molecules are self-assembled into one-dimensional supramolecular double-chains, which are mediated by C–H···O, C–H···N hydrogen bonding and π–π stacking interactions. The perspective view of the supramolecular architecture, along with the geometric parameters of the H-bonds, is depicted in Figure S4. Two triazine rings of adjacent A and B asymmetric units are stacked with *Cg*1···*Cg*2 centroid-to-centroid distance of 3.3646(1) Å and a perpendicular distance of 3.2756(9) Å. Another significant π–π stacking interaction are observed between *Cg*3 (the centroid of C1B–C6B ring) and *Cg*4 (the centroid of C1A–C6A ring) with *Cg*3···*Cg*4 distance of 3.9108(1) Å and a perpendicular distance of 3.2836(2) Å. The main crystal structure motif can be characterized as the parallel packing of the above-mentioned supramolecular aggregates with no more intermolecular contacts significantly below the sum of the van der Waals radii for the corresponding atoms.

*2-Ethynyl-4,6-dimethoxy-1,3,5-triazine (**1**)*

Under inert atmosphere, a solution of propioloyl chloride **11** (6.28 g, 70.9 mmol) in anhydrous CH_2Cl_2 (35 mL) was added dropwise over 30 min to a stirred mixture of zinc dimethyl imidodicarbonimidate (16.47 g, 49.6 mmol) and powdered 4 Å molecular sieves in anhydrous CH_2Cl_2 (120 mL). After the complete addition, the mixture was stirred at room temperature for 24 h. The mixture was then filtered, the solid washed with CH_2Cl_2 and the filtrate washed with H_2O and $NaHCO_3$ saturated



Scheme 3 Synthesis of dimethoxytriazines **15** and **16**, of unexpected addition product **17** and of addition product **18**. *Reagents and conditions*: *i N*-hydroxysuccinimide (1.2 equiv), carbonyldiimidazole (CDI) for **15** and **17** or 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) (1.2 equiv) for **16**, CH_2Cl_2, RT, 24 h; *ii* zinc dimethylimidodicarbonimidate **3b** (0.7 equiv), powdered 4 Å molecular sieves, CH_2Cl_2, RT, 24 h; *iii* CH_2Cl_2, RT, 15 h

solution, until complete neutralization of resulting HCl. Following drying (Na_2SO_4) then evaporation in vacuo, the obtained solid was recrystallized from absolute ethanol to give pure 2-ethynyl-4,6-dimethoxy-1,3,5-triazine **1** as a beige solid in 55 % yield (6.43 g); mp (EtOH) 160–162 °C; $Rf = 0.55$ (EtOAc:*n*-heptane, 1:1). IR ν cm^{-1}: 3163, 2116, 1537, 1464, 1403, 1346, 1231, 1128. ^1H NMR ($CDCl_3$, 400 MHz): δ (ppm) 3.22 (s, 1H, C*H*), 4.06 (s, 6H, 2OCH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 55.7 (2CH_3), 78.8 (CH), 80.2 (C), 161.8 (C), 172.4 (2C). Elemental analysis calcd (%) for $C_7H_7N_3O_2$: C 50.91, H 4.27, N 25.44; found: C 51.26, H 3.97, N 25.71.

*2-((E)-2-Chlorovinyl)-4,6-dimethoxy-1,3,5-triazine (**14**)* By-product from the synthesis of triazine **1** isolated from the ethanol residue of the recrystallization of compound **1**. Beige solid; 30 % yield (4.28 g); mp (EtOH) 88–90 °C; $Rf = 0.7$ (EtOAc:*n*-heptane, 1:1). IR ν cm^{-1}: 3085, 2941, 1544, 1496, 1465, 1352, 1236,

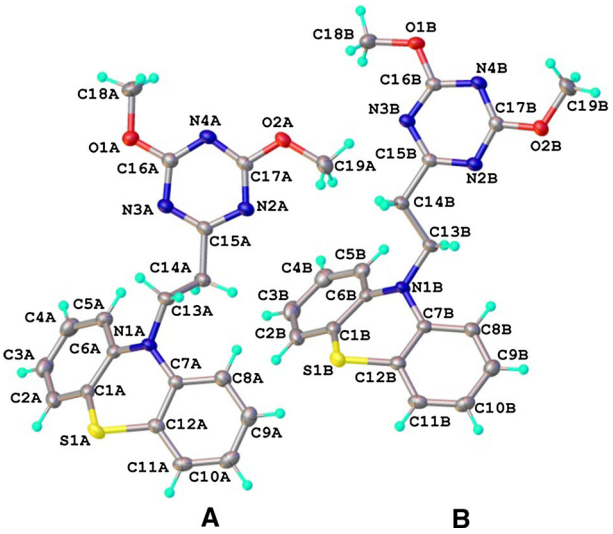


Fig. 3 View of the asymmetric unit in the crystal structure **16**. Thermal ellipsoids are drawn at 50 % probability level

1196. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 4.05 (s, 6H, 2OCH_3), 6.73 (d, J = 13.4 Hz, 1H, C*H*), 7.78 (d, J = 13.4 Hz, 1H, C*H*). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 55.3 (2CH_3), 131.1 (CH), 135.4 (CH), 172.5 (2C), 172.6 (C). Elemental analysis calcd (%) for $C_7H_8ClN_3O_2$: C 41.70, H 4.00, N 20.84; found: C 41.54, H 4.31, N 21.13.

*General procedure for the synthesis of triazinyl–triazoles (**2a–i**)*

A mixture of 2-ethynyl-4,6-dimethoxy-1,3,5-triazine (**1**) (1 equiv) and appropriate azide **5** was dissolved (1 equiv) in *t*-BuOH:MeCN, 10:4. Sodium ascorbate (0.3 equiv), and water and hydrated copper (II) sulfate (0.1 equiv) were then added to the reaction mixture. The resulting medium was stirred at room temperature for 24 h. The obtained solid was filtered and washed with water and ammonium hydroxide, and then with ethanol to provide pure triazinyl–triazoles (**2a–i**).

*1-(4-Fluorophenyl)-2-(4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-1H-1,2,3-triazol-1-yl)ethanone (**2a**)* The general procedure was followed using 2-ethynyl-4,6-dimethoxy-1,3,5-triazine **1** (0.19 g, 1.12 mmol), 2-azido-1-(4-fluorophenyl)ethanone **5a** (0.2 g, 1.12 mmol), $CuSO_4{\cdot}5H_2O$ (0.03 g, 0.1 mmol), sodium ascorbate (0.07 g, 0.3 mmol), *t*-BuOH (10 mL), MeCN (4 mL) and H_2O (4 mL) to provide triazole **2a** as a white solid in 52 % yield (0.2 g); mp (EtOH) 200–203 °C; Rf = 0.05 (EtOAc:*n*-hexane, 1:1). IR ν cm^{-1}: 1702, 1536, 1500, 1391, 1347, 1227, 1202, 1162. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 4.04 (s, 6H, 2OCH_3), 6.32 (s, 2H, CH_2), 7.47 (t, J = 8.4 Hz, 2H, Ar*H*), 8.19 (m, 2H, Ar*H*), 8.88 (s, 1H, Ar*H*). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 55.0 (2CH_3), 56.1 (CH_2), 116.1 (d, J = 88.0 Hz, 2CH), 116.2 (CH), 130.3 (CH), 130.7 (d, J = 12.0 Hz, C), 131.3 (d, J = 36.0 Hz,

2CH), 131.3 (CH), 164.3 (C), 166.8 (C), 168.4 (C), 172.4 (2C), 174.4 (C), 190.4 (C). Elemental analysis calcd (%) for $C_{15}H_{13}FN_6O_3$: C 52.33, H 3.81, N 24.41; found: C 52.60, H 3.62, N 24.67.

*1-(4-Chlorophenyl)-2-(4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-1H-1,2,3-triazol-1-yl)ethanone (**2b**)* The general procedure was followed using 2-ethynyl-4,6-dimethoxy-1,3,5-triazine **1** (0.16 g, 1 mmol), 2-azido-1-(4-clorophenyl)ethanone **5b** (0.2 g, 1 mmol), $CuSO_4 \cdot 5H_2O$ (0.02 g, 0.1 mmol), sodium ascorbate (0.06 g, 0.3 mmol), *t*-BuOH (10 mL), MeCN (4 mL) and H_2O (4 mL) to provide triazole **2b** as a beige solid in 65 % yield (0.21 g); mp (EtOH) 189–192 °C; *Rf* = 0.06 (EtOAc:*n*-hexane, 1:1). IR *v* cm^{-1}: 1705, 1536, 1500, 1387, 1360, 1225, 1199, 1086. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 4.04 (s, 6H, 2OCH_3), 6.32 (s, 2H, CH_2), 7.71 (d, J = 9.2 Hz, 2H, Ar*H*), 8.11 (d, J = 9.2 Hz, 2H, Ar*H*), 8.88 (s, 1H, Ar*H*). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 55.0 (2CH_3), 56.2 (CH_2), 129.1 (2CH), 130.1 (2CH), 130.3 (CH), 132.7 (C), 139.2 (C), 144.3 (C), 168.4 (C), 172.4 (2C), 190.9 (C). Elemental analysis calcd (%) for $C_{15}H_{13}ClN_6O_3$: C 49.94, H 3.63, N 23.30; found: C 50.32, H 3.90, N 23.53.

*1-(4-Bromophenyl)-2-(4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-1H-1,2,3-triazol-1-yl)ethanone (**2c**)* The general procedure was followed using 2-ethynyl-4,6-dimethoxy-1,3,5-triazine **1** (0.16 g, 1 mmol), 2-azido-1-(4-brorophenyl)ethanone **5c** (0.24 g, 1 mmol), $CuSO_4 \cdot 5H_2O$ (0.02 g, 0.1 mmol), sodium ascorbate (0.06 g, 0.3 mmol), *t*-BuOH (10 mL), MeCN (4 mL) and H_2O (4 mL) to provide triazole **2c** as a beige solid in 58 % yield (0.22 g); mp (EtOH) 176–180 °C; *Rf* = 0.06 (EtOAc:*n*-hexane, 1:1). IR *v* cm^{-1}: 1705, 1534, 1498, 1387, 1358, 1284, 1249, 1222, 1198. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 4.03 (s, 6H, 2OCH_3), 6.31 (s, 2H, CH_2), 7.85 (d, J = 9.2 Hz, 2H, Ar*H*), 8.30 (d, J = 9.2 Hz, 2H, Ar*H*), 8.88 (s, 1H, Ar*H*). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 55.8 (2CH_3), 56.8 (CH_2), 129.1 (C), 130.8 (2CH), 130.9 (CH), 132.7 (2CH), 133.7 (C), 144.9 (C), 169.1 (C), 173.0 (2C), 191.8 (C). Elemental analysis calcd (%) for $C_{15}H_{13}BrN_6O_3$: C 44.46, H 3.23, N 20.74; found: C 44.72, H 3.45, N 20.91.

*2-(4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-1H-1,2,3-triazol-1-yl)-1-p-tolylethanone (**2d**)* The general procedure was followed using 2-ethynyl-4,6-dimethoxy-1,3,5-triazine **1** (0.24 g, 1.42 mmol), 2-azido-1-*p*-tolylethanone **5d** (0.25 g, 1.42 mmol), $CuSO_4 \cdot 5H_2O$ (0.035 g, 0.14 mmol), sodium ascorbate (0.08 g, 0.43 mmol), *t*-BuOH (10 mL), MeCN (4 mL) and H_2O (4 mL) to provide triazole **2d** as a beige solid in 38 % yield (0.18 g); mp (EtOH) 148–151 °C; *Rf* = 0.07 (EtOAc:*n*-hexane, 1:1). IR *v* cm^{-1}: 1696, 1580, 1542, 1496, 1461, 1391, 1344, 1287, 1253, 1225, 1182, 1105. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 2.43 (s, 3H, CH_3), 4.03 (s, 6H, 2OCH_3), 6.36 (s, 2H, CH_2), 8.12 (d, J = 8.4 Hz, 2H, Ar*H*), 8.24 (d, J = 8.4 Hz, 2H, Ar*H*), 8.87 (s, 1H, Ar*H*). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 21.3 (CH_3), 55.1 (2CH_3), 56.1 (CH_2), 128.3 (2CH), 129.5 (2CH), 130.3 (CH), 131.5 (C), 144.2 (C), 144.9 (C), 168.5 (C), 172.4 (2C), 191.2 (C). Elemental analysis calcd (%) for $C_{16}H_{16}N_6O_3$: C 56.47, H 4.74, N 24.69; found: C 56.86, H 4.98, N 24.83.

*4-(2-(4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-1H-1,2,3-triazol-1-yl)acetyl)benzonitrile (**2e**)* The general procedure was followed using 2-ethynyl-4,6-dimethoxy-1,3,5-triazine **1** (0.16 g, 1 mmol), 4-(2-azidoacetyl)benzonitrile **5e** (0.2 g, 1 mmol), $CuSO_4{\cdot}5H_2O$ (0.025 g, 0.10 mmol), sodium ascorbate (0.06 g, 0.32 mmol), *t*-BuOH (10 mL), MeCN (4 mL) and H_2O (4 mL) to provide triazole **2e** as a beige solid in 43 % yield (0.16 g); mp (EtOH) 230–231 °C; $Rf = 0.03$ (EtOAc:*n*-hexane, 1:1). IR ν cm^{-1}: 2225, 1706, 1552, 1498, 1382, 1355, 1249, 1227, 1199, 1156. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 4.03 (s, 6H, 2OCH_3), 6.36 (s, 2H, CH_2), 8.12 (d, $J = 8.4$ Hz, 2H, Ar*H*), 8.24 (d, $J = 8.4$ Hz, 2H, Ar*H*), 8.87 (s, 1H, Ar*H*). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 55.6 (2CH_3), 57.0 (CH_2), 116.5 (C), 118.5 (C), 129.3 (2CH), 130.7 (CH), 133.4 (2CH), 137.7 (C), 144.8 (C), 168.9 (C), 172.9 (2C), 191.9 (C). Elemental analysis calcd (%) for $C_{16}H_{13}N_7O_3$: C 54.70, H 3.73, N 27.91; found: C 54.51, H 3.59, N 27.77.

*2-(4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-1H-1,2,3-triazol-1-yl)-1-(3,4,5-trimethoxyphenyl)ethanone (**2f**)* The general procedure was followed using 2-ethynyl-4,6-dimethoxy-1,3,5-triazine **1** (0.29 g, 1.73 mmol), 2-azido-1-(3,4,5-trimethoxyphenyl)ethanone **5f** (0.43 g, 1.73 mmol), $CuSO_4{\cdot}5H_2O$ (0.04 g, 0.17 mmol), sodium ascorbate (0.10 g, 0.52 mmol), *t*-BuOH (10 mL), MeCN (4 mL) and H_2O (4 mL) to provide triazole **2f** as a white solid in 79 % yield (0.56 g); mp (EtOH) 241–243 °C. IR ν cm^{-1}: 2948, 1676, 1545, 1496, 1449, 1411, 1392, 1363, 1240, 1196, 1127. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 3.94 (s, 6H, 2OCH_3), 3.96 (s, 3H, OCH_3), 4.14 (s, 6H, 2OCH_3), 5.92 (s, 2H, CH_2), 7.25 (s, 2H, Ar*H*), 8.87 (s, 1H, Ar*H*). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 29.7 (CH_2), 55.5 (2CH_3), 56.1 (2CH_3), 61.1 (CH_3), 105.8 (2CH), 128.7 (2C), 130.4 (CH), 144.1 (C), 153.5 (2C), 172.9 (2C), 174.5 (C), 188.4 (C). Elemental analysis calcd (%) for $C_{18}H_{20}N_6O_6$: C 51.92, H 4.84, N 20.18; found: C 52.16, H 5.01, N 20.15.

*2-(4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-1H-1,2,3-triazol-1-yl)-1-(10H-phenothiazin-10-yl)ethanone (**2g**)* The general procedure was followed using 2-ethynyl-4,6-dimethoxy-1,3,5-triazine **1** (0.2 g, 1.21 mmol), 2-azido-1-(10*H*-phenothiazin-10-yl)ethanone **5 g** (0.34 g, 1.21 mmol), $CuSO_4{\cdot}5H_2O$ (0.03 g, 0.12 mmol), sodium ascorbate (0.07 g, 0.36 mmol), *t*-BuOH (10 mL), MeCN (4 mL) and H_2O (4 mL) to provide triazole **2 g** as a white solid in 61 % yield (0.31 g); mp (H_2O) 136–140 °C; $Rf = 0.07$ (EtOAc:*n*-hexane, 1:1). IR ν cm^{-1}: 1687, 1545, 1498, 1460, 1353, 1282, 1256, 1180. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 4.10 (s, 6H, 2OCH_3), 5.42 (s, 2H, CH_2), 7.31(t, $J = 7.6$ Hz, 2H, Ar*H*), 7.39 (t, $J = 7.6$ Hz, 2H, Ar*H*), 7.51 (d, $J = 7.6$ Hz, 2H, Ar*H*), 7.60 (d, $J = 7.6$ Hz, 2H, Ar*H*), 8.57 (s, 1H, Ar*H*). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 52.2 (CH_2), 55.7 (2CH_3), 126.7 (2CH), 127.6 (2CH), 128.2 (2CH), 129.7 (2CH), 130.2 (CH), 130.9 (2C), 137.8 (C), 144.6 (2C), 164.2 (C), 169.0 (C), 173.0 (2C). Elemental analysis calcd (%) for $C_{21}H_{17}N_7O_3S$: C 56.37, H 3.83, N 21.91; found: C 56.67, H 3.94, N 22.07.

*1-(2-Chloro-10H-phenothiazin-10-yl)-2-(4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-1H-1,2,3-triazol-1-yl)ethanone (**2h**)* The general procedure was followed using

2-ethynyl-4,6-dimethoxy-1,3,5-triazine **1** (0.1 g, 0.61 mmol), 2-azido-1-(2-chloro-10*H*-phenothiazin-10-yl)ethanone **5 h** (0.2 g, 0.61 mmol), $CuSO_4{\cdot}5H_2O$ (0.015 g, 0.06 mmol), sodium ascorbate (0.035 g, 0.18 mmol), *t*-BuOH (10 mL), MeCN (4 mL) and H_2O (4 mL) to provide triazole **2 h** as a white solid in 83 % yield (0.23 g); white solid; mp (H_2O) 155–158 °C; *Rf* = 0.1 (EtOAc:*n*-hexane, 1:1). IR *v* cm^{-1}: 1689, 1543, 1501, 1458, 1353, 1286, 1249, 1179, 1103. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 4.02 (s, 6H, 2OCH_3), 5.77 (s, 2H, CH_2), 7.39-7.51 (m, 3H, Ar*H*), 7.65 (d, *J* = 7.2 Hz, 2H, Ar*H*), 7.80-7.88 (m, 2H, Ar*H*), 8.82 (s, 1H, Ar*H*). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 52.7 (CH_2), 56.1 ($2CH_3$), 127.9 (CH), 128.6 (CH), 128.9 (2CH), 129.3 (CH), 130.3 (CH), 131.2 (2CH), 133.7 (2C), 139.2 (C), 140.1 (C), 145.1 (2C), 165.7 (C), 169.4 (C), 173.4 (2C). Elemental analysis calcd (%) for $C_{21}H_{16}ClN_7O_3S$: C 52.34, H 3.35, N 20.35; found: C 52.48, H 3.40, N 20.58.

*1-(1H-Indazol-1-yl)-2-(4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-1H-1,2,3-triazol-1-yl)ethanone (**2i**)* The general procedure was followed using 2-ethynyl-4,6-dimethoxy-1,3,5-triazine **1** (0.1 g, 0.61 mmol), 2-azido-1-(2-chloro-10*H*-phenothiazin-10-yl)ethanone **5i** (0.2 g, 0.61 mmol), $CuSO_4{\cdot}5H_2O$ (0.015 g, 0.06 mmol), sodium ascorbate (0.035 g, 0.18 mmol), *t*-BuOH (10 mL), MeCN (4 mL) and H_2O (4 mL) to provide triazole **2i** as a white solid in 26 % yield (0.14 g); white solid; mp (EtOH) 195–199 °C; *Rf* = 0.08 (EtOAc:*n*-hexane, 1:1). IR *v* cm^{-1}: 1708, 1582, 1557, 1468, 1434, 1385, 1344, 1331, 1234, 1173, 1149, 1121. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 4.13 (s, 6H, 2OCH_3), 6.19 (s, 2H, CH_2), 7.43 (t, *J* = 7.6 Hz, 1H, Ar*H*), 7.60 (t, *J* = 7.6 Hz, 1H, Ar*H*), 7.90 (d, *J* = 7.6 Hz, 1H, Ar*H*), 8.25 (s, 1H, Ar*H*), 8.32 (d, *J* = 7.6 Hz, 1H, Ar*H*), 8.64 (s, 1H, Ar*H*). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 52.4 (CH_2), 55.4 ($2CH_3$), 115.0 (CH), 121.4 (CH), 125.5 (CH), 129.1 (CH), 130.3 (CH), 141.7 (CH), 145.7 (2C), 164.5 (2C), 168.8 (2C), 172.8 (C). Elemental analysis calcd (%) for $C_{16}H_{14}N_8O_3$: C 52.46, H 3.85, N 30.59; found: C 52.80, H 4.11, N 30.82.

*Propioloyl chloride (**11**)*

Propiolic acid **10** (5.0 g, 70.9 mmol) was added to a suspension of PCl_5 (14.77 g, 70.9 mmol) in anhydrous CH_2Cl_2 (30 mL), under nitrogen. The obtained solution was stirred at room temperature for 1 h, then concentrated in vacuo and used without further purification to provide the propioloyl chloride **11** in 90 % yield among with compounds **12** and **13**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 3.76 (d, *J* = 13.3 Hz, 1H, C*H*).

*(E)-3-Chloroacryloyl chloride (**12**)* By-product from the synthesis of propioloyl chloride **11**.

7 % NMR yield. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 6.52 (d, *J* = 13.3 Hz, 1H, C*H*), 7.68 (d, *J* = 13.3 Hz, 1H, C*H*).

*(Z)-3-Chloroacryloyl chloride (**13**)* By-product from the synthesis of propioloyl chloride **11**.

3 % NMR yield. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 6.60 (dd, $J = 7.8$, 1.2 Hz, 1H, C*H*), 6.87 (dd, $J = 7.8$, 1.2 Hz, 1H, C*H*).

ω-Bromo or chloroacetophenones **4a–e** were commercially available.

2-Bromo-1-(3,4,5-trimethoxyphenyl)ethanone (**4f**) and ω-chloroacetophenones **4g** and **4h** were prepared as described previously by our team [43, 45].

*1-(Chloroacetyl)-1H-indazole (**4i**)*

To a solution of 1*H*-indazole (1.00 g, 8.47 mmol), and triethylamine (1.41 mL, 10.17 mmol) in toluene (20 mL), chloroacetyl chloride (1.01 mL, 12.71 mmol) was added dropwise. The obtaining mixture was stirred at room temperature for 4 h. The solvent was removed in vacuo and the resulting solid was recrystallized from ethanol to give pure acylated indazole **4i** as a cream solid in 78 % yield (1.28 g) with the same physico-chemical properties as described in the literature [54, 55]; cream solid; mp (EtOH) 75–78 °C; $Rf = 0.61$ (EtOAc:*n*-hexane, 1:1). IR ν cm^{-1}: 1742, 1497, 1386, 1356, 1336, 1270, 1203, 1131, 1063, 953, 897, 777. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 5.01 (s, 2H, CH_2), 7.41 (t, $J = 8.0$ Hz, 1H, Ar*H*), 7.61 (t, $J = 8.0$ Hz, 1H, Ar*H*), 7.76 (d, $J = 8.0$ Hz, 1H, Ar*H*), 8.17 (s, 1H, Ar*H*), 8.44 (d, $J = 8.0$ Hz, 1H, Ar*H*). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 43.1 (CH_2), 115.5 (CH), 121.3 (CH), 125.3 (CH), 126.4 (C), 130.2 (CH), 139.3 (C), 141.1 (CH), 166.0 (C). Elemental analysis calcd (%) for $C_9H_7ClN_2O$: C 55.54, H 3.63, N 14.39; found: C 55.86, H 3.84, N 14.61.

Azides **5a–e**, **5g** and **5h** were prepared as described previously by our team [45, 56–58].

*General procedure for the synthesis of azides **5f** and **5i***

To a solution of ω-bromoacetophenone **4f** or of ω-chloroacetophenone **4i** (1 equiv) in chloroform, sodium azide (NaN_3) (1.1–1.2 equiv), water, and tetrabutylammonium bromide (TBAB) (0.11–0.15 equiv) were added. The reaction mixture was stirred vigorously at room temperature for 24 h and then separated. The organic layer was washed with water, dried (Na_2SO_4), and concentrated in vacuo. The residue was titrated with ethanol and the resulting solid recrystallized from absolute ethanol to give pure azide **5f** or **5i**.

*2-Azido-1-(3,4,5-trimethoxyphenyl)ethanone (**5f**)* The general procedure was used with 2-bromo-1-(3,4,5-trimethoxyphenyl)ethanone **4f** (0.5 g, 1.73 mmol), NaN_3 (0.2 g, 3.07 mmol) and TBAB (0.12 g, 0.37 mmol) in chloroform (10 mL) and water (10 mL) to provide pure azide **5f** as a beige solid in 82 % yield (0.36 g); mp (EtOH): 92–94 °C. $Rf = 0.78$ (EtOAc:*n*-C_6H_{12} = 1:1). IR ν cm^{-1}: 2942, 2100, 1694, 1584, 1502, 1469, 1413, 1348, 1319, 1283, 1229, 1158, 1125, 1062. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 4.03 (s, 6H, 2OCH_3), 4.04 (s, 3H, OCH_3), 4.64 (s, 2H, CH_2), 7.25 (s, 2H, Ar*H*). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 54.7 (CH_2), 56.4 (2CH_3),

61.0 (CH_3), 105.6 (2CH), 129.5 (C), 143.6 (C), 153.3 (2C), 192.1 (C). Elemental analysis calcd (%) for $C_{11}H_{15}N_3O_4$: C 52.17, H 5.97, N 16.59; found: C 52.42, H 6.09, N 16.81.

*1-(Azidoacetyl)-1H-indazole (**5i**)* The general procedure was used with 1-(chloroacetyl)-1*H*-indazole **4i** (1.5 g, 7.71 mmol) in chloroform (15 mL), NaN_3 (0.6 g, 9.23 mmol), water (15 mL), and TBAB (0.37 g, 1.15 mmol) to give pure 1-(azidoacetyl)-1*H*-indazole **5i** as a beige solid in 69 % yield (1.1 g); mp (EtOH) 81–83 °C; *Rf* = 0.53 (EtOAc:*n*-hexane,1:1). IR ν cm^{-1}: 2121, 1702, 1504, 1469, 1430, 1346, 1265, 1215, 1168, 1146. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 4.77 (s, 2H, C*H*$_2$), 7.39 (t, *J* = 7.2 Hz, 1H, Ar*H*), 7.59 (t, *J* = 7.2 Hz, 1H, Ar*H*), 7.75 (d, *J* = 8.0 Hz, 1H, Ar*H*), 8.14 (s, 1H, Ar*H*), 8.41 (dd, *J* = 8.4, 0.4 Hz, 1H, Ar*H*). ^{13}C NMR ($CDCl_3$, 100 MHz): 51.7 (CH_2), 115.1 (CH), 121.2 (CH), 125.1 (CH), 126.1 (C), 129.9 (CH), 138.0 (C), 141.0 (CH), 167.4 (C). Elemental analysis calcd (%) for $C_9H_9N_5O$: C 53.20, H 4.46, N 34.47; found: C 53.47, H 4.68, N 34.55.

*General procedure for the synthesis of ethyl 1,2,3-triazole-4-carboxylates (**6a–d**)*

A mixture of ethyl propiolate (1 equiv) and appropriate azide **5a–d** was dissolved in *t*-BuOH:MeCN = 10:4. Sodium ascorbate (0.2 equiv), water and copper sulfate (0.1 equiv) were then added. The reaction was stirred at room temperature for 24 h. The obtained solid was filtered and washed with water and ammonium hydroxide, and then with ethanol to provide pure triazoles (**6a–d**).

*Ethyl 1-[2-(4-fluorophenyl)-2-oxoethyl]-1H-1,2,3-triazole-4-carboxylate (**6a**)* The general procedure was followed using ethyl propiolate (0.97 mL, 8.45 mmol), 2-azido-1-(4-fluorophenyl)ethanone **5a** (1.51 g, 8.45 mmol), $CuSO_4{\cdot}5H_2O$ (0.21 g, 0.84 mmol), sodium ascorbate (0.34 g, 1.62 mmol), *t*-BuOH (10 mL), MeCN (4 mL) and H_2O (4 mL) to provide triazole **6a** as a white solid in 71 % yield (2.02 g) with the same properties as described in the literature [47]; *Rf* = 0.38 (EtOAc:*n*-hexane,1:1). ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 1.32 (t, *J* = 6.8 Hz, 3H, $CO_2CH_2CH_3$), 4.34 (q, *J* = 6.8 Hz, 2H, $CO_2CH_2CH_3$), 6.27 (s, 2H, C*H*$_2$), 7.46 (t, *J* = 8.8 Hz, 2H, Ar*H*), 8.18 (q, *J* = 6.4 Hz, 2H, Ar*H*), 8.71 (s, 1H, Ar*H*). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 14.1 (CH_3), 56.1 (CH_2), 60.5 (CH_2), 116.1 (d, *J* = 86.8 Hz, 2CH), 130.6 (d, *J* = 74.3 Hz, C), 131.3 (d, *J* = 40.0 Hz, 2CH), 138.8 (2C), 160.2 (CH), 164.3 (C), 166.8 (C), 190.2 (C).

*Ethyl 1-[2-(4-chlorophenyl)-2-oxoethyl]-1H-1,2,3-triazole-4-carboxylate (**6b**)* The general procedure was followed using ethyl propiolate (0.83 mL, 8.2 mmol), 2-azido-1-(4-chlorophenyl)ethanone **5b** (1.61 g, 8.2 mmol), $CuSO_4{\cdot}5H_2O$ (0.21 g, 0.82 mmol), sodium ascorbate (0.33 g, 1.6 mmol), *t*-BuOH (15 mL), MeCN (6 mL) and H_2O (6 mL) to provide triazole **6b** as a beige solid in 33 % yield (0.79 g) with the same physico-chemical properties as described in the literature [47, 48]; mp (EtOH) 181–184 °C; *Rf* = 0.33 (EtOAc:*n*-hexane,1:1). ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 1.32 (t, *J* = 7.2 Hz, 3H, $CO_2CH_2CH_3$), 4.33 (q, *J* = 7.2 Hz,

2H, $CO_2CH_2CH_3$), 6.27 (s, 2H, CH_2), 7.71 (d, $J = 8.4$ Hz, 2H, Ar*H*), 8.09 (d, $J = 8.8$ Hz, 2H, Ar*H*), 8.70 (s, 1H, Ar*H*). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 14.1 (CH_3), 56.1 (CH_2), 60.5 (CH_2), 129.1 (2CH), 130.0 (2CH), 130.6 (CH), 132.6 (C), 138.8 (C), 139.2 (C), 160.2 (C), 190.7 (C).

*Ethyl 1-[2-(4-bromophenyl)-2-oxoethyl]-1H-1,2,3-triazole-4-carboxylate (**6c**)* The general procedure was followed using ethyl propiolate (1.16 mL, 11.43 mol), 2-azido-1-(4-bromophenyl)ethanone **5c** (2.75 g, 11.4 mol), $CuSO_4{\cdot}5H_2O$ (0.29 g, 1.14 mol), sodium ascorbate (0.45 g, 2.28 mol), *t*-BuOH (20 mL), MeCN (8 mL) and H_2O (8 mL) to provide triazole **6c** as a beige solid in 64 % yield (2.47 g) with the same physico-chemical properties as described in the literature [47]; mp (EtOH) 180–184 °C; *Rf* = 0.3 (EtOAc:*n*-hexane,1:1).

*Ethyl 1-[2-(4-methylphenyl)-2-oxoethyl]-1H-1,2,3-triazole-4-carboxylate (**6d**)* The general procedure was followed using ethyl propiolate (1.68 mL, 16.66 mol), 2-azido-1-*p*-tolylethanone **5d** (2.91 g, 16.66 mol), $CuSO_4{\cdot}5H_2O$ (0.42 g, 1.66 mol), sodium ascorbate (0.66 g, 3.33 mol), *t*-BuOH (20 mL), MeCN (8 mL) and H_2O (8 mL) to provide triazole **6d** as a yellow solid in 54 % yield (2.99 g) with the same physico-chemical properties as described in the literature [48]; mp (EtOH) 157–160 °C; *Rf* = 0.23 (EtOAc:*n*-hexane, 1:1). ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 1.32 (t, $J = 6.8$ Hz, 3H, $CO_2CH_2CH_3$), 2.43 (s, 3H, $ArCH_3$), 4.33 (q, $J = 6.8$ Hz, 2H, $CO_2CH_2CH_3$), 6.23 (s, 2H, CH_2), 7.42 (d, $J = 8.0$ Hz, 2H, Ar*H*), 7.98 (d, $J = 8.0$ Hz, 2H, Ar*H*), 8.72 (s, 1H, Ar*H*). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 14.1 (CH_3), 21.2 (CH_3), 56.0 (CH_2), 60.5 (CH_2), 128.2 (2CH), 129.5 (2CH), 130.7 (CH), 131.3 (C), 138.7 (C), 144.9 (C), 160.2 (C), 191.0 (C).

*General procedure for the synthesis of 1,2,3-triazole-4-carboxylic acids (**7a–d**)*

KOH aq solution (30 %) was added to a suspension of ethyl 1,2,3-triazole-4-carboxylate **6a–d** (1 equiv) in EtOH. The resulting solution was heated at reflux for 15 min. After cooling to RT, the pH was adjusted to 4 with citric acid. The resulting white solid was filtered and washed with ethanol to provide pure carboxylic acids **7a–d**.

*1-[2-(4-Fluorophenyl)-2-oxoethyl]-1H-1,2,3-triazole-4-carboxylic acid (**7a**)* The general procedure was followed using ethyl 1,2,3-triazole-4-carboxylate **6a** (1.25 g, 4.2 mol), KOH (2.28 g, 40 mol), H_2O (10 mL) and EtOH (20 mL) to provide carboxylic acid **7a** as a white solid in 97 % yield (1.12 g); mp (EtOH) 223–224 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 6.26 (s, 2H, CH_2), 7.47 (t, $J = 8.6$ Hz, 2H, Ar*H*), 8.62 (q, $J = 8.6$ Hz, 2H, Ar*H*), 8.62 (s, 1H, Ar*H*), 13.0 (s, 1H, COO*H*). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 56.5 (CH_2), 116.6 (d, $J = 21.8$ Hz, 2CH), 131.1 (d, $J = 20.3$ Hz, CH), 131.8 (d, $J = 20.3$ Hz, CH), 140.2 (C), 162.1 (C), 164.8 (CH), 167.3 (C), 190.8 (C). Elemental analysis calcd (%) for $C_{11}H_8FN_3O_3$: C 53.02, H 3.24, N 16.86; found: C 53.39, H 3.41, N 17.02.

*1-[2-(4-Chlorophenyl)-2-oxoethyl]-1H-1,2,3-triazole-4-carboxylic acid (**7b**)* The general procedure was followed using ethyl 1,2,3-triazole-4-carboxylate **6b** (0.8 g, 2.6 mol), KOH (1.35 g, 24.2 mol), H_2O (5 mL) and EtOH (10 mL) to provide carboxylic acid **7b** as a white solid in 84 % yield (0.6 g); mp (EtOH) 231–234 °C. IR ν cm^{-1}: 3121, 2998, 2954, 1682, 1589, 1542, 1438, 1232, 1092, 990. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 6.26 (s, 2H, CH_2), 7.71 (d, J = 8.6 Hz, 2H, Ar*H*), 8.10 (d, J = 8.6 Hz, 2H, Ar*H*), 8.61 (s, 1H, Ar*H*), 13.25 (s, 1H, COO*H*). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 56.6 (CH_2), 129.6 (2CH), 130.6 (2CH), 131.0 (C), 133.1 (C), 139.7 (CH), 140.4 (C), 162.2 (C), 191.3 (C). Elemental analysis calcd (%) for $C_{11}H_8ClN_3O_3$: C 49.73, H 3.04, N 15.82; found: C 49.98, H 3.32, N 15.97.

*1-[2-(4-Bromophenyl)-2-oxoethyl]-1H-1,2,3-triazole-4-carboxylic acid (**7c**)* The general procedure was followed using ethyl 1,2,3-triazole-4-carboxylate **6c** (2.32 g, 6.85 mol), KOH (3.45 g, 61.6 mol), H_2O (10 mL) and EtOH (20 mL) to provide carboxylic acid **7c** as a white solid in 91 % yield (1.93 g); mp (EtOH) 231–234 °C. IR ν cm^{-1}: 3121, 2999, 2953, 1682, 1586, 1542, 1438, 1233, 1071. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 6.25 (s, 2H, CH_2), 7.85 (d, J = 8.4 Hz, 2H, Ar*H*), 8.01 (d, J = 8.8 Hz, 2H, Ar*H*), 8.61 (s, 1H, Ar*H*), 13.15 (s, 1H, COO*H*). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 56.5 (CH_2), 128.9 (CH), 130.6 (2CH), 131.0 (C), 132.6 (2CH), 133.4 (C), 140.3 (C), 162.1 (C), 191.5 (C). Elemental analysis calcd (%) for $C_{11}H_8BrN_3O_3$: C 42.60, H 2.60, N 13.55; found: C 42.81, H 2.51, N 13.74.

*1-[2-(4-Methylphenyl)-2-oxoethyl]-1H-1,2,3-triazole-4-carboxylic acid (**7d**)* The general procedure was followed using ethyl 1,2,3-triazole-4-carboxylate **6d** (2.79 g, 0.01 mol), KOH (5.15 g, 0.09 mol), H_2O (10 mL) and EtOH (20 mL) to provide carboxylic acid **7d** as a yellow solid in 87 % yield (2.5 g); mp (EtOH) 233–235 °C. IR ν cm^{-1}: 3120, 1696, 1676, 1604, 1540, 1439, 1343, 1226, 1182, 1055, 949. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 2.42 (s, 3H, CH_3), 6.22 (s, 2H, CH_2), 7.43 (d, J = 8.2 Hz, 2H, Ar*H*), 7.98 (d, J = 8.2 Hz, 2H, Ar*H*), 8.62 (s, 1H, Ar*H*), 13.16 (s, 1H, COO*H*). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 21.7 (CH_3), 56.4 (CH_2), 128.8 (2CH), 130.0 (2CH), 131.1 (C), 131.9 (C), 140.1 (C), 145.4 (CH), 162.2 (C), 191.6 (C). Elemental analysis calcd (%) for $C_{12}H_{11}N_3O_3$: C 58.77, H 4.52, N 17.13; found: C 58.63, H 4.38, N 17.40.

*General procedure for the synthesis of triazines **15** and **16***

Under an inert atm, triethylamine (1 equiv) was added dropwise to a homogeneous mixture of activated ester **20** or **22** (1 equiv) and zinc dimethylimidodicarbonimidate **3b** (0.6 equiv) in dichloromethane. After the complete addition, the mixture was stirred at room temperature for 18 or 24 h. Then, the reaction mixture was washed with water. The combined organic layers were dried (Na_2SO_4), filtered, and the solvent evaporated. After evaporation, *n*-hexane was added to the crude residue. The resulting solid was collected by filtration and recrystallized in *n*-hexane or purified by flash chromatography to provide pure triazine **15** or **16**.

*2,4-Dimethoxy-6-(pyridin-2-yl)-1,3,5-triazine (**15**)* The general procedure was used with triethylamine (0.25 mL, 1.82 mmol), activated ester **20** (0.4 g, 1.82 mmol) and zinc dimethylimidodicarbonimidate **3b** (0.38 g, 1.09 mmol) in dichloromethane (40 mL). After the complete addition, the mixture was stirred at room temperature for 18 h. After evaporation of dichloromethane, *n*-hexane was added to the crude residue. The resulting solid was collected by filtration to provide triazine **15** as a white solid in 46 % yield (0.18 g); mp (*n*-C_6H_{14}) 93–96 °C; *Rf* = 0.15 (EtOAc:*n*-hexane, 2:1). IR *v* cm^{-1}: 1540, 1505, 1468, 1343, 1295, 1232, 1193, 1108, 1093. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 4.15 (s, 6H, 2OC*H*$_3$), 7.49 (t, *J* = 7.2 Hz, 1H, Ar*H*), 7.88 (t, *J* = 7.2 Hz, 1H, Ar*H*), 8.53 (d, *J* = 7.2 Hz, 1H, Ar*H*), 8.85 (d, *J* = 7.2 Hz, 1H, Ar*H*). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 55.5 ($2CH_3$), 124.6 (2CH), 126.6 (CH), 137.2 (C), 150.2 (CH), 152.3 (C), 173.2 (2C). Elemental analysis calcd (%) for $C_{10}H_{10}N_4O_2$: C 55.04, H 4.62, N 25.68; found: C 55.21, H 4.91, N 25.96.

*10-(2-(4,6-Dimethoxy-1,3,5-triazin-2-yl)ethyl)-10H-phenothiazine (**16**)* The general procedure was used with triethylamine (0.11 mL, 0.81 mmol), activated ester **22** (0.3 g, 0.81 mmol) and zinc dimethyl imidodicarbonimidate **3b** (0.16 g, 0.49 mmol) in dichloromethane. The mixture was stirred at room temperature for 24 h. The residue obtained upon evaporation was purified by chromatography (EtOAc:*n*-hexane = 1:1) to provide pure 10-(2-(4,6-dimethoxy-1,3,5-triazin-2-yl)ethyl)-10*H*-phenothiazine **16** as colorless crystals in 72 % yield (0.21 g); mp (EtOAc:*n*-hexane) 89–92 °C; *Rf* = 0.68 (EtOAc:*n*-hexane, 1:1). IR *v* cm^{-1}: 1559, 1547, 1496, 1459, 1443, 1382, 1353, 1251, 1232, 1194, 1114. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 3.27 (t, *J* = 7.2 Hz, 2H, C*H*$_2$), 3.98 (s, 6H, 2OC*H*$_3$), 4.38 (bs, 2H, C*H*$_2$), 6.82–7.05 (m, 4H, Ar*H*), 7.05-7.22 (m, 4H, Ar*H*). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 36.1 (CH_2), 44.7 (CH_2), 55.5 ($2CH_3$), 115.4 (2CH), 122.6 (2CH), 124.9 (2C), 127.3 (2CH), 127.4 (2CH), 144.7 (2C), 172.4 (2C), 180.6 (C). Elemental analysis calcd (%) for $C_{19}H_{18}N_4O_2S$: C 62.28, H 4.95, N 15.29; found: C 62.32, H 5.04, N 15.48.

*General procedure for the synthesis of activated esters (**17**, **20** and **22**)*

N-hydroxysuccinimide (1.2 equiv) and EDCI (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride) or CDI (carbonyldiimidazole) (1.2 equiv) were added to a suspension of carboxylic acid **10**, **19** or **21** (1 equiv) in dichloromethane. The reaction mixture was stirred at room temperature for 24 h. After concentration under vacuum of the reaction media, the crude residue was recrystallized from ethanol to give pure activated ester **17**, **20** or **22**.

*1-{[(2E)-3-(1H-imidazol-1-yl)prop-2-enoyl]oxy}pyrrolidine-2, 5-dione (**17**)* The general procedure was used with CDI (1,1′-Carbonyldiimidazole) (0.50 g, 3.05 mmol), *N*-hydroxysuccinimide (0.35 g, 3.06 mmol) and propiolic acid **10** (0.25 g, 2.55 mmol) in dichloromethane (35 mL). After complete homogenization of the reaction medium, a white precipitate was formed. This was filtered and

washed with CH_2Cl_2 to afford pure compound **17** as a white solid in 39 % yield (0.23 g); mp (CH_2Cl_2) 209–211 °C. IR ν cm^{-1}: 1770, 1727, 1651, 1495, 1305, 1223, 1188. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 2.87 (s, 4H, 2CH_2), 6.80 (d, J = 14.0 Hz, 1H, C*H*), 7.16 (s, 1H, Ar*H*), 7.93 (s, 1H, Ar*H*), 8.28 (s, 1H, Ar*H*), 8.51 (d, J = 14.0 Hz, 1H, C*H*). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 25.5 ($2CH_2$), 99.5 (CH), 117.3 (CH), 131.4 (CH), 139.6 (CH), 141.9 (CH), 162.3 (C), 170.3 (2C). MS (EI) *m/z* (%): 235 (5) [M+], 138 (4), 121 (100), 93 (22).

*1-[(2-Pyridinylcarbonyl)oxy]-2,5-pyrrolidinedione (**20**)* The general procedure was used with *N*-hydroxysuccinimide (1.12 g, 9.76 mmol), CDI (carbonyldiimidazole) (1.58 g, 9.76 mmol) and picolinic acid **19** (1.0 g, 8.13 mmol) in dichloromethane (50 mL). After concentration of the reaction medium under vacuum, the crude residue was recrystallized from ethanol to give pure activated ester **20** as a white solid in 17 % yield (0.3 g) with the same physico-chemical properties as described in the literature [59]; mp (EtOH) 172–175 °C. IR ν cm^{-1}: 1796, 1775, 1737, 1439, 1357, 1284, 1255, 1196. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 2.93 (s, 4H, 2CH_2), 7.84 (t, J = 7.6 Hz, 1H, Ar*H*), 8.14 (t, J = 7.6 Hz, 1H, Ar*H*), 8.24 (d, J = 7.6 Hz, 1H, Ar*H*), 8.86 (d, J = 7.6 Hz, 1H, Ar*H*). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 25.6 ($2CH_2$), 126.6 (CH), 128.4 (CH), 137.3 (CH), 144.0 (C), 150.4 (CH), 160.4 (C), 168.8 (2C).

*1-{[3-(10H-Phenothiazin-10-yl)propanoyl]oxy}pyrrolidine-2,5-dione (**22**)* The general procedure was used with *N*-hydroxysuccinimide (1.02 g, 8.82 mmol), EDCI (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride) (1.69 g, 8.82 mmol) and carboxylic acid **21** (2.0 g, 7.38 mmol) in dichloromethane (30 mL). After concentration of the reaction medium under vacuum, the crude residue was recrystallized from ethanol to give pure activated ester **22** as a white solid in 90 % yield (2.44 g) with the same physico-chemical characteristics as described in the literature [45, 60, 61]; mp (EtOH) 159–160 °C. IR ν cm^{-1}: 1814, 1779, 1735, 1456, 1363, 1323, 1200, 1124, 1069, 997, 931, 838, 746. ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 2.81 (s, 4H, 2CH_2), 3.14 (t, J = 6.4 Hz, 2H, CH_2), 4.28 (t, J = 6.4 Hz, 2H, CH_2N), 7.0 (t, J = 7.6 Hz, 2H, Ar*H*), 7.06 (d, J = 8.0 Hz, 2H, Ar*H*), 7.18–7.26 (m, 4H, Ar*H*). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 25.4 ($2CH_2$), 28.9 (CH_2), 41.5 (CH_2), 115.5 (2CH), 123.0 (2CH), 124.0 (2C), 127.3 (2CH), 127.7 (2CH), 144.1 (2C), 167.4 (C), 170.0 (2C).

*(E)-N,N-Diethyl-2-(4,6-dimethoxy-1,3,5-triazin-2-yl)ethenamine (**18**)*

To a solution of 2-ethynyl-4,6-dimethoxy-1,3,5-triazine **1** (0.58 g, 3.52 mmol) in CH_2Cl_2 (25 mL), diethylamine (0.36 mL, 3.42 mmol) was added. The obtained solution was stirred at room temperature for 15 h. The residue obtained upon evaporation was purified by flash chromatography (EtOAc/*n*-heptane) to provide pure (*E*)-*N*,*N*-diethyl-2-(4,6-dimethoxy-1,3,5-triazin-2-yl)ethenamine **18** as an yellow oil in 70 % yield (0.58 g); *Rf* = 0.18 (EtOAc:*n*-heptane, 4:6). IR ν cm^{-1}: 2974, 2941, 2874, 1629, 1539, 1512, 1445, 1404, 1338, 1255, 1193, 1115, 971. ^{1}H NMR

($CDCl_3$, 400 MHz): δ (ppm) 1.21 (t, $J = 7.1$ Hz, 6H, $2CH_3$), 3.30 (q, $J = 6.7$ Hz, 4H, $2CH_2$), 3.97 (s, 6H, $2OCH_3$), 5.13 (dd, $J = 13.0$, 1.6 Hz, 1H, C*H*), 8.03 (dd, $J = 13.0$, 1.6 Hz, 1H, C*H*). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 11.4 (CH_3), 14.6 (CH_3), 42.2 (CH_2), 50.2 (CH_2), 54.0 ($2CH_3$), 91.8 (CH), 149.8 (CH), 171.2 (C), 176.1 (2C). Elemental analysis calcd (%) for $C_{11}H_{18}N_4O_2$: C 55.44, H 7.61, N 23.51; found: C 55.73, H 7.44, N 23.79.

Biological evaluation

Farnesyltransferase assay Assays were conducted in 96-well plates, prepared with a Biomek NKMC and a Biomek 3000 from Beckman Coulter and read on a Wallac Victor fluorimeter from Perkin–Elmer. Per well, 20 µL of farnesylpyrophosphate (10 µM) was added to 180 µL of a solution containing 2 µL of varied concentrations of potential inhibitors (dissolved in DMSO) and 178 µL of a solution composed by 10 µL of partially purified recombinant human FTase (1.5 mg/mL) and 1.0 mL of Dansyl-GCVLS peptide [in the following buffer: 5.6 mM DTT, 5.6 mM $MgCl_2$, 12 µM $ZnCl_2$ and 0.2 % (w/v) octyl-b-D-glucopyranoside, 52 mM Tris/HCl, pH 7.5]. Fluorescence development was recorded for 15 min (0.7 s per well, 20 repeats) at 30 °C with an excitation filter to 340 nm and an emission filter of 486 nm. Each measurement was realized twice, in duplicate or in triplicate. The kinetic experiments were realized under the same conditions, either with FPP as varied substrate with a constant concentration of Dns-GCVLS of 2.5 µM, or with Dns-GCVLS as varied substrate with a constant concentration of FPP of 10 µM. Nonlinear regressions were performed by KaleidaGraph 4.03 software [62].
Cell proliferation assay Compounds **2a–i** have been tested on a panel of 60 human cancer cell lines at the National Cancer Institute, Rockville, MD. The cytotoxicity studies were conducted using a 48 h exposure protocol using the sulforhodamine B assay [63, 64].

Results and discussion

The activity of newly triazin–triazoles **2a–i** was evaluated on human farnesyltransferase (FTase) [62] and on the NCI-60 human tumor cell lines [63, 64]. The results are summarized in Tables 2 and 3. Interestingly, triazin–triazole **2c**, substituted by a *para*-bromophenyl unit, showed the best inhibitory activity on farnesyltransferase ($IC_{50} = 38.62 \pm 1.7$ µM) in the current study (Table 3). The *para*-chloro analogue, compound **2b**, showed decreased biological potential ($IC_{50} = 72.05 \pm 6.9$ µM), confirming the structure–activity relationships previously established [45]. The other investigated substituents (F, CN, Me or OMe) at the *para*-position of the phenyl ring in compounds **2a**, **2d–f** or the replacement of the phenyl unit by a phenothiazine or an indazole moiety in compounds **2g–i** abolished the inhibitory activity toward FTase. In conclusion, the investigation of triazin–triazoles **2a–i** as potential inhibitors of human FTase furnished compounds with decreased inhibitory potency relative to our previously described triazoles [45].

Table 3 Results of the human farnesyltransferase assay

Compound	% Inh (FTase)[a,b]	IC_{50} (μM) ± SD	Compound	% Inh (FTase)[a,b]	IC_{50} (μM) ± SD
2a	19	–	**2f**	0	–
2b	59	72.05 ± 6.9	**2g**	0	–
2c	73	38.62 ± 1.7	**2h**	35	–
2d	37	–	**2i**	24	–
2e	48	–			

SD standard deviation

[a] Inhibition ratio of protein farnesyltransferase at a 100 μM concentration

[b] Values represent mean of two experiments

Table 4 Results of the NCI cell growth inhibition assay. In vitro percentage growth inhibition (GI%) caused by the selected compounds against some tumor cell lines in the single-dose assay[a]

Compound	Leukemia			Non-small cell lung cancer			Renal cancer	
	K-562	MOLT-4	SR	HOP-62	HOP-92	NCI-H522	A498	UO-31
2a	21	7	17	12	13	11	n.a.	21
2b	n.a.[b]	n.a.	6	6	n.a.	n.a.	20	16
2c	n.a.	n.a.	10	3	12	n.a.	18	13
2d	n.a.	5	9	6	13	n.a.	19	21
2e	n.a.	n.a.	8	5	13	n.a.	18	14
2f	10	10	16	12	7	n.a.	n.a	28
2g	9	13	14	n.a.	20	11	26	26
2h	13	13	14	n.a.	24	11	25	28
2i	n.a.	6	14	10	n.a.	6	n.a.	26

[a] Data obtained from NCI's in vitro disease-oriented human tumor cell screen at 10 μM concentration

[b] No inhibitory effect

The results of the NCI single-dose assay at a 10 μM concentration of each compound indicated that triazin–triazoles **2a–i** have low cytostatic effects (Table 4). The most interesting growth inhibition potentials have been registered for compounds **2f** and **2h** on UO-31 renal cancer cell lines (28 % inhibition).

Conclusion

The investigation of the triazin–triazole unit as a potential chelating unit of the zinc atom of human farnesyltransferase resulted in the discovery of a new series of compounds **2a–i** with unprecedented biological activity. The best compound in the current study showed an IC_{50} value against FTase of 38.6 μM. This activity is

moderate comparing to known families of inhibitors of protein farnesyltransferase. However, this new class of triazin–triazoles deserves further chemical modulations on different points of the skeleton that could valuably lead to significant biological results.

From a synthetic strategy point of view, 2-ethynyl-4,6-dimethoxy-1,3,5-triazine **1** proved to be a new heterocycle with interesting properties. Indeed, in this compound, the triazine group, which is substituted by two strongly electro-donating methoxy groups proves to be strongly withdrawing,[3] able to activate the triple bond toward nucleophile addition (Cl^-, imidazole or diethylamine). Ethynyldimethoxy-triazine **1** is a significant platform-compound for developing new chemical entities for anticancer research and for other biological applications. It is recognized that alkynes constitute privileged structural units in medicinal chemistry and chemical biology [67].[4] Consequently, further chemical and biological studies are necessary to highlight the great importance of molecule **1**.

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References

1. R. Kumar, L. Gupta, P. Pal, S. Khan, N. Singh, S.B. Katiyar, S. Meena, J. Sarkar, S. Sinha, J.K. Kanaujiya, S. Lochab, A.K. Trivedi, P.M.S. Chauhan, Eur. J. Med. Chem. **45**, 2265 (2010)
2. B.B. Baldaniya, P.K. Patel, Eur. J. Chem. **6**, 673 (2009)
3. L. Almeida, B. Aquila, C.E. Chuaqui, H.P. Guan, S. Huang, S. Ioannidis, M. Lamb, B. Peng, J. Shi, M. Su, Q.B. Su, WO2009016410 (2009)
4. L. Almeida, B. Aquila, C.E. Chuaqui, H.P. Guan, S. Huang, S. Ioannidis, M. Lamb, B. Peng, J. Shi, M. Su, Q.B. Su, Chem. Abstr. **153**, 456713 (2010)
5. N.M. Niyaz, K.A. Guenthenspberger, R. Hunter, A.V. Brown, J.S. Nugent, US 20090093481 (2009)
6. N.M. Niyaz, K.A. Guenthenspberger, R. Hunter, A.V. Brown, J.S. Nugent, Chem. Abstr. **150**, 398582 (2009)
7. B. Liu, Y. Lee, J.M. Zou, H.M. Petrassi, R.W. Joseph, W.C. Chao, E.L. Michelotti, M. Bukhtiyarova, E.B. Springman, B.D. Dorsey, Bioorg. Med. Chem. Lett. **20**, 6592 (2010)
8. A. Solankee, K. Kapadia, A. Ciric, M. Sokovic, I. Doytchinova, A. Geronikaki, Eur. J. Med. Chem. **45**, 510 (2010)
9. P.K. Pareek, P.D. Mithlesh, M. Chaudhary, A. Pareek, R. Kant, K.G. Ojha, Main Group Chem. **10**, 63 (2011)
10. H. Ojha, P. Gahlot, A.K. Tiwari, M. Pathak, R. Kakkar, Chem. Biol. Drug Des. **77**, 57 (2011)
11. C. Fanelli, A.A. Fabbri, S. Monti, R. Menicagli, D. Pini, S.M. Rapaccini, S. Samaritani, P. Salvadori, *7th International Congress of Plant Pathology* (Edinburgh, 1998)
12. S. Samaritani, A.A. Fabbri, C. Fanelli, R. Menicagli, P. Salvadori, *XXV Convegno Nazionale della Divisione di Chimica Organica* (Folgaria, 1998)
13. A. Ricelli, A.A. Fabbri, C. Fanelli, R. Menicagli, S. Samaritani, D. Pini, S.M. Rapaccini, P. Salvadori, Restaurator **20**, 97 (1999)
14. G.H. Singhal, H. Roebke, DE 2115318 A (1971)

[3] For additional proof on the electronic behavior of acetylene group of compound **1**, see [65].

[4] For activating groups in accelerating reactivity of compounds bearing a triple bond group, see [66].

15. G.H. Singhal, H. Roebke, Chem. Abstr. **76**, 72560 (1972)
16. M. Just, I. Glase, DD 248590 (1987)
17. M. Just, Chem. Abstr. **108**, 131864 (1988)
18. K. Koizumi, O. Yamashita, K. Wakabayashi, K. Tomono, H. Sasayama, WO 9720825 (1997)
19. K. Koizumi, O. Yamashita, K. Wakabayashi, K. Tomono, H. Sasayama, Chem. Abstr. **127**, 95296 (1997)
20. A.S. Gajare, S.B. Bhawsar, D.B. Shinde, M.S. Shingare, Indian J. Chem. Sect. B **37B**, 510 (1998)
21. H. Zhao, Y. Liu, Z. Cui, D. Beattie, Y. Gu, Q. Wang, J. Agric. Food Chem. **59**, 11711 (2011)
22. L.M. Abell, J.V. Schloss, A.R. Rendina, in *Target-side Directed Herbicide Design;* ACS Symposium Series, vol 524 (1993), p. 16
23. L. Ballantine, J. McFarland, D.Hackett, in *Triazine Herbicides: Risk Assessments*; ACS Symposium Series, vol 683 (1998), p. xi
24. G. Rembarz, E. Fischer, K.Ch. Roeber, R. Ohff, H. Crahmer, J. Prakt. Chem. **311**, 889 (1969)
25. G.C. Chiang, US Patent 4933450 A (1990)
26. G.C. Chiang, Chem. Abstr. **113**, 231414 (1990)
27. P.S. Gates, G.P. Jones, D. E. Saunders, PCT Int. Appl. WO 9309099 A2 (1993)
28. P.S. Gates, G.P. Jones, D.E. Saunders, Chem. Abstr. **119**, 180810 (1993)
29. O. Voss, P.J. Dudfield, K. Bauer, H. Bieringer, C. Rosinger, M. J. Ford, D. Green, Ger. Offen. 19521355 **(1996)**
30. O. Voss, P.J. Dudfield, K. Bauer, H. Bieringer, C. Rosinger, M.J. Ford, D. Green, Chem. Abstr. **126**, 144293 (1997)
31. U. Burckhardt, M. Zimmermann, DE 2209470 (1972)
32. U. Burckhardt, M. Zimmermann, Chem. Abstr. **77**, 164744 (1972)
33. S. Weiss, H. Krommer, US Patent 6342600 (2002)
34. S. Weiss, H. Krommer, Chem. Abstr. **136**, 134795 (2002)
35. R. Menicagli, S. Samaritani, G. Signore, F. Vaglini, L.J. Dalla Via, Med. Chem. **47**, 4649 (2004)
36. C. Courme, S. Gillon, N. Gresh, M. Vidal, C. Garbay, J.-C. Florent, E. Bertounesque, Tetrahedron Lett. **49**, 4542 (2008)
37. R. Menicagli, S. Samaritani, S. Gori, Tetrahedron Lett. **40**, 8419 (1999)
38. S. Samaritani, R. Menicagli, Tetrahedron **58**, 1381 (2002)
39. S. Oudir, B. Rigo, J.P. Hénichart, P. Gautret, Synthesis **17**, 2845 (2006)
40. L. Lucescu, P. Gautret, S. Oudir, B. Rigo, D. Belei, E. Bîcu, A. Ghinet, Synthesis **45**, 1333 (2013)
41. J. Sun, M.A. Blaskovich, D. Knowles, Y. Qian, J. Ohkanda, R.D. Bailey, A.D. Hamilton, S.M. Sebti, Cancer Res. **59**, 4919 (1999)
42. C.-M. Abuhaie, A. Ghinet, A. Farce, J. Dubois, P. Gautret, B. Rigo, D. Belei, E. Bîcu, Eur. J. Med. Chem. **59**, 101 (2013)
43. C.-M. Abuhaie, A. Ghinet, A. Farce, J. Dubois, B. Rigo, E. Bîcu, Bioorg. Med. Chem. Lett. **23**, 5887 (2013)
44. G.-M. Dumitriu, A. Ghinet, E. Bîcu, B. Rigo, J. Dubois, A. Farce, D. Belei, Bioorg. Med. Chem. Lett. **24**, 3180 (2014)
45. D. Belei, C. Dumea, A. Samson, A. Farce, J. Dubois, E. Bîcu, A. Ghinet, Bioorg. Med. Chem. Lett. **22**, 4517 (2012)
46. F.L. Zhang, P.J. Casey, Ann. Rev. Biochem. **65**, 241 (1996)
47. K. Kónya, S. Fekete, A. Ábrahám, T. Patonay, Mol. Divers. **16**, 91 (2012)
48. M. Boominathan, N. Pugazhenthiran, M. Nagaraj, S. Muthusubramanian, S. Murugesan, N. Bhuvanesh, ACS Sustainable Chem. Eng. **1**, 1405 (2013)
49. B. Quan, S.X. Zhuang, C.C. Li, H.S. Dong, Indian J. Chem. **44B**, 1717 (2005)
50. G. Biagi, I. Giorgi, O. Livi, A. Nardi, F. Pacchini, V. Scartoni, A. Lucacchini, Eur. J. Med. Chem. **38**, 983 (2003)
51. D.R. Buckle, J. Heterocycl. Chem. **22**, 77 (1985)
52. H. Schirok, C. Alonso-Alija, M. Michels, Synthesis **18**, 3085 (2005)
53. W.J. Balfour, C.C. Greig, S. Visaisouk, J. Org. Chem. **39**, 725 (1974)
54. K. von Auwers, E. Frese, Justus Liebigs Anna. Chem. **450**, 273 (1926)
55. K. von Auwers, H.G. Allardt, Justus Liebigs Anna. Chem. **438**, 20 (1924)
56. D. Belei, E. Bicu, P.G. Jones, M.L. Birsa, J. Heterocycl. Chem. **48**, 129 (2011)
57. D. Belei, E. Bicu, M.L. Birsa, Acta Chem. Iasi **17**, 197 (2009)
58. A.N. Gritsenko, S.V. Zhuravlev, V.A. Skorodumov, *Uchenye Zapiski Inst. Farmakol. I 90 Kimioterapii Akad. Med. Nauk. USSR* **1**, 13 (1958)

59. J.B. Christensen, Molecules **6**, 47 (2001)
60. G. Lang, G. Plos, PCT Int. Appl. (2002) WO 2002007689 A1 20020131
61. G. Lang, G. Plos, Chem. Abstr. **136**, 139633 (2002)
62. L. Coudray, R.M. De Figueiredo, S. Duez, S. Cortial, J.J. Dubois, Enz. Inhib. Med. Chem. **24**, 972 (2009)
63. R.B. Boyd, The NCI in vitro anticancer drug discovery screen, in *Anticancer Drug Development Guide: Preclinical Screening, Clinical Trials, and Approval*, ed. by B. Teicher (Humana Press Inc., Totowa, NJ, 1997), pp. 23–42
64. P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesh, S. Kenney, M.R. Boyd, J. Natl. Cancer Inst. **82**, 1107 (1990)
65. S. Diring, P. Retailleau, R. Ziessel, Synlett **19**, 3027 (2007)
66. R. Frei, D. Wodrich, D.P. Hari, P.-A. Borin, C. Chauvier, J. Waser, J. Am. Chem. Soc. **136**, 16563 (2014)
67. F. Diederich, P.J. Stang, R.R. Tykwinski, in *Acetylene Chemistry: Chemistry, Biology and Material Science* (Wiley-VCH: Weinheim, 2005)