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Barbiturate- and Thiobarbituarte-Based *s*-Triazine Hydrazone Derivatives with Promising Antiproliferative Activities

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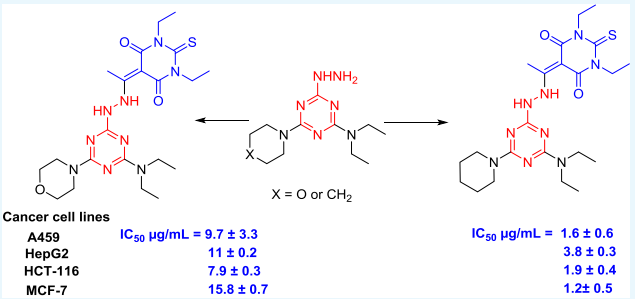
ABSTRACT: A new class of compounds, which include *s*-triazine with pyrimidinetrione or thiopyrimidinedione moiety through a hydrazone linkage, were synthesized and characterized. The newly synthesized *s*-triazine hydrazone derivatives were evaluated *in vitro* against four cancer cell lines: A549, HepG2, HCT-116, and MCF-7. Several derivatives showed growth inhibition activity in the low microgram range. The results reveal that the barbiturate derivatives showed poor to no activity, while thiobarbiturate derivatives showed better activity than the analogues barbiturate derivatives. The substituents on the s-triazine moiety have a great effect on the antiproliferative activity, where derivatives with the piperidino and diethylamino on the *s*-triazine ring (**5h**) showed the highest activity against all of the tested cell lines (IC_{50} 1.6 ± 0.6, 3.8 ± 0.3, 1.9 ± 0.4, and 1.2± 0.5 μg/mL for the tested cell lines A549, HepG2, HCT-116, and MCF-7, respectively). These results indicate that thiobarbiturates-*s*-triazine hydrazone derivatives may provide an excellent scaffold for the development of an anticancer drug candidate.



INTRODUCTION

Chemotherapy is one of the major strategies for the treatment of cancer. It is estimated that cancer is the second leading cause of morbidity and mortality after the clinical practice has been increasingly limited due to their risk of toxicity, drug resistance, and lack of specificity. This scenario thus calls for a new class of agents for the treatment of cancer and converting it into a chronic disease.

Barbituric acid (BA) and thiobarbituric (TBA) derivatives have become increasingly attractive to medicinal chemists as they possess a wide range of biological activities, such as the capacity to act as enzyme inhibitors,[1] as well as antibacterial, anticancer, antiangiogenic, immunomodulatory, antifungal, and antioxidant agents.[2−5] These activities are associated with several structural changes at positions N^1, N^3, and C5, the latter being the most effective/disrupting position.[6−12] Among the most important C5-functionalized BAs and TBAs are the 5-benzylidene or 5-methylene derivatives, which have been biologically evaluated, resulting in several compounds of this class being found to possess xanthine oxidase (XO) inhibitory effect[13] and antimicrobial activity as well as against fungal strains.[14−18]

On the other hand, 1,3,5-triazine (*s*-triazine) is extensively studied because it shows a wide range of biological activities, including antimicrobial,[19,20] antiviral,[21] and anti-inflammatory[22] effects. Moreover, many of *s*-triazine derivatives showed great promise for further development as new hits for antitumor agents.[23−38]

Recently, Bai et al. identified a series of *s*-triazine hydrazone derivatives as dual-effective inhibitors against both wild-type (WT) and mutant EGFR TKIs.[39] Of these derivatives, the compound represented in Figure 1 shows the most potent activity against epidermal growth factor receptor (WTEGFR, IC_{50} = 25.9 μM) and mutant epidermal growth factor receptor (EGFR T790M/L858R, IC_{50} = 6.5 μM). Moreover, it also exhibited considerable antiproliferative activity against A549,

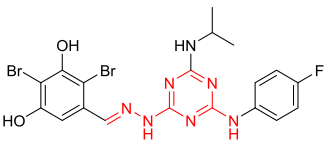


Figure 1. *s*-Triazine hydrazone derivatives as dual-effective inhibitors against both wild-type (WT) and mutant EGFR TKIs.

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A431, and NCIH1975 cell lines, with IC_{50} = 7.7, 8.0, and 10.5 μM, respectively.

In our recent publications,[37,40] we reported the synthesis of several *s*-triazine hydrazone derivatives (Figure 2) and their

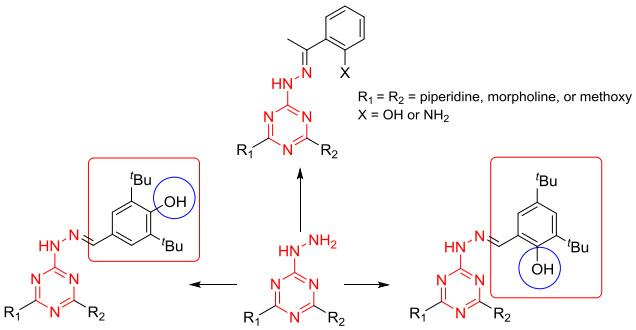


Figure 2. Structure of *s*-triazine hydrazone derivatives with anticancer activity.

corresponding antiproliferative activities against lung carcinoma A549, hepatocyte carcinoma HepG2,[40] breast cancer (MCF-7), and colon cancer (HCT-116).[37] Our results showed that the substituents at both the *s*-triazine and benzylidine moieties have a great effect on the antiproliferative activities.

Based on our previous results and searching for new compounds with promising anticancer activity, we set about developing a new class of compounds (Figure 3) bearing the *s*-

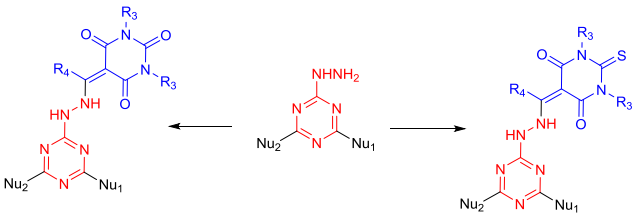


Figure 3. Structure of the target products based on previous reported results.

triazine core and either a pyrimidinetrione (barbiturate) or thiopyrimidinedione (thiobarbiturate) moiety attached *via* a hydrazone linkage. The antiproliferative activities of these new compounds against four cancer cell lines, namely, hepatocyte carcinoma HepG2, colon carcinoma HCT-116, lung carcinoma A549, and breast cancer cell lines MCF-7, were studied.

RESULTS AND DISCUSSION

Chemistry. The synthesis of the target compounds was achieved in several consecutive steps as outlined in Scheme 1. Cyanuric chloride **1** was reacted first with a different amine in a one-pot method, as described previously,[32] to afford the disubstituted *s*-triazine derivatives. The chloro derivatives **2a−g** were then treated with hydrazine hydrate to afford the 2-hydrazino-4,6-disubstituted-*s*-triazine derivatives **3a−g** in excellent yields and purities.[40]

Compounds **3a−g** were reacted with 1,3-dimethyl-5-propionyl pyrimidin-2,4,6-trione **4a**, 1,3-dimethyl-5-acetyl pyrimidin-2,4,6-trione **4b**, or 5-acetyl-1,3-diethyl-2-thioxodihydropyrimidine-4,6 (1*H*,5*H*)-dione **4c** in ethanol (EtOH) in the presence of drops of glacial acetic acid as shown in Scheme 1 to afford the target products **5a−k** in excellent yields and purities as indicated from their spectral data (Figures S1−S12, Supporting Information).

The NMR data for all of the target products **5a−k** show that they are in the enhydrazine **a** form rather than the hydrazone **b** form (Figure 4), where the two peaks in the range of δ 90.0

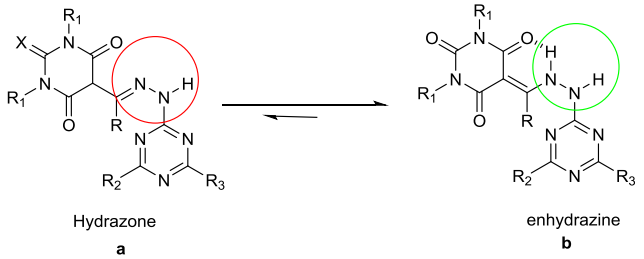
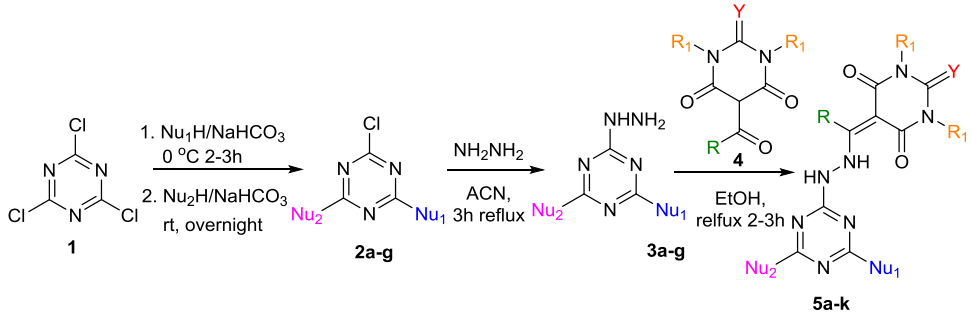


Figure 4. Tautomeric structure of hydrazone-enhydrazine.

and 198.0 ppm are related to the two carbon atoms similar to those of the enhydrazine form (C=C−NH−NH−). In addition, the hydrogen bond in the enhydrazine form stabilizes structure **b** more than it does the hydrazone isomer **a** (Figure 4). This observation is also in good agreement with our

Scheme 1. Synthesis of Pyrimidinetrione/Thiopyrimidimetrione-*s*-Triazine Derivatives



	Nu_1	Nu_2	Y	R_1	R
5a	Morpholine	Morpholine	O	CH_3	C_2H_5
5b	Morpholine	Piperidine	O	CH_3	C_2H_5
5c	Benzyl	Morpholine	O	CH_3	C_2H_5
5d	Diethylamine	Morpholine	O	CH_3	C_2H_5
5e	Benzyl	Piperidine	O	CH_3	C_2H_5
5f	Diethylamine	Piperidine	O	CH_3	C_2H_5
5g	Diethylamine	Piperidine	O	CH_3	CH_3
5h	Diethylamine	Piperidine	S	C_2H_5	CH_3
5i	Diethylamine	Morpholine	S	C_2H_5	CH_3
5j	Morpholine	Piperidine	S	C_2H_5	CH_3
5k	Piperidine	Piperidine	S	C_2H_5	CH_3

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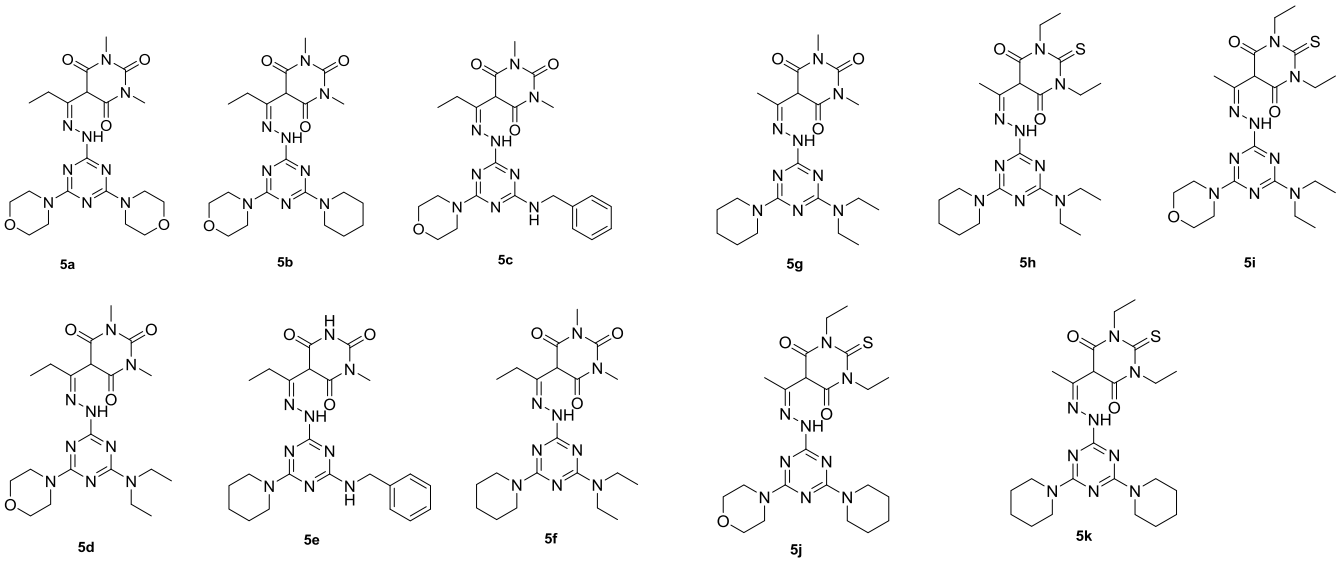

Figure 5. Structure of the target products.

previously reported data[40] and those reported by Giziroglu et al.[41]

Antiproliferative Activities. The antiproliferative activities of the target compounds (Figure 5) were tested against four cancer cell lines at concentrations ranging between 616 pg/mL and 1 mg/mL using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The results are represented in Table 1.

Table 1. IC_{50} Values of the Derivatives in Three Cancer Cell Lines, as Measured Using the MTT Assay

	A549	HepG2	HCT-116	MCF-7
compound	IC_{50} (μg/mL)	IC_{50} (μg/mL)	IC_{50} (μg/mL)	IC_{50} (μg/mL)
5a	ND[a]	ND[a]	ND[a]	ND[a]
5b	48.2 ±1.8	ND[a]	15.5 ± 2.1	34.5 ± 2.3
5c	ND[a]	ND[a]	ND[a]	54.6 ± 3.5
5d	71.5 ± 2.3	60.3 ± 7.9	41.6 ± 2.0	48.4 ± 2.1
5e	16.1 ± 3.1	13.8 ± 1.0	14.2 ± 0.3	12.9 ± 0.3
5f	6.9 ± 0.6	10.5 ± 0.3	3.9 ± 0.4	16.1 ± 1.5
5g	9.4 ± 7.2	15.2 ± 1.8	13.1 ± 1.2	19.1 ± 0.7
5h	1.6 ± 0.6	3.8 ±0.3	1.9 ± 0.4	1.2 ± 0.5
5i	9.7 ± 3.3	11.0 ± 0.2	7.9 ± 0.3	15.8 ± 0.7
5j	18.2 ± 1.8	20.2 ± 0.9	15.5 ± 0.2	24.5 ± 2.3
5k	12.2 ± 1.5	13.2 ± 0.9	10.5 ± 2.1	18.2 ± 2.3

[a]ND not active.

The barbiturate series **5a−g** showed poor to no activity against the four cancer cell lines (Table 1). Only derivatives **5f** and **5g** showed moderate activity against the four tested cell lines. These two derivatives contained both a diethylamine and a piperidine moiety on the *s*-triazine ring with a methyl or an ethyl group at the azomethene group (C═N−NH−) in the hydrazone moiety. Based on these results, it can be concluded that the presence of diethylamine with the piperidine on the *s*-triazine moiety enhances the antiproliferative activities, as shown in **5h** (thio series) and **5f** and **5g** (oxa series). Moreover, the piperidine core clearly emerges as preferable to the structurally similar morpholine one (**5b** *vs* **5a**, **5e** *vs* **5c**, **5f** *vs* **5d**, **5h** *vs* **5i**, **5k** *vs* **5j**). These results indicate that the substituent on the *s*-triazine core has a great effect on the antiproliferative activity.

The presence of thiopyrimidinetrione increased the activity of both, as shown in Table 1. Compound **5h** with the piperidine-diethylamine substituent on the *s*-triazine ring gave excellent results for the four cancer cell lines (Table 1) and showed better performance than compound **5i** with the morpholine as shown in Table 1.

The obtained results here agreed with the previously reported results, where the presence of piperidine ring on the triazine moiety increases the activity against the cancer cell line. In addition, the dipiperidino derivatives showed higher activity than the morpholino-piperidino derivatives **5k** *vs* **5j**, respectively. These observations agreed also with our reported results for derivatives shown in Figure 2[35−37,40]

These results led us to study the induction of apoptosis in HepG2 cells after treatment with the most potent derivatives **5h**. The percentages of early and late apoptotic cells were determined by double staining with annexin V and propidium iodide (PI) *via* flow cytometry. The positioning of quadrants on dot plots was designated, and living cells (annexin V−/PI−), early apoptotic cells (annexin V+/PI−), late apoptotic cells (annexin V+/PI+), and necrotic cells (annexin V−/PI+) were identified. Representative results are shown in Figure 6. These data demonstrate that incubation of HepG2 cells with **5h** at concentrations of 15 and 5 μg/mL, respectively, for 24 h markedly decreased the population of viable cells and increased the percentage of apoptotic cells. In the untreated controls and solvent controls, the percentage of apoptotic cells was negligible. When HepG2 cells were exposed to 5 μg/mL (about 5× IC_{50} value) of **5h** for 24 h, the percentage of early apoptotic cells increased from 1.23% in the dimethyl sulfoxide (DMSO) control (Figure 6b) to 15.8% in the treated cells (Figure 6c) and that of late apoptotic cells increased from 1.08% in the DMSO control (Figure 6b) to 4.5% (Figure 6c) in the treated cells.

■ CONCLUSIONS

In summary, the newly synthesized *s*-triazine hydrazone pyrimidinetrione (thiopyrimidinetrione) derivatives described in this study showed weak to high activity against the tested

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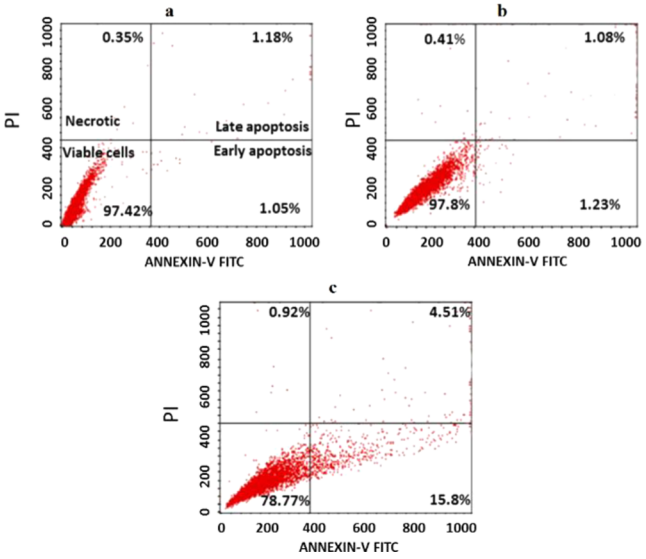

Figure 6. Flow cytometric analysis (annexin V-FTIC/PI assay) of HepG2 cells exposed for 24 h to 15 μg/mL of **5h** (c): (a) untreated control and (b) vehicle control (DMSO). The represented dot plots showing percentage of viable, early apoptotic, late apoptotic, and necrotic cells.

four cancer cell lines. The results reveal that the pyrimidinetrione (barbituric) series show poor to no activity against cancer cell lines. However, only one compound **5f** (with the diethylamino-pieridino derivatives on the triazine moiety from the barbituric series) inhibited the growth of the four cell lines, being the most effective and more selective to HCT-116 and having IC_{50} of 3.9 ± of 0.4 μg/mL. This observation indicates that the substituent on the *s*-triazine core has a great effect on the antiproliferative activity of these compounds. Indeed, the combination between the piperidino and diethylamine on the *s*-triazine core is the optimal configuration.

Thiobarbiturate showed better activity than the analogous barbiturate derivatives (**5h−k** *vs* **5a−g**). The piperidino-benzyl derivatives **5e** showed higher activity than its analogues morpholino-benzyl **5c** derivatives. These results agreed with our previous results. Derivatives with the piperidino and diethylamino on the *s*-triazine ring showed promising and highest activity against all tested cell lines. Again, the piperidino derivatives have higher activity than the morpholino derivatives, *e.g.*, **5h** showed IC_{50} of 1.6 ± 0.6, 3.8 ± 0.3, 1.9 ± 0.4, and 1.2 ± 0.5 μg/mL, while **5i** showed IC_{50} of 9.7 ± 3.3, 11 ± 0.2, 7.9 ± 0.3, and 15.8 ± 0.7 μg/mL for the tested cell lines A549, HepG2, HCT-116, and MCF-7, respectively. The obtained results agreed with the previously reported results, where the presence of piperidine on the *s*-triazine ring increases the activity against the cancer cell. In addition, the dipiperidino derivatives showed higher activity than the morpholino-piperdino derivatives **5k** *vs* **5j**, respectively. These observations agreed also with our previously reported results[35−37,40] for the derivatives shown in Figure 2.

Finally, our findings demonstrate that the derivative **5h** at concentrations of 15 and 5 μg/mL, respectively, is able to induce apoptosis in HepG2 cells. These results indicate that *s*-triazine may provide an excellent scaffold for the development of anticancer drug candidates.

MATERIALS AND METHODS

All solvents were used directly from the vendor. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a JEOL 400 MHz spectrometer (JEOL, Ltd., Tokyo, Japan); at room temperature in $CDCl_3$ and/or DMSO-d_6 using an internal standard, δ values were expressed in ppm. Elemental analyses were performed on PerkinElmer 2400 elemental analyzer (PerkinElmer, Inc. 940 Winter Street, Waltham, MA). Melting points were recorded on a Gallenkamp melting point apparatus (Sigma-Aldrich Chemie GmbH, 82024 Taufkirchen, Germany) and are uncorrected.

Chemistry. *Synthesis of Compounds **3a−g**.* Compounds **3a−g** were synthesized as previously described by our group[42] and characterized and used without further purification.

*5-Acetyl-1,3-diethyl-2-thioxodihydropyrimidine-4,6-(1H,5H)-dione **(4c)**.* Compound **4c** was prepared following the reported method for 4a,b[33] and obtained as an off-white solid from EtOH in yield 78%; mp 58−59 °C, ^{1}H-NMR ($CDCl_3$) δ = 1.23−1.30 (m, 6H, $2CH_3$), 2.71 (s, 3H, CH_3), 4.47−4.55 (m, 4H, $2CH_2$), 17.73 (s, 1H, OH); ^{13}C-NMR ($CDCl_3$) δ = 11.89, 12.19, 25.41, 42.92, 43.25, 97.29, 158.76, 167.57, 177.32, 198.08. Anal. calcd for $C_{10}H_{14}N_2O_3S$ (242.29): C, 49.57; H, 5.82; N, 11.56. Found C, 49.81; H, 5.98; N, 11.82.

*General Method for the Synthesis of 4,6-Disubstituted 1,3,5-Triazine-2-hydrazone Derivatives (**5a−k**).* To a solution of pyrimidinetrione (or thiopyrimidinetrione) **4a−c** (5 mmol) in EtOH (10 mL) containing two to three drops of acetic acid, 4,6-disubstituted 1,3,5-triazine-2-hydrazino **3a−g** (5 mmol) was added and the reaction mixture was stirred under reflux for 2−3 h. The solvent was reduced under vacuum and the precipitated product was filtered off and dried at room temperature. The products were collected by simple filtration and then recrystallized to afford the target compounds.

*5-(1-(2-(4,6-Dimorpholino-1,3,5-triazin-2-yl)hydrazinyl)-propylidene)-1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione (**5a**).* White solid from EtOH in yield 88%; mp 143−145 °C; ^{1}H-NMR ($CDCl_3$) δ = 1.24 (t, 3H, J = 7.6 Hz, CH_3), 3.29 (m, 8H, CH_2, $2CH_3$), 3,68 (m, 8H, $4CH_2$-N), 3.75 (m, 8H, $4CH_2$-O), 14.21 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ = 11.6, 22.4, 27.7, 43.8, 66.6, 88.5, 151.3, 162.1, 163.8, 164.3, 166.3, 175.5. Anal. calcd for $C_{20}H_{29}N_9O_5$ (475.51): C, 50.52; H, 6.15; N, 26.51. Found C, 50.66; H, 6.31; N, 26.76.

*1,3-Dimethyl-5-(1-(2-(4-morpholino-6-(piperidin-1-yl)-1,3,5-triazin-2-yl)hydrazinyl)propylidene)pyrimidine-2,4,6-(1H,3H,5H)-trione (**5b**).* Yellow solid from ethylacetate in yield 87%; mp 210−212 °C; ^{1}H-NMR ($CDCl_3$) δ = 1.24 (t, 3H, J = 7.8 Hz, CH_3), 1.5−1.61 (m, 6H, $3CH_2$), 3.29 (m, 8H, CH_2, $2CH_3$), 3,68−3.77 (m, 12H, $6CH_2$), 14.2 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ = 11.6, 22.4, 24.2, 27.7, 43.8, 59.8, 66.8, 88.7, 151.2, 162.2, 163.6, 164.5, 166.3, 175.6. Anal. calcd for $C_{21}H_{31}N_9O_4$ (473.54): C, 53.27; H, 6.60; N, 26.62. Found C, 53.51; H, 6.73; N, 26.88.

*5-(1-(2-(4-(Benzylamino)-6-morpholino-1,3,5-triazin-2-yl)hydrazinyl)propylidene)-1,3-dimethylpyrimidine-2,4,6-(1H,3H,5H)-trione (**5c**).* White solid from ethylacetate in yield 82%; mp 222−223 °C; ^{1}H-NMR ($CDCl_3$) δ = 1.23 (t, 3H, J = 7.2 Hz, CH_3), 3.27 (m, 8H, CH_2, $2CH_3$), 3.67 (brs, 8H, $4CH_2$), 3,78 (brs, 8H, $4CH_2$), 4.56 (d, 2H, J = 6.0 Hz, CH_2-Ph), 7.26−7.30 (m, 5H, C_6H_5-), 14.2 (brs, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ = 11.7, 22.4, 27.9, 44.9, 59.8, 66.6, 88.8, 127.5, 128.6, 151.2, 162.2, 163.6, 164.5, 166.3, 175.6. Anal.

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calcd for $C_{23}H_{29}N_9O_4$ (495.54): C, 55.75; H, 5.90; N, 25.44. Found C, 55.99; H, 5.83; N, 25.69.

*5-(1-(2-(4-(Diethylamino)-6-morpholino-1,3,5-triazin-2-yl)hydrazinyl)propylidene)-1,3-dimethylpyrimidine-2,4,6-(1H,3H,5H)-trione (**5d**).* Pale yellow solid from EtOH in 85% yield; mp 171−173 °C; ^{1}H-NMR ($CDCl_3$) δ = 1.13 (t, 6H, J = 6.8 Hz, $2CH_3$), 1.26 (t, 3H, J = 7.2 Hz, CH_3), 3.29−3.34 (m, 8H, CH_2, $2CH_3$), 3.52−3.54 (m, 4H, $2CH_2$), 3,70 (m, 4H, $2CH_2$-N), 3.77 (m, 4H, $2CH_2$), 14.2 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ = 11.7, 12.9, 22.4, 27.7, 41.6, 43.8, 66.7, 88.4, 151.2, 162.2, 169.5, 175.6. Anal. calcd for $C_{20}H_{31}N_9O_4$ (461.53): C, 52.05; H, 6.77; N, 27.31. Found C, 52.29; H, 6.96; N, 27.56.

*5-(1-(2-(4-(Benzylamino)-6-(piperidin-1-yl)-1,3,5-triazin-2-yl)hydrazinyl)propylidene)-1,3-dimethylpyrimidine-2,4,6-(1H,3H,5H)-trione (**5e**).* Off-white solid from ethylacetate in 86% yield; mp 226−227 °C; ^{1}H-NMR (DMSO-d_6) δ = 1.11 (t, 3H, J = 6.4 Hz, CH_3), 1.46 (brs, 4H, $2CH_2$), 1.58 (brs, 2H, CH_2), 3.67−3.9 (m, 6H, $3CH_2$), 4.46 (brs, 2H, CH_2-Ph), 7.26−7.30 (m, 5H, C_6H_5−), 14.85 (brs, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ = 11.2, 21.4, 24.2, 25.5, 27.2, 44.0, 78.6, 78.9, 79.2, 126.8, 127.4, 128.2, 150.8, 162.2, 161.5, 163.6, 166.3, 174.9. Anal. calcd for $C_{24}H_{31}N_9O_3$ (493.57): C, 58.40; H, 6.33; N, 25.54. Found C, 58.65; H, 6.54; N, 25.80.

*5-(1-(2-(4-(Diethylamino)-6-(piperidin-1-yl)-1,3,5-triazin-2-yl)hydrazono)propyl)-1,3-dimethylpyrimidine-2,4,6-(1H,3H,5H)-trione (**5f**).* Off-white solid from EtOH in 83% yield; mp 135−137 °C; ^{1}H-NMR ($CDCl_3$) δ = 1.12 (t, 6H, J = 7.2 Hz, $2CH_3$), 1.55 (brs, 4H, $2CH_2$), 2.75(s, 3H, CH_3), 3.29 (s, 6H, $2CH_3$), 3.52 (q, 4H, J = 6.8, 7.2 Hz, $2CH_2$), 3.70 (t, 4H, J = 6.0 Hz, $2CH_2$-N), 14.37 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ = 13.2, 16.8, 24.7, 25.7, 27.7, 41.6, 44.5, 89.0, 151.5, 162.4, 169.7, 175.6, 189.0. Anal. calcd for $C_{20}H_{31}N_9O_3$ (455.53): C, 53.92; H, 7.01; N, 28.30. Found C, 54.12; H, 7.20; N, 28.55.

*5-(1-(2-(4-(Diethylamino)-6-morpholino-1,3,5-triazin-2-yl)hydrazinyl)ethylidene)-1,3-diethyl-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (**5g**).* Pale yellow solid from EtOH in 88% yield; mp 149−150 °C; ^{1}H-NMR ($CDCl_3$) δ = 1.15 (t, 6H, J = 7.2 Hz, $2CH_3$), 1.26 (t, 6H, J = 7.2 Hz, $2CH_3$), 2.78 (s, 3H, CH_3), 3.52−3.57(q, 4H, J = 6.4, 7.6 Hz, $2CH_2$), 3.70 (m, 4H, $2CH_2$−N), 3.79 (m, 4H, $2CH_2$−O), 4.54 (q, 4H, J = 7.6, 6.4 Hz, $2CH_2$), 14.8 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ = 12.3, 17.2, 41.9, 43.1, 43.9, 66.6, 91.3, 162.9, 169.5, 177.4; Anal. calcd for $C_{21}H_{33}N_9O_3S$ (491.24): C, 51.31; H, 6.77; N, 25.64; O; found: C, 51.16; H, 6.51; N, 25.88.

*5-(1-(2-(4-(Diethylamino)-6-(piperidin-1-yl)-1,3,5-triazin-2-yl)hydrazono)ethyl)-1,3-diethyl-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (**5h**).* Yellow solid from ethylacetate in 81% yield; mp 222−224 °C; ^{1}H-NMR ($CDCl_3$) δ = 1.17 (t, 6H, J = 7.6 Hz, $2CH_3$), 1.26 (t, 6H, J = 7.2 Hz, $2CH_3$), 1.59 (brs, 4H, $2CH_2$), 1.65 (brs, 2H, CH_2), 3.58 (brs, 4H, $2CH_2$), 3.75 (brs, 4H, $2CH_2$), 4.55 (q, 4H, J = 7.2, 6.8 Hz, $2CH_2$), 15.11 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ = 12.3, 17.2, 24.5, 25.7, 42.1, 43.1, 44.9, 91.2, 151.5, 162.4, 169.7, 177.3. Anal. calcd for $C_{22}H_{35}N_9O_2S$ (489.64): C, 53.97; H, 7.21; N, 25.75. Found C, 54.18; H, 7.44; N, 25.98.

*5-(1-(2-(4-(Diethylamino)-6-morpholino-1,3,5-triazin-2-yl)hydrazono)ethyl)-1,3-diethyl-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (**5i**).* Yellow crystals from EtOH in 90% yield; mp 198−200 °C; ^{1}H-NMR ($CDCl_3$) δ = 1.17 (t, 6H, J = 7.2 Hz, $2CH_3$), 1.26 (t, 6H, J = 6.8 Hz, $2CH_3$), 2.78 (s, 3H, CH_3), 3.52−3.57 (m, 4H, $2CH_2$), 3.69 (brt, 4H, $2CH_2$), 3.71 (brt, 4H, $2CH_2$), 4.54 (q, 4H, 2CH2), 14.89 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ = 12.3, 17.1, 41.9, 43.1, 43.9, 66.6, 91.3, 162.9, 177.3. Anal. calcd for $C_{21}H_{33}N_9O_3S$ (491.24): C, 51.31; H, 6.77; N, 25.64. Found C, 51.43; H, 6.91; N, 25.82.

*1,3-Diethyl-5-(1-(2-(4-morpholino-6-(piperidin-1-yl)-1,3,5-triazin-2-yl)hydrazono)ethyl)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (**5j**).* Yellow powder from ethylacetate in 89% yield; mp 199−203 °C; ^{1}H-NMR ($CDCl_3$) δ = 1.16−1.23 (m, 6H, $2CH_3$), 1.57−1.64 (m, 6H, $3CH_2$), 2.72 (s, 3H, CH_3), 3.66 (m, 12H, $6CH_2$), 4.51 (q, 4H, J = 6.4 Hz, $2CH_2$), 14.89 (brs, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ = 12.3, 17.2, 18.4, 24.5, 25.7, 43.1, 43.9, 44.8, 58.4, 66.6, 91.4, 168.2, 177.4. Anal. calcd for $C_{22}H_{33}N_9O_3S$ (503.63): C, 52.47; H, 6.60; N, 25.03. Found C, 52.54; H, 6.83; N, 25.25.

*5-(1-(2-(4,6-Di(piperidin-1-yl)-1,3,5-triazin-2-yl)-hydrazono)ethyl)-1,3-diethyl-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (**5k**).* Yellow powder from ethylacetate in 92% yield; mp 189−193 °C; ^{1}H-NMR ($CDCl_3$) δ = 1.16−1.24 (m, 6H, $2CH_3$), 1.53−1.60 (m, 6H, $3CH_2$), 2.74 (s, 3H, CH_3), 3.64 (m, 4H, $2CH_2$), 4.51 (q, 4H, J = 6.4 Hz, $2CH_2$), 14.89 (brs, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ = 12.3, 17.2, 18.4, 24.6, 25.7, 43.1, 44.8, 58.4, 91.2, 167.4, 177.3. Anal. calcd for $C_{23}H_{35}N_9O_2S$ (501.65): C, 55.07; H, 7.03; N, 25.13. Found C, 55.25; H, 7.29; N, 25.39.

Antiproliferative Activity. *Cell Proliferation Assay.* As described previously,[32] growth inhibitions were measured in 96-well plates. Aliquots of 120 μL of the suspended cells (50 000 mL^{-1}) were added to 60 μL of a serial dilution of the inhibitor (S) and incubated for five days. After the incubation time, growth was determined the MTT assay. Briefly, 20 μL of MTT (5 mg/mL in phosphate-buffered saline (PBS)) was added to each well, and the plates were incubated for 2 h at 37 °C, and 5% CO_2-atmosphere in the cell incubator. The supernatants were removed, and the cells were washed two times, each with 100 μL of PBS. The formed water-insoluble formazan crystals were dissolved in 200 μL of isopropanol/HCl mixture. The absorbance was read at 595 nm using a microplate reader (Thermo Scientific). The metabolic activities of cells were calculated by dividing the absorbance of the treated cells by the absorbance of the control cells multiplied by 100%. The IC_{50} values were defined as the sample concentration inhibiting 50% of cell growth.

Detection of Apoptosis by Flow Cytometry. Apoptosis was measured in HepG2 cells by annexin V−propidium iodide (PI) double staining method using the annexin V-FITC apoptosis detection kit (BD Biosciences, San Diego). Briefly, the cells were exposed to **5h** at a concentration of about 5× IC_{50} values in six-well plates for 24 h. At the end of the exposure, the cells were washed with cold PBS, trypsinized, and centrifuged at 1000 rpm; then, the cell pellet was washed again with PBS and resuspended in 100 μL of 1× binding buffer (1×10^6 cells/mL). Then, 5 μL each of annexin V-FITC and PI were added to the cell suspension and the cells were gently vortexed. After incubation for 20 min at room temperature (25 °C) in the dark, the samples were diluted by adding 400 μL of 1× binding buffer. Annexin V/PI fluorescence was analyzed for each sample using a BD FACSCalibur flow cytometer. A total of 10 000 events were acquired for each sample, and the data were analyzed using Cell Quest Pro software (BD Biosciences).

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■ ASSOCIATED CONTENT

Supporting Information

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Figures S1−S12 present the NMR (^{1}H and ^{13}C) spectra for the prepared compounds **5a−k** (PDF)

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Author Contributions

The synthesis was carried out by H.A.R., K.D., and A.S.; the series was designed and supervised by A.E.-F., B.G.d.l.T., and F.A.; and the antiproliferative activities were carried by E.S. All authors contributed to the results and discussion. The first draft of the manuscript was prepared by H.A.R., K.D., A.S., and E.S.; all authors contributed in the final version.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Wang, J.; Radomski, M. W.; Medina, C.; Gilmer, J. F. MMP inhibition by barbiturate homodimers. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 444−447.

(2) Shaker, R. M.; Ishak, E. A. Barbituric acid utility in multicomponent reactions. *Z. Naturforsch.* **2011**, *66*, 1189−1201.

(3) Ziarani, G. M.; Aleali, F.; Lashgari, N. Recent applications of barbituric acid in multicomponent reactions. *RSC Adv.* **2016**, *6*, 50895−50922.

(4) Mahmudov, K. T.; Kopylovich, M. N.; Maharramov, A. M.; Kurbanova, M. M.; Gurbanov, A. V.; et al. Pombeiro, Barbituric acids as a useful tool for the construction of coordination and supramolecular compounds, A.J.L. *Coord. Chem. Rev.* **2014**, *265*, 1−37.

(5) Kobra, N.; Zahra, K. Barbituric acids in organic transformations, an outlook to the reaction media. *Mini-Rev. Org. Chem.* **2017**, *14*, 143−173.

(6) Barakat, A.; Islam, M. S.; Al-Majid, A. M.; Soliman, S. M.; Mabkhot, Y. N.; Al-Othman, Z. A.; Ghabbour, H. A.; Fun, H.-K. Synthesis of novel 5-monoalkylbarbiturate derivatives: new access to 1,2-oxazepines. *Tetrahedron Lett.* **2015**, *56*, 6984−6987.

(7) Bayat, M.; Bayat, Y.; Asayesh, S. S. One-pot synthesis of 2*H*-pyrano[2,3-*d*]pyrimidine derivatives. *Monatsh. Chem. − Chem. Mon.* **2012**, *143*, 479−483.

(8) Ghahremanzadeh, R.; Fereshtehnejad, F.; Bazgir, A. Chromeno-[2,3-*d*]pyrimidine-triones synthesis by a three-component coupling reaction. *Chem. Pharm. Bull.* **2010**, *58*, 516−520.

(9) Ismail, M. A.; Al-Shihry, S.; Arafa, R. K.; El-Ayaan, U. Synthesis, antimicrobial activity and molecular modeling study of substituted 5-aryl-pyrimido[5,4-*c*]quinoline-2,4-diones. *J. Enzyme Inhib. Med. Chem.* **2013**, *28*, 530−538.

(10) Pati, A.; Majumdar, P.; Garnayak, S.; Behera, A. K.; Behera, R. K. Regiospecific ring closure reactions of 1,3-diphenylthiobarbituric acid and dimedone: Formation of spiro *vs* fused heterocycles. *Indian J. Chem., Sect. B: Org. Med. Chem.* **2013**, *53*, 384−391.

(11) Girgis, A. S.; Farag, H.; Ismail, N. S. M.; George, R. F. Synthesis, hypnotic properties and molecular modeling studies of 1,2,7,9-tetraaza-spiro[4.5]dec-2-ene-6,8,10-triones. *Eur. J. Med.Chem.* **2011**, *46*, 4964−4969.

(12) Zhao, H.-W.; Tian, T.; Li, B.; Yang, Z.; Pang, H.-L.; Meng, W.; Song, X.-Q.; Chen, X.-Q. Diastereoselective synthesis of dispirobarbiturates through Et3N-catalyzed [3+2] cycloaddition of barbiturate-based olefins with 3-isothiocyanato oxindoles. *J. Org. Chem.* **2015**, *80*, 10380−10385.

(13) Khan, K. M.; Khan, M.; Karim, A.; Taha, M.; Ambreen, N.; Gojayev, A.; Perveen, S.; Choudhary, M. I. Xanthine oxidase inhibition by 5-aryledene *N*,*N*′-dimethylbarbituric acid derivatives. *J. Chem. Soc. Pak.* **2013**, *35*, 495−498.

(14) Khan, K. M.; Khan, M.; Ahmad, A.; Irshad, A.; Kardono, L. B. S.; Rahim, F.; Haider, S. M.; Ahmed, S.; Parveen, S. Antibacterial and antifungal activities of 5-arylidene-*N*,*N*-dimethylbarbiturates derivatives. *J. Chem. Soc. Pak.* **2014**, *36*, 1153−1157.

(15) Aly, H. M.; Kamal, M. M. Efficient one-pot preparation of novel fused chromeno[2,3-d]pyrimidine and pyrano[2,3-d]pyrimidine derivatives. *Eur. J. Med. Chem.* **2012**, *47*, 18−23.

(16) Figueiredo, J.; Serrano, J. L.; Cavalheiro, E.; Keurulainen, L.; Yli-Kauhaluomac, J.; Moreira, V. M.; Ferreira, S.; Domingues, F. C.; Silvestre, S.; Almeida, P. Trisubstituted barbiturates and thiobarbiturates: synthesis and biological evaluation as xanthine oxidase inhibitors, antioxidants, antibacterial and anti-proliferative agents. *Eur. J. Med. Chem.* **2018**, *143*, 829−842.

(17) Rauf, A.; Shahzad, S.; Bajda, M.; Yar, M.; Ahmed, F.; Hussain, N.; Akhtar, M. N.; Khan, A.; Jonczyk, J. Design and synthesis of new barbituric- and thiobarbituric acid derivatives as potent urease inhibitors: Structure activity relationship and molecular modeling studies. *Bioorg. Med. Chem.* **2015**, *23*, 6049−6058.

(18) Neumann, D. M.; Cammarata, A.; Backes, G.; Palmer, G. E.; Jursic, B. S. Synthesis and antifungal activity of substituted 2,4,6-pyrimidinetrione carbaldehyde hydrazones. *Bioorg. Med. Chem.* **2014**, *22*, 813−826.

(19) Bahar, A. A.; Liu, Z.; Garafalo, M.; Kallenbach, N.; Ren, D. Controlling persister and biofilm cells of gram-negative bacteria with a new 1, 3, 5-triazine derivative. *Pharmaceuticals* **2015**, *8*, 696−710.
(20) Yu, K.; Pieterse, C. M. J.; Bakker, P. A. H. M.; Berendsen, R. L. Beneficial microbes going underground of root immunity. *Plant Cell Environ* **2019**, *42*, 2860−2870.
(21) Mibu, N.; Yokomizo, K.; Aki, H.; Ota, N.; Fujii, H.; Yuzuriha, A.; Saneyoshi, S.; Tanaka, A.; Koga, A.; Zhou, J.; et al. Synthesis and Antiviral Evaluation of Some C3-Symmetrical Trialkoxy-Substituted 1, 3, 5-Triazines and Their Molecular Geometry. *Chem. Pharm. Bull.* **2015**, *63*, 935−944.
(22) Zacharie, B.; Abbott, S. D.; Bienvenu, J.-F. O.; Cameron, A. D.; Cloutier, J. E.; Duceppe, J.-S.; Ezzitouni, A.; Fortin, D.; Houde, K.; Lauzon, C.; et al. 2, 4, 6-trisubstituted triazines as protein a mimetics for the treatment of autoimmune diseases. *J. Med. Chem.* **2010**, *53*, 1138−1145.
(23) Liu, B.; Sun, T.; Zhou, Z.; Du, L. A systematic review on antitumor agents with 1, 3, 5-triazines. *Med. Chem.* **2015**, *5*, 131−148.
(24) Zhao, H.; Liu, Y.; Cui, Z.; Beattie, D.; Gu, Y.; Wang, Q. Design, synthesis, and biological activities of arylmethylamine substituted chlorotriazine and methylthiotriazine compounds. *J. Agric. Food. Chem.* **2011**, *59*, 11711−11717.
(25) Shah, D. R.; Modh, R. P.; Chikhalia, K. H. Privileged s-triazines: structure and pharmacological applications. *Future Med. Chem.* **2014**, *6*, 463−477.
(26) Hu, Z.; Ma, T.; Chen, Z.; Ye, Z.; Zhang, G.; Lou, Y.; Yu, Y. Solid-phase synthesis and antitumor evaluation of 2, 4-diamino-6-aryl-1, 3, 5-triazines. *J. Comb. Chem.* **2009**, *11*, 267−273.
(27) Zheng, M.; Xu, C.; Ma, J.; Sun, Y.; Du, F.; Liu, H.; Lin, L.; Li, C.; Ding, J.; Chen, K.; et al. Synthesis and antitumor evaluation of a novel series of triaminotriazine derivatives. *Biorg. Med. Chem.* **2007**, *15*, 1815−1827.
(28) Kuo, G.-H.; DeAngelis, A.; Emanuel, S.; Wang, A.; Zhang, Y.; Connolly, P. J.; Chen, X.; Gruninger, R. H.; Rugg, C.; Fuentes-Pesquera, A. Synthesis and identification of [1, 3, 5] triazine-pyridine biheteroaryl as a novel series of potent cyclin-dependent kinase inhibitors. *J. Med. Chem.* **2005**, *48*, 4535−4546.
(29) Sączewski, F.; Bułakowska, A.; Bednarski, P.; Grunert, R. Synthesis, structure and anticancer activity of novel 2, 4-diamino-1, 3, 5-triazine derivatives. *Eur. J. Med. Chem.* **2006**, *41*, 219−225.
(30) Sączewski, F.; Bułakowska, A. Synthesis, structure and anticancer activity of novel alkenyl-1, 3, 5-triazine derivatives. *Eur. J. Med. Chem.* **2006**, *41*, 611−615.
(31) Perspicace, E.; Jouan-Hureaux, V.; Ragno, R.; Ballante, F.; Sartini, S.; La Motta, C.; Da Settimo, F.; Chen, B.; Kirsch, G.; Schneider, S.; et al. Design, synthesis and biological evaluation of new classes of thieno [3, 2-d] pyrimidinone and thieno [1, 2, 3] triazine as inhibitor of vascular endothelial growth factor receptor-2 (VEGFR-2). *Eur. J. Med. Chem.* **2013**, *63*, 765−781.
(32) Bojarski, J. T.; Mokrosz, J. L.; Bartoń, H. J.; Paluchowska, M. H. Recent progress in barbituric acid chemistry. *Adv. Heterocycl. Chem.* **1985**, *38*, 229−297.
(33) Neumann, D. M.; Cammarata, A.; Backes, G.; Palmer, G. E.; Jursic, B. S. Synthesis and antifungal activity of substituted 2, 4, 6-pyrimidinetrione carbaldehyde hydrazones. *Bioorg. Med. Chem.* **2014**, *22*, 813−826.
(34) Rageot, D.; Bohnacker, T.; Keles, E.; McPhail, J. A.; Hoffmann, R. M.; Melone, A.; Borsari, C.; Sriramaratnam, R.; Sele, A. M.; Beaufils, F.; Hebeisen, P.; Fabbro, D.; Hillmann, P.; Burke, J. E.; Wymann, M. P. (S)-4-(Difluoromethyl)-5-(4-(3-methylmorpholino)-6-morpholino- 1,3,5-triazin-2-yl)pyridin-2-amine (PQR530), a potent, orally bioavailable, and brain-penetrable dual inhibitor of class I PI3K and mTOR Kinase. *J. Med. Chem.* **2019**, *62*, 6241−6261.
(35) El-Faham, A.; Farooq, M.; Almarhoon, Z.; Abd Alhameed, R.; Wadaan, M. A. M.; de la Torre, B. G.; Albericio, F. Di- and tri-substituted *s*-triazine derivatives: Synthesis, characterization, anti-cancer activity in human breast-cancer cell lines, and developmental toxicity in zebrafish embryos. *Bioorg. Chem.* **2020**, *94*, No. 103397.
(36) Farooq, M.; Sharma, A.; Almarhoon, Z.; Al-Dhfyan, A.; El-Faham, A.; Abu Taha, N.; Wadaan, M. A. M.; de laTorre, B. G.; Albericio, F. Design and synthesis of mono-and di-pyrazolyl-*s*-triazine derivatives, their anticancer profile in human cancer cell lines, and *in vivo* toxicity in zebrafish embryos. *Bioorg. Chem.* **2019**, *87*, 457−464.
(37) Barakat, A.; El-Senduny, F. F.; Almarhoon, Z.; Al-Rasheed, H. H.; Badria, F. A.; Al-Majid, A. M.; Ghabbour, H. A.; El-Faham, A. Synthesis, X-ray crystal structures, and preliminary anti-proliferative activities of new *s*-triazine-hydroxybenzylidene hydrazone derivatives. *J. Chem.* **2019**, *2019*, No. 9403908.
(38) Srivastava, G. K.; Alonso-Alonso, M. L.; Fernandez-Bueno, I.; Garcia-Gutierrez, M. T.; Rull, F.; Medina, J.; Coco, R. M.; Pastor, J. C. Comparison between direct contact and extract exposure methods for PFO cytotoxicity evaluation. *Sci. Rep.* **2018**, *8*, No. 1425.
(39) Bai, F.; Liu, H.; Tong, L.; Zhou, W.; Liu, L.; Zhao, Z.; Liu, X.; Jiang, H.; Wang, X.; Xie, H.; et al. Discovery of novel selective inhibitors for EGFR-T790M/L858R. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1365−1370.
(40) El-Faham, A.; Soliman, S. M.; Ghabbour, H. A.; Elnakady, Y. A.; Mohaya, T. A.; Siddiqui, M. R.; Albericio, F. Ultrasonic promoted synthesis of novel s-triazine-Schiff base derivatives; molecular structure, spectroscopic studies and their preliminary anti-proliferative activities. *J. Mol. Struct.* **2016**, *1125*, 121−135.
(41) Giziroglu, E.; Aygün, M.; Sarikurkcu, C.; Kazar, D.; Orhan, N.; Firinci, E.; Soyleyici, H. C.; Gokcen, C. Synthesis, characterization and antioxidant activity of new dibasic tridentate ligands: X-ray crystal structures of DMSO adducts of 1, 3-dimethyl-5-acetyl-barbituric acid o-hydroxybenzoyl hydrazone copper (II) complex. *Inorg. Chem. Commun.* **2013**, *36*, 199−205.
(42) Sharma, A.; Jad, Y.; Ghabbour, H.; de la Torre, B.; Kruger, H.; Albericio, F.; El-Faham, A. Synthesis, crystal structure and DFT studies of 1,3-dimethyl-5-propionylpyrimidine-2,4,6(1H,3H,5H)-trione. *Crystals* **2017**, *7*, No. 31.

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