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Original article

Cyclocondensation reaction of heterocyclic carbonyl compounds, Part XIII: Synthesis and cytotoxic activity of some 3,7-diaryl-5-(3,4,5-trimethoxyphenyl)pyrazolo[4,3-*e*][1,2,4]triazines ☆

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Abstract

A series of the 3,7-diaryl-5-(3,4,5-trimethoxyphenyl)pyrazolo[4,3-*e*][1,2,4]triazines (**17a–20f**) have been synthesized in five steps. The cytotoxic activity of all of the newly synthesized compounds has been tested in vitro against five cancer cell lines. Several compounds demonstrated significant broad cytotoxic activity in low micromolar range, while others were selectively active against lung adenocarcinoma cell line A549.

Keywords: Pyrazolo[4,3-*e*][1,2,4]triazines; Cytotoxic activity

1. Introduction

The pyrazolo[4,3-*e*][1,2,4]triazines has been less studied heterocyclic compounds so far in comparison with other condensed heterocycles which bear [1,2,4]triazine ring. There has been found a few pyrazolo[4,3-*e*][1,2,4]triazines of the natural origin. *Pseudoiodinine* and *Normethylpseudoiodinine* and some other related compounds are produced by the microorganisms of the genus *Pseudomonas fluorescens var. pseudoidininum* [2–4] and *Nostocin A* is produced by the cyanobacter *Nostoc spongiaeforme* [5]. These natural compounds have presented slight or moderate antineoplastic and antibacterial activity.

In our previous works we have described a few series of 3,5-disubstituted, and 3,5,7-trisubstituted pyrazolo[4,3-*e*]-[1,2,4]triazines [6–8] and tested their cytotoxic activity which was, however, very low. For this reason we have decided to perform another study of synthesis and cytotoxic activity of novel series of 3,7-diaryl-5-(3,4,5-trimethoxyphenyl)pyrazolo[4,3-*e*][1,2,4]triazines (**17a–20f**) as well as the study of cytotoxic activity of the key intermediates, 2-acylamino-3-(3,4,5-trimethoxyphenyl)acrylic acid hydrazides (**5–8**), 3-substituted-5-(3,4,5-trimethoxybenzyl)[1,2,4]triazine-6-ones (**9–12**) and arylhydrazones of 3-substituted-5-(3,4,5-trimethoxybenzoyl)[1,2,4]triazine-6-ones (**13a–16f**).

The 3,4,5-trimethoxyphenyl group on position 5 of the triazine ring could elevate the cytotoxic activity since a large number of molecules bearing the 3,4,5-trimethoxyphenyl group show various biological activity. The vicinal trimethoxy group is common to *Colchicine* [9], *Demecolcine*[10] and *Podophyllotoxin* [11] which show antimitotic activity as well as for *combrestatins* [12] and their analogs [13,14] which similarly to *Colchicine* and *Demecolcine* inhibit tubulin polymerisation and also in several synthetic compounds like *Trimethoprim* [15], *Duocarmine* analogs [16–19] and some

☆ See Ref. [1].

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HIV-1 reverse transcriptase inhibitors [20]. The antineoplastic activity of ansa-titanocene trimethoxy derivatives has been reported as well [21].

The target of this work was to find the relationship between structure and cytotoxic activity in the series of studied 3,7-diaryl-5-(3,4,5-trimethoxyphenyl)pyrazolo[4,3-*e*][1,2,4]triazines and the intermediates.

2. Chemistry

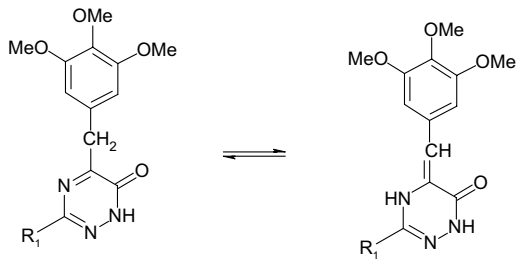
The starting (4*Z*)-2-substituted-4-(3,4,5-trimethoxybenzylidene)-1,3-oxazol-5(4*H*)-ones (**1**–**4**) were prepared by the modified *Erlenmeyer's* reaction. Compounds **2**, **3** and **4** have not been described in the literature so far. It is probable that oxazolone derivatives **1**–**4** form *Z* isomers only which has been proven for other similar compounds synthesized under similar conditions [25].

The hydrazinolysis of oxazolone ring under mild conditions gave the *N*-[(*Z*)-1-(hydrazinocarbonylvinyl)-2-(3,4,5-trimethoxyphenyl)]acylamides (**5**–**8**). The stereochemistry on the exocyclic double bond does not change during hydrazinolysis of oxazolone ring.

The cyclization reaction of the hydrazides **5**–**8** in boiling sodium hydroxide solution proceeded smoothly to the 3-substituted-5-(3,4,5-trimethoxybenzyl)[1,2,4]triazin-6(1*H*)-ones (**9**–**12**). We investigated also the tautomerism of [1,2,4]triazines (**9**–**12**) which can form tautomers **A** and **B**. We have proven that both of the possible forms were present in dimethylsulfoxide solution. This fact is in agreement with previously reported findings [22] which were focused on the study of tautomerism of analogous 3-substituted-5-arylmethylene-[1,2,4]triazin-6(1*H*)-ones. We have assigned the ratio of forms A and B by the comparison of relative intensities of methylene group hydrogens of form A and methine group hydrogen of form B (see Scheme 1 and Table 1).

The arylhydrazones of 3-substituted-5-(3,4,5-trimethoxybenzoyl)[1,2,4]triazin-6(1*H*)-ones (**13a**–**16f**) were obtained by the coupling reaction on active methylene group of compounds (**9**–**12**) with aryldiazonium salts in pyridine in high yields and purity. The stereochemistry of the arylhydrazones **13a**–**16f** has not been studied and is difficult to be supposed.

These arylhydrazones were cyclized to the target 3,7-diaryl-5-(3,4,5-trimethoxyphenyl)pyrazolo[4,3-*e*][1,2,4]triazines (**17a**–**20f**) by the action of phosphorous oxychloride with catalytic amount of pyridine (see Scheme 2).



9A - 12A 9B-12B

Scheme 1.

Table 1
Selected NMR data of compounds **9**–**12**

Compound	δ (CH_2)	Rel. int. (CH_2)	δ (=CH)	Rel. int. (=CH)	Ratio A/B
9	4.109	2.0	6.349	0.78	1.28
10	4.095	2.0	6.333	0.63	1.58
11	4.114	2.0	6.368	0.49	2.03
12	4.104	2.0	6.351	0.66	1.52

3. Pharmacology

The cytotoxic activities were examined under in vitro conditions using MTT assays towards five tumour cell lines [CEM – T-lymphoblastic leukemia, CEM DNR Bulk – T-lymphoblastic leukemia, daunorubicin resistant, K-562 (myeloid leukemia), K-562-Tax (myeloid leukemia, paclitaxel resistant) and A549 (lung adenocarcinoma)]. Those cell lines we routinely use for characterization of new compounds and were characterized previously [24]. The presented data represent mean values from three independent experiments, the standard deviation did not usually exceed 15% on the mean.

4. Results and discussion

The hydrazides (**5**–**8**) have shown only very slight cytotoxic activity against all of the tested cell lines and no structure–activity relationship could be found.

The cytotoxic activity of the 3-aryl-5-(3,4,5-trimethoxybenzyl)-1,6-dihydro-[1,2,4]triazine-6-ones **9**–**12** is interesting and among them **9** showed significant and broad activity against all tested cell lines.

There has been found significant cytotoxic activity at the arylhydrazones **13a**–**13f**, **14d**, **15a**, **15c**, **15d**, **15f**, **16a**, **16d** and **16f**, however, we were not able to identify any structure–activity relationship among these compounds.

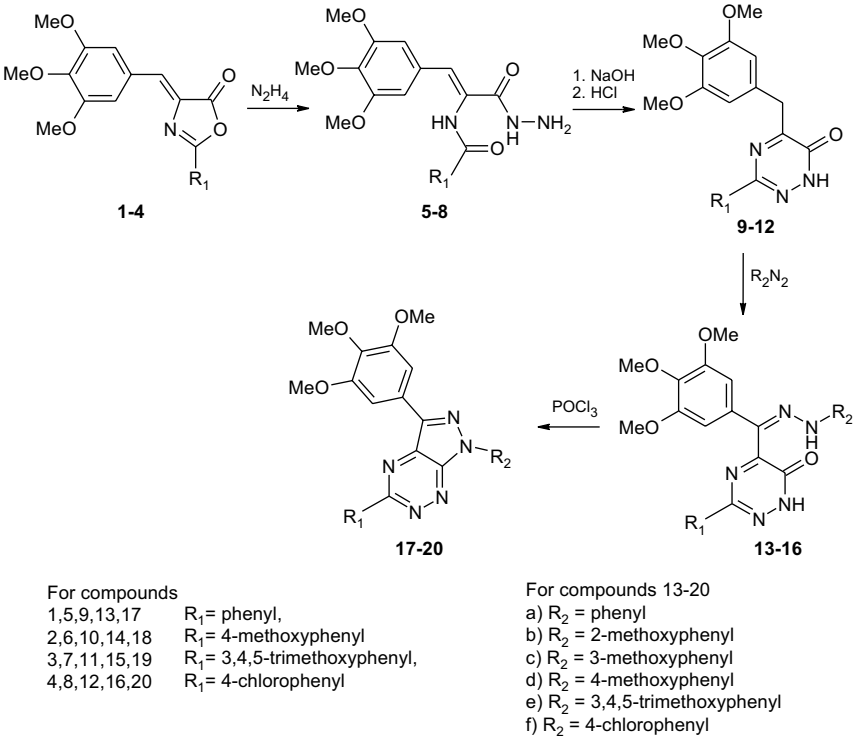
The most interesting, 3,7-diaryl-5-(3,4,5-trimethoxyphenyl)pyrazolo[4,3-*e*][1,2,4]triazines (**17b**, **17c**, **17e**, **18b**, **19b**, **19f**, **20b**, **20c** and **20e**) have exhibited significant and relative selective inhibitory activity against A549 cell line, while they were generally less active against leukemia cell lines, including otherwise highly chemosensitive CEM lymphoblasts. Those data suggest that this class of compounds could be predominantly active in epithelial cancers and deserves further experimental verification.

The results are summarized in Table 2.

5. Experimental

5.1. Apparatus and methods

Melting points were determined on a Boetius stage and are not corrected. ^{1}H NMR spectra were measured in DMSO-d_6 and $CDCl_3$ at 300 K on a Bruker Avance 300 spectrometer (300 MHz) with TMS as an internal standard; chemical shifts are reported in ppm, and coupling constants in Hz. Mass spectra were recorded using an LCQ ion trap mass spectrometer (Finnigan MAT, San Jose, CA, USA). Elemental analyses were performed using an EA 1108 Elemental Analyzer (Fison Instruments).



Scheme 2.

5.2. Chemistry

5.2.1. (4Z)-2-Substituted-4-(3,4,5-trimethoxybenzyli dene)-1,3-oxazol-5(4H)-ones **1–4**

5.2.1.1. General procedure. The *N*-acylglycine (56.00 mmol), 3,4,5-trimethoxybenzaldehyde (11.00 g, 56.00 mmol), acetic anhydride (16.0 ml, 167.5 mmol) and anhydrous pyridine (4.50 ml, 56.00 mmol) was heated on an oil bath at temperature 80 °C for 2 h. After cooling to room temperature, the mixture was allowed to stand at 0 °C overnight. The precipitate was filtered and washed three times with ice-cooled ethanol (10 ml). The crude product was crystallized from toluene.

5.2.2. (4Z)-2-Phenyl-4-(3,4,5-trimethoxybenzyli dene)-1,3-oxazol-5(4H)-one **1**

See Ref. [23].

5.2.3. (4Z)-2-(4-Methoxyphenyl)-4-(3,4,5-trimetho xybenzylidene)-1,3-oxazol-5(4H)-one **2**

Yield: 68%, mp: 166–167 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.77 (s, 3H, OCH_3), 3.87 (s, 6H, OCH_3), 3.90 (s, 3H, OCH_3), 7.18 (d, $J = 8.7$, 2H, ArH), 7.21 (s, 1H, =CH), 7.72 (s, 2H, ArH), 8.05 (d, $J = 8.7$, 2H, ArH), ms: 370.40 (MH^+). Anal. Calcd for $C_{20}H_{19}NO_6$ (369.37): C, 65.03; H, 5.18; N, 3.79. Found: C, 65.23; H, 5.11; N, 3.57.

5.2.4. (4Z)-2-(3,4,5-Trimethoxyphenyl)-4-(3,4,5-trimethoxy benzylidene)-1,3-oxazol-5(4H)-one **3**

Yield: 58%, mp: 194–196 °C, ^{1}H NMR (300 MHz, DMSO-d_6): 3.77 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.89 (s, 12H, OCH_3), 7.29 (s, 1H, =CH), 7.38 (s, 2H, ArH), 7.79 (s, 2H, ArH), ms: 430.40 (MH^+). Anal. Calcd for $C_{22}H_{23}NO_8$ (429.43): C, 61.53; H, 5.39; N, 3.26. Found: C, 61.77; H, 5.73; N, 3.30.

5.2.5. (4Z)-2-(4-Chlorophenyl)-4-(3,4,5-trimethoxy benzylidene)-1,3-oxazol-5(4H)-one **4**

Yield: 73%, mp: 178–180 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.78 (s, 3H, OCH_3), 3.88 (s, 6H, OCH_3), 7.32 (s, 1H, =CH), 7.70 (d, $J = 8.7$, 2H, ArH), 7.76 (s, 2H, ArH), 8.09 (d, $J = 8.7$, 2H, ArH), ms: 374.90 (MH^+). Anal. Calcd for $C_{19}H_{16}NO_5Cl$ (373.84): C, 61.05; H, 4.31; N, 3.75. Found: C, 60.97; H, 4.54; N, 3.53.

5.2.6. N-[(Z)-1-(Hydrazinocarbonylvinyl)-2-(3,4,5-trime thoxyphenyl)]acylamides **5–8**

5.2.6.1. General procedure. The (4*Z*)-2-substituted-4-(3,4,5-trimethoxybenzylidene)-1,3-oxazol-5(4*H*)-one (14.00 mmol) was suspended in absolute ethanol (30 ml) and 99% hydrazine hydrate (0.825 ml, 17.00 mmol) was added. The mixture was refluxed for 3 min and filtered while hot. The filtrate was cooled to room temperature and allowed to stand at 0 °C for 2 h. The white precipitate was filtered and washed twice with ice-cooled ethanol (10 ml). The crude product was crystallized from ethanol.

5.2.7. N-[(Z)-1-(Hydrazinocarbonylvinyl)-2-(3,4,5-trimetho xyphenyl)]benzamide **5**

Yield: 78%, mp: 170–172 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.57 (s, 6H, OCH_3), 3.04 (s, 3H, OCH_3), 4.40

Table 2
Results of MTT cytotoxic activity tests (IC_{50} in μmol/l)

	CEM	CEM DNR Bulk	K562	K562 tax	A549
5	124	121	162	162	164
6	144	147	172	167	134
7	123	126	160	153	103
8	134	130	166	174	161
9	2.71	3.21	1.17	3.88	3.46
10	3.15	13.3	3.07	19.9	9.31
11	38.4	129	82.1	138	117
12	12.9	14.6	9.63	24.7	15.8
13a	10.6	18.2	11.5	19.3	7.14
13b	13.7	43.2	148	145	3.63
13c	8.60	19.3	12.7	13.7	8.30
13d	4.16	9.46	6.27	11.0	3.06
13e	12.2	33.2	7.40	27.6	3.19
13f	25.1	46.8	12.0	38.4	8.55
14a	9.81	39.8	18.4	31.8	23.7
14b	54.6	141	148	250	250
14c	68.7	132	162	250	250
14d	6.43	9.80	2.73	8.45	6.20
14e	24.2	47.4	45.5	137	3.33
14f	20.2	50.1	41.3	238	18.5
15a	5.06	95.5	44.9	8.60	3.30
15b	61.9	203	144	186	75.2
15c	7.83	140	112	22.1	3.38
15d	5.71	34.6	18.7	8.91	3.39
15e	53.8	158	94.3	118	162
15f	2.25	175	246	128	1.18
16a	10.8	37.7	17.5	33.1	22.1
16b	54.5	36.6	54.1	250	60.5
16c	11.5	133	41.8	26.3	8.32
16d	9.49	9.16	7.50	8.33	2.34
16e	42.1	139	50.1	159	200
16f	7.67	37.7	10.2	12.5	3.81
17a	109	108	169	216	86.6
17b	109	118	182	94.3	5.41
17c	8.41	99.3	117	159	0.61
17d	117	100	84.4	150	39.0
17e	65.2	129	52.6	99.9	2.16
17f	150	156	250	173	57.3
18a	114	114	166	203	78.8
18b	105	119	178	155	6.58
18c	54.5	184	115	174	124
18d	124	118	186	229	113
18e	212	117	230	246	160
18f	115	118	203	232	113
19a	142	109	183	233	92.3
19b	126	125	182	156	15.0
19c	48.3	109	164	141	163
19d	36.9	118	66.2	161	75.6
19e	246	201	241	250	194
19f	82.2	115	158	196	8.26
20a	11.6	113	220	225	130
20b	53.8	35.7	48.7	20.7	3.62
20c	54.7	108	192	219	2.86
20d	226	139	248	250	113
20e	99.9	117	192	210	15.0
20f	68.9	126	190	222	155

(br s, 2H, NH_2), 6.92 (s, 2H, ArH), 7.25 (s, 1H, =CH), 7.49 (t, $J = 7.5$, 2H, ArH), 7.58 (t, $J = 7.5$, 1H, ArH), 8.04 (d, $J = 7.5$, 2H, ArH), 9.51 (br s, 1H, NH), 9.81 (br s, 1H, NH), ms: 372.38 (MH^+). Anal. Calcd for $C_{19}H_{21}N_3O_5$ (371.39): C, 61.45; H, 5.70; N, 11.31. Found: C, 61.15; H, 5.70; N, 5.95.

5.2.8. N-[2-(3,4,5-Trimethoxyphenyl)-1-hydrazino carbonylvinyl]-(4-methoxy)benzamide ***6***

Yield: 90%, mp: 184–186 °C, ^1H NMR (300 MHz, DMSO-d_6): δ 3.58 (s, 6H, OCH_3), 3.63 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 4.38 (br s, 2H, NH_2), 6.92 (s, 2H, ArH), 7.02 (d, $J = 9.0$, 2H, ArH), 7.21 (s, 1H, =CH), 8.01 (d, $J = 9.0$, 2H, ArH), 9.45 (br s, 1H, NH), 9.65 (br s, 1H, NH), ms: 402.43 (MH^+). Anal. Calcd for $C_{20}H_{23}N_3O_6$ (401.42): C, 59.84; H, 5.77; N, 10.47. Found: C, 60.00; H, 6.01; N, 10.50.

5.2.9. N-[(Z)-1-(Hydrazinocarbonylvinyl)-2-(3,4,5-trimetho xyphenyl)](3,4,5-trimethoxy)benzamide ***7***

Yield: 95%, mp: 188–190 °C, ^1H NMR (300 MHz, DMSO-d_6): δ 3.62 (s, 6H, OCH_3), 3.65 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 3.83 (s, 6H, OCH_3), 4.41 (br s, 2H, NH_2), 6.95 (s, 2H, ArH), 7.23 (s, 1H, =CH), 7.40 (s, 2H, ArH), 9.48 (s, 1H, NH), 9.78 (s, 1H, NH), ms: 462.50 (MH^+). Anal. Calcd for $C_{22}H_{27}N_3O_8$ (461.47): C, 57.26; H, 5.90; N, 9.11. Found: C, 57.38; H, 6.10; N, 8.94.

5.2.10. N-[(Z)-1-(Hydrazinocarbonylvinyl)-2-(3,4,5-trimeth oxyphenyl)](4-chloro)benzamide ***8***

Yield: 95%, mp: 163–164 °C, ^1H NMR (300 MHz, DMSO-d_6): δ 3.59 (s, 6H, OCH_3), 3.64 (s, 3H, OCH_3), 4.41 (br s, 2H, NH_2), 6.91 (s, 2H, ArH), 7.23 (s, 1H, =CH), 7.59 (d, $J = 8.7$, 2H, ArH), 8.06 (d, $J = 8.7$, 2H, ArH), 9.54 (s, 1H, NH), 9.90 (s, 1H, NH), ms: 406.93 (MH^+). Anal. Calcd for $C_{19}H_{20}N_3O_5Cl$ (405.88): C, 56.23; H, 4.97; N, 10.36. Found: C, 56.19; H, 4.85; N, 10.16.

5.2.11. 3-Substituted-5-(3,4,5-trimeth oxybenzyl)[1,2,4]triazin-6(1H)-ones ***9–12***

5.2.11.1. General procedure. The *N*-[2-(3,4,5-trimethoxyphen yl)-1-hydrazinocarbonylvinyl]acylamide **5–8** (7.50 mmol) was refluxed in 1 M sodium hydroxide water solution for 5 min. After cooling to room temperature the solution was slowly neutralized with acetic acid. The yellow precipitate was filtered and washed with water (30 ml). The crude product was crystallized from ethanol.

5.2.12. 3-Phenyl-5-(3,4,5-trimethoxybenzyl)[1,2,4]tr iazin-6(1H)-one ***9***

Yield: 87%, mp: 169–171 °C, ^1H NMR (300 MHz, DMSO-d_6): **9A**: 3.639 (s, 3H, OCH_3), 3.749 (s, 6H, OCH_3), 4.109 (s, 2H, CH_2), 6.708 (s, 2H, ArH), 7.456–7.481 (m, 3H, ArH), 8.007–8.040 (m, 2H, ArH), 13.502 (s, 1H, NH), **9B**: 3.683 (s, 3H, OCH_3), 3.868 (s, 6H, OCH_3), 6.349 (s, 1H, =CH), 7.456–7.481 (m, 3H, ArH), 7.742–7.774 (m, 2H, ArH), 9.823 (s, 1H, NH), 11.139 (s, 1H, NH), ^{13}C NMR: 55.80, 59.91, 104.53, 105.56, 107.01, 126.01, 126.45, 126.60, 128.26, 128.68, 129.78, 130.03, 130.31, 131.22, 131.79, 133.98, 136.38, 141.84, 146.72, 152.63, 152.81, 154.09, 157.16, 169.42, ms: 354.20 (MH^+). Anal. Calcd for $C_{19}H_{19}N_3O_4$ (353.38): C, 64.58; H, 5.42; N, 11.89. Found: C, 64.53; H, 5.21; N, 12.01.

5.2.13. 3-(4-Methoxyphenyl)-5-(3,4,5-trimetho xybenzyl)[1,2,4]triazin-6(1H)-one ***10***

Yield: 95%, mp: 192–194 °C, ^{1}H NMR (300 MHz, DMSO-d_6): **12A**: 3.639 (s, 3H, OCH_3), 3.748 (s, 6H, OCH_3), 4.104 (s, 2H, CH_2), 6.698 (s, 2H, ArH), 7.535–7.564 (m, 2H, ArH), 8.020 (d, $J = 8.7$, 2H, ArH), 11.178 (s, 1H, NH), **12B**: 3.682 (s, 3H, OCH_3), 3.860 (s, 6H, OCH_3), 6.351 (s, 1H, =CH), 6.908 (s, 2H, ArH), 7.535–7.564 (m, 2H, ArH), 7.759 (d, $J = 8.7$, 2H, ArH), 9.890 (s, 1H, NH), 13.567 (s, 1H, NH), ms: 384.45 (MH^+). Anal. Calcd for $C_{20}H_{21}N_3O_5$ (383,4): C, 62.65; H, 5.52; N, 10.96. Found: C, 62.74; H, 5.76; N, 10.84.

5.2.14. 3-(3,4,5-Trimethoxyphenyl)-5-(3,4,5-trimetho xybenzyl)[1,2,4]triazin-6(1H)-one ***11***

Yield: 94%, mp: 157–160 °C, ^{1}H NMR (300 MHz, DMSO-d_6): **10A**: 3.642 (s, 3H, OCH_3), 3.751 (s, 6H, OCH_3), 3.802 (s, 3H, OCH_3), 4.095 (s, 2H, CH_2), 6.702 (s, 2H, ArH), 7.037 (d, $J = 8.7$, 2H, ArH), 7.956 (d, $J = 8.7$, 2H, ArH), 11.060 (s, 1H, NH), **10B**: 3.685 (s, 3H, OCH_3), 3.810 (s, 3H, OCH_3), 3.868 (s, 6H, OCH_3), 6.333 (s, 1H, =CH), 6.909 (s, 2H, ArH), 7.010 (d, $J = 8.7$, 2H, ArH), 7.783 (d, $J = 8.7$, 2H, ArH), 9.726 (s, 1H, NH), 13.381 (s, 1H, NH), ms: 444.13 (MH^+). Anal. Calcd for $C_{22}H_{25}N_3O_7$ (443.46): C, 59.59; H, 5.68; N, 9.48. Found: C, 59.47; H, 5.74; N, 9.61.

5.2.15. 3-(4-Chlorophenyl)-5-(3,4,5-trimetho xybenzyl)[1,2,4]triazin-6(1H)-one ***12***

Yield: 96%, mp: 179–181 °C, ^{1}H NMR (300 MHz, DMSO-d_6): **11A**: 3.632 (s, 3H, OCH_3), 3.698 (s, 3H, OCH_3), 3.745 (s, 6H, OCH_3), 3.772 (s, 6H, OCH_3), 4.114 (s, 2H, CH_2), 6.692 (s, 2H, ArH), 7.271 (s, 2H, ArH), 11.112 (s, 1H, NH), **11B**: 3.677 (s, 3H, OCH_3), 3.710 (s, 3H, OCH_3), 3.821 (s, 6H, OCH_3), 3.839 (s, 6H, OCH_3), 6.368 (s, 1H, =CH), 6.901 (s, 2H, ArH), 7.082 (s, 2H, ArH), 9.763 (s, 1H, NH), 13.432 (s, 1H, NH), ^{13}C NMR: 55.61, 55.78, 55.87, 56.02, 59.88, 59.98, 60.06, 103.33, 104.52, 106.08, 107.24, 126.48, 127.26, 129.33, 130.23, 131.19, 136.28, 136.66, 139.05, 139.30, 141.62, 146.22, 152.59, 152.81, 152.99, 154.00, 156.9, 169.40, ms: 388.92 (MH^+). Anal. Calcd for $C_{19}H_{18}N_3O_4Cl$ (387.87): C, 58.84; H, 4.68; N, 10.83. Found: C, 58.66; H, 4.94; N, 10.56.

5.2.16. 3-Substituted-5-[(3,4,5-trimethoxyphenyl) (arylhydrazono)methyl][1,2,4]triazin-6(1H)-ones ***13a–16f***

5.2.16.1. General procedure. The appropriate aromatic amine (3.10 mmol) was dissolved in the mixture of water (4.5 ml) and conc. hydrochloric acid (0.5 ml) and the solution was cooled to 0 °C. Then a solution of sodium nitrite (0.23 g, 3.30 mmol) in water (3.0 ml) was slowly added with stirring so that the temperature was maintained between 0 and 5 °C. The solution of aryldiazonium salt was stirred at 0 °C for further 20 min.

The 3-substituted-5-(3,4,5-trimethoxybenzyl)-1*H*-[1,2,4]triazine-6-one (3.00 mmol) was dissolved in pyridine (15 ml) and cooled to 0 °C. Then the solution of aryldiazonium salt was slowly added with stirring so that the temperature was maintained between 0 and 5 °C. Then the reaction mixture was allowed to stand at 0 °C overnight. After warming to room temperature was the solution diluted with water (25 ml) and the precipitate was filtered off and washed with water (50 ml). The crude product was used for further reaction without crystallisation.

5.2.17. 3-Phenyl-5-[(3,4,5-trimethoxyphenyl) (phenylhydrazono)methyl][1,2,4]-triazin-6(1H)-one ***13a***

Yield: 87%, mp: 207–209 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.70 (s, 3H, OCH_3), 3.77 (s, 6H, OCH_3), 6.84 (t, $J = 7.2$, 1H, ArH), 6.89 (s, 2H, ArH), 7.19 (t, $J = 7.5$, 2H, ArH), 7.27 (t, $J = 7.2$, 2H, ArH), 7.40 (t, $J = 7.5$, 1H, ArH), 7.51 (d, $J = 7.2$, 2H, ArH), 8.09 (d, $J = 7.5$, 2H, ArH), 10.11 (s, 1H, NH), 13.87 (s, 1H, NH), ms: 458.49 (MH^+). Anal. Calcd for $C_{25}H_{23}N_5O_4$ (457.49): C, 65.63; H, 5.07; N, 15.31. Found: C, 65.74; H, 5.07; N, 15.45.

5.2.18. 3-Phenyl-5-[(3,4,5-trimethoxyphenyl)(2-meth oxyphenylhydrazono)methyl][1,2,4]-triazin-6(1H)-one ***13b***

Yield: 86%, mp: 203–205 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.70 (s, 3H, $-OCH_3$), 3.77 (s, 3H, $-OCH_3$), 3.80 (s, 6H, $-OCH_3$), 6.89 (s, 2H, ArH), 6.95–7.04 (m, 3H, ArH), 7.57–7.65 (m, 4H, ArH), 8.17 (d, $J = 8.4$, 2H, ArH), 11.89 (br s, 1H, NH), 13.75 (br s, 1H, NH), ms: 488.13 (MH^+). Anal. Calcd for $C_{26}H_{25}N_5O_5$ (487.51): C, 64.06; H, 5.17; N, 14.37. Found: C, 64.28; H, 5.31; N, 14.09.

5.2.19. 3-Phenyl-5-[(3,4,5-trimethoxyphenyl)(3-metho xyphenylhydrazono)methyl][1,2,4]-triazin-6(1H)-one ***13c***

Yield: 83%, mp: 185–187 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.73b (s, 3H, $-OCH_3$), 3.76 (s, 6H, $-OCH_3$), 3.80 (s, 3H, $-OCH_3$), 6.41 (d, $J = 8.9$, 1H, ArH), 6.75 (d, $J = 9.3$, 2H, ArH), 6.88 (s, 2H, ArH), 7.40 (t, $J = 8.1$, 1H, ArH), 7.51–7.56 (m, 3H, ArH), 8.08 (d, $J = 8.1$, 2H, ArH), 10.08 (s, 1H, NH), 13.89 (br s, 1H, NH), ms: 488.13 (MH^+). Anal. Calcd for $C_{26}H_{25}N_5O_5$ (487.51): C, 64.06; H, 5.17; N, 14.37. Found: C, 64.19; H, 5.12; N, 14.35.

5.2.20. 3-Phenyl-5-[(3,4,5-trimethoxyphenyl)(4-methoxy phenylhydrazono)methyl][1,2,4]-triazin-6(1H)-one ***13d***

Yield: 94%, mp: 175–177 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.69 (s, 3H, OCH_3), 3.77 (s, 6H, OCH_3), 3.81 (s, 3H, OCH_3), 6.83 (t, $J = 7.2$, 1H, ArH), 6.87 (s, 2H, ArH), 7.07 (d, $J = 9.0$, 2H, ArH), 7.18 (d, $J = 7.2$, 2H, ArH), 7.26 (t, $J = 7.2$, 2H, ArH), 8.02 (d, $J = 9.0$, 2H, ArH), 10.09 (s, 1H, NH), 13.74 (s, 1H, NH), ms: 488.28 (MH^+). Anal. Calcd for $C_{26}H_{25}N_5O_5$ (487.51): C, 64.06; H, 5.17; N, 14.37. Found: C, 63.96; H, 5.31; N, 14.25.

5.2.21. 3-Phenyl-5-[(3,4,5-trimethoxyphenyl)(3,4,5-trimetho xyphenylhydrazono)methyl][1,2,4]-triazin-6(1H)-one ***13e***

Yield: 79%, mp: 134–136 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.60 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3), 3.77 (s, 6H, OCH_3), 3.81 (s, 6H, OCH_3), 6.50 (s, 2H, ArH), 6.91

(s, 2H, ArH), 7.40 (t, $J = 7.2$, 1H, ArH), 7.51 (t, $J = 7.2$, 2H, ArH), 8.09 (d, $J = 7.2$, 2H, ArH), 10.05 (s, 1H, NH), 13.12 (s, 1H, NH), ms: 548.49 (MH^+). Anal. Calcd for $C_{28}H_{29}N_5O_7$ (547.57): C, 61.42; H, 5.34; N, 12.79. Found: C, 61.26; H, 5.51; N, 12.55.

5.2.22. 3-Phenyl-5-[(3,4,5-trimethoxyphenyl)(4-chlorop henylhydrazono)methyl][1,2,4]-triazin-6(1H)-one ***13f***

Yield: 68%, mp: 127–128 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.70 (s, 3H, OCH_3), 3.78 (s, 6H, OCH_3), 6.89 (s, 2H, ArH), 7.19 (d, $J = 9.0$, 2H, ArH), 7.29 (d, $J = 9.0$, 2H, ArH), 7.38 (t, $J = 7.8$, 2H, ArH), 7.78 (t, $J = 7.8$, 1H, ArH), 8.57 (d, $J = 7.8$, 2H, ArH), 10.14 (s, 1H, NH), 13.23 (s, 1H, NH), ms: 492.95, 494.02 (MH^+). Anal. Calcd for $C_{25}H_{22}N_5O_4Cl$ (491.98): C, 61.04; H, 4.51; N, 14.24. Found: C, 61.18; H, 4.73; N, 14.30.

5.2.23. 3-(4-Methoxyphenyl)-5-[(3,4,5-trimethoxyphenyl)(3-phenylhydrazono)methyl][1,2,4]-triazin-6(1H)-one ***14a***

Yield: 71%, mp: 184–186 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.69 (s, 3H, OCH_3), 3.77 (s, 6H, OCH_3), 3.81 (s, 3H, OCH_3), 6.83 (t, $J = 7.2$, 1H, ArH), 6.87 (s, 2H, ArH), 7.07 (d, $J = 9.0$, 2H, ArH), 7.18 (d, $J = 7.2$, 2H, ArH), 7.26 (t, $J = 7.2$, 2H, ArH), 8.02 (d, $J = 9.0$, 2H, ArH), 10.09 (s, 1H, NH), 13.74 (s, 1H, NH), ms: 488.48 (MH^+). Anal. Calcd for $C_{26}H_{25}N_5O_5$ (487.52): C, 64.06; H, 5.17; N, 14.37. Found: C, 64.31; H, 5.08; N, 14.62.

5.2.24. 3-(4-Methoxyphenyl)-5-[(3,4,5-trimethoxyphenyl) (2-methoxyphenylhydrazono)methyl][1,2,4]-triazin-6 (1H)-one ***14b***

Yield: 75%, mp: 203–205 °C, ms: 518.36 (MH^+). Anal. Calcd for $C_{27}H_{27}N_5O_6$ (517.55): C, 62.66; H, 5.26; N, 13.53. Found: C, 62.64; H, 5.17; N, 13.34.

5.2.25. 3-(4-Methoxyphenyl)-5-[(3,4,5-trimethoxyphenyl) (3-methoxyphenylhydrazono)methyl][1,2,4]-triazin-6 (1H)-one ***14c***

Yield: 72%, mp: 100–105 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.73 (s, 3H, $-OCH_3$), 3.76 (s, 6H, $-OCH_3$), 3.80 (s, 3H, $-OCH_3$), 6.41 (d, $J = 8.9$, 1H, ArH), 6.75 (d, $J = 9.3$, 2H, ArH), 6.88 (s, 2H, ArH), 7.40 (t, $J = 8.1$, 1H, ArH), 7.51–7.56 (m, 3H, ArH), 8.08 (d, $J = 8.1$, 2H, ArH), 10.08 (s, 1H, NH), 13.89 (br s, 1H, NH), ms: 518.46 (MH^+). Anal. Calcd for $C_{27}H_{27}N_5O_6$ (517.55): C, 62.66; H, 5.26; N, 13.53. Found: C, 62.42; H, 5.34; N, 13.62.

5.2.26. 3-(4-Methoxyphenyl)-5-[(3,4,5-trimethoxyphenyl) (4-methoxyphenylhydrazono)methyl][1,2,4]-triazin-6 (1H)-one ***14d***

Yield: 87%, mp: 183–185 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.69 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 3.76 (s, 6H, OCH_3), 3.82 (s, 3H, OCH_3), 6.85 (s, 2H, ArH), 6.88 (d, $J = 9.0$, 2H, ArH), 7.07 (d, $J = 8.7$, 2H, ArH), 7.13 (d, $J = 8.7$, 2H, ArH), 8.02 (d, $J = 9.0$, 2H, ArH), 10.09 (s, 1H, NH), 13.70 (s, 1H, NH), ms: 518.36 (MH^+). Anal. Calcd for $C_{27}H_{27}N_5O_6$ (517.55): C, 62.66; H, 5.26; N, 13.53. Found: C, 62.47; H, 5.31; N, 13.54.

5.2.27. 3-(4-Methoxyphenyl)-5-[(3,4,5-trimethoxyphenyl) (3,4,5-trimethoxyphenylhydrazono)methyl] [1,2,4]-triazin-6(1H)-one ***14e***

Yield: 65%, mp: 162–165, ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.59 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3), 3.76 (s, 6H, OCH_3), 3.77 (s, 6H, OCH_3), 3.82 (s, 3H, OCH_3), 6.50 (s, 2H, ArH), 6.89 (s, 2H, ArH), 7.06 (d, $J = 8.7$, 2H, ArH), 8.02 (d, $J = 8.7$, 2H, ArH), 10.03 (s, 1H, NH), 13.74 (s, 1H, NH), ms: 578.61 (MH^+). Anal. Calcd for $C_{29}H_{31}N_5O_8$ (577.60): C, 60.31; H, 5.41; N, 12.12. Found: C, 60.19; H, 5.27; N, 11.94.

5.2.28. 3-(4-Methoxyphenyl)-5-[(3,4,5-trimethoxyphenyl) (4-chlorophenylhydrazono)methyl][1,2,4]-triazin-6 (1H)-one ***14f***

Yield: 86%, mp: 110–112 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.81 (s, 3H, OCH_3), 3.77 (s, 6H, OCH_3), 3.69 (s, 3H, OCH_3), 6.88 (s, 2H, ArH), 7.06 (d, $J = 9.0$, 2H, ArH), 7.18 (d, $J = 8.7$), 7.29 (d, $J = 9.0$, 2H, ArH), 8.02 (d, $J = 8.7$, 2H, ArH), 10.13 (s, 1H, NH), 13.25 (s, 1H, NH), ms: 523.00, 524.94 (MH^+). Anal. Calcd for $C_{26}H_{24}N_5O_5Cl$ (521.96): C, 59.83; H, 4.63; N, 13.42. Found: C, 59.59; H, 4.42; N, 13.54.

5.2.29. 3-(3,4,5-Trimethoxyphenyl)-5-[(3,4,5-trimetho xyphenyl)(phenylhydrazono)methyl][1,2,4]-triazin-6 (1H)-one ***15a***

Yield: 56%, mp: 128–130 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.69 (s, 6H, OCH_3), 3.78 (s, 6H, OCH_3), 3.81 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 7.12 (s, 2H, ArH), 7.21–7.31 (m, 3H, ArH), 7.36 (s, 2H, ArH), 7.56 (d, $J = 7.8$, 2H, ArH), 10.15 (s, 1H, NH), 13.25 (s, 1H, NH) ms: 548.36 (MH^+). Anal. Calcd for $C_{28}H_{29}N_5O_7$ (547.57): C, 61.42; H, 5.34; N, 12.79. Found: C, 61.13; H, 5.11; N, 12.56.

5.2.30. 3-(3,4,5-Trimethoxyphenyl)-5-[(3,4,5-trimethoxy phenyl)(2-methoxyphenylhydrazono)methyl] [1,2,4]-triazin-6(1H)-one ***15b***

Yield: 77%, mp: 106–108 °C, ms: 578.56 (MH^+). Anal. Calcd for $C_{29}H_{31}N_5O_8$ (577.59): C, 60.31; H, 5.41; N, 12.12. Found: C, 60.05; H, 5.36; N, 11.95.

5.2.31. 3-(3,4,5-Trimethoxyphenyl)-5-[(3,4,5-trimethoxy phenyl)(3-methoxyphenylhydrazono)methyl] [1,2,4]-triazin-6(1H)-one ***15c***

Yield: 59%, mp: 194–196 °C, ms: 578.11 (MH^+). Anal. Calcd for $C_{29}H_{31}N_5O_8$ (577.60): C, 60.31; H, 5.41; N, 12.12 Found: C, 60.52; H, 5.29; N, 12.15.

5.2.32. 3-(3,4,5-Trimethoxyphenyl)-5-[(3,4,5-trimethoxy phenyl)(4-methoxyphenylhydrazono)methyl] [1,2,4]-triazin-6(1H)-one ***15d***

Yield: 63%, mp: 152–155 °C, ms: 578.13 (MH^+). Anal. Calcd for $C_{29}H_{31}N_5O_8$ (577.60): C, 60.31; H, 5.41; N, 12.12 Found: C, 60.48; H, 5.19; N, 11.94.

5.2.33. 3-(3,4,5-Trimethoxyphenyl)-5-[(3,4,5-trimethoxy phenyl)(3,4,5-trimethoxyphenylhydrazono)methyl] [1,2,4]-triazin-6(1H)-one ***15e***

Yield: 52%, mp: 95–98 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.67 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3), 3.73 (s, 6H, OCH_3), 3.75 (s, 3H, OCH_3), 3.78 (s, 6H, OCH_3), 3.81 (s, 3H, OCH_3), 6.71 (s, 2H, ArH), 6.88 (s, 2H, ArH), 7.12 (s, 2H, ArH), 10.13 (s, 1H, NH), 13.16 (s, 1H, NH), ms: 638.47 (MH^+). Anal. Calcd for $C_{31}H_{35}N_5O_{10}$ (637.65): C, 58.39; H, 5.53; N, 10.98. Found: C, 58.46; H, 5.26; N, 11.15.

5.2.34. 3-(3,4,5-Trimethoxyphenyl)-5-[(3,4, 5-trimethoxyphenyl)(4-chlorophenylhydrazono) methyl][1,2,4]-triazin-6(1H)-one ***15f***

Yield: 58%, mp: 207–209 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.67 (s, 3H, OCH_3), 3.69 (s, 6H, OCH_3), 3.78 (s, 6H, OCH_3), 3.81 (s, 3H, OCH_3), 6.72 (s, 2H, ArH), 6.88 (s, 2H, ArH), 7.19 (d, $J = 9.0$, 2H, ArH), 7.30 (d, $J = 9.0$, 2H, ArH), 10.17 (s, 1H, NH), 13.30 (s, 1H, NH), ms: 583.05 (MH^+). Anal. Calcd for $C_{28}H_{26}N_5O_7Cl$ (582.02): C, 57.78; H, 4.85; N, 12.03. Found: C, 57.59; H, 4.67; N, 12.14.

5.2.35. 3-(4-Chlorophenyl)-5-[(3,4,5-trimethoxyphenyl) phenylhydrazonomethyl][1,2,4]-triazin-6(1H)-one ***16a***

Yield: 73%, mp: 228–230 °C, ms: 493.00, 494.95 (MH^+). Anal. Calcd for $C_{25}H_{22}N_5O_4Cl$ (491.98): C, 61.04; H, 4.51; N, 14.24. Found: C, 61.15; H, 4.36; N, 14.51.

5.2.36. 3-(4-Chlorophenyl)-5-[(3,4,5-trimethoxyphenyl) (2-methoxyphenylhydrazono)methyl][1,2,4]-triazin-6 (1H)-one ***16b***

Yield: 96%, mp: 215–217 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.70 (s, 3H, OCH_3), 3.78 (s, 6H, OCH_3), 3.82 (s, 3H, OCH_3), 6.89 (s, 2H, ArH), 6.94–6.99 (m, 2H, ArH), 7.61 (d, $J = 8.7$, 1H, ArH), 7.66 (d, $J = 7.8$, 2H, ArH), 7.85 (d, $J = 8.7$, 1H, ArH), 8.15 (d, $J = 7.8$, 2H, ArH), 11.81 (s, 1H, NH), 13.47 (s, 1H, NH), ms: 583.05 (MH^+). Anal. Calcd for $C_{28}H_{26}N_5O_7Cl$ (580.04): C, 57.78; H, 4.85; N, 12.03. Found: C, 57.59; H, 4.67; N, 12.14.

5.2.37. 3-(4-Chlorophenyl)-5-[(3,4,5-trimethoxyphenyl) (3-methoxyphenylhydrazono)methyl] [1,2,4]-triazin-6(1H)-one ***16c***

Yield: 92%, mp: 198–200 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.69 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 3.77 (s, 6H, OCH_3), 6.89 (s, 2H, ArH), 7.13 (t, $J = 8.7$, 1H, ArH), 7.48 (d, $J = 8.7$, 1H, ArH), 7.58 (d, $J = 8.5$, 2H, ArH), 7.83 (d, $J = 8.7$, 1H, ArH), 8.09 (d, $J = 8.5$, 1H, ArH), 10.07 (s, 1H, NH), 13.92 (s, 1H, NH), ms: 522.13, 524.07 (MH^+). Anal. Calcd for $C_{26}H_{24}N_5O_5Cl$ (580): C, 59.83, H, 4.63, N, 13.42. Found: C, 59.92 C, H, 4.58, N, 13.55.

5.2.38. 3-(4-Chlorophenyl)-5-[(3,4,5-trimethoxyphenyl) (4-methoxyphenylhydrazono)methyl][1,2,4]-triazin-6 (1H)-one ***16d***

Yield: 92%, mp: 188–190 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.76 (s, 6H, OCH_3), 3.78 (s, 3H, OCH_3), 7.79 (s, 3H, OCH_3), 6.85 (s, 2H, ArH), 6.88 (d, $J = 8.4$, 2H, ArH), 7.12 (d, $J = 8.4$, 2H, ArH), 7.58 (d, $J = 8.1$, 2H, ArH), 8.08 (d, $J = 8.1$, 2H, ArH), 10.08 (s, 1H, NH), 13.30 (br s, 1H, NH), ms: 523.00, 525.05 (MH^+). Anal. Calcd for $C_{26}H_{24}N_5O_5Cl$ (522.01): C, 59.83; H, 4.63; N, 13.42. Found: C, 59.64; H, 4.92; N, 13.20.

5.2.39. 3-(4-Chlorophenyl)-5-[(3,4,5-trimethoxyphenyl) (3,4,5-trimethoxyphenylhydrazono)methyl] [1,2,4]-triazin-6(1H)-one ***16e***

Yield: 93%, mp: 168–170 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.59 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3), 3.77 (s, 6H, OCH_3), 3.79 (s, 6H, OCH_3), 6.49 (s, 2H, ArH), 6.91 (s, 2H, ArH), 7.58 (d, $J = 8.4$, 2H, ArH), 8.09 (d, $J = 8.4$, 2H, ArH), 10.03 (s, 1H, NH), 13.39 (s, 1H, NH), ms: 582.07, 584.93 (MH^+). Anal. Calcd for $C_{28}H_{28}N_5O_7Cl$ (582.06): C, 57.78; H, 4.85; N, 12.03. Found: C, 57.56; H, 4.59; N, 11.78.

5.2.40. 3-(4-Chlorophenyl)-5-[(3,4,5-trimethoxyphenyl) (4-chlorophenylhydrazono)methyl] [1,2,4]-triazin-6(1H)-one ***16f***

Yield: 82%, mp: 210–212 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.69 (s, 3H, OCH_3), 3.77 (s, 6H, OCH_3), 6.89 (s, 2H, ArH), 7.18 (d, $J = 8.7$, 2H, ArH), 7.29 (d, $J = 8.7$, 2H, ArH), 7.58 (d, $J = 8.7$, 2H, ArH), 8.08 (d, $J = 8.7$, 2H, ArH), 10.12 (s, 1H, NH), 13.95 (s, 1H, NH), ms: 523.13 (MH^+). Anal. Calcd for $C_{26}H_{24}N_5O_5Cl$ (522.01): C, 59.83; H, 4.63; N, 13.42. Found: C, 59.64; H, 4.92; N, 13.20.

5.2.41. 3,7-Disubstituted-5-(3,4,5-trimethoxyphenyl) pyrazolo[4,3-e][1,2,4]triazines ***17a–20f***

5.2.41.1. General procedure. The mixture of 3-substituted-5-[(3,4,5-trimethoxyphenyl)(arylhydrazono)methyl][1,2,4]-triazin-6(1*H*)-one (1.00 mmol), phosphorous oxychloride (2.50 ml) and anhydrous pyridine (100 μL) was heated on an oil bath at 115 °C for 2 h. After cooling to room temperature the solution was poured over crushed ice (30 g) and left to stand at room temperature for 30 min. The brown precipitate was filtered off and washed with water (100 ml). The crude product was crystallized from an appropriate solvent, resp. mixture of solvents (see text).

5.2.42. 3,7-Diphenyl-5-(3,4,5-trimethoxyphenyl) pyrazolo[4,3-e][1,2,4]triazine ***17a***

Yield: 92%, mp: 197–198 °C (ethanol–toluene (2:1)), ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.99 (s, 3H, OCH_3), 4.04 (s, 6H, OCH_3), 7.40 (t, $J = 7.2$, 1H, ArH), 7.56–7.63 (m, 5H,

ArH), 7.92 (s, 2H, ArH), 8.49 (d, $J = 8.1$, 2H, ArH), 8.62–8.64 (m, 2H, ArH), ms: 440.50 (MH^+). Anal. Calcd for $C_{25}H_{21}N_5O_3$ (439.47): C, 68.32; H, 4.82; N, 15.94. Found: C, 68.15; H, 4.76; N, 15.78.

5.2.43. 7-(2-Methoxyphenyl)-3-phenyl-5-(3,4,5-trimethoxy phenyl)pyrazolo[4,3-e][1,2,4]triazine ***17b***

Yield: 92%, mp: 186–187 °C (ethanol–toluene (3:1)), ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.78 (s, 3H, $-OCH_3$), 3.79 (s, 3H, $-OCH_3$), 3.95 (s, 6H, $-OCH_3$), 7.26 (t, $J = 7.5$, 1H, ArH), 7.41 (d, $J = 7.8$, 1H, ArH), 7.63–7.68 (m, 3H, ArH), 7.73 (d, $J = 7.8$, 1H, ArH), 7.85 (s, 2H, ArH), 8.58 (d, $J = 7.8$, 2H, ArH), ms: 470.13 (MH^+). Anal. Calcd for $C_{26}H_{23}N_5O_4$ (469.5): C, 66.51; H, 4.94; N, 14.92. Found: C, 66.78; H, 4.99; N, 14.85.

5.2.44. 7-(3-Methoxyphenyl)-3-phenyl-5-(3,4,5-trimethoxy phenyl)pyrazolo[4,3-e][1,2,4]triazine ***17c***

Yield: 80%, mp: 171–172 °C (ethanol–toluene (2:1)), ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.79 (s, 3H, $-OCH_3$), 3.92 (s, 3H, $-OCH_3$), 3.99 (s, 6H, $-OCH_3$), 7.26 (t, $J = 7.5$, 1H, ArH), 7.41 (d, $J = 7.8$, 1H,ArH), 7.63–7.68 (m, 3H, ArH), 7.73 (d, $J = 7.8$, 1H, ArH), 7.85 (s, 2H, ArH), 8.58 (d, $J = 7.8$, 2H, ArH), ms: 470.12 (MH^+). Anal. Calcd for $C_{26}H_{23}N_5O_4$ (469.50): C, 66.51; H, 4.94; N, 14.92. Found: C, 66.39; H, 4.69; N, 14.71.

5.2.45. 7-(4-Methoxyphenyl)-3-phenyl-5-(3,4,5-trimethoxy phenyl)pyrazolo[4,3-e][1,2,4]triazine ***17d***

Yield: 94%, mp: 183–184 °C (ethanol–toluene (2:1)), ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.78 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.98 (s, 6H, OCH_3), 7.26 (d, $J = 8.7$, 2H, ArH), 7.66 (d, $J = 7.2$, 2H, ArH), 7.92 (s, 2H, ArH), 8.28 (d, $J = 8.7$, 2H, ArH), 8.61 (d, $J = 7.2$, 2H, ArH), ms: 470.13 (MH^+). Anal. Calcd for $C_{26}H_{23}N_5O_4$ (469.5): C, 66.51; H, 4.94; N, 14.92. Found: C, 66.73; H, 4.88; N, 14.66.

5.2.46. 3-Phenyl-5-(3,4,5-trimethoxyphenyl)-7-(3,4,5-trime thoxyphenyl)pyrazolo[4,3-e][1,2,4]triazine ***17e***

Yield: 62%, mp: 173–174 °C (ethanol–toluene (1:1)), ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.96 (s, 3H, $-OCH_3$), 3.99 (s, 3H, $-OCH_3$), 4.06 (s, 6H, $-OCH_3$), 4.08 (s, 6H, $-OCH_3$), 7.59–7.62 (m, 3H, ArH), 7.78 (s, 1H, ArH), 7.96 (s, 1H, ArH), 8.65–8.69 (m, 2H, ArH), ms: 530.32 (MH^+). Anal. Calcd for $C_{28}H_{27}N_5O_6$ (529.55): C, 63.51; H, 5.14; N, 13.22. Found: C, 63.50; H, 4.97; N, 13.45.

5.2.47. 7-(4-Chlorophenyl)-3-phenyl-5-(trimethoxyphenyl) pyrazolo[4,3-e][1,2,4]triazine ***17f***

Yield: 83%, mp: 241–242 °C, ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.99 (s, 3H, $-OCH_3$), 4.07 (s, 6H, $-OCH_3$), 7.56–7.60 (m, 5H, ArH), 7.95 (s, 2H, ArH), 8.50 (d, $J = 9.0$, 2H, ArH), 8.64–8.67 (m, 2H, ArH), ms: 459 (MH^+). Anal. Calcd for $C_{25}H_{20}N_5O_3Cl$ (473.96): C, 63.36; H, 4.25; N, 14.78. Found: C, 63.26; H, 3.97; N, 14.55.

5.2.48. 3-(4-Methoxyphenyl)-7-phenyl-5-(3,4,5-trime thoxyphenyl)pyrazolo[4,3-e][1,2,4]triazine ***18a***

Yield: 95%, mp: 218–219 °C, ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.92 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 4.06 (s, 6H, OCH_3), 7.05 (d, $J = 7.5$, 2H, ArH), 7.40 (t, $J = 7.5$, 1H, ArH), 7.60 (t, $J = 7.5$, 2H, ArH), 7.93 (s, 2H, ArH), 8.49 (d, $J = 8.7$, 2H, ArH), 8.57 (d, $J = 8.7$, 2H, ArH), ms: 470.50 (MH^+). Anal. Calcd for $C_{26}H_{23}N_5O_4$ (469.50): C, 66.51; H, 4.94; N, 14.92. Found: C, 66.73; H, 4.81; N, 14.78.

5.2.49. 3-(4-Methoxyphenyl)-7-(2-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)pyrazolo[4,3-e][1,2,4]triazine ***18b***

Yield: 98%, mp: 176–177 °C, ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.94 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 4.08 (s, 6H, OCH_3), 6.97 (m, 2H, ArH), 7.09 (d, $J = 8.7$, 2H, ArH), 7.51 (t, $J = 8.1$, 1H, ArH), 7.98 (s, 2H, ArH), 8.12 (d, $J = 8.1$, 1H, ArH), 8.61 (d, $J = 8.7$, 2H, ArH), ms: 500.33 (MH^+). Anal. Calcd for $C_{27}H_{25}N_5O_5$ (469.50): C, 64.92; H, 5.04; N, 14.02. Found: C, 64.90; H, 4.89; N, 13.85.

5.2.50. 3-(4-Methoxyphenyl)-7-(3-methoxyphenyl)-5-(3,4, 5-trimethoxyphenyl)pyrazolo[4,3-e][1,2,4]triazine ***18c***

Yield: 96%, mp: 142–145 °C, ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.94 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3) 3.99 (s, 3H, OCH_3), 4.08 (s, 6H, OCH_3), 7.10 (d, $J = 8.7$, 2H, ArH), 7.57 (d, $J = 8.7$, 2H, ArH), 7.95 (s, 2H, ArH), 8.50 (d, $J = 8.7$, 2H, ArH), 8.60 (d, $J = 8.7$, 2H, ArH), ms: 500.42 (MH^+). Anal. Calcd for $C_{27}H_{25}N_5O_5$ (469.50): C, 64.92; H, 5.04; N, 14.02. Found: C, 64.77; H, 5.16; N, 13.96.

5.2.51. 3-(4-Methoxyphenyl)-7-(4-methoxyphenyl)-5-(3,4, 5-trimethoxyphenyl)pyrazolo[4,3-e][1,2,4]triazine ***18d***

Yield: 97%, mp: 194–196 °C, ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.92 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 4.07 (s, 6H, OCH_3), 7.07 (d, $J = 8.4$, 2H, ArH), 7.12 (d, $J = 8.4$, 2H, ArH), 7.95 (s, 2H, ArH), 8.35 (d, $J = 9.0$, 2H, ArH), 8.59 (d, $J = 9.0$, 2H, ArH), ms: 500.50 (MH^+). Anal. Calcd for $C_{27}H_{25}N_5O_5$ (469.50): C, 64.92; H, 5.04; N, 14.02. Found: C, 65.08; H, 4.87; N, 13.93.

5.2.52. 3-(4-Methoxyphenyl)-7-(3,4,5-trimethoxyphenyl)-5-(3,4,5-trimethoxyphenyl)pyrazolo[4,3-e][1,2,4]triazine ***18e***

Yield: 95%, mp: 192–193 °C, ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.93 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 4.04 (s, 6H, OCH_3), 4.06 (s, 6H, OCH_3), 7.07 (d, $J = 9.0$, 2H, ArH), 7.76 (s, 2H, ArH), 7.92 (s, 2H, ArH), 8.58 (d, $J = 9.0$, 2H, ArH), ms: 500.46 (MH^+). Anal. Calcd for $C_{29}H_{29}N_5O_7$ (559.58): C, 62.25; H, 5.22; N, 12.52. Found: C, 62.38; H, 4.07; N, 12.31.

5.2.53. 3-(4-Methoxyphenyl)-7-(4-chlorophenyl)-5-(3,4,5-tri methoxyphenyl)pyrazolo[4,3-e][1,2,4]triazine ***18f***

Yield: 93%, mp: 255–256 °C, ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.99 (s, 3H, OCH_3), 4.08 (s, 6H, OCH_3), 7.44 (t, $J = 7.5$, 1H, ArH), 7.58 (d, $J = 8.7$, 2H, ArH), 7.64 (t, $J = 7.5$, 2H, ArH), 7.97 (s, 2H, ArH), 8.52 (d, $J = 7.5$, 2H,

ArH), 8.62 (d, $J = 8.7$, 2H, ArH), ms: 505.00, 505.94 (MH^+). Anal. Calcd for $C_{26}H_{22}N_5O_4Cl$ (503.95): C, 61.97; H, 4.40; N, 13.90. Found: C, 61.90; H, 4.56; N, 13.76.

5.2.54. 7-Phenyl-3-(3,4,5-trimethoxyphenyl)-5-(3,4,5-trimethoxyphenyl)pyrazolo[4,3-e][1,2,4]triazine ***19a***

Yield: 94%, mp: 209–211 °C, ^1H NMR (300 MHz, $CDCl_3$): δ 3.96 (s, 6H, OCH_3), 3.98 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 4.03 (s, 6H, OCH_3), 7.40 (t, $J = 7.2$, 1H, ArH), 7.60 (t, $J = 7.2$, 2H, ArH), 7.91 (s, 2H, ArH), 7.93 (s, 2H, ArH), 8.48 (d, $J = 7.2$, 2H, ArH), ms: (MH^+). Anal. Calcd for $C_{27}H_{28}N_5O_6$: C, 63.51; H, 5.14; N, 13.22; Found: C, 63.74; H, 5.19; N, 13.11.

5.2.55. 7-(2-Methoxyphenyl)-(3,4,5-trimethoxyphenyl)-5-(3,4,5-trimethoxyphenyl)pyrazolo[4,3-e][1,2,4]triazine ***19b***

Yield: 95%, mp: 278–279 °C, ^1H NMR (300 MHz, $CDCl_3$): δ 3.85 (s, 3H, $-OCH_3$), 3.97 (s, 3H, $-OCH_3$), 3.98 (s, 3H, $-OCH_3$), 4.01 (s, 6H, $-OCH_3$), 4.05 (s, 6H, $-OCH_3$), 7.19–7.24 (m, 2H, ArH), 7.55–7.61 (d, $J = 6.0$, 1H, ArH), 7.68 (d, $J = 6.6$, 1H, ArH), 7.98 (s, 2H, ArH), 8.02 (s, 2H, ArH), ms: 560.57(MH^+). Anal. Calcd for $C_{29}H_{29}N_5O_7$ (559.58): C, 62.25; H, 5.22; N, 12.52. Found: C, 62.55; H, 5.11; N, 12.74.

5.2.56. 7-(3-Methoxyphenyl)-(3,4,5-trimethoxyphenyl)-5-(3,4,5-trimethoxyphenyl)pyrazolo[4,3-e][1,2,4]triazine ***19c***

Yield: 86%, mp: 216–218 °C, ^1H NMR (300 MHz, $CDCl_3$): δ 3.85 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 3.97 (s, 6H, OCH_3), 4.01 (s, 6H, OCH_3), 4.05 (s, 3H, OCH_3), 7.19–7.28 (m, 2H, ArH), 7.58 (t, $J = 6.3$, 1H, ArH), 7.68 (d, $J = 7.2$, 1H, ArH), 7.98 (s, 2H, ArH), 8.02 (s, 2H, ArH), ms: 560.07(MH^+). Anal. Calcd for $C_{29}H_{29}N_5O_7$ (559.58): C, 62.25; H, 5.22; N, 12.52. Found: C, 62.48; H, 5.13; N 12.34.

5.2.57. 7-(4-Methoxyphenyl)-(3,4,5-trimethoxyphenyl)-5-(3,4,5-trimethoxyphenyl)pyrazolo[4,3-e][1,2,4]triazine ***19d***

Yield: 94%, mp: 251–252 °C, ^1H NMR (300 MHz, DMSO-d_6): δ 3.98 (s, 3H, $-OCH_3$), 3.97–3.99 (m, 12H, $-OCH_3$), 4.06 (s, 3H, $-OCH_3$), 7.56 (d, $J = 9.0$, 2H, ArH), 7.93 (s, 2H, ArH), 7.95 (s, 2H, ArH), 8.49 (d, $J = 9.0$, 2H, ArH), ms: 470(MH^+). Anal. Calcd for $C_{29}H_{29}N_5O_7$ (559.58): C, 62.25; H, 5.22; N, 12.52. Found: C, 62.28; H, 5.51; N 12.43.

5.2.58. 3,5,7-Tri-(3,4,5-trimethoxyphenyl)pyrazolo[4,3-e][1,2,4]triazine ***19e***

Yield: 95%, mp: 223–225 °C, ^1H NMR (300 MHz, $CDCl_3$): δ 3.96 (s, 3H, $-OCH_3$), 3.98 (s, 3H, $-OCH_3$), 3.99 (s, 3H, $-OCH_3$), 4.02 (s, 6H, $-OCH_3$), 4.06 (s, 6H, $-OCH_3$), 4.07 (s, 6H, $-OCH_3$), 7.76 (s, 1H, ArH), 7.95 (s, 2H, ArH), 7.98 (s, 2H, ArH), ms: 620.13(MH^+). Anal. Calcd for $C_{31}H_{33}N_5O_9$ (619.63): C, 60.09; H, 5.37; N, 11.30. Found: C, 60.01; H, 5.28; N, 11.15.

5.2.59. 7-(4-Chlorophenyl)-3,5-di-(3,4,5-trimethoxyphenyl)pyrazolo[4,3-e][1,2,4]triazine ***19f***

Yield: 92%, mp: 266–267 °C, ^1H NMR (300 MHz, $CDCl_3$): δ 3.98 (s, 3H, $-OCH_3$), 3.97–3.99 (m, 12H, $-OCH_3$), 4.06 (s, 3H, $-OCH_3$), 7.56 (d, $J = 9.0$, 2H, ArH), 7.93 (s, 2H, ArH), 7.95 (s, 2H, ArH), 8.49 (d, $J = 9.0$, 2H, ArH), ms: 564.73 (MH^+). Anal. Calcd for $C_{28}H_{26}N_5O_6Cl$ (564.00): C, 60.09; H, 5.37; N, 11.30. Found: C, 60.01; H, 5.28; N, 11.15.

5.2.60. 3-(4-Chlorophenyl)-7-(phenyl)-5-(3,4,5-trimethoxyphenyl)pyrazolo[4,3-e][1,2,4]triazine ***20a***

Yield: 59%, mp: 233–235 °C, ^1H NMR (300 MHz, $CDCl_3$): δ 3.99 (s, 3H, OCH_3), 4.08 (s, 6H, OCH_3), 7.44 (t, $J = 7.5$, 1H, ArH), 7.57 (d, $J = 8.7$, 2H, ArH), 7.64 (t, $J = 7.5$, 2H, ArH), 7.97 (s, 2H, ArH), 8.52 (d, $J = 7.5$, 2H, ArH), 8.62 (d, $J = 8.7$, 2H, ArH), ms: 475.13(MH^+). Anal. Calcd for $C_{25}H_{20}N_5O_3Cl$ (473.96): C, 63.36; H, 4.25; N, 14.78. Found: C, 63.59; H, 4.28; N, 14.96.

5.2.61. 3-(4-Chlorophenyl)-7-(2-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)pyrazolo[4,3-e][1,2,4]triazine ***20b***

Yield: 96%, mp: 213–215 °C, ^1H NMR (300 MHz, $CDCl_3$): δ 3.77 (s, 3H, $-OCH_3$), 3.78 (s, 3H, $-OCH_3$), 3.95 (s, 6H, $-OCH_3$), 7.25 (t, $J = 7.9$, 1H, ArH), 7.39 (t, $J = 7.9$, 1H, ArH), 7.65–7.74 (m, 4H, ArH), 7.84 (s, 2H, ArH), 8.58 (d, $J = 8.1$, 2H, ArH), ms: 504.07(MH^+). Anal. Calcd for $C_{26}H_{22}N_5O_4Cl$ (503.99): C, 61.97; H, 4.40; N, 13.90. Found: C, 61.83; H, 4.51; N, 13.85.

5.2.62. 3-(4-Chlorophenyl)-7-(3-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)pyrazolo[4,3-e][1,2,4]triazine ***20c***

Yield: 99%, mp: 182–184 °C, ^1H NMR (300 MHz, $CDCl_3$): δ 3.77 (s, 3H, $-OCH_3$), 3.78 (s, 3H, $-OCH_3$), 3.95 (s, 6H, $-OCH_3$), 7.25 (t, $J = 7.9$, 1H, ArH), 7.39 (t, $J = 7.9$, 1H, ArH), 7.65–7.74 (m, 4H, ArH), 7.84 (s, 2H, ArH), 8.58 (d, $J = 8.1$, 2H, ArH), ms: 504.13, 506.05 (MH^+). Anal. Calcd for $C_{26}H_{22}N_5O_4Cl$ (503.99): C, 61.97; H, 4.40; N, 13.90. Found: C, 61.73; H, 4.28; N, 13.97.

5.2.63. 3-(4-Chlorophenyl)-7-(4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)pyrazolo[4,3-e][1,2,4]triazine ***20d***

Yield: 98%, mp: 233–235 °C, ^1H NMR (300 MHz, $CDCl_3$): δ 3.92 (s, 3H, $-OCH_3$), 3.98 (s, 3H, $-OCH_3$), 4.07 (s, 6H, $-OCH_3$), 7.12 (d, $J = 9.0$, 2H, ArH), 7.55 (d, $J = 8.4$, 2H, ArH), 7.92 (s, 2H, ArH), 8.33 (d, $J = 9.0$ 2H, ArH), 8.58 (d, $J = 8.4$, 2H, ArH), ms: 504.13, 506.05 (MH^+). Anal. Calcd for $C_{26}H_{22}N_5O_4Cl$ (503.99): C, 61.97; H, 4.40; N, 13.90. Found: C, 62.05; H, 4.58; N, 13.72.

5.2.64. 3-(4-Chlorophenyl)-5-(3,4,5-trimethoxyphenyl)-7-(3,4,5-trimethoxyphenyl)pyrazolo[4,3-e][1,2,4]triazine ***20e***

Yield: 96%, mp: 168–170 °C, ^1H NMR (300 MHz, $CDCl_3$): δ 3.77 (s, 3H, $-OCH_3$), 3.79 (s, 3H, $-OCH_3$), 3.94 (s, 6H, $-OCH_3$), 3.98 (s, 6H, OCH_3), 7.71 (s, 2H, ArH), 7.73 (d, $J = 7.5$, 2H, ArH), 7.89 (s, 2H, ArH), 8.59 (d, $J = 7.5$, 2H, ArH), ms: 565.06, 567.05 (MH^+). Anal. Calcd

for $C_{28}H_{26}N_5O_6Cl$ (564.04): C, 59.63; H, 4.65; N, 12.42. Found: C, 59.81; H, 4.47; N, 12.23.

5.2.65. 3-(4-Chlorophenyl)-7-(4-chlorophenyl)-5-(3,4,5-trimethoxyphenyl)pyrazolo[4,3-e][1,2,4]triazine ***20f***

Yield: 96%, mp: 303–304 °C, ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.99 (s, 3H, $-OCH_3$), 4.07 (s, 6H, $-OCH_3$), 7.55–7.60 (m, 4H, ArH), 7.95 (s, 2H, ArH), 8.49 (d, $J = 9.0$, 2H, ArH), 8.65 (d, $J = 9.0$, 2H, ArH), ms: 509.13, 511.05 (MH^+). Anal. Calcd for $C_{25}H_{19}N_5O_3Cl_2$ (508.46): C, 59.07; H, 3.77; N, 13.78. Found: C, 59.26; H, 3.49; N, 13.51.

5.3. Biological activity

5.3.1. Cell lines

All cells were purchased from the American Tissue Culture Collection (ATCC), unless otherwise indicated. The daunorubicin resistant subline of CEM cells (CEM DNR Bulk) and paclitaxel resistant subline K-562-tax were selected in our laboratory by the cultivation of maternal cell lines in increasing concentrations of daunorubicin or paclitaxel, respectively [24]. The cells were maintained in Nunc/Corning 80 cm^2 plastic tissue culture flasks and cultured in cell culture medium (DMEM/RPMI 1640 with 5 g/L glucose, 2 mM glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 10% fetal calf serum, and $NaHCO_3$).

5.3.2. Cytotoxic MTT assay

Cell suspensions were prepared and diluted according to the particular cell type and the expected target cell density (2500–30,000 cells/well based on cell growth characteristics). Cells were added by pipette (80 μL) into 96-well microtiter plates. Inoculates were allowed a pre-incubation period of 24 h at 37 °C and 5% CO_2 for stabilisation. Fourfold dilutions, in 20-μL aliquots, of the intended test concentration were added to the microtiter plate wells at time zero. All test compound concentrations were examined in duplicate. Incubation of the cells with the test compounds lasted for 72 h at 37 °C, in a 5% CO_2 atmosphere at 100% humidity. At the end of the incubation period, the cells were assayed using MTT. Aliquots (10 μL) of the MTT stock solution were pipetted into each well and incubated for further 4 h. After this incubation period the formazan produced was dissolved by the addition of 100 μL/well of 10% aq SDS (pH = 5.5), followed by a further incubation at 37 °C overnight. The optical density (OD) was measured at 540 nm with a Labsystem iEMS Reader MF. Tumour cell inhibitory concentration (IC) was calculated using the following equation: $IC = (OD_{\text{drug-exposed well}}/\text{mean } OD_{\text{control wells}}) \times 100\%$. The IC_{50} value, the drug concentration lethal to 50% of the tumour cells, was calculated from appropriate dose–response curves.

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