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Research Article

Synthesis, cytotoxic and antimicrobial activity of some new 3,4,5-trimethoxybenzyl-1,2,4-triazines and their condensed derivatives

Azza M. Kadry* and Amany M. Al-Mahmoudy

Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt

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Keywords: 3,4,5-Trimethoxybenzyl-1,2,4-triazine, thieno[3,2-*e*][1,2,4]triazines, [1,2,4]triazolo[3,4-*f*][1,2,4]triazine, [1,2,4]triazino[6,1-*c*][1,2,4]triazine, cytotoxic activity, antimicrobial activity

Abstract: A series of 3,4,5-trimethoxybenzyl-1,2,4-triazine derivatives have been synthesized and converted to thieno[3,2-*e*][1,2,4]triazines, [1,2,4]triazolo[3,4-*f*][1,2,4]triazine, and [1,2,4]triazino[6,1-*c*][1,2,4]triazine derivatives. The cytotoxic activity of some of the newly synthesized compounds have been tested against two cell lines, breast carcinoma (MCF7) and colon carcinoma (HCT 116) and some showed significant activity. Thieno[3,2-*e*][1,2,4]triazines and [1,2,4]triazino[6,1-*c*][1,2,4]triazines proved to be potential leads for further cytotoxic activity studies. Also, some of the newly synthesized compounds exhibited a high antimicrobial activity against either Gram-positive or Gram-negative bacteria and fungi.

Introduction

Many compounds containing the 1,2,4-triazine (6-azapyrimidine) nucleus exhibit significant biological importance[1] and are used as antimicrobial,[2] antibacterial,[1-6] antifungal,[6,7] antiviral[1,7] and anticancer agents.[1, 7-9] 3,4,5-Trimethoxybenzyl derivative of pyrazino-1,2,4-triazine **A** (Figure 1) exhibited broad spectrum antimicrobial activity.[7] Recently 3,7-diaryl-5-(3,4,5-trimethoxyphenyl) pyrazolo [4,3-*e*][1,2,4] triazines **B** (Figure 1) have been synthesized and found to exhibit significant broad cytotoxic activity in low micromolar range against lung adenocarcinoma cell line A549.[9]

A series of 1,3,5-triazine derivatives have also been synthesized and shown to exhibit interesting cytotoxic and antibacterial activity.[10] Also, some derivatives of 7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-one **C** (Figure 1) exhibited comparable

* Corresponding author.
E-mail addresses: azzamokadry@yahoo.com

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antibacterial potencies in vitro to that of ampicillin and antiviral and anticancer activity.[6] In continuation of our interest[11] for the synthesis of 3,4,5-trimethoxyphenyl substituted heterocyclic compounds of potential biological activity we report in the present work the synthesis of a series of 3,4,5-trimethoxybenzyl-1,2,4-triazine derivatives and their conversion to their corresponding thieno[3,2-*e*][1,2,4]triazines, [1,2,4]triazolo[3,4-*f*][1,2,4]triazine, and [1,2,4]triazino[6,1-*c*][1,2,4]triazine derivatives and to investigate their potential cytotoxic and antimicrobial activity.

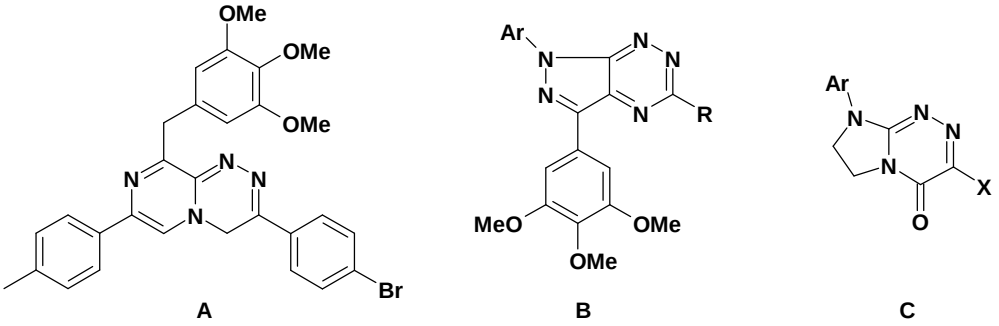

Figure 1: Examples of condensed 1,2,4-triazines with antimicrobial, cytotoxic and antibacterial activities

Results and Discussion

The starting 3-aryl-5-(3,4,5-trimethoxybenzyl)-1,2,4-triazin-6(1*H*)-ones **1a-c** were prepared as described recently.[9] Compounds **1a-c** were then converted into their corresponding Mannich bases **2a-c** or bis-Mannich bases **3a-c** upon treatment with HCHO and morpholine in methanol. Thiation of **3a-c** with phosphorus pentasulfide in pyridine gave the corresponding thieno[3,2-*e*][1,2,4]triazines **7a-c** in *ca*. 55% overall yields from **1a-c** to **7a-c**. In the present study also, two more efficient methods are described for the synthesis of **7a-c** following reported procedures for the synthesis of this ring system.[12,13] Thus, heating **1a-d** at 100 ^{o}C with *N*,*N*-dimethylformamide dimethyl acetal (DMF-DMA) for 5 minutes gave the corresponding dimethylenamines **4a-c**. Longer reaction time led to further methylation with the formation of 1-methyl derivatives **5a-c**. Heating **4a-c** with phosphorus pentasulfide in pyridine under reflux gave the corresponding thieno[3,2-*e*][1,2,4]triazines **7a-c**. Alternatively, thiation of **1a-c** gave the corresponding 3-aryl-5-(3,4,5-trimethoxybenzyl)-1,2,4-triazine-6(1*H*)-thiones **6a-c**. Heating the latter at 100 ^{o}C with DMF-DMA for 30 minutes gave the corresponding thienotriazines **7a-c**.

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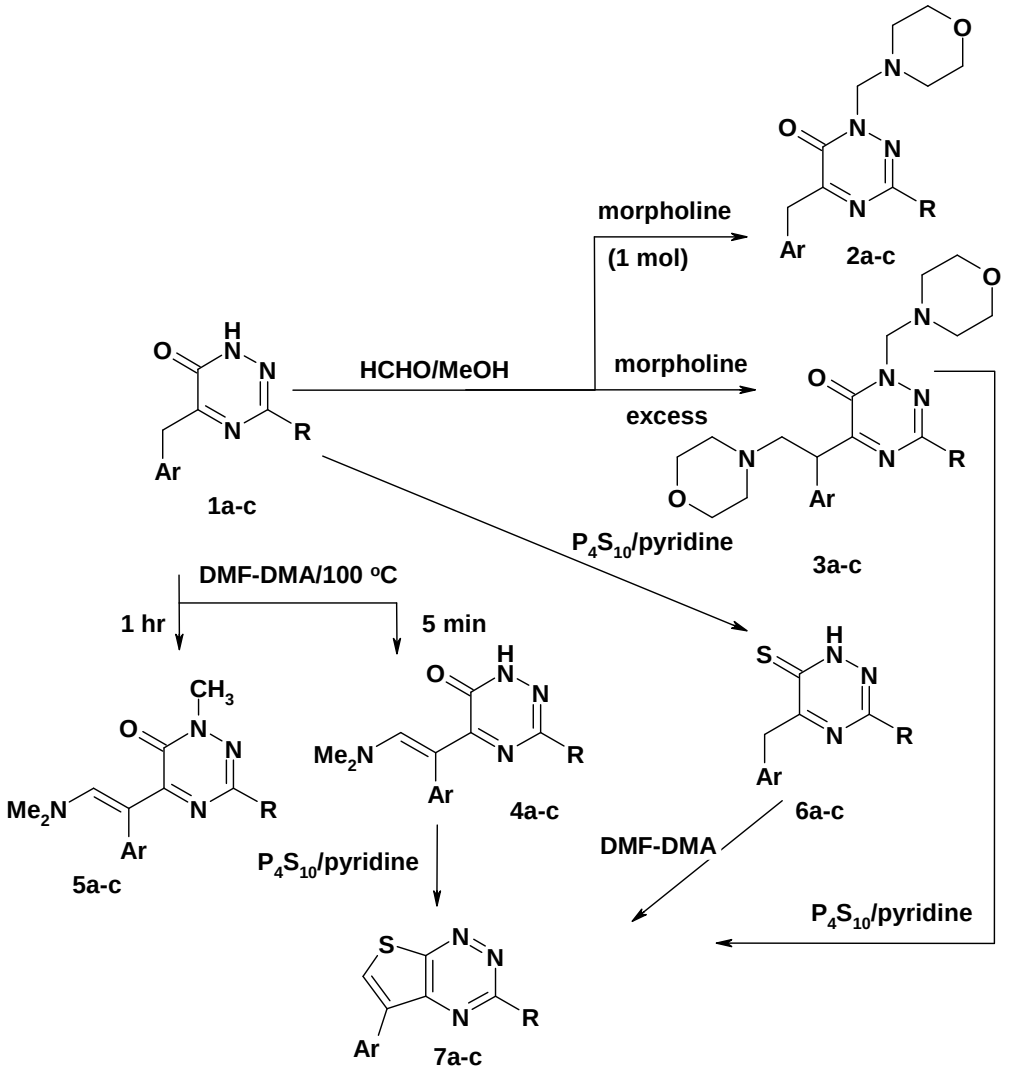

Scheme 1

Treatment of compounds **1a,b** with phosphorous oxychloride gave the corresponding 6-chloro derivatives **8a,b**. Treatment of the latter with the appropriate amine or hydrazine hydrate gave the corresponding 6-amino derivatives **9-11** or 6-hydrazino derivatives **12a,b**. The latter were condensed with formic acid, carbon disulfide or pyruvic acid to give the corresponding [1,2,4]triazolo[3,4-*f*][1,2,4]triazines **13a,b**, [1,2,4]triazolo[3,4-*f*][1,2,4]triazine-3(2*H*)-thiones **14a,b** and [1,2,4]triazino[6,1-*c*][1,2,4]triazin-4-one **15b** respectively.

Alkylation of the thioxotriazines **6a-c** with the appropriate alkyl halide in DMF or acetone in the presence of anhydrous K_2CO_3 gave the corresponding thioether derivatives **16-20**.

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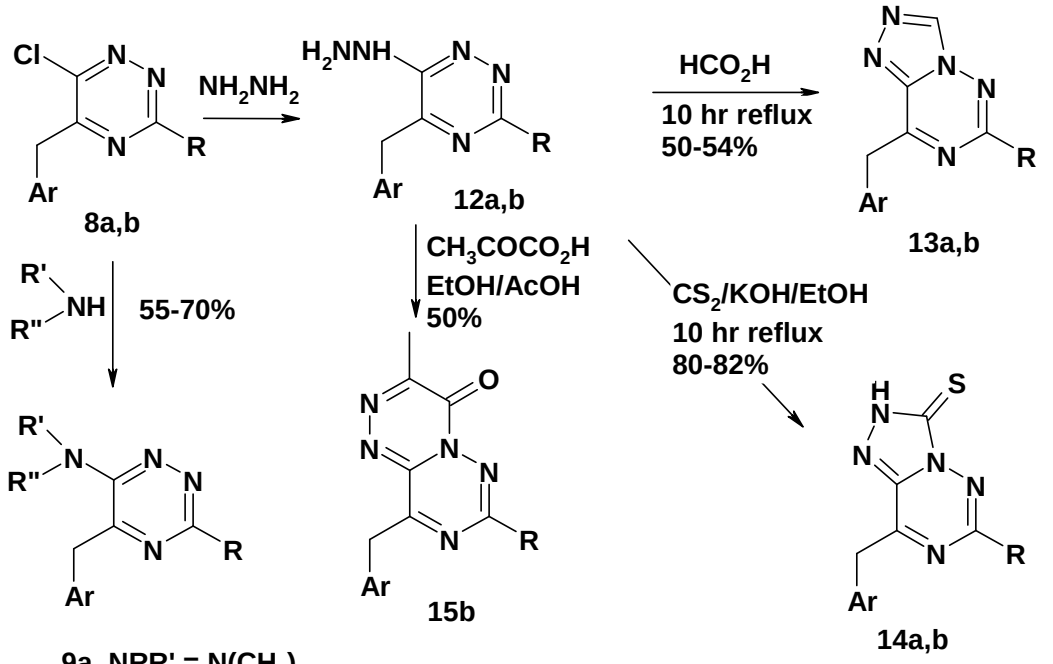
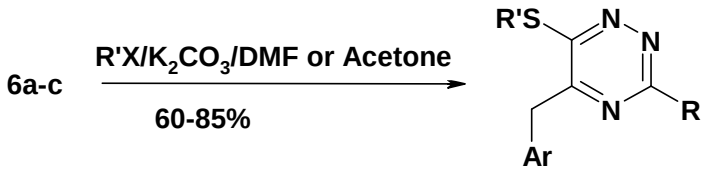


9a, NRR' = $N(CH_3)_2$
10a, NRR' = NHC_6H_4Cl-*p*
10b, NRR' = NHC_6H_4Cl-*p*
11b, NRR' = $NHC_6H_4CH_3$-*p*



16a-c, R' = CH_2CO_2Et
17a-c, R' = CH_2CONH_2
18a,b, R' = CH_3
19a,b, R' =CH_2CH_3
20a,c, R' =$CH_2C_6H_5$

Ar = 3,4,5-trimethoxyphenyl
a, R = C_6H_5, b, R = *p*-ClC_6H_4, c, R = *p*-$CH_3OC_6H_4$

Scheme 2

Cytotoxic activity evaluation

The cytotoxic activity of some of the newly synthesized compounds have been tested against two cell lines, breast carcinoma (MCF7) and colon carcinoma (HCT 116) and the results are summarized in Table 1. The Sulforhodamine B (SRB) assay of Skehan[14] was used to evaluate the

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cytotoxic activity of the newly synthesized compounds **4a, 5c, 6c, 7b, 8b, 12a, 13a, 14b, 15b** and **16c** against two cell lines, breast carcinoma (MCF7) and colon carcinoma (HCT 116). It was observed through analysis of Table 1 that:

- Novel 1,2,4-triazines containing *p*-chlorophenyl as substituent at position 3 (compounds **7b** and **8b**) had the highest and the same cytotoxic activity against breast carcinoma (MCF7) cell line (IC_{50} = 0.743 ug/ml) but compound **7b** showed higher cytotoxic activity against colon carcinoma cell line than compound **8b** as shown in Table 1.
- Triazinotriazine derivative **15b** was the most active one against colon carcinoma cell line (IC_{50} = 0.629 ug/ml) had IC_{50} higher than that of standard drug and showed high activity against breast carcinoma cell line (IC_{50} = 0.896 ug/ml).
- Triazinone bearing 4-methoxyphenyl moiety **5c** and 3-thioxotriazolotriazine **14b** showed high and the same activity against breast carcinoma cell line (IC_{50} = 0.858 ug/ml). Also, compound **5c** showed high activity against colon carcinoma cell line but **14b** showed moderate activity against colon carcinoma cell line.
- Compounds **4a**, **12a** and **16c** showed moderate and the same activity against breast carcinoma cell line (IC_{50} = 0.934 ug/ml). Compounds **4a** and **16c** showed moderate activity against colon carcinoma cell line but the hydrazino derivative **12a** was the least active one against colon carcinoma cell line (IC_{50} = 1.62 ug/ml).
- 6-Thioxotriazine bearing 4-methoxyphenyl moiety **6c** was the least active one against breast carcinoma cell line (IC_{50} =1.85 ug/ml) but it showed higher and the same cytotoxic activity as compound **4a** against colon carcinoma cell line (IC_{50} = 0.858 ug/ml).
- Triazolotriazine derivative **13a** showed low activity against the two cell lines as seen from their IC_{50} values (Table 1).
- From the above results, among the uncondensed 1,2,4-triazines tested **4a, 5c, 6c, 8b, 12a,16c,** the 6-chloro derivative **8b** was the most active against breast carcinoma (MCF7) cell line (IC_{50} = 0.743 ug/ml) while the 6-oxo derivative **5c** was the most active against colon carcinoma cell line (IC_{50} = 0.781 ug/ml).
- Among the tested condensed 1,2,4-triazines **7b, 13a, 14b, 15b,** the thienotriazine **7b** was the most active against breast carcinoma (MCF7) cell line (IC_{50} = 0.743 ug/ml) and the second most active against colon carcinoma cell line (IC_{50} = 0.781 ug/ml) while the triazinotriazine **15b** was the most active against colon carcinoma cell line (IC_{50} = 0.628 ug/ml) with higher activity than the reference used Doxorubicin (IC_{50} = 0.743 ug/ml).

In conclusion, the results presented in Table 1 showed that compounds **7b** and **8b** were the most active ones while compound **6c** was the least active one against breast carcinoma cell line (MCF7). Meanwhile compound **15b** was the most active while compound **12a** was the least active one against colon carcinoma cell line (HCT 116). Thus, thieno[3,2-*e*][1,2,4]triazines and [1,2,4]triazino[6,1-*c*][1,2,4]triazines proved to be potential leads for further cytotoxic activity studies which will be pursued further in our research work.

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Table 1: Cytotoxicity of the synthesized compounds against breast carcinoma (MCF7) and colon carcinoma (HCT 116) cell lines.

Compound No.	IC_{50} (ug/ml)	
	Breast carcinoma (MCF7) cell line	Colon carcinoma (HCT 116) cell line
4a	0.934	0.858
5c	0.858	0.781
6c	1.85	0.858
7b	0.743	0.781
8b	0.743	0.896
12a	0.934	1.62
13a	1.66	1.54
14b	0.858	1.16
15b	0.896	0.629
16c	0.934	0.896
Doxorubicin	0.629	0.743

- IC_{50} is a dose required to inhibit the cell growth by 50%

Antimicrobial activity:

The antimicrobial activity of some of the newly synthesized compounds was tested against each of the mentioned ATCC reference strains in Table 2. Different bacteria and fungi were subjected to susceptibility testing on Muller-Hinton agar medium by the disc agar diffusion method.[15] The strains used were: *S. aureus* ATCC 25923, *B. subtilis* ATCC 6633, *E. coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 27736, *Aspergillus spp.* ATCC 16404, *Mucor spp.* ATCC 7941 and *C. albicans* ATCC 10231. The in vitro antimicrobial activity of the tested compounds summarized in Table 2 revealed the following:

- Compound **15b** showed marked activity against *S. aureus*. Also, Compounds **3c, 4a, 7c** and **14b** showed high activity against *S. aureus*.
- Compounds **15b, 19b** and **20a** were effective against *B. subtilis*.
- Compounds **4a, 6c, 19b, 20a** and **20c** showed high activity against Gram-negative rods (*E. coli*).
- Compounds **6c, 8b, 12a, 15b, 17c** and **18a** exhibited high activity against Gram-negative bacteria (*Klebsiella pneumonia*).
- Compounds **6c, 7c, 14b, 15b, 18a** and **19b** showed high antifungal activity against *Aspergillus spp*.
- Compounds **7b, 8b, 12a, 9a** and **19b** exhibited high activity against *Mucor spp*.
- Compounds **6c, 7b, 13a, 9a** and **17c** were highly active against fungi (*C. albicans*).

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Table 2: Antimicrobial activity of the synthesized compounds determined by disc agar diffusion method.*

Compound No.**	Cram-positive		Gram-negative		Fungi		
	S. aureus	*B. subtilis*	*E.coli*	*Klebsiella pneumoniae*	*Aspergillus spp.*	*Mucor spp.*	*C. albicans*
DMSO (control)	6	6	6	6	6	6	6
2b	15	8	19	11	8	9	6
3c	18	13	17	10	8	6	6
4a	20	11	20	8	7	8	8
5c	11	12	17	17	6	8	13
6c	13	15	22	19	9	10	15
7b	15	11	18	12	6	11	14
7c	19	6	16	14	9	6	9
8b	10	10	15	20	7	12	10
12a	8	9	18	22	8	14	13
13a	12	17	11	15	6	8	15
14b	18	13	12	16	9	6	11
15b	26	20	15	23	10	6	12
10a	6	11	19	11	5	7	8
9a	11	8	10	6	6	13	17
16c	8	10	14	11	6	8	10
17c	10	15	19	20	8	9	16
18a	16	14	13	19	10	10	13
19b	17	19	20	15	11	12	12
20a	13	20	23	14	4	10	11
20c	15	12	22	16	6	8	10
Ofloxacin	24	23	25	25	-	-	-
Amphotericin B	-	-	-	-	12	16	19

*Values under each strain indicate the diameter of inhibition zones (mm).

** Stock solution of each of the tested compounds 0.5 mg/mL in DMSO.

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Experimental Section

General. Melting points were determined on a Gallen Kamp Digital melting point apparatus and are uncorrected. IR spectra were determined as KBr discs on a Shimadzu FTIR 8000 Spectrophotometer. ^{1}H NMR spectra were carried out on Varian Gemini-200, 200 MHz spectrometer. Mass spectra (EIMS) were run on Hewlett Packard 5988 spectrometer. Elemental analyses were carried out at the Microanalytical Center, Faculty of Science, Cairo University, Cairo, Egypt and the Microanalytical Center, National Research Center, Dokki, Egypt. Cytotoxic activity was carried out in the Cancer Biology Department, Pharmacology Unit, National Cancer Institute, Cairo, Egypt. Progress of the reaction was monitored by TLC using sheets precoated with UV fluorescent silica gel Merck 60 F254. Compounds **1a-c** were prepared as reported recently.[9]

3-Aryl-1-morpholinomethyl-5-(3,4,5-trimethoxybenzyl)-1,2,4-triazin-6(1*H*)-ones (Mannich bases) 2a-c. To a stirred cold (5 °C) solution of each of **1a-c** (2 mmol) in methanol (20 mL) was added formaldehyde (2 mL) and morpholine (2 mmol). Stirring was continued at 5 °C for 2 h and then at room temperature overnight. The solid precipitated was collected and recrystallized from methanol to give the corresponding Mannich products **2a-c** (Table 3).

IR of **2a**: 3001, 2937, 2837, 1660, 1585, 1128; IR of **2b**: 3073, 2998, 2939, 2837, 1668, 1590, 1128; IR of **2c**: 2935, 2837, 1658, 1590, 1127. ^{1}H NMR ($CDCl_3$) of **2a**: δ 2.78 (m, 4H), 3.59 (m, 4H), 3.68 (m, 4H), 3.80 (s, 3H, *p*-OCH_3), 3.82 (s, 6H, 2 *m*-OCH_3), 7.46 (s, 2H, ArH), 8.17 (m, 5H, ArH); ^{1}H NMR ($CDCl_3$) of **2b**: δ 2.58 (m, 2H), 3.63 (s, 3H, *p*-OCH_3), 3.71 (s, 6H, 2 *m*-OCH_3), 3.88 (s, 2H), 4.81 (m, 4H), 5.27 (m, 4H), 7.35 (d, 2H, *J* = 8.0, ArH), 7.92 (m, 2H, ArH), 8.09 (d, 2H, *J* = 8.0, ArH).

3-Aryl-5-{[1-(3,4,5-trimethoxybenzyl)-2-morpholino]ethyl}-1-morpholinomethyl-1,2,4-triazin-6(1*H*)-ones (Mannich bases) 3a-c. To a stirred cold (5 ^{o}C) solution of each of **1a-c** (2 mmol) in methanol (20 mL) was added formaldehyde (2mL) and morpholine (2 mL). Stirring was continued at 5 ^{o}C for 2 h and then at room temperature overnight. The solid precipitated was collected and recrystallized from ethanol to give the corresponding Mannich products **3a-c** (Table 3).

IR of **3a**: 3116, 3063, 2947, 2854, 1658, 1585, 1127; IR of **3b**: 3003, 2933, 2889, 2835, 1661, 1585, 1126; IR of **3c**: 3067, 2934, 2833, 1662, 1587, 1125. ^{1}H NMR ($CDCl_3$) of **3a**: δ 2.45 (m, 4H), 2.79 (t, 4H, *J* = 4.6), 3.66 (m, 5H, $2CH_2$, CH), 3.68 (t, 4H, *J* = 4.6), 3.80 (s, 3H, *p*-OCH_3), 3.85 (s, 6H, 2 *m*-OCH_3), 4.93 (d, 2H, *J* = 13.0), 5.15 (d, 2H, *J* = 13.0), 7.47 (s, 2H, ArH), 8.18 (m, 5H, ArH); ^{1}H NMR ($CDCl_3$) of **3b**: δ 2.45 (m, 2H), 2.65 (m, 2H), 2.80 (t, 4H, *J* = 4.0), 3.55 (m, 5H, $2CH_2$, CH), 3.68 (t, 4H, *J* = 4.0), 3.78 (s, 3H, *p*-OCH_3), 3.85 (s, 6H, 2 *m*-OCH_3), 4.93 (d, 2H, *J* = 12.0, CH_2), 5.11 (d, 2H, *J* = 12.0, CH_2), 7.44 (s, 2H, ArH), 7.46 (d, 2H, *J* = 8.0, ArH), 8.13 (d, 2H, *J* = 8.0, ArH).

3-Aryl-5-[2-dimethylamino-1-(3,4,5-trimethoxybenzyl)ethenyl]-1,2,4-triazin-6(1*H*)-ones 4a-c. A mixture of each of **1a-c** (1 mmol) and DMF-DMA (1 mL) was heated at 100 ^{o}C (steam bath) for 5 min. After cooling and adding EtOH (5 mL), the precipitate was collected and recrystallized

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to give the corresponding products **4a-c** (Table 3).

IR of **4a**: 3260, 3173, 3059, 3008, 2934, 1649, 1593, 1121; IR of **4b**: 3198, 3157, 2996, 2923, 1645, 1591, 1121; IR of **4c**: 3212, 3054, 2932, 2830, 1642, 1590, 1124. ^{1}H NMR ($CDCl_3$) of **4a**: δ 2.90 (br, 6H, $N(CH_3)_2$), 3.84 (s, 6H, 2 *m*-OCH_3), 3.92 (s, 3H, *p*-OCH_3), 7.27-7.31 (m, 5H, ArH), 7.85 (s, 2H, ArH), 9.47 (s, 1H, =CH), 10.85 (br, 1H, NH); ^{1}H NMR ($CDCl_3$) of **4b**: δ 2.88 (br, 6H, $N(CH_3)_2$), 3.83 (s, 6H, 2 *m*-OCH_3), 3.92 (s, 3H, *p*-OCH_3), 7.26 (s, 2H, ArH), 7.80 (d, 2H, *J* = 8.0, ArH), 7.82 (d, 2H, *J* = 8.0, ArH), 9.45 (s, 1H, =CH), 10.57 (br, 1H, NH).

3-Aryl-5-[2-dimethylamino-1-(3,4,5-trimethoxybenzyl)ethenyl]-1-methyl-1,2,4-triazin-6(1*H*)-ones 5a-c. General procedure. A mixture of **1a-c** (1 mmol) and DMF-DMA (1 mL) was heated at 100^{o}C (steam bath) for 1 h. After cooling and adding EtOH (5 mL), the precipitate was collected and recrystallized from EtOH to give yellow crystals of **5a-c** (Table 3).

IR of **5a**: 3062, 2995, 2937, 1654, 1589, 1124; IR of **5b**: 3069, 2994, 2934, 2832, 1635, 1594, 1124; IR of **5c**: 3091, 2936, 1660, 1586. ^{1}HNMR ($CDCl_3$) of **5a**: δ 2.88 (br, 6H, $N(CH_3)_2$), 3.68 (s, 3H, *p*-OCH_3), 3.70 (s, 6H, 2 *m*-OCH_3), 3.85 (s, 3H, NCH_3), 7.48 (s, 2H, ArH), 8.23-8.24 (m, 5H, ArH), 10.43 (s, 1H, =CH); ^{1}H NMR (DMSO-d_6) of **5b**: δ 2.84 (br, 6H, $N(CH_3)_2$), 3.51 (s, 3H, *p*-OCH_3), 3.66 (s, 6H, 2 *m*-OCH_3), 3.91 (s, 3H, NCH_3), 7.39 (d, 2H, *J* = 8.0, ArH), 7.57 (s, 2H, ArH), 7.77 (d, 2H, *J* = 8.0, ArH), 9.44 (s, 1H, =CH). MS of **5c**: m/z = 453 (M^+, 2.2%).

3-Aryl-5-(3,4,5-trimethoxybenzyl)-1,2,4-triazine-6(1*H*)-thiones 6a-c. A solution of each of **1a-c** (2 mmol) and phosphorus pentasulfide (0.7 g, 3 mmol) in anhydrous pyridine (15 mL) was heated under reflux for 3 h. After cooling the precipitate was collected and recrystallized from ethanol to give the corresponding 1,2,4-triazine-6(1H)-thiones **6a-c** (Table 3).

IR of **6a**: 3244, 2936, 2824, 1588, 1124; IR of **6b**: 3309, 2935, 2834, 1590, 1125; IR of **6c**: 3060, 2931, 2832, 1631, 1575, 1122. ^{1}H NMR (DMSO-d_6) of **6a**: δ 3.65 (s, 3H, *p*-OCH_3), 3.75 (s, 6H, 2 *m*-OCH_3), 4.40 (s, 2H), 7.52 (s, 2H, ArH), 7.79-8.79 (m, 5H, ArH), 12.91 (br, 1H, NH); ^{1}H NMR ($CDCl_3$) of **6b**: δ 3.74 (s, 3H, *p*-OCH_3), 3.83 (s, 6H, 2 *m*-OCH_3), 4.45 (s, 2H), 7.41 (s, 2H, ArH), 7.42 (d, *J* = 8.0, 2H, ArH), 8.05 (d, *J* = 8.0, 2H, ArH), 8.55 (s, 1H, NH).

Thieno[3,2-*e*][1,2,4]triazines 7a-c.

Method A: A solution of each of **3a-c** (2 mmol) and phosphorus pentasulfide (0.7 g, 3 mmol) in pyridine (10 mL) was heated under reflux for 3 h. After cooling the precipitate was collected and recrystallized from the proper solvent to give the corresponding products **7a-c** in 78-85% yields (Table 3).

Method B: A solution of each of **4a-c** (1 mmol) and phosphorus pentasulfide (0.34 g, 1.5 mmol) in pyridine (10 mL) was heated under reflux for 2 h. After cooling the precipitate was collected and recrystallized from the proper solvent to give the corresponding products **7a-c** in 82-86% yields (Table 3).

Method C: A mixture of each of **6a-c** (1 mmol) and DMF-DMA (1 mL) was heated under reflux for 0.5 h. After cooling and triturating with ethanol the precipitate was collected and recrystallized from the proper solvent to give the corresponding products **7a-c** in 80-83% yields (Table 3).

IR of **7a**: 3075, 2998, 2935, 2832, 1583, 1533, 1124; IR of **7b**: 3068, 2935, 2832,

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1635, 1585, 1533, 1126; IR of **7c**: 3091, 3001, 2950, 2836, 1627, 1582, 1124. ^{1}H NMR ($CDCl_3$) of **7a**: δ 3.95 (s, 3H, *p*-OCH_3), 4.00 (s, 6H, 2 *m*-OCH_3), 7.39 (s, 2H, ArH), 7.40-7.56 (m, 5H, ArH), 8.31 (s, 1H, =CH). ^{1}H NMR ($CDCl_3$) of **7b**: δ 3.76 (s, 3H, *p*-OCH_3), 3.90 (s, 6H, 2 *m*-OCH_3), 7.18 (s, 2H, ArH), 7.43 (d, 2H, *J* = 8.0, ArH), 8.23 (s, 1H, =CH), 8.52 (d, 2H, *J* = 8.0, ArH).

Table 3: Yield mp and C, H, N analysis of compounds 2-7.

Compd. No.	Yield %	m.p. °C	Mol. Form. (Mol. Wt.)	Analysis (%) Calcd./Found		
				C	H	N
2a	66	95-7	$C_{24}H_{28}N_4O_5$ (452.5)	63.70 63.43	6.24 6.11	12.38 12.73
2b	60	102-4	$C_{24}H_{27}ClN_4O_5$ (487.0)	59.20 59.45	5.59 5.61	11.51 11.50
2c	65	112-4	$C_{25}H_{30}N_4O_6$ (482.5)	62.23 62.39	6.27 6.56	11.61 11.47
3a	68	115-7	$C_{29}H_{37}N_5O_6$ (551.7)	63.14 62.95	6.75 6.45	12.70 12.45
3b	70	100	$C_{29}H_{36}ClN_5O_6$ (586.1)	59.43 59.72	6.18 6.39	11.95 12.09
3c	66	121	$C_{30}H_{39}N_5O_7$ (581.7)	61.95 61.65	6.76 6.44	12.04 11.78
4a	85	226	$C_{22}H_{24}N_4O_4$ (408.5)	64.69 64.39	5.92 5.65	13.72 13.69
4b	90	235	$C_{22}H_{23}ClN_4O_4$ (442.9)	59.66 59.95	5.23 5.03	12.65 12.68
4c	88	253	$C_{23}H_{26}N_4O_5$ (438.5)	63.00 63.18	5.97 5.65	12.78 12.60
5a	86	286	$C_{23}H_{26}N_4O_4$ (422.5)	65.39 65.66	6.20 6.09	13.26 13.55
5b	88	311	$C_{23}H_{25}ClN_4O_4$ (456.9)	60.46 60.60	5.51 5.60	12.26 12.15
5c	80	>300	$C_{24}H_{28}N_4O_5$ (452.5)	63.70 63.78	6.23 5.96	12.38 12.67
6a	80	221	$C_{19}H_{19}N_3O_3S$ (369.5)	61.77 61.73	5.18 4.92	11.37 11.30
6b	85	233	$C_{19}H_{18}ClN_3O_3S$ (403.9)	56.50 56.80	4.49 4.40	10.40 10.56
6c	72	228	$C_{20}H_{21}N_3O_4S$ (399.5)	60.14 60.44	5.30 5.00	10.52 10.82
7a	80	211	$C_{20}H_{17}N_3O_3S$ (379.4)	63.31 63.38	4.52 4.82	11.07 11.27
7b	83	216	$C_{20}H_{16}ClN_3O_3S$ (413.9)	58.04 58.32	3.90 3.60	10.15 10.35
7c	85	220	$C_{21}H_{19}N_3O_4S$ (409.5)	61.60 61.53	4.68 4.96	10.26 10.54

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3-Aryl-6-chloro-5-(3,4,5-trimethoxybenzyl)-1,2,4-triazines 8a,b. A mixture of each of 1a,b (2 mmol) and phosphorus oxychloride (5 mL) was heated on a boiling water bath for 1 hr. The excess phosphorus oxychloride was removed under reduced pressure and the residue was poured over crushed ice. The precipitated solid was collected, washed with water and crystallized from absolute ethanol to give the corresponding **8a,b** (Table 4).

IR of **8a**: 3062, 3009, 2954, 2933, 2838, 1622, 1579,; IR of **8b**: 2938, 2838, 1633, 1572, 1128. ^{1}H NMR ($CDCl_3$) of **8a**: δ 3.48 (s, 3H, *p*-OCH_3), 3.62 (s, 6H, 2 *m*-OCH_3), 3.77 (s, 2H), 7.26 (s, 2H, ArH), 7.76 (m, 5H, ArH); ^{1}H NMR ($CDCl_3$) of **8b**: δ 3.74 (s, 3H, *p*-OCH_3), 3.92 (s, 6H, 2 *m*-OCH_3), 4.35 (s, 2H), 7.37 (s, 2H, ArH), 7.41 (d, 2H, *J* = 7.0, ArH), 7.98 (d, 2H, *J* = 7.0, ArH).

6-Substituted amino-3-aryl-5-(3,4,5-trimethoxybenzyl)-1,2,4-triazines 9-12. A solution of each of **8a** or **8b** (3 mmol) in absolute ethanol (20 mL) was refluxed with the appropriate amine (6 mmol) or hydrazine hydrate (80%, 1 mL) for 6-8 hr (monitored by TLC). The solvent was removed under reduced pressure and the residue was collected and crystallized from ethanol to give the corresponding 6-substituted amino derivatives **9-12** (Table 4).

IR of **9a**: 3062, 3011, 2987, 2954, 2934, 1622, 1579, 1130; IR of **10a**: 3309, 3059, 2970, 2938, 2830, 1644, 1580, 1125; IR of **10b**: 3289, 3000, 2946, 2835, 1629, 1580; IR of **11b**: 3309, 3059, 2970, 2938, 2830, 1644, 1580, 1125. ^{1}H NMR ($CDCl_3$) of **9a**: δ 2.56 (s, 6H, $N(CH_3)_2$), 3.62 (s, 9H, 3 OCH_3), 4.52 (s, 2H), 7.26-7.83 (m, 7H, ArH); ^{1}H NMR (DMSO-d_6) of **10b**: δ 3.52 (s, 3H, *p*-OCH_3), 3.56 (s, 6H, 2 *m*-OCH_3), 3.63 (s, 2H), 6.84 (s, 2H, ArH), 7.53 (d, 2H, *J* = 8.0, ArH), 7.64 (d, 2H, *J* = 8.0, ArH), 8.07 (d, 2H, *J* = 8.0, ArH), 8.19 (d, 2H, *J* = 8.0, ArH), 13.55 (s, 1H, NH, exchangeable). IR of **12a**: 3330-3200 (NH-NH_2), 2925, 2853, 1602, 1507. IR of **12b**: 3380, 3278, 3178, 2684, 1617, 1470, ^{1}H NMR (DMSO-d_6) of **12b**: δ 3.62 (s, 6H, 2 *m*-OCH_3), 3.67 (s, 3H, *p*-OCH_3), 4.41 (s, 2H), 7.28 (s, 2H, ArH), 7.62 (d, *J* = 8.0, 2.0, ArH), 8.05 (d, *J* = 8.0, 2.0, ArH), 9.54-9.90 (m, 3H, NH-NH_2).

6-Substituted-8-(3,4,5-trimethoxybenzyl)-[1,2,4]triazolo][4,3-*f*][1,2,4]triazines 13a,b. A solution of each of **12a** or **12b** (1 mmol) in formic acid (10 mL) was refluxed for 8 h. After cooling and dilution with water, the formed precipitate was collected and crystallized from ethanol as white crystals of **13a,b** (Table 4).

IR of **13a**: 2933, 1587, 1499, 1120; IR of **13b**: 2935, 2834, 1590, 1489, 1126. ^{1}H NMR (DMSO-d_6) of **13a**: δ 3.56 (s, 9H, 3 OCH_3), 4.69 (s, 2H), 7.32-7.67 (m, 7H, ArH), 8.54 (s, 1H, =CH).

6-Substituted-8-(3,4,5-trimethoxybenzyl)-[1,2,4-triazolo][4,3-*f*][1,2,4]triazine-3(2H)-thiones 14a,b. To an ice cooled solution of each **12a** or **12b** (2 mmol) in absolute ethanol (20 mL) containing potassium hydroxide (2 mmol), carbon disulfide (4 mmol) was added dropwise with stirring. The mixture was diluted with absolute ethanol (10 mL) and was refluxed for 10 hrs. The reaction mixture was filtered, concentrated, diluted with water and neutralized with acetic acid. The precipitated product was crystallized from dioxane as yellow crystals of **14a,b** (Table 4).

IR of **14a**: 3251, 2933, 2832, 1579, 1126; IR of **14b**: 3289, 3073, 2999, 2958, 2936, 2833, 1649, 1125. ^{1}H NMR (DMSO-d_6) of **14a**: δ 3.79, (s, 6H, 2 *m*-OCH_3), 3.88 (s, 3H, *p*-OCH_3), 3.96 (s, 2H), 7.63-8.59 (m, 7H, ArH), 9.14 (s, 1H, NH).

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Table 4: Yield mp and C, H, N analysis of compounds 8-15.

Compd. No.	Yield %	m.p. °C	Mol. Form. (Mol. Wt.)	Analysis (%) Calcd./Found		
				C	H	N
8a	70	133	$C_{19}H_{18}ClN_3O_3$ (371.8)	61.38 61.09	4.88 4.76	11.30 11.21
8b	75	111	$C_{19}H_{17}Cl_2N_3O_3$ (406.3)	56.17 55.90	4.22 4.50	10.34 10.54
9a	70	124	$C_{21}H_{24}N_4O_3$ (380.5)	66.30 66.31	6.36 6.31	14.73 14.56
10a	50	140	$C_{25}H_{23}ClN_4O_3$ (462.9)	64.86 64.76	5.01 4.75	12.10 12.12
10b	55	100	$C_{25}H_{22}Cl_2N_4O_3$ (497.4)	60.37 60.18	4.46 4.27	11.26 11.21
11b	62	147	$C_{26}H_{25}ClN_4O_3$ (477.0)	65.47 65.71	5.28 5.22	11.57 11.44
12a	68	261	$C_{19}H_{21}N_5O_3$ (367.4)	62.11 62.10	5.76 5.61	19.06 19.23
12b	62	281	$C_{19}H_{20}ClN_5O_3$ (401.9)	56.79 56.62	5.02 5.26	17.43 17.71
13a	50	216	$C_{20}H_{19}N_5O_3$ (377.4)	63.65 63.45	5.07 4.89	18.56 18.38
13b	54	230	$C_{20}H_{18}ClN_5O_3$ (411.9)	58.33 58.62	4.41 4.59	17.00 16.85
14a	80	243	$C_{20}H_{19}N_5O_3S$ (409.5)	58.67 58.71	4.68 4.80	17.10 17.32
14b	82	253	$C_{20}H_{18}ClN_5O_3S$ (443.9)	54.11 54.34	4.09 4.12	15.78 16.01

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7-*p*-Chlorophenyl-3-methyl-9-(3,4,5-trimethoxybenzyl)-4*H*-[1,2,4]-triazino[6,1-*c*][1,2,4]triazin-4-one 15b A mixture of **12b** (2 mmol) and pyruvic acid (3 mmol) in absolute ethanol (20 mL) containing few drops of glacial acetic acid was refluxed for 10 hrs. After cooling, the formed precipitate was collected and crystallized from DMF to give buff crystals of **15b**. Yield: 50%, mp >300 ^{o}C, IR: 3096, 2996, 2937, 1678, 1588, 1126, ^{1}H NMR ($CDCl_3$): δ 2.46 (s, 3H, CH_3), 3.54 (s, 9H, 3 OCH_3), 4.75 (s, 2H), 6.42 (d, 2H, *J* = 8.0, ArH), 7.14 (s, 2H, ArH), 7.86 (d, 2H, J = 8.0, ArH). Anal. Calcd for $C_{22}H_{20}ClN_5O_4$ (453.9): C, 58.22; H, 4.44; N, 15.43. Found: C, 57.98; H, 4.68; N, 15.62.

3-Aryl-6-(ethoxycarbonylmethylthio)-5-(3,4,5-trimethoxybenzyl)-1,2,4-triazines16a-c. A mixture of each of **6a-c** (1 mmol), ethyl bromoacetate (1 mmol) and anhydrous K_2CO_3 (1.5 mmol) in dry acetone (20 mL) was refluxed for 20 h. The reaction mixture was filtered while hot, concentrated and the obtained solid was crystallized from ethanol to give **16a-c** (Table 5).
IR of **16a**: 2976, 2931, 1725, 1577, 1123, 853; IR of **16b**: 2928, 2848, 1725, 1587, 1499, 1124, 835, 755; IR of **16c**: 2943, 2930, 1727, 1579, 1125, 843, 750.

3-Aryl-5-(3,4,5-trimethoxybenzyl)-1,2,4-triazine-6-thioacetamides 17a-c. A mixture of each of **6a-c** (1 mmol) and chloroacetamide (1 mmol) in dimethylformamide (15 mL) was refluxed for 20 h, cooled and diluted with water. The resulting solid was crystallized from ethanol to give **17a-c** (Table 5).
IR of **17a**: 3385, 3293, 3185, 3105, 2959, 1670, 1617; IR of **17b**: 3220, 3193, 2938, 2838, 1681, 1592, 1126, 761, 725; IR of **17c**: 3380, 3202, 2937, 1669, 1590, 1109, 762, 721. ^{1}H NMR (DMSO-d_6) of **17c**: 3.06 (s, 2H), 3.78 (s, 6H, 2 *m*-OCH_3), 3.82 (s, 6H, 2 *p*-OCH_3), 3.97 (s, 2H), 6.90 (s, 2H, NH_2), 7.08 (s, 2H, ArH), 7.16 (d, 2H, *J* = 8.0, ArH), 8.52 (d, 2H, *J* = 8.0, ArH).

6-Alkylsulfanyl-3-aryl-5-(3,4,5-trimethoxybenzyl)-1,2,4-triazines 18-20. A mixture of each of **6a-c** (2 mmol) and the appropriate alkyl halide (3 mmol) in dimethylformamide (10 mL) and anhydrous potassium carbonate (0.5 g) was stirred overnight. The reaction mixture was diluted with water and the formed product was collected and crystallized from ethanol to give the corresponding alkylthio derivatives **18-20** (Table 5).
IR of **18a**: 2935, 2839, 1602, 1505, 1123; IR of **18b**: 3093, 3003, 2949, 2838, 1624, 1581, 1128, 817; IR of **19a**: 3003, 2951, 2838, 1624, 1580, 1128; IR of **19b**: 3064, 2960, 2929, 1589, 1126, 840; IR (KBr) of **20a**: 3059, 2934, 2833, 1633, 1590, 1124; IR of **20c**: 2928, 2835, 1583, 1124, 847. ^{1}H NMR ($CDCl_3$) of **20a**: δ 3.55 (s, 9H, 3 OCH_3), 4.64 (s, 2H), 4.67 (s, 2H, SCH_2), 7.30-8.51 (m, 12H, ArH).

Table 5: Yield mp and C, H, N analysis of compounds 16-20.

Compd. No.	Yield %	m.p. ^{o}C	Mol. Form. (Mol. Wt.)	Analysis (%) Calcd./Found		
				C	H	N
16a	60	1112	$C_{23}H_{25}N_3O_5S$ (455.5)	60.64 60.94	5.53 5.28	9.22 9.46
16b	67	114	$C_{23}H_{24}ClN_3O_5S$ (489.0)	56.38 56.43	4.94 5.12	8.58 8.64
16c	70	118	$C_{24}H_{27}N_3O_6S$	59.37	5.60	8.65

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			(485.6)	59.45	5.78	8.81
17a	76	241	$C_{21}H_{22}N_4O_4S$ (426.5)	59.14 59.33	5.20 4.95	13.14 13.10
17b	79	256	$C_{21}H_{21}ClN_4O_4S$ (460.9)	54.72 55.01	4.59 4.33	12.16 11.89
17c	70	264	$C_{22}H_{24}N_4O_5S$ (456.5)	57.88 57.82	5.30 5.49	12.27 12.44
18a	85	167	$C_{20}H_{21}N_3O_3S$ (383.5)	62.64 62.36	5.52 5.29	10.96 10.93
18b	80	131	$C_{20}H_{20}ClN_3O_3S$ (417.9)	57.48 57.75	4.82 4.99	10.05 10.08
19a	76	122	$C_{21}H_{23}N_3O_3S$ (397.5)	63.46 63.75	5.83 6.03	10.57 10.32
19b	70	127	$C_{21}H_{22}ClN_3O_3S$ (431.9)	58.39 58.25	5.13 5.06	9.73 10.03
20a	64	143	$C_{26}H_{25}N_3O_3S$ (459.6)	67.95 68.08	5.48 5.19	9.14 8.85
20c	69	138	$C_{27}H_{27}N_3O_4S$ (489.6)	66.24 66.27	5.56 5.46	8.58 8.87

Cytotoxic activity. The Sulforhodamine B (SRB) assay of Skehan[14] was used to evaluate the cytotoxic activity of the newly synthesized compounds **4a, 5c, 6c, 7b, 8b, 12a, 13a, 14b, 15b** and **16c** against two cell lines, breast carcinoma (MCF7) and colon carcinoma (HCT 116). Cells were plated in 96-multiwell plates (10^4cells/well) for 24h before treatment with the compound to allow attachment of the cells to the wall of the plate. Different concentrations of the compounds under test (0, 1, 2.5, 5 and 10 mg/ml) were added to the cell monolayer. Triplicate wells were prepared for each dose. Monolayer cells were incubated with the compounds under test for 48 h at 37°C and atmosphere of 5% CO_2. After 48h, cells were fixed, washed and stained with Sulforhodamine B stain. Excess stain was washed with acetic acid and then after attached stain was recovered with tris EDTA buffer. Color intensity was measured in an ELISA reader. IC_{50} value was determined for each tumor cell line for the specified compounds, calculated by an available computerized program, which was defined as the concentration of drug to produce a 50% reduction in the viability relative. The control given compounds were recorded in (Table 1). Given compounds were considered significantly inactive when their IC_{50} values are higher than 10 ug/mL.

Antimicrobial activity. Different bacteria and fungi were subjected to susceptibility testing on Muller-Hinton agar medium by the disc agar diffusion method.[15] The antimicrobial activity of some of the newly synthesized compounds was tested against each of the mentioned ATCC reference strains in Table 2. The strains used were: S. aureus ATCC 25923, B. subtilis ATCC 6633, E. coli ATCC 25922, Klebsiella pneumoniae ATCC 27736, Aspergillus spp. ATCC 16404, Mucor spp. ATCC 7941 and C. albicans ATCC 10231. Overnight culture was streaked on the surface of Muller-Hinton agar plate. Sterile filter paper disc

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was saturated with 10µl of 0.5 mg/ml w/v solution of the compound under investigation in DMSO. The plates and discs were then incubated at 37 °C for 24 h and examined for inhibition zones to determine the activity of the tested compounds. Control testing using DMSO was used to determine the solvent effect.

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