An efficient synthesis of new fused 1,2,4-triazines as potential antimicrobial and anticancer agents

Ibrahim M. El-Deen[1], Jehan A. Hasanen [2] and Ahmed M. Hefny [2]

[1] *Chemistry department, faculty of science, Port-Said University, Port-Said, Egypt, postal code 42526*
[2] *Chemistry department, faculty of science, Suez Canal University, Ismailia, Egypt, postal code 41522*

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Abstract

The objective of this work is synthesis of some fused 1,2,4-triazine derivatives and study the fields of applications for the synthesized compounds (their antimicrobial and anticancer activities). Methods; new series of fused 1,2,4-triazine derivatives (**4**, **5** and **7**) were prepared via the cyclization of 5-substituted-3-phenyl-2-(amino)thiocarbonyl-1,2,4-triazine -6- one (**3**) with acetic anhydride, phenacyl bromide and ethyl choloroacetate under reflux. Condensation of **7** with aromatic aldehydes yielded the corresponding arylidene derivatives (**10**$_{a,b}$). Acetylation of compounds **5**, **7** and **10**$_{a,b}$ with acetic anhydride afforded the formation of N-acetyl derivatives (**6**, **8** and **11**$_{a,b}$) 1,5-diphenyl-3-acetyl-4-thioxo-7-(3,4,5-trimethoxybenzylidene)-triazino[2,1-a]-1,2,4-triazine-8-one(**6**),3-acetyl-4-thioxo-5-phenyl-7-(3,4,5-trimethoxy benzylidene) -triazino[2,1-a]-1,2,4-triazine-1,8-dione (**8**) and 2,7-di(arylidene)-4-thioxo-5-phenyl-7-(3,4,5-trimethoxy benzylidene)-triazino [2,1-a]-1,2,4-triazine-1,8-dione (**11**$_{a,b}$), respectively. Acetylation of **7** with acetic anhydride in the presence of fused sodium acetate gives diacetyl derivative **9**. The structures of the prepared compounds were confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, MS and elemental analysis. Key findings; Some compounds selected as potential agents hepatocellular carcinoma (HCC) were then evaluated in vitro for their biological activity on HCC-derived cell lines (the compounds show a promising inhibitory growth efficacy with compared standard anticancer drugs).In conclusion, some fused 1,2,4-triazine derivatives might be potentially useful in the field of cancer treatment, finally compounds **3**, **7, 9,** and **11**$_{a,b}$ can be suggested as potent candidates for liver cancer treatment

Introduction

Triazine derivatives have occupied a unique position in medicinal chemistry. Triazine derivatives have attracted considerable pharmaceutical interest due to their antitumor [1-5], anticonvulsant [6] and antileukemic [7, 8] activities and cytotoxic effects [9]

Triazine has been used to form many types of functional groups other than amines and heterocycles and used as protecting groups in natural product synthesis. Thus, they are reactive groups, which are adaptable to different synthetic transformations.

1,2,4-Triazines play a vital role in many biological processes and as synthetic drugs. Furthermore, many heterocyclic systems bearing 1,2,4-triazines are found to exhibit remarkable pharmacological effects such as antimicrobial, antifungal, antibacterial, anticancer, anti-HIV, anti-inflammatory, anti-tuberculosis, antimalarial, etc [10-18].

Nitrogen containing heterocyclics have attracted huge interest over the past decades because of their diverse pharmacological activities, including protein kinase inhabitation. Being involved in nearly all aspects of life at the cellular level, protein kinase have become the most important targets of drugs for various indications, such as cancers and inflammations [18-23].

Herein we describe the synthesis and in vitro biological activity of fused 1, 2, 4-triazine derivatives. The new compounds were screened for antimicrobial and anticancer activates and some of them were found active both in vitro.

1. Experimental section

1.1. Chemistry

The Melting points were determined in capillaries with a MEL-TEMP II laboratory apparatus, USA, and uncorrected. The infra-red spectrum was recorded on a Perkin-Elmer 337 spectrophotometer KBr wafers. Proton and carbon NMR spectra were recorded on a BRUKER EM 360 spectrometer using solution in hexadeuteriodimethyl sulfoxide with tetramethylsilane as the internal standard. Mass spectra was recorded on VG AUTSPEC GEIFAB and a Hewlett-Packard MS Engine Thermospray and ionization by electron impact to 70 eV, the accelerating voltage was 6 KV, the temperature of the ion source was ~ 200°C, and the emission current ~ 100 mA. Microanalyses were conducted using a PERKIN-ELMER 2408 CHN analyzer.

1.1.1 General experimental procedure for the synthesis of triazines

The synthesis pathway leading to the title compounds is outlined in **scheme 1** and **2**. 5-(3,4,5-Trimethoxy benzylidene-2-phenyl-3,1-oxazol-4-one (**2**) was prepared via the condensation of N-benzoyl glycine (**1**) with 3,4,5-trimethoxy benzaldehyde in the presence of fused sodium acetate and acetic anhydride under fusion.

The oxazolinone (**2**) is considered to be a useful starting material for further synthesis, thus compound **2** was treated with thiosemicarbazide in glacial acetic acid to give the corresponding 5- (3,4,5-trimethoxybenzylidene)-3-phenyl-2-(amino)thiocarbonyl-1,2,4-triazine-6-one (**3**). The structure of the novel compound **3** was confirmed by the spectral data.

Acetylation of 1,2,4-triazine-6-one derivative (**3**) with acetic anhydride under reflux led to the formation of 6-(3,4,5-trimethoxybenzylidene)-4- phenyl-3-thioxo-1-methyl-triazolo[2,1-a]-1,2,4-triazine-7-one (**4**). The structure of compound **4** was confirmed by the spectral data. The ^{1}H-NMR spectrum shows a signal for three protons at δ, 2.44 (CH_3) ppm and two signals for three protons and six protons at 3.78, 3.87 ppm due to the three methoxy groups and also, multiplet at 7.30-8.11 ppm due to aromatic ring (8H) and olefinic proton (1H). The mass spectrum of **4** shows molecular ion peak at m/z 436.

Cyclization of 5-(3,4,5-trimethoxybenzylidene)-3-phenyl-2-(amino) thiocarbonyl-1,2,4-triazine -6-one (**3**) either by phenylacyl bromide and ethyl choloroacetate in the presence of fused sodium acetate in acetic acid under reflux, furnished the novel fused triazino [2,1a]-1,2,4-triazine derivatives (**5** and **7**) .

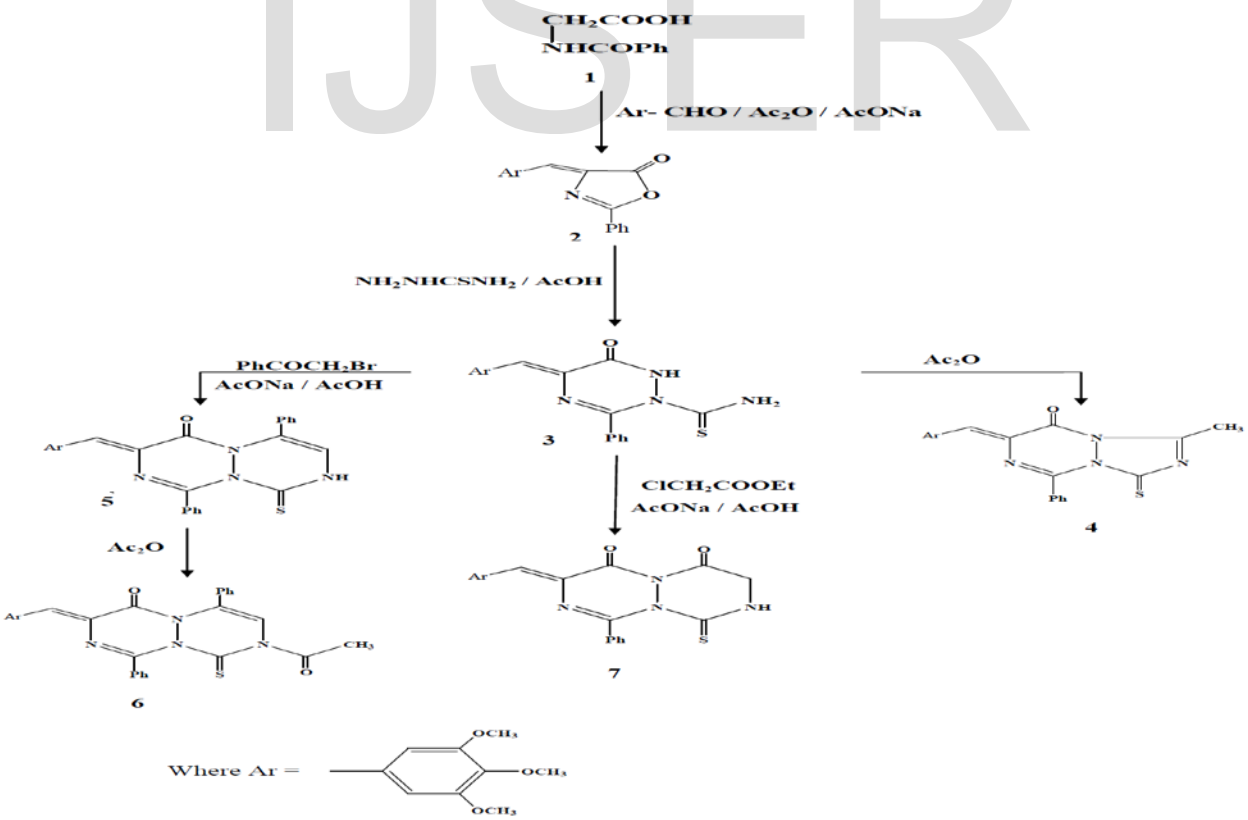
The structures and purity of the newly synthesized fused triazino [2,1a]-1,2,4-triazine derivatives (**5** and **7**) were characterized by using ^{1}H- and ^{13}C NMR spectroscopy, HPLC-MS and elemental analysis.

Acetylation of compounds **5** and **7** with acetic anhydride under reflux affording 1,5-diphenyl-3- acetyl-4-thioxo-7-(3,4,5-trimethoxybenzylidene)-triazino[2,1-a]-1,2,4-triazine-8-one (**6**) and 3 - acetyl - 4- thioxo - 5 - phenyl - 7 - (3,4,5-trimethoxybenzylidene) - triazino[2,1-a] -1,2,4-triazine-1,8-dione (**8**), respectively.

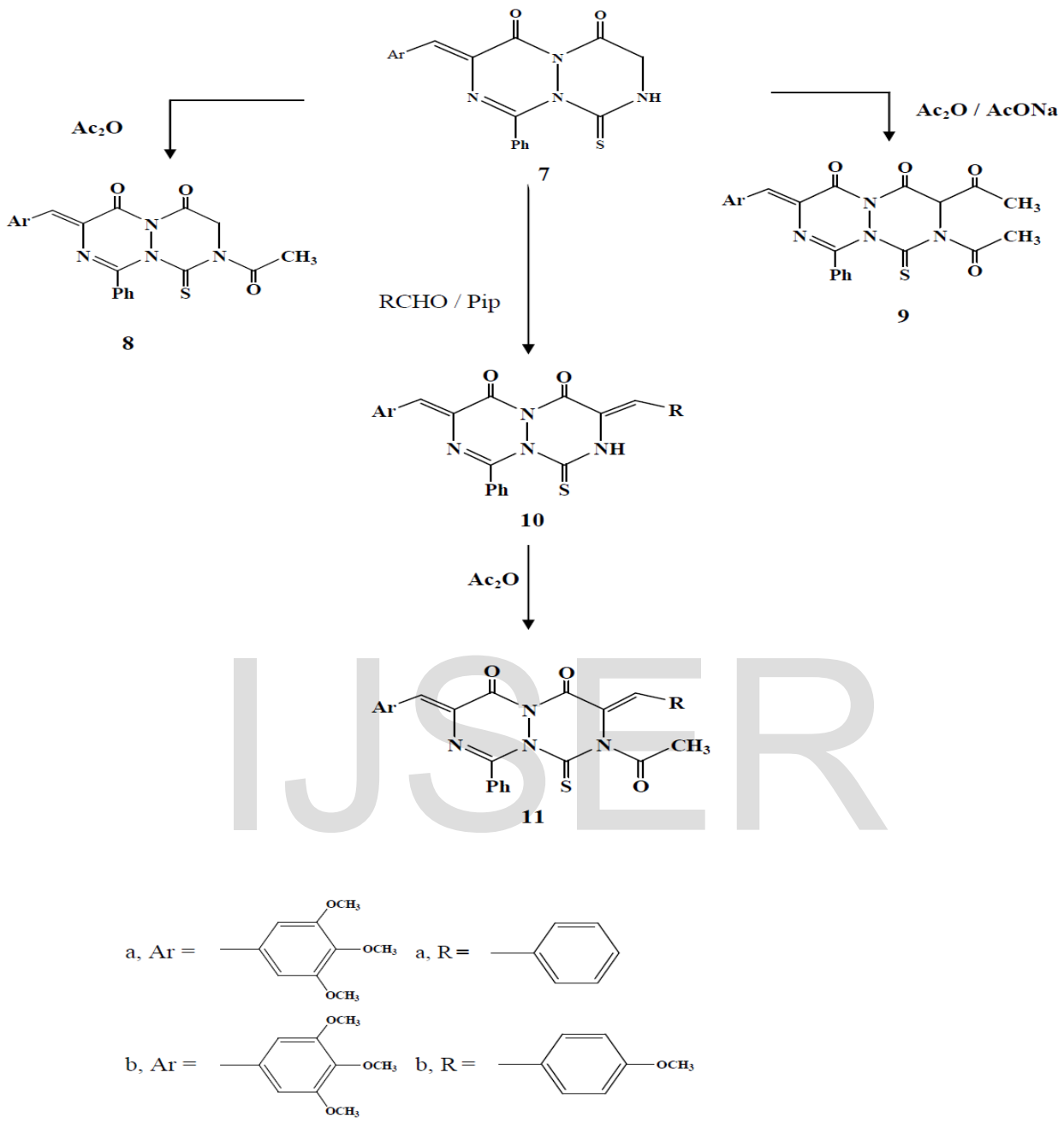
2,3- Diacetyl - 4 - thioxo -5- phenyl - 7 - (3,4,5-trimethoxybenzylidene) - triazino [2,1-a] - 1,2,4-triazine - 1,8 - dione (**9**) was obtained by Acetylation of compound **7** with acetic anhydride in the presence of fused sodium acetate.

2–Arylidene-4-thioxo-5-phenyl-7-(3,4,5-trimethoxybenzylidene)-triazino[2,1-a]-1,2,4-triazine-1,8-dione(**10**$_{a,b}$)were prepared by the fusion between compound **7** with aromatic aldehydes (such as benzaldehyde and anisaldehyde) using a catalytic amount of piperidine.

Boiling of compound **10** with acetic anhydride afforded the N - acetyl - 2,7 - di (arylidene) - 4 - thioxo - 5 - phenyl - 7 - (3,4,5-trimethoxybenzylidene) - triazino [2,1-a] - 1,2,4 - triazine -1,8 - dione (**11**$_{a,b}$).



Scheme 1: synthetic pathway for the preparation of 1,2,4-triazines (**3-7**)



Scheme 2: synthetic pathway for the preparation of 1,2,4-triazines (**8-11**) from the novel fused triazino [2,1a]-1,2,4-triazine derivatives (**7**) .

5-(3,4,5-trimethoxy benzylidene)-2-phenyl-3,1-oxazol-4-one (2)

A mixture of N-benzoyl glycine (**1**, 0.01 mole), 3,4,5-trimethoxy benzaldehyde (0.01 mole), fused sodium acetate (0.03 mole) and acetic anhydride (5 ml) was fused on a hot plate for 2-3 min. The reaction mixture was heated on a water bath for 2 hr, and then was cooled and was poured on to ice water. The precipitate that formed was collected by filtration, was washed with hot water, was dried and purified by recrystalization with benzene to give **2** as yellow crystals.

5-(3,4,5-trimethoxybenzylidene)-3-phenyl-2-(amino)thiocarbonyl-1,2,4-triazine-6-one (3)

A mixture of oxazolinone (**2**, 0.01 mole), thiosemicarbazide (0.01 mole) in acetic acid (30 ml) was heated under reflux for 4 hrs, and then the reaction mixture was cooled and poured into water. The solid product that formed was collected by filtration, was washed with hot water, was dried and purified by recrystalization from ethanol to give **3** as yellow crystals.

6 - (3,4,5-trimethoxybenzylidene)-4–phenyl-3-thioxo-1-methyl-triazolo[2,1-a]-1,2,4-triazine -7-one (4).

A solution of **3** (0.01 mole) in acetic anhydride (20 ml) was heated under reflux for 3 hr, then was cooled and poured into ice-water. The solid obtained was filtered off, was washed with water, was dried and was purified by recrystalization from benzene to give **4** as pale yellow crystals.

1,5-Diphenyl-4-thioxo-7-(3,4,5-trimethoxybenzylidene)-triazino[2,1-a]-1,2,4-triazine-8-one (5)
7-(3,4,5-trimethoxybenzylidene)-5-phenyl-4-thioxo-2,3-dihydro-triazino[2,1-a]-1,2,4-triazine-1,8-dione (7)

A mixture of **3** (3, 0.01 mole) and phenacyl bromide and/or ethylchloroacetate (0.01 mole) in acetic acid (30 ml) in presence of fused sodium acetate (0.03 mole) was heated under reflux for 4 hrs. Then the reaction mixture was cooled and poured into water. The resulting solid was filtered off, was washed with water, was dried and was purified by recrystalization from a suitable solvent to give **5** and **7**. Compound **5** as pale yellow crystals and compound **7** as yellow crystals

1,5-Diphenyl-3-acetyl-4-thioxo-7-(3,4,5-trimethoxybenzylidene)-triazino[2,1-a]-1,2,4-triazine-8-one (6)
3-acetyl-4-thioxo-5-phenyl-7-(3,4,5-trimethoxybenzylidene)-triazino[2,1-a]-1,2,4-triazine-1,8-dione (8)

A solution of **5** and/or **7** (0.01 mole) in acetic anhydride (25 ml) was heated under reflux for 2 hrs. Then the reaction mixture was cooled and poured into ice water. The resulting solid was filtered off, was washed with water, was dried and purified by recrystalization from benzene to give **6** and **8**. Compound **6** as pale yellow crystals and compound **8** as pale yellow crystals.

2,3-diacetyl-4-thioxo-5-phenyl-7-(3,4,5-trimethoxybenzylidene)-triazino[2,1-a]-1,2,4-triazine-1,8-dione (9)

A mixture of **7** (0.01 mole) and fused sodium acetate (0.03 mole) in acetic anhydride (25 ml) was heated under reflux for 3 hrs. Then the reaction mixture was cooled and poured into water. The solid formed was collected by filtration, was washed with water, was dried and purified by recrystalization from benzene to give **9** as pale yellow crystals.

2 - Arylidene-4-thioxo-5-phenyl-7-(3,4,5-trimethoxybenzylidene)-triazino[2,1-a]- 1,2,4-triazine-1,8-dione ($10_{a, b}$)

A mixture of **7** (0.01 mole), aromatic aldehydes (namely, benzaldehyde and anisaldehyde) (0.01 mole) and piperidine (1 ml) was fused for 1 hr at 130-140 ºc. Then the reaction mixture was cooled and acidified with dilute hydrochloric acid (2 %). The solid formed was collected by filtration, was washed with water, was dried and purified by recrystalization with ethanol to give $\mathbf{10_{a,b}}$.

*2-Benzylidene-4-thioxo-5-phenyl-7-(3,4,5-trimethoxybenzylidene)-triazino[2,1-a]-1,2,4-triazine-1,8-dione (**10_a**)* as yellow crystals.

*2-(4-Methoxy)benzylidene-4-thioxo-5-phenyl-7-(3,4,5-trimethoxybenzylidene)-triazino[2,1-a]-1,2,4-triazine-1,8-dione (**10_b**)* as yellow crystals.

2-Arylidene-3-acetyl-4-thioxo-5-phenyl-7-(3,4,5-trimethoxybenzylidene)-triazino [2,1-a]-1,2,4-triazine-1,8-diones ($11_{a, b}$).

A solution of $\mathbf{10_{a,b}}$ (0.01 mole) in acetic anhydride (20 ml) was heated under reflux for 2 hrs. Then the reaction mixture was cooled and poured into ice-water. The solid formed was filtered off, was washed with water, was dried and purified by recrystalization from benzene to give $\mathbf{11_{a,b}}$

*2-Benzylidene-3-acetyl-4-thioxo-5-phenyl-7-(3,4,5-trimethoxybenzylidene)-triazino[2,1-a]-1,2,4-triazine-1,8-diones (**11_a**)* as pale yellow crystals.

*2-(4-Methoxy)benzylidene-3-acetyl-4-thioxo-5-phenyl-7-(3,4,5-trimethoxybenzylidene)-triazino [2,1-a]-1,2,4-triazine-1,8-diones (**11_b**)* as pale yellow crystals.

1.2. Biological screening

1.2.1. Antibacterial screening

The antibacterial activity of 1,2,4-triazine derivatives (**3-11**) were screened against Gram positive bacteria (Bacillus Subtilis, Streptococcus Pneumoniae and Staphylococcus aureus) and gram negative bacteria (such as E-Coli and Pseudomonas sp.) in DMSO by the well diffusion method using standard Mueller Hinton agar as the medium . The zone of inhibition was measured in mm, and was compared with standard drug. DMSO was used as a blank and Streptomycin was used as antibacterial drug standard [24, 25].

Sensitivity plates were inoculated with Gram positive and gram negative bacteria and the well was loaded with (1 mg/ml) of test compound solution using a micropipette. The incubation was done for 24 hrs at 37ºC. During this period, the test solution diffused zones of inhibition which were recorded using Vernier callipers; the radius of the zone is the measure of antibacterial activity.

1.2.2. Antifungal screening

The antifungal activity of 1,2,4-triazine derivatives (**3-11**) were evaluated by using the Sabouraud dextrose agar diffusion method Wells were made (8 mm diameter) with a sterile cork borer. To these wells 1 mg/ml of the test stock solution compounds were added and the plates were allowed to cool for an hour to facilitate the diffusion. The plates were then incubated at 37ºC for 72 hrs. At the end of the incubation period, the diameter of inhibition around the wells was measured [26, 27].

1.2.3. Cytotoxic assay of 1,2,4-triazine derivatives

In this study, the antitumor activity of the 9 prepared fused triazines derivatives has been evaluated on human cancer cell lines, representing liver cancer. The cytotoxic activities of prepared compounds were tested against HepG-2 cell line according to method of Mosmann and Vijayan et al [28, 29]. The inhibitory activity against liver carcinoma cells (HepG-2 cell line) was detected by using different concentrations of the tested samples (50, 25, 12.5, 6.25, 3.125 and 1.56 µg) and cell viability cell (%) was determined by colorimetric method ,the data is summarized in **table 6**. The IC_{50} was calculated from **table 6** and **Figures 1**, **2**, **3** and **4**.

1.2.3.1. Cell lines and culture

Human hepatocellular carcinoma (HepG-2) cells were obtained from the American type culture collection (ATCC, Rock Villa, and MD). The cells were grown on RPMI-1640 medium supplemented with 10 % inactivated fetal calf serum and 50 µg/ml gentamycin. The cells were maintained at 37ºC in humidified atmosphere with 5 % CO2 and were subcultured two to three times a week.

1.2.3.2. Evaluation of the antitumor activity of 1,2,4-triazine derivatives

The antitumor activity was evaluated on HepG-2 cell. The cells were grown a monolayers in growth RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50 µg/ml gentamycin. The monolayers of 10000 cells adhered at the bottom of the wells in a 96-well micro titer plate incubated for 24 hrs at 37ºC in humidified atmosphere with 5 % CO2.the monolayers were then washed with sterile phosphate buffered saline (0.01 M pH 7.2) and simultaneously the cells were treated with 100 µl from different dilutions of the test sample in fresh maintenance medium and incubated at 37ºC. A control of untreated cells was made in the absence of the test sample. Six wells were used for each concentration of the test sample. Every 24 hrs the observation under the inverted microscope was made. The number of the surviving cells was determined by staining the cells with crystal violet followed by cell lysine using 33 % glacial acetic acid and read the absorbance at 490 nm using ELISA reader (Sun Rise, TECAN, Inc, USA) after well mixing. The absorbance values from untreated cells were considered as 100 % proliferation. The number of viable cells was determined using ELISA reader as previously mentioned before and the percentage of viability was calculated as [1 – (ODt /ODC)] *100 %, where; OD_t is the mean optical density of wells treated with the test sample, OD_c is the mean optical density of untreated cells and The 50 % inhibitory concentration (IC_{50}): the concentration required to cause toxic effect in 50 % of inactivated cells, was estimated from graphic plots.

2. Results and discussion

2.1. Chemistry

The spectral data for the newly synthesized compounds (**2-11**) are given in table 2 and 3. The elemental analysis, some properties and data for these compounds are given below in table 1.

Table 1: some properties and data of synthesize compounds

Product	M. P.(°c)	Mol. Formula (mol. Wt.)	Yields (%)	Elemental analysis (%)			
					C	H	N
2	160-161	**$C_{19}H_{17}NO_5$ (339)**	67.0	Calcd.	67.25	5.01	4.13
				found	67.03	4.98	4.01
3	230-231	**$C_{20}H_{20}N_4O_4S$ (412)**	71.9	Calcd.	58.25	4.85	13.59
				found	58.08	4.63	13.39
4	155-156	**$C_{22}H_{20}N_4O_4S$ (436)**	56.0	Calcd.	60.55	4.59	12.84
				found	60.33	4.23	12.58
5	91-92	**$C_{28}H_{24}N_4O_4S$ (512)**	65.0	Calcd.	65.62	4.69	10.94
				found	64.46	4.49	10.73
6	70-71	**$C_{30}H_{26}N_4O_5S$ (554)**	63.0	Calcd.	64.98	4.69	10.11
				found	64.78	4.39	10.02
7	209-210	**$C_{22}H_{20}N_4O_5S$ (452)**	67.0	Calcd.	58.41	4.42	13.39
				found	58.23	4.24	12.26
8	199-200	**$C_{24}H_{22}N_4O_6S$ (494)**	59.0	Calcd.	58.30	4.45	11.33
				found	58.09	4.33	11.11
9	180-181	**$C_{26}H_{24}N_4O_7S$ (536)**	61.0	Calcd.	58.21	4.47	10.45
				found	58.01	4.52	10.23
10_a	248-249	**$C_{29}H_{29}N_4O_5S$ (540)**	71.0	Calcd.	64.44	4.44	10.37
				found	64.22	4.18	10.17
10_b	258-259	**$C_{30}H_{26}N_4O_6S$ (570)**	69.0	Calcd.	63.14	4.56	9.82
				found	63.03	4.34	9.59
11_a	135-136	**$C_{31}H_{26}N_4O_6S$ (582)**	63.0	Calcd.	63.92	4.47	9.62
				found	63.79	4.27	9.43
11_b	214-215	**$C_{32}H_{28}N_4O_7S$ (612)**	61.0	Calcd.	62.74	4.57	9.15
				found	62.54	4.39	9.03

Table 2: Spectral characteristics of compounds synthesized

Compd. No.	IR spectrum (U, cm^{-1})	^1H, ^{13}C NMR spectrum, δ, ppm
2	1785 (C=O of oxazol ring), 1683 (C=N), 1581(C=C), 1234-1124 (C-O)	3.78 (s, 3H, OCH3), 3.85(s, 6H, 2 OCH3), 6.89-7.81 (m, 8H, Ar-H and H-olefinic)
3	3361-3153 (NH_2), 3221 (NH), 1729 (C=O), 1643 (C=N), 1581 (C=C), 1452(C=S), 1118-1000(C-O)	3.59 (s, 2H, NH_2), 3.70 (s, 3H, OCH_3), 3.87(s, 6H, 2 OCH_3), 7.09-8.09(m, 8H, Ar-H and H-olefinic), 10.06 (s, 1H, NH) 182.49(C=S), 168.63(C=O), 158.64 - 153.61 (3C-O), 140.35 (N=C-N), 136.03(=C-N), 134.05, 132.41, 130.13, 129.95, 129.41, 129.10, 128.92, 128.27, 127.90-110.57(C-Aromatic), 60.73(OCH3), 56.38(2 OCH3)
4	1730 (C=O), 1635 (C=N), 1577 (C=C), 1452(C=S), 1128-1002(C-O)	2.44 (s, 3H, CH_3), 3.78 (s, 3H, OCH_3), 3.87(s, 3H, OCH_3), 3.88(s, 3H, OCH_3), 7.30-8.11 (m, 8H, Ar-H and H-olefinic)
5	3235 (NH), 1722 (C=O), 1641 (C=N), 1581 (C=C), 1455(C=S), 1128-1000 (C-O)	3.75 (s, 3H, OCH_3), 3.84(s, 3H, OCH_3), 3.87(s, 3H, OCH_3), 6.98-8.16 (m, 14H, Ar-H, H-olefinic and H-triazine), 10.70 (s, 1H, NH)

6	1721-1702 (br C=O), 1641 (C=N), 1579 (C=C), 1455(C=S), 1240 -1000 (C-O)	2.45 (s, 3H, $COCH_3$), 3.78(s, 3H, OCH_3), 3.83(s, 3H, OCH_3), 3.86(s, 3H, OCH_3), 7.21-8.11 (m, 14H, Ar-H, H-olefinic and H-triazine)
		171.50(C=S), 170.14&168.58(C=O), 158.33, 153.80&153.70(C-O), 148.54(N=C=N), 140.60(CH-N), 134.49, 134.08, 133.76, 132.47, 131.52, 129.87, 129.30, 129.71, 129.17, 128.63, 127.71, 126.27, 125.81, 125.61, 114.52, 112.03, 111.13, 110.85, 107.21&105.61(C-Aromatic, C-olefinic and triazine), 60.77(OCH_3), 56.44(2 OCH_3), 22.86($COCH_3$)
7	3321 (NH), 1731-1673 (br C=O), 1623 (C=N), 1605-1498 (C=C), 1452(C=S), 1124-1000 (C-O)	2.50(s, 2H, NCH_2CO), 3.78(s, 3H, OCH_3), 3.86(s, 3H, OCH_3), 3.88(s, 3H, OCH_3), 7.20-8.07 (m, 8H, Ar-H, H-olefinic), 10.53 (s, 1H, NH).
		172.57-166.23(C=O), 158.84, 153.33-153.27(C-O), 140.51(N=C=N), 136.30, 132.40, 130.29, 129.87, 129.52, 129.36, 129.21, 128.93, 128.78, 128.49-110.69(C-Aromatic), 61.53(OCH_3), 56.35(2 OCH_3), 35.05(NCH_2CO)
8	1735-1717 (br. C=O), 1641 (C=N), 1569 (C=C), 1498 – 1454 (C=S), 1122-1000 (C-O)	
9	1733-1710 (br. C=O), 1639 (C=N), 1577 (C=C), 1452(C=S), 1124-1000 (C-O)	2.36 (s, 3H, $COCH_3$), 2.44(s, 3H, OCH_3), 3.83-3.88(br. s, 6H, 2 OCH_3), 6.99-7.13 (m, 9H, Ar-H, H-olefinic and H-triazine)
		172.48(C=S), 171.89, 168.10, 167.65&167.34(C=O), 156.85, 153.35& 153.27(C-O), 149.17(N=C=N), 141.31(-C-N), 134.02, 133.16, 129.89, 129.29, 129.21, 127.84, 127.77, 127.19, 111.14- 101.29 (C-Aromatic and C-olefinic), 60.77(OCH_3), 56.40(2 OCH_3), 21.49($COCH_3$), 21.08($COCH_3$)
10a	3235(NH), 1710-1705 (br. C=O), 1629 (C=N), 1604-1577 (C=C), 1450(C=S), 1128&1000 (C-O)	3.78(s, 3H, OCH_3), 3.87(s, 6H, 2 OCH_3), 7.19-8.09 (m, 14H, Ar-H and H-olefinic), 10.02(S, 1H, NH)
		193.70(C=S), 167.55&166.02(C=O), 158.67& 153.29(C-O), 140.67(N=C=N), 136.03(N-C=), 135.05, 133.32, 132.76, 131.59, 130.82, 130.53, 129.95, 129.83, 129.62, 129.54, 129.46, 129.37, 129.11, 128.32, 121.91&110.77 (C-Aromatic and C-olefinic), 60.74(OCH_3), 56.40(2 OCH_3)
10b	3267(NH), 1706 (br. C=O), 1639 (C=N), 1610-1592 (C=C), 1454(C=S), 1124-1027 (C-O)	3.78(s, 6H, 2 OCH3), 3.87(s, 6H, 2 OCH3), 7.07-8.07 (m, 13H, Ar-H and H-olefinic), 9.87(S, 1H, NH)
11a	1735-1720 (br. C=O), 1631 (C=N), 1598 (C=C), 1452(C=S), 1126-1000 (C-O)	1.92(S, 3H, $COCH_3$)3.78(s, 3H, OCH_3), 3.88(s, 6H, 2 OCH_3), 7.18-8.09 (m, 14H, Ar-H and H-olefinic), 10.02(S, 1H, NH)

		172.47(C=S), 167.76, 166.06 - 165.90(C=O), 158.29, 153.29 - 153.06(O-C Ar), 140.67(N=C=N), 136.99(=C-N), 136.06, 133.37, 132.73, 131.64, 131.45, 131.11, 130.76, 130.50, 130.26, 129.94, 129.80, 129.51, 129.44, 129.31, 129.11, 128.35, 128.02, 127.68, 127.36, 126.91, 122.08 - 110.76 (C-Aromatic and C-olefinic), 60.72(OCH_3), 56.38(2 OCH_3), 21.50($COCH_3$)
11b	1737-1720 (br. C=O), 1635 (C=N), 1587 (C=C), 1452(C=S), 1255, 1151-1022 (C-O)	1.91(S, 3H, $COCH_3$)3.75(s, 6H, 2 OCH_3), 3.88(s, 6H, 2 OCH_3), 7.09-8.07 (m, 13H, Ar-H and H-olefinic)
		172.47(C=S), 167.77, 166.36 & 162.36(C=O), 158.67, 156.79, 153.34 - 153.29(O-C Ar), 141.29(N=C=N), 139.30(=C-N), 136.07, 133.78, 133.34, 132.73, 132.60, 132.26, 131.84, 129.82, 129.44, 129.23, 128.35, 127.87, 127.03, 125.81, 123.32, 118.66, 115.62, 114.97- 110.47 (C-Aromatic and C-olefinic), 60.77(OCH_3), 56.41(2 OCH_3), 55.87(OCH_3), 21.50($COCH_3$)

The ^{1}H and ^{13}C NMR spectra were taken in DMSO- d_6 for all compounds

The mass spectral decomposition modes of the prepared heterocyclic compounds containing triazine ring have been investigated.

Compound 3

The mass spectrum of compound 3 (figure 2d) showed an intense molecular ion peak at m/z 412, corresponding to the molecular formula C20H20N4O4S. The molecular ion of compound 3 (scheme 11) underwent fragmentation to produce a peak at m/z 353 by losing NHCS group. The loss of NH group from the ion with m/z 353 resulted in an ion at m/z 338, which lost carbonyl group (CO) to give peak at m/z 310. The ion at m/z 310 underwent fragmentation to produce stable peaks at m/z 206 and 104. The ion at m/z 104 underwent loss of hydrogen cyanide (HCN) and acetylene molecule to give peaks at m/z 77 and m/z 51, respectively. Also the ion at m/z 104 underwent loss of hydrogen atom to give peaks at m/z 103.Also the ion at m/z 412 underwent fragmentation with rearrangement to produce peak at m/z 395. The ion at m/z 395 underwent loss of (N2CS) to give peaks at m/z 323. The stable ion at m/z 323 underwent fragmentation with rearrangement to produce peak at m/z 103, which underwent loss of cyanide and acetylene molecule to give peaks at m/z 77 and m/z 51, respectively.

Compound 4

From the mass spectrum of compound **4** (figure 3_c), it was concluded that the molecular ion was at m/z 436, corresponding to the molecular formula $C_{22}H_{20}N_4O_4S$. The loss of methyl nitrile (CH_3CN), isocyanate group (NCO) and nitrogen atom (N) from the molecular ion peak at m/z 436 gave a peak at m/z 339. The ion at m/z 339 underwent fragmentation with rearrangement to produce peak at m/z 148, which lost thiocarbonyl (C=S) group to give peak at m/z 104. The stable peak at m/z 77 was obtained by loss of hydrogen cyanide from the ion of m/z 104. The stable ion at m/z 77 underwent loss of acetylene molecule to give peaks at m/z 51

Compound 7, 8 and 9

The mass spectrum of compounds **7**, **8** and **9** (figure 6_d, 7_b and 8_d) showed the molecular ion peaks at m/z 452, m/z 494 and m/z 536 corresponding to the molecular formula $C_{22}H_{20}N_4O_5S$, $C_{24}H_{22}N_4O_6S$ and $C_{26}H_{24}N_4O_7S$, respectively. The molecular ion peak of diacetyl derivative (**9**) (figure 8_d) was observed at m/z 536 corresponding to the molecular formula $C_{26}H_{24}N_4O_7S$. The loss of ketene molecule (CH_2=C=O) from the molecular ion at m/z 536 gave a fragment ion of m/z 494, corresponding to the

molecular ion of compound **8** (figure 7_d). The molecular ion of compound 8 of m/z 494 underwent fragmentation to produce a peak at m/z 452, corresponding to the molecular ion of compound **7**(figure 6_d) by losing ketene molecule (CH_2CO)

Compounds $10_{a, b}$ and $11_{a, b}$

The mass spectrum of compounds10a, 10b, 11a and 11b (figures 9d, 10c, 11d and 12d) showed the molecular ion peaks at m/z 540, m/z 570, m/z 582 and m/z 612 corresponding to the molecular formula C29H29N4O5S, C30H26N4O6S, C31H26N4O6S and C32H28N4O7S, respectively. The molecular ion peak of 11b (figure 12d) was observed at m/z 612 corresponding to the molecular formula C32H28N4O7S. The loss of ketene molecule (CH2=C=O) from the molecular ion at m/z 612 gave a fragment ion of m/z 570, corresponding to the molecular ion of compound 10b (figure 10c). The loss of ketene molecule (CH2=C=O) and (CH2=O) from the molecular ion at m/z 582 gave a fragment ion of m/z 540, corresponding to the molecular ion of compound 10a. Also the loss of (CH2 =O) from the molecular ion at m/z 570 gave a fragment ion of m/z 540, corresponding to the molecular ion of compound 10a. The molecular ion of compound 10a at m/z 540 underwent fragmentation with rearrangement to produce peaks at m/z 339, m/z 338 and m/z 323, respectively. The ion at m/z 339 underwent fragmentation with rearrangement to produce peak at m/z 148, which lost thiocarbonyl (C=S) group to give peak at m/z 104. The ion at m/z 104 underwent loss of hydrogen cyanide (HCN) and acetylene molecule to give peaks at m/z 77 and m/z 51, respectively. Also the ion at m/z 338 underwent fragmentation to produce stable peaks at m/z 206 and 104. The stable ion at m/z 323 underwent fragmentation with rearrangement to produce peak at m/z 103, which underwent loss of cyanide and acetylene molecule to give peaks at m/z 77 and m/z 51, respectively

Table 3: Mass spectra of compounds

Compd. No.	m/z (%)
2	340 (M^++1, 6.24), 339 (M^+, 31.98), 207 (1.09), 206(4.69), 198(1.24), 197(3.28), 196(29.02), 182(2.18), 181(14.49), 175(1.07), 173(3.05), 166(1.31), 165(1.71), 162(1.36), 161(2.97), 160(1.24), 154(1.05), 153(2.44), 152(1.29), 151(1.43), 148(1.51), 146(2.65), 136(1.79), 135(2.43), 134(1.85), 130(1.29), 125(5.06), 123(1.80), 121(1.56), 120(1.50), 119(1.46), 118(1.05), 117(1.02), 110(5.30), 109(1.17), 107(2.18), 106(7.83), 105(100), 103(1.82), 93(3.43), 92(1.37), 91(1.03), 78(2.92), 77(24.05), 76(3.84), 65(2.73), 64(1.16), 63(1.94), 54(3.32), 53(2.16), 52(12.80), 51(4.12).
3	413 (M^++1, 2.66), 412 (M^+, 8.78), 397 (3.69), 395 (17.70), 380(2.42), 378(4.48), 354(3.24), 353(10.31), 339(31.58), 338(100), 324(19.89), 323(31.35), 322(4.19), 310(2.66), 308(3.13), 307(3.78), 306(2.62), 294(2.52), 293(2.56), 292(6.19), 281(2.49), 280(2.49), 279(2.40), 235(3.17), 234(4.85), 207(4.05), 206(11.67), 204(2.41), 193(7.44), 192(44.82), 181(6.47), 178(4.04), 177(4.69), 176(4.92), 173(5.22), 165(2.97), 164(7.26), 163(4.02), 162(7.28), 161(16.26), 154(2.37), 153(2.86), 151(2.60), 149(7.13), 148(5.27), 146(8.01), 145(4.51), 136(3.10), 134(5.77), 132(3.91), 131(3.97), 121(3.66), 120(6.42), 119(18.69), 118(4.45), 117(3.80), 116(2.68), 106(7.30), 105(55.04), 104(60.59), 103(22.86), 92(3.38), 91(4.01), 89(3.72), 78(6.18), 77(40.81), 76(9.95), 65(2.99), 64(7.64), 63(4.20), 61(5.68), 60(6.61), 54(4.69), 53(4.61), 52(12.84), 51(6.30).
4	437 (M^++1, 9.02), 436 (M^+, 9.15), 392 (9.12), 390 (9.24), 374(9.67), 370(9.19), 367(9.33), 365(9.27), 361(9.18), 358(9.89), 355(9.27), 350(10.99), 344(10.35), 340(11.21), 339(26.02), 338(9.55), 337(9.18), 329(12.23), 313(11.61), 307(13.46), 291(15.28), 284(14.44), 273(17.03), 260(19.99), 256(10.10), 247(9.98), 232(13.15), 208(54.64), 206(12.29), 192(9.12), 181(9.42), 176(9.05), 173(10.22), 164(9.52), 162(9.24), 161(10.26), 157(64.21), 148(9.12), 146(9.76), 135(83.80), 134(4.36), 130(9.24), 121(9.39), 120(9.62), 119(9.42), 117(9.39), 115(9.02), 107(9.12), 106(15.12), 105(100), 104(12.35), 103(10.59), 102(9.22), 92(9.18), 91(9.21), 90(9.21), 89(9.52), 78(9.74), 77(28.80), 75(10.47), 65(9.49), 64(9.95), 63(9.39), 58(17.37), 55(9.56), 53(9.55), 52(15.15), 51(10.59).
5	513 (M^++1, 10.49), 512 (M^+, 22.98), 370 (6.03), 369 (11.13), 354(5.69), 339(8.71), 338(29.43), 337(22.15), 324(5.27), 323(15.14), 321(5.66), 307(6.10), 304(5.80), 279(6.65), 234(5.54), 207(7.86), 206(10.43), 203(7.06), 195(8.71), 194(7.88), 193(15.11), 192(42.90), 191(6.90), 190(7.03), 189(7.60), 183(7.57), 182(6.07), 181(16.19), 178(12.06), 177(18.31),

	176(100), 175(13.53), 174(10.35), 173(7.83), 167(5.46), 165(5.08), 164(7.23), 162(6.37), 161(6.07), 149(7.27), 148(12.01), 147(5.35), 135(7.45), 134(32.25), 133(6.23), 129(5.98), 121(6.14), 119(5.51), 106(5.57), 105(22.62), 104(27.21), 103(11.56), 102(6.54), 91(6.98), 90(6.66), 89(7.17), 77(16.44), 53(6.07), 52(14.23), 51(11.26).
6	554 (M^+, 8.83), 519 (8.70), 499 (11.13), 474 (15.52), 432(25.12), 429(8.91), 349(100), 348(9.45), 344(9.08), 338(8.80), 323(10.87), 317(9.20), 294(10.31), 288(9.14), 287(9.03), 267(9.66), 256(25.81), 222(9.51), 218(10.50), 200(48.97), 192(8.85), 183(8.95), 181(13.49), 178(67.23), 176(16.71), 168(13.65), 148(9.52), 144(22.15), 134(14.05), 121(31.08), 105(16.58), 104(13.22), 103(9.45), 102(9.29), 89(72.86), 80(8.74), 77(20.05), 75(10.82), 66(11.71), 65(11.31), 64(20.39), 63(11.88), 57(12.80), 56(9.10), 53(9.68), 52(23.09), 51(9.74).
7	453 (M^++1, 20.29), 452 (M^+, 100), 437 (4.08), 339 (3.89), 338(24.44), 337(5.61), 324(3.71), 323(7.54), 307(3.68), 305(3.74), 304(6.03), 276(3.13), 274(5.18), 234(6.65), 220(4.04), 219(8.20), 218(62.37), 217(4.35), 207(5.88), 206(30.91), 192(10.64), 191(3.69), 190(7.12), 181(4.87), 177(3.92), 176(4.95), 164(4.51), 163(3.78), 162(5.97), 161(9.63), 149(7.41), 148(23.58), 147(4.24), 146(10.91), 145(6.17), 144(7.43), 134(6.48), 133(4.42), 132(4.68), 131(6.05), 129(3.78), 121(4.07), 120(5.01), 119(7.12), 118(4.82), 117(11.66), 116(8.50), 115(5.58), 106(5.88), 105(33.96), 104(71.86), 103(55.92), 92(3.30), 91(5.58), 90(5.12), 89(7.29), 87(44.08), 78(4.73), 77(22.35), 76(10.09), 65(3.04), 63(6.94), 61(3.03), 52(8.79), 51(5.95).
8	495 (M^++1, 5.99), 494 (M^+, 6.99), 487 (7.74), 486 (7.74), 453(6.53), 452(15.78), 451(7.79), 392(8.27), 388(8.92), 366(7.88), 365(10.05), 363(7.60), 340(10.33), 339(27.97), 338(80.00), 337(29.13), 324(21.77), 323(36.89), 322(11.07), 321(11.60), 307(9.04), 306(15.64), 305(3.39), 293(8.23), 292(11.32), 279(9.76), 278(11.86), 277(8.09), 275(10.67), 235(10.95), 234(22.04), 233(9.56), 219(6.99), 218(0.84), 217(6.04), 204(7.99), 203(11.09), 196(7.48), 195(8.53), 192(58.75), 191(44.65), 190(13.33), 182(12.10), 181(23.58), 178(12.87), 177(11.09), 174(13.01), 173(11.18), 165(21.79), 164(89.40), 163(21.79), 151(14.51), 150(21.47), 149(56.47), 148(23.89), 146(19.95), 135(19.34), 134(33.21), 133(14.28), 121(34.82), 120(21.61), 119(14.70), 117(14.36), 116(17.18), 115(10.79), 110(10.04), 106(14.61), 105(33.74), 104(100), 103(46.40), 102(10.42), 92(10.07), 91(16.59), 90(14.82), 89(10.97), 78(13.03), 77(39.15), 76(18.68), 65(8.76), 64(10.80), 63(11.95), 58(14.83), 57(12.19), 56(11.39), 53(14.21), 52(26.54), 51(27.80).
9	537 (M^++1, 7.36), 536 (M^+, 76.90), 494 (42.03), 452 (55.40), 339(10.46), 338(38.45), 337(100), 323(12.15), 321(6.46), 307(12.52), 306(11.45), 277(3.97), 276(15.70), 264(6.09), 263(5.04), 235(5.89), 234(15.29), 208(5.88), 207(18.18), 206(46.88), 193(8.64), 192(44.81), 181(12.44), 174(4.31), 173(19.63), 162(7.98), 161(17.04), 160(4.91), 149(6.10), 146(12.98), 144(11.72), 134(6.88), 120(7.04), 119(6.74), 117(6.63), 116(6.26), 105(19.26), 104(45.80), 103(34.65), 92(3.38), 91(4.74), 90(3.72), 89(3.74), 78(7.73), 77(30.48), 76(10.63), 65(4.95), 64(9.75), 63(6.30), 53(3.69), 52(12.04), 51(7.10), 47(11.59).
10_a	542.15 (M^++2, 4.30), 540 (M^+, 6.30), 523 (11.46), 520 (4.17), 516(5.08), 454(5.99), 443(7.55), 400(5.34), 399(4.56), 339(10.16), 338(40.36), 324(9.64), 323(11.07), 293(5.60), 292(12.11), 285(14.58), 268(7.55), 266(7.42), 208(8.47), 206(6.77), 205(7.94), 204(17.84), 202(13.93), 193(3.39), 192(14.19), 182(3.91), 181(28.13), 177(8.07), 169(14.84), 162(14.58), 148(13.15), 135(14.58), 134(100), 121(10.94), 118(8.33), 117(10.16), 116(5.08), 105(34.24), 104(76.82), 103(27.08), 102(6.12), 91(27.60), 90(19.68), 89(16.93), 78(11.72), 77(36.90), 76(10.55), 64(16.28), 63(3.52), 59(13.28), 57(14.19), 56(11.20), 53(29.30), 52(28.26), 51(10.55), 48(19.40), 47(18.80).
10_b	571 (M^++1, 11.14), 570 (M^+, 14.35), 569 (M^+-1, 6.07), 542 (6.63), 541(7.11), 540(10.46), 452(6.37), 353(10.60), 352(10.02), 340(8.85), 339(18.29), 338(45.61), 337(10.23), 324(17.76), 323(22.19), 322(6.07), 235(9.07), 234(13.51), 227(6.79), 226(10.25), 207(8.53), 206(9.72), 196(10.18), 195(8.05), 193(14.11), 192(30.36), 191(44.90), 190(11.70), 183(9.26), 182(7.39), 181(18.38), 175(8.26), 174(10.75), 166(12.50), 165(28.53), 164(100), 163(18.51), 161(19.61), 151(13.92), 150(21.80), 149(58.43), 148(12.70), 147(10.91), 146(15.67), 145(10.17), 135(17.02), 134(20.12), 133(13.18),

	132(12.38), 121(29.05), 120(11.95), 119(12.27), 118(10.13), 117(11.12), 105(26.14), 104(56.59), 103(33.69), 102(6.68), 91(18.47), 90(9.78), 89(17.11), 78(6.88), 77(42.06), 76(13.12), 69(12.75), 65(9.81), 64(10.47), 63(7.29), 57(12.92), 56(12.34), 54(10.91), 52(19.60), 47(19.54).
11_a	583 (M^++1, 1.38), 582 (M^+, 1.11), 540 (1.11), 510 (4.93), 509(10.94), 508(1.31), 452 (2.01), 451(2.52), 426(3.85), 425(3.38), 399(4.74), 398(12.95), 397(27.08), 396(4.52), 384(2.97), 383(3.35), 382(3.52), 381(4.30), 367(3.74), 363(2.02), 362(2.08), 361(4.42), 354(2.62), 353(3.13), 351(2.67), 348(3.01), 340(3.60), 339(20.61), 338(33.55), 324(5.75), 323(14.61), 322(4.11), 307(7.730, 306(10.99), 305(19.53), 304(6.47), 297(4.02) 296(6.99), 295(3.53), 281(3.80), 280(5.55), 279(3.37), 278(3.52), 270(3.13), 269(11.64), 262(4.53), 261(10.24), 259(5.30), 254(4.28), 253(3.47), 248(5.10), 247(3.53), 246(3.06), 207(97.30), 206(8.92), 205(6.02), 204(5.53), 196(4.87), 195(10.38), 193(8.60), 192(19.98), 191(5.76), 179(5.84), 178(11.77), 177(8.19), 176(5.30), 174(15.71), 173(14.05), 172(23.61), 165(10.09), 164(8.66), 163(6.10), 162(10.80),161(20.44), 160(5.96), 147(5.55), 146(6.42), 145(5.06), 135(9.75), 134(46.81), 133(7.51), 121(8.28), 120(9.54), 119(8.30), 118(7.60), 117(8.98), 116(10.25), 115(8.05), 106(14.50), 105(100), 104(58.58), 103(46.32), 102(26.46), 91(30.20), 90(16.38), 89(14.73), 84(41.17), 78(7.75), 77(40.79), 76(13.05), 69(10.30), 65(5.13), 64(7.62), 63(3.61), 57(12.08), 56(7.24), 52(21.27), 51(9.77), 47(15.40).
11_b	612 (M^+, 17.20), 586 (17.20), 571(25.60), 570(13.20), 555(16.20), 554(23.23), 553(14.20), 540(52.55), 539(13.68), 497(10.01), 488(9.350, 487(20.03), 393(11.96), 381(10.56), 380(13.75), 379(10.99), 370(10.49), 365(10.44), 363(10.44), 354(10.18), 340(10.44), 339(18.46), 338(46.72), 337(28.71), 335(10.29), 325(16.22), 324(21.67), 323(31.61), 322(16.10), 321(10.44), 307(14.03), 306(15.15), 305(11.30), 292(14.32), 291(13.99), 285(11.16), 262(10.78), 261(10.37), 254(11.73), 253(10.89), 236(13.20), 235(12.35), 234(14.46), 206(19.65), 205(17.08), 204(15.56), 203(10.16), 195(10.94), 193(14.70), 192(35.80), 191(37.38), 167(12.11), 165(21.33), 164(72.36), 163(19.72), 162(17.03), 161(63.30), 158(10.51), 153(12.13), 152(10.63), 151(17.95), 150(21.76), 149(59.23), 147(16.51), 146(19.74), 135(25.17), 134(83.92), 133(28.28), 121(34.47), 120(15.87), 119(26.86), 118(17.10), 117(14.65), 105(50.09), 104(100), 103(45.03), 102(16.03), 91(21.96), 90(21.30), 89(25.74), 78(16.30), 77(46.10), 76(18.40), 75(12.02), 65(10.25), 64(11.02), 63(23.20), 57(13.30), 56(14.39), 52(37.50), 51(31.50), 47(17.44).

2.2. Biological activity

2.2.1 Antibacterial activity

The *in vitro* results of antibacterial activity of the newly synthesized compounds **3-11** are presented in **table 4** as zone of inhibition. Some of the compounds displayed moderate to good inhibition. Here, the Gram-positive bacteria, B. Subtilis, St. Pneumonia and *S. aureus* showed relative high sensitivity toward the fused *s*-triazine derivatives **7**, **8** and **9** whereas the compound **10_b** and**11_b** exhibited moderate activity against the same organism. With regard to the activity against Gram-negative bacteria, E-Coli and Pseudomonas Sp., the best activity was displayed by compounds **7**, **8** and **11_b** While, compounds **9 and 10_b** exhibited moderate activity against the same organism.

Table 4: Antibacterial activities of tested 1,2,4-triazine derivatives (**3-11**)

Compd.	The inhibition zones of the investigated compounds, mm				
	Gram positive bacteria			Gram negative bacteria	
	B. Subtilis	St. Pneumonia	St. Aureas	E-Coli	Pseudomonas Sp.
3	6	1	2	5	4
4	5	3	8	2	8
5	9	5	12	7	3
6	4	3	9	11	12

Compd.	The inhibition zones of the investigated compounds, mm				
	Gram positive bacteria			Gram negative bacteria	
	B. Subtilis	St. Pneumonia	St. Aureas	E-Coli	Pseudomonas Sp.
7	19	15	18	20	23
8	16	19	21	19	24
9	18	20	17	20	18
10a	4	13	5	13	14
10b	10	15	16	14	19
11a	3	9	11	14	12
11b	8	11	18	20	24
Streptomycin	18	17	20	22	27

2.2.2 *Antifungal activity*

Concerning the antifungal activity of the tested compounds presented in **table 5**, only 1,2,4-triazine derivatives namely, 7**, 8, 9, 10$_a$** and **11b** showed sensitivity against Aspergillus Niger, Penicillium sp. and Candida albicans, whereas the rest of the derivatives were reasonably active or insensitive. The 1,2,4-triazine derivatives **7** and **8** displayed higher sensitive against mentioned fungal strains. Moreover compounds **9, 10$_a$** and **11$_b$** were also found to be moderately active against the same strain.

Table 5: Antifungal activities of tested 1,2,4-triazine derivatives (**3-11**)

Compd.	The inhibition zones of the investigated compounds, mm		
	Aspergillus niger	Penicillium sp.	Candida albicans
3	2	3	-
4	5	7	1
5	5	9	7
6	8	8	12
7	17	15	22
8	19	23	21
9	11	17	18
10a	11	13	12
10b	9	11	15
11a	8	11	12
11b	15	18	17
Ketoconazole	18	21	21

2.2.3 Cytotoxicity evaluation of fused triazines derivatives

The activity of new compounds against hepatocellular carcinoma cell line (HepG-2) reported in **table 6** and the results of 50% inhibitory concentration (IC_{50}) data are summarized in **table 7** suggests that only triazines derivatives compounds (**3**, **7**, **9** and **11$_{a,b}$**) were active in terms of IC_{50}, in which the compound **11$_a$** showed highest activity at value of 2.01. However, **3**, **7**, **9** and **11$_b$** also indicated significant activity against liver cancer HepG-2 cell line at IC_{50} value of 4.57, 4.58, 2.89 and 3.43, respectively.

Also compounds **8** and **10**$_{a,b}$ were observed less active against HepG-2 cell line than standard antitumor drugs(Vinblastine and doxorubicin) while compound **4** is inactive.

Table 6: Evaluation of cytotoxicity of fused triazines derivatives **3**, **4** and **7-11**

Compound		Sample conc. (µg)						
		50	**25**	**12.5**	**6.25**	**3.125**	**1.56**	**0**
Viability%	**3**	11.48	20.66	31.89	42.63	56.32	71.54	100
	4	56.88	69.32	86.91	92.74	98.08	100	100
	7	8.31	15.96	23.43	33.68	64.21	85.59	100
	8	19.54	26.16	41.38	80.81	91.17	96.94	100
	9	8.12	14.69	19.86	30.64	46.97	65.18	100
	10a	14.78	26.57	33.95	65.53	88.19	96.32	100
	10b	13.74	25.37	53.96	68.82	79.21	93.98	100
	11a	6.83	10.65	15.49	20.27	29.42	58.38	100
	11b	7.18	13.86	21.43	34.92	51.66	78.51	100
	Vinblastine standard	14.38	16.13	24.25	45.13	55.0	72.13	100
	Doxorubian standard	10.95	14.29	16.9	21.03	30.32	48.25	100

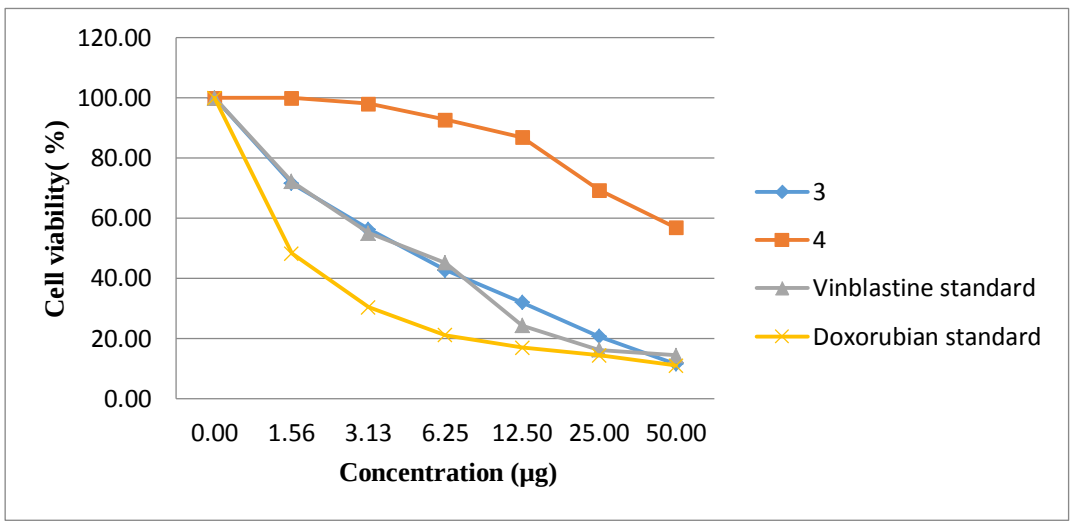


Fig. 1: The inhibitory activities of compounds **3** and **4**

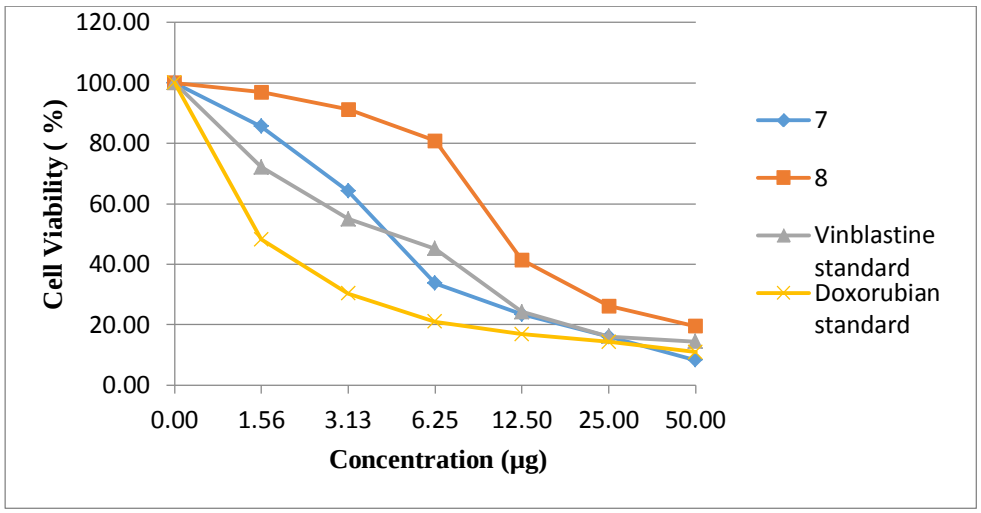


Fig. 2: The inhibitory activities of compounds **7** and **8**

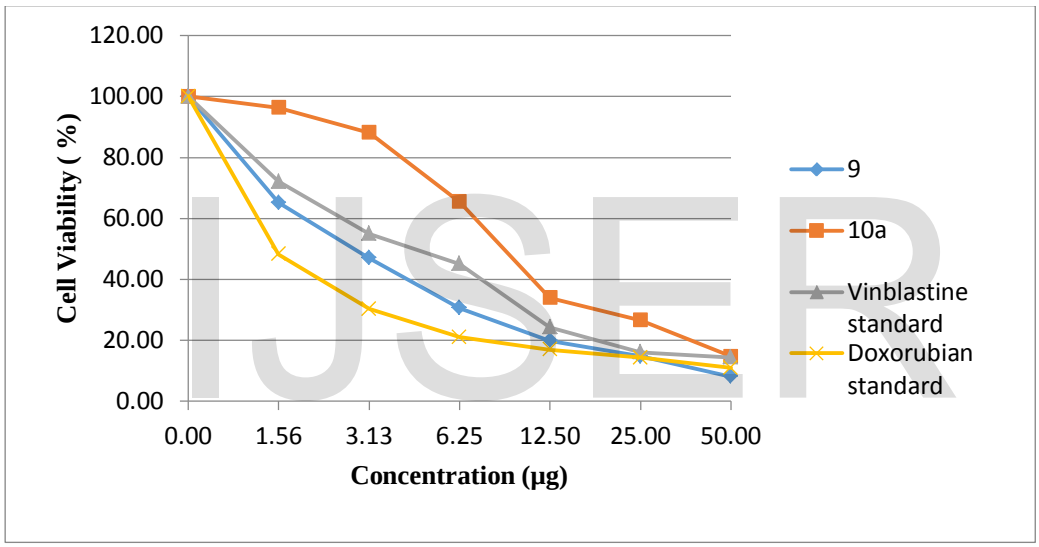


Fig. 3: The inhibitory activities of compounds **9** and **10a**

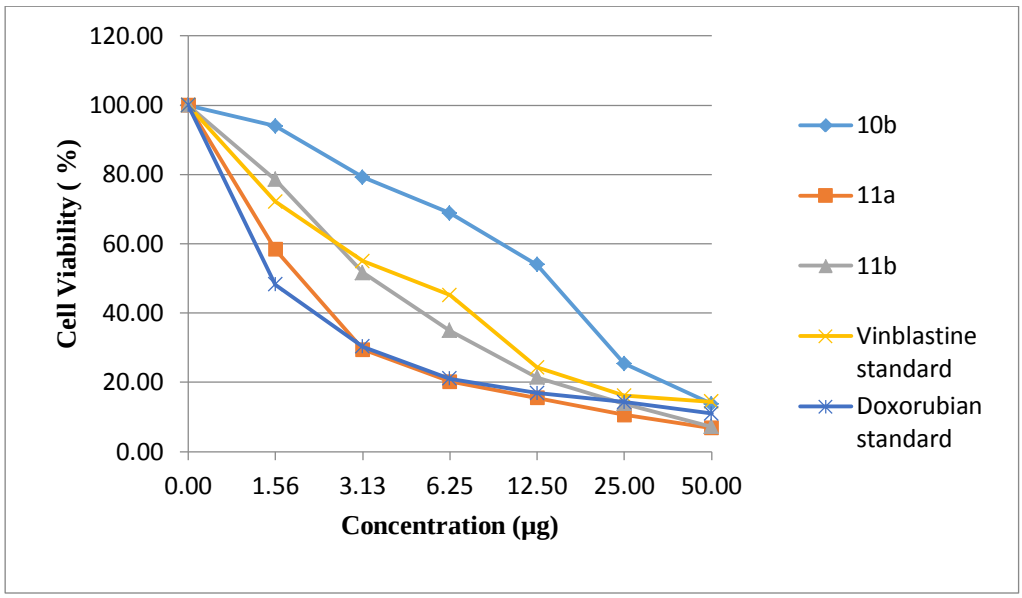


Fig. 4: The inhibitory activities of compounds $\mathbf{10_b}$ and $\mathbf{11_{a,b}}$

Table 7: The results of 50% inhibitory concentration (IC50) data of fused triazines derivatives

Compound No.	3	4	7	8	9	10_a	10_b	11_a	11_b	Vinblastine standard	Doxorubicin standard
HepG-2 cell line	4.57	>50	4.58	11.1	2.89	9.32	14.2	2.01	3.43	4.60	1.20

3 *Conclusion*

In conclusion, we report an efficient synthesis of New series of fused 1,2,4-triazine derivatives and the structures of these compounds were confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, MS and elemental analysis. The bioassay results revealed that, most of the 1,2,4-triazine derivatives compounds displayed an exceptional *in vitro* antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria and fungal strains. Also reported that some of 1,2,4-triazine derivatives compounds displayed anticancer activity for liver cancer cells (the compounds show a promising inhibitory growth efficacy with compared standard anticancer drugs), finally compounds **3**, **7**, **9**, and $\mathbf{11_{a,b}}$ can be suggested as potent candidates for liver cancer treatment.

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