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Synthesis of Some New 1,2,4-Triazines Bearing Pyridoyl Moiety as Potential Antimicrobial and Anticancer Agents

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ABSTRACT

In this work, novel organic based compounds 1,2,4-triazine derivatives were synthesized and their antimicrobial and anticancer activities were investigated. A new series of 1,2,4-triazine derivatives (**IVa-c**) containing pyridine ring were prepared *via* reaction of N-benzoyl glycine with aromatic aldehydes in presence of fused sodium acetate and acetic anhydride to give oxazolinone derivatives (**IIa-c**), followed by condensation of compound **II** with nicotinic acid hydrazide in glacial acetic acid. 3-Substituted pyridinium acetate (**Va-c**) was obtained *via* acetylation of compounds (**IVa-c**) with acetic anhydride. The structures of the synthesized 1,2,4-triazine derivatives (**IV** and **V**) were confirmed by IR, ^{1}H NMR, ^{13}C NMR, MS and elemental analysis. Antimicrobial and cytotoxicity activities of some synthesized compounds have been investigated. The previous compounds, selected as potential agent′s hepatocellular carcinoma were then evaluated *in vitro* for their biological activity on HCC-derived cell lines. The compounds **IVa**, **Va** and **Vb** show a promising inhibitory growth efficiency (IC_{50} 4.30, 4.70 and 4.00 μM) with compared standard antitumor drug (IC_{50} 4.60 μM).

KEYWORDS

Pyridoyl moiety, 1,2,4-Triazines, Antimicrobial activity, Cytotoxicity activity.

INTRODUCTION

Cancer is one of the biggest health problems in the world. Especially, the number of deaths from colon and liver cancer is increasing gradually worldwide. Pharmaceutical industries always struggling for the development of effective agents in the diagnosis and treatment of cancer. Therefore, many organic based cytotoxic agents have been discovered and they are extensively applied for treatment of cancer [1,2].

Triazine derivatives have occupied a unique position in medicinal chemistry. Nitrogen heterocycles have attracted considerable pharmaceutical interest due to their antitumor [3-7], anticonvulsant [8], antileukemic activities [9-11] and cytotoxic effects [12]. Among the compounds having good antimicrobial properties [13], S-triazine derivatives constituted an important class of compounds possessing diverse pharmacological activities including broadly active triazine compounds. Herein, we describe the synthesis and *in vitro* biological activity of 3-pyridoyl with a 1,2,4-triazine core. The new compounds were shown to exhibit selective antimicrobial and cytotoxicity activities.

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EXPERIMENTAL

Melting points were determined on a Met-Temp. apparatus. ^{1}H and ^{13}C NMR spectra were recorded on a BRUKER spectrometer (400 MHz), chemical shift values were expressed in ppm relative to tetramethylsilane as an internal standard. The relative integrates of peak areas agreed with those expected for the assigned structures. The molecular weights of the final compounds were determined by electrospray ionization mass spectrometry (ESI/MS) performed using a Joel JMS D-300 spectrometer operating at 70 eV. The elemental analysis was performed on a Perkin Elmer 2400 series II CHNSXO elemental analyzer.

5-Arylidene-2-phenyl-3,1-oxazole-4-ones (IIa-c): A mixture of *N*-benzoyl glycine (**1**) (0.01 mol), aromatic aldehydes (such as 4-fluorobenzaldehyde, 4-nitrobenzaldehyde and 3,4,5-trimethoxybenzaldehyde) (0.01 mol), fused sodium acetate (0.03 mol) and acetic anhydride (5 mL) was fused on a hot-plate for 3-5 min. The reaction mixture was heated on a water-bath for 2 h, then cooled and poured into water. The solid formed was filtered off, washed with hot water, dried and purified by recrystallization from suitable solvent.

5-(4-Fluorobenzylidene)-2-phenyl-3,1-oxazole-4-one (IIa): Yellow crystals (benzene), yield 87 %, m.p. 134 ºC; IR (KBr, ν_{max}, cm^{-1}):1773 (C=O), 1632 (C=N), 1610, 1585 (C=C), 1215, 1093 (C-O). ^{1}H NMR (DMSO-d_6; δ ppm): 77.31-8.12 (m, 10H, Ar-H and H-olefinic) ppm. MS (*m/z*, %) = 267 (M^+, 23.10), 105 (100 %). Anal. calcd. (found) % for $C_{16}H_{10}NO_2F$: C, 71.91 (71.73); H, 3.74 (3.57); N, 5.24 (5.05).

5-(4-Nitrobenzylidene)-2-phenyl-3,1-oxazole-4-one (IIb): Yellow crystals (AcOH), yield 83 %, m.p. 200-201 ºC; IR (KBr, ν_{max}, cm^{-1}): 1778 (C=O), 1630 (C=N), 1605, 1578 (C=C), 1210, 1007 (C-O); ^{1}H NMR (DMSO-d_6; δ ppm): 7.21-8.11 (m, 10H, Ar-H and H-olefinic) ppm. MS (*m/z*, %) = 294 (M^+, 17.30), 105 (100). Anal. calcd. (found) % for $C_{16}H_{10}N_2O_4$: C, 65.31 (65.23); H, 3.40 (3.21); N, 9.52 (9.36).

5-(3,4,5-Trimethoxybenzylidene)-2-phenyl-3,1-oxazole-4-one (IIc): Yellow crystals (Benzene), yield 81 %, m.p. 137-138 ºC; IR (KBr, ν_{max}, cm^{-1}): 1774 (C=O), 1631 (C=N), 1607, 1593 (C=C), 1305, 1207, 1019 (C-O) ; ^{1}H NMR (DMSO-d_6; δ ppm): 3.78 (s, 6H, 2×OCH_3), 3.85 (s, 3H, OCH_3), 7.1-7.81 (m, 8H, Ar-H and H-olefinic) ppm. MS (*m/z*, %) = 339 (M^+, 21.30), 105 (100). Anal. calcd. (found) % for $C_{19}H_{17}NO_5$: C, 67.26 (67.03); H, 5.01 (4.97); N, 4.13 (4.03).

5-Arylidene-3-phenyl-2-(3-pyridoyl)-1,2-dihydro-1,2,4-triazine-6(5H)-ones (IVa-c): A mixture of compound **II** (0.01 mol), nicotinic acid hydrazide (3, 0.01 mol) and fused sodium acetate (0.03 mol) in glacial acetic acid (30 mL) was heated under reflux for 3-4 h. The reaction mixture was cooled and poured into ice-water. The resulting solid was filtered off, washed with water, dried and purified by recrystallization from ethanol.

5-(4-Fluorobenzylidene)-3-phenyl-2-(3-pyridoyl)-1,2-dihydro-1,2,4-triazine-6(5H)-one (IVa): Yellow crystals, yield 71 %, m.p. 120 ºC; IR (KBr, ν_{max}, cm^{-1}) 3251 (NH), 1695-1685 (br. C=O), 1635 (C=N), 1605, 1589 (C=C); ^{1}H NMR (DMSO-d_6; δ ppm): 7.32-8.36 (m, 10H, Ar-H and olefinic-H), 8.40-9.06 (m, 4H, pyridine-H), 11.97 (s, 1H, NH). ^{13}C NMR (400 MHz, DMSO-d_6): 167.46, 163.08 (C=O), 162.26, 160.54 (C=N), 158.28(C-N), 153.25, 150.22, 136.91, 135.67 (C-pyridine), 135.40, 134.83, 134.45, 133.65, 132.45, 129.56, 129.36, 128.53, 127.81, 127.23, 126.57, 123.75, 116.29 (C-aromatic and olefinic). MS (*m/z*, %) = 386 (M^+, 12.30), 106 (100). Anal. calcd. (found) % for $C_{22}H_{15}N_4O_2F$ (%): C, 68.34 (68.23); H, 3.88 (3.69); N, 14.51 (14.27).

5-(4-Nitrobenzylidene)-3-phenyl-2-(3-pyridoyl)-1,2-dihydro-1,2,4-triazine-6(5H)-one (IVb): Yellow crystals, yield 68 %, m.p. 240-241 ºC; IR (KBr, ν_{max}, cm^{-1}): 3267 (NH), 1701-1695 (br. C=O), 1635 (C=N), 1610, 1578 (C=C); ^{1}H NMR (DMSO-d_6; δ ppm): 7.46-8.36 (m, 10H, Ar-H and olefinic-H), 8.52-9.07 (m, 4H,pyridine-H), 12.06 (s, 1H, NH). ^{13}C NMR (400 MHz, DMSO-d_6): 168.07, 166.39 (C=O), 164.69, 162.63 (C=N), 160.33, 147.80 (C-N), 147.72, 147.51, 138.50, 137.33 (C-pyridine), 136.19, 136.12, 133.45, 129.40, 128.88, 127.96, 126.89, 126.20, 125.02, 124.71, 124.61, 123.80, 123.72 (C-aromatic and olefinic). MS (*m/z*, %) = 413 (M^+, 21.70), 104 (100). Anal. calcd. (found) % for $C_{22}H_{15}N_5O_4$ (%): C, 63.92 (63.76); H, 3.63 (3.43); N, 16.95 (16.81).

5-(3,4,5-Trimethoxybenzylidene)-3-phenyl-2-(3-pyridoyl)-1,2-dihydro-1,2,4-triazine-6(5H)-one (IVc): Yellow crystals, yield 73%, m.p. 195 ºC. IR (KBr, ν_{max}, cm^{-1}): 3227 (NH), 1702-1695 (br. C=O), 1631 (C=N), 1608, 1589 (C=C), 1205, 1009 (C-O); ^{1}H NMR (DMSO-d_6; δ ppm): 3.77 (s, 6H, 2×OCH_3), 3.88 (s, 3H, OCH_3), 7.30-7.85 (m, 8H, Ar-H and olefinic-H), 8.41-9.05 (m, 4H, pyridine-H), 11.98 (s,1H, NH). ^{13}C NMR (400 MHz, DMSO-d_6): 168.23, 164.07 (C=O), 162.45, 159.44 (C=N), 152.81, 152.79 (C-aromatic-O), 148.43 (C-N), 140.45, 135.48, 133.54, 131.90 (C-pyridine), 131.03, 129.33, 129.03, 128.70, 127.98, 127.28, 125.09, 123.99, 110.33, 109.87 (C-aromatic and olefinic), 60.22 (OCH_3), 55.87, 55.86 (OCH_3). MS (*m/z*, %) = 458 (M^+, 18.30), 104 (100). Anal. calcd. (found) % for $C_{25}H_{22}N_4O_5$:C, 65.50 (65.35); H, 4.80 (4.71); N, 12.23 (12.09).

3-(1-Acetyl-3-phenyl-5-arylidene-6-oxo-1,2-dihydro-1,2,4-triazine-2-carbonyl)pyridineium acetates (Va-c): A solution of compound **IV** (0.01 mol) in acetic anhydride (20 mL) was heated under reflux for 1-2 h. then cooled and poured into ice-water. The resulting solid was obtained after evaporating the solvent, dried and purified by recrystallization from ethanol.

3-[(1-Acetyl)-3-phenyl-5-(4-fluorobenzylidene)-6-oxo-1,2-dihydro-1,2,4-triazine-2- carbonyl]-pyridinium acetate (Va): Pale yellow crystals, yield 63 %, m.p. 130 ºC; IR (KBr, ν_{max}, cm^{-1}): 3430-2980 (br. OH), 1705-1693 (br. C=O), 1631 (C=N), 1605, 1593 (C=C); ^{1}H NMR (DMSO-d_6; δ ppm): 1.97 (s, 3H, $COCH_3$), 2.43 (s, 3H, $COCH_3$), 7.30-8.14 (m, 10H, Ar-H and olefinic-H), 8.40-9.01 (m, 4H, pyridine-H), 11.05 (s, 1H, NH). ^{13}C NMR (400 MHz, DMSO-d_6): 170.35, 168.61, 167.97, 163.09 (C=O), 162.27, 160.57 (C=N), 158.29 (C-N), 153.25, 150.22, 136.91, 135.69 (C-pyridine), 135.41, 135.32, 135.03, 134.95, 134.84, 134.74, 134.46, 133.66, 132.71, 132.64, 132.25, 129.57, 129.53, 129.33, 129.28, 129.03, 128.82, 128.16, 127.96, 127.70, 127.29, 127.19, 126.57, 125.05, 123.74, 116.29, 116.26, 116.07, 116.03 (C-aromatic and olefinic), 24.49, 20.20 ($COCH_3$). MS (*m/z*, %) = 488 (M^+, 13.30), 118(100). Anal. calcd. (found) % for $C_{26}H_{21}N_4O_5F$:C, 63.93 (63.90); H, 4.30 (4.11); N, 11.47 (11.58).

3-[(1-Acetyl)-3-phenyl-5-(4-nitrobenzylidene)-6-oxo-1,2-dihydro-1,2,4-triazine-2-carbonyl]pyridinium acetate (Vb): Pale yellow crystals, yield 68 %, m.p. 200 ºC; IR (KBr, ν_{max}, cm^{-1}): 3451-2980 (br. OH), 1703-1697 (br. C=O), 1636 (C=N), 1605, 1583 (C=C); ^{1}H NMR (DMSO-d_6; δ ppm): 1.98 (s, 3H, $COCH_3$), 2.45 (s, 3H, $COCH_3$), 7.39-8.36 (m, 10H, Ar-H and olefinic-H), 8.54-9.10 (m, 4H, pyridine-H), 11.13 (s, 1H, NH): ^{13}C NMR (400 MHz, DMSO-d_6): 170.34, 168.63, 168.14, 168.02 (C=O), 166.36, 164.79 (C=N), 162.65, 160.35(C-N), 147.81, 147.73, 147.52, 138.52 (C-pyridine), 137.34, 137.03, 136.90, 136.13, 133.47, 133.17, 132.87, 129.40, 129.35, 128.89, 128.42, 128.35, 127.98, 126.98, 126.90, 126.79, 126.21, 125.03, 124.73, 123.81, 123.73 (C-aromatic and olefinic), 24.48, 20.12 ($COCH_3$). MS (*m/z*, %) = 515 (M^+, 12.20), 49 (100). Anal. calcd. (found) % for $C_{26}H_{21}N_5O_7$: C, 60.58 (60.46); H, 4.08 (3.97); N, 13.59 (13.33).

3-[(1-Acetyl)-3-phenyl-5-(3,4,5-trimethoxybenzylidene)-6-oxo-1,2-dihydro-1,2,4-triazine-2-carbonyl]pyridinium acetate (Vc): Pale yellow crystals, yield 58 %, m.p. 110 ºC; IR (KBr, ν_{max}, cm^{-1}): 3435-2960 (br. OH), 1706-1645 (br. C=O), 1633 (C=N), 1607, 1588 (C=C), 1205, 1015 (C-O); ^{1}H NMR (DMSO-d_6; δ ppm): 1.96 (s, 3H, $COCH_3$), 2.44 (s, 3H, $COCH_3$), 3.78 (s, 6H, 2×OCH_3), 3.88 (s, 3H, OCH_3), 7.31-77.86 (m, 8H, Ar-H and olefinic-H), 11.10 (s, 1H, NH). ^{13}C NMR (400 MHz, DMSO-d_6): 170.34, 168.63, 168.21, 164.03 (C=O), 162.44, 159.43 (C=N), 152.71, 152.69 (C-aromatic-O), 148.47 (C-N), 140.46, 135.46, 133.93, 131.12 (C-pyridine), 131.10, 131.02, 129.82, 129.62, 129.34, 129.02, 128.70, 128.32, 127.97, 127.72, 127.43, 127.29, 127.03, 125.05, 123.96, 123.41, 110.32, 109.85 (C-aromatic and olefinic), 60.22 (OCH_3), 55.86, 55.84 (OCH_3), 24.47, 20.15 ($COCH_3$). MS (*m/z*, %) = 560 (M^+, 14.20). Anal. calcd. (found) % for $C_{29}H_{28}N_4O_8$:C, 62.14 (62.02); H,5.00 (4.87); N, 10.00 (N, 9.90).

Biological Assay: The antibacterial activity of 1,2,4-triazine derivatives (**IV** and **V**), using concentration 1 mg/mL, were screened against gram positive bacteria (*Bacillus subtilis*, *Streptococcus pneumonia* and *Staphylococcus aureus*) and gram negative bacteria (*E. coli* and *Pseudomonas* sp.) in DMSO by well diffusion method using standard Mueller-Hinton agar as the medium. The incubation was done for 24 h at 37 ºC. After this period, the zones of inhibition were recorded using vernier callipers. The radius of the zone is the measure of antibacterial activity. Streptomycin was used as standard.

Antifungal screening: Antifungal activity of 1,2,4-triazine derivatives (**IV** and **V**) evaluated against three fungal strains (*Aspergillus niger*, *Penicillium* sp. and *Candida albicans*) using sabouraud dextrose agar diffusion method. Wells were made (8 mm diameter) with a sterile cork borer. To these wells 7 µL from 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 37 ºC for 48 h. At the end of the incubation period, diameter of zone of inhibition around the wells was measured and ketoconazole was used as standard antifungal.

Cytotoxicity

Cell lines and culture: Human hepatocellular carcinoma (HepG-2) cells were obtained from the American type culture collection ATCC, Rockville, 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 a humidified atmosphere with 5 % CO_2 and sub-cultured two to three times a weak.

Evaluation of antitumor activity: The cells were grown as 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 h at 37 ºC in a humidified incubator with 5 % CO_2. 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 37 ºC. 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. After every 24 h, 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 lysing 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 follows:

$$1-\frac{OD_t}{OD_c}\times 100\ \%$$

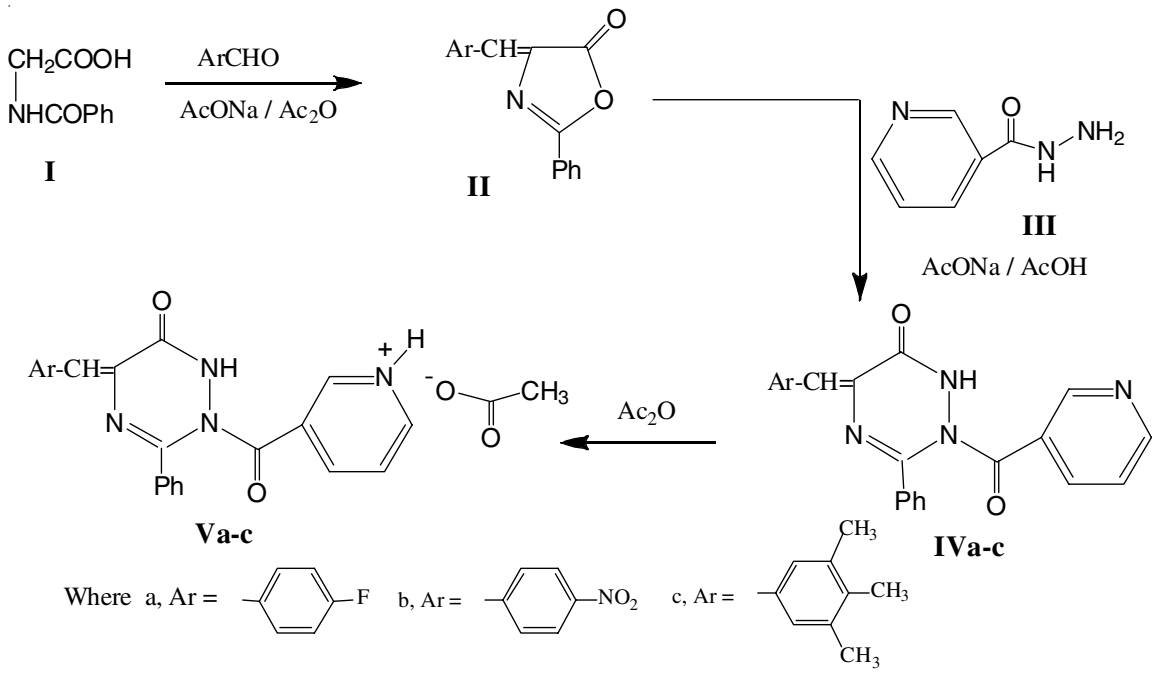
where, OD_t is the mean optical density of wells treated with the test sample and OD_c is the mean optical density of untreated cells.

The 50 % inhibitory concentration (IC_{50}), the concentration required to cause toxic effect in 50 % of inactivated cells, was estimated from graphic plots.

RESULTS AND DISCUSSION

In the present work, the synthetic pathway is outlined in **Scheme-I**. *N*-Benzoyl glycine (**1**) was condensed with aromatic aldehydes (such as 4-fluorobenzaldehyde, 4-nitrobenzaldehyde and 3,4,5-trimethoxybenzaldehyde) in presence of fused sodium acetate and acetic anhydride under fusion yielded the corresponding 5-arylidene-2-phenyl-3,1-oxazole-4-ones (**IIa-c**) [14,15]. The condensation of 5-arylidene-2-phenyl-3,1-oxazole-4-ones (**IIa-c**) with pyridoyl-3-acid hydrazide (**III**) in presence of sodium acetate and glacial acetic acid as reaction solvent yielded the corresponding 5-arylidene-3-phenyl-2-(3-pyridoyl)-1,2-dihydro-1,2,4-triazine-6(5*H*)-ones (**IVa-c**) [16]. Acetylation of 1,2,4-triazine derivatives (**IVa-c**) with acetic anhydride under reflux yielded the corresponding 3-(1-acetyl-3-phenyl-5-arylidene-6-oxo-1,2-dihydro-1,2,4-triazine-2-carbonyl)-pyridinium acetate (**Va-c**) [17-21].

Antimicrobial activity: The antibacterial activity of 1,2,4-triazine derivatives (**IV** and **V**), using concentration 1 µg/mL, were screened against gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and gram negative bacteria (*E. coli* and *Pseudomonas* sp.). Streptomycin was used as bacterial activity positive control by well diffusion method using standard



Scheme-I

Mueller-Hinton agar as medium [22]. Antifungal activity of 1,2,4-triazine derivatives (**IV** and **V**) evaluated against two fungal strains (*Penicillium* sp. and *Candida albicans*) using the sabouroud dextrose agar diffusion method [23]. Ketoconazole was used as standard antifungal.The compounds were tested at different concentrations (100 and 50 µg/100 mL DMSO) and the activity was determined by measuring the zone of inhibition (mm) and compared with the inhibition zones of streptomycin and ketoconazole (25 µg/100 mL) was used as a positive control. The screening results are listed in Table-1.

It is well noticed that the compounds **IVa**, **IVc**,**Va** and **Vc** showed considerably higher potency against Gram positive bacteria (MIC range = 5- 28 µg/100 mL) and Gram negative bacteria (MIC range = 8- 30 µg/100 mL) and fungal employed (MIC range = 8-27 µg/100 mL) compared with the reference standard. The compounds **IVb** and **Vb** showed lower potency against all bacteria compared with the reference standard.The MIC study of synthesized compounds confirmed that the potency of the title compounds based on the presence of electron donating group such as methoxy group and halogen atom increased the antimicrobial activity, while the presence of nitro group decreased the antimicrobial activity, while It is well noticed that compounds **IVa**, **IVc** and **Va** exhibited maximum antifungal activities compared with the reference standard.

Cytotoxicity: In this study, the anticancer activity of five synthesized 1,2,4-triazine derivatives had been evaluated on human cancer cell line, representing liver cancer. The cytotoxic activities of prepared compounds were tested against HepG-2 cell line according to method of Masmann and Vijayen *et al.* [24,25]. The inhibitory activity against liver carcinoma cells (HepG-2) was detected by using different concentrations of tested samples (50, 25, 12.5, 6.25, 3.125 and 1.56 µM) and the survival cell (%) were determined by colorimetric method. The IC_{50} was calculated from Figs. 1 and 2.

The results of inhibitory concentration in 50 % population (IC_{50}) data are summarized in Table-2.

TABLE-1
ANTIMICROBIAL ACTIVITY OF COMPOUNDS **IV-V** CHARACTERIZED BY ZONE INHIBITION (mm)

Product	Gram-positive bacteria		Gram-negative bacteria		Antifungal activity	
	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas* sp.	*Penicillium* sp.	*Candida albicans*
IVa	24	23	25	29	22	25
IVb	5	11	13	8	8	10
IVc	20	21	23	30	25	23
Va	28	24	27	28	21	27
Vb	10	14	15	11	5	9
Vc	24	23	25	31	17	19
Streptomycin	18	20	22	27	–	–
Ketoconazole	–	–	–	–	21	23

Notes: Reported is the zone of inhibition expressed in mm; – no activity

TABLE-2
CYTOXICITY VALUES OF 1,2,4-TRIAZINE DERIVATIVES (AFTER 24 h CONTINUOUS EXPOSURE OF HepG-2 TUMOR CELL LINE)

Compd. No.	**Iva**	**Ivb**	**Ivc**	**Va**	**Vb**	Vinblastine standard
IC_{50} (µM)	4.30	9.60	11.00	4.70	4.00	4.60

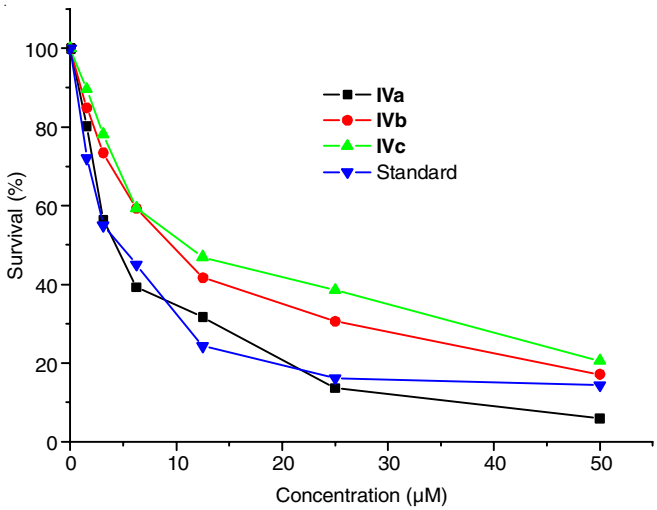


Fig. 1. Inhibitory activities of compounds **IVa-c** against HepG-2 cell lines

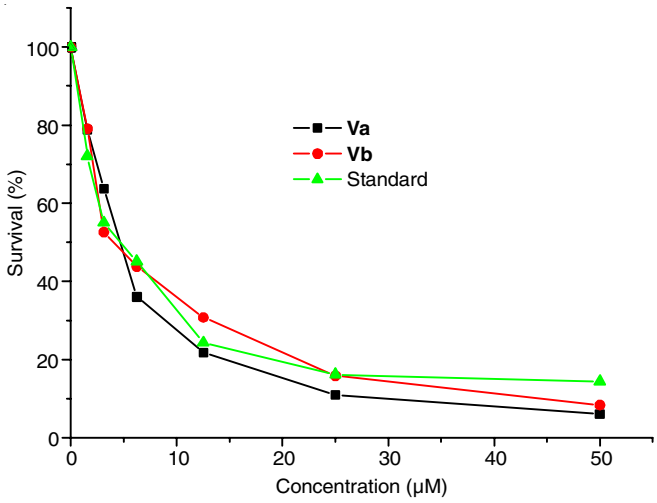


Fig. 2. Inhibitory activities of compounds **Va,b** against HepG-2 cell lines

According to the results of cell culture studies, the compounds **IVa**, **Va** and **Vb** showed antitumor activity more than the standard antitumor drug against (HepG-2) cell line. In comparison with standard antitumor drug vinblastine, compounds **4b** and **4c** have cytotoxic and antitumor activity less than standard antitumor drug against hepatocellular carcinoma cell line (HepG-2). The IC_{50} values for the cancer cell line deduced and varied in the range of 4.00-11.00 μM (Table-2).

Conclusion

A new series of 1,2,4-triazine derivatives (**IV** and **V**) containing pyridine ring were screened for their antibacterial and antifungal activities. The tested compounds **IVa**, **IVc**, **Va** and **5c** demonstrated excellent antimicrobial activity. Also, anticancer activities of synthesized 1,2,4-triazine derivatives were evaluated on liver cancer cell lines. As a result of the cell culture studies, all of 1,2,4-triazine derivatives (**IV** and **V**) have shown anticancer activity for liver cancer cells. Finally compounds **IVa** and **Va-b** can be suggested as potent candidates for liver cancer drug. The tested compounds **IVa**, **IVc**, **Va** and **Vc** demonstrated excellent antimicrobial activity. Also, anticancer activities of synthesized 1,2,4-triazine derivatives were evaluated on liver cancer cell lines. As a result of the cell culture studies, all of 1,2,4-triazine derivatives (**IV** and **V**) have shown anticancer activity for liver cancer cells.

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