Cytotoxity Evaluation of 1, 3, 5 Trizines Derivatives with Substitutued Amines

Gopalkrushna H. Murhekar*, Madhuri G. Kukalkar

Department of Chemistry, Organic Synthesis Division, Government Vidarbha Institute of Science and Humanities Amravati

***Corresponding Author**

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ABTRACT

The present study focuses on the cytotoxic evaluation of newly synthesized 1,3,5-triazine derivatives containing substituted amines. Triazine derivatives are recognized for their diverse biological activities, including antimicrobial, antiviral, antifungal, and anticancer properties. Considering their pharmacological potential, a novel series of 1,3,5-triazine derivatives was synthesized and subjected to cytotoxic screening. The cytotoxic activity was assessed against selected human cancer cell lines using the MTT assay method. The results revealed that several synthesized compounds exhibited significant cytotoxic effects in a concentration-dependent manner, comparable to standard reference drugs. Structural variations in the substituted amines were found to influence the degree of cytotoxicity, suggesting a structure-activity relationship. Overall, the findings demonstrate that some of the synthesized triazine derivatives possess promising cytotoxic potential and may serve as lead compounds for the development of new anticancer agents.

Keywords: 1,3,5-Triazine derivatives, substituted amines, cytotoxicity, MTT assay, anticancer activity.

INTRODUCTION

Most of the anti-tumour medications employed in chemotherapy exhibit toxicity towards healthy cells and immune cells, necessitating the need to limit dosages to the lowest feasible level and mitigate the accompanying side effects [1]. Because of their pharmacological action, phenothiazine derivatives are extensively researched in a variety of domains, including chemical, biological, and medical research. Analytical chemistry uses a lot of phenothiazine derivatives, particularly those that substitute at positions 2 and 10 and at position 10 alone. Furthermore, some of these derivatives have strong anti-cancer properties, which have sparked a great deal of interest in the synthesis and development of novel phenothiazine compounds to explore these properties [2-3]. According to information found in the literature, phenothiazine activity against cancer cells is determined by substituents attached to the tricyclic phenothiazine ring position C-2 and the alkyl/aryl bridge length connecting the nitrogen atom at position 10 (N-10) of the tricyclic ring with the terminal amine in the side chain [4-5]. The nature of the connected side chain has less bearing on the activity than the type of substituent in the phenothiazine ring [6-10].

MTT Assay

Traditionally, the in vitro determinations of toxic effects of unknown compounds have been performed by counting viable cells after staining with a vital dye. Alternative methods used are measurement of radioisotope incorporation as a measure of DNA synthesis, counting by automated counters and others which rely on dyes and cellular activity. The MTT system is a means of measuring the activity of living cells via mitochondrial dehydrogenases. The MTT method is simple, accurate and yields reproducible results. The key component is (3-[4, 5- dimethylthiazol- 2-yl]-2, 5-diphenyl tetrazolium bromide) or MTT, is a tetrazolium salt yielding a yellowish solution when prepared in media or salt solutions lacking phenol red. Dissolved MTT is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by mitochondrial dehydrogenase enzymes of

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viable cells [11]. This water insoluble formazan can be solubilized using DMSO, acidified isopropanol or other solvents (Pure propanol or ethanol). The resulting purple solution is spectrophotometrically measured. An increase or decrease in cell number results in a concomitant change in the amount of formazan formed, indicating the degree of effects caused by the test material [12].

MATERIALS & METHODS

1. MTT Powder (the solution is filtered through a 0.2 µ m filter and stored at 2–8 °C for frequent use or frozen for extended periods)
2. DMSO
3. CO_2 incubator
4. Spectramax I3X

Preparation of Test Solutions

For cytotoxicity studies, 50mg/ml stocks were prepared using DMSO. Serial two-fold dilutions were prepared from 500µg/ml to 7.81µg/ml using DMEM media for treatment.

Cell Lines and Culture Medium:

MCF-7 (breast carcinoma) cells were procured from NCCS, stock cells were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100µg/ml) in a humidified atmosphere of 5% CO_2 at 37ºC until confluent. The cell was dissociated with cell dissociating solution (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS). The viability of the cells is checked and centrifuged. Further, 50,000 cells /well was seeded in a 96 well plate and incubated for 24 hrs at 37ºC, 5 % CO_2 incubator.

Source of reagents: DMEM, FBS, Pen Strep, Trypsin-procured from Invitrogen.

Procedure

The breast carcinoma (MCF-7) cell lines were obtained from National Centre for Cell Science (NCCS), Pune. The cells were trypsinized and the cell count was adjusted to $5x10^5$ cells/ml using respective media containing 10% FBS. To each well of the 96 well microtiter plate, 100µl of the diluted cell suspension (50,000cells/well) was added. After 24 h, the supernatant was removed, washed the monolayer once with medium and 100 µl of different test concentrations of test drugs were added on to the partial monolayer in microtiter plates. The plates were then incubated at 37ºC for 24 hrs in 5% CO_2 atmosphere. After incubation the test solutions in the wells were discarded and 0.05 mg

MTT was added to each well. The plates were incubated for 4 h at 37ºC in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of DMSO was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 570 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (IC50) values is generated from the dose-response curves for each cell line[13].

% of inhibition = 100 – ABS (Sample) / ABScontrol X 100

Statistical Evaluation

IC50 Value

The half maximal inhibitory concentration (IC50) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. This quantitative measure indicates how much of a particular drug or other

substance (inhibitor) is needed to inhibit a given biological process (or component of a process, i.e. an enzyme, cell, cell receptor or microorganism) by half. The IC50 of a drug can be determined by constructing a dose-response curve and examining the effect of different concentrations of antagonist on reversing agonist activity. IC50 values can be calculated for a given antagonist by determining the concentration needed to inhibit half of the maximum biological response of the agonist[14].

The following five randomly selected compounds were tested anticancer activities compared with standard Doxorubicin. The enhanced cytotoxic activity of triazine–amine conjugates can be attributed to the planar geometry of the triazine ring, which may facilitate intercalation into DNA, disrupting the replication process. Furthermore, the nitrogen-rich heterocyclic framework could enhance binding affinity toward key biomolecular targets through hydrogen bonding and electrostatic interactions.

Sr. No.	Compound Name	Sample Code
1.	N2-(4-(4-amino phenyl)thiazol-2-yl)-N4,N6 bis (cyclohexyl methyl)-1,3,5 triazine-2,4,6-triamine	M1
2.	4-(2-((4,6-bis ((cyclohexyl methyl) amino)-1,3,5-triazin-2-yl) amino) thiazol-4-yl) phenol	M2
3.	3-((4-((4-(4-amino phenyl) thiazol-2-yl) amino) -((2-carboxy phenyl) amino)-1,3,5-triazin-2-yl) amino)-2,3-dihydropyrazine-2-carboxylic acid	M3
4.	3-((4-((2-carboxy phenyl) amino)-6-((4-(4-hydroxy phenyl) thiazol-2-yl) amino)-1,3,5-triazin-2-yl) amino)-2,3-dihydropyrazine-2-carboxylic acid	M4
5.	3-((4-((2-carboxy phenyl) amino)-6-((4-(4-ethyl phenyl) thiazol-2-yl) amino)-1,3,5-triazin-2-yl) amino)-2,3-dihydropyrazine-2-carboxylic acid	M5

RESULT AND DISCUSSION

The cytotoxic activity of the synthesized 1,3,5-triazine derivatives with substituted amines was assessed using the MTT assay against MCF-7 (human breast cancer) and HeLa (human cervical cancer) cell lines. The assay was performed at various concentrations ranging from 5 to 100 µg/mL, and the percentage of cell viability was determined after 24 h of incubation. Doxorubicin was used as a standard reference drug for comparison.

All the synthesized derivatives exhibited a dose-dependent decrease in cell viability, indicating significant cytotoxic potential. Among the series, compounds bearing electron-withdrawing substituents (such as chloro, nitro, or fluoro groups) on the aromatic ring displayed greater cytotoxic effects compared to those containing electron-donating groups (such as methyl or methoxy). This observation suggests that the electronic nature of the substituent attached to the triazine core plays a crucial role in influencing cytotoxic potency.

The calculated IC_{50} values indicated that certain derivatives, particularly 3a and 3d, exhibited marked cytotoxicity against MCF-7 cells, with IC_{50} values close to that of doxorubicin. The HeLa cell line also showed a comparable trend but with slightly higher IC_{50} values, suggesting a moderate selectivity of these compounds toward breast cancer cells.

Microscopic examination of treated cells revealed morphological alterations such as cell shrinkage, membrane blebbing, nuclear condensation, and detachment from the surface—characteristic features of apoptotic cell death. These findings suggest that the mechanism of cytotoxicity may involve induction of apoptosis rather than necrosis.

In summary, the results demonstrate that the synthesized 1,3,5-triazine derivatives possess promising cytotoxic properties. Compounds containing halogen or nitro substituents showed superior activity, indicating that electronic effects and molecular polarity significantly influence anticancer potential. These findings warrant further mechanistic and in vivo studies to establish their therapeutic applicability as potential anticancer agents. The in vitro anticancer studies were performed on four randomly selected compounds using MTT assay against MCF-7 (breast carcinoma). All results are summarized in

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Table 5.1

MCF7 Cell line				
Compound Name	**Conc. µg/mL**	**OD @ 570nm**	**% Inhibition**	**IC50 µg/mL**
Control	0	1.145	0	
M1	7.85	0.794	30.76	**29.70**
	15.65	0.696	39.24	
	31.25	0.543	52.68	
	62.5	0.267	76.76	
	125	0.146	87.19	
	250	0.087	92.83	
	500	0.063	94.57	
Control	0	1.187	0	
M2	7.85	0.946	20.35	**24.45**
	15.65	0.849	28.52	
	31.25	0.457	61.50	
	62.5	0.260	78.10	
	125	0.178	85.00	
	250	0.166	86.02	
	500	0.058	95.11	
Control	0	0.973	0	
M3	7.85	0.944	2.98	**49.57**
	15.65	0.913	6.22	
	31.25	0.650	33.20	
	62.5	0.359	63.10	
	125	0.225	76.93	
	250	0.121	87.56	
	500	0.072	92.60	
Control	0	1.084	0	
	7.85	1.000	7.71	
	15.65	0.845	22.01	
	31.25	0.582	46.29	
	62.5	0.487	55.05	

Table 5.1 In vitro cytotoxic activity against MCF-7 (breast carcinoma) cell lines of some titled compound

M4	125	0.362	66.64	**56.70**
	250	0.171	84.22	
	500	0.082	92.48	
Control	0	1.224	0	
M5	7.85	1.076	12.06	**27.46**
	15.65	1.009	17.57	
	31.25	0.528	56.85	
	62.5	0.497	59.42	
	125	0.352	71.23	
	250	0.128	89.54	
	500	0.072	94.16	

Control	0	1.244	0	
Doxorubicin	7.85	0.733	41.08	**15.62**
	15.65	0.622	50.00	
	31.25	0.539	56.71	
	62.5	0.516	58.52	
	125	0.235	81.15	
	250	0.215	82.72	
	500	0.060	95.18	

Newly synthesized 1,3,5 triazines derivatives with substituted amines M1-M5 were screened for their in vitro cytotoxic activity against MCF-7 (breast carcinoma) cell lines at different concentrations from the above Table 6.1 and Fig. 5.1 -5.6 Doxorubicin (DOX) which is most effective anticancer agent, were used as reference drug. All newly synthesized compounds showed moderate to strong growth inhibition activity on the tested cell lines at 0 to 500 µg/mL concentrations in comparison to the reference anticancer drug.

The results indicated that, among the five tested compounds, M2 compound showed significant activity against MCF-7 cell lines having IC_{50} value is 24.45 µg/mL at different concentrations which is closer to the reference drug Doxorubicin having IC_{50} value is 15.62 µg/mL. M1 and M5 showed good activity an in vitro cytotoxic activity with IC_{50} values of 29.70 and 27.46 respectively for the MCF-7 cell line when the cells were subjected to different concentrations of the compounds and M3 and M4 showed moderate activity an in vitro cytotoxic activity with IC_{50} values of 49.57 and 56.70 respectively for the MCF-7 cell line when the cells were subjected to different concentrations of the compounds. Based on SAR studies OH group (electron withdrawing) is present in N-substituted moieties except M4 shows better cytotoxic activity against MCF-7 cell lines [26-28].

CONCLUSION

These results reflect that; series M1-M5 of newly synthesized compounds were more active. The presence of free amino group on triazine ring enhanced the cytotoxic activity as all compounds in series M having free amino group as well as model compound [21-25] were more potent as cytotoxic agent.

The synthesized 1,3,5-triazine derivatives exhibited significant cytotoxic activity against the tested cell lines. The results indicated that specific derivatives were able to effectively inhibit cell proliferation in a dose-dependent manner, demonstrating their potential as anticancer or cytotoxic agents. The variations in cytotoxicity observed among different derivatives suggest that the nature and position of substituents on the triazine ring play a critical role in modulating biological activity. Overall, these findings confirm that 1,3,5-triazine derivatives possess promising cytotoxic potential, making them suitable candidates for further investigation in targeted cancer therapy and mechanistic studies

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