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Synthesis, Docking Studies, and *in-vitro* Anti Tumour Evaluation of Novel 1,2,4-Triazine Derivatives

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ABSTRACT

1,2,4-Triazine occupies a prominent position in the therapeutic front , possessing diverse range of biological activities. A series of novel derivatives of 1,2,4-Triazine were synthesized, characterized by means of spectral and chromatographic methods and their docking scores as an inhibitor of the enzyme CMet Kinase was calculated. The synthesized compounds were evaluated for their in vitro anti tumour activity by Trypan Blue exclusion method. The compound Ethyl [(5,6-diphenyl 3-(4-fluorophenyl)-2-(3-oxo-1,2,4-triazin- 2(3H)-yl)] prop-2-enoate (Va) exhibited significant in vitro antitumour activity. Other derivatives like Ethyl [(5,6-diphenyl-3-(4-choro phenyl)-2-3-oxo-1,2,4-Triazine-2(3H)yl)] acetate (Vc), Ethyl [(5,6-diphenyl-3-(2-choro phenyl)-2-3-oxo-1,2,4-Triazine-2(3H)yl)] acetate. (Vh) and Ethyl [(5,6-diphenyl-3-(4-bromo phenyl)-2-3-oxo-1,2,4-Triazine-2(3H) yl)] acetate. (Vd) exhibited comparable anti-cancer properties. It can be concluded that 1,2,4-Triazine derivatives offers potential scope to drug research community for developing novel compounds of high therapeutic interest.

Keywords: 1,2,4-Triazine; Argus Lab; Docking; Tumour; Trypan blue exclusion method

INTRODUCTION

The design, synthesis and evaluation of molecules with therapeutic values, remain one of the main objective of organic and medicinal chemists. Synthesis of nitrogen containing heterocyclic compounds has been attracting increased interest because of their utility for various biological receptors with a high degree of binding affinity. The triazine moiety with a multi potential biological profile has been matured into an indispensable heterocyclic scaffold that makes it one of the extensively studied heterocyclic compounds [1]. Triazine is a heterocyclic ring, analogues to the six membered benzene ring but with three carbons replaced by Nitrogen. The three isomers of Triazine are distinguished each other by the positions of their nitrogen atom and are referred to as 1,2,3-Triazine, 1,2,4-Triazine and 1,3,5-triazine. Triazine is weaker bases than pyridine. The isomers of Triazine are widely used in organic reactions that offers access to a multitude of useful molecules due to its specific structure and electronic properties. Owing to the immense synthetic importance and varied bioactivities, efforts have been made from time to time to generate libraries of these compounds. Nitrogen containing triazine heterocyclic moiety is the, Master Key, as acting at different targets to elicit varied pharmacological properties by inhibiting the action of an inducible membrane protein that normally function to increase the efflux of the cytotoxic agents. The triazine scaffold provides the basis for the design of biologically relevant molecules with widespread applications like anticancer, antiviral and antimicrobial. Triazine is also the basic structure of some herbicides like amitrole, atrazine, cyanazine, simazine, trietazine, and resin modifiers like melamine and benzoguanamine. Among the triazine isomers, the 1,2,4-Triazine scaffold occupies a prominent position, possessing a broad range of biological activities. 1,2,4-Triazines are six membered heterocyclic compounds possessing three nitrogen in its structure with general formula $C_3H_3N_3$.1,2,4-Triazine is found in many potent biologically active molecules with promising biological potential like anti-cancer, anti-inflammatory, anti-mycobacterial, anti- viral, anxiolytic. Which makes it an attractive scaffold for the design and development of new drugs the wide spectrum of biological activity of this moiety has attracted considerable attention to it from medicinal chemists. Due to these biological activities, their structure

activity relationship has generated interest among medicinal chemists and this has culminated in the discovery of several lead molecules. The outstanding development of triazine derivatives in diverse diseases within very short span of time proves its magnitude for medicinal chemistry research. In this article, we have analysed therapeutic potential of 1,2,4-triazine derivatives against tumour by molecular docking studies followed by synthesis of few novel derivatives possessing triazine ring, characterization of the synthesized derivatives and *in vitro* evaluation of its anti-cancer potential [2].

MATERIALS AND METHODS

Docking

The crystal structure of the enzymes was downloaded from protein data bank. The protein preparation was then conducted using the software Argus Lab 4.0.1 version. Molecular docking studies were performed on the α-chain of the receptor and the β-chain was removed by protein editing. The non-essential water molecules were removed and polar hydrogen was merged. The PDB files were minimized using Argus Lab by utilizing force field based approaches. Then the modified protein structure was saved in PDF format and used for all further docking studies [3]. All the final synthesized derivatives were built by using ACDLABS Chemsketch 12.0 version software. The obtained structures were saved in MDL mol format and can be imported into the workspace of Argus Lab 4.0.1 version. The geometry optimization was performed by UFF molecular mechanics method. Hydrogen bonds were added to each molecule and the hybridization level and valency of atoms in ligand is checked. Clean hybridization was done to make sure of the exact hybridization pattern of the molecules. Final geometry optimization was done by using semi empirical Quantum mechanics PM3 method. The energy minimized structures were saved in pdb format for docking studies. Anticancer docking studies of the synthesized derivatives were carried out on Argus Lab Software using the C met kinase [PDB id: 3FD2] as the receptor. The ability of the synthesized analogues to inhibit the enzyme was recorded as docking scores. Argus Lab 4.0.1 version was employed in the docking between protein and ligand using Argus dock with a fast and simplified Potential Mean Force (PMF). Docking the ligand into binding site was done by "Argus Dock" as the docking engine. Dock was selected as the calculation type, "flexible" for the ligand and "A score" was used for the scoring function.

The binding site box was automatically calculated by the software based on the size of the binding group for enclosing the entire active binding site of the protein with a grid resolution of 0.4 A°. The process of docking is repeated until a constant value of docking score is obtained. The resulting final docked structures were saved in pdb format and the docking snap shots were imported into Mole grow Molecular Viewer version 2012.2.5.0. This programme can generate the amino acid residue around each segment of final synthesized derivatives and interpreting the different drug-receptor interactions such as hydrogen bonding, hydrophobic stearic interaction and electrostatic interaction. The docking scores of the compounds and the reference compound methotrexate are presented in table. The snap shot of the docked conformation is presented in figure (Table 1 and Figure 1) [4].

Table 1: Docking scores of synthesized compounds.

S. NO	Codes of compounds	Docking score (Kcal/Mol)
1	Va	-6.71
2	Vb	-1.18
3	Vc	-4.32
4	Vd	-1.04
5	Ve	-3.37
6	Vf	-4.86
7	Vg	-4.56
8	Vh	-3.32
9	Vi	-1.33
10	Vj	-2.12
11	Methotrexate	18.43

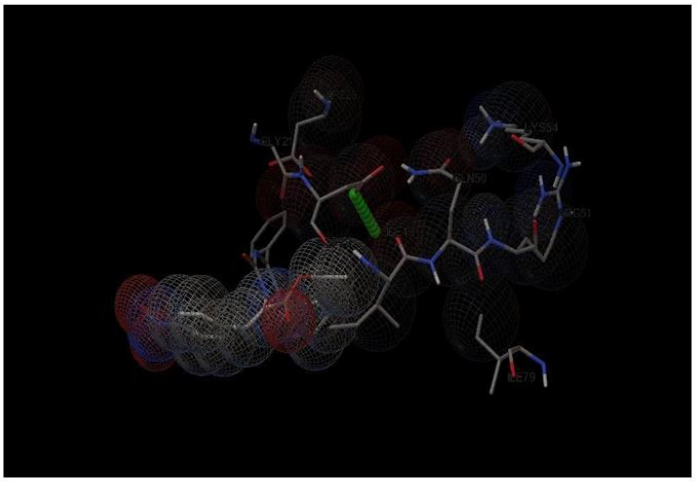

Figure 1: Docked conformation of Va with Cmet Kinase.

Synthesis

All the reagents were of A.R grade and were purchased from Merck and Alfa Aesar. The melting points of the compound were determined using melting point apparatus and are uncorrected. The purity of the compounds wereestablished by means of TLC using n-hexane and chloroform as mobile phase. IR spectra were recorded using PerkinElmer FT-IR Model [5]. 400. 1H NMR was recorded using Bruker DPX 400 FTNMR at 400 MHz. Mass spectra was recorded using Joel JMS 600 H mass spectrometer. The physical characterization data of synthesized compounds are depicted in table number 2.

Step: 1. Synthesis of 5,6-Diphenyl-1,2,4-Triazine-3(2H)-one-1,2.4-Triazine-2(3H)-yl acetate: 0.05 mol of benzyl (I) and equimolar amount of semicarbazide (II) were dissolved in 30 ml of acetic acid. The reaction mixture was then refluxed for 8-10 hours. After the completion of reaction, the reaction mixture was poured on to the crush ice. The solid mass precipitated was filtered, washed with water and recrystallized from ethanol to yield compound [6].

MP: 185 C, yield -68%.
CHN analysis cald: C-72%,H-4.5%,N-17%,O-6.4%.found C-71.56%,H-4.34 %,N-16.89%,O-6.34%.
IR: KBrcm-13152 (N-H str), 1647(C=O str)
NMR: 7.391-7880 (m.A-.H), 8.10 s(1H,NH), $\mathbf{M}^{+}$498

Step: 2. Synthesis of Ethyl (5,6-diphenyl-3-oxo-1,2,4-Triazine-2(3H)yl) acetate: Compound 1(0.056 Mol) was dissolved in pyridine (30 ml) and the solution was chilled on an icebath then chloroethylacetate (0.112 mol) was added drop wise with constant shaking and the mixture was further stirred for 1 hour and poured into ice cold water. The solid separated out was filtered and washed with water. The product was recrystallized from ethanol [7].

M.P: 202 C, yield -68%.
IR: KBrcm-13152(N-H str), 1647(C=O str)
NMR: 7.478 -7.789 (mA-.H), 8.21(s Ar-H,NH),3.89(q,CH3). $\mathbf{M}^{+}$ 319
CHN analysis cald: C-71.4%, H-5.37%, N-13.16%, O-10.2% found C-72.16%, H-5.27%, N-13.24%, O-10.35%.

Step: 3. Ethyl [(5,6-diphenyl 3-(4-fluorophenyl)-2-(3-oxo-1,2,4-triazin-2(3H)-yl)]prop-2-enoate: An equimolar quantity of compound B (0.014) and 4-fluoro benzaldehyde in methanol (20 ml) were taken in a flask. To this 40% W/V methanolic potassium hydroxide was added and stirred for 2 hours, then left overnight. The mixture was poured onto crushed ice, filtered, washed, dried, and recrystallized from ethanol to yield corresponding triazine derivatives, [Ethyl (5,6-diphenyl 3-(4-fluorophenyl)-2-(3-oxo-1,2,4-triazin-2(3H)-yl)prop-2-enoate].

- **MP:** 185 C, yield -68%.
- **IR:** KB rcm-13186 (N-H str), 1647(C=O str), 1389 (C-F str)
- **NMR:** 7.491-7780 (mA-.H), 3.1(s Ar-H,NH),6.82(1H,CH) $\mathbf{M}^{+}$: 425,M+1: 426
- **Elemental analysis cald:** C-73.40% H-4.74% F-4.47% N-9.88% O-7.52% Found C-72.85% H-4.65% N-9.23% O-7.09%

Synthesis of Ethyl [(5,6-diphenyl-3-(4-nitro phenyl)-2-3-oxo-1,2,4-Triazine-2(3H)yl)] acetate: An equimolar quantity of compound (0.014) and 4-nitro benzaldehyde in methanol (20 ml) weretaken in a flask. To this 40% W/V methanolic potassium hydroxide was added and stirred for 2 hours, then left overnight. The mixture was poured onto crushed ice, filtered, washed, dried, andre crystallized from ethanol to yield corresponding triazine derivatives. [Ethyl (5,6-diphenyl (2E)-3-(4-fluorophenyl)-2-(3-oxo-1,2,4-triazin-2(3H)-yl) prop-2-enoate]

- **M.P:** 185 C, yield -68%.
- **IR:** KBrcm-13172 (N-H str),1647(C=O str),
- **NMR:** 7.371-7870(m,A-.H),3.45(s Ar-H,NH),6.832(s,1H,CH) $\mathbf{M}^+$: 452 M+2:454

Elemental analysis cald: C-69.02% H-4.46% N-12.38% O-14.14% found C-70.1% H-4.12% N-12.57% O-14.46%

Synthesis of Ethyl [(5,6-diphenyl-3-(4-choro phenyl)-2-3-oxo-1,2,4-Triazine- 2(3H)yl)]acetate: An equimolar quantity of compound (0.014) and 4-chloro benzaldehyde in methanol (20 ml) were taken in a flask. To this 40% W/V methanolic potassium hydroxide was added and stirred for 2 hours, then left overnight. The mixture was poured onto crushed ice, filtered, washed, dried, and recrystallized from ethanol to yield corresponding triazine derivatives. [Ethyl (5,6-diphenyl (2E)-3-(4-fluorophenyl)-2-(3-oxo-1,2,4-triazin-2(3H)-yl)prop-2-enoate][8].

- **MP:** 185 C, yield -68%, IR: KBrcm-13152(N-H str),1647(C=O str),
- **NMR:** 7.491-7780(mA-.H),3.67(s,Ar-H,NH),6.752(s,1H,CH) $\mathbf{M}^+$: 441,
- **Elemental analysis cald:** C-70.67%, H-4.56%, Cl- 8.02% N-9.51%, O-7.24% Found C--70.07%, H-4.23 %, Cl- 7.92% N-9.23 %, O-7.18%

Synthesis of Ethyl [(5,6-diphenyl-3-(4-bromo phenyl)-2-3-oxo-1,2,4-Triazine- 2(3H)yl)]acetate: An equimolar quantity of compound B (0.014) and 4-bromobenzaldehyde in methanol (20 ml) weretaken in a flask. To this 40% W/V methanolic potassium hydroxide was added and stirred for 2 hours, then left overnight. The mixture was poured onto crushed ice, filtered, washed, dried, and recrystallized from ethanol to yield corresponding triazine derivatives. [Ethyl (5,6-diphenyl (2E)-3-(4-fluorophenyl)-2-(3-oxo-1,2,4-triazin-2(3H)-yl)prop-2-enoate]

- **MP:** 185 C, yield -68%.
- **IR:** KBrcm-13190 (N-H str),1647(C=O str),
- **NMR:** 7.392-758 (m, A-H), 3.667(s, Ar-H,NH), 6.65(s, 1H,CH) $\mathbf{M}^+$: 485.
- **Elemental analysis Cald:** C-64.21%, H-4.14%, Br-16.43%, N-8.64%, O-6.58% found C-64.28% H-4.32%, Br-16.65%, N-8.34%, O-7.06%

Synthesis of Ethyl [(5,6-diphenyl-4-tolyl-2-3-oxo-1,2,4-Triazine-2(3H)yl)]acetate: An equimolar quantity of compound B (0.014) and 4-methyl benzaldehyde in methanol (20 ml) were taken in a flask. To this 40% W/V methanolic potassium hydroxide was added and stirred for 2 hours, then left overnight. The mixture was poured onto crushed ice, filtered, washed, dried, and recrystallized from ethanol to yield corresponding triazine derivatives. [Ethyl(5,6-diphenyl (2E)-3-(4-fluorophenyl)-2-(3-oxo-1,2,4-triazin-2(3H)-yl)]prop-2-enoate [9].

- **MP:** 185 C, yield -68%.
- **IR:** KBrcm-13201 (N-H str), 1647(C=O str), NMR: 7.491-7880 (mA-.H), 3.567(s Ar-H, NH), 6.65(s,1H,CH) $\mathbf{M}^+$: 421.
- **Elemental analysis cald:** C-76.94%, H-5.50% ,N-9.97% ,O-7.59% Found C-76.12%, H-5.23%, N-9.14%, O-7.23%

Synthesis of Ethyl [(5,6-diphenyl-4-ethyl phenyl-3-oxo-1,2,4-Triazine-2(3H) yl)] acetate: An equimolar quantity of compound B (0.014) and 4-ethyl benzaldehyde in methanol (20 ml) were taken in a flask. To this 40% W/V methanolic potassium hydroxide was added and stirred for 2 hours, then left overnight. The mixture was poured onto crushed ice, filtered, washed, dried, and recrystallized from ethanol to yield corresponding triazine derivatives. [Ethyl (5,6-diphenyl (2E)-3-(4-fluorophenyl)-2-(3-oxo-1,2,4-triazin-2(3H)-yl)prop-2-enoate]

- **MP:** 185 C, yield-68%.
- **IR:** KBrcm-13166 (N-H str),1647 (C=O str),
- **NMR:** 7.491-7780 (mA-H), 3.567 (s Ar-H, NH), 6.35, (s,1H,CH) $\mathbf{M}^+$: 435, M-1:433.
- **Elemental analysis cald:** C-77.22%, H-5.79%, N-9.65%, O-7.35% Found-C-77.13%, H-5.87%, N-9.24%,O-7.23%

Ethyl [(5,6-diphenyl 3-(2-fluorophenyl)-2-(3-oxo-1,2,4-triazin-2(3*H*)-yl)]prop-2-enoate: An equimolar quantity of compound B (0.014) and 2-fluoro benzaldehyde in methanol (20 ml) were taken in a flask. To this 40% W/V methanolic potassium hydroxide was added and stirred for 2 hours, then left overnight. The mixture was poured onto crushed ice, filtered, washed, dried, and recrystallized from ethanol to yield corresponding triazine derivatives. [Ethyl (5,6-diphenyl (2E)-3-(4-fluorophenyl)-2-(3-oxo-1,2,4-triazin-2(3H)-yl)prop-2-enoate][10].

- **MP:** 185 C, yield -68%.
- **IR:** KBrcm-13182(N-H str), 1647 (C=O str), 1260 (C-F str)
- **NMR:** 7.341-7.780 (mA-.H), 3.67 (s Ar-H,NH), 6.05(s,1H,CH)M^+: 425.
- **Elemental analysis cald:** C-73.40%, H-4.74%, F-4.47%, N-9.88%, O-7.52%, found C-73.13%, H-4.69%, F-4.67%, N-9.36%, O-7.09%

Synthesis of Ethyl [(5,6-diphenyl 3(2-bromo phenyl)-oxo-1,2,4-Triazine-2(3H) yl) acetate: An equimolar quantity of compound B (0.014) and 2-bromo benzaldehyde in methanol (20 ml) were taken in a flask. To this 40% W/V methanolic potassium hydroxide was added and stirred for 2 hours, then left overnight. The mixture was poured onto crushed ice, filtered, washed, dried, and recrystallized from ethanol to yield corresponding triazine derivatives. [Ethyl (5,6-diphenyl (2E)-3-(4-fluorophenyl)-2-(3-oxo-1,2,4-triazin-2(3H)-yl)prop-2-enoate].

MP: 185 C, yield -68%.
IR: KBrcm-13162 (N-H str),1647(C=O str),
NMR: 7.391-7.890 (m, A-.H),3.541 (s Ar-H,NH),6.234 (s,1H,CH)$\mathbf{M}^+$: 485. $\mathbf{M}^+$1:486.
Elemental analysis Cald: C-64.21%, H-4.14%, Br-16.43%, N-8.64%, O-6.58% found C-63.28%, H-4.56%, Br-16.78%, N-8.12%, O-6.23%

Synthesis of Ethyl [(5,6-diphenyl-3-(2-choro phenyl)-2-3-oxo-1,2,4-Triazine-2(3H)yl)] acetate: An equimolar quantity of compound B (0.014) and 4-choloro benzaldehyde in methanol (20ml) were taken in a flask. To this 40% W/V methanolic potassium hydroxide was added and stirred for 2 hours, then left overnight. The mixture was poured onto crushed ice, filtered,washed, dried, and recrystallized from ethanol to yield corresponding

triazine derivatives. [Ethyl (5,6-diphenyl (2*E*)-3-(4-fluorophenyl)-2-(3-oxo-1,2,4-triazin-2(3*H*)-yl)prop-2-enoate][11].

- **M.P:** 185 C, yield -68%.
- **IR:** KBrcm-13133 (N-H str),1647(C=O str),
- **NMR:** 7.391-7880 (mA-.H), 3.567 (10 H CH2), 6.095 (s,1H,CH) $\mathbf{M}^{+}$: 441, M+2:443.
- **Elemental analysis cald:** C-70.67%, H-4.56%, Cl- 8.02% N-9.51%, O-7.24%

Synthesis of Ethyl [(5,6-diphenyl-2-ethyl phenyl -3-oxo-1,2,4-Triazine- 2(3H)yl)]acetate: An equimolar quantity of compound B (0.014) and benzaldehyde in methanol (20 ml) were taken in a flask. To this 40% W/V methanolic potassium hydroxide was added and stirred for 2 hours, then left overnight. The mixture was poured onto crushed ice, filtered, washed, dried, and recrystallized from ethanol to yield corresponding triazine derivatives. [Ethyl (5,6-diphenyl (2E)-3-(4-fluorophenyl)-2-(3-oxo-1,2,4-triazin-2(3H)-yl)prop-2-enoate]

- **MP:** 185 C, yield -68%
- **IR:** KBrcm-13143 (N-H str), 1647(C=O str),
- **NMR:** 7.391-7880 (mA-.H), 3.567(s Ar-H,NH), 6.15(s,1H,CH) $\mathbf{M}^{+}$: 435,
- **Elemental analysis cald:** C-77.22%, H-5.79%, N-9.65%, O-7.35% Found C-76.94%, H-5.17%, N-9.24%, O-7.15%

Synthesis of Ethyl [(2,5,6-triphenyl-3-oxo-1,2,4-Triazine-2(3H)yl)] acetate: An equimolar quantity of compound B (0.014) and benzaldehyde in methanol (20 ml) weretaken in a flask. To this 40% W/V methanolic potassium hydroxide was added and stirred for 2 hours, then left overnight. The mixture was poured onto crushed ice, filtered, washed, dried, and recrystallized from ethanol to yield corresponding triazine derivatives. [Ethyl (5,6-diphenyl (2E)-3-(4-fluorophenyl)-2-(3-oxo-1,2,4-triazin-2(3H)-yl)] prop-2-enoate[12].

- **M.P:** 185 C, yield -68%.
- **IR:** KBrcm^{-1}3172 (N-H str), 1647 (C=O str),
- **NMR:** 7.391-7880 (mA-H), 3.567 (s Ar-H,NH), 6.15(s,1H, CH) M^{+}: 407
- **Elemental analysis cald:** C-76.64%, H-5.19%, N-10.31%, O-7.85% found C-76.23%, H-5.26%, N-10.65%, O8.11. (Table2)

Table 2: Physical characterization of synthesized compounds.

Sl. No	Codes of compounds	Melting points	Molecular formula	Molecular weight	Rf Value
1	Va	276	$C_{26}H_2OFN_3O_3$	441.46	0.78
2	Vb	260	$C_{28}H_25N_3O_3$	451.53	0.67
3	Vc	254	$C_{26}H_2ON4O_5$	468.47	0.72
4	Vd	256	$C_{26}H_2OBrN_3O_3$	476.37	0.7
5	Ve	256	$C_{27}H_23N_3O_3$	437.5	0.75
6	Vf	270	$C_{26}H_2OClN_3O_3$	457.42	0.65
7	Vg	278	$C_{26}H_2OFN_3O_3$	441.46	0.69
8	Vh	235	$C_{26}H_2OBrN_3O_3$	476.37	0.75
9	Vi	245	$C_{27}H_23N_3O_3$	437.5	0.65
10	Vj	266	$C_{26}H_21N_3O_3$	449.11	0.56

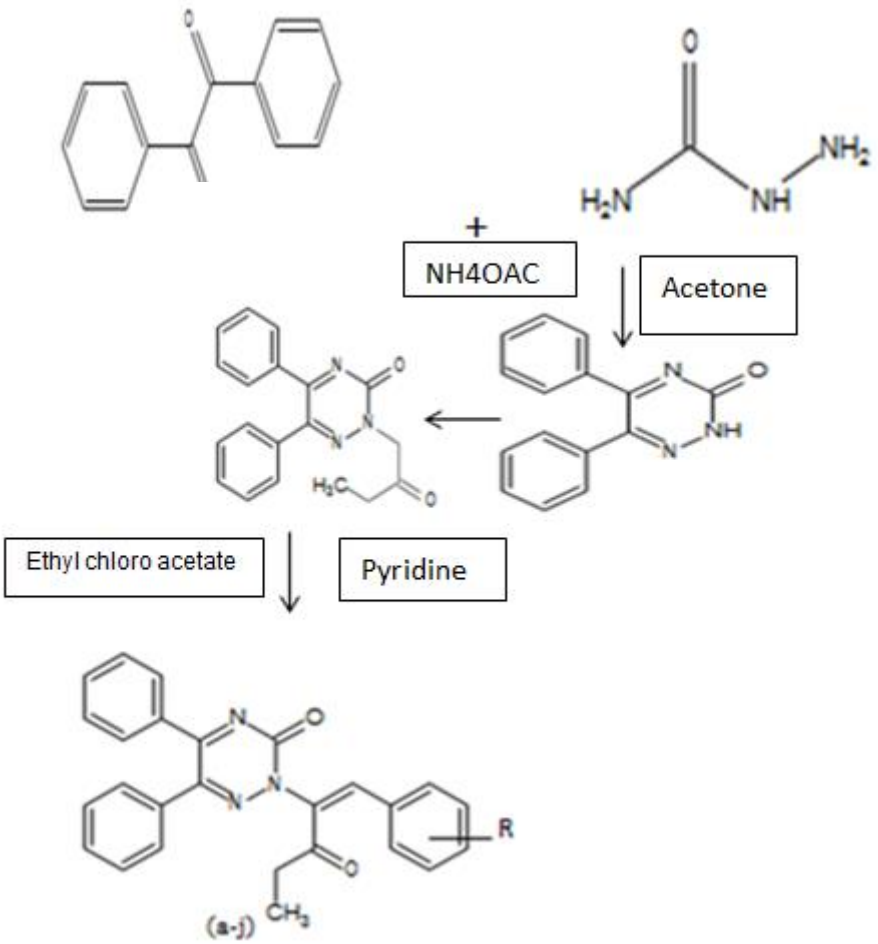

Figure 2: Synthesis of 1,2,4-Triazine Derivative.

RESULTS AND DISCUSSION

Anticancer activity: Synthesized compounds having high docking scores were tested for their short term *in-vitro* cytotoxicity using Daltons Lymphoma Ascites cells (DLA) by means of Trypan blue exclusion method. The tumour cells aspirated from the peritoneal cavity of tumour bearing mice were washed thrice with PBS or normal saline. Cell viability was determined by Trypan blue exclusion method. Viable cell suspension (1 × 106 cells in 0.1 ml) was added to tubes containing various concentrations (10, 20, 50, 100, 200) µg of the test compounds and the volume was made up to 1ml using Phosphate Buffered Saline (PBS). Control tube contained only cell suspension. These assay mixture were incubated for 3 hrs at 37ºc. Further cell suspension was mixed with 0.1 ml of 1% trypan blue and kept for 2-3 mints and loaded on a haemocytometer. Dead cells take up the blue colour of trypan blue while live cells don't take up the dye. The numbers of stained and unstained cells were counted separately.

% cytotoxicity = Number of dead cells × 100. Number of live cell+Number of dead cells. The anticancer activity of the compounds was represented as percentage cell death. The percentage cell death of DLA cells corresponding to the various concentrations (200 µg/ml. 100 µg/ml, 50 µg/ml, 25 µg/ml and 10 µg/ml) of the compounds was reported and summarized in Table 3 [13].

Table 3: Antitumour activity of compounds determined using Trypan Blue exclusion method.

Sl. No	Codes of compounds	Concentration of compounds (µg/ml)	% Cell death
1	Va	200	65
		100	38

		50	18
		25	9
2	Vb	200	31
		100	14
		50	10
		25	3
		10	0
3	Vc	200	48
		100	20
		50	11
		25	2
		10	0
4	Vd	200	52
		100	23
		50	12
		25	10
		10	3
5	Ve	200	34
		100	9
		50	2
		25	0
		10	0
6	Vf	200	58
		100	32
		50	12
		25	4
		10	1
7	Vg	200	46
		100	21
		50	7
		25	2
		10	0
8	Vh	200	44
		100	26
		50	15
		25	3
		10	0
9	Vi	200	42
		100	16
		50	9
		25	2
		10	0
10	Vj	200	42
		100	17
		50	6
		25	0
		10	0

Strucutures of ten novel compounds possessing 1,2,4-Triazine were docked using Argus lab on crystal structure of Cmet Kinase and their docking scores were calculated. The results indicated that the anti tumour activity of the compound Va showed more activity than other derivatives. The compounds were then synthesized. The characterization of the synthesized compounds showed that the compounds were obtained pure. The IR, NMR and mass spectral data confirmed the structures of 1,2,4-Triazine derivatives. *In vitro* anti tumour activities of the synthesized compounds were done using Trypan Blue exclusion method. The results of anti tumour activity were in accordance of the docking scores [14].

The compound Ethyl [(5,6- diphenyl 3-(4-fluorophenyl)-2-(3-oxo-1,2,4-triazin-2(3H)-yl)]prop-2-enoate (Va) exhibited significant *in vitro* anti tumour activity. Other derivatives like Ethyl [(5,6-diphenyl-3-(4- choro phenyl)-2-3-oxo-1,2,4-Triazine-2(3H)yl)]acetate (Vc), Ethyl [(5,6-diphenyl-3-(2- choro phenyl)-2-3-oxo-1,2,4-Triazine-2 (3H) yl)] acetate. (Vh) and Ethyl [(5,6-diphenyl-3-(4- bromo phenyl)-2-3-oxo-1,2,4-Triazine-2(3H)yl)] acetate. (Vd) exhibited comparable anti-cancer properties [15].

CONCLUSION

1,2,4-Triazine nucleus a privileged structure in the field of drug discovery, All the synthesized 1,2,4-Triazine derivatives were docked using Argus lab score and then were evaluated for their *in vitro* anti tumour activity using Trypan Blue exclusion method. The results of *in viva* anti tumour activities were in accordance to the docking scores. From this research it can be concluded that the anticancer activity of the triazine derivatives with electron attracting groups in their structure were the more active compounds and that, among the derivatives synthesized the one possessing fluoro group in its structure was the most active compound. Thus it can be finally concluded that 1,2,4-triazine is a promising scaffold for the development of novel anti tumour agents.

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