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Screening of *in-vitro* Anti Mitotic Activity of Some Newly Synthesized s- Triazine Derivatives

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ABSTRACT

A variety of s- triazine derivatives- 4, 6-dichloro-N-(3-substituted phenyl)-1, 3, 5-triazin-2-amine derivatives (B1 to B6) and 4, 6-dichloro-N-(4-substituted phenyl)-1, 3, 5-triazin-2-amine derivatives (C1 to C6)- were prepared by reacting cyanuric chloride with amine substituted acetophenone and bromination of the ketone group followed by cyclization using urea/ thiourea/ thiosemicarbazole/ amino guanidine. HCl/ acetamide/ benzamide to prepare the respective derivatives. The structures of all the compounds were confirmed by spectral analysis. The possibility of using the germinating mung beans (Vigna radiata). Linn, for rapid and inexpensive and preliminary screening of drugs exhibiting cytotoxic properties has been recently reported. The newly synthesized compounds were evaluated for antimitotic activity and were found to have minimal activity compared to standard. All the compounds showed dose dependant activity.

Keywords: s- triazine, anti mitotic activity.

INTRODUCTION

Cytotoxic properties of plant extracts and drugs being developed for cancer treatment are usually evaluated by a variety of *in vivo* and *in vitro* tests carried out in animal or plant based models [1]. Despite the current availability of a multiplicity of anticancer agents, there is a continuous search for new compounds that may be more effective and safe. However, there is greater emphasis on *in vitro* studies for the initial screening that is followed by validation in an animal model. The possibility of using the germinating mung beans (*Vigna radiata)*. Linn, for rapid and inexpensive and preliminary screening of drugs exhibiting cytotoxic properties has been recently reported [1,2]. In the present study we have evaluated the response of germinating mung beans, *Vigna radiata* (syn. *Phaseolus aureus*) to newly synthesized derivatives of s-triazines and standard drug cisplatin.

To randomly explore the novel compounds, our idea was to combine cyanuric chloride with amine substituted acetophenone and bromination of the ketone group followed by cyclization with urea/ thiourea/ thiosemicarbazole/ amino guanidine. HCl/ acetamide/ benzamide to synthesize the respective derivatives. Substituted s-triazine derivatives remain attractive, with their significant biological activities [3] like

antibacterial, fungicidal, antiretroviral, antiviral, antiulcer, antiarthritic, local anesthetic, anticonvulsant, algaecide and disinfectant, hypoglycemic, analgesic, sedative, anthelmintic, antitubercular, anticancer [4], antitubulin [5], antitrypanosomal [6], antileishmanial [7], antiinflammatory [8], thymidine phosphorylase inhibitors [9], anti malarial [10], anti tmv [11], phosphorescent organic light emitting devices [12], electron transport-type host materials for highly efficient green phosphorescent OLEDs [13].

MATERIALS AND METHODS

All the melting points were taken in open capillary tube and are uncorrected. The purity of the compounds was checked routinely by TLC using silica gel coated plates and spots were visualized by exposing the dry plates in iodine vapors. IR spectra (λ_{max} in cm^{-1}) were recorded on FT-IR-Spercle Elmer DHF1FT-IR using KBr technique. The ^{1}HNMR and ^{13}C NMR spectra of the compounds were carried out in Bruker AMX 400 MHz NMR instrument using $CDCl_3$ or DMSO-d_6 as solvent and TMS as internal reference (chemical shifts in δ ppm). The mass spectra of the compounds were carried out in Agilent 1100 series LC-MSD.

Synthesis of 4,6- dichloro-N- (3 or 4- substituted phenyl)- 1,3,5-triazin-2-amine 1-(3 or 4 substituted-(4,6-dichloro-1,3,5-triazin-2-ylamino) phenyl) ethanone (1): Amine substituted acetophenone (0.01 mol) was added slowly to cyanuric chloride (0.01 mol) in acetone (30 ml) with constant stirring over a period of 4 hr at 0 to 5^0 C. Then, sodium carbonate (0.005 mol) dissolved in water (10 ml) was added drop wise to neutralize HCl evolved during the reaction. Finally, the contents were poured into crushed ice. The solid was separated out by filtration and washed with water. The product is dried, recrystallized from alcohol to give the product (**1**).

Synthesis of 1-(3 or 4 substituted-(4,6-dichloro-1,3,5-triazin-2-ylamino)phenyl)-2-bromoethanone (2): To a solution of compound **1** (0.095 mol) in 150 ml of glacial acetic acid, bromine (0.1 mol) in 20 ml glacial acetic acid was added with stirring for 0.5 hr at room temperature. The mixture was then warmed to decompose an addition product. The mixture was heated for 15 min on a water bath to expel most of the hydrogen bromide, cooled and filtered. It was then poured into ice cold water and the solid separated out was filtered, washed with water and dried. The product was washed with ether and recrystallized from alcohol.

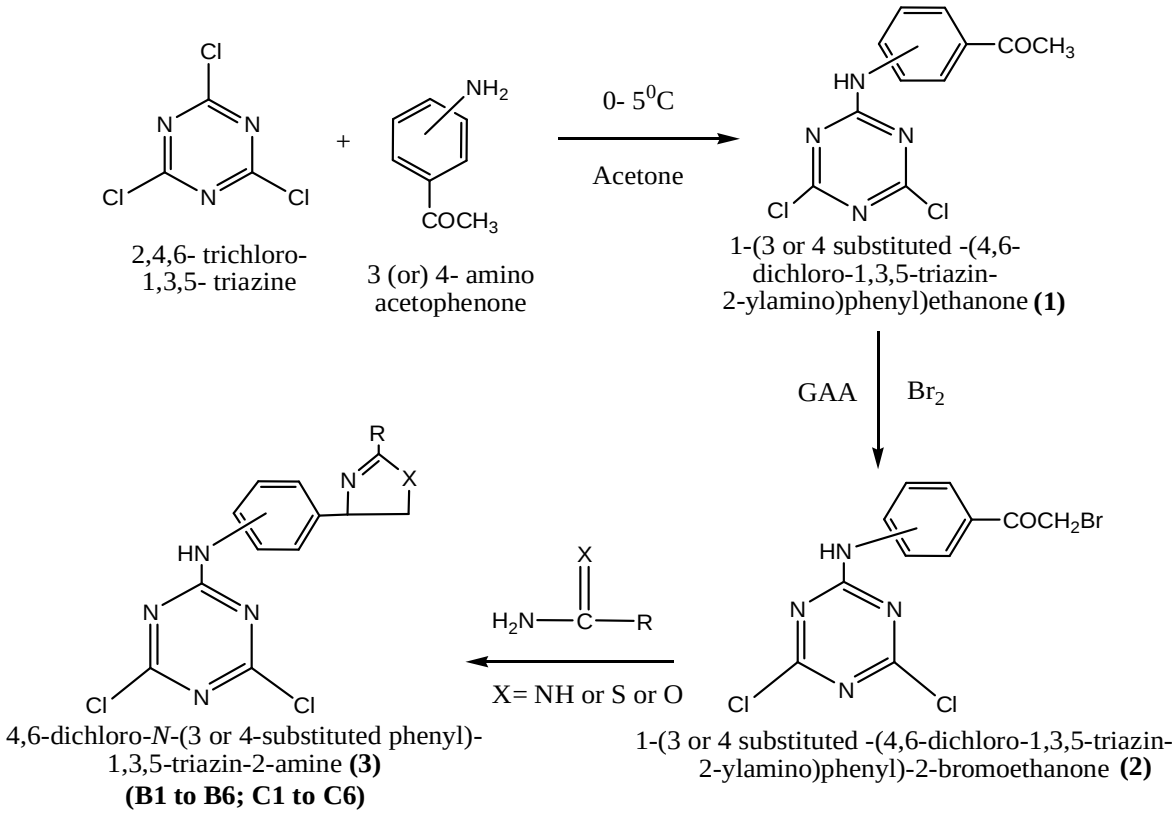
Synthesis of 4,6- dichloro- N- (3 or 4-substituted phenyl)-1,3,5- triazin-2-amine (3):A suspension of compound **2** (0.056 mol) in 15 ml of hot ethanol was treated with urea/ thio urea/ thio semicarbazole/ amino guanidine. HCl/ acetamide/ benzamide (0.06 mol) a mild exothermic reaction took place, gave a clear solution that soon deposited as crystals. The deposit was removed, washed with ethanol and then boiled with water containing sodium acetate which yielded the derivative compound **3 (B1 to B6** and **C1 to C6)**. The product was recrystallized with absolute ethanol. The characterization data of the compounds is shown in table 1.

Table 1: Characterization data of the synthesized s-Triazine derivatives

Compound code	X	R	Mol. formula	Mol. Weight (g mol^{-1})	Melting point (^{0}C)	% yield	R_f value*
B1	O	NH_2	$C_{12}H_{10}Cl_2N_6O$	325.15	170-178	49.32	0.53
B2	S	NH_2	$C_{12}H_{10}Cl_2N_6S$	341.22	260-264	17.25	0.55
B3	S	$NHNH_2$	$C_{12}H_{11}Cl_2N_7S$	356.23	180-183	54.16	0.46
B4	O	CH_3	$C_{13}H_{11}Cl_2N_5O$	324.17	164-170	54.54	0.57
B5	O	C_6H_5	$C_{18}H_{13}Cl_2N_5O$	386.23	168-170	37.03	0.59

B6	NH	$NHNH_2$	$C_{12}H_{12}Cl_2N_8$	339.18	166-168	46.95	0.50
C1	O	NH_2	$C_{12}H_{10}Cl_2N_6O$	325.15	240-242	46.94	0.60
C2	S	NH_2	$C_{12}H_{10}Cl_2N_6S$	341.22	180-185	45.58	0.56
C3	S	$NHNH_2$	$C_{12}H_{11}Cl_2N_7S$	356.23	248-250	48.34	0.90
C4	O	CH_3	$C_{13}H_{11}Cl_2N_5O$	324.17	255-258	38.46	0.55
C5	O	C_6H_5	$C_{18}H_{13}Cl_2N_5O$	386.23	280-284	23.52	0.61
C6	NH	$NHNH_2$	$C_{12}H_{12}Cl_2N_8$	339.18	292-294	18.66	0.54

*mobile phase = Ethylacetate: methanol (8:2)



Scheme 1: Synthetic route to title compounds

Spectral data of the synthesized s- triazine derivatives

B1: IR (in KBr) υ, cm^{-1}: 1083.73 (Ar C –Cl, ;Str), 1159.90 (secondary N –C ;Str), 1293.75 (Ar N ;Str), 1531.95 (primary N –H ;Bend), 3301.86 (secondary N –H ;Str), 3399.45 (primary N –H ;Str)
^{1}HNMR (in DMSO d_6) δ, ppm: 4.47 (d, 2H, $-CH_2$), 5.10 (t, 1H, CH), 5.63 (s, 3H, -NH, $-NH_2$), 7.71 (d, 1H, Ar- H), 7.79 (t, 1H, –CH), 8.2 (d, 1H, Ar –H), 8.32 (s, 1H, Ar –H)
C^{13}NMR: (δ in DMSO, ppm): 65.26 (^{11}C), 66.36 (^{10}C), 120.18 (^{7}C), 124.30 (^{8}C), 125.04 (^{4}C), 129.50 (^{5}C), 137.51 (^{6}C), 152.65 (^{9}C), 163.93 (^{12}C), 169.84 (^{1,3}C), 171.92 (^{2}C)
B2: IR (in KBr) υ, cm^{-1}: 1044.67 (Ar C –Cl, ;Str), 1172.49 (secondary N –C ;Str), 1339.34 (Ar N ;Str), 1505.91 (primary N –H ;Bend), 3285.19 (secondary N –H ;Str), 3771.91 (primary N –H ;Str)

^{1}HNMR (in DMSO d_6) δ, ppm: 3.32 (d, 2H, -CH_2), 5.10 (t, 1H, -CH), 5.53 (s, 3H, -NH), 7.32- 7.41 (m, 2H, Ar –H), 7.51- 7.93 (m, 2H, Ar –H)
B3: IR (in KBr) υ, cm^{-1}: 1018.61 (Ar C –Cl, ;Str), 1217.78 (secondary N –C ;Str), 1308.08 (Ar N ;Str), 1535.71 (Aliphatic primary N –H ;Bend), 3061.12 (secondary N –H ;Str)
^{1}HNMR (CDC13) δ, ppm: 3.31 (d, 2H, -CH_2), 4.83- 4.86 (t, 1H, –CH), 6.01 (s, 4H, -NH), 7.26- 7.65 (m, 4H, Ar –H)
B4: IR (in KBr) υ, cm^{-1}: 1015.04 (Ar C –Cl, ;Str), 1160.06 (C –O –C ;Asymm. str), 1218.65 (secondary N –C ;Str), 1296.34 (Ar N ;Str), 3303.24 (secondary N –H ;Str)
^{1}HNMR (CDC13) δ, ppm: 2.64 (s, 3H, -CH_3), 4.2 (d, 2H, -CH_2), 5.23 (t, 1H, -CH), 7.55- 7.65 (m, 2H, Ar-H), 7.79- 7.87 (m, 2H, Ar –H)
B5: IR (in KBr) υ, cm^{-1}: 1014.54 (Ar C –Cl, ;Str), 1159.54 (C –O –C ;Asym. Str), 1219.03 (secondary N –C ;Str), 1295.90 (Ar N ;Str), 3302.14 (secondary N –H ;Str)
^{1}HNMR (CDC13) δ, ppm: 4.47 (d, 2H, -CH_2), 5.24 (t, 1H, -CH), 7.51- 7.58 (m, 5H, Ar –H), 7.66- 7.68 (m, 2H, Ar –H), 7.79- 7.87 (m, 2H, Ar –H)
MS: m/z 385 (M –)
B6: IR (in KBr) υ, cm^{-1}: 1010.86 (Ar C –Cl, ;Str), 1223.75 (secondary N –C ;Str), 1322.32 (Ar N ;Str), 1549.62 (primary N –H ;Bend), 3267.92 (secondary N –H ;Str), 3775.21 (primary N –H ;Str)
^{1}HNMR (MeOD) δ, ppm: 4.21 (d, 2H, -CH_2), 5.10- 5.27 (t, 1H, -CH), 5.63 (s, 5H, –NH), 7.40- 7.75 (m, 2H, Ar –H), 7.79 (s, 2H, Ar –H)
C1: IR (in KBr) υ, cm^{-1}: 1062.19 (Ar C –Cl, ;Str), 1182.60 (secondary N –C ;Str), 1322.03 (Ar N ;Str), 1548.98 (primary N –H ;Bend), 3298.90 (primary N –H ;Str)
^{1}HNMR (in DMSO d_6) δ, ppm: 4.20 (d, 2H, -CH_2), 5.10- 5.16 (t, 1H, -CH), 5.42 (s, 3H, –NH), 7.72- 7.84 (m, 4H, Ar –H)
C2: IR (in KBr) υ, cm^{-1}: 1063.29 (Ar C –Cl, ;Str), 1184.76 (secondary N –C ;Str), 1326.32 (Ar N ;Str), 1554.87 (primary N –H ;Bend), 3298.81 (secondary N –H ;Str), 3763.57 (primary N –H ;Str)
^{1}HNMR (in DMSO d_6) δ, ppm: 3.76 (d, 2H, -CH_2), 4.76 (s, 2H, –NH), 6.08 (t, 1H, -CH), 7.75- 7.84 (m, 4H, Ar –H)
C3: IR (in KBr) υ, cm^{-1}: 1012.71 (Ar C –Cl, ;Str), 1224.75 (secondary N –C ;Str), 1323.12 (Ar N ;Str), 1550.16 (primary N –H ;Bend), 3137.23 (primary N –H ;Str), 3266.91 (secondary N –H ;Str)
^{1}HNMR (in DMSO d_6) δ, ppm: 3.42 (d, 2H, -CH_2), 4.47- 4.52 (t, 1H, –CH), 5.15 (s, 4H, -NH), 7.76- 8.13 (m, 4H, Ar –H)
C4: IR (in KBr) υ, cm^{-1}: 1011.76 (Ar C –Cl, ;Str), 1165.30 (secondary N –C ;Str), 1322.08 (Ar N ;Str), 1384.37 (-CH_3 ;Sym. bend), 1425.40 (-CH_3 ;Asym. bend), 3268.07 (secondary N –H ;Str)
^{1}HNMR (CDC13) δ, ppm: 2.0 (s, 3H, -CH_3), 4.01 (d, 2H, -CH_2), 5.30 (t, 1H, -CH), 6.63 (s, 1H, -NH), 7.68- 8.19 (m, 4H, Ar –H)
C5: IR (in KBr) υ, cm^{-1}: 1011.53 (Ar C –Cl ;Str), 1184.84 (secondary N –C ;Str), 1322.80 (Ar N ;Str), 3268.29 (secondary N –H ;Str)
^{1}HNMR(in DMSO d_6) δ, ppm:4.22 (d,2H,–CH_2),5.14 (t, 1H, –CH),7.72- 8.13(m, 9H, Ar-H)
C6: IR (in KBr) υ, cm^{-1}: 1010.86 (Ar C –Cl ;Str), 1223.75 (secondary N –C ;Str), 1322.32 (Ar N ;Str), 1549.62 (Aliphatic primary N –H ;Bend), 3267.92 (secondary N –H ;Str), 3775.21 (primary N –H ;Str)
^{1}HNMR (CDC13) δ, ppm: 3.50 (d, 2H, -CH_2)**,** 5.50 (t, 1H, –CH), 6.63 (s, 5H, –NH), 7.68- 8.19 (m, 4H, Ar-H)
MS: m/z 340 (M +)

Anti Mitotic Activity [1,2] : Mung beans used in this study were obtained from the local market. Drug solutions of different concentrations (1 mg ml^{-1}, 500 µg ml^{-1}, 100µg ml^{-1}) were prepared and mung beans of equal weight are weighed and soaked in each concentration respectively for 6 h. Standard is prepared with the same concentrations, with the anti cancer agent cisplatin. Control is prepared with mung beans soaked in tap water for 6h. The water or the drug solution (test/ standard) was drained and the seedlings were kept moist (either with tap water or the drug solutions in a covered Petri dish) until the radicle in the

control group had grown to 1.0 - 3 cm (time 0, T_0). At T_0, the weight of seedlings and length of radicle were recorded both in the control and test groups. The seedlings were maintained at room temperature under moist conditions for an additional period of 48 h (T_{48}). The weight of the seedlings was measured again at T_{48}. Percentage inhibition is calculated using the formula:

$$\% \text{ inhibition} = \frac{\text{Wet weight of seeds in control group} - \text{Wet weight of seeds in sample group}}{\text{Wet weight of seeds in control group} - \text{Wet weight of seeds in standard group}} \times 100$$

The stalk length could not be measured since it is bent. The change in weight and gain in radicle length between T_0 and T_{48} were calculated. The seeds that did not germinate were simply weighed and no other parameters could be measured on these seeds.

RESULTS AND DISCUSSION

All the synthesized compounds were evaluated for anti mitotic activity using mung beans (*Vigna radiata)* at concentration level 100 µg ml^{-1}, 500 µg $ml^{-1}$and 1000 µg ml^{-1}. Cisplatin was used as standard and tap water is control. All the reports were shown in the table 2 (a-b**)** and fig. 1- 3 (a-d). Out of the derivatives B1 to B6, B6 was found to have highest inhibition of 64.8% at 1000 µg ml^{-1} concentration, followed by B2 and B3. Out of the derivatives C1 to C6, C4 was observed to have the highest inhibiting activity of 60.73% at 1000 µg ml^{-1}concentration, followed by C3 and C5. The standard drug cisplatin was found to have 100 % inhibition at all the three concentrations. All the compounds showed dose dependant activity.

Table 2 (a): Anti mitotic activity studies of the synthesized s- Triazine derivatives

Comp code	Wt. of dry beans (g)	Wt. of soaked beans (gm)			Wt. of beans at (T_0) (gm)			Length of beans at (T0) (cm)		
		100 µg ml^{-1}	500 µg ml^{-1}	1000 µg ml^{-1}	100 µg ml^{-1}	500 µg ml^{-1}	1000 µg ml^{-1}	100 µg ml^{-1}	500 µg ml^{-1}	1000 µg ml^{-1}/
B1	1.80	3.62	4.06	3.87	5.65	5.56	4.85	2.2	1.9	1.3
B2	1.80	4.39	3.86	4.02	5.85	5.38	5.08	2.2	1.9	1.7
B3	1.80	3.71	3.80	3.78	5.78	5.36	4.69	2.5	1.6	1.5
B4	1.80	4.34	3.95	3.91	6.35	5.51	4.95	2.5	1.6	0.8
B5	1.80	4.48	4.15	4.09	6.12	5.89	5.15	2.3	1.7	1.3
B6	1.80	3.64	4.14	3.98	6.68	5.15	4.58	2.5	1.7	1.9
C1	1.80	3.99	3.97	3.68	7.01	5.47	4.65	2.0	1.7	1.6
C2	1.80	3.68	4.52	4	6.71	6.05	5.1	2.3	1.9	1.9
C3	1.80	4.17	4.27	4.32	6.21	5.79	5.25	2.4	1.8	1.3
C4	1.80	3.94	4.08	3.9	6.98	4.51	4.95	2.3	1.8	1.5
C5	1.80	4.06	4.30	4.1	6.16	5.87	5.15	2.0	1.9	1.7
C6	1.80	3.65	4.07	4.07	6.68	5.57	5.1	2.5	1.7	1.3
Std	1.80	4.01	3.42	3.84	4.34	3.86	4.04	0	0	0
Ctrl	1.80	4.12			5.90			3		

Table 2 (b): Anti mitotic activity studies of the synthesized s- triazine derivatives

Comp code	Wt. of beans after 48 hrs (T_{48}) (gm)			% inhibition at (T_{48}) ±SEM^n		
	100 µg/ml	500 µg/ml	1000 µg/ml	100 µg/ml	500 µg/ml	1000 µg/ml
B1	12.00	10.75	9.25	1.31 ±1.5	17.41 ± 1.8	36.21 ± 1.2
B2	10.00	8.75	6.25	27.6 ± 1.6	43.22 ± 0.9	64.33 ± 0.9
B3	10.50	9.50	7.25	21.08 ± 1.9	33.5 ± 1.2	61.62 ± 1.9
B4	11.25	9.50	7.5	11.19 ± 1.3	33.5 ± 1.6	58.44 ± 1.3
B5	10.25	8.75	6.5	24.37 ± 1.2	43.22 ± 0.9	60.11 ± 1.7
B6	9.75	8.25	7	30.96 ± 1.7	49.67 ± 0.8	64.8 ± 1.5
C1	12.00	9.98	8.75	1.31 ± 1.3	27.35±1.4	42.56±1.3
C2	9.98	9.50	8.25	27.93±1.6	33.54±1.9	48.91±1.9
C3	10.32	9.25	7.75	23.45±1.9	36.77±1.5	55.27±0.9
C4	11.50	9.62	7.32	7.90 ±1.6	32.00 ± 1.4	60.73±0.8
C5	11.75	8.53	7.75	4.611±1.3	46.06±1.7	55.27±1.5
C6	10.75	9.09	8	17.78±1.7	38.83±0.9	52.09±1.7
Std	4.51	4.35	4.23	100	100	100
Ctrl	12.10			0		

n= triplicate SEM = Standard Error Mean Std: Cisplatin Ctrl: Tap water

Figure 1: Length of the radical of the mung beans at (T0)

Figure 2: Mung beans at (T48)

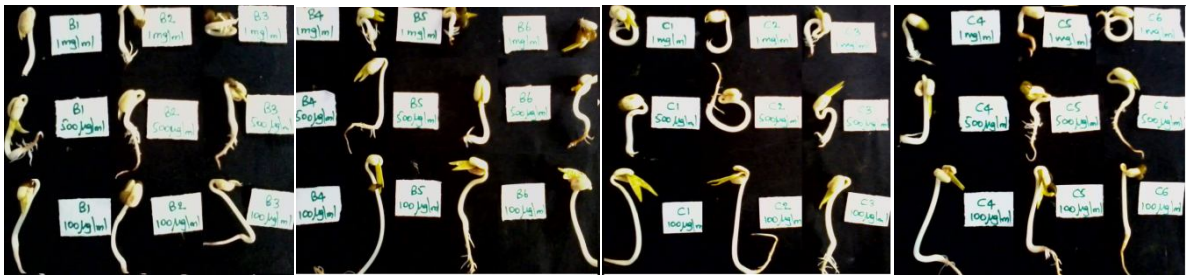
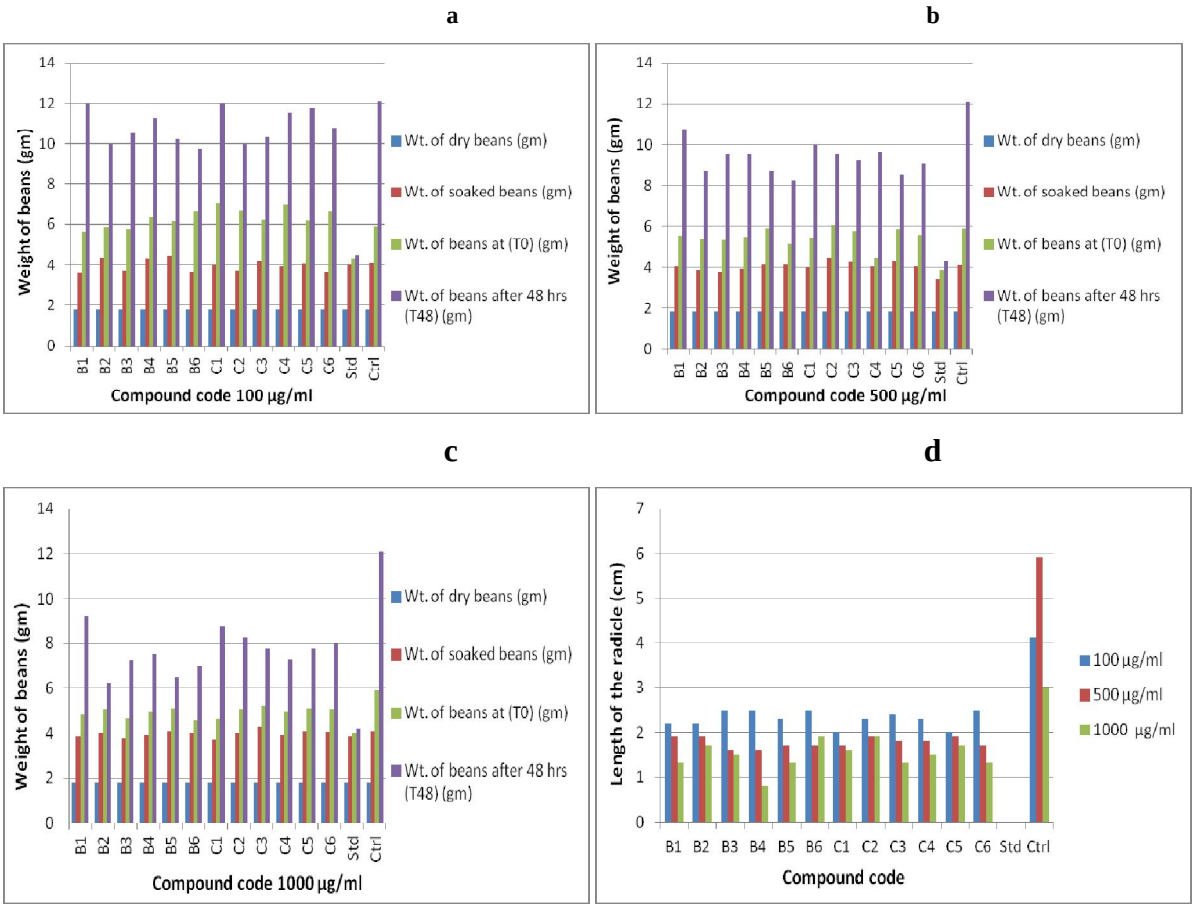


Figure 3 (a-d): Antimitotic activity studies of the synthesized s- Triazine derivatives



APPLICATIONS

The determination of anti mitotic activity using mung beans is useful in screening the anti mitotic activity of synthesized substituted s- triazine derivatives.

CONCLUSIONS

Substituted s-triazine derivatives, B1 to B6 and C1 to C6 were synthesized and characterized for their structure elucidation. All the derivatives were tested for anti mitotic activity using the germinating mung beans (*Vigna radiata*. Linn), and were found to have minimal activity compared to standard. All the compounds showed dose dependant activity.

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