ejpmr, 2018, 5(11), 259-267

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

SYNTHESIS, CHARACTERIZATION AND ANTICANCER EVALUATION OF NOVEL SUBSTITUTED-[1, 3, 4]-THIADIAZOLE DERIVATIVES

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Article Received on 01/09/2018 **Article Revised on 22/09/2018** **Article Accepted on 13/10/2018**

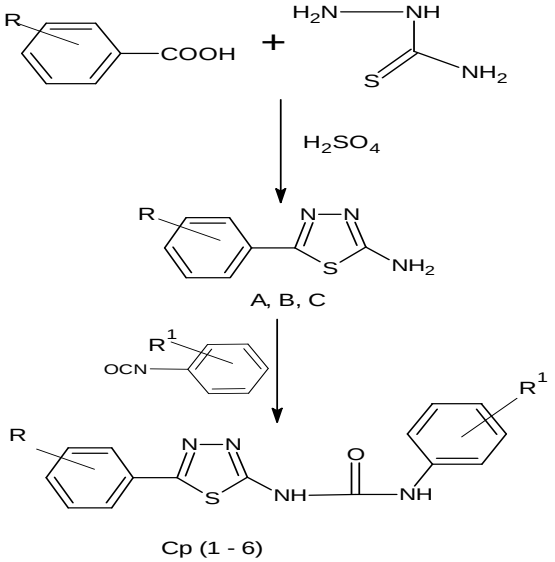
ABSTRACTS

A series of 1-(substituted phenyl)-3-[5-(2-substitutedphenyl)-1, 3, 4-thiadiazol-2yl] urea derivatives Cp1-Cp6 have been prepared by reaction of 5-(substituted phenyl)-2-amino - [1, 3, 4]-thiadiazole and an appropriate phenyl isocyanate. Further *meta* and *para*- chloro phenyl derivatives were synthesized in order to study the effect of these substituents on antitumor activity. Structures of these compounds were established by IR, 1HNMR, Mass spectroscopy and CHN analysis. All six compounds were used for anticancer activity by using EAC cells (0.2 ml of 2 x 10^6 cells/mice) panel. Among the compounds tested, *N-(4-chlorophenyl)-3-[5-(2-hydroxy phenyl)-1,3,4-thiadiazol-2-yl]urea*,Cp2 was found to be the most active candidate of the series at five dose level screening with degree of selectivity toward EAC cancer cell line.

KEYWORDS: Synthesis, 1, 3, 4-thiadiazole, anti tumor agents, EAC cell.

1. INTRODUCTION

Malignancy is a noteworthy issue all through the world and is a class of sicknesses in which cell, or a gathering of cells show uncontrolled development, intrusion, and once in a while metastasis. Ebb and flow authoritative opinion expresses that growth is a multi-quality, multi-step sickness beginning from a solitary strange cell (clonal origin) with a changed DNA-arrangement (transformation). Uncontrolled expansions of these unusual cells are trailed by a moment transformation prompting the somewhat atypical stage. Progressive rounds of transformation and specific extension of these cells brings about the arrangement of a tumor mass. It influences individuals at all ages with the danger of most sorts expanding with age. American Cancer Society distributed some factual information in 2013, that growth caused around 23.3% of every single human passing and is the second driving reason for mortality in developed countries.[1] It is imperative to grow new anticancer medications, in light of the fact that the greater parts of the chemotherapeutic medications are dangerous at ideal measurement and are costly. This reality elevated us to blended new mixes. Substituted 1,3,4-thiadiazoles have pulled in extensive enthusiasm inferable from their wide range natural movement, including antimicrobial, antituberculosis, soporific, antithrombotic, anticonvulsant, antihypertensive, anti-inflammatory and antiulcer properties. And furthermore different 1, 3, 4-thiadiazole subsidiaries have been found to show noteworthy anticancer properties against extensive variety of growths.[2, 3, 4] In the present work, we have concentrated on 1-(substituted phenyl) - 3-[5-(2-substitutedphenyl)- 1, 3, 4-thiadiazol-2yl] urea because of their huge pharmacological profiles with the expectation that the focused on subsidiary will demonstrate higher viability with the lesser poisonous quality and we report the in-vivo anticancer and danger investigations of the six 1, 3, 4-thiadiazole subordinates, in EAC cells. Amid the most recent couple of decades, an impressive consideration has been given to the combination of 1, 3, 4-thiadiazole subordinates show assorted pharmacological exercises conceivably because of quality of N=C=S moiety. Besides, mixes with thiadiazole ring have created as antibacterial[5], calming[6], fungicidal[7] and anticancer specialists.[8] Their capacity to hinder tumor advancement has been recorded in various *in-vitro*[9,10,11] and *in-vivo*[12] thinks about. Their mechanism of activity contrasts, contingent upon the kind of change of the thiadiazole ring.[13, 14, 15] Likewise found that 2-amino-1, 3, 4-thiadiazole are utilized as antitumour medications and their acetazolamide subordinates demonstrate diuretic movement and some of their subsidiaries utilized as carbonic anhydrase inhibitors. The subsidiaries indicated significant guarantee as solutions for contamination in the gastrointestinal tract. 1, 3, 4-thiadiazoles are additionally utilized as pesticidal, herbicidal, amoebicidal, CNS depressant, and antiviral.



Scheme 1.

2. MATERIALS AND METHODS

2.1. Experimental

Aromatic carboxylic acids and thiosemicarbazide were subjected to customary strategies (Scheme 1). Liquefying purposes of all blended mixes were dictated by open capillary tube strategy communicated in ^{0}C and the information were considered as uncorrected. Virtue of all synthesized compounds was observed by thin layer chromatography technique (0.2 mm thickness of silica gel GF plates) and iodine was utilized as visualizing agent. Ultraviolet visible spectroscopy analysis has been done in UV– SPECORD® 50 PLUS-232H1004 UV-visible spectrophotometer. IR spectra were recorded on THERMO NICOLET iS10 FT-IR spectrometer utilizing KBr disc technique in the scope of 4000-400 cm^{-1}. C, H, N estimation was acknowledged on a Euro EA (CHN) basic analyser. FT-IR and Elemental examination was completed at Central Analytical Instrument Facility (CAIF), the 1H-NMR spectra were recorded at 400-MHz on BRUKER spectrometer in dimethylsulfoxide-d6 as dissolvable and tetramethylsilane (TMS) as interior standard and synthetic move was communicated in δ (delta esteems).The mass spectra were recorded on SHIMADZU 2010A LC-MS spectrometer.

2.1.1 Synthesis of 1-(substituted phenyl)-3-[5-(2-substitutedphenyl)-1, 3, 4-thiadiazol-2yl] urea, Cp1-Cp6.

1-(substituted phenyl)-3-[5-(2-substitutedphenyl)- 1, 3, 4-thiadiazol-2yl]-urea were acquired to scheme 1. *m* and *p*-chlorophenyl isocyanate (2 mmole) was gradually added to an all around blended arrangement of 2-amino-5(substituted phenyl)- 1, 3, 4-thiadiazole (2 mmole), 5-(substituted phenyl)- 2-amino - [1, 3, 4]-thiadiazole (A, B and C) in dry acetonitrile (12 ml). After total expansion, the response blend was kept for 30 min at room temperature; the blend was warmed on water shower under reflux until finish (TLC location) of the reaction. The residue was cooling; the precipitated was isolated and recrystallized from ethanol to give the crystalline product. Purity of the mixes was investigated by n-Hexane: methanol (9:1) as mobile phase.[16] The spectral information of the representative compounds were as per the following.

N-(3-chlorophenyl)-3-[5-(2-hydroxy phenyl)-1,3,4-thiadiazol-2-yl]urea* (Cp1)**: % yield 78; Mp 202-204^{0}C; Rf 0.745; ^{1}H NMR (400MHz, DMSO-*d*6) δ 6.88-7.31 (m, 4H, ArH); 7.47-7.79 (m, 4H, ArH); 10.10 (s, 1H, OH); 5.89 (s, 2H, CH_2-NH); 5.84 (s, 1H, CONH); **IR(cm^{-1}):**3500.02 (O-H, st.); 667.61 (C-S-C, st.); 3424.54 (N-H, st.); 1653.93 (C=N, st.); 1745.65 (C=O, st.); 3272.44 (CON-H, st.); 1440.52 (aryl C=C, st.); 3037.58 (aryl C-H, st.); 771.20 (C-Cl); ***(m/z) 346(M$^+$); **λ-max** 359; (CHN analysis) Calculated: 51.95, 3.20, 16.16 ; Found: 50.95, 3.12, 16.67.

N-(4-chlorophenyl)-3-[5-(2-hydroxy phenyl)-1,3,4-thiadiazol-2-yl]urea* (Cp2)**: %yield 85; Mp 212-214^{0}C; Rf 0.619; ^{1}H NMR (400MHz, DMSO-*d*6) δ 7.30-7.56 (m, 4H, ArH); 7.91-7.94 (m, 4H, ArH); 10.15 (s, 1H, OH); 6.15 (s, 2H, CH_2NH); 6.13 (s, 1H, CONH);) ; **IR(cm^{-1}):** 3510.17 (O-H, st.); 682.01 (C-S-C, st.); 3411.75 (N-H, st.); 1624.11 (C=N, st.); 1491.79 (C-N, st.); 1701.17 (C=O, st.); 3295.76 (CON-H, st.); 1425.36 (aryl C=C, st.); 2985.06 (aryl C-H, st.); 761.30, 775.30 (C-Cl); ***(m/z) 347(M+1)$^+$; **λ-max** 270; (CHN analysis) Calculated: 51.95, 3.20, 16.16; Found: 51.78, 3.22, 16.06.

N-(3-chlorophenyl)-3-[5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl] urea* (Cp3)**: %yield 90;Mp 170-172^{0}C; Rf 0.594; ^{1}H NMR (400MHz, DMSO-*d*6) δ 7.00-7.31 (m, 4H, ArH); 7.52-7.94 (m, 4H, ArH); 6.00 (s, 2H, CH_2N<u>H</u>); 5.90 (s, 1H, CONH); **IR(cm^{-1}):** 763.27, 790.17 (C-Cl); 683.56 (C-S-C, st.); 3372.75 (N-H, st.); 1478.97 (C-N, st.); 3289.59 (CO N-H, st.); 1455.78 (aryl C= C, st.); 3039.79 (aryl C-H, st.); ***(m/z) 365(M$^+$); **λ-max** 260; (CHN analysis) Calculated: 49.33, 2.76, 15.34; Found:49.30, 2.96, 15.14.

N-(4-chlorophenyl)-3-[5-(4-chlorophenyl)-1, 3,4-thiadiazol-2-yl]urea* (Cp4)**:%yield 85;Mp 245-248^{0}C; Rf 0.595; ^{1}H NMR (400MHz, DMSO-*d*6) δ 7.45-7.55 (m, 4H, ArH); 7.90-7.94 (m, 4H, ArH); 5.54 (s, 2H, CH_2N<u>H</u>) ; 5.53 (s, 1H, CON<u>H</u>); **IR(cm^{-1}):** 712.80, 762.25 (C-Cl); 640.03, 682.10 (C-S-C, st.); 3372.75 (N-H, st.); 1491.67 (C-N, st.); 1681.45 (C=O, st.); 3193.61 (CON-H, st.); 1424.57 (aryl C=C, st.); 3093.83 (aryl C-H, st.); ***(m/z) 364(M-1)$^+$; **λ-max** 313; (CHN analysis) Calculated: 49.33, 2.76, 15.34 ; Found:48.73, 2.60, 14.94.

N-(3-chlorophenyl)-3-[5-(2-nitrophenyl)-1, 3, 4-thiadiazol-2-yl]urea* (Cp5)**: %yield 66; Mp 224-216 ^{0}C; Rf 0.739;^{1}H NMR (400MHz, DMSO-*d*6) δ 7.60-8.45 (m, 4H, ArH); 7.00-7.31 (m, 4H, ArH); 5.99 (s, 2H, CH_2N<u>H</u>); 5.78 (s, 1H, CON<u>H</u>); **IR(cm^{-1}):** 790.03 (C-Cl); 687.92 (C-S-C, st.); 3424.01 (N-H, st.); 1700.38 (C=O, st.); 3158.73 (CON-H, st.); 1406.71 (aryl C=C, st.); 1535.07 (NO_2); ***(m/z) 374(M-1)$^+$; **λ-max** 308; (CHN

analysis) Calculated: 47.94, 2.68, 18.64; Found: 47.34, 2.98, 17.66.

N-(4-chlorophenyl)-3-[5-(2-nitrophenyl)-1,3,4-thiadiazol-2-yl]urea*(Cp6)**: %yield 78 ;Mp 230-232^{0}C; Rf 0.519; ^{1}H NMR (400MHz, DMSO-*d*6) δ 7.03-7.62 (m, 4H, ArH); 7.70-8.29 (m, 4H, ArH); 6.12 (s, 2H, CH_2NH); 5.02 (s, 1H, CONH) ; **IR(cm^{-1}):** 752.52, 776.42 (C-Cl); 624.66, 691.03 (C-S-C, st.); 3423.95 (N-H, st.); 1490.78 (C-N, st.); 1600.81 (C=N, st.); 1690.50 (C=O, st.); 3159.61 (CON-H, st.); 1438.89 (aryl C=C, st.); 2979.91 (aryl C-H, st.); 1535.91 (NO_2); ***(m/z) 375(M^+), 374(M-1)$^+$; **λ-max** 292; (CHN analysis) Calculated: 47.94, 2.68, 18.64; Found:46.94, 1.98, 18.60.

2.2. Biological evaluation

2.2.1. *In-vivo* anticancer activity-EAC liquid tumor model

2.2.1.1. Selection of animals: Swiss albino mice (eight weeks old, 25-30 g) inbred at Jadavpur University, Kolkata, India were used in the study. The animal experiments were performed as per the regulations of the Institutional Animal Ethics Committee (AEC/PHARM/1502/11/2015).

2.2.1.2. Acute toxicity study: Acute toxicity studies were were led to decide the safe dose by up and down stair case method. Animals were divided into groups consisting of 6 animals in each group. Drug suspension was readied utilizing 0.25 % CMC. Test compounds were given orally as suspension at a solitary dosage of 400, 500,750, 850, 1000 and 1250 mg/kg body weight. Control group was treated with 0.25 % CMC. Animals were continuously observed for the initial 72 h and after that for 7 days for behavioral changes, toxicity and mortality.[19] The animals treated with 750, 850 and1000 mg/kg of the test samples were found be safe. In this way, 1/10th of the more secure dose was chosen for the activity.

Procedure: The compounds which indicated noteworthy in-vitro anticancer action were additionally chosen for *in-vivo* anticancer activity.[31,32,33] The animals were weighed and divided into nine groups, each containing of 6 animals. EAC cells (0.2 ml of 2 x 10^6 cells/mice) were injected by intraperitoneal route to each mouse of each group with the exception of the normal control (Group I). This was taken as day 0. Treatment with test compounds and 5-flurouracil (standard) were given alternatively for 7 days from day 1. On fourteenth day, after 24 h of the last dose, half the numbers of animals from each group were sacrificed for concentrate the accompanying parameters.

2.2.1.3. Parameters monitored

2.2.1.3.1. % increase in weight as compared to day "0" weight*:* Upon weighing the animals on the day of inoculation and after once in 2 days of the post inoculation period, the increase in weight was calculated as follows

$$\left(\frac{\text{wt on respective day}}{\text{wt on day 0}} - 1\right) \text{ X } 100$$

2.2.1.3.2. Percentage increase in life span*:* The effect of test compounds on percentage increases in life span was calculated on the basis of mortality of the experimental mice.[17]

$$\text{ILS(\%)} = \left[\frac{\text{Mean survival time of the treated group}}{\text{Mean survival time of the control group}} - 1\right] \text{X } 100$$

Mean survival time (in terms of number of days) = [First Death + Last Death] / 2.

2.2.1.3.3. Tumor weight: The tumor weight was estimated by taking the weight of the mice when the accumulation of the ascetic fluid from peritoneal cavity.

2.2.1.3.4. Tumor volume: The ascitic liquid was gathered from the peritoneal pit. The volume was estimated by taking it in a graduated centrifuge tube.

2.2.1.3.5. Tumor cell count: The ascitic liquid was taken in a WBC pipette and weakened 100 times. At that point a drop of the weakened cell suspension was set on the Neubauer's counting chamber and the quantities of cells in the 64 little squares were tallied.

2.2.1.3.6. Viable and nonviable tumor cell count: The **Viablility** and nonviability of the cell was observed by trypan blue measure. The cells were recolored with trypan blue (0.4 % in typical saline) color. The cells were said to be **viable** cell in the event that it didn't take up the color where as those that took the color were nonviable cell. The quantities of reasonable and nonviable cells were then tallied by utilizing following articulation.
Cell tally = Number of cells × dilution factor /Area × thickness of fluid film.

2.2.1.3.7. Assurance of Protein Content: After examining at 280nm the tubes with critical absorbance were pooled and a quantitative protein assurance was finished by the adjusted biuret (end point test) strategy utilizing the diagnostic reagent kit.[18]

2.2.1.3.8. Hematological parameters[19, 20]: On fourteenth day, in the wake of giving up the animals, blood tests were gathered keeping in mind the end goal to find out the red blood cells (RBC), white platelets (WBC) and Hemoglobin (Hb) counts which are the critical parameters to evaluate the impact of the synthesized compounds on EAC cells bearing tumor mice.

2.2.1.3.9. Evaluation of serum particular markers compounds: Blood was collected from mice via cardiac puncture and were kept for 15 min for coagulating and afterward centrifuged at 5000 rpm for 10 min. The supernatants (serum) were collected and total protein,

glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), Alkaline Phosphatase (ALP) were resolved utilizing the total protein, by utilizing the auto analyzer instrument.[21-24]

2.2.1.3.10. Biochemical methodology: Livers was extracted and quickly solidified in dry-ice and put away at 20^0C. Frozen tissue from each mouse was blended continually for 15 min. before being diluted with 0.5 ml of distilled water. After centrifugation for 10 min. at 1600 g, the light yellow supernatant was isolated from the protein precipitate and was utilized to examine LPO and SOD catalyst movement. All protein action estimations were resolved utilizing a Shimatzu UV-obvious spectrophotometer and were completed in copy. Crude GSH movement data were standardized utilizing lysate expanded to 5 g hemoglobin for every 100 ml lysate. LPO and SOD action was estimated by the technique described by Ohkawa[25] and Kakkar.[26] One unit of action is characterized as the measure of compound important to diminish the rate of cytochrome c decrease to half of maximum at 25^0C and pH 7.8 (utilizing xanthine and xanthine-oxidase as the superoxide source). The action of CAT was controlled by the rate of hydrogen peroxide disappearance measured at 240 nm, as indicated by Aebi.[27] One unit of CAT action is characterized as the measure of compound that breaks down 1µmol hydrogen peroxide for every moment at 25^0C and pH 7.0. The activity of GSH was controlled by the GSH- dependent reduction of t-butyl hydroperoxide, utilizing a modification of the assay depicted by Ellman.[28] This assay depends on NADPH oxidation accompanying with GSH lessening. One unit of GSH activity is characterized as the oxidation of 1 nmol NADPH every moment at 37^0C and pH 7.6.

3. RESULTS AND DISCUSSION

3.1. Chemistry

Synthesis and structural formula of 5-(substituted phenyl)-2-amino - [1, 3, 4]-thiadiazole, (A, B, C) have been described previously.[29] Totally, six novel 2, 5-substituted-1, 3, 4-thiadiazole derivatives were synthesized (Table 1) and characterized by UV, IR, 1HNMR and Mass spectral analysis.

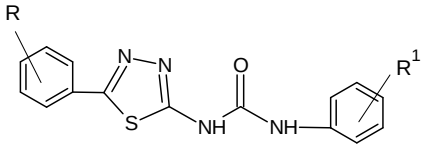
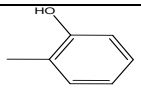
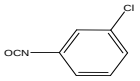
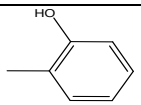
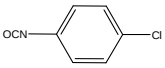
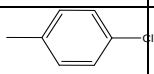
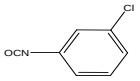
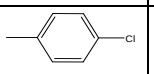
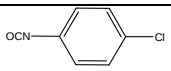
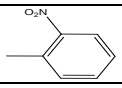
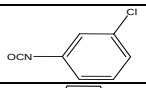
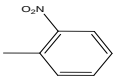
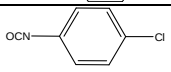


Table 1: List of the 1, 3, 4-thiadiazole derivatives synthesized.

Code	R	R1	Mol. formula	Mol. wt
Cp1			$C_{15}H_{11}ClN_4O_2S$	346.79
Cp2			$C_{15}H_{11}ClN_4O_2S$	346.79
Cp3			$C_{15}H_{10}Cl_2N_4OS$	365.23
Cp4			$C_{15}H_{10}Cl_2N_4OS$	365.23
Cp5			$C_{15}H_{10}ClN_5O_3S$	375.78
Cp6			$C_{15}H_{10}ClN_5O_3S$	375.78

All compounds showed characteristic peaks as expected. The IR spectra of all the 1,3,4-thiadiazole derivatives were recorded in the 667-3510 cm^{-1} range on THERMO NICOLET iS10 FT-IR using KBr pellets. The absorption bands in the regions 1600-1653 cm^{-1} and 1535 cm^{-1} indicated the presence of C=N and NO_2 groups, respectively. The aromatic C=C stretching showed a peak in the region 1406-1455 cm^{-1} and the presence of C-N stretch was observed in the range between 1491-1478 cm-1. Also the absorption bands in the regions 624-691 cm^{-1} and 1681-1745cm^{-1} indicated the presence of C-S-C and C=O groups, respectively. In addition, compounds containing O-H group exhibited a peak at 3510 cm^{-1}.The 1H NMR spectra were recorded on Bruker Avance-400-MHz using *d*-DMSO as solvent. The chemical shifts were reported as parts per million downfield from tetramethylsilane (Me4Si). The protons of the CONH group in the Cp 1-6 series showed a singlet between δ 5.02 - 6.13ppm and aromatic protons near δ 6.88-7.00 and 7.3-8.45, respectively. The results were found

satisfactory. In addition, to support the above data, Mass spectra were recorded on the SHIMADZU 2010A LC-MS. The molecular ion peak of the reference compounds, **Cp1-Cp6** were observed respectively at m/z, 346, 347, 365, 364, 374 and 375 and the mass spectral data of the compounds were satisfactory. Hence, all the above spectral data and UV-visible Spectrum as well as CHN analysis were confirmed the proposed structure of the synthesized compounds.

3.2. Biological evaluation: All the test compounds were taken to test their potency *in vivo* by EAC model. The impacts of the compounds **Cp1-Cp6** on mean survival time, increase in life span, tumor volume, packed cell volume, tumor weight, haematological and liver antioxidant parameters were contrasted with control animals with decide their adequacy. The anticancer potential was assessed by the parameters MST and % ILS. Results appeared in Table 2 affirmed the promising anticancer activity of Cp2 (100mg/kg). It showed increase in life span of 66.66 % which was comparable with standard (79.16 %). It demonstrated increment in life span of 66.66 % which was equivalent with standard (79.16 %). From the above outcomes, it was discovered that among the integrated compound (Cp2) was the most potential compound against tumor.

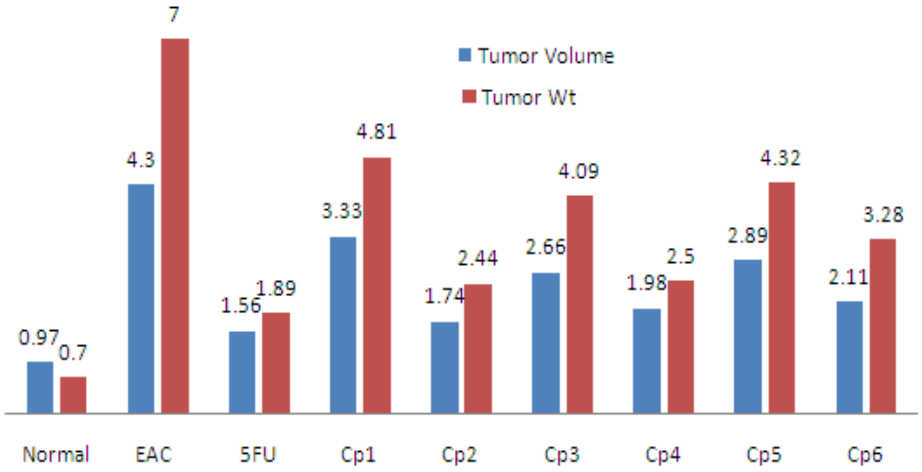
Table 2: Effect of the selected compounds on Mean Survival Time (MST) and % Increase in Life Span (ILS) (mean ± S.E.M, n=6)

Code	MST (days)	%ILS
EAC control	24±2.312	00.00
5-FU	43±0.062*	79.16
Cp1	26±1.071	8.33
Cp2	40±2.806*	66.66
Cp3	30±0.835**	25.00
Cp4	39±1.980*	62.50
Cp5	28±1.523	16.66
Cp6	33±0.067*	37.50

**P < 0.05 **P < 0.01 ***P < 0.001*

On fourteenth day, animals were weighed before and after collecting the peritoneal fluid to calculate the tumor weight.

The outcomes appeared in Figure 1 demonstrated the impact of compound/s on the tumor weight decrease. Among the six tested compounds, Cp2 and Cp4 displayed more lessening in tumor weight. Though, compound Cp1, having 3-chloro phenyl moiety connected with the urea linkage indicates unimportant impact on diminishment of the tumor weight as contrasted and the control. Be that as it may, whatever is left of the other two compounds diminished the tumor weight moderately. The peritoneal liquid which is available in the peritoneal cavity is the immediate hotspot for the tumor cells.[30] In this way, the reduction in the tumor volume shows the anticancer action of the test compounds. In this investigation, compounds Cp2 and Cp4, bearing 2-hydroxy and 4-chloro phenyl substitution at the fifth position and 4-chloro phenyl moiety connected with the urea linkage on the 1,3,4-thiadiazole framework diminished the aggregate tumor volume about the 5-flurouracil treatment and their aggregate tumor volume was estimated as 1.98±0.07 and 1.74±0.86mL, respectively. Though, the 5-flurouracil treated animals demonstrated 4.3±0.096 mL. Notwithstanding, alternate compounds showed direct action when contrasted and the standard. In this manner, the lessening in the tumor volume could fill in as an indication of antitumor action.

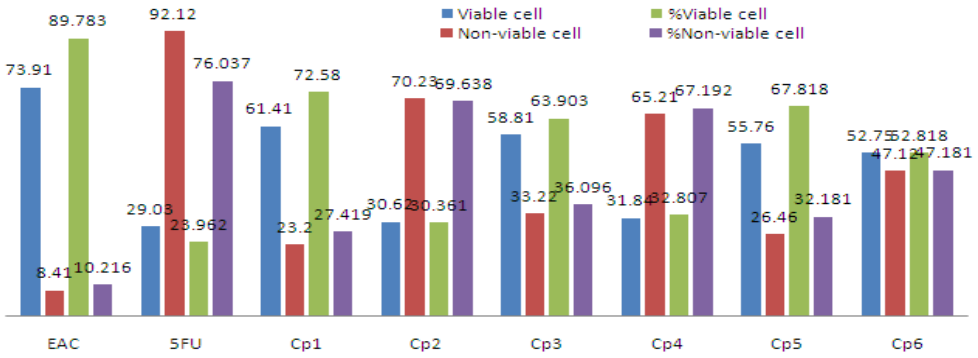


**P < 0.05 **P < 0.01 ***P < 0.001*

Figure1: Comparative analysis of Tumour volume and Tumour weight of the EAC cells bearing mice (mean ± S.E.M, n=6).

The compound Cp2 rose as the most dynamic against the packed cell volume at (mean± SE) 1.10±0.33, as appeared in Figure1.

The outcomes (Figure 2) uncovered that, all the chose compounds were indicated significant increment in the proportion of non-viable to viable cells contrasted with control. Especially, the impact of compound Cp2 on tumor bearing mice was observed to be pretty much equal with that of the 5-flurouracil treatment; the non-viable and viable cell counts of the Cp2 treatment was observed to be 70.23±0.037x 10^6 and 30.62±0.06x 10^6 cells/mouse, individually.5-flurouracil, being the standard, displayed huge anticancer activity with 92.12±0.34 million non-viable cells and 29.03±0.58 million viable cells /mouse.



**P < 0.05 **P < 0.01 ***P < 0.001*

Figure 2: Comparative analysis of viable and nonviable tumor cell countin EAC bearing mice (mean ± S.E.M, n=6).

Further, compound **Cp2**, showed better activity when compared with the rest of the compounds. The viable and non-viable cell count for this compound was calculated as 31.84±0.10 and 65.21±0.33 million cells, respectively. Whereas, the other three compounds such as, Cp-1, 3, 5 and 6 were not found to show great increase in the non-viable cell count.

Table 3: Effect of different test compounds on different biochemical parameter in EAC bearing mice.

Code	Total Protein (gm/ dl)
Normal Saline	17.143±0.02
EAC Control	6.324±0.01$^{€}$
5FU	15.672±0.08*
Cp1	11.501±0.32**
Cp2	13.029±0.085*
Cp3	12.152±1.38*
Cp4	12.741±0.47*
Cp5	11.966±0.068**
Cp6	12.431±0.066*

**P < 0.05 **P < 0.01 ***P < 0.001*

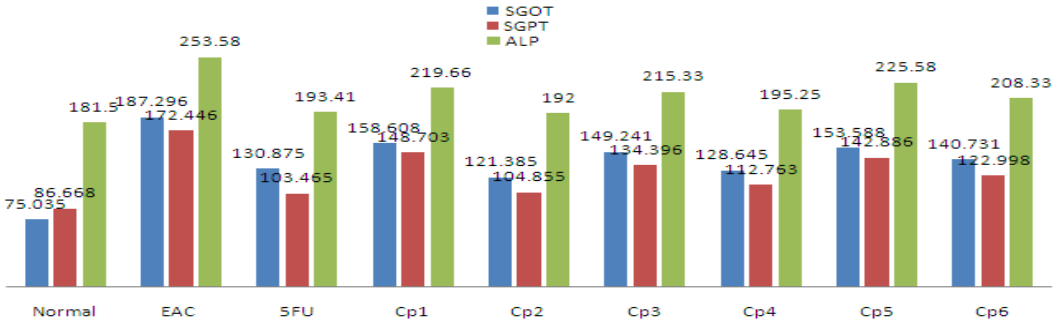
The protein content was essentially reduced in EAC control group when contrasted with control animals. A 36.88% lessening in protein content was noted in EAC control animals.

The tested compounds treated group fundamentally raised the protein content when contrasted with EAC control animals. Compounds Cp2 and Cp4 at dosages of 100 mg/kg were observed to be firmly raised the protein content to around 76.00% and 74.32 % respectively. (Table3).

The present investigation demonstrated that the presentation of 1, 3, 4-Thiadiazole drivatives caused decreasing the activity of serum glutamic oxaloacetic acid transaminase (SGOT), serum glutamic pyruvic acid transaaminase (SGPT), and alkaline phosphate (ALP). The compound (Cp2) was observed to be the most dynamic against SGOT, SGPT and ALP, while compound (Cp4) indicated direct activity against these tests. A significant reduction in the serum SGOT (121.385±0.01) and SGPT (104.855±0.87) IU/ml levels were found in the compound (Cp2) treated mice, by contrasted and control, the consequences of such examinations are given in Figure 3.

The marked elevation of **ALP** level in the serum of mice were significantly decreases in the Thiadiazole, the SGOT and SGPT levels respectively dropped from 187.296±0.012 IU/ml to 121.385±0.01 IU/ml regarding compound (**Cp2**) and from 187.296±0.012 IU/ml to 128.645±0.053 IU/ml regarding compound (**Cp4**), as shown in Figure 3.

Liver enzymes, SGOT and SGPT were chosen to assess liver function. The enzyme is absorbed by the organism and passes through the blood to fulfill a systemic activity. Thus, it inhibits the production of prostaglandins which provoke inflammations. The decrease in inflammatory phenomenon results in the retrogression of liver deterioration. The results establish the decrease in those two enzymes and ALP. On the whole, the use of thiadiazole derivatives made the SGPT and SGOT rates increase the liver function.



P < 0.05 **P < 0.01 ***P < 0.001

Figure 3: Comparative analysis of test compounds in liver enzymes (mean±S.E.M, n=6).

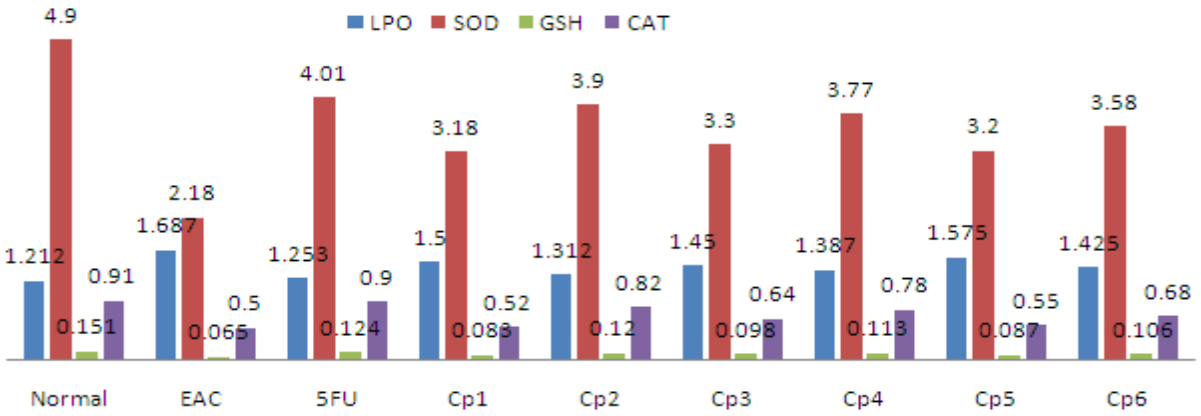
Superoxide dismutase (SOD) levels were reduced (55.51%) in animals of EAC control aggregate when contrasted with control animals. At the point when an comparison is made between Cp1-Cp6, it was discovered that compounds with electron giving groups are more dynamic than the compound having halogen group and in addition electron pulling back substituent on the primary phenyl ring (substituted phenyl ring on the C5 position of the nucleus) which additionally was affirmed by looking at the information for compounds Cp2, Cp4 and Cp6.

It was observed to be the compound Cp2 which have the most noteworthy action (78.89%) and compound Cp4 have significant action (72.93%) though compounds Cp6 and Cp3 (64.22% and 51.37% separately) showed direct exercises when contrasted with 5-FU treated group (83.94 %) (Figure 4).The outcomes uncovered that most of the integrated compound demonstrated shifting level of lessened glutathione (GSH) activity against the EAC cell.

Glutathione levels were fundamentally decreased in EAC control group animals (56.69%) when contrasted with control group animals. Though the incorporated compound Cp2 indicated critical bring up in glutathione level to around 84.61% when contrasted with EAC control group and the esteem was observed to be closer to the GSH level of the standard 5-FU (90.76.5%).

However the compound Cp2 demonstrated most encouraging activity (73.84%) when contrasted with the standard one (Figure 4). The in-vivo inhibitory impact of compounds Cp2 and Cp4 on LPO level at dosages of 100 mg/kg was observed to be more stronger (28.57% and 21.16% separately) when contrasted with that of the control (39.23%) might be because of the nearness of a p-chloro gather in the phenyl ring connected to the 1, 3, 4-thiadiazole moiety at second position (Figure 4) unmistakably EAC instigated oxidative pressure caused an improved lipid peroxidation.

The group treated with Cp2 and Cp4 demonstrated huge level of security against oxidative harm caused by EAC by decreasing lipid peroxidation. The catalase (CAT) was observed to be essentially lessened in EAC control gather when contrasted with control group. A 45.05% decreasing was noted when contrasted with the control group. Among every one of the compound, compound Cp2 indicated most increment in catalase level of around 64% when contrasted with EAC control group while compound Cp4 have demonstrated promising consequence of around 56% (Figure 4).



P < 0.05 **P < 0.01 ***P < 0.001

Figure 4: Comparative analysis of SOD, LPO, GSH and Catalase level in EAC cells induced hepatotoxicity study(mean±S.E.M, n=6).

Anemia is one of the significant symptoms detailed in the cancer chemotherapy. Along these lines, furthermore, the impacts of the compounds on hematological parameters were likewise contemplated and the outcomes were appeared in Figure 5.

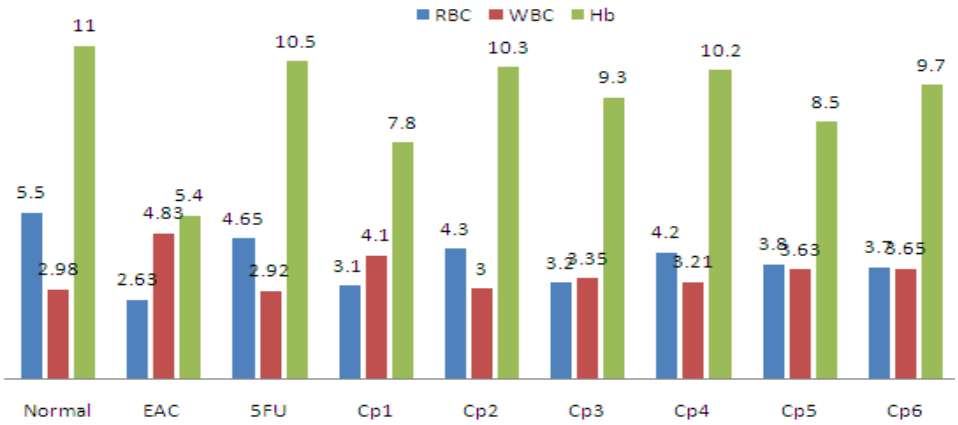
On fourteenth day, blood samples were collected by retro orbital puncture and broke down for the hematological parameters.

Tumor bearing mice (EAC Control) appeared, the lessening in the RBC and Hemoglobin (Hb) levels, though, the WBC tally was observed to be increased.

The outcomes appeared in the Figure 5 showed that, the RBC tallies of the compound treated animals were increased.

Especially, compound Cp2 treatment demonstrated RBC cell count of 4.35±0.86 million cells, though, in the control group, it was observed to be 2.63±0.27 million cells.

In addition, the Hb levels were additionally increased and it was seen as 10.3±0.017 and 5.4±0.13 g/dL, respectively for Cp2 and EAC control, which is connoting the anticancer impact of Cp2. Be that as it may, different compounds likewise displayed promising activity.



P < 0.05 **P < 0.01 ***P < 0.001

Figure 5: Comparative analysis of RBC, WBC counts and Hemoglobin level (mean±S.E.M, n=6).

The reduction in WBC cell counts also an important parameter to assess the anticancer potential of the compounds. Here again, compound **Cp2** showed greater reduction in the WBC cell count when compared with that of the EAC control. Further, it also showed similar effect as 5-Fluro uracil treatment (Standard Group). The WBC cell count for this compound, **Cp2** was observed as $3.0\pm0.12x10^3$ as shown in Figure5.

3.3. Stastistical analysis

All the data were analyzed by using one way analysis of variance (ANOVA) and results were expressed as mean ± SEM. Statistical significance (p) calculated by one-way ANOVA followed by Dunnett's post hoc test of significance where *p<0.05, *p<0.01 and p<0.001 considered to be significant and highly significant, respectively.

4. CONCLUSION

A series of 2, 5-substituted-1, 3, 4-thiadiazole (**Cp1-Cp6**) were synthesized and evaluated for *in vitro* and *in-vivo* anticancer studies. The *in-vitro* cytotoxicity of compounds **Cp2** and **Cp4** were found to be comparable with that of 5-Flurouracil. The *in vivo* anticancer activity of compound **Cp2** was notable on liquid tumor (Ehrlich's Ascites Carcinoma; EAC) induced mice as indicated by decrease in progressive gain in body weight as well as increase in life span when compared to control group animals.

6. ACKNOWLEDGEMENTS

We wish to thank to Dr. N. N Dutta for his encouragements, advice and for providing all the necessary facilities to carry out this study.

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