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Study on Blood Parameters of Tetrahydropyrimidine Carboxamide Derivatives on Breast Cancer

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Abstract

Pyrimidine is one of the most important heterocycles exhibiting remarkable pharmacological activities. Pyrimidine is a heterocyclic aromatic organic compound containing 1,2,3,4-Tetrahydropyrimidine carboxamide derivatives ring which shows wide range of biological activities. Numerous methods for the synthesis of pyrimidine and also their diverse reactions offer enormous scope in the field of medicinal chemistry. The potential antitumor activities of 1,2,3,4-Tetrahydropyrimidine carboxamide derivatives were prepared and used for the anti Cancer treatment. Inhibitory action of the tumor of this compound was shown significantly improved blood parameter includes hemoglobin (HB), RBC, platelets and WBC. *In-vivo* studies results showed substantial improved blood parameters in compared to untreated control rats. The newly synthesized compound exhibited significant effects on the treated animals as compared to DMBA induced animals.

Keywords: 1,2,3,4-Tetrahydropyrimidine carboxamide derivatives; anticancer activity; WBC; RBC; PCV

Introduction

Cancer is a terrible disease which is the leading death of the human population in some areas of the world. Cancer may affect people at all ages, even fetuses, but the risk for most varieties with age. Cancer has three main methods of treatments: chemotherapy, surgery, and radiation therapy. In recent years chemotherapy is becoming more important therapeutic method. So synthesizing a new designed anticancer drug with high efficiency activity is a significant study area today.

In addition, the biological and analgesic activities of many heterocyclic compounds containing a sulfur atom have been reviewed [1,2]. On the other hand, thienopyrimidine and thioxopyrimidine derivatives have promising biological [3] and anticancer activities [4,5]. Recently, some medicinal value of new pyrimidine and their derivatives have been synthesized and used significant effects among various heterocycles, as they are found to possess antiviral, antibiotic, anti-inflammatory [6], antimicrobial [7], antifungal [8] and analgesic [9] antibacterial [10,11] as analgesic, anticonvulsant, and anticancer agents [12].

Cancer chemotherapy causes myelosuppression and anemia [13,14] because of the reduction of both red blood cell (RBC) content and hemoglobin percentage. In support of this anticancer study, hematological parameters have also been studied accordingly. Thus the aim of the present study was to synthesize some 1,2,3,4 Tetrahydro pyrimidine carboxamide derivative using para toluene sulphonic acids as a catalyst. The present study carried out to evaluate pyrimidine derivatives for blood parameters and anticancer activity.

Materials and methods

Procedure

The melting points were determined using an Electro thermal IA 9000 digital melting point apparatus and are uncorrected. Elemental analysis was carried out at IIT Madras. Infrared (IR) spectra were recorded on a FT/IR 6000, Fourier transform, infrared spectrometer (Japan) using the KBr disc technique. The ^{1}H-NMR spectra were recorded at 300 MHz with a Bruker instrument and DMSO-d_6 used as a solvent, TMS (tetramethylsilane) as internal standard. The mass spectra (MS) were measured with a JEOL GC mate mass spectrometer and for ^{13}C-NMR spectral analysis DMSO-d_6 was used as a solvent. The formation of the compounds were checked by TLC using silica gel – pre-coated aluminum sheets (Type 60 F254, Merck, Darmstadt, Germany) and the spots were detected by using UV lamp at λ_{254} nanometer for few seconds.

Synthesis of 3-OXO-O-N-(2-OXO-1, 2-dihydropyrimidin-4-yl) butanamide

Preparation of 3-OXO-O-N-(2-OXO-1,2-dihydropyrimidin-4-yl)butanamide was carried out as per Scheme 1. Ethyl acetoacetate (0.01 M) and Cytosine (0.01 M) were mixed and refluxed for about 3 hr under solvent free conditions (28). The yellowish liquid formed was then heated on a water bath for 30 min. The reaction mixture was allowed to cool. The crude solid obtained was filtered and washed with ether. The product was re-crystallized from aqueous alcohol. Pale Yellow. m.f. $C_8H_9N_3O_3$; IR(KBr, υ_{max} cm^{-1}): 3450(N-H), 1656(C=O), 1623(C=C), 1545(C=N), 762 (primidine ring skeleton vib); ^{1}H-NMR (300 MHz, DMSO-d_6):δ_H:2.9(s, 3H, CH_3), 3.65-3.67(d, 2H, CH_2), 4.84-4.86(d, 1H,), 7.3-7.4(m,1H), 8.31-8.33(d, 2H, NH); ^{13}C-NMR(300 MHz, DMSO-d_6):δ_c: 186.03, 169.91, 165.70, 158.67, 128.29, 93.23, 52.46, 26.32; MS(m/z): EI+ calculated:195.17, found:195.46.

Synthesis of compound

Preparation of the compound (2) by one-pot multi component reaction was carried out as per Scheme 2. The mixture of appropriate β-keto ester, 3-OXO-O-N-(2-OXO-1,2-dihydropyrimidin-4-yl)

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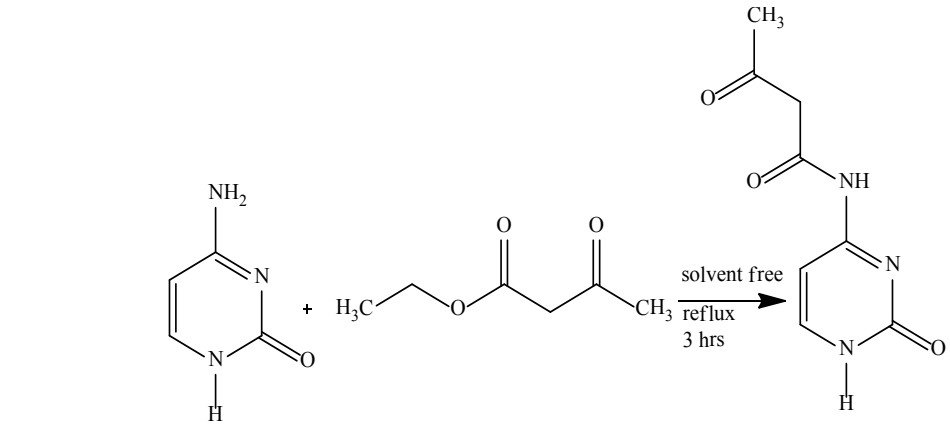
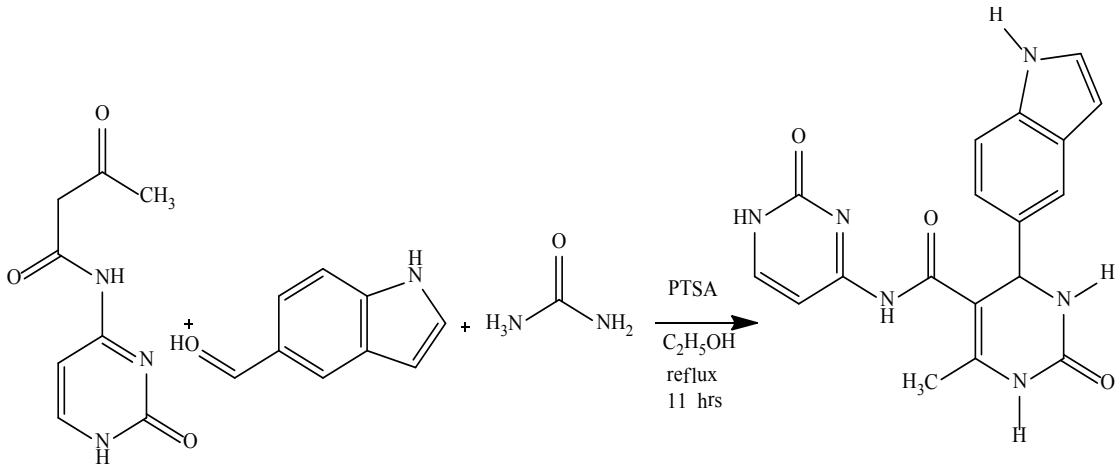


Scheme 1: Synthesis of compound 1.



Scheme 2: Synthesis of compound 2.

butanamide (0.005 M), urea (0.0075 M), and indol-5-yl carb- aldehyde (0.005 M) with catalytic amount of PTSA (0.025 M) was transferred to a round bottom flask containing 15 ml of ethanol to serve as a solvent. The round bottom flask was stirred to dissolve the reactants. The mixture was heated at reflux temperature for 11 hours to form the compound 2. The reactions were monitored through TLC. After the completion of reaction, the mixture was allowed to cool. The solid product formed was filtered, washed with water to remove the unreacted urea and dried. Product was further purified by recrystallization with ethanol.

Experimental animals

Adult Female Sprague-Dawley rats with an average body weight of 200 ± 20 g, Sprague-Dawley (SD) rats (n=30) were used in this study. Thirty rats were divided into five groups each group contain six animals as follow Group 1 considered normal control, Group 2 induced with DMBA, Group 3: DMBA induced animals treated with Tetrahydro pyrimidine carboxamide (TPC), Group 4: DMBA induced + Doxorubicin, Group 5: Treated with compound TPC. The animals were housed under standard laboratory conditions (12 hr light/12 hr dark) in a room with controlled temperature (24 ± 3°C). The rats were fed with standard commercial rat chow and drinking water. The protocol used in this study for the use of rats as the animal model for research was approved by the Institution Animal Ethical Committee (1182/PO/C/08).

Acute toxicity study

Acute toxicity study was carried out using OECD guide lines No. 423. Three rats of the same age group and weight were taken in a single dose (TPC) up to the highest dose 2000 mg/kg orally. The animals were observed for 1 hr continuously and then hourly for 4 hr, and finally after every 24 hr upto 15 days, animals mortality or gross behavioral changes was not observed.

Statistical analysis

Statistical analysis was carried out by using Graph Pad Prism software (version 4.03). One way ANOVA was used, followed by Newman-Keuls multiple comparison test. The data were representing mean ± SEM and the minimum level of significance was set at $p<0.001$ and $p<0.05$.

Results

Biological evaluation

Hematological parameters: Hematological parameters and survival times were among the most important parameters for evaluation of synthesized compounds. Tetrahydropyrimidine carboxamide were studied compared with control (non-treated) and experimentally induced DMBA rats. The results showed significant changes when compared with control (non-treated) rat as shown

in Figures 1-6. The effects of the test compound on tumor-bearing rats are shown in Figures 2, 3 and 4. The animal's body weight and hematological parameters significantly varied from their normal animals along with tumor growth. The hemoglobin content and RBC counts decreased, whereas WBC counts increased after induction of DMBA animals. In this study currently observed treatment with newly synthesized test compound (TPC) at the previously specified doses, the parameters were restored moderately only at a high TPC dose (50 mg/kg).

Hematological examination of the positive control rats shown in Figures 1-3 extremely significant increase in RBC's count average ($p<0.001$), with change (6.3%). WBC's count average shown significant decrease ($p<0.001$) with change (12.5 cumm). Platelets count average showed a significant decrease ($p<0.01$) with change (80.1%). Hemoglobin average shown significant increase ($p<0.001$) as compared DMBA induced animals (12.72 mg%). HCT (%) average showed significant decrease ($p<0.05$) with change (35.85%). TPC treated animals showed significant increase ($p<0.001$) with change (63.24%).

Hematological examination of the Tetrahydropyrimidine carboxamide derivative treated rats showed Figures 4, 5 and 6 significant increase in PCV count average ($p<0.05$), with change % (37.32%) as compared with DMBA induced animals. Leukocyte count average showed significant increase ($p<0.05$) with change % (83.24). Platelets count average shown significant decrease, change % was (-80.48%). MCHC average showed significant increase ($p<0.01$) with change % (55.95%). MCH (%) average showed significant decrease ($p<0.01$) with change % (20.26%).

Discussion

One mechanism whereby mitochondrial dysfunction could result in alterations in nucleotide pools is the mitochondrial dependence of a key enzyme in de novo pyrimidine biosynthesis, dihydroorotate dehydrogenase [15] Direct pharmacologic inhibition of the electron transport chain results in decreased pyrimidine biosynthesis [16] and a pharmacologic inhibitor of dihydroorotate dehydrogenase, leflunomide, has been reported to cause an anemia with megaloblastic features [17]. The effects of the test compound at varies level of hematological parameters were normal shown in Figures 5 and 6. The test compound showed slight toxicity to the host during the treatment period, but these parameters were almost restored back to normal values after the treatment. The treatment with the test compound at increasing doses resulted in an increase of the normal peritoneal cells and macrophages. In this study to confirm *in vivo* blood parameters was carried out using DMBA induced rat. The reliable criteria for judging the value of any anticancer drug (TPC) are prolongation of decrease of WBC and increase hemoglobin [18].

Hematological parameters were found to be altered from normal values along with treatment of test compound. Hemoglobin content, RBC counts were found to be increased and WBC counts were found to be decreased during treatment period. Decrease in RBC and hemoglobin content result from the hemolyzing power of the compounds [18]. The increase in WBC count can be correlated with an increase in antibody production which helps in survival and recovery of the host. After the treatment period with help of synthesized test compounds, it was observed that the parameters restored almost towards normal. The effects of the test compound on hematological parameters of are shown in Figures 1-6. For DMBA induced rats, the tumor weight has been found to increase rapidly with time. The treatment with the test compound (TPC) reduced the tumor growth rate. Similar trend has been found in cell growth inhibition ability. The lifespan of the DMBA induced rats increased remarkably when treated with the test compound. The prolongation of lifespan of cancer bearing rats is a very important and reliable criterion [18] for judging the potency of any drug as anticancer agent. The positive effect of the compound on DMBA induced cancer bearing rats has further been verified by monitoring the change in hematological and biological Both the RBC and hemoglobin content of DMBA cancer bearing rats were found to be decreased gradually with time as found in normal rats. This is probably owing to the deficiency of iron in hemolytic or myclopathic condition [19]. The treatment with TPC compound has reversed back RBC and hemoglobin contents towards normal. With the growth of tumor, WBC level increased with time. The rise of WBC count of the treated DMBA induced cancer bearing rats was slower than that in untreated EAC-bearing rats. Parallel hematological experiments have been studied with normal animals to evaluate the host toxic effect of the compound. A very slight deterioration in such parameters has been observed during the treatment period. Similar toxic effect observed in doxorubicin [0.25 mg/kg (i.p.)] treated rats.

The cytotoxic and immunological effect of the current studied compound in fresh healthy animals have been performed by counting peritoneal macrophages, which has been provided further support for the potency of the compound as anticancer agent. This compound

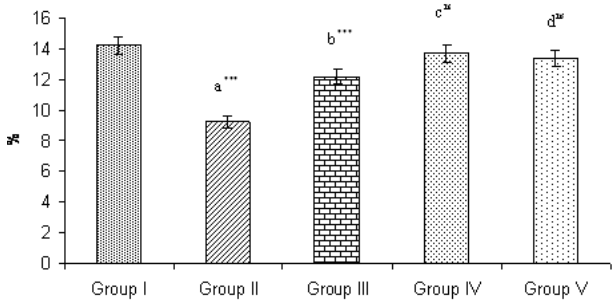


Figure 1: HB after two month of treatment by TPC in DMBA induced Rats. Rats were treated with TPC (50 mg/ kg., i.p.). Hematological and histopathological studies were assessed. Results are expressed as mean ± standard deviation (S.D.), where n=6. The results were considered statistically significant, when $p \leq 0.001$. (a) Significance compared to normal control and standard rats. (b) Significance compared to TPC -treated rats. Comparisons were between: a: Group I vs. II, b: Group II vs. III, c: Group I vs. III and IV, d: Group III vs. V, Symbols represent statistical significance: ***: $p<0.001$, **: $p<0.01$, *: $p<0.05$, ns: non-significant.

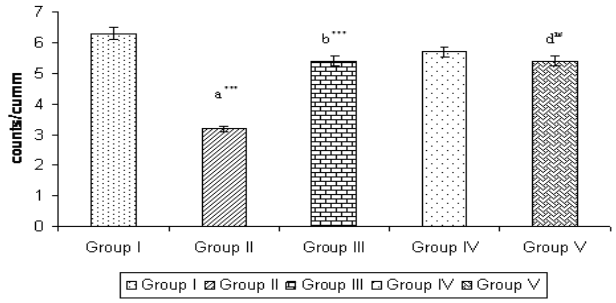


Figure 2: WBC's Count after two month of Treatment by TPC in TAA-Induced HCC Rats. Rats were treated with TPC (50 mg/ kg., i.p.). Hematological and histopathological studies were assessed. Results are expressed as mean ± standard deviation (S.D.), where n=6. The results were considered statistically significant, when $p \leq 0.001$. (a) Significance compared to normal control and standard rats. (b) Significance compared to TPC -treated rats. Comparisons were between: a: Group I vs. II, b: Group II vs. III, c: Group I vs. III and IV, d: Group III vs. V, Symbols represent statistical significance: ***: $p<0.001$, **: $p<0.01$, *: $p<0.05$, ns: non-significant.

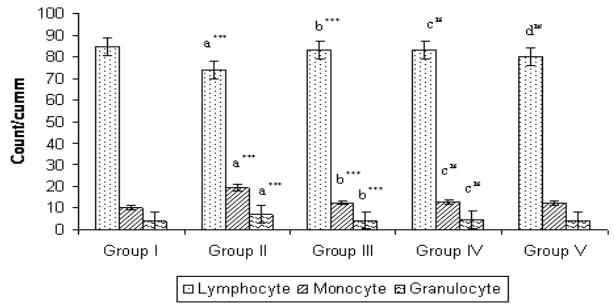


Figure 3: WBC's Count after two month of Treatment by TPC in DMBA induced SD Rats. Rats were treated with TPC (50 mg/ kg., i.p.). Hematological and histopathological studies were assessed. Results are expressed as mean ± standard deviation (S.D.), where n=6. The results were considered statistically significant, when p ≤ 0.001. (a) Significance compared to normal control and standard rats. (b) Significance compared to TPC -treated rats. Comparisons were between: a: Group I vs. II, b: Group II vs. III, c: Group I vs. III and IV, d: Group III vs. V, Symbols represent statistical significance: ***: p<0.001, **: p<0.01, *: p<0.05, ns: non-significant.

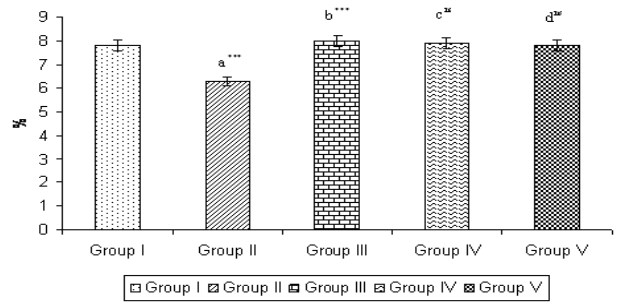


Figure 4: PLT's Count after two month of Treatment by TPC in DMBA induced SD Rats. Rats were treated with TPC (50 mg/ kg., i.p.). Hematological and histopathological studies were assessed. Results are expressed as mean ± standard deviation (S.D.), where n=6. The results were considered statistically significant, when p ≤ 0.001. (a) Significance compared to normal control and standard rats. (b) Significance compared to TPC -treated rats. Comparisons were between: a: Group I vs. II, b: Group II vs. III, c: Group I vs. III and IV, d: Group III vs. V, Symbols represent statistical significance: ***: p<0.001, **: p<0.01, *: p<0.05, ns: non-significant.

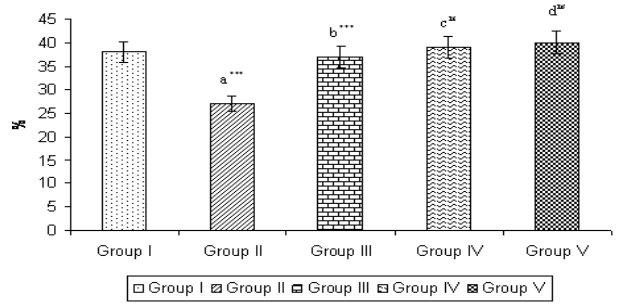


Figure 5: PCV Count after two month of Treatment by TPC in DMBA induced SD Rats. Rats were treated with TPC (50 mg/ kg., i.p.). Hematological and histopathological studies were assessed. Results are expressed as mean ± standard deviation (S.D.), where n=6. The results were considered statistically significant, when p ≤ 0.001. (a) Significance compared to normal control and standard rats. (b) Significance compared to TPC -treated rats. Comparisons were between: a: Group I vs. II, b: Group II vs. III, c: Group I vs. III and IV, d: Group III vs. V, Symbols represent statistical significance: ***: p<0.001, **: p<0.01, *: p<0.05, ns: non-significant.

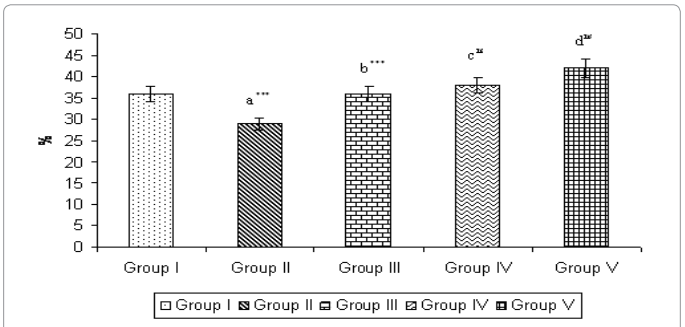


Figure 6: MCHC Count after 45 days of Treatment by TPC in DMBA Induced SD Rats. Rats were treated with H2PPS (50 mg/ kg., i.p.). Hematological and histopathological studies were assessed. Results are expressed as mean ± standard deviation (S.D.), where n=6. The results were considered statistically significant, when p value ≤ 0.05. (a) Significance compared to normal control rats. (b) Significance compared to TAA -toxicated control rats. Comparisons were between: a: Group I vs. II, b: Group II vs. III, c: Group I vs. III and IV, d: Group III vs. V, Symbols represent statistical significance: ***: p<0.001, **: p<0.01, *: p<0.05, ns: non-significant.

has distinguished increased number of macrophages. This enhancement might produce some cytokinetic products, such as tumor necrosis factor, interleukins, interferon's etc. which in turn may be responsible in destroying tumor cells [20,21]. Based on the above results, it can be concluded that the test compound showed pronounced activity as an anticancer agent against DMBA induced SD rats. Many more investigation has to be carried out with this compound and its derivatives using various cancer cell lines and higher animal models.

Conclusions

Pyrimidine is a distinct and unique place in our life since it is an essential constituent of all living cells. In this heterocyclic moiety has great biological and medicinal significance. The biological profiles of this new generation of Pyrimidine represent much progress with regard to the older compounds. We hope that in the future many new biological profiles will be added to it and more investigations must be carried out to evaluate more activities of Pyrimidine for many diseases whose treatment are challenging in the field of medical sciences. The newly synthesized Tetrahydro Pyrimidine carboxamide derivative was tested for their anticancer activity against EAC bearing rat were found to increase the life span the tumor hosts also found to bring the altered Hemoglobin and RBC values of the EAC bearing rat to near normal. The versatile synthetic applicability and biological activity of these heterocycles will help to the medicinal chemists to plan, organize and implement new approaches towards discovery of novel drugs.

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