RESEARCH ARTICLE

Synthesis and Biological activity of new 5-Methyl-3-Oxo-N_2 [5'-Carbonyl-(4'-Aryl-6'methyl)-1',2',3',4'-Tetrahydropyrimidine-2'-One]Pyrazolidines

Radhika C*, Venkatesham A, Anantha Krishna Chaitanya D

Jawaharlal Nehru Technological University, Hyderabad-500085, Andhra Pradesh, India.

J. Adv. Pharm. Edu. & Res.

ABSTRACT

A number of new pyrimidine fused pyrazolidine derivatives have been synthesized starting from the preparation of tetrahydro pyrimidine derivatives which utilised the involvement of biginelli reaction.Finally, clinical candidates were obtained by the reaction of ary hydrazine with ethyl acetoacetate,P-toluene sulfonic acid and ethanol . The structure of compounds was confirmed by IR, and (1) H-NMR data. The synthesized compounds were evaluated for their anti-inflammatory activity against Formalin-induced pedal inflammation in albino rats at a dose of 50.75 and 100mg/kg. The most active compound of this series is 5-methyl-3-oxo-N_2[5'-carbonyl-(4-(4-methoxy phenyl)-6'-methyl)-1',2',3',4'-tetrahydro pyrimidine-2'-one] pyrazolidine 4a) was found to be most potent, which has shown higher percent of inhibition of oedema than than the standard drug Diclofenac sodium.

Keywords: Biginelli Reaction, P-toluene sulfonic acid, Anti-inflammatory activity, Formalin, Diclofenac sodium

INTRODUCTION

In recent years, synthesis of combination towards various heterocyclic rings has been interest for the source of lead molecules possesses wide range of pharmacological activities. Most of the compounds having pyrimidine and pyrazolidinone nucleus exhibits pharmacological action. [1-3].A broad spectrum of biological activities like anti-inflammatory [4], antibacterial [5], antifungal [6], antitubercular [7], analgesic and hypothermic [8] are found to be associated with compounds having pyrimidine moiety. 3-Pyrazolidinones and their fused analogues, 1*H*,5*H*-pyrazolo[1,2–*a*]pyrazol-1-ones, are interesting classes of heterocyclic compounds which, since the beginning of a systematic work in this area more than four decades ago, found a widespread use in various applications.[9] For example, phenindone and its derivatives and analogues are used in photographic applications [10] and as inhibitors of cyclooxygenase, lipooxygenase, and γ-aminobutyrate transferase.[11,12] Bicyclic pyrazolo [1,2–*a*]pyrazol-1-ones have been prepared as scaffolds for β-turn mimics.[13] Such an example is Lilly's bicyclic pyrazolidinone LY 186826 exhibiting antibiotic activity which is larger than that of several penicillins and cephalosporins.[14]

One of such fused ring compounds are Pyrazolo[3,4-d]pyrimidines which were identified as a general class of adenosine receptors due to the similarity between their structures and purines.[15] Pyrazolo[3,4-d]pyrimidines have been reported to possess antimicrobial and anticancer activities.[16] 4,7-Dihydro-4-ethyl-2-phenylpyrazolo-[1,5-a]pyrimidin-7-one (FPP028) is the prototype of a class of pyrazolo[1,5-a]pirimidine derivatives that has been shown to possess marked antiinflammatory and analgesic properties.[17] The aim of study was to synthesize new series of 5-methyl-3-oxo-n_2 [5'-carbonyl-(4'-aryl-6'methyl)-1',2',3',4'–tetrahydropyrimidine-2'-one]pyrazolidines derivatives and evaluate for its antiinflammatory activity as most of the compounds having pyrimidine or pyrazolidine nucleus possess pharmacological action.[1-3]

Address for correspondence

Radhika C
Jawaharlal Nehru Technological University,
Hyderabad-500085
Andhra Pradesh, India
Email id: cradhika8@gmail.com

Access this article online
www.japer.in

MATERIALS AND METHODS

All melting points were determined in open capillary tubes and were uncorrected. IR spectra were recorded in KBr pellets on Thermo Nicolet Nexus 670 spectrometer-NMR spectra were recorded. The purity of the compounds were verified by TLC.

Step 1 :Synthesis of 2-oxo-6-methyl-5-carbethoxy-4-aryl-1,2,3,4-tetrahydropyrimidine-2-one(1):

Aldehyde (3mmol), Ethyl acetoacetate (3mmol), Urea (4.5 mmol) and Iodine (0.3 mmol) were mixed in a 100ml RBF with a magnetic stirring bar and dipped in preheated oil bath at 90^0c (bath temperature). The contents were stirred till the solution mixture converted to solid mass (15 min-1h). The solid mass was heated under another 5 min and ice cold saturated Sodium thiosulphate solution (10ml) was added. There after, the crude solid product was filtered off, washed with ice-cold water (20ml) and dried. The crude product was purified by recrystallisation from ethanol.[18]

Step 2:Synthesis of 4-aryl-5(hydrazinocarbomoyl)-6-methyl-1,2,3,4-tetra hydro pyrimidine-2-one (2):

Weigh the required quantity of step 1 product particularly in a fine powder form (0.01mol) is transferred into a 500ml RBF containing excess amount of pure ethanol (100ml). Now add excess amount of 0.0l mole of Hydrazine hydrate (99%) into it and keep the reaction mixture under reflux at a constant temperature (100^oc) in a preheated oil bath with magnetic stirring or nearly about 6 hrs. After the completion of reaction by confirmation from TLC; stop the reaction and cool the reaction contents by keeping it a side. The excess solvent was then distilled off and the resulting solid was dried and recrystallised from ethanol.

Step 3: Synthesis of 5-mehyl-3-oxo-N_2 [5'-carbonyl-(4'-aryl-6'methyl)-1`,2`,3`,4'-tetrahydropyrimidine-2'one] pyrazolidine (3):

A mixture of the aryl hydrazine (0.01 mol) (Step 2 product) and ethyl acetoacetate (0.01 mol) with PTSA (P-toluene sulfonic acid) (0.1g) and ethanol (100ml). Now the reaction mixture was kept under reflux at constant temperature of 90^oc in a preheated oil bath with magnetic stirring for 8hrs. on cooling, compound got separated out, which was filtered and recrystallised from ethanol. The melting point and other physical data were summarized in Table-1

In view of varied biological and pharmacological importance of different series of pyrimidine fused thiazolidinone, it is felt worthwhile to evaluate them for possible activities. These compounds therefore were screened for anti-inflammatory activity

Acute toxicity studies:

Healthy adult male albino swiss mice weighing between 50-100 gm were used in this investigation. The animals were fasted overnight prior to the experiment and fixed dose (OECD) guide line no.425 method of (CPCSEA) was adopted for toxicity studiesand divided into groups of six animals each. The test compounds, suspended in sodium carboxy methyl cellulose solution (0.1%) were administrated, intraperitoneally in doses of 100mg to 1000mg per kg (b.w). The control groups of animals received only the vehicle (0.1% sodium CMC). The animals were observed for 48 hours from the time of administration of test compound to record the mortality.[19]

Atiinflammatory activity:

Procedure:

Albino rats of either sex weighing 100-150gms were used as a test animal for the experiment. Initial volume of hind paw of the test animals were measured on Plethysmograph apparatus. In the present assay, formalin was employed for producing edema in hind paw of the rat. The rats were divided into 3 groups of three animals in a each group. One group is served as a control and received only vehicle (Tween 80). In other group the test compounds in the form of suspension of 1% tween 80 in a dose of 20mg per kg body weight. The method used (Plethysmographic) was based on the method of Gregory Crosby.[20] The phylogistic agent used was 3.7% formalin prepared in sterile 0.9%Nacl a volume of 0.05 ml of formalin was injected, 60 min after the

administration of test compound subcutaneously into the planar region of each hind paw. The volume of the hind paw was recorded, four hrs after formalin injection. The difference in the initial and final volume of the hind paw indicates the volume of inflammation. The volume of paw edema of the treated rats with the test compounds and untreated rats were compared and the %inhibition of edema was calculated for each of the test compound using formula given below.

%inhibition of edema= 100[1-V_t/V_c]

Where V_t= volume of paw of treated animal

V_c= volume of paw of the controlled animal.

Diclofenac sodium was taken as standard drug to study the relative anti-inflammatory effect of the compounds of the present investigation.The percentage inhibition of edema of compounds were presented in the given Table –2

RESULTS AND DISCUSSION

In this present study, derivatives of pyrimidine fused pyrazolidinones (Scheme-1) were synthesized by using Ethylacetoacetate, Urea, Aromatic aldehydes, Iodine (BIGINELLI CONDENSATION REACTION) (Step–1) as starting materials. By this biginelli reaction it concludes that there are two advantages i.e., the % yield of dihydropyrimidinones (60-80%) was more than the % yield obtained by the Ethanol-Reflux method (20-30%) and also time taken for the completion of the reaction is low (10min-30min) than the Ethanol-Reflux method (3-5h). In step 2 reaction low amount of hydrazine fails to formation of corresponding hydrazine derivative. So it has been concluded that an excess amount of hydrazine and ethanol is added to facilitate the easy formation of corresponding hydrazine derivative. Finally, A mixture of the aryl hydrazine (0.01 mol) (Step 2 product) and ethyl acetoacetate (0.01 mol) with PTSA (P-toluene sulfonic acid) (0.1g) and ethanol (100ml). All synthesized compounds were identified by their melting point, physical data and their spectral data (IR, ^{1}H NMR).

5-methyl-3-oxo-N_2[5'-carbonyl-(4-(4-methoxy phenyl)-6'-methyl)-1',2',3',4'-tetrahydro pyrimidine-2'-one] pyrazolidine(3a) : 178^{0}C, 67%,R_f value : 0.42,Ir (KBr, cm^{-1}) : 3241 (N-H), 3113 (Ar-CH), 2954 (-CH_3), 1705 (C=O), 1649 (Ar-C=C), 1225 (C-N), 1281, 1031 (C-O-C)(Ar-OCH_3);^{1}H-NMR (δ ppm) :1.1 (s, 3H, CH_3-6), 2.24 (s, 3H, CH_3-5'), 3.32-3.33 (d, *J*=7.4Hz, 2H, CH_2-4'), 3.72 (s, 3H, OCH_3-4 Ar), 3.96-4.01 (d, *J*=7.2Hz, 1H, CH-5'), 5.09 (s, 1H, CH-4), 6.86-6.89 (d, *J*=6.8Hz, 2H, CH-3&5 Ar), 7.14-7.16 (d, *J*=8.2Hz, 2H, CH-2&6 Ar).

5-methyl-3-oxo-N_2[5'-carbonyl-(4'-phenyl-6'-methyl)-1',2',3',4'-tetrahydro pyrimidine-2'-one] pyrazolidine(3b): 187^{0}C; 61% ;R_f value : 0.31;Ir (KBr, cm^{-1}) : 3243 (N-H), 3115 (Ar-CH), 2977 (-CH_3), 1725 (C=O), 1645 (Ar-C=C), 1223 (C-N) ; ^{1}H-NMR (δ ppm) : 1.09 (s, 3H, CH_3-6), 2.268 (s, 3H, CH_3-5'), 3.41-3.42 (d, *J*=7.2Hz, 2H, CH_2-4'), 3.96-4.02 (d, *J*=8.8Hz, 1H, CH-5'), 5.16 (s, 1H, CH-4), 7.22-7.26 (t, *J*=10.4Hz, 3H, CH-3,4&5 Ar), 7.31-7.34 (d, *J*=8.6Hz, 2H, CH-2&6 Ar).

All the compounds have been found to be safe even upto a dose of 1000 mg/kg i.p in experimental animals. From the results, it was noticed that all the pyrimidine fused pyrazolidine derivatives tested showed considerable antiinflammatory activity. Among all the compounds tested, compound having phenyl ring without substituents are found to be more potent and remaining compound are found to be moderate potent which is compared to that of standard Diclofenac sodium.In addition, it has been found that 3a showed maximum activity when compared to diclofenac sodium and this may be due to the presence of phenyl moiety of pyrimidine ring.

CONCLUSIONS

From the results there is a need for further advanced studies to establish the efficacy of some of these compounds as antiinflammatory agents and also have excellent scope for further development as commercial anti-inflammatory drugs.

Table 1: Physical data of 5-methyl-3-oxo-N_2 [5'-carbonyl-(4'-aryl-6'methyl)-1',2',3',4'–tetra hydro pyrimidine-2'-one] pyrazolidine.

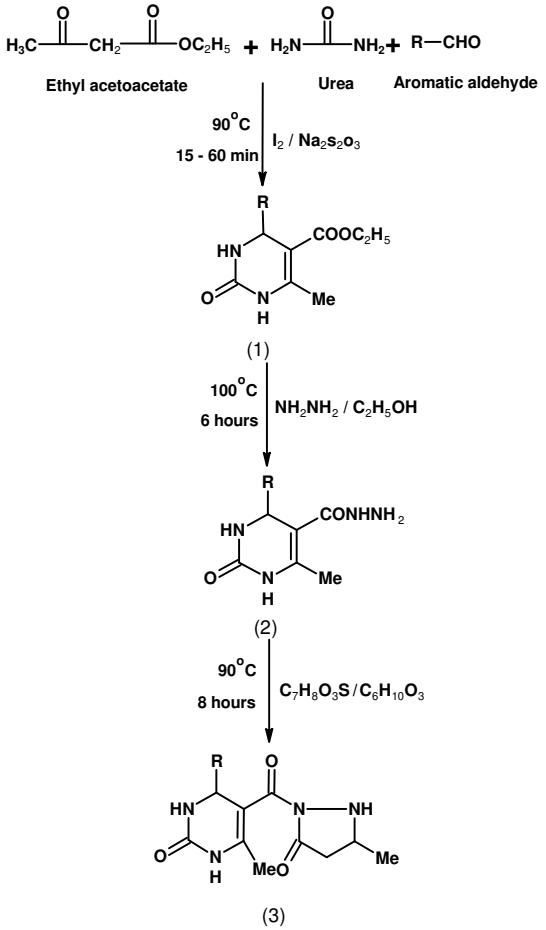
S. No	Ar	M.Formula	M.Wt	M.P. (°C)	Yield (%)	R_f
3a	C_6H_5	$C_{16}H_{18}N_4O_3$	314.3	178	67	0.42
3b	P-OCH_3-C_6H_4	$C_{17}H_{20}N_4O_4$	344.37	187	61	0.51

Mobile phase = Chloroform: Methanol :: 8:2

Table 2: Anti inflammatory-activity of 5-methyl-3-oxo-N_2 [5'-carbonyl-(4'-aryl-6'methyl)-1',2',3',4'–tetrahydropyrimidine-2'-one]pyrazolidine.

Sl. No.	Ar	% inhibition at different concentration		
		50mg	75mg	100mg
1	C_6H_5	30	32	35
2	P-OCH_3- C_6H_4	29	33	34
3	Control	0	0	0
4	Diclofenac sodium	44	46	50

SCHEME-I: SYNTHESIS OF PYRIMIDINE FUSED PYRAZOLIDINONE DERIVATIVES



REFERENCES

1. Dave C.G. Shukla. Pyridopyrimidines: Part IX - Synthesis and antibacterial activity of 2-methylthio-6-phenylazo-5,7-dimethylpyrido[2,3-*d*]pyrimidin-4(3*H*)-ones. Indian. J. Chem. B. 2000; 39B:210-214.
2. Patel R.B.,Desai P.S.,Desai K.R.,Chikhalia K.H. Synthesis of pyrimidine based thiazolidinones and azetidinones:Antimicrobial and antitubercular agents. Indian. J. Chem B.2006; 45B:773-778.
3. Singh A.K., Birendra Kumar M. Synthesis of some ThiazolidinoPyrimidines. Asian. J. Chem. 1997; 9: 234-238.
4. Menozzi G., Mosti L., Schenone P., D'Amico M., Falciani M., Filippelli W.1-Aryl-1H-pyrazole-5-acetic acids with antiinflammatory, analgesic and other activities.Farmaco 1994; 49:115-119.
5. Barot V.M. Synthesis and antibacterial activity of some new 2-aminopyrimidines and 2-(1H) pyrimidinones. Asian. J. Chem. 1996; 8: 802-804.
6. Khan M. H., Nizamuddin J. Pesticidal activities of some 3-aryl-4-methylpyrazolo [3,4-b][1,5] benzodiazepines and 4-aryl-2-imino-5-methyl-1,3-thiazino [4,5-b][1,5] benzodiazepines. J. Agr. Food. Res.1995; 43: 2719-2721.
7. Gangiee A., Adaer G. Pneumocystis carinii and Toxoplasma gondiidihydrofolate reductase inhibitors and antitumor agents: synthesis and biological activities of 2,4-diamino-5-methyl-6-[(monosubstitutedanilino)methyl] pyrido[2,3-d]pyrimidines.J. Med Chem. 1999; 42: 2447-2455.
8. Ahluwalia V.K., Gupta C. Synthesis of 1,3-Diaryl-1,3-dihydro-4,5-dioxo-2-thioxofuro[2,3-*d*]pyrimidines and 1,3-Diaryl-1,3-dihydro-2-thioxo-5-(thiazol-4-l)-2*H*,5*H*-pyrimidine-4,6-diones. Heterocycles. 1991; 32: 907-914.
9. Dorn H. Advances in the chemistry of pyrazolidones, iminopyrazolidines, and amino- and hydroxypyrazoles. Chem. Heterocycl. Comp. 1981; 17(1): 1-24.
10. Kendall J.D. Photographic developers,Brit. J. Phot.1951; 539-543
11. Moon C., Ahn M., Wie M.B., Kim H.M., Koh C.S., Hong S.C., Kim M.D., Tanuma N., Matsumoto Y., Shin T. Phenidone, a dual inhibitor of cyclooxygenases and lipoxygenases, ameliorates rat paralysis in experimental autoimmune encephalomyelitis by

suppressing its target enzymes. Brain Res. , 2005;28:206-10.

12. Cusan C., Spalluto G., Prato M., Adams M., Bodensieck A., Bauer R., Tubaro A., Bernardi P., Da Ros T. Synthesis and biological evaluation of new phenidone analogues as potential dual cyclooxygenase (COX-1 and COX-2) and human lipoxygenase (5-LOX) inhibitors. Farmaco.2005;60(1):7-13
13. Hanessian S., McNaughton Smith G., Lombart H.G., Lubell W.D. Design and synthesis conformationally constrained amino acids as versatile scaffolds and pepticmimetics. Tetrahedron. 1997; 53: 12789-12850
14. Jungheim., Louis N., Sigmund., Sandra K. 1,3-Dipolar cycloaddition reactions of pyrazolidinium ylides with acetylenes. Synthesis of a new class of antibacterial agents. J. Org. Chem.1987;52(18): 4007-13.
15. Harriet W. Hamilton, Daniel F. Ortwine ., Donald F. Worth., James A. Bristol. Synthesis and structure-activity relationships of pyrazolo [4,3-d]pyrimidin-7-ones as adenosine receptor antagonist. *J. Med. Chem*. 1987; 30 (1): 91–96.
16. Fathalla O.A., Mohamad N.A., Abbas E.M.,ShIAbd-Elmoez. , Soliman A.M.Synthesis and evaluation of some new pyrazolopyrimidine and thiazolidin-4-one derivatives as antimicrobial and anticancer.World journal of chemistry.2009;4(2):141-148.
17. Perretti F., Pirisino R., Pecori Vettori L., Auzzi G., Bruni F., Costanzo A., Matucci M., Longo G., Messori A. Pharmacological activity of some pyrrolo and pyrazolo pyrimidine derivatives. Farmaco Sci.1987;42(11):793-804
18. Dolly Kataki, Pravina Chakraborthy. Pubaleesarmah. Phukan P. Scalable synthesis of 3, 4-dihydropyrimidin-2(1H)-ones under solvent free condition. Ind. J. Chem .tech. 2006; 13: 519-521.
19. Litchfield J.T., Wilcoxon F. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther.1949; 96: 99-113
20. Lee O.K.,Gregory Crosby. Halothane effect on formalin-induced paw edema and flinching in rat. J. Korean Med. Sci. 1999; 14: 34-38

How to cite this article: Radhika C*, Venkatesham A, Anantha Krishna Chaitanya D; Synthesis and Biological activity of new 5-Methyl-3-Oxo-N_2 [5'-Carbonyl-(4'-Aryl-6'methyl)-1', 2', 3', 4' – Tetrahydropyrimidine-2'-One] Pyrazolidines; J. Adv. Pharm. Edu. & Res. 2014: 4(1): 66-70.

Source of Support: Nil, **Conflict of Interest:** Nil