International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS™ | Volume 8 | Issue 4 | OCT-DEC | 2018 | 539-543
Research Article | Pharmaceutical Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

SYNTHESIS AND ANTI-INFLAMMATORY POTENTIAL OF OXADIAZOLE DERIVATIVES OF NSAIDS

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ABSTRACT

The present investigation was undertaken with the aim to synthesize oxadiazole derivatives of NSAIDs, by selecting ketoprofen as drug moiety. Ketoprofen was too converted into its conversion to hydrazide. The synthesized hydrazide of the ketoprofen was then treated with $POCL_3$ and certain selected (aliphatic and aromatic) acids were substituted for the designing of four compounds viz. A, B, C, D. All the synthesized derivatives were then characterized on the basis of UV, FTIR and ^{1H}NMR spectral data. The 4 analogues were synthesized to ascertain the affectivity of the substituted oxadiazole derivatives on anti-inflammatory efficacy that illustrated good anti-inflammatory activity as compared to the standard drug. Evaluating the synthesized analogues pharmacologically assured the activity of derivatives.

KEY WORDS

Oxadiazole, Ketoprofen, NSAIDs, Anti-inflammatory, Characterization.

INTRODUCTION:

NSAIDs are very widely used for the treatment of anti-inflammatory disorders. However, prolonged intake of NSAIDs is related to gastro intestinal bleeding and nephrotoxicity [1,2]. In both severity and frequency these reactions are from considerably mild too much serious and potentially life treating such as many gastrointestinal related problems [3-5]. NSAIDs pharmacological activity is through inhibition of enzyme prostaglandin biosynthesis through arachidonic acid which is known as (COX) [6,7]. The COX exhibits in two isoform, COX-1 and COX-2, these are managed and expressed differently [8-10]. Cytoprotection is provided cytoprotection to gastrointestinal tract through (COX-1), whereas inflammatory signals are selectively mediated by (COX-2) [11-13]. Since majority of the presently used NSAIDs shows greater preference towards COX-1 than COX-2, [14] excessive use of NSAIDs, including ketoprofen, may cause considerable GI irritation, bleeding and ulceration [15]. It has been revealed through literature survey that several leads aiming at the discovery of new anti-inflammatory has been pushed. The synthetic studies include work on various aryl and hetero aryl alkanoic acid, other acids and their derivatives. A range of heterocyclic systems, in isolation and fused with other system, have been synthesized, oxadiazole being one of them. NSAIDs are used in treatment of many inflammatory disorders for instance osteoarthritis, spondylitis etc. But causes adverse effects due to presence of free carboxylic group (-COOH) in molecule [1,16]. Thus, in ongoing study the (-COOH) group of ketoprofen was temporarily masked by converting it into the substituted oxadiazole derivatives to impart good anti- inflammatory activity.

MATERIAL AND METHODS:

Chemicals and equipment's used- Ketoprofen, Ethanol, Petroleum ether, Carrageenan, Methanol, Carbon tetra

chloride, Sodium hydrogen carbonate, Sodium sulphate, Chloroform, Hydrazide, Ethyl Acetate, Acetic acid, 4-Amino benzoic acid, 4- Nitro benzoic acid, 3, 5- Dinitro benzoic acid and Phosphorous oxychloride. Digital weight balance, heating mental, UV-spectrophotometer, FTIR, NMR.

Manufacturer- Molychem, Yarrow Chem Products, Loba Chemie Laboratory, Rankem laboratory Reagents, Himedia Laboratory, Ambassdor, Perkin Elmer, Shimadzu, Bruker Daltonics esquire 400.

Various steps for the synthesis of compounds: [17]

1) Step-1: Synthesis of Ester
2) Step-2: Synthesis of hydrazide
3) Step-3: Synthesis of oxadiazole derivatives of ketoprofen

***Synthetic Scheme is given in Figure 1.1**

Step-1: Synthesis of Ester: 10 grams of ketoprofen, 1.45 ml of H_2SO_4 and 32.5 ml of dry methanol was placed in a 250 ml of round bottom flask; the mixture was refluxed under anhydrous condition for 7 hours and was monitored through TLC, by using petroleum ether and ethyl acetate in ratio (80:20) as solvent. On completion of the reaction excess of the methanol was evaporated using water bath it was allowed to cool, after cooling it was poured into separating funnel and was extracted out using 20-25 ml of CCl_4, and was washed using 10-12 ml of strong solution of sodium hydrogen carbonate till all the free acids are removed and no further evaluation of CO_2, excess of moisture was removed through sodium sulphate and later it was filtered off hence, pure ester was obtained.

Step-2: Synthesis of hydrazide of ketoprofen:

8 ml of ester and 2.63 ml of hydrazine hydrate was placed into 100 ml of round bottom flask with 15 ml of methanol (dried) and was refluxed under anhydrous condition for 7 hours and was monitored through TLC utilizing chloroform and methanol in ratio of (95:5) TLC was kept in iodine chamber to detect spot. The excess of methanol was evaporated, and the solution was placed in freezer, after white crystals occurred, they were collected and recrystallized using ethyl alcohol and pure crystals were collected.

Step-3: Synthesis of substituted oxadiazole derivatives

(A) 2-(3- benzoyl phenyl)-5-methyl-1,3,4 oxadiazole

The hydrazide and acetic acid were placed for reflux with (15ml) of phosphorous oxychloride and was given reflux for 8-10 hrs. After the reaction was completed the mixture it was poured into crushed ice along with sodium carbonate solution (7%) and resulting solid was filtered, dried and recrystallized using ethanol: DMF.

Step-3: (B) 2-(3-benzoyl phenyl)-4-nitro-1,3,4-oxadiazole:

The hydrazide and 4- nitro benzoic acid was placed for reflux with (15ml) of phosphorous oxychloride and was given a reflux for 8-10 hrs. After completion of reaction it was poured into crushed ice along with sodium carbonate solution (7%) and resulting solid was filtered, dried and recrystallized using ethanol: DMF.

Step-3: (C) 2-(3-benzoyl phenyl)-4-amino-1,3,4-oxadiazole

The hydrazide and 4- amino benzoic acid were placed for reflux with (15ml) of phosphorous oxychloride and was given a reflux for 8-10 hrs. After the completion of reaction, it was poured into crushed ice along with sodium carbonate solution (7%) and resulting solid was filtered, dried and recrystallized using ethanol: DMF.

Step-3: (D) 2-(3-benzoyl phenyl)-3,5-dinitro-1,3,4-oxadiazole

The hydrazide and 3, 5- dinitro benzoic acid were placed for reflux with (15ml) of phosphorous oxychloride and was given a reflux for 8-10 hrs. After the completion of reaction, it was poured onto crushed ice along with sodium carbonate solution (7%) and resulting solid was filtered, dried and recrystallized using ethanol: DMF.

Pharmacological Study:

The rats were weighed and divided into groups and a mark was made on the left hind paw beyond tibio tarsal junction, to ensure that the paw is dipped only uptill that mark. The initial volume of individual rat after the administration of 0.1% of carrageenan in sodium C.M.C by injecting into the sub-planter region of paw. Standard drug (5mg/kg b.wt.) and the test drug sample at dose level (5mg/kg b.wt.) were administered orally by gavage, 30 minutes before to carrageenan administration [18]. The rat paw volume was treated by drug and control and was recorded through Digital Plethysmograph. Edema was calculated and Inhibition that was calculated by comparing the change in swelling in the paw between the treated and non-treated rats. Anti-inflammatory activity was determined as the percentage of inhibition later. The formula that was used to calculate percentage inhibition:

% inhibition=Mean paw inflammation of control-Mean paw inflammation of test/ Mean paw inflammation of control × 100

RESULT AND DISCUSSION:

Spectral data including UV, IR AND ^{1H}NMR of the 4 oxadiazole derivatives are following:

Step-3: (A) 2-(3-benzoyl phenyl)-5-methyl-1,3,4-oxadiazole

I.R. ν max cm^{-1}: 2935 (C-H stretching); 1646 (C=O stretching); 1495(C-N stretching)

^{1}HNMR MeOH-D_4 (300MHz): δ 1.50 (d, 3H CH-CH_3), 3.11, (S 2H NH_2), 2 3.40, (q 1H CH-CH_3), 7.86, (br S 9H Ar-C=O-Ar), 8.36-8.52, (S3H) at 2.2 CH_3.

λmax = 254.50.

Step-3: (B) 2-(3-benzoyl phenyl)-4-nitro-1,3,4-oxadiazole

I.R. V_{max} cm^{-1}= 3112 (C-H aromatic); 3060 (N-H stretching); 1701 (C=O stretching); 1462 (C=N stretching)

^{1}H NMR MeOH-D_4 (300MHz) δ: 1.50 (d, 3H (CH-CH_3), 3.11, (q 1H (CH-CH_3), 7.86, br S 9H (AR-C=O-AR) 8.12, (br S 4H Ar-NO_2),

λmax: 397.80

Step-3: (C) 2-(3-benzoyl phenyl)-4-amino-1,3,4-oxadiazole

I.R. V_{max} cm^{-1}= 3390 (N-H stretching); 2934 (C-H stretching); 1496 (C-N stretching); 1646 (C=C stretching);

^{1}H NMR MeOH-D_4 (300MHz) δ: 1.50 (d, 3H, CH-CH_3), 3.11, (q1H CH-CH3), 7.86 (br S 9H Ar-C=O-Ar) 8.36-8.52 (br S4H Ar-NH_2)

λmax: 316.

Step-3: (D) 2-(3-benzoyl phenyl)-3,5-dinitro-1,3,4-oxadiazole

I.R. V_{max}cm^{-1}= 3108 (C-H aromatic); 1659 (C=O stretching); 1462 (C-N stretching)

^{1}H NMR MeOH-D_4 (300MHz) δ: 1.5d 3H, (CH-CH_3), 3.5 (q ^{1}H CH-CH_3), 7.93 (br S 9H (Ar C=O-Ar); 8.09 (br S 3H Ar $2NO_2$)

λmax: 389.60, 336.60.

Compounds that were synthesized were distinguished on the basis of TLC, U.V, I.R and ^{1}HNMR spectral data. U.V spectrum of compounds exhibited **λmax** at peaks. Acetic Acid: **254.50**; 4- Nitro benzoic acid: **397.80**; 4-Amino benzoic acid: **316**; 3,5- Dinitro benzoic acid: **389.60,336.60**.

Characterization of benzene and carbonyl chromophore using U.V spectrum.

^{1}HNMR data showed characterization signal of aromatic proton in range of 7.2-8.36 along with doublet at 1.50, quadrate at 3.11 and characteristic of methyl and methane proton (CH-CH_3) present in ketoprofen nucleus.

I.R spectrum indicated presence of (C=O stretching) **1701**; (C=N stretching) **1462**; (C-N stretching) **33390**; (C-H aromatic) **3108**. (N-H stretching) **3060**. All the spectral data are well in agreement with the documented structures of synthesized compound.

The spectral data are summarized in experimental methods. Further, compounds were screened for pharmacological activity.

Effect of ketoprofen standard and oxadiazole derivatives (T1, T2, T3 and T4) on paw volume in carrageenan induced inflammation.

Results are summarized in table 1.1 indicate the affectivity of Ketoprofen standard and oxadiazole derivatives on mean paw volume in carrageenan induced paw edema in rats.

Results indicate that administration of carrageenan (0.1 ml of 1% solution) caused significant (P<0.01) increase in inflammation in rats. However, paw volume found to be maximum on 120 minute of carrageenan administration (0.86±0.025) as compared to 15th minute (0.32±0.025).

Administration of standard drug ketoprofen at a dose of 5mg/kg caused significant decrease (P<0.01) in paw volume as compared to the control group. The decline in paw volume was maximum on 120 min (0.28±0.0469) but the paw volume of control group was (0.86±0.025).

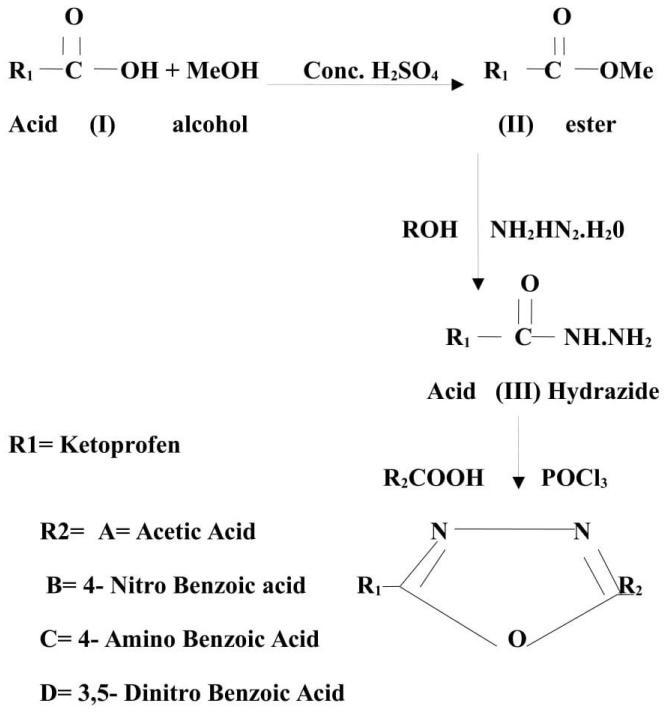
Table 1.1: Effect of Ketoprofen and oxadiazole derivatives on mean paw volume inflammation in rats.

GROUPS	TIME INTERVAL IN MINUTES			
	15 mint	30 mint	60 mint	120 mint
Normal (Distilled Water 1 ml/kg, p.o.)	0.23±0.0391	-	-	-
Control (Carragenan 1%, 0.1 ml in hind paw)	0.32±0.025	0.47±0.026	0.74±0.0316	0.86±0.025
Standard (Ketoprofen, 5mg/kg, s.c.)	0.23±0.0816	0.25±0.035	0.34±0.0125	0.28±0.469
Test 1 (5mg/kg, o.d)	0.23±0.0408	0.22±0.0129	0.33±0.221	0.30±0.0403
Test 2 (5mg/kg, o.d)	0.24±0.0506	0.22±0.015	0.34±0.033	0.30±0.0222
Test 3 (5mg/kg, o.d)	.29±0.0141	0.34±0.984	0.47±0.0170	0.49±0.0129
Test 4 (5mg/kg, o.d)	0.28±0.015	0.33±0.0403	0.38±0.0129	0.37±0.0129

Table 1.2 Effect of ketoprofen and oxadiazole derivatives on percentage inhibition of inflammation.

GROUPS	% INHIBITION			
	15 mint	30 mint	60 mint	120 mint
Standard (Ketoprofen)	29%	49.3%	54%	67.4%
Test 1	27%	52%	55%	66%
Test 2	26%	53.2%	54%	65.1%
Test 3	10.7%	27%	36.2%	40%
Test 4	13.8%	30.5%	48%	56.5%

Figure1.1: Synthetic Scheme: Followed for the preparation of derivatives:



Results show that administration of oxadiazole derivatives i.e. Test1, Test2, Test3 and T4 at a dose of 5 mg/kg, p.o. also caused decline in paw volume. However, Test1 induced more significant decline ($P<0.01$) in paw volume followed by decline in paw volume by Test2 ($P<0.05$). The decline in paw volume by Test 1 was comparable to that by the standard drug. Test1 causes maximum decrease in paw volume at 120 minutes (0.29±0.0403) as compared to carrageenan treated rats. Followed by T2 causing maximum decline in paw volume at 120 minutes (30±0.0222)

Effect of standard ketoprofen and oxadiazole derivatives on percentage inhibition of inflammation:

Results given in the table 1.2 indicate that standard drug ketoprofen produced maximal inhibition of the inflammation at 120 minutes (67%). Test1 also caused maximal inhibition of inflammation at 120 minute (66%) which was comparable to that of standard drug.

Test2 also caused good inhibition of inflammation (65%). However, T3 does not cause notable decline in inflammation in carrageenan induced inflammation and T4 also causes significant inhibition.

Hence as the results indicate it can be presumed that both the standard and the oxadiazole derivative (test1) and (test2) produced noteworthy decline in inflammation and have alike anti- inflammatory effect. As the derivatives lacks –COOH group which is replaced by oxadiazole moiety, hence they will not be causing ulcer as did to ketoprofen, when taken for a longer

duration of time, while it is equally potent as ketoprofen.

So it can be stated that by the substitution of the –COOH group by oxadiazole ring and other selected different substituted benzoic acids minimizes the occurrence of ulceration and this will help in producing a better anti inflammatory drug with less gastro intestinal toxicity.

CONCLUSION:

In the present study some new oxadiazole derivatives 2-(3-benzoyl phenyl) -5- methyl- 1,3,4- oxadiazole (T1), 2-(3-benzoyl phenyl)- 4-nitro-1,3,4-oxadiazole (T2), 2-(3-benzoyl phenyl)-4–amino-1,3,4-oxadiazole, (T3), 2-(3-benzoyl phenyl)–3,5–dinitro-1,3,4- oxadiazole, (T4) were synthesized these oxadiazole derivatives T1, T2, T3, T4 were identified on the basis of TLC, U.V, I.R, ^{1}HNMR spectral analysis. These compounds have been evaluated for anti- inflammatory activity by comparing with ketoprofen as standard drug using hind paw oedema method in Albino rats some of these oxadiazole derivative, exhibited remarkable anti-inflammatory activity on testing. The anti-inflammatory is associated with various side effects therefore, the current work was conducted for the search of better, anti-inflammatory drugs, free from side effects and for better therapeutic activity.

ACKNOWLEDGEMENTS:

I pay my respect and gratitude towards Ms. Urmi Chaurasia Controller of Examination, S.B.S University for her extensive support throughout my project work. Special remembrance Mrs. Amita Rana, Dr. Archana Negi and Janmejay Pant sir for their guidance.

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Received:08.08.18, Accepted: 09.09.18, Published:01.10.2018

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