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Design, Synthesis and Biological Evaluation of Novel 1, 3, 4-Oxadiazole Bearing Pyridine Moiety

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ABSTRACT

Series of novel substituted Synthesis of N-{[5-(substituted)-1,3,4-oxadiazole-2-yl] carbamothioyl} derivatives containing 1,3,4-oxadiazole moiety were synthesized by microwave as a green chemistry method and conventional method by using pyridine 3- carboxylic acid as a starting material. The structures of the synthesized compounds were characterized by physicochemical data, IR, Mass spectra and 1HNMR. All the newly synthesized compound screened for their antimicrobial and In-vivo and In-vitro Anti-inflammatory studies. Anti-inflammatory studies revealed that compound 4f showed significant in-vivo and in-vitro anti-inflammatory activity as well potent antimicrobial activity.

Keywords : Pyridine 3- carboxylic acid, 1,3,4-oxadiazole, Anti-inflammatory activity.

I. INTRODUCTION

It is stable neutrally aromatic molecules and other aromatic molecule are 1,3,4-oxadiazolium captions. They are also referred to be non-aromatic reduced systems 2,5-dihydro-1,3,4-oxadiazole and 2,3,4,5-tetrahydro-1,3,4-oxadiazole. Simple 2,5-dialkyl and 2-alkyl derivatives absorb slightly above 200nm. It is containing 3 or more conjugated rings having different applications as luminescent compounds.

The azole when attached to a Thiourea functional group forms the building block for pharmaceutical agents. They exhibit a wide spectrum of pharmaceutical activity. In the design of new Thiourea and 1,3,4- oxadiazole derivatives, this novel dual inhibitory activity of enzyme pathway holds promise as anti-inflammatory agent with an improved efficacy and safety profile. It possesses different chemical and biological applications and this nucleus having important in medicinal and organic chemistry. It having number of activities like antioxidant, antimicrobial, anticonvulsant, antitumor, DENV2 inhibitory activity, analgesic etc.

II. RESULTS AND DISCUSSION

The starting synthesized compound, 5-(Pyridine-4-yl)-1,3,4-oxadiazole-2-amine was prepared by the reaction of pyridine-4-carboxylic acid with hydrazine hydrate and CNBr in presence of methanol and conc. H_2SO_4. and synthesis of substituted benzoyl isothiocyanate was prepared by substituted benzoyl chloride and ammonium thiocyanate in the presence of acetone as a solvent resulted in the formation of the second compound the final desired compound N-{[5-(substituted)-1,3,4-oxadiazole-2-l]carbamothioyl}derivatives was prepared by mixture of 5-(Pyridine-4-yl)-1,3,4-oxadiazole-2-amine and substituted benzoyl isothiocyanate in presence of

acetone. The experimental procedure is very simple. The process is under green chemistry.

The structures of these synthesized compounds were confirmed by their Physical and spectral analysis. In general, IR spectral data (cm^{-1}) revealed bands at 3500-3300 (NH, stretching), 1600-1500 (C=C, stretching), 1300-1100 (C=S stretching), 1900-1700 (C=O), 2800-3300 (C-H str. of Aromatic ring). In Nuclear magnetic resonance spectra (1H NMR, δ ppm), the signals of the respective protons at 7.00-8.02 (Benzene ring, C(=O) N, NH-Secondary amide, 1,3,4- oxadiazole), 3.49-4.51 (NH-Aromatic). Further, the molecular ion recorded MS (m/z+) [M+] in the mass spectrum is also in agreement with the molecular mass of the compounds.

Table No 1: *In vitro* anti-inflammatory activity of synthesized compounds measuring the percentage inhibition. (2a-f)

Compound	% of inhibition of protein denaturation		Viscosity (cps)	
	50µg/ml	100 µg/ml	50 µg/ml	100 µg/ml
3a	66.07	68.01	89.95	87.70
3b	66.15	65.11	86.98	86.58
3c	64.15	64.35	86.22	86.28
3d	61.50	66.89	85.02	87.27
3e	42.25	70.70	85.2	88.73
3f	71.00	75.21	88.85	90.47
Standard (Diclofenac)	72.53	75.73	89.44	90.67
Viscosity of control = 0.95 cps				

Table 2. *In vivo* anti-inflammatory activity of synthesized compounds (3f) measuring the percentage inhibition. (2a-f)

Sr. no	Compound	% inhibition of Paw Volume						
		0 Min	30 Min	60 Min	90 Min	120 Min	150 Min	180 Min
1.	Control (Normal)	3.7	-	-	-	-	-	-

2.	Standard (Diclofenac)	0.0	13.84	22.97	27.16	39.18	52.70	60.81
3.	2a	0.0	10.25	18.19	22.45	30.15	48.25	55.89
4.	2b	0.0	11.45	15.44	23.18	29.33	49.25	52.34
5.	2c	0.0	09.18	17.33	20.14	33.15	47.14	55.92
6.	2d	0.0	12.17	22.44	25.12	30.11	49.00	58.90
7.	2e	0.0	13.78	21.08	24.34	34.59	51.35	61.89
8.	2f	0.0	11.44	20.16	22.45	32.65	49.70	60.44

The statistical significance of difference across the groups is determined using ANOVA followed by Dunnett's multiple comparisons test. **** p < 0.0001 [one-way ANOVA, Dunnett's multiple comparisons].

Pharmacology

The *in vitro* anti-inflammatory activity of the test compounds was performed by protein denaturation method. The carrageenan-induced rat paw edema method was used for the evaluation of anti-inflammatory activity of oxadiazole derivatives. Animals Wistar rats (100–120gm) of either sex used in this study were purchased from the Animal House of the National Institute of Biosciences, Pune. The animals were maintained under standard laboratory conditions (12 h light/dark cycles at 22 ± 2◦C), humidity 45-55% and fed standard rodent pellets (National Institute of Biosciences, Pune, Maharashtra) and water. The protocol and procedure was approved by the CPCSEA. [1].

Experimental

Chemicals and solvents were procured from commercial sources, purified and dried using standard procedures from literature whenever required the reagents were purchased from S.D fine, Research laboratory Mumbai and MERCK laboratory Mumbai. The melting points (^{0}C) of synthesized compound were determined in open capillary tube method and are uncorrected. Thin layer chromatography was used confirmation of reaction and the purity of the intermediate and the final compounds by applying a single spot on TLC plate (silica gel G) using various solvents such as toluene, acetone, ethanol system. TLC plates were visualized under iodine chamber. IR spectra were recorded on ATR JASCO FTIR-4600. H1NMR spectra were performed in DMSO solution using Bruker 300 MHz and their chemical shift are reported in δ unit with respect to TMS as internal standard. Mass spectra were recorded on Pe sciex (model no. API 2000) software analyst 1.4.2 mode: Q1MS Q1/AUTO INJECTION.

4.1 General procedure for the synthesis of 5-(Pyridine-4-yl)-1,3,4-oxadiazole-2-amine :

Methyl carboxylate was prepared by using 0.01 mole of pyridine-4-carboxylic acid in 35ml methanol. Carboxylic acid is esterified with methanol, further

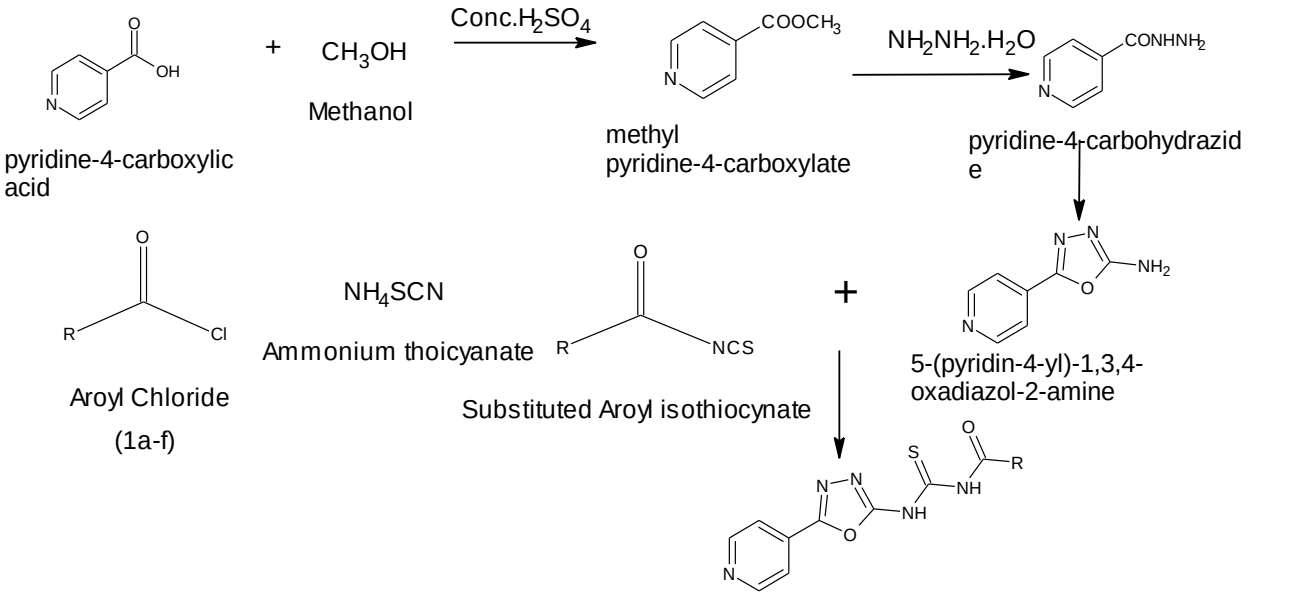
reaction is processed by refluxing the mixture for 5-6 hours by adding few drops of H_2SO_4 as catalyst. After completion of reaction solid was formed which was used for next step for the preparation of substituted carbohydrazide. The mixture of above compound (0.01mol) and 4 ml 99% hydrazine hydrate was refluxed for 4-5 hours. After completion of reaction checked by TLC and solid precipitate was dried and recrystallize from methanol. The mixture of above substituted carbohydrazide (0.01 mol) in 20ml methanol and add cynogen bromide (0.01 mol) this reaction mixture was stirred and refluxed at 55-56^{0}C for 2 hour, after completion of reaction checked by TLC. The solution was cooled and neutralized with NAHCO3. The solid precipitate was washed, dried and recrystallize from ethanol.

General procedure for the synthesis of substituted benzoyl isothiocyanate :

A solution of substituted benzoyl chloride (10mmol) in acetone (50ml) was added dropwise to ammonium thiocyanate (10mmol) in absolute acetone (30ml). the reaction mixture was heated (50^{0}C) under refluxed for 30 min. after completion of reaction checked by TLC. The reaction mixture was cooled to room temperature and the formed precipitate (NH4Cl) was filtered off. To the freshly prepared solution of aroyl isothiocyanate derivative.

General procedure for the synthesis of N-{[5-(substituted)-1,3,4-oxadiazole-2-yl]carbamothioyl} derivatives:

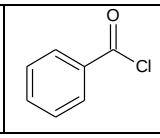
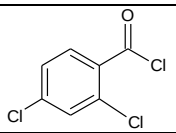
A solution of 5-(pyridine-4-yl)-1,3,4-oxadiazole-2-amine (10mmol) in acetone (10ml) was added and the resulting mixture was stirred with refluxed for 2-3hr. after completion of reaction checked by TLC. The solid prod was washed with water and purified by washing with ethanol absolute.

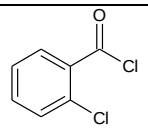
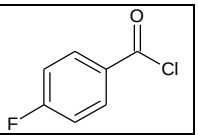
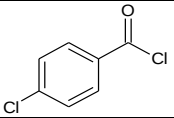
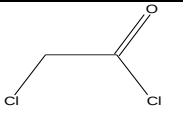


R= Where,

R		R	

(2a-f)

A		D	

B		E	
C		F	

(2a). *N*-{[5-(pyridine-4-yl)1,3,4-oxadiazole-2-yl] carbamothioyl}benzamide:

This compound isolated as brown colour solid with 76% yield, m.p. 134-136 ^{0}C; IR (KBr cm^{-1}): 3500 (NH- str.), 1718 (C=O), 1376 (C=S), 1509 (C=C str.), 3262 (C-H str. of Aromatic ring), 1660 (C=N str.), 1263 (C-C) : 1H NMR (400 MHz, DMSO-d6) : 7.46-7.52 (5H, Benzene ring, C(=O)N); 7.63-7.69 (4H, 4-Pyridine, 1,3,4-oxadiazole), 7.957 (1H, NH Secondary amide), 4.00 (1H, NH Aromatic) : MS (m/z+) [M+] 324; For $C_{15}H_{11}N_5O_2S$.

(2d). 2,4-dichloro-N-{[5-(pyridine-4-yl)1,3,4-oxadiazole-2-yl] carbamothioyl}benzamide:

This compound isolated as brown colour solid with 74% yield, m.p. 166-168^{0}C; IR (KBr cm^{-1}): 3407 (NH str.), 1670 (C=C str.), 1105 (C=S), 1922 (C=O str.), 2981 (C-H str. of Aromatic ring), 1201 (C-C), 1536 (C=N str.), 763 (C-Cl) : 1H NMR (400 MHz, DMSO-d6) : 7.46-7.51 (3H, Benzene ring, C(=O)N); 7.58-7.93 (4H, 4-Pyridine, 1,3,4-Oxadiazole), 7.96 (1H, NH Secondary amide), 3.809 (1H, NH Aromatic) : MS (m/z+) [M+] 394; For $C_{15}H_9Cl_2N_5O_2S$.

(2e). 4-fluro -N-{[5-(pyridine-4-yl)-1,3,4-oxadiazole-2-yl] carbamothioyl}benzamide:

This compound isolated as brown colour solid with 82% m.p. 112-114^{0}C; IR (KBr cm^{-1}): 3585 (NH str.), 1600 (C=C str.), 1160 (C=S), 1882 (C=O), 2873 (C-H str. of Aromatic), 1203 (C-C), 1533 (C=N str.), 811 (C-F) : 1H NMR (400 MHz, DMSO-d6) : 7.28- 7.50 (4H, Benzene ring, C(=O) N, F), 7.95-8.00 (4H, 4-Pyridine, 1,3,4-Oxadiazole), 8.027 (1H-NH Secondary amide), 3.499 (1H, NH Aromatic) : MS (m/z+) [M+] 344; For; $C_{15}H_{10}FN_5O_2S$.

(2f). 2-chloro-N-{[5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl] carbamothioyl}acetamide:

This compound isolated as brown colour solid with 80%.m.p 126-128^{0}C; IR (KBr cm^{-1}): 3345 (NH str.), 1577 (C=C str.), 1295 (C=S), 1722 (C=O), 1508 (C=N str.), 719 (C-Cl) : 1H NMR (400 MHz, DMSO-d6) : 7.40-7.52 (4H, 4-Pyridine, 1,3,4-Oxadiazole), 7.94 (1H, NH Secondary amide), 4.51 (1H, NH-Aromatic), 3.42 (1H, Cl) : MS (m/z+) [M+] 296; For $C_{10}H_8ClN_5O_2S$.

Anti-inflammatory activity:

a) In vitro Anti-inflammatory evaluation

The compounds have been bought from research lab, Mumbai, India. And the solvent were purified by distillation process. The test of 1,3,4-oxadaizole derivatives were synthesized in our laboratory. DMSO as a solvent Diclofenac sodium (50 µg/ml) as a standard 50 µg/ml, 100 µg/ml as test compound UV spectrophotometer instrument for absorbance.

Method: Synthesized compound's In vitro anti-inflammatory action by protein denaturation method. (2a-f)

The sample mixture (10ml) consist of 0.4ml of albumin egg (from new egg), 0.6ml of phosphate buffered solution (pH 6.4) and 4ml of changing concentration of test samples so that final concentration become 50 µg/ml ,100 µg/ml. same volume of DMSO act as control. Then the sample incubation of compounds (7°c ± 2) for 15-20 minutes. and then heat for 5min at 700C. After cooling sample, its absorption was recorded at 660nm (JASCO UV Spectrophotometer) by using a blank carrier. Their viscosity was determined with the support of

Ostwald viscometer. Sodium Diclofenac at last level of 50µg/ml ,100 µg/ml was used for the conventional medicine and similar treatment for absorption and viscosity determination. by making use of the corresponding formula, the percent protein cell lysis inhibition calculated.

$$\% \text{ inhibition protein denaturation} : \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

a) In vivo- Anti-inflammatory evaluation of synthesized compound.

Animals Used for anti-inflammatory activity

Animals Wistar rats (100–120gm) of either sex was purchased from the National Biomedical Research center Animal House in Pune. The animals the normal experimental terms (12 hours) were preserved light/dark cycles at 22 ± 2?C), humidity 45-55% and feeding normal pellets for animals (National Institute of Biosciences, Pune, Maharashtra) and water. The protocol and procedure was approved by the CPCSE (Reg.No. 1290/PO/Re/S/09/CPCSEA 16/03/19.)

In vivo- Anti-Inflammatory Activity. (2a-f)

Anti-inflammatory activity was assessed by carrageenan-induced paw edema test using group of Wistar rats weighing 100-120gm each and 2 rats per group as standard, test and control. In each rat, the hind paw edema was caused by 0.1mL of carrageenan suspension (1.0 percent w / v in 0.9 percent saline) sub-plantar injection 1 h going to follow oral administration sample compounds and conventional drug. The linear circumference of the paw was evaluated immediately prior to injection, using the cotton thread technique at an average of 30 min for 4 h. Anti-inflammatory activity is determined by assessing the edema size decrease and determining the percentage of edema inhibition. An indication of anti-inflammatory activity is a decrease in edema relative to control and an increase in percent inhibition in the processed groups. The mean % inhibition of Diclofenac and tested compound at 25 mg kg-1 concentration Control was compared and repeated measures ANOVA with Dunnet's test.

b) Antimicrobial activity

The filter paper disc method was performed in nutrient agar for bacterial and Sabraud's agar for fungi. These agar media were inoculated with 0.5 mL of the 24 hr liquid culture. Filter paper disc (5 mm diameter) saturated with each compound solution (500µg/mL of DMSO) were placed on indicated agar media. The incubation time was 48hr at 36^{0}c. Amoxicillin (10mg/mL of DMSO) was used as standard against Fluconazole (10mg/ML of DMSO) was used as standard against fungi. The diameter of inhibition zone in mm were measured and recorded in Tables 3 and 4 as antibacterial and antifungal, respectively. [2,7]

Table No. 3 Antimicrobial activity of the synthesis compounds (a-f) against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Candida albicans.

Compounds	Zone of inhibition (mm)				
	Staphylococcus aureus	Bacillus subtilis	Escherichia coli	Pseudomonas aeruginosa	Candida albicans

2a	++	+++	++	-	++
2b	-	++	+++	+	-
2c	+	++	++	+	+
2d	++	-	-	++	-
2e	-	+	++	+	-
2f	+++	+	++	++	++
Amoxicillin	+++	++	++	+	+++
Fluconazole	-	-	-	-	++

*Highly active = +++ (Inhibition zone > 12 mm)
Moderately active = ++ (Inhibition zone 9-12 mm)
Slightly active = + (Inhibition zone 6-9 mm)
Inactive = - (Inhibition zone < 6 mm)

III.CONCLUSION

This investigation proposes, economical, cheaper and useful method for the N-{[5-(substituted)-1,3,4-oxadiazole-2-yl] carbamothioyl} derivatives possessing anti-inflammatory , antimicrobial properties. Exhaustive pharmacological studies have been conducted with the 1, 3, 4-oxadiazole derivative. The 5-position are an extremely important site of molecular modification, which play a dominant role in determining the pharmacological activates of 1, 3, 4- oxadiazole derivatives. This new class of heterocycles, exhibit a significant anti-inflammatory activity. Hence, it can concluded that, this new class of compounds certainly holds a greater promise in discovering a potent anti-inflammatory agent. The compound 2f shows potent antimicrobial, in vivo anti-inflammatory and in vitro anti-inflammatory activity.

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V. REFERENCES

[1]. Allison MC, Howatson AG, Torrance CJ, Lee FD, Russell IG., 1992. N Engl J Med. 327, 749-754.
[2]. Bauer AW, Kirby W.W.M, Sherris J.C, Turck M., Clin Am. J., 1996. Pathol. 45, 493.
[3]. Bekhit AA, Tarek AA., 2004. Bioorg Med Chem.12, 1935-1945.
[4]. Bhansali S, Kulkarni V., 2015. Der Pharma Chemica. 7(1): 156.
[5]. Chopade A, Sayyad F, Naikwade N., 2014. Pharmacol Rep. 353-362.
[6]. Chopade A, Sayyad F., 2015. Phytother Res Int. 29, 1202–1210.
[7]. Kalyoncuoglu N, Rollas S, Sur-Altiner D, Yegenoglu, Ang O., 1992. Pharmazie, 47, 769.
[8]. Raju G, Swathi K, Padma K, Sravani Y, Rao N., 2015. Asian J. Pharm. Sci. 3(1), 20-30.
[9]. Ranian K, Parmar S, Salzman S., 1998. J. Pharm. Sci. 78(12), 999-1002.
[10]. Rathore A, Sudhakar R, Ahsan M, Ali A, Subbarao N, Jadav S, Umar S, Shahar Yar M., 2016. Bioorg Med Chem. 66, 1-35.

[11]. Ravindra KC, Vagdevi HM, Vaidya VP, Padmashali B., 2006. Indian J Chem B. 45, 2506-2511.
[12]. Singh AK, Parthsarthy R, Lohani M., 2012. J Chem Pharm Res. 4, 779-782.
[13]. Sondhi M, Dinodia M, Singh J, Rani R., 2007. Curr Bioact Compd. 3, 91-108.
[14]. Talley JJ, Brown DL, Carter JS, Graneto MJ, Koboldt CM, Masferrer JL, Perkins WE, Rogers RS, Shaffer AF, Zhang YY, Zweifel BS, Seibert K., 2000. J Med Chem. 43, 775-777.
[15]. Vishwanathan B, B.M. Gurupadayya., 2015. Int. J. Pharm. 2, 41-51.

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