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Research Article

Synthesis, Characterization and *invitro* Anti- inflammatory activity of 1, 3, 4-Oxadiazole derivatives

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ABSTRACT

Oxadiazole derivatives have played a vital part in the development of heterocyclic compounds. In this present work, a series of 5-(2-aminophenyl)-1,3,4-oxadiazole-2(3*H*)-thione derivatives (1-10) have been synthesized by Mannich reaction. The reaction progress of the synthesized compounds was checked by TLC. The structures of the newly synthesized compounds were confirmed by IR and ^{1}H NMR spectral data. The *in-vitro* anti-inflammatory activity of 1, 3, 4-oxadiazole compounds (1-10) was assessed by HRBC Membrane Stabilization Method. Among the newly synthesized 1,3,4-oxadiazole derivatives, compounds OFP, OAP, OBNP, OPBNP, ORP, OUP, OPClBP, OFD, OAD and OBND possessed highly significant anti-inflammatory activity at a dose of 1000µg/ml when compared with standard, Diclofenac potassium.

Keywords: 1,3,4-Oxadiazole, Mannich reaction, HRBC Membrane Stabilization Method, Anti-inflammatory, Diclofenac potassium.

INTRODUCTION

The Oxadiazole types of heterocyclic compounds contain oxygen and two nitrogen atoms. Various 1,3,4-oxadiazoles have been reported to have a broad biological activities including analgesic[1], anti-inflammatory[2], anticancer[3], anti-HIV[4], anti-parkinson[5], antibacterial[6], antifungal[7] and anti-tubercular[8] agents. These observations prompted to synthesis 5-(2-aminophenyl)-1,3,4-oxadiazole-2(3H)-thione and followed by a novel series of mannich bases of 5-(2-aminophenyl)-1,3,4-oxadiazole-2(3H)-thione.

Oxadiazole is a heterocyclic nucleus and is considered to be derived from furan by replacement of two methane (-CH=) group in furan replaced by two pyridine type nitrogen. Among the methods employed in synthesis of 1,3,4-oxadiazole, cyclization reaction of acid hydrazide and its derivatives were prepared by incorporation of secondary amines with aldehydes.

Materials and Methods

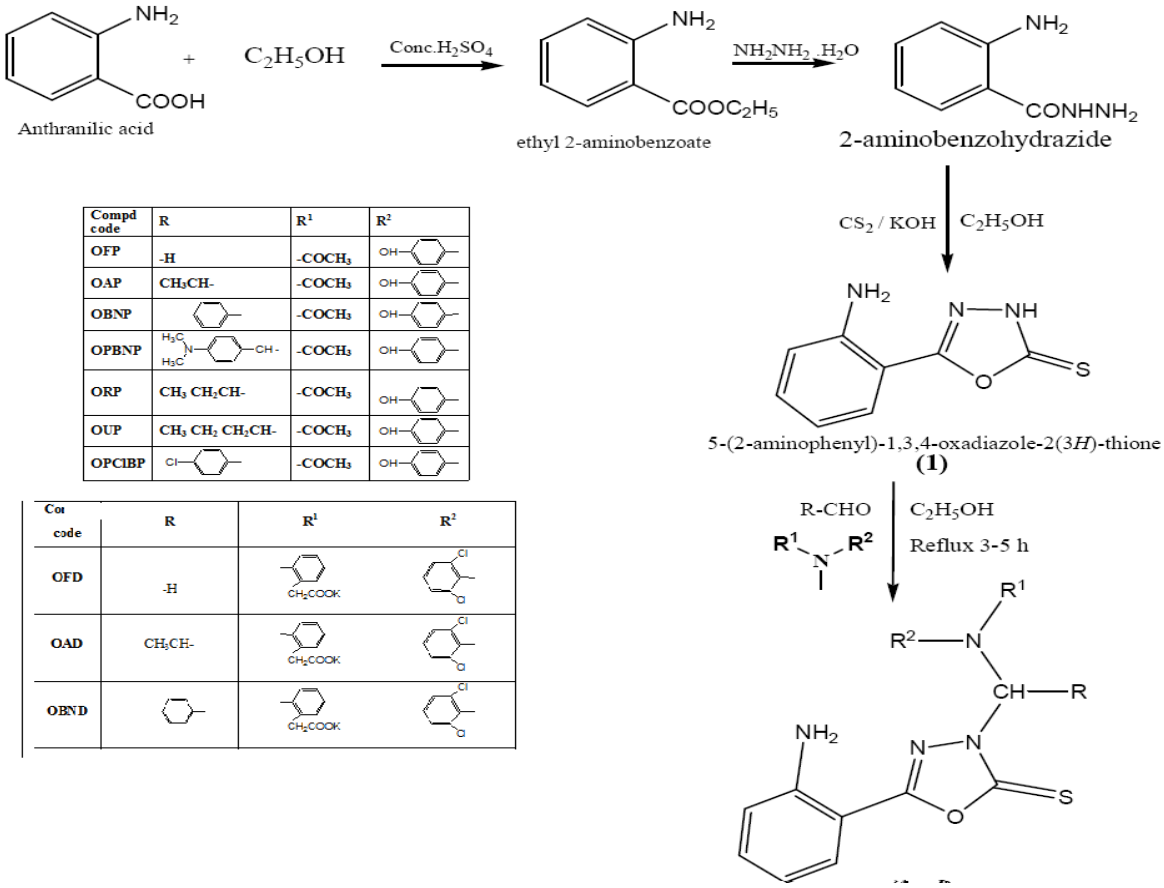
Synthesis of substituted 1,3,4-oxadiazole[9]

Anthranilic acid (0.01 mol) was dissolved in 20 ml ethanol. To this, concentrated sulphuric acid was added drop wise until the white precipitate was formed and it was dried. Then the residue was dissolved in ethanol and hydrazine hydrate (0.5 mol) was added to this solution with constant shaking for 10 min. The white precipitate obtained was collected by filtration and dissolved in ethanol. Potassium hydroxide (0.56 mol) was added into the above solution followed by carbon disulphide solution (0.76 mol) drop wise with constant shaking until the formation of yellow precipitate of substituted oxadiazole (1) and it was recrystallized from ethanol.

Synthesis of Mannich base substituted 1,3,4-oxadiazoles [9]

Equimolar quantities (0.01mol) of substituted oxadiazole and respective compounds containing secondary amine such as N-(4-hydroxyphenyl) acetamide, N-(2,3-xylyl) anthranilic acid and potassium 2-(2-(2,6-dichloro anilino) phenyl) acetate were dissolved in ethanol (30 ml). To the above mixture, the corresponding aldehyde (0.01mol) such as formaldehyde and acetaldehyde was added and reflux for 3-5 h. The content was kept overnight in the freezer. The respective compound obtained was recrystallised from ethanol.

Melting point was determined on electrical melting point apparatus by open-ended capillary tube. The purity of the compounds were checked by TLC using Silica Gel as stationary phase and chloroform-methanol (8:2) as eluent and the spots were visually detected in an Iodine chamber[10]. The structure of the synthesized compounds was elucidated by IR spectra in υmax (cm^{-1}) on FT-IR (Shizmadu-8400 series) using KBr disc technique [11,12] and ^1H NMR spectra in δ units (ppm) relative to an internal standard of tetramethylsilane on ^1H NMR (Brucker 400 MHz) in DMSO-d6 [13]. The synthetic method is depicted below.



Compd code	R	R^1	R^2
OFP	-H	$-COCH_3$	OH
OAP	CH_3CH-	$-COCH_3$	OH
OBNP		$-COCH_3$	OH
OPBNP	H_3C, H_3C, N, CH-	$-COCH_3$	OH
ORP	CH_3 CH_2CH-	$-COCH_3$	OH
OUP	CH_3 CH_2 CH_2CH-	$-COCH_3$	OH
OPClBP	Cl	$-COCH_3$	OH

Co code	R	R^1	R^2
OFD	-H	CH_2COOK	Cl, Cl
OAD	CH_3CH-	CH_2COOK	Cl, Cl
OBND		CH_2COOK	Cl, Cl

5-(2-aminophenyl)-1,3,4-oxadiazole-2(3H)-thione

$C_8H_7N_3OS$; Yield: 85.5%; mp: 155-157^{0}C; Rf: 0.45; IR (KBr,υmax cm^{-1}): 3482.24 (NH_2), 3154 (NH), 1624.32 (N –N), 1224.21 (C=S), 1224.21 (C=S); ^{1}H NMR (δ ppm): 3.8 (s,NH_2, 2H), 6.0 (s, NH, 1H), 6.6 – 7.8 (m, ArH, 4H).

N-((5-(2-aminophenyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl)methyl)-N-(4-hydroxyphenyl) acetamide

$C_{17}H_{16}N_4O_3S$; Yield: 71.78%; mp: 178-180^{o}C; Rf 0.581.IR (KBr, υmax cm^{-1}): 3426 (OH), 3343 (NH_2), 1651 (N –N),1457(CH_3), 1426 (CH_2) 1120 (C=S); ^{1}H NMR (δ ppm): 2.2(s,3H, CH_3), 3.5 (s, 2H, NH_2), 4.7(s, 2H, CH_2), 6.6 – 7.4 (m,8H, ArH), 9.8 (s, 1H, OH).

N-(1-(5-(2-aminophenyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl)ethyl)-N-(4-hydroxyphenyl) acetamide

$C_{18}H_{18}N_4O_3S$; Yield: 70.76%; m.p.198-200^{o}C; Rf 0.641. IR (KBr, υmax cm^{-1}): 3386 (OH), 3286 (NH_2), 2084 (CH),1605(N-N), 1454 (CH_3), 1422 (CH_2), 1122 (C=S); ^{1}H NMR (δppm): 1.28 (d, 3H, CH_3), 1.98 (s, 3H, CH_3), 3.42 (s, 2H,NH_2), 4.64(q, 1H, CH), 6.46 – 7.44 (m, 8H, ArH), 9.62 (s,1H, OH).

Potassium-2-(2-(((5-(2-aminophenyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl)methyl)(2,6-dichloro phenyl) amino)phenyl) acetate

$C_{23}H_{17}N_4O_3SCl_2K$; Yield: 69.19%; m.p. 210-212^{o}C; Rf 0.612.IR (KBr, υmax cm^{-1}): 3257 (NH_2), 1650 (C=O), 1615 (N-N),1436(CH_2), 1120(C=S); 1H NMR (δ ppm) : ^{1}H NMR (δppm): 3.44 (s, 2H, CH_2), 4.62 (s, 2H, CH), 4.26 (s, 2H, NH_2),6.52 – 7.48 (m, 11H, ArH).

Potassium-2-(2-((1-(5-(2-aminophenyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl)ethyl)(2,6-dichloro phenyl) amino)phenyl) acetate

$C_{24}H_{19}N_4O_3SCl_2K$; Yield: 59.18%; m.p. 186-188^{o}C; Rf 0.812.IR (KBr, υmax cm^{-1}): 3272 (NH_2), 1649 (C=O), 1622 (N-N),1456 (CH_3), 1449 (CH_2), 1121(C=S); ^{1}H NMR (δ ppm): 1.26(d, 3H, CH_3), 3.52 (s, 2H, CH_2), 3.98 (q, 1H, CH), 4.12 (s, 2H,NH_2), 6.36 – 7.24 (m, 11H, ArH), 9.62 (s, 1H, OH).

2-(((5-(2-aminophenyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl)methyl)(2,3-dimethylphenyl) amino benzoic acid

$C_{24}H_{22}N_4O_3S$; Yield: 68.70 %; m.p 254-256^{o}C; Rf 0.840. IR (KBr, υmax cm^{-1}):.3335(OH), 3226(NH_2), 1675(C=O), 1615 (N-N), 1459 (CH_3), 1439 (CH_2), 1190 (C=S); ^{1}H NMR(δ ppm) : 2.28 (s, 3H, CH_3), 4.46(s, 2H, CH_2), 3.82 (s, 2H,NH_2), 6.16 – 7.64 (m, 11H, ArH), 10.46 (br, 1H, OH).

2-((1-(5-(2-aminophenyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl)ethyl)(2,3-dimethylphenyl) amino) benzoic acid

$C_{25}H_{24}N_4O_3S$; Yield: 70.76 %; m.p. 244-246^{o}C; Rf 0.770. IR(KBr, υmax cm^{-1}):. 3343 (OH), 3286 (NH_2), 1650 (C=O),1628 (N-N), 1449 (CH_3), 1428 (CH_2), 1123 (C=S); ^{1}H NMR (δppm) 1.24 (d, 3H, CH_3), 2.26 (s, 3H, CH_3), 3.72 (s, 2H,NH_2), 6.08 – 8.24 (m, 11H, ArH), 10.64 (br, 1H, OH).

PHARMACOLOGICAL STUDIES

The *In-Vitro* Anti-Inflammatory Effect of Novel Oxadiazole Derivatives By HRBC Membrane Stabilisation Method[14, 15, 16]

Inflammation is a tissue-reaction to infection, irritation or foreign substance. It is a part of the host defense mechanism but when it becomes great it is hopeless condition. Aging is also considered to be an inflammatory response. There are several tissue factors or mechanisms that are known to be involved in the inflammatory reactions such as release of histamine, bradykinin and prostaglandins. The development of non-steroidal anti-inflammatory agents in recent years have contributed a lot in not only overcoming the human sufferings such as arthiritis but also has helped in understanding the tissue mechanism of action of anti-inflammatory drugs. The synthesized compounds were used for this study. They were made into doses of 1000µg/ml with 1% acacia solution. Diclofenac sodium is taken as standard.

The reaction mixtures (4.5ml) consisted of 2ml of hypotonic saline (0.25% NaCl), 1ml of 0.15 M phosphate buffer (pH 7.4, 0.15 M), and 1ml of test solution (1000µg /ml) in normal saline. 0.5 ml of 10% HRBC in normal saline was added. For control tests, 1ml of isotonic saline was used instead of test solution while product control tests lacked red blood cells. The mixtures were incubated at 56^{0}C for 30 minutes. Then they were cooled under running tap water and centrifuged at 3000rpm for 20 minutes.

The absorbance of the supernatants was read at 560nm. Percentage membrane stabilizing activity was calculated as follows.

$$\text{Percentage stabilization} = \frac{100 - (\text{OD of sample} - \text{OD of product control}) \times 100}{\text{OD of test control}}$$

The control represents 100% lysis.

HRBC Membrane Stabilization Method[15, 16]:

Alseviers Solution: 2gm dextrose + 0.8gm sodium citrate + 0.05gm Citric acid + 0.42gm sodium chloride

↓

Make up with distilled water to 100ml

↓

0.5ml OF 10% HRBC Suspension: 3ml of blood + 3ml of Alseviers solution

Centrifuge at 3000 rpm for 20 minutes

↓

Packed cells were washed with isotonic saline

↓

10% v/v Suspension of the packed cells was made with isotonic saline.

Hypotonic Saline: 0.36 gm of sodium chloride in 100ml of distilled water.

Isotonic Saline: 0.85gm of sodium chloride in 100ml of distilled water.

Phosphate Buffer (pH 7.4, 0.15 M): 2.38gm of disodium hydrogen phosphate + 0.19gm of potassium dihydrogen phosphate + 8gm of sodium chloride

↓

Make up with 100ml of distilled water

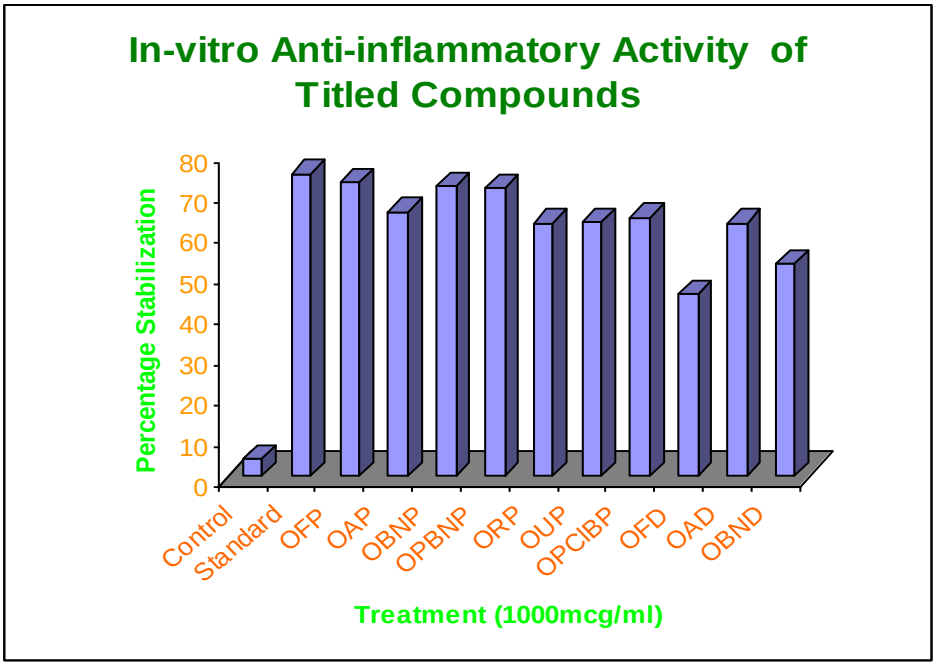
Table 1 ***In Vitro*** **ANTI INFLAMMATORY ACTIVITY OF TITLED COMPOUNDS**

S.No	Treatment	Percentage Stabilization (Mean ± SEM)
1.	OFP	72.50 ± 0.9400**
2.	OAP	65.16 ± 0.5416**
3.	OBNP	71.50 ± 0.8146**
4.	OPBNP	71.16 ± 0.5416**
5.	ORP	62.30 ± 0.9583**
6.	OUP	62.66 ± 0.9166**
7.	OPClBP	63.78 ± 0.5426**
8.	OFD	44.80 ± 0.9400**
9.	OAD	62.30 ± 0.6666**
10.	OBND	52.40 ± 0.7812**
	Control	4.3262 ± 0.6780
	Standard	84.50 ± 0.5000**

P < 0.001 indicates the significant difference compared with control
** Highly significant

BIO STATISTICAL ANALYSIS

All the data (Mean ± SEM) were evaluated by student's "*t*" test[17, 18] and the probability was determined for all the newly synthesized compounds and the results were tabulated in the following Table. 1



RESULTS AND DISCUSSION

The substituted oxadiazole, 5-(2-aminophenyl)-1,3,4-oxadiazole-2(3H)-thione was synthesized by reacting anthranilic acid with ethanol in presence of sulphuric acid followed by reacting with hydrazine hydrate, carbon disulphide and alkali. The titled oxadiazole derivatives were synthesized by making substitution at free N-(3H) position of substituted oxadiazole, 5-(2-aminophenyl)-1,3,4-oxadiazole-2(3H)-thione through Mannich condensation with secondary amine bearing compounds such as N-(4-hydroxyphenyl) acetamide, N-(2,3-xylyl) anthranilic acid, potassium 2-(2-(2,6-dichloro anilino) phenyl) acetate and series of aldehydes like formaldehyde, acetaldehyde. The synthesized compounds were characterized by various methods such as melting point, IR Spectroscopy, NMR spectroscopy. The compounds purity was further established by chromatographic method (TLC).

In-Vitro Anti-Inflammatory Activity:

Among these synthesized compounds, compounds OFP, OAP, OBNP, OPBNP, ORP, OUP, OPClBP, OFD, OAD and OBND were screened for *in-vitro* anti-inflammatory activity by HRBC Membrane Stabilization Method at a concentration of 1000µg/ml. All the above evaluated compounds possessed highly significant anti-inflammatory activity at a dose of 1000 µg/ml when compared with standard, Diclofenac potassium 1000µg/ml.

CONCLUSION

The titled oxadiazole derivatives were synthesized by making substitution at free N-(3H) position of 5-(2-aminophenyl)-1,3,4-oxadiazole-2(3H)-thione through Mannich condensation. All the evaluated compounds possessed highly significant anti-inflammatory activity at a dose of 1000µg/ml when compared with standard, Diclofenac potassium 1000µg/ml.

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