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Synthesis and *in vitro* screening of some 1,3,4-oxadiazole derivatives as lipoxygenase inhibitors

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ABSTRACT

1,3,4-oxadiazole derivatives of some classical Non Steroidal Anti-inflammatory Drugs were synthesized and investigated for the lipoxygenase inhibitory activity *in vitro*. The target compounds were obtained by cyclodesulfurization of the corresponding thiosemicarbazides using I_2/NaOH. The intermediates were readily accessible through conversion of the carboxylic acid group to the respective acid hydrazides followed by treatment with phenyl isothiocyanate to yield corresponding thiosemicarbazides. The constitution of the products was confirmed by spectroscopic and elemental analysis. All the synthesized oxadiazole derivatives exhibited significant lipoxygenase inhibitory activity.

Key words: 1,3,4-oxadiazole; Lipoxygenase inhibition; NSAIDs.

INTRODUCTION

Non Steroidal Anti-inflammatory Drugs (NSAIDs) are widely used in the treatment of rheumatoid arthritis and inflammatory disease. However in addition to cyclo-oxygenase inhibition which is the principle mechanism for analgesic and anti-inflammatory properties of NSAIDs, they have also been associated with nephrotoxicity and gastrointestinal side effects[1]. Leukotrienes derived from lipoxygenase also play an important in the initiation of inflammation and pain along with prostaglandins

Literature reveals that the replacement of carboxylic acid functionality of some NSAIDs with tetrazole group not only retained cyclo-oxygenase inhibitory activity of the parent drug but also introduced lipoxygenase inhibition[2,3]. Several other heterocyclic compounds including di-ter-butyl phenyl thiadiazole, oxazole, thiazole, imidazole, and substituted oxadiazole derivatives have been proved to be potent[4-10].

In the light of these findings, the present study was undertaken in which attempts were made to convert the carboxylic acid functionality of some classical NSAIDs to corresponding 1,3,4-oxadiazole system and to explore the lipoxygenase inhibitory activity *in vitro*.

MATERIAL AND METHODS

In present investigation the gift sample of novel NSAIDs were procured from the respective manufacturer and the enzyme hporygenase was procured from Sigma Chemical Co. USA.

Lipoxygenase inhibitory activity[12]

The lipoxidase activity was determined by the measurement of spectral absorbance of the conjugated hydroperoxides produced by lipoxidase catalysis. The lipoxygenase inhibitory activity was determined by *in vitro* method. A direct spectrophotometric assay employing increase in absorbance at 234 nm as a function of time where soyabean lipoxidase as a representative of

5-lipoxygenase enzyme and linoleic acid as the substrate were used. For calculating the enzyme activity maximum absorbance (A) at 234 nm per minute between 1 – 3 minute intervals was noted and the enzyme activity was calculated by formula,

$$\text{Enzyme activity(unit mg solid)} = \frac{\text{A at 234/minute}}{0.004 \times \text{mg enzyme/3.0mL reaction mixture}}$$

The percent lipoxygenase activity inhibited in the presence of 1,3,4-oxadiazole derivatives is presented in Fig 1.

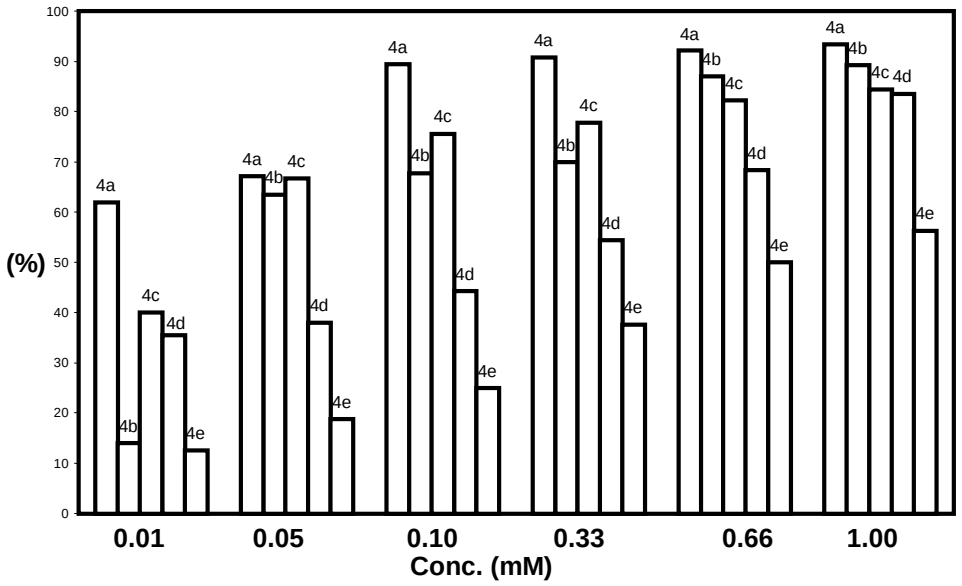


Fig. 1: Lipoxygenase inhibitory activity of synthesized compounds 4a – 4e

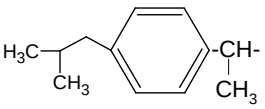
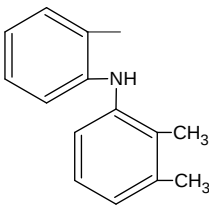
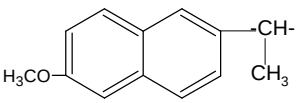
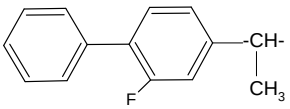
EXPERIMENTAL

Melting points were determined in open capillaries using paraffin bath and are uncorrected. Purity of the compounds was checked by TLC using silica gel-G plates. IR spectra (KBr disc) were recorded on Perkin Elmer RXI-FTIR system. Proton Magnetic Resonance spectra (^{1}HNMR) were recorded on Bruker AC-300F NMR spectrometer (300MHz) using $CDCl_3$ and DMSO-d_6 as solvent and Tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on Jeol SX 102/DA-6000 mass spectrometer/ data system using Argon / Xenon (6Kv, 10mA) as FAB gas. The accelerating voltage was 10 kV and the spectra were recorded at room temperature using *m*-nitrobenzyl alcohol (NBA) matrix. Elemental analysis was carried out with Carlo Erba 1108 analyzer; all the compounds gave satisfactory elemental analysis within ±0.4% of the theoretical values.

Synthesis of methyl ester 1: General procedure[11]

To a solution of appropriate acidic NSAIDs (0.025 mol) in methanol (20 mL) p-toluene sulphonic acid (1 gm) was added and the reaction mixture was refluxed for 4 hours, the reaction was monitored by TLC. After completion of reaction the mixture was cooled to room temperature and then was poured

Table 1: Characterization table for the compounds 4a – 4e

Comp.	Ar	Yield (%)	m.p. (^{o}C)	R_f	λ max	% Nitrogen Found (Cal.)
4a		78	194 – 196	0.55	329	13.84 (13.62)
4b		74	150 – 152	0.62	337	13.05 (13.07)
4c		50	215 – 218	0.80	340	15.65 (15.71)
4d		56	233 – 235	0.88	334	12.66 (12.61)
4e		50	218 – 222	0.70	341	11.43 (11.69)

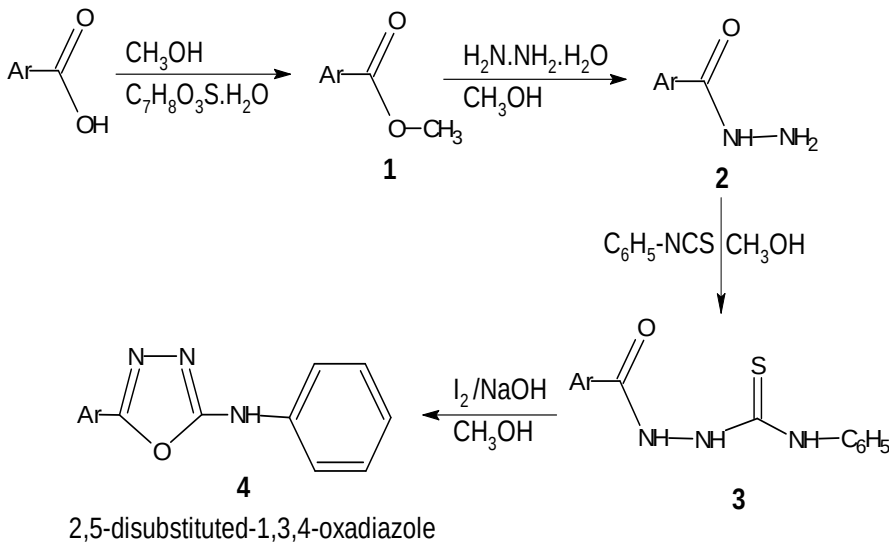
in sufficient quantity of ice-water. The solids that separated were filtered and dissolved in sufficient quantity of ice-water. The solids that separated were filtered and dissolved in sufficient quantity of ehteter. The filtrate was also extracted with sufficient ether; the combined ethereal extracts were washed with 10% sodium bicarbonate solution. The crude product obtained after removal of ether was recrystallized by using methanol.

Methyl 2-(2-(2,6-dichlorophenyl amino)phenyl acetate 1a

Yield 90%, m.p. 104 ^{O}C R_f 0.91; IR (KBr, cm^{-1}) 3325 (N-H str), 2870 (C-H str), 1740 (C=O str), 1410 (C-O str). ; ^{1}HNMR ($CDCl_3$/ $DMSO_{d6}$): V 3.1 (s, 3H, OCH_3), 4.3 (s, 2H, CH_2), 6.5 – 7.1 (m, 7H, Ar-H), 8.3 (s, 1H, NH)

Synthesis of acid hydrazide 2: General procedure[5]

To a mixture of corresponding methyl ester (0.01 mol) in methanol (15mL), 99% hydrazine hydrate (3.0 gm; 0.06 mol) was added and the reaction mixture was refluxed for 3 hours, the reaction was monitored by TLC. After completion of reaction the resulting clear solution was then poured onto 200gm of crushed ice. The separated solids were filtered, washed thoroughly with cold water, dried and recrystallized from methanol.



Scheme 1.

2-(2-(2,6-dichlorophenylamino)acetohydrazide 2a

Yield 71%, m.p. 155 °C R_f 0.42; IR (KBr, cm^{-1}) 3410 (N-H str), 2860 (C-H str), 1735 (C=O str). ; ^{1}HNMR ($CDCl_3$/ $DMSO_{d6}$): V 3.4 (s, 2H, -CH_2), 4.5 (t, 1H, -NH), 6.1 (d, 2H, -NH_2), 6.8 – 7.5 (m, 7H, Ar-H).

Synthesis of substituted thiosemicarbazide 3: General procedure[13]

To a solution of corresponding acid hydrazide (0.01 mol) in hot methanol (25 mL) was added equimolar amount of phenyl isothiocyanate in methanol (5 mL) and the mixture was refluxed with stirring for 2 hours, the reaction was monitored by TLC. After completion of reaction the mixture was cooled in ice bath, the solids separated were filtered, dried and recrystallized from methanol / toluene mixture (1:1).

1-(2-(2-(2,6-dichlorophenylamino)phenyl) acetyl) -4-phenylthiosemicarbazide 3a

Yield 70%, m.p. 165 °C R_f 0.60; IR (KBr, cm^{-1}) 3460 (N-H str), 1725 (C=O str0, 1310 (C=S str); ^{1}HNMR ($CDCl_3$/ $DMSO_{d6}$): V 3.5 (s, 2H, -CH_2), 6.2 – 7.9 (m, 12H, Ar-H), 8.6 (d, 3H, >NH).

Synthesis of 2,5-disubstituted-1,3,4-oxadiazole 4 : General procedure

To a stirred and cooled (0–5 °C) solution of respective thiosemicarbazides (0.01 mol) in methanol (50 mL) was added 2N sodium hydroxide solution until the solution acquired pH 9.0. Iodine in potassium iodide solution (5%) was added drop wise with stirring at room temperature until the yellow colour of iodine persisted. The reaction mixture was cooled in ice bath and the solids precipitated were filtered washed with cold water, dried and recrystallized from methanol: hexane (1:1) mixture.

5-(2-(2,6-dichlorophenylamino)benzyl)-N-phenyl-1,3,4-oxadiazole-2-amine 4a

Yield 78%, m.p. 194 -196 °C R_f 0.55; IR (KBr, cm^{-1}) 3367 (NH str), 1622 (>C=N str), 1565 (NH bend), 1290 (C-N str), 1089 (C-O str) ; ^{1}HNMR ($CDCl_3$): V 3.7 (s, 2H, -CH_2), 6.9 (s, 2H, -NH-), 7.1 – 8.2 (m, 12H, Ar-H) ; Mass (FAB): 411 (M^+, 33.3 %), 236 (Base peak 100 %) ; UV (Acetone) : 329 nm.

5-(1-(4-isobutylphenyl) ethyl-N-phenyl-1,3,4-oxadiazol-2-amine 4b

Yield 74%, m.p. 150-152 °C, R_f 0.62; IR (KBr, cm^{-1}) 3343 (NH str), 1625 (>C=N str), 1544 (NH bend), 1311 (C-N str), 1067 (C=O str); ^{1}HNMR ($CDCl_3$ / $DMSO_{d6}$): V 1.4 (d, 6H, -CH_3), 2.2 (m, 1H, >CH-), 4.1 (q, 1H, >CH-CH_3), 6.4 (s, 1H, -NH-), 6.8 – 7.9 (m, 9H, Ar-H); Mass (FAB): 321 (M^+, 15 %), 188 (Base peak 100 %) ; UV (Acetone) : 337 nm

5-(2-(2,3-dimethylphenylamino) -N-phenyl-1,3,4-oxadiazol-2-amine 4c

Yield 50%, m.p. 215-218 °C, R_f 0.80; IR (KBr, cm^{-1}) 3215 (NH str), 1654 (>C=N str), 1546 (NH bend), 1255 (C-N str), 1067 (C=O str) ; ^{1}HNMR ($CDCl_3$/ $DMSO_{d6}$): V 2.4 (s, 4H, -CH_3), 6.7 (s, 2H, -NH-), 7.2 – 8.1 (m, 12H, Ar-H) ; Mass (FAB): 356 (M^+, 12 %), 209 (Base peak 100 %) ; UV (Acetone) : 340 nm

5-(1-(2-methoxynapthalen-6-yl) ethyl) -N-phenyl-1,3,4-oxadiazol-2-amine 4d

Yield 56%, m.p. 233-235 °C, R_f 0.88; IR (KBr, cm^{-1}) 3229 (NH str), 1603 (>C=N str), 1549 (NH bend), 1330 (C-N str), 1091 (C=O str) ; ^{1}HNMR ($CDCl_3$/ $DMSO_{d6}$): V 1.6 (d, 3H, -CH_3), 3.5 (s, 3H, -OCH_3), 4.2 (q, 1H, >CH-), 6.2 (s, 1H, -NH-), 6.5 – 7.9 (m, 11H, Ar-H) ; Mass (FAB): 345 (M^+, 9 %), 160 (Base peak 100 %) ; UV (Acetone) : 334 nm

5-(2-(2-flurobiphenyl-4-yl) ethyl) -N-phenyl-1,3,4-oxadiazol-2-amine 4e

Yield 50%, m.p. 218-222 °C, R_f 0.70; IR (KBr, cm^{-1}) 3212 (NH str), 1658 (>C=N str), 1546 (NH bend), 1312 (C-N str), 1090 (C=O str) ; ^{1}HNMR ($CDCl_3$/ $DMSO_{d6}$): V 1.8 (d, 3H, -CH_3), 4.6 (q, 1H, >CH-), 6.8 (s, 1H, -NH-), 7.1 – 8.2 (m, 13H, Ar-H) ; Mass (FAB): 359 (M^+, 20 %), 171 (Base peak 100 %) ; UV (Acetone) : 341 nm

RESULTS AND DISCUSSION

The 2,5-disubstituted-1,3,4-oxadiazole derivatives of various Non Steroidal Anti-inflammatory Drugs were evaluated for *in vitro* activity in model of 5-lipoxygenase enzyme. All the oxadiazole derivatives were found to exhibit lipoxygenase inhibition with 4a showing maximum inhibition of 62%, 4c showed 40% inhibition, 4d showed 36% inhibition, 4b showed 14% inhibition, , and 4e showed 12% inhibition at 0.01 mM concentration. The IC-50 values of these compounds varied in the range of 0.006 to 0.6 mM concentrations, which were found from the obtained dose response curve.

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