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Synthesis and characterization and anti-inflammatory activity of 1,3,4 oxadiazole derivative

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Abstract

In the present work newer anti inflammatory agents based on oxadiazole scaffold were synthesized and evaluated. The synthesized compounds were characterized for the physicochemical properties such as melting point, colour and solubility. All the compounds were yellowish to brown in colour and were obtained in 62-69% yields using the optimized reaction conditions. The compounds were insoluble in water, methanol, soluble in chloroform and DMSO. The confirmation of the structure of the synthesized compounds was done by IR, ^{1}HNMR and mass spectral studies. All the compounds exhibited the absorption bands of C=O, C=N, C-H, C=C stretching in the IR spectra. The ^{1}HNMR spectra of all the compounds exhibited chemical shifts of aromatic protons and characteristic proton of the functional groups. The mass spectra of the compounds were examined for the presence of molecular ion peak or the isotopic peaks to confirm the formation of the compounds. The compounds were evaluated for anti-inflammatory potential using albumin denaturation method (*in vitro*). The results obtained led to the conclusion that the activity of the oxadiazole derivatives as anti-inflammatory depends on type of substitution present in the scaffold.

Keywords: Oxadiazole, anti-inflammatory, synthesis, in vitro, albumin denaturation

Introduction

Oxadiazoles are five membered heterocyclic ring systems containing two carbons, two nitrogen and one oxygen atom. They are known to exist in different isomeric forms viz., 1,2,4-Oxadiazole, 1,3,4-oxadizole, 1,2,5-oxadiazole and 1,2,3-oxadiazole (Figure 1.1) [1]. Due to a broad spectrum of activities exhibited oxadiazole has a special position in medicinal chemistry [2]. The isomer 1,2,3-oxadiazole is a slightly unstable and gets converted into a diazoketone tautomer [3]. Amongst all the isomers, 1,3,4-oxadiazole has high significance in the area of research [4].

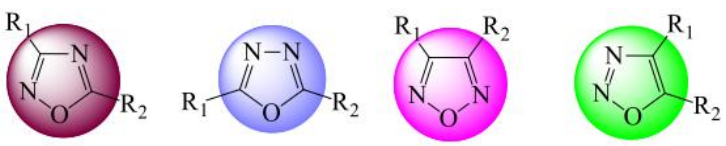


Figure 1.1Isomers of Oxadiazole

Several methods can be reported in literature for preparation of 1,3,4-oxadiazole. Commonly used method involves cyclodehydration of acid and hydrazide derivatives in the presence of dehydrating agents like phosphorus oxychloride ($POCl_3$), trifluoroacetic anhydride, thionyl chloride, polyphosphoric acid [5] (,figure 1.2).

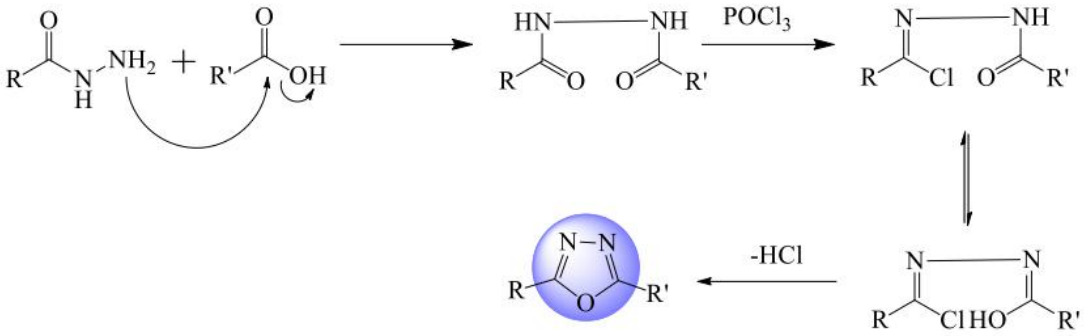
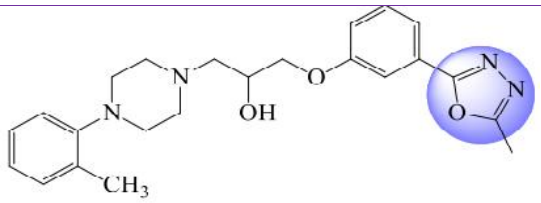
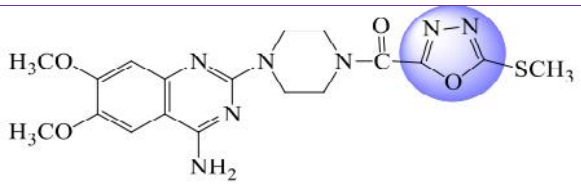
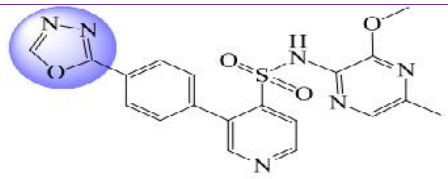
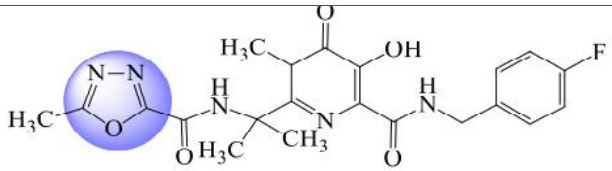


Figure 1.2 Mechanism of formation of oxadiazole

This moiety is well known to demonstrate a wide spectrum of pharmacological activities. This array includes antitubercular, analgesic, anti-inflammatory, antimicrobial, antimalarial, anti-oxidant, anticancer, antiviral and many more. This particular isoform is also found in a number of commercially available medicinal agents for treatment of several ailments (Table1).

Table 1.1 Drugs containing oxadiazole nucleus

S.No.	Drug	Structure	Therapeutic Use
1	Nesapidil		Antihypertensive
2	Furamizole		Antibacterial
3	Tiodazosin		Antihypertensive
4	Zibotentan		Anticancer
5	Raltegravir		Antireteroviral

Apart from the presence of 1,3,4-oxadiazole in these commercially available drugs, researchers are actively involved in design and development of novel 1,3,4-oxadiazole based compounds.

Experimental Work

2.1 Materials

Mefenamic acid was purchased from Yarrow Pharmaceuticals, Mumbai. All other reagents and chemicals used were of synthesis grade, purchased from Oxford Fine Chemicals, Mumbai and were used without further purification.

2.2Methods

The scheme for the synthesis of the oxadiazole derivatives was adapted from the procedures reported by Amir et al [6] and Mishra et al [7] and the scheme is depicted in Figure 2.1.

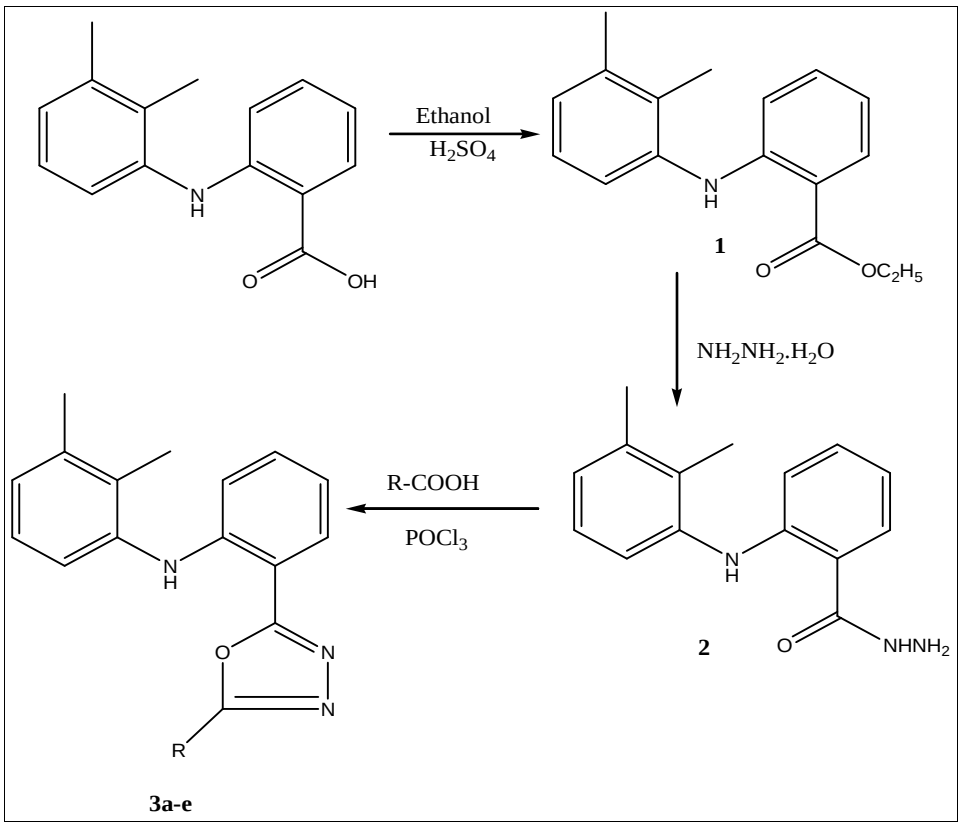


Figure 2.1Scheme for synthesis of oxadiazoles

The scheme consists of three steps leading towards the synthesis of the oxadiazole derivatives.

2.2.1 Synthesis of ethyl 2-(2,3-dimethylphenylamino)benzoate

0.1 moles of mefenamic acid was dissolved in 25 ml ethanol and the mixture was refluxed for 5h in presence of 5 drops of concentrated H_2SO_4. On cooling, a solid separated which was filtered and recrystallised using ethanol to give the product **1**. Completion of the reaction was monitored by TLC.

2.2.2 Synthesis of 2-(2,3-dimethylphenylamino)benzohydrazide

The hydrazide derivative of the mefenamic acid was synthesized by the reaction of **1** by hydrazine hydrate in presence of ethanol with catalytic amount of concentrated sulfuric acid. To 0.1 mole of the product **2** in 20 ml ethanol, 0.1 mole of hydrazine hydrate was added. To the mixture, catalytic amount of concentrated sulfuric acid was added. The mixture was refluxed until the completion of the reaction (approximately 2 hours). On cooling, a solid separated, which was recrystallized from ethanol to give the product **2**.

2.2.3 Synthesis of N-(2,3-dimethylphenyl)-2-(1,3,4-oxadiazol-2-yl)-5-substituted benzenamine

Compound 2 (0.001 mol) and the appropriate aromatic acid (0.001 mol) were dissolved in phosphorus oxychloride and refluxed for 18–26 h. The reaction mixture was slowly poured over crushed ice and kept overnight. The solid thus precipitated was filtered, washed with water, dried and recrystallized from ethanol to obtain compounds **3a-e.**

2.2.3.1 Synthesis of 3a

Compound 2 (0.001 mol) and coumaric acid (0.001 mol) were dissolved in phosphorus oxychloride and refluxed for 19 h. The reaction mixture was slowly poured over crushed ice and kept overnight. The solid thus precipitated was filtered, washed with water, dried and recrystallized from ethanol to obtain compounds **3a.**

2.2.3.2 Synthesis of 3b

Compound 2 (0.001 mol) and cinnamic acid (0.001 mol) were dissolved in phosphorus oxychloride and refluxed for 19 h. The reaction mixture was slowly poured over crushed ice and kept overnight. The solid thus precipitated was filtered, washed with water, dried and recrystallized from ethanol to obtain compounds **3b.**

2.2.3.3 Synthesis of 3c

Compound 2 (0.001 mol) and gallic acid (0.001 mol) were dissolved in phosphorus oxychloride and refluxed for 19 h. The reaction mixture was slowly poured over crushed ice and kept overnight. The solid thus precipitated was filtered, washed with water, dried and recrystallized from ethanol to obtain compounds **3c.**

2.2.3.4 Synthesis of 3d

Compound 2 (0.001 mol) and 2,4-dihydroxybenzoic acid (0.001 mol) were dissolved in phosphorus oxychloride and refluxed for 19 h. The reaction mixture was slowly poured over crushed ice and kept overnight. The solid thus precipitated was filtered, washed with water, dried and recrystallized from ethanol to obtain compounds 3d.

2.2.3.5 Synthesis of 3e

Compound 2 (0.001 mol) and 2,5-dihydroxybenzoic acid (0.001 mol) were dissolved in phosphorus oxychloride and refluxed for 19 h. The reaction mixture was slowly poured over crushed ice and kept overnight. The solid thus precipitated was filtered, washed with water, dried and recrystallized from ethanol to obtain compounds **3e.**

2.3 Chemical Characterization

All the synthesized compounds were characterized for melting point, solubility, yield and elucidation of the structure. The structure elucidation was performed by spectroscopic analysis (NMR, Mass and IR).

2.3.1 Melting point

The melting points were determined by open capillary method and are uncorrected using a electrically heated melting point determination apparatus.

2.3.2 Thin Layer Chromatography

The purity and homogeneity of the compounds was determined by thin layer chromatography, using silica gel G as the stationary phase on glass plates. Iodine vapors were used for development of the chromatogram. The solvent system used for performing the TLC of compounds was hexane: methanol in the ratio 7:3.

2.3.3 Solubility

The solubility of all the synthesized compounds was qualitatively determined in different solvents. A small amount of the sample was shaken in 1 mL of solvent in a test tube and was visually inspected for the absence of the solid particles in the test tube.

2.4 Evaluation of anti-inflammatory potential

2.4.1 Inhibition of albumin denaturation

Preparation of Phosphate Buffer Saline (PBS)

A solution of PBS was prepared by dissolving an accurately weighed quantity of 8 g NaCl, 0.2 g KCl, 1.44 g disodium hydrogen phosphate and 0.24 g potassium dihydrogen phosphate in deionized water to produce 1 L of solution.

The technique of inhibition of albumin denaturation reported by Singh and Mishra [8] was was reduced to half its volume.

The synthesized molecules were individually dissolved in DMSO and appropriately diluted to prepare solutions of 100, 200, 300, 400 and 500 µg/mL concentrations. A solution of 1% BSA in deionized water was prepared for the test. Ibuprofen solution of concentration 1 µg/mL was used as the positive control.

The reaction vessel was filled with 200 µL of BSA, 1400 µL of PBS and 1000 µL of the test solution. Ibuprofen solution was used in the positive control and distilled water was used in the negative control vessels instead of test solution.

The reaction mixtures were incubated at 37°C for 15 min and then heated at 70°C for 5 min. The mixtures were then allowed to cool to room temperature and the absorbance of constituent of each vessel were analyzed in UV-Visible spectrophotometer at 660 nm. The inhibition of percent denaturation of albumin was determined using the following formula:

$$\% \text{ Denaturation inhibition} = (1-D/C) \times 100\%$$

Where D is the absorbance reading of the test sample, and C is the absorbance reading without test sample (negative control).

Results and Discussion

The synthesis of all the compounds was achieved using the scheme depicted in Figure 3.1. The results of characterization of the synthesized compounds are presented in the present section.

Chemical Characterization

The synthesized compounds were subjected to determination of yield, melting point, solubility and structure elucidation. The physicochemical properties are shown in Table 3.1, 3.2 and 3.3.

Table 3.1Yield and color

Compound code	Aromatic acid Used	Yield (%)	Color
3a	Coumaric acid	68	Yellow
3b	Cinnamic acid	65	Yellow
3c	Gallic acid	62	Yellow
3d	2,4-dihydroxy benzoic acid	69	Yellow
3e	2,5-dihydroxy benzoic acid	62	Brown

Table 3.2 Physical Properties of the synthesized compounds

Compound code	Molecular Formula	Molecular Weight	R_f Value	Melting point (°C)
3a	$C_{24}H_{21}N_3O_2$	356.5	0.61	246-249
3b	$C_{24}H_{21}N_3O$	341.5	0.57	235-238
3c	$C_{22}H_{19}N_3O_4$	386.4	0.69	218-222
3d	$C_{22}H_{19}N_3O_3$	371.4	0.55	256-259
3e	$C_{22}H_{19}N_3O_3$	371.4	0.57	263-265

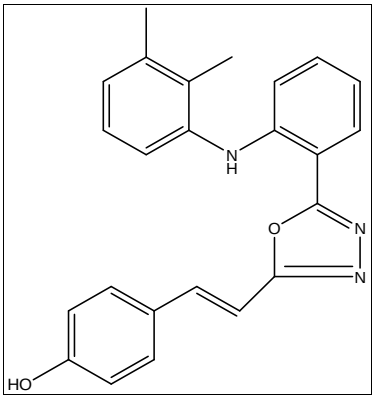
Table 3.3 Physical Properties of the synthesized compounds

Compound code	Water	Methanol	Chloroform	DMSO
3a	Insoluble	Insoluble	Soluble	Soluble
3b	Insoluble	Insoluble	Soluble	Soluble
3c	Insoluble	Insoluble	Soluble	Soluble
3d	Insoluble	Insoluble	Soluble	Soluble
3e	Insoluble	Insoluble	Soluble	Soluble

Structure Elucidation

The structure elucidation of the synthesized compounds was confirmed by interpretation of the IR, ^{1}HNMR and Mass spectra of the compounds. The IR spectra were observed for the characteristic peaks obtained due to the presence of the functional groups. All the compounds exhibited the peaks of aromatic C=C stretching, C-H stretching, C-N and C=N stretching and C-O stretching. The occurrence of absorption bands for C-O and C=N may occur at the same frequency and Fermi resonance peaks may be the diagnostic of a carbonyl group in the compounds. The ^{1}HNMR spectra of all the compounds exhibited chemical shifts of aromatic hydrogen. They also exhibited any peak that may arise due to certain functional groups like –OH and NH protons. The mass spectra of the compounds were examined for the presence of molecular ion peak or the isotopic peaks to confirm the formation of the compounds.

Elucidation of 3a



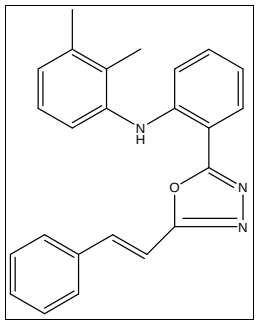
IUPAC Name - (E)-4-(2-(5-(2-(2,3-dimethylphenylamino)phenyl)-1,3,4-oxadiazol-2-yl)vinyl)phenol

Table 3.4 IR and 1H NMR data of 3a

S. No.	NMR signals (ppm relative to TMS)	Wave number (cm^{-1})	Due to…
1	7.9-6.8 Ar H, 2.996 CH_3, 3.969 NH	3104.67	Ar/Het C-H Str
2		2970.38	Ar C-C Str
3		1639.00	C=N Str
4		1417.36	C-N Str

MS – 356.1 (M^+)

Elucidation of 3b



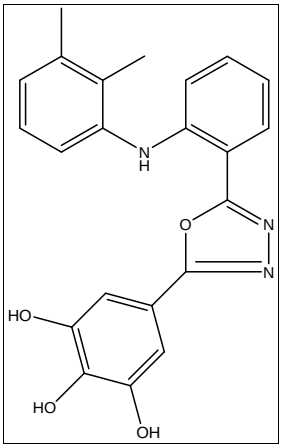
IUPAC Name - (E)-N-(2,3-dimethylphenyl)-2-(5-styryl-1,3,4-oxadiazol-2-yl)benzenamine

Table 3.5 IR and 1H NMR data of 3b

S. No.	NMR signals (ppm relative to TMS)	Wave number (cm^{-1})	Due to…
1	7.9-6.8 Ar H, 2.641 CH_3, 4.063 NH	3554.90	N-H stretching
2		3107.54	Ar/Het C-H Str
3		3039.13	Ar C-C Str
4		1653.56	C=N Str
5		1393.03	C-N Str

MS – 341.5 (M^+)

Elucidation of 3c



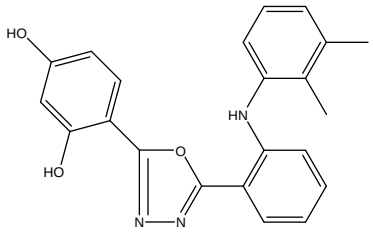
IUPAC Name - 5-(5-(2-(2,3-dimethylphenylamino)phenyl)-1,3,4-oxadiazol-2-yl)benzene-1,2,3-triol

Table 3.6 IR and 1H NMR data of 3c

S. No.	NMR signals (ppm relative to TMS)	Wave number (cm^{-1})	Due to…
1	7.9-6.8 Ar H, 2.993 CH_3, 3.974 NH, 5.008 OH	3100.40	Ar/Het C-H Str
2		2970.97	Ar C-C Str
3		1639.54	C=N Str
4		1289.17	C-N Str

MS – 386.3 (M^+)

Elucidation of 3d



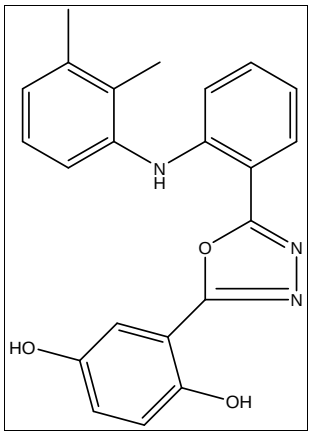
IUPAC Name - 4-(5-(2-(2,3-dimethylphenylamino)phenyl)-1,3,4-oxadiazol-2-yl)benzene-1,3-diol

Table 3.7 IR and 1H NMR data of 3d

S. No.	NMR signals (ppm relative to TMS)	Wave number (cm^{-1})	Due to…
1	8.001-8.025 Ar, 7.9-6.8 Ar H, 2.991 CH_3, 3.974 NH,	3705.25	O-H str
2		3104.67	Ar/Het C-H Str
3		2970.38	Ar C-C Str
4		1639.00	C=N Str
5		1289.63	C-N Str
6		1082.70	C-O Str

MS – 371.1 (M^++1)

Elucidation of 3e



IUPAC Name - 2-(5-(2-(2,3-dimethylphenylamino)phenyl)-1,3,4-oxadiazol-2-yl)benzene-1,4-diol

Table 3.8 IR and 1H NMR data of 3e

S. No.	NMR signals (ppm relative to TMS)	Wave number (cm^{-1})	Due to…
1	7.9-6.8 Ar H, 2.993 CH_3, 3.974 NH	3099.37	Ar/Het C-H
2		2992.19	Ar C-C
3		1687.76	C=N
4		1299.77	C-N

MS – 371.1 (M^++1)

Inhibition of albumin denaturation

All the synthesized compounds (**3a-e**) showed dose dependent inhibition of albumin denaturation (Table 5.9, Figure 5.1). The 500 µg/mL concentration of 3**b** has shown the greatest inhibition capacity (71.89±3.899%) whereas the lowest inhibition capacity was exhibited by 100 µg/mL of **3e** (2.98±0.695%). The inhibition protein denaturation by 100 µg/mL solution of standard drug Ibuprofen was found to be 78.73 ± 3.561%.

Protein denaturation has been significantly correlated with the occurrence of the inflammatory response and may lead to various inflammatory diseases including arthritis [9]. According to Opie [10], tissue injury during life might be due to denaturation of the protein constituents of cells or of intercellular substance. Hence, the ability of a substance to inhibit the denaturation of protein signifies obvious potential for anti-inflammatory activity.

Table 3.9 Albumin denaturation inhibition activity

Treatment	100 µg/mL	200 µg/mL	300 µg/mL	400 µg/mL	500 µg/mL
Ibuprofen	78.73±3.561	-	-	-	-
3a	12.13±3.291	21.53±2.657	30.59±2.194	44.87±3.167	52.38±2.869
3b	15.17±2.165	29.36±2.243	43.22±3.128	59.45±3.692	71.81±3.899
3c	11.96±1.783	22.17±2.165	31.27±2.791	43.33±2.695	56.48±2.194
3d	13.18±1.695	15.61±1.899	21.69±2.369	26.97±2.165	32.76±2.375
3e	2.98±0.695	4.35±0.310	8.25±0.160	11.25±0.975	16.91±1.333

Results are expressed as mean ± SEM

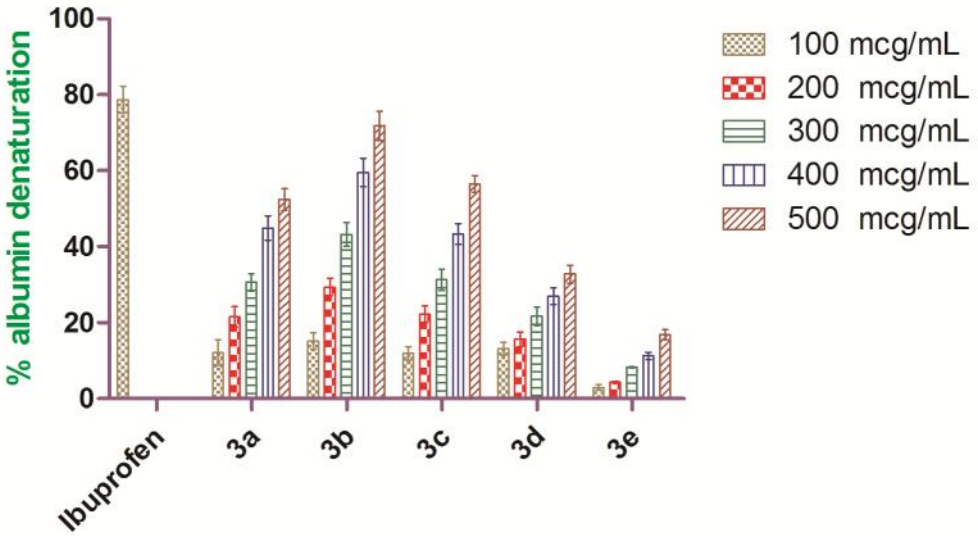


Figure 3.1 % albumin denaturation exhibited by the synthesized

Conclusion

The objective of the present investigation was to develop newer antiinflammatory molecules based on oxadiazole scaffold. It was accomplished by converting the carboxyl group of mefenamic acid to oxadiazole nucleus. The synthesized compounds presented anti-antiinflammatory activity comparable to that of the standard drug.

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