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SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF CERTAIN NOVEL OXADIAZOLE DERIVATIVES CONTAINING SUBSTITUTED-PHENYLIMIDAZOLE MOIETY

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ABSTRACT
A series of Oxadiazole derivatives (4a-d) have been synthesized from (2, 4, 5-triphenyl-imidazole-1-yl)-acetic acid hydrazide under various reaction conditions. Elemental analysis, IR, ^{1}H NMR and mass spectral data confirmed the structure of the newly synthesized compounds. All the synthesized Oxadiazole derivatives have been investigated for their anti-inflammatory activity showing moderate to good activity.
Keywords: 2, 4, 5-Triphenyl imidazole, oxadiazole, anti-inflammatory agents.

INTRODUCTION

Asprin and others NSAIDs are one of the most widely used class of drugs in treatment of inflammatory diseases. NSAIDs are believed to act through inhibition of prostaglandin (PG) synthesis secondary to their inhibition of enzyme cylcooxyenase. The most frequent complication associated with NSAID uses are those involving the gastrointestinal tract and and nephrotoxicity[1]. GI bleeding, ulceration and perforation are major cause of morbidity and mortality in patients who are treated with these agents. A new approach to avoiding NSAID-induced complication became feasible with the discovery that there are 2 isoforms of COX, COX-1 and COX-1. COX-2 is expressed primarily in response to inflammation, but to some extent in other tissues including kidneys and brain. The tendency of many acidic drugs to accumulate in the stomach walls soon after oral absorption, as evidenced by radio autography, has been considered as a contributory factor for GI irritation[2]. It has been reported in the literature that certain five membered heterocyclic compounds possess interesting anti-inflammatory activity with lesser GI effect[3,4]. Among heterocyclic compounds, imidazole bearing nucleus have been reported to have significant anti-inflammatory activity[5,6]. Furthermore, oxadiazole were also reported to exhibit anti-inflammatory and antimicrobial activity[7-12,27]. Encouraged by these observations and in continuation of the research programme on the synthesis of five membered heterocyclic compounds, herein is reported the synthesis of various oxadiazole derivatives bearing 2, 4, 5-triphenyl imidazole nucleus. The reaction sequence leading to the formation of the desired heterocyclic compounds are outlined in Scheme I. (2,4,5-Triphenyl-imidazole-1-yl)-acetic acid hydrazide 3 was prepared by treating 2,4,5-triphenylimidazole with ethyl chloroacetate in the presence of anhydrous acetone and potassium carbonate followed by reaction with hydrazine hydrate in absolute ethanol. Hydrazide 3 on treatment with various substituted phenylisothiocynate gave N^1-(2, 4, 5-triphenyl-imidazol-1-yl-ethanoyl)-N^4-substitutedphenyl thiosemicarbazide 3a-d. The thiosemicarbazides were cyclised with 2% aqueous NaOH and Potassium Iodide to 1-{[2-(4-substituted phenyl amino)-(1, 3, 4-oxadiazol-5-yl)] methyl}-2, 4, 5-triphenylimidazole 4a-d. The IR spectrum of the compound 4a showed absorption peaks at 1600-1695 cm^{-1} and 1600-1695 cm^{-1} due to C=C and C=N stretching vibration. The peak at 3310-3390 cm^{-1} appeared due to NH stretching vibration. The ^{1}H NMR spectra of compound 4a displayed a singlet at δ 3.73 showing the presence of a methoxy group. The NCH_2 proton appeared as a singlet at δ 4.45. A broad singlet at δ 12.69 indicated the presence of NH proton attached to oxadiazole ring. The IR spectrum of the compound 4b showed absorption peaks at 3310-3390 cm^{-1} (NH) stretching, and NH bending at (1530-1575) cm^{-1}, C-O-C group stretching at (3310-3395) cm^{-1}, C=C and C=N appears as a strong band at (1600-1695) cm^{-1} and (1600-1695) cm^{-1}. The ^{1}H NMR spectra of compound 4b displayed a singlet at δ 2.34 showing the presence of a methyl group. The NCH_2 proton appeared as a singlet at δ 4.45. A broad singlet at δ 12.13 indicated the presence of NH proton attached to oxadiazole ring. The IR spectrum of the compound 4c showed absorption peaks at 3310-3389 cm^{-1} (NH) stretching, and NH bending at (1530-1575) cm^{-1}, C-O-C group stretching at (3310-3390) cm^{-1}, C=C and C=N appears as a strong band at (1600-1695) cm^{-1} and (1600-1695) cm^{-1}. The ^{1}H NMR spectra of compound 4b displayed a singlet at δ 2.34 showing the presence of a methyl group. The NCH_2 proton appeared as a singlet at δ 4.45. A broad singlet at δ 12.57 indicated the presence of NH proton attached to oxadiazole ring. The IR spectrum of the compound 4d showed absorption peaks at 3308-3390 cm^{-1} (NH) stretching, and NH bending at (1529-1573) cm^{-1}, C-O-C group stretching at (3310-3390) cm^{-1}, C=C and C=N appears as a strong band at (1600-1695) cm^{-1} and (1600-1695) cm^{-1}. The ^{1}H NMR spectra of compound 4b displayed a singlet at δ 2.44 showing the presence of a methyl group. The NCH_2 proton appeared as a singlet at δ 4.45. A broad singlet at δ 12.57 indicated the presence of NH proton attached to oxadiazole ring.

Biological Studies

Adult male Wister strain rats of either sex, weighing 180-200 g were used. The animals were allowed food and water *ad libitum*. They were housed in a room at 25 ± 2°C, and 50 ± 5 % relative humidity with 12 h light/dark cycle. The animals were randomly allocated into groups at the beginning of all the experiment. All the test compounds and reference drug were administered orally, suspended in 0.5 % carboxymethyl cellulose (CMC) solution.

Anti-inflammatory Activity

The test was performed by the method for winter *et al*.[16] on the group of six animals in each. The animals were maintained under standard environmental conditions and had fee access to standard diet and water. Carrageenan solution (0.1 % in sterile 0.9 % NaCl solution) in a volume of 0.1 mL was injected subcutaneously into the sub plantar region of the right hind paw of each rat, 1 h after the administration of test compounds and standard drug indomethacin (10 mg/kg, p.o). One group was kept as control, receiving only 0.5 % carboxymethyl cellulose solution. The right hind paw volume was measured before and after 4 h of carrageenan treatment by means of a plethysmometer. The percentage anti-inflammatory activity was calculated according to the following formula.

Percentage anti-inflammatory activity = (1-Vt/Vc) × 100,
Where Vt represents the mean in paw volume in rats tested with test compounds and Vc represents the mean increase in paw volume in control group of rats

Data are expressed as mean ± SEM. The student t-test was applied to determine the significance of the difference between the control group and rats treated with the test compounds. Carrageenan induced rat paw edema is used widely as a working model of inflammation in the search for new anti-inflammatory drug. The anti-inflammatory activity of the newly synthesized compounds 4a-d was compared with the standard drug indomethacin. At the same oral dose, indomethacin showed 71.56 % inhibition of rat paw edema whereas the tested compounds showed inhibition ranging from 40.81 % to 83.40 % after 4 h (Table 1). The oxadiazole derivatives 4a showed anti-inflammatory activity ranging from 40.81 %. The compounds 4c and 4d having 4-chloro and 4-flouro groups showed moderate activity (53.90 and 54.01 %) in comparison to standard drug indomethacin (71.56 %). The results of anti-inflammatory activity clearly indicated that oxadiazole ring have moderate anti-inflammatory activity.

Experimental Section

All the chemicals used in the synthesis were supplied by E. Merck and S. D. Fine Chemicals. Melting point was determined by open capillary tube method and is uncorrected. Homogeneity of the compounds was checked on thin layer chromatography using iodine vapors as visualizing agent. IR spectra were obtained on a Perkin-Elmer 1720 FT-IR spectrometer (KBr pellets). ^{1}H NMR spectra were obtained on a Bruker DRX-300 (300 MHz FT NMR) spectrometer in $CDCl_3$ using tetramethysilane (TMS) as the internal reference (chemical shifts in δ, ppm). Mass spectra were recorded on a Jeol SX-102 spectrometer. Synthesis of 2, 4, 5-triphenyl-1H-imidazole 1 was carried out by the method reported in literature[18].

Synthesis of (2, 4, 5-triphenyl-imidazole-1-yl)-acetic acid ethyl ester 2

A mixture of 2, 4, 5-triphenylimidazole (0.01 mole) and ethyl chloroacetate (0.01 mole) in dry acetone (40 mL) was refluxed on a heating mantle for 30 h. The reaction mixture was cooled to RT. The crystals thus obtained was filtered, washed with water, dried and purified by recrystallization from ethanol to yield colourless crystalline compound 2. Yield 65 %; m.p. 260°C; IR (KBr): 1668 (C=O), 1595 cm^{-1} (C=N); ^{1}H NMR ($CDCl_3$): δ 1.31-1.37 (t, 3H, CH_3), 4.08-4.16 (q, 2H, CH_2), 4.48 (s, 2H, NCH_2), 7.20-7.45 (m, 9H, ArH), 7.54, 7.66 and 8.10 (6H, 3d, ArH); MS: m/z 382 (M^+). Anal. Calcd for $C_{25}H_{22}N_2O_2$: C, 78.51; H, 5.8; N, 7.32. Found: C, 78.31; H, 5.69; N, 7.16 %.

Synthesis of (2, 4, 5-triphenyl-imidazole-1-yl)-acetic acid hydrazide 3

A mixture of (2, 4, 5-triphenyl-imidazol-1-yl)-acetic acid ethyl ester (0.01 mole) and hydrazine hydrate (0.05 mole) in ethanol (50 mL) was refluxed on water bath for 12 h. The reaction-mixture was cooled to RT. The crystals thus obtained were filtered, washed with water, dried and purified by recrystallization from ethanol to yield colourless crystalline compound 3. Yield 66 %; m.p. 240°C; IR (KBr): 3038 (NH), 1694 (C=O), 1597 cm^{-1} (C=N); ^{1}H NMR ($CDCl_3$): δ 4.35 (s, 2H, NCH_2), 4.71 (d, 2H, NH_2), 7.15-8.12 (m, 15H, ArH), 12.78 (bs, 1H, s, NH); MS: m/z 368 (M^+). Anal. Calcd for $C_{23}H_{20}N_4O$: C, 74.98; H, 5.47; N, 15.21. Found: C, 74.73; H, 5.29; N, 15.28 %.

General for synthesis of N^1-(2, 4, 5-triphenyl-imidazol-1-yl-ethanoyl)-N^4-substitutedphenyl thiosemicarbazide 3a-d

A mixture of acid hydrazide (0.01 mole) and substitutedphenyl isothiocynate (0.01 mole) in absolute ethanol (50 mL) was refluxed on a water bath for 6 h. The reaction-mixture was concentrated and allowed to stand at RT overnight. The needle shaped crystals of thiosemicarbazide thus obtained were filtered, washed with petroleum ether and purified by recrystallization from ethanol.

N^1-(2, 4, 5-triphenyl-imidazol-1-yl-ethanoyl)-N^4-4-methoxyphenyl thiosemicarbazide, 3a

Yield 69 %; m.p. 230°C; IR (KBr): 3238 (NH), 1683 (C=O), 1597 (C=N), 1099 cm^{-1} (C=S); ^{1}H NMR ($CDCl_3$): δ 3.74 (s, 3H, OCH_3), 4.48 (s, 2H, NCH_2), 7.2-7.5 (m, 19H, ArH), 8.12 (bs, 1H, NH) 10.91 (bs, 1H, CSNH), 12.70 (bs, 1H, CONH); MS: m/z 533 (M^+). Anal. Calcd for $C_{31}H_{27}N_5O_2S$: C, 69.77; H, 5.10; N, 13.12. Found: C, 69.81; H, 5.11; N, 12.92 %.

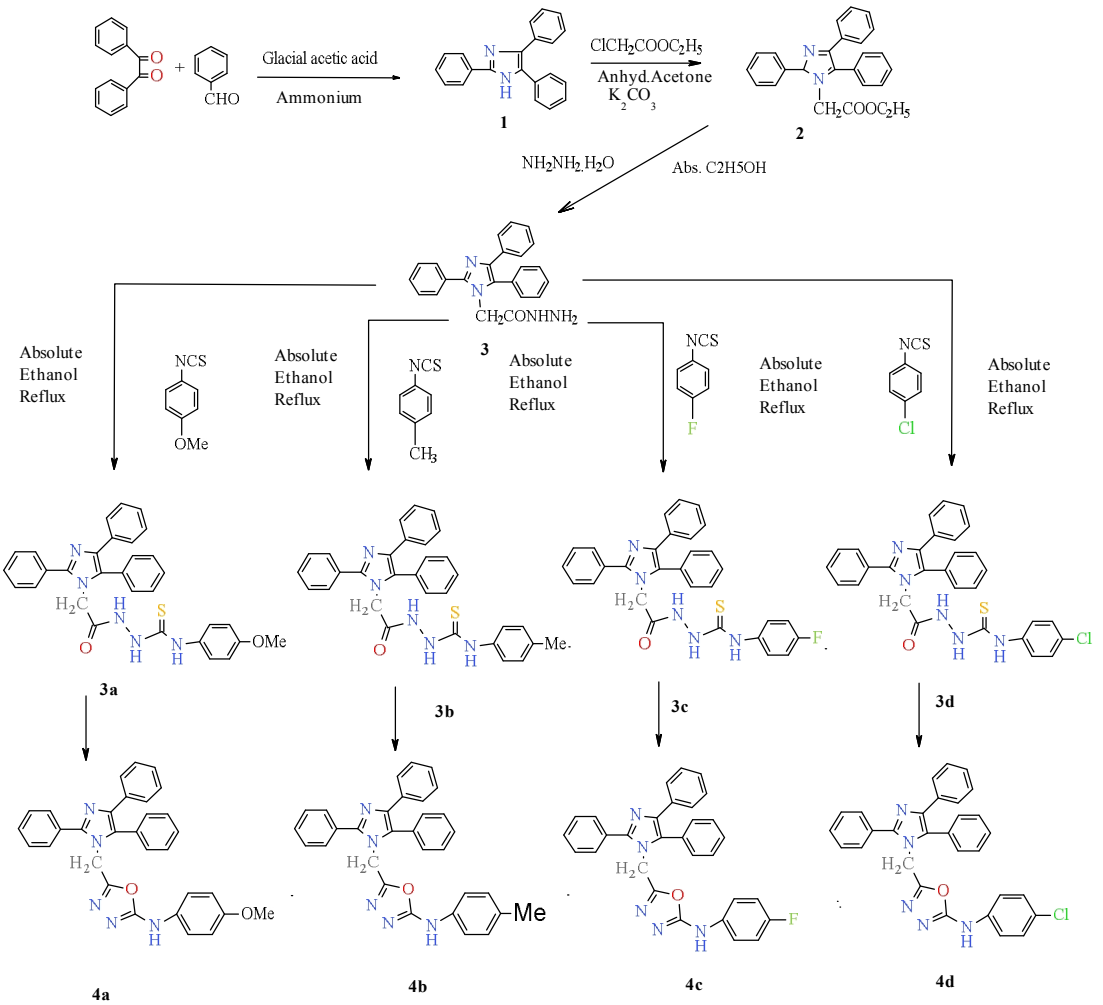
N^1-(2, 4, 5-triphenyl-imidazol-1-yl-ethanoyl)-N^4-4-methylyphenyl thiosemicarbazide, 3b

Yield 70 %; m.p. 238°C; IR (KBr): 3238 (NH), 1668 (C=O), 1595 (C=N), 1091 cm^{-1} (C=S); ^{1}H NMR ($CDCl_3$): δ 2.28 (s, 3H, CH_3), 4.53 (s, 2H, NCH_2), 7.12-7.54 (m, 19H, ArH), 8.08 (bs, 1H, NH), 12.41 (bs, 1H, CSNH), 12.59 (bs, 1H, CONH); MS: m/z 517 (M^+). Anal. Calcd for $C_{31}H_{27}N_5OS$: C, 71.93; H, 5.28; N, 13.53. Found: C, 71.83; H, 5.12; N, 13.29 %.

Table 1: Anti-inflammatory activity of compounds 4a-d

Compound	Mean values (± SEM) of edema Volume		Anti-inflammatory activity	(% inhibition ± SEM)
	3 h	4 h	3 h	4 h
Control	1.74 ± 0.076	1.62 ± 0.082	----	---
Indomethacin	0.50 ± 0.026	0.46 ± 0.029	71.10 ± 1.42	71.56 ± 1.42
4a	1.06 ± 0.048	0.97 ± 0.047	39.32 ± 1.37*	40.81 ± 1.40*
4b	1.05 ± 0.042	0.94 ± 0.043	40.73 ± 1.87*	41.90 ± 1.21*
4c	0.82 ± 0.031	0.74 ± 0.035	53.00 ± 1.67*	53.90 ± 1.39*
4d	0.83 ± 0.028	0.75 ± 0.028	52.44 ± 1.28*	54.01 ± 1.24*

Anti-inflammatory activity of the test compounds were compared w. r. t. standard drug. Data were analyzed by student's t test for n = 6; *p < 0.0001, **p < 0.01, ***p < 0.5



Scheme I

N^1-(2, 4, 5-triphenyl-imidazol-1-yl-ethanoyl)-N^4-4-flourophenyl thiosemicarbazide, 3c

Yield 67 %; m.p. 251°C; IR (KBr): 3196 (NH), 1651 (C=O), 1606 (C=N), 1096 cm^{-1} (C=S); ^1H NMR ($CDCl_3$): δ 4.52 (s, 2H, NCH_2), 7.21-7.51 (m, 19H, ArH), 8.14 (bs, 1H, NH), 11.08 (bs, 1H, CSNH), 12.80 (s, 1H, CONH); MS: m/z 521 (M^+); Anal. Calcd for $C_{30}H_{24}FN_5OS$: C, 69.97; H, 4.64; N, 13.43. Found: C, 69.78; H, 4.57; N, 13.26 %.

N^1-(2, 4, 5-triphenyl-imidazol-1-yl-ethanoyl)-N^4-4-chlorophenyl thiosemicarbazide, 3d

Yield 65 %; m.p. 223°C; IR (KBr): 3216 (NH), 1664 (C=O), 1591 (C=N), 1101 cm^{-1} (C=S); ^1H NMR ($CDCl_3$): δ 4.49 (s, 2H, NCH_2), 7.22-7.67 (m, 19H, ArH), 11.16 (bs, 1H, NH), 12.69 (bs,1H, CSNH), 12.83 (bs, 1H, CONH); MS: m/z 538 (M^+), 540 (M^++2). Anal. Calcd for $C_{30}H_{24}Cl\ N_5OS$: C, 66.97; H, 4.50; N, 13.02. Found: C, 66.81; H, 4.39; N, 12.86 %.

General method for synthesis of 1-{[2-(4-substitiutedphenyl amino)-(1, 3, 4-oxadiazol-5-yl)] methyl}-2, 4, 5-triphenylimidazole, 4a-d

In a 100 ml round bottom flask, thiosemicarbazide (0.02 mole) and ethanol (25 ml) was taken. To it, sodium hydroxide solution (5N, 2 ml) was added and mixture was cooled with continuous stirring for half an hour. To this mixture iodine in potassium iodide was added drop wise till the colour of iodine persisted at room temperature. The reaction mixture was refluxed for one hour on a water bath. When reaction was completed, reaction mass was poured over crushed ice in a beaker. The precipitated solid thus obtained was washed with sodium thiosulphate solution and recrystallized from absolute ethanol.

1-{[2-(4-methoxyphenyl amino)-(1, 3, 4-oxadiazol-5-yl)] methyl}-2, 4, 5-triphenylimidazole, 4a

Yield 65 %; m.p. 190°C; IR (KBr): 3310-3390(NH) stretching, and N-H bending at (1530-1575) cm^{-1}, C-O-C group stretching at (3310-3390) cm^{-1}, C=C and C=N appears as a strong band at (1600-1695) cm^{-1} and (1600-1695) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 3.73 (s, 3H, OCH_3), 4.45 (s, 2H, N-CH_2), 6.90-7.11 (m, 4H, ArH), 7.22-7.53 (m, 15H, ArH), 12.69 (s, 1H, NH); MS: m/z 515 (M^+).

1-(4-methylphenyl amino)-(1, 3, 4-oxadiazol-5-yl)] methyl}-2, 4, 5-triphenylimidazole, 5b

Yield 64 %; m.p. 1910°C; IR (KBr): 3310-3390(NH) stretching, and N-H bending at (1530-1575) cm^{-1}, C-O-C group stretching at (3310-3390) cm^{-1}, C=C and C=N appears as a strong band at (1600-1695) cm^{-1} and (1600-1695) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.34 (s, 3H, CH_3), 4.45 (s, 2H, N-CH_2), 6.90-7.11 (m, 4H, ArH), 7.22-7.53 (m, 15H, ArH), 12.69 (s, 1H, NH); MS: m/z 514 (M^+).

1-{[2-(4-fluorophenyl amino)-(1, 3, 4-oxadiazol-5-yl)] methyl}-2, 4, 5-triphenylimidazole, 4c

Yield 68 %; m.p. 193°C; IR (KBr): 3310-3390(NH) stretching, and N-H bending at (1530-1575) cm^{-1}, C-O-C group stretching at (3310-3390) cm^{-1}, C=C and C=N appears as a strong band at (1600-1695) cm^{-1} and (1600-1695) cm^{-1}; ^{1}H NMR ($CDCl_3$): 4.45 (s, 2H, N-CH_2), 6.90-7.11 (m, 4H, ArH), 7.22-7.53 (m, 15H, ArH), 12.69 (s, 1H, NH); MS: m/z 514 (M^+).

1-{[2-(4-chlorophenyl amino)-(1, 3, 4-oxadiazol-5-yl)] methyl}-2, 4, 5-triphenylimidazole, 4d

Yield 68 %; m.p. 193°C; IR (KBr): 3310-3390(NH) stretching, and NH bending at (1530-1575) cm^{-1}, C-O-C group stretching at (3310-3390) cm^{-1}, C=C and C=N appears as a strong band at (1600-1695) cm^{-1} and (1600-1695) cm^{-1}; ^{1}H NMR ($CDCl_3$): 4.54 (s, 2H, N-CH_2), 6.90-7.11 (m, 4H, ArH), 7.22-7.53 (m, 15H, ArH), 12.69 (s, 1H, NH); MS: m/z 520 (M+), 522 (M++2).

CONCLUSION

The anti-inflammatory activity of the newly synthesized compounds was found to have moderate activity in comparison to existing drugs in market. The oxadiazole derivative with 4-methoxy substitiuon is least activity. Whereas, 4-chloro and 4-flouro groups substitution showed moderate activity (53.90 and 54.01 %) in comparison to standard drug indomethacin (71.56 %). The results of anti-inflammatory activity clearly indicated that oxadiazole ring have moderate anti-inflammatory activity.

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