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Original Article

DESIGN, SYNTHESIS, *IN VITRO* ANTIOXIDANT AND *IN VIVO* ANTI-INFLAMMATORY ACTIVITIES OF NOVEL OXADIAZOLE DERIVATIVES

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ABSTRACT

Objective: In the present study, a series of novel 1,3,4-oxadiazole derivatives (3a-3q) were designed, synthesized and evaluated for antioxidant and anti-inflammatory activities.

Methods: The title compounds were designed and docked onto the COX-2 enzyme (3LN1) protein using SYBYLX 2.1. 2-substituted-5-(5-nitrobenzofuran-2-yl)-1,3,4-oxadiazole derivatives (3a-3p) were synthesized from acid catalyzed dehydrative cyclization of 5-nitrobenzofuran-2-carbohydrazide (2) with various heteroaryl/aryl/aliphatic carboxylic acid derivatives. And 5-(5-nitrobenzofuran-2-yl)-1,3,4-oxadiazole-2-thiol (3q) was synthesized on reacting the hydrazide derivative 2 with carbon disulfide. The synthesized compounds were evaluated for *in vitro* antioxidant property by DPPH radical scavenging assay method and *in vivo* anti-inflammatory activity by carrageenan induced paw edema method.

Results: The synthesized 1,3,4-oxadiazole derivatives (3a-3q) were characterized on the basis of LCMS, ^{1}HNMR [13]CNMR, IR and elemental analysis. The title compounds 3a-3q exhibited significant antioxidant efficacy ranging from 34 to 86%and the results of anti-inflammatory evaluation revealed that compounds 3c, 3e and 3d exhibited substantial anti-inflammatory activity of 72, 68 and 65%, respectively, at a dose of 50 mg kg^{-1}.

Conclusion: A significant correlation was observed between the *in silico* study and the anti-inflammatory results. The anti-inflammatory results highlight the synthesized compounds 3c, 3e and 3d could be considered as possible hit as therapeutic agents.

Keywords: Benzofuran, Oxadiazole, Antioxidant, Anti-inflammatory.

INTRODUCTION

Non steroidal anti-inflammatory drugs (NSAID's) are the choice of drugs for the treatment/management of rheumatoid arthritis. Osteoarthritis and other pain conditions [1,2]. NSAID's tops the lists of self medication and are also the frequently prescribed medication for all clinical conditions associated with pain and inflammation. NSAID's act via suppression of prostaglandin biosynthesis by inhibiting the enzyme prostaglandin endoperoxidase, known as cyclooxygenase (COX), COX mediates the production of prostaglandins, prostacyclins and thromboxanes from arachidonic acid [3].

COX exists in two isotherms, COX-1 and COX-2, which are regulated and expressed differently [4-6]. COX-2 isoenzyme selectively mediates inflammatory signals and COX-1 isoenzyme regulates the mucus formation and thromboxan production, and provides cytoprotection in the gastrointestinal tract (GIT) [7,8]. Most of the currently available NSAIDs in clinical use like diclofenac, mefenamic acid, ibuprofen etc., are mainly non-selective COX inhibitors or exhibit greater selectivity for COX-1 than COX-2 [3]. Thus, with increased frequency and chronic use of these non-selective NSAID's, secondary clinical condition like dyspepsia, gastroduodenal ulcers, gastritis bleeding and renal disorders are precipitated [9-11]. Whereas, COX-2 selective NSAIDs like celecoxib, rofecoxib, valdecoxib etc., are associated with serious cardiovascular problems due to the increased platelet aggregation [11,12].

Literature studies emphasize the importance of inhibition of 5-lipoxygenase (5-LOX) enzyme, as the leucotrienes play an important role in blood coagulation and GIT irritation [13,14]. Thus, inhibition of 5-LOX will be helpful in attenuating the formation of gastric ulcer during long term therapy of non-selective and COX-1 selective NSAID's [15], and, ameliorate the prothrombotic tendency resulting from COX-2 inhibition by the NSAID's [16].

Studies on 1,3,4-oxadiazoles highlight their importance in inhibiting 5-LOX, thus reducing gastric ulcer formation, apart from suppressing prostaglandin anabolism from arachidonic acid by inhibiting the COX enzyme, thus highlighting its importance as anti-inflammatory agent and nonulcerogenic nature [3,17,18]. Hence, 1,3,4-oxadiazoles have been widely explored for its anti-inflammatory property [19-30].1,3,4-oxadiazole derivatives are also reported for their broad spectrum of biological activitieslike anticonvulsant [31,32], antidepressant [33], anticancer [34,35], analgesic [36,37], antibacterial [38-41], antifungal [42,43], antimycobacterial [29,44,45], anthelmintic [25], hypoglycemic [46] and antiangiogenic [47] etc., indicating 1,3,4-oxadiazole is a physiologically active nucleus and an imperative scaffold on which therapeutic molecules are designed and developed. In view of these observations, seventeen novel 2-substituted-5-(5-nitrobenzofuran-2-yl)-1,3,4-oxadiazole derivatives (3a-3p) were designed as potential COX-2 inhibitors and predicted to have strong therapeutic benefit as anti-inflammatory agents. The designed molecules were synthesized and evaluated for their anti-inflammatory activity.

MATERIALS AND METHODS

The chemicals used were purchased from Fluka Chemicals. Mumbai, India, and the solvents were purified by distillation if necessary and residual water was removed. Melting points were determined in open capillary tube and are uncorrected. TLC was used to assess the progress/completion of the reactions and the purity of the synthesized compounds using ethyl acetate and hexane (8:2) as solvent system and iodine vapors as visualizing agent. The IR spectra were recorded using Shimadzu FTIR-8400, Japan, by KBr disc pellet method and only noteworthy absorption levels (cm^{-1}) are listed, ^{1}HNMR spectra were recorded using Brucker AC-400 MHz FT NMR spectrophotometer at 400 MHz with deuteriated dimethyl sulfoxide (DMSO-d_6) as solvent and tetramethylsilane (TMS) as internal standard (chemical shifts in δ, ppm), [13]CNMR spectra were recorded using Bruker DSX 300 MHz FT NMR spectrophotometer at 100 MHZ with deuteriated dimethyl sulfoxide (DMSO-d_6) as solvent and mass spectra were recorded using JEOL GCMATE II GC-MS. Elementary analysis of final compounds were recorded using Thermo Finnigan FLASH EA 1112 CHNS analyser and all the compounds gave satisfactory elemental analysis.

Synthesis of ethyl 5-nitrobenzofuran-2-carboxylate(1)

To the solution of 2-hydroxy-5-nitrobenzaldehyde (16.7 g, 0.10 mol) in dry acetone (50 mL), ethyl 2-chloroacetate (18.3 mL, 0.15 mol) and activated anhydrous potassium carbonate (12.0 g) were added and the mixture was heated to reflux with stirring for 6 h. The progress of the reaction was monitored by TLC. On completion of the reaction, the reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated and poured in to the crushed ice to obtain the solid product 3. The product was recrystallized using rectified sprit as solvent. Yield: 72 %, mp 121-123 °C, R_f: 0.71, IR (KBr) 3030 (C=C), 1737 (C=O),1529 (N-O, NO_2), 1432 (C-O-C); ^{1}HNMR (400 MHz, DMSO-d_6,) δ 7.6-7.4 (m, 4H, benzofuran), 2.3 (d, *J* = 9.4 Hz, 2H, $COOCH_2$), 1.8 (t, *J* = 12.6 Hz, 3H, $COOCH_2CH_3$).

Synthesis of 5-nitrobenzofuran-2-carbohydrazide (2)

To the solution of ester derivative 1 (23.5 g, 0.1 mol) in ethanol (30 mL), hydrazine monohydrate (10.0 mL, 0.20 mol) was added and the mixture was heated to reflux for 8 h. The progress of the reaction was monitored by TLC. On completion of the reaction, the reaction mixture was cooled to room temperature and allowed to cool in the refrigerator overnight to precipitate the acid hydrazide2. Then the product was recrystallized using rectified sprit as solvent. Yield: 67 %, mp154-155 °C, R_f: 0.74, IR (KBr) 3398-3450 (NH_2, NH), 3045 (C=C), 1718 (C=O), 1521 (N-O, NO_2),1432 (C-O-C) cm^{-1}; ^{1}HNMR (400 MHz, DMSO-d_6,) δ 11.8 (s, 1H, CONH), 7.8-7.3 (m, 4H, ArH), 4.7 (s, 2H, $NHNH_2$); [13]C NMR (100 MHz, DMSO-d_6): δ 113.4 (C_3), 121.3 (C_7), 124.9 (C_4), 125.9 (C_6), 132.4 (C_9),146.3 (C_2), 159.7 (C_5), 163.6 (C_8), 174.8 (-CONH).

General procedure for the synthesis of 2-substituted-5-(5-nitrobenzofuran-2-yl)-1,3,4-oxadiazole derivatives (3a-3p)

The acid hydrazide 2 was condensed with various heteroaryl/aryl/aliphatic carboxylic acids to afford the title oxadiazole derivatives.

To the solution of acid hydrazide 2 (2.2 g, 0.01 mol) in ethanol (25.0 mL), carboxylic acid derivatives (0.01 mol) and phosphorous oxychloride (1.6 mL, 0.01 mol) were added slowly in an exhaustion chamber and heated to reflux for 8 -12 h. The progress of the reaction was monitored by TLC. On completion of the reaction, the reaction mixture was cooled to room temperature and crushed ice was added to obtain oxadiazole derivative (3a-3q). The product was recrystallized using rectified sprit as solvent.

2-(5-nitrobenzofuran-2-yl)-1,3,4-oxadiazole(3a): Yield: 82 %, mp162-163 °C, R_f: 0.75, IR(KBr) 3026 (C=C), 1592 (C=N), 1523 (N-O, NO_2),1424 (C-O-C) cm^{-1}; ^{1}HNMR(400 MHz, DMSO-d_6) δ 8.2 (s, 1H, CH, oxadizole),7.1-6.9 (m, 4H); [13]C NMR (100 MHz, DMSO-d_6): δ 121.2 (C_3), 123.1 (C_7), 124.5 (C_4), 124.9 (C_6), 131.7 (C_9),147.8 (C_2), 161.1 (C_5), 164.8 (C_8), 169.3 (C_5, oxadiazole), 171.9 (C_2, oxadiazole). MS m/z(%):231.0291 (24) [M^+]; Anal. Calcd for $C_{10}H_5N_3O_4$: C 51.96, H 2.18, N 18.18,Found C 51.92, H 2.14, N 18.13.

2-methyl-5-(5-nitrobenzofuran-2-yl)-1,3,4-oxadiazole(3b): Yield: 86 %, mp 167-168 °C, R_f: 0.65, IR (KBr) 3016 (C=C), 1613 (C=N), 1526 (N-O, NO_2), 1425 (C-O-C) cm^{-1}; ^{1}HNMR (400 MHz, DMSO-d_6) δ 7.9-7.4 (m, 4H), 1.2 (s, 3H, CH_3); [13]C NMR (100 MHz, DMSO-d_6) δ26.7 (CH_3),119.2 (C_3), 121.1 (C_7), 124.7 (C_4), 126.3 (C_6), 129.7 (C_9),148.8 (C_2), 159.1 (C_5), 161.4 (C_8), 168.2 (C_5, oxadiazole), 171.4 (C_2, oxadiazole).; MS m/z(%): 245 (35) [M^+]; Anal. Calcd for $C_{11}H_7N_3O_4$: C 53.88, H 2.88, N 17.14, Found C 53.79, H 2.72, N 17.08.

2-ethyl-5-(5-nitrobenzofuran-2-yl)-1,3,4-oxadiazole (3c): Yield: 78 %, mp 181-183 °C, R_f: 0.69, IR (KBr) 3021 (C=C), 1643 (C=N), 1542 (N-O, NO_2), 1431 (C-O-C) cm^{-1}; ^{1}HNMR (400 MHz, DMSO-d_6) δ 7.7-7.3 (m, 4H), 1.4 (d, *J* = 11.7 Hz, 2H, CH_2), 0.8 (t, *J* = 12.8 Hz, 3H, CH_3); [13]C NMR (100 MHz, DMSO-d_6) δ23.4 (CH_3),34.2 (CH2), 114.7 (C_3), 118.3 (C_7), 122.6 (C_4), 124.3 (C_6), 127.6 (C_9),143.3 (C_2), 157.6 (C_5), 159.2 (C_8), 163.4 (C_5, oxadiazole), 168.2 (C_2, oxadiazole).;MS m/z(%): 259 (20) [M^+]; Anal. Calcd for $C_{12}H_9N_3O_4$: C 55.60, H 3.50, N 16.21, Found C 55.46, H 3.39, N 16.13

5-(5-nitrobenzofuran-2-yl)-2-propyl-1,3,4-oxadiazole (3d): Yield: 76 %, mp 189-191 °C, R_f: 0.74, IR (KBr) 3019 (C=C), 1607 (C=N), 1519(N-O, NO_2), 1436 (C-O-C) cm^{-1}; ^{1}HNMR (400 MHz, DMSO-d_6) δ7.4-7.1 (m, 4H), 1.6 (d, *J* = 11.4 Hz, 4H, $(CH_2)_2$), 1.1 (t, *J* = 14.6 Hz, 3H, CH_3); [13]C NMR (100 MHz, DMSO-d_6) δ19.8 (CH_3),29.7 (2C, CH_2), 119.2 (C_3), 121.8 (C_7), 123.3 (C_4), 126.7 (C_6), 131.1 (C_9),147.2 (C_2), 157.1 (C_5), 160.4 (C_8), 165.6 (C_5, oxadiazole), 169.4 (C_2, oxadiazole); MS m/z(%): 273 (20) [M^+]; Anal. Calcd for $C_{13}H_{11}N_3O_4$: C 57.14, H 4.06, N 15.38, Found C 57.21, H 4.19, N 15.46.

1-[5-(5-nitrobenzofuran-2-yl)-1,3,4-oxadiazol-2-yl]ethanol (3e): Yield: 68 %, mp 176-177 °C, R_f: 0.59, IR (KBr) 3353 (OH), 3024 (C=C), 1589 (C=N), 1522 (N-O, NO_2),1416 (C-O-C) cm^{-1}; ^{1}HNMR (400 MHz, DMSO-d_6) δ9.3 (s, 1H, OH), 7.8-7.4 (m, 4H), 2.1 (d, *J* = 19.2 Hz, 1H, CH), 1.8 (t, *J* = 14.8 Hz, 3H, CH_3); [13]C NMR (100 MHz, DMSO-d_6) δ26.2 (CH_3),34.1 (CH), 117.1 (C_3), 119.3 (C_7), 121.7 (C_4), 127.1 (C_6), 132.4 (C_9),148.8 (C_2), 159.4 (C_5), 163.1 (C_8), 167.2 (C_5, oxadiazole), 171.1 (C_2, oxadiazole); MS m/z(%): 275 (42) [M^+]; Anal. Calcd for $C_{12}H_9N_3O_5$: C 52.37, H 3.30, N 15.27, Found C 52.46, H 3.39, N 15.42.

[5-(5-nitrobenzofuran-2-yl)-1,3,4-oxadiazol-2-yl]methanamine (3f): Yield: 76 %, mp 213-214 °C, R_f: 0.62, IR (KBr) 3324 (NH_2), 3249 (NH), 3021 (C=C), 1612 (C=N), 1516 (N-O, NO_2),1266 (C-O-C) cm^{-1}; ^{1}HNMR (400 MHz, DMSO-d_6) δ7.9-7.4 (m, 4H), 4.9 (s, 2H, CH_2NH_2), 2.1 (d, *J* = 12.6 Hz, 2H, CH_2); [13]C NMR (100 MHz, DMSO-d_6) δ31.2 (CH_2), 119.2 (C_3), 121.9 (C_7), 124.3 (C_4), 127.8 (C_6), 131.9 (C_9),146.1 (C_2), 161.1 (C_5), 165.5 (C_8), 169.7 (C_5, oxadiazole), 173.6 (C_2, oxadiazole);MS m/z(%): 360 (25) [M^+]; Anal. Calcd for $C_{11}H_8N_4O_4$: C 50.77, H 3.10, N 21.53, Found C 50.89, H 3.36, N 21.78.

5-(5-nitrobenzofuran-2-yl)-2-phenyl-1,3,4-oxadiazole (3g): Yield: 69 %, mp 217-218 °C, R_f: 0.55, IR (KBr) 3027 (C=C), 1593 (C=N), 1509 (N-O, NO_2),1411 (C-O) 1278 (C-O-C) cm^{-1}; ^{1}HNMR (400 MHz, DMSO-d_6) δ 8.1-7.8 (m, 9H); [13]C NMR (100 MHz, DMSO-d_6) δ117.2 (C_3), 121.1 (C_7), 123.5 (C_4), 125.4 (C_1 phenyl), 127.1 (2C, C_2 and C_6 phenyl), 127.8 (C_6), 129.2 (2C, C_3 and C_5 phenyl), 129.8 (C_4 phenyl), 134.2 (C_9),148.4 (C_2), 163.7 (C_5), 166.4 (C_8), 169.2 (C_5 oxadiazole), 172.1 (C_2 oxadiazole); MS m/z (%): 307 (18) [M^+]; Anal. Calcd for $C_{16}H_9N_3O_4$: C 62.54, H 2.95, N 13.68, Found C 62.78, H 3.11, N 13.82.

5-(5-nitrobenzofuran-2-yl)-2-(2-nitrophenyl)-1,3,4-oxadiazole (3h): Yield: 58 %, mp 227-228 °C, R_f: 0.67, IR (KBr) 3021 (C=C), 1632 (C=N), 1526, 1521(N-O, NO_2),1425 (C-O), 1264 (C-O-C) cm^{-1}; ^{1}HNMR (400 MHz, DMSO-d_6) δ8.1-7.9 (m, 8H); [13]C NMR (100 MHz, DMSO-d_6) δ120.9 (C_3), 122.7 (C_7), 126.1 (C_1nitrophenyl),127.1 (C_4), 128.8 (C_6), 131.4 (C_4nitrophenyl), 133.9 (C_5nitrophenyl),134.1 (C_9), 135.7 (C_3nitrophenyl), 136.5 (C_5nitrophenyl), 147.8 (C_2), 159.3 (C_2nitrophenyl), 163.6 (C_5), 168.2 (C_8), 169.7 (C_5, oxadiazole), 173.4 (C_2, oxadiazole); MS m/z(%): 352 (20) [M^+]; Anal. Calcd for $C_{16}H_8N_4O_6$: C 54.55, H 2.29, N 15.91, Found C 54.64, H 2.35, N 16.16.

2-(3,5-dinitrophenyl)-5-(5-nitrobenzofuran-2-yl)-1,3,4-oxadiazole (3i): Yield: 61 %, mp 288-289 °C, R_f: 0.48, IR (KBr) 3029 (C=C), 1622 (C=N), 1524, 1516 (N-O, NO_2), 1424 (C-O), 1261 (C-O-C) cm^{-1}; ^{1}HNMR (400 MHz, DMSO-d_6) δ8.2-7.9 (m, 7H); [13]C NMR (100 MHz, DMSO-d_6) δ123.2 (C_3), 124.1 (C_7), 126.7 (C_4), 127.1 (C_1 phenyl),128.7 (C_6), 133.9 (2C, C_2 and C_6dinitrophenyl), 134.4 (C_9), 148.1 (C_2), 148.2 (C_4dinitrophenyl), 158.7 (2C, C_3 and C_5dinitrophenyl), 166.1 (C_5), 168.4 (C_8), 169.9 (C_5, oxadiazole), 173.2 (C_2, oxadiazole); MS m/z(%): 397 (34) [M^+]; Anal. Calcd for $C_{16}H_7N_5O_8$: C 48.37, H 1.78, N 17.63, Found C 48.31, H 1.65, N 17.66.

5-(5-nitrobenzofuran-2-yl)-2-(4-nitrophenyl)-1,3,4-oxadiazole (3j): Yield: 67 %, mp 234-235 °C, R_f: 0.60, IR (KBr) 3027 (C=C), 1611 (C=N), 1522, 1517 (N-O, NO_2),1409 (C-O) 1272 (C-O-C) cm^{-1}; ^{1}HNMR (400 MHz, DMSO-d_6) δ 7.9-7.6 (m, 8H); [13]C NMR (100 MHz, DMSO-d_6) δ121.6 (C_3), 123.8 (C_7), 126.2 (C_4), 126.8 (C_1 phenyl), 128.2 (C_6), 129.8 (2C, C_2 and C_6nitrophenyl), 134.9 (C_9),135.1 (2C, C_3 and C_5nitrophenyl), 148.1 (C_2), 158.3 (C_4nitrophenyl), 164.3 (C_5), 167.9 (C_8), 169.6 (C_5, oxadiazole), 171.8 (C_2, oxadiazole); MS m/z(%): 352 (26) [M^+]; Anal. Calcd for $C_{16}H_8N_4O_6$: C 54.55, H 2.29, N 15.91, Found C 54.31, H 2.15, N 15.66.

5-(5-nitrobenzofuran-2-yl)-2-styryl-1,3,4-oxadiazole (3k): Yield: 58 %, mp 246-247 °C, R_f: 0.55, IR (KBr) 3026 (C=C), 1593 (C=N), 1523 (N-O, NO_2), 1416 (C-O) 1271 (C-O-C) cm^{-1}; ^{1}HNMR (400 MHz, DMSO-d_6) δ7.8 (d, *J* = 14.6Hz, 1H, CH=CHC$_6$H$_5$), 7.7-7.2 (m, 9H), 6.3 (d, *J* = 14.2Hz, 1H, CHC$_6$H$_5$); [13]C NMR (100 MHz, DMSO-d_6) δ119.2

(CH=CHC_6H_5), 121.6 (C_3), 122.6 (C_7), 124.9 (C_4), 127.4 (2C, C_2 and C_6 phenyl), 127.8 (C_6), 128.3 (2C, C_3 and C_5 phenyl), 129.4 (C_4 phenyl), 134.2 (C_1 phenyl),136.1 (C_9), 147.4 (C_2),148.1 (CH=CHC_6H_5), 165.2 (C_5), 169.1 (C_8), 171.7 (C_5 oxadiazole), 173.6 (C_2 oxadiazole); MS m/z (%): 333 (18) [M^+]; Anal. Calcd for $C_{18}H_{11}N_3O_4$: C 64.86, H 3.33, N 12.61, Found C 64.97, H 3.41, N 12.87.

2-(2-aminophenyl)-5-(5-nitrobenzofuran-2-yl)-1,3,4-oxadiazole (3l): Yield: 60 %, mp 257-258 °C, R_f: 0.69, IR (KBr) 3318 (NH_2), 3251 (NH), 3024 (C=C), 1631 (C=N), 1526 (N-O, NO_2), 1422 (C-O), 1267 (C-O-C) cm^{-1}; ^{1}HNMR (400 MHz, DMSO-d_6) δ 8.1-7.9 (m, 8H), 5.6 (s, 2H, NH_2); [13]C NMR (100 MHz, DMSO-d_6) δ121.6 (C_3), 123.4 (C_7), 125.2 (C_1aminophenyl), 126.7 (C_4), 129.3 (C_6), 133.7 (C_4aminophenyl), 134.6 (C_5aminophenyl), 135.2 (C_9), 136.7 (C_3aminophenyl), 138.1 (C_5aminophenyl), 149.8 (C_2), 161.1 (C_2aminophenyl), 167.8 (C_5), 169.3 (C_8), 171.4 (C_5 oxadiazole), 176.1 (C_2 oxadiazole); MS m/z(%): 322 (15) [M^+]; Anal. Calcd for $C_{16}H_{10}N_4O_4$: C 59.63, H 3.13, N 17.38, Found C 59.64, H 3.15, N 17.42.

2-(4-aminophenyl)-5-(5-nitrobenzofuran-2-yl)-1,3,4-oxadiazole (3m): Yield: 68 %, mp 234-236 °C, R_f: 0.74, IR (KBr) 3309 (NH_2), 3247 (NH), 3019 (C=C), 1629 (C=N), 1518 (N-O, NO_2), 1434 (C-O-C) cm^{-1};^{1}HNMR (400 MHz, DMSO-d_6) δ 7.9-7.7 (m, 8H), 5.4 (s, 2H, NH_2); [13]C NMR (100 MHz, DMSO-d_6) δ119.8 (C_3), 122.2 (C_7), 124.7 (C_4), 126.2 (C_1aminophenyl), 127.8 (C_6), 129.3 (2C, C_2 and C_6aminophenyl), 132.6 (C_9),134.3 (2C, C_3 and C_5aminophenyl), 147.3 (C_2), 156.1 (C_4aminophenyl), 159.8 (C_5), 162.3 (C_8), 167.8 (C_5, oxadiazole), 168.4 (C_2, oxadiazole); MS m/z(%): 322 (25) [M^+]; Anal. Calcd for $C_{16}H_8N_4O_6$: C 59.63, H 3.13, N 17.38 Found C 59.78, H 3.25, N 17.66.

2-(2-hydroxyphenyl)-5-(5-nitrobenzofuran-2-yl)-1,3,4-oxadiazole(3n): Yield: 52 %, mp 241-242 °C, R_f: 0.68, IR (KBr) 3326 (OH), 3017 (C=C), 1617 (C=N), 1525 (N-O, NO_2), 1272 (C-O-C) cm^{-1}; ^{1}HNMR (400 MHz, DMSO-d_6) δ10.7 (s, 1H, OH), 8.1-7.6 (m, 8H); [13]C NMR (100 MHz, DMSO-d_6) δ118.2 (C_3), 121.7 (C_7), 124.8 (C_1hydroxyphenyl), 127.1 (C_4), 129.6 (C_6), 134.2 (C_4hydroxyphenyl), 135.1 (C_5hydroxyphenyl), 136.4 (C_9), 138.4 (C_3hydroxyphenyl), 142.7 (C_5hydroxyphenyl), 147.4 (C_2), 163.1 (C_2hydroxyphenyl), 169.2 (C_5), 170.3 (C_8), 173.7 (C_5 oxadiazole), 176.9 (C_2 oxadiazole); MS m/z(%): 323 (32) [M^+]; Anal. Calcd for $C_{16}H_9N_3O_5$: C 59.45, H 2.81, N 13.00, Found C 59.58, H 3.06, N 13.21.

2-(4-hydroxyphenyl)-5-(5-nitrobenzofuran-2-yl)-1,3,4-oxadiazole (3o): Yield: 56 %, mp 218-219 °C, R_f: 0.72, IR (KBr) 3335 (OH), 3023 (C=C), 1597 (C=N), 1514 (N-O, NO_2), 1478 (C-O-C) cm^{-1}; ^{1}HNMR (400 MHz, DMSO-d_6) δ10.1 (s, 1H, OH), 7.9-7.6 (m, 8H);[13]C NMR (100 MHz, DMSO-d_6) δδ116.4 (C_3), 120.6 (C_7), 121.6 (C_4), 127.1 (C_1hydroxyphenyl), 128.2 (C_6), 129.8 (2C, C_2 and C_6hydroxyphenyl), 133.7 (C_9),136.2 (2C, C_3 and C_5hydroxyphenyl), 149.1 (C_2), 157.8 (C_4hydroxyphenyl), 159.3 (C_5), 163.5 (C_8), 167.2 (C_5, oxadiazole), 169.3 (C_2, oxadiazole); MS m/z(%): 323 (25) [M^+]; Anal. Calcd for $C_{16}H_9N_3O_5$: C 59.45, H 2.81, N 13.00, Found C 59.72, H 2.97, N 13.16.

5-(5-nitrobenzofuran-2-yl)-2-(pyridin-3-yl)-1,3,4-oxadiazole (3p): Yield: 69 %, mp 267-269 °C, R_f: 0.74, IR (KBr) 3025 (C=C), 1621, 1624 (C=N), 1524 (N-O, NO_2), 1455 (C-O-C) cm^{-1}; ^{1}HNMR (400 MHz, DMSO-d_6) δ 8.3-7.7 (m, 8H); [13]C NMR (100 MHz, DMSO-d_6) δ120.5 (C_3), 121.7 (C_7), 124.6 (C_4), 126.2 (C_5pyridin-3-yl), 127.8 (C_6), 132.6 (C_3pyridin-3-yl), 134.2 (C_9), 137.1 (C_4pyridin-3-yl), 148.4 (C_2), 159.1 (C_6 pyridin-3-yl), 163.4 (C_5), 165.9 (C_8), 167.9 (C_2 pyridin-3-yl), 169.4 (C_5 oxadiazole), 172.1 (C_2 oxadiazole);MS m/z(%): 308(38) [M^+]; Anal. Calcd for $C_{15}H_8N_4O_4$: C 58.45, H 2.62, N 18.18, Found C 58.72, H 2.86, N 18.31.

Synthesis of 5-(5-nitrobenzofuran-2-yl)-1,3,4-oxadiazole-2-thiol (3q)

To a solution of the acid hydrazide 2 (2.2 g, 0.01 mol) in ethanol (30.0 mL), equimolar quantities of carbon disulphide (3.8 mL, 0.05 mol) and potassium hydroxide (2.8 g, 0.05 mol) were added. The contents were heated to reflux for 4 h. Distilled water was added followed by neutralization with dilute HCl, to obtain a solid mass product **3q**. The product separated out, was filtered and recrystallized using methanol as solvent. Yield: 58 %, mp 283-284 °C, R_f: 0.53, IR (KBr) 3087 (C=C), 2587 (SH), 1620 (C=N), 1527 (N-O, NO_2),1283 (C-O-C) cm^{-1}; ^{1}HNMR (400 MHz, DMSO-d_6) δ 12.9 (s, 1H, SH), 8.1-7.6 (m, 4H); [13]C NMR (400 MHz, DMSO-d_6) δ 119.6 (C_3), 122.4 (C_7), 123.8 (C_4), 126.1 (C_6), 133.2 (C_9),147.2 (C_2), 162.7 (C_5), 163.4 (C_8), 168.9 (C_5, oxadiazole), 173.3 (C_2, oxadiazole); MS m/z(%): 263 (45) [M^+]; Anal. Calcd for $C_{10}H_5N_3O_4S$: C 45.63, H 1.91, N 15.96, S 12.18, Found C 45.69, H 2.07, N 16.11, S 12.39.

***In vitro* antioxidant activity**

The *in vitro* antioxidant activity was carried out by DPPH radical scavenging assay method with suitable modification [51]. In brief, the assay was carried out using UV spectrophotometer at 517 nm. To the 1 mL solution of synthesized compounds (10^{-4} M), 1 mL DPPH solution (25 µM) was added into the test tube. The solution was incubated at 37 °C for 30 min and the absorbance of each solution was measured at 517 nm against the reagent blank solution. The ascorbic acid (25 µM) was used as the reference antioxidant. The experimental values summarized for DPPH radical scavenging assays are expressed as the mean ± standard error of mean (SEM). The percent free radical scavenging activity was calculated by the formula given below.

$$\% \text{ Scavenging} = \frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}} \times 100$$

Pharmacology

All the animal experimental procedures and protocols adapted in the study were reviewed and approved by the Institutional Animal Ethics Committee. The experimental procedures and protocols were in accordance with the guidelines of the CPCSEA, Ministry of Forests and Environment, Govt. of India. The animals were obtained from the JSS Medical college, Mysore, India, and were maintained in colony cages at 25 ± 2°C, relative humidity of 45-55%, under a 12 h light and dark cycle; they were fed standard animal feed. All the animals were acclimatized for a week before use. Animals were deprived of food 12 h prior to experiment and only water was allowed *ad libitum*.

Anti-inflammatory evaluation

Anti-inflammatory activity was evaluated by carrageenan-induced paw edema test using groups of albino rats weighing 100-120 g each, 6 rats per group, as per the reported method [19]. The first group was injected with 0.1 mL of carrageenan (1% solution in normal saline) in the plantar tissue of the right hind paw and served as untreated control. The positive control group was given 10 mg kg^{-1} indomethacin one hour before carrageenan injection. The test compounds (3a–3q) were suspended in 0.5% carboxy methyl cellulose (CMC) and given to the rats orally at a dose of 50 mg kg^{-1} one hours prior to carrageenan injection.

Four hours after carrageenan administration, the paw volumes (mL) were measured using the mercury displacement technique with the help of a plethysmograph. The percent inhibition of paw edema was calculated by using the following formula.

$$\% \text{ Inhibition} = \left(\frac{a - x}{b - y}\right) \times 100$$

Where x is the mean paw volume of rats before the administration of carrageenan and/or test compounds or reference compound (test group), a is the mean paw volume of rats after the administration of carrageenan in the test group (drug/compound treated), b is the mean paw volume of rats after the administration of carrageenan in the control group and y is the mean paw volume of rats before the administration of carrageenan in the control group.

The mean percent inhibition of indomethacin and tested compounds at 10 mg kg^{-1} concentrations were compared with control using repeated measures ANOVA with Dunnet's test. The data obtained is expressed as mean ± standard error of mean (SEM).

Molecular docking studies

Crystallographicdata of the mouse COX-2 enzyme (PDB-ID: 3LN1) was used for docking studies, since the data on murine COX-2 have been reported in many studies [16]. The x-ray crystal structure of 3LN1 was downloaded from (http: //www. pdb. org).

The protein structure of 3LN1 is well established with a hydrophobic active site and was determined at 2.4 Å resolution. The protein was prepared using a protein preparation module in SYBYLX 2.1, where bond orders were assigned. Water and other residues were removed and the protein model was charged with AMBER7 FF99. Ligands, 1,3,4-oxadiazoles 3a-3q were drawn in ChemDraw and molecules were converted into *. sdf using Open Babel software.

Using the ligand preparation utility of SYBYLX 2.1, 3D structures were generated from 2D and the ligands were energy minimized using minimize module of SYBYL X 2.1 by applying molecular mechanics force fields (MMFF94s) and charged using Gasteiger-Marsili method.

Molecular docking simulations were performed in order to distinguish the basic receptor-ligand interactions. The docking experiments were carried using the SYBYL X 2.1. The bound conformation of celecoxib was used as controls in order to define the active site in COX-2, and the optimized 3D-structuresof oxadiazole series 3a-3q was docked within 10 Å radius to find the most optimal binding pose of each ligand. The docking algorithm performs a series of hierarchical searches for locations of possible ligand affinity within the binding site of the enzyme. The docked molecules are ranked with total scores, highlighting the affinity to the enzyme core. This total score is a model energy function that combines empirical and force field based terms.

RESULTS AND DISCUSSION

Chemistry

Synthesis of intermediate and target compounds was accomplished according to the steps depicted in s*cheme* 1. The starting material ethyl 5-nitrobenzofuran-2-carboxylate (1) was synthesized by condensing 2-hydroxy-5-nitrobenzaldehyde with ethyl 2-chloroacetate in the presence of anhydrous potassium carbonate, and a catalytic amount of potassium iodide, with modification of the reported method [48]. The ^{1}HNMR spectra of compound 1, accounts for nine protons, of which four protons are of benzofuran moiety, which have resonated as multiplet between 7.46 and 7.61 ppm. A doublet at 1.81 ppm was attributed to the methyl protons of the ester, and the methylene protons of ester have resonated as a triplet at 2.34 ppm. The conversion of the ester to hydrazide function was carried out as per the reported method [49]. The ester derivative 1 was treated with excess of hydrazine monohydrate in absolute ethanol to undergo nucleophilic addition to yield the acid hydrazide derivative, 5-nitrobenzofuran-2-carbohydrazide (2). In the [13]CNMR of the acid hydrazide derivative 2, the chemical signal at 174.8 ppm was attributed to the –CONH function of the hydrazide. In the ^{1}HNMR of the acid hydrazide derivative 2, two isolated singlet at 11.82 and 4.79 ppm were attributed to the NH and NH_2 protons of the hydrazide function, respectively and the mutiplet between 7.36 to 7.82 ppm was assigned to the four protons of the benzofuran moiety.

A total of seventeen 1,3,4-oxadiazole derivatives 3a-3q were synthesized, the acid hydrazide derivative 2 was condensed with various hetroaryl/aryl/aliphatic carboxylic acids in presence of phosphorous oxychloride to undergo dehydrative cyclization as per the reported method [50]. On the other hand, the mercapto derivative 3q was prepared by treating the acid hydrazide 2 with carbon disulphide in the presence of potassium hydroxide as per the reported method [30].

The ^{1}HNMR spectra of compound 3a accounts for five protons, a mutiplet between 6.92 to 7.11 ppm can be attributed to the protons of the benzofuran moiety. A singlet 8.21 ppm can be attributed to the proton at C_2 of the oxadiazole moiety. In the [13]CNMR spectra of compound 3b, the chemical signal at 26.71 ppm can be attributed to the methyl substitution at C_2 position of the oxadiazole moiety and the same methyl group protons have resonated as an isolated singlet at 1.21 ppm in the ^{1}HNMR spectra. The proton NMR spectra of compound 3c, with doublet at 1.43 ppm and a triplet at 0.86 ppm can be attributed for the methylene and the methyl protons of the ethyl group substitution at C_2 position of the oxadiazole moiety, respectively. The rest of the compounds synthesized were also characterized similarly and data were found in agreement with the proposed structures.

In vitro antioxidant evaluation

The newly synthesized 1,3,4-oxadiazole derivatives (3a-3q) were evaluated for their free radical scavenging activity by 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical assay method using ascorbic acid as standard. The compounds 3a-3q exhibited significant scavenging activity ranging from 34 to 86% and the data are expressed in Mean ± SEM in Table 1.

Table 1: *In vitro* antioxidant activity of the test compounds

S. No.	Compound	*Percentage free radical scavenging activity
1.	3a	68.64 ± 2.17
2.	3b	61.42 ± 1.76
3.	3c	56.11 ± 1.47
4.	3d	61.31 ± 2.04
5.	3e	72.61 ± 1.34
6.	3f	64.93 ± 1.85
7.	3g	34.86 ± 2.12
8.	3h	46.67 ± 2.31
9.	3i	36.21 ± 1.81
10.	3j	43.16 ± 2.07
11.	3k	39.33 ± 1.57
12.	3l	79.42 ± 3.09
13.	3m	74.87 ± 2.54
14.	3n	86.13 ± 1.84
15.	3o	81.42 ± 3.12
16.	3p	82.42 ± 1.34
17.	3q	41.67 ± 1.27
18.	Ascorbic Acid	89.17 ± 1.86

*Results are expressed as the mean values from three independent experiments ± SEM.

In vivo anti-inflammatory evaluation

The title compounds, with significant antioxidant property were evaluated for *in vivo* anti-inflammatory activity at a dose of 50 mg kg^{-1} by carrageenan induced paw edema method. Indomethacin at 10 mg kg^{-1} was used as reference standard and CMC as control. The result of the anti-inflammatory screening at the end of four hours after the administration of carrageenan highlighted compounds 3a-3q with edema reduction ranging from 9.1 to 72.5%, in comparison to 48.7% edema reduction seen with standard indomethacin.

The result of anti-inflammatory evaluation highlighted that compound 3c (ethyl), 3e (ethan-1-ol), 3d (propyl) and 3f (methanamine)exhibited superior anti-inflammatory activity than that of indomethacin (48.7%) with an edema reduction of 72.5, 68.4, 65.2 and 58.9%, respectively in comparison to the other set of oxadiazole derivatives (3a, 3b, 3g-3q).

Interestingly, derivatives with aryl substitutionat position C2 of the oxadiazole series (3g-3p), compounds with electron donating substituents on the phenyl moiety; namely 3l (2-aminophenyl), 3m (4-aminophenyl), 3n (2-hydroxyphenyl) and 3f (4-hydroxyphenyl) exhibited substantial activity ranging from 32.7 to 44.9% edema reduction. On the other hand, compound 3p with pyridin-3-yl substitution proved to be the major exception of all the tested compounds with edema reduction of only 9.1%. Whereas, compounds 3b(methyl) and 3q (thiol) exhibited edema reduction of only 21.4 and 26.7%, respectively.

The results of antioxidant and anti-inflammatory studies reveal correspondence between free radical scavenging efficacy and inhibition of induction of edema (percentage protection). The statistical analysis of the anti-inflammatory data obtained by Dunnet's test revealed that the tested compounds exhibited significant (*P< 0.001*) anti-inflammatory activity compared to control. The percentage inhibition of inflammation by the test compounds at dose of 50 mg kg^{-1} at the end of four hours time intervals are expressed in mean ± SEM in Table 2.

Table 2: *In vivo* anti-inflammatory activity of the test compounds at 50 mg kg^{-1} by carrageenan induced paw edema method at the end 4 h

S. No.	Compound	[a,b]Percentage Protection
19.	3a	17.25 ± 0.72***
20.	3b	24.41 ± 1.14***
21.	3c	72.51 ± 1.16***
22.	3d	65.19 ± 2.07***
23.	3e	68.43 ± 2.16***
24.	3f	58.92 ± 2.10***
25.	3g	24.46 ± 1.15***
26.	3h	16.85 ± 1.24***
27.	3i	18.21 ± 2.19***
28.	3j	12.05 ± 0.82***
29.	3k	25.68 ± 1.38***
30.	3l	42.08 ± 1.36***
31.	3m	44.9 ± 0.66***
32.	3n	38.85 ± 1.24***
33.	3o	32.71 ± 2.19***
34.	3p	09.14 ± 0.73***
35.	3q	26.68 ± 1.38***
36.	Indomethacin (10 mg kg^{-1})	48.72 ± 1.61***

[a] Results are expressed as the mean values from three independent experiments ± SEM.

[b] Data was analyzed by Dunnet's test. n = 3; (***) equals P<0.001

Docking Studies

The molecular modeling technique was used to explore, predict and understand the protein/enzyme interactions with our designed 1,3,4-oxadiazole library; and also to visualize the probable binding. The docking study was performed using theSYBYL X 2.1., wherein, all the seventeen 2,5-disubstituted 1,3,4-oxadizoles were docked into the active site of the enzyme COX-2 (PDB: 3LN1). The crystal structure of the enzyme along with its co crystallized ligand celecoxib, was downloaded from the RCSB protein data bank (http: //www. pdb. org). The binding site of 3LN1, a typical cylindrical core of 23 Å between Ser516A, Ser339B and Asp510A concentrating the hydrophobic interaction. The ligand, celecoxib exhibited a docking score of 9.6292, with the main hydrophobic interactions with the surrounding residues His75A, Leu345A and Leu517A, strongly contributed to the stabilization with 3LN1. And Tyr371A, Trp373A and Val335A residues are involved in Vanderwall's interaction with the akyl/haloakyl chain present in the celecoxib. The hydrogen bonding interaction of the amino group protons of the celecoxib were with the residues Gln178OE1 and His75. NE2 at a distance of 2.392 and 2.249 Å, respectively. The sulfonyl oxygen of the ligand celecoxib was involved in hydrogen bonding interaction with Arg499. HH11 and Phe504H residues at a distance of 2.114 and 2.295 Å, respectively (*Figure 1*).

The docking study revealed that the 1,3,4-oxadiazole derivatives 3a-3q, possessed high affinity towards 3LN1 (*Figure 2*). Among the series, compound 3d (2-propyl derivative) generated a good docking score (7.1399), the nitro group substitution at position C5 of the benzofuran moiety was involved showed hydrogen bond interaction with Arg499HH11 and Phe504H at a distance of 2.173 and 2.211 Å, respectively. The compound 3d was well aligned in the hydrophobic core of the 3LN1 (*Figure 3*).

Compound 3e, 3c and 3f exhibited a docking score of 6.6198, 6.3832 and 6.0524, respectively. Wherein, the nitro function at C_5 of the benzofuran moiety is involved in the hydrogen bond interaction with Arg499HH11 and Phe504H residues. Apart from the nitro group interaction, the compound 3c and 3f also exhibited hydrogen bond interaction between the nitrogen of the oxadiazole moiety and AArg106HH12 residue.

Compound 3k (2-styryl), 3h (2-nitro phenyl), 3g (phenyl), 3o (4-hydroxy phenyl), 3m (2-amino phenyl) and 3j (4-nitro phenyl) exhibited crash score of -4.7634, -4.7654, -4.9915, -5.4487, -7.443 and -8.0054 kcal mol^{-1}, respectively. A crash score > -4.5 kcal mol^{-1}, indicates inappropriate penetration of the ligand into the binding site of COX-2; 3LN1 resulting in the decreased forces of interaction with the amino acid. The results of docking studies and anti-inflammatory results reveal the correspondence between the importance of docking studies and anti-inflammatory activity.

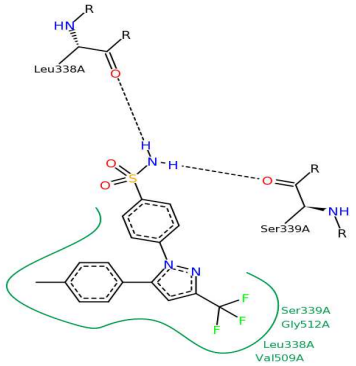

Fig. 1: Graphical representation of the ligand; celecoxib binding conformation onto 3LN1

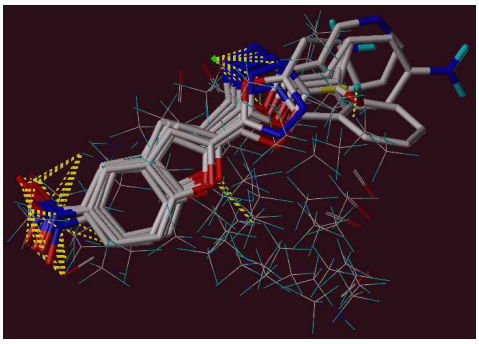

Fig. 2: Energy minimized and conformationally analyzed structures alignedagainst common atoms interacting with protomol.

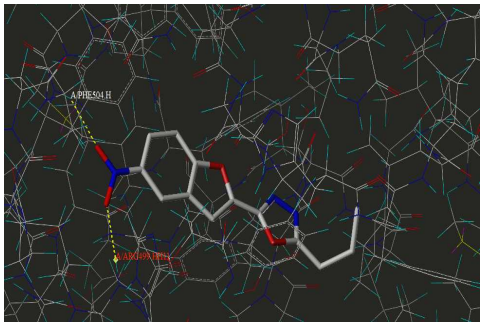

Fig. 3: Docking of 5-(5-nitrobenzofuran-2-yl)-2-propyl-1,3,4-oxadiazole (3d) into the active site of 3LN1(yellow dotted line represents the hydrogen bonding interaction).

CONCLUSION

A series of novel benzofuran encompassing oxadiazoles (3a-3q) was designed with the help of molecular modelling and docking studies. The designed library of oxadiazoles was synthesized, characterized and evaluated for *in vitro* antioxidant property and *in vivo* anti-inflammatory activity. The results of free radical scavenging activity by DPPH radicalassay method highlighted that the tested compounds 3a-3q exhibited significant scavenging activity. The *in vivo* anti-inflammatory activity was evaluated by carrageenan

induced paw edema method at dose of 50 mg kg^{-1}. The anti-inflammatory results highlight the synthesized compounds 3c, 3e and 3d could be considered as possible hit as therapeutic agents. It can be concluded that these compounds 3c, 3e and 3d certainly holds great promise towards good active leads in medicinal chemistry.

CONFLICT OF INTEREST

None

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