Design, Synthesis, Pharmacological Evaluation and Molecular Docking Studies of Substituted Oxadiazolyl-2-Oxoindolinylidene Propane Hydrazide Derivatives

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The manuscript describes design and synthesis of novel oxadiazolyl-2-oxoindolinylidene propane hydrazides as amide tethered hybrids of indole and oxadiazole and their evaluation for anti-inflammatory and analgesic activity. The compounds were synthesized following five step reaction to yield fifteen derivatives as 3-(5-substituted-1,3,4-oxadiazol-2-yl)-N’-[2-oxo-1,2-dihydro-3H-indol-3-ylidene]propane hydrazides. The final derivatives 3-[5-(4-hydroxyphenyl)-1,3,4-oxadiazol-2-yl]-N’-[2-oxo-1,2-dihydro-3H-indol-3-ylidene]propane hydrazide and 3-[5-(4-methylphenyl)-1,3,4-oxadiazol-2-yl]-N’-[2-oxo-1,2-dihydro-3H-indol-3-ylidene]propane hydrazide were found to be highly promising molecules with severity index of 0.35 and 0.56, respectively, which is promising for an analgesic compound. The hydroxy and methyl substitution on phenyl ring system provided with active anti-inflammatory compounds having increase in reaction time of 84.11 and 83.17%, respectively compared to standard drug at 85.84%. Molecular docking studies exhibit comparable interaction with synthesized derivatives and standard drug having a dock score of −4.44 by the K-nearest neighbour genetic algorithm method.

Keywords: hybrid approach, indole, anti-inflammatory, analgesic, molecular docking

Introduction

Discovery of new molecules within a short possible time has become a focal point in current medicine. Need for new and better drugs with less toxicity and more selectivity are major criteria for the designing of a molecule. Recently, many new approaches are observed to be practiced for development of newer biologically active molecules. Special focus is given on structure based drug discovery, fragment based drug discovery, protein-protein interaction inhibitor study, proteomics and pharmacogenomics and these are becoming more and more popular to medicinal chemists. Still the earlier methods are persistent and yielding good results in form of newer and safer molecules. One of such approach is hybrid approach. It involves development of better, synergistic molecules on hybridization of two or more active biomolecules or ligands to develop newer derivative that possess good pharmacological activity. The success of this approach has been unequivocal with reports of development of anticancer hybrid agents, several nonsteroidal anti-inflammatory drugs (NSAIDs) and antiviral agents. Many drugs that are developed on basis of hybrid approach are used in therapy today and many more are in pipelines and yet to come on market.

The success of hybrid approach is depicted by several molecules such as cediranib, which is a hybrid of quinazoline and indole; ateviridine, a hybrid of pyridine and indole; indalpine, a hybrid of indole; and

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piperazine and pravadoline, which is a hybrid of indole and morpholine, respectively (Figure 1). The success of this approach is well defined in discovery of NSAIDs such as the pravadoline, which acts as cyclooxygenase (COX) inhibitor but also exhibit a potent cannabinoid agonist effect. The need for efficient and nontoxic NSAIDs has made us to design and develop newer and better molecules. The unwanted gastrointestinal (GI) ulceration, bleeding, nephrotoxicity observed in conventional COX inhibitors and the failure of selective COX inhibitors due to adverse cardiac effects suggest for better drugs devoid of these severe side effects. In view of this scenario, it was decided to employ the hybrid approach for development of novel molecules for their analgesic and anti-inflammatory activity. To achieve this goal, we selected to design and synthesize a hybrid molecule from indole and oxadiazole nuclei. Indole derivatives have been found to exhibit variety of pharmacological activities and have raised considerable interest because of their potential beneficial effects on human health. They have been reported to possess antibacterial, anticonvulsant, antifungal, antiviral, anticancer and anti-inflammatory properties.
Indole derivative such as indomethacin is an established NSAID but it cause severe gastrointestinal side effect. The oxadiazole is found to poses versatile activity like the antiviral, anticancer and anticystic activity; compounds bearing 1,3,4-oxadiazole nucleus are also known to exhibit unique antiedema and anti-inflammatory potential. In the past, many indole and 1,3,4-oxadiazole derivatives showed potential for anti-inflammatory and analgesic activity in animal model of inflammation and pain.

Scheme 1. Synthesis of N’-(2-oxoindolin-3-ylidene)-3-(5-substituted phenyl-1,3,4-oxadiazol-2-yl)propane hydrazide derivatives 49-63.
The need for newer drug molecule and success of hybrid approach compelled us for development of some new NSAIDs. The target moieties selected for formation of a hybrid drug was based on our previous literature study, and it was found that indole and oxadiazole nuclei possess analgesic and anti-inflammatory properties and thus it was found worth to prepare a hybrid derivative from these moieties. To achieve this goal, we synthesized fifteen different hybrids of indole and oxadiazole as 5-substituted-1,3,4-oxadiazole-2-yl-(2-oxo-1,2-dihydro-indol-3-ylidene) propane hydrazide derivatives. Scheme 1 explains the synthesis of these derivatives that involves two different steps. In the first step, substituted aryl acid hydrazides were obtained from various substituted benzoic acids with formation of intermediate benzoates. In the second step, the indole-2,3-dione was reacted with hydrazine hydrate to form isatin-3-hydrazone, which in the subsequent reaction with succinic anhydride produces indolyl hydrazinyl butonic acid. This acid on reaction with substituted aryl acid hydrazides obtained from earlier steps yields the final derivatives as substituted oxadiazolyl indolyl propane hydrazides.

These newly synthesized compounds were tested initially for their in vitro characteristic as active or inactive molecules by the egg albumin denaturation test. Further, these compounds were subjected for their analgesic and anti-inflammatory activity by the carrageenan induced rat paw edema model and Eddy’s hot plate method, respectively. After evaluation for pharmacological studies, it becomes necessary to determine the safety of synthesized compounds with respect to ulcerogenesis study or the determination of severity index with respect to reference drug such as indomethacin. Further, to determine the possible interaction of most potent compound and receptor in silico, studies need to be carried out. This was achieved by molecular docking studies of the ligands on the COX-II receptor.

The series of final derivatives \( N'-(2\text{-oxoindolin-3-ylidene})-3-(5\text{-substituted phenyl-1,3,4-oxadiazol-2-yl}) \) propane hydrazides 49-63 as described in this study is outlined in Scheme 1. The products were obtained following two different steps; in the first step various substituted benzoic acids 1-15 were obtained commercially; these substituents were reacted with ethanol in presence of sulphuric acid to derive corresponding benzoates 16-30 under conditions of nucleophilic substitution reaction. These benzoates were further treated with hydrazine hydrate to yield various substituted aryl acid hydrazides 31-45, which were further employed in the reaction system. In the second step to the reaction scheme, isatin 46 was treated with hydrazine hydrate under ambient conditions to yield the isatin-3-hydrazone 47. It was further substituted by succinic anhydride to yield the oxindolyldiene hydrazinyl butanoic acid 48. Up to this moiety, the step two provides with reactant for the final reaction, in which the various substituted aryl acid hydrazides 31-45 and oxoindolyldiene hydrazinyl butanoic acid 48 react to undergo the cyclization reaction in presence of phosphorous oxychloride, yielding various \( N'-(2\text{-oxoindolin-3-ylidene})-3-(5\text{-substituted phenyl-1,3,4-oxadiazol-2-yl}) \) propane hydrazides 49-63 in 40-80% yield.

Experimental

Materials and methods

Chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA), S D Fine-Chem (Mumbai, MH, India) and Merck (Darmstadt, Germany), unless specified. Melting points (m.p.) were detected with open capillaries using ThermoNik precision melting point cum boiling point apparatus (model C-PMB-2, Mumbai, MH, India) and are uncorrected. Infrared (IR) spectra (KBr) were recorded on FTIR-8400s spectrophotometer (Shimadzu, Tokyo, Japan) at the Department of Pharmaceutical Sciences, Rashtrasant Tukadoii Maharai (RTM) Nagpur University. Proton (\( ^1\text{H} \)) and carbon 13 (\( ^{13}\text{C} \)) nuclear magnetic resonance (NMR) were obtained using a Bruker Avance II 400 MHz spectrometer (Billerica, MA, USA), using tetramethylsilane (TMS) as internal standard. All chemical shift values were recorded as \( \delta \) (ppm), coupling constant value \( J \) is measured in hertz, the peaks are presented as s (singlet), d (doublet), t (triplet), brs (broad singlet), dd (double doublet), m (multiplet). The purity of compounds was controlled by thin layer chromatography (silica gel HF254e361, type 60, 0.25 mm; Merck, Darmstadt, Germany). Electrospray ionization mass spectrometry (ESI-MS) was recorded at Waters Q-TOF spectrometer (Waters, Milford, MA, USA) and Merck (Darmstadt, Germany), unless specified.

Synthesis

General methods for synthesis of substituted ethyl benzoates (16-30)

To a solution of substituted benzoic acid (1-15) (0.246 mol) in dry ethanol (2.5 mol), concentrated sulphuric acid (0.5 mL) was added. The reaction mixture was refluxed for 8 h. Excess of ethanol was distilled off and the content was allowed to cool. The residue was poured into separating funnel containing 60 mL of water. Carbon-tetrachloride (5-10 mL) was added to obtain sharp separation of aqueous and ester layer. Ester layer was washed with sodium hydrogen carbonate solution. The esters (16-30) were
collected and recrystallized from ethanol. Details of these compounds are available in Supplementary Information.

General methods for synthesis of substituted aryl acid hydrazides (31-45)²⁴

The substituted ethyl benzoates (16-30) (0.01 mol) dissolved in dry ethanol (25 mL), hydrazine hydrate (99%, 0.01 mol) was added and the mixture was refluxed for 6 h. The reaction mixture was cooled and the solid obtained was filtered and recrystallized from dilute ethanol or from water. Details of these compounds are available in Supplementary Information.

Procedure for synthesis of 3-hydrazinylidene-1,3-dihydro-2H-indol-2-one (47)²⁵

A mixture of isatin (46) (1 mmol) and hydrazine hydrate (99%, 0.055 g, 1.1 mmol) in absolute methanol (25 mL) was refluxed for 1 h and then cooled to room temperature. The precipitate of hydrazones was filtered and dried. The crude product was recrystallized from ethanol to give hydrazones (47).

Yield: 1.0 g (70%); m.p. 248-250 °C; Rf 0.39 (methanol:toluene, 1:4); IR (KBr) ν / cm⁻¹ 3411, 2916, 1655, 1618; ¹H NMR (DMSO) δ 7.63 (s, 1H, Ar-H), 7.45 (s, 1H, Ar-H), 7.31 (s, 1H, Ar-H), 7.20 (s, 1H, Ar-H), 7.0 (s, 1H, NH), 3.34 (s, 2H, NH); ¹³C NMR (100 MHz, DMSO-d₆) δ 166.25, 152.64, 130.73, 125.24, 120.75, 120.61, 117.55, 78.81; El-MS m/z [M + H]+ 162.24.

Procedure for synthesis of 4-oxo-4-[2-oxo-1,2-dihydro-3H-indol-3-ylidene]-propane hydrazinecarboxylic acid (48)²⁶

The mixture of compound (47) (0.01 mol), succinic anhydride (0.01 mol) and trimethylamine (1 mmol) in dichloromethane (DCM) was stirred at room temperature for 3 h and diluted with DCM. The solution was washed with sodium bicarbonate and brine twice. The product thus obtained was dried over sodium sulphate and the excess of solvent was distilled off. The resulting solid was recrystallized from ethanol.

Yield: 0.08 g (80%); m.p. 238-240 °C; Rf 0.58 (toluene); IR (KBr) ν / cm⁻¹ 3394, 3059, 2916, 1655, 1618; ¹H NMR (DMSO) δ 12.44 (s, 1H, OH), 7.99 (t, 1H, J 6.7 Hz, Ar-H), 7.97 (s, 1H, Ar-H), 7.44 (d, 1H, J 8.2 Hz, Ar-H), 7.31 (d, 1H, J 7.2 Hz, Ar-H), 6.30 (s, 1H, NH), 3.78 (s, 2H, CH₂), 2.23 (s, 2H, CH₂); ¹³C NMR (100 MHz, DMSO-d₆) δ 166.22, 157.59, 148.41, 131.35, 126.12, 123.58, 121.71, 120.58, 118.78, 106.41, 51.01, 30.31; El-MS m/z [M + H]+ 262.04.

General procedure for synthesis of 3-(5-substituted-1,3,4-oxadiazol-2-yl)-N’-[2-oxo-1,2-dihydro-3H-indol-3-ylidene]propane hydrazide derivatives (49-63)²⁷

To a solution of compound (31-45) (0.01 mol) in phosphorous oxychloride (15-20 mL), compound (48) (0.01 mol) was added. The reaction mixture was refluxed for 5 h. The mixture was cooled to room temperature and poured onto crushed ice and neutralized the contents with sodium hydroxide solution (1 mol L⁻¹). The product was filtered, washed with water, dried and recrystallized using methanol to get 49-63.

3-(5-Phenyl-1,3,4-oxadiazol-2-yl)-N’-[2-oxo-1,2-dihydro-3H-indol-3-ylidene]propane hydrazide (49)

Yield: 2.03 g (78%); m.p. 208-210 °C; Rf 0.83 (chloroform); λ max 448 nm; IR (KBr) ν / cm⁻¹ 3254, 3059, 2916, 1655, 1618; ¹H NMR (DMSO) δ 8.91 (s, 1H, Ar-H), 8.59 (s, 1H, Ar-H), 8.0 (s, 1H, Ar-H), 7.98 (d, 2H, J 7.9 Hz, Ar-H), 7.76 (t, 2H, J 8.3 Hz, Ar-H), 7.45 (dd, 1H, J 7.4 Hz, Ar-H), 7.32 (dd, 1H, J 7.6 Hz, Ar-H), 3.58 (s, 2H, CH₂), 2.38 (s, 2H, CH₂); ¹³C NMR (100 MHz, DMSO-d₆) δ 160.53, 156.17, 148.33, 131.52, 126.20, 123.76, 121.78, 120.72, 33.98; El-MS m/z [M + H]+ 362.58; anal. calcd. for C₁₆H₁₃N₂O₃; C, 63.1; H, 4.15; N, 19.37; found: C, 63.3; H, 4.17; N, 19.39.

3-(5-(4-Hydroxyphenyl)-1,3,4-oxadiazol-2-yl)-N’-[2-oxo-1,2-dihydro-3H-indol-3-ylidene]propane hydrazide (50)

Yield: 2.08 g (80%); m.p. 210-212 °C; Rf 0.8 (chloroform); λ max 442 nm; IR (KBr) ν / cm⁻¹ 3581, 3211, 2849, 2978, 1711, 1605; ¹H NMR (DMSO) δ 9.49 (s, 1H, OH), 7.72 (t, 2H, J 6.9 Hz, Ar-H), 7.40 (t, 2H, J 8.1 Hz, Ar-H), 7.21 (t, 1H, J 6.9 Hz, Ar-H), 7.18 (d, 1H, J 7.4 Hz, Ar-H), 6.65 (t, 1H, J 7.8 Hz, Ar-H), 6.36 (s, 1H, Ar-H), 2.31 (s, 2H, CH₂), 1.23 (s, 2H, CH₂); ¹³C NMR (100 MHz, DMSO-d₆) δ 166.11, 162.44, 157.29, 153.76, 137.59, 132.14, 128.25, 126.72, 122.81, 115.18, 112.36, 102.89, 50.84, 23.17; El-MS m/z [M + H]+ 378.11; anal. calcd. for C₁₆H₁₃N₂O₃; C, 63.4; H, 4.13; N, 19.33; found: C, 63.6; H, 4.17; N, 19.35.

3-(5-(4-Methylphenyl)-1,3,4-oxadiazol-2-yl)-N’-[2-oxo-1,2-dihydro-3H-indol-3-ylidene]propane hydrazide (51)

Yield: 1.82 g (70%); m.p. 200-202 °C; Rf 0.47 (chloroform); λ max 357 nm; IR (KBr) ν / cm⁻¹ 3249, 2849, 2918, 1614, 1598; ¹H NMR (DMSO) δ 7.75 (t, 1H, J 8.1 Hz, Ar-H), 7.46 (t, 1H, J 7.6 Hz, Ar-H), 7.34 (t, 1H, J 5.9 Hz, Ar-H), 7.19 (d, 1H, J 7.8 Hz, Ar-H), 7.02 (m, 2H, J 7.9 Hz, Ar-H), 6.82 (t, 1H, J 8.4 Hz, Ar-H), 6.43 (s, 1H, Ar-H), 3.34 (s, 4H, CH₂), 2.32 (s, 3H, CH₃); ¹³C NMR (100 MHz, DMSO-d₆) δ 166.37, 166.05, 162.55, 158.97, 153.97, 152.79, 137.52, 133.74, 130.88, 128.22, 126.76, 122.81, 120.82, 117.68, 113.56, 112.36, 102.79, 55.90, 54.94, 23.20; El-MS m/z [M + H]+ 376.31; anal. calcd. for C₁₇H₁₄N₂O₃; C, 64.1; H, 4.6; N, 18.7; found: C, 64.6; H, 4.8; N, 18.91.
3-[5-[(3-Nitrophenyl)-1,3,4-oxadiazol-2-yl]-N'-[2-oxo-1,2-dihydro-3H-indol-3-ylidene]propane hydrazide (52)

Yield: 1.87 g (72%); m.p. 248–250 °C; Rf 0.72 (chloroform); \( \delta_{\text{max}} \) 447 nm; IR (KBr) \( \nu / \text{cm}^{-1} \) 3084, 2918, 1655, 1618, 2835, 1425; \(^1\)H NMR (DMSO-\( \text{d}_6 \)) \( \delta \) 7.73 (s, 1H, Ar−H), 7.21 (2H, \( J = 7.4 \text{ Hz, Ar−H} \)), 7.19 (1H, Ar−H), 7.05 (s, 1H, Ar−H), 6.76 (t, 1H, \( J = 7.9 \text{ Hz, Ar−H} \)), 6.66 (s, 1H, Ar−H), 4.07 (t, 2H, \( J = 7.9 \text{ Hz, CH}_2 \)), 3.40 (s, 2H, CH\( _2 \)), 2.50 (s, 3H, O−CH\( _3 \)), 1.22 (d, 3H, J = 7.8 Hz, O−CH\( _3 \)); \(^1\)C NMR (100 MHz, DMSO-\( \text{d}_6 \)) \( \delta \) 165.64, 162.36, 153.50, 147.05, 146.48, 137.67, 137.76, 126.71, 122.80, 119.49, 115.51, 112.52, 112.23, 90.27, 56.38, 55.57, 23.08, 14.13; EI-MS m/z [M + H]+ 407.72; anal. calcd. for \( \text{C}_{18}\text{H}_{17}\text{N}_{2}\text{O}_5 \): C, 56.3; H, 3.5; N, 20.7.

3-[5-[(2,3-Dimethoxyphenyl)-1,3,4-oxadiazol-2-yl]-N'-[2-oxo-1,2-dihydro-3H-indol-3-ylidene]propane hydrazide (53)

Yield: 2.16 g (83%); m.p. 230–232 °C; Rf 0.69 (chloroform); \( \delta_{\text{max}} \) 525 nm; IR (KBr) \( \nu / \text{cm}^{-1} \) 3107, 2918, 1648, 1603, 1210, 2849; \(^1\)H NMR (DMSO-\( \text{d}_6 \)) \( \delta \) 7.73 (t, 1H, \( J = 7.4 \text{ Hz, Ar−H} \)), 7.49 (d, 1H, \( J = 7.9 \text{ Hz, Ar−H} \)), 7.19 (2H, \( J = 8.5 \text{ Hz, Ar−H} \)), 7.03 (d, 1H, \( J = 8.3 \text{ Hz, Ar−H} \)), 6.75 (d, 1H, \( J = 7.4 \text{ Hz, Ar−H} \)), 6.65 (s, 1H, Ar−H), 6.37 (s, 1H, Ar−H), 3.71 (s, 2H, CH\( _2 \)), 3.35 (s, 2H, CH\( _2 \)), 2.50 (s, 3H, CH\( _3 \)), \(^1\)C NMR (100 MHz, DMSO-\( \text{d}_6 \)) \( \delta \) 200.36, 166.12, 162.48, 153.70, 147.14, 146.49, 137.65, 136.71, 126.74, 123.93, 122.80, 119.31, 115.50, 112.50, 111.41, 108.24, 56.29, 55.58, 50.82, 23.13; EI-MS m/z [M + H]+ 404.09; anal. calcd. for \( \text{C}_{18}\text{H}_{17}\text{N}_{2}\text{O}_2 \): C, 62.48; H, 4.28; N, 17.42.

3-[5-[(4-Acetylphenyl)-1,3,4-oxadiazol-2-yl]-N'-[2-oxo-1,2-dihydro-3H-indol-3-ylidene]propane hydrazide (56)

Yield: 2.08 g (80%); m.p. 166–170 °C; Rf 0.65 (chloroform); \( \delta_{\text{max}} \) 3105 nm; IR (KBr) \( \nu / \text{cm}^{-1} \) 3017, 3107, 2918, 1649, 1603, 1210, 2849; \(^1\)H NMR (DMSO-\( \text{d}_6 \)) \( \delta \) 7.73 (t, 1H, \( J = 7.4 \text{ Hz, Ar−H} \)), 7.49 (d, 1H, \( J = 7.9 \text{ Hz, Ar−H} \)), 7.19 (2H, \( J = 8.5 \text{ Hz, Ar−H} \)), 7.03 (d, 1H, \( J = 8.3 \text{ Hz, Ar−H} \)), 6.75 (d, 1H, \( J = 7.4 \text{ Hz, Ar−H} \)), 6.65 (s, 1H, Ar−H), 6.37 (s, 1H, Ar−H), 3.71 (s, 2H, CH\( _2 \)), 3.35 (s, 2H, CH\( _2 \)), 2.50 (s, 3H, CH\( _3 \)); \(^1\)C NMR (100 MHz, DMSO-\( \text{d}_6 \)) \( \delta \) 200.36, 166.12, 162.48, 153.70, 147.14, 146.49, 137.65, 136.71, 126.74, 123.93, 122.80, 119.31, 115.50, 112.50, 111.41, 108.24, 56.29, 55.58, 50.82, 23.13; EI-MS m/z [M + H]+ 404.09; anal. calcd. for \( \text{C}_{18}\text{H}_{17}\text{N}_{2}\text{O}_2 \): C, 62.48; H, 4.28; N, 17.42.
3-[5-(2-Nitrophenyl)-1,3,4-oxadiazol-2-yl]-N-[2-oxo-1,2-dihydro-3-H-indol-3-ylidene]propane hydrazide (59)

Yield: 2.22 g (89%); m.p. 242-244 °C; Rf 0.71 (chloroform); λ_max 438 nm; IR (KBr) v / cm⁻¹ 3212, 2916, 1645, 1615, 2749, 1522; ¹H NMR (DMSO) δ 8.31 (s, 1H, Ar−H), 7.87 (d, 1H, J 7.6 Hz, Ar−H), 7.78 (d, 1H, J 7.4 Hz, Ar−H), 7.61 (dd, 2H, J 5.3 Hz, Ar−H), 7.21 (t, 2H, J 7.4 Hz, Ar−H), 6.72 (t, 1H, J 7.1 Hz, Ar−H), 3.63 (s, 2H, CH₂), 2.34 (s, 2H, CH₃); ¹³C NMR (100 MHz, DMSO-d₆) δ 165.75, 163.11, 155.20, 147.82, 143.43, 137.15, 133.34, 130.43, 127.01, 124.34, 123.33, 123.02, 122.80, 121.50, 112.37, 101.87, 51.11, 23.38; EI-MS m/z [M + H]+ 407.4; anal. calcd. for C₅₁H₄₁N₁₉O₇ Br: C, 60.31; H, 3.78; N, 18.52; found: C, 60.38; H, 3.78; N, 18.52.

3-[5-(3-Chlorophenyl)-1,3,4-oxadiazol-2-yl]-N-[2-oxo-1,2-dihydro-3-H-indol-3-ylidene]propane hydrazide (60)

Yield: 1.1 g (43%); m.p. 235-237 °C; Rf 0.66 (chloroform); λ_max 432 nm; IR (KBr) v / cm⁻¹ 3229, 2743, 1713, 1538, 2902, 725; ¹H NMR (DMSO) δ 8.56 (s, 1H, Ar−H), 8.14 (s, 1H, Ar−H), 7.87 (d, 2H, J 8.4 Hz, Ar−H), 7.65 (t, 2H, J 7.6 Hz, Ar−H), 7.45 (dd, 1H, J 7.5 Hz, Ar−H), 7.25 (dd, 1H, J 7.8 Hz, Ar−H), 3.35 (s, 2H, CH₃), 2.80 (s, 2H, CH₂); ¹³C NMR (100 MHz, DMSO-d₆) δ 172.16, 159.17, 144.48, 136.60, 125.16, 123.26, 123.17, 121.85, 121.70, 117.27, 56.10, 33.33; EI-MS m/z [M + H]+ 397.02; anal. calcd. for C₅₃H₄₅ClN₁₉O₇Br: C, 56.3; H, 3.48; N, 20.68; found: C, 56.6; H, 3.5; N, 20.69.

3-[5-(2-Fluorophenyl)-1,3,4-oxadiazol-2-yl]-N-[2-oxo-1,2-dihydro-3-H-indol-3-ylidene]propane hydrazide (61)

Yield: 2.11 g (82%); m.p. 180-182 °C; Rf 0.65 (chloroform); λ_max 368 nm, IR (KBr) v / cm⁻¹ 3297, 2916, 1655, 1605, 1097; ¹H NMR (DMSO) δ 8.10 (d, 2H, J 8.3 Hz, Ar−H), 7.68 (t, 2H, J 7.6 Hz, Ar−H), 7.26 (dd, 1H, J 7.6 Hz, Ar−H), 7.24 (dd, 1H, J 7.3 Hz, Ar−H), 6.81 (t, 2H, J 6.9 Hz, Ar−H), 3.73 (s, 2H, CH₂), 2.50 (s, 2H, CH₃); ¹³C NMR (100 MHz, DMSO-d₆) δ 164.52, 163.31, 161.31, 160.36, 135.94, 129.96, 129.88, 127.96, 124.35, 124.17, 115.94, 115.72, 114.50, 103.80, 79.49, 57.01, 51.68; EI-MS m/z [M + H]+ 380.16; anal. calcd. for C₅₀H₄₇F₁₁N₁₉O₇Br: C, 60.31; H, 3.71; N, 18.48; found: C, 60.38; H, 3.78; N, 18.52.

3-[5-(4-Bromophenyl)-1,3,4-oxadiazol-2-yl]-N-[2-oxo-1,2-dihydro-3-H-indol-3-ylidene]propane hydrazide (62)

Yield: 2.64 g (82%); m.p. 174-176 °C; Rf 0.65 (chloroform); λ_max 398 nm, IR (KBr) v / cm⁻¹ 2982, 2906, 1655, 1685; ¹H NMR (DMSO) δ 8.91 (s, 1H, Ar−H), 8.59 (s, 1H, Ar−H), 8.08 (s, 1H, Ar−H), 7.98 (d, 1H, J 7.9 Hz, Ar−H), 7.76 (t, 2H, J 8.3 Hz, Ar−H), 7.45 (dd, 1H, J 7.4 Hz, Ar−H), 7.32 (dd, 1H, J 7.6 Hz, Ar−H), 3.58 (s, 2H, CH₂), 2.38 (s, 2H, CH₃); ¹³C NMR (100 MHz, DMSO-d₆) δ 160.53, 156.17, 148.33, 131.52, 126.20, 123.76, 121.78, 120.72, 33.98; EI-MS m/z [M + H]+ 441.32; anal. calcd. for C₅₀H₄₅Br₁₁N₁₉O₇: C, 60.31; H, 3.71; N, 18.48; found: C, 60.38; H, 3.78; N, 18.52.

In vitro evaluation for anti-inflammatory activity by protein albumin denaturation method

All synthesized compounds were screened for anti-inflammatory activity by using in vitro method reported earlier by Mizushima and Kobayashi with slight modification. Accordingly, inhibition of albumin denaturation technique was studied, a 5.0 mL reaction mixture was prepared consisting of 0.2 mL of egg albumin (obtained from fresh hen’s egg), 2.8 mL phosphate buffered saline (pH 6.4) and 2.0 mL of varying concentration of test compounds, so that final concentrations become 25, 50, 100 and 200 µg mL⁻¹. Similar volume of double distilled water served as control. Then, the mixtures were incubated at 37±2 °C in an incubator for 15 min and then heated at 70°C for 5 min. After cooling, their absorbance was measured at 660 nm by using vehicle as blank. Indomethacin with final concentration of 50 and 100 µg mL⁻¹ was used as reference drug and treated similarly for determination of absorbance. The reading for this activity was taken on the same day and percent inhibition of protein denaturation was calculated by equation 1, as follows:

\[
\text{%Inhibition} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} \times 100
\]  

In vivo evaluation

Anti-inflammatory activity

The anti-inflammatory activity was carried out according to the method of Halen et al. and employed...
with some modifications. Sprague Dawley rats were used for this study; seventeen groups with six rats per group were formed. All test samples were administered to animals at 100 mg kg\(^{-1}\) dosage, as suspension in 0.5% carboxymethyl cellulose in water. The samples were administered orally; after 60 min of drug dose, injection of 0.1 mL solution of carrageenan (0.5 mg 25 mL\(^{-1}\)) was injected into the sub-plantar tissue of the left hind paw of each rat. Out of this, one group was treated with standard drug indomethacin (100 mg kg\(^{-1}\)). The initial volume of paw was measured within 30 s after carrageenan injection. Later on paw volume was measured after 1-5 h, respectively. The relative increase in the paw volume was calculated in the individual animal of the control, test, and standard groups, respectively. The percentage of inhibition of edema was calculated by the equation 2, as follows:

\[
\text{Anti-inflammatory activity (\% inhibition)} = \left(1 - \frac{D_t}{D_c}\right) \times 100
\]  

(2)

where \(D_t\) means relative change in paw volume in test group and \(D_c\) means relative change in paw volume in control group.

Analgesic activity

Analgesic activity test was performed following the method of Eddy and Leimbach.\(^21\) Analgesic activities of all synthesized compounds were quantified \textit{in vivo} by Eddy’s hot plate method using analgesiometer. Albino wistar mice were used for this study; seventeen groups with six rats per group were formed. All the test compounds were suspended in 0.5% of carboxymethylcellulose sodium (CMC) and administered orally. The albino wistar rats were treated with the newly synthesized derivatives (100 mg kg\(^{-1}\), p.o.) and standard drug indomethacin (100 mg kg\(^{-1}\), p.o.). The animals were individually placed on the hot plate maintained at 55 °C, one hour after their respective treatments. The response time was noted as the time at which animals reacted to the pain stimulus either by paw licking or jump response. The relative increase in reaction time was measured at an interval of 0, 30, 60 and 90 min in the individual animal of the control, test and the standard group (Table 1). The percent increase in reaction time was calculated using equation 3 as follows,

\[
\% \text{ Increase in reaction time} = \left(1 - \frac{I_t}{I_0}\right) \times 100
\]  

(3)

where \(I_t\) is the reaction time at time \(t\) and \(I_0\) is the reaction time at time zero (0 min).

Ulcerogenic activity

Acute ulcerogenesis was done according to Cioli \textit{et al.}\(^28\) and modifications standardized in our laboratory.\(^18\) Albino rats (150-200 g) were divided into seven different groups

### Table 1. Anti-inflammatory, analgesic, ulcerogenic index and molecular docking studies of synthesized compounds (49-63)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Anti-inflammatory activity</th>
<th>Analgesic activity</th>
<th>Ulcerogenic index</th>
<th>Dock score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>\textit{In vitro}</td>
<td>\textit{In vivo}</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Absorbance(^a)</td>
<td>Inhibition of denaturation / %</td>
<td>Inhibition of paw edema(^b) / %</td>
<td>Increase in reaction / %</td>
</tr>
<tr>
<td>49</td>
<td>0.0401</td>
<td>61.64</td>
<td>11.6</td>
<td>13.7</td>
</tr>
<tr>
<td>50</td>
<td>0.0409</td>
<td>66.59</td>
<td>35.8</td>
<td>40.9</td>
</tr>
<tr>
<td>51</td>
<td>0.0407</td>
<td>66.19</td>
<td>38.4</td>
<td>43.1</td>
</tr>
<tr>
<td>52</td>
<td>0.0403</td>
<td>65.58</td>
<td>13.4</td>
<td>14.1</td>
</tr>
<tr>
<td>53</td>
<td>0.0402</td>
<td>65.07</td>
<td>30.3</td>
<td>30.5</td>
</tr>
<tr>
<td>54</td>
<td>0.0398</td>
<td>62.13</td>
<td>20.4</td>
<td>21.2</td>
</tr>
<tr>
<td>55</td>
<td>0.0403</td>
<td>65.63</td>
<td>20.2</td>
<td>21.4</td>
</tr>
<tr>
<td>56</td>
<td>0.0401</td>
<td>61.64</td>
<td>35.1</td>
<td>37.6</td>
</tr>
<tr>
<td>57</td>
<td>0.0402</td>
<td>63.18</td>
<td>35.5</td>
<td>40.0</td>
</tr>
<tr>
<td>58</td>
<td>0.0403</td>
<td>65.87</td>
<td>21.2</td>
<td>22.6</td>
</tr>
<tr>
<td>59</td>
<td>0.0397</td>
<td>61.58</td>
<td>33.4</td>
<td>35.56</td>
</tr>
<tr>
<td>60</td>
<td>0.0373</td>
<td>54.12</td>
<td>22.5</td>
<td>25.8</td>
</tr>
<tr>
<td>61</td>
<td>0.0403</td>
<td>65.12</td>
<td>23.1</td>
<td>25.2</td>
</tr>
<tr>
<td>62</td>
<td>0.0404</td>
<td>64.16</td>
<td>28.5</td>
<td>31.8</td>
</tr>
<tr>
<td>63</td>
<td>0.0402</td>
<td>62.13</td>
<td>23.5</td>
<td>24.8</td>
</tr>
<tr>
<td>Standard drug(^c)</td>
<td>0.0510</td>
<td>82.14</td>
<td>28.5</td>
<td>28.7</td>
</tr>
</tbody>
</table>

\(^a\)Readings at 660 nm; \(^b\)increase in paw volume measured after 3 h from administration of samples; \(^c\)indomethacin. NT: Not treated.
Accordingly, the mean score of each treated group minus the mean score of the control group was considered to determine the severity index of gastric damage for compounds under study.

Molecular docking study

For docking purpose, the three-dimensional structure of COX-2 (protein data bank, PDB code 4Z0L) was obtained from RCSB PDB. The receptor molecule was refined and validated on the basis of Ramachandran plot using Biopredicta© module on the Vlife MDS Molecular Modeling software, version 4.3.1. The Vlife MDS suit uses k-nearest neighbour genetic algorithm (KNN-GA) method for molecular docking. docking calculations and energy minimization were set in the Biopredicta© module, most of the parameters were set default with 10000 cycles per molecule for the active site cavity No. 1. Since this receptor is a homodimer nearly default with 10000 cycles

Results and Discussion

The reported investigation deals with synthesis and characterization of several hybrid derivatives from indole and 1,3,4-oxadiazole nucleuses linked via hydrazide chain to form final fifteen derivatives. To achieve these, two different steps were carried out to provide with substituted aryl acid hydrazides and indolidene hydrazinyl butanoic acids, which were further reacted to yield the final derivatives. In the first step, various substituted benzoic acids esterifies in presence of ethanol and concentrated sulphuric acid to yield corresponding ethyl benzoates. These compounds were treated with hydrazine hydrate to yield the substituted aryl acid hydrazide. These compounds were not characterised on basis of NMR and mass analysis because they are reported in literature; hence, the physicochemical parameters were used and carry out the confirmation of these compounds. All the compounds were analysed on the basis of melting point and TLC and found to be in understanding with the reported molecules.

In the second step, isatin (1H-indole-2,3-dione) was reacted with hydrazine hydrate in presence of methanol under conditions of reflux to yield the 3-hydrazinylidene-1,3-dihydro-2H-indol-2-one. NMR spectra of this compound exhibited prominent signals at 3.34 ppm corresponding to the secondary amide proton, the aromatic protons belonging to fused benzene ring was exhibited around 7.0 to 7.7 ppm presenting four protons.

This compound was confirmed on the basis of molecular weight 162.24 on the ESI-MS in positive mode. In the next step, was reacted with succinic anhydride under basic condition in an aprotic solvent such as dichloromethane to obtain 4-oxo-4-[2-(2-oxo-1,2-dihydro-3H-indol-3-ylidene)-hydrazinyl]butanoic acid. This compound was confirmed from mass spectra in positive mode. In the next step, was reacted with succinic anhydride under basic condition in an aprotic solvent such as dichloromethane to obtain 4-oxo-4-[2-(2-oxo-1,2-dihydro-3H-indol-3-ylidene)-hydrazinyl]butanoic acid.

This compound was confirmed on the basis of mass spectral study which reflects the molecular weight of 162.24 on the ESI-MS in positive mode. In the next step, was reacted with succinic anhydride under basic condition in an aprotic solvent such as dichloromethane to obtain 4-oxo-4-[2-(2-oxo-1,2-dihydro-3H-indol-3-ylidene)-hydrazinyl]butanoic acid.

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is indicated at 1655 cm\(^{-1}\) and the C=N bond is reflected around 1618 cm\(^{-1}\). These groups are common to all the molecules from final derivatives. The \(^1\)H NMR spectra of these compounds exhibited several characteristic NMR shifts. The ethylene protons were observed around \(\delta\ 2.00\) to 3.85 ppm; the aromatic protons were observed within the range of \(\delta\ 7.00\) to 8.50 ppm, representing the phenyl ring and the indole aromatic nucleus. Compound 50 presented with a hydroxyl proton at \(\delta\ 9.49\) ppm. These compounds were also analysed for their mass spectral characteristic and was found to be in good agreement with the results obtained for compound 50, which exhibited molecular mass of [M + H]\(^{+}\) 378.11 in the electron spray ionisation with positive mode of mass spectrometry.

All the synthesized compounds of the final series 49-63 were screened for \textit{in vitro} anti-inflammatory activity based on assay developed by Mizushima and Kobayashi. This assay is also known as the protein albumin denaturation test as it involves the determination of percentage from denaturation of protein and thus its stabilization ultimately the inhibition of inflammation. The relativity of absorbance between test samples (compounds 49-63) with respect to control, designated the stabilization of protein and thus the inhibition of heat-induced protein (albumin) denaturation or the inhibition by derivatives and reference drug indomethacin. The increase in absorbance in the test compounds indicated better stabilization of proteins compared to standard drug indomethacin and the blank. Compounds 50 and 51 exhibited absorbance of 0.0409 and 0.0407 accounting for a 66.59 and 66.19\% of denaturation, respectively. These are highest in this series of compounds followed by 65.87\% (0.0403) for 58, then 65.63\% (0.0403) for 55, and 65.07\% (0.0402) for 53 (Table. 1). Other derivatives have also shown better percent of denaturation, which is in the range of 54.12 to 64.16\%; the standard drug indomethacin has exhibited percent of denaturation of 82.14\% (0.0510). These results indicate that all the compounds have potential anti-inflammatory activity since the least active compound is also exhibiting a percent denaturation of 54.12\%, with more than fifty percent, whereas the most active compound presents inhibition of 66.59\%. \textit{In vitro} assay displayed some features of this series with respect to substitutions; methyl, hydroxy, methoxy, chloro and nitro substituted derivatives, such as 50, 51, 58 and 55, possess good anti-inflammatory activity. Since these compounds were studied at various concentrations (25, 50, 100 and 200 \(\mu\)g mL\(^{-1}\)), it was decided to perform \textit{in vivo} activity on all the derivatives to determine their anti-inflammatory activity.

Compounds were evaluated for their \textit{in vivo} anti-inflammatory activity by carrageenan induced paw edema method. The protocol of animal experiments was approved by the Institutional Animal Ethics Committee (IAEC). The compounds were tested at 100 mg kg\(^{-1}\) oral dose and were compared with the standard drug indomethacin at 100 mg kg\(^{-1}\) oral dose. The tested compounds showed anti-inflammatory activity ranging from 13.9 to 45.5\% after 3 h (Table 1). The anti-inflammatory results revealed that compounds 50, 51 and 58 exhibited good anti-inflammatory activity whereas compounds 53 and 55 showed moderate activity, and compounds 63, 62, 57, 54 and 49 showed low activities when compared with standard drug (indomethacin). These results illustrate that compounds substituted with methyl 51, hydroxy 50, methoxy 53 and nitro 55 at position 5 of the 1,3,4-oxadiazole ring system showed good anti-inflammatory activity, having maximum percentage of inhibition in edema. Surprisingly, the halogen derivatives such as chloro 54, fluoro 57, bromo 63 and acetyl 56 showed low activity. This indicates that the compound having \(p\)-substituted electron withdrawing groups may enhance anti-inflammatory activity and electron releasing groups diminished the activity. The activity declines with replacement of electronegative group by electropositive group. It is noteworthy that NO\(_2\) substitution of 3-nitrophenyl in 52 and 4-nitrophenyl in 55 induce remarkable change in the activity, i.e., compound substituted with 3-nitrophenyl 52 showed low activity while compound substituted with 4-nitrophenyl 55 showed good activity. So, \(p\)-substituted derivatives favour good activity than \(m\)-substituted derivatives.

The analgesic activity of the synthesized compounds was evaluated by hot plate test according to Eddy and Leimbach. The compounds were tested at 100 mg kg\(^{-1}\) oral dose and were compared with the standard drug indomethacin at 100 mg kg\(^{-1}\) oral dose. The tested compounds showed analgesic activity ranging from 25.13 to 84.11\% (Table 1) after 90 min time cycle for the series of compounds under study. The analgesic screening results revealed that compounds 50, 51 and 52 showed good analgesic activity whereas compounds 49 and 61 showed moderate activity and compounds 56 and 57 showed low activity when compared with standard drug. The results illustrate that compounds substituted with 3-nitrophenyl 52 at position 5 of the 1,3,4-oxadiazole ring system showed good analgesic activity having elevated percentage increase in reaction time following the compounds 50 and 51 with maximum activity. In addition, compounds substituted with phenyl 49 and 2-fluorophenyl 61 showed moderate activities while compound substituted with 2,3-dimethoxy 53, 4-chlorophenyl 54 and 4-nitrophenyl 55 showed low activity. This indicates that the compounds having \(p\)-substituted and \(m\)-substituted electron withdrawing
groups may enhance analgesic activity and electron releasing groups diminish the activity. From the above discussion, it is clear that compound substituted with 4-fluorophenyl 57 was found to be a fair anti-inflammatory agent with poor analgesic activity. These results suggest that unlike the substitution on indomethacin, the 1,3,4-oxadiazole ring system behaves differently and suggests for substitution with electron donating group (EDG) on the ring system.

The ulcerogenic activity was performed according to Cioli et al. and reports published earlier from our lab. Those compounds, which showed good results in anti-inflammatory and analgesic activity, were screened for their ulcerogenic activity. The tested compounds exhibited activity in the range of 0.14 to 0.56, whereas standard drug indomethacin presented very high severity index of 1.25. Results of this activity indicate that compound 50 and 51 have a severity index of 0.35 and 0.56, respectively. Compounds 49 and 61 exhibit severity index of 0.24 and 0.39, respectively, which is the lowest compared to other tested compounds; these compounds lack good anti-inflammatory activity. This experiment clearly indicates that 50 and 51 are better tolerated derivative of this series and are far safer than the standard drug indomethacin with respect to the ulcerogenic effect.

Indole is a very well-known nucleus and its derivative indomethacin is known to possess anti-inflammatory activity and used in therapy since last forty years. Accordingly, we proposed and derived several hybrids consisting of indole and oxadizole nuclei as inhibitor of the COX receptor, which is the target receptor for nearly all the NSAIDs. On the basis of in vitro and in vivo studies, it was found that compounds 50 and 51 exhibit good anti-inflammatory and analgesic activity. In order to investigate the molecular interactions and binding modes of some of the synthesized derivatives like 50, 51, 53, 55 and 62, we docked these derivatives with COX enzyme (PDB code 4Z0L) using licensed version of Vlife MDS 4.32 software tools. This COX-2 receptor was used for our study because of two reasons: first, this complex provides with an indole derivative, which can act as a reference molecule for docking; and the second reason was the similarity with our animal model. Prior to carrying out docking the COX-2 receptor was prepared for docking and the Van der Waals forces interactions. Three compounds, 50, 51 and 53, have exhibited good activity with 50 and 51 being most active followed by the other. This was also observed with the results of molecular docking; the energies for 50 and 51 were found to be −4.44 and −4.37, respectively, which is highest in the series of synthesized derivatives and comparable to standard drug indomethacin with score of −4.47 (Table 1). Compound 50 revealed that it binds to the active site of COX-2 receptor by forming hydrogen bond with GLY536 (bond length: 2.125 Å) and TYR373 (bond length: 2.325 Å; Figure 2a, 50). Hydrophobic interactions were found to be mostly between GLN374 with bond length of 4.635, 4.538 and 4.045, ARG376 (Figure 2b, 50). It also formed multiple Van der Waal interactions with the receptor amino acids having bond length between 2 to 3.9 Å (Figure 2c, 50).

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The Van der Waal interactions were observed between the receptor and indole nucleus plus its substituted oxadiazole moiety. This molecule seemed to lay on both the chains as A and B of the homodimer, it is illustrated by fact that interactions were observed with GLY536A, PHE142B, TRP139B, PRO538A, ASN375A, GLN374A, ASN375A, ARG376B, ASN373B, GLY536B, TYR373B, PRO127A, PRO538B and ASN537B with bond length between 2.342 and 4.828 (Figure 2b, 51). It also formed multiple Van der Waal interactions with the receptor amino acids having bond length between 2.0 and 3.9 Å (Figure 2c, 51).

The docking was carried out for all synthesized compounds. Figure 2 exhibits the docking mode of compound 50 and 51 with all three important interactions like hydrogen bonding, hydrophobic interaction and the Van der Waals forces interactions. Three compounds, 50, 51 and 53, have exhibited good activity with 50 and 51 being most active followed by the other. This was also observed with the results of molecular docking; the energies for 50 and 51 were found to be −4.44 and −4.37, respectively, which is highest in the series of synthesized derivatives and comparable to standard drug indomethacin with score of −4.47 (Table 1). Compound 50 revealed that it binds to the active site of COX-2 receptor by forming hydrogen bond with GLY536 (bond length: 2.125 Å) and TYR373 (bond length: 2.325 Å; Figure 2a, 50). Hydrophobic interactions were found to be mostly between GLN374 with bond length of 4.635, 4.538 and 4.045, ARG376 (Figure 2b, 50). It also formed multiple Van der Waal interactions with the receptor amino acids having bond length between 2 to 3.9 Å (Figure 2c, 50).

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as the bromine. It is observed that presence of methyl or hydroxyl groups causes rise in activity as well as it can be seen that nitro substitution on the 1,3,4-oxadiazole causes increase in the activity. The docking of newly designed molecules has also presented their entry into deep sited region of the receptor, probably on the confluence of both the chains. Thus, it is considered that presence of indole and oxadiazole ring in the single molecule is beneficial for the activity, but the presence of halogen such as fluorine or chlorine on the 1,3,4-oxadiazole reduces the activity. Further, it is also noted that the position of substitution on the phenyl ring has great contribution towards the activity as an anti-inflammatory and analgesic compound.

In this preliminary communication, we have presented synthesis and pharmacological evaluation of novel N’-(2-oxoindolin-3-ylidene)-3-(5-substituted phenyl-1,3,4-oxadiazol-2-yl)propane hydrazide derivatives 49-63, obtained following a five step reactions. The reactions were simple and follow-up procedure resulted in pure compounds with satisfactory yield. In vitro and in vivo anti-inflammatory activity revealed that compounds 50 and 51 exhibit comparable inhibition to standard drug indomethacin with added advantage of very less possibility of ulceration. These compounds were found to be good analgesic agents also when compared with standard drug. These molecules exhibited that methyl and hydroxy substituted molecules are better tolerated as well as present better activity when compared to their chloro and fluoro counterparts. The molecular docking studies on these molecules also verified the wet lab results, compounds 50 and 51 exhibited hydrogen bonding, hydrophobic interactions as well as Van der Waals interactions. The dock scores and binding energy were found to be in good agreement with the pharmacological results. This work need to be further elaborated with respect to number of molecules, their versatility, animal models, and structure activity relationship studies and most importantly with respect to the pK/pD studies.

Conclusions

In conclusion, we have described the design and synthesis of novel oxadiazolyl-2-oxoindolinylidene propane hydrazides as amide tethered hybrids of indole and oxadiazole and their evaluation for anti-inflammatory and analgesic activity. The compounds were successfully synthesised following five step reaction to yield fifteen
derivatives as 3-(5-substituted-1,3,4-oxadiazol-2-yl)-N’-[2-oxo-1,2-dihydro-3H-indol-3-ylidene]propane hydrazides. In vitro and in vivo studies have exhibited final derivatives 50 and 51 as highly promising molecules with severity index of 0.35 and 0.56, respectively, encouraging for an analgesic compound. The hydroxy and methyl substitutions on phenyl ring system provided with active compounds having percentage of inhibition of 84.11 and 83.17%, respectively, compared to standard the drug at 85.84%. Molecular docking studies are also in agreement with the pharmacological evaluation with potent compounds exhibiting dock score of −4.44. It can be stated that these compounds can be further studied for their structure-activity relationship (SAR) studies and developed into potential lead molecules.

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br as PDF file.

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References


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