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Synthesis and Biological Evaluation of 1*H*-(Indole-5-yl)-3-Substituted-1,2,4-Oxadiazoles as Novel 5-Lox Inhibitors

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Abstract: A series of indolyloxadiazoles were synthesized from amidoxime and indole 3-carboxaldehyde using CAN as a catalyst and PEG as a solvent. In *vitro,* a 5-LOX inhibitory assay has been performed for all the synthesized compounds. Among the tested compounds, **4bf** showed the highest potency (IC_{50} 18.78 µg/ml). The synthesized compound carried out docking on the 5-LOX enzyme protein crystal structure. Compound (**4bf**) docked snugly into the receptor site with a score of -9.1 Kcal/mol, and it showed strong hydrogen bond interactions with two key amino acids, **His368** and **Asn555**.

Keywords: 1,2,4-oxadiazoles, Indolyloxadiazoles, 5-LOX inhibitors.

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1. INTRODUCTION

Over the past decades, the synthesis of heterocyclic compounds has become the cornerstone of synthetic organic chemistry due to the wide variety of their applications in medicinal and pharmaceutical chemistry (1). The key field of medicinal chemistry is the investigation of heterocycles as favored structures in drug discovery (2). Among them, oxadiazole and indole ring systems are ubiquitous structural units and important pharmacophores in several alkaloids and many biologically active compounds (3). Some of the recent studies have shown that 1,2,4-oxadiazole and its derivatives were reported to possess peptide inhibitory activity (4), anti-hyperglycemic (5), antiparasitic (6), anti-inflammatory (7), muscarinic (8), anticancer (9), antifungal (10), antibacterial (11), antitumor (12), histemic-H_3 (13), and signal transduction (14) activities.

Indole derivatives have a wide range of biological and pharmacological actions (15). Specifically, 3-substituted indole derivatives exhibit a variety of pharmacological effects, including antibacterial (16), anti-inflammatory (17), antitumor (18), anticancer (19), antihypertensive (20), antidepressant (21), antiviral (22) and anti-HIV (23) activities. Oxadiazole and indole-containing substances exhibit a diversity of biological roles. Therefore, the linked molecules of 3-substituted indole and oxadiazole frame structures, indole-based oxadiazoles, are useful physiologically active agents. Indole-substituted 1,2,4-oxadiazoles exhibit a broad spectrum of biological activities, including anticancer activity (24). Indole-substituted 1,2,4-oxadiazoles also act as 5-HT3 antagonists (25). Recently, oxadiazole ring containing indole alkaloids such as phidianidines A and B **(Fig. 1)** have been isolated by Marianna, C. et al., 2011 from the marine opisthobranch *mollusk phidianamilitaris* (26).

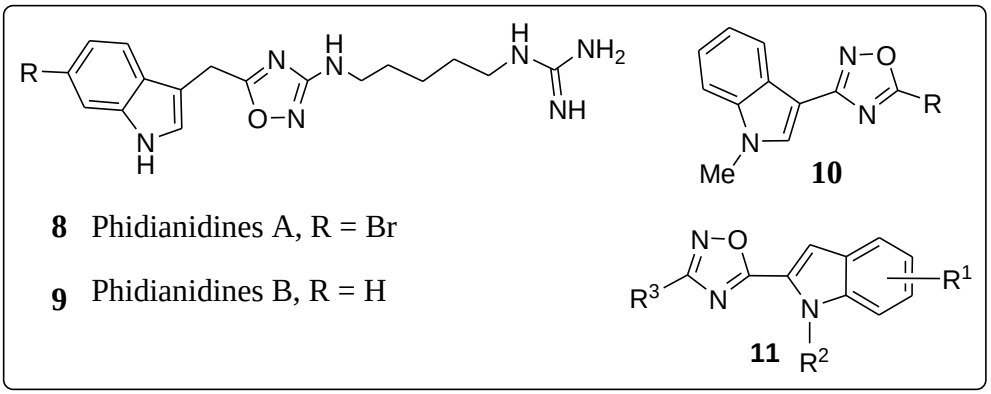


Figure 1: Bioactive indole substituted 1,2,4-oxadizoles

Lipoxygenases (LOXs), which are widely distributed in both the plant and animal kingdoms, belong to a class of non-heme iron-containing enzymes that catalyze the hydroperoxidation reaction of fatty acids to peroxides (27). In recent years, multi-functional inhibitors of 5-LOX and other enzymes in the arachidonic acid metabolic network have been paid much attention. Leukotrienes (LTs) have been identified as mediators of a variety of inflammatory and allergic reactions, including asthma, rheumatoid arthritis, inflammatory bowel disease, psoriasis, allergic rhinitis (28), stroke (29) and anticancer drugs (30). 5-LOX inhibitors structural and functional diversity of naturally occurring indole has made it a privileged pharmacophore in drug development where indole-based natural and synthetic compounds (31) find myriad properties as antibacterial anti-inflammatory (32), antitumor (33), anticancer (34), activities.

This is prompted by the wide range of activities of indole-substituted oxadiazoles and due to insufficient effective synthetic methods for synthesizing indole-substituted oxadiazoles and primarily focused on synthesizing a series of 1H-(indole-5-yl)-3-substituted 1,2,4-oxadiazoles and examining their 5-lipoxygenase inhibitory effects.

2. EXPERIMENTAL

All the chemicals used were of synthetic grade procured from Sigma Aldrich. Progress of the reactions was monitored by analytical thin layer chromatography (TLC) using E-Merck 0.25 mm silica gel plates using ethyl acetate/hexane as a solvent system. Visualization was accomplished with UV light (256 nm) and an iodine chamber. Synthesized compounds were purified by column chromatography (silica gel 100-200 mesh) using a hexane and ethyl acetate mixture. Melting points were measured in open capillary tubes and were uncorrected; all the ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$/DMSO-d_6 solvent (400 MHz for ^{1}H and 100 MHz for ^{13}C) relative to TMS internal standard.

2.1. General procedure for the synthesis of 1H-(indole-5-yl)-3-substituted 1,2,4-oxadiazoles (4aa-4ah; 4ba-4bf)

In a 50-mL round-bottom flask equipped with a magnetic bar, benzonitriles (**1**) (1.0 mmol), hydroxylamine hydrochloride (0.5 mmol, 34.7 mg), triethylamine (0.9 mmol) and EtOH (1.5 mL) were added. The reaction mixture was heated to 80 °C under vigorous stirring. The reaction progress was monitored by TLC. After completion, the mixture (**2a**) cooled to room temperature and concentrated by using a rotary evaporator to remove ethanol solvent and then 1*H*-indole-3-carbaldehyde (**3a**) (1.2 mmol), CAN (0.5 mmol) and PEG (1.5 mL) were added to the round-bottom flask, and the mixture was stirred at 80 °C. The reaction progress was monitored by TLC; after completion, the reaction mixture was cooled to room temperature, and then the solution was extracted with ethyl acetate. The obtained organic layer was dried over anhydrous Na_2SO_4 and concentrated by using a rotary evaporator to get crude compound **4aa**, which was further purified by column chromatography on silica gel using petroleum ether/ethyl acetate as the eluent to give the desired target product (**4aa-4ah; 4ba-4bf**)

2.1.1. 3-(3-Phenyl-1,2,4-oxadiazol-5-yl)-1H-indole (4aa) (35)

White Solid; Yield: 83%; Mp: 170-172 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.29 (d, 1H), 8.45 (d, 1H), 8.25 (d, 2H, J = 8 Hz), 8.15 (d, 1H, J = 2.8 Hz), 7.61 (t, 4H), 7.32 (d, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.7, 168.0, 137.0, 131.7, 131.3, 129.6, 127.5, 127.2, 124.8, 123.5, 122.2, 120.6, 113.1, 100.3; LC-MS: *m/z* = 262.1 $[M+H]^+$; Anal. Calcd. For $C_{16}H_{11}N_3O$: C, 73.57; H, 4.23; N, 16.07; Found: C, 73.54; H, 4.25; N, 16.01.

2.1.2. 3-(3-p-Tolyl-1,2,4-oxadiazol-5-yl)-1H-indole (4ab)

Yellow Solid; Yield: 81%; Mp: 137-139 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.95 (d, 1H), 8.45 (d, 1H), 8.43 (d, 2H, *J* = 8 Hz), 7.82 (d, 1H), 7.52-7.50 (d, 1H, *J* = 8 Hz), 7.39-7.36 (m, 4H, *J* = 12 Hz), 2.46 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.1, 168.4, 141.2, 136.1, 134.8, 129.5, 128.4, 127.4, 124.5, 123.8, 122.4, 121.3, 111.7, 102.8, 21.6; LC-MS: *m/z* = 276.8 $[M+H]^+$; Anal. Calcd. For $C_{17}H_{13}N_3O$: C, 74.17; H, 4.73; N, 5.79; Found: C, 74.09; H, 4.77; N, 5.75.

2.1.3. 3-(3-m-Tolyl-1,2,4-oxadiazol-5-yl)-1H-indole (4ac)

Yellow Solid; Yield: 84%; Mp: 204-206 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.95 (d, 1H), 8.45 (d, 1H), 8.14 (d, 2H), 7.82 (d, 1H), 7.74 (d, 1H), 7.36 (m, 4H), 2.46 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.1, 168.4, 141.2, 136.4, 134.8, 130.5, 128.6, 127.5, 124.8, 123.6, 122.4, 121.3, 119.7, 115.8, 111.7, 102.8, 21.9; LC-MS: *m/z* = 276.7 $[M+H]^+$; Anal. Calcd. For $C_{17}H_{13}N_3O$: C, 74.17; H, 4.73; N, 5.79; Found: C, 74.09; H, 4.75; N, 5.77.

2.1.4. 3-(3-(4-Methoxyphenyl)-1,2,4-oxadiazol-5-yl)-1H-indole (4ad)

Blue Solid; Yield: 82%; Mp: 121-122 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.2 (d, 1H), 8.23 (d, 1H, *J* = 2.4 Hz), 8.22 (d, 1H *J* = 3.2 Hz), 8.065-8.06 (d, 2H, *J* = 2 Hz), 7.572-7.553 (d, 3H, *J* = 8 Hz), 7.292-7.273 (d, 2H *J* = 7.6 Hz), 3.84 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.4, 167.7, 162.0, 136.9, 134.8, 131.1, 129.2, 124.8, 123.8, 122.1, 120.6, 119.5, 113.1, 100.4, 55.8; LC-MS: *m/z* = 292.8 $[M+H]^+$; Anal. Calcd. For $C_{17}H_{13}N_3O_2$: C, 70.06; H, 4.47; N, 14.41; Found: C, 70.03; H, 4.49; N, 14.38.

2.1.5. 3-(3-(4-Chlorophenyl)-1,2,4-oxadiazol-5-yl)-1H-indole (4ae) (36)

White Solid; Yield: 78%; Mp: 158-160 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.29 (d, 1H), 8.43(d, 1H), 8.21 (d, 2H, *J* = 6.8 Hz), 8.05 (d, 1H, *J* = 8.1 Hz), 7.65 (d, 3H), 7.27 (d, 2H, *J* = 7.5 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 174.0, 167.0, 136.9, 134.6, 134.3, 131.7, 129.0, 126.8, 125.1, 124.7, 123.8, 122.1, 120.5, 113.3; LC-MS: *m/z* = 296.08 $[M+H]^+$; Anal. Calcd. For $C_{16}H_{10}ClN_3O$: C, 64.96; H, 3.40; N, 14.20; Found: C, 64.95; H, 3.45; N, 14.17.

2.1.6. 3-(3-(3-Chlorophenyl)-1,2,4-oxadiazol-5-yl)-1H-indole (4af)

Colorless Solid; Yield: 83%; Mp: 110-112 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.30 (d, 1H), 8.23 (d, 1H, *J* = 4 Hz), 8.22 (s, 1H, *J* = 4 Hz), 8.21 (d, 1H, *J* = 6.4 Hz), 7.65 (m, 4H, *J* = 4 Hz), 7.64 (m, 2H, *J* = 4 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 174.0, 167.0, 136.9, 134.9, 134.3, 131.6, 129.1, 127.1, 126.1, 124.7, 123.8, 122.1, 120.5, 118.8, 113.3, 100.1; LC-MS: *m/z* = 296.9 $[M+H]^+$; Anal. Calcd. For $C_{16}H_{10}ClN_3O$: C, 64.96; H, 3.40; N, 14.20; Found: C, 64.95; H, 3.44; N, 14.15.

2.1.7. 3-(3-(4-Bromophenyl)-1,2,4-oxadiazol-5-yl)-1H-indole (4ag)

Yellow Solid; Yield: 75%; Mp: 155-157 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.28 (d, 1H), 8.43 (d, 1H), 8.14 (d, 1H, *J* = 4 Hz), 8.13 (d, 2H, *J* = 4 Hz), 7.78 (d, 2H *J* = 8 Hz), 7.59 (d, 1H), 7.31 (t, 2H, *J* = 2.4 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.9, 167.3, 136.9, 132.7, 132.6, 131.4, 129.6, 127.5, 126.4, 124.8, 122.5, 120.5, 113.2, 100.2; LC-MS: *m/z* = 341.2 $[M+H]^+$; Anal. Calcd. For $C_{16}H_{10}BrN_3O$: C, 56.46; H, 2.96; N, 12.38; Found: C, 56.41; H, 2.98; N, 12.35.

2.1.8. 3-(3-(3-Bromophenyl)-1,2,4-oxadiazol-5-yl)-1H-indole (4ah)

Colorless Solid; Yield: 68%; Mp: 174-176 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.28 (d, 1H), 8.43 (s, 1H), 8.25 (d, 1H), 8.11 (d, 2H), 7.75 (d, 2H), 7.53 (t, 1H), 7.31 (t, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.9, 167.3, 136.9, 132.7, 131.4, 130.6, 128.5, 127.5, 124.8, 122.3, 120.5, 118.8, 113.1, 100.2; LC-MS: *m/z* = 341.2 $[M+H]^+$; Anal. Calcd. For $C_{16}H_{10}BrN_3O$: C, 56.46; H, 2.96; N, 12.34; Found: C, 56.44; H, 2.99; N, 12.32.

2.1.9. 5-Bromo-3-(3-phenyl-1,2,4-oxadiazol-5-yl)-1H-indole (4ba)

White Solid; Yield: 76%; Mp: 211-213 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.44 (d, 1H), 8.46-8.44 (s, 1H, *J* = 8 Hz), 8.31 (d, 1H, *J* = 3.2 Hz), 8.11-8.10 (d, 2H, *J* = 4 Hz), 7.58 (d, 2H), 7.54-7.53 (m, 2H, *J* = 4 Hz), 7.43-7.42 (m, 1H, *J* = 4 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.0, 168.1, 135.7, 132.4, 131.8, 129.6, 127.5, 127.0, 126.5, 126.2, 122.6, 115.2, 114.9, 100.0; LC-MS: *m/z* = 341.2 $[M+H]^+$; Anal. Calcd. For $C_{16}H_{10}BrN_3O$: C, 56.47; H, 2.96; N, 12.34; Found: C, 56.42; H, 3.01; N, 12.30.

2.1.10. 5-Bromo-3-(3-p-tolyl-1,2,4-oxadiazol-5-yl)-1H-indole (4bb)

White Solid; Yield: 72%; Mp: 217-219 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.40 (d, 1H), 8.44 (s, 1H), 8.30 (d, 1H), 8.11 (d, 2H), 7.54 (d, 2H), 7.40 (d, 2H), 2.40 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.0, 168.1, 138.7, 135.7, 132.4, 129.5, 127.8, 126.9, 126.5, 125.7, 124.7, 121.6, 115.2, 114.6, 100.1, 21.5; LC-MS: *m/z* = 355.8 $[M+H]^+$; Anal. Calcd. For $C_{17}H_{12}BrN_3O$: C, 57.62; H, 3.41; N, 11.86; Found: C, 57.59; H, 3.45; N, 11.82.

2.1.11. 5-Bromo-3-(3-m-tolyl-1,2,4-oxadiazol-5-yl)-1H-indole (4bc)

White Solid; Yield: 70%; Mp: 216-218 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.40 (d, 1H), 8.446-8.442 (s, 1H, *J* = 1.6 Hz), 8.436-8.433 (d, 1H, *J* = 2 Hz), 7.54-7.53 (s, 2H, *J* = 4 Hz), 7.45 (d, 1H, *J* = 3.6 Hz), 7.45-7.40 (t, 3H, *J* = 8 Hz), 2.50 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.0, 168.1, 138.9, 135.7, 132.4, 129.5, 127.8, 126.9, 126.5, 126.2, 124.7, 122.6, 115.2, 114.9, 100.0, 21.3; LC-MS: *m/z* = 355.2 $[M+H]^+$; Anal. Calcd. For $C_{17}H_{12}BrN_3O$: C, 57.60; H, 3.39; N, 11.85; Found: C, 57.58; H, 3.42; N, 11.83.

2.1.12. 5-Bromo-3-(3-(4-methoxyphenyl)-1,2,4-oxadiazol-5-yl)-1H-indole (4bd)

Yellow Solid; Yield: 73%; Mp: 190-192 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.33 (d, 1H, *J* = 1.6 Hz), 8.32 (d, 1H), 8.072-8.05 (d, 2H, *J* = 8.8 Hz), 7.57-7.55 (d, 3H, *J* = 8 Hz), 7.409-7.404 (d, 2H, *J* = 1.6 Hz), 3.85 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.8, 167.8, 162.0, 136.3, 135.7, 132.4, 129.2, 126.6, 122.6, 121.1, 119.3, 115.5, 100.0, 55.8; LC-MS: *m/z* = 371.8 $[M+H]^+$; Anal. Calcd. For $C_{17}H_{12}BrN_3O_2$: C, 55.12; H, 3.25; N, 11.35; Found: C, 55.10; H, 3.30; N, 11.32.

2.1.13. 5-Bromo-3-(3-(3-chlorophenyl)-1,2,4-oxadiazol-5-yl)-1H-indole (4be)

White Solid; Yield: 72%; Mp: 215-217 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.43 (d, 1H), 8.48-8.46 (s, 1H, *J* = 8Hz), 8.298-8.293 (s, 1H, *J* = 2Hz), 8.206-8.202 (s, 1H, *J* = 8Hz), 8.115-8.095 (d, 1H, *J* = 8Hz), 7.816-7.814 (d, 1H, *J* = 8Hz), 7.58-7.56 (d, 2H, *J* = 8Hz), 7.446-7.442 (m, 1H, *J* = 1.6Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.3, 166.9, 135.7, 134.6, 132.7, 131.9, 129.8, 129.2, 126.5, 126.4, 126.3, 122.7, 122.6, 115.3, 115.0, 99.8; LC-MS: *m/z* = 375.7 $[M+H]^+$; Anal. Calcd. For $C_{16}H_9BrClN_3O$: C, 51.28; H, 2.42; N, 11.21; Found: C, 51.26; H, 2.44; N, 11.17.

2.1.14. 5-Bromo-3-(3-(3-bromophenyl)-1,2,4-oxadiazol-5-yl)-1H-indole (4bf)

Yellow Solid; Yield: 68%; Mp: 127-129 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.48-12.47 (d, 1H, *J* = 4Hz), 8.496-8.488 (s, 1H, *J* = 3.2Hz), 8.306-8.301 (s, 1H, *J* = 2Hz), 8.078-8.068 (d, 2H, *J* = 4Hz), 7.652-7.641 (d, 3H, *J* = 4.4Hz), 7.57-7.55 (t, 1H, *J* = 8Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.4, 167.1, 135.7, 134.3, 132.8, 131.7, 129.0, 127.0, 126.5, 126.3, 126.2, 122.7, 122.6, 115.3, 115.0, 99.8; LC-MS: *m/z* = 420.11 $[M+H]^+$; Anal. Calcd. For $C_{16}H_9Br_2N_3O$: C, 45.84; H, 2.16; N, 10.03; Found: C, 45.77; H, 2.20; N, 10.01.

3. RESULTS AND DİSCUSSİON

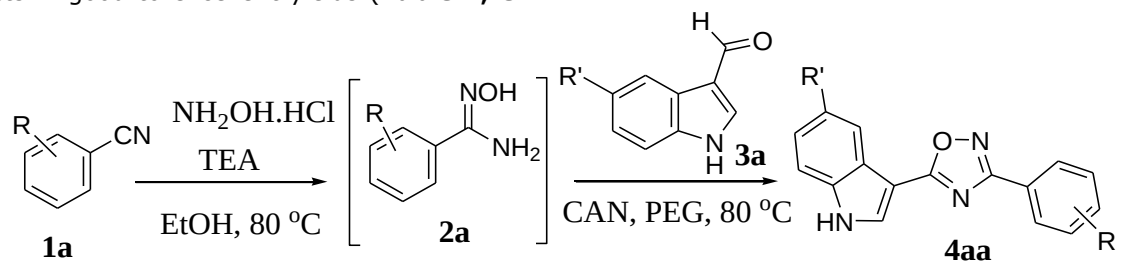
A series of amidoximes from corresponding benzonitriles (**1a**) was prepared by reacting with hydroxylamine hydrochloride, TEA, in ethanol under refluxing conditions. These amidoximes were further treated with 1*H*-indole-3-carboxaldehyde to afford the 1*H*-(indole-5-yl)-3-substituted-1,2,4-oxadiazoles. In a model study, benzamidoxime **(2a)** was treated with 1*H*-indole-3-carboxaldehyde **(3a)** in the presence of cerium ammonium nitrate (CAN) and polyethylene glycol (PEG) solvent at 80 °C, and it is formed the desired product **4aa** (scheme 1), albeit in a low yield of 60% (**Table 1, entry 1**). After the formation of product **4aa**, the reaction conditions were optimized in order to increase the yields. Thus, different solvents were screened, and the results are summarized in **Table 1**. PEG solvent was found to be the most superior in terms of product yields (**Table 1, entry 1**). The product yields maxi-mazed from 60% to 85% when the amount of CAN increases from (2 mol% to 5 mol%) (**Table 1, entry 5**). Further, increasing the quantity of CAN to 10 mol % decreased yields to 80% (**Table 1, entry 6**). The effect of temperature on the reaction rate, as well as on the yields of the products, was also investigated. On increasing the temperature from 80 °C to 120 °C, the product yields decreased (**Table 1, entries 7, 8**). Therefore, the subsequent reactions of several substituted benzamidoximes with 1*H*-indole-3-carboxaldehydes were carried out in the presence of CAN (5 mol%) as catalyst, PEG solvent, and temperature at 80 °C. The progress of the reactions was monitored by TLC analysis (using EtOAc-hexane as the eluent).

Table 1: Optimization of reaction conditions 4**aa**[a]

S.No	Solvent	Catalyst (mol %)	T (°C)	Yield[b] (%)
1	PEG	CAN (2 %)	80	60
2	CH_3CN	CAN (2 %)	80	40
3	DMF	CAN (2 %)	80	35
4	THF	CAN (2 %)	80	25
5	**PEG**	**CAN (5 %)**	**80**	**85**
6	PEG	CAN (10%)	80	80
7	PEG	CAN (5 %)	100	80
8	PEG	CAN (5 %)	120	78

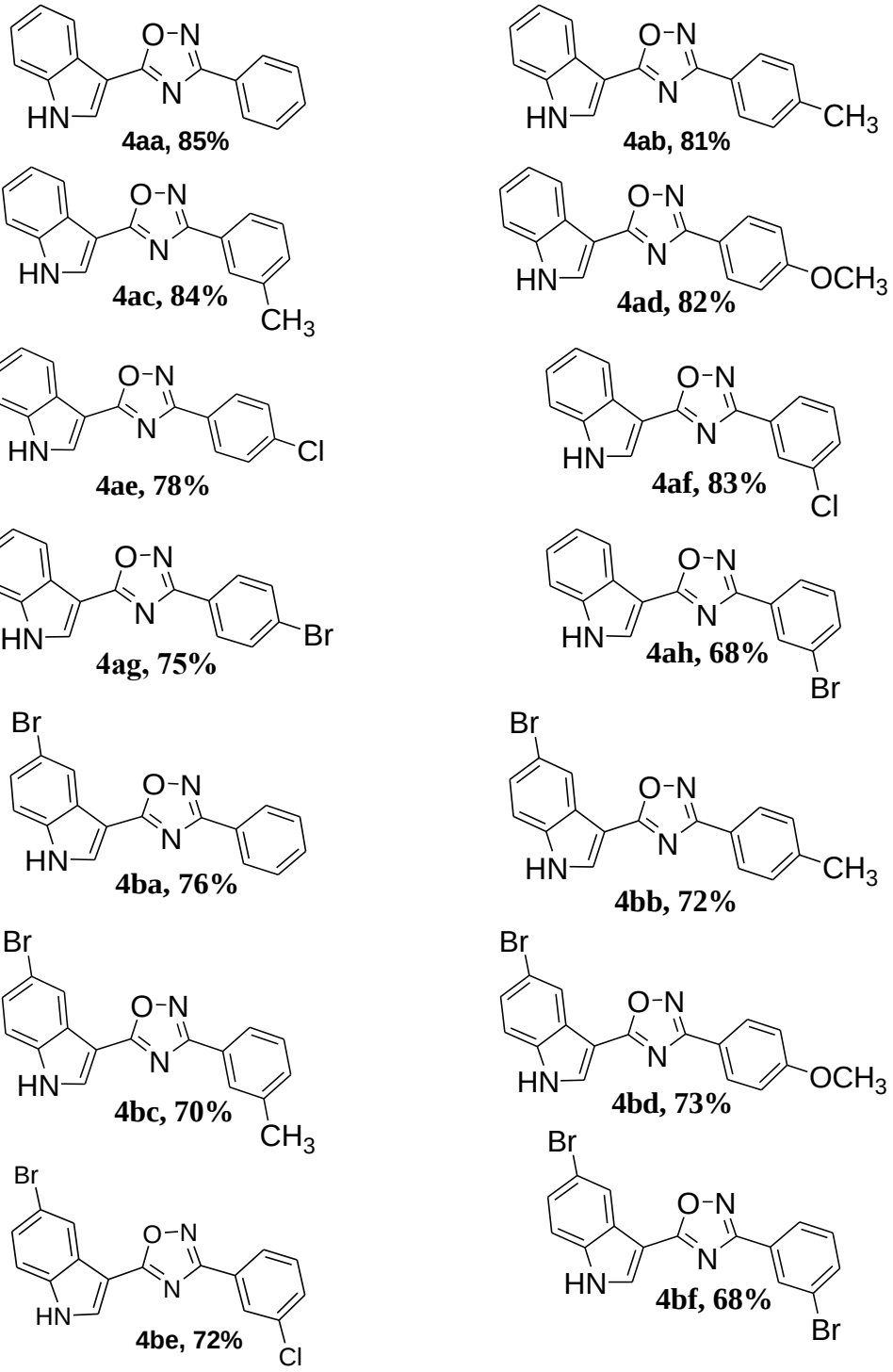
[a]Reaction conditions: **1a** (1.5 equiv.), **2a** (1.2 equiv.), **3a** (1.2 equiv.), CAN (x equiv.), TEA (2 equiv.), $NH_2OH.HCl$, (3 equiv.), solvent (1.5 mL), and temperature 80 °C, [b]Isolated yields.

With optimized conditions in hand, the scope of the reaction was investigated, and the results are summarized in **Table 2**. As expected, various benzamidoximes were reacted smoothly with 1*H*-indole-3-carboxaldehyde to give the corresponding products in good to excellent yields (**Table 2, entries 4ab**-**4ah**). 5-Bromo-1*H*-indole-3-carboxaldehyde also undergoes smooth transformation with different substituted benzamidoximes and affords the desired products (**Table 2, entries 4ba**-**4bf**) in good yields.



Scheme 1: Synthesis of oxadiazoles: Reagents and conditions: (i) $NH_2OH.HCl$, aqueous ethanol, reflux, 7 h; (ii) 1*H*-indole-3-carboxaldehyde, CAN, PEG, 80°C

Table 2: Structures and Yields of the Synthesized Compounds[a]



[a]Reaction conditions: **1a** (1.5 equiv.), **2a** (1.2 equiv.), **3a** (1.2 equiv.), CAN (x equiv.), TEA (2 equiv.), $NH_2OH.HCl$ (3 equiv.), solvent (1.5 mL), and temperature 80 °C.

3.1. Biological Activity

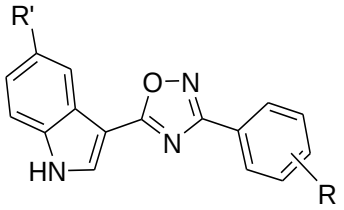
3.1.1. 5-Lipoxygenase enzyme inhibitory activity

All the synthesized 1*H*-(indole-5-yl)-3-substituted 1,2,4-oxadiazoles were evaluated for 5-lipoxygenase (5-LOX) assay and found to have a significant 5-LOX enzyme inhibitory activity with IC_{50} range from 18.78 µg/ml to >100 µg/ml (**Table 3**). Compounds **4af** and **4bc** showed moderate enzyme inhibitory activity with IC_{50} of 25.78, 26.72, and 27.86 µg/ml, respectively. Bromo-substituted indolyl compounds **4ag, 4bb**, and **4bd** showed effective enzyme inhibitory activity with IC_{50} of 23.13, 20.86, 23.51, and 20.48 µg/ml, respectively. Amongst the synthesized compounds, **4bf** showed the highest potency with IC_{50} of 18.78 µg/ml in the in *vitro* 5-lipoxygenase inhibitory as-

say. Nordihydroguaiaretic acid (positive control) inhibited 5-LOX with IC_{50} of 36.49 µg/ml.

The docking studies with the protein crystal structure of the 5-LOX enzyme further evaluated the anti-inflammatory potential of the synthesized compounds. The docking score and dock pose were analyzed to gain clear insight into probable interactions of synthesized compounds with the enzyme. Docking simulations were performed on X-ray crystallographic structures available for 5-LOX (3V99.pdb). The protein structures were thoroughly verified for breaks or missing residues, and necessary corrections were made in the pdb files. Docking simulations were performed by considering the entire protein as a receptor to obtain information regarding all possible interaction sites. The 5-LOX enzyme contains a relatively large and flexible receptor site. The catalytic iron is held in place with the help of three histidines (HIS551, 368, and 373), ASN555, and ILE674. The docking simulations were performed with the help of Auto Dock Vina. Partial flexibility allowed for the amino acids present close to the active site.

Table 3: Inhibitory activities of 1*H*-(indole -5-yl)-3-substituted 1,2,4-oxadiazoles



Entry	Compound	R'	R (3/4)	IC_{50} µg/ml
1	4aa	H	H	31.07
2	4ab	H	4-CH_3	30.13
3	4ac	H	3-CH_3	30.70
4	4ad	H	4-OCH_3	37.89
5	4ae	H	4-Cl	>100
6	4af	H	3-Cl	25.78
7	4ag	H	4-Br	23.13
8	4ah	H	3-Br	>100
9	4ba	Br	H	>100
10	4bb	Br	4-CH_3	20.86
11	4bc	Br	3-CH_3	26.72
12	4bd	Br	4-OCH_3	23.51
13	4be	Br	3-Cl	27.86
14	4bf	Br	3-Br	18.78
	Standard *			36.49

IC_{50} represents the concentration of a drug that is required for 50% inhibition expressed in µg/ml.
*Nordihydroguaiaretic acid as a positive control.

Amongst the synthesized compounds, **4bf** showed the highest potency (IC_{50} 18.78 µg/ml) in the in *vitro* 5-LOX inhibitory assay. The compound docked snugly into the receptor site with a score of -9.1 Kcal/mol. It showed strong hydrogen bond interactions with two key amino acids His368 and Asn555. Additionally, it also showed non-covalent

interactions with several other lipophilic amino acids present in the receptor channel. The oxadiazole moiety was found to be critical for the bioactivity as it has formed very strong salt bridge interactions with Asn555. This series showed A similar trend for other bioactive compounds, which showed potent enzyme inhibition (MIC<30 µg/ml). The introduction of a halogen on *C*-3 of benzene significantly potentiated the bioactivity.

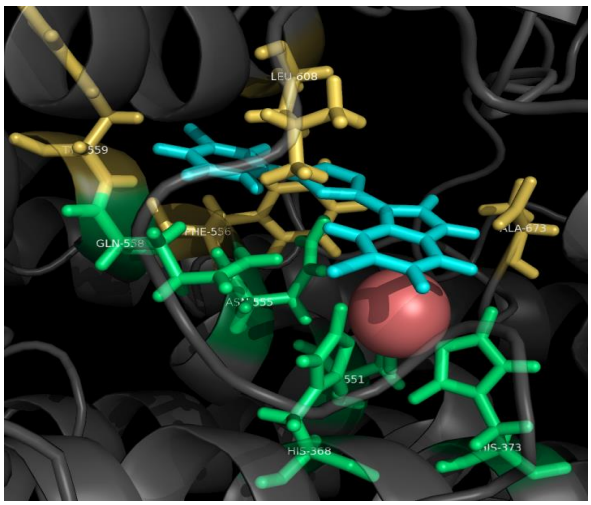


Figure 2: Docking poses of the compound 4bf. Ligand (aqua blue), Fe (salmon red), and active site residues making polar contacts (light yellow)

4bf showed the highest potency (IC_{50} 18.78 µg/ml) in the in *vitro* 5-LOX inhibitory assay. Amino acid interactions of compound **4bf** is Gly175, Val176, Phe178, **His368**, Ile407, **Asn555**, Phe556, Gln558, Tyr559, Val605, Leu608, Phe611, Ala673. Dock Score of -9.1 Kcal/mol.

4. CONCLUSION

In conclusion, a series of indolyloxadiazoles from amidoxime and indole 3-carboxaldehyde using CAN as a catalyst and PEG as a solvent were synthesized, and an In *vitro* 5-LOX inhibitory assay was performed. Among the tested compounds, **4bf** showed the highest potency (IC_{50} 18.78 µg/ml). The synthesized compound carried out docking on the 5-LOX enzyme protein crystal structure. The compound (**4bf**) docked snugly into the receptor site with a score of -9.1 Kcal/mol, and it showed strong hydrogen bond interactions with two key amino acids, **His368** and **Asn555**. The obtained dock scores and the bioactivity findings correlated well.

5. CONFLICT OF INTEREST

The authors agree there are no conflicts to declare.

6. ACKNOWLEDGMENT

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