

Design and Synthesis of Novel 3-(2-methylbenzofuran-3-yl)-5-aryl-1,2,4-oxadiazole derivatives: Antimicrobial Evaluation

Santhosh Kumar Sirisilla¹, Jagram Anterbody^{1,2}, Rajesh Alukucha^{1,3}, Govu Ramana¹,
Gajula Naveen¹, Santosh Kumar Konda⁴, Thalari Gangadhar^{1*}

¹Natural Products Laboratory, Department of Chemistry, Osmania University, Hyderabad, 500007.

²Associate Professor, Department of Chemistry, Government Degree College for Men, Adilabad 504001.

³Assistant Professor, Department of Chemistry, Girraj Government College, Nizamabad 503002.

⁴Associate Professor, Department of Chemistry, Geethanjali College of Engineering and Technology, Cheeryal (V), Keesara (M), Medchal Dist. 501301

*Corresponding author mail: gangadharchemou@gmail.com

ABSTRACT

An efficient synthesis of 3-(2-methylbenzofuran-3-yl)-5-phenyl-1,2,4-oxadiazoles (**7a-o**) obtained by Condensation of N'-hydroxy-2-methylbenzofuran-3-carboximidamides (**5a-c**) with benzaldehydes (**6a-e**). All synthesized analogs analysed by NMR, FT-IR and mass spectroscopy. The antibacterial potential of all the produced compounds was assessed against selected bacterial and fungus species. Biological screening showed that several compounds had remarkable antibacterial activity with some of the derivatives displaying equivalent or better efficacy than the conventional medicines.

Keywords: Benzofuran, oxadiazoles, carboximidamide.

How to cite this article: Santhosh Kumar Sirisilla¹ Jagram Anterbody^{1,2} Rajesh Alukucha^{1,3} Govu Ramana¹. Design and Synthesis of Novel 3-(2-methylbenzofuran-3-yl)-5-aryl-1,2,4-oxadiazole derivatives: Antimicrobial Evaluation. Int J Drug Deliv Technol. 2026;16(84s):104-111. DOI: 10.25258/ijddt.16.84s.11.

INTRODUCTION

The 1,2,4-oxadiazoles are unique five-membered aromatic heterocycles of the azole family with one oxygen atom and two nitrogen atoms, and they exhibit remarkable chemical and biological properties. The 1,2,4-oxadiazole ring is a significant ring in synthetic chemistry, but also exists in a variety of biologically active natural molecules, testifying its importance in nature and in the laboratory.¹ Natural compounds containing oxadiazoles are common, which indicates their distinct and important biological activities that can be employed as a template for the design and synthesis of new analogs for therapeutic applications.

The oxadiazole ring can serve as a bio-isostere of the hydrogen bonding and spatial orientation of the amide and ester groups, which are prevalent motifs in physiologically active molecules. This is a very valuable trait in drug design, since it allows scientists to change existing drug molecules to improve their pharmacological qualities²⁻⁵ (potency, selectivity, metabolic stability, and bioavailability) without losing their biological activity. A benefit of the oxadiazole scaffold is its hydrolytic and metabolic stability.⁶

The 1,2,4-oxadiazole ring system has become an important scaffold in material science, medicinal chemistry and the pharmaceutical sector.⁷ Its chemical stability, rigid aromatic framework, and ease of functionalization account for its great popularity for inclusion into a wide variety of functional materials, medicines and biologically active compounds. The

oxadiazole ring possesses unusual photophysical⁸⁻¹⁰ and electrochemical¹¹⁻¹³ capabilities due to its electrical structure, and has been an interesting building block for the construction of organic light-emitting diodes (OLEDs), liquid crystals and other innovative materials. 1,2,4-oxadiazoles are of particular interest in medicinal chemistry as bio isosteres of esters and amides.¹⁴

The 1,2,4-oxadiazole ring has been utilized in the design of innovative functional materials in materials science due to its unique electrical and photophysical characteristics. Modern synthetic techniques permit efficient access to the oxadiazole cores and their diversification affording rapid access to libraries of novel compounds for screening and optimization. The development of oxadiazole-containing medications has also been assisted by computational chemistry and molecular modeling which predict their interactions with biological targets and their pharmacokinetic features. Some of natural products containing oxadiazole substance are phidianidines A and B, quisqualic acid.

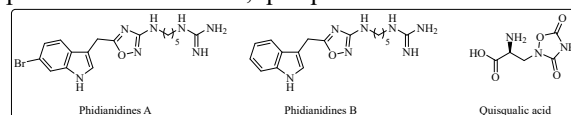


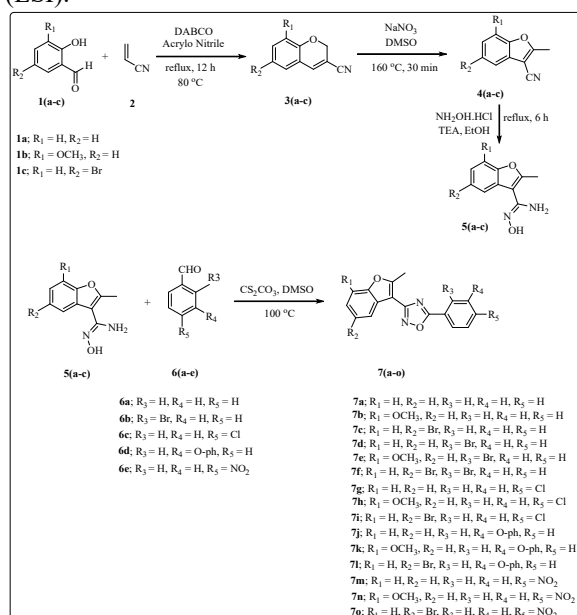
Fig.1.1. 1,2,4-oxadiazole core containing natural products.

The biological features of 1,2,4-oxadiazole derivatives such as anticancer¹⁵⁻¹⁶, anti-inflammatory¹⁷⁻¹⁸, antibacterial¹⁹⁻²⁰, anti-Alzheimer

disease²¹⁻²², antioxidant²³⁻²⁴, insecticidal²⁵, antifungal²⁶⁻²⁷ and antidiabetic²⁸⁻²⁹ makes them intriguing candidates for novel drug discovery. Present work focus on synthesis of benzofuran-assisted phenyl-1,2,4-oxadiazoles (**7a-o**) by the condensation of carboximidamides (**5a-c**) with benzaldehydes (**6a-e**).

2 EXPERIMENTAL

All reagents, and solvents were purchased from Spectrochem, Sigma-Aldrich, Merck. Reactions were monitored by TLC purified by recrystallization or silica-gel column chromatography (60–120 mesh). FT-IR spectra recorded using KBr pellets. ¹H and ¹³C-NMR spectras were recorded on a Bruker 400 MHz using CDCl₃ with TMS as the internal standard. Mass spectra were obtained using electrospray ionization (ESI).



Scheme 1: Synthesis of 3-(2-methylbenzofuran-3-yl)-5-phenyl-1,2,4-oxadiazole derivatives

2.1 Synthesis of 2H-chromene-3-carbonitriles (**3a-c**)

2H-Chromene-3-carbonitrile (**3a**) O-Salicylaldehyde (**1a**) (10.0 g, 81.886 mmol) and acrylonitrile (**2**) (100 mL). To the mixture was added DABCO (1.837 g, 16.377 mmol). The reaction was carried out by shaking the solution at 80 °C for twelve hours. At the end of the reaction the surplus acrylonitrile was removed by mild heating under reduced pressure. The rest of the thick mixture was dissolved in 500 millilitres of ethyl acetate and washed with weak hydrochloric acid to get rid of the rest of the constituents. Finally, the mixture was rinsed again with brine. The organic solution was dried over anhydrous sodium sulfate. Finally the product was purified by column chromatography on silica gel using ethyl acetate and hexane (1:10) as a solvent. The

product was 2H-chromene-3-carbonitrile (**3a**), yellow solid (8.5 g).

2.2 Synthesis of 2-methylbenzofuran-3-carbonitriles (**4a-c**)

To a stirred solution of 2H-chromene-3-carbonitrile (**3a**) (8 grams, 50.903 millimoles) in DMSO (20 millilitres) was added sodium azide (3.971 grams, 61.084 millimoles). The resulting mixture was heated at 160 degrees Celsius for thirty minutes. When the reaction was complete (TLC), the reaction mixture was poured into ice water (200 mL) and extracted with ethyl acetate (300 mL). The organic layer was dried over anhydrous Na₂SO₄ and purified by column chromatography on silica gel 60-120 mesh. The organic layer was then eluted with EtOAc:Hexane (1:10) to provide 4.8 g of a white solid of 2-methylbenzofuran-3-carbonitrile (**4a**).

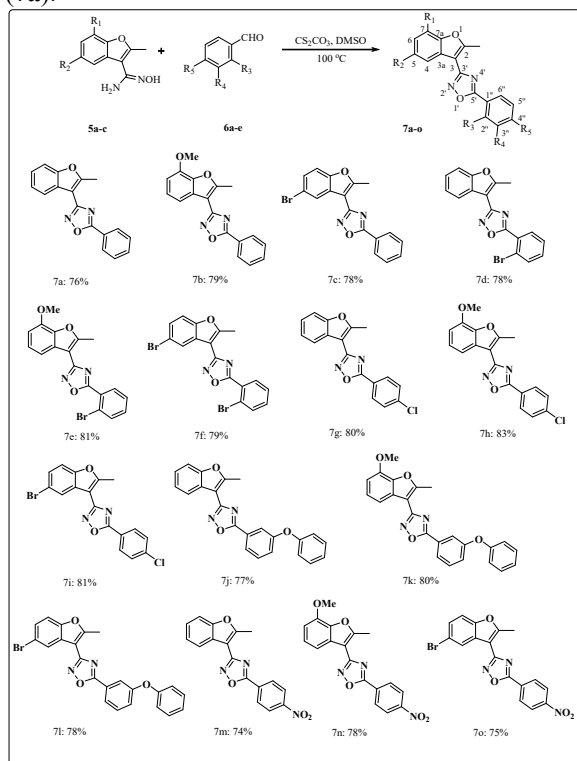
2.3 Synthesis of N'-hydroxy-2-methylbenzofuran-3-carboximidamides (**5a-c**)

Synthesis of amidoxime derivatives, 2-Methylbenzofuran-3-carbonitrile (**4a**) (4 g, 25.450 mmol) was mixed with hydroxylamine hydrochloride (2.122 g, 30.540 mmol) and 10 mL of triethylamine in 50 mL of ethanol reflux for 2 hours. The course of the reaction was followed by TLC. The mixture was heated under lower pressure to eliminate the surplus ethanol and triethylamine. The brown solid obtained was added to 20 ml of water, the solid was filtered to remove unreacted starting ingredients or byproducts included in the liquid. The substance was rinsed well with water to remove any traces of contaminants or salts. Finally, the product was dried at 50 °C to give the pure amidoxime as a brown solid for further purification or analysis.

2.4 Synthesis of 3-(2-methylbenzofuran-3-yl)-5-phenyl-1,2,4-oxadiazole derivatives (**7a-o**)

A mixture of N'-hydroxy-2-methylbenzofuran-3-carboximidamide (**5a**) (0.200 g, 1.052 mmol), benzaldehyde (**6a**) (0.134 g, 0.126 mL, 1.262 mmol) in 20 mL of dimethyl sulfoxide (DMSO) was added and compound (**7a**) was synthesized. Cesium carbonate (Cs₂CO₃) (0.685 g, 2.104 mmol) was used as base and the reaction was carried out under nitrogen atmosphere to avoid any undesired oxidation. The mixture was heated at 100 °C and agitated for 6 hours to effect base catalysed oxidative cyclization of the reactants to give the desired oxadiazole ring system. After completion, DMSO was removed in vacuo to assist in product isolation. The residue was taken up in ethyl acetate and rinsed with 50 mL water to eliminate any residual salts or side-products. The organic layer was dried over anhydrous sodium sulphate, filtered and the solvent was evaporated. The crude product was refined by column chromatography using 5% ethyl acetate in n-hexane as eluent to give pure 3-(2-

methylbenzofuran-3-yl)-5-phenyl-1,2,4-oxadiazole (7a).



3 SPECTRAL DATA:

3.1 3-(2-methylbenzofuran-3-yl)-5-phenyl-1,2,4-oxadiazole (7a)

White solid, Yield 76 %, M.P. 182-184 °C, IR (KBr) 3065, 2924, 1608, 1512 cm^{-1} , ^1H -NMR (400MHz, CDCl_3) δ 8.17 (d, J = 8.0 Hz, 2H), 7.68 (d, J = 7.6 Hz, 1H), 7.56–7.49 (m, 3H), 7.39 (dd, J = 8.4, 2.0 Hz, 1H), 7.28 (d, J = 2.0 Hz, 1H), 2.52 (s, 3H). ^{13}C -NMR (100MHz, CDCl_3) δ 164.9, 156.8, 153.2, 137.5, 130.9, 129.6, 128.7, 127.8, 125.9, 120.6, 112.4, 13.2. ESI-HRMS m/z 277.0958 $[\text{M}+\text{H}]^+$

3.2 3-(7-methoxy-2-methylbenzofuran-3-yl)-5-phenyl-1,2,4-oxadiazole (7b)

White solid, Yield 79 %, M.P. 188 - 190 °C, IR (KBr) 3058, 2922, 1605, 1507 cm^{-1} , ^1H -NMR (400MHz, CDCl_3) δ 8.15 (d, J = 8.0 Hz, 2H), 7.62 (d, J = 7.0 Hz, 1H), 7.54–7.48 (m, 3H), 7.14 (d, J = 2.0 Hz, 1H), 6.98 (dd, J = 8.4, 2.0 Hz, 1H), 3.92 (s, 3H), 2.51 (s, 3H). ^{13}C -NMR 100MHz, CDCl_3) δ 173.7, 164.0, 159.5, 157.3, 154.1, 130.3, 128.7, 127.2, 125.8, 124.4, 122.3, 121.5, 114.6, 110.5, 104.3, 56.2, 14.3, MS (ESI) m/z 307.1031 $[\text{M}+\text{H}]^+$

3.3 3-(5-bromo-2-methylbenzofuran-3-yl)-5-phenyl-1,2,4-oxadiazole (7c)

White solid, Yield 78 %, M.P. 201-203 °C, IR (KBr) 3070, 2920, 1612, 1510 cm^{-1} , ^1H -NMR

(400MHz, CDCl_3) δ 8.14 (d, J = 8.0 Hz, 2H), 7.69 (d, J = 7.6 Hz, 1H), 7.57–7.50 (m, 3H), 7.40 (d, J = 2.0 Hz, 1H), 7.11 (dd, J = 8.4, 2.0 Hz, 1H), 2.55 (s, 3H). ^{13}C -NMR (100MHz, CDCl_3) δ 164.7, 156.7, 153.0, 138.5, 131.0, 129.8, 128.6, 127.7, 125.5, 120.1, 114.3, 113.5, 13.3. MS (ESI) m/z 355.0063 $[\text{M}+\text{H}]^+$

3.4 5-(2-bromophenyl)-3-(2-methylbenzofuran-3-yl)-1,2,4-oxadiazole (7d)

White solid, Yield 78 %, M.P. 196 - 198 °C, IR (KBr) 3068, 2923, 1610, 1508 cm^{-1} , ^1H -NMR (400MHz, CDCl_3) δ 8.06 (d, J = 8.0 Hz, 1H), 7.83 (dd, J = 7.8, 1.8 Hz, 1H), 7.61–7.52 (m, 3H), 7.46 (dd, J = 8.4, 2.0 Hz, 1H), 7.34 (d, J = 2.0 Hz, 1H), 7.19 (td, J = 7.5, 1.3 Hz, 1H), 2.54 (s, 3H). ^{13}C -NMR (100MHz, CDCl_3) δ 164.5, 156.5, 153.1, 138.7, 136.1, 131.3, 130.4, 129.9, 128.4, 127.6, 126.8, 120.5, 114.1, 113.2, 13.3, MS (ESI) m/z 355.0063 $[\text{M}+\text{H}]^+$

3.5 5-(2-bromophenyl)-3-(7-methoxy-2-methylbenzofuran-3-yl)-1,2,4-oxadiazole (7e)

White solid, Yield 81 %, M.P. 204-206 °C, IR (KBr) 3072, 2924, 1606, 1514 cm^{-1} , ^1H -NMR (400MHz, CDCl_3) δ 8.08 (d, J = 8.0 Hz, 1H), 7.87 (dd, J = 7.8, 1.8 Hz, 1H), 7.67–7.54 (m, 3H), 7.14 (d, J = 2.0 Hz, 1H), 7.00 (dd, J = 8.4, 2.0 Hz, 1H), 7.20 (td, J = 7.5, 1.3 Hz, 1H), 3.94 (s, 3H), 2.53 (s, 3H). ^{13}C -NMR (100MHz, CDCl_3) δ 164.6, 156.3, 153.6, 151.2, 137.2, 136.0, 131.1, 130.1, 129.7, 128.3, 127.8, 120.4, 114.2, 111.7, 55.7, 13.2. ESI-HRMS m/z 385.1169 $[\text{M}+\text{H}]^+$

3.6 3-(5-bromo-2-methylbenzofuran-3-yl)-5-(2-bromophenyl)-1,2,4-oxadiazole (7f)

White solid, Yield 79 %, M.P. 216-218 °C, IR (KBr) 3075, 2922, 1615, 1516 cm^{-1} , ^1H -NMR (400MHz, CDCl_3) δ 8.13 (d, J = 8.0 Hz, 1H), 7.85 (dd, J = 7.8, 1.8 Hz, 1H), 7.65–7.53 (m, 3H), 7.43 (d, J = 2.0 Hz, 1H), 7.17 (td, J = 7.5, 1.3 Hz, 1H), 7.08 (dd, J = 8.4, 2.0 Hz, 1H), 2.56 (s, 3H). ^{13}C -NMR (100MHz, CDCl_3) δ 164.9, 156.9, 153.5, 138.2, 136.4, 131.5, 130.2, 129.8, 128.2, 127.9, 125.8, 120.9, 114.5, 113.8, 13.4. ESI-HRMS m/z 433.9169 $[\text{M}+\text{H}]^+$

3.7 5-(4-chlorophenyl)-3-(2-methylbenzofuran-3-yl)-1,2,4-oxadiazole (7g)

White solid, Yield 80 %, M.P. 186 - 188 °C, IR (KBr) 3065, 2921, 1607, 1510 cm^{-1} , ^1H -NMR (400MHz, CDCl_3) δ 8.09 (d, J = 8.4 Hz, 2H), 7.69 (d, J = 7.6 Hz, 1H), 7.62 (d, J = 8.4 Hz, 2H), 7.55–7.48 (m, 1H), 7.38 (dd, J = 8.4, 2.0 Hz, 1H), 7.27 (d, J = 2.0 Hz, 1H), 2.52 (s, 3H). ^{13}C -NMR (100MHz, CDCl_3) δ 164.8, 156.4, 153.2, 137.6, 134.4, 130.9, 129.8,

- 128.5, 127.7, 125.8, 120.8, 112.6, 13.2. MS (ESI) m/z 293.0720 $[M+H]^+$
- 3.8 5-(4-chlorophenyl)-3-(7-methoxy-2-methylbenzofuran-3-yl)-1,2,4-oxadiazole (7h)**
White solid, Yield 83 %, M.P. 192 - 194 °C, IR (KBr) 3068, 2923, 1609, 1513 cm^{-1} , ^1H -NMR (400MHz, CDCl_3) δ 8.10 (d, J = 8.4 Hz, 2H), 7.68 (d, J = 7.6 Hz, 1H), 7.62 (d, J = 8.4 Hz, 2H), 7.12 (d, J = 2.0 Hz, 1H), 6.98 (dd, J = 8.4, 2.0 Hz, 1H), 3.93 (s, 3H), 2.51 (s, 3H). ^{13}C -NMR (100MHz, CDCl_3) δ 164.9, 156.2, 153.5, 151.3, 137.8, 134.2, 130.8, 129.7, 128.3, 127.8, 120.5, 112.5, 111.6, 55.6, 13.1. MS (ESI) m/z 323.0826 $[M+H]^+$
- 3.9 3-(5-bromo-2-methylbenzofuran-3-yl)-5-(4-chlorophenyl)-1,2,4-oxadiazole (7i)**
White solid, Yield 81 %, M.P. 209 - 211 °C, IR (KBr) 3071, 2926, 1613, 1515 cm^{-1} , ^1H -NMR (400MHz, CDCl_3) δ 8.11 (d, J = 8.4 Hz, 2H), 7.72 (d, J = 7.6 Hz, 1H), 7.61 (d, J = 8.4 Hz, 2H), 7.42 (d, J = 2.0 Hz, 1H), 7.09 (dd, J = 8.4, 2.0 Hz, 1H), 2.54 (s, 3H). ^{13}C -NMR (100MHz, CDCl_3) δ 164.7, 156.8, 153.4, 138.3, 134.5, 131.2, 130.7, 129.6, 128.6, 127.9, 125.7, 120.7, 114.6, 113.7, MS (ESI) m/z 371.9169 $[M+H]^+$
- 3.10 3-(2-methylbenzofuran-3-yl)-5-(3-phenoxyphenyl)-1,2,4-oxadiazole (7j)**
White solid, Yield 77 %, M.P. 178 - 180 °C, IR (KBr) 3062, 2924, 1607, 1514 cm^{-1} , ^1H -NMR (400MHz, CDCl_3) δ 8.12 (d, J = 8.0 Hz, 1H), 7.80 (d, J = 7.6 Hz, 1H), 7.61–7.54 (m, 3H), 7.45 (dd, J = 8.4, 2.0 Hz, 1H), 7.35–7.21 (m, 6H), 7.11 (d, J = 2.0 Hz, 1H), 2.54 (s, 3H). ^{13}C -NMR (100MHz, CDCl_3) δ 164.8, 156.3, 153.4, 154.6, 153.0, 137.2, 131.1, 130.7, 129.5, 128.3, 127.8, 125.9, 123.8, 120.6, 119.5, 116.1, 114.7, 112.6, 13.3, MS (ESI) m/z 369.1211 $[M+H]^+$
- 3.11 3-(7-methoxy-2-methylbenzofuran-3-yl)-5-(3-phenoxyphenyl)-1,2,4-oxadiazole (7k)**
White solid, Yield 80 %, M.P. 182–184 °C, IR (KBr) 3064, 2931, 1602, 1517 cm^{-1} , ^1H -NMR (400MHz, CDCl_3) δ 8.13 (d, J = 8.8 Hz, 1H), 7.77 (d, J = 7.6 Hz, 1H), 7.59–7.54 (m, 2H), 7.49–7.44 (m, 2H), 7.38–7.28 (m, 4H), 7.12 (d, J = 2.0 Hz, 1H), 6.97 (dd, J = 8.4, 2.0 Hz, 1H), 6.92–6.90 (m, 2H), 3.92 (s, 3H), 2.52 (s, 3H). ^{13}C -NMR (100MHz, CDCl_3) δ 164.9, 156.3, 153.7, 151.4, 154.4, 153.1, 137.3, 131.2, 130.8, 129.6, 128.5, 127.9, 125.8, 123.9, 120.7, 119.4, 116.2, 114.8, 112.4, 111.5, 55.7, 13.2. MS (ESI) m/z 389.1316 $[M+H]^+$
- 3.12 3-(5-bromo-2-methylbenzofuran-3-yl)-5-(3-phenoxyphenyl)-1,2,4-oxadiazole (7l)**
White solid, Yield 78 %, M.P. 192 - 194 °C, IR (KBr) 3073, 2928, 1614, 1518 cm^{-1} , ^1H -NMR (400MHz, CDCl_3) δ 8.15 (d, J = 8.8 Hz, 1H), 7.81 (d, J = 7.8 Hz, 1H), 7.61–7.55 (m, 2H), 7.50–7.43 (m, 2H), 7.39–7.27 (m, 4H), 7.44 (d, J = 2.0 Hz, 1H), 7.09 (dd, J = 8.4, 2.0 Hz, 1H), 6.93–6.91 (m, 2H), 2.54 (s, 3H). ^{13}C -NMR (100MHz, CDCl_3) δ 165.0, 156.5, 153.4, 154.6, 153.1, 138.4, 131.4, 130.9, 129.7, 128.7, 128.0, 125.9, 123.7, 120.8, 119.6, 116.3, 114.7, 113.8, 13.3. MS (ESI) m/z 437.9268 $[M+H]^+$
- 3.13 3-(2-methylbenzofuran-3-yl)-5-(4-nitrophenyl)-1,2,4-oxadiazole (7m)**
Yellow solid, Yield 74 %, M.P. 210–212 °C, IR (KBr) 3082, 2928, 1598, 1519, 1347 cm^{-1} , ^1H -NMR (400MHz, CDCl_3) δ 8.40 (d, J = 8.8 Hz, 2H), 8.18 (d, J = 8.8 Hz, 2H), 7.75 (d, J = 7.6 Hz, 1H), 7.56 (dd, J = 8.4, 2.0 Hz, 1H), 7.49 (d, J = 2.0 Hz, 1H), 2.54 (s, 3H). ^{13}C -NMR (100MHz, CDCl_3) δ 164.8, 156.1, 153.4, 149.6, 147.2, 137.2, 131.1, 130.2, 128.5, 127.7, 125.9, 120.8, 112.5, 13.2. MS (ESI) m/z 336.0776 $[M+H]^+$
- 3.14 3-(7-methoxy-2-methylbenzofuran-3-yl)-5-(4-nitrophenyl)-1,2,4-oxadiazole (7n)**
Yellow solid, Yield 78 %, M.P. 214–216 °C, IR (KBr) 3084, 2929, 1596, 1518, 1345 cm^{-1} , ^1H -NMR (400MHz, CDCl_3) δ 8.42 (d, J = 8.8 Hz, 2H), 8.20 (d, J = 8.8 Hz, 2H), 7.79 (d, J = 7.6 Hz, 1H), 7.13 (d, J = 2.0 Hz, 1H), 6.98 (dd, J = 8.4, 2.0 Hz, 1H), 3.94 (s, 3H), 2.54 (s, 3H). ^{13}C -NMR (100MHz, CDCl_3) δ 164.9, 156.3, 153.6, 151.4, 149.7, 147.3, 137.5, 131.2, 130.4, 128.6, 127.9, 120.9, 112.4, 111.7, 55.6, 13.1. MS (ESI) m/z 366.0882 $[M+H]^+$
- 3.15 3-(5-bromo-2-methylbenzofuran-3-yl)-5-(4-nitrophenyl)-1,2,4-oxadiazole (7o)**
Yellow solid, Yield 75 %, M.P. 218–220 °C, IR (KBr) 3085, 2927, 1599, 1522, 1348 cm^{-1} , ^1H -NMR (400MHz, CDCl_3) δ 8.44 (d, J = 8.8 Hz, 2H), 8.22 (d, J = 8.8 Hz, 2H), 7.80 (d, J = 7.6 Hz, 1H), 7.43 (d, J = 2.0 Hz, 1H), 7.09 (dd, J = 8.4, 2.0 Hz, 1H), 2.56 (s, 3H). ^{13}C -NMR (100MHz, CDCl_3) δ 165.1, 156.6, 153.7, 149.9, 147.4, 138.6, 131.5, 130.5, 128.8, 127.9, 125.7, 120.9, 114.5, 113.7, 13.4. MS (ESI) m/z 414.9382 $[M+H]^+$

4 Biological Activities

4.1 Antibacterial activity:

The synthesized compounds **7(a-o)** were tested for antibacterial activity against a panel of bacterial strains including Gram-negative *Klebsiella pneumonia*, *Escherichia coli* and Gram-positive *Bacillus subtilis*

and *Staphylococcus aureus*³⁰⁻³¹. The antibacterial assays were performed by agar disc diffusion method³² at two concentrations (10 µg/mL and 20 µg/mL). Fresh bacterial cultures were produced and diluted in 5% sterile saline to an inoculation density of approximately 105–106 CFU/mL. The test compounds were dissolved in dimethyl sulfoxide (DMSO) to obtain the concentrations desired for the biological testing. Sterile discs (6 mm diameter) were impregnated with 20 µL of each test solution and allowed to dry in air to allow full saturation. These disks were then placed on the surface of Müller-Hinton agar plates that had been uniformly seeded with the respective bacterial strains. Plates were incubated at 37 °C for 24–48 h and then the zones of inhibition were determined (in mm). All tests were done in triplicate and the antibacterial activity of the synthesized compounds was evaluated with standard antibiotic gatifloxacin at the same dosages (10 and 20 µg/mL). Average zone of inhibition data was noted and tabulated.

4.2 Results of Antibacterial activity:

Table 1. Antibacterial activity of compounds **7(a-o)**

Compound	Zone of inhibition (mm)							
	Gram positive bacteria				Gram negative bacteria			
	<i>S. aureus</i>		<i>B. subtilis</i>		<i>K. pneumoniae</i>		<i>E. coli</i>	
	10 µg/mL	20 µg/mL	10 µg/mL	20 µg/mL	10 µg/mL	20 µg/mL	10 µg/mL	20 µg/mL
	L	L	L	L	L	L	L	L
7a	10.8	17.2	9.2	18.1	9.6	14.4	9.8	16.8
7b	11.4	20.3	10.8	21.4	10.6	15.7	10.8	19.3
7c	10.9	19.1	10.2	20.2	9.7	15.6	11.1	17.3
7d	9.4	18.5	9.8	19.3	10.8	16.8	10.9	18.3
7e	10.2	22.6	10.7	21.4	9.4	15.2	10.8	17.8
7f	10.6	20.2	10.1	20.1	10.4	17.6	11.8	18.1
7g	11.1	19.0	9.7	20.0	10.5	15.3	9.8	17.0
7h	10.6	20.4	11.3	21.6	9.6	18.5	12.0	18.1
7i	11.8	21.1	10.7	19.2	8.9	17.4	10.3	19.0
7j	10.4	20.9	10.0	21.8	10.5	16.3	11.9	17.9
7k	10.6	21.4	9.2	22.1	10.4	15.2	10.8	19.2

7l	11.8	20.1	10.5	21.2	9.1	16.9	9.5	19.6
7m	12.0	19.6	10.6	21.7	10.4	17.3	10.8	20.0
7n	13.7	23.3	12.1	25.2	12.5	20.2	13.6	21.1
7o	13.4	23.2	12.0	25.1	12.3	20.1	13.4	20.9
Gatifloxacin	13.9	23.5	12.4	25.4	12.8	20.4	13.9	21.4

The newly synthesized title compounds **7a–o** were screened for their in-vitro antibacterial activity against Gram-positive strains *Bacillus subtilis* (MTCC 121), *Staphylococcus aureus* (MTCC 96) and Gram-negative strains *Escherichia coli* (MTCC 43), *Klebsiella pneumoniae* (MTCC 530) at concentration of 10 µg/mL and 20 µg/mL. The zone of inhibition was determined in mm using Gatifloxacin as a reference medication (Table 1).

Among the synthesized derivatives, compound **7n** exhibited the most potent antibacterial activity against *S. aureus* and *B. subtilis* with inhibition zones of 13.7 mm and 23.3 mm (*S. aureus*, 10 and 20 µg/mL) and 12.1 mm and 25.2 mm (*B. subtilis*, 10 and 20 µg/mL), which was nearly comparable to that of Gatifloxacin. Compound **7o**, in particular, exhibited considerable antibacterial activity against *B. subtilis* (25.1 mm at 20 µg/mL) and *E. coli* (20.9 mm at 20 µg/mL).

Both compounds **7n** and **7o** demonstrated significant inhibitory activity against *E. coli* and *K. pneumoniae* at the concentration of 20 µg/mL (21.1 mm and 20.9 mm against *E. coli*, 20.2 mm and 20.1 mm against *K. pneumoniae*, respectively) which were very close to the reference medication Gatifloxacin (21.4 mm and 20.4 mm) for Gram-negative bacteria. Generally, the halogen substituted compounds at different positions (**7b–7d**) showed moderate antibacterial activity as contrast to nitro and methoxy substituted analogues (**7e**, **7f**, **7h–7l**) which showed moderate to good activity particularly at higher doses.

To summarize, compounds **7n**, **7o** and **7m** displayed antibacterial activity comparable to or slightly lower than Gatifloxacin indicating their potential as promising antibacterial agents to be explored further.

4.3 Antifungal activity:

The antifungal activity of produced compounds **7(a–o)** was determined against three harmful fungus *Aspergillus niger*, *Aspergillus flavus* and *Fusarium oxysporum* using poison plate technique at concentration 50 µg/mL³³⁻³⁴. For antifungal test fresh mycelium was obtained by incubating three different types of fungus in PDA for 5 days at 25±1 °C. Then, with a sterile inoculation needle, a mycelial disc of

approximately 0.45 cm in diameter was cut from the culture media and injected in the middle of the PDA plate³⁵. Test chemicals were dissolved in 5 mL of DMSO and added to Potato Dextrose Agar medium (PDA, 20 mL). The final concentration of the added chemicals to the medium was 50 µg/mL. Inoculation plates were incubated for five days at 25±1 °C. The experiment was repeated three times for each treatment. The control was the sterile distilled water diluted with acetone and the standard control was clotrimazole (50 µg/mL). On the 5th day, the radial growth of the fungal colonies was measured.

4.4 Results of Antifungal activity

Table 2. Antifungal activity of compounds **7(a-o)**

Compound Conc. (50 µg/mL)	Zone of Inhibition (mm)		
	<i>A. niger</i>	<i>A. flavus</i>	<i>F.oxysporu m</i>
7a	14.6	12.3	13.1
7b	15.8	13.1	14.0
7c	14.3	12.9	13.5
7d	13.6	14.3	14.9
7e	12.2	13.6	15.3
7f	13.7	13.1	14.9
7g	14.2	13.8	16.4
7h	13.0	14.5	14.1
7i	15.5	12.0	15.0
7j	12.9	13.3	16.8
7k	14.0	13.5	15.4
7l	15.3	14.9	14.9
7m	15.6	13.2	16.3
7n	16.9	15.9	17.8
7o	16.5	15.6	17.7
Clotrimazole	17.3	16.7	18.2

All the synthesized compounds **7a–7o** were screened for their in vitro antifungal activity against *Aspergillus niger*, *Aspergillus flavus* and *Fusarium oxysporum* at a concentration of 50 µg/mL. The results of antifungal activity are given in Table 2 and compared with conventional medicine Clotrimazole. The antifungal data analysis showed that compound **7n** demonstrated the highest inhibition activity against *A. niger* (16.9 mm) followed by **7o** (16.5 mm), **7b** (15.8 mm), **7m** (15.6 mm) and **7i** (15.5 mm).

In contrast, **7e** was the least active against *A. niger* (12.2 mm). The same pattern was found against *A. flavus* where **7n** (15.9 mm) and **7o** (15.6 mm) had the greatest antifungal activity than other derivatives. **7i** had the lowest inhibition (12.0 mm, *A. flavus*). The compounds **7n** (17.8 mm) and **7o** (17.7 mm) exhibited the highest activity among the test compounds against *F. oxysporum*, comparable to Clotrimazole (18.2 mm). Other compounds like **7j** (16.8 mm) and **7g** (16.4 mm) also exhibited considerable inhibition. In general, compounds **7n** and **7o** exhibited the most similar antifungal properties to Clotrimazole against all three

fungi tested, which demonstrated their potential as antifungal drugs for further investigation.

5 RESULTS AND DISCUSSION

Scheme 1, synthesis of 3-(2-methylbenzofuran-3-yl)-5-phenyl-1,2,4-oxadiazole derivatives outlined, synthetic route of 2Hchromene-3-carbonitriles³⁶ (**3a–c**) were obtained from substituted salicylaldehydes (**1a–c**) by using Baylis-Hillman reaction, enables the synthesis of highly functionalized molecules under mild conditions. In this approach, the activated double bond of acrylonitrile is connected directly to the modified salicylaldehydes (**1a–c**). DABCO reacts with acrylonitrile (**2**) via Michael addition to yield azaenolate that reacts with salicylaldehyde (**1a–c**) to give intermediate. DABCO catalyzed nucleophilic addition, cyclization and elimination of DABCO from the intermediate to give the chromene ring with a nitrile substituent at the 3-position followed by dehydration to give 2H-chromene-3-carbonitrile (**3a**). Compounds (**4a–c**) were prepared from compounds (**3a–c**) in DMSO at 160 °C for 30 min under catalyst free conditions using sodium azide. The azide ion serves as a base and abstracts a proton from the methyl group adjacent to the chromene oxygen, leading to ring opening and rearrangement to give the benzofuran core. This facile and efficient catalyst free transformation provides desired 2-methylbenzofuran-3-carbonitriles (**4a–c**) in a short reaction time³⁷.

2-methylbenzofuran-3-carbonitriles (**4a–c**) reacts with hydroxylamine hydrochloride in ethanol, in the presence of trimethylamine under reflux for 6 h, hydroxylamine undergoes nucleophilic addition to the electrophilic nitrile carbon, forming the amidoxime group at the 3-position, while triethylamine neutralizes the generated HCl and facilitates the reaction to afford the corresponding N'-hydroxy-2-methylbenzofuran-3-carboximidamides (**5a–c**) as intermediates.

Condensation of N'-hydroxy-2-methylbenzofuran-3-carboximidamides (**5a–c**) with benzaldehyde (**9a–e**) in DMSO in the presence of cesium carbonate as a base. The reaction mixture was heated at 100 °C for 6 h (Scheme 4). Cesium carbonate deprotonates the amidoxime group boosting its nucleophilicity toward the carbonyl carbon of benzaldehyde. The so formed intermediate cyclizes intramolecularly followed by dehydration. This sequence gives an effective access to the required 3-(2-methylbenzofuran-3-yl)-5-phenyl-1,2,4-oxadiazoles (**7a–o**).

6 CONCLUSIONS

In summary, novel 3-(2-methylbenzofuran-3-yl)-5-aryl-1,2,4-oxadiazoles (**7a–o**) synthesized by Condensation of N'-hydroxy-2-methylbenzofuran-3-carboximidamides (**5a–c**) with substituted benzaldehydes (**6a–e**) with efficient yields, all title

compounds characterized by NMR spectroscopy IR, Mass spectroscopy.

7 ACKNOWLEDGEMENTS

The authors Dr. Gangadhar Thalari and Mr. Santhosh Kumar Sirisilla profoundly thank ANRF, (SERB, under DST, Government of India) for providing the financial support under SURE scheme (File No. SUR/2022/003151). We are thankful to Department of Chemistry, Osmania University, Hyderabad for providing the laboratory facilities, CFRD, Osmania University, for providing analytical support.

8 CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. Jochims, J. C., et al. "Comprehensive Heterocyclic Chemistry II." Elsevier Science, Oxford (1996): 179-228.
2. Atmaram, Upare Abhay, and Selvaraj Mohana Roopan. "Biological activity of oxadiazole and thiadiazole derivatives." *Applied microbiology and biotechnology* 106.9 (2022): 3489-3505.
3. Kučerová-Chlupáčová, Marta. "Systematic review on 1, 2, 3-oxadiazoles, 1, 2, 4-oxadiazoles, and 1, 2, 5-oxadiazoles in the antimycobacterial drug discovery." *ChemMedChem* 20.9 (2025): e202400971.
4. Sulman, Abu, et al. "Oxadiazole Derivatives: A Comprehensive Review of Their Chemistry, Synthesis, and Pharmacological Potential." *Journal of Pharma and Biomedics* 3.2 (2025): 37-50.
5. Mohan, Magesh, et al. "A pharmacological update of oxadiazole derivatives: A review." *Current Topics in Medicinal Chemistry* 25.20 (2025): 2367-2384.
6. Clapp, L. B. "Adv. Heterocycl. Chem." (1976): 65-116.
7. Mokenapelli, Sudhakar, et al. "Synthesis, cytotoxicity, and molecular docking of substituted 3-(2-methylbenzofuran-3-yl)-5-(phenoxyethyl)-1, 2, 4-oxadiazoles." *Archiv der Pharmazie* 353.6 (2020): 2000006.
8. Badawy, Safa A., et al. "Oxadiazole-based fluorescent dyes: photophysical characterization and dual application in latent fingerprint detection and security inks." *Journal of Fluorescence* 35.12 (2025): 12361-12372.
9. Sharahili, Mohammed D., et al. "Multifunctional 1, 3, 4-oxadiazole-based donor-acceptor fluorophores exhibiting advanced photophysical properties for forensic and security applications and in silico anticancer evaluation." *Journal of Photochemistry and Photobiology A: Chemistry* (2026): 117186.
10. Ravi, C., et al. "Synthesis and solvent-sensitive studies of symmetrical 1, 3, 4-oxadiazole derivative: Experimental and computational insights for optoelectronic applications." *Journal of the Indian Chemical Society* (2025): 102075.
11. Kurowska, Aleksandra, et al. "Effect of donor to acceptor ratio on electrochemical and spectroscopic properties of oligoalkylthiophene 1, 3, 4-oxadiazole derivatives." *Physical Chemistry Chemical Physics* 19.44 (2017): 30261-30276.
12. Yalazan, Halise, et al. "Simultaneous electrochemical and spectroelectrochemical properties of novel peripheral 1, 3, 4-oxadiazole bearing CoII, CuII MnIIICl phthalocyanines." *Inorganica Chimica Acta* (2026): 123200.
13. Sharma, Deepak, et al. "A convenient synthesis, electrochemical profiling, and morphological studies of a pyridine-based 1, 3, 4-oxadiazole hybrid: a promising study for corrosion mitigation of mild steel in strongly acidic environment." *Inorganic Chemistry Communications* 158 (2023): 111554.
14. Maftai, Catalin-Vasile, et al. "Synthesis of 3-tert-butyl-5-(4-vinylphenyl)-1, 2, 4-oxadiazole using two different pathways." *Rev. Roum. Chim* 55.11-12 (2010): 989-994.
15. Arya, Girish Chandra, Kamalpreet Kaur, and Vikas Jaitak. "Isoxazole derivatives as anticancer agent: A review on synthetic strategies, mechanism of action and SAR studies." *European journal of medicinal chemistry* 221 (2021): 113511.
16. Kaur, Kamalneet, et al. "Isoxazoline containing natural products as anticancer agents: A review." *European journal of medicinal chemistry* 77 (2014): 121-133.
17. Abu-Hashem, Ameen A., and Mohamed El-Shazly. "Synthesis of new isoxazole-, pyridazine-, pyrimidopyrazines and their anti-inflammatory and analgesic activity." *Medicinal Chemistry* 14.4 (2018): 356-371.
18. Mota, Fernanda Virginia Barreto, et al. "Evaluation of anti-inflammatory activity and molecular docking study of new azabicyclic isoxazoline acylhydrazone derivatives." *Medchemcomm* 10.11 (2019): 1916-1925.
19. Aarjane, Mohammed, et al. "Synthesis, biological evaluation, molecular docking and in silico ADMET screening studies of novel

- isoxazoline derivatives from acridone." *Arabian Journal of Chemistry* 14.4 (2021): 103057.
20. Shaik, Afzal, et al. "Antimicrobial, antioxidant, and anticancer activities of some novel isoxazole ring containing chalcone and dihydropyrazole derivatives." *Molecules* 25.5 (2020): 1047.
21. Rastegari, Arezoo, et al. "Synthesis and evaluation of novel arylisoxazoles linked to tacrine moiety: In vitro and in vivo biological activities against Alzheimer's disease." *Molecular Diversity* 26.1 (2022): 409-428.
22. Patil, Pooja, et al. "Natural products and their derivatives as multifunctional ligands against Alzheimer's disease." *Drug development research* 81.2 (2020): 165-183.
23. Gul, Melek, and Serpil Eryılmaz. "Synthesis, antioxidant activity and theoretical investigation of isoxazolines derivatives of monoterpenoids." *Letters in Organic Chemistry* 16.6 (2019): 501-510.
24. Pothuri, Vijai Varma, P. V. S. Machiraju, and V. Samba Siva Rao. "Synthesis and Biological Activity of Some Novel Derivatives of 4-[5-(2, 3-Dihydrobenzo [b][1, 4] dioxin-7-yl) isoxazole-3-yl] benzoic Acid." *Russian Journal of General Chemistry* 90.5 (2020): 889-894.
25. Huang, Shi-sheng, et al. "Design, synthesis, and insecticidal and fungicidal activities of quaternary ammonium salt derivatives of a triazolylphenyl isoxazoline insecticide." *Pest management science* 78.5 (2022): 2011-2021.
26. Trefzger, Ozildéia S., et al. "Design, synthesis, antileishmanial, and antifungal biological evaluation of novel 3, 5-disubstituted isoxazole compounds based on 5-nitrofurans scaffolds." *Archiv der Pharmazie* 353.2 (2020): 1900241.
27. Zhang, Tao, et al. "Synthesis and antifungal activity of novel pyrazolines and isoxazolines derived from cuminaldehyde." *Journal of Pesticide Science* 44.3 (2019): 181-185.
28. Li, Zheng, et al. "Identification of highly potent and orally available free fatty acid receptor 1 agonists bearing isoxazole scaffold." *Bioorganic & Medicinal Chemistry* 26.3 (2018): 703-711.
29. Fettach, Saad, et al. "Biological, toxicological and molecular docking evaluations of isoxazoline-thiazolidine-2, 4-dione analogues as new class of anti-hyperglycemic agents." *Journal of Biomolecular Structure and Dynamics* 41.3 (2023): 1072-1084.
30. Douglas, Edward JA, et al. "Improved antibacterial activity of 1, 3, 4-oxadiazole-based compounds that restrict *Staphylococcus aureus* growth independent of LtaS function." *ACS infectious diseases* 9.11 (2023): 2141.
31. Glomb, Teresa, and Piotr Świątek. "Antimicrobial activity of 1, 3, 4-oxadiazole derivatives." *International journal of molecular sciences* 22.13 (2021): 6979.
32. Mollea, Chiara, Francesca Bosco, and Davide Fissore. "Agar plate methods for assessing the antibacterial activity of thyme and oregano essential oils against *S. epidermidis* and *E. coli*." *Antibiotics* 11.12 (2022): 1809.
33. Hao, Feng, et al. "Design, synthesis and antifungal activity of novel 1, 3, 4-Oxadiazole derivatives against *Botrytis cinerea*." *Pest Management Science* 82.7 (2026): 6256-6271.
34. Yu, Lili, et al. "Synthesis and antifungal activity of 1, 2, 4-oxadiazole derivatives." *Molecules* 30.8 (2025): 1851.
35. Espinoza-Hicks, José C., et al. "Synthesis of cyclic amino acid baikiain via asymmetric phase transfer catalysis." *Journal of Heterocyclic Chemistry* 60.6 (2023): 1027-1031.
36. Ramana, G., Madhu, G., Rao, Y. J., Rao, C. H. P., et al. "Design, Synthesis of 5-(2-Methylbenzofuran)-2-aryl-2H-tetrazole Derivatives via Cross Coupling of N-H Free Tetrazoles with Boronic Acids." *Asian Journal of Chemistry* 35, (2023), 2001-2006.
37. Santhosh Kumar Sirisilla, Jagram Anterbedy, Rajesh Alukucha, Govu Ramana, Gajula Naveen, Thalari Gangadhar. "Design and Synthesis of Novel 3-(2-methylbenzofuran-3-yl)-5-arylisoxazole derivatives: Antimicrobial Evaluation". *The bioscan*. 20(1): S.I (1), (2025), 158-172.