Research Article

Synthesis and Biological Evaluation of (4-Fluorophenyl) (1-(5-phenyl-1,3,4-oxadiazol-2-yl)indolizin-3-yl)methanone Derivatives as Anti-cancer and Antimicrobial Agents

Mahanthesha G. 1, Suresh T. 1, T. R. Ravikumar Naik 2*

1Department of Chemistry, Vijayanagara Sri Krishnadevaraya University, Jnana Sagar, Vinayakanagar, Cantonment, Ballari, Karnataka-583105, India.
2Department of P. G. Studies in Chemistry, Veerashaiva College, Vijayanagara Sri Krishnadevaraya University, Cantonment, Ballari, Karnataka-583105, India

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A B S T R A C T

A novel series of (4-Fluorophenyl) (1-(5-phenyl-1,3,4-oxadiazol-2-yl)indolizin-3-yl)methanone derivatives 9(a-n) were synthesized by the coupling reaction of 3-(4-fluorobenzoyl)indolizine-1-carboxylic acid and substituted benzohydrazide followed by intramolecular cyclization. The structures of the compounds were characterized by 1H NMR, 13C NMR, LCMS, FT-IR, and elemental analyses. The compounds 9(a-n) anti-cancer activity was evaluated against the MCF-7 cell line (HTB-22, Homo sapiens, Breast carcinoma). Compound 9j (IC50 = 21.57 µM), and 9n (IC50 = 8.52 µM) exhibited the most potent cytotoxicity activity compared with standard drug doxorubicin (IC50 = 25.71). The antibacterial activity was evaluated against Staphylococcus aureus ATCC 6538 and Escherichia coli ATCC 8739. The compounds ZOI=16mm) and 9i (ZOI=18 mm) exhibited moderate antibacterial activity compared with standard drug ciprofloxacin. The antifungal activity was evaluated against Candida albicans ATCC 10231. Most compounds exhibited moderate antifungal activity compared with the standard drug Itraconazole.

I N T R O D U C T I O N

Indolizine ring system scaffolds are compounds with vast application in the life science and medicine sector. Some of the naturally occurring indolizidines (Fig. 1) has induced much attention due to its anti-inflammatory, anti-angiogenic activity, anti-cancer, larvicidal agents, COX-2 inhibitors and anti-tubercular activity. These have also been used as photographic sensitizing and fabric brightening agents.

Graphical Abstract

Fig. 1: Naturally occurring indolizidine alkaloids.

*Corresponding Author: T. R. Ravikumar Naik
Address: Department of P. G. Studies in Chemistry, Veerashaiva College, Vijayanagara Sri Krishnadevaraya University, Cantonment, Ballari, Karnataka-583105, India
Email: naikravi7@gmail.com

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Cancer is one of the leading causes of death after heart disease, making it one of the world’s most challenging and deadly diseases. The discovery of selective and less toxic anti-cancer agents is the need of the hour. 1,3,4-oxadiazole forms an important motif on a heterocyclic system due to its thermal stability at the second position. It is also evident that existing clinical candidates like Ratelgravin an antiretroviral drug and Zibotentan an anti-cancer drug contain a 1,3,4-oxadiazole ring. The anti-cancer activity of 1,3,4-oxadiazole encouraged us to incorporate it into the indolizine derivatives. Several methods have been reported to prepare oxadiazole on a heterocyclic system. To the best of our knowledge, there is no previous report for the synthesis of Indolizine incorporated 1,3,4-oxadiazole derivatives. The present work involves the synthesis of 4-fluoro substituted indolizine derivatives appended with 1,3,4-oxadiazole compounds with an active functional group and are expected to increase the biological activity of the compound. The structural activity relationship study is an important part of any research work in synthetic chemistry to understand the biological efficacy of various substituents in a drug molecule and explore efficient pharmacological activities of the compounds.

The drug’s efficacy may decrease due to resistance built by the organisms over an extended period of usage. Hence, the need to be replaced by newer drugs periodically. In this need, the discovery of new anti-cancer agents is the need of the hour. 1,3,4-oxadiazole derivatives. Several methods have been reported to prepare oxadiazole on a heterocyclic system. However, to the best of our knowledge, there is no previous report for the synthesis of Indolizine incorporated 1,3,4-oxadiazole derivatives. The present work involves the synthesis of 4-fluoro substituted indolizine derivatives appended with 1,3,4-oxadiazole compounds with an active functional group and are expected to increase the biological activity of the compound. The structural activity relationship study is an important part of any research work in synthetic chemistry to understand the biological efficacy of various substituents in a drug molecule and explore efficient pharmacological activities of the compounds.

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General procedure for N-Benzoyl-3-(4-fluorobenzoyl) indolizine-1-carbohydrazide derivatives 8(a-n)

To a solution of compound 6 (0.96 mmol) in dry CH₂Cl₂ (8 mL), triethylamine (1.92 mmol) and T3P (1.44 mmol) were added at room temperature. Then was added 7(a-n) (1.15 mmol) was added to the reaction mixture and stirred at room temperature for 12 hours. The reaction mixture was quenched with ice-cold water and extracted with CH₂Cl₂ (2 x 50 mL). The organic layer was washed with brine solution (2x10 mL), dried over anhydrous sodium sulfate, and concentrated to give compounds 8(a-n) red gum, and purified by column chromatography on silica gel using CH₂Cl₂/MeOH (9:1) to achieve compounds 8(a-n).

N-Benzoyl-3-(4-fluorobenzoyl) indolizine-1-carbohydrazide (8a)

Yellow solid; Yield: 65%; MP: 154.5-155.3°C; IR (νmax cm⁻¹): 3213 (N-H), 3014 (Aromatic, C-H), 1520 (C=C); 1H NMR (400 MHz, DMSO-d₆) δ (ppm): 10.43 (s, 1H, NH), 10.32 (s, 1H, NH), 9.87 (d, J = 7.20 Hz, 1H, Ar-H), 8.51 (d, J = 8.80 Hz, 1H, Ar-H), 8.17 (s, 1H, Ar-H), 7.94 -7.91 (m, 4H, Ar-H), 7.62-7.58 (m, 2H, Ar-H), 7.55-7.51 (m, 2H, Ar-H), 7.44 (t, J = 8.80 Hz, 2H, Ar-H), 7.33-7.29 (m, 1H, Ar-H); 13C NMR (100 MHz, DMSO-d₆) δ (ppm): 107.4, 115.7, 115.9, 116.3, 119.7, 121.7, 126.0, 127.9, 123.8, 128.8, 128.9, 131.9, 132.0, 133.1, 136.5(2C), 139.7, 140.0, 163.2(2C), 165.6, 166.5, 183.5. Anal.cald.for: C₉_H₁₆_F₂_N₂_O₂; C, 68.82%; H, 4.02%; N, 10.47%. Found: C, 68.81%; H, 4.01%; N, 10.46%. LCMS (EI, m/z): 402.1 [M+H]^+.

3-(4-Fluorobenzoyl)-N¹-(3,4-methoxybenzoyl) indolizine-1-carbohydrazide (8b)

Yellow solid; Yield: 56%; MP: 152.3-153.6°C; IR (νmax cm⁻¹): 3219 (N-H), 2964 (Aromatic, C-H), 1603(C=O): 1504, 1477 (C=C); 1H NMR (400 MHz, DMSO-d₆) δ (ppm): 10.29 (d, J = 7.20 Hz, 2H, NH-NH), 9.87 (d, J = 6.80 Hz, 1H, Ar-H), 8.51 (d, J = 8.80 Hz, 1H, Ar-H), 8.18 (d, J = 7.20 Hz, 1H, Ar-H), 7.94 -7.86 (m, 4H, Ar-H), 7.60 (t, J = 8.00 Hz, 1H, Ar-H), 7.44 (t, J = 8.80 Hz, 2H, Ar-H), 7.31 (t, J = 6.80 Hz, 1H, Ar-H), 7.06 (d, J = 8.80 Hz, 2H, Ar-H), 3.84 (s, 3H, methoxy); Anal.cald.for: C₁₉_H₁₈_F₂_N₂_O₆; C, 66.82%; H, 4.21%; N, 9.74%. Found: C, 66.81%; H, 4.20%; N, 9.73%. LCMS (EI, m/z): 431.9[M+H]^+.

3-(4-Fluorobenzoyl)-N¹-(5,3,5-dimethylbenzoyl) indolizine-1-carbohydrazide (8c)

Off-white solid; Yield: 65%; MP: 151.0-152.5°C; IR (νmax cm⁻¹): 3221 (N-H), 3005 (Aromatic, C-H), 1518(C=C); 1H NMR (400 MHz, DMSO-d₆) δ (ppm): 10.39 (s, 1H, NH), 10.31 (s, 1H, NH), 9.87 (d, J = 7.20 Hz, 1H, H), 8.52 -8.50 (m, 1H, Ar-H), 8.16 (s, 1H, Ar-H), 7.94 -7.87 (m, 2H, Ar-H), 7.65-7.59 (m, 1H, Ar-H), 7.46 -7.40 (m, 2H, Ar-H), 7.33 -7.29 (m, 1H, Ar-H), 7.08 (d, J = 2.40 Hz, 2H), 6.72 (t, J = 2.40 Hz, 1H), 3.81 (s, 6H, methoxy); Anal.cald.for: C₁₉_H₂₅_F₂_N₂_O₂; C, 65.07%; H, 4.37%; N, 9.11%. Found: C, 65.06%; H, 4.36%; N, 9.10%. LCMS (EI, m/z): 462.0
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2H, Ar-H), 7.61 (t, J = 8.00 Hz, 1H, Ar-H), 7.44 (t, J = 8.80 Hz, 2H, Ar-H), 7.38-7.22 (m, 4H, Ar-H), 2.41 (s, 3H, methyl); ^13^C NMR (100 MHZ, DMSO-d_6) δ (ppm): 19.9, 107.2, 114.4, 115.9, 116.3, 117.2, 119.8, 121.7, 126.0, 128.4, 128.9, 131.9, 132.0, 132.9, 133.0, 136.5, 136.8, 139.8, 159.0, 163.1, 163.2, 165.6, 167.8, 183.5. Anal.cald.for: C_{23}H_{12}F_{2}N_{2}O_{2}; C, 65.87%; H, 3.61%; N, 10.02%. Found: C, 65.85%; H, 3.59%; N, 10.00%. LCMS (El, m/z): 420.0 [M+H]^+.

3-(4-Fluorobenzoyl)-N1-(4-chlorobenzoyl) indolizine-1-carboxylic acid (8l)

Off-white solid; Yield: 61%; MP: 216.6 -217.7°C; IR (v_{max} cm^{-1}): 3252 (N-H), 3196, 3127, 3046 (Aromatic, C-H), 1611(C=O), 1525 (C=C); ^1^H NMR (400 MHZ, DMSO-d_6) δ (ppm): 10.53 (s, 1H, NH), 10.35 (s, 1H, NH), 9.87 (d, J = 6.80 Hz, 1H, Ar-H), 8.51 (d, J = 8.80 Hz, 1H, Ar-H), 8.16 (s, 1H, Ar-H), 7.96-7.91 (m, 4H, Ar-H), 7.63-7.59 (m, 3H, Ar-H), 7.44 (t, J = 8.40 Hz, 2H, Ar-H), 7.31 (t, J = 6.80 Hz, 1H, Ar-H), 7.16 (t, J = 8.80 Hz, 1H, Ar-H), 7.06 (m, 4H, Ar-H), 3.27 (s, 3H, methyl); Anal.cald.for: C_{23}H_{12}ClF_{2}N_{2}O_{2}; C, 63.38%; H, 3.47%; N, 9.64%. Found: C, 63.37%; H, 3.46%; N, 9.63%. MS (El, m/z): 436.0[M+H]^+.

3-(4-Fluorobenzoyl)-N1-(4-fluorobenzoyl) indolizine-1-carboxylic acid (8m)

Off-white solid; Yield: 63%; MP: 177.1-178.9°C; IR (v_{max} cm^{-1}): 3255 (N-H) 2916 (Aromatic, C-H), 1595 (C=O); ^1^H NMR (400 MHZ, DMSO-d_6) δ (ppm): 10.52 (s, 1H, NH), 10.34 (s, 1H, NH), 9.85 (d, J = 6.80 Hz, 1H, Ar-H), 8.48 (d, J = 8.80 Hz, 1H, Ar-H), 8.14 (s, 1H, Ar-H), 7.92-7.83 (m, 4H, Ar-H), 7.74 (t, J = 7.60 Hz, 2H, Ar-H), 7.60 (t, J = 7.60 Hz, 1H, Ar-H), 7.42 (t, J = 8.40 Hz, 2H, Ar-H), 7.30 (t, J = 6.80 Hz, 1H, Ar-H); Anal.cald.for: C_{23}H_{13}BrF_{2}N_{2}O_{2}; C, 57.52%; H, 3.15%; N, 8.75%. Found: C, 57.51%; H, 3.14%; N, 8.74%. LCMS (El, m/z): 479.9 [M+H]^+.

3-(4-Fluorobenzoyl)-N1-(3,4-difluorobenzoyl) indolizine-1-carboxylic acid (8n)

Brown solid; Yield: 50%; MP: 235.3-236.1°C; IR (v_{max} cm^{-1}): 3236 (N-H), 3009 (Aromatic, C-H), 1614(C=O), 1514 (C=C); ^1^H NMR (400 MHZ, DMSO-d_6) δ (ppm): 10.57 (s, 1H, NH), 10.38 (s, 1H, NH), 9.87 (d, J = 7.20 Hz, 1H, Ar-H), 8.50 (d, J = 9.20 Hz, 1H, Ar-H), 8.16 (s, 1H, Ar-H), 7.99 -7.91 (m, 3H, Ar-H), 7.83 (t, J = 7.60 Hz, 2H, Ar-H), 7.64-7.59 (m, 2H, Ar-H), 7.44 (t, J = 8.40 Hz, 2H, Ar-H), 7.32 (t, J = 6.80 Hz, 1H, Ar-H); Anal.cald.for: C_{23}H_{13}F_{4}N_{2}O_{2}; C, 63.16%; H, 3.23%; N, 9.61%. Found: C, 63.14%; H, 3.21%; N, 9.59%. LCMS (El, m/z): 438.0[M+H]^+.

General procedure for synthesis of (4-Fluorophenyl) (1-(5-phenyl-1,3,4-oxadiazol-2-yl)indolizin-3-yl)methanone (9a-n).

To a solution of compounds 8(a-n) (0.498 mmol) in dry CH_2Cl_2 (10 mL), was added pyridine (0.996 mmol) and triflic anhydride (0.747 mmol) at 0°C, and stirred at 0°C for 2h. After completion, the reaction was quenched with ice-cold water and the mixture was extracted with CH_2Cl_2 (2x50 mL). The organic layer was washed brine, dried over sodium sulfate, and concentrated with a vacuum to afford compound 9(a-n).
(4-Fluorophenyl)(1-(5-(3,4-dimethylphenyl)-1,3,4-oxadiazol-2-yl)indolin-3-yl)methanone (9e)

Off-white solid; Yield: 65%; MP: 211.0-212.6°C; IR (νₘₐₓ cm⁻¹): 3095, 3039 (C-H, stretching, aromatic ring), 1630 (C=O), 1575 (C=N), 1476 (C=C), 1227 (C-O-C stretching, oxadiazole ring); ¹H NMR (400 MHz, CDCl₃) δ (ppm): 10.02-10.00 (m, 1H, Ar-H), 8.63-8.60 (m, 1H, Ar-H), 7.95-7.91 (m, 3H, Ar-H), 7.57 (s, 1H, Ar-H), 7.88-7.86 (m, 1H, Ar-H), 7.59-7.55 (m, 1H, Ar-H), 7.31-7.26 (m, 3H, Ar-H), 7.22-7.18 (m, 1H, Ar-H), 2.39 (s, 6H methyl); ¹³C NMR (100 MHz, CDCl₃) δ (ppm): 19.7, 20.0, 99.7, 115.5, 115.7 (2C), 119.5, 121.4, 124.9, 124.3, 124.8, 127.6, 127.7, 129.2, 130.2, 131.3, 131.4, 136.0, 136.1, 137.5, 140.7, 160.8, 163.3, 166.1, 183.7; Anal. calcd. for C₂₅H₁₅FN₂O₂: C, 72.98%; H, 4.41%; N, 10.21%; Found: C, 72.97%; H, 4.40%; N, 10.20%; LCMS (EI, m/z): 412.1[M+H]^⁺.

(4-Fluorophenyl)(1-(5-(4-fluorophenyl)-(1-(5-(4-(trifluoromethoxy)phenyl)-1,3,4-oxadiazol-2-yl)indolin-3-yl)methanone (9d)

Off-white solid; Yield: 82%; MP: 222.4-223.1°C; IR (νₘₐₓ cm⁻¹): 3129 (C-H, stretching, aromatic ring), 1626 (C=O), 1586 (C=N), 1480 (C=C), 1265 (C-O-C stretching, oxadiazole ring); ¹H NMR (400 MHz, CDCl₃) δ (ppm): 10.03-10.01 (m, 1H, Ar-H), 8.62-8.59 (m, 1H, Ar-H), 8.22-8.19 (m, 2H, Ar-H), 7.95-7.91 (m, 2H, Ar-H), 7.87 (s, 1H, Ar-H), 7.60-7.56 (m, 1H, Ar-H), 7.40 (dd, J = 0.80, 8.80 Hz, 2H, Ar-H), 7.30-7.29 (m, 1H, Ar-H), 7.28-7.25 (m, 1H, Ar-H), 7.23-7.21 (m, 1H, Ar-H); ¹³C NMR (100 MHz, CDCl₃) δ (ppm): 99.2, 115.5, 115.8, 119.4, 121.2, 121.6, 122.5, 123.1, 124.8, 127.8, 128.5, 129.2, 131.3, 131.4, 136.0 (2C), 137.6, 151.3, 151.4, 161.4, 162.0, 163.6, 166.1, 183.7; Anal. calcd. for C₂₅H₁₅F₂N₂O₂: C, 61.68%; H, 2.80%; N, 8.99%; Found: C, 61.67%; H, 2.79%; N, 8.98%; LCMS (EI, m/z): 467.9 [M+H]^⁺.
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3.64%; N, 10.12%; Found: C, 69.38%; H, 3.63%; N, 10.11%; LCMS (EI, m/z): 416.0[M+H]+.

(1-(5-(5-Chloro-2-methylphophenyl)-1,3,4-oxadiazol-2-yl)indolizin-3-yl)(4-fluorophenyl)methanone (9h)

Off-white solid; Yield: 56%; MP: 218.9-220.1°C; IR (νmax, cm⁻¹): 3099, 3042 (C-H, stretching, aromatic ring), 1623 (C=O), 1596 (C-N), 1482 (C=C), 1231 (C-O-C stretching, oxadiazole ring); 1H NMR (400 MHz, CDCl₃) δ (ppm): 10.04-10.11 (m, 1H, Ar-H), 8.64-8.62 (m, 1H, Ar-H), 8.00 (d, J = 2.40 Hz, 1H, Ar-H), 7.95-7.92 (m, 2H, Ar-H), 7.88 (s, 1H, Ar-H), 7.61-7.56 (m, 1H, Ar-H), 7.43-7.40 (m, 1H, Ar-H), 7.34 (d, J = 8.40 Hz, 1H, Ar-H), 7.31-7.26 (m, 2H, Ar-H), 7.23-7.20 (m, 1H, Ar-H), 2.78 (s, 3H, methyl); 13C NMR (100 MHz, CDCl₃) δ (ppm): 21.7, 116.0, 119.5, 125.1, 127.0 (2C), 128.1, 128.3, 128.9, 129.3, 129.8, 130.8 (2C), 131.6, 131.8, 133.1, 134.8, 136.9, 137.8, 141.6, 141.6, 154.0, 160.5, 183.4.

C₂₃H₁₉F₂NO₃: C, 68.75%; H, 3.50%; N, 9.73%. Found: C, 66.74%, H, 3.49%, N, 9.72%. LCMS (EI, m/z): 432.0[M+H]+.

(1-(5-(5-Bromo-2-methylphophenyl)-1,3,4-oxadiazol-2-yl)indolizin-3-yl)(4-fluorophenyl)methanone (9i)

Off-white solid; Yield: 58%; MP: 226.5-227.7°C; IR (νmax, cm⁻¹): 3098, 3041 (C-H, stretching, aromatic ring), 1622 (C=O), 1596 (C-N), 1485 (C=C), 1232 (C-O-C stretching, oxadiazole ring); 1H NMR (400 MHz, CDCl₃) δ (ppm): 10.03-10.01 (m, 1H, Ar-H), 8.64-8.62 (m, 1H, Ar-H), 8.14 (d, J = 2.00 Hz, 1H, Ar-H), 7.94-7.92 (m, 2H, Ar-H), 7.89 (s, 1H, Ar-H), 7.61-7.55 (m, 2H, Ar-H), 7.30-7.26 (m, 3H, Ar-H), 7.23-7.21 (m, 1H, Ar-H), 2.77 (s, 3H, methyl); 13C NMR (100 MHz, CDCl₃) δ (ppm): 21.7, 116.0, 119.5, 125.1, 127.0 (2C), 128.1, 128.3, 128.9, 129.3, 129.8, 130.8 (2C), 131.6, 131.8, 133.1, 134.8, 136.9, 137.8, 141.6, 141.6, 154.0, 160.5, 183.4.

C₂₃H₁₉BrFNO₃: C, 60.52%; H, 3.61%; N, 8.82%. Found: C, 60.50%; H, 3.15%; N, 8.80%. LCMS (EI, m/z): 475.8 [M+H]+.

(4-Fluorophenyl)(1-(5-(3-fluorophenyl)-1,3,4-oxadiazol-2-yl)indolizin-3-yl)methanone (9j)

Off-solid; Yield: 61%; MP: 209.8-210.5°C; IR (νmax, cm⁻¹): 3135, 3046 (C-H, stretching, aromatic ring), 1623 (C=O), 1584 (C-N), 1479 (C=C), 1228 (C-O-C stretching, oxadiazole ring); 1H NMR (400 MHz, CDCl₃) δ (ppm): 10.02-10.01 (m, 1H, Ar-H), 8.62-8.59 (m, 1H, Ar-H), 8.59-8.58 (m, 1H, Ar-H), 8.59-8.58 (m, 2H, Ar-H), 7.95-7.91 (m, 2H, Ar-H), 7.87 (s, 1H, Ar-H), 7.60-7.53 (m, 3H, Ar-H), 7.30-7.25 (m, 2H, Ar-H), 7.23-7.21 (m, 1H, Ar-H); 13C NMR (100 MHz, CDCl₃) δ (ppm): 99.3, 115.5, 115.8, 119.4, 122.8, 123.1, 124.8, 126.1, 127.8, 128.1, 129.2, 131.3, 134.2, 134.2, 136.0 (2C), 137.6, 161.3, 162.3 (2C), 163.6, 166.1, 183.7; Anal. calcld. for C₂₃H₁₉BrFNO₃: C, 59.76%; H, 2.83%; N, 9.09%; Found: C, 59.74%; H, 2.81%; N, 9.07%; LCMS (EI, m/z): 463.9 [M+H]+.

(4-Fluorophenethyl)(1-(5-(3,4-difluorophenyl)-1,3,4-oxadiazol-2-yl)indolizin-3-yl)methanone (9k)

Brown solid; Yield: 62%; MP: 211.1-212.3°C; IR (νmax, cm⁻¹): 3128, 3086 (C-H, stretching, aromatic ring), 1615 (C=O), 1583 (C-N), 1481 (C=C), 1225 (C-O-C stretching, oxadiazole ring); 1H NMR (400 MHz, CDCl₃) δ (ppm): 10.03-10.00 (m, 1H, Ar-H), 8.61-8.58 (m, 1H, Ar-H), 7.92-7.89 (m, 4H, Ar-H), 7.86 (s, 1H, Ar-H), 7.60-7.56 (m, 1H, Ar-H), 7.37-7.30 (m, 1H, Ar-H), 7.29-7.20 (m, 3H, Ar-H); 13C NMR (100 MHz, CDCl₃) δ (ppm): 99.2, 115.5, 115.8 (2C), 119.4, 121.2, 121.6, 122.5, 123.1, 124.8, 127.8, 128.5, 128.2, 129.3, 131.3, 136.0.
137.6, 151.3, 161.4, 162.0, 163.6, 166.1, 183.7. Anal. calcd for C_{23}H_{12}F_{3}N_{3}O_{2}: C, 65.87%; H, 2.88%; N, 10.02%. Found: C, 65.86%, H, 2.87%, 10.01%. LCMS (EI, m/z): 419.9 [M+H]^+.

**Biological Procedures**

In-vitro anti-cancer activity bioassay

For Cytotoxicity studies, 10mM stocks were prepared using Chloroform. Serial two-fold dilutions were prepared from 100µM to 3.125µM using DMEM media for treatment.

Cell lines and Culture

MCF-7 cell line was procured from ATCC, stock cells were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100µg/ml) in a humidified atmosphere of 5% CO₂ at 37°C until confluent. The cell was dissociated with cell dissociating solution (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS). The viability of the cells is checked and centrifuged. Further, 50,000 cells/well were seeded in a 96 well plate and incubated for 24 h at 37°C, 5% CO₂ incubator.

MTT assay

The monolayer cell culture was trypsinized and the cell count was adjusted to 5 x 10⁵ cells/ml using respective media containing 10% FBS. To each well of the 96 well Microtiter plate, 100µl of the diluted cell suspension (50,000cells/well) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium, and 100 µl of different test concentrations of test drugs were added to the partial monolayer in Microtiter plates. The plates were then incubated at 37°C for 24 h in a 5% CO₂ atmosphere. After incubation, the test solutions in the wells were discarded and 100 µl of MTT (5mg/10 ml of MTT in PBS) was added to each well. The plates were incubated for 4 h at 37°C in a 5% CO₂ atmosphere. The plates were removed and 100µl of DMSO was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 590 nm. The percentage growth inhibition was calculated using the following formula and the concentration of test drug needed to inhibit cell growth by 50% (IC₅₀) values is generated from the dose-response curves for each cell line.[23-26]

In-vitro Antibacterial Activity Assay

The newly synthesized novel compounds were screened for their antibacterial activity against Staphylococcus aureus and Escherichia coli strains by the well diffusion method. The cell suspension was prepared and grown on Tryptic soya broth, and cultures were incubated for 24 h at 37°C for bacteria. Cell density is adjusted to 1 x 10⁶ cells/ml using 0.5 McFarland standards. The cell suspensions of all the cultures were adjusted to 1-2 x 10⁵ cells/ml and inoculated on Soya bean Casein Digested agar plates. Test compounds 9a-n (20 µL), Standard Ciprofloxacin (20 µL) for S. aureus and E. coli were added to the 5mm well on agar plates. The treated plates are incubated in an anaerobic chamber at 37°C for 24 hours. The plates were observed for the zone of inhibition around the wells.

In-vitro Antifungal Activity Assay

All compounds were screened for antifungal activity against Candida albicans by the well diffusion method. Cells were grown on Potato dextrose broth and cultures were incubated for 24 h 35°C for candida. Cell density was adjusted to 1-2 x 10⁵ cells/ml and inoculated on Soya bean Casein Digested agar plates. Test compounds 9a-n (20 µL), Standard Itracanazole (20 µL) for C. Albicans was added to the 5mm well on agar plates and incubated in the aerobic chamber at 35°C for 24 hours. The plates were observed for the zone of inhibition around the wells.[27-31]

**Results and Discussion**

Chemistry

The synthetic approach for preparing target compounds is illustrated in Scheme 1. The pyridinium salt 3, was

![Scheme 1: Synthesis of (4-Fluorophenyl)[1-(5-phenyl-1,3,4-oxadiazol-2-yl)indolizin-3-yl]methanone derivatives.](image)

Reagents and Conditions: a) Acetone, rt, 0.5 h; b) TEA, CH₃Cl₂, rt, 12h, 60%; c) Citric acid, THF/H₂O (4:1), Reflux, 48h; d) T₂P, TEA, CH₂Cl₂, rt, 12h; e) Triflic anhydride, Pyridine, CH₂Cl₂, rt, 2h.
synthesized by commercially available 2-Bromo-1-(4-fluorophenyl)ethanone 1 with pyridine 2. The construction of indolizine ring 5 was achieved by the cycloaddition reaction of the corresponding ylide, generated in situ by the treatment of pyridinium salt 3 with ethyl propiolate in the presence of triethylamine. Compound 5 in the presence of citric acid and THF/H₂O afforded acid 6, which on reaction with substituted hydrazide 7(a-n) in presence of T₃P, triethylamine in CH₂Cl₂ afforded N-Benzoyl-3-(4-fluorobenzoyl)indolizine-1-carbohydrazide derivatives 8(a-n). Finally, the title compounds 9(a-n) were synthesized by cyclization of carbohydrazide derivatives 8a-n in the presence of pyridine and triflic anhydride in CH₂Cl₂.

**Biological Activity**

**In-vitro Anti-cancer Activity**

The analogue of 9(a-n) was screened for their in-vitro cytotoxicity activity against MCF-7 cell line and the obtained results IC₅₀ value tabulated in Table 2. Among the synthesized compounds 9c (IC₅₀ = 26.48 µM), 9j (IC₅₀ = 21.57 µM), 9k (IC₅₀ = 28.85 µM) and exhibited potent cytotoxicity activity compared with standard drug doxorubicin (IC₅₀=25.71 µM) showed in Fig. 2(a), Fig. 2(b) and Fig.3 and 9n (IC₅₀ = 8.52 µM) considered to be the best candidate of the series.

**In-vitro Antimicrobial Activity**

The in-vitro antimicrobial activity was carried out by well diffusion method and the obtained results are
The analog 9a-n was tested for their antibacterial activity. The compounds 9h (ZOI=16mm) and 9i (ZOI=18 mm) exhibited significant inhibition compared with standard drug ciprofloxacin. Most of the compounds in this series showed moderate inhibition on fungi strains compared with the standard drug Itraconazole.

**Table 3: In-vitro antimicrobial activity of 9(a-n) derivatives.**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>S. aureus</th>
<th>E. coli</th>
<th>C. albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td>9a</td>
<td>8</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>9b</td>
<td>7.5</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>9c</td>
<td>9</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>9d</td>
<td>9.5</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>9e</td>
<td>13</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>9f</td>
<td>13</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>9g</td>
<td>12</td>
<td>8.5</td>
<td>11</td>
</tr>
<tr>
<td>9h</td>
<td>16</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>9i</td>
<td>18</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>9j</td>
<td>8.5</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>9k</td>
<td>8.5</td>
<td>8.5</td>
<td>9</td>
</tr>
<tr>
<td>9l</td>
<td>9</td>
<td>9</td>
<td>9.5</td>
</tr>
<tr>
<td>9m</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>9n</td>
<td>11</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Ciprofloxacin(2.0µg/well)</td>
<td>21.2 ± 0.44</td>
<td>21.2 ± 0.83</td>
<td>-</td>
</tr>
<tr>
<td>Itraconazole (20ug/well)</td>
<td>-</td>
<td>-</td>
<td>21 ± 1.22</td>
</tr>
</tbody>
</table>

**Fig. 4: In-vitro antimicrobial activity images of 9(a-n) derivatives.**
Synthesis and Biological Evaluation of (4-Fluorophenyl)(1-(5-phenyl-1,3,4-oxadiazol-2-yl)indolizin-3-yl)methanone... 

CONCLUSION

The present research focused on the efficient synthesis and biological applications of indolizines appended 1,3,4-oxadiazole ring by intramolecular cyclization using triflic anhydride. The reactions were performed keeping green-chemistry principles in mind and were carried out at room temperature. A series of novel compounds (4-Fluorophenyl)(1-(5-phenyl-1,3,4-oxadiazol-2-yl)indolizin-3-yl)methanone 9(a-n) were screened for their in-vitro anticancer activity. Results indicate that among the synthesized compounds, 9c (IC$_{50}$ = 26.48 µM), 9j (IC$_{50}$ = 21.57 µM) and 9k (IC$_{50}$ = 28.85 µM) exhibited significant anticancer activity compared with standard drug doxorubicin (IC$_{50}$ = 25.71 µM). 9n (IC$_{50}$ = 8.52 µM) is considered a good candidate for the series. This could be a good starting point to develop new lead compounds in the fight against cancer.

Supporting Information Summary

Details of Characterization data for synthesized compounds, $^1$H NMR, $^{13}$C-NMR, and Mass spectra, have been provided in the supporting information file.

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