Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

Available online at www.ijpsdronline.com

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

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ARTICLE INFO

Article history:
Received: 30 March, 2021
Revised: 08 December, 2021
Accepted: 20 December, 2021
Published: 30 January, 2022

Keywords:
1,3,4-Oxadiazole, Anticancer, Antibacterial activity, Antifungal activity, Indolizines.

DOI:
10.25004/IJPSDR.2022.140102

ABSTRACT

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 ^{1}H NMR, ^{13}C 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 (IC_{50} = 21.57 µM), and 9n (IC_{50} = 8.52 µM) exhibited the most potent cytotoxicity activity compared with standard drug doxorubicin (IC_{50}= 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.

Introduction

Indolizine ring system scaffolds are compounds with vast application in the life science and medicine sector.[1,2] These are isoelectronic with indole and have shown a significant impact on drug design and drug development, which were developed over several decades.[3] Coldham in 2009 has published a synthesis of nitrogen-containing heterocycles, which was applied to the total synthesis of new indolizidine alkaloid crispine A.[4] Some of the naturally occurring indolizidines (Fig. 1) has induced much attention due to its anti-inflammatory,[5] anti-angiogenic activity,[6] anti-cancer,[7-9] larvicidal agents,[10] COX-2 inhibitors,[11] and anti-tubercular activity.[12] These have also been used as photographic sensitizing and fabric brightening agents.[13,14]

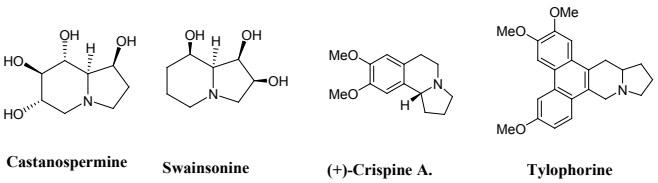
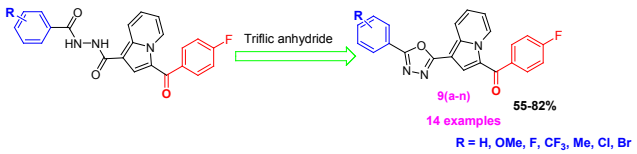


Fig. 1: Naturally occurring indolizidine alkaloids [15].

Graphical Abstract



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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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.[16] It is also evident that existing clinical candidates like Raltegravir 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 hetero polycyclic system.[17] 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.[18] 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 perspective, we proposed synthesizing and developing 4-Fluoro-aroylindolizines incorporated 1,3,4-oxadiazole unit targeting anti-cancer,[19] antimicrobial.[20-22] In continuation of our research, the main objective is to discover new anti-cancer agents.

Materials and Methods

Chemistry

All the chemicals and solvents were procured from commercial supplier's viz., Sigma Aldrich, Chempure, and Sony industries. All reactions were carried out in hot-air dried glassware under a nitrogen atmosphere using dry solvents. Thin-layer chromatography was performed on aluminum-backed silica plates and visualized by UV–light. The melting point was determined on a Thomas Hoover capillary melting point apparatus with a digital thermometer. FT-IR spectra recorded on FT-IR Shimadzu 8300 spectrophotometer, ^{1}H NMR spectra on a Bruker 400 MHz NMR spectrophotometer in $CDCl_3$ and DMSO-d_6 the chemical shifts were recorded in parts ppm and was referenced with TMS. LC-MS was performed on Agilent LC-1200 series coupled with 6140 single quad mass spectrometer with EI +ve, IR spectra were recorded on Brucker alpha FT-IR spectrometer.

1-(2-(4-Fluorophenyl)-2-oxoethyl) pyridinium bromide (3)

To a solution of 2-Bromo-1-(4-flulorophenyl) ethanone (20 g, 92.14 mmol) in acetone (200 mL) was added pyridine (7.18 g, 92.14 mmol) and stirred at room temperature for 1-hour. The obtained precipitate was filtered and dried to afford **1** as a white solid.

White solid; Yield; 98%; MP: 209.6-210.9°C; IR (ν_{max},cm^{-1}): 3461, 3400, 3051(Aromatic, C-H), 1681, (C=O); ^{1}H NMR (400 MHZ, DMSO-d_6) δ (ppm): 9.04 (d, *J*= 6.00 Hz, 2H, Ar-H), 8.77-8.73 (m, 1H, Ar-H), 8.31-8.28 (m, 2H, Ar-H), 8.19-8.15 (m, 2H, Ar-H), 7.53 (t, *J* = 9.20 Hz, 2H, Ar-H), 6.53 (s, 2H, CH2); ^{13}C NMR (100 MHZ, DMSO-d_6) δ (ppm): 66.7, 127.4, 128.3, 128.4, 131.6, 134.4, 134.7 (2C), 135.9, 146.7 (2C), 147.0, 190.4. Anal. cald. for: $C_{13}H_{11}BrFNO$: C, 52.73%; H, 3.74%; N, 4.73%. Found: C, 52.72%; H, 3.73%, N, 4.72%. LCMS (EI, m/z): 218.1 $[M+H]^+$.

Ethyl 3-(4-Fluorobenzoyl) indolizine carboxylate (5)

To a solution of (2-(4-Flulorophenyl)-2-oxoethyl) pyridinium bromide (50.65 mmol) **3** in CH_2Cl_2 (150 mL) was added triethylamine (12.78 g, 126.62 mmol) and stirred for 5 minutes. Then ethylpropiolate (7.44 g, 75.97 mmol) **4** was added dropwise at 0°C, the resulting reaction mixture was stirred at room temperature for 12 hours. After completion of the reaction was concentrated in a vacuum. The crude product was purified by column chromatography (EtOAc/Hexane 1: 9) to afford compound **5** as yellow solid.

Yellow solid; Yield: 60%; MP: 110.8-111.3°C; IR (ν_{max}, cm^{-1}): 3064 (Aromatic, C-H), 1693 (C=O); ^{1}H NMR (400 MHZ, DMSO-d_6) δ (ppm): 9.83 (d, *J* = 0.80 Hz, 1H, Ar-H), 8.33 (d, *J* = 1.20 Hz, 1H, Ar-H) 7.90-7.87 (m, 2H, Ar-H), 7.70-7.66 (m, 1H, Ar-H), 7.63 (s, 1H, Ar-H), 7.45-7.40 (m, 2H, Ar-H), 7.37-7.33 (m, 1H, Ar-H), 4.31 (q, *J* = 7.20 Hz, 2H, CH_2 of ester 1.32 (t, *J* = 7.20 Hz, 3H, methyl); ^{13}C NMR (100 MHZ, DMSO-d_6) δ (ppm): 14.7, 60.2, 106.0, 116.7, 119.3, 122.1, 127.7, 128.4, 128.5, 129.2, 129.4, 130.9, 131.8, 133.8, 139.6, 141.7, 163.3, 183.2. $C_{18}H_{14}FNO_3$: C, 69.45%; H, 4.53%; N, 4.50%. Found: C, 69.44%; H, 4.52%, N, 4.49%. LCMS (EI, m/z): 312.1$[M+H]^+$.

3-(4-Fluorobenzoyl) indolizine-1-carboxylic acid (6)

To a solution of ethyl 3-(4-Flulorobenzoyl) indolizine carboxylate **5** (5 g, 16.06 mmol) in THF/H2O (50/10 mL), was added citric acid solution (12.34 g, 64.24 mmol) and refluxed for 48 hours. The reaction mixture was concentrated and acidified pH < 4 with 6N HCl. The obtained precipitate was filtered and dried with a vacuum to afford compound 6 as an off-white solid.

Off-white solid; Yield; 65%; MP: 245.7-246.8°C; IR (ν_{max},cm^{-1}): 3050(Aromatic, C-H), 1679 (C=O); 1624 (C=C); ^{1}H NMR (400 MHZ, DMSO-d_6:D_2O) δ (ppm): 9.77 (d, *J*= 7.2 Hz, 1H, Ar-H), 8.31 (d, *J*=8.80 Hz, 1H, Ar-H), 7.84-7.75 (m, 2H, Ar-H), 7.62-7.56 (m, 2H, Ar-H), 7.37-7.33 (m, 2H, Ar-H), 7.27-7.22 (m, 1H, Ar-H); ^{13}C NMR (100 MHZ, DMSO-d_6) δ (ppm): 107.1, 116.5, 119.5, 121.9, 127.8, 128.5, 128.9, 129.1, 129.1, 130.9, 131.7, 133.7, 139.8, 141.8, 164.9, 183.1. Anal.cald.for: $C_{16}H_{10}FNO_3$: C, 67.84%; H, 3.56%; N, 4.94%. Found: C, 67.83%; H, 3.55%; N, 4.93%. LCMS (EI, m/z): 284.0 $[M+H]^+$.

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

To a solution of compound **6 (0.96 mmol)** in dry CH_2Cl_2 (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 CH_2Cl_2 (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 CH_2Cl_2/MeOH (9:1) to achieve compounds **8(a-n)**.

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

Yellow solid ; Yield: 65%; MP: 154.5-155.3°C; IR (ν_{max}, cm^{-1}): 3213 (N-H), 3014 (Aromatic, C-H), 1520 (C=C); ^{1}H NMR (400 MHZ, DMSO-d_6) 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); ^{13}C NMR (100 MHZ, DMSO-d_6) δ (ppm): 107.4, 115.7, 115.9, 116.3, 119.7, 121.7, 126.0, 127.9, 128.3, 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: $C_{23}H_{16}FN_3O_3$: 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)-N^1-(4-methoxybenzoyl) indolizine-1-carbohydrazide (8b)

Yellow solid; Yield: 56%; MP: 152.3-153.6°C; IR (ν_{max}, cm^{-1}): 3219 (N-H), 2964 (Aromatic, C-H), 1603(C=O); 1504, 1477 (C=C); ^{1}H NMR (400 MHZ, DMSO-d_6) 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: $C_{24}H_{18}FN_3O_4$: 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-(3,5-dimethoxybenzoyl)indolizine-1-carbohydrazide (8c)

Off-white solid Yield: 65%; MP: 151.0-152.5°C; IR (ν_{max}, cm^{-1}): 3221 (N-H), 3005 (Aromatic, C-H), 1518(C=C); ^{1}H NMR (400 MHZ, DMSO-d_6) δ (ppm): 10.39 (s, 1H, NH), 10.31 (s, 1H, NH), 9.87 (d, *J* =7.20 Hz, 1H), 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: $C_{25}H_{20}FN_3O_5$: 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

3-(4-Fluorobenzoyl)-N^1-(4-trifluoromethoxy) benzoyl) indolizine-1-carbohydrazide (8d)

Off-white solid; Yield: 75%; MP: 150.4-151.9°C; IR (ν_{max},cm^{-1}): 3225 (N-H), 3010 (Aromatic, C-H), 1600(C=O); 1524 (C=C); ^{1}H NMR (400 MHZ, DMSO-d_6) δ (ppm): 10.56 (s, 1H, NH), 10.37 (s, 1H, NH), 9.87 (d, *J* = 7.20 Hz, 1H, Ar-H), 8.51 (d, *J* = 9.20 Hz, 1H, Ar-H), 8.17 (s, 1H), 8.06 (d, *J* = 8.80 Hz, 2H, Ar-H), 7.95 -7.91 (m, 2H, Ar-H), 7.63-7.59 (m, 1H, Ar-H), 7.54 (d, *J* = 8.00 Hz, 2H, Ar-H), 7.44 (t, *J* = 9.20 Hz, 2H, Ar-H), 7.33-7.30 (m,1H, Ar-H); ^{13}C NMR (100 MHZ, DMSO-d_6) δ (ppm): 107.3, 115.7, 115.9, 116.3, 119.7, 121.3, 121.7, 121.8, 126.0, 127.9, 128.4, 128.8, 130.3, 131.9, 132.0, 132.2, 136.4, 136.5, 139.8, 151.1, 163.2, 165.3, 165.7, 183.5. Anal.cald. for: $C_{24}H_{15}FN_3O_4$: C, 59.39%; H, 3.11%, N, 8.66%. Found C, 59.37%; H, 3.09%; N, 8.64%. LCMS (EI, m/z): 486.0 $[M+H]^+$.

3-(4-Fluorobenzoyl)-N^1-(3,4-dimethylbenzoyl) indolizine-1-carbohydrazide (8e)

Off-white solid; Yield: 62%; MP: 126.2-127.3°C; IR (ν_{max},cm^{-1}): 3224 (N-H), 2962 (Aromatic, C-H), 1600(C=O); 1524 (C=C); ^{1}H NMR (400 MHZ, DMSO-d_6) 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), 8.17 (s, 1H, Ar-H), 7.94 -7.91 (m, 2H, Ar-H), 7.72 (s, 1H, Ar-H), 7.66 (d, *J* = 7.60 Hz, 1H, Ar-H), 7.60 (t, *J* = 7.60 Hz, 1H, Ar-H), 7.44 (d, *J* = 8.80 Hz, 2H, Ar-H), 7.30 (q, *J* = 8.40 Hz, 2H, Ar-H), 2.30 (s, 6H, methyl); ^{13}C NMR (100 MHZ, DMSO-d_6) δ (ppm): 19.8(2C), 107.5, 115.7, 115.9, 116.3, 119.7, 121.7, 125.3, 126.0, 128.3, 128.9, 129.9, 130.6, 131.9, 132.0, 136.5, 136.8, 139.7, 140.9, 163.2, 163.3, 165.6, 166.5, 183.5. Anal.cald.for: $C_{25}H_{20}FN_3O_3$: C, 69.92%; H, 4.69%N, 9.78%. Found: C, 69.91%; H, 4.67%; N, 9.78%. LCMS (EI, m/z): 430.1 $[M+H]^+$.

3-(4-Fluorobenzoyl)-N^1-(4-fluoro-2-methyl benzoyl) indolizine-1-carbohydrazide (8f)

Brown solid; Yield: 45%; MP: 241.5-242.3°C; IR (ν_{max},cm^{-1}): 3671 (N-H), 3216, 2996 (Aromatic, C-H), 1610(C=O); 1519 (C=C); ^{1}H NMR (400 MHZ, DMSO-d_6) δ (ppm): 10.33 (s, 1H, NH), 10.14 (s, 1H, NH), 9.87 (d, *J* = 7.20 Hz, 1H, Ar-H), 8.54 (d, *J* = 8.80 Hz, 1H, Ar-H), 8.17 (s, 1H, Ar-H), 7.94 -7.91 (m, 2H, Ar-H), 7.61 (t, *J* = 8.40 Hz, 1H, Ar-H), 7.52-7.42 (m, 3H, Ar-H), 7.31 (t, *J* = 7.20 Hz, 1H, Ar-H), 7.16 (q, *J* = 8.80 Hz, 2H, Ar-H), 2.46 (s, 3H, methyl); ^{13}C NMR (100 MHZ, DMSO-d_6) δ (ppm): 19.8, 107.3, 112.7, 115.9, 116.3, 117.5, 117.7, 119.8, 121.7, 126.0, 128.3, 128.8, 130.2, 131.9, 132.0, 136.5, 139.8, 139.9, 140.0, 161.8, 163.1, 163.2, 168.3, 183.5. Anal.cald. for: $C_{24}H_{17}F_2N_3O_3$: C, 66.51%; H, 3.95%; N, 9.70%. Found: C, 66.50%; H, 3.94%; N, 9.69%. LCMS (EI, m/z): 434.0 $[M+H]^+$.

3-(4-Fluorobenzoyl)-N^1-(5-fluoro-2-methylbenzoyl) indolizine-1-carbohydrazide (8g)

Off-white solid; Yield: 48%; MP: 234.4 -235.3°C; IR (ν_{max}, cm^{-1}): 3218 (N-H), 2999 (Aromatic, C-H), 1504 (C=C); ^{1}H NMR (400 MHZ, DMSO-d_6) δ (ppm): 10.36 (s, 1H, NH), 10.22 (s, 1H, NH), 9.87 (d, *J* = 7.20 Hz, 1H, Ar-H), 8.54 (d, *J* = 8.80 Hz, 1H, Ar-H), 8.17 (s, 1H, Ar-H), 7.93 (t, *J* = 7.60 Hz,

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: $C_{24}H_{17}F_2N_3O_3$: C, 66.51%; H, 3.95%; N, 9.70%. Found: C, 66.50%; H, 3.94%; N, 9.69%. MS (EI, m/z): 434.0 $[M+H]^+$.

N^1-(5-Chloro-2-methylbenzoyl)-3-(4-flurobenzoyl) indolizine-1-carbohydrazide (8h)

Off-white solid; Yield: 46%; MP: 175.6-176.9°C; IR (ν_{max},cm^{-1}): 3278 (N-H), 3196, 2966, (Aromatic, C-H), 1613(C=O); 1598 (C=C); ^{1}H NMR (400 MHZ, DMSO-d_6) δ (ppm): 10.37 (s, 1H, NH), 10.25 (s, 1H, NH), 9.87 (d, *J* = 6.80 Hz, 1H, Ar-H), 8.54 (d, *J* = 8.80 Hz, 1H, Ar-H), 8.17 (s, 1H, Ar-H), 7.93 (t, *J* = 8.00 Hz, 2H, Ar-H), 7.61 (t, *J* = 8.80 Hz, 1H, Ar-H), 7.49-7.41 (m, 4H, Ar-H), 7.39-7.27 (m, 2H, Ar-H), 2.42 (s, 3H, methyl); Anal.cald.for: $C_{24}H_{17}ClFN_3O_3$: C, 64.08%; H, 3.81%; N, 9.34%. Found: C, 64.07%; H, 3.80%; N, 9.34%. LCMS (ESI, m/z): 450.0$[M+H]^+$.

N1-(5-Bromo-2-methylbenzoyl)-3-(4-fluorobenzoyl) indolizine-1-carbohydrazide(8i)

Off-white solid; Yield: 49%; MP: 147.0-148.5°C; IR (ν_{max},cm^{-1}): 3278 (N-H), 2984 (Aromatic, C-H), 1686(C=O); 1597 (C=C); ^{1}H NMR (400 MHZ, DMSO-d_6) δ (ppm): 10.35 (s, 1H, NH), 10.24 (s, 1H, NH), 9.84 (d, *J* = 7.2 Hz, 1H, Ar-H), 8.51 (d, *J* = 8.80 Hz, 1H, Ar-H), 8.13 (s, 1H, Ar-H), 7.90 (t, *J* = 6.8 Hz, 2H, Ar-H), 7.62-7.57 (m, 3H, Ar-H), 7.44-7.40 (m, 2H, Ar-H), 7.32-7.27 (m, 2H, Ar-H), 2.37(s, 3H, methyl); Anal.cald.for: $C_{24}H_{17}ClBrN_3O_3$: C, 58.31%; H, 3.47%; N, 8.50%. Found: C, 58.30%; H, 3.46%; N, 8.50%. LCMS (EI, m/z): 493.9 $[M+H]^+$.

3-(4-Fluorobenzoyl)-N^1-(3-fluorobenzoyl)indolizine-1-carbohydrazide (8j)

Off-white solid; Yield: 56%; MP: 200.0-201.9°C; IR (ν_{max}, cm^{-1}): 3234 (N-H), 3121, 3008 (Aromatic, C-H), 1615(C=O); 1526 (C=C); ^{1}H NMR (400 MHZ, DMSO-d_6) δ (ppm): 10.54 (s, 1H, NH), 10.37 (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, 2H, Ar-H), 7.79 (d, *J* = 7.60 Hz, 1H, Ar-H), 7.71 (d, *J* = 9.60 Hz, 1H, Ar-H), 7.63-7.58 (m, 2H, Ar-H), 7.50-7.42 (m, 3H, Ar-H), 7.31 (t, *J* = 7.20 Hz, 1H, Ar-H); Anal. cald.for: $C_{23}H_{15}F_2N_3O_3$: C, 65.87%; H, 3.61%; N, 10.02%. Found: C, 65.85%; H, 3.59%; N, 10.00%. LCMS (EI, m/z): 420.0$[M+H]^+$.

3-(4-Fluorobenzoyl)-N^1-(4-fluorobenzoyl) indolizine-1-carbohydrazide (8k)

Off-white solid; Yield: 59%; MP: 195.3-196.8°C; IR (ν_{max},cm^{-1}): 3260 (N-H), 3196, 3126 (Aromatic, C-H), 1612(C=O), 1527 (C=C); ^{1}H NMR (400 MHZ, DMSO-d_6) δ (ppm): 10.46 (s, 1H, NH), 10.33 (s, 1H, NH), 9.87 (d, *J* = 9.20 Hz, 1H, Ar-CH), 8.50 (d, *J* = 11.60 Hz, 1H, Ar-CH), 8.16 (s, 1H, Ar-H), 8.02 -7.88 (m, 4H, Ar-H), 7.61 (t, *J* = 10.80 Hz, 1H, Ar-H), 7.47-7.29 (m, 5H, Ar-H); Anal.cald.for: $C_{23}H_{15}F_2N_3O_3$: C, 65.87%; H, 3.61%; N, 10.02%. Found: C, 65.85%; H, 3.59%; N, 10.00%. LCMS (EI, m/z): 420.0 $[M+H]^+$.

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

Off-white solid; Yield: 61%; MP: 216.6 -217.7°C; IR (ν_{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); ^{13}C NMR (100 MHZ, DMSO-d_6) δ (ppm): 107.3, 115.7, 115.9, 116.3, 119.7, 121.7, 125.9, 128.4, 128.8, 129.1, 129.8, 131.9, 132.0, 136.4, 136.5, 137.1, 139.7 140.9, 163.2, 163.3, 165.6, 166.4, 183.3. Anal.cald.for: $C_{23}H_{15}ClFN_3O_3$: C, 63.38%; H, 3.47%, N, 9.64%. Found: C, 63.37%; H, 3.46%, N, 9.63%. MS (EI, m/z): 436.0$[M+H]^+$.

N^1-(4-Bromobenzoyl)-3-(4-fluorobenzoyl) indolizine-1-carbohydrazide (8m)

Off-white solid; Yield: 63%; MP: 177.1-178.9°C; IR (ν_{max}, cm^{-1}): 3255 (N-H) 2916 (Aromatic, C-H), 1595 (C=C); ^{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 (d, *J* = 7.6 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* = 6.8 Hz, 1H, Ar-H); Anal.cald.for: $C_{23}H_{15}BrFN_3O_3$: C, 57.52%; H, 3.15%; N, 8.75%. Found: C, 57.51%; H, 3.14%; N, 8.74%. LCMS (EI, m/z): 479.9 $[M+H]^+$.

3-(4-Fluorobenzoyl)-N^1-(3, 4-difluorobenzoyl) indolizine-1-carbohydrazide (8n)

Brown solid; Yield: 50%, MP: 235.3-236.1°C; IR (ν_{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 (s,1H, Ar-H), 7.68-7.59 (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: $C_{23}H_{14}F_3N_3O_3$: C, 63.16%; H, 3.23%; N, 9.61%. Found: C, 63.14%; H, 3.21%; N, 9.59%. LCMS (EI, 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 9(a-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-phenyl-1,3,4-oxadiazol-2-yl) indolizin-3-yl)methanone (9a)

Yellow solid; Yield: 71%; MP: 197.6-198.3°C; IR (ν_{max},cm^{-1}): 3055, 2922 (stretching, aromatic ring), 1633 (C=O), 1578 (C=N), 1477 (C=C), 1226 (C-O-C stretching, oxadiazole); ^{1}H NMR (400 MHZ, $CDCl_3$) δ (ppm): 10.03-10.01 (m, 1H, Ar-H), 8.63-8.61 (m, 1H, Ar-H), 8.18-8.15 (m, 2H, Ar-H), 7.95-7.91 (m, 2H, Ar-H), 7.89 (s, 1H, Ar-H), 7.60-7.54 (m, 4H, Ar-H), 7.30-7.25 (m, 2H, Ar-H), 7.22-7.19 (m, 1H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 99.8, 115.9, 119.5, 122.8, 123.9, 125.1, 126.8, 127.0 (2C), 128.0, 128.8, 129.0, 129.3 (2C), 129.8, 131.51, 131.53, 134.7, 137.7, 141.5, 161.0, 163.1, 183.4. Anal. calcd. for $C_{23}H_{14}FN_3O_2$: C, 72.06%; H, 3.68%; N, 10.96%; Found: C, 72.05%; H, 3.67%; N, 10.95%; LCMS (EI, m/z): 383.9 $[M+H]^+$.

(4-Fluorophenyl)(1-(5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl)indolizin-3-yl)methanone (9b)

Yellow solid; Yield: 68%; MP: 212.4-213.9°C; IR (ν_{max},cm^{-1}): 3073 (C-H, stretching, aromatic ring) 1497 (C=C), 1227 (C-O-C stretching, oxadiazole ring); ^{1}H NMR (400 MHZ, DMSO-d_6) δ (ppm): 9.90 (d, *J* = 6.80 Hz, 1H, Ar-H), 8.51 (d, *J* = 8.80 Hz, 1H, Ar-H), 8.11 (d, *J* = 2.00 Hz, 2H, Ar-H), 7.98-7.94 (m, 3H, Ar-H), 7.76-7.72 (m, 1H, Ar-H), 7.48-7.39 (m, 3H, Ar-H), 7.16 (d, *J* = 8.80Hz, 2H, Ar-H), 3.87 (s, 3H, methoxy); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 55.4, 100.0, 114.4, 115.8, 116.4, 119.5, 122.7, 124.9, 127.0 (2C), 127.8, 128.5, 128.8, 129.2, 129.8(2C), 131.4, 134.7, 137.6, 141.6, 160.5, 162.1, 163.0, 183.3. Anal. calcd. for $C_{24}H_{16}FN_3O_3$: C, 69.73; H, 3.90; N, 10.16 %; Found: C, 69.71; H, 3.88; N, 10.14%. LCMS (EI, m/z): 414.0 $[M+H]^+$.

(4-Fluorophenyl)(1-(5-(3,5-dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)indolizin-3-yl)methanone (9c)

Off-white solid; Yield: 69%; MP: 227.9-228.6°C; IR (ν_{max}, cm^{-1}): 3118, 2966 (C-H, stretching, aromatic ring) 1482 (C=C), 1233 (C-O-C stretching, oxadiazole ring); ^{1}H NMR (400 MHZ, $CDCl_3$) δ (ppm): 10.03-10.01 (m, 1H, Ar-H), 8.62-8.59 (m, 1H, Ar-H), 7.95-7.91 (m, 2H, Ar-CH), 7.88 (s, 1H, Ar-H), 7.59-7.55 (m, 1H, Ar-H), 7.31-7.26 (m, 4H, Ar-H), 7.22-7.20 (m, 1H, Ar-H), 6.65 (t, *J* = 2.40 Hz, 1H, Ar-H), 3.92 (s, 6H, methoxy); ^{13}C NMR (100 MHZ, $CDCl_3$) δ (ppm): 55.7, 99.7, 103.7, 104.7, 115.9, 119.5, 122.8, 125.1, 125.4, 127.0(3C), 128.0, 128.8, 129.3(2C), 129.8, 131.5(2C), 134.7, 137.7, 141.5, 161.13, 161.18, 163.0, 183.4. Anal. calcd. for $C_{25}H_{18}FN_3O_4$: C, 67.72%; H, 4.09%; N, 9.48%. Found C, 67.71%; H, 4.08%, N, 9.47%. LCMS (EI, m/z): 443.9 $[M+H]^+$.

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

Off-white solid; Yield: 82%; MP: 222.4-223.1°C; IR (ν_{max}, cm^{-1}): 3129 (C-H, stretching, aromatic ring), 1626 (C=O), 1586 (C=N), 1480 (C=C), 1265 (C-O-C stretching, oxadiazole ring); ^{1}H NMR (400 MHZ, $CDCl_3$) 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); ^{13}C NMR (100 MHZ, $CDCl_3$) 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 $C_{24}H_{13}F_4N_3O_3$: 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 $[M+H]^+$.

(4-Fluorophenyl)(1-(5-(3,4-dimethylphenyl)-1,3,4-oxadiazol-2-yl)indolizin-3-yl)methanone (9e)

Off-white solid; Yield: 65%; MP: 211.0-212.6°C; IR (ν_{max},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); ^{1}H NMR (400 MHZ, $CDCl_3$) 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); ^{13}C NMR (100 MHZ, $CDCl_3$) δ (ppm): 19.7, 20.0, 99.7, 115.5, 115.7(2C), 119.5, 121.4, 122.9, 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 $C_{25}H_{18}FN_3O_2$: 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-fluoro-2-methylphenyl)-1,3,4-oxadiazol-2-yl)indolizin-3-yl)methanone (9f)

Brown solid; Yield: 59%; MP: 196.9-198.1°C; IR (ν_{max},cm^{-1}): 3100, 3044 (C-H, stretching, aromatic ring), 1478 (C=C), 1225 (C-O-C stretching, oxadiazole ring); ^{1}H NMR (400 MHZ, $CDCl_3$) δ (ppm): 10.03-10.01 (m, 1H, Ar-H), 8.63-8.60 (m, 1H, Ar-H), 8.04-8.00 (m, 1H, Ar-H), 7.94-7.91 (m, 2H, Ar-H), 7.87 (s, 1H, Ar-H), 7.59-7.55 (m, 1H), 7.29-7.25 (m, 2H, Ar-H), 7.23-7.19 (m, 1H, Ar-H), 7.13-7.07 (m, 2H, Ar-H), 2.81 (s, 3H, methyl); ^{13}C NMR (100 MHZ, $CDCl_3$) δ (ppm): 21.6, 99.2, 115.8, 117.7, 119.4, 123.1, 124.2, 124.9, 127.8, 129.2, 131.3, 133.3, 133.4, 134.1, 134.2, 135.9, 136.0, 137.6, 159.6, 160.8, 162.0, 163.6, 166.1, 183.8; Anal. calcd. for $C_{24}H_{15}F_2N_3O_2$: C, 69.39%; H, 3.64%; N, 10.12%. Found: C, 69.37%; H, 3.63%; N, 10.11%; LCMS (EI, m/z): 416.0$[M+H]^+$.

(4-Fluorophenyl)(1-(5-(5-fluoro-2-methylphenyl)-1,3,4-oxadiazol-2-yl)indolizin-3-yl)methanone (9g)

Off-white solid; Yield: 55%; MP: 211.2-212.9°C; IR (ν_{max},cm^{-1}): 3102, 3045 (C-H, stretching, aromatic ring), 1634 (C=O), 1581 (C=N), 1479 (C=C), 1232 (C-O-C stretching, oxadiazole ring); ^{1}H NMR (400 MHZ, $CDCl_3$) δ (ppm): 10.03-10.01 (m, 1H, Ar-CH), 8.63-8.60 (m, 1H, Ar-H), 7.95-7.91 (m, 2H, Ar-H), 7.58 (s, 1H, Ar-H), 7.74 (dd, *J* = 2.80, 9.40 Hz, 1H, Ar-H), 7.60-7.56 (m, 1H, Ar-H), 7.39-7.35 (m, 1H, Ar-H), 7.30-7.29 (m, 1H, Ar-H), 7.28-7.26 (m, 1H, Ar-H), 7.23-7.19 (m, 1H, Ar-H), 7.18-7.16 (m, 1H, Ar-H), 2.78 (s, 3H, methyl); ^{13}C NMR (100 MHZ, $CDCl_3$) δ (ppm): 21.6, 99.2, 115.8, 117.7, 119.4, 123.1, 124.2, 124.9, 127.8, 129.2, 131.3, 133.3, 133.4, 134.1, 134.2, 135.9, 136.0, 137.6, 159.6, 160.8, 162.0, 163.6, 166.1, 183.8; Anal. calcd. for $C_{24}H_{15}F_2N_3O_2$: C, 69.39%; H,

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-methylphenyl)-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 (ν_{max}, 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); ^{1}H NMR (400 MHZ, $CDCl_3$) δ (ppm): 10.04-10.01 (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); ^{13}C NMR (100 MHZ, $CDCl_3$) 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. $C_{24}H_{15}ClFN_3O_2$: C, 66.75%; 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-methylphenyl)-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 (ν_{max}, 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); ^{1}H NMR (400 MHZ, $CDCl_3$) 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); ^{13}C NMR (100 MHZ, $CDCl_3$) 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. Anal.cald. for: $C_{24}H_{15}BrFN_3O_2$: C, 60.52%; H, 3.17%; 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: 200.8 - 210.5˚C; IR (ν_{max},cm^{-1}): 3070 (C-H, starching, aromatic ring), 1487 (C=C), 1225 (C-O-C stretching, oxadiazole ring); ^{1}H NMR (400 MHZ, $CDCl_3$) δ (ppm): 10.01 (d, *J* = 1.20 Hz, 1H, Ar-H), 8.61 (d, *J* = 8.80 Hz, 1H, Ar-H), 7.97-7.93 (m, 3H, Ar-H), 7.92 (s, 1H, Ar-H), 7.90 (d, *J* = 12.40 Hz, 1H, Ar-H), 7.59-7.53 (m, 2H, Ar-H), 7.30-7.27 (m, 3H, Ar-H), 7.26-7.21 (m, 1H, Ar-H); ^{13}C NMR (100 MHZ, $CDCl_3$) δ (ppm): 99.2, 113.6, 113.9, 115.6, 115.8, 118.4, 118.6, 119.4, 122.5, 123.1, 124.9, 125.8, 127.8, 129.2, 130.8, 131.3, 136.0 , 137.6, 161.4, 162.0, 163.6, 166.1, 183.8; Anal. calcd. for $C_{23}H_{13}F_2N_3O_2$: C, 68.83; H, 3.26; N, 10.47; Found: C, 68.82%; H, 3.25%; N, 10.46%; LCMS (EI, m/z): 402.0[M+H]$^+$.

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

Off- solid; Yield: 66%; MP: 202.1-203.6˚C; IR (ν_{max},cm^{-1}): 3075 (C-H, stretching, aromatic ring), 1488 (C=C), 1229 (C-O-C stretching, oxadiazole ring); ^{1}H NMR (400 MHZ, $CDCl_3$) δ (ppm): 10.03-10.01 (m, 1H, Ar-H), 8.63-8.60 (m, 1H, Ar-H), 8.18-8.14 (m, 2H, Ar-H), 7.94-7.90 (m, 2H, Ar-H), 7.87 (s, 1H, Ar-H), 7.59-7.53 (m, 1H, Ar-H), 7.30-7.27 (m, 4H, Ar-H), 7.26-7.21 (m, 1H, Ar-H); ^{13}C NMR (100 MHZ, $CDCl_3$) δ (ppm): 99.2, 113.6, 113.9, 115.6, 115.8, 118.4, 118.6, 119.4, 122.5, 123.1, 124.9, 125.8, 127.8, 129.2, 130.8, 131.3, 136.0 , 137.6, 161.4, 162.0, 163.6, 166.1, 183.8. Anal. calcd. for $C_{23}H_{13}F_2N_3O_2$: C, 68.83%; H, 3.26%; N, 10.47%; Found: C, 68.82%; H, 3.25%; N, 10.46%; LCMS (EI, m/z): 401.9[M+H]$^+$.

(4-Fluorophenyl)(1-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)indolizin-3-yl)methanone (9l)

Off-white solid; Yield: 59%; MP: 208.5-209.8˚C; IR (ν_{max},cm^{-1}): 3137, 3073 (C-H, stretching, aromatic ring), 1626 (C=O), 1585 (C=N), 1481(C=C), 1229 (C-O-C stretching, oxadiazole ring); ^{1}H NMR (400 MHZ, $CDCl_3$) δ (ppm): 10.03-10.01 (m, 1H, Ar-H), 8.62-8.59 (m, 1H, Ar-H), 8.11-8.09 (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); ^{13}C NMR (100 MHZ, $CDCl_3$) δ (ppm): 99.6, 116.0, 119.5, 122.4, 122.9, 125.0, 127.0, 128.1, 128.9, 129.3(2C), 129.4, 131.5, 131.7, 137.7, 137.8(2C), 141.5, 137.6, 161.2, 162.3(2C), 183.4; Anal. calcd. for $C_{23}H_{13}ClFN_3O_2$: C, 66.12%; H, 3.14%; N, 10.06%. Found: C, 66.11%; H, 3.13%, N, 10.05%. LCMS (EI, m/z): 418.0 [M+H]$^+$.

(4-Fluorophenyl)(1-(5-(4-bromophenyl)-1,3,4-oxadiazol-2-yl)indolizin-3-yl)methanone (9m)

Brown solid; Yield: 67%; MP: 209.8-210.9˚C; IR (ν_{max}, 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); ^{1}H NMR (400 MHZ, $CDCl_3$) δ (ppm): 10.02-10.01 (m, 1H, Ar-H), 8.62-8.59 (m, 1H, Ar-H), 8.59-8.01 (m, 2H, Ar-H), 7.95-7.91 (m, 2H, Ar-H), 7.87 (s, 1H, Ar-H), 7.71-7.69 (m, 2H, Ar-H), 7.60-7.56 (m, 1H, Ar-H), 7.30-7.25 (m, 2H, Ar-H), 7.23-7.21(m, 1H, Ar-H); ^{13}C NMR (400 MHZ, $CDCl_3$) 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, 131.4, 132.3, 136.0 (2C), 137.6, 161.3, 162.3(2C), 163.6, 166.1, 183.7; Anal. calcd. for $C_{23}H_{13}BrFN_3O_2$: 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-Fluorophenyl)(1-(5-(3,4-difluorophenyl)-1,3,4-oxadiazol-2-yl)indolizin-3-yl)methanone (9n)

Brown solid; Yield: 62%; MP: 211.1-212.3˚C; IR (ν_{max},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); ^{1}H NMR (400 MHZ, $CDCl_3$) δ (ppm): 10.03-10.00 (m, 1H, Ar-H), 8.61-8.58 (m, 1H, Ar-H) 7.98-7.90 (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); ^{13}C NMR (100 MHZ, $CDCl_3$) 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.cald.for $C_{23}H_{12}F_3N_3O_2$: 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% CO_2 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% CO_2 incubator.

MTT assay

The monolayer cell culture was trypsinized and the cell count was adjusted to 5 x 10^5 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 100µl 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% CO_2 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% CO_2 atmosphere. The supernatant was 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% (IC_{50}) 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 10^8 cells/ml using 0.5 McFarland standards. The cell suspensions of all the cultures were adjusted to 1-2 x10^5 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.

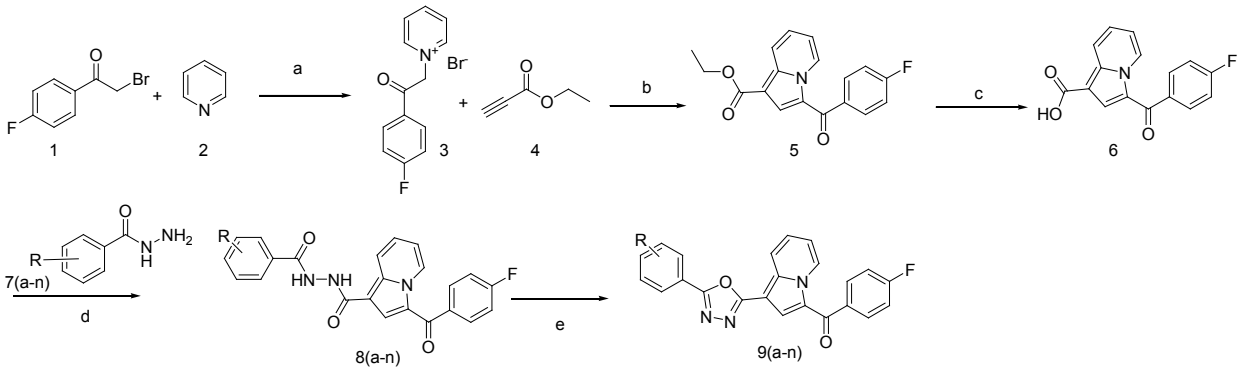
In-vitro Antifungal Activity Assay

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 to1x 10^8 cells/ml using a 0.5 McFarland standard. The cell suspensions of all the cultures were adjusted to 1-2x10^5cells/ml. The strain was inoculated of n Potato dextrose agar plate (90 mm). 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

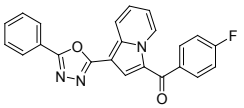
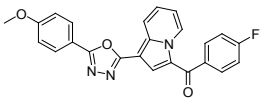
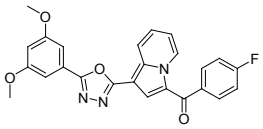
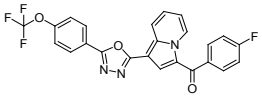
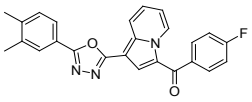
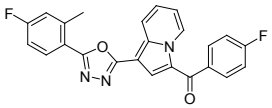
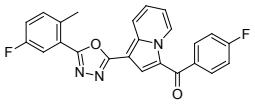
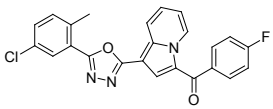
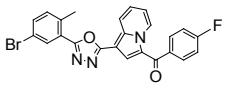
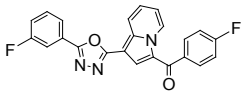
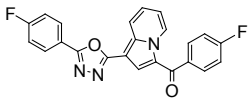
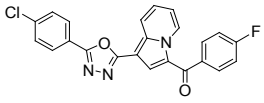
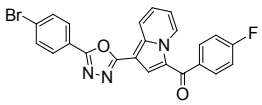
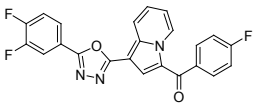
Chemistry

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.
Reagents and Conditions: a) Acetone, rt, 0.5 h; b) TEA, CH_2Cl_2, rt, 12h, 60%; c) Citric acid, THF/H_2O (4:1), Reflux, 48h; d) T_3P, TEA, CH_2Cl_2, rt, 12h; e) Triflic anhydride, Pyridine, CH_2Cl_2, rt, 2h.

Table 1: Structure of **9(a-n)** derivatives.

Compounds	*Structure*	*Isolated yield in %*
9a		71
9b		68
9c		69
9d		82
9e		65
9f		59
9g		55
9h		56
9i		58
9j		61
9k		66
9l		59
9m		67
9n		62

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/H_2O afforded acid **6,** which on reaction with substituted hydrazide **7(a-n)** in presence of T_3P, triethylamine in CH_2Cl_2 afforded N-Benzoyl-3-(4-flulorobenzoyl) 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 CH_2Cl_2.

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 IC_{50} value tabulated in Table 2. Among the synthesized compounds **9c** (IC_{50} = 26.48 µM), **9J** (IC_{50} = 21.57 µM), **9k** (IC_{50} = 28.85 µM) and exhibited potent cytotoxicity activity compared with standard drug doxorubicin (IC_{50}=25.71 µM) showed in Fig. 2(a), Fig. 2 (b) and Fig. 3 and **9n** (IC_{50} = 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

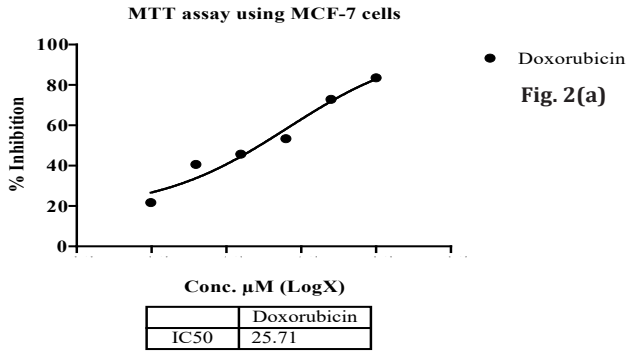


Fig. 2(a)

	Doxorubicin
IC50	25.71

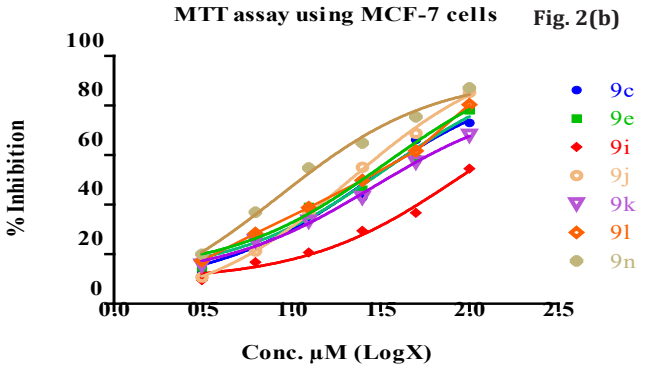


Fig. 2(b)

Fig. 2(a) and 2(b): Dose-response curve

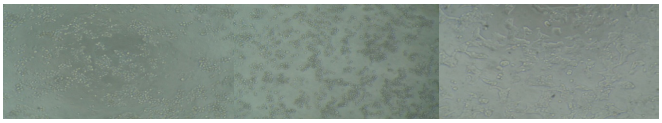
Table 2: *In-vitro* anticancer activity of **9(a-n)** derivatives.

	Conc. µM OD @ 590nm												
Comp. No	*3.125*	*6.25*	*12.5*	*25*	*50*	*100*	*% Inhibition*						IC_{50} *µM*
9a	0.856	0.841	0.724	0.674	0.634	0.589	1.75	8.41	15.42	21.26	25.93	31.19	-
9b	0.786	0.724	0.645	0.596	0.524	0.511	8.18	15.42	24.65	30.37	38.79	38.79	-
9c	0.745	0.625	0.556	0.489	0.289	0.231	12.97	26.99	35.05	42.87	66.24	73.01	26.48
9d	0.823	0.789	0.743	0.682	0.631	0.561	3.86	7.83	13.20	20.33	26.29	34.46	-
9e	0.730	0.620	0.521	0.462	0.356	0.187	14.72	27.57	39.14	46.03	58.41	78.15	34.01
9f	0.821	0.799	0.725	0.688	0.652	0.533	4.09	6.66	15.30	19.63	23.83	37.73	-
9g	0.801	0.763	0.641	0.556	0.512	0.487	6.43	10.86	25.12	35.05	40.17	43.11	-
9h	0.823	0.789	0.745	0.700	0.646	0.613	3.86	7.83	12.97	18.22	24.53	28.39	-
9i	0.774	0.712	0.679	0.604	0.542	0.389	9.58	16.82	20.68	29.44	36.68	54.56	86.97
9j	0.764	0.674	0.541	0.384	0.267	0.125	10.75	21.26	36.80	55.14	68.81	85.40	21.57
9k	0.723	0.638	0.571	0.489	0.367	0.274	15.54	25.47	33.29	42.87	57.13	67.99	28.85
9l	0.707	0.613	0.524	0.430	0.328	0.168	17.41	28.39	38.79	49.77	61.68	80.37	33.76
9m	0.779	0.724	0.654	0.632	0.523	0.487	9.00	15.42	23.60	26.17	38.90	43.11	-
9n	0.685	0.540	0.387	0.301	0.210	0.110	19.98	36.92	54.79	64.84	75.47	87.15	8.527
Doxorubicin	0.670	0.508	0.465	0.399	0.232	0.141	21.73	40.65	45.68	53.39	72.90	83.53	25.71

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

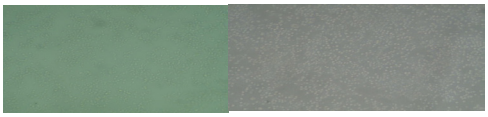
	Zone of inhibition(mm)		
	S. aureus	*E. coli*	*C. albicans*
Compounds	*Concentration per well(400µg)*		
9a	8	11	10
9b	7.5	9	11
9c	9	9	10
9d	9.5	-	7
9e	13	-	7
9f	13	8	9
9g	12	8.5	11
9h	16	9	11
9i	18	9	11
9J	8.5	9	10
9k	8.5	8.5	9
9l	9	9	9.5
9m	10	10	10
9n	11	12	11
Ciprofloxacin(2.0µg/well)	21.2 ± 0.44	21.2 ± 0.83	-
Itraconazole (20ug/well)	-	-	21 ± 1.22

summarized in Table 3 and the zone of inhibitions was also shown in Fig. 4. 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.



9c 9e 9i

9j 9k 9l



9n Doxorubicin

Fig. 3: Anti-proliferative images.

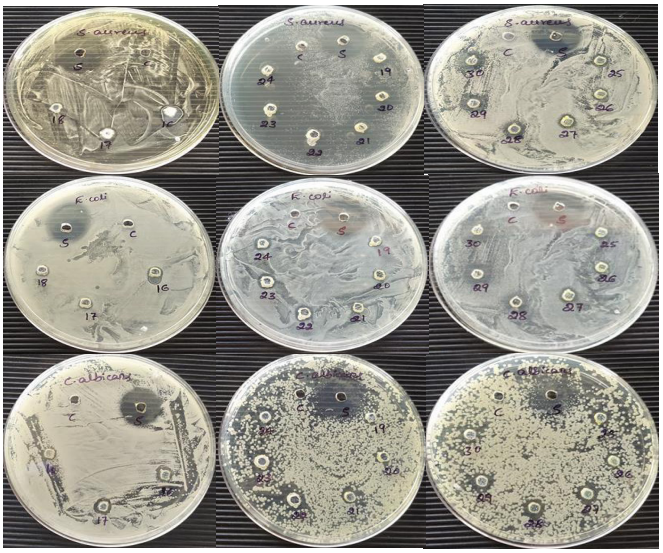


Fig. 4: *In-vitro* antimicrobial activity images of **9(a-n)** derivatives.

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.

Acknowledgment

The authors express sincere gratitude to the Department of Chemistry, VSK University, Ballari, for providing the laboratory facilities. The author Mahanthesha G is also highly thankful to the Department of Science and Technology, Govt. of India, for providing DST-FIST (SR/FST/CSI-274/2016) to conduct the research work.

References

1. Sadowski B, Klajn J, Gryko DT. Recent advances in the synthesis of indolizines and their π-expanded analogues. Org Biomol Chem. 2016; 14:7804 -7828.
2. Kuntz DA, Nakayama S, Shea K, Hori H, Uto Y, Nagasawa H, Rose DR. Structural investigation of the binding of 5-substituted swainsonine analogues to Golgi alpha-mannosidase II. Chem Biochem. 2010; 11:673-680.
3. Niu T, Huang L, Wu T, Zhang Y. FeCl3-promoted alkylation of indoles by enamides. Org Biomol Chem. 2011; 9: 273-277.
4. Coldham I, Jana S, Watson L, Martin NG. Cascade condensation, cyclization, intermolecular dipolar cycloaddition by multi-component coupling and application to a synthesis (±) crispine A. Orga Biomol Chem. 2009; 7:1674-1679.
5. Shrivastava SK, Srivastava P, Bandresh R, Tripathi PN, Tripathi A. Design, synthesis, and biological evaluation of some novel indolizine derivatives as dual cyclooxygenase and lipoxygenase inhibitor for anti-inflammatory activity Bioorg Med Chem. 2017; 25:4424-4432.
6. Arvin-Berod M, Desroches-Castan A, Bonte S, Brugière S, Couté Y, Guyon, L, Jean-Jacques F, Isabelle Baussanne B, Demeunynck M. Indolizine-Based Scaffolds as Efficient and Versatile Tools: Application to the Synthesis of Biotin-Tagged Anti-angiogenic Drugs. ACS Omega, 2017; 2:9221–9230.
7. Saraswati S, Kanaujia PK, Kumar S, Kumar R, Alhaider AA. Tylophorine, a Phenanthraindolizidine alkaloid isolated from Tylophora indica exerts anti-angiogenic and antitumor activity by targeting vascular endothelial growth factor receptor 2–mediated angiogenesis. Mol cancer. 2013; 12:1-16.
8. Boot A, Brito1 A, Wezel TV, Morreau H, Costa M, Proença F. Anticancer Activity of Novel pyrido[2,3-*b*]indolizine Derivatives: The Relevance of Phenolic Substituents. Anticancer Research. 2014; 34:1673-1678.
9. De Fatima Pereira M, Rochais C, Dallemagne P. Recent Advances in Phenanthroindolizidine and Phenanthroquinolizidine Derivatives with Anticancer Activities. Anti-Cancer Agents in Med Chem. 2015; 15:1080-1091.
10. Sandeep C, Venugopala KN, Gleiser RM, Chetram A, Padmashali B, Kulkarni RS, Venugopala R, Odhav B, Greener synthesis of indolizine analogue using water as a base and solvent: Study for larvicidal activity against Anopheles arabiensis. Chem Biology & Drug Design, 2016; 88:899-904.
11. Sandeep C, Venugopala KN, Khedr MA, Padmashali B, Kulkarni RS, Venugopala R, Odhav B. Design and Synthesis of Novel Indolizine Analogues as COX-2 Inhibitors: Computational Perspective and in vitro Screening. Ind J Pharma Education and Research. 2017; 51:452-460.
12. Gundersen LL, Charnock C, Negussie A.H, Rise F, Teklu S. Synthesis of indolizine derivatives with selective antibacterial activity against Mycobacterium tuberculosis. European J Pharm Sciences. 2007; 30:26-35.
13. Flitsch W. Pyrroles with Fused Six-membered Heterocyclic Rings: (i) a-Fused. Comprehensive Heterocyclic Chemistry. 1984; 443-495.
14. Flitsch W. Fused Five- and Six-membered Rings with Ring Junction Heteroatoms. Comprehensive Heterocyclic Chemistry II. 1996; 237-248.
15. Ghinea IO, Dinica RM. Breakthroughs in Indole and Indolizine Chemistry – New Synthetic Pathways, New Applications. Org Pharma Perspective. 2016; 115-142
16. Nagaraj, Chaluvaraju KC, Niranjan MS, kiran S. 1, 3, 4 oxadiazole: a potent drug candidate with various pharmacological activities. International J Pharmacy and Pharma Sciences. 2011; 3:9-16.
17. De Oliveira CS, Lira BF, Barbosa-Filho JM, Lorenzo JGF, de Athayde-Filho PF. Synthetic Approaches and Pharmacological Activity of 1,3,4-Oxadiazoles: A Review of the Literature from 2000-2012. Molecules.2012; 17:10192-10231.
18. Gurupadaswamy HD, Girish V, Kavitha CV, Raghvan SC, Khanum SA. Synthesis and evaluation of 2, 5-di(4-aryloylaryloxymethyl)-1,3,4-oxadiazoles as anti-cancer agents. European J Med Chem. 2013; 63:536-543.
19. Agarwal M, Singh V, Sharma SK, Sharma P, Ansari MY, Jadav SS, Ahsan MJ. Design and synthesis of new 2, 5-disubstituted-1, 3, 4-oxadiazole analogues as anti-cancer agents. Med Chem Research. 2016; 25:2289-2303
20. Kandemir H, Ma C, Kutty SK, Black DS, Griffith R, Lewis PJ, Kumar N. Synthesis and biological evaluation of 2,5-di(7-indolyl)-1,3,4-oxadiazoles, and 2- and 7-indolyl 2-(1,3,4-thiadiazolyl)ketones as antimicrobials. Bioorg Med Chem. 2014; 22:1672-1679.
21. Franski R. Biological activities of the compound Bearing 1,3,4-Oxa(thia)diazole ring: A Review. Asian J Chem. 2005; 17:2063-2075.
22. Anjali B, Sowmyasree K, Archana A, Shweta B, Saritha P, Bhagvan Raju M. Synthesis and evaluation of some novel heterocyclic compounds containing an oxadiazole moiety. GSC Bio Pharma Sciences. 2019; 06: 9-20.
23. Crouch SPM, Kozlowski R, Slater KJ, Fletcher J. The use of ATP bioluminescence as a measure of cell proliferation and cytotoxicity. Journal of Immunol Methods. 1993; 160: 81-88.
24. Gonzalez RJ, Tarloff JB. Evaluation of hepatic subcellular fractions for Alamar blue and MTT reductase activity. Toxicol in Vitro. 2001; 15:257-259.
25. Hussain RM, Nouri AME, Oliver RTD. A new approach for measurement of cytotoxicity using Colorimetric assay. J Immunol Methods. 1993; 160:89-96.
26. He Y, Zhu Q, Chen M, Huang Q, Wang W, Li Q, Huang Y, Di W. The changing 50% inhibitory concentration (IC_{50}) of cisplatin: a pilot study on the artifacts of the MTT Assay and the precise measurement of density-dependent chemoresistance in ovarian cancer. Oncotarget, 2016; 7: 70803-70821.
27. Bergeron MG, Brusch JL, Barza M, Weinstein L. Bactericidal Activity and Pharmacology of Cefazolin. Antimicrobial Agents and Chemotherapy. 1973; 4:396-401.