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Synthesis and biological screening of 4-(5-alkyl-2-isoxazolin-3-yl)-2-aryl-2,3-dihydro-1*H*-1,5-benzodiazepines

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ABSTRACT

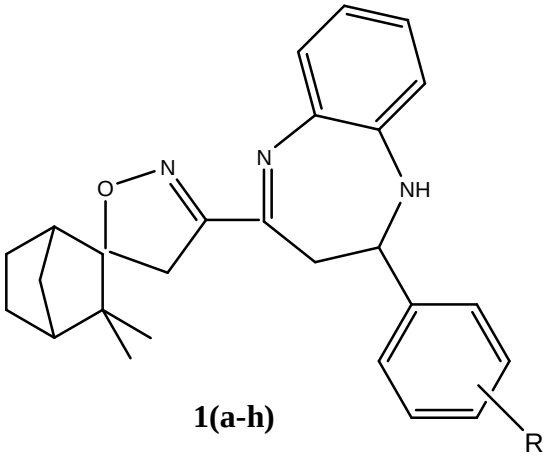
A Series of novel 4-(5-alkyl-2-isoxazolin-3-yl)-2-aryl-2,3-dihydro-1H-1,5-benzodiazepines were synthesized under solvent less conditions and screened for their antimicrobial and antifungal activity.

Keywords: 1,5-benzodiazepine, lipophilic, solid supported reagents, *o*-phenylenediamine.

INTRODUCTION

Isoxazoles and benzodiazepines are important scaffolds for the pharmaceutical industry. Isoxazoles have been used extensively in pharmaceutical as antibacterial [1], antifungal [2], immunosuppressant [3], antiviral [4], analgesic [5], antimicrobial [6], anticonvulsant [7]. Some derivatives of these are known to be antitumor [8] and hypoglycemic [9] in nature. Benzodiazepines are bicyclic heterocyclic compounds and have shown sufficient pharmacological and clinical activity including hypnotic, sedative, anticonvulsant, anti-inflammatory, muscle relaxing properties, amnesic properties [10], antiplatelet and antileukemic activity [11].

In our ongoing efforts towards the synthesis of heterocyclic systems [12] , hybrid molecules incorporating isoxazole and benzodiazepine moieties of general structure **1** were synthesized [12f] in our laboratory and screened for their antimicrobial activity. The dimethyl bicycloheptyl being lipophilic [13] in nature was expected to help in permeation through the lipid membrane by interacting with the hydrophobic interiors of the membrane. After screening these compounds **(1a-h)** for antibacterial (*Staphylococcus aureus* ATCC 29313, methicillin resistant *S. Aureus*, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) and antifungal (*Aspergillus fumigates* LSI-II, *Aspergillus niger* ATCC 16404) activities, it was found that none of the compounds exhibited any effect upto concentrations of 180 μg/ml.



1(a-h)

Figure 1.

R: a = H; b = 4-OCH_3; c = 3-NO_2; d = 4-CH_3; e = 4-Cl; f = 2-Cl; g = 3-Br; h = 3,4-$(OCH_3)_2$

In **1** the lipophilic portion derived from the camphene, is rigid. To extend this study further, replacement of the rigid lipophilic moiety (dimethyl bicycloheptyl) with a flexible long chain (C_8, C_{10}, C_{12}) moieties leads to structure **2**, which we wish to synthesise to study their antimicrobial activity.

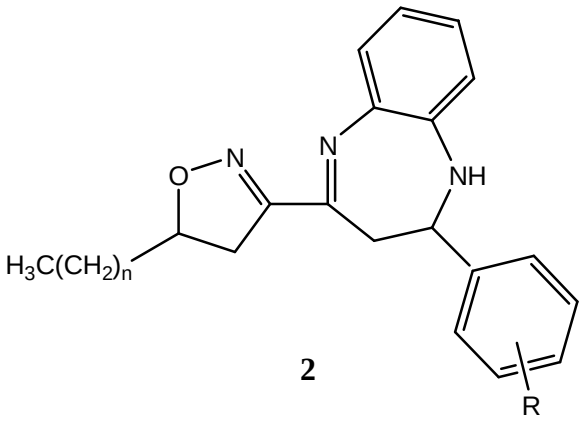


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Figure 2.

a : n = 5; b : n = 7; c : n = 9

R: a = H; b = 4-OCH_3; c = 3-NO_2; d = 4-CH_3; e = 4-Cl; f = 2-Cl; g = 3-Br; h = 3,4-$(OCH_3)_2$

EXPERIMENTAL SECTION

General Experimental. Melting points were measured in open capillaries on perfit melting points apparatus and are uncorrected. Infrared spectra on KBr were recorded on Brucker-4800 infrared spectrometer. NMR and EIMS spectra were recorded on Brucker AC-400(400 MHz and 100MHz and JEOL D-300 mass spectrometer, respectively. Elemental analysis was carried out by Heraeus CHNS rapid analyzer. ^{1}H and ^{13}C chemical shifts are reported in parts per million (ppm) from tetramethylsilane as internal standard. All experiments were performed in oven dried glass apparatus. SISCO silica was used as adsorbent for thin layer chromatography (TLC) (0.5 mm). The column chromatography was performed over silica gel (60-120 mesh) with graded solvent systems of ethylacetate-petroleum ether (60-80).

Procedure and Spectral data

Procedure for the Synthesis of isoxazole (4b): A mixture of 1-Decene (1.4g, 10.0 mmol) and iron(III) nitrate nonahydrate (4.04g, 10.0 mmol) in acetone (50 mL) was refluxed with stirring for 6h. The reaction mixture was filtered through celite under suction. The filtrate was concentrated *in vacuo*. The residue was dissolved in ethylacetate (100 mL) and washed with aqueous $NaHCO_3$ (1 × 20 mL), water (3 × 20 mL), and brine (1 × 20 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed to give **4b** as pale yellow oil.

3-Acetyl-5-octylisoxazoline-2 (4b): Pale yellow oil, yield 70%, **IR** (KBr) (υ_{max}, cm^{-1}): 1688 (C=O), 1577 (C=N). **^{1}H NMR** (400MHz, $CDCl_3$), δ_H 0.99 (3H, t, $^3J_{HH}$ = 6.7Hz, CH_3), 1.24-1.44 (6H, m, $3CH_2$), 1.60-1.74 (4H, m, $2CH_2$), 1.80-1.92 (4H, m, $2CH_2$), 2.60 (3H, s, $COCH_3$), 2.75 (1H, dd, $^2J_{HH}$ = 17.6Hz, $^3J_{HH}$ = 8.6Hz, CH_2, isoxazoline), 3.16 (1H, dd, J = 17.6Hz, $^3J_{HH}$ = 11.0 Hz, CH_2 isoxazoline), 4.72-4.82 (1H, m, OCH). **^{13}C NMR** (100 MHz, $CDCl_3$), δ_C 14.0, 22.5, 25.1, 26.6(4C, octyl), 27.5(1C, CH_3), 29.3, 29.7, 31.7 (3C, octyl), 35.2 (1C, isoxazoline), 36.7 (1C, octyl), 84.9, 158.3 (2C, isoxazoline), 193.4 (C=O). Anal Calcd. for $C_{13}H_{23}N_3$: C, 69.29; H, 10.29; N, 6.22%, Found: C, 69.44; H, 10.11; N, 6.05%. MS: m/z = 226$(M+H)^+$

Procedure for the Synthesis of 3-Cinnamoyl-5-octylisoxazoline-2 (6ba)

To an ethanolic solution (10 mL) of 3-acetyl-5-octylisoxazoline-2 **4** (0.22g, 1.0 mmol) and benzaldehyde (0.10g, 1.0 mmol), was added an aqueous solution (0.5 mL) of NaOH (0.04g, 1.0 mmol) with stirring at room temp, till the completion of reaction (TLC). On cooling, the reaction mixture solid product was obtained , which was filtered, washed with cold water and recrystallized from hot ethanol.

3-Cinnamoyl-5-octylisoxazoline-2 (6ba): Amorphous off-white solid , yield 72%, mp 45-47 °C, **IR** (KBr) (υ_{max}, cm^{-1}) : 2957, 2852, 1660, 1631, 1605. **^{1}H NMR** (400 MHz, $CDCl_3$), δ_H 0.98 (3H, t, $^3J_{HH}$ = 6.7Hz, CH_3), 1.30-1.37 (6H, m, $3CH_2$), 1.56-1.70 (4H, m, $2CH_2$), 1.77-1.90 (4H, m, $2CH_2$), 2.85 (1H, dd, $^2J_{HH}$ = 17.6Hz, $^3J_{HH}$ = 8.4Hz, CH_2 isoxazoline), 3.20 (1H, dd, $^2J_{HH}$ = 17.6Hz, $^3J_{HH}$ = 11.0Hz, CH_2 isoxazoline), 4.76-4.84 (1H, m, OCH), 7.30-7.42 ($2H_{aro}$, 1H COCH, m, 3CH), 7.54-7.62 ($3H_{aro}$, m, 3CH), 7.73 (1H, d, $^3J_{HH}$ = 16Hz, CHC_6H_5). **^{13}C NMR** (100 MHz, $CDCl_3$), δ_C 14.0, 22.5, 25.1, 26.6, 29.1, 29.4, 32.1(7C, octyl), 35.2(1C, isoxazoline), 36.7(1C, octyl), 84.9(1C, isoxazoline), 126.5, 126.7, 128.0, 128.1, 128.2(5C, ArCH), 129.0 (1C, COCH=CH), 136.7(1C, ArCH), 143.0(1C, COCH=CH), 158.3(1C, isoxazoline), 193.4(C=O, Carbonyl) Anal Calcd. for $C_{20}H_{27}NO_2$: C, 76.64; H, 8.68; N, 4.47% Found: C, 76.50; H, 8.51; N, 4.61%. MS: m/s = 314$(M+H)^+$

Procedure for the Synthesis of 4-(5-octyl-2-isoxazolin-3-yl)-2-phenyl-2,3-dihydro-1*H*-1,5-Benzodiazepine (2ba): A mixture of 3-cinnamoyl-5-octylisoxazoline-2 **6** (0.31g, 1.0 mmol) and *o*-phenylene diammine (0.01g, 1.0mmol) in a round bottom flask, was added of $KHSO_4/SiO_2$ (0.10g, 20.0 mol%) and heated on an oil bath at 100°C for 1h or till the completion of reaction (TLC). On completion of reaction , the reaction mixture was taken in ethylacetate (20 mL) and filtered off, residue was given washing with ethylacetate (2 × 5 mL). The combined organic layers were washed with water (2× 10 mL), saturated aqueous NaCl (1× 10 mL) and dried over Na_2SO_4 and concentrated *in vacuo* to give the crude product , which was purified by column chromatography, using mixtures of ethylacetate and petroleum ether as eluents.

4-(5-Octyl-2-isoxazolin-3-yl)-2-phenyl-2,3-dihydro-1*H*-1,5-benzodiazepine (2ba): viscous oil yield 70% **IR**(KBr) (υ_{max}, cm^{-1}): 2960, 2872, 1607, 1569, 1482. **^{1}H NMR** (400MHz, $CDCl_3$), δ_H 0.99(3H, t, $^3J_{HH}$ = 6.7Hz, CH_3), 1.24-1.44(6H, m, $3CH_2$), 1.60-1.74(4H, m, $2CH_2$), 1.80-1.92(4H, m, $2CH_2$), 2.79-2.86(4H, m, $2CH_2$), 4.2-4.6(2H, m, OCH, NH), 5.4(1H, m, CH), 6.58-6.72($1H_{aro}$, d, $^3J_{HH}$ = 7.7Hz, CH), 6.83-7.0($4H_{aro}$, m, 4CH), 7.20-7.33($4H_{aro}$, m, 4CH). **^{13}C NMR** (100MHz, $CDCl_3$), δ_C 14.0, 22.5, 25.1, 26.6, 29.3, 29.7, 31.7(7C, octyl), 32.1(1C,Benzodiazepine) 35.2(1C, isoxazoline), 36.7(1C, octyl), 68.9(1C, Benzodiazepine), 84.9(1C, isoxazoline),120.8, 120.9, 126.2, 127.2, 127.8, 128.2, 128.7, 131.2, 137.5, 139.2(10C, ArCH), 141.1, 144.5, 160.5(3C, Benzodiazepine), 163.7(1C, isoxazoline). Anal calcd for $C_{26}H_{33}N_3O$: C, 71.38; H, 8.24; N, 10.41%, Found: C, 71.19; H, 8.41; N, 10.18%. MS: m/z = 404$(M+H)^+$

3-(*p*-Methoxycinnamoyl)-5-octylisoxazoline-2 (6bb): Yellow oil; yield 75% ; **IR**(KBr) (υ_{max}, cm^{-1}): 2957, 2840, 1655, 1632, 1605. **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 0.98 (3H, t, $^3J_{HH}$ = 6.7Hz, CH_3), 1.30-1.37 (6H, m, $3CH_2$), 1.56-1.70 (4H, m, $2CH_2$), 1.77-1.90 (4H, m, $2CH_2$), 2.83 (1H, dd, $^2J_{HH}$ = 17.6Hz, $^3J_{HH}$ = 8.4Hz, CH_2 isoxazoline), 3.1 (1H, dd, $^2J_{HH}$ = 17.6Hz, $^3J_{HH}$ =11.0Hz, CH_2 isoxazoline), 3.8 (3H, s, OCH_3), 4.76-4.84 (1H, m, OCH), 6.93 (1H, d, $^3J_{HH}$ = 8.6Hz, COCH), 7.46-7.64 ($4H_{aro}$, m, 4CH), 7.81 (1H, d, $^3J_{HH}$ = 16Hz, CHC_6H_5). **^{13}C NMR** (100MHz, $CDCl_3$), δ_C 14.1, 22.8, 26.2, 26.9, 29.3, 29.7, 31.1(7C, octyl), 35.2(1C, isoxazoline), 37.7(1C, octyl), 83.2(1C, isoxazoline), 120.9, 123.1, 129.6, 129.7, 130.1(5c, ArCH), 132.4(1C, COCH=CH), 151.(1C, ArCH), 159.2(1C, COCH=CH), 160.3(1C, isoxazoline), 191.2(C=O, Carbonyl) Anal Calcd. for $C_{21}H_{29}NO_3$: C, 73.44; H, 8.51; N, 4.08%.Found: C, 73.20; H, 8.79; N, 4.29%. MS: m/s = 344$(M+H)^+$

4-(5-Octyl-2-isoxazolin-3-yl)-2-(*p*-methoxyphenyl)-2,3-dihydro-1*H*-1,5-benzodiazepine (2bb): Yellow oil yield 76% **IR**(KBr) (υ_{max}, cm^{-1}): 2960, 2873, 1607, 1478. **^{1}H NMR**(400 MHz, $CDCl_3$), δ_H 0.99(3H, t, $^3J_{HH}$ = 6.7Hz, CH_3), 1.24-1.44(6H, m, $3CH_2$), 1.60-1.74(4H, m, $2CH_2$), 1.80-1.92(4H, m, $2CH_2$), 2.80-2.89(4H, m, $2CH_2$), 3.91(3H, s, OCH_3), 4.2-4.6(2H, m, OCH, NH), 5.4(1H, m, CH), 6.53-6.68($1H_{aro}$, d, $^3J_{HH}$ = 7.3Hz, CH), 6.78-7.26($7H_{aro}$, m, 7CH),). **^{13}C NMR** (100MHz, $CDCl_3$), δ_C 14.0, 22.5, 25.1, 26.6, 29.3, 29.7, 31.1(7C, octyl), 32.1(1C,Benzodiazepine), 35.2(1C, isoxazoline), 36.7(1C, octyl), 60.7(1C, OCH_3), 65.3(1C, Benzodiazepine), 83.2(1C, isoxazoline), 120.8, 120.9, 126.2, 127.8, 129.7, 130.7, 137.5, 138.1(8C,ArCH), 140.1(1C, Benzodiazepine), 141.0(1C, ArCH), 144.7(1C, Benzodiazepine), 152.0(1C, ArCH), 158.1(1C, Benzodiazepine), 161.0(1C, isoxazoline), Anal calcd for $C_{27}H_{35}N_3O_2$: C, 74.89 ; H, 8.41; N, 9.69%, Found: C, 74.69; H, 8.20; N, 9.45%. MS: m/z = 434$(M+H)^+$

3-(*m*-Nitrocinnamoyl)-5-octylisoxazoline-2 (6bc): Yellow solid, yield 76%, mp 57-59 °C, **IR**(KBr) (υ_{max}, cm^{-1}): 2949, 2851, 1660, 1630, 1605; **^{1}H NMR**(400 MHz, $CDCl_3$) δ_H 0.98(3H, t, $^3J_{HH}$ = 6.7Hz, CH_3), 1.30-1.36(6H, m, $3CH_2$), 1.57-1.70(4H, m, $2CH_2$), 1.77-1.90(4H, m, $2CH_2$), 2.83(1H, dd, $^2J_{HH}$ = 17.6Hz, $^3J_{HH}$ = 8.4Hz, CH_2 isoxazoline), 3.1(1H, dd, $^2J_{HH}$ = 17.6Hz, $^3J_{HH}$ = 11.0Hz, CH_2 isoxazoline), 4.76-4.84(1H, m, OCH), 7.54-7.83($2H_{aro}$, 1H COCH, m, 3CH), 7.89-7.92($2H_{aro}$, m, 2CH), 8.22(1H, d, $^3J_{HH}$ = 16Hz, CHC_6H_5). **^{13}C NMR** (100 MHz, $CDCl_3$), δ_C 14.1, 22.5, 25.1, 26.6, 29.2, 29.9, 31.5(7C, octyl), 35.9(1C, isoxazoline), 37.8(1C, octyl), 82.1(1C, isoxazoline), 120.3, 121.3, 121.5, 129.5, 130.1(5C, ArCH), 132.4(1C, COCH=CH), 145.5(1C, ArCH), 150.5(1C, COCH=CH), 159.2(1C, isoxazoline), 189.9(C=O, Carbonyl). Anal Calcd for $C_{20}H_{26}N_2O_4$: C, 67.02; H, 7.31; N, 7.82%. Found: C, 67.29; H, 7.09; N, 7.69%. MS: m/z = 359$(M+H)^+$

4-(5-Octyl-2-isoxazolin-3-yl)-2-(*m*-nitrophenyl)-2,3-dihydro-1*H*-1,5-benzodiazepine (2bc): Yellow oil yield: 75% **IR**(KBr) (υ_{max}, cm^{-1}): 2962, 2881, 1605, 1570, 1345. **^{1}H NMR** (400MHz, $CDCl_3$), δ_H 0.99(3H, t, $^3J_{HH}$ = 6.7Hz, CH_3), 1.24-1.44(6H, m, $3CH_2$), 1.60-1.74(4H, m, $2CH_2$), 1.80-1.92(4H, m, $2CH_2$), 2.80-2.89(4H, m, $2CH_2$), 4.25-4.63(2H, m, OCH, NH), 5.4(1H, m, CH), 6.83-6.85($1H_{aro}$, d, $^3J_{HH}$ = 6.2Hz, CH), 7.0($1H_{aro}$, d, $^3J_{HH}$ = 1.3Hz, CH), 7.03($1H_{aro}$, d, $^3J_{HH}$ = 8.1Hz, CH), 7.23-7.25($1H_{aro}$, m, CH), 7.4($1H_{aro}$, m, CH), 7.67($1H_{aro}$, m, CH), 8.0($1H_{aro}$, m, CH), 8.4($1H_{aro}$, s, CH). **^{13}C NMR** (100MHz, $CDCl_3$), δ_C 14.0, 22.5, 25.1, 26.6, 29.3, 29.7, 31.1(7C, octyl), 32.3(1C,Benzodiazepine) 35.2(1C, isoxazoline), 37.7(1C, octyl), 68.2(1C, Benzodiazepine), 82.0(1C, isoxazoline), 120.5, 120.8, 121.0 121.6, 126.7, 129.3, 129.7, 132.5, 138.1(9C, ArCH), 142.3(1C, Benzodiazepine), 146.0(1C, ArCH), 150.2, 152.9(2C, Benzodiazepine), 159.8(1C, isoxazoline). Anal calcd for $C_{26}H_{32}N_4O_3$: C, 69.62; H, 7.19; N, 12.49%, Found: C, 69.32; H, 7.37; N, 12.25%. MS: m/z = 449$(M+H)^+$

3-(*p*-Methylcinnamoyl)-5-octylisoxazoline-2 (6bd): Creamish yellow solid, yield: 78%, mp 78-80 °C, **IR**(KBr)(υ_{max}, cm^{-1}): 2953, 2918, 2851, 1660, 1631, 1605; **^{1}H NMR**(400 MHz, $CDCl_3$), δ_H 0.98(3H, t, $^3J_{HH}$ = 6.7Hz, CH_3), 1.30-1.37(6H, m, $3CH_2$), 1.56-1.70(4H, m, $2CH_2$), 1.77-1.90(4H, m, $2CH_2$), 2.3(3H, s, CH_3), 2.85(1H, dd, $^2J_{HH}$ = 17.6Hz, $^3J_{HH}$ = 8.4Hz, CH_2 isoxazoline), 3.2(1H, dd, $^2J_{HH}$ = 17.6Hz, $^3J_{HH}$ = 11.0Hz, CH_2 isoxazoline), 4.76-4.84(1H, m, OCH), 7.17-7.25($1H_{aro}$, 1H COCH, m, 2CH), 7.50-7.57($3H_{aro}$, m, 3CH), 7.74-7.82(1H, d, $^3J_{HH}$ = 15.9Hz, CHC_6H_5). **^{13}C NMR** (100 MHz, $CDCl_3$), δ_C 14.1, 22.5, 24.2(3C, octyl), 25.8(1C, CH_3), 26.5, 29.4, 29.7, 30.1(4C, octyl), 33.9(1C, isoxazoline), 37.7(1C, octyl), 85.0(1C, isoxazoline), 120.9, 126.7, 126.8, 129.1, 130.1(5C, ArCH), 132.4(1C, COCH=CH), 141.9(1C, ArCH), 151.7(1C, COCH=CH), 159.3(1C, isoxazoline), 192.9(C=O, Carbonyl). Anal Calcd for $C_{21}H_{29}NO_2$: C,77.02; H, 8.93; N, 4.28%. Found: C, 77.22; H, 8.64; N, 4.09%. MS: m/z = 328$(M+H)^+$

4-(5-Octyl-2-isoxazolin-3-yl)-2-(*p*-methylphenyl)-2,3-dihydro-1*H*-1,5-benzodiazepine (2bd): Pale yellow oil yield: 74% **IR**(KBr)(υ_{max}, cm^{-1}): 2962, 2878, 1617,1570. **^{1}H NMR**(400 MHz, $CDCl_3$), δ_H 0.99(3H, t, $^3J_{HH}$ = 6.7Hz, CH_3), 1.24-1.44(6H, m, $3CH_2$), 1.60-1.74(4H, m, $2CH_2$), 1.80-1.92(4H, m, $2CH_2$), 2.20(3H, s, CH_3), 2.81-2.89(4H, m, $2CH_2$), 4.2-4.6(2H, m, OCH, NH), 5.4(1H, m, CH), 6.75-6.76($1H_{aro}$, d, $^3J_{HH}$ = 7.1Hz, CH), 6.80-7.26($7H_{aro}$, m, 7CH),). **^{13}C NMR** (100MHz, $CDCl_3$), δ_C 14.1, 22.8, 25.1(3C, octyl), 25.8(1C, CH_3), 26.6, 29.3, 29.7, 31.1(4C,octyl), 32.1(1C,Benzodiazepine), 35.2(1C, isoxazoline), 36.7(1C, octyl), 66.1 (1C, Benzodiazepine), 85.0(1C, isoxazoline),120.8, 120.9, 126.2, 126.7, 127.8, 128.9, 129.7, 130.2, 132.8, 139.2(10C, ArCH), 142.3, 149.1, 160.6(3C, Benzodiazepine), 161.2(1C, isoxazoline). Anal calcd for $C_{27}H_{35}N_3O$: C, 77.66; H, 8.45; N, 10.06%, Found: C, 77.46; H, 8.14; N, 10.24%. MS: m/z = 418$(M+H)^+$

3-(*p*-Chlorocinnamoyl)-5-octylisoxazoline-2 (6be): Yellow solid, yield 70%, mp 62-65 °C, **IR**(KBr) (υ_{max}, cm^{-1}): 2958, 2919, 2846, 1659, 1631, 1605. **^{1}H NMR** (400MHz, $CDCl_3$), δ_H 0.98(3H, t, $^3J_{HH}$ = 6.7Hz, CH_3), 1.30-1.37(6H, m, $3CH_2$), 1.56-1.70(4H, m, $2CH_2$), 1.77-1.90(4H, m, $2CH_2$), 2.85(1H, dd, $^2J_{HH}$ = 17.6Hz, $^3J_{HH}$ = 8.4Hz, CH_2 isoxazoline), 3.2(1H, dd, $^2J_{HH}$ = 17.6Hz, $^3J_{HH}$ = 11.0Hz, CH_2 isoxazoline), 4.76-4.84(1H, m, OCH), 7.34-7.38($1H_{aro}$, 1H COCH, m, 2CH), 7.50-7.68($3H_{aro}$, m, 3CH), 7.71-7.79(1H, d, $^3J_{HH}$ = 15.9Hz, CHC_6H_5). **^{13}C NMR** (100 MHz, $CDCl_3$), δ_C 14.1, 22.8, 25.3, 26.6, 29.2, 29.8, 31.7(7C, octyl), 35.2(1C, isoxazoline), 36.7(1C, octyl), 84.9(1C, isoxazoline), 122.0, 129.5, 130.3, 130.5, 132.4, (5C, ArCH), 132.7(1C, COCH=CH), 145.7(1C, ArCH), 153.3(1C, COCH=CH), 160.4(1C, isoxazoline), 186.0(C=O, Carbonyl). Anal Calcd for $C_{20}H_{26}ClNO_2$: C, 69.05; H, 7.53; N, 4.03%, Found: C, 69.29; H, 7.37; N, 4.26%. MS: m/z = 348(100), 350(33) $(M+H)^+$

4-(5-Octyl-2-isoxazolin-3-yl)-2-(*p*-chlorophenyl)-2,3-dihydro-1*H*-1,5-benzodiazepine (2be): Viscous oil yield: 75% **IR**(KBr)(υ_{max}, cm^{-1}): 2960, 2931, 2878, 1610, 1599. **^{1}H NMR**(400 MHz, $CDCl_3$), δ_H 0.99(3H, t, $^3J_{HH}$ = 6.7Hz, CH_3), 1.24-1.44(6H, m, $3CH_2$), 1.60-1.74(4H, m, $2CH_2$), 1.80-1.92(4H, m, $2CH_2$), 2.83-2.88(4H, m, $2CH_2$), 4.2-4.6(2H, m, OCH, NH), 5.39-5.42(1H, m, CH), 6.73-6.75($1H_{aro}$, d, $^3J_{HH}$ = 7.1Hz, CH), 6.7-7.7($7H_{aro}$, m, 7CH). **^{13}C NMR** (100 MHz, $CDCl_3$), δ_C 14.1, 22.8, 25.3, 26.6, 29.3, 29.7, 31.7(7C,octyl), 33.0(1C, Benzodiazepine), 35.3(1C, isoxazoline), 36.7(1C, octyl), 68.9(1C, Benzodiazepine), 85.0(1C, isoxazoline), 120.8, 120.9, 124.1, 126.3, 128.8, 128.9, 129.7, 130.2, 134.9, 137.6(10C, ArCH), 141.4, 145.1, 160.5(3C, Benzodiazepine), 162.6(1C, isoxazoline). Anal calcd for $C_{26}H_{32}ClN_3O$: C,71.29; H, 7.34; N, 9.59%, Found: C, 71.09; H, 7.04; N, 9.69%. MS: m/z = 438 (100), 440 (33) $(M+H)^+$.

3-(*o*-Chlorocinnamoyl)-5-octylisoxazoline-2 (6bf): Yellow solid, yield 70% mp 59-60 °C **IR**(KBr) (υ_{max}, cm^{-1}): 2937, 2919, 2845, 1659, 1631, 1605. **^{1}H NMR**(400MHz, $CDCl_3$): δ_H 0.98(3H, t, $^3J_{HH}$ = 6.7Hz, CH_3), 1.30-1.37(6H, m, $3CH_2$), 1.56-1.70(4H, m, $2CH_2$), 1.77-1.90(4H, m, $2CH_2$), 2.85(1H, dd, $^2J_{HH}$ = 17.6Hz, $^3J_{HH}$ = 8.4Hz, CH_2 isoxazoline), 3.2(1H, dd, $^2J_{HH}$ = 17.6Hz, $^3J_{HH}$ = 11.0Hz, CH_2 isoxazoline), 4.76-4.84(1H, m, OCH), 7.34-7.38($1H_{aro}$, 1H COCH, m, 2CH), 7.50-7.68($3H_{aro}$, m, 3CH), 7.70-7.77(1H, d, $^3J_{HH}$ = 15.9Hz, CHC_6H_5). **^{13}C NMR** (100 MHz, $CDCl_3$), δ_C 14.1, 22.8, 25.3, 26.7, 29.2, 29.7, 31.7(7C, octyl), 35.2(1C, isoxazoline), 36.7(1C, octyl), 85.0(1C, isoxazoline), 122.0, 129.5, 130.3, 130.4, 132.4, (5C, ArCH), 132.7(1C, COCH=CH), 146.7(1C, ArCH), 160.3(1C, COCH=CH), 160.4(1C, isoxazoline), 186.0(C=O, Carbonyl). Anal Calcd for $C_{20}H_{26}ClNO_2$: C, 69.05; H, 7.53; N, 4.03%. Found: C, 69.27 ; H, 7.74; N, 4.21%. MS: m/z = 348 (100), 350 (33) $(M+H)^+$

4-(5-Octyl-2-isoxazolin-3-yl)-2-(*o*-chlorophenyl)-2,3-dihyro-1*H*-1,5-benzodiazepine (2bf): viscous oil yield: 73% **IR**(KBr) (υ_{max}, cm^{-1}): 2933, 2877, 1605, 1569. **^{1}H NMR**(400MHz, $CDCl_3$), δ_H 0.99(3H, t, $^3J_{HH}$ = 6.7Hz, CH_3), 1.24-1.44(6H, m, $3CH_2$), 1.60-1.74(4H, m, $2CH_2$), 1.80-1.92(4H, m, $2CH_2$), 2.83-2.88(4H, m, $2CH_2$), 4.2-4.6(2H, m, OCH, NH), 5.39-5.42(1H, m, CH), 6.75-6.75($1H_{aro}$, d, $^3J_{HH}$ = 7.1Hz, CH), 6.8-7.7($7H_{aro}$, m, 7CH),). **^{13}C NMR** (100MHz, $CDCl_3$), δ_C 14.1, 22.8, 25.3, 26.6, 29.3, 29.7, 31.7(7C,octyl), 32.9(1C,Benzodiazepine), 35.3(1C, isoxazoline), 36.7(1C, octyl), 68.8(1C, Benzodiazepine), 84.9(1C, isoxazoline), 120.8, 120.9, 124.1, 126.3, 127.8, 128.2, 129.7, 130.2, 134.9, 139.9(10C, ArCH), 140.0, 145.0, 159.0(3C, Benzodiazepine), 161.0(1C, isoxazoline). Anal calcd for $C_{26}H_{32}ClN_3O$: C, 71.30; H, 7.36; N, 9.59%, Found: C, 71.18; H, 7.20; N, 9.79%. MS: m/z = 438(100), 440(33) $(M+H)^+$.

3-(*m*-Bromocinnamoyl)-5-octylisoxazoline-2 (6bg): Viscous oil yield 72% **IR**(KBr) ($\upsilon_{max,}$ cm^1) : 2957, 2852, 1660, 1631, 1605. **^{1}H NMR**(400MHz, $CDCl_3$): δ_H 0.98(3H, t, $^3J_{HH}$ = 6.7Hz, CH_3), 1.30-1.37(6H, m, $3CH_2$), 1.56-1.70(4H, m, $2CH_2$), 1.77-1.90(4H, m, $2CH_2$), 2.85(1H, dd, $^2J_{HH}$ = 17.6Hz, $^3J_{HH}$ = 8.4Hz, CH_2 isoxazoline), 3.2(1H, dd, $^2J_{HH}$ = 17.6Hz, $^3J_{HH}$ = 11.0Hz, CH_2 isoxazoline), 4.76-4.84(1H, m, OCH), 7.22-7.30($1H_{aro}$, 1H COCH, m, 2CH), 7.47-7.65($3H_{aro}$, m, 3CH), 7.78-7.80(1H, d, $^3J_{HH}$ = 15.9Hz, CHC_6H_5). **^{13}C NMR** (100 MHz, $CDCl_3$), δ_C 14.1, 22.5, 25.9, 26.9, 29.2, 29.9, 31.9(7C, octyl), 35.9(1C, isoxazoline), 36.9(1C, octyl), 85.9(1C, isoxazoline), 120.9, 129.6, 130.1, 130.2, 132.3, (5C, ArCH), 132.8(1C, COCH=CH), 142.7(1C, ArCH), 156.6(1C, COCH=CH), 160.4(1C, isoxazoline), 190.3 (C=O, Carbonyl). Anal Calcd for $C_{20}H_{26}BrNO_2$: C, 61.23; H, 6.68; N, 3.57%, Found: C, 61.47; H, 6.41; N, 3.20%. MS: m/z = 392 (100), 394 (90) $(M+H)^+$

4-(5-Octyl-2-isoxazolin-3-yl)-2-(*m*-bromophenyl)-2,3-dihydro-1*H*-1,5-benzodiazepine (2bg): Viscous oil yield: 69% **IR**(KBr) (υ_{max}, cm^{-1}): 2955, 2880, 1605, 1480. **^{1}H NMR**(400MHz, $CDCl_3$), δ_H 0.99(3H, t, $^3J_{HH}$ = 6.7Hz, CH_3), 1.23-1.46(6H, m, $3CH_2$), 1.60-1.75(4H, m, $2CH_2$), 1.80-1.92(4H, m, $2CH_2$), 2.78-2.86(4H, m, $2CH_2$), 4.2-4.6(2H, m, OCH, NH), 5.44 (1H, m,

CH), 6.80-6.82(1H_{aro}, d, $^3J_{HH}$ = 1.6Hz, CH), 6.97-7.26(5H_{aro}, m, 5CH), 7.31-7.52(2H_{aro}, m, CH). **^{13}C NMR** (100MHz, $CDCl_3$), δ_C 14.1, 22.8, 25.9, 26.9, 29.2, 29.9, 31.7(7C,octyl), 32.0(1C,Benzodiazepine), 35.3(1C, isoxazoline), 36.7(1C, octyl), 68.8(1C, Benzodiazepine), 84.9(1C, isoxazoline), 120.8, 121.8, 123.1, 125.1, 128.1, 129.1, 130.0, 130.7, 131.2, 138.9(10C, ArCH), 141.9, 145.0, 158.2(3C, Benzodiazepine), 160.8(1C, isoxazoline). Anal calcd for $C_{26}H_{32}BrN_3O$: C, 64.73; H, 6.69; N, 8.71%, Found: C: 64.42; H, 7.10; N, 8.55%. MS; m/z = 482 (100), 484 (90) $(M+H)^+$

3-(*m,p*-Dimethoxycinnamoyl)-5-octylisoxazoline-2 (6bh): Yellow oil yield 60% **IR**(KBr) (υ_{max}, cm^{-1}): 2951, 2850, 1660, 1631, 1605 **^{1}H NMR**(400MHz, $CDCl_3$): δ_H 0.98(3H, t, $^3J_{HH}$ = 6.7Hz, CH_3), 1.30-1.37(6H, m, 3CH_2), 1.56-1.70(4H, m, 2CH_2), 1.77-1.90(4H, m, 2CH_2), 2.85(1H, dd, $^2J_{HH}$ = 17.6Hz, $^3J_{HH}$ = 8.4Hz, CH_2 isoxazoline), 3.2(1H, dd, $^2J_{HH}$ = 17.6Hz, $^3J_{HH}$ = 11.0Hz, CH_2 isoxazoline), 3.83(3H, s, OCH_3), 3.90(3H, s, OCH_3), 4.76-4.84(1H, m, OCH), 6.42-6.52(1H_{aro}, 1H COCH, m, 2CH), 7.52-7.61(2H_{aro}, m, 2CH), 8.01-8.08(1H, d, $^3J_{HH}$ = 15.9Hz, CHC_6H_5). **^{13}C NMR** (100 MHz, $CDCl_3$), δ_C 14.1, 22.7, 25.1, 26.6, 29.0, 29.7, 31.7(7C, octyl), 35.2(1C, isoxazoline), 36.9(1C, octyl), 59.3, 59.5(2C, OCH_3), 85.0(1C, isoxazoline), 107.3, 109.1, 120.1, 120.5, 129.0(5C, ArCH), 130.9(1C,COCH=CH), 143.1(1C, ArCH), 144.9(1C,COCH=CH), 155.9(1C, isoxazoline), 185.2(C=O, Carbonyl). Anal Calcd for $C_{22}H_{31}NO_4$: C, 70.75; H, 8.37; N,3.75%. Found: C, 70.23; H, 8.50; N, 3.53%. MS: m/z = 374$(M+H)^+$

4-(5-Octyl-2-isoxazolin-3-yl)-2-(*m,p*-dimethoxyphenyl)-2,3-dihydro-1*H*-1,5-benzodiazepine (2bh): Oil yield 70% **IR**(KBr) (υ_{max}, cm^{-1}): 2931, 2878, 1607, 1569 **^{1}H NMR**(400MHz, $CDCl_3$), δ_H 0.99(3H, t, $^3J_{HH}$ = 6.7Hz, CH_3), 1.23-1.46(6H, m, 3CH_2), 1.60-1.75(4H, m, 2CH_2), 1.80-1.92(4H, m, 2CH_2), 2.83-2.88(4H, m, 2CH_2), 3.88(3H, s, OCH_3), 3.90(3H, s, OCH_3) 4.2-4.6(2H, m, OCH, NH), 5.46 (1H, m, CH), 6.42-6.54(4H_{aro}, m, 4CH), 7.54-7.61(3H_{aro}, m, 3CH). **^{13}C NMR** (100MHz, $CDCl_3$), δ_C 14.1, 22.8, 25.9, 26.6, 29.0, 29.9, 31.7(7C, octyl), 32.0(1C,Benzodiazepine), 35.3(1C, isoxazoline), 36.7(1C, octyl), 59.3, 59.5(2C, OCH_3), 68.8(1C, Benzodiazepine), 84.9(1C, isoxazoline), 120.7, 120.9, 123.5, 125.2, 128.0, 129.0, , 130.0, 138.9 (8C, ArCH), 142.9, 144.9 (2C, Benzodiazepine), 146.2, 148.1(2C, ArCH), 157.0(1C, Benzodiazepine), 159.5(1C, isoxazoline). Anal calcd for $C_{28}H_{37}N_3O_3$: C, 72.54; H, 8.04; N, 9.06%, Found: C 72.74; H, 8.25; N, 9.31%. MS: m/z = 464$(M+H)^+$

The compounds 4a , 4c, 6aa, 6ca, 2aa, 2ca were prepared as per the procedure adopted for 4b, 6ba, 2ba. All other compounds enlisted in the table **1** gave satisfactory spectral data.

General Procedure and Antimicrobial Activity Screening

Antibacterial and antifungal activities of benzodiazepine analogues were performed using microdilution method[17,18,19] against two gram positive strains (*Staphylococcus aureus* ATCC 29313, Methicillin resistant *S. Aureus*), two gram negative strains (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853), two yeast strains (*C. albicans* ATCC 22019, *C. albicans* V-01-27853), and two filamentous fungi(*Aspergillus fumigates* LSI-II, *Aspergillus niger* ATCC 16404). Antibacterial testing was performed in Muller Hinton Broth (Becton Dickenson, Cockeysville, MD) Where as for antifungal testing RPMI 1640 with L-glutamine (Sigma-Aldrich, St. Louis, MO) buffered to pH 7.0 supplemented with 0.165 M 3-(N-morpholino) propanesulfonic acid (Sigma-Aldrich) was used. The stock solution of the compounds was prepared in dimethyl sulphoxide. The minimum inhibitory concentration (MIC) of the compounds was determined by serial 2-fold diluting the solution in the 100 μL volume of aforementioned media in a 96-well U bottom microtitre plate. The final concentrations of compounds ranged from 128 to 0.25 μg/mL. Amphotetricin B and ciprofloxacin (16-0.03 μg/mL) (both from Sigma-Aldrich) were used as

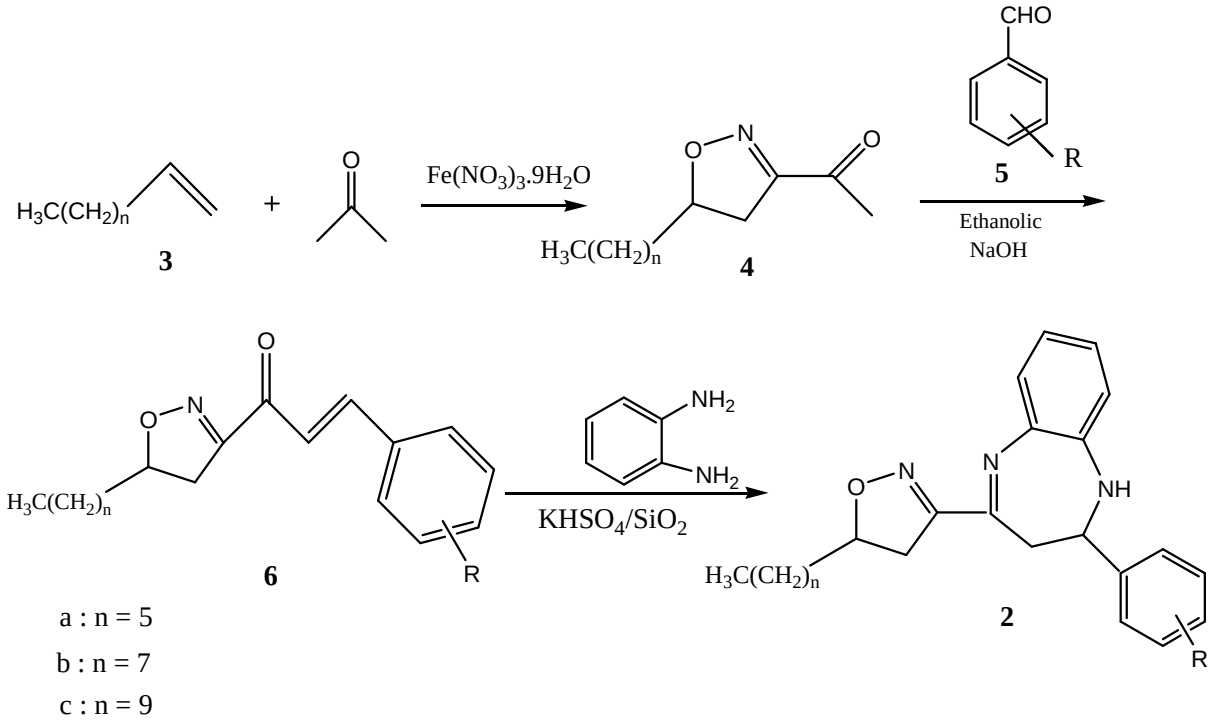
standard antifungal and antibacterial agents, respectively. The bacterial and fungal suspension of the overnight grown bacterial and fungal was prepared in sterile normal saline, and the density was adjusted to 0.5 Mcfarland. The bacterial cultures were further diluted and added in 100 μL volume at final inoculums of 1×10^3 CFU/mL was used. The plates were incubated at 37°C for 24hr for bacterial cultures and 48h for fungal cultures. The plates were read visually, and the minimum concentration of the compound showing no turbidity was recorded as MIC.

RESULT AND DISCUSSION

The target compound **2** was synthesised following the sequence of reactions as given below:

(i) Condensation [14] of alkenes (octene, decene, dodecene) and acetone in presence of $Fe(NO_3)_3$ to yield the respective 3-Acetyl-5-alkylisoxazolines-2 **4**
(ii)
(iii)**4** upon Claisen-Schmidt condensation [15] with araldehydes gives 3-Cinnamoyl-5-alkylisoxazoline-2 **6**
(iv)
(v) **6** upon condensation [16] with *o*-phenylenediammine in presence of $KHSO_4/SiO_2$ generates the target molecule **2**. The structure of the synthesised compounds were assigned by spectral means (*vide experimental*).

Scheme 1. Preparation of 4-(5-octyl-2-isoxazolin-3-yl)-2-aryl-2,3-dihydro-1*H*-1,5-benzodiazepines.



R: a =H; b = 4-OCH_3; c = 3-NO_2; d = 4-CH_3; e = 4-Cl; f = 2-Cl; g = 3-Br; h = 3,4-$(OCH_3)_2$

Table 1. Synthesis of 4-(5-alkyl-2-isoxazolin-3-yl)-2-aryl-2,3-dihydro-1*H*-1,5-benzodiazepines.

S. No	Compound No.	n	R
1	2aa	5	H
2	2ba	7	H
3	2ca	9	H
4	2ab	5	4-OCH_3
5	2bb	7	4-OCH_3
6	2cb	9	4-OCH_3
7	2ac	5	3-NO_2
8	2bc	7	3-NO_2
9	2cc	9	3-NO_2
10	2ad	5	4-CH_3
11	2bd	7	4-CH_3
12	2cd	9	4-CH_3
13	2ae	5	4-Cl
14	2be	7	4-Cl
15	2ce	9	4-Cl
16	2af	5	2-Cl
17	2bf	7	2-Cl
18	2cf	9	2-Cl
19	2ag	5	3-Br
20	2bg	7	3-Br
21	2cg	9	3-Br
22	2ah	5	3,4-$(OCH_3)_2$
23	2bh	7	3,4-$(OCH_3)_2$
24	2ch	9	3,4-$(OCH_3)_2$

CONCLUSION

In summary, we have demonstrated a novel, mild, efficient method for the synthesis of 4-(5-alkyl-2-isoxazolin-3-yl)-2-aryl-2,3-dihydro-1*H*-1,5-benzodiazepines.This method has several advantages such as solvent less condition, simple work-up, easy handling.

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