Synthesis and Biological Screening of 4-(3,3-Dimethylspiro{bicyclo[2.2.1]heptan-2,5′-isoxazoline-2}-3′-yl)-2-aryl-2,3-dihydro-1*H*-1,5-benzodiazepines

Tahira Naqvi,[a] Deepali Mahajan,[a] Rattan Lal Sharma,[a] Inshad Ali Khan,[b] and Kamal K. Kapoor[a]*

[a]Department of Chemistry, University of Jammu, Jammu 180006, India
[b]Department of Biotechnology, Indian Institute of Integrative Medicine (CSIR), Jammu 180001, India
*E-mail: k2kapoor@yahoo.com

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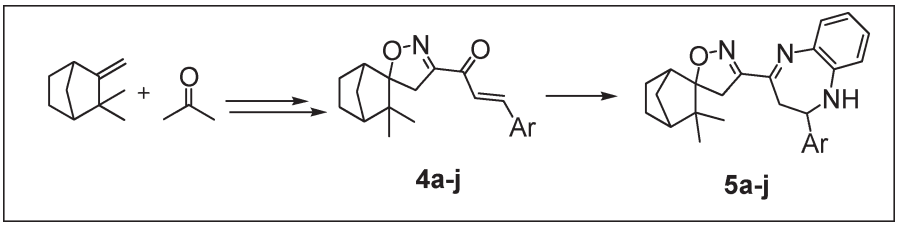

A series of novel 4-(3,3-dimethylspiro{bicyclo[2.2.1]heptan-2,5′-isoxazoline-2}-3′-yl)-2-phenyl-2,3-dihydro-1*H*-1,5-benzodiazepines were synthesized. These molecules were screened *in vitro* for their antifungal and antibacterial activity, and none of the tested compounds showed promising antimicrobial or antifungal activity.

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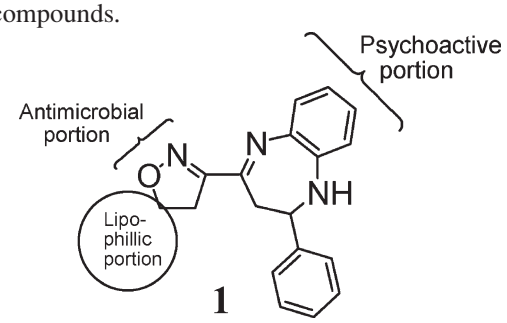
INTRODUCTION

Synthesis of hybrid molecules with more than one bioactive moiety as part of their structure is an area of intense research interest these days [1], because these molecules can be used to act against single target or they can be made to perform in a way, so that these pharmacophores act collectively on two different targets, or they can enhance each others activity.

Isoxazoles are an important class of heterocyclic compounds of pharmacological importance and so are the benzodiazepines. Isoxazoles possess a plethora of biological activities, for instance, bactericidal, antifungal [2], antileprous, anti-inflammatory, and antiviral [3]. They are also known to act as immunosuppressant [4]. Benzodiazepines exhibit wide spectrum of pharmacological activity, including hypnotic, sedative, anxiolytic, anticonvulsant, muscle relaxant, and amnesic properties, which are mediated by slowing down the central nervous system [5]. Benzodiazepines are well known for their strong muscular relaxing properties and can be useful in the treatment of muscular spasms for example tetanus or spastic disorders [6], and much recently, they are found to have antiplatelet and antileukemic activity [7]. Studies [8] have shown that lipophilicity of a molecule is a very important parameter in terms of its biological activity, because molecules having lipophilic substituent permeate easily through the lipid membrane by interacting with the hydrophobic interiors of the membrane.

In view of the pharmaceutical activities associated with isoxazoles and diazepines, we wished to synthesize a hybrid molecule of type **1** incorporating these moieties as its part structures, with a dimethyl bicycloheptyl as a lipophillic portion hooked on probably to ease the entry into the cells, and to increase the lipid solubility of the title compounds.



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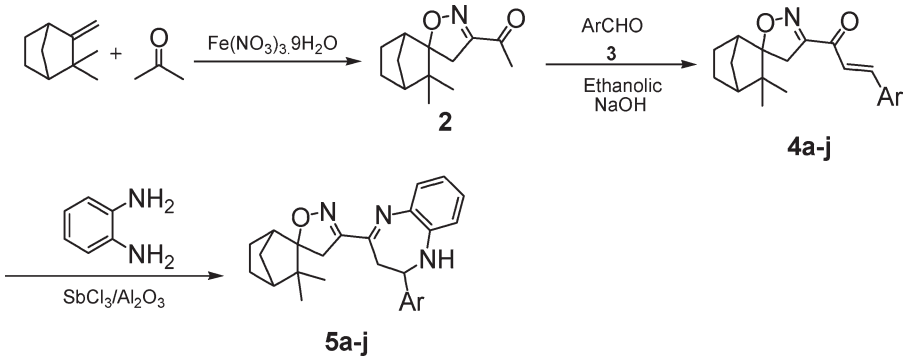
RESULTS AND DISCUSSION

3′-Aceto-3,3-dimethylspiro{bicyclo[2.2.1]heptan-2,5′-isoxazoline-2} (**2**), obtained [9] from the reaction of camphene and acetone, upon Claisen-Schmidt condensation [10] with aromatic aldehyde **(3a)** generated 3′-cinnamoyl-3,3-dimethylspiro{bicyclo{2.2.1]heptan-2,5′-isoxazoline-2} (**4a**). **4a** was further condensed [11] with *o*-phenyl enediamine to yield target compound 4-(3,3-dimethylspiro {bicyclo[2.2.1]heptan-2,5′-isoxazoline-2}-3′-yl)-2-phenyl-2, 3-dihydro-1H-1,5-benzodiazepine (**5a**). Its formation was supported by the disappearance of a resonance at

Scheme 1. Preparation of 4-(3,3-dimethylspiro{bicyclo[2.2.1]heptan-2,5′-isoxazoline-2}-3′-yl)-2-aryl-2,3-dihydro-1H-1,5-benzodiazepines. Aryl: a = C_6H_5; b = 4-$OCH_3C_6H_4$; c = 3-$NO_2C_6H_4$; d = 4-CH_3 C_6H_4; e = 4-Cl C_6H_4; f = 2-Cl C_6H_4; g = 3-Br C_6H_4; h = 3, 4-$(OCH_3)_2$ C_6H_3; i = 3, 4-OCH_2O—C_6H_3; j = α-$C_{10}H_9$.



δ 8.5 (d, J = 16 Hz), characteristic for **4a** in the ^{1}H NMR spectrum of **5a**, coupled with the appearance of signals at δ 3.0–3.2 (4H, CH_2 at C-3 of the benzodiazepine ring and CH_2 at C-4 of the isoxazoline ring), a broad singlet at δ 3.9 (for NH) and a doublet at δ 5.1 (1H, CH at C-2 of benzodiazepine ring). Following the similar protocol, a range of compounds (**5a-j**) was synthesized using various aldehydes (**3**) (Scheme 1).

The synthesized compounds [3′-cinnamoyl-3,3-dimethylspiro{bicyclo{2.2.1]heptan-2,5′-isoxazoline-2} **(4)** and 4-(3,3-dimethylspiro{bicyclo[2.2.1]heptan-2,5′-isoxazoline-2}-3′-yl)-2-aryl-2,3-dihydro-1H-1,5-benzodiazepines **(5)** were screened for their antibacterial and antifungal activities but did not show any effect upto concentrations of 180 μg/mL.

EXPERIMENTAL

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/HRMS spectra were recorded on Brucker AC-400(400 MHz and 100 MHz) 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 thick plates), and TLC plates were eluted with 1:9 ratio of ethyl acetate and *n*-hexane. The column chromatography was performed over silica gel (60–120 mesh) with graded solvent systems of ethyl acetate-petroleum ether (60–80).

General Procedure for the synthesis of isoxazole 2. A mixture of camphene (1.36 g, 10.0 mmol) and iron (III) nitrate nonahydrate (4.04 g, 10.0 mmol) in acetone (50 ml) was refluxed with stirring for 6 h. The reaction mixture was filtered through celite under suction. The filtrate was concentrated *in vacuo*, the residue was dissolved in ethyl acetate (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 (pet. ether-ethyl acetate) to give **2** as colorless oil.

General Procedure for the synthesis of chalcones 4a-j. To an ethanolic solution (10 mL) of 3′-aceto-3,3-dimethylspiro{bicyclo[2.2.1]heptan-2,5′-isoxazoline-2} **2** (1.0 mmol) and aromatic aldehyde (1.0 mmol) was added an aqueous solution (0.5 mL) of NaOH (0.04 g, 1.0 mmol) with stirring at room temperature, 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.

General Procedure for the synthesis of 1,5 benzodiazepines 5a-j. A mixture of 3′-cinnamoyl-3,3-dimethylspiro{bicyclo{2.2.1]heptan-2,5′-isoxazoline-2} **4** (1.0 mmol) and *o*-phenylene diamine (0.10 g, 1.0 mmol) in a round bottom flask, was added of $SbCl_3/Al_2O_3$ (0.12g, 10.0 mol %) and heated on an oil bath at 100°C for 1 h or till the completion of reaction (TLC). On completion of reaction, the reaction mixture was taken in ethyl acetate (20 mL) and filtered off, residue was given washings with ethyl acetate (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 ethyl acetate and petroleum ether as eluents.

3′-Aceto-3,3-dimethylspiro{bicyclo[2.2.1]heptan-2,5′-isoxazoline-2} (2). This compound was isolated as colorless oil; yield 69%; IR (KBr) υ (cm^{-1}): 1720, 1400; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.01 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.29–1.37 (4H, m, CH, CH_2), 1.55–1.60 (4H, *m*, CH_2), 2.40 (3H, *s*, CH_3), 2.90 (2H, *s*, CH_2); ^{13}C NMR (50 MHz, $CDCl_3$): δ 18.5, 21.9, 22.9, 23.2, 26.1, 31.5, 32.5, 39.4, 46.5, 47.4; Anal. Calcd. for $C_{13}H_{19}NO_2$: C, 70.56; H, 8.65; N, 6.33. Found: C, 70.84; H, 8.77; N, 6.14. MS: m/z = 221(M^+); R_f = 0.73.

3′-Cinnamoyl-3,3-dimethylspiro{bicyclo{2.2.1]heptan-2,5′-isoxazoline-2} (4a). This compound was isolated as amorphous off-white solid; mp 142–144°C; yield 74%; IR (KBr) υ (cm^{-1}): 2932, 1655, 1592, 1566, 1508; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.01 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.29–1.37 (4H, m, CH, CH_2), 1.55 (4H, m, CH_2), 3.10 (2H, s, CH_2), 7.37–7.44 (3H, m, CH), 7.56–7.66 (3H, m, CH), 7.77–7.85 (1H, d, J = 16 Hz, CH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 18.5,

21.9, 22.9, 26.2, 31.2, 39.5, 46.5, 106.7, 119.3, 126.5, 126.8, 132.4, 136.7, 141.7, 156.6, 181.8; Anal. Calcd for $C_{20}H_{23}NO_2$: C, 77.64; H, 7.49; N, 4.53. Found: C, 77.50; H, 7.63; N, 4.69. MS: $m/z = 309(M^+)$; $R_f = 0.68$.

3′-(*p*-Methoxycinnamoyl)-3,3-dimethylspiro{bicyclo[2.2.1] heptan-2,5′-isoxazoline-2} (4b). This compound was isolated as amorphous yellow solid; mp 134–136°C; yield 79%; IR (KBr) υ (cm^{-1}): 2920, 1675, 1590, 1566, 1518; ^1H NMR (200 MHz, $CDCl_3$): δ 1.01 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.29–1.37 (4H, m, CH, CH_2), 1.55 (4H, m, CH_2), 3.1 (2H, s, CH_2), 3.9 (3H, s, OCH_3), 6.95 (1H, d, $J = 8.7$ Hz, CH), 7.48–7.66 (4H, m, CH), 7.79–7.87 (1H, d, $J = 16$ Hz, CH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.6, 23.8, 24.7, 25.6, 25.9, 34.1, 35.4, 48.8, 49.1, 102.4, 120.9, 129.6, 130.1, 132.4, 151.7, 160.4, 170.3, 174.1; Anal. Calcd. for $C_{21}H_{25}NO_3$: C, 74.31; H, 7.42; N, 4.13. Found: C, 74.05; H, 7.65; N, 4.33. MS: $m/z = 339(M^+)$; $R_f = 0.63$.

3′-(m-Nitrocinnamoyl)-3,3-dimethylspiro{bicyclo[2.2.1] heptan-2,5′-isoxazoline-2} (4c). This compound was isolated as off white solid; mp 150–152°C; yield 80%; IR (KBr) υ (cm^{-1}): 2902, 1665, 1572, 1566, 1500, 1335; ^1H NMR (200 MHz, $CDCl_3$): δ 1.01 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.29–1.37 (4H, m, CH, CH_2), 1.55 (4H, m, CH_2), 3.1 (2H, s, CH_2), 7.56–7.85 (3H, m, ArCH, CH), 7.90–7.94 (2H, m, ArCH), 8.23–8.27 (1H, d, $J = 16$ Hz, CH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.8, 22.6, 24.2, 26.6, 25.8, 34.8, 35.3, 48.0, 49.3, 102.3, 120.3, 129.5, 130.1, 132.4, 141.7, 144.4, 159.2, 184.4; Anal. Calcd. for $C_{20}H_{22}N_2O_4$: C, 67.78; H, 6.26; N, 7.90. Found: C, 67.91; H, 6.48; N, 7.72. MS: $m/z = 354(M^+)$. $R_f = 0.58$.

3′-(p-Methylcinnamoyl)-3,3-dimethylspiro{bicyclo{2.2.1] heptan-2,5′-isoxazoline-2} (4d). This compound was isolated as creamish yellow solid; mp 118–120°C; yield 80%; IR (KBr) υcm^{-1}: 2932, 1655, 1592, 1566, 1508; ^1H NMR (200 MHz, $CDCl_3$): δ 1.01 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.29–1.37 (4H, m, CH, CH_2), 1.55 (4H, m, CH_2), 2.38 (3H, s, CH_3), 3.1 (2H, s, CH_2), 7.19–7.27 (2H, m, CH), 7.51–7.59 (3H, m, CH), 7.75–7.83 (1H, d, $J = 15.9$ Hz, CH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.9, 22.7, 24.5, 25.5, 25.9, 34.2, 35.4, 48.8, 49.1, 102.4, 120.9, 129.1, 130.1, 132.4, 141.7, 144.4, 159.3, 184.4; Anal. Calcd. for $C_{21}H_{25}NO_2$: C, 77.98; H, 7.79; N, 4.33. Found: C, 77.80; H, 7.93; N, 4.58. MS: $m/z = 323(M^+)$; $R_f = 0.61$.

3′-(p-Chlorocinnamoyl)-3,3-dimethylspiro{bicyclo{2.2.1] heptan-2,5′-isoxazoline-2} (4e). This compound was isolated as yellow solid; mp 159–160°C; yield 69%; IR (KBr) υ (cm^{-1}): 2922, 1645, 1592, 1566, 1548; ^1H NMR (200 MHz, $CDCl_3$): δ 1.01 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.29–1.37 (4H, m, CH, CH_2), 1.55 (4H, m, CH_2), 3.1 (2H, s, CH_2), 7.36–7.40 (2H, m, CH), 7.52–7.60 (3H, m, CH), 7.71–7.79 (1H, d, $J = 16$ Hz, CH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 22.9, 23.8, 24.5, 25.5, 25.8, 34.1, 35.4, 48.8, 49.1, 102.4, 120.9, 129.1, 130.1, 132.3, 151.7, 154.4, 160.3, 174.6; Anal. Calcd. for $C_{20}H_{22}ClNO_2$: C, 69.86; H, 6.45; N, 4.07. Found: C, 69.99; H, 6.66; N, 4.24. MS: $m/z = 343(M^+)$; $R_f = 0.61$.

3′-(o-Chlorocinnamoyl)-3,3-dimethylspiro{bicyclo{2.2.1] heptan-2,5′-isoxazoline-2} (4f). This compound was isolated as viscous oil; yield 65%; IR (KBr) υ (cm^{-1}): 2949, 1657, 1592, 1566, 1508; ^1H NMR (200 MHz, $CDCl_3$): δ 1.01 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.29–1.37 (4H, m, CH, CH_2), 1.55 (4H, m, CH_2), 3.1 (2H, s, CH_2), 7.36–7.40 (2H, m, CH), 7.52–7.60 (3H, m, CH), 7.71–7.79 (1H, d, $J = 16$ Hz, CH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.9, 21.8, 23.5, 24.5, 25.0, 34.2, 36.4, 48.8, 49.1, 102.4, 120.9, 129.5, 130.1, 133.4, 152.7, 161.4, 160.3, 166.1; Anal. Calcd. for $C_{20}H_{22}ClNO_2$: C, 69.86; H, 6.45; N, 4.07. Found: C, 70.01; H, 6.30; N, 4.25; MS: $m/z = 343(M^+)$; $R_f = 0.62$.

3′-(m-Bromocinnamoyl)-3,3-dimethylspiro{bicyclo{2.2.1] heptan-2,5′-isoxazoline-2} (4g). This compound was isolated as cream colored solid; mp 138–140°C; yield 76%; IR (KBr) υ (cm^{-1}): 2899, 1645, 1592, 1566, 1510; ^1H NMR (200 MHz, $CDCl_3$): δ 1.01 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.29–1.37 (4H, m, CH, CH_2), 1.55 (4H, m, CH_2), 2.03–2.15 (2H, m,CH), 3.10 (2H, s, CH_2), 7.23–7.31 (2H, m, CH), 7.48–7.66 (4H, m, CH), 7.79–7.87 (1H, d, $J = 15.9$ Hz, CH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 22.6, 23.3, 24.7, 26.5, 25.9, 34.1, 35.4, 48.8, 49.1, 102.4, 120.9, 129.6, 131.0, 132.4, 151.7, 160 .5, 171.3, 175.1; Anal. Calcd. for $C_{20}H_{22}BrNO_2$: C, 61.86; H, 5.71; N, 3.61. Found: C, 61.99; H, 5.90; N, 3.79; MS: $m/z = 388(M^+)$; $R_f = 0.63$.

3′-(3,4-Dimethoxycinnamoyl)-3,3-dimethylspiro{bicyclo {2.2.1]heptan-2,5′isoxazoline-2} (4h). This compound was isolated as white solid; mp 119–121°C; yield 60%; IR (KBr) υ (cm^{-1}): 2982, 1645, 1599, 1576; ^1H NMR (200 MHz, $CDCl_3$): δ 1.01 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.29–1.37 (3H, m, CH, CH_2), 1.55 (4H, m, CH_2), 2.03–2.15 (2H, m), 3.10 (2H, s, CH_2), 3.85 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 6.44–6.54 (2H, m, CH), 7.54–7.62 (2H, m, CH), 8.02–8.10 (1H, d, $J = 16$ Hz, CH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 20.6, 22.8, 23.7, 25.6, 25.9, 34.1, 35.4, 48.8, 49.1, 101.4, 119.9, 128.6, 130.1, 132.4, 151.7, 160.4, 169.3, 172.1; Anal. Calcd. for $C_{22}H_{27}NO_4$: C, 71.52; H, 7.37; N, 3.79. Found: C, 71.70; H, 7.59; N, 3.95; MS: $m/z = 369(M^+)$; $R_f = 0.62$.

3′-(3-(1,3-Benzodioxolyl-5)propen-2-oyl)-3,3-dimethylspiro {bicyclo{2.2.1]heptan-2,5′isoxazoline-2} (4i). This compound was isolated as off white solid; mp 146–148°C; yield 68%; IR (KBr) υ (cm^{-1}): 2980, 1655, 1562, 1556, 1518; ^1H NMR (200 MHz, $CDCl_3$): δ 1.01 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.29–1.37 (4H, m, CH, CH_2), 1.55 (4H, m, CH_2), 3.10 (2H, s, CH_2), 6.02 (2H, s, CH), 6.81–6.84 (1H, d, $J = 8.1$ Hz, CH), 7.09–7.13 (2H, m, CH), 7.38–7.46 (1H, d, $J = 15.9$ Hz, CH), 7.68–7.76 (1H, d, $J = 16$ Hz, CH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 22.9, 24.5, 25.5, 25.9, 34.2, 35.4, 44.1, 48.8, 49.1, 102.1, 107.3, 109.1, 120.1, 126.0, 144.1; Anal. Calcd. for $C_{21}H_{23}NO_4$: C, 71.37; H, 6.56; N, 3.96. Found: C, 71.55; H, 6.73; N, 3.79; MS: $m/z = 353(M^+)$; $R_f = 0.58$.

3′-(3-(α-Naphthyl)-prop-2-enoyl)-3,3-dimethylspiro {bicyclo {2.2.1]heptan-2,5′isoxazoline-2} (4j). This compound was isolated as pale yellow oil; yield 72%; IR (KBr) υ (cm^{-1}): 2992, 1645, 1582, 1566, 1508; ^1H NMR (200 MHz, $CDCl_3$): δ 1.01 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.29–1.37 (4H, m, CH, CH_2), 1.55 (4H, m, CH_2), 3.20 (2H, s, CH_2), 7.40–7.74 (4H, m, CH), 7.85–7.92 (4H, m, CH), 8.63–8.71 (1H, d, $J = 15.8$ Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.1, 22.9, 25.9, 27.7, 34.2, 35.5, 48.8, 49.1, 102.6, 123.8, 124.3, 125.9, 126.7, 127.4, 129.0, 131.6, 132.2, 134.2, 141.0, 159.2, 184.3; Anal. Calcd. for $C_{24}H_{25}NO_2$: C, 80.19; H, 7.01; N, 3.90. Found: C, 80.37; H, 7.19; N, 4.03; MS: $m/z = 359(M^+)$; $R_f = 0.56$.

4-(3,3-Dimethylspiro{bicyclo[2.2.1]heptan-2,5′-isoxazoline-2} -3′-yl)-2-phenyl-2,3-dihydro-1H-1,5-benzodiazepine (5a). This compound was isolated as pale yellow solid; mp 120–122°C; yield 80%; IR (KBr) υ (cm^{-1}): 3375, 2931, 2878, 1607, 1569, 1482; ^1H NMR (200 MHz, $CDCl_3$) δ 1.01 (3H, s, CH_3), 1.10

(3H, s, CH_3), 1.29–1.37 (4H, m, CH, CH_2), 1.55 (4H, m, CH_2), 2.9–3.0 (2H, m, CH_2), 3.20–3.30 (2H, m, CH), 3.95 (1H, brs, NH), 5.11–5.15 (1H, d, J = 8.7 Hz, CH), 6.78–6.82 (1H, d, J = 7.7 Hz, ArCH), 6.93–7.12 (4H, m, ArCH), 7.20–7.33 (4H, m, ArCH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 22.5, 23.4, 23.1, 25.5, 28.8, 35.1, 35.3, 37.5, 48.1, 49.2, 68.9, 105.3, 120.8, 120.9, 126.2, 127.8, 128.7, 131.2, 137.5, 139.2, 142.3, 160.5, 163.6; Anal. Calcd. for $C_{26}H_{29}N_3O$: C, 78.16; H, 7.32; N, 10.52. Found: C, 78.35; H, 7.57; N, 10.69. MS: m/z = 399(M^+); R_f = 0.57.

4-(3,3-Dimethylspiro{bicyclo[2.2.1]heptan-2,5′-isoxazoline-2}-3′-yl)-2-(*p*-methoxyphenyl)-2,3-dihydro-1H-1, 5-benzodiazepine (5b). This compound was isolated as pale yellow solid; mp 118–120°C; yield 82%; IR (KBr) υ (cm^{-1}): 3335, 2930, 2878, 1607; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.01 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.29–1.37 (4H, m, CH, CH_2), 1.55 (4H, m, CH_2), 2.92–3.30 (4H, m, CH_2), 3.90 (3H, s, OCH_3), 3.93 (1H, brs, NH), 5.05–5.14 (1H, m, CH), 6.76–6.77 (1H, d, J = 1.3 Hz, ArCH), 6.80–7.28 (7H, m, ArCH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.5, 23.8, 23.1, 25.5, 28.8, 35.1, 35.3, 37.5, 48.1, 49.2, 68.9, 105.3, 120.8, 120.9, 126.2, 127.8, 129.7, 130.2, 137.5, 139.2, 142.3, 160.5, 161.6; Anal. Calcd. for $C_{27}H_{31}N_3O_2$: C, 75.49; H, 7.27; N, 9.78. Found: C, 75.64; H, 7.44; N, 9.96; MS: m/z = 429(M^+); R_f = 0.57.

4-(3,3-Dimethylspiro{bicyclo[2.2.1]heptan-2,5′-isoxazoline-2}-3′-yl)-2-(m-nitrophenyl)-2,3-dihydro-1H-1,5-benzodiazepine (5c). This compound was isolated as orange color solid; mp 168–170°C; yield 78%; IR (KBr) υ (cm^{-1}): 3385, 2921, 2878, 1607, 1570, 1345; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.01 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.29–1.37 (4H, m, CH, CH_2), 1.55 (4H, m, CH_2), 3.05–3.10 (4H, m, CH_2), 4.0 (1H, brs, NH), 5.30–5.39 (1H, m, CH), 6.85–6.86 (1H, d, J = 7.9 Hz, ArCH), 7.02 (1H, d, J = 1.5 Hz, ArCH), 7.03 (1H, d, J = 8.3 Hz, ArCH), 7.26–7.27 (1H, m, ArCH), 7.5 (1H, m, ArCH), 7.69 (1H, m, ArCH), 8.1 (1H, m, ArCH), 8.3 (1H, s, ArCH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 22.1, 22.2, 22.6, 24.1, 25.0, 25.4, 43.3, 43.5, 48.1, 48.4, 48.6, 48.7, 48.8, 68.9, 100.9, 120.5, 120.6, 121.0, 121.6, 122.7, 127.8, 129.4, 129.6, 129.7, 132.4, 138.2, 146.6, 148.4, 159.7; Anal. Calcd. for $C_{26}H_{28}N_4O_3$: C, 70.25; H, 6.35; N, 12.60. Found: C, 70.44; H, 6.51; N, 12.82. MS: m/z = 444(M^+); R_f = 0.55.

4-(3,3-Dimethylspiro{bicyclo[2.2.1]heptan-2,5′-isoxazoline-2}-3′-yl)-2-(p-methylphenyl)-2,3-dihydro-1H-1,5-benzodiazepine (5d). This compound was isolated as pale yellow solid; mp 138–140°C; yield 77%; IR (KBr) υ (cm^{-1}):3395, 2941, 2878, 1617, 1579; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.01 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.29–1.37 (4H, m, CH, CH_2), 1.55 (4H, m, CH_2), 2.20 (3H, s, CH_3), 2.92–3.30 (4H, m, CH_2), 3.93 (1H, brs, NH), 5.05–5.14 (1H, m, CH), 6.76–6.77 (1H, d, J = 7.3 Hz, ArCH), 6.80–7.28 (7H, m, ArCH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.5, 23.8 23.1, 25.5, 28.8, 35.1, 35.3, 37.5, 48.1, 49.2, 68.9, 105.3, 120.8, 120.9, 126.2, 127.8, 129.7, 130.2, 137.5, 139.2, 142.3, 160.5, 161.6; Anal. Calcd. for $C_{27}H_{31}N_3O$: C, 78.42; H, 7.56; N, 10.16. Found: C, 78.59; H, 7.71; N, 10.28; MS: m/z = 413(M^+); R_f = 0.58.

4-(3,3-Dimethylspiro{bicyclo[2.2.1]heptan-2,5′-isoxazoline-2}-3′-yl)-2-(p-chlorophenyl)-2,3-dihydro-1H-1,5-benzodiazepine (5e). This compound was isolated as pale yellow solid; mp 141–143°C; yield 79%; IR (KBr) υ (cm^{-1}): 3354, 2931, 2878, 1610, 1599; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.01 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.29–1.37 (4H, m, CH, CH_2), 1.55 (4H, m, CH_2), 2.92–3.30 (4H, m, CH_2), 3.93 (1H, brs, NH), 5.05–5.14 (1H, m, CH), 6.76–6.77 (1H, d, J = 7.3 Hz, ArCH), 6.8–7.28 (7H, m, ArCH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 19.5, 22.8, 23.1, 26.0, 28.8, 35.1, 35.6, 37.5, 47.1, 48.2, 68.9, 105.3, 120.8, 120.9, 126.2, 127.8, 129.7, 137.5, 139.2, 142.3, 160.5; Anal. Calcd. for $C_{26}H_{28}ClN_3O$: C, 71.96; H, 6.50; N, 9.68. Found: C, 72.11; H, 6.66; N, 9.47; MS: m/z = 433(M^+); R_f = 0.56.

4-(3,3-Dimethylspiro{bicyclo[2.2.1]heptan-2,5′-isoxazoline-2}-3′-yl)-2-(o-chlorophenyl)-2,3-dihydro-1H-1,5-benzodiazepine (5f). This compound was isolated as pale yellow thick liquid; yield 78%; IR (KBr) υ (cm^{-1}): 3365, 2933, 2877, 1667, 1599; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.01 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.29–1.37 (4H, m, CH, CH_2), 1.55 (4H, m, CH_2), 2.92–3.30 (4H, m, CH_2), 3.93 (1H, brs, NH), 5.05–5.14 (1H, m, CH), 6.76–6.77 (1H, d, J = 7.3 Hz, ArCH), 6.80–7.28 (7H, m, ArCH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.5, 22.8, 24.1, 25.5, 28.8, 35.1, 35.6, 37.5, 48.1, 49.2, 68.9, 105.3, 120.8, 126.2, 127.8, 129.7, 130.2, 137.5, 140.2, 143.3, 170.6, 182.7; Anal. Calcd. for $C_{26}H_{28}ClN_3O$: C, 71.96; H, 6.50; N, 9.68. Found: C, 71.79; H, 6.68; N, 9.45; MS: m/z = 433(M^+). R_f = 0.57.

4-(3,3-Dimethylspiro{bicyclo[2.2.1]heptan-2,5′-isoxazoline-2}-3′-yl)-2-(m-bromophenyl)-2,3-dihydro-1H-1,5-benzodiazepine (5g). This compound was isolated as thick viscous liquid; yield 81%; IR (KBr) υcm^{-1}: 3380, 2951, 1607, 1559; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.01 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.29–1.37 (4H, m), 1.55 (4H, m), 3.02–3.25 (4H, m, CH_2), 3.95–4.0 (1H, brs, NH), 5.13–5.18 (1H, m, CH), 6.84–6.85 (1H, d, J = 1.4 Hz, ArCH), 6.99–7.27 (5H, m, ArCH), 7.30–7.50 (2H, m, ArCH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 22.5, 24.5, 34.8, 35.0, 43.6, 44.0, 48.5, 48.5, 49.1, 68.9, 98.3, 120.7, 120.8, 123.1, 125.1, 128.0, 129.0, 130.0, 131.2, 138.9, 147.3, 159.8; Anal. Calcd. for $C_{26}H_{28}BrN_3O$: C, 65.27; H, 5.90; N, 8.78. Found: C, 65.09; H, 6.11; N, 8.94; MS: m/z = 478(M^+). R_f = 0.56.

4-(3,3-Dimethylspiro{bicyclo[2.2.1]heptan-2,5′-isoxazoline-2}-3′-yl)-2-(m,p-dimethoxyphenyl)-2,3-dihydro-1H-1,5-benzodiazepine (5h). This compound was isolated as thick viscous liquid; yield 81%; IR (KBr) υ (cm^{-1}):3375, 2931, 2878, 1607, 1569; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.01 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.29–1.37 (4H, m, CH, CH_2), 1.55 (4H, m, CH_2), 3.10–3.35 (4H, m, CH_2), 3.88 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 4.0–4.10 (1H, brs, NH), 5.01–5.25 (1H, m, CH) 6.44–6.54 (2H, m, ArCH), 7.54–7.62 (4H, m, ArCH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 20.5, 24.5, 34.8, 35.0, 35.4, 43.6, 44.0, 48.5, 48.5, 49.1, 68.9, 98.3, 120.7,123.1, 125.1, 128.0, 129.0, 130.0, 130.7, 138.9, 147.32, 159.8; Anal. Calcd for $C_{28}H_{33}N_3O_3$: C, 73.18; H, 7.24; N, 9.14. Found: C, 73.37; H, 7.45; N, 9.33; MS: m/z = 459(M^+). R_f = 0.54.

4-(3,3-Dimethylspiro{bicyclo[2.2.1]heptan-2,5′-isoxazoline-2}-3′-yl)-2-(1,3-benzodioxolyl-5)-2,3-dihydro-1H-1,5-benzodiazepine (5i). This compound was isolated as thick liquid; yield 80%; IR (KBr) υ (cm^{-1}): 3335, 2942, 2888, 1607, 1569; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.01 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.29–1.37 (4H, m, CH, CH_2), 1.55 (4H, m, CH_2), 2.85–3.01 (1H, m, CH), 3.23–3.28 (3H, m, CH_2), 4.10 (1H, brs, NH), 5.03–5.07 (1H, m, CH), 5.89 (2H, s, CH_2), 6.68–6.86 (4H, m, ArCH), 6.92–7.24 (3H, m, ArCH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 22.8, 23.1, 24.6, 25.6, 26.9, 35.4, 37.4, 48.6, 49.2, 69.2 101.5, 106.8, 119.6, 120.8, 121.1, 127.9, 130.0, 139.4, 148.2, 161.7; Anal. Calcd. for $C_{27}H_{29}N_3O_3$: C, 73.11;

H, 6.59; N, 9.47. Found: C, 73.27; H, 6.81; N, 9.65; MS: $m/z = 443$ (M^+). $R_f = 0.53$.

4-(3,3-Dimethylspiro{bicyclo[2.2.1]heptan-2,5′-isoxazoline-2}-3′-yl)-2-(α-naphthyl)-2,3-dihydro-1H-1,5-benzodiazepine (5j). This compound was isolated as thick viscous liquid; yield 74%; IR (KBr) υ (cm^{-1}): 3375, 2931, 2878, 1627, 1549; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.01 (3H, s, CH_3), 1.1 (3H, s, CH_3), 1.29–1.37 (4H, m, CH, CH_2), 1.55 (4H, m, CH_2), 3.20–3.43 (4H, m, CH_2), 4.1 (1H, brs, NH), 5.03–5.07 (1H, m, CH), 7.40–7.74 (6H, m, ArCH), 7.85–7.92 (6H, m, ArCH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.1, 22.9, 25.9, 27.7, 34.2, 35.5, 48.8, 49.1, 102.6, 123.8, 124.3, 125.9, 126.7, 127.4, 129.0, 131.6, 132.2, 134.2, 141.0, 159.2, 184.3; Anal. Calcd. for $C_{30}H_{31}N_3O$: C, 80.14; H, 6.95; N, 9.35. Found: C, 80.33; H, 7.09; N, 9.52. MS: $m/z = 449(M^+)$. $R_f = 0.56$.

General Procedure for Antimicrobial Activity Screening. Antibacterial and antifungal activities of benzodiazepine analogues were performed using microdilution method [12–14] 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 fumigatus* 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 sulfoxide. 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 1×10^5 CFU/mL. For fungal cultures, 1×10^3 CFU/mL was used. The plates were incubated at 37°C for 24 h for bacterial cultures and 48 h for fungal cultures. The plates were read visually, and the minimum concentration of the compound showing no turbidity was recorded as MIC.

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