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Research Article

Anticancer and Antimicrobial Activity of Some New 2,3-Dihydro-1,5-benzodiazepine Derivatives

Felix Odame,[1] **Tatenda Madanhire**,[2] **Clement Tettey**,[3] **David Neglo**,[1] **Francisca Adzaho**,[3] **Daniel Sedohia**,[4] **and Eric C. Hosten**[2]

[1]*Department of Basic Sciences, University of Health and Allied Sciences, PMB 31, Ho, Ghana*
[2]*Department of Chemistry, Nelson Mandela University, P.O. Box 77000, Gqeberha 6031, South Africa*
[3]*Department of Biomedical Sciences, University of Health and Allied Sciences, PMB 31, Ho, Ghana*
[4]*Department of Clinical Microbiology, Kwame Nkrumah University of Science and Technology, University Post Office, Kumasi, Ghana*

Correspondence should be addressed to Felix Odame; felixessah15@gmail.com

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A series of 2,3-dihydro-1,5-benzodiazepine derivatives have been synthesized and characterized using IR, NMR, GC-MS, single crystal XRD, and microanalysis. The results of their antibacterial activity against methicillin-resistance *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Streptococcus mutans*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Streptococcus pyrogens* indicated that most of the compounds were bacteriostatic (0.125–4 mg/mL) and also exhibited good biofilm inhibition (0.21–72.69%). The compounds were found to be synergistic when used in combination with other antibiotics. The antiproliferative and cytotoxic effects were also investigated against PC-3 prostate cancer and RAW 264.7 macrophage cell lines, respectively, using the MTT assay. Apart from compounds **6** and **7**, a good number of the compounds (**1**, **2**, **3**, **4**, **5,** and **8**) were selectively toxic to the prostate cancer cells at 20 μM, whilst sparing the normal cells. Compound **3** demonstrated the highest antiprostate cancer effect by reducing the viability of PC-3 cells to (13.75%), which was followed by compounds **1** (47.72%), **2** (48.18%), **4** (62.61%), **5** (66.70%), and **8** (69.55%).

1. Introduction

The synthesis of a series of 2,3-dihydro-1,5-benzodiazepines by the condensation reaction of *o*-phenylenediamine and a group of substituted chalcones in the presence of DMF as a solvent has been reported. When screened for their antibacterial and antifungal activities, the compounds demonstrated significant antimicrobial activity, moderate antibacterial and antifungal activity, and high potency against *E. coli*, *S. typhi*, and *A. oryzae* [1]. A series of benzodiazepines have also been synthesized and screened for their anti-inflammatory, analgesic, and antibacterial activities. Some of the compounds showed potent anti-inflammatory, antibacterial, and analgesic activities, the bioactivity of these compounds was attributed to the presence of the oxadiazole ring [2].

The condensation of β-diketones or β-ketoesters with *o*-phenylenediamine has been used to access some benzodiazepine derivatives. When tested against *S. aureus*, *K. pneumoniae*, *A. niger*, and *C. albicans* the compounds showed good antimicrobial activity [3]. The Pd-catalyzed intramolecular Heck reaction has been used to obtain some benzodiazocine-annulated heterocycles in excellent yields [4]. $KAl(SO_4)_2.12\,H_2O$ has been used to catalyze the synthesis of (3-(4-1H-Indol-3-yl)-2,3-dihydro-1,5-benzodiazepin-2-yl)-2H-chromen-2-one) which showed significant antimicrobial activity against *B. subtilis*, *P. aeruginosa*, *E. coli*, *M. luteus*, and *S. aureus* [5]. Different methods have been used to synthesize 1,5-benzodiazepine derivatives and tested for their biological activity, benzodiazepines have been reported as an extremely versatile pharmacophore [6]. Alkyne/nitrile oxide cycloaddition has been used to synthesize some 2-(3,5-

disubstitutedisoxazolyl)-1,5-benzodiazepines and 2,4-bis-(3,5-disubstitutedisoxazolyl)-1,5-benzodiazepine derivatives which were found to have significant antimicrobial and antityrosinase activities [7]. Some 1,5-benzodiazepine derivatives have been accessed from chalcones, the compounds exhibited good anti-inflammatory activity [8]. 1-Phenyl-3-(2-(tosyloxy)phenyl)propane-1,3-dione, *N*,*N*-dimethylformamide dimethylacetal, hydrazine, hydroxylamine hydrochloride, 2-aminothiophenol, 2-aminophenol, benzene-1,2-diamine have been used to synthesize some benzodiazepine, benzothiazepine, and azole derivatives. The compounds showed good antibacterial, antifungal, and anti-inflammatory activities [9]. Benzodiazepines are known to act by blocking the action of the nerves in the brain and the central nervous system when taken in large amounts. Some benzodiazepines have been synthesized, characterized, and tested for their biological properties. The DFT-computed properties of the compounds have also been discussed [10].

We herein report the synthesis, characterization, antimicrobial, and anticancer activity of some 2,3-dihydro-1,5-benzodiazepine derivatives. A discussion on the single-crystal X-ray diffraction data of (*E*)-3-(2-(2,2-dimethyl-2,3-dihydro-1-5-benzodiazepin-4-yl)vinyl)phenol has also been presented to provide some insight into the structural properties of this compound.

2. Experimental

2.1. Materials. The reagents and solvents used in the synthesis were all of analytical grade. *o*-Phenylenediamine, 4-methylpent-3-en-2-one, 4-hydroxybenzaldehyde, 3-nitrobenzaldehyde, 3-chlorobenzaldehyde, 4-(*N*,*N*-diethylamino)benzaldehye, 4-(*N*,*N*-diethyl-2-hydroxy benzaldehye, 4-methoxybenzaldehyde, benzaldehyde, and 3-hydroxybenzaldehyde were obtained from Sigma Aldrich (USA) whilst the solvent (ethanol, acetone, and methanol) were sourced from Merck Chemicals (SA). The reagents were used without any further purification.

Thin layer chromatography was used to follow the reaction using ethyl acetate: petroleum ether (1 : 1). A Bruker Avance AV 400 MHz spectrometer was used to obtain the ^{1}H NMR and ^{13}C NMR spectra operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C with dimethyl sulfoxide as solvent and tetramethylsilane as internal standard. The chemical shifts were expressed in ppm. A Bruker Platinum ATR Spectrophotometer Tensor 27 was used to obtain the FT-IR spectral data. The elemental analysis was performed using a Vario Elementar Microcube ELIII. A Stuart Lasec SMP30 was used to determine the melting points, and were reported uncorrected. To obtain the masses of the compounds PerkinElmer GC Clarus 580 Gas Chromatograph interfaced to a Mass Spectrometer PerkinElmer (Clarus SQ 8 S) equipped with ZB-5HTMS (5% diphenyl/95% dimethyl poly siloxane) fused capillary column (30 × 0.25 μm ID × 0.25 μm DF) was used polysiloxane.

2.2. Synthesis and Characterization

*2.2.1. 2,2,4-Trimethyl-2,3-dihydro-1,5-benzodiazepine (**1**).* *o*-Phenylene diamine (0.030 mol, 3.27 g) was added to 4-methylpent-3-en-2-one (0.030 mol, 3.003 g) in methanol and heated under reflux for 8 h. The reaction mixture was transferred into a beaker and allowed to stand overnight in the fume hood. It recrystallized as a light brown solid from ethanol. Yield = 88%, m.p. 124–125°C. IR: (ν_{max}, cm^{-1}) 3309 (N-H), 2966 (C-H), 1607 (C=N), 1555 (C=C), 1434 (C-N). ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 6.95 (d, J = 7.6 Hz, 1H), 6.90 (d, J = 7.2 Hz, 1H), 6.83 (m, 2H), 4.69 (s, N-H), 2.23 (s, 3H), 2.16 (s, 2H), 1.24 (s, 6H). (100 MHz, DMSO-d6): δ 170.93 (C=O), 139.34 (C), 126.84 (CH), 125.03 (CH), 121.03 (CH), 119.86 (CH), 66.94 (C), 45.16 (CH_3), 30.10 (CH_3), 29.37 (CH_3). Anal calcd. for $C_{12}H_{22}N_2$: % C, 76.60; H, 8.51; N, 14.89. Found: for $C_{12}H_{22}N_2$ = C, 76.54; H, 8.45; N, 14.8, Found: LRMS (m/z, M^+): 188.13, Expected mass = 188.27. This compound has been synthesized by different methods) [11–14].

*2.2.2. (E)-4-(4-Methoxystyryl)-2,2-dimethyl-2,3-dihydro-1,5-benzodiazepine (**2**).* 4-Methoxybenzaldehyde (0.010 mol, 1.362 g) was added to 2,2,4-trimethyl-2,3-dihydro-1,5-benzodiazepine (0.010 mol, 1.883 g) in methanol and heated under reflux for 8 h. The reaction mixture was transferred into a beaker and allowed to stand overnight in the fume hood. The product was recrystallized from ethanol : acetone (1 : 1) as a yellow solid. Yield = 71%, mp = 115–117°C. IR (ν_{max}, cm^{-1}): 3247 (N-H), 2926 (C-H), 2833 (C-H), 1601 (C=N), 1557 (C=C), 1509 (C=C), 1476 (C-N), 1454 (C-N). ^{1}H NMR (400 MHz, DMSO-d6): δ 7.69 (d, J = 8 Hz, 2H), 7.41 (d, J = 16 Hz, 1H), 7.08 (m, 5H), 6.93 (d, J = 8 Hz, 2H), 4.94 (s, N-H), 3.87 (s, 3H), 2.58 (s, 2H), 1.36 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d6): δ 167.7 (C=N), 159.9 (C-N), 139.7 (C), 139.3 (C), 136.0 (C), 129.7 (CH), 128.8 (CH), 128.0 (CH), 125.5 (CH), 121.0 (CH), 120.0 4(CH), 114.3 (CH), 66.8 (C), 55.4 (CH_3), 39.7 (CH_2), 30.0 (C_2H_6). Anal. calcd. for $C_{20}H_{22}N_2O$: % C, 68.75; H, 3.97; N, 12.25. Found for $C_{20}H_{22}N_2O$ = C, 68.70; H, 3.94; N, 12.20, LRMS (m/z, M^+): 228.44, Expected mass = 228.68.

*2.2.3. (E)-4-(2-(2,2-Dimethyl-2,3-dihydro-1,5-benzodiazepin-4-yl)vinyl)phenol (**3**).* 4-hydroxybenzaldehyde (0.010 mol, 1.220 g) was added to 2,2,4-trimethyl-2,3-dihydro-1,5-benzodiazepine (0.010 mol, 1.883 g) in methanol and heated under reflux for 8 h. The dark brown solid obtained upon evaporation of the solvent at room temperature was recrystallized as a light brown solid from ethanol : acetone (1 : 1) Yield = 75%, mp = 196–197°C. IR (ν_{max}, cm^{-1}): 3313 (N-H), 3043 (C-H), 2973 (C-H), 1595 (C=C), 1573 (C=C), 1472 (C-N), 1456 (C-N). ^{1}H NMR (400 MHz, DMSO-d6): δ 9.87 (s, OH), 7.59 (d, J = 8 Hz, 2H), 7.36 (d, J = 16.4 Hz, 1H), 7.09 (d, J = 7.2 Hz, 1H), 6.99 (d, J = 16.4, 2H), 6.89 (m, 4H),

4.90 (s, N-H), 2.57 (s, 2H), 1.35 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d6): δ 167.9 (C=N), 158.5 (C), 139.9 (CH), 139.2 (C), 136.3 (CH), 129.0 (CH), 128.7 (CH), 127.4 (CH), 127.2 (C), 125.4 (CH), 121.0 (CH), 120.0 (CH), 115.7 (CH), 66.9 (C), 39.5 (CH_2), 30.2 (CH_3). Anal. calcd. for $C_{19}H_{20}N_2O$: % C, 78.05; H, 6.89; N, 9.54. Found for $C_{19}H_{20}N_2O$ = C, 78.00; H, 6.85; N, 9.50%: LRMS (*m/z*, M^+):293.22. Expected mass = 293.37.

2.2.4. (E)-2,2-Dimethyl-4-(3-nitrostyryl)-2,3-dihydro-1,5-benzodiazepine (4). 3-Nitrobenzaldehyde (0.010 mol, 1.510 g) was added to a methanolic solution of 2,2,4-trimethyl-2,3-dihydro-1,5-benzodiazepine (0.010 mol, 1.883 g) and the mixture was refluxed for 8 h. Evaporation of the solvent yielded a light brown solid that was recrystallized from ethanol : acetone (1 : 1). Yield = 77%, mp = 160–161°C. IR (ν_{max}, cm^{-1}): 3313 (N-H), 3043 (C-H), 2973 (C-H), 1595 (C=C), 1573 (C=C), 1472 (C-N), 1456 (C-N). ^{1}H NMR (400 MHz, DMSO-d6): δ 8.56 (s, 1H), (t, *J* = 8, 13.6 Hz, 2H), 7.78 (t, *J* = 7.6, 8 Hz, 1H), 7.63 (d, *J* = 16.4 Hz, 1H), 7.37 (d, *J* = 16.4 Hz, 1H), 7.16 (d, *J* = 7.6 H, 1H), 7.05 (t, *J* = 7.2 Hz, 1H), 6.93 (d, *J* = 8 Hz, 2H), 5.16 (s, 1H, N-H), 2.59 (s, 2H), 1.39 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d6): δ 166.8 (C=N), 148.3 (C), 139.5 (C), 138.7 (C), 138.2 (C), 134.5 (CH), 133.6 (CH), 132.7 (CH), 130.3 (CH), 128.3 (CH), 126.2 (CH), 123.0 (CH), 122.3 (CH), 120.8 (CH), 119.6, 66.3 (CH_3), 39.9 (CH_2), 30.4 (CH_3). Anal. calcd. for $C_{19}H_{19}N_3O_2$: % C, 71.01; H, 5.96; N, 13.08; Found for $C_{19}H_{19}N_3O_2$: C, 70.96; H, 5.92; N, 13.03%; Found: 321.18 LRMS (*m/z*, M^+): Expected mass = 321.37.

*2.2.5. (E)-4-(2-Chlorostyryl)-2,2-dimethyl-2,3-dihydro-1,5-benzodiazepine (**5**).* 2-Chlorobenzaldehyde (0.010 mol, 1.400 g) was added to a solution of 2,2,4-trimethyl-2,3-dihydro-1,5-benzodiazepine (0.010 mol, 1.883 g) in methanol and heated under reflux for 8 h. The yellow solid was formed after evaporation of methanol, followed by recrystallization from ethanol : acetone (1 : 1) Yield = 70%, mp = 136–137°C. IR (ν_{max}, cm^{-1}): 3051 (C-H), 2957 (C-H), 1594 (C=C), 1572 (C=C), 1469 (C-N), 1443 (C-N). ^{1}H NMR (400 MHz, DMSO-d6): δ 8.00 (d, *J* = 6.8 Hz, 1H), 7.66 (s, 1H), 7.62 (d, *J* = 8.8 Hz, 1H), 7.47 (m, 2H), 7.22 (s, 1H), 7.16 (t, *J* = 7.6, 9.6 Hz, 1H), 7.05 (t, *J* = 7.2, 7.6 Hz, 1H), 6.96 (t, *J* = 8, 8.4 Hz, 2H), 5.04 (s, 1H, N-H), 2.17 (s, 2H), 1.38 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d6): δ 167.0 (C=N), 139.3 (CH), 139.2 (C), 134.9 (CH), 133.9 (C), 132.8 (C), 131.0 (CH), 130.2 (CH), 130.0 (CH), 128.0 (CH), 127.7 (CH), 127.5 (CH), 126.1 (CH), 121.1 (CH), 120.0 (CH), 67.0 (CH_3), 40.3 (CH_2), 30.1 (CH_3). Anal. calcd. for $C_{19}H_{19}ClN_2$: % C, 73.42; H, 6.16; Cl, 11.41; N, 9.01. Found for $C_{19}H_{19}ClN_2$: % C, 73.38; H, 6.11; Cl, 11.36; N, 9.05. Found: LRMS (*m/z*, M^+): 310.61 Expected mass = 310.82.

*2.2.6. (E)-4-(2-(2,2-Dimethyl-2,3-dihydro-1,5-benzodiazepin-4-yl)vinyl)-N,N-diethyl Aniline (**6**).* 4-(*N*,*N*-diethylamino)benzaldehyde (0.010 mol, 1.774 g) was added to a methanolic solution of 2,2,4-trimethyl-2,3-dihydro-1,5-benzodiazepine (0.010 mol, 1.883 g). After refluxing for 8 h, followed by evaporation of methanol to give a dark brown solid. The product was recrystallized from ethanol : acetone (1 : 1). Yield = 63%, mp = 99–101°C. IR (ν_{max}, cm^{-1}): 3293 (N-H), 2965 (C-H), 2908 (C-H), 1659 (C=N), 1592 (C=C), 1548 (C=C), 1474 (C-N), 1432 (C-N). ^{1}H NMR (400 MHz, DMSO-d6): δ 9.67 (s, 1H), 7.70 (d, *J* = 7.6 Hz, 2H), 6.92 (m, 3H), 6.79 (t, *J* = 8.4, 9.2 Hz, 6H), 4.71 (s, N-H), 3.00 (s, 4H), 2.16 (s, 2H) 1.24 (s, 12H). ^{13}C NMR (100 MHz, DMSO-d6): δ 190.0 (C=N), 170.9 (C-N), 154.0 (C), 139. (C), 131.5 (CH), 126.9 (CH), 125.0 (CH), 124.6 (C), 121.0 (CH), 119.9 (CH), 111.0 (CH), 66.6 (CH), 45.5 (CH_2), 39.9 (CH_2), 30.0 (CH_3), 29.4 (CH_3). Anal. calcd. for $C_{23}H_{29}N_3$: % C, 79.50; H, 8.41; N, 12.09. Found: for $C_{23}H_{29}N_3$: % C, 79.45; H, 8.36; N, 12.03%. LRMS (*m/z*, M^+): 347.35 Expected mass = 347.50.

*2.2.7. (E)-5-(Diethylamino)-2-(2-(2,2-dimethyl-2,3-dihydro-1,5-benzodiazepin-4-yl)vinyl)phenol (**7**).* 4-(*N*,*N*-diethyl-2-hydroxybenzaldehye (0.010 mol, 1.930 g) was added to 2,2,4-trimethyl-2,3-dihydro-1,5-benzodiazepine (0.010 mol, 1.883 g) in methanol and heated under reflux for 8 h. The reaction mixture was transferred into a beaker and allowed to stand overnight in the fume hood. The product was recrystallized and obtained as a dark brown solid from ethanol : acetone (1 : 1) Yield = 68%, mp = 95–96°C. IR (ν_{max}, cm^{-1}): 3052 (C-H), 2848 (C-H), 1587 (C=C), 1472 (C-N), 1448 (C-N). ^{1}H NMR (400 MHz, DMSO-d6): δ 9.65 (s, 1H), 7.42 (s, 1H), 6.92 (s, 2H), 6.82 (s, 2H), 6.37 (s, 1H), 6.05 (s, 1H), 4.72 (s, N-H), 3.33 (s, 2H), 2.33 (s, 2H), 2.17 (s, 2H), 1.24 (s, 9H), 1.12 (1H). ^{13}C NMR (100 MHz, DMSO-d6): δ 171.3 (C=N), 139.9 (C), 128.0 (CH), 125.5 (CH), 121.5 (CH), 120.6 (CH), 67.4 (C), 45.8 (CH_2), 44.5 (CH_2), 30.6 (CH_6), 29.9 (CH_3), 12.90 (CH_3). Anal. calcd. for $C_{23}H_{29}N_3O$: % C, 76.00; H, 8.04; N, 11.56; Found for $C_{23}H_{29}N_3O$: C, 75.95; H, 8.00; N, 11.50%; Found: 363.36 LRMS (*m/z*, M^+): Expected mass = 363.51.

*2.2.8. (E)-2,2-Dimethyl-4-styryl-2,3-dihydro-1,5-benzodiazepine (**8**).* Benzaldehyde (0.01 mol, 1.06 g) and 2,2,4-trimethyl-2,3-dihydro-1,5-benzodiazepine (0.01 mol, 1.883 g) dissolved in methanol was heated under reflux for 8 h, after which the solvent was evaporated at room temperature. Recrystallization of the product from ethanol : acetone (1 : 1) yielded a yellow solid. Yield = 74%, mp = 132–133°C. IR (ν_{max}, cm^{-1}): 3053 (N-H), 3022 (C-H), 2924 (C-H), 1627 (C=N), 1576 (C=C), 1560 (C=C), 1456 (C-N). ^{1}H NMR (400 MHz, DMSO-d6): δ 7.69 (d, *J* = 7.2 Hz, 2H), 7.40 (t, *J* = 7.2 Hz, 2H), 7.36 (s, 1H), 7.12 (s, 1H), 7.06 (t, *J* = 7.6, 9.6 Hz, 1H), 6.95 (t, *J* = 7.2 Hz, 1H), 8.87 (t, *J* = 8.4 Hz, 2H), 4.95 (s, N-H), 2.55 (s, 2H), 1.30 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d6): δ 168.0 (C=N), 139.8 (C), 136.5 (CH), 132.4 (CH), 129.4 (CH), 128.6 (CH), 127.8 (CH), 126.3 (CH), 121.5 (CH), 120.5 (CH), 67.2 (C), 40.2 (CH_2), 30.6 (CH_3). Anal. calcd. for $C_{19}H_{20}N_2$: % C, 82.57; H, 7.29; N, 10.14. Found for $C_{19}H_{20}N_2$: C, 82.52; H, 7.23; N, 10.90. Found: LRMS (*m/z*, M^+): 276.23. Expected mass = 276.38. Compound **8** has been previously reported [15, 16].

*2.2.9. (E)-3-(2-(2,2-Dimethyl-2,3-dihydro-1,5-benzodiazepin-4-yl)vinyl)phenol (**9**).* 3-Hydroxybenzaldehyde (0.01 mol, 1.22 g) and 2,2,4-trimethyl-2,3-dihydro-1,5-benzodiazepine (0.01 mol, 1.883 g) were dissolved in methanol and heated under reflux for 8 h. The product obtained after evaporation at room temperature was recrystallized as a yellow solid from ethanol : acetone (1 : 1) Yield = 78%, mp = 174–176°C. IR (ν_{max}, cm^{-1}): 3342 (N-H), 3053 (C-H), 2973 (C-H), 1630 (C=N), 1580 (C=C), 1472 (C-N), 1455 (C-N). ^{1}H NMR (400 MHz, DMSO-d6): δ (ppm): 9.55 (s, 1H, OH), 7.30 (d, J = 16 Hz, 1H), 7.21 (t, J = 7.6 Hz, 1H), 7.10 (d, J = 7.6 Hz, 1H), 7.03 (t, J = 7.2, 12.4 Hz, 2H), 6.94 (t, J = 7.2 Hz, 2H), 6.86 (t, J = 8–8.4 Hz, 2H), 6.78 (d, J = 8 Hz, 1H), 4.90 (s, N-H), 2.58 (s, 2H), 1.28 (s, 6H), ^{13}C 2NMR (100 MHz, DMSO-d6): δ (ppm): 167.7 (C=N), 157.7 (C=N), 139.4 (C), 139.3 (C), 137.4 (C), 136.3 (CH), 131.6 (CH), 129.8 (CH), 127.8 (CH), 125.8 (CH), 121.0 (CH), 119.9 (CH), 118.4 (CH), 116.1 (CH), 113.7 (CH), 66.7 (C), 48.9 (CH_3), 39.7 (CH_2) 30.3 (CH_3). Anal. calcd. for $C_{19}H_{20}N_2O$: % C, 78.05; H, 6.89; N, 9.58. Found for $C_{19}H_{20}N_2O$ = C, 78.05; H, 6.89; N, 9.58; Found: LRMS (m/z, M^+): 292.25. Expected mass = 292.37.

2.3. X-Ray Crystallography. The single crystal X-ray diffraction analysis of compound **9** was carried out at 296 K with the aid of a Bruker Kappa Apex II diffractometer with monochromated Mo Kα radiation (λ = 0.71073 Å). Data collection was carried out using APEXII and whilst SAINT was used for cell refinement and data reduction [17], the numerical method implemented in SADABS was used for the correction of absorption effects [17]. Dual-space methods using SHELXT–2014/7 [18] were used to solve the structure and refined by least-squares procedures using SHELXL-2014/7 [19], with SHELXLE [20] as a graphical interface. Refinement of all nonhydrogen atoms was achieved anisotropically. Carbon-bound H atoms were placed in calculated positions (C-H 0.95 Å for aromatic carbon atoms and C-H 0.99 Å for methylene groups) and were included in the refinement in the riding model approximation, with *U*iso (H) set to 1.2*U*eq (C). The H atoms of the methyl groups were allowed to rotate with a fixed angle around the C-C bond to best fit the experimental electron density (HFIX 137 in the SHELXL program [19], with *U*iso (H) set to 1.5*U*eq (C). The H atoms of the hydroxyl groups were allowed to rotate with a fixed angle around the C-O bonds to best fit the experimental electron density (HFIX 147 in the SHELXL program [19], with *U*iso (H) set to 1.5*U*eq(O). Nitrogen-bound H atoms were located on a different Fourier map and refined freely.

2.4. Antibacterial Potentials of Benzodiazepines (Test Organisms). The test bacterial strains involved in this study were obtained from the Microbiology Laboratory, Department of Biomedical Sciences, School of Basic and Biomedical Sciences, UHAS, based on their implications in most contagions. These included methicillin-resistance *Staphylococcus aureus* (NCTC 29212), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (NCTC 13440), *Bacillus subtilis* (ATCC 10004), *Streptococcus mutans* (ATCC 700610), *Pseudomonas aeruginosa* (ATCC 4853), *Salmonella typhi* (ATCC 14028), and *Streptococcus pyrogens* (Clinical).

2.5. Determination of Antibacterial Activity of the Benzodiazepines. The antibacterial activity of the benzodiazepine derivatives was evaluated using the following assays: Kirby–Bauer agar well diffusion, and the broth microdilution [21–23].

2.6. Agar Well Diffusion. About 20 mL of sterile Muller-Hinton agar was poured out into Petri dishes to set. These agar plates were then inoculated with the test bacteria of concentrations 1×10^8 colony forming units (CFU)/mL. Eleven (11) wells were immediately made in each plate using a cork borer (No. 3, 5 mm). Next, these wells were filled with 100 μL of 5 mg/mL stock solution of each compound prepared with 20% methanol to give a final concentration of 0.5 mg or 500 µg per hole. Methanol was used as a negative control, whereas chloramphenicol (30 μg/disc) was used as a positive control in this study. The agar plates were finally subjected to incubation at 37°C for 24 h, and the zones of inhibitions were recorded. The procedure was carried out in triplicates.

2.7. Determination of Minimum Inhibitory Concentrations (MICs). The minimum inhibitory concentrations (MIC) of the test benzodiazepines were obtained by the micro broth dilution method using 96-well microtiter plates according to the protocol previously outlined by the Clinical and Laboratory Standards Institute, 2011 [22, 23], with slight modifications. A stock solution of 2 mg/mL of each compound in methanol as a diluent was used to prepare 10 different concentrations using serial dilution by mixing them with double-strength Mueller Hinton Broth (Oxoid Limited, United Kingdom) in the 96-well plates (Citotest Labware Manufacturing Co. Ltd., Jiangsu, China) to arrive at concentrations ranging from 0.0039 to 1.0 mg/mL per well. Wells 11 and 12 on each row of the plates served as positive control (broth + organism only) and negative control (broth with no organism), respectively, for each bacterial strain on each column. This procedure was executed for the antibiotics; ciprofloxacin, tetracycline, ampicillin, chloramphenicol, fluconazole, and nystatin at concentrations ranging from 128.0 to 0.125 μg/mL in separate plates against all the bacteria. The addition of 100 μL of each of the 0.5 McFarland standardized test organisms followed, after which the plates were subjected to incubation at 37°C for 24 hours for all the bacterial strains. The MIC values were then noted and recorded by visual analysis after tetrazolium chloride (TTC), (0.1 g/mL) dye for 10 minutes, and the MIC was recorded as the least concentration which did not change colour from colourless/light yellow to red/pink.

2.8. Determination of Minimum Bactericidal Concentration (MBC). In order to confirm if the synthesized benzodiazepine derivatives would be able to kill the different bacterial strains, the MBCs against the test strains were determined.

Aliquots from each well from susceptibility testing assays were pipetted into the plates containing nutrient agar and then incubated for 24 hours at 37°C. The plates were then checked for the presence or absence of bacterial growth in the nutrient agar [24].

2.9. Evaluation of Synergistic Effects of the Test Benzodiazepines and Test Drugs. Combinatory effects of the benzodiazepines and drugs/antibiotics were assessed using the checkerboard test against the strains of bacteria under study with slight modification from the method reported [25–27]. Briefly, solutions with different proportions of each benzodiazepine (final volume of 200 μL) were prepared from the MIC solutions of each test benzodiazepine and the individual drugs/antibiotics (1 mg/mL). The antibacterial activity of each compound and antibiotics combination was determined as described for the MIC determination. The fractional inhibitory concentration index (FICI) was obtained according to the following equation:

$$\text{FIC index} = \frac{[\text{MIC of drug in combination}]}{[\text{MIC of drug alone}]} + \frac{[\text{MIC of benzodiazepine in combination}]}{[\text{MIC of benzodiazepine alone}]}. \quad (1)$$

The interaction between the test benzodiazepines and drug/antibiotics was considered synergistic if the FIC index was ≤0.5, partial synergistic if FIC index was >0.5 and <1, additive if FIC index was =1, no difference if the FIC index was >1 and ≤4, and antagonistic if the FIC index was >4.0.

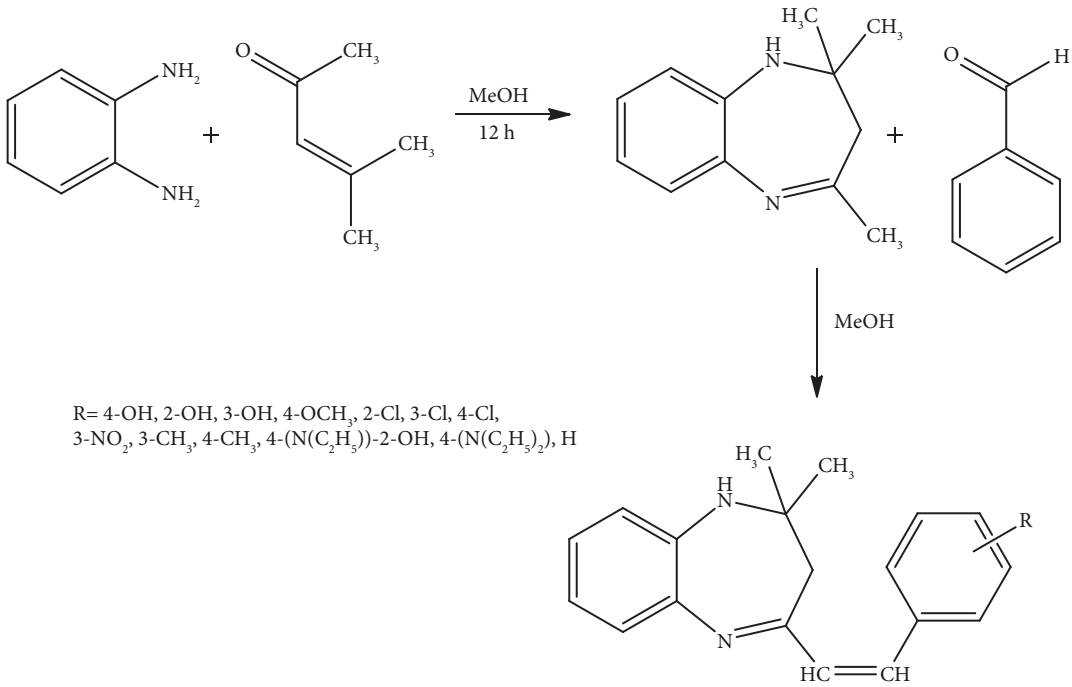
2.10. Determination of Antibiofilm Activity of Benzodiazepines. The activity of the benzodiazepines against bacterial biofilms (inhibition of biofilm formation) was evaluated using a 96-well microtiter plate for bacterial biofilm formation and susceptibility testing [28, 29]. In brief, the media, double-strength Mueller Hinton broth (Oxoid Limited, United Kingdom) was dispensed with multipipette into each well of the flat-bottom 96-well microplate (Citotest Labware Manufacturing Co. Ltd., Jiangsu, China). Each benzodiazepine compound (100 μL) was added to column 1 and diluted until column 10 at concentrations ranging from 0.0039 to 1.0 mg/mL. Afterwards, 100 μL of the microbial suspension of 1×10^6 cells/mL was pipetted into the wells of columns 1–11 to arrive at a final volume of 200 μL. To each of the solutions in the microplate wells, 2 μL of sterile-filtered TTC, 5% (wt/vol) solution was added to attain final TTC concentrations of 0.05% (wt/vol). The microtiter plates were then subjected to incubation for 24 h at 37°C. Later, the mixtures were judiciously enunciated, ensuring that there was no interaction with the preformed biofilm, which was then flowed over with PBS (100 μL) two times in order to take away planktonic and nonadherent cells. The metabolic activity after the antibacterial (benzodiazepines) treatment was assessed by the TTC (CDH) reduction assay [28, 29]. Finally, plates were taken through spectrophotometry at an OD of 492 nm using a microtiter plate reader, and the percentage of biofilm inhibition was determined using equation (2). The IC_{50}s values were then calculated.

$$\%\text{biofilm inhibition} = \left(\text{optical density (OD) of control} - \frac{\text{OD of treatment}}{\text{OD of control}}\right) \times 100. \quad (2)$$

2.11. Determination of Antiprostate Cancer Effect of Benzodiazepines. Human (PC-3) prostate cancer cells (2×10^5 cells/well) and RAW 264.7 mouse macrophage cells (2×10^5 cells/well) were plated in 96-well plates and kept at 37°C in a humidified atmosphere of 5% CO_2 and 95% air for 24 h, after which the cells were treated with 20 μM of each compound for 48 h. 100 nM of paclitaxel was used as the positive control. Twenty microliters (20 μL) of MTT solution was added to each well, and incubated for 4 h after which its absorbance was measured at 517 nm (reference wavelength at 670 nm) using a microplate reader (DNM-9602). Cell viability was expressed as a percentage of untreated controls (100%).

3. Results and Discussion

3.1. Chemistry. The synthesized compounds were obtained by heating the reagents in methanol for 8 h under reflux. Scheme 1 gives a synthetic scheme for the synthesis of the 2,3-dihydro-1,5-benzodiazepine derivatives. The unsubstituted benzodiazepine derivative which was the precursor for the other compounds was obtained by the reaction of *o*-phenylene diamine with 4-methypent-3-en-2-one. The reaction was followed by thin-layer chromatography until the disappearance of the spot for the starting material. Most synthesized benzodiazepines have their substituents attached to different sites on the diazepine moiety. In this



Scheme 1: Reaction scheme for 2,3-dihydro-1,5-benzodiazepine derivatives.

work, we have explored the effect of introducing a two-carbon rigid (alkene) gap between different substituents and the benzodiazepine ring and studied their activity. The synthesis of 3-aceyl-bearing-benzodiazepines has been achieved by microwave irradiation. Some of the synthesized compounds have a rigid alkane as part of the substituent. The compounds were tested for their metal-scavenging activity [30]. Palladium (0) has been used to catalyze a series of 1,4-benzodiazepinones *via* a domino sequence by converting *N*-allenamides and aryl halides in the presence 3-Iodopyridine to give products with an alkene rigid frame as part of the substituents attached to the benzodiazepine ring [31]. Compound **1** reacted with different aldehydes in methanol to give the final products. Different reagents were used to confirm the pathway to the product. In that study, the products were accessed *via* a one-pot synthesis [32]. The use of electron-withdrawing groups and electron-donating groups were tested for the ability of the different compounds to give the alkene-containing benzoxazepines. The conversion of aldehydes to alkenes is widely reported and the mechanism or pathway is well known [33–39]. In this work, we present a conventional method for making alkene-linked benzodiazepines and their antimicrobial and anticancer activities. Generally, among these compounds the derivatives with electron-donating groups gave lower yields. The synthesized compounds are listed in Table 1.

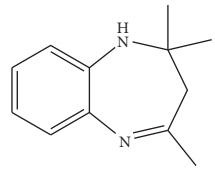
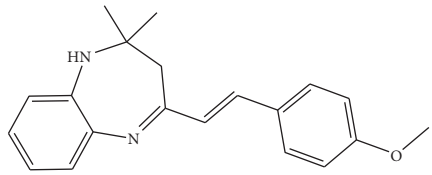
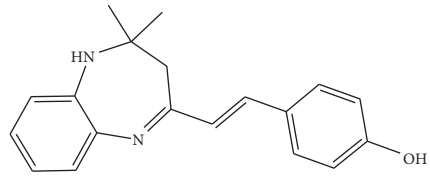
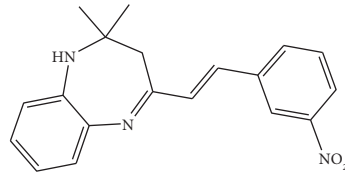
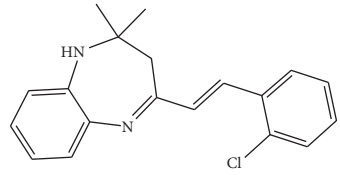
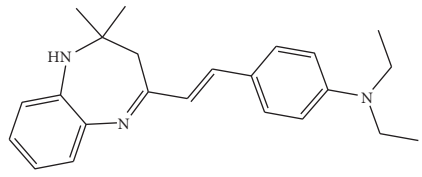
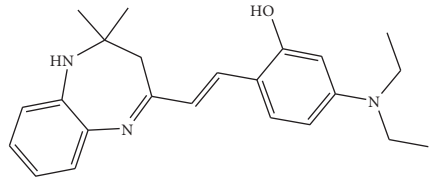
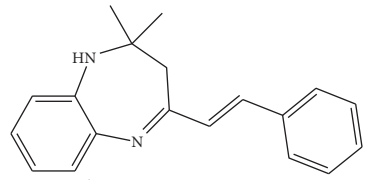
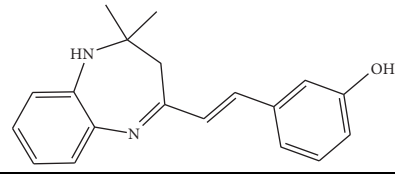
Figure 1 gives the ^{1}H NMR spectrum for compound **1**, three signals were observed for the 4 protons at 6.95, 6.90, and 6.83 ppm, a signal for a methyl group was observed at 2.23 ppm whilst a signal for the methylene group was observed at 2.16 ppm. Upon addition of 4-methoxybenzaldehyde to compound **1** in methanol and heating under reflux compound **2** was obtained. Five signals were observed for 10 protons at 7.69, 7.41, 7.06, and 6.93 ppm, confirming the incorporation of six more aromatic protons (Figure 2). A signal for a methoxy group was observed at 3.87 ppm, accompanied by the loss of the signal for the methyl group in Figure 1 confirming the incorporation of the 4-methoxybenzaldehyde into the molecule to form compound **2**.

The method of synthesis was optimized, the condition and time that gave the best yields were used for subsequent derivatives. Table 2 gives the scope and yields of the synthesized compounds.

The proposed reaction mechanism for the synthesis of 2,3-dihydro-1,5-benzodiazepine derivatives is presented in Scheme 2. The reaction is proposed to proceed by a proton abstraction from the methyl group connected to the imine on the benzodiazepine ring by the methoxide produced from methanol in **2a**, the loss of a proton leads to the formation of a carbanion. The carbanion formed attacks the carbonyl carbon of the aldehyde in **2b,** leading to the formation of a hydroxyl group in **2c**. Abstraction of a proton from the methylene group by the methoxide leads to **2d** an intermediate, with the partial formation of the double bond. The subsequent loss of an OH leads to the formation of the final product **2e**. A similar mechanism has been reported and the path to the product is the same regardless of the heteroatom used [32].

The infrared spectrum of compound **1** showed the presence of characteristic absorption peaks at 3309 and 2966 cm^{-1} for the amine (N-H) and aliphatic C-H stretches, respectively. Peaks were observed at 1607, 1555, and 1434 cm^{-1} for the C=N, C=C, and C-N absorptions, respectively. Compound **1** has been reported to be synthesized by different methods [30]. For compounds **1**–**9** (Table S1 in

Table 1: List of synthesized compounds.

Compound	Structure
1	
2	
3	
4	
5	
6	
7	
8	
9	

supplementary information), the N-H stretches appear in the range 3342–3053 cm^{-1}, while aliphatic C-H, C=N and C=C stretches lie in the range 3053–2833, 1630–1601, and 1595–1509 cm^{-1}, respectively. The bands at ~1476–1432 cm^{-1} of target compounds are assigned to contributions from the stretching vibrations of C-N groups. The ^{1}H NMR spectra of the synthesized compounds indicated the formation of the methylene groups of the seven-membered rings with singlet signals having chemical shifts distributed through a range of 2.16 (compound **1**) to 2.59 (compound **4**) ppm. The presence of methylene groups was confirmed by peaks at 39.5–40.3 ppm in the ^{13}C NMR and DEPT spectra. The appearance of a methoxy group peak at 55.4 ppm in the ^{13}C NMR spectrum of compound **2**, as well as the resonance of the phenolic proton (singlet) at 9.87 ppm in compound **3**, confirmed the incorporation of the aldehyde moieties. The proton NMR spectrum of **6** revealed peaks belonging to the 4-(*N*,*N*-diethylamino) moiety, with signals found at 3.00 and 1.24 ppm (observed at 3.33 and 2.33 ppm in **7**). This was further ascertained in the ^{13}C NMR and DEPT spectra at 45.5 and 30.0 ppm for the methylene and methyl protons of the 4-(*N*,*N*-diethylamino) group, respectively. In compound **7**, the incorporation of the 4-(*N*,*N*-diethylamino) moiety was shown by resonances at 45.8 and 30.0 ppm in the ^{13}C NMR and DEPT spectra. For compound **8,** in the ^{1}H NMR spectrum, a singlet was observed for two protons of a methylene group at 2.55 ppm, this was confirmed in the ^{13}C NMR and DEPT spectra at 40.2 ppm. The ^{1}H NMR spectrum of compound **9** gave a signal at 2.58 ppm for the methylene protons. This was also confirmed in the DEPT and ^{13}C NMR spectra at 39.7 ppm. Figures S1–S41 give the IR, ^{1}H NMR, ^{13}C NMR, DEPT, and the GC-MS of the synthesized compounds.

3.2. Crystal Structure of Compound 9. Compound **9** was recrystallized from ethanol : acetone (1 : 1) and was obtained as a yellow solid. The crystallographic data, selected bond lengths and bond angles for the crystal structure of compound **9** are provided in Tables 3 and 4. The ORTEP diagram for compound 9 is presented in Figure 3. The compound crystallized in the monoclinic space group *P*21/*c*. The bond distances of N1-C11 and N2-C12 are 1.409(2) and 1.406(2) Å are consistent with the bond length of an imine [40], whilst the bond distances of C1-C2 and C2-C3 which were 1.504(2) and 1.532(2) Å, respectively, are typical C-C single bonds [40]. The bond distance of O1-C23 which is 1.364(2) Å is consistent with the hydroxyl bond length [41]. The bond angles of N1-C11-C12, N2-C12-C13, and N2-C12-C11 were 122.3(1), 121.2(1) and 120.3(1)°,respectively, this confirmed that these carbon atoms are sp^2 hybridized [41].

3.3. Antibacterial Susceptibility Testing of the Test Benzodiazepines. The study began with a preliminary test of the different benzodiazepines against the test bacteria, the results as shown in Figure 4 indicated that all the compounds were good antibacterial agents when compared to the standard control, chloramphenicol. At concentrations of 500 μg/well for the compounds, different zones of inhibition were obtained and compared to the control at 30 μg/mL to determine their activity. The zones of inhibition ranged from

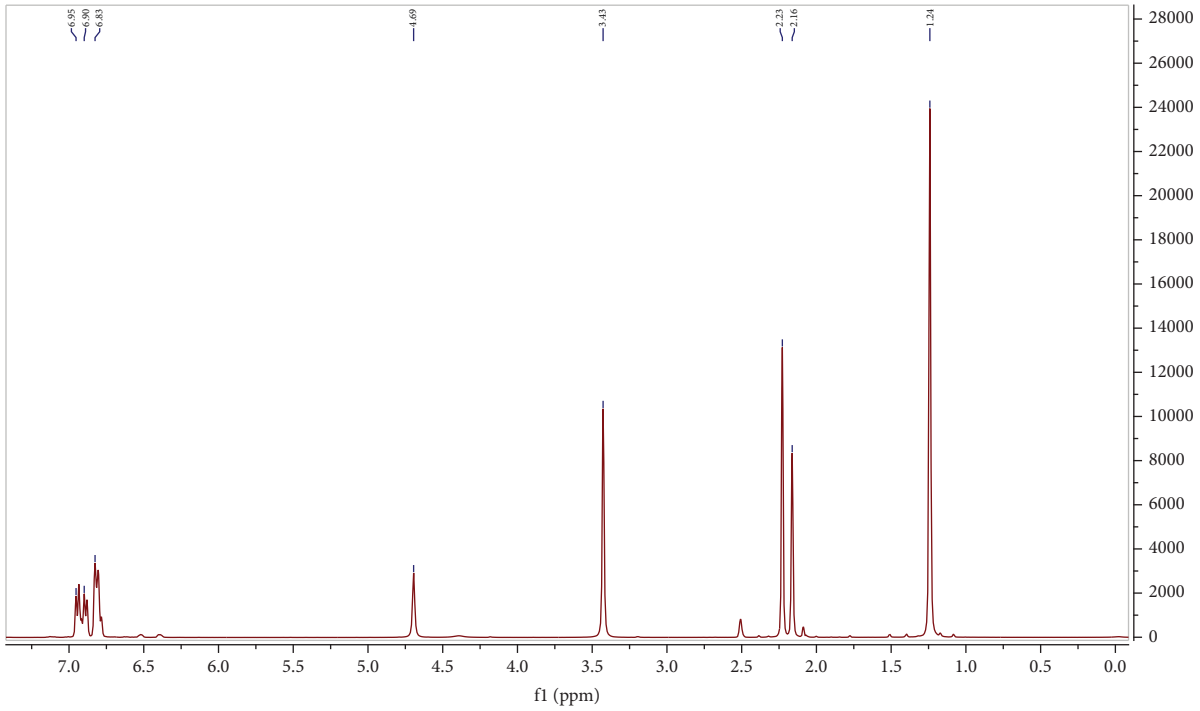


Figure 1: ^{1}H NMR spectrum of 2,2,4-trimethyl-2,3-dihydro-1, 5-benzodiazepine (**1**).

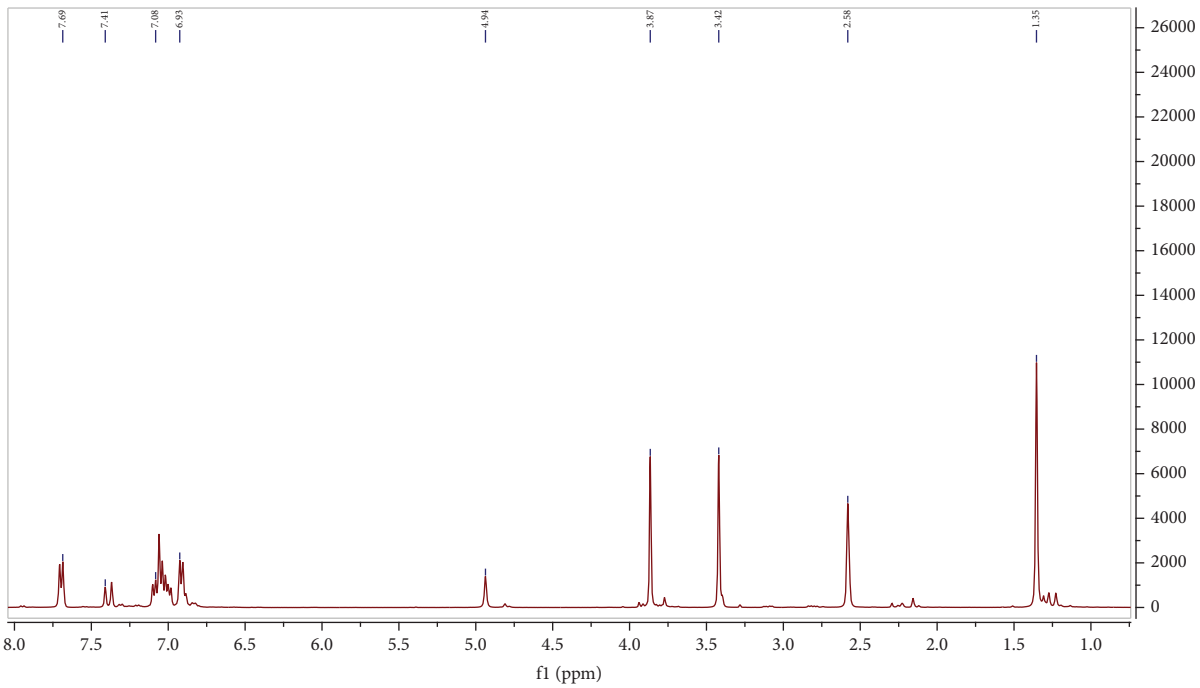


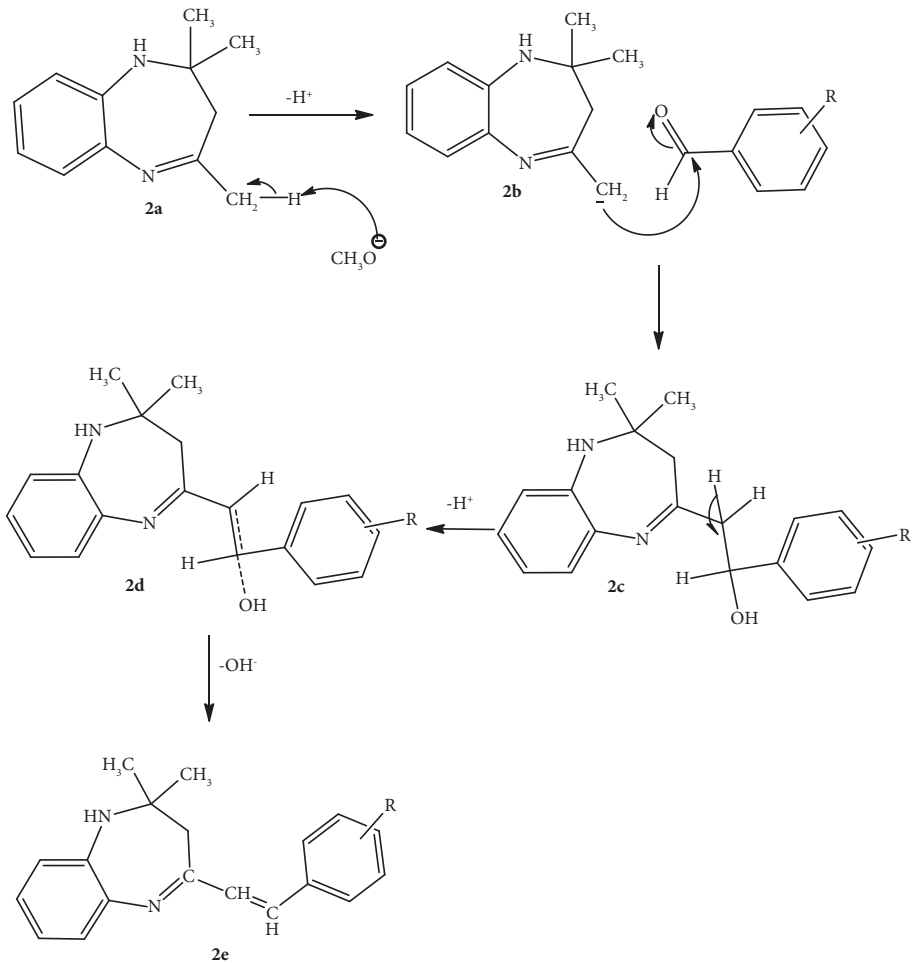
Figure 2: ^{1}H NMR of spectrum (*E*)-4-(4-methoxystyryl)-2,2-dimethyl-2,3-dihydro-1,5 benzodiazepine (**2**).

0.00 to 16.33 mm. The absence of the zones of inhibition in some instances was attributable to the inability of some of the compounds to dissolve completely in the solution. These results are consistent with findings reported in the literature [9].

3.4. Minimum Inhibitory/Bactericidal Concentrations (MICs/MBCs) and Synergistic Potentials of the Antibiotics and the Test Benzodiazepine. In exploring the scope of activity of the compounds (benzodiazepines) against the test strains, their MICs, MBCs, and synergistic potentials were determined.

Table 2: Scope and yields of synthesized benzodiazepines.

Entry	Condition/time (h)	Yield (%)
1	6	63
	8	88
	10	82
2	6	58
	8	71
	12	65
3	8	75
4	8	77
5	8	70
6	8	63
7	8	68
8	8	74
9	8	78



Scheme 2: Proposed reaction mechanism for the synthesis of 2,3-dihydro-1,5-benzodiazepine derivatives.

The MICs obtained were done in a dose-dependent manner. Most of the benzodiazepines displayed a good antibacterial potential with MIC values between 0.125 and 4 mg/mL (Tables S2–S12). This has been affirmed by studies that have indicated that similar compounds gave strong microbial inhibition with MIC values equal to or lower than 500 μg/mL

Table 3: Crystallographic data and structure refinement summary for compounds **9**.

Property	Compound 9
Formula	$C_{19}H_{20}N_2O$, CH_4O
CCDC	2141381
Formula weight	324.41
Crystal system	Monoclinic
Space group	*P*21/*c*
a (Å)	12.4123 (7)
b (Å)	7.3789 (3)
c (Å)	19.6257 (11)
α (°)	90
β (°)	98.609 (2)
γ (°)	90
V (Å^3)	1777.25 (16)
Z	4
ρ (g/cm^3)	1.212
μ (mm^{-1})	0.079
F (000)	696
Crystal size (mm)	$0.43 \times 0.50 \times 0.50$
Temperature (K)	296
MoKa radiation (Å)	0.71073
Theta min-max (°)	1.7, 28.3
Dataset	−16 : 15; −8 : 9; −22 : 26
Tot., uniq. data, R (int)	31222, 4349, 0.031
Observed data ($I > 2.0$ sigma (I))	3532
Nref	4349
Npar	226
R	0.0466
$_wR_2$	0.13090
S	1.04
Max. and av. shift/error	0.00, 0.00
$\Delta\rho$min, $\Delta\rho$max (e Å-3)	−0.33, 0.42
Max. resd. dens. (e/Å^3)	0.42

Table 4: Selected bond lengths (Å) and bond angles (°) for compound **9**.

Bond lengths (Å)		Bond angles (°)	
C11-C12	1.410 (2)	C1-N1-C11	120.1 (1)
N1-C1	1.293 (2)	C3-N2-C12	120.9 (1)
N1-C11	1.409 (2)	N1-C1-C2	121.5 (1)
N2-C3	1.474 (2)	N1-C1-C6	117.0 (1)
N2-C12	1.406 (2)	N2-C3-C2	107.6 (1)
C1-C2	1.504 (2)	N2-C3-C4	111.3 (1)
C1-C6	1.459 (2)	N1-C11-C16	118.1 (1)
C2-C3	1.532 (2)	N1-C11-C12	122.3 (1)
C3-C4	1.528 (2)	N2-C12-C11	120.3 (1)
C3-C5	1.526 (2)	N2-C12-C13	121.2 (1)
C6-C7	1.328 (2)	N2-C3-C5	107.4 (1)
C7-C21	1.464 (2)	C2-C1-C6	121.5 (1)
O1-C23	1.364 (2)	C1-C2-C3	113.5 (1)
O2-C8	1.396 (2)	C2-C3-C5	111.2 (1)
C11-C16	1.394 (2)	C4-C3-C5	110.3 (1)
C13-C14	1.377 (2)	C2-C3-C4	109.0 (1)
C14-C15	1.377 (3)	C1-C6-C7	125.1 (1)

[42, 43] thus the MIC values recorded for most of these test benzodiazepines in this study, especially with respect to compounds **2** and **7** suggest that they can serve as strong microbial inhibitory agents. The MICs of the test drugs/antibiotics determined ranged from 0.98 to 1000 mg/mL (Table S2). In addition to the MIC determination, the MBCs for the benzodiazepines indicated that they were bactericidal against the test strains used except benzodiazepines **4** and **6** which exhibited bacteriostatic activities against *S. mutans*, *S. pyrogens*, and *P. aeruginosa*, respectively, (Tables S2–S12). This falls in line with the literature, which indicated that the antimicrobial activity of compounds benzodiazepines against the microbes can be classified as strong with MIC <2 mg/mL or good with MIC range of (2–10) mg/mL). The description of a bactericidal agent is one with the ratio of MBC/MIC ≤4, while a bacteriostatic agent has an MBC/MIC ratio of >4 [44, 45]. The results of the antimicrobial assay as shown in Tables S5–S12 agree with the findings in the literature [9]. In addition, the synergistic activities of the test compounds with some antibiotics (Ampicillin, Ciprofloxacin, and Tetracycline) were carried out. The compounds showed indifferent interaction with Ampicillin and Ciprofloxacin. The compounds were found to be antagonistic to Tetracycline except for a few compounds that had indifferent interactions with Tetracycline. These results show that benzodiazepines can be used to enhance the efficacy of standard antibiotics. A similar study has been reported on the use of benzodiazepine as respiratory depressants [46].

3.5. Antibiofilm Formation Potential of Test Benzodiazepines. With several studies reporting that biofilm formation by most microbes has been contributing to the occurrence of most infectious diseases that have been difficult to treat lately, many clinically vital microbes including Gram-positive methicillin-resistant *S. aureus* biofilms have been singled out for many nosocomial infections [47]. Moreover, recent studies have shown that the pathogenicity of these many organisms mainly depends on their virulence factors, such as adherence and invasion, hyphal and biofilm formation, cell wall integrity, and hydrolase secretion [48].

In effect, the quest to find a lasting solution to this resistance factor such as biofilm formation of most organisms implicated in most infections has become imperative. In this regard, the antibiofilm formation activity of our compounds determined has been found to be appreciable with concentrations ranging between 0.02 and 1 mg/mL. The percentage inhibition recorded against the biofilms ranged from 0.21 to 72.69% against the test organisms, respectively, (Figures 5(a) and 5(b)). The IC_{50} determined for each of the compounds when compared to the standard antibiotic against the bacterial biofilms gave IC_{50} values between 0.03 and 0.94 mg/mL. However, the IC_{50}s of some of the compounds could not be determined due to their low activity at

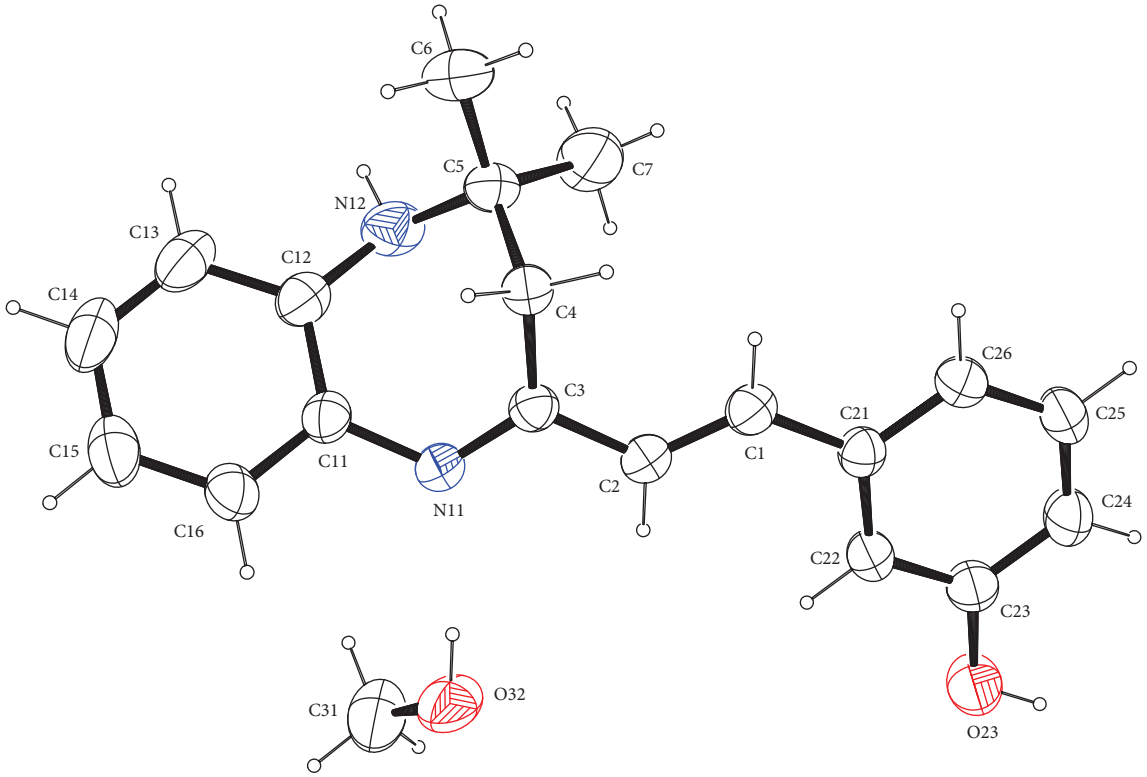


Figure 3: An ORTEP view of (E)-3-(2-(2,2-dimethyl-2,3-dihydro-1,5-benzodiazepin-4-yl)vinyl)phenol (**9**) showing 50% probability displacement ellipsoids and atom labelling.

determined concentrations (Table S10). This variation is expected because studies have shown that biofilm formation depends on the structure, nature, and composition of the organism in question [49].

In a related report recorded, similar benzodiazepines tested have been found to be effective against similar microbial biofilms [50, 51]. These abilities of the benzodiazepines have been alluded to by the variation in the sensitivity of the biofilm to these benzodiazepine derivatives. More importantly, the biofilm inhibition mechanisms of these benzodiazepines may also be attributed to the release of ions in the internal structures of the test bacteria to disrupt cells walls and essential structure, thereby enhancing cell-to-cell adhesion [52]. Again, it must be noted that biofilm formation is largely affected by cell surface hydrophobicity, extracellular appendages such as flagella, and extracellular polymeric substances, and these properties may diverge from cell to cell hence the transformation in strength of biofilm formation as a result [53]. Therefore, the fact established about the inhibition of microbial biofilms by benzodiazepines as reported in many studies has been their possession of abilities to go into the biofilm matrix to subvert bacterial cell walls [54].

Compound **1** was the most active against *E. coli* with an IC_{50} of 0.1 mg/mL, whilst compound 2 was most active against methicillin-resistance *S. aureus* and *K. pneumoniae* with IC_{50}s of 0.07 and 0.2 mg/mL, respectively. Against *S. mutans* compound **4** was the most active with an IC_{50} of 0.14 mg/mL. The most active compounds against *B. subtilis* were compounds **4** and **9** were with an IC_{50} of 0.08 mg/mL whilst compounds **7** and **9** were the most active against *S. pyrogens* with an IC_{50} of 0.11 mg/mL. For *S. typhi* the most active compounds were compound 4 and 7 with an IC_{50} of 0.07 mg/mL. Compound 8 was most active against *P. aeruginosa* with an IC_{50} of 0.11 mg/mL. Figures S42–S45 give pictures of the zones of inhibition for all the compounds and organisms.

3.6. Antiprostate Cancer Effect of Compounds. There are various methods of treating cancers, which include chemotherapy, radiotherapy, surgery, and immunotherapy. Although there are several limitations associated with these methods, drugs offer the only approach in treating cases where the disease has spread through the body. A good number of chemotherapeutic drugs are available for the treatment of cancers, but for most of them the applications are limited due to adverse effects including anaemia, fatigue, hair loss, nephrotoxicity, hepatotoxicity, and skin infections [55].

There is therefore the need to search for novel agents with little or no side effects for the treatment of cancers. Some benzodiazepine derivatives containing the thiochromeno moiety have been synthesized and tested for their anticancer, antimicrobial, and antitubercular activities. The incorporation of the thiochromeno moiety provides further rigidity to the compound and allows it to fit into a wider space than is possible in most benzodiazepine compounds. It was also observed that the presence of the thiochromeno and the

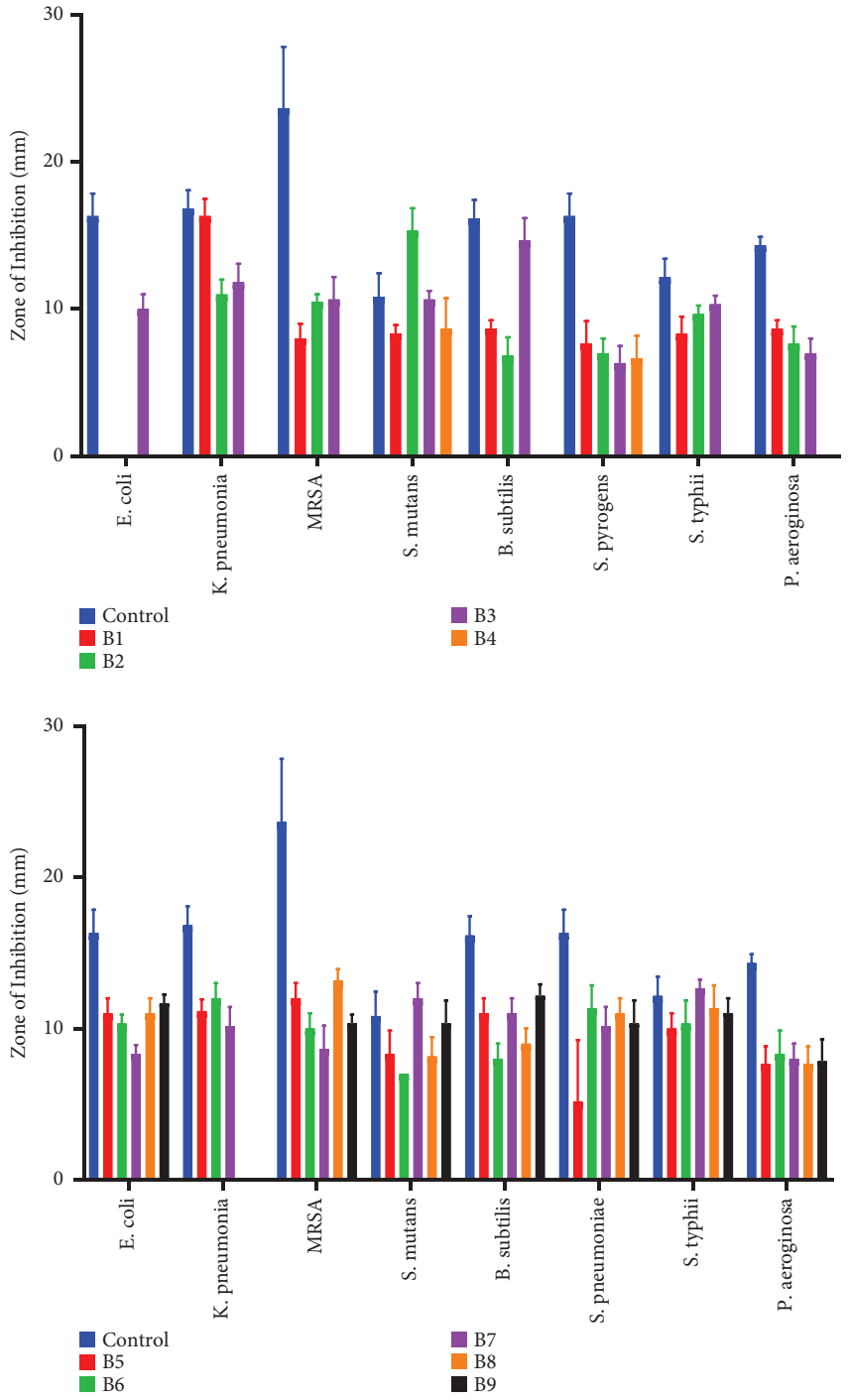
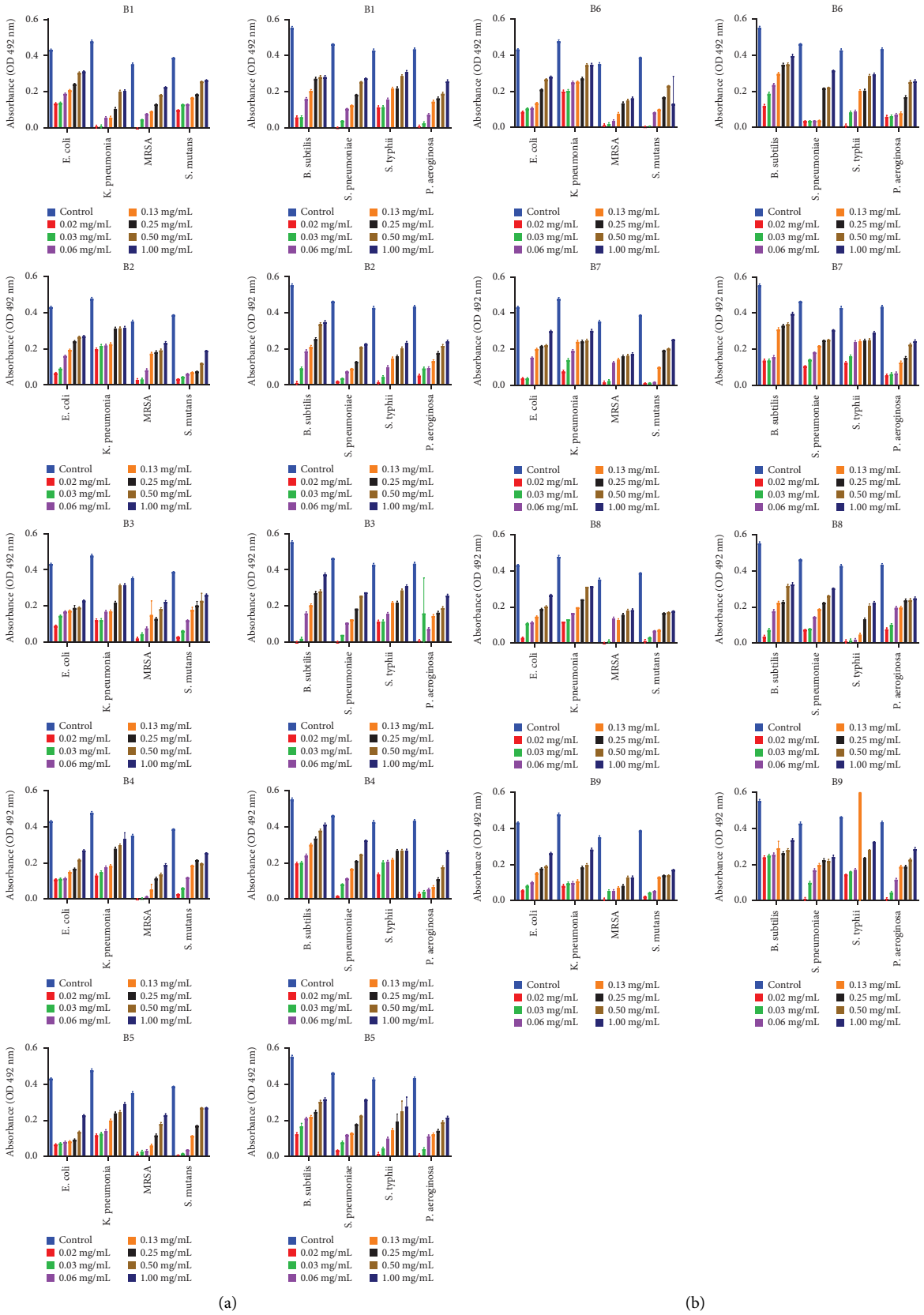


Figure 4: Antibacterial susceptibility testing of test benzodiazepine.

benzothiepino derivatives had higher activity against the test organisms [56]. The selective anticancer activity of some benzodiazepine and benzothiazepine derivatives has been reported. 2-Methoxy-4-(4-phenyl-*1H*-1,5-benzodiazepin-2-yl)phenol which is a benzodiazepine gave the best anticancer activity. The higher activity was attributable to the benzodiazepine scaffold [57]. The synthesis of some chloro- and fluoro-substituted 5-aryl-1,4-benzodiazepines has been achieved. The compounds were tested for their anti-inflammatory, myeloperoxidase, and anticancer properties. 7-Chloro-5-(2-chlorophenyl)-1H-benzo[e] [1,4]diazepin-2(3H)-one gave the best activity amongst the compounds tested, the activity was attributable to the presence of the chloro group on the benzodiazepine ring [58].

Apart from compounds **6** and **7**, the compounds did not demonstrate significant toxicity to normal cells and had no negative effect on their viability at 20 μM compared to the untreated controls (100%) (Figure 6). Compounds **1–5** and **8**



(a) (b)

Figure 5: (a) Antibiofilm inhibition of some benzodiazepine against test strains of bacteria. (b) Antibiofilm inhibition of various benzodiazepine against test strains of bacteria.

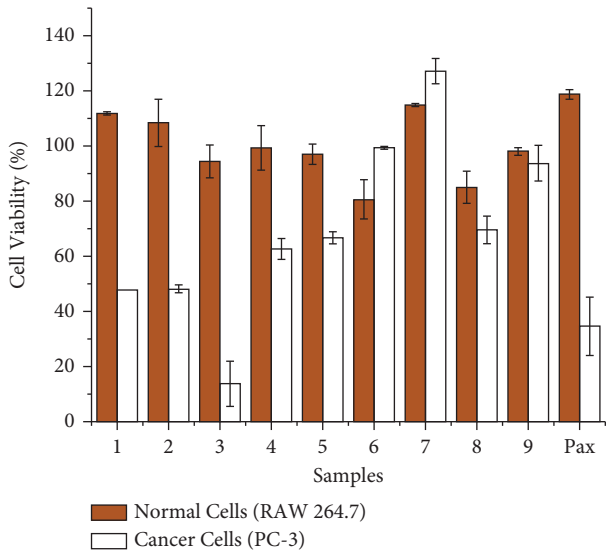


Figure 6: Anticancer activity and cell viability tests of benzodiazepines.

were selectively toxic to the prostate cancer (PC-3) cells, whereas sparing the normal (RAW 264.7) cells. Compound **3** demonstrated the highest antiprostate cancer effect by reducing the viability of PC-3 cells to a minimum (13.75%), indicating that the presence of the hydroxyl group at position 4 on the aromatic ring leads to a higher anticancer activity amongst these compounds. Compound **1** was moderately active (47.72%), confirming that the benzodiazepine frame was active on its own as an anticancer scaffold. The moderate activity of compound **2** (48.18%) also confirmed the anticancer effect of the compound when a methoxy group is at position 4 on the aromatic ring. The activity of compounds **4** (62.61%), **5** (66.70%), and **8** (69.55%) (Figure 6) confirmed that substitution at position 3 with a nitro group, position 2 with a chloro group or the aromatic ring without any substituent leads to mild activity amongst these compounds. Compounds **6** and **7,** however, did not reduce the viability of PC-3 cells significantly, indicating that the presence of the *N*,*N*-diethylamino group at position 4 on the aromatic ring makes the compounds less active than the benzodiazepine frame. Although compound **9** did not demonstrate a significant antiprostate cancer effect, it still appears to be selectively toxic to the PC-3 cell line and hence may exhibit mild to moderate antiprostate cancer effects at a higher dose.

Prostate cancer is the second-deadliest malignancy in males after skin cancer and also the most diagnosed cancer type in men [59]. More than 1,400,000 new cases of prostate cancer are diagnosed annually with 375,000 deaths worldwide [60]. The search for novel therapies for the treatment of prostate cancers is therefore warranted. Benzodiazepines are noted to have a variety of therapeutic effects including antimicrobial, antiviral, and antioxidant effects [61]. However, research into the anticancer effect of benzodiazepines is scanty. Despite a handful of studies on the anticancer activities of benzodiazepines, research into the antiprostate cancer effect of these compounds remains limited. The result of this study is therefore imperative as it gives insight into the possibility of exploring the antiprostate cancer effects of benzodiazepines. Figure S46 gives a pictorial presentation of the anticancer results.

4. Conclusion

These synthesized compounds were evaluated for their antibacterial activity using agar well diffusion, microdilution, and biofilm inhibition assays. Subsequently, the determination of the combined antimicrobial activity of these benzodiazepines with antibiotics (ampicillin, tetracycline, and ciprofloxacin) against microbial strains was evaluated by checkerboard microdilution assay. Results from the study indicated that the antimicrobial activity of most of these compounds was bacteriostatic with their MICs ranging from 0.125 to 4 mg/mL. Interestingly, all the compounds were proven as good biofilm inhibitors with percentage inhibition ranging from 0.21 to 72.69%. The combination interaction of the benzodiazepine derivatives with antibiotics gave results ranging from synergy to antagonism according to the parameters used. The results showed that these benzodiazepines have significant antibacterial properties. Furthermore, benzodiazepines alone or in combination with the tested antibiotics could provide a promising approach to the management of microbial infections caused by drug-resistant strains. The interaction of the compounds with other antibacterial agents would be helpful in combating common infections caused by methicillin-resistance *Staphylococcus aureus* (NCTC 29212), *Escherichia coli* (ATCC25922), *Klebsiella pneumoniae* (NCTC 13440), *Bacillus subtilis* (ATCC 10004), *Streptococcus mutans* (ATCC 700610), *Pseudomonas aeruginosa* (ATCC 4853), *Salmonella typhi* (ATCC14028), and *Streptococcus pyrogens* (Clinical).

This study has demonstrated the effect of some 2,3-dihydro-*1H*-benzo[b] [1,4]diazepine derivatives against PC-3 prostate cancer cells, a good number of which were found to be selectively toxic to the PC-3 cells, whilst sparing the normal macrophage cells. These compounds are therefore promising candidates for further research into their mechanism of action against the proliferation of PC-3 prostate cancer cells.

Data Availability

Spectra for the characterization of all the compounds have been included in the Supplementary Information. Crystallographic data for the structure reported in this manuscript have been deposited with the Cambridge Crystallographic Data Centre under the CCDC number: 2141381. Copies of these data can be obtained free of charge from https://www.ccdc.cam.ac.uk/data_request/cif.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Felix Odame conceptualized the project, contributed to synthesis and characterization of compounds (all

characterization except NMR), contributed to crystal structure discussion, and wrote the first draft, and contributed to copy editing. Tatenda Madanhire carried out the NMR and reviewed the manuscript. Clement Tettey contributed to discussion of anticancer results and reviewed the manuscript. David Neglo carried out antimicrobial tests and reviewed the manuscript. Francisca Adzaho carried out the anticancer assay and reviewed the manuscript. Daniel Sedohia carried out the analysis of antimicrobial results and reviewed the manuscript. Eric C. Hosten carried out the single crystal XRD determination and reviewed the manuscript.

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Supplementary Materials

The supplementary information contains the IR, ^{1}H NMR, ^{13}C NMR, DEPT, and the GC-MS of the synthesized compounds (Figures S1–S41). The zones diffusion for all the compounds and organisms (Figures S42–S45) and the anticancer results (Figure S46). Whilst Tables S1–S12 give the tabulated results of the antimicrobial tests. (*Supplementary Materials*)

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