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Studies on 6,11-Bisthiatetracyclic- and pentacyclic Steroidal Analogues: Syntheses, Characterization, Antimicrobial-, Antituberculosis-, Antitumor- and DNA Cleavage Activity of New Pyrazole-, Isoxazole-, Pyrimidine-, and Benzodiazepine Frameworks

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ABSTRACT

A series of new heterocyclic steroidal analogues has been synthesized from the reactions of the key intermediates **5** and **6**. All these compounds were evaluated for bioactivity. In the benzodiazepine series **17**-**20**, the 1,2-Diphenylethane-1,2-diamine substituted compounds **19** and **20** displayed the highest activity with IC_{50} equal to 13 and 12 µM for HeLa cell, and 12 and 10 µM for HCT116 cell. Similarly these compounds (**19** and **20)** showed higher activity than chloroamphenicol against *Klebsiella pneumoniae* and *Escherichia coli* and behaved as the most promising being active against *M. tuberculosis* (H37Rv) with MIC 7.7 and 7.3 µM respectively. Moreover, the compounds were subjected for DNA cleavage potential by gel electrophoresis method.

Key words: Tetracyclic, pentacyclic, 6,11-bisthia steroids, biological activity

INTRODUCTION

The recent pharmaceutical interest in steroids bearing heterocycles [1–5] has lead us to synthesize steroids fused with pyrazole-, isoxazole- and pyrimidine ring systems in the 2,3- and 16,17-positions. Several types of steroidal[3,2-c] pyrazoles such as androst-4-eno- and androstano [3,2-c]pyrazoles [6], 5α-pregnano- and pregn-4-eno[3,2-c]pyrazoles [7] were synthesized by formylation at the 2-position and the reaction of the resulting 2-hydroxymethylene-3-ketosteroids with hydrazine or substituted hydrazines. The preparation of steroidal heterocycles containing an isoxazole ring fused to the 2,3-position of the steroid nucleus was described by Clinton and co-workers [8]. Clinton *et al* also reported [9] the synthesis of the steroidal [3,2-d]pyrimidines by condensing the 2-hydroxymethylene compounds with acetamidine hydrochloride in the presence of triethylamine and ethanol. Rapole *et al* [10] reported the synthesis of steroidal pyrimidines from the corresponding 16-arylidene-17-oxo-5-androsten -3β-yl acetates and urea. Steroidal heterocycles having pyrazole-, thiophene-, and thiazole moieties are an interesting group of compounds, many of which possessing widespread pharmacological properties such as analgesic-, antipyretic-, and antiandrogenic activities [11-14]. Pyrazoles also possess antidepressant-, anti-inflammatory- and antirheumatic activities [15–17]. Nitrogen heterocycles have recently been reported to exhibit anticancer- [18–20], antimicrobial- [21, 22] *etc.* activities. Several steroidal analogues are known to behave as antiproliferative agents against fungi, yeast, and protozoa [23, 24]. In view of the above mentioned facts and in continuation of our interest in the synthesis of heterocyclics [25a, 25b], we investigated the synthesis, characterization, antimicrobial-, antituberculosis-, and antitumor activity of some new pyrazole-, isoxazole-, pyrimidine-, and benzodiazepine steroidal analogues based on 2,3-dihydro thiopyrano [3,2-c]thiochroman-4(5*H*)-one and 2,3,5,6-tetrahydro-4*H*-thiopyrano[3,2-d][1]benzothiepin-4-one.

EXPERIMENTAL

Analysis and instruments: Melting points were obtained on a TECHNICO melting point apparatus and are uncorrected. IR spectra were recorded as thin films (for oils) or KBr (for solids) with a JASCO FT- IR spectrophotometer and are expressed in cm^{-1}. All NMR spectra were taken on

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a Brucker Advance 400 FT-NMR spectrometer with ^{1}H and ^{13}C observed at 400 MHz. Chemical shifts for ^{1}H and ^{13}C-NMR spectra were reported in δ or ppm downfield from TMS [$(CH_3)_4Si$]. ESI mass spectra were obtained on an Agilent 6520 ES+2000 spectrometer. All reactions involving air or moisture-sensitive compounds were performed under nitrogen atmosphere. Separation of compounds was carried out by column chromatography using silica gel. All materials, solvents, and reagents were obtained commercially.

Synthesis:

3-(Hydroxymethylene)-2,3-dihydrothiopyrano[3,2-c]thiochromen-4(5H)-one (5): A solution of ethylformate (15 mmol) in anhydrous toluene (6 mL), was added to freshly prepared sodium methoxide (5 mmol) in the same solvent (8 mL) and cooled to 0-5ºC. Then a solution of 2,3-dihydrothiopyrano[3,2-c]thiochromen-4(5H)-one (**3**) (7.5 mmol) in anhydrous toluene (10 mL) was added dropwise with stirring, to the above ice cold mixture under nitrogen atmosphere. Stirring was continued at room temperature for 24h to give the sodium salt of **5**, which was collected and treated with water. The solution obtained was acidified with cold hydrochloric acid (1:1) to give the product **5** (0.724g, 68% yield). An analytical product of **5** was obtained by recrystallization from ethanol, mp 116-119^{0}C; FT-IR 1214, 1508, 1627, 1880, 3548 cm^{-1}; ^{1}H-NMR($CDCl_3$) δ 3.4 (s, 2H, -S-C<u>H</u>$_2$, at C_7), 3.5 (s, 2H, -S-C<u>H</u>$_2$, at C_{12}), 6.1 (s, 1H, =C<u>H</u>), 7.2-7.5 (m, 4H, aromatic), 14.3 (bs, 1H, OH); ^{13}C-NMR ($CDCl_3$) δ 22.42, 33.41, 114.63, 121.63, 125.43, 126.42, 127.53, 128.23, 133.24, 138.24, 161.25, 175.52, 186.36. ESI-MS m/z: 262.03 and Anal. Calcd for $C_{13}H_{10}O_2S_2$; C, 59.52; H, 3.84; S, 24.44, Found: C, 59.31; H, 3.66, and S, 24.32%.

Pyrazole-, isoxazole-, pyrimidine- and benzodiazepine derivatives (7, 9, 11, 13, 15, 17, and 19) from 3-(hydroxymethylene)-2,3-dihydrothiopyrano[3,2-c]thiochromen-4(5H)-one (5):

General Procedure: Phenyl hydrazine hydrochloride/ hydrazine hydrochloride/ hydroxylamine hydrochloride/ urea/ thiourea/ o-phenylenediamine or 1,2-diphenylethane-1,2-diamine was added to a solution 3-(hydroxymethylene)-2,3-dihydrothiopyrano[3,2-c] thiochromen-4(5H)-one (**5**) in anhydrous methanol (15 mL). The reaction mixture was stirred at room temperature for 24h and then refluxed for 5h. After cooling, the solid, if present, was collected and the solution was evaporated under reduced pressure. The solid and the residue were washed with an ice cold ethanol solution to give crude pyrazole-, isoxazole-, pyrimidine- and benzodiazepine derivatives (**7**, **9**, **11**, **13**, **15**, **17**, and **19**), which were purified by column chromatography on silica gel using petroleum ether (40-60º C):ethyl acetate as the eluting system.

15,16-Bisaza-15-phenyl-6,11-bisthiagona-1,3,5(10),8,13,16-hexaene (7): Compound **7** (0.62g, 64%) was obtained as a yellow solid. mp 162-165 ^{0}C; FT-IR 1064, 1211, 1513, 1633, 2814, 2385cm^{-1}; ^{1}H-NMR($CDCl_3$) 3.4 (s, 2H, -S-C<u>H</u>$_2$, at C_7), 4.1 (s, 2H, -S-C<u>H</u>$_2$, at C_{12}), 7.1-7.4 (m, 9H, aromatic), 8.2 (s, 1H, C<u>H</u>=N); ^{13}C-NMR($CDCl_3$) 39.15, 43.61, 117.54, 118.64, 120.43, 125.36, 126.48, 126.53, 126.76, 126.86, 128.63, 129.43, 133.42, 139.25, 140.53, 141.63. ESI-MS m/z: 334.04 and Anal. Calcd for $C_{19}H_{14}N_2S_2$; C, 68.23; H, 4.22; N, 8.38; S, 19.17, Found: C, 68.13; H, 4.09; N, 8.21; and S, 19.08%.

15,16-Bisaza-6,11-bisthiagona-1,3,5(10),8,13,16-hexaene (9): Compound **9** (0.58g, 61%) was obtained as a colourless solid. mp 148-151^{0}C; FT-IR, 1061, 1214, 1524, 2816, 2930cm^{-1}; ^{1}H-NMR($CDCl_3$) 3.5 (s, 2H, -S-C<u>H</u>$_2$, at C_7), 4.2 (s, 2H, -S-C<u>H</u>$_2$, at C_{12}), 7.1-7.5 (m, 4H, aromatic), 7.9 (s, 1H, C<u>H</u>=N), 13.3 (bs, 1H, NH); ^{13}C-NMR ($CDCl_3$) 39.15, 43.52, 114.63, 117.32, 125.42, 126.35, 126.45, 128.63, 132.42, 133.35, 133.42, 138.63, 141.53. ESI-MS m/z: 258.02 and Anal. Calcd for $C_{13}H_{10}N_2S_2$; C, 60.43; H, 3.90; N, 10.84; S, 24.82, Found: C, 60.35; H, 3.82; N, 10.68; and S, 24.69%.

15-Oxa-16-aza-6,11-bisthiagona-1,3,5(10),8,13,16-hexaene (11): Compound **11** (0.53g, 58%) was formed as a colourless solid. mp 149-152 ^{0}C; FT-IR 942, 1070, 1486, 1528, 2815, 2385cm^{-1}; ^{1}H-NMR($CDCl_3$) 3.4 (s, 2H, -S-C<u>H</u>$_2$, at C_7), 4.1 (s, 2H, -S-C<u>H</u>$_2$, at C_{12}), 7.1-7.5 (m, 4H, aromatic), 8.7 (s, 1H, C<u>H</u>=N); ^{13}C-NMR ($CDCl_3$) 39.42, 44.12, 100.32, 117.52, 125.46, 125.63, 125.73, 128.42, 133.42, 138.42, 141.53, 150.42, 158.63. ESI-MS m/z: 259.02 and Anal. Calcd for $C_{13}H_9NOS_2$; C, 60.20; H, 3.50; N, 5.40; S, 24.73, Found: C, 60.15; H, 3.43; N, 5.38; and S, 24.68%.

15,16-Bisaza-6,11-bisthiagona-1,35(10),8,13,17-hexaene-16-one (13): Compound **13** (0.53g, 58%) was obtained as a colourless solid. mp 162-165 ^{0}C; FT-IR 1055, 1234, 1476, 1642, 1872, 1927, 2386, 2815, 2861, 2880, 3480 cm^{-1}; ^{1}H-NMR(DMSO-d_6) 3.4 (s, 2H, -S-C<u>H</u>$_2$, at C_7), 3.5 (s, 2H, -S-C<u>H</u>$_2$, at C_{12}), 7.2-7.5 (m, 4H, aromatic), 7.5 (s, 1H, C<u>H</u>=N), 8.2 (bs, 1H, NH); ^{13}C-NMR(DMSO-d_6) 22.43, 34.57, 100.32, 117.52, 125.23, 125.24, 125.36, 128.47, 133.42, 138.24, 138.42, 140.52, 156.42 (C=O). ESI-MS m/z: 286.35 and Anal. Calcd for $C_{14}H_{10}N_2OS_2$; C, 58.72; H, 3.52; N, 9.78; S, 22.39, Found: C, 58.63; H, 3.49; N, 9.64; and S, 22.21%.

15,17-Bisaza-6,11-bisthiagona-1,3,5(10),8,13,17-hexaene-16-thione (15): Compound **15** (0.53g, 58%) was got as a colourless solid. mp 171-173 ^{0}C; FT-IR 1045, 1110, 1143, 1218, 1475, 1553, 1580, 2390, 2815, 2868, 2884, 3140, 3480 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.0 (bs, 1H, NH), 3.4 (s, 2H, -S-C<u>H</u>$_2$, at C$_7$), 3.5 (s, 2H, -S-C<u>H</u>$_2$, at C$_{12}$), 7.2-7.6 (m, 4H, aromatic) 7.9 (s, 1H, C<u>H</u>=N); ^{13}C-NMR (DMSO-d_6) δ 22.41, 34.51, 98.35, 117.42, 125.82, 126.42, 126.85, 128.64, 133.54, 138.65, 140.32, 151.42, and 176.53 (C=S). ESI-MS m/z: 302.01 and Anal. Calcd for $C_{14}H_{10}N_2S_3$; C, 55.60; H, 3.33; N, 9.26; S, 31.81, Found: C, 55.59; H, 3.26; N, 9.12; and S, 31.67%.

15,17a-Bisaza-6,11-thiagona-1,3,5(10),8,13,17a-hexaene-(15, 17a)-benzodiazepines (17): Compound **17** (0.53g, 58%) was obtained as a yellow solid. mp 183-185 ^{0}C; FT-IR 1206, 1553, 1570, 2206, 2788, 2865, 2883, 2916, 3452, 3780 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 3.3 (s, 2H, -S-C<u>H</u>$_2$, at C$_7$), 3.4 (s, 2H, -S-C<u>H</u>$_2$, at C$_{12}$), 4.0 (bs, 1H, NH), 6.5-7.2 (m, 8H, aromatic) δ 7.7 (s, 1H, C<u>H</u>=N); ^{13}C-NMR (DMSO-d_6) 23.42, 34.63, 96.45, 117.52, 120.42, 123.42, 125.43, 125.84, 128.35, 128.63, 133.42, 137.42, 138.63, 140.53, 145.32, and 163.52. ESI-MS m/z: 334.04 and Anal. Calcd for $C_{19}H_{14}N_2S_2$; C, 68.23; H, 4.22; N, 8.38; S, 19.17, Found: C, 68.04; H, 4.16; N, 8.31; and S, 19.13%.

15,17a-Bisaza-16,17-diphenyl-6,11-bisthiagona-1,3,5(10),8,13, 17a-hexaene- (15.17b)-benzodiazepines (**19):** Compound **19** (0.53g, 58%) was obtained as a yellow solid. mp 214-217 ^{0}C; FT-IR 1108, 1904, 1442, 1481, 1514, 1536, 1580, 1591, 1856, 2380, 2811, 3458 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.0 (bs, 1H, NH), 3.4 (s, 2H, -S-C<u>H</u>$_2$, at C$_7$), 3.4 (d, 1H, C<u>H, *J=5.6*</u>), 3.5 (s, 2H, Ar-S-C<u>H</u>$_2$, at C$_{12}$), 3.5 (dd, 1H, C<u>H, *J=5.6 and 6.0*</u>), 6.9-7.5 (m, 14H, aromatic), δ 7.7 (s, 1H, C<u>H</u>=N); ^{13}C-NMR(DMSO-d_6) δ 24.63, 34.25, 61.24, 65.34, 96.45, 117.52, 125.46, 126.65, 126.76, 126.86, 127.56, 127.83, 128.56, 128.63, 128.86, 133.46, 138.42, 140.53, 143.25, 151.53, 163.47. ESI-MS m/z: 438.10 and Anal. Calcd for $C_{27}H_{22}N_2S_2$; C, 73.94; H, 5.06; N, 6.39; S, 14.62, Found: C, 73.64; H, 4.98; N, 6.32; and S, 14.51%.

2,3,5,6-tetrahydro-3-(hydroxymethylene)4H-thiopyrano[3,2-d][1]benzothiepin-4-one (6): An analogues procedure for **5** was carried out to give **6** (0.689, 62% yield). An analytical product of **6** was obtained by recrystallization from ethanol, mp 118-121^{0}C; FT-IR 1209, 1503, 1617, 1869, 3542 cm^{-1}; ^{1}H-NMR(CDCl$_3$) 2.3 (t, 2H, -S-CH$_2$-C<u>H</u>$_2$, at C$_{7a}$), 2.8 (t, 2H, -S-C<u>H</u>$_2$-CH$_2$, at C$_7$), 4.2 (s, 2H, -S-C<u>H</u>$_2$, at C$_{12}$), 6.1 (s, 1H, C<u>H</u>=N), 7.2-7.5 (m, 4H, aromatic); ^{13}C-NMR(CDCl$_3$) 22.86, 28.35, 34.52, 115.63, 121.43, 125.63, 126.75, 127.86, 128.63, 134.84, 139.54, 161.53, 173.36, 187.63. ESI-MS m/z: 276.01 and Anal. Calcd for $C_{14}H_{12}O_2S_2$; C, 60.84; H, 4.38; S, 23.20, Found: C, 60.72; H, 4.29, and S, 23.16%.

New pyrazole-, isoxazole-, pyrimidine- and benzodiazepine derivatives (8, 10, 12, 14, 16, 18, and 20) from 2,3,5,6-tetrahydro-3-(hydroxymethylene)4H-thiopyrano[3,2-d][1]benzothiepin-4-one:

General Procedure: A similar procedure as in 5. 2. 2 afforded the crude pyrazole-, isoxazole-, pyrimidine- and benzodiazepine derivatives (**8**, **10**, **12**, **14**, **16**, **18**, and **20**), which were purified by column chromatography on silica gel using petroleum ether (40-60^o C): ethyl acetate as the eluting system.

B-Homo-16,17-bisaza-16-phenyl-6,11-bisthiagona-1,3,5(10),9,14,17-hexaene (8): Compound **8** (0.63g, 61%) was obtained as a yellow solid. mp 166-169 ^{0}C; FT-IR 1061, 1209, 1508, 1628, 2808, 2378 cm^{-1}; ^{1}H-NMR(CDCl$_3$) 2.2 (t, 2H, -S-CH$_2$-C<u>H</u>$_2$, at C$_{7a}$), 2.8 (t, 2H, -S-C<u>H</u>$_2$-CH$_2$, at C$_7$), 4.1 (s, 2H, -S-CH$_2$, at C$_{12}$), 7.1-7.5 (m, 9H, aromatic), 8.3 (s, 1H, CH=N); ^{13}C-NMR (CDCl3) 35.28, 38.64, 43.96, 117.85, 119.46, 120.63, 120.86, 125.69, 126.64, 126.78, 126.86, 128.74, 129.58, 129.76, 133.67, 138.75, 139.85, 140.96, and 142.35. ESI-MS m/z: 348.06 and Anal. Calcd for $C_{20}H_{16}N_2S_2$; C, 68.93; H, 4.63; N, 8.04; S, 18.40, Found: C, 68.82; H, 4.53; N, 7.98; and S, 18.36%.

B-Homo-16,17-bisaza-6,11-bisthiagona-1,3,5(10),9,14,17-hexaene (10): Compound **10** (0.56g, 58%) was got as a colourless solid. mp 151-153 ^{0}C; FT-IR 1056, 1206, 1518, 2810, 2928 cm^{-1}; ^{1}H-NMR(CDCl$_3$) δ 2.3 (t, 2H, -S-CH$_2$-C<u>H</u>$_2$, at C$_{7a}$), 2.8 (t, 2H, -S-C<u>H</u>$_2$-CH$_2$, at C$_7$), 4.3 (s, 2H, Ar-S-CH$_2$, at C$_{12}$), 7.2-7.5 (m, 4H, aromatic), 7.8 (s, 1H, CH=N); ^{13}C-NMR(CDCl$_3$) δ 34.62, 38.33, 44.86, 113.63, 117.83, 126.53, 127.58, 127.59, 129.63, 133.62, 134.25, 134.63, 138.25, 1425.63. ESI-MS m/z: 272.02 and Anal. Calcd for $C_{14}H_{12}N_2S_2$; C, 61.73; H, 4.44; N, 10.28; S, 23.54, Found: C, 61.68; H, 4.32; N, 10.13; and S, 23.49%.

B-Homo-16-oxa-17-aza-6,11-bisthiagona-1,3,5(10),9,14,17-hexaene (12): Compound **12** (0.51g, 56%) appeared as a colourless solid. mp 155-158 ^{0}C; FT-IR 938, 1065, 1482, 1524, 2810, 2381cm^{-1}; ^{1}H-NMR(CDCl$_3$) δ 2.3 (t, 2H, -S-CH$_2$-C<u>H</u>$_2$, at C$_{7a}$), 2.8 (t, 2H, -S-C<u>H</u>$_2$-CH$_2$, at C$_7$), 4.2 (s, 2H, -S-CH$_2$, at C$_{12}$), 7.2-7.5 (m, 4H, aromatic), 8.9 (s, 1H, CH=N); ^{13}C-NMR (CDCl$_3$) 35.63, 38.42, 43.62, 99.86, 118.96, 125.35, 126.63, 126.75, 129.86, 133.76, 137.96, 142.63, 150.52, 159.86.

ESI-MS m/z: 273.02 and Anal. Calcd for $C_{14}H_{11}NOS_2$; C, 61.51; H, 4.06; N, 5.12; S, 23.46, Found: C, 61.53; H, 3.96; N, 5.03; and S, 23.39%.

B-Homo-16,18-bisaza-6,11-bisthiagona-1,3,5(10),9,14,17-hexaene-17-one (14): Compound **14** (0.42g, 49%) was got as a colourless solid. mp 167-170 ^{0}C; FT-IR 1051, 1230, 1471, 1654, 1863, 1924, 2381, 2810, 2856, 2877, 3478 cm^{-1}; ^{1}H-NMR (DMSO-d_6) 2.3 (t, 2H, -S-CH$_2$-C$\underline{H}_2$, at C_{7a}), 2.8 (t, 2H, -S-C$\underline{H}_2$-CH$_2$, at C_7), 3.5 (s, 2H, -S-CH$_2$, at C_{12}), 7.2-7.8 (m, 4H, aromatic), 7.6 (s, 1H, CH=N), 8.3 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ 23.86, 29.57, 101.12, 117.86, 125.76, 125.86, 126.76, 126.86, 128.62, 133.48, 133.56, 139.12, 139.42, 141.23, 157.63. ESI-MS m/z: 300.03 and Anal. Calcd for $C_{15}H_{12}N_2OS_2$; C, 59.97; H, 4.03; N, 9.33; S, 21.35, Found: C, 59.86; H, 3.98; N, 9.24; and S, 21.26%.

B-Homo-16,18-bisaza-6,11-bisthiagona-1,3,5(10),9,14,17-hexaene-17-thione (16): Compound **16** (0.53g, 58%) was obtained as a colourless solid. mp 177-180 ^{0}C; FT-IR 1040, 1108, 1146, 1214, 1470, 1549, 1578, 2383, 2810, 2864, 2881, 3136, 3476 cm^{-1}; ^{1}H-NMR (DMSO-d_6) 2.0 (s, 1H, NH), 2.2 (t, 2H, -S-CH$_2$-C$\underline{H}_2$, at C_{7a}), 2.7 (t, 2H, -S-C$\underline{H}_2$-CH$_2$, at C_7), 3.5 (s, 2H, -S-CH$_2$, C_{12}), 7.2-7.9 (m, 4H, aromatic), 8.7 (s, 1H, CH=N); ^{13}C-NMR (DMSO-d_6) 13.54, 29.75, 35.64, 99.36, 117.86, 125.63, 126.46, 126.58, 128.75, 133.56, 133.86, 138.96, 140.86, 152.46, 177.85. ESI-MS m/z: 316.02 and Anal. Calcd for $C_{15}H_{12}N_2S_3$; C, 56.93; H, 3.82; N, 8.85; S, 30.40, Found: C, 56.83; H, 3.76; N, 8.69; and S, 30.36%.

B-Homo-16,17b-bisaza-6,11-bisthiagona-1,3,5(10),9,14,17a-hexaene-(16,17b)-benzdiazepines (18): Compound **18** (0.51g, 56%) was got as a yellow solid. mp 189-192 ^{0}C; FT-IR 1202, 1548, 1567, 2201, 2784, 2861, 2879, 2912, 3448, 3778 cm^{-1}; ^{1}H-NMR (DMSO-d_6) 2.2 (t, 2H, -S-CH$_2$-C$\underline{H}_2$, at C_{7a}), 2.8 (t, 2H, -S-C$\underline{H}_2$-CH$_2$, at C_7), 3.5 (s, 2H, -S-CH$_2$, at C_{12}), 4.1 (s, 1H, NH), 7.1-7.5 (m, 8H, aromatic), 7.8 (s, 1H, CH=N) ; ^{13}C-NMR (DMSO-d_6) δ 23.52, 29.83, 33.68, 97.62, 118.62, 120.53, 123.52, 125.96, 126.57, 126.75, 128.86, 129.86, 133.54, 139.76, 139.85, 140.64, 141.63, 144.98, and 164.52. ESI-MS m/z: 348.06 and Anal. Calcd for $C_{20}H_{16}N_2S_2$; C, 68.93; H, 4.63; N, 8.04; S, 18.40, Found: C, 68.85; H, 4.58; N, 7.98; and S, 18.31%.

B-homo-16,17b-bisaza-17,17a-diphenyl-6,11-bisthiagona-1,3,5(10),9,14,17a-hexaene-(16,17b)-diazepines (20): Compound **20** (0.48g, 51%) appeared as a yellow solid. mp 221-224 ^{0}C; FT-IR 1104, 1901, 1438, 1476, 1509, 1530, 1575, 1587, 1851, 2377, 2809, 3454 cm^{-1}; ^{1}H-NMR(DMSO-d_6) 1.91 (s, 1H, NH), 2.2 (t, 2H, -S-CH$_2$-C$\underline{H}_2$, at C_{7a}), 2.8 (t, 2H, -S-C$\underline{H}_2$-CH$_2$, at C_7), 3.4 (d, 1H, CH, *J= 5.6*), 3.5 (s, 2H, -S-CH$_2$, at C_{12}), 4.5 (dd, 1H, CH, *J=5.6 and 6.0*), 6.9-7.5 (m, 14H, aromatic) 7.8 (s,1H, CH=N); ^{13}C-NMR(DMSO-d_6) δ 29.64, 33.48, 35.67, 61.98, 66.75, 96.86, 117.64, 125.86, 126.45, 126.46, 126.57, 126.75, 127.64, 127.84, 128.63, 128.87, 128.91, 133.72, 138.64, 140.54, 143.74, 151.86, 164.56. ESI-MS m/z: 452.02 and Anal. Calcd for $C_{28}H_{24}N_2S_2$; C, 74.30; H, 5.34; N, 6.19; S, 14.17, Found: C, 74.18; H, 5.21; N, 6.01; and S, 14.03%.

Antimicrobial evaluation: Standard sterilized filter paper discs (5 mm diameter) impregnated with a solution of the test compound in DMSO (1.0 µg/mL) were placed on an agar plate seeded with the appropriate test organism in triplicates. The utilized test organisms were *Staphylococcus aureus* and *Streptococcus pneumoniae*, as examples of Gram-positive bacteria and *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli* as examples of Gram-negative bacteria. They were also evaluated for their *in vitro* antifungal activity against *Candida albicans*, *Aspergillus flavus*, and *Aspergillus niger* strains. Chloroamphenicol and amikacin were used as standard antibacterial agents and clotrimazole was used as a standard antifungal agent. DMSO alone was used as a control at the above mentioned concentration. The plates were incubated at 37 ^{0}C for 24h for bacteria and 28 ^{0}C for 48h for fungi. Compounds that showed significant growth inhibition zones (>20 mm) using the two-fold serial dilution technique were further evaluated for their minimal inhibitory concentrations (MICs).

Minimal inhibitory concentration (MIC) measurement: Broth dilution test was used to determine the MIC of the above mentioned samples [33, 34]. The micro dilution susceptibility test was used for the determination of antibacterial- and antifungal activity. Stock solutions of the tested compounds, and those of chloroamphenicol, amikacin, and clotrimazole were prepared in DMSO at concentrations of 1000 µg/mL followed by a two-fold dilution at concentrations of 500, 250, ….. 3.125 µg/mL. All the plates were incubated at 37 ^{0}C for 24h for bacteria and at 28 ^{0}C for 48h for fungi. Control experiments were also carried out.

Antituberculosis activity: All the compounds were screened for their *in vitro* antituberculosis activity against MTB. The antituberculosis activity of the compounds was tested by 'resazurin microplate assay (REMA)' as per Martin *et al* [35, 36]. MTB H_{37}Rv was grown in Middlebrook 7H11 broth medium supplemented with 10% OADC

(oleic acid, albumin, dextrose, and catalase (1, 10, 100 µg/L). After incubation at 37 ºC for 7 days, 15.0 µL of 0.01% resazurin (Sigma, St. Louis. MO, USA) solution in sterile water was added to the first growth control wells and incubated for 24h. Once the first set of growth controls turned pink, the dye solution was added to the second set of growth controls and the test wells, and incubated for 24h at 37 ºC. Blue color in the wells containing the test compounds would indicate inhibition of growth and pink would indicate lack of inhibition of growth of *M. tuberculosis*. The minimal inhibitory concentration (MIC) is defined as the minimum concentration of compound required for 99.9% inhibition of bacterial growth.

Antitumor activity: The *in vitro* antitumor activity was analyzed by MTT assay method [37, 38]. The human cervical cancer cell line (HeLa) and colon cancer cell line (HCT116) were obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagle's Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). The cells were maintained at 37 ^{0}C, 5% CO_2, 95% air, and 100% relative humidity. Maintenance cultures were passaged weekly and the culture medium was changed twice a week.

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions. The viable cells were counted using a hemocytometer and diluted with a medium containing 5% FBS to give the final density of 1x105 cells/mL. One hundred microlitres per well of cell suspension were seeded into 96-well plates at a plating density of 10,000 cells/well and incubated to allow for the cell attachment at 37 ^{0}C, 5% CO_2, 95% air, and 100% relative humidity. After 24h, the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat dimethylsulfoxide (DMSO) to prepare the stock (200 mM) and stored frozen prior to use. At the time of drug addition, an aliquot of the frozen concentrate was thawed and diluted twice the desired final maximum test concentration with serum-free medium. Additional three, two-fold serial dilutions were made to provide a total of four drug concentrations. Aliquots of 100 µL of these different drug dilutions were added to the appropriate wells already containing 100 µL of the medium, resulting in the required final drug concentrations. Following the drug addition, the plates were incubated for an additional 48h at 37 ^{0}C, 5% CO_2, 95% air, and 100% relative humidity. The medium without compound samples served as control and a triplicate was maintained for all concentrations.

MTT is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. The amount of formazan produced is directly proportional to the number of viable cells. After 48h of incubation, 15.0 µL of MTT (5.0 µg/mL) in phosphate buffered saline (PBS) was added to each well and incubated at 37 ^{0}C for 4h. The medium with MTT was then flicked off and the precipitated formazan crystals were solubilized in 100.0 µL of DMSO and the absorbance measured at 570nm using micro plate reader.

The % cell inhibition was determined using the following formula.

% cell inhibition = 100-Abs (sample)/Abs (control) x100

Nonlinear regression graph was plotted between % cell inhibition and log10 concentration and IC50 was determined using Graph Pad Prism software.

DNA cleavage activity: The synthesized compounds were added separately to the CT-DNA sample. The mixtures were incubated at 37 °C for 2 h. The efficiency of DNA cleavage was studied from agarose gel electrophoresis. Nutrient broth media was used (peptone 10 gL^{-1}, NaCl 10 gL^{-1} and yeast extract 5 gL^{-1}). The electrophoresis of the samples was done according to the reported procedure [39]. Briefly, 250 mg of agarose was dissolved in 25 mL of tris- acetate-EDTA (TAE) buffer (4.84 g Tris base, pH 8.0, 0.5 M EDTA L^{-1}) by boiling, when the gel attains ~55°C, it was poured into the gel cassette fitted with comb. The gel was allowed to solidify and then carefully the comb was removed. The gel placed in the electrophoresis chamber flooded with TAE buffer. 20.0 µL of DNA sample (mixed with bromophenol blue dye in 1:1 ratio) was loaded carefully into the wells, along with standard DNA marker with the constant 50 V of electricity for 45 min. Later, the gel was removed carefully and stained with ethidium bromide (ETBR) solution (10 µg mL^{-1}) for 10-15 min and the bands were observed under UV gel documentation system.

RESULTS AND DISCUSSION

Chemistry: Preparation of the pyrazole-, isoxazole-, pyrimidine-, and benzodiazepine derivatives was performed following the synthetic route described in **Scheme 1**. The starting ketones **3** and **4** were prepared following the reported method [26]. The starting key intermediates 3-(hydroxymethylene)-2,3-dihydrothiopyrano[3,2-c]thiochroman-4(5H)-one (**5**) and 2,3,5,6-tetrahydro-3-(hydroxymethylene)4H-thiopyrano[3,2-d][1]benzothiepin-4-one (**6**) were obtained reacting ethylformate with **3** and **4**. Condensation of compounds **5** and **6** with phenylhydrazine hydrochloride, hydrazine

hydrochloride, hydroxylamine hydrochloride, urea, thiourea, o-phenylenediamine, and 1,2-diphenylethane-1,2-diamine afforded the target series **7**-**20**.

Reaction of 1 equiv of the compound **5** with 1.15 equiv of phenylhydrazine hydrochloride/hydrazine hydrochloride under reflux in ethanol afforded the pyrazole derivatives **7** and **9**. Compounds **8** and **10** were obtained from **6** in a similar way. The isoxazole moiety in compounds **11** and **12** was built from the compounds **5** and **6** with 1.5 equiv of hydroxylamine hydrochloride and the pyrimidine derivatives **13**, **14**, **15**, and **16** were prepared from the compounds **5** and **6** with 1.5 equiv of urea/thiourea. The benzodiazepine derivatives **17**, **18**, **19**, and **20** were prepared from the condensation of the compounds **5** and **6** with 1.5 equiv of *o*-phenylenediamine/1,2-diphenylethane-1,2-diamine.

The structures of compounds **7**-**20** have been arrived at on the basis of their IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral data (*vide* supplementary material). The IR spectra of pyrazole derivatives (**7**, **8**, **9**, and **10**) show the pyrazole ring absorption bands at ≈ 1206-1214 cm^{-1}. The ^{1}H-NMR spectra of **7** and **9** have two singlets each at ≈ 3.4 (2H, -S-CH_2 at C_7) and 4.1 (2H, -S-CH_2 at C_{12}) ppm, while compounds **8** and **10** display two triplets each at 2.2 (2H, -S-CH_2-CH_2 at C_{7a}) and 2.8 (2H, -S-CH_2-CH_2 at C_7) ppm and a singlet each at 4.1 (s, 2H, -S-CH_2 at C_{12}). The C=C and C=N absorb in the region 141.5-141.6 and 138.6-140.5 ppm respectively in the ^{13}C-NMR.

In the IR spectra of the isoxazole derivatives **11** and **12,** a characteristic strong band in the range ≈ 938-942 cm^{-1} is assigned to the stretching of the isoxazole ring. The ^{1}H-NMR of compound **11** showed two singlets at 3.4 (2H, -S-CH_2 at C_7) and 4.1 (2H, S-CH_2 at C_{12}) ppm, while compound **12** showed two triplets at 2.2 (2H, -S-CH_2-CH_2 at C_{7a}) and 2.8 (2H, -S-CH_2-CH_2 at C_7) ppm and a singlet at 4.2 (s, 2H, -S-CH_2 at C_{12}). The ^{13}C-NMR displays the C=<u>C</u>-O and <u>C</u>=N signals in the range 158.6-159.8 and 150.4-150.5 ppm respectively for **11** and **12**.

IR spectra of the pyrimidine derivatives (**13**, **14**, **15**, and **16**) showed the pyrimidine ring absorption bands at ≈ 1045-1055cm^{-1}. Compounds **13** and **14** displayed $\upsilon_{C=O}$ at 1642-1654cm^{-1}, while compounds **15** and **16** displayed $\upsilon_{C=S}$ at 1143 and 1146 cm^{-1} respectively. The ^{1}H-NMR spectra of compounds **13** and **14** showed a two singlets at 3.4 (2H, S-CH_2 at C_7) and 3.5 (2H, S-CH_2 at C_{12}) ppm respectively, while compounds **15** and **16** showed two triplets at 2.3 (2H, -S-CH_2-C<u>H</u>$_2$ at C_{7a}) and 2.8 (2H, -S-C<u>H</u>$_2$-CH_2 at C_7) ppm and a singlet at 3.5 (s, 2H, -S-CH_2 at C_{12}). The <u>C</u>=O absorbs at 156.45 and 157.6 ppm (**13** and **14**), the NH<u>C</u>O at 140.5 and 141.2 ppm (**13** and **14**), and the <u>C</u>=S (**15** and **16**) absorbs at 176.5 and 177.8 ppm [25c] respectively in the ^{13}C-NMR.

IR spectra of the benzodiazepine derivatives (**17**, **18**, **19**, and **20**) showed benzodiazepine ring absorption band in the range ≈ 1108-1110 cm^{-1}. The ^{1}H-NMR spectra of compounds **17** and **19**, show a two singlets each at 3.4 (2H, -S-CH_2 at C_7) and 3.5 (2H, S-CH_2 at C_{12}) ppm, while compound **18** and **20** showed two triplets at 2.2 (2H, -S-CH_2-C<u>H</u>$_2$ at C_{7a}) and 2.8 (-S-C<u>H</u>$_2$-CH_2 at C_7) ppm and a singlet each at 3.5 (s, 2H, -S-CH_2 at C_{12}). The methine protons of compounds **19** and **20** resonate in the range 3.4-4.5 ppm and the NH proton absorbed at 1.9 ppm. The C=C (C_{13}-C_{14}) of **17**, **18**, **19**, and **20** absorbs in the range 151.3-151.8 ppm and <u>C</u>=N of **17**, **18**, **19**, and **20** absorbs in the range 163.4-164.5 ppm respectively in the ^{13}C-NMR.

Biological evaluation

Antimicrobial evaluation: All the fourteen newly synthesized compounds (**7**-**20**) were evaluated for their *in vitro* antibacterial activity against *Staphylococcus aureus*, and *Streptococcus pneumoniae*, as examples of Gram-positive bacteria and *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli* as examples of Gram-negative bacteria. They were also evaluated for their *in vitro* antifungal activity against *Candida albicans*, *Aspergillus flavus*, and *Aspergillus niger*. Agar-diffusion method was used for the determination of the preliminary antibacterial and antifungal activity. Chloroamphenicol, amikacin, and clotrimazole were used as reference drugs. The results were recorded for each tested compound as the average diameter of inhibition zones (IZ) of bacterial or fungal growth around the discs in mm. The minimal inhibitory concentration (MIC) measurement for these compounds showed significant growth inhibition zones (>20 mm) by a twofold serial dilution method [27]. The MIC (µg/mL) and inhibition zone diameter values are recorded in Table 1.

The results depicted in Table 1 revealed that most of the tested compounds displayed variable inhibitory effects on the growth of the tested Gram-positive and Gram-negative bacterial strains, and also against fungal strains. In general, these compounds revealed better activity against the Gram-negative rather than the Gram-positive bacteria. It would also be noticed that the pyrazole-, isoxazole-, and pyrimidine derivatives exhibited better antibacterial ability than the benzodiazepine derivatives.

Regarding the structure-activity-relationship (SAR) against Gram-negative bacteria, the results revealed that the pyrazole-, isoxazole-, pyrimidine-, and benzodiazepine derivatives exhibited broad spectrum antibacterial profile against the tested organisms. In this view, 1,2-diphenylethane-1,2-diamine substituted 2,3-dihydrothiopyrano [3,2-c] thiochromen-4(5H)-one (**19**) and 2,3,5,6-tetrahydro-4H-thiopyrano[3,2-d][1]benzothiepin-4-one (**20**) derivatives were found to exhibit higher activity (MIC 3.125 µg/mL) than that of the standard drug, chloroamphenicol (MIC 6.25 µg/mL), against *Klebsiella pneumoniae* and *Escherichia coli*. The benzodiazepine derivatives displayed higher activity than amikacin in inhibiting the growth of *Escherichia coli* (MIC 3.125 µg/mL). The results indicated that the isoxazole-, and pyrimidine derivatives showed a comparable activity against clotrimazole, whereas the benzodiazepine derivatives are equipotent to clotrimazole in inhibiting the growth of *Candida albicans* (MIC 3.125 µg/mL).

***In vitro* antituberculosis activity:** All the synthesized compounds were screened for their *in vitro* antituberculosis activity against MTB ($H_{37}Rv$). The primary screening was carried out by agar dilution method using two fold dilution techniques. Isoniazid (INH) was used as a standard drug. The benzodiazepine derivatives displayed better antituberculosis activity compared to the pyrazole-, isoxazole-, and pyrimidine derivatives. The observed data on the antituberculosis activity of the title compounds and the standard drug are given in **Table 2**. Ten compounds were found to be active with minimum inhibitory concentrations of 6.5-16 µM. The 1,2-diphenylethane-1,2-diamine substituted 2,3-dihydrothiopyrano[3,2-c] thiochromen-4(5H)-one (**19**) and 2,3,5,6-tetrahydro-4H-thiopyrano[3,2-d][1]benzothiepin-4-one derivatives (**20**) showed good inhibitory activity against MTB at MIC 7.7 and 7.3 µM, while compounds **17** and **18** showed moderate inhibitory activity against MTB at MIC 10-11 µM (**Table 2**).

***In vitro* antitumor activity:** The newly synthesized compounds **7-20** were initially screened at a single concentration of two fold dilution using the colorimetric MTT to test their *in vitro* cytotoxicity against HeLa (cervical cancer cells) and HCT116 (colon cancer cells). Doxorubicin was used as the reference drug in this study. The cytotoxicity of the tested compounds was estimated in terms of percent growth inhibition compared to untreated control cells. All the compounds effected >70% inhibition and were retested by a two-fold dilution from 6.25 to 100 µM. The results are expressed as IC_{50} (inhibitory concentration 50%), the concentration of the compound which inhibits the tumor cell growth by 50%, and the data are presented in **Table 2** and **Figs. 1** and **2**.

Cell growth inhibition was analyzed by MTT assay and the results show that compounds **7-20** exhibit an inhibitory effect on the proliferation of HeLa and HCT116 cells in a dose-dependent manner (**Table 2**). Compounds **19** and **20** were found to exhibit higher cytotoxic potency (13, 12 µM and 12, 10 µM) than that of doxorubicin (21 and 19 µM) against HeLa cell and HCT116 cell. The 1,2-diphenylethane-1,2-diamine-substituted thiochromeno- and benzothiepeno derivatives (**19** and **20** respectively) have higher ability than the rest on both the cancer cell lines. The *o*-phenylenediamine- substituted compounds **17** and **18** show comparable IC_{50} values than the other substituted compounds on both the cells. Neverthales, many of the IC_{50} values for HCT116 cells are lower than those for the corresponding HeLa cells.

Benzodiazepine core-unit ('privileged structure') is an example of a G-protein coupled receptor (GPCR) [28, 29]. Data summarized above (**Table 2**) regarding compounds **9** and **10** is in accordance with the results that compounds containing the N-C-C-N pharmacophore site act significantly as highly selective κ-opioid agonists [30], antischistosomal drugs *etc.* [32]. Synthesis of structurally such similar compounds is under progress in our laboratory.

DNA Cleavage activity: The cleavage reaction was monitored by gel electrophoresis. The electrophoresis clearly revealed that, the compound 7-20 have acted on DNA as there was difference in the bands of lanes 7–20 compared to the control DNA (Fig. 3). In this study, the CT-DNA gel electrophoresis experiment was conducted at 35 ºC using the synthesized compounds in the presence of H_2O_2 as an oxidant. From Fig. 3, it is observed that the control CT-DNA (lane C) does not show any significant cleavage even on longer exposure of time. Compounds **17-20** have completely cleaved and compounds **7-16** have partially cleaved the DNA. As these compounds were observed to cleave the DNA, it can be concluded that they inhibit the growth of the pathogenic organism by cleaving their genome.

CONCLUSION

We have synthesized and investigated the antimicrobial-, antituberculosis-, and antitumor activity of some new pyrazole-, isoxazole-, pyrimidine- and benzodiazepine derivatives

containing 3-(hydroxymethylene)-2,3-dihydro thiopyrano[3,2-c]thiochromen-4(5H)-one (**5**) and 2,3,5,6-tetrahydro-3-(hydroxymethylene) 4H-thiopyrano [3,2-d][1] benzothiepin-4-one (**6**) moieties. Results obtained clearly revealed that the 1,2-diphenylene-1,2-diamine substituted 2,3-dihydrothiopyrano[3,2-c]thiochromen-4(5H)-one (**19**) and 2,3,5,6-tetrahydro-4H-thiopyrano[3,2-d][1]benzothiepin-4-one derivatives (**20**) exhibited better antimicrobial activity than their counterparts. Similarly compounds **19** and **20** displayed more antimicrobial-, antituberculosis-, antitumor-, and DNA cleavage activity compared to the other derivatives. In general, the benzodiazepine analogues (**17-20**) exhibited higher activity than the pyrazole-, isoxazole-, and pyrimidine analogues.

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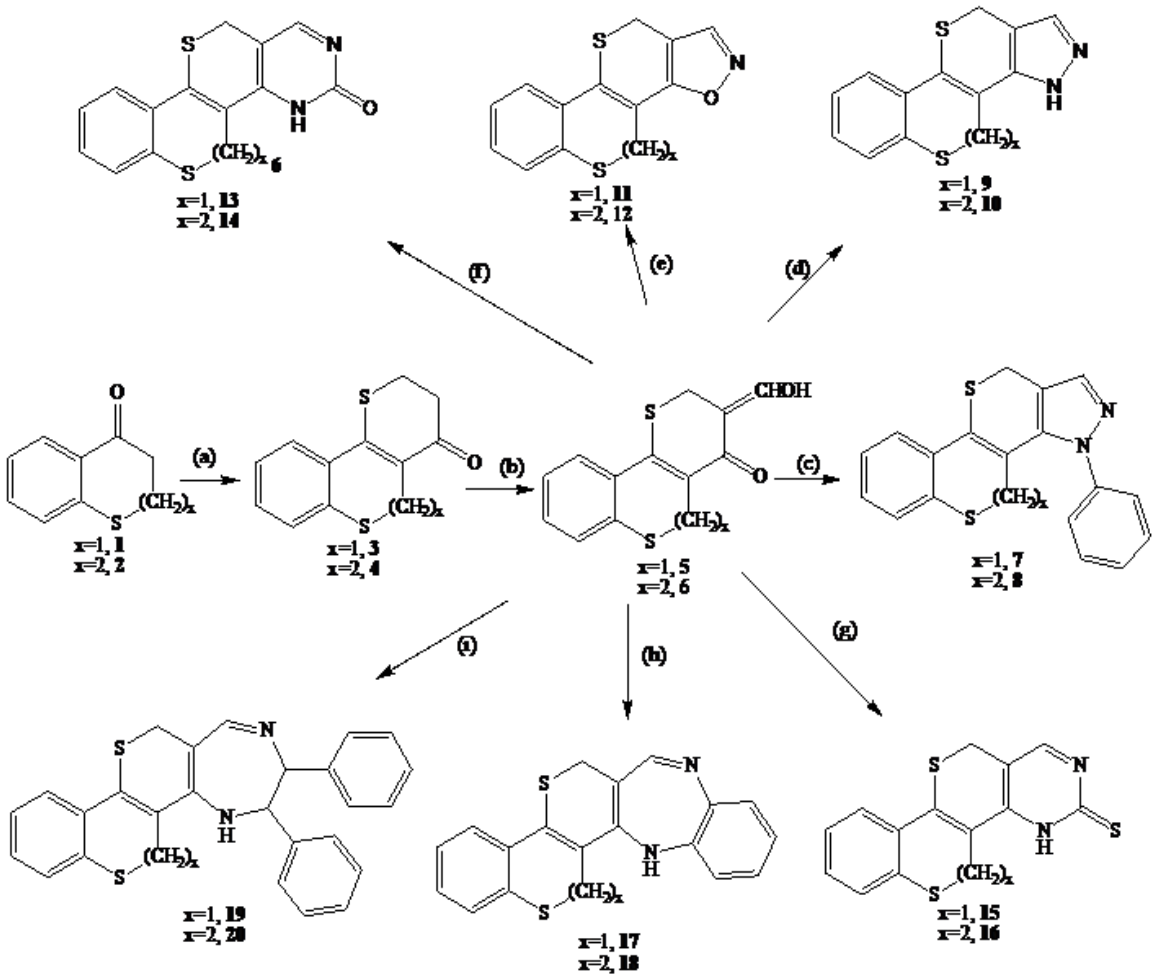

Scheme 1 Synthesis of heterocyclic steroidal analogues (**7-20**). Reagents and conditions:
(a) $ClCH_2CH_2COOH$/NaOH; PPA (b) NaOEt, dry toluene, $HCOOC_2H_5$ (c) $C_6H_5NHNH_2$. HCl, EtOH
(d) NH_2NH_2.HCl, EtOH (e) NH_2OH.HCl, EtOH, (f) urea, CH_3COOH (g) thiourea, CH_3COOH
(h) o-phenylenediamine, CH_3COOH (i) 1,2-diphenylethane-1,2-diamine, CH_3COOH

Table 1. Minimal inhibitory concentrations (MIC, mg/mL) and inhibition zones (mm) of compounds **7-20**

Compound	MIC in µg/mL, and zone of inhibition (mm)							
	Bacteria					Fungi		
	Gram-positive bacteria		Gram-negative bacteria					
	Staphylococcus aureus	*Streptococcus pneumoniae*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Candida albicans*	*Aspergillus flavus*	*Aspergillus niger*
7	25 (30-33)	25 (28-31)	25(25-28)	12.5 (25-28)	25 (26-29)	25 (25-28)	50(24-27)	25(27-30)
9	25 (31.34)	25 (26-29)	25 (29-32)	12.5 (30-33)	25 (31-34)	12.5 (29-32)	25 (26-29)	25 (27-30)
11	25 (29-32)	25 (27-30)	12.5 (28-31)	12.5 (29-32)	25 (28-31)	25 (24-27)	25 (27-30)	12.5 (28-31)
13	25 (28-31)	12.5 (31-34)	25 (27-30)	12.5 (28-31)	6.25 (27-30)	12.5 (28-31)	12.5(26-29)	6.25 (29-32)
15	25 (27-30)	12.5 (29-32)	25 (26-29)	12.5 (30-33)	6.25 (28-31)	25 (29-32)	12.5 (27-30)	6.25 (28-31)
17	25 (31-34)	25 (31-34)	12.5 (27-30)	12.5 (27-30)	25 (27-30)	25 (27-30)	12.5 (28-31)	12.5 (25-28)
19	6.25 (33-35)	6.25 (27-30)	3.125 (31-33)	6.25 (28-31)	3.125 (26-28)	3.125 (31-34)	6.25 (28-31)	3.125 (24-27)
8	25 (31-34)	25 (30-33)	25(27-30)	12.5 (27-30)	25 (28-31)	25 (28-31)	50(26-29)	25(28-31)
10	25 (32-35)	25 (27-30)	25 (29-32)	12.5 (32-35)	25 (33-36)	12.5 (31-34)	25 (28-31)	25 (28-31)
12	25 (30-33)	25 (28-31)	12.5 (29-32)	12.5 (31-34)	25 (30-33)	25 (28-31)	25 (32-35)	12.5 (33-36)
14	25 (31-34)	12.5 (32-35)	25 (28-31)	12.5 (32-35)	6.25 (31-34)	12.5 (30-33)	12.5(27-30)	6.25 (31-34)
16	25 (31-34)	12.5 (30-33)	25 (31-34)	12.5 (32-35)	6.25 (30-33)	25 (31-34)	12.5 (30-33)	6.25 (29-32)
18	25 (32-35)	25 (33-36)	12.5 (28-31)	12.5 (29-32)	25 (29-32)	25 (30-33)	12.5 (30-33)	12.5 (27-30)
20	6.25 (34-37)	6.25 (29-32)	3.125 (33-36)	6.25 (32-35)	3.125 (30-33)	3.125 (32-35)	6.25 (29-32)	3.125 (28-31)
Chloramphe-nicol	3.125 (38-41)	6.25 (38-41)	6.25 (32–35)	6.25 (36–39)	6.25 (34-37)	NT	NT	NT
Amikacin	6.25 (35-38)	6.25 (34-37)	3.125 (37-40)	6.25 (29–32)	6.25 (36–39)	NT	NT	NT
Clotrimazole	NT	NT	NT	NT	NT	6.25 (29-32)	6.25 (28-31)	3.125 (27-30)

NT-Not Tested

Table 2. Antitumor activity and antituberculosis activity of compounds **7-20**

Compound	Antitumor activity IC_{50}(µM)[a]		Antituberculosis activity MIC (µM)
	HeLa cell	HCT116	
7	33	31	15
9	32	28	13
11	27	26	12
13	25	23	13
15	24	21	12
17	20	19	11
19	13	12	7.7
8	30	27	13
10	28	26	12
12	25	24	11
14	23	21	12
16	22	20	11
18	18	17	10
20	12	10	7.3
INH	---[b]	---[b]	8.6
Doxorubicin[c]	21	19	---[b]

Negative control DMSO, no activity.

[a] The IC_{50} value is defined as the concentration at which 50% survival of cells was observed.

[b] Not Tested

[c] Used as a positive control

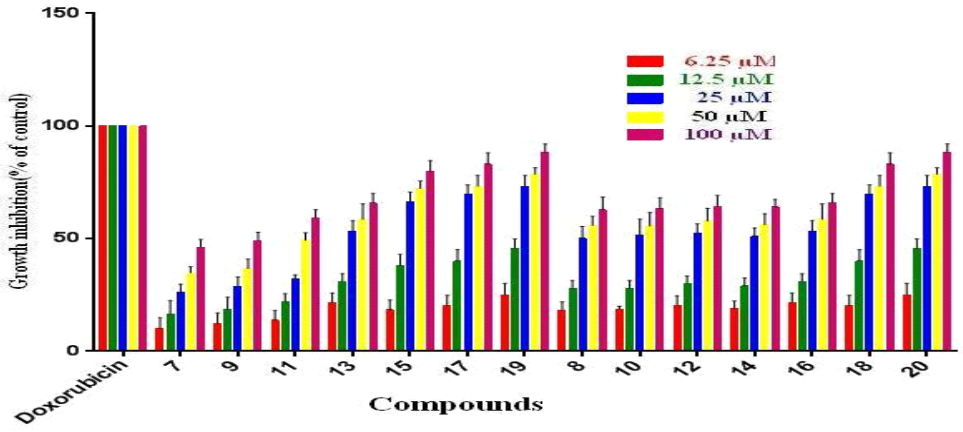

Fig. 1 Effect of the compounds **7-20** on the growth inhibition based on the concentrations on HeLa cells.

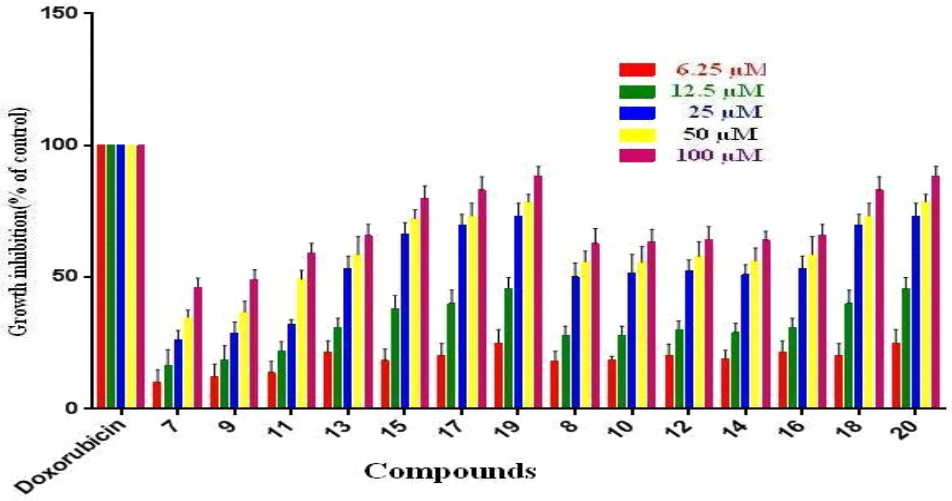

Fig. 2 Effect of the compounds **7-20** on the growth inhibition based on the concentrations on HCT 116 cells.

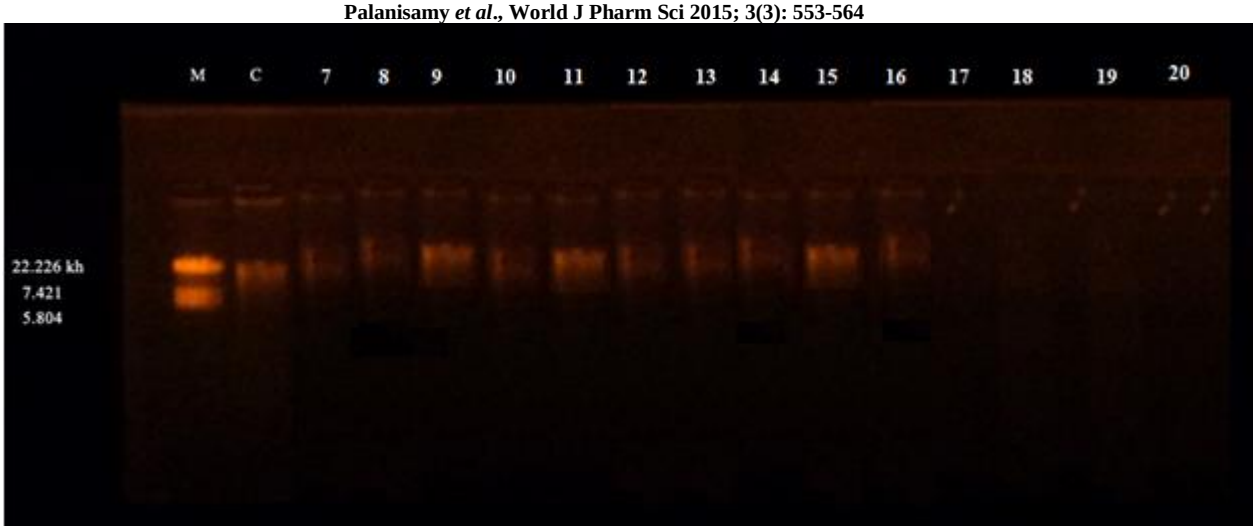

Fig.3 Gel electrophoresis picture of compounds **7-20** showing the effect on CT-DNA.
Lane M: DNA marker; Lane C: untreated DNA.

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