Synthesis, Spectrochemical and Antimicrobial Activity of 2-Amino-7,9-diaryl-6-methyl-8-(2'-amino-1'3'4'-thiadiazolyl)methyl-thia-3,4,8-triazaspiro[4,5]dec-2-ene

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New heterocyclic ketoesters dithiosemicarbazone and corresponding spiro-1,3,4-thiadiazolines are prepared using 2,6-diaryl piperidones as substrates. The synthesized compounds are characterised by elemental analysis, spectral and analytical data. They are also screened for their antibacterial and antifungal activity against *E. coli* and *A. niger*, respectively.

Keywords: Piperidones, Dithiosemicarbazones, Spiro-1,3,4-thiadiazolines, Heterocyclization, Antimicrobial activity.

INTRODUCTION

Complexity of spiro molecules are represented by the quarternary carbon centre and two fused rings^{1,2}. Spirocyclane structure was found in wide range of natural compounds isolated from various sources. A large number of spiro heterocycles are synthesized and their applicability in various fields are tested. 1,3,4-Thiadiazole are associated with diverse biocidal activities by virtue of the toxophonic N=C-S grouping. A large number of thiadiazolines have been reported to be antifungal³, antibacterial⁴, antileukemia agents⁵, optically active liquid crystals⁶, photographic materials, diuretic⁷ and CNS depressants⁸. One of the methods to prepare 1,3,4-thiadiazoline involve cyclization of thiosemicarbazones. 2,6-Diaryl-4-piperidones are reported as substrates for preparing spiro heterocycles. The introduction of an inbuilt spiro system in an heterocyclic ring may cause an increase in the potency of the system. These observations led us to prepare the title compounds and study their antimicrobial activity.

The present work deals with the preparation of title compounds *i.e.* spiro-1,3,4-thiadiazolines from 2,6-diaryl piperidin-4-one as substrates *via* the formation of ketoesters and dithiosemicarbazones⁹⁻¹¹. 2,6-diaryl piperidin-4-one (**1a-c**) are N-substituted by refluxing them with BrCH₂COOC₂H₅ in the presence of acetone and anhydrous K₂CO₃ to form ethyl(3-methyl-4-oxo-2,6-diarylpiperidin-1-yl)acetate (**2a-e**). These ketoesters formed are subjected to nucleophilic addition with thiosemicarbazones (**3a-e**). Title compounds spiro-1,3,4-thiadiazolines (**4a-e**) are prepared by heterocyclizing the various dithiosemicarbazones using sulphuric acid as cyclizing agent followed by neutralization with ammonia.

EXPERIMENTAL

Melting point of the compounds are determined using an open capillary in a Tempo apparatus and are uncorrected. IR spectra is recorded on Perkin Elmer FT-IR 1600 series spectrometer and varian 300 instrument. ¹H NMR and ¹³C NMR are recorded on VARIAN 300 MHZ spectrometer. All chemical shifts are reported in δ ppm using TMS as internal standard. Mass spectra on varian Eric, 410 Proster Binary LC with 500 Ms IT DDA Detector. Elemental analysis was done on CHNS (O) analyser. Model FLASH EA 1112 series.

Ethyl-2-(3-methyl-4-oxo-2,6-diarylpiperidin-1-yl)acetate (2a-e): To a solution of 2,6-diaryl-3-methylpiperidin-4-one (0.01 m) in acetone, ethyl bromoacetate (2 mL) was added and anhydrous K₂CO₃ (2 g) was added to act as a base. The mixture was refluxed for about 15 h on a steam bath and filtered hot. Excess solvent was removed and then poured into ice, filtered, washed with water and recrystallized repeatedly from methanol gave compounds (**2a-e**).

Ethyl-2-(3-methyl-4-oxo-2,6-diphenylpiperidine-1-yl)acetate (2a): m.f. C₂₂H₂₅NO₃; m.p. 59 °C, N (3.98 % calc.); IR (KBr, $\nu_{\max}$, cm⁻¹): 1750-1700 (C=O of COOEt), 1697 (C=O of ring); 3100-3000, 1600 (C=C aryl); 1450 (N=CH₂); ¹H NMR (δ ppm)-0.81 (d, 3H-CH₃) 0.99-1.21 (t-3H, >N-CH₂COOCH₂CH₃), 2.13-2.4 (m, 3H C₃H, C₅H) 3.3-3.6 (m, 2H, C₂(H) and C₆(H)), 3.78 (s, 2H, >N-CH₂-COOEt) 4.11 (t, 3H-CH₂ of ester), 7.3-7.7 (m- 8H aryl H's); ¹³C NMR (δ ppm) 12.3, 13.9, 32.8, 36.8, 50.4 to 52.3, 43.8 to 44.7, 61, 64, 126.9, 129.6, 174.2, 200.7. Mass (*m/e*) 351 (M⁺) 295, (21.9 %) 223 (100 %) 146 (59).

Ethyl-2-(3-methyl-4-oxo-2,6-di(p-anisyl)-piperidin-1-yl)acetate (2b): m.f. C₂₄H₂₉NO₅; m.p. 110 °C; N 7.06 (7.24) %, IR (KBr, $\nu_{\max}$, cm⁻¹): 1750-1700 (C=O of COOEt), 1697 (C=O of ring); 3100-3000, 1600 (C=C aryl); 1450 (N=CH₂); ¹H NMR (δ ppm)-0.81 (d, 3H-CH₃) 0.99-1.21 (t-3H, >N-CH₂COOCH₂CH₃), 2.13-2.4 (m, 3H C₃H, C₅H) 3.3-3.6 (m, 2H, C₂(H) and C₆(H)), 3.78 (s, 2H, >N-CH₂-COOEt) 4.11 (t, 3H-CH₂ of ester), 7.3-7.7 (m- 8H aryl H's); ¹³C NMR (δ ppm) 12.3, 13.9, 32.8, 36.8, 50.4 to 52.3, 43.8 to 44.7, 61, 64, 126.9, 129.6, 174.2, 200.7. Mass (*m/e*) 351 (M⁺) 295, (21.9 %) 223 (100 %) 146 (59).

calc (obs) IR (KBr, $\nu_{\max}$, cm^{-1}): 3020-2929 (C-H aryl) 2841 (C-H ali) 1744 (C=O ester); 1685 (C=O ring) 1604 (C=C aryl); 1425 (N-CH₂); 1263 (C-O of aryether). ¹H NMR. (1.2 to 1.3, 3H, CH₃), 3.01 (2H, C₃-H, C₅-H) 3.23 (2H, C₆-H) 3.1 (S, 6H, OCH₃) 4.2 to 4.3 (9.1, CH₂ of ester and N-CH₂) 6.7 to 7.7 (m Ar-H) ¹³C NMR 11.0, 13.9, 20.6, 20.7, 49.7 to 50.1, 59.4, 63.9, 70.5, 127.2 to 138.8 (Ar-C) 169.9 (< C=O of ester 208.2 (C=O of ring)).

Ethyl-2(3-methyl-4-oxo-2,6-di(*p*-N,N-dimethylamino-phenyl)piperidin-1-yl)acetate (2c): m.f. C₂₆H₃₅N₃O₅; m.p. 93-95 °C; N 3.98 (3.92) %, calc (obs). IR (KBr, $\nu_{\max}$, cm^{-1}): 2914.4 (C-H aryl) 2822 (C-H ali) 1710 (C=O ester) 1661 (C=O ring) 1600 (C=C aryl), 1432 (N-CH₂). ¹H NMR 0.8 (3H, CH₃) 1.8 (t, 3H, CH₃ of ester) 3.9 (2H, NCH₂) 5.0 to 5.6 (q, 2H, CH₂ of ester) 6.8 to 8 (Ar-H).

Ethyl-2(3-methyl-4-oxo-2,6-di(*p*-tolyl)-piperidin-1-yl)acetate (2d): m.f. C₂₄H₂₉NO₃; m.p. 140-142 °C; N 3.98 (3.88) %, calc(obs) IR (KBr, $\nu_{\max}$, cm^{-1}): 3100-3000 (C-H aryl) 2820-2800 (C-H ali) 1766-1760 (C=O of ester) 1722-1602 (C=O of ring), 1438.5 (N-CH₂) ¹H NMR δ ppm 0.9 (3H, CH₃) 1.1-1.5 (t, 3H, COOCH₂CH₃), 2.0-2.5 (m, C₃-H, C₅-H, aryl CH₃), 3.8 (q, 2H, COOCH₂CH₃), 3.9 (2H, NH₂), 6.8 to 8 (Ar-H). ¹³C NMR δ ppm 10.4 (CH₃ at C₃), 21.05, 23.77 (Ar-CH₃), 29.6 (COOCH₂CH₃), 63.2 (COOCH₂CH₃), 67.0 (N-CH₂), 125.5-141.4 (Ar-C), 166.8 (C=O of ester), 174.6 (C=O of ring).

Ethyl-2(3-methyl-4-oxo-2,6-di(*p*-chlorophenyl)-piperidin-1-yl)acetate (2e): m.f. C₂₂H₂₃NO₃Cl₂; m.p. 133 °C; N 3.98 (3.86) %, calc (obs) IR (KBr, $\nu_{\max}$, cm^{-1}): 3046.4 (C-H aryl), 2900 (C-H ali) 1685.6 (C=O of ester) 1641.2 (C=O of ring), 1602.5 (C=C aryl), 1490.6 (N-CH₂), ¹³C NMR δ ppm 10.4 (CH₃ at C₃), 29.6 (COOCH₂CH₃), 63.2 (COOCH₂CH₃), 67.0 (N-CH₂), 125.5-141.4 (Ar-C), 166.8 (C=O of ester), 174.6 (C=O of ring).

General procedure for preparation of aceto-2-(3-methyl-2,6-diaryl-4-thiosemicarbazido-piperidin-5-yl)thiosemicarbazide (3a-e): Michael condensation of >C=O group and thiosemicarbazide led to the formation of the dithiosemicarbazones (3a-e).

A mixture of corresponding ester (0.01 m) and thiosemicarbazide (0.02 m) in ethanol (30 mL) were refluxed for 6 h in a steam bath. It was concentrated, cooled and poured into crushed ice. The solid separated was filtered, washed and recrystallized repeatedly from methanol to get half-white crystals of (3a-e).

Aceto-2-(3-methyl-2,6-diphenyl-4-thiosemicarbazido-piperidin-1-yl)thiosemicarbazide (3a)^{12,13}: m.f. C₂₂H₂₇N₇O₂S₂; m.p. 170 °C; N 20.89 (21.02) %, calc (obs) IR¹² (KBr, $\nu_{\max}$, cm^{-1}): 3422 (NH₂), 3252 (2°NH), 3090 (C-H aryl), 2971 (C-H ali), 1709 (C=O), 1588 (C=C aryl), 1493 (C=N), 1453 (N=CH₂), 1258 (C=S). ¹H NMR (δ ppm), 1.2 (d, 3H, CH₃), 2.49 (C₃-H), C₅-H), 3.32-3.81 (C₂-H, C₆-H) 3.79 (2H, N-CH₂), 6.99-7.69 (Ar-H), 8.32 (NH of NHCSNH₂). ¹³C NMR (δ ppm) 10.47 (CH₃), 21.0, 23.7 (C₃, C₅) 38.9, 45.8 (C₂, C₆), 62.9 (N-CH₂) 122.5 to 133.9 (Aryl C's) 157.1 (C=N) 166.8 (-C=S) 174.6 (> C=O) Mass 469 (M⁺), 438 (100 %), 421 (70 %), 304 (65 %), 275 (55 %).

Aceto-2-(3-methyl-2,6-di-*p*-anisyl-1,4-thiosemicarbazido-piperidin-1-yl)thiosemicarbazide (3b): m.f. C₂₄H₃₁N₇O₃S₂; m.p. 226 °C; N 18.52 (18.66) %; S 12.1 (12.36) %, calc(obs). IR (KBr, $\nu_{\max}$, cm^{-1}): 3450-3250 (NH₂, NH), 3049 (C-H aryl) 2925 (C-H ali), 1726 (C=O), 1493 (C=N), 1243 (C=S).

Aceto-2-(3-methyl-2,6-di-*p*-N,N-dimethylaminophenyl)-4-thiosemicarbazido-piperidin-1-yl thiosemicarbazide (3c): ¹H NMR (δ ppm), 0.69-0.79 (d, 3H, CH₃), 3.03-3.12 (6H, OCH₃), 2.49-2.26 (C₃-H), 2.66-2.80 (C₅-H), 2.8-3.4 (6H, N(CH₃)₂), 3.7 (2H, N-CH₂), 3.71-3.86 (C₂-H, C₆-H), 7.16-8.08 (Ar-H), 9.8-10.4 (NH), 10.53 (NH₂). ¹³C NMR (δ ppm) 12 (CH₃), 30.6-40.1 (C₃, C₅), 59.3 (C₂, C₆), 69 (N-CH₂) 126.9-129.2 (Aryl C's) 145 (C=N) 162 (-C=S) 180 (> C=O).

Aceto-2-(3-methyl-2,6-di-*p*-tolyl)-4-thiosemicarbazido-piperidin-1-yl thiosemicarbazide (3d): m.f. C₂₄H₃₁N₇O₂S₂; m.p. 195 °C; N (20.97 (21.02) %; S 10.64 (10.62) %, calc (obs) IR (KBr, $\nu_{\max}$, cm^{-1}): 3443 (NH₂), 3000-2950 (N-H), 2950 (C-H aryl) 2876 (C-H ali), 1726 (C=O), 1445 (C=N), 1247 (C=S) ¹H NMR (δ ppm), 0.69-0.79 (d, 3H, CH₃), 2.07-2.28 (6H, aryl, CH₃), 3.31 (2H, NCH₂), 7.16-8.08 (Ar-H), 9.8-10.4 (NH), 10.53 (NH₂). ¹³C NMR (δ ppm) 13-15 (CH₃), 20.7 (aryl CH₃), 70 (N-CH₂), 126.9-129.2 (Aryl C's) 138 (C=N), 177 (-C=S), 180(C=O).

Aceto-2-(3-methyl-2,6-di-*p*-chlorophenyl)-4-thiosemicarbazido-piperidin-1-yl thiosemicarbazide (3e): m.f. C₂₂H₂₅N₇SO₂Cl₂; m.p. 239 °C; N (20.97(20.98) %, S 10.64 (11.02) %, IR (KBr, $\nu_{\max}$, cm^{-1}): 3434 (NH₂), 3287.1 (N-H), 3027.3 (C-H aryl), 2940.9 (C-H ali), 1718 (C=O), 1596.6 (C=C aryl), 1515(C=N), 1459.1 (N=CH₂), 1280 (C=S) ¹H NMR (δ ppm), 0.92 (3H, CH₃), 2.0-2.4 (C₃-H, C₅-H), 3.8 (2H, NCH₂), 5.0-5.4 (2H, C₂-H, C₆-H), 6.8-8.1 (m, Ar-H), 10.02-10.49 (NH, NH₂). ¹³C NMR (δ ppm) 13 (CH₃), 30 (C₃, C₅), 42 (C₂-C₆), 127.5-132.9 (Ar-C), 144.8 (C=N), 160.16 (-C=S), 177.8 (C=O).

General procedure for the preparation of 2-amino-6-methyl-7,9-diaryl-8(2'-amino-1',3',4'-thiadiazolyl)methyl-1-thia-3,4,8-triaza-spiro[4,5]dec-2-ene(4a-4e): Title compounds (4a-4e) were obtained from their corresponding dithiosemicarbazones (3a-3e) using sulphuric acid as cyclizing agent, neutralized with ammonia and poured into crushed ice. The precipitates formed were filtered and recrystallized repeatedly from methanol to get pure samples.

2-Amino-7,9-diphenyl-6-methyl-8(2'-amino-1',3',4'-thiadiazolyl)methyl-1-thia-3,4,8-triaza-spiro[4,5]dec-2-ene (4a): m.f. C₂₆H₂₉N₇S₂; m.p 102 °C; N 18.31 (18.32) %, S 11.96 (11.92) %, calc (obs) IR (KBr, $\nu_{\max}$, cm^{-1}): 3500 (N-H), 2976 (C-H aryl) 2900 (C-H ali), 1601.7 (C=C aryl), 1492.3 (C=N), 1454.2 (N-CH₂), 1028 (N=N), 754.3 (C-S-C), 1601.79, 1155.72, 1028.11, 754.3 (thiadiazole ring^{14,15}). ¹H NMR (δ ppm), 0.98 (d, 3H, CH₃), 2.35-3 (C₆-H, C₁₀H), 3.34-4.39 (m, C₇H, C₉H), 3.78, 3.93 (N-CH₂), 7.31-7.25 (Ar-H), 10.25-10.4 (NH₂, NH). ¹³C NMR (δ ppm)^{16,17} 13 (CH₃), 21.22 (C₆, C₁₀), 52, 52 (C₇, C₉), 60 (NHCOCH₃) 62 (N-CH₂), 82, 84 (C₅), 121-130 (ArylC's), 140-148 (C=N), 170 (C=S). Mass 561 (M⁺), 530 (9 %), 444 (20 %), 360 (12.25 %), 338.7 (15 %), 326 (35 %), 254 (100 %), 161 (27.5 %), 136 (22.5 %).

2-Amino-7,9-di-(*p*-anisyl)-6-methyl-8(2'-amino-1',3',4'-thiadiazolyl)methyl-1-thia-3,4,8-triaza-spiro[4,5]dec-2-ene (4b): m.f. C₂₈H₃₃N₇O₂S₂; m.p. 103 °C; N (17.34 (17.32) %, S10.75 (10.92) %, calc (obs) IR¹² (KBr, $\nu_{\max}$, cm^{-1}): 3300-3100 (N-H), 2978 (C-H aryl), 2836.09 (C-H ali), 1604.32 (C=C aryl), 1496.99 (C=N), 1439.42 (N-CH₂), 1256.39 (C-OCH₃), 1110.60, 1053.62, 1018.86, 814.9, 616.84 (thiadiazole ring). ¹H NMR (δ ppm), 1.2 (3H, CH₃), 1.8-2.4 (bm, 5H, C₆-H, C₁₀H), 2.8-3.0 (C₇-H, C₉-H), 3.4-4.8H, OCH₃ and NCH₂), 6.8-7.0 (m,

aryl H's), 8.7-9.9-N-H. ^{13}C NMR (δ ppm) 12 (CH_3), 36, 37 ($\text{C}_6, \text{C}_{10}$), 45 (C_7, C_9), 56 (OCH_3), 61 (N-CH_2), 70 (C_5), 127-130 (ArylC's), 142 (C=N), 180 (-C=O), 156 (C=O).

2-Amino-7,9-di-(*p*-N,N-dimethyl aminophenyl)-6-methyl-8(2'-amino-1',3',4',thiadiazolyl)methyl-1-thia-3,4,8-triaza-spiro[4,5]dec-2-ene(4c). m.f. $\text{C}_{30}\text{H}_{39}\text{N}_9\text{S}_2$; m.p. 130°C ; N 21.39 (21.42) %, S 10.86 (10.92) %, calc (obs) IR (KBr, ν_{max} , cm^{-1}): 3422 (N-H), 2922 (C-H aryl), 1622 (C=C aryl), 1548 (C=N), 1384 (N-CH_2), 1246, 1125, 1020, 813, 744, 619, 519 (thiadiazole ring). ^1H NMR (δ ppm), 1.32 (3H, CH_3), 1.678-2.4 ($\text{C}_6\text{-H}, \text{C}_{10}\text{H}$), 3.10-3.19 ($\text{N-(CH}_3)_2$), 3.80 (N-CH_2), 6.28 ($\text{C}_7\text{-H}, \text{C}_9\text{-H}$), 732-776.0 (aryl H's), 9.7-9.9 NH_2 , NH. ^{13}C NMR (δ ppm) 11.02 (CH_3), 13.9 (NHCOCH_3), 30.6 ($\text{-N-(CH}_3)_2$), 20.6, 20.7 ($\text{C}_6, \text{C}_{10}$), 50.1-50.7 (C_7, C_9), 59.4 (N-CH_2), 70.5 (C_5), 127.2-129.9 (ArylC's), 137.1 (C=N), 208 (C=O).

2-Amino-7,9-di(*p*-tolyl)-6-methyl-8(2'-amino-1',3',4'-thiadiazolyl)methyl-1-thia-3,4,8-triazaspiro[4,5]dec-2-ene (4d): m.f. $\text{C}_{22}\text{H}_{27}\text{N}_7\text{S}_2$; m.p. 102°C ; N (21.57) %, found), S (14.18 %, found); IR (KBr, ν_{max} , cm^{-1}): 3418 (NH_2), 3142 (NH), 1587 (C=C aryl), 1493 (C=N), 1454 (N-CH_2), 1289.74, 1073.44 (N-C-S), 751.67 (C-S-C), 699.18 (thiadiazole ring). ^1H NMR (δ ppm), 0.9 (3H, CH_3), 1.2, 1.5 ($\text{C}_6\text{-H}, \text{C}_{10}\text{H}$), 3.5 (2H, N-CH_2), 3.1-3.8 ($\text{C}_7\text{-H}, \text{C}_9\text{-H}$), 6.7-7.4 (aryl H's), 10.25-10.4 (NH_2 and NH). ^{13}C NMR (δ ppm) 14 (CH_3), 39.9, 39.7 ($\text{C}_6, \text{C}_{10}$), 49.7-50.1 (C_7, C_9), 64.3 (N-CH_2), 70.8 (C_5), 126.5-128.8 (ArylC's), 141.8 (C=N). Mass. 451 (M^+), 530 (9 %), 394 (19.1 %), 338 (100 %), 263.1 (31.7 %).

2-Amino-7,9-di(*p*-chlorophenyl)-6-methyl-8(2'-amino-1',3',4'-thiadiazolyl)methyl-1-thia-3,4,8-triazaspiro[4,5]dec-2-ene (4e): m.f. $\text{C}_{24}\text{H}_{31}\text{N}_7\text{O}_2\text{S}_2$; m.p. 63°C ; N (19.98 %, found), S (12.52 %, found), IR (KBr, ν_{max} , cm^{-1}): 3429.7 (NH_2), 2915.4 (C-H aryl), 2849 (C-H ali), 1622.1 (C=C aryl), 1459 (C=N), 1224.9, 1038.5, 837.9, 761.5 (thiadiazole ring). ^1H NMR (δ ppm), 0.8 (d, 3H, CH_3), 1.0-1.6, 1.5 ($\text{C}_6\text{-H}, \text{C}_{10}\text{H}$), 3.32, 3.81 (2H, N-CH_2), 4.0-4.5 ($\text{C}_7\text{-H}, \text{C}_9\text{-H}$), 6.99-7.69 (aryl H's), 8.3-10.0 (NH_2 and NH). ^{13}C NMR (δ ppm) 10.11 (CH_3), 30 ($\text{C}_6, \text{C}_{10}$), 40, 41 (C_7, C_9), 65 (N-CH_2), 84 (C_5), 124-132 (ArylC's), 141-146 (C=N at $\text{C}_2, \text{C}_2', \text{C}_3'$)

RESULTS AND DISCUSSION

Present work describes the synthesis, characterization and antimicrobial activity of novel spiroheterocycles 2-acylamino-6-methyl-7,9-diaryl-8(2'-amino-1',3',4',thiadiazolyl)-1-thia-3,4,8-triazaspiro[4,5]dec-2-ene(**4a-4c**) using 3-methyl 2,6-diarylpiperidin-4-one(**1a-e**) following literature procedure¹⁸. Compound (**1a-e**) were converted into keto esters (**2a-e**) on reaction with ethylbromoacetate in presence of anhydrous K_2CO_3 and acetone as solvent. These ketoesters were condensed with thiosemi-carbazide to form dithiosemicarbazones (**3a-e**) which in turn were heterocyclized to form spirothiadiazolines (**4a-e**) using sulphuric acid as the cyclising agent followed by neutralization with NH_3 . All the synthesized compounds were characterized by elemental analysis and spectral data.

Structures of the keto-esters (**2a-e**) were assigned on the basis of IR, ^1H NMR, ^{13}C NMR and Mass spectral data. Keto-esters showed the presence of two carbonyl groups in the region $1750\text{-}1650\text{ cm}^{-1}$ in IR spectra. Also they showed frequencies corresponding to -N-CH_2 in the region $1450\text{-}1400\text{ cm}^{-1}$. ^{13}C NMR signals were obtained for both carbonyl carbons in the range above $160\text{ }\delta$ ppm.

Dithiosemicarbazone (**3a-e**) showed the absence of carbonyl group and presence of N and S as characteristic elements on elemental analysis. IR spectra of dithiosemicarbazones showed absorption corresponding to C=N , NH and NH_2 of -NNHCSNH_2 moiety. In ^1H NMR, NH and NH_2 proton signals were obtained between $8\text{-}10.4\text{ }\delta$ ppm for all the compounds. ^{13}C NMR spectra showed absorption corresponding due to C=S in the region $160\text{-}175\text{ }\delta$.

Spiro compounds (**4a-e**) were characterized by IR, ^1H NMR, ^{13}C NMR spectra. IR data showed frequencies corresponding to 1° NH_2 at $3450\text{-}3100\text{ cm}^{-1}$ and thiadiazole nucleus in the region $1600\text{ to }750\text{ cm}^{-1}$. The various frequencies corresponding to N-N stretch at 1100 cm^{-1} , N-C-S stretch at $1100\text{-}1000\text{ cm}^{-1}$, C-S-C stretch at $720\text{-}690\text{ cm}^{-1}$, C=N stretch at $1450\text{-}1500\text{ cm}^{-1}$ were observed for all the compounds. ^1H NMR showed adsorption signals corresponding 1° NH_2 protons

in the range 9.0 to 10.4 δ , aryl protons in the range 6.7 to 7.4 δ . ^{13}C NMR signals were obtained at 60 to 70 δ for $-\text{NCH}_2$, 65 to 84 δ for spiro carbons, 140 δ for $\text{C}=\text{N}$ and 150-170 δ for $\text{C}=\text{S}$ carbons.

Compounds (**3a-e**) and spiro-thiazolins (**4a-e**) were tested for microbial activity against the gram negative bacteria, *E. coli* and fungus *Aspergillus niger* by the parer disc diffusion method. The screening was done at two different concentrations 50 ppm and 100 ppm in DMSO¹⁹ in Table-1. All the compounds showed positive inhibition activity against both bacteria and fungus at both concentrations as the activity was found to be higher in higher concentrations.

TABLE-1
ANTIMICROBIAL ACTIVITY AGAINST *E. coli* AND *A. niger*

Compound	<i>E. coli</i>		<i>A. niger</i>	
	50 ppm	100 ppm	50 ppm	100 ppm
3a	19	20	15	17
3b	19	20	16	18
3c	21	22	15	16
4a	22	24	17	18
4b	20	25	15	17
4c	18	22	14	16

Conclusion

Synthesis of ketoesters, dithiosemicarbazones and amino-7,9-di-(*p*-diaryl)-6-methyl-8-(2'-amino-1',3',4'-thiadiazolyl)-methyl-1-thia-3,4,8-triaza-spiro[4,5]dec-2-ene are discussed.

All the assigned structures are confirmed by spectral and analytical data. Selected compounds are screened for antimicrobial activity against *E. coli* and *A. niger* and results showed that they are antimicrobial compounds.

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