Asian Journal of Chemistry; Vol. 26, No. 20 (2014), 6823-6826

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.16951

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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Received: 28 December 2013; *Accepted*: 9 April 2014; *Published online*: 25 September 2014; AJC-16018

New heterocyclic ketoesters dithiosemicarbazone and corresponding spiro-1,3,4-thiadiazolines are prepared using 2,6-diaryl piperidones as substrates. The synthesized compounds are charactersised 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 antifungal[3], antibacterial[4], antilukemia agents[5], optically active liquid crystals[6], photographic materials, diuretic[7] and CNS depressants[8]. 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 acvtivity.

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 dithiosemicarbazones[9-11]. 2,6-diaryl piperidin-4-one (**1a-c**) are N-substituted by refluxing them with $BrCH_2COOC_2H_5$ in the presence of acetone and anhydrous K_2CO_3 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 heterocycling the various dithiosemicarbazones using sulphuric acid as cycling 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. ^{1}H NMR and ^{13}C 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 K_2CO_3 (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.$C_{22}H_{25}NO_3$; m.p. 59 °C, N (3.98 % calc.); IR (KBr, ν_{max}, cm^{-1}): 1750-1700 (C=O of COOEt), 1697 (C=O) of ring); 3100-3000, 1600 (C=C aryl); 1450 (N=CH_2); ^{1}H NMR (δ ppm)-0.81 (d, 3H-CH_3) 0.99-1.21 (t-3H, > N-$CH_2COOCH_2CH_3$), 2.13-2.4 (m, 3H C_3H,C_5H) 3.3-3.6 (m, 2H, C_2(H) and C_6(H), 3.78 (S, 2H, > N-CH_2-COOEt) 4.11 (t, 3H-CH_2 of ester), 7.3-7.7 (m- 8H aryl H's); ^{13}C 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. $C_{24}H_{29}NO_5$; m.p. 110 °C; N 7.06 (7.24) %,

calc (obs) IR (KBr, ν_{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-CH_2); 1263 (C-O of arylether). ^1H NMR. (1.2 to 1.3, 3H, CH_3), 3.01 (2H, C_3-H,C_5-H) 3.23 (2H, C_6-H) 3.1 (S, 6H, OCH_3) 4.2 to 4.3 (9.1, CH_2 of ester and N-CH_2) 6.7 to 7.7 (m Ar-H) ^{13}C 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-dimethylaminophenyl)piperidin-1-yl acetate (2c): m.f. $C_{26}H_{35}N_3O_3$; m.p. 93-95 °C; N 3.98 (3.92) %, calc (obs). IR (KBr, ν_{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-CH_2). ^1H NMR 0.8 (3H, CH_3) 1.8 (t, 3H, CH_3 of ester) 3.9 (2H, NCH_2) 5.0 to 5.6 (q, 2H, CH_2 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. $C_{24}H_{29}NO_3$; m.p.140-142 °C; N 3.98 (3.88) %, calc(obs) IR (KBr, ν_{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-CH_2) ^1H NMR δ ppm 0.9 (3H, CH_3) 1.1-1.5 (t, 3H, $COOCH_2CH_3$), 2.0-2.5 (m,C_3-H,C_5-H, aryl CH_3), 3.8 (q, 2H, $COOCH_2CH_3$), 3.9 (2H, NH_2), 6.8 to 8 (Ar-H). ^{13}C NMR δ ppm 10.4 (CH_3 at C_3), 21.05,23.77 (Ar-CH_3), 29.6 ($COOCH_2CH_3$), 63.2 ($COOCH_2CH_3$), 67.0 (N-CH_2), 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-ylacetate (2e): m.f. $C_{22}H_{23}NO_3Cl_2$; m.p. 133 °C; N 3.98 (3.86) %, calc (obs) IR (KBr, ν_{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-CH_2), ^{13}C NMR δ ppm 10.4 (CH_3 at C_3), 29.6 ($COOCH_2CH_3$), 63.2 ($COOCH_2CH_3$), 67.0 (N-CH_2), 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-thiosemicarbazo-piperidin-5-yl)thiosemisemicarbazide (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-thiosemicarbazo-piperidin-1yl)thiosemicarbazide (3a)[12,13]: m.f. $C_{22}H_{27}N_7OS_2$; m.p. 170 °C; N 20.89 (21.02) %, calc (obs) IR[12] (KBr, ν_{max}, cm^{-1}): 3422 (NH_2), 3252 (2°NH), 3090 (C-H aryl), 2971 (C-H ali), 1709 (C=O), 1588 (C=C aryl), 1493 (C=N), 1453 (N=CH_2), 1258 (C=S). ^1H NMR (δ ppm), 1.2 (d,3H,CH_3), 2.49 (C3-H), C5-H), 3.32-3.81 (C_2-H,C_6-H) 3.79 (2H, N-CH_2), 6.99-7.69 (Ar-H), 8.32 (NH of $NHCSNH_2$). ^{13}C NMR (δ ppm) 10.47 (CH_3), 21.0, 23.7 (C_3,C_5) 38.9,45.8 (C_2,C_6), 62.9 (N-CH_2) 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-thiosemicarbazide-piperidin-1-yl)thiosemicarbazide (3b): m.f. $C_{24}H_{31}N_7O_3S_2$; m.p. 226 °C; N 18.52 (18.66) %,; S 12.1(12.36) %, calc(obs). IR (KBr, ν_{max}, cm^{-1}): 3450-3250 (NH_2, 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,CH_3), 3.03-3.12 (6H,OCH_3), 2.49-2.26 (C_3-H), 2.66-2.80 (C_5-H), 2.8-3.4 (6H, $N(CH_3)_2$), 3.7 (2H, N-CH_2), 3.71-3.86 (C_2-H,C_6-H), 7.16-8.08 (Ar-H), 9.8-10.4 (NH), 10.53 (NH_2). ^{13}C NMR (δ ppm) 12 (CH_3), 30.6-40.1 (C_3,C_5), 59.3 (C_2,C_6), 69 (N-CH_2) 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-thiosemicarbazo-piperidin-1-yl thiosemicarbazide (3d): m.f. $C_{24}H_{31}N_7OS_2$; m.p. 195 °C; N (20.97 (21.02) %; S 10.64 (10.62) %, calc (obs) IR (KBr, ν_{max}, cm^{-1}): 3443 (NH_2), 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,CH_3), 2.07-2.28 (6H, aryl, CH_3), 3.31 (2H, NCH_2), 7.16-8.08 (Ar-H), 9.8-10.4 (NH), 10.53 (NH_2). ^{13}C NMR (δ ppm) 13-15 (CH_3), 20.7 (aryl CH_3), 70 (N-CH_2), 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-thiosemicarbazo-piperidin-1-yl thiosemicarbazide (3e): m.f. $C_{22}H_{25}N_7SO_2Cl_2$; m.p. 239 °C; N (20.97(20.98) %, S 10.64 (11.02) %, IR (KBr, ν_{max}, cm^{-1}): 3434 (NH_2), 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=CH_2), 1280 (C=S) ^1H NMR (δ ppm), 0.92 (3H, CH_3), 2.0-2.4 (C_3-H,C_5-H), 3.8 (2H,NCH_2), 5.0-5.4 (2H, C_2-H, C_6-H), 6.8-8.1 (m,Ar-H), 10.02-10.49 (NH, NH_2). ^{13}C NMR (δ ppm) 13 (CH_3), 30 (C_3,C_5), 42 (C_2-C_6), 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. $C_{26}H_{29}N_7S_2$; m.p 102 °C; N 18.31 (18.32) %, S 11.96 (11.92) %, calc (obs) IR (KBr, ν_{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-CH_2), 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,CH_3), 2.35-3 (C_6-H,C_{10}H), 3.34-4.39 (m, C_7H, C_9H), 3.78, 3.93 (N-CH_2), 7.31-7.25 (Ar-H), 10.25-10.4 (NH_2, NH). ^{13}C NMR (δ ppm)[16,17] 13 (CH_3), 21,22 (C_6,C_{10}), 52,52 (C_7,C_9), 60 ($NHCOCH_3$) 62 (N-CH_2), 82, 84 (C_5), 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. $C_{28}H_{33}N_7O_2S_2$; m.p. 103 °C; N (17.34 (17.32) %, S10.75 (10.92) %, calc (obs) IR[12] (KBr, ν_{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-CH_2),1256.39 (C-OCH_3), 1110.60, 1053.62, 1018.86, 814.9, 616.84 (thiadiazole ring. ^1H NMR (δ ppm), 1.2 (3H,CH_3),1.8-2.4 (bm, 5H,C_6-H, C_{10}H), 2.8-3.0 (C_7-H,C_9-H), 3.4-48H, OCH_3 and NCH_2), 6.8-7.0 (m,

aryl H's), 8.7-9.9-N-H. ^{13}C NMR (δ ppm) 12 (CH_3), 36, 37 (C_6,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. $C_{30}H_{39}N_9S_2$; 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). ^{1}H NMR (δ ppm), 1.32 (3H,CH_3),1.678-2.4 (C_6-H,C_{10}H), 3.10-3.19 (N-(CH_3)$_2$), 3.80 (N-CH_2), 6.28 (C_7-H,C_9-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 (-N-(CH_3)$_2$), 20.6, 20.7 (C_6,C_{10}), 50.1-50.7 (C_7,C_9), 59.4 (N-CH_2), 70.5 (C_5), 127.2-129-(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. $C_{22}H_{27}N_7S_2$; 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). ^{1}H NMR (δ ppm), 0.9 (3H,CH_3), 1.2,1.5 (C_6-H,C_{10}H), 3.5 (2H, N-CH_2), 3.1-3.8 (C_7-H, C_9-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 (C6,C10), 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. $C_{24}H_{31}N_7O_2S_2$; 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=Caryl), 1459 (C=N), 1224.9, 1038.5, 837.9, 761.5 (thiadiazole ring). ^{1}H NMR (δ ppm), 0.8 (d, 3H, CH_3), 1.0-1.6, 1.5 (C_6-H, C_{10}H), 3.32, 3.81 (2H, N-CH_2), 4.0-4.5 (C_7-H,C_9-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(C_6,C_{10}), 40,41 (C_7, C_9), 65 (N-CH_2), 84 (C_5), 124-132 (ArylC's), 141-146 (C=N at C_2,C_2',C_3')

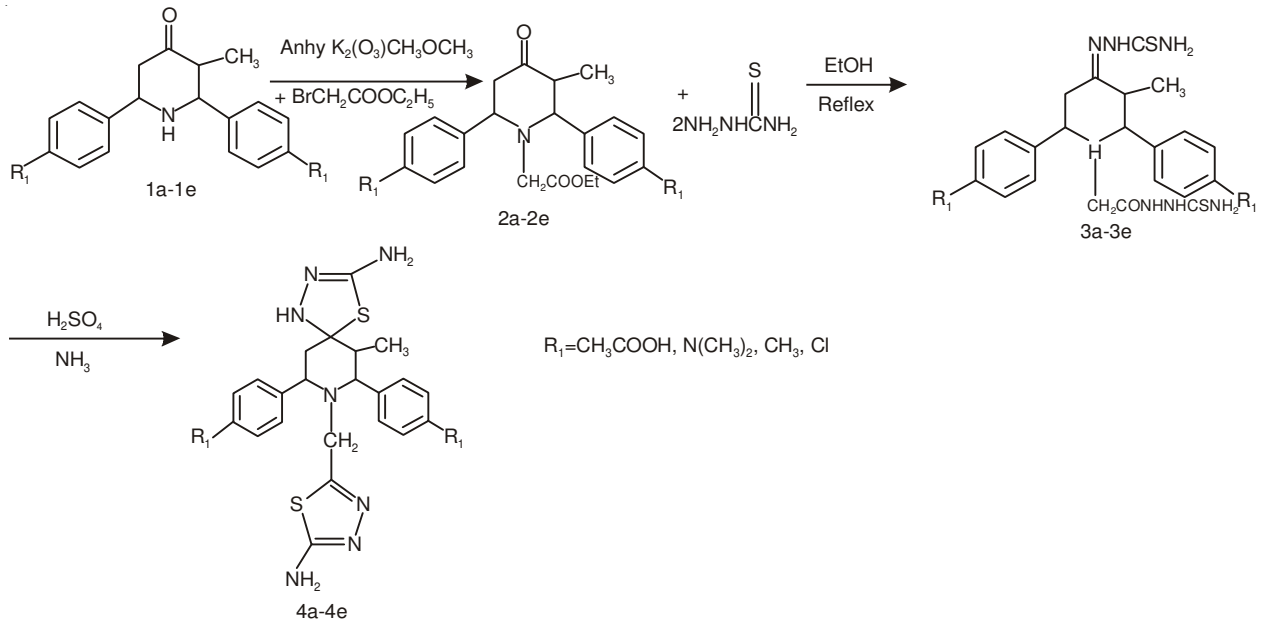
RESULTS AND DISCUSSION

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 procedure[18]. 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 heterocycled to form spirothiad-iazolines (**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, ^{1}H NMR, ^{13}C NMR and Mass spectral data. Keto-esters showed the presence of two carbonyl groups in the region 1750-1650 cm^{-1} in IR spectra. Also they showed frequencies corresponding to -N-CH_2 in the region 1450-1400 cm^{-1}. ^{13}C NMR signals were obtained for both carbonyl carbons in the range above 160 δ 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 ^{1}H NMR, NH and NH_2 proton signals were obtained between 8-104 δ ppm for all the compounds. ^{13}C NMR spectra showed absorption corresponding due to C=S in the region 160-175 δ.

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



in the range 9.0 to 10.4 δ, aryl protions in the range 6.7 to 7.4 δ. ^{13}C NMR signals were obtained at 60 to 70 δ for -NCH_2, 65 to 84 δ for spiro carbans, 140 δ for C=N and 150-170 δ for C=S carbons.

Compounds (**3a-e**) and spiro-thiazolins (**4a-e**) were tested for microbial activity against 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 DMSO[19] 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	*E. coli*		*A. niger*	
	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 anti-microbial activity against *E. coli* and *A. niger* and results showed that they are antimicrobial compounds.

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