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http://heteroletters.org

ISSN: 2231 – 3087(print)
ISSN: 2230 – 9632 (Online)
Coden HLEEAI
Vol. 4: (4), 2014, 537-547

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NOVEL SPIRO 4-THIAZOLINONE DERIVATIVES AS ANTIMICROBIAL AGENTS

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Abstract: In the present communication, novel spiro 4-thiazolinone derivatives were synthesized by superficial and swift method. The newly synthesized compounds were characterized by IR, NMR, MS and elemental analyses. All newly synthesized compounds were screened for their antibacterial (*Staphylococcus aureus*, *Streptococcus viridans* and *Escherichia coil*) and antifungal (*Gibberela*, *Cercospora arachidicola*, *Physolospora piricola* and *Fusarium oxysporum*) studies. Their results revealed that the present work provided a novel class of spiro-based 4-thiazolinone derivatives with potent microbial activities for further optimization against the tested microorganisms.

Keywords: Spiro 4-thiazolidinone, thiazolopyrimidine, pyrazolothiazole, thiazolopyridine, bis-thiazolopyrimidine, antimicrobial activities.

INTRODUCTION

Chemical alteration of bioactive component is one of the most common approaches in drug discovery with enhanced therapeutic effect[i] and the wide incidence of the heterocyclics moieties in bioactive natural products and pharmaceuticals has made them as central synthetic targets.

Small ring containing hetereocyclics atoms like nitrogen, sulfur and oxygen have been under investigation for a long time because of their vital medicinal possessions and also gave to the society from biological and industrial point which helps to understand life processes[ii]. Among these types of heterocyclic molecules, 4-thiazolidinones have been shown to have a variety of vital biological activities such as anti bacterial,antifungal, antiviral, diuretic, antituberculostatic, anti-HIV, antihistaminic, anticancer, anticonvulsant, anti inflammatory and analgesic properties[iii-v]. 4-Thiazolidinone derivatives display high activity in vitro against mycobacterium tuberculosis (TB) and as drugs to treat HIV and cancer[v-vii]. They were also accounted as novel inhibitors of the Mur B enzyme, integral component in bacterial peptidoglycan biosynthesis, at the low micro molar level[viii]. In recent times, 2-aryl-4-thiazolidinone has been synthesized and found to display potent selective antiplatelet activating

factors both in vitro and in vivo and anti-inflammatory[ix], antibacterial[x], anticancer[xi] and anti-HIV-1 activities[xii]. Spiro heterocyclic compounds together with thiazolidine moiety have antimicrobial activity[xiii]. Both pyrazolothiazole and thiazolopyrimidine moieties have effective kinase modulators[xiv] and are used in pharmaceutical compositions[xv]. The second compound has analgesic and anti-Parkinson activities[xvi] and inhibits the growth of parasite *Trypanosoma cruzi*[xvii]. Therefore, such medicinal properties associated with these heterocyclic molecules turn into them as valuable structural units in drug research. These findings encouraged us to synthesize various spiro heterocyclic derivatives of 4-thiazolidinone, for the investigation of an antimicrobial activity profile. From this approach, the purpose of the present communication encompasses the synthesis of series of new compounds having novel spiro cyclohexanone-thiazolidine system. The synthetic approach is shown in Fig. 1.

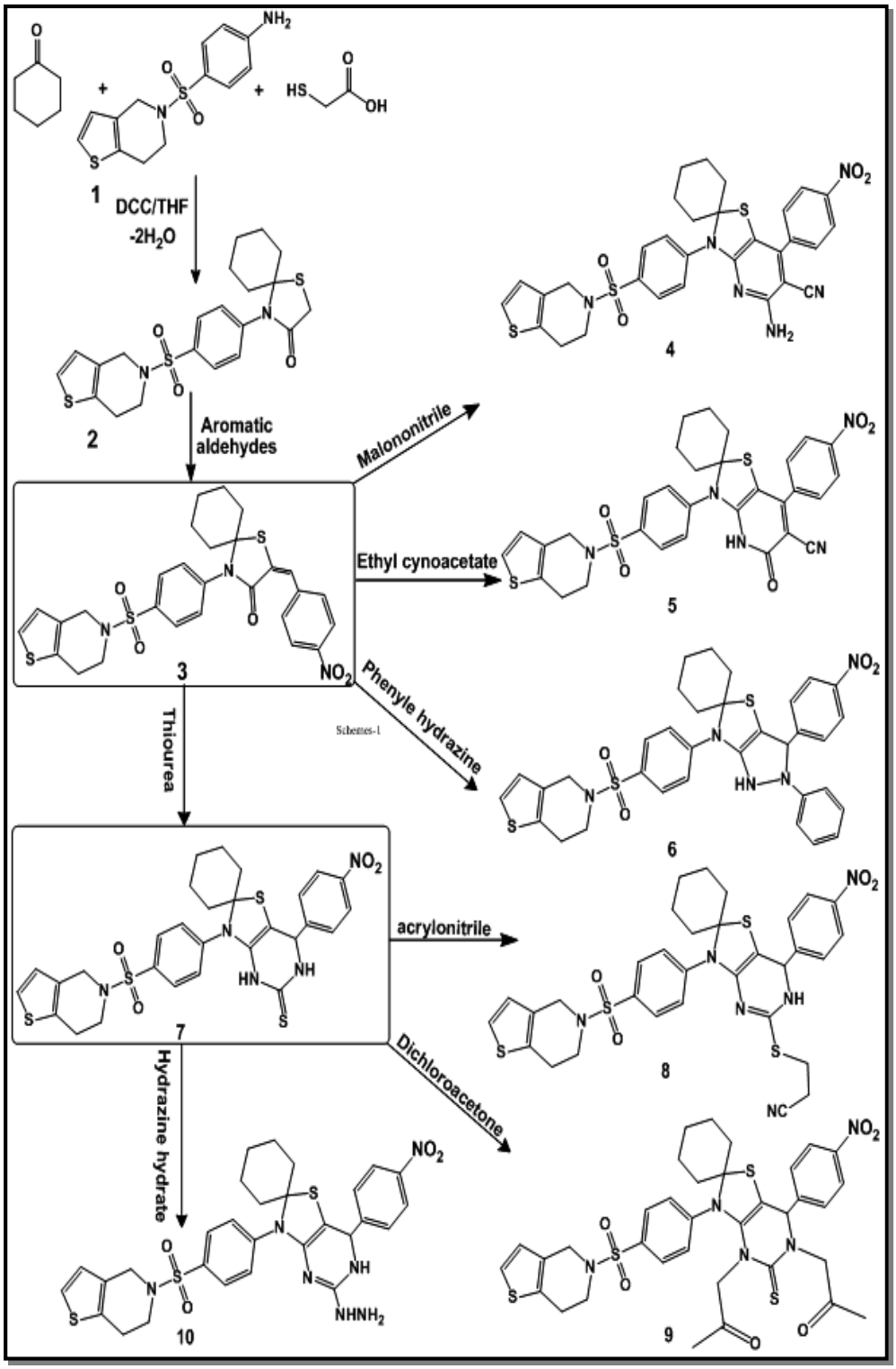

Fig.1.synthetic approach

EXPERIMENTAL

^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO-d_6 or $CDCl_3$ solutions on a BRUKER 400-MHz spectrometer, and chemical shifts were expressed as part per million (ppm; *ð* values)

against tetramethylsilane as an internal reference (TMS). The Infrared spectra (νcm^{-1}) were obtained with a Perkin-Elmer 1650 FTIR spectrometer in KBr pellets. Mass spectra (MS) were recorded on EI +Q1 MSLMR UPLR. Elemental analyses were performed on an ECS 4010 Elemental Combustion System and the results were within the accepted range (±0.40) of the calculated values. All melting points were determined on an Electro-thermal IA 9100 apparatus and were uncorrected. All the reagents and solvents were of the commercial quality and purchased from Merck, Fluka and localize companies.

Synthesis

The synthetic routes of the target compounds **2** to **10** were shown in Scheme 1. The starting compounds 4-(6,7-dihydro thieno[3,2-*c*]pyridin-5(4H)-ylsulfonyl)aniline **1** were synthesized by our previously reported routes[xviii].

4-(4-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-ylsulfonyl) phenyl)-1-thia-4-azaspiro[4.5]-decan-3-one 2

According to the reported procedure[xix], 4-(4-(6,7-dihydro thieno[3,2-*c*]pyridin-5(4*H*)-yl sulfonyl)phenyl)-1-thia-4-aza spiro[4.5]decan-3-one **2**, was prepared by stirring 4-(6,7-dihydro thieno[3,2-*c*]pyridin-5(4*H*)-ylsulfonyl) aniline **1** (1.47g, 5 mmole) with cyclohexanone (0.98 g, 10 mmole) in THF at an ice-bath for 5 min, followed by addition of mercapto acetic acid (1.38 g, 15 mmole). After 5 min, DCC (1.2 mmole) was added to the reaction mixture at 0°C and the reaction mixture stirred for additional 2-3 hours at room temperature. Formed DCU was removed by filtration, filtrate was concentrated to dryness under reduced pressure and the residue was extracted with ethyl acetate, and washed with 5 % aq. citric acid, water, 5 % aq. sodium hydrogen carbonate and then with brine. The organic layer was dried over anhydrous sodium sulfate and the solvent removed under vacuum to give the crude product, the purity of product was checked by TLC and purified by column chromatography to afford compound **2**. Yield 78%;mp166-167ºC;*Anal.*Calcd. for $C_{21}H_{24}N_2O_3S_3$(448.62): C, 56.22;H,5.39;N,6.24;S,21.44. Found:C,56.23,H,5.37,N,6.25; S, 21.47; IR (KBr, ν,cm^{-1}): 1695(CO, thiozolidinone),1338-1142(SO_2). ^{1}H-NMR(400 MHz,DMSO-d_6,ð/ppm):1.34(brd.,6H,$C_{21\text{-}23\text{-}H}$),1.78(t,4H,$C_{20\text{-}H}$,$C_{24\text{-}H}$,*J*=2.40 Hz), 2.45(t,2H,$C_{7\text{-}H}$,*J* = 6.2 Hz), 3.12(t,2H,$C_{6\text{-}H}$,*J*=6.2Hz),3.62(s,2H,$C_{17\text{-}H}$),3.71(t,2H,$C_{4\text{-}H}$), 6.72(d,1H,$C_{3\text{-}H}$,J=4.6Hz),7.34(d,1H,$C_{2\text{-}H}$,*J*=4.8Hz),7.48(d, 2H,$C_{11\text{-}H}$,$C_{13\text{-}H}$,*J*=8.6Hz),7.78 (d, 2H, $C_{10\text{-}H}$,$C_{14\text{-}H}$,*J*=8.6Hz). ^{13}C NMR (100 MHz, DMSO-d_6, ð/ ppm): 23.7, 24.3, 25.4, 32.4, 34.7, 47.9, 52.3, 68.2, 121.5, 124.0, 124.6, 128.7, 132.7, 134.9, 135.3, 143.7, 170.2. MS (EI+) : 448.24.

4-(4-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-ylsulfonyl) phenyl)-2-(4-nitrobenzylidene)-1-thia-4-azaspiro[4.5] decan-3-one 3

To a well-stirred solution of compound **2** (2.34 g, 4 mmol) in acetic acid (35 mL) buffered with sodium acetate (0.65 g, 8 mmol), the 4-nitrobenzaldehyde (0.90g,6mmol) was added[xx]. The solution was heated at reflux for 4 hours and then poured into ice-cold water. The precipitate was filtered and washed with water, and the resulting crude product was purified by recrystallization from dioxane to give compound **3**. Yield 68%; mp 189-190ºC;*Anal.* Calcd. for $C_{28}H_{27}N_3O_5S_3$ (581.73)C,57.81;H,4.68;N,7.22;S,16.54.Found:C,57.83;H,4.65;N,7.23;S,16.52;IR(KBr, ν,cm^{-1}): 1698(CO),1364,1528(NO_2),1330-1146(SO_2);^{1}H–NMR(400MHz,DMSO-d_6,ð/ppm):1.28 (brd., 6H, $C_{21\text{-}23\text{-}H}$), 1.82(t,4H,$C_{20,24\text{-}H}$,J=2.36Hz), 2.48(t,2H,$C_{7\text{-}H}$,J=6.2Hz),3.14(t,2H,$C_{6\text{-}H}$,J=6.2 Hz),

3.76 (t,2H, $C_{4\text{-}H}$),6.64(s,1H,$C_{25\text{-}H}$),6.74(d,1H,$C_{3\text{-}H}$,J=4.6 Hz),7.33 (d,1H, $C_{2\text{-}H}$,J=4.8Hz), 7.42 (d,2H,$C_{28,30\text{-}H}$,J=8.0Hz),7.51(d,2H,$C_{11,13\text{-}H}$, J=8.6Hz),7.71(d,2H,$C_{27,31\text{-}H}$,J=8.0 Hz),7.82 (d,2H, $C_{10,14\text{-}H}$, J= 8.6 Hz); ^{13}C NMR (100 MHz, DMSO-d_6, ð/ ppm): 20.9, 22.8, 24.7, 32.4, 39.4, 45.6, 48.5, 72.8, 120.8, 122.8, 124.7, 125.9, 126.5, 127.4, 128.6, 129.7, 130.2, 133.4, 134.7, 137.0, 142.7, 167.2; MS (EI+): 580.

5'-amino-3'-(4-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-ylsulfonyl)phenyl)-7'-(4-nitro phenyl)- 3'H-spiro[cyclo hexane-1,2'-thiazolo[4,5-b]pyridine]-6'-carbonitrile 4

A mixture of compound **3** (1.88 g, 5 mmol), malononitrile (0.66 g, 10 mmol), and ammonium acetate (1.53 g, 20 mmol) was heated at reflux in glacial acetic acid (40 ml) for 30 hours. The reaction mixture was cooled and poured into water. The formed solid was filtered off, dried, and recrystallized from methanol to give compound **4** in 50% yield;mp 222-223ºC;*Anal.*Calcd.for $C_{31}H_{28}N_6O_4S_3$ (644.79): C, 57.74; H,4.38;N,13.03;S,14.92.Found : C, 57.76; H, 4.37; N, 13.05; S,14.90.IR(KBr, ν, cm^{-1}): 3418-3224(NH_2), 2216 (CN),1360,1524(NO_2), 1339,1145(SO_2);^{1}H-NMR (400 MHz, DMSO-d_6,ð/ppm):1.23(brd.,6H,$C_{21\text{-}23\text{-}H}$),1.79(t,4H,$C_{20,\ 24\text{-}H}$, J =2.36Hz), 2.50(t, 2H,$C_{7\text{-}H}$,J=6.2Hz),3.17(t,2H,$C_{6\text{-}H}$,J= 6.2 Hz), 3.79(t,2H,$C_{4\text{-}H}$),6.75(d,1H,$C_{3\text{-}H}$,J=4.6 Hz), 7.30 (d,1H,$C_{2\text{-}H}$,J = 4.8 Hz), 7.40 (d,2H,$C_{28,30\text{-}H}$,J = 8.0 Hz), 7.55 (d,2H,$C_{11,13\text{-}H}$, J = 8.6 Hz), 7.73(d, 2H, $C_{27,31\text{-}H}$,J = 8.0 Hz), 7.80 (d,2H,$C_{10,14\text{-}H}$,J = 8.6 Hz), 7.96(s,2H,NH_2, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6, ð/ ppm):23.5, 24.8, 25.2, 32.7, 47.6, 52.5, 60.4, 121.4, 123.8, 124.2, 124.9, 126.7, 127.2, 128.3, 128.7, 129.2, 132.3, 134.2, 135.3, 138.4, 141.7, 167.2; MS (EI+): 642.

3'-(4-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-ylsulfonyl)phenyl)-7'-(4-nitrophenyl)-5'-oxo hexa hydro-3'H-spiro [cyclohexane-1,2'-thiazolo[4,5-b]pyridine]-6'-carbonitrile 5

A mixture of compound **3** (1.88 g, 5 mmol), ethyl cyano acetate (1.13 g, 10 mmol), and anhydrous ammonium acetate (1.60 g, 20 mmol) was heated at reflux in glacial acetic acid (40 ml) for 20 hours. The reaction mixture was cooled and poured into water. The formed solid was filtered off, dried, and recrystallized from acetic acid to give compound **5** in 55% yield; mp 251-252 ºC;*Anal.* Calcd. for $C_{31}H_{27}N_5O_5S_3$ (645.77): C, 57.66; H, 4.21; N, 10.84; S, 14.90. Found : C, 57.67; H, 4.18; N, 10.82; S, 14.93;IR (KBr, ν, cm^{-1}): 3152 (NH), 2217 (CN), 1685 (CO)1367,1527 (NO_2), 1338, 1143 (SO_2); ^{1}H -NMR(400 MHz,DMSO-d6,ð/ppm):1.20(brd., 6H, $C_{21\text{-}23\text{-}H}$), 1.78(t, 4H,$C_{20,24\text{-}H}$,J =2.36 Hz),2.50(t,2H,$C_{7\text{-}H}$,J=6.2 Hz),3.16(t,2H,$C_{6\text{-}H}$,J= 6.2 Hz,), 3.81 (t,2H,$C_{4\text{-}H}$),6.74(d,1H,$C_{3\text{-}H}$,J = 4.6 Hz,), 7.33(d,1H,$C_{2\text{-}H}$, J =4.8 Hz),7.42 (2H,d, $C_{28,\ 30\text{-}H}$, J = 8.0 Hz), 7.58(2H, d, $C_{11,13\text{-}H}$,J=8.6 Hz),7.77(d,2H,$C_{27,31\text{-}H}$,J = 8.0 Hz),7.80(d,2H, $C_{10,14\text{-}H}$,J= 8.6Hz),10.23 (s,1H, NH,D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6, ð / ppm):21.7, 23.8, 25.0, 32.7, 47.8, 50.5, 71.4, 122.4, 123.6, 124.5, 124.9, 126.4, 127.5, 128.0, 128.7, 129.2, 132.7, 134.0, 135.7, 138.2, 167.2, 170.8; MS (EI+): 645.

6'-(4-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-ylsulfonyl) phenyl)-3'-(4-nitrophenyl)-2'-phenyl-1',2',3',6'-tetra hydrospiro[cyclohexane-1,5'-pyrazolo[3,4-d]thiazole] 6

A mixture of compound **3** (1.88 g, 5 mmol) and phenyl hydrazine (1.06mL,10 mmol) was heated at reflux in absolute ethanolic (50 ml) for 4 hours. The reaction mixture was cooled, and the solid substance was filtered off, dried, and recrystallized from dioxane to give compound **5b** in 80% yield; mp 263-264 ºC; *Anal.* Calcd. for $C_{34}H_{33}N_5O_4S_3$ (671.85): C, 60.78; H, 4.95; N, 10.42; S, 14.32. Found : C, 60.75; H, 4.97; N, 10.44; S, 14.30; IR (KBr, ν, cm^{-1}): 3153 (NH), 1356,1532(NO_2)1328,1147(SO_2); ^{1}H -NMR(400 MHz DMSO-d6,ð/ppm):1.17(brd.,6H,$C_{21\text{-}23\text{-}}$

$_{H}$),1.82(t,4H,$C_{20,24-H}$,J =2.36 Hz),2.48(t,2H,C_{7-H},J=6.2 Hz),3.17(t,2H,C_{6-H},J=6.2Hz), 3.83(t,2H, C_{4-H}), 4.57(s,1H,pyrazoleH),6.72–8.24(m,15H,Ar-H), 9.32(s,1H,NH,D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6, ð / ppm):23.6, 24.0, 24.7, 30.7, 46.6, 52.2, 56.6, 60.1, 121.0, 123.7, 125.7, 124.0, 124.8, 125.7, 126.3, 127.7, 128.2,128.9, 129.0, 129.5,132.2, 132.6, 134.8, 135.0,136.4, 138.7, 141.4; MS (EI+): 670.

3'-(4-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-ylsulfonyl)phenyl)-7'-(4-nitrophenyl)-6',7'-di hydro -3'H-spiro[cyclo hexane-1,2'-thiazolo[4,5-d]pyrimidine]-5'(4'H)-thione 7

A mixture of compound **3** (1.88 g, 5 mmol) and thiourea (0.76 g, 10 mmol) was heated at reflux in ethanolic sodium hydroxide (1 g, 30 ml) for 4 hours. The reaction mixture was cooled and poured into water (100 ml). The formed solid was filtered off, dried, and recrystallized from dioxane to give compound **7** in 56% yield; mp 273-274 ºC; *Anal.* Calcd. for $C_{29}H_{29}N_5O_4S_4$ (639.83): C, 54.44; H, 4.57; N, 10.95; S, 20.05. Found : C, 54.43; H, 4.54; N, 10.93; S, 20.07; IR(KBr,v,cm^{-1}): 3170, 3120 (2NH), 1360, 1525(NO_2), 1331,1142(SO_2),1213(CS);^{1}H - NMR(400MHz,DMSO-d_6,ð/ppm):1.21(6H,brd.,$C_{21-23-H}$),1.84(t,4H,$C_{20,24-H}$,J=2.36Hz), 2.43(t,2H, C_{7-H}, J=6.2Hz), 3.17(t,2H,C_{6-H},J=6.2 Hz),3.80(t, 2H,C_{4-H}),4.58(s,1H,pyrimidine H), 6.77(d,1H, C_{3-H},J=4.6Hz), 7.28(d,1H,C_{2-H},J=4.8 Hz),7.42(d,2H,$C_{28,30-H}$,J= 8.0 Hz), 7.54 (d,2H,$C_{11,13-H}$,J = 8.6Hz),7.70(d,2H,$C_{27,31-H}$,J=8.0Hz),7.78(d,2H,$C_{10,14-H}$,J=8.6Hz),8.87(s,1H,NH,D_2O exchange able), 11.18 (1H, s, NH, D_2O exchangeable); ^{13}CNMR (400MHz, DMSO-d_6, ð/ppm): 22.3, 23.9, 25.0, 31.9, 43.5, 47.2, 52.1, 72.7, 120.6, 122.9, 124.4, 124.9, 126.2, 127.4, 128.0, 128.7, 129.0, 132.5, 134.6, 135.6, 138.7, 139.7, 172.5; MS (EI+): 637.

3-(3'-(4-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-ylsulfonyl) phenyl)-7'-(4-nitrophenyl)-6',7'-dihydro-3'H-spiro[cyclo hexane-1,2'-thiazolo[4,5-d]pyrimidine]-5'-ylthio) propanenitrile 8

A mixture of compound **4** (1.67 g, 2.5 mmol) and acrylonitrile (0.13 mL, 2.5 mmol) was heated at reflux in absolute ethanol (30mL) for 3 hours. The formed solid was filtered off, dried, and recrystallized from ethanol to give compound **8** in 70% yield; mp 172-173 ºC; *Anal.* Calcd. for $C_{32}H_{32}N_6O_4S_4$ (692.89):C,55.47;H,4.65;N,12.13;S,18.51. Found : C, 55.44; H, 4.67; N,12.15; S, 18.48;IR (KBr,v,cm^{-1}): 3187(NH), 2219 (CN)1368, 1522(NO_2), 1328,1137 (SO_2);^{1}H-NMR(100MHz, DMSO-d_6,ð/ppm): 1.16(brd., 6H, $C_{21-23-H}$),1.83(t,4H,$C_{20,24-H}$, J =2.36Hz), 2.30(t,2H, J=5.8Hz), 2.47(t,2H,C_{7-H},J=6.2 Hz), 2.63(t,2H,J=5.8 Hz) 3.15(t,2H,C_{6-H},J=6.2Hz), 3.83(t,2H,C_{4-H}),4.58(s,1H,pyrimidine H),6.76(d, 1H,C_{3-H},J=4.6Hz),7.33(1H,d,C_{2-H},J=4.8Hz), 7.47(d,2H,$C_{28,30-H}$,J=8.0Hz),7.56(d,2H,$C_{11,13-H}$,J=8.6Hz),7.78(2H,d,$C_{27,31-H}$,J =8.0Hz), 7.86(d,2H, $C_{10,14-H}$,J=8.6Hz),9.62(s,1H,NH,D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6, ð / ppm):23.2, 24.6, 26.2, 32.1,42.7, 47.3, 53.5, 60.1, 71.6, 121.6, 123.4, 124.0, 124.7, 126.1, 128.2, 128.8, 129.7, 130.2, 132.7, 134.4, 135.5, 138.0, 141.4, 172.0; MS (EI+): 691.

1,1'-(3'-(4-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl sulfonyl)phenyl)-7'-(4-nitrophenyl)-5'-thioxo-3'H-spiro[cyclohexane-1,2'-thiazolo[4,5-d]pyrimidine]-4',6'(5'H,7'H) -diyl)-dipropan -2-one 9

A mixture of compound **4** (1.67 g, 2.5 mmol) and dichloro acetone (0.73 g, 50 mmol) was heated at reflux in ethanolic sodium hydroxide (0.4 g, 30 ml) for 3 hours. The reaction mixture was cooled and poured into water. The formed solid was filtered off, dried, and recrystallized from ethanol to give compound **9** in 70% yield; mp 182-183 ºC; *Anal.* Calcd. for $C_{35}H_{37}N_5O_6S_4$ (751.96): C, 55.90; H, 4.96;N,9.31;S,17.06.Found:C,55.87;H,4.97;N,9.34;S,17.04 ; IR(KBr, v,cm^{-1}):1703,1709(CO),1524(NO_2),1360,1330, 1147 (SO_2),1202(CS); ^{1}H-NMR(400 MHz,

DMSO -d_6,ð/ppm): 1.17 (brd., 6H, $C_{21\text{-}23\text{-}H}$), 1.83(t, 4H,$C_{20,24\text{-}H}$,J=2.36Hz), 2.47(t, 2H, $C_{7\text{-}H}$,J =6.2 Hz), 3.19 (t,2H,$C_{6\text{-}H}$,J=6.2Hz),3.87(t,2H,$C_{4\text{-}H}$), 4.51(4H,s,2CH_2),4.63(s,1H, pyrimidineH), 6.30 (s, 4H, 2CH_2), 6.74(d,1H,$C_{3\text{-}H}$,J=4.6 Hz), 7.38(d,1H,$C_{2\text{-}H}$,J=4.8Hz),7.44 (d, 2H, $C_{28,30\text{-}H}$,J=8.0Hz), 7.58(d, 2H,$C_{11,13\text{-}H}$,J=8.6Hz), 7.72(d, 2H, $C_{27,31\text{-}H}$,J=8.0Hz),7.80(d,2H, $C_{10,14\text{-}H}$,J=8.6Hz); ^{13}CNMR (100MHz,DMSO-d_6,ð/ppm): 21.9, 24.3,25.4,31.9, 35.9,46.7, 49.8, 52.5,62.3, 70.4, 123.1, 123.8, 124.4, 125.4, 126.6, 127.6, 128.5, 128.9, 129.4, 132.5, 134.7, 135.1, 138.0, 141.4, 169.8; MS (EI+): 750.

3'-(4-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-ylsulfonyl)phenyl)-5'-hydrazinyl-7'-(4-nitro phenyl)-6',7'-dihydro-3'H-spiro[cyclohexane-1,2'-thiazolo[4,5-d]pyrimidine] 10

A mixture of compound **4** (1.67 g, 2.5 mmol) and hydrazine hydrate 99% (20 mL) was heated at reflux for 8 hours. The reaction mixture was cooled and poured into water. The formed solid was filtered off, dried, and recrystallized from methanol to give compound 10 in 65% yield;mp167-168 ºC; *Anal.* Calcd. for $C_{29}H_{31}N_7O_4S_3$(637.80):C,54.61;H, 4.90; N, 15.37;S,15.08.Found: C, 54.59;H,4.92;N,15.34;S,15.06;IR(KBr,ν,cm^{-1}):3310-3313(NH_2),3156-170(NH),1364, 1528(NO_2), 1336,1140(SO_2);^{1}H-NMR(400MHz,DMSO-d_6,*ð*/ppm):1.23 (brd.,6H,$C_{21\text{-}23\text{-}H}$),1.76 (t,4H,$C_{20,24\text{-}H}$,J=2.36Hz),2.51(t,2H,$C_{7\text{-}H}$,J=6.2Hz,),3.15(t,2H,$C_{6\text{-}H}$,J=6.2Hz),3.80(t,2H,$C_{4\text{-}H}$),4.26 (s, 2H,$NH_2$, D_2Oexchangeable),4.87(s,1H,pyrimidine),5.61(s,1H, NH,D_2O exchangeable), 6.74 (d,1H,$C_{3\text{-}H}$,J=4.6Hz),7.29(d,1H,$C_{2\text{-}H}$,J=4.8Hz,),7.42(d,2H,$C_{28,30\text{-}H}$,J=8.0Hz), 7.59(d,2H,$C_{11,13\text{-}H}$, J=8.6 Hz), 7.74 (d,2H,$C_{27,31\text{-}H}$,J= 8.0 Hz), 7.87(d,2H,$C_{10,14\text{-}H}$, J=8.6Hz),9.60(s,1H,NH,D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6, *ð* / ppm):20.6, 23.8, 27.6, 32.7, 48.2, 52.5, 70.4, 121.9, 122.8, 123.5, 123.9, 125.9, 128.0, 128.5, 129.2, 129.8, 131.3, 133.7, 135.8, 138.7, 142.7; MS (EI+): 637.

ANTIMICROBIAL TESTS

All the newly synthesized compounds were evaluated for their in vitro antibacterial activity against *Staphylococcus aureus*, *Streptcoccus viridans* and *Escherichia coli*. Disk diffusion method [xxi-xxii] was used for determination of the preliminary antibacterial activity. Disks measuring 6.25 mm in diameter were punched from Whatman No. 1 filter paper. Batches of 100 disks were dispensed to each screw-capped bottle and sterilized by dry heat at 140°C for an hour. The test compounds were prepared with different concentrations using DMF.One milliliter containing 100 times the amount of chemical in each disk was added to each bottle, which contained 100 disks. Disks of each concentration were placed in triplicate in nutrient agar medium seeded with fresh bacteria separately. The incubation was carried out at 37°C for 24 h. Ampicillin trihydrate was used as a standard drug. Solvent and growth controls were kept and zones of inhibition were noted. The MIC (lg/mL) values of the tested compounds against the tested bacteria strains are recorded in Table I.

On the other hand, the newly prepared compounds were also screened for their in vitro antifungal activity against *Cibberela*, *Cercospora arachidicola*, *Physolospora piricola* and *Fusarium oxysporum* in DMSO by the serial plate dilution method[xxiii-xxiv]. All the fungal strains were clinical isolates, identified with conventional morphological and biochemical methods. Fluconazole (antifungal) was used as reference drug. Sabouraud's agar media were prepared by dissolving peptone (1 g), D-glucose (4 g), and agar (2 g) in distilled water (100 ml) and adjusting the pH to 5.7. Normal saline was used to make a suspension of the spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 ml saline to get a suspension of

the corresponding species. Agar media (20 ml) was poured into each petri dish. Excess suspension was decanted and the plates were dried by placing in an incubator at 37°C for 1 hour. Using an agar punch wells were made into each well labeled. A control was also prepared in triplicate and maintained at 37°C for 3-4 days. Antifungal activity was determined by measuring the diameter of the inhibition zone. The MIC (μg/mL) values of the tested compounds against the tested fungal strains are recorded in Table 1.

Table 1. Antibacterial and antifungal data for the synthesized compounds

Comp. No.	**Antibacterial activity data in MIC (lg/mL)**			**Antifungal activity data in MIC (lg/mL)**			
	SA	***SV***	***E***	***G***	***CA***	***PP***	***FA***
3	10	11	11	20	18	26	24
4	11	12	14	24	30	27	28
5	24	27	30	38	28	36	30
6	15	20	17	20	22	34	29
7	40	37	53	57	49	51	46
8	29	28	36	40	31	42	30
9	32	29	38	47	33	38	31
10	34	30	43	50	35	45	36
Ampicillin trihydrate (std.)	16	5	21	23	12	10	34
Fluconazole (std.)	38	32	27	15	29	26	31

Where **SA**= *S. aureus ;**SV**= S. viridans ;* **E**=*E. coli ; **G**=Gibberela ;**CA**=C.arachidicola ; **PP**=P.piricola ; **FA**=F.oxysporum*

RESULTS AND DISCUSSION

Chemistry

In the present communication, a novel series of spiro 4-thiazolinone derivatives of 4-(4-(6,7-dihydrothieno[3,2-*c*]pyridin-5(4*H*)-ylsulfonyl)phenyl)-1-thia-4-azaspiro[4.5] decan-3-one **2** were synthesized by facile and fast procedure, which further underwent condensation with 4-nitro benzaldehyde to afford **3**. Compound **3** was used as precursor for the preparation of some fused heterocyclic compounds **4**, **5**, **6** and **7**. Among them, compound **7** was alkylated using dichloroacetone to afford **9**. Also, it reacted with acrylonitrile and hydrazine hydrate to afford **8** and **10**, respectively. All the newly synthesized compounds were characterized by IR, NMR, MS spectra and elemental analyses and also screened for their antimicrobial studies. Their results revealed that the present work provided a novel class of spiro-based 4-thiazolinone derivatives with potent microbial activities for further optimization.

Characterization of the synthesized compounds

The structures of the resulting compounds were established by elemental analysis, IR, NMR and MS spectral data. The proposed structure given to 4-(4-(6,7-dihydro thieno[3,2-

c]pyridin-5(4*H*)-ylsulfonyl)phenyl)-1-thia-4-aza spiro[4.5] decan-3-one **2** was support by IR analysis which showed band at 1695 cm^{-1} (thiazolidinone, CO)[xxv] and 1338-1142 cm^{-1} (SO_2)[xxvi]. Its ^1H NMR spectrum revealed signals at ð 2.45 ppm, ð 3.12 ppm and ð 3.71 ppm attributed to piperidine protons, signal at ð 3.62 ppm attributed to thiazole ring protons and two doublets at ð 7.48 ppm and ð 7.78 ppm corresponding to aromatic protons of thiophene. Furthermore, the mass spectra gave a molecular ion peak at m/z 448.24.

Also, compound **2** was confirmed chemically via condensation with *p*-nitro benzaldehyde to afford **3**. The structures of the latter compounds were elucidated from their correct spectral data (cf. the Experimental section). For example, the IR spectrum of compound **3** showed an absorption band at 1698 cm^{-1} (thiazolidinone CO) due to conjugation, ^1H NMR spectrum showed absence of thiazolomethylene protons, and its mass spectrum showed the molecular ion peak at m/z 581.73, all of which support its molecular formula. Compound **3** was used as starting material for further synthesis of other heterocyclic compounds. It reacted with malononitrile, ethyl cyanoacetate, phenyle hydrazine and thiourea to afford compounds **4-7**, respectively.

The structures of these compounds were confirmed from their correct spectral data (cf. the Experimental section). For example, the IR spectrum of compound **7** showed the absence of the band characteristic for (thiazolidinone CO) and the presence of absorption bands at 3170, 3120 cm^{-1}(NH), and 1213 cm^{-1}(CS). Also, it's ^1H NMR spectrum revealed signals at 4.58 ppm characteristic of the pyrimidine ring and at ð 8.87 ppm and ð 11.18 ppm for 2NH protons that are D_2O exchangeable. Mass spectra showed M^+ peak at m/z 639.83, which supports its molecular formula.

On the other hand, compound **7** was allowed to react with acrylonitrile via Michael addition to afford **8** and halo compound, namely dichloroacetone to afford compound **9**. Also, compound **7** was reacted with hydrazine hydrate to afford hydrazine pyrimidine derivative **10** (Scheme 1). The structures of all the synthesized compounds were confirmed by their correct spectral data (cf. the Experimental section).

Antimicrobial studies

All the newly synthesized compounds were screened for their antibacterial and antifungal activity. For antibacterial studies microorganisms employed were *S. aureus*, *Streptococcus viridans* and *Escherichia coil*. For antifungal, *Gibberela*, *C. arachidicola*, *P. piricola* and *F. oxysporum* were used as microorganisms. Both antimicrobial studies were assessed by minimum inhibitory concentration (MIC). The data are summarized in Table I and show that all compounds display certain activity against the tested microorganisms.

From analyzing these data, we can see that the antibacterial and antifungal activity of the synthesized compounds may be due to the presence of the versatile pharmacophore which might increase the lipophilic character of the molecules, which facilitate the crossing through the biological membrane of the microorganism and thereby inhibit their growth.

CONCLUSION

The preparation procedure follow in this work for synthesis of the title compounds offers reduction in the reaction time, operation simplicity, cleaner reaction, easy work-up and improved yields. All spectroscopic analysis confirmed the proposed structures of these compounds. Biological activity data have shown that the synthesized compounds have a significant biological activity against the tested microorganisms.

In conclusion, a series of novel 4-(4-(6,7-dihydro thieno[3,2-*c*]pyridin-5(4*H*)-ylsulfonyl)phenyl)-1-thia-4-aza spiro[4.5]decan-3-one 5 derivatives have been synthesized and evaluated for their antibacterial(MIC) activity and antifungal (MIC) activity against various bacteria and fungi. Many of the synthesized compounds showed good activity against the test bacteria and fungi.

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Received on June 23,2014.