Synthesis and *In Vitro* Antibacterial Activity of Novel Steroidal (6R)-Spiro-1,3,4-thiadiazoline Derivatives

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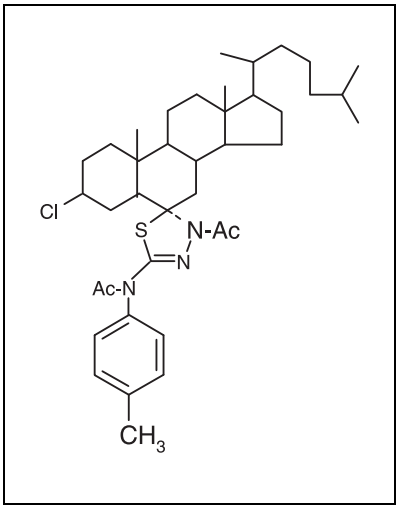

Novel steroidal (6R)-spiro-1,3,4-thiadiazoline derivatives were synthesized by the cyclization of steroidal thiosemicarbazones with acetic anhydride, screened *in vitro* against antibacterial activity using disc-diffusion method and the minimum inhibitory concentration. The results showed that steroidal thiadiazoline derivatives exhibited better antibacterial activity than the steroidal thiosemicarbazone derivatives. Chloro and acetoxy substituents on the 3β-position of the steroidal thiadiazoline ring increased the antibacterial activity. Among all the compounds, compound **7** and **8** were found better inhibitors of both types of bacteria (Gram-positive and Gram-negative) as compared to the respective drug amoxicillin. All the synthesized compounds were well characterized by spectroscopic methods such as IR, ^{1}H-NMR, ^{13}C-NMR mass, and elemental analysis and their stereochemistry was also discussed.

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INTRODUCTION

Infectious diseases are the main cause of mortality in the world and the resistance of pathogenic bacteria towards available antibiotics is rapidly becoming a major worldwide problem. Hence, the design of new compounds to deal with resistant bacteria has become one of the most important areas of antibacterial research today. Food poisoning, rheumatic, salmonellosis, and diarrhea are the second leading cause of death from bacterial disease world-wide [1]. More than 50 million people worldwide are infected and up to 150,000 die every year because of these bacterial infections [2]. Moreover, drug resistance in food poisoning, rheumatic, salmonellosis, and diarrhea can be attributed to the use of drugs (amoxicillin, norfloxacin, ciprofloxacin, and chloramphenicol) for treatment and to the adaptation of the bacterial parasite by developing alternate pathways for survival [3,4]. Hence, the present work is aimed towards developing novel molecules with improved potential for treating bacterial infections and with decreased probability for developing drug resistance. Five member heterocyclic compounds with sepal references of sulfur canting such as thiazole, thiazolidinone, and thiadiazoline are abundant in nature and are of great significance to life because their structural subunits exist in many natural products such as vitamins, hormones, antibiotics etc. Compounds with a thiadiazoline structure are known to possess tranquilizing, muscle relaxing, psychoanaleptic, hypnotic, ulcerogenic, antidepressant, antibacterial, antifungal, analgesic, and antiinflammatory properties [5–10]. Recently a number of thiazolidinone derivatives were synthesized and their potential antibacterial activity has been studied in our laboratory [11]. It is evident from the literature that no work has been done on steroidal

(cholesterol) thiadiazoline derivative screening on bacteria. Considering the facts, that nearly all the classes of the cyclic thiosemicarbazones are biologically active, and as a part of our continuous efforts towards the development of more potent antibacterial agents, we herein report the synthesis, characterization, and *in vitro* antibacterial activity of novel spiro-1,3,4-thiadiazoline derivatives.

RESULTS AND DISCUSSION

Chemistry. The starting material steroidal thiosemicarbazones were prepared by condensing the steroidal ketones with *p*-toludinethiosemicarbazide in the presence of catalytic amount of conc. HCl [12]. 3β-acetoxycholest-6-one [13], 3β-chloro-cholest-6-one [14], and 5α-cholest-6-one [15] were prepared according to the published methods. The 1,3,4-thiadiazoline derivatives were synthesized according to the literature procedure by the acetylation of steroidal thiosemicarbazone derivatives as shown in Scheme 1 [16]. All the compounds were soluble in DMSO and ethanol. The structures of all the compounds were established on the basis of spectral studies such as IR, ^{1}HNMR, FAB mass spectra, and the elemental analyses were carried out to check the purity of the compounds.

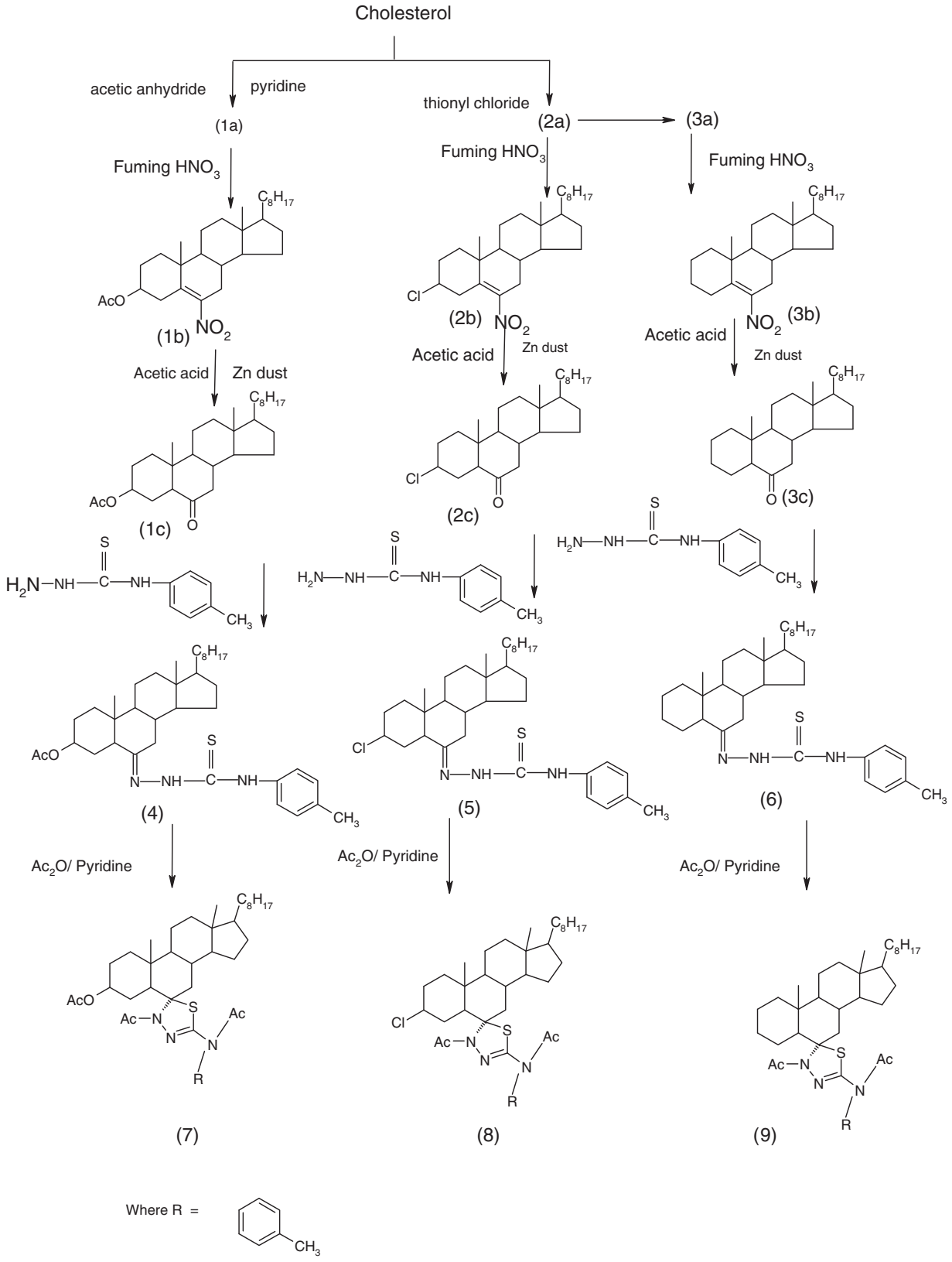
Assignments of selected characteristic IR band positions provide significant indication for the formation of the cyclized thiadiazoline analogs of thiosemicarbazones. All the compounds showed *v* (C═N) stretch at 1652–1658 cm^{-1} due to the ring closure. In addition, the absorption band at 1162–1168 cm^{-1} was attributed to the *v* (C—N) stretch vibrations. The compounds showed intense bands at 642–648 cm^{-1} due to *v* (C—S) stretch, which also confirm the formation of thiazole ring in all the compounds. Further evidence for the formation of thiadiazoline compounds was obtained from the ^{1}H-NMR spectra, which provide diagnostic tools for the positional elucidation of the protons. Assignments of the signals are based on the chemical shifts and intensity patterns. The acetylation proton thiadiazoline ring of all the compounds is shown as singlet in the range 2.12–2.26 ppm. ^{13}C NMR spectra of the compounds **(7-9)** were taken in $CDCl_3$ and the signal obtained further confirmed the proposed structures. All the compounds showed a signal at (146-7-155.8) ppm due to (N═C—S) and (47.5–55.8) ppm due to N—C6—S indicates the cyclization of thiocarbamoyl carbon. The characteristic peaks observed within the ^{13}C NMR spectra of thiadizazoline derivatives are given in Section 6.Characteristic peaks were observed in the mass spectra of compounds **7–9**, which followed the similar fragmentation pattern. The spectrum of compound **7** showed a molecular ion peak (M^+) at m/z 691, compound **8** showed a molecular ion peak (M^+) at m/z 668 and compound **9** showed a molecular ion peak (M^+) at m/z 634. Further fragmentation pattern of these compounds has given in the in experimental section.

Stereochemistry of spiro1,3,4-thiadiazolines. All the 1,3,4-thiadiazolines derivatives (**7–9**) have C6—S bond axially and C6—NAc bond as equatorially oriented. Although, sulfur atom is more bulky than the nitrogen but NAc group becomes more bulky than the sulfur atom, so during cyclization (Scheme 1) the thiadiazoline ring preferably closes at C-6 from the front (β, axial) side by the attack of sulfur (C═S) of the thiosemicarbazone moiety and as the bulky group, already attached at C-6, is moved toward back (α, equatorial) side to avoid the 1,3-diaxial interactions mainly because of C10β-Me, and leaving front (β, axial) side for the attack of incoming group. So the compounds (**7-9**) of this reaction have R geometry at C-6 in which C6—S and C6—NAc bonds are oriented axially (β) and equatorially (α), respectively. This arrangement (geometry) provides greater stability to the molecule as they have less 1,3-diaxial interactions.

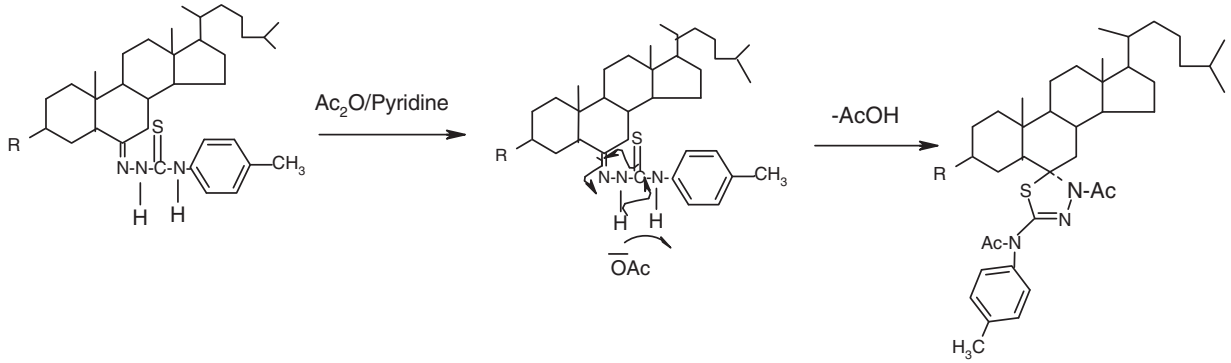
The mechanism (Scheme 2) for the formation of 1,3,4-thiadiazolines can also explained on the basis of the hard soft acid and base principle [17]. The harder acetylating reagent reacts with the harder nitrogen atom rather than the softer sulfur atom and this acetylation favors cyclization of thiosemicarbazones to 1,3,4-thiazolines.

Antibacterial activity. ***Disc–diffusion and micro dilution assay.*** The compounds (**4–9**) were tested for their antibacterial activities by disc-diffusion method using nutrient broth medium [contained (g/L): beef extract 3 g; peptone 5 g; pH 7.0] [18]. The Gram-positive bacteria and Gram-negative bacteria utilized in this study consisted of *Staphylococcus aureus, Streptococcus pyogenes, Salmonella typhimurium*, and *Escherichia coli*. In the disc-diffusion method, sterile paper discs (0.5 mm) impregnated with compound dissolved in dimethylsulfoxide (DMSO) at concentration 100 μg/mL were used. Then, the paper discs impregnated with the solution of the compound tested were placed on the surface of the media inoculated with the microorganism. The plates were incubated at 35°C for 24 h. After incubation, the growth inhibition zones are shown in Table 1. The thiadiazoline derivatives were further checked by minimum inhibitory concentration (MIC) method. The results are presented in Table 2. The molecular structure of these active compounds showed enhanced activity. The distinct differences in the antibacterial property of these compounds further justify the purpose of this study. The importance of such work lies in the possibility that the new compound might be more efficacious drugs against bacteria for which a thorough investigation regarding the structure-activity relationship, toxicity, and their biological effects, which could be helpful in designing more potent antibacterial agents for therapeutic use.

Scheme 1. Schematic diagram showing the synthesis of compounds **7**, **8**, and **9**. compound **1a**: 3β-acetoxycholest-5-ene; compound **2b**: 3β-chloro-cholest-5-ene; compound **3a**: cholest-5-ene.



Scheme 2. Mechanism of steroidal thiadiazoline derivatives.



CONCLUSION

This research involves the synthesis of novel steroidal (6R)-spiro-1,3,4-thiadiazoline derivatives by the cyclization of steroidal thiosemicarbazones. The antibacterial activity of these compounds was carried out by disc-diffusion method and the MIC, against culture of bacteria. The biological behavior of these compounds revealed that chloro- and acetoxy-substituents on the 3β-position of the steroidal thiadiazoline ring increased the antibacterial activity. Among all the six compounds compound **7** and **8** showed better antibacterial activity than the respective drug amoxicillin. These results identified that steroidal (6R)-spiro-1,3,4-thiadiazoline derivatives are new leads in antibacterial chemotherapy. The study suggests the beneficial potential of these leads that need to be further explored in order to discover and develop better and yet safer therapeutic agents for bacterial infections.

EXPERIMENTAL

All chemicals were purchased from Aldrich Chemical Company (USA) and were used without further purification. Precoated aluminum sheets (silica gel 60 F_{254}, Merck Germany) were used for thin-layer chromatography (TLC) and spots were visualized under UV-light. All melting points were measured with a capillary apparatus and are uncorrected. All the compounds were routinely checked by IR, ^{1}H NMR, and mass spectrometry. IR spectra were recorded on a Perkin-Elmer model 1620 FTIR spectrophotometer as KBr discs. ^{1}H NMR spectra were recorded on Brucker spectroscopin DPX-400 MHz spectrophotometer in $CDCl_3$ and DMSO. The following abbreviations were used to indicate the peak multiplicity s-singlet, d-doublet, t-triplet, and m-multiple. FAB mass spectra were recorded on a JEOL SX102 mass spectrometer using Argon/Xenon (6 kV, 10 mB gas. Column chromatography was performed on silica gel (merck). Anhydrous sodium sulfate was used as a drying agent for the organic phase.

Synthesis of steroidal thiosemicarbazones (4–6): A general method. Steroidal thiosemicarbazones was synthesized by refluxing the solution of thiosemicarbazide (0.03 mol) in methanol and the alcoholic solution of steroidal ketones (0.03 mol) at 60°C for 5 h with continuous stirring after cooling the compounds were filtered and recrystallized from methanol.

3β-acetoxy-5α-cholestan-6-one-*p*-toludinethiosemicarbazone (4). Yield: 68%; m.p. 156°C; IR (KBr) λ_{max} cm^{-1}: 3245 (N—H), 1732 (AcO), 1562 (C═N), 1621 (C═C), 1124 (C—N), 1034 (C═S); ^{1}H NMR (DMSO) δ_H: 10.46 (2H, s, —NH), 7.32–8.22 (4H, m, aryl protons), 4.76 (br, m, 1H J = 18 Hz, C3α axial), 1.92 (s, 3H, CH_3), 1.08 (s, 3H, 10—CH_3), 0.96 (s, 3H, 18—CH_3),

Table 1

Antibacterial activity of steroidal thiosemicarbazones (**4–6**) and steroidal thiadiazoline derivatives (**7–9**) (positive control amoxicillin and negative control DMSO) measured by the halo zone test (mm).

	Corresponding effect on microganisms			
Compounds	*S. aureus*	*S. pyogenes*	*S. typhimurium*	*E. coli*
4	10.8 ± 0.4	11.5 ± 0.3	11.2 ± 0.2	12.6 ± 0.3
5	10.6 ± 0.5	11.2 ± 0.3	10.3 ± 0.2	10.2 ± 0.5
6	9.8 ± 0.5	9.4 ± 0.2	10.4 ± 0.2	9.6 ± 0.3
7	17.5 ± 0.5	21.4 ± 0.5	19.4 ± 0.4	20.5 ± 0.5
8	21.7 ± 0.4	20.5 ± 0.4	22.8 ± 0.3	21.6 ± 0.6
9	13.6 ± 0.5	14.4 ± 0.5	14.2 ± 0.4	13.8. ± 0.4
Amoxycillin	17.0 ± 0.5	18.2 ± 0.4	17.2 ± 0.8	20.0 ± 0.2
DMSO	–	–	–	–

Table 2

Minimum inhibition concentration (MIC) of steroidal thiadiazoline derivatives (positive control amoxicillin).

MIC (μg mL^{-1}), Strain	Compounds 7	8	9	Positive control
S. aureus	32	32	128	32
S. Pyogenes	64	32	64	32
S. typhimurium	64	64	128	32
E. coli	128	32	512	32

0.88 (s, 3H, 19—CH_3) and 0.78 (s, 3H, 13—CH_3); Mass spectra ($M^{+\cdot}$) at m/z 608, 593 (M—CH_3), 517 (M—C_7H_7), 502 (M—C_7H_8N), 458 (M—C_8H_8NS), 443 (M—$C_8H_9N_2S$), 549 (M—AcO) Anal. Calc. for $C_{37}H_{57}N_3O_2S$: C, 73.14; H, 9.39; N, 6.91. Found: C, 73.09; H, 9.25; N, 6.55.

3β-chloro-5α-cholestan-6-one-*p*-toludinethiosemicarbazone (5). Yield: 72%; m.p. 136 °C; IR (KBr) λ_{max} cm^{-1}: 3242 (N—H), 1566 (C═N), 1628 (C═C), 1122 (C—N), 1032 (C═S), 716 (C—Cl); ^{1}H NMR (DMSO) δ_H: 10.42 (2H, s, —NH), 7.31–8.36 (4H, m, aryl protons), 4.54 (br, m, 1H w1/2 = 17 Hz, C3α—H, axial), 1.91 (s, 3H, —CH_3), 1.06 (s, 3H, 10—CH_3), 0.95 (s, 3H, 18—CH_3), 0.86 (s, 3H, 19—CH_3), and 0.74 (s, 3H, 13—CH_3); Mass spectra ($M^{+\cdot}$) at m/z 584, 569 (M—CH_3), 493 (M—C_7H_7), 478 (M—C_7H_8N), 458 (M—C_8H_8NS), 419 (M—$C_8H_9N_2S$), 549 (M—Cl); Anal. Calc. for $C_{35}H_{54}N_3SCl$: C, 72.04; H, 9.26; N, 7.20. Found: C, 71.96; H, 9.16; N, 7.18.

5α-cholestan-6-one-*p*-toludinethiosemicarbazone (6). Yield: 75%; m.p. 210°C; IR (KBr) λ_{max} cm^{-1}: 3252 (N—H), 1572 (C═N), 1632 (C═C), 1126 (C—N), 1028 (C═S); ^{1}H NMR (DMSO) δ_H: 10.46 (2H, s, —NH), 7.30–8.42 (4H, m, aryl protons), 1.91 (s, 3H, —CH_3), 1.05 (s, 3H, 10—CH_3), 0.97 (s, 3H, 18—CH_3), 0.86 (s, 3H, 19-CH_3) and 0.76 (s, 3H, 13-CH_3); Mass spectra ($M^{+\cdot}$) at m/z 550, 535, (M—CH_3), 459 (M—C_7H_7), 441 (M—C_7H_8N), 400 (M—C_8H_8NS), 385 (M—$C_8H_9N_2S$); Anal. Calc. for $C_{35}H_{55}N_3S$: C, 76.50; H, 10.01; N, 7.65. Found: C, 75.52; H, 9.96; N, 7.62.

Oxidative cyclization of steriodal 6-ketone thiosemicarbazones (4-6). Steroidal 6R-Spiro-1′,3′,4′-thiadiazolines. General procedure. Steroidal thiosemicarbazones 4–6 (1.0 mmol) were dissolved in chloroform (25 mL) and treated with freshly distilled acetic anhydride (11.0 mmol) and pyridine (2.5 mmol) and the mixture was stirred for 3–4 h over an oil bath at 80°C. Reaction progress was monitored by TLC. After completion, solvent was removed under reduced pressure and the residue was purified by column chromatography over silica gel (light petroleum:diethyl ether, 8:2) to give the respective steroidal (6R)-spiro-1′,3′,4′-thiadiazolines 7–9.

3β-acetoxy-5α-cholestan-(6R)-spiro-6, 4′-acetyl-2′-(acetylaminomethylbenzene)-Δ$^{2'}$-1′,3,4′-thiadiazoline (7). Yield: 74%; m.p. 132°C; IR ν_{max} (KBr) cm^{-1}: 2965 (C—H), 1722, 1692 (amide), 1656 (C═N), 1735 (OCOCH_3), 1162 (C—N), 648 (C—S). ^{1}H NMR ($CDCl_3$): δ/ppm: 4.86 (m, $W_{1/2h}$ 18 Hz, C3 α—H), 2.26, 2.12 (each, s, 3H Ac), 7.34–8.32 (4H, m, aryl protons), 1.94 (s, 3H, CH_3), 0.75 (s, 3H, 13-CH_3), 0.96 (s, H, 18-CH_3), 1.12 (s, 3H, 10-CH_3), and 0.82 (s, 3H, 19-CH_3); ^{13}C NMR ($CDCl_3$): δ/ppm: 173.6, 168.0, 155.2, 136.6, 132.2, 128.4, 52.3, 45.2, 29.3, 24.5, 21.2, 18.7; Mass spectra ($M^{+\cdot}$) at m/z 691, 677 (M—CH_3), 601 (M—C_7H_7), 650 (M—CH_3CO), 545 (M—$C_9H_{10}NO$), 428 (M—$C_{12}H_{13}N_3SO_2$), 632 (M—CH_3COO); Anal. Calc. for $C_{41}H_{61}N_3O_4S$: C, 71.20; H, 8.82; N, 6.07. Found: C, 70.95; H, 8.78; N, 6.07.

3β-chloro-5α-cholestan-(6R)-spiro-6,4′-acetyl-2′-(acetylaminomethylbenzene)-Δ$^{2'}$-1′,3,4′-thiadiazoline (8). Yield: 62%; greenish semi-solid IR ν_{max} (KBr) cm^{-1}: 2962 (C—H), 1732, 1696 (amide), 1658 (C═N), 1168 (C—N), 722 (C—Cl), 646 (C—S). ^{1}H NMR ($CDCl_3$): δ/ppm: 4.52 (m, $W_{1/2h}$ 18 Hz, C3 α—H), 2.24, 2.14 (each, s, 3H Ac), 7.36–8.34 (m, aryl protons), 1.96 (s, 3H, CH_3), 0.74 (s, 3H, 13-CH_3), 0.97 (s, H, 18-CH_3), 1.14 (s, 3H, 10-CH_3), and 0.84 (s, 3H, 19-CH_3); ^{13}C NMR ($CDCl_3$): δ/ppm: 174.4, 165.4, 154.2, 137.2, 136.4, 127.5, 54.3, 46.5, 27.3, 25.7, 22.5, 19.8; Mass spectra ($M^{+\cdot}$) at m/z 668, 653 (M—CH_3), 577 (M—C_7H_7), 625 (M—CH_3CO), 520 (M—$C_9H_{10}NO$), 405 (M—$C_{12}H_{13}N_3SO_2$), 533 (M—Cl); Anal. Calc. for $C_{39}H_{58}O_2N_3SCl$: C, 70.16; H, 8.69; N, 6.29. Found: C, 69.98; H, 8.45; N, 6.08.

5α-cholestan-(6R)-spiro-6,4′-acetyl-2′-(acetylaminomethylbenzene)-Δ$^{2'}$-1′,3,4′-thiadiazoline (9). Yield: 73%; semi-solid; IR ν_{max} (KBr) cm^{-1}: 2962 (C—H), 1726, 1698 (amide), 1652 (C═N), 1166 (C—N), 642 (C—S); ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm: 4.56 (m, $W_{1/2h}$ 18 Hz, C3 α—H), 2.26, 2.16 (each, s, 3H Ac), 7.38–8.38 (m, aryl protons), 1.92 (s, 3H, CH_3), 0.76 (s, 3H, 13-CH_3), 0.98 (s, H, 18-CH_3), 1.12 (s, 3H, 10-CH_3), and 0.86 (s, 3H, 19-CH_3); ^{13}C NMR ($CDCl_3$): δ/ppm: 175.6, 164.2, 153.5, 135.2, 134.5, 126.5, 53.5, 44.2, 28.6, 23.8, 19.2, 20.8, Mass spectra ($M^{+\cdot}$) at m/z 634, 619 (M—CH_3), 543 (M—C_7H_7), 591 (M—CH_3CO), 486 (M—$C_9H_{10}NO$), 371 (M—$C_{12}H_{13}N_3SO_2$). Anal. Calc. for $C_{39}H_{59}O_2N_3S$: C, 73.93; H, 9.30; N, 6.63. Found: C, 73.85; H, 9.18; N, 6.45.

Organism culture and *in vitro* screening. Antibacterial activity was carried out by the disc-diffusion method with minor modifications. *S. aureus, S. pyogenes, S. typhimurium*, and *E. coli* were sub cultured in BHI medium and incubated for 18 h at 37°C, and then the bacterial cells were suspended, according to the McFarland protocol in saline solution to produce a suspension of about 10^{-5} CFU mL^{-1}: 10 μL of this suspension was mixed with 10 mL of sterile antibiotic agar at 40°C and poured onto an agar plate in a laminar flow cabinet. Five paper discs (0.5 mm diameter) were fixed onto nutrient agar plate. One milligram of each test compound was dissolved in 100 μL DMSO to prepare stock solution and from stock solution different concentration 10, 20, 25, 50, and 100 μg/μL of each test compound were prepared. These compounds of different concentration were poured over disc plate on to it. amoxicillin (30 μg/disc) was used as standard drug (positive control). DMSO poured disc was used as negative control. The susceptibility of the bacteria to the test compounds was determined by the formation of an inhibitory zone after 18 h of incubation at 36°C. (Table 1) reports the inhibition zones (mm) of each compound and the controls. The minimum inhibitory concentration (MIC) was evaluated by the macro dilution test using standard inoculums of 10^{-5} CFL mL^{-1}. Serial dilutions of the test compounds, previously dissolved in dimethyl sulfoxide (DMSO) were prepared to final concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2, and 1 μg/mL to each tube was added 100 μL of a 24 h old inoculum. The MIC, defined as the lowest concentration of the test compound, which inhibits the visible growth after 18 h, was determined visually after incubation for 18 h, at 37°C, and the

result are presented in Table 2. Tests using DMSO and amoxicillin as negative and positive controls.

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REFERENCES AND NOTES

[1] Qadri, F.; Svennerholm, A. M.; Faruque, A. S. G.; Sack, R. B. Clin Microbiol Rev 2005, 18, 465.

[2] Zhang, W.; Berberoy, E. M.; Freeling, J.; He, D.; Moxley, R. A.; Francis, D. H. Infect Immun 2006, 74, 3107.

[3] Butler, C. C.; Hillier, S.; Roberts, Z.; Dunstan, F.; Howard, A.; Palmer, S. Br J Gen Pract 2006, 56, 686.

[4] Puerto, S. A.; Fernandez, G. J.; Castillo, L. D. J.; Jose, M.; Pinoa, S.; Anguloa, P. G. Microbiol Infect Dis 2006, 42, 1513.

[5] Gupta, A.; Mishra, P.; Pandeya, S. N.; Kashaw, S. K.; Kashaw, V.; Stables, J. P. Eur J Med Chem 2009, 44, 1100.

[6] Zhan, P.; Liu, X.; Cao, Y.; Wang, Y.; Pannecouque, C.; Clercq, E. D. Bioorg Med Chem Lett 2006, 18, 5368.

[7] Hagiwara, K.; Hashimoto, S.; Shimoda, S. J Pesticide Sci 1992, 17, 251.

[8] Satyanarayana, K.; Rao, M. N. A. J Pharm Sci 1995, 84, 263.

[9] Satyanarayana, K.; Rao, M. N. A. Eur J Med Chem 1995, 30, 641.

[10] Kavali, J. R.; Badami, B. V. IL Farmaco 2000, 55, 406.

[11] Khan, S. A.; Yusuf, M. Eur J Med Chem 2009, 44, 2597.

[12] Khan, S. A.; Saleem, K.; Khan, Z. Eur J Med Chem 2007, 42, 103.

[13] Callow, R. K.; James, V. H. T. J Chem Soc 1956, 4744.

[14] Millurn, A. H.; Trutr, E. V. J Chem Soc 1956, 1736.

[15] Dauben, G. W.; Takemura, K. H. J Am Chem Soc 1953, 75, 6302.

[16] Bekhit, A. A.; Ashour, H. M. A.; Ghany, Y. S. A.; Bekhit, A. A.; Pearson, R. G.; Sngstah, J. J. A. Chem Soc 1967, 89, 1827.

[17] Baraka, A. Eur J Med Chem 2008, 43, 456.

[18] Bauer, W. A.; Kirby, M. W.; Sherris, C. J.; Turck, M. Am J Clin Pathol 1966, 45, 493.