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Short communication

Synthesis, characterization and *in vitro* antibacterial activity of new steroidal thiazolo quinoxalines

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Abstract

Herein, we report the synthesis of different steroidal thiazolo quinoxaline derivatives as antibacterial agents against *Escherichia coli*. Steroidal ketone thiosemicarbazones (**4**–**6**) were obtained from corresponding ketones (**1**–**3**) by refluxing with thiosemicarbazide. The thiosemicarbazones on reaction with 2,3-dichloroquinoxalines at 80 °C gives 3β-acetoxy-5α-cholestan-6-[thiazolo(4,5-*b*)quinoxaline-2-yl-hydrazone] (**7**), 3β-chloro-cholestan-6-[thiazolo(4,5-*b*)quinoxaline-2-yl-hydrazone] (**8**), and 5α-cholestan-6-[thiazolo(4,5-*b*)quinoxaline-2-yl-hydrazone] (**9**). The structures of the compounds were evident by elemental, IR, ^{1}H NMR and FAB mass spectral analyses. The antibacterial activities of these compounds were evaluated by disk diffusion method against the culture of *E. coli* and the results were compared with the standard drug amoxicillin. The results reveal that the compounds are better antibacterial agents as compared to amoxicillin. Among all the three compounds (**7**–**9**), compound **8** showed better zone of inhibition.

Keywords: Thiosemicarbazone; Thiazolo quinoxalines; Antibacterial activity

1. Introduction

Morbidity and mortality due to enteric bacterial infection remain important health problems worldwide mainly in developing countries and regions such as the Indian sub-continent, part of South America and tropical part of Africa [1,2]. Invasive dysentery and diarrhea caused by *Escherichia coli* are the world's most prevalent and fatal infectious diseases [3,4]. Patients usually show wide range of symptoms such as stomachache, cramps, bloating or tenderness [5]. Abscess of the brain is a dreadful complication of *E. coli* infection [6]. Amoxicillin, norfloxacin and ciprofloxacin are the most common drugs used for *E. coli* infection [7] but are associated with severe side effects. Toxicity and resistance to the drugs also play important role in the treatment failure [8]. Therefore, there is an urgent need to screen new compounds for the development of new antibacterial agents against *E. coli* infection.

The study of quinoxaline derivatives has become of much interest in recent years on account of their antibacterial, antiviral, anti-cancer, anti-fungal, anti-helmithic and insecticidal [9,10] activities. Thiazolo quinoxaline ring dramatically increases the diversity of certain biological properties such as antibacterial, antiviral and antiamoebic activities [11–14]. Recently, the compounds obtained by the cyclization of thiosemicarbazones have been reported as antibacterial agents [15]. In this paper the steroidal thiazolo quinoxaline derivatives have been synthesized by the condensation of the steroidal thiosemicarbazone with 2,3-dichloroquinoxaline. The activities of these compounds were screened *in vitro* against *E. coli*.

2. Results and discussion

The yield of the product of thiosemicarbazones is in the range of 60–75%. The thiazolo(4,5-*b*)quinoxaline-2yl-hydrazone

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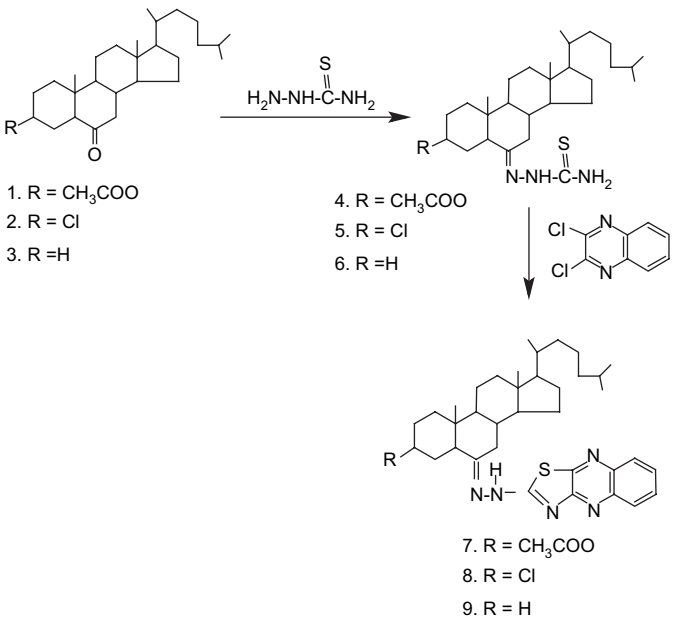
derivatives were synthesized by using the literature procedure [16,17]. Under the reaction condition, the reaction mixture contained only unreacted starting material and cyclized product in good yield. All the three compounds (**7**–**9**) were prepared by refluxing an equimolar ratio of thiosemicarbazones and 2,3-dicholoro quinoxaline in dry ethanol and gave 65–80% yield (Scheme 1). The obtained compounds are stable in the solid state as well as in the solution state.

2.1. IR spectral studies

Assignments of selected characteristic IR band positions provide significant indication for the formation of steroidal thiazolo(4,5-*b*)quinoxaline-2-yl-hydrazone. All the compounds (**7**–**9**) showed intense band in the region of 1555–1561 cm^{-1} due to the ν (C═N) stretch. IR spectra of all the compounds showed ν (C–S) stretching at 615–635 cm^{-1} due to the thiazolo ring closure. In addition, the absorption band at 1130–1180 cm^{-1} was attributed to the ν (C–N) stretching vibration, which also confirms the formation of desired thiazolo quinoxaline ring in all the compounds. All the compounds showed additional sharp band in the region of 3412–3418 cm^{-1} due to the ν (NH) stretch.

2.2. Nuclear magnetic resonance spectral studies

Further evidence for the formation of steroidal thiazolo(4,5-*b*)quinoxaline-2-yl-hydrazone compounds was obtained from the ^{1}H NMR spectra, which proved to be a diagnostic tool for the positional elucidation of the proton. Assignments of the signals are based on the chemical shift and intensity pattern. The aromatic protons of thiazolo quinoxaline are in the range 7.2–7.6 ppm for compound **7**, 7.5–7.9 ppm for compound **8**, and 6.5–7.3 ppm for compound **9**. A singlet due to NH proton in compounds **7**–**9** was observed at 8.2, 8.4 and 8.08 ppm, respectively.



Scheme 1. Schematic diagram showing the synthesis of compounds **7**–**9**.

2.3. FAB mass analysis

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 ($M^{+\bullet}$) at *m*/*z* 643, compound **8** showed a molecular ion peak ($M^{+\bullet}$) at *m*/*z* 621 and compound **9** showed a molecular ion peak ($M^{+\bullet}$) at *m*/*z* 585. Other fragments within the mass spectra of thiazolo(4,5-*b*)quinoxaline-2-yl hydrazones (compounds **7**–**9**) are given in Section 4.

2.4. In vitro antimicrobial activity

The *in vitro* antibacterial activities of thiazolo(4,5-*b*)quinoxaline-2-yl-hydrazone (**7**–**9**) were carried out using the culture of *E. coli* by the disk diffusion method [18]. Amoxicillin (30 μg) was used as the standard drug, whereas DMSO poured disk was used as negative control. Compounds **7**–**9** have 3β-acetoxy, 3β-chloro and cholestan groups, respectively. The *in vitro* study results showed that compound **8** with chloro group at 3β position was found to be more active among all the three compounds. The molecular structure of compound **8** showed enhanced activity and its zone of inhibition was 1.6 cm (Figs. 1a and 2a for compound **7**, Figs. 1b and 2b for compound **8** and Figs. 1c and 2c for compound **9**). The results significantly showed that the three compounds are antibacterial agents against *E. coli* compared to standard drug amoxicillin as indicated in Figs. 1d, e and 2d). The distinct difference in the antibacterial potency of the thiazolo(4,5-*b*)quinoxaline-2-yl-hydrazones (**7**–**9**) further justifies the purpose of this study. The importance of such work lies in the possibility that the new compound might be a more efficacious drug against bacteria for which a thorough investigation regarding the structure activity relationship, toxicity and its biological effects is essential, which could be helpful in designing more potent antibacterial agents for therapeutic use.

3. Conclusion

Literature survey revealing the biological importance of steroids and their derivatives prompted us to synthesize new steroidal thiazolo quinoxaline derivatives (**7**–**9**). To the best of our knowledge, this is the first report on steroidal quinoxaline derivatives and it shows very encouraging results of *in vitro* activity against *E. coli*. We have examined the antibacterial activities of all the three compounds having 3β-acetoxy, 3β-chloro and cholestan groups, using disk diffusion method. It is inferred from the biological studies that among all the three compounds, compound **8** with chloro group at 3β position was found to be a better inhibitor of *E. coli*.

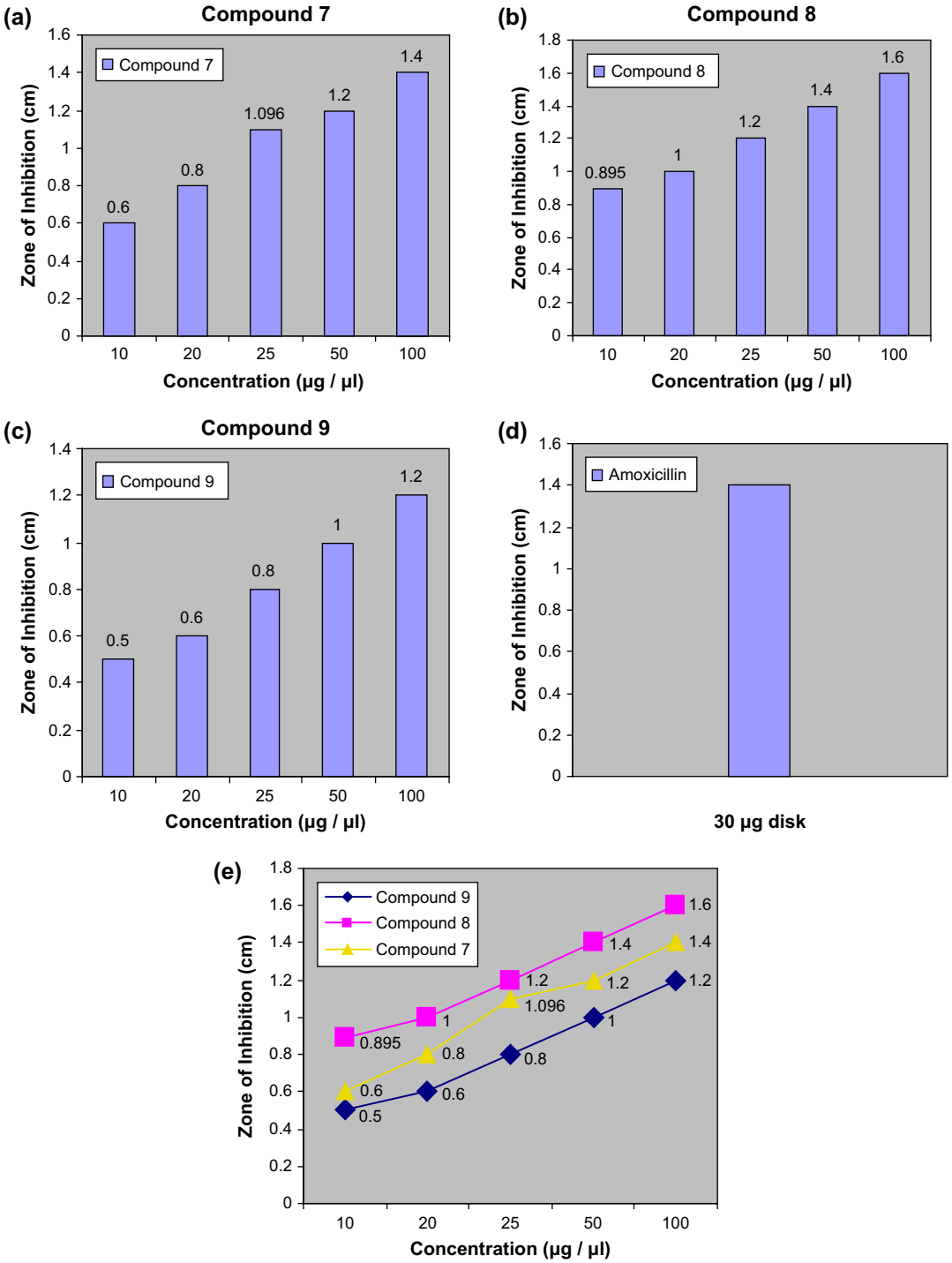


Fig. 1. (a), (b), and (c) depict the graphical representation of antibacterial activities of synthesized compounds **7–9**, (d) represents the antibacterial activity of amoxicillin, and (e) shows the graphical representation of all the three compounds **7–9**.

4. Experimental

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 spectrometries. IR spectra were recorded in KBr on a Perkin–Elmer model 1620 FTIR spectrophotometer. ^{1}H NMR spectra were recorded at ambient temperature using a Brucker spectroscopin DPX-300 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 – multiplet. 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). Thin layer chromatography (TLC) was carried out on 2.5 × 7.5 cm plates with a large thickness of 0.25 mm using the indicator

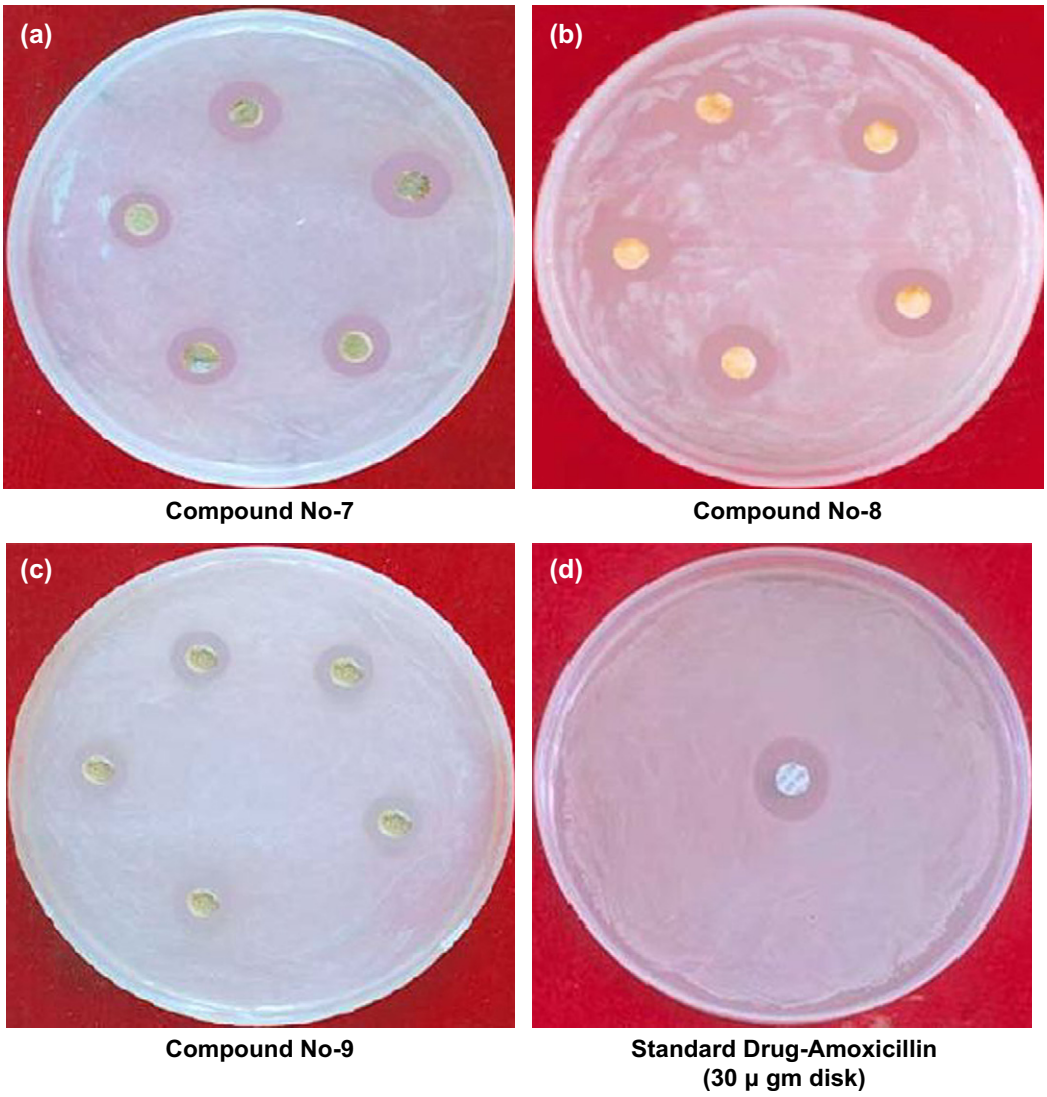


Fig. 2. (a), (b), (c) and (d) show the snaps depicting the antibacterial activities of synthesized compounds **7**, **8** and **9** and standard drug amoxicillin.

elements. Anhydrous sodium sulfate was used as a drying agent for the organic phase. Compounds 3β-acetoxy-5α-cholestan-6-one (**1**) [19], 3β-chloro-5α-cholestan-6-one (**2**) [20–22] and 5α-cholestan-6-one (**3**) [23] were prepared according to the published methods.

4.1. General method for the synthesis of thiosemicarbazone

To a boiling solution of ketone (**1**, **2** or **3**) (4.49 mmol) in 20 ml ethanol, few drops of concentrated HCl were added followed by thiosemicarbazide (0.41 g, 4.56 mmol) in ethanol (15 ml) with stirring. The reaction mixture was refluxed for 1 h and then cooled. The heavy precipitate thus obtained was collected by filtration and purified by recrystallization from methanol to give corresponding thiosemicarbazone.

*4.1.1. 3β-Acetoxy-5α-cholestan-6-one-thiosemicarbazone (**4**)*

White crystalline solid (ethanol); yield: 95%; m.p. 128 °C; Anal. calc. for $C_{30}H_{51}N_3O_2S$: C, 69.59; H, 9.93; N, 8.12. Found: C, 69.55; H, 9.98; N, 8.08%. IR (KBr): ν_{max} cm^{-1}: 3500 (NH_2), 3355 (N–H), 1735 ($OCOCH_3$), 1590 (C=N), 1175 (C=S), 1040 (C–O). ^1H NMR ($CDCl_3$) (δ): 9.50 (s, 1H, NH), 6.52 (s, 2H, NH_2), 4.70 (br, m, 1H, $w1/2 = 18$ Hz, $C_{3\alpha}$–H, axial), 2.01 (s, 3H, $OCOCH_3$), 1.18 (C_{10}–CH_3), 0.72, (C_{13}–CH_3), 0.96 and 0.86 (remaining methyl protons).

*4.1.2. 3β-Chloro-5α-cholestan-6-one-thiosemicarbazone (**5**)*

Spongy yellow solid (ethanol); yield: 85%; m.p. 136–138 °C; Anal. calc. for $C_{28}H_{48}N_3SCl$: C, 68.05; H, 9.79; N, 8.5. Found: C, 68.00; H, 9.79; N, 8.47%. IR (KBr) ν_{max} cm^{-1}: 3515 (NH_2), 3350 (NH), 1580 (C=N), 1170 (C=S), 710 (C–C_1). ^1H NMR ($CDCl_3$) (δ): 9.20 (s, 1H, NH), 6.46 (s, 2H, NH_2), 3.90 (br, m, 1H, $w1/2 = 16$ Hz, $C_{3\alpha}$–H, axial), 1.12 (C_{10}–CH_3), 0.68 (C_{13}–CH_3), 0.93 and 0.82 (remaining methyl protons).

*4.1.3. 5α-Cholestan-6-one-thiosemicarbazone (**6**)*

White solid (ethanol); yield: 88%; m.p. 110–112 °C; Anal. calc. for $C_{28}H_{49}N_3S$: C, 73.14; H, 10.74; N, 9.14. Found: C,

73.07; H, 10.79, N, 9.18%. IR (KBr) ν_{max} cm^{-1}: 3510 (NH_2), 3370 (N–H), 1590 (C=N), 1180 (C=S). ^1H NMR ($CDCl_3$) (δ): 8.80 (s, 1H, NH), 6.38 (s, 2H, NH_2), 1.12 (C_{10}–CH_3), 0.69 (C_{13}–CH_3), 0.93 and 0.83 (remaining methyl protons).

4.2. 3β-Acetoxy-5α-cholestan-6-[thiazolo(4,5-b)quinoxaline-2-yl-hydrazone] (7)

A mixture of 3β-acetoxy-5α-cholestan-6-one-thiosemicarbazone (0.01 mol) and 2,3-dichloroquinoxaline (0.01 mol) in anhydrous ethanol (15 ml) was refluxed for 24 h. Progress of the reaction was monitored by TLC. After completion of the reaction, the solvent was removed under reduced pressure and the residue obtained was purified by column chromatography (20:80, diethyl ether:petroleum ether). Light orange solid obtained was recrystallized with methanol (DMSO); yield: 70%; m.p. 156–158 °C; Anal. calc. for $C_{38}H_{53}N_5SO_2$: C, 70.91; H, 8.24; N, 10.80. Found: C, 70.82; H, 8.20; N, 10.76%. IR (KBr) ν_{max} cm^{-1}: 3412 (N–H), 2960 (C–H), 1555 (C=N), 1145 (C–N), 618 (C–S). ^1H NMR (DMSO-d_6) (δ): 8.20 (s, 1H, NH), 7.20–7.60 (m, 4H, aromatic), 2.01 (s, 3H, $OCOCH_3$), 1.25 (C_{10}, CH_3), 0.63 (C_{13}, CH_3), 0.11 and 0.82 for other methyl protons. Mass spectra (M^+•) at *m/z* 643, 584 (M − AcO), 530 (M − side chain), 457 (M − $C_9H_4N_3S$), 442 (M − $C_9H_5N_4S$).

4.3. 3β-Chloro-5α-cholestan-6-[thiazolo(4,5-b)quinoxaline-2-yl-hydrazone] (8)

A mixture of 3β-chloro-5α-cholestan-6-one-thiosemicarbazone (0.01 mol) and 2,3-dicholoro quinoxaline (0.01 mol) in anhydrous ethanol 15 ml was refluxed for 24 h. Progress of the reaction was monitored by TLC. After completion of the reaction, solvent was removed under reduced pressure and the residue obtained was recrystallized from methanol. Dark brown solid (DMSO); yield: 70%; m.p. 172–174 °C; Anal. calc. for $C_{36}H_{50}N_5SCl$: C, 69.73; H, 8.07; N, 11.29. Found: C, 69.65; H, 8.00; N, 11.18%. IR (KBr) ν_{max} cm^{-1}: 3413 (N–H), 2944 (C–H), 1561 (C=N), 1180 (C–N), 615 (C–S). ^1H NMR (DMSO-d_6) (δ): 8.40 (s, 1H, NH), 7.50–7.90 (m, 4H, aromatic), 0.62, 0.82, 1.04, 1.20 (CH_3-methylene proton). Mass spectra (M^+•) at *m/z* 621, 599 (M − $C_9H_5N_4S$), 584 (M − Cl), 510 (M − side chain), 434 (M − $C_9H_4N_3S$).

4.4. 5α-Cholestan-6-[thiazolo(4,5-b)quinoxaline-2-yl-hydrazone] (9)

A mixture of 6α-cholestan-6-one-thiosemicarbazone (0.01 mol) and 2,3-dicholoro quinoxaline (0.01 mol) in anhydrous ethanol 15 ml was refluxed for 24 h. Progress of the reaction was monitored by TLC. After completion of the reaction, solvent was removed under reduced pressure and the residue obtained was recrystallized from methanol. Orange solid (DMSO); yield: 80%; m.p. 148 °C; Anal. calc. for $C_{36}H_{51}N_5S$: C, 73.84; H, 8.71; N, 11.96. Found: C, 73.80; H, 8.56; N, 11.84%. IR (KBr) ν_{max} cm^{-1}: 3418 (N–H), 2934 (C–H), 1560 (C=N), 1130 (C–N), 635 (C–S). ^1H NMR (DMSO) δ: 8.08 (s, 1H, NH), 6.90–7.30 (m, 4H, aromatic), 0.60, 0.80, 1.08, 1.28 (CH_3-methylene proton). Mass spectra (M^+•) at *m/z* 585, 472 (M − side chain), 399 (M − $C_9H_4N_3S$), 384 (M − $C_9H_5N_4S$).

4.5. Organism culture and in vitro screening

Preliminary experiments were carried out to determine the antibacterial activities of steroidal thiazolo quinoxaline derivatives *in vitro* culture against *E. coli* as previously described in Ref. [18]. The *E. coli* were sub-cultured in broth agar and incubated for 18 h at 37 °C and then freshly prepared bacterial cells were spread onto nutrient agar plate in a laminar flow cabinet. Five paper disks (6.0 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 concentrations 10, 20, 25, 50 and 100 μg/μl of each compound were prepared. These compounds of different concentrations were poured over disk plate. Amoxicillin was used as a standard drug (positive control). DMSO poured disk was used as a negative control. Plates were incubated at 37 °C for 24 h. The diameter of zone of inhibition was measured for each concentration in centimeter as shown in graphs and snaps.

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