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Short communication

Synthesis, structure elucidation and antibacterial evaluation of new steroidal -5-en-7-thiazoloquinoxaline derivatives

Salman Ahmad Khan, Kishwar Saleem*, Zaheer Khan

Department of Chemistry, Jamia Millia Islamia, Jamia Nagar, New Delhi 110025, India

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Abstract

Some heterocyclic systems namely cholest-5-en-7-thiazolo[4,5-*b*]quinoxaline-2-yl-hydrazone] were synthesized by the reaction of cholest-5-en-7-one-thiosemicarbazone with 2,3-dichloroquinoxaline at 80 °C in high yield. The thiosemicarbazone derivatives were obtained by the condensation of the thiosemicarbazide with steroidal ketones. All the compounds have been characterized by means of elemental analyses, IR, ^{1}H NMR and mass spectroscopic data. The in vitro antibacterial activity was evaluated by disk diffusion method and then the minimum inhibitory concentration (MIC) of compounds was determined against the culture of *Escherichia coli*. The results were compared with the standard drug chloramphenicol. The results showed that compounds **7** and **8** are better antibacterial agents as compared to chloramphenicol.

Keywords: Thiosemicarbazone; Thiazoloquinoxalines; Antibacterial activity

1. Introduction

Diarrhea is a serious health problem; moreover drug resistance in diarrhea and dysentery can be attributed to the use of drug (amoxicillin, norfloxacin, ciprofloxacin and chloramphenicol) for treatment and to the adaptation of the bacterial parasite by developing alternate pathways for survival. Hence, the present strategy for new drug development is directed towards developing new steroidal molecules to inhibit the growth of parasite [1,2]. The importance of heterocyclic compounds has long been recognized in the field of synthetic organic chemistry. It is well known that a number of heterocyclic compounds containing nitrogen and sulphur exhibited a wide variety of biological activities. Quinoxaline derivatives are important moieties of several pharmacologically active compounds [3–8]. Although rarely described in nature, synthetic quinoxaline ring is a part of a number of antibiotics such as echinomycin and actinomycin, which are known to inhibit the growth of Gram-positive bacteria and are active against various transplantable tumors [9–11]. There are many examples of biologically active quinoxalines, which showed very interesting pharmacological properties such as antibacterial, antiviral, anticancer, antifungal, antihelmintic and insecticidal [12–15]. Thiazoloquinoxaline ring dramatically increases the diversity of certain biological properties such as antibacterial, antiviral and antiamoebic activities [16–19]. Recently, the compounds obtained from thiosemicarbazone have been reported as antibacterial, antitumor, antiviral and antimalarial agents [20–24]. Steroidal thiosemicarbazones dramatically increase the diversity of certain biological properties [25–27].

The present work is aimed towards developing novel molecules with improved potential for treating bacterial infection (*Escherichia coli*). It is proposed to achieve this by generating a common pharmacophore from the structures of potent antibacterial belonging to different classes by synthesizing novel molecules and evaluating them for antibacterial activity. In this paper the steroidal thiazoloquinoxaline 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*.

* Corresponding author. Tel.: +91 11 26981717/3253, fax: +91 11 26980229/1232.
E-mail address: kishwarsaleem2003@yahoo.co.in (K. Saleem).

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2. Results and discussion

Thiosemicarbazones prepared by condensing the steroidal ketones with thiosemicarbazide gave yields 70–80%. All the steroidal ketones were prepared by the reported method. The thiosemicarbazone derivatives were used as a starting material for the preparation of thiazoloquinoxalines. The thiazolo[4,5-*b*]quinoxaline-2-yl-hydrazone derivatives were synthesized by the literature procedure [28,29] as indicated in Scheme 1. Thiosemicarbazones were heated at reflux with 2,3-dichloroquinoxaline for 24 h and after that solvent was removed under reduced pressure and crystallization was done in ethanol. All the compounds were soluble in DMSO and ethanol. The structures of all the compounds were established by means of their IR, ^{1}H NMR, FAB mass spectra and the elemental analysis were carried out to check the purity of the compounds.

2.1. IR spectral studies

Assignments of selected characteristic IR band positions provide significant indication for the formation of the cyclized thiazoloquinoxaline analogues of thiosemicarbazones. All the compounds showed sharp band in the region 3412–3422 cm^{-1} due to the ν (N–H) stretch. The IR spectra of all the compounds showed ν (C=N) stretch at 1542–1555 cm^{-1} due to the ring closure. In addition, the absorption bands at 1140–1174 cm^{-1} were attributed to the ν (C–N) stretch vibrations. The compounds showed intense bands at 610–632 cm^{-1} due to ν (C–S) stretch, which also confirm the formation of thiazolo ring in all the compounds.

2.2. Nuclear magnetic resonance spectral studies

Further evidence for the formation of thiazoloquinoxaline 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 aromatic protons of thiazoloquinoxaline are shown as multiplet in the range 7.4–7.9 ppm for compound **7**, 7.6–8.1 ppm for compound **8** and 6.9–7.3 ppm for compound **9**. A singlet due to NH proton in compounds **7**, **8** and **9** was observed at 8.42, 8.2 and 8.08 ppm, respectively.

2.3. FAB mass analysis

Characteristic peaks were observed in the mass spectra of compounds **7**, **8** and **9**, which followed similar fragmentation pattern. The spectrum of compound **7** showed a molecular ion peak (M^{+}•) at $m/z = 642$, compound **8** showed a molecular ion peak (M^{+}•) at $m/z = 619$ and compound **9** showed a molecular ion peak (M^{+}•) at $m/z = 585$. The characteristic peaks observed within the mass spectra of thiazolo[4,5-*b*]quinoxaline-2-yl-hydrazone compounds are given in Section 4.

2.4. In vitro antimicrobial activity

The in vitro antibacterial activities of thiosemicarbazones **4–6**, thiazoloquinoxalines **7–9**, cholesterol (**a**) and 2,3-dichloroquinoxaline (**b**) were carried out using the culture of *E. coli* by the disk diffusion method [30] and then the minimum inhibitory concentration (MIC) of all the compounds was determined. Chloramphenicol (30 μg) was used as the standard drug, whereas DMSO poured disk was used as negative control. Minimum inhibitory concentration (MIC) was evaluated by the macro dilution test using standard inoculums of 10^{-5} CFU 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. The thiosemicarbazone derivatives and thiazoloquinoxaline **4**, **7** and **5**, **8** have acetoxy and chloro group at 3β-position, respectively. The in vitro studies' results showed that the compounds chloro and acetoxy derivatives of thiazoloquinoxaline were found to be more active among all the compounds. 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. The inhibition zones (mm) of each compound are reported in Table 1. These active compounds were further checked by MIC method. The results are presented in Table 2.

The molecular structure of these active compounds (**7** and **8**) showed enhanced activity. The distinct difference in the antibacterial property of these compounds further justifies 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 in their biological effects which could be helpful in designing more potent antibacterial agents for therapeutic use.

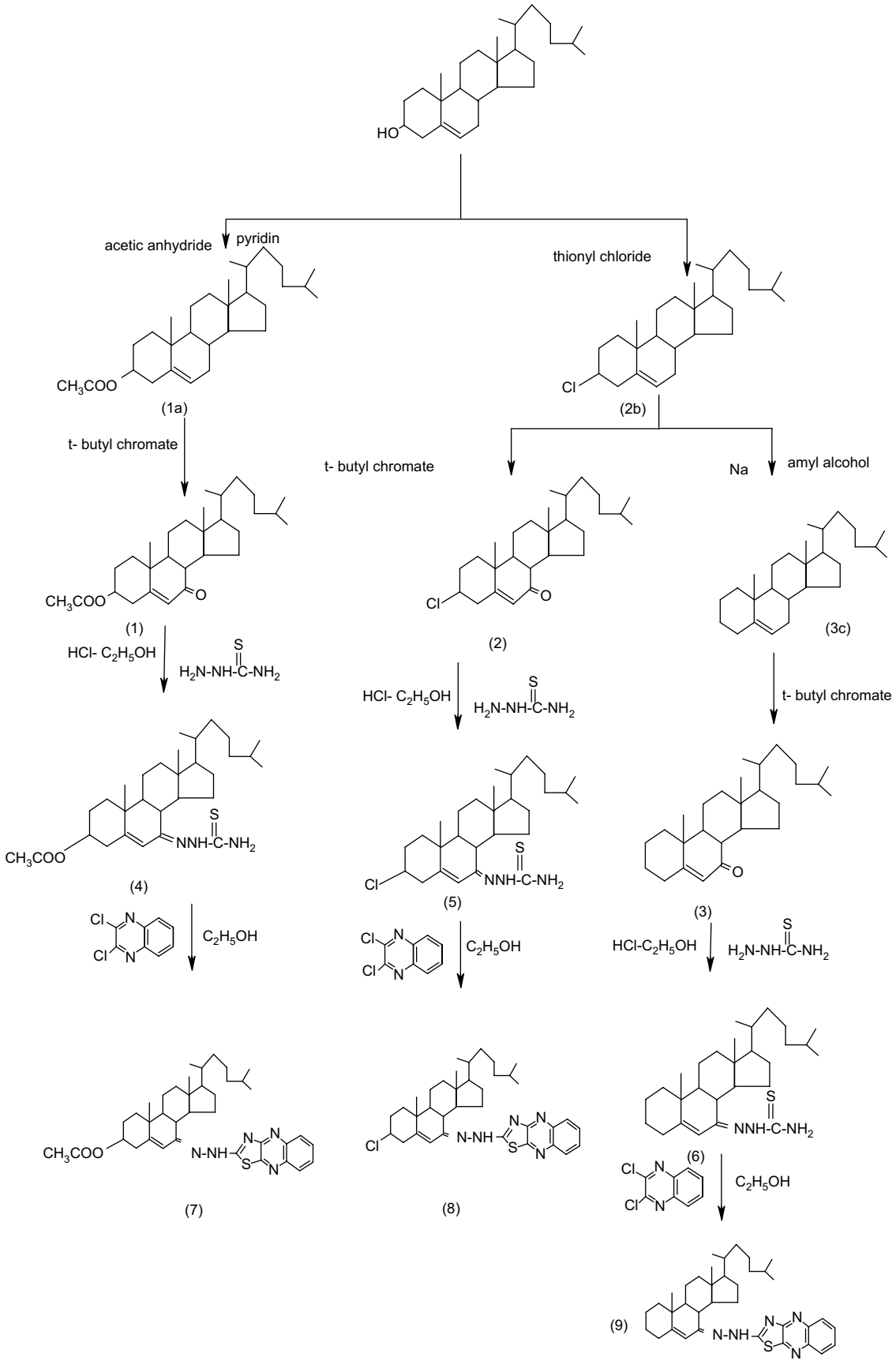
3. Conclusion

This research examined the antibacterial activities of new cyclized steroidal thiazoloquinoxaline prepared by the reaction of thiosemicarbazone with 2,3-dichloroquinoxalines at 80 °C. In vitro antibacterial activities of these compounds were carried out against culture of *E. coli*. The biological behaviour of the compounds revealed that chloro and acetoxy substituents on the 3β-position of the steroidal ring increased the antibacterial activity. Among all the six compounds derivatives **7** and **8** showed better antibacterial activity than their respective drug (chloramphenicol).

4. Experimental

4.1. Materials and methods

All the chemicals were purchased from Aldrich Chemical Company (U.S.A.) and were used without further purification.



Scheme 1. Showing the synthesis of compounds **7**, **8** and **9**.

Table 1
Antibacterial activity of steroidal compounds, 2,3-dichloroquinoxaline, positive control (chloramphenicol), and negative control (DMSO) measured by the halo zone test (unit, mm)

Compound	*E. coli*
(**a**)	5.8 ± 0.3
(**b**)	6.2 ± 0.4
4	13.2 ± 0.2
5	14.8 ± 1.6
6	9.8 ± 0.4
7	20.8 ± 0.4
8	22.4 ± 0.4
9	14.0 ± 0.4
Chloramphenicol	20.0 ± 0.2
DMSO	–

Compounds (**a**) and (**b**) are cholesterol and 2,3-dichloroquinoxaline.

The reactions were monitored by precoated aluminium silica gel 60F 254 thin layer plates procured from Merck (Germany). 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 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, 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. 3β-Acetoxy-cholest-5-en-7-one [31], 3β-chloro-cholest-5-en-7-one [32] and 5α-cholest-5-en-7-one [33] were prepared according to published methods.

4.2. General method for the preparation of thiosemicarbazone

To a solution of steroidal ketones (5.25 mmol) in methanol (30 mL) a few drops of conc. HCl were added followed by thiosemicarbazide (5.25 mmol) in methanol (15 mL) with stirring. The reaction mixtures were heated at reflux for 1.5 h and cooled. The heavy precipitate thus obtained was collected by filtration and purified by recrystallization from methanol to give thiosemicarbazone.

4.2.1. 3β-acetoxy-cholest-5-en-7-one-thiosemicarbazone (*4*)

Light orange crystals (ethanol); yield: 73%; m.p. 119 °C. Anal. calc. for ($C_{30}H_{49}N_3O_2S$): C, 69.84, H, 9.57, N, 8.15; found: C, 69.84, H, 9.48, N, 8.12; IR ν_{max} cm^{-1}; 3485 (NH_2), 3345 (NH), 1725 ($OCOCH_3$), 1625 (C=C weak), 15,859 (C=N), 1172 (C=S); ^{1}H NMR ($CDCl_3$)/ppm; 9.4 (s, 1H, NH), 6.48 (s, 2H, NH_2), 5.72 (s,1H, C6-H), 4.92 (br m, $w = -17$ Hz, C3α-H axial), 2.02 (s, 3H, $OCOCH_3$), 1.18 (C10-CH_3), 0.72 (C13-CH_3) 0.47, 0.86 other methyl protons.

Table 2
Minimum inhibitory concentration (MIC) of steroidal thiosemicarbazone, thiazoloquinoxaline derivatives and positive control (chloramphenicol)

MIC (μg mL^{-1})	Compounds								Positive
Strain	**4**	**5**	**6**	**7**	**8**	**9**	(**a**)	(**b**)	Control
E. coli	128	64	256	64	32	128	512	512	32

Compounds (**a**) and (**b**) are cholesterol and 2,3-dichloroquinoxaline.

4.2.2. 3β-chloro-cholest-5-en-7-one-thiosemicarbazone (*5*)

Orange brown crystals (methanol); yield: 78%; m.p. 141 °C. Anal. calc. for ($C_{28}H_{46}N_3SCl$): C, 68.32, H, 9.41, N, 8.54; found: C, 68.26, H, 9.37, N, 3.52; IR ν_{max} cm^{-1} 3457 (NH_2), 3340 (NH), 1620 (C=C), 1580 (C=N), 1164 (C=S), 725 (C–Cl); ^{1}H NMR ($CDCl_3$)/ppm; 8.76 (s, 1H, NH), 6.42 (s, 2H, NH_2), 5.62 (s, 1H, C6-H), 4.52 (br m, $w = 15$ Hz, C3α-axial), 1.8 (C10-CH_3), 0.69 (C13-CH_3), 0.94, 0.80 (remaining methyl protons).

4.2.3. 5α-cholest-5-en-7-one-thiosamecarbazone (*6*)

White crystals (methanol); yield: 70%; m.p. 102–103 °C. Anal. calc for ($C_{28}H_{47}N_3S$): C, 73.47, H, 10.30, N, 9.16; found: C, 73.47, H, 10.35, N, 9.18; IR ν_{max} cm^{-1} 3505 (NH_2), 3375 (NH), 1622 (C=C), 1575 (C=N), 1175 (C=S); ^{1}H NMR ($CDCl_3$)/ppm; 8.62 (s, 1H, NH), 6.40 (s, 2H, NH_2), 5.42 (s, 1H, C6-H), 1.16 (C10-CH_3), 0.68 (C13-CH_3), 0.92, 0.83 other methyl protons.

4.2.4. 3β-acetoxycholest-5-en-7-[thiazolo(4,5-b)quinoxaline-2-yl-hydrazone] (*7*)

A mixture of 3β-acetoxy-cholest-5-en-7-one-thiosemiarbzone (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 reaction, the solvent was removed under reduced pressure and the residue obtained was purified by column chromatography (15:85, diethyl ether:petroleum ether). Light orange solid obtained was recrystallized with ethanol (DMSO); yield: 70%; m.p. 148 °C. Anal. calc. for ($C_{38}H_{51}O_2SN_5$): C, 71.13, H, 7.94, N, 10.90; found: C, 69.8, H, 8.89, N,10.74; IR ν_{max} cm^{-1}; 3416 (N–H), 2942 (C–H), 1542 (C=N), 1622 (C=C), 1145 (C–N), 625 (C–S); ^{1}H NMR (DMSO-d_6)/ppm; 8.42 (s, 1H, NH), 7.40–7.90 (m, 4H, aromatic), 5.28 (s, 1H, C6-H), 4.4 (br m, $w1/2 = 17$ Hz, C3α-axial), 2.02 (s, 3H, $OCOCH_3$), 1.17 (C10, CH_3), 0.71 (C13, CH_3), 0.95, 0.87 for other methyl protons; mass spectra (M^{+}•); at *m/z* 642, 583 (M-AcO), 529 (M-side chain), 514 (M-$C_8H_4N_2$), 482 ($C_8H_4N_2S$), 456 (M-$C_9H_4N_3S$), 441 (M-$C_9H_5N_4S$).

4.2.5. 3β-chlorocholest-5-en-7-[thiazolo(4,5-b)quinoxaline-2-yl-hydrazone] (*8*)

A mixture of 3β-chloro-cholest-5-en-7-one-thiosemicarbazone (0.01 mol) and 2,3-dichloroquinoxaline (0.01 mol) in anhydrous ethanol 15 mL was refluxed for 24 h. Progress of reaction was monitored by TLC. After completion of the reaction solvent was removed under reduced pressure and residue was recrystallized from ethanol. Dark brown solid (DMSO); yield: 70%; m.p. 166 °C. Anal. calc. for ($C_{36}H_{48}N_5SCl$): C, 69.90, H, 7.77, N, 11.33; found: C, 69.8, H, 8.00, N, 11.31; IR ν_{max} cm^{-1}; 3412 (N–H), 2928 (C–H), 1554 (C=N),

1158 (C–N), 610 (C–S); ^{1}H NMR (DMSO-d_6)/ppm; 8.2 (s, 1H, NH), 7.6–8.1 (m, 4H, aromatic), 5.28 (s, 1H, C6-H), 3.85 (br m, 1H, $w1/2 = 15$ Hz C3α-H axial), 1.19 (C10-CH_3), 0.72 (C10-CH_3), 0.82, 1.04 (other methyl protons); mass spectra (M^+•) at *m/z* 619, 584 (M-Cl), 506 (M-side chain), 491 (M-$C_8H_4N_2$), 459 ($C_8H_4N_2S$), 433 (M-$C_9H_4N_3S$), 418 (M-$C_9H_5N_4S$).

4.2.6. 5α-cholest-5-en-7-[thiazolo(4,5-b)quinoxaline-2-yl-hydrazone] (**9**)

A mixture of 6α-cholest-5-en-7-one-thiosemicarbazone (0.01 mol) and 2,3-dichloroquinoxaline (0.01 mol) in anhydrous ethanol 15 mL was refluxed for 24 h. Progress of reaction was monitored by TLC. After completion of the reaction solvent was removed under reduced pressure and residue was recrystallized from ethanol. Dark yellow solid (DMSO); yield: 80%; m.p. 134 °C. Anal. calc for ($C_{36}H_{49}N_5S$): C, 73.84, H, 8.71, N, 11.96; found: C, 73.88, H, 8.76, N, 11.92; IR ν_{max} cm^{-1}; 3422 (N–H), 2932 (C–H), 1560 (C=N), 1130 (C–N), 635 (C–S); ^{1}H NMR (DMSO)/ppm 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), 457 (M-$C_8H_4N_2$), 425 ($C_8H_4N_2S$), 399 (M-$C_9H_4N_3S$), 384 (M-$C_9H_5N_4S$).

4.3. Organism culture and in vitro screening

Antibacterial activity was done by the disk diffusion method with minor modifications. *E. coli* was subcultured 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 disks (6.0 mm diameter) were fixed onto nutrient agar plate. Each test compound (1 mg) 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 test compound were prepared. These compounds of different concentrations were poured over disk plate. Chloramphenicol (30 μg/disk) was used as standard drug (positive control). DMSO poured disk 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} CFU 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 results are presented in Table 2.

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