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Short communication

Synthesis, spectral studies and in vitro antibacterial activity of steroidal thiosemicarbazone and their palladium (Pd (II)) complexes

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Abstract

We investigated the antibacterial activity of some new steroidal thiosemicarbazone and their Pd(II) metal complexes. Metal complexes were prepared from the reaction of steroidal thiosemicarbazone with $[Pd(DMSO)_2Cl_2]$. Coordination via the thionic sulphur and the azomethine nitrogen atom of the thiosemicarbazone to the metal ion, the thiosemicarbazone derivatives were obtained by the thiosemicarbazide with steroidal ketones. All the compounds have been confirmed by spectral data. The antibacterial activity of these compounds was first tested in vitro by the disk diffusion assay against two gram-positive and two gram-negative bacteria, and then the minimum inhibitory concentration (MIC) was determined. The results showed that steroidal complexes are better inhibit growth as compared to steroidal thiosemicarbazones of both types of the bacteria (gram-positive and gram-negative); compound **Ia** is better antibacterial agent as compared to amoxicillin.

Keywords: Thiosemicarbazone; Palladium(II); Antibacterial activity

1. Introduction

A wide range of biological activities possessed by substituted thiosemicarbazones and their metal complexes include cytotoxic, antibacterial, antitumor and antileukemic properties [1–6]. They are well known chelating ligands coordinating to the metal ion through the sulphur and one of the hydrazinic nitrogen atoms (N2 or N1). Coordination through N2 results in an unusual four membered chelate ring [7–9] while with N1 hydrazinic nitrogen a more stable five membered chelate ring results [10–13]. However, on incorporation of an additional donor site into the thiosemicarbazones, their coordination behavior becomes unpredictable. If there is intramolecular hydrogen bonding between either N2 nitrogen or N1 nitrogen and phenolic OH which reduces the availability of lone pair on the imines nitrogen preventing its coordination which might lead to the formation of either unusual four member rings or more stable five member rings. The satiric hindrance created by the bulky ligands in six coordinated complexes is not present in four coordinated square planar complexes [10]. Though the versatility of thiosemicarbazone ligands for binding to the metal ion has been well established, there remains an ambiguity in predicting the actual coordination mode of thiosemicarbazones. The biological activities of thiosemicarbazones are considered to be due to their ability to form chelates with heavy metal. Biological activities of metal complexes differ from those of either ligands or the metal ions and increased and/or decreased biological activities are reported for several transition metal complexes, such as copper(II) and nickel(II). The present work deals with synthesis of new thiosemicarbazones (Fig. 1) and their Pd(II) complexes (Fig. 2). The structures of all compounds were elucidated by IR, ^{1}H NMR, ^{13}C NMR, Fab mass and elemental analyses. These compounds were tested in vitro against bacteria such as *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella typhimuurium* and *Escherichia coli*.

2. Results and discussion

Reaction of steroidal thiosemicarbazones with $[Pd(DMSO)Cl_2]$ gave amorphous solid compounds. All the compounds were isolated in good yields and were stable

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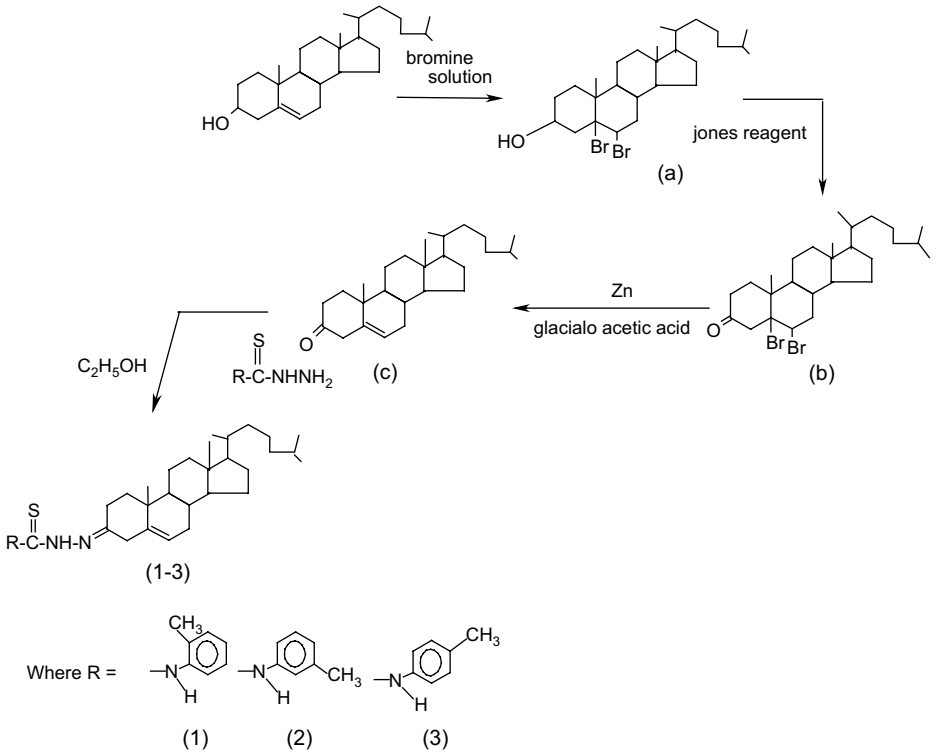


Fig. 1. Synthesis of compounds **1**, **2**, **3**: **1** – cholest-5-en-3-one-*o*-toludinethiosemicarbazone; **2** – cholest-5-en-3-one-*m*-toludinethiosemicarbazone; **3** – cholest-5-en-3-one-*p*-toludinethiosemicarbazone.

both in the solid and solution state. Analytical data of these compounds were in good agreement with their composition (see Section 4). The complexes were insoluble in water, methanol and ethanol, soluble in DMF and DMSO. The complexes do not undergo any weight loss up to 245 °C, which suggest their fair thermal stability. The structures of the ligands and complexes presented in Figs. 1 and 2a,b were established by comparing spectral data (IR, ^{1}H NMR, ^{13}C NMR and Fab mass) with the free ligand along with their thermo gravimetric analysis.

2.1. IR spectral studies

Assignments of selected characteristic IR band positions provide significant indication for the formation of steroidal thiosemicarbazones and their complexes. The thiosemicarbazones

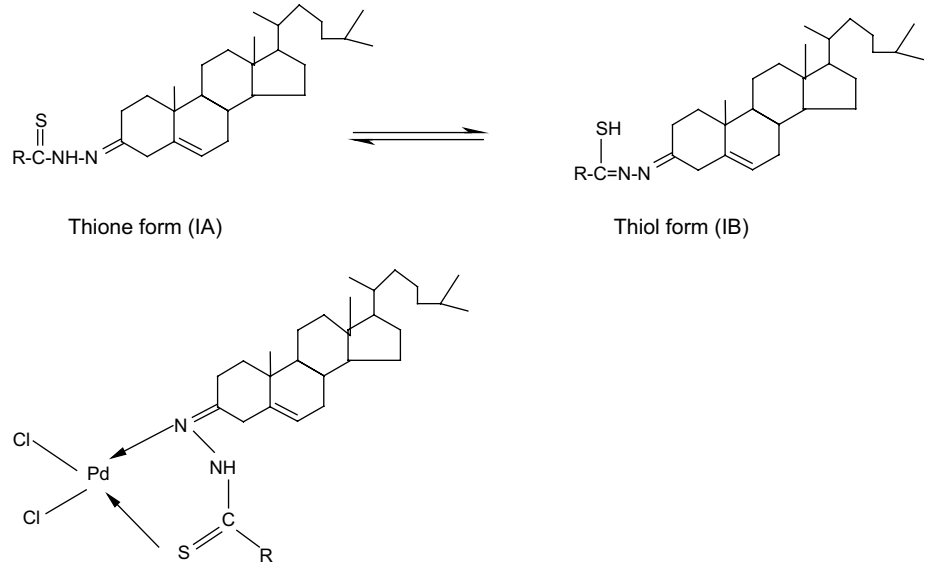
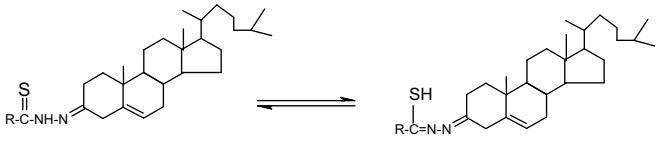


Fig. 2. (a) Structure of palladium(II) complexes: (**1a**) R = –NHC_7H_7; (**2a**) R = –NHC_7H_7; (**3a**) R = –NHC_7H_7. (b) Synthesis of complexes **1a**, **2b**, **3c** (TSCN = thiosemicarbazones).

can exist as thione and thioltautomeric from IA and IB, respectively.



Thione form (IA) Thiol form (IB)

However, the existence of a strong in the region 1034–1042 cm^{-1} due to ν (C=S) and absence of any band in the region 2500–2600 cm^{-1} due to ν (C–SH) suggesting that all the thiosemicarbazones remain in their thione form.

The negative shift (18–33 cm^{-1}) of ν (C=N) band observed in all complexes indicates the involvement of azomethine nitrogen upon complexation. This was further supported by the upward shift of N–N band of ligand on coordination. The strong band at 1028–1082 cm^{-1} ascribed to ν (C=S) of ligands is shifted to lower frequency (12–28 cm^{-1}) indicating the bonding of metal through thionic sulphur. The broad band observed in region 3226–3252 cm^{-1} due to ν (N–H) stretch is only slightly affected on the coordination.

2.2. ^1H NMR spectral analysis

Further evidence for the formation of thiosemicarbazones and their metal complexes was obtained from the ^1H NMR, which provides diagnostic tools for the positional elucidation of the protons. Assignments of the signals are based on the chemical shifts and intensity patterns. The ^1H NMR spectra of thiosemicarbazones (**1**–**3**) recorded in DMSO-d_6 exhibit a broad peak at 9.82–10.42 ppm due to –NH proton, which indicate that even in a polar solvent they remain in the thione form. The N–H proton signal of the thiosemicarbazones usually shifts to upfield and appears at 3.32–3.84 ppm in their respective complexes. This information suggests the adjustment of electronic current upon coordination of >C=S group to the metal ion.

The ^{13}C NMR spectra of ligand (**I**) were recorded in DMSO and the spectral signals are in good agreement with the probable structures. The ligand (**I**) showed two signals at 187.6 and 156.7 ppm assigned to (C=S) and (C=N), respectively and their complex (**Ia**) at 185.6 and 155.4 ppm assigned to (C=S) and (C=N), respectively. Other carbons in this complex resonate nearly at the same region as that of free ligands is given in Section 4.

2.3. TGA analysis

The TGA (under nitrogen, 10% min) profiles of complexes along with the % weight at different temperatures were recorded. These complexes do not lose weight up to 245 °C. Further increment of temperature causes decomposition of the complexes in two steps being 245–315 °C where loss of mixed fragment was observed. The second step starts immediately after one and continues until the complete decomposition of the ligand and formation of MS [M = Pd(II)] as the end product. The total % weight loss corresponds to the loss of the respective ligand after considering the transfer of one sulphur atom to the metal ion and residue corresponds to the metal sulphide.

2.4. FAB mass analysis

Characteristic peaks were observed in the mass spectra of ligand and their metal complexes, which followed the similar fragmentation pattern. The spectrum of compound (**1**) showed a molecular ion peak ($M^{+\bullet}$) at $m/z = 548$ and its complex compound (**1a**) showed a molecular ion peak ($M^{+\bullet}$) at $m/z = 725$. The characteristic peaks observed within the mass spectra of thiosemicarbazones and their metal complexes are given in Section 4.

2.5. In vitro evaluation of antibacterial

The in vitro antibacterial activities of thiosemicarbazone derivatives (**1**–**3**) and their metal complexes (**1a**–**3a**) were carried out using the culture of *S. aureus*, *S. pyogenes*, *S. typhimurium*, and *E. coli* by the disk diffusion method [18] and then the minimum inhibitory concentration (MIC) of all the compounds were determined. Amoxicillin (30 μg) was used as the standard drug, where as DMSO poured disk was used as negative control and then minimum inhibitory concentration (MIC) was evaluated by the macrodilution 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 24 h old inoculums. 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 in vitro studies result showed that the compound *o*-toludinethiosemicarbazone(I) derivative of steroidal was found to be more active among the all thiosemicarbazon compounds and their complex (**Ia**) is better antibacterial agent as compared to amoxicillin. 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

Table 1
Antibacterial activity of steroidal thiosemicarbazons and their complexes, positive control A (amoxicillin), and negative control (DMSO) measured by the Halo Zone Test (unit, mm)

Compound	Corresponding effect on microorganisms			
	S. aureus	*S. pyogenes*	*S. typhimurium*	*E. coli*
1	14.2 ± 0.4	12.8 ± 0.4	12.4 ± 0.2	14.2 ± 0.5
2	12.4 ± 0.2	10.6 ± 0.2	10.6 ± 0.4	11.2 ± 0.5
3	12.6 ± 0.4	11.5 ± 0.5	10.2 ± 0.5	10.2 ± 0.4
1a	18.4 ± 0.2	19.8 ± 0.4	21.5 ± 0.5	23.2 ± 0.2
2a	16.2 ± 0.4	14.4 ± 0.8	18.2 ± 0.4	15.6 ± 0.8
3a	16.8 ± 1.1	14.8 ± 0.6	18.8 ± 0.4	19.2 ± 0.8
A	21.0 ± 0.5	22.2 ± 0.4	25.2 ± 0.8	20.0 ± 0.2
DMSO	–	–	–	–

Table 2
Minimum inhibition concentration (MIC) of steroidal thiosemicarbazons and their complexes and positive control (amoxicillin)

Strain	MIC (μg/mL)						Positive control
	1	**2**	**3**	**1a**	**2a**	**3a**	
S. auras	64	128	256	32	64	64	32
S. pyogenes	64	128	256	32	64	64	32
S. typhimurium	128	128	128	64	64	128	32
E. coli	128	256	128	64	128	64	32

compound and the result is presented in (Table 2). Tests using DMSO and amoxicillin as negative and positive controls. The molecular structure of these active compounds showed enhanced activity. The distinct difference in the antibacterial property of the thiosemicarbazones and their metal complexes 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

Although the synthesis of thiosemicarbazones and their metal complexes was reported several years ago, very little is known about their antibacterial activity. This research examined the biological activities of the new thiosemicarbazones from steroids and their Pd(II) complexes. *o*-Toludinethiosemicarbazone (**I**) derivative of steroidal thiosemicarbazines was found to be more active among the all thiosemicarbazon compounds and their complex (**Ia**) is better antibacterial agent as compared to amoxicillin.

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 mass spectrometry and elemental analysis. 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 – 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 elements. Anhydrous sodium sulfate was used as a drying agent for the organic phase. Compounds **a**, **b** and **c** were prepared according to published methods [14].

4.1. Synthesis of thiosemicarbazides: a general method

Carbon disulphide (50 mmol) was added drop wise, a solution of amine and potassium hydroxide in water/ethanol (1:3) mixture. The temperature of the reaction was maintained below 10 °C. Sodium chloroacetate (50 mmol) was added and the reaction mixture was left overnight at room temperature. Addition of conc. hydrochloric acid (to pH ~ 1) precipitated substituted thioglycolic acid was recrystallized from methanol. A solution of thioglycolic acid (40 mmol) in water (15 mL) containing sodium hydroxide (40 mmol) and hydrazine hydrate (40 mmol) was refluxed for 2 h with continuous stirring. The compound separated out during the reaction or on cooling at 0 °C for12 h. The product was filtered and crystallized from methanol [15].

4.2. Synthesis of thiosemicarbazones: a general method

Steroidal thiosemicarbazone was synthesized (Fig. 1) 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 [16].

4.2.1. Compound *1*

Yield: 64%; m.p. 210 °C; Anal. Calc. for $C_{35}H_{53}N_3S$: C, 76.78; H, 9.68; N, 7.67. Found: C, 75.65; H, 8.82: N, 7.35. IR (KBr) ν_{max} cm^{-1}: 3246 (N–H), 1568 (C=N), 1618 (C=C), 1124 (C–N), 1034 (C=S). ^{1}H NMR (DMSO) (δ): 10.42 (2H, s, –NH), 1.96 (3H, s, CH_3), 7.20–8.04 (6H, m, aryl protons), 5.36 (1H, s, C6–H), 1.08 (C10–CH_3), 0.76 (C13–CH_3), 0.88, 0.96 (other methyl protons). ^{13}C NMR (DMSO) (δ): 187.6 (C=S), 156.7 (C=N), 135.5 (C–NH), 21.4 (C10–CH_3), 19.6 (C13–CH_3), 18.6, 18.4 (remaining methyl carbon), 16.4 (CH_3 of *o*-toludine). Mass spectra ($M^{+\bullet}$) at *m/z* 548, 533 ($M - CH_3$), 457 ($M - C_7H_7$), 442 ($M - C_7H_8N$), 398 ($M - C_8H_8NS$), 383 ($M - C_8H_9N_2S$).

4.2.2. Compound *2*

Yield: 54%; m.p. 218 °C; Anal. Calc. for $C_{35}H_{53}N_3S$: C, 76.78; H, 9.68; N, 7.67. Found: C, 74.85; H, 9.24; N, 7.82. IR (KBr) ν_{max} cm^{-1}: 3226 (N–H), 1572 (C=N), 1522 (C=C), 1138 (C–N), 1038 (C=S). ^{1}H NMR (DMSO) (δ): 9.62 (2H, s, –NH), 7.08–8.62 (6H, m, aryl protons), 5.48 (1H, s, C6–H), 2.42 (3H, s, CH_3), 1.12 (C10–CH_3), 0.78 (C13–CH_3), 0.92, 0.98 (other methyl protons).

4.2.3. Compound *3*

Yield: 58%; m.p. 232 °C; Anal. Calc. for $C_{35}H_{53}N_3S$: C, 76.78; H, 9.68; N, 7.67. Found: C, 76.55; H, 97.92; N, 6.55. IR (KBr) ν_{max} cm^{-1}: 3252 (N–H), 1546 (C=N), 1513 (C=C), 1148 (C–N), 1042 (C=S). ^{1}H NMR (DMSO) (δ): 9.80 (2H, s, –NH), 6.80–7.92 (6H, m, aryl protons), 5.62 (1H, s, C6–H), 2.64 (3H, s, CH_3), 1.18 (C10–CH_3), 0.84 (C13–CH_3), 0.98, 1.02 (other methyl protons).

4.3. Preparation of palladium(II) complexes

All the complexes were prepared by mixing the equimolar ratio of ligand and $[Pd(DMSO)_2Cl_2]$ in refluxing methanol. The solution was kept at 0 °C overnight, the product was separated by filtration and finally washed with methanol. Recrystallization was effected in methanol/DMF (6:4) [17].

4.3.1. $[Pd(C_{35}H_{53}N_3S)Cl_2]$ (**1a**)

Yield: 72%; m.p. 248 °C; Anal. Calc. for $[Pd(C_{35}H_{53}N_3S)Cl_2]$: C, 58.01; H, 7.32; N, 5.80. Found: C, 57.02; H, 7.32; N, 5.80. IR (KBr) ν_{max} cm^{-1}: 3446 (N–H), 1535 (C=N), 1522 (C=C), 1148 (C–N), 1022 (C=S), 432 (M–N, M–S). ^1H NMR (DMSO) (δ): 9.60 (2H, s, –NH), 7.26–8.14 (6H, m, aryl protons), 5.52 (1H, s, C6–H), 3.72 (1H, s, –NH), 2.10 (3H, s, CH_3), 1.14 (C10–CH_3), 0.84 (C13–CH_3), 0.96, 1.04 (other methyl protons). ^{13}C NMR (DMSO) (δ): 185.6 (C=S), 155.4 (C=N), 134.8 (C–NH), 21.2 (C10–CH_3), 19.5 (C13–CH_3), 18.2, 18.5 (remaining methyl carbon), 16.2 (CH_3 of *o*-toludine). Mass spectra ($M^{+\bullet}$) at *m/z* 725, 689 (M – Cl), 653 (M – Cl_2), 548 (M – Pd), 533 (M – CH_3), 457 (M – C_7H_7), 442 (M – C_7H_8N), 398 (M – C_8H_8NS), 383 (M – $C_8H_9N_2S$).

4.3.2. $[Pd(C_{35}H_{53}N_3S)Cl_2]$(**2a**)

Yield: 68%; m.p. 262 °C; Anal. Calc. for $[Pd(C_{35}H_{53}N_3S)Cl_2]$: C, 58.01; H, 7.32; N, 5.80. Found: C, 56.86; H, 6.82; N, 5.20. IR (KBr) ν_{max} cm^{-1}: 3452 (N–H), 1552 (C=N), 1518 (C=C), 1146 (C–N), 1014 (C=S), 498, 445 (M–N, M–S). ^1H NMR (DMSO) (δ): 9.28 (2H, s, –NH), 7.40–8.08 (6H, m, aryl protons), 5.32 (1H, s, C6–H), 3.84(1H, s, NH), 2.12 (3H, s, CH_3), 1.16 (C10–CH_3), 0.76 (C13–CH_3), 0.94, 0.96 (other methyl protons).

4.3.3. $[Pd(C_{35}H_{53}N_3S)Cl_2]$ (**3a**)

Yield: 78%; m.p. 278 °C; Anal. Calc. for $[Pd(C_{35}H_{53}N_3S)Cl_2]$: C, 58.01; H, 7.32; N, 5.80. Found: C, 56.45; H, 6.20; N, 4.50. IR (KBr) ν_{max} cm^{-1}: 3468 (N–H), 1528 (C=N), 1478 (C=C), 1146 (C–N), 1018 (C=S), 428, 447 (M–N, M–S). ^1H NMR (DMSO) (δ): 9.48 (2H, s, –NH), 7.26–8.12 (6H, m, aryl protons), 5.38 (1H, s, C6–H), 3.32 (1H, s, NH), 2.05 (3H, s, CH_3), 1.14 (C10–CH_3), 0.78 (C13–CH_3), 0.96, 0.98 (other methyl protons).

4.4. Organism culture and in vitro screening

Antibacterial activity was done by the disk 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 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 test compound were prepared. These compounds of different concentrations were poured over disk plate onto it. Amoxicillin (30 μg) 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 results of minimum inhibitory concentration (MIC) are presented in Table 2. Tests using DMSO and amoxicillin as negative and positive controls.

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