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Short communication

Synthesis, characterization and *in vitro* antibacterial activity of new steroidal 5-en-3-oxazolo and thiazoloquinoxaline

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Abstract

Steriodal heterocyclic systems namely cholest-5-en-3-oxazolo and thiazoloquinoxaline have been synthesized *via* the reaction of cholest-5-en-3-one semicarbazone/thiosemicarbazone with 2,3-dichloroquinoxaline at 80 °C in high yield. Cholest-5-en-3-one semicarbazone is obtained by the condensation of cholest-5-en-3-one with semicarbazide in the presence of AcONa in ethanol and cholest-5-en-one thiosemicarbazone is obtained by the condensation of cholest-5-en-3-one with thiosemicarbazide in ethanol in the presence of a few drops of HCl. The structures of these compounds were evident by elemental analysis, IR, ^{1}H NMR and FAB mass spectral analysis. These synthesized compounds were investigated for antibacterial activity first by the disk-diffusion assay against two Gram-positive and two Gram-negative bacteria and then the minimum inhibitory concentration (MIC) of these compounds were determined and the results were compared with the standard drug Amoxicillin. The results showed that these compounds oxazolo/thiazoloquinoxaline are better antibacterial agents as compared to the standard drug Amoxicillin.

Keywords: Semicarbazone; Oxazoloquinoxaline; Antibacterial activity

1. Introduction

Bacterial infections such as food poisoning, rheumatic, salmonellosis and diarrhea are caused by multidrug-resistant Gram-positive and Gram-negative pathogens. Principal players among these problematic organisms are isolates of methicillin-resistant *Staphylococcus aureus*, *Staphylococcus pyogenes*, *Salmonella typhimurium* and *Escherichia coli* [1]. Million of people in the subtropical regions of the world are infected and 20,000 deaths every year due to these parasitic bacterial infections. Amoxicillin, norfloxacin, ciprofloxacin are the principal drugs of choice in the treatment of bacterial infection since they are effective against extraintestinal and intestinal wall infection [2], but these are associated with several side effects such as nausea, metallic taste, dizziness, hypertension, etc. as well as resistance have been reported [3]. The present strategy for new drug development is directed towards identifying the essential enzyme systems in the bacterial and developing molecules to inhibit them on our going medicinal chemistry research activity. We have found that quinazolines and condensed quinazolines exhibit potent anti-microbial [4] and central nervous system (CNS) activities like analgesic and anti-inflammatory [5] activities. Recent reports have shown that thienopyrimidines (bioisotere of quinazoline) possess CNS and antibacterial activities [6–8]. Steroids bearing heterocycles fused to the A-ring of the steroid nucleus have been of pharmaceutical interest. Several methods are reported for the preparation of the steroids with the pyrazole, isoxazole, pyridine or pyrimidine ring fused to the 2,3-position of the nucleus [9–18]. Many of these steroidal heterocycles have been found to possess potent biological activities, such as anti-microbial, anti-estrogenic, anti-inflammatory, hypertensive, anabolic and cardiovascular activities [19–22]. In view of the therapeutic importance of these steroidal heterocycles, the goal is to investigate the synthesis of steroidal oxazolo/thiazoloquinoxaline as substrates. The present work is an extension of our ongoing efforts towards the development and

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identification of new steroidal molecules to act as antibacterial agents. Herein we describe the synthesis of new steroidal oxazolo/thiazoloquinoxaline derivative from cholesterol and screened for antibacterial.

2. Chemistry

Under the reaction conditions, the reaction mixture contained only unreacted starting material and cyclized product in good yield. The yield of the product of semicabazone/thiosemicarbazone was 75%. The oxazolo/thiazoloquinoxaline were synthesized by the literature procedure [23,24]. The obtained compounds are stable in the solid as well as in the solution state. The IR spectrum of compounds **2** and **3** exhibited a characteristic band at 1422, 1542 cm^{-1} and that of **4** and **5** at 1435, 1416 cm^{-1}, respectively, due to ν(C=N) stretch. IR spectra of compound **3** showed ν(C–O) stretch at 1252 cm^{-1} due to the oxazolo ring closure and compound **5** showed ν(C–S) stretch at 624 cm^{-1} due to the thiazolo ring closure. Compounds **2** and **3** showed additional sharp band in the region 3355 and 3420 cm^{-1}, respectively, due to the ν(N–H) stretch. There is additional sharp band in the region 3445 cm^{-1} for compound **4** and 3425 cm^{-1} for compound **5** due to the ν(N–H) stretch. ^{1}H NMR spectrum of compounds **2** and **3** showed characteristic peaks as a one proton singlet at δ 6.8 for compound **2** and δ 6.1 for compound **3**. The aromatic proton of oxazolo quinoxaline is in the range δ (7.4–7.9) for compound **3** and one proton singlet at δ 6.5 for compound **4** and 6.1 for compound **5**. Another singlet was observed at δ 5.8 and δ 5.6. The aromatic proton of thiazoloquinoxaline is in the range 7.2–7.6 for compound **5**. Characteristic peaks were observed in the mass spectra of compound **3** with a molecular ion peak ($M^{+\bullet}$) at $m/z = 568$ and compound **5** showed a molecular ion peak ($M^{+\bullet}$) at $m/z = 584$. The characteristic peaks observed in the mass spectra of compound **3** and **5** are given in Section 5.

3. Microbiological evaluation

3.1. Disc-diffusion assay

Compounds (**2**–**5**) were tested for their antibacterial activities by disc-diffusion method [25] using nutrient broth medium (contained (g/L): beef extract 3 g; peptone 5 g; pH 7.0). The Gram-positive bacteria utilized in this study consisted of *S. aureus* and *S. pyogenes*. The Gram-negative bacteria included *S. typhimurium* and *E. coli*. In the disc-diffusion method, sterile paper discs (0.5 mm) impregnated with compound dissolved in dimethylsulfoxide (DMSO) at concentration 200 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.

Table 1
Antibacterial activity of steroidal derivatives, positive control (Amoxicillin) and negative control (DMSO) measured by the Halo Zone Test (unit, mm)

Compounds	Corresponding effect on microorganisms			
	S. aureus	*S. pyogenes*	*S. typhimurium*	*E. coli*
a	9.2 ± 0.2	7.8 ± 0.3	8.2 ± 0.2	9.0 ± 0.2
2	14.6 ± 0.5	13.4 ± 0.4	15.5 ± 0.3	14.2 ± 0.2
4	15.2 ± 0.6	13.5 ± 0.4	14.5 ± 0.6	15.5 ± 0.6
3	22.5 ± 0.5	23.4 ± 0.4	24.5 ± 0.4	23.6 ± 0.6
5	21.2 ± 0.4	23.2 ± 0.5	20.4 ± 0.6	21.4 ± 0.8
Amoxicillin	17.0 ± 0.5	18.2 ± 0.4	17.2 ± 0.8	20.0 ± 0.2
DMSO	–	–	–	–

Compound **a** is cholesterol.

3.2. Microdilution assay

The minimum inhibitory concentration (MIC) values for compounds (**2**–**5**) defined as the lowest concentration of the compound preventing the visible growth were determined by using the microdilution both method [26]. The incocula of microorganisms were prepared from 24 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The test compounds dissolved in dimethylsulfoxide (DMSO) were first diluted to the highest concentration (400 g/mL) to be tested. Then serial twofold dilution was made in concentration range from 0.1 to 400 g/mL in 10 mL sterile tubes. A prepared suspension of the standard microorganisms was added to each dilution in a 1:1 ratio. Growth (or its lack) of microorganisms was determined visually after incubation for 24 h at 37 °C. The lowest concentration at which there was no visible growth (turbidity) was taken as the MIC. The concentrations affording 50% inhibition of bacteria growth (IC_{50}) was computed from the dose response curves. MIC and IC_{50} values were studied for the same bacterial strains in the disc-diffusion assay and are given in Table 2. Amoxicillin was used as reference drug. All disc-diffusion and microdilution experiments were preformed in duplicate and repeated three times.

4. Conclusion

This research examined the antibacterial activities of new cyclized steroidal oxazolo/thiazoloquinoxaline prepared by the reaction of semicarbazone/thiosemicarbazone with 2,3-dichloroquinoxaline at 80 °C. *In vitro* antibacterial activities of these compounds were carried out against culture of bacteria and the biological behavior of these compounds revealed that oxazolo/thiazoloquinoxaline steroidal compounds are better antibacterial agents as compared to their respective drugs.

5. 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

Table 2
Antibacterial of activities of the tested compounds and standard drug Amoxicillin using the microdilution method expressed as MIC and IC_{50} (μg/mL)

Compound	Gram-positive bacteria				Gram-negative bacteria			
	S. aureus		*S. pyogenes*		*S. typhimurium*		*E. coli*	
	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}
2	6.25	3.65	6.25	3.68	3.12	1.94	3.12	1.88
4	6.25	3.68	6.25	3.66	6.25	3.72	6.25	3.68
3	0.78	0.48	0.78	0.54	0.78	0.48	0.39	0.26
5	0.78	0.54	0.78	0.52	0.39	0.28	0.39	0.24
Amoxicillin	3.12	1.82	3.12	1.80	3.12	1.85	3.12	1.90

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 **1** were prepared according to published methods [27].

5.1. Synthesis of cholest-5-en-3-one semicarbazone (*2*)

To a solution of cholest-5-en-3-one 1 (5.19 mmol) in ethanol (50 mL) was added a mixture of semicarbazide hydrochloride (6.58 mmol) and sodium acetate (3.0 g) in ethanol (20 mL). The reaction mixture was refluxed for 2 h on a steam bath and cooled. The separated solid was filtered, washed with water and recrystallized from methanol to give compound **2**. Yield 71%; m.p. 224 °C; Anal. calc. for $C_{28}H_{47}N_3O$: C, 76.13; H, 10.73; N, 9.51; found C, 75.12; H, 10.65; N, 9.42%; IR: ν_{max}/cm^{-1}: 3355 (NH), 3125 (NH_2), 1625 (C═C), 1422 (C═N), 1721 (C═O); ^1H NMR ($CDCl_3$): δ_H 6.8 (s, 1H, NH), 6.4 (s, 2H, NH_2), 1.15, 0.91, 0.85 and 0.65 (angular and side chain methyl protons).

5.2. Choles-5-en-3-oxazolo [4,5-b] quinoxaline-2-yl-hydrazone (*3*)

A mixture of cholest-5-en-3-one semicarbazone (**2**) (0.01 mol) and 2,3-dicholoro quinoxaline (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 reduce pressure and residue obtained was purified by column chromatography (10:90, diethyl ether:-petroleum ether). Orange solid obtained was recrystallized with ethanol. Yield: 82%; m.p. 256 °C; Anal. calc. for $C_{36}H_{49}N_5O$: C, 76.19; H, 8.64; N, 12.34; found: C, 76.24; H, 8.56; N, 12.12%; IR: ν_{max}/cm^{-1}: 3420 (N–H), 2934 (C–H), 1612 (C═C), 1542 (C═N), 1156 (C–N), 1252 (C–O); ^1H NMR (DMSO): δ_H 7.4–7.9 (m, 4H, aromatic), 6.1 (s, 1H, NH), 1.14, 0.99, 0.85, 0.76 (angular and side chain methyl protons); mass spectra ($M^{+\bullet}$) at *m/z* 568, 455 (M − side chain), 398 (M − $C_9H_4N_3O$), 382 (M − $C_9H_5N_4O$), 369 (M − $C_9H_5N_5O$).

5.3. Synthesis of cholest-5-en-3-one thiosemecarbazone (*4*)

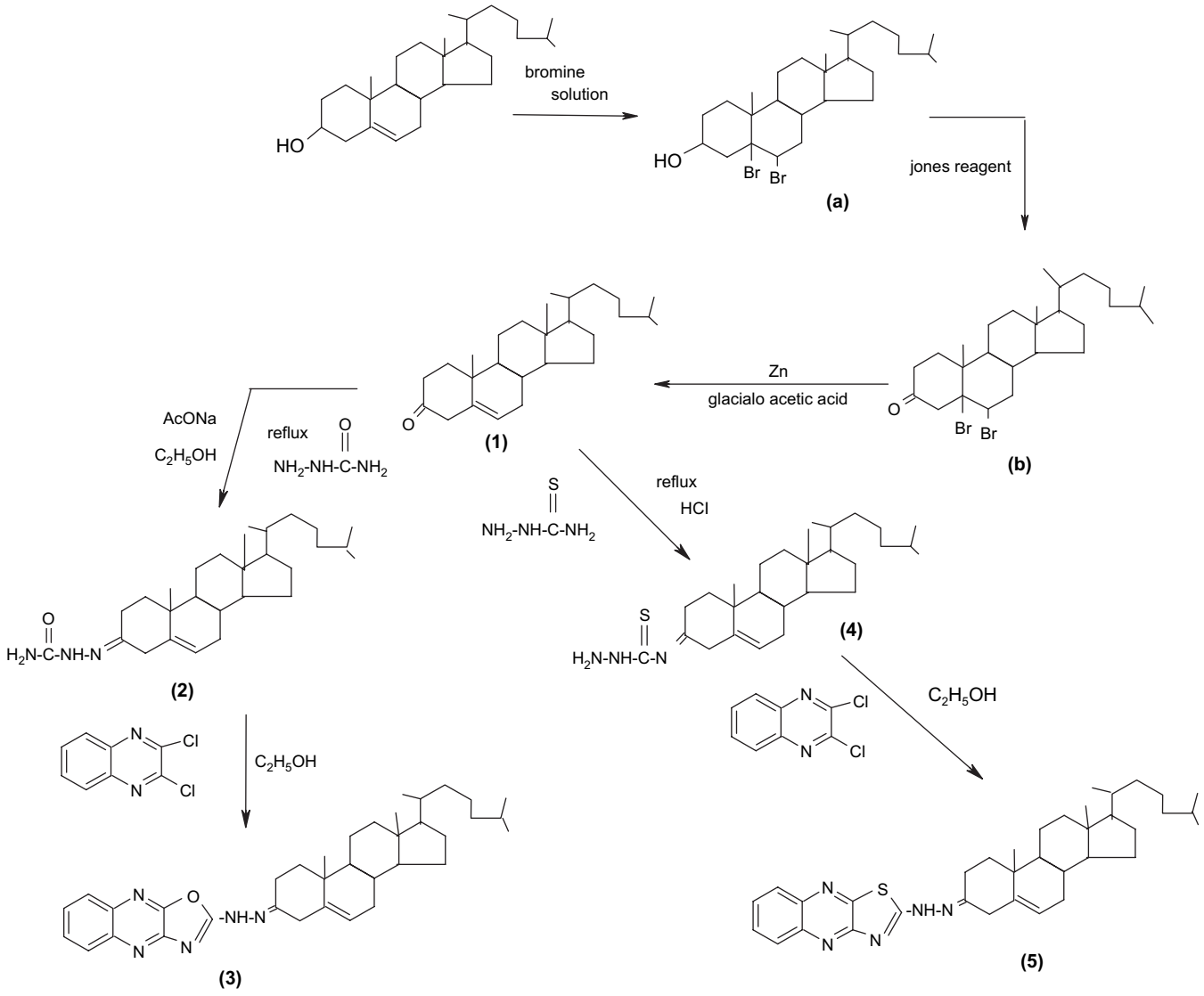
To a solution of cholest-5-en-3-one (5.19 mmol) in ethanol (35 mL) was added thiosemicarbazide (6.58 mmol) and conc. HCl (1.0 mL). The reaction mixture was refluxed for half an hour. The progress of the reaction was monitored by TLC. After completion of the reaction the solvent was removed under reduced pressure and the solid left after removal of the solvent was dissolved in chloroform and extract was washed successively with water, sodium bicarbonate solution (5%) and water, and dried over anhydrous sodium sulfate. The solution was crystallized from chloroform/methanol to give **4** (Scheme 1). Yield: 71%; m.p. 196 °C; Anal. calc. for $C_{28}H_{47}N_3S$: C, 73.44; H, 10.35; N, 9.18; found: C, 73.44; H, 10.36; N, 9.18%; IR (KBr) ν_{max}/cm^{-1}: 3450 (NH), 3125 (NH_2), 1625 (C═C), 1435 (C═N), 1080 (C═S); ^1H NMR ($CDCl_3$): δ 6.5 (s, 1H, NH), 5.8 (s, 1H, C4–H), 1.15, 0.91, 0.85 and 0.65 (angular and side chain methyl protons).

5.4. Choles-5-en-3[thiazolo [4,5-b] quinoxaline-2-yl-hydrazone (*5*)

A mixture of cholest-5-en-3-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 reaction was monitored by TLC. After completion of the reaction solvent was removed under reduced pressure and residue obtained was purified by column chromatography (20:80, diethyl ether:petroleum ether). Orange solid obtained was recrystallized with methanol (DMSO). Yield: 80%; m.p. 186 °C; Anal. Calc. for $C_{36}H_{49}N_5S$: C, 74.05; H, 8.40; N, 12.00; found: C, 73.9; H, 8.32; N, 11.83%; IR: ν_{max}/cm^{-1}: 3425 (N–H), 2934 (C–H), 1612 (C═C), 1416 (C═N), 1124 (C–N), 624 (C–S); ^1H NMR (DMSO): δ 7.2–7.6 (m, 4H, aromatic), 6.1 (s, 1H, NH), 1.11, 0.89, 0.82, 0.62 (angular and side chain methyl protons); mass spectra ($M^{+\bullet}$) at *m/z* 584, 471 (M − side chain), 398 (M − $C_9H_4N_3S$), 383 (M − $C_9H_5N_4S$), 369 (M − $C_9H_5N_5S$).

5.5. Bioactivity

The *in vitro* antibacterial activity of the structurally promising steroidal oxazolo/thiazoloquionoxaline derivatives



Scheme 1. Schematic diagram showing synthesis of compounds **4** and **5**.

(**2**–**5**) against two strains of Gram-positive bacteria and two strains of Gram-negative bacteria was investigated using disc-diffusion and microdilution methods in comparison to the reference drugs Amoxicillin. The results of the disc-diffusion methods are shown in Table 1 and that of microdilution method, for estimation of minimal inhibitory concentration (MIC) and the concentration affords 50% inhibition of bacteria growth (IC_{50}) values, are shown in Table 2. All results clearly revealed that, all tested compounds in the present study were found to have highly statistically significant antibacterial activity against the used strains of Gram-positive and Gram-negative bacteria ($P < 0.05$). In particular, steroidal oxazolo/thiazoloquinoxalines exhibits the greatest significant activity followed by steroidal semicarbazone/thiosemicarbazone. Moreover, all the tested compounds are significantly more potent than reference drugs as depicted in Tables 1 and 2. This excellent effectiveness makes these substances attractive antibacterial candidates. Studies to establish their *in vitro* efficacy and safety are being planned for their further development.

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