SINGLE-STEP SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL STUDIES OF NOVEL STEROIDAL 1', 2', 3'- THIADIAZOLES

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ABSTRACT

A convenient one-step synthesis of steroidal thiadiazoles **7-9** from steroidal 6-ketone semicarbazones **1-3** with thionyl chloride has been reported. The steroidal 6-ketone thiosemicarbazones **4-6** under similar reaction conditions also provided the same products **7-9**. The structures of these newly synthesized compounds have been established on the basis of their IR, ¹H NMR and ¹³C NMR, elemental and analytical data. The synthesized compounds were screened for in vitro antimicrobial activity against different bacterial (gram positive and gram negative) and fungal strains. All the synthesized compounds showed significant activity against various micro-organisms.

Keywords: Steroidal thiadiazoles, Semicarbazones, thiosemicarbazones, Thionyl chloride, Antimicrobial activity.

INTRODUCTION

Recent studies reveals that incorporation of hetero atom (N/O/S) enhances the biological activities of steroidal molecules. The importance of heterocyclic compounds has long been recognized in the field of synthetic organic chemistry and thiadiazoles are well documented, especially after the discovery that some thiadiazoles are associated with wide variety of physiological activities such as antiaflatoxic¹, antimicrobial²⁻⁶, anti-phage⁷, tuberculostatic^{8,9}, anticonvulsant¹⁰, activity as well as fungicidal¹¹, and amoebicidal¹² properties. Besides this thiadiazoles are useful as plant growth regulator^{13,14}, antidiabetics¹⁵, and chemotherapeutic¹⁶ agents. They have also been used as a drug intermediate¹⁷. These medicinal properties prompted us to undertake their preparation and evaluate their antimicrobial properties. We now report a convenient, efficient and one-step preparation of some steroidal thiadiazoles **7-9**, their characterization and antimicrobial activity, with quantitative yields.

EXPERIMENTAL

All melting points reported were determined in open capillary tubes and are uncorrected. The IR spectra were recorded on a Perkin Elmer 1600 FTIR spectrometer using Nujol and KBr pellets. ¹H NMR and ¹³C NMR spectra were recorded in CDCl₃ solutions on a Bruker instrument at 300 MHz and 100 MHz, respectively, using TMS as an internal standard (chemical shifts in δ , ppm). The mass spectra were run on a Jeol JMS D-300 spectrometer. Elemental analyses were performed on a Heraeus CHN Rapid Analyser. Column chromatography purifications were carried out using silica gel (60-120 mesh). Thionyl chloride was freshly distilled.

General procedure for preparation of steroidal thiadiazoles **7-9**.

Steroidal semicarbazone¹⁸ **1-3** (0.0020 mol) was added portion wise to thionyl chloride¹⁹ (6 mL) at ice bath temperature and kept for sometime at room temperature. Then the reaction mixture was stirred at 50°C and the reaction progress was monitored by TLC. After completion of the reaction, dichloromethane (30 mL) was added to the reaction mixture and decomposed with ice cold sodium carbonate solution, till the reaction slowed down. The organic layer was washed with water (4-5 times each with 30mL) and dried over anhydrous sodium sulphate. The crude product obtained after removal of the solvent was purified by column chromatography over silica gel column. Elution with a mixture of light petroleum and diethyl ether (19:1) provided the respective steroidal thiadiazoles **7-9**. Compound **7** was recrystallized from methanol, while compounds **8** and **9** were found as non-crystallizable semi-solids.

Under similar reaction conditions steroidal 6-ketone thiosemicarbazones²⁰ **4-6** gave the same products **7-9**.

5 α -Cholest-6-eno [6, 7-d] thiadiazole (7**)** was obtained by the procedure described above by stirring the 5 α -cholestan-6-one semicarbazone **1** with thionyl chloride at 50°C for one hour. Yield 72.92%, mp 110-112°C; IR: 1652 (C=C), 1585 (N=N), 1383 (C-N), 682cm⁻¹ (C-S); ¹H NMR: 0.68 (s, 3H, 18-CH₃), 0.80 (d, J=1.28Hz, 3H, 26-CH₃), 0.87 (d, J=1.3Hz, 3H, 27-CH₃), 0.91 (d,

J=6.3Hz, 3H, 21-CH₃), 1.10 (s, 3H, 19-CH₃); ¹³C NMR (CDCl₃): δ 27.2 (C-3), 23.1 (C-4), 45.7 (C-5), 155.1 (C-6), 109.5 (C-7), 30.7 (C-8), 48.1 (C-9), 38.5 (C-10), 42.8 (C-13), 56.3 (C-17), 19.8 (C-18), 20.1 (C-19); MS *m/z* 428 [M⁺]; Anal. calcd. for C₂₇H₄₄N₂S: C, 75.64; H, 10.35; N, 6.54. Found C, 75.67; H, 10.38; N, 6.51%.

On stirring the 5 α -cholestan-6-one thiosemicarbazone **4** with thionyl chloride at 50 °C for 3 hours also provided **7** in 65.80% yield with similar m. p. and spectral data.

3 β -Acetoxy-5 α -cholest-6-eno [6,7-d] thiadiazole (8**)** was obtained from 3 β -acetoxy-5 α -cholestan-6-one semicarbazone **2** by stirring with thionyl chloride at 50 °C for 6 hours as semi-solid. Yield 75.4%; IR: 1736, 1237 (CH₃COO), 1633 (C=C), 1540 (N=N), 1378 (C-N), 674 cm⁻¹(C-S); ¹H NMR: 0.71 (s, 3H, 18-CH₃), 0.81 (d, J=1.28Hz, 3H, 26-CH₃), 0.86 (d, J=1.3Hz, 3H, 27-CH₃), 0.93 (d, J=6.3Hz, 3H, 21-CH₃), 1.11 (s, 3H, 19-CH₃), 2.01 (s, 3H, CH₃COO), 4.7 (br m, 1H, C3 α -H, W 1/2 = 16Hz, axial); ¹³C NMR: δ 71 (C-3), 30.1 (C-4), 53.5 (C-5), 155.2 (C-6), 109.5 (C-7), 30.4 (C-8), 48.3 (C-9), 38.2 (C-10), 42.5 (C-13), 56.6 (C-17), 19.3 (C-18), 21.0 (C-19), 170.2 (CH₃COO), 21.5 (CH₃COO); MS *m/z* 486[M⁺]; Anal. calcd. for C₂₉H₄₆N₂O₂S: C, 71.56; H, 9.52; N, 5.75. Found: C, 71.61; H, 9.55; N, 5.72%.

Here also the treatment of 3 β -acetoxy-5 α -cholestan-6-one thiosemicarbazone **5** with thionyl chloride at 50 °C for 10 hours provided the same compound **8** as semi-solid in 69.3% yield, with identical spectral and physical data.

3 β -Chloro-5 α -cholest-6-eno [6, 7-d] thiadiazole (9**)** was obtained from 3 β -chloro-5 α -cholestan-6-one semicarbazone **3** by stirring with thionyl chloride at 50 °C for 2 hours as semi-solid. Yield 70.10%; IR: 1628 (C=C), 1485 (N=N), 1380 (C-N), 740 (C-Cl), 645 cm⁻¹ (C-S); ¹H NMR: δ 0.65 (s, 3H, 18-CH₃), 0.83 (d, J=1.2Hz, 3H, 26-CH₃), 0.85 (d, J=1.27Hz, 3H, 27-CH₃), 0.95 (d, J=6.3Hz, 3H, 21-CH₃), 1.15 (s, 3H, 19-CH₃), 4.15 (br m, C3 α -H, W 1/2 = 17 Hz, axial); ¹³C NMR: δ 60.3 (C-3), 38.0 (C-4), 39.8 (C-5), 155.7 (C-6), 109.3 (C-7), 30.3 (C-8), 48.8 (C-9), 39.1 (C-10), 43.0 (C-13), 57.0 (C-17), 19.5 (C-18), 20.5 (C-19); MS *m/z* 462/464 [M⁺]; Anal. Calcd for C₂₇H₄₃N₂SCl: C, 70.01; H, 9.36; N, 6.05. Found: C, 70.05; H, 9.31; N, 6.06%. Beilstein test positive.

Further stirring **6** with thionyl chloride under similar reaction conditions for 7 hours also provided **9** as semi-solid in 64.0% yield.

In vitro antibacterial activity

The in vitro antimicrobial activities of corresponding steroidal thiadiazoles **7-9** were

screened for their antibacterial activity against the bacterial cultures of *staphylococcus aureus* (ATCC29213), *Staphylococcus epidermidis* (ATCC-29887), *Escherichia coli* (ATCC-8739) and *Pseudomonas aeruginosa* (ATCC 25619) by disc diffusion method^{21,22}. Standard inoculums (1 $\times$ 10⁷-2 $\times$ 10⁷) c.f.u. ml⁻¹ (0.5 McFarland standards) was introduced onto the surface of sterile agar plates and a sterile glass spreader was used for even distribution of the inoculums. 1 mg of every trial compound was dissolved in 100 μ l DMSO to prepare stock solution and from stock solutions diverse concentrations 10, 20, 25, 50, and 100 μ g/ μ l of every trial compound were prepared. After that the compounds of diverse concentrations were poured over disk plate onto

it. The discs measuring 6 mm in diameter were prepared from Whatman No. 1 filter paper and sterilized by dry heat at 140 °C for 1 h. The sterile discs previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. Solvent and growth controls were also kept. Gentamicin was used as positive control. While the disc poured in DMSO was used as negative control. The plates were inverted and incubated for 24 h at 37 °C. The susceptibility was assessed on the basis of diameter of zone of inhibition against different strains of bacteria. Inhibition zones were measured and compared with standard drug. The bacterial zones of inhibition values are given in Table 1.

Table1: Zones of inhibition of compounds 7-9, standard (gentamicin) and negative control (DMSO) with different bacterial strains.

Compound	Zone of inhibition (mm)			
	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
7	18	25	13	15
8	13	18	16	20
9	12	16	15	18
Gentamicin	20	28	24	22
DMSO	—	—	—	—

In vitro antifungal activity

For assaying antifungal activity, different fungal strains like *Claviceps purpurea* (MTCC-1479) and *Aspergillus niger* (MTCC-281) were taken and antifungal activity was done by agar diffusion method^{23, 24}. Sabourand agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 ml) and adjusting pH to 5.7. Normal saline was used to make a suspension of spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 ml saline to get a suspension of corresponding species. 20 ml of agar media was poured into each petri dish. Excess of suspension was decanted and the plates were dried by placing in an incubator at 37 °C for 1 h. using an agar punch, wells were made and each well was labelled. 1 mg of every trial compound was dissolved in 100 µl DMSO to prepare stock solution and from stock solutions diverse concentrations 10, 20, 25, 50, and 100 µg/µl of every trial compound were prepared. After that the compounds of diverse concentrations were poured over disk plate onto it. A control was also prepared in triplicate and maintained at 37 °C for 3–4 days. The antifungal activity of each compound was compared with Ketoconazole as standard drug. Inhibition zones were measured and compared with the controls. The fungal zones of inhibition values are given in Table 2.

Table 2: Zones of inhibition of compounds 7-9, standard (ketoconazole) and negative control (DMSO) with different fungal strains.

Compound	Zone of inhibition (mm)	
	<i>C. purpuria</i>	<i>A. niger</i>
7	15	16
8	15	18
9	17	18
Ketoconazole	24	23
DMSO	—	—

RESULTS AND DISCUSSION

Chemistry

We describe in this paper a simple method for the synthesis of steroidal thiadiazoles 7-9 from corresponding steroidal semicarbazones 1-3 and thiosemicarbazones 4-6 using thionyl chloride. The reaction was carried out by stirring respective semicarbazones and thiosemicarbazones with thionyl chloride at 50 °C (scheme-1). The products were obtained through treatment with dichloromethane and sodium carbonate solution, then washing with water and removal of the solvent. The crude product obtained was purified by elution with light petroleum-diethyl ether.

The steroidal 6-ketone semicarbazone 1 on reaction with the excess of thionyl chloride gave the corresponding steroidal 1', 2', 3'- thiadiazole 7 in high yield. The IR spectrum (cm⁻¹) of the compound 1 displayed primary bands at 3440 (NHCO), 3341 (CONH₂), 1721 (C=O) and 1432 (C=N). While the IR spectrum (cm⁻¹) of the product 7 showed characteristic bands at 1652 (C=C), 1585 (N=N), 1383 (C-N) and 682 (C-S). Thus the appearance of bands in the latter and disappearance of bands of 1 confirmed the formation of 7.

In the ¹H NMR spectrum of 1, a sharp singlet at δ 6.5 integrating for one

proton was assigned to NH proton (exchangeable with deuterium), a broadened singlet appeared at δ 5.9 integrating for two protons was ascribed to NH₂ protons and a two-proton doublet for C7-H₂ was observed at δ 3.3. These peaks were again not found in the ¹H NMR spectrum of compound 7, which further provided the evidence for the formation of 5α-cholest-6-eno[6,7-d] thiadiazole 7.

In ¹³C NMR of 1 a characteristic peak at around δ 33.0 was observed for C-7, a peak at δ 160.4 was assigned to carbonyl carbon (CONH₂) of semicarbazone moiety and another peak at δ 158.6 was given for C-6. Especially the ¹³C NMR spectrum of 7 strongly supported its formation. In the ¹³C NMR of 7, a well marked characteristic peak appeared at δ 109.5, which was assigned to C-7 and the reason for this peak may be the change in the hybridization (from sp³ to sp²) of C-7 due to formation of double bond between C6-C7. There was a peak at δ 155.1 also which was assigned to C-6. The peaks at δ 27.2 and δ 45.7 were assigned to C-3 and C-5 respectively. These spectral values provided strong evidence of the formation of 5α-cholest-6-eno[6,7-d] thiadiazole 7.

In the same way the formation of 8 and 9 from corresponding steroidal semicarbazone 2 and 3 was also confirmed by their spectral studies (data given in Table 3 and experimental section).

It was found that steroidal 6-ketone thiosemicarbazones 4-6 (Table 3) under similar reaction conditions on treatment with excess of thionyl chloride provided the products which were found to contain the same spectral data as 7-9 (identical m.p., mixed m.p., TLC).

Thus the study of physical, analytical and spectral data (mixed m.p., IR, ¹H NMR, ¹³C NMR, mass spectrometry and elemental analysis) revealed the formation of same products 7-9 from compounds 1-3 as well as from compounds 4-6.

Antibacterial activity

The *in vitro* antimicrobial activities of steroidal thiadiazoles 7-9 were screened for their antibacterial activity against the bacterial cultures of *staphylococcus aureus* (ATCC29213), *Staphylococcus epidermidis* (ATCC-29887), *Escherichia coli* (ATCC-8739) and *Pseudomonas aeruginosa* (ATCC 25619) by disc diffusion method. Standard inoculums (1 × 10⁷–2 × 10⁷ c.f.u ml⁻¹, 0.5 McFarland standards) were introduced onto the surface of sterile agar plates and a sterile glass spreader was used for even distribution of the inoculums. The susceptibility was assessed on the basis of diameter of zone of inhibition against different strains of bacteria. Inhibition zones were measured and compared with standard drug, Gentamicin. The bacterial zones of inhibition values are given in Table 1. The results indicated that the compound 7 has shown the significant activity against *S. aureus* and *S. epidermidis* while compound 8 showed marked activity against *P. aeruginosa*. Other compounds showed moderate to good antibacterial activity.

Antifungal activity

For assaying antifungal activity, different fungal strains like *C. purpurea* and *A. niger* were taken and antifungal activity was done by agar diffusion method. The antifungal activity of each compound was compared with Ketoconazole as standard drug. Inhibition zones were measured and compared with the controls. The fungal zones of inhibition values are given in Table 2. The antifungal screening data also showed moderate to good antifungal inhibition. Among the screened compounds, the compound 9 showed good zones of inhibition against both the strains in comparison with the reference drug ketoconazole.

CONCLUSION

The present procedure of oxidative cyclization of steroidal semicarbazones and thiosemicarbazones to steroidal thiadiazoles by thionyl chloride is a simple, straight forward and can be easily scaled up. Besides this the reagent is cheap and easily available. Thus by this method we can synthesize steroidal thiadiazoles 7-9 in quantitative yields. The antimicrobial activities including antibacterial and antifungal properties of the synthesized compounds 7-9 showed a significant activity as compared with standard drugs.

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Table 3: Characterization data of compounds 1-6.

Compound	Mol. Formula	I R(cm ⁻¹)	¹ H NMR (δ , ppm)	¹³ C NMR (δ , ppm)
1	C ₂₈ H ₄₉ N ₃ O	3440(NHCO), 3341(CONH ₂), 1721(C=O), 1432(C=N)	6.5(s,1H,NH), 5.9(s,2H,NH ₂), 3.3(d,2H,C7-H ₂)	C6=158.6, C7=33.0, CONH ₂ =160.4
2	C ₃₀ H ₅₁ N ₃ O ₃	3435(NHCO),3343 (CONH ₂),1714(C=O), 1412(C=N)	6.3(s,1H,NH), 5.8(s,2H,NH ₂), 2.9(d,2H,C7-H ₂)	C6=158.8, C7=33.5, CONH ₂ =160.0
3	C ₂₈ H ₄₈ N ₃ OCl	3420(NHCO),3246 (CONH ₂),1705(C=O), 1395(C=N),770(C-Cl)	6.1(s,1H,NH), 5.5(s,2H,NH ₂), 2.5(d,2H,C7-H ₂)	C6=158.4, C7=33.1, CONH ₂ =160.2
4	C ₂₈ H ₄₉ N ₃ S	3510(NHCS),3370 (CSNH ₂),1590(C=N), 1180(C=S)	8.8(s,1H,NH), 6.3(s,2H,NH ₂), 2.5(d,2H,C7-H ₂)	C6=158.2, C7=35.3, CSNH ₂ =183.0
5	C ₃₀ H ₅₁ N ₃ O ₂ S	3500(NHCS),3355 (CSNH ₂),1735(OCO CH ₂),1590(C=N), 1175(C=S),1040 (C-O)	9.2(s,1H,NH), 6.5(s,2H,NH ₂), 3.0(d,2H,C7-H ₂)	C6=158.6, C7=35.0, CSNH ₂ =183.2
6	C ₂₈ H ₄₈ N ₃ SCl	3515(NHCS),3350 (CSNH ₂),1580(C=N), 1170(C=S),750(C-Cl)	9.2(s,1H,NH), 6.4(s,2H,NH ₂), 3.5(d,2H,C7-H ₂)	C6=158.2, C7=35.7, CSNH ₂ =183.6

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