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Synthesis and spectroscopic studies of Ru(II) complexes of steroidal thiosemicarbazones by multi step reaction: As anti-bacterial agents

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ABSTRACT

Ru(II) steroidal metal complexes were synthesized by the reaction of dichlorodicarbonyl ruthenium(II) $[Ru(CO)_2Cl_2]_n$ with Steroidal thiosemicarbazones. Coordination via the thionic sulfur and the azomethine nitrogen atom of the thiosemicarbazone to the Ru(II) metal. Steroidal thiosemicarbazone derivatives were obtained by the thiosemicarbazide with steroidal ketones. Structures of the steroidal thiosemicarbazone and their metal complexes were confirmed by the FT-IR, ^{1}H NMR, ^{13}C NMR, Fab-Mass spectroscopy and elemental analysis. The antibacterial activity of these compounds were 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 Ru(II) complexes are better inhibit growth as compared to steroidal thiosemicarbazones of both types of the bacteria (gram-positive and gram-negative).

1. Introduction

Sulfur containing compound such as thiosemicarbazones are generally synthesized by reaction of aldehydes/ketones with thiosemicarbazide [1]. Its biologically active molecule including antiviral [2], antitumor [3], and antimicrobial properties [4], as well as other industrially important activities, including anticorrosion [5] and antifouling [6] effects. Different derivative of the thiosemicarbazide by the reaction of different amine increase the bioactivities. Thiosemicarbazones used as intermediate for the formation of heterocyclic compounds such as pyrazolines, thiadiazolines, thiazoloquinoxaline, thiazolidinones etc. [7,8]. Due to presence of lone pair of electron to sulfur and nitrogen in the thiosemicarbazones can be applicable in the field of inorganic chemistry use as chelate for the formation of metal complexes by the various transition metals [9]. Last few decades the medicinal chemist has been working on bioinorganic chemistry that means the relationship between the metal ions and their complexes as anti-tumor and anti-bacterial agents [10]. In-vitro have indicated that some biologically active compounds may become more carcinostatic and bacteriostatic upon chelation [11]. Such interactions of transition metal ions with amino acids, peptides and nucleic acids, are of immense biological importance [12]. Thiosemicarbazone and its metal complexes are important compounds due to their wide range of industrial applications [13]. Thiosemicarbazone and metal complexes are used in the photostabilization of poly (vinyl chloride) polymers against photodegradation by ultraviolet radiation [14]. On the other hand, thiosemicarbazone derivative and their metal complexes are widely used in materials science fields, such as, third order non-linear optics (NLO) [15], optical switching [16], electrochemical sensing, Langmuir films and photoinitiated polymerization [17]. On the basis of literature survey we find that lot of work have been done on thiosemicarbazone and their Ru(II) metal complex as anti-bacterial agent but nobody worked on steroidal thiosemicarbazones and its Ru(II) metal complexes. In this paper we are reporting synthesis of steroidal thiosemicarbazone by multi steps reaction and its ruthenium metal complexes as anti-bacterial agents.

2. Experimental

2.1. General

All melting points was measured with a capillary apparatus and are uncorrected. All the compounds were routinely checked by IR, ^{1}H NMR, ^{13}C NMR, FAB 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 Bruker spectroscopy 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

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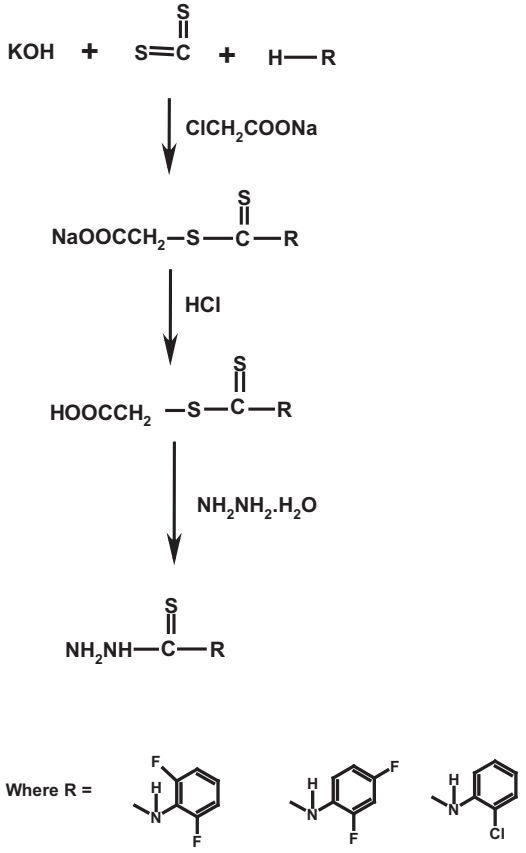

Scheme 1. Synthesis of thiosemicarbazides.

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. Compound **a**, **b** and **c** was prepared according to published methods [18].

2.2. Synthesis of thiosemicarbazides: A general method

Carbon disulphide (50 mmol) was added drop wise, a solution of different 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 over night at room temperature. Addition of conc. hydrochloric acid (to $P_H \sim 1$) 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 for 12 h. The product was filtered and crystallized from methanol (Scheme 1) [19].

2.3. Synthesis of thiosemicarbazones: A general method

Steroidal thiosemicarbazones were 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 80 °C for 5 h with continuous stirring after cooling the compounds were filtered and recrystallized from methanol and chloroform (Scheme 2) [20].

2.3.1. Cholest-5-en-3-one 2,6-difluoroaniline thiosemicarbazone (1)

Yield: 73%; m. p. 227 °C; IR (KBr) ν_{max} cm^{-1}: 3253 (N—H), 1622 (C═N), 1234 (C—F), 1129 (C—N), 1047 (C═S); ^{1}H NMR (DMSO) (δ): 9.86 (s, 1H, -NH), 7.82 (d, 1H, CHaromatic, J = 5.6 Hz), 7.76 (dd, 1H, CHaromatic, J = 5.2, 4.8 Hz), 7.32 (d, 1H, CHaromatic), 6.62 (s, 1H, NH), 1.36 (C1, m, 2H, C$\underline{H}_2$), 1.62 (C2, m, 2H, C$\underline{H}_2$), 1.48 (C4, m, 2H, C$\underline{H}_2$), 1.26 (C5, m, 1H, C$\underline{H}$), 1.23 (C7, m, 2H, C$\underline{H}_2$), 1.34 (C8, m, 1H, CH), 1.28 (C9, m, 1H, CH), 1.32 (C11, m, 2H, C$\underline{H}_2$), 1.33 (C12, m, 2H, C$\underline{H}_2$), 1.40 (C14, m, 1H, C$\underline{H}$), 1.44 (C15, m, 2H, C$\underline{H}_2$), 1.46 (C16, m, 2H, C$\underline{H}_2$), 1.38 (C17, m, 1H, C$\underline{H}$), 1.12 (C18, s, 3H, C13-C$\underline{H}_3$), 0.82 (C19, s, 3H, C10-C$\underline{H}_3$), 1.67 (C20, m, 1H, C$\underline{H}$), 0.90 (C21, s, 3H, C20-C$\underline{H}_3$), 1.08 (C22, m, 2H, C$\underline{H}_2$), 1.19 (C23, m, 2H, C$\underline{H}_2$), 1.13 (C24, m, 2H, C$\underline{H}_2$), 1.69 (C25. m, 1H, C$\underline{H}$), 0.73 (m, C26, -CH_3), 0.77 (m, C27, -CH_3); ^{13}C NMR (DMSO-d_6) (δ): 186.6 (C═S), 155.6 (C═N), 134.6 (C—NH), 23.2 (C18-CH_3), 18.8 (C19-CH_3), 18.6 (C26), 18.2 (C27), 137.4, 136.8, 129.8, 127.6, 126.7, 125.2 62.6, 55.4, 54.3, 53.4, 42.6, 41.2, 39.6, 37.8, 36.4, 35.2, 34.6, 33.8, 32.8, 29.6, 28.2, 27.4, 27.0, 25.4, 24.8, 22.5, 21.2; Mass spectra (M^+) at *m/z* 571, 552 (M-F), 533 (M-F_2), 556 (M-CH_3), 458 (M-$C_6H_3F_2$), 443 (M- $C_6H_4NF_2$), 399 (M- $C_7H_4NSF_2$), 384 (M- $C_7H_5N_2SF_2$); Anal. Calc. for $C_{34}H_{49}F_2N_3S$: C, 71.66; H, 8.67; N, 7.37. Found: C, 71.56; H, 8.53: N, 7.28.

2.3.2. Cholest-5-en-3-one 2,4-difluoroaniline thiosemicarbazone (2)

Yield: 65%; m. p. 235 °C; IR (KBr) ν_{max} cm^{-1}: 3248 (N—H), 1618 (C═N), 1238 (C—F), 1132 (C—N), 1037 (C═S); ^{1}H NMR (DMSO) (δ): 9.85 (s, 1H, -NH), 7.81 (d, 1H, CHaromatic, J = 5.6 Hz), 7.74 (dd, 1H, CHaromatic, J = 5.4, 4.5 Hz), 7.34 (d, 1H, CHaromatic), 6.66 (s, 1H, NH), 1.35 (C1, m, 2H, C$\underline{H}_2$), 1.65 (C2, m, 2H, C$\underline{H}_2$), 1.47 (C4, m, 2H, C$\underline{H}_2$), 1.24 (C5, m, 1H, C$\underline{H}$), 5.58 (1H, s, C6-H), 1.22 (C7, m, 2H, C$\underline{H}_2$), 1.33 (C8, m, 1H, CH), 1.27 (C9, m, 1H, CH), 1.31 (C11, m, 2H, C$\underline{H}_2$), 1.36 (C12, m, 2H, C$\underline{H}_2$), 1.41 (C14, m, 1H, C$\underline{H}$), 1.43 (C15, m, 2H, C$\underline{H}_2$), 1.45 (C16, m, 2H, C$\underline{H}_2$), 1.37 (C17, m, 1H, C$\underline{H}$), 1.10 (C18, s, 3H, C13-C$\underline{H}_3$), 0.81 (C19, s, 3H, C10-C$\underline{H}_3$), 1.66 (C20, m, 1H, C$\underline{H}$), 0.91 (C21, s, 3H, C20-C$\underline{H}_3$), 1.07 (C22, m, 2H, C$\underline{H}_2$), 1.18 (C23, m, 2H, C$\underline{H}_2$), 1.14 (C24, m, 2H, C$\underline{H}_2$), 1.67 (C25. m, 1H, C$\underline{H}$), 0.74 (m, C26, -CH_3), 0.76 (m, C27, -CH_3); ^{13}C NMR (DMSO-d_6) (δ): 187.5 (C═S), 157.2 (C═N), 134.8 (C—NH), 23.8 (C18-CH_3), 18.2 (C19-CH_3), 18.7 (C26), 18.5 (C27), 138.2, 137.5, 128.8, 127.3, 126.3, 125.8 62.2, 55.2, 54.3, 52.6, 42.2, 41.5, 39.3, 37.5, 36.5, 35.4, 34.3, 33.5, 32.5, 29.3, 28.4, 27.7, 27.05, 26.4, 25.03, 23.6, 21.89; Mass spectra (M^+) at *m/z* 571, 552 (M-F), 533 (M-F_2), 556 (M-CH_3), 458 (M-$C_6H_3F_2$), 443 (M- $C_6H_4NF_2$), 399 (M- $C_7H_4NSF_2$), 384 (M- $C_7H_5N_2SF_2$); Anal. Calc. for $C_{34}H_{49}F_2N_3S$: C, 71.66; H, 8.67; N, 7.37. Found: C, 71.48; H, 8.57: N, 7.29.

2.3.3. Cholest-5-en-3-one chloroaniline thiosemicarbazone (3)

Yield: 69%; IR (KBr) ν_{max} cm^{-1}: 3252 (N—H), 1632 (C═N), 1126 (C—N), 1032 (C═S), 722 (C—Cl); ^{1}H NMR (DMSO) (δ): 10.04 (s, 1H, -NH), 7.78 (d, 1H, CHaromatic, J = 7.8 Hz), 7.73 (dd, 1H, CHaromatic, J = 7.2, 6.8 Hz), 7.12 (dd, 1H, CHaromatic, J = 7.2, 7.6 Hz), 7.8 (d, 1H, CHaromatic, J = 5.6 Hz), 6.65 (s, 1H, NH), 1.34 (C1, m, 2H, C$\underline{H}_2$), 1.66 (C2, m, 2H, C$\underline{H}_2$), 1.45 (C4, m, 2H, C$\underline{H}_2$), 1.23 (C5, m, 1H, C$\underline{H}$), 1.25 (C7, m, 2H, C$\underline{H}_2$), 1.34 (C8, m, 1H, CH), 1.28 (C9, m, 1H, CH), 1.32 (C11, m, 2H, C$\underline{H}_2$), 1.35 (C12, m, 2H, C$\underline{H}_2$), 1.42 (C14, m, 1H, C$\underline{H}$), 1.41 (C15, m, 2H, C$\underline{H}_2$), 1.44 (C16, m, 2H, C$\underline{H}_2$), 1.36 (C17, m, 1H, C$\underline{H}$), 1.11 (C18, s, 3H, C13-C$\underline{H}_3$), 0.80 (C19, s, 3H, C10-C$\underline{H}_3$), 1.65 (C20, m, 1H, C$\underline{H}$), 0.92 (C21, s, 3H, C20-C$\underline{H}_3$), 1.03 (C22, m, 2H, C$\underline{H}_2$), 1.21 (C23, m, 2H, C$\underline{H}_2$), 1.16 (C24, m, 2H, C$\underline{H}_2$), 1.68 (C25. m, 1H, C$\underline{H}$), 0.76 (m, C26, -CH_3), 0.75 (m, C27, -CH_3); ^{13}C NMR (DMSO-d_6) (δ): 188.8 (C═S), 158.6 (C═N), 135.4 (C—NH), 23.5 (C18-CH_3), 18.6 (C19-CH_3), 18.5 (C26), 18.1 (C27), 138.5, 137.2, 129.4, 127.6, 126.6, 125.2, 63.2, 55.4, 54.1, 52.2, 43.5, 42.8, 39.1, 37.8, 36.2, 35.2, 35.1, 33.2, 32.6, 30.2, 29.5, 28.2, 27.3, 26.2, 25.6, 24.2, 21.7; Mass spectra (M^+) at *m/z* 572, 557 (M-CH_3), 538 (M-Cl), 460 (M-C_6H_4Cl), 445 (M- C_6H_5NCl), 401 (M- C_7H_5NSCl), 386 (M- $C_7H_6N_2SCl$); Anal. Calc. for $C_{34}H_{50}N_3SCl$: C, 71.86; H, 8.87; N, 7.39. Found: C, 71.84; H, 8.83: N, 7.32.

2.4. Preparation of ruthenium(II) complexes

All the complexes were prepared by mixing the equimolar ratio of