Steroidal compounds exhibit particular physiological activities. In this paper, some steroidal thiosemicarbazones platinum (Pt(II)) complexes were synthesized by the condensation of steroidal ketones with thiosemicarbazide using estrone, chenodeoxycholic acid, and 7-deoxycholic acid as starting materials and complexation of steroidal thiosesemicarbazones with Pt(II). The complexes were characterized by IR, NMR, and MS, and their antiproliferative activities were evaluated. The results showed that some steroidal thiosemicarbazones platinum (Pt(II)) complexes displayed moderate cytotoxicity to HeLa and Bel-7404 cells. Thereinto, complex 6 showed an excellent inhibited selectivity to HeLa cells with an IC$_{50}$ value of 9.2 $\mu$M and SI value of 21.7. At the same time, all compounds were almost inactive to HEK293T (normal kidney epithelial cells). The information obtained from the studies may be useful for the design of novel chemotherapeutic drugs.

1. Introduction

In the late 1960s, anticancer activity of cisplatin was found by Rosenberg [1–4]. Subsequently, cisplatin had become a metal anticancer drug for the treatment of human cancers. But in the process of treatment, cisplatin showed a high toxicity to patients and led to some strong side effects [5]. In accordance with the traditional structure-activity relationship of platinum complexes, the synthesis of new platinum anticancer drugs with the same mode of action has difficulty in achieving a major breakthrough. Because steroid hormones play an important role in the biochemistry of many cancers, a number of steroidal complexes connected to a metal pharmacophore had been designed and synthesized by many research groups, and their physiological activities were evaluated [6–15]. Steroidal metal complexes are transferred to cancer cells by steroidal carrier, selectively accumulated in cancer cells, and combined with specific DNA to reduce the destruction of normal cells. Not only can the metal pharmacophores of steroidal metal complexes enhance DNA damage by its space hinder, but also they play the role of steroid hormones and interfere with cancer cell growth regulating process. Therefore, maintaining and improving the two parts of the activity of the steroidal part and platinum pharmacophore are the key to the molecular design strategy of anticancer steroidal platinum complexes [16–18]. Thiosemicarbazones have received considerable attention since the discovery of their cytotoxic activity against cancer cells and bacteriostatic effects, and the biological properties of thiosemicarbazone complexes are often related and modulated by the metal ion of coordination [19]. Khan and Yusuf [20] and Murugkar et al. [21] investigated the bioactivity of some new steroidal thiosemicarbazones and their Pd(II) or Pt(II) metal complexes and discovered that some compounds had better antibacterial or antineoplastic activity.

In the present study, some novel steroidal thiosemicarbazone platinum (Pt(II)) complexes were synthesized by condensing steroidal ketones with thiosemicarbazide using estrone, chenodeoxycholic acid, and 7-deoxycholic acid as starting materials and complexing steroidal thiosemicarbazone with Pt(II). Their antiproliferative activities against HeLa (human cervical carcinoma), Bel-7404 (human liver...
carcinoma), and HEK293T (normal kidney epithelial cells) cells were evaluated.

2. Materials and Methods

2.1. Materials. The sterols were purchased from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China. All chemicals and solvents were of analytical grade from commercial sources. All solvents were used without further purification unless otherwise specified.

2.2. Instrumentation and Methods. Melting points were determined on an X4 apparatus (Beijing Tech Instrument Co., Ltd., Beijing, China) and were uncorrected. The 1H and 13C NMR spectra were recorded in CDCl3 on a Bruker AV-600 spectrometer at working frequencies 600 and 150 MHz and a Bruker AV-300 spectrometer at working frequencies 300 and 75 MHz, respectively. Chemical shifts are expressed in parts per million (δ) values and coupling constants (J) in Hertz. Infrared spectra were measured with a Thermo Scientific Nicolet IS-10 Spectrophotometer (Thermo Scientific, America). HREIMS was measured on an Agilent 6210 TOFMS instrument (Agilent Technologies, America). The cell proliferation assay was undertaken by a MTT method using 96-well plates on a MLLTISKAN MK3 analysis spectrometer (Thermo Scientific, Shanghai, China).

2.3. Synthesis. Compound 7 was prepared according to the method of [22] and compound 12 was prepared following the method of [23].

2.3.1. General Procedure for Preparation of Steroidal Thiosemicarbazon. A mixture of steroidal ketone (1 mmol), thiosemicarbazide (1 mmol), and a few drops of glacial acetic acid in 2.52 mmol) in 10 min at room temperature. After no starting material was observed (the progress of the reaction was monitored by TLC, petroleum ether/ethyl acetate = 1:1), the reaction was stopped. The solution was neutralized with 1 M HCl. After evaporation of the majority of MeOH under reduced pressure, proper water was added. The residue

Methyl 3-Thiosemicarbazonyl-7-oxocholedeoxycholate (8, L8) Light yellow solid, Yield: 52.5%, m.p. 285–286°C; IR (KBr) ν/cm⁻¹: 2947, 2946, 1706, 1624, 1434, 1329, 1170, 1023; 1H NMR (CDCl3, 600 MHz): 0.65 (3H, s, 18-CH3), 0.89 (1.2H, d, J = 6.6, 21-CH3, 3Z), 0.90 (1.8H, d, J = 6.6, 21-CH3, 3E), 1.22 (1H, s, 19-CH3, 3E), 1.23 (1.2H, s, 19-CH3, 3Z), 2.31 (0.6H, dd, dd, J = 10.2, 5.4, 4-C=βH, 3E), 2.34 (0.4H, dd, J = 10.2, 5.4, 4-C=βH, 3Z), 2.84 (0.6H, dd, J = 13.2, 6.0, 26-CH3, βH), 2.89 (0.4H, dd, J = 13.2, 6.0, 26-CH3, βZ), 3.63 (3H, s, OCH3), 6.52 (1H, br s, -NH2), 7.19 (0.4H, d, J = 4.2, -NH2, 3Z), 7.19 (0.6H, dd, J = 4.2, -NH2, 3E), 8.93 (0.6H, s, -NH-, 3E), 8.97 (0.4H, s, -NH-, 3Z); 13C NMR (CDCl3, 150 MHz): 211.7 (7-C, 3Z), 215.1 (7-C, 3Z), 178.9 (C=O), 174.8 (24-C, 3E), 155.4 (3-C, 3E), 155.3 (3-C, 3Z), 54.9 (17-C), 51.7 (O-CH3), 49.6 (9-C), 49.0 (8-C, 3E), 48.9 (8-C, 3Z), 47.7 (14-C), 46.8 (5-C, 3Z), 45.0 (5-C, 3E), 43.1 (13-C), 42.8 (10-C), 42.7 (6-C, 3E), 38.9 (12-C), 36.5 (20-C), 35.9 (4-C, 3E), 35.3 (4-C, 3-Z), 31.2 (22-C), 31.1 (23-C), 29.8 (29-C, 3E), 28.7 (2-3-CZ), 28.4 (16-C), 24.9 (1-C, 3E), 24.8 (1-C, 3Z), 22.9 (15-C), 22.1 (11-C), 18.5 (21-C), 14.3 (19-C), 12.2 (18-C); HREIMS: m/z 476.2941 [M+H]+ (calcd for C26H27N2O10S, 476.2947).

Methyl 3-Thiosemicarbazonyl-12-oxo-7-deoxycholate (13, L12). Light yellow solid, Yield: 52.5%, m.p. 125–127°C; IR (KBr) ν/cm⁻¹: 3431, 1736, 1706, 1591, 1494; 1H NMR (CDCl3, 300 MHz): 0.84 (3H, d, J = 6.3, 21-CH3, 3Z), 1.034 (3H, s, 18-CH3), 1.062 (3H, s, 18-CH3), 2.60–2.49 (2H, m, C11-H), 3.657 (3H, s, OCH3), 4.649 (1H, br s, -NH2), 7.214 (1H, br s, -NH2), 8.897 (1H, s, -NH-); 13C NMR (CDCl3, 150 MHz): 117.8 (18-C), 186.9 (19-C), 22.3 (21-C), 24.3 (15-C), 25.6 (16-C), 27.5 (1-C), 29.8 (2-3-C), 30.5 (6-C), 31.3 (7-C), 35.4 (22-C), 35.6 (23-C), 36.0 (4-C), 37.0 (20-C), 38.3 (11-C), 42.3 (8-C), 43.7 (10-C), 44.4 (13-C), 46.5 (5-C), 51.5 (OCH3), 57.5 (9-C), 58.4 (17-C), 58.5 (14-C), 156.9 (3-C), 174.6 (24-C), 178.7 (C=O), 214.3 (12-C); HREIMS: m/z 476.2942 [M+H]+ (calcd for C26H26N2O9S, 476.2947).

2.3.2. Methyl 3-Thiosemicarbazonyl-7-hydroxycholedeoxycholate (10, L10). To the stirred solution of 8 (674 mg, 1.42 mmol) in CH3OH (30 mL) was added NaBH4 (96 mg, 2.52 mmol) in 10 min at room temperature. After no starting material was observed (the progress of the reaction was monitored by TLC, petrol ether/ethyl acetate = 1:1), the reaction was stopped. The solution was neutralized with 1 M HCl. After evaporation of the majority of MeOH under reduced pressure, proper water was added. The residue
was extracted with ethyl acetate. The organic layer was washed with cold water, saturated NaHCO₃ solution, and saturated brines. After drying over anhydrous sodium sulfate, the solvent was removed under reduced pressure. A crude product was chromatographed on silica gel (elution: Vpetroleum ether : Vzylene acetate = 2:1) to give 285 mg of compound 10 (42.3%) as a white solid. IR (KBr) ν/cm⁻¹: 1165, 1434, 1501, 1589, 1733, 2930, 3429; ¹H NMR (CDCl₃, 600 MHz): 0.655 (IH, s, 18-CH₃), 0.896 (3H, d, J = 6.6, 21-CH₃), 0.946 (3H, s, 19-CH₃), 2.36-2.31 (1H, m, C23-H), 2.55-2.52 (2H, m, C23-H), 2.894 (IH, br s, OH), 3.060 (1H, dd, J = 15.6, 13.2, C2-β-H), 3.636 (3H, s, OCH₃), 3.932 (1H, br s, C7-H), 6.309 (1H, d, J = 4.2, -NH₂), 7.281 (1H, d, J = 4.2, -NH₂), 9.135 (1H, s, -NH-); ¹³C NMR (CDCl₃, 150 MHz): 11.9 (18-C), 18.4 (19-C), 21.2 (21-C), 22.4 (11-C), 23.8 (15-C), 28.2 (1-C), 30.2 (2-C), 31.0 (16-C), 31.1 (23-C), 31.2 (22-C), 33.2 (4-C), 33.7 (20-C), 35.5 (6-C), 35.9 (12-C), 37.4 (10-C), 39.6 (8-C), 39.7 (9-C), 42.3 (13-C), 42.8 (5-C), 50.3 (14-C), 51.6 (-OCH₃), 55.9 (17-C), 68.7 (7-C), 158.7 (3-C), 174.9 (24-C), 178.4 (C=S); HREIMS: m/z 478.3108 [M+H]+ (calcd for C₂₆H₄₅N₃O₃S, 478.3103).

### 2.3.3. Preparation of Platinum(II) Complexes.

Solution of [PtL₈Cl₂](Compound 3). Light yellow solid, Yield: 48.5%; m.p. 297–299°C; IR (KBr) ν/cm⁻¹: 3416, 2927, 1609, 1584, 1499, 1409, 1160, 878; ¹H NMR (300 MHz, DMSO) δ: 0.90 (3H, s, 18-CH₃), 0.94 (0.9H, s, 18-CH₃), 2.77–2.57 (2H, m, C16-H), 6.46 (1H, d, J = 8.4, C4-H), 6.52 (1H, dd, J = 8.4, 2.4, C2-H), 7.03 (0.3H, d, J = 9.0, Cl-H), 7.07 (0.69H, d, J = 8.4, Cl-H), 8.38 (1H, s, -NH₂), 8.99 (1H, s, -NH-), 10.77 (1H, s, -NH-); ¹³C NMR (75 MHz, DMSO) δ: 12.5 (18-C), 14.2 (11-C), 20.9 (11-C), 23.6 (15-C), 26.3 (16-C), 26.8 (7-C), 29.1 (6-C), 31.8 (16-C), 35.2 (12-C), 35.4 (12-C), 37.5 (9-C), 40.1 (8-C), 43.1 (13-C), 49.4 (13-C), 53.6 (14-C), 59.9 (14-C), 112.9 (15-C), 115.0 (4-C), 126.0 (1-C), 130.1 (10-C), 137.1 (5-C), 151.3 (3-C), 170.5 (17-C), 185.9 (C=S); HREIMS: m/z 607.0728 [M+H]+ (calcd for C₁₉H₁₅Cl₂O₂NiP₁S₁, 607.0665).  

### 2.4. Cytotoxicity Assay In Vitro.

The antiproliferative activity of all steroidal thiosemicarbazones and their Pt(II) metal complexes and cisplatin on Bel 7404 (human liver carcinoma), HeLa (human cervical carcinoma), and HEK293T (normal kidney epithelial cells) cell lines was determined by using the MTT method. The detailed procedure has been reported in our previous work [24].

### 3. Results and Discussion

#### 3.1. Synthesis and Characterization.

The synthetic route and the structures of complexes 3 and 6 were outlined in Scheme 1. Steroidal thiosemicarbazones 2 and 5 were
obtained as an (E)-configuration by reacting estrone with thiosemicarbazide, and the reaction of compounds 2 and 5 with K₂PtCl₆ gave steroidal platinum (Pt(II)) complexes 3 and 6 as (S)-configuration, respectively. The structures of 3 and 6 were confirmed by analysis of UV, IR, NMR, and HRMS.

In order to investigate the effect of position of pharmacophore on the antiproliferative activity of complexes, we prepared complexes 9 and 11 using chenodeoxycholic acid as a starting material (Scheme 2). Starting from compound 7, compound 8 with 3-thiosemicarbazone group was yielded as a mixture of (3E)- and (3Z)-isomer (ratio: 0.6:0.4, ¹H
NMR data) by controlling an appropriate molar ratio of 7 and thiosemicarbazide because 3-carbonyl group was more active than 7-carbonyl, and thiosemicarbazide was selectively reacted with 3-carbonyl. The reaction of compound 8 with $K_2PtCl_4$ afforded further complex 9 as a mixture of ($R$)- and ($S$)-configuration isomer ($9-R : 9-S = 3:2$, $^1H$ NMR data).

Next, the 7-carbonyl of 8 was converted to 7-hydroxyl of compound 10 by the reduction of NaBH$_4$, but the 3-thiosemicarbazone group was still kept in compound 10. The reaction of 10 with $K_2PtCl_4$ gave complex 11. Complex 11 was a mixture of ($R$)- and ($S$)-configuration isomer also ($9-R : 9-S = 7:3$, $^1H$ NMR data).

The structures of complexes 9 and 11 had been determined by analysis of IR, UV, NMR, and HRMS.

Using 7-deoxycholic acid as a starting material, another steroidal thiosemicarbazone platinum (Pt(II)) complex 14 was synthesized (Scheme 3). Similarly, complex 14 was a mixture of ($R$)- and ($S$)-configuration isomer ($14-R : 14-S = 0.54:0.46$, $^1H$ NMR data) and their structures had been confirmed by analysis of IR, UV, NMR, and HRMS.

### 3.2. Cytotoxic Activity In Vitro

The antiproliferative activities of all steroidal thiosemicarbazones and their Pt(II) metal complexes were determined in vitro on Bel 7404, HeLa, and HEK293T. The MTT method was used to assay the antiproliferative activity and cisplatin was used as a positive control. The results are summarized as IC$_{50}$ values in $\mu$M in Table I.

As shown in Table I, steroidal platinum (Pt(II)) complexes 9, 11, and 14 are almost inactive against Bel 7404 and HeLa cells. However, complexes 3 and 6 exhibited an obvious cytotoxicity to HeLa and Bel 7404 cells. In particular, complex 6 showed an excellent antiproliferative activity against HeLa cells with the IC$_{50}$ values of 9.2 $\mu$M and had a better cytotoxicity compared with its precursor.

### Table I: Cytotoxicity$^a$ of steroidal thiosemicarbazones and their Pt-complexes in vitro (IC$_{50}$; $\mu$M)$^b$.

| Compounds | Bel-7404 | HeLa | HEK293T |
|-----------|----------|------|----------|
| 2         | 19       | 34   | ND       |
| 3         | 39       | 23   | >200     |
| 5         | >200     | 42   | >200     |
| 6         | 86       | 9.2  | >200     |
| 8         | >200     | 96   | >200     |
| 9         | >200     | 156  | >200     |
| 10        | >200     | 142  | >200     |
| 11        | >200     | >200 | >200     |
| 13        | >200     | >200 | >200     |
| 14        | 103      | 102  | >200     |
| Cisplatin | 23.2     | 10.1 | 10.3     |

$^a$Cytotoxicity as IC$_{50}$ for each cell line is the concentration of compound which reduced by 50% the optical density of treated cells with respect to untreated cells using the MTT assay.

$^b$Data represent the mean values of three independent determinations.

Comparing the antiproliferative activity of estrone-17-thiosemicarbazone platinum(II) with that of methyl chenodeoxycholate-thiosemicarbazone platinum(II) and methyl 7-deoxycholate-thiosemicarbazone platinum(II), we can see that estrone-17-thiosemicarbazone platinum(II) shows a better inhibiting activity than methyl chenodeoxycholate-thiosemicarbazone platinum(II) and methyl 7-deoxycholate-thiosemicarbazone platinum(II). A reason is that estrone-17-thiosemicarbazone platinum(II) with the structure of steroidal nucleus of estrone may be connected with the metabolism of estrogen.
Here, the positive control cisplatin and complex 6 displayed similar cytotoxicity against HeLa cells but cisplatin had obvious cytotoxicity to normal kidney epithelial cells HEK293T and complex 6 was almost inactive. The Selectivity Index (SI) was defined as the ratio of the cytotoxicity of a compound with respect to normal cells (IC\textsubscript{50} HEK293T) versus cancer cells and used to determine the criterion of effectiveness of the compounds. The SI values of the complexes are listed in Table 2.

One important criterion for a therapeutic drug for cancer is to have minimal or no side effects to normal body cells of patients undergoing chemotherapy. Considering that a higher SI corresponds to greater overall anticancer activity, we can affirm that complex 6 is an excellent selective inhibitor against HeLa cells, which deserve further study (SI value: complex 6 21.7, cisplatin 1.0).

4. Conclusion

In conclusion, we had prepared some steroidal thiosemicarbazone platinum(II) complexes and assayed their antiproliferative activities. The results showed that estrone-17'-thiosemicarbazone platinum(II) displayed a better inhibiting activity than methyl chenodeoxycholate-thiosemicarbazone platinum(II) and methyl 7-deoxycholate-thiosemicarbazone platinum(II). Among them, complex 6 based on the structure of estrone was found to be a valuable selective inhibitor against HeLa cells possessing the IC\textsubscript{50} values of 9.2 μM and SI value of 21.7. The result may be useful for the design of novel chemotherapeutic drugs.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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