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Phosphorus, Sulfur, and Silicon and the Related Elements

Publication details, including instructions for authors and subscription information:

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Facile Synthesis and in Vitro Cytotoxic Evaluation of Novel Thiadiazole, Pyrazole, and Dithiole-Androstane Derivatives

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Online publication date: 23 March 2010

To cite this Article Mohamed, Nadia R. , Elmegeed, Gamal A. , Abdelhalim, Mervat M. and Rady, Hanaa M.(2010) 'Facile Synthesis and in Vitro Cytotoxic Evaluation of Novel Thiadiazole, Pyrazole, and Dithiole-Androstane Derivatives', *Phosphorus, Sulfur, and Silicon and the Related Elements*, 185: 4, 848 — 856

To link to this Article: DOI: 10.1080/10426500903008914

URL: <http://dx.doi.org/10.1080/10426500903008914>

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FACILE SYNTHESIS AND IN VITRO CYTOTOXIC EVALUATION OF NOVEL THIADIAZOLE, PYRAZOLE, AND DITHIOLE-ANDROSTANE DERIVATIVES

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In the aim of identifying new steroidal cytotoxic agents with potential antiproliferative activity against hepatoma cell lines (Hep-G₂), we synthesized modified steroids containing the thiadiazole, pyrazole, or dithiole moiety. Epiandrosterone 1 reacted with carbon disulfide and sodium hydride to furnish α -oxoketene dithio-disodium salt 2. Treatment of 2 with the hydrazonoyl halides 5a–d produced the thiadiazole anellated androstanone 7a–d, respectively. The reaction of 1 with hydrazine hydrate produced the hydrazide adduct 8, which cyclized upon reflux in acetic acid to form the condensed pyrazoloandrostanone derivative 9. Interaction of 8 with carbon disulfide and sodium hydride formed the disodium salt 10, which reacted with ethylchloroacetate to furnish the final adduct, dithioandrostanone derivative, 13. Compounds 7a, 7d, 9, and 13 were examined for their cytotoxicity against a panel of hepatoma cell lines (Hep-G₂) using MTT assay. The results provide that, at incubation time 72 h, in DMSO, compound 7d (50 μ mol/mL) showed the most significant cytotoxic effect at $P < 0.05$. The higher dose (100 μ mol/mL) of compound 7d, at 48 h incubation, reversed the effect causing resistance and the growth rate return to the control level.

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Keywords Cytotoxicity; hepatoma; hydrazonoyl halides; pyrazole; steroids; thiadiazole

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide. It accounts for about 6% of all human cancers annually.¹ Because of the multifocal nature of liver carcinoma, most cancer patients are considered non-resectable. In these patients, chemotherapy is the only choice of treatment. Unfortunately, the development of drug resistance in the tumor after treatment is always a major obstacle to the successful

Received 24 February 2009; accepted 30 April 2009.

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management of liver cancer.² Thus, developing new therapeutic agents that can overcome drug resistance has become an urgent need for cancer patients.

Hybrid anticancer agents, which combine two active compounds in one, such as steroidal alkylators, contain the steroidal moiety as biological vectors for cytotoxic agents in order to diminish toxicity and to enhance specificity.³ These agents attain a duplicate effect on cancer cells. A variety of steroids with unusual and interesting structures has been synthesized and evaluated for their antitumor activity.^{4–6} In addition, the antitumor activities of many compounds containing a heterocyclic ring have been reviewed.^{7,8} Pyrazole, thiadiazole, and dithiole rings represent molecular frameworks that serve as a platform for developing pharmaceutical agents for various applications. Many derivatives of pyrazole, thiadiazole, and dithiole have been proven as antitumor agents.^{9–11}

In view of the above facts, we set a goal to prepare some new steroid derivatives by combining them with a heterocyclic moiety of potent cytotoxic activity. In continuation of our investigation of new aza and thia steroids and their condensed heterocyclic derivatives as biologically active molecules,^{12–14} we report in this article the facile synthesis of some new thiol, thiadiazole, pyrazole, and dithiole steroid derivatives. Furthermore, some compounds were examined for their cytotoxicity against a panel of hepatoma cell lines (Hep-G₂) using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay.

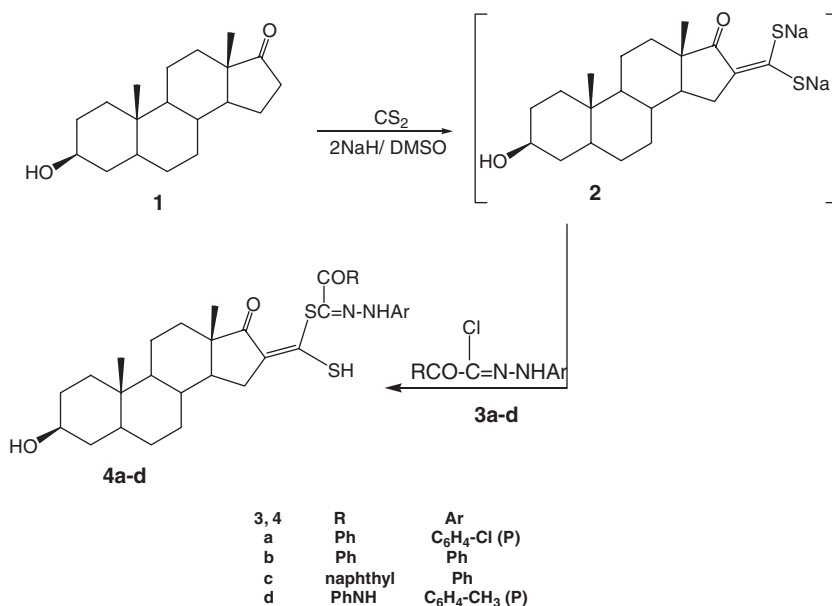
RESULTS AND DISCUSSION

Chemistry

In a recent communication from this laboratory, we have reported the synthesis of the non-isolable α -oxoketen dithio-disodium salt **2** via the reaction of 3 β -hydroxy-5 α -androstane-3-one (epiandrosterone) **1** with carbon disulfide and 2 equivalent moles of sodium hydride in dimethyl sulfoxide (DMSO) at 0°C.¹³ The addition of the hydrazonoyl halide, *c*-benzoyl-*N*-(4-chlorophenyl)hydrazonoyl chloride (**3a**) to the non-isolable salt **2** produced the corresponding androstane thiol derivative **4a** (Scheme 1). The structure of **4a** was supported with the spectroscopic and microanalytical data. The ¹H NMR spectrum of structure **4a** revealed beside the characteristic signals of androstane moiety, a singlet at $\delta = 1.23$ ppm for the (SH), two multiplets at 7.20–7.60 and 7.67–7.90 ppm for the aromatic protons, and two singlets at 11.30 and 11.80 for the (OH) and (NH) groups, respectively. To generalize such a methodology, the previous reaction was carried out by the utility of the hydrazonoyl halides **3b–d** to form the corresponding androstan-ethanhydrazonothioate derivatives **4b–d**, respectively. All the spectroscopic and microanalytical data of compounds **4b–d** were in accordance with the suggested structures (see the Materials and Methods section). Many trials to cyclize compounds **4a–d** under different conditions failed.

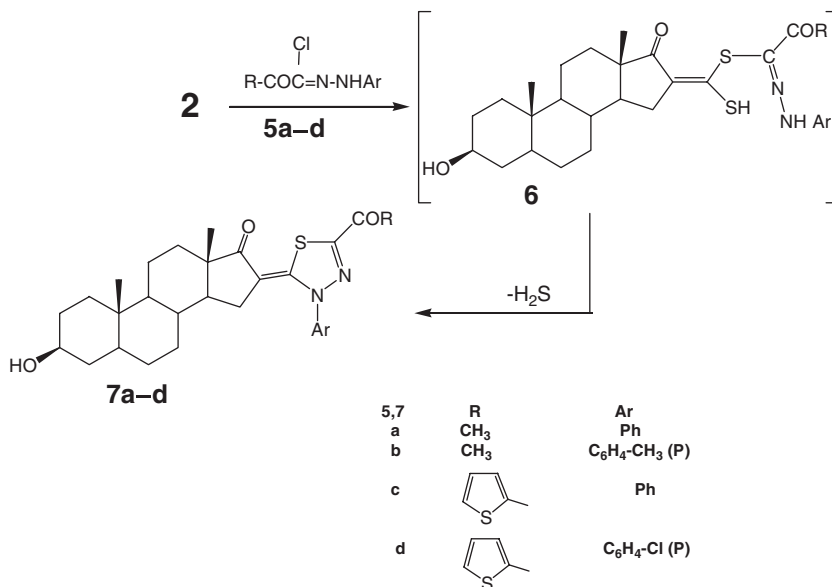
Carrying out the previous reaction with other types of hydrazonoyl halides including electron-rich alkyl groups or a thiophene ring led to the formation of the corresponding thiadiazole steroidal derivatives **7a–d**, respectively (Scheme 2). The ¹H NMR of the suggested structures **7a–d** reflect the disappearance of the characteristic signals of the (SH) and (NH) groups. For example, the ¹H NMR spectrum of **7a** showed the appearance of singlet at $\delta = 2.63$ ppm for (CH₃) group, multiplet at $\delta = 7.10$ –7.78 ppm for the aromatic protons and a singlet at 11.58 for the (OH) group (see the Materials and Methods section).

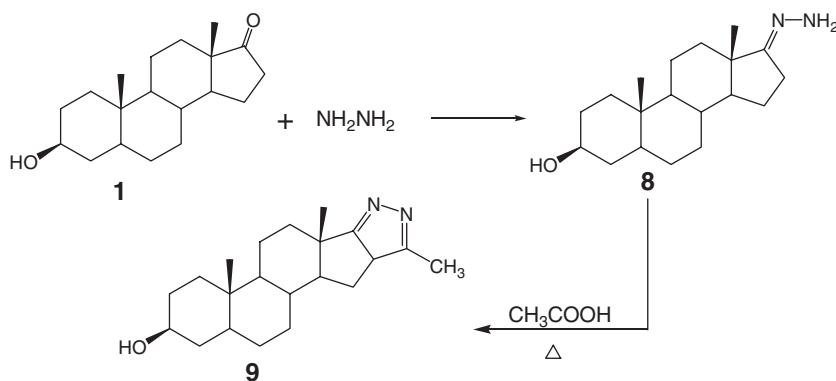
With the aim to obtain the pyrazolo anellated androstane derivative, the 17-hydrazonoandrostane derivative **8** was synthesized via the reaction of epiandrosterone **1**

Scheme 1 Synthesis of compounds **4a-d**.

with hydrazine hydrate under reflux.¹⁵ The pyrazoloandrosterone derivative **9** was obtained through the cyclization of compound **8** by heating in acetic acid under reflux (Scheme 3).

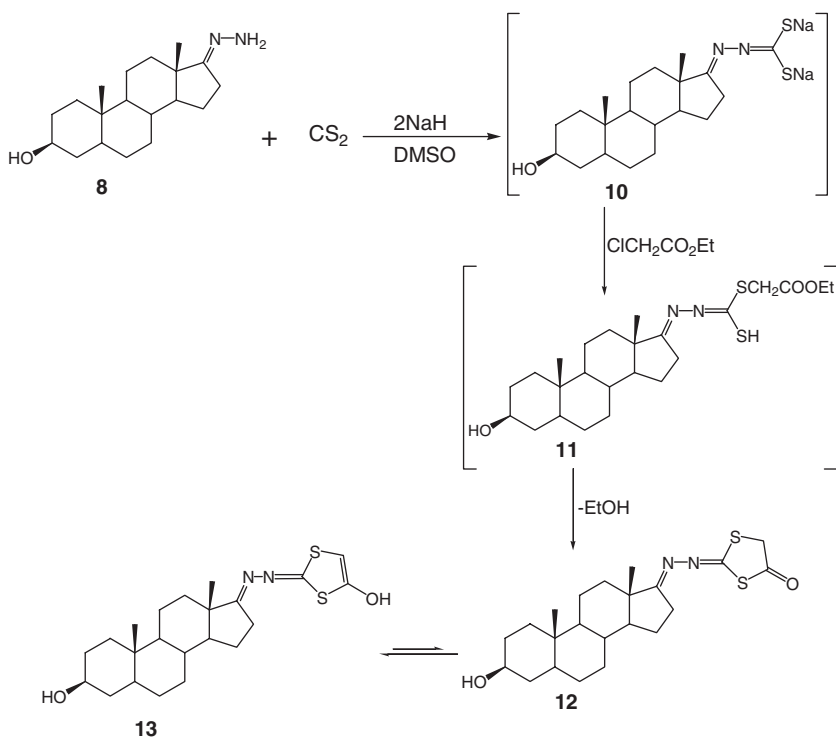
Interaction of hydrazonoandrosterone derivative **8** with carbon disulfide and 2 equivalent moles of sodium hydride in DMSO at 0°C furnished the non-isolable dithio-disodium

Scheme 2 Synthesis of compounds **7a-d**.



Scheme 3 Synthesis of compound 9.

salt **10**. Addition of ethylchloroacetate to the non-isolable salt **10** yielded the corresponding intermediate **11**, which cyclized via the elimination of ethanol to give the dithioleandrostane derivative **12** (Scheme 4). The structure of compound **12** was proved to isomerize to the final form **13**, since, in addition to the absence of CO group stretching in the IR spectrum, the ^1H NMR spectrum of the product revealed two singlets at 10.63 and 10.87 ppm for the two OH groups.



Scheme 4 Synthesis of compound 13.

In Vitro Cytotoxic Activity

The cytotoxic effect of the tested compounds **7a**, **7d**, **9**, and **13**, against hepatoma cell line (HepG₂), was performed using the MTT assay, which is widely used as a screening method to measure cell viability and proliferation.¹⁶ The results are discussed in the Supplemental Materials (available online, Figures S1 and S2)

There have been reported data that steroids at low concentrations exhibited increased absorbance values in the MTT test over the control that could indicate a stimulating effect of these compounds on cell proliferation and mitochondrial hydrogenase activity.¹⁷

CONCLUSION

In this study, we have described a straightforward and efficient synthesis of novel steroid derivatives containing thiadiazole, pyrazole, or dithiole nucleus. We investigated also the importance of incorporating heterocyclic moiety to the steroid nucleus to form new effective anticancer hybrid molecules. The androstan-16-[(thiophene-2''-carbonyl)-3H-[1,3,4]thiadiazol-2'-ylidene] derivative **7d** was the more potent cytotoxic agent against hepatoma cell line (HepG₂).

EXPERIMENTAL

The starting steroid (epiandrosterone) was purchased from Sigma Company, USA. All anhydrous reactions were carried out under argon atmosphere, and solvents were dried by distillation prior to use. All melting points were measured using an electrothermal apparatus and are uncorrected. Hydrazonoyl halides were recrystallized and dried before use. The IR spectra were recorded in (KBr discs) on a Shimadzu FT-IR 8201 PC spectrometer and expressed in cm⁻¹. The ¹H NMR and ¹³C NMR spectra were recorded with a Jeol instrument (Japan), at 270 and 125 MHz respectively, in DMSO-d₆ or CDCl₃ as solvent, and chemical shifts were recorded in ppm relative to TMS. Mass spectra were recorded on a GCMS-QP 1000 Ex spectra mass spectrometer operating at 70 eV. Elemental analyses were carried out by the Microanalytical Data Unit at National Research Center, Giza, Egypt. Reactions were monitored on Merck aluminum thin layer chromatography (TLC) plates and visualized by UV light (254 nm). For the nomenclature of steroid derivatives, we used the definitive rules for the nomenclature of steroids published by the Joint Commission on the Biochemical Nomenclature (JCBN) of IUPAC.^{18,19} The ¹H NMR spectra of all compounds showed the published characteristic data of the androstane series.^{20,21} The starting compound **8** was prepared according to the published procedure.¹⁵

Synthesis of Compounds **4a-d** and **7a-d**

To a stirred solution of epiandrosterone **1** (1.45 g, 5 mmol) in anhydrous DMSO (30 mL), sodium hydride (60% suspension in mineral oil, 0.24 g, 10 mmol) and carbon disulfide (0.38 g, 5 mmol) were added. The reaction mixture was stirred for 3 h at 0°C, and then the appropriate hydrazonoyl halide **3a-d** (5 mmol) was added dropwise with stirring. After completion of the addition, the mixture was stirred for further 3 h, left overnight at 0°C, poured into an ice-water mixture, and finally the resulting solid product was collected by filtration and crystallized from the appropriate solvent.

3 β -Hydroxy-5 α -androstan-16-ylidene-[(sulfanyl)methyl-(2'-oxo-2'-phenyl-N-(p-chlorophenyl)ethanhydrazonothioate)]-17-one (4a). Orange crystals from ethanol, yield 68% (2.12 g), mp 234–235°C. IR (KBr, cm⁻¹): ν = 3469–3340 (OH, NH), 2929, 2828 (CH₃, CH₂), 2590 (SH), 1729, 1685 (2C=O), 1642 (C=C); ¹H NMR (DMSO, ppm): δ = 0.81 (s, 3H, CH₃-19), 1.08 (s, 3H, CH₃-18), 1.23 (s, 1H, SH), 7.22–7.60 (m, 5H, aromatic protons), 7.67–7.90 (m, 4H, aromatic protons), 11.30 (s, 1H, OH), 11.80 (s, 1H, NH); MS (EI): m/z (%): 622 (M⁺, 30), 105 (C₆H₅CO, 100); Calc for C₃₄H₃₈O₃N₂S₂Cl (622.27): C, 65.62; H, 6.15; N, 4.50; S, 10.30; Found: C, 65.43; H, 6.08; N, 4.63; S, 10.16%.

3 β -Hydroxy-5 α -androstan-16-ylidene[(sulfanyl)methyl-(2'-oxo-N,2'-diphenyl-ethanhydrazonothioate)]-17-one (4b). Yellow crystals from ethanol, yield 78% (2.28 g), mp 196–197°C; IR (KBr, cm⁻¹): ν = 3480–3350 (OH, NH), 2997, 2950 (CH₃, CH₂), 2587 (SH), 1719, 1676 (2C=O), 1649 (C=C); ¹H NMR (CDCl₃, ppm): δ = 0.78 (s, 3H, CH₃-19), 1.05 (s, 3H, CH₃-18), 1.22 (s, 1H, SH), 7.24–7.30 (m, 5H, aromatic protons), 7.45–7.95 (m, 5H, aromatic protons), 11.40 (s, 1H, OH), 12.14 (s, 1H, NH); MS (EI): m/z (%): 586 (M⁺–1, 37.2), 105 (C₆H₅CO, 100); Calc for C₃₄H₃₉O₃N₂S₂ (587.83): C, 69.74; H, 6.63; N, 4.76; S, 10.91; Found: C, 69.43; H, 6.48; N, 4.93; S, 11.12%.

3 β -Hydroxy-5 α -androstan-16-ylidene[(sulfanyl)methyl-(2'-naphthyl-2'-oxo-N-phenylethanhydrazonothioate)]-17-one (4c). Red crystals from methanol, yield 68% (2.2 g), mp 68–70°C; IR (KBr, cm⁻¹): ν = 4780–3420 (OH, NH), 2954, 2890 (CH₃, CH₂), 2592 (SH), 1720, 1678 (2C=O), 1646 (C=C); ¹H NMR (DMSO, ppm): δ = 0.81 (s, 3H, CH₃-19), 1.19 (s, 3H, CH₃-18), 1.23 (s, 1H, SH), 7.02–7.32 (m, 5H, aromatic protons), 7.59–8.07 (m, 6H, naphthyl protons), 8.50 (s, 1H, naphthyl proton), 11.49 (s, 1H, OH), 12.20 (s, 1H, NH). MS (EI): m/z (%): 638 (M⁺, 23), 154 [(naphthyl-CO)-1, 100], 77 (C₆H₅, 37.5); Calc for C₃₈H₄₂O₃N₂S₂ (638.90): C, 71.43; H, 6.62; N, 4.38; S, 10.03; Found: C, 71.65; H, 6.43; N, 4.62; S, 10.21%.

3 β -Hydroxy-5 α -androstan-16-ylidene[(sulfanyl)methyl-(2'-oxo-2'-phenylimino-N-(p-methylphenyl)ethanhydrazonothioate)]-17-one (4d). Yellow crystals from ethanol, yield 84% (2.52 g), mp 75–77°C; IR (KBr, cm⁻¹): ν = 3468–3360 (OH, NH), 2928, 2866 (CH₃, CH₂), 2590 (SH), 1731, 1677 (2C=O), 1642 (C=C); ¹H NMR (CDCl₃, ppm): δ = 0.90 (s, 3H, CH₃-19), 1.17 (s, 3H, CH₃-18), 1.24 (s, 1H, SH), 2.61 (s, 3H, CH₃), 7.18–7.37 (m, 5H, aromatic protons), 7.59–7.63 (m, 4H, aromatic protons), 11.73 (s, 1H, OH); MS (EI): m/z (%): 602 (M⁺, 28), 120 (C₆H₅NHCO, 100); Calc for C₃₄H₄₀O₃N₃S₂ (602.84): C, 67.79; H, 6.68; N, 6.47; S, 10.63; Found: C, 65.43; H, 6.08; N, 4.63; S, 10.16%.

3 β -Hydroxy-5 α -androstan-16-(5'-acetyl-3'-phenyl-3'H-[1',3',4']thiadiazol-2'-ylidene)-17-one (7a). Pale brown crystals from 1,4-dioxan, yield 73% (1.79 g), mp 135–137°C; IR (KBr, cm⁻¹): ν = 3468–3248 (OH, NH), 2931, 2860 (CH₃, CH₂), 1728, 1684 (2C=O), 1648 (C=C); ¹H NMR (CDCl₃, ppm): δ = 0.79 (s, 3H, CH₃-19), 1.10 (s, 3H, CH₃-18), 2.63 (s, 3H, CH₃), 7.10–7.78 (m, 5H, aromatic protons), 11.58 (s, 1H, OH); ¹³C NMR (DMSO-d₆, ppm): δ = 27.8 (C-1), 30.7 (C-2), 67.9 (C-3), 33.7 (C-4), 36.1 (C-5), 25.4 (C-6), 25.9 (C-7), 29.6 (C-8), 43.4 (C-9), 37.6 (C-10), 21.1 (C-11), 27.5 (C-12), 54.3 (C-13), 39.3 (C-14), 11.3 (C-15), 126.9 (C-16), 202.4 (C-17), 17.2 (C-18), 21.2 (C-19), 136.2 (C-2'), 152.8 (C-5'), 200.3 (C=O), 18.0 (CH₃-acetyl), 146.0, 116.5, 129.1, 117.8 (C-phenyl). MS (EI): m/z (%): 491 (M⁺–1, 32.7), 288 (C₁₉H₂₈O₂, 100); Calc for C₂₉H₃₆O₃N₂S (492.68): C, 70.69; H, 7.36; N, 5.68; S, 6.50; Found: C, 65.43; H, 6.08; N, 4.63; S, 10.16%.

3 β -Hydroxy-5 α -androstan-16-(5'-acetyl-3'-(p-chlorophenyl)-3'H-[1',3',4']thiadiazol-2'-ylidene)-17-one (7b). Yellow crystals from ethanol, yield 67% (1.69 g), mp 198–200°C; IR (KBr, cm⁻¹): ν = 3405 (OH), 3023, 2920 (CH₃, CH₂), 1704, 1678 (2C=O), 1640 (C=C); ¹H NMR (CDCl₃, ppm): δ = 0.82 (s, 3H, CH₃-19), 1.08 (s, 3H, CH₃-18), 2.20 (s, 3H, CH₃), 2.52 (s, 3H, CH₃), 7.17 (d, 2H, aromatic protons), 7.34 (d, 2H, aromatic protons), 11.45 (s, 1H, OH), MS (EI): m/z (%): 506 (M⁺, 47), 255 (C₁₉H₂₇, 100); Calc for C₃₀H₃₈O₃N₂S (506.71): C, 71.42; H, 7.55; N, 5.52; S, 6.32; Found: C, 65.43; H, 6.08; N, 4.63; S, 10.16%.

3 β -Hydroxy-5 α -androstan-16-(3'-phenyl-5'-(thiophene-2''-yl-methanone)-3'H-[1',3',4']thiadiazol-2'-ylidene)-17-one (7c). Pale yellow crystals from methanol, yield 67% (1.87 g), mp 180–181°C; IR (KBr, cm⁻¹): ν = 3400 (OH), 2985, 2957 (CH₃, CH₂), 1716, 1678 (2C=O), 1649 (C=C); ¹H NMR (CDCl₃, ppm): δ = 0.76 (s, 3H, CH₃-19), 1.07 (s, 3H, CH₃-18), 7.05–7.25 (m, 2H, aromatic protons), 7.40–7.60 (m, 3H, thiophene protons), 7.80–7.95 (m, 3H, aromatic protons), 11.45 (s, 1H, OH); ¹³C NMR (DMSO-d₆, ppm): δ = 27.2 (C-1), 31.2 (C-2), 67.5 (C-3), 34.7 (C-4), 37.0 (C-5), 25.5 (C-6), 26.3 (C-7), 29.0 (C-8), 42.8 (C-9), 37.4 (C-10), 20.4 (C-11), 28.0 (C-12), 55.6 (C-13), 39.8 (C-14), 12.6 (C-15), 125.0 (C-16), 203.6 (C-17), 16.5 (C-18), 20.5 (C-19), 136.0 (C-2'), 154.4 (C-5'), 184.3 (C=O), 145.7, 137.6, 129.5, 135.9 (C-thiophene), 146.2, 115.3, 129.4, 118.3 (C-phenyl); MS (EI): m/z (%): 560 (M⁺, 48.3), 111 [thiophen-CO (C₅H₃SO) 100]; Calc for C₃₂H₃₆O₃N₂S₂ (560.78): C, 68.53; H, 6.47; N, 4.99; S, 11.43; Found: C, 68.74; H, 6.25; N, 4.73; S, 11.19%.

3 β -Hydroxy-5 α -androstan-16-{3'-(p-chlorophenyl)-5'-(thiophene-2''-yl-methanone)-3'H-[1',3',4']thiadiazol-2'-ylidene}-17-one (7d). Orange crystals from ethanol, yield 70% (2.28 g), mp 248–250°C; IR (KBr, cm⁻¹): ν = 3405 (OH), 2985, 2978 (CH₃, CH₂), 1716, 1678 (2C=O), 1645 (C=C); ¹H NMR (DMSO): δ = 0.78 (s, 3H, CH₃-19), 1.03 (s, 3H, CH₃-18), 7.14–7.27 (m, 2H, aromatic protons), 7.42–7.56 (m, 3H, thiophene protons), 7.95–8.04 (m, 2H, aromatic protons), 11.50 (s, 1H, OH); ¹³C NMR (DMSO-d₆, ppm): δ = 26.2 (C-1), 31.5 (C-2), 66.9 (C-3), 33.7 (C-4), 37.4 (C-5), 25.9 (C-6), 26.3 (C-7), 29.5 (C-8), 42.6 (C-9), 37.4 (C-10), 20.0 (C-11), 28.6 (C-12), 56.4 (C-13), 39.3 (C-14), 12.8 (C-15), 124.6 (C-16), 201.6 (C-17), 17.5 (C-18), 21.5 (C-19), 135.3 (C-2'), 154.6 (C-5'), 185.2 (C=O), 145.6, 137.9, 128.5, 136.4 (C-thiophene), 144.2, 116.3, 129.6, 123.0 (C-phenyl); MS (EI): m/z (%): 595 (M⁺, 58), 111 [thiophen-CO (C₅H₃SO) 100]; Calc for C₃₂H₃₅O₃N₂S₂Cl (595.23): C, 64.57; H, 5.92; N, 4.70; S, 10.77; Found: C, 65.43; H, 6.08; N, 4.63; S, 10.16%.

(9) Synthesis of 3 β -Hydroxy-5'-methyl-5 α -androstan[16,17:3',4']pyrazole

To 17-hydrazoneandrostan derivative **8** (1.52 g, 5 mmol), 20 mL of glacial acetic acid was added dropwise with stirring. The reaction was heated under reflux for 3 h until all the starting materials had disappeared as indicated by TLC, and then poured into an ice/water mixture (60 mL). The solid product that formed was collected by filtration, dried, and crystallized from ethanol to give compound **9**, pale brown crystals, yield 73% (1.19 g), mp 60–62°C; IR (KBr, cm⁻¹): ν = 3450 (OH), 2942, 2851 (CH₃, CH₂); ¹H NMR (DMSO): δ = 0.78 (s, 3H, CH₃-19), 1.03 (s, 3H, CH₃-18), 2.80 (s, 3H, CH₃); ¹³C NMR (DMSO-d₆, ppm): δ = 27.4 (C-1), 30.2 (C-2), 67.0 (C-3), 34.9 (C-4), 36.8 (C-5), 25.3 (C-6), 25.9 (C-7), 29.4 (C-8), 42.5 (C-9), 39.0 (C-10), 22.4 (C-11), 27.6 (C-12), 45.6 (C-13), 43.8 (C-14),

25.6 (C-15), 34.0 (C-16), 167.2 (C-17), 16.3 (C-18), 21.2 (C-19), 164.0 (C-5'); MS (EI): m/z (%): 328 (M^+ , 45.6), 55 ($C_2H_3N_2$, 100); Calc for $C_{21}H_{32}ON_2$ (328.50): C, 76.78; H, 9.81; N, 8.52; Found: C, 77.01; H, 9.67; N, 8.73%.

Synthesis of 3 β -Hydroxy-5 α -androstan-17-hydrazono-N-[(1',3')dithiol-2'-ylidene]-4'-ol (**13**)

Sodium hydride (60% suspension in mineral oil, 0.24 g, 10 mmol) and carbon disulfide (0.38 g, 5 mmol) were added to a stirred solution of 17-hydrazonoandrostan derivative **8** (1.52 g, 5 mmol) in anhydrous DMSO (40 mL). The reaction mixture was stirred for 4 h at 0°C, and then ethyl chloroacetate (0.62 g, 5 mmol) was added dropwise with stirring. After completion of the addition, the reaction mixture was stirred for further 2 h, left overnight at 0°C, poured into an ice/water mixture, and the resulting solid product was collected by filtration and crystallized from ethanol to yield 66% (1.38 g) of dithioandrostan derivative **13**, yellow crystals, mp 195–197°C; IR (KBr, cm^{-1}): ν = 3393 (OH), 2927, 2856 (CH_3 , CH_2), 1649 ($C=C$); 1H NMR (DMSO): δ = 0.78 (s, 3H, CH_3 -19), 1.12 (s, 3H, CH_3 -18), 10.63, 10.87 (2s, 2H, 2OH); ^{13}C NMR (DMSO- d_6 , ppm): δ = 28.0 (C-1), 30.2 (C-2), 68.0 (C-3), 34.5 (C-4), 36.7 (C-5), 25.4 (C-6), 26.7 (C-7), 29.4 (C-8), 43.1 (C-9), 38.6 (C-10), 20.8 (C-11), 27.5 (C-12), 42.5 (C-13), 46.8 (C-14), 26.6 (C-15), 23.8 (C-16), 165.6 (C-17), 16.8 (C-18), 21.0 (C-19), 163.4 (C-2'), 151.7 (C-4'), 87.3 (C-5'); MS (EI): m/z (%): 420 (M^+ , 38.7), 255 ($C_{19}H_{27}$, 100); Calc for $C_{22}H_{32}O_2N_2S_2$ (420.64): C, 62.81; H, 7.66; N, 6.65; S, 15.24; Found: C, 63.07; H, 7.85; N, 6.83; S, 15.47%.

In Vitro Cytotoxic Activity

The Supplemental Materials (available online) detail the materials and methods used for the cytotoxic activity testing.²²

Statistics

Statistical evaluation of the results was done with the analysis of variance (ANOVA) using the MSTATC program.²³ When significant differences ($P < 0.05$) were detected, the least significant difference (LSD) test was used to separate the main values.²⁴

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