Synthesis and anticancer activity of 4-azasteroidal-20-oxime derivatives

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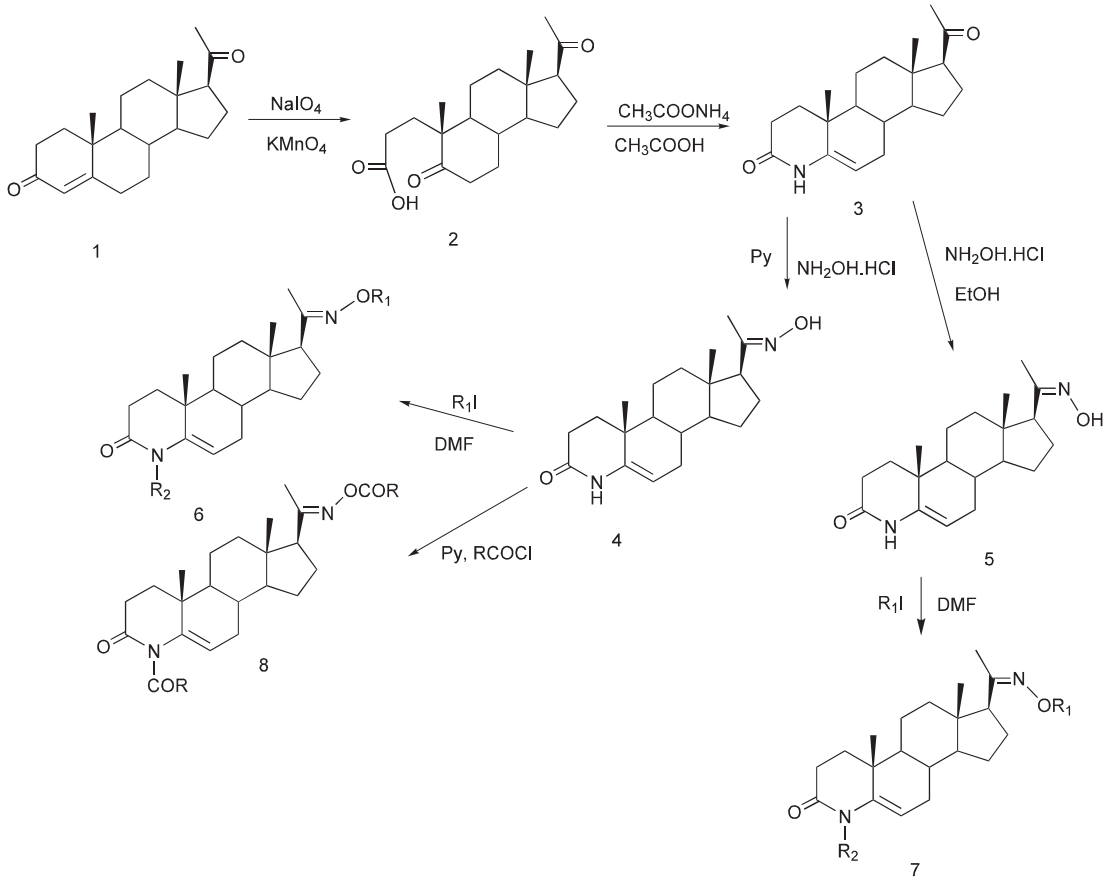
A new series of 4-azasteroidal-20-oxime derivatives have been synthesised from progesterone. All the new compounds have been characterised by analysis and spectroscopic data and subsequently evaluated for their anticancer activity *in vitro* against T24 cell. The studies show that the methyl and ethyl oxime-ethers exhibited higher activity than the aryl substituted oxime-esters.

Keywords: progesterone, synthesis, configuration, anticancer activity

Cancer is a major cause of death in both developing and developed countries according to the WHO.[1] A number of natural products and their semi synthetic analogues have been discovered and evaluated for their role in the treatment and cure of cancer and other fatal diseases.[2,3] Steroids and their derivatives elicit diverse biological effects though various functional groups located around the periphery of their rigid tetracyclic core.[4,5] It has been reported that 4-azasteroidal analogues may lead to compounds having anticancer activity or 5α-reductase inhibitor activity.[6-8] In several studies, derivatives of steroidal oximes have been tested for a variety of anticancer, antimicrobial and antioxidant activities.[9,10] The advantage of employing hydrophobic steroid units with an oxime group enhances their ability to interact with cell membranes and thus paves the way for biological activity of such hybrid molecules. There are few reports of the synthesis of oximes of 4-azasteroids and their biological screening. We have synthesised novel 4-azasteroidal-20-oxime derivatives from the commercially available progesterone and evaluated their anticancer activities against the T24 cell.

The synthesis of 3-oxo-4-aza-5-pregnene-20-oxime derivatives are shown in Scheme 1. Starting from progesterone (**1**), ring A was oxidatively cleared by treatment withsodium periodate and potassium permanganate to give 5,20-dioxo-A-nor-3,5-secopregnane-3-oic acid (**2**) in a yield of 92%.[11,12] Ozonolysis of progesterone followed by oxidative work-up(H_2O_2–NaOH) also produced compound **2**, but the yield was 80% and the procedure was complex.[13] This compound was treated with ammonium formate to afford 4-aza-5-pregnene-3,20-dione (**3**) in 91.5% yield,[14] and this was converted into its 20-oxime analogue **4** in



6a $R^1=R^2=CH_3$; **6b** $R^1=R^2=Et$; **6c** $R^1=H$, $R^2=Et$; **7a** $R^1=R^2=CH_3$; **7b** $R^1=R^2=Et$; **7c** $R^1=H$, $R^2=Et$; **8a** $R=CH_3$; **8b** R=Ph; **8c** R=*p*-CH_3-Ph; **8d** R=*p*-OCH_3-Ph; **8e** R=*p*-Cl-Ph.

Scheme 1 Synthesis of 3-oxo-4-substituted-4-aza-5-pregnene-20-oxime derivatives.

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67% yield using hydroxylamine in the presence of pyridine.[15] In the IR spectrum of **4**, the characteristic oxime bands were observed at 3402 cm^{-1}(OH) and 1,654 cm^{-1} (C=N), whilst the 20-C=O carbon signal (209.4 ppm) of compound **3** was not observed in ^{13}C NMR spectrum of compound **4**. Instead the 20-C=NOH carbon signal was identified at 155.5ppm. Furthermore, its structure was confirmed by ESI mass spectra. With ethanol as the solvent, a different oxime **5** was obtained in 82% yield.[8] The configuration of the 20-oxime was determined using the 2D-NOESY spectrum. The NOESY spectrum of compound **5** did not exhibit correlation between the peak of the 21-CH_3 (1.73ppm) and the proton 20-oxime group (10.34ppm) while compound **4** showed this NOE effect. [16] Therefore compound **5** was identified as *Z*-isomer. In the formation of the ethers, compound **4** reacts faster than **5** because of the influence of steric hindrance. This indirectly confirmed its configuration as the *E*-isomer.

The oxime was treated with the alkyl iodide under basic conditions to afford 3-oxo-4-substituted-4-aza-5-pregnene-20-oxime ether derivatives. The ESI mass spectra of **6a–c** and **7a–c** were characterised by their molecular ion peaks. The ^1H NMR spectrum of **6c** displayed the signals at 4.06–4.10, which were assigned to the proton of –N–O–CH_2, while the –NH signal appeared at 7.22 ppm. The expected singles for 18-CH_3 (δ = 0.65 ppm), 19-CH_3(δ = 1.09 ppm), 21-CH_3(δ = 1.82 ppm) and double bond (δ = 4.81ppm) were observed. Under these reaction conditions, we concluded that the 20*E*-oxime was more reactive than the 20*Z*-oxime. A reaction using KOH to obtain the ethyl ester of 3-oxo-4-substituted-4-aza-5-pregnene-20*Z*-oxime did not succeed. Consequently, potassium *t*-butoxide was used to afford the target products. However, using iodopropane and other reactants, the reaction was so complex that the products could not be isolated. Because of steric hindrance, only compound **4** was treated with the acyl chlorides in a mixture of pyridine and dichloromethane as the solvent to afford the 4-acyl-3-oxo-4-aza-5-pregnene-20*E*-oxime ester derivatives.[16]

The novel compounds were evaluated for their anticancer activity *in vitro* against T24 (human bladder carcinoma) cell. The inhibition of test compounds was determined using the MTS assay. The anticancer activity was indicated in terms of IC_{50} (μM) value and the results were presented in Table 1.

From the data shown in Table 1, it is evident that the oxime-ether derivatives have a higher activity against T24 than the oxime-ester derivatives. In the oxime-ester derivatives, only compound **8a** which contained the acetyl group had significant influence on the cytotoxicity. In contrast, for compounds **8b–e** the introduction of aryl group resulted in a marked decrease in potency compared to **8a**. Compounds in which the configuration of oxime was *E* displayed strong cell growth inhibitory activity against T24, with IC_{50} values of 2.41μM. It is interesting that compound **6a** bearing methyl groups at the 4N and 20C=N⁻O⁻ positions demonstrated the best anticancer activity against T24 cells, with the IC_{50} values of 1.94μM. However, compounds **6b** and **6c** bearing the same substituent at C20-position exhibited low inhibitory activity against the tested cell. Compound **5** exhibited no inhibitory activity of these cells, while its oxime-ether derivatives **7b** and **7c** showed significant influence on the cytotoxic activity with the IC_{50} values of 1.99 and 2.62 μM respectively, suggesting that substituent at the 4N-position of the steroidal moiety also affected the anticancer activity.

Table 1 The *in vitro* anticancer activity of 4-azasteroidal-20-oxime derivatives

Compound	IC_{50}/μM	Compound	IC_{50}/μM
4	2.41	**7c**	2.62
5	>100	**8a**	1.90
6a	1.94	**8b**	80.89
6b	50.45	**8c**	91.34
6c	>100	**8d**	>100
7a	>100	**8e**	>100
7b	1.99		

In conclusion, we have reported the synthesis and anticancer activity against T24 cell of 4-azasteroidal-20-oxime derivatives. The results from MTS-assays showed that some compounds had excellent activity against T24 (compound **4, 6a, 7b, 7c, 8a**). It was evident that introduction of aryl groups resulted in a marked decrease in potency (compounds **8b–e**).

Experimental

All the reagents were obtained commercially and used without further purification. Melting points were measured on an X4 apparatus and uncorrected. IR spectra were determined as KBr pellets on a FTS-135 spectrophotometer. ^1H NMR spectra were measured with a Varian INOVA 500 spectrometer at 500 MHz in $CDCl_3$ as the solvent with TMS as internal standard. ESI-MS were performed on LCQ Advantage MAX spectrometer. Elementary analyses were performed by a Carlo-Erba CHNO-S analyser.

5,20-Dioxo-A-nor-3,5-secopregnane-3-oic acid (**2**): A mixture of progesterone (15.0 g, 47.6 mmol) in *tert*-butanol (450 mL), anhydrous sodium carbonate (6.1 g, 57.6 mmol) and H_2O (20 mL) was heated to reflux, and a solution of sodium periodate (61.2 g, 286.0 mmol) and potassium permanganate (0.45 g, 2.9 mmol) in hot water (300 mL) was added dropwise to a refluxing mixture. This was then refluxed for another 3 h. All inorganic material was filtered off, and washed with water and methylene chloride. The filtrate was acidified with dilute hydrochloric acid and extracted with dichloromethane (150 mL×3). The organic layer was washed with brine, dried over anhydrous sodium sulfate, and evaporated under reduced pressure. Compound **2** (14.7 g, 92.1%) was obtained as pale yellow solid, which was used directly without further purification; m.p. 176–178 °C (lit.[6] 177–179 °C). IR (KBr), ν/cm^{-1}: 3148(OH), 1734(CO), 1698(3-CO); ESI-MS *m/z*: 333(M-H)⁻; 1H NMR ($CDCl_3$, 500 MHz), δ 2.13 (3H, s, 21-CH_3), 1.13 (3H, s, 19-CH_3), 0.69 (3H, s, 18-CH_3).

4-Aza-5-pregnene-3,20-dione (**3**): The mixture of 5,20-dioxo-A-nor-3, 5-secopregnane-3-oic acid (5.0 g, 15.0 mmol), and ammonium acetate (3.5g, 45.1 mmol) in acetic acid (50 mL) was heated to reflux for 4 h. After removal of the acetic acid under reduced pressure, the residue was poured into water. The precipitate was filtered and washed with water to give **3**(4.3g, 91.5%) as pale yellow solid; m.p. 273–275°C (274–276°C[12]). IR(KBr), σ/cm^{-1}: 2938(⁻NH), 1702(20-C=O), 1667(3-C=O); ESI-MS (*m/z*): 316.4(M+H)⁺; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.65 (s, 3H, 18-CH_3), 1.08 (s, 3H, 19-CH_3), 2.15 (s, 3H, 21-CH_3), 4.85 (m, 1H, 6-H), 7.76 (s, 1H, 4-H); ^{13}C NMR (125 MHz, DMSO) δ 209.4, 169.3, 140.0, 103.0, 63.5, 56.6, 47.8, 44.0, 38.5, 34.3, 31.6, 31.5, 31.4, 29.6, 28.5, 24.4, 22.8, 21.0, 18.7, 13.3.

*3-Oxo-4-aza-5-pregnene-20*E*-oxime* (**4**): Hydroxylamine hydrochloride (3.7 g, 48.6 mmol) was added to a stirred soluion of 4-aza-5-pregnene-3,20-dione (5.0g , 15.8 mmol) in pyridine (120 mL). The mixture was stirred for 12 h at 80–90°C, and then poured into water (150 mL), extracted with dichloromethane (50 mL×3). The organic layer was washed with brine, dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel and eluted with petroleum ether/ethyl acetate to afford compound **4** (3.2 g, 60.7%) as a white solid; m.p. 230–231 °C; IR(KBr), σ/cm^{-1}: 3350.18, 2928.18, 1654.09, 1368.00, 1217.6, 930.51; ESI-MS (*m/z*): 331.4(M+H)⁺; ^1H NMR (500 MHz, DMSO) δ 0.58 (s, 3H, 18-CH_3), 1.02 (s, 3H, 19-CH_3), 1.74 (s, 3H, 21-CH_3), 5.31 (s, 1H, 6-H), 9.71 (s, 1H, ⁻NH), 10.35 (s, 1H, ⁻OH); ^{13}C NMR (125 MHz, DMSO) δ 162.3, 155.5, 142.3, 101.3, 56.6, 55.8, 48.5, 43.6, 38.4, 36.2, 31.3, 31.2, 29.8, 28.9, 24.2, 23.1, 21.1, 19.3,

15.7, 13.6. Anal. calcd for $C_{20}H_{30}N_2O_2$: C, 72.69; H, 9.15; N, 8.48; found: C, 72.73; H, 9.34; N, 8.60%.

3-Oxo-4-aza-5-pregnene-20Z-oxime (**5**): Hydroxylamine hydrochloride (1.0 g, 14.1 mmol) and triethylamine (2.1 mL, 15.0 mmol) were added to a stirred solution of 4-aza-5-pregnene-3,20-dione (3.3 g, 10.0 mmol) in ethanol (100 mL). Then the mixture was heated to reflux for 12 h, cooled to room temperature, poured into water (80 mL), extracted with dichloromethane (50 mL×3). The organic layer was washed with brine, dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel and eluted with petroleum ether/ethyl acetate to afford compound **5** (2.7 g, 81.8%) as white solid; m.p. 243–245°C; IR (KBr), σ/cm^{-1}: 3316.16, 2928.18, 1664.98, 1368.00, 1056.48; ESI-MS (*m/z*): 347.4 $(M+NH_3)^+$; ^{1}H NMR (500 MHz, DMSO) δ 0.58 (s, 3H, 18-CH_3), 0.99 (s, 3H, 19-CH_3), 1.73 (s, 3H, 21-CH_3), 4.84 (s, 1H, 6-H), 9.27 (s, 1H, –NH), 10.34 (s, 1H, –OH); ^{13}C NMR (125 MHz, DMSO) δ 168.3, 155.5, 141.3, 101.6, 56.6, 55.9, 48.2, 43.6, 38.4, 34.0, 31.3, 31.8, 31.7, 29.7, 28.7, 24.3, 23.1, 21.0, 19.0, 15.7, 13.6. Anal. calcd for $C_{20}H_{30}N_2O_2$: C, 72.69; H, 9.15; N, 8.48; found: C, 72.89; H, 9.02; N, 8.50%.

*Synthesis of 3-oxo-4-substituted-4-aza-5-pregnene-20*E*-oxime-ether derivatives (***6a–c***); general procedure*

A mixture of compound **4** (0.21 g, 0.64 mmol) in N,N-dimethylformamide (DMF, 30mL), containing potassium hydroxide (KOH, 0.1g, 1.78 mmol) was stirred for 0.5h at room temperature, then iodomethane or iodoethane (0.2 mL) was added dropwise. The mixture was heated to 110–115°C for 5 h, poured into water (10 mL), and extracted with dichloromethane (20 mL×3). The organic layer was washed with water (50mL×3), dried over anhydrous sodium sulfate, andevaporated under reduced pressure. The crude was purified by column chromatography on silica gel and eluted with petroleum ether/ethyl acetate to afford compounds **6a–c**.

*3-Oxo-4-methyl-4-aza-5-pregnene-20*E*-oxime-N–O-methyl ether* (**6a**): Yield 66.4%; m.p. 177–179 °C; ESI-MS (*m/z*): 375.4 $(M+NH_3)^+$; 1H NMR (500 MHz, $CDCl_3$) δ 0.65 (s, 3H, 18-CH_3), 1.09 (s, 3H, 19-CH_3), 1.85 (s, 3H, 21-CH_3), 3.72 (s, 3H, 4-NCH_3), 3.84 (s, 3H, –N–O–CH_3), 5.39 (s, 1H, 6-H). Anal. calcd for $C_{22}H_{34}N_2O_2$: C, 73.70; H, 9.56; N, 7.81; found: C, 73.64; H, 9.47; N, 7.62%.

*3-Oxo-4-ethyl-4-aza-5-pregnene-20*E*-oxime-N–O-ethyl ether* (**6b**): Yield 16.9%; ESI-MS (*m/z*): 385.4(M-H)$^-$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.67 (s, 3H, 18-CH_3), 1.09 (s, 3H, 19-CH_3), 1.83 (s, 3H, 21-CH_3), 3.87 – 3.99 (m, 2H, 4 – N – CH_2), 4.03–4.10 (m, 2H, –N–O–CH_2), 5.39 (s, 1H, 6-H). Anal. calcd for $C_{24}H_{38}N_2O_2$: C, 74.57; H, 9.91; N, 7.25; found: C, 74.64; H, 9.97; N, 7.12%.

3-Oxo-4-aza-5-pregnene-20E-oxime-N–O-ethyl ether (**6c**): Yield 46.1%; m.p. 178–180°C; ESI-MS (*m/z*): 381.3(M+Na)$^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.65 (s, 3H, 18-CH_3), 1.09 (s, 3H, 19-CH_3), 1.82 (s, 3H, 21-CH_3), 4.06–4.10 (m, 2H, –N–O–CH_2), 4.81 (s, 1H, 6-H), 7.22 (s, 1H, –NH). Anal. calcd for $C_{22}H_{34}N_2O_2$: C, 73.70; H, 9.56; N, 7.81; found: C, 73.89; H, 9.57; N, 7.88%.

*4-Acyl-3-oxo-4-aza-5-pregnene-20*E*-oxime-ester derivatives (***8a–e***); general procedure*

A stirred solution of compound **4** (0.21 g, 0.64 mmol) in a mixture of pyridine (3 mL) and dichloromethane (3 mL) was treated with the acyl chloride (1.92 mmol) at 0 °C. The mixture was then stirred for 0.5 h at room temperature, poured into water (10 mL), and extracted with dichloromethane (20 mL × 3). The organic layer was washed with dilute hydrochloric acid, saturated sodium carbonate solution, brine, dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel. Elution with petroleum ether/ethyl acetate afforded compounds **8a–e** as white solids.

*4-Acetyl-3-keto-4-aza-5-pregnene-20*E*-oxime-N–O-acetate ester* (**8a**): Yield 64.1%; m.p. 175–176 °C; ESI-MS (*m/z*): 437.3 (M + Na)$^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.61 (s, 3H, 18-CH_3), 1.18 (s, 3H, 19-CH_3), 1.89 (s, 3H, 21-CH_3), 2.11 (s, 3H, OCO – CH_3), 2.21 (s, 3H, 4-N – CO – CH_3), 5.08 (s, 1H, 6-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.24, 166.75, 166.10, 162.05, 141.39, 101.43, 60.09, 56.67, 55.84, 48.26, 44.05, 38.18, 37.09, 31.16, 29.43, 29.05, 24.09, 23.10, 22.80, 19.89, 18.95, 18.18, 16.99, 13.37. Anal. calcd for $C_{24}H_{34}N_2O_4$: C, 69.54; H, 8.27; N, 6.76; found: C, 69.65; H, 8.37; N, 6.78%.

*4-Benzoyl-3-oxo-4-aza-5-pregnene-20*E*-oxime-N–O-benzoate ester* (**8b**): Yield 54.6%; m.p. 260–262 °C; ESI-MS (*m/z*): 477.9 (M + K)$^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.79 (s, 3H, 18-CH_3), 1.58 (s, 3H, 19-CH_3), 2.10 (s, 3H, 21-CH_3), 5.25 (s, 1H, 6-H), 7.45–7.51 (m, 4H, Ph), 7.58–7.66 (m, 2H, Ph), 8.06–8.08 (m, 2H, Ph), 8.13–8.14 (m, 2H, Ph); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.60, 163.83, 163.03, 162.24, 141.57, 134.20, 133.11, 130.12, 129.52, 128.75, 128.51, 101.44, 56.84, 55.89, 48.35, 48.33, 44.24, 38.24, 37.30, 37.28, 31.22, 31.20, 29.47, 29.27, 29.25, 24.17, 23.23, 20.86, 19.04, 17.14, 13.89, 13.49. Anal. calcd for $C_{34}H_{38}N_2O_4$: C, 75.81; H, 7.11; N, 5.20; found: C, 75.89; H, 7.21; N, 5.29%.

*4-*p*-Methylbenzoyl-3-oxo-4-aza-5-pregnene-20*E*-oxime-N–O-*p*-methylbenzoate ester* (**8c**): Yield 51.9%; m.p. 188–189 °C; ESI-MS (*m/z*): 605.5 (M + K)$^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.78 (s, 3H, 18-CH_3), 1.58 (s, 3H, 19-CH_3), 2.09 (s, 3H, 21-CH_3), 2.43 (s, 6H, 2Ph-CH_3), 5.24 (s, 1H, 6-H), 7.25 – 7.30 (m, 4H, Ph), 7.95 – 7.96 (m, 2H, Ph), 8.01 – 8.03 (m, 2H, Ph); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.39, 163.91, 163.08, 162.24, 145.15, 143.81, 141.60, 140.53, 130.16, 129.55, 129.44, 129.20, 126.72, 101.40, 56.83, 55.90, 48.36, 48.35, 44.23, 38.24, 37.28, 37.27, 31.22, 31.20, 29.48, 29.46, 29.26, 29.25, 24.17, 23.22, 21.83, 21.70, 20.85, 19.02, 17.09, 13.49. Anal. calcd for $C_{36}H_{42}N_2O_4$: C, 76.29; H, 7.47; N, 4.94; found: C, 76.39; H, 7.57; N, 4.88.

*4-*p*-Methoxybenzoyl-3-oxo-4-aza-5-pregnene-20*E*-oxime-N–O-*p*-methoxybenzoate ester* (**8d**): Yield 48.7%; m.p. 186–188 °C; ESI-MS (*m/z*): 637.4 (M + K)$^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.78 (s, 3H, 18-CH_3), 1.55 (s, 3H, 19-CH_3), 2.08 (s, 3H, 21-CH_3), 3.87 (s, 3H, Ph-OCH_3), 3.88 (s, 3H, Ph-OCH_3), 5.24 (s, 1H, 6-H), 6.93 – 6.97 (m, 4H, Ph), 8.2 – 8.04 (m, 2H, Ph), 8.08 – 8.10 (m, 2H, Ph); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.19, 164.35, 163.60, 163.49, 162.70, 162.33, 141.62, 140.57, 132.30, 131.56, 121.75, 114.07, 113.79, 101.39, 56.83, 55.89, 55.56, 55.47, 48.36, 48.12, 44.22, 38.23, 37.04, 37.03, 31.22, 31.20, 29.49, 29.48, 29.26, 24.17, 23.22, 21.69, 20.84, 19.02, 17.07, 13.45. Anal. calcd for $C_{36}H_{42}N_2O_6$: C, 72.22; H, 7.07; N, 4.68; found: C, 72.32; H, 7.17; N, 4.78%.

*4-*p*-Chlorobenzoyl-3-oxo-4-aza-5-pregnene-20*E*-oxime-N–O-*p*-chlorobenzoate ester* (**8e**): Yield 49.7%; m.p. 170–172 °C; ESI-MS (*m/z*): 645.4 (M + K)$^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.78 (s, 3H, 18-CH_3), 1.55 (s, 3H, 19-CH_3), 2.08 (s, 3H, 21-CH_3), 5.21 (s, 1H, 6-H), 7.43–7.45 (m, 4H, Ph), 7.99–8.01 (m, 4H, Ph); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.93, 163.13, 162.40, 162.31, 141.68, 140.96, 139.71, 131.02, 129.29, 129.01, 128.07, 101.58, 56.95, 56.01, 48.44, 48.43, 44.38, 38.34, 37.45, 37.36, 31.34, 31.32, 29.57, 29.55, 29.36, 29.35, 24.28, 23.36, 20.98, 19.16, 17.26, 13.62. Anal. calcd for $C_{34}H_{36}Cl_2N_2O_4$: C, 67.21; H, 5.97; N, 4.61; found: C, 67.35; H, 5.89; N, 4.58%.

*3-Oxo-4-methyl-4-aza-5-pregnene-20*Z*-oxime-N–O-methyl ether* (**7a**): A mixture of compound **5**(0.11 g, 0.31 mmol) in N,N-Dimethylformamide(DMF, 15mL), containing potassium hydroxide (KOH, 0.1 g, 1.78 mmol) was stirred for 0.5h at room temperature, and then iodomethane (0.1mL) was added dropwise. The mixture was heated to 95–100°C for 12 h, poured into water (15 mL), and extracted with dichloromethane (30 mL×3). The organic layer was washed with water (50 mL×3), dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel eluted with petroleum ether/ethyl acetate to afford compound **7a**; yield 66.4%; m.p. 197–199°C; ESI-MS (*m/z*): 375.4(M+NH_3)$^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.66 (s, 3H, 18-CH_3), 1.10 (s, 3H, 19-CH_3), 1.87 (s, 3H, 21-CH_3), 3.12 (s, 3H, 4-NCH_3), 3.84 (s, 3H, –N–O–CH_3), 5.05 (s, 1H, 6-H). Anal. calcd for $C_{22}H_{34}N_2O_2$: C, 73.70; H, 9.56; N, 7.81; found: C, 73.65; H, 9.49; N, 7.72%.

*3-Oxo-4-substituted-4-aza-5-pregnene-20*Z*-oxime-ether derivatives (***7b–c***); general procedure*

A mixture of compound **5**(0.81 g, 2.40 mmol) in N,N-Dimethylformamide(DMF, 85mL), containing potassium *t*-butoxide (from *t*-BuOH, 0.82 g, 6 mmol) was stirred for 0.5h at room

temperature, and then iodoethane (2.0mL) was added dropwise. The mixture was heated to 100-110°C for 10 h, poured into water (100 mL), and extracted with dichloromethane (100 mL×3). The organic layer was washed with water (100 mL×3), dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The crude was purified by column chromatography on silica gel eluted with petroleum ether/ethyl acetate to afford compounds **7b–c**.

3-Oxo-4-ethyl-4-aza-5-pregnene-20Z-oxime-N–O-ethyl ether (**7b**): Yield 15.2%; m.p. 97–99°C; ESI-MS (*m/z*): 409.0(M+Na)$^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.66 (s, 3H, 18-CH_3), 1.10 (s, 3H, 19-CH_3), 1.86 (s, 3H, 21-CH_3), 3.83–3.91 (m, 2H, 4–N–CH_2), 3.98–4.08 (m, 2H, –N–O–CH_2), 5.03 (s, 1H, 6-H). Anal. calcd for $C_{24}H_{38}N_2O_2$: C, 74.57; H, 9.91; N, 7.25; found: C, 74.66; H, 9.95; N, 7.46%.

3-Oxo-4-aza-5-pregnene-20Z-oxime-N–O-ethyl ether (**7c**): Yield 39.7%; m.p. 113–115°C; ESI-MS (*m/z*): 359.3(M+H)$^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.66 (s, 3H, 18-CH_3), 1.10 (s, 3H, 19-CH_3), 1.87 (s, 3H, 21-CH_3), 4.05–4.10 (m, 2H, –N–O–CH_2), 4.83 (s, 1H, 6-H), 7.54 (s, 1H, –NH). Anal. calcd for $C_{22}H_{34}N_2O_2$: C, 73.70; H, 9.56; N, 7.81; found: C, 73.99; H, 9.59; N, 7.68%.

Bioactivity

All the synthetic new 20-oxime derivatives were subjected to *in vitro* cytotoxic evaluation against T24 (human bladder carcinoma) cell. The anticancer potency of test compounds was measured using the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetazolium) assay. For the test procedure, cells were distributed in the wells of 96-well plates (about 10,000 cells per well). The cell were cultured in RPMI 1640 medium with 10% fetal bovine serum, 100 U penicillin and 100 μg mL^{-1} streptomycin at 37℃ with 5% CO_2 in a humidified incubator. After 48 h of incubation at 37℃ and 5% CO_2 to allow cell attachment, the cells were treated with various concentrations of test samples. Thereafter, 20 μL of MTS solution was added to each well, and incubated for 2.5 h at 37℃. The medium was removed, 150 μL of DMSO per well was added to dissolve the purple formazan crystals formed and plates were gently shaken for 10 min on a mechanical shaker. The optical density of solubilised formazan was measured at 570 nm with an automatic microplate reader. All the data of the experiment were expressed as the IC_{50} (μM) values.

The authors appreciate the financial support from Natural Science Foundation of Hebei Province (B2012208036, B2012208051, B2013208016), XiaoLi Foundation of Hebei University of Science and Technology (YY14).

Received 3 July 2015; accepted 7 August 2015
Paper 1503465 doi: 10.3184/174751915X14401666837860
Published online: 1 September 2015

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