Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Medicinal Chemistry 38 (2003) 1025–1034
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
www.elsevier.com/locate/ejmech

Short communication

Synthesis and antineoplastic activity of 2-alkylaminoethyl derivatives of various steroidal oximes

Dharam Paul Jindal, Raja Chattopadhaya, Sheetal Guleria, Ranju Gupta *

University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh 160014, India

Received 20 June 2003; received in revised form 11 September 2003; accepted 12 September 2003

This paper is dedicated to our esteemed and beloved guide Professor D.P. Jindal

Abstract

Various steroidal oxime ether derivatives in androstene and estrane series have been synthesized and evaluated for the antineoplastic activity at National Cancer Institute, Bethesda, Maryland, USA. *O*-Alkylation of the oximes by various alkylaminoethyl halides gave the oxime ether derivatives. The 17α-ethynylandrostene derivatives **29** (DPJ-684), **30** (DPJ-685), **31** (DPJ-686) and estrane derivatives **35** (DPJ-531) and **36** (DPJ-532) were among the small percentage of compounds, which have been screened by NCI for in vivo hollow fiber assay by virtue of their activity against one or more human tumour cell lines in 60 cell line in vitro prescreen. The preliminary in vivo reports of hollow fiber assays have been referred to the Biological Evaluation Committee for Cancer Drugs for considering these compounds for further detailed in vivo testing.

Keywords: steroidal oximes and oxime ethers; tamoxifen; 6-hydroxiimino derivatives; alkylation; 60 cell line; hollow fiber assay; antineoplastic activity

1. Introduction

Breast cancer is the most common cause of death from cancer in women [1]. Although, overall survival has improved with earlier diagnosis by mammography and with adjuvant therapy, it still represents a leading cause of cancer related deaths [2,3]. There are several approaches for the therapy of breast cancer but the most effective way to treat hormone-dependent breast cancer is to deprive the cancer cells of estrogens by inhibiting their biosynthesis [4]. This is achievable by inhibiting the key enzyme in oestrogen biosynthesis i.e. aromatase cytochrome P_{450} (P_{450} arom) by making use of aromatase inhibitors [5–7]. The commercially available non-steroidal aromatase inhibitors include aminoglutethimide (**1**) [8] and fadrozole (**2**) [9]. Among steroidal aromatase inhibitors, 4-hydroxyandrostenedione (4-OHA, Formestane) (**3**) [10] has been approved for clinical use in the treatment of breast cancer in several countries. Oximino derivatives, 6-hydroxiimino-4-en-3-ones (**4**) and (**5**) also show a high affinity for human placental aromatase, and function as competitive inhibitors of this enzyme [11].

Selective oestrogen receptor modulators (SERMs) such as tamoxifen (**6**), which act through the oestrogen receptor (ER), is the most widely used antioestrogen for the management of breast cancer [12]. However, prolonged treatment causes endometrial cancer [13]. It has been reported that the combination therapy of tamoxifen with aromatase inhibitors in the treatment of advanced breast cancer leads to interactions and affects antitumour efficacy [14,15] (Fig. 1).

The reported aromatase inhibitory activity of oximes (**4**, **5**) and the importance of 2-alkylaminoethyl side chain of tamoxifen (**6**) and other SERMs [12] with ER modulating activity prompted us to prepare alkylated oxime ethers. Consequently, we embarked on the synthesis of a number of substituted steroidal oximes. Of these **29** (DPJ-684), **30** (DPJ-685), **31** (DPJ-686), **35** (DPJ-531) and **36** (DPJ-532) came out with significant

* Corresponding author.
E-mail address: ranju29in@yahoo.co.in (R. Gupta).

0223-5234/03/$ - see front matter
doi:10.1016/j.ejmech.2003.09.002

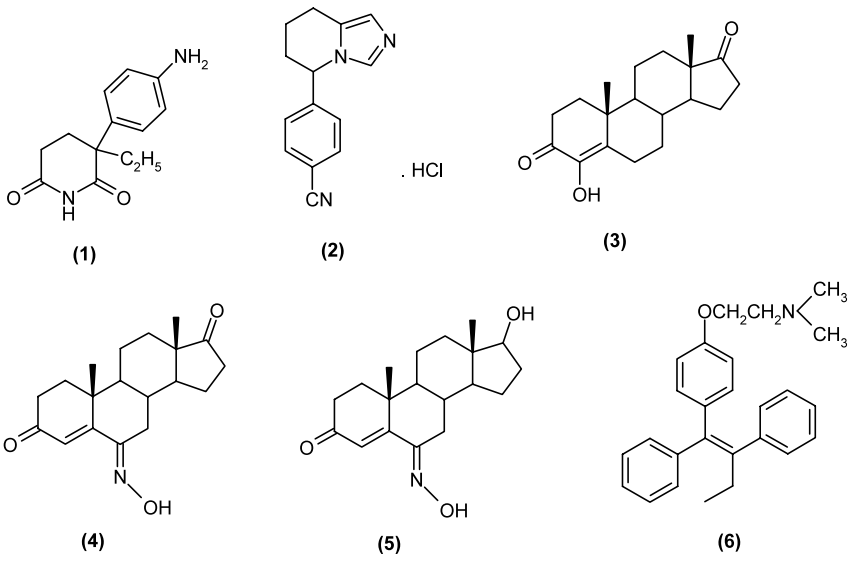

Fig. 1. Structures of compounds **1**–**6**. (**1**) Aminoglutethimide, (**2**) Fadrozole, (**3**) Formestane, (**4**, **5**) 6-Hydroxiimino-4-en-3-ones, (**6**) Tamoxifen.

activity in the preliminary in vivo hollow fiber assay, which we report herein.

2. Chemistry

Alkylaminoethyl derivatives of various steroidal oximes have been synthesized according to Figs. 2–6. For the synthesis of 17-oximino steroidal derivatives, dehydroepiandrosterone acetate (**7**) was subjected to oximation using hydroxylamine hydrochloride–sodium acetate in aldehyde-free ethanol. The reaction mixture was processed to obtain the oxime **8** [16], which was condensed with hydrochlorides of 2-dimethylaminoethyl chlorides, 2-diethylaminoethyl chloride, 2-pyrrolidinoethyl chloride and 2-piperidinoethyl chloride in dry ethyl methyl ketone containing anhydrous potassium carbonate–sodium hydroxide at refluxing temperature to obtain the desired steroidal oximino derivatives **9**–**12**, respectively (Fig. 2). The ^{1}H-NMR spectrum exhibited signals at δ 2.28 (s, 6H, –N(CH_3)$_2$) for **9** and 2.57 (q, 4H, –N(CH_2CH_3)$_2$) for compound **10**. *N*-Methylenes of pyrrolidino and piperidino functionality appeared in ^{1}H-NMR spectrum as multiplet at δ 2.58 and 2.46 for compounds **11** and **12**, respectively. Triplets for –CH_2N< and –OCH_2 appeared in the ^{1}H-NMR spectra of all four compounds.

Testosterone acetate **13** was used as the starting material for the preparation of 3-[*O*-(2-alkylaminoethyl)]oximino compounds. The oxime **14** was prepared by heating **13** and hydroxylamine hydrochloride in pyridine

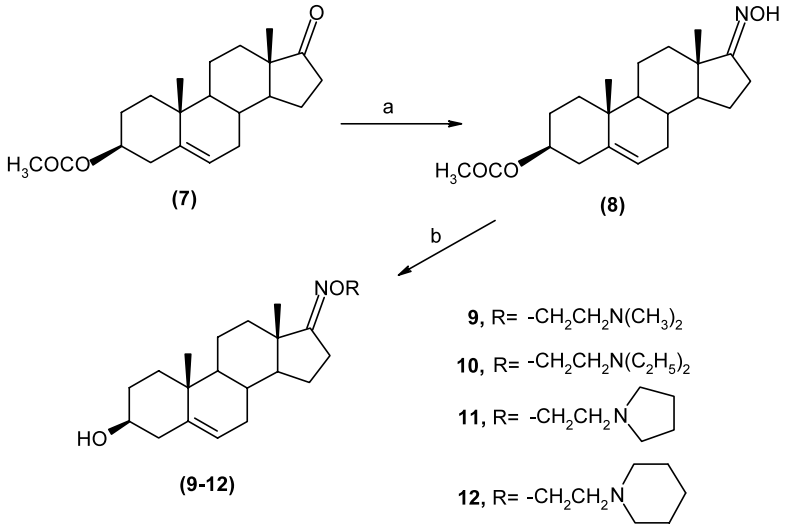

Fig. 2. Synthetic routes to compounds **8**–**12**. Reagents and conditions: (a) hydroxylamine hydrochloride/sodium acetate, aldehyde free alcohol, reflux; (b) dry ethyl methyl ketone/anhydrous potassium carbonate/sodium hydroxide/R-Cl.HCl, reflux.

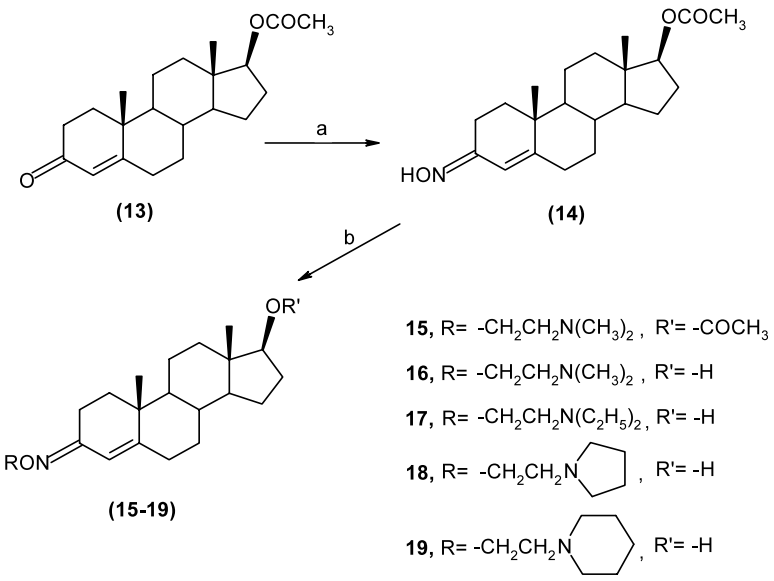

Fig. 3. Synthetic routes to compounds **14**–**19**. Reagents and conditions: (a) hydroxylamine hydrochloride/pyridine (moist), heat; (b) dry ethyl methyl ketone/anhydrous potassium carbonate/potassium hydroxide/R-Cl.HCl, reflux.

on steam bath as reported earlier [17]. Condensation of hydrochlorides of 2-dimethylaminoethyl chloride, 2-diethylaminoethyl chloride, 2-pyrrolidinoethyl chloride and 2-piperidinoethyl chloride with **14** was carried out in dry ethyl methyl ketone containing anhydrous potassium carbonate–sodium hydroxide to afford **16**–**19**, respectively (Fig. 3). During the preparation of 2-dimethylaminoethyl derivative **16**, we were also able to isolate the 17-acetoxy derivative **15** by fractional crystallization from solvent ether–hexane. The mother liquor, which was left after removing **16**, was concentrated to give an oily residue **15**, which was characterized as an oxalate salt. ^{1}H-NMR signal δ 2.91 (d, $-N^+H(CH_3)_2$] confirmed the formation of oxalate salt. The peak for the acetoxy function was absent in the IR spectrum of **16**. The ^{1}H-NMR spectrum of 2-diethylaminoethoxy derivative **17** exhibited signal at δ 2.59 [$-N(CH_2CH_3)_2$]. Oxalate salts of 2-pyrrolidinoethoxy derivative **18** and 2-piperidinoethoxy derivative **19** were also prepared and their structures were confirmed using various spectroscopical techniques.

The conversion of 17α-methyl testosterone **20** to its 3-oxime **21** [17] was accomplished by employing hydroxylamine hydrochloride in moist pyridine. 17α-Methyl-3-oximino-4-androsten-17β-ol **21** underwent a clean substitution with respective hydrochlorides of alkylaminoethyl chlorides to give the oxime ethers **22**–**25** (Fig. 4). There appeared triplets for $-CH_2N<$ and $-OCH_2-$

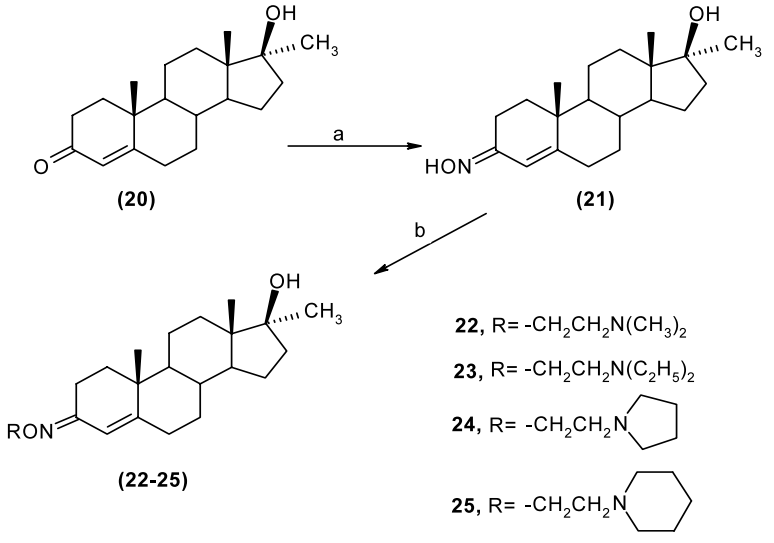

Fig. 4. Synthetic routes to compounds **21**–**25**. Reagents and conditions: (a) hydroxylamine hydrochloride/pyridine (moist), heat; (b) dry ethyl methyl ketone/anhydrous potassium carbonate/potassium hydroxide/R-Cl.HCl, reflux.

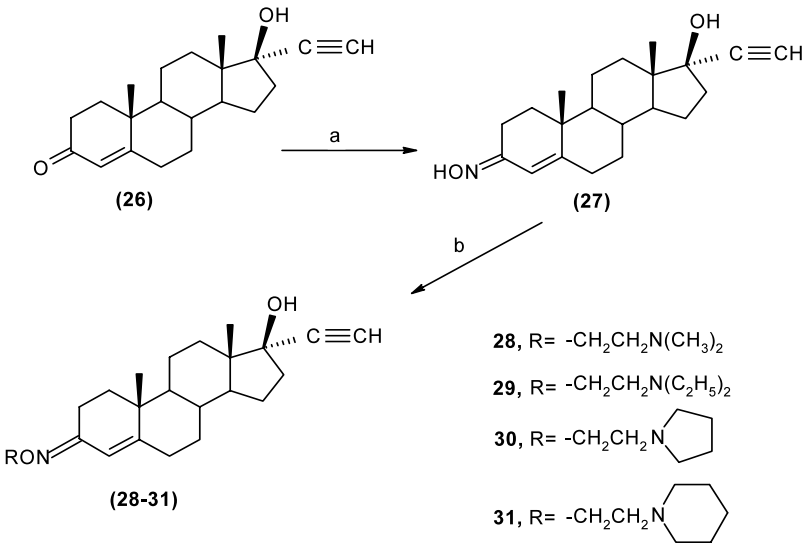

Fig. 5. Synthetic routes to compounds **27**–**31**. Reagents and conditions: (a) hydroxylamine hydrochloride/pyridine (moist), heat; (b) dry ethyl methyl ketone/anhydrous potassium carbonate/potassium iodide/R-Cl.HCl, reflux.

in the ^{1}H-NMR spectra of all the four compounds **22**–**25**.

Ethisterone **26** was subjected to oximation at the 3 position by using the known procedure [17] to obtain the oxime **27**. Similar treatment of oxime **27** with respective hydrochlorides of alkylaminoethyl chlorides in the presence of a catalytic amount of potassium iodide led to the formation of different 3-substituted alkoximino derivatives **28**–**31** (Fig. 5). The compound **28** showed ^{1}H-NMR signals at δ 2.29 [s, 6H, –N(CH_3)$_2$]. *N*-Methylenes of the compounds **29**–**31** appeared at their respective positions in the NMR spectra.

Finally, the above reaction sequence was applied for the synthesis of alkylated oximino analogues of estrone methyl ether. The 17-oximino derivative **33** of 3-methoxy-1,3,5(10)-estratrien-17-one (estrone methyl ether, **32**), was prepared in refluxing aldehyde-free ethanol as reported earlier [18]. Condensation of the hydrochlorides of different alkylaminoethyl chlorides with **33** in refluxing dry ethyl methyl ketone and in the presence of catalytic amount of sodium hydroxide gave respective 17-substituted alkylaminoethoximino derivatives **34**–**37** (Fig. 6). Subsequent purification and work-up yielded oily residues and attempts to crystallize proved unsuccessful. Hydrochloride salts were prepared

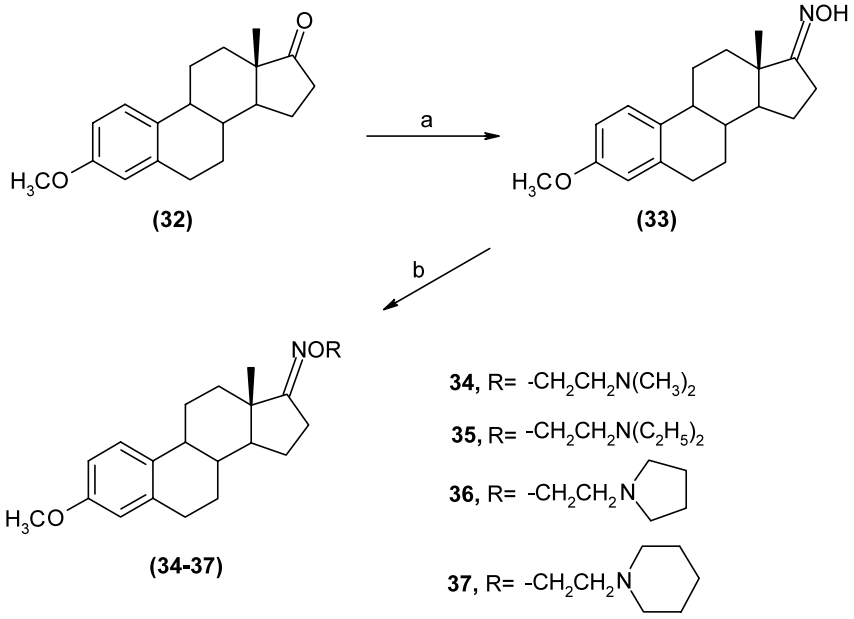

Fig. 6. Synthetic routes to compounds **33**–**37**. Reagents and conditions: (a) hydroxylamine hydrochloride/sodium acetate, aldehyde free alcohol, reflux; (b) dry ethyl methyl ketone/anhydrous potassium carbonate/sodium hydroxide/R-Cl.HCl, reflux.

by passing dry hydrochloric acid gas through the solutions of oily residues **34**–**37** in dry acetone. The structures of all the compounds were confirmed by various spectral and elemental analyses.

The stereochemistry of the oximino functionality for all the derivatives has been assigned *anti* (*E*) on the basis of ^{1}H-NMR spectroscopy as described earlier [17].

3. Pharmacology

Out of the numerous synthesized steroidal oximino derivatives, 15 compounds were selected for screening by Drug Synthesis and Chemistry Branch, National Cancer Institute, Bethesda, Maryland, USA based, in general, on the degree of novelty of the structure and computer modelling techniques. The compounds were tested for in vitro antineoplastic activity against the cell panel consisting of 60 cell lines at a minimum of five concentrations at 10-fold dilutions [19,20]. The compounds **29** (DPJ-684), **30** (DPJ-685), **31** (DPJ-686), **35** (DPJ-531) and **36** (DPJ-532) were found to be active in the in vitro assay by virtue of their activity against one or more human tumour cell lines and were further screened for preliminary in vivo hollow fiber assay [21].

4. Results and discussion

Out of the 15 selected steroidal oximino derivatives, ethynyl derivatives **29** (DPJ-684), **30** (DPJ-685), **31** (DPJ-686) and estrone methyl ether derivatives **35** (DPJ-531) and **36** (DPJ-532) have exhibited significant results and were further selected as leads by virtue of their activity from the large scale in vitro 60 cell line screen. Mean log dose response parameters such as GI50 (drug concentration resulting in a 50% reduction in the net protein increase), TGI (drug concentration of total growth inhibition) and LC50 (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) are summarized in Table 1. These compounds were then screened for preliminary in vivo testing using hollow fiber assay and have shown interesting IP and SC scores. Data summarizing the in vivo hollow fiber assay performed by the Developmental Therapeutic Program on the compounds is shown in Table 2. In addition, these oximino derivatives lie outside the category of adequately studied class of anti-tumour agents and are among the small percentage, which have been selected for further testing in the in vivo hollow fiber assays. Therefore, these results have been referred to Biological Evaluation Committee for Cancer Drugs to select these compounds for further detailed in vivo testing.

17α-Ethynyl derivatives have exhibited significant anticancer activity for further studies in contrast to the compounds with 17β-acetoxy or 17α-methyl substituents in androstene series. Oxime ether derivatives in estrone methyl ether series have also shown appreciable antineoplastic activity. This can be attributed to its binding with ERs because of aromatic A ring. Data further highlight the importance of oximino functionality for the development of leads as antineoplastic agents. Combining the alkylaminoethyl side chain with oxime seems to be a right step for the development of potent antineoplastic agents for the treatment of breast cancer.

5. Experimental

5.1. Chemistry

The m.p.s reported are uncorrected, ^{1}H-NMR spectra were recorded on Bruker AC-300F, 300 MHz instrument using Me_4Si (TMS) as the internal standard (chemical shifts in δ, ppm). The IR and UV spectra were recorded on Perkin–Elmer 882 and Lambda 15 spectrophotometer models, respectively. The purity of the compounds was established by thin layer chromatography and elemental analyses (C, H, N). Elemental analyses were carried out on a Perkin–Elmer 2400. Mass spectra were recorded on a V6-11-250J70 S and CEC-21-110B Finnigan Mat 1210 or MICRO MASS 7070 at 70 eV using a direct inlet system. Ultraviolet spectra were recorded in methanol (λ_{max} in nm). IR spectra were obtained with potassium bromide pellets (ν_{max} in cm^{-1}). Plates for TLC were prepared according to Stahl (E. Merck) using EtOAc as solvent (activated at

Table 1
Reports of in vitro 60 cell line screen

Compound	NSC No.	Mean Log_{10} GI50 (molar)	Mean Log_{10} TGI (molar)	Mean Log_{10} LC50 (molar)
29 (DPJ-684)	701573	−5.35	−4.77	−4.23
30 (DPJ-685)	701574	−4.99	−4.55	−4.13
31 (DPJ-686)	701575	−5.41	−4.85	−4.34
35 (DPJ-531)	680094	−5.83	−5.36	−4.77
36 (DPJ-532)	680095	−5.66	−5.21	−4.76

Table 2
Data summarizing the preliminary in vivo hollow fiber assay reports

S. No.	Compound	NSC NO.	IP Score	SC Score	Total score	Cell kill
1.	**29** (DPJ-684)	701573	0	4	4	No
2.	**30** (DPJ-685)	701574	4	0	4	No
3.	**31** (DPJ-686)	701575	4	2	6	No
4.	**35** (DPJ-531)	680094	0	4	4	No
5.	**36** (DPJ-532)	680095	2	4	6	No

110 °C for 30 min) and were visualized by exposure to iodine vapours. Anhydrous sodium sulphate was used as a drying agent. All solvents were dried and freshly distilled prior to use according to standard procedures.

*5.1.1. General procedure for the synthesis of 17E-[O-(2-alkylaminoethyl)]oximino-5- androstene derivatives **9**–**12***

To a solution of 17*E*-oximino-5-androsten-3β-yl acetate **8** [16] (1.0 g, 2.9 mmol) in dry C_4H_8O (100 mL), anhydrous K_2CO_3 (4.0 g, 28.9 mmol) was added. The reaction mixture was refluxed with stirring for 1 h. Respective hydrochlorides of alkylaminoethyl chlorides (10.0 mmol) and NaOH (0.8 g, 20 mmol) were then added and it was further refluxed for 18 h with stirring. The completion of the reaction was determined by TLC. The slurry was filtered and the solvent was removed under reduced pressure to obtain the oily residue. Distilled water was added to the oily residue and allowed to stand overnight. The solid residue obtained was washed with distilled water, dried and crystallized to give the alkylaminoethyloximino derivatives **9**–**12**.

*5.1.1.1. 17E-[O-(2-Dimethylaminoethyl)]oximino-5-androsten-3β-ol (**9**).* Yield: 46.12%, m.p.: 135–137 °C (from $C_4H_{10}O$) (lit. [22] 119–120 °C, from hexane); UV_{max}: 219.8 nm (log ε 4.61); IR: 3272, 2910, 2810, 1472, 1450, 1350, 1245, 1180, 1070, 1030; ^{1}H-NMR ($CDCl_3$): δ 0.93 (s, 3H, 18-C*H*$_3$), 1.03 (s, 3H, 19-C*H*$_3$), 2.28 [s, 6H, –N(C*H*$_3$)$_2$], 2.57 (t, 2H, *J* = 6 Hz, –OCH$_2$C*H*$_2$N<), 3.51 (m, 1H, 3α-C*H*), 4.13 (t, 2H, *J* = 6 Hz, –OC*H*$_2$–), 5.35 (m, 1H, 6-C*H*); MS: *m*/*z* 374 [M$^+$]. Anal. Calc. for $C_{23}H_{38}N_2O_2$: C, 73.75; H, 10.23; N, 7.48. Found: C, 73.72; H, 10.51; N, 7.28%.

*5.1.1.2. 17E-[O-(2-Diethylaminoethyl)]oximino-5-androsten-3β-ol (**10**).* Yield: 45.90%, m.p.: 96–98 °C (from $C_4H_{10}O$); UV_{max}: 219.8 nm (log ε 4.80); IR: 3580, 3400, 2900, 1480, 1430, 1360, 1045, 907; ^{1}H-NMR ($CDCl_3$): δ 0.92 (s, 3H, 18-C*H*$_3$), 1.03 [t, 6H, *J* = 7 Hz, –N(CH$_2$C*H*$_3$)$_2$, and s, 3H, 19-C*H*$_3$ (merged)], 2.57 [q, 4H, *J* = 7 Hz, –N(C*H*$_2$CH$_3$)$_2$], 2.73 (t, 2H, *J* = 6 Hz, –OCH$_2$C*H*$_2$N<), 3.52 (m, 1H, 3α-C*H*), 4.11 (t, 2H, *J* = 6 Hz, –OC*H*$_2$–), 5.36 (m, 1H, 6-C*H*); MS: *m*/*z* 402 [M$^+$]. Anal. Calc. for $C_{25}H_{42}N_2O_2$: C, 74.58; H, 10.51; N, 6.96. Found: C, 74.28; H, 10.79; N, 6.95%.

*5.1.1.3. 17E-[O-(2-Pyrrolidinoethyl)]oximino-5-androsten-3β-ol (**11**).* Yield: 34.50%, m.p.: 140–142 °C (from $C_4H_{10}O$); UV_{max}: 220 nm (log ε 4.51); IR: 3140, 2923, 2822, 1479, 1468, 1379, 1130, 873; ^{1}H-NMR ($CDCl_3$): δ 0.92 (s, 3H, 18-C*H*$_3$), 1.03 (s, 3H, 19-C*H*$_3$), 2.58 (m, 4H, *N*-methylenes of pyrrolidino functionality), 2.74 (t, 2H, *J* = 6 Hz, –OCH$_2$C*H*$_2$N<), 3.52 (m, 1H, 3α-C*H*), 4.14 (t, 2H, *J* = 6 Hz, –OC*H*$_2$–), 5.35 (m, 1H, 6-C*H*); MS: *m*/*z* 400 [M$^+$]. Anal. Calc. for $C_{25}H_{40}N_2O_2$: C, 74.95; H, 10.07; N, 6.99. Found: C, 74.93; H, 10.10; N, 6.93%.

*5.1.1.4. 17E-[O-(2-Piperidinoethyl)]oximino-5-androsten-3β-ol (**12**).* Yield: 54.17%, m.p.: 157–159 °C (from $C_4H_{10}O$); UV_{max}: 219.2 nm (log ε 4.84); IR: 3168, 2920, 2836, 1480, 1440, 1308, 1043, 991; ^{1}H-NMR ($CDCl_3$): δ 0.91 (s, 3H, 18-C*H*$_3$), 1.03 (s, 3H, 19-C*H*$_3$), 2.46 (m, 4H, *N*-methylenes of piperidino functionality), 2.62 (t, 2H, *J* = 6 Hz, –OCH$_2$C*H*$_2$N<), 3.51 (m, 1H, 3α-C*H*), 4.16 (t, 2H, *J* = 6 Hz, –OC*H*$_2$–), 5.35 (m, 1H, 6-C*H*); MS: *m*/*z* 414 [M$^+$]. Anal. Calc. for $C_{26}H_{42}N_2O_2$: C, 75.31; H, 10.21; N, 6.76. Found: C, 75.21; H, 9.81; N, 6.44%.

*5.1.2. General procedure for the synthesis of 3E-[O-(2-alkylaminoethyl)]oximino-4-androstene derivatives **15**–**19***

A slurry of 3*E*-oximino-4-androsten-17β-yl acetate **14** [17] (1.0 g, 2.9 mmol) in dry C_4H_8O (100 mL) and anhydrous K_2CO_3 (4.0 g, 28.9 mmol) was added and refluxed for 2 h. Respective hydrochlorides of alkylaminoethyl chlorides (10.0 mmol) and KOH (0.8 g, 14.3 mmol) were added and the reaction mixture was further refluxed for 48 h with stirring. The completion of the reaction was determined by TLC. The slurry was filtered, the solvent was removed under reduced pressure, distilled water was added and allowed to stand at low temperature overnight. The solid residue obtained was filtered, washed with distilled water, dried and crystallized to give the products **15**–**19**. Compounds **15**, **18** and **19** could not be crystallized and were characterized as their oxalate salts.

*5.1.2.1. 3E-[O-(2-Dimethylaminoethyl)]oximino-4-androsten-17β-yl acetate (**15**) oxalate.* Yield: 34.78%,

m.p.: 122–125 °C (from dry C_3H_6O); UV_{max}: 248.2 nm (log ε 4.35); IR: 3455, 2944, 2711, 1734, 1619,1471, 1242, 879; ^{1}H-NMR ($CDCl_3$): δ 0.82 (s, 3H, 18-CH_3), 1.06 (s, 3H, 19- CH_3); 2.04 (s, 3H, 17β-OCOCH_3), 2.91 [d, 6H, $J = 2$ Hz, –N(CH_3)$_2$], 3.46 (br, 2H, –OCH$_2$CH_2N<), 4.40 (br, 2H, –OCH_2–), 4.58 (t, 1H, $J = 9$ Hz, 17α-CH) 5.76 (s, 1H, 4-CH). Anal. Calc. for $C_{27}H_{42}N_2O_7$: C, 64.01; H, 8.36; N, 5.53. Found: C, 64.24; H, 8.00; N, 5.37%.

*5.1.2.2. 3E-[O-(2-Dimethylaminoethyl)]oximino-4-androsten-17β-ol (**16**).* Yield: 38.37%, m.p.: 145–146 °C (from $C_4H_{10}O$–hexane); UV_{max}: 248.8 nm (log ε 4.39); IR: 3222, 2941, 2881, 1538, 1370, 1297, 1059, 878; ^{1}H-NMR ($CDCl_3$): δ 0.77 (s, 3H, 18-CH_3), 1.05 (s, 3H, 19-CH_3): 2.29 [s, 6H, –N(CH_3)$_2$], 2.62 (t, 2H, $J = 5$ Hz, –OCH$_2$CH_2N<), 3.63 (t, 1H, $J = 4$ Hz, 17α-CH), 4.13(t, 2H, $J = 5$ Hz, –OCH_2–), 5.77 (s, 1H, 4-CH); MS: *m/z* 375 [M$^+$]. Anal. Calc. for $C_{23}H_{38}N_2O_2$: C, 73.75; H, 10.23; N, 7.48. Found: C, 73.73; H, 10.47; N, 7.30%.

*5.1.2.3. 3E-[O-(2-Diethylaminoethyl)]oximino-4-androsten-17β-ol (**17**).* Yield: 41.70%, m.p.: 102–104 °C (from $C_4H_{10}O$); UV_{max}: 248.8 nm (log ε 4.59); IR: 3257, 2969, 2837, 1631, 1292, 874; ^{1}H-NMR ($CDCl_3$): δ 0.78 (s, 3H, 18-CH_3), 1.04 [t, 6H, $J = 7$ Hz, –N(CH$_2$CH_3)$_2$], 1.06 (s, 3H, 19-CH_3), 2.59 [q, 4H, $J = 7$ Hz, –N(CH_2CH$_3$)$_2$], 2.77 (t, 2H, $J = 6$ Hz, –OCH$_2$CH_2N<),), 3.64 (t, 1H, $J = 8$ Hz, 17α-CH), 4.13 (t, 2H, $J = 6$ Hz, –OCH_2–), 5.77 (s, 1H, 4-CH); MS: *m/z* 401 [(M − H)$^+$]. Anal. Calc. for $C_{25}H_{42}N_2O_2$: C, 74.58; H, 10.52; N, 6.96. Found: C, 74.35; H, 10.65; N, 6.75%.

*5.1.2.4. 3E-[O-(2-Pyrrolidinoethyl)]oximino-4-androsten-17β-ol (**18**) oxalate.* Yield: 23.94%, m.p.: 146–148 °C (from dry C_3H_6O); UV_{max}: 248.2 nm (log ε 4.4); IR: 3385, 2948, 1717, 1624, 1278, 878, ^{1}H-NMR ($CDCl_3$): δ 0.82 (s, 3H, 18-CH_3), 1.06 (s, 3H, 19-CH_3); 2.89 (m, 4H, *N*-methylenes of pyrrolidino functionality), 3.46 (t, 2H, $J = 8$ Hz, –OCH$_2$CH_2N<), 3.64 (t, 1H, $J = 8$ Hz, 17α-CH), 4.36 (t, 2H, $J = 8$ Hz, –OCH_2–) 5.72 (s, 1H, 4-CH): Anal. Calc. for $C_{27}H_{42}N_2O_6$: C, 66.09; H, 8.63; N, 5.71. Found: C, 66.14; H, 8.89; N, 5.33%.

*5.1.2.5. 3E-[O-(2-Piperidinoethyl)]oximino-4-androsten-17β-ol (**19**) oxalate.* Yield: 16.43%, m.p.: 158–160 °C (from dry C_3H_6O); UV_{max}: 248.2 nm (log ε 4.4); IR: 3445, 2938, 2661, 1724, 1615, 1281, 960; ^{1}H-NMR ($CDCl_3$): δ 0.79 (s, 3H, 18-CH_3), 1.06 (s, 3H, 19-CH_3), 2.89 (m, 4H, *N*-methylenes of piperidino functionality), 3.38 (m, 2H, –OCH$_2$CH_2N<), 3.63 (t, 1H, $J = 8$ Hz, 17α-CH), 4.37 (m, 2H, –OCH_2–), 5.72 (s, 1H, 4-CH): Anal. Calc. for $C_{28}H_{44}N_2O_6$: C, 66.64; H, 8.79; N, 5.55. Found: C, 66.55; H, 8.92; N, 5.82%.

*5.1.3. General procedure for the synthesis of 17α-methyl-3E-[O-(2-alkylaminoethyl)]oximino-4-androstene derivatives **22**–**25***

To a solution of 17α-methyl-3*E*-oximino-4-androsten-17β-ol (**21**) [17] (1.0 g, 3.1 mmol) in dry C_4H_8O (100 mL), anhydrous K_2CO_3 (4.0 g, 28.9 mmol) was added and refluxed for 2 h. Then, respective hydrochlorides of alkylaminoethyl chlorides (10.0 mmol) and KOH (0.8 g, 14.3 mmol) were added and the reaction mixture was refluxed for 48 h with stirring. The completion of the reaction was determined by TLC. The slurry was filtered, the solvent was removed under reduced pressure, distilled water added and allowed to stand at low temperature overnight. The solid residue so obtained was washed with distilled water, dried and crystallized to afford **22**–**25**.

*5.1.3.1. 17α-Methyl-3E-[O-(2-dimethylaminoethyl)]-oximino-4-androsten-17β-ol (**22**).* Yield: 45.75%, m.p.: 143–145 °C (from $C_4H_{10}O$); UV_{max}: 249.2 nm (log ε 4.42); IR: 3206, 2936, 1629, 1465,1296, 1038, 968; ^{1}H-NMR ($CDCl_3$): δ 0.89 (s, 3H, 18-CH_3), 1.06 (s, 3H, 19-CH_3), 1.20 (s, 3H, 17α-CH_3), 2.29 (s, 6H, –N(CH_3)$_2$), 2.61 (t, 2H, $J = 6$ Hz, –OCH$_2$CH_2N<), 4.15 (t, 2H, $J = 6$ Hz,–OCH_2–), 5.78 (s, 1H, 4-CH); MS: *m/z* 388 [M$^+$]. Anal. Calc. for $C_{24}H_{40}N_2O_2$: C, 74.18; H, 10.38; N, 7.21. Found: C, 74.12; H, 10.25; N, 7.29%.

*5.1.3.2. 17α-Methyl-3E-[O-(2-diethylaminoethyl)]-oximino-4-androsten-17β-ol (**23**).* Yield: 36.30%, m.p.: 100–102 °C (from hexane); UV_{max}: 249.3 nm (log ε 4.30); IR: 3473, 2937, 1623, 1442, 1295, 1062; ^{1}H-NMR ($CDCl_3$): δ 0.89 (s, 3H, 18-CH_3), 1.04 (t, 6H, $J = 7$Hz, –N(CH$_2$CH_3)$_2$ and s, 3H, 19-CH_3 (merged)], 1.20 (s, 3H, 17α-CH_3), 2.59 [q, 4H, $J = 7$ Hz, –N(CH_2CH$_3$) $_2$], 2.76 (t, 2H, $J = 6$ Hz, –OCH$_2$CH_2N<),), 4.14 (t, 2H, $J = 6$ Hz, –OCH_2–), 5.77 (s, 1H, 4-CH); MS: *m/z* 416 [M$^+$]. Anal. Calc. for $C_{26}H_{44}N_2O_2$: C, 74.95; H, 10.64; N, 6.73. Found: C, 75.16; H, 10.48; N, 6.68%.

*5.1.3.3. 17α-Methyl-3E-[O-(2-pyrrolidinoethyl)]-oximino-4-androsten-17β-ol (**24**).* Yield: 42.46%, m.p.: 136–138 °C (from $C_4H_{10}O$–hexane); UV_{max}: 249.0 nm (log ε 4.20); IR:3222, 2940, 2880, 1637, 1438, 1297, 1060, 878; ^{1}H-NMR ($CDCl_3$): δ 0.89 (s, 3H, 18-CH_3), 1.06 (s, 3H, 19-CH_3), 1.20 (s, 3H, 17α-CH_3), 2.57 (m, 4H, *N*-Methylenes of pyrrolidino functionality), 2.78 (t, 2H, $J = 6$ Hz, –OCH$_2$CH_2N<), 4.19 (t, 2H, $J = 6$ Hz, –OCH_2), 5.77 (s, 1H, 4-CH); MS: *m/z* 414 [M$^+$]. Anal. Calc. for $C_{26}H_{42}N_2O_2$: C, 75.31; H, 10.21; N, 6.76. Found: C, 75.27; H, 10.35; N, 6.85%.

*5.1.3.4. 17α-Methyl-3E-[O-(2-piperidinoethyl)]-oximino-4-androsten-17β-ol (**25**).* Yield: 25.54%, m.p.: 134–136 °C (from $C_4H_{10}O$); UV_{max}: 249.4 nm (log ε

4.30); IR: 3547, 2929, 1628, 1447, 1295, 1061, 852; ^{1}H-NMR ($CDCl_3$): δ 0.89 (s, 3H, 18-CH_3), 1.06 (s, 3H, 19-CH_3), 1.21 (s, 3H, 17α-CH_3), 2.47 (m, 4H, *N*-methylenes of piperidino functionality), 2.66 (t, 2H, $J = 6$ Hz, –$OCH_2CH_2N<$), 4.18 (t, 2H, $J = 6$ Hz, –OCH_2–), 5.77 (s, 1H, 4-C*H*); MS: *m*/*z* 429 [M^+]. Anal. Calc. for $C_{27}H_{44}N_2O_2$: C, 75.65; H, 10.35; N, 6.54. Found: C, 75.30; H, 10.48; N, 6.48%.

5.1.4. General procedure for the synthesis of 17α-ethynyl-3E-[O-(2-alkylaminoethyl)]oximino-4-androstene derivatives **28**–**31**

To a solution of 17α-ethynyl-3*E*-oximino-4-androsten-17β-ol (**27**) (1.0 g, 3.1 mmol) in dry C_4H_8O (100 mL), anhydrous K_2CO_3 (4.0 g, 28.9 mmol) was added and refluxed for 2 h. Respective hydrochlorides of alkylaminoethyl chlorides (10.0 mmol) and KI (0.015 g, 0.09 mmol) were added and the reaction mixture was further refluxed for 48 h with stirring. The completion of the reaction was determined by TLC. The slurry was filtered, the solvent was removed under reduced pressure, distilled water was added and allowed to stand at low temperature overnight. The solid residue so obtained was washed with distilled water, dried and crystallized to give compounds **28**–**31**.

5.1.4.1. 17α-Ethynyl-3E-[O-(2-dimethylaminoethyl)]-oximino-4-androsten-17β-ol (**28**).

Yield: 24.65%, m.p.: 200–203 °C (from $C_4H_{10}O$–C_3H_6O); UV_{max}: 247.6 nm (log ε 4.10); IR: 3241, 2946, 1633, 1459, 1026, 967, 876; ^{1}H-NMR ($CDCl_3$): δ 0.89 (s, 3H, 18-CH_3), 1.06 (s, 3H, 19-CH_3], 2.29 [s, 6H, –$N(CH_3)_2$], 2.56 (s, 1H, 17α-C≡C*H*), 2.62 (t, 2H, $J = 6$ Hz, –$OCH_2CH_2N<$), 4.16 (t, 2H, $J = 6$ Hz, –OCH_2–) 5.77 (s, 1H, 4-C*H*); MS: *m*/*z* 399 [M^+]. Anal. Calc. for $C_{25}H_{38}N_2O_2$: C, 75.33; H, 9.61; N, 7.03. Found: C, 75.19; H, 9.57; N, 7.11%.

5.1.4.2. 17α-Ethynyl-3E-[O-(2-diethylaminoethyl)]-oximino-4-androsten-17β-ol (**29**).

Yield: 85.50%, m.p.: 120–122 °C (from $C_4H_{10}O$–hexane); UV_{max}: 248.4 nm (log ε 4.53); IR: 3260, 2925, 1620, 1436, 1050, 985; ^{1}H-NMR ($CDCl_3$): δ 0.88 (s, 3H, 18-CH_3), 1.06 [t, 6H, $J =$ 7 Hz, –$N(CH_2CH_3)_2$ and s, 3H, 19-CH_3 (merged)], 2.59 [m, 5H, –$N(CH_2CH_3)_2$ and 17α-C≡C*H*, (merged)], 2.77 (t, 2H, $J = 6$ Hz, –$OCH_2CH_2N<$), 4.15 (t, 2H, $J = 6$ Hz, –OCH_2–), 5.77 (s, 1H, 4-C*H*); MS: *m*/*z* 426 [M^+]. Anal. Calc. for $C_{27}H_{42}N_2O_2$: C, 76.01; H, 9.92; N, 6.57. Found: C, 76.06; H, 10.08; N, 6.51%.

5.1.4.3. 17α-Ethynyl-3E-[O-(2-pyrrolidinoethyl)]-oximino-4-androsten-17β-ol (**30**).

Yield: 80.20%, m.p.: 129–132 °C (from $C_4H_{10}O$); UV_{max}: 247.2 nm (log ε 4.52); IR: 3310, 3285, 2950, 1630, 1492, 1396, 1065; ^{1}H-NMR ($CDCl_3$): δ 0.89 (s, 3H, 18-CH_3), 1.08 (s, 3H, 19-CH_3), 2.57 (t, 5H, $J = 4$ Hz, 17α-C≡C*H*, *N*-methylenes of pyrrolidino functionality), 2.79 (t, 2H, $J = 5$ Hz, –$OCH_2CH_2N<$), 4.2 (t, 2H, $J = 5$ Hz, –OCH_2–) 5.75 (s, 1H, 4-C*H*); MS: *m*/*z* 425 [M^+]. Anal. Calc. for $C_{27}H_{40}N_2O_2$: C, 76.37; H, 9.50; N, 6.60. Found: C, 76.46; H, 9.27; N, 6.75%.

5.1.4.4. 17α-Ethynyl-3E-[O-(2-piperidinoethyl)]-oximino-4-androsten-17β-ol (**31**).

Yield: 97.05%, m.p.: 118–122 °C (from $C_4H_{10}O$); UV_{max}: 242.4 nm (log ε 4.5); IR: 3420, 3250, 2945, 1650, 1436, 1392, 1035, 994; ^{1}H-NMR ($CDCl_3$): δ 0.88 (s, 3H, 18-CH_3), 1.06 (s, 3H, 19-CH_3), 2.46 (m, 4H, *N*-methylenes of piperidino functionality), 2.56 (s, 1H, 17α-C≡C*H*), 2.65 (t, 2H, $J = 6$ Hz, –$OCH_2CH_2N<$), 4.18 (t, 1H, $J = 6$ Hz, –OCH_2–), 5.76 (s, 1H, 4-C*H*); MS: *m*/*z* 438 [M^+]. Anal. Calc. for $C_{28}H_{42}N_2O_2$: C, 76.66; H, 9.65; N, 6.39. Found: C, 76.62; H, 10.01; N, 6.47%.

5.1.5. General procedure for the synthesis of 17E-[O-(2–alkylaminoethyl)]oximino-3-methoxy -1,3,5-(10)-estratriene derivatives **34**–**37**

To a solution of 3-methoxy-17*E*-oximino-1,3,5-(10)-estratriene **33** [18] (1.0 g, 3.18 mmol) in dry C_4H_8O (100 mL), anhydrous K_2CO_3 (4.0 g, 28.9 mmol) was added and refluxed for 1 h. Then, respective hydrochlorides of alkylaminoethyl chlorides (10.0 mmol) and NaOH in the catalytic amount were added and the reaction mixture was refluxed for 22 h with stirring. The completion of the reaction was determined by TLC. The slurry was filtered, the solvent was removed under reduced pressure, distilled water added and allowed to stand. The aqueous suspension was acidified with dilute hydrochloric acid (10 mL) and extracted with solvent ether (2 × 20 mL). The aqueous solution was basified with potassium hydroxide solution (5%, 10 mL) and extracted with chloroform (3 × 50 mL). The combined chloroform extract was washed with water, dried and the solvent was removed under reduced pressure to give orange coloured oil, which could not be crystallized. The hydrochloride salts were prepared by passing dry hydrochloric acid gas through the solution of oily residues in dry acetone. The precipitate so obtained was filtered and crystallized to afford the hydrochloride salts of **34**–**37**.

5.1.5.1. 17E-[O-(2-Dimethylaminoethyl)]oximino-3-methoxy-1,3,5-(10)-estratriene (**34**) hydrochloride.

Yield: 95.63%, m.p.: 230–232 °C (from dry $C_5H_{10}O_2$); IR: 3335, 2600, 2432, 1593, 1436, 1220, 1024; ^{1}H-NMR ($CDCl_3$): δ 0.93 (s, 3H, 18-CH_3), 2.89 [s, 6H, –$N(CH_3)_2$], 3.39 (t, 2H, $J = 3$ Hz, –$OCH_2CH_2N<$), 3.78 (s, 3H, 3-OCH_3), 4.49 (t, 2H, $J = 3$ Hz, –OCH_2–), 6.64 (d, 1H, $J = 3$ Hz, 4-C*H*), 6.72 (dd, 1H, $J = 3$ Hz and 9 Hz, 2-C*H*) and 7.21 (d, 1H, $J = 9$ Hz, 1-C*H*); Anal. Calc. for $C_{23}H_{35}N_2O_2Cl$: C, 67.87; H, 8.67; N, 6.88. Found: C, 67.57; H, 8.76; N, 6.67%.

*5.1.5.2. 17E-[O-(2-Diethylaminoethyl)]oximino-3-methoxy-1,3,5-(10)-estratriene (**35**) hydrochloride.* Yield: 72.26%, m.p.: 160–163 °C (from dry $C_5H_{10}O_2$); IR: 3412, 2935, 2640, 2423, 1600, 1498, 1230,1028; ^{1}H-NMR ($CDCl_3$): δ 0.93 (s, 3H, 18-CH_3), 1.45 (t, 6H, J = 6Hz, –N(CH_2CH_3)$_2$), 3.23 [m, 4H, –N(CH_2CH_3)$_2$], 3.40 (t, 2H, J = 4 Hz, –OCH_2CH_2N<), 3.78 (s, 3H, 3-OCH_3), 4.49 (t, 2H, J = 4 Hz, –OCH_2–), 6.64 (d, 1H, J = 3 Hz, 4-CH), 6.72 (dd, 1H, J = 3 Hz and 9 Hz, 2-CH), 7.21(d, 1H, J = 9 Hz, 1-CH) and 12.01 (s, 1H, hydrochloride-H); Anal. Calc. for $C_{25}H_{39}N_2O_2Cl$: C, 69.02; H, 9.04; N, 6.44. Found: C, 68.82; H, 9.01; N, 6.21%.

*5.1.5.3. 17E-[O-(2-Pyrrolidinoethyl)]oximino-3-methoxy-1,3,5-(10)-estratriene (**36**) hydrochloride.* Yield: 40.79%, m.p.: 215–218 °C (from dry $C_5H_{10}O_2$); IR: 3410, 2942, 2600, 1590, 1513, 1435, 1225, 1025; ^{1}H-NMR ($CDCl_3$): δ 0.92 (s, 3H, 18-CH_3), 2.87 (m, 4H, J = 4 Hz, N-methylenes of pyrrolidino functionality), 3.40 (t, 2H, J = 5 Hz, –OCH_2CH_2N<), 3.78 (s, 3H, 3-OCH_3), 4.50 (t, 2H, J = 5 Hz, –OCH_2–), 6.64 (d, 1H, J = 3 Hz, 4-CH), 6.72 (dd, 1H, J = 3 Hz and 9 Hz, 2-CH), 7.21(d, 1H, J = 9 Hz, 1-CH) and 12.36 (s, 1H, hydrochloride-H); Anal. Calc. for $C_{25}H_{37}N_2O_2Cl$: C, 69.34; H, 8.61; N, 6.47. Found: C, 69.06; H, 8.87; N, 6.35%.

*5.1.5.4. 17E-[O-(2-Piperidinoethyl)]oximino-3-methoxy-1,3,5-(10)-estratriene (**37**) hydrochloride.* Yield: 68.31%, m.p.: 200–202 °C (from dry $C_5H_{10}O_2$); IR: 3458, 2917, 2640, 2536, 1505, 1460, 1258,1030; ^{1}H-NMR ($CDCl_3$): δ 0.93 (s, 3H, 18-CH_3), 2.86 (m, 4H, N-methylenes of piperidino functionality), 3.34 (t, 2H, J = 4 Hz, –OCH_2CH_2N<), 3.78 (s, 3H, 3-OCH_3), 4.53 (t, 2H, J = 4 Hz, –OCH_2–), 6.64 (d, 1H, J = 3 Hz, 4-CH), 6.72 (dd, 1H, J = 3 Hz and 9 Hz, 2-CH), 7.21(d, 1H, J = 9 Hz, 1-CH); Anal. Calc. for $C_{26}H_{39}N_2O_2Cl$: C, 69.85; H, 8.79; N, 6.27. Found: C, 69.48; H, 8.97; N, 6.06%.

5.2. Antineoplastic activity

The compounds were evaluated for antineoplastic activity at National Cancer Institute, Bethesda, Maryland, USA. The in vitro assays were performed against the cell panel consisting of 60 cell lines at a minimum of five concentrations at 10-fold dilutions. A 48 h continuous drug exposure protocol was used, and a sulforhodamine B (SRB) protein assay was used to estimate cell viability or growth [19,20].

A standard panel of 12 tumour cell lines was used for the preliminary in vivo hollow fiber screening of the in vitro actives [21]. These include NCI-H23, NCI-H522, MDA-MB-231, MDA-MB-435, SW-620, COLO 205, LOX, UACC-62, OVCAR-3, OVCAR-5, U251 and SF-295. A total of three different tumour lines are prepared for each experiment so that each mouse receives three intraperitoneal (IP) implants (one for each tumour line) and three subcutaneous (SC) implants (one of each tumour line). Each compound is assessed in a total of four experiments (3 cell lines/experiment × 4 experiments = 12 cell lines). The test compound was administered into athymic nude mice implanted with 12 selected human tumour cell lines encased in hollow fibers. Vehicle controls consist of six mice receiving the compound diluent only. After 6–8 days, the fibers were collected, cells were removed and growth inhibition was measured using MTT assay. The percent net growth for each cell line in each treatment group was calculated and compared to the percent net growth in the vehicle treated controls. A 50% or greater reduction in percent net growth in the treated samples compared to the vehicle control samples is considered a positive result. Each positive result is given a score of 2 and all of the scores are totalled for a given compound. Generally a compound is referred for xenograft testing if it has a combined IP (intraperitoneal)+SC (subcutaneous) score of 20 or greater, a SC score of 8 or greater, or produces cell kill of any cell line at either dose level evaluated. The total pattern of activity of the compounds is also taken into consideration by the Biological Evaluation Committee for Cancer Drugs for further in vivo evaluation.

Acknowledgements

The financial support of the project by the CSIR, New Delhi, India and the research facilities provided by Panjab University, India are gratefully acknowledged. The authors express appreciation to National Cancer Institute, Bethesda, Maryland, USA for evaluation of compounds for antineoplastic activity, and Cipla Ltd., Bombay, India for the generous supply of steroids.

References

[1] J.F. Tomera, Drugs Today 30 (1994) 565–574.
[2] E.A. Grunfeld, A.J. Ramirez, M.S. Hunter, M.A. Richards, Br. J. Cancer 86 (2002) 1373–1378.
[3] R. Peto, J. Boreham, M. Clarke, C. Davies, V. Beral, Lancet 355 (2000) 1822.
[4] P.A. Cole, C.H. Robinson, J. Med. Chem. 33 (1990) 2933–2942.
[5] V.C.O. Njar, A.M.H. Brodie, Drugs 58 (1999) 233–255.
[6] P.E. Goss, K.M.E.H. Gwyn, J. Clin. Oncol. 12 (1994) 2460–2470.
[7] J.M. O'Reilly, R.W. Bruggemeier, Curr. Med. Chem. 3 (1996) 11–22.
[8] A.B. Foster, M. Jarman, C.-S. Leung, M.G. Rowlands, G.N. Taylors, R.G. Plevey, R. Sampson, J. Med. Chem. 28 (1985) 200–204.
[9] A.U. Buzdar, Drugs Fut. 22 (1997) 63.
[10] C. Royce, Drugs Fut. 18 (1993) 599.

[11] H.L. Holland, S. Kumaresan, L. Tan, V.C.O. Nzar, J. Chem. Soc. Perkin Trans. 1 13 (1992) 585–587.
[12] M.J. Meegan, R.B. Hughes, D.G. Lloyd, D.C. Williams, D.M. Zisterer, J. Med. Chem. 44 (2001) 1072–1084.
[13] P.E. Goss, K. Strasser, Drugs 62 (2002) 957–966.
[14] G.-E. Seraloni, S. Moslemi, Mol. Cell. Endocrinol. 178 (2001) 117–131.
[15] M. Dowsett, C. Pfister, S.R. Johnson, Clin. Cancer Res. 5 (1999) 2338–2343.
[16] E.B. Hershberg, J. Org. Chem. 13 (1948) 542–546.
[17] R.H. Mazur, J. Org. Chem. 28 (1963) 248–250.
[18] B.M. Regan, F.N. Hayes, J. Am. Chem. Soc. 78 (1956) 639–643.
[19] M.R. Boyd, K.D. Paull, Drug Dev. Res. 34 (1995) 91–109.
[20] M.C. Alley, D.A. Scudiero, P.A. Monks, M.L. Hursey, M.J. Czerwinski, D.L. Fine, B.J. Abbott, J.G. Mayo, R.H. Shoemaker, M.R. Boyd, Cancer Res. 48 (1988) 589–601.
[21] M. Hellingshead, M.C. Alley, R.F. Camalier, M.J. Abbott, J.G. Mayo, L. Malspeis, M.R. Grever, Life Sci. 57 (1995) 131–141.
[22] J.V. Frank, R.F. Tavares, C.A. Ellis, J. Pharm. Sci. 58 (1969) 138–140.