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Pregnenolone derivatives as potential anticancer agents

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ABSTRACT

Pregnenolone (**1**) was used as a template to develop new anticancer compounds. Ring-D modification of **1** resulted in the synthesis of benzylidenes **2**–**17**, pyrazolines **18**–**76**, pyrazoles **85**–**91**, hydrazones **77**–**84**, and oximes **92**–**107** derivatives. The structure of compound **107** was also deduced through single crystal X-ray diffraction studies. The inclusion of furanyl and pyridyl rings to pregnenolone skeleton increases the cytotoxicity of all compounds significantly. Among benzylidene derivatives, only heterocyclic enone **8** (IC_{50} = 0.74 μM/mL against HepG2), and **17** (IC_{50} = 4.49 μM/mL against HepG2, IC_{50} = 5.01 μM/mL against MDA-MB-230 cancer cell line) exhibited a significant activity. The cytotoxicity data of pyrazoline derivatives **18**–**76** revealed that only furanyl bearing pyrazolines **40**, **42**–**44**, **48**, and **49** exhibited significant activities. While all (*O*-carboxymethyl) oximes, hydazones, and pyrazoles derivatives of pregnenolone did not show any significant activity against both the cell lines. Thus the furanyl bearing enone **8** (IC_{50} = 0.74 μM/mL against HepG2), and its pyrazoline derivative **48** (IC_{50} = 0.91 μM/mL against MDA-MB-230 cancer cell lines) were identified as the most active compounds in all derivatives of pregnenolone.

1. Introduction

Cancer has been a major threat to public health for many years. Despite recent advances in early diagnosis, prevention, and therapy, cancer still affects millions of people worldwide and is one of the leading causes of death. Breast cancer is one of the most common cancers among women in both developed and developing countries. It is the malignancy with the highest incidence and death rate in women [1,2]. On the other hand, hepatocellular carcinoma is the fifth most common form of cancer, and increasing globally due to increased incidence of hepatitis [1]. However, the efficacy of the existing drugs for the treatment of various cancers is rather limited, and there is an urgent need to develop new therapeutic agents to overcome the limitations with the current therapy. *In vitro* studies, using a variety of human cell lines, have been employed to evaluate the effectiveness of new medicinal compounds against these cancers.

Steroids form an important class of biologically active compounds which exhibit diverse biological activities. Upto now, several steroidal derivatives have been investigated as new curative agents for cancers, and other diseases [3–5]. Several modified steroids, bearing heterocyclic systems as a part of rings-A- and -D, have exhibited diverse biological activities, such as anti-microbial, anti-inflammatory, hypotensive, hypocholesterolemic, and diuretic activities. Among these derivatives, 16,17-condensed steroidal pyrazolines have immense pharmacological significance [6–9,16].

Pregnenolone (**1**), a naturally occurring steroid, is known as a precursor to other hormones, including cortisone, estrogen, testosterone, and progesterone [10,11]. Previously different pregnenolone derivatives have been synthesized, and evaluated for various biological activities. The pregnenolone analogs, hydroxylated at C-20, are known to effect calcium-dependent processes, and also affect the degree of depolarization of smooth muscles [12]. The hemisuccinate of pregnenolone-derivative significantly increases the perfusion pressure, and vascular resistance in isolated rat heart [13]. The nitrochlorambucil ester of pregnenolone exhibited a significant cytotoxic activity towards brain posterior fossa, medullablastoma (Daoy), and lung large cell carcinoma (H460) cell lines [14].

In a recent report, benzylidene, and pyrazoline derivatives of pregnenolone were synthesized, and activity of pyrazoline derivatives against the HT-29, HCT-15, 502713 cancer cell lines, has been evaluated [16]. This study, conducted by Banday et al. has shown that pregnenolone pyrazoline derivatives (10 compounds) possess significant cytotoxicity against HT-29, and HCT-15, 502713 cell

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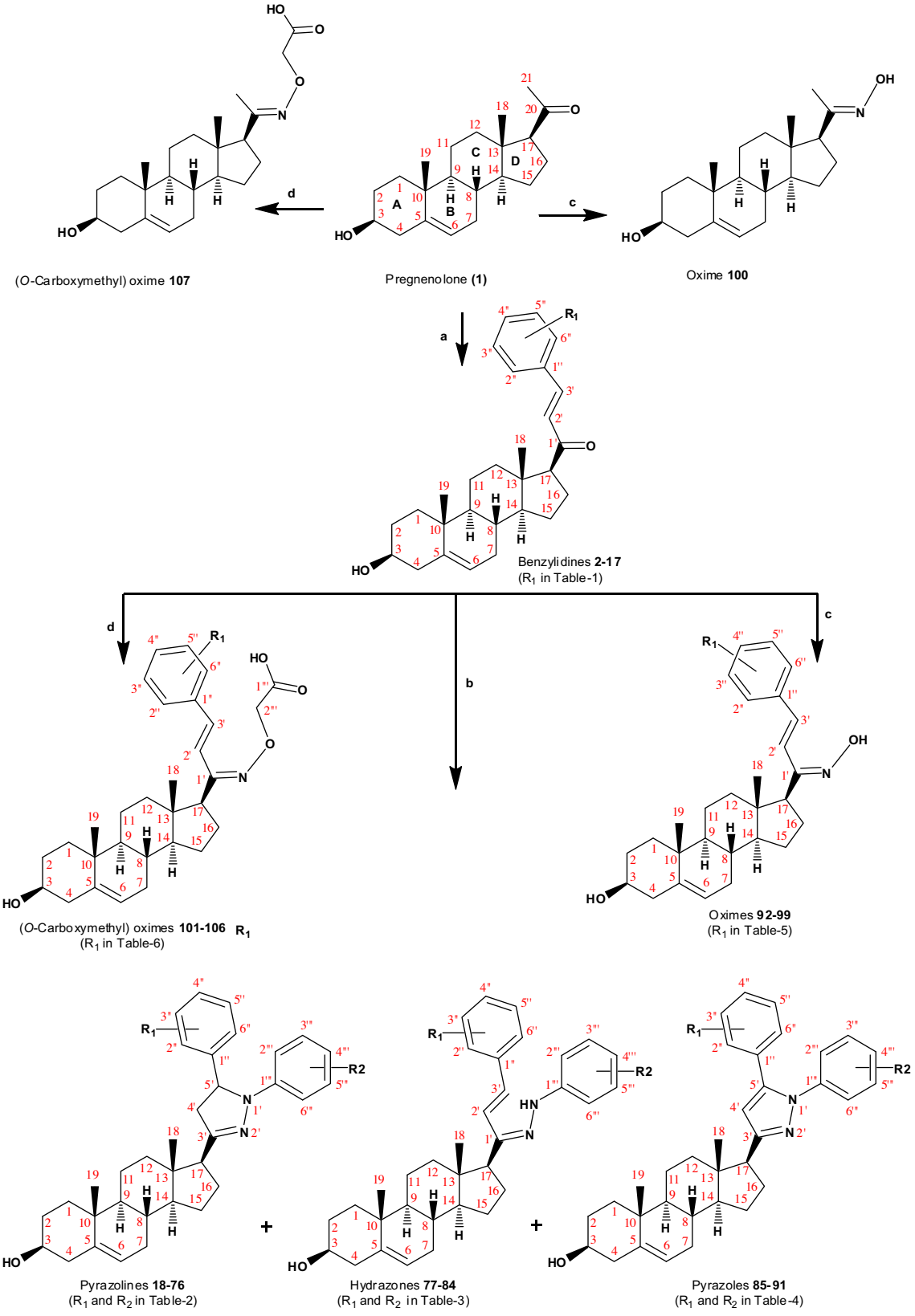
lines. It was further demonstrated that the cytotoxicity of the compounds against cancer cell lines increases significantly with the changes in the substitutions on the aromatic ring [16]. To further evaluate the potential of pyrazoline, and other heterocylic analogs of pregnenolone, the present study was conducted in which pyrazolines **18–76**, hydrazones **77–84**, pyrazoles **85–91**, and oximes



Scheme 1. Synthesis of pregnenolone derivatives. Reagents and conditions: (a) EtOH (25 mL), NaOH (3 mL, 4 M, in H_2O), R_1CHO (0.6 mmol) r.t., 3 h; (b) EtOH (06 mL), phenylhydrazine 1.5 eq. molar, HCl, 90 °C, 22 h; (c) NH_2OH, pyridine, stirring for 22 h; (d) $NH_2OC.H_2CO_2H.HCl$, pyridine, stirring for 24 h.

92–**107**, and benzylidene **2**–**17** derivatives of pregnenolone were synthesized, and evaluated against HepG2, and MDA-MB-230 cell lines.

2. Results and discussion

By using pregnenolone as a template, libraries of pregnenolone derivatives were created by parallel synthesis (Scheme 1). From the screening of a total of 107 compounds, furanyl-bearing enone **8**, and its oxime derivative **95** were found to be significantly active against the Hep2 cell lines, while its pyrazoline derivative **48**, was strongly active against the MDA-MB-230 cell line.

3. Benzylidene derivatives 2–17

The synthesis of benzylidene derivatives of pregnenolone has previously been reported [15,16]. In the present study, pregnenolone (**1**) was initially converted into a series of benzylidene derivatives **2**–**17** by parallel synthesis. These derivatives were evaluated against HepG2, and MDA-MB-231 cancer cell lines (Table 1). Derivatives **2**–**17** were used in the synthesis of pregnenolone derivatives **18**–**106**.

4. Pregnenolone pyrazoline derivatives 18–76

The synthesis of 17-pyrazolinyl derivatives of pregnenolones has previously been reported [15,16]. Banday et al. has also reported the cytotoxicity of 17-pyrazolinyl derivatives of pregnenolones against a panel of seven human cancer cell lines [16]. In the reported study, a series of 10 *N*-acetyl pyrazolinyl derivatives of pregnenolone were synthesized by reacting different benzylidene derivatives of pregnenolone with hydrazine in the presence of acetic acid. However in the present study, 59 pyrazoline derivatives of pregnenolone **18**–**76** (Table 2) were synthesized by treating differently substituted phenylhydrazines with 16 different benzylidene derivatives **2**–**17**, in the presence of hydrochloric acid through parallel synthesis approach (Scheme 1). The pyrazoline derivatives **18**–**76** were obtained in moderate yields (40–60%). Analytical and spectral data (IR, 1D, 2D-^{1}H NMR and ^{13}C NMR) of these derivatives was in good agreement with the proposed structures. In the ^{1}H NMR spectra of all pyrazolines, H-4a′ and H-4b′ resonated as a pair of a doublet of doublets between δ 4.2–3.1 ($J_{4a',\,5'}$ = 10.5 Hz, $J_{4a',\,4b'}$ = 16.5–17.5 Hz), and 3.3–3.5 ($J_{4b',\,5'}$ = 10.5–11.5 Hz, $J_{4b',\,4a'}$ = 16.5–17.5 Hz), respectively. H-5′ of the pyrazoline ring was resonated as a double doublet between δ 4.8–5.6 ($J_{5',\,4a'}$ = 8.2–9.3 Hz, $J_{5',\,4b'}$ = 10.5–11.2 Hz).

Reactions of benzylidene derivatives **2**–**17** with aromatic hydrazines, such as 2-nitro- and 2,4-dinitrophenyl hydrazines, produced hydrazones **77**–**84** (Scheme 1; Table 3). The structures of all hydrazone derivatives were deduced from the IR and ^{1}H NMR spectra. No pyrazoline formation was detected in cases of dinitro derivatives due to the strong electron withdrawing effect of NO_2 groups in phenylhydrazine, which decreases the electron density at NH, and inhibits the formation of pyrazoline ring from hydrazone derivatives.

The reactions of benzylidene derivatives **2**–**17** with phenylhydrazines bearing electron-donating groups such as 4-methoxy-, and 2-ethyl groups, and unsubstituted phenylhydrazine produced pyrazole derivatives **85**–**91** (Table 4). These products were characterized by IR and ^{1}H NMR spectroscopy. In the ^{1}H NMR spectra of the pyrazoles, H-4′ of the pyrazole ring was resonated as a singlet between δ 6.2–6.4.

5. Pregnenolone oxime derivatives 92–100

Oxime derivatives **92**–**100** (Table 5) and the (*O*-carboxymethyl) oxime derivatives **101**–**107** of pregnenolone (**1**) were synthesized by adding hydroxylamine, and the (*O*-carboxymethyl) hydroxylamine, respectively, in the presence of pyridine to the solution of corresponding benzylidene derivatives **2**–**17**, as shown in Scheme 1. After the completion of the reaction, the mixtures were poured in the acidic water to neutralize the pyridine. The products were extracted with dichloromethane from acidic solution. The analytical, and spectral data (^{1}H NMR, and EI-MS) of all the products were in good agreement with the proposed structures.

6. X-ray structure of compound 107

Single-crystal X-ray diffraction analysis was carried out to deduce the structure of compound **107** (Scheme 1). The asymmetric unit comprised on two independent molecules of **107**. The ORTEP diagrams of **107** (Fig. 1) showed four *trans* fused rings A, B, C, and D with *chair*/*half chair*/*chair*, and *envelop* conformation, respectively. The C-3 OH exists in equatorial orientation, while C-20/N-1

Table 1
Benzylidene derivatives **2**–**17**.

Product	R_1	Yield %	IC_{50} (μM)	
			HepG2	MDA-MB-231
2	Ph	94	16.25	>40.0
3	4-Phenoxyphenyl	72	>40.0	>40.0
4	4-Methylphenyl	93	20.91	>40.0
5	3-Nitrophenyl	85	18.57	30.83
6	4-Methoxyphenyl	47	>40.0	>40.0
7	3-Chlorophenyl	42	>40.0	>40.0
8	2-Furyl	89	0.74	>40.0
9	2-Naphthalenyl	43	>40.0	>40.0
10	2-Thienyl	56	>40.0	>40.0
11	3,4,5-Trimethoxyphenyl	78	>40.0	>40.0
12	4-Ethoxyphenyl	73	>40.0	>40.0
13	2-Chlorophenyl	72	>40.0	>40.0
14	3,4-Difluorophenyl	81	20.02	>40.0
15	3,4-Dichlorophenyl	89	12.49	15.80
16	2,4-Dichlorophenyl	87	>40.0	>40.0
17	2-Pyridinyl	88	4.49	5.01
Pregnenolone (1)			28.97	>40.0
Doxorubicin			0.63	1.23

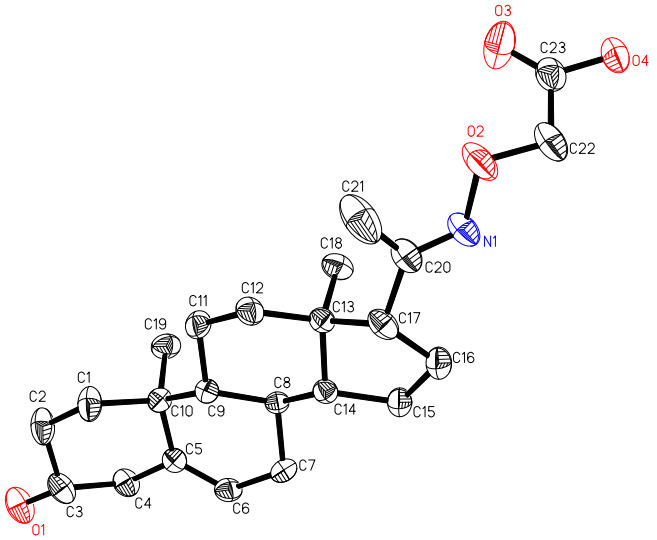


Fig. 1. ORTEP diagram of final X-ray model of compound **107** (H-atoms are omitted for clarity).

oxime bond exists in *Z* configuration. All the bond angles and lengths were within the normal range.

7. SAR study

Pregnenolone (**1**) showed cytotoxicity against the HepG2 (IC_{50} = 28.97 μM/mL), while it was found to be inactive against MDA-MB-230 cancer cell lines. Among the enone derivatives, compound **8** with a furanyl group was most active against HepG2 cell line (IC_{50} = 0.74 μM/mL). It was found to be inactive against human breast cancer cell line (MDA-MB-231). On the other hand, compound **17** bearing a pyridinyl moiety, was highly cytotoxic against both the cell lines.

The cytoxocity data of pyrazoline derivatives revealed that pyrazoline derivatives bearing furanyl ring (**40**, **42**–**44**, and **49**–**50)** were active compounds. While the pyrazoline **48** bearing furanyl ring, was the most active compound (IC_{50} = 0.91 μM/mL) against human breast cancer cell MDA-MB-231. All other pyrazolines, derived from 4-methylpheny, 3-nitrophenyl, 3-chlorophenyl, 4-ethoxyphenyl, 2-chlorophenyl, and 3,4-dichlorophenyl-bearing benzylidenes, were found to be inactive (Table 2). On the other hand, all pyrazole and hydrazone derivatives did not show any significant activity against both cancer cell lines (Tables 3 and 4).

Among the oxime derivatives (Table 5), compound **95** was selectively active against the HepG2 cell line, while compound **98** showed activity against both the cancer cell lines. Interestingly all (*O*-carboxymethyl) oxime derivatives were found to be inactive against both the cancer cell lines (Table 6).

The purity level of cytotoxic compounds was determined by Shimadzo analytical HPLC 10 A using UV Detector at the wavelength of 256 nm (Table 7).

In conclusion, the synthesis and biological evaluation of a new series of pregnenolone derivatives is reported. Among the enone, pyrazoline, oxime, pyrazole, and hydrazone derivatives of pregnen-

Table 2
Pyrazolines functionalized pregnenolones **18**–**76**.

No.	R_1	R_2	IC50 (μM)	
			HepG2	MDA-MB-231
18	Phenyl	4-Bromophenyl	>40.0	>40.0
19	Phenyl	4-Chlorophenyl	33.77	20.83
20	Phenyl	2-Bromophenyl	>40.0	>40.0
21	Phenyl	4-Flourophenyl	14.85	11.06
22	Phenyl	2-Chlorophenyl	24.42	26.95
23	Phenyl	Phenyl	14.05	9.65
24	Phenyl	2-Nitrophenyl	26.80	19.86
25	3-Nitrophenyl	4-Flourophenyl	16.45	16.45
26	3-Nitrophenyl	4-Chlorophenyl	>40.0	>40.0
27	3-Nitrophenyl	4-Bromophenyl	>40.0	>40.0
28	3-Nitrophenyl	4-Methoxyphenyl	32.34	>40.0
29	3-Nitrophenyl	2-Chlorophenyl	>40.0	>40.0
30	3-Nitrophenyl	2-Bromophenyl	26.73	30.67
31	3-Nitrophenyl	Phenyl	30.34	28.93
32	4-Methylphenyl	4-Flourophenyl	>40.0	>40.0
33	4-Methylphenyl	4-Chlorophenyl	>40.0	>40.0
34	4-Methylphenyl	4-Bromophenyl	>40.0	>40.0
35	4-Methylphenyl	4-Methoxyphenyl	>40.0	>40.0
36	4-Methylphenyl	2-Chlorophenyl	27.69	22.08
37	4-Methylphenyl	2-Bromophenyl	>40.0	>40.0
38	4-Methylphenyl	Phenyl	>40.0	>40.0
39	4-Methylphenyl	2-NitroPhenyl	22.81	33.24
40	2-Furyl	4-Flourophenyl	14.25	12.88
41	2-Furyl	4-Chlorophenyl	>40.0	>40.0
42	2-Furyl	4-Bromophenyl	6.63	11.12
43	2-Furyl	2-Chlorophenyl	8.82	18.87
44	2-Furyl	2-Bromophenyl	18.25	6.65
45	3-Chlorophenyl	4-Flourophenyl	>40.0	32.42
46	3-Chlorophenyl	4-Chlorophenyl	>40.0	>40.0
47	3-Chlorophenyl	4-Bromophenyl	>40.0	>40.0
48	**2-Furyl**	**Phenyl**	**>40.0**	**0.91**
49	2-Furyl	2,4-Dichlorophenyl	11.03	12.93
50	2-Furyl	4-Methylphenyl	13.49	10.44
51	4-Ethoxyphenyl	4-Flourophenyl	>40.0	>40.0
52	4-Ethoxyphenyl	2-Chlorophenyl	29.10	29.10
53	4-Ethoxyphenyl	2-Bromophenyl	>40.0	>40.0
54	4-Ethoxyphenyl	2-Nitrophenyl	>40.0	>40.0
55	2-Chlorophenyl	4-Flourophenyl	>40.0	>40.0
56	2-Chlorophenyl	4-Methoxyphenyl	>40.0	>40.0
57	2-Chlorophenyl	2-Chlorophenyl	>40.0	>40.0
58	2-Chlorophenyl	2-Bromophenyl	>40.0	>40.0
59	2-Chlorophenyl	Phenyl	>40.0	>40.0
60	3,4-Dichlorophenyl	4-Flourophenyl	>40.0	>40.0
61	3,4-Dichlorophenyl	4-Chlorophenyl	>40.0	>40.0
62	3,4-Dichlorophenyl	4-Bromophenyl	>40.0	>40.0
63	3,4-Dichlorophenyl	4-Methoxyphenyl	>40.0	>40.0
64	3,4-Dichlorophenyl	2-Chlorophenyl	>40.0	>40.0
65	3,4-Dichlorophenyl	2-Bromophenyl	>40.0	>40.0
66	3,4-Dichlorophenyl	Phenyl	>40.0	>40.0
67	3,4-Dichlorophenyl	2-Ethylphenyl	>40.0	>40.0
68	3,4-Dichlorophenyl	2,4-Dichlorophenyl	>40.0	>40.0
69	2-Pyridinyl	4-Flourophenyl	16.12	16.12
70	2-Pyridinyl	4-Chlorophenyl	32.47	32.47
71	2-Pyridinyl	4-Bromophenyl	23.56	23.56
72	2-Pyridinyl	4-Methoxyphenyl	>40.0	>40.0
73	2-Pyridinyl	2-Chlorophenyl	25.95	25.95
74	2-Pyridinyl	2-Bromophenyl	21.55	23.21
75	2-Pyridinyl	Phenyl	18.40	18.38
76	2-Pyridinyl	2,4-Dichlorophenyl	27.91	18.49

Table 3
Hydrazone derivatives **77**–**84**.

No.	R_1	R_2	HepG2	MDA-MB-231
			IC_{50} (μM)	
77	2-Furyl	2-Nitrophenyl	29.68	19.66
78	2-Furyl	2,4-Dichlorophenyl	>40.00	>40.00
79	Phenyl	2,4-Dinitrophenyl	>40.00	>40.00
80	3-Nitrophenyl	2-Nitrophenyl	>40.00	>40.00
81	3-Nitrophenyl	2,4-Dinitrophenyl	>40.00	>40.00
82	4-Methylphenyl	2,4-Dinitrophenyl	>40.00	>40.00
83	3,4-Dichlorophenyl	2-Nitrophenyl	>40.00	>40.00
84	2-Pyridinyl	2-Nitrophenyl	16.28	16.28

Table 4
Pyrazole derivatives **85**–**91**.

No.	R_1	R_2	HepG2	MDA-MB-231
			IC_{50} (μM)	
85	Phenyl	4-Methoxyphenyl	>40.00	>40.00
86	Phenyl	Phenyl	25.57	18.69
87	2-Furyl	4-Chlorophenyl	24.43	18.64
88	2-Furyl	4-Methoxyphenyl	>40.00	>40.00
89	2-Furyl	Phenyl	>40.00	>40.00
90	3,4-Dichlorophenyl	4-Methoxy	>40.00	>40.00
91	2-Pyridinyl	2-Ethylphenyl	17.94	17.94

Table 5
Cytotoxic activity of oximes **92**–**100**.

S. no	*R*	IC_{50} (μM)	
		HepG2	MDA-MB-231
92	Phenyl	13.30	26.69
94	3-Nitrophenyl	25.32	>40.00
95	2-Furyl	5.09	>40.00
96	2-Chlorophenyl	–[a]	–[a]
97	3,4-Difluorophenyl	18.05	19.83
98	3,4-Dichlorophenyl	4.50	6.76
99	2-Pyridinyl	>40.00	>40.00
100	–	13.77	29.30
Pregnenolone (1)		28.97	>40.00
Doxorubicin		0.63	1.23

[a] Not tested.

Table 6
Cytotoxicity of (*O*-carboxymethyl) oximes derivatives **101**–**106**.

S. no.	*R*	IC_{50} (μM)	
		HepG2	MDA-MB-231
101	4-Methylphenyl	>40.00	>40.00
102	2-Chlorophenyl	>40.00	>40.00
103	3,4-Difluorophenyl	24.52	34.52
104	3,4-Dichlorophenyl	>40.00	>40.00
105	2-Pyridinyl	>40.00	>40.00
106	2,4-Dichlorophenyl	16.44	26.92
107	–	>40.00	>40.00
Pregnenolone (1)		28.97	>40.00
Doxorubicin		0.63	1.23

Table 7
The purity level of the cytotoxic compounds.

Compound no.	% Purity
8	91.8
17	87.7
48	89.3
98	98.0

olone (**1**), the heterocyclic enone **8**, and its pyrazoline derivative **48** were found to be the most active compounds. All (*O*-carboxymethyl) oxime, hydazones and pyrazole derivatives of pregnenolone did not show any significant activity against both cell lines.

8. General experimental method

Parallel synthesizer (Smart Start Synthesizer with 16 reaction vessels) was purchased from Chemspeed Ltd., Switzerland. Reagents were purchased from Wako Pure Chemical Industries Ltd., Japan. Pregnenolone (**1**) was supplied by Acros Organics, New Jersey, USA, and CD_3OD were purchased from the E. Merck (Germany). The other organic solvents were obtained from Fisher Scientific Limited, UK, and were used as received. Absolute ethanol was obtained by distillation of ethanol over anhydrous calcium oxide. Flash chromatography was performed on E. Merck (60 230–400 mesh silica gel). Thin layer chromatography was performed on 0.25 mm silica gel plates (E. Merck, $60F_{254}$). Visualization of the TLC plates was achieved under the UV light at 254 and 366 nm and by spraying with ceric sulfate reagent. The solvent system *n*-hexane-ethyl acetate (3:2) was used as eluent. The IR spectra were recorded on a Shimadzu FTIR-8900 spectrophotometer. Melting point was taken on BUCHI 535. $[\alpha]_D$ was recorded on Hitachi U-3200 spectrophotometer. EI- and HREI-MS were recorded on JMS HX 110 and on JMS-DA 500 mass spectrometers. The ^1H and ^{13}C NMR spectra were recorded on Bruker NMR spectrometers, operating at 300, 400, and 500 (75, 100, and 125 for ^{13}C). The chemical shifts values are reported in ppm (δ) units, and the coupling constants (J) are given in Hz. Single-crystal X-ray diffraction data was collected on Bruker Smart APEX II, CCD 4-K area detector diffractometer [17]. Data reductions was performed by using SAINT program. The structure was solved by direct method [18], and refined by full-matrix least squares on F2 by using the SHELXTL-PC package [19]. The figures were plotted with the aid of ORTEP program [20].

8.1. General procedure for benzylidene derivatives *2*–*17*

The ethanol (25 mL) and NaOH (4 M, 3 mL) were mixed, cooled in an ice bath, and then compound **1** (0.3 mmol, 100 mg) was added. The aromatic aldehyde (0.6 mmol) was added dropwise. The mixture was stirred for three hrs, and then neutralized by adding 2 M HCl. The precipitates were filtered, washed with acidic water (10 mL × 2), and neutral water (20 mL), and dried in vacuum.

8.2. General procedure for pyrazoline derivatives *18*–*76*

One drop of hydrochloric acid (11.0 mol) was added to the solution of each benzylidene derivatives **2**–**17**, each 100 mg) in ethanol (6 mL). Phenyl hydrazine (1.5 eq molar) was added to the resulting solution, and refluxed overnight at 90 °C. After completion of reaction, the solvent was evaporated under *vacuo*, and purified by SiO_2 gel column chromatography.

8.3. General procedure for oxime derivatives *92*–*99*

Each benzylidene derivative (100 mg) in pyridine (2.2 mL) was combined with hydroxylamine hydrochloride (2.5 eq) and stirred for 22 h. Reaction mixture was poured into a solution containing pre-cooled 10% aq. HCl (10 mL). The product was extracted with dichloromethane (3 × 15 mL). The organic extracts were combined, and washed with saturated aqueous NaCl (3 × 10 mL), dried over anhydrous magnesium sulfate, and the solvent was removed in *vacuo*. Oximes **92**–**99** were purified by thin layer chromatography.

8.4. (O-Carboxymethyl) oxime derivatives of enones *100*–*106*

The benzylidene derivative (250 mg) in pyridine (100 mL) was combined with amino oxy-acetic acid hemihydrochloride (280 mg), and stirred at 23 °C. After 24 h, the reaction mixture was poured into a solution containing 10% aq HCl (30 mL), and precoold in ice. The product was extracted with ethyl acetate (3 × 50 mL). The organic extract was washed with saturated solution of NaCl (3 × 30 mL), dried over anhydrous $MgSO_4$, and concentrated in *vacuo*.

Crystal data of **107**: $C_{23}H_{36}NO_4$, Mr = 390.53, monoclinic, space group $P21$, $a = 6.5950(11)$ Å, $b = 11.3801(18)$ Å, $c = 29.257(4)$ Å, $V = 2182.9(6)$ Å^3, $Z = 4$, $\rho_{calc} = 1.185$ mg/m^3, $F(000) = 848$, μ (Mo Kα) = 0.71073 Å, max/min transmission 0.9960/0.9641 crystal dimensions 0.46 × 0.23 × 0.05, $0.70 < \theta < 25.5$, 12,988 reflections were collected, of which 4240 reflections were judged ($R_{int} = 0.0477$). The R values were: $R_1 = 0.0505$, $wR_2 = 0.1165$ for $I > 2\sigma(I)$, and $R_1 = 0.0910$, $wR_2 = 0.1476$ for all data; max/min residual electron density: 0.223/−0.136 eÅ^{-3}. Crystallographic data for compound **107** has been deposited in the Cambridge Crystallographic Data Center. These information can directly be obtained free of charge from CCDC data center (CCDC 836728 reference code).

8.5. Cytotoxicity assay

Human breast (MDA-MB-231), and liver (HepG2) cancer cell lines were obtained from the American Type Culture Collection (ATCC), USA. Cell viability was measured by the MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazoliumbromide] colorimetric method. After drug treatment, the attached cells were incubated for 4 h with MTT (0.5 mg/mL, 1 h). The medium was removed, and DMSO was added to dissolve the formazan crystals. The absorbance at 550 nm was then measured by using an ELISA microplate reader.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.steroids.2011.09.006.

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