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# Synthesis and *In vitro* cytotoxic activity evaluation of (*E*)-16-(substituted benzylidene) derivatives of dehydroepiandrosterone

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## Abstract

**Background and the purpose of the study:** Modified androsterone derivatives are class of steroidal compounds with potential anticancer properties. Various steroidal derivatives containing substitution at position 16 have shown diversified pharmacological activities. In the present study, a new series of cytotoxic 16-(substituted benzylidene) derivatives of dehydroepiandrosterone (DHEA) were synthesized and evaluated against three different cancer cell lines.

**Methods:** The cytotoxic 16-(substituted benzylidene) derivatives of DHEA were synthesized via aldol condensation of DHEA with corresponding benzaldehyde derivatives. The cytotoxic activity of synthesized derivatives was evaluated against three different cancer cells including KB, T47D and SK-N-MC cell lines by MTT reduction colorimetric assay.

**Results:** The results indicated that 16-(substituted benzylidene) derivatives of DHEA could be served as a potent anti-cancer agent. The 3-chloro benzylidene derivatives of DHEA was the most potent synthesized derivative especially against KB and T47D cell lines (IC<sub>50</sub> values were 0.6 and 1.7  $\mu$ M; respectively).

**Conclusion:** The cytotoxic potential of novel benzylidene derivatives of DHEA is mainly attributed to the position and nature of the substituted group on the benzylidene pendant.

**Keywords:** Synthesis, Dehydroepiandrosterone (DHEA), MTT assay, Cytotoxic activity

## Introduction

Steroidal derivatives are important class of synthetic and naturally occurring compounds, which have exhibited different biological properties [1-3] and attracted profound attention for development of potent pharmacological agents for treatments of various diseases [4] including: cardiovascular disease [5], adrenal insufficiencies [6], autoimmune disorders [7], fungal and microbial infections [8,9]. Furthermore, different steroidal derivatives have been considered as potent anti-cancer agents

for the treatment of leukemia [4], breast cancer [10-12], prostate cancer [13] and brain tumors [14].

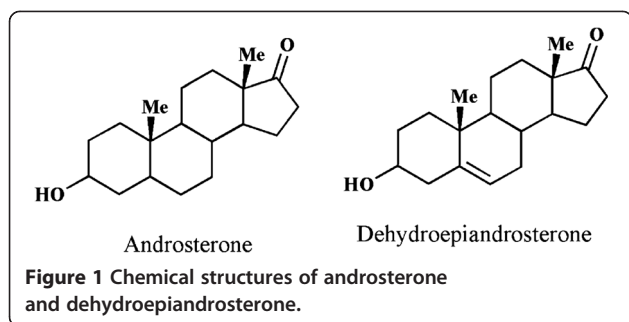
Several natural and modified steroidal derivatives have been previously described in the literatures as potent cytotoxic agents [15-17]. In this regard, different derivatives of androsterone (3 $\alpha$ -hydroxy-5 $\alpha$ -androstane-17-one) have been excessively studied as potent anti-cancer agents (Figure 1) [18,19]. Recently, the significant cytotoxic and aromatase inhibitory potential of a large number of androsterone derivatives containing substitution at position 16 have been reported [12,20,21]. Bansal et. al demonstrated the effectiveness of different 16E-arylidene steroids as potential anticancer and anti-aromatase scaffold against estrogen-dependent breast

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cancer and different human tumor cell lines [12,20]. The cytotoxic mechanistic study of  $\alpha,\beta$ -unsaturated carbonyl derivatives revealed that compounds containing described functional group can cause alteration and misfolding of proteins through the formation of adducts with reactive thiol groups of proteins [22]. For this reason, the  $\alpha$ - $\beta$  unsaturated androsterone derivatives containing exocyclic double bond at C16 position could be served as potent chemotherapeutic agents. Dehydroepiandrosterone (DHEA), also known as androstenedione ( $3\beta$ -hydroxyandrost-5-en-17-one) is an androsterone derivative and important endogenous steroid hormone which plays an important role as intermediate for biosynthesis of androgens and estrogen hormones [23]. Apart from its different biological potential, DHEA demonstrated antiproliferative and antiapoptotic effects on different cancer cell lines [24-26].

In the course of our ongoing study for the synthesis and biological evaluation of potential anticancer agents [27-34], herein, we investigate the synthesis and cytotoxic activity evaluation of a new series of 16-(substituted benzylidene) derivatives of DHEA taking into account the structural necessities for cytotoxic activity of these derivatives. The aim of this study was to investigate the structural requirements affecting the cytotoxic potential of modified steroidal compounds.

## Material and methods

### Chemistry

All starting materials, reagents, and solvents were prepared from Merck AG (Germany). Thin layer chromatography (TLC) using various solvents of different polarities was applied for determination of the purity of the synthesized compounds. Melting points were determined on a Kofler hot stage apparatus (Vienna, Austria) and are uncorrected.  $^1\text{H-NMR}$  spectra were recorded using a Bruker 400 spectrometer (Bruker, Rheinstatten, Germany), and chemical shifts are expressed as  $\delta$  (ppm) with tetramethylsilane (TMS) as

internal standard. The IR spectra were recorded using a Shimadzu 470 (Shimadzu, Tokyo, Japan) spectrophotometer (potassium bromide disks). The mass spectra were recorded on a Finnigan TSQ-70 spectrometer (Finnigan, USA) at 70 eV.

### General procedure for the preparation of the (E)-16-(substituted benzylidene) dehydroepiandrosterone derivatives 1a-m using aldol Condensation

The appropriate aldehyde was added to a mixture of DHEA (1.0 g, 3.47 mmol) and NaOH (1.75 g) in methanol (20 ml). The reaction mixture was stirred for 1 h at room temperature. The completion of reaction was confirmed using analytical thin layer chromatography. After completion, the reaction mixture was poured into ice-water. The final precipitate was filtered; washed with cold water, dried under reduced pressure and crystallized in methanol.

#### (E)-16-(2-Chlorobenzylidene)-1,3,4,7,8,9,10,11,12,13,15,16-dodecahydro-3-hydroxy-10,13-dimethyl-2H-cyclopenta[a]phenanthren-17(14H)-one (1a)

Yield: 23%; mp=213-214°C; IR (KBr,  $\nu_{\text{max}}$ ,  $\text{cm}^{-1}$ ): 3475 (OH), 1725(C=O).  $^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ ): 1.00(s, 3H,  $\text{CH}_3$ ), 1.07 (s, 3H,  $\text{CH}_3$ ), 3.52-3.63(m, 1H,  $\text{CH-OH}$ ), 5.40(s, 1H,  $\text{H}_{\text{vinyl}}$ ), 7.28-7.32(m, 2H,  $\text{H}_{\text{phenyl}}$ ), 7.42-7.46 (m, 1H,  $\text{H}_{\text{phenyl}}$ ), 7.52-7.56(m, 1H,  $\text{H}_{\text{phenyl}}$ ). MS (EI)  $m/z$  (%): 412 ( $\text{M}^+ + 2$ , 31), 410( $\text{M}^+$ , 100).

#### (E)-16-(3-Chlorobenzylidene)-1,3,4,7,8,9,10,11,12,13,15,16-dodecahydro-3-hydroxy-10,13-dimethyl-2H-cyclopenta[a]phenanthren-17(14H)-one (1b)

Yield: 30%; mp= 199-202°C; IR (KBr,  $\nu_{\text{max}}$ ,  $\text{cm}^{-1}$ ): 3219 (OH), 1708 (C=O).  $^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ ): 0.99(s, 3H,  $\text{CH}_3$ ), 1.07(s, 3H,  $\text{CH}_3$ ), 3.48-3.60(m, 1H,  $\text{CH-OH}$ ), 5.41(s, 1H,  $\text{H}_{\text{vinyl}}$ ), 7.33-7.43(m, 4H,  $\text{H}_{\text{phenyl}}$ ). MS (EI)  $m/z$  (%): 412 ( $\text{M}^+ + 2$ , 5), 410 ( $\text{M}^+$ , 15).

#### (E)-16-(4-Chlorobenzylidene)-1,3,4,7,8,9,10,11,12,13,15,16-dodecahydro-3-hydroxy-10,13-dimethyl-2H-cyclopenta[a]phenanthren-17(14H)-one (1c)

Yield: 27%; mp=229-231°C; IR (KBr,  $\nu_{\text{max}}$ ,  $\text{cm}^{-1}$ ): 3416 (OH), 1710(C=O).  $^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ ): 0.98(s, 3H,  $\text{CH}_3$ ), 1.07(s, 3H,  $\text{CH}_3$ ), 3.53-3.54(m, 1H,  $\text{CH-OH}$ ), 5.40(s, 1H,  $\text{H}_{\text{vinyl}}$ ), 7.39(dd, 1H,  $\text{H}_{\text{phenyl}}$ ,  $J = 8.5\text{Hz}$ ), 7.46 (dd, 1H,  $\text{H}_{\text{phenyl}}$ ,  $J = 8.5\text{Hz}$ ). MS (EI)  $m/z$  (%): 412 ( $\text{M}^+ + 2$ , 10), 410 ( $\text{M}^+$ , 28), 378(18), 351(4), 300(19), 268(10), 214(22), 150(100), 91(87), 79(100).

#### (E)-16-(2,4-Dichlorobenzylidene)-1,3,4,7,8,9,10,11,12,13,15,16-dodecahydro-3-hydroxy-10,13-dimethyl-2H-cyclopenta[a]phenanthren-17(14H)-one (1d)

Yield: 35%; mp=203-205°C; IR (KBr,  $\nu_{\text{max}}$ ,  $\text{cm}^{-1}$ ): 3472 (OH), 2929(CH aliphatic), 1710(C=O).  $^1\text{H-NMR}$  (DMSO-

*d*<sub>6</sub>): <sup>1</sup>HNMR (400 MHz, CDCl<sub>3</sub>): 1.03(s, 3H, CH<sub>3</sub>), 1.11 (s, 3H, CH<sub>3</sub>), 3.55-3.56(m, 1H, CH-OH), 5.40(s, 1H, H<sub>vinyl</sub>), 7.48-7.49(m, 2H, H<sub>phenyl</sub>), 7.48(s, 1H, H<sub>phenyl</sub>), 7.70-7.72(m, 1H, H<sub>phenyl</sub>). MS (EI) *m/z* (%): 445(M<sup>+</sup>,10), 410(100), 343(18), 297(10), 213(11), 186(18), 105(9), 57 (18).

**(E)-16-(4-Fluorobenzylidene)-1,3,4,7,8,9,10,11,12,13,15,16-dodecahydro-3-hydroxy-10,13-dimethyl-2H-cyclopenta[a]phenanthren-17(14H)-one (1e)**

Yield: 46%; mp=234-236°C; IR (KBr,  $\nu_{\max}$ , cm<sup>-1</sup>): 3426 (OH), 1716(C=O). <sup>1</sup>HNMR (400 MHz, CDCl<sub>3</sub>): 0.98(s, 3H, CH<sub>3</sub>), 1.07(s, 3H, CH<sub>3</sub>), 3.48-3.58(m, 1H, CH-OH), 5.40(s, 1H, H<sub>vinyl</sub>), 7.10(t, 1H, H<sub>phenyl</sub>, *J*= 8.5 Hz), 7.52(t, 1H, H<sub>phenyl</sub>, *J*= 8.5Hz). MS (EI) *m/z* (%): 395(M<sup>+</sup>, 38), 377(10), 284(10), 232(32), 203(39), 145(25), 134(100), 109(25), 82(12).

**(E)-16-(3-Bromobenzylidene)-1,3,4,7,8,9,10,11,12,13,15,16-dodecahydro-3-hydroxy-10,13-dimethyl-2H-cyclopenta[a]phenanthren-17(14H)-one (1f)**

Yield: 28%; mp=222-234°C, IR (KBr,  $\nu_{\max}$ , cm<sup>-1</sup>): 3471 (OH), 1709 (C=O). <sup>1</sup>HNMR (400 MHz, CDCl<sub>3</sub>):0.95(s, 3H, CH<sub>3</sub>), 1.04(s, 3H, CH<sub>3</sub>), 3.48-3.60 (m, 1H, CH-OH), 5.40(s, 1H, H<sub>vinyl</sub>), 7.29(t, 1H, H<sub>phenyl</sub>, *J*= 6.4Hz), 7.35(s, 1H, H<sub>phenyl</sub>), 7.46(d, 1H, H<sub>phenyl</sub>, *J*= 6.4Hz), 7.49(d, 1H, H<sub>phenyl</sub>, *J*= 6.4Hz). MS (EI) *m/z* (%): 456(M<sup>+</sup>, 26), 454(M<sup>+</sup>, 26), 436(46), 424(32), 343(26), 315(18), 263(32), 213 (38).

**(E)-16-(5-Bromo-2-hydroxybenzylidene)-1,3,4,7,8,9,10,11,12,13,15,16-dodecahydro-3-hydroxy-10,13-dimethyl-2H-cyclopenta[a]phenanthren-17(14H)-one (1g)**

Yield: 46%; mp=208-210°C; IR (KBr,  $\nu_{\max}$ , cm<sup>-1</sup>): 3464 (OH), 1709(C=O). <sup>1</sup>HNMR (400 MHz, CDCl<sub>3</sub>): 1.00(s, 3H, CH<sub>3</sub>), 1.07 (s, 3H, CH<sub>3</sub>), 3.42-3.60(m, 1H, CH-OH),5.40(s, 1H, H<sub>vinyl</sub>), 6.83(dd, 1H, H<sub>phenyl</sub>, *J*= 8.4Hz), 7.32(dd, 1H, H<sub>phenyl</sub>, *J*= 8.4Hz), 7.52(d, 1H, H<sub>phenyl</sub>, *J*= 8.4Hz). MS (EI) *m/z* (%): 472 (M<sup>+</sup>+2, 95), 470(M<sup>+</sup>, 95).

**(E)-16-(2-(Trifluoromethyl)benzylidene)-1,3,4,7,8,9,10,11,12,13,15,16-dodecahydro-3-hydroxy-10,13-dimethyl-2H-cyclopenta[a]phenanthren-17(14H)-one (1h)**

Yield: 34%; mp = 208-210°C; IR (KBr,  $\nu_{\max}$ , cm<sup>-1</sup>): 3456(OH), 1724(C=O).<sup>1</sup>HNMR (400 MHz, CDCl<sub>3</sub>): 1.00(s, 3H, CH<sub>3</sub>), 1.06 (s, 3H, CH<sub>3</sub>), 3.48-3.60(m, 1H, CH-OH), 5.38(s, 1H, H<sub>vinyl</sub>), 7.42-48(m, 1H, H<sub>phenyl</sub>), 7.56-7.60 (m, 2H, H<sub>phenyl</sub>), 7.70-7.75(m, 2H, H<sub>phenyl</sub>-H<sub>vinyl</sub>). MS (EI) *m/z* (%): 445 (M<sup>+</sup>+1, 14), 444(M<sup>+</sup>, 100).

**(E)-16-(4-(Trifluoromethyl)benzylidene)-1,3,4,7,8,9,10,11,12,13,15,16-dodecahydro-3-hydroxy-10,13-dimethyl-2H-cyclopenta[a]phenanthren-17(14H)-one (1i)**

Yield: 27%; mp=244-246°C; IR (KBr,  $\nu_{\max}$ , cm<sup>-1</sup>): 3215 (OH), 1708(C=O). <sup>1</sup>HNMR (400 MHz, CDCl<sub>3</sub>): 0.99(s, 3H, CH<sub>3</sub>), 1.08(s, 3H, CH<sub>3</sub>), 3.48-3.60(m, 1H, CH-OH), 7.40(s, 1H, H<sub>vinyl</sub>), 7.38-7.46(m, 2H, H<sub>phenyl</sub>), 7.64(dd, 2H, H<sub>phenyl</sub>, *J*=8.8Hz). MS (EI) *m/z* (%): 445 (M<sup>+</sup>+1, 21), 444(M<sup>+</sup>, 100).

**(E)-16-(4-Methylbenzylidene)-1,3,4,7,8,9,10,11,12,13,15,16-dodecahydro-3-hydroxy-10,13-dimethyl-2H-cyclopenta[a]phenanthren-17(14H)-one (1j)**

Yield: 78%; mp=238-240°C; IR (KBr,  $\nu_{\max}$ , cm<sup>-1</sup>): 3421 (OH), 1716(C=O). <sup>1</sup>HNMR (400 MHz, CDCl<sub>3</sub>): 0.98(s, 3H, CH<sub>3</sub>), 1.07(s, 3H, CH<sub>3</sub>), 1.57(s, 3H, CH<sub>3</sub>), 3.45-3.50 (m, 1H, CH-OH), 5.42(s, 1H, H<sub>vinyl</sub>), 7.25(dd, 1H, H<sub>phenyl</sub>, *J*=8.4Hz), 7.44(dd, 1H, H<sub>phenyl</sub>, *J*=8.4 Hz). MS (EI) *m/z* (%): 391 (M<sup>+</sup>+1, 9), 390 (M<sup>+</sup>, 44), 376(4), 131 (100).

**(E)-16-(4-Methoxybenzylidene)-1,3,4,7,8,9,10,11,12,13,15,16-dodecahydro-3-hydroxy-10,13-dimethyl-2H-cyclopenta[a]phenanthren-17(14H)-one(1k)**

Yield: 25%; mp =225-227°C; IR (KBr,  $\nu_{\max}$ , cm<sup>-1</sup>): 3454 (OH), 1712(C=O). <sup>1</sup>HNMR (400 MHz, CDCl<sub>3</sub>): 0.97(s, 3H, CH<sub>3</sub>), 1.07(s, 3H, CH<sub>3</sub>), 3.85(s, 3H, OCH<sub>3</sub>), 3.50-3.60(m, 1H, CH-OH), 5.40(s, 1H, H<sub>vinyl</sub>), 6.94(dd, 1H, H<sub>phenyl</sub>, *J*=8.4Hz), 7.40(s, 1H, H<sub>vinyl</sub>), 7.51(dd, 1H, H<sub>phenyl</sub>, *J*=8.4Hz). MS (EI) *m/z* (%): 407(M<sup>+</sup>+1, 7), 406(M<sup>+</sup>, 29), 408(100).

**(E)-16-(2,3,4-Trimethoxybenzylidene)-1,3,4,7,8,9,10,11,12,13,15,16-dodecahydro-3-hydroxy-10,13-dimethyl-2H-cyclopenta[a]phenanthren-17(14H)-one (1l)**

Yield: 20%; mp=199-201°C; IR (KBr,  $\nu_{\max}$ , cm<sup>-1</sup>): 3429 (OH), 1717(C=O).<sup>1</sup>HNMR (400 MHz, CDCl<sub>3</sub>): 0.98(s, 3H, CH<sub>3</sub>), 1.07(s, 3H, CH<sub>3</sub>), 3.90(s, 9H, 3OCH<sub>3</sub>), 5.39(s, 1H, H<sub>vinyl</sub>), 6.72(d, 1H, H<sub>phenyl</sub>, *J*=8.4Hz), 7.27(d, 1H, H<sub>phenyl</sub>, *J*=8.4Hz). MS (EI) *m/z* (%):467(M<sup>+</sup>+1, 28), 466 (M<sup>+</sup>, 100).

**(E)-16-(4-(Dimethylamino)benzylidene)-1,3,4,7,8,9,10,11,12,13,15,16-dodecahydro-3-hydroxy-10,13-dimethyl-2H-cyclopenta[a]phenanthren-17(14H)-one (1m)**

Yield: 35%; mp=216-218°C; IR (KBr,  $\nu_{\max}$ , cm<sup>-1</sup>): 3521 (OH), 1721(C=O). <sup>1</sup>HNMR (400 MHz, CDCl<sub>3</sub>): 0.96(s, 3H, CH<sub>3</sub>), 1.07(s, 3H, CH<sub>3</sub>), 3.03(s, 6H, 2CH<sub>3</sub>), 3.42-3.60 (m, 1H, CH-OH), 5.42(s, 1H, H<sub>vinyl</sub>), 6.71(d, 1H, H<sub>phenyl</sub>, *J*= 8.3Hz), 7.47(d, 1H, H<sub>phenyl</sub>, *J*= 8.3Hz). MS (EI) *m/z* (%): 420(M<sup>+</sup>+1, 32), 419(M<sup>+</sup>, 10).

## Biological assay

### Cell lines and cell culture

The synthesized compounds were tested against three different human cancer cell lines including KB (human nasopharyngeal epidermoid carcinoma), T47D (human breast cancer) and SK-N-MC (human neuroblastoma) cells. The cell lines were purchased from the National Cell Bank of Iran (NCBI). The cells were grown in RPMI- 1640 medium (Gibco BRL) supplemented with 10% heat inactivated fetal calf serum (Gibco BRL), 100 µg/mL streptomycin, and 100 U/mL penicillin, in a humidified air atmosphere at 37°C with 5% CO<sub>2</sub>.

### In vitro cytotoxicity assay

The *in vitro* cytotoxic activity of each synthesized derivatives 1a-m was investigated using MTT colorimetric assay [35]. Briefly, each cell line in log-phase of growth was harvested by trypsinization followed by resuspension in complete growth medium to give a total cell count of  $5 \times 10^4$  cells/ml. The resulted cell suspension was seeded into the wells of 96-well plates (Nunc, Denmark). The plates were incubated overnight in a humidified air atmosphere at 37°C with 5% CO<sub>2</sub>. After the incubation period, 5 µL of the media containing various concentrations of the compounds was added per well in triplicate followed by further incubation for 24 h. The final maximum concentration of DMSO was 0.1%. Etoposide was used as positive control for cytotoxic activity, while three different wells containing evaluated cancer cells cultured in 200 µL of complete medium were used as negative controls of cell viability. After incubation, the medium was discarded and 200 µl phenol red-free RPMI containing MTT (final concentration 1 mg/ml), was added to each well. The test plate was incubated for 4h. The culture medium was then replaced with 100 µL of DMSO and the absorbance of each well was measured by using a micro plate reader (Gen5, Power wave xs2, BioTek, America) at 492 nm. Each set of experiments was independently performed three times. The concentration causing 50% cell growth

inhibition (IC<sub>50</sub>) compared with the control was calculated using concentration-response curves by regression analysis.

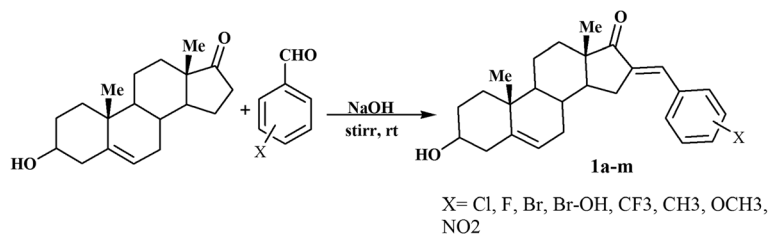
## Results and discussion

The benzylidene-substituted DHEA derivatives 1a-m were synthesized through the aldol condensation [36] of DHEA with corresponding benzaldehyde derivatives (Figure 2).

The *in vitro* cytotoxic activity of synthesized compounds 1a-m was investigated against three different cancer cell lines including KB, T47D and SK-N-M cells. The percentage of growth inhibition was assessed using MTT reduction assay versus controls not treated with test compounds. The 50% growth inhibitory concentration (IC<sub>50</sub>) for each compound was determined and presented in Table 1. The data for etoposide was also included.

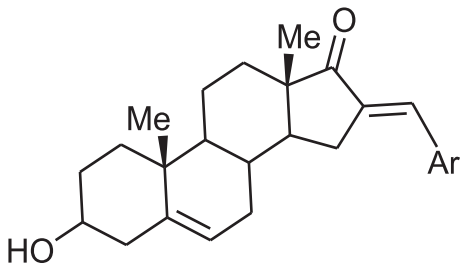
The results of cytotoxic data indicate that most of synthesized compounds showed moderate to strong cytotoxic potential in all three cell lines. Based on the cytotoxic data, the following structure-activity relationship may be developed:

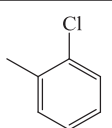
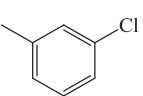
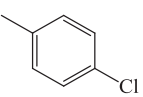
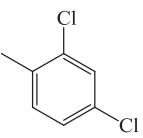
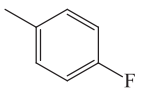
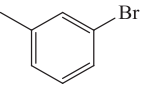
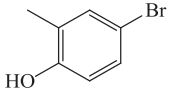
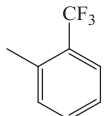
- Introduction of different substitutes such as chlorine, trifluoromethyl, methoxy and methyl groups into the *ortho* or *meta* position of benzylidene moiety, resulted in enhanced cytotoxic potential of benzylidene derivatives of DHEA.
- The compounds containing chlorine, nitro and fluorine substitutes at *para* position of benzylidene pendant, were almost inactive against all three evaluated cell lines (IC<sub>50</sub>>100 µM). Whereas, substitution of methoxy, methyl and trifluoromethyl groups into the *para* position (compounds 1i-j), resulted in enhanced cytotoxic potential of corresponding derivatives, e.g. the corresponding IC<sub>50</sub> values of *para*-methyl



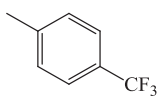
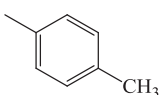
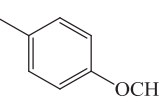
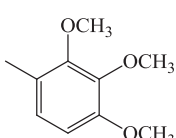
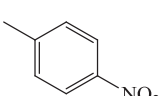
**Figure 2** Synthetic protocol for compounds 1a-m.

**Table 1 Chemical structures and in vitro cytotoxic activity of compounds 1a-m assessed by MTT reduction assay**



Compound	Ar	IC <sub>50</sub> (μM) <sup>a</sup>		
		KB	T47D	SK-N-MC
1a		2.9(±10.1)	9.6 ±3.1	13.2± 2.2
1b		0.6(±2.0)	1.7±14.8	10.0± 3
1c		>100	>100	>100
1d		>100	>100	3.6±13.26
1e		>100	>100	>100
1f		6.5(±21.1)	>100	3.6±40.7
1g		>100	>100	16.3(±55.9)
1h		1.2(±3.3)	3.6(±7.3)	2.7(±3.6)

**Table 1 Chemical structures and in vitro cytotoxic activity of compounds 1a-m assessed by MTT reduction assay (Continued)**

1i		2.5(±8.3)	2.4(±9.6)	2.0(±5.3)
1j		1.7(±12.5)	7.6(±17.4)	1.0(±17.4)
1k		1.3(±18)	4.1(±12.3)	5.9(±14.1)
1l		>100	>100	>100
1m		>100	>100	>100
<b>Etoposide</b>	-	2.8(±16.8)	1.2(±8)	3.9(±8.3)

<sup>a</sup> Values in parentheses represent the average of 3–4 experiments ± S.E.M.

benzylidene derivative 1j in KB, T47D and SK-N-MC cell lines were 1.7, 7.6 and 1.0 μM, respectively.

- The 3-chloro benzylidene derivatives of DHEA, compound 1b, was the most potent synthesized derivative especially against KB and T47D cell lines (IC<sub>50</sub> values were 0.6 and 1.7 μM; respectively) which were comparable with etoposide (IC<sub>50</sub>= 2.8 and 1.2 μM; respectively).

Based on the above finding it might be deduced that 16-(substituted benzylidene) derivatives of DHEA could be served as a potent anti-cancer agents. The cytotoxic potential of described compounds is mainly attributed to the position and nature of the substituted group on the benzylidene pendant. The *ortho* or *meta* positions of the benzylidene group could well accommodate different substitute in order to afford potent cytotoxic derivatives of this types.

## Conclusion

A new series of cytotoxic 16-(substituted benzylidene) derivatives of DHEA were synthesized and evaluated

against three different cancer cells including KB, T47D and SK-N-MC cell lines by MTT reduction colorimetric assay. The cytotoxic potential of these novel benzylidene derivatives of DHEA is mainly attributed to the position and nature of the substituted group on the benzylidene pendant.

#### Competing interests

The authors declare that they have no competing interests.

#### Authors' contributions

MV: Design and synthesis of target compounds. HY: Synthesis of some intermediates and target compounds, KD: performing the biological tests, HS: Synthesis of some target compounds, MS: performing the cytotoxic test, SKA: Supervision of biological tests, NM: collaboration in identification of synthesized compounds, NE: collaboration in identifying of the structures of target compounds, manuscript preparation, MK: collaboration in manuscript preparation, AS: Collaboration in identifying the structures of target compounds, AF: Design of target compounds and supervision of the synthetic and pharmacological parts. All authors read and approved the final manuscript.

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