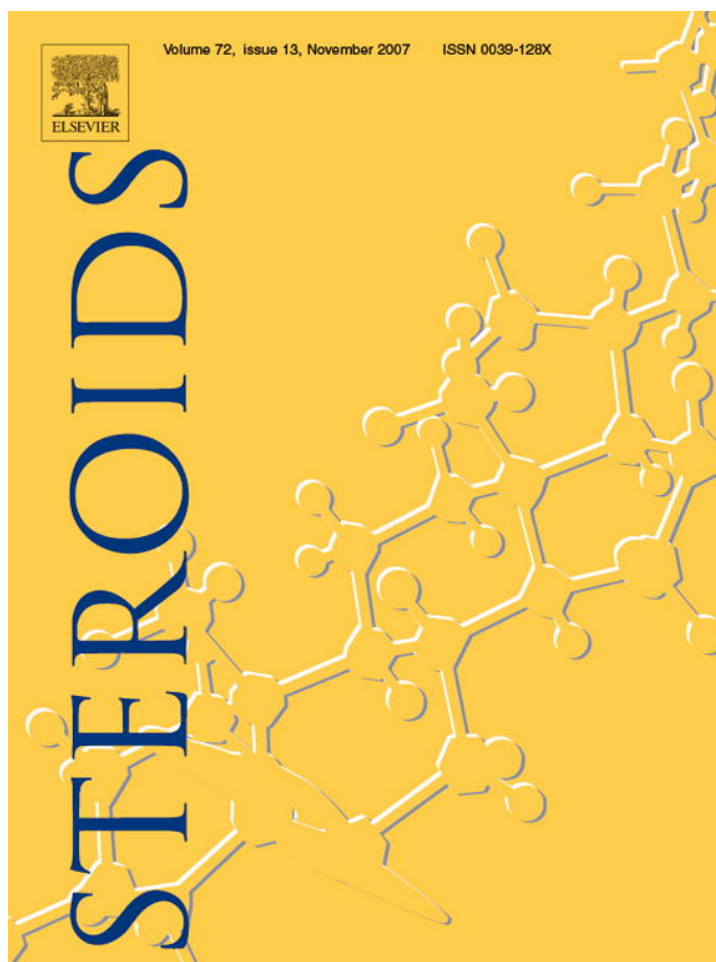




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Synthesis of chalcone derivatives on steroidal framework and their anticancer activities[☆]

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ABSTRACT

Chalcone derivatives on estradiol framework have been synthesized. Some of the derivatives showed potent anticancer activity against some human cancer cell lines. Compounds **9** and **19** showed potent activity against MCF-7, a hormone dependent breast cancer cell line. Chalcone **7** was further modified to the corresponding indanone derivative (**19**) using the Nazarov reaction, which showed better activity than the parent compound against the MCF-7 breast cancer cell line. Active anticancer derivatives were also evaluated for osmotic hemolysis using the erythrocyte as a model system. It was observed that chalcone derivatives showing cytotoxicity against cancer cell lines did not affect the fragility of erythrocytes and hence may be considered as non-toxic to normal cells.

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1. Introduction

Chalcone moieties are common substructures in numerous natural products belonging to the flavonoid family [1–4]. Chalcone derivatives are very versatile as physiologically active compounds and substrates for the evaluation of various organic syntheses. These compounds have been reported to possess several biological activities, such as cytotoxic [5–8] antimalarial [9,10], antileishmanial [11,12], anti-inflammatory [13,14], anti-HIV [15], antifungal [16] and as tyrosine kinase inhibitors [17]. Having such varied pharmacological activities, these molecules have attracted medicinal chemists and therefore several strategies have been developed to synthesize them.

Breast cancer is one of the most common cancers in women. Due to various reasons, the estrogen receptors are over-expressed in these tumour cells and, hence, estrogenicity is enhanced by many folds leading to excessive proliferation [18]. Steroids are a fundamental class of biological signaling molecules with profound chemical, clinical and scientific significance [19]. Steroids elicit their diverse biological actions via different functional groups located around the periphery of their rigid tetracyclic core. A major research target today is the preparation of novel steroid molecules with reactions chemically simpler and easier to prepare. This would also provide a platform to approach the synthesis of new drugs for tackling important biological problems. In the recent past, two steroidal molecules, 2-methoxyestradiol and fulvestrant

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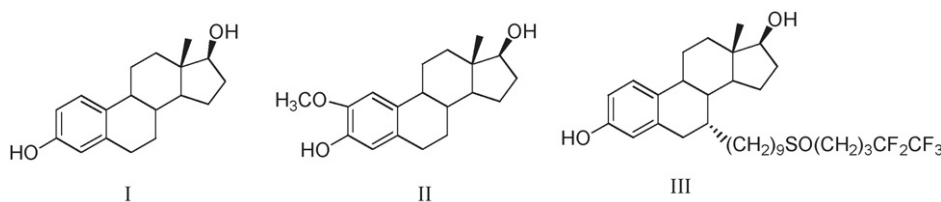


Fig. 1 – Structures of estradiol (I), 2-methoxyestradiol (II) and fulvestrant (III).

(Fig. 1), have emerged as potent anticancer agents against breast cancer [20]. 2-Methoxyestradiol has successfully completed Phase II clinical trials [21]. It acts as a tubulin polymerisation inhibitor by interacting at the colchicine site [22] and also as an antiangiogenic agent by acting at the tumour vascular system. On the other hand, fulvestrant, which acts as a pure antiestrogen competing with estradiol for its receptor binding [23], has already completed phase III clinical trials [24]. US-FDA approved fulvestrant in 2002 for the treatment of hormone dependent advanced breast cancer, while the European Union approved it in 2004 for the same purpose.

The anticancer activity of various chalcone molecules is well studied and chalcone with a trimethoxyphenyl unit has been reported to be the most cytotoxic (compound 1; $IC_{50}=0.21\text{ nM}$) derivative synthesized so far [25]. Synthesis of chalcones on steroids has not been studied so far. Thus, we synthesized various chalcone derivatives on a steroid frame. Some of them showed potent cytotoxicity against the MCF-7 hormone dependent breast cancer cells (Fig. 2). The effect of these chalcones was also studied on the erythrocytes osmotic fragility, a model system used to evaluate the effect of chalcones on membrane stability of a cell at the same concentration at which anticancer activity was observed.

2. Experimental

2.1. General

Estrone was procured from Sigma chemicals, USA. Melting points were determined on Toshniwal melting point (electrothermal) apparatus and were uncorrected. Dry solvents were prepared as per reported methods. All the reactions were performed as per standard procedures and monitored on Merck aluminium thin layer chromatography (TLC, $UV_{254\text{ nm}}$) plates. TLC visualization was accomplished by spraying with a solution of 2% ceric sulphate in 10% aqueous sulphuric acid and charring at $100\text{--}110^\circ\text{C}$. Column chromatography was carried out on silica gel (60–120 mesh, MERCK chemicals). NMR experiments were obtained on Bruker Avance-300 MHz instrument with tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in δ ppm values. All the ^1H and necessary ^{13}C spectral data are reported. EI mass spectra were recorded on Perkin-Elmer TurboMass after dissolving the compounds in methanol, while ESI mass spectra were recorded on Shimadzu LC-MS after dissolving the compounds in methanol. FT-IR spectra were recorded on Perkin-Elmer SpectrumBX. Nomenclature of steroid derivatives has been given as per the

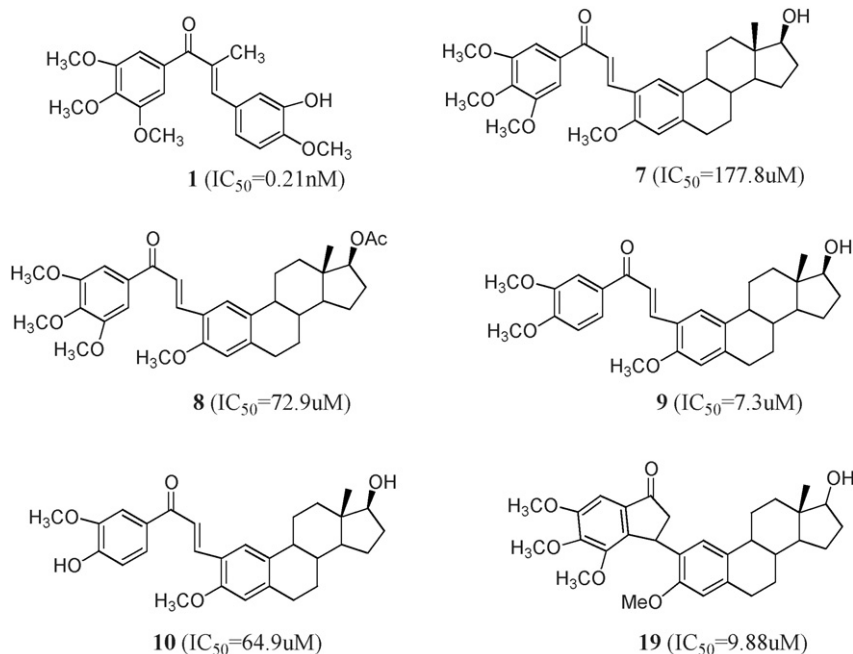


Fig. 2 – Most active chalcone (1) reported earlier and some of the potent steroidal chalcones and indanone against MCF-7 breast cancer cell lines.

recommendations published by the Joint Commission on the Biochemical Nomenclature (JCBN) of IUPAC [26]. All the compounds were screened against five human cancer cell lines by MTT assay for cytotoxic evaluation and compounds showing potent cytotoxicity were further evaluated for erythrocyte osmotic fragility test to determine their toxicities.

2.2. Chemical syntheses

2.2.1. Synthesis of 2-formyl,3-methoxyestra-1,3,5(10)-trien-17 β -acetate (6)

Estra 1,3,5(10)-trien-3,17 β -diol-3-methyl ether, 17 β -acetate (5), (100 mg, 0.35 mmol) was taken in dry dimethylformamide (0.2 mL, 2.59 mmol). The reaction mixture was stirred at 5–10 °C by using an ice bath. After 10 min, phosphorus oxychloride (0.2 mL, 2.26 mmol) was added dropwise to the reaction mixture. It was kept at this temperature for 40 min and then heated at 80 °C for 3 h. After completion, the reaction mixture was poured into crushed ice and extracted with chloroform (3 $\times$ 25 mL). The organic layer was washed with water, dried over anhydrous sodium sulphate and concentrated in vacuum. The residue thus obtained was purified through a silica gel (60–120 mesh) column by eluting with 8–10% hexane–ethyl acetate. Compound 6 was recrystallised with hexane–chloroform (4:1) to get a creamish white solid (39 mg).

Yield 36.0%, m.p. 170–171 °C. ¹H NMR (CDCl₃, 300 MHz): δ 0.76 (s, 3H, 18-CH₃), 1.99 (s, 3H, OAc), 1.19–2.28 (m, 13H, rest of the 5XCH₂ and 3XCH of steroidal ring), 2.85 (bs, 2H, 6-CH₂), 3.81 (s, 3H, OCH₃), 4.61 (t, 1H, 17-CH, J = 8.33 Hz), 6.60 (s, 1H, 4-CH), 7.68 (s, 1H, 1-CH), 10.32 (s, 1H, CHO). ¹³C NMR (CDCl₃, 75 MHz): δ 12.3, 21.2, 23.6, 26.5, 27.3, 28.0, 30.7, 37.2, 38.9, 43.3, 44.0, 50.4, 56.0, 82.9, 112.3, 123.6, 125.9, 133.6, 146.2, 160.3, 171.1, 189.5. Electrospray mass (MeOH): 357.0 [M + H]⁺, 378.9 [M + Na]⁺, 735.1 [2M + Na]⁺. IR: 2933, 2870, 1733, 1674, 1608, 1495, 1270, 1027.

2.2.2. General procedure for the synthesis of chalcones

2.2.2.1. Synthesis of 1-(3,4,5-trimethoxyphenyl)-3-[methoxyestra-1,3,5(10)-trien-17 β -ol,2-yl]-2-propen-1-one (7). Potassium hydroxide solution (7% (w/v), 15 mL) in ethanol–water (1:2) was cooled to 10 °C. Compound 6 (100 mg, 0.28 mmol) and 3,4,5-trimethoxy acetophenone (84 mg, 0.4 mmol) were added to this stirred solution. The reaction mixture was further stirred at this temperature for 3 h and then kept in refrigerator (5–10 °C) overnight. Next day, the reaction mixture was poured into crushed ice, acidified with diluted HCl (10%, v/v), extracted with chloroform (3 $\times$ 20 mL) and washed with water. The organic layer was dried over anhydrous sodium sulphate and evaporated to dryness. The residue thus obtained was column chromatographed over silica gel (60–120 mesh) and eluted with 15% ethyl acetate–hexane to obtain the desired chalcone (7). It was recrystallised with chloroform–hexane (1:4) to get a light yellowish solid.

Yield 73.0%, m.p. 179–182 °C. ¹H NMR (CDCl₃, 300 MHz): δ 0.74 (s, 3H, 18-CH₃), 1.21–1.95 (m, 13H, rest of the 5XCH₂ and 3XCH of steroidal ring), 2.84 (bs, 2H, 6-CH₂), 3.69 (t, 1H, 17-CH, J = 8.35 Hz), 3.83 (s, 3H, OCH₃), 3.88 (s, 3H, OCH₃), 3.89 (s, 6H, 2X OCH₃), 6.60 (s, 1H, 4-CH of steroidal ring), 7.20 (s, 2H, 2'' & 6''-CH of benzoyl moiety), 7.43–7.49 (d, 1H, =CH–CO, J = 15.80 Hz), 7.46 (s, 1H, 1-CH of steroidal ring), 7.93–7.98 (d, 1H, CH=C–CO,

J = 15.85 Hz). ¹³C NMR (CDCl₃, 75.47 MHz): δ 11.4, 23.5, 26.7, 27.5, 30.5, 31.0, 37.1, 39.2, 43.7, 44.2, 50.6, 55.9, 56.8, 56.8, 61.2, 82.1, 107.1, 112.0, 122.0, 122.8, 133.4, 134.5, 141.5, 141.7, 143.0, 153.5, 157.3, 190.9. Electrospray Mass (MeOH): 507.1 [M + H]⁺, 529.0 [M + Na]⁺. IR: 3448, 2930, 1656, 1580, 1502, 1251, 1124.

2.2.2.2. Synthesis of 1-(3,4,5-Trimethoxyphenyl)-3-[methoxyestra-1,3,5(10)-trien-17 β -acetate,2-yl]-2-propen-1-one (8). Substrate 7 (100 mg, 0.19 mmol) in dry pyridine:acetic anhydride (2:1) was left overnight at room temperature. On completion, the reaction mixture was poured into cold water to get 8 as a creamish white solid.

Yield 86%, m.p. 133–35 °C. ¹H NMR (CDCl₃, 300 MHz): δ 0.84 (s, 3H, 18-CH₃), 1.25–2.32 (m, 13H, rest of the 5XCH₂ and 3XCH of steroidal ring), 2.06 (s, 3H, OAc), 2.90 (bs, 2H, 6-CH₂), 3.84 (t, 1H, 18-CH, J = 8.35 Hz), 3.87 (s, 3H, 2X OCH₃), 3.93 (s, 3H, OCH₃), 4.69 (t, 1H, 17-CH, J = 8.92 Hz), 6.65 (s, 1H, 4-CH of steroidal ring), 7.25 (s, 2H, 2'' & 6''-CH of benzoyl moiety), 7.48–7.54 (d, 1H, =CH–CO, J = 15.75 Hz), 7.50 (s, 1H, 1-CH of steroidal ring), 7.98–8.03 (d, 1H, CH=C–CO, J = 15.80 Hz). EI Mass (MeOH): 548 [M]⁺, 533, 518, 517, 488, 458, 457. IR: 2938, 1732, 1678, 1581, 1502, 1248.

2.2.2.3. Synthesis of 1-(3,4-dimethoxyphenyl)-3-[methoxyestra-1,3,5(10)-trien-17 β -ol,2-yl]-2-propen-1-one (9). Same procedure as for 7. Yield 60.0%, oil, ¹H NMR (CDCl₃, 300 MHz): δ 0.79 (s, 3H, 18-CH₃), 1.23–2.10 (m, 13H, rest of the 5XCH₂ and 3XCH of steroidal ring), 2.90 (bs, 2H, 6-CH₂), 3.75 (t, 1H, 17-CH, J = 8.4 Hz), 3.88 (s, 3H, OCH₃), 3.97 (s, 6H, 2XOCH₃), 6.65 (s, 1H, 4-CH of steroidal ring), 6.94 (d, 1H, 5-CH of benzoyl moiety, J = 8.3 Hz), 7.53 (s, 1H, 1-CH of steroidal ring), 7.59–7.64 (d, 1H, =CH–CO, J = 15.74 Hz), 7.62 (d, 1H, 2-CH of benzoyl moiety, J = 1.78 Hz), 7.67 (dd, 1H, 6-CH of benzoyl moiety, J = 8.36, 1.82 Hz), 8.01–8.06 (d, 1H, CH=C–CO, J = 15.75 Hz). EI Mass (MeOH): 476 [M]⁺, 458, 428. IR: 3426, 2925, 1652, 1595, 1511, 1263, 1024.

2.2.2.4. Synthesis of 1-(3-methoxy,4-hydroxyphenyl)-3-[methoxyestra-1,3,5(10)-trien-17 β -ol,2-yl]-2-propen-1-one (10). Same procedure as for 7. Yield 37%, m.p. 78 °C. ¹H NMR (CDCl₃, 300 MHz): δ 0.80 (s, 3H, 18-CH₃), 1.25–2.16 (m, 13H, rest of the 5XCH₂ and 3XCH of steroidal ring), 2.89 (bs, 2H, 6-CH₂), 3.78 (t, 1H, 17-CH, J = 8.4 Hz), 3.88 (s, 3H, OCH₃), 3.99 (s, 3H, OCH₃), 6.65 (s, 1H, 4-CH of steroidal ring), 6.99 (d, 1H, 5-CH of benzoyl moiety, J = 8.64 Hz), 7.52 (s, 1H, 2-CH of benzoyl moiety), 7.58–7.64 (d, 1H, =CH–CO, J = 15.78 Hz), 7.65 (s, 1H, 1-CH of steroidal ring), 8.01–8.06 (d, 1H, CH=C–CO, J = 15.78 Hz). EI Mass (MeOH): 462 [M]⁺, 400, 399. IR: 3321, 2929, 1661, 1606, 1516, 1290, 1192, 1026.

2.2.2.5. Synthesis of 1-(3,4-methylenedioxyphenyl)-3-[methoxyestra-1,3,5(10)-trien-17 β -ol,2-yl]-2-propen-1-one (11). Same procedure as for 7. Yield 44.0%, oil, ¹H NMR (CDCl₃, 300 MHz): δ 0.82 (s, 3H, 18-CH₃), 1.23–2.37 (m, 13H, rest of the 5XCH₂ and 3XCH of steroidal ring), 2.89 (bs, 2H, 6-CH₂), 3.75 (t, 1H, 17-CH, J = 7.85 Hz), 3.88 (s, 3H, OCH₃), 6.06 (s, 2H, –O–CH₂–O–), 6.64 (s, 1H, 4-CH of steroidal ring), 6.89 (d, 1H, 4-CH of benzoyl moiety, J = 8.1 Hz), 7.53–7.58 (d, 1H, =CH–CO, J = 15.54 Hz), 7.63 (s, 1H, 2-CH of benzoyl moiety), 7.63–7.66 (dd, 1H, 6-CH of benzoyl moiety), 8.01–8.07 (d, 1H, CH=C–CO, J = 15.72 Hz). EI

Mass (MeOH): 460 [M]⁺, 445, 427. IR: 3402, 2921, 1656, 1605, 1251, 1034.

2.2.2.6. Synthesis of 1-(4-methylphenyl)-3-[methoxyestra-1,3,5(10)-trien-17 β -ol,2-yl]-2-propen-1-one (12). Same procedure as for 7. Yield 52.0%, oil, ¹H NMR (CDCl₃, 300 MHz): δ 0.80 (s, 3H, 18-CH₃), 1.26–2.36 (m, 13H, rest of the 5XCH₂ and 3XCH of steroidal ring), 2.89 (bs, 2H, 6-CH₂), 3.72 (bt, 1H, 17-CH), 3.88 (s, 3H, OCH₃), 6.67 (s, 1H, 4-CH of steroidal ring), 7.29 (d, 2H, 3 & 5-CH of benzoyl moiety, J =8.0 Hz), 7.52 (s, 1H, 1-CH of steroidal ring), 7.59–7.61 (d, 1H, =CH–CO, J =15.8 Hz), 7.92 (d, 2H, 2 & 6-CH of benzoyl moiety, J =8.1 Hz), 8.03–8.08 (d, 1H, CH=C–CO, J =15.8 Hz). ¹³C NMR (CDCl₃, 75.47 MHz): δ 11.4, 21.9, 23.5, 26.7, 27.5, 30.4, 31.0, 37.1, 39.2, 43.7, 44.2, 50.6, 56.0, 82.2, 112.1, 122.1, 122.7, 127.0, 129.3, 129.3, 129.5, 129.5, 133.3, 136.7, 141.0, 141.5, 143.3, 157.3, 191.3. Electrospray mass (MeOH): 431.1 [M+H]⁺, 883.2 [2M+Na]⁺. IR: 3429, 2926, 1656, 1605, 1590, 1499, 1258, 1025.

2.2.2.7. Synthesis of 1-(2,4-dimethylphenyl)-3-[methoxyestra-1,3,5(10)-trien-17 β -ol,2-yl]-2-propen-1-one (13). Same procedure as for 7. Yield 46.0%, m.p. 164–166 °C. ¹H NMR (CDCl₃, 300 MHz): δ 0.78 (s, 3H, 18-CH₃), 1.26–2.21 (m, 13H, rest of the 5XCH₂ and 3XCH of steroidal ring), 2.37 (s, 3H, CH₃), 2.42 (s, 3H, CH₃), 2.87 (bs, 2H, 6-CH₂), 3.74 (t, 1H, 17-CH, J =8.38 Hz), 3.83 (s, 3H, OCH₃), 6.62 (s, 1H, 4-CH of steroidal ring), 7.07 (d, 1H, 5-CH of benzoyl moiety, J =7.3 Hz), 7.22 (d, 1H, =CH–CO, J =16.1 Hz), 7.26 (s, 1H, 3-CH), 7.41 (s, 1H, 1-CH of steroidal ring), 7.44 (d, 1H, 6-CH of benzoyl moiety), 7.73–7.78 (d, 1H, CH=C–CO, J =16.2 Hz). ¹³C NMR (CDCl₃, 75.46 MHz): δ 11.4, 20.6, 21.6, 23.5, 26.7, 27.5, 30.4, 31.0, 37.1, 39.2, 43.7, 44.2, 50.6, 55.9, 82.2, 112.1, 121.9, 126.5, 126.7, 127.1, 128.9, 132.4, 133.4, 137.4, 140.7, 141.7, 141.7, 157.2, 197.0. Electrospray Mass (MeOH): 445.1 [M+H]⁺, 467 [M+Na]⁺, 911.2 [2M+Na]⁺. IR: 3492, 2924, 1650, 1608, 1500, 1256, 1026.

2.2.2.8. Synthesis of 1-(4-chlorophenyl)-3-[methoxyestra-1,3,5(10)-trien-17 β -ol,2-yl]-2-propen-1-one (14). Same procedure as for 7. Yield 41.0%, oil, ¹H NMR (CDCl₃, 300 MHz): δ 0.796 (s, 3H, 18-CH₃), 1.31–2.37 (m, 13H, rest of the 5XCH₂ and 3XCH of steroidal ring), 2.90 (bs, 2H, 6-CH₂), 3.73 (distorted t, 1H, 17-CH), 3.88 (s, 3H, OCH₃), 6.65 (s, 1H, 4-CH of steroidal ring), 7.29 (s, 1H, 1-CH of steroidal ring), 7.46 (d, 2H, 3 & 5-CH of benzoyl moiety, J =8.4 Hz), 7.52–7.57 (d, 1H, =CH–CO, J =16 Hz), 7.95 (d, 2H, 2 & 6-CH of benzoyl moiety, J =8.43 Hz), 8.02–8.07 (d, 1H, CH=C–CO, J =15.78 Hz). Electrospray Mass (MeOH): 451.0 [M+H]⁺. IR: 3506, 2925, 1656, 1591, 1498, 1257, 1092.

2.2.2.9. Synthesis of 1-(2,4-dichlorophenyl)-3-[methoxyestra-1,3,5(10)-trien-17 β -ol,2-yl]-2-propen-1-one (15). Same procedure as for 7. Yield 35.0%, oil, ¹H NMR (CDCl₃, 300 MHz): δ 0.78 (s, 3H, 18-CH₃), 1.22–2.14 (m, 13H, rest of the 5XCH₂ and 3XCH of steroidal ring), 2.86 (bs, 2H, 6-CH₂), 3.69 (distorted t, 1H, 17-CH), 3.83 (s, 3H, OCH₃), 6.60 (s, 1H, 4-CH of steroidal ring), 7.15–7.21 (d, 1H, =CH–CO, J =16.2 Hz), 7.33 (dd, 1H, 5-CH of benzoyl moiety, J =8.2 & 1.7 Hz), 7.44 (s, 1H, 1-CH of steroidal ring), 7.40 (d, 1H, 6-CH of benzoyl moiety, J =8.2 Hz), 7.47 (d, 1H, 3-CH of benzoyl moiety, J =1.7 Hz), 7.67–7.73 (d, 1H, CH=C–CO, J =16.2 Hz). Electrospray Mass (MeOH): 485.0 [M–1]⁺. IR: 3506, 2925, 1656, 1591, 1498, 1257, 1092.

2.2.2.10. Synthesis of 1-(2,4-dichlorophenyl)-3-[methoxyestra-1,3,5(10)-trien-17 β -acetate,2-yl]-2-propen-1-one (16). Proceeded as per synthesis of substrate 8. Yield 77.0%, oil, ¹H NMR (CDCl₃, 300 MHz): δ 0.81 (s, 3H, 18-CH₃), 2.06 (s, 3H, OAc), 1.28–2.90 (m, 13H, rest of the 5XCH₂ and 3XCH of steroidal ring), 2.88 (bs, 2H, 6-CH₂), 4.68 (t, 1H, 17-CH), 3.83 (s, 3H, OCH₃), 6.62 (s, 1H, 4-CH of steroidal ring), 7.15–7.20 (d, 1H, =CH–CO, J =16.1 Hz), 7.33 (dd, 1H, 5-CH of benzoyl moiety, J =8.2 & 1.8 Hz), 7.40 (s, 1H, 6-CH of benzoyl moiety), 7.47 (d, 1H, 3-CH of benzoyl moiety, J =1.8 Hz), 7.69–7.75 (d, 1H, CH=C–CO, J =16.3 Hz). EI Mass (MeOH): 528 [M]⁺, 495, 381. IR: 2931, 1737, 1642, 1603, 1498, 1245, 1102, 1025.

2.2.2.11. Synthesis of 1-(4-fluorophenyl)-3-[methoxyestra-1,3,5(10)-trien-17 β -ol,2-yl]-2-propen-1-one (17). Same procedure as for 7. Yield 43.0%, oil, ¹H NMR (CDCl₃, 300 MHz): δ 0.797 (s, 3H, 18-CH₃), 1.25–2.12 (m, 13H, rest of the 5XCH₂ and 3XCH of steroidal ring), 2.89 (bs, 2H, 6-CH₂), 3.73 (distorted t, 1H, 17-CH), 3.88 (s, 3H, OCH₃), 6.65 (s, 1H, 4-CH of steroidal ring), 7.12 (d, 2H, 2 & 6-CH of benzoyl moiety, J =7.6 Hz), 7.18 (d, 2H, 3 & 5-CH of benzoyl moiety, J =8.6 Hz), 7.52 (s, 1H, 1-CH of steroidal ring), 7.54–7.59 (d, 1H, =CH–CO, J =15.8 Hz), 8.02–8.08 (d, 1H, CH=C–CO, J =15.8 Hz). EI Mass (MeOH): 434 [M]⁺, 416, 403, 385. IR: 3432, 2926, 1678, 1599, 1503, 1227, 1155, 1026.

2.2.2.12. Synthesis of 1-(4-bromophenyl)-3-[methoxyestra-1,3,5(10)-trien-17 β -ol,2-yl]-2-propen-1-one (18). Same procedure as for 7. Yield 34.0%, oil, ¹H NMR (CDCl₃, 300 MHz): δ 0.796 (s, 3H, 18-CH₃), 1.28–2.16 (m, 13H, rest of the 5XCH₂ and 3XCH of steroidal ring), 2.89 (bs, 2H, 6-CH₂), 3.75 (distorted t, 1H, 17-CH), 3.88 (s, 3H, OCH₃), 6.65 (s, 1H, 4-CH of steroidal ring), 7.51–7.56 (d, 1H, =CH–CO, J =15.56 Hz), 7.63 (d, 2H, 2 & 6-CH of benzoyl moiety, J =8.57 Hz), 7.85–7.88 (d, 2H, 3 & 5-CH of benzoyl moiety, J =8.47 Hz), 8.02–8.07 (d, 1H, CH=C–CO, J =15.78 Hz). EI Mass (MeOH): 494 [M]⁺. IR: 3428, 2923, 1680, 1586, 1501, 1258, 1069.

2.2.3. Synthesis of 4,5,6-trimethoxy-3-[3-methoxyestra-1,3,5(10)-trien-17-acetate-2yl]-indan-1-one (19)

In a Borosil test tube, 8 (200 mg, 0.36 mmol) was taken in tri-fluoroacetic acid (0.5 mL) and the tube was sealed properly. The reaction mixture was heated at 120 °C for 4 h. The reaction was poured into crushed ice and extracted with ethyl acetate, the organic layer was washed with water, dried over anhydrous sodium sulphate and evaporated in vacuum. The crude residue thus obtained was purified through column chromatography on silica gel using ethyl acetate–hexane as an eluent. The desired indanone 19 was obtained as an oil.

Yield 23.0%, oil, ¹H NMR (CDCl₃, 300 MHz): 0.866 (s, 3H, 18-CH₃), 2.83 (bs, 2H, 6-CH₂), 4.89 (bs, 1H, 17-CH), 2.57–2.65 (dd, 1H, 2-CH of Indanone ring), 3.09–3.18 (dd, 1H, 2-CH of Indanone ring), 3.72 (s, 6H, 2 X OCH₃), 3.81 (s, 6H, 3 X OCH₃), 4.83–4.89 (bs, 1H, H-3 of Indanone ring), 6.58 (s, 1H, 4-CH of steroid ring), 6.81 (s, 1H, 1-CH of steroid ring), 7.11 (s, 1H, 7-CH of Indanone ring). ¹³C NMR (CDCl₃, 75.46 MHz): δ 11.4, 14.4, 23.0, 29.7, 31.0, 32.3, 44.0, 44.4, 46.3, 50.0, 55.9, 55.9, 60.4, 60.4, 101.4, 113.1, 125.9, 129.7, 132.0, 133.2, 133.3, 144.5, 148.8, 150.8, 154.9, 155.5, 206.3. Electrospray Mass (MeOH): 507.2 [M+H]⁺. IR: 3447, 2930, 1709, 1601, 1504, 1218, 1162, 1031.

2.3. Biological methods

2.3.1. Cell culture

The ATCC (American type of cell culture collection) human cancer cell lines MCF-7 (hormone dependent breast cancer cell line), KB (oral and mouth), HepG2 (liver cells), CaCO2 (Colon cancer) and WRL68 (liver cancer) were obtained from NCCS Pune, India. Cells were cultured in DMEM with HEPES-25 mM, 0.22% NaHCO₃ and 10% FBS.

2.3.2. *In vitro* anticancer activity using MTT assay

In vitro cytotoxicity testing was performed as described [27]. 2×10^3 cells/well were incubated in a 5% CO₂ incubator for 24 h to enable them to adhere properly to the 96 well polystyrene microplate (Grenier, Germany). The test compound, dissolved in dimethyl sulphoxide (DMSO, Merck, Germany) in at least five concentrations, was added into the wells and left for 4 h. After the incubation, the compound plus media was replaced with fresh media and the cells were incubated for another 48 h in the CO₂ incubator at 37 °C. The concentration of DMSO was always kept below 1.25%, which was found to be non-toxic to the cells. Then, 10 μ L of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was added to each well and plates were incubated at 37 °C for 4 h. 100 μ L of DMSO was added to all wells and mixed thoroughly to dissolve the dark blue crystals. The plates were read on a SpectraMax 190 Microplate reader (Molecular Devices Inc. USA) at 570 nm within 1 h of DMSO addition.

2.3.3. Determination of osmotic hemolysis of erythrocytes

Blood from healthy human male volunteers ($n=3$) with informed consent was collected for experiments using Heparin (10 units/mL) as the anti-coagulant. The collected blood was stored at 4 °C and used for experiments within four hours of collection [28].

Experiments were carried *in vitro* by adding heparinized blood to hypotonic solutions of varying concentrations of phosphate buffered saline (0.85–0.10%). Phosphate buffered saline stock (10%) was prepared by dissolving 5 g of sodium chloride, 1.3655 g of disodium hydrogen orthophosphate and 0.243 g of sodium dihydrogen orthophosphate in 100 mL of autoclaved double distilled water. From this stock, working standards of 0.85–0.10% were prepared. The tubes were incubated at 37 °C for 60 min with mild shaking and the extent of hemolysis was measured colorimetrically at 540 nm [29,30]. Results are expressed in terms of mean erythrocyte fragility (MEF₅₀), which is the level of hemolysis of the erythrocytes at 50% saline concentrations. Similarly, prior to the experiment, heparinized blood was incubated with effective concentration of chalcones and indanone (19.7–182.4 μ M) at 37 °C for 60 min. Only those concentrations at which the compounds showed anticancer activity (or higher) were chosen.

Aliquots of saline solutions, with concentrations ranging from 10 to 1 g/L were prepared as described by Papart et al. [31]. The chalcone treated erythrocytes were then transferred to the tubes containing decreasing concentrations of saline solutions. After careful mixing, the cell suspensions were left to equilibrate for 30 min and then centrifuged at 3000 rpm for 5 min. The absorbance of supernatants was read at 540 nm, standardized against a blank assay (10 g/L saline supernatant

correspond to 0% hemolysis). The recorded optical density (OD) of the supernatant reflects the degree of hemolysis of the erythrocytes. The lysis percentage was calculated by dividing the OD of the supernatant obtained from a particular saline concentration by the OD of the standard (1 g/L) representing 100% hemolysis. Osmotic fragility curves were constructed by plotting the lysis percentage against the concentration of saline solutions. The MEF₅₀ (mean erythrocyte fragility) value, which is the saline concentration at which 50% of the cells hemolyse at standard pH and temperature [32], was then obtained from the curve.

2.3.4. Data analysis

The experiment was done in triplicate and the inhibitory concentration (IC) values were calculated from a dose response curve. IC₅₀ is the concentration in μ g/mL required for 50% inhibition of cell growth as compared to that of untreated control.

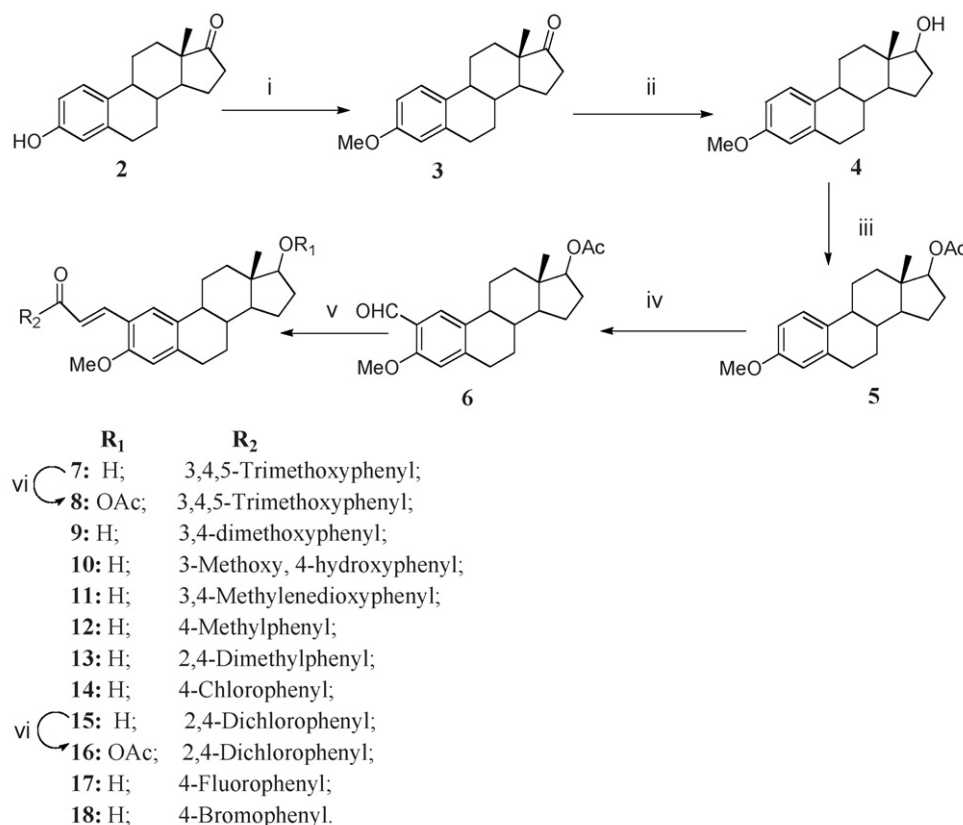
Complete hemolysis of the erythrocyte suspension occurred in the 0.1% NaCl solution, for which the hemolysis was defined as 100%. Hemolysis of the erythrocytes did not occur in the 0.85% NaCl solution, for which the hemolysis was defined as 0%. The effective concentration of the NaCl solution inducing 50% hemolysis (MEF₅₀) of the applied erythrocytes was calculated from the hemolysis curve by using a straight-line equation between the points immediately adjacent to 50%. All values are expressed as the mean of three experiments in replicates.

3. Results and discussion

3.1. Chemistry

Aldehyde and acetophenone moieties were condensed together to synthesize these chalcone derivatives (Scheme 1). The steroidal framework was chosen as the aldehydic substrate and thus, estrone (2) was used as the starting material. To synthesize the aldehydic substrate (6), the phenolic hydroxyl of 2 was first protected by methylating with dimethyl sulphate/anhydrous potassium carbonate in dry acetone under refluxing conditions to get estrone 3-methyl ether (3) in a 91% yield. The 17-Keto group of the substrate 3 was reduced to the corresponding 17-alcohol by using sodium borohydride in a methanol–chloroform (2:1) mixture to get estradiol 3-methyl ether (4) in a 94% yield. The 17-hydroxyl of 4 was acetylated with acetic anhydride in dry pyridine to get estradiol 3-methyl ether 17-acetate (5). The aldehydic substrate 6 was obtained from estradiol, 3-methyl ether, 17 β -acetate by performing the Vilsmeier formylation reaction, using dimethyl formamide and phosphorus oxychloride at 80 °C [33]. 2-Formyl estradiol 3-methyl ether 17-acetate (6) was treated with different acetophenone derivatives in 7% aqueous-alcoholic potassium hydroxide solution to get the various chalcones on the steroidal moiety with 17-acetate deprotected. Some of the deprotected chalcones were further acetylated with acetic anhydride and dry pyridine.

One of the chalcone derivatives was further modified to the corresponding 1-indanone derivative (19) using the trifluoroacetic acid catalyzed Nazarov cyclisation reaction (Scheme 2). Chalcone 8 was heated with trifluoroacetic acid for 4 h in a sealed tube yielding indanone 19 in a 23% yield [34].



Reagents and conditions: (i) Dimethyl sulphate, K₂CO₃, dry acetone-DCM, reflux, 5h, 91%; (ii) NaBH₄, MeOH-DCM (2:1), 50°C, 1h, 94%; (iii) Pyridine:acetic anhydride (2:1), room temperature, overnight, 93%; (iv) DMF-POCl₃, 0–10°C for 1h, then heat 80°C for 3h, 36%; (v) Acetophenones as per R₂, 7% KOH solution in ethanol:water (1:2) at 10°C, acetophenone, overnight, 34–86%; (vi) substrate, acetic anhydride: dry pyridine (1:2), RT, overnight.

Scheme 1 – Synthesis of chalcone derivatives on estradiol framework.

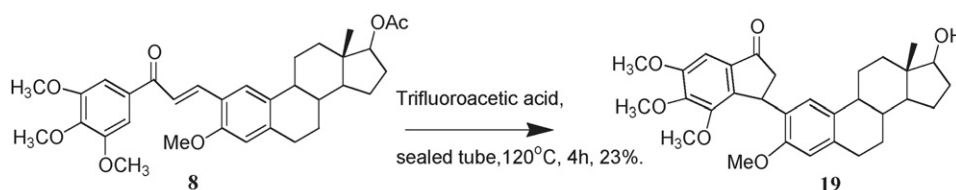
Chalcones represent an important class of anticancer molecules. Hence, various types of chalcones have been synthesized as anticancer agents. In most cases, the anticancer activities reported were found in μ M concentrations. Chalcone 1 has been reported to be the most active synthesized chalcone so far, having IC₅₀ value at 0.21 nM concentration against K562 human leukemia cells [25]. We synthesized various estradiol chalcone derivatives, which may act as anticancer agents. One of the chalcone derivatives (8) has been modified to an indanone derivative (19). All these chalcones were obtained in good yields after chromatographic separations (Isolated yields 34–86%) and showed high levels of cytotoxicities (Table 1).

3.2. Bioactivity

The anticancer activity of all the synthesized chalcones and indanone was evaluated against various human cancer cell

lines MCF-7, KB, HepG2, CaCO2 and WRL68 using the MTT assay. Tamoxifen, Paclitaxel (Taxol) and Podophyllotoxin were used as reference compounds.

Although these compounds were screened for various human cancer cell lines, our main interest was in their activity against MCF-7 breast cancer cells. Among these chalcones, 9 was found to be the most active (IC₅₀ = 7.3 μ M) followed by indanone derivative 19 (IC₅₀ = 9.8 μ M) against the MCF-7 hormone dependent breast cancer cell line where tamoxifen is the only drug of choice. Chalcones 8, 10 and 13 also possessed moderate level of cytotoxicity against MCF-7 cancer cells having IC₅₀ values of 72.9 μ M, 64.9 μ M and 56.3 μ M, respectively. The remaining chalcones exhibited low cytotoxicity levels (IC₅₀ values higher than 100 μ M), except 12 (IC₅₀ = 697 μ M, inactive). Among these, 7 also possessed high levels of cytotoxicity against WRL68, HepG2, and CaCO2 cancer cells, having IC₅₀ values of 49.5 μ M, 49.5 μ M, and 16.7 μ M, respectively.



Scheme 2 – Conversion of steroidal chalcone to a steroidal indanone derivative.

Table 1 – Cytotoxicity of different compounds against various human cancer cell lines by the MTT assay

S. no.	Compound no.	Human cancer cells				
		MCF-7 IC ₅₀ μ M	KB IC ₅₀ μ M	HepG2 IC ₅₀ μ M	CaCO2 IC ₅₀ μ M	WRL68 IC ₅₀ μ M
1.	7	177.86	– ^a	49.4	16.7	49.4
2.	8	72.9	–	127.7	100.3	145.9
3.	9	7.3	–	^a	189.0	189.0
4.	10	64.9	^a	194.8	140.7	^a
5.	11	^a	^a	206.5	141.3	184.7
6.	12	^a	174.4	162.7	116.2	197.6
7.	13	56.3	180.1	^a	168.9	191.4
8.	14	200.0	^a	^a	200.0	^a
9.	15	154.9	^a	144.6	185.9	^a
10.	16	^a	^a	^a	142.0	160.9
11.	17	138.2	161.2	^a	126.7	172.8
12.	18	151.8	^a	^a	^a	^a
13.	19	9.88	138.3	3.9	167.9	128.4
14.	Tamoxifen	0.027	0.013	2.28	0.40	0.027
15.	Paclitaxel (Taxol)	0.006	0.001	0.009	0.008	0.005
16.	Podophyllotoxin	8.45	20.5	4.83	0.002	4.83

^a Means inactive (IC₅₀ values > 200 μ M); IC₅₀ values mean of three experiments in replicate.

The structure and activity relationship (SAR) of these steroidal chalcones against the MCF-7 cancer cells line was studied. Earlier reports [25] on SAR of these class of compounds showed that the chalcone (1) with the 3,4,5-trimethoxy benzoyl unit was the most cytotoxic one whereas, in the case of steroidal chalcones, compound 9, containing the 3,4-dimethoxy benzoyl unit, was the most active (nearly 24fold higher activity than 7, which has a 3,4,5-trimethoxy benzoyl unit). Using the Nazarov reaction, compound 8 yielded indanone derivative 19, which showed a better cytotoxic activity than the parent compound and comparable to 9. The cytotoxicity was reduced when one of the methoxy group of 9 was replaced with a phenolic hydroxyl (10). When the 3,4-dimethoxy benzoyl unit of 9 was replaced with the 3,4-methylenedioxy benzoyl unit in chalcone 11 the anticancer activity was lost. 4-Halogented (F/Cl/Br) chalcone derivatives (14, 15, 16, 17 & 18) were either inactive or poorly active against the MCF-7 human cancer cell line.

Chalcones have been reported to act as tubulin polymerization inhibitors. Having structural similarity with colchicine, these are believed to bind to the same site of tubulin [35]. Tubulin exists as a heterodimer of alpha and beta-subunits. This dimer can couple together to make protofilaments consisting of alternating alpha and beta subunits. Several protofilaments (12 or more) can bind together to form pipe like structures known as microtubulin (polymer of tubulin). The structure plays a vital role in various biochemical processes of cell survival and growth. One of these is the formation of the mitotic spindle, without which mitosis would not take place.

Hsu et al. [7] studied the molecular mechanism of some of the chalcones against the MCF-7 cancer cells and found that chalcones were involved in the; (1) induction of apoptosis, (2) blockage of cell cycle progression by cell cycle-related factor regulation, (3) initiation of Fas/Fas ligand pathway, (4) trigger of mitochondrial pathway, and (5) modulation of Bcl-2 family protein. Thus, the authors demonstrated that chalcones may be a promising chemopreventive agent for breast cancer treatment.

The osmotic fragility profiles of the control and *in vitro* chalcone(s)-treated erythrocytes are shown in Fig. 3. Various reference compounds were also used, namely, curcumin as a positive control, hydrogen peroxide as a negative control, tamoxifen and podophyllotoxin as standard anticancer molecules. *In vitro* treatment of erythrocytes with chalcone(s) resulted in an altered osmotic fragility profile, which is evident by the shift of the curve to the left and a decrease in MEF values, representing decreased cell lysis. The MEF₅₀ values of the control, the reference compounds and the *in vitro* chalcone(s)-treated erythrocytes are tabulated in Table 2. Erythrocyte osmotic fragility is used as a marker of erythrocyte tensile strength and is related to cellular deformability, useful in the clinical detection of many diseases including anemia [36–40]. The significance of this parameter lies in the fact that it gives information about the metabolism and membrane sta-

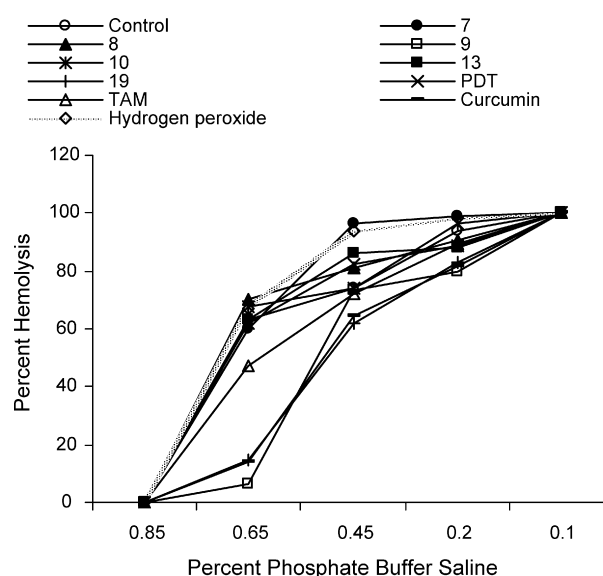
**Fig. 3 – Osmotic hemolysis curve of erythrocyte.**

Table 2 – Evaluation of the effect of chalcones on MEF values of erythrocyte osmotic fragility

S. no.	Condition	Mean erythrocyte fragility ^a	Concentration (μM)
1.	Control	0.69	–
2.	7	0.685	177.8
3.	8	0.71	182.4
4.	9	0.52	7.35
5.	10	0.70	64.9
6.	13	0.69	56.3
7.	19	0.55	19.7
8.	Curcumin	0.51	25.8
9.	Podophyllotoxin	0.69	241.5
10.	Tamoxifen	0.65	1.34
11.	Hydrogen peroxide	0.77	2000

^a Values are mean of three experiments in replicate.

bility of the erythrocytes. The present study was undertaken to study the effect of chalcone(s) on the osmotic hemolysis profile of erythrocytes. The decrease in the erythrocytes' osmotic hemolysis observed suggests that the chalcone derivatives, which showed cytotoxicity against a cancer cell line, are non-toxic to erythrocytes since they did not affect the fragility of these cells. However, further work is needed to understand how these chalcones interact and affect the fluidity and permeability of the erythrocyte membrane.

In conclusion, the chalcone derivatives synthesized on a steroidal framework showed good anticancer activity against hormone dependent breast cancer cells and were found to be non-toxic to erythrocytes. Thus, steroidal chalcones may be developed as potent anticancer agents. These derivatives may be further studied for antiestrogenic activity and receptor binding affinity in order to better understand their mode of action. The present study already gives some insight about its further optimization for better activity.

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