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Article

Design, Synthesis, Characterization and Computational Evaluation of Novel Isobutychalcones as Cytotoxic Agents: Part-A

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Abstract: A series of novel isobutylchalcones (**A1-A20**) were prepared, evaluated for their cytotoxic activity and characterized by FTIR, ^{1}H NMR, ^{13}C NMR, and elemental analysis data. The logic behind the design is to synthesize and compare chalcones containing electron releasing lipophilic isobutyl substituent on aromatic ring A and the B ring with aromatic ring containing a range of electron releasing and electron withdrawing groups as well as heteroaromatic rings for their cytotoxic activity. The compounds were tested against **HT-29** (colon cancer), **MCF-7** (breast cancer) and **DU-145** (prostate cancer) cell lines using methotrexate (IC_{50} 12 ± 1 (HT-29), 9 ±1 (MCF-7) 5 ± 1 (DU-145)) as reference standard. Compound **A6** having 2,4-difluorphenyl moiety was most potent of the series against all the three cell lines and notably **A6** was mainly effective against DU-145 cell lines with an IC_{50} value of 18 µg/mL. The critical structural features required for the activity against all the cell lines were identified through pharmacophore model using PHASE^{TM} which has recognised a 5 point AHHRR model and is consistent with the cytotoxic activity of the tested compounds.

Keywords: chalcone; cytotoxic activity; pharmacophore model

1. Introduction

Cancer is uncontrolled growth of cells, which can invade and spread to distant sites of the body and is one of the most difficult afflictions in the world [1, 2]. According to World Health Organization (WHO) cancer figures among the leading causes of morbidity and mortality worldwide, with approximately 14 million new cases and 8.2 million cancer related deaths in 2012 [3] and the number of new cases is expected to rise by about 70% over the next 2 decades. Among men, the 5 most common sites of cancer diagnosed in 2012 were lung, prostate, colorectal, stomach, and liver cancer and among the women were breast, colorectal, lung, cervix, and stomach cancer. Despite of the availability of advanced chemotherapeutic agents, the treatment of cancer is challenging because of objectionable side effects of existing cytotoxic agents and also lack of selectivity for tumour cells as a dose of anticancer drug sufficient to kill tumour cells is often toxic to the normal tissue and leads to many side effects, which in turn, limits its treatment efficacy. Development of resistance against the existing anticancer drugs keeps research window open in the search of newer

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chemotherapeutics [4]. Hence, there is a hunger for the development of novel compounds with high usefulness, fewer side effects, devoid of resistance and superior selectivity.

Chalcones are a class of natural α, β-unsaturated enones [5-7] biosynthesized by means of polyketide pathway and are the intermediates for flavonoids biosynthesis [8]. These compounds possess broad array of pharmacological activities [9, 10] and specifically emerged as potential anticancer agents in the last 15 years [11]. Several pure chalcones have been approved for clinical use in humans. Clinical trials have shown that these compounds reached reasonable plasma concentration, well-tolerated [12] and have less chance to interact with DNA, which omits the risk of mutagenicity, a key problem with many anticancer agents [13]. They are absorbed through daily diet and show promising cancer chemopreventive role [14]. Cytotoxic and anticancer activities are mediated by modulating important molecular pathways or targets including, P-glycoprotein-mediated multidrug resistance, m-TOR pathway, β-catenin degradation, STAT3, tumour vasculature, cell death induction, tubulin polymerization inhibition, NF-kappa B pathway, androgen and estrogen receptor signalling, p53 pathway etc., [15]. Aforementioned properties motivated us to synthesize and evaluate chalcones as potential cytotoxic agents.

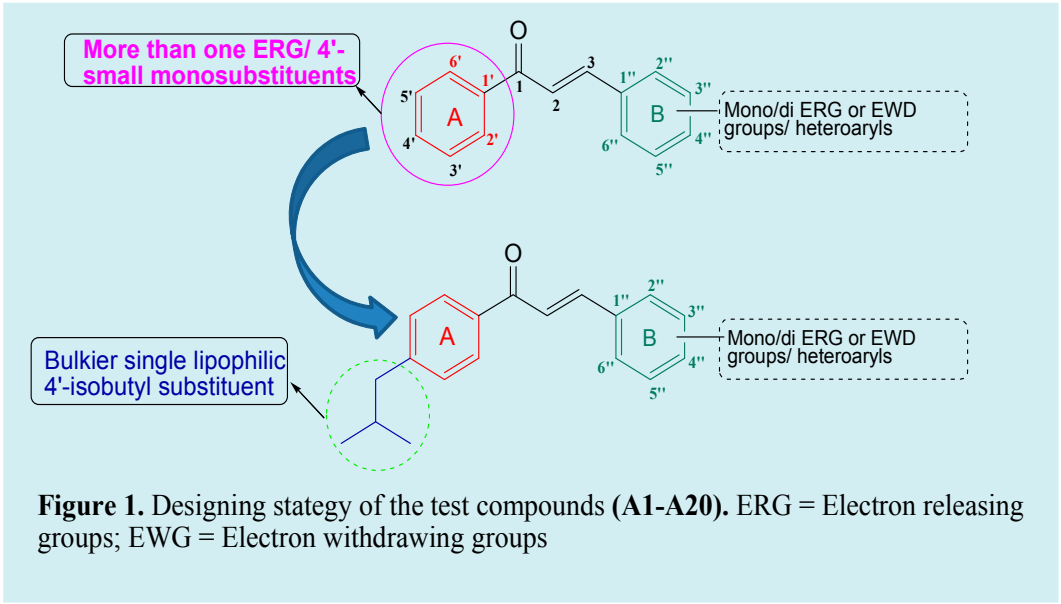

Figure 1. Designing stategy of the test compounds **(A1-A20).** ERG = Electron releasing groups; EWG = Electron withdrawing groups

A range of chalcones with altered functionalities linked to α, β-unsaturated carbonyl system, proved as active anticancer agents. In particular incorporation of multiple electron releasing groups on ring-A with single or multiple electron releasing (or withdrawing) groups on ring-B [16], replacement of aryl rings with heteroaryl(s) [17], rigidification of keto vinyl arrangement to form chalconoids [18]. To the best of our knowledge most of the chalcones reported with anticancer action, both from the nature and synthesis typically contain more than one electron releasing group on ring-A [19-24], and even if monosubstitution is present it is either a simple $-NH_2$, $-OH$, $-OCH_3$ and $-CH_3$ [25-27], but not a bulkier hydrophobic isobutyl functionality. Lipophilicity plays a crucial role in cell permeability and presence of such groups increase the penetrability and inhibitory effects of compounds against cancer cells. Hence we premeditated to design and study the effect of monosubstituted ring-A with 4'-isobutyl by conserving the same with changing ring-B portion (**Fig. 1**).

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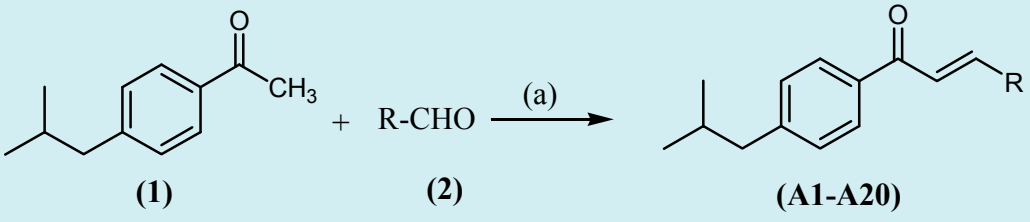
2. Materials and methods

2.1 General

All chemical reagents and solvents were purchased from S.D Fine Chem. Ltd, Mumbai, India. 4-isobutylacetophenone was purchased from Aldrich Chemical Co. (Melwaukee, Wisconsin, USA). Reactions were monitored by TLC using silica gel-G (Merck grade) as the adsorbent. All the melting points were determined in open capillaries, using Boitus melting point apparatus, expressed in ^{o}C and are uncorrected. The IR spectra were recorded on Bruker Vertex 80v spectrometer using potassium bromide disks. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AMX 400 MHz NMR spectrophotometer using TMS as an internal standard and the chemical shifts (δ) are expressed in ppm. Elemental analyses were carried out using a Carlo Erba 1108 elemental analyzer for C, H, and N and the results are within ± 0.4% of the calculated values. **HT-29** (colon), **MCF-7** (breast) and **DU-145** (prostate) cancer cell lines were obtained from National Centre for Cell Science (NCCS), Pune, India. DMEM (Dulbeccos Modified Eagels Medium), MEM (Minimum Essential Media Eagle), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], Trypsin, EDTA were purchased from Sigma chemicals (St.Louis, MO). Fetal bovine serum (FBS) was purchased from Arrow Labs and 96 well flat bottom tissue culture plates were purchased from Tarsons Products Pvt. Ltd, Kolkata, India.

2.2 Chemistry

2.2.1 General method of synthesis of isobutylchalcones: A mixture of 1-(4-isobutylphenyl)ethanone (0.001 mole) and the appropriate aryl or heteroaryl aldehyde (0.001 mole) was stirred in ethanol (7.5 mL) and an aqueous solution of KOH (50%, 7.5 mL) was added drop wise. The mixture was set aside for 24 h at room temperature, acidified with mixture of hydrochloric acid and water (1:1), to attain the precipitate of chalcones (**A1-A20**). The chalcones were then filtered under vacuum, washed with water and dried. Purity of the compounds was checked using TLC and impure chalcones were recrystallized from ethanol to obtain the pure compounds (**Scheme 1**).



(1) **(2)** **(A1-A20)**

Scheme 1 Synthesis of chalcones A1-A20. Reagents and conditions: (a) ethanol, KOH, room temparature; (1) 1-(4-isobutylphenyl)ethanone (2) aromatic or heteroaromatic aldehyde

2.2.2. (E)-1-(4'-isobutylyphenyl)-3-(4''-chlorophenyl)-2-propen-1-one **(A1): Yield** 92%; **m.p.** 136-138 ^{o}C; **IR** (KBr, cm^{-1}): 1659 (C=O), 1585 (C=C of Ar), 1505 (CH=CH), 835 (C-Cl), 3050 (Ar C-H), 2833 (Alkyl C-H); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 7.39 (1H, d, J = 17 Hz, -CO-CH=), 7.74 (1H, d, J =17 Hz, =CH-Ar), 7.19-7.91 (8H, Ar-H), 0.92 (6H, d, J = 8 Hz, -$(CH_3)_2$), 1.75-1.95 (1H, m, -CH-), 2.72 (2H, d, J = 8 Hz, -CH_2-); **^{13}C NMR** (400 MHz,

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$CDCl_3$, ppm): δ 189.77 (C-1), 122.65 (C-2), 142.79 (C-3), 129.41 (C-2' and C-6'), 129.52 (C-3' and C-5'), 135.79 (C-1'), 147.53 (C-4'), 133.58 (C-1''), 138.12 (C-4''), 128.50 (C-2'' and C-6''), 129.22 (C-3'' and C-5''), 22.33 (-CH_3, C of isobutyl group at C-4''), 30.12 (-CH-, C of isobutyl group at C-4''), 45.45 (-CH_2-, C of isobutyl group at C-4''); **Anal. Calcd** for: $C_{19}H_{19}ClO$: C, 76.37; H, 6.41; Found: C, 76.40; H, 6.44.

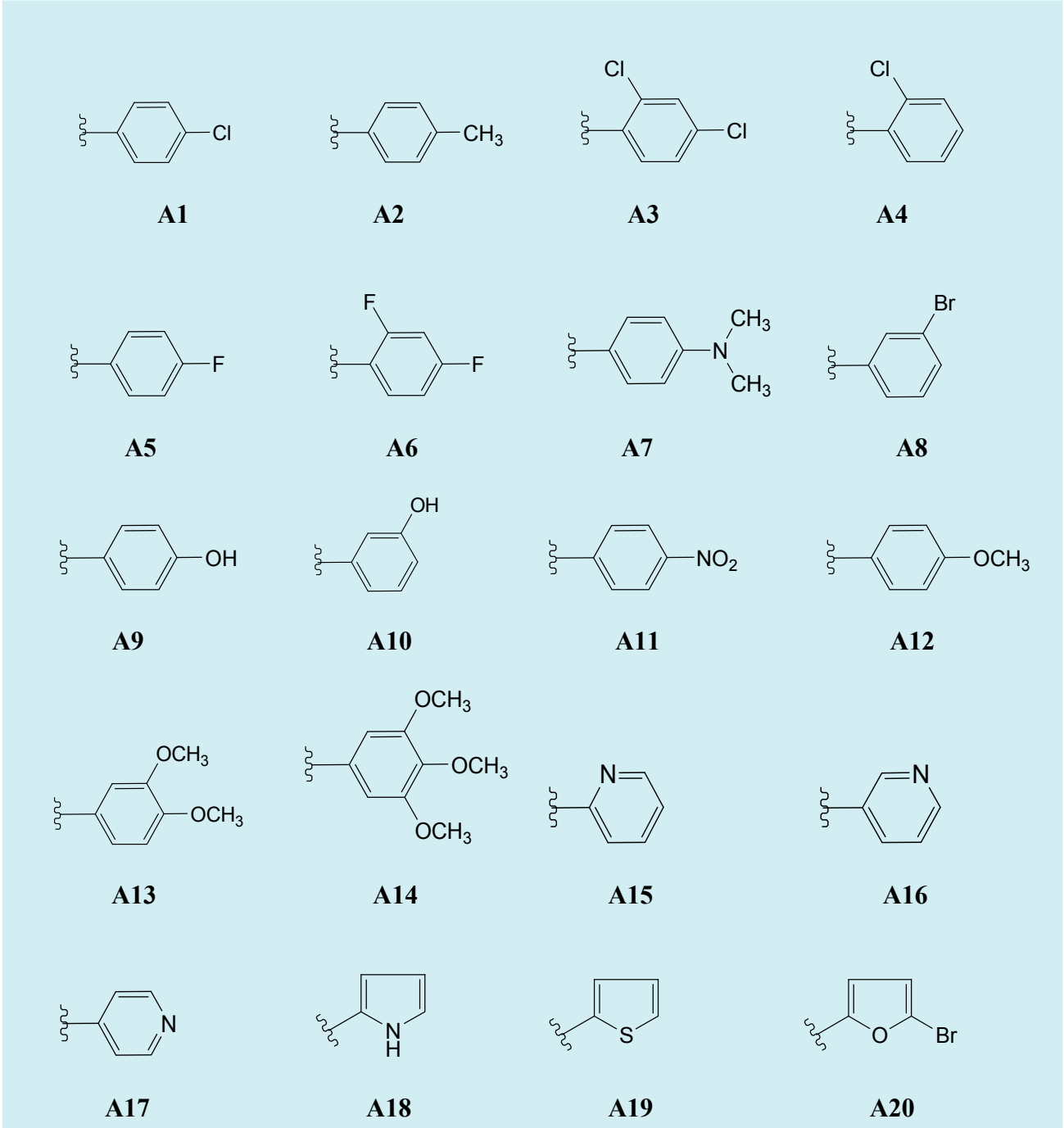

Figure 2. Different aldehydes selected for synthesis; **A1** to **A20** are the codes of 20 chalcones.

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2.2.3. (E)-1-(4'-isobutylphenyl)-3-(4''-methylphenyl)-2-propen-1-one **(A2): Yield** 87%; **m.p**. 128-130 °C; **IR** (KBr, cm^{-1}): 1655 (C=O), 1602 (C=C of Ar), 1505 (CH=CH), 3010 (Ar C-H), 2921 (Alkyl C-H); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 2.30 (3H, s, Ar-CH_3), 7.25 (1H, d, J = 17 Hz, -CO-CH=), 7.65 (1H, d, J =17 Hz, =CH-Ar), 6.83-7.82 (8H, Ar-H), 0.89 (6H, d, J = 8 Hz, -$(CH_3)_2$), 1.70-1.92 (1H, m, -CH-), 2.65 (2H, d, J = 8 Hz, -CH_2-); **^{13}C NMR** (400 MHz, $CDCl_3$, ppm): δ 188.67 (C-1), 121.65 (C-2), 140.97 (C-3), 126.51 (C-2' and C-6'), 128.22 (C-3' and C-5'), 132.12 (C-1'), 137.63 (C-4'), 134.89 (C-1''), 145.12 (C-4''), 127.09 (C-2'' and C-6''), 129.1 (C-3'' and C-5''), 22.80 (-CH_3, C of isobutyl group at C-4''), 29.12 (-CH-, C of isobutyl group at C-4''), 45.72 (-CH_2-, C of isobutyl group at C-4''), 24.35 (-CH_3 C at C-4''); **Anal. Calcd** for: $C_{20}H_{22}O$: C, 86.29; H, 7.97; Found: C, 86.32; H, 7.99.

2.2.4. (E)-1-(4'-isobutylphenyl)-3-(2'',4''-dichlorophenyl)-2-propen-1-one **(A3): Yield** 85%; **m.p.** 149-151 °C; **IR** (KBr, cm^{-1}): 1655 (C=O), 1581 (C=C of Ar), 1510 (CH=CH), 833 (C-Cl), 3057 (Ar C-H), 2877 (Alkyl C-H); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 7.42 (1H, d, J = 17 Hz, -CO-CH=), 7.84 (1H, d, J =17 Hz, =CH-Ar), 7.20-8.20 (7H, Ar-H), 1.11 (6H, d, J = 8 Hz, -$(CH_3)_2$), 1.99-2.13 (1H, m, -CH-), 2.73 (2H, d, J = 8 Hz, -CH_2-); **^{13}C NMR** (400 MHz, $CDCl_3$, ppm): δ 190.11 (C-1), 121.78 (C-2), 141.21 (C-3), 130.03 (C-2' and C-6'), 130.35 (C-3' and C-5'), 134.25 (C-1'), 147.63 (C-4'), 132.71 (C-1''), 135.27 (C-4''), 132.69 (C-2''), 129.23 (C-6''), 130.79 (C-3''), 126.72 (C-5''), 23.11 (-CH_3, C of isobutyl group at C-4''), 29.31 (-CH-, C of isobutyl group at C-4''), 45.91 (-CH_2-, C of isobutyl group at C-4''); **Anal. Calcd** for: $C_{19}H_{18}Cl_2O$: C, 68.48; H, 5.44; Found: C, 68.53; H, 5.49.

2.2.5. (E)-1-(4'-isobutylyphenyl)-3-(2''-chlorophenyl)-2-propen-1-one **(A4): Yield** 65%; **m.p.** 140-142 °C; **IR** (KBr, cm^{-1}): 1652 (C=O), 1583 (C=C of Ar), 1502 (CH=CH), 833 (C-Cl), 3120 (Ar C-H), 2920 (Alkyl C-H); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 7.31 (1H, d, J = 17 Hz, -CO-CH=), 7.74 (1H, d, J =17 Hz, =CH-Ar), 6.87-7.91 (8H, Ar-H), 1.02 (6H, d, J = 8 Hz, -$(CH_3)_2$), 2.22-2.44 (1H, m, -CH-), 2.66 (2H, d, J = 8 Hz, -CH_2-); **^{13}C NMR** (400 MHz, $CDCl_3$, ppm): δ 189.44 (C-1), 121.42 (C-2), 145.15 (C-3), 128.5 (C-2' and C-6'), 129.5 (C-3' and C-5'), 133.22 (C-1'), 146.71 (C-4'), 133.91 (C-1''), 129.55 (C-4''), 128.85 (C-2''), 130.64 (C-6''), 128.8 (C-3''), 126.2 (C-5''), 22.21 (-CH_3, C of isobutyl group at C-4''), 29.11 (-CH-, C of isobutyl group at C-4''), 45.71 (-CH_2-, C of isobutyl group at C-4''); **Anal. Calcd** for: $C_{19}H_{19}ClO$: C, 76.37; H, 6.41; Found: C, 76.42; H, 6.50.

2.2.6. (E)-1-(4'-isobutylphenyl)-3-(4''-fluorophenyl)-2-propen-1-one **(A5): Yield** 85%; **m.p.** 142-144 °C; **IR** (KBr, cm^{-1}): 1664 (C=O), 1580 (C=C of Ar), 1524 (CH=CH), 928 (C-F), 3127 (Ar C-H), 2954 (Alkyl C-H); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 7.57 (1H, d, J = 17 Hz, -CO-CH=), 7.87 (1H, d, J =17 Hz, =CH-Ar), 7.33-8.12 (8H, Ar-H), 1.00 (6H, d, J = 8 Hz, -$(CH_3)_2$), 1.80-2.04 (1H, m, -CH-), 2.75 (2H, d, J = 8 Hz, -CH_2-); **^{13}C NMR** (400 MHz, $CDCl_3$, ppm): δ 190.21 (C-1), 124.52 (C-2), 145.29 (C-3), 129.44 (C-2' and C-6'), 129.67 (C-3' and C-5'), 135.92 (C-1'), 144.76 (C-4'), 131.8 (C-1''), 163.12 (C-4''), 129.11 (C-2'' and C-6''), 118.98 (C-3'' and C-5''), 22.82 (-CH_3, C of isobutyl group at C-4''), 29.57 (-CH-, C of isobutyl group at C-4''), 45.91 (-CH_2-, C of isobutyl group at C-4''); **Anal. Calcd** for: $C_{19}H_{19}FO$: C, 80.82; H, 6.78; Found: C, 80.86; H, 6.85.

2.2.7. (E)-1-(4'-isobutylphenyl)-3-(2'',4''-difluorophenyl)-2-propen-1-one **(A6): Yield** 79%; **m.p.** 163-165 °C; **IR** (KBr, cm^{-1}): 1655 (C=O), 1581 (C=C of Ar), 1510 (CH=CH), 925 (C-F), 926 (C-F), 3040 (Ar C-H), 2933 (Alkyl C-H); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 7.49 (1H, d, J = 17 Hz, -CO-CH=), 7.99 (1H, d, J =17 Hz, =CH-Ar), 7.11-8.20 (7H, Ar-H), 1.19 (6H, d, J = 8 Hz, -$(CH_3)_2$), 2.10-2.41 (1H, m, -CH-), 2.91 (2H, d, J = 8 Hz, -CH_2-); **^{13}C NMR** (400 MHz, $CDCl_3$, ppm): δ 190.23 (C-1), 122.1 (C-2), 146.2 (C-3), 134.8 (C-2' and C-6'), 129.52 (C-3' and C-5'), 134.55 (C-1'), 147.29 (C-4'), 134.11 (C-1''), 165.42 (C-4''), 159.51 (C-

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2''), 129.6 (C-6''), 109.29 (C-3''), 112.0 (C-5''), 23.21 (-CH_3, C of isobutyl group at C-4''), 30.12 (-CH-, C of isobutyl group at C-4''), 45.81 (-CH_2-, C of isobutyl group at C-4''); **Anal. Calcd** for: $C_{19}H_{19}FO$: C, 75.98; H, 6.04; Found: C, 76.03; H, 6.06.

2.2.8. (E)-1-(4'-isobutylphenyl)-3-(4''-dimethylaminophenyl)-2-propen-1-one **(A7): Yield** 82%; **m.p.** 138-140 °C; **IR** (KBr, cm^{-1}): 1650 (C=O), 1586 (C=C of Ar), 1505 (CH=CH), 1178 (-$N(CH_3)_2$), 3198 (Ar C-H), 2940 (Alkyl C-H); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 3.10 (6H, s, -$N(CH_3)_2$), 7.29 (1H, d, J = 17 Hz, -CO-CH=), 7.75 (1H, d, J =17 Hz, =CH-Ar), 6.64-8.10 (8H, Ar-H), 0.98 (6H, d, J = 8 Hz, -$(CH_3)_2$), 2.19-2.33 (1H, m, -CH-), 2.58 (2H, d, J = 8 Hz, -CH_2-); **^{13}C NMR** (400 MHz, $CDCl_3$, ppm): δ 186.61 (C-1), 120.10 (C-2), 144.65 (C-3), 130.11 (C-2' and C-6'), 128.45 (C-3' and C-5'), 135.67 (C-1'), 146.81 (C-4'), 133.31 (C-1'') 167.22 (C-4''), 159.51 (C-2''), 129.61 (C-6''), 109.29 (C-3''), 111.03 (C-5''), 23.10 (-CH_3, C of isobutyl group at C-4''), 30.33 (-CH-, C of isobutyl group at C-4''), 45.99 (-CH_2-, C of isobutyl group at C-4''), 40.33 (-$N(CH_3)_2$,); **Anal. Calcd** for: $C_{21}H_{25}NO$: C, 82.04; H, 8.20; N, 4.56; Found: C, 82.09; H, 8.23; N, 4.59.

2.2.9. (E)-1-(4'-isobutylphenyl)-3-(3''-bromophenyl)-2-propen-1-one **(A8): Yield** 80%; **m.p.** 107-109 °C; **IR** (KBr, cm^{-1}): 1650 (C=O), 1605 (C=C of Ar), 1502 (CH=CH), 969 (C-Br), 3155 (Ar C-H), 2836 (Alkyl C-H); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 7.36 (1H, d, J = 17 Hz, -CO-CH=), 7.79 (1H, d, J =17 Hz, =CH-Ar), 7.19-8.09 (8H, Ar-H), 1.01 (6H, d, J = 8 Hz, -$(CH_3)_2$), 2.02-2.20 (1H, m, -CH-), 2.58 (2H, d, J = 8 Hz, -CH_2-); **^{13}C NMR** (400 MHz, $CDCl_3$, ppm): δ 189.37 (C-1), 123.09 (C-2), 146.27 (C-3), 131.21 (C-2' and C-6'), 129.27 (C-3' and C-5'), 136.13 (C-1'), 146.28 (C-4'), 137.42 (C-1''), 131.12 (C-4''and C-5''), 130.54 (C-2''), 124.43 (C-6''), 127.67 (C-3''), 22.92 (-CH_3, C of isobutyl group at C-4''), 30.12 (-CH-, C of isobutyl group at C-4''), 45.61 (-CH_2-, C of isobutyl group at C-4''); **Anal. Calcd** for: $C_{19}H_{19}BrO$: C, 66.48; H, 5.58; Found: C, 66.53; H, 5.63.

2.2.10. (E)-1-(4'-isobutylphenyl)-3-(4''-hydroxyphenyl)-2-propen-1-one **(A9): Yield** 73%; **m.p.** 156-158 °C; **IR** (KBr, cm^{-1}): 3460 (O-H), 1648 (C=O), 1606 (C=C of Ar), 1505 (CH=CH), 3060 (Ar C-H), 2852 (Alkyl C-H); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 4.92 (1H, Ar-OH), 7.29 (1H, d, J = 17 Hz, -CO-CH=), 7.80 (1H, d, J =17 Hz, =CH-Ar), 7.61-8.02 (8H, Ar-H), 1.02 (6H, d, J = 8 Hz, -$(CH_3)_2$), 1.92-2.01 (1H, m, -CH-), 2.40 (2H, d, J = 8 Hz, -CH_2-); **^{13}C NMR** (400 MHz, $CDCl_3$, ppm): δ 188.52 (C-1), 122.87 (C-2), 148.32 (C-3), 131.26 (C-2' and C-6'), 128.91 (C-3' and C-5'), 134.99 (C-1'), 145.56 (C-4'), 133.54 (C-1''), 159.35 (C-4''), 129.11 (C-2'' and C-6''), 120.21 (C-3'' and C-5''), 23.45 (-CH_3, C of isobutyl group at C-4'), 29.92 (-CH-, C of isobutyl group at C-4'), 44.99 (-CH_2-, C of isobutyl group at C-4'); **Anal. Calcd** for: $C_{19}H_{19}O_2$: C, 81.40; H, 7.19; Found: C, 81.45; H, 7.24.

2.2.11. (E)-1-(4'-isobutylphenyl)-3-(3''-hydroxyphenyl)-2-propen-1-one **(A10)**: **Yield** 65%; **m.p.** 152-154 °C; **IR** (KBr, cm^{-1}): 3520 (O-H), 1648 (C=O), 1612 (C=C of Ar), 1505 (CH=CH), 3111 (Ar C-H), 2928 (Alkyl C-H); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 4.80 (1H, Ar-OH), 7.26 (1H, d, J = 17 Hz, -CO-CH=), 7.71 (1H, d, J =17 Hz, =CH-Ar), 6.85-8.00 (8H, Ar-H), 1.01(6H, d, J = 8 Hz, -$(CH_3)_2$), 2.19-2.31 (1H, m, -CH-), 2.45 (2H, d, J = 8 Hz, -CH_2-); **^{13}C NMR** (400 MHz, $CDCl_3$, ppm): δ 188.96 (C-1), 123.13 (C-2), 148.77 (C-3), 132.11 (C-2' and C-6'), 129.56 (C-3' and C-5'), 135.43 (C-1'), 146.12 (C-4'), 136.62 (C-1''), 115.21 (C-2''), 159.43 (C-3''), 118.46 (C-4''), 130.13 (C-5''), 120.14 (C-6''), 22.67 (-CH_3, C of isobutyl group at C-4'), 29.39 (-CH-, C of isobutyl group at C-4'), 44.17 (-CH_2-, C of isobutyl group at C-4'); **Anal. Calcd** for: $C_{19}H_{19}O_2$: C, 81.40; H, 7.19; Found: C, 81.45; H, 7.24.

2.2.12. (E)-1-(4'-isobutylphenyl)-3-(4''-nitrophenyl)-2-propen-1-one **(A11):** Yield 95%; m.p.190-192 °C; IR (KBr, cm−1): 1652 (C=O), 1610 (C=C of Ar), 1502 (CH=CH), 1541

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(N=O, asymmetric), 1346 (N=O, symmetric), 3092 (Ar C-H), 2951 (Alkyl C-H). ^{1}H NMR(400 MHz, CDCl3, δ) : 7.35 (1H, d, J = 17 Hz, -CO-CH=), 7.84 (1H, d, J =17 Hz, =CH-Ar), 7.05-7.95 (8H, Ar-H), 0.91 (6H, d, J = 8 Hz, -$(CH_3)_2$), 2.10-2.13 (1H, m, -CH-), 2.33 (2H, d, J = 8 Hz, -CH_2-); **^{13}C NMR** (400 MHz, CDCl3): δ 190.22 (C-1), 123.68 (C-2), 147.56 (C-3), 133.13 (C-2' and C-6'), 131.58 (C-3' and C-5'), 137.02 (C-1'), 148.31 (C-4'), 142.41 (C-1''), 152.32 (C-4''), 128.23 (C-2'' and C-6''), 122.11 (C-3'' and C-5''), 22.18 (-CH_3, C of isobutyl group at C-4'), 28.98 (-CH-, C of isobutyl group at C-4') and 43.87 (-CH_2-, C of isobutyl group at C-4'); Anal. Calcd for: $C_{19}H_{19}NO_3$: C, 73.77; H, 6.19; N, 4.53; Found: C, 73.80; H, 6.24; N, 4.59.

2.2.13. (E)-1-(4'-isobutylphenyl)-3-(4''-methoxyphenyl)-2-propen-1-one **(A12):** Yield 79%; **m.p.** 149-151 ºC; **IR** (KBr, cm^{-1}): 1655 (C=O), 1605 (C=C of Ar), 1508 (CH=CH), 1125 (-OCH_3), 3054 (Ar C-H), 2956 (Alkyl C-H); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 3.90 (3H,s,Ar-OCH_3), 7.19 (1H, d, J = 17 Hz, -CO-CH=), 7.74 (1H, d, J =17 Hz, =CH-Ar), 6.71-8.08 (8H, Ar-H), 0.80 (6H, d, J = 8 Hz, -$(CH_3)_2$), 1.62-1.84 (1H, m, -CH-), 2.09 (2H, d, J = 8 Hz, -CH_2-); **^{13}C NMR** (400 MHz, $CDCl_3$, ppm): δ 189.05 (C-1), 121.12 (C-2), 142.97 (C-3), 127.85 (C-2' and C-6'), 129.51 (C-3' and C-5'), 133.53 (C-1'), 143.32 (C-4'), 134.89 (C-1'') 145.12 (C-4''), 127.09 (C-2'' and C-6''), 115.51 (C-3'' and C-5''), 22.31 (-CH_3, C of isobutyl group at C-4''), 28.91 (-CH-, C of isobutyl group at C-4''), 44.91 (-CH_2-, C of isobutyl group at C-4''), 55.99 (-OCH_3 C at C-4''); **Anal. Calcd** for: $C_{20}H_{22}O_2$: C, 81.60; H, 7.53; Found: C, 81.65; H, 7.57.

2.2.14. (E)-1-(4'-isobutylphenyl)-3-(3'',4''-dimethoxyphenyl)-2-propen-1-one **(A13): Yield** 66%; **m.p.** 146-148 ºC; **IR** (KBr, cm^{-1}): 1655 (C=O), 1605 (C=C of Ar), 1500 (CH=CH), 1130 (-OCH_3), 3066 (Ar C-H), 2839 (Alkyl C-H); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 3.95 (6H, s, 2x Ar-OCH_3), 7.21 (1H, d, J = 17 Hz, -CO-CH=), 7.80 (1H, d, J =17 Hz, =CH-Ar), 6.91-8.12 (6H, Ar-H), 1.00 (6H, d, J = 8 Hz, -$(CH_3)_2$), 2.21-2.42 (1H, m, -CH-), 2.55 (2H, d, J = 8 Hz, -CH_2-); **^{13}C NMR** (400 MHz, $CDCl_3$, ppm): δ 188.55 (C-1), 121.01 (C-2), 142.31 (C-3), 129.61 (C-2' and C-6'), 130.74 (C-3' and C-5'), 134.20 (C-1'), 144.53 (C-4'), 128.29 (C-1'') 112.22 (C-2''), 149.90 (C-3'' and C-4''), 115.51 (C-5''), 119.84 (C-6''), 21.71 (-CH_3, C of isobutyl group at C-4''), 28.58 (-CH-,C of isobutyl group at C-4''), 43.82 (-CH_2-, C of isobutyl group at C-4''), 56.71 (-OCH_3 C at C-3'' and C-4''); **Anal. Calcd** for: $C_{20}H_{22}O_2$: C, 77.75; H, 7.46; Found: C, 77.77; H, 7.47.

2.2.15. (E)-1-(4'-isobutylphenyl)-3-(3'',4'',5''-trimethoxyphenyl)-2-propen-1-one **(A14): Yield** 70%; **m.p.** 180-182 ºC; **IR** (KBr, cm^{-1}): 1652 (C=O), 1585 (C=C of Ar), 1462 (CH=CH), 1127 (-OCH_3), 3110 (Ar C-H), 2853 (Alkyl C-H); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 3.90 (3H, s, Ar-OCH_3), 3.92 (6H, s, 2x Ar-OCH_3), 7.22 (1H, d, J = 17 Hz, -CO-CH=), 7.53 (1H, d, J =17 Hz, =CH-Ar), 6.85-8.07 (6H, Ar-H), 1.08 (6H, d, J = 8 Hz, -$(CH_3)_2$), 2.29-2.45 (1H, m, -CH-), 2.65 (2H, d, J = 8 Hz, -CH_2-); **^{13}C NMR** (400 MHz, $CDCl_3$, ppm): δ 188.11 (C-1), 121.33 (C-2), 141.86 (C-3), 128.11 (C-2' and C-6'), 130.99 (C-3' and C-5'), 134.73 (C-1'), 146.24 (C-4'), 129.93 (C-1''), 102.91 (C-2'' and C-6''), 151.04 (C-3'' and C-5''), 139.29 (C-4''), 21.91 (-CH_3, C of isobutyl group at C-4''), 29.34 (-CH-,C of isobutyl group at C-4''), 45.56 (-CH_2-, C of isobutyl group at C-4''), 56.71 (-OCH_3 C at C-3'', C-4'' and C-5''); **Anal. Calcd** for: $C_{20}H_{22}O_2$: C, 74.55; H, 7.39; Found: C, 74.56; H, 7.43.

2.2.16. (E)-1-(4'-isobutylphenyl)-3-(2''-pyridinyl)-2-propen-1-one **(A15)**: Yield 76%; **m.p.** 132-134 ºC; **IR** (KBr, cm^{-1}): 1651 (C=O), 1581 (C=N), 1604 (C=C of Ar), 1505 (CH=CH), 1368 (C-N), 3006 (Ar C-H), 2799 (Alkyl C-H); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 7.15 (1H, d, J = 17 Hz, -CO-CH=), 7.51 (1H, d, J =17 Hz, =CH-Ar), 6.32-8.41 (8H, Ar-H), 1.91

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(6H, d, J = 8 Hz, -(CH_3)$_2$), 1.89-2.09 (1H, m, -CH-), 2.33 (2H, d, J = 8 Hz, -CH_2-); **^{13}C NMR** (400 MHz, $CDCl_3$, ppm): δ 189.70 (C-1), 127.73 (C-2), 140.32 (C-3), 128.39 (C-2' and C-6'), 129.05 (C-3' and C-5'), 134.94 (C-1'), 147.11 (C-4'), 155.75 (C-2''), 122.02 (C-3''), 137.39 (C-4''), 123.09 (C-5''), 149.16 (C-5''), 22.53 (-CH_3, C of isobutyl group at C-4''), 29.88 (-CH-, C of isobutyl group at C-4''), 45.99 (-CH_2-, C of isobutyl group at C-4''); **Anal. Calcd** for: $C_{18}H_{19}NO$: C, 81.47; H, 7.22; N, 5.28; Found: C, 81.51; H, 7.25; N, 5.33.

2.2.17. 1-(4'-isobutylphenyl)-3-(3''-pyridinyl)-2-propen-1-one **(A16):** Yield 86%; **m.p.** 143-145 ºC; **IR** (KBr, cm^{-1}): 1645 (C=O), 1590 (C=N), 1603 (C=C of Ar), 1502 (CH=CH), 1370 (C-N), 3098 (Ar C-H), 2937 (Alkyl C-H); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 7.17 (1H, d, J = 17 Hz, -CO-CH=), 7.55 (1H, d, J =17 Hz, =CH-Ar), 6.23-8.15 (8H, Ar-H), 0.99 (6H, d, J = 8 Hz, -(CH_3)$_2$), 1.90-2.13 (1H, m, -CH-), 2.59 (2H, d, J = 8 Hz, -CH_2-); **^{13}C NMR** (400 MHz, $CDCl_3$, ppm): δ 188.20 (C-1), 127.31 (C-2), 143.32 (C-3), 127.91 (C-2' and C-6'), 129.59 (C-3' and C-5'), 134.77 (C-1'), 146.52 (C-4'), 151.25 (C-2''), 132.26 (C-3''), 133.53 (C-4''), 123.85 (C-5''), 149.99 (C-5''), 22.11 (-CH_3, C of isobutyl group at C-4''), 29.59 (-CH-, C of isobutyl group at C-4''), 45.12 (-CH_2-, C of isobutyl group at C-4''); **Anal. Calcd** for: $C_{18}H_{19}NO$: C, 81.47; H, 7.22; N, 5.28; Found: C, 81.51; H, 7.25; N, 5.33.

2.2.18. (E)-1-(4'-isobutylphenyl)-3-(4''-pyridinyl)-2-propen-1-one **(A17):** Yield 89%; **m.p.** 165-167 ºC; **IR** (KBr, cm^{-1}): 1650 (C=O), 1581 (C=N), 1605 (C=C of Ar), 1505 (CH=CH), 1373 (C-N), 3101 (Ar C-H), 2811 (Alkyl C-H); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 7.26 (1H, d, J = 17 Hz, -CO-CH=), 7.61 (1H, d, J =17 Hz, =CH-Ar), 6.21-8.59 (8H, Ar-H), 0.93 (6H, d, J = 8 Hz, -(CH_3)$_2$), 2.12-2.17 (1H, m, -CH-), 2.62 (2H, d, J = 8 Hz, -CH_2-); **^{13}C NMR** (400 MHz, $CDCl_3$, ppm): δ 188.59 (C-1), 127.77 (C-2), 143.91 (C-3), 128.26 (C-2' and C-6'), 128.88 (C-3' and C-5'), 134.96 (C-1'), 146.97 (C-4'), 149.35 (C-2''), 121.75 (C-3''), 144.31 (C-4''), 120.92 (C-5''), 149.97 (C-5''), 22.11 (-CH_3, C of isobutyl group at C-4''), 29.59 (-CH-, C of isobutyl group at C-4''), 45.12 (-CH_2-, C of isobutyl group at C-4''); **Anal. Calcd** for: $C_{18}H_{19}NO$: C, 81.47; H, 7.22; N, 5.28; Found: C, 81.51; H, 7.25; N, 5.33.

2.2.19. (E)-1-(4'-isobutylphenyl)-3-(2''-pyrrolyl)-2-propen-1-one (A18)**:** Yield 82%; **m.p.** 189-191 ºC; **IR** (KBr, cm^{-1}): 1652 (C=O), 1588 (C=N), 1605 (C=C of Ar), 1506 (CH=CH), 1375 (C-N), 3121 (Ar C-H), 2935 (Alkyl C-H); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 5.10 (1H, s, -NH), 7.24 (1H, d, J = 17 Hz, -CO-CH=), 7.60 (1H, d, J =17 Hz, =CH-Ar), 6.94-7.72 (7H, Ar-H), 0.95 (6H, d, J = 8 Hz, -(CH_3)$_2$), 1.85-2.07 (1H, m, -CH-), 2.55 (2H, d, J = 8 Hz, -CH_2-); **^{13}C NMR** (400 MHz, $CDCl_3$, ppm): δ 187.51 (C-1), 126.23 (C-2), 132.74 (C-3), 129.17 (C-2' and C-6'), 129.88 (C-3' and C-5'), 134.61 (C-1'), 146.97 (C-4'), 129.51 (C-2''), 112.56 (C-3''), 108.26 (C-4''), 119.39 (C-5''), 23.12 (-CH_3, C of isobutyl group at C-4''), 30.63 (-CH-, C of isobutyl group at C-4''), 47.99 (-CH_2-, C of isobutyl group at C-4''); **Anal. Calcd** for: $C_{17}H_{19}NO$: C, 80.60; H, 7.56; N, 5.53; Found: C, 80.64; H, 7.59; N, 5.54.

2.2.20. (E)-1-(4'-isobutylphenyl)-3-(2''-thienyl)-2-propen-1-one **(A19):** Yield 86%; **m.p.** 179-181 ºC; **IR** (KBr, cm^{-1}): 1655 (C=O), 1610 (C=C of Ar), 1505 (CH=CH), 624 (C-S), 3119 (Ar C-H), 2954 (Alkyl C-H); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 7.34 (1H, d, J = 17 Hz, -CO-CH=), 7.82 (1H, d, J =17 Hz, =CH-Ar), 6.85-8.30 (7H, Ar-H), 0.85 (6H, d, J = 8 Hz, -(CH_3)$_2$), 1.71-2.09 (1H, m, -CH-), 2.99 (2H, d, J = 8 Hz, -CH_2-); **^{13}C NMR** (400 MHz, $CDCl_3$, ppm): δ 189.98 (C-1), 127.95 (C-2), 134.26 (C-3), 130.76 (C-2' and C-6'), 129.76 (C-3' and C-5'), 135.84 (C-1'), 147.38 (C-4'), 138.85 (C-2''), 128.21 (C-3''), 129.15 (C-4''), 130.19 (C-5''), 22.91 (-CH_3, C of isobutyl group at C-4''), 30.11 (-CH-, C of isobutyl group at C-4''), 47.12 (-CH_2-, C of isobutyl group at C-4''); **Anal. Calcd** for: $C_{17}H_{18}SO$: C, 75.51; H, 6.71; Found: C, 75.55; H, 6.73.

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2.2.21. (E)-1-(4'-isobutylphenyl)-3-(5''-bromofuran-2''-yl)-2-propen-1-one **(A20):** Yield 85%; **m.p.** 149-151 ^{o}C; **IR** (KBr, cm^{-1}): 1652 (C=O), 1585 (C=C of Ar), 1503 (CH=CH), 2959 (Ar C-H), 2713 (Alkyl C-H); **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 7.19 (1H, d, J = 17 Hz, -CO-CH=), 7.79 (1H, d, J =17 Hz, =CH-Ar), 6.89-7.85 (7H, Ar-H), 1.01 (6H, d, J = 8 Hz, -$(CH_3)_2$), 2.05-2.22 (1H, m, -CH-), 2.62 (2H, d, J = 8 Hz, -CH_2-); **^{13}C NMR** (400 MHz, $CDCl_3$, ppm): δ 189.88 (C-1), 127.58 (C-2), 131.43 (C-3), 130.21 (C-2' and C-6'), 128.95 (C-3' and C-5'), 135.13 (C-1'), 146.88 (C-4'), 154.81 (C-2''), 114.38 (C-3''), 114.67 (C-4''), 123.12 (C-5''), 22.65 (-CH_3, C of isobutyl group at C-4''), 29.81 (-CH-, C of isobutyl group at C-4''), 46.66 (-CH_2-, C of isobutyl group at C-4''); **Anal. Calcd** for: $C_{17}H_{17}BrO_2$: C, 61.28; H, 5.14; Found: C, 61.33; H, 5.17.

2.3. In vitro cytotoxicity assays

Compounds (**A1 to A20**) were evaluated for cytotoxic activity against **HT-29**, **MCF-7** and **DU-145** cell lines by means of MTT [3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyl-tetrazoliumbromide] cell proliferation assay (Mosmann 1983). This is a colorimetric assay that measures the reduction of yellow MTT by mitochondrial reductase to an insoluble, dark purple coloured formazan. The cells are then treated with DMSO to solubilize formazan which is measured spectrophotometrically at 570 nm. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells [28, 29].

HT-29 and **DU-145** cell lines were grown as adherent in DMEM media, whereas **MCF-7** was grown in MEM media supplemented with 10% fetal bovine serum. The cultures were maintained in a humidified atmosphere with 5% CO_2. Stock solutions of test compounds (**A1 to A20)** were prepared (10 mg/mL) in DMSO and from them various dilutions were made with sterile water to get the final drug concentrations of 10, 50, 100 and 200 mg/mL.

Cell lines were seeded in 96 well plates at a concentration of 1x10^4/well and incubated for 24 h at 37 ^{0}C, and then the medium was replaced with fresh media containing different dilutions of the test compounds and incubated for additional 48 h at 37 ^{0}C in DMEM/MEM with 10% FBS medium. Subsequently the medium was replaced with 90 µL of fresh DMEM without FB. Above wells were treated with 10 µL of MTT reagent (5 mg/mL of stock solution in DMEM without FBS) and incubated at 37 ^{0}C for 3-4 h. The formed blue formazan crystals were dissolved in 200 µL of DMSO. The absorbance at 570 nm was measured on a spectrophotometer. Anticancer agent methotrexate (Mtx) was used as positive control. Assay was performed in triplicate for three independent determinations. The cytotoxicity was expressed as IC_{50} (µg/mL).

2.4. Computational evaluation

A set of 20 synthesized chalcones were selected to build the pharmacophore using PHASE™ v 3.1 (Schrödinger LLC, Portland, Oregon, USA; http://www.schrodinger.com/). The IC_{50} values emerged out of the cytotoxicity studies on the three cancer cell lines **HT-29**, **MCF-7** and **DU-145** were used to perform the required study with PHASE™. The 3D-structure of the ligands were built and minimized with ChemDraw Ultra™ v 10.0 (CambridgeSoft Corporation, Cambridge, MA, USA; http://www.cambridgesoft.com/) and were incorporated into PHASE™ and then cleaned by the PHASE's LipPreg™ module. The set of new chalcones were divided

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into active or inactive according to their –log10 (IC_{50}) values. Tree-based partitioning technique was applied for the identification of pharmacophores that are common to a set of active compounds which have a specific number of pharmacophore sites. Thus in this study, the total number of required active compounds and the number of pharmacophores were reduced to 4. By following the above process, we obtained a list of different variants for different pharmacophore models which were the result of the combinations of five pharmacophore features including, one H-bond acceptor (A), two hydrophobic groups (H) and aromatic rings (R) respectively. The hypothesis identified by Phase was scored according to how the active ligands superimpose on features associated with that hypothesis.

3. Results and Discussion

3.1. Chemistry.

The designed novel target isobutylchalcones were synthesized by conventional base-catalyzed Claisen-Schmidt condensation of 1-(4-isobutylphenyl)ethanone and aromatic or heteroaromatic aldehyde as illustrated in **Scheme 1**. Most of the compounds were pure as evidenced by their TLC profiles and the impure compounds were purified by recrystallization using ethanol. Structures of the purified compounds **(A1-A20)** were explicitly unravelled on the basis of spectroscopic data (IR, ^{1}H NMR, ^{13}C NMR) and the results were consistent with the proposed structures of the compounds. Two intense characteristic IR absorption bands in the range 1645-1660 cm^{-1} (-C=O) and 1450-1520 cm^{-1} (-C=C-) respectively confirmed the formation of chalcone bridge. Additional -C=C- and -C-H stretching bands in the range 1580-1610 cm^{-1} and 3010-3150 cm^{-1} had confirmed the presence of aromatic rings. A characteristic band appearing in the range of 2750-2850 cm^{-1} corresponds to the alkyl –C-H stretching of the isobutyl group. The ^{1}H NMR spectrum of these compounds showed the characteristic resonance of -CO-CH= (α-H) around δ 6.7-7.4 ppm and δ 7.3-7.8 =CH-Ar (β-H) as doublets with coupling constant **(*J* =17 HZ)** respectively confirming the *trans* (*E*) geometry at the ethylenic double bond of the molecule. The peaks in between δ 6-8 accounts for the other aromatic protons. Further the doublet between δ 0.80-1.10 integrated for the six protons of two methyl groups, multiplet around δ 1.60-2.20 integrated for one methine (methanetriyl) proton and a doublet between 2.30-2.80 integrated for two methylene protons of the isobutyl group. Other protons exhibited additional resonance signals typically present in each compound. ^{13}C NMR of compounds exhibited the diagnostic signals around δ 186-191 (C-1), 120-128 (C-2) and 131-142 (C-3). The composition of the synthesized compounds was confirmed by elemental analysis and the results were also in close agreement with those of the calculated values.

3.2. In vitro cytotoxicity assays and structure activity relationships

As noticeable from **Table 1** (IC_{50} in µg/ml), most of the compounds possess cytotoxic activity but less compared to the standard methotrexate. All the compounds were active against **DU-145** and some being inactive against the other two cell lines. Among the three cell lines the compounds were more active against **DU-145** compared to **HT-29** and **MCF-7**. 2″,4″-difluorophenyl chalcone **A6**, exhibited maximum activity against all the cell lines with an IC_{50} (µg/ml) values of 42 (**HT-29**), 38 (**MCF-7**) and 18 (**DU-145**) whereas **A3** containing 2″,4″-

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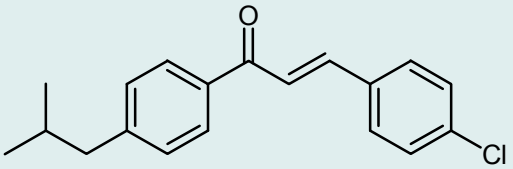
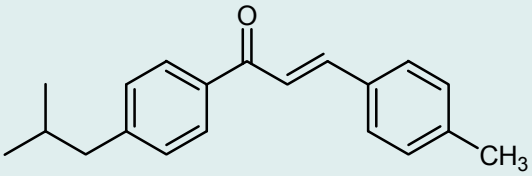
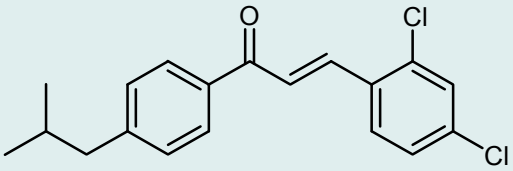
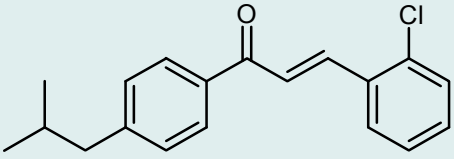
dichlorophenyl moiety was next in potency to **A6** against **HT-29** (67 µg/ml), **MCF-7** (58 µg/ml) and **DU-145** (23 µg/ml) respectively. Chalcones **A8**, **A14**, and **A19** containing 3″-bromophenyl, 3″,4″,5″-trimethoxyphenyl and 2″-thienyl respectively were inactive against both **HT-29** and **MCF-7** while **A2**, **A7** and **A20** with 4″-methylphenyl, 4″-dimethylaminophenyl and 5″-bromofuran-2″-yl were inactive only against **MCF-7**.

Against **HT-29** chalcones with electron releasing groups 4″-fluorophenyl (**A5**), 4″-chlorophenyl (**A1**), 4″-nitrophenyl (**A11**) were active with IC_{50} values 68, 76 and 85 µg/ml respectively subsequent to **A3** and **A6**. Intriguingly compounds **A2** with electron releasing 4″-methylphenyl and **A17** with heteroaryl 4″-pyridinyl were also active with IC_{50} of 90 and 93 but less than that of the substituents with electron withdrawing groups. It suggests that electron withdrawing group at 4″-position was more essential for activity than electron releasing groups and heteroaryl scaffold. Substitution with electron withdrawing groups only at 2″- and 3″-positions or with five membered heterocycles makes the compounds less active or inactive. Hence six membered aryl heteroaryl with more number of electron withdrawing groups can be synthesized for further escalating the potency. The other compounds were active at concentrations higher than 100 µg/ml.

Interestingly, against **MCF-7** chalcone **A17** with 4″-pyridinyl system was more active than **A5** with 4″-fluorophenyl and **A11** with 4″-nitrophenyl. Nearly equal potencies of the three compounds again represented the importance of electron withdrawing group at 4″-position and six membered heteroaryl scaffolds for activity.

Table 1. Cytotoxicity of compounds **A1-A20** against different cell lines in comparison to methotrexate (IC_{50} in µg/ml)

Compound	Structure	HT-29	MCF-7	DU-145
A1		76 ± 2	83 ± 1	70 ± 2
A2		90 ± 2	NA	72 ± 1
A3		67 ± 2	58 ± 2	23 ± 1
A4		137±2	183 ± 2	144 ± 1

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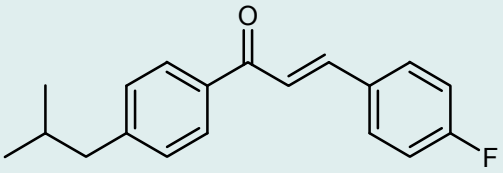
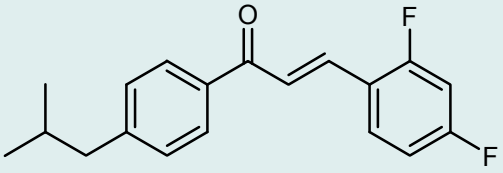
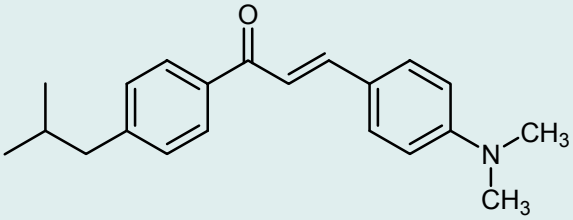
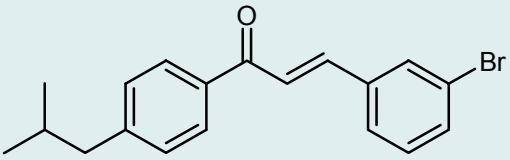
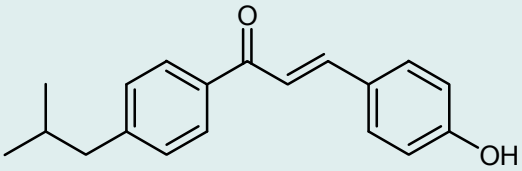
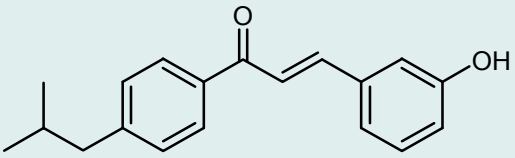
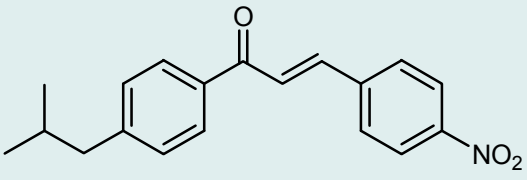
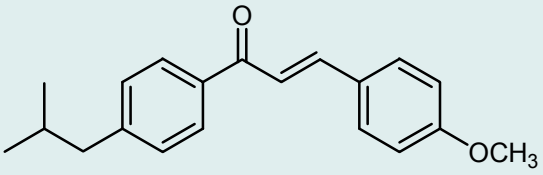
A5		68 ± 2	89 ± 1	43 ± 2
A6		42 ± 2	38 ± 1	18 ± 1
A7		110 ± 2	NA	101 ± 2
A8		NA	NA	155 ± 2
A9		146 ± 2	167 ± 2	120 ± 1
A10		164 ± 2	182 ± 2	176 ± 2
A11		85 ± 2	92 ± 2	68 ± 3
A12		124 ± 2	133 ± 1	78 ± 2

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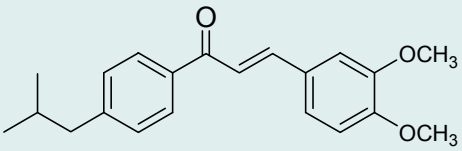
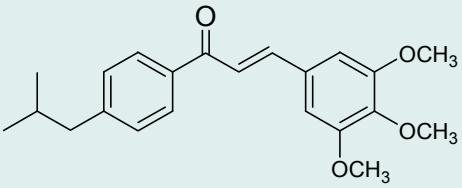
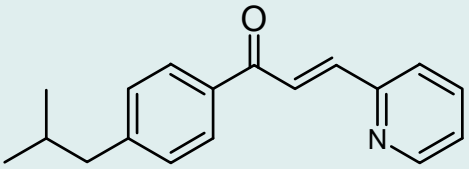
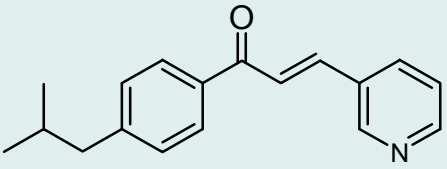
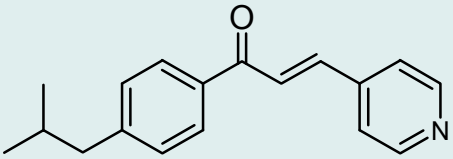
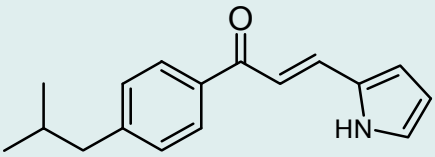
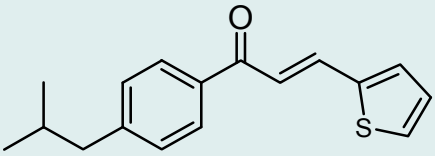
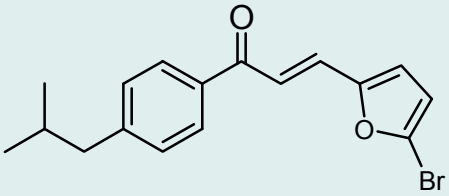
A13		146 ± 2	153 ± 2	82 ± 2
A14		NA	NA	174 ± 2
A15		120 ± 1	172 ± 2	105 ± 2
A16		132 ± 1	117 ± 2	105 ± 2
A17		93 ± 2	88 ± 1	74 ± 2
A18		NA	148 ± 2	107 ± 2
A19		NA	NA	123 ± 2
A20		190 ± 2	NA	116 ± 1
Mtx		12 ± 1	9 ± 1	5 ± 1

NA= No activity

DU-145 cell line was most susceptibile of the three cell lines. In this case the chalcones **A2** with 4″-methyl, **A12** with 4″-methoxyphenyl and **A13** with 3″,4″- methoxyphenyl possessed potencies below 100. It seems that for cytotoxic activity against **DU-145** cell line

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chalcones with electron releasing/withdrawing/heteroaryl were important. Compounds with five membered heterocycles also possess some inhibitory activity.

In summary, for chalcones to be act as potential cytotoxic agents against the cell lines, it could be observed that chalcone bridge with isobutyl phenyl ring-A is essential and the structural requirements of ring-B (**Figs. 3** and **4**) are as follows.

a) **HT-29**: 6-membered aryl rings with 2",4"-di/4-mono EWGs or 6-membered heteroaryl.
b) **MCF-7**: 6-membered aryl rings with 2",4"-di/4-mono EWGs or 6-membered heteroaryl.
c) **DU-145**: (i) 6-membered aryl rings with EWGs at 2",4"- or 4"-positions.
(ii) 6-membered aryl rings with ERGs at 4" or 3" - and 4"-positions.
(iii) 6-membered heteroaryl rings.

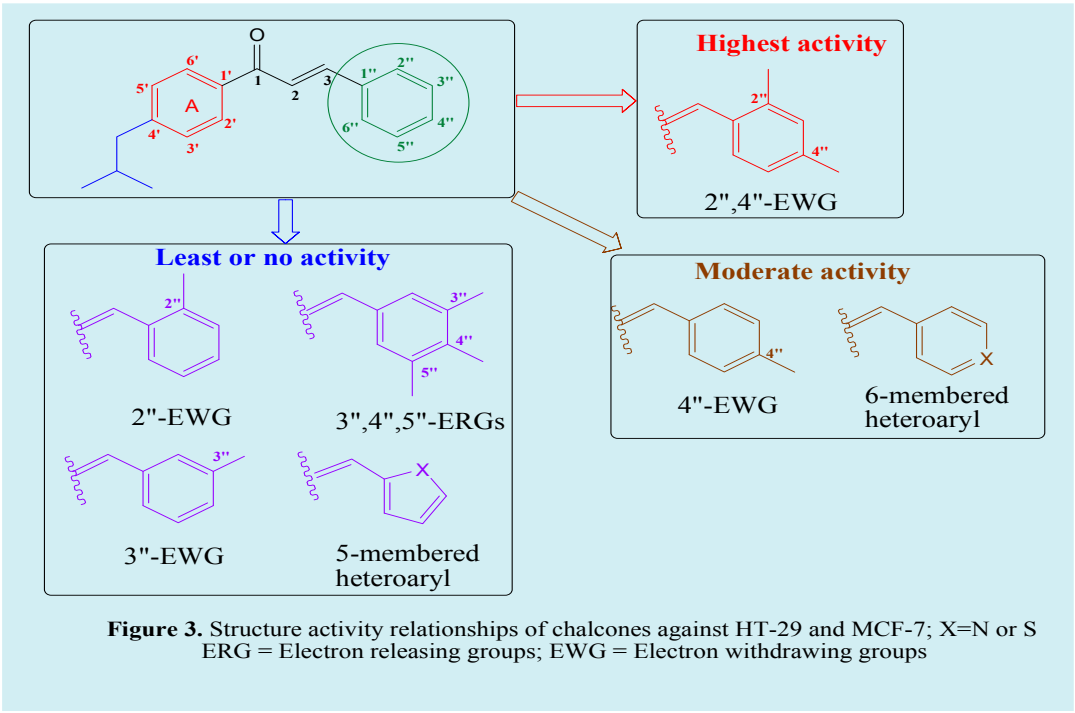

Figure 3. Structure activity relationships of chalcones against HT-29 and MCF-7; X=N or S
ERG = Electron releasing groups; EWG = Electron withdrawing groups

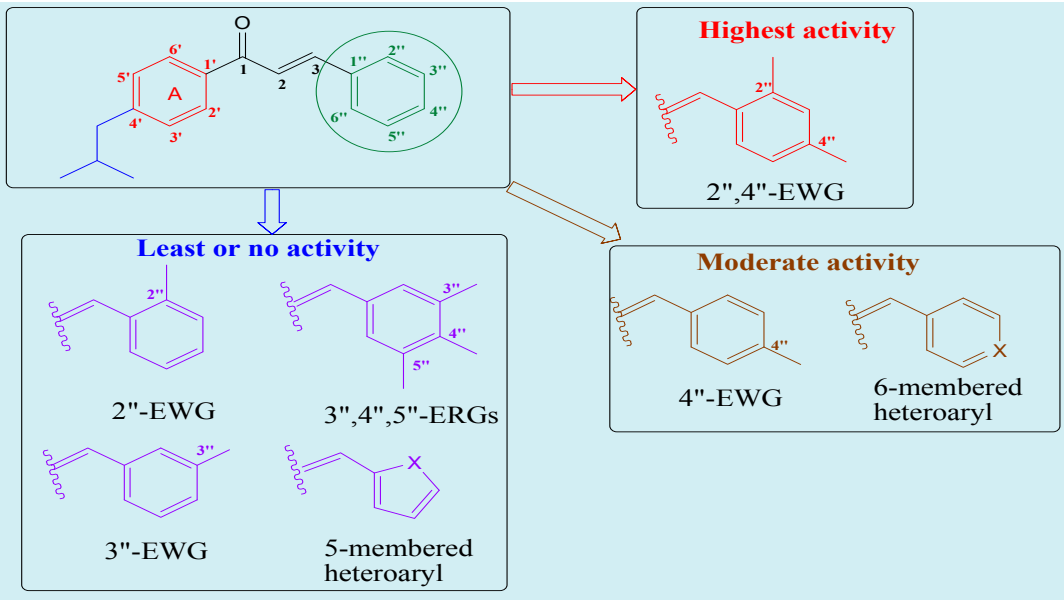

Figure 4. Structure activity relationships of chalcones against HT-29 and MCF-7; X=N or S
ERG = Electron releasing groups; EWG = Electron withdrawing groups

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3.3. Computational evaluation

From the pharmacophore modelling results, it was able to identify a common pharmacophore with a five point model AHHRR (**Fig. 5**). The parameters of the five point model are presented in **Table 2**. Two pharmacophore hypotheses were chosen. The pharmacophore hypothesis-1, **AHHRR.43*** and hypothesis-2, **AHHRR.25*** that were selected belong to a box that had survived the partitioning process as characterized by five sites (*i.e.,* the hypothesis contained one hydrogen bond acceptor (A), two hydrophobic groups (H) and two aromatic rings (R)) at a specific intersite distance and specific bond angles as shown in **Tables 3, 4, 5** and **6** respectively. These hypotheses were selected on the basis of the active and the inactive compounds (*i.e.* the less active compounds), because the subsequent inactive compounds were used to penalize. Thus, we chose the hypothesis with the highest survival score after the penalization with the inactive compounds. Phase generated pharmacophore hypothesis-1 and 2 are shown in **figures 6** and **8**. The pharmacophore hypothesis-1 was generated from the chalcone **A6** (**Fig. 7**) having a fitness factor 3 and the IC_{50} values 42±2, 38±1, 18±1 on cell lines **HT-29**, **MCF-7** and **DU-145** respectively whereas the chalcone **A3** was utilized to generate pharmacophore hypothesis-2 (**Fig. 9**) having fitness factor 3 and the IC_{50} values 67±2, 58±1, 23±1 on cell lines **HT-29**, **MCF-7** and **DU-145** respectively. These results can be considered as moderate when compared with the standard methotrexate (IC_{50} values 12±1, 9±1, 5±1 on cell lines **HT-29**, **MCF-7** and **DU-145** respectively). The pharmacophore models are in correlation with the *invitro* activity data and clearly explained the importance of ring-A with 4'-isobutyl substituent and ring-B with electron withdrawing groups.

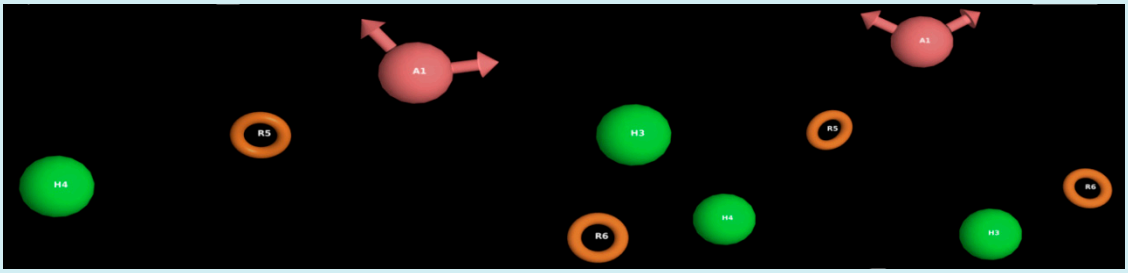

Figure 5. Common pharmacophore hypothesis generated using the set of all 20 chalcones illustrating hydrophobic groups (H, Green spheres), aromatic rings (R, Orange spheres) and hydrogen bond acceptor (A, Pink sphere).

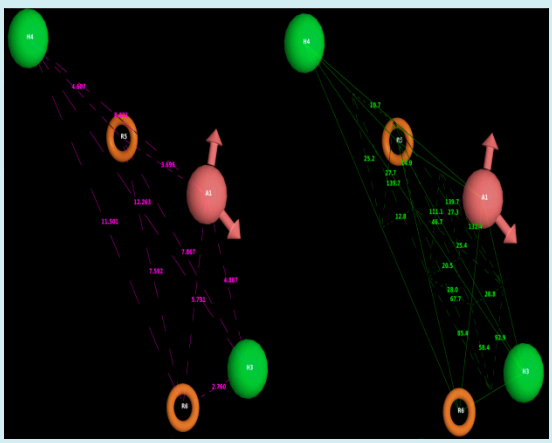

Figure 6. Phase generated pharmacophore hypothesis-1 AHHRR.43*and distance/angle between pharmacophoric sites.

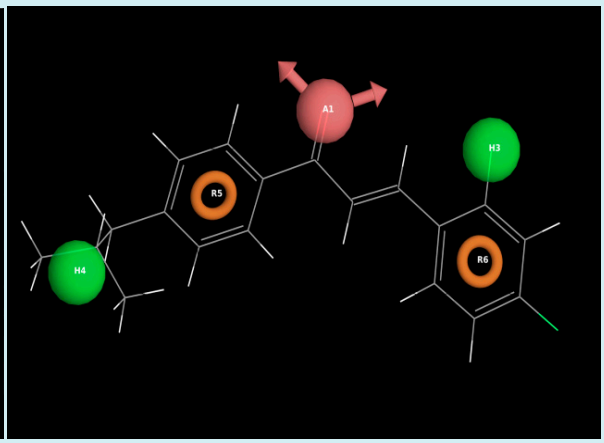

Figure 7. Phase generated best pharmacophore hypothesis-1 AHHRR.43* aligned with molecule A6.

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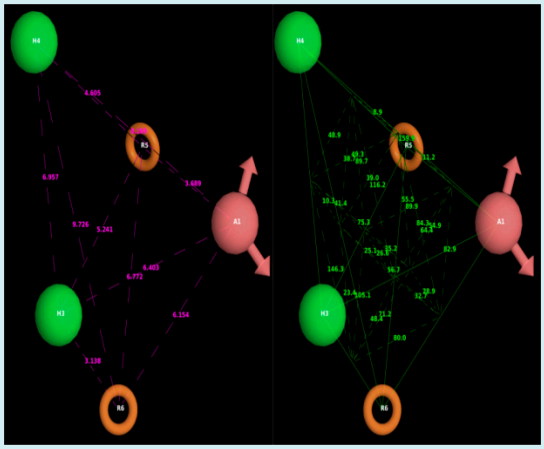

Figure 8. Phase generated pharmacophore hypothesis-2 AHHRR.25*and distance/angle between pharmacophoric sites. All distances are in Å unit.

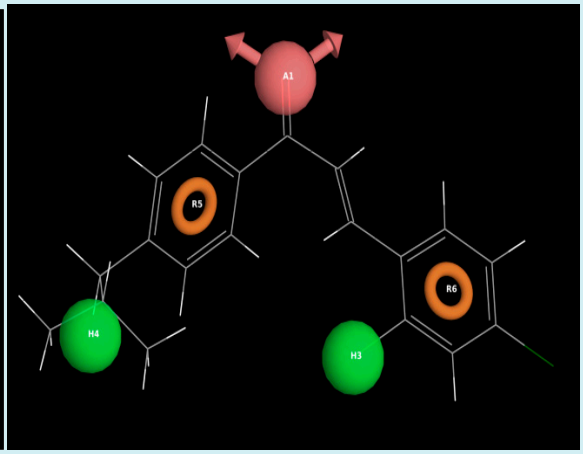

Figure 9. Phase generated best pharmacophore hypothesis-2 AHHRR.25* aligned with molecule A3.

Table 2. Parameters of the five featured pharmacophore

S.No	ID	Survival	Site	Vector	Volume	Selectivity	Energy
1.	**AHHRR.43***	**3.696**	**0.81**	**0.995**	**0.887**	**1.93**	**0.25**
2.	AHHRR.5	3.682	0.8	0.995	0.885	1.928	0.251
3.	AHHRR.23	3.682	0.8	0.995	0.885	1.922	0.53
4.	AHHRR.3	3.68	0.78	0.992	0.906	1.982	0.107
5.	AHHRR.4	3.68	0.78	0.992	0.906	1.979	0.044
6.	**AHHRR.25***	**3.677**	**0.78**	**0.991**	**0.903**	**2.002**	**0.18**
7.	AHHRR.26	3.677	0.78	0.991	0.903	2	0.09
8.	AHHRR.65	3.657	0.77	0.996	0.887	1.997	0.25
9.	AHHRR.72	3.657	0.77	0.996	0.887	1.99	0.528
10.	HHHRR.51	3.654	0.79	0.993	0.872	1.969	0.25
11.	HHHRR.54	3.654	0.79	0.993	0.872	1.955	0.528
12.	AHHHR.111	3.652	0.76	0.999	0.888	1.828	0.25
13.	AHHHR.112	3.652	0.76	0.999	0.888	1.825	0.528
14.	AHHHR.101	3.644	0.73	0.999	0.917	1.908	0
15.	AHHHR.102	3.644	0.73	0.999	0.917	1.91	0
16.	AHHHR.107	3.644	0.76	0.993	0.891	1.878	0.25
17.	AHHHR.108	3.644	0.76	0.993	0.891	1.874	0.528
18.	AHHHR.83	3.636	0.75	0.999	0.888	1.825	0.251
19.	HHHRR.52	3.635	0.77	0.993	0.875	1.968	0.251
20.	HHHRR.56	3.635	0.77	0.993	0.875	1.95	0.53
*selected pharmacophore hypothesis							

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Table 3. Distances between different sites of pharmacophore model AHHRR.43*

Entry	Site1	Site2	Distance
AHHRR.43	A1	H3	4.887
AHHRR.43	A1	H4	8.121
AHHRR.43	A1	R5	3.695
AHHRR.43	A1	R6	5.731
AHHRR.43	H3	H4	12.263
AHHRR.43	H3	R5	7.867
AHHRR.43	H3	R6	2.760
AHHRR.43	H4	R5	4.607
AHHRR.43	H4	R6	11.501
AHHRR.43	R5	R6	7.592

Table 4. Distances between different sites of pharmacophore model AHHRR.25*

Entry	Site1	Site2	Distance
AHHRR.25	A1	H3	6.403
AHHRR.25	A1	H4	8.169
AHHRR.25	A1	R5	3.689
AHHRR.25	A1	R6	6.154
AHHRR.25	H3	H4	6.957
AHHRR.25	H3	R5	5.241
AHHRR.25	H3	R6	3.138
AHHRR.25	H4	R5	4.605
AHHRR.25	H4	R6	9.726
AHHRR.25	R5	R6	6.772

Conclusions

A series of twenty chalcones were prepared by simple Claisen-Schmidt condensation reaction of isobutylacetophenone with aromatic aldehydes containing electron releasing or withdrawing substituents on other aryl or heteroaryl rings (B) for comparing the *in vitro* cytotoxic activity. Among the compounds tested for cytotoxic activity the chalcone **A6** with electron withdrawing 2",4"-difluorophenyl moiety was found to be most potent against all the three cancer cell lines. The compound **A3** with 2",4"-dichlorophenyl moiety also possessed comparable cytotoxic potency. From the SAR studies it could be inferred that the chalcone bridge linked to 4'-isobutyphenyl ring and ring-B with electron withdrawing substituents in *ortho* and *para* positions are very much essential for cytotoxic activity. All the twenty chalcones were subjected to pharmacophore modeling using PHASE^{TM} software. The computational results revealed the most potent nature of chalcones **A6** having a 2,4-difluorophenyl moiety and **A3** having a 2,4-dichlorophenyl moiety against the tested cell lines. The pharmacophores generated from these two compounds by the two proposed hypotheses (as discussed under results) accounts for the said cytotoxicity and further these models can be used as a reference to identify features required for newly synthesized chalcones for their cytotoxicity.

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Table 5. Angles between different sites of model AHHRR.43*.

Entry	Site1	Site2	Site3	Angle
AHHRR.43	H3	A1	H4	139.7
AHHRR.43	H3	A1	R5	132.4
AHHRR.43	H3	A1	R6	28.8
AHHRR.43	H4	A1	R5	13.4
AHHRR.43	H4	A1	R6	111.1
AHHRR.43	R5	A1	R6	105.2
AHHRR.43	A1	H3	H4	25.4
AHHRR.43	A1	H3	R5	20.3
AHHRR.43	A1	H3	R6	92.9
AHHRR.43	H4	H3	R5	8.1
AHHRR.43	H4	H3	R6	67.7
AHHRR.43	R5	H3	R6	74.1
AHHRR.43	A1	H4	H3	14.9
AHHRR.43	A1	H4	R5	10.7
AHHRR.43	A1	H4	R6	27.7
AHHRR.43	H3	H4	R5	13.8
AHHRR.43	H3	H4	R6	12.8
AHHRR.43	R5	H4	R6	25.2
AHHRR.43	A1	R5	H3	27.3
AHHRR.43	A1	R5	H4	155.8
AHHRR.43	A1	R5	R6	46.7
AHHRR.43	H3	R5	H4	158.1
AHHRR.43	H3	R5	R6	20.5
AHHRR.43	H4	R5	R6	139.7
AHHRR.43	A1	R6	H3	58.4
AHHRR.43	A1	R6	H4	41.2
AHHRR.43	A1	R6	R5	28.0
AHHRR.43	H3	R6	H4	99.5
AHHRR.43	H3	R6	R5	85.4
AHHRR.43	H4	R6	R5	15.0

Table 6. Angles between different sites of model AHHRR.25*.

Entry	Site1	Site2	Site3	Angle
AHHRR.25	H3	A1	H4	55.5
AHHRR.25	H3	A1	R5	54.9
AHHRR.25	H3	A1	R6	28.9
AHHRR.25	H4	A1	R5	11.2
AHHRR.25	H4	A1	R6	84.3
AHHRR.25	R5	A1	R6	82.9
AHHRR.25	A1	H3	H4	75.3
AHHRR.25	A1	H3	R5	35.2
AHHRR.25	A1	H3	R6	71.2
AHHRR.25	H4	H3	R5	41.4
AHHRR.25	H4	H3	R6	146.3
AHHRR.25	R5	H3	R6	105.1
AHHRR.25	A1	H4	H3	49.3
AHHRR.25	A1	H4	R5	8.9
AHHRR.25	A1	H4	R6	39.0
AHHRR.25	H3	H4	R5	48.9
AHHRR.25	H3	H4	R6	10.3
AHHRR.25	R5	H4	R6	38.7
AHHRR.25	A1	R5	H3	89.9
AHHRR.25	A1	R5	H4	159.9
AHHRR.25	A1	R5	R6	64.4
AHHRR.25	H3	R5	H4	89.7
AHHRR.25	H3	R5	R6	26.6
AHHRR.25	H4	R5	R6	116.2
AHHRR.25	A1	R6	H3	80.0
AHHRR.25	A1	R6	H4	56.7
AHHRR.25	A1	R6	R5	32.7
AHHRR.25	H3	R6	H4	23.4
AHHRR.25	H3	R6	R5	48.4
AHHRR.25	H4	R6	R5	25.1

Conflict of Interests

There is no conflict of interests.

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