FABAD J. Pharm. Sci., 40, 1-16, 2015

RESEARCH ARTICLES

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

Afzal Basha SHAIK[*o], Y. Rajendra PRASAD[*], Shaik SHAHANAAZ[**]

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

SUMMARY

A series of novel isobutylchalcones (A1-A20) were prepared, evaluated for their cytotoxic activity and characterized by FTIR, 1H NMR, 13C NMR, and elemental analysis data. The logic behind the design was 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 (IC50 12 ± 1 (HT-29), 9 ±1 (MCF-7) 5 ± 1 (DU-145)) as reference standard. Compound A6 having 2,4-difluorophenyl moiety was the most potent of the series against all the three cell lines and notably A6 was mainly effective against DU-145 cell lines with an IC50 value of 18 µg/mL. The critical structural features required for the activity against all the cell lines were identified through pharmacophore model using PHASETM which has recognised a 5 point AHHRR model and is consistent with the cytotoxic activity of the tested compounds.

Key Words: *Chalcone, Cytotoxic activity, Pharmacophore model, PHASETM, AHHRR model*

Sitotoksik bileşikler olarak yeni izobütilşalkonların dizaynı, kolay sentezi, karakterizasyonu ve bilgisayarlı değerlendirilmesi-Kısım A

ÖZET

Yeni bir seri izobütilşalkon (A1-A20) hazırlandı, sitotoksik aktiviteleri için değerlendirildi ve FTIR, 1H-NMR, 13C-NMR ve elementel analiz verileriyle karekterize edildi. Bileşiklerin dizaynındaki amaç sitotoksik aktiviteye sahip heteroaromatik halkalar kadar elektron çeken ve sunan grupları taşıyan A ve B aromatik halkaları üzerinde lipofilik özelllikte elektron sunan izobutil sübstitüenti taşıyan şalkonların sentezlenmesi ve etkilerinin karşılaştırılmasıdır. Bileşikler metotreksat referans standardına (IC50 12 ± 1 (HT-29), 9 ±1 (MCF-7) 5 ± 1 (DU-145)) karşı HT-29 (kolon kanseri), MCF-7 (meme kanseri) ve DU-145 (prostat kanseri) hücre hatlarına karşı test edildi. 2,4-Difluorofenil artığı taşıyan bileşik A6 bütün hücre hatlarına karşı en etkin bileşiktir ve esas olarak A6 bileşiğinin DU-145 hücre hatlarına karşı 18 µg/mL IC50 değeri dikkat çekicidir. Bütün hücre hatlarına karşı aktivite için gerekli olan kritik yapısal özellik PHASE TM modeli kullanılarak farmakofor tayini üzerinden tespit edilmiştir ve test edilen bileşiklerin sitotoksik etkileri ile uyumludur.

Anahtar kelimeler: *Şalkon, Sitotoksik aktivite, Farmakofor model, PHASETM, AHHRR model*

Received: 16.12.2016
Revised: 29.12.2016
Accepted: 27.01.2017

*A.U College of Pharmaceutical Sciences, Andhra University, 53000, Andhra Pradesh, India.
**Victoria College of Pharmacy, 522001, Andhra Pradesh, India.

[o]Corresponding Author:
E-mail: bashafoye@gmail.com

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 (Fadeyi *et al*., 2008; Rostom *et al*., 2006). According to World Health Organization (WHO) cancer figures among the leading causes of morbidity and mortality worldwide, there were approximately 14 million new cases and 8.2 million cancer related deaths in 2012 (WHO/Cancer-World Health Organisation., 2013) 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 and among women were breast, colorectal, lung, cervix, and stomach cancer. Despite 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 chemotherapeutics (Sierra *et al*., 2010). 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 (Soliman *et al*., 2005; Nowakowska, 2007; Go *et al*., 2005) biosynthesized by means of polyketide pathway and are the intermediates for flavonoid biosynthesis (Thomas, 2010). These compounds possess broad array of pharmacological activities (Yazdan *et al*., 2015; Batovska *et al*., 2010) and specifically emerged as potential anticancer agents in the last 15 years (Sylvie, 2007). Several pure chalcones have been approved for clinical use in humans. Clinical trials have shown that these compounds reached reasonable plasma concentration, well-tolerated (Sahu *et al*., 2012) and have less chance to interact with DNA, which omits the risk of mutagenicity, a key problem with many anticancer agents (Dimmock *et al*., 1999). They are absorbed through daily diet and show promising cancer chemopreventive role (Lahsasni *et al*., 2013). 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., (Jandial *et al*., 2014). Aforementioned properties motivated us to synthesize and evaluate chalcones as potential cytotoxic agents.

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 (Wu *et al*., 2012), replacement of aryl rings with heteroaryl(s) (Sharma *et al*., 2013), rigidification of keto vinyl arrangement to form chalconoids (Letafat *et al*., 2013). 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 (Tang *et al*., 2008; Nishimura *et al*., 2007; Tang *et al*., 2010; Ivanova *et al*., 2008; Ducki *et al*., 2009; Cho *et al*., 2013), and even if monosubstitution is present it is either a simple $-NH_2$, –OH, $-OCH_3$ and $-CH_3$ (Mai *et al*., 2014; Syam *et al*., 2012; Hieu *et al*., 2012), 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). Most of the compounds prepared are new. However, some of the compounds A1, A2, A5, A12, A14 and A19 used in the present were previously evaluated for antiinflammatory, antibacterial and antifungal activities but not cytotoxic activity (Keche *et al*., 2010; Turkar *et al*., 2010; Abdellatif *et al*., 2015).

MATERIALS AND METHODS

GENERAL

All chemical reagents and solvents were purchased from S.D Fine Chem. Ltd, Mumbai, India. 4-isobutylacetophenone was purchased from Aldrich Chemical Co. (Milwaukee, 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

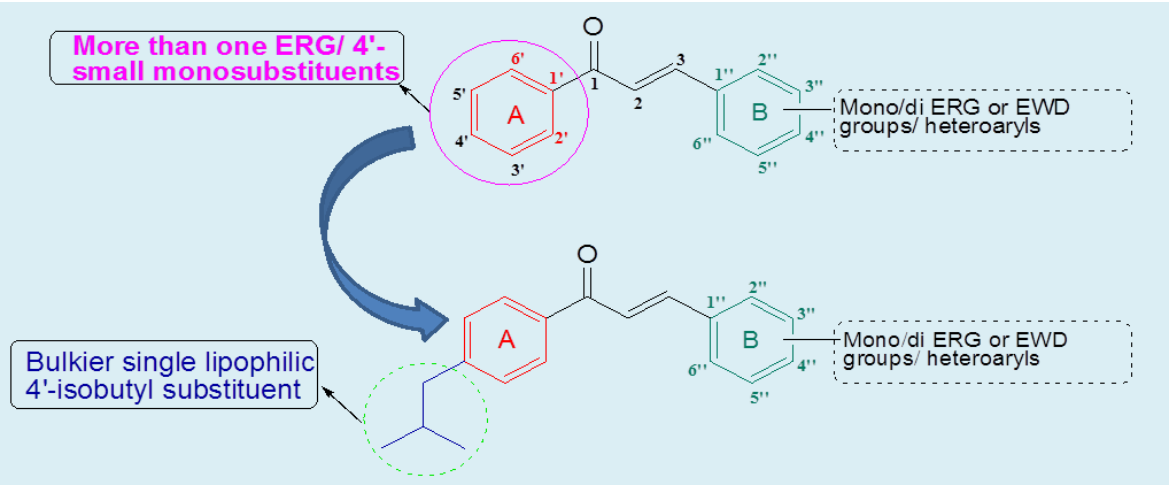
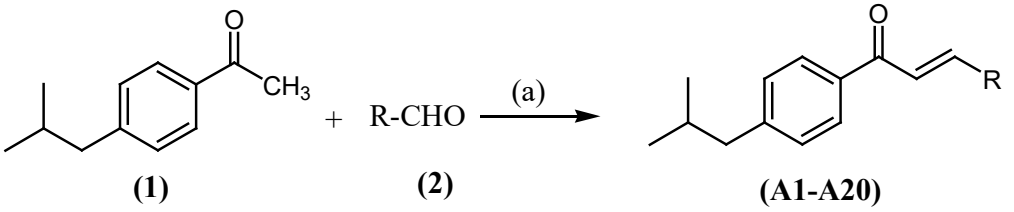


Figure 1. Designing strategy of the test compounds (A1-A20). ERG = Electron releasing groups; EWG = Electron withdrawing groups.

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.

CHEMISTRY

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 dropwise. 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).



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

(E)-1-(4′-isobutylyphenyl)-3-(4”-chlorophenyl)-2-propen-1-one **(A1): Yield** 92%; **m.p.** 136-138 °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** (100 MHz, $CDCl_3$, 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.

(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** (100 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.

(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** (100 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.

(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** (100 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.

(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** (100 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.

(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** (100 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-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.

(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** (100 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** (100 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.

(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** (100 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.

(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** (100 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** (100 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.

(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** (100 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.

(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 (N=O, asymmetric), 1346 (N=O, symmetric), 3092 (Ar C-H), 2951 (Alkyl C-H). ^{1}H NMR (400 MHz, $CDCl_3$, 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** (100 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.

(E)-1-(4′-isobutylphenyl)-3-(4”-methoxyphenyl)-2-propen-1-one **(A12):** Yield 79%; **m.p.** 149-151 °C; **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** (100 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.

(E)-1-(4′-isobutylphenyl)-3-(3”,4”-dimethoxyphenyl)-2-propen-1-one **(A13): Yield** 66%; **m.p.** 146-148 °C; **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** (100 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.

(E)-1-(4′-isobutylphenyl)-3-(3”,4”,5”-trimethoxyphenyl)-2-propen-1-one **(A14): Yield** 70%; **m.p.** 180-182 °C; **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** (100 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.

***(E)-1-(4′-isobutylphenyl)-3-(2”-pyridinyl)-2-propen-1-one* (A15)**: **Yield** 76%; **m.p.** 132-134 °C; **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 (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** (100 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.

***(E)-1-(4′-isobutylphenyl)-3-(3”-pyridinyl)-2-propen-1-one* (A16): Yield** 86%; **m.p.** 143-145 °C; **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** (100 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.

***(E)-1-(4′-isobutylphenyl)-3-(4”-pyridinyl)-2-propen-1-one* (A17): Yield** 89%; **m.p.** 165-167 °C; **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** (100 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.

***(E)-1-(4′-isobutylphenyl)-3-(2”-pyrrolyl)-2-propen-1-one (A18)*: Yield** 82%; **m.p.** 189-191 °C; **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** (100 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.

***(E)-1-(4′-isobutylphenyl)-3-(2”-thienyl)-2-propen-1-one* (A19): Yield** 86%; **m.p.** 179-181 °C; **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** (100 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.

***(E)-1-(4′-isobutylphenyl)-3-(5”-bromofuran-2”-yl)-2-propen-1-one* (A20): Yield** 85%; **m.p.** 149-151 °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** (100 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.

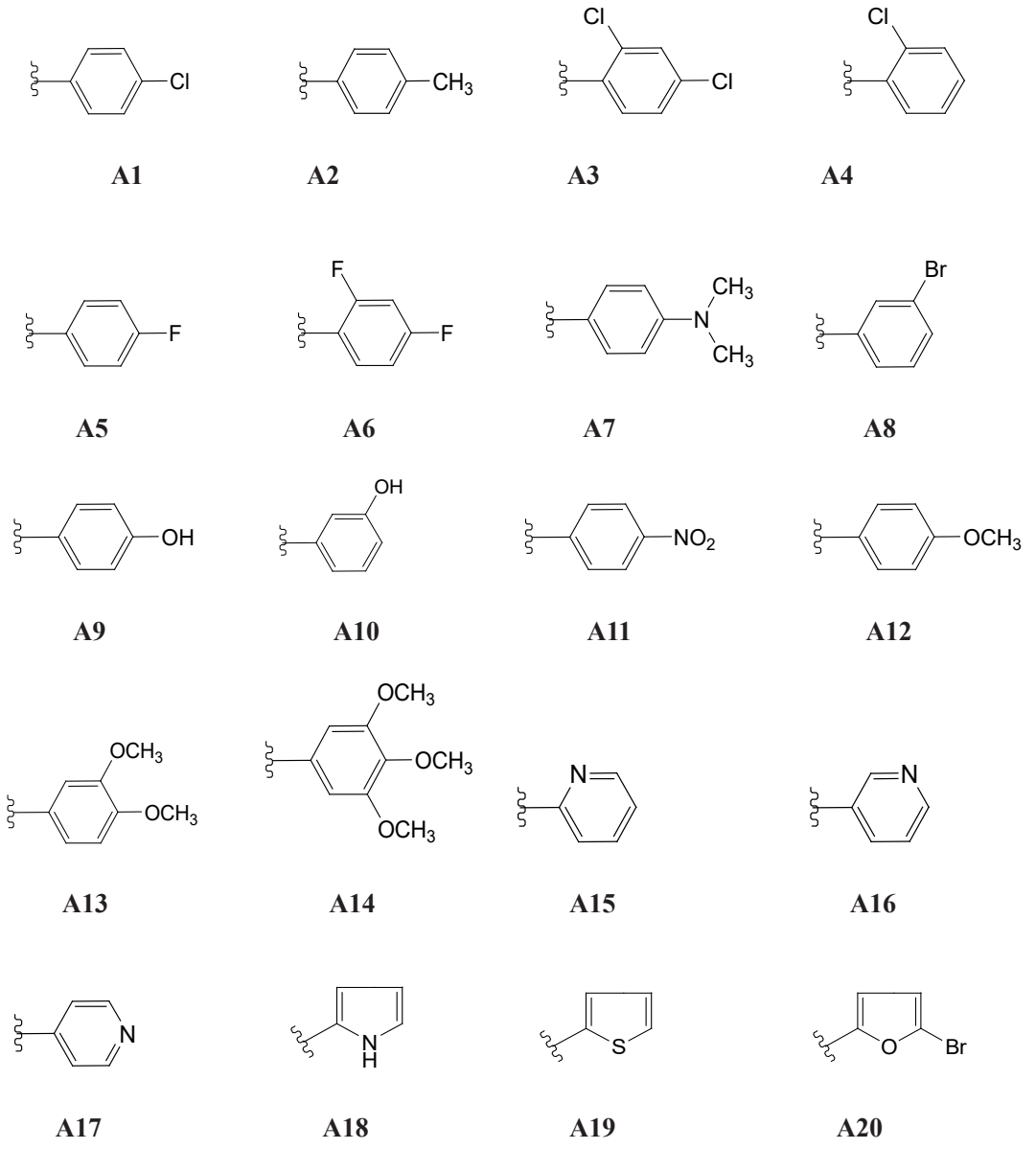


Figure 2. Different aldehydes selected for synthesis; **A1** to **A20** are the codes of 20 chalcones.

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 (Wilson, 2000; Alley *et al*., 1998).

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 µg/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 FBS. 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).

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 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.

RESULTS AND DISCUSSION

CHEMISTRY

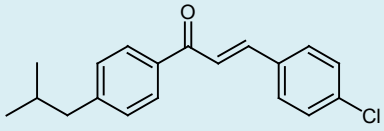
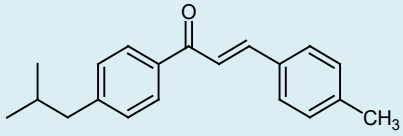
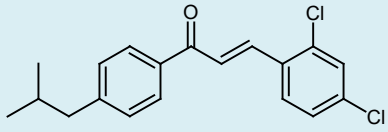
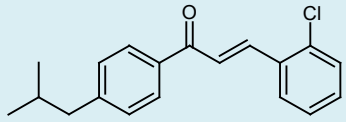
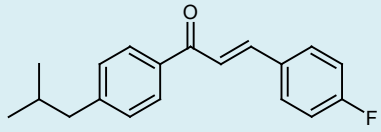
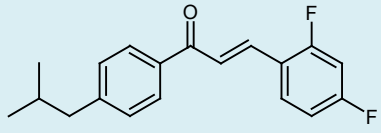
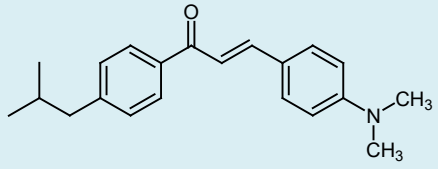
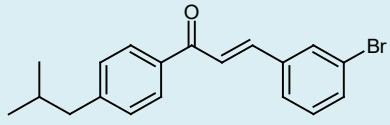
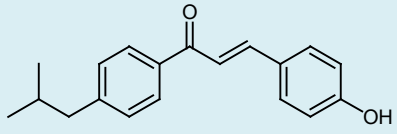
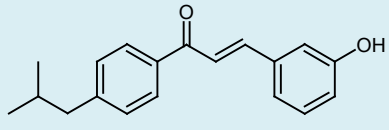
The designed novel target isobutylchalcones were synthesized by conventional base-catalyzed Claisen-Schmidt condensation of 1 - (4 - isobutylphenyl) ethanone and aromatic or heteroaromatic aldehyde as illustrated in Scheme 1. Most of the compounds were pure as evidenced by their TLC profiles and the 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 between chemical shift 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 within ± 0.4 % of the calculated values.

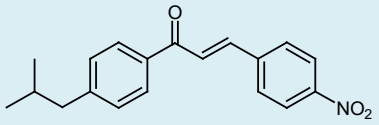
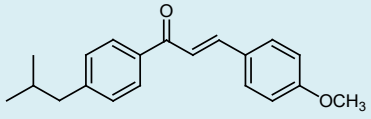
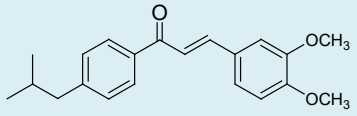
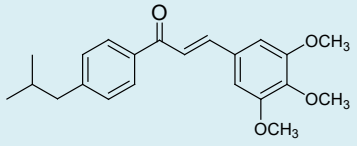
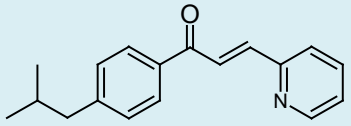
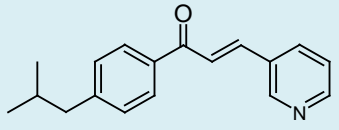
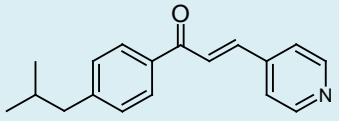
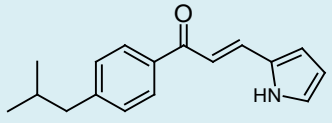
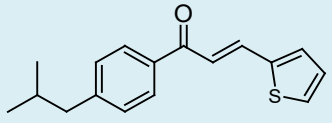
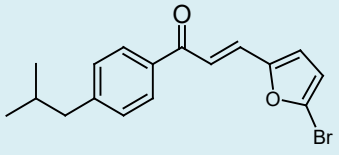
IN VITRO CYTOTOXICITY ASSAYS AND STRUCTURE ACTIVITY RELATIONSHIPS

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 exhibited activity 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 IC_{50} (µg/ml) values 42 (HT-29), 38 (MCF-7) and 18 (DU-145) whereas A3 containing 2",4"-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,

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
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
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

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 µg/ml 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 or 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.

DU-145 cell line was most susceptible 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 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 (Fig. 3) 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.

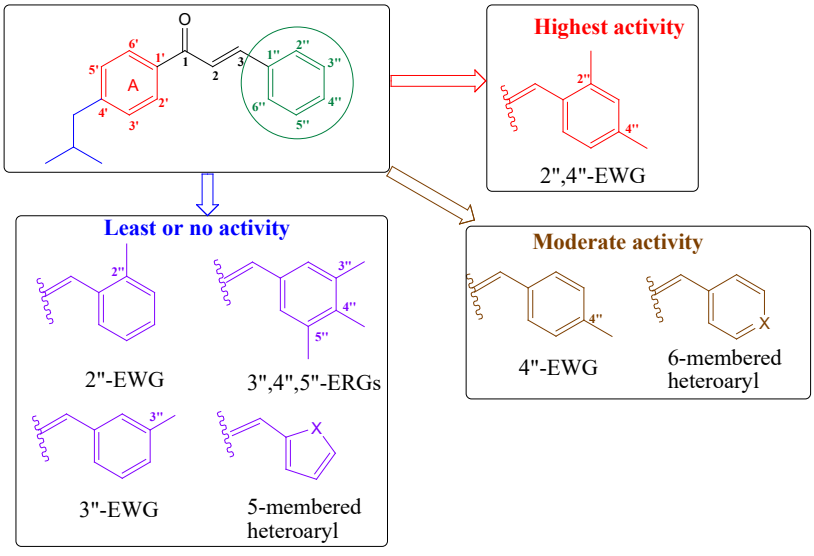


Figure 3. Structure activity relationships of chalcones against HT-29, MCF-7 and DU-145 cell lines;

X=N or S ERG = Electron releasing groups; EWG = Electron withdrawing groups

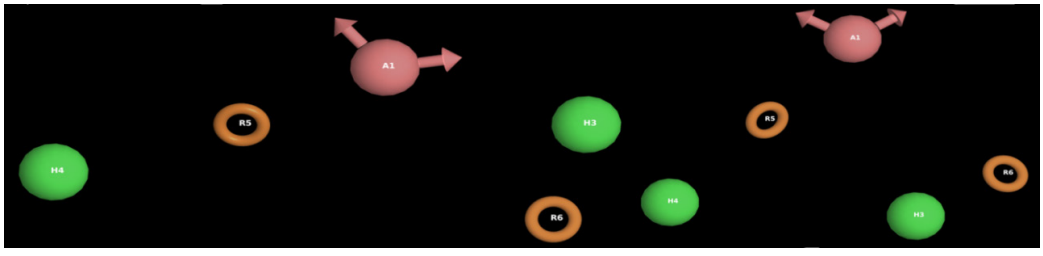


Figure 4. Common pharmacophore hypothesis generated using the set of all 20 chalcones illustrating hydrophobic groups (H, Green spheres), aromatic rings (R, Orange spheres) and hydrogen bond acceptor (A, Pink sphere).

COMPUTATIONAL EVALUATION

From the pharmacophore modelling results, it was able to identify a common pharmacophore with a five point model AHHRR (Fig. 4). 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, 6 and 7 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 5 and 7. The pharmacophore hypothesis-1 was generated from the chalcone A6 (Fig. 6) 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. 8) 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 *in vitro* activity data and clearly explained the importance of ring-A with 4′-isobutyl substituent and ring-B with electron withdrawing groups.

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. None of the compounds were as active as methotrexate. 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™ 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.

CONFLICT OF INTERESTS

There is no conflict of interests.

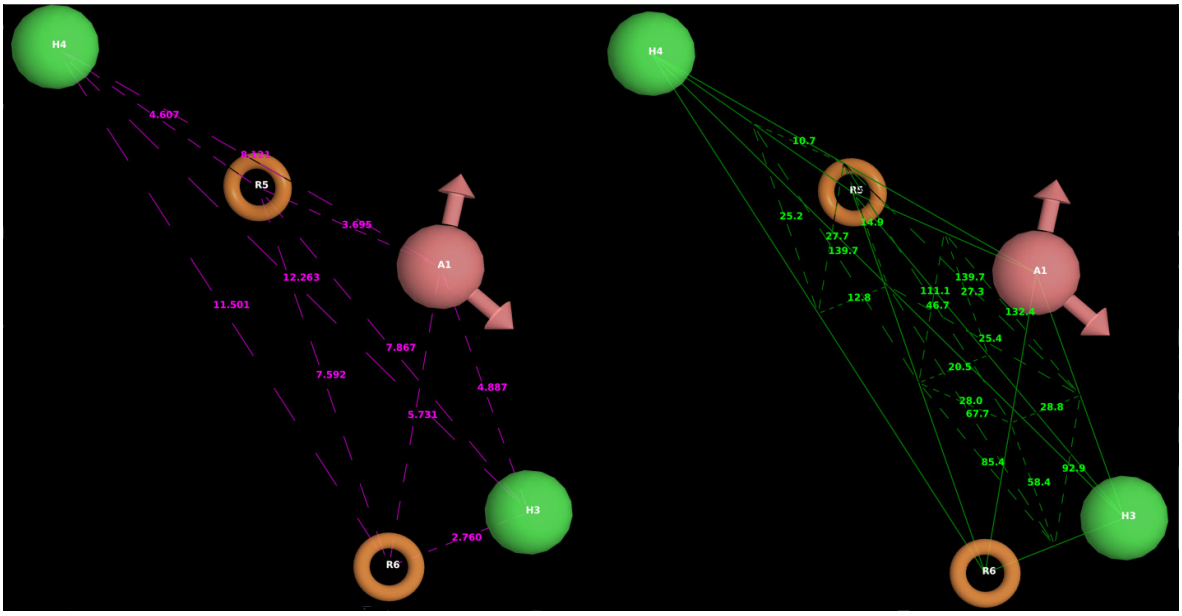


Figure 5. Phase generated pharmacophore hypothesis-1 AHHRR.43* and distance/angle between pharmacophoric sites. All distances in °A unit

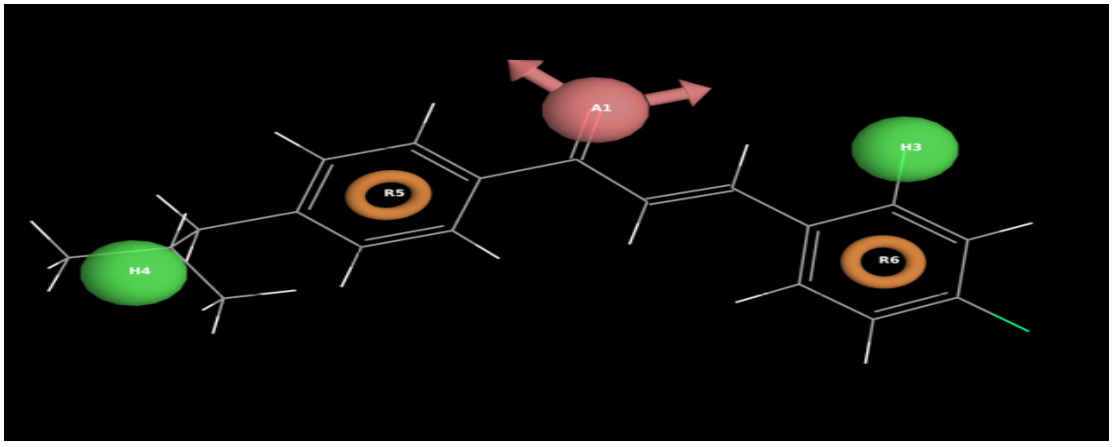


Figure 6. Phase generated best pharmacophore hypothesis-1 AHHRR.43* aligned with molecule A6

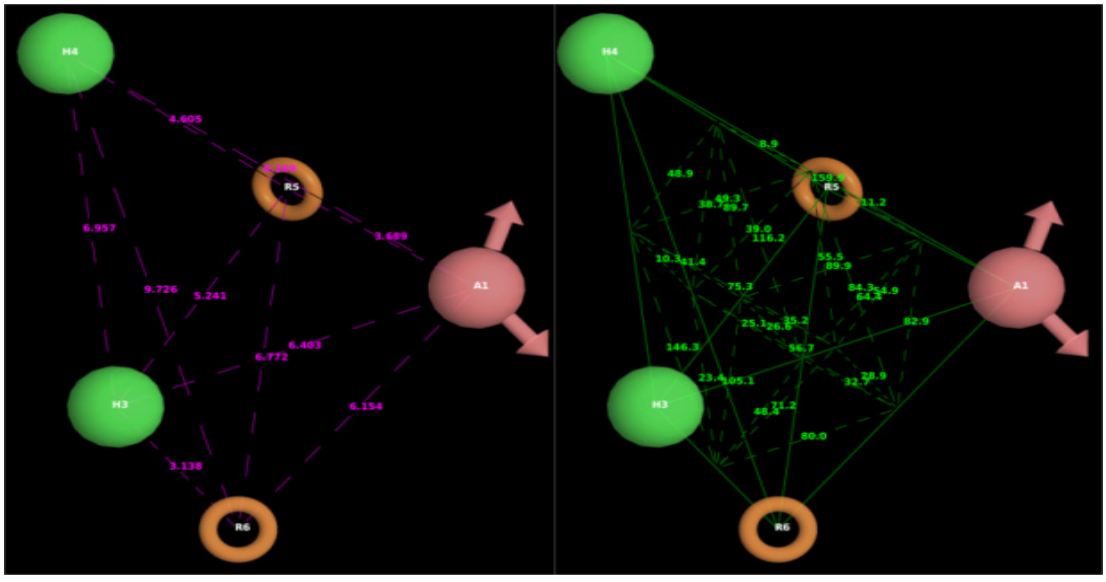


Figure 7. Phase generated pharmacophore hypothesis-2 AHHRR.25* and distance/angle between pharmacophoric sites. All distances in °A unit

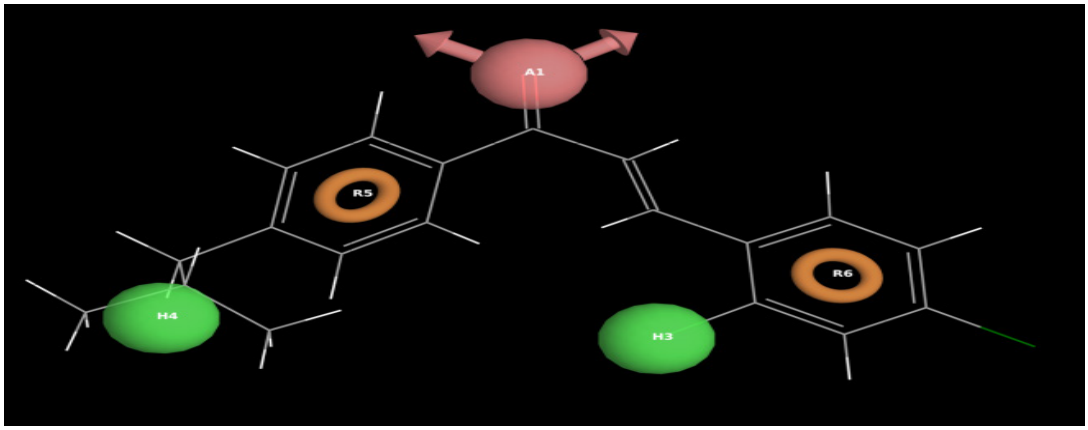


Figure 8. Phase generated best pharmacophore hypothesis-1 AHHRR.25* aligned with molecule A3

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

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

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

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 7. Distances between different sites of 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

ACKNOWLEDGEMENTS

The authors like to acknowledge Ministry of Minority Affairs, Indian Government, for providing UGC- Maulana Azad National Fellowship.

REFERENCES

Abdellatif, K.R.A., Abdelgawad, M.A., Elshemy, H.A.H., Alsayed, S.S.R., & Kamel, G. (2015). Synthesis and anti-inflammatory evaluation of new 1,3,5-triaryl-4,5-dihydro-1H-pyrazole derivatives possessing an aminosulphonyl pharmacophore. *Archives of Pharmacal Research*, 38, 1932-1942.

Alley, M.C., Scudiero, D.A., Monks, A., Hursey, M.L., Czerwinski, M.J., Fine, D.L., Abbott, B.J., Mayo, J.G., Shoemaker, R.H., & Boyd, M.R. (1998). Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. *Cancer Res*, 48, 589-601.

Batovska, D.I., & Todorova, I.T. (2010). Trends in utilization of the pharmacological potential of chalcones. *Curr. Clin. Pharmacol.* 5, 1-29.

Cho, M., Ryu, M, Jeong, Y., Chung, Y.H., Kim, D.E., Cho, H.S., Kang, S., Hans, J.S., Chang, M.Y., Lee, C.K., Jin, M., Kim, H.J., & Oh, S. (2009). Cardamonin suppresses melanogenesis by inhibition of Wnt/β-catenin signalling. *Biochem. Biophys. Res. Commun.* 390, 500-505.

Dimmock, J.R., Elias, D.W., Beazely, M.A., & Kandepu, N.M. (1999). Bioactivities of chalcones. *Curr. Med. Chem.* 6, 1125–1149.

Ducki, S., Rennison, D., Woo, M., Kendall, A., Chabert, J.F., McGown, A.T., & Lawrence, N.J. (2009). Combretastatin-like chalcones as inhibitors of microtubule polymerization. Part 1: synthesis and biological evaluation of antivascular activity. *Bioorg. Med. Chem*. 17, 7698–7710.

Fadeyi, O.O., Adamson, S.T., Myles, E.L., & Okoro, C.O. (2008). Novel fluorinated acridone derivatives. Part 1: Synthesis and evaluation as potential anticancer agent. *Bioorg Med Chem Lett.* 18, 4172–4176.

Go, M.L., Wu, X., & Liu, X.L. (2005). Chalcones: an update on cytotoxic and chemoprotective properties. *Curr Med Chem.* 12, 481-499.

Hieu, B.T., Thuy, L.T., Thuy, V.T., Tien, H.X., Chinh, L.V., Hoang, V.D., & Vu, T.K. (2012). Design, Synthesis and In Vitro Cytotoxic Activity Evaluation of New Mannich Bases. *Bull. Korean Chem. Soc.* 33, 1586-1592.

Ivanova, A., Batovska, D., Engi, H., Parushev, S., Ocsovszki I., Kostova I., & Molnar J. (2008). MDR-reversal activity of chalcones. *In Vivo*. 22, 379–384.

Jandial, D.D., Blair, C.A., Zhang, S., Krill, L.S., Zhang, Y., & Zi, X. (2014). Molecular targeted approaches to cancer therapy and prevention using chalcones. *Curr. Canc. Drug Targets.* 14, 181-200.

Keche, A.P., Thakur, S.D., Rodge, A.H., Patil, K., Shubhangi, G., & Turkar, S.S. (2010). Synthesis and study of anti-bacterial and anti-fungal activities of novel chalcones. *Acta Ciencia Indica*, *Chemistry*, 36, 191-198.

Lahsasni S.A., Korbi, F.H.A., & Aljaber, N.A. (2013) Synthesis, characterization and evaluation of antioxidant activities of some novel chalcones analogues. *Chem. Cent. J*. 8, 1-10.

Letafat, B., Shakeri, R., Emami, S., Noushini, S., Mohammadhosseini, N., Shirkavan, N., Ardestani, S.K., Safavi, M., Samadizadeh, M., Letafat, A., Shafie, A., & Foroumadi, A. (2013) Synthesis and In Vitro cytotoxic activity of novel chalcone-like agents. *Iran. J. Basic. Med. Sci.* 16, 1155-1162.

Mai, C.W., Yaeghoobi, M., Abd-Rahman, N., Kang, Y.B., & Pichka M.R. (2014). Chalcones with electron-withdrawing and electron-donating substituents: anticancer activity against TRAIL resistant cancer cells, structure-activity relationship analysis and regulation of apoptotic proteins. *Eur. J. Med. Chem*. 77, 378-387.

Nishimura, R., Tabata, K., Arakawa, M., Ito, Y., Kimura, Y., Akihisa, T., Sakuma, A., Kohno, H., & Suzuki, T. (2007). Isobavachalcone, a chalcone constituent of *Angelica keiskei*, induces apoptosis in neuroblastoma. *Biol. Pharm. Bull*. 30, 1878-1883.

Nowakowska Z. (2007). A review of anti-infective and anti-inflammatory chalcones. *Eur J Med Chem*. 42, 125.

Rostom, S.A.F. (2006) Synthesis and in vitro antitumor evaluation of some indeno[1,2-c] pyrazol(in)es substituted with sulfonamide, sulfonylurea(-thiourea) pharmacophores, and some derived thiazole ring systems. *Bioorg Med Chem*. 14, 6475–6485.

Sahu N.K., Balbhadra, S.S., Choudhary, J., & Kohli, D.V. (2012) Exploring pharmacological significance of chalcone scaffold: a review. *Curr. Med. Chem*. 19, 209-225.

Sharma, V., Kumar, V., & Kumar, P. (2013). Heterocyclic chalcone analogues as potential anticancer agents. *Anticancer Agents. Med. Chem.* 13, 422-432.

Sierra, J.R., Cepero, V., & Giordano. S. (2010). Molecular mechanisms of acquired resistance to tyrosine kinase targeted therapy. *Mol Can.* 9, 75–88.

Soliman, K., Ohad, N., Ramadan, M., Maayan, S., Snait, T., & Jacob, V. (2005). Chalcones as potent tyrosinase inhibitors: the importance of a 2,4-substituted resorcinol moiety. *Bioorg. Med. Chem.* 13, 433-441.

Syam, S., Abdelwahab, S.I., Al-Mamary, M.A., & Mohan, S. (2012) Synthesis of chalcones with anticancer activity. *Molecules*. 17, 6179-6195.

Sylvie, D. (2007). The development of chalcones as promising anticancer agents. *IDrugs*. 10, 42-46.

Tang, Y., Simoneau, A.R., Xie, J., Shahandeh, B., & Zi, X. (2008). Effects of the Kava Chalcone Flavokawain A Differ in Bladder Cancer Cells with Wild-type versus Mutant p53. *Cancer Prev Res.* 1, 439–451.

Tang, Y., Li, X., Liu, Z., Simoneau, A.R., Xie, J., & Zi, X. (2010). Flavokawain B, a kava chalcone, induces apoptosis via up-regulation of death-receptor 5 and Bim expression in androgen receptor negative, hormonal refractory prostate cancer cell lines and reduces tumor growth. *Int. J. Cancer*. 127, 1758–1768.

Thomas, V. (2010). Phenylpropanoid biosynthesis. *Mol. Plant.* 3, 2-20.

Turkar, S.S., Rodge, A.H., Hatnapure, G.D., Keche, A.P., & Gaikwad, G.S. (2010) Synthesis and antibacterial, antifungal activity of some novel chalcone derivatives. *JOCPR*. 2, 348-355.

WHO/Cancer-World Health Organisation (2013) http://www.who.int/topics/cancer/ Accessed on 17 January 2016.

Wilson, A.P. (2000). Cytotoxicity and viability assays. In: J.R.W. Masters (Eds.), Animal cell culture: a practical approach (pp.175–219). 3rd ed. Oxford, UK: Oxford University Press.

Wu, J., Wang, C., Cai, Y., Peng, J., Liang, D., Zhao, Y., Yang, S., Li, X., Wu, X., & Liang, G. (2012) Synthesis and crystal structure of chalcones as well as on cytotoxicity and antibacterial properties. *Med. Chem. Res.* 21, 444–452.

Yazdan S.K., Sagar, G.V., & Afzal, B.S. (2015). Biological and synthetic potentiality of chalcones: A review. *J. Chem. Pharm. Res*. 7, 829-842.