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Synthesis of newer piperidinyl chalcones and their anticancer activity in human cancer cell lines

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Abstract Newer tetrahydropyridine chalcones were synthesized and tested for their antioxidant and anticancer activity. These molecules showed significant anticancer activity at $IC_{50} < 50$ μM in human cancer cell lines such as A549 (lung adenocarcinoma), HepG2 (hepatocellular liver carcinoma), HeLa (cervical cancer), HCT-116 (colon carcinoma cells), MCF-7 (breast cancer cell line), and MDA MB 231 (breast cancer cells) cancer cells. The test compounds significantly inhibited cancer cell migrations in scratch wound assay for angiogenesis. Further, the test compounds bearing naphthalene substituents on the main ring showed in vitro antioxidant activity at <100 μM by 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation, superoxide radical scavenging, *O*-phenonthroline, and lipid peroxidation assays. In conclusion, two test compounds bearing naphthalene substitutions exhibited potent anticancer activity that could be attributed to their ability to induce apoptosis, inhibit cancer cell migrations, provide antiangiogenic properties, and also partly by reducing oxidative stress by scavenging free radicals.

Keywords Piperidinyl chalcones · Anticancer · Human cancer cell lines · Bio-isosterism · Antioxidant

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Introduction

Cancer is a multifactorial disease characterised by loss of the normal control mechanisms that govern cell survival, proliferation, and differentiation. Globally, around 5.1 % of disease burden and 12.5 % of deaths are attributed to cancer [1]. The toxicities and resistance of anticancer cells limit the available anticancer drugs in therapeutics. Naturally occurring chalcones such as licochalcone A, butein, cardamonin, xanthoangelol, isoliquiritigenin, and flavokawain-A/B are reported to be potential leads as anticancer agents. Hence, synthetic chalcones could become a useful scaffold for anticancer activity [2].

Chalcones have the chemical structure 1,3-diaryl-2-propen-1-ones (Fig. 1), and the anticancer activity is mainly attributed to the interaction of the enone system with the thiol residues, making them free from mutagenic and carcinogenic effects that are associated with many alkylating agents [3]. To substantiate this, some of the open ring chalcones, such as curcumin, and closed ring structures of chalcones (cyclic chalcones), such as colchicine [4] and flavopiridol [5], have shown anticancer activity. Further, a number of modifications are made on the basic α, β unsaturated ketones by introducing Mannich base [6], amino [7], nitro, hydroxyl, and methoxy groups and have been evaluated for their anticancer potential. Another approach used in exploring the anticancer potential of chalcones is in replacing the existing phenyl group in B-ring with a heterocyclic moiety of the chalcone. Selection of the heterocyclic moiety is based on the molecular properties resembling that of a phenyl group. For example, a phenyl ring can be conveniently replaced with a thiophene moiety, as they are ring counterparts. In other words, they are isosteric to each other. This strategy has been used to generate newer series of chalcones, such as thiophene [8], pyrrole [9], pyrazoline [10], piperidyl, indolyl [11], and naphthyl chalcones [12]. Most of these compounds were found to be superior in anticancer activity compared to the corresponding benzylidenechalcones, presumably because of the introduction of a smaller heterocyclic group as mentioned above, which might cause variations in the hydrophobicity of chalcones. Liu and Go [13] have demonstrated the effect of the piperidinyl group in the A-ring of chalones and suggested that the position of the nitrogen provides selectivity and is also responsible for the cell cycle arrests.

Our present work was planned by keeping the position of the nitrogen atom intact, along with the double bond in the tetrahydropyridine ring, which is further reduced in piperidinyl chalcones [12], and introducing various heterocyclic moieties in the B-ring of chalcone. Introduction of such groups may cause variations in the

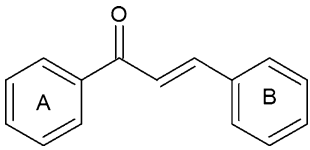

Fig. 1 Chemical structure of chalcone: 1,3-diaryl-2-propen-1-ones

hydrophobicity of the chalcones. This in turn might facilitate better penetration for the test compounds into the cancer cells. It is of interest to elucidate the contribution of the nature of different heterocyclic rings on the biological activity of such piperidinyl chalcones. Thus, in our present study, the effect of isomeric and isosteric substitutions on the B-ring of chalcone was studied for anticancer activity.

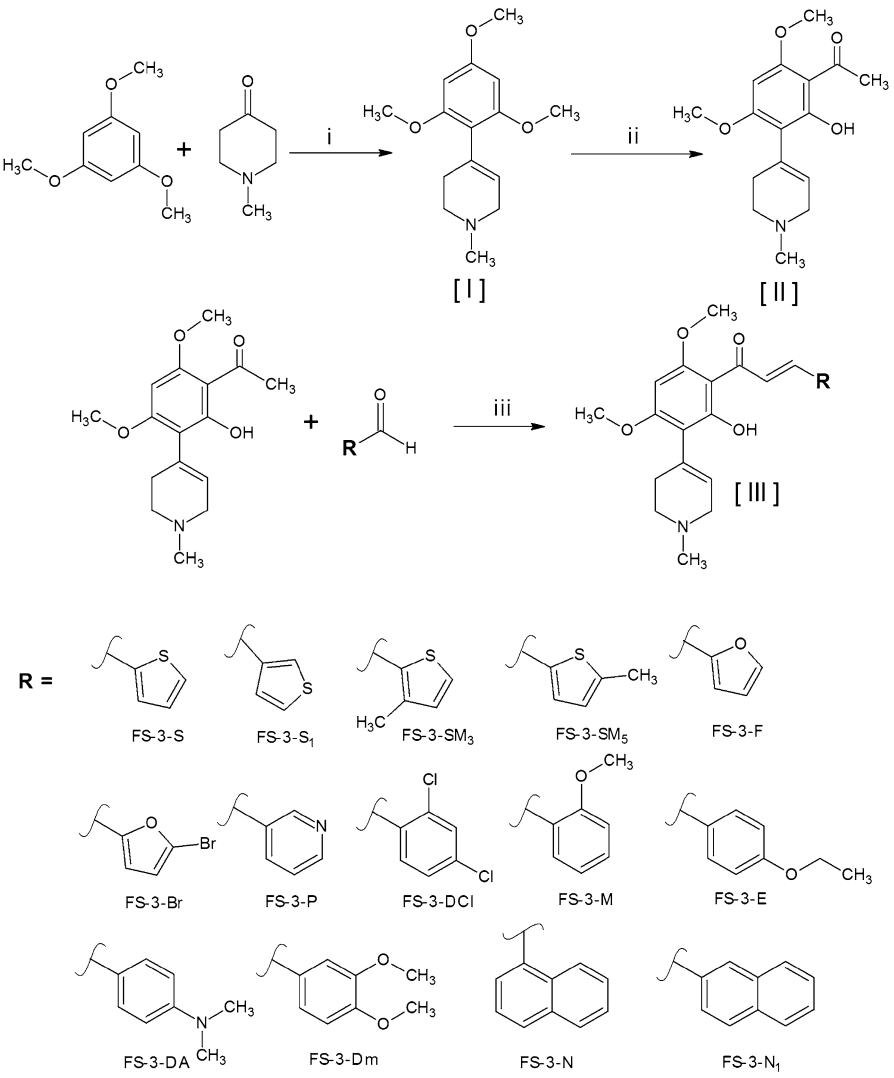

Fig. 2 Synthesis of newer chalcones analogues. Reagents: (*i*) Hydrochloric acid, Glacial acetic acid, rt 24 h, 95–100 °C, 3 h. (*ii*) Boron triflouride ethereate, acetic anhydride, dichloromethane, rt, 24–72 h. (*iii*) 10 % w/v sodium hydroxide, methanol, rt, 12–24 h

Materials and methods

Chemicals, reagents, and instruments

All the chemicals required for the synthesis and purification of the intermediates and final compounds were purchased from Sigma-Aldrich and Merck. Melting points were determined using a melting point apparatus from Shital Scientific Industries and are uncorrected. The reactions were monitored by TLC plates, precoated with Kieselgel 60 F254 (Merck), and spots were detected using short- and long-UV. Column chromatography was performed on silica gel (120–200 mesh, 230–400 mesh). λ_{max}, ε_{max} for the synthesized compounds were established by UV–visible spectrophotometer UV-1650 PC (Shimadzu, Japan). The IR studies were determined using Shimadzu FTIR 8310. ^{1}HNMR were recorded on Bruker Avance II and III and a 400 MHz spectrometer, using TMS as internal standard; all values are reported in parts per million. Mass spectral studies were done using GC–MS (Shimadzu GCMS QP5050) and ESI MS [Applied Biosystem, QSTAR Elite (Q-TOF)]. The test compounds are dissolved in dimethyl sulfoxide (DMSO) for testing of biological activity.

Synthesis

General Procedure for the synthesis of 1-methyl-4-(2,4,6-trimethoxyphenyl)-1,2,3,6-tetrahydropyridine (**I**; Fig. 2, reaction **i**) 1-Methyl-4-piperidone (0.005 mol) was added to a solution of 1,3,5-trimethoxybenzene (0.005 mol) in glacial acetic acid (2 mL) with constant stirring, and the temperature was maintained below 25 °C. Further, concentrated HCl (0.75 mL) was added drop-wise. The reaction mixture was then stirred for 24 h at 25 °C, and later at 95–100 °C for 3 h. It was then allowed to cool to 40 °C, poured over crushed ice, and stirred for 10 min. The precipitated 1,3,5-trimethoxy benzene was filtered off. Filtrate was basified at below 10 °C, with pH set at 11–12 using 50 % aqueous sodium hydroxide. The white solid obtained was filtered, washed with water, and dried. Recrystallization was done with acetone/water (1:2), which gave 1,2,3,6-tetrahydro-4-(2,4,6-trimethoxyphenyl)-1-methylpyridine (**I**).

General Procedure for the synthesis of 1-(3-(1,2,3,6-tetrahydro-1-methylpyridin-4-yl)-2-hydroxy-4,6-dimethoxyphenyl)ethanone (**II**; Fig. 2, reaction **ii**) Boron trifluoride diethyl ethereate (3.5 mL, 0.065 mol) was added drop-wise to a previously cooled solution of compound **I** (0.8 g, 0.003 mol) in dichloromethane (15 mL) under constant stirring. After stirring for 2–4 h in an ice bath, acetic anhydride (5 mL, 0.049 mol) was added drop-wise to the solution, and the stirring was continued for 24–72 h at room temperature. The concentrated reaction mixture was diluted with cold water and rendered alkaline with sodium carbonate (reaction **ii**). The precipitate obtained was then filtered and dried. It was further purified by column chromatography using solvents such as chloroform and methanol in gradient flow (up to 3.5 % methanol) to obtain the acetylated product.

General Procedure for the synthesis of (3-(1,2,3,6-tetrahydro-1-methylpyridin-4-yl)-2-hydroxy-4,6-dimethoxyphenyl)prop-2-en-1-one analogues (**III**; Fig. 2, reaction **iii**) A solution of aldehyde (1.2 mmol) in methanol (5 mL) was added drop-wise to a stirred solution of acetophenone-**II** (1 mmol) dissolved in 10 % (w/v) NaOH in methanol (20 mL). Alternatively, the base was added drop-wise to a solution of different aldehydes and acetophenones under stirring. The same was stirred at room temperature (28 °C) for 12–24 h, and then the solvent was removed under pressure. The resulting residue was then dissolved in 1 M HCl and extracted with ether. The aqueous layer was rendered alkaline with saturated sodium carbonate solution to give a precipitate that was filtered, washed with water, and dried. Final purification was done by column chromatography, solvent as chloroform:methanol; gradient flow (up to methanol 3.5 %).

Antioxidant activity

The ABTS radical scavenging assays were performed as per the procedure explained in the literature [14]. The superoxide generated by alkaline DMSO was assessed in the presence and absence of test compounds. The reduction of nitroblue tetrazolium (NBT) by superoxide was measured spectrophotometrically at 560 nm. Similarly, the chelating iron by *O*-phenanthroline and lipid peroxidation assay was performed in rat brain homogenate as per the previously reported method [15].

Anticancer activity

Reagents and media

The media for cell culture (MEM and DMEM) were purchased from Sigma-Aldrich. MTT reagent [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] and fetal bovine serum (FBS) was purchased from Himedia. Gentamycin sulphate was procured from Kasturba Hospital, Manipal.

Cell lines

The human cancer cell lines A549 (lung adenocarcinoma epithelial cell line), HepG2 (hepatocellular liver carcinoma cell line), HeLa (cervical cancer cell line), HCT-116 (colon carcinoma cell line), MCF-7 (breast cancer cell line—ER-positive), MDA MB 231 (breast carcinoma cell line—ER-negative), and one normal cell line [Vero cells (money kidney epithelial cells/normal cells)] were procured from National Centre for Cell Sciences, Pune, India. The cells were cultured in MEM medium supplemented with 10 % FBS, 1 % L-glutamine, and 50 μg/mL gentamicin sulphate in a humidified atmosphere of 5 % CO_2 and 95 % air.

MTT Assay

This assay was carried out as explained in the literature [16] with minor modifications. Exponentially growing cell lines were harvested from 25 cm^2 tissue

culture flasks and a stock cell suspension (1×10^5 cell/mL) was prepared with respective media. A 96-well flat bottom tissue culture plate was seeded with 1×10^4 cells in 0.1 mL of MEM and DMEM medium supplemented with 10 % FBS and allowed to attach for 24 h. Test compounds were prepared just prior to the experiment in 0.4 % DMSO and serially diluted with medium to get working stocks of 100, 50, 25, 1.0 μmol/mL. After 24 h of incubation, cells were treated with 100 μL of test compounds, and the plates were again incubated for 48 h. The cells in the control group received only the medium containing the 0.1 % DMSO (vehicle). Each treatment was performed in triplicates. After the treatment, test compounds/standard drug containing-media was removed by inverting the plate onto a tissue paper. To each well of the 96-well plate, 100 μL of MTT reagent (stock: 1.0 mg/mL in PBS) was added and incubated for 4 h at 37 °C. After 4 h of incubation the plates were inverted on tissue paper to remove the MTT reagent. To solubilize formazan crystals in the wells, 100 μL of 100 % DMSO was added to each well. The optical density (OD) was measured by an ELISA plate reader (Biotek FLX800) at 540 nm. The percentage anticancer activity was calculated using the formula

$$\%\text{anticancer activity} = \frac{(\text{Control OD} - \text{Blank OD}) - (\text{Test OD} - \text{Blank OD})}{(\text{Control OD} - \text{Blank OD})} \times 100$$

The IC_{50} was calculated from the graph plotted by taking concentration against % anticancer activity using Microsoft Excel.

Chromatin condensation studies (Hoechst 33342 staining)

Condensation of chromatin is a late event in apoptosis and can be detected by Hoechst 33342 dye staining. Hoechst 33342 stain easily crosses the membrane and binds nuclear adenine–thymine-rich regions of DNA and emits blue fluorescence at UV range [17]. HepG2 cells were grown in culture media with a suitable concentration of DMSO in 96-well plates and subjected to the treatment with test compounds at their IC_{50} concentrations (lowest IC_{50} in MTT assay). After 24-h incubation, the media containing the test samples was removed and 100 μL of 1 % paraformaldehyde solution was added and incubated for 2 h at room temperature. The excess paraformaldehyde solution was removed, and the plate was dried. Fifty microliters of Hoechst 33342 stain was added, and plate was wrapped in aluminium foil and incubated at 60 °C for 15 min. The plate was then washed with PBS/HBSS, dried and observed under Nikon eclipse TS100 inverted microscope with excitation filter 480/530 nm and emission filter 535/540 nm. The chromatin condensation and apoptotic bodies were viewed and comparison was made with DMSO-treated control and doxorubicin-treated positive control.

Scratch wound anti-migration assay

A scratch wound in a monolayer is made with the help of the micro-tip of predefined size and images are captured at zero time point and then onwards depending upon

the cell cycle time. Closures of the scratch are compared and the images are snapped to quantify the migration rate of the cells. This method has been used to measure the migration of individual cells in the leading edge of the scratch [18, 19]. HeLa cell suspension containing 1×10^7 cells/mL was prepared in DMEM. The tissue culture grade 6-well plate was seeded with 2 mL of the above cell suspension, and 1 mL of fresh medium was added into each well. The cells were allowed to attach for 24 h. The spent medium was removed by decantation and by using a micro-pipette and 1.5 mL of low-serum medium (medium containing 0.5–0.1 % FBS) was added to each well and starved overnight. After 24 h, medium from each well was removed, the cells were washed with 2 mL of sterile PBS and a wound was created with a sterile 200 μL micro tip. The test compounds/standards were suitably diluted with the medium to get IC_{50} concentration (lowest IC_{50} of MTT) and 1.5 mL of the diluted test sample solution was added to each well. Images were taken in ×10 magnification using an inverted microscope at 0, 6, 12, 24 h after incubation with test samples. The wound area was roughly measured using a scale in a standard magnification of the image and the mean wound width was expressed in centimeters is compared with standard drug methotrexate.

Results

Chemistry

The synthesis of all the test compounds was achieved in three steps as previously described [13, 20]. The first step involved an electrophilic substitution on 1,3,5-trimethoxy benzene in the presence of hydrochloric acid by the carbonyl carbon of N-methyl piperidone to get intermediate compound **(I)**. The melting point was 121–123 °C; the spectral details were as follows.

IR: 2937 (C–H), 1585 (C=C), 1126 (C–O); ^{1}HNMR (DMSO-d_6) δ 6.19 (2H, s), 5.32 (1H, s), 3.75 (3H, s), 3.68 (6H, s), 2.89 (2H, m), 2.5 (2H, m), 2.15 (3H, s), 2.147 (2H, m); MS: *m/z* $[M]^+$ 263.

Compound **I**, upon acylation using boron triflouride as a catalyst for demethylation of the adjacent methoxy group gave corresponding acetophenone **(II)**. The purified acetophenone (**II**; mp 170–171 °C) was subjected to spectral characterization.

IR: 3441 (O–H), 2976 (C–H), 1626 (C=O), 1587 (C=C), 1130 (C–O); ^{1}HMNR (DMSO-d_6) δ 14 (1H, s), 6.28 (1H, s), 5.49 (1H, s), 3.99 (3H, s), 3.87 (3H, s), 3.68 (2H, m), 3.24-3.32 (2H, m), 2.77 (3H, s), 2.57 (3H, s), 2.42–2.5 (2H, m); MS: *m/z* $[M]^+$291.

Finally, the desired chalcones **(III)** were obtained by Claisen-Schmidt condensation of the synthesized acetophenone (**II**) with various aldehydes (Fig. 2, reaction **iii**).

All the synthesised test compounds showed a characteristic bathochromic shift and exhibited λ_{max} above 330 nm when compared to that of acetophenone at λ_{max} 272 nm. This bathochromic shift indicated the formation of α-β unsaturated carbonyl functional group, characteristic for chalcones [21]. The IR spectra of all the compounds clearly showed a sharp C=O peak at around 1630 cm^{-1} and a broad

peak at above 3300 cm^{-1} indicating the presence of the hydroxyl group. The mass spectra of all the test compounds showed the molecular ion peak as M + 1, which was also the base peak, indicating the stability of the synthesised chalcones. The ^{1}HNMR spectra of all the test compounds showed the presence of the phenolic hydroxyl proton at around δ 14 along with the aromatic and aliphatic protons. The chalcone characteristic H_a and H_b protons showed two sharp doublets at around δ 7.67–8.62 and δ 7.2–7.95, respectively. As the coupling constant for these two adjacent protons is around 15.2–15.6 Hz, the protons are trans to each other and the synthesised chalcones are assigned 'E' configuration [22] as shown in Fig. 3. Similarly, the IR, ^{1}HNMR and mass spectral details are given below along with the physical parameters such as melting point, λ_{max} and ε_{max} under the sub-headings of each test compounds.

Physicochemical and spectral details of the newly synthesized chalcones

FS-3-S: *1-(3-(1,2,3,6-tetrahydro-1-methylpyridin-4-yl)-2-hydroxy-4,6-dimethoxyphenyl)-3-(thiophen-2-yl)prop-2-en-1-one* Yield: 68 %; mp: 155–156 °C; λ_{max}: 347 nm; ε_{max}: 19635; IR: 3414 (O–H), 1631 (C=O), 1564 (C=C), 1126 (C–O); ^{1}HNMR (DMSO-d_6) δ 13.8 (1H,s), 7.93 (1H, d, J = 15.6 Hz), 7.77 (1H, d, J = 15.6 Hz), 7.65 (1H, br), 7.58–7.6 (1H, m), 7.17–7.2 (1H, m), 6.295 (1H, s), 5.44 (1H, s), 3.99 (3H, s), 3.87 (3H, s), 3.07 (2H, m), 2.78 (2H, m), 2.24 (3H, s), 1.91 (2H, m); MS: *m/z* $[M + 1]^+$ 386.17.

FS-3-S$_1$: *1-(3-(1,2,3,6-tetrahydro-1-methylpyridin-4-yl)-2-hydroxy-4,6-dimethoxyphenyl)-3-(thiophen-3-yl)prop-2-en-1-one* Yield: 82 %; mp: 155–156 °C; λ_{max}: 349 nm; ε_{max}: 20,650; IR: 3437 (O–H), 1624 (C=O), 1568 (C=C), 1124 (C–O); ^{1}HNMR ($CDCl_3$) δ 14.03 (1H, s), 7.82 (1H, d, J = 15.52 Hz), 7.72 (1H, d, J = 15.52 Hz), 7.57 (1H, s), 7.36–7.39 (2H, m), 6.02 (1H, s), 5.6 (1H, br, s), 3.98 (3H, s), 3.87 (3H, s), 3.2 (2H, m), 2.75–2.78 (2H, m), 2.48 (3H, s), 2.43 (2H, m); MS: *m/z* $[M + 1]^+$ 386.17.

FS-3-SM$_3$: *1-(3-(1,2,3,6-tetrahydro-1-methylpyridin-4-yl)-2-hydroxy-4,6-dimethoxyphenyl)-3-(3-methylthiophen-2-yl)prop-2-en-1-one* Yield: 50 %; mp: 121–122 °C; λ_{max}: 375 nm; ε_{max}: 22,155; IR: 3433 (O–H), 1614 (C=O), 1556 (C=C), 1120 (C–O); ^{1}HNMR ($CDCl_3$) δ 14.06 (1H, s), 8 (1H, d, J = 16 Hz), 7.67 (1H, d, J = 15.52 Hz), 7.2 (1H, d, J = 2.8 Hz), 6.89 (1H, d, J = 4.8 Hz), 6 (1H, s), 5.59 (1H, s), 3.96 (3H, s), 3.85 (3H, s), 3.19 (2H, m), 2.7 (2H, m), 2.46 (3H, s), 2.41 (2H, m), 2.38 (3H, s); MS: *m/z* $[M + 1]^+$ 400.15.

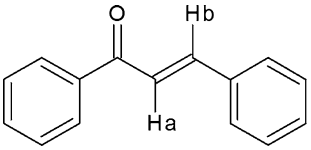

Fig. 3 Configuration of chalcones; characteristic Ha and Hb protons shows two sharp doublets at around δ 7.67–8.62 and δ 7.2–7.95, respectively, in NMR spectral studies

FS-3-SM$_5$: *1-(3-(1,2,3,6-tetrahydro-1-methylpyridin-4-yl)-2-hydroxy-4,6-dimethoxyphenyl)-3-(5-methylthiophen-2-yl)prop-2-en-1-one* Yield: 57 %; mp: 124–125 °C; λ_{max}: 380 nm; ε_{max}: 21450; IR: 3419 (O–H), 1614 (C=O).1556 (C=C), 1120 (C–O); ^{1}HNMR ($CDCl_3$) δ 14.23 (1H, s), 7.88 (1H, d, $J = 15.52$ Hz), 7.6 (1H, d, $J = 15.16$ Hz), 7.12–7.15 (1H, m), 6.7 (1H, d, $J = 3.56$ Hz), 6.02 (1H, s), 5.58 (1H, br, s), 3.98 (3H, s), 3.88 (3H, s), 3.36 (2H, m), 2.9 (2H, m), 2.6 (3H, s), 2.5 (3H, s), 2.18 (2H, m); MS: *m/z* $[M + 1]^+$400.15.

FS-3-F: *1-(3-(1,2,3,6-tetrahydro-1-methylpyridin-4-yl)-2-hydroxy-4,6-dimethoxyphenyl)-3-(furan-2-yl)-prop-2-en-1-one* Yield: 71 %; mp: 146–147 °C; λ_{max}: 366 nm; ε_{max}: 19965; IR: 3435 (O–H), 1624 (C=O), 1595 (C=C), 1126 (C–O); ^{1}HNMR ($CDCl_3$) δ 14.07 (1H, s), 7.77 (1H, d, $J = 15.2$ Hz), 7.59, (1H, d, $J = 15.2$ Hz), 7.5–7.51 (1H, m), 6.66 (1H, d, $J = 3.6$ Hz), 6.48–6.5 (1H, m), 6 (1H, s), 5.58 (1H, s), 3.96 (3H, s), 3.85 (3H, s), 3.15 (2H, m), 2.71 (2H, m), 2.43 (3H, s), 2.39–2.4 (2H, m); MS: *m/z* $[M + 1]^+$ 370.15.

FS-3-Br: *1-(3-(1,2,3,6-tetrahydro-1-methylpyridin-4-yl)-2-hydroxy-4,6-dimethoxyphenyl)-3-(5-bromofuran-2-yl)-prop-2-en-1-one* Yield: 40 %; mp: 135–135 °C; λ_{max}: 372 nm; ε_{max}: 22600; IR: 3435 (O–H), 2935 (C–H), 1624 (C=O), 1124 (C–O); ^{1}HNMR ($CDCl_3$) δ 14.01 (1H, s), 7.75 (1H, d, $J = 4$ Hz), 7.71 (1H, d, $J = 4$ Hz), 6.59 (1H, s), 6.43 (1H, m), 5.99 (1H, s), 5.58 (1H, m), 3.97 (3H, s), 3.85 (3H, s), 3.1 (2H, m), 2.7 (2H, m), 2.3 (3H, s), 2.1 (2H, m); MS: *m/z* $[M + 1]^+$ 448.06.

FS-3-P: *1-(3-(1,2,3,6-tetrahydro-1-methylpyridin-4-yl)-2-hydroxy-4,6-dimethoxyphenyl)-3-(pyridin-3-yl)prop-2-en-1-one* Yield: 38 %; mp: 160–161 °C; λ_{max}: 339 nm; ε_{max}: 18652; IR:3435 (O–H), 1631 (C=O), 1566 (C=C), 1124 (C–O); ^{1}HNMR ($CDCl_3$) δ 13.87 (1H, s), 8.87 (1H, s), 8.62 (1H, d, $J = 15.7$ Hz), 7.95 (1H, d, $J = 15.72$ Hz), 7.34–7.37 (1H, m), 3.99 (3H, s), 3.88 (3H, s), 3.18 (2H, m), 2.72–2.75 (2H, m), 2.46 (3H, s), 2.19 (2H, m); MS: *m/z* $[M + 1]^+$ 381.17.

FS-3-DCl: *1-(3-(1,2,3,6-tetrahydro-1-methylpyridin-4-yl)-2-hydroxy-4,6-dimethoxyphenyl)-3-(2,4-dichlorophenyl)-prop-2-en-1-one* Yield: 72 %; mp: 186–187 °C; λ_{max}: 373 nm; ε_{max}: 19665; IR: 3433 (O–H), 2933 (C–H), 1620 (C=O), 1122 (C–O); ^{1}HNMR (DMSO-d_6) δ 7.5–7.96 (5H, m), 6.3 (1H, s), 5.44 (1H, s), 3.98 (3H, s), 3.87 (3H, s), 2.98 (2H, s), 2.57 (2H, m), 2.3 (3H, s), 2.21 (2H, s); molecular weight: 448; MS: *m/z* $[M + 1]^+$ 448.10.

FS-3-M: *1-(3-(1,2,3,6-tetrahydro-1-methylpyridin-4-yl)-2-hydroxy-4,6-dimethoxyphenyl)-3-(2-methoxyphenyl)prop-2-en-1-one* Yield: 71 %; mp: 126–127 °C; λ_{max}: 366 nm; ε_{max}: 20560; IR: 3423 (O–H), 1620 (C=O), 1564 (C=C), 1116 (C–O); ^{1}HNMR ($CDCl_3$) δ 14 (1H, s), 7.9 (2H, m), 7.7 (1H, m), 7.4 (1H, m), 7.1 (1H, m), 7 (1H, m), 6.2 (1H, s), 5.4 (1H, s), 3.8–3.9 (9H, s), 3.12 (2H, br), 2.7 (2H, br), 2.39 (3H, s), 2.26 (2H, br); MS: *m/z* $[M + 1]^+$ 410.18.

FS-3-E: *1-(3-(1,2,3,6-tetrahydro-1-methylpyridin-4-yl)-2-hydroxy-4,6-dimethoxyphenyl)-3-(4-ethoxyphenyl)-prop-2-en-1-one* Yield: 73 %; mp: 155–156 °C; λ_{max}: 368 nm; ε_{max}: 23210; IR: 3425 (O–H), 1627 (C=O), 1556 (C=C), 1122 (C–O); ^{1}HNMR (DMSO-d_6) δ 7.67–7.77 (4H, m), 6.99–7.01 (2H, m), 6.35 (1H, s), 5.48

(1H, s), 4.08 (2H, q, $J = 6.08$ Hz), 4.02 (3H, s), 3.9 (3H, s), 3.21 (2H, br), 2.69 (2H, br), 2.38 (3H, s), 2.26 (2H, br), 1.34 (3H, t, $J = 12.8$ Hz); MS: *m/z* $[M + 1]^+$ 424.20.

FS-3-DA: *1-(3-(1,2,3,6-tetrahydro-1-methylpyridin-4-yl)-2-hydroxy-4,6-dimethoxyphenyl)-3-(4-(dimethylamino)phenyl)-prop-2-en-1-one* Yield: 30 %; mp: 145–146 °C; λ_{max}: 335 nm; ε_{max}: 24255; IR: 3435 (O–H), 2935 (C–H), 1614 (C=O), 1126 (C–O); ^{1}HNMR ($CDCl_3$) δ 14.03 (1H, s), 7.76 (1H, d, $J = 15.4$ Hz), 7.65 (1H, d, $J = 15.4$ Hz), 7.45 (2H, d, $J = 8.8$ Hz), 6.63 (2H, d, $J = 8.8$ Hz), 5.93 (1H, s), 5.5 (1H, s), 3.89 (3H, s), 3.84 (3H, s), 3.09 (2H, m), 2.9 (6H, s), 2.66 (2H, m), 2.36 (3H, s), 2.1 (2H, m); MS: *m/z* $[M + 1]^+$ 423.22.

FS-3-Dm: *1-(3-(1,2,3,6-tetrahydro-1-methylpyridin-4-yl)-2-hydroxy-4,6-dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one* Yield: 62 %; mp: 168–169 °C; λ_{max} 374 nm; ε_{max}: 22140; IR: 3435 (O–H), 1625 (C=O), 1566 (C=C), 1126 (C–O); ^{1}HNMR (DMSO-d_6) δ7.78 (1H, d, $J = 15.6$ Hz), 7.7 (1H, d, $J = 15.32$ Hz), 7.27–7.32 (2H, m), 7.02 (1H, m), 6.28 (1H, s), 5.43 (1H, s), 3.99 (3H, s), 3.86 (3H, s), 3.83 (3H, s), 3.79 (3H, s), 2.98 (2H, m), 2.56 (2H, m), 2.29 (3H, s), 2.08 (2H, m); MS: *m/z* $[M + 1]^+$ 440.19.

FS-3-N:*1-(3-(1,2,3,6-tetrahydro-1-methylpyridin-4-yl)-2-hydroxy-4,6-dimethoxyphenyl)-3-(naphthalen-1-yl)prop-2-en-1-one* Yield: 78 %; mp: 160–161 °C; λ_{max}:

Table 1 Antioxidant activity of the synthesised test compounds

	Radical scavenging activity (IC_{50}) in μM ± SEM				
	Compound code	ABTS radical	Superoxide radical	*O*-Phenanthroline	Lipid peroxidation
1	**FS-3-S**	81.21 ± 3.25	73.42 ± 1.39	33.42 ± 1.27	46.43 ± 0.41
2	**FS-3-S_1**	92.13 ± 2.15	98.32 ± 2.03	48.23 ± 2.30	54.53 ± 1.35
3	**FS-3-SM_3**	134.2 ± 3.62	142.3 ± 5.23	78.43 ± 4.21	82.30 ± 2.31
4	**FS-3-SM_5**	130.5 ± 4.55	139.6 ± 3.34	80.03 ± 3.50	85.20 ± 2.42
5	**FS-3-F**	80.19 ± 0.29	107.9 ± 1.86	22.50 ± 1.16	47.09 ± 1.85
6	**FS-3-Br**	83.20 ± 1.24	110.3 ± 1.05	25.35 ± 1.25	49.10 ± 2.31
7	**FS-3-P**	79.47 ± 1.61	79.59 ± 1.04	53.61 ± 1.56	46.44 ± 2.44
8	**FS-3-DCl**	82.55 ± 1.35	80.50 ± 2.02	55.65 ± 1.24	50.05 ± 3.00
9	**FS-3-M**	68.45 ± 1.45	64.22 ± 2.23	28.33 ± 1.46	14.09 ± 1.65
10	**FS-3-E**	71.99 ± 1.06	79.98 ± 1.31	30.31 ± 1.13	15.47 ± 1.80
11	**FS-3-DA**	69.45 ± 2.12	75.29 ± 1.43	28.23 ± 2.03	14.27 ± 2.08
12	**FS-3-Dm**	66.35 ± 1.55	65.02 ± 1.43	27.50 ± 2.04	13.90 ± 1.51
13	**FS-3-N**	73.24 ± 2.06	77.28 ± 2.01	32.03 ± 2.23	14.91 ± 2.05
14	**FS-3-N_1**	72.50 ± 1.56	75.44 ± 1.45	29.45 ± 1.54	17.55 ± 2.90
	Ascorbic acid	37.00 ± 1.76	27.25 ± 1.56	50.31 ± 1.63	–
	Quercetin	–	–	–	56.25 ± 2.70

374 nm; ε_{max}: 19660; IR: 3435 (O–H), 1618 (C=O), 1566 (C=C), 1120 (C–O); ^{1}HNMR ($CDCl_3$) δ 13.93 (1H, s), 8.54 (1H, d, $J = 15.6$ Hz), 8.25 (1H, d, $J = 15.6$ Hz), 7.4–7.9 (7H, m), 5.95 (1H, s), 5.54 (1H, br, s), 3.9 (3H, s), 3.8 (3H, s), 3.1 (2H, m), 2.7 (2H, m), 2.37 (3H, s), 2.36 (2H, m); MS: *m/z* $[M + 1]^+$ 430.19.

***FS-3-N*$_1$**: *1-(3-(1,2,3,6-tetrahydro-1-methylpyridin-4-yl)-2-hydroxy-4,6-dimethoxyphenyl)-3-(naphthalen-2-yl)prop-2-en-1-one* Yield: 73 %; mp: 172–173 °C; λ_{max}: 356 nm; ε_{max}: 19885; IR: 3435 (O–H), 1624 (C=O), 1556 (C=C), 1118 (C–O); ^{1}HNMR ($CDCl_3$) δ 14 (1H, s), 7.99 (1H, br), 7.95–7.96 (2H, m), 7.82–7.89 (3H, m), 7.74–7.76 (1H, m), 7.49–7.53 (2H, m), 6.03 (1H, s), 5.61 (1H, s), 4 (3H, s), 3.87 (3H, s), 3.17 (2H, m), 2.75 (2H, m), 2.4 (2H, m), 1.8 (3H, s); MS: *m/z* $[M + 1]^+$ 430.19.

Antioxidant activity

The antioxidant activity of the synthesised test compounds are expressed in terms of IC_{50} values as shown in Table 1. The test compounds have considerable free radical scavenging activity when they were tested against ABTS and superoxide radical

Table 2 Anticancer activity of the synthesised test compounds

No	Compound code	IC_{50} (μM)					
		A549	HepG2	HeLa	HCT-116	MCF-7	MDA MB 231
1	**FS-3-S**	26.05	17.50	56.35	45.33	48.65	49.21
2	**FS-3-S$_1$**	23.07	10.20	48.23	39.21	45.35	45.63
3	**FS-3-SM$_3$**	33.50	6.75	44.32	37.23	42.57	43.26
4	**FS-3-SM$_5$**	34.48	8.00	45.31	35.34	40.38	42.91
5	**FS-3-F**	43.84	15.00	41.83	32.23	38.47	40.28
6	**FS-3-Br**	46.01	11.50	49.16	40.34	44.43	46.19
7	**FS-3-P**	24.12	6.60	67.29	56.45	59.18	60.48
8	**FS-3-DCl**	22.07	7.00	41.11	34.54	37.73	41.65
9	**FS-3-M**	23.80	6.75	42.12	35.56	38.63	40.46
10	**FS-3-E**	28.34	6.75	42.12	33.63	36.71	36.54
11	**FS-3-DA**	–	28.0	54.31	45.72	47.16	48.43
12	**FS-3-Dm**	27.07	7.00	41.23	32.38	35.21	37.32
13	**FS-3-N**	4.69	7.25	35.23	23.34	25.36	27.54
14	**FS-3-N$_1$**	4.28	6.50	33.21	21.80	23.45	25.80
Standards	Methotrexate	0.7	–	–	–	–	–
	Cisplatin	–	10.00	–	–	–	–
	Doxorubicin	–	–	3.9	0.07	0.12	3.2

IC_{50} values are calculated at 95% confidence interval (CI) and coefficient of variation <10

A549 human lung adenocarcinoma epithelial cell line, *HepG2* human hepatocellular carcinoma cell line, *HeLa* human cervical cancer cell line, *HCT-116* human colon carcinoma cell line, *MCF-7* human breast cancer cell line (ER-positive), *MDA MB 231* human breast carcinoma cell line (ER-negative)

scavenging assay. Similarly, the test compounds showed good iron chelating abilities in *O*-phenanthroline assay. The test compounds also showed considerable inhibition of ferric induced lipid peroxidation in vitro. Further, all the test compounds showed an overall free radical scavenging activity at $IC_{50} < 100$ μM in all the tests performed as shown in Table 1.

Toxicity on Vero cells

When the test compounds were tested on Vero cells, they showed IC_{50} above 800 μM suggesting the selective toxicity of the synthesized compounds for cancer cells.

Anticancer activity

The results of anticancer activity of the test compounds performed by MTT assay are presented in Table 2. Test compounds **FS-3-S** and **FS-3-S$_1$**, bearing a thienyl substitution, **FS-3-SM$_3$** and **FS-3-SM$_5$** possessing methyl thienyl group and compounds such as **FS-3-N** and **FS-3-N$_1$** having naphthalene rings were isomeric to each other. However, there was no significant difference in the anticancer activity in two isomers of a particular substitution as shown in Table 2. Further, the test compounds **FS-3-N** and **FS-3-N$_1$** exhibited potent anticancer activity comparable to that of other molecules in all the human cancer cell lines.

The bio-isosteric replacement of the thienyl ring with that of furanyl moiety was another objective of the present study, where the test compounds **FS-3-S** and **FS-3-S$_1$** with thienyl ring were conveniently replaced by sulphur with oxygen in the B-ring. Here, bio-isosteric modification was used as a tool in lead modification, where one sulphur atom becomes equivalent to one oxygen atom (non-classical bio-isosteres). With this rationale, the furanyl substituted compounds were designed, speculating for their anticancer activity. From our studies it was found that the furanyl substituted test compounds such as **FS-3-F** and **FS-3-Br**, resulted in a slightly decreased activity against cell lines studied. Further, the six-membered substitutions, namely, 3-pyridyl in test compound **FS-3-P** and other non-hetero aromatic compounds such as **FS-3-DCl**, **FS-3-M**, **FS-3-E**, and **FS-3-Dm**, showed potency equivalent to that of those bearing a thiophene nucleus, or even more, as seen against the cancer cell lines studied. Test compounds **FS-3-N** and **FS-3-N$_1$**

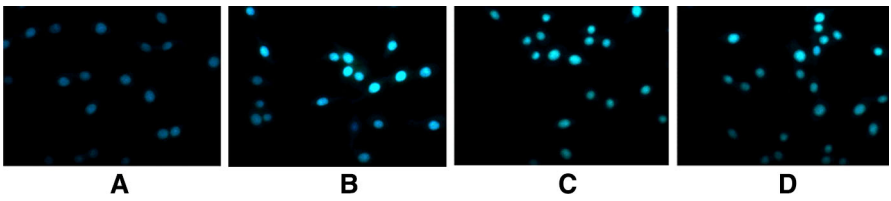

Fig. 4 Hoechst 33342 staining to study chromatin condensation in HepG2 cells, **a** control DMSO-treated; **b** positive control doxorubicin-treated; **c** compound **SF-3-N**-treated; **d** **SF-3-N$_1$** -treated

bearing a naphthalene moiety were the most active compounds among the entire group as shown in Table 2.

Hoechst staining technique was used for observing apoptosis in cancer cell lines. The stained apoptotic cells would show a bright fluorescence. The Fig. 4 represents the apoptosis induced by compound **FS-3 N** and **FS-3-N$_1$**. The chromatin condensations in apoptotic cells would show high fluorescence. This test inferred the strong evidence for apoptosis induction by our test compounds.

Scratch wound antimigration assay was also used to assess the inhibition of invasion and angiogenesis by tumour cells. The test compounds exhibited antimigration activity at 24 h incubations. All the test compounds were equally potent in inhibiting cell migrations compared to the control and the standard methotrexate (Table 3). The Fig. 5 represents the photograph taken at different time interval after control, standard and test compounds **SF-3-N** and **SF-3-N$_1$** inoculation.

Discussion

Chalcones have been reported for their anticancer activity by multimodal mechanisms, such as inhibition of tubulin assembly, inhibition of angiogenesis, induction of apoptosis, anti-estrogenic activity, and reversal of multidrug resistance,

Table 3 Scratch wound antimigration assay

Sl no.	Treatment[a]	Wound width in mm (mean ± SEM)			
		0 h	6 h	12 h	24 h
1.	Control	0.83 ± 0.037	0.46 ± 0.057	0.16 ± 0.02	0.06 ± 0.057
2.	MTX	0.75 ± 0.04	0.72 ± 0.03	0.68 ± 0.01	0.66 ± 0.042
3.	**FS-3-S**	0.60 ± 0.01	0.50 ± 0.03	0.44 ± 0.01	0.41 ± 0.045
4.	**FS-3-S$_1$**	0.64 ± 0.02	0.54 ± 0.05	0.46 ± 0.03	0.43 ± 0.05
5.	**FS-3-SM$_3$**	0.65 ± 0.03	0.54 ± 0.06	0.45 ± 0.05	0.42 ± 0.03
6.	**FS-3-SM$_5$**	0.67 ± 0.05	0.56 ± 0.03	0.46 ± 0.06	0.43 ± 0.02
7.	**FS-3-F**	0.80 ± 0.04	0.70 ± 0.041	0.65 ± 0.02	0.63 ± 0.015
8.	**FS-3-Br**	0.76 ± 0.06	0.72 ± 0.06	0.66 ± 0.07	0.44 ± 0.02
9.	**FS-3-P**	0.64 ± 0.04	0.58 ± 0.01	0.53 ± 0.01	0.45 ± 0.042
10.	**FS-3-DCl**	0.76 ± 0.03	0.68 ± 0.03	0.54 ± 0.02	0.43 ± 0.034
11.	**FS-3-M**	0.77 ± 0.02	0.74 ± 0.04	0.55 ± 0.05	0.42 ± 0.06
12.	**FS-3-E**	0.73 ± 0.01	0.68 ± 0.03	0.53 ± 0.01	0.44 ± 0.042
13.	**FS-3-DA**	0.76 ± 0.03	0.64 ± 0.04	0.58 ± 0.03	0.48 ± 0.021
14.	**FS-3-Dm**	0.79 ± 0.04	0.65 ± 0.05	0.59 ± 0.04	0.52 ± 0.031
15.	**FS-3-N**	0.73 ± 0.07	0.65 ± 0.02	0.63 ± 0.03	0.60 ± 0.032
16.	**FS-3-N$_1$**	0.72 ± 0.05	0.68 ± 0.03	0.65 ± 0.02	0.63 ± 0.022

Inhibition of closure of scratch wound in HeLa cell line; *MTX* methotrexate

[a] At concentration equal to the IC_{50} determined by MTT in HeLa cell lines

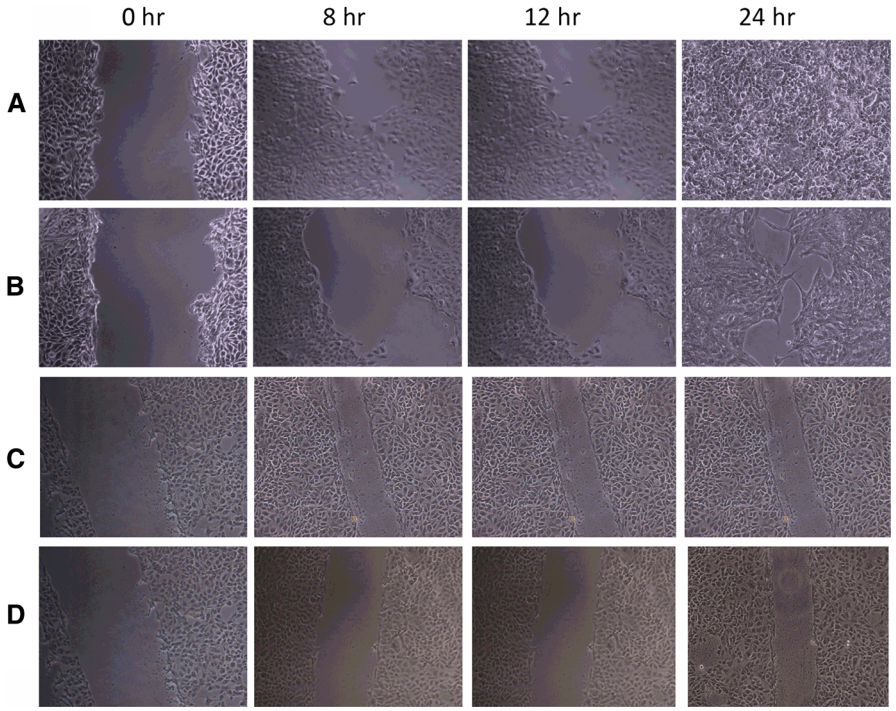

Fig. 5 Scratch wound anti-migration assay; Photomicrograms of scratch wounds on HeLa confluent cell layer and their closure by exposure without treatment and with test compounds for 8, 12, and 24 h; **a** control treated with solvent DMSO; **b** methotrexate treated; **c**, **d** compound **FS-3-N** and **FS-3-N$_1$** treated

or a combination of these mechanisms [2]. A recent report on newer 3-phenylquinolinylchalcone derivatives, which showed anticancer activity by polymerization of tubulins, induced G2/M cell cycle arrest via modulation of the cyclin B1, cdk1, and CDC25 [17]. The pyrazole chalcones and heterocyclic diamides have been reported for anticancer activity in MCF-7 and HeLa cell lines [23]. The newly synthesized test compounds have shown anticancer activity in human cancer cell lines by induction of apoptosis. Hoechst stain enters the plasma membrane and binds to apoptotic DNA, making the cell nucleus highly fluorescent, and confirms the mechanism of action of test compounds as apoptotic agent [19]. However, further studies have to be carried out to find alternative pathways for understanding the apoptotic mechanism for anticancer activity in these test compounds.

Cell migration is an indicator of proliferation of tumors or tumor invasion and metastasis [24]. The scratch wound healing assay is proven to be a good model to screen anti-invasive or antimetastatic molecules. The scratch wound assay is also an indicator for angiogenesis during tumor growth [18]. The inhibition of angiogenesis has multiple therapeutic implications other than cancer [25]. Many of the synthetic chalcones are reported for inhibition of angiogenesis and prevention of tumour growth [26]. Thus, in the present study, considerable inhibition of scratch wound

closure by test compounds indicates their applications as antimetastatic or antiangiogenic potentials.

Plant-derived and dietary phenolic antioxidants were proven to have anticancer properties with different mechanisms along with their antioxidant activity [27]. However, the relevance of antioxidant molecule for anticancer activity has recently been established. Along with intracellular free radical scavenging activity, the majority of antioxidant molecules activates transcription factor Nrf2 (nuclear factor-E2-related factor 2), which acts through antioxidant reactive element (NRE) signaling pathways in cancer cells and increases antioxidant enzymes by gene expression, whereby they contribute for anticancer activity [28]. In line with this, there are reports of novel synthetic chalcones for anticancer activity by activation of Nrf2. These chalcones have increased expression of Nrf2 dependent antioxidant genes in human lung epithelial cells and in mouse model of cancer [29]. It has been observed from our studies that these test compounds possesses antioxidants and this could attributed to their anticancer activity hence it warrants further mechanistic study.

Conclusions

The piperidinyl chalcones having naphthalene substitutions on the B-ring in chalcone showed antioxidant activity in vitro and anticancer activity against human cancer cell lines. The bio-isosteric replacement of the thienyl substituent with furanyl moiety resulted in decreased anticancer activity. However, non-hetero aromatic substitutions were equally potent as compared to their thienyl ring counterpart. Furthermore, there was no significant difference in the activity of the isomeric compounds bearing a thienyl group, methyl thienyl group and naphthalene moiety. The tetrahydropyridine substituted chalcones, with the retention of double bond in the piperidinyl ring were the newer bioisosterically modified derivatives of piperidinyl chalcones, and could be considered as useful leads for further anticancer investigations.

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Compliance with ethical standards

Conflict of Interest Authors declare that there is no conflict of interest.

References

1. R. Siegel, D. Naishadham, A. Jemal, CA: Cancer J. Clin. **63**, 11–30 (2013)
2. S. Ducki, IDrugs. **10**, 42–46 (2007)

3. A.M. Katsori, D. Hadjipavlou-Litina, Curr. Med. Chem. **16**, 1062–1081 (2009)
4. D. Passarella, B. Peretto, R. Blasco y Yepes, G. Cappelletti, D. Cartelli, C. Ronchi, J. Snaith, G. Fontana, B. Danieli, J. Borlak, Eur. J. Med. Chem. **45**, 219–226 (2010)
5. W. Huang, Y. Ding, Y. Miao, M.-Z. Liu, Y. Li, G.-F. Yang, Eur. J. Med. Chem. **44**, 3687–3696 (2009)
6. M.V. Reddy, C.R. Su, W.F. Chiou, Y.N. Liu, R.Y. Chen, K.F. Bastow, K.H. Lee, T.S. Wu, Bioorg. Med. Chem. **16**, 7358–7370 (2008)
7. A. Jha, C. Mukherjee, A.J. Rolle, E. De Clercq, J. Balzarini, J.P. Stables, Bioorg. Med. Chem. Lett. **17**, 4545–4550 (2007)
8. R. Romagnoli, P.G. Baraldi, M.D. Carrion, C.L. Cara, O. Cruz-Lopez, D. Preti, M. Tolomeo, S. Grimaudo, A. Di Cristina, N. Zonta, J. Balzarini, A. Brancale, T. Sarkar, E. Hamel, Bioorg. Med. Chem. **16**, 5367–5376 (2008)
9. R. LeBlanc, J. Dickson, T. Brown, M. Stewart, H.N. Pati, D. VanDerveer, H. Arman, J. Harris, W. Pennington, H.L. Holt Jr, M. Lee, Bioorg. Med. Chem. **13**, 6025–6034 (2005)
10. M. Johnson, B. Younglove, L. Lee, R. LeBlanc, H. Holt Jr, P. Hills, H. Mackay, T. Brown, S.L. Mooberry, M. Lee, Bioorg. Med. Chem. Lett. **17**, 5897–5901 (2007)
11. D. Kumar, N.M. Kumar, K. Akamatsu, E. Kusaka, H. Harada, T. Ito, Bioorg. Med. Chem. Lett. **20**, 3916–3919 (2010)
12. E. Winter, L.D. Chiaradia, C.A. de Cordova, R.J. Nunes, R.A. Yunes, T.B. Creczynski-Pasa, Bioorg. Med. Chem. **18**, 8026–8034 (2010)
13. X. Liu, M.L. Go, Bioorg. Med. Chem. **15**, 7021–7034 (2007)
14. T. Ak, İ. Gülçin, Chem. Biol. Interact. **174**, 27–37 (2008)
15. A. Shirwaikar, K.S. Prabhu, I.S.R. Punitha, Indian J. Exp. Biol. **44**, 993–996 (2006)
16. S.N. Manjula, N. Malleshappa Noolvi, K. VipanParihar, S.A. Manohara Reddy, V. Ramani, A.K. Gadad, G. Singh, N. Gopalan Kutty, C. Mallikarjuna Rao, Eur. J. Med. Chem. **44**, 2923–2929 (2009)
17. C.H. Tseng, C.C. Tzeng, C.Y. Hsu, C.M. Cheng, C.N. Yang, Y.L. Chen, Eur. J. Med. Chem. **97**, 306–319 (2015)
18. M. Pilatova, L. Varinska, P. Perjesi, M. Sarissky, L. Mirossay, P. Solar, A. Ostro, J. Mojzis, Toxicol. In Vitro **24**, 1347–1355 (2010)
19. D. Sunil, A.M. Isloor, P. Shetty, K. Satyamoorthy, A.S. Bharath Prasad, Arabian J. Chem. **3**, 211–217 (2010)
20. H. Venkatachalam, Y. Nayak, B.S. Jayashree, APCBEE Procedia. **3**, 209–213 (2012)
21. H.H. Szmant, A.J. Basso, J. Am. Chem. Soc. **74**, 4397–4400 (1952)
22. V. Opletalová, J. Hartl, K. Palát Jr, A. Patel, J. Pharm. Biomed. Anal. **23**, 55–59 (2000)
23. U. Sankappa Rai, A.M. Isloor, P. Shetty, K.S.R. Pai, H.K. Fun, Arabian J. Chem. **8**, 317–321 (2015)
24. A.B. Kunnumakkara, P. Anand, B.B. Aggarwal, Cancer Lett. **269**, 199–225 (2008)
25. P. Carmeliet, R.K. Jain, Nature **473**, 298–307 (2011)
26. J. Mojzis, L. Varinska, G. Mojzisova, I. Kostova, L. Mirossay, Pharmacol. Res. **57**, 259–265 (2008)
27. F.M.F. Roleira, E.J. Tavares-Da-Silva, C.L. Varela, S.C. Costa, T. Silva, J. Garrido, F. Borges, Food Chem. **183**, 235–258 (2015)
28. J.D. Hayes, M. McMahon, S. Chowdhry, A.T. Dinkova-Kostova, Antioxid. Redox Signal. **13**, 1713–1748 (2010)
29. V. Kumar, S. Kumar, M. Hassan, H. Wu, R.K. Thimmulappa, A. Kumar, S.K. Sharma, V.S. Parmar, S. Biswal, S.V. Malhotra, J. Med. Chem. **54**, 4147–4159 (2011)