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Synthesis and Cytotoxic Activity of New Chalcones and their Flavonol Derivatives

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Abstract

A series of chalcones and respective flavonols have been synthesized to explore their anti-cancer activities. All the chalcones were synthesized by the reaction between aldehydes and substituted acetophenones in typical base carried Claisen–Schmidt condensation and their corresponding flavonols were synthesized by hydrogen peroxide oxidized Flynn-Algar-Oymada epoxidation and cyclization. The synthesized compounds were characterized by FTIR, ^{1}H NMR, ^{13}C NMR and Mass spectrometry and subjected for cytotoxicity test on MCF-7, HT-29 and HeLa cell lines. Maximum number of compounds demonstrated anti-proliferative activity with IC_{50} in the range of 18.67-174.3 μM. Compound **3h** with a chloro group and 1-phenyl-3(4-methoxy phenyl)-4-pyrazolyl moiety and the flavonol **4a** with 3-thienyl group were found to be most potent compounds among all the tested compounds against MCF-7 cell lines with IC_{50} 18.67 and 23.79 μM respectively. The most active compound **3h** also showed high docking score of -8.825.

Keywords: Chalcone; Flavonol; Anti-proliferative activity

Introduction

Cancer is a major health problem in developing as well as underdeveloped countries recognized by uncontrolled cell growth [1,2]. Despite, intensive advancement in the treatment of cancer, the present chemotherapy is ineffective because of drug resistance and inability of many drugs to differentiate between normal cells and the cancerous cells [3]. According to the WHO report, cancer is the second leading cause of death globally and was responsible for 8.8 million deaths in 2015. Approximately 70% of deaths occur in low and middle income countries due to cancer [3]. Therefore, development of new molecules with less toxicity, better efficacy and improved selectivity provide an important contribution for the development of safer drugs useful in the chemotherapy of cancer.

Targeted agents have more effective and less toxicity profiles over conventional chemotherapeutic agents. Natural compounds, like chalcones (Figure 1), have been shown to be relatively nontoxic, and certain chalcone moieties can target key molecular events that may lead to carcinogenesis [4]. Chalcones are important bioactive molecules belong to the flavonoid family found in many natural compounds [5]. Chalcones are the precursors of flavonoids and isoflavonoids are an important pharmacophore of various natural products [6]. Chemically, chalcones are 1,3-diaryl-2-propen-1-ones in which two aromatic rings or substituted aromatic rings are joined together by a three carbon α, β unsaturated carbonyl system.

Molecular hybridization approach has been gaining special attention from medicinal chemistry, in which two pharmacophores are combined to yield a single molecule with additive biological properties. Literature survey reveals that chalcone-heterocycle hybrids with substituted pyrazole [7], pyridine [8], thiophene [9] moieties exhibited potent anticancer activities in *in vitro* studies. Chalcone-triazole-coumarin hybrids were reported by Pingaew et al. as anticancer and antimalarial agents [10]. It has been reported that chalcones possess many important biological activities including anti-oxidant [11], anti-cancer [12], anti-bacterial [13], anti-fungal [14], antiparasitic [15], antivascular [16] and anti-inflammatory [17]. Recently Tatiana et al. reported the anticancer activities of chalcone and flavonol derivatives [18]. Due to their anticancer activities, considerable efforts have been dedicated to identify new potential chalcone based drug candidates with in the field of oncology [19]. Keeping in view the biological importance of chalcones with various heterocycles such as pyrazole, pyridyl, thienyl moieties, herein, it is proposed to carry out the synthesis, docking and anticancer evaluation of some chalcone-hybrids with various heterocycles such as pyridyl, furyl, thienyl, substituted pyrazolyl groups and their flavonol derivatives (Figures 1 and 2).

Hence we proposed to synthesize molecules with 2-hydroxy phenyl group and various heterocyclic moieties as part of the chalcone system. Similarly the chalcones were converted to their corresponding flavonols and all the synthesized compounds were evaluated for their anticancer activity. The molecular docking studies were also performed for the synthesized compounds and the results are all presented here.

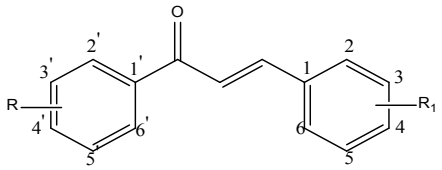

Figure 1: General structure of chalcones.

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Materials and Methods

All chemicals and solvents were purchased from commercial sources (Sigma Aldrich, Hymedia and SD Fine) used without further purification. All compounds were characterized by spectroscopic data and compared with the data available in the literature. The NMR spectra were recorded in DMSO-d_6 or $CdCl_3$. ^{1}H NMR spectra were obtained on a Bruker Advance 3400 (^{1}H: 400 MHz and ^{13}C: 100 MHz) and deuterated DMSO was used as solvent. The chemical shifts were expressed in values parts per million (ppm scale) and the J values were reported in Hertz (Hz). The peak patterns were indicated as follows: s, singlet; d, doublet; t, triplet; m, multiplet. The reactions were monitored by Thin Layer Chromatography (TLC) using silica gel 60 F254 plates (Merck). The melting points were determined on a Stuart SMP3 melting point apparatus. Elemental analyses were performed on Elementar Vario MICRO CHNS instrument.

Chemicals and cell culture the 3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT), DMEM (Dulbecco's modified Eagles medium), antibiotic/antimycotic solution, trypsin, EDTA and phosphate buffered saline (PBS) were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Fetal bovine serum (FBS) was bought from Gibco. 25 cm^2 and 75 cm^2 flask and 96 well plates were purchased from Eppendorf India.). All other reagents were of analytical grade. MCF-7 (Breast adenocarcinoma cancer cell line) and HT-29 cells (human colorectal cancer cell line), HeLa (Human Cervical Carcinoma Cell line) were purchased from NCCS, Pune.

Experimental

General procedure for the synthesis of substituted hydroxychalcone derivatives (3a-h)

To a solution of KOH (1.12 g, 0.02 mol) in methanol (50 ml) was added substituted hydroxyacetophenones **2a-c** (1.36 g,0.01 mol) and substituted hetero aromatic aldehydes **1a-e** (2.62 g, 0.01 mol) at 0-5°C [14]. The reaction mixture was stirred overnight at room temperature (Scheme 1). Then, this reaction mixture was poured over crushed ice and acidified with dil. HCl. The solid formed was filtered and washed with distilled water and dried. The crude product was crystallized with ethanol to afford pure 2-hydroxychalcones.

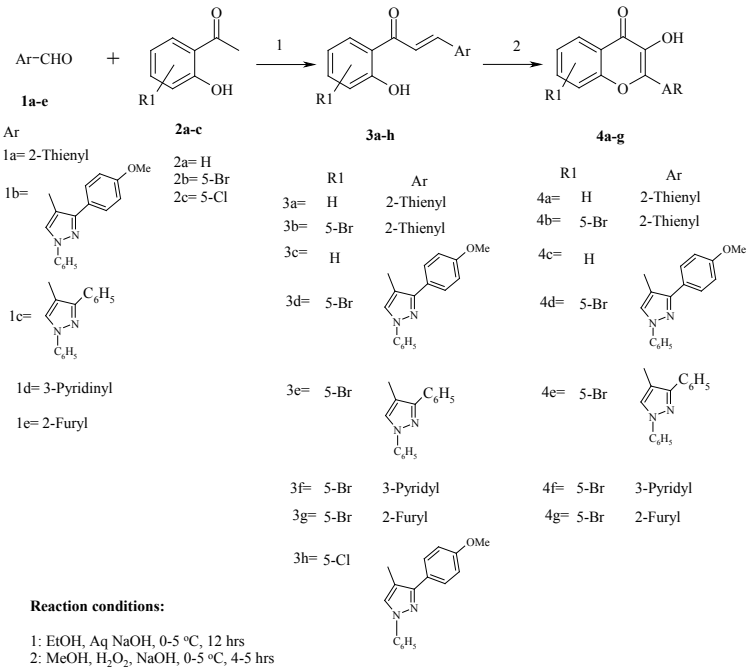
General procedure for the synthesis of substituted chromone derivatives (4a-g)

To a well-stirred solution of substituted hydroxychalcones **3** (2.0 g, 0.007 mol) in MeOH (20 ml) and aq.KOH (10 ml, 20%), cooled at 5-10°C, was added 30% H_2O_2 (10 ml) drop wise over 1 hr. The reaction mixture was further stirred for 4-5 hrs and the resulting light yellow reaction mixture was poured on crushed ice and neutralized with dilute hydrochloric acid [14]. The light yellow solid thus obtained was filtered, washed with water and dried. The crude product was crystallized with ethanol to afford pure hydroxychromone derivatives.

Biological assays

Cell culture: The cell lines (MCF-7, HT-29 and HeLa) were maintained in culture with MEM supplemented with 10% FBS and the antibiotics penicillin/streptomycin (0.5 mL^{-1}), in atmosphere of 5% CO_2 and 95% air at 37°C. Stock solutions of synthesized chalcones **(3a-h)** and flavonols **(4a-g)** were made in DMSO and kept in aliquots at -20°C. For MTT assay, each test compound was weighed separately and dissolved in DMSO, made up the final concentration with media to 1 mg/ ml and the cells were treated with series of concentrations from 10 to 100 µm.

MTT assay: Inhibition of cell proliferation by chalcones and flavonols were determined using the methyl thiazolyltetrazolium (MTT) cell viability assay with three independent experiments with six concentrations of compounds in triplicates. MCF- 7, HT-29 and HeLa cells were trypsinized and preformed the tryphan blue assay to know viable cells in cell suspension. Cells were counted by haemocytometer



Scheme 1: Synthetic routes of chalcone and flavonol derivatives.

and seeded at density of 5.0 × 10^3 cells / well in 100 µl media in 96 well plate culture medium and incubated overnight at 37°C. After incubation, the old media was taken off and added with fresh media 100 µl with different concentrations of test compound in respective 96 well plate. After 48 hrs, the drug solution discarded and the fresh media with MTT solution (0.5 mg/ml) was added to each well and plates were incubated at 37°C for 3 hrs. At the end of incubation time, precipitates are formed as a result of the reduction of the MTT salt to chromophore formazan crystals by the cells with metabolically active mitochondria. The optical density of solubilized crystals in DMSO was measured at 570 nm on a microplate reader. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% values is generated from the dose-response curves for each cell line [20].

%Inhibition=100(Control-Treatment)/Treatment

Docking

Dataset preparation: Glide, version 6.1, (Schrodinger, LLC, New York, NY, 2013.Inc. 2012) was used for docking studies. The structure of fifteen synthesized compounds consisting both 2-hydroxychalcones and flavonols were drawn by considering all possible tautomers and were subjected for minimization using OPLS-2005 force field using water as solvent in the GB/SA continuum solvation model in Schrodinger module.

The drawn structures were minimized using Polak-Ribiere Conjugate Gradient (PRCG) method with maximum of 5000 iterations. Van der Waal interaction (8.0), electrostatic (20), and hydrogen bond (4.0) were included in extended contribution. The extensive conformational search was carried out with Mixed torsional/Low-mode sampling method with the use of 100 steps per rotatable bond, maximum number of steps 1000, energy window for saving structures with 5.02 kcal/mol, eliminate the redundant conformers with maximum atom deviation cut-off 0.5 Å and saved 100 structures for each search.

Protein preparation: The 3D coordinates of crystal structure of tubulin was obtained from protein data bank (PDB ID: 1SA0) with resolution of 2.5 Å and co-crystal ligand. Usually deposited crystal structures lack hydrogens, which were added and also adjusted the bond orders using protein preparation wizard of Schrödinger software graphical user interface Maestro v9.3. Also, refining the structure and minimizing is a major step involved to ensure a stable protein formation. Finally selective protein was minimized to relieve unwanted interactions using OPLS-2005 force field with converge heavy atoms to RMSD 0.3 Å relative to original protein structure.

Docking studies: This step involves actual docking where the given ligands are checked for their binding capability and interactions. The grid and the prepared ligands were placed in respective slots. Further, the process was run as per the requirement. HTVS (high throughput virtual screening), SP (standard precision) was chosen. The output was also selected as pose viewer file.

Structure characterization

(E)-1-(2-hydroxyphenyl)-3-(thiophen-2-yl)prop-2-en-1-one (3a): IR νmax (KBr) cm^{-1}: 3308 (OH), 1640 (C=O), 1560 (olefinic C=C). ^{1}HNMR ($CdCl_3$) δ (ppm): 6.96-6.93 (t, J=7.5Hz, 1H), 7.03-7.01 (d, J=8.4Hz, 1H), 7.13-7.10 (t, J=4.2Hz, 1H), 7.42-7.41 (d, J=2.8Hz, 1H), 7.51-7.46 (m, 3H), 7.89-7.87 (d, J=7.6 Hz, 1H), 8.07-8.03(d, J=15.4 Hz, 1H, olefinic), 12.84 (s, 1H). Elemental analysis: calculated for $C_{13}H_{10}O_2S$; (230); calculated: C, 67.80; H, 4.38; found: C, 67.68; H, 4.46. MS: (m/z); 231 $[M+1]^+$.

(E)-1-(5-bromo-2-hydroxyphenyl)-3-(thiophen-2-yl) prop-2-en-1-one (3b): IR νmax (KBr) cm^{-1}: 3290 (OH), 1610(C=O), 1558 (olefinic C=C). ^{1}HNMR ($CdCl_3$) δ (ppm): 6.94-6.92 (d, J=8.8 Hz, 1H), 7.14-7.12 (dt, J=4.8 Hz, 5.2 Hz, 1H), 7.34-7.31 (d, J=15.2 Hz, 1H, olefinc), 7.52-7.43 (m, 2H), 7.57-7.55 (dd, J=8.8 Hz, 2.4 Hz, 1H), 7.966-7.960 (d, J=2.4 Hz, 1H), 8.09-8.05 (d, J =14.8 Hz, 1H, olefinic), 12.78 (s, 1H); Elemental analysis: calculated for $C_{13}H_9BrO_2S$ (309); calculated: C, 50.50; H, 2.93; found: C, 50.58; H, 2.85. MS: (m/z); 311 $[M+2]^+$.

(E)-1-(2-hydroxyphenyl)-3-(4-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-3-yl)prop-2-en-1-one (3c): IR νmax (KBr) cm^{-1}: 3310 (OH), 1637(C=O), 1564 (olefinic C=C). ^{1}HNMR ($CdCl_3$) δ (ppm): 3.91(s, 3H, OCH_3), 6.94 (m, 1H), 7.04-7.02 (d, J=8.4 Hz, 1H), 7.08-7.06 (d, J=8.7 Hz, 2H), 7.39-7.41 (d, J=8.4 Hz, 1H), 7.48-7.55 (m, 4H), 7.67-7.69 (d, J=8.7Hz, 2H), 7.82-7.84 (m, 3H), 8,02-7.88 (d, J=15.3Hz,1H, olefinic), 8.4 (s, 1H), 12.94 (s, 1H, OH), Elemental analysis: calculated for $C_{25}H_{20}N_2O_3$ (396); calculated; C, 75.74; H, 5.08; N, 7.07; found: C, 75.91; H, 5.19; N, 7.01. MS: (m/z); 397 $[M+1]^+$.

(E)-1-(5-bromo-2-hydroxyphenyl)-3-(4-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-3-yl)prop-2-en-1-one (3d): IR νmax (KBr) cm^{-1}: 3294 (OH), 1634 (C=O), 1545 (olefinic C=C). ^{1}HNMR ($CdCl_3$) δ (ppm): 3.89 (s, 3H, OCH_3), 6.93-6.91 (d, J=8.8Hz, 1H), 7.08-7.06 (d, J=8.8Hz, 2H), 7.40-7.36 (m, 2H), 7.56-7.49 (m, 3H), 7.65-7.63 (d, J=8.8Hz , 2H), 7.83-7.8 (d, J=8.0Hz, 2H), 7.87-7.86 (d, J=2.0Hz, 1H), 8.03-7.99 (d, J=15.2 Hz, 1H, olefinic), 8.41(s, 1H), 12.84 (s, 1H). Elemental analysis: calculated for $C_{25}H_{19}BrN_2O_3$ (475) calculated: C, 63.17; H, 4.03; N, 5.89; found: C, 63.25; H, 3.94; N, 5.93. MS: (m/z); 477 $[M+2]^+$.

(E)-1-(5-bromo-2-hydroxyphenyl)-3-(1,4-diphenyl-1H-pyrazol-3-yl)prop-2-en-1-0ne prop-2-en-1-one (3e): IR νmax (KBr) cm^{-1}: 3320 (OH), 1648 (C=O), 1593 (olefinic C=C). ^{1}HNMR ($CdCl_3$) δ (ppm): 6.93-6.91 (d, J=8.8Hz, 1H), 7.40-7.34 (m, 2H), 7.57-7.47 (m, 6H), 7.72-7.70 (m, 2H), 7.85-7.82 (m, 3H), 8.03-7.99 (d, J=15.2Hz, 1H, olefinic), 8.43 (s, 1H), 12.80 (s, 1H). Elemental analysis: calculated for $C_{24}H_{17}BrN_2O_2$ (445); calculated; C, 64.73; H, 3.85; N, 6.29; found: C, 64.65; H, 3.92; N, 6.23. MS: (m/z); 447 $[M+2]^+$.

(E)-1-(5-bromo-2-hydroxyphenyl)-3-(pyridine-3-yl) prop-2-en-1-one (3f): IR νmax (KBr) cm^{-1}: 3298 (OH), 1636 (C=O), 1560 (olefinic C=C). ^{1}HNMR ($CdCl_3$) δ (ppm): 6.98 - 6.96 (d, J=8.9Hz, 1H), 7.45-7.42 (m, 1H), 7.67-7.58 (m, 2H), 7.95-7.91 (d, J=15.52Hz, 1H, olefinic), 8.05-8.00 (m, 2H), 8.70-8.69 (d, J=4.03Hz, 1H), 8.91 (s, 1H), 12.58 (s, 1H). ^{13}CNMR (100MHZ, DMSO-d_6): δ 192.15, 162.58, 151.50, 150.20, 142.53, 139.34, 134.97, 131.83, 130.24, 123.99, 121.54, 121.00, 120.78, 110. 62 ppm. Elemental analysis: calculated for $C_{14}H_{10}BrNO_2$ (304); calculated; C, 55.29; H, 3.31; N, 4.61; found: C, 55.37; H, 3.24; N, 4.68. MS: (m/z); 306 $[M+2]^+$.

(E)-1-(5-bromo-2-hydroxyphenyl)-3-(furan-2-yl) prop-2-en-1-one (3g): IR νmax (KBr) cm^{-1}: 3300 (OH), 1635 (C=O), 1516 (olefinic C=C). ^{1}HNMR ($CdCl_3$) δ (ppm): 6.57-6.55 (m, 1H), 6.83- 6.81 (d, J=3.30Hz, 1H), 6.94-6.91 (d, J=8.92Hz, 1H), 7.47-7.43 (d, J=15.03Hz, 1H, olefinic), 7.59-7.55 (m, 2H), 7.71-7.68 (d, J=15.03Hz, 1H, olefinic), 8.01-8.00 (d, J=2.20Hz, 1H), 12.82 (s, 1H). ^{13}CNMR (100MHZ, DMSO-d6): δ 192.14, 162.46, 151.36, 145.82, 138.85, 131.97, 131.83, 121.31, 120.57, 117.87, 116.92, 113.07, 110.45 ppm. Elemental analysis: calculated for $C_{13}H_9BrO_3$ (293); calculated; C, 53.27; H, 3.09; found: C, 53.19; H, 2.98. MS: (m/z); 295 $[M+2]^+$.

(E)-1-(5-chloro-2-hydroxyphenyl)-3-(4-(4-methoxyphenyl)-1-

phenyl-1H-pyrazol-3-yl)prop-2-en-1-one (3h): IR νmax (KBr) cm^{-1}: 3300 (OH), 1640 (C=O), 1516 (olefinic C=C). ^{1}HNMR ($CdCl_3$) δ (ppm): 3.90 (s, 3H, OCH_3), 7.10-6.98 (m, 3H), 7.43-7.38 (m, 2H), 7.55-7.51 (m, 2H), 7.68-7.65 (m, 1H), 7.75 (m, 1H), 7.86-7.80 (m, 4H), 8.05-8.01(d, J=16Hz, 1H, olefinic), 8.54 (s, 1H), 12.83 (s, 1H). Elemental analysis: calculated for $C_{25}H_{19}ClN_2O_3$ (430); calculated; C, 69.69; H, 4.44; N, 6.50; found: C, 69.73; H, 4.52; N, 6.43. MS: (m/z); 432 $[M+2]^+$.

3-hydroxy-2-(thiophen-2-yl)-4H-chromen-2-one (4a): IR νmax (KBr) cm^{-1}: 3310 (OH), 1610 (C=O). ^{1}HNMR ($CdCl_3$) δ (ppm): 6.93 (bs, 1H), 7.25 – 7.23 (m, 1H), 7.43-7.39 (m, 1H), 7.59-7.56 (d, J=8.4HZ, 1H), 7.63-7.61 (m, 4.8Hz, 1H), 7.72-7.67 (m, 1H), 8.03-8.02 (m, 1H), 8.25-8.23 (m, 1H). Elemental analysis: calculated for $C_{13}H_8O_3S$ (244); calculated; C, 63.92; H, 3.30; found: C, 63.86; H, 3.38. MS: (m/z); 245 $[M+1]^+$.

6-bromo-3-hydroxy-2-(thiophen-2-yl)-4H-chromen -2-one (4b): IR νmax (KBr) cm^{-1}: 3370 (OH), 1612 (C=O). ^{1}HNMR ($CdCl_3$) δ (ppm): 7.24-7.22 (t, J=3.54Hz, 1H), 7.36 (s, 1H), 7.48-7.46 (d, J=8.80Hz, 1H), 7.63-7.62 (d, J=4.64Hz, 1H), 7.76-7.74 (d, J=8.68Hz, 1H), 8.02-8.01 (d, J=2.56Hz 1H), 8.34 (s, 1H). Elemental analysis: calculated for $C_{13}H_7BrO_3S$ (323); calculated; C, 48.32; H, 2.18; found: C, 48.22; H, 2.10. MS: (m/z); 325 $[M+2]^+$.

3-hydroxy-2-(4-4-methoxyphenyl)-1-phenyl-1H-pyrazol-3-yl)-4H-chromen-4-one (4c): IR νmax (KBr) cm^{-1}:3298 (OH), 1613 (C=O). ^{1}HNMR ($CdCl_3$) δ (ppm): 3.88 (s, 3H, OCH_3), 6.99-6.97 (d, J=8.8Hz, 1H), 7.04-7.02 (d, J=8.8Hz, 2H) 7.41-7.37 (m, 2H), 7.53-7.47 (m, 4H), 7.80-7.77 (m, 3H), 7.87-7.85 ((d, J=8.8Hz, 1H), 8.52 (s, 1H), 10.04 (s, 1H) Elemental analysis: calculated for $C_{25}H_{18}N_2O_4$ (410); calculated; C, 73.16; H, 4.42; N, 6.83; found: C, 73.22; H, 4.18; N, 6.96. MS: (m/z); 411 $[M+1]^+$.

6-bromo-3-hydroxy-2-(4-4-methoxyphenyl)-1-phenyl-1H-pyrazol-3-yl)-4H-chromen-4-one (4d): IR νmax (KBr) cm-1:3300 (OH), 1610 (C=O). ^{1}HNMR ($CdCl_3$) δ (ppm): 3.89 (s, 3H, OCH_3), 6.90-6.88 (m, 1H), 7.03-7.00 (m, 2H), 7.41-7.39 (m, 1H), 7.55-7.50 (m, 2H), 7.67-7.63 (m, 2H), 7.81-7.79 (m, 1H),7.89-7.87 (m, 2H), 8.38-8.37 (d, J=2.4Hz, 1H), 8.58 (s, 1H), 8.85 (s, 1H). Elemental analysis: calculated for $C_{25}H_{17}BrN_2O_4$ (489); calculated; C, 61.36; H, 3.50; N, 5.72 found: C, 61.15; H, 3.62; N, 5.84. MS: (m/z); 491 $[M+2]^+$.

6-bromo-2-(1,4-diphenyl-1H-pyrazol-3-yl)-3-hydroxy-4H-chromen-4-one (4e): IR νmax (KBr) cm^{-1}: 3402 (OH), 1609 (C=O). ^{1}HNMR ($CdCl_3$) δ (ppm): 6.77-6.75 (m, 1H), 7.42-7-7.38 (m, 1H), 7.56-7.45 (m, 5H), 7.64-7.61(m, 1H), 7.70-7.68 (m, 1H), 7.82-7.80 (m,1H), 7.92-7.86 (m, 2H), 8.37-8.36 (m, 1H), 8.60 (s, 1H), 8.87 (s, 1H). Elemental analysis: calculated for $C_{24}H_{15}BrN_2O_3$ (459); calculated; C, 62.76; H, 3.29; N, 6.10 found: C, 62.84; H, 3.20; N, 6.19. MS: (m/z); 46 $[M+2]^+$.

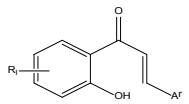
6-bromo-3-hydroxy-2-(pyridine-3-yl)-4H-chromen-4-one (4f): IR νmax (KBr) cm^{-1}: 3352 (OH), 1609 (C=O). ^{1}HNMR ($CDCl_3$) δ (ppm): 7.54-7.40 (m, 2H), 7.81-7.79 (d, J=8.55 Hz, 1H), 8.37 (s, 1H), 8.68-8.58(m, 3H), 9.45(s, 1H).^{13}CNMR (100 MHZ, DMSO-d_6): 172.20, 154.22, 150.74, 148.56, 142.94, 139.29, 137.05, 134.99, 128.06, 127.14, 123.44, 122.01, 120.27, 118.22 ppm. Elemental analysis: calculated for $C_{14}H_8BrNO_3$ (318); calculated; C, 52.86; H, 2.53; N, 4.40 found C, 52.95; H, 2.46; N, 4.48. MS: (m/z); 320 $[M+2]^+$.

6-bromo-2-(furan-2-yl)-3-hydroxy-4H-chromen-4-one (4g): IR νmax (KBr) cm^{-1}: 3284 (OH), 1613 (C=O). ^{1}HNMR ($CdCl_3$) δ (ppm): 6.80-6.79 (t, J=1.4Hz, 1H), 7.31-7.30 (d, J=3.2Hz, 1H), 7.70-7.68 (d, J=8Hz, 1H), 7.92-7.89 (m, 1H), 8.04 (m, 1H), 8.167-8.162 (d, J=2Hz, 1H), 10.197 (s, 1H, OH, D_2O Exchangeable). Elemental analysis: calculated for $C_{13}H_7BrO_4$ (307); calculated; C, 50.84; H, 2.30 found C, 50.72; H, 2.41. MS: (m/z); 309 $[M+2]^+$.

Results and Discussion

Chemistry

All the chalcones **3a-h** were synthesized by base catalyzed aldol condensation type reaction by using appropriate heterocyclic aldehydes **1a-e** and 2-hydroxy acetophenones **2a-c**. 3M sodium hydroxide was employed for the removal of proton from 2-hydroxy acetophenones and all the reactions were carried at 0-5°C and stirred at room temperature. Upon acidification of the reaction mixture, desired chalcones were obtained, which were purified by recrystalization to afford chalcones (**3a-h**) in 50-80% yield. All the 2-hydroxy chalcones except **3h** in Flynn-Algar-Oymada epoxidation and cyclization using alkaline hydrogen peroxide resulted in cyclized flavonols **4a-g** which were purified by recrystallization to get the desired flavonols in 54-90% yield. All the details of characterization have been included in Tables 1 and 2.



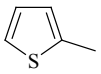
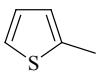
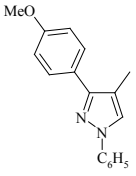
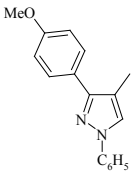
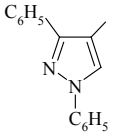
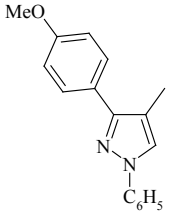
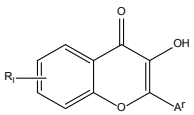
Comp.	R1	Ar	Yields (%)	MP (°C)	R_f
3a	-H		65	85-87	0.71
3b	-Br		78	77-79	0.66
3c	-H		70	170-172	0.78
3d	-Br		84	180-182	0.74
3e	-Br		65	184-186	0.68
3f	-Br		77	132-134	0.64
3g	-Br		50	75-77	0.55
3h	-Cl		61	144-146	0.74

Table 1: Physical data of synthesized chalcone derivatives.

The IR spectra of hydroxychalcones showed carbonyl absorption in the range of 1600-1650 cm^{-1} and an olefinic C=C in the range 1516-1593 cm^{-1}. Flavanol showed OH absorption in the range of 3284-3402 cm^{-1} and C=O in the range 1609-1613 cm^{-1} respectively. In the ^1H NMR spectra of hydroxylchalcones, OH peak appeared as singlet in the range of δ 12.58-12.80, olefinic protons H-a and H-b appeared as doublets or multiplets in the range of δ 7.31-7.34 and δ 8.05-8.09 respectively. Trans-stereochemistry of propenone moiety of chalcones was confirmed by coupling constant of vinyl hydrogen (14.8-16 Hz) indicating formation of trans chalcones. In flavonols OH peak appeared as a singlet in the range of δ 6.93-10.19. The parent ion peak appeared on the positive mode in the mass spectrum confirms the chalcones and flavonols.

Cytotoxicity activity

The cytotoxicity studies of eight chalcone and seven flavonol derivatives were evaluated on three human cancer cell lines namely



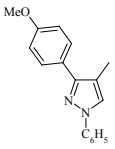
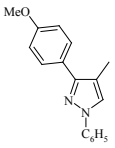
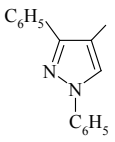
Comp.	R1	Ar	Yields (%)	MP (°C)	R_f
4a	-H		68	199-201	0.73
4b	-Br		76	210-212	0.81
4c	-H		70	161-163	0.66
4d	-Br		90	180-182	0.69
4e	-Br		60	170-172	0.61
4f	-Br		67	200-202	0.76
4g	1 Br		54	205-207	0.72

Table 2: Physical data of synthesized flavonol derivatives.

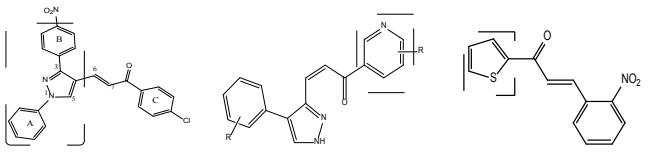


Figure 2: Biologically active substituted chalcone derivatives from literature reports.

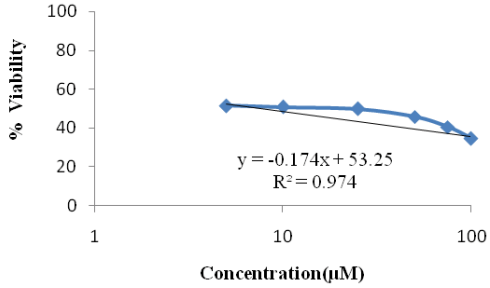
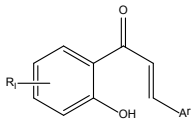


Figure 3: Dose response relationship of **3h** in the number of MCF-7 viable cells. The IC_{50} concentration of tested compounds is also depicted.



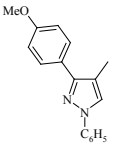
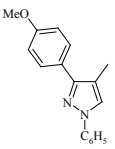
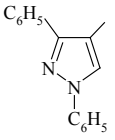
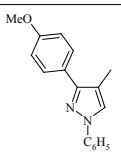
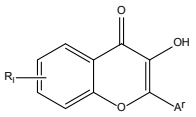
Comp.	R1	Ar	MCF-7	HT-29	HeLa	Docking score
3a	-H		33.29	52.63	112.36	-6.768
3b	-Br		44.15	42.02	114.02	-6.913
3c	-H		150.10	100.23	-	-6.478
3d	-Br		85.51	156.22	77.52	-7.874
3e	-Br		23.48	78.13	158.61	-8.595
3f	-Br		28.17	89.08	146.20	-6.328
3g	-Br		156.22	104.04	173.67	-6.363
3h	-Cl		18.67	99.38	-	-8.825
Cisplatin			4.05	6.05	4.05	

Table 3: Anticancer activity of synthesized chalcone derivatives (IC_{50} in μM).

MCF-7, HT-29, HeLa using MTT assay *in vitro* [20]. Semi log plot of concentration of compounds versus % inhibition of cell lines corroborated the determination of IC_{50} values of compounds using Microsoft Excel Figure 3. The cytotoxicity of all the tested compounds were compared against Cisplatin as standard compound which showed cytotoxic activity with IC_{50} value of 4.05 μM against MCF-7 and HeLa

cell lines and 6.05 µM against HT-29 Cell lines. The results of anticancer activity of all tested compounds are summarized in Table 3 and 4.

Among all tested compounds, cyclized flavanols have demonstrated enhanced activity over their corresponding 2-hydroxy chalcones against MCF-7 and HT-29 cell lines which indicates the restricted binding site which aides in accommodating flat structures. This is evidenced by the activity of profiles of flavonols, compounds **4a, 4b, 4c, 4d** and **4g** were found to be more active than corresponding 2-hydroxy chalcones (**3a, 3b, 3c, 3d** and **3g**). Only two compounds **3e** and **3f** were more active than corresponding flavonols against MCF-7 and HT-29 cell lines. Maximum 2-hydroxy chalcones were active in cytotoxicity trials against HeLa cell lines except **3c**.



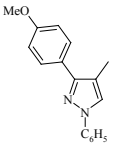
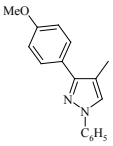
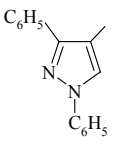
Comp.	R1	Ar	MCF-7	HT-29	HeLa	Docking score
4a	-H		23.79	77.54	148.82	-6.388
4b	-Br		32.08	89.13	-	-6.140
4c	-H		40.01	92.11	118.54	-5.953
4d	-Br		55.31	109.34	174.73	-5.623
4e	-Br		28.08	62.88	-	-6.369
4f	-Br		37.17	80.08	-	-5.877
4g	-Br		95.51	110.11	-	-5.953
Cisplatin			4.05	6.05	4.05	

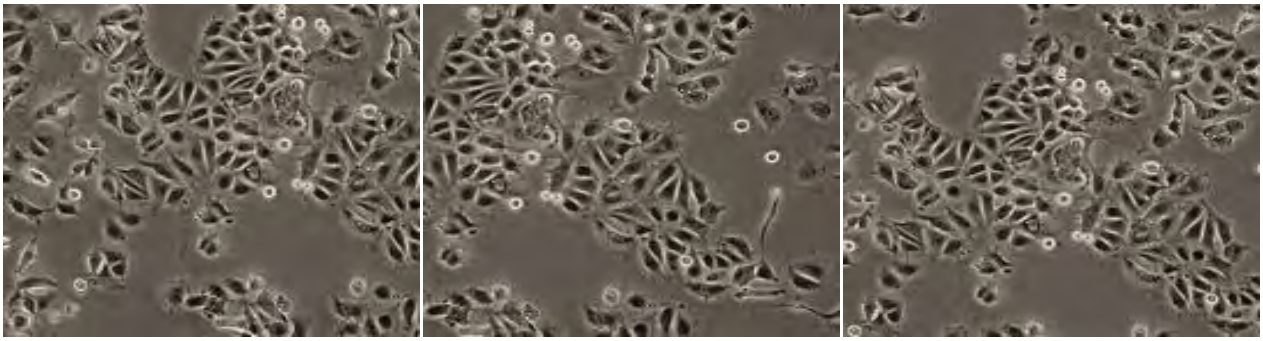
Table 4: Anticancer activity of synthesized flavonol derivatives (IC_{50} in µM).

Lone chloro derivative compound **3h** is the most potent against MCF-7 cell line with IC_{50} value of 18.67 µM (Figure 4) while **3b** is most potent against HT-29 cell line (IC_{50} 42.02 µM). Both **3a** and **4a** are more potent than their corresponding bromo derivatives **3b** and **4b**. However this claim is untrue for **3d** (bromo derivative) which is more potent than **3c** against MCF-7 cell line. Three 2-hydroxy chalcone derivatives **3e**, **3f** and **3h** are found to be highly potent against MCF-7 with IC_{50} less than 30 µM and 100 µM against HT-29cell line. Among the bromo derivatives, five membered ring at Ar showed varied activity, 2-thienyl moiety as in **3b** found to be moderately active and when it is replaced by pyrazole scaffold, nearly as in **3d**, the activity was reduced by half and improved as in the case of **3e**. Only one compound was synthesized with 2-furyl moiety (**3g**), which is found to be least active among all the compounds tested against MCF-7 cell line. None of the compounds were highly potent against HeLa cell line, all the compounds either were moderately active or poorly active and only compound **3d** showed cytotoxicity activity with IC_{50} 77.52 µM.

A similar profile of activity could also be noticed with flavonol derivatives **4a-g**. Unsubstituted compound **4a** with 2-thienyl ring was found to be highly potent against both MCF-7 and HT-29 with IC_{50} 23.79 and 77.54 µM respectively. Substitution at R1 by bromo leads to **4b**, which exhibited a little less potency. Replacement of 2-thienyl group by another five membered ring pyrazole improved the activity as in **4e**, but resulted in decrease in activity with 4-methoxy substitution on phenyl ring as in **4d** against MCF-7 cell line and the same activity profile could be seen against HT-29 cell line for **4e** as that of **4a**. 2-Furyl derivative **4g**, was least active against all the three cell lines and only 3-pyridyl derivative **4f** was nearly equal potent like **4b** (Figures 5 and 6).

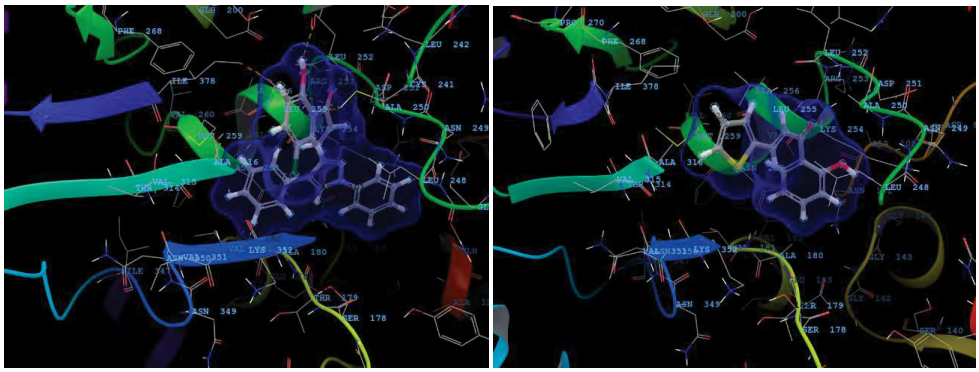
Molecular docking

The tubulin is involved in many cellular functions and its dynamic activity is controlled by many of compounds and proteins, including colchicine and stathmin family proteins, which provoked us to study the targeting of this protein by synthesized molecules. Ravelli et al. [21] reported that the structure, at 3.5 Å resolution, of tubulin in complex with colchicine and with the stathmin-like domain (SLD) of RB3 [22]. It shows the interaction of RB3-SLD with two tubulin heterodimers in a curved complex capped by the SLD amino-terminal domain, which



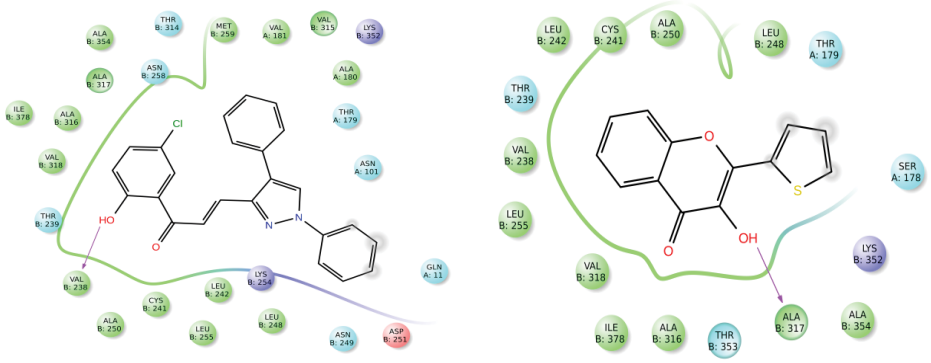
5 µM **10 µM** **25 µM**

Figure 4: Effect of compound **3h** on cell morphology during study incubation with **MCF-7** cells.



a b

Figure 5: The binding orientation of the Ligands to tubulin protein is shown. **a)** Surface view of the active compound **3h** with tubulin receptor for the highest docking score. **b)** Surface view of the active compound **4b** with tubulin receptor.



a b

Figure 6: 2D ligand interaction diagrams of compound 3h **(a)** and 4b **(b)** with tubulin.

prevents the incorporation of the complexed tubulin into microtubules. A comparison with the structure of tubulin in protofilaments shows changes in the subunits of tubulin as it switches from its straight conformation to a curved one. These changes correlate with the loss of lateral contacts and provide a rationale for the rapid microtubule depolymerization characteristic of dynamic instability. Hence targeting this receptor with the novel compounds for enhancing the desired activities is one of the possible options. The Molecular docking of synthetic compounds revealed the binding interactions with receptor (PDB: 1AS0). Among all, the synthesized compounds, **3h** [(E)-1-(5-chloro-2-hydroxyphenyl)-3-(4-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-3-yl)prop-2-en-1-one] and **4b**, [3-hydroxy-2-(thiophen-2-yl)-4H-chromen-2-ones] have shown high docking scores of -8.825 and -6.388 than other compounds (Figures 5 and 6; Tables 3 and 4).

Conclusion

The present study revealed the synthesis of chalcones from different aldehydes and corresponding hydrogen peroxide cyclized flavanols. All the purified compounds were screened for their anti-cancer activity against MCF-7, HT-29 and HeLa cell lines. Compounds **3h** and **3b** in chalcone series exhibited potent cytotoxic activity against MCF-7 and HT-29 cell lines with IC_{50} of 18.67 and 42.02 µM respectively. Among the flavonols, compounds **4a** and **4e** demonstrated potent cytotoxicity with IC_{50} activity of 23.79 and 62.88 µM. Further synthesis and cytotoxicity studies of more such derivatives and other supportive studies like appoptosis studies probably infer probable mechanism of cyctotoxic activities of chalcones and flavanols. The docking result of the most active compound in chalcone series **3h** is in accordance with its potency.

Conflict of Interest

Author RRK is thankful to AICTE for the award of fellowship under Quality Improvement Program.

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References

1. Marwa F, Ahmed I, Mahmoud Y (2013) Synthesis and biological evaluation of a novel series of 6, 8-Dibromo-4(3H)quinazolinone derivatives as anticancer agents. Arch Pharm Chem Life Sci 346: 610-617.
2. Alaa AM, Abdel A (2007) Novel and versatile methodology for synthesis of cyclic imides and evaluation of their cytotoxic, DNA binding, apoptotic inducing activities and molecular modeling study. Eur J Med Chem 42: 614- 626.
3. Yadav P, Lal K, Kumar A, Guru SK, Jaglan S, et al. (2017) Green synthesis and anticancer potential of chalcone linked-1,2,3-triazoles. Eur J Med Chem 126: 944-953.
4. Danielle DJ, Christopher AB, Saiyang Z, Lauren SK, Yan-Bing Z, et al. (2014)

Molecular targeted approaches to cancer therapy and prevention using chalcones. Curr Cancer Drug Targets 14: 181-200.

5. Rajendra S, Govindha B, Maruthan K, Jayachitra A, Padmini V (2016) Multiple biological activities and molecular docking studies of newly synthesized 3-(pyridin-4-yl)-1H-pyrazole-5-carboxamide chalcone hybrids. Bioorg Med Chem Lett 26: 5624-5630.
6. Hui Z, Jia JL, Jian S, Xian Y, Ting Z, et al. (2012) Design, synthesis and biological evaluation of novel chalcone derivatives as antitubulin agents. Bioorg Med Chem 20: 3212-3218.
7. Braulio I, Alexis T, Fabian O, Jairo Q, Rodrigo A, et al. (2010) Synthesis of novel pyrazolic analogues of chalcones and their 3-aryl-4-(3-aryl-4,5-dihydro-1H-pyrazol-5-yl)-1-phenyl-1H-pyrazole derivatives as potential antitumor agents. Bioorg Med Chem 18: 4965-4974.
8. Sankappa RU, Isloor AM, Fun HK, Shetty P (2015) Synthesis and in vitro biological evaluation of new pyrazole chalcones and hetero cyclic diamides as potential anticancer agents. Arab J Chem 8: 317-321.
9. Romagnoli R, Baraldi PG, Carrion MD, Cara CL, Olga CL, et al. (2008) Design, synthesis, and biological evaluation of thiopheneanalogues of chalcones. Bioorg Med Chem 61: 5367-5376.
10. Pingaew R, Saekee A, Mandi P, Nantasenamat C, Ruchirawat S, et al. (2014) Synthesis, biological evaluation and molecular docking of novel chalcone-coumarin hybrids as anticancer and antimalarial agents. Eur J Med Chem 85: 65-76.
11. Babasaheb PB, Shrikant S, Gawande B, Ragini G, Bodade C, et al. (2010) Synthesis and biological evaluation of simple methoxylated chalconesas anticancer, anti-inflammatory and antioxidant agents. Bioorg Med Chem 18: 1364-1370.
12. Saiharish R, Prasath M, Balasubramanian KK, Ganesh V, Krishna Kumari GN (2015) Synthesis and anticancer activity of chalcones derived from vanillin and isovanillin. Med Chem Res 24: 4157-4165.
13. Prakash O, Kumar R, Rakesh S (2009) Synthesis and antibacterial activity of some new 2,3-dimethoxy-3-hydroxy-2-(1-phenyl-3-aryl-4-pyrazolyl)chromones. Eur J Med Chem 44: 1763 - 1767.
14. Prakash O, Kumar R, Prakash V (2008) Synthesis and antifungal activity of some new 3-hydroxy-2-(1-phenyl-3-aryl-4-pyrazolyl)chromones. Eur J Med Chem 43: 435 - 440.
15. Montes-Avila J, Camacho SD, Josefina SF, Francisco DV, Rivero IA (2009) Solution-phase parallel synthesis of substituted chalcones and their antiparasitary activity against Giardia lamblia. Bioorg Med Chem 17: 6780-6785.
16. Ducki S, Rennison D, Woo M, Kendall A, Jeremie FC, et al. (2009) Combretastatin-like chalcones as inhibitors of microtubule polymerization. Part 1: Synthesis and biological evaluation of antivascular activity. Bioorg Med Chem 17: 7698-7710.
17. Bano S, Javed K, Ahmed S, Rathish IG, Singh S (2013) Synthesis of some novel chalcones, flavanones and flavones andevaluation of their anti-inflammatory activity. Eur J Med Chem 65: 51-59.
18. Dias TA, Duarte CL, Lima CF, Fernanda P, Cristina PW (2013) Superior anticancer activity of halogenated chalcones and flavonols over the natural flavonol quercetin. Eur J Med Chem 65: 500-510.
19. Dyrager C, Wickstrom M, Friden-Saxin M, Annika F, Kristian D, et al. (2011) Inhibitors and promoters of tubulin polymerization: Synthesise and biological evaluation of chalcones and related dienones as potential anticancer agents. Bioorg Med Chem 19: 2659-2665.
20. Venkanna A, Siva B, Poornima B, Rao PR, Vadaparthi K, et al. (2014) Phytochemical investigation of sesquiterpenes from the fruits of Schisandrachinensis and their cytotoxic activity, Fitoterapia 95: 102-108.
21. Ravelli RBG, Gigant B, Curmi PA, Jourdain I, Lachkar S, et al. (2004) Insight into tubulin regulation from a complex with colchicine and a stathmin-like domain. Nature 428: 198-202.
22. Friesner RA, Murphy RB, Repasky MP, Frye LL, Greenwood JR, et al. (2006) Extra Precision Glide: Docking and Scoring Incorporating a Model of Hydrophobic Enclosure for Protein-Ligand Complexes. J Med Chem 49: 6177-6196.