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# Synthesis, Docking Studies and Evaluation of Antioxidant Activity of Some Chromenone Derivatives.

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**ABSTRACT**

Many chromen-4-one and coumarin derivatives have been reported to possess antiproliferative activity. 1,5-benzodiazepine compounds also endowed with potential bioactivities. In view of this, the synthesis and antioxidant screening of chalcones of 3-formyl chromen-4-ones and 8-formyl-7-hydroxy coumarins, 7-hydroxy-3-(4-substituted phenyl-2,3-dihydro-1H-1,5-benzodiazepin-2-yl)-4H-chromen-4-ones and 7-hydroxy-4-methyl-8-(2- substituted phenyl-2,3-dihydro-1H-1,5-benzodiazepin-4-yl)-2H-chromen-2-ones was investigated. Antioxidant activity was carried out with DPPH assay. Docking studies of compounds was carried out using Autodock4.2 on epidermal growth factor receptor protein to investigate the potential of these compounds as cytotoxic agents. Some chalcones and 1,5-benzodiazepine derivatives bearing 7-hyroxy coumarin substitution were found to possess moderate antioxidant activity.

**Keywords:** Chromenes, coumarin, chalcone, antioxidant activity, DPPH assay, Autodock4.2

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## INTRODUCTION

Chromenones are important class of bioactive molecules consisting of benzene and pyran fused ring called benzopyrans. The flavones, isoflavones, flavanoids and coumarins have been extensively studied phyto constituents containing benzopyran ring. Chromen-4-ones and chromen-2-ones (commonly called as coumarins) possess diverse pharmacological activities including antitumor [1, 2], antivascular [3], antimicrobial [4, 5], antioxidant [6], TNF-α inhibitor [7], antifungal [8], anticoagulant [9], antispasmolytic [10, 11], estrogenic [12, 13], antiviral [14], anthelminthic [15], anti-HIV [16], antitubercular [17, 18], anti-inflammatory [19, 20], herbicidal [21], analgesic [22] and anticonvulsant [23,24] activity. Substituted 1,-5 benzodiazepines possess antimicrobial [25], antioxidant [26] and CNS modulating activities [27]. Protein tyrosine kinases (PTKs) are key mediators of the cellular signalling cascade which performs key roles in diverse biological processes like growth, differentiation, metabolism and apoptosis [28]. They are classified as receptor tyrosine kinases (RTKs) and non receptor tyrosine kinases (NRTKs). RTK's like epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGFR), fibroblast growth factor (FGF), platelet derived growth factor (PDGF) and nerve growth factor (NGF) have been identified as targets in various cancers. Genistein, a soya isoflavone, is well known protein tyrosine kinase (PTK) inhibitor which inhibits EGFR autophosphorylation at 2.6 µM concentration [29]. Coumarin derivatives like Daphnetin have been identified as EGFR-PTK inhibitors [30]. Computer docking technique helps in finding the important binding modes of ligand with its target protein. The analysis of important interactions like hydrogen bonds formed with important residues, hydrophobic interactions facilitate drug design process. In the present paper, we report the synthesis, characterization and docking studies of hybdrid molecules of 1,5-benzodiazepines with coumarin and chromone-4-one. In the present study, prompted by the potential of substituted coumarins, chromen-4-ones and 1,5-benzodiazepines, the synthesis and antioxidant screening of 7-hydroxy-3-(4-substituted phenyl-2,3-dihydro-1H-1,5-benzodiazepin-2-yl)-4H-chromen-4-ones and 7-hydroxy-4-methyl-8-(2- substituted phenyl-2,3-dihydro-1H-1,5-benzodiazepin-4-yl)-2H-chromen-2-ones was investigated.

## MATERIALS AND METHODS

### Docking studies

In the present study, the X-ray crystal structure of the epidermal growth factor receptor (EGFR) protein in complex with 4-anilinoquinazoline inhibitor erlotinib was obtained from the RCSB Protein Data Bank (PDB ID: 1M17). Resolution of protein structure with 333 amino acid residues was 2.60 $A^0$. The protein was processed by removing water, erlotinib and energy minimization in UCSF Chimera with Amber ff12SB force field. Combination of 10,000 steepest descent and conjugate gradient steps with 0.02 $A^0$ step size were used during energy minimization. The energy minimized protein structure was used for docking procedure. 2D structures of all the synthesized compounds were drawn and converted to 3D structures using Marvin Sketch (a structure drawing program). Geometry optimization was carried out in ArgusLab 4.0.1 (from Thomson and Planaria Software LLC) on semi empirical quantum mechanical basis with parameterized model number 3 (PM3) hamiltonian, until restricted closed shell hartree-fock self consistent field formalism converses to $10^{-10}$ kcal/mol and steepest descent geometry search criteria until gradient converses to $10^{-6}$ kcal/mol. Gasteiger partial atomic charges of optimized molecules were computed in UCSF chimera and were updated in 3D structures. Docking simulation was carried out in Autodock4.2 using MGLtools [31].

### Chemistry

The reagents used for synthesis were of laboratory grade and solvents were of analytical grade obtained from Thomas Baker and Loba Chemie respectively. The melting point of the compound was determined by open capillary method, expressed in °C. The reactions were monitored by preparative TLC's from Merck with the solvent system chloroform: methanol in the ratio of 9:1. Microwave assisted synthesis was carried out using Catalyst Microwave oven, Pune, at 140-700 watt. Infra Red spectra were recorded on Shimadzu FT-IRAffinity-1 spectrophotometer by KBr pellet technique and are expressed in $cm^{-1}$. $^1$H-NMR spectra were recorded on Bruker Avance 300 MHz FT-NMR spectrophotometer using $CDCl_3$ as solvent and TMS as internal standard. The chemical shifts are expressed in δ ppm and splitting patterns are designated as s: singlet; d: doublet; q: quartet; m: multiplet. Free radical scavenging assay by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was carried out on Jasco 630 UV-Spectrophotometer. **Scheme 1 and 2** depicts the strategy adopted to synthesize the compounds. The physical data of synthesised compounds are given in **Table 1**.

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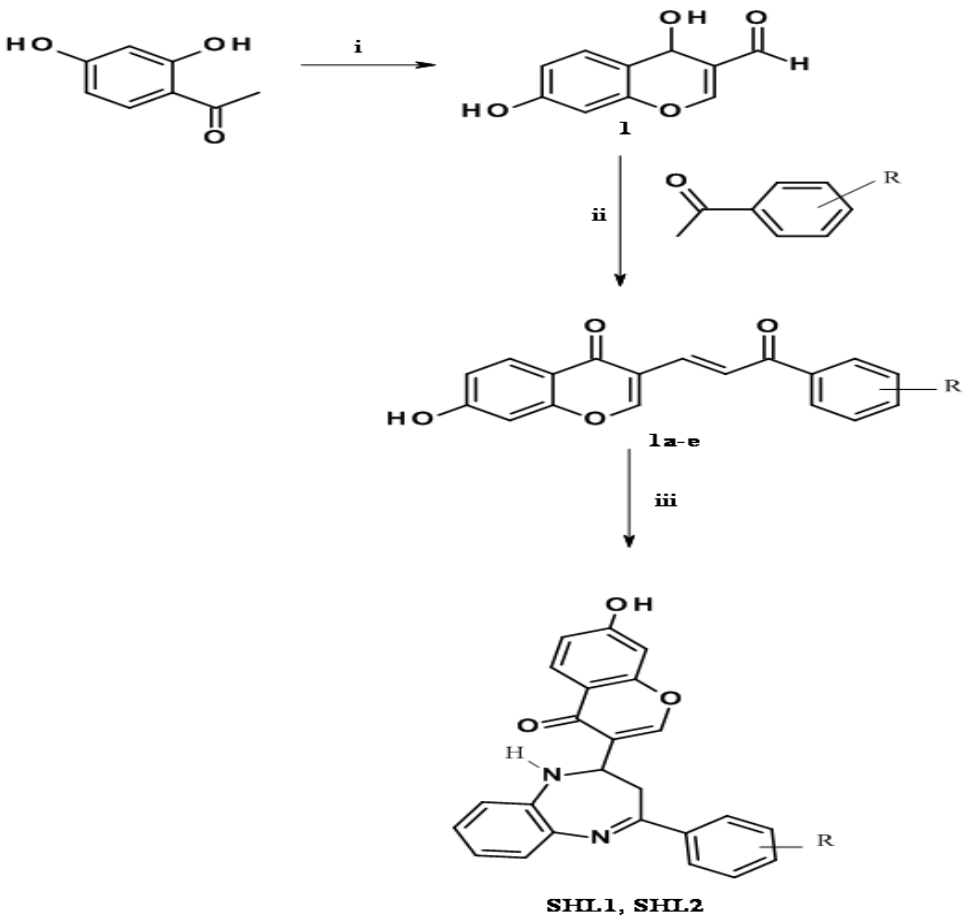
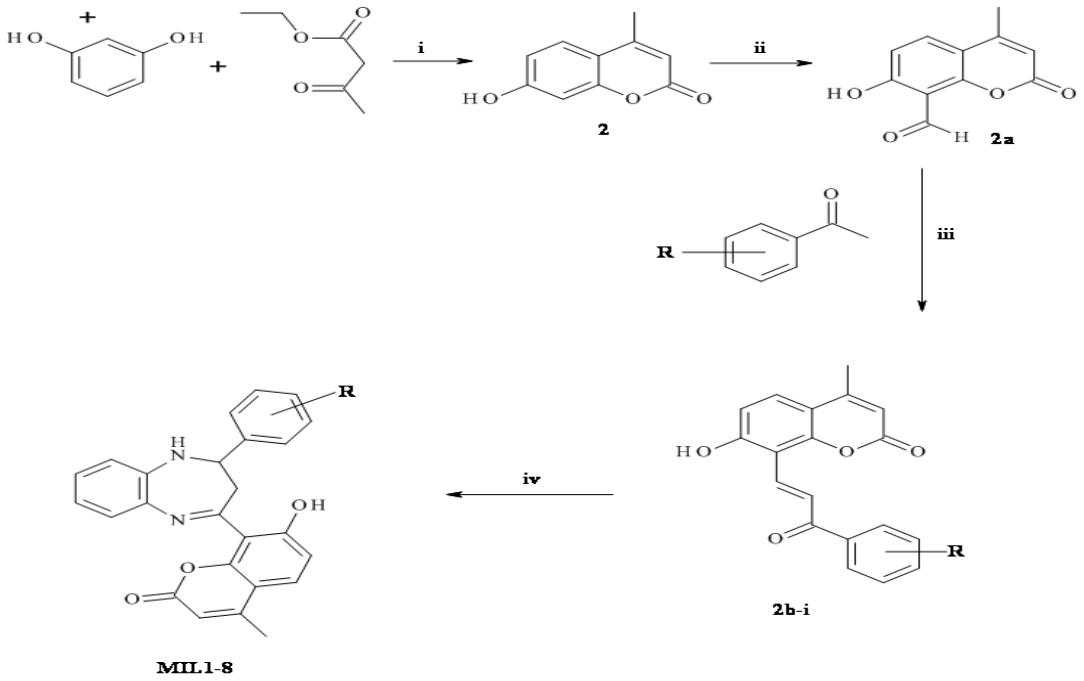



**Scheme 1: Synthesis of 7-hydroxy-3-(4-substituted phenyl-2,3-dihydro-1H-1,5-benzodiazepin-2-yl)-4H-chromen-4-one; (i) DMF, $POCl_3$, (ii) Piperidine, (iii) Microwave irradiation at 160 W for 15 min, Glacial acetic acid**




**Scheme 2: Synthesis of 7-hydroxy-4-methyl-8-(2-substituted phenyl-2,3-dihydro-1H-1,5-benzodiazepin-4-yl)-2H-chromen-2-one; (i) Conc. $H_2SO_4$, (ii) Hexamethylene tetramine, 100$^0$C, (iii) Piperidine, (iv) Microwave irradiation at 160 W for 15 min, Glacial acetic acid.**

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**Table 1: Physical data of synthesised compounds**

| Comp. code | R | Nature | Molecular formula | Mol. Wt. | Yield | Melting point ($^oC$) | Rf* |
|---|---|---|---|---|---|---|---|
| 1a | H | Yellow solid | $C_{18}H_{12}O_4$ | 292 | 60% | 289-291 | 0.59 |
| 1b | 2-OH, 4-OH | Brown solid | $C_{18}H_{12}O_6$ | 324 | 50% | 266-267 | 0.56 |
| 1c | 4-F | Brown solid | $C_{18}H_{11}O_4F$ | 371 | 20% | 295-297 | 0.53 |
| 1d | 4-Cl | Brown solid | $C_{18}H_{11}O_4Cl$ | 323 | 50% | 281-283 | 0.61 |
| 1e | 4-OH | Yellow solid | $C_{18}H_{12}O_4$ | 308 | 60% | 261-263 | 0.57 |
| SHL1 | 4-Cl | Yellow solid | $C_{24}H_{17}ClN^{O_3}$ | 416.85 | 60% | 315-316 | 0.59 |
| SHL2 | 4-F | Brown solid | $C_{24}H_{17}FN^{O_3}$ | 400.40 | 50% | 325-327 | 0.56 |
| 2b | H | Brown solid | $C_{19}H_{14}O_4$ | 306.31 | 50% | 178-180 | 0.62 |
| 2c | 4-OH | Yellow solid | $C_{19}H_{14}O_5$ | 316.31 | 60% | 172-174 | 0.59 |
| 2d | 2-OH, 4-OH | Brown solid | $C_{19}H_{14}O_6$ | 332.31 | 50% | 176-178 | 0.56 |
| 2e | 4-F | Brown solid | $C_{19}H_{13}O_4F$ | 323.32 | 20% | 180-182 | 0.53 |
| 2f | 4-Cl | Brown solid | $C_{19}H_{13}O_4Cl$ | 306.31 | 50% | 173-175 | 0.61 |
| 2g | 2-OH | Yellow solid | $C_{19}H_{14}O_5$ | 316.31 | 60% | 173-175 | 0.57 |
| 2h | 4-$NH_2$ | Orange solid | $C_{19}H_{16}O_4N$ | 332.31 | 70% | 179-181 | 0.56 |
| 2i | 3-$NO_2$ | Yellow solid | $C_{19}H_{13}O_6N$ | 323.32 | 60% | 180-182 | 0.62 |
| MIL1 | H | Brown solid | $C_{25}H_{20}N_2O_3$ | 396.43 | 30% | 178-180 | 0.62 |
| MIL2 | 4-OH | Yellow solid | $C_{25}H_{20}N_2O_4$ | 412.43 | 35% | 172-175 | 0.59 |
| MIL3 | 2-OH, 4-OH | Brown solid | $C_{25}H_{20}N_2O_5$ | 428.43 | 30% | 176-178 | 0.56 |
| MIL4 | 4-F | Brown solid | $C_{25}H_{19}N2O3F$ | 414.42 | 45% | 180-182 | 0.53 |
| MIL5 | 4-Cl | Brown solid | $C_{25}H_{19}N_2O_3Cl$ | 430.88 | 50% | 178-180 | 0.61 |
| MIL6 | 2-OH | Yellow solid | $C_{25}H_{20}N_2O_4$ | 412.43 | 50% | 172-174 | 0.57 |
| MIL7 | 4-$NH_2$ | Orange solid | $C_{25}H_{21}N_3O_3$ | 411.45 | 70% | 176-180 | 0.56 |
| MIL8 | 3-$NO_2$ | Yellow solid | $C_{25}H_{19}N_3O_5$ | 441.43 | 60% | 180-182 | 0.62 |

* Mobile phase: Chloroform: Methanol (9:1)

**Synthesis of 7-hydroxy-3-formyl chromen-4-one (1)** [32]

In dry dimethyl formamide (DMF) (60 ml) in three neck flask, $POCl_3$ (37.5 ml) was added slowly with vigorous stirring at 50 $^0$C. Heating and stirring was continued for 2 hrs at 45-55 $^0$C. The solution of resacetophenone (9.12 gm) in DMF (12.5 ml) was then slowly added with stirring at 50 $^0$C and stirring was continued for 2 hrs. The mixture was kept overnight at room temperature and diluted slowly by adding ice cold water (250 ml) and was stirred for 6hrs. The red crystalline product separated was filtered and recrystallised from ethanol. Yield 85%.

**General procedure for the synthesis of 3-[3-oxo-3-substituted phenylprop-1-en-1-yl]-4H-chromen-4-ones (chalcones) (1a-e)**

A mixture of **1** (0.01 mol), substituted acetophenones (0.012 mol), ethanol and 2-4 drops of piperidine was refluxed for 2hrs. Upon completion reaction, mixture cooled to 0 $^0$C. Solid obtained was filtered, washed with distilled water, dried and crystrallized from ethanol. Yield 20 – 60%.

**General procedure for the synthesis of 7-hydroxy-3-(4-substituted phenyl-2,3-dihydro-1H-1,5-benzodiazepin-2-yl)-4H-chromen-4-one (SHL1, SHL2)**

Chalcones (1c/1d) (0.00342 mol) was dissolved in DMF 15 ml. *o*-Phenylenediamine (0.00342 mol) and catalytic amount of glacial acetic acid was added and the mixture was irradiated in catalyst microwave oven for 15 minutes at 160 W. Upon cooling the reaction mixture, crushed ice was added to obtain solid compounds which were subsequently filtered, washed with water and recrystallized from ethanol. Yield 50 - 60%.

**Synthesis of 7-Hydroxy-4-Methyl Coumarin (2)** [33]

A mixture of resorcinol (0.1 mol) and ethylacetoacetate (0.1 mol) in 50 ml sulphuric acid solution (85%) was heated on a water bath for 3 hr. The resulting reddish brown coloured solution was decomposed by adding 500 g of crushed ice. The separated bright yellow coloured solid was washed with excess of cold water, dried and crystallized from methanol to get the pure product. Yield: 80%; m.p. 180-182 ºC.

**Synthesis of 8-formyl-7-hydroxy-4-methyl coumarin (2a)** [34]

A mixture of **(2)** (5 gm) and hexamethylene tetramine (10 gm) in glacial acetic acid (40 ml) was heated on a water bath for 6 hr. The hexamine adduct formed was hydrolyzed with 20% HCl (75 ml) and the mixture was heated for another 30 min. After cooling, the mixture was extracted with diethyl ether. The ether layer was evaporated to afford pale yellow coloured solution which was poured on crushed ice to obtain pale yellow solid. The crude 8-formyl-7-hydroxy-4-methyl coumarin was recrystallized from ethanol and dioxan mixture. Yield: 22%; m.p. 176-178 ºC.

**General procedure for the synthesis of 7-hydroxy-4-methyl-8-[3-oxo-3-substituted phenylprop-1-en-1-yl]-2H-chromen-2-ones (chalcones) (2b-i)**

Compound **(2a)** (0.1 mol) was dissolved in 10 ml absolute ethanol and substituted acetophenones (0.1 mol) were added. The mixture was initially irradiated in microwave for 2 minutes at 120 W. Few drops of NaOH (60%) were added and the mixture was further irradiated in microwave for 5 minutes at 120 W. Upon cooling the mixture, crushed ice was added and dilute HCl was added to neutralize the mixture. The solids obtained were washed with water, filtered and recrystalized with ethanol. Yield 20-70%.

**General procedure for the synthesis of 7-hydroxy-4-methyl-8-(2-substituted phenyl-2,3-dihydro-1H-1,5-benzodiazepin-4-yl)-2H-chromen-2-one (MIL1-8)**

Chalcones **(2b-i)** (0.00342 mol) was dissolved in DMF 15 ml. *o*-Phenylenediamine (0.00342 mol) and catalytic amount of glacial acetic acid was added and the mixture was irradiated in catalyst microwave oven for 15 minutes at 160 W. Upon cooling the reaction mixture, crushed ice was added to obtain solid compounds which were subsequently filtered, washed with water and recrystallized from ethanol. Yield 30-70%.

**Biological evaluation**

**Radical scavenging assays by the DPPH**

The DPPH assay protocol was adapted from [35]. Briefly the assay included preparation of fresh DPPH stock solution at a concentration of 0.004% on each day of analysis which was stored in dark place until used in analysis. As a positive control, L-ascorbic acid (50 mg/50 ml) was prepared in distilled water and serial dilutions [10, 20, 30, 40, 50 µg/ml] were made from this stock solution. Test solutions (50 mg / 50 ml) of synthesised compounds were prepared in ethanol and dilutions were made so as to match final concentration (10, 20, 30, 40, 50 µg/ml). Test solutions were allowed to react with DPPH solution at room temperature in dark place after 30 min and the absorbance values were measured at 517 nm against blank. The radical scavenging activity (% inhibition) was expressed as % of DPPH radical elimination calculated according to following equation; % inhibition = (absorbance of control – absorbance of test/absorbance of control) × 100; where absorbance of control is absorbance of the blank without sample.

## RESULTS AND DISCUSSION

**Docking studies**

In docking studies, Erlotinib, a quinazoline EGFR inhibitor, shows interaction with Met769. In docking studies of **1a-e, SHL1-2, 2b-i & MIL1-8**, all the compounds except **MIL1, 3, 4, 5, 6 SHL1 and SHL2** show interaction with Met769. **MIL1, 3, 4, 5, 6, SHL1 and SHL2** show interactions with side by residue Lys721. The interaction of some selected compounds under study with important residue is shown in **figure 1**. The docking scores in terms of binding free energy in Kcal/mol and inhibitory concentration calculated in Autodock4.2 are

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presented in **Table 2**. The docking studies showed that the compounds under investigation can be promising protein tyrosine kinase inhibitors.

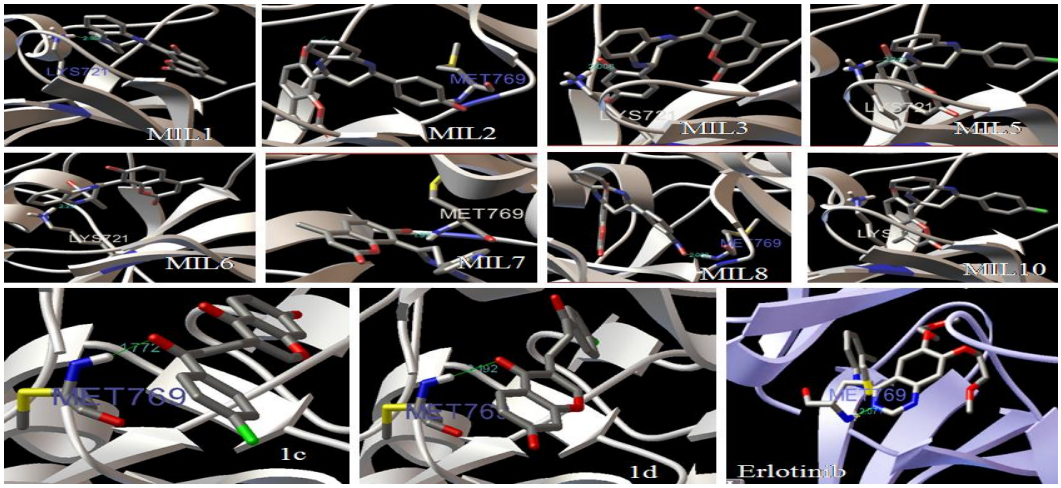


**Figure 1: Hydrogen bond interaction between Met769 and heteroatom of compounds.**

**Table 2: The docking scores in terms of binding free energy in Kcal/mol and inhibitory concentration (Ki in µM concentration)**

| Sr. No. | Comp. Code | Autodock Docking scores (Kcal/mol) | Inhibition constants (µM) |
|---|---|---|---|
| 1 | 1a | -9.97 | 40.11 |
| 2 | 1b | -10.07 | 41.47 |
| 3 | 1c | -8.36 | 750.27 |
| 4 | 1d | -8.5 | 591.93 |
| 5 | 1e | -8.07 | 1.22 |
| 6 | SHL1 | -10.57 | 17.98 |
| 7 | SHL2 | -10.09 | 40.39 |
| 8 | 2b | -8.91 | 296.03 |
| 9 | 2c | -9.01 | 250.13 |
| 10 | 2d | -9.57 | 96.73 |
| 11 | 2e | -8.91 | 296.86 |
| 12 | 2f | -9.20 | 179.35 |
| 13 | 2g | -9.02 | 245.97 |
| 14 | 2h | -8.9 | 297.14 |
| 15 | 2i | -9.46 | 117.01 |
| 16 | MIL1 | -9.74 | 74.37 |
| 17 | MIL2 | -10.35 | 35.12 |
| 18 | MIL3 | -9.9 | 55.43 |
| 19 | MIL4 | -10.25 | 30.91 |
| 20 | MIL5 | -10.76 | 13.03 |
| 21 | MIL6 | -10.4 | 23.72 |
| 22 | MIL7 | -8.83 | 36.59 |
| 23 | MIL8 | -11.58 | 3.25 |

**Chemistry**

The starting materials **1** and **2** were synthesised by conventional Vilsmeier Haack reaction and Pechmann condensation reaction respectively. The chalcones of **1** were prepared by piperidine catalysed aldol condensation reaction. The formyl derivative **2a** was prepared by hexamethylene tetramine catalysed Duff reaction on **2**. Chalcones of **2a** were prepared by piperidine catalysed reaction. In both reaction schemes, target 1,5-benzodiazepine derivatives were prepared by condensation of respective chalcones with *o*-phenylenediamine in presence of catalytic amount of glacial acetic acid. Under conventional condition reaction was found too sluggish. This diverted us to adopt microwave assisted method employing irradiation of mixture

in Catalyst microwave for 15 minutes at 160 W. All the synthesised compounds were characterised by FT-IR and $^1$H NMR.

**7-hydroxy-3-[3-(4-hydroxyphenyl)-3-oxoprop-1-en-1-yl]-4H-chromen-4-one (1a)**

IR (KBr) ν ($cm^{-1}$): 1705 (C=O), 3100 (Ar C-H), 3400 (O-H); $^1$H NMR ($CDCl_3$): (ppm) 2.32 (2H, d, -CH-CH-), 6.21 (1H, s, pyran-H), 6.54 - 7.74 (7H, m, Ar- H), 9.30 (2H, d, Ar–OH)

**3-[3-(2,4-dihydroxyphenyl)-3-oxoprop-1-en-1-yl]-7-hydroxy-4H-chromen-4-one (1b)**

IR (KBr) ν ($cm^{-1}$): 3350 (O-H), 1715 (C=O), 1650 (C=C), 3100 (Ar-C-H); $^1$H NMR ($CDCl_3$): (ppm) 2.5 (2H, dd, -CH=CH-), 6.25 (1H, s, pyran-H), 7.79 – 8.52 (6H, m, Ar-H), 10.45 (3H, s, Ar-OH).

**7-hydroxy-3-[(1E)-3-oxo-3-phenylprop-1-en-1-yl]-4H-chromen-4-one (1c)**

IR (KBr) ν ($cm^{-1}$): 3400 (O-H), 1705 (C=O), 1650 (C=C), 3100 (Ar-C-H); $^1$H NMR ($CDCl_3$): (ppm) 2.32 (2H, dd, -CH=CH-), 6.05 (1H, s, pyran-H), 7.15 – 8.47 (8H, m, Ar-H), 11.3 (1H, s, Ar-OH).

**3-[3-(4-chlorophenyl)-3-oxoprop-1-en-1-yl]-7-hydroxy-4H-chromen-4-one (1d)**

IR (KBr) ν ($cm^{-1}$): 3350 (O-H), 1715 (C=O), 1650 (C=C), 3100 (Ar-C-H); $^1$H NMR ($CDCl_3$): (ppm) 3.33 (2H, dd, -CH=CH-), 6.25 (1H, s, pyran-H), 7.18 – 8.07 (7H, m, Ar-H), 9.5 (1H, s, Ar-OH).

**3-[3-(4-fluorophenyl)-3-oxoprop-1-en-1-yl]-7-hydroxy-4H-chromen-4-one (1e)**

IR (KBr) ν ($cm^{-1}$): 3400 (O-H), 1720 (C=O), 1650 (C=C), 3100 (Ar-C-H); $^1$H NMR ($CDCl_3$): (ppm) 2.31 (2H, dd, -CH=CH-), 6.03 (1H, s, pyran-H), 6.83 – 7.65 (7H, m, Ar-H), 9.29(1H, s, Ar-OH).

**3-[4-(4-chlorophenyl)-2,3-dihydro-1H-1,5-benzodiazepin-2-yl]-7-hydroxy-4H-chromen-4-one (SHL1)**

IR (KBr) ν ($cm^{-1}$): 3350 (N-H stretch), 1745 (C=O), 1620 (N-H bend), 1500 (Ar-C=C), 3350(O-H); $^1$H NMR ($CDCl_3$): (ppm) 1.8 (2H, s, -$CH_2$-), 3.3 (1H, s, -NH), 6.3 (1H, s, pyran-H), 7.07 – 7.88 (11H, m, Ar-H), 12.66 (1H, s, Ar-OH).

**3-[4-(4-fluorophenyl)-2,3-dihydro-1H-1,5-benzodiazepin-2-yl]-7-hydroxy-4H-chromen-4-one (SHL2)**

IR (KBr) ν ($cm^{-1}$): 3430 (N-H stretch), 1730 (C=O), 1600 (N-H bend), 1400 (Ar-C=C), 3400 (O-H); $^1$H NMR ($CDCl_3$): (ppm) 1.8 (2H, s, -$CH_2$-), 3.5 (1H, s, -NH), 5.0 (1H, s, pyran-H), 7.4 – 7.9 (11H, m, Ar-H), 8.2 (1H, s, Ar-OH).

**7-hydroxy-4-methyl-8-[3-oxo-3-phenylprop-1-en-1-yl]-2H-chromen-2-one (2b)**

IR (KBr) ν ($cm^{-1}$): 3350 (O-H), 1720 (C=O, Lactone), 1650 (C=O), 1510 (-C=C), 1450 (Ar-C=C), 2950 (-C-H); $^1$H NMR ($CDCl_3$): (ppm) 2.4 (2H, s, -CH=CH-), 2.5 (1H, s, pyran-H), 2.9 (3H, s, -$CH_3$), 6.4 – 8.8 (7H, m, Ar-H), 12.0 (1H, s, Ar-OH)

**7-hydroxy-8-[3-(4-hydroxyphenyl)-3-oxoprop-1-en-1-yl]-4-methyl-2H-chromen-2-one (2c)**

IR (KBr) ν ($cm^{-1}$): 3300 (O-H), 1720 (C=O, Lactone), 1650 (C=O), 1570 (C=C), 1386 (Ar-C=C), 2854 (C-H); $^1$H NMR ($CDCl_3$): (ppm) 1.2 (2H, s, -CH=CH-), 1.8 (1H, s, pyran-H), 2.4 (3H, s, -$CH_3$), 5.6 (2H, s, -$NH_2$), 6.2-8.0 (6H, m, Ar-H), 10.2 (2H, s, Ar-OH).

**8-[3-(2, 4-dihydroxyphenyl)-3-oxoprop-1-en-1-yl]-7-hydroxy-4-methyl-2H-chromen-2-one (2d)**

IR (KBr) ν ($cm^{-1}$): 3167 (O-H), 1750 (C=O, lactone type in coumarin), 1650 (C=O), 1590 (-C=C), 1479 (Ar-C=C), 2920 (C-H); $^1$H NMR ($CDCl_3$): (ppm) 1.3 (2H, s, -CH=CH-), 2.0 (1H, s, pyran-H), 2.25 (3H, s, -$CH_3$), 6.6 – 7.7 (5H, m, Ar-H), 12.6 (3H, s, Ar-OH)

**8-[3-(4-fluorophenyl)-3-oxoprop-1-en-1-yl]-7-hydroxy-4-methyl-2H-chromen-2-one (2e)**

IR (KBr) ν ($cm^{-1}$): 1745 (C=O, Lactone), 1654 (C=O), 1587 (C=C), 1481 (Ar-C=C), 2924 (C-H); $^1$H NMR ($CDCl_3$): (ppm) 2.4 (2H, s, -CH=CH-), 2.5 (1H, s, pyran-H), 2.9 (3H, s, $-CH_3$), 6.4 – 8.8 (7H, m, Ar-H), 9.0 (1H, s, Ar-OH).

**8-[3-(4-chlorophenyl)-3-oxoprop-1-en-1-yl]-7-hydroxy-4-methyl-2H-chromen-2-one (2f)**

IR (KBr) ν ($cm^{-1}$): 3230 (O-H), 1720 (C=O, Lactone), 1650 (C=O), 1600 (C=C), 1567 (Ar-C=C), 2935 (C-H); $^1$H NMR ($CDCl_3$): (ppm) 1.2 (2H, s, -CH=CH-), 1.8 (1H, s, pyran-H), 2.4 (3H, s, $-CH_3$), 5.6 (2H, s, $-NH_2$), 6.2-8.0 (6H, m, Ar-H), 10.2 (2H, s, Ar-OH)

**7-hydroxy-8-[3-(2-hydroxyphenyl)-3-oxoprop-1-en-1-yl]-4-methyl-2H-chromen-2-one (2g)**

IR (KBr) ν ($cm^{-1}$): 3300 (O-H), 1720 (C=O, Lactone), 1650 (C=O), 1570 (C=C), 1386 (Ar-C=C), 2854 (C-H); $^1$H NMR ($CDCl_3$): (ppm) 1.2 (2H, s, -CH=CH-), 1.8 (1H, s, pyran-H), 2.4 (3H, s, $-CH_3$), 5.6 (2H, s, $-NH_2$), 6.2-8.0 (6H, m, Ar-H), 10.2 (2H, s, Ar-OH)

**8-[3-(4-aminophenyl)-3-oxoprop-1-en-1-yl]-7-hydroxy-4-methyl-2H-chromen-2-one (2h)**

IR (KBr) ν ($cm^{-1}$): 1745 (C=O, Lactone), 1654 (C=O), 1587 (C=C), 1481 ( Ar-C=C), 2924 (C-H); $^1$H NMR ($CDCl_3$): (ppm) 1.5 (2H, s, -CH=CH-), 2.5 (1H, s, pyran-H), 3.5 (3H, s, $-CH_3$), 5.6 (2H, s, $-NH_2$), 6.0-8.0 (6H, m, Ar-H), 9.3 (1H, s, Ar-OH)

**7-hydroxy-4-methyl-8-[3-(3-nitrophenyl)-3-oxoprop-1-en-1-yl]-2H-chromen-2-one (2i)**

IR (KBr) ν ($cm^{-1}$): 3230 (O-H), 1720 (C=O, Lactone), 1650 (C=O), 1600 (C=C), 1567 (Ar-C=C), 2935 (C-H); $^1$H NMR ($CDCl_3$): (ppm) 2.4 (2H, s, -CH=CH-), 2.5 (1H, s, pyran-H), 2.9 (3H, s, $-CH_3$), 6.4 – 8.8 (7H, m, Ar-H), 9.0 (1H, s, Ar-OH)

**7-hydroxy-4-methyl-8-(2-phenyl-2,3-dihydro-1H-1,5-benzodiazepin-4-yl)-2H-chromen-2-one (MIL1)**

IR (KBr) ν ($cm^{-1}$): 3400 (N-H stretch), 1730 (C=O), 1610 (N-H bend), 1300 (-C-N), 1400 (C=C), 1630 (-C=N); $^1$H NMR ($CDCl_3$): (ppm) 2.0 (2H, s, $-CH_2$), 2.8 (3H, s, $-CH_3$), 4.1 (1H, s, -OH), 6.0-8.0 (11H, m, Ar-H).

**7-hydroxy-8-[2-(4-hydroxyphenyl)-2,3-dihydro-1H-1,5-benzodiazepin-4-yl]-4-methyl-2H-chromen-2-one (MIL2)**

IR (KBr) ν ($cm^{-1}$): 3500 (N-H stretch), 1730 (C=O), 1610 (N-H bend), 1350 (-C-N), 1400 (C=C), 1630 (-C=N); $^1$H NMR ($CDCl_3$): (ppm) 1.19 (2H, s, $-CH_2$), 2.32 (3H, s, $-CH_3$), 2.8 (2H, s, $-CH_2$), 6.0-8.0 (10H, m, Ar-H), 9.1 (1H, s, -NH), 11.3 (2H, s, -OH).

**8-[2-(2,4-dihydroxyphenyl)-2,3-dihydro-1H-1,5-benzodiazepin-4-yl]-7-hydroxy-4-methyl-2H-chromen-2-one (MIL3)**

IR (KBr) ν ($cm^{-1}$): 3430 (N-H stretch), 1710 (C=O), 1600 (N-H bend), 1350 (-C-N), 1400 (C=C), 1620 (-C=N); $^1$H NMR ($CDCl_3$): (ppm) 2.4 (3H, s, $-CH_3$), 3.0 (1H, s, -N-H), 6.2-7.8 (9H, m, Ar-H), 11.2 (3H, s, Ar-OH).

**8-[2-(4-fluorophenyl)-2,3-dihydro-1H-1,5-benzodiazepin-4-yl]-7-hydroxy-4-methyl-2H-chromen-2-one (MIL4)**

IR (KBr) ν ($cm^{-1}$): 3430 (N-H stretch), 1730 (C=O), 1600 (N-H bend), 1360 (-C-N), 1400 (C=C), 1620 (C=N); $^1$H NMR ($CDCl_3$): (ppm) 1.5 (2H, s, $-CH_2$), 2.5 (3H, t, $-CH_3$), 3.5 (1H, s, pyran-H), 6.4-8.9 (11H, m, Ar-H), 11.8 (1H, s, -OH).

**8-[2-(4-chlorophenyl)-2,3-dihydro-1H-1,5-benzodiazepin-4-yl]-7-hydroxy-4-methyl-2H-chromen-2-one (MIL5)**

IR (KBr) ν ($cm^{-1}$): 3350 (N-H stretch), 1740 (C=O), 1610 (N-H bend), 1350 (-C-N), 1500 (C=C); $^1$H NMR ($CDCl_3$): (ppm) 1.5 (2H, s, $-CH_2$), 2.5 (3H, t, $-CH_3$), 3.5 (1H, s, pyran-H), 6.4-8.9 (11H, m, Ar-H), 11.8 (1H, s, -OH).

**7-hydroxy-8-[2-(2-hydroxyphenyl)-2,3-dihydro-1H-1,5-benzodiazepin-4-yl]-4-methyl-2H-chromen-2-one (MIL6)**

IR (KBr) ν ($cm^{-1}$): 3500 (N-H stretch), 1730 (C=O), 1610 (N-H bend), 1350 (-C-N), 1400 (C=C), 1630 (-C=N); $^1H$ NMR ($CDCl_3$): (ppm) 1.19 (2H, s, $-CH_2$), 2.32 (3H, s, $-CH_3$), 2.8 (2H, s, $-CH_2$), 6.0-8.0 (10H, m, Ar-H), 9.1 (1H, s, -NH), 11.3 (2H, s, -OH).

**8-[2-(4-aminophenyl)-2,3-dihydro-1H-1,5-benzodiazepin-4-yl]-7-hydroxy-4-methyl-2H-chromen-2-one (MIL7)**

IR (KBr) ν ($cm^{-1}$): 3430 (N-H stretch), 1730 (C=O), 1600 (N-H bend), 1360 (-C-N), 1400 (C=C), 1620 (C=N); $^1H$ NMR ($CDCl_3$): (ppm) 1.5 (2H, s, $-CH_2$), 2.34 (3H, s, $-CH_3$), 6.0-7.4 (10H, m, Ar-H), 11.25 (1H, s, -OH).

**7-hydroxy-4-methyl-8-[2-(3-nitrophenyl)-2,3-dihydro-1H-1,5-benzodiazepin-4-yl]-2H-chromen-2-one (MIL8)**

IR (KBr) ν ($cm^{-1}$): 3350 (N-H stretch), 1740 (C=O), 1610 (N-H bend), 1350 (-C-N), 1500 (-C=C); $^1H$ NMR ($CDCl_3$): (ppm) 1.5 (2H, s, $-CH_2$), 2.5 (3H, t, $-CH_3$), 3.5 (1H, s, pyran-H), 6.4-8.9 (11H, m, Ar-H), 11.8 (1H, s, -OH).

**Antioxidant activity**

DPPH free radical scavenging assay revealed that most of the chalcones were moderately active as compared to the positive control L-ascorbic acid. The results are expressed in terms of % inhibition which is reduction of capability of DPPH radicals by the decrease in its absorbance induced by antioxidants. IC50 values were computed by taking the logarithm of the concentration scale used and extrapolating the concentration required to cause 50% inhibition. The results of antioxidant activity are presented in **Figure 2** and **Table 3**. Compounds **1e, 2c, 2f, 2g, MIL3, MIL4, MIL7 & MIL8** were found to possess IC50 below 100 µM. In case of L-ascorbic acid, **MIL2** and **MIL6** the concentrations chosen were found insufficient and instead IC75 value which is concentration required to produce 75 % inhibition was calculated. Compounds **MIL2** and **MIL6** may possess good antioxidant property.

**Table 3: IC50 values of compounds and L-ascorbic acid**

| Compound code | IC50 in µM |
|---|---|
| 1a | 16,270.00 |
| 1b | 206.0. |
| 1c | 82.47 |
| 1d | 119.12 |
| 1e | 31.00 |
| SHL1 | 1173.81 |
| SHL2 | 895.36 |
| 2b | 137.43 |
| 2c | 29.53 |
| 2d | 895.36 |
| 2e | 206.01 |
| 2f | 72.06 |
| 2g | 31.00 |
| 2h | 119.12 |
| 2i | 810.40 |
| MIL1 | 297.30 |
| MIL2 | 8.11* |
| MIL3 | 22.13 |
| MIL4 | 43.95 |
| MIL5 | 109.47 |
| MIL6 | 7.75* |
| MIL7 | 82.47 |
| MIL8 | 15.86 |
| L-Ascorbic acid | 10.52* |

* IC75 calculated which is concentration required to produce 75% inhibition.

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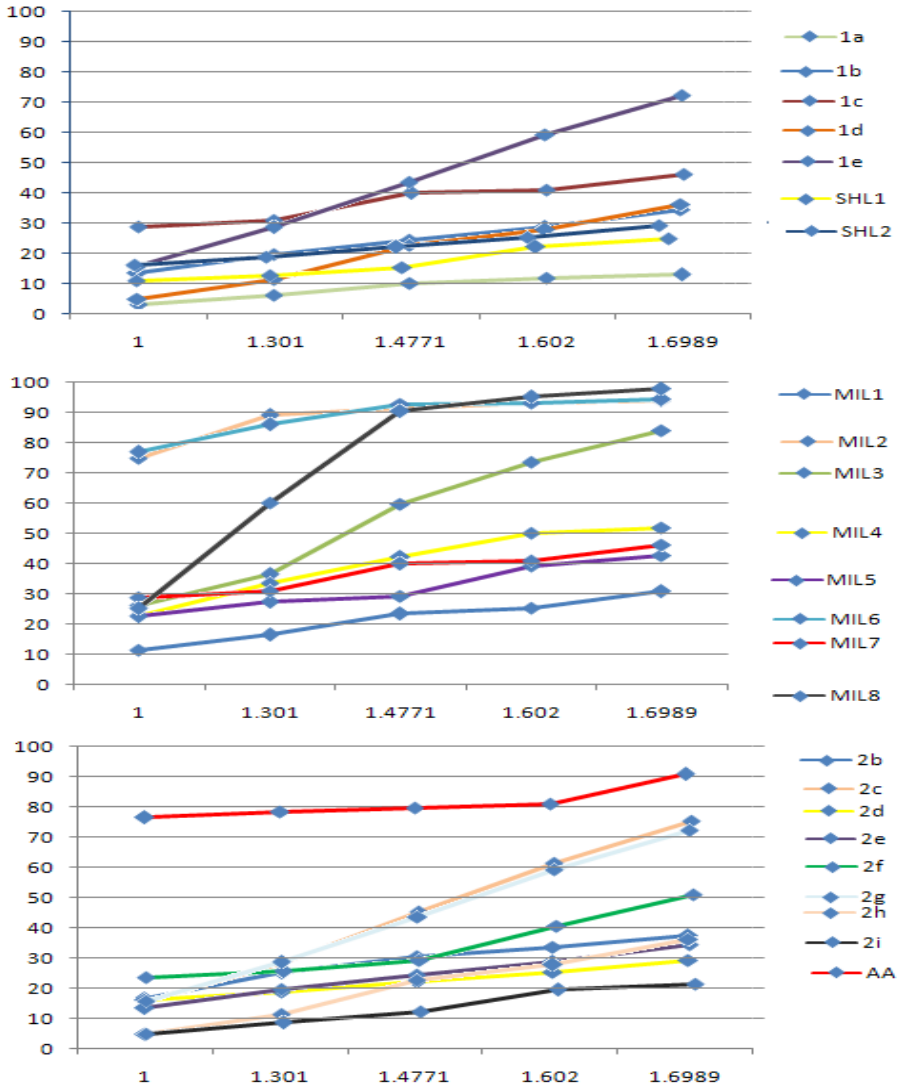


**Figure 2: Antioxidant activity of synthesised compounds (X-axis: log concentration of 10, 20, 30, 40 and 50 µg/ml; Y-axis: 5 inhibitions)**

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