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Synthesis of new 2,2-dimethyl-2*H*-chromen derivatives as potential anticancer agents

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The synthesis of some new heterocyclic derivatives comprising imidazothaidiazole, diaryl ketone and chromen as starting compound has been reported. The new series of chromen analogues have been synthesized. The reaction has been monitored by Thin Layer Chromatography (TLC) using suitable mobile phase. The R_f values have been compared and the melting points of derivatives determined. Further, these derivatives have been characterized and confirmed by IR, ^{1}H NMR and mass spectral (MS) studies. All the selected compounds submitted to National Cancer Institute (NCI) for *in vitro* anticancer assay have been evaluated for their anticancer activity.

Keywords: Anticancer drugs, imidazothaidiazole, diaryl ketone, 2,2-dimethyl-2*H*-chromen

Cancer is the second leading cause of death in the world. Now a days, chemotherapy, surgery, radiotherapy and endocrine therapy have been the standard treatment available for patients[1-3]. It is an urgent need to develop newer more effective therapies to improve patient outcome. In the present study we have reported the synthesis of some new heterocyclic derivatives comprising imidazothaidiazole, diaryl ketone and chromen as starting compound[4-43]. Chromen are of interest because of their diverse biological activities and clinical applications. We have reported the new series of chromen analogues to target HIF (Hypoxia Inducing Factor) inhibitors. The proposed molecule is structurally similar to the drug Seselin, which also inhibits the hypoxia inducible factor in anti cancer treatment (Figure 1). By using seselin, proposed molecule is modified and to synthesize the derivatives which have also gives potent anticancer activity.

Material and methods

For the synthesis of chromen derivatives the chemical are used 4-hydroxybenzaldehyde, 3-chloro-3-methyl-1-butyne, DMF (Di methyl formamide), NaOH, Ddiethyl ether, brine, NMP (N-Methyl-2-pyrrolidone), magnesium sulphate, ethylacetate, N-hexane, 2,2-dimethyl-2*H*-chromen-6-carbaldehyde, thiosemicarbazide, ethanol, $FeCl_3$, citric acid, sodium citrate, ammonia, salisaldehyde, ethylacetoacetate, piperidine, KBr pellets. These all chemical are purchased from Alfa Asar, Rankem, Finar, Chemdynes, Chinachangshu yangyuan chemicals, Avra, Lobal chemie, Astron.

The purity of the compound was confirmed by Thin Layer Chromatography using precoated TLC plates (MACHEREY-NAGEL SIL G/UV254), Melting points of synthesized compounds were performed in one end open capillary on Veego (VMP-PM) melting point apparatus, Infrared (IR) were recorded for the synthesized compounds on Shimadzu

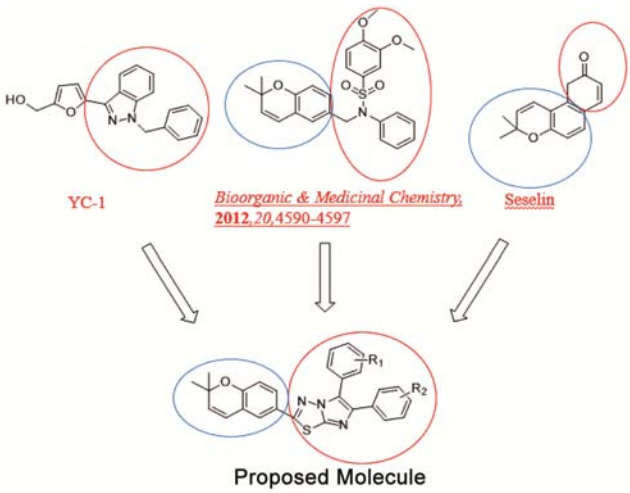


Figure 1 — Design strategy of proposed molecule

FTIR-8400 (4000-400 ν max in cm-1) Spectrophotometer in KBr disc. Mass spectra (GC-MS) of synthesized compounds were performed on SHIMADZU QP-2010. ^{1}H NMR spectra of synthesized compounds were recorded on Bruker AVANCE-III 400 MHz FT-NMR instrument by using DMSO-d_6 as solvent, TMS (Tetra Methyl Silane) as internal standard and chemicals shifts in parts per million (ppm).

Procedure for synthesis of 2, 2-dimethyl-2*H*-chromen-6-carbaldehyde

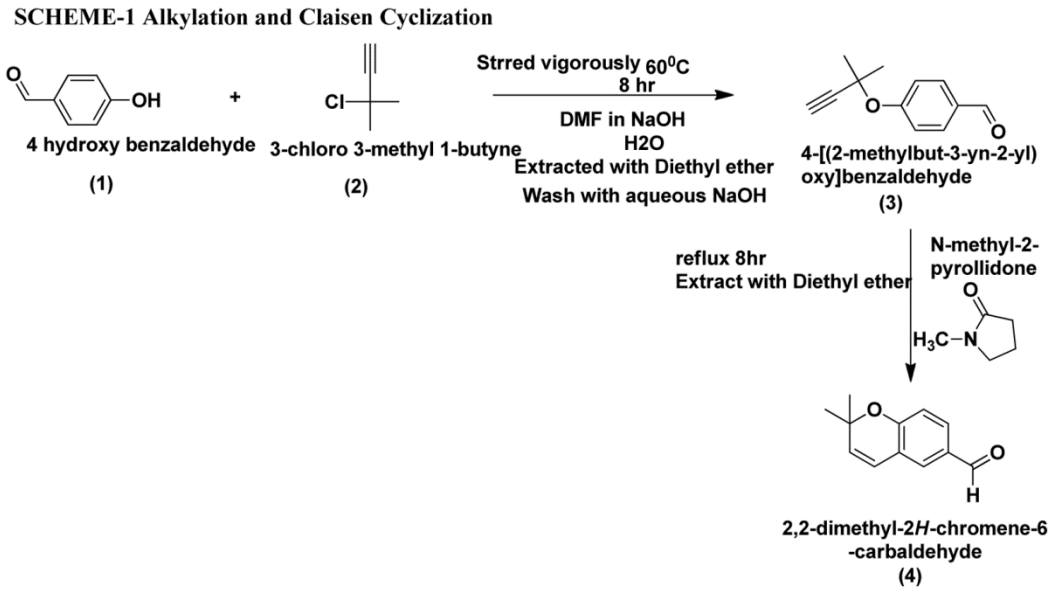
A solution of 4-hydroxybenzaldehyde (20 mmole) and 3-chloro-3-methyl-1-butyne (10 mmole) in 8 ml of DMF was taken in reaction flask. This solution was mixed with 5 ml (4 N) aqueous NaOH solution. The mixture was stirred vigorously at 60°C for 20 h. After cooling 20ml of water was added to the reaction mixture, which was extracted with 30ml of diethyl ether three times for consuming maximum product. The organic layer was washed with 40ml (1 N) aqueous NaOH Solution and brine. Then it was dried with magnesium sulphate. A pale yellow viscous liquid was produced as a crude product. A crude product was dissolved in 20ml of N-methyl-2-pyrrolidone, NMP (BP 202°C) and then it was refluxed for 18 h. After cooling 40ml of water was extracted with 60ml diethyl ether three times for the desired conversion of maximum product. The combined organic layer was than washed with brine solution and also it was dried with magnesium sulphate. The product was recrystallised with n-hexane (Scheme I).

Procedure for synthesis of 5-(2,2-dimethyl-2*H*-chromen-6-yl)-1,3,4-thiadiazol-2-amine

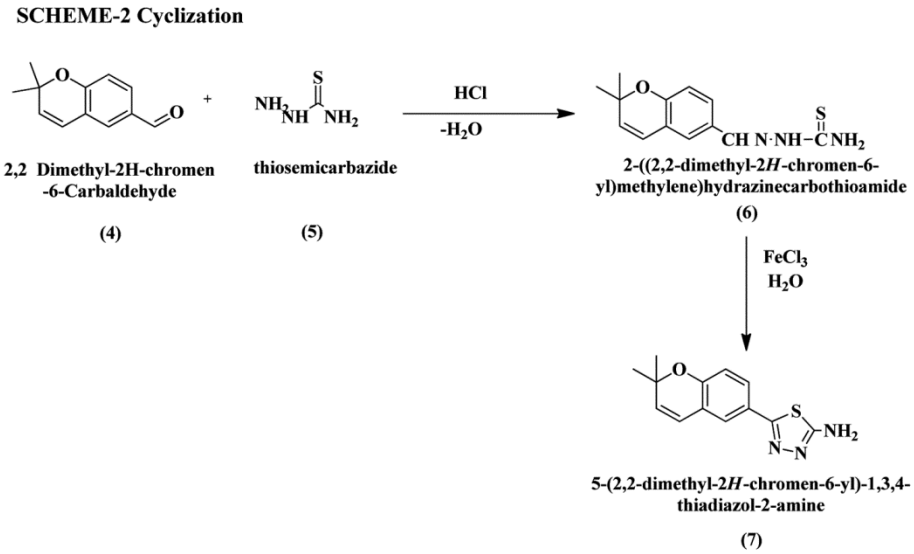
2,2-Dimethyl-2*H*-chromen-6-carbaldehyde (0.01mol) in ethanol 10ml is added into thiosemicarbazide (0.01mol) into 20ml hot ethanol over a period of 10min with continuous stirring. The reaction mixture was reflux for 2 h and allows cooling where a shiny yellow compound began to separate. It was filtered and then washed with ethanol and then dried. The compound was recrystallized from hot ethanol, giving light yellow like crystals. Then Thiosemicarbazone VI (0.05M) was suspended in 300ml warm water, $FeCl_3$ (0.15M) in 300ml water was added quantitatively slowly with constant stirring. The contents were heated at 80-90°C for 45 min. This solution was hot filtered and then citric acid (0.11M) and sodium citrate (0.05M) were added. The resulting mixture was neutralized with ammonia (10%). The required amine separated out, filtered and dried. And also recrystallized with appropriate solvent (Scheme II).

Procedure for synthesis of 2-bromo-2-(4-fluorophenyl)-1-(4-methoxyphenyl) ethanone

To a mixture of phenyl acetic acid/p-substituted phenyl acetic acid (7.3 mmol), substituted aromatic hydrocarbon (one of 8.8 mmol) and 88–93% orthophosphoric acid (8.8 mmol) was added trifluoroaceticanhydride (29.5 mmol) rapidly with



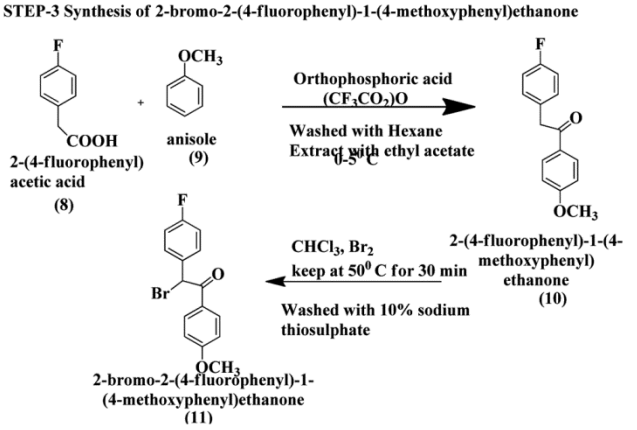
Scheme I — Scheme for synthesis of 2, 2-dimethyl-2*H*-chromen-6-carbaldehyde



Scheme II — Scheme for synthesis of 5-(2,2-dimethyl-2h-chromen-6-yl)-1,3,4-thiadiazol-2-amine

vigorous stirring at 25°C. The mixture turned into a dark colored solution with vigorous exothermic reaction. The reaction mixture was stirred for 1 min at the same temperature and poured into ice cold water (50 ml) with stirring. Then it was washed with cold hexane (2×10 ml) to obtain desired ethanone derivatives as solid.

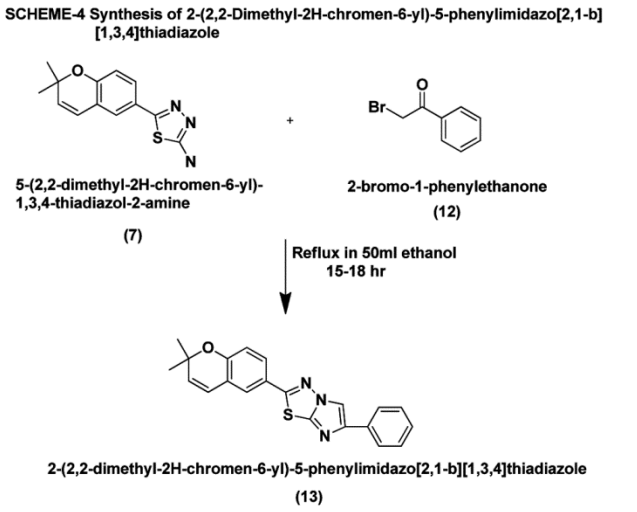
To a solution of synthesized ethanone derivatives (200 mmol) in chloroform (30 ml) kept at 50°C was added dropwise bromine (220 mmol) with stirring. After being stirred at 50°C for 0.5 h, the mixture was washed successively with aqueous 10% sodium thiosulfate solution and water. The solvent was removed in vacuum to obtain the title compounds either as oil/solid mass/crystalline compounds (Scheme III).



Scheme III — Scheme for synthesis of 2-bromo-2-(4-fluorophenyl)-1-(4-methoxyphenyl) ethanone

Procedure for synthesis of 2-(2,2-dimethyl-2*H*-chromen-6-yl)-5-phenylimidazo[2,1-b][1,3,4]thiadiazole

5-(2, 2-dimethyl-2H-chromen-6-yl)-1,3,4-thiadiazol-2-amine(10 mmol) and 2-bromo-1-phenylethanone (10 mmol) were added in RBF. This mixture is refluxed in dry ethanol (50 ml) for 15-18 hr. then resulting mixture was cooled at room temperature for 24 hr and excess solvent was removed at reduced pressure. The solid mass was washed by cold ethanol and also neutralized by cold aqueous solution of Na_2CO_3, so it gives the derivatives of thiadiazole (Scheme IV).



Scheme IV — Scheme for synthesis of 2-(2,2-dimethyl-2*H*-chromen-6-yl)-5-phenylimidazo[2,1-b][1,3,4]thiadiazole

Procedure for synthesis of 2-(2,2-dimethyl-2*H*-chromen-6-yl)-5-(4-fluorophenyl)-6-(4-methoxyphenyl) imidazo [2, 1-b][1,3,4]thiadiazole

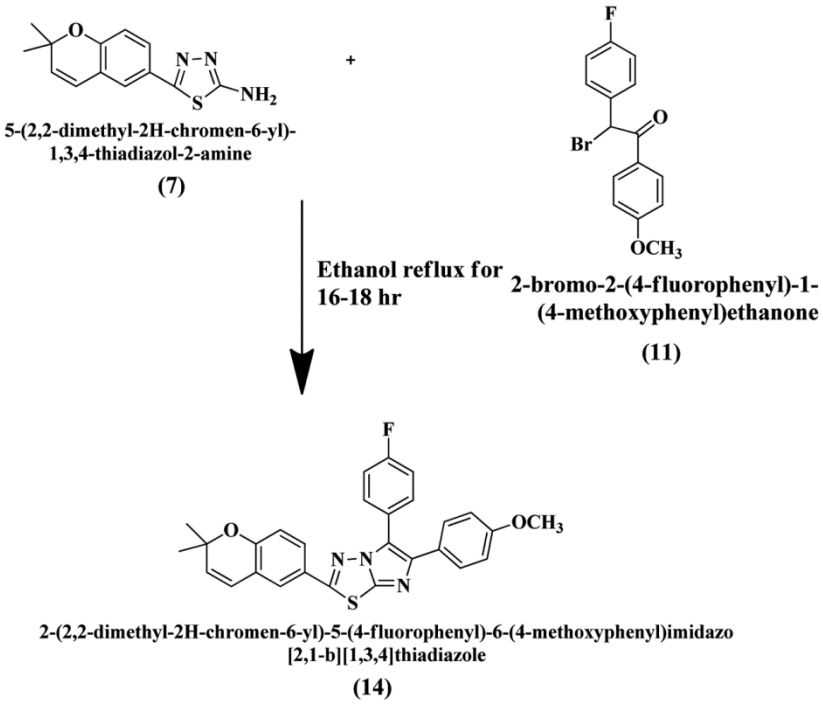
5-(2,2-Dimethyl-2*H*-chromen-6-yl)-1,3,4-thiadiazol -2-amine (10 mmol) and 2-bromo-2-(4-fluorophenyl)-

1-(4-methoxyphenyl)ethanone(10 mmol) were added in RBF. This mixture is refluxed in dry ethanol (50 ml) for 15-18 hr. then resulting mixture was cooled at room temperature for 24 hr and excess solvent was removed at reduced pressure. The solid mass was washed by cold ethanol and also neutralized by cold aqueous solution of Na_2CO_3, so it gives the thiadiazole derivatives 2-(2,2-dimethyl-2H-chromen-6-yl)-5-(4-fluorophenyl)-6-(4-methoxyphenyl)imidazo [2,1-b][1,3,4]thiadiazole. It was recrystallised by dry ethanol (Scheme V).

Anticancer screening by MTT (3-(4,5-dimethylthiazol -2-yl)-2,5-diphenyl tetrazolium bromide) assay[44]

First to prepare MTT solution (stock solution) was 5 mg in 1 ml of PBS (Phosphate Buffer Saline). The cell line used for the study was MCF-7. The MCF-7 (Michigan Cancer Foundation-7) cell line was maintained in 96 wells microtiter plate containing MEM (Minimum Essential Medium) media supplemented with 10% heat inactivated fetal calf serum (FCS), containing 5% of mixture of Gentamicin (10ug), Penicillin (100 Units/ ml) and Streptomycin (100µg/ml) in presence of 5% CO_2 incubated at 37°C for 48-72 hours. *In-vitro* growth inhibition effect of test compound was assessed by calorimetric or spectrophotometric determination of conversion of MTT into "Formazan blue" by living cells. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilised with an organic solvent (eg. DMSO, Isopropanol) and the released, solubilised formazan reagent is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells. So MTT assay performed by the remove the supernatant from the plate and add fresh MEM solution and treat with different concentrations of extract or compound appropriately diluted with DMSO. Control group contains only DMSO. In our study, 10, 20, 25, 30 and 50 µl of the stock solution (10mg / ml prepared in DMSO) were added to respective wells containing 100 µl of the medium. So, the final concentrations were 10, 20, 25, 30 and 50 µg / ml. After 48hrs incubation at 37ºC in a humidified atmosphere of 5% CO_2, stock solution of MTT was added into each well (20µl, 5mg per ml in sterile PBS) for further 4 hrs incubation. The supernatant carefully aspirated, the

SCHEME-5 Synthesis of 2-(2,2-dimethyl-2H-chromen-6-yl)-5-(4-fluorophenyl)-6-(4-methoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazole



Scheme V — Scheme for synthesis of 2-(2, 2-dimethyl-2*H*-chromen-6-yl)-5-(4-fluorophenyl)-6-(4-methoxyphenyl) imidazo [2, 1-b][1,3,4]thiadiazole

precipitated crystals of "Formazan blue' were solubilised by adding DMSO (100µl) and optical density was measured at wavelength of 570nm by using ELISA plus. The results represent the mean of five readings. The concentration in which the OD of treated cells was reduced by 50% with respect to the untreated control, it is known as IC_{50}value.

The % of Surviving cells which calculated by the formula that given below.

$$\text{Surviving cells (\%)} = \frac{\text{Mean OD of test compound}}{\text{Mean OD at control}} \times 100$$

The % of Inhibition cells which calculated by the formula that given below.

$$\text{Inhibition cells (\%)} = 100 - \text{Surviving cells (\%)}$$

IC_{50} value was calculated by using graph pad software. In this software plot the graph of % inhibition *vs*. concentration and then software calculate the IC_{50} value (Figure 2).

Results

MTT assay

Biological activities of 2,2-dimethyl-2H-chromen derivatives were carried out on MCF-7 cancer cell line at Maratha College, Belgaum. The synthesized compound is 13 screened for the anticancer activity on MCF-7 cancer cell line. The resulting data is mentioned in Table I.

Discussion

Physicochemical and spectral characterization

During study, 2,2-dimethyl-2*H*-chromen containing derivatives were synthesized by conventional method. The synthesised 2,2-dimethyl-2*H*-chromen containing derivatives were characterized by the physicochemical properties and spectral data. The purity of 2,2-dimethyl-2*H*-chromen containing derivatives was confirmed by single spot TLC. 2,2-dimethyl-2*H*-chromen containing derivatives were also confirmed by spectral data (FT-IR, Mss, $_1$H NMR).

The FT-IR spectrum of the 4-hydroxybenzaldehyd (1) showed hydroxyl stretching vibrational bands at 3194 cm^{-1} , strong carbonyl stretching vibrational band at 1667 cm^{-1} and bands around 3171 cm^{-1} assigned to aromatic carbon-hydrogen single bond.

The FT-IR spectrum of 2,2-dimethyl-2*H*-chromen-6-carbaldehyde (4) showed strong carbonyl stretching vibrational band at 1665 cm^{-1}, strong ether stretching vibrational band at 1399 cm^{-1} and bands around 3168 cm^{-1} assigned to aromatic carbon-hydrogen single bond.

The FT-IR spectrum of 2-(2,2-dimethyl-2*H*-chromen -6-yl)-5-phenylimidazo[2,1-b][1,3,4-thiadiazole **13** showed strong carbon-carbon stretching vibrational band at 1458 cm^{-1}, strong ether stretching vibrational band at 1395 cm^{-1} , showed carbon-nitrogen stretching vibrational band at 1247 $cm^{-1}$and bands around 3158 cm^{-1} assigned to aromatic carbon-hydrogen single bond.

Table I — MTT assay of 2,2-dimethyl-2*H*-chromen derivative

S. No.	Sample Code	Concentration (µg/ml)	Absorbance (nm)	Observation	IC_{50} (µg)
1		2.5	0.610	No lysis	
2		5	0.452	No lysis	
3	Doxorubicin	7.5	0.438	25% lysis	10
4		10	0.358	50% lysis	µg
5		Control	0.668	No lysis	
6		10	0.921	No lysis	
7		20	0.864	No lysis	
8	13	25	0.799	No lysis	50
9		30	0.729	No lysis	µg/
10		50	0.572	50 % lysis	ml
11		Control	1.131	-	

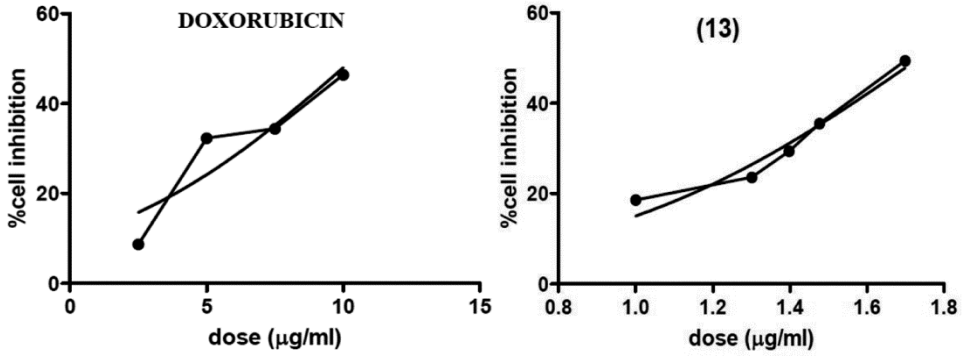


Figure 2 — Graph of % cell inhibition vs dose (µg/ml) for doxorubicin and **13**

The mass spectrum of 2-(2,2-dimethyl-2*H*-chromen-6-yl)-5-phenylimidazo[2,1-b][1,3,4-thiadiazole **13** showed molecular ion peak (M^+) at 359 m/e, based on molecular ion peak (M^+) which confirmed the compound **13**.

In the ^{1}H NMR spectrum of 2-(2,2-dimethyl-2H-chromen-6-yl)-5-phenylimidazo[2,1-b][1,3,4-thiadiazole **13** showed six proton of chromen C_2 singlet at 1.232 (d), one proton of chromen C_3 multiplet at 6.774 - 6.845 (d), six proton of chromen $C_{4\text{-}6\text{-}7}$ and phenyl $C_{10\text{-}11}$ multiplet at 7.274 – 7.862 (d),one proton of chromen C_5 singlet at 8.356 (d), two proton of phenyl C_9 and C_{13} multiplet at 8.556 – 8.682 (d),one proton of imidazo C_6 singlet at 9.123 (d).

Biological activity

2,2-Dimethyl-2*H*-chromen containing final compound **13** was screened for anticancer activity on MCF-7 cancer cell line. During the course of study we observed that compound **13** showed 50% lysis of cancer cell. We observed that 2,2 dimethyl-2*H*-chromen compound **13** showed IC_{50} value 50 µg/ml. It was compared with standard drug doxorubicin which showed 50% lysis at 10 µg concentration.

Conclusion

The 2,2 dimethyl-2*H*-chromen derivatives was synthesized by using conventional method and then it was also confirmed by spectral data.

The anticancer activities of synthesized compound **13** were evaluated on MCF-7 cancer cell-line by MTT assay. We concluded that compound **13** has potent cytotoxicity with 50 µg/ml IC_{50} on MCF-7 cancer cell-line. Compound **13** is comparable with standard drug doxobubicin with IC_{50} 10 µg/ml. So, we can conclude that the chromen containing derivative has good anticancer activity based on docking and MTT assay results.

Acknowledgement

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Conflict of interest

The authors declare no conflict of interest.

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