Original Article

Thai Journal of Pharmaceutical Sciences

Docking, synthesis of some novel flavonoid analogues, and their biological evaluation

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Received: September 28, 2021
Accepted: February 07, 2022
Published: January 23, 2023

ABSTRACT

Cancer remains a major class of morbidity and mortality despite basic and clinical research and trials of promising new therapies. In the current era, many drugs are marketed with flavonoid nucleus. Based on this information, this study aims to synthesize and screen flavones for their anticancer activity. Docking studies for cytotoxic activity of flavonoid derivatives targeting tankyrase enzyme were performed. A new series of flavonoid derivatives were prepared from chalcones. Chalcones were prepared between substituted benzaldehyde and 2-hydroxy acetophenone in the presence of potassium hydroxide as catalyst. The flavone derivatives were characterized based on IR, ^{1}H NMR, and Mass spectral data. All the new compounds were subjected to antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl and nitric oxide free radical scavenging activity. Cytotoxic activity was evaluated by MTT assay on two different cell lines. The antioxidant result reveals the potent antioxidant activity of compounds f4 and f6. Compounds mf6 and mf11 possess moderate activity. Cytotoxic activity results revealed that compound f10 have moderate activity and compound f4 shows potent cytotoxic activity on both MCF-7 and Hep-G2 cell line. The *in vitro* results suggest that some of the compounds showed potent antioxidant and cytotoxic activity. Compound f4 may be a promising cytotoxic molecule.

Keywords: Antioxidant, chalcones, cytotoxic, docking, flavonoid

INTRODUCTION

Cancer, a malignancy, is a disease that is characterized by the uncontrollable proliferation of cells leading to the destruction of body tissues. Cancer has now become one of the most carving diseases that the population suffers because these cells have the potential to invade and spread to other parts of the body. Cancer remains a major class of morbidity and mortality despite basic and clinical research and trials of promising new therapies. It is the third leading cause of death worldwide after a heart attack and infectious disease.

Polycyclic organic compounds that result from the fusion of benzene ring and heterocyclic pyran ring are known as benzopyran, whereas benzopyrone is a ketone derivative of benzopyran which is an outcome of benzene fused with pyran-4-one. These benzopyrone form the core skeleton of many flavonoids, coumarins, and 4-quinolone compounds. Many structures have been widely used as an effective template in medicinal chemistry for drug discovery. Among which chalcone is the common and simple scaffold used. Due to their convenience in the synthesis, they are taken as starting point for drug discovery. Chalcones are nothing but benzene rings connected by olefinic linkages.

The main reason for selecting chalcone as a starting point is due to its simple chemistry, ease of hydrogen atom manipulation, straightforward synthesis, and variety of promising biological properties.[1] Chalcones and their heterocyclic analogs belong to the family of flavonoids which owns numerous significant biological activities such

as antioxidant, cytotoxic,[2] antimicrobial, antiprotozoal, antiulcer, antihistaminic, and anti-inflammatory.[3]

Flavonoids are polyphenolic having a general structure of fifteen carbon skeletons which consist of two phenyl rings and a pyran ring which are widely present in plants. The main function of flavonoids is to regulate cellular activity and fight against free radicals that cause oxidative stress to our body. Most of the flowers, fruits, and seeds have their color due to flavonoids. Flavonoids are also called as free radical scavengers due to the ability of flavonoids, to donate hydrogen atoms.

Flavonoids have various pharmacological actions such as antioxidant,[4] sedative, antidepressant, anticonvulsant, antiproliferative,[5] anti-inflammatory,[6] antimicrobial,[7] anticancer,[8] cardioprotective, antihypertensive, antiulcerogenic, antidiabetic[9], and hepatoprotective.

Due to these findings, it was thought to synthesize flavonoid analogs, hoping that these compounds might possess certain anticancer and antioxidant activity.

MATERIALS AND METHODS

Molecular Docking Study

Molecular docking was carried out on Schrodinger 2019-4 suite device Maestro 11.7.012, (Ligprep, Glide XP docking, QikProp), this software package programmed on DELL Inc.27" workstation machine running on Intel Core i7-7700 CPU@ 3.60 GHz x8, a processor with 8GB RAM, and 1000 GB hard disk with Linux –X6_64 as the operating system. Docking scores were calculated using Maestro (Schrodinger) software. The binding affinity was assessed in terms of binding free energies (S-score, kcal/mol). All synthesized compounds were docked in the groove of the binding site present in tankyrase 4HKI.

Laboratory grade chemicals and reagents were used to synthesize all the reported compounds. The IR spectra were recorded using Alpha Bruker IR spectrometer using a thin film on KBr pellet technique and frequencies are expressed in cm^{-1}. ^{1}H-NMR spectra were recorded on Bruker Avance-II 400 MHz NMR spectrometer. All spectra were obtained in $CDCl_3$ and DMSO. Chemical shift values are reported in ppm relative to TMS ($\delta = 0$) as the internal standard. The mass spectrometry was recorded on LC-MS Shimadzu 2020 series in electrospray ionization mode. Melting points were determined by the open capillary method using Equitronics EQ 730 digital melting point apparatus and are uncorrected. Pre-coated silica gel plates (Merck, Silica gel 60 F254) were used for analytical TLC, and UV radiations were used to visualize the spots.

General Procedure for the Synthesis of 2-hydroxy Chalcones[10]

About 50% potassium hydroxide solution (10 mL) was added dropwise to a mixture of 2-hydroxy acetophenone (28 mmol) and selected benzaldehyde (28 mmol) in ethanol (50 mL) and the mixture was stirred at room temperature overnight. The reaction mixture was then poured into ice and acidified with HCl. The precipitate was filtered with chloroform and crystallized from ethanol.

General Procedure for the Synthesis of Flavonols[11]

About 30% hydrogen peroxide (7 mL) solution was added dropwise to a mixture of chalcone (0.01 mol) and sodium hydroxide (5 g) in 50% ethanol (50 mL) and stirred overnight and then poured into ice and acidified with HCl. The precipitate was filtered and crystallized from methanol as given in Table 1.

General Procedure for the Synthesis of Methoxy Flavones[12]

A mixture of flavonols (1.8 mmol), dry potassium carbonate (1.8 mmol), and dimethyl sulfate (1.8 mmol) in acetone (50 mL) was refluxed for 7–9 h and filtered. The solvent was evaporated and the residue was crystallized from ethanol as given in Table 2.

General Procedure for the Synthesis of Flavones from Chalcones[13]

A mixture of chalcone (0.5 g) and selenium dioxide (0.5 g) in dry isoamyl alcohol (15 mL) was refluxed in an oil bath for 6 h. The reaction mixture was filtered to remove the precipitate of selenium dioxide and the filtrate was kept for evaporation to remove isoamyl alcohol. A dark brown solid along with some pasty mass was formed. This was then filtered, dried, and extracted with benzene. The solid obtained after the removal of benzene was recrystallized from ethanol, producing yellowish-brown needles.

Cytotoxic Activity

Since there is a need for effective anticancer agents, we are evaluating the cytotoxic potential of the synthesized compounds by MTT assay using MCF-7 cell line for breast cancer and Hep-G2 cell line for lung cancer.

MTT Assay[14]

Preparation of MTT reagent

Aseptically add 6ml of cell-based assay buffer in one MTT vial and completely dissolve the powder. MTT powder dissolves slowly in a buffer. Vigorous vortexing is needed to dissolve the powder completely. MTT solution should appear bright yellow.

Assay Procedure

10 µL of cell suspension was seeded in a 96-well microliter plate at the required cell density, with or without the cell growth modifying agent. The plate was incubated at 37°C in a 5% CO_2 atmosphere for the required period. After the incubation period, the plate was removed from the incubator and the MTT reagent was added to the final conc. of 10% of total volume. This volume should be the same as the volume used while determining cell density.

The plated was, then, wrapped with aluminum foil to avoid exposure to light. The plates were returned to the incubator and incubated for 2–4 h. After an incubation period, 10 µL of solubilization solution is added to each well. The absorbance was read on a spectrophotometer or an ELISA reader at 570 nm with a reference wavelength of 630 nm. The average 570 nm absorbance value of the control wells was subtracted

from the average 570 nm absorbance values of corresponding experimental wells. A graph was plotted with absorbance value on Y-axis and experimental parameter on X-axis.

Antioxidant Activity

Antioxidants are a class of chemical substances that can prevent or decrease the oxidative stress of the physiological system. Oxidation is a chemical reaction that can generate free radicals, resulting in chain reactions that might damage the cells of organisms. Free radicals may be superoxide, hydroxyl, and nitric oxide (NO) which are oxygen-centered free radicals and are also known as reactive oxygen species.

Out of the different methods available for evaluating antioxidant potential, 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity, and NO free radical scavenging activity methods were used here.

DPPH Radical Scavenging Assay[15]

The free radical scavenging activity of the synthesized flavonoid derivatives was measured using the DPPH method. 0.2 mM solution of DPPH in methanol was prepared and 100 µL of this solution was added to various concentrations of the sample (10–50 µg/mL) and allowed to stand for 30 min, absorbance was measured at 517 nm. Ascorbic acid was used as the standard drug. The percentage inhibition was calculated by comparing the absorbance values of both control and test samples with the standard.

$$\% \textbf{ inhibition} = \frac{Abs_{control} - Abs_{test}}{Abs_{control}} \times 100$$

Where $Abs_{control}$ is the control absorbance and Abs_{test} is the test absorbance.

NO Radical Scavenging Activity[16]

NO radicals are generated from sodium nitroprusside solution at physiological pH. Sodium nitroprusside (1 mL of 10 mM) is mixed with 1 mL of flavonoid derivatives (10–50 µg/mL) in a phosphate buffer of pH 7.4. The mixture was incubated at 25°C for 150 min. To 1 mL of the incubated solution, 1 mL of Griess's reagent (1% sulfanilamide, 2% o-phosphoric acid, and 0.1% naphthyl ethylene diamine dihydrochloride) was added and it was allowed to stand for 30 min, absorbance was taken at 546 nm. % inhibition of the synthesized compounds was calculated using the formula mentioned earlier. Ascorbic acid was used as standard drug material.

$$\% \textbf{ inhibition} = \frac{Abs_{control} - Abs_{test}}{Abs_{control}} \times 100$$

Where $Abs_{control}$ is the control absorbance and Abs_{test} is the test absorbance.

RESULTS AND DISCUSSION

Spectral Data

2-(4-(dimethylamino)phenyl)-4H-chromen-4-one (f4)

IR (cm^{-1}): 3240 (Ar C-H str), 1460 (Ar C=C str), 1682 (C=O str), 1341 (C-C str), 1165 (C-N str)

^{1}**H NMR** (400 MHz, DMSO-d_6): δ 7.72-7.90 (m, 9H, Ar-H)

Mass (m/z): 265 (M^+).

2-(4-nitrophenyl)-4H-chromen-4-one (f8)

IR (cm^{-1}): 3243 (Ar C-H str), 1688 (C=O str), 1340 (NO_2 str), 1166 (C-N str)

^{1}**H NMR** (400 MHz, DMSO-d_6): δ 7.75-7.96 (m, 9H, Ar-H)

Mass (m/z): 267 (M^+).

2-(2,4-dichlorophenyl)-4H-chromen-4-one (f10)

IR (cm^{-1}): 2916 (Ar C-H str), 1465 (Ar C=C str), 1637 (C=O str), 1204 (C-O str), 862 (C-Cl str)

^{1}**H NMR** (400 MHz, DMSO-d_6): δ 7.00-7.77 (m, 8H, Ar-H)

Mass (m/z): 291 (M^+).

2-(3-nitrophenyl)-4H-chromen-4-one (f11)

IR (cm^{-1}): 2912 (Ar C-H str), 1460 (Ar C=C str), 1336 (NO_2 str), 1630 (C=O str), 1208 (C-O str),

^{1}**H NMR** (400 MHz, DMSO-d_6): δ 7.00-7.67 (m, 9H, Ar-H)

Mass (m/z): 267 (M^+).

2-(4-(dimethylamino)phenyl)-3-methoxy-4H-chromen-4-one (mf4)

IR (cm^{-1}): 2920 (Ar C-H str), 1519 (Ar C=C str), 1659 (C=O str), 1227 (C-N str), 1154 (C-O str)

^{1}**H NMR** (400 MHz, DMSO-d_6): δ 7.68-7.70 (m, 8H, Ar-H), 3.05-3.34 (m, 6H, CH_3)

Mass (m/z): 295 (M^+).

3-methoxy-2-(4-methoxyphenyl)-4H-chromen-4-one (mf6)

IR (cm^{-1}): 2923 (Ar C-H str), 1510 (Ar C=C str), 1618 (C=O str), 1252 (C-O str)

^{1}**H NMR** (400 MHz, DMSO-d_6): δ 7.46-7.94 (m, 8H, Ar-H), 3.77-3.97 (m, 6H, OCH_3)

Mass (m/z): 282 (M^+).

2-(2,4-dichlorophenyl)-3-methoxy-4H-chromen-4-one (mf10)

IR (cm^{-1}): 2928 (Ar C-H str), 1520 (Ar C=C str), 1630 (C=O str), 1257 (C-O str), 865 (C-Cl str)

^{1}**H NMR** (400 MHz, DMSO-d_6): δ 7.40-7.96 (m, 7H, Ar-H), 3.72-3.95 (m, 3H, OCH_3)

Mass (m/z): 321 (M^+).

3-methoxy-2-(3-nitrophenyl)-4H-chromen-4-one (mf11)

IR (cm^{-1}): 2935 (Ar C-H str), 1516 (Ar C=C str), 1638 (C=O str), 1255 (C-O str), 1345 (NO_2 str)

^{1}**H NMR** (400 MHz, DMSO-d_6): δ 7.46-7.94 (m, 8H, Ar-H), 3.70-3.92 (m, 3H, OCH_3)

Mass (m/z): 292 (M^+).

Chemistry

The main aim of the work was to synthesize substituted flavonoid derivatives and to evaluate their *in vitro* antioxidant and cytotoxic activities. The reaction sequence is outlined in Figure 1. Structures of the compounds were confirmed based on IR, ^{1}H-NMR, and Mass spectral data. The purity of the compounds was assessed by TLC. The IR spectrum of the compounds showed the presence of an enone group which indicates the formation of chalcone. All the synthesized compounds showed absorption bands at 1300–1100 cm^{-1} and 1550–1500 cm^{-1} indicating the presence of C-N and C=C, respectively.

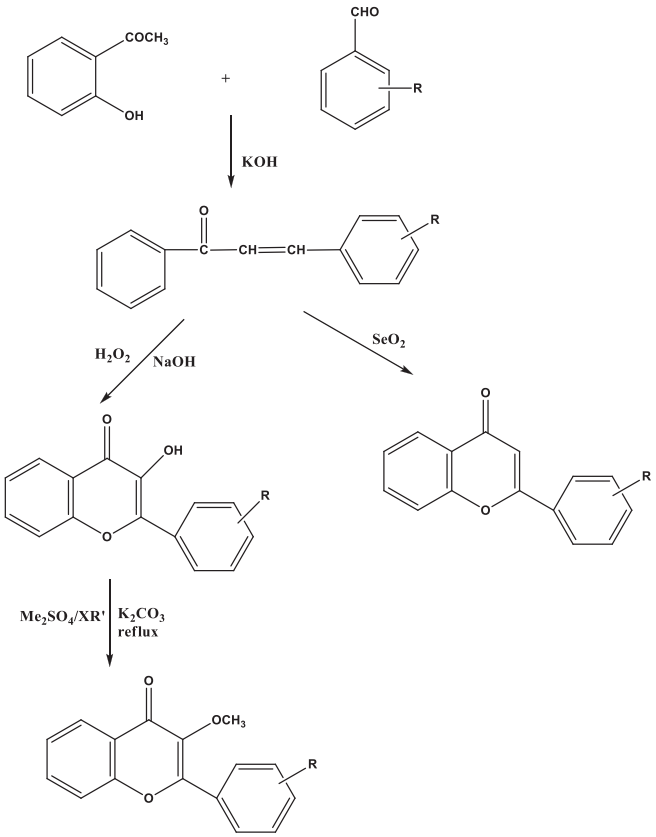

Figure 1: Scheme for the synthesis of flavone derivatives

The ^{1}H-NMR spectrum of the compounds showed the appearance of multiplets in the region δ 8.94–6.31 integrating for the presence of aromatic protons. A single peak in the region δ 3.9–2.5 indicates the presence of CH_3 protons of compounds.

In the mass spectrum of compound f10, the molecular ion peak was observed at 292.25 (M+1), which was in agreement with the assigned molecular formula $C_{15}H_8Cl_2O_2$. The other compounds are also conformity with the molecular formula.

The RMSD value was calculated in molecular docking to compare the docked conformation of the reference ligand and to validate the protein bound ligand docked in the same pocket to check the deviations. Lower the RMSD value, greater the efficiency of docking. In our study, RMSD value was found to be 0.079 Å. The values found had the least RMSD and were chosen for further studies.

The synthesized compounds have the binding free energy in the range of -10.492 to –4.868 kcal/mol with tankyrase. The active residues of 4HKI are Phe 1061, Tyr 1060, Ala 1062, Hid 1031, Gly 1032, Ser 1032, Pro 1014, Phe 1035, Lys 1067, Ser 1068, Tyr 1071, Arg 1047, Hid 1041, Ala 1049, Tyr 1050, Ile 1051, Ile 1075, Phe 1032, Het 1054.

Compounds f4 and f8 have the best binding energy of −10.498 with the highest affinity among the entire compounds docked with 4HKI. Compound f6 and mf4 fit into the binding cleft of 4HKI with dock score −9.648 kcal/mol and −8.948 kcal/mol, respectively. The docking orientations

Table 1: Physical data of methoxy flavone derivatives

Compound	Substitution	Molecular formula	Molecular weight	Melting Point (°C)	Percentage Yield (%)
mf2	p-Cl	$C_{16}H_{11}ClO_3$	286.70	265–267	70.43
mf4	p-N $(CH_3)_2$	$C_{18}H_{17}NO_3$	295.33	280–282	81.29
mf6	p-OCH_3	$C_{17}H_{14}O_4$	282.29	253–255	72.22
mf8	p- NO_2	$C_{16}H_{11}NO_5$	297.26	379–381	80.91
mf10	2,4-$(Cl)_2$	$C_{16}H_{10}Cl_2O_3$	321.15	308–310	88.23

Table 2: Physical data flavone derivatives

Compound	Substitution	Molecular formula	Molecular weight	Melting Point (°C)	Percentage Yield (%)
f2	p-Cl	$C_{15}H_9ClO_2$	256.68	169–171	76.09
f4	p-N $(CH_3)_2$	$C_{17}H_{15}NO_2$	265.30	244–246	78.98
f6	p-OCH_3	$C_{16}H_{12}O_3$	252.26	223–225	89.19
f8	p-NO_2	$C_{15}H_9NO_4$	267.23	333–335	70.98
f10	2,4-$(Cl)_2$	$C_{15}H_8Cl_2O_2$	291.12	262–264	86.19

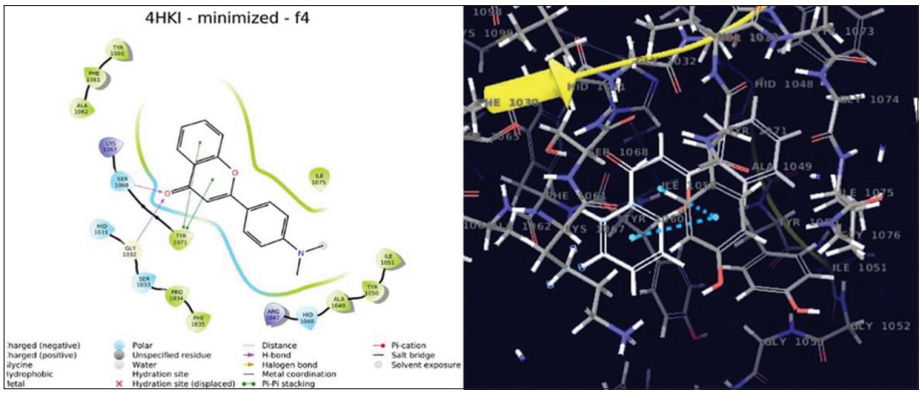

Figure 2: 2D and 3D interactions of compound f4 with 4HKI

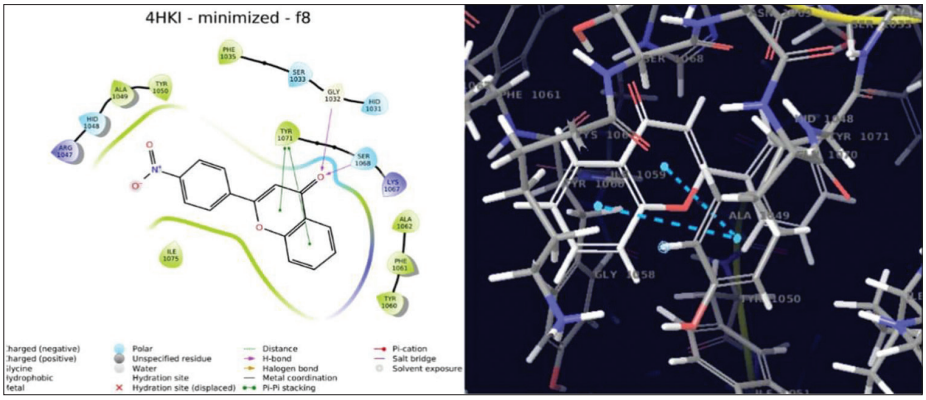

Figure 3: 2D and 3D interactions of compound f8 with 4HKI

Table 3: Docking score of flavone derivatives with 4HKI

Compound	Docking score
f4	−10.492
f8	−10.179
f10	−10.158
f2	−10.105
f11	−10.053
f6	−9.648
mf4	−8.948
mf8	−8.765
mf11	−8.680
mf22	−8.546
mf6	−8.229
mf10	−4.868

of compounds f4 and f8 with 4HKI receptor are represented in Figures 2 and 3 respectively and Table 3.

Cytotoxic Activity

The *in vitro* cytotoxic activity was evaluated by MTT assay. MTT assay was performed on two cell lines which are MCF-7and Hep-G2. All the compounds have been tested at 10–50 µg/mL using cisplatin as standard. Test results revealed the potent anticancer potential of compound f4 on both the cell lines similarly compound f10 showed moderate activity, as given in Tables 4 and 5.

Table 4: Data of MTT assay of flavonoid derivatives for MCF-7 cell line

Conc (µg/mL)	Percentage inhibition				
	Cisplatin	f4	f8	f10	mf4
10	12.83	38.29	25.89	30.85	25.89
20	41.94	41.7	29.92	34.72	29.99
30	73.2	46.66	36.43	39.06	36.43
40	79.88	51.62	38.75	42.63	38.75
50	83.21	53.79	42.17	44.8	42.17
IC_{50}	18.74	1.29	1.88	1.58	1.5

Table 5: Data of MTT assay of flavonoid derivatives for Hep-G2 cell line

Conc (µg/ml)	Percentage inhibition				
	Cisplatin	f4	f8	f10	mf4
10	12.83	48.45	41.7	42.37	39.48
20	41.94	49.64	46.39	48.14	44.22
30	73.2	53.29	46.7	51.75	46.28
40	79.88	55.67	49.27	54.12	48.55
50	83.21	57.01	50.92	55.25	51.85
IC_{50}	18.74	1.03	1.16	1.12	1.23

Table 6: Data of DPPH free radical scavenging activity of flavonoid derivatives

Concentration (µg/mL)	Percentage inhibition										
	Standard	mf4	mf6	mf8	mf10	mf11	f4	f6	f8	f10	f11
10	1.02	17.49	57.64	41.18	26.36	37.52	59.28	66.13	23.3	68	25.03
20	28.64	25.37	62.9	44.46	37.78	46.57	66.95	76.6	31.23	75.57	47.86
30	85.67	41.18	71.65	48.72	53.55	56.91	75.05	80.56	40.71	79.36	58.85
40	89.64	58.98	78.84	64.15	60.7	66.3	94.7	85.82	47.78	84.01	75.91
50	91.3	80.87	94.78	75.57	61.39	71.73	95.43	92.97	65.14	91.25	90.73
IC_{50}	16.16	3.8	0.89	1.32	1.67	1.28	0.85	0.72	2.26	0.71	1.74

Table 7: Data of nitric oxide free radical scavenging activity of flavonoid derivatives

Concentration (µg/ml)	Percentage inhibition										
	Standard	mf4	mf6	mf8	mf10	mf11	f4	f6	f8	f10	f11
10	12.37	18.62	48.84	36.74	6.66	54.73	35.97	61.26	49.8	47.63	50.89
20	77.91	30.21	54.28	45.26	10.24	57.29	41.48	62.35	59.34	52.72	55.95
30	83	36.36	55.69	52.24	36.49	67.67	49.61	63.63	60.75	54.16	60.81
40	94.9	48.59	67.73	59.15	45.83	71.83	57.23	64.91	63.95	63.7	67.22
50	97.81	60.69	71.51	60.81	55.88	77.59	67.34	99.59	66.64	75.16	70.42
IC_{50}	7.64	2.66	1.03	1.28	11.87	0.93	1.42	0.82	0.94	1.08	0.97

Antioxidant Activity

The *in vitro* antioxidant activity of the synthesized compounds was evaluated by DPPH radical scavenging method and NO free radical scavenging method using ascorbic acid as the standard. The result shows that f4 and f6 compounds showed potent activity. Similarly, mf6 and mf11 possess moderate activity, as given in Tables 6 and 7. All the other compounds have shown moderate antioxidant activity in both models.

CONCLUSIONS

In this present work, a novel series of flavonoid derivatives have been synthesized, characterized by spectral data, and evaluated for their *in vitro* antioxidant and anticancer activities. Based on *in-vitro* studies and chemistry, the final synthesized compounds might be useful as a lead molecule for pharmaceutical industries, so the current work requires further structural modification to get better pharmacological actions.

ACKNOWLEDGMENTS

The authors are thankful to authorities of NGSM Institute of Pharmaceutical Sciences, Nitte (Deemed to be University), Mangaluru for providing all the necessary facilities.

CONFLICTS OF INTEREST

Authors declare no conflicts of interest in this study.

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