Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2018, 10(8): 25-32

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

In Silico Design, Synthesis and Anticancer Evaluation of 7-O-Substituted Isoflavones [3-(4'-Methoxyphenyl)-4h-Chromene-4-One 7-Yl Derivatives

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ABSTRACT

Isoflavonoids constitute an important class of oxygen heterocycles. The structure of an isoflavone is composed of a 3-phenylchromen-4-one skeleton. Isoflavones exhibit a large number of diverse biologic effects such as anticance, antioxidant, anti-inflammatory, antimicrobial activities etc. This work is aimed to insilico design, synthesis & anticancer evaluation of Isoflavone derivatives. Insilico design were done using Argus, Schrodinger, Molinspiration softwares of twenty analogues .Out of these, based on the docking scores, five analogues were selected for synthesis. Phenyl acetic acid &resorcinol were selected as starting materials. These analogues were purified by TLC and column chromatography and further charectirized by FT-IR, 1H NMR and Mass spectral studies. The anticancer activity was done using Trypan blue dye exclusion method. Among the synthesized derivatives (1AC),Showed significant anticancer activity. Natural isoflavonoid compounds have so far proven to be of limited utility in the clinic, synthetic isoflavonoid compounds continue to attract the interest of the pharmaceutical industry. These novel analogues could specifically target anticancer activities and can be considered as suitable lead compounds for further research & development.

Keywords: 3-phenyl chromene-4-one; Isoflavonoid; Deoxybenzoin; Anticancer activity; Trypanblue dye exclusion method

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INTRODUCTION

Isoflavonoids exhibit a large number of diverse biologic effects, both in vivo and in vitro that may be associated with anticancer activities [1]. The isoflavonoids bind with estrogen receptorsis related to the inhibition of cell cycle and leads to produce anti-cancer activity. As Isoflavonoids have various pharmacological activities, such as antioxidant activity, antiaromatase (CYP19) activity, and inhibition of endothelial proliferation, these should act along the initiation, promotion and progression phase of carcinogenesis. In case of breast cancer, transfer of Isoflavonoids from mother to foetus could affect the preventive effect to mammary gland after birth. It may suppress the development of mammary buds, which leads to the decreased risk of breast cancer in adulthood [2]. Administration of Isoflavonoids showed decreased plasma estradiol level and can reduce the risk of endometrial and ovarian cancer. As dietary soy-phytoestrogens decrease testosterone level and prostate weight, low prostatic cancer incidence occurs. Inhibitory effect of Isoflavonoids on 5-alpha- reductase was considered to be related to prevent prostatic cancer. The proposed mechanisms by which isoflavones may inhibit cancer are, inhibition of DNA topoisomerase, suppression of angiogenesis, induction of differentiation in cancer cell lines, Induction of apoptosis. The antioxidant effect of isoflavonoids reduce the risk of artherosclerosis, hypertension, hyperlipidemia, cardiac infarction and also used for the prevention of osteoporosis [3-5].

In contrast to the flavonoids, the distributions of isoflavonoids are relatively limited, most likely because of the sporadic occurrence of the isoflavone synthase enzyme, which is only produced by plants when required. Although there are several examples of naturally occurring isoflavonoids with potent activity against several ailments, their use as medicaments has been limited due to the following reasons: i) Low abundance of these compounds in the plant material, ii) Tedious extraction and purification techniques which often require extraction with very large quantities of solvents, multiple chromatographic purifications, occasionally including HPLC purifications iii) Unavailability of appropriate biological data. One of the possible solutions to these problems is the development of efficient synthetic methodologies, which can produce not only the natural products but also their synthetic analogues for pharmacological applications [6-10].Focusing these properties ,we aim to design and synthesize a novel series of derivatives of isoflavonoids and screened for anticancer activity. Figure 1 represents the scheme of the synthesis.

EXPERIMENTAL SECTION

Materials and Methods

All the chemicals and reagents used in this research work were of analytical or synthetic grade from Sigma Aldrich, E-Merck and Chemco (India). All the chemicals were dried and purified according to standard methods before use whenever necessary. Software used in this study include Chemdraw Ultra, Molinspiration, PASS, Schrodinger and Argus Lab 4.1.0etc. All the reaction courses and product mixtures were routinely monitored by aluminium coated TLC plates 60 F245 (E-Merck) and visualized with UV light or Iodine chamber. The melting point of synthetic compounds were determined on a Lab India MR-VIS visual melting point apparatus & are corrected. The FTIR spectra were recorded using FTIR/FT-FIR Perkin Elmer Spectrum 400.1HNMR Spectrophotometer (Bruker AVANCE DPX400FTNMR) and Chemical shifts are expressed as delta (ppm) using TMS as internal standard in DMSO-D6.The mass spectra of the compounds were done with Mass Spectrometer(JEOL JMS600H). Anticancer docking studies of the synthesized derivatives were carried out on Argus lab software/Schrodinger software using the enzyme human protein kinase /Akt1 ligand complex (PDB:3CQW) or human tyrosine kinase receptor. The ability of the synthesized analogues to inhibit the enzyme was recorded as docking scores.

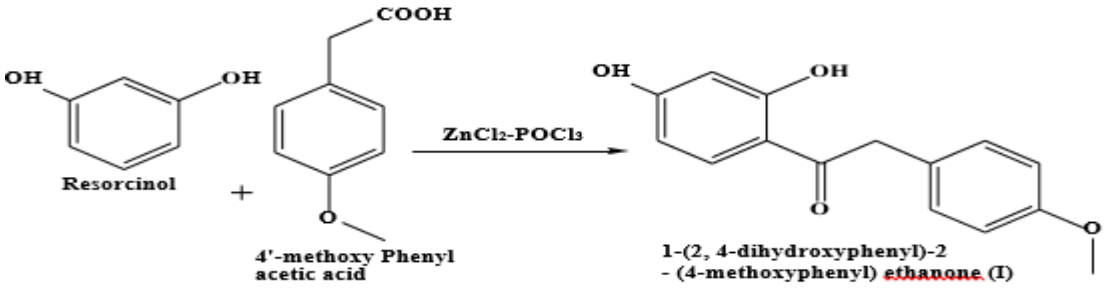
In-Silico Drug Design

Molecular modeling studies: In silico design of drug is a computer aided program that represents a fast and cost-effective tool for computationally screening compound databases in search for novel drug leads. The modeling of the proposed derivatives was carried out by using different computational softwares like Molinspiration, ChemSketch, ChemDraw Ultra, PASS, ArgusLab, Molegrow molecular viewer and Q-site finder for the determination of different molecular descriptors profile and docking analysis.

Docking studies: Molecular docking is a key tool in structural molecular biology and computer-assisted drug design. It predicts the binding orientation of small molecule drug candidates with the protein targets and thus the affinity and activity of the small molecule. It is useful to know the binding strength (binding energy) of a group of compounds or derivatives to determine which derivative is the best binder or inhibitor. Molecular docking was done by using MEASTRO version 8.5GUI Schrodinger software suite 2008 in Tyrosine protein kinase receptor (PDB ID-2SRC) which is complexed with the ligand, phosphoramino phosphonic acid-adenylate ester. Docking was also performed using Argus lab 4.0.1version using the enzyme Tyrosine protein kinase receptor for anticancer activity.

Synthetic Methods

Step1: Synthesis of Deoxybenzoin [1-(2, 4-dihydroxyphenyl)-2-(4-methoxyphenyl) ethanone]



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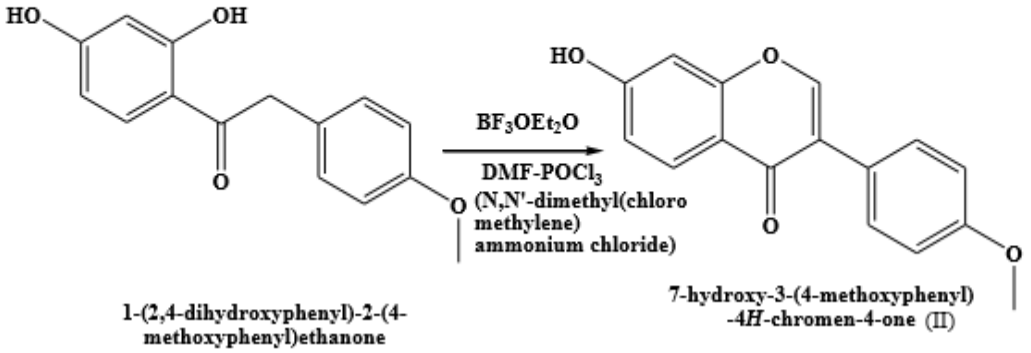
Step 2: Synthesis of Isoflavone (7-hydroxy 3-(4'-methoxyphenyl)-4H-chromen-4-one)



Step 3: Synthesis of 7-O substituted Isoflavone [3-(4'-methoxyphenyl)-4-H-chromen-4-ones]

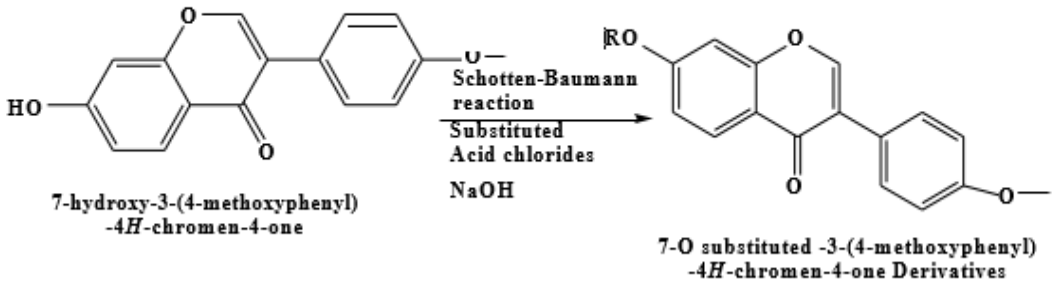


Figure 1: Scheme of synthesis

Synthetic procedure:

Step 1: Synthesis of deoxybenzoin [1-(2, 4-dihydroxyphenyl)-2-(4-methoxyphenyl) ethanone]

In a 500ml RB flask fitted with a reflux condenser and drying tube, para methoxy phenyl acetic acid (5g, 0.03mol), resorcinol (3.32g,0.03mol), anhydrous zinc chloride (20g,0.15mol) and phosphorousoxychloride(13.97ml, 0.15mol) were stirred with heating at 700C for 1 hour. The completion of the reaction was monitored by TLC using chloroform: ethyl acetate (7:2) as solvent. After cooling the reaction mixture was poured slowly in to 12% sodium acetate solution and the precipitated product was filtered off, washed with water and air dried. The product was further purified by recrystallization from aqueous methanol to yield white shining crystals. Yield is 95%w/w.

Step 2: Synthesis of 7-hydroxy-3-(4'-methxoyphenyl)-4H-chromen-4-one (Isoflavone)

In a 500ml three necked RB flask fitted with a reflux condenser, a dropping funnel and a stopper, 1-(2,4-dihydroxyphenyl)-2-(4-methoxyphenyl)ethanone (5g,0.019mol) was dissolved in Borontrifluride etherate(7.5ml, 0.059mol) with stirring and then cooled to 100C. To this cooled solution DMF (15ml) was added drop-wise with stirring. In another 500ml RB flask fitted with a drying tube, DMF (25ml) was cooled to 100C. To this cooled DMF phosphorous pentachloride (6.3g, 0.3mol) was added in small portions and the mixture was allowed to stand at 550C for 10minutes. This pale pink colored mixture containing N, N-dimethyl (chloromethylene) ammonium chloride was then added to the above reaction mixture and the mixture was stirred at room temperature for 1 hour. The light

yellow colored solution was then poured slowly into 0.1N dilute HCl with vigorous stirring and allowed to stand for 30minutes.During the process, the yellow precipitate slowly become white with the formation of 7-hydroxy-3-(4-methxoyphenyl)-4H-chromen-4-one. The product was further purified by aqueous methanol. Yield: 55%w/w.

Step 3: Synthesis of 7-O-substituted-3-(4'-methoxyphenyl)-4H-chromen-4-one derivatives

To a well stirred solution of synthesized 7-hydroxy-3-(4ˇ-methxoyphenyl)-4Hchromen-4-one (0.01 mol) and aqueous NaOH solution, substituted acid chlorides were added slowly. The reaction mixture was heated to reflux for 15-45minutes. Completion of reaction was monitored by TLC (Chloroform: methanol, 9:1).After cooling the reaction mixture was poured into crushed ice. Desired product was obtained on cooling. The obtained yield is 79%w/w.

Five different acid chlorides were Benzoyl Chloride(1BC),Acetyl Chloride(1AC),Chloro acetyl chloride,,Benzene sulphonyl chloride and P-toluene sulphonyl chloride.

Anticancer Studies

The anticancer activities of the synthesized compounds were studied at Amala Cancer Research Center, Thrissur. The test compounds were studied for short term in-vitro cytotoxicity using Dalton's Lymphoma Ascites cells (DLA) by means of Trypan blue exclusion method.

RESULTS AND DISCUSSION

Insilico Molecular Modelling

The In-silico molecular modeling studies were carried out on 20 proposed analogues of Isoflavones using different softwares for the selection of suitable candidates prior to wet lab synthesis. Among the 20 designed analogues, five analogues were find to obey Lipinski rule of five and their drug likeness were predicted by Molinspiration software. These selected analogues with desired physico- chemical parameters were selected for wet lab synthesis. Table 1 represents analysis of Lipinski rule of five for selected analogues and Table 2 represents analysis of drug likeness score for selected analogues

Table 1: Analysis of Lipinski rule of five for selected analogues

Compound code	Log P	Molecular weight	nON	nOHNH	nrotb	nviolations
IBC	4.442	372.376	5	0	5	0
IAC	3.12	310.305	5	0	4	0
ICAC	3.52	344.75	5	0	5	0
IBSC	4.53	408.431	6	0	5	0
ITSC	4.78	422.45	6	0	5	0

Table 2: Analysis of drug likeness score for selected analogues

Compound code	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear Receptor ligand	Protease inhibitor	Enzyme inhibitor
IBC	-0. 30	-0.60	-0.23	-0.02	-0.57	-0.14
IAC	-0.32	-0.71	-0.28	-0.03	-0.64	-0.10
ICAC	-0.37	-0.77	-0.23	-0.02	-0.58	-0.14
IBSC	-0.23	-0.54	-0.23	-0.25	-0.33	-0.00
ITSC	-0.26	-0.58	-0.26	-0.27	-0.37	-0.05

Molecular Docking Studies

Docking studies of the synthesized derivatives were done by using the software's Schrodinger and Argus Lab.

Using Argus Lab: Anticancer docking studies of the synthesized derivatives were carried out on Argus lab software using the enzyme human protein kinase/ Akt1 ligand complex [PDB-id: 3CQW]. The ability of the synthesized analogues to inhibit the enzyme was recorded as docking scores. The docking score of all derivatives is represented in Table 3. Figure 2 represents docking image of 1AC on 3CQW using Argus Lab.

Table 3: Docking scores using Argus lab

Compd.code	Docking score
IBC	-9.92
IAC	-10.35
ICAC	-9.64
IBSC	-8.75
ITSC	-8.88

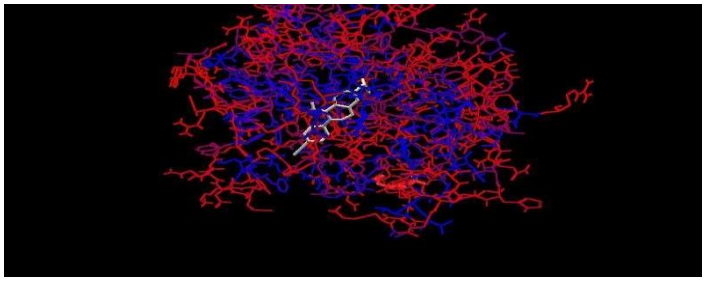


Figure 2: Docking image of 1AC on 3CQW

Using Schrodinger software: Anticancer docking studies of the synthesized derivatives were carried out on the enzyme human tyrosine kinase receptor, C-SRC [PDB- id: 2SRC]. Table 4 represents docking scores using Schrodinger Software. Figure 3 represents docking image of 1AC on 2SRC.

Table 4: Docking scores using Schrodinger Software

Ligand	Glide score	Glide energy
IBC	-4.15	-24.52
IAC	-4.97	-37.22
ICAC	-2.34	-25.89
IBSC	-1.24	-35.29
ITSC	-1.89	-34.9

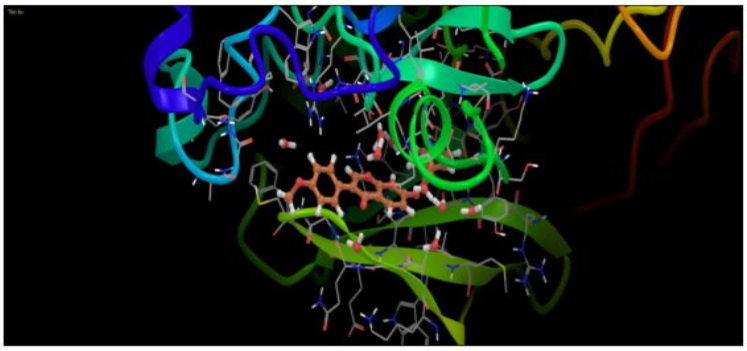


Figure 3: Docking image of 1AC on 2SRC

Synthetic Methods

The synthetic scheme involved a three step reaction (scheme 1). The synthesized derivatives were recrystalised and purified by TLC and column chromatography. 5 new derivatives were synthesized which are 1BC,1AC, 1CAC, 1BSC, 1TSC. The characterized data for the synthesized derivatives is given in the Table 5.

Table 5: Characterization data of Synthesized derivatives

Compound code	R	Molecular Formula	Formula Weight	Melting Point(0C)	% yield	Rf	colour
1BC	Benzoyl chloride	C23H16O5	372.37	110-113	79%	0.72	Off white
1AC	Acetyl chloride	C18H14O5	310.30	162-165	72%	0.76	white
1CAC	Chloro acetyl chloride	C18H13O5Cl	344.75	140-143	65%	0.74	Pale yellow
1BSC	Benzene sulfonyl chloride	C22H16O6S	408.43	187-189	79%	0.69	Brown
1TSC	p-toluene sulfonyl chloride	C23H16O6S	420.48	182-185	71%	0.67	Light brown

Spectral Analysis of Synthesized Derivatives

The newly synthesized derivatives were further characterized by FT-NMR, 1HNMR and Mass spectral studies. Table 6 represents the characteristic IR absorption peak of selected analogues. Table 7 represents 1H NMR of derivative 1BC and Table 8 represents Mass Spectra of derivative 1BC. Figure 4 represents cytotoxicity of compound 1AC.

Table 6: Characteristic IR absorption peak of selected analogues

Compound	IR values
1AC	3050.23 (C-H), 1606.85(C=O), 1509.99(C=C), 1350.99(-OCH3), 1173.66(C=C-CO),1239.53,1104.68,1128.94,2951.02
1BC	3049.88 (C-H), 1674.62 (C=O), 1322.85 (OCH3), 1452.71,1420.53 (C=C), 1288.57 (C-O-C), 2970.1

Table 7: 1H NMR of derivative 1BC

Compound code	Signal position
1BC	3.791 (OCH3), 4.243 (=CH), 7.258, 7.468, 7.487, (Ar-H)

Table 8: Mass Spectra of derivative 1BC

Molecular ion peak	372.0946
Base peak	122.1711

Anticancer Evaluation Of Synthesized Derivatives

All the synthesized derivatives were tested for their short term in vitro cytotoxicity using Dalton's Lymphoma Ascites cells (DLA) by means of Trypan blue exclusion method. The anti-cancer activities of the compounds were represented as percentage cell death. The percentage cell death of DLA cells corresponding to the various concentrations (200g/ml, 100g/ml, 50g/ml) of the compounds is represented in Table 9.

Table 9: Anti-cancer activity results

Compound code	Sample con.(□g/ml)	Percentage Cell Death(DLA)
IBC	200	57
	100	30
	50	16
IAC	200	60
	100	32
	50	20
ICAC	200	56
	100	32
	50	20
ITSC	200	52
	100	34
	50	19
+ ve Control (5-Fluro Uracil)	200	100
	100	100
	50	80
Control (DMSO)	200	00
	100	00
	50	00

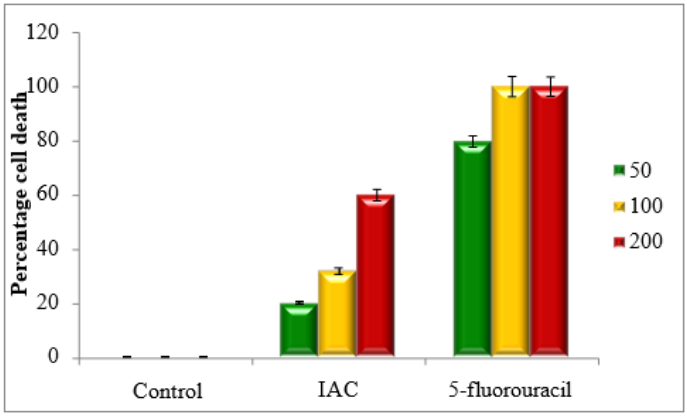


Figure 4: Cytotoxicity of compound 1AC

CONCLUSION

This research work was focused in the design and development of novel isoflavanoid derivatives as anticancer agents. We have designed twenty analogues and after insilico modelling and docking studies, five were selected for wet lab synthesis. Docking studies for the anti-cancer activity of synthesized compounds were done by using ArgusLab and Schrodinger software for determining the analogue which shows better interaction with enzyme receptor. The results indicate that the compound IAC and 1BC shows better binding affinity. These synthesized derivatives were evaluated for anticancer activity. The invitro cytotoxicity studies were carried out using Dalton's Lymphoma Ascites cells (DLA) by means of Trypan blue exclusion method. Acetyl derivative (1AC) possess

significant cytotoxicity at higher concentration (200g/ml).The synthesized compounds were characterized by FT-IR, 1HNMR and Mass spectral data. The structure, functional groups and molecular weights of the compounds were confirmed. This present study would prove extremely useful to synthesize novel Isoflavone analogues in future which would eventually be beneficial for optimizing the lead molecule for the cytotoxic action.

ACKNOWLEDGEMENT

The authors gratefully acknowledge to the institution St. Josephs college of Pharmacy, Cherthala for providing financial support to carry out the research. The authors also acknowledge to the Amala Cancer Centre, Thrissur for providing the results of anticancer studies of the research.

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