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SYNTHESIS AND ANTICANCER STUDY OF CHALCONE LINKED 1, 3, 4-OXADIAZOLE DERIVATIVES

C K Thasneem *[1], C R Biju[1], Babu G[1]

[1]Department of pharmaceutical chemistry, Devaki Amma Memorial College of Pharmacy, Chelembra, Malappuram, India – 673634

*Corresponding Author Email: thasneeemck@gmail.com

ABSTRACT

Synthesis of chalcone linked 1, 3, 4 – oxadiazole were carried out by clubbing of substituted chalcone and substituted oxadiazole. Purity of the compounds ascertained consistency by TLC and melting point determination. The structure of newly synthesized compounds were characterized by IR, HNMR, MAS Spectral analysis and evaluated for anticancer activity on human breast cancer cell line MCF 7. The derivatives showed significant activity on MCF 7 cell line.

KEY WORDS

Chalcone, 1, 3, 4 – Oxadiazole, MCF 7, MTT, Anticancer.

INTRODUCTION

Chalcones, considered to be a precursor of flavanoids and isoflavanoids, are abundant in edible plants. They consist of open chain flavanoids in which two aromatic rings are joined by three carbon α and β-unsaturated carbonyl system. Chalcones shows various biological activities like antibacterial, antifungal, antitumour, anti inflammatory, antioxidant and anti cancer etc.

1, 3, 4-oxadiazoles represent an important class of heterocyclic compounds that have wide applications in therapeutic area, oxadiazole moiety shows anticancer and anti-inflammatory activity and suitably substituted 1,3,4-oxadiazole having biological activities like anticancer and other biological activities. This work focused to develop efficient synthetic strategies to afford a structurally diverse chalcone derivatives having an improved anticancer activities by attaching other lead structure like oxadiazole heterocyclic moeties.

MATERIALS AND METHODS

1. **Synthesis and characterization:**

   All the chemicals and reagents used in this research work were analytical or practical grade. Melting point of the synthesized compounds were determined by open capillary method and are uncorrected. Infra Red spectra of the synthesized compounds are recorded using Perkin Elmer FT-IR spectrophotometer. Proton NMR spectra of synthesized compounds are recorded in $CDCl_3$ on Bruker ultra shield DPX 400 spectrophotometer. The reactions were monitored by TLC over precoated preactivated glass plates with solvent system chloroform: methanol (6:4)

2. **Pharmacological activity (MTT ASSAY) :**

   The human breast cancer cell line (MCF 7) was obtained from National Center for Cell Science (NCCS), Pune. All the lines were grown Dulbecco's modified eagles medium containing 10% fetal bovine serum (FBS)

and maintained at 37°C, 5%CO2, 95% air and 100% relative humidity.

MTT is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore the amount of formazan produced is directly proportional to the number of viable cells.

After 48 hrs of incubation, 15µl of MTT (5gm/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37°Cfor 4 hrs. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using microplate reader. The % cell inhibition was determined using the following formula.

% cell inhibition = 100-Abs(sample) /Abs(control) x 100

Nonlinear regression graph was plotted between % cell inhibitions and log 10 concentration and IC50 was determined using graph pad prism software.

3. Experimental section

Synthetic Procedure:

Step 1: Preparation of P- chloro chalcone (C1)

Dissolved 0.01 mol chloro benzaldehyde and 0.01 mol acetophenone in 10 ml of 95% ethanol in 25 ml Erlen mayer flask and equipped with magnetic stirrer bar. 3.5 ml of sodium hydroxide solution was added to the reaction flask and stirred for 10 minutes. Cooled the mixture until the crystal formation was completed. Added 2 ml ice cold water to the flask. Filtered, washed with 5 ml ice cold ethanol. Allowed to air dry, recrystallised from 95% ethanol.

Step 2: synthesis of 4-Hydroxybenzohydrazides (OX1)

Dissolved equimolar quantity of p-hydroxy methyl benzoate and hydrazine hydrate in 25 ml ethanol and refluxed for four hours. Reaction mixture is poured in to ice cold water. Stirred for some time. White crystalline product obtained. Filtered, washed with ice cold water. Dried and recrystallized from ethanol.

Step 3 : synthesis of 4-[5-(4-hydroxy phenyl) -1,3,4-oxadiazole -2-yl] benzoic acid (OX2)

0.01mol OX 1 and 0.01 mol benzoic acid mixed with 0.06 ml phosphorous oxy chloride and refluxed for 3 hours. Pour the reaction mixture in to crushed ice. Stirred well. Filtered the product obtained. Washed with water, dried and recrystallised from ethanol.

Step 4 : Synthesis of 1-phenyl-3-(4-(4-(5-phenyl-1,3,4-oxadiazole-2-yl) phenoxy) phenyl) Prop-2-en-1-one. (COX 29)

0.01 mol C 1 and 0.01 mol OX 2 was taken in a round bottom flask. To this mixture 10 ml acetone and 1 gm potassium carbonate was added. Refluxed for 8 hrs. Reaction mixture poured in to ice water. Filtered the product obtained. Washed with water. Dried and recrystallised from ethanol.

Synthesis of 1-(4-hydroxyphenyl)-3-(4-(4-(5-phenyl-1,3,4-oxadiazole-2-yl)phenoxy) phenyl) prop-2-en-1-one (COX 1)

Dissolved 0.01 mol chloro benzaldehyde and 0.01 mol P-hydroxy acetophenone in 10 ml of 95% ethanol in 25 ml Erlen mayer flask and equipped with magnetic stirrer bar. 3.5 ml of sodium hydroxide solution was added to the reaction flask and stirred for 10 minutes. Cooled the mixture until the crystal formation was completed. Added 2 ml ice cold water to the flask. Filtered, washed with 5 ml ice cold ethanol. Allowed to air dry, recrystallised from 95% ethanol (C 1a).

0.01 mol C 1a and 0.01 mol OX 2 was taken in a round bottom flask. To this mixture 10 ml acetone and 1 gm potassium carbonate was added. Refluxed for 8 hrs. Reaction mixture poured in to ice water. Filtered the product obtained. Washed with water, dried and recrystallised from ethanol.

Synthesis of 1 - (4- chlorophenyl) -3- (4 -(4 -(5- phenyl-1, 3, 4- oxadiazole- 2 -yl) phenoxy) phenyl) prop- 2- en-1-one. (COX 2)

Dissolved 0.01 mol chloro benzaldehyde and 0.01 mol P-chloro acetophenone in 10 ml of 95% ethanol in 25 ml Erlen mayer flask and equipped with magnetic stirrer bar. 3.5 ml of sodium hydroxide solution was added to the reaction flask and stirred for 10 minutes. Cooled the mixture until the crystal formation was completed. Added 2 ml ice cold water to the flask. Filtered, washed with 5 ml ice cold ethanol. Allowed to air dry, recrystallised from 95% ethanol. (C 1b).

0.01 mol C 1b and 0.01 mol OX 2 was taken in a round bottom flask. To this mixture 10 ml acetone and 1 gm potassium carbonate was added. Refluxed for 8 hrs. Reaction mixture poured in to ice water. Filtered the product obtained. Washed with water, dried and recrystallised from ethanol.

Synthesis of 3-(4-(4-(5-(4-nitrophenyl)-1,3,4-oxadiazole-2-yl)phenoxy) phenyl)-1-phenyl prop-2-en-1-one (COX 25)

0.01mol OX 1 and 0.01 mol nitro benzoic acid mixed with 0.06 ml phosphorous oxychloride and refluxed for 3 hours. Pour the reaction mixture in to crushed ice. Stirred well. Filtered the product obtained. Washed with water, dried and recrystallised from ethanol (OX 2a).

0.01 mol C 1 and 0.01 mol OX 2a was taken in a round bottom flask . To this mixture 10 ml acetone and 1 gm potassium carbonate was added. Refluxed for 8 hrs. reaction mixture poured in to ice water. Filtered the product obtained. Washed with water, dried and recrystallised from ethanol.

Synthesis of 3-(4-(4-(5-(4-hyroxyphenyl)-1,3,4-oxadiazole-2-yl)phenoxy) phenyl)-1-phenyl prop-2-en-1-one. (COX 26)

0.01mol OX1 and 0.01 mol hydroxy benzoic acid mixed with 0.06 ml phosphorous oxy chloride and refluxed for 3 hours. Pour the reaction mixture in to crushed ice. Stirred well. Filtered the product obtained. Washed with water, dried and recrystallised from ethanol. (OX 2b).

0.01 mol C 1 and 0.01 mol OX 2b was taken in a round bottom flask . To this mixture 10 ml acetone and 1 gm potassium carbonate was added. Refluxed for 8 hrs. Reaction mixture poured in to ice water. Filtered the product obtained. Washed with water, dried and recrystallised from ethanol.

Synthesis of 3-(4-(4-(5-(4-aminophenyl)-1,3,4-oxadiazole-2-yl)phenoxy) phenyl)-1-phenyl prop-2-en-1-one (COX 27)

0.01mol OX 1 and 0.01 mol amino benzoic acid mixed with 0.06 ml phosphorous oxy chloride and refluxed for 3 hours. Pour the reaction mixture in to crushed ice. Stirred well. Filtered the product obtained. Washed with water, dried and recrystallised from ethanol. (OX 2c).

0.01 mol C 1 and 0.01 mol OX 2c was taken in a round bottom flask . To this mixture 10 ml acetone and 1 gm potassium carbonate was added. refluxed for 8 hrs. Reaction mixture poured in to ice water. Filtered the product obtained. Washed with water, dried and recrystallised from ethanol.

Synthesis of 3-(4-(4-(5-(2,4-dihyroxyphenyl)-1,3,4-oxadiazole-2-yl)phenoxy) phenyl)-1-phenyl prop-2-en-1-one. (COX 28)

0.01mol OX 1 and 0.01 mol dihydroxy benzoic acid mixed with 0.06 ml phosphorous oxy chloride and refluxed for 3 hours. Pour the reaction mixture in to crushed ice. Stirred well. Filtered the product obtained. Washed with

water, dried and recrystallised from ethanol. (OX 2d).

0.01 mol C 1 and 0.01 mol OX 2d was taken in a round bottom flask. To this mixture 10 ml acetone and 1 gm potassium carbonate was added. Refluxed for 8 hrs. Reaction mixture poured in to ice water. Filtered the product obtained. Washed with water, dried and recrystallised from ethanol.

Synthetic scheme

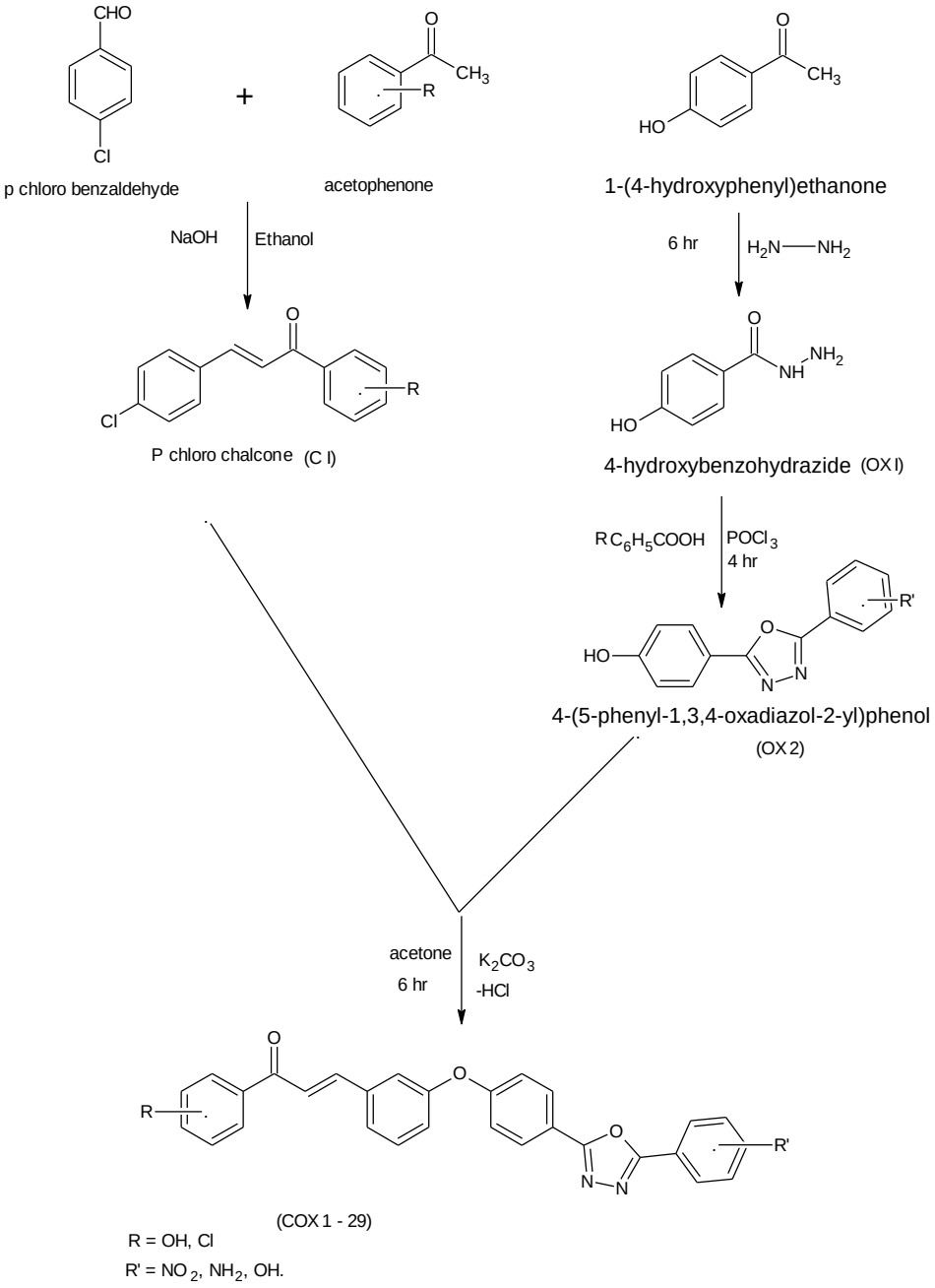
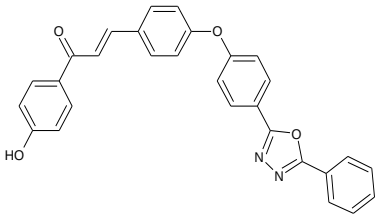
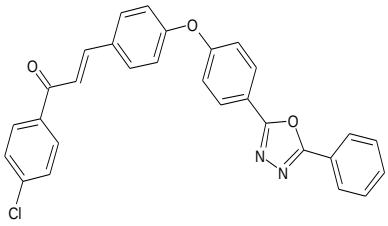
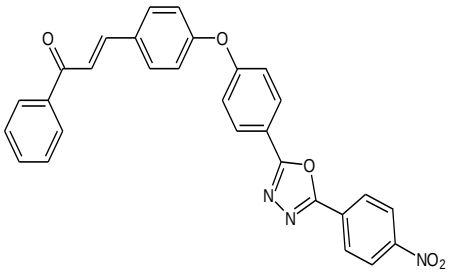
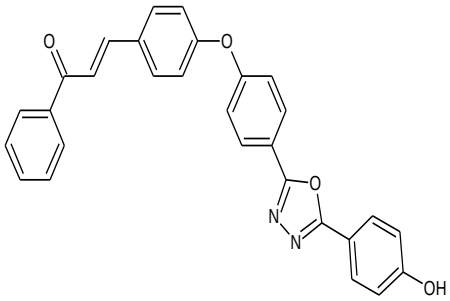
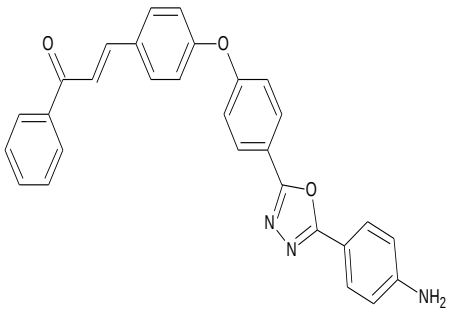
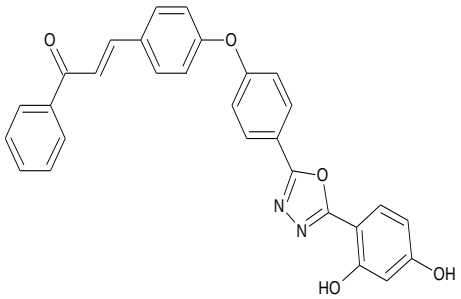


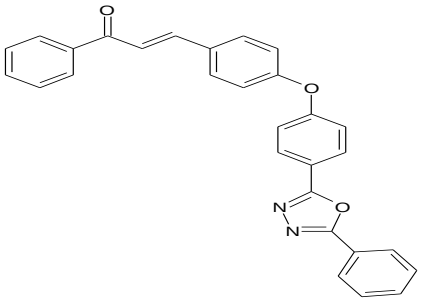
Table 1: List of derivatives

Compounds	Structure
COX 1	
COX 2	
COX 25	
COX 26	
COX 27	

COX 28



COX 29



RESULTS

Table 2: Preliminary characterization of synthesized compound

Compound code	Molecular formula	Molecular weight	Melting point (°C)	Percentage yield (%)	Rf value
COX 1	$C_{29}H_{20}N_2O_4$	460.48	220	55	0.67
COX 2	$C_{29}H_{19}ClN_2O_3$	478.92	190	42	0.72
COX 25	$C_{29}H_{19}N_3O_5$	489.47	220	60	0.63
COX 26	$C_{29}H_{20}N_2O_4$	460.48	200	54	0.66
COX 27	$C_{29}H_{20}N_2O_5$	459.49	180	48	0.74
COX 28	$C_{29}H_{20}N_2O_5$	476.47	200	40	0.61
COX 29	$C_{29}H_{20}N_2O_3$	444.4	190	62	0.74

Table 3: Spectral data of synthesized compounds

Compounds	Mass value	IR spectra	^{1}HNMR spectra
COX 25	490.75	1658(C=C str); 1592(C=O str); 1486(Ar-NO_2 str); 1444(C=N str); 1210(C-O-C str);	8.38 (2H of phenyl), 8.16 (2H(s) of phenyl), 8.024 (1H(s) of alkene), 7.775 (2H(s) of phenyl), 7.57(1H(m) of alkene), 7.40(1H(s) of phenyl), 7.38 (2H of phenyl), 7.32-7.34(4H of phenyl) , 7.26(4H (s) of phenyl)
COX 26	461.10	3738(free phenol); 1654(C=Cstr); 1596(C=O str); 1211(C-O-C str);	7.492 ($2H_{(m)}$ of phenyl), 7.407-7.39 ($4H_{(m)}$ of phenyl), 7.26($2H_{(s)}$ of phenyl), 7.745-7.777($2H_{(d)}$) of alkene, 7.499-7.526 ($5H_{(m)}$ of phenyl)
COX 27	460.55	3668(N-N str); 3333(N-H str); 1655(C=O str); 1595(C=N str); 1312(Ar-N str); 1172(C-O-C str);	7.514-7.527($4H_{(m)}$ phenyl), 7.39-7.408($4H_{(m)}$ phenyl), 7.74($2H_{(s)}$of phenyl), 7.58($2H_{(m)}$ of phenyl), 7.53($1H_{(m)}$ of phenyl), 8.007-8.010($2H_{(m)}$ of alkene), 7.576 ($4H_{(m)}$ of phenyl), 5.142 ($2H_{(s)}$ of amino)
COX 29	444.51	3055(ArC-H str); 1657(C=C str); 1596(C=O str); 1486(C=N str); 1214(C-O-C str)	7.492 ($2H_{(m)}$ of phenyl), 7.407-7.39 ($4H_{(m)}$ of phenyl), 7.26($2H_{(s)}$ of phenyl), 7.745-7.777($2H_{(d)}$) of alkene, 7.499-7.526 ($5H_{(m)}$ of phenyl)

Pharmacological activity

All the synthesized compounds showed significant cytotoxic activity against human MCF 7 cell lines. Among them COX 26 showed excellent activities with IC_{50} Value 6.8 µM against MCF 7 Cell lines.

Table 4:IC_{50} values of synthesised derivatives on MCF 7

Compounds code	% Cell inhibition					IC_{50} Value (µM)
	0.1 µM	1 µM	10 µM	50 µM	100 µM	
COX 25	3.381	3.633	12.456	85.553	99.826	22.92
COX 26	1.816	3.373	67.993	99.913	100	6.8
COX 27	0.086	3.373	31.833	100	100	11.4
COX 29	1.730	6.228	26.470	97.750	99.134	14.2

Anticancer studies of derivatives on MCF 7 cell line

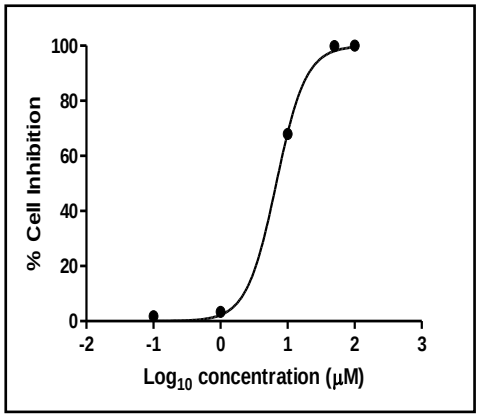


Fig 1 percentage inhibition Curve of COX 26

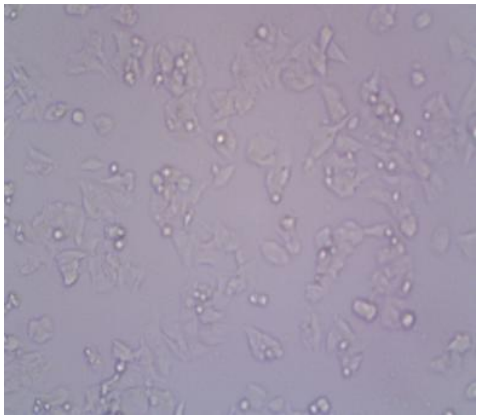


Fig 2 anticancer studies of COX 26 on MCF 7

DISCUSSION

The synthesis of substituted chalcone was carried out by the condensation of substituted benzaldehyde and acetophenone in the presence of sodium hydroxide and ethanol. Substituted Oxadiazole was synthesized by the cyclization reaction of 4-hydroxy benzohydrazide in the presence of substituted benzoic acid and phosphorous oxychloride. Finally substituted oxadiazole and substituted chalcone were combined by the removal hydrogen chloride.

The purity of the compounds was ascertained consistency by TLC as well as melting point determination. The structure of newly synthesized compounds were characterized by IR, ^{1}HNMR and MAS Spectral analysis. By considering the IR, ^{1}HNMR, MASS spectra of the synthesized compounds, we can confirmed that the expected structure of the derivatives were confirmed.

Anticancer activity of synthesized compounds were carried out by MTT assay in cell line MCF7 cell line. Four synthesized derivatives are randomly selected and send for anticancer activity. COX26 showed significant anticancer activity against MCF 7 cell line. It has an IC_{50} value 6.8 µM which is a significant value. It indicates this is the most potent drug. Other compounds COX 27 and COX 29 were also shows significant anti cancer activity and possessing an IC_{50} value of 11.4µM and 14.2µM respectively. COX 25 shows the better anticancer activity having the IC_{50} value of 22.9 µM.

CONCLUSION

This research mainly focused on the study of chalcone linked 1, 3, 4 – oxadiazole derivatives and its anticancer activity on MCF 7 cell lines.

Synthesis of chalcone linked 1, 3, 4 – oxadiazole were carried out by clubbing of substituted chalcone and substituted oxadiazole. Purity of the compounds ascertained consistency by TLC and melting point determination. The structure of newly synthesized compounds were characterized by IR, HNMR, MAS Spectral analysis.

Results of these study revealed that all the synthesized derivatives showed promisingly significant activity on MCF 7cell line and we can conclude that they are anticancer agents. By comparing the results of all compounds we reached in a conclusion that COX 26 was considered as a potent anticancer agents.

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***Corresponding Author:**
C.K THASNEEM*