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RESEARCH ARTICLE

Synthesis, characterisation and *invitro* anticancer activity of thiazolidine-2,4-dione derivatives

T. T Akshaya*, V. B Arunlal, Dr. G. Babu, Dr. Biju. C. R

Department of Pharmaceutical Chemistry, Devaki Amma Memorial College of Pharmacy, Pulliparamba (P.O), Chelembra, Malappuram-673634, Kerala, India

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ABSTRACT

Thiazolidine-2,4-diones exhibit broad spectrum of biological activities like antimicrobial, anticancer, anti-inflammatory, anti diabetic etc.The aim and objective of present study was to develop novel thiazolidine-2,4-diones hybrid analogues in favour of the molecular docking studies and to evaluate their cytotoxic activities. The molecular docking studies of the proposed derivatives were carried out using softwares - Argus lab, CORINA,swiss PDB and Molegro molecular viewer. The anti cancer targets used is PPAR-γ and those with better scores were selected and subjected for the wet lab synthesis. In this study, 5 novel thiazolidine-2,4-dione analogues were synthesized by the Knoevenangel condensation reaction of thiazolidine-2,4-diones with substituted aromatic aldehydes using L-Tyrosine as catalyst to yield 5-arylidine thiazolidine-2,4-diones,which on further undergo mannich reaction with aromatic amines to yield mannich bases. The purity of the newly synthesized compounds was ascertained by consistency in the TLC as well as melting point determination and was characterized by means of FTIR,^{1}HNMR and MASS spectral analysis. Among the synthesized compounds, POD 1,POD 2, POD 3 with better scores were tested for *in-vitro* cytotoxicity studies by MTT assay on MCF-7 cell lines. Among this POD 1 and POD 2 exhibited significant activity on the cell line. Finally it was concluded that novel thiazolidine-2,4-diones hybrid analogues can be considered as the future lead molecule for drug discovery process.

Key words: Thiazolidine analogues, anticancer activity, Knoevenangel condensation.

INTRODUCTION:

Heterocyclic compounds and their derivatives has been an interesting field in medicinal chemistry because of their biological and pharmacological properties[1]. Thiazolidine-2,4-diones nucleus is one of the most important heterocyclic that has received much attention due to its diversified molecular design and remarkable optical and electronic properties[2]. According to literature survey, thiazolidine-2,4-diones were reported to posess anti-bacterial[3], anti-tubercular[4], anti-cancer[5,6], anti-diabetic[7], anti-oxidant activities. The Knoevenangel condensation reaction of 5-arylidine thiazolidine-2,4-diones,which on further undergo mannich reaction with aromatic amines to yield mannich bases. This may results in the formation of worthwhile molecule with promising biological activity. From the above observations it was aimed to synthesize novel thiazolidine-2,4-dione analogues. The proposed molecule 5-(4-substituted)-3-{[(4-substituted)amino]methyl}-1,3-thiazolidine-2,4-dione that envisages a meaningful exploration for newer anticancer activities with minimum toxicity and high potency.

MATERIALS AND METHODS:

All the chemicals and reagents used in this research work were of analytical or synthetic grade. Melting point of the synthesized compounds was determined by open capillary method and is uncorrected. The IR spectra of the synthesized compounds were recorded using Perkin Elmer FT-IR Spectrophotometer in the range of 3500-500cm^{-1}. ^{1}H NMR of the synthesized compounds were recorded in DMSO on Bruker Ultra Shield DPX 500. Chemical shifts were reported in δ (ppm) relative to Tetra Methyl Silane (TMS) as internal standard. The reactions were monitored by thin layer chromatography with solvent system chloroform: methanol (9:1).

SYNTHETIC PROCEDURE:

Step 1: synthesis of thiazolidine-2,4-dione

A mixture of 56.5 g (0.6 M) of chloroacetic acid in 60 ml of water and45.6 g (0.6 M) of thiourea dissolved in 60 ml of water was taken in a 250 ml round bottom flask. The mixture was stirred for 15 min to obtain a white precipitate, accompanied by considerable cooling. To the content of the flask then added slowly 60 ml of concentrated hydrochloric acid. The mixture was then

**Corresponding author: T. T Akshaya || E-mail: akshayattpkv@gmail.com*

reflux for 8-10 hr at 100-110 °C. On cooling, the contents of the flask solidified into a cluster of white needles. The product was filtered and washed with water to remove traces of hydrochloric acid and dried.

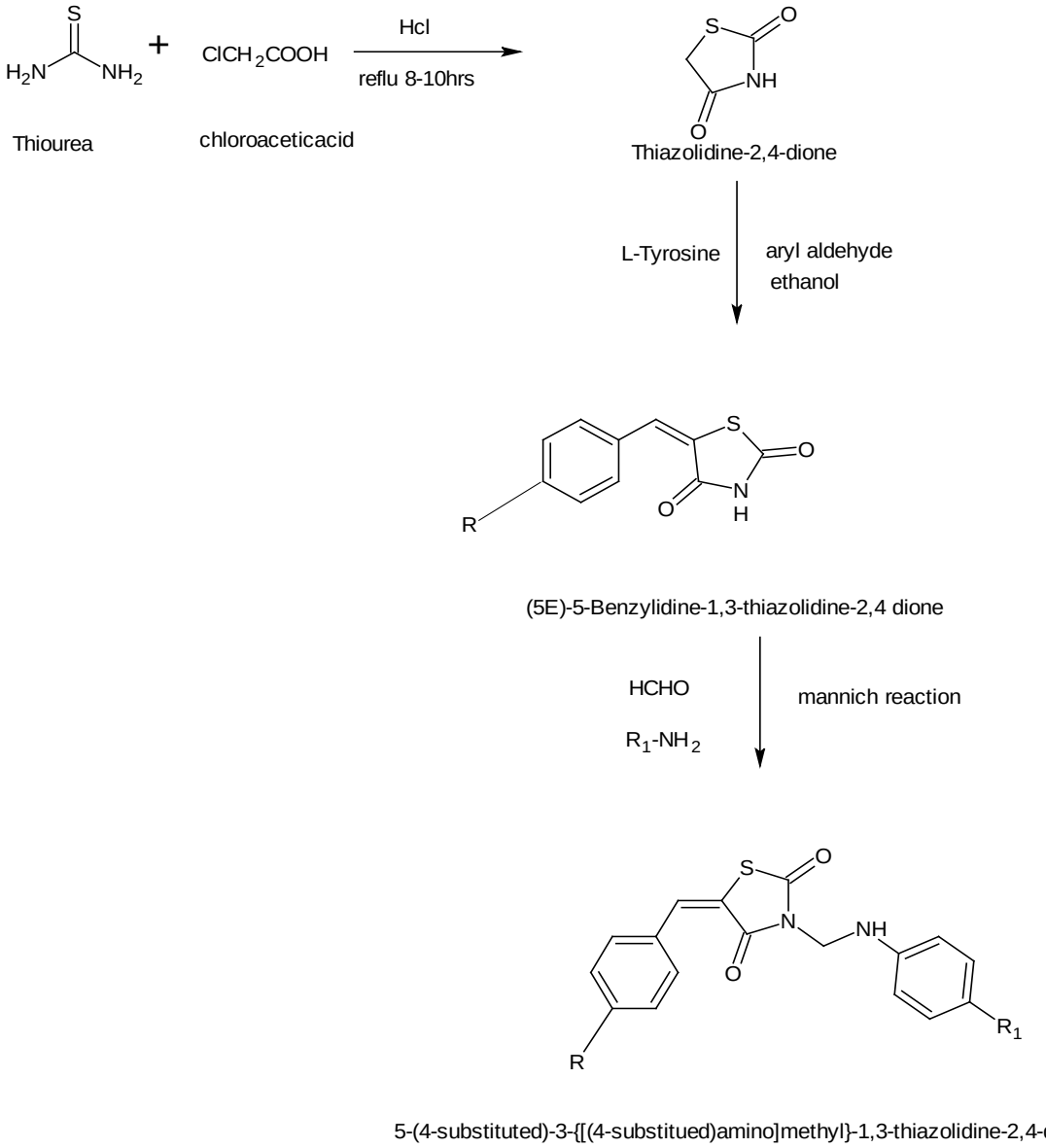
Step 2: Preparation 5-arylidine-thiazolidine-2,4-dione

A mixture of 2,4-thiazolidinedione (10 mmol) and L-tyrosine (2 mmol) was stirred in aqueous medium at room temperature for 10-15 min. After completion of reaction, the mixture was poured into ice-cold water (50 ml). The separated solid was filtered, washed with water(100 ml) and dried to obtain crude product. The latter were then recrystallised from ethanol to afford pure.

Step 3: Preparation of 5-(4-substituted)-3-{[(4-substituted) amino]methyl}-1,3-thiazolidine-2,4-dione

A mixture of 5-arylidene thiazolidine-2,4-diones(0.01mol), 2ml of methanol, formaldehyde (0.02mol), aromatic amine(0.02mol) and 1-2 drops of hydrochloric acid were refluxed for 2-4 hrs. The solvent was distilled off and the residue poured into crushed ice. The resulting solids were filtered off, dried and purified by re crystallisation from ethanol

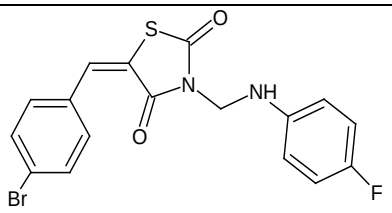
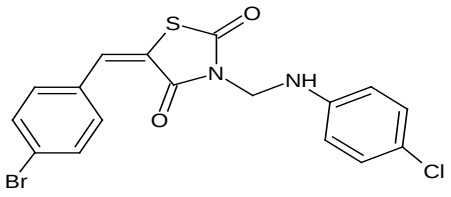
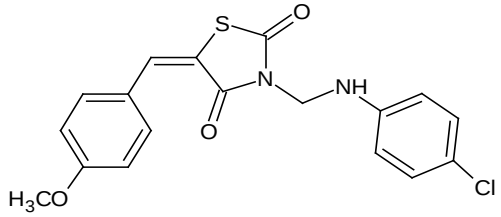
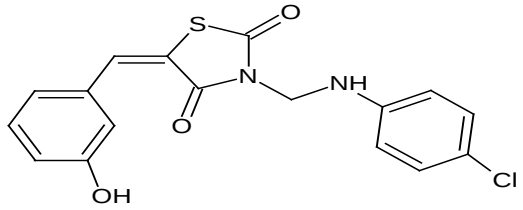
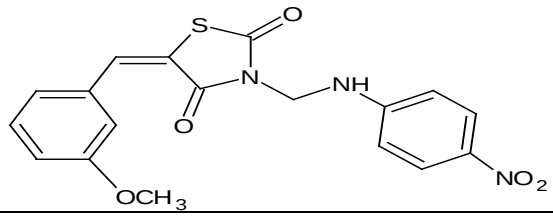
SYNTHETIC SCHEME



R= Cl, Br, I, OCH_3, OH

R_1= NO_2, Cl, F

Table 01: List of Synthesized compound

Compound code	Name of the compound	Structure of the compound
POD 1	5-(4-bromobenzylidene)-3-{[(4-flurophenyl)amino] methyl}-1,3-thiazolidine-2,4-dione	
POD 2	5-(4-bromobenzylidene)-3-{[(4-chlorophenyl)amino] methyl}-1,3thiazolidine-2,4-dione	
POD 3	5-(4-methoxybenzylidene)-3-{[(4-chlorophenyl) amino]methyl}-1,3-thiazolidine-2,4-dione	
POD 4	5-(3-hydroxybenzylidene)-3-{[(4chlorophenyl)amino] methyl}-1,3-thiazolidine-2,4-dione	
POD 5	5-(3-methoxybenzylidene)-3{[(4-nitrophenyl)amino] methyl}-1,3-thiazolidine-2,4-dione	

PHARMACOLOGICAL SCREENING:

The extract and fractions were studied for its cytotoxic effects on human breast carcinoma (MCF 7). MCF 7 was grown in Dulbecco's modified eagle's medium (DMEM) containing 10% foetal bovine serum. All cells were maintained at 37^0c, 5% CO_2, 95% air and 100% relative humidity. MTT is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living 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 propotional to the number of viable cells. After 48hr of incubation, 15 µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0c for 4hr. The medium with MTT was then flicked off and the formed formazan crystals were solubilised in 100µl of DMSO and then measured the absorbance at 570nm using micro plate reader. The percentage cell inhibition was determined using the following formula and the IC 50 was calculated using Graph Pad Prism software.

%Cell Inhibition = 100-Abs_{sample} / $Abs_{control}$×100

RESULTS:

Table 02: Preliminary characterization of newly synthesized compound

Compounds code	Molecular formula	Molecular weight	Melting point(^{0}c)	Percentage yield (%)	R_f value
POD 1	$C_{17}H_{12}BrFN_2O_2S$	434.26	158	65	0.87
POD 2	$C_{17}H_{12}BrClN_2O_2S$	419.29	176	78	0.86
POD 3	$C_{18}H_{15}ClN_2O_3S$	405.26	193	82	0.97
POD 4	$C_{17}H_{13}ClN_2O_3S$	407.25	120	88	0.88
POD 5	$C_{18}H_{15}N_3O_5S$	423.7	115	79	0.99

Table 03: spectral data of the synthesized compound

Compound code	IR	^{1}H NMR
POD 1	C=O Str(1566),C-N Str(1379),N-H Str(3344), C-S Str(808) C=C Str (1646),C-H Str(2852), C-Br Str(602), C-F Str(1379)	δ 7.5-7.387(m, 5H, ArH), δ 4.93(s,1H, NH, D_2O exchangeable), δ 1.718(d, 2H, CH_2 of $NHCH_2$),
POD 2	C=O Str(1563),C-N Str(1376),N-H Str(2914), C-S Str(805),C=CStr(1685), C-H Str(2847),C-Br Str (566),C-ClStr(676)	δ 1.253(d, 2H, CH_2 of $NHCH_2$), δ 7.25-7.61(m, 5H, ArH), δ 7.34-7.80(m, 5H, ArH)

PHARMACOLOGICAL SCREENING:

All the synthesized compounds showed moderate cytotoxic activity towards both cell lines. Among them POD 1 and POD 2 showed significant activity against MCF-7 cell lines.

Table 04: IC_{50} values of tested compounds on MCF-7

Compounds code	% cell inhibition					IC_{50} value (µg/ml)
	0.5µg/ml	1µg/ml	1.5µg/ml	2µg/ml	2.5µg/ml	
POD 1	1.69	3.65	11.35	38.27	74.84	51.4
POD 2	1.54	2.25	9.87	30.02	68.51	56.5
POD 3	1.36	3.16	11.33	54.57	64.85	82

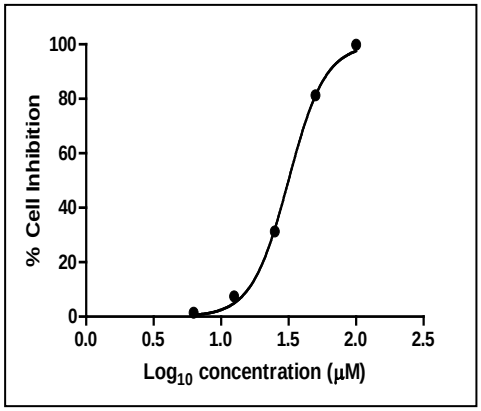


Figure: 1 Percentage growth inhibition curve of POD 1 on MCF7

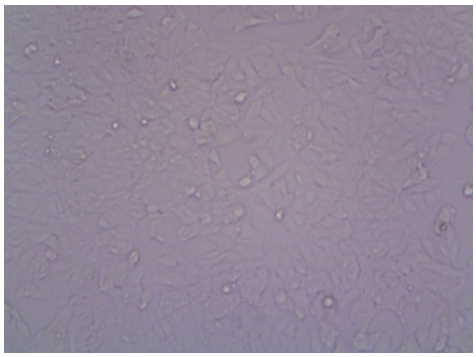


Figure: 2 Images of anticancer studies of POD 1 on MCF-7

DISCUSSION:

The preliminary *insilico* screening of various 2,4-thiazolidinedione analogues were performed to assess the drug like properties using Molinspiration software. Molecular structure were drawn using Chemsketch and various molecular properties like Molar volume, Parachor, Polarizability etc were generated. Drug likeness properties of the proposed analogues were studied and calculated. All the compounds obeyed the Lipinski rule of five. Docking studies of 27 different analogues were carried out against cancer targets like PPAR-γ using softwares, CORINA, Argus lab and Molegro molecular viewer. Among the docked molecules, POD 1 which is a p-bromo benzaldehyde derivative attached with a p-fluro aniline through mannich reaction showed better score. In this reaction, substitution occurs at the third position of the 2,4- thiazolidinedione ring. Since amines are strong electron withdrawing groups and easily susceptible to electrophilic substitution reaction. The literature review also revealed that, incorporation of amino groups showed a wide range of altered pharmacological activities. *In-vitro* cytotoxicity studies of the synthesized compounds were carried out by MTT assay on MCF-7 cell lines. The values obtained were plotted against % Growth inhibition and Concentration gives a non-linear graph which results in the IC_{50} values of each compound. Results revealed that POD 1 and POD 2 showed significant activity among the analogues as it exerts strong inductive electron withdrawing effect. Fluorine and chlorine is quite electro negative and has non-bonding valence electron that participate in conjugation and their strong inductive effect predominates.

CONCLUSION:

The compounds which showed better docking scores were screened for *invitro* anticancer activity carried out by MTT assay on MCF-7 cell lines. Among this POD 1 and POD 2 exhibited significant activity on the cell lines.Thus it was observed that POD 1 and POD 2 contain strong electron withdrawing atoms Bromine-Fluorine and Bromine-Chlorine respectively enhance the potency of drug while in POD 3 contain electron releasing substituent methoxy group have a reverse effect. So in future, by incorporating more electronegative atoms or ring systems into these derivatives, their activity can be enhanced and can be marketed as a good anticancer drug. So we can concluded that novel thiazolidine-2,4-diones hybrids analogues were considered as the future lead molecule for drug discovery.

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