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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME NEW COUMARINYL THIAZINE DERIVATIVES

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Abstract

A series of new substituted 3-(2-amino-6-phenyl-6H-1, 3-thiazin- 4-yl) -2H-chromen-2-one (PT1-PT5) were synthesized upon refluxing ethanolic solution of substituted 3-cinnamoyl-2H-chromen-2-one with thiourea in presence of 20% NaOH. The intermediate chalcones substituted 3-cinnamoyl-2H-chromen-2-one were synthesized by condensing 3-acetyl coumarin with various substituted benzaldehydes in presence of 20% NaOH. The structures of the final synthesized compounds were characterized by IR spectra. The antibacterial activity of the synthesized compounds was performed by tube dilution method. The turbidity was observed for the synthesized compounds. Few compounds showed promising minimum inhibitory concentration by tube dilution method.

Key words: 3-acetyl coumarin, chalcones, thiazine, antibacterial activity, minimum inhibitory concentration

Introduction

Coumarins owe their class name to 'Coumarou', the vernacular name of the tonka bean (Dipteryx odorata Willd., Fabaceae), from which coumarin itself was isolated in 1820. Coumarin (2*H*-1-Benzopyran-2-one) derivatives belong to one of the most widespread classes of natural compounds. They have been also found to exhibit antitumour (Rosskopf F *et al.*, 1992), antioxidant, anti-inflammatory (Kontogiorgis CA *et al*., 2005), antimicrobial (Hamdi M *et al*., 2008) and antidiabetic activities. The antibacterial activity of coumarin and other 45 coumarin derivatives have been tested against strains of *Bacillus cereus* MIP 96016, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923. Coumarin has fungicidal properties as well. These biological activities make coumarin compounds more attractive and testing as novel therapeutic compounds. Coumarin compounds can be synthesised by Pechmann, Knoevenagel, Perkin, Witty condensation methods. Chalcones are versatile molecules used for the synthesis of different heterocyclic compounds like thiazines, pyrazole, oxazine etc. Thiazine is a heterocyclic compound having four carbon atoms and one nitrogen and sulphur atom at varied positions in the six membered ring exist as 1, 2; 1, 3; 1, 4 -thiazines and subsequently their derivatives having N-C-S linkage have been used as antitubercular (Koketsu M *et al*., 2002), antibacterial (Mohamed JE *et al*., 2012), antitumor (Malah TE *et al*., 2018), insecticidal, fungicidal, herbicidal agents, tranquilizers and various dyes etc. 1, 3-thiazines are of great importance because they form part of the framework of cephalosporins (3, 6-dihydro-2*H*, 1, 3-thiazine). Based on the above observation it is worthwhile to prepare newer compounds for their antimicrobial activity. Herein an attempt is made towards the incorporation of thiazine with coumarin moiety to probe how this combination could influence the biological activity.

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Materials and Methods

All the chemicals were of analytical grade: substituted salicylaldehyde, ethylacetoacetate, absolute ethanol, piperidine, thiourea and substituted benzaldehyde.

Melting points were determined by open capillary method and are uncorrected. The purity of the compounds was monitored by thin layer chromatography (TLC) using

silica gel G plates. The spots were visualized under UV light. The homogeneity of the compounds were checked on silica gel-G coated plate by using n-Hexane:Ethylacetate (7 : 3) as solvent. All IR spectra were recorded in Alpha Bruker using ATR method.

Synthesis of 3-acetyl coumarin

A mixture of salicylaldehyde (0.05 mol) and ethylacetoacetate was added to 250ml conical flask. It was then condensed by adding sufficient piperidine dropwise with stirring in ice cold condition. The reaction mixture was then kept overnight in refrigerator. The solid lumps were broken in cold ethanol. The resulting yellow colored solid mass was then filtered and washed with cold ethanol to remove the excess piperidine. It was then recrystallised from ethanol to give white needle shaped crystals.

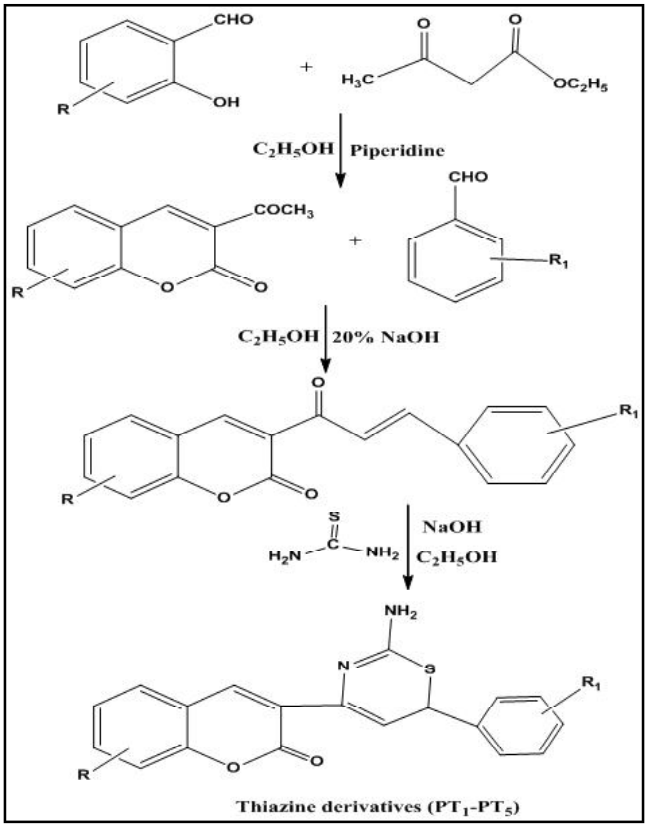

Fig. 1: Scheme for synthesis of coumarin incorporated thiazine derivatives.

Table 1: Physicochemical data of Substituted 1,3-Thiazine (PT1-PT5).

Comp. code	R	R_1	Mol. formula	Mol. wt	M.P °C	R_f Value	% Yield
PT1	H	4-Cl	$C_{19}H_{13}ClN_2O_2S$	368	198-200	0.70	78
PT2	4-OCH_3	3-NO_2	$C_{20}H_{15}N_3O_5S$	409	206-208	0.76	75
PT3	5-NO_2	4-Cl	$C_{19}H_{12}ClN_3O_4S$	413	210-212	0.68	69
PT4	5-Cl	4-Cl	$C_{19}H_{12}Cl_2N_2O_2S$	402	216-218	0.60	76
PT5	5-Cl	3-NO_2	$C_{19}H_{12}ClN_3O_4S$	413	226-228	0.64	58

Table 2: Antimicrobial activity of the compounds (PT1-PT5) by tube dilution method.

Comp-ound	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.125 µg/ml	MIC (µg/ml)
AO1	+	-	-	-	-	-	100
AO2	+	+	-	-	-	-	50
AO3	+	+	+	+	-	-	12.5
AO4	+	+	-	-	-	-	50
AO5	+	+	+	-	-	-	25

Synthesis of substituted 3-cinnamoyl-2*H*-chromen-2-one (Kotra V *et al*., 2011)

A mixture of 3-acetyl coumarin (0.01 mol) and different substituted benzaldehyde (0.01 mol) in 20 ml absolute ethanol was stirred together at room temperature for 24 hours in the presence of 20% NaOH. The completion of the reaction was monitored by TLC. The reaction mixture was then poured into crushed ice and acidified with 2N HCl with stirring. The product obtained was filtered, washed with water and recrystallised from ethanol.

3-(2-amino-6-phenyl-6*H*-1,3-thiazin-4-yl)-2*H*-chromen-2-one (PT1-PT5) (Joseph L *et al*., 2017)

A mixture of chalcones (0.01 mol), thiourea (0.01 mol) were dissolved in 10 ml of ethanolic NaOH and stirred for 5-6 hrs with a magnetic stirrer. The completion of the reaction was monitored by TLC. After the completion of the reaction, the reaction mixture was poured into crushed ice. The precipitated solid was filtered, washed with cold water and recrystallised from ethanol.

Determination of minimum inhibitory concentration by tube dilution method (Andrews JM., 2008)

The evaluation of all the synthesized compounds were done to evaluate the MIC by tube dilution method. Different concentrations were prepared to test the synthesized compounds along with the amoxicillin. The liquid medium was used for the dilution of the test samples that was inoculated with the standard number of organisms and was incubated for 24 hrs. The MIC is determined by selecting the lowest concentration that has turbidity in which growth does not occur.

Double concentration of the nutrient broth were prepared. Distribute each 5ml into 6 test tubes and label them A1 to A6 and sterilize for 30 min. in autoclave. Distribute 5ml of sample solution in one test tube and 5ml of solution from 1st test tube to second. Serial dilution of the same was performed (100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.12 µg/ml). Inoculate microorganism *E. coli* into each test tube by aseptic

method. Keep it in incubator for 24 hrs and then observe the turbidity.

Results and Discussion

Spectral data

3-(2-amino-6-(4-chlorophenyl)-6*H*-3-thiazin-4-yl)-2*H*-chromen-one (PT1)

IR KBr (cm^{-1}): 1557 (Ar C=C str), 1664 (C=O str), 1231 (C-S-C str), 3382 (N-H str), 752 (C-Cl str).

3-(2-amino-6-(3-nitrophenyl)-6*H*-1,3-thiazin-4-yl)-5-methoxy-2*H*-chromen-one (PT2)

IR KBr (cm^{-1}): 1527 (Ar C=C str), 1682 (C=O str), 1275 (C-S-C str), 3392 (N-H str), 1349 (Ar-NO_2 str)

3-(2-amino-6-(3-nitrophenyl)-6*H*-1,3-thiazin-4-yl)-6-chloro-2*H*-chromen-one (PT5)

IR KBr (cm^{-1}): 1528 (Ar C=C str), 1698 (C=O str), 1229 (C-S-C str), 3360 (N-H str), 1394 (Ar-NO_2 str), 735 (C-Cl str).

Antibacterial activity

The different coumarinyl 1, 3-thiazine derivatives were evaluated for their antibacterial activity by tube dilution method. In negative control no growth was observed and in positive control growth was observed. Compound PT3 and PT5 showed significant antibacterial activity with MIC value of 12.5 µg/ml and 25 µg/ml respectively**.** The presence of electron withdrawing groups like nitro and chloro resulted in increased antibacterial activity.

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