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Screening of antibacterial activity of some newly synthesised N-(4'oxo-2'-(aryl/ alkyl substituted) thiazolidin-2-one) -3-carboxamido-2H-chromen-2-ones

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ABSTRACT

The synthesis of a range of N-[4'-oxo-2'-(substituted phenyl)-thiazolidin-3'-yl]-3-carboxamido-2H-chromen-2-one (IV a-b, d, f, h-m) by condensation of N-(substituted benzylidene)-3-carbohydrazide-2H-chromen-2-one (III a-c, d, f, h-m) with thioglycolic acid in dimethyl formamide and Aluminium trichloride (0.05gms) and their evaluation of antibacterial activity by agar disc diffusion method. All the newly synthesized compounds tested for their Minimum Inhibitory Concentration (MIC) against all tested Gram positive and Gram-negative bacteria by micro dilution Broth method. Parent compound showed good activity. All compounds revealed better results against Gram positive as compared to Gram-negative bacteria. IV-b and IV-d were exhibited higher antibacterial activity when compared with all the synthesized analogues against all the tested micro organisms. Some of the coumarin derivatives showed more or less same activity indicating that number and position of substituted groups are not important regarding antibacterial activity. In addition IV-j was found most potent compound showing broad spectrum of antibacterial activity.

Key words: Coumarin, Thiazolidinones, Antibacterial activity, Disc diffusion method, Benzylidene derivatives.

INTRODUCTION

The increasing number of pathogenic bacteria that are resistant to the commonly used therapeutic agents is a major worldwide health problem. For all the reasons the search for new antibacterial agents with novel modes of action represents a major target in chemotherapy.[1-2] Coumarin

derivatives play a vital role in biological fields such as anticoagulant [3] [William R Sullivan, et.al., (1943)], Antimicrobial[4] [Mandour A. H, et.al., (2004)], CNS Depressant [5] [Imthyaz khan, et.al., (1999)], Platelet aggregation Inhibitors [6] [Chen Yeh Long et.al.,(1996)], Antitumor [7] [Mladen Kornick T, et.al.,(1998)] activities. 3-Substituted coumarin derivatives have been found to be biologically most potent. Thiazolidinones represent an essential group of natural as well as synthetic products and some of them possess wide range of pharmacological activity such as antibacterial and anticoagulant [8] [Mulwad V V et.al., (2002)], antifungal [9] [Min Ji, et.al., (2001)], antitubercular [10][Hankare P P, et.al., (2001)], anthelmintics [11] [Sonal Shah, et.al., (1999)], antimicrobial [12] [Aditya Vardhan V, et.al., (1999)]. The presence of reactive keto group in coumarin is found to be responsible for their biological activity. In the present work coumarin thiazolidinones derivatives have been prepared according to Knoevenagal condensation by condensing salicylaldehyde with dimethyl maloanate. The structures of the synthesized compounds were elucidated on the basis of their elemental analysis, IR, 1H NMR spectroscopic data. These compounds were screened for their antibacterial activity by using standard drug Amoxycillin and Ciprofloxacin at the concentration of 10 $\mu g/$ disc at the concentration of 100 and 250 $\mu g/$ disc.

MATERIALS AND METHODS

1.0. Chemistry

The reagents / chemicals / solvents used during the course of these Studies were obtained from Merck (India), SD Fine and CDH Laboratories and were of the laboratory grade. The solvents were purified by distillation before their use. The solvent systems used for Thin Layer Chromatography were given in the experimental procedure. Silica Gel G used for TLC was CDH brand. Iodine chamber and UV lamps were used for visualization of TLC spots. Whatmann filter paper (No. 1, England) was used for filtration (vacuum or ordinary). Melting points of all the compounds were recorded in liquid paraffin bath in open capillary tubes and are uncorrected.

Synthetic work was started from reacting salicylaldehyde with diethyl maloanate in presence of piperidine and glacial acetic acid in ethanol by Knoevenagal condensation method into 3-carbethoxy coumarin, prepared coumarin was converted into its corresponding acid hydrazide derivative by using hydrazine hydrate in ethanol and finally it was converted to expected compound using appropriate reagents. In this work mentioned the preparation of N-(4'oxo-2'-(substituted aryl/heteryl thiazolidin-2-one)-3-carboxamido-2H-chromen-2-one (IV $_{a\text{-}b,\ d,\ f,\ h\text{-}m}$) from N-(substituted aryl / heteryl)-3-carbohydrazide-2H-chromen-2-ones (III $_{a\text{-}d,\ f,\ h\text{-}m}$) and screened for their antibacterial activity against tested bacteria by following standard methods.

2.0. Invitro Antibacterial Activity: Antibacterial activity of the newly synthesized Compounds were screened for their antibacterial activity against the entire tested organism by comparing with standard compounds like Amoxycillin and Ciprofloxacin (10 µgm /disc) at various concentrations and determined Minimum Inhibitory Concentration by Mueller Hinton Broth serial dilution method. MIC values were considered to be the lowest concentration that may inhibit growth on agar plates.

2.1.1. Preparation of the medium: Mueller – Hinton broth gelled by the addition of 2% Agar [Bacteriological grade]. Casein Enzymic Hydrolysate (17.5 gm/lit), Beef infusion (300 gm / lit),

Soluble Starch (1.5 gm/lit) were dissolved in distilled water with the aid of heat, pH was adjusted to 7.2 – 7.6 using alkali or dilute acid.

2.1.2. Compounds and solvents: Newly synthesized compounds of (III $_{a\text{-}c,\ d,\ f,\ h\text{-}m}$) and (IV $_{a\text{-}b,\ d,\ f,\ h\text{-}m}$) were screened for antibacterial activity against *Staphylococcus aureus* MTCC 1044, *E.coli* MTCC 1089, *Bacillus subtilis* MTCC 121 and *P.aeruginosa* MTCC 1034 using Amoxycillin and Ciprofloxacin (10 $\mu g/$ disc) as standard at the concentration of 100 and 250 $\mu g/$ disc.

2.1.3. Sterilization: 15 – 20 ml of Mueller – Hinton agar was transferred to test tubes and sealed with non absorbent cotton. It was then autoclaved at a pressure of 15 psi (121 ^{0} C) for 15 minutes.

2.1.4. Organisms Used: *Staphylococcus aureus* MTCC 1044, *E.coli* MTCC 1089, *Bacillus subtilis* MTCC 121 and *P.aeruginosa* MTCC 1034 include remaining which were collected from National Chemical Laboratory, Pune. The strain was confirmed for its purity and identity by Gram's staining method and its characteristic biochemical reactions. The selected strain was preserved by sub culturing them periodically on agar slants and storing them under frozen conditions. For the study, fresh 24 hrs broth cultures were used. The entire work was done using horizontal laminar flow hood so as to provide aseptic conditions. Before commencement of the work, air sampling was carried out using a sterile nutrient agar plat and exposing it to the environment inside the hood. On incubation it was checked for the growth of microorganism and absence of growth confirmed aseptic working conditions.

2.1.5. Preparation of Inoculums: The inoculums for the experiment were prepared fresh in Mueller Hinton Broth from preserved frozen slant culture. It was kept incubated at 37^o C for 24 hrs. Mueller – Hinton agar plates were prepared aseptically to get a thickness of 5- 6 mm. The plates were allowed to solidify and inverted to prevent the concentrate falling on the agar surface. The plates were dried at 37^0 C before Inoculation. The organisms are inoculated in the plates prepared earlier, by dipping a sterile swab in the previously standardized inoculum, removing the excess of inoculum by pressing and rotating the swab firmly against the sides of the culture tube, above the level of the liquid and finally streaking the swab all over the surface of the medium three times, rotating the plates through an angle of 60^0 C after each application. Finally press the swab round the edge of the agar surface. Leave it to dry at room temperature with the lid closed. The sterile disc containing test drugs, standard and blank were placed on the previously inoculated surface of the Mueller – Hinton agar plate and were kept in the refrigerator for one hour to facilitate uniform diffusion of the drug. Plates were prepared in triplicate and it was then incubated for 18 – 24 hours. Observations were made for zone of inhibition around the drugs and compared with that of standard. All the compounds synthesized were tested for antibacterial activity against gram (+) ve and gram (-) ve bacteria.

3.0. Determination of minimum inhibitory concentration

3.1.1. Strain Selection and Preservation: The non - pathogenic strain used for the study *Staphylococcus aureus* MTCC 1044, *E.coli* MTCC 1089, *Bacillus subtilis* MTCC 121 and *P.aeruginosa* MTCC 1034 were collected and the strain was confirmed for its purity and identity by Gram's staining method, and its characteristic biochemical reactions. The selected strain was

preserved by sub culturing them periodically on agar slants and storing them under frozen conditions. For the study, fresh 24 hrs broth cultures were used.

3.1.2. Nutrient Media Preparation: 21.0 gms of dehydrated Mueller- Hinton Media broth was dissolved in 1000 ml distilled water by boiling. The pH was Adjusted to 7.4 ± 0.2. Sterilization was done by autoclaving at 15 psi pressure (121˚C) for 15 minutes.

3.1.3. Sterilization of Apparatus and Media used: The Mueller Hinton media were Sterilized by moist heat (autoclaving) at 121˚C for 15 minutes at 15 psi. The glass Petri dishes, pipettes, culture tubes etc were sterilized by dry heat (Hot air oven at 160˚ C for 2 hour).

3.1.4. Working Conditions: The entire work was done using horizontal laminar flow hood so as to provide aseptic condition. Before commencement of the work, air sampling was carried out using a sterile nutrient agar plate and exposing it to the environment inside the hood. On incubation it was checked for the growth of microorganism and absence of growth confirmed aseptic working conditions.

3.1.5. Preparation of Inoculum: The inoculum for the experiment was prepared fresh in Mueller Hinton broth from preserved frozen slant culture .It was kept incubated at 37˚C for 24 hours and used for the study after dilution to give 1:10 or 1:100 dilutions.

3.1.6. Standardization of Inoculum: The test organism used was diluted to 1: 100 after Overnight incubation and standard drop (0.01ml) was used for the Minimum Inhibitory Concentration determination.

4.0. *In vitro* Determination of Minimum Inhibitory Concentration of IV-b: *In vitro* Determinations of MIC of Thiazolidinones (IV a, b, d, f, h-m) were carried out by serial dilution technique in Mueller-Hinton broth using *Staphylococcus aureus* MTCC 1044, *E.coli* MTCC 1089, *Bacillus subtilis* MTCC 121 and *P.aeruginosa* MTCC 1034. The concentration range of all the newly synthesized compounds were screened for MIC determination was 512 µg/ml to 2.0 µg/ml. The test organisms used was diluted to 1:100 after overnight incubation and a standard drop (0.01ml) was used for the Minimum Inhibitory Concentration determination.

4.1.1. Procedure: Test tubes were labeled with number 1 to 9 and 0.5 ml of Mueller Hinton broth was added to each tube and 0.5ml of diluted stock solution [1024 µg/ml] was added to the first tube and serially 0.5 ml were transferred through tube No. 8 to obtain the quantities indicated. Each pipette was discarded after transfer and a fresh pipette was used for mixing and transferring to the next tube. 0.5ml was discarded from the 8 th tube. The 9 th was used for the control. With a standardized micro pipette a drop of the diluted broth culture of the test organism (approximately 0.01ml) was added to all the tubes, including the control and blank. Mixed gently and Incubated at 37 ˚C for 16 to 18 hrs. Readings were observed and a duplicate was performed. Blank and positive blank were also kept to confirm proper sterilization and suitable conditions of growth of microorganism respectively. The Minimum Inhibitory Concentration was interpreted as the highest dilution of the compound which shows clear fluid with no development of turbidity.

RESULTS AND DISCUSSION

In this work Series of synthesized compounds (III $_{a\text{-}c,\ d,\ f,\ h\text{-}m}$) and (IV $_{a\text{-}b,\ d,\ f,\ h\text{-}m}$) were dissolved in dimethyl sulphoxide and tested against both gram positive and gram negative bacteria by conventional agar disc diffusion method and listed in Table No-1 and 2.

Antibacterial Screening:

i. N-(substituted benzylidene) -3- carbo hydrazide - 2H - chromen - 2 - ones derivatives (III $_{a\text{-}c,\ d,\ f,\ h\text{-}m}$) at 100 µg/ disc: Among the test compounds in N-(substituted benzylidene)-3-carbohydrazide-2H-Chromen-2-ones, compound III $_b$ and III $_m$ showed maximum zone of inhibition of 24 mm and 22 mm and were considered to be sensitive against gram positive bacteria *S. aureus* and it is slightly sensitive to *P. aeruginosa.* On the other hand, compound III $_b$ showed good activity against gram negative bacteria *E. coli* with zone of inhibition of 20 mm where as compound III $_m$ fails to exhibit activity against *E. coli*. Hence, compound III $_b$ could be considered as superior in antibacterial activity against both gram positive and negative bacteria and where as compound III $_m$ is only superior in its activity against gram positive bacteria. Compound III $_f$ showed zone of inhibition of 17 mm against *S. aureus* and considered to be slightly sensitive and remaining compounds are moderate in its activity against *S. aureus* but compound III $_l$, III $_i$, III $_k$ exhibited 18, 17 & 16 mm zone of inhibition respectively against *P. aeruginosa*. Compound III $_j$ showed maximum inhibition of 17 mm against gram negative bacteria *E. coli* followed by compound III $_f$ (14 mm, zone of inhibition) and both these compounds were considered to be slightly sensitive against *E. coli*. Compound III $_d$, III $_i$, III $_l$ and III $_m$ were found to be least active compound against gram negative bacteria which are showed 8-9 mm zone of inhibition. All the screened compounds were slightly active against *B.subtilis* at the tested concentration.

N-(substituted benzylidene) -3- carbo hydrazide - 2H - chromen - 2 - ones derivatives (III $_{a\text{-}c,\ d,\ f,\ h\text{-}m}$) at 250 µg/ disc: Most of the compounds did not show any increase in the zone of inhibition against gram positive bacteria *S. aureus, P. aeruginosa* and also against gram negative bacteria *E. coli* when compared to the zone of inhibition of test compounds at 100 µg/ disc except few compounds. Compound III $_m$ showed maximum zone of inhibition of 24 & 23 mm against gram positive bacteria *S. aureus* and *P. aeruginosa* where as compound III $_b$ showed zone of inhibition of 24 & 19 mm against *S. aureus* and *P. aeruginosa.* Both these compounds could be considered as sensitive to gram positive bacteria followed by III $_l$, III $_m$ and III $_k$. Remaining all compounds exhibited moderate in action against gram positive bacteria. Compound III $_b$ & III j showed 18 mm zone of inhibition against gram negative bacteria *E coli*, considered to be slightly sensitive to the organism. Other compounds showed very less sensitivity towards the gram negative bacteria. All the screened compounds were moderatively active against *B.subtilis* at the tested concentration.

ii. **N-[4'- oxo - 2' - (substituted aryl/ heteryl) - thiazolidin-3'-yl] – 3 - carboxamido- 2H-chromen-2-ones (IV $_{a\text{-}b,\ d,\ f,\ h\text{-}m}$) at 100 and 250 µg/ disc:** All the synthesized N-[4'- oxo - 2' - (substituted aryl/ heteryl) - thiazolidin-3'-yl] – 3 - carboxamido- 2H-chromen-2-one (IV $_{a\text{-}b,\ d,\ f,\ h\text{-}m}$) derivatives were sensitive to the gram positive and gram negative bacteria at a concentration of 100 and 250 µg/ disc and not sowed any differences in the zone of inhibition between the concentration but their activity was not comparable to the standard drug. The standard drug,

Amoxycillin showed 35 – 45 mm zone of inhibition against gram positive bacteria *S. aureus* & *P. aeruginosa* and 26 – 29 mm of zone of inhibition against gram negative bacteria *E. coli* at a concentration of 10 µg/ disc. Among the Carboxamido-2H-chrome-2-one derivatives, compound IV $_b$ and IV $_m$ showed zone of inhibition of 19 mm and 18 mm respectively and could be considered as slightly sensitive to the organism *S. aureus* but compound IV $_f$ & IV $_j$ showed zone of inhibition of 24 mm against *P. aeruginosa* and highly active against *B.subtilis* followed by compound IV $_k$, IV $_d$, IV $_b$ and IV $_l$ showed 18 – 22 mm zone of inhibition. All these compounds are considered to be sensitive to gram positive bacteria *P. aeruginosa*. Compound IV $_j$ considered to be superior in activity against gram negative bacteria *E. coli*, which showed zone of inhibition of 23 mm at 250 µg/ disc followed by IV $_d$ and IV $_b$ showed 19-21 mm of zone of inhibition for both the concentrations. Remaining all the compounds showed moderate action against gram negative bacteria. Compound IV $_k$ is considered to be the least active in this series and showed only 10-12 mm zone of inhibition against bacteria.

SCHEME

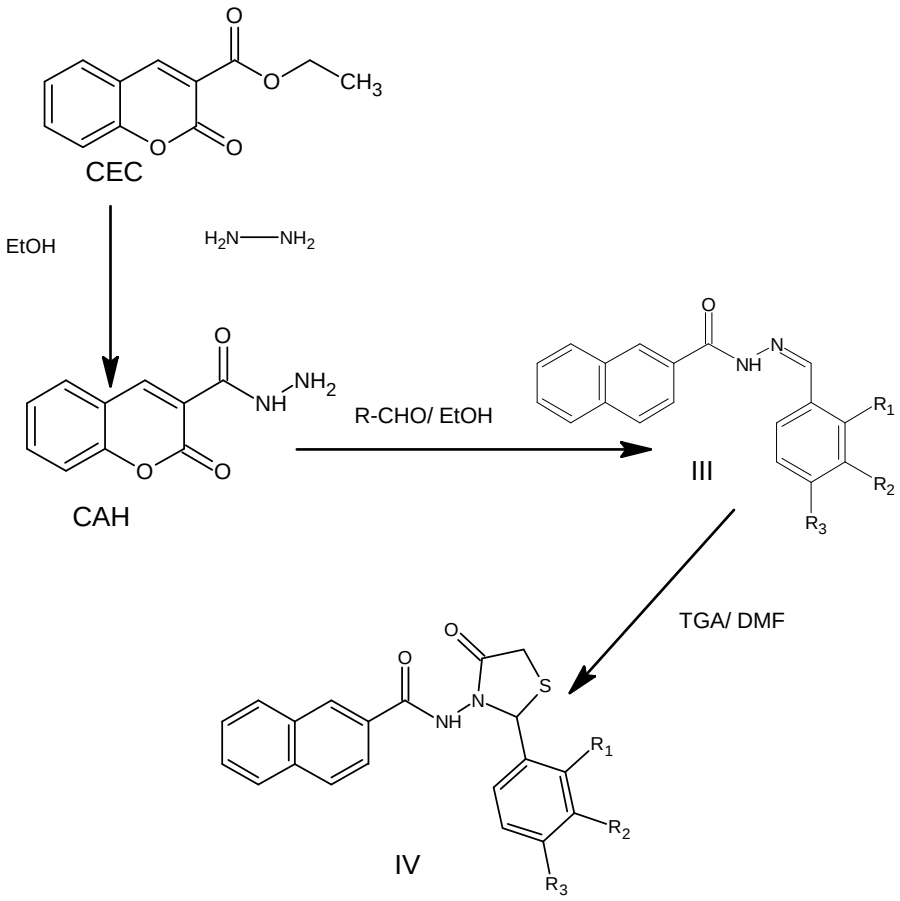

Table No.: 1 ANTIBACTERIAL ACTIVITY OF N-(SUBSTITUTED BENZYLIDENE)-3-CARBOHYDRAZIDE-2H-CHROMEN-2-ONE DERIVATIVES (III $_{a- d, f, h-m}$)

Compound No	Conc. (µg/ml)	Zone of Inhibition (in mm)							
		S. aureus Gram +ve		*Bacillus subtilis Gram +ve*		*E. coli Gram -ve*		*P.aeruginosa Gram -ve*	
		Test	*Std* 10µg / disc	*Test*	*Std* 10µg/ disc	*Test*	*Std* 10µg/ disc	*Test*	*Std* 10µg/ disc
III $_a$	100µg/ disc	08	39	11	28	14	34	12	35
	250µg/ disc	12		10		15		12	
III $_b$	100µg/ disc	24	39	14	28	20	34	16	35
	250µg/ disc	24		15		18		20	
III $_c$	100µg/ disc	13	39	09	28	11	34	09	35
	250µg/ disc	15		13		13		15	
III $_d$	100µg/ disc	13	38	11	28	09	34	11	35
	250µg/ disc	14		10		10		15	
III $_f$	100µg/ disc	17	37	11	28	14	34	11	36
	250µg/ disc	19		12		15		16	
III $_h$	100µg/ disc	12	8	14	28	11	34	14	36
	250µg/ disc	15		14		15		18	
III $_i$	100µg/ disc	11	39	12	28	09	29	17	34
	250µg/ disc	13		14		12		19	
III $_j$	100µg/ disc	14	36	15	28	17	29	14	34
	250µg/ disc	13		16		18		18	
III $_k$	100µg/ disc	12	36	13	28	11	29	16	34
	250µg/ disc	13		15		13		18	
III $_l$	100µg/ disc	14	36	14	28	08	29	18	34
	250µg/ disc	15		14		10		19	
III $_m$	100µg/ disc	22	39	12	28	09	29	19	34
	250µg/ disc	24		14		10		23	
Blank	DMF	-		-		-		-	

Table No.: 2 ANTIBACTERIAL ACTIVITY OF N-[4'-OXO-2'-(SUBSTITUTED ARYL / HETERYL)-THIAZOLIDIN -3'-YL]-3-CARBOXAMIDO-2H-CHROMEN-2-ONES (IV $_{a\text{-}b,\ d,\ f,\ h\text{-}m}$)

Compound No	Conc. (µg/ml)	Zone of Inhibition (in mm)							
		S. aureus Gram +^{ve}		*Bacillus subtilis Gram +^{ve}*		*E. coli Gram -^{ve}*		*P.aeruginosa Gram -^{ve}*	
		Test	*Std* 10µg / disc	*Test*	*Std* 10µg/ disc	*Test*	*Std* 10µg/ disc	*Test*	*Std* 10µg/ disc
IV $_{a}$	100µg/ disc	10	41	10	28	12	29	18	38
	250µg/ disc	12		12		14		19	
IV $_{b}$	100µg/ disc	19	38	12	28	19	29	18	38
	250µg/ disc	19		14		21		22	
IV $_{d}$	100µg/ disc	13	39	09	28	20	29	20	37
	250µg/ disc	15		11		21		22	
IV $_{f}$	100µg/ disc	14	38	12	28	18	29	24	36
	250µg/ disc	15		14		15		24	
IV $_{h}$	100µg/ disc	15	39	10	28	11	26	20	36
	250µg/ disc	16		12		13		18	
IV $_{i}$	100µg/ disc	10	41	12	28	16	26	15	35
	250µg/ disc	10		14		15		15	
IV $_{j}$	100µg/ disc	11	45	12	28	21	26	24	24
	250µg/ disc	11		13		23		24	
IV $_{k}$	100µg/ disc	11	43	14	28	11	26	21	38
	250µg/ disc	12		17		10		22	
IV $_{l}$	100µg/ disc	15	41	14	28	12	26	18	37
	250µg/ disc	16		15		12		22	
IV $_{m}$	100µg/ disc	18	36	12	28	13	26	18	37
	250µg/ disc	18		14		13		20	
Blank	DMF	-		-		-		-	-

***In vitro* Antibacterial activity**

iii. Coumarin and thiazolidinones derivatives are very interesting components in terms of their biological properties. Newly synthesized compounds were tested against a panel of microorganisms including gram positive and gram negative bacteria by using micro dilution broth method. The MIC values for these compounds IV- (a-b, d, f, h-m) was evaluated by comparison to standard drug cefoxime as reference drug. Newly synthesized compounds were tested against organism displayed a significant antibacterial activity with wide degree of variation as show in Table- 3. This test was performed at different concentrations of synthesized compound (512 µg/ml to 2.0 µg/ml) in Mueller Hinton broth medium.

Minimum Inhibitory Concentration of IV- (a-b, d, f, h-m) was determined against *P.aeruginosa* MTCC 1034**,** *E.coli* MTCC 1089 and *Staphylococcus aureus* MTCC 1044 and *Bacillus subtilis* MTCC 121. Nitro group containing compound (IV-j) was found to be active against all tested gram positive and gram negative bacteria than other group containing compounds. MIC of the synthesized compounds was listed in Table No-3.

Table No.: 3 *INVITRO* ANTIBACTERIAL ACTIVITY OF N-[4'-OXO-2'-(SUBSTITUTED ARYL / HETERYL)-THIAZOLIDIN -3'-YL]-3-CARBOXAMIDO-2H-CHROMEN-2-ONES (IV $_{a\text{-}b,\ d,\ f,\ h\text{-}m}$)

Compound code	*Gram Positive Bacteria*		*Gram Negative Bacteria*	
	Staph. aureus	***Bacillus subtilis***	***E. coli***	***P.aeruginosa***
CEC	128	256	256	256
CAH	128	256	256	64
IV $_a$	256	256	256	256
IV $_b$	8	8	32	4
IV $_d$	8	8	8	4
IV$_f$	128	128	32	64
IV $_h$	128	256	64	256
IV $_i$	256	128	256	128
IV $_j$	8	8	8	256
IV$_k$	32	128	32	4
IV $_l$	256	64	256	128
IV$_m$	32	64	32	128
Standard Cefoxime	2	4	2	2

CONCLUSION

The synthetic route of thiazolidinones from benzylidene is presented in the scheme. The antibacterial activities of the molecules are reported, In this study, we compared the antibacterial activity of coumarin acid hydrazide and all its thiazolidinones derivatives was found to be IV-j most bacteriocidal properties and active compound against all tested gram (+) and gram (-) organism among the entire series. From the antibacterial screening it was observed that all the compounds exhibited activity against all the organisms employed at the concentration of 100 and 250 µg/ disc. Looking at the structure activity relationship, marked inhibition in bacteria was observed in the compounds bearing Nitro phenyl and hydroxy phenyl substituent where as other compounds showed moderate to good activity. All the newly synthesized compounds were effective against all of the tested non pathogenic microorganisms. However, IV-a, IV-g & IV-i

had no appreciable inhibitory activity against all screened bacteria. As we consider all results obtained from antibacterial tests together we can say that entire compounds tested are active towards bacteria. From this study it can be concluded that many coumarin derivatives possess antibacterial activity.

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