Synthesis and Biological Activity Evaluation of Some Azetidinone and Thiazolidinone Derivatives of Coumarins

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ABSTRACT: Keeping in view the pharmacological potential of azetidinones, thiazolidinones and coumarins, the title compounds containing these nuclei were synthesized. The 4-methyl -7-hydroxy coumarin (**1)** on treatment with hydrazine hydrate affords 2-hydrazo- 4-methyl -7-hydroxy coumarin (**2**). The N- (2'-imino-4'-methyl-7'-hydroxy coummarinyl)-imino substituted benzene (**3**) was synthesized by reaction of compound **2** with various aromatic aldehydes. Condensation of compound **3** with chloroacetyl chloride in presence of 1,4-dioxan and triethyl amine yields the 3-chloro-4- (substituted)-1-(2'-imino-4'-methyl-7'-hydroxy coumarinyl) azetidin-2-one (**4a-d**). Further more condensation of **3** with thioglycollic acid in presence of 1,4-dioxan and anhydrous aluminium chloride gives 2-(substituted phenyl)-3-(2'-imino-4'-methyl-7'-hydroxy coumarinyl)-1,3-thiazolidinone (**4'a-d**). Elemental and spectral characterization established the identity of these compounds. All the products were screened *in vitro* for their anti microbial activity against different strains of urinary tract pathogens. All compounds exhibited significant antimicrobial activity compared to the standard drug nitrofurantoin.

Kew words: Azetidinones, thiazolidinone, coumarin, nitrofurantoin, *E. coli, P. auregenosa, K. pneumoniae, P. mirabilis E. faecalis,* and *S.aureus*.

INTRODUCTION

Azetidinones and thiazolidinone are well known for various biological activities such as antimicrobial,[1] anticancer,[2-3] antidiabetics[4-5] etc. Further, more coumarin nucleus[6-7] has proves to be of great importance in exhibiting and enhancing the biological activities. Thus, with an effort to capitalise the pharmacological potential of the above heterocylic nucleus and to synthesise biologically potential compounds, the title compounds have been investigated. The compounds 3-chloro-4- (substituted)-1-(2'-imino-4'-methyl-7'-hydroxy coumarinyl) azetidin-2-one **(4a-d)** and 2- (substituted phenyl)-3-(2'-imino-4'-methyl-7-'hydroxy coumarinyl)-1,3-thiazolidinone **(4'a-d)** were synthesized. The structure of the compounds has been supported by elemental analysis, IR, and ^{1}H NMR spectral study. All the compounds have been screened *in vitro* for their antimicrobial activity against different strains of gram negative *E. coli, P. auregenosa, K. pneumoniae, P. mirabilis* and gram positive *E. faecalis* and *S.aureus*.

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MATERIALS AND METHODS

Melting points were determined in open capillary tubes and are uncorrected. Infrared spectra were recorded on Perkin Elmer model 600 spectrophotometer using KBr pellet. ^{1}H NMR spectra

were recorded on a Bruker DRX-300 NMR spectrophotometer (300 MHz) using TMS as internal standard. The CHN elemental analysis was carried out using elemental analyzer supplied by EURO-EA. Nitrofurantoin was used as the reference drug for antimicrobial activity comparison. The results obtained are expressed in mean ± S.E.M. values.

Synthesis of N- (-2'-imino-4'-methyl-7'-hydroxycoumarinyl) substituted benzyl imines (3a-d). An equimolar (0.1 mol) amount of resorcinol and ethyl acetoacetate were mixed. To this 100 ml of cold sulfuric acid at 10°C was added with constant stirring. The mixture was kept for 20 hrs at room temperature and was poured with vigorous stirring onto crushed ice. The product separated was filtered, washed and recrystallised from alcohol to yield 4-methyl-7-hydroxy- coumarin **(1)**. Hydrazine hydrate (0.057 mol) and compound **1** (0.057 mol) were mixed with 100 ml of ethanol. Few drops of glacial acetic acid were added to it and the mixture was refluxed for 2 hrs. The mixture was then cooled and mixed with ice-cold water. The precipitate formed was filtered dried and recrystallised from 1,4-dioxan to yield 2-hydrazo-4-methyl-7-hydroxy coumarin **(2)**. To the compound **2** (0.02 mol) equimolar aromatic aldehydes were added followed by addition of few drops of glacial acetic acid. The mixture was refluxed for 2 hrs. The products so formed were cooled, dried and recrystallised from 1, 4-dioxan to produce the compounds, **3a-d**.

Synthesis of 3-chloro-4- (-o-hydroxy phenyl)-1-(2'-imino-4'-methyl-7'-hydroxycoumarinyl) azetidn-2-one (4a). An equimolar mixture of compound 3a and chloroacetyl chloride was taken in 100 ml of 1,4-dioxan. To this 4 to 5 drops of triethylamine was added and the mixture was stirred for 4 hrs. The reaction mixture was kept at room temperature for two days. It was then poured onto crushed ice and water. The product, 4a was filtered, washed, dried and recrystallised from dichloro-methane. The other compounds of the series (4a-d) were prepared in the above manner.

Synthesis of 2-(o-hydroxyphenyl)-3-(2'-imino-4'-methyl-7'-hydroxycoumarinyl)-1,3-thiazolidin-4-one (4'a). To the mixture of compound 3a (0.01 mol) and thioglycollic acid (0.01 mol) in 100ml of 1,4-dioxan few milligrams of anhydrous aluminum chloride was added. The mixture was refluxed for 12 hrs. After cooling it was triturated with an excess of 10% sodium bicarbonate solution. The product 4'a was filtered, washed, dried and recrystallised from 1, 4-dioxan. The other target compounds of the series 4'a-d were synthesized following the above process.

3-chloro-4- (o-hydroxyphenyl)-1-(2'-imino-4'-methyl-7'-hydroxycoumarinyl) azetidn-2-one 4a. Molecular weight- 371.5, color-white, mp-170°C IR, (KBr cm^{-1}): 3497.52 (aromatic-OH stretching), 2924.92 (C-H stretching), 1672.51 (C=O stretching). ^{1}H NMR (δ, ppm) 6.94-6.99 (m, 1H, aromatic-OH), 1.12 (d, 3H, $-CH_3$) 7.258-7.765 (m, 7H, aromatic protons). Calculated for $C_{19}H_{14}N_2O_4Cl$, C-61.37, H-3.77, N-8.07; found C-60.78, H-3.68, N-7.85.

3-chloro-4-(o-chlorophenyl)-1-(2'-imino-4'-methyl -7'-hydroxy coumarinyl) azetidn-2-one 4b. Molecular weight-391.00, color-white, mp-169°C IR, (KBr, cm^{-1}): 3494.32 (aromatic-OH stretching), 2919.02 (C-H stretching), 1673.59 (C=O stretching), 1601.20 (aromatic C=C stretching).^{1}HNMR (δ, ppm) 6.91-6.99 (m,1H, aromatic-OH), 0.98 (d,3H,-CH3) 7.25-7.415 (m, 7H aromatic protons), 4.87-4.97 (m, 1H, vinylic proton). Calculated for $C_{19}H_{14}N_2O_3Cl_2$, C-58.3, H-3.58,N-7.67; found C-58.18, H-3.45,N-7.61.

3-chloro-4-phenyl-1-(2'-imino-4'-methyl-7'-hydroxycoumarinyl) azetidn-2-one 4c. Molecular weight-356.50, color-white, mp-172°**C** IR, (KBr, cm^{-1}): 3487.02 (aromatic -OH stretching), 2921.52 (C-H stretching), 1673.89 (C=O stretching), 1598.20 (aromatic C=C stretching). ^{1}H NMR (δ, ppm) 6.14-6.89 (m, 1H,aromatic-OH), 1.04 (d, 3H, -CH3) 7.26-7.69 (m, 7H, aromatic protons), 4.82-4.92 (m, 1H,vinylic proton). Calculated for $C_{19}H_{14}N_2O_3Cl_2$, C-63.95, H-4.2, N-8.41; found C-63.81, H-4.15,N-7.59.

3-chloro-4-(-p-chlorophenyl)-1-(2'-imino-4'-methyl-7'-hydroxycoumarinyl) azetidn-2-one 4d. Molecular weight-391.00, color-white, mp-168°C IR, (KBr, cm^{-1}): 3493.32(aromatic-OH stretching), 2919.23 (C-H stretching), 1671.59 (C=Ostretching), 1601.20 (aromatic C=C stretching). ^{1}H NMR (δ, ppm) 6.87-6.99 (m, 1H, aromatic-OH), 1.08 (d, 3H, -CH3) 7.25-7.415 (m, 7H aromatic protons), 4.89-4.95 (m, 1H, vinylic proton). Calculated for $C_{19}H_{14}N_2O_3Cl_2$, C-58.3, H-3.58, N-7.67; found C-58.18, H-3.45, N-7.61.

2-(o-hydroxy phenyl)-3-(2'-imino-4'-methyl-7'-hydroxy coumarinyl)-1,3-thiazolidin-4-one (4'a). Molecular weight-370, color-light yellow, mp-265°C IR, (KBrcm^{-1}): 3485.02 (aromatic–OH stretching), 2924.6 (C-H stretching), 1689.98 (C=O stretching), 1614.48 (aromatic C=C stretching). ^{1}H NMR (δ, ppm) 6.82-6.94 (m, 1H, aromatic-OH), 1.11 (d, 3H, -CH3) 7.26-7.69 (m, 7H, aromatic protons), 3.4 (s, 2H, -CH_{2of} thiazolidinone), 5.8 (s, 1H, -CH of thiazolidinone). Calculated for $C_{19}H_{16}N_2O_4S$, C-61.62, H-4.3, N-8.1; found C-61.54, H-3.95, N-7.51.

2-(o-chloro phenyl)-3-(2'-imino-4'-methyl-7'-hydroxycoumarinyl)-1,3-thiazolidin-4-one (4'b). Molecular weight-388.5, color-light yellow, mp-236°C IR, (KBr, cm^{-1}): 3461.41 (aromatic-OH stretching), 2918.47 (C-H stretching), 1702.66 (C=O stretching), 1617.72 (aromatic C=C stretching). ^{1}H NMR (δ, ppm) 6.82-6.89 (m, 1H, aromatic-OH), 1.09 (d, 3H, -CH3) 7.26-7.33 (m, 7H, aromatic protons), 3.24 (s, 2H, -CH_{2of} thiazolidinone), 5.69 (s, 1H, -CH of thiazolidinone). Calculated for $C_{19}H_{15}N_2O_3SCl$, C-64.4, H-3.86, N-7.72; found C-63.92, H-3.82, N-6.97.

2-phenyl-3-(2'-imino-4'-methyl-7'-hydroxy-coumarinyl)-1,3-thiazolidin-4-one (4'c). Molecular weight-354, color-light yellow, mp-230°C IR, (KBr, cm^{-1}): 3497.41 (aromatic –OH stretching), 2907.6 (C-H stretching), 1695.36 (C=O stretching), 1614.24 (aromatic C=C stretching). ^{1}H NMR (δ, ppm) 6. 86-6.92 (m, 1H,aromatic-OH), 1.12 (d, 3H, -CH3) 7.26-7.38 (m, 7H,aromatic protons), 3.31(s, 2H, -$CH_2$of thiazolidinone), 5.49 (s, 1H, -CH of thiazolidinone). Calculated for $C_{19}H_{16}N_2O_3S$ C-64.4, H 4.5, N-8.47; found C-63.92, H-4.62, N-7.88.

2-(p-chlorophenyl)-3-(2'-imino-4'-methyl-7'-hydroxycoumarinyl)-1, 3-thiazolidin-4-one (4'd). Molecular weight-388.5, color-light yellow, mp-234°C IR, (KBr, cm^{-1}): 3457.86 (aromatic–OH stretching), 2914.35 (C-H stretching), 1688.23 (C=O stretching), 1613.53 (aromatic C=C stretching). ^{1}HNMR (δ, ppm) 6.78-6.87 (m, 1H, aromatic-OH), 1.12 (d, 3H, -CH3) 7.26-7.42 (m, 7H, aromatic protons), 3.4 (s, 2H, -CH_2 of thiazolidinone), 5.59 (s, 1H, -CH of thiazoli-dinone). Calculated for $C_{19}H_{15}N_2O_3SCl$, C-64.4, H-3.86, N-7.72; found C-63.96, H-3.68, N-6.99.

Antimicrobial Activity. The antibacterial activity assay was carried out using the disc diffusion method by measuring the zones of inhibition in mm. All the compounds were screened *in vitro* for their antimicrobial activity against selected pathogens causing urinary tract infections such as gram negative *E. coli*, *P.auregenosa*, *K. pneumoniae*, *P.mirabilis* and gram positive *E. faecalis*, and *S. aureus*. The test compounds 4a-d were dissolved in 50% DMF and 4'a-d were dissolved in distilled water to make solution of 125, 250, 500 and 1000 µg/ml for each test compound. The sterile discs (6 m diameter) were impregnated with 10ml of the prepared solution and placed in the inoculated agar. The standard disc (Himedia, Mumbai) of nitrofurantoin (NF, 300 µg/disc) was used as reference. Controls were prepared using the same solvents employed to dissolve the test compounds. The inoculated plates with the tests and standard discs on them were incubated at 37 ± 1°C for 24 hrs.

RESULTS AND DISCUSSION

By analyzing the zone of inhibition (Table 1) it can be concluded that, **4b, 4c, 4d, 4'a, 4'b, 4'c** and **4'd** showed variable antimicrobial activity at different test concentrations. Their relative potency (Figure 1) against the standard drug nitrofurantoin was found to be comparable and in cases more against the above strains. Compound **4b** and **4c**

Table 1. In *vitro* Antibacterial Activity of Synthesized Compounds.

Drug	Conc (µg/ml)	*E. coli*	*Zone Of Inhibition (in mm)*				
			P. auriginosa	*K. pneumenio*	*P. mirabilis*	*E. faecalis*	*S. aureus*
Solvent		-	-	-	-	-	-
4a	100	-	-	-	-	-	-
	250	-	-	-	-	-	-
	500	-	-	-	-	-	-
	1000	-	-	-	-	-	-
4b	100	-	-	-	-	-	16 ±1
	250						20 ±1.2
	500	10 mm	-	-	-	-	26.1 ±0.5
	1000	13 mm	-	-	-	-	30 ±0.55
4c	100	-	-	-	12 ±0.55	-	11 ±1.41
	250	-	-	-	15.2 ±0.89	-	13 ±1.2
	500	-	-	-	20 ±0.25	-	
	1000	-	-	-	23 ±0.35	-	24 ±0.71
4d	100	-	-	-	-	-	-
	250	-	-	-	-	-	-
	500	-	-	-	-	-	11 ±1.21
	1000	-	-	-	15 ±0.87	-	13 ±0.49
4'a	100	-	-	9 ±0.76	10 ±0.85	8 ±0.55	12 ±0.8
	250	-	-	15 ±0.65	15 ±0.89	12.4 ±0.53	13.9 ±0.7
	500	-	-	19 ±0.76	18.1 ±0.79	17.1 ±0.6	17 ±0.59
	1000	-	-	27.1 ±0.69	22 ±0.75	25 ±0.49	22 ±0.43
4'b	100	-	-	10 ±0.5	12.5 ±1.1	-	-
	250	-	-	13 ±0.8	17.35 ±1.5	-	-
	500	-	-	15.2 ±0.75	23.1 ±1.0	-	-
	1000	-	-	19.25 ±0.89	25 ±0.9	-	-
4'c	100	-	-	10 ±0.55	15 ±0.44	15 ±1.0	12 ±0.23
	250	-	-	16 ±0.63	17 ±0.39	16.2 ±1.1	13 ±0.021
	500	-	-	18 ±0.72	20 ±0.29	19.1 ±0.9	20.1 ±0.25
	1000	-	-	22 ±0.59	25 ±0.41	21.4 ±1.2	22 ±0.20
4'd	100			7 ±0.5	-	-	-
	250			10 ±0.52	-	-	-
	500			17 ±0.63	12 ±0.54	9 ±0.58	8 ±0.82
	1000			19 ±0.59	15 ±0.26	14 ±0.29	12 ±0.25

"-" Indicates no zone of inhibition. All the values are mean ±standard deviation of three determiners. Values showed Significant difference from solvent control at P<0.001

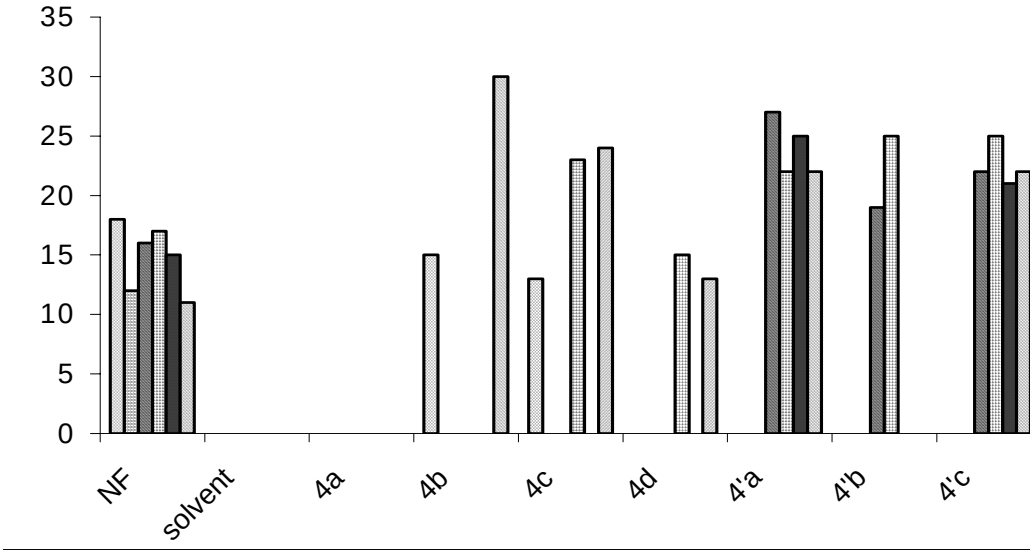
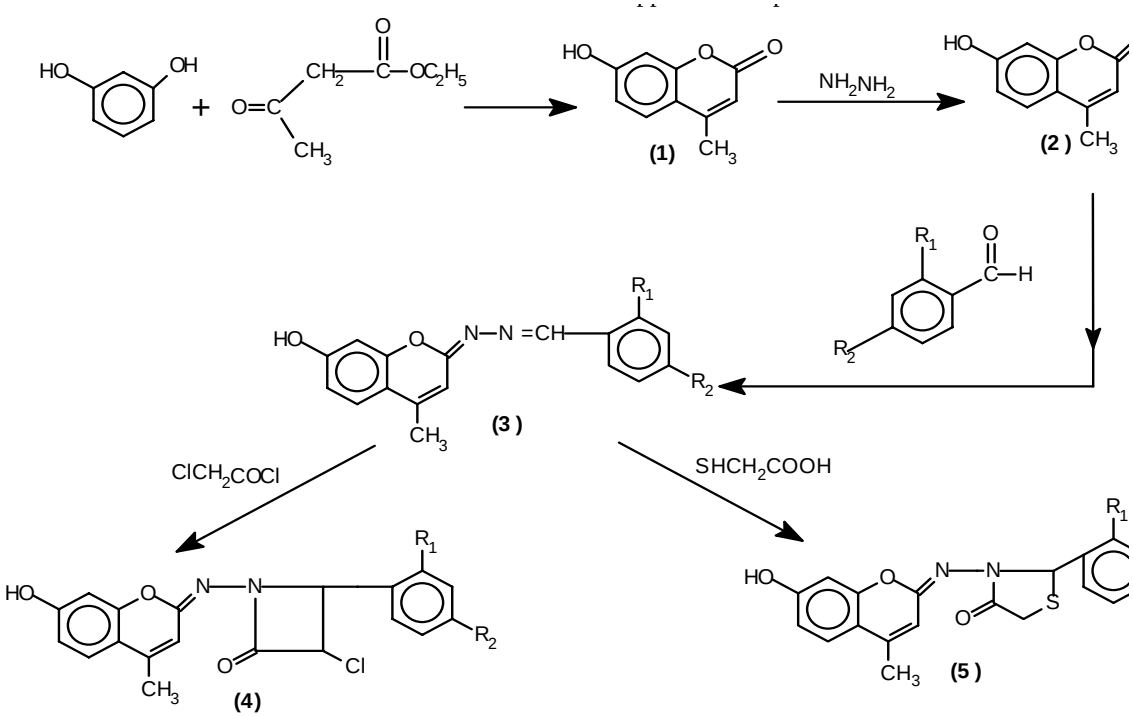


Figure 1. Relative antibacterial activity of title compounds
NF-Nitrofurantoin ( ) E.coli ( ) P.aureginosa ( ) K.pneumoniae ( ) P.mirabilis ( ) E.faecalis ( ) S.aureus g



	R_1	R_2		R_1	R_2
4a	OH	H	4'a	OH	H
4b	H	H	4'b	H	H
4c	Cl	H	4'c	Cl	H
4d	H	Cl	4'd	H	Cl

exhibited significantly higher activity against *S.aureus.* In comparison to the standard drug the antimicrobial effect of compound **4'a,** and **4'c** was found to be more against *K. neumoniae, P.mirabilis, E.faecalis*, and *S.aureus*. Relating activity to the structure of the compounds it can be proposed that remarkable inhibition was observed in compounds bearing thiazolidinone group irrespective of the substitution on the phenyl group.

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