SYNTHESIS OF BIOLOGICALLY ACTIVE AMINO COUMARIN AND ITS DERIVATIVES

Report of the Proposal

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Submitted by

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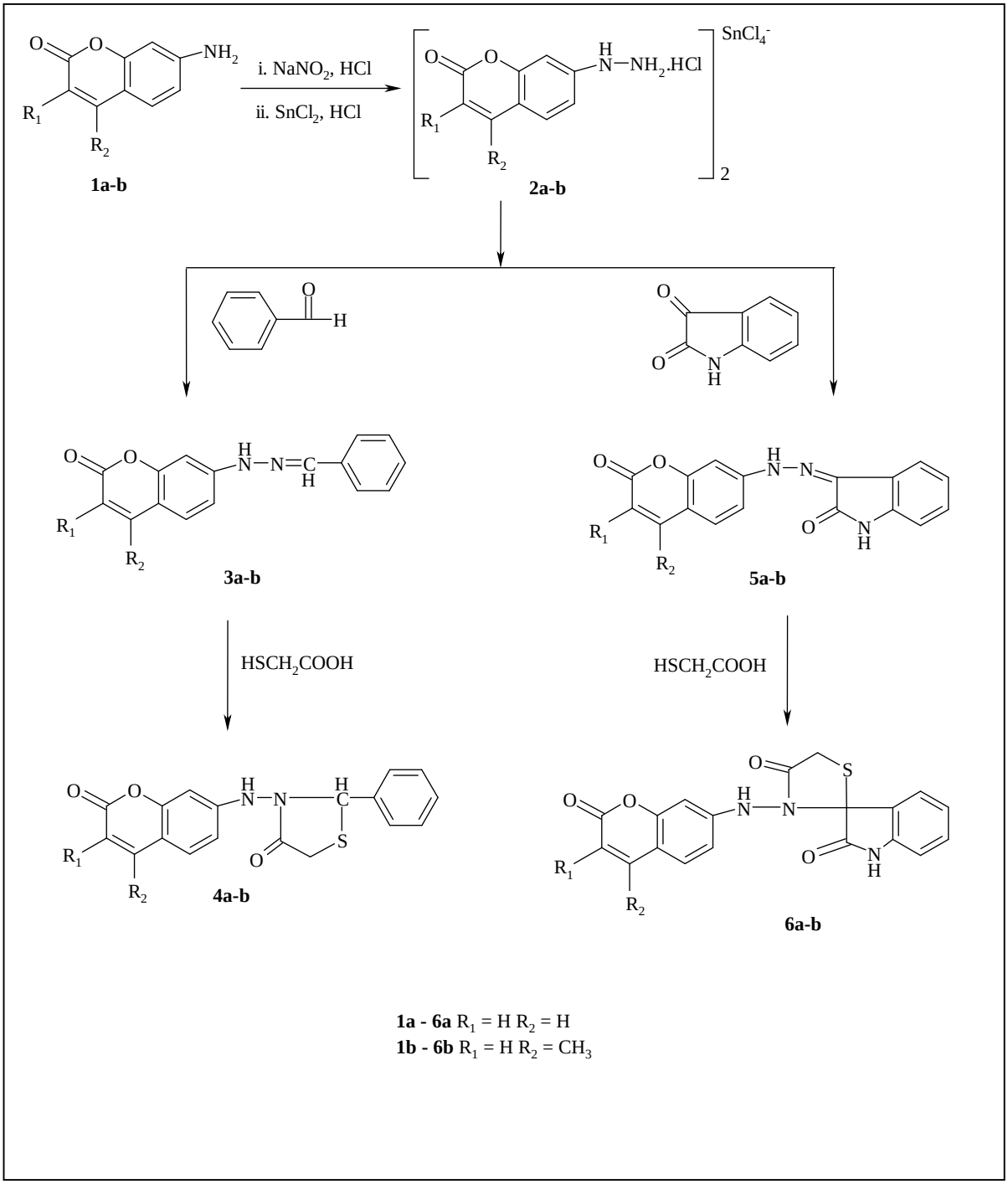
Scheme 1



1a - 6a R_1 = H R_2 = H
1b - 6b R_1 = H R_2 = CH_3

1.1 Introduction:

Coumarins are the important class of heterocyclic compounds and have gained considerable synthetic and pharmacological interest for a long time because of their various biological activities[1], such as, antihelminitic, anti-HIV activity, antioxidation, the synthesis and study of the biological activities of coumarin derivatives has been the aim of many researchers.[2-5] An *N*-acyl derivative of some 7-Amino coumarin serves as fluorescent marker for detection of proteinase.[6]

Thiazolodin-4-one have considerable commercial importance. A large number of thiazolidinone derivatives are associated with a wide range of industrial importance as stabilizers for polymeric materials and pharmaceuticals activities such as nematocidal,[7] fungicidal,[8-13] pesticidal,[14-16] antitubercular,[17-18] anticonvulsant,[19-22] local anesthetic,[23] antibacterial,[24-25] antiviral,[26-27] etc.

Isatin also known as indole-2,3-dione can be *o*-quinone of 2,3-dihydoindole. It is a unique molecule possessing both amide and ketone carbonyl groups. It exists in tautomeric form and these functional characteristics plays an important role in governing various reaction of the molecule. The C_3 carbonyl group of isatin is strongly electrophilic and is readily involved in condensation and addition reaction with carbanion type nucleophile-3-substituted oxindoles.[28] 2-(3-phenyl-1,8-naphthyridin-2-ylhydrazone)-2-indolinone[29] and Phenyl indolo-3-carboaldehyde-3-carbonylhydrazones[30] are found to be active against Gram-positive and Gram-negative bacteria such as *E. Coli, P. auruginosa, B. subtilis, B. mycoids*.

1.2 Chemistry:

Coumarin-7-ylhydrazine hydrochlorides **(2a-b)** were synthesized from 7-aminocoumarin **(1a-b)**. For this purpose 7-aminocoumarin **(1a-b)** was diazotized by sodium nitrite and conc. HCl at 0^{O}C and reduced by the addition of stannous chloride in conc. HCl at the same temperature. This solution of Coumarin-7-ylhydrazine hydrochlorides **(2a-b)** was later condensed *in situ* with benzaldehyde and isatin in ethanol and catalytic amount of glacial acetic acid to yield [2-oxo-2H-benzopyran-7-yl] hydrazono-1-arylmethanes **(3a-b)** and [2-oxo-2H-benzopyran-7-yl] hydrazono indolin-$2^{/}$-one **(5a-b)** respectively.

[2-oxo-2*H*-benzopyran-7-yl] hydrazono-1-arylmethanes **(3a-b)** was treated with mercaptoacetic acid in 1,4-dioxane in presence of catalytic amount of anhydrous zinc chloride to yield [$2^{/}$-oxo-$2^{/}H$-benzopyran-$7^{/}$-ylamino]-2-aryl-4*H*-thiazolidine-4-ones **(4a-b)**.

Similarly [2-oxo-2*H*-benzopyran-7-ylamino] spiro [$3^{/}H$-indole-($1^{/}H$, $2^{/}H$)-$3^{/}$, $2^{/}$ ($4^{/}H$) thaizolidine] -$2^{/}$,$4^{//}$-dione **(6a-b)** was obtained by [2-oxo-2H-benzopyran-7-yl] hydrazono indolinone **(5a-b)** treated with mercaptoacetic acid in 1,4-dioxane in presence of catalytic amount of anhydrous zinc chloride.

The structures of the compounds **(3a-b)** to **(6a-b)** were confirmed on the basis of spectral and analytical data. All the above compounds were screened for their antimicrobial activities.

1.3 Experimental Details:

1.3.1 Experimental

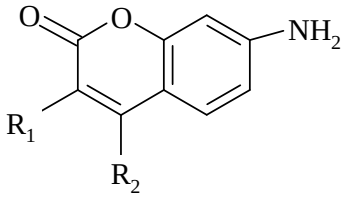
All compounds were confirmed by their spectral data and physical properties and all yields refer to the isolated yields. Melting points were taken in open capillaries and are uncorrected. Purity of the compounds was checked on TLC. FT-IR spectra (*vmax* in cm^{-1}) were recorded on a Perkin Elmer 400 spectrometer using KBr. ^{1}H-NMR spectra were recorded on JEOL NMR AL300 (300 MHz) using TMS as standard and mass spectra on a Shimadzu GC-MS QP-2010.

1.3.2 General Procedure of 7-amino coumarin **(1a-b)**

It was prepared according to the method of Bailey & Bostner.[31]

A mixture of m-cresol (50 gm) and concentrated sulfuric acid (150 mL) was heated at 130-135°C and then malic acid (50 gm) was added portion wise to it with gradual stirring over a period of 1-2 hrs. The solution was maintained for 1.0 hr then cooled to room temperature and poured into ice water, solid separated was filtered, washed with water till neutral and dried to give (26 gm) 7-methylcoumarin and was crystallized from methanol.

m. p. = 126-28°C (Reported [52] m. p. = 124-128°C)



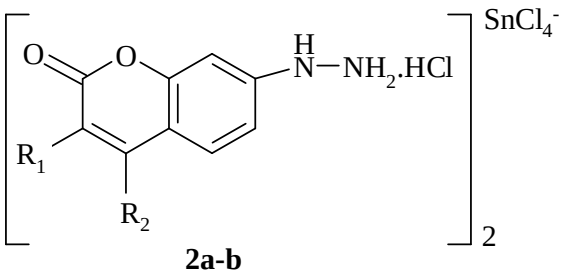
1a-b

1a R_1 = H R_2 = H
1b R_1 = H R_2 = CH_3

1.3.3 *General Procedure of Coumarin-7-ylhydrazine hydrochlorides* **(2a-b)**

It was prepared according to the method described by Morgan et. al.[32]

7-Aminoacoumarin **(1a-b)** (0.01 mole) was dissolved in conc. HCl (5 mL) and cooled to 0-5^{o}C, it was then diazotized by sodium nitrite solution (0.01 mole $NaNO_2$ in 3 mL H_2O) at the same temperature. This diazonium salt solution was later reduced by addition of the solution of stannous chloride (0.01 mole) in conc. HCl (5 mL) to obtain the coumarin-7-ylhydrazine hydrochlorides **(2a-b)** which was kept in refrigerator for overnight (minimum 4 hrs.) and later used directly (*in situ*) for next reaction.

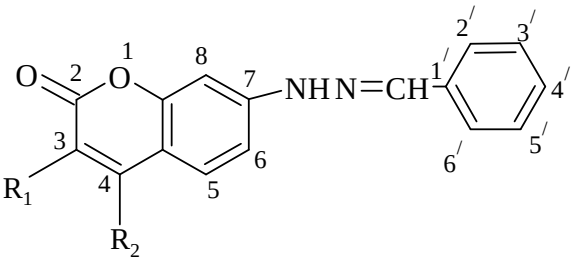


2a-b

2a R_1 = H R_2 = H
2b R_1 = H R_2 = CH_3

1.3.4 *General Procedure of [2-oxo-2H-benzopyran-7-yl] hydrazono-1-arylmethanes* **(3a-b)**

To a suspension of coumarin-7-ylhydrazine hydrochlorides **(2a-b)** in ethanol (25 mL), benzaldehyde (0.01 mole) and catalytic amount of glacial acetic acid (3-4 drops) was added and refluxed on water-bath for 4 hrs. The reaction mixture was then cooled and poured on crushed ice and water. The product separated was filtered, washed, dried and recrystalized from ethanol.



3a-b

3a R_1 = H R_2 = H
3b R_1 = H R_2 = CH_3

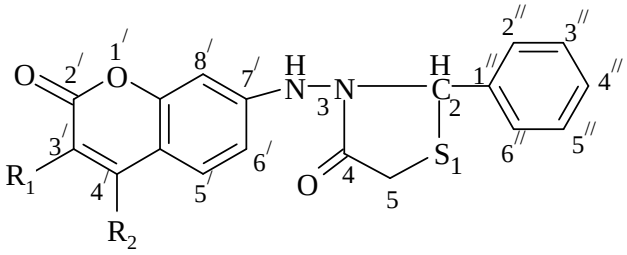
Comp	m.p.^{0}C	Yield %	Spectral data
3a	143-145	72	**IR (KBr cm^{-1}):** 3422(>NH), 3010 & 2980 (-CH), 1721 (C=O), 1600, 1562, 1500, 1425 etc. **^{1}H NMR (DMSO-*d*$_6$, δ, ppm):** δ 6.19 (d, 1H, *J=9.50 Hz* C_3-H), 10.32 (s, 1H, -NH, D_2O exchangeable), 7.10 (s, 1H, -CH=N-), 7.20-7.39 (m, 8H, Ar-H), 7.56 (s, 1H, *J=9.50 Hz* C_4-H).
3b	189-191	63	**IR (KBr cm^{-1}):** 3431 (>NH), 2950 (-CH), 1735 (C=O), 1625, 1551, 1420, 1224, 1053 etc. **^{1}H NMR (DMSO-*d*$_6$, δ, ppm):** δ 2.15 (s, 3H, C_4-CH_3), 10.30 (s, 1H, -NH, D_2O exchangeable), 6.25 (s, 1H, C_3-H), 7.12 (s, 1H, -CH=N-), 7.22-7.40 (m, 8H, Ar-H). **^{13}C NMR (DMSO-*d*$_6$, δ, ppm):** 18.10 (C_4-CH_3), 116.40 (C_3), 122-138 (Aromatic Carbons), 143.50 (C_4), 158.00 (-CH= N-), 160.00 (C_2 >C=O)

Analytical data of compounds (3a-b)

Comp	Substitution		Molecular Formula	Elemental Analysis Found (calculated)		
	R_1	R_2		%C	%H	%N
3a	H	H	$C_{16}H_{12}N_2O_2$	72.61 (72.72)	4.49 (4.58)	10.62 (10.60)
3b	H	CH_3	$C_{17}H_{14}N_2O_2$	73.40 (73.37)	5.00 (5.07)	10.12 (10.07)

1.3.5 *General Procedure of [2′-oxo-2′H-benzopyran-7′-ylamino]-2-aryl-4H-thiazolidine -4-ones* **(4a-b)**

[2-oxo-2H-benzopyran-7-yl] hydrazono-1-arylmethanes **(3a-b)** (0.01 mole) was refluxed with mercaptoacetic acid (0.01 mole) in dry 1,4-dioxane (25 mL) in presence of catalytic amount of $ZnCl_2$ for 7 hrs. The mixture was filtered, dried and recrystalised from ethanol.



4a-b

4a R_1 = H R_2 = H
4b R_1 = H R_2 = CH_3

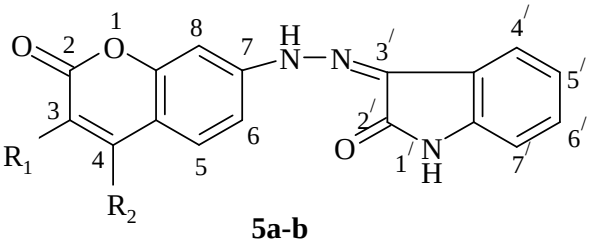
Comp	m.p.^{0}C	Yield %	Spectral data
4a	172-174	69	**IR (KBr cm^{-1}):** 3400 (>NH), 3070 & 2919 (-CH), 1725 (>C=O), 1619, 1552, 1446, 1376, 1300, 1190, 1112, 1005 etc. **^{1}H NMR (DMSO-*d*$_6$, δ, ppm):** δ 3.60 (s, H, H_2C-S), 5.92 (s, 1H, C_2-H), 6.20 (d, 1H, *J*=7.50 Hz $C_3^{/}$-H), 6.80- 7.10 (m, 8H, Ar-H), 7.35 (d, 1H, *J*=7.50Hz, $C_4^{/}$), 11.02 (s, 1H, NH, D_2O Exchangeable)
4b	192-194	60	**IR (KBr cm^{-1}):** 3424(>NH), 3073 & 2925 (-CH), 1722 (>C=O), 1616, 1554, 1451etc. **^{1}H NMR (DMSO-*d*$_6$, δ, ppm):** δ 2.30 (s, 1H, $C_4^{/}$-CH_3), 3.64 (s, 2H, H_2C-S), 5.90 (s, 1H, C_2-H), 6.25 (s, 1H, $C_3^{/}$-H), 6.78-7.04 (m, 8H, Ar-H), 11.10 (s, 1H, NH, D_2O Exchangeable) **^{13}C NMR (DMSO-*d*$_6$, δ, ppm):** 17.23 ($C_4^{/}$-CH_3), 43.50 (C_2), 66.00 (C_5), 115.90 ($C_3^{/}$), 120.00-142.20 (Aromatic-Carbons),143.28 ($C_4^{/}$), 161.00 ($C_2^{/}$), 181.50 (C_4).

Analytical data of compounds (4a-b)

Comp	Substitution		Molecular Formula	Elemental Analysis Found (calculated)			
	R_1	R_2		%C	%H	%N	%S
4a	H	H	$C_{18}H_{14}N_2O_3S$	63.80 (63.89)	4.05 (4.17)	8.19 (8.28)	9.42 (9.48)
4b	H	CH_3	$C_{19}H_{16}N_2O_3S$	64.70 (64.76)	4.60 (4.58)	7.85 (7.95)	9.07 (9.10)

1.3.6 *General Procedure of [2-oxo-2H-benzopyran-7-yl] hydrazono indolinone* **(5a-b)**

To a suspension of coumarin-7-ylhydrazine hydrochlorides **(2a-b)** in ethanol (25 mL), isatin (0.01 mole) and catalytic amount of glacial acetic acid (3-4 drops) was added and refluxed on water-bath for 3 hrs. The reaction mixture was then cooled and poured on crushed ice and water. The product separated was filtered, washed, dried and recrystalized from ethanol.



5a-b

5a R_1 = H R_2 = H
5b R_1 = H R_2 = CH_3

Comp	m.p.^{0}C	Yield %	Spectral data
5a	181-183	78	**IR (KBr cm^{-1}):** 3400 (>NH), 3025 (-CH), 1721 (>C=O), 1610 (>C=N-), 1552, 1462, 1383, 1290 etc. **^{1}H NMR (DMSO-*d*$_6$, δ, ppm):** δ 6.22 (d, 1H, *J*=9.0 Hz C_3-H), 6.80- 6.93 (m, 7H, Ar-H), 7.29 (d, 1H, *J*=9.0 Hz C_4-H), 11.10 (s, 1H, NH, D_2O exchangeable), 12.89 (s, 1H, indole >NH, D_2O exchangeable)
5b	195-197	68	**IR (KBr cm^{-1}):** 3441 (>NH), 3050 (-CH), 1700 (>C=O), 1619 (>C=N-), 1560, 1455, 1420, 1341, etc. **^{1}H NMR (DMSO δ, ppm):** δ 2.36 (s, 1H, C_4-CH_3), 6.25 (s, 1H, C_3-H), 6.92- 7.10 (m, 7H, Ar-H), 11.12 (s, 1H, NH, D_2O

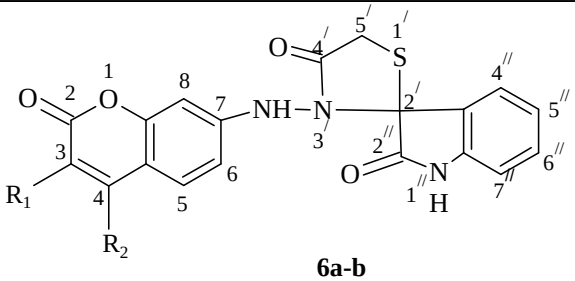
			exchangeable), 12.91 (s, 1H, indole >NH, D_2O exchangeable) **^{13}C NMR (DMSO δ, ppm):** 17.00 (C_4-CH_3), 116.00 (C_3), 120.00-146.25 (Aromatic-Carbons), 143.00 (C_4), 161.00 (C_3'), 162.20 (C_2), 175.10 (C_2').

Analytical data of compounds (5a-b)

Comp	Substitution		Molecular Formula	Elemental Analysis Found (calculated)		
	R_1	**R_2**		**%C**	**%H**	**%N**
5a	H	H	$C_{17}H_{11}N_3O_3$	66.81 (66.88)	3.68 (3.63)	13.25 (13.16)
5b	H	CH_3	$C_{18}H_{13}N_3O_3$	67.76 (67.71)	3.92 (4.10)	13.10 (13.16)

1.3.7 *General Procedure of [2-oxo-2H-benzopyran-7-ylamino] spiro [3′H-indole-(1′H, 2′H)-3′, 2′ (4′H) thaizolidine] -2′,4″-dione* **(6a-b)**

[2-oxo-2H-benzopyran-7-yl] hydrazono indolinone **(5a-b)** (0.01 mole) and mercaptoacetic acid (0.01 mole) was refluxed with in dry 1,4-dioxane (25 mL) in presence of catalytic amount of $ZnCl_2$ for 6 hrs. The mixture was filtered, dried and recrystalised from ethanol.



6a-b

6a R_1 = H R_2 = H
6b R_1 = H R_2 = CH_3

Comp	m.p.^{0}C	Yield %	Spectral data
6a	229-231	69	**IR (KBr cm^{-1}):** 3407 (>NH), 2920 & 2835 (-CH), 1724 (>C=O), 1612, 1552, 1462, 1405 etc. **^{1}H NMR (DMSO-*d*$_6$, δ, ppm):** δ 3.62 (s, H, -S-CH_2), 6.40 (d, 1H, *J*=8.0 Hz, C_3-H), 6.65-7.15 (m, 7H, Ar-H), 7.26 (d, 1H, *J*=8.0 Hz, C_4-H), 11.13 (s, 1H, NH, D_2O Exchangeable), 12.89 (s, 1H, indole >NH, D_2O Exchangeable).
6b	245-247	56	**IR (KBr cm^{-1}):** 3427 (>NH), 2922 & 2853 (-CH), 1720 (>C=O), 1610, 1551, 1458, 1400 etc. **^{1}H NMR (DMSO δ, ppm):** δ 2.40 (s, 1H, C_3-H), 3.60 (s, H, -S-CH_2), 6.23 (s, 1H, C_3-CH_3), 6.69-7.21 (m, 7H, Ar-H), 11.10 (s, 1H, NH, D_2O Exchangeable), 12.92 (s, 1H, indole >NH, D_2O Exchangeable) **^{13}C NMR (DMSO δ, ppm):** 17.25 (C_4-CH_3), 42.00 ($C_2^{/}$), 66.00 ($C_5^{/}$), 116.00 (C_3), 120.00-145.15 (Aromatic-Carbons), 143.50 (C_4), 162.20 (C_2), 175.10 ($C_2^{//}$), 182.10 ($C_4^{/}$).

Analytical data of compounds (6a-b)

Comp	Substitution		Molecular Formula	Elemental Analysis Found (calculated)			
	R_1	R_2		%C	%H	%N	%S
6a	H	H	$C_{19}H_{13}N_3O_4S$	60.10 (60.15)	3.40 (3.45)	11.12 (11.08)	8.49 (8.45)
6b	H	CH_3	$C_{20}H_{15}N_3O_4S$	61.10 (61.06)	3.90 (3.84)	10.75 (10.68)	8.04 (8.15)

1.4 Biological Screening:

All the synthesized compound **(3a-b), (4a-b), (5a-b)** and **(6a-b)** were screened for their antibacterial activity by drug diffusion method by preparing the paper discs of the drug.[33] The activity was tested against three bacterial strains *S. aureus, S.typhi and E.coil* at two concentration (50 µg/mL and 100 µg/mL) using DMSO as solvent. The activities of compounds were compared with streptomycin as antibacterial standard.

The bacteria were cultured on nutrient broth containing peptone (0.6%), yeast extract, sodium chloride prepared in distilled water and autoclaved at 15 lbs pressure at 121^0C for 20 min. For drug diffusion, nutrient agar was prepared in sterile Petri plate. Agar Agar (1.2%) was used as solidifying agent. Paper discs (6.35 mm) were prepared using Whatmann Filter Paper No. 1 were soaked in sterile compounds under study and were placed onto the nutrient agar on which the bacteria were inoculated by spread plate technique. The plates were incubated at 37^0C for 24 hrs.

The extent of inhibition was observed by measuring zone of inhibition in mm. As DMSO also has antimicrobial activity, Black DMSO also used as blank and its zone of inhibition also measured. For all the compounds the zone of inhibition produced by **(3b), (4b), (5b)** and **(6b)** is significant.

From the antimicrobial screening of the compounds compound **(3a-b), (4a-b), (5a-b)** and **(6a-b)** it could observe that **(3b), (4b), (5b)** and **(6b)** were found to be more active which due to presence of methyl group at C_4 of coumarin moiety.

Antibacterial activity of compounds compound **(3a-b), (4a-b), (5a-b)** and **(6a-b)**

Compound	**Zone of inhibition in mm**					
	E. coli		*S. typhi*		*S. aureus*	
	50µg	100µg	50µg	100µg	50µg	100µg
3a	12	14	15	16	15	16
3b	15	19	16	18	17	19
4a	14	15	14	16	16	18
4b	16	19	16	19	18	21
5a	15	18	15	18	17	19
5b	18	20	18	20	20	22
6a	15	18	17	19	17	19
6b	18	20	19	21	18	21

Disc size: 6.35mm Standard: Streptomycin Control: DMSO
Duration: 24 hrs. resistant (11mm/less) intermediate(12-14mm)
sensitive(15mm/more)

1.5 References:

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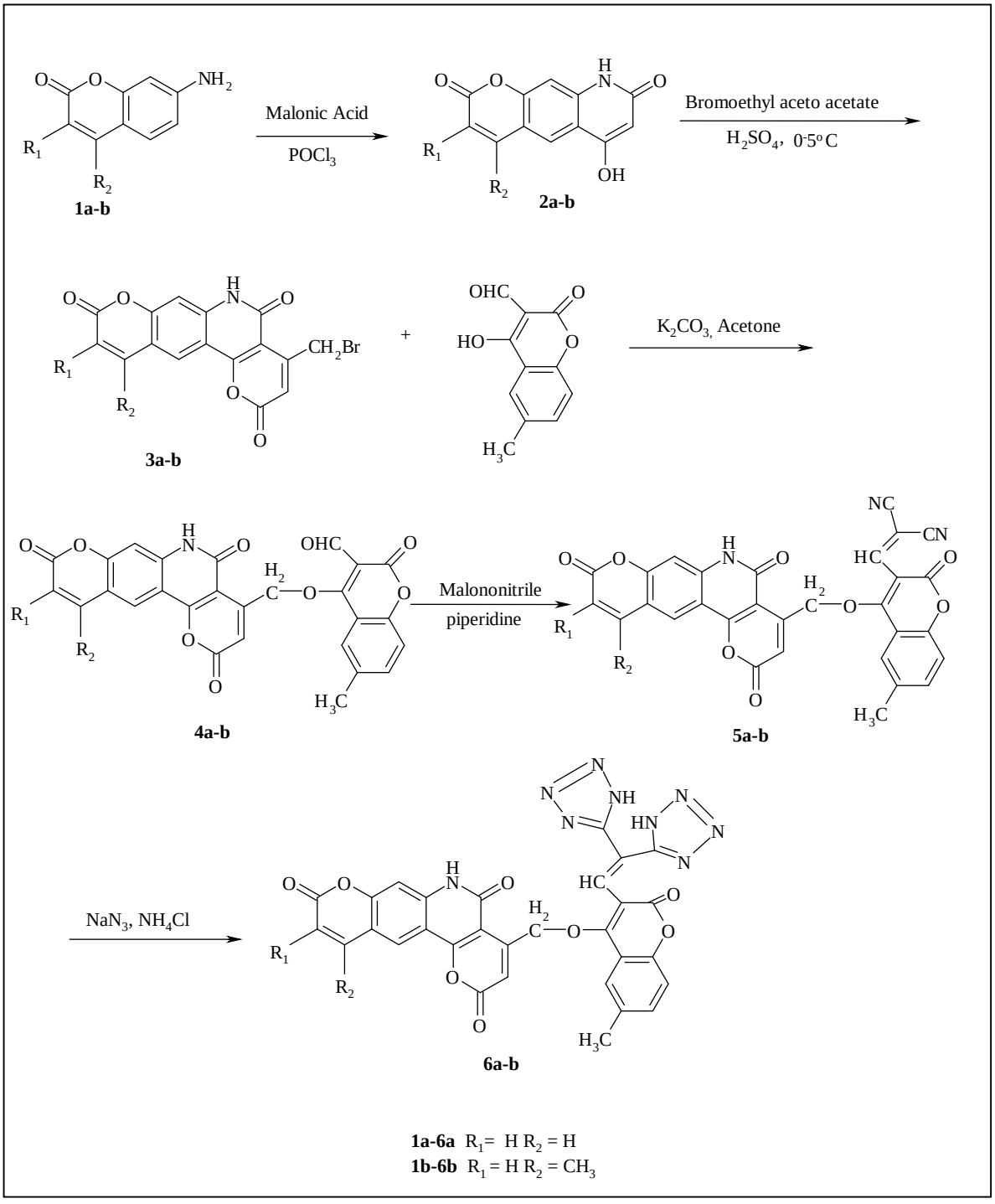
Scheme 2


Malonic Acid
POCl3
Bromoethyl aceto acetate
H2SO4, 0-5°C
K2CO3, Acetone
Malononitrile
piperidine
NaN3, NH4Cl
1a-b
2a-b
3a-b
4a-b
5a-b
6a-b
1a-6a R1= H R2 = H
1b-6b R1 = H R2 = CH3

2.1 Introduction:

Tetrazoles and its derivatives have their own class of drugs. They are reputed as CNS drugs and have found wide application as sedatives[1]. They are also known to possess antimicrobial[2], antilipemic[3,4], antiallergic, antihypotensive, antiinflammatory[5] and hormonal activity[6]. Tetrazoles are also known for their non-steroid oestrogens, bronchodilating effects, anticholinergic activity, diuretics activity and radioprotective agents[6]. Tetrazoles with aryl and heteroaryl substituent are reputed effective agents for regulating plant growth and inhibitors of top growth of vegetables, fruits, trees, cereals and canes.[6,7,8] Particularly 2-tetrazol-5-yl chromones[9,10] are known for their useful activity in preventing the release of spasmogens, which gives rise to allergies and are potentially useful in treatment of asthama.[11]

2.2 Chemistry:

Keeping in the view the biological importance of pyranoquinolines and the coumarins, we synthesized the formyl derivative of methoxy benzopyrane and subsequently to its tetrazole derivative. For this 3*H*, 6*H*, 6*H*, 10*H*-8-bromomethyl- 3, 7, 10-trioxo-dipyrano [2,3-*f*; 2,3-*c*] quinoline **(3a-b)** on treatment with 3-formyl-4-hydroxy-2-oxo-2*H*-[1]-benzopyrane in anhydrous K_2CO_3 gave 3*H*, 6*H*, 7*H*, 10*H*-8-[(3′-formyl-6′-methyl-2′-oxo-2′*H*-[1]-4′-benzopyranoxy)methyl]-3,7,10-trioxo-dipyrano [2,3-*f*; 2,3-*c*] quinoline **(4a-b).** This formyl derivative is converted to 1″,1″-dicyano-2″-{3*H*,6*H*,7*H*, 10*H*-8-[(6′-methyl-2′-oxo-2′*H*-[1]-4′-benzopyranoxy)methyl]-3,7,10-trioxo-dipyrano [2,3-*f*; 2,3-*c*] quinoline}ethane **(5a-b)** via Knovengeal condensation by using active methylene compound like malanonitrile in presence of piperidine. This 1,1- dicyno derivative **(5a-b)** undergoes 1,3- dipolar reaction with sodium azide in presence of ammonium chloride via Hantzsch synthesis to give 1″,1″-

di(tetrazol)-2$^{//}$-{3*H*,6*H*,7*H*,10*H*-8-[(6$^{/}$-methyl-2$^{/}$-oxo-2$^{/}$*H*-[1]-4$^{/}$-enzopyranoxy) methyl] -3,7,10-trioxo-dipyrano [2,3-*f*; 2,3-*c*] quinoline}ethane **(6a-b)** with an interest to have some of the above biological property.

2.3 Experimental Details:

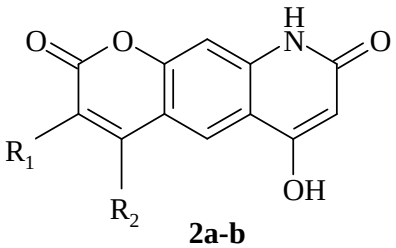
2.3.1 Experimental

All compounds were confirmed by their spectral data and physical properties and all yields refer to the isolated yields. Melting points were taken in open capillaries and are uncorrected. Purity of the compounds was checked on TLC. FT-IR spectra (*vmax* in cm^{-1}) were recorded on a Perkin Elmer 400 spectrometer using KBr. ^{1}H-NMR spectra were recorded on JEOL NMR AL300 (300 MHz) using TMS as standard and mass spectra on a Shimadzu GC-MS QP-2010.

2.3.2 General Procedure of 2H, 8H, 9H, 2,9-dioxo-6-hydroxy pyrano[3,2-9]quinoline **(2a-b)**

It was prepared according to the method already reported.

To a mixture of 7- aminocoumarin (0.01 mole), malonic acid (0.01 mole) and naphthalene (0.01 mole) was added phosphorus oxychloride (4 ml) and mixture was heated on a water bath for 30 minutes. The mixture was then cooled and diluted with water. The solution was basified with NaOH to pH 9 and filtered. The filtrate was acidified to pH 2 with conc. HCl. The product obtained was filtered, washed with water and later recrystallised from ethanol.

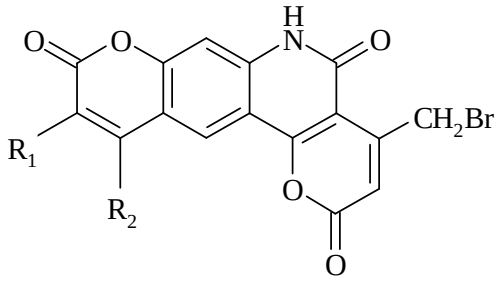


2a-b

2a R_1 = H R_2 = H
2b R_1 = H R_2 = CH_3

2.3.3 *General Procedure of 3H, 6H, 6H, 10H-8-bromomethyl- 3, 7, 10-trioxo-dipyrano [2,3-f; 2,3-c] quinoline* **(3a-b)**

A mixture of equimolar quantity of 2*H*, 8*H*, 9*H*, 2,9-dioxo-6-hydroxy pyrano [3,2-9] quinoline (0.01 mole) and 1-bromoethylaceto-acetate (0.01 mole) was added to conc. H_2SO_4 (30 ml) with stirring at 0-5^0 C. the reaction mixture was kept in ice overnight. Deep red colour solution was poured over crush ice. Solid separated was filtered and washed with NaOH and cold ethanol and purified by recrystallisation from acetic acid to give compound **(3a-b).**

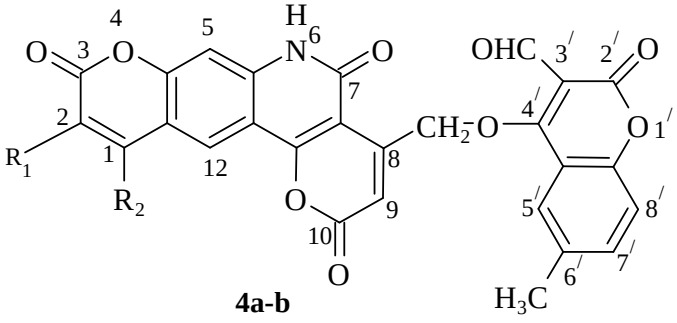


3a-b

3a R_1 = H R_2 = H
3b R_1 = H R_2 = CH_3

2.3.4 *General Procedure of 3H, 6H, 7H, 10H-8-[(3′-formyl-6′-methyl-2′-oxo-2′H-[1]-4′-benzopyranoxy) methyl]-3,7,10-trioxo-dipyrano [2,3-f; 2,3-c] quinoline* **(4a-b)**

To a compound (**3a-b)** (0.01 mole), 3-formyl-4-hydroxy-2-oxo-2*H*-[1]-benzopyrane (0.01 mole) and anhydrous K_2CO_3 (0.01 mole) was added in dry acetone and reflux for 8 hrs. The reaction mixture was filtered hot and the filtrate was concentrated to half the volume and poured over crushed ice and neutralized with dil. HCl. Solid separated was filtered and washed with water and purified by recrystallisation from ethanol to give compound (**4a-b).**



4a-b

4a R_1 = H R_2 = H
4b R_1 = H R_2 = CH_3

Comp	m.p.^{0}C	Yield %	Spectral data
4a	152-154	70	**IR (KBr cm^{-1}):** 3440 (>NH), 2955 (-CH), 1720 (C=O), 1685 (-CONH), 1630, 1545, 1415, 1218, 1020, etc **^{1}H NMR (DMSO-d_6, δ, ppm):** δ 2.28 (s, 3H, C_6'-CH_3), 4.62 (s, 2H, -CH_2), 6.11 (s, 1H, C_9-H), 6.40 (d,1H, *J*=9Hz, C_2-H), 6.82-7.15 (m, 5H, Ar-H), 7.60 (d, 1H, *J*=9Hz C_1-H), 9.25 (s, 1H, -CHO), 9.87 (s, 1H, -NH, D_2O exchangeable).
4b	158-160	60	**IR (KBr cm^{-1}):** 3431 (>NH), 2950 (-CH), 1735 (C=O), 1625, 1551, 1420, 1224, 1053 etc.

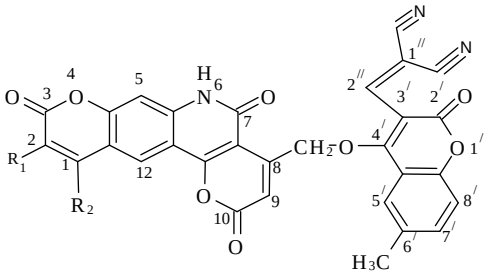
			^{1}H NMR (DMSO-d_6, δ, ppm): δ 2.12 (s, 3H, C_1-CH_3), 2.29 (s, 3H, C_6'-CH_3), 4.61 (s, 2H, -CH_2), 6.10 (s, 1H, C_9-H), 6.35 (s,1H, C_2-H), 7.02-7.39 (m, 5H, Ar-H), 9.23 (s, 1H, CHO), 9.85 (s, 1H, -NH, D_2O exchangeable). **^{13}C NMR (DMSO-d_6, δ, ppm):** 18.10 (C_1-CH_3), 21.25 (C_6'-CH_3), 66.80 (CH_2-O-), 110.20-135.40 (Aromatic Carbons), 152.89 (C_1), 158.57 (C_9), 160.80 (C_2', >C=O), 160.95 (C_3, >C=O), 161.75(C_7, >C=O), 175.60 (C_4'), 187.34 (-CHO).

Analytical data of compounds (4a-b)

Comp	Substitution		Molecular Formula	Elemental Analysis Found (calculated)		
	R_1	R_2		%C	%H	%N
4a	H	H	$C_{27}H_{15}NO_9$	65.10 (65.20)	3.10 (3.04)	2.75 (2.82)
4b	H	CH_3	$C_{28}H_{17}NO_9$	65.70 (65.76)	3.30 (3.35)	2.70 (2.74)

2.3.5 *General Procedure of 1″,1″-dicyano-2″-{3H,6H,7H,10H-8-[(6′-methyl-2′-oxo-2′H-[1]-4′-benzopyranoxy)methyl]-3,7,10-trioxo-dipyrano[2,3-f;2,3-c]quinoline} ethane* **(5a-b)**

To compound **(4a-b)** (0.01 mole) malononitrile (0.01 mole) and few drops of piperidine was added in alcohol. The reaction mixture was stirred for 6 hrs. at room temperature, left overnight. The resulting solution was added to crushed ice. Solid separated was washed with water and purified by recrystallisation from 1,4-Dioxane to give compound **(5a-b).**



5a-b

5a R_1 = H R_2 = H

5b R_1 = H R_2 = CH_3

Comp	m.p.^{0}C	Yield %	Spectral data
5a	202-204	67	**IR (KBr cm^{-1}):** 3300 (-NH), 2215 (CN) 1705 (>C=O), 1620, 1560, 1440, 1375, 1260, 1058, 955 etc. **^{1}H NMR (DMSO-d_6, δ, ppm):** δ 2.30 (s, 3H, $C_6^{/}$-CH_3), 7.60 (d, 1H, *J*=8.5 Hz C_1-H), 4.60 (s, 2H, -CH_2), 6.38 (s, 1H, C_9-H), 6.30 (d, 1H, *J*=8.5 Hz, C_2-H), 6.80 (s, 1H, $C_2^{//}$-H), 7.15-7.29 (m, 5H, Ar-H), 9.85 (s, 1H, -NH, D_2O exchangeable).

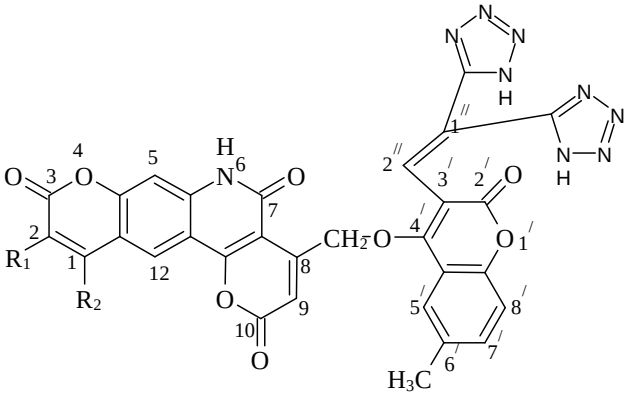
5b	223-225	55	**IR (KBr cm^{-1}):** 3309 (-NH), 2207 (CN) 1715 (>C=O), 1617, 1561, 1446, 1383, 1262, 1067, 959, 807 etc.
			^{1}H NMR (DMSO-d_6, δ, ppm): δ 2.03 (s, 3H, C_1-CH_3), 2.36 (s, 3H, $C_6^{/}$-CH_3), 4.83 (s, 2H, -CH_2O), 6.32 (s, 1H, C_2-H), 6.41 (s, 1H, C_9-H), 6.84 (s, 1H, $C_2^{//}$-H), 7.24-7.45 (m, 5H, aromatic), 9.83 (s, 1H, NH, D_2O exchangable).
			^{13}C NMR (DMSO-d_6, δ, ppm): 18.90 (C_1-CH_3), 21.95 ($C_6^{/}$-CH_3), 67.25 (CH_2-O-), 78.17 ($C_1^{//}$), 101.09 ($C_4^{/}$), 109.10 (-CN), 110.11 (C_9), 110.75-138.60 (Aromatic Carbons), 146.23 ($C_4^{/}$), 152.91 (C_1), 150.19 (C_8), 150.88 ($C_2^{//}$), 158.31 (C_{10}, >C=O), 160.10 ($C_2^{/}$, >C=O), 160.24 (C_3, >C=O), 161.53 (C_7, >C=O).

Analytical data of compounds (5a-b)

Comp	Substitution		Molecular Formula	Elemental Analysis Found (calculated)		
	R_1	R_2		%C	%H	%N
5a	H	H	$C_{30}H_{15}N_3O_8$	66.10 (66.06)	2.70 (2.77)	7.72 (7.70)
5b	H	CH_3	$C_{31}H_{17}N_3O_8$	66.50 (66.55)	3.15 (3.06)	7.45 (7.51)

2.3.6 *General Procedure of* 1″,1″-di(tetrazol)-2″-{3*H*,6*H*,7*H*,10*H*-8-[(6′-methyl-2′-oxo-2′*H*-[1]-4′-enzopyranoxy) methyl] -3,7,10-trioxo-dipyrano [2,3-*f*; 2,3-*c*] quinoline}ethane **(6a-b)**

To a compound **(5a-b)** (0.01 mole), NaN_3 (0.02 mole) and NH_4Cl (0.01 mole) was added in DMF and refluxed on oil-bath for 16 hrs. the reaction mixture was added to crushed ice. Solid separated was filtered and washed with water and purified by recrystallisation from ethanol to give compound **(6a-b).**



6a-b

6a R_1 = H R_2 = H

6b R_1 = H R_2 = CH_3

Comp	m.p.^{0}C	Yield %	Spectral data
6a			**IR (KBr cm^{-1}):** 3400 (>NH), 2940 (-CH), 1732 (C=O), 1620, 1540, 1406, 1350, 1256, 830 etc. **^{1}H NMR (DMSO-*d*$_6$, δ, ppm):** δ 2.30 (s, 3H, $C_6^{/}$-CH_3), 7.65 (d, 1H, *J*=8.5Hz C_1-H), 4.60 (s, 2H, -CH_2), 6.11 (s, 1H, C_9-H), 6.42 (d,1H, *J*=8.5Hz, C_2-H), 6.95-7.22 (m, 5H, Ar-H), 9.70 (s, 1H, -NH, D_2O exchangeable), 10.00 (s, 1H, 2 NH tetrazole, D_2O exchangeable).

6b			**IR (KBr cm^{-1}):** 3400 (>NH), 2950 (-CH), 1730 (C=O), 1615, 1530, 1400, 1356, 1250, 827 etc.
			^{1}H NMR (DMSO-*d*$_6$, δ, ppm): δ 2.10 (s, 3H, C_1-CH_3), 2.35 (s, 3H, $C_6^{/}$-CH_3), 4.63 (s, 2H, -CH_2O), 6.15 (s, 1H, C_9-H), 6.36 (s, 1H, C_2-H), 6.90 (s, 1H, $C_2^{//}$-H), 7.15-7.38 (m, 5H, aromatic), 9.77 (s, 1H, NH, D_2O exchangable), 10.08 (s, 1H, 2 NH tetrazole, D_2O exchangeable).
			^{13}C NMR (DMSO-*d*$_6$, δ, ppm): 18.95 (C_1-CH_3), 21.88 ($C_6^{/}$-CH_3), 67.10 (CH_2-O-), 95.69 ($C_1^{//}$), 101.12 ($C_4^{/}$), 110.10 (C_9), 115.75-138.95 (Aromatic Carbons), 145.80 ($C_2^{//}$), 146.20 ($C_4^{/}$), 150.10 (C_8), 152.95 (C_1), 154.19 (Tetrazole Carbons), 158.09 (C_{10}, >C=O), 160.22 ($C_2^{/}$, >C=O), 160.10 (C_3, >C=O), 161.50 (C_7, >C=O).

Analytical data of compounds (6a-b)

Comp	Substitution		Molecular Formula	Elemental Analysis Found (calculated)		
	R_1	R_2		%C	%H	%N
6a	H	H	$C_{30}H_{17}N_9O_8$	57.10 (57.06)	2.78 (2.71)	19.90 (19.96)
6b	H	CH_3	$C_{31}H_{19}N_9O_8$	57.60 (57.68)	2.90 (2.97)	19.59 (19.53)

2.4 Biological Screening:

All the synthesized compound **(4a-b), (5a-b)** and **(6a-b)** were screened for their antibacterial activity by drug diffusion method by preparing the paper discs of the drug.[12] The activity was tested against three bacterial strains *S. aureus, S.typhi and E.coil* at two concentration (50 µg/mL and 100 µg/mL) using DMSO as solvent. The activities of compounds were compared with streptomycin as antibacterial standard.

The bacteria were cultured on nutrient broth containing peptone (0.6%), yeast extract, sodium chloride prepared in distilled water and autoclaved at 15 lbs pressure at 121^0C for 20 min. For drug diffusion, nutrient agar was prepared in sterile Petri plate. Agar Agar (1.2%) was used as solidifying agent. Paper discs (6.35 mm) were prepared using Whatmann Filter Paper No. 1 were soaked in sterile compounds under study and were placed onto the nutrient agar on which the bacteria were inoculated by spread plate technique. The plates were incubated at 37^0C for 24 hrs.

The extent of inhibition was observed by measuring zone of inhibition in mm. As DMSO also has antimicrobial activity, Black DMSO also used as blank and its zone of inhibition also measured. For all the compounds the zone of inhibition produced by **(4b), (5b)** and **(6b)** is significant.

From the antimicrobial screening of the compounds compound **(4a-b), (5a-b)** and **(6a-b)** it could observe that **(4b), (5b)** and **(6b)** were found to be more active which due to presence of methyl group at C_4 of coumarin moiety.

Antibacterial active ity of compounds compound **(4a-b), (5a-b)** and **(6a-b)**

Compound	**Zone of inhibition in mm**					
	E. coli		*S. typhi*		*S. aureus*	
	50µg	100µg	50µg	100µg	50µg	100µg
4a	12	14	13	15	16	17
4b	14	18	16	17	18	20
5a	13	16	13	17	15	18
5b	17	18	17	20	20	21
6a	15	19	16	19	18	19
6b	18	20	19	21	19	21

Disc size: 6.35mm
Duration: 24 hrs.

Standard: Streptomycin
resistant (11mm/less)
sensitive(15mm/more)

Control: DMSO
intermediate(12-14mm)

2.5 References:

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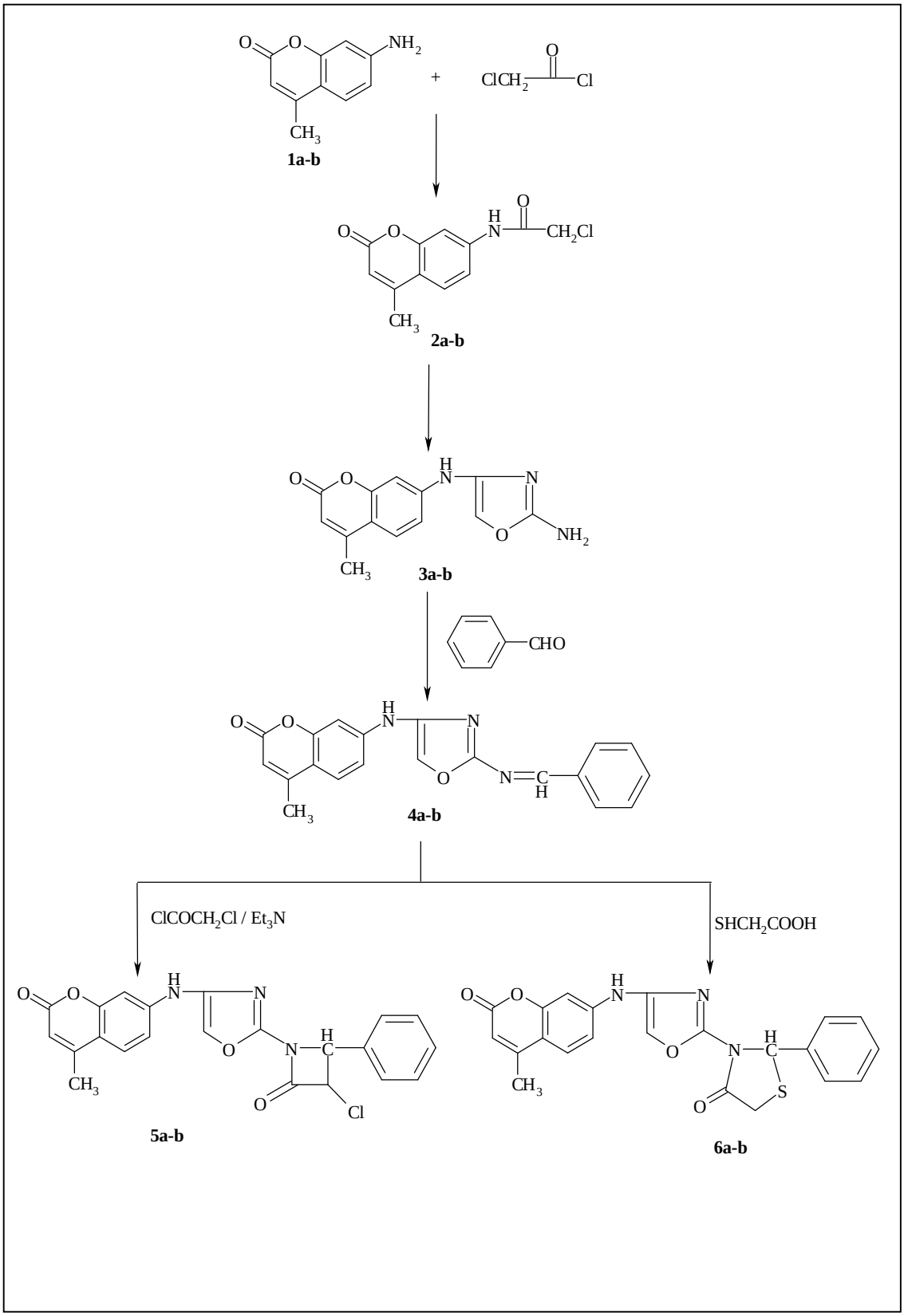
Scheme 3



3.1 Introduction:

Amino coumarin has been reported to possess anti-viral activity and especially effective against HIV.[1] An *N*-acyl derivative of some 7-Amino coumarin serves as fluorescent marker for detection of proteinase.[2] J. Berdy[3] has discussed antibiotics containing pyran and benzopyran moieties.

Moreover, coumarin moiety is widely distributed in nature and many natural products, which contain this subunit, shows useful and diverse biological activity as antifungal,[4] anticoagulants[5] and compounds active against psoriasis,[6] carcinogen,[7] antibacterial[8] and insecticidals.[9]

Thiazole nucleus has been a seat of diverse biological properties, this moiety present in most important naturally occurring substances like Vit-B_1, Penicillin and in close related antibiotics. Thiazole is also part of celebrated drug like Sulphathiazole, Nitrazole, Tetramisole and Schistomicidazole etc.

2-Amino-thiazole derivatives are also exhibited by various physiological activities like anti-thyroid, anti-spasmodic, analgesic, anti-inflammatory, antibacterial and fungicidal properties.[10,11]

3.2 Chemistry:

7-Amino coumarin **(1a-b)** on acetylation by using acetyl chloride in dry benzene gives 7-(2-chloro-acetyl) amino coumarin **(2a-b)** which was treated with urea in dry acetone to yield 7-(2′-amino-1′, 3′-oxazol-4′-yl)-amino-coumarin **(3a-b)**. Further **(3a-b)** on treatment with benzaldehyde gives the schiff's base 7-(arylidenimino-1′,3′-oxazol-4′-yl)-amino coumarin **(4a-b).** This schiff's base on further treatment with chloroacetyl chloride in presence of triethyl amine gives 7-[2′-(3″-chloro-2″-oxo-4″-phenyl-1″-azetidinyl)-1′,3′-oxazol-4′-yl]amino coumarin **(5a-b)** and with thioglycollic

acid in presence of anhydrous zinc chloride to give 7-[$2^{/}$-($2^{//}$-phenyl-$4^{//}$-thiazolidinone-$3^{//}$-yl)-$1^{/}$,$3^{/}$-thiazole-$4^{/}$-yl]amino coumarin **(6a-b)**.

The structures of the compounds **(2a-b)** to **(6a-b)** were confirmed on the basis of spectral and analytical data. Compounds **(5a-b)** and **(6a-b)** were screened for their antimicrobial activities.

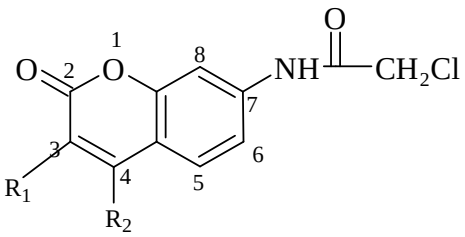
3.3 Experimental Details:

3.3.1 Experimental

All compounds were confirmed by their spectral data and physical properties and all yields refer to the isolated yields. Melting points were taken in open capillaries and are uncorrected. Purity of the compounds was checked on TLC. FT-IR spectra (*vmax* in cm^{-1}) were recorded on a Perkin Elmer 400 spectrometer using KBr. ^{1}H-NMR spectra were recorded on JEOL NMR AL300 (300 MHz) using TMS as standard and mass spectra on a Shimadzu GC-MS QP-2010.

3.3.2 General Procedure of 7-(2-chloro-acetyl) amino coumarin **(2a-b)**

Chloroacetylchloride (0.02 mole) was added to a solution of 7-amino coumarin (0.02 mole) in dry benzene (60 mL) at 0-5 ^{0}C under stirring, which was subsequently refluxed for 6 hr. on water bath. The completion of the reaction was monitored by TLC. The solvent was removed under reduced pressure and the residue was poured into water. The solid obtained was recrystallised by ethyl acetate-hexane to yield compound **(2a-b)**.



2a R_1 = H R_2 = H
2b R_1 = H R_2 = CH_3

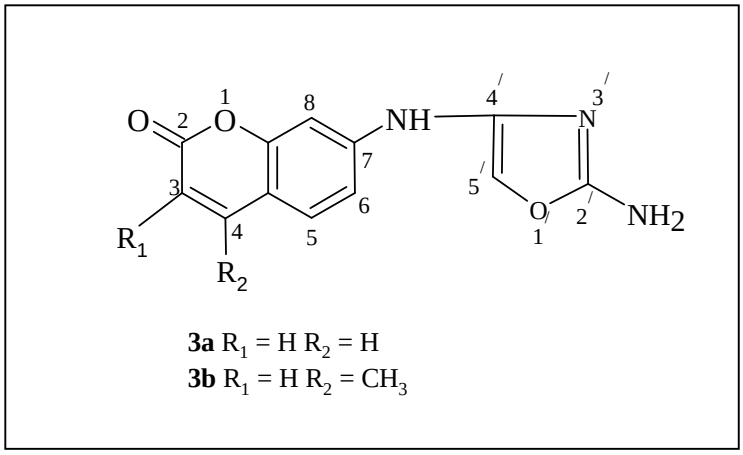
Comp	m.p.^{0}C	Yield %	Spectral data
2a	164-166	68	**IR (KBr cm^{-1}):** 3370 (-NH stretch.), 2950 (-CH), 1722 (>C=O), 1680 (NH-C=O), 1375, 1150, 1060, 870, 760, 685 etc. **^{1}H NMR ($CDCl_3$, δ, ppm):** δ 4.20 (s, 2H, CH_2), 6.42 (d, 1H, *J*=9Hz, C_3-H), 6.86 (d, 1H, *J*=9Hz, C_6-H), 7.20 (s, 1H, C_8-H), 7.22 (d, 1H, *J*=9Hz, C_5-H), 7.77 (d, 1H, *J*=9Hz, C_4-H), 8.30 (s, 1H, NH, D_2O-exchangable).
2b	205-207	54	**IR (KBr cm^{-1}):** 3375 (-NH), 2950 (-CH), 1720 (C=O), 1680 (NH-C=O), 1400, 1380, 1250, 1085, etc. **^{1}H NMR ($CDCl_3$, δ, ppm):** 2.42 (s, 3H, CH_3), 4.35 (s, 2H, CH_2), 6.20 (s, 1H, C_3-H), 6.80 (d, 1H, *J*=9Hz, C_6-H), 7.21 (s, 1H, C_8-H), 7.18 (d, 1H, *J*=9Hz, C_5-H), 8.40 (s, 1H, -NH, D_2O-exchangable).

Analytical data of compounds (2a-b)

Comp	Substitution		Molecular Formula	Elemental Analysis Found (calculated)		
	R_1	R_2		%C	%H	%N
2a	H	H	$C_{11}H_8NO_3Cl$	55.55 (55.60)	3.45 (3.39)	5.80 (5.89)
2b	H	CH_3	$C_{12}H_{10}NO_3Cl$	57.30 (57.27)	4.00 (4.01)	5.50 (5.57)

3.3.3 *General Procedure of 7-(2′-amino-1′, 3′-oxazol-4′-yl)-amino-coumarin* **(3a-b)**.

A mixture of **(2a-b)** (0.02 mole) and urea (0.02 mole) in dry acetone (60 mL) was refluxed for 8 hrs. The excess of acetone was distilled off and the residue obtained was poured into crushed ice, filtered, dried and recrystallised from methanol.



3a R_1 = H R_2 = H
3b R_1 = H R_2 = CH_3

Comp	m.p.^{0}C	Yield %	Spectral data
3a	189-191	59	**IR (KBr cm^{-1}):** 3380 (NH), 3010 (-CH), 1720 (>C=O), 1350, 1220, 1028, etc. **^{1}H NMR (DMSO-d_6, δ, ppm):** δ 6.15 (s, 2H, NH_2, D_2O-exchangable), 6.30 (d, 1H, *J*=8.5Hz, C_3-H), 6.80 (d, 1H, *J*=8.5Hz, C_6-H), 7.21 (s, 1H, C_8-H), 7.29 (d, 1H, *J*=8.5Hz, C_5-H) 7.35 (s, 1H, CH- oxazole), 7.75 (d, 1H, *J*=8.5Hz, C_4-H), 9.21 (s, 1H, NH, D_2O Exchangable).

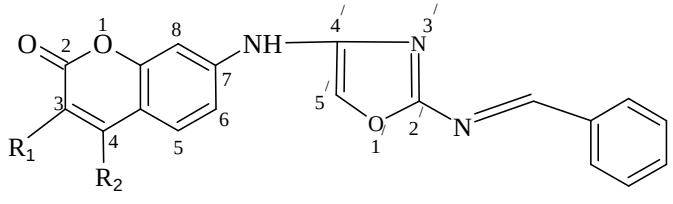
3b	222-224	50	**IR (KBr cm^{-1}):** 3381 (NH), 2990 (-CH), 1723 (>C=O), 1500, 1310, 1265, 1050, etc. **^{1}H NMR (DMSO-d_6, δ, ppm):** 2.30 (s, 3H, CH_3), 6.00 (s, 2H, NH_2, D_2O exchangeable), 6.25 (s, 1H, C_3-H), 6.85 (d, 1H, *J*=8.5Hz, C_6-H), 7.25 (s, 1H, C_8-H), 7.39 (s, 1H, CH-oxazole), 7.74 (d, 1H, *J*=8.5Hz, C_5-H), 9.15 (s, 1H, NH, D_2O exchangable).

Analytical data of compounds (3a-b)

Comp	Substitution		Molecular Formula	Elemental Analysis Found (calculated)		
	R_1	R_2		%C	%H	%N
3a	H	H	$C_{12}H_9N_3O_3$	59.20 (59.26)	3.79 (3.73)	17.35 (17.28)
3b	H	CH_3	$C_{13}H_{11}N_3O_3$	60.76 (60.70)	4.24 (4.31)	16.40 (16.33)

3.3.4 *General Procedure of 7-(arylidenimino-1′,3′-oxazol-4′-yl)-amino coumarin* **(4a-b).**

To a solution of **(3a-b)** (0.02 mole) in absolute ethanol (40 mL) benzaldehyde (0.02 mole) was added and 2-3 drops of acetic acid were also added, the mixture was refluxed for 10 hrs. The resulting mixture was cooled, excess of alcohol was removed by distillation and the residue obtained was poured into crushed ice, the solid obtained was filtered washed with water, dried and recrystallised from methanol to give (**4a-b).**



4a R_1 = H R_2 = H
4b R_1 = H R_2 = CH_3

Comp	m.p.^{0}C	Yield %	Spectral data
4a	189-191	59	**IR (KBr cm^{-1}):** 3240 (-NH), 3015 (–CH), 1722 (>C=O), 1610 (C=N), 1420, 1200, 1115, 1050, etc. **^{1}H NMR (CDCl$_3$, δ, ppm):** δ 6.35 (d, 1H, *J*=9Hz, C_3-H), 7.32 (s, 1H, -CH-oxazole), 7.15-7.28 (m, 8H, Ar-H), 7.72 (d, 1H, *J*=9Hz, C_4-H), 8.05 (s, 1H, N=CH), 8.90 (s, 1H, NH, D_2O exchangable).
4b	222-224	50	**IR (KBr cm^{-1}):** 3250 (-NH), 3020 (-CH), 1710 (>C=O), 1615 (C=N), 1425, 1210, 1110, 1050, etc. **^{1}H NMR (CDCl$_3$, δ, ppm):** 2.28 (s, 3H, -CH_3), 6.20 (s, 1H, C_3-H), 6.80-7.06 (m, 8H, Ar-H), 7.30 (s, 1H, -CH-oxazole), 8.00 (s,1H, N=CH), 9.06 (s, 1H, NH, D_2O exchangable).

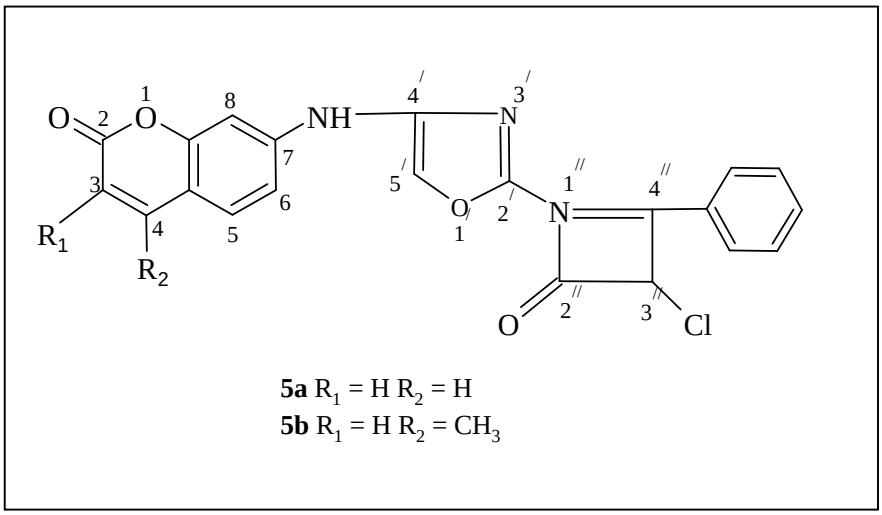
Analytical data of compounds (4a-b)

Comp	Substitution		Molecular Formula	Elemental Analysis Found (calculated)		
	R_1	R_2		%C	%H	%N
4a	H	H	$C_{19}H_{13}N_3O_3$	68.80 (68.88)	3.90 (3.95)	12.75 (12.68)
4b	H	CH_3	$C_{20}H_{15}N_3O_3$	69.52 (69.56)	4.30 (4.38)	12.10 (12.17)

3.3.5 *General Procedure of 7-[2′-(3″-chloro-2″-oxo-4″-phenyl-1″-azetidinyl)-1′,3′-oxazol-4′-yl]amino coumarin* **(5a-b)**

To a solution of **(4a-b)** (0.01 mole) in 1,4 dioxane (30 mL), chloroacetyl chloride (0.01 mole) and triethylamine (0.01 mole) was added drop wise with constant stirring. The reaction mixture was then refluxed on water bath and excess of dioxane was distilled out and resulting mixture was poured in ice-cold water

containing HCl, the solid obtained was filtered washed with water, dried and recrystallised from ethanol to give the desired product.



5a R_1 = H R_2 = H
5b R_1 = H R_2 = CH_3

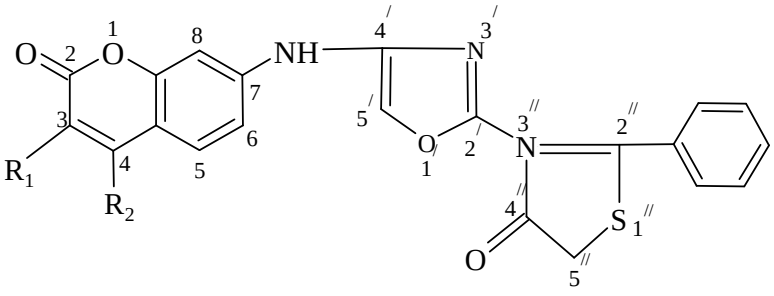
Comp	m.p.^{0}C	Yield %	Spectral data
5a	172-174	50	**IR (KBr cm^{-1}):** 3250 (-NH), 3017 (-CH), 1740 (>C=O), 1721 (>C=O of lactone), 1612 (-C=N), 1510, 1480, 1450, 1335, 1309, 1165, etc. **^{1}H NMR (DMSO-*d*$_6$, δ, ppm):** δ 3.88 (d, 1H, *J*=9Hz, CH-N-), 5.30 (d, 1H, *J*= 9Hz, CH-Cl), 6.26 (d, 1H, *J*=9Hz, C_3-H), 7.31 (s, 1H, CH-oxzole), 7.35-7.49 (m, 8H, Ar-H), 7.79 (d, 1H, *J*=9Hz, C_4-H), 9.12 (s, 1H, NH, D_2O exchangable)
5b	198-200	42	**IR (KBr cm^{-1}):** 3245 (-NH), 3010 (-CH), 1736 (>C=O), 1720 (>C=O of lactone), 1610 (-C=N), 1510, 1480, 1455, 1325, 1300, 1160, etc. **^{1}H NMR (DMSO-*d*$_6$, δ, ppm):** 2.25 (s, 3H, -CH_3), 3.80 (d, 1H, *J*=8.5Hz, CH-N-), 5.32 (d, 1H, *J*= 8.5Hz, CH-Cl), 6.24 (d, 1H, *J*=8.5Hz, C_3-H), 7.28 (s, 1H, CH-oxzole), 7.32-7.43 (m, 8H, Ar-H), 9.10 (s, 1H, NH, D_2O exchangable)

Analytical data of compounds (5a-b)

Comp	Substitution		Molecular Formula	Elemental Analysis Found (calculated)		
	R_1	R_2		%C	%H	%N
5a	H	H	$C_{21}H_{14}N_3O_4Cl$	61.80 (61.85)	3.50 (3.46)	10.38 (10.30)
5b	H	CH_3	$C_{22}H_{16}N_3O_4Cl$	62.58 (62.64)	3.75 (3.82)	9.90 (9.96)

3.3.6 *General Procedure of 7-[2′-(2″-phenyl-4″-thiazolidinone-3″-yl)-1′,3′-oxazole-4′-yl]amino coumarin* **(6a-b)**.

A mixture of compound **(4a-c)** (0.01 mole), thioglycollic acid (0.01 mole) and anhydrous zinc chloride (2 gm.) was refluxed in absolute ethanol (40 mL) for 8 hrs. The excess of alcohol was removed by distillation and the residue poured into crushed ice, the solid obtained was filtered washed with water, dried and recrystallised from ethanol to give (**6a-b).**



6a R_1 = H R_2 = H
6b R_1 = H R_2 = CH_3

Comp	m.p.^{0}C	Yield %	Spectral data
6a	195-197	53	**IR (KBr cm^{-1}):** 3250 (-NH), 3035 (-CH), 1690 (>C=O of thiazolidinone), 1560 (C=N), 1720 (>C=O), 1495, 1495, 1220, etc. **^{1}H NMR (DMSO-d_6, δ, ppm):** δ 3.70 (s, 2H, CH_2), 5.90 (s, 1H, -CH thazolodinone), 6.32 (d, 1H, *J*=9Hz, C_3-H), 7.24 (s, 1H, CH- oxazole ring), 7.29-7.35 (m, 8H, Ar-H), 7.82 (d, 1H, *J*=9Hz, C_4-H), 9.42 (s, 1H, -NH, D_2O exchangeable).
6b	208-210	45	**IR (KBr cm^{-1}):** 3245 (-NH), 3025 (-CH), 1680 (>C=O of thiazolidinone), 1565 (-C=N), 1715 (>C=O), 1490, 1480, 1210, etc. **^{1}H NMR (DMSO-d_6, δ, ppm):** 2.28 (s, 3H, -CH_3), 3.72 (s, 2H, CH_2), 5.95 (s, 1H, -CH thazolodinone), 6.30 (d, 1H, *J*=9Hz, C_3-H), 7.28 (s, 1H, CH- oxazole ring), 7.25-7.38 (m, 8H, Ar-H), 9.40 (s, 1H, -NH, D_2O exchangeable).

Analytical data of compounds (6a-b)

Comp	Substitution		Molecular Formula	Elemental Analysis Found (calculated)			
	R_1	R_2		%C	%H	%N	%S
6a	H	H	$C_{21}H_{15}N_3O_4S$	62.28 (62.21)	3.79 (3.73)	10.31 (10.36)	7.83 (7.91)
6b	H	CH_3	$C_{22}H_{17}N_3O_4S$	63.08 (63.00)	4.15 (4.09)	10.11 (10.02)	7.58 (7.64)

3.4 Biological Screening:

The synthesized compound **(5a-b)** and **(6a-b)** were screened for their antibacterial activity by drug diffusion method by preparing the paper discs of the drug.[12] The activity was tested against three bacterial strains *S. aureus, S.typhi and E.coil* at two concentration (50 µg/mL and 100 µg/mL) using DMSO as solvent. The activities of compounds were compared with streptomycin as antibacterial standard.

The bacteria were cultured on nutrient broth containing peptone (0.6%), yeast extract, sodium chloride prepared in distilled water and autoclaved at 15 lbs pressure at 121^0C for 20 min. For drug diffusion, nutrient agar was prepared in sterile Petri plate. Agar Agar (1.2%) was used as solidifying agent. Paper discs (6.35 mm) were prepared using Whatmann Filter Paper No. 1 were soaked in sterile compounds under study and were placed onto the nutrient agar on which the bacteria were inoculated by spread plate technique. The plates were incubated at 37^0C for 24 hrs.

The extent of inhibition was observed by measuring zone of inhibition in mm. As DMSO also has antimicrobial activity, Black DMSO also used as blank and its zone of inhibition also measured. For all the compounds the zone of inhibition produced by **(5b)** and **(6b)** is significant.

From the antimicrobial screening of the compounds compound **(5a-b)** and **(6a-b)** it could observe that **(5b)** and **(6b)** were found to be more active which due to presence of methyl group at C_4 of coumarin moiety.

Antibacterial activity of compounds compound **(5a-b)** and **(6a-b)**

Compound	**Zone of inhibition in mm**					
	E. coli		*S. typhi*		*S. aureus*	
	50µg	100µg	50µg	100µg	50µg	100µg
5a	13	14	15	18	16	19
5b	15	18	17	19	18	20
6a	15	19	16	18	17	19
6b	17	20	18	20	19	21

Disc size: 6.35mm
Duration: 24 hrs.

Standard: Streptomycin
resistant (11mm/less)
sensitive(15mm/more)

Control: DMSO
intermediate(12-14mm)

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