International Journal for Pharmaceutical Research Scholars (IJPRS)

V-5, I-3, 2016 ISSN No: 2277 - 7873

REVIEW ARTICLE

Synthesis, Characterization, Anti-Microbial Activities and Conductance of Narrative Coumarine Compounds

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Manuscript No: IJPRS/V5/I3/00120, Received On: 17/07/2016, Accepted On: 25/07/2016

ABSTRACT

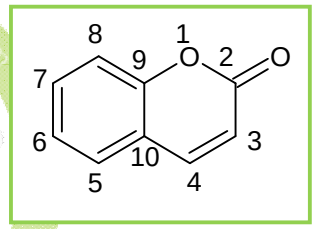
An unimpeachable and well-organized method for synthesis of 3-acetyl-4-hydroxy-2H-chromen-2-one derivatives was accomplished from different substituted-4-hydroxy-2H-chromen-2-one, acetic acid and POCl3 using with refluxed and good yield and no auxiliary purification requirement for compound. The structures of the products were supported by FTIR, 1HMR and mass spectral data and microbiological activity completed of all compounds.

KEYWORDS

Gels, Nanoparticle, Polymers, Control and Sustained Release, Bioavailability

INTRODUCTION

Coumarins are the best known aromatic lactones. The isolation of coumarin was first reported by Vogel2 in Munich in 1820. He associated the pleasant odour of the tonka bean from Guiana with that of clover, Melilotous officinalis, which gives rise to the characteristic aroma of new-mown hay. Vogel then concluded that the long colourless crystals which he discovered on slicing open tonka beans and which crystallized as glistening needles from aqueous alcohol were identical with similar crystals he obtained, albeit in much lower yield, by extracting fresh clover blossoms3.The name coumarin originated4 from a Caribbean word ‘coumarou’ for the tonka tree, which was known botanically at one time as Coumarouna odorata Aubl.Coumarin is now well accepted trivial name. The IUPAC nomenclature of the coumarin ring system is 2H-1-benzopyran-2-one.



The coumarin ring system has an easy acceptability in the biological system compared to its isomeric chromones and flavones nucleus5 and is widely distributed in nature. An excellent account of these naturally occurring coumarins is presented by R D H Murray and S A Brown.

The reaction of aldol condensation has been widely used in organic synthesis. In order to synthesize coumarin acidichromic dyes, 7-substituted *3*-acetyl-*4*-hydroxycoumarin has beencondensed with *4-N,N*-dimethylaminobenzaldehyde in benzene, giving products of the aldol reaction with very good yields [1]. The reaction of *3*-acetylcoumarin with *3*- pyridylaldehyde in butanol in presence of piperidine gave two products - a product of self aldol condensation and a product of mixed condensation [2]. Different coumarin chalcones have been synthesized by aldol reaction of 3-

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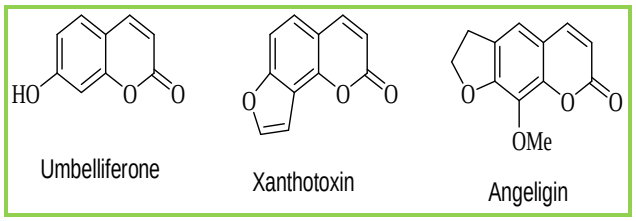
acetyl-4- hydroxy-8-isopropyl-5-methylcoumarin and 3- acetyl-4-hydroxy-6-chloro-7-methylcoumarin with a variety of aromatic aldehydes with very good yields [3, 4]. Microwave-assisted synthesis has been also used for similar reaction intermediate and synthesis of pyrazolinylcoumarins with possible antioxidant activity [5]. Coumarin chalcones and bis-3-coumarinylpyridines have been synthesized by a series of two steps of aldol reactions in solventless conditions [6,7], using the catalyst Bi(NO3)3, immobilized onalumina.

The BF3-catalyzed aldol condensations of 5-methyl-2,3-dihydrofuran The mechanism of formation of hemiacetal group - compound (3-methoxy-3-hydroxy-2-(2'-hydroxy-5'-nitro)-benzylydenebutanoic acid).-*2*-one with RCHO (R = Ph, halo, nitro,trifluoromethyl, methyl, methoxy or(methylenedioxi)phenyl, thienyl, furyl, cyclohexyl) has given acetyltetrahydrofuranones**,** which show cardiovascular activity [8]. Efficient aldol dimerization of ketones occuredwhen the neat ketone is absorbed on basic Al2O3, followed, when necessary, by heating [9]. This could be a reason for forming dimers of acetophenone, *1*-indanone and *1*-tetralone. Coumarins containing electron withdrawing group (-CN, -CONH2, etc.) at third position and tertiary amino group at seventh position in the coumarin ring, usefull as laser dyes, were prepared by Vilsmeyer formylation of trimethylsililated *3*- *N,N*-substituted aminophenols, hydrolysis and basecatalyzed aldol condensation with activated methylene compounds [10]. Proline has been used as a catalyst of the aldol reaction for asymmetric direct intermolecular aldol reaction. [11]. Strong solvent influence on the enantiopurity has been observed; anhydrous DMSO has been found to be the most suitable solvent. 4-Hydroxycoumarin has been involved in the aldol type of reaction with benzaldehyde in the presence of NaOH in ultrasonic bath [12]. The product was obtained in high yield. The aim of the present investigation was to explore the influence of different substituents(electron donating or electron withdrawing) in thebenzene ring of 3-acetylcoumarin derivatives on thecourse of the self-aldol condensation and the possible side products.

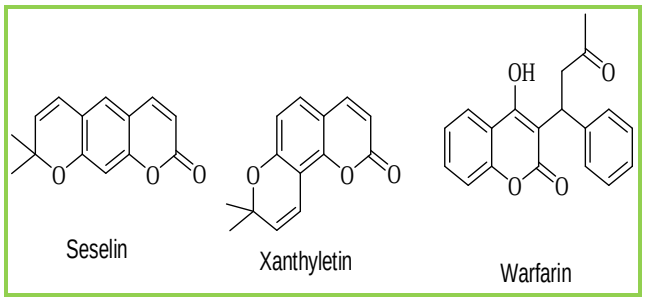
The coumarin unit is a chromophore with an absorption maximum at 306 – 320 nm. The presence of auxochromic substituents may result in a bathochromic shift with the appearance of absorption in the visible spectral region. With substituents exhibiting acidbase properties, the wavelength at which an absorption maximum is observed depends on the degree of deprotonation. It is known that in an alkaline medium, 6-acetyl-5-hydroxy-4-methylcoumarin is bright yellow, while in an acid medium the solution is colourless. 3-Carbethoxy-4-[2-[4-(dimethylamino) phenyl] ethenyl]coumarin reveals several colour transitions and its application in titrimetric analysis is quite promising.

The attention of organic chemists has been directed towards the field of heterocyclic chemistry, because of their valuable utilities in the synthesis of variety of biologically active derivatives. Nitrogen, oxygen and sulfur are the most common heteroatoms, but heterocyclic rings containing other hetero atoms are also widely known. A vast number of heterocyclic compounds are known and this number is increasing rapidly. Among the various classes of heterocycles, we are selected coumarin, pyrazole, pyrans, oxazines, oxadiazole and thiadiazoles for the present study.

Coumarins are classified based on their chemical composition such as, simple coumarins which are hydroxylated, alkoxylated or alkylated on the benzene ring (e.g. Umbelliferone), Furanocoumarins, that contain a five membered furan ring attached to the coumarin moiety such as linear furanocoumarins (e.g. Xanthotoxin) and angular furanocoumarins (e.g. Angeligin).



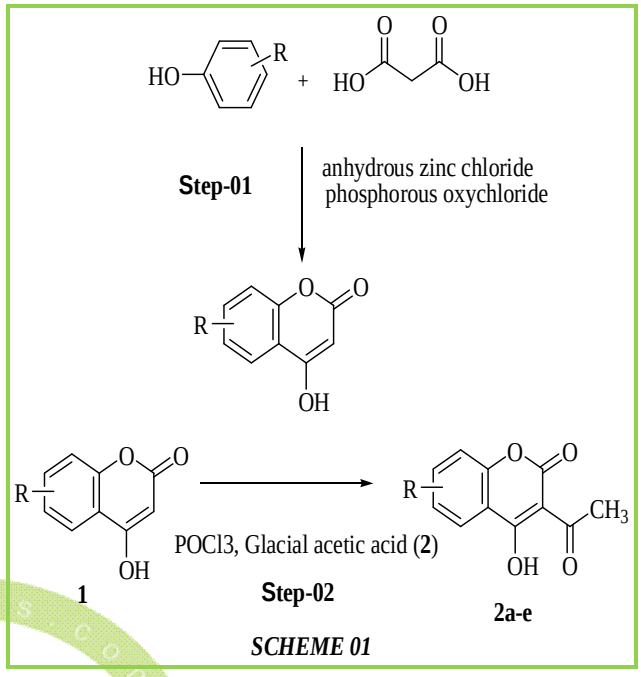
Pyrano coumarins containing a six membered ring attached to the coumarin moiety (e.g. Seselin and Xanthyletin).Coumarins with substituents in the pyrone ring (e.g. Warfarin).



Coumarin and its derivatives considered as the most active classes of heterocycles, which possess a broad spectrum of biological activity. They have been proven to be active as antibacterial, antifungal, anti-inflammatory, antidepressant, anti-HIV and antitumour agents. Moreover, coumarin and its related derivatives have been used as inhibitors of lipoxygenase (LOX) and cyclooxygenase (COX) pathways of arachidonic acid meyabolism[8]. Besides the biological applications, the literature embodies their applications from the material view point such as additives in food, perfumes, cosmetics, optical brighteners and would dispersed fluorescent and laser dyes. Optical applications such as laser dyes, nonlinear optical chromophores, fluorescent whiteners, polymer science and solar energy collectors associated with coumarins have been extensively studied.

Coumarins are also found in selective microorganisms. Members of coumarins isolated from microbial sources are novobiocin from *streptomycin* and aflatoxin from *Aspergillus* species. They are used as enhancing agent in cosmetic products like soap, toothpaste, perfumes and alcoholic beverages. It is also used as a neutralizer in rubber and plastic materials and also in paints and sprays to dilute the unpleasant odors.

We have developed a new diffidence for the synthesis **3-acetyl-4-hydroxy-2H-chromen-2-one and its derivatives *(2a-e)*** with the advantage of fine yield and environmentally easiness (**Scheme-1**).



SCHEME 01

METHOD

STEP 01

Phenol and malonic acid were added to a mixture of phosphorous oxychloride and anhydrous zinc chloride which is preheated to 600^{O}C and the reaction mixture was heated on a water bath at 70^{O}C for 36 hrs. It was cooled and decomposed with ice and water to afforded solid mass, which was filtered and washed with water. It was then treated with 10% sodium bicarbonate and filtered. The filterate was slowly acidify with dilute hydrochloric acid. At the neutral point, some oily product was separated which was filtered and washed with water, dried and recrystallized from ethanol.

STEP 02

A 4-hydroxy coumarin derivative was mixed with glacial acetic acid, and phosphorous oxychloride was added slowly, the mixture was further reflux for 2-3 hrs. And then poured on crushed ice with stirring. The solid separated out was filtered, washed with water and crystallize from alcohol.

RESULTS & DISCUSSION

3-acetyl-4-hydroxy-2H-chromen-2-one (2a)

Yield: 65%; mp 145ºC; Anal. Calcd. for $C_{11}H_8O_4$: C, 64.71; H, 3.95; O, 31.34; Found: C, 64.76; H, 3.90; O, 31.30%; IR (cm^{-1}): 3471 (O-H stretching), 3180 (C-H stretching of aromatic ring), 2980 (C-H asymmetrical stretching of CH_3 group), 2852 (C-H symmetrical stretching of CH_3 group), 1716 (C=O stretching of ketone), 1600, 1537 & 1496 (C=C stretching of cyclic), 1396 (C-H asymmetrical deformation of CH_3 group), 1346 (C-H symmetrical deformation of CH_3 group), 1274 (C-O-C stretching), 920 (para-substituted), 734 (C-H in out plane deformation of aromatic ring); ^1H NMR (DMSO) δ ppm: 2.50-2.51 (s, 3H, H), 7.32-7.37 (dd', 2H, H), 7.62-7.66 (dd', 1H, H),7.81-7.83(dd', 1H, H), 12.58 (s, 1H, H), MS: *m/z* 204.

3-acetyl-4-hydroxy-6-nitro-2H-chromen-2-one (2b)

Yield: 57%; mp 160ºC; Anal. Calcd. for $C_{11}H_7NO_6$: C, 53.02; H, 2.83; N, 5.62; O, 38.53; Found: C, 53.03; H, 2.87; N, 5.60; O, 38.50%; MS: *m/z* 249.

3-acetyl-6-bromo-4-hydroxy-2H-chromen-2-one (2c)

Yield: 50%; mp 159ºC; Anal. Calcd. for $C_{11}H_7BrO_4$: C, 46.67; H, 2.49; Br, 28.23; O, 22.61; Found: C, 46.69; H, 2.47; Br, 28.29; O, 22.55%; MS: *m/z* 283.

3-acetyl-7-Bromo-4-hydroxy-2H-chromen-2-one (2d)

Yield: 58%; mp 154ºC; Anal. Calcd. for $C_{11}H_7BrO_4$: C, 46.67; H, 2.49; Br, 28.23; O, 22.61; Found: C, 46.65; H, 2.55; Br, 28.20; O, 22.60%; MS: *m/z* 283.

3-acetyl-4,7-dihydroxy-2H-chromen-2-one (2e)

Yield: 61%; mp 160ºC; Anal. Calcd. for $C_{11}H_8O_5$: 60.00; H, 3.66; O, 36.33; Found: , 60.09; H, 3.60; O, 36.30%; MS: *m/z* 220.

CONDUCTANCE

The synthesized complexes are stable at room temperature, insoluble in water, soluble in methanol and ethanol, but highly soluble in DMF and DMSO. molar conductance data given in (Table 01)

The measured conductance (k) of each solution after correction was used to determine the specific conductance (κ), which is then used for the calculation of equivalent conductance (λ_c).

The equations used for calculating specific conductance (κ) and equivalent conductance (λ_c) are:

$$\kappa = k\theta$$

$$\lambda_c = 1000\frac{\kappa}{C}$$

where θ is the cell constant (= 0.93 cm^{-1}) and c is the concentration (g.equi./lit.) of solution.

These equivalent conductance values of all the 3-acetyl-4-hydroxycoumarins in DMF and DMSO at 303.15 K are reported along with measured conductance (k) except for DMSO solutions of 2a and 2c, where conductance is almost same as that of pure solvent. The variation of conductance with concentration in both the solvents. Conductivities of all studied compounds are observed to be less in DMF than those in DMSO. Further, for all the systems studied, conductance increases with concentration. There is sharp increase in conductance with concentration. However, at higher concentration, the values increase very slowly.

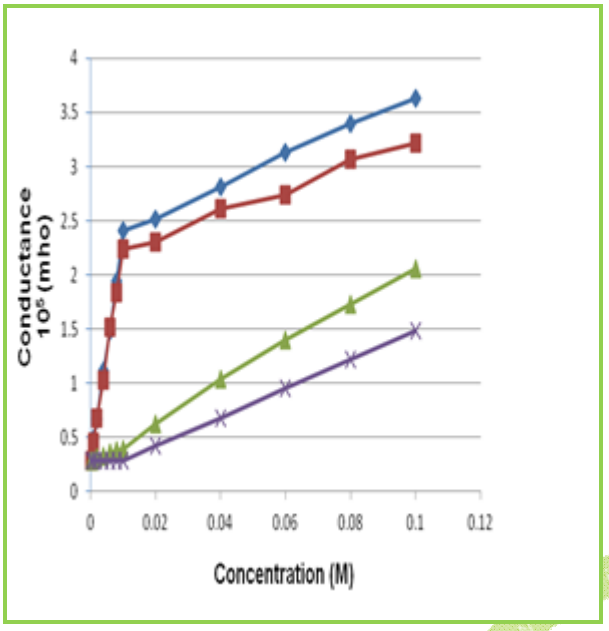
The conductance (k) and equivalent conductance of GK-01 to GK-04 in DMF at 303.15 K.

Conc. C (g/lit)	$k.10^5$ $(\Omega)^{-1}$	λ_C (cm^2/Ω.equiv.)	$k.10^5$ $(\Omega)^{-1}$	λ_C (cm^2/Ω.equiv.)
	2a		2b	
0.000	0.28	---	0.28	---
0.001	0.49	1.8512	0.46	1.7440
0.002	0.67	1.8513	0.68	1.7442
0.004	1.11	1.8756	1.03	1.7443
0.006	1.49	1.8685	1.52	1.7449
0.008	1.94	1.8632	1.84	1.7454
0.010	2.41	1.8532	2.24	1.7464
0.020	2.52	0.9894	2.30	0.9264
0.040	2.81	0.5610	2.61	0.5214
0.060	3.13	0.4157	2.74	0.3801
0.080	3.40	0.3424	3.07	0.3116
0.100	3.63	0.2983	3.22	0.2704
	2c		**2d**	
0.001	0.29	0.0979	0.28	0.0000
0.002	0.32	0.1424	0.28	0.0044
0.004	0.33	0.1426	0.29	0.0089
0.006	0.36	0.1291	0.29	0.0090
0.008	0.38	0.1103	0.28	0.0090
0.010	0.39	0.0996	0.28	0.0089
0.020	0.62	0.1455	0.42	0.0632
0.040	1.04	0.1709	0.68	0.0893
0.060	1.40	0.1660	0.95	0.0997
0.080	1.73	0.1624	1.22	0.1041
0.100	2.06	0.1587	1.48	0.1072

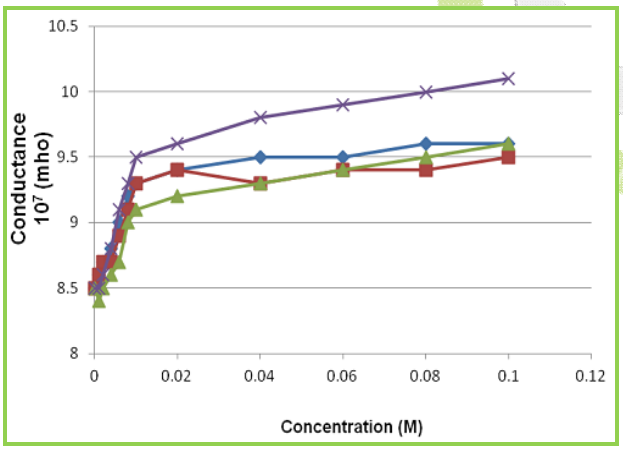
The conductance (k) and equivalent conductance of GK series in DMSO at 303.15 K.

Conc. c (g/lit)	**$k.10^7$ $(\Omega)^{-1}$**	**λ_C (cm^2/Ω.equiv.)**	**$k.10^7$ $(\Omega)^{-1}$**	**λ_C (cm^2/Ω.equiv.)**
	2a		**2b**	
0.000	8.50	---	8.50	---
0.001	8.60	8.8000	8.60	8.8000
0.002	8.70	8.8000	8.70	4.4400
0.004	8.80	8.8000	8.70	6.6850
0.006	9.00	8.8000	8.90	7.4167
0.008	9.20	8.9000	9.10	7.7875
0.010	9.30	8.9000	9.30	8.0100
0.020	9.40	4.4500	9.40	4.0050
0.040	9.50	2.4475	9.30	2.0024
0.060	9.50	1.6317	9.40	1.4833
0.080	9.60	1.3350	9.40	1.1124
0.100	9.60	1.0680	9.50	0.9791
	2a		**2d**	
0.001	8.40	8.8000	8.50	8.9000
0.002	8.50	8.8000	8.60	8.9000
0.004	8.60	6.6740	8.80	8.9000
0.006	8.70	5.9331	9.10	10.3733
0.008	9.00	6.6750	9.30	10.0125
0.010	9.10	7.1201	9.50	10.6800
0.020	9.20	3.5600	9.60	5.3400
0.040	9.30	2.0026	9.80	3.1150
0.060	9.40	1.4833	9.90	2.2240
0.080	9.50	1.2237	10.00	1.7800
0.100	9.60	1.1570	10.10	1.6020

The variation of conductance (k) with concentration for compound in DMF at 303.15 K.



The variation of Conductance (k) with concentration for compounds in DMSO at 303.15 K.



This typical behaviour may be due to interactions within the molecule thereby causing constriction within the molecule or due to association between solute with solvent molecules. Similar behaviour was observed by Singh et al.[13]. Haffner et al.[14] have also reported such abnormal behaviour in the studied systems and interpreted the results in terms of aggregation or complex formation at lower concentration range. This suggests that all the studied compounds exhibit weak electrolytic behaviour in DMF and DMSO solutions. So the equivalent conductance at infinite dilution for these compounds cannot be evaluated.

Antimicrobial evaluation

Total of the Prepared compounds **(2a-e)** were experienced for their antibacterial and antifungal activity (MIC) *in vitro* by broth dilution method [15-17] with two Gram-positive bacteria ***Staphylococcus aureus*** MTCC-96, ***Streptococcus pyogenes*** MTCC 443, two Gram-negative bacteria ***Escherichia coli*** MTCC 442, ***Pseudomonas aeruginosa*** MTCC 441 and three fungal strains ***Candida albicans*** MTCC 227, ***Aspergillus Niger*** MTCC 282, ***Aspergillus clavatus*** MTCC 1323 taking **gentamycin, ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, nystatin and greseofulvin** as regular drugs.

The minimal inhibitory concentration (MIC) values for all the newly synthesized compounds, definited as the lowly concentration of the compound preventing the observable growth, were determined by using micro dilution broth method according to NCCLS standards.

Minimal Inhibition Concentration [MIC]:-
The main advantage of the 'Broth Dilution Method' for MIC determination lies in the fact that it can readily be converted to determine the MIC as well.

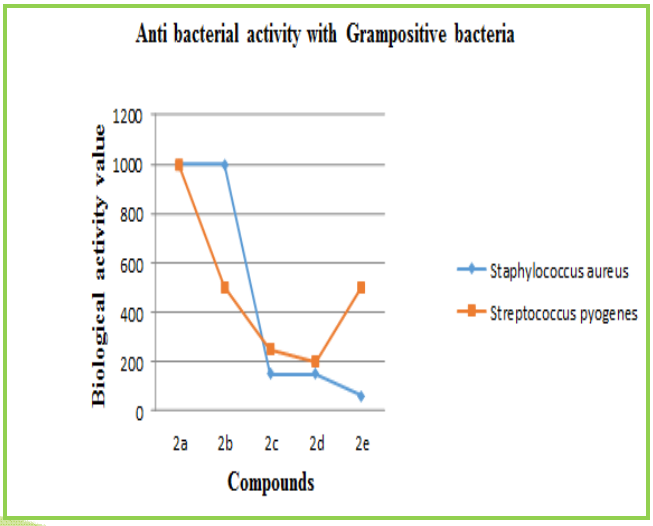
- Serial dilutions were prepared in primary and minor screening.
- The control tube containing no antibiotic is immediately subcultured by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37 ^{0}C overnight.
- The MIC of the control organism is read to check the accuracy of the drug concentrations.
- The lowest concentration inhibiting growth of the organism is recorded as the MIC.
- The amount of growth from the control

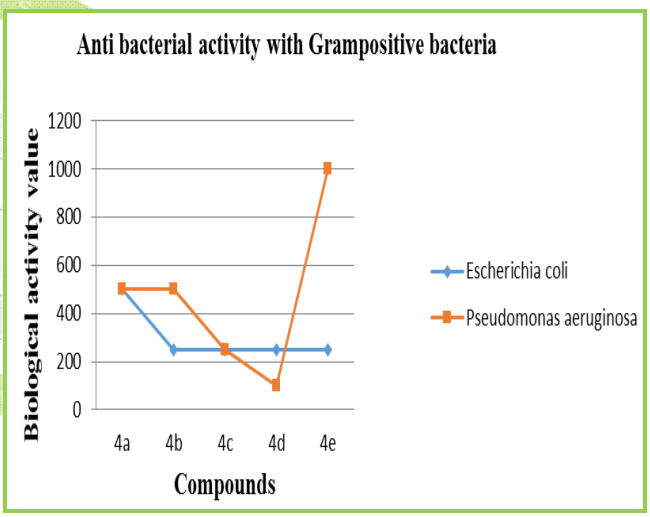
tube before incubation (which represents the original inoculums) is compared.

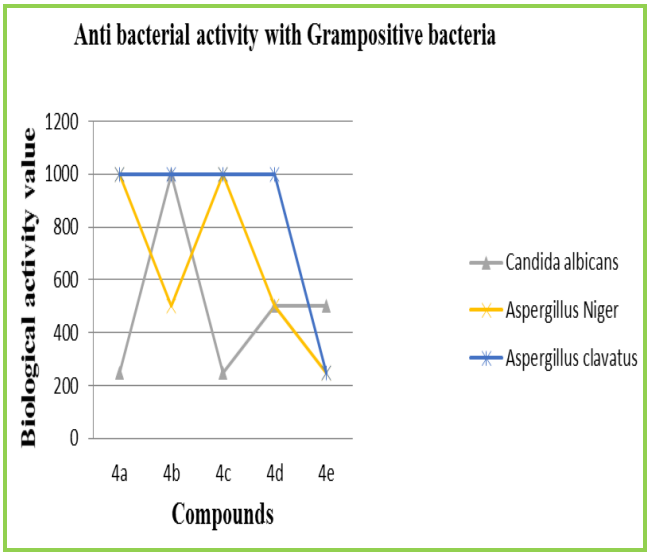
Antibacterial and antifungal activity of synthesized compounds (2a-e)

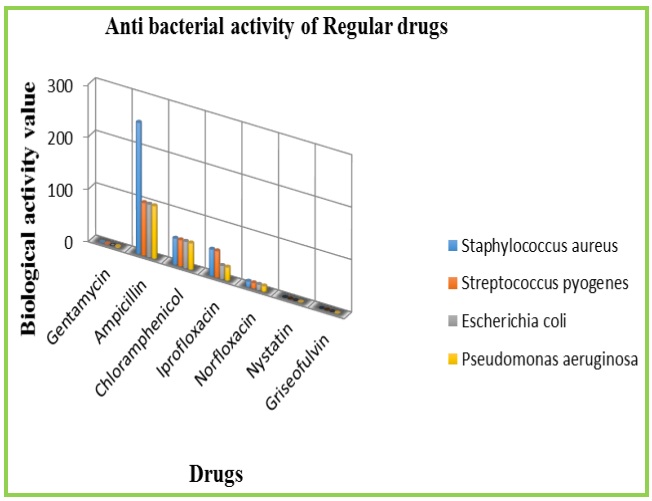
Code	Minimal inhibition concentration (µg mL^{-1})						
	Gram-positive		Gram-negative		Fungal species		
	S.a.	*S. p.*	*E. c.*	*P. a.*	*C. a.*	*A. n.*	*A. c.*
2a	1000	1000	500	500	250	1000	1000
2b	1000	500	250	500	1000	500	1000
2c	150	250	250	250	250	1000	1000
2d	150	200	250	100	500	500	1000
2e	62.5	500	250	1000	500	250	250
Gentamycin	0.25	0.5	0.05	1	-	-	-
Ampicillin	250	100	100	100	-	-	-
Chloramphenicol	50	50	50	50	-	-	-
Iproflo xacin	50	50	25	25	-	-	-
Norflo xacin	10	10	10	10	-	-	-
Nystatin	-	-	-	-	100	100	100
Griseofulvin	-	-	-	-	500	100	100

Antibacterial and antifungal activities draw by graph









CONCLUSION

In height, we include synthesized of novel **3-acetyl-4-hydroxy-2H-chromen-2-one** derivatives using easy and proper method. This method produces these products in unmatched yields and difficulty-free workup. Product is isolated by smooth filtration. The isolated products are very pure and do not need any purification. This study opens up a new area of useful synthesis of potentially biologically active novel pyrimidine derivatives compounds.

ACKNOWLEDGEMENT

Authors are thankful to the department of chemistry, TOLANI COLLEGE OF ARTS & SCIENCE, ADIPUR (KUTCH), India for providing laboratory facilities. One of us Dr Govind J. Kher is thankful to UNIVERSITY GRANTS COMMISSION, BAHADUR SHAH ZAFAR MARG, NEW DELHI for providing a research project [(**47-609/13(WRO)** dated **20 MAR 2014**].

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