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SYNTHESIS OF COUMARIN HETEROCYCLIC DERIVATIVES WITH *IN-VITRO* ANTITUBERCULER ACTIVITY

Godge Rahul [1*], Kunkulol Rahul [2]

[1*] Pravara Rural College of Pharmacy, Loni Bk, Tal-Rahata, Ahmednagar (M.S.), 413736-India

[2] Pravara Institute of Medical Sciences, Loni BK - Rahata, Ahmednagar (M.S.), 413736-India

ABSTRACT

In last few decades, though significant progress has been made in the treatment and control strategies of tubercular infections by introducing new diagnostic and monitoring tools and combination therapy, it still continues to be severe problem. The need of study was only because of there are many drugs in market to treat infection but most of the drugs are showing resistance because of the same it is difficult to treat the infection. In this study we chosen coumarin nucleus for study. Thus with the aim of developing novel molecule with improved potency for treating Mycobacterium tuberculosis H37Rv strain infections and with decreased probability of developing drug resistance**.** The synthesis of coumarin derivatives, starting from salicyaldehyde and ethyl acetoacetate, by conventional organic reaction and results of investigations of their anti-mycobacterial activity. MICs of the synthesized compounds are compared with existing drugs Cytotoxicity. Many compounds have shown promising activity while some were inactive. It was found that Compound A_1, A_2, B_1, B_2, C_1, C_2 have shown promising anti tubercular activity against std. Streptomycin

Keywords: Coumarin derivative, well diffusion method, antituberculer activity.

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***Address for Correspondence:** Mr. Rahul Keshav Godge, Pravara Rural College of Pharmacy, Loni BK, Tal-Rahata, Dist-Ahmednagar, Maharashtra-413736, India

INTRODUCTION

Microbial infections remain the major cause of death over the world. Emergence of multi-drug resistant to different infectious organisms like M. tuberculosis made the condition most alarming. Tuberculosis, MTB, or TB is a deadly infectious disease caused by various strains of mycobacteria; usually Mycobacterium tuberculosis. According to World Health Organization (WHO) TB is a global pandemic, which has become an important world-wide public health menace with one-third of the world's population infected by the TB bacillus. Most infections do not have symptoms, known as latent tuberculosis and about one in ten latent infections eventually progresses to active disease which, if left untreated, kills more than 50% of those so infected. People with weak immune systems (those with HIV/AIDS, those receiving immunosuppressive drugs and chemotherapy) are at a greater risk for developing TB disease.

In this research work we had synthesized novel heterocyclic compound and evaluated it for antituberculer activity which focuses on finding new anti mycobacterial agent to treat tuberculosis as well to overcome resistance caused by most of drugs. Coumarin (2H-Lbenzopyran-2-one) and its derivatives possess a wide range of various biological and pharmaceutical activities. They have a wide range of applications as antitumor[1, 2], anti-HIV [3, 4], anticoagulant [5, 6], antimicrobial[7, 8], antioxidant[9, 10], and anti-inflammatory[11, 12] agents. The antitumor activities of coumarin compounds have been extensively examined[13–16]. Although most of the existing natural coumarins have been isolated from higher plants, some of them have been discovered in microorganisms, for example, aminocoumarin antibiotics: novobiocin,

coumermycin A1, and chlorobiocin (produced by the actinomycete Streptomyces niveus) [17]. Synthetic coumarin derivatives have been obtained by chemical modification of the coumarin ring. Recently, density functional theory (DFT) has been accepted by the quantum chemistry community as a cost-effective approach for the computation of molecular structure, vibration frequencies, and energies of chemical reactions. Many studies have shown that the molecular structures and vibration frequencies calculated by DFT methods are more reliable than MP2 methods[18–26]. While there is sufficient evidence that DFT provides accurate description of the electronic and structural properties of solids, interfaces, and small molecules, relatively little is known about the symmetric performance of DFT applications to their molecular associates.

Structure activity relationships of coumarin derivatives have revealed that the presence of substituted amino derivatives is an essential feature of their pharmacological action. Based on these findings, we try to describe the synthesis of some compounds featuring different heterocyclic rings fused onto the coumarin moiety with the aim of obtaining more potent pharmacologically active compounds. The need of new antimycobacterial is only because of microorganisms is being resistant to the present drugs available in the clinical use. Worldwide researchers are trying to synthesize new drugs with better pharmacokinetic and dynamic activity with less adverse effects. The literature survey suggests that coumarins have proved to be good bioactive molecules, and holds broad potential for various activities like anti-bacterial, anti-fungal, anti-inflammatory, anti-tubercular, anti-HIV, and anticancer agents. In this coumarin derivatives synthesized and evaluated for antituberculer activity. Structural determination was carried out by Infra Red spectroscopy, ^{1}H-NMR, and preliminary tests as Physical constant determination, TLC, and Elemental analysis.

MATERIALS AND METHODS

General

The nucleus and its derivatives were analyzed by different ways. The melting points were recorded on electrothermal apparatus and are uncorrected. (IR) spectra were determined on Bruker IFS-66 FTIR (Bruker Bioscience, USA) using Potassium bromide (KBr) pallets and wave number (ί) was reported in cm-1. ^{1}H NMR spectra on a Bruker Avance 300 MHz instrument using DMSO as solvent using TMS as internal standard; the chemical shifts (δ) were reported in ppm with coupling constants (*J*) are given in Hz. Signal multiplicities were represented by s (singlet), d (doublet), t (triplet), ds (double singlet), dd (double doublet), m (multiplet) and bs (broad singlet. Elemental analysis was performed on a Hera-cus CHN-Rapid Analyzer. Analysis indicated by the symbols of the elements of functions was within ±0.4% of the theoretical values. The purity of the compounds was checked on silica gel coated Al plates (Merck).

1. Synthesis of 3-acetyl coumarin

To a cold mixture of Salicylaldehydehyde (0.2M) and ethyl acetoacetate (0.2M), 2ml of piperidine was added by rapid stirring. After 20 min thee yellowish solid separated was filtered off subsequently washed with ethanol and was recrystallised from water: ethanol (3:7), M.P 120^0 C and yield was 83.6%.

2. Preparation of 3-aryl-1-(3-coumarinyl) propan-1-ones:

A mixture of 3-acetyl coumarin and various substituted aldehydes(0.012 M) were dissolved in 10ml of n-butanol under heating; then 0.3ml glacial acetic acid and the same quantity of piperidine were added. The reaction mixture was refluxed for 4 hours and then solvent was removed in vacuum. The residue was triturated with 10ml ethanol until a precipitate formed. The precipitate was filtered off and recrystallized from appropriate solvent.

3. Synthesis of 5-(substituted) aryl-3-(3-coumarinyl)-1-phenyl-2- pyrazoline

3-aryl-1-(3-coumarinyl) -1-propan-1-ones, 0.05M and phenyl hydrazine (0.2M) were dissolved in pyridine (30ml) and refluxed for 6hrs. Reaction mixture was poured on to the crushed ice and neutralized with 2N hydrochloric acid. The precipitated solid was filtered, dried and recrystallised from appropriate solvent to afford the title compound.

Spectral Data

A_1- IR (KBr): 3650(Secondary amine), 3150 (C=C str), 3016 (Aromatic CH), 1950-1450 (Double bonded functional group), 1706 (CO str), 1639 (CN str), 1410 (Co str), 3000 (NH bending), 1250 (NH bending),

NMR: δ 10.0-10.1 1H, (NH, Pri. amine), 8.8-8.9 4H, (CH, Pyridine), 7.8-7.9 5H, (CH, Benzene), 4.6-4.8 1H, (NH, Sec. amine)

A_2- IR (KBr): 3645(Secondary amine), 3150 (C=C str), 3016 (Aromatic CH), 1950-1450 (Double bonded functional group), 1700 (CO str), 1620 (CN str), 1410 (Co str), 1400 (SO_2 bending), 3000 (NH bending), 1250 (NH bending)

A_3- IR (KBr): 3650(Secondary amine), 3150 (C=C str), 3010 (Aromatic CH), 1950-1450 (Double bonded functional group), 1706 (CO str), 1639 (CN str), 1410 (Co str), 3000 (NH bending), 1250 (NH bending)

B_1- IR (KBr): 3650(Secondary amine), 3150 (C=C str), 3016 (Aromatic CH), 1950-1450 (Double bonded functional group), 1706 (CO str), 1639 (CN str), 1410 (Co str), 3000 (NH bending), 1250 (NH bending)

B_2- IR (KBr): 3650(Secondary amine), 3150 (C=C str), 3016 (Aromatic CH), 1950-1450 (Double bonded functional group), 1706 (CO str), 1639 (CN str), 1410 (Co str), 1400 (SO_2 bending),3000 (NH bending), 1250 (NH bending)

B_3- IR (KBr): 3650(Secondary amine), 3150 (C=C str), 3016 (Aromatic CH), 1950-1450 (Double bonded functional group), 1706 (CO str), 1639 (CN str), 1410 (Co str), 3000 (NH bending), 1250 (NH bending)

C_1- IR (KBr): 3650(Secondary amine), 3150 (C=C str), 3016 (Aromatic CH), 1950-1450 (Double bonded functional group), 1706 (CO str), 1639 (CN str), 1410 (Co str), 3000 (NH bending), 1250 (NH bending)

NMR: δ 10.0-10.1 1H, (NH, Pri. amine), 8.8-8.9 4H, (CH, Pyridine), 7.8-7.9 5H, (CH, Benzene), 4.6-4.8 1H, (NH, Sec. amine)

C_2- IR (KBr): 3650(Secondary amine), 3150 (C=C str), 3016 (Aromatic CH), 1950-1450 (Double bonded functional group), 1706 (CO str), 1639 (CN str), 1410 (Co str), 1400 (SO_2 bending) 3000 (NH bending), 1250 (NH bending)

C_3- IR (KBr): 3650(Secondary amine), 3150 (C=C str), 3016 (Aromatic CH), 1950-1450 (Double bonded functional group), 1706 (CO str), 1639 (CN str), 1410 (Co str), 3000 (NH bending), 1250 (NH bending)

Antituberculer activity:

The compounds were tested in-vitro for their antituberculer activity against H_{37}Rv Strain.

Method:

Alamar Blue Dye

The antitubercular screening was carried out by Middle brook 7H9 agar medium against H_{37}Rv Strain. Middle brook 7H9 agar medium was inoculated with *Mycobacterium tuberculosis* of H_{37}Rv Strain. The inoculated bottles were incubated for 37°C for 4 weeks. At the end of 4 weeks they were checked for growth.

RESULT

Scheme: (A_1-A_3, B_1-B_3, C_1-C_3)

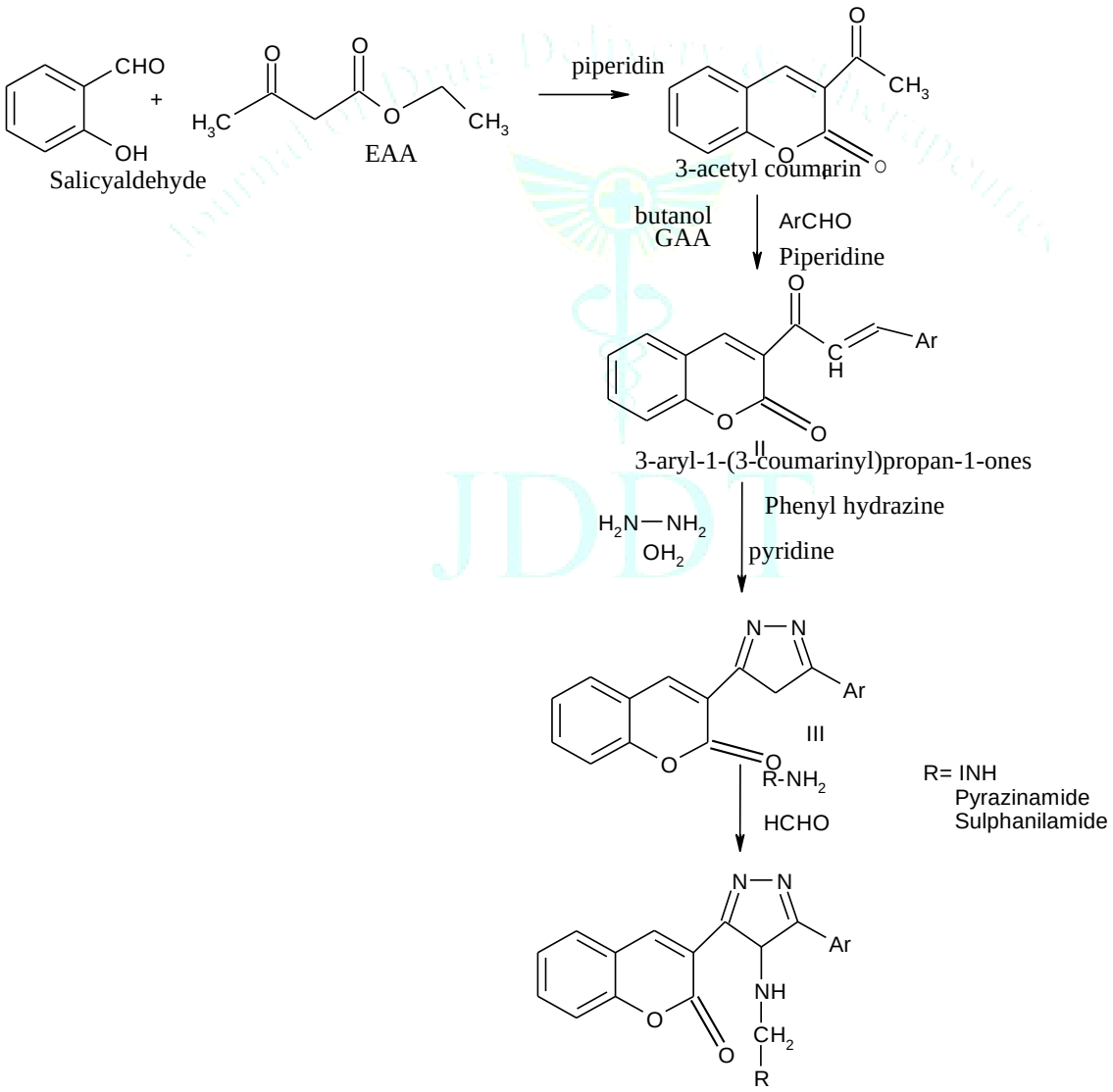


Table 1: Derivatives

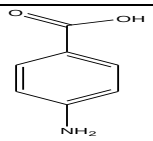
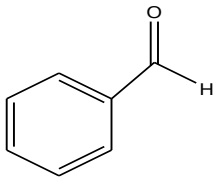
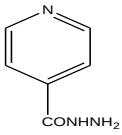
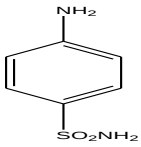
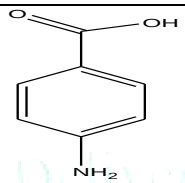
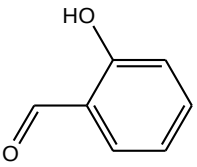
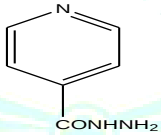
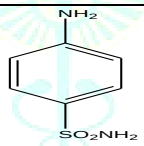
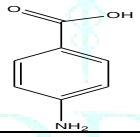
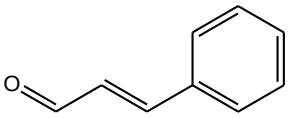
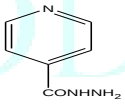
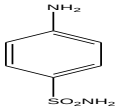
Compound	R	Ar
A_1		
A_2		
A_3		
B_1		 salicaldehyde
B_2		
B_3		
C_1		 Cinnamaldehyde
C_2		
C_3		

Table 2: Analytical data of the compound

Comp.	Mol. formula	Mol. wt	Melting Point (^{0}C)	R_f Value	Yield %	Elemental analysis Calculated				
						C	H	N	O	S
A1	C27H19N3O5	465.45	198-200	0.57	84.67	69.67	4.11	9.03	17.19	
A2	C26H19N5O4	465.46	175-177	0.69	82.77	67.09	4.11	15.05	13.75	
A3	C26H20N4O5S	500.52	167-169	0.62	77.19	62.39	4.03	11.19	15.98	6.41
B1	C27H19N3O6	481.45	199-202	0.55	71.57	67.36	3.98	8.73	19.94	
B2	C26H19N5O5	481.45	202-204	0.78	81.29	64.86	3.98	14.55	16.62	
B3	C26H20N4O5S	500.52	273-275	0.75	84.23	62.39	4.03	11.19	15.98	6.41
C1	C29H21N3O5	491.49	198-200	0.8	79.83	70.87	4.31	8.55	16.28	
C2	C28H21N5O4	491.49	195-198	0.64	82.91	68.42	4.31	14.25	13.02	
C3	C28H22N4O4S	510.56	198-200	0.57	82.79	65.87	4.34	10.97	12.53	6.28

Table 3: Anti-tuberculer activity of 5-(substituted) aryl-3-(3-coumarinyl)-1-phenyl-2- pyrazoline compounds

Comp ID	100	50	25	12.5	6.25	3.125	1.6	0.8	0.4	0.2
A_1	S	S	S	S	S	S	S	R	R	R
A_2	S	S	S	S	S	S	R	R	R	R
A_3	S	S	S	S	R	R	R	R	R	R
B_1	S	S	S	S	S	S	S	S	R	R
B_2	S	S	S	S	S	S	R	R	R	R
B_3	S	S	S	S	S	R	R	R	R	R
C_1	S	S	S	S	S	S	S	R	R	R
C_2	S	S	S	S	S	S	R	R	R	R
C_3	S	S	S	R	R	R	R	R	R	R
Streptomycin	S	S	S	S	S	R	R	R	R	R

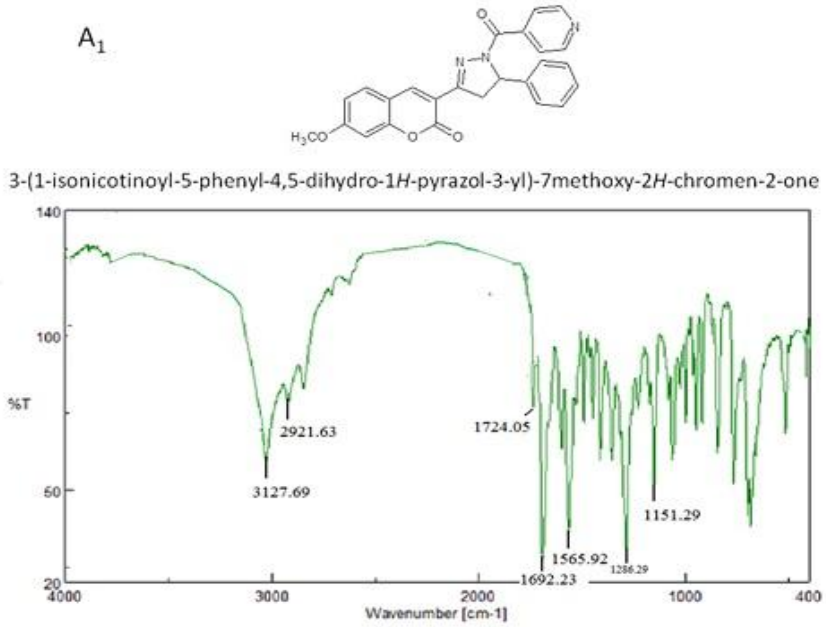


Figure 1: IR Spectra of A_1

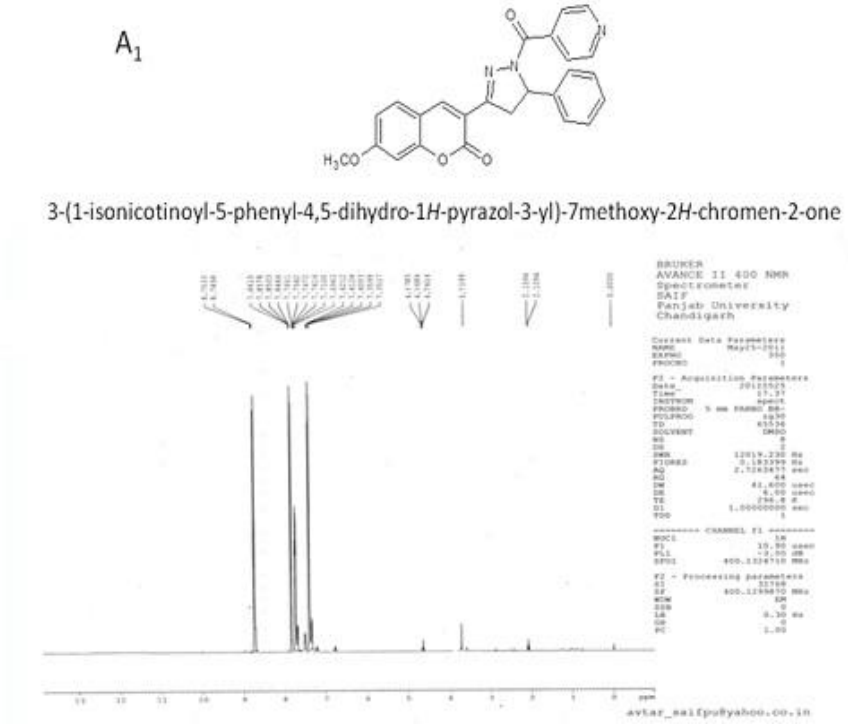


Figure 2: NMRSpectra of A_1

DISCUSSION

In the present research work, we have synthesized 9 new 5-(substituted) aryl-3-(3-coumarinyl)-1-phenyl-2-pyrazoline derivatives as explained in the scheme. The purity of the compounds was checked by TLC and melting point. Structures of these compounds were confirmed by IR, 1HNMR and elemental analysis. The synthesized compounds were subjected to anti tubercular activity by Alamar Blue Dye method against the standard streptomycin.

Compound A_1, A_2, B_1, B_2, C_1, C_2 have shown promising antitubercular activity against streptomycin at concentration of 1.6 mcg/ml by interpreting data of MIC. With the suitable molecular modification and manipulation with possible SAR studies of these compounds, promising anti tubercular agents can be obtained[26-27].

CONCLUSION

The practice of medicinal chemistry is devoted to discovery and development of new agents for treating diseases. Most of the activity in this discipline is directed to new natural or synthetic organic compounds. Heterocyclic compounds have a major place in therapy and they being increasingly specific biological and pharmacological activities are clearly the dominant force. Thousands of heterocyclic compounds are prepared annually throughout the world, and many of them enter into pharmacological screening to determine if they have useful biological activities.

The present work proposed to study on design, synthesis and biological evaluation of coumarin heterocycles. Around nine compounds were synthesized by conventional and efficient method. The detailed review of literature and survey was carried out for the synthesis of coumarin derivatives for antituberculer activity. A series of 5-(substituted) aryl-3-(3-coumarinyl)-1-phenyl-2- pyrazoline (A_1-C_3) were designed and synthesized.

The purity of synthesized compounds was ascertained by TLC. Melting points were determined by open capillary method and are uncorrected. The structures of the synthesized compounds were confirmed on the basis of elemental analysis and spectral studies detailed below.

1. IR spectra were recorded on JASCO-420-FTIR spectrometer by KBr pellet technique.
2. ^{1}H-NMR spectra were recorded on Bruker Avance II 400 NMR spectrometer.

The compounds were subjected to selected biological and pharmacological activities. The synthesized compounds were screened for the antituberculer activity against streptomycin between concentration ranges of 2µg/ml - 100µg/ml by Alarm blue method. Most of the compounds were active against selected microorganisms and inhibited the growth.

ACKNOWLEDGEMENT

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