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Synthesis and Evaluation of Anti-Tubercular Activity of Schiff Base Divalent Metal Complexes of 7-Amino-4-Methyl-Benzopyran-2-One Derivatives.

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ABSTRACT

Background: *The anti-tubercular characteristics of coumarin-metal complexes have received greater research attention than their other biological attributes because of the urgency of TB treatment. This study focuses on the synthesis and anti-tubercular activity of Schiff base manganese metal complexes of derivatives of 7-amino-4-methyl-benzopyran-2-one. Five of the title compounds have been synthesized. These were made by mixing m-aminophenol, ethyl acetate, and ethyl chloroformate together to create substituted urethane. The resultant substance was further condensed with sulphuric acid and ethyl acetoacetate to produce 7-carbethoxyamino-4-methyl benzopyran-2-one, which was then converted to 7-amino-4-methyl-benzopyran-2-one.*

Results: *This was then used to synthesize the (S1-5) Schiff base compounds by reacting it with a series of substituted aromatic aldehydes. Metal complexes (M1–5) of benzopyran-2-one Schiff derivatives were generated by stirring at 25°C, followed by filtering. TLC was used to determine the purity of the compounds. Spectroscopic measurements verified the structures of these substances.*

Conclusion: *The consequently produced title compounds are next characterized by IR and 1H NMR spectrum analyses. The IR characteristic peaks validated the production of the desired chemicals. A thorough 1H NMR analysis of the products also confirmed the process. The MABA method was used to examine all of these recently synthesized compounds M1–M5 for their in vitro anti-tubercular activity.*

Keywords: *Coumarin, Manganese metal complex, anti-tubercular activity, benzopyran-one, Schiff base.*

BACKGROUND

Tuberculosis which is also known as 'white plaque' is the most serious infectious disease globally(Hu et al., 2017). It is the 13th most significant cause of death and the second leading infectious disease. Approximately 10.4 million cases of tuberculosis were reported worldwide, with 5 million (56%) men, 3.5 million (34%) women, and 1 million (10%) children (WHO, 2017(Khan et al., 2019). Robert Koch first identified *Mycobacterium tuberculosis* as the causative agent of human tuberculosis (TB) nearly a century ago. However, this bacterium is still a threat to human health. Aside from producing tuberculosis, there is growing evidence that M. *tuberculosis* is linked to a variety of other human diseases, including pulmonary complications, autoimmune diseases, and metabolic syndromes(Chai et al., 2018). The other causative organisms of tuberculosis are M. *africanum*, M. *bovis*, M. *caprae*, M. *microti*, M. *pinnipedii*

and M. canettii(Hu et al., 2017). Tuberculosis is quite prominent among poor socioeconomic groups and underprivileged members of the community. It is definitively diagnosed by demonstrating *M. tuberculosis* bacilli using microbiological, cytopathological, or histopathological methods. Tuberculosis (TB) has evolved from an incurable to a curable disease due to the development of drugs such as(Natarajan et al., 2020)

- First line – Isoniazid, Rifampicin, Rifabutin, Pyrazinamide, Ethambutol.
- Second line – Amikacin, Kanamycin, Streptomycin, Capreomycin, Gatifloxacin, Levofloxacin, Para-amino-salicylic acid, Cycloserine, Ethionamide.
- Third line – Clarithromycin, Amoxicillin/clavulanic acid, Linezolid(Fenando, 2012).

But the most problematic issue is that the TB-bacteria are becoming resistant to almost all these drugs which lead to multidrug-resistant TB (MDR-TB), extensively drug-resistant TB (XDR-TB) and in rare cases totally drug-resistant TB (TDR-TB), hence there is necessary to increase research efforts to develop anti-tubercular medications.

Natural substances have always drawn the attention of medicinal chemists because of their enormous medical benefits. Among naturally occurring substances, coumarin (2H-1-benzopyran-2-one) is a well-known bioactive substance that is mostly found in plants and is renowned for its wide range of biological activity(Reddy et al., 2021). It was first isolated by Vogel in 1820 from *dipteryx odorata*, popularly known as cumaru and more than 1300 coumarins were identified from natural sources, mainly green plants(Pereira et al., 2018). Coumarin and its derivatives, constitute an important class of benzopyrones that play an vital role in chemistry attributing to their ability to exert noncovalent interactions (π-π, hydrophobic, electrostatic interactions, hydrogen bonds, metal coordination and van der Waals force etc.) with the various active sites in organisms(Hu et al., 2017). In the field of medicine, there has been an incredible development of coumarin compounds as anticancer(Abdizadeh et al., 2017; Al-Wahaibi et al., 2018; Garro et al., 2016; Huang et al., 2011), anti-inflammatory(Bansal et al., 2013), antimalarial(Pingaew et al., 2014), antimicrobial(Chimenti et al., 2010; El-Wahab et al., 2014; Nagamallu et al., 2016), antioxidant(Kostova et al., 2012; Niu et al., 2017), antiviral(Mishra et al., 2020; Zaheen et al., 2020) and antidiabetic agents(Pan et al., 2022; Tegginamath et al., 2016). This broad spectrum of biological activity and successful usage of coumarin based drugs in the medical field, further inspires more research work that allows developing a large number of structurally diverse coumarin derivatives(Hu et al., 2017).

Metal ions play a significant role in many biological applications(Patel et al., 2014). Transition metal complexes are linked to a variety of biomolecules that are crucial to the human body's vital physiological processes. In pursuit of searching new anti-TB agents, numerous metal complexed coumarin derivatives have been synthesized and screened for their anti-TB activity, as coumarin derivatives have a tendency to give coordination compounds with different transition metal ions.

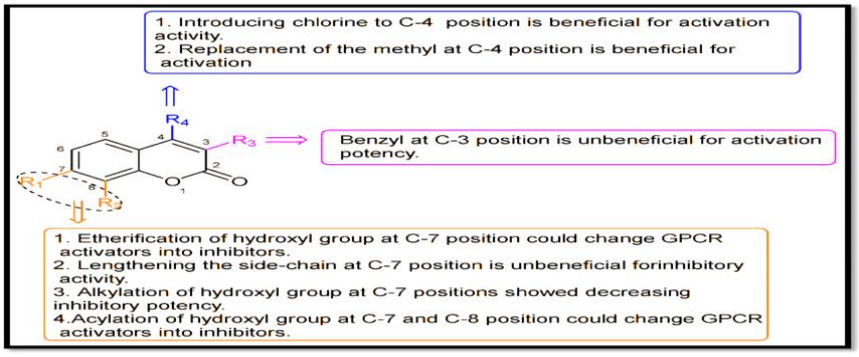


Figure No: 01 General structure-activity relationship of Benzopyran-2-one(Singh et al., 2019)

MATERIAL AND METHODS:

Chemicals:

m-aminophenol, Ethyl acetate, Ethyl chloroformate, Conc. Sulphuric acid, Ethyl acetoacetate, Hydrazine hydrate (99%), Acetic anhydride (Fischer scientific Co.), The aromatic aldehydes (S.D. Fine Chem. Ltd., Mumbai and Sigma Aldrich Co.) and Every chemical is of the reagent-grade variety.

Instruments:

The open capillary method was used to determine the uncorrected melting points of the synthesized compounds, KBr pellets were used to record FT-IR spectra on a Shimadzu spectrophotometer and 400mHz NMR spectroscopy.

Chemical synthesis:

The title Schiff base metal complexes of benzopyran-2-one were synthesized by 5 steps method. In order to create 3-hydroxy phenyl urethane, m-aminophenol was condensed with ethyl chloroformate to start the process. In order to create 7-carbethoxyamino-4-methyl coumarin, this product is treated with 75% conc. sulphuric acid in the presence of ethyl acetoacetate in the second stage. The resultant compound produces 7-amino-4-methyl benzopyran-2-one after being hydrolyzed with sulfuric acid and glacial acetic acid. These 7-amino-4-methyl benzopyran-2-one derivatives were further processed with different substituted aromatic aldehydes to produce Schiff bases, which were then processed with manganese sulphate and diluted ammonia solution to produce Schiff base metal complexes.

Preparation of Schiff bases of 7-amino-4methyl-benzopyran-2-one (S1-S10):

The 7-amino-4-methyl-benzopyran-2-one Schiff bases were created by refluxing a mixture of 7-amino-4-methylcoumarin (1.5 g, 0.085 mol), substituted aromatic aldehyde (0.017 mol), and pure alcohol in 25 ml for 6 hours. A lower pressure was used to extract the solvent. The resultant crude product was then recrystallized using ethyl acetate to produce cyclohexane after being rinsed with cold water (1:2). TLC was used to verify the compounds' purity.

Preparation of metal complexes of coumarin Schiff base (M1-M10):

By combining Schiff bases of 7-amino-4-methyl-benzopyran-2-one with metal sulphates that were dissolved in methanol in amounts that matched the molar ratio of the metal to the ligand, the metal complexes were produced (1:2). By gradually adding drops of a diluted ammonia solution, the pH was increased. After the metal (1 mmol) and ligand (2 mmol) were gradually added, the reaction mixture was stirred at 25°C using an electromagnetic stirrer. Precipitate was produced as the solutions were being mixed. The mixture was filtered after being agitated for a specific amount of time.

ANTI-TB ACTIVITY USING ALAMAR BLUE DYE

Procedure

- The anti-Mycobacterial activity of compounds were assessed against M.tuberculosis using microplate Alamar Blueassay (MABA).
- This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method.
- Briefly, 200µl of sterile deionized water was added to all outerperimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation.
- The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate.
- The final drug concentrations tested were100 to 0.2µg/ml.
- Plates were covered and sealed with para film and incubated at 37°C for five days.
- After this time, 25µl of freshly prepared 1:1mixture of Alamar Blue reagent and 10% tween80 was added to the plate and incubated for 24hrs.

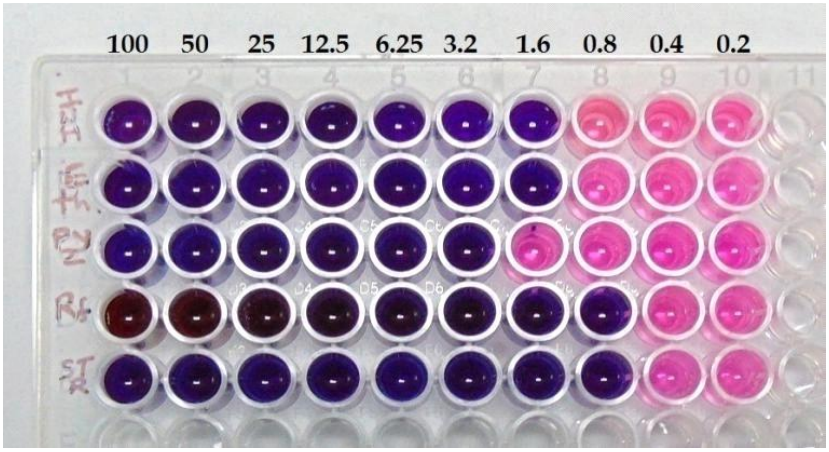
- A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth.
- The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.

Standard Strain used: ***Mycobacteria tuberculosis*** (Vaccinestrain,H37RVstrain):ATCCNo-27294.

StandardvaluesfortheAnti-Tbtestwhichwasperformed.

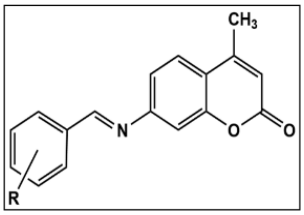
Isoniazid – 1.6µg/ml
Ethambutol – 1.6µg/ml
Pyrazinamide- 3.125µg/ml
Rifampicin – 0.8µg/ml
Streptomycin- 0.8µg/ml

Standard Drug Photograph



RESULTS

Table No 01: Physical data of N-substituted Schiff bases of 7-amino-4-methyl-benzopyran-2-one.



Sr. No	Compound Code	R	% Yield	Melting Point (℃)	Molecular Formula	Rf Value
1	S1	2-Cl	62.6	160	$C_{17}H_{12}ClNO_2$	0.75
2	S2	3-Br	80	154	$C_{18}H_{15}O_2N$	0.72
3	S3	4-NO_2	83.4	156	$C_{17}H_{12}N_2O_4$	0.43
4	S4	2,3-Cl_2	62.98	152	$C_{17}H_{11}O_2NCl_2$	0.51
5	S5	2,5-OCH_3	62.4	164	$C_{19}H_{17}NO_4$	0.72

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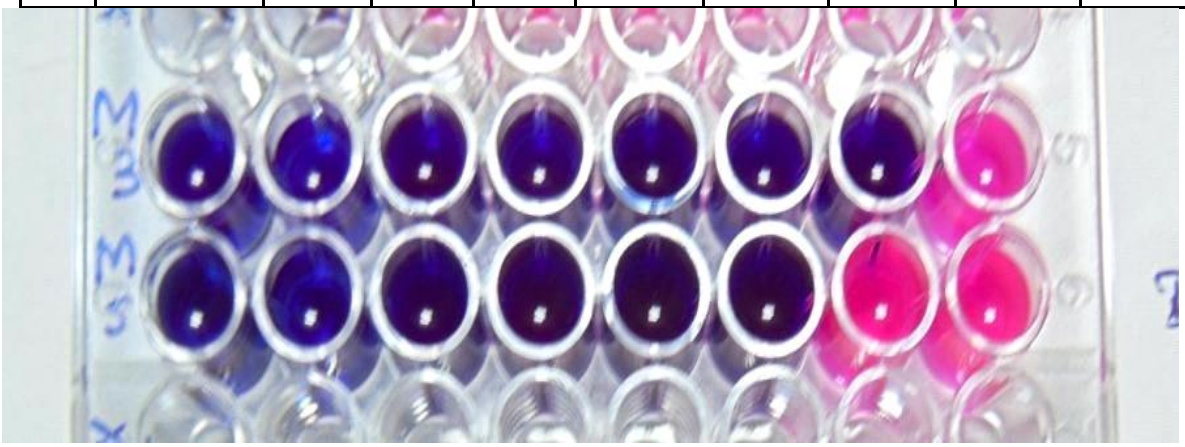
Table No 02: Physical data of Manganese metal complexes of n-substituted Schiff bases of 7-amino-4-methyl-benzopyran-2-one derivatives.

Sr. No	Compound Code	R	% Yield	Melting Point (℃)	Molecular Formula	Rf Value
1	M1	2-Cl	62	254	$C_{34}H_{24}O_4N_2Cl_2Mn$	0.72
2	M2	3-Br	64.20	278	$C_{34}H_{24}O_4N_2Br_2Mn$	0.74
3	M3	4-NO_2	71.2	238	$C_{34}H_{24}O_8N_4Mn$	0.54
4	M4	2,5-Cl_2	64.28	282	$C_{34}H_{22}O_4N_2Cl_4Mn$	0.51
5	M5	2,5-OCH_3	81.3	262	$C_{38}H_{34}O_8N_2Mn$	0.71

Table No 03: The sensitivity and resistance data showed by synthesized compounds towards *Mycobacterium tuberculosis*.

Sr. No.	Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.2 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
01	M1	S	S	S	S	S	R	R	R
02	M2	S	S	S	S	S	R	R	R
03	M3	S	S	S	S	S	S	S	R
04	M4	S	S	S	S	R	R	R	R
05	M5	S	S	S	S	S	S	R	R



Photograph of the compounds which are showing good activity(M3 & M5).

Note:

S- Sensitive
R- Resistant

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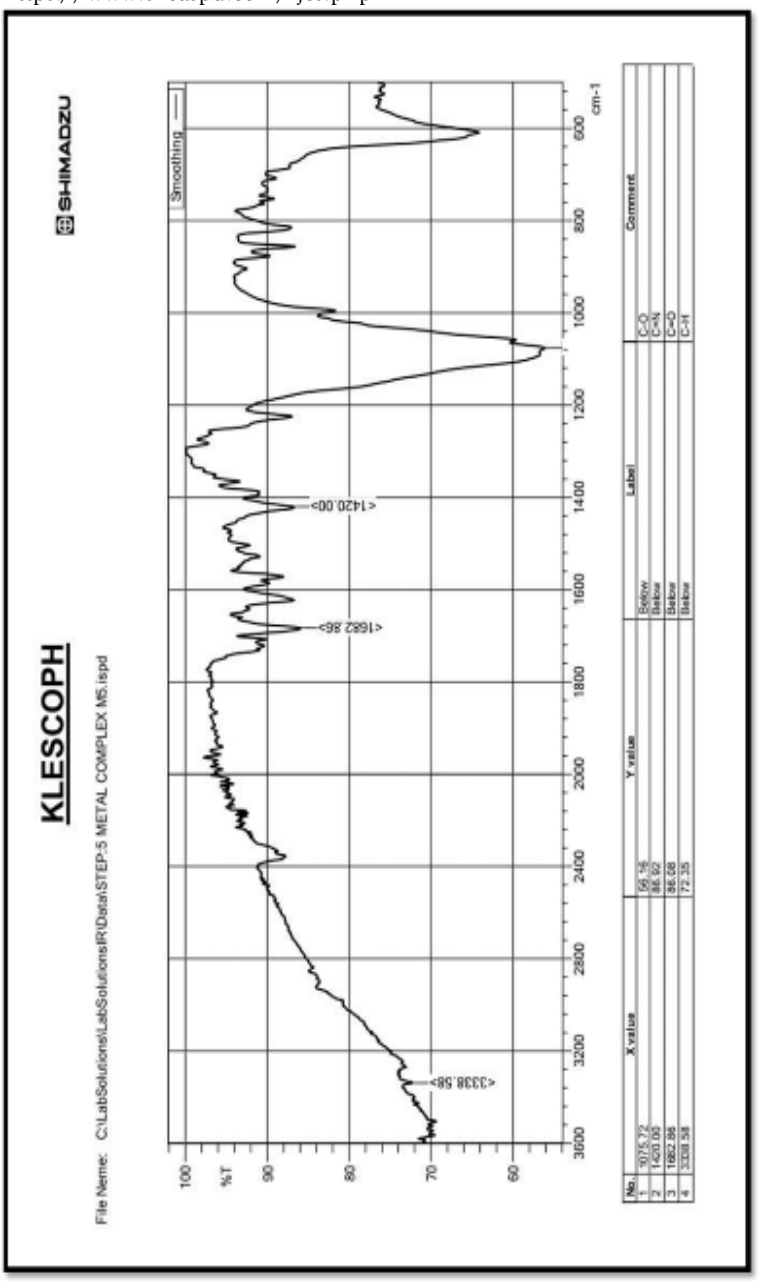
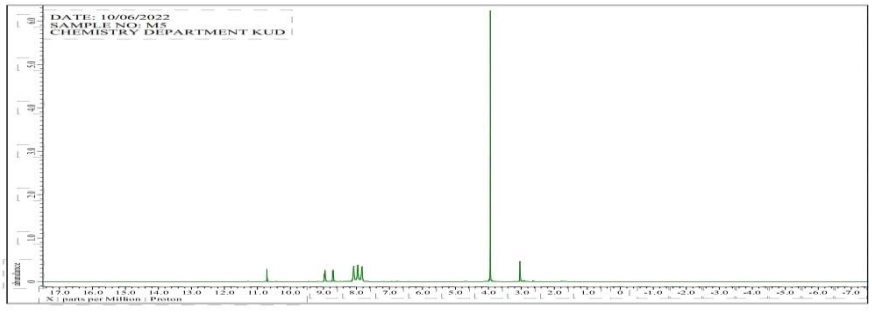


Spectrum No 01: IR of Manganese metal complex of Schiff base of 4-nitro benzaldehyde.



Spectrum No 03: NMR Of Manganese metal complex of Schiff base of 2,5-Dimethoxy Benzaldehyde.

DISCUSSION

The goal of the work is to produce N-substituted Schiff bases of manganese metal complexes of 7- amino-4-methyl benzopyran-2-one.

The second molecule was created by combining the first substance, 7-amino-4-methyl coumarin, with substituted aromatic aldehydes. The first chemical was created by hydrolyzing 7-carboxyamino-4-methyl coumarin with sulfuric acid. N-substituted Schiff bases of 7-amino-4-methyl coumarin were converted to manganese complexes by treating the molecule with manganese sulphate (M1 – M5).

Metal complexes (M1-M5) exhibited good anti-tubercular activity, among which the metal complexes M3 and M5 showed potent activity with significant sensitivity towards *M. Tuberculosis* at various concentrations.

CONCLUSION:

The 7-hydroxy-4-methyl-benzopyran-2H-chromen-2-one or 7-hydroxy-4-methyl-coumarin was produced using Pechmann condensation. Sulfuric acid is necessary for the condensation between m-aminophenol and ethyl acetoacetate to occur. First, 3-hydroxyphenylurethane was created by treating the m-aminophenol with ethyl acetate in the presence of ethyl chloroformate. This compound was then condensed with ethyl acetoacetate in the presence of 75% sulfuric acid to create 7-carbethoxyamino-4-methylcoumarin. Additionally, the 7-carbethoxyamino-4-methylcoumarin was treated with an equal volume of sulphuric acid and glacial acetic acid, which results in the production of 7-amino-4-methylcoumarrin when made alkaline with sodium hydroxide and sodium carbonate. By reacting the 7-amino-4-methyl-benzopyran-2H-chromen-2-one with substituted aldehydes that are dissolved in absolute alcohol in the presence of acetic anhydride, the Schiff bases are produced. Using manganese sulphate, the 7-amino-4-methylcoumarin Schiff bases are converted into the corresponding manganese metal complexes. The consequently produced title compounds are next characterized by IR and 1H NMR spectrum analyses. The IR characteristic peaks validated the production of the desired chemicals. A thorough 1H NMR analysis of the products also confirmed the process.

The MABA method was used to examine all of these recently synthesized compounds M1–M5 for their in vitro anti-tubercular activity.

Acknowledgement

Authors express their heartfelt gratitude to Discipline of KLE College of Pharmacy Hubli, for their constant support, encouragement and financial assistance. Authors sincerely thank to, Dr. Arun for in vitro anti-tubercular activity characterization. And also, thanks to Mr. Pavan for NMR studies.

LIST OF ABBREVIATIONS

℃ - Degree centigrade

CDCl3 - Deuterated chloroform

Conc. - Concentrated

DMSO - Dimethyl sulfoxide

FT- IR - Fourier Transform Infrared

gm - Grams

hr - Hour

M.P. - Melting point

min - Minutes

Mol - Mole

NMR - Nuclear magnetic resonance

pH - Hydrogen ion concentration

ppm - Parts per million

Rf - Retardation factor

TLC - Thin Layer Chromatography

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