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PRELIMINARY EVALUATION OF ANTI TUBERCULI ACTIVITY OF NOVEL SYNTHESIZED HYDRAZONE DERIVATIVES

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ABSTRACT

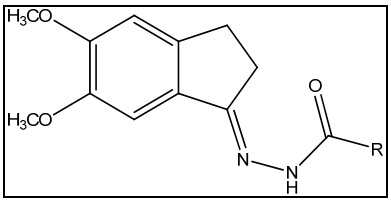
Series of newly synthesized hydrazone derivatives were obtained and evaluated for their *in vitro* antimicrobial activity using conventional quantitative methods, to find out minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) using macrodilution and microdilution techniques according to CLSI standards for anti-tubercular and anti-microbial evaluation respectively. From the results of MIC and MBC it is concluded that synthesized hydrazone derivatives shows significant anti tubercular as well as antimicrobial activity. Amongst all, test drug no. V0102, V0104 and V201 were found more effective against *Mycobacterium tuberculosis*. (MIC= 100µg/ml). While, for antimicrobial activity almost all drugs shows more or less inhibitory activity. (specifically against *E. coli*. strain).

INTRODUCTION

Tuberculosis is a chronic infectious disease caused by Mycobacteria [1], and presently regarded as the most dangerous infective disease worldwide. It is one of the major infection associated with AIDS.[2] The increase in drug-resistant Mycobacterium tuberculosis isolates during recent years presents a therapeutic challenge to physicians selecting antimicrobial agents. [3]That is why, search for new anti -tuberculosis agent is required, in spite of the availability of effective antibiotics. In literature, it is revealed that hydrazones possessing anti tubercular, antimicrobial, anticonvulsant, analgesic, anti inflammatory, anti platelet, anti tumoral activity and having an azometine -NHN=CH- proton constitute, serves an important class of compounds for new drug development. These observations have been guiding for the development of new hydrazones that possess varied biological activities [4]. So, the present was undertaken to evaluate preliminary Anti Microbial activity of some novel synthesized Hydrazone derivatives with special emphasis on their activity against *Mycobacterium tuberculosis*. Samples for the study was obtained as a gift from Mr. Vimal Patel (From Dept. of Pharmaceutical Chemistry, Saurashtra Uni.). Structural characteristics of these compounds are listed in Table 1:

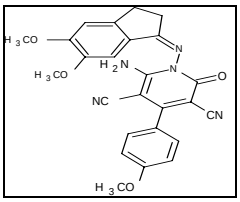
Table 1: Series of novel synthesized Hydrazone Derivatives.

(A) Series -1



Comp no.	R
V0101	$-CH_2CN$
V0102	$-C_6H_5$
V0103	$-CH_2CH_3$
V0104	$-CH_2OC_6H_5$
V0105	$-C_6H_5NO_2$
V0106	$-C_6H_50H$

(B) Series-2



V0201

MATERIALS AND METHODS

Standard & Test Drugs:

Standard Drugs: Isoniazid, Gentamycin, Ampicillin, Chloramphenicol, Ciprofloxacin, Norfloxacin, Nystatin, Greseofulvin.

Test Drugs: All listed above in Table 1 were taken as test drugs.

Microbial Strains & Media:

Acid fast Bacilli: *Mycobacterium tuberculosis H37Rv*

Gram-positive bacteria: *Staphylococcus aureus, Streptococcus pyogenus, Streptococcus pneumoniae, Bacillus subtillis.*

Gram-negative bacteria: *Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa.*

Fungi: *Candida albicans, Aspergillus fumigates.*

Bacteria and fungi strains were procured from the stock cultures of the Institute of Microbial Technology, Chandigarh, India. Strains of mycobacteria, bacteria (Gram-positive as well as Gram-negative) and fungi were maintained on Lowenstein-Jensen medium, Muller Hinton agar medium and Sebouraud-dextrose agar medium respectively.

EVALUATION

The Antimicrobial activity with special emphasis on the Anti Tuberculi activity was determined by using turbidometric analysis. The inhibitory effect of the test drugs were estimated by serial two fold dilution technique (MIC and MBC). For Anti Tuberculi activity, macrodilution technique and for Anti Microbial activity, microdilution technique was used. *In vitro* susceptibility of these organisms was carried out using NCCLS method.[5] The test drugs were dissolved in Dimethyl Sulphoxide (DMSO 2%) and then diluted to the highest concentration(1000 µg/ml). Subsequently two fold serial dilutions were made in two different concentration range from 1000-7.8 µg/ml and 100-0.375 µg/ml. For anti tubercular activity, the dilutions were prepared by dispensing into each tube, 1 ml of respective nutrient broth and 1 ml of each test drug to achieve the concentrations of 1000µg/ml and 100µg/ml and then serially diluted and for anti microbial activity 0.1 ml of these dilutions was transferred to 96-well round bottomed microtitre plate, and 0.1 ml of respective broth media was added to each well. Suspensions of organisms were prepared as per 0.5 McFarland standard. 50µl of freshly prepared inoculum ($1*10^6$ cells/ml) was added to each tube including control and then incubated at 37ºC for 24 hours for determination of MIC.

For determination of MBC, a loopful of the culture from the tube exhibiting MIC was streaked on sterile Nutrient agar and incubated overnight at 37ºC. The plate showing no growth of organism after incubation was determined as MBC.

RESULTS

Table 3: *In Vitro* anti-tubercular activity of hydrazone derivatives V0101-V0106 and V0201[a].

Comp.	R	MIC (µg/ml)
V0101	$-CH_2CN$	1000
V0102	$-C_6H_5$	100
V0103	$-CH_2CH_3$	500
V0104	$-CH_2OC_6H_5$	100
V0105	$-C_6H_5NO_2$	200
V0106	$-C_6H_5OH$	1000
V0201		100

a:Standard drug: Isoniazid MIC: 0.20 µg/ml

Fig:3:Anti tubercular activity of test drug V0104 at MIC of 100µg/ml.

Table 3a:Activity against Gram-positive bacteria.

MINIMAL INHIBITORY CONCENTRATION (µg/ml)					
SR.NO	**CODE NO.**	***S.aureus***	***S.pyogenus***	***S.pneumoniae***	***B.subtilis***
		MTCC 96	**MTCC 442**	**MTCC 1936**	**MTCC441**
1	VO101	125	62.5	125	100
2	VO102	250	200	200	62.5
3	VO103	100	100	250	125
4	VO104	200	200	200	250
5	VO105	500	500	200	200
6	VO106	125	250	250	250
7	VO201	250	250	250	200
Standard					
	Gentamycin	0.25	0.5	0.5	1
	Chloramphenicol	50	50	50	50
	Ciprofloxacin	50	50	50	50
	Norfloxacin	10	10	10	100

MINIMAL BACTERICIDAL CONCENTRATION (µg/ml)					
SR.NO	**CODE NO.**	***S.aureus***	***S.pyogenus***	***S.pneumoniae***	***B.subtilis***
		MTCC 96	**MTCC 442**	**MTCC 1936**	**MTCC441**
1	VO101	125	100	250	125
2	VO102	250	250	250	125
3	VO103	125	125	500	200
4	VO104	250	200	250	250
5	VO105	500	500	500	250
6	VO106	125	500	250	500
7	VO201	250	250	250	250

Table 3b:**Activity against Gram-negative bacteria.**

MINIMAL BACTERICIDAL CONCENTRATION (µg/ml)				
SR.NO	**CODE NO.**	***E.COLI***	***P.AERUGINOSA***	***S.TYPHI***
		MTCC 443	**MTCC 1688**	**MTCC98**
1	VO101	125	250	500
2	VO102	25	125	200
3	VO103	250	125	250
4	VO104	200	200	250
5	VO105	250	500	200
6	VO106	100	125	500
7	VO201	125	200	250

MINIMAL INHIBITORY CONCENTRATION (µg/ml)				
SR.NO	**CODE NO.**	***E.COLI***	***P.AERUGINOSA***	***S.TYPHI***
		MTCC 443	**MTCC 1688**	**MTCC98**
1	VO101	100	250	250
2	VO102	12.5	125	125
3	VO103	200	125	250
4	VO104	100	100	200
5	VO105	250	500	125
6	VO106	62.5	100	250
7	VO201	100	125	200
Standard				
	GENTAMYCIN	0.05	1	5
	AMPICILLIN	100	--	100
	CHLORAMPHENICOL	50	50	50
	CIPROFLOXACIN	25	25	25
	NORFLOXACIN	10	10	10

Table 3c: **Activity against fungus strain**

MINIMAL INHIBITORY CONCENTRATION (µg/ml)			
SR.NO	**CODE NO.**	***C.ALBICANS***	***A.NIGER***
		MTCC 227	**MTCC 282**
1	VO101	500	1000
2	VO102	250	1000
3	VO103	500	1000
4	VO104	1000	500
5	VO105	>1000	250
6	VO106	>1000	500
7	VO201	500	500
Standard			
	NYSTATIN	100	100
	GRESEOFULVIN	500	100

MINIMAL BACTERICIDAL CONCENTRATION (µg/ml)			
SR.NO	**CODE NO.**	***C.ALBICANS***	***A.NIGER***
		MTCC 227	**MTCC 282**
1	VO101	1000	1000
2	VO102	500	>1000
3	VO103	>1000	1000
4	VO104	>1000	1000
5	VO105	>1000	250
6	VO106	>1000	500
7	VO201	>1000	1000

DISCUSSION

Antitubercular activity of synthesized hydrazone derivatives (V0101, V0102, V0103, V0104, V0105, V0106 and V0201.) was determined in terms of MIC by using macrodilution technique against *Mycobacterium tuberculosis* $H_{37}RV$ strain MTCC-200. Isoniazid was taken as standard (MIC=0.20 µg/ml) and MIC of all test drugs were determined (1000, 100, 500, 100, 200, 1000 and 100µg/ml respectively) using L. J. medium. The drugs showing MIC of 100 µg/ml or less than 100 µg/ml were considered effective against T.B. strain (according to CLSI standards). According to the standards, amongst the stated test drugs, V0102, V0104 and V0201 were found to have effective anti-tubercular activity. However, the activity was significantly lower than that of Isoniazid.

Our results are in agreement with the structure-activity relationship of hydrazone derivatives having similarity with Isoniazid. Here, in the first series, the pyridine ring has been replaced with different alkyl/aryl groups in addition to substitution at N 2. Data from earlier study suggests retention of activity, though potency may be significantly lower than Isoniazid. Our results show that 2 of the compounds, V0102 and V0104 from this series retained antitubercular activity however; the MIC was much higher than Isoniazid. In other words, they had lesser potency. Earlier data also suggests that such substitution is associated with a lower toxicity profile, probably owing to lower formation of toxic metabolic products in the Liver. Hence, these compounds and their derivatives may be tested further as anti tubercular moieties with a lower toxicity profile. [24]

In the second series, V0201 was also found to have similar activity as the other 2 compounds from first series. However, no data is available on the SAR (anti TB) of compounds with similar structure. Hence, further work on other compounds of this series (not available here) needs to be done to ascertain their potential as anti tubercular leads.

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