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ANTITUBERCULAR ACTIVITY OFTHIOSENIICARBAZONES AND SEMICARBAZONES

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Thiosemicarbazones and semicarbazones of (±) 3-menthone and aminoacetophenone have been prepared and screened for antitubercular activity. The MIC has been compared against rifampin and clarithromycin vs. M. tuberculosis. Compound 8 has shown more than 90% inhibition against M tuberculosis and M. avium (MIC more than 12.5μg/ml).

Keywords: *Antitubercular, thiosemicarbazone, semicarbazone, synthesis*

Introduction

A brief review by World Health Organization estimating the global scenario regarding tuberculosis and HIV gives explosive figures of the epidemic. Countries affected by this epidemic are Africa, Latin America, Asia including India(l). This is even more alarming by the threat of multidrug resistance (MDR.) which might result in an unbeatable epidemic that could spread globally. The introduction of drugs, the most prominent of which were isoniazid and rifampicin, the mainstay of short course chemotherapy today. Synthetic analogues of rifampicin such as rifabutin, rifapentine, and most recently, KRM-1648(2) have been prepared, but none has yet had an impact on therapy. 4-Alkylthiopyridine 2-carbothioamide(3), 2,6-bis (alkyl\thio) -4-pyridine carboxamides and carboxthioamides(4) have shown antitubercular activity. The attributes of the perfect TB therapy would include, activity against all bacteria present during an infection, activity against all MDR strains, an orally active formulation, no toxic side effects, long duration of action to make intermittent therapy possible and ability to cure an infection in a very short space of time. The current generation of drugs exert their effect by targeting essential processes in the mycobacterial cell, such as biosynthesis of the cell wall polymers. New agents with similar rapid mycobacterial properties but with new and different modes of action are urgently required to counter the threat posed by drug-resistant bacteria.

From the literature it is known that a thiosemicarbazide derivative (thiacetazonc, A)(5) and a thiourea derivative (thiocarlide, B)(6) are clinically used. They have been also helpful in drug resistant mycobacteria. Therefore, we have recently synthesized new compounds with thiosemicarbazone and thiourea derivatives. Both of these moieties have been incorporated in a single molecule to increase the lipophilicity of a molecule. These compounds have been investigated for antimycobacterial activity. The compounds have been synthesized according to schemes 1 and 2. Thiosemicarbazones and semicarbazones were synthesized by treating (±)3-menthone and aminoacetophenone with thiosemicarbazide and semicarbazide, respectively. (±)3-Menthone hydrazone and aminoacetophenone scmicarbazone and thiosemicarbazone were condensed with alkyi / aryl isothiocyanates.

Materials and Methods

Melting points are reported uncorrected, infrared spectra were obtained with Jasco FT1R 5300 using KBr disc. ^{1}H-NMR spectra were determined with Jcol FX 90Q multinuclear spectrometer and are reported as δppm values in $CDCI_3$ /DMSO-d_6, with TMS as the internal standard. Elemental analysis were performed by Perkin Elmer Model 240C analyser and were within ±0.4% of the theoretical values.

The reaction of thioscmicarbazide and scmicarbazidc with (±)3-menthone and aminoacetophenone led to the formation of 1, 2 and 3 respectively (scheme 1). Condensation of (±)3-incnthonc hydrazone and aminoacetophenone semicarbazone with aryl/alkyl isothiocyanates gave 4-14 (scheme 2).

± 3-Menthone thiosemicarbazone 1: A solution of thiosemicarbazide (1.82g, 0.02 mol) in ethanol (10 ml) was added slowly to a stirring solution of (±)3-menthone (0.02 mol) in ethanol (30 ml) and acetic acid (4 ml). The reaction was carried out for 1 h and the product collected. It was washed with ether and water. Compound was recrystallized from ethanol. 1R (KBr : cm^{-1}) 3250 (N-H), 2950 (C-H), 1595 (N-H), 1450 (C=N), 1245 (C=S); ^{1}H-NMR (90 MHz, $CDCI_3$: δppm) 1.08 (s,4H), 1.3 (s,lH), 1.78 (s,H), 3.2 (s,9H), 4.5 (s,2H), 5.2 (s,lH), 7.32 (s,2H, D_2O exchangeable), 11.2 (s.lH, D_2O exchangeable). Similarly 3-aminoacetophenone thiosemicarbazone and 4-aminoacetophenone thiosemicarbazone were prepared. Physical data are given in Table 1.

4-Aminoacetophenone semicarbazone: Semicarbazide hydrochloride (1.11g, 0.01 mol) and sodium acetate (0.82g, 0.01 mol) were dissolved in water (10 ml). Then the solution was added slowly to a stirred solution of 4-aminoacetophenone (1.35g, 0.01 mol) in ethanol (30 ml). The reaction mixture was stirred at room temperature for 1 h. The precipitate was collected, washed with ether and water and dried. Recrystallization from ethanol gave semicarbazone with melting point 248°C, yield 84%(8). IR (KBr: cm^{-1}) 3475 (N-H), 2895 (C-H), 1690 (C=0), 1570 (N-H), 1510 (Ar-11), 1430 (C=N), 830 (para disubstituted); ^{1}H-NMR (90 MHz, DMSO-d_6: δppm) 2.1 (s,3H), 3.5 (s,2H, D;0 exchangeable), 6.9 (d,2H), 7.7 (d,2H), 8.72 (s,lH, D_2O exchangeable), 9.2 (s,2H, D_2O exchangeable).

4-Methylphenyl thioureidoacetophenone semicarbazone 10: 4-Methylphcnylisothiocyanate (1.49 ml, 0.01 mol) was added to the solution of 4-amino acetophenone semicarbazone (1.92g, 0.01 mol) in ethanol (40 ml). The reaction mixture was refluxed for 4h. The solvent was evaporated and solid was washed with petroleum ether (40-60°C). Recrystallised from ethanol. IR (KBr: cm^{-1}) 3350 (N-H), 2824 (C-H), 1680 (CO), 1580 (N-H), 1525 (Ar-H), 1447 (C=N), 1111 (C=S), 810 (para disubstituted); ^{1}H-NMR (90 MHz, DMSO-d_6: δppm) 2.15 (s,3H), 2.6 (s,3H), 6.85 (bs, 2H, D_2O exchangeable), 7.2 (q,4H), 7.4 (d,2H), 7.6 (d,2H), 9.1 (s,2H, D_2O exchangeable), 11.2 (s,1H, D_2O exchangeable). Similarly other thioureido derivatives were prepared taking appropriate isothiocyanates. Physical data are given in Table 1.

Biological Evaluation

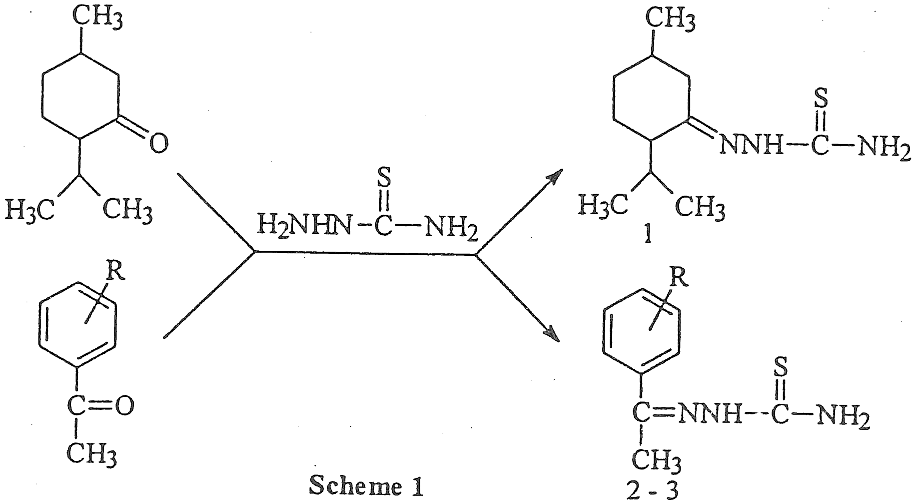
In vitro antimycotibacterium tuberculosis activity.

Antituberculosis activity was determined using the BACTEC 460 system (9, 10, ll) as modified below. Stock solution of test compounds were prepared in dimethylsulphoxide (DMSO) at ling/ml and sterilized by passage through 0.22 mm PFTE filters (Millex-FG, Millipore, Bedford, MA).

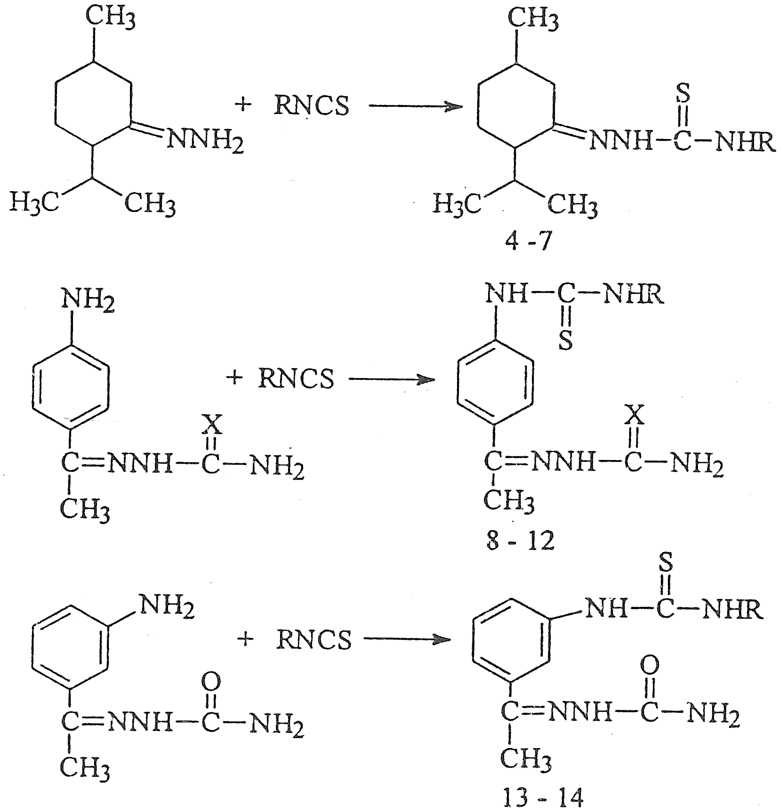
Table 1 : Some characteristics of the compounds

Comp.	R	X	m.p. °C	Mol.formula	IRcm^{-1}
1	-	S	155	$C_{11}H_{21}N_3S$	3250, 2950, 1595, 1450,1245
2	3-NH_2	-	105	$C_9H_{12}N_4S$	3225, 2950, 1500, 1490. 1460, 1240, 1080, 760
3	4-NH_2	-	180	$C_9H_{12}N_4S$	3300, 2825, 1500, 1480, 1415, 1280, 1180, 820
4	C_6H_5	-	90	$C_{17}H_{25}N_3S$	3182, 2949, 1593, 1587, 1446, 1138, 767
5	C_6H_4Cl(p)	-	163	$C_{17}H_{24}N_3SCl$	3466, 2924, 1529, 1500, 1439, 1120, 690
6	C_6H_4Cl(m)	-	72	$C_{17}H_{24}N_3SCl$	3280, 2884, 1550, 1510, 1430, 1050, 776
7	$C_6H_4OCH_3$(p)	-	104	$C_{18}H_{27}N_3SO$	3398, 2924, 1541, 1448, 1132, 1032, 758
8	C_6H_5	S	165	$C_{16}H_{17}N_5S_2$	3225, 2850, 1540, 1520, 1480, 1290,1090, 840,720
9	$C_6H_4CH_3$(p)	S	180	$C_{17}H_{19}N_5S_2$	3150, 2950, 1560, 1500, 1400, 1230, 1100, 820
10	$C_6H_4CH_3$(p)	O	140	$C_{17}H_{19}N_5SO$	3350, 2824, 1680, 1580, 1525, 1447, 1111, 810
11	$C_6H_4OCH_3$(p)	S	186	$C_{17}H_{19}N_5S_2O$	3150, 2950, 1560, 1500, 1400, 1240, 1160, 1100, 820
12	C_4H_9(n)	S	175	$C_{14}H_{21}N_5S_2$	3190, 2910, 1540, 1510, 1450, 1300, 1110, 840
13	$C_6H_4OCH_3$(p)	-	157	$C_{17}H_{19}N_5SO_2$	3200, 2950, 1680, 1605, 1500, 1460, 1180, 1100, 825
14	C_4H_9(n)	-	187	$C_{14}H_{21}N_5SO$	3500, 2885, 1680, 1659, 1500, 1480, 1120, 770

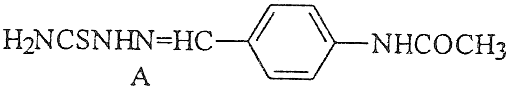
Percentage yeilds between 72-89%.



Scheme 1



Scheme 2



A

B

Fifty microliters were added to 4ml radiometric 7H12 broth (BACTEC 12BHATTACHARYA; Becton Dickinson Diagnostic Instrument Systems, Sparkes, MD) to achieve a final concentration of 12.5 Hg/ml. Controls received 50 ml DMSO. Rifampin (Sigma Chemical Co. St. Louis, MO) was included as a positive drug control. Rifampin was solubilized and diluted in DMSO and added to BACTEC 12BHATTACHARYA broth to achieve a range of concentrations for determination of minimum inhibitory concentration (MIC, lowest concentration inhibiting 99% of the inoculum). *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294; American Type Culture Collection, Rockville, MD) was cultured at 37°C on a rotary shaker in Middlebrook 7H9 broth (Difco Laboratories, Detroit MI) supplemented with 0.2% v/v glycerol and 0.05% v/v Tween 80 until the culture turbidity achieved an optical density 0.45-0.55 at 550 nm. Bacteria were then pelleted by centrifugation, washed twice and resuspended in one-fifth of the original volume in Dulbecco's phosphate-buffered saline (PBS, Irvine Scientific, Santa Ana, CA). Large bacterial clumps were removed by passage through an 8-mm filter (Nalgene, Rochester, NY) and samples were frozen at -80°C. Cultures were thawed and an appropriate dilution performed such that a BACTEC 12BHATTACHARYA vial inoculated with a 0.1 ml would reach a growth index (GI) of 999 in five days. One-tenth millilitre of the diluted inoculum was used in inoculate 4 ml fresh BACTEC 12BHATTACHARYA broth containing test compounds. An additional control vial was included which received a further 1:100-diluted inoculum (as well as 50 ml DMSO) for use in calculating the MIC ofrifampin by established procedures(11). Cultures were incubated at 37°C and the GI determined daily until control cultures achieved a GI of 999. Assays were usually completed in 5-8 days. Precent inhibition was defined as (1-GI of test sample/GI of control) x 100. Minimum inhibitory concentration of rifampin was defined as the lowest concentration of compound effecting a reduction in the daily change in GI which was less than that observed with a 1:100-diluted control culture on the day the latter reached a 01 of at least 30.

Results and Discussion

The compounds were synthesized using the methodologies outlined in schemes 1-2. All the compounds were examined for antimycobactcrial activity. The screening of antimycobacterial activity has been done by tuberculosis antimicrobial acquisition and coordinating facility. Southern Research Institute, Alabama, USA. The results are presented in Table 2.

All the derivatives resulting from these reactional sequences were evaluated in vitro against *Mycobaclerium tuberculosis.* Rifampin was used as the reference (MIC=0.25 µg/ml). Among the fourteen derivatives tested in a preliminary screening, seven exhibited percantage of inhibition ranging between 90-96% at a concentration lower than 12.5 µg/ml

Table 2 : The MIC values and % Inhibition of Compounds 1-14

Comp.	*M.tuberculosis* (μg/ml)	%Inhibition	*M.avium* (μg/ml)	%Inhibition	$H_{37}Rv$ (μg/ml)
1	>12.5	-28	-	-	-
2	>12.5	-87	-	-	-
3	>12.5	+92	<12.5	+99	>12.5
4	>12.5	- 4	-	-	-
5	<12.5	+96	-	-	6.25
6	>12.5	-62	-	-	-
7	>12.5	-24	-	-	-
8	>12.5	+90	<12.5	+99	>12.5
9	<12.5	+93	-	-	3.13
10	>12.5	-84	-	-	-
11	>12.5	+91	<12.5	+99	>12.5
12	<12.5	+96	<12.5	+99	>12.5
13	<12.5	+94	<12.5	+99	>12.5
14	>12.5	-60	-	-	-
Rifampin	0.25	-	-	-	0.06
Clarithromycin	-	-	2	-	-

- Dashes indicate commpounds not tested.

(Table 1). Those compounds demonstrating >90% inhibition (MIC >12.5 μg/ml) were further evaluated against *Mycobacterium avium.* Clarithromycin was used as the reference (MIC=2 μg/ml). All the five derivatives tested against *Mycobacterium avium* gave 99% inhibition at a concentration lower than 12.5 μg/ml. Compounds demonstrating at least 90% inhibition in the primary screen are re-tested (secondary level) at lower concentrations against *Mycobacterium tuberculosis* $H_{37}RV$ to determine the actual minimum inhibitory concentration (MIC) in the MABA (Microplate Alamar Blue Assay). The MIC is defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls. Rifampin was used as the reference (MIC=0.06 μg/ml). Compounds 5 and 9 exhibit MIC in BACTEC assay 6.25 AND 3.13 μg/ml respectively. All the compounds tested in secondary level showed MIC >12.5 μg/ml. The following structure activity relationship has been derived:

1. Thiosemicarbazones are more active than semicarbazones.
2. Compounds containing (±)3-menthone nucleus were generally less active than aminoacetophenone derivatives.
3. In the two isomers of 3 and 4-aminuacetophcnone thiosemicarbazones the 4-substituted derivatives were more active as compared to 3-substituted derivatives.
4. In the series of aminoacetophenone thiosemicarbazones and semicarbazones introduction of p-tolyl and p-anisyi thioureido groups enhances the activity.
5. In (±)3-menthone series p-chlorophenyl substituted derivative is more active than others.

Compound 8 showed MIC >12.5μg/ml and 99% inhibition. Thus this compound could be a lead compound for further molecular manipulation.

Conclusion

In conclusion the results of the present study indicate great potential for antitubercular activity of thiosemicarbazones and semicarbazones. An attempt will be made to incorporate further lipophilic moieties in the structure to increase the antitubercular activity.

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