

Synthesis, Molecular Structure and Antibacterial Activity of 1-(4-Methoxybenzaldehyde)-4-Methylthiosemicarbazone

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Abstract

A novel ligand, 1-(4-methoxybenzylidene)-4-methylthiosemicarbazone (MBT) derived from 4-methoxybenzaldehyde was synthesized and characterized by spectroscopic methods (IR, ¹H NMR, ¹³C NMR), single crystal X-ray diffraction technique and physical means. MBT showed antibacterial activity *in vitro* with MICs of 64 µg/mL (a strain of *Salmonella typhi* (ATCC 6539)), 128 µg/mL (*Salmonella typhi*), 64 µg/mL (*Salmonella paratyphi A*), 128 µg/mL (*Salmonella paratyphi B*) and 64 µg/mL (*Salmonella typhimurium*). The results showed that MBT demonstrated moderate *in vitro* antibacterial activity against a strain of *Salmonella typhi* (ATCC 6539), *Salmonella paratyphi A*, and *Salmonella typhimurium*, indicating its promise as a possible lead for the discovery of antibacterial drugs.

Keywords

Novel Schiff Base, Antibacterial Activity, Crystal Structure

1. Introduction

4-Methoxybenzaldehyde (also: *p*-anisaldehyde, anise aldehyde) is an organic compound that is commonly encountered in fragrances, both synthetic and natural [1]. It is a clear liquid with a strong aroma. Anisaldehyde is prepared commercially by oxidation of methoxytoluene (*p*-cresyl methyl ether) using manganese dioxide. It can also be produced by oxidation of anethole, a related fra-

grance that is found in some alcoholic beverages [1]. Thiosemicarbazones are useful in the medicinal and pharmaceutical fields because of their great significant pharmacological properties such as antibacterial [2] [3] [4], antifungal [2], anti-HIV [5] [6] [7] anticancer [8] [9], antineoplastic [10], anti-inflammatory [11], tuberculostatic [12], antimalarial [13] [14] and their variable bonding nature. In continuation with our earlier work on heterocyclic thiosemicarbazone derivatives [15], the present paper reports on the synthesis and antibacterial studies of 1-(4-methoxybenzylidene)-4-methylthiosemicarbazone (MBT).

Typhoid, paratyphoid A and B fevers are caused by *Salmonella typhi*, *Salmonella paratyphi* A and *Salmonella paratyphi* B respectively [16]. Typhoid fever continues to pose a marked public health problem in developing countries, where it is endemic [17]. In addition to these currently used expensive antibiotics, cases of resistance have been encountered with some strains of these bacteria [18]. We therefore tested our compound on these bacterial strains.

2. Experimental

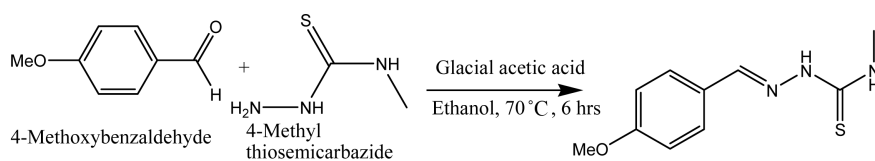
2.1. Materials

4-Methoxybenzaldehyde, 4-methylthiosemicarbazide, ethanol and acetic acid were used as purchased without further purification. IR spectrum was obtained from a Perkin-Elmer System 2000 FT-IR spectrophotometer using KBr pellets. NMR spectra were run in CDCl_3 on a 400 MHz spectrometer. Melting point was recorded on a Meltemp II Apparatus and is uncorrected. Elemental analysis was performed on a Thermo Flash EA-1112 Series CHNS-O Elemental Analyzer. Details of X-ray techniques and biological materials are described under their respective sections below.

2.2. Synthesis of 1-(4-Methoxybenzylidene)-4-Methylthiosemicarbazone (MBT)

A sample of 4-methylthiosemicarbazide (210 mg, 0.002 mol) dissolved in 15 ml ethanol was added to a solution of 4-methoxybenzaldehyde (0.28 mL, 0.002 mol). Five (05) drops of glacial acetic acid were added to the mixture (Scheme 1). The reaction mixture was allowed to reflux for 6 hours at a temperature of 70°C . The clear solution which was allowed to cool, formed suitable crystals which were filtered and washed several times with ethanol for X-ray diffraction studies.

Yield: 85%. *Mp.* $181 - 182^\circ\text{C}$. *Anal. Calcd* for $\text{C}_{10}\text{H}_{13}\text{N}_3\text{OS}$: C 53.78%; H 5.87%; N 18.82%; O 7.17% and S 14.36%. *Experimental:* C 54.14%; H 5.65%; N



Scheme 1. Synthesis of 1-(4-methoxybenzylidene)-4-methylthiosemicarbazone.

19.11%; O 7.18 and S 13.90%. IR (KBr, cm^{-1}): 3319 and 3157 (2N-H); 1600 and 1507 (C=C of the aromatic ring); 1543 (C=N); 1177 (C=S); 850 (N-N) and 828 (p-disubstituted benzene ring). ^1H NMR (δ ppm): 3.18 ($\text{CH}_3\text{-N}$); 3.77 (CH_3O); 6.85 (2H, $J = 11$ Hz, meta H); 7.45 (2H, $J = 11$ Hz, ortho H); 7.20 (N-H at position 4); 7.70 (CH=N) and 9.40 (N-H and position 2). ^{13}C NMR (δ ppm): 178.3 (C=S); 161.6 (C=N); 142.3 (para C); 128.7 (ortho C); 125.9 (C-1') 114.4 (meta C); 55.4 ($\text{CH}_3\text{-O}$) and 31.2 ($\text{CH}_3\text{-N}$).

2.3. Single Crystal X-Ray Diffraction Analysis and Structure Determination

This was performed using standard procedures as reported by Nfor *et al.* [19]. Briefly, suitable single crystal of **MBT** was mounted in air onto a loop. The data collection for **MBT** was carried out with a Bruker DUO APEX II CCD diffractometer at temperature controlled using an Oxford cryostream-700. Data reduction and cell refinement were performed using SAINT-Plus, and the space group was determined from systematic absences by XPREP and further justified by the refinement results. Graphite monochromated $\text{MoK}\alpha$ ($\lambda = 0.71073$ Å) radiation was used. The X-ray diffraction data have corrected for Lorentz-polarization factor and scaled for absorption effects by multi-scan using SADABS. The structure was solved by direct method, implemented in SHELXS-97. Refinement procedure by full-matrix least-square method based on F^2 values against reflections have been performed by SHELX-97, including anisotropic displacement parameters for all non-H atoms. The positions of hydrogen atoms belonging to the carbon atoms were geometrically optimized by applying a riding model. CCDC 1556045 contains the supplementary crystallographic data for this paper. Calculations concerning the molecular geometry, the affirmation of chosen space groups and the analysis of hydrogen bonds were performed with PLATON. The molecular graphic was done with ORTEP-3 and Mercury (version 3.0).

2.4. Biological Screening

2.4.1. Test Bacteria and Culture Media

The test microorganisms, including *Salmonella typhi* (ST), *Salmonella paratyphi* A (SPA), *Salmonella paratyphi* B (SPB), *Salmonella typhimurium* (STM) isolates were obtained from Pasteur Centre, Yaoundé, Cameroon. One strain of *Salmonella typhi* (ATCC 6539) obtained from the American Type Culture Collection (ATCC) was also used. The culture media used, namely Salmonella-Shigella Agar (SSA) and Mueller Hinton Broth (MHB) were manufactured by Accu-mixTM (Belgium). SSA was used for the activation of the bacteria species and for the screening of contaminants from the inoculum. Mueller Hinton Broth (MHB) was used for sensibility (antibacterial) test (Minimal Inhibitory Concentrations (MICs) and Minimal Bactericidal Concentrations (MBCs)).

2.4.2. Antimicrobial Assay

The minimum inhibitory concentrations (MICs) of **MBT** were determined using

serial microdilution in the rapid *p*-iodonitrotetrazolium chloride (INT) (Sigma-Aldrich, St Quentin Fallavier, France) colorimetric assay. The tests were carried out in 96-micro well sterile plates as previously described by Mativandlela *et al.* [20]. For this, **MBT** was dissolved in DMSO/MHB (v/v, 5%). This solution was then serially introduced in the different well of the microplate, which previously contains 100 μL /well of MHB. One hundred microliters of 10^6 CFU/ml bacterial suspensions prepared in MHB were added to the respective wells. The plates were covered with a sterile plate sealer, then agitated to mix the contents of the wells using a shaker, and incubated at 37°C for 18 h. Wells containing MHB, 100 μL of bacterial suspensions and DMSO at a final concentration of 2.5% served as a negative control. Ciprofloxacin was used as reference antibiotics. The MICs of sample were detected after 18 h of incubation at 37°C , following addition of 40 μL of 0.2 mg/mL INT and incubation at 37°C for 30 min [21]. Viable bacteria reduced the yellow dye of *p*-iodonitrotetrazolium to pink. MIC was defined as the lowest sample concentration that prevented this colour change and exhibited inhibition of microbial growth.

The Minimum Bactericidal Concentrations (MBCs) were determined by adding 50 μL aliquots of the preparations (without INT), which did not show any visible colour change after incubation during MIC assays, into 150 μL of fresh Mueller Hinton broth. These preparations were further incubated at 37°C for 48 h and bacterial growth was revealed by the addition of INT as above. The lowest concentration at which no visible colour change was observed was considered as the MBC. These tests were performed in triplicates at three different occasions.

3. Results and Discussion

The condensation reaction between 4-methoxybenzaldehyde and 4-methylthiosemicarbazide gave 1-(4-methoxybenzylidene)-4-methylthiosemicarbazone in good yield (**Scheme 1**).

3.1. Elemental Analyses

The elemental analysis for C, H, N and S revealed that the calculated and experimental data for the Schiff base are in good agreement suggesting the high percent purity of the compound.

3.2. Infrared Spectrum

The infrared spectrum (**Figure S1**) was taken in $4000 - 400 \text{ cm}^{-1}$ region. Two bands between 3319 and 3157 cm^{-1} represent stretching frequencies for the two N-H groups. A band at 828 cm^{-1} indicating the presence of a *p*-disubstituted benzene ring. Other important bands were observed at 1543 cm^{-1} (C=N) and 1177 cm^{-1} (C=S).

3.3. ^1H NMR Spectrum

The ^1H NMR spectrum (**Figure S2**) of 1-(4-methoxybenzylidene)-4-methylthio-

osemicarbazone was recorded in CDCl_3 . Prominent peaks were observed at 3.18 which was assigned to $\text{CH}_3\text{-N}$, 3.77 attributed to the methoxy group (CH_3O), 6.85 (2H, $J = 11$ Hz, meta H), 7.45 (2H, $J = 11$ Hz, ortho H) and 7.70 ($\text{CH}=\text{N}$).

3.4. ^{13}C NMR Spectrum

The ^{13}C NMR spectrum (**Figure S3**) of 1-(4-methoxybenzylidene)-4-methylthiosemicarbazone was recorded in CDCl_3 . The most deshielded peak appeared at 178.8 ppm and was attributed to $\text{C}=\text{S}$, followed by a peak at 161.6 ppm which was assigned to $\text{C}=\text{N}$. Signals for the aromatic carbon atoms were observed in the range, 142.3 - 125.9 ppm while the methoxy carbon atom resonated at 55.4 ppm and $\text{CH}_3\text{-N}$ appeared at 31.2 ppm.

3.5. Single Crystal X-Ray Crystallography

The molecular structure of the Schiff base is shown in **Figure 1** along with the atomic numbering scheme. The Schiff base crystallises in the orthorhombic system in space group $Pbca$. The unit cell dimensions are $a = 13.3144$ (13) Å, $b = 8.9064$ (9) Å and $c = 18.6349$ (18) Å with the cell angles being $\alpha = 90^\circ$, $\beta = 90^\circ$ (2) and $\gamma = 90^\circ$. The Z value is equal to 8.

The crystal structure of the molecule is in line with the IR, NMR and elemental analysis data of the molecule. The bond distances and angles are listed in **Table 1** and are comparable to those reported in the literature for similar compounds [22]. The crystal structure refinement data for MBT are listed on **Table 2**.

Table 1. Selected bond lengths (Å) and angles ($^\circ$) for MBT.

Bond lengths	MBT
C(1)-N(1)	1.455 (2)
C(2)-N(1)	1.327 (2)
C(2)-S(1)	1.691 (18)
C(3)-C(4)	1.460 (3)
C(4)-C(5)	1.378 (3)
C(7)-O(1)	1.359 (2)
C(8)-O(1)	1.426 (2)
N(2)-N(3)	1.383 (2)
C(2)-N(2)	1.349 (2)
C(3)-N(3)	1.282 (2)
Bond angles	
N(1)-C(2)-N(2)	117.58 (16)
N(2)-C(2)-S(1)	118.97 (13)
C(2)-N(1)-C(1)	123.35 (17)
C(3)-N(3)-N(2)	114.20 (15)
C(7)-O(1)-C(8)	117.94 (14)
N(3)-C(3)-C(4)	123.76 (17)

Table 2. Crystal structure refinement data for C₁₀H₁₃N₃OS (MBT).

Chemical formula	C ₁₀ H ₁₃ N ₃ OS	
Formula weight	223.29 g/mol	
Temperature	100 (2) K	
Wavelength	0.71073 Å	
Crystal size	0.068 × 0.180 × 0.708 mm	
Crystal habit	Clear light colourless plate	
Crystal system	Orthorhombic	
Space group	P b c a	
Unit cell dimensions	a = 13.3144 (13) Å	α = 90°
	b = 8.9064 (9) Å	β = 90°
	c = 18.6349 (18) Å	γ = 90°
Volume	2209.8 (4) Å ³	
Z	8	
Density (calculated)	1.342 g/cm ³	
Absorption coefficient	0.270 mm ⁻¹	
F (000)	944	
Theta range for data collection	2.19° to 26.38°	
Index ranges	-16 ≤ h ≤ 16, -11 ≤ k ≤ 11, -23 ≤ l ≤ 23	
Reflections collected	23,947	
Independent reflections	2263 [R(int) = 0.0653]	
Max. and min. transmission	1.0000 and 0.9270	
Refinement method	Full-matrix least-squares on F ²	
Refinement program	SHELXL-2014 (Sheldrick, 2014)	
Function minimized	Σw(F _o ² - F _c ²) ²	
Data/restraints/parameters	2263/0/188	
Goodness-of-fit on F²	1.139	
Δ/σ_{max}	0.001	
Final R indices	1843 data; I > 2σ (I)	R1 = 0.0412, wR2 = 0.1018
	All data	R1 = 0.0534, wR2 = 0.1085
Weighting scheme	w = 1/[σ ² (F _o ²) + (0.0620P) ²] where P = (F _o ² + 2F _c ²)/3	
Largest diff. peak and hole	0.592 and -0.228 eÅ ⁻³	
R.M.S. deviation from mean	0.064 eÅ ⁻³	

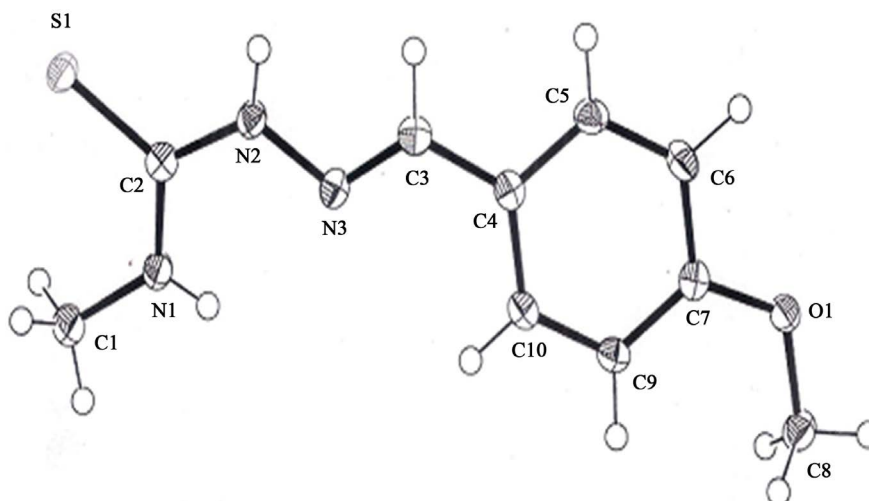


Figure 1. An ORTEP view of the molecule (MBT), with displacement ellipsoids drawn at the 50% probability level.

3.6. Antibacterial Activity of 1-(4-Methoxybenzylidene)-4-Methylthiosemicarbazone (MBT)

MBT was tested against four isolates of *Salmonella* species, namely *Salmonella typhi* (ST), *Salmonella paratyphi* A (SPA), *Salmonella paratyphi* B (SPB), *Salmonella typhimurium* (STM) and one strain of *Salmonella typhi* (ATCC 6539).

The data obtained from this test (**Table 3**) showed that **MBT** exhibited the various *Salmonella* species, with the MIC values ranging from 64 to 128 $\mu\text{g/mL}$. STs, SPA and STM presented MBCs equal to 128 $\mu\text{g/mL}$ and MBCs/MICs ratio equal to 2.

In fact, according to the criteria described by Kuete [23], activities of isolated product are significant when $\text{MIC} \leq 10 \mu\text{g/mL}$, moderate when $10 < \text{MIC} \leq 100 \mu\text{g/mL}$, weak when $\text{MIC} > 100 \mu\text{g/mL}$. Therefore, **MBT** is active and possesses moderate inhibitory potential vis-à-vis STs, SPA and STM. Antimicrobial substances are considered as bactericidal agents when the ratio $\text{MBC/MIC} \leq 4$, and bacteriostatic when the ratio $\text{MBC/MIC} > 4$ [16] [24]. For **MBT**, the ratio MBC/MIC was < 4 against most of the bacteria tested, suggesting that this product may be classified as bactericidal agent against these bacteria.

4. Conclusion

A novel ligand, 1-(4-methoxybenzylidene)-4-methylthiosemicarbazone (MBT) derived from 4-methoxybenzaldehyde and 4-methylthiosemicarbazide was synthesized and characterized by spectroscopic methods (IR, ^1H NMR, ^{13}C NMR). MBT showed moderate antibacterial *in vitro* activity with MICs of 64 $\mu\text{g/mL}$ against a strain of *Salmonella typhi* (ATCC 6539), *Salmonella paratyphi* A and *Salmonella typhimurium* and weak activity with MICs of 128 $\mu\text{g/mL}$ against *Salmonella typhi* and *Salmonella paratyphi* B, indicating its promise as a possible lead for the discovery of antibacterial drugs.

Table 3. *In vitro* antibacterial activity of 1-(4-methoxybenzylidene)-4-methylthiosemicarbazone (MBT).

Products	Study parameters	Strain and isolates				
		STs	ST	SPA	SPB	STM
MBT	MICs ($\mu\text{g/mL}$)	64	128	64	128	64
	MBCs ($\mu\text{g/mL}$)	128	/	128	/	128
	MBCs/MICs	2	/	2	/	2
Positive control (ciprofloxacin)	MICs ($\mu\text{g/mL}$)	0.5	0.5	0.25	0.5	0.5
	MBCs ($\mu\text{g/mL}$)	0.5	1	0.5	0.5	1
	MBCs/MICs	1	2	2	1	2

ST: *Salmonella typhi*, SPA: *Salmonella paratyphi* A, SPB: *Salmonella paratyphi* B, STM: *Salmonella typhi* murium, STs: strain of *Salmonella typhi* (ATCC 6539), MIC = Minimal Inhibitory Concentration, MBC = Minimal Bactericidal Concentration.

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Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

This work was carried out in collaboration between all the authors. J. A. M. and J. N. Y. designed the work, A. G. A. synthesized the compound, E. N. N. interpreted the X-ray crystallographic data, N. K. and D. G. performed biological screening of the compound. All authors drafted the manuscript.

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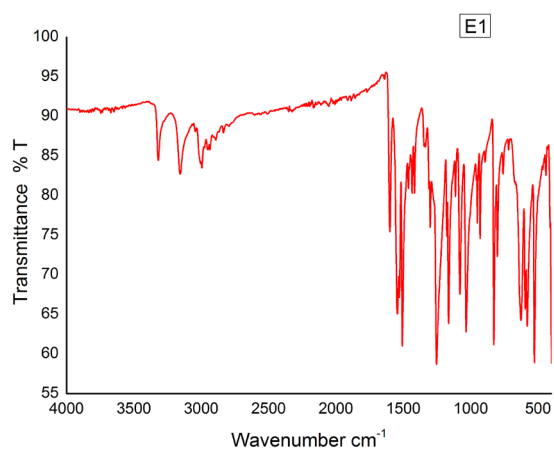
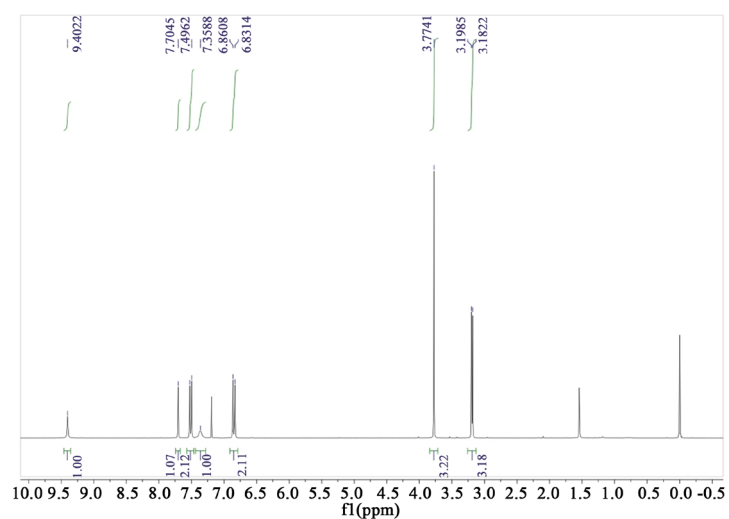
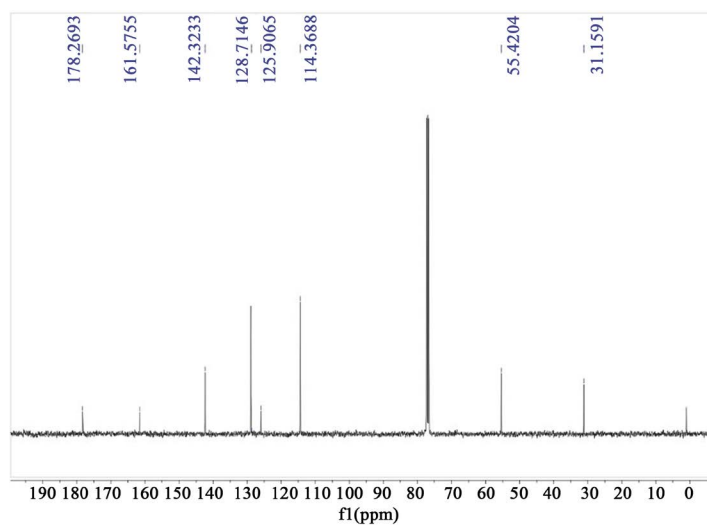


Figure S1. IR spectrum of MBT.

Figure S2. ^1H NMR of MBT.Figure S3. ^{13}C NMR of MBT.

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