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Research Article

SYNTHESIS, CHARACTERISATION AND ANTIMICROBIAL PROPERTIES OF SOME SEMICARBAZONES AND THIOSEMICARBAZONES

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ABSTRACT

Semicarbazones and thiosemicarbazones are inhibitors of DNA replication. This activity, which is explained by their chelating property of certain metal ions cofactors of enzymes involved in the replication of DNA, justifies their use as antimicrobials. Our work was aimed at synthesizing and studying the action of certain semicarbazones and thiosemicarbazones on strains of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*.

After their synthesis, the semicarbazones and thiosemicarbazones of six arylketones were identified by their IR, ^{1}H and ^{13}C NMR spectra and in mass spectrometry. The biological activity of these compounds was tested *in vitro* against the growth of strains of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*. Thiosemicarbazones **1b** and **5b**, the most lipophilic compounds, had reproducibly exerted an inhibitory effect on the growth of the studied bacteria.

Keywords: Semicarbazones ; Thiosemicarbazones and spectrometric identification.

1. INTRODUCTION

In organic chemistry, semicarbazones are derivatives of aldehydes or ketones formed by condensation reaction with a semicarbazide. Many semicarbazones are crystalline solids used for the identification of the corresponding aldehydes or ketones by melting point (**Williamson et al., 1999**). However, in pharmaceutical chemistry, these small molecules (compared to peptides) and their derivatives are of interest because they are inhibitors of DNA replication. This activity, which is explained by their chelating property vis-à-vis certain metal ions involved in this DNA replication (**French et al., 1966**), justifies the importance of the interest they are given in the fight against microbial diseases.

Indeed, antimicrobial resistance is considered a serious threat to health worldwide (**Laxminarayan et al., 2013; Antibiotic resistance coalition 2014**). It is estimated that it is already causing 700,000 deaths each year and, in the absence of effective action, it is

expected that it causes 10 million deaths a year by 2050. However, humanity has a limited number of effective antibiotics. It is then necessary to broaden the spectrum of antimicrobials (**Review on antimicrobial resistance 2014**).

Subsequent work had shown that semicarbazones and thiosemicarbazones have antiviral activities (**García et al., 2004 ; Rogolino et al., 2015 ; Soraires et al., 2017**), antifungal (**Kovač et al., de Araújo et al., 2017**), antibacterial (**San et al., Rebolledo et al., Kasuka et al., 2003; Rodriguez-Argüelles et al 2005 ; Rosu et al., 2010**), antimalarial (**Klayman et al., 1984**), antitumor (**Quiroga et al., 1998 ; Perez et al., 1999 ; Easmon et al., 2001 ; Hall et al., 2002 ; Kovala-Demertzi et al., 2002 ; Afrasiabi et al., 2003 ; Afrasiabi et al., 2004 ; Altıntop et al 2016**), anticonvulsant [**Pandeya et al., 1998 ; Pandeya et al., 1999**] etc ... Several methods of synthesis in the laboratory had allowed their obtaining.

The aim of this work is to synthesize, to confirm the structures by spectrometric methods and then to test the antimicrobial activity of certain semicarbazones and thiosemicarbazones of aromatic ketones on microbes. Its interest lies in the fact that it will make it possible to see if drugs based on semicarbazones, thiosemicarbazones and their derivatives could constitute an alternative to the antibiotics usually used in the antimicrobial fight.

2. MATERIAL AND METHODS

2.1. Chemistry

Melting points were determined using Köfler's bench and were not corrected. They are reported in Table 1 with the yields for each compound. Spectroscopic data were recorded with the following instruments: IR, Perkin Elmer FTIR 286; ^{1}H NMR and ^{13}C NMR, Bruker 300; SM, Xcalibur.

Semicarbazones and thiosemicarbazones are synthesized in one step (Scheme 1).

Semicarbazone

To a stirred mixture of 0.01 mole of ketone in 5 ml of 95 ° ethanol and 0.01 mole of semicarbazide hydrochloride dissolved in 2 ml of water, ten drops of triethylamine were added. Stirring was continued at room temperature until the appearance of precipitate and was maintained for another fifteen minutes.

The precipitate was then filtered and then recrystallized in ethanol at 95 °.

Thiosemicarbazone

A mixture of 0.01 mol of ketone in ethanol, 0.216 mol of thiosemicarbazide dissolved in 15 ml of ethanol and 5 ml of 1N hydrochloric acid was prepared. This mixture was stirred until the crystals of thiosemicarbazone were obtained. Stirring was maintained for another fifteen minutes, then, the precipitate was filtered and recrystallized in ethanol.

2.2. Antimicrobial test

The method used was that of dilution in a liquid medium. The solutions of semicarbazones and thiosemicarbazones were carried out at an initial concentration of 20 mg / ml in acetone. The bacterial suspensions were carried out at a colony for 5 ml respectively in LB medium (Luria Bertani) for *Escherichia coli* and *Staphylococcus aureus* and in TRIPTON-YEAST medium for *Pseudomonas aeruginosa* and *Enterococcus faecalis*.

Three series of eight wells initially containing 100 µl of distilled water were made. 100 µl of solution of semicarbazones or thiosemicarbazones were added to the first well; and a 2-to-2 dilution from one row to another until the eighth set of wells. The microbial suspensions were then added and the wells incubated in an oven at 37 ° C.

After 18 hours of incubation, 40 µl of a 0.2 mg / ml solution of p-iodonitrotetrazolium violet (p-INT) were added to each well and the whole was incubated for one hour.

Iodonitrotetrazolium is a reagent for the detection of enzymatic activity. In the medium, it was reduced by mitochondrial enzymes and stains red; thus marking the presence of life and enzymatic activity in the environment. Wells stained red were those in which the concentration of synthetic products was insufficient to inhibit bacterial growth. The MIC corresponds to the concentration of the undyed red well in which there was the lowest amount of semicarbazone or thiosemicarbazone. The reading was done in comparison with the control wells. It should also be noted that a series of positive controls has been performed with equivalent concentrations of gentamycin.

3. RESULTS AND DISCUSSIONS

Our synthesis method of semicarbazone used triethylamine instead of pyridine (**Fieser et al., 1967**). This method that we had developed, is very fast, leads to better yields and reduces the risks of toxicity related to pyridine (Scheme 1). Table 1 shows the physical and spectral data of the compounds.

The data in this table are consistent with the proposed structures for the different semicarbazones and their thiosemicarbazones. IR spectra show bands in the region of 3341.16-3495.80 cm-1 due to the N-H elongation

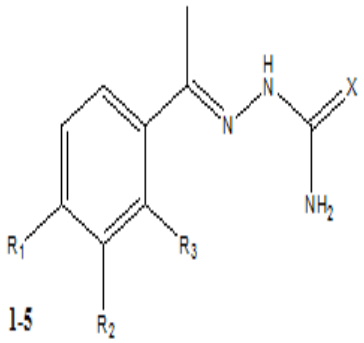
vibration. The C=O elongation vibration of semicarbazones occurs in the 1678.11 - 1750.23 cm^{-1} region. The elongation band of C=N appears in the region 1563.85-1608.78 cm^{-1}. In the spectrums ^1H NMR, the most unshielded proton that is bound to the nitrogen atom of the In light of these results, we see that only thiosemicarbazones **1b** and **5b** show interesting antimicrobial activity against all bacterial strains. All other compounds have moderate activity on gram-negative bacteria however they are inactive against Gram-positive bacteria. The activity of compounds **1b** and **5b** is due to their good lipophilicity.

4. CONCLUSION

In total, six semicarbazones were synthesized using triethylamine in the reaction medium. This method proved to be much more reactive than the sodium acetate method generally used and less risky than that using pyridine. The corresponding thiosemicarbazones were also synthesized. After their synthesis, the structures of these semicarbazones and thiosemicarbazones were clearly identified by infrared spectroscopy, proton and carbon 13 nuclear magnetic resonance and mass spectrometry. Only the most lipophilic thiosemicarbazones, that is, acetophenone and 2-chloroacetophenone, reproducibly exert an inhibitory effect.

medium appears as a widened singlet between 8.9 and 10.3 ppm. The synthesized compounds were tested for antimicrobial activity. The results of the test by the diffusion method in liquid medium are reported in the following Table 2.

Table 1: Physical, analytical and spectral data of synthesized semicarbazones and thiosemicarbazones



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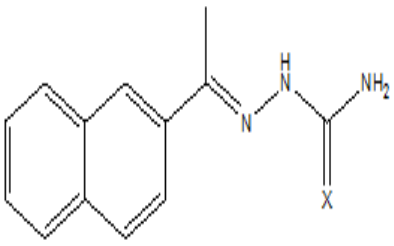


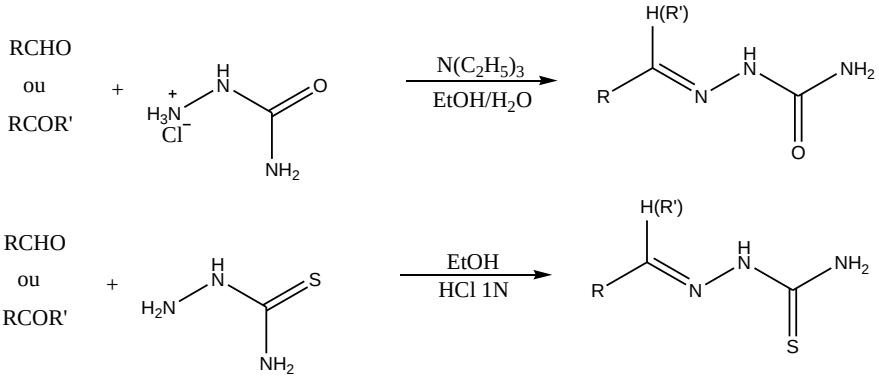
6a-b

SC et TSC[a]	R_1	R_2	R_3	X	M (g/mol)[b]	Pf[c]	Rend[d] %	^{1}H RMN (δ, ppm)	^{13}C RMN (δ, ppm)	IR (cm^{-1})	SM[e]
1a	H	H	H	O	177	198	83	2,2 (s ; 3H) ; 6,5 (s ; 2H) ; 7,4 (s ; 3H) ; 7,8 (s ; 2H) ; 9,3 (s ; 1H)	157,07 ; 143,37; 138,09 ; 127,90; 125,35 ; 128,22 ; 125,35 ; 127,90 ; 13,1	3480,14 ; 1740,70 ; 1583,84 ; 1118,70	178,06 ; 161,19 ; 135,21 ; 118,19 ; 120,26
1b	H	H	H	S	193	120	85	2,3 (s ; 3H) ; 6,5 (s ; 2H) ; 7,2 (s ; 1H) ; 7,4 (s ; 2H) ; 7,7 (s ; 2H); 8,9 (s ; 1H)	177 ; 146 ; 135 ; 127 ; 124 ; 128,55 ; 124 ; 127 ; 12,28	3408,23 ; 1587,19	194,04 ; 160,16 ; 177,08 ; 135,13 ; 118,15 ; 120,13
2a	OCH_3	H	H	O	207	201	86	2,1 (s ; 3H) ; 3,8 (s ; 3H) ; 6,5 (s ; 2H) ; 6,9 (s ; 2H) ; 7,8 (s ; 2H) ; 9,3 (s ; 1H)	158,58 ; 143,37 ; 129,32 ; 126,33 ;112,49 ;156, 37 ;112,49 ;126,33 ; 54,13	3481,66 ; 1698,81 ; 1581,30 ; 1119,56	208,08 ; 191,12 ; 165,13 ; 148,18 ; 149,11 ; 150,15
2b	OCH_3	H	H	S	223	178	89	2,3 (s ; 3H); 3,8 (s ; 3H) ; 6,9 (s ; 2H) ; 7,9 (s ; 2H) ; 8,2 (s ; 2H) ; 10,1 (s ; 1H)	178,02 ; 147,21 ; 129,45 ; 127,51 ; 112,91 ; 159,61 ; 112,91 ; 127,51 ; 54,50 ; 13,34	3400,66 ; 1608,78	224,03 ; 190,16 ; 207,07 ; 165,00 ; 148,20 ; 150,18
3a	OCH_3	OCH_3	H	O	237	218	80	2,3 (s ; 3H); 3,7 (s ; 3H) 3,8 (s ; 3H) ; 6,5 (s ; 1H) ; 6,9(d ; 1H) ; 7,3 (d ; 1H) ; 7,5 (s ; 2H) ; 9,2 (s ; 1H)	155,27 ; 147,43 ; 129,39 ; 108,32-107,32 ; 146,43 ; 142,13 ; 116,36 ; 53,40/53,53 ; 11,26	3495,80 ; 1678,11 ; 1563,85 ; 1134,60	238,09 ; 221,18 ; 195,23 ; 178,25 ; 180,28
3b	OCH_3	OCH_3	H	S	253	225	82	2,3 (s ; 3H) ; 3,8 (s ; 3H) ; 3,9 (s ; 3H) ; 6,9 (s ; 1H) ; 7,3 (s ; 1H) ; 7,5 (s ; 1H); 7,9 (s ; 1H) 8,2 (s ; 1H) ; 10,1 (s ; 1H)	177,52 ;147,18-143,19 ;129,19 ; 108,6 ;109,9 ; 149,12 ; 147,18-143,19 ; 118,91 ; 54,65-54,66; 54,12-35,65-54,66	3376,55 ; 1600,74	254,04 ; 220,15 ; 237,10 ; 195,23 ; 178,22 ; 180,22
4a	OCH_3	OCH_3	OCH_3	O	267	179	71			3417,12 ; 1681,40 ; 1588,14 ; 1129,08	268,13 ; 251,00 ; 225,00 ; 210,36
4b	OCH_3	OCH_3	OCH_3	S	283	215	74	2,3 (s ; 3H) ; 3,7 (s ; 3H) ; 3,8 (s ; 6H) ; 7,2 (s ; 2H) ; 7,9-8,3 (d ; 2H) ; 10,1 (s ; 1H)	176,60 ; 146,67 ; 134,11 ; 102,20 ; 150,31 ; 136,70 ; 150,31 ; 102,20 ; 37,91 ; 53,83 ; 37,91 ; 12,25	3341,16 ; 1585,79	284,97 ; 250,10 ; 267,00 ; 209,30 ; 210,29
5a	H	H	Cl	O	211,5	180	40	2,2 (s ; 1H) ; 6,3 (s ; 2H) ; 7,4 (m ; 4H) ; 9,4 (s ; 1H)		3488,04 ; 1729,57 ; 1581,31 ; 1105,58	212,04 ; 195,27 ; 169,27 ; 152,00 ; 154,25
5b	H	H	Cl	S	227,5	157	45			3400,13 ; 1587,15	228,14 ; 194,29 ; 211,23 ; 169,00 ; 152,06 ; 154,21
6a	- -	- -	- -	O	227	225	86	2,3 (s ; 3H) ; 6,8 (s ; 2H) ; 7,6-8,3 (m ; 7H) ; 9,4 (s ; 1H)	161,98 ; 148,48	3483,24 ; 1750,23 ; 1584,65 ; 1130,65	228,10 ; 211,18, 185,21 ; 168,21

6b	- -	- -	- -	S	243	180	89	2,4 (s ; 3H) ; 6,5 (s ; 2H) ; 7,5-8,3 (m ; 7H); 10,3 (s ; 1H)	177,05 ; 145,71	3435,11 ; 1606,63	244,04 ; 210,20 ; 227,10 ; 185,25 ; 168,24 ; 170,23

Table 2: Antimicrobial activity of semicarbazones and thiosemicarbazones

Composés	CMI (mg /ml)			
	E. coli (gram -)	*S. aureus (gram +)*	*P. aeruginosa (gram -)*	*E. faecalis (gram +)*
1a	1.25	>5	>5	>5
1b	0.3125	0.3125	0.3125	1.25
2a	0.625	>5	1.25	>5
2b	2.5	>5	0.625	>5
3a	0.625	>5	>5	>5
3b	5	>5	>5	>5
4a	2.5	5	0.625	>5
4b	2.5	>5	5	>5
5a	1.25		0.625	>5
5b	0.625			
6a	2.5	>5	1.25	>5
6b	1.25	>5	>5	>5



Scheme. 1: Synthetic route of semicarbazones and thiosemicarbazones

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