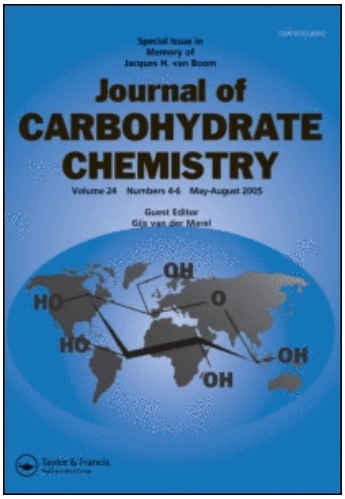

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SYNTHESIS AND SOD-LIKE ACTIVITY OF MONOSACCHARIDE DERIVED THIOSEMICARBAZONES

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ABSTRACT

The synthesis of new *O*-β-D-glucopyranosyl- and *O*-β-D-galactopyranosyl-2-hydroxyacetaldehyde thiosemicarbazones (**5a,b**) is reported. Oxidation of allyl glycoside **1** with $KMnO_4$ followed by $NaIO_4$ cleavage of the resulting diol **2** afforded aldehyde **3**, which was then condensed with thiosemicarbazide and deprotected to give the target compounds (**5a,b**). Compounds **5a,b** showed hydrolytic stability in neutral solution with half-life periods greater than 70 h. The SOD-mimetic activity was determined for the Cu(II) and Mn(II) complexes of **5a,b** (IC_{50} = 0.2-0.8 μM). This activity is higher by two orders of magnitude than copper (II) bisthiosemicarbazones. The ESR and UV-Vis data of Cu(II)-**5a** suggest a tetrahedral distortion of the coordination sphere around the central copper atom which could be the reason for their high SOD-mimetic activity.

INTRODUCTION

Thiosemicarbazones (R_1R_2C=N-NH-C(S)-NH_2) are important due to their wide applications in industry, medicine and analytical determination of various metal ions. These compounds exhibit antitumor,[1] bactericide[2] and fungicide[3] properties. In general,

thiosemicarbazones can react as chelating ligands with transition metal ions by bonding through the thioketo sulfur and hydrazine nitrogen atoms. Therefore, this type of compound can coordinate *in vivo* to metal ions. As a consequence of such coordination, the thiosemicarbazone moiety undergoes a steric reorientation that can favor its biological activity.[4] The biological activity of thiosemicarbazones is also considered to involve the inhibition of ribonucleotide reductase, an obligatory enzyme in DNA synthesis.

The extreme water insolubility of most thiosemicarbazones makes them difficult to their oral administer orally. Substitution of a thiosemicarbazone, with an unprotected carbohydrate moiety, should increase its water solubility and, at the same time, its cell membrane permeability. In this paper, the synthesis, characterization and superoxide dismutase (SOD) activity of D-glucose and D-galactose derived thiosemicarbazones are reported.

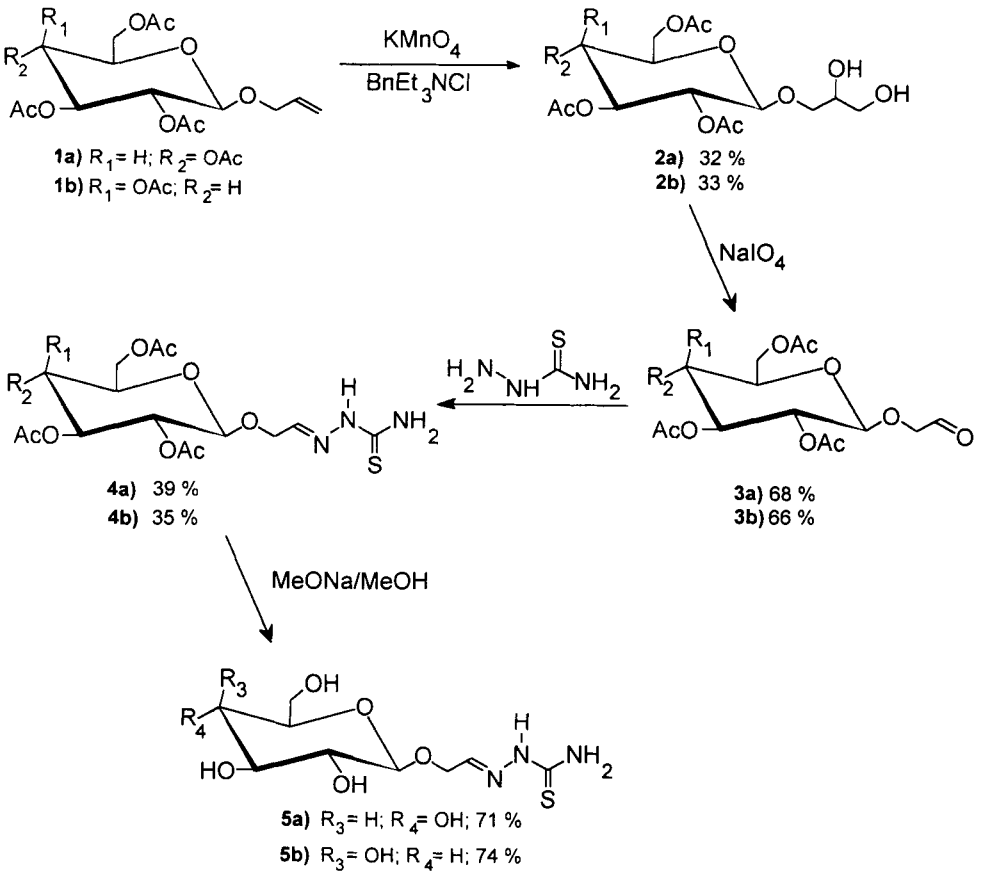
RESULTS AND DISCUSSION

Synthesis of the target compounds

The synthesis of **5a,b** was performed in five steps according to Scheme 1. The required aldehyde function for the synthesis of the target thiosemicarbazones was introduced through the oxidation of allyl glycosides (**1a,b**).

The reaction of acetobromo-glucose and galactose with allyl alcohol in the presence of mercuric oxide gave the corresponding allyl glycosides in good yields after recrystallization from ethanol. The second step of the synthesis involved the well-known permanganate oxidation of olefins to 1,2-diols[5] under phase transfer conditions. These products (**2a,b**) were purified by column chromatography and characterized by ^{1}H and ^{13}C NMR spectroscopy. Oxidation of the resulting 1,2-diols with $NaIO_4$ afforded the corresponding aldehydes **3a,b**. Their ^{1}H and ^{13}C spectra showed the characteristic aldehyde signals around 9.7 ppm and 200 ppm respectively.

Acetylated thiosemicarbazones (**4a,b**) were prepared by condensation of thiosemicarbazide with the corresponding aldehyde **3a,b** and were fully characterized by NMR spectroscopy and elemental analysis. The resulting products were deacetylated with sodium methoxide in methanol without decomposition of the thiosemicarbazone moiety to give the target compounds **5a,b**.



Scheme 1

The free monosaccharide moiety increases the water solubilty of the thiosemicarbazones, as was expected. This synthetic route should also be useful in preparation of water soluble thiosemicarbazones derived from other monosaccharides.

There are only a few reports on thiosemicarbazones and their analogs bearing carbohydrate moieties. El Khadem[6] reported the synthesis of D-*arabino*-hexos-2-ulose disemicarbazone by a different route. More recently, Horton[7] reported the synthesis of 3-deoxyaldos-2-ulose bisthiosemicarbazones, by the reaction of the corresponding monosaccharide with thiosemicarbazide in the presence of *p*-toluidine. A rearrangement of the Amadori type takes place in this reaction yielding 3-deoxyaldos-2-uloses which react

with two molecules of thiosemicarbazide to give stable derivatives having a short polyol chain. In our work, the synthetic pathway allowed us to conserve the cyclic structure of the carbohydrate, which is important for its cell membrane permeability.

Hydrolysis Studies

The hydrolysis constants of the two synthesized thiosemicarbazones are given in Table 1. The half-life periods for the two compounds were 88 and 70 hours respectively. However, the half-life periods decrease exponentially in acid medium and at pH 5 both thiosemicarbazones were hydrolyzed within a few minutes. The hydrolytic stability of these compounds above pH 7 and their water solubility may permit preparation as their aqueous solutions for parenteral use in clinical practice.

SOD-like Activity

In the interpretation of the biological properties of copper(II) thiosemicarbazones, it has been established that they can catalyze the dismutation of superoxide radical[8] following a cyclic two-step mechanism. In this process, the central copper atom undergoes a geometric rearrangment from square planar to tetrahedral as depicted in Figure 1. The easier this transformation takes place, the higher the catalytic activity to be expected. Therefore, complexes with tetrahedral distortion should be more active than square planar complexes.

In this work, we determined the SOD-like activity of the copper(II) complexes of the studied thiosemicarbazones and correlated their activity with ESR data. The SOD-like activity of the copper(II) and manganese(II) complexes with **4a**, **4b**, **5a**, **5b** and 3-deoxy-D-*erythro*-hexo-2-ulose bisthiosemicarbazone (GluTSC) is reported in Table 2. No significant difference is observed between the IC_{50} values of the acetylated (**4a**,**b**) and deacetylated (**5a**,**b**) monothiosemicarbazone complexes. These results indicate that the hydroxyl groups do not interact with the superoxide radical during its approach to the divalent metal. The obtained IC_{50} values are two orders of magnitude higher than those obtained for the corresponding complexes of GluTSC. This difference may be attributed to geometric factors.

Electron spin resonance (ESR) spectroscopy provides a very useful tool to study the geometry of the coordination sphere of copper(II) complexes. Characteristics of the ESR

Table 1. UV Spectral Data and Hydrolysis Constants at pH 7 for **5a,b**.

COMPOUND	λ (nm)	ε ($cm^{-1}\cdot mol^{-1}\cdot L$)	k (h^{-1})
5a	264	13000	7.9×10^{-3}
5b	265	12000	9.9×10^{-3}

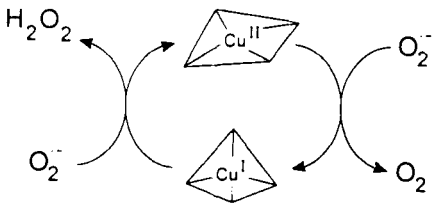


Figure 1. Coordination geometry changes undergone by copper(II) thiosemicarbazones during superoxide radical dismutation.

spectra of the synthesized thiosemicarbazones indicate bidentate coordination through ^{1}N and sulfur atoms. The ESR parameters of the copper complex of **5a** (Cu-**5a**), obtained in 50% aq DMSO are reported in Table 3. A similar set of parameters was observed for the copper complex of **5b**. For comparison, the ESR parameters of 3-deoxy-D-*erythro*-hexo-2-ulose bisthiosemicarbazonate copper(II) (Cu-GluTSC),[9] which coordinates in tetradentate fashion through ^{1}N and sulfur atoms of both thiosemicarbazone moieties, are also reported in Table 3. The $g_{\|}/A_{\|}$ ratio measured from the ESR spectra of frozen solutions may be considered as an empirical index of tetrahedral distortion. Values lower than 135 cm have been observed for square-planar structures and those higher than 150 cm for tetrahedrally distorted complexes.[10] The $g_{\|}/A_{\|}$ value for bovine Cu,ZnSOD is 160 cm, revealing a distortion from the ideal square planar geometry, as evidenced by the X-ray structural determination.[11] The differences between the ESR data of Cu-**5a** and Cu-GluTSC indicate that their coordinating spheres do not have the same distortion patterns. The $g_{\|}/A_{\|}$ value of 141 cm for Cu-**5a** is close to those observed for tetrahedrally distorted complexes, while

Table 2. IC_{50} Values[a] for the Studied Metal(II) Complexes.

	LIGAND				
METAL	**4a**	**4b**	**5a**	**5b**	GluTSC
Cu(II)	0.21	0.31	0.20	0.22	>10
Mn(II)	0.44	0.85	0.21	0.31	7.1

a. Values in μmol/L.

Table 3. ESR Data for the Copper(II) Complexes of **5a** and GluTSC[a].

	298 K		77 K				
COMPLEX	g_{iso}	A_{iso} (cm^{-1})	$g_{\|}$	$g_{\perp}$	$A_{\|}$ (cm^{-1})	$A_{\perp}$ (cm-1)	$g_{\|}/A_{\|}$ (cm)
Cu-**5a**	2.11	0.0079	2.24	2.02	0.0159	0.0039	141
Cu-GluTSC	2.06	0.0083	2.13	2.03	0.0164	0.0033	130

a. In 50 % aqueous DMSO.

CuGluTSC shows a value of 130 cm, suggesting a square planar geometry. The same conclusion arises from the analysis of the ESR parameters of Cu-**5a** and Cu-GluTSC measured in solution at room temperature. Higher g_{iso} and lower A_{iso} values are found in tetrahedrally distorted copper(II) complexes than in square-planar complexes. There are also differences in the electronic spectra of Cu-**5a** and Cu-GluTSC (Table 4). Cu-GluTSC shows the characteristic band of square-planar Cu(II) complexes around 460 nm. However, the d-d band of Cu-**5a** is red-shifted. This low energy band is characteristic for tetrahedrally distorted Cu(II) complexes.[12] Therefore, the differences between the ESR and the UV-Vis data of Cu-**5a** and Cu-GluTSC indicate that their coordinating spheres do not have the same distortion patterns. This may be the reason why the copper complexes of **5a** and **5b** exhibit a higher SOD-like activity than bisthiosemicarbazone complexes. In the latter compounds the geometric rearrangement is more constrained than in the former complexes. We have

Table 4. UV-Vis Data for the Copper(II) Complexes of **5a** and GluTSC in Solution.

COMPLEX	λ_{max} (nm)	log ε	λ_{max} (nm)	log ε
Cu-**5a**	263 319	4.29 3.99	720	2.98
Cu-GluTSC	252 304	4.26 4.38	467	3.77

recently observed[13] the same influence of the distortion on the SOD-like activity for the copper(II) complex of glutamic acid dithiocarbamate.

These results allow us to consider the monosaccharide derived thiosemicarbazone complexes as good SOD models. Electrochemical studies of the copper complexes of these thiosemicarbazones are in progress in our laboratory in order to complement the present results.

EXPERIMENTAL

General Methods. NMR spectra were recorded on a Bruker AC 250 spectrometer (250.13 MHz for ^1H and 62.89 MHz for ^{13}C) in $CDCl_3$, DMSO-d_6 or D_2O and were referenced to internal tetramethylsilane (TMS) for $CDCl_3$ and DMSO-d_6 or sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) for D_2O. Assignments were verified by 2D HH-COSY and CH-COSY experiments. Electronic spectra were recorded on an Ultrospec III (Pharmacia-LKB) spectrophotometer in 1 cm quartz cells. IR spectra (KBr tablets) were recorded on a PU9600 FT-IR spectrometer (Philips). ESR spectra were recorded on a Bruker ER 200D-SRC spectrometer operating in the X band (ν = 9.78 GHz) in 50% aq DMSO at rt and 77 K. TLC was run on glass sheets precoated with silica gel $60F_{254}$. Detection of spots was carried out by charring the plates after spraying with 1% $FeCl_3$ in *n*-BuOH (for thiosemicarbazones) or 20% aq H_2SO_4. Column chromatography was performed using silica gel 60G (Merck, 230-400 mesh). Elemental analyses were performed using an EAGER 200 instrument. Melting points were determined on an Electrothermal 9100 apparatus and are uncorrected.

Allyl 2,3,4,6-tetra-*O*-acetyl-ß-D-glucopyranoside (**1a**) and allyl 2,3,4,6-tetra-*O*-acetyl-ß-D-galactopyranoside (**1b**) were synthesized following standard literature procedures.[14]

General procedure for oxidation of the allyl group.[5] $KMnO_4$ (1 g), 1 % aq $KMnO_4$ (5 mL) and benzyltriethylammonium chloride (1 g) were added to a stirred solution of **1a** or **1b** (1 g, 2.6 mmol) in EtOAc (15 mL) at room temperature. Stirring was continued for 1 h. The reaction mixture was washed with EtOAc (10 x 10 mL) and filtered. The organic phase was dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo*. The residues **2a** or **2b** were obtained as colorless syrups after drying *in vacuo* and used without further purification in the next step.

1-*O*-(2,3,4,6-Tetra-*O*-acetyl-ß-D-glucopyranosyl)glycerol (2a). (340 mg, 32 %). ^1H NMR ($CDCl_3$): δ 2.06 (4s, 12H, CH_3), 2.8-3.8 (m, 7H, CH_2, OH), 3.9 (m, 1H, H-5), 4.23 (m, 1H, H-6), 4.4 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 4.5-5.0 (m, 3H, H-2,3,4). ^{13}C NMR ($CDCl_3$): δ 20.5 (CH_3), 61.3, 63.2, 67.0, 68.7, 70.4, 70.5, 70.6, 70.7, 71.7, 72.3 (CH_2, CH, C-2,3,4,5,6), 101.2 (C-1), 169.9 (C=O).

1-*O*-(2,3,4,6-Tetra-*O*-acetyl-ß-D-galactopyranosyl)glycerol (2b). (350 mg, 33%). ^1H NMR ($CDCl_3$): δ 2.05 (4s, 12H, CH_3), 3.1-3.8 (m, 7H, CH_2, OH), 3.94 (m, 1H, H-5), 4.13 (m, 1H, H-6), 4.5 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1), 5.02 (dd, 1H, $J_{3,4}$ = 4.3, H-3), 5.16 (dd, 1H, $J_{2,3}$ = 10.6, H-2), 5.38 (dd, 1H, H-4). ^{13}C NMR ($CDCl_3$): δ 20.5 (CH_3), 61.3, 63.3, 66.9, 68.8, 70.4, 70.5, 70.6, 70.8, 71.7, 72.3 (CH_2, CH, C-2,3,4,5,6), 101.7 (C-1), 170.1 (C=O).

General procedure for oxidation of 1,2-diols. Sodium periodate (350 mg, 1.54 mmol) was added to a solution of **2a** or **2b** (400 mg, 0.82 mmol) in 1,4-dioxane-water (3:1 v/v, 12 mL) and the resulting suspension was stirred for 24 h at room temperature. The mixture was filtered, concentrated to dryness and a chloroform-water (2:1 v/v) mixture was added to the residue. The organic phase was washed with water, dried over anhydrous sodium sulfate, filtered and concentrated to dryness. **3a** or **3b** were each obtained as a colorless syrup. The aldehyde function was detected by NMR and TLC ($CHCl_3/Me_2CO$, 2:1) using the aniline phthalate reagent. No further purification was attempted and the product was immediately used in the next step.

***O*-(2,3,4,6-Tetra-*O*-acetyl-ß-D-glucopyranosyl)-2-hydroxyacetaldehyde (3a).** (250 mg, 68%). ^1H NMR ($CDCl_3$) δ 9.6 (br, 1H, HC=O); ^{13}C NMR ($CDCl_3$) δ 199.9 (HC=O).

***O*-(2,3,4,6-Tetra-*O*-acetyl-ß-D-galactopyranosyl)-2-hydroxyacetaldehyde (3b).** (240 mg, 66%). ^{1}H NMR ($CDCl_3$) δ 9.7 (br, 1H, HC=O); ^{13}C NMR ($CDCl_3$) δ 199.8 (HC=O).

***O*-(2,3,4,6-Tetra-*O*-acetyl-ß-D-glucopyranosyl)-2-hydroxyacetaldehyde Thiosemicarbazone (4a).** A solution of freshly prepared **3a** (110 mg, 0.30 mmol) and thiosemicarbazide (32 mg, 0.35 mmol) in 10% AcOH in methanol (10 mL) was refluxed for 3 h. The mixture was cooled to room temperature and the resulting white solid was filtered and recrystallized from methanol (51 mg, 39%): mp 165 °C; IR (KBr) 1752 ν(C=O), 1594 ν(C=N) + δ(NH_2), 825 ν(C=S) cm^{-1}; UV (H_2O) λ_{max} 264 (log ε = 4.11); ^{1}H NMR (DMSO-d_6) δ 2.00, 2.04, 2.06, 2.08 (4s, 12H, CH_3), 3.98 (m, 1H, H-5), 4.23 (m, 2H, H-6, H-6'), 4.30 (2q, 2H, $J_{7,7'}$ = 12.3 Hz, $J_{7,8}$ = 4.9 Hz, CH_2), 4.82 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.89 (t, 1H, $J_{2,3}$ = 8.2 Hz, H-2), 5.01 (dd, 1H, $J_{4,5}$ = 10 Hz, H-4), 5.29 (t, 1H, $J_{3,4}$ = 9.3 Hz, H-3), 7.35 (bs, 2H, NH_2), 7.43 (t, 1H, H-8), 11.32 (bs, 1H, NH); ^{13}C (DMSO-d_6) δ 20.12, 20.18, 20.26, 20.32 (CH_3), 61.49 (C-6), 67.91 (C-4), 68.25 (C-7), 70.74 (C-2), 70.82 (C-5), 72.05 (C-3), 99.37 (C-1), 141.58 (HC=N), 168.72, 168.86, 169.23, 169.81 (C=O), 178.43 (C=S).

Anal. Calcd for $C_{17}H_{25}O_{10}N_3S$ (463.3): C, 44.1; H, 5.4; N, 9.1; S, 6.9. Found: C, 43.7; H, 5.4; N, 7.3; S, 6.3.

***O*-(2,3,4,6-Tetra-*O*-acetyl-ß-D-galactopyranosyl)-2-hydroxyacetaldehyde Thiosemicarbazone (4b).** Compound **4b** was prepared following the same procedure described for **4a**. The reaction mixture was concentrated *in vacuo* and the crude product was washed several times with ethanol. A colorless syrup was obtained after drying *in vacuo* (46 mg, 35%): UV (H_2O) λ_{max} 265 (log ε = 4.08); ^{1}H NMR (DMSO-d_6) δ 1.94, 2.04, 2.06, 2.14 (4s, 12H, CH_3), 4.11 (m, 2H, H-6, H-6'), 4.15 (m, 1H, H-5), 4.29 (2q, 2H, $J_{7,7'}$ = 13.3 Hz, $J_{7,8}$ = 4.8 Hz, CH_2), 4.72 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 5.06 (t, 1H, $J_{2,3}$ =10.4 Hz, H-2), 5.17 (dd, 1H, $J_{3,4}$ = 3.4 Hz, H-3), 5.35 (dd, 1H, $J_{4,5}$ = 3.4 Hz, H-4), 7.31 (bs, 2H, NH_2), 7.44 (t, 1H, $J_{7,8}$ = 4.8 Hz, H-8), 11.33 (bs, 1H, NH); ^{13}C (DMSO-d_6) δ 20.53, 20.59, 20.68, 20.77 (CH_3), 61.27 (C-6), 67.26 (C-4), 68.59 (C-7), 68.70 (C-2), 70.31 (C-5), 70.53 (C-3), 100.26 (C-1), 142.10 (HC=N), 169.21, 169.55, 169.92, 170.00 (C=O), 178.75 (C=S).

Anal. Calcd. for $C_{17}H_{25}O_{10}N_3S \cdot 0.5\ CH_3CH_2OH$ (508.2): C, 40.1; H, 4.9; N, 8.3; S, 6.3. Found: C, 39.7; H, 5.0; N, 7.8; S, 5.9.

***O*-(ß-D-glucopyranosyl)-2-hydroxyacetaldehyde Thiosemicarbazone (5a).** Sodium methoxide solution (1 mL) was added dropwise to a cold solution of **4a** (200 mg)

in dry methanol (4 mL) and the solution was stirred for 3 h at rt. The mixture was then neutralized with Dowex 50W-X8 resin and filtered. The solvent was removed *in vacuo* and the product was recrystallized from hot methanol to give a colorless solid (90 mg, 71%). ^{13}C NMR (D_2O) δ 61.88 (C-6), 69.57 (CH_2), 70.77 (C-4), 74.25 (C-2), 76.93, 77.21 (C-3,5), 103.26 (C-1), 146.74 (HC=N), 178.12 (C=S).

Anal. Calcd for $C_9H_{17}O_6N_3S$ (295.1): C, 36.6; H, 5.8; N, 14.2; S, 10.8. Found: C, 36.3; H, 5.9; N, 13.8; S, 9.6.

***O*-(ß-D-galactopyranosyl)-2-hydroxyacetaldehyde Thiosemicarbazone (5b).** This compound was prepared as described above for **4a** and was obtained as a hygroscopic colorless syrup after drying *in vacuo* (95 mg, 74 %). ^{13}C NMR (D_2O) δ 62.22 (C-6), 69.50 (CH_2), 69.87, 71.96, 73.98, 76.49 (C-2,3,4,5), 103.85 (C-1), 146.92 (HC=N), 178.12 (C=S).

Anal. Calcd for $C_9H_{17}O_6N_3S \cdot H_2O$ (313.1): C, 34.5; H, 5.4; N, 13.4; S, 10.2. Found: C, 34.3; H, 5.6; N, 12.8; S, 8.9.

Stability Study in Solution. The hydrolysis constants were determined in a 10 mM pH 7 phosphate buffer solution. The studies were performed on an Ultrospec III (Pharmacia-LKB) spectrophotometer using *Wavescan* software. Changes in absorbance at λ_{max} of the thiosemicarbazones were measured hourly.

SOD-like Activity. The method used to study the SOD-like activity is similar to that described by Fridovich,[15] with the same specifications as reported elsewhere,[16] using nitroblue tetrazolium chloride (NBT) as indicator, and the xanthine-xanthine oxidase system as the superoxide radical generator in a pH 7.8 phosphate buffer solution. NBT reduction by $O_2^{\cdot-}$ was spectrophotometrically monitored at 560 nm. The IC_{50} values were determined by regression analysis and interpolation of the % inhibition vs assay concentration curve for at least five experimental points for each system, with inhibition values within the range of 10 to 75 %. In all cases a linearity greater than 0.960 was achieved.

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