SYNTHESIS, CHARACTERIZATION and ANTIMICROBIAL PROPERTIES OF THIOSEMICARBAZONE DERIVED FROM *α*-CHLORALOSE

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Abstract – 1,2-*O*-(*R*)-Trichloroethylidene-*α*-D-*xylo*-1,4-furanodialdose hydrate was obtained from *α*-chloralose via conventional sodium metaperiodate cleavage-oxidation reaction. 1,2-*O*-(*R*)-trichloroethylidene-*α*-D-*xylo*-1,4-furanodialdose thiosemicarbazone was obtained from the reaction of 1,2-*O*-(*R*)-trichloroethylidene-*α*-D-*xylo*-1,4-furanodialdose hydrate with thiosemicarbazide in the presence of an acid catalyst. The structure of thiosemicarbazone product was characterized with NMR and FTIR spectroscopic methods. This new compound (**5**) was evaluated for its antimicrobial activity against Gram-positive, Gram-negative bacteria and *C. albicans* using the well diffusion and microdilution method. The thiosemicarbazone product shows significant growth inhibitory activity against bacteria *Staphylococcus aureus*, *Escherichia coli*, *Kocuria rhizophila*, *Bacillus cereus*, *Enterobacter aerogenes* and showed moderate activity against *Candida albicans*. The minimal inhibitory concentrations (MIC) experiments revealed that tested compound exhibited variable MICs and selective antimicrobial activity, depending on the microbial strains.

Keywords: Thiosemicarbazone; chloralose; pentadialdofuranose; trichloroethylidene; antimicrobial activity.

1. Introduction

Arthur Heffter firstly synthesized chloralose in 1889 from the condensation of D-glucose and trichloroacetaldehyde (chloral) in the presence of an acid catalyst[1]. A mixture of two optical isomers were obtained from glucose which *α*-glucochloralose (*α*-chloralose) and *β*-glucochloralose (*β*-chloralose). If the trichloromethyl (**CCl_3**) substituent has axial orientation, this compound is called *α*-chloralose and equatorial orientation is *β*-chloralose (**Fig. 1**). (*α*-) and (*β*-) terms show the configuration on the acetal carbon. Afterwards, the syntheses of *β*-xylochloralose[2] from D-xylose, *β*-arabinochloralose[3] from D-arabinose, *β*-galactochloralose[4] from D-galactose, *β*-mannochloralose[5] from

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D-mannose were reported via same synthesis method but only *β*-product from the possible configuration of optical isomers was obtained. Actually, chloraloses are furanose-type cyclic acetals of pentoses and hexoses containing to 1,2-*O*-trichloroethylidene ring. Trichloroethylidene rings are highly stable in acidic and mildly basic conditions[6] but it is unstable in strongly basic condition as a potassium *tert*-butoxide[3,5]. Cyclic ketene acetal was occurred by HCl elimination from trichloroethylidene ring of chloralose with alkoxide (strong base)[3,5]. The removing of trichloroethylidene group is only method when hydrogenation reaction using of Ranel nickel, followed by acidic hydrolysis[7].

Trichloroethylidene acetals are potential biologically active compounds; such as *α*-chloralose (1,2-*O*-(*R*)-trichloroethylidene-*α*-D-glucofuranose) is a hypnotic drug has been used as an anesthetic agent in laboratory animals[8]. It also was used in humans until the early part of the twentieth century[9]. Used as a commercial drug *α*-chloralose is also widely used in bird repellent, rodenticide, neuroscience and veterinary medicine as an anesthetic and sedative[10,11]. Overton[9] found that anesthetic differences between *α*-chloralose and its structural isomer *β*-chloralose were hard to explain. The phenomena of narcosis with *α*-chloralose are not very easy to interpret *β*-chloralose, which is only very slightly soluble in water in most solutions, has no narcotic effect (**Fig. 1**). It has been characterized as a molecule possessing potent *central nervous system* activity, and has been evaluated in human and animal models, for its therapeutic properties[12,13].

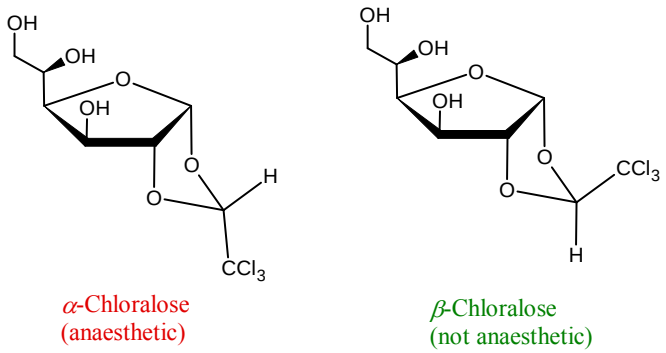


Figure 1. Molecular structures of *α*-chloralose and *β*-chloralose.

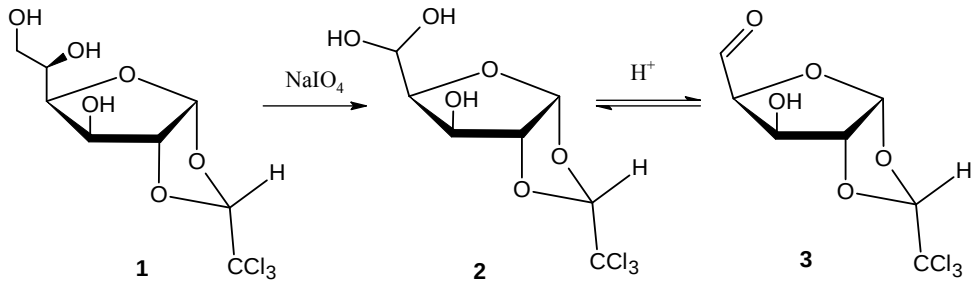
In addition, anesthesia effects well-known arabinochloralose has been used as an intermediate compound for the development of new anti-tuberculosis drugs in pharmaceutical research[14]. *Spiro*-endoperoxide chloraloses were synthesized and investigated antimicrobial activity against some microorganisms[15,16].

Thiosemicarbazones (R_1R_2C=N-NH-C(*S*)-NH_2) are important due to their wide applications in industry and medicine. Thiosemicarbazones are obtained from condensation reaction of an aldehyde or ketone with thiosemicarbazide and use of acid catalyst or microwave. Thiosemicarbazones, a class of compounds possessing a wide spectrum of potential medicinal applications, have been studied for their antitumor, antiviral, antituberculosis, antibacterial, antimalarial, antifungal, antiinflammatory and *anti*-HIV activities[17-20].

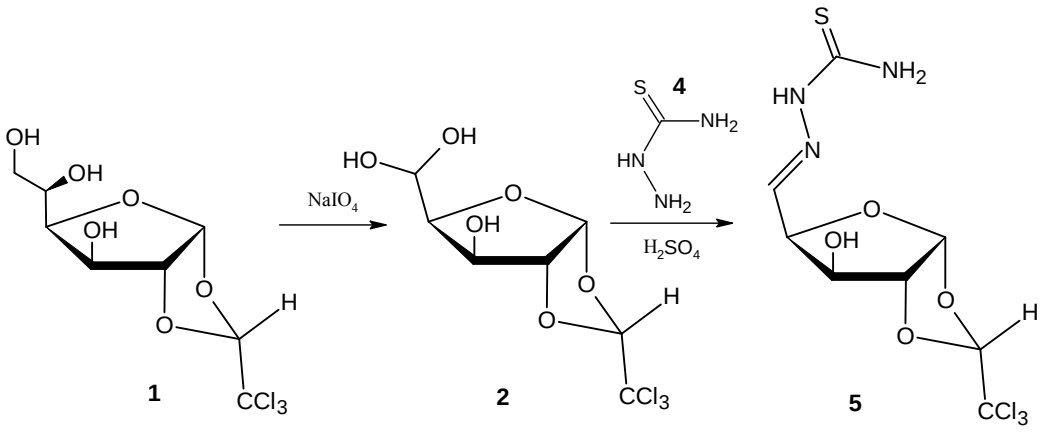
Carbohydrate derivatives have been extensively investigated, including synthesis, characterization and biological activity, partly due to the facts that many naturally occurring saccharides and synthesized analogues exhibit various and potent biological activities. The free[21-23] and protected[24-28] monosaccharide derivative thiosemicarbazones have been reported in literature. This thiosemicarbazone has been used in preparation of metal complexes[21,22,29] and potential biological active thiadiazole[23-28] derivatives.

2. Results and Discussion

1,2-*O*-(*R*)-trichloroethylidene-*α*-D-*xylo*-1,4-furanodialdose (**3**) was obtained as a hydrate form (**2**) from *α*-chloralose (**1)** via periodate oxidation. The hydrate form (**2**) is stable in room temperature and converted to aldehyde form (**3**) in organic solvents in acidic condition at heating.



1,2-*O*-(*R*)-Trichloroethylidene-*α*-D-*xylo*-1,4-furanodialdose thiosemicarbazone has been synthesized from *α*-chloralose as figured in following reactions:



Structure has been confirmed by using spectral methods (FTIR, ^{1}H-NMR, ^{13}C-NMR). The IR spectrum (**Fig. 2**) of 1,2-*O*-(*R*)-trichloroethylidene-*α*-D-*xylo*-1,4-furanodialdose thiosemicarbazone shows bands in 3408 and 3461 cm^{-1} due to the asymmetric and symmetric stretching frequencies for NH_2, while the absorption for NH is present in 3290 cm^{-1}. An absorption band for C=N appears in 1606 cm^{-1} regions and OH-alcohol absorption in carbohydrate part is observed absorption bands in 3178 cm^{-1}.

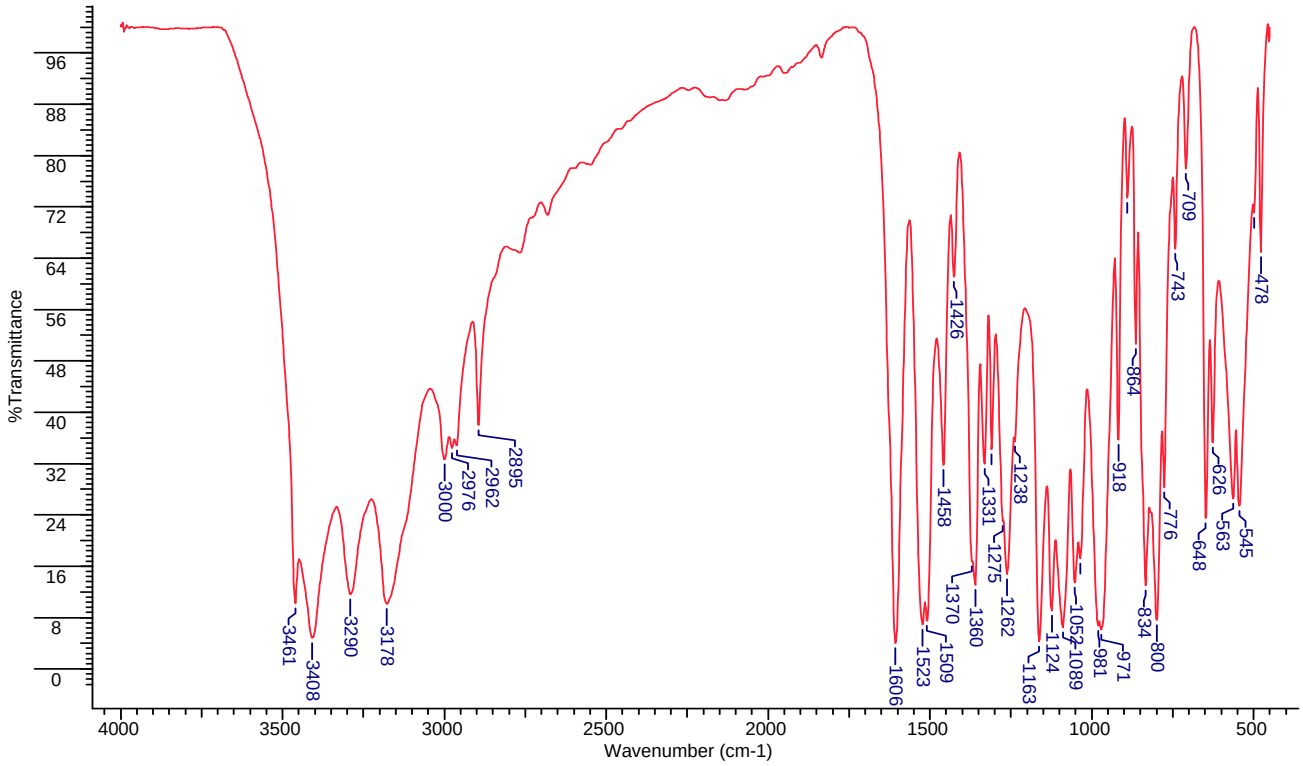


Figure 2. FTIR spectrum of the compound **5** in KBr

^{1}H-NMR (**Fig. 4**) and ^{13}C-NMR spectral data of compound **5** are shown in **Table 1**. Azomethine proton was observed doublet at 7.33 ppm. The proton of hydroxyl is specified by broad singlet chemical shifts at 5.73 ppm. Primer amine protons were observed two singlet at 8.07 and 7.68 ppm and proton of secondary amine was observed singlet at 11.31 ppm. This finding was previously explained because of a twisted conformation of the furanose ring causing the *endo*-trichloromethyl group to approach the H-4 hydrogen hence shifting it downfield[7].

^{13}C NMR spectrum is also consistent with the proposed structure (**Fig. 3**), exhibiting two double heterogeneous bond carbons C=N (141.9 ppm) and C=S (179.1 ppm).

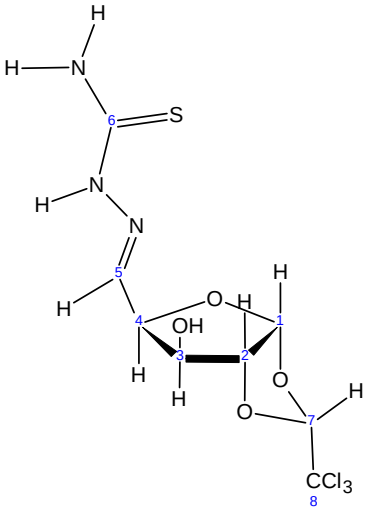


Figure 3. Molecular structure of the compound **5**

Table 1. ^{1}H- and ^{13}C-NMR spectral data of compound **5**

Location of atoms	^{1}H NMR (δ)	H and *J* couplings (Hz)	^{13}C NMR (δ)
6	-	-	179.1
5	7.33 d	1 H, $J_{4,5}$=6.8	141.9
4	4.81 dd	1 H, $J_{3,4}$=3.2	**88.1, 82.6, 75.8**
3	4.22 d	1 H	
2	4.70 d	1 H	
1	6.11 d	1 H, $J_{1,2}$=4.0	**106.8, 106.4**
7	5.42 s	1 H	
8	-	-	97.8
NH_2	8.07 s, 7.68 s	2 H, H_a and H_b	-
NH	11.31 s	1 H	-
OH	5.73 bs	1 H	-

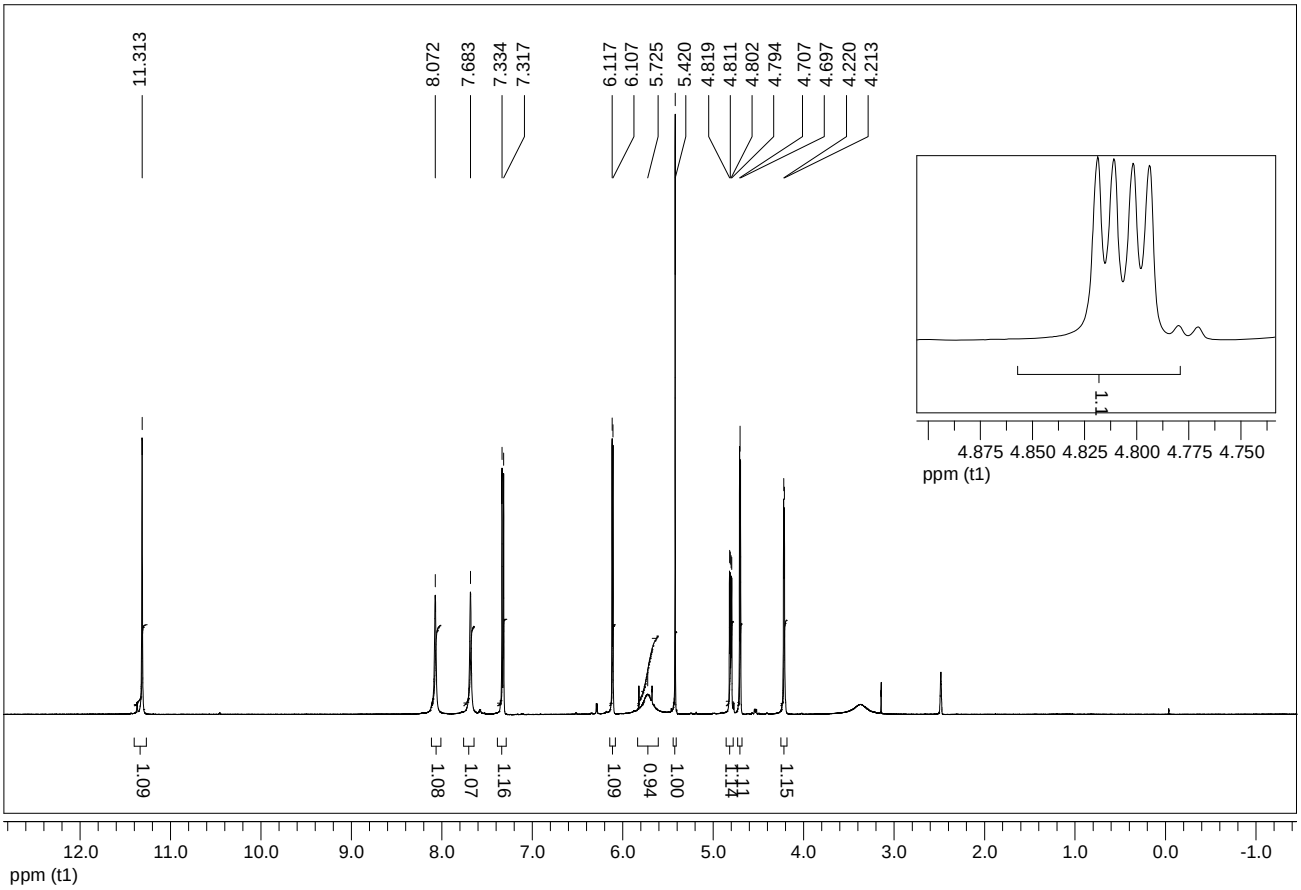


Figure 4. ^{1}H-NMR spectrum of the compound **5**

OSIRIS is online open access software in internet and estimated drug-relevant properties of chemical molecule structures[30]. Thiosemicarbazone molecule structure (compound **5**) is drawn in this software, and the results of theoretical calculations showed that the drug is likely to be (**Fig. 5**).

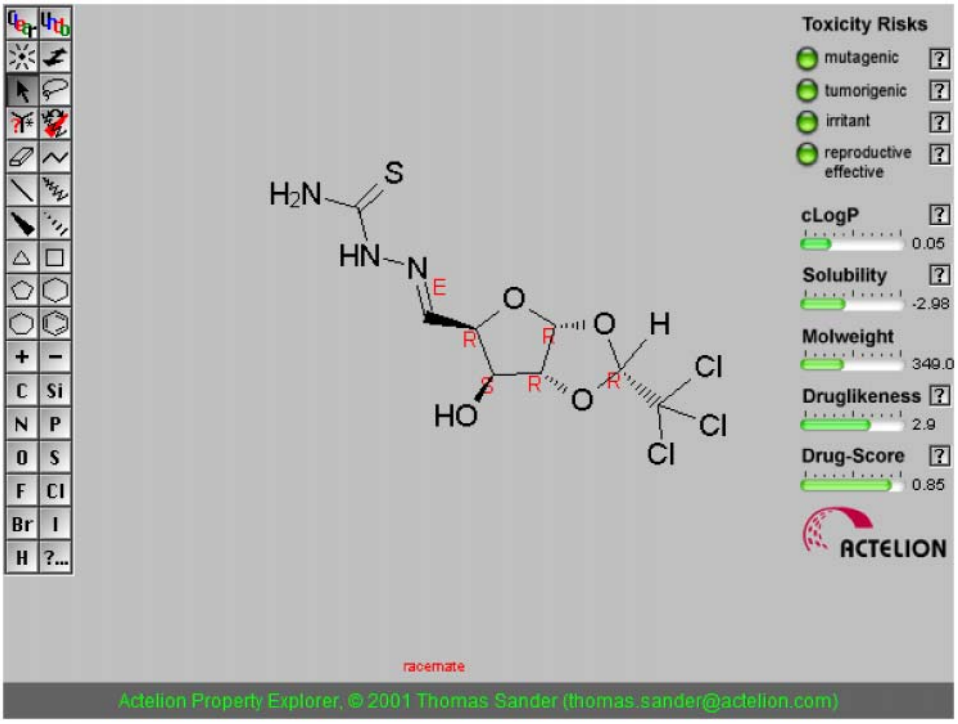


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Figure 5. The predicted properties of OSIRIS software for the compound **5**

Natural antibiotic compounds have become indispensable to the current health care system, assisting and complementing the natural immune system against microbial pathogens. However, because conventional antibiotics are often overused to treat microbial infections, some microorganisms have developed resistance to many of these antibiotics[35].

Nowadays it is known that thiosemicarbazones and their metal complexes show *anti*-tumor, *anti*-viral, *anti*-fungal, *anti*-bacterial, and *anti*-malarial activities. More over metal complexes of thiosemicarbazone often display enhanced activities when compared with the uncomplexed thiosemicarbazone activity. Literature survey reveals that metal complexes derived from thiosemicarbazide and different carbonyl containing compounds received much attention by researchers[29,35].

The antimicrobial screening data of the compound **5** and some control antibiotics against eleven-test microorganism are presented in **Table 2**. It shows that the new compound exhibits antimicrobial activities with an inhibition zones ranging from 22 to 12 mm and it is important to note that this compound showed greater activity than the standard antibiotic penicillin but exhibited lower activity than chloramphenicol and nystatin. In addition, its anti-candidal activity lowers than both nystatin and its anti-bacterial effects. The thiosemicarbazone (**5**) showed a wide spectrum of effective antimicrobial activities against the test bacteria, especially against *Staphylococcus aureus*, *Kocuria rhizophila*, *Enterobacter aerogenes* and *Escherichia coli*.

According to the primary antimicrobial results, six microorganisms were selected for MIC determination to find out the efficiency of compound **5** and to compare with some standard antibiotics. The MICs against selected microorganisms are reported in **Table 3**. Obtained results showed that compound **5** was the more active against *S. aureus* with the best MIC (0.833 mg/mL) comparable with neomycin (27.08 µg/mL) and gentamycin (3.907 µg/mL). On the other hand, this compound was not selective activity between Gram-negative and Gram-positive bacteria but more potent to last one. Thiosemicarbazone MIC against *C. albicans* was 6.67 mg/mL and the result shows very low anti-candidal activity compared with control antibiotic nystatin (MIC=0.488 µg/mL).

Table 2. Antimicrobial activity of synthesized compound 5 against test microorganisms.

	Microorganisms[a]										
Compound	*SA*[d]	*EC*	*KR*	*BC*	*BS*	*STYP*	*PV*	*EF*	*EA*	*ECLO*	*CA*
5	**22**[b]	**18**	**20**	**16**	**18**	**10**	**12**	**16**	**20**	**14**	**12**
P[c]	24	6^{R}	20^{R}	10^{R}	8^{R}	6^{R}	10^{R}	24	6^{R}	12^{R}	ND
NA	20	26	10^{R}	28	30	6^{R}	12^{R}	28	26	10^{R}	ND
CLH	20	26	30	26	28	40	16	28	12^{R}	28	ND
NYS	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	22
NC (EtOH)	4	0	6	$4^{\pm}$	0	$6^{\pm}$	$6^{\pm}$	0	0	0	$4^{\pm}$

[a]Test microorganisms: SA, *Staphylococcus aureus* ATCC 6538P; EC, *Escherichia coli* ATCC 39628; KR, *Kocuria rhizophila* ATCC 9341; BC, *Bacillus cereus* CCM 99; BS, *Bacillus subtilis* ATCC 6633; STYP, *Salmonella typhimurium* CCM 5445; PV, *Proteus vulgaris* ATCC 8427; EF, *Enterococcus faecalis* ATCC 29212; EA, *Enterobacter aerogenes* ATCC 13048; ECLO, *Enterobacter cloacae* ATCC 13047D; CA, *Candida albicans* ATCC 10231. [b]Inhibition zone diameter in millimeters, including well and disc diameter (6 mm). [c]Standard antibiotics, P – Penicillin (10 IU); NA – Nalidixic acid (30 µg/disc); CHL – Chloramphenicol (30 µg/disc); NYS – Nystatin (10 µg/disc). [d]Bacteria tested in MHA medium, yeast in PDA; mean values, n=3; ND, not determined; [R], resistant, NC, negative control (Ethanol, 60 µL), [±] partially inhibition. Applied compounds dose, 1.2 mg/well.

Table 3. MIC values of the tested compound **5** and standard antibiotics against selected microorganisms

	Tested compound (mg/mL) and Standard antibiotics (μg/mL)			
MO[a]	**5**	**NEO[b]**	**GE**	**NYS**
SA	0.833 ± 0.323[c] (0.625 – 1.25)	27.08 ± 9.88 (18.75 – 37.5)	3.907 ± 1.172 (2.344 – 4.688)	ND
EC	2.083 ± 0.645 (1.25 – 2.50)	5.730 ± 2.067 (4.688 – 9.375)	6.250 ± 2.344 (4.688 – 9.375)	ND
KR	0.938 ± 0.342 (0.625 – 1.25)	3.386 ± 1.235 (2.344 – 4.688)	2.865 ± 1.034 (2.344 – 4.688)	ND
BS	1.667 ± 0.645 (1.25 – 2.50)	6.250 ± 2.420 (4.688 – 9.375)	4.569 ± 1.913 (4.688 – 9.375)	ND
EA	1.042 ± 0.323 (0.625 – 1.25)	7.813 ± 2.420 (4.688 – 9.375)	4.297 ± 0.957 (2.344 – 4.688)	ND
CA	6.67 ± 2.58 (5.0 – 10.0)	ND	ND	0.488 ± 0.293 (0.293 – 1.172)

[a] MO: Microorganisms: SA – *Staphylococcus aureus*; EC – *Escherichia coli*; KR – *Kocuria rhizophila*; BS – *Bacillus subtilis*; EA – *Enterobacter aerogenes*; CA – *Candida albicans*. [b] Standard antibiotics: NEO – Neomycin; GE – gentamycin; NYS – Nystatin. ND – not determined. [c] Minimum and maximum values are shown in parentheses. Data presented as the mean value of six determinations ± standard deviation.

3. Experimental Part

Melting points were measured by using Electrothermal AI 9200 melting point apparatus in capillary and uncorrected. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) were recorded on a Varian AS 400 NMR spectrometer and optical rotation measurements were carried out on a Rudolph Research Analytical Autopol II automatic polarimeter in Ege University. IR spectra were recorded on a Perkin Elmer Spectrum 100 FTIR spectrometer. Elemental analyses were performed on a LECO CHNS 932 analyzer in İnönü University. TLC and column chromatography were performed on precoated aluminium plates (Merck 5554), respectively. All solvent removals were carried out under reduced pressure.

3.1. Chemical synthesis

3.1.1. *1,2-O-(R)-Trichloroethylidene-α-D-xylo-1,4-furanodialdose hydrate*[16] (**3**)

Commercial *α*-chloralose (**1**) contains *β*-chloralose impurity and soluble in cold methanol but *β*-chloralose insoluble. *α*-Chloralose was purified in cold methanol and solvent was removed under reduced pressure. Periodate oxidation of (*R*)-1,2-*O*-trichloroethylidene-*α*-D-glucofuranose (*α*-chloralose) was made according to literature[16]. A hot solution of *α*-chloralose (10 g, 0.33 mol) in CH_3OH (150 mL) was mixed with a solution of $NaIO_4$ (8 g, 0.038 mol) in H_2O (200 mL) and stirred

until a clear solution gained. The solution was allowed to stand at room temperature for 4 h. The mass of $NaIO_3$ crystals, which formed, was filtered and washed with CH_3OH. The filtrate and the washings were combined and concentrated under reduced pressure to give a solid product. The solid was extracted with CH_2Cl_2 and evaporated under reduced pressure. The syrupy product solidified in air over night as the hydrate form. White solid, 3.2 g (65 %), mp 139-142 °C (decomp.) (lit.[16] mp 138-142 °C decomp.).

3.1.2. *1,2-O-(R)-Trichloroethylidene-α-D-xylo-1,4-furanodialdose thiosemicarbazone* (**5**)

A solution of compound **3** (3 g, 0.01 mol) in absolute ethanol (50 mL) was mixed with thiosemicarbazide (**4**) (1.11 g, 0.012 mol) and two drops H_2SO_4 and stirred. The solution was allowed to stand under reflux for 2 h. TLC (toluene-methanol, 8:2) showed a single product. The mixture was concentrated with evaporation under reduced pressure. The mixture was poured in pure water (200 mL). The solid product was filtered and washed with water, dried in air, white powder (**5**), 2.56 g (72%), mp 179 ^oC (decomp.), $[\alpha]_D^{27}$ -21.6 (*c* 2.893 in CH_3OH). Anal. Calcd. for $C_8H_{10}Cl_3N_3O_4S$: C, 27.41; H, 2.87; N, 11.98; S, 9.15. Found: C, 27.18; H, 2.89; N, 11.79; S, 9.29.

3.2. Antimicrobial spectrum

3.2.1. Agar well diffusion assay

Antimicrobial studies were carried out by the agar well diffusion method[31] against test microorganisms (see **Table 2**.). Bacterial strains grown on nutrient agar (37 ^oC for 24 h) and *C. albicans* grown on Potato Dextrose Agar (30 ^oC for 48 h), were suspended in a saline solution (0.85 % NaCl) and adjusted to a turbidity of 0.5 McFarland standards (10^6 Colony Forming Units/mL). Then, 50 µL inoculums was added to 25 mL melted Mueller Hinton Agar (MHA) for bacteria and Potato Dextrose Agar (PDA) (Oxoid, Basingstoke, UK) for *C. albicans* medium cooled at 45 °C. These were then poured into 90 mm diameter Petri dishes and maintained for 1 h at room temperature. 6 mm diameter wells were cut in the agar plate and 60 µL of compound (1.2 mg/well) and solvent as a negative control (Ethanol, 60 µL) were loaded individually in the wells. The dishes were preincubated at 4 °C for 2 h to uniform diffusion into the agar. After preincubation, the plates were incubated at 37 °C for 24 h for bacteria and 30 °C for 48 h for yeast. The antimicrobial activity was determined by measuring the inhibition zone diameter around the wells. In addition, commercial antibiotics such as nalidixic acid (30 µg), chloramphenicol (30 µg) and nystatin (10 µg) were used as positive control to determine the sensitivity of the strains[32]. Experiments were repeated three times, and the results were expressed as average values.

3.2.2. Determination of minimum inhibition concentration (MIC)

The microtiter broth dilution technique was performed by using the CLSI standards[33,34]. A sterile 96 round-bottom well plate was labeled. A volume of 100 µL of antimicrobial compound solution was

pipetted into the first row of the plate. To all other wells 50 µL of double strength Mueller Hinton broth or Potato Dextrose broth was added. Serial dilutions were performed using a micropipette (A1-A10). Tips were discarded after use such that each well had 50 µL of the test material in serially descending concentrations. Then, 50 µl of broth containing bacterial suspension (5 x 10^6 cfu/mL) or yeast (5 x 10^5 cfu/mL) was added to each well. Each column of wells contained a single antimicrobial agent in progressive dilutions and was inoculated with a single microorganism. Each plate had a set of both a growth (A11) and sterility control (A12). Plates were sealed with clean film to ensure that microorganisms did not become dehydrated. The plates were prepared in triplicate, and placed in an incubator set at 37 °C for 18–24 h and at 30 °C for 48 h, respectively for bacteria and *C. albicans*. After incubation, added 10 µL of 0.2% 2,3-5 Triphenyl tetrazolium chloride (TTC) solution to each well of microtitre plate. The plates containing TTC were incubated one h at 37 °C for reaction. The color change was then assessed visually. Any color changes from purple to pink, which showed the growth of organism. MIC was defined as that the lowest inhibitory concentration of the antimicrobial agent contained in the microtiter well in which the absence of visual color change (colorless) first observed. The average of six values was calculated and that was the MIC for the test material and microbial strain.

4. Conclusion

Possible biological active 1,2-*O*-(*R*)-trichloroethylidene-*α*-D-*xylo*-1,4-furanodialdose thiosemicarbazone has been synthesized in good yield from *α*-glucochloralose. Stable trichloroethylidene acetal group of *α*-chloralose is not hydrolyzed with acid catalyst in thiosemicarbazone formation reaction. Structure of thiosemicarbazone has been confirmed using modern spectroscopic methods.

Resistance to drugs in clinical use is a major concern and our results suggest that this class of thiosemicarbazones derived from *α*-chloralose present an interesting antimicrobial profile against clinically significant Gram-negative and Gram-positive bacteria. The results of the present work could be an interesting strategy for preparing new antimicrobial agents against multi-drug resistant bacteria and suggest new possibilities for future investigation.

5. References

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