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Surface and biocidal activity of some synthesized metallo azobenzene isothiouronium salts

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Abstract—A novel series of azobenzene isothiouronium salts of different alkyl chains (propyl, hexyl and dodecyl) were synthesized by reaction of 4-((4-methylphenyl)azo)phenol with 1,3-dibromopropane, 1,6-dibromohexane and 1,12-dibromododecane, respectively. These salts were reacted with copper (II) halide to give their corresponding metallo complexes. The surface tension measurements for the synthesized compounds show that the metallo complexes have adsorption and micellization better than that of the parent azobenzene isothiouronium salts. The pathogenic Gram-negative bacteria, Gram-positive bacteria, fungi and yeast were used to determine the biocidal activity of these compounds using gradient plate technique. The results indicate that the copper complexes of the synthesized azobenzene isothiouronium salts have a relatively better biocidal activity than the parent salts.

1. Introduction

Isothiouronium salts show a variety of biocidal activities.[1–3] In the quest for biologically active sulfur compounds, studies included isolation, characterization and synthesis of novel compounds that can be used in medicine. A wide variety of reactions to explore the synthetic utility of these compounds were carried out to synthesize more potent biocidal derivatives.[4] Many of the publications describe the synthesis, structural activity relationship, mode of action and pharmacological effects such as germicidal, antibacterial, antifungal and anticancer activities of these compounds. Thiadiazole isothiouronium derivatives have been synthesized and their antifungal and antibacterial activities were tested.[5] A series of thiourea and isothiouronium salt derivatives of the aglycone of teicoplanin was prepared by reaction of the terminal amino group with isothiocyanates, followed by S-alkylation of the thiourea compounds. Unexpectedly, the two classes of derivatives showed a similar in vitro antibacterial activity against Gram-positive bacteria. Thiourea compounds, due to the lack of apositively charged N-terminus group, have a 10-fold lower binding constant to Ac-D-Ala-D-Ala, a bacterial cell-wall model, than the parent antibiotic and isothiouronium salt derivatives.[3] Sjouke et al. described the properties of a novel family of aromatic isothiouronium derivatives.[6] These isothiouronium derivatives appear to be promising candidates for further development as affinity labels of cation-binding domains, for kinetic analysis of isoforms or mutated Na/K pumps, or as probes of other cation transport proteins.[6] The structures of the new gamma-lactam derivatives (N^{15}-isothiouronium) were determined by using ^{1}H NMR, IR and fast atom-bombardment mass spectra. The cyclization mechanism was interpreted on the basis of the identification of the intermediate structure. The poor in vitro antibacterial activity of the new cyclic compounds and the negligible affinity for the synthetic peptidoglycan model Ac2-L-Lys-D-Ala-D-Ala are probably due to the lack of the N-17 amidic proton and to the lack of the basic character of the nitrogen in position (15).[7] The antimicrobial and antifungal activities of the isothiouronium derivatives, *S-sec*-butyl isothiouronium 2-pyrimidinyl sulfonamide; *S-sec*-butyl isothiouronium 2-(4, 6-dimethyl pyrimidinyl) sulfonamide and *S-sec*-butyl isothiouronium 3-(6-methoxy) pyrydazinyl sulfonamide, were investigated.[8] Several publications have investigated the biocidal activity of copper (II) complexes and show that the copper complexes have a better biocidal activity than the uncomplexed compounds.[9–12] In this paper, we investigate the surface properties and the

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biocidal activity of a series of azobenzene isothiouronium salts and their complexes with copper metal.

2. Results and discussion

2.1. Surface properties

The surface tension (γ) of the synthesized azobenzene isothiouronium salts and their corresponding copper complexes against different concentrations at 25 °C is plotted in Figure 1. The results show that the copper complexes have lower surface tension than the parent azobenzene isothiouronium salts. The critical micelle concentration (CMC) values were determined from the plot of surface tension and concentration as shown in Table 3. It is observed from Table 3 that the CMC values of copper complexes with the exception of **C3m** are lower than those of the parent azobenzene isothiouronium salts which reflect greater surface properties. These results explain that the complexes retain its unity of structure in their solutions, which increased their volume in the aqueous media with repulsion between the hydrophobic chains and water molecules. This repulsion facilitates two processes at the same time. The adsorption process of the molecules of metal complex at the air/water interface of extremely lower concentrations (below CMC) and the micellization process of these molecules at concentrations lower than their parent azobenzene isothiouronium salts.[13] Table 3 reveals that the CMC values decrease as the alkyl chain moiety increases. This is due to the decrease in the solubility of the prepared azobenzene isothiouronium salts and their complexes as the alkyl chain in the hydrophobic moiety increases. Effectiveness (Π_{CMC}) is calculated according to the following equation as shown in the previous publication.[14]

$$\Pi_{CMC} = \gamma_o - \gamma$$

where γ_o measures the surface tension of pure water at the appropriate temperature and γ is the surface tension at critical micelle concentration (CMC).

The data of effectiveness are summarized in Table 3. The effectiveness of the prepared compounds increases as the alkyl chain length increases. This indicates that the ability of the synthesized compounds to adsorb at air/water interface and decrease the surface tension. The decreas-

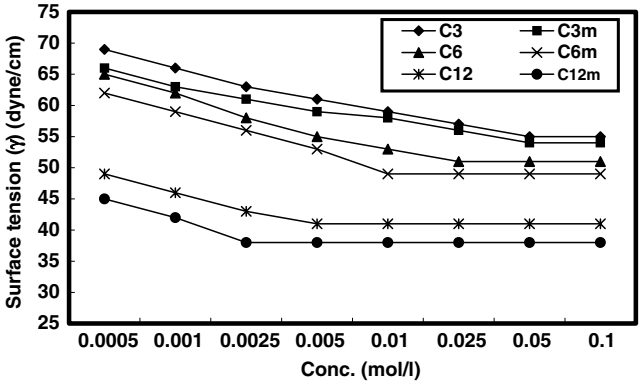


Figure 1. The relation between the surface tension and concentration of the prepared isothiouronium salts and their complexes at 25 °C.

Table 3. Critical micelle concentration (CMC) and surface parameters for the synthesized azobenzene isothiouronium salts and their copper complexes

Compound	CMC (mol/l)	Surface tension at CMC (γ_{CMC}) dyne/cm	Effectiveness (E_{CMC}) dyne/cm
C3	0.05	55	17
C6	0.025	51	21
C12	0.005	41	31
C3m	0.05	54	18
C6m	0.01	49	23
C12m	0.0025	38	34

Table 1. The elemental analysis of the synthesized azobenzene isothiouronium salts and their copper complexes

Compound	M_W	% C		% H		% N		% S		% Br		% Cl	
		Calcd	Found	Calcd	Found	Calcd	Found	Calcd	Found	Calcd	Found	Calcd	Found
C3	409	49.88	49.86	5.17	5.15	13.69	13.67	7.83	7.81	19.55	19.53	—	—
C6	451	53.21	53.19	6.03	6.01	12.41	12.40	7.09	7.10	17.73	17.70	—	—
C12	535	58.31	58.30	7.34	7.31	10.46	10.44	5.98	5.99	14.95	14.97	—	—
C3m	952.5	42.83	42.80	4.40	4.37	11.75	11.77	6.71	6.72	16.79	16.77	7.45	7.42
C6m	1036.5	46.30	46.32	5.20	5.17	10.80	10.81	6.17	6.20	15.43	15.41	6.84	6.87
C12m	1204.5	51.80	51.82	6.47	6.45	9.29	9.30	5.31	5.29	13.28	13.29	5.89	5.88

Table 2. The FTIR spectroscopy of the synthesized azobenzene isothiouronium salts and their copper complexes

Compound	CH_3	CH_2	C–S	C–O–C	Para substituted benzene ring
C3	2913 cm^{-1}	2848 cm^{-1}	710 cm^{-1}	1026 cm^{-1}	1005–825 cm^{-1}
C6	2920 cm^{-1}	2840 cm^{-1}	716 cm^{-1}	1027 cm^{-1}	1005–822 cm^{-1}
C12	2935 cm^{-1}	2835 cm^{-1}	734 cm^{-1}	1028 cm^{-1}	1005–820 cm^{-1}
C3m	2947 cm^{-1}	2860 cm^{-1}	722 cm^{-1}	1025 cm^{-1}	1005–840 cm^{-1}
C6m	2960 cm^{-1}	2872 cm^{-1}	711 cm^{-1}	1026 cm^{-1}	1005–835 cm^{-1}
C12m	2955 cm^{-1}	2865 cm^{-1}	720 cm^{-1}	1027 cm^{-1}	1005–825 cm^{-1}

Table 4. Biocidal activity for the prepared azobenzene isothiouronium salts

Sample	Inhibition zone diameter (mm/mg sample)			
	Pseudomonae aeruginosa (G^-)	*Staphylococcus aureus* (G^+)	*Aspergillus flavus* (fungus)	*Candida albicans* (yeast)
Control: DMSO	0.0	0.0	0.0	0.0
C3	13	13	0.0	14
C6	12	13	0.0	13
C12	13	14	0.0	13

G, Gram reaction.
Solvent: DMSO.

Table 5. Biocidal activity for the prepared azobenzene isothiouronium copper complexes

Sample	Inhibition zone diameter (mm/mg sample)			
	Pseudomonae aeruginosa (G^-)	*Staphylococcus aureus* (G^+)	*Aspergillus flavus* (fungus)	*Candida albicans* (yeast)
Control: DMSO	0.0	0.0	0.0	0.0
C3m	15	14	14	16
C6m	13	14	12	14
C12m	14	13	13	13

G, Gram reaction.
Solvent: DMSO.

ing in the surface tension is increase as the alkyl chain length increase. The synthesized copper complexes (**C3m**, **C6m** and **C12m**) show higher Π_{CMC} than their parent azobenzene isothiouronium salts (**C3**, **C6** and **C12**) indicating that the copper complexes have more efficiency towards adsorption at air/water interface than their parent azobenzene isothiouronium salts.

2.2. Biocidal activity

The biological activity of the synthesized azobenzene isothiouronium salts and their copper complexes against Gram-negative bacteria (*Pseudomonae aeruginosa*), Gram-positive bacteria (*Staphylococcus aureus*), *Aspergillus flavus* fungus and *Candida albicans* yeast are represented in Tables 4 and 5. The biocidal activity of the compounds under investigation (**C3**, **C6**, **C12**, **C3m**, **C6m** and **C12m**) agrees with their ability for adsorption at the water/cell membrane interface. This adsorption increases solubility through the cell membrane increasing its permeability towards the media ingredients. Thus biological reactions disturb within the cell cytoplasm.[13] Tables 4 and 5 show that, the prepared azobenzene isothiouronium salts and their copper complexes have a good biocidal activity against bacteria, fungi and yeast used in this investigation. Table 4 shows that, compounds **C3** and **C12** give relatively better inhibition zones against bacteria and fungi than compound **C6**. It is noticed that, compounds **C3**, **C6** and **C12** show negative biological activity against *A. flavus* fungus. The comparison of the data in Tables 4 and 5 indicates that, the copper complexes have a better biological activity towards bacteria, fungi and yeast under study than their parent azobenzene isothiouronium salts. Also the results show that, the synthesized 4-methyl-4′-propyloxy-azobenzene isothiouronium dibromo-dichlorocuparate complex (**C3m**) compound gives the highest inhibition zones against Gram-negative bacteria (*P. aeruginosa*), Gram-positive bacteria (*S. aureus*), *A. flavus* fungus and *C. albicans* yeast. This behaviour could be explained through their ability towards adsorption at the cell membrane of the microorganisms under investigation. The increase in biocidal activity of the copper complexes is attributed to the lower electronegativity and large volume of copper ions, which increase their molecular area. Hence, the effective area of the complex molecules on the cell membrane increases resulting in a higher biocidal action.[13]

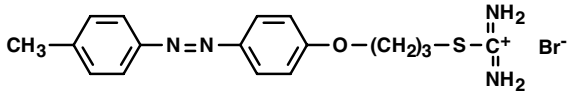
3. Conclusions

1. The copper complexes have more effectiveness towards adsorption at air/water interface than their parent azobenzene isothiouronium salts.
2. The azobenzene isothiouronium salts and their copper complexes have a good biological activity against Gram-positive and Gram-negative bacteria, fungi and yeast.
3. The biocidal activity of the copper complexes is greater than that of their parent azobenzene isothiouronium salts.

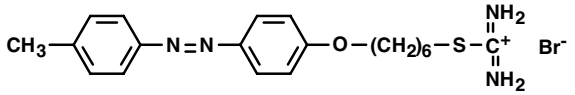
4. Experimental technique

4.1. Materials

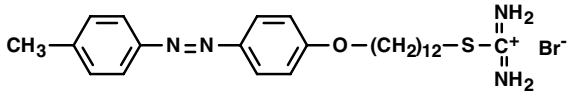
4.1.1. Synthesis of azobenzene isothiouronium salts. A series of azobenzene isothiouronium salts (**C3**, **C6 and C12**) of different alkyl chains (propyl, hexyl and dodecyl) was synthesized by refluxing 4-((4-methylphenyl)azo)phenol with 1,3-dibromopropane, 1,6-dibromohexane or 1,12-dibromododecane, respectively, in acetone for 6 h in presence of potassium carbonate. The refluxing product was crystallized from 2-propanol to afford the corresponding bromoazobenzene



4-methyl -4`- propyloxy-azobenzene isothiouronium bromide (C3)



4-methyl - 4`- hexyloxy-azobenzene isothiouronium bromide (C6)



4-methyl - 4`- dodecyloxy-azobenzene isothiouronium bromide (C12)

Scheme 1. The chemical structure of the prepared azobenzene isothiouronium salts.

compounds which were converted to isothiouronium salt by refluxing with thiourea in ethanol for 5 h.[15] The chemical structure of the synthesized azobenzene isothiouronium salts (**C3**, **C6** and **C12**) is shown in Scheme 1.

4.1.2. Synthesis of copper azobenzene isothiouronium salts. Copper complexes of azobenzene isothiouronium salts (**C3m**, **C6m** and **C12m**) were prepared by refluxing two moles of azobenzene isothiouronium salts and one mole $CuCl_2$ in ethyl alcohol for 3 h.[16] The resulting crystals were washed twice with petroleum ether, recrystallized from methanol and dried under vacuum at 50 °C for 4 h (Scheme 2).

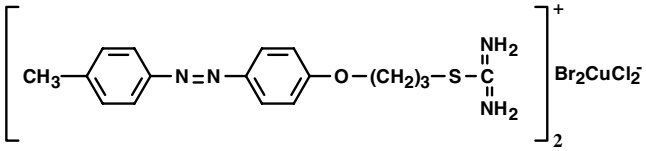
The elemental analysis for the synthesized azobenzene isothiouronium salts and their complexes is shown in Table 1. The FTIR spectroscopy for these compounds was carried out using ATI Malttson Genesis Series FTIR™ and the data are represented in Table 2. The chemical structure of the synthesized azobenzene isothiouronium salts and their complexes was confirmed using the elemental analysis and the FTIR spectroscopy data.

4.2. Surface tension (γ) measurements

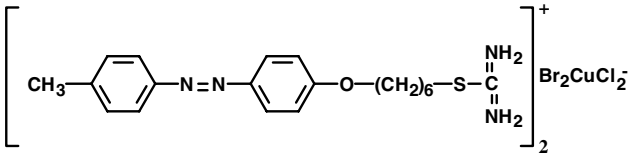
Surface tension values of the azobenzene isothiouronium salts and their corresponding copper complexes were determined at 25 °C using processor Tensiometer (Kruss Type K100) by plate method.

4.3. Biocidal activity

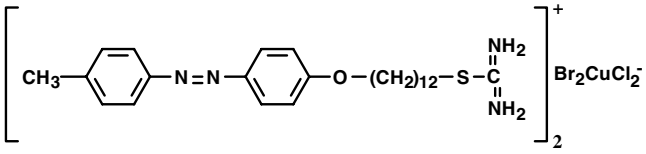
The synthesized azobenzene isothiouronium salts and their corresponding copper complexes were screened for their biocidal activity using diffusion disc method. A filter paper sterilized disc saturated with measured quantity of sample (20 mg in 1 ml DMSO) is placed on plate containing solid bacterial medium (nutrient agar broth) or fungal medium (Dox's medium) which has been heavily seeded with the spore suspension of the tested organism. After inoculation, the diameter of



4-methyl -4`- propyloxy-azobenzene isothiouronium dibromo-dichloro cuparate complex (C3m)



4-methyl -4`- hexyloxy-azobenzene isothiouronium dibromo-dichloro cuparate complex (C6m)



4-methyl - 4`- dodecyloxy-azobenzene isothiouronium dibromo-dichloro cuparate complex (C12m)

Scheme 2. The chemical structure of the prepared azobenzene isothiouronium complexes.

the clear zone of inhibition surrounding the sample is taken as a measure of the inhibitory power of the sample against the particular test organism.[17–20]

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