IN VITRO ANTIMICROBIAL ACTIVITY STUDIES OF THIOETHOXY-AND THIOPHYENOXYHALOBENZENE DERIVATIVES

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Abstract: The in vitro antibacterial and antifungal activities of thioethoxy- and thiophyenoxyhalobenzene derivatives were investigated. Thioethoxy- and thiophyenoxyhalobenzenedervatives synthesized and identified by spectroscopic means IR and NMR and elemental analysis. The antibacterial and antifungal activities were measured by Minumum inhibition concentration (MIC) method against gram-positive bacteria i.e. Staphylococcus aureus ATCC 25923, Bacillus subtilis ATCC 6633; Gram-negative bacteria as Yersinia enterocolitica ATCC 1501, Escherichia coli ATCC 11230, Klebsiella pneumoniae and fungus as Candida albicans from our strain collection. Antimicrobial activies of these compounds tended to increase with size and numerous and kinds of halogene and thiogroups substitutents.
Keywords: Antibacterial activity, antifungal activity, thioethoxy- and thiophyenoxyhalobenzene, Minimum Inhibition Concentration, Ampicillin and Fluconazole

Introduction

Human beings are exposed to an increasing number and increasing amounts of organobromine and organochlorine compounds , both man made and natural origine.There are many of these compounds as a commercial product, all of which exert well-documented toxic effects on mammalian cells (1). In the last three decades the environmental impact of halogenated chemicals has become increasingly apparent. Similarly many other haloarens are important environmental pollutants. The accumulation of organic pollutants in fish is a matter of especially concern, because fish serve as good for many species including humans (2, 3, 4)

The hexachlorocyclohexanes (HCHs) constitue a major group of organochlorinated compounds that have widely been used as insecticides. Thanks to environmental concerns, the production and use of HCHs declined quickly in the developed countries but more slowly in the developing areas (5, 6, 7, 8,9).

In mammals, polybrominated biphenyls cause loss of weight, chloracne, edema, hepatic- hypertrophy, porphyria, estrogenic activity and immunosupression (10, 11, 12).

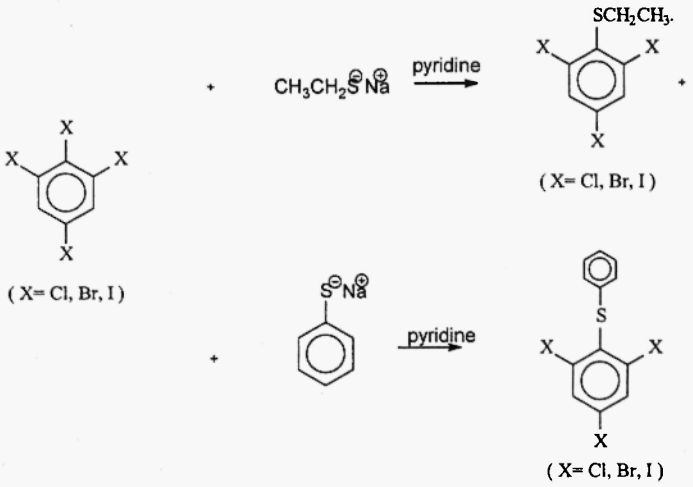
In the literature, with the regard to thio- substituent over benzene derivatives, there is no antimicrobial activity research. In our previous work, we studied with tetrasubstituted halogenes benzene and dipiperidino- and pyrrolidinohalogene benzenes derivatives (13, 14).

In this work halogenes and thio groups are together. The presence of halogenes we tried to find relation between thio- groups, halogens and structure for antimicrobial activity. In this work, we report on the synthesis of thiohalo benzene derivatives and on the biological activities of these compounds against *S. aureus, B. subtilis, Y. enterocolitica, E. coli K. pneumoniae* and C. *albicans.*

Material and Methods

Synthesis of Chemicals

The thioethoxy- and thiophyenoxyhalobenzenederivatives were prepared from 1,2,3,5-tetrahalogene benzenes according to the literature Tüzün, C., et. al. (15, 16). 1,2,3,5-tetrahalogene benzenes dissolved in pyridine then ethylmercaptane or thiophynole and sodiumhydroxide added to give thioethoxy- and thiophyenoxyhalobenzene derivatives. Reaction mixed under reflux at15 h, 60-70 ^{0}C. Reaction scheme were given at Figure-1.



Reaction scheme of thioethoxy- and thiophyenoxyhalobenzene

Figure-1

Microbiological Studies

Test Microorganisms and Medium

The bacterial subcultures for *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Yersinia enterocolitica* ATCC 1501 and *Escherichia coli* ATCC 11230 were standard bacterial strains. *K. pneumoniae* and yeast-like fungus *Candida albicans* were obtained from Department of Microbiology, Faculty of Medicine, Abant İzzet Baysal University, TURKEY.

All tests were performed in Mueller-Hinton Broth (MHB). Bacterial strains were cultured overnight at 37 °C in Brain Heart Infusion broth (BHI) and the yeast were cultured overnight at 30 °C in Sabouraud Dextrose Broth (SDB).The inoculum densities were 5x 10^5 cfu/ ml for bacteria and fungus.

Method

Minimum inhibitory concentrations (MICs) were determined by macrodilution broth method following the procedures recommended by the National Committee for Clinical Laboratory Standards (17).

MICs were defined as the lowest concetrations of the antimicrobial agents that inhibited visible growth of the microorganism. For the determination of antibacterial activities two gram-positive *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633and two gram-negative *Yersinia enterocolitica* ATCC 1501 and *Escherichia coli* ATCC 11230, *K. pneumoniae* bacteria were used as test bacteria. For testing antifungal activity of the compounds were used *Candida albicans* (18)

The compounds under the test were dissolved in dimethylsulphoxide (DMSO) and the final two-fold concentrations were prepared from 10245 μg/ ml to 1 μg/ ml. Ampicilline and fluconazole were used as antibiotics reference powders for bacteria and fungus, respectively. The doubling concentrations used for broth of them were 512-0.25 μg/ ml.

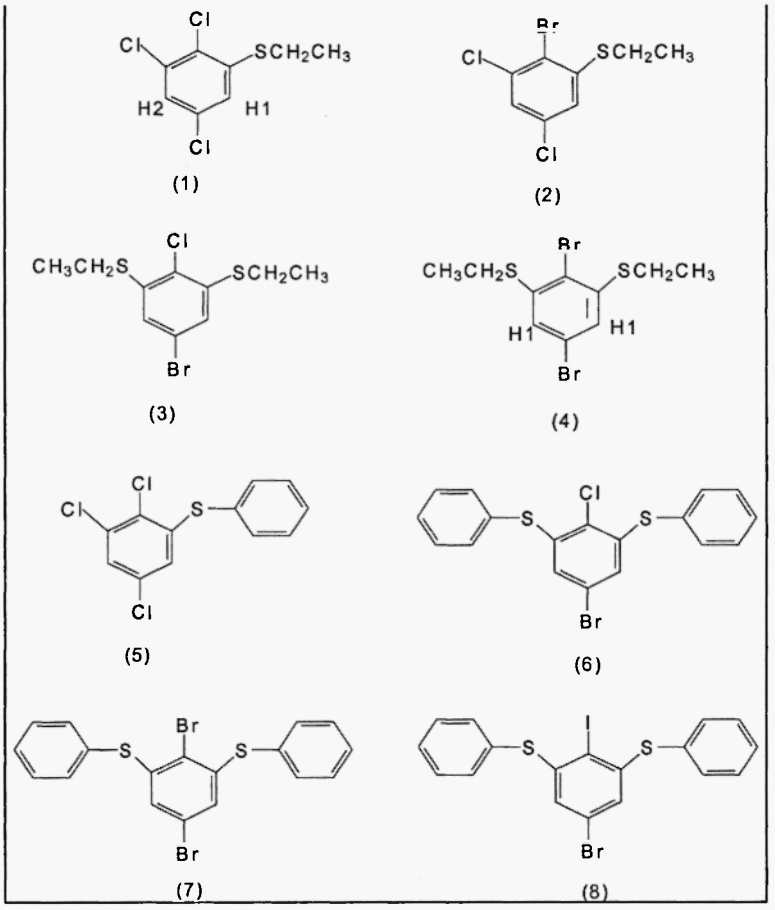
Microtiter plates were incubated for 18-24 h at 37 °C for testing bacteria strains and yeast-like fungus. The MIC values of compounds and standards (ampicillin and flucanazole) are presented in Table II.

Results and Discussions

Synthesis

The thioethoxy- and thiophyenoxyhalobenzenedervatives were 2-tioethoxy-1,4,6-trichloro benzene **(1);** 2-thioethoxy-1-bromo-4-choloro benzene **(2)**; 2,6-dithioethoxy-1-choloro-4-bromo benzene **(3)**; 2,6-dithioethoxy-1,4-dibromo benzene **(4)**; 2-thiophenoxy-1,4,6-tricholoro benzene **(5)**; 2,6-dithiophenoxy-1-choloro-4-bromo benzene **(6);** 2,6-dithiophenoxy-1,4-dibromo benzene **(7)**; 2,4-dithiophenoxy-1-iodo-4-bromo benzene **(8)**. These compounds were prepared from 2,4,6-tribromo and triiodo anilines according to the literature(15). The structures of the compounds prepared were identified with IR and NMR spectra (NMR spectra were recorded on a 100 M Hz spectrometer.). The structures, NMR, IR spectral datas and elemental analysis results of all thiohalogenebenzene derivatives were given in Table-1 and Table-2, Table-3 and Table-4, respectively.

Table-1: The Structures of thioethoxy- and thiophyenoxyhalobenzene derivatives



[2-tioethoxy-1,4,6-trichloro benzene **(1)**;2-tioethoxy-1-bromo-4,6-dichloro benzene **(2)**; 2,6-dithioethoxy-1-bromo-4-choloro benzene **(3)**; 2,6-dithioethoxy-1,4-dibromo benzene **(4)**; 2-thiophenoxy-1,4,6-tricholoro benzene **(5)**; 2,6-dithiophenoxy-1-choloro-4-bromo benzene **(6)**;2,6-dithiophenoxy-1,4-dibromo benzene **(7)**; 2,4-dithiophenoxy-1-iodo-4-bromo benzene **(8)**]

Table-2: NMR spectral data of the thioethoxy- and thiophyenoxyhalobenzenederivatives

Compound → / Peak (ppm) ↓	1	2	3	4	5	6	7	8
Aliphatic -CH_3	1.38	1.38	1.32	1.32				
Aliphatic *CH_2-*	2.9	2.9	2.84	2.84				
Aromatic H1	6.92	6.92	6.09	6.09	6.38	6.46	6.46	6.46
Aromatic H2	7.16	7.16			7.05			
Ar-*S*-C_6H_5					7.20	7.20	7.20	7.20

Table-3: IR spectral data of the thioethoxy- and thiophyenoxyhalobenzenederivatives

Group	Peak cm^{-1}
Ar-S-CH_2-CH_3	1270
Ar-CH-	3060
CH_2.CH_3 (Aliphatic)	2950
C-Cl	790
C-Br	665
C-I	490

Table-4: Elemental analysis of the thioethoxy- and thiophyenoxyhalobenzenederivatives

Compound Number	Formula	Yield %	m.p ^0C	Counted C %	Founded C %	Counted H %	Founded H %
1	$C_8H_7SCl_3$	33.3	46	39.77	40.79	2.92	2.74
2	$C_8H_8SCl_2Br$	Low	52	33.45	33.38	2.78	2.65
3	$C_{10}H_{12}S_2ClBr$	24.6	73	38.53	38.29	3.88	3.93
4	$C_{10}H_{12}S_2Br_2$	21	76	33.72	32.69	3.39	3.48
5	$C_{12}H_7SCl_3$	55.3	81-83	49.74	48.84	2.41	2.28
6	$C_{18}H_{12}S_2ClBr$	83.3	110-114	53.00	52.75	2.94	2.86
7	$C_{18}H_{12}S_2Br_2$	84.8	133	47.78	48.24	2.65	2.54
8	$C_{18}H_{12}S_2lBr$	Low	149	43.29	43.18	2.40	2.35

Antimicrobialactivities

Thioethoxy- and thiophyenoxyhalobenzene dervatives were assayed in vitro for their ability to inhibit the growth of representative bacteria; Gram-positive (*Staphylococcus aureus Bacillus subtilis)* and Gram-negative (*Yersinia enterocolitica, E.coli , K. pneumoniae) and the fungus (Candida albicans).* The susceptibilities of certain strains of bacteria and fungus to the thiohalo benzene dervatives cause the inhibition of a visible growth of the microorganism. The MIC of ampicillin and fluconazole was individually determined in parallel experiments in order to control the sensitivity of the test organisms. MIC values of the compounds and the standards (ampicillin and flucanazole) are presented in Table-5.

Table-5: The Minimum inhibition concentrations (MIC) of the tested compounds*.

Compounds (μg/ml) → Bacteria and fungus ↓	**1**	2	3	4	**5**	**6**	**7**	**8**	**Ampicillin**	**Fluconazole**
S. aureus	256	128	-	-	512	-	-	64	2	-
B. subtilis	256	256	-	-	512	-	-	512	8	-
E. coli	512	512	-	-	1024	-	-	512	4	-
Y.enterocolitica	512	512	-	-	512	-	-	512	8	-
K. pneumoniae	512	512	-	-	1024	-	-	512	64	-
C. albicans	128	64	-	-	64	-	-	32	-	128

*Values are given as μg/ ml for the compounds.

From the results we can say that the compound 1and 2 are more active against to our test bacteria if the bacteria is gram-negative as E.coli and Y. Enterocolitica and K. pneumoniae. Quite suprisingly, compound 3, 4, 6,7 the antibacterial activity is zero against to our test bacteria and fungus. Compound 5 is the most active among to studied compounds against to all of our microorganisms especilly to *K. pneumoniae* and *E.coli* .This means that if the compound has siterik hidered groups like –thioethyl and –thiophenyl, it will cause no activity against to our test bacteria. Compound 8 has the unexpected results in this experiment. Although two -thiophenyl groups over compound 8, antibacterial activity is increasing compared with compounds 6, 7.

Apparently a chloro on the benzene ring is the most active substitutent over our previous work in tetrasubtituebenzene derivatives [Logoglu, E., et. al. Communication (in press)]. In this work seem that still chloro is highly active group over benzene as in the compound 5, 1, 2 if compounds have not many thioethyl and thiophenyl groups .

Compound 8 activity shows diffrentations against different bacteria. Ampicillin antibiotic was found to have more antibacterial activity against to *B. substilis* and *Y. Entorocolitica* and less that than *E. coli and S. aureus*. Floconazole was found to have highly antifungal activity against to *C. Albicans*.

The lower concentration of compounds 2,5 and 8 have more active than fluconazole concentration against to C. Albicans. In future, the interesting results of these chemicals could able to use as a drug row-materials. Further studies with other similar structures would better clarify this issue.

Acknowledgements

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