

ANTIMICROBIAL ACTIVITY OF TETRA SUBSTITUTED BENZENE DERIVATIVES

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ABSTRACT

The *in vitro* antibacterial and antifungal activities of tetrasubstituted benzene derivatives [1-chloro (or bromo or iodo)-2,4,6-trichloro and 1-chloro (or bromo or iodo)-2, 4,6-tribromo and 1-chloro (or bromo or iodo)-2, 4, 6-triiodo benzenes] derived from 2,4,6-trichloro tribromo and triiodo anilines were investigated. Tetrasubstituted benzene derivatives synthesized and identified by spectroscopic means IR and NMR. The antibacterial and antifungal activities were measured by Minimum inhibition concentration (MIC) method against gram-positive bacteria i.e. *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633; Gram-negative ones as *Yersinia enterocolitica* ATCC 1501 *Escherichia coli* ATCC 11230 and fungus as *Candida albicans* from our strain collection. Antimicrobial activities of these compounds tended to decrease with increasing size of halogen substituents.

Keywords: Antimicrobial activity, Tetra-substituted benzene derivatives, MIC, Ampicillin and Fluconazole

1. INTRODUCTION

In the last three decades the environmental impact of halogenated chemicals has become increasingly apparent. Similarly many other haloarenes are important environmental pollutants. The accumulation of organic pollutants in fish

is a matter of especially concern, because fish serve as food for many species including humans¹.

The effects on hexachlorobenzene (HCB) dechlorination of several factors, including electron donors, electron acceptors, and microbial inhibitors (including bromoethane sulfonic acid [BESA], vancomycin, and molybdate-recognized as selective inhibitors of metlianogen, eubactefia, and sulfate-reducing bacteria, respectively². Polychlorinated benzenes (PcBzs) are substances widely used in industry. They are used as solvents and starting materials or intermediates in the synthesis of many other substances e. g. phenols, dyestuffs in chemical industry and as pesticides and fungicides in agriculture. Hexachloro benzene (HcBz) has many uses in industry e. g. as a plasticizer for PVC as a fungicide in agriculture³. Hexachloro benzene may enter into the human diet via the food chain or respiration. The hexachlorocyclohexanes (HCHs) constitute 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 areas⁴.

Hexabromobenzene and its metabolites are present in water, fish, birds, sediments and human tissues. Among polybromobenzenes hexabromobenzene has been used most widely. The products that result from the hexabromobenzene debromination (penta-, tetra-, tribromobenzenes) are formed by means of environmental degradation or through metabolisms of various organisms⁵⁻⁷. They are more volatile and water soluble than the parent compound. Dibromobenzenes found in natural environments mainly originates from their use as fumigants, additives to cleaning agents, or as intermediates in the production of pharmaceutical preparations⁸. In mammals, polybrominated biphenyls cause loss of weight, chloracne, edema, hepatic hypertrophy, porphyria, estrogenic activity and immunosuppression⁹.

For hepatocarcinogenicity, As to the methods of determination, some scientist focus sed the on the bioassay to assess the concentration of 1,2,4,5-tetrachlorobenzene and 1,4-dichlorobenzene using a medium-term liver¹⁰⁻¹¹.

In this work, we report on the synthesis of tetrasubstituted benzene derivatives and on the biological activities of these compounds against *S. aureus*, *B. subtilis*, *Y. enterocolitica*, *E. coli* and *C. albicans*.

2. RESULTS AND DISCUSSIONS

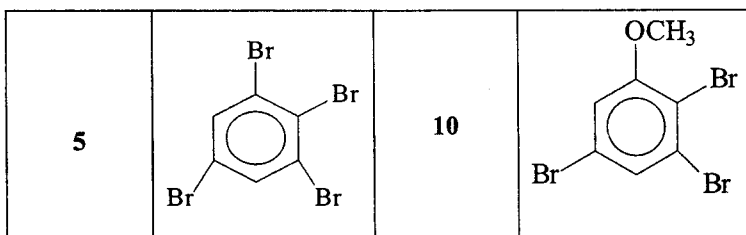
Synthesis of tetrasubstitued benzene derivatives

The ten tetrasubstitued benzene derivatives were 1,2,3,5-Tetrachlorobenzene (1); 1,3,5-trichloro-2-bromobenzene (2); 1,3,5-trichloro-2-iodobenzene (3); 1,3,5-tribromo-2-chlorobenzene (4); 1,2,3,5-Tetrabromobenzene (5); 1,3,5-tribromo-

2-iodobenzene (6); 2,3,5-trichloro anisole (7); 2-bromo-3,5-dichloro anisole (8); 2-chloro-3,5-dibromo anisole (9); 2,3,5-tribromo anisole (10)¹². These compounds were prepared from 2,4,6-trichloro, tribromo and triiodo anilines according to the methods given in literature¹³. The structures of all tetrasubstituted benzene derivatives were given in Table 1. The structures of the compounds prepared were identified with IR and NMR. (NMR spectra were recorded on a 100 M Hz spectrometer.)

Table 1: The structures of ten tetrasubstituted benzene derivatives

Compound No	Structures	Compound No	Structures
1		6	
2		7	
3		8	
4		9	



Antimicrobial activity

Tetrasubstitued benzene derivatives were assayed in vitro for their ability to inhibit the growth of representative Gram-positive (*Staphylococcus aureus* *Bacillus subtilis*) and Gram- negative (*Yersinia enterocolitica*, *E.coli*) bacteria and the fungus (*Candida albicans*). The susceptibilities of certain strains of bacteria and fungus to the tetrasubstitue benzene derivatives 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 are presented in Table II.

Table II. Antimicrobial activity of the compounds

Comp	Bacteria (MIC ^a)				Fungus (MIC ^a)
	S. aureus	B. subtilis	Y. enterocolitica	E. coli	C. albicans
	ATCC25923	ATCC 6633	ATCC 1501	ATCC11230	
1	256	256	256	256	128
2	256	256	128	256	256

3	256	256	256	256	256
4	512	256	128	256	256
5	512	256	256	512	512
6	Na ^b	Na	Na	Na	Na
7	256	256	256	256	256
8	512	Na	Na	Na	256
9	Na	Na	Na	Na	Na
10	Na	Na	Na	Na	Na
Ampicilin	2	8	8	4	Nt ^c
Fluconazole	Nt	Nt	Nt	Nt	128

^a MIC, minimum inhibitory concentration. Values are given as $\mu\text{g/ml}$ for the compounds,

^b Na, not active, ^c Nt, not tested

From the results we can say that the bromo substituent seem to contribute to the activity against our test bacteria. Bromo and iodo substituents seem to cause a decrease in the activity if these are present together. Quite suprisingly the antibacterial activity is almost zero. Apparently a chloro on the benzene ring is the most active substituent and if one of chloro substituents, in polychlorobenzenes, is replaced by another halogene the activity does not change dramatically.

If iodo is together with chloro as in compound 3, the compound has better activity compared to the bromo-iodo combination. as in compound 6.

Methoxy group is an activity decreasing group. If we consider compound 8 and 9 antibacterial and fungal activity differenci comes from again bromo and chloro substituent. For compound 8 activity shows highly differentiation against different bacteria. Further studies with other similar structures would better clarify this issue.

Ampicillin antibiotic was found to have more antibacterial activity against *B. subtilis* and *Y. Enterocolitica* and less against to than *E. coli* and *S. aureus*. Floconazole was found to have highly antifungal activity against to *C. Albicans*.

3. EXPERIMENTAL

Synthesis of Chemicals

The tetrasubstituted benzene derivatives were prepared using well-known procedures¹². Tetrasubstitue benzene derivatives, 1-chloro (or bromo or iodo)-2,4,6-trichloro-1-chloro (or bromo or iodo)-2, 4,6-tribromo- and 1-chloro (or bromo or iodo)-2, 4, 6-triiodo benzenes were synthesized for trial. 2,4,6-trichloro tribromo and triiodo anilines were used as the starting material and the procedures were carried out according to the methods given in literature¹³.

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 obtained from İzzet Baysal University, Biology Department. *Candida albicans* was from our strain collection . 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) for antibacterial and antifungal activity tests. Test strains were suspended in MHB to give a final density of 5×10^5 cfu/ ml.

Method

Minimum inhibitory concentrations (MICs) were determined by macrodilution broth method following the procedures recommended by the National Committee for Clinical Laboratory Standards¹⁴. MICs were defined as the lowest concentrations 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 6633 and two gram-negative *Yersinia enterocolitica* ATCC 1501 and *Escherichia coli* ATCC 11230 bacteria were used as test bacteria. For testing antifungal activity of the compounds were used *Candida albicans*¹⁵.

All tests were performed in Mueller-Hinton Broth (MHB). The compounds under the test were dissolved in analytically pure dimethylsulphoxide (DMSO) and geometric dilutions ranging from 0.5 µg/ ml to 512 µg/ ml of the compounds were

prepared including one growth control and one sterility control. Tubes were incubated for 24 h at 37 °C for the bacteria and for 48 h at 30 °C for the yeast. The MIC of ampicillin and fluconazole was individually determined in parallel experiments in order to control the sensitivity of the test organisms.

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ÖZET

2,4,6-triklor tribrom and triiyot anilinlerden sentezlenmiş olan [1-klor (veya brom veya iyot)-2,4,6-triklor ve 1-klor (veya brom veya iyot)-2, 4,6-tribrom ve 1-klor (veya brom veya iyot)-2, 4, 6-triyot benzen] gibi tetra substitue benzen türevlerinin *in vitro* antibakteriyel ve antifungal özellikleri incelenmiştir.

Tetrasubstitue benzen türevleri sentezlenmiş, IR ve NMR gibi spektroskopik yöntemlerle yapıları aydınlatılmıştır. Antibakteriyel ve antifungal aktiviteler; Minimum inhibisyon konsantrasyonu (MIC) metodu ile gram-pozitif bakteriler (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633) ve gram-negatif bakteriler (*Yersinia enterocolitica* ATCC 1501 *Escherichia coli* ATCC 11230) ve kendi koleksiyonumuzdan olan maya (*Candida albicans*) bakterilerine karşı ölçülmüştür. Bu bileşiklerde halojen substitüentin büyüklüğü arttıkça antimikrobiyal aktivite azalmaktadır.

REFERENCES

1. Lu, X., Tao, S., Hu, H., Dawson, R-W. Chemosphere 41 1675-1688, 2000.
2. Chang, BV., Su, CJ., Yuan SY. Chemosphere, Vol, 36, No:13, pp. 2721-2730, 1998.
3. Nowakowska, M., Szczubialka, K. Chemosphere, 39, 1, 71-80 1999.
4. Otero, R., Santiago-Silva, M., Grimalt, O. Joan. Journal of Chromatography A 778, 87-94, 1997.
5. Watanabe, I., Tatsukawa, R. Proceedings, Workshop on Brominated Aromatic Flame Reterdants, Skokloster, Sweden, pp.63-71. 1989.
6. Kashimoto, T and Tatsukawa, R. Bull Environ Contam. Toxicol 36, 778-784, 1986.
7. de Roode, D. F., Klomp, A. V.et. al.Environmental Toxicology and Pharmacology 12147-156, 2002.
8. Colacci, A., Bartoli, S., Bonova, B. et al. Toxicol Lett 54: 121, 1990.
9. Freeman, P. K., Haugen, M. C. J. Chem. Technol. Biotechnol. 72, 45-49, 1998.

10. Gustafson D. L., Coulson, A. L., Feng, L., Pott W. A., Thomas, R. S., Chubb, L. S., Saghir, S. A., Benjamin, S. A., and Yang, R. S. H., *Cancer Letters*, Volume 129, Issue 1, Pages 39-44, 3 July 1998.
11. Great lakes fishery Commissions Technical report No: 11 The relation between molecular structure and biological activity among mononitrofenols containing halogens p: 9-19 1451 Green road Ann arbor, michigan December, 1966.
12. Tüzün, C., and Öktemer, A. *Communication de la Faculte des Sciences de L'Universite d'Ankara*, Tome 25, Anne 1979.
13. Hodgson, H. H., and Mahadeven,, A. P. *J. Chem. Soc*, 173-4, 1974.
14. NCCLS (National Committee for Clinical Laboratory Standards), *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*, Approved Standard, M7- A4, Wayne, Pa. 1997.
15. NCCLS (National Committee for Clinical Laboratory Standards), *Reference method for broth dilution antifungal susceptibility testing of yeasts*, Approved Standard, M27, 1997.