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# Synthesis and Biological Activity Studies of Di- and Mono-halogenofluoro Benzenes

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In this work, di- and mono-halogenofluorobenzene dervatives synthesized and identified by spectroscopic means $^{1}H$ NMR and $^{19}F$ NMR. Di- and mono-halogenofluorobenzene dervatives were 2-fluoro-3-chlorobromobenzene (**1**), 1,3-dibromo-2-fluorobenzene (**2**), 2-fluoro-3-iodobromobenzene (**3**), 2-bromofluorobenzene (**4**), 2-iodofluorobenzene (**5**), 2-chlorofluorobenzene (**6**). The antibacterial and antifungal activities of di- and mono- halogenofluorobenzene derivatives studied against bacteria *i.e. Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 6633, *Morganella morganii* (clinical isolate), *Escherichia coli* ATCC 27853, *Micrococcus flavus* (clinical isolate), *Candida albicans* (clinical isolate). To measure zone diameter, the antibacterial and antifungal activities were measured by Minumum Inhibition Concentration (MIC) method and disc-diffusion method used against to that gram-positive and gram-negative bacteria and fungus. All the bacteria and fungus studied against to antibiotics like ketoconazole, ampicillin, tetracycline, penicillin, chloramphenicol and oxacillin to compare with our chemicals zone diameters.

**Key Words: Di- and mono-halogenofluoro benzenes, Antimicrobial activity, MIC.**

## INTRODUCTION

Halogenated organic compounds are frequently employed as solvents, pesticides and refrigerants and for the study of electron transfer reactions in radiation and electrochemical investigations[1-6]. These compounds are toxic in nature if metabolized in living species[7-9]. The more using of halogenated organic compounds have toxicological and environmental implications[10,11].

The products obtained from effects on hexachlorobenzenes by dechlorination depends on several factors, including electron donors, electron

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acceptors and microbial inhibitors (*i.e.* bromoethane sulfonic acid, vancomycin and molybdate-recognized) as selective inhibitors of metlianogen and sulfate-reducing bacteria, respectively[12]. Polychlorinated benzenes 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. Hexachlorobenzenes have many uses in industries like plasticizer for PVC and fungicide in agriculture[13].

Some scientists focussed the on the bioassay to assess the concentration of 1,2,4,5-tetrachlorobenzene and 1,4-dichlorobenzene using a medium-term liver for hepatocarcinogenicity[14,15].

Among polybromobenzenes, hexabromobenzene has been used most widely. The products that result from the hexabromobenzene by debromination (penta-, tetra- and tribromobenzenes) are formed by means of environmental degradation or through metabolism of various organisms[16-18]. Dibromobenzenes found in natural environments mainly originates from their use as fumigants, additives to cleaning agents or as intermediates in the production of pharmaceutical preparations[19].

The antimicrobial and antifungal activities of 2,4-dihalogenofluoro benzenes and tetrasubstituted benzenes and piperidine and pyrrolidine substituted halogenobenzenes were studied against to some bacteria and fungus[20-22].

Further studies with a wider range of compounds would need to be under taken to establish the importance of the different functional groups on the benzene skeleton to structure-activity relationship of the compounds.

## EXPERIMENTAL

The di- and mono-halogenofluoro benzenes were 2-fluoro-3-chlorobromobenzene (**1**) [yield: 39 %; b.p.: 190ºC, 1,3-dibromo-2-fluorobenzene (**2**) [yield: 38 %; m.p.: 42-45ºC], 2-fluoro-3-iodobromobenzene (**3**) [yield: 30 %, m.p.: 53-55ºC], 2-bromofluorobenzene (**4**) [yield: 19 %, b.p.:155ºC], 2-iodofluorobenzene (**5**) [yield: 25 %, b.p.: 188ºC], 2-chlorofluorobenzene (**6**) [yield: 36 %, b.p.: 137ºC].

**Syntheses of 2,4-dihalogenoanilines:** These compounds were prepared from aniline according to the methods given in literature[23]. Aniline, conc. HCl and water in flask (500 mL) stirred until aniline was dissolved in solvent. Acetic anhydride was added over solutions which aniline-hydrochloride is formed during stirring in flask and the mixture was stirred and followed by the addition of sodium acetate. This mixture was cooled while stirring. The synthesized crude compound was recrystallized with acetanilide. The halogen gas was flushed to this mixture in order to obtain 2,4-dihalogeno anilines.

**Schieman reactions of 2,4-dihalogenoanilins:** 2,4-Dihalogeno aniline was reacted with $NaNO_2$ and $NaBF_4$ to prepare 2,4-dihalogeno-

benzenediazonium tetrafluoroborate compounds. 2,4-dihalogenobenzenediazonium tetrafluoroborate compounds give reaction to form di- and monohalogenofluoro benzene compounds at 150-170ºC.

The general reaction scheme of all of 2,4-dihalogenanilines and their Scheiman reactions were given in Fig. 1.

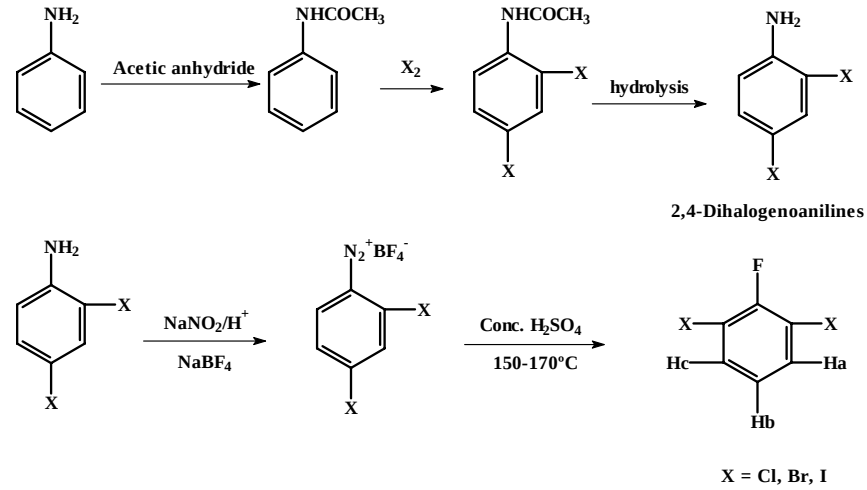



Fig. 1. Di- and mono-halogenofluoro benzenes derivatives

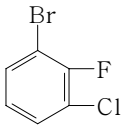
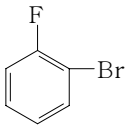
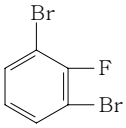
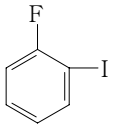
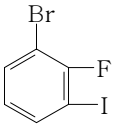
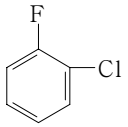
The bacterial subcultures for *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 6633, *Morganella morganii* (clinical isolate), *Escherichia coli* ATCC 27853, *Micrococcus flavus* (clinical isolate), *Candida albicans* (clinical isolate) were obtained from Biology Department of Pamukkale and Gazi University. Bacterial strains were cultured overnight at 37°C in Nutrient Broth and the yeast were cultured overnight at 30°C in YEPD Broth for antibacterial and antifungal activity tests. These stock cultures were stored in the dark at 4°C during the survey.

**Determination of MIC's:** Minimum inhibitory concentrations (MIC's) were determined by microdilution broth method following the procedures recommended by the National Committee for Clinical Laboratory Standards[24,25].

All the tests were performed in Mueller-Hinton Broth (MHB) and YEPD Broth. The compounds under the test were dissolved in AR-grade DMSO and geometric dilutions ranging from 300 µg/mL to 3000 µg/mL of the compounds.

**Screening of antimicrobial activity:** Antimicrobial activity of compounds was determined by the disc diffusion method[26,27]. The antimicrobial screening was performed using Mueller-Hinton Agar and YEPD Agar for a yeast. The culture suspensions were prepared and adjusted by comparing against 0.3 Mc Farland turbidity standard tubes.

TABLE-1
STRUCTURES OF DI- AND MONO-HALOGENOFLUORO BENZENE DERIVATIVES

| Compd. No. | Structures | Compd. No. | Structures |
|---|---|---|---|
| **I** |  2-Fluoro-3-chlorobromobenzene | **IV** |  2-Bromofluorobenzene |
| **II** |  1,3-Dibromo-2-fluorobenzene | **V** |  2-Iodofluorobenzene |
| **III** |  2-Fluoro-3-iodobromobenzene | **VI** |  2-Chlorofluorobenzene |

Muller- Hinton and YEPD agar (20 mL) were poured into each sterile petri dish after injecting cultures (100 µL) of microorganisms and distributing medium in petri dish homogeneously. Compounds were filtered with a pore size of 0.45 µm. All the compounds were dissolved in DMSO of 5 mg/mL. Empty sterilized discs of 6 mm (Schleicher and Schuell, No. 2668, Germany) were each impregnated with 50 mL of compounds. Discs were placed on agar plates, and the plates were incubated at 37ºC for 24 h for bacteria and 48 h for *C. albicans*. Inhibition zones formed on the medium were evaluated in mm. The solvent control (DMSO) did not show any antimicrobial activity. Studies performed in duplicate and the inhibition zones were compared with those of reference discs. Reference discs used for control are as follows: ketoconazole (50 µg), ampicillin (10 µg), tetracycline (30 µg), penicillin (10 U), chloramphenicol (30 µg) and oxacillin (1 µg).

**Statistical analysis:** Data were analyzed and treatments compared using the one-way ANOVA with 95% confidence limits ($p < 0.05$) (SPPS 9.0 for windows).

## RESULTS AND DISCUSSION

Di- and mono-halogenofluoro benzene derivatives (Table-1) synthesized and identified by $^{1}H$ NMR and $^{19}F$ NMR. Spectrum of compounds

were obtained from Bruker-Spectrospin Avance DPX 400 Ultra-Shield in Tubitak - The Scientific and Technological Research Council of Turkey and Middle East Technical University Analysis Laboratory.

**2-Fluoro-3-chlorobormobenzene:** JHa-Hb: *ortho*, 9.23 Hz , JHa-F: *meta* 4.45 , JHa-Hc: *meta* 1.54 Ha:multiplet, 7.4 ppm, JHb-Ha: 9.1 Hz, JHb-Hc; *ortho* 8.02 Hz, JHb-F: 1.12 Hz, Hb: multiplet, 6.9 ppm: JHc-Ha: *meta*, 1.53 Hz, JHc-Hb: *ortho* 7.94 Hz , JHc-F: 5.94 Hz , Hc: 7.7 ppm; $^{19}$F NMR: F: -93.6 ppm.

**1,3-Dibromo-2-fluorobenzene:** JHa-Hb: *ortho*, 8.12 Hz , JHa-F: *meta* 6.52 Hz, Ha and Hc are the equal protones: multiplet, 7.25 ppm, JHb-Ha: *ortho* 8.9 Hz, JHb-Hc; *ortho* 8.9 Hz , JHb-F: 1.6 Hz, Hb: multiplet 6.95 ppm.

**2-Fluoro-3-iodobromobenzene:** JHa-Hb: *ortho*, 7.45 Hz , JHa-F: *meta*, 5.5 Hz : JHa-Hc: *meta* 1.43 Hz Ha: 7.6 ppm, JHb-Ha: *ortho*, 7.99 Hz, JHb-Hc; *ortho* 7.99 Hz, Hb: triplet, 6.85 ppm, JHc-Hb: *ortho*, 8.11 Hz , JHc-F: *meta*, 6.69 Hz, Hc: multiplet, 7.4 ppm.

**2-Bromofluorobenzene:** JHa-Hb: *ortho* 8.33 Hz , JHa-F: *meta* 7.06 Hz, JHa-Hc; *meta* 1.64 Hz Ha: 7.6 ppm, JHb-Ha: *ortho*, 8.32 Hz, JHb-Hc; *ortho* 7.75 Hz, JHb-Hd: *meta*, 1.37 Hz, Hb: 7.05 ppm, JHc-Ha: *meta* 1.62 Hz, JHc-Hb: *ortho*, 7.56 Hz , JHc-Hd: *ortho*, 9.76 Hz, JHc-F: *ortho*, 4.96 Hz, Hc: 7.3 ppm, JHd-Ha: *para*, 1.46 Hz, JHd-Hc: *ortho*, 8.60 Hz, Jdc-F: *ortho*, 9.30 Hz, Hd: multiplet, 7.2 ppm $^{19}$F NMR: F: -107.1 ppm.

**2-Iodofluorobenzene:** JHa-Hb: *ortho* 7.22 Hz , JHa-F: *meta* 6.55 Hz, JHa-Hc; *meta* 1.51 Hz Ha: multiplet, 7.8 ppm, JHb-Ha: *ortho*, 8.27 Hz, JHb-Hc; *ortho* 7.84 Hz, JHb-Hd: *meta*, 1.18 Hz, Hb: multiplet 6.95 ppm, JHc-Ha: *meta* 1.54 Hz, JHc-Hb: *ortho*, 7.52 Hz, JHc-Hd: *ortho*, 8.23 Hz, JHc-F: *meta*, 5.18 Hz, Hc: multiplet, 7.35 ppm, JHd-Hc: *ortho*, 8.21 Hz, JHd-F: *ortho*, 8.79 Hz, Hd: multiplet, 7.1 ppm.

**2-Chlorofluorobenzene:** JHa-Hb: *ortho* 8.56 Hz, JHa-F: *meta* 7.76 Hz, JHa-Hc; *meta* 1.68 Hz Ha: multiplet, 7.45 ppm, JHb-Ha: *ortho*, 8.73 Hz, JHb-Hc; *ortho* 8.45 Hz, JHb-Hd: *meta*, 1.43 Hz, JHb-F: 0.58 Hz, Hb: multiplet 7.15 ppm, JHc-Ha: *meta* 1.67 Hz, JHc-Hb: *ortho*, 7.27 Hz, JHc-Hd: *ortho*, 9.89 Hz, JHc-F: *meta*, 4.87 Hz, Hc: multiplet, 7.3 ppm, JHd-Hb: *meta*, 1.55 Hz, JHd-Hc= *ortho*, 8.34 Hz, JHd-F: *ortho*, 9.00 Hz, Hd: multiplet, 7.11 ppm, $^{19}$F NMR: F: -115.4 ppm.

In the present studies, the antimicrobial activities of compounds were determined and given Tables 2 and 3. According to Table-2, the activity was showed in terms of the minimum concentration (MIC) inhibiting the microbial growth. All the compounds except compound **VI** were showed antibacterial activity (Table-3) against *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 6633, *Morganella morganii* (clinical isolate), *Escherichia coli* ATCC 27853, *Micrococcus flavus* (clinical isolate) and

*Candida albicans* (clinical isolate) and were found to be more effective on tested Gram-positive bacteria. Although *E. coli* and *M. morgani* were the most resistant gram-negative bacteria, we had determined that the tested bacteria were sensitive to compound **I**, **III**, **IV** and **V**. These also showed antifungal activity against to *Candida albicans* at the same concentration (Table-2).

TABLE-2
MIC'S OF THE TESTED COMPOUNDS

| Compd. | MIC (µg/mL) | | | | | |
|---|---|---|---|---|---|---|
| | *E. coli* ATCC27853 | *M. morgani* (clinical isolate) | *M. flavus* (clinical isolate) | *St. aureus* ATCC 25923 | *B. cereus* ATCC 6633 | *C. albicans* (clinical isolate) |
| **I** | 2000 | 2000 | 1200 | 2000 | 2500 | 2500 |
| **II** | 2000 | 2000 | 1200 | 2500 | 2000 | 2500 |
| **III** | 2000 | 2000 | 1000 | 1500 | 2500 | 2500 |
| **IV** | 2500 | 2500 | 1500 | 2500 | 2500 | 2500 |
| **V** | 2500 | 2500 | 1100 | 2500 | 2500 | 2500 |
| **VI** | 2500 | 2500 | 1500 | 2500 | 2500 | 2500 |

TABLE-3
INHIBITION ZONES DIAMETERS OF COMPOUNDS AND REFERENCE ANTIBIOTICS DISCS AGAINST THE TEST MICROORGANISMS

| Compd. | Inhibition zone (mm) | | | | | |
|---|---|---|---|---|---|---|
| | *E. coli* ATCC27853 | *M. morgani* (clinical isolate) | *M. flavus* (clinical isolate) | *S. aureus* ATCC 25923 | *B. cereus* ATCC 6633 | *C. albicans* (clinical isolate) |
| **I** | 15 | 15 | 21 | 13 | 14 | 12 |
| **II** | - | - | 30 | - | 10 | 19 |
| **III** | 12 | 12 | 15 | 15 | 12 | 17 |
| **IV** | 5 | 10 | - | - | - | - |
| **V** | - | 12 | 19 | - | 14 | 20 |
| **VI** | - | - | - | - | - | - |
| Penicillin | 19 | - | - | - | - | - |
| Chloramphenicol | - | 15 | - | - | - | - |
| Tetracycline | - | - | 20 | - | - | - |
| Ampicillin | - | - | - | 9 | - | - |
| Oxacillin | - | - | - | - | 9 | - |
| Ketoconazole | - | - | - | - | - | 16 |

In this work, the activities of compounds varied because of different substituent like -Br, -Cl and -I together with fluoro over the benzene skeleton.

Compared to reference discs, the compound **I** and penicillin have showed same activity against to *E. coli*, compound **II** and chloramphenicol have showed same activity against *M. morganii*. On the otherside, compound **III**, **IV**, **V** were more active against to *M. flavus, S. aureus, B. cereus* and *C. albicans* than reference antibiotics (tetracycline, ampicillin,

oxacillin, ketoconazole) and compound **VI** were not active against all test micro-organisms.

In present studies, antimicrobial screening clearly indicate that the present compounds show more antibacterial activities for gram-positive bacteria and yeast than gram-negative bacteria. At the same time, if we evaluate as statistical effects of these compounds on the test microorganisms, it was found significantly different activity against to test microorganisms ($F = 5.004$, $df = 5$, $p < 0.05$).

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