

## Antimicrobial activity studies on some piperidine and pyrrolidine substituted halogenobenzene derivatives

SEZA ARSLAN<sup>1</sup>, ELİF LOĞOĞLU<sup>2</sup>, & ATILLA ÖKTEMER<sup>3</sup>

<sup>1</sup>Abant İzzet Baysal University, Faculty of Arts and Sciences, Department of Biology, 14280, Gököy/Bolu, Turkey, <sup>2</sup>Gazi University, Faculty of Arts and Sciences, Department of Chemistry, 06500, Teknikokullar/Ankara, Turkey, and <sup>3</sup>Ankara University, Science Faculty, Department of Chemistry, 06100, Tandoğan/Ankara, Turkey

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### Abstract

The in vitro antibacterial and antifungal activities of the compounds synthesised from some 1,2,3,5-tetrahalogeno benzenes in presence of sodium piperidide and sodium pyrrolidide (2,6-dipiperidino-1,4-dihalogenobenzenes; 2,6-dipyrrolidino-1,4-dibromobenzene; 2,4,6-tripyrrolidino chlorobenzene; and 1,3-dipyrrolidino benzene) were investigated. The in vitro antimicrobial activities were screened against the standard strains: *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633 as Gram positive, *Yersinia enterocolitica* ATCC 1501, *Escherichia coli* ATCC 11230 and *Klebsiella pneumoniae* as Gram negative, and *Candida albicans* as yeast-like fungus. Compounds (3, 5, 6, 7) inhibited the growth of all the test strains at MIC values of 32–512 µg/ml. None of the four compounds (1, 2, 4, 8) studied showed antimicrobial activity against any of the test strains within the MIC range 0.25–512 µg/ml.

**Keywords:** Antimicrobial activity, Piperidine derivatives, Pyrrolidine derivatives, Halogeno benzenes, MIC

### Introduction

Piperidine, an alicyclic base is used as a raw material for pharmaceuticals, agrochemicals, rubber chemicals, surface active agents and other organic chemicals. Pyrrolidine, a similar base found in tobacco leaves with a strong piperidine-like odor provides derivatives used in pharmaceuticals, especially as modifiers of quinolone antibacterial agents [1,2]. Numerous biological compounds possess pyrrolidine and piperidine rings in their framework, and some of them are pharmaceutically important [3,4]. In the last several decades, halogenated aromatic hydrocarbons have been used extensively as intermediates in the chemical industry and agriculture. Some of these chemicals are carcinogenic and have deleterious effects on organisms [5,6].

Pharmaceuticals have a history of success in controlling morbidity and mortality caused by infectious diseases but as a consequence of frequent

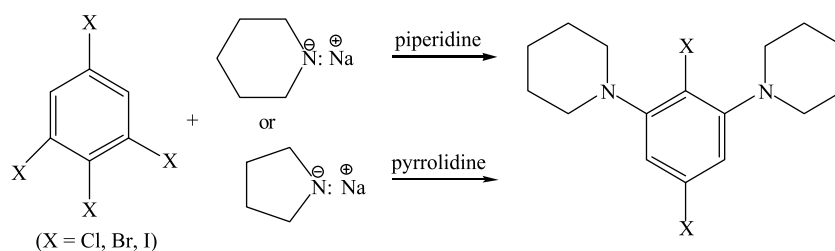
use, bacterial resistance to known classes of antibiotics has become a severe global problem in recent years [7,8]. There are serious concerns that untreatable pathogens may develop at an alarming rate in the near future. In this respect, we screened the antimicrobial activities of some piperidine and pyrrolidine substituted halogenobenzene derivatives.

### Materials and methods

#### Synthesis

The tetrasubstituted benzene derivatives were prepared using well-known procedures [9]. Tetrasubstituted 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 as reactants. 2,4,6-trichloro, tribromo and triiodo anilines were used as the starting material and the procedures were

Correspondence: Seza Arslan, Ph.D., Abant İzzet Baysal University, Faculty of Arts and Sciences, Department of Biology, 14280, Gököy/Bolu, Turkey. Tel: + 903742534511. Fax: + 903742534642. E-mail: arslan\_s3@ibu.edu.tr



Scheme 1. Synthesis of the compounds.

carried out according to the methods given in literature. In nucleophilic substitution experiments, the sodium salts of piperidine and pyrrolidine were prepared with sodium amide and the reactions of these derivatives were successful [9].

#### Microbiological assays

Minimum inhibitory concentrations (MICs) were determined by broth microdilution following the procedures recommended by the National Committee for Clinical Laboratory Standards [10,11]. All the compounds were tested for their *in vitro* growth inhibitory activity against *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633 as Gram positive bacteria, *Yersinia enterocolitica* ATCC 1501, *Escherichia coli* ATCC 11230 and *Klebsiella pneumoniae* as Gram negative bacteria and *Candida albicans* as a yeast-like fungus. *K. pneumoniae* and *C. albicans* were obtained from the Department of Microbiology, Faculty of Medicine, Abant Izzet 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 was cultured overnight at 30°C in Sabouraud Dextrose Broth (SDB). Test strains were suspended in MHB to give a final density of  $5 \times 10^5$  cfu/ml. The compounds under test were dissolved in 100% DMSO and the final twofold dilutions gave concentrations from 1–512 µg/ml. Ampicillin and fluconazole were used as reference antibiotics for the bacteria and fungus, respectively 0.25–512 µg/ml. Microtiter plates were incubated for 18–24 h at 37°C for the bacterial tests and for the yeast-like fungus, MICs were determined after incubation for 48 h at 30°C. The lowest concentration of the compounds that prevented visible growth was considered to be the minimal inhibitory concentration (MIC). MIC values for the compounds and the standards are presented in Table IV.

## Results and discussion

### Synthesis

The compounds synthesised were 2,6-Dipiperidino-4-chlorobromobenzene (1); 2,6-Dipiperidino-4-

chloriodobenzene (2); 2,6-Dipiperidino-4-bromochlorobenzene (3); 2,6-Dipiperidino-1,4-dibromobenzene (4); 2,6-Dipiperidino-4-bromiodobenzene (5); 2,4,6-Tripyrrolidinochlorobenzene (6); 2,6-Dipyrrolidino-1,4-dibromobenzene (7); 1,3-Dipyrrolidinobenzene (8) [9] (Scheme 1). These compounds were prepared from sodium piperidide and sodium pyrrolidide and 2,4,6-trichloro-, tribromo- and triiodoanilines according to the literature methods [9]. The structures of all the tetrasubstituted benzene derivatives are given in Table II and were confirmed by IR, NMR (100 M Hz spectrometer) and elemental analysis (Tables I, II and III).

### Antimicrobial activity

The *in vitro* antibacterial screening of the compounds against *S. aureus*, *B. subtilis*, *Y. enterocolitica*, *E. coli* and *K. pneumoniae* and the antifungal screening against *C. albicans* was carried out by the minimum inhibitory concentration method [10,11]. The antimicrobial activities (MIC values) are given in Table IV, which also contains those for ampicillin and fluconazole used as reference compounds as comparison and a check on the reliability of the method used.

In this study, eight derivatives were tested against standard strains of gram-positive bacteria, gram-negative bacteria and fungus. Compounds 3, 5, 6 and 7 inhibited the growth of all microorganisms, include fungus, with MICs 32–512 µg/ml (Table IV). Among the tested compounds, 5, 6 and 7 showed good antifungal activity against *C. albicans* with MICs 32–64 µg/ml, which was comparable with that for fluconazole (128 µg/ml). Four compounds (1, 2, 4, 8) did not show antimicrobial activity against any of the test strains within the range MICs 0.25–512 µg/ml. The inhibitory compounds had the

Table I. IR spectral data for the compounds.

| Bond                       | Peak (cm <sup>-1</sup> ) |
|----------------------------|--------------------------|
| Aromatic C-H               | 3025                     |
| Alifatic C-H               | 2940                     |
| Aromatic primary Amine C-N | 1340                     |
| Halogen C-Cl               | 790                      |
| Halogen C-Br               | 665                      |
| Halogen C-I                | 490                      |

Table II. NMR spectral data of the Dipiperidino- and Pyrrolidinobenzenes.

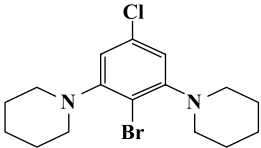
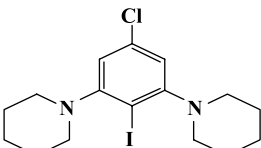
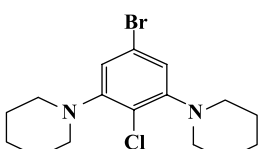
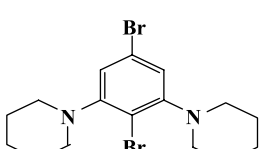
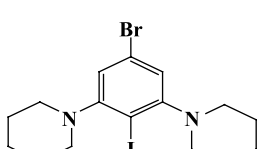
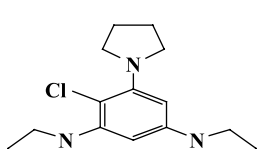
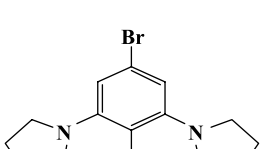
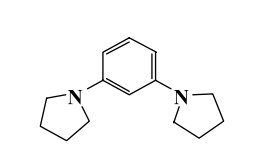
| Compounds | Structures  | Group peaks (ppm)  |                      |                        |                         |                        |
|-----------|---|--------------------|----------------------|------------------------|-------------------------|------------------------|
|           |   | -CH <sub>2</sub> - | -CH <sub>2</sub> -N- | Aromatic               |                         |                        |
|           |   |                    |                      | N-CH <sub>(1)</sub> -N | N-CH <sub>(2)</sub> -CH | N-CH-CH <sub>(3)</sub> |
| 1         |    | 1.58               | 3.0                  |                        | 5.9                     |                        |
| 2         |    | 1.53               | 3.0                  |                        | 5.9                     |                        |
| 3         |    | 1.58               | 3.0                  |                        | 5.9                     |                        |
| 4         |   | 1.58               | 3.0                  |                        | 5.9                     |                        |
| 5         |  | 1.58               | 3.0                  |                        | 5.9                     |                        |
| 6         |  | 1.94               | 3.24                 |                        | 5.1                     |                        |
| 7         |  | 1.94               | 3.2                  |                        | 5.0                     |                        |
| 8         |  | 1.90               | 3.2                  | (H1) 5.5               | (H2) 5.74               | (H3) 6.79              |

Table III. Physical and chemical data for the compounds.

| Comp. No | Empirical formula  | Found %C | Calc. %C | Found %H | Calc. %H | Yield (%) | M.p. (°C) |
|----------|--|----------|----------|----------|----------|-----------|-----------|
| 1        | C <sub>16</sub> H <sub>22</sub> BrClN <sub>2</sub>             | 53.37    | 53.70    | 6.12     | 6.15     | 37.8      | 179–182   |
| 2        | C <sub>16</sub> H <sub>22</sub> IClN <sub>2</sub>              | 47.23    | 47.46    | 5.28     | 5.43     | 20        | 170–173   |
| 3        | C <sub>16</sub> H <sub>22</sub> BrClN <sub>2</sub>             | 53.55    | 53.70    | 5.99     | 6.15     | 37.8      | 170–172   |
| 4        | C <sub>16</sub> H <sub>22</sub> Br <sub>2</sub> N <sub>2</sub> | 47.36    | 47.76    | 5.33     | 5.47     | 35        | 180–183   |
| 5        | C <sub>16</sub> H <sub>22</sub> IBrN <sub>2</sub>              | 42.35    | 42.76    | 4.78     | 4.89     | 26.6      | 177       |
| 6        | C <sub>18</sub> H <sub>26</sub> ClN <sub>3</sub>               | 67.45    | 67.60    | 8.04     | 8.13     | 40        | 173–179   |
| 7        | C <sub>14</sub> H <sub>18</sub> Br <sub>2</sub> N <sub>2</sub> | 44.78    | 44.91    | 4.76     | 4.81     | 34        | 172–175   |
| 8        | C <sub>14</sub> H <sub>20</sub> N <sub>2</sub>                 | 77.49    | 77.77    | 9.23     | 9.26     | 92.6      | 94        |

Table IV. The MICs (µg/ml) of the tested compounds.

| Compounds   | <i>S. aureus</i> | <i>B. subtilis</i> | <i>E. coli</i> | <i>Y. enterocolitica</i> | <i>K. pneumoniae</i> | <i>C. albicans</i> |
|-------------|------------------|--------------------|----------------|--------------------------|----------------------|--------------------|
| 1           | na <sup>a</sup>  | na                 | na             | na                       | na                   | na                 |
| 2           | na               | na                 | na             | na                       | na                   | na                 |
| 3           | 64               | 256                | 256            | 512                      | 512                  | 128                |
| 4           | na               | na                 | na             | na                       | na                   | na                 |
| 5           | 64               | 256                | 512            | 512                      | 512                  | 64                 |
| 6           | 32               | 128                | 256            | 256                      | 256                  | 32                 |
| 7           | 32               | 32                 | 64             | 128                      | 256                  | 32                 |
| 8           | na               | na                 | na             | na                       | na                   | na                 |
| Ampicillin  | 2                | 8                  | 4              | 8                        | 64                   | nt <sup>b</sup>    |
| Fluconazole | nt               | nt                 | nt             | nt                       | nt                   | 128                |

<sup>a</sup> na = not active; <sup>b</sup> nt = not tested.

highest antimicrobial activities against *S. aureus* and *C. albicans* with MICs of 32–128 µg/ml, but poor activity of the compounds was observed against gram-negative bacteria such as *Y. enterocolitica* ATCC 1501, *E. coli* ATCC 11230 and *K. pneumoniae*, which are highly pathogenic to humans. Indeed, the bacterium *K. pneumoniae* showed lower microbial susceptibility compared to the other tested microorganisms. The compounds tested here generally show lower antimicrobial activity against gram-negative bacteria. The antimicrobial activity against gram-positive bacteria and fungus may depend on the differences between the cell structures of these microorganisms.

The results (Table IV) show that 1,3-dipyrrolidino-benzene (8) had no activity against any of the test microorganisms so we can say that the bioactivities of compounds 6 and 7 (pyrrolidine derivatives) are mainly correlated with the halogen substituents. Here the bromo, chloro and pyrrolidino substituents in combination showed inhibitory activity against the microorganisms (Table IV). However, the results of the antimicrobial tests with compounds 1–5 indicated that the type and the position of the halogen and piperidine tetrasubstituents had a significantly different effects on the growth of microorganisms. In conclusion, we report that some of the compounds (piperidine and pyrrolidine substituted halogeno-benzene derivatives) had antimicrobial activity against some hazardous microbes.

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