

Scientific paper

# Synthesis, characterization and antimicrobial activity of fluoro-containing aroylhydrazones

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## Abstract

2-Fluorobenzohydrazide reacted with various benzaldehydes afforded five new aroylhydrazones, which were characterized by elemental analysis, IR, UV-Vis and <sup>1</sup>H NMR spectroscopy. Detailed structures of four compounds were further determined by single crystal X-ray diffraction. Antibacterial activities of reported compounds on *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas fluorescens*, and antifungal activities on *Candida albicans* and *Aspergillus niger* were assayed. The biological assay indicated that the presence of electron-withdrawing groups in the benzaldehydes can improve antimicrobial activities.

**Keywords:** Aroylhydrazone; antimicrobial activity; crystal structure; X-ray diffraction

## 1. Introduction

Aroylhydrazones derived from the condensation reactions of aldehydes with benzohydrazides belong to class of Schiff bases. The compounds bearing CH=N-NH-C(O) functional groups have shown interesting biological activities, such as antibacterial,<sup>1</sup> antifungal,<sup>2</sup> antitumor,<sup>3</sup> anti-inflammatory,<sup>4</sup> and cytotoxic.<sup>5</sup> It was reported that the compounds bearing one or more halo-atoms in the aromatic rings can improve their biological activities especially for the antibacterial and antifungal activities.<sup>6</sup> Rai and co-workers reported a series of fluoro, chloro, bromo, and iodo-substituted compounds, and found that they have significant antimicrobial activities.<sup>7</sup> Aroylhydrazones are also a kind of interesting ligands in coordination chemistry. A number of copper, zinc, nickel, vanadium, and molybdenum complexes with aroylhydrazone ligands have been reported and shown potential biological activities.<sup>8</sup> Recently, our research group has reported a series of fluoro-substituted aroylhydrazones, which indicated that the electron-withdrawing groups, such as nitro-, chloro- and fluoro-, are important substitutes for the designation of effective antibacterial agents. The trifluoro and chloro substitutes at the benzene rings of benzohydrazides have

the most effective antibacterial activities.<sup>9</sup> As a continuation of work on the exploration of new antimicrobial agents,<sup>9,10</sup> in the present paper, a series of new aroylhydrazones derived from 2-fluorobenzohydrazide were synthesized and evaluated for their antimicrobial activities. Structure-activity relationship was also investigated.

## 2. Experimental

### 2.1. Materials and measurements

2-Fluorobenzohydrazide, 3-bromo-5-chlorosalicylaldehyde, 2,3-dichlorobenzaldehyde, 3-bromo-5-nitrosalicylaldehyde, 3,4-dibromobenzaldehyde and 2-bromo-4-chlorobenzaldehyde with AR grade were purchased from Sigma-Aldrich Co. Ltd, and used as received. Elemental analyses were performed on a Perkin-Elmer 240C elemental analyzer. IR spectra were recorded on a Jasco FT/IR-4000 spectrometer with KBr pellets. UV-Vis spectra were recorded on a Lambda 35 spectrometer. <sup>1</sup>H NMR spectra were recorded on a Bruker instrument at 300 MHz. X-ray diffraction was carried out at a Bruker SMART 1000 CCD area diffractometer equipped with MoK $\alpha$  radiation.

## 2.2. General method for the synthesis

Equimolar quantities (1.0 mmol each) of 2-fluorobenzohydrazide with various aldehydes were dissolved in methanol (30 mL) and stirred at room temperature for 30 min to give clear solution. X-ray quality single crystals were formed by slow evaporation of the solution in air for a few days.

### 2.2.1. *N'*-(3-Bromo-5-chloro-2-hydroxybenzylidene)-2-fluorobenzohydrazide (1)

Colorless crystals. Yield: 0.26 g (70%). Anal. calcd. for  $C_{14}H_9BrClFN_2O_2$ : C, 45.25; H, 2.44; N, 7.54; found C, 45.03; H, 2.51; N, 7.62%. Characteristic IR data ( $cm^{-1}$ ): 3452 (w), 3238 (w), 1656 (s), 1612 (m). UV-Vis data in methanol ( $\lambda$ ,  $\epsilon$ ): 220 nm,  $1.83 \cdot 10^4$  L mol $^{-1}$  cm $^{-1}$ ; 290 nm,  $1.97 \cdot 10^4$  L mol $^{-1}$  cm $^{-1}$ ; 338 nm,  $9.02 \cdot 10^3$  L mol $^{-1}$  cm $^{-1}$ ; 400 nm,  $9.17 \cdot 10^2$  L mol $^{-1}$  cm $^{-1}$ .  $^1H$  NMR (DMSO- $d_6$ ):  $\delta$ : 12.51 (s, 1H, NH), 8.48 (s, 1H, CH=N), 7.76 (s, 1H, ArH), 7.74 (s, 1H, ArH), 7.72–7.63 (m, 2H, ArH), 7.43–7.35 (m, 2H, ArH).

### 2.2.2. *N'*-(2,3-Dichlorobenzylidene)-2-fluorobenzohydrazide (2)

Colorless crystals. Yield: 0.26 g (84%). Anal. calcd. for  $C_{14}H_9Cl_2FN_2O$ : C, 54.04; H, 2.92; N, 9.00; found C, 54.23; H, 3.01; N, 8.87%. Characteristic IR data ( $cm^{-1}$ ): 3432 (w), 3240 (w), 1658 (s), 1612 (m). UV-Vis data in methanol ( $\lambda$ ,  $\epsilon$ ): 230 nm,  $2.02 \cdot 10^4$  L mol $^{-1}$  cm $^{-1}$ ; 293 nm,  $2.11 \cdot 10^4$  L mol $^{-1}$  cm $^{-1}$ .  $^1H$  NMR (DMSO- $d_6$ ):  $\delta$ : 12.18 (s, 1H, NH), 8.77 (s, 1H, CH=N), 8.48 (d, 1H, ArH), 8.00 (d, 1H, ArH), 7.75–7.61 (m, 2H, ArH), 7.50–7.31 (m, 3H, ArH).

### 2.2.3. *N'*-(3-Bromo-2-hydroxy-5-nitrobenzylidene)-2-fluorobenzohydrazide (3)

Yellow crystals. Yield: 0.33 g (87%). Anal. calcd. for  $C_{14}H_9BrFN_3O_4$ : C, 44.00; H, 2.37; N, 11.00; found C, 43.83; H, 2.32; N, 10.89%. Characteristic IR data ( $cm^{-1}$ ): 3440 (w), 3328 (w), 1679 (s), 1612 (m), 1517 (m), 1340 (s). UV-Vis data in methanol ( $\lambda$ ,  $\epsilon$ ): 212 nm,  $2.38 \cdot 10^4$  L mol $^{-1}$  cm $^{-1}$ ; 293 nm,  $1.97 \cdot 10^4$  L mol $^{-1}$  cm $^{-1}$ ; 376 nm,  $9.85 \cdot 10^3$  L mol $^{-1}$  cm $^{-1}$ .  $^1H$  NMR (DMSO- $d_6$ ):  $\delta$ : 12.72 (s, 1H, NH), 8.66 (s, 1H, CH=N), 8.64 (s, 1H, ArH), 8.50 (s, 1H, ArH), 7.79–7.75 (t, 1H, ArH), 7.69–7.64 (t, 1H, ArH), 7.46 (d, 1H, ArH), 7.38 (d, 1H, ArH).

### 2.2.4. *N'*-(3,4-Dibromobenzylidene)-2-fluorobenzohydrazide (4)

Colorless crystals. Yield: 0.30 g (75%). Anal. calcd. for  $C_{14}H_9Br_2FN_2O$ : C, 42.03; H, 2.27; N, 7.00; found C,

42.27; H, 2.15; N, 6.93%. Characteristic IR data ( $cm^{-1}$ ): 3435 (w), 3238 (w), 1656 (s), 1612 (m). UV-Vis data in methanol ( $\lambda$ ,  $\epsilon$ ): 225 nm,  $2.11 \cdot 10^4$  L mol $^{-1}$  cm $^{-1}$ ; 298 nm,  $2.36 \cdot 10^4$  L mol $^{-1}$  cm $^{-1}$ .  $^1H$  NMR (DMSO- $d_6$ ):  $\delta$ : 12.08 (s, 1H, NH), 8.29 (s, 1H, CH=N), 8.11 (d, 1H, ArH), 7.88 (d, 1H, ArH), 7.78–7.75 (d, 1H, ArH), 7.71–7.62 (m, 2H, ArH), 7.41–7.33 (m, 2H, ArH).

### 2.2.5. *N'*-(2-Bromo-4-chlorobenzylidene)-2-fluorobenzohydrazide (5)

Colorless crystals. Yield: 0.27 g (77%). Anal. calcd. for  $C_{14}H_9BrClFN_2O$ : C, 47.29; H, 2.55; N, 7.88; found C, 47.43; H, 2.45; N, 7.78%. Characteristic IR data ( $cm^{-1}$ ): 3429 (w), 3233 (w), 1656 (s), 1615 (m). UV-Vis data in methanol ( $\lambda$ ,  $\epsilon$ ): 230 nm,  $1.86 \cdot 10^4$  L mol $^{-1}$  cm $^{-1}$ ; 300 nm,  $2.73 \cdot 10^4$  L mol $^{-1}$  cm $^{-1}$ .  $^1H$  NMR (DMSO- $d_6$ ):  $\delta$ : 12.19 (s, 1H, NH), 8.68 (s, 1H, CH=N), 8.02 (d, 1H, ArH), 7.89 (d, 1H, ArH), 7.83 (d, 1H, ArH), 7.70 (t, 1H, ArH), 7.61 (s, 1H, ArH), 7.58 (d, 1H, ArH), 7.39 (t, 1H, ArH).

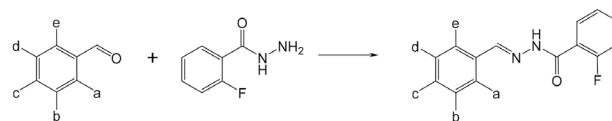
## 2.3. Antimicrobial assay

The compounds were tested against the bacterial strains *B. subtilis*, *S. aureus*, *E. coli* and *P. fluorescence* using Mueller-Hinton medium, and against the fungi strains *C. albicans* and *A. niger* using RPMI-1640 medium. The MIC values were determined by a colorimetric method using MTT.<sup>11</sup> A stock solution of the tested compounds (150  $\mu$ M) in DMSO was prepared and graded quantities (75  $\mu$ M, 37.5  $\mu$ M, 18.8  $\mu$ M, 9.4  $\mu$ M, 4.7  $\mu$ M, 2.3  $\mu$ M, 1.2  $\mu$ M, 0.59  $\mu$ M) were incorporated in specified quantity of the corresponding sterilized liquid medium. A specified quantity of the medium containing the compounds was poured into micro-titration plates. Suspension of the microorganism was prepared to contain  $10^5$  cfu mL $^{-1}$  and applied to micro-titration plates with serially diluted compounds in DMSO to be tested and incubated at 37 °C for 24 h and 48 h for bacteria and fungi, respectively. Then the MIC (minimum inhibitory concentration) values were visually determined on each of the micro-titration plates, 50  $\mu$ L of PBS (phosphate buffered saline 0.01 M, pH = 7.4) containing 2 mg of MTT mL $^{-1}$  was added to each well. Incubation was continued at room temperature for 4–5 h. The content of each well was removed, and 100  $\mu$ L of isopropanol containing 5% 1 M HCl was added to extract the dye. After 12 h of incubation at room temperature, the optical density was measured with a microplate reader at 550 nm.

## 2.4. Crystal structure determination

Diffraction intensities for compounds **1**, **3**, **4** and **5** were collected at 298(2) K using a Bruker SMART 1000 CCD area-detector diffractometer with Mo K $\alpha$  radiation ( $\lambda$  = 0.71073 Å). The collected data were reduced with SAINT program,<sup>12</sup> and multi-scan absorption correction

was performed using SADABS program.<sup>13</sup> The structures of the compounds were solved by direct methods, and refined against  $F^2$  by full-matrix least-squares method using SHELXTL.<sup>14</sup> All of the non-hydrogen atoms were refined anisotropically. The amino and phenol H atoms in the compounds were located from difference Fourier maps and refined isotropically, with N–H and O–H distances restrained to 0.90(1) and 0.85(1) Å, respectively. The remaining hydrogen atoms were placed in calculated positions and constrained to ride on their parent atoms. The crystallographic data for the compounds are summarized in Table 1.



Scheme 1. Synthesis of the compounds.

Table 1. Crystallographic and experimental data for the compounds 1, 3, 4 and 5

| Compound                                         | 1                                                                 | 3                                                               | 4                                                                | 5                                                    |
|--------------------------------------------------|-------------------------------------------------------------------|-----------------------------------------------------------------|------------------------------------------------------------------|------------------------------------------------------|
| Formula                                          | C <sub>14</sub> H <sub>9</sub> BrClFN <sub>2</sub> O <sub>2</sub> | C <sub>14</sub> H <sub>9</sub> BrFN <sub>3</sub> O <sub>4</sub> | C <sub>14</sub> H <sub>9</sub> Br <sub>2</sub> FN <sub>2</sub> O | C <sub>14</sub> H <sub>9</sub> BrClFN <sub>2</sub> O |
| Mr                                               | 371.59                                                            | 382.15                                                          | 399.04                                                           | 354.58                                               |
| Crystal system                                   | Triclinic                                                         | Monoclinic                                                      | Monoclinic                                                       | Monoclinic                                           |
| Space group                                      | <i>P</i> –1                                                       | <i>P</i> 2 <sub>1</sub> / <i>c</i>                              | <i>P</i> 2 <sub>1</sub> / <i>n</i>                               | <i>P</i> 2 <sub>1</sub> / <i>c</i>                   |
| <i>a</i> (Å)                                     | 10.1089(11)                                                       | 8.4675(5)                                                       | 9.5787(12)                                                       | 7.2991(4)                                            |
| <i>b</i> (Å)                                     | 17.1740(15)                                                       | 12.2533(8)                                                      | 5.0364(8)                                                        | 26.4778(15)                                          |
| <i>c</i> (Å)                                     | 17.4040(15)                                                       | 13.9123(9)                                                      | 29.256(1)                                                        | 8.1883(5)                                            |
| $\alpha$ (°)                                     | 91.099(1)                                                         | 90                                                              | 90                                                               | 90                                                   |
| $\beta$ (°)                                      | 103.949(1)                                                        | 99.828(1)                                                       | 94.467(1)                                                        | 115.566(1)                                           |
| $\gamma$ (°)                                     | 100.220(1)                                                        | 90                                                              | 90                                                               | 90                                                   |
| <i>V</i> (Å <sup>3</sup> )                       | 2879.8(5)                                                         | 1422.3(2)                                                       | 1407.1(3)                                                        | 1427.56(1)                                           |
| <i>Z</i>                                         | 8                                                                 | 4                                                               | 4                                                                | 4                                                    |
| <i>D<sub>c</sub></i> (g cm <sup>–3</sup> )       | 1.714                                                             | 1.785                                                           | 1.884                                                            | 1.650                                                |
| <i>m</i> (Mo–Ka) (mm <sup>–1</sup> )             | 3.056                                                             | 2.927                                                           | 5.768                                                            | 3.073                                                |
| <i>F</i> (000)                                   | 1472                                                              | 760                                                             | 772                                                              | 700                                                  |
| Reflections collected                            | 17386                                                             | 13602                                                           | 12747                                                            | 13057                                                |
| Unique reflections                               | 10688                                                             | 2650                                                            | 2605                                                             | 2527                                                 |
| Observed reflections ( $I \geq 2\sigma(I)$ )     | 5390                                                              | 2201                                                            | 1999                                                             | 1771                                                 |
| Parameters                                       | 781                                                               | 214                                                             | 184                                                              | 184                                                  |
| Restraints                                       | 15                                                                | 2                                                               | 1                                                                | 1                                                    |
| Goodness-of-fit on $F^2$                         | 1.005                                                             | 1.077                                                           | 1.034                                                            | 1.050                                                |
| $R_1, wR_2$ [ $I \geq 2\sigma(I)$ ] <sup>a</sup> | 0.0611, 0.1183                                                    | 0.0253, 0.0589                                                  | 0.0352, 0.0765                                                   | 0.0485, 0.1055                                       |
| $R_1, wR_2$ (all data) <sup>a</sup>              | 0.1462, 0.1556                                                    | 0.0359, 0.0629                                                  | 0.0556, 0.0844                                                   | 0.0826, 0.1205                                       |

<sup>a</sup>  $R_1 = \sum ||F_o| - |F_c|| / \sum |F_o|$ ,  $wR_2 = [\sum w(F_o^2 - F_c^2)^2 / \sum w(F_o^2)^2]^{1/2}$ .

### 3. Results and discussion

#### 3.1. Synthesis and general characterization

The aroylhydrazones were facile synthesized by condensation reaction of equimolar quantities of 2-fluorobenzohydrazide with 3-bromo-5-chlorosalicylaldehyde, 2,3-dichlorobenzaldehyde, 3-bromo-5-nitrosalicylaldehyde, 3,4-dibromobenzaldehyde and 2-bromo-4-chlorobenzaldehyde, respectively in methanol (Scheme 1). The compounds were crystallized as well-shaped single crystals, which are soluble in methanol, ethanol, acetonitrile, chloroform, DMF and DMSO, but insoluble in water. The C, H, N analyses were in accordance with the chemical formulae proposed by the single crystal X-ray analysis.

#### 3.2. IR and electronic spectra

The intense bands in the range 1656–1679 cm<sup>–1</sup> are generated by  $\nu(\text{C}=\text{O})$ , whereas the bands in the range 1612–1615 cm<sup>–1</sup> are assigned to  $\nu(\text{C}=\text{N})$ .<sup>15</sup> The broad absorptions in the range 3429–3452 cm<sup>–1</sup> can be attributed to the hydrogen-bonded phenol groups. The sharp bands in the range 3228–3240 cm<sup>–1</sup> are assigned to  $\nu(\text{N}-\text{H})$ . The bands indicative of the  $\nu_{as}(\text{NO}_2)$  and  $\nu_s(\text{NO}_2)$  vibrations in the spectrum of compound 3 are observed at 1517 and 1340 cm<sup>–1</sup>.<sup>14</sup>

In the electronic spectra of the compounds, the absorptions at 210–230 nm can be assigned to  $\pi-\pi^*$  transitions in the aromatic and intra-ligand  $\pi-\pi^*$  transitions.<sup>16</sup> The absorptions at 290–300 nm may be assigned to  $n-\pi^*$  transitions of the azomethine and carbonyl groups.<sup>17</sup>

### 3.3. $^1\text{H}$ NMR spectra

The  $^1\text{H}$  NMR spectra of the compounds show no signal of the amino group ( $\text{NH}_2$ ) characteristic to the starting material (hydrazide). The spectra show singlet at 12–13 ppm range, which may be assigned to the N–H protons.<sup>18</sup> The singlet at 8.2–8.8 ppm range is assigned to the azomethine proton ( $\text{CH}=\text{N}$ ).<sup>19</sup> The multiplets in the 7.3–8.7 ppm range are attributed to aromatic protons.<sup>20</sup>

### 3.4. Crystal structure description

The molecular structures of compounds **1**, **3**, **4** and **5** are shown in Figures 1–4, respectively. Selected bond lengths and angles are listed in Table 2. All the molecules of the compounds adopt *E* configuration with respect to the methyldiene units. The distances of the methyldiene bonds, ranging from 1.26 to 1.28 Å, confirm them as typical double bonds. The shorter distances of the C–N bonds and the longer distances of the C=O bonds for the  $-\text{C}(\text{O})-\text{NH}-$  units than usual, suggest the presence of conjugation effects in the molecules. All the bond lengths in the compounds are comparable to each other and are within normal values.<sup>21</sup> The aromatic rings of the compounds form dihedral angles of  $49.2(6)^\circ$  (**1**),  $5.3(2)^\circ$  (**3**),  $2.7(3)^\circ$  (**4**) and  $9.0(4)^\circ$  (**5**). Crystal structures of the compounds are stabilized by intermolecular hydrogen bonds (Table 3).

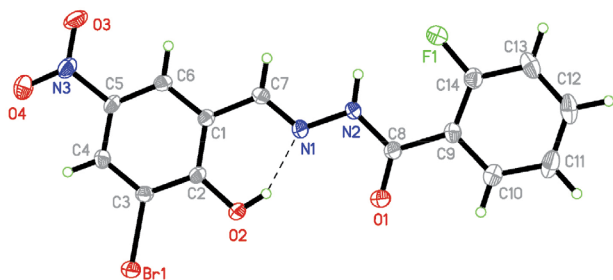


Fig. 1. Molecular structure **1** with the atom labeling scheme. Thermal ellipsoids are drawn at the 30% probability level. Hydrogen bond is shown as a dashed line.

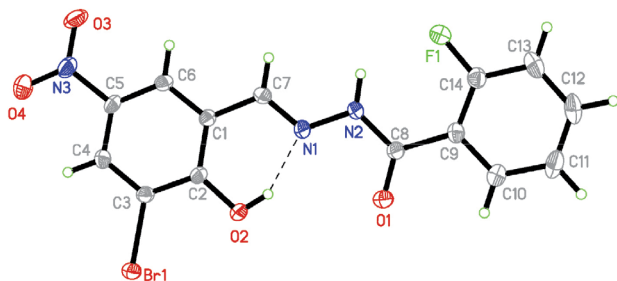


Fig. 2. Molecular structure **3** with the atom labeling scheme. Thermal ellipsoids are drawn at the 30% probability level. Hydrogen bond is shown as a dashed line.

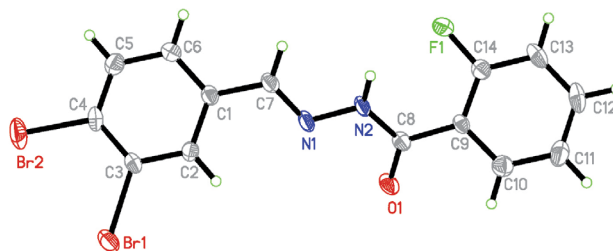


Fig. 3. Molecular structure **4** with the atom labeling scheme. Thermal ellipsoids are drawn at the 30% probability level.

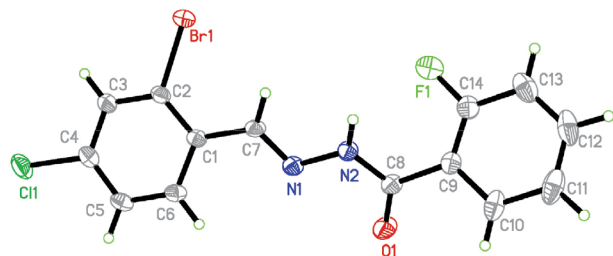


Fig. 4. Molecular structure **5** with the atom labeling scheme. Thermal ellipsoids are drawn at the 30% probability level.

Table 2 Selected bond lengths/Å and angles/ $^\circ$  for the compounds **1**, **3**, **4** and **5**

|          | <b>1</b> | <b>3</b>   | <b>4</b> | <b>5</b> |
|----------|----------|------------|----------|----------|
| C7–N1    | 1.271(7) | 1.275(3)   | 1.270(4) | 1.262(5) |
| N1–N2    | 1.376(6) | 1.369(2)   | 1.382(4) | 1.382(5) |
| N2–C8    | 1.351(8) | 1.364(3)   | 1.345(4) | 1.341(6) |
| C8–O1    | 1.222(7) | 1.209(3)   | 1.223(4) | 1.214(5) |
| C7–N1–N2 | 115.9(5) | 120.87(18) | 115.8(3) | 115.2(3) |
| N1–N2–C8 | 119.7(5) | 115.68(17) | 119.0(3) | 119.9(3) |
| N2–C8–C9 | 116.6(6) | 118.06(18) | 117.2(3) | 115.7(4) |
| N2–C8–O1 | 122.5(6) | 120.72(19) | 122.3(3) | 122.9(4) |

### 3.5. Antimicrobial activity

The compounds were screened for antibacterial activity against two Gram (+) bacterial strains *Bacillus subtilis* and *Staphylococcus aureus*, and two Gram (–) bacterial strains *Escherichia coli* and *Pseudomonas fluorescence* by MTT method. The MIC values are listed in Table 4. Kanamycin and penicillin G were used as reference drugs. Compounds **2** and **5** showed the most effective activities against *B. subtilis*, and good activities against *S. aureus* and *E. coli*, while weak activity against *P. fluorescence*. Compound **1** showed good activity against *B. subtilis*, medium activity against *S. aureus*, weak activity against *E. coli*, and no activity against *P. fluorescence*. Compound **3** showed good activity against *B. subtilis*, weak activity against *S. aureus* and *E. coli*, and no activity against *P. fluorescence*.

**Table 3** Hydrogen bond distances (Å) and bond angles (°) for the compounds 1, 3, 4 and 5

| <i>D</i> – <i>H</i> ... <i>A</i> | <i>d</i> ( <i>D</i> – <i>H</i> ) | <i>d</i> ( <i>H</i> ... <i>A</i> ) | <i>d</i> ( <i>D</i> ... <i>A</i> ) | Angle<br>( <i>D</i> – <i>H</i> ... <i>A</i> ) |
|----------------------------------|----------------------------------|------------------------------------|------------------------------------|-----------------------------------------------|
| <b>1</b>                         |                                  |                                    |                                    |                                               |
| O2–H2A...N1                      | 0.85(1)                          | 1.92(5)                            | 2.639(6)                           | 142(7)                                        |
| N2–H2...O5 <sup>i</sup>          | 0.90(1)                          | 2.03(2)                            | 2.901(7)                           | 164(6)                                        |
| N8–H8...F2                       | 0.90(1)                          | 2.17(6)                            | 2.708(6)                           | 118(5)                                        |
| N8–H8...F4                       | 0.90(1)                          | 2.48(4)                            | 3.196(6)                           | 138(5)                                        |
| N8–H8...O7                       | 0.90(1)                          | 2.36(4)                            | 3.145(7)                           | 146(6)                                        |
| O10–H10...N7                     | 0.85(1)                          | 1.83(4)                            | 2.594(6)                           | 148(7)                                        |
| N4–H4...O1                       | 0.90(1)                          | 2.10(2)                            | 2.981(7)                           | 164(6)                                        |
| O6–H6...N3                       | 0.85(1)                          | 1.87(4)                            | 2.616(6)                           | 146(7)                                        |
| N6–H6A...O11 <sup>ii</sup>       | 0.90(1)                          | 1.99(3)                            | 2.855(6)                           | 160(6)                                        |
| O8–H8A...N5                      | 0.85(1)                          | 1.81(5)                            | 2.543(7)                           | 143(7)                                        |
| <b>3</b>                         |                                  |                                    |                                    |                                               |
| N2–H2...F1                       | 0.90(1)                          | 2.12(2)                            | 2.707(2)                           | 124(2)                                        |
| N2–H2...O3 <sup>iii</sup>        | 0.90(1)                          | 2.21(2)                            | 3.008(2)                           | 150(2)                                        |
| O2–H2A...N1                      | 0.85(1)                          | 1.79(2)                            | 2.552(2)                           | 149(3)                                        |
| <b>4</b>                         |                                  |                                    |                                    |                                               |
| N2–H2...O1 <sup>iv</sup>         | 0.90(1)                          | 2.02(2)                            | 2.882(3)                           | 162(3)                                        |
| <b>5</b>                         |                                  |                                    |                                    |                                               |
| N2–H2...O1 <sup>v</sup>          | 0.90(1)                          | 2.19(2)                            | 3.040(5)                           | 157(4)                                        |

Symmetry codes: (i)  $-1 + x, y, z$ ; (ii)  $1 + x, y, z$ ; (iii)  $-x, -y, -1 - z$ ; (iv)  $x, 1 + y, z$ ; (v)  $x, \frac{1}{2} - y, \frac{1}{2} + z$ .

Compound **4** showed good activity against *B. subtilis* and *S. aureus*, weak activity against *E. coli*, and no activity against *P. fluorescens*. After careful analysis between structures and activities, we noticed that the presence of chloro substitute groups in the compounds might play an important role in the antibacterial activities. In addition, the presence of bromo substitute groups gives positive action. Our previous work indicates that the electron-withdrawing group (NO<sub>2</sub>) in the benzene ring of hydrazide part can improve the antibacterial activity.<sup>9</sup> However, in this work, the nitro substitute group in the benzene ring of aldehyde part did not enhance the bacterial activity. The chloro groups are known as important substituents for antibacterial activities.<sup>22</sup> It is interesting that compound **2** has stronger activity against *B. subtilis* than kanamycin

and penicillin G, and similar activity against *S. aureus* as compared to kanamycin. Compound **5** has similar activity against *B. subtilis* than kanamycin and penicillin G. The results indicated that the electron-withdrawing groups Cl and Br of the aromatic rings may be important for the designation of antibacterial materials.

The antifungal activities of the compounds were also evaluated against two fungal strains *Candida albicans* and *Aspergillus niger* by MTT method. Ketoconazole was used as a reference drug. As a result, compound **2** has medium activity against *C. albicans*, and compound **5** has weak activity against *C. albicans*. All the compounds are negative against *A. niger*.

## 4. Conclusions

In summary, five new substituted aroylhydrazones with electron-withdrawing groups (Cl, Br, F, NO<sub>2</sub>) were synthesized and characterized. The antimicrobial activities against the bacteria *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas fluorescens*, and the fungal *Candida albicans* and *Aspergillus niger* were determined. *N*'-(2,3-dichlorobenzylidene)-2-fluorobenzohydrazide and *N*'-(2-bromo-4-chlorobenzylidene)-2-fluorobenzohydrazide showed the most effective antimicrobial activities against *B. subtilis* and *S. aureus*. The electron-withdrawing groups Cl, Br and F are important substitutes for the designation of effective antibacterial agents. The compounds could be useful as a template for future development through modification to explore more effective antimicrobial materials.

## 5. Supplementary material

CCDC-2494751 for **1**, 1035480 for **3**, 2494753 for **4**, 2494754 for **5** contain the supplementary crystallographic data for this paper. These data can be obtained free of charge at <http://www.ccdc.cam.ac.uk/const/retrieving.html> or from the Cambridge Crystallographic Data Centre (CCDC), 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44(0)1223-336033 or e-mail: [deposit@ccdc.cam.ac.uk](mailto:deposit@ccdc.cam.ac.uk).

**Table 4** The MIC values (μM) of the compounds 1–5

| Tested material | <i>B. subtilis</i> | <i>S. aureus</i> | <i>E. coli</i> | <i>P. fluorescens</i> | <i>C. albicans</i> | <i>A. niger</i> |
|-----------------|--------------------|------------------|----------------|-----------------------|--------------------|-----------------|
| <b>1</b>        | 4.7                | 18.8             | 75             | >150                  | >150               | >150            |
| <b>2</b>        | 0.16               | 2.3              | 9.4            | 18.8                  | 18.8               | >150            |
| <b>3</b>        | 9.4                | 37.5             | 75             | >150                  | >150               | >150            |
| <b>4</b>        | 2.3                | 9.4              | 37.5           | >150                  | >150               | >150            |
| <b>5</b>        | 0.62               | 4.7              | 9.4            | 37.5                  | 37.5               | >150            |
| Kanamycin       | 0.59               | 2.3              | 4.7            | 4.7                   | >150               | >150            |
| Penicillin G    | 2.3                | 4.7              | >150           | >150                  | >150               | >150            |
| Ketoconazole    | >150               | >150             | >150           | >150                  | 4.7                | 18.8            |

## 6. References

- (a) Y. R. Sable, R. A. Shinde, H. K. A. Yasin, N. Ghanwate, S. N. Mali, S. K. Ghotekar, D. P. Gawari, D. A. Sasane, V. A. Adole, *RSC Adv.* **2025**, *15*, 30001–30025; DOI:10.1039/D5RA04238K  
(b) G.-X. He, L.-W. Xue, *Acta Chim. Slov.* **2021**, *68*, 567–574; DOI:10.17344/acsi.2020.6333  
(c) O. O. Ajani, C. A. Obafemi, O. C. Nwinyi, D. A. Akinpelu, *Bioorg. Med. Chem.* **2010**, *18*, 214–221. DOI:10.1016/j.bmc.2009.10.064
- (a) G. Visbal, G. San-Blas, A. Maldonado, A. Alvarez-Aular, M. V. Capparelli, J. Murgich, *Steroids* **2011**, *76*, 1069–1081. DOI:10.1016/j.steroids.2011.04.012
- (a) D. Zeng, K. H. Li, A. P. Xing, J. Wang, Y. T. Hao, Z. Q. Zhang, J. Yuan, *J. Mol. Struct.* **2025**, *1348*, 143435; DOI:10.1016/j.molstruc.2025.143435  
(b) T. Horiuchi, J. Chiba, K. Uoto, T. Soga, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 305–308. DOI:10.1016/j.bmcl.2008.11.090
- (a) M. A. A. El-Sayed, N. I. Abdel-Aziz, A. A. M. Abdel-Aziz, A. S. El-Azab, Y. A. Asiri, K. E. H. ElTahir, *Bioorg. Med. Chem.* **2011**, *19*, 3416–3424; DOI:10.1016/j.bmc.2011.04.027  
(b) S. M. Sondhi, M. Dinodia, A. Kumar, *Bioorg. Med. Chem.* **2006**, *14*, 4657–4663. DOI:10.1016/j.bmc.2006.02.014
- (a) P. Krishnamoorthy, P. Sathyadevi, A. H. Cowley, R. R. Buttorac, N. Dharmaraj, *Eur. J. Med. Chem.* **2011**, *46*, 3376–3387; DOI:10.1016/j.ejmech.2011.05.001  
(b) P. G. Avaji, C. H. V. Kumar, S. A. Patil, K. N. Shivananda, C. Nagaraju, *Eur. J. Med. Chem.* **2009**, *44*, 3552–3559. DOI:10.1016/j.ejmech.2009.03.032
- (a) L. C. Felton, J. H. Brewer, *Science* **1947**, *105*, 409–410; DOI:10.1126/science.105.2729.409  
(b) M. Gopalakrishnan, J. Thanusu, V. Kanagarajan, R. Govindaraju, *J. Enzyme Inhib. Med. Chem.* **2009**, *24*, 52–58; DOI:10.1080/14756360801906632  
(c) L. Shi, H.-M. Ge, S.-H. Tan, H.-Q. Li, Y.-C. Song, H.-L. Zhu, R.-X. Tan, *Eur. J. Med. Chem.* **2007**, *42*, 558–564. DOI:10.1016/j.ejmech.2006.11.010
- (a) N. P. Rai, V. K. Narayanaswamy, T. Govender, B. K. Manuprasad, S. Shashikanth, P. N. Arunachalam, *Eur. J. Med. Chem.* **2010**, *45*, 2677–2682; DOI:10.1016/j.ejmech.2010.02.021  
(b) N. P. Rai, V. K. Narayanaswamy, S. Shashikanth, P. N. Arunachalam, *Eur. J. Med. Chem.* **2009**, *44*, 4522–4527.
- (a) E.-C. Liu, W. Li, X.-S. Cheng, *Acta Chim. Slov.* **2019**, *66*, 971–977; DOI:10.17344/acsi.2019.5220  
(b) H.-Y. Qian, *Acta Chim. Slov.* **2019**, *66*, 995–1001; DOI:10.17344/acsi.2019.5247  
(c) Y. Li, L. Xu, M. Duan, J. Wu, Y. Wang, K. Dong, M. Han, Z. You, *Inorg. Chem. Commun.* **2019**, *105*, 212–216; DOI:10.1016/j.inoche.2019.05.011  
(d) X.-Q. Luo, Y.-J. Han, L.-W. Han, *Acta Chim. Slov.* **2020**, *67*, 853–859; DOI:10.17344/acsi.2020.5819  
(e) Z. You, H. Yu, B. Zheng, C. Zhang, C. Lv, K. Li, L. Pan, *Inorg. Chim. Acta* **2018**, *469*, 44–50; DOI:10.1016/j.ica.2017.09.011  
(f) Y.-L. Sang, X.-S. Lin, W.-D. Sun, *Acta Chim. Slov.* **2020**, *67*, 581–585; DOI:10.17344/acsi.2019.5595  
(g) H. Zhao, X.-P. Tan, Q.-A. Peng, C.-Z. Shi, Y.-F. Zhao, Y. Cui, *Acta Chim. Slov.* **2020**, *67*, 638–643. DOI:10.17344/acsi.2019.5644
- F.-M. Wang, L.-J. Li, G.-W. Zang, T.-T. Deng, Z.-L. You, *Acta Chim. Slov.* **2021**, *68*, 541–547. DOI:10.17344/acsi.2020.6051
- (a) R.-H. Hui, P. Zhou, Z.-L. You, *Indian J. Chem. A* **2009**, *48*, 1102–1106;  
(b) Z.-L. You, H.-L. Zhu, *Z. Anorg. Allg. Chem.* **2006**, *632*, 140–146; DOI:10.1002/zaac.200500308  
(c) Z.-L. You, Q.-Z. Jiao, *Synth. React. Inorg. Met.-Org. Nano-Met. Chem.* **2006**, *36*, 713–717. DOI:10.1080/15533170601028165
- J. Meletiadis, J. F. Meis, J. W. Mouton, J. P. Donnelly, P. E. Verweij, *J. Clin. Microbiol.* **2000**, *38*, 2949–2954. DOI:10.1128/JCM.38.8.2949-2954.2000
- Bruker, SMART (Version 5.625) and SAINT (Version 6.01). Bruker AXS Inc., Madison, Wisconsin, USA, 2007.
- G. M. Sheldrick. *SADABS. Program for Empirical Absorption Correction of Area Detector*, University of Göttingen, Germany, 1996.
- G. M. Sheldrick. *SHELXTL V5.1 Software Reference Manual*, Bruker AXS, Inc., Madison, Wisconsin, USA, 1997.
- M. Zhang, D.-M. Xian, H.-H. Li, J.-C. Zhang, Z.-L. You, *Aust. J. Chem.* **2012**, *65*, 343–350. DOI:10.1071/CH11424
- M. F. R. Fouda, M. M. Abd-Elzaher, M. M. Shakhdoza, F. A. El-Saied, M. I. Ayad, A. S. El-Tabl, *J. Coord. Chem.* **2008**, *61*, 1983–1996. DOI:10.1080/00958970701795714
- R. Gup, B. Kirkan, *Spectrochim. Acta A* **2005**, *62*, 1188–1195. DOI:10.1016/j.saa.2005.04.015
- U. O. Ozmen, G. Olgun, *Spectrochim. Acta A* **2008**, *70*, 641–645. DOI:10.1016/j.saa.2007.08.012
- M. R. Maurya, S. Khurana, C. Schulzke, D. Rehder, *Eur. J. Inorg. Chem.* **2001**, *3*, 779–788. DOI:10.1002/1099-0682(200103)2001:3<779::AID-EJIC779>3.3.CO;2-R
- P. N. Remya, C. H. Suresh, M. L. P. Reddy, *Polyhedron* **2007**, *26*, 5016–5022. DOI:10.1016/j.poly.2007.07.020
- (a) S. N. Podyachev, I. A. Litvinov, R. R. Shagidullin, B. I. Buzykin, I. Bauer, D. V. Osyanina, L. V. Avvakumova, S. N. Sudakova, W. D. Habicher, A. I. Konovalov, *Spectrochim. Acta Part A* **2007**, *66*, 250–261;  
(b) S. M. S. V. Wardell, M. V. N. de Souza, J. L. Wardell, J. N. Low, C. Glidewell, *Acta Crystallogr.* **2005**, *C61*, o683–o689;  
(c) X.-L. Wang, Z.-L. You, C. Wang, *J. Chem. Crystallogr.* **2011**, *41*, 621–624. DOI:10.1016/j.saa.2006.02.049
- O. O. Guven, T. Erdogan, H. Goker, S. Yildiz, *Bioorg. Med. Chem.* **2007**, *17*, 2233–2236.

## Povzetek

2-Fluorobenzohidrazid je pri reakciji z različnimi benzaldehidi tvoril pet novih aroilhidrazonov, ki smo jih okarakterizirali z elementarno analizo, IR, UV-Vis in  $^1\text{H}$  NMR spektroskopijo. Strukture štirih spojin so bile dodatno določene z monokristalno rentgensko difrakcijo. Antibakterijske aktivnosti spojin smo testirali na *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* in *Pseudomonas fluorescense*, ter protiglivične aktivnosti na *Candida albicans* in *Aspergillus niger*. Biološki testi so pokazali, da lahko v benzaldehidih prisotnost elektronakceptorskih skupin izboljša protimikrobne aktivnosti.



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