



## SYNTHESIS AND BIOLOGICAL EVALUATION OF HYDRAZONE DERIVATIVES OF ORTHO-AMINO BENZOIC ACID

Dachepally Raju<sup>a\*</sup>, J. Sreeramulu<sup>a</sup>, P. Malleswarareddy<sup>b</sup> and Nagaraju<sup>c</sup>

Department of Chemistry, Sri Krishnadevaraya University, Anantapuramu, Andhra Pradesh, India-515003.

Corresponding author: J. Sreeramulu, Department of Chemistry, Sri Krishnadevaraya University, Anantapur. Andhra Pradesh, India—515003. [pmeshvarreddy@gmail.com](mailto:pmeshvarreddy@gmail.com)

**Abstract:** In this present investigation author synthesised Hydrazone derivatives of o-Aminobenzoic acid (**5a-j**) successfully with good yield and their structures were confirmed by the spectroscopic techniques such as <sup>1</sup>H NMR, mass and FTIR data. The synthesized Hydrazone derivatives of o-Aminobenzoic acid (**5a-5j**) were tested against Gram negative strains of (i) *Escherichia coli* (MTCC 443) and (ii) *Pseudomonas aeruginosa* (MTCC 424) and Gram-positive strains of (iii) *Staphylococcus aureus* (MTCC 96) and (iv) *Streptococcus pyogenes* (MTCC 442) by agar diffusion method using ciprofloxacin as standard antibiotic. All the compounds shown moderate anti-bacterial activity.

**Key words:** o-Aminobenzoic acid; Hydrazone derivatives; agar diffusion method; ciprofloxacin.

### Introduction

Ortho-Aminobenzoic acid is a constituent of many bioactive compounds that exhibits a variety of biological activities. In particular, the nucleus of o-Aminobenzoic acid is the biochemical precursor to the amino acid tryptophan and its derivatives as well as to the constituents of quite a few alkaloids. The biological activities of this essential moiety have been well reported. Some of them like mefenamic acid and meclofenamate, both *N*-phenyl o-Aminobenzoic acid derivatives, have been used as anti-inflammatory agents.<sup>I</sup> O-Aminobenzoic acid is a good pharmacophore that is recurrently used in drug discovery programmes.<sup>II, III</sup> O-Aminobenzoic acid derivatives were shown to possess diverse biological activities such as anti-cancer,<sup>IV</sup> antibacterial,<sup>V</sup> antifungal,<sup>VI</sup> antidiabetic,<sup>VII</sup> anti-hepatitis-C,<sup>VIII</sup> CCK<sub>1</sub> receptor antagonists,<sup>IX</sup> partial FXR agonists<sup>X</sup> etc.

Hydrazone nucleus is found in natural and synthetic products of biological interest. Literature studies revealed that hydrazones and various substituted hydrazones are associated with a broad spectrum of biological activities such as antimicrobial activity,<sup>XI</sup> anti-tumor activity,<sup>XII</sup> antimycobacterial activity,<sup>XIII, XIV</sup> anti-inflammatory<sup>XV</sup> and anti-platelet activity.

The need of new anti-microbial agents is justified because more microorganisms are

being resistant to the present drugs available in the market. Worldwide researchers are trying to synthesize new drugs with better pharmacokinetic and pharmacodynamic properties with less adverse effects.

Prompted by the significance of *o*-Aminobenzoic acid in biological systems, an attempt was made to design and synthesize some new hydrazone derivatives of *o*-Aminobenzoic acid and evaluate them for their potential antibacterial activities. Hydrazone derivatives of *o*-Aminobenzoic acid **5a-j** were synthesized successfully with good yield and their structures were confirmed by the spectroscopic techniques such as <sup>1</sup>H NMR, mass and FTIR data.

### Materials and methods

Chemicals and solvents were purchased from Sigma-Aldrich and Merck. All the reagents were of analytical grade. Thin-layer chromatography (TLC) was performed on E. Merck AL silica gel 60 F254 plates and visualized under UV light. IR spectra were recorded as KBr pellets with Perkin-Elmer Spectrum GX FT-IR instrument and only diagnostic and/or intense peaks were reported. <sup>1</sup>H-NMR spectra were recorded in DMSO- *d*<sub>6</sub> with Varian Mercury plus 400 MHz instrument. Signals due to the residual protonated solvent (<sup>1</sup>H-NMR) served as the internal standard. All the chemical shifts were reported in δ (ppm) using TMS as internal standard. The <sup>1</sup>H-NMR chemical shifts and coupling constants were determined assuming first-order behavior. Mass spectra were recorded with a PE Sciex model API 3000 instrument. All the reactions were carried out under nitrogen atmosphere.

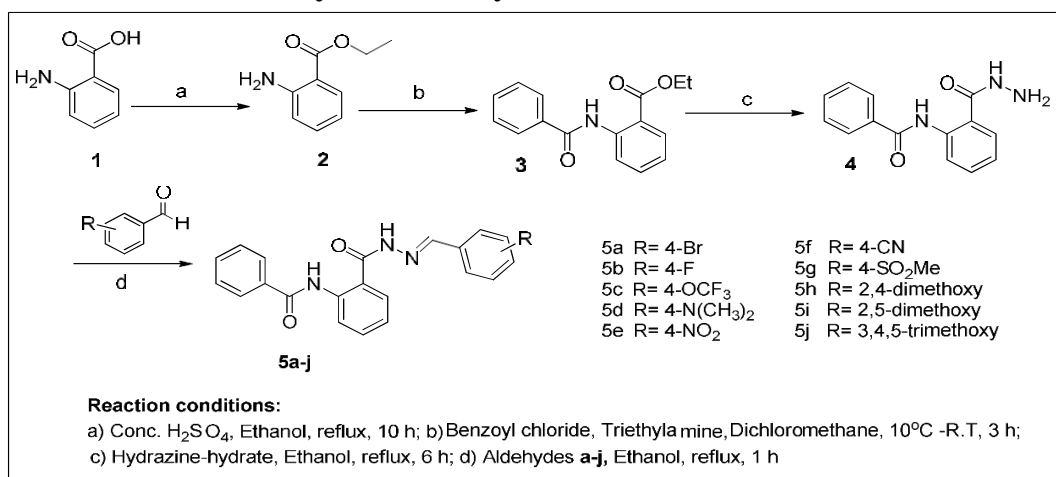
### Antibacterial bioassay

The synthesized (*E*)-*N'*-(substituted-benzylidene)-2-(benzamido) benzo hydrazides (**5a-5j**) were tested against Gram negative strains of (i) *Escherichia coli* (MTCC 443) and (ii) *Pseudomonas aeruginosa* (MTCC 424) and Gram-positive strains of (iii) *Staphylococcus aureus* (MTCC 96) and (iv) *Streptococcus pyogenes* (MTCC 442) by agar diffusion method using ciprofloxacin as standard antibiotic and test solutions of concentration 50 μg ml<sup>-1</sup> in DMSO.

### Results and Discussion

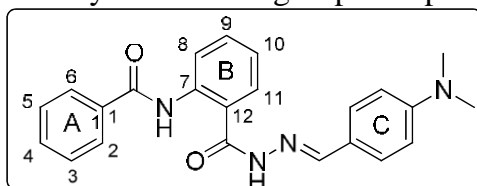
#### Synthesis

Synthesis of the title compounds is outlined in **Scheme-1**. Esterification of *o*-Aminobenzoic acid in the presence of conc. H<sub>2</sub>SO<sub>4</sub> and ethanol and refluxing for 10 h resulted in ethyl 2-aminobenzoate (**2**). Amide coupling of compound **2** was accomplished by reacting with benzoyl chloride in the presence of triethylamine in dichloromethane at room temperature for 3h which gave ethyl 2-(benzamido) benzoate (**3**). Hydrazinolysis of benzoate **3** in the presence of hydrazine hydrate in ethanol and reflux for 6h resulted in 2-(benzamido) benzo hydrazide (**4**). Condensation of compound **4** with various aldehydes, **a-j**, was done in ethanol with reflux for 1h which resulted in the formation of (*E*)-*N'*-(substituted-benzylidene)-2-(benzamido) benzo hydrazides (**5a-j**).

**Scheme-1- Scheme of synthesis of hydrazone derivatives of *o*-Aminobenzoic acid****Spectral interpretation**

The structures of newly synthesized benzohydrazide derivatives **5a-j** were ascertained by <sup>1</sup>H NMR, mass and IR spectral data. <sup>1</sup>H NMR spectra of hydrazones **5a-j** displayed the characteristic singlets in the regions δ 12.57-12.11 ppm, 12.00-10.21 ppm and 8.81- 8.32 ppm corresponding to protons of hydrazone NH, amide NH and azomethine (N=CH) groups respectively. Aromatic protons appeared in the region 6.77-8.67 ppm and the aliphatic protons appeared in the expected region. The mass spectra of the compounds showed (M+1) peaks and are in agreement with their molecular formulae. The IR spectra of the compounds **5a-j** represented the characteristic bands that comply with the desired functional groups in the structure.

Spectral details of the (*E*)-*N'*-(4-(dimethylamino) benzylidene)-2-(benzamido) benzo hydrazide (**5d**) are as follows: <sup>1</sup>H NMR spectrum of compound **5d** displayed characteristic singlets at δ 12.11 ppm, 11.86 ppm and 8.32 ppm corresponding to the CO-NH protons of hydrazone moiety and amide group and proton of azomethine (N=CH)



group respectively. Doublets at δ 7.57 ppm (*J* = 8.7Hz) and 6.77 ppm (*J* = 8.7Hz) each with two protons integration, correspond to 4 protons of aromatic ring **C**. Absorption signals at δ 8.61 (d, *J* = 8.3 Hz, 1H), 7.90 (d, *J* = 7.6Hz, 1H) and 7.27 (t, *J* = 7.6 Hz, 1H) are due to H-8, H-11 and H-10 protons of ring **B**. Doublet with two proton integration at δ 7.97 ppm is due to H-1 and H-6 protons of ring **A**. A multiplet between δ 7.70-7.59 ppm is due to protons H-3, H-4, H-5 and H-9. Protons of two methyl groups resonate at δ 2.99 ppm. IR spectrum of compound **5a** displayed characteristic band at 3192 cm<sup>-1</sup> which corresponds to NH stretching and bands at 1663 cm<sup>-1</sup> and 1645 cm<sup>-1</sup> correspond to C=O and C=N stretching of hydrazone moiety respectively. ESI-MS spectrum of compound **5d** showed M+1 peak at 386.9 in agreement with its molecular formula.

**Stereochemistry of synthesized compounds**

The <sup>1</sup>H NMR spectra of all synthesized compounds **5a-j** showed very minute signals around δ 11.6 ppm and 10.6 ppm. These peaks are very weak (4-5%) and hence negligible

to consider for integration. However, the consistent appearance of the minute peaks indicates that an equilibrium mixture of *syn*- and *periplanar* conformational isomers may exist in the final product with very small fraction of minor rotamer.

#### Antibacterial activity

The newly synthesized compounds **5a-j** were evaluated for *in-vitro* antimicrobial activity against two Gram negative and two Gram positive bacteria, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pyogenes* by agar diffusion method. The antibacterial evaluation results of compounds **5a-j** are presented in **Table .** Compounds **5b** (R = 4-F), **5c** (R = 4-OCF<sub>3</sub>), **5g** (4-SO<sub>2</sub>CH<sub>3</sub>) and **5j** (3,4,5-OMe) exhibited good antibacterial activity against *E.coli* and *P.aeruginosa* with inhibition zone 21-24 mm while the compounds **5d** (N(CH<sub>3</sub>)<sub>2</sub>), **5h** (R = 2,4-OMe) and **5i** (R = 2,5-OMe) showed moderate activity with zone of inhibition 15-17 mm. These compounds exhibited similar pattern of antimicrobial activity against *S.aureus* and *S.pyogenes* with variation in zone of inhibition i.e. 18-23 mm for good activity, 12-17 mm for moderate activity. The remaining compounds such as **5a** (R=4-Br), **5e** (R = 4-NO<sub>2</sub>) and **5f** (R= 4-CN) were observed to be less active towards all the above tested pathogens

**Table :1. Antibacterial activity of compounds 5a-j (Concentration used 50 µg mL<sup>-1</sup> of DMSO)**

| Compound No                           | R                                  | Gram negative bacteria     |                                 | Gram positive bacteria     |                               |
|---------------------------------------|------------------------------------|----------------------------|---------------------------------|----------------------------|-------------------------------|
|                                       |                                    | <i>E. coli</i><br>MTCC 443 | <i>P.aeruginosa</i><br>MTCC 424 | <i>S.aureus</i><br>MTCC 96 | <i>S.pyogenes</i><br>MTCC 442 |
| Zone of inhibition <sup>b</sup> in mm |                                    |                            |                                 |                            |                               |
| <b>5a</b>                             | 4-Br                               | 10                         | 9                               | 9                          | 12                            |
| <b>5b</b>                             | 4-F                                | 22                         | 22                              | 21                         | 19                            |
| <b>5c</b>                             | 4-OCF <sub>3</sub>                 | 24                         | 21                              | 20                         | 18                            |
| <b>5d</b>                             | -N (CH <sub>3</sub> ) <sub>2</sub> | 16                         | 15                              | 12                         | 13                            |
| <b>5e</b>                             | 4-NO <sub>2</sub>                  | 11                         | 11                              | 11                         | 10                            |
| <b>5f</b>                             | 4-CN                               | 10                         | 10                              | 9                          | 8                             |
| <b>5g</b>                             | 4-SO <sub>2</sub> CH <sub>3</sub>  | 23                         | 22                              | 23                         | 20                            |
| <b>5h</b>                             | 2,4-OMe                            | 16                         | 17                              | 17                         | 14                            |
| <b>5i</b>                             | 2,5-OMe                            | 15                         | 16                              | 15                         | 14                            |
| <b>5j</b>                             | 3,4,5-OMe                          | 24                         | 23                              | 20                         | 19                            |
| <sup>a</sup> Standard Drug            | -                                  | 28                         | 24                              | 21                         | 21                            |

<sup>a</sup> Ciprofloxacin (50 µg mL<sup>-1</sup> of DMSO); <sup>b</sup> Zone of inhibition - good activity: 18-24 mm; moderate activity: 12-17 mm; weak activity: < 12 mm;

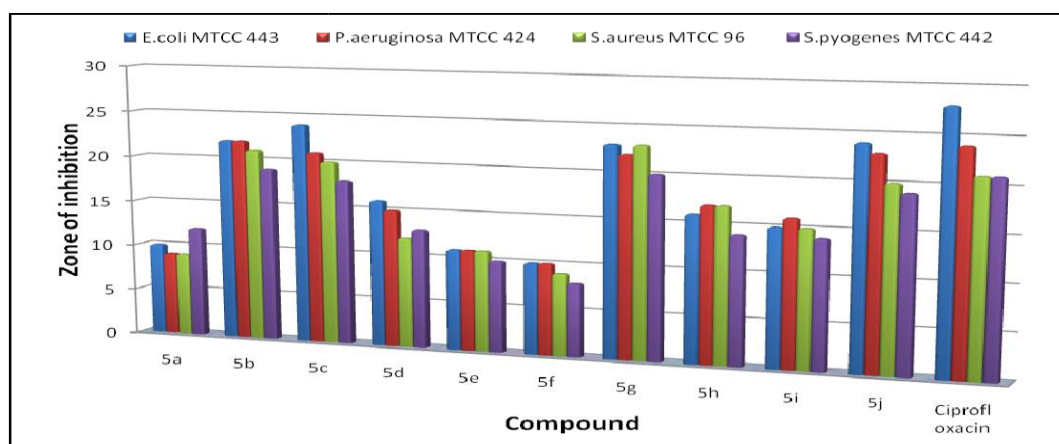


Fig.2: Graphical representation of antibacterial activity of compounds 5a-j

## Experimental Procedure and Characterization Data

### Preparation of ethyl 2-aminobenzoate (2)

To a stirred solution of 2-amino-benzoic acid (1.5g, 10.94 mmol) in ethanol (15.0 mL) was added conc.  $\text{H}_2\text{SO}_4$  (0.2 mL) and refluxed for 10 h. After completion of the reaction (monitored by T.L.C), the reaction mixture was diluted with water and extracted with ethyl acetate (25 mL). The organic layer was washed with aqueous saturated  $\text{NaHCO}_3$  solution, water and brine solution. The organic layer was separated, dried over  $\text{Na}_2\text{SO}_4$ , filtered and evaporated to obtain compound **2** as a pale yellow liquid. Yield: 1.5g, 83%. The crude compound was utilized in the next step without further purification.

### Preparation of ethyl 2-(benzamido) benzoate (3)

To a solution of ethyl 2-aminobenzoate **2** (1.5g, 9.08 mmol) in dichloromethane, cooled to  $10^\circ\text{C}$ , was added triethyl amine (1.10g, 10.90 mmol) followed by drop wise addition of benzoyl chloride (1.3g, 9.25 mmol). The reaction mixture was stirred at room temperature for 3h and then diluted with water followed by ethyl acetate (50 mL). The organic layer was separated, washed with water and brine solution, dried over  $\text{Na}_2\text{SO}_4$ , filtered and evaporated to obtain ethyl 2-(benzamido) benzoate **3** as a pale yellow viscous liquid; Yield: 1.5g, 60%; IR (KBr):  $\nu_{\text{max}}$  3245 (NH str), 1687 (C=O str, ester), 1671 (C=O str, amide), 1531 (NH def)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  11.64 (s, 1H, NH), 8.57 (d,  $J = 8.8$  Hz, 1H, Ar), 8.03 (dd,  $J = 8.0, 1.4$  Hz, 1H, Ar), 8.01 – 7.94 (m, 2H, Ar), 7.74 – 7.54 (m, 4H, Ar), 7.26 (t,  $J = 7.7$  Hz, 1H), 4.36 (q,  $J = 7.1$  Hz, 2H,  $\text{CH}_2$ ), 1.32 (t,  $J = 7.1$  Hz, 3H,  $\text{CH}_3$ ); ESI MS:  $m/z$  269.8 ( $\text{M}+\text{H}$ ) $^+$ .

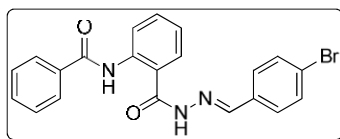
### Preparation of 2-(benzamido)benzo hydrazide (4)

To a stirred solution of compound **3** (1.5g, 5.60 mmol) in 15mL ethanol, hydrazine hydrate (2.29 g, 45.80 mmol) was added and refluxed for 6 h. The reaction mixture was cooled to room temperature and the precipitated solid was filtered, washed with pet-ether and dried under vacuum to obtain 2-(benzamido)benzo hydrazide (**4**) as white solid. Yield: 1.10g, 80%; M.p: 185-186  $^\circ\text{C}$ ; IR (KBr):  $\nu_{\text{max}}$  3317 (NH str), 1649 (C=O str), 1634 (C=O str), 1537 (NH def)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  12.57 (s, 1H, NH), 10.21 (s, 1H, NH), 8.67 (d,  $J = 8.4$  Hz, 1H, Ar), 7.97 (d,  $J = 7.3$  Hz, 2H, Ar), 7.79 (d,  $J = 7.8$  Hz, 1H, Ar), 7.62 (m, 4H, Ar), 7.18 (t,  $J = 7.8$  Hz, 1H, Ar), 4.69 (s, 2H,  $\text{NH}_2$ ); ESI MS:  $m/z$  255.8 ( $\text{M}+\text{H}$ ) $^+$ .

### General experimental procedure for the preparation of hydrazone derivatives 5a-j

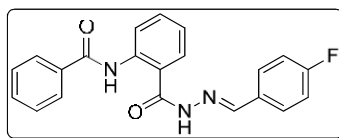
To a stirred solution of compound **4** (100 mg, 0.392 mmol) in 1.0 mL ethanol, was added the corresponding aromatic aldehydes **a-j** (0.392 mmol) and refluxed for 1 h. The reaction mixture was cooled to room temperature and the solid precipitated was filtered and dried to obtain the hydrazone derivatives **5a-j**. Yields of the products varied between 85 to 95%.

**Spectral characteristics of (*E*)-*N'*-(4-bromobenzylidene)-2-benzamido)benzo hydrazide (5a)**



White solid; Yield: 90%; M.p: 92-93°C ; IR (KBr):  $\nu_{\max}$  3328 (NH str), 1652 (C=O & C=N str), 1530 (NH def)  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  12.21 (s, 1H, NH), 11.89 (s, 1H, NH), 8.56 (d,  $J = 8.3$  Hz, 1H, Ar), 8.44 (s, 1H, N=CH), 7.96 (d,  $J = 7.2$  Hz, 2H, Ar), 7.91 (d,  $J = 7.6$  Hz, 1H, Ar), 7.76- 7.57 (m, 8H, Ar), 7.30 (t,  $J = 7.6$  Hz, 1H, Ar); ESI MS:  $m/z$  421.8 (M+H) $^+$  and 423.8 (M+2+H) $^+$ .

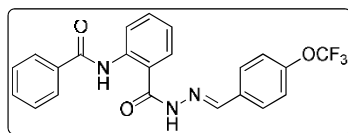
**Spectral characteristics of (*E*)-*N'*-(4-fluorobenzylidene)-2-(benzamido) benzo hydrazide (5b)**



White solid; Yield: 88%; M.p: 112-113°C ;  $^1\text{H NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  12.16 (s, 1H, NH), 11.93 (s, 1H, NH), 8.57 (d,  $J = 8.5$  Hz, 1H, Ar), 8.46 (s, 1H, N=CH), 7.97 (d,  $J = 7.4$  Hz, 2H, Ar), 7.91 (d,  $J = 7.6$  Hz, 1H, Ar), 7.83 (t,  $J = 7.0$  Hz, 2H, Ar), 7.69-7.56 (m, 4H, Ar), 7.34- 7.27 (m, 3H, Ar); ESI MS:  $m/z$  361.9 (M +H) $^+$ ;

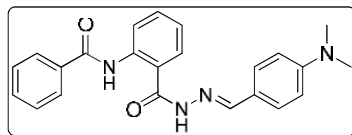
**Spectral characteristics of (*E*)-*N'*-(4-(trifluoromethoxy) benzylidene)-2 (benzamido) benzohydrazide (5c)**

White solid; Yield: 90%; M.p: 88-89°C; IR (KBr):  $\nu_{\max}$  3323 (NH str), 1660 (C=O str), 1650



(C=N str), 1525 (NH def)  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  12.23 (s, 1H, NH), 11.89 (s, 1H, NH), 8.56 (d,  $J = 8.3$  Hz, 1H, Ar), 8.49 (s, 1H, N=CH), 7.97 (d,  $J = 7.6$  Hz, 2H, Ar), 7.94-7.82 (m, 3H, Ar), 7.71-7.56 (m, 4H, Ar), 7.48 (d,  $J = 8.2$  Hz, 2H, Ar), 7.30 (t,  $J = 7.6$  Hz, 1H, Ar); ESI MS:  $m/z$  427.9 (M+H) $^+$ .

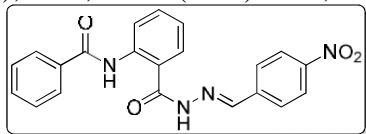
**Spectral characteristics of (*E*)-*N'*-(4-(dimethylamino) benzylidene)-2-(benzamido) benzohydrazide (5d)**



White solid; Yield: 88%; M.p: 76-77°C; IR (KBr):  $\nu_{\max}$  3192 (NH str), 1663 (C=O str), 1645 (C=N str), 1523 (NH def)  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  12.11 (s, 1H, NH), 11.86 (s, 1H, NH), 8.61 (d,  $J = 8.3$  Hz, 1H, Ar), 8.32 (s, 1H, N=CH), 7.97 (d,  $J = 7.2$  Hz, 2H, Ar), 7.90 (d,  $J = 7.6$  Hz, 1H, Ar), 7.70 - 7.59 (m, 4H, Ar), 7.57 (d,  $J = 8.7$  Hz, 2H, Ar), 7.27 (t,  $J = 7.6$  Hz, 1H, Ar), 6.77 (d,  $J = 8.7$  Hz, 2H, Ar), 2.99 (s, 6H, NCH $_3$ ); ESI MS:  $m/z$  386.9 (M+H) $^+$ .

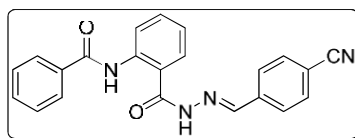
**Spectral characteristics of (*E*)-*N*'-(4-nitrobenzylidene)-2-(benzamido)benzo hydrazide (5e)**

White solid; Yield: 96%; M.p: 119-120°C; IR (KBr):  $\nu_{\max}$  3263 (NH str), 1667 (C=O str), 1651 (C=N str), 1519 (NH def), 1518, 1340 (NO<sub>2</sub>) cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  12.42



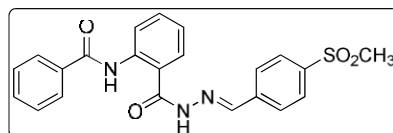
(s, 1H, NH), 11.79 (s, 1H, NH), 8.56 (s, 1H, N=CH), 8.52 (d, *J* = 8.3 Hz, 1H, Ar), 8.33 (d, *J* = 8.6 Hz, 2H, Ar), 8.03 (d, *J* = 8.6 Hz, 2H, Ar), 7.97 (d, *J* = 7.1 Hz, 2H, Ar), 7.92 (d, *J* = 7.6 Hz, 1H, Ar), 7.71 – 7.54 (m, 4H, Ar), 7.32 (t, *J* = 7.6 Hz, 1H, Ar); ESI MS: *m/z* 388.9 (M+H)<sup>+</sup>.

**Spectral characteristics of (*E*)-*N*'-(4-cyanobenzylidene)-2-(benzamido)benzo hydrazide (5f)**



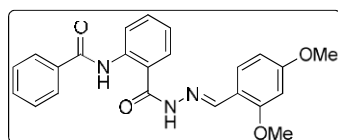
White solid; Yield: 84%; M.p: 126-128°C ; IR (KBr):  $\nu_{\max}$  3263 (NH str), 1665 (C=O str), 1654 (C=N str), 1521 (NH def), 2221 (C≡N) cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  12.36 (s, 1H, NH), 11.81 (s, 1H, NH), 8.53 (d, *J* = 8.5 Hz, 1H, Ar), 8.51 (s, 1H, N=CH), 8.02 – 7.86 (m, 7H, Ar), 7.69 – 7.55 (m, 4H, Ar), 7.31 (t, *J* = 7.5 Hz, 1H, Ar); ESI MS: *m/z* 368.9 (M+H)<sup>+</sup>.

**Spectral characteristics of (*E*)-*N*'-(4-(methyl sulfonyl) benzylidene)-2-(benzamido)benzo hydrazide (5g)**



White solid; Yield: 86%; M.p: 132-133°C ; IR (KBr):  $\nu_{\max}$  3354 (NH str), 1667 (C=O str), 1650 (C=N str), 1531 (NH def) cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  12.36 (s, 1H, NH), 11.81 (s, 1H, NH), 8.54 (s, 1H, N=CH), 8.53 (d, *J* = 8.2 Hz, 1H, Ar), 8.02 (s, 4H, Ar), 7.97 (d, *J* = 7.2 Hz, 2H, Ar), 7.92 (d, *J* = 7.6 Hz, 1H, Ar), 7.73 – 7.47 (m, 4H, Ar), 7.31 (t, *J* = 7.6 Hz, 1H, Ar), 3.27 (s, 3H, SCH<sub>3</sub>); ESI MS: *m/z* 421.9 (M+H)<sup>+</sup>.

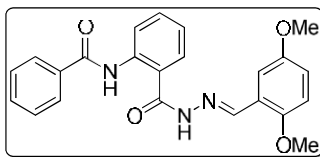
**Spectral characteristics of (*E*)-*N*'-(2,4-dimethoxybenzylidene)-2-(benzamido)benzo hydrazide (5h)**



White solid; Yield: 88%; M.p: 141-142°C ; IR (KBr):  $\nu_{\max}$  3246 (NH str), 1674 (C=O str), 1636 (C=N str), 1534 (NH def) cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  12.12 (s, 1H, NH), 12.00 (s, 1H, NH), 8.74 (s, 1H, N=CH), 8.61 (d, *J* = 8.3 Hz, 1H, Ar), 7.97 (d, *J* = 7.4

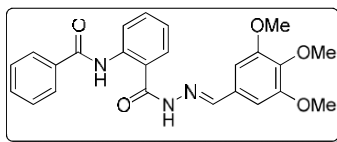
Hz, 2H, Ar), 7.93 (d,  $J = 7.6$  Hz, 1H, Ar), 7.88 – 7.81 (m, 1H, Ar), 7.70 – 7.57 (m, 4H, Ar), 7.27 (t,  $J = 7.6$  Hz, 1H, Ar), 6.69 – 6.63 (m, 2H, Ar), 3.87 (s, 3H, OCH<sub>3</sub>), 3.84 (s, 3H, OCH<sub>3</sub>); ESI MS:  $m/z$  404.0 (M+H)<sup>+</sup>.

**Spectral characteristics of (*E*)-N'-(2, 5-dimethoxybenzylidene)-2-(benzamido) benzohydrazide (5i)**



White solid; Yield: 92%; M.p: 100-101°C; IR (KBr):  $\nu_{\max}$  3204 (NH str), 1650 (C=O, C=N str), 1525 (NH def) cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  12.13 (s, 1H, NH), 11.96 (s, 1H, NH), 8.81 (s, 1H, N=CH), 8.57 (d,  $J = 8.3$  Hz, 1H, Ar), 7.97 (d,  $J = 7.4$  Hz, 2H, Ar), 7.93 (d,  $J = 7.6$  Hz, 1H, Ar), 7.70 – 7.56 (m, 4H, Ar), 7.40 (d,  $J = 3.2$  Hz, 1H, Ar), 7.28 (t,  $J = 7.6$  Hz, 1H, Ar), 7.08 (d,  $J = 8.9$  Hz, 1H, Ar), 7.04 (dd,  $J = 8.9, 3.2$  Hz, 1H, Ar), 3.83 (s, 3H, OCH<sub>3</sub>), 3.77 (s, 3H, OCH<sub>3</sub>); ESI MS:  $m/z$  404.0 (M+H)<sup>+</sup>.

**Spectral characteristics of (*E*)-N'-(3, 4, 5-trimethoxybenzylidene)-2-(benzamido) benzohydrazide (5j)**



White solid; Yield: 95%; M.p: 118-119°C; IR (KBr):  $\nu_{\max}$  3219 (NH str), 1665 (C=O str), 1649 (C=N str), 1513 (NH def) cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  12.11 (s, 1H, NH), 11.82 (s, 1H, NH), 8.52 (d,  $J = 8.3$  Hz, 1H, Ar), 8.38 (s, 1H, N=CH), 7.96 (d,  $J = 7.6$  Hz, 2H, Ar), 7.89 (d,  $J = 7.6$  Hz, 1H, Ar), 7.70 – 7.56 (m, 4H, Ar), 7.30 (t,  $J = 7.6$  Hz, 1H, Ar), 7.04 (s, 2H, Ar), 3.85 (s, 6H, OCH<sub>3</sub>), 3.72 (s, 3H, OCH<sub>3</sub>); ESI MS:  $m/z$  434.0 (M+H)<sup>+</sup>.

**Conflict of interest:** None

**Conclusion:** The newly synthesized Hydrazone derivatives of *o*-Aminobenzoic acid (**5a-5j**) were tested against Gram negative strains of (i) *Escherichia coli* (MTCC 443) and (ii) *Pseudomonas aeruginosa* and Gram-positive strains of (iii) *Staphylococcus aureus* and (iv) *Streptococcus pyogenes* by agar diffusion method using ciprofloxacin as standard antibiotic. All the compounds shown moderate anti-bacterial activity. These are new addition to the hydrazone chemistry.

**References**

- I. Rani, P.; Srivastava, P.K.; Kumar, A. Indian J. Chem. 2003, 42B, 1729–1733.
- II. Thongtan, J.; Saenboonrueng, J.; Rachtawee, P.; Isaka, M. J. Nat. Prod. 2006, 69(4), 713-714.
- III. De Luca, S; Saviano, M.; Lassiani, L.; Yannakopoulou, K.; Stefanidou, P.; Aloj, L.; Morelli, G.; Varnavas, A. J. Med. Chem. 2006, 49(8), 2456-2462.
- IV. Cocco, M.T.; Congiu, C.; Lilliu, V.; Onnis, V. Bioorg. Med. Chem. Lett. 2004, 14(23), 5787-5791.
- V. Shou, Q.; Banbury, L.K.; Maccarone, A.T.; Renshaw, D.E.; Mon, H.; Griesser, S.;



- Griesser, H.J.; Blanksby, S.J.; Smith, J.E.; Wohlmuth, H. *Fitoterapia* 2014, 93, 62-66.
- VI. Kar, A.; Gugnani, H.C.; Madumere, U.A. *Pharmazie* 1980, 35, 466-468.
- VII. Shearer, B.G.; Patel, H.S.; Billin, A.N.; Way, J.M.; Winegar, D.A.; Lambert, M.H.; Xu, R.X.; Leesnitzer, L.M.; Merrihew, R.V.; Huet, S.; Wilson, T.M. *Bioorg Med Chem Lett.* 2008, 18, 5018–5022.
- VIII. Jonathan, D.B.; Nyack, N.Y.; Thomas, R.B.; Phoenixville, P.A. US patent: US 20080269333A1, 2008.
- IX. Lassiani, L.; Pavan, M.V.; Berti, F. *Bioorg. Med. Chem.* 2009, 17(6), 2336-2350.
- X. Merk, D.; Gabler, M.; et al. *Bioorg. Med. Chem.* 2014, 22(8), 2447-2460.
- XI. Demirbas, N.; Karaoglu, S.; Demirbas, S.A.; Demirbas, A.; Sancak, K. *Eur. J. Med. Chem.* 2004, 39(9), 793-804.
- XII. Mohareb, R.M.; Fleita, D.H.; Sakka, O.K. *Molecules* 2011, 16, 16-27.
- XIII. Sriram, D.; Yogeewari, P.; Madhu, K. *Bioorg. Med. Chem. Lett.* 2005, 15(20), 4502-4505. 14. Sriram, D.; Yogeewari, P.; Madhu, K. *Bioorg. Med. Chem.* 2006, 14(4), 876- 878.
- XIV. Moldovan, C.M.; Oniga, O.; Parvu, A. *Eur. J. Med. Chem.* 2011, 46(2), 526–534.
- XV. Todeschini, A.R.; Ana Luisa, P.; da Miranda, Kelly Christine, M.; da Silva, S.; Parrini, C.; Eliezer Barreiro, J. *Eur. J. Med. Chem.* 1998, 33(3), 189-199.

Received on November 11, 2021.