

# Antibacterial Screening of the Synthesized (Z) -3- (2-phenylhydrazono) benzofuran-2 (3H) against Gram positive and Gram negative Bacteria

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**ABSTRACT:** The catalytic olefination of salicylphenylhydrazone was carried out and NMR and X-ray methods were used to prove the conversion of the reaction product dichlorodiazobutadiene to (Z) -3- (2-phenylhydrazono) benzofuran-2 (3H) -one. It is clearly revealed from the results that, (Z) -3- (2-phenylhydrazono) benzofuran-2 (3H) -one showed better results in case of *A.baumannii* BDU32 while compound was less sensitive against *P.aeruginosa* BDU49 and *K.pneumoniae* BDU44 at all tested concentrations.

**KEYWORDS:** antibacterial activity, gram positive bacteria, gram negative bacteria, benzofuran derivatives

## I. INTRODUCTION

The increasing and emerging bacterial infections as well as resistance, render it important to discover new strategies and searching antimicrobial agents to develop the next generation of drugs for treatment of microbial infections [1-3].

Derivatives of benzofuran have several biological properties such as inflammatory, antimicrobial, antifungal, antihyperglycemic, analgesic, antiparasitic and antitumor activities. According to a wide range of biological properties, benzofuran derivatives are increasingly drawing much interest as pharmacological agents against several bacterial infections [4-9]. In order to great biological importance of this scaffold, investigation of different methods for synthesis and structural modification of benzofuran derivatives is the main goal of some research groups [10-11].

In the literature data, in recent years, have several methods for the synthesis of benzofuranone rings. Among them are: an effective Pd-catalyzed hydroesterification of alkenylphenols with phenyl formate as CO surrogate enables the synthesis of various lactones [12]. Pd(II)-catalyzed C-H activation of phenylacetic acids followed by an intramolecular C-O bond formation afforded benzofuranones. A metal-free tandem Friedel-Crafts/lactonization reaction to 3,3-diaryl or 3-alkyl-3-aryl benzofuranones is catalyzed by HClO<sub>4</sub>. The reaction of various tertiary  $\alpha$ -hydroxy acid esters with substituted phenols affords the desired products in rich diversity [13]. Enantioselective activation of C-H

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phenylacetic acids catalyzed by the Pd (II) catalyst and subsequent intramolecular formation of the C-O bond led to the formation of chiral benzofuranones [14].

Reaction of 3 - ((dimethylamino) methylene) - and 3-ethoxymethylenebenzofuran-2 (3H) -one with various amine derivatives produced a series of substituted 3-R-aminomethylenebenzofuranones of interest in the search for biologically active compounds [15]. Reaction of 3 - [(dimethylamino) methylene- and 3-ethoxymethylene] benzofuran-2- (3H) -one with various amine derivatives was used to synthesize a series of substituted 3-R-aminomethylenebenzofuranones. Synthesized compounds are a promising basis for the search for biologically active substances.

Taking in account all this information, we became interested to synthesize benzofuran derivatives by new methods and also screening for antibacterial activity against gram positive (*Staphylococcus aureus* BDU23) and gram negative bacteria (*Escherichia coli* BDU12, *Pseudomonas aeruginosa* BDU49, *Klebsiella pneumonia* BDU44 and *Acinetobacter baumannii* BDU32).

## II. MATERIAL AND METHODS

NMR <sup>1</sup>H and <sup>13</sup>C spectra were recorded on a Bruker Advance 300 spectrometer (operating frequency 300.1 MHz and 100.6 MHz) in CDCl<sub>3</sub> and DMSO-d<sub>6</sub>. SiMe<sub>4</sub> is used as an internal standard. NTX was conducted on the Silufol UB-254 board, and for the apparent visibility of emerging spots an acidified KMnO<sub>4</sub> solution and UV lamps are used. Column chromatography was carried out on Merk silica gel (63-200).

### *Synthesis of phenylhydrazones (general procedure):*

In a three-neck flat bottom flask to phenylhydrazine (2 mmol), ethanol (20-50 ml) and acetic acid (1 ml) are added. The flask is equipped with a reflux condenser, a dropping funnel and a thermometer. Using a dropping funnel, 2.1 mmol of aldehyde was added to the mixture and the reaction mixture was heated with constant stirring. When the temperature reaches 78 ° C, the mixture is boiled for another 5-10 minutes. The reaction mixture was then cooled to room temperature, and 50 ml of water was added thereto. Intensively stirring the temperature is again increased to 60 ° C and the heating is stopped. The reaction mixture, cooled to room temperature, is filtered. As necessary, the product residue was washed with water. The resulting hydrazone is dried at room temperature (15-20 hours). NMR <sup>1</sup>H and <sup>13</sup>C spectra correspond to the literature data.

### *Synthesis of (Z) -3- (2-phenylhydrazone) - benzofuran-2 (3H) -one derivatives (general procedure):*

2 mmol of phenylhydrazone are added first, followed by 20 ml of DMSO, then (581 mg, 2.5 mol / eq) of TMEDA. After all this, CuCl (6 mg, 3 mole%) is added. And at the very end, add CCl<sub>4</sub> (7-10 mol / eq, 2.2-3 g). The magnetic stirrer is brought into operation. The reaction is checked by means of an NTX. Usually, the reaction stops within 1.5-3 hours. The reaction mixture is transferred to a separate funnel, add 50-60 ml of water. It is extracted with methylene chloride (3 \* 15 ml). The organic phase is first washed with water (3 \* 50 ml), and then once with a saturated solution of NaCl (1 \* 50 ml). It is dried with Na<sub>2</sub>SO<sub>4</sub> (MgSO<sub>4</sub>), filtered and dichloromethane is distilled using a rotary evaporator in a vacuum rotor. Dichlorodiazabutadiene as a viscous mass is heated by dissolving in ethyl alcohol and upon cooling of the solution in the flask, corresponding crystals of the benzofuran-2-one derivatives are formed. The yield is 67%.

### *Bacterial strains and growth conditions*

*Escherichia coli* BDU12, *Staphylococcus aureus* BDU23, *Pseudomonas aeruginosa* BDU49, *Klebsiella pneumoniae*BDU44 and *Acinetobacter baumannii* BDU32 strains were grown in Brain Heart Infusion (BHI) medium (Conda) with shaking at 200 rpm, or on BHI agar, at 37 °C.

### *Determination of antibacterial activity*

The antibacterial screening of benzofuran is evaluated against gram positive bacteria, *Staphylococcus aureus* BDU23 and gram negative bacteria *Escherichia coli* BDU12, *Pseudomonas aeruginosa* BDU49, *Klebsiella*

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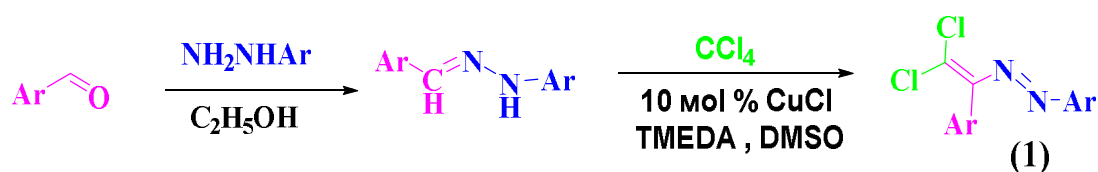
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*pneumoniae* BDU44, *Acinetobacter baumannii* BDU32 by agar well diffusion method [16-17]. The test cultures were grown in autoclaved Brain Heart Infusion (BHI) media and 0.5 McFarland bacterial culture ( $1.5 \times 10^8$  cfu/ml) were swabbed onto the agar plate. The 8 mm wells were made in the center of the plate with a sterile cork borer. Each wells filled with the decreasing concentrations (0.5%, 0.2%, 0.1%, and 0.05%) of substance which was solved in the ethanol and solvent used as a control during investigation. The experiment was carried out in triplicate. The antimicrobial activity was determined by measuring the diameter of the complete inhibition (mm) zones after incubation of plates at 37 °C for 24 h.

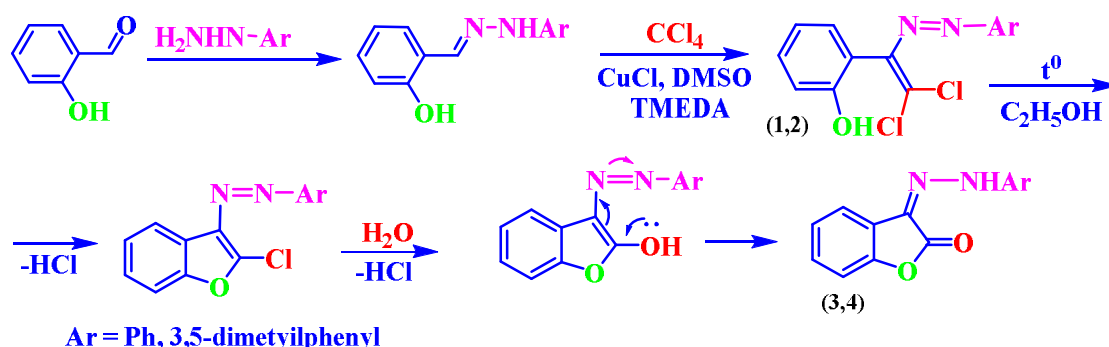
### III. EXPERIMENTAL RESULTS

In previous studies, we investigated the catalytic olefination reaction of the corresponding phenylhydrazones of many aldehydes and by NMR and X-ray methods, it was shown that dichlorodiazabutadiene (1) was obtained as reaction products (Scheme 1) [18 - 23].



**Scheme 1.** General reaction scheme for catalytic olefination

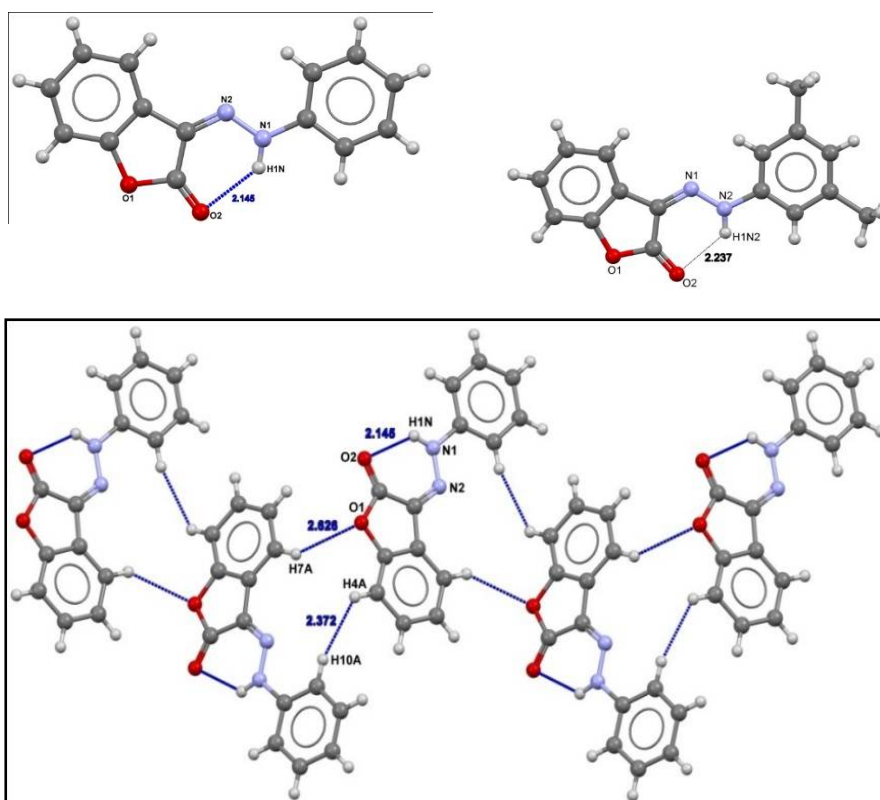
Considering the fact that synthesized compounds play the role of the most important synthons in organic synthesis, and also have high physiological activity, the reaction of catalytic olefination with salicylic aldehyde was continued. During the analysis of the reaction product, a high probability of converting the corresponding synthesized dichlorodiazabutadiene (1) to the compound (Z) -3- (2-phenylhydrazono) benzofuran-2 (3H) -one according to the following scheme (Scheme 2).



**Scheme 2.** Probable scheme for the synthesis of (Z) -3- (2-phenylhydrazono) benzofuran-2 (3H) -ones.

Single crystals of synthesized compounds were obtained and the presence of intra- and intermolecular hydrogen bonds was established by X-ray methods.

Thus, the conversion of dichlorodiazabutadienes (1,2), synthesized on the basis of the catalytic olefination reaction from phenylhydrazones of salicylic aldehyde to (Z) -3- (2-phenylhydrazono) benzofuran-2 (3H) -ones (3,4), has been proved by NMR and X-ray methods, the presence of intra- and intermolecular hydrogen bonds in the crystal was established and the physiological activity of the compound was checked (3).



**Fig. 1.** Molecular structure of (Z) -3- (2-phenylhydrazono) benzofuran-2 (3H) -ones. Hydrogen bonds are shown in dashed lines

### Antibacterial studies of synthesized compound

Antibacterial activity was carried out for synthesized benzofuran derivative. The sample was tested at the concentrations ranging from 0.5% to 0.05% in ethanol (95%) using agar well diffusion method. Solvent (ethanol 95%) was used as a control in order to exclude the possible bacterial inhibition during experiments and few effects were determined against tested strains. As shown in table (table 1) inhibition zones of the tested chemical substance were different not only depending on the investigated bacterial strains and but also concentration of the compound.

**Table**  
**Antibacterial activity results for (Z) -3- (2-phenylhydrazono) benzofuran-2 (3H) –one**

Bacterial strains	Concentration (in %) and inhibition zone (in mm) of tested compound				Control (ethanol 95%)
	0.5	0.2	0.1	0.05	
<i>Escherichia coli</i> BDU12	19	17	13	10	0
<i>Staphylococcus aureus</i> BDU23	21	17	12	11	2
<i>Pseudomonas aeruginosa</i> BDU49	16	14	12	11	2
<i>Klebsiella pneumoniae</i> BDU 44	17	16	12	0	4
<i>Acinetobacter baumannii</i> BDU32	22	19	16	12	3

\*The standard error for the experiments was  $\pm 0.001$  cm

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From the results it has been observed that, the compound at the concentration of 0.5 % showed promising activity against test cultures as compared to other concentrations. (Z) -3- (2-phenylhydrozono) benzofuran-2 (3H) –one is more active against *S.aureus* BDU23 while, less activity was detected when compound tested with *P.aeruginosa* BDU49.

The highest zone of inhibition 19 mm was detected in case of *A.baumannii* BDU32 at the concentration of 0.2 %. Compound showed same inhibition zones (17 mm) against *E.coli* BDU12 and *S.aureus* BDU23.

Compound at the concentration of 0.1% showed better result (16 mm) when tested with the *A.baumannii* BDU32 while at the concentration of 0.05% zones of inhibition were similar for all studied pathogens except *K. pneumoniae* BDU 44. So, any inhibition was not detected against *K.pneumoniae* BDU44 when the effectiveness of the compound was tested at concentration of 0.05%. It is clearly revealed from the results that our compound showed moderate inhibitory activity against tested cultures

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