Synthesis, Characterization and the Antimicrobial Activity of 3-(2-phenylhydrazinyl)-2-benzofuran-1(3H)-one

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Abstract:

High yield efficiency and minimum steps are important strategies in synthetic organic chemistry. In this study, 3-(2-phenylhydrazinyl)-2-benzofuran-1(3H)-one was synthesized in one step, with high yield, through the condensation of two components. 2-carboxybezaldehyde (A) and phenylhydrazine (B) in methanol. The chemical structur was confirmed using a molecular spectroscopy tools (^{1}H,^{13}C-NMR, IR). The in vitro antibacterial and antifungal activities of this product was screened against two fungal strains (Candida albicans and Thielaviopsis paradoxa) and

against three bacterial strains (Staphylococcus aureus, Escherichia coli and Bacillus subtilis). Results showed that the compound has antifungal and antibacterial activity.

Introduction:

The Phthalide (2-benzofuran-1(3*H*)-one) ring system of a cyclic lactone is still of interest due to its exhibiting diverse biological and pharmacological properties, such as fungicidal, bactericidal, herbicidal, analgesic, pesticidal, hypotensive, anorexic, anti-inflammatory, antioxidant, antifungal, anti-platelet, anticonvulsant, anti HIV and vasorelaxant activities.

In addition, phthalide derivatives are useful in the treatment of circulatory and heart-related diseases[1-16].

On the other hand. Some of phthalide derivatives appear forming part of the structure of natural products such as fuscinarin isolated from soil fungus was found to compete effectively with macrophage inflammatory protein (MIP)-1α for binding to human CCR5. An important anti HIV-1 target that interferes with HIV entry into cells[17], noscapine[18] has antitussive drug and anti-tumor properties, typhaphthalide[19] isolated from Typha capensis, and synthetic compounds like some spirolactones[20] inhibitors of the influenza virus type B.

3-Butylphthalides isolated from the basidiomycete Phanerochaete velutina CL6387 appear to be specific for Helicobacter pylori[21] and Cytosporone E has antifungal activities[22].

(-)-Alcyopterosin E which contains phthalides bone shows mild cytotoxicity toward Hep-2 (human larynx carcinoma) cell line[22] and many

phthalides-containing plants have been used worldwide as herbal remedies in traditional and folk medicines, dietary supplements and food flavorings.

It has been reported that derivatives of phthalides show potent antigonistic effect[23] on acetylcholine - and histamine – induced tracheal muscle constriction. And some of them can be used as a blood viscosity reducing agent[24] .

Moreover, 3 -Arylphthalides, are useful intermediates for the synthesis of tri and tetracyclic natural products such as anthracycline antibiotics[25]. On the other hand, Phenylhydrazine itself is an antipyretic drug[26].

The varied biological and pharmacological properties of phthalide derivatives motivated us to prepare a new compound via a condensation of two components 2-carboxybezaldehyde (A) and phenylhydrazine (B). This method involves the same reaction conditions used previously[27].

Experimental:

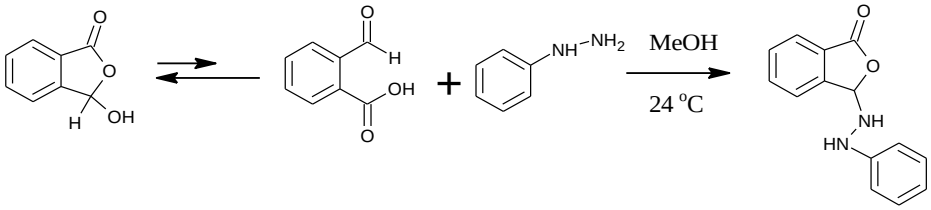
All melting points were measured on electrotherrmal melting point and were uncorrected, infrared (IR) spectra were measured using Pye-Unicam SP- 300 spectrophotometer as a potassium bromide disc. ^{1}H, ^{13}C - NMR were measured using a Bruker operating at 300 MHz spectrometer. Thin layer chromatography was performed using silica gel 60 F 254 pre coated almunium sheets from Merck. The progress of the reaction was monitored by TLC. Reagents and solvents for the synthesis were obtained from the Aldrich Chemical Co., and were used without additional purification.

Reaction of 2-carboxybenzaldehyde with phenyl hydrazine.

Equimolar quantities of 2-carboxybenzaldehyde (1.5g, 0.01mole) and phenyl hydrazine (1.08g, 0.01mole) were stirred in methanol(15ml) at room temperature for one hour. Then the separated solid was collected and the recrystallized from methanol afforded the target compound as a white needles.

General Equation:

Reaction of 2-carboxybenzaldehyde with phenyl hydrazine:



(A) (B)

Results and Discussion:

In our previous work[27] we have used derivatives of hydrazine (2,4-dinitrophenylhydrazine) and 2-carboxybenzaldehyde in stirring methanol, resulted in formation of one single compound of phthalide derivatives.

In the present study, using phenyhydrazine itself with 2-carboxybenzaldehyde applying the same reaction conditions afforded a crystalline solid product. TLC of the crude product indicating the presence of one single component, the analytical data of isolated phthalide are given in (table-1). The isolated product formed was confirmed by spectral analysis. Infrared spectra(Fig.**3)** shows, broad peak at 1736cm^{-1} suggested presence of five member lactone ring, and the peak at 3335 cm^{-1} attributed

to stretching vibrations of the amino group (NH). The ^{1}H-NMR spectra ($CDCl_3$) of the target molecule, shows the presence of two protons as a broad singlet exchanges with deuterated water (D_2O), in the range of 1.2-1.9 ppm due to two NH protons and one proton a singlet in the 7.4 ppm is due to phthalidyl proton (fig.1 and table-2), the rest of spectrum due to nine aromatic protons in the range of 7.4-8.53 ppm.

The ^{13}C -NMR(d^6-DMSO) spectrum (Fig.**2)** showes twelve resolved carbon signals, the carbonyl carbon signal was observed at δ 159.45. The aniline carbon (C-NH) signal was observed at δ142.10 and the rest of the resolved carbon signals are corresponding to the aromatic carbon atoms of the product. These Observations confirm the formation of 3-[2-(phenyl)hydrazinyl]-2-benzofuran-1(3*H*)-one). This compound can only arise if the starting amines react with the lactol form of the acid (**A**). Through nucleophilic substitution reaction (S_N2) on C-3. The deshielding of H-3 in this compound, compared with CH-N alkyl analogs can be attributed to the anisotropic effect caused by the hetero-aromatic ring.

Bacterial strains:

The following strains were used in antibacterial study; B.subtilis ATCC 6633, S. aureus ATCC 25923, E, coli ATCC 25922 and in antifungal study C.albicans, Thielaviopsis paradoxa.

Screening for biological activity:

The Antimicrobial Activities of synthesized compound 3-(2-phenylhydrazinyl)-2-benzofuran-1(3H)-one was screened in vitro for their antibacterial activity against Bacillus subtilis, Escherichia coli and Staphylococcus aureus by the ditch-plate technique[28]. The synthesized

compound was also tested for antifungal activity against Candida albicans and Thielaviopsis paradoxa by paper-disc diffusion method[29] using concentrations of 5 mg/mL. Nutrient agar was employed as culture media and DMF was used as solvent control for both antibacterial and the results of antimicrobial activity showed clearly that all tested compound exhibited antifungal activity against yeast-like fungi, The antibacterial activity of the compound showed a good activity against four used strains(table-4).

Table -1

Analytical data of 3-(2-phenylhydrazinyl)-2-benzofuran1(3*H*)- one.

Phthalide	Molecular Formula	Color	Melting Point (°C)	Yield (%)	Solvent for Crystallization
3-(2-phenylhydrazinyl)-2-benzofuran-1(3*H*)-one	$C_{14}H_{12}N_2O_2$	White	208-210	90	Methanol

Table -2

Spectral data (C^{13} and ^{1}H-NMR) of 3-(2-phenylhydrazinyl)-2-benzofuran-1(3*H*)-one.

Phthalide	^{13}C- NMR (DMSO-d_6,TMS, ppm)	^{1}H-NMR ($CDCl_3$,TMS, ppm)
3-[2-phenylhydrazinyl]-2-benzofuran-1(3*H*)-one.	125.98,126.39, 127.52,128.06,128.79,129.04, 129.75, 132.28,133.77,138.75,142.10,159.45.	1.20(br,s,NH), 1.66(br,s,NH), 7.4(br,s,H-3), 7.48-8.53 (m,10H, aromatic protons)

Table-3

Spectral data (IR) of 3-(2-phenylhydrazinyl)-2-benzofuran-1(3H)-one.

Phthalide	**IR (KBr, cm^{-1})**
3-(2-phenylhydrazinyl)-2-benzofuran-1(3*H*)-one.	3332 (NH), 2099 (C-H Aromatic), 1739(C = O), 1592(C = C). 1072 (C-N)

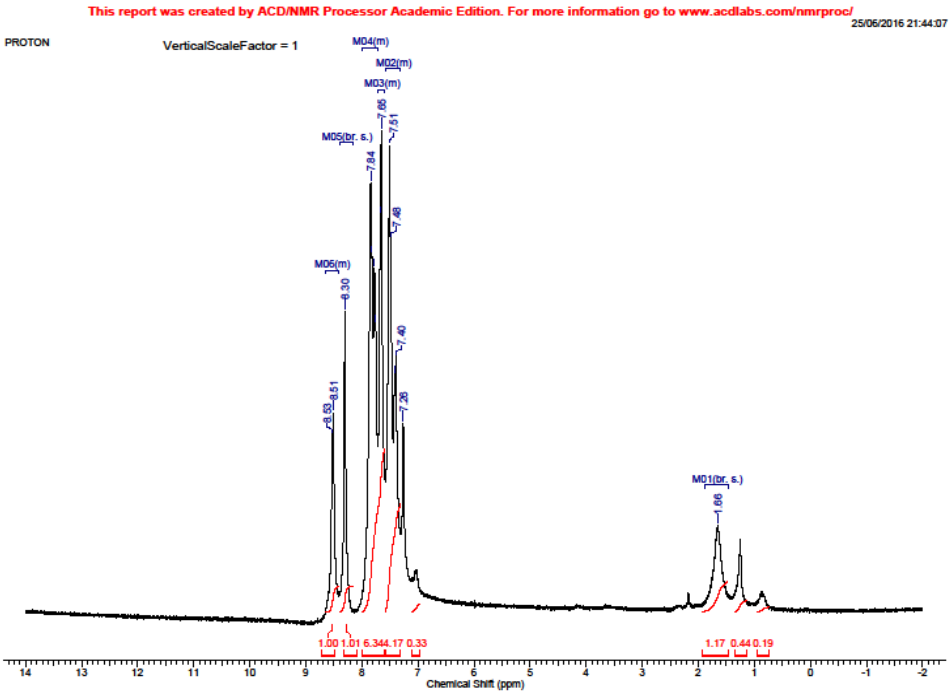


Fig.1. ^{1}H -NMR spectrum of 3-(2-phenylhydrazinyl)-2-benzofuran-1(3*H*)-one in $CDCl_3$.

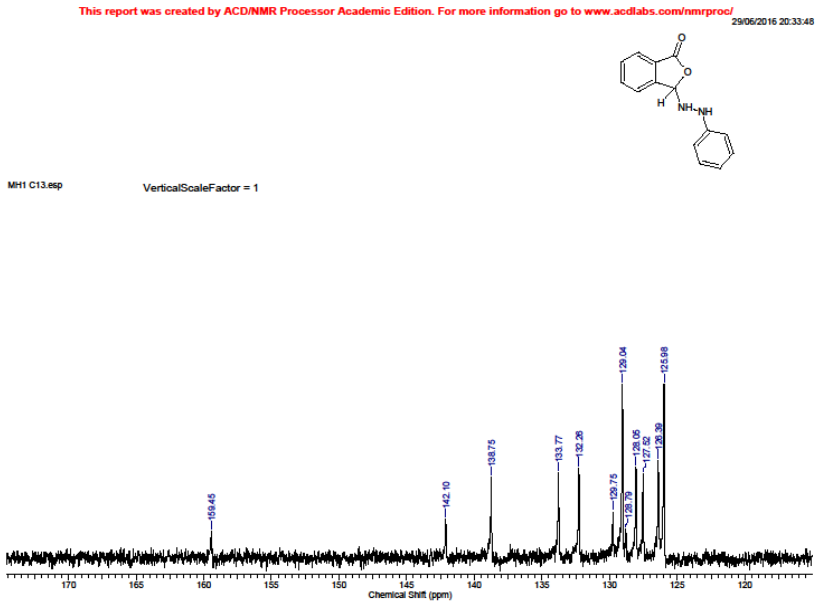


Fig. 2. ^{13}C -NMR spectrum of 3-(2-phenylhydrazinyl)-2-benzofuran-1(3*H*)-one in $CDCl_3$.

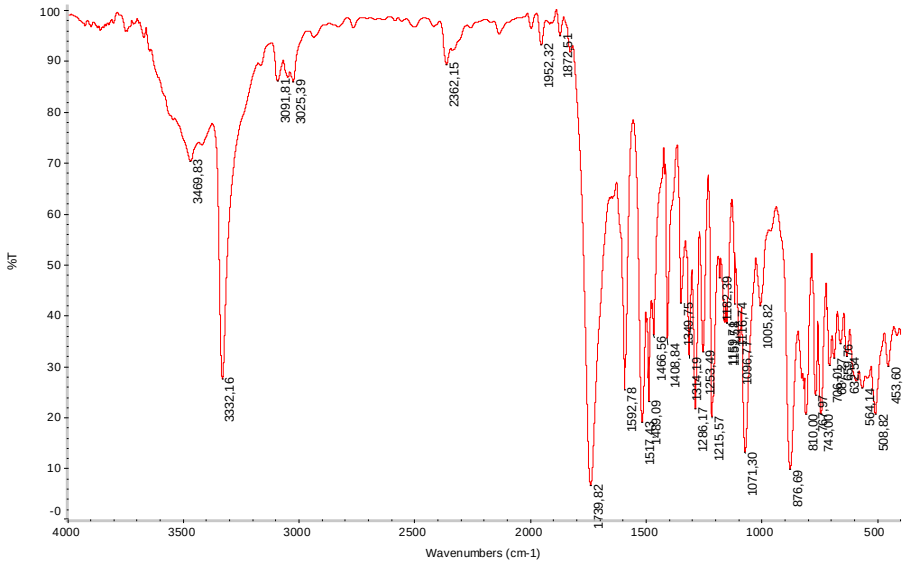


Fig.3. IR spectrum of 3-(2-phenylhydrazinyl)-2-benzofuran-1(3*H*)-one.

Table – 4
Antifungal and Antibacterial activities (conc.5mg/ml) of 3-(2-phenylhydrazinyl)-2-benzofuran-1(3*H*)-one.

Candida albicans	Thielaviopsis paradoxa	Eschericha coli	Bacillus subtilis	Staphylococcus Aureus
+	+	+	+	+

Inhibition zone diameter in mm: (-) < 11; (+) 11-14

Conclusion

In the present study, 3-(2-phenylhydrazinyl)-2-benzofuran-1(3*H*)-one was synthesized in a single step. The compound, which was synthesized as potential antimicrobial agents was found to exhibit both antibacterial and antifungal action.

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