Synthesis, Characterization and Bioactivity of Three Carboxylic Arylhydrazone Compounds

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Summary: Three novel carboxylic arylhydrazone compounds named 2- oxo propionic acid terephthalal acyl dihydrazone **(1)**, 2-ketoglutaric acid terephthalal acyl dihydrazone **(2)** and 2-ketoglutaric acid salicyl- hydrazone **(3)** were prepared and characterized by elemental analysis, IR and ^{1}H NMR. The antibacterial activities of **1** and **2** against wheat rust and coliform were investigated. The results showed that **1** had more excellent antibacterial properties than **2** against both wheat rust and coliform. In addition, the title compounds interaction with calf-thymus DNA (CT-DNA) were measured by fluorescence spectra method which indicating that they combined with CT-DNA by groove binding through hydrogen bonds.

Keywords: Arylhydrazone; Bioactivity; Calf-thymus DNA; Wheat rust; Coliform.

Introduction

Because of its excellent biological activity, strong coordination ability and various coordination methods, arylhydrazone has been paid more attention in the fields of pesticide, medicine and analytical reagent.[1-3] As early as 1983, the antibacterial activity of several kinds of phenyl hydrazones toward Puccinia triticinia were reported.[4] R. B. Johari found that benzaldehyde salicylhydrazone showed significant antibacterial activity against Aspergillus niger and Aspergillus nedus.[5] 1-methyl-2-benzimidazole hydrazone and dihydrazone have potential anticancer activity.[6] 4-acetyl pyridine-2-benzothiazolizone and 3-acetoiso- quinoline-2- phenylpropanoxazole hydrazone can act as a new type of anticancer agents, they present an unusual inhibitory effect on leukemia, melanoma, lung cancer and renal cancer.[7] The bacteriostasis of salicylic salicylhydrazone on Phytophthora capsici, cotton Fusarium Wilt and Nicotiana Nicotiana were studied by Shuiyang He group. The results showed that salicylsalicylhydrazone had different inhibitory effects on the three kinds of bacteria.[8] It is also found that the metabolites of arylhydrazones containing peptide bond in the large number of bioactive hydrazones are of low toxic or nontoxic.[9] However, the water solubility of aryl hydrazone compounds is very small. Therefore, it is of great significance to construct aromatic acylhydrazone compounds containing hydrophilic groups. [10, 11] Three arylhydrazone compounds containing carboxylic groups were designed and synthesized, and antimicrobial activities were investigated.

Experimental

Materials and methods

Pyruvic acid, 2-ketoglutaric acid and calf thymus DNA were biochemical reagents, terephthalic acid dimethyl ester, methyl salicylate, hydrazine hydrate and ethanol were AR grade. Elemental analysis of the target compounds were measured on PE-2400 elemental analyzer. Emission spectrum were recorded on Hitachi F-4600 fluorescent spectrophotometer. Absorption spectroscopy were tested by CARY300 type ultraviolet visible spectrophotometer. ^{1}H NMR spectra were recorded on VARIAN INOVA 400MHz superconducting NMR instrument using $CHCl_3$. IR were recorded in KBr pellets using Nicolet iS10.

Preparation of the target compounds

The synthetic routes of the target compounds were shown in Scheme-1.

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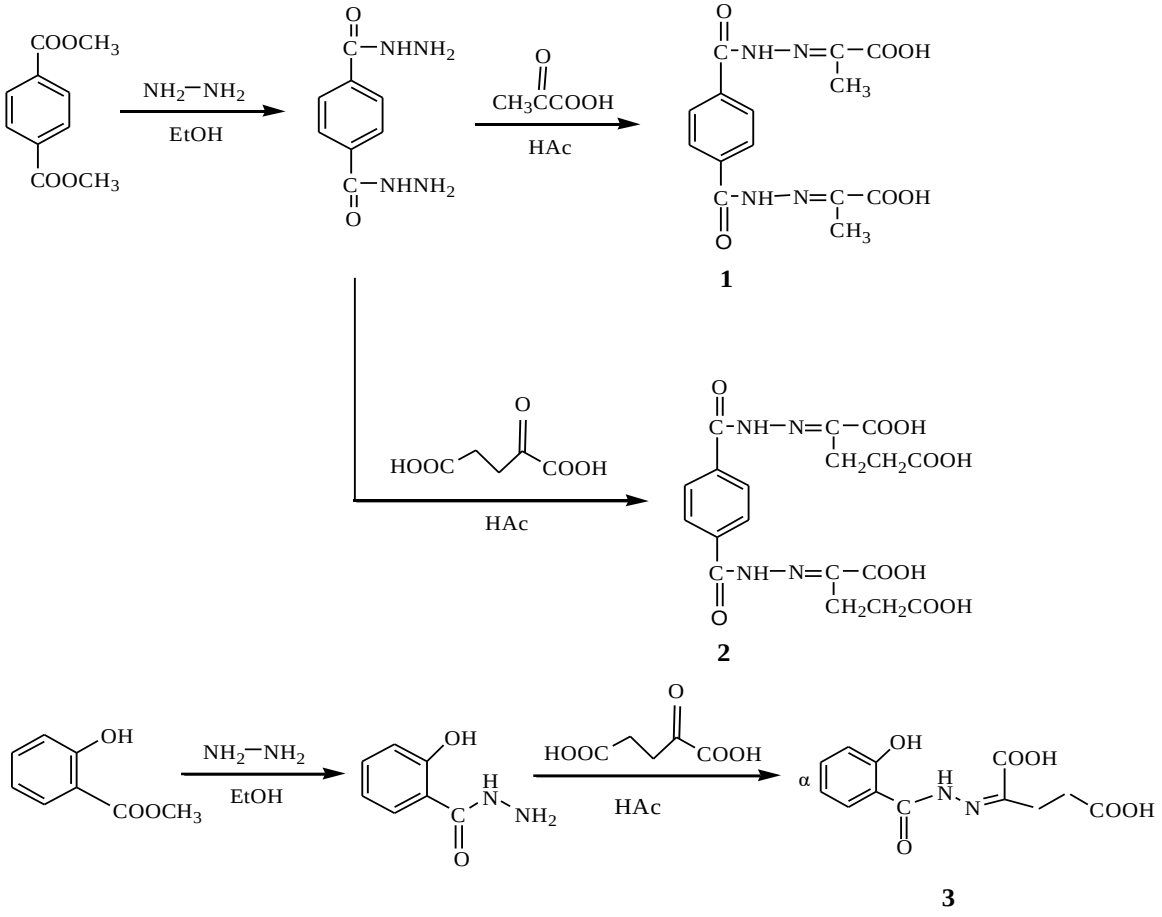
Synthesis of [N-(Propionic acid)] terephthalal acyl dihydrazone, **1**

The synthesis *of* 1 was according to literature [12]. Firstly, a proper amount of terephthalic acid dimethyl ester(0.1942 g, 1 mmol) and hydrazine hydrate (0.5 mL, 2.1 mmol) were refluxed in 50 mL absolute alcohol for 5 h with the presence of triethylamine as catalyzer. The product terephthalic dihydrazide was filtered, recrystallized and dried. Secondly, a proper amount of terephthalic dihydrazide(0.1945g, 1 mmol) and pyruvic acid (0.13 mL, 2.5 mmol) were refluxed in alcohol for 6 h. After the reaction was finished, white precipitate [N-(Propionic acid)] terephthalal acyl dihydrazone **1** was filtered, washed with water and ethanol, recrystallized from the mixed solvent of dimethylformamide and water(V:V, 1:10), filtered, washed and dried. Then white powder was obtained with a yield of 75%. Anal. calcd for **1**: C 50.30, H 4.22, N 16.76; Found C 50.65, H 4.34, N 16.54. IR (KBr, cm^{-1}): ν_{COOH} 1726, $\nu_{C=O}$ 1665, $\nu_{C=N}$ 1533.

Synthesis of 2-ketoglutaric acid terephthalal acyl dihydrazone, **2**

The synthesis route about **2** was similar to 1. A proper amount of terephthalic dihydrazide(0.1945g, 1 mmol) and 2-ketoglutaric acid(0.3069 g, 2.1 mmol) were refluxed in alcohol for about 8 h. After the reaction was finished, the crude product of **2** was obtained. Then, the crude product of **2** was washed, recrystallized, and dried to obtain a white powder product. The yield was about 70%. Anal. calcd for **2**: C 48.01, H 4.03, N 12.44; Found C 48.12, H 4.28, N 12.65. IR (KBr, cm^{-1}): ν_{COOH} 1711, $\nu_{C=O}$ 1638, $\nu_{C=N}$ 1533.



Scheme-1: Synthetic routs for target compounds.

Synthesis of 2-ketoglutaric acid Salicylhydrazone, **3**

The synthesis of 3 was according to literature [13], but using 2-ketoglutaric acid instead of Pyruvic acid. Anal. calcd for **3**: C 51.43, H 4.32, N 10.00; Found C 51.72, H 4.68, N 9.85. IR (KBr, cm^{-1}): ν_{COOH} 1694, $\nu_{C=O}$ 1644, $\nu_{C=N}$ 1537.

Antimicrobial Activity for wheat rust

The antimicrobial activities of **1** and **2** against wheat rust were determined by spore sprout method. Suspensions of microorganism spore were prepared by mixing the wheat rust pathogenic microbe and 5 mL of 0.1% dextrose solution evenly. Solutions of 1 and 2 of different concentrations were prepared from diluting **1** and **2** stock solution with distilled water. A drop of mixed liquor of 2 mL of suspension and 2 mL of stock solution was dripped on a concavity slide. The concavity slide was inverted on the keep-wet shelf and incubated for 24 h at 12 ℃. The sprout number of the spore was counted if the germinal tube exceeded the half of the spore diameter.

Antimicrobial Activity for coliform

The bioactivities of **1** and **2** against coliform was tested by turbidimetry/spectrometry method [14], and the inhibitor rate curves in the presence and absence of 1 and 2 were studied. The LB culture medium was prepared by mixing 2 g peptone, 1 g yeast, 1 g NaCl, and 200 mL H_2O. The experimental route was as follows: mixing 10 mL LB culture medium, 10 mL coliform solution, and 1 mL solution of **1** or **2** (0.25%) together, placing them on the rocking bed, cultivating at 37℃, measuring OD of each sample every 2 h, calculating inhibition rate, and drawing inhibitor rate curves. The method is:

$$\text{Inhibitor rate (\%)}=[OD_{(CK)}- OD_{(sample)}]/ OD_{(CK)}\times 100\%$$

Results and Discussion

^{1}H-NMR

The proton labeling of compound **1**, **2** and **3** were shown in Fig. 1, and ^{1}H NMR data were listed in Table-1. The ^{1}H NMR data of target 1 were taken as an example. There were four types of hydrogen in 1. They were benzene ring hydrogen, -NH hydrogen, $-CH_3$ hydrogen and –COOH hydrogen, respectively. The chemical shift of benzene ring hydrogen, -NH hydrogen, $-CH_3$ hydrogen and –COOH hydrogen were at 7.952 ppm, 11.040 ppm, 2.156 ppm and 13.795 ppm, respectively. At the same time, their area ratio was about 2:1:3:1.

Table-1: ^{1}H-NMR data for the target compounds.

Type of H	1	2	3
α-Ar	7.95	7.95	6.96-7.89
β-N-H	11.04	11.41	11.04
γ-NH_2	—	—	—
δ-CH_2	2.16	2.30	2.32
ε-COOH	13.80	13.80	13.86
ζ-CH_2	—	2.33	2.33
η-COOH	—	12.69	13.21
θ-OH	—	—	11.40

Antimicrobial Activity for wheat rust

The antimicrobial activity of **1** against wheat rust can be seen in literature [12]. The data of **2** against wheat rust by spore sprout method were listed in Table-2. It showed that **2** had excellent antibacterial activity against wheat rust. The inhibitory rate can reach to 100% when the concentration was 250 μg/mL. The inhibitory rates fell gradually with the concentration decreasing. Compared with 1, the antimicrobial activity against wheat rust of 2 was a little weaker. It was very exciting that inhibitory rate can still maintain 98.5% when the concentration decreased to 25 μg/mL.

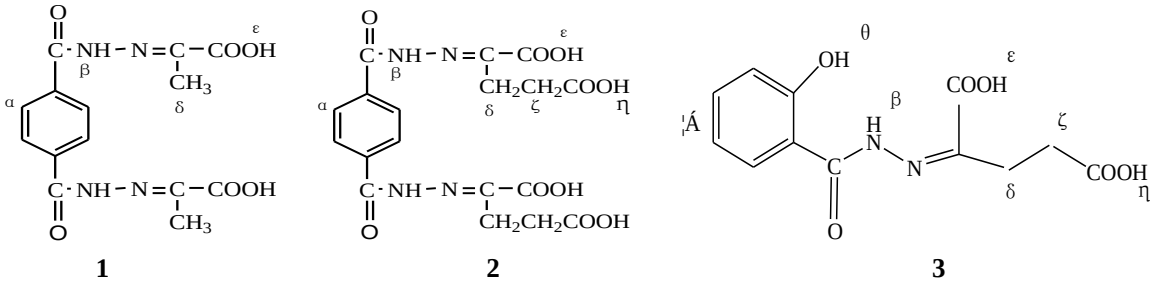


Fig. 1: Proton labeling of Compound **1, 2** and **3.**

Table-2: The data of **2** against wheat rust.

Concentration/(μg·mL^{-1})	Average sprout rate(%)	Inhibitory rate(%)
250	0	100
25	1.5	98.5
2.5	22.3	77.7
0.25	69.3	30.7

Antimicrobial Activity for coliform

Fig. 2 shows the inhibitor rate curves of **1** and **2** against coliform. It can be seen that the inhibitory rates increased quickly at the beginning (0–4 h), and then inhibitory rates almost kept in a level (6–12 h). The inhibitor rate of **1** was always higher than that of **2**. The maximum inhibitor rates of 1 and 2 were 99.85% and 86.65%, respectively.

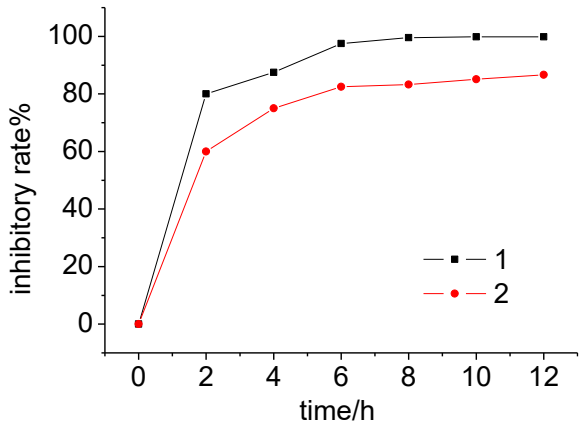


Fig. 2: Inhibitor rate curves of **1** and **2**.

DNA-binding study

Absorption spectroscopy is one of the most convenient and commonly used technology to study the interaction between compounds and DNA. If small molecules interact with DNA by intercalation, absorption spectrum will occur strong hypochromic effect (>35%) and significant red shift(>15 nm). If by groove- binding, the hypochromism (<10%) and red shift (<8 nm) will be a little smaller. Fig. 3 was the absorption spectrum of **1, 2** and **3** in the presence (----) and absence (—)of CT-DNA. The absorption spectrum of all the compounds showed a small hypochromism(<10%) and the red shift was not observed. So 1, 2 and 3 may interact with CT - DNA by external groove surface. [15]

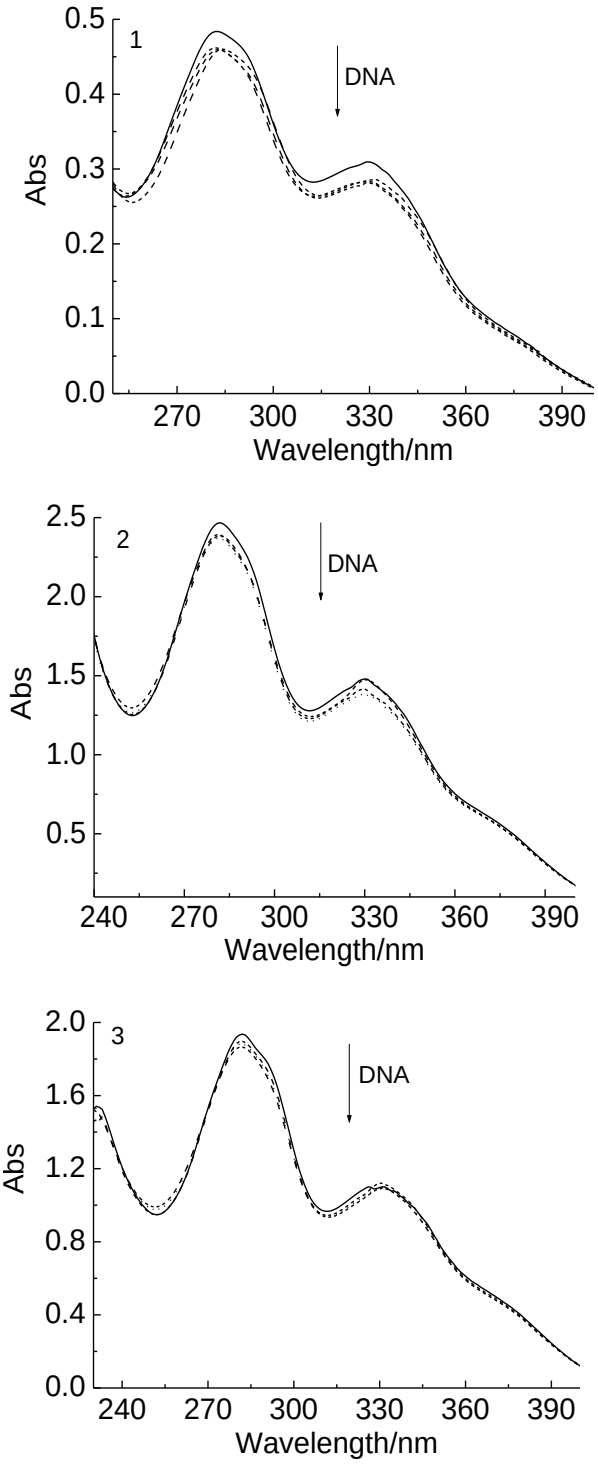
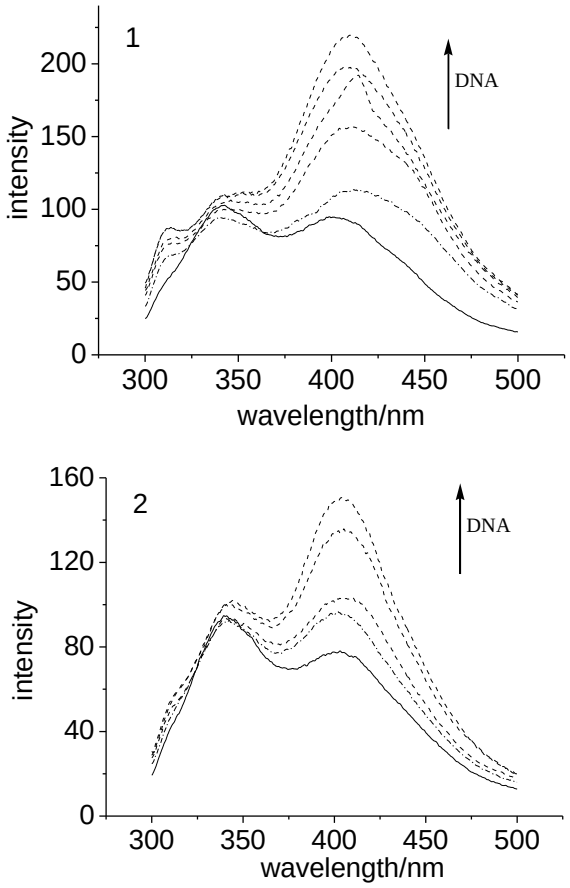


Fig. 3: Absorption spectrum of 1, 2 and 3 in the presence (----)and absence (—)of CT-DNA. Arrow shows the absorption intensities changes with increasing DNA concentration.

DNA react with small molecules not only can enhance its luminescence, but also can quench its luminescence. Therefore, in the presence of DNA, the enhancement or reduction of luminescence may be the evidence for the combination of this substance and DNA. If the of emission spectrum intensity of the original luminescent material changed very small in the presence of DNA, so it can be used as a criterion that matter has no obvious effect on DNA. The emission spectrum of **1, 2** and **3** in the presence and absence of DNA were shown in Fig. 4. The fluorescence intensities had a greater degree of enhancement with the addition of DNA to solutions of **1, 2**, and **3**. At the same time, the peak pattern of the fluorescence spectra changed greatly, and the change trend of the three compounds were very similar. The variation of the intensity around 340 nm was small, and the intensity at 410 nm varies greatly from scratch to strong. This was due to hydrogen bonds formed between oxygen atoms and nitrogen atoms of the title compounds and oxygen atoms on phosphoric acid and nitrogen atoms on guanine of CT-DNA. So the three compounds were bonded to DNA grooves through hydrogen bonds [16-18].



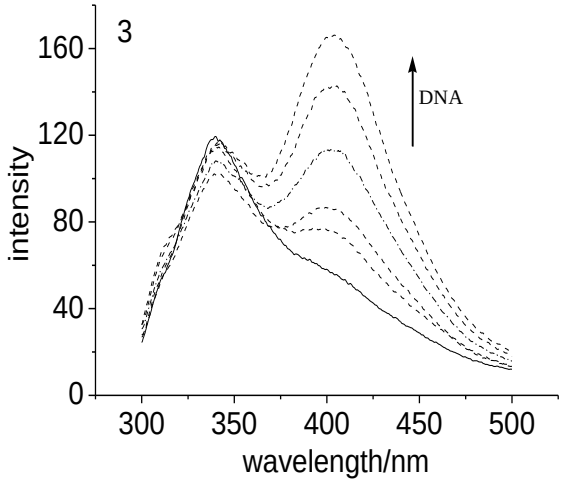


Fig. 4: Emission spectra of **1, 2** and **3** in the presence (----)and absence (—) of CT-DNA. Arrow shows the emission intensities changes with DNA concentration increased.

Conclusions

Three carboxylic arylhydrazone compounds had been prepared and characterized by elemental analysis, IR and ^{1}H NMR. The results of bioactivity experiment showed that both 1 and 2 possessed excellent inhibitory activities against coliform and wheat rust. The DNA binding properties of **1, 2** and **3** were studied by fluorescence spectrum and the results indicated that they all bonds to DNA by groove binding through hydrogen bonds.

Acknowledgements

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