THE SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF ISATIN-3-(4'-HYDROXY)BENZOYLHYDRAZONE

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Isatin is an endogenous compound and acts as a important precursor for the synthesis of various pharmacologically active heterocyclic compounds. Isatin and its derivatives possess a different activity such as antimicrobial, antiviral, anti-inflammatory, anticonvulsive, antitumor, anti-HIV, etc. In the present work, isatin-3-(4'-hydroxy)benzoylhydrazone was synthesized and identified by elemental microanalysis as well as FTIR, UV/VIS and MS methods. The compound was tested for antimicrobial activity against *Staphylococcus aureus*, *Serratia marcescens*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterococcus faecalis* and *Candida albicans*.

Keywords: Isatin, synthesis, spectroscopic analysis, antimicrobial activity

Introduction

Isatin and its derivatives as biological and pharmaceutical active components are very important intermediates in different organic synthesis [1]. Isatin is an endogenous compound, which can be found in plants of the genus *Isatis*, in *Calanthe discolor* LINDL and *Couroupita guianensis* Aubl [2]. It is a consistent component of the secretion from the parotid gland of *Bufo* frogs and a metabolic derivative of adrenaline in humans [2].

Isatin is a building unit of various numbers of alkaloids, drugs, pesticides and dyes [3]. Isatin and its derivatives possess an antimicrobial, antiviral, anticonvulsive, antitumor, anti-HIV, anti-inflammatory and antimycobacterial activity [4].

Hydrazones also possess antibacterial, anticancer, tuberculostatic and fungicidal properties and have a wide application in medicine and pharmacy [5].

The present paper describes the synthesis and identification of isatin-3-(4'-hydroxy)benzoylhydrazone. The synthesized compound was tested for antibacterial activity against *Staphylococcus aureus* ATCC 6538, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 43895, *Klebsiella pneumoniae* ATCC 13883, *Serratia marcescens* ATCC 39006 and *Candida albicans* ATCC 10231 by the standard minimum inhibitory concentration (MIC) of a serial dilution test.

Further purification. They were purchased from Sigma Aldrich (Munich, Germany).

Instruments

Carbon, hydrogen and nitrogen were determined by microanalysis using a Carlo Erba 1106 microanalyser. The melting point was determined in open capillary tubes in a Thomas-Hoover point apparatus and was uncorrected. The purity of the compound was obtained by TLC on silica gel using benzene:chloroform = 55:45, the compound being visualized by iodine vapors. The FTIR spectrum was recorded with a MichaelsonBomen MB-series spectrophotometer, using the KBr pellet (1 mg/100 mg) technique. The UV/VIS spectrum was recorded on a Perkin-Elmer Lambda 15 UV/Vis spectrophotometer, using 10^{-5} mol·dm⁻³ solutions in 95% ethanol. MALDI-TOF mass spectrum was acquired on a Voyager Biospectrometry DE Pro Workstation (Perseptive Biosystems, Framingham, MA, USA). The instrument is equipped with a 20Hz N₂ laser emitting at 337 nm. The spectrum was acquired without a low mass gate and the spectrum represents an average of at least 400 single laser shots. The spectrum was recorded from the methanolic solution of 1 µL, without a matrix.

Methods

Synthesis of isatin-3-(4'-hydroxy)benzoylhydrazone
Isatin-3-(4'-hydroxy)benzoylhydrazone was prepared by the reaction of the equimolar amount of isatin (1.47g) and 4-hydroxybenzoylhydrazide (1.52g) in 95 % ethanol

Experimental

Chemicals

All chemicals were of reagent grade and used without

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following the standard procedure [6,7]. The mixture was refluxed at 50 °C for 60 min. The product, precipitated as a yellow solid, was collected by filtration and washed with using a solvent.

Antimicrobial activity

The compound was evaluated for its in vitro antimicrobial activity against Gram-positive bacteria *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212, Gram-negative bacteria *Serratia marcescens* ATCC 39006, *Pseudomonas aeruginosa* ATCC 43895, *Klebsiella pneumoniae* ATCC 13883, and the yeast *Candida albicans* ATCC 10231. The minimum inhibitory concentration (MIC) was assessed by the agar dilution method. The minimum inhibitory concentration (MIC) represents the concentration of antimicrobial at which there is a complete inhibition of the growth of organism. A series of different concentrations of the com-

pound (25 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml and 400 µg/ml) with Mueller Hinton broth for bacteria and RPMI 1640 medium for the yeast was inoculated and incubated at 37 °C for 24 h (bacteria) and 48–72 h for the yeast [6-9].

Results and discussion

Isatin-3-(4'-hydroxy)benzoylhydrazone was synthesized by using the carbonyl-amine condensation reaction of isatin and 4-hydroxybenzoylhydrazide (Figure 1). Although the primary amines are suitable nucleophilic, the reaction required an acid catalyst to activate a carbonyl group, speed reaction and to allow the water elimination. The C-3 position in isatin is very susceptible to the nucleophilic attack, while C-2 reacts with nucleophiles only under specific conditions due to a negative inductive effect of the amido group [2].

Figure 1. Synthesis of isatin-3-(4'-hydroxy)benzoylhydrazone using the carbonyl-amine condensation reaction

Table 1. Physico-chemical characteristics of isatin-3-(4'-hydroxy)benzoylhydrazone

Compound	Color	Melting Point (°C)	C	Analysis ^a (%)		Solubility
				H	N	
Isatin-3-(4'-hydroxy)benzoylhydrazone	Yellow	245	64.02 (64.06)	3.96 (3.94)	14.99 (14.94)	Ethanol, Methanol, DMF, DMSO

a – Found (Calculated)

The yield of the synthesized compound is significant (94.30%). The experimental and calculated values for the elemental analysis are in a good accordance, which confirms the suggested molecular formula $C_{15}H_{11}N_3O_3$ of isatin-3-(4'-hydroxy)benzoylhydrazone (Table 1).

UV/VIS spectral analysis of isatin-3-(4'-hydroxy)benzoylhydrazone

The UV/VIS spectra of isatin, 4-hydroxybenzoylhydrazide and isatin-3-(4'-hydroxy)benzoylhydrazone were recorded in the solution of 95% ethanol (Figure 2). The numerical data are given in Table 2. Comparing to electronic spectra of started components, the spectrum of isatin-3-(4'-hydroxy)benzoylhydrazone shows a new band at λ_{max} = 342 nm. This is a characteristic absorp-

tion band for the azomethine (HC=N) group and also the confirmation of the isatin derivative formation.

Figure 2. UV/VIS spectra of isatin, 4-hydroxybenzoylhydrazide and isatin-3-(4'-hydroxy)benzoylhydrazone

Table 2. UV/VIS spectral data of isatin, 4-hydroxybenzoylhydrazide and isatin-3-(4'-hydroxy)benzoylhydrazone

Compound	$\lambda(\text{nm}) / \varepsilon \cdot 10^3 (\text{mol}^{-1} \cdot \text{dm}^{-3} \cdot \text{cm}^{-1})$	Band assignment
Isatin	212.0/1.1575	$\pi \rightarrow \pi^*$
	243.0/2.0561	$\pi \rightarrow \pi^*$
	300.0/0.3758	$n \rightarrow \pi^*$
4-hydroxybenzoylhydrazide	212.0/2.6675	$\pi \rightarrow \pi^*$
	255.0/3.2910	$\pi \rightarrow \pi^*$
	258.0/3.2487	$\pi \rightarrow \pi^*$
isatin-3-(4'-hydroxy)benzoylhydrazone	204.0/1.0437	$\pi \rightarrow \pi^*$
	246.0/0.6390	$\pi \rightarrow \pi^*$
	342.0/0.6593	$\pi \rightarrow \pi^*$

FTIR spectral analysis of isatin-3-(4'-hydroxy)benzoylhydrazone

The main IR spectral bands and their numerical data are given in Figure 3 and Table 3. Comparing to spectra of isatin and 4-hydroxybenzoylhydrazide, the spectrum of isatin-3-(4'-hydroxy)benzoylhydrazone contains a band at 1610 cm^{-1} , due to a newly formed $\nu(\text{C}=\text{N})$ stretching vibration. The spectrum of 4-hydroxybenzoylhydrazide contains symmetrical and asymmetrical NH_2 stretching bands at 3316 cm^{-1} and 3274 cm^{-1} , which cannot be found in the spectrum of isatin-3-(4'-hydroxy)benzoylhydrazone. The position of other bands are slightly bathochromic shifted due to extended conjugation within isatin-3-(4'-hydroxy)benzoylhydrazone

MS spectral analysis of isatin-3-(4'-hydroxy)benzoylhydrazone

Mass spectrum of the synthesized compound is given in Figure 4. The spectrum of isatin-3-(4'-hydroxy)benzoylhydrazone contains the characteristic molecular ion (M^+) at $m/z = 281$. The position of this peak is in accordance with the molecular mass of the compound with molecular formula $\text{C}_{15}\text{H}_{11}\text{O}_3\text{N}_3$. It also followed the „nitrogen rule“, by which the odd number of nitrogen means the odd molecular mass of the molecule. The spectrum con-

tains a peak near a molecular one which is of low intensity and belongs to $\text{M}+1$ ion. This is also a characteristic of the compounds with nitrogen atoms, i.e. isotopes ^{14}N and ^{15}N . The spectrum contains high-intensity peaks at $m/z = 149$ [$\text{C}_8\text{H}_5\text{N}_3\text{O}$] $^+$ and $m/z = 121$ [$\text{C}_7\text{H}_5\text{NO}$] $^+$ which belong to fragmentation ions.

Antimicrobial activity

Experimental results of the antimicrobial activity are given in Table 4. MIC values show that isatin-3-(4'-hydroxy)benzoylhydrazone possesses a different activity against the tested microorganisms.

The best results are shown against the Gram-positive bacteria *Enterococcus faecalis* and yeast *Candida albicans* in the concentration range of $25\text{--}50 \mu\text{g}\cdot\text{cm}^{-3}$. Isatin-3-(4'-hydroxy)benzoylhydrazone shows a medium activity against Gram-negative bacteria *Klebsiella pneumoniae*, *Serratia marcescens* and *Pseudomonas aeruginosa* in the concentration range of $50\text{--}100 \mu\text{g}\cdot\text{cm}^{-3}$, while the lowest activity is achieved against the Gram-positive bacteria *Staphylococcus aureus* in the concentration of $<100 \mu\text{g}\cdot\text{cm}^{-3}$.

Figure 3. FTIR spectrum of isatin, 4-hydroxybenzoylhydrazide and isatin-3-(4'-hydroxy)benzoylhydrazone

Figure 4. MS spectrum of isatin-3-(4'-hydroxy)benzoylhydrazone

Table 3. FTIR spectral data of isatin, 4-hydroxybenzoylhydrazide and isatin-3-(4'-hydroxy)benzoylhydrazone

Band assignation (cm ⁻¹)	Isatin	4-hydroxybenzoylhydrazide	isatin-3-(4'-hydroxy)benzoylhydrazone
v(OH)	-	3560	3450
vs(NH ₂) + vas(NH ₂)	-	3316, 3274	-
v(NH)	3189	3196	3251, 3176
v(C=O)	1740, 1720	1620	1715, 1686
v(C=N)	-	-	1610
δ(OH)	-	1249	1265
δ(NH) ₂	-	1590	-
δ(NH)	1460	1510	1537, 1491
v(C-O)	1201	1173	1192

Table 4. MIC values (μg-cm³) of isatin-3-(4'-hydroxy)benzoylhydrazone

Microorganism	Concentration of isatin-3-(4'-hydroxy)benzoylhydrazone (μg-cm ⁻³)				
	25	50	100	200	400
<i>Staphylococcus aureus</i>	-	-	-	+	+
<i>Klebsiella pneumoniae</i>	-	-	+	+	+
<i>Serratia marcescens</i>	-	-	+	+	+
<i>Pseudomonas aeruginosa</i>	-	-	+	+	+
<i>Enterococcus faecalis</i>	-	+	+	+	+
<i>Candida albicans</i>	-	+	+	+	+

(+) active

(-) neactive

Conclusion

The synthesis of isatin-3-(4'-hydroxy)benzoylhydrazone was done by using the carbonyl-amine condensation reaction of isatin and 4-hydroxybenzoylhydrazide in 95% ethanol. The structure and molecular formula $C_{15}H_{11}O_3N_3$ of the synthesized compound was established by the elemental microanalysis as well as FTIR, UV/VIS and MS methods. The compound shows a different antimicrobial activity against *Staphylococcus aureus*, *Serratia marcescens*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterococcus faecalis* and *Candida albicans*. The best results were shown against the Gram-positive bacteria *Enterococcus faecalis* and yeast *Candida albicans* in the concentration range of 25-50 $\mu\text{g}\cdot\text{cm}^{-3}$.

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Izvod

SINTEZA I ANTIMIKROBNA AKTIVNOST IZATIN-3-(4'-HIDROKSI)BENZOILHIDRAZONA

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Izatin je endogeno heterociklično jedinjenje koje predstavlja veoma važan prekursor u sintezi farmaceutski aktivnih komponenti. Izatin i njegovi derivati poseduju različitu biološku aktivnost, kao što je antimikrobna, antiviralna, antiinflamatorna, antikonvulzivna, antitumorna, anti-HIV, itd. U ovom radu je izvršena sinteza izatin-3-(4'-hidroksi)benzoilhidrazona i jedinjenje je identifikovano pomoću elementarne mikroanalize, FTIR, UV/VIS i MS metode. Sintetisanom derivatu izatina je određena antimikrobna aktivnost dilucionom metodom, odnosno određivanjem MIC vrednosti u odnosu na mikroorganizme *Staphylococcus aureus*, *Serratia marcescens*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterococcus faecalis* i *Candida albicans*. Najbolji rezultati su postignuti u odnosu na Gram-pozitivnu bakteriju *Enterococcus faecalis* i kvasac *Candida albicans* u koncentraciji 25-50 $\mu\text{g}\cdot\text{cm}^{-3}$.

Ključne riječi: Izatin, sineza, spektroskopska analiza, antimikrobna aktivnost