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SCIENTIFIC PAPER

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SYNTHESIS, X-RAY AND ANTIMICROBIAL ACTIVITY OF ISATIN-3-PHENYLHYDRAZONE

Isatin-3-phenylhydrazone was synthesized and X-ray crystal structure of this compound has been solved. Its structure was also established using FTIR, UV/Vis and ^{1}H-NMR spectroscopic method. The compound was tested for antimicrobial activity against Staphylococcus aureus, Bacillus subtilus, Enterococcus D, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa and Candida albicans. Stability of isatin-3-phenylhydrazone toward UV-A irradiation has been studied in this work. Isatin derivative undergoes bleaching following first-order kinetics.

Key words: isatin-3-phenylhydrazone, crystal structure, antimicrobial activity, photolytic stability.

Isatin is an endogeous compound isolated in 1988 [1] and reported to participate in a wide range of the central nervous system activities. Isatin derivatives are reported to show a variety of biological activities such as antibacterial, antimicrobial, antifungal and anti-HIV activities [2]. Isatin-3-phenylhydrazone was previously synthesized [3-5], but no database contains its crystal structure. Therefore, in our work we verify the structure of this isatin derivative.

Though being very stable in the dark, these solid compounds become very unstable upon dissolution, especially when being irradiated by UV-light; this is not a surprise because they are known as the excellent UV-absorbers [9]. Since UV-light is one of the major agents leading to melanoma skin cancer [10], Schiff bases have been used for some time in the skin protection cosmetics and pharmaceutical formulations. Yet, the basic mechanisms related to their interactions with UV-light are still being investigated. For such a purpose the stability of isatin-3-thiosemicarbazone toward UV-A irradiation has been studied in this work.

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MATERIALS AND METHODS

Preparation of isatin-3-phenylhydrazone

Isatin-3-phenylhydrazone was prepared by the reaction of equimolar amounts of isatin and phenylhydrazone in 95 % ethanol, following the standard procedure [3-5]. The product precipitated as yellow crystals in 30 min, and was collected by filtration and washed with cold 95 % ethanol. The melting point of isatin-3-phenylhydrazone is 201 °C, the yield is 95 %, and the Rf value is 0.820.

Microanalysis for carbon, hydrogen and nitrogen was carried out with Carlo Erba 1106 microanalyser. Melting point was determined in open capillary tubes in a Thomas-Hoover point apparatus and uncorrected. The purity of the compound was gained by TLC on silica gel, using benzene:chloroform = 55:45, and visualized by iodine vapors. The Fourier transformation infrared spectra (FTIR) were recorded with a Michaelson Bomen MB-series spectrophotometer, using the KBr pellet (1 mg/100 mg) technique. The electro-

nic spectra (UV/Vis) were recorded on a Perkin-Elmer Lambda 15 UV/Vis spectrophotometer, using 10^{-3} mol·dm^{-3} solutions in DMF. The nuclear magnetic resonance (^{1}H-NMR) spectra were obtained in DMSO solution with a Gemini-200 "HF NMR".

X-ray structure measurements

The X-ray structure of isatin-3-phenylhydrazone was solved at 293 K. The collection of X-ray reflections was performed on an Enraf Nonius CAD4 single crystal diffractometer using the graphite monochromatic MoKα radiation. Data reduction was carried out by the XCAD4 program [11]. The initial structure model was found by a direct method using SHELXS 97 [12]. The full-matrix-least-squares structure refinement on F^2 was done by SHELXL 97 [13]. The anisotropic displacement parameters were refined for all non-hydrogen atoms. The positions of H-atoms were generated from the assumed geometry and checked in different Fourier maps. For nitrogen hydrogens H1 and H3, the fractional coordinates and isotropic displacement parameters were refined. Final R factor was R = 0.0456 for 172 parameters using the F^2 values of 1564 ($I > 2\sigma$ (I)) reflections. The details of the structure solution and refinement are given in Table 1.

Photodegradation

The continuous irradiation of the Schiff base in DMF (10^{-6} mol·dm^{-3}) was performed in a cylindrical photochemical reactor "Rayonnet", with 14 symmetrically placed lamps with the emission maximum at 350 nm (UV-A), and the total measured energy flux of about 18 W·m^{-2}. The sample was irradiated in quartz cuvettes (1x1x4.5 cm^3) placed on a rotating circular holder.

Antimicrobial activity

The preliminary antibacterial screening was performed by the agar diffusion method using a paper disc. The sterilized (autoclaved at 120 °C for 30 min) agar was inoculated (1cm^3/100 cm^3 medium) with the suspension of the microorganism (matched to a McFarland barium sulphate standard) and poured into a Petra dish. The paper discs impregnated with isatin-3-phenylhydrazone (500 mg·cm^{-3}) in N,N-dimethylformamide (DMF) were placed on the solidified medium. The plates were incubated at 37 °C for 24 h [14-16].

A series of glass tubes containing different concentrations of the isatin-3-phenylhydrazone with Müller Hinton broth was inoculated with the required amount of the inoculums to obtain a suspension of microorganism, which contains 10^5 colony forming units per ml. One growth control tube was prepared with the addition of the compound and one blank tube was prepared without the addition of the microorganism. The tubes were incubated at 37 °C for 24 h. The turbidity produced in each tube was observed. The minimum inhibitory concentration (MIC in μg·cm^{-3}) was considered to be the lowest concentration, which exhibited the same turbidity as the blank tube [17,18].

RESULTS AND DISCUSSION

X-ray crystal structure

Crystals of isatin-3-phenylhydrazone are monoclinic with the P21/c space group. A perspective view of the compound is presented in Fig. 1.

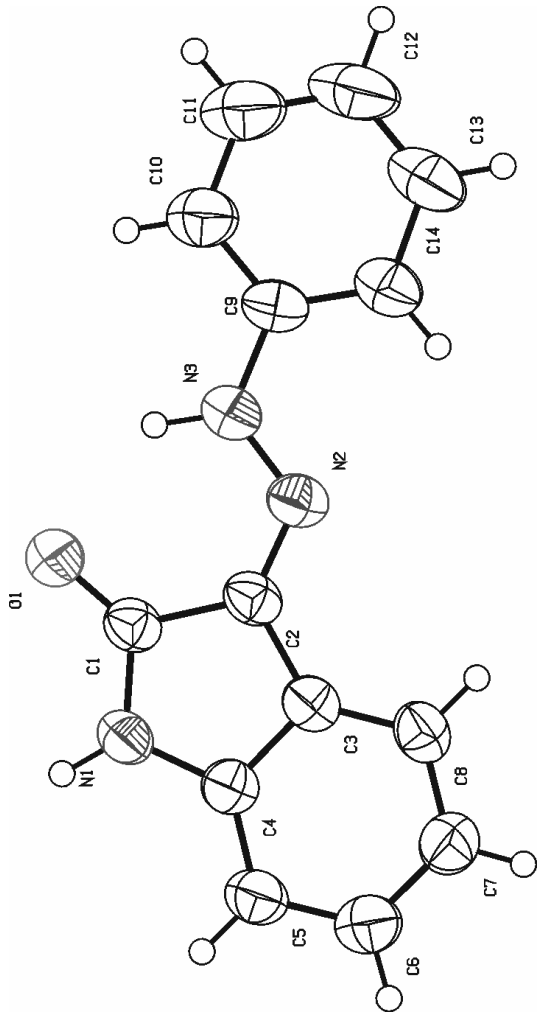


Figure 1. A perspective view of isatin-3-phenylhydrazone from X-ray data

Table 1 shows a summary of crystal data and X-ray analysis information for isatin-3-phenylhydrazone, and relevant bond distances and angles are listed in Table 2.

The six- and five-membered rings of the isatin moiety and the six-membered ring of phenylhydrazone are very nearly planar. The dihedral angles between

the mean planes of both isatin rings are only 0.6°, and phenylhydrazone is 0.9°. The planarity of the molecules was stabilized by a strong intramolecular hydrogen bond N(3)-H(3)···O(1) = 2.754(2) Å leading to the formation of the pseudo six-membered ring. The phenylhydrazone moiety is in Z configuration in respect to C(2) = N(2) double bond (torsion angle C(1)-C(2)-N(2)-N(3) = -0.2°), as a consequence of formation of the intramolecular N(3)-H(3)···O(1), and intermolecular hydrogen bond N(1)-H(1)···O(1) = 2.858(2) Å. The configuration around N(3)-C(9) is E, since the torsion angle between the atoms N(2)-N(3)-C(9)-C(10) is 177.5°. These results are very similar to those of the previously published isatin compound [17,18].

Table 1. Crystal data and structure refinement of isatin-3-phenylhydrazone

Empirical formula	$C_{14}H_{11}N_3O$
Formula weight	237.26
Melting point (K)	474
Temperature (K)	293
Radiation wavelength (MoKα), λ (Å)	0.71070
Crystal system	Monoclinic
Space group	P21/c (No. 14)
a (Å)	12.677(4)
b (Å)	5.6026(12)
c (Å)	17.825(5)
α, β, γ (°)	90, 109.166(15), 90
V (Å^3)	1195.8(6)
Z	4
D (calc) (mg/m^3)	1.318
Absorption coefficient, μ (mm^{-1})	0.087
F(000)	496
Crystal size (mm)	0.42x0.46x0.60
θ range for data collection (°)	$3.4 \leq \theta \leq 27.5$
Index range	$-16 \leq h \leq 15$; $-7 \leq k \leq 6$; $-23 \leq l \leq 23$
Reflection collected/unique	11227/2676 [R_{int} = 0.047]
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	2676/0/172
Reflections I > 2 σ(I)	1564
Finale R indices (R, wR_2)	0.0456, 0.1296
Goodness of fit on F^2	1.16
Maximum, mean shift/esd	0.00, 0.00
Largest differential peak and hole (eÅ^{-3})	0.17 and -0.16

Spectral characterization

Isatin-3-phenylhydrazone was also identified by its ^{1}H-NMR spectra (Figure 2, Table 3).

In the spectra, the signal at δ 11.00 is assigned to N(1)-H based on the position of this peak in the spectrum of the parent isatin molecule. The assignment of the peak at δ 12.75 as N(3)-H is supported by its location at lower field (indicative of an intramolecularly hydrogen-bonded proton) and by the greater dependency of its position on the nature of the N(4) substituent [17].

The FTIR spectra (Table 3, Figure 3) confirm the existing structure. The frequencies of ν(NH) and ν(C=O) vibrations (3169, 3130 and 1686 cm^{-1}) is lower than usually due to the intramolecular hydrogen bonding between N(3)-H and O(1).

The UV/Vis spectrum of isatin-3-phenylhydrazone was recorded in 95 % ethanol solution and DMF solution (Table 4, Figure 4).

Although isatin-3-phenylhydrazone is soluble in both solvents, it seems that DMF is a better solvent than ethanol. The UV/Vis spectra show strong π-$\pi*$ bands at 207.9 and 258.5 nm and n-$\pi*$ band at 398.5 nm. This band has bathochromic shift for 370 cm^{-1} (6 nm) in DMF, since this solvent has a greater dipole moment than ethanol (1.71 for ethanol and 3.86 for DMF).

UV-A irradiation

UV/Vis absorption spectra of isatin-3-phenylhydrazone after the continuous prolonged irradiation (0, 5, 15, 30, 45 and 60 min) with UV-A light are shown in Fig. 5.

Both the absorption maxima (λ_{max}= 348 nm and λ_{max}= 355 nm) decrease, and a slight bathochromic shift have been detected, at the end of any particular UV-irradiating period. The log values of the absorbance maxima plotted against irradiation time yielded a linear plot, suggesting the involved kinetics to be probably of pseudo-first order, depending on the isatin-3-phenylhydrazone concentration only.

Table 2. Bond distances (Å) and bond angles (°) for Isatin-3-phenylhydrazone

Bond distances			
N(1)-C(1)	1.354(3)	C(9)-C(10)	1.380(3)
N(1) -C(4)	1.398(2)	C(10)-C(11)	1.381(4)
N(2)-N(3)	1.327(2)	C(11)-C(12)	1.361(4)
N(2)-C(2)	1.302(3)	C(12) -C(13)	1.378(4)
N(3)-C(9)	1.394(3)	C(13) -C(14)	1.380(3)
N(10-H(1)	0.885(18)	C(5) -H(5)	0.9302
N(3)-H(3)	0.94(2)	C(6) -H(6)	0.9296
C(1)-C(2)	1.483(2)	C(7) -H(7)	0.9295
C(2) -C(3)	1.449(3)	C(8) -H(8)	0.9304
C(3) -(C8)	1.381(3)	C(10 -H(10)	0.9303
C(3) -C(4)	1.401(3)	C(11 -H(11)	0.9300
C(4) -C(5)	1.370(3)	C(12) -H(12)	0.9295
C(5) -C(6)	1.389(3)	C(13) -H(13)	0.9296
C(6)-C(7)	1.380(3)	C(14) -H(14)	0.9301
C(7)-C(8)	1.384(3)		
Bond angles			
C1 -N1 -C4	111.50(16)	C10 -C9 -C14	120.22(19)
N3 -N2 -C2	118.16(16)	C9 -C10 -C11	119.6(2)
N2 -N3 -C9	120.62(16)	C10 -C11 -C12	120.6(3)
C4 -N1 -H1	124.6(14)	C11 -C12 -C13	119.9(3)
C1 -N1 -H1	123.6(14)	C12 -C13 -C14	120.7(2)
N2 -N3 -H3	118.2(13)	C9 -C14 -C13	118.9(2)
C9 -N3 -H3	121.2(13)	C4 -C5 -H5	121.41
O1 -C1 -N1	127.15(16)	C6 -C5 -H5	121.38
O1 -C1 -C2	126.62(17)	C5 -C6 -H6	119.28
N1 -C1 -C2	106.23(16)	C7 -C6 -H6	119.34
N2 -C2 -C1	127.18(18)	C6 -C7 -H7	119.50
N2 -C2 -C3	126.08(16)	C8 -C7 -H7	119.43
C1 -C2 -C3	106.71(16)	C3 -C8 -H8	120.80
C2 -C3 -C8	133.69(17)	C7 -C8 -H8	120.82
C4 -C3 -C8	119.76(18)	C9 -C10 -H10	120.17
C2 -C3 -C4	106.53(15)	C11 -C10 -H10	120.20
C3 -C4 -C5	122.20(17)	C10 -C11 -H11	119.72
N1 -C4 -C3	109.03(17)	C12 -C11 -H11	119.68
N1 -C4 -C5	128.77(16)	C11 -C12 -H12	120.11
C4 -C5 -C6	117.21(17)	C13 -C12 -H12	120.01
C5 -C6 -C7	121.4(2)	C12 -C13 -H13	119.58
C6 -C7 -C8	121.07(18)	C14 -C13 -H13	119.68
C3 -C8 -C7	118.37(17)	C9 -C14 -H14	120.55
N3 -C9 -C10	118.27(17)	C13 -C14 -H14	120.52
N3 -C9 -C14	121.51(18)		

The mechanism leading to isatin-3-phenylhydrazone concentration decrease certainly involves a hydrogen abstraction from DMF by a triplet state of isatin C=O group, a well-known reactive chromophore, playing a sensitizing role [5]; the other potential mechanisms (like an electron-transfer mechanism) - but more related to use of UV-B or UV-C light - are probably minor or excluded in this case. The suggested UV-induced initiation step - emphasizing a crucial role of carbonyl chromophore - is shown in Figure 6. The consequent, radicals mediated events (the ketyl-radical and DMF-radical), can only be speculated, since the flash techniques were not available.

Antimicrobial activity

Isatin-3-phenylhydrazone was tested for its *in vitro* antimicrobial activity against 6 pathogenic bacteria and one yeast [3-7] (Table 5). Tha data for amoxycillin and norfloxacin are included for comparison for bacteria and clortrimazole for yeast [3-5]. Isatin-3-phenylhydrazone possesses the best activity against *Bacillus subtilis*. Compared to parameters such as amoxicillin and norfloxacin, the situatuion is quite

different. As a matter of fact, isatin-3-phenylhydrazone is more active against *Escherichia coli*, *Pseudomonas aureginosa* and *Proteus* than amoxycillin. Also, it is more active than norfloxacin against *Staphylococcus aureus*, but has lowest activity against *Candida albicans*, considered tha data obtained for clortrimazole as a standard.

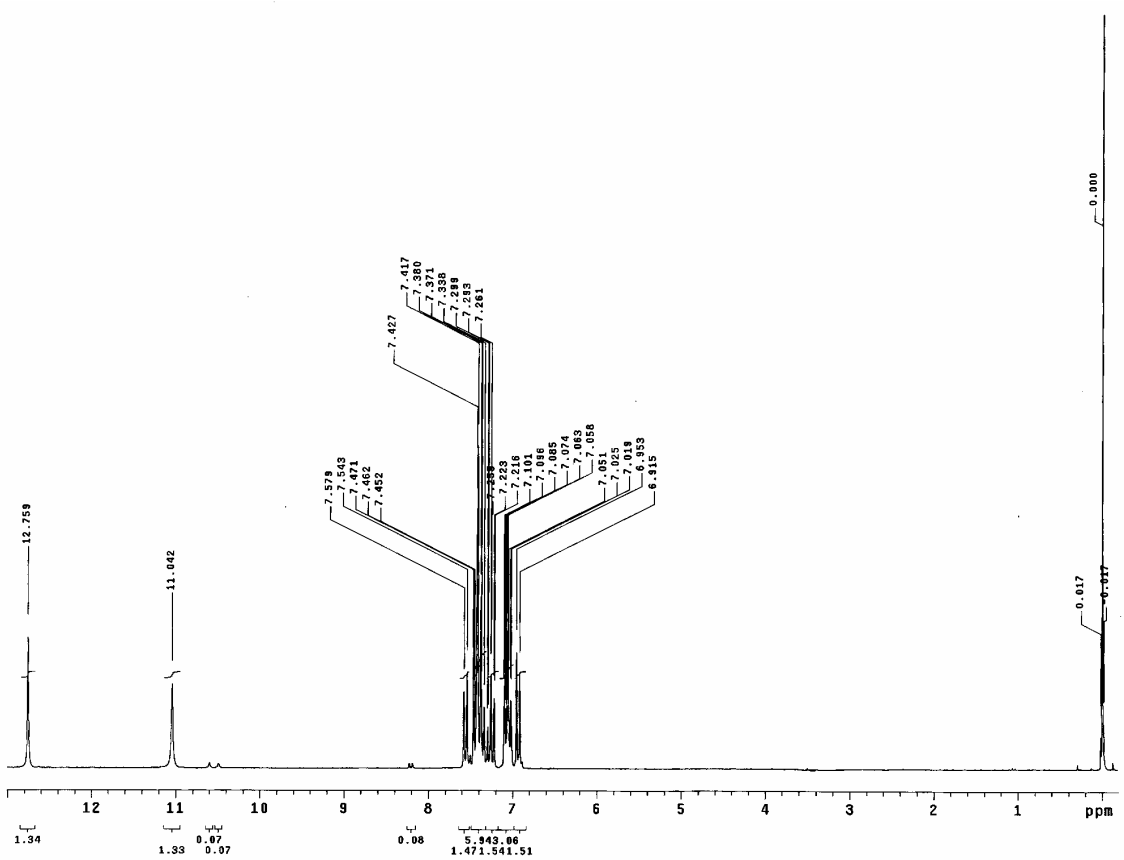


Figure 2. ^{1}H-NMR spectra of isatin-3-phenylhydrazone

Table 3. FTIR and ^{1}H-NMR data of the isatin-3-phenylhydrazone

σ (cm^{-1})	Assignments	δ / ppm	Assignments
3402	ν(NH)	N(3)-H	12.75 (*s*, 1H)
3169	ν(NH)	N(1)-H	11.00 (*s*, 1H)
3130	ν(NH)	C(8)-H	7.45-7.58 (*d*, 1H)
3057	ν(CH)	C(10,11,12,13,14)	7.22-7.42 (*m*, 4H)
1686	ν(C=O)	C(5,6,7)	6.92-7.22 (m, 3H)
1617	ν(C=C)+ ν(C=N)		
1597	δ(NH)		
1555	ν(C=N) + ν(CC)		
1490	ν(CC)		
1465	ν(C-NH)		
1345	δ(CH)		
1300	ν(CC)		
1242	δ(CH)		
1166	ν(NN)		
1092	ν(CNNC)		
987	ν(CC)		
888	δ(CH)		
789	δ(CH)		
747	π(CH)		
689	δ(CH)		
663	π(NH)		
626	π(CC)		
549			

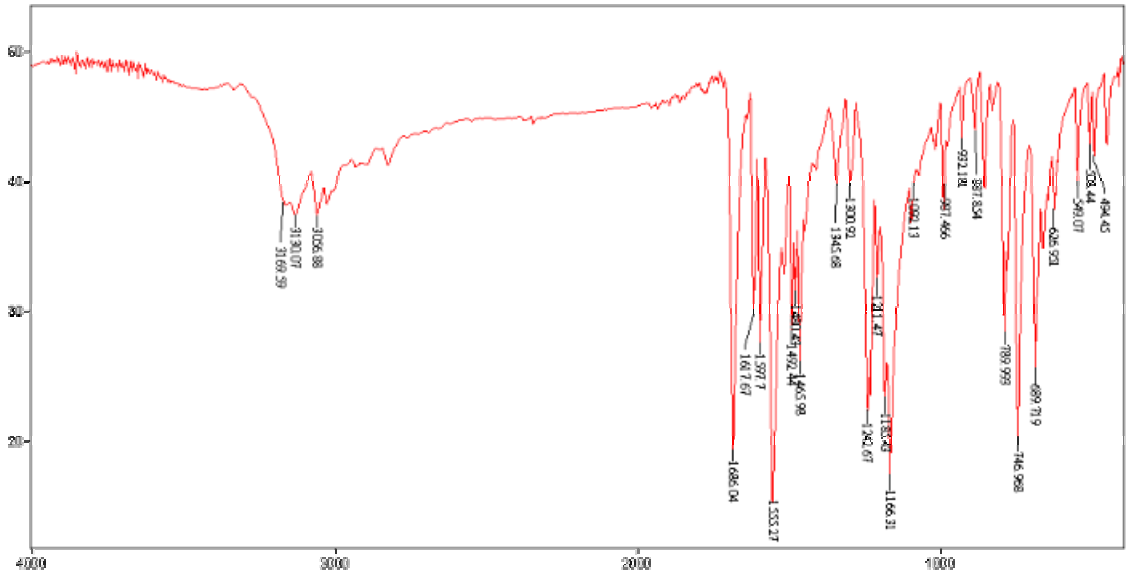


Figure 3. FTIR spectra of isatin-3-phenylhydrazone

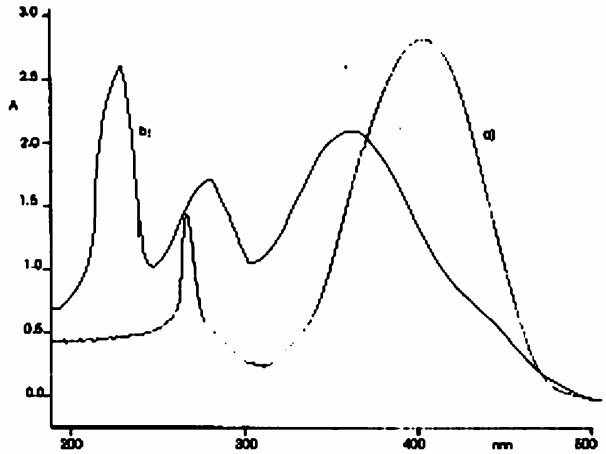
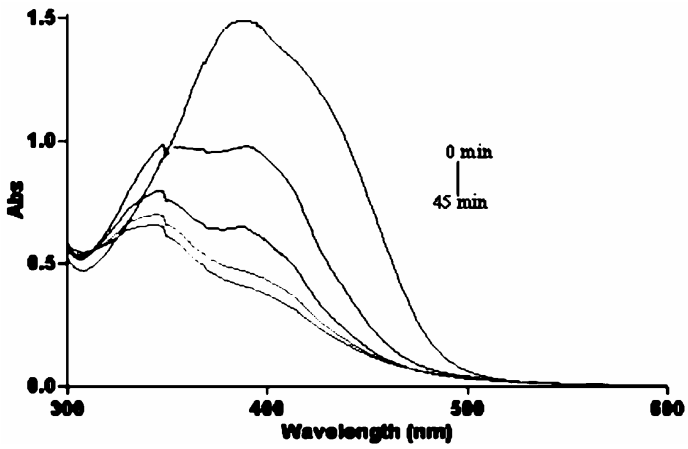


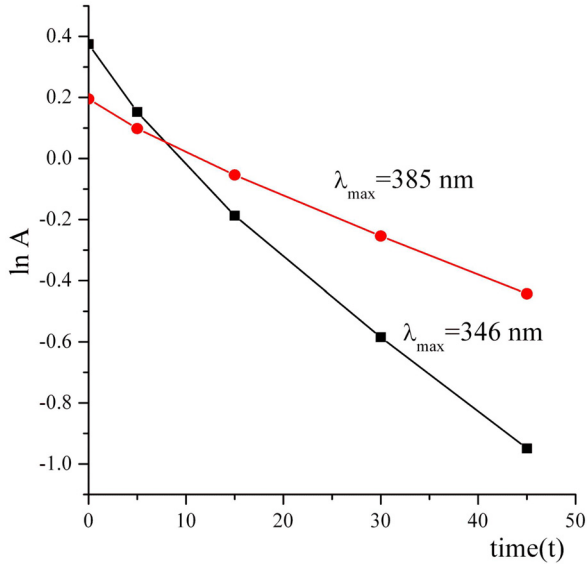
Figure 4.UV/Vis spectra of isatin-3-phenlhydrazone in a)ethanol and b)DMF

Table 4. Electronic spectral data in 95 % ethanol and DMF, λ_{max} (nm) / ε_{max}·(10^3 mol^{-1}·dm^3·cm)

Solvent	Isatin-3-phenylhydrazone			
	I	II	III	III
Ethanol	207.5/2.91	257.5/1.63	398.5/2.26	24700/2.82
DMF	-	267.6/2.8164	-	404.0/1.4227



(a)



(b)

Figure 5. a) The changes of the absorption spectra of isatin-3-phenylhydrazone after the continuous prolonged irradiation (0, 5, 15, 30 and 45 min.); b) The kinetics log plot of isatin-3-phenylhydrazone in DMF, during the increasing UV-A irradiation

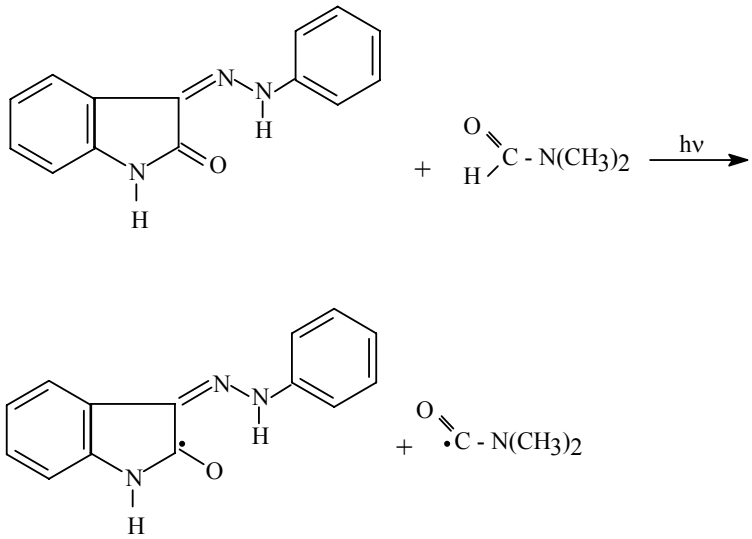


Figure 6. Reaction scheme for isatin-3-phenylhydrazone photoreduction

Table 5. The inhibition zones (mm) and MIC values (μg·cm^{-3}) of the isatin-3-phenylhydrazone

	MIC values (μg·cm^{-3})						
Compound	*S. aureus* ATCC 6538	*B. subtilis* ATCC 6051	E. group D	*E. coli* ATCC 95	*P. aeruginosa* ATCC 2853	*P. vulgaris*	*C. albicans* ATCC 10231
isatin-3-phenylhydrazone	140	45	160	100	240	200	160
Amoxycillin	16	2	2	128	1020	500	-
Norfloxacin	2500	1.22	≤0.018	0.3	19.53	≤0.018	-
Clortrimazole	-	-	-	-	-	-	0.3

CONCLUSIONS

Isatin-3-phenylhydrazone was synthesized and X-ray crystal structure was solved. The planarity of the molecules is stabilized by a strong intramolecular hydrogen bond N(3)-H(3)···O(1) = 2.754(2) Å leading to the formation of the pseudo-six-membered ring. Its structure was also established using FTIR, UV/Vis and ^{1}H-NMR spectroscopic method. Studying the stability of isatin-3-phenylhydrazone toward UV-A irradiation shows that isatin derivative undergoes bleaching following first-order kinetics. Isatin-3-phenylhydrazone was tested for its *in vitro* antimicrobial activity and it is more active against *Escherichia coli*, *Pseudomonas aureginosa* and Proteus than amoxycillin and against *Staphylococcus aureus* than norfloxacin.

Acknowledgments

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