Research Article

Synthesis, Crystal Structure, and Biological Activity of *cis/trans* Amide Rotomers of (Z)-N'-(2-Oxoindolin-3-ylidene)formohydrazide

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(Z)-N'-(2-Oxoindolin-3-ylidene)formohydrazide (**2**) was synthesized by the reaction of (Z)-3-hydrazonoindolin-2-one (**1**) with formic acid under reflux. The structure of **2** was characterized by IR, Mass, ¹H NMR, and X-ray crystal structure determination. Interestingly, compound **2** appeared in DMSO-*d*₆ as *cis* and *trans* amide rotomers in 25% and 75%, respectively. The X-ray analysis showed the *Z* geometrical isomer of **2** around -C=N- for *cis* and *trans* amide rotomers. The crystal of **2** belongs to monoclinic, space group *P*2₁/*c*, with *a* = 4.5206 (1) Å, *b* = 22.4747 (7) Å, *c* = 17.3637 (5) Å, β = 103.752 (1)°, *Z* = 8, *V* = 1713.57 (8) Å³, *D*_c = 1.467 Mg m⁻³, μ = 0.11 mm⁻¹, *F*(000) = 784, *R* = 0.047, and *wR* = 0.123 for 3798 observed reflections with *I* > 2σ(*I*). Compound **2** exhibited a moderate activity in its antimicrobial evaluation against *E. coli* and *P. aeruginosa* and a good activity against *S. aureus* close to that of the standard drug ciprofloxacin. The *in vitro* anticancer activity of **2** was evaluated against two human tumor cell lines, namely, HepG2 hepatocellular carcinoma and MCF-7 breast cancer. HepG2 cancer cell line was more susceptible to compound **2** than MCF-7.

1. Introduction

Indoline-2,3-dione or indole-1*H*-2,3-dione, commonly known as isatin, is a well-known natural product found in plants of genus *Isatis* and *Couroupita guianensis* Aubl. It is also isolated as a metabolite of adrenaline in humans [1]. Due to the importance of isatin, it has received extensive investigations [2]. Isatin is a versatile precursor in a large number of pharmacologically active agents [3] with antifungal [4], antiviral [5], anti-HIV [6], antiprotozoal [7], antitubercular [8], antimalarial [9], antileishmanial [10], and antiepileptic inhibition activities [11]. Antibacterial [12–15], antitumor, and antineoplastic properties of isatin

derivatives have also been reported [16, 17]. The isatin derivative, sunitinib, is an anticancer drug for the treatment of gastrointestinal stromal cancers, metastatic renal cell carcinoma, and pancreatic neuroendocrine tumors [18–20]. In addition, isatin-based derivative SU9516 was reported as a potential inhibitor of cyclin-dependent kinases (CDKs) with apoptotic activity against colon carcinoma cells [21]. Furthermore, we have reported the synthesis of *N,N'*-hydrazono-bis-isatins as active agents against multidrug-resistant cancer cells [22] and the synthesis of isatin-based chromene hydrazones with good cytotoxic activity against leukemia K562, breast MDA-MB-468, and colon HT-29 cell lines [23]. In view of our findings and in continuation of

our interest in chemistry and biological activity of isatins [22–24], we report herein the synthesis, crystal structure, antimicrobial activity, and cytotoxic properties of the title compound.

2. Experimental

2.1. Chemistry

2.1.1. General. Melting point of **2** was measured with a Gallenkamp apparatus and was uncorrected. Infrared (IR) spectrum of **2** was recorded as KBr disk using the Perkin Elmer FT-IR Spectrum BX apparatus. The NMR spectra of **2** were recorded on a Bruker NMR spectrometer. ^1H spectrum was run at 500 MHz and ^{13}C spectrum was run at 125 MHz in deuterated dimethylsulfoxide ($\text{DMSO}-d_6$). Chemical shifts are expressed in δ values (ppm) using the solvent peak as internal standard. Mass spectrum was measured on an Agilent Triple Quadrupole 6410 QQQ LC/MS equipped with an ESI (electrospray ionization) source.

2.1.2. Synthesis of (Z)-N'-(2-Oxoindolin-3-ylidene)formohydrazide (2). A stirred solution of formic acid (20 mL) and (Z)-3-hydrazonoindolin-2-one (**1**) [25] (1.61 g, 10 mmol) was refluxed for 1 h. The reaction mixture was then left to cool. Then formic acid was removed by distillation. The solid obtained was recrystallized from acetic acid to give compound **2** in 58% yield, mp 190–192°C; IR ν 3482–3412 (2NH), 2820 (CH aldehydic), 1735, 1696 (2C=O), 1617 (C=N) cm^{-1} ; ^1H NMR ($\text{DMSO}-d_6$) (the ratio of *cis/trans* = 25/75) δ 6.94–6.97 (m, 1H, ArH), 7.08–7.11 (m, 1H, ArH), 7.38–7.41 (m, 1H, ArH), 7.53–7.55 (m, 1H, ArH), 8.44 (s, 1H, $-\text{CH}=\text{O}$ *cis* rotomer), 8.85 (s, 1H, $-\text{CH}=\text{O}$ *trans* rotomer), 11.30 (br. s, 1H, D_2O exchangeable, NH isatin), 12.70 (s, 1H, NH, D_2O exchangeable, *trans* rotomer), 12.90 (s, 1H, NH, D_2O exchangeable, *cis* rotomer); ^{13}C NMR ($\text{DMSO}-d_6$) δ 111.69, 119.97, 121.11, 123.15, 132.21, 137.02, 143.07, 162.62, 168.23; MS (ESI) m/z 190.11 ($\text{M}^+ + 1$).

2.2. X-Ray Crystallography. X-ray data was collected on a Bruker D8 Venture area diffractometer equipped with graphite monochromatic $\text{MoK}\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$) at 293 (2) K.

Single crystal of **2**, which is suitable for X-ray analysis, was grown by slow evaporation from acetic acid. Computing details: APEX II [26]; cell refinement: SAINT; data reduction: SAINT; program(s) used to solve structure: SHELXTL [27]; program(s) used to refine structure: SHELXTL; molecular graphics: SHELXTL; software used to prepare material for publication: SHELXTL and PLATON [28].

2.3. Antimicrobial Activity

2.3.1. Microorganisms and Media. In this study, we used five reference strains: *Escherichia coli* ATCC 10536, *Pseudomonas aeruginosa* ATCC 15442, *Staphylococcus aureus* ATCC 6538, *Candida albicans* ATCC 90029, and *Candida parapsilosis*

ATCC 22019. The bacterial strains were grown on Mueller-Hinton agar medium (MHA, Becton Dickinson) at 37°C for 24 h and the yeast on Sabouraud agar medium for 48 h.

2.3.2. Antimicrobial Screening. Antimicrobial activity of the synthesized compound was determined by the agar well diffusion method [29]. This method was used to assess the susceptibility of the reference microorganisms to the tested compounds. Petri dishes were prepared with a base layer of Mueller-Hinton agar medium (MHA, Becton Dickinson) and Sabouraud agar. The inoculum was prepared using plate cultures of the reference microbial strains. The colonies were suspended in 0.85% saline and the turbidity was compared with the 0.5 McFarland standards, to produce a suspension of 1.5×10^8 CFU/mL. The suspension was loaded on a sterile cotton swab for streaking over the entire sterile agar surface to ensure a uniform distribution of inoculum. After drying, small wells (6 mm in diameter) were made in the agar plates by sterile cork borer. 100 μL of each compound was loaded into the different wells. Finally, compound **2** was dissolved in dimethylsulfoxide (DMSO) to a final concentration of 10 mg/mL. Ciprofloxacin (50 $\mu\text{g/mL}$) and fluconazole (25 $\mu\text{g/mL}$) were used as standards for antibacterial and antifungal activities, respectively, as positive controls. Negative controls were prepared using DMSO. The plates were incubated at 37°C for 24 h and 48 h for bacterial strains and yeast, respectively. The antibacterial activity was expressed as the mean of inhibition diameters (mm) produced. The experiment was carried out in triplicate and the average zone of inhibition was calculated.

2.3.3. Minimal Inhibitory Concentration. The quantitative assay of the antimicrobial activity of compound **2** was determined by microplate assay (in 96-well plate) using the twofold serial dilution technique as described in (CLSI) [30]. Compound **2** was prepared in DMSO and the correct volume was put in the first microplate well with Mueller-Hinton broth medium, ensuring the concentration to be 1000 $\mu\text{g/mL}$ in that well. The inoculum suspension was prepared in 0.85% saline, with an optical density equivalent to 0.5 McFarland standard, and diluted in Mueller-Hinton broth to obtain final concentration 6×10^5 CFU/mL. This suspension was inoculated in each well of a microdilution plate previously prepared with the tested compound to give concentrations from 1000 $\mu\text{g/mL}$ down to 1.95 $\mu\text{g/mL}$. The last two wells were reserved for inoculum viability and DMSO effect. The control drug for each ATCC strain was ciprofloxacin dissolved in DMSO. The plate was covered and incubated for 24 h at 37°C. Growth was assayed by absorbance measurement at 623 nm. The MIC was defined as the lowest concentration at which the optical density (OD) was reduced to 90% of the OD in the growth control well as measured by spectrophotometer. Results were analyzed visually and spectrophotometrically.

2.4. Anticancer Activity. HepG2 liver cancer and MCF-7 breast cancer cell lines were obtained from the National Cancer Institute (Cairo, Egypt). HepG2 cells were grown in DMEM while MCF-7 was grown in RPMI-1640. Media were

supplemented with 10% heat-inactivated FBS, 50 units/mL of penicillin, and 50 g/mL of streptomycin and maintained at 37°C in a humidified atmosphere containing 5% CO₂. The cells were maintained as “monolayer culture” by serial subculturing. Cytotoxicity was determined using the SRB method as previously described by Skehan et al. [31]. Exponentially growing cells were collected using 0.25% trypsin-EDTA and seeded in 96-well plates at 1000–2000 cells/well in supplemented DMEM medium. After 24 h, cells were incubated for 72 h with various concentrations of the tested compounds as well as doxorubicin as the reference compound. Following 72 h of treatment, the cells were fixed with 10% trichloroacetic acid for 1 h at 4°C. Wells were stained for 10 min at room temperature with 0.4% SRB dissolved in 1% acetic acid. The plates were air-dried for 24 h, and the dye was solubilized with Tris-HCl for 5 min on a shaker at 1600 rpm. The optical density (OD) of each well was measured spectrophotometrically at 564 nm with an ELISA microplate reader (ChroMate-4300, FL, USA). The IC₅₀ values were calculated according to the equation for Boltzmann sigmoidal concentration-response curve using the nonlinear regression models (Graph Pad, Prism Version 5). The results reported are means of at least three separate experiments. Significant differences were analyzed by one-way ANOVA wherein the differences were considered to be significant at $P < 0.05$.

3. Results and Discussion

3.1. Chemistry. The title compound **2** was synthesized in 58% yield by the reaction of (Z)-3-hydrazonoindolin-2-one (**1**) with formic acid under reflux (Figure 1). Conformational isomerism occurs when the rotation about a single bond is relatively unhindered. Such isomers are known as conformational isomers, rotomers, or conformers. However, rotations about single bonds are restricted by a rotational energy barrier which must be small enough to overcome the interconversion of one rotomer to another [32]. In solution phase, the compounds having arylidene-hydrazide structure (–C=N–NH–C=O) may exist, depending on the solvent, as *Z* geometrical isomers around –C=N– bond and as *cis* and *trans* amide rotomers (conformers) around amide N–H single bond [33–36]. In this study, the ¹H NMR (DMSO-*d*₆) spectrum of compound **2** showed the presence of *cis* and *trans* amide rotomers of compound **2** in 25% and 75%, respectively (Figure 2). The X-ray analysis showed the *Z* geometrical isomer of **2** around –C=N– for *cis* and *trans* amide rotomers (X-ray section, Figure 3).

The ¹H NMR spectrum of **2** showed the signals belonging to *cis* and *trans* amide rotomers (conformers) in 25% and 75%, respectively, (Figure 2). It revealed the aldehydic proton at δ 8.44 and 8.85 for *cis* and *trans* rotomers, respectively, whereas D₂O exchangeable signal of hydrazone NH group appeared at δ 12.7 and δ 12.9 for *trans* and *cis* rotomers, respectively (Figure 2).

In addition, the IR spectrum of compound **2** exhibited two absorption bands in the region 3482–3412 cm^{–1} for 2NH groups in addition to the two absorption bands of two carbonyl groups at 1735 and 1696 cm^{–1}. The absorption

TABLE 1: Experimental details of X-ray analysis for compound **2**.

Crystal data	
Chemical formula	C ₉ H ₅ N ₃ O ₂
<i>M_r</i>	187.16
Crystal system, space group	Monoclinic, <i>P</i> 2 ₁ / <i>c</i>
Temperature (K)	293
<i>A</i> , <i>b</i> , <i>c</i> (Å)	4.5206 (1), 22.4747 (7), 17.3637 (5)
<i>B</i> (°)	103.752 (1)
<i>V</i> (Å ³)	1713.57 (8)
<i>Z</i>	8
Radiation type	Mo <i>K</i> α
μ (mm ^{–1})	0.11
Crystal size (mm)	0.71 × 0.15 × 0.08
Data collection	
Diffractometer	Bruker APEX-II D8 venture
Absorption correction	multiscan, SADABS (Bruker, 2012)
No. of measured, independent, and observed (<i>i</i>) reflections	80166, 5269, 3798
<i>R</i> _{int}	0.038
Refinement	
<i>R</i> [<i>F</i> ² > 2σ(<i>F</i> ²)], <i>wR</i> (<i>F</i> ²), <i>S</i>	0.065, 0.127, 4.80
No. of reflections	5269
No. of parameters	253
No. of restraints	0
<i>H</i> -atom treatment	H-atom parameters constrained
$\Delta\rho_{\max}$, $\Delta\rho_{\min}$ (e Å ^{–3})	0.68, –0.26

band of aldehydic proton appeared at 2820 cm^{–1} in the IR of compound **2**. The mass spectrum of **2** revealed a peak corresponding to the molecular ion at *m/z* = 190.11.

3.2. X-Ray Crystallography. The crystal of **2** belongs to monoclinic, space group *P*2₁/*c*, with *a* = 4.5206 (1) Å, *b* = 22.4747 (7) Å, *c* = 17.3637 (5) Å, β = 103.752 (1)°, *Z* = 8, *V* = 1713.57 (8) Å³, *D_c* = 1.467 Mg m^{–3}, μ = 0.11 mm^{–1}, *F*(000) = 784, *R* = 0.047, and *wR* = 0.123 for 3798 observed reflections with *I* > 2σ(*I*) (Table 1).

The asymmetric unit of the title compound, C₉H₇N₃O₂, contains two molecules *cis* and *trans* conformers of amide function for *Z* geometrical isomer around the C2=N2 double bond [37] (Figure 3).

Although, the *trans* conformer of **2** is the kinetically favored candidate in free space, a network of intermolecular hydrogen bonds supported the formation of the *cis* conformer in the crystalline state. The single bond N2–N3 is clearly characterized by the distance of 1.36 Å. The double bond of C2=N2 is characterized by the distance of 1.28 Å. Selected bond distances (Å) and bond angles (°) of compound **2** are illustrated in Table 2.

The torsion angles N2A–N3A–C9A–O2A, N2A–N3A–C9A–H5, H3NA–N3A–C9A–O2A, and H3NA–N3A–C9A–H5 are –175.97°, 4.01°, 3.2°, and 176.82°, respectively. The torsion

FIGURE 1: The synthesis of compound 2 which exists as *cis* and *trans* amide rotomers in DMSO- d_6 (*cis* : *trans* = 25 : 75). The X-ray analysis showed the Z geometrical isomer of 2 around -C=N- for *cis* and *trans* amide rotomers.

FIGURE 2: ^1H NMR (DMSO- d_6) of compound 2.

angles N2B-N3B-C9B-O2B, N2B-N3B-C9B-H10, H3NB-N3B-C9B-O2B, and H3NB-N3B-C9B-H10 are -0.63° , 179.36° , 178.09° , and -1.95° , respectively. The crystal packing is stabilized by intermolecular interactions forming a three-dimensional network (Figure 4 and Table 3).

3.3. Antimicrobial Activity. The synthesized compound 2 is evaluated for its antimicrobial activity against five reference strains: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, and *Candida parapsilosis* using the agar well diffusion method and broth microdilution methods. Compound 2 exhibited a moderate activity against *E. coli* and *P. aeruginosa* while its inhibition zone value is close

to that of the standard drug ciprofloxacin in case of *S. aureus* (Table 4).

According to the results obtained by the well diffusion method, the minimal inhibitory concentration (MIC) value of compound 2 determined for *S. aureus* was $15.6 \mu\text{g/mL}$ compared to ciprofloxacin which is $0.5 \mu\text{g/mL}$.

3.4. Anticancer Activity. Antiproliferative activity of (Z)-N'-((2-oxindol-3-ylidene)hydrazono)indole-3-carboxamide (2) was examined in two human tumor cell lines, namely, HepG2 hepatocellular carcinoma and MCF-7 breast cancer using Sulforhodamine B (SRB) colorimetric assay as described by Skehan et al. [31]. Doxorubicin was included in the experiments as a reference cytotoxic drug. The results were expressed as

FIGURE 3: The asymmetric unit of compound **2** showing the atomic numbering and 40% probability displacement ellipsoids, *cis* : *trans* = 1 : 1.TABLE 2: Selected bond distances (Å) and bond angles (°) of compound **2**.

Bond distances			
O1A–C1A	1.221 (2)	N2A–C2A	1.281 (2)
O2A–C9A	1.215 (3)	N3A–C9A	1.349 (2)
O1B–C1B	1.215 (2)	N1B–C8B	1.410 (2)
O2B–C9B	1.221 (3)	N1B–C1B	1.357 (2)
N1A–C1A	1.372 (2)	N2B–N3B	1.377 (2)
N1A–C8A	1.406 (2)	N2B–C2B	1.275 (2)
N2A–N3A	1.3712 (19)	N3B–C9B	1.343 (2)
Bond angles			
C1A–N1A–C8A	110.35 (13)	N1A–C8A–C7A	128.91 (15)
N3A–N2A–C2A	117.63 (13)	N1A–C8A–C3A	110.21 (13)
N2A–N3A–C9A	114.53 (14)	O2A–C9A–N3A	125.08 (18)
C1B–N1B–C8B	110.41 (13)	O1B–C1B–N1B	127.54 (15)
N3B–N2B–C2B	116.62 (14)	O1B–C1B–C2B	125.83 (15)
N2B–N3B–C9B	117.81 (15)	N1B–C1B–C2B	106.63 (14)
O1A–C1A–C2A	126.91 (15)	N2B–C2B–C1B	128.35 (15)
O1A–C1A–N1A	126.56 (16)	N2B–C2B–C3B	125.65 (15)
N1A–C1A–C2A	106.53 (14)	N1B–C8B–C3B	110.11 (14)
N2A–C2A–C1A	129.10 (14)	N1B–C8B–C7B	128.52 (15)
N2A–C2A–C3A	124.87 (14)	O2B–C9B–N3B	125.67 (19)

TABLE 3: Hydrogen-bond geometry (Å, °) of compound **2**.

<i>D</i> –H... <i>A</i>	<i>D</i> –H	H... <i>A</i>	<i>D</i> ... <i>A</i>
N1AA–H1NA...O2BB	0.87 (2)	2.1980	2.981 (2)
N1AA–H1NA...N2BB	0.87 (2)	2.6140	3.368 (2)
N1AB–H1NA...O2BA	0.891 (19)	2.2010	3.034 (19)
N1AB–H1NA...N2BA	0.891 (19)	2.5800	3.392 (2)
N1BA–H1NA...O2BD	0.960 (5)	2.3200	3.014 (2)

TABLE 4: Antibacterial activity of **2** and its mean inhibition diameters (mm).

	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>C. albicans</i>	<i>C. parapsilosis</i>
Compound 2	10	12	18	8	—
Ciprofloxacin	23	24	20		
Fluconazole	—	—	—	29	27

FIGURE 4: Portion of the crystal packing of compound **2** showing hydrogen bonds as dashed lines.TABLE 5: *In vitro* cytotoxic activities of the compound **2** against hepatocellular carcinoma (HepG2) and breast cancer (MCF-7) cell lines.

	IC_{50} (μM) ^a	
	HepG2	MCF-7
Compound 2	105	183
Doxorubicin	29.5	3.3

^a IC_{50} values are the mean $\pm$ S.D. of three separate experiments.

growth inhibitory concentration (IC_{50}) values which represent the compound concentrations required to produce a 50% inhibition of cell growth after 72 h of incubation compared to untreated controls (Table 5). From the results, it was obvious that compound **2** displayed moderate growth inhibitory activity against HepG2 hepatocellular carcinoma cell line. It was found to be that compound **2** was less potent than doxorubicin by approximately 3.5-fold with IC_{50} of 105 μM , while doxorubicin showed IC_{50} of 29.5 μM . On the other hand, compound **2** displayed weak cytotoxic activity against MCF-7 breast cancer cell line with IC_{50} of 183 μM comparable to doxorubicin which showed IC_{50} of 3.3 μM .

4. Conclusion

In conclusion, the title compound **2** appeared as *cis* and *trans* amide rotomers in 25% and 75%, respectively, in DMSO- d_6 . X-ray single crystal analysis illustrates the interesting property of compound **2** in crystalline state as a *Z* geometrical isomer around $-C=N-$ bond with *cis* and *trans* amide rotomers due to hydrogen bonds even though in free space the *trans* conformer is the kinetically favoured candidate. Compound **2** showed moderate-good activity against five microbial species in its antimicrobial evaluation and a good potency against HepG2 and MCF-7 cell lines in its anticancer evaluation.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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