Synthesis, Characterization and X-Ray Crystal Structure of the New Schiff Base and Anticandidal Evaluation

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Summary: A new Schiff base, namely 2-methoxy-6-((2-(4 nitrophenyl) hydrazineylidene) methyl)phenol was synthesized and characterized by melting points, elemental analysis, thermogravimetric analysis and spectroscopic techniques (FT-IR, ^{1}H-NMR and UV-VIS spectra). The chemical structure of compound was further confirmed by single crystal structural X-ray diffraction. The Schiff base is crystallized in the triclinic space group P-1. In the crystal, molecules are linked by O-H…O hydrogen bonds between the hydroxy "-O-" atom and the methoxy "-O-" atom. Furthermore, the synthesized Schiff base was tested for the *in vitro* anticandidal activities using CLSI broth microdilution method against human pathogenic *Candida albicans*, *C. parapsilosis* and *C. krusei* standard strains. In the anticandidal activity test results, the new Schiff base was found to be effective at 1 mg/mL - 0.25 mg/mL concentrations. (The last line omitted) (The sentence marked in red will be deleted)

Key words: Schiff base; Crystal structure; Methoxy; Human pathogenic; Anticandidal activity.

Introduction

Schiff bases have potential sites such as nitrogen and other donors; it may be attributed to their stability and applications in both synthetic and structural research [1, 2]. Also they are very popular, due to their versatile application in many areas. Schiff bases can be used in industrial [3, 4] and chemical applications [5-7], depending on their structural properties. In addition, Schiff bases and their complexes are also known for their antimicrobial, anti-cancer and anti-inflammatory activities and properties [8-12].

The Super Bug (multidrug resistant organisms) has become a significant threat to global health. At the same time, the incidence of fungal infections has increased significantly [13]. The current increase has led to research on new antifungal agents [14]. Schif bases are known to possess promising antifungal activities [15-19].

In this present study, a new Schiff base ligand was synthesized and characterized using different spectroscopic and analytical methods such as melting points, elemental, thermogravimetric, FT-IR, ^{1}H-NMR and UV-Vis analyses, respectively. To the best of our knowledge the crystal structure was also established for the first time. *In vitro* biological screenings of the synthesized ligand were carried out against the human pathogenic *Candida* spp.

Experimental

Synthesis of 2-methoxy-6-((2-(4-nitrophenyl)hydrazineylidene)methyl)phenol

4-Nitrophenylhydrazine (10 mmol) was dissolved by heating in 25 ml of methanol. The solution of 2-Hydroxy-3-methoxybenzaldehyde (10 mmol) in 25 ml of methanol was then added to the solution of 4-Nitrophenylhydrazine by addition funnel. The resulting solution was heated with stirring at 60 ºC for 2 h. Progress of the reaction was monitored by thin-layer chromatography (TLC). The completion of the reaction was monitored through TLC. R_f: 0.67 [*n*-hexane/ethyl acetate (2:1)]. After completion of the reaction, the resulting suspension was cooled to ambient temperature. Red precipitate was collected by filtration and dried under vacuum. The red solid was recrystallized from a minimum volume methanol. Yield 2.78 g (97 %), mp: 212 ºC. Elemental analysis found: C, 58.38 (58.53), H, 4.43 (4.53), N, 14.47 (14.63) % calculated for $C_{14}H_{13}N_3O_4$. See Scheme-1 for the preparation reaction of compound 1. The compound is soluble in organic solvents (*e.g.* methanol, ethanol, dimethyl sulfoxide, ethyl acetate, chloroform, dichloromethane, dimethylformamide, acetonitrile), however, insoluble in water.

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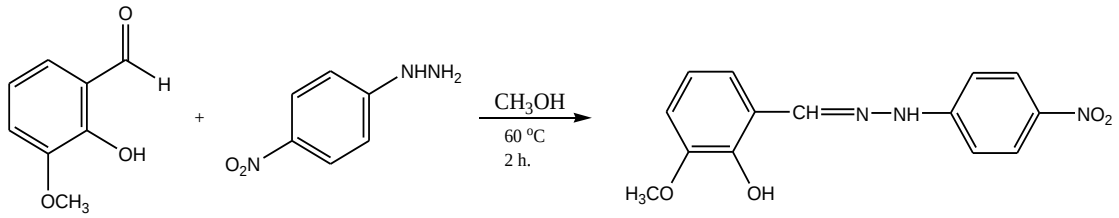

2-hydroxy-3-methoxybenzaldehyde (4-nitrophenyl)hydrazine 2-methoxy-6-((2-(4-nitrophenyl)hydrazineylidene)methyl)phenol

Scheme-1: Preparation reaction scheme of compound **1.**

Physical measurements

Melting point was determined using a Stuart SMP 30 melting points apparatus and reported results are uncorrected. Elemental analysis (C, H, N) was carried out using a LECO-932 CHNS-O analyzer. FT-IR spectra were recorded (as KBr pellets) on an IR-Affinity-1S Shimadzu FT-IR Spectrometer.^{1}H-NMR was recorded at ambient temperature; on a Agilent 400 MHz NMR Magnet Spectrometer in deuterated dimethyl sulfoxide (DMSO-d_6). The UV-VIS spectra were carried out with a Shimadzu UV-1700 PharmaSpec UV-VIS Spectrophotometer in the range 900-190 nm. The thermal analysis was performed in nitrogen atmosphere using a Shimadzu TG-60H thermal analyzer with a heating rate of 20 °C/min in the temperature range 25–800 ºC. X-ray diffraction was carried out on a Bruker D8 QUEST diffractometer.

X-ray data collection and structure refinement

X-ray diffraction study was performed on a Bruker D8 QUEST diffractometer equipped with a graphite-monochromatic Mo-K_α radiation (λ=0.71073 Å) at 296 K. The structure was solved by direct methods using the solution program SHELXS-97 and refined by full-matrix least-squares methods on F^2 using with SHELXL-97 [20]. The H-atoms were placed in idealized positions and constrained to ride on their parent atoms with distances in the range of N-H = 0.86 Å, Csp^2-H = 0.93 Å and C(methyl)-H= 0.96 Å; U_{iso}(H)= 1.2Ueq(C,N) or U_{iso}(H)=1.5U_{eq}(C_{methyl}). Hydrogen atoms of -OH group were localized from difference electron density synthesis and refined independently in isotropic approximation. Molecular diagrams were created using ORTEP-3 for Windows program [21]. Details of crystallographic parameters, data collection and refinements for compound **1** are given in Table-1. Crystallographic data for the structures reported in this manuscript have been deposited with the Cambridge Crystallographic Data Centre under the CCDC numbers: **1535350** compound **1.** Copy of this information can be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK fax (+44 1123 336 033, email: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

Table-1: Crystal data and structure refinement details for compounds **1**.

	1
Empirical formula	**$C_{14}H_{13}N_3O_4$**
Formula weight	**287.27**
T [K]	**296(2)**
Wavelength (Å)	**0.71073**
Crystal system, space group	**Monoclinic, P c**
Unit cell dimensions (Å, º)	
a	**4.544(2)**
b	**11.373(6)**
c	**13.188(7)**
α	**90.00**
β	**92.496(18)**
γ	**90.00**
V (Å^3)	**680.9(6)**
Z	**2**
Absorbtion coefficient (mm^{-1})	**0.105**
D_{calc} (Mg /m^3)	**1.401**
F(000)	**300**
Crystal dimensions (mm)	**0.18x0.14x0.11**
θ range for data collection (º)	**3.09-28.00**
Index ranges	**-5≤ h ≤6,-14≤ k ≤15,-17≤ l ≤17**
Reflections collected	**4830**
Independent reflections	**3265**
Data/parameters	**1640/194**
Max. and min. transmission	**0.983, 0.989**
Final R indices [I≥2σ(I)]	**R_1 = 0.0532**
	wR_2 = 0.1331
R indices (all data)	**R_1 = 0.1102**
	wR_2 = 0.1602
Goodness-of-fit on F^2	**1.025**
Largest difference in peak and hole (e Å^{-3})	**0.307/ -0.272**

Anticandidal activity assay

Candida albicans ATCC 90028, *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 standard strains were obtained from the American Type Culture Collection. All microorganisms were stored at -85 °C in 15 % sterile glycerol. *Candida* strains were refreshed on Potato Dextrose Agar

(PDA, Merck) plates and RPMI medium (Sigma-Aldrich) at 37 °C. Thereafter, all microorganisms were standardized versus McFarland No: 0.5 (1-5 x 10^6 CFU/mL) in sterile saline (0.85%), turbidimetrically [22].

The *in vitro* broth microdilution assay according to CLSI standards [23, 24] was used to determine the anticandidal activity of the new Schiff base (Compound 1). Stock solution of the test sample was prepared in ethanol and diluted with sterile distilled water. Sample dilution series were prepared from 10 mg/mL to 0.002 mg/mL in 96 well microtiter plates. 1:1000 diluted *Candida* suspensions (100 µL) were then added to each well. After incubation at 37 °C for 18-24 h, for staining of viable microorganisms, 20 µL of resazurin solution of 0.01 % was added to all the plate. The first blue well was determined as the minimal inhibitory concentration (MIC, mg/mL). The last row containing medium with microorganism was used as negative control and medium served as a growth control. Amphotericin B (Sigma, Germany) was used as standard antimicrobial agent at concentration range $1.6x10^{-5}$-3.2×10^{-2} mg/mL. All experiments were repeated in triplicate and average MICs are given in Table-2.

Table-2: Antimicrobial activities of the Schiff base (compound) and standard antimicrobial (MIC, mg/mL).

	Candida albicans ATCC 90028	*C. parapsilosis* ATCC 22019	*C. krusei* ATCC 6258
Compoun 1	**1**	**0.25**	**1**
Amphotericin B	**0.064×10^{-3}**	**0.064×10^{-3}**	**0.064×10^{-3}**

Results and Discussion

FT-IR measurements

The IR spectrum of compound 1 (Fig. 1) shows broad band at 3478 cm^{-1} is attributed to intramolecular hydrogen bonded -OH group [25]. A medium absorption band at 3258 cm^{-1} is due to stretching vibration of the - NH (secondary amine) group [26]. A strong absorption band at 1595 cm^{-1} is due to C=N (imine) stretching vibration frequency [27]. A medium absorption band at 645 cm^{-1} is due to bending vibration of the NH (secondary amine) group [26]. The absorption bands at 1552-1374 cm^{-1} are due to NO asymmetric and symmetric stretching vibrations of $-NO_2$ [26]. A strong absorption band at 1249 cm^{-1} is due to C-O stretching vibration phenolic -OH group. Strong absorption band at 1274 and 1078 cm^{-1} is due to C-O stretching vibration C-O-C group [26]. The different aromatic -CH vibrations are observed at 3084 cm^{-1}, 3044 cm^{-1}, 1574 cm^{-1}, 1494 cm^{-1}, 1105 cm^{-1} and 731 cm^{-1}. The aliphatic -CH stretching vibrations are observed at 2964 cm^{-1}, 2869 cm^{-1} and 2838 cm^{-1}[28].

^{1}H NMR measurement

^{1}H-NMR spectrum is recorded at ambient temperature; using a Agilent 400 MHz NMR Magnet Spectrometer in deuterated dimethyl sulfoxide (DMSO-d_6). The spectrum (Fig. 2) exhibits a multiplet at 6.77-8.10 ppm for the hydrogens of the aromatic rings. The seconder amine hydrogen (-NH-) leads to a singlet of integration intensity equivalent to one hydrogen at 9.46 ppm. The azomethine hydrogen (-CH=N-) leads to a singlet of integration intensity equivalent to one hydrogen at 8.33 ppm. The spectrum displays signals at 3.78 ppm (s, 3H) and 11.23 ppm (s, 1H) due to the hydrogens of the $-OCH_3$ and –OH (phenolic) groups respectively.

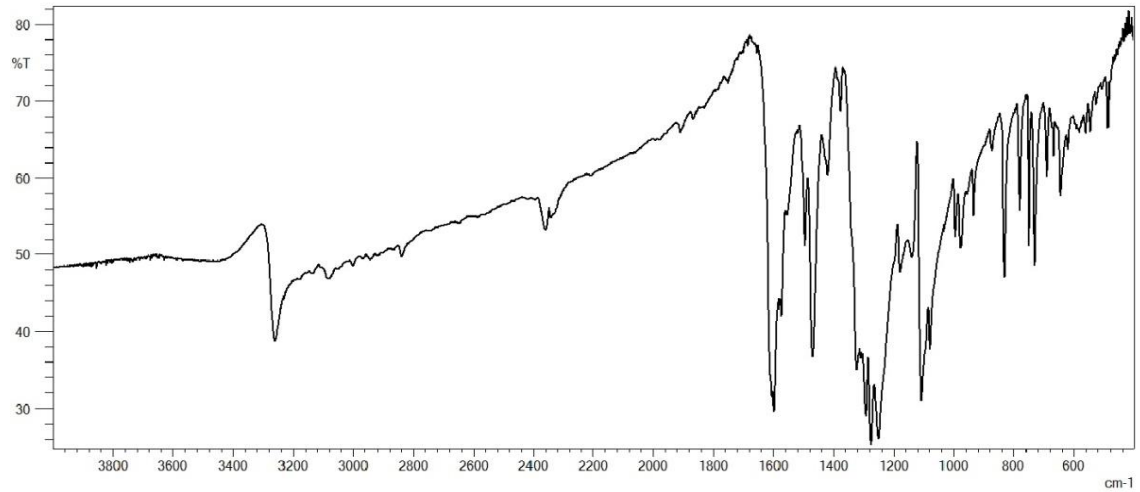

Fig. 1: FT-IR Spectrum of compound **1.**

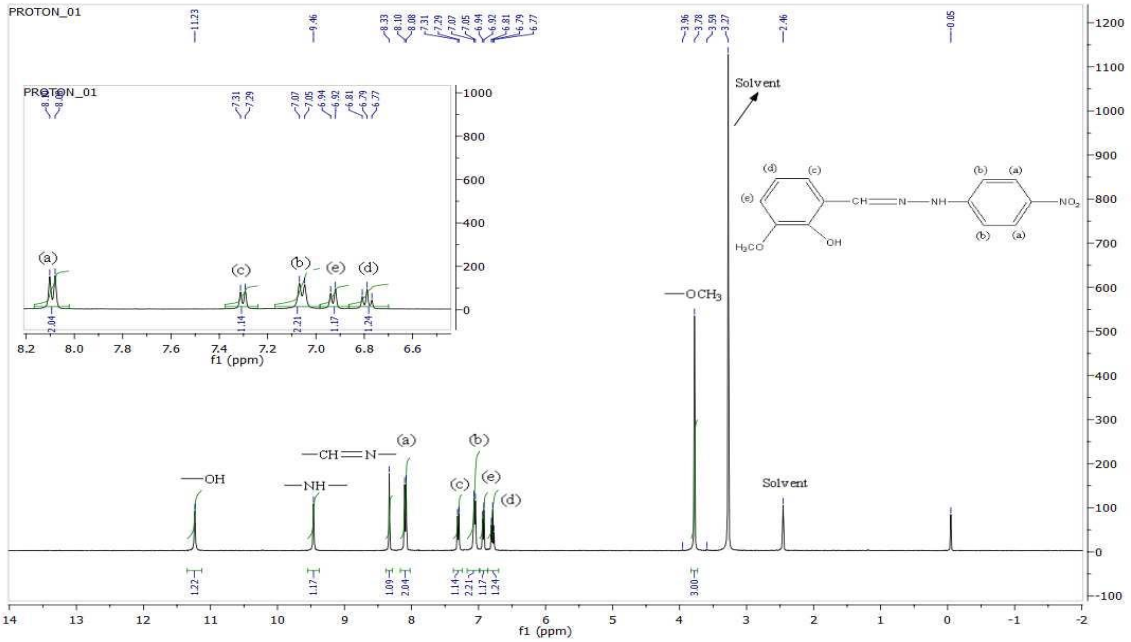

Fig. 2: ^{1}H NMR (400 MHz) spectrum of compound 1 in DMSO-d_6.

UV–VIS Spectra

The electronic spectra of the compound 1 was recorded in CH_3CN (1 x 10^{-5}M) at room temperature. The electronic absorption spectra of the compound 1 exhibit mainly four bands (Fig. 3). The two bands on the higher energy side, at 213 nm and 298 nm are due to the excitation of the π electrons (π-π* transitions) of the aromatic rings. The third band within the 328 nm range is assigned to the π-π* transition within the (-CH=N-) group, while the longer wavelength band at 399 nm is due to an intramolecular charge transfer (CT transition) involving the whole molecule [29].

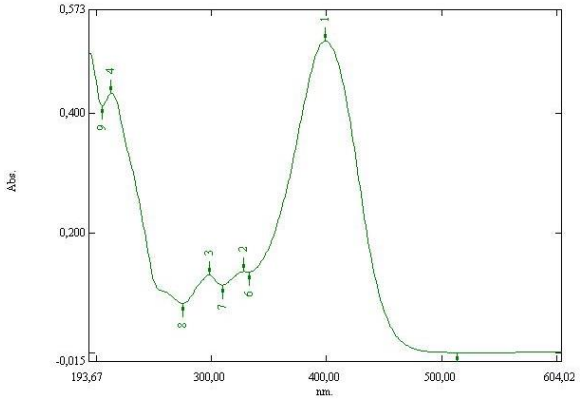

Fig. 3: UV-VIS absorption spectrum of compound **1.**

Thermal analysis

The TGA curve of compound 1 (Fig. 4) indicates that the compound begins to decompose at 234 ºC. The TGA curve for compound displays three stages of mass loss within the temperature range of 234-800 °C (Table-3). The first stage is at 234-416 ºC, and exhibits a mass loss of 39.15%, corresponding to the loss of $C_7H_7O_2$ (calc. 42.82%) [30]. The second stage occurs at 416-632 ºC, with a mass loss of 11.45%, corresponding to the loss of CHN (calc. 9.40%). The third stage of decomposition occurs at the temperature range 632-800 ºC, with a mass loss of 6.60%, corresponding to the loss of NH (calc. 5.22%).

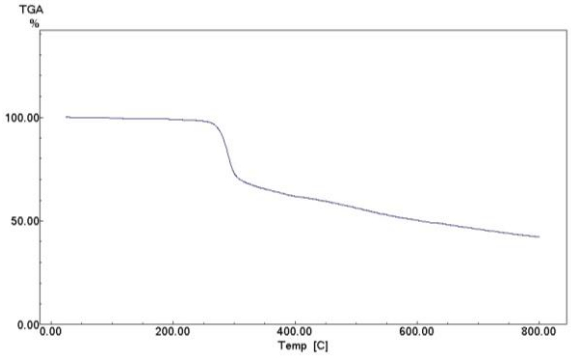

Fig. 4: TGA spectrum of the compound **1.**

Table-3: TGA data of compound **1.**

Temperature range, °C	Weight loss		Assignment
	Found, %	Calculated, %	
234-800	39.15	42.82	$C_7H_7O_2$
416-632	11.45	9.40	CHN
632-800	6.60	5.22	NH

Crystal structure of compound 1

The molecular structure of 1, is shown in Fig. 5. The details of the crystal structure solutions are summarized in Table-1 and the selected bond lengths and angles are listed in Table-4. The compound is soluble in organic solvents (*e.g.* methanol, ethanol, dimethyl sulfoxide, ethyl acetate, chloroform, dichloromethane, dimethylformamide, acetonitrile), however, insoluble in water. (The all sentence will be deleted). Compound 1, $C_{14}H_{13}N_3O_4$, crystallizes in the monoclinic crystal system with Pc space group. The molecule adopts an E geometry with respect to the C=N double bond, with an C4-N2-N3-C7 torsion angle of 179.4(6)° as seen in Fig. 5. The methoxybenzene and the nitrobenzene rings are roughly planar, however, with the two benzene rings slightly twisted with respect to each other by a dihedral angle of 9.11(9)° and these two planes make dihedral angles with the central bridge (N2/N3/C7) of 6.01(10) and 3.27(8)°, respectively. The N2-N3 [1.389(9) Å], O1-N1[1.247(9) Å] and O2-N1 [1.236(10) Å] bond distances are appreciably close to that of a C=N double bond (1.28 Å). All the bond distances (Table-4) in the molecule are within normal ranges comparable to those of the similar Schiff base complex [31-37].

Table-4: Selected bond distances [Å] and angles [°] of compounds **1.**

$C_{14}H_{13}N_3O_4$					
O(1)-N(1)	1.247(9)	O(4)-C(12)	1.350(9)	N(2)-C(4)	1.345(11)
O(2)-N(1)	1.236(10)	O(4)-C(14)	1.402(9)	N(2)-N(3)	1.389(9)
O(3)-C(13)	1.359(9)	N(1)-C(1)	1.399(11)	N(3)-C(7)	1.287(10)
O(1)-C(1)-C(2)	119.0(3)	N(1)-C(2)-C(1)	122.9(3)	C(2)-C(1)-C(5)	119.9(3)
O(1)-C(1)-C(5)	121.1(3)	N(2)-C(2)-C(1)	126.3(3)		

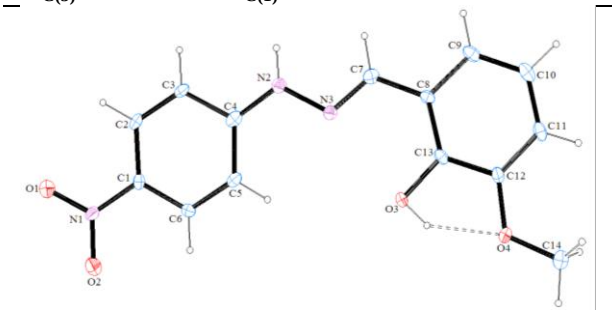

Fig. 5: The molecular structure of Compound 1 and atomic labeling scheme. Displacement ellipsoids are drawn at the 40% probability level. Hydrogen-bonding interaction is shown as dashed lines.

In the structure, the intramolecular hydrogen bonds involving the hydroxy O3 atom and methoxy O4 atom plays important role in stabilizing the crystal structure. The parameters for hydrogen bonding interaction in the complex are as follows: H…O3 1.90(16) Å, O3…O4 2.598(7) Å, O3-H3'…O4 135(17)° (Fig. 5).

Anticandidal activity

In this study, *in vitro* anticandidal activity of Schiff base compound1 and standard antmicrobial Amphotericin B were tested comparatively against 3 different human pathogenic *Candida* standard strains. As reported, the best anticandidal effect of the Schiff base was observed at the concentration of 0.25 mg/ml against *C. parapsilosis* strain. Inhibition concentrations of 1 mg/mL were determined against the other strains, *C. albicans* and *C. krusei*, respectively. The preliminary anticandidal activity results suggest rather moderate inhibitory activity when compared to the standard antimicrobial reference substance.

Conclusion

In the present work, the new Schiff base (1) was synthesized and characterized by many different techniques. It is roughly planar, with the two benzene rings twisted slightly with respect to each other by a dihedral angle of 6.90(9)º. In the crystal, molecules are linked by O-H…O hydrogen bonds between hydroxy -O- atom and methoxy -O- atom. All measurements show good agreement with the structure. Preliminary findings from this study showed that the new Schiff base is *in vitro* moderately anticandidal effective at the tested concentration range.

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