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Original Research Article

X-ray Molecular Structure of ({[(1*E*)-3-(1*H*-Imidazol-1-yl)-1-phenylpropylidene]amino} oxy)(3,4,5-trimethoxyphenyl)-methanone: A Potential Anti-*Candida* Agent

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Abstract

Purpose: *To elucidate the solid-state conformation as well as the imine double bond configuration of a potential anti-Candida agent ({[(1E)-3-(1H-imidazol-1-yl)-1-phenylpropylidene]amino}oxy)(3,4,5-trimethoxyphenyl)methanone.*

Methods: *Acetophenone was used as a starting material to prepare the target oximino ester in a four-step reaction sequence. Nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR) and mass spectrometry were used to confirm the chemical structure of the synthesized compounds. Thereafter, x-ray crystallography was performed on single crystals of the target compound. The solid-state conformation of the target molecule and the (E)-configuration of its imine double bond were determined via the investigation of its single crystal x-ray molecular structure.*

Results: *The titled compound crystallized in the triclinic space group P-1 with a = 11.0719 (7) Å, b = 14.6602 (9) Å, c = 14.8530 (9) Å, α = 67.205 (4)°, β = 80.388 (5)º, γ = 70.100 (5)°, V = 2088.2 (2) Å^{3}, and Z = 4. Individual molecules were packed in the crystal by three weak non-classical intermolecular hydrogen interactions, including C9A—H9AA•••O3A, C9B—H9BA•••O3B, C18B—H18C•••O2A and C20B—H20B•••O4B.*

Conclusion: *The results of the single crystal x-ray molecular structure of the titled anti-Candida agent unequivocally confirmed its (E)-configuration.*

Keywords*: Molecular structure, X-ray crystallography, Synthesis, Azole, Anti-Candida*

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INTRODUCTION

A substantial increase in fungal infections has been observed over the past few decades. Invasive and nosocomial fungal infections are primarily caused by *Candida* and *Aspergillus* species, where *Candida albicans* is responsible for the majority of invasive and superficial *Candida* infections.

The azole antifungal agents constitute a major antifungal class and contain an azole pharmacophoric moiety. In spite of the fact that there is a growing list of new antifungal agents,

the treatment of fungal infections remains unsatisfactory in many cases. Furthermore, the use of many antifungal drugs is often complicated by clinical obstacles, including a suboptimal spectrum of activity, hazardous drug-drug interactions, toxicity, and limited bioavailability [1-6]. Consequently, the search for novel antifungal agents is urgently needed for clinical therapy.

The molecular structure of the anti-*Candida* oximino ester, namely ({[(1*E*)-3-(1*H*-imidazol-1-yl)-1-phenylpropylidene]amino}oxy)(3,4,5-trimethoxyphenyl)methanone (**4**) has not been previously investigated. Herein, we report the molecular structure as well as the configuration of the imine moiety of the oximino ester **4** using x-ray crystallography as an unambiguous analytical tool.

EXPERIMENTAL

General

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. NMR spectra were carried out on a Bruker NMR spectrometer operating at 500 MHz for ^{1}H and 125.76 MHz for ^{13}C at the Research Center, College of Pharmacy, King Saud University, Saudi Arabia. Tetramethylsilane (TMS) was used as internal standard and chemical shift values were recorded in ppm on δ scale. ^{1}H-NMR data were represented as follows: chemical shifts, multiplicity (s. singlet, d. doublet, t. triplet, m. multiplet) and number of protons. The ^{13}C-NMR data were represented as chemical shifts and type of carbon. Mass spectra were measured on Agilent Triple Quadrupole 6410 QQQ LC/MS with an electrospray ionization (ESI) source. X-ray diffraction measurements of the target oximino ester **4** were performed using Bruker SMART APEXII CCD diffractometer. Crystallographic data of the title anti-*Candida* agent **4** has been deposited with the Cambridge Crystallographic Data Center (supplementary publication numbers CCDC-1048685). Copies of the data can be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (deposit@ccdc.cam.ac.uk).

Chemistry

Preparation of 3-(1H-imidazol-1-yl)-1-phenyl-propan-1-one (2)

A catalytic amount of concentrated hydrochloric acid (0.1 mL) was added to a mixture of acetophenone (2.4 g, 20 mmol), dimethylamine hydrochloride (2.2 g, 27 mmol) and paraformaldehyde (0.81 g, 9 mmol). The reaction mixture was refluxed in absolute ethanol (5 mL) for two hours. Acetone (20 mL) was added to the cooled reaction mixture in order to precipitate the Mannich base hydrochloride **1**. A solution containing compound **1** (3.7 g, 17.4 mmol) and imidazole (2.4 g, 34.8 mmol) in water (10 mL) was refluxed for five hours. Cooling the reaction mixture led to precipitation of the ketone **2** which was filtered off to give 2.7 g (77%) of **2** mp 368-370 K [7]. Ketone **2** was used in the next step without any further purification, and its chemical structure was confirmed *via* ^{1}H and ^{13}C NMR.

Preparation of (1E)-N-hydroxy-3-(1H-imidazol-1-yl)-1-phenylpropan-1-imine (3)

Hydroxylamine hydrochloride (1.39 g, 20 mmol) was added to a solution containing ketone **2** (2.00 g, 10 mmol) and KOH (1.12 g, 20 mmol) in ethanol (10 mL). The reaction was stirred under reflux for 18 h, cooled to ambient temperature, and filtered. After concentrating the filtrate under reduced pressure, the residue was poured onto ice-cold water (15 mL). The precipitated solid was filtered, dried and re-crystallized from ethanol to yield 1.51 g (70 %) of compound **3** as colorless crystals with mp 428-430 K [8]. The assigned chemical structure of oxime **3** was confirmed *via* ^{1}H and ^{13}C NMR and mass spectral data.

Preparation of ({[(1E)-3-(1H-imidazol-1-yl)-1-phenylpropylidene]amino}oxy)(3,4,5-trimeth-oxyphenyl)methanone (4)

1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI.HCl, 1.40 g, 7.3 mmol) was added to a stirred solution containing 3,4,5-trimethoxybenzoic acid (1.49 g, 7 mmol) and 4-dimethylaminopyridine (DMAP, 400 mg) in dichloromethane (75 mL). Thereafter, oxime **3** (1.49 g, 6.9 mmol) was added and the reaction mixture was stirred at ambient temperature for 18 h. The reaction mixture was successively washed with water (2 x 20 mL), a 10 % $NaHCO_3$ solution (2 x 15 mL), and water (2 x 15 mL). The organic phase was separated, dried (Na_2SO_4), and evaporated under vacuum. The residue was re-crystallized (isopropanol) to yield 1.50 g (53 %) of the target oximino ester **4** as colorless crystals with mp 408-410 K [8]. The assigned chemical structure of compound **4** was confirmed *via* ^{1}H and ^{13}C NMR and mass spectral data.

Crystal structure determination

The slow evaporation of the isopropanol solution containing pure oximino ester **4** afforded its colorless block single crystals. A single crystal

with dimensions of 0.32 × 0.23 × 0.05 mm was selected for x-ray diffraction analysis. Data were collected on a Bruker APEX-II CCD area diffractometer equipped with graphite monochromatic CuKa radiation (λ = 1.54178 Å) at 296 (2) K.

RESULTS

Synthetic reactions

Oximino ester **4** was obtained as shown in Scheme 1. Briefly, acetophenone was subjected to a Mannich reaction followed by substitution of the resulting Mannich base hydrochloride **1** with imidazole to furnish the pivotal ketone **2**. Subsequently, the ketone function in compound **2** was transformed to an oxime *via* reaction with hydroxylamine hydrochloride in the presence of potassium hydroxide to yield the oxime **3**. Esterification of the hydroxyl moiety in compound **3** with 3,4,5-trimethoxybenzoic acid in the presence of the coupling agent EDCI.HCl and DMAP provided the target oximino ester **4**.

3-(1H-Imidazol-1-yl)-1-phenylpropan-1-one (2)

^{1}H-NMR ($CDCl_3$): δ 3.44 (t, *J* = 6.5 Hz, 2H, -C*H*$_2$-CH_2-N), 4.43 (t, *J* = 6.5 Hz, 2H, -CH_2-C*H*$_2$-N), 6.98 (s, 1H, -N-C*H*=CH-N=), 7.03 (s, 1H, -N-CH=C*H*-N=), 7.45–7.49 (m, 2H, Ar-H), 7.56-7.61 (m, 2H, Ar-H, -N-C*H*=N-), 7.92 (d, *J* = 7.5 Hz, 2H, Ar-H); ^{13}C-NMR ($CDCl_3$): δ 39.9 (-*C*H_2-CH_2-N), 41.5 (-CH_2-*C*H_2-N), 119.1 (-N-*C*H=CH-N=), 127.9, 128.8, 129.6 (-N-CH=*C*H-N=, Ar-CH), 133.8, 136.2 (Ar-CH, Ar-C), 137.5 (-N-*C*H=N-), 196.6 (C=O).

(1E)-N-Hydroxy-3-(1H-imidazol-1-yl)-1-phenylpropan-1-imine (3)

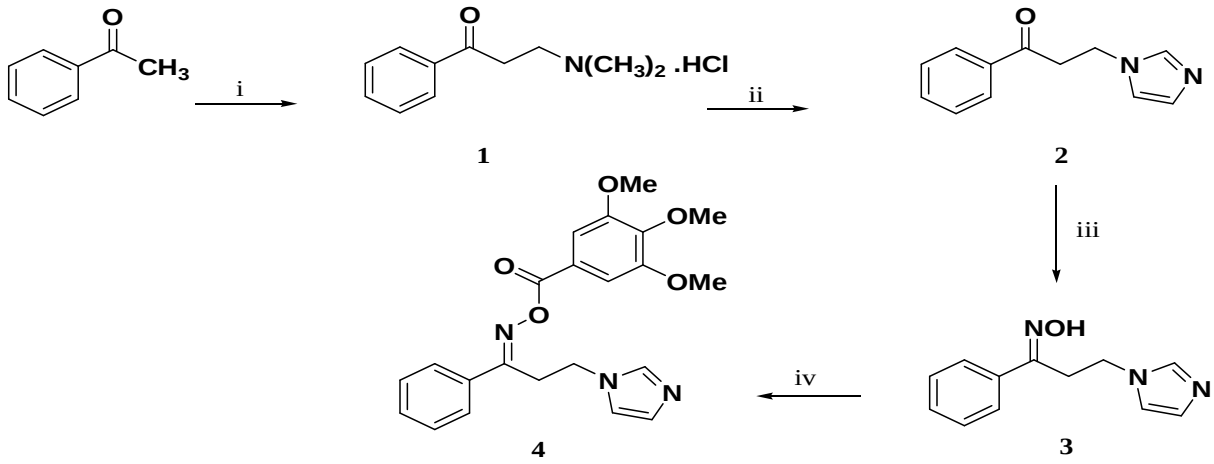
^{1}H-NMR ($CDCl_3$): δ 3.31 (t, *J* = 7.1 Hz, 2H, -C*H*$_2$-CH_2-N), 4.28 (t, *J* = 7.1 Hz, 2H, -CH_2-C*H*$_2$-N), 6.96 (s, 1H, -N-C*H*=CH-N=), 7.07 (s, 1H, -N-CH=C*H*-N=), 7.29–7.49 (m, 5H, Ar-H), 7.58 (s, 1H, -N-C*H*=N-); ^{13}C-NMR ($CDCl_3$): δ 28.3 (-*C*H_2-CH_2-N), 41.8 (-CH_2-*C*H_2-N), 119.1 (-N-*C*H=CH-N=), 126.1, 128.8, 128.9 (-N-CH=*C*H-N=, Ar-CH), 135.1, 137.0 (Ar-C), 139.5 (-N-*C*H=N-), 155.4 (C=N-OH); MS *m/z* (ESI): 216.0 $[M + 1]^+$ [8].

({[(1E)-3-(1H-Imidazol-1-yl)-1-phenylpropylidene]amino}oxy)(3,4,5-trimethoxyphenyl) methanone (4)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.45 (t, *J* = 6.8 Hz, 2H, -C*H*$_2$-CH_2-N), 3.92 (s, 6H, 2 × OC*H*$_3$), 3.94 (s, 3H, OC*H*$_3$), 4.28 (t, *J* = 6.8 Hz, 2H, -CH_2-C*H*$_2$-N), 6.91 (s, 1H, -N-C*H*=CH-N=), 7.02 (s, 1H, -N-CH=C*H*-N=), 7.27–7.50 (m, 6H, -N-C*H*=N-, Ar-H), 7.67 (d, *J* = 7.0 Hz, 2H, Ar-H); ^{13}C-NMR ($CDCl_3$): δ 30.9 (-*C*H_2-CH_2-N), 43.6 (-CH_2-*C*H_2-N), 56.5 (2 × O*C*H_3), 61.0 (O*C*H_3), 106.9 (Ar-*C*H), 118.6 (-N-*C*H=CH-N=), 123.5, 127.3, 129.1, 130.1, 131.3, 132.9 (-N-CH=*C*H-N=, Ar-CH, Ar-C), 136.8 (-N-*C*H=N-), 142.9, 153.2 (Ar-C), 163.2 (C=N), 163.6 (C=O); MS *m/z* (ESI): 410.1 $[M + 1]^+$ [8].

Crystal structure of the target oximino ester 4

Cell refinement and data reduction were performed using Bruker SAINT [9]. SHELXS-97 [10] was used to solve and refine the structure. The final refinement was performed using the



Scheme 1: Synthesis of the title compound **4**. Reagents and conditions: i) $HN(CH_3)_2$.HCl, $(CH_2O)_n$, conc. HCl, ethanol, reflux, 2 h; ii) Imidazole, water, reflux, 5 h; iii) H_2NOH.HCl, KOH, ethanol, reflux, 18 h; iv) 3,4,5-Trimethoxybenzoic acid, EDCI.HCl, DMAP, DCM, rt, 18 h.

full-matrix least-squares techniques with anisotropic thermal data for non-hydrogen atoms on F2. All hydrogen atoms were placed in calculated positions and constrained to ride on their parent atoms. Multi-scan absorption correction was applied using SADABS software [9]. The crystallographic data and refinement information are summarized in Table 1, and the selected bond lengths and angles are listed in Table 2. The labeled displacement ellipsoid plot is shown in Figure 1, in which the minor disordered component has been omitted for clarity. Figure 2 depicts the packing of the molecules in the crystal structure.

Table 1: Crystallographic data and refinement information.

Molecular formula	$C_{22}H_{23}N_3O_5$
Mr	409.43
Crystal system, space group	Triclinic, P
Temperature (K)	296
a, b, c (Å)	11.0719 (7), 14.6602 (9), 14.8530 (9)
α, β, γ (°)	67.205 (4), 80.388 (5), 70.100 (5)
V (Å3)	2088.2 (2)
Z	4
Radiation type	Cu Kα
μ (mm−1)	0.77
Crystal size (mm)	0.32 × 0.23 × 0.05
Data collection	
Diffractometer	Bruker APEX-II CCD diffractometer
Absorption correction	Multi-scan SADABS Bruker 2014
Tmin, Tmax	0.790, 0.962
No. of measured, independent and observed [I > 2σ(I)] reflections	25011, 6450, 2607
Rint	0.105
Refinement	
R[F2 > 2σ(F2)], wR(F2), S	0.065, 0.189, 0.83
No. of reflections	6450
No. of parameters	548
No. of restraints	0
H-atom treatment	H atoms treated by a mixture of independent and constrained refinement
Δρmax, Δρmin (e Å−3)	0.24, −0.27

Table 2: Selected geometric parameters, bond length and bond angles (Å, °).

Atom	**Bond length (Å)**	**Atom**	**Bond angles (°)**
O1A—C2A	1.358 (5)	C2A—O1A—C8A	118.0 (4)
O1A—C8A	1.411 (7)	C3A—O2A—C9A	113.2 (3)
O2A—C3A	1.383 (5)	C4A—O3A—C10A	116.8 (3)
O2A—C9A	1.430 (6)	N1A—O5A—C7A	113.7 (3)
O3A—C4A	1.359 (5)	C2B—O1B—C8B	117.6 (4)
O3A—C10A	1.413 (5)	C3B—O2B—C9B	113.3 (4)
O4A—C7A	1.193 (5)	C4B—O3B—C10B	118.1 (4)
O5A—N1A	1.434 (4)	N1B—O5B—C7B	114.5 (3)
O5A—C7A	1.358 (5)	O5A—N1A—C11A	109.2 (3)
O1B—C2B	1.371 (6)	C19A—N2A—C20A	127.5 (4)
O1B—C8B	1.433 (7)	C19A—N2A—C22A	126.6 (4)
O2B—C3B	1.383 (5)	C20A—N2A—C22A	105.7 (5)
O2B—C9B	1.429 (7)	C21A—N3A—C22A	109.9 (5)
N1B—C11B	1.274 (5)	C20B—N2B—C22B	106.6 (4)
N2B—C19B	1.455 (6)	C21B—N3B—C22B	110.1 (4)
N2B—C20B	1.344 (6)	O1A—C2A—C3A	115.7 (4)
N2B—C22B	1.378 (6)	O1A—C2A—C1A	124.5 (4)
N3B—C22B	1.391 (7)	O2A—C3A—C4A	120.1 (4)
N3B—C21B	1.361 (7)	O2A—C3A—C2A	118.6 (4)

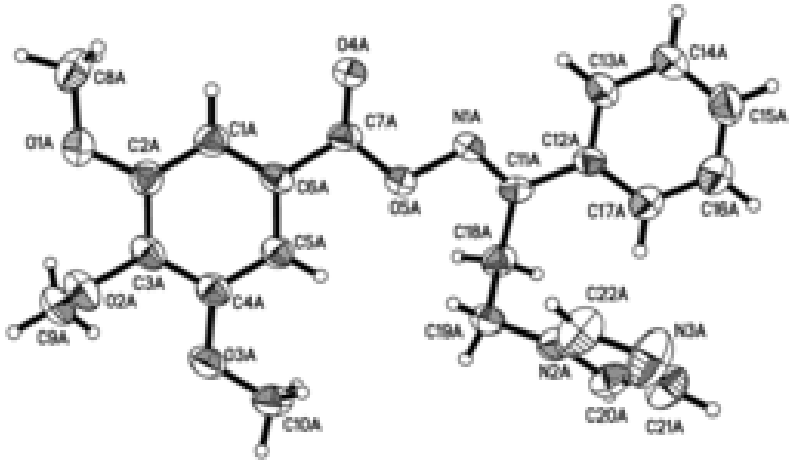


Figure 1: ORTEP diagram of the titled compound **4** drawn at 50 % ellipsoids for non-hydrogen atoms.

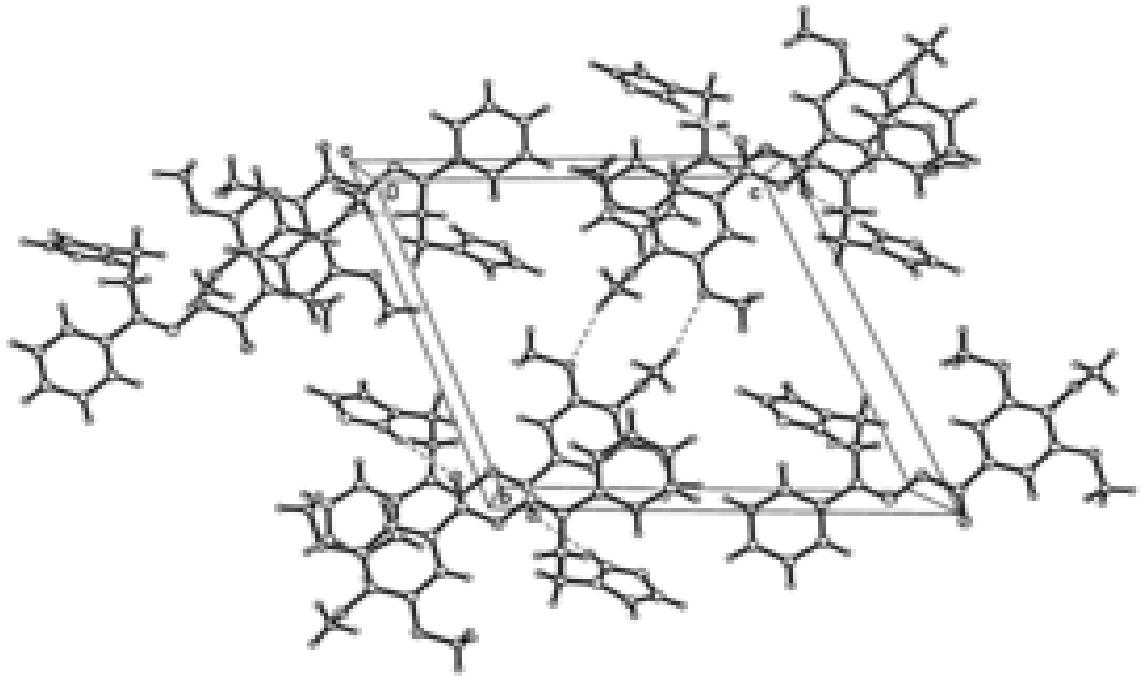


Figure 2: Crystal packing showing intermolecular C—H•••O hydrogen bonds as dashed lines along the *c* axis.

DISCUSSION

The chemical structures of the synthesized compounds were confirmed *via* spectral analysis including ^{1}H NMR, ^{13}C NMR, and mass spectral data. The target oximino ester **4** has exhibited potential *in vitro* anti-*Candida* activity and has been evaluated using clinical isolates of *C. albicans* and *C. tropicalis*. It has a MIC value of 0.3053 µmol/mL against both *C. albicans* and *C. tropicalis* and is approximately 5-fold more potent than the gold standard antifungal agent fluconazole (MIC >1.6325 µmol/mL) [8].

The configuration of the anti-*Candida* oximino ester **4** was confirmed *via* x-ray crystallography approach as an analytical tool to unequivocally assign its structure. In this manner, the imine group in the target compound **4** was assigned an (*E*)-configuration.

The crystal structure of **4** contains two molecules in the asymmetric unit. The phenyl ring (C1-C6) forms dihedral angles of 8.76 (3)° and 12.13 (2)° with the benzene ring (C12-C17), in the two molecules. The phenyl ring (C1-C6) also forms dihedral angles of 70.75(1)° and 65.80(2)° with the imidazole ring (N2-C20-C21-N3-C22), in the two molecules. Furthermore, the phenyl ring (C12-C17) forms dihedral angles of 64.63 (1)° and 64.76(2)° with the imidazole ring (N2-C20-C21-N3-C22) in the two molecules. The crystal

structure is stabilized by four C-H…O non-classical hydrogen bonds along the c axis, where the length between C9A—H9AA is 0.96 Å, C9B—H9BA is 0.96 Å, C18B—H18C is 0.97 Å, and C20B—H20B is 0.93 Å. The angles between C9A—H9AA•••O3A(i), C9B—H9BA•••O3B(ii), C18B—H18C•••O2A(i), and C20B—H20B••• O4B(iii) are 169.00°, 162.00°, 165.00°, and 163.00°, respectively, with the following respective symmetry codes: (i) −x, −y+2, −z; (ii) −x−1, −y+3, −z−1; (iii) −x−1, −y+2, −z.

CONCLUSION

An x-ray single crystal molecular structure of the anti-*Candida* agent, namely (({[(1*E*)-3-(1*H*-imidazol-1-yl)-1-phenylpropylidene]amino}oxy) (3,4,5-trimethoxyphenyl)methanone (**4**), has been obtained in this study. The assigned (*E*)-configuration of the imine moiety in the target oximino ester **4** was confirmed *via* single-crystal x-ray crystallography as a decisive analytical tool. Compound **4** may be a promising new anti-*Candida* lead agent bearing an imidazole pharmacophore.

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