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Original Research Article

Single-Crystal X-ray Structure of the Anti-*Candida* Agent, (*E*)-3-(1*H*-Imidazol-1-yl)-1-phenylpropan-1-one *O*-3-Chlorobenzoyl Oxime

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Abstract

Purpose: *To determine the conformation as well as imine double bond configuration of the anti-Candida oximino ester, 3-(1H-imidazol-1-yl)-1-phenyl- propan-1-one O-3-chlorobenzoyl oxime.*
Methods: *The titled compound was synthesized in a four-step reaction sequence using acetophenone as a starting material. Spectral analysis, viz, nuclear magnetic resonance (1H NMR and 13C NMR spectroscopy) and mass spectrometry (MS) confirmed the chemical structure of the synthesized compounds. Subsequently, single crystals of the titled compound were subjected to x-ray crystallographic analysis.*
Results: *The single crystal x-ray crystallography of the investigated anti-Candida agent revealed its conformation and the (E)-configuration of its imine double bond. The titled compound crystallizes in the monoclinic space group $P2_{1/c}$ with a = 11.1894 (2)Å, b = 19.5577 (4)Å, c = 8.2201 (2)Å, β = 104.919 (2)º, V = 1738.24 (6)Å^3, Z = 4. The molecules are packed in crystal structure by weak non-classical intermolecular hydrogen C2—H2A•••O2 interactions.*
Conclusion: *X-ray crystallography analysis confirms the (E)-configuration of the titled compound.*

Keywords: *X-ray crystallography, Synthesis, Anti-Candida, Configuration, Conformation, Single crystal*

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INTRODUCTION

The increased incidence of fungal infections in the last half-century has raised this class of infections one of the most current common distress. Invasive nosocomial and systemic fungal infections are among the main causes of morbidity and mortality, especially in patients with compromised immune systems such as cancer or AIDS, steroid treatments and in organ transplant cases [1-3]. *Candida* species appear to be the main causative agent for nosocomial fungal infections with *Candida albicans* being the cause of the majority of invasive candidiases with about 30 – 40 % of mortality [4].

Azole antifungal agents bearing either imidazole or 1, 2, 4-triazole moieties constitute the major group of the currently available anti-*Candida* agents [5,6]. The titled compound **4** is an azole-containing anti-*Candida* agent having the imidazole pharmacophore moiety.

There is no previous research pertaining to explore the configuration of the anti-*Candida* oxime ester **4**. Therefore, x-ray crystallography, as a doubtless analytical tool, was used to determine the configuration of the imine double bond of the titled compound **4** as well as its conformation.

EXPERIMENTAL

General

Melting points were determined on a Gallenkamp melting point apparatus and were uncorrected. NMR spectra were obtained from 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. TMS was used as internal standard and chemical shift values were recorded in ppm on δ scale. The ^{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. The X-ray diffraction measurements of compound **4** were performed using Bruker SMART APEXII CCD diffractometer. Crystallographic data of compound **4** has been deposited with the Cambridge Crystallographic Data Center (supplementary publication number CCDC-1050446). Copies of the data may be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (deposit@ccdc.cam.ac.uk).

Preparation of 3-(1*H*-imidazol-1-yl)-1-phenylpropan-1-one (2)

A mixture containing acetophenone (2.4 g, 20 mmol), paraformaldehyde (0.81 g, 9 mmol), dimethylamine hydrochloride (2.2 g, 27 mmol) and catalytic amount of concentrated hydrochloric acid (0.1 mL) was heated to reflux for two hours in absolute ethanol (5 mL). The reaction mixture was cooled to ambient temperature and acetone (20 mL) was added to precipitate the Mannich base hydrochloride **1**. Imidazole (2.4 g, 34.8 mmol) was added to a solution of compound **1** (3.7 g, 17.4 mmol) in water (10 mL) and the reaction mixture was heated at reflux temperature for five hours. The reaction mixture was cooled and the precipitated solid was filtered off to furnish compound **2** (2.7 g, 77%) mp 368-370 K [7] which was used in the next step without any further purification.

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

The ketone **2** (2.00 g, 10 mmol), hydroxylamine hydrochloride (1.39 g, 20 mmol), and KOH (1.12 g, 20 mmol) in ethanol (10 mL) were refluxed under stirring for 18 h. The reaction mixture was cooled to room temperature and the insoluble materials were filtered off. The filtrate was concentrated under vacuum and the residue was poured onto ice-cold water (15 mL). The precipitated solid was filtered, dried, and recrystallized from ethanol to give 1.51 g (70 %) of the oxime **3** as colourless crystals mp 428 - 430 K [8].

Preparation of (*E*)-3-(1*H*-imidazol-1-yl)-1-phenylpropan-1-one *O*-3-chlorobenzoyl oxime (4)

A solution of the 3-chlorobenzoic acid (1.1 g, 7 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI.HCl, 1.40 g, 7.3 mmol) and 4-dimethylaminopyridine (DMAP, 400 mg) in dichloromethane (75 mL) was stirred at ambient temperature. The oxime **3** (1.49 g, 6.9 mmol) was added and the reaction mixture was further stirred for 18 h at ambient temperature. The reaction mixture was washed successively with water (2 x 20 mL), 10 % $NaHCO_3$ solution (2 x 15 mL), and water (2 x 15 mL). The organic layer was separated, dried (Na_2SO_4), evaporated under reduced pressure and the residue was re-crystallized (isopropanol) to give 1.51 g (61 %) of **4** as colourless crystals melting point of 383-385 K [8].

Evaluation of anti-*Candida* activity

The MIC values of fluconazole and/or compound **4** were determined with a microdilution test (M27-A2 Protocol), according to the reference method of the CLSI [9]. Aliquots of the previously prepared *Candida* inocula (100 µL) were added to each well of the 96-well microdilution plates; each well contained 100 µL of twofold serial dilutions of fluconazole or compound **4** (1 µg/mL to 500 µg/mL) in RPMI 1640 medium. The plates were incubated at 35 °C for 48 h and the observed turbidity of each well was measured at 490 nm with a microplate ELISA reader. The MICs for fluconazole and/or compound **4** were determined with 80 % growth inhibition at the end point relative to the turbidity of the growth control.

RESULTS

Synthesised compounds

The target compound **4** was synthesized as depicted in Scheme 1. The ketone **2** was obtained from acetophenone *via* Mannich reaction and subsequent substitution of the produced Mannich base hydrochloride **1** with imidazole to give the pivotal ketone **2**. Compound **2** was allowed to react with hydroxylamine hydrochloride in the presence of potassium hydroxide to give the oxime **3**. Esterification of the hydroxyl functionality of compound **3** with 3-chlorobenzoic acid was successfully achieved using EDCI. HCl and DMAP to yield the target compound **4**.

3-(1*H*-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).

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

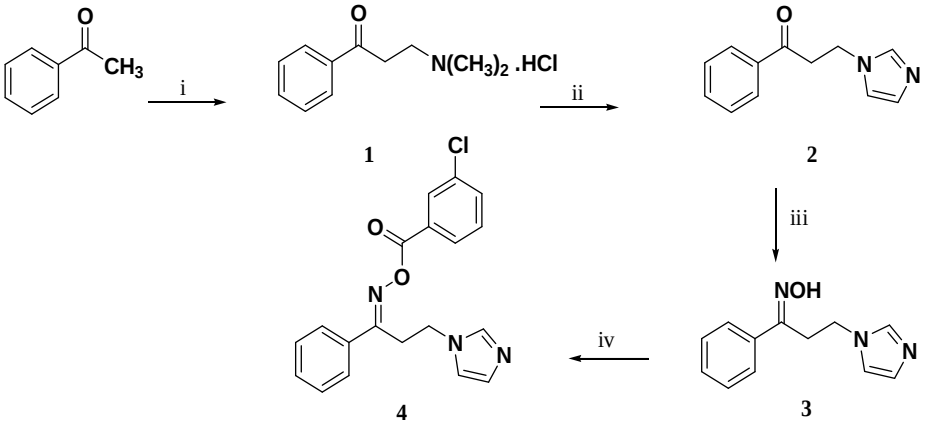
^{1}H-NMR ($CDCl_3$): δ 3.31 (t, *J* = 7.1 Hz, 2H, -C*H*$_2$-CH_2-N), 4.28 (t, *J* = 7.1 Hz, 2H, -CH_2-C*H*$_2$-N), 6.96 (s, 1H, -N-C*H*=CH-N=), 7.07 (s, 1H, -N-CH=C*H*-N=), 7.29–7.49 (m, 5H, Ar-H), 7.58 (s, 1H, -N-C*H*=N-); ^{13}C-NMR ($CDCl_3$): δ 28.3 (-*C*H2-CH2-N), 41.8 (-CH_2-*C*H_2-N), 119.1 (-N-*C*H=CH-N=), 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 $[M + 1]^+$.

(*E*)-3-(1*H*-Imidazol-1-yl)-1-phenylpropan-1-one *O*-3-chlorobenzoyl oxime (4)

^{1}H-NMR (DMSO-d_6): δ (ppm) = 3.52 (br. s, 2H, -C*H*$_2$-CH_2-N), 4.30 (br. s, 2H, -CH_2-C*H*$_2$-N), 6.80 (s, 1H, -N-C*H*=CH-N=), 7.17 (s, 1H, -N-CH=C*H*-N=), 7.51–7.82 (m, 8H, -N-C*H*=N-, Ar-H), 8.00 (d, *J* = 1.5 Hz, 2H, Ar-H,); ^{13}C-NMR (DMSO-d_6): δ 30.1 (-*C*H_2-CH_2-N), 43.0 (-CH_2-*C*H_2-N), 119.3 (-N-*C*H=CH-N=), 127.3, 128.1, 128.5, 128.9, 130.3, 130.9, 131.1, 132.9, 133.6, 133.7 (-N-CH=*C*H-N=, Ar-CH, Ar-C), 137.1 (-N-*C*H=N-), 161.7 (C=N), 164.8 (C=O); MS *m/z* (ESI): 354.1 $[M]^+$ [8].

Crystal structure of the titled compound 4

A single crystal of dimensions, 0.58 mm X 0.39 mm X 0.21 mm, was selected for x-ray diffraction analysis. Data were collected on a Bruker APEX-II CCD area diffractometer equipped with graphite monochromatic CuK\a radiation (λ = 1.54178 Å) at 296 (2) K. Cell refinement and data reduction were done by Bruker SAINT [10]. SHELXS-97 [11] was used to solve and refine the titled structure. The final refinement was conducted by full- matrix least-squares techniques with anisotropic thermal data for non-hydrogen atoms on F2 [12]. All the hydrogen atoms were placed in calculated positions and constrained to ride on their parent atoms [12]. Multi-scan absorption correction was applied by the use of SADABS software [10]. The crystallographic data and refinement information are listed in Table 1. The selected bond lengths and bond angles are summarized in Table 2.



Scheme 1: Synthesis of the target 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-Chlorobenzoic acid, EDCI.HCl, DMAP, DCM, rt, 18 h.

The labelled displacement ellipsoid plot of this molecule is shown in Figure 1 in which 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	$C_{19}H_{16}ClN_3O_2$
M_r	353.80
Crystal system, space group	Monoclinic, $P2_1/c$
Temperature (K)	296(2)
a, *b*, *c* (Å)	11.1894 (2), 19.5577 (4), 8.2201 (2)
β (°)	104.919 (2)
V (Å^3)	1738.24 (6)
Z	4
Radiation type	Cu *K*α
μ (mm^{-1})	2.09
Crystal size (mm)	0.58 × 0.39 × 0.21
Data collection	
Diffractometer	Bruker *APEX*-II CCD diffractometer
Absorption correction	Multi-scan *SADABS* Bruker 2014
T_{min}, T_{max}	0.379, 0.664
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	3245, 3245, 2664
R_{int}	0.0000
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, *S*	0.047, 0.142, 1.05
No. of reflections	3245
No. of parameters	291
No. of restraints	0
H-atom treatment	H atoms treated by a mixture of independent and constrained refinement
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.43, −0.43

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

Atoms	bond lengths (Å)	Atoms	bond angles (°)
Cl1—C16	1.736 (2)	N1—O1—C13	113.28 (14)
O1—N1	1.433 (2)	O1—N1—C7	110.18 (15)
O1—C13	1.354 (2)	C9—N2—C10	126.8 (4)
O2—C13	1.190 (3)	C9—N2—C12	129.0 (6)
N1—C7	1.262 (2)	N1—C7—C6	115.83 (17)
N2—C9	1.462 (5)	N1—C7—C8	123.6 (2)
N2—C10	1.365 (8)	N2—C9—C8	111.3 (3)
N2—C12	1.326 (12)	N2—C10—C11	105.1 (7)
N2A—C12A	1.36 (3)	N3—C11—C10	110.7 (11)
N2A—C9A	1.451 (15)	N2—C12—N3	115.6 (10)
N2A—C10A	1.31 (3)	O1—C13—O2	124.75 (19)
N3—C12	1.268 (15)	O1—C13—C14	110.28 (15)
N3—C11	1.325 (13)	O2—C13—C14	124.94 (19)

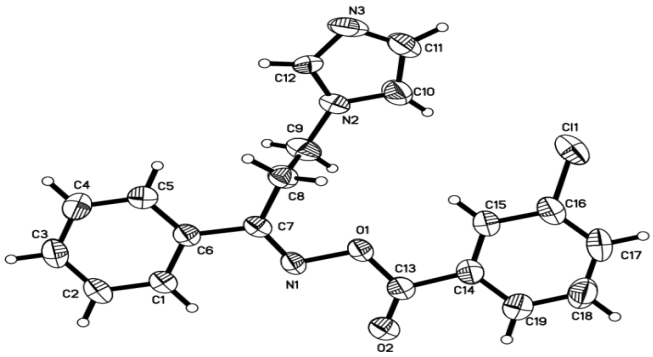


Figure 1: ORTEP diagram of the titled compound **4** drawn at 50% ellipsoids for non-hydrogen atoms.

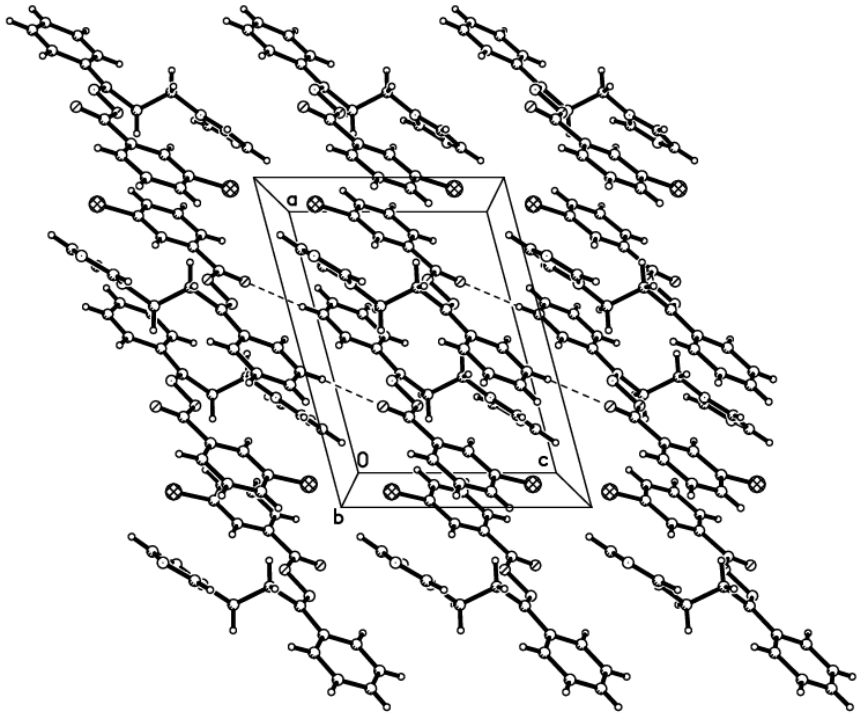


Figure 2: Crystal packing showing intermolecular C—H•••O hydrogen bonds as dashed lines along the *c* axis. Minor disordered component have been omitted for clarity.

DISCUSSION

The target compound **4** displayed potential *in vitro* anti-*Candida* activity and it has been examined using clinical isolates of *C. albicans* and *C. tropicalis*. These clinical isolates were practically considered resistant to the gold standard antifungal drug, fluconazole (MIC > 1.6325 µmol/mL). The test compound **4** exhibited MIC values of 0.0221 and 0.7069 µmol/mL being about 74 and two folds more potent than fluconazole against *C. albicans* and *C. tropicalis*, respectively [8].

X-ray crystallography is a crucial analytical tool which can confirm the configuration of the titled oxime ester **4**. Accordingly, the assigned (*E*)-configuration of the titled compound **4** was established *via* its single crystal x-ray structure. The crystal structure of the titled compound contains one molecule in the asymmetric unit. The imidazole ring and ethyl side chain are statistically disordered over two conformations with a site-occupancy ratio of 0.785 (6): 0.215 (5). Similarity, (SAME) restraint was used for the major and minor components of the disordered imidazole ring and ethyl side chain (C8-C9-N2-C10-C11-N3-C12). The phenyl ring (C1-C6) forms dihedral angles of 1.54 (3)° with the major component of 3-chloro-benzene ring (C14-C19). The phenyl ring (C1-C6) also forms dihedral angles of 27.00° and 15.86(2)° with the major and minor components of the imidazole ring (N2-C10-C11-N3-C13) respectively. The crystal structure is stabilized by C-H…O non-classical hydrogen bonds along the c axis, where the length between C2—H2A is 0.93 Å and between H2A•••O2 is 2.51 Å and the angle between C2—H2A•••O2 is 138.00° with symmetry code: −x+1, −y+1, −z+2.

CONCLUSION

Single-crystal x-ray structure of the anti-*Candida* agent, namely (*E*)-3-(1*H*-imidazol-1-yl)-1-phenyl-propan-1-one *O*-3-chlorobenzoyl oxime (**4**) is reported. The assigned (*E*)-configuration of the title compound **4** is confirmed *via* its doubtlessly single-crystal x-ray structure. The oxime ester **4** could be considered as an imidazole-bearing new anti-*Candida* lead prodrug.

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