Structural study and antioxidant activity determination of (2E)-*N*-[2-(morpholin-4-yl)ethyl]-cinnamide

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Abstract

Herein we report the structural study and antioxidant activity of (2*E*)-*N*-[2-(morpholin-4-yl) ethyl)-cinnamanilide (**1**) and its salt (**1-HCl**). Compound **1** crystallizes as orthorhombic P_{bca} system while its salt is monoclinic $P2_1/n$. The supramolecular structures are held in shape by classical (D—H···A) and non classical (C—H···A) interactions. The antioxidant capability of both compounds shows a structural relationship, compound **1** has moderate capability, meanwhile compound **1-HCl** increases its properties owing to the presence of $N\text{-}H^+$ in the structure.

Keywords: Solid state study, NMR, X-ray analysis, antioxidant activity, (2*E*)-*N*-[2-(morpholin-4-yl)ethyl]-cinnamide

Introduction

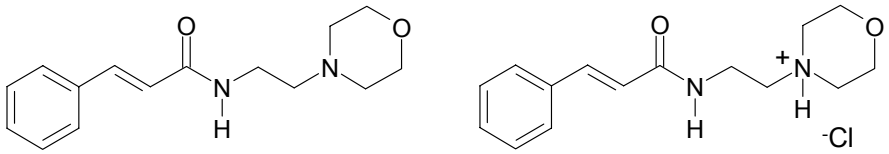
Preservation of industrialized food containing polyunsaturated fatty acids, such as eicosapentaenoic (20:5ω-3) and docosahexaenoic (22:6ω-3) acids, has been subject of growing interest because of importance in human nutrition. ω-3-Polyunsaturated fatty acids are believed

to have several health benefits, i.e. in cardiovascular disease, immune disorders, inflammation, allergies, and diabetes[1]. In foods, fatty acids oxidation is one of the most important reactions leading to a deterioration of sensorial features, shelflife and general quality. The products of oxidation cause unpleasant flavors and structural molecular changes that in turn lead to rejection of products by the consumer[2]. Several compounds have been used to retard the oxidative reaction: the butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylhydroquinone (TBHQ) have been used commonly as food antioxidants. However, a growing interest exists for the use of new antioxidant compounds focused on expanding the shelf half life of foods. These food additives could eventually be used by the food industry to prevent lipid peroxidation. However, it has been reported that some of these molecules possess toxic and carcinogenic effects on health[3]. Efforts have been made to search for novel antioxidant additives leading to slow down fatty acids and lipids oxidation. Oxidative rancidity is initiated by oxygen free radical species, by the action of molecular oxygen on pre-formed free radical species from polyunsaturated fatty acids or by presence of trace amounts of metallic ions[2,4]

The antioxidant activity is known to inhibit lipid peroxidation, to scavenge free radicals and active oxygen, to chelate iron and to inactivate lipoxygenase[5]. Interestingly, the same antioxidant compound may work by a number of different pathways. All of these mechanisms play a role in the antioxidant compound behaving as a stabilizing agents in foods lipids[6].

Recently, it has been reported that cinnamic acids and other compounds behave as good free radical scavengers. Furthermore, cinnamic acids derivatives have demonstrated antibacterial and antifungic activities as well as inhibitory activity against low density lipoprotein (LDL) and acetyl-CoA oxidation.[7-9] It has been demostrated that caffeic acid (a cinnamic acid analogue) possesses antioxidant properties and may prevent and/or revert acute or chronic hepatic damage caused by CCl_4 administration[10]. Anti oxidant compounds can diminish the oxidative rancidity in foods, as well as prevent several related diseases, such as cancer, diabetes, Alzheimer's and Parkinson's diseases[11].

In this contribution, structural characterization and antioxidant activity of (2*E*)-*N*-[2-(morpholin-4-yl)ethyl]-cinnamide and its **HCl** salt are reported (Scheme 1).



Scheme 1

Results and Discussion

Structure analysis

The ^{1}H and ^{13}C NMR spectra of compounds **1** and **1-HCl** are in agreement with the proposed structures (Table 1).

Table 1. ^{1}H and ^{13}C NMR chemical shifts ($CDCl_3$) for compounds **1** and **1-HCl**

			^{1}H NMR					
Comp.	$O(CH_2)_2$	$N(CH_2)_2$	$NHCH_2$	NCH_2	COCH (^{3}J/Hz)	PhCH (^{3}J/Hz)	NH	NH^+
1[a]	3.25 (m)	2.49 (m)	3.51 (dt)	2.57 (t)	6.25 d (15.6)	7.65 d (15.6)	6.28	---
1-HCl[b]	3.95-4.35 (m)	3.28-3.58 (m)	3.89 (dt)	2.95 (t)	6.63 d (15.6)	7.63 d (15.6)	8.40	12.4
			^{13}C NMR					
	$O(CH_2)_2$	$N(CH_2)_2$	$NHCH_2$	NCH_2	COCH	PhCH	CO	---
1[c]	66.8	53.2	35.6	56.9	120.5	140.9	165.8	---
1-HCl[d]	63.4	52.8	58.2	33.6	120.2	141.9	166.8	---

δ for Ph protons: (a) *ortho* and *meta* 7.34-7.36, *para* 7.36; (b) 7.36-7.40 pm. δ for Ph carbons: (c) *ipso* 134.8, *ortho* 128.7, *meta* 127.7, *para* 129.6 ppm; (d) *ipso* 134.5, *ortho* 128.7, *meta* 128.0, *para* 129.9 ppm.

The characteristic ^{1}H NMR signals for compound **1** are the amide NH at 6.28 ppm and vinyl signals, the latter appear as an AX system at 6.45 and 7.65 ppm with $^{3}J_{H\text{-}H}$ = 15.6 Hz, characteristic of a *trans* coupling. In ^{13}C NMR spectrum, amide carbonyl appears at 165.8 ppm and vinyl signals at 120.6 and 140.9 ppm, whereas the methylene $HNCH_2$ and NCH_2 of the pendant $NHCH_2CH_2N$ fragment are at 35.6 and 56.9 ppm, respectively, and morpholine ring carbon atoms are at 66.8 and 53.2 ppm. Salt formation goes on with few changes in both ^{1}H and ^{13}C NMR spectra; in the former, a new signal at 12.4 ppm appears corresponding to the NH^+ proton. In ^{13}C NMR the most significant change occurs at the $O(CH_2)_2$ and $NHCH_2$ carbon atoms from morpholine and $NHCH_2CH_2N$ fragments, respectively, which are shifted 3.4 and 1.9 ppm, respectively, to lower frequencies.

Compounds **1** and **1-HCl** crystallized from chloroform solutions, the former as an orthorhombic system space group P_{bca}, whereas the last crystallized as a monoclinic system space group $P2_{1/n}$, a summary of bond distances and angles are listed in Table 3, and molecular structures are shown in Figures 1(a) and 2(a), respectively.

Table 2. Bond distances and angles of molecules **1** and **1-HCl**

Distances/Å			Angles/°		
Atoms	**1**	**1-HCl**	Atoms	**1**	**1-HCl**
O(1)—C(1)	1.243(4)	1.230(6)	C(1)N(10)C(11)	122.6(3)	122.6(4)
N(10)—C(1)	1.344(4)	1.350(5)	C(12)N(13)C(14)	111.2(2)	110.8(3)
N(10)—C(11)	1.452(4)	1.451(6)	C(12)N(13)C(18)	111.0(2)	114.4(3)
N(13)—C(12)	1.460(4)	1.499(5)	O(1)C(1)N(10)	122.5(3)	121.1(4)
N(13)—C(14)	1.460(4)	1.508(5)	O(1)C(1)C(2)	122.5(3)	123.7(4)
N(13)—C(18)	1.459(5)	1.495(6)	O(1)C(1)N(10)C(11)	-1.1(5)	8.7(6)
C(1)—C(2)	1.481(4)	1.488(7)	C(2)C(1)N(10)C(11)	177.4(3)	-169.4(4)
C(2)—C(3)	1.327(4)	1.306(6)	O(1)C(1)C(2)C(3)	-6.7(5)	-8.8(7)
C(3)—C(4)	1.462(4)	1.457(6)			
C(11)—C(12)	1.521(5)	1.530(6)			

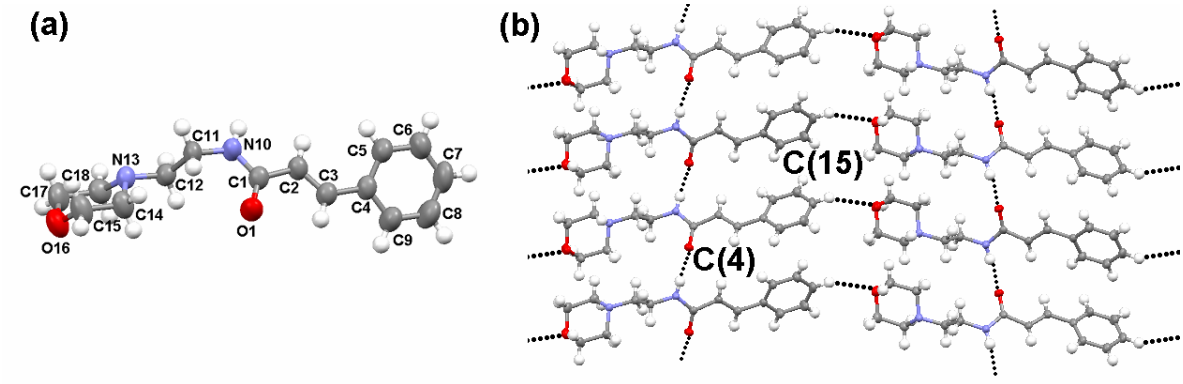


Figure 1. Compound **1**: (a) molecular structure, ORTEP diagram at 30% probability level; (b) supramolecular structure viewed along the *c* axis direction.

As most of NH amides, the NH and CO groups are positioned antiperiplanar with an O1C1N10C11 torsion angle of -1.1(5)° and 8.7(6)°, for **1** and **1-HCl**, respectively. Protonation does not significantly affect either amide bond distances and angles or morpholine ring conformation. Its main effect is related to the supramolecular arrangement. Molecule **1** forms zig-zagging C(4) and C(15) chains[12] through N(10)—H(10)···O(1)^{i} and C(7)—H(7)^{ii}···O(16) hydrogen bonding interactions with neighboring molecules, to develop a zig-zagging plane [Figure 1(b)]. The N(10)···O(1) distance of 2.908(3) and N(10)—H(10)···O(1) angle of 174°, are in the range for strong hydrogen bonding interactions[13] . The hydrogen bonding geometry for **1** and **1-HCl** is summarized in Table 3. In molecule **1-HCl**, the chlorine atom is the strongest hydrogen bonding acceptor, thus it forms centrosymmetric $\mathbf{R}_4^2(14)$ rings that pile along the *a* axis, through the interaction with both the amide N(10)H and morpholinium N(13)H protons (Figure 2). The latter occupies an appropriate axial position in the morpholine ring. In addition,

each chlorine atom adopts a tetrahedral geometry by short contacts with two C(sp^3)—H donors[14] of a neighboring molecule: C(18)—H(18B)···Cl and C(11)—H(11A)···Cl, to develop a zig-zagging chain along the *a* axis. Finally, the third dimension is given by the interaction of amide carbonyl with two C(sp^3)—H donors: C(14)—H(14A)···O(1) and C(18)—H(18A)···O(1), that join the centrosymmetric pairs forming $\mathbf{R}_4^2\mathbf{(16)}$ rings.

Table 3. Hydrogen bonding and contact geometry of compounds **1** and **1-HCl**

Compound	D—H···A	D—H	H···A	D···A	D—H···A
1	N(10)—H(10)···O(1)^{i}	0.86	2.05	2.908	174
	C(7)—H(7)^{ii}···O(16)	0.97	2.622	3.468	151
1-HCl	N(10)—H10···Cl1	0.86	2.40	3.211(4)	156
	N13—H12···Cl1^{iii}	0.91	2.14	3.049(3)	177
	C11—H11A···Cl1^{iv}	0.91	2.93	3.698(4)	135
	C18—H18B···Cl1^{iv}	0.97	2.81	3.773(4)	172
	C14—H14A···O1^{v}	0.97	2.50	3.367(5)	149
	C18—H18A···O1^{v}	0.93	2.680	3.494(5)	142

Symmetry codes: (i) 3/2 – x, ½ + y, z] (ii) x, ½ -y, -1/2 +z; (iii) 1 -x, -y, 2 -z; (iv) 1 +x, y, z; (v) 1 –x, -y, 1 –z.

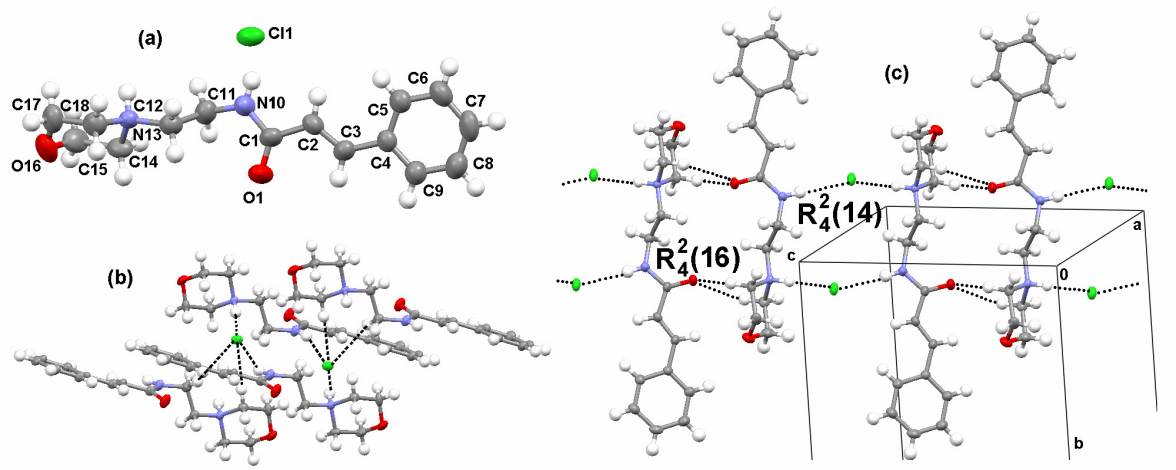


Figure 2. Compound **1-HCl**: (a) molecular structure, ORTEP diagram at 30% probability level; (b) tetrahedral geometry around Cl atom; (c) centrosymmetric dimmers propagating along the *a* axis.

Anti-oxidant analysis

The measured antiradical DPPH (1,1-diphenyl-2-picryl-hydrazyl) activity of compound **1-HCl** was 203 meqTrolox/g, it means 51% of BHT antiradical activity, whereas compound **1** was inactive. The reported antiradical activity for BHT, the commercially most used antioxidant, is

395 meqTrolox/g measured, at similar concentration[15]. The test for protection against β-carotene-linoleic acid emulsion bleaching gives a measure of the protective capacity of compounds against oxidation. It was found that compound **1** does not provide any protection whereas compound **1-HCl** reaches the same protective capacity of BHT (1 mg/mL) at 11.5 mg/mL. The bleaching kinetics of β-carotene-linoleic acid emulsion are shown in Figure 3. Compound **1-HCl** was as effective as BHT to avoid the β-carotene deterioration, thus the absorbance versus time graph remained constant, in contrast, compound **1** showed no activity. Finally, the antioxidant activity of compounds **1** and **1-HCl** is not related to their chelating capability, since the test for **1** was 29% effective (35.8% for BHT[16]) and for **1-HCl**, it was 0%. Chelating capabilities are not involved in the mode of action of **1-HCl**.

These results are in agreement with previously reported studies in which the capacity for donating electrons seems to play a more significant role in stabilizing the antioxidant efficiency of hydroxycinnamic acids than the ability for chelating metals[17]. The above results can be explained in terms of the structure: the morpholine NH^+ proton in **1-HCl** can be easily transferred to interrupt the chain reactions of free radicals, which are responsible for lipid oxidation.

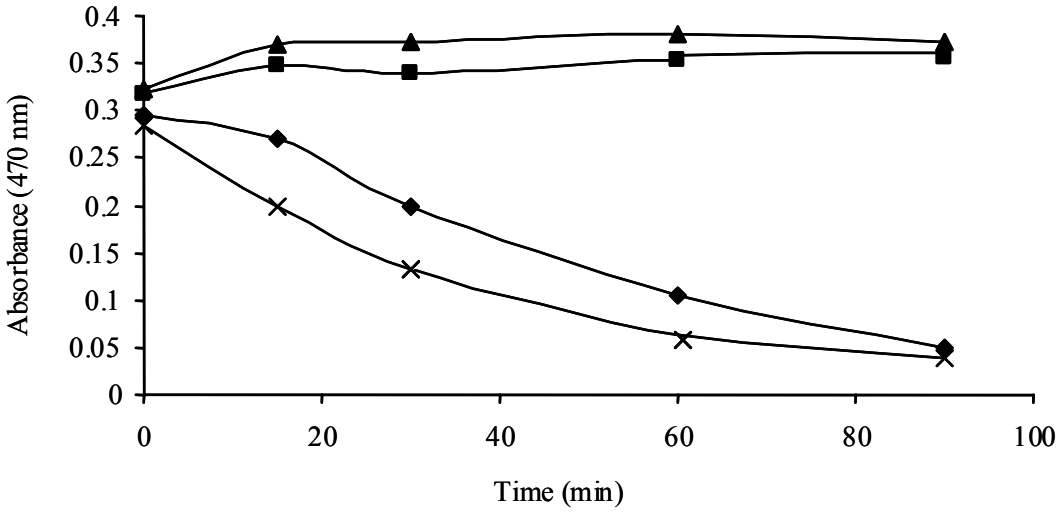


Figure 3. Reduction in absorbance during bleaching β-carotene-linoleic acid emulsion. —▲— Compound **1-HCl**, —×— Compound **1**, —■— BHT, —◆— Control.

Conclusions

The NMR analysis of (2*E*)-*N*-[2-(morpholin-4-yl)ethyl]-cinnamanilide (**1**) and its chlorhydrate salt (**1-HC**l) showed no significant differences between the two compounds. However, x-ray diffraction demonstrated that the main effect of protonation is related to the supramolecular structure in the solid state, as well as in their antioxidant capabilities. (2*E*)-*N*-[2-(morpholin-4-

yl)ethyl]-cinnamide chlorhydrate (**1-HCl**) showed moderate antioxidant properties in comparison with BHT, whereas the free base was not active. The above results can be explained in terms of structure the morpholine NH^+ proton in **1-HCl** can be easily transferred to interrupt the chain reactions of free radicals.

Experimental Section

General Procedures. All chemicals and solvents were of reagent grade and used as received. Melting points were measured on a Electrothermal IA 9100 apparatus and were uncorrected. IR spectra were recorded for KBr disks using a Perkin-Elmer 16F PC IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury 300 (^{1}H, 300.08; ^{13}C, 75.46 MHz) instrument in $CDCl_3$ solution, measured with $SiMe_4$ as internal reference, following standard techniques. Elemental analyses were determined on a Perkin-Elmer Series II CHNS/O analyzer 2400 instrument.

Single-crystal X-ray diffraction data for molecules **1** and **1-HCl** were collected on a Bruker Smart 6000 diffractometer at 298 K with Mo Kα radiation, λ = 0.71073 Å). A semiempirical absorption correction was applied using SADABS[18], and the program SAINT[19] was used for integration of the diffraction profiles. The structures were solved by direct methods using SHELXS[19] program of WinGX package[20]. The final refinement was performed by full-matrix least-squares methods on F^2 with SHELXL[19] program. Collected data and refinement parameters are listed in Table 4. Complete crystallographic data, as a CIF has been deposited with at Cambridge Crystallographic Data Centre (CCDC No. 649381 and 649382 compounds **1** and **1-HCl**, respectively.

(2E)-*N*-[2-(Morpholin-4-yl)ethyl]-cinnamide (1). Compound **1** was prepared in 78% yield as reported in the literature[21] and recrystallized from chloroform to give 0.78 g. mp. = 122-123 °C. *Anal*. Calcd. $C_{15}H_{20}N_2O_2 \cdot 1.4\ H_2O$: C, 63.09; H, 8.03; N, 9.80. Found: C, 63.52; H, 7.93; N, 9.61; IR ν_{KBr} (cm^{-1}): IR ν_{KBr} (cm^{-1}): 3403 (NH); 1610 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.25 (2H, m), 2.49 (2H, m), 3.51 (2H, dt), 2.57 (2H, t,) 6.25 (1H, d, *J* = 15.6Hz), 7.65 (1H, d, *J* = 15.6 Hz) 6.28 (1H, br), 7.34-7.36 (5H, m, Ph); ^{13}C NMR (75.4 MHz , $CDCl_3$) δ 66.8, 53.2, 35.6, 56.9, 120.5, 140.9, 165.8, Ph; 134.8, 128.7, 127.7, 129.6.

(2E)-*N*-[2-(morpholin-4-yl)ethyl]-cinnamide chlorhydrate (**1-HCl**). A hydrochloric acid solution (3.8 mmol, 0.5 ml, HCl 33%) was added slowly to a suspension of compound **1** (1.0 g, 3.8 mmol) in toluene with stirring. The solvent was evaporated and the residue was crystallized from chloroform to give 0.52 g (46%) mp. = 199-200 °C. *Anal*. Calcd. For $C_{15}H_{21}N_2O_2Cl \cdot 0.7\ H_2O$: C, 58.22; H, 7.24; N, 9.00. Found: C, 58.41; H, 7.19; N, 8.46; IR ν_{KBr} (cm^{-1}): 3224 (NH); 1663 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.95-4.35 (2H, m), 3.28-3.58 (2H, m), 3.89 (2H, dt), 2.95 (2H, t), 6.63 (1H, d, *J* = 15.6 Hz), 7.63 (1H, d, *J* = 15.6 Hz), 8.40 (1H, br), 12.4 (1H, br), 7.36-

7.40 (5H, m, Ph); ^{13}C NMR (75.4 MHz , $CDCl_3$) δ 63.4, 52.8, 58.2, 33.6, 120.2, 141.9, 166.8, Ph: 134.5, 128.7, 128.0, 129.9.

Antioxidant activity

Antioxidant properties were analyzed by three different methods:

1. Measurement of free radical scavenging activity. The antiradical activity of samples was evaluated according to the procedure reported by Sumaya-Martinez et al. 2005[22]. An aliquot of sample (200 μL) was added to 1 ml of a daily-prepared solution of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) in ethanol (74 mg/L). The mixture was homogenized in a vortex, incubated at room temperature for 1 h at 25°C and then centrifuged at 10 000 rpm/5 min. 200 μL of each supernatant were placed in a UV-VIS microplate spectrophotometer and the absorbance was determined at 520 nm. For all experiments deionized water instead of sample solution was used as blank. Trolox solution (mmol equiv/L) was used as the control.

2. Antioxidant activity measured by the bleaching of β-Carotene.[23] A solution of β-Carotene is prepared dissolving 2 mg of β-carotene in 10 mL of chloroform. 2 mL of this solution is pippeted into 500 ml RB flask, after chloroform was removed under vacuum (at 40°C), 40 μL of linoleic acid, 400 μL of Tween-20 and 100 mL of deionized water were added. The mixture was vigorously shaken until the formation of an emulsion. Aliquots (5 ml) of this emulsion are added to test tubes containing 200 μL of the samples solutions. BHT solution in ethyl alcohol at 0.1 mg/ml was used for comparison purposes. The tubes were homogenized in a vortex and placed in a water bath at 50°C. The course of the color disappearance of β-Carotene was followed at 0, 30, 60 and 90 min. Absorbance was measured on UV-VIS microplate spectrophotometer at 470 nm.

Table 4. Crystal and experimental data for compounds **1** and **1-HCl**

	1	**1-HCl**
Data CCDC	649381	649382
Empirical formula	$C_{15}H_{20}N_2O_2$	$C_{15}H_{21}N_2O_2Cl$
Formula weight	260.3	296.8
Temperature[K]	298	298
Wavelength	0.71073	0.71073
Crystal system	orthorhombic	monoclinic
Space group	P bca	P 2_1/c
Unit cell dimensions		
a [Å]	9.6480(14)	6.3494(8)
b [Å]	9.1027(18)	24.7590(30)
c [Å]	33.4120(67)	11.7953(13)
α [º], β [º], γ [º]	90, 90, 90	90, 120.083(3), 90
Volume [Å^3]	2934.34(14)	1604.52(65)
Z	8	4
Density (calculated) [Mg/m^3]	1.18	1.23

Absortion coefficient [mm^{-1}]	0.079	0.241
F(000)	1119.8	631.9
Crystal size [mm]	0.20x0.17x0.15	0.40x0.30x0.30
Diffractometer	Bruker-Smart CCD	Bruker-Smart CCD
θ min/max for data collection [º]	1.2/23.3	1.6/23.3
Index ranges		
	-10≤h≤10	-7≤h≤7
	-10≤k≤10	-25≤k≤27
	-30≤l≤37	-10≤l≤13
Reflections collected	14096	8280
Reflections independent	2107(0.096)	2297(0.103)
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data/restraints/parameters	2107/0/172	2297/0/181
Goodness-of-fit on F^2	0.851	0.886
Final R indices [I>2σ(I)]	R1=0.043, wR2=0.093	R1=0.052, wR2=0.110
R indices (all data)	R1=0.108, wR2=0.141	R1=0.111, wR2=0.138
Largest diff. Peak and hole[e/ $Å^3$]	0.133 and -0.166	0.255 and -0.166

3. Metal chelating activity. The chelation of ferrous ions was estimated as described by Dorman (2003)[24]. Briefly, a 200 μL aliquot of sample solution was added to 100 μL of 2.0 mM aqueous of $FeCl_2 \cdot 4H_2O$ and 900 μL of methyl alcohol, the mixtures were homogenized and after 5 min incubation, the reaction was initiated by adding 400 μL of 5.0 mM of Ferrozine solution. After a 10 min equilibrium period, the absorbance at 562 nm was recorded. The controls contained all the reaction reagents except the sample solution or positive control substance (EDTA at 1%). The Fe^{2+} chelating activity was reported according with: Inhibition %= $[(A_{EDTA} - A_S)/A_{EDTA}]100$; ($A_{EDTA}$ = absorbance of EDTA solution, A_S = absorbance of sample solutions).

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