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Synthesis, Crystal Structure and Anti-Breast Cancer Activity of Some Enaminone Derivatives

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The present work reports the synthesis of some enaminone derivatives bearing a biologically active 3,4-dimethoxyphenyl (**3**) or 3,4,5-trimethoxyphenyl moieties (**5** and **7**), respectively. The trimethoxybenzene moiety has been previously reported to confer cytotoxic activity. The structure of the newly synthesized compounds was verified by elemental analyses, IR, ^{1}H NMR, ^{13}C NMR spectra and X-ray analysis. The anti-breast cancer activity of the enaminone derivatives were evaluated. Compounds **3**, **5** and **7** showed excellent anti-breast cancer activity with IC_{50} values (55.2, 79.06 and 50.49 μM) compared with doxorubicin with IC_{50} value (71.80 μM) as reference drug.

Keywords: Synthesis, Enaminones, X-ray analysis, Anti-breast cancer activity.

INTRODUCTION

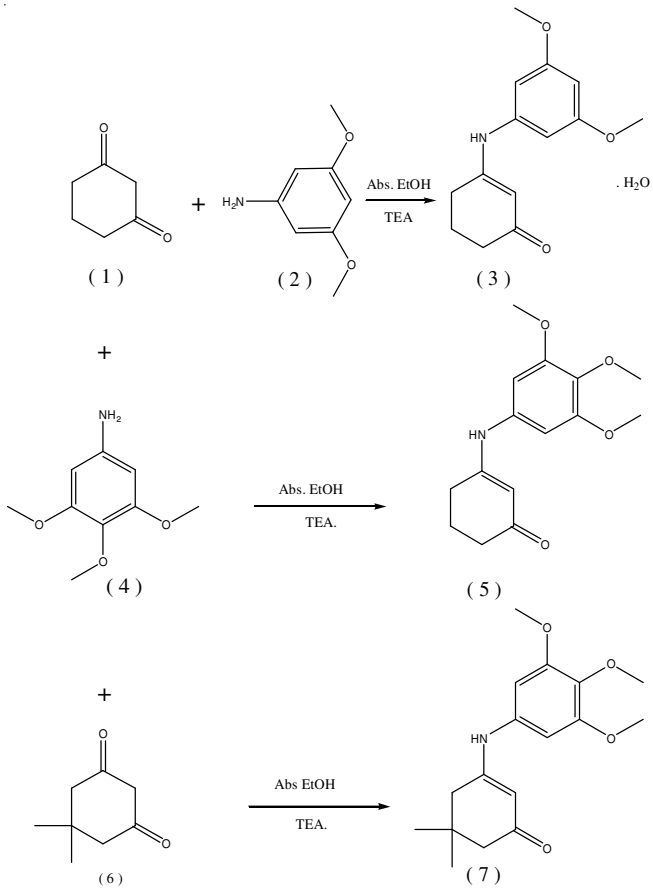
Enaminones are a group of organic compounds carrying the conjugated system N-C=C-C=O[1]. The chemistry of enaminones, their physicochemical properties and biological activities has been extensively reported in the literatures[2-10]. In addition, cyclohex-2-enone exhibited a wide range of biological activities such as anticancer[11] and antimicrobial activities[12]. On the other hand, enaminones have been used as key intermediates in organic synthesis[13-18] and the chemistry of these compounds have been reviewed[19]. In particular they have been employed as synthons of a wide variety of biologically active heterocyclic compounds[20], as pharmaceutical compounds having anticancer[21], antibacterial[22], antiinflammatory[23] and other therapeutic agents[24-26]. During the last decade we have been involved in a program aimed at exploring the potentials of enaminone as a building block for heteroaromatics[27] and we have successfully synthesized and reported the corresponding quinoline derivatives[13-18] utilizing enaminones as starting materials. Based on the above information and as a continuation of previous work on anticancer agents[28-33], we report here the synthesis, spectral characterization and X-ray crystal structures of the three enaminone derivatives of expected anticancer activity.

EXPERIMENTAL

The starting materials cyclohexane-1,3-dione, 5,5-dimethylcyclohexane-1,3- dione, 3,4-dimethoxyaniline and 3,4,5-trimethoxyaniline were purchased from Sigma- Aldrich and used without further purification. Melting points were determined on an electrothermal melting point apparatus (Stuart Scientific, Stone) and were uncorrected. Precoated Silica gel plates (Kiesel gel 0.25 mm, 60 GF 254, Merck) were used for thin layer chromatography (TLC). The developing solvent system was chloroform/methanol (10:3) and the spot were detected by ultraviolet light. The infrared (IR) spectra (KBr disc) were recorded on FT- IR spectrophotometer (Perkin Elmer) at the Research Center, College of Pharmacy, King Saud University, Saudi Arabia. ^{1}H-NMR spectra were scanned in dimethylsulfoxide (DMSO-d_6) on a NMR spectrophotometer (Bruker AXS Inc.) operating at 500 MHz for ^{1}H and 125.76 MHz for ^{13}C at the aforementioned Research Center. Chemical shifts are expressed in δ-values (ppm) relative to tetramethylsilane (TMS) as an internal standard. Exchangeable protons were confirmed by addition of drop of D_2O. Elemental analyses were done on a model 2400 CHNSO analyzer (Perkin Elmer).

X-ray crystallography: Single crystal suitable for X-ray diffraction was mounted on glass fibres. The diffraction data were collected on Bruker APEX-II CCD area-detector diffractometer with CuK_α radiation (λ = 1.54178 Å) at a temperature of 296(2) K. The data for these compounds were processed with SAINT and corrected for absorption using multi-scan[34]. The structures were solved by direct methods using the program SHELXTL97[35] and refined by full-matrix least squares technique on F^2 using anisotropic displacement parameters using SHELXTL program[35]. All geometrical calculations were carried out using the program PLATON[36]. Molcular graphics

were drawn using SHELXTL[35] and PLATON[36] programs. All the hydrogen atoms were fixed at calculated positions with a common isotropic displacement parameters set to 1.2 (1.5 for methyl groups) times the equivalent isotropic U values of the parent carbon atoms. A rotating group model was applied to the methyl groups. The crystallographic data are given in Table-1. Hydrogen bonding interactions are shown in Table-2. The selected bond lengths and bond angles are listed in Table-3. The scheme of the synthesis of the compounds is shown in **Scheme-I**.



Scheme-I: Formation of enaminone derivatives

Synthesis of 3-(3,5-dimethoxyphenylamino) cyclohex-2-enone (3): A mixture of cyclohexane-1,3-dione **1** (1.22 g, 0.01 mol) and 3,5- dimethoxyaniline **2** (1.53 g, 0.01 mol) in absolute ethanol (30 mL) containing 3 drops of triethylamine was refluxed for 8 h. The reaction mixture was cooled and the solid obtained was recrystallized from dioxane. Crystals suitable for X-ray diffraction were obtained after a week on slow evaporation the solution. Yield 94 %; m.p. 138-140 °C; IR (KBr, ν_{max}, cm^{-1}): 3298 (NH), 3086 (CH arom.), 2972, 2876 (CH aliph.), 1652 (C=O). ^{1}H-NMR spectrum in (DMSO-d_6): 1.39, 1.87, 2.99 [m, 6H, $3CH_2$ cyclo.], 3.70 [s, 6H, 2 OCH_3], 5.7 [s, 1H, CH cyclo.], 5.9-6.3 [m, 3H, Ar-H], 9.7 [s, 1H, NH, D_2O-exchangeable]. ^{13}C-NMR spectrum in (DMSO-d_6): 19.6, 28.3, 38.8, 56.4 (2), 91.2, 92.6, 101.6, 145.9, 161.4, 163.7 (2), 200.6 (C=O). Anal. Calcd for $C_{14}H_{17}NO_3$ (247.29): C, 68.00; H, 6.93; N, 5.66. Found: C, 68.31; H, 6.64; N, 5.93 %.

Synthesis of 3-(3,4,5-trimethoxyphenylamino) cyclohex-2-enone (5): A mixture of cyclohexane-1,3-dione **1** (1.12 g, 0.01 mol) and 3,4,5-trimethoxyaniline **4** (1.83 g, 0.01 mol) in absolute ethanol (30 mL) containing 3 drops of triethylamine was refluxed for 6 h. The reaction mixture was cooled and the solid obtained was recrystallized from ethanol. Crystals were harvested after a month on slow evaporation the solution. Yield 89 %; m.p. 205-207 °C; IR (KBr, ν_{max}, cm^{-1}): 3270 (NH), 3100 (CH arom.), 2966, 2836 (CH aliph.), 1653 (C=O). ^{1}H NMR spectrum in (DMSO-d_6): 1.8-2.4 [m, 6H, $3CH_2$ cyclo.], 3.70, 3.78 [2s, 9H, 3 OCH_3], 5.2 [s, 1H, CH cyclo.], 6.4 [s, 2H, Ar-H], 8.7 [s, 1H, NH, D_2O- exchangeable]. ^{13}C-NMR spectrum of **5** in (DMSO-d_6): 21.5, 28.4, 36.3, 55.8 (2), 60.0, 98.0 (2), 101.1, 134.4, 135.6, 153.0 (2), 162.2, 195.7 (C=O). Anal.[13] Calcd for $C_{15}H_{19}NO_4$ (277.32): C, 64.97; H, 6.91; N, 5.05. Found: C, 64.69; H, 6.59; N, 5.31.

Synthesis of 3-(3,4,5-trimethoxyphenylamino)-5,5-dimethylcyclohex-2-enone (7): A mixture of 5,5- dimethyl-cyclohexane-1,3-dione **6** (1.40 g, 0.01 mol) and 3,4,5- trimethoxyaniline **4** (1.83 g, 0.01 mol) in absolute ethanol (30 mL) containing three drops of triethylamine was refluxed for 6 h. The reaction mixture was cooled and the obtained solid was recrystallized from dioxane. Crystals suitable for X-ray diffraction was obtained after a week on slow evaporation of the solution[14].

***in vitro* Cytotoxic activity:** The human tumor breast cancer cell line (MCF7) was obtained from the National Cancer Institute, Cairo, Egypt. The cytotoxic activity of the synthesized compounds was measured by the Sulfo-Rhodamine-B stain (SRB) assay as reported by Alsaid *et al.*[29]. Cells were plated in 96-multiwell plate (10^4 cells per well) for 24 h before treatment with the compounds to allow attachment of cell to the wall of the plate. Tested compounds were dissolved in DMSO and diluted with saline. Different concentrations of the compounds under test (5, 12.5, 25 and 40 µmol L^{-1}) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h at 37 °C and in atmosphere of 5 % CO_2. After 48 h, cells were fixed, washed and stained for 0.5 h. with 0.4 % (*m/v*) SRB dissolved in 1 % acetic acid. Unbounded dye was removed by four washes with 1 % acetic acid and attached stain was recovered with *tris*-EDTA buffer. Colour intensity was measured in an ELISA reader (sunostick medical technology, SPR-960B, U.K.). Negative control was added by using the cell lines with the solvent without drug. The relation between surviving fraction and drug concentration was plotted to get the survival curve of each tumor cell line after the specified time. The concentration required for 50 % inhibition of cell viability (IC_{50}) was calculated and compared with the reference drug doxorubicin and the results are given in Table-1.

RESULTS AND DISCUSSION

The aim of the present work was to design, synthesis elucidation of some enaminone derivatives carrying a biologically active 3,5-dimethoxyphenyl moiety **3** and 3,4,5-trimethoxyphenyl moieties **5** and **7** with expected anticancer activity (**Scheme-I**). 3-(3,5-Dimethoxyphenyl-amino)-cyclohex-2-enone **3** was obtained in good yield *via* reaction of cyclohexane-1,3-

TABLE-1
in vitro CYTOTOXIC ACTIVITY OF SOME NEWLY SYNTHESIZED COMPOUNDS AGAINST HUMAN BREAST CANCER CELL LINE (MCF7)

Compd. No.	Control	Compound Concentration (μmol L^{-1})				IC_{50}
		5	12.5	25	40	
		Surviving Fraction (mean ± SE)[a]				
DOX	1.00	0.721 ± 0.020	0.546 ± 0.020	0.461 ± 0.010	0.494 ± 0.030	71.8
3	1.00	0.614 ± 0.002	0.519 ± 0.009	0.365 ± 0.028	0.279 ± 0.047	55.2
5	1.00	0.806 ± 0.008	0.631 ± 0.033	0.418 ± 0.032	0.332 ± 0.081	79.06
7	1.00	0.577 ± 0.044	0.421 ± 0.012	0.369 ± 0.014	0.211 ± 0.065	50.49

[a]n = 3

dione **1** with 3,5- dimethoxyaniline **2** in refluxing ethanol containing a few drops of triethylamine as catalyst (**Scheme-I**). The structure of compound **3** was supported by elemental analysis, IR, ^{1}H-NMR, ^{13}C-NMR spectra and X-ray data. IR spectrum of **3** revealed the presence of characteristic bands for NH at 3310 cm^{-1}, (CH arom.) at 3096 cm^{-1}, (CH aliph.) at 2977, 2866 cm^{-1} and (C=O) at 1647 cm^{-1}. Also, ^{1}H-NMR spectrum in (DMSO-d_6) indicated the presence of a signals at 3,71 ppm which could be assigned to two methoxyl groups, 5.7 ppm due to CH cyclo. and 9.7 ppm for NH of enaminone **3**. ^{13}C-NMR spectrum of **3** in (DMSO-d_6) showed signals at 19.6, 28.3, 38.8, 56.4 (2), 91.2, 92.6 (2), 101.6, 145.9, 161.4, 163.7 (2), 200.6 (C=O).

In addition, interaction of **1** with 3,4,5-trimethoxyaniline **4** in refluxing ethanol[13] afforded the corresponding 3-(3,4,5-trimethoxyphenylamino)-cyclohex-2- enone **5** in good yield. The structure of **5** was confirmed from its microanalysis, IR, ^{1}H-NMR, ^{13}C-NMR and X-ray data. Thus, IR spectrum of **5** exhibited the presence of characteristic bands for NH, CH aromatic, CH aliphatic and (C=O). ^{1}H-NMR spectrum of **5** revealed signals at 3.70, 3.78 ppm corresponding to three methoxy groups, 5.2 ppm due to CH cyclo and 8.7 ppm for NH group. ^{13}C-NMR spectrum of **5** in (DMSO-d_6) showed signal at 195.7 (C=O). On the other hand, condensation of 5,5-dimethyl-cyclohexane-1,3-dione (**6**) with 3,4,5-trimethoxy-aniline (**4**) yielded the corresponding 3-(3,4,5- trimethoxy-phenylamino)-5,5- dimethylcyclohex-2-enone (**7**) in good yield[14]. The structure of compound **7** was proved on the basis of elemental analysis, IR, ^{1}H-NMR, ^{13}C-NMR and X-ray analysis. The IR sectrum of **7** showed bands for (NH), (CH aromatic), (CH aliphatic) and (C=O). Also, ^{1}H-NMR spectrum in (DMSO-d_6) indicated the presence of a singlet at 8.7 ppm which could be assigned to NH of enaminone **3**.

Crystal structure of 3-(3, 5-dimethoxyphenylamino) cyclohex-2-enone monohydrate (3): In the crystal structure of **3** (Fig. 1), the asymmetric unit comprises of one molcule of 3-(3, 5-dimethoxyphenylamino) cyclohex-2-enone and a water molcule **3**. The mean plane of dimethoxyphenylamino unit and the mean plane of cyclohex-2-enone unit are twisted by an angle of 38.23(12) °. The phenyl ring is essentially planar with the maximum deviation from planarity being 0.014(2) Å for the atom C9. In cyclohex-2-enone unit, the atom C4 deviates from planarity of -0.313(3) Å. The cyclohex-2-enone unit adopts the envelope conformation with the puckering amplitude of Q = 0.440(3) Å; θ = 51.9(4)°; ϕ = 176.4(4)° [37]. The C1-C6 ring has an envelope on C4. Symmetry related molcules are linked together by N-H…O hydrogen bonds [symmetry code: x,-1 + y, z] to form infinite one dimensional chain along the [100] direction (Fig. 2). Symmetry related O–H···O [symmetry code: 1-x, 2-y, 1-z] hydrogen bonds further strengthen the hydrogen bonding. The molcules are stacked along the a-axis (Fig. 3). Even though the molcules are stacked along the a-axis from the packing mode, there is no significant π-π stacking interaction. The geometrical bonding of the hydrogen is shown in Table-2.

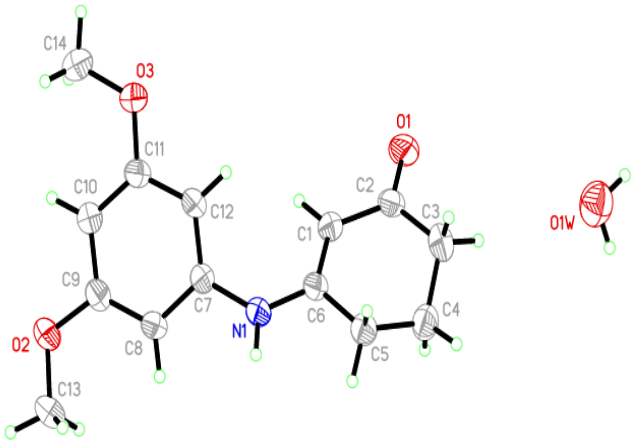


Fig. 1. Thermal ellipsoidal plot of compound **3**, ellipsoids are drawn at the 50 % probability level

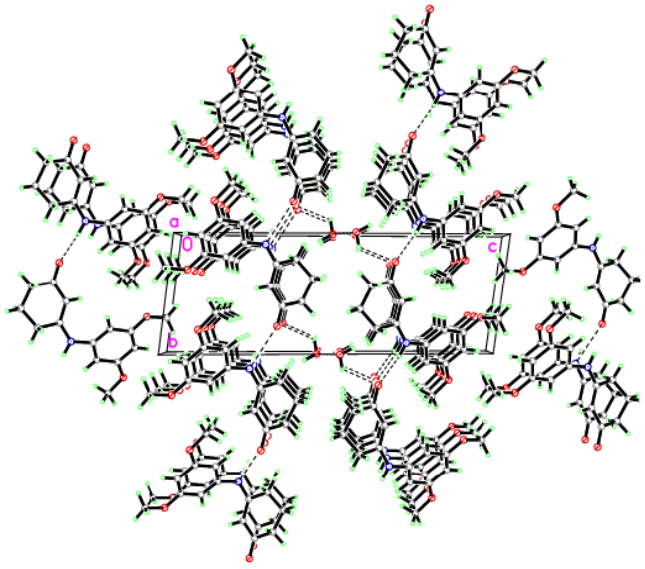


Fig. 2. Perspective view of the packing diagram of compound **3**, the molcules are stacked along the a-axis

TABLE-2
CRYSTAL DATA AND PARAMETERS FOR STRUCTURE REFINEMENT OF COMPOUNDS **3**, **5** AND **7**

Parameters	Compound **3**	Compound **5**	Compound **7**
CCDC deposition numbers	922316	922317	922318
Empirical formula	$C_{14}H_{19}NO_4$	$C_{15}H_{19}NO_4$	$C_{17}H_{23}NO_4$
Formula weight	265.30	277.31	305.36
Temperature	296(2) K	296(2) K	296(2) K
Wavelength	1.54178 Å	1.54178 Å	1.54178Å
Crystal system, space group	Triclinic P-1	Triclinic P-1	Triclinic P-1
Unit cell dimensions	a = 4.792(3) Å b = 7.209(3) Å c = 202(10) Å α = 97.169(3)° β = 95.977(4)° γ = 95.030(4)°	a = 7.173(10) Å b = 10.258(10) Å c = 11.325(10) Å α = 113. 5(10)° β = 107. 5(10)° γ = 93.83(10)°	a = 7.048(2) Å b = 11.849(3) Å c = 11.876(3) Å α = 115.712(10)° β = 104. 136(2)° γ = 96.427(2)°
Volume	685.29(6) $Å^3$	711.17(17) $Å^3$	839.31(4) $Å^3$
Z, calculated density	2, 1.286 Mg m^{-3}	2, 1.295 Mg m^{-3}	2, 1.208 Mg m^{-3}
Absorption coefficient	0.776 mm^{-1}	0.773 mm^{-1}	0.699 mm^{-1}
F(000)	284	296	328
Θ Range for data collection	4.44-64.96°	4.82-64.98°	4.37-64.99°
Reflections collected/Unique	8107/2286	6674/2347	8605/2775
Rint	0.0890	0.0191	0.0201
Completeness to Θ	64.96 97.3	64.98 96.7 %	64.99, 97.2 %
Absorption correction	Semi-empirical from equivalents	Semi-empirical from equivalents	Semi-empirical from equivalents
Max and Min Transmission	0.9405/0.6920	0.7311/0.6888	0.8683/0.6740
Refinement method	Full matrix least squares on F^2	Full matrix least squares on F^2	Full matrix least squares on F^2
Data/restraints/parameters	2286/0/174	2347/0/183	2775/0/209
Goodness of fit on F^2	1.096	1.117	1.094
R_1, wR_2 [$I \geq 2\sigma(I)$]	0.0536, 0.1464	0.0656, 0.1803	0.0441, 0.1325
R_1 and wR_2 (all data)	0.0752, 0.1599	0.0683, 0.1834	0.0484, 0.1370
Largest diff. peak and hole	0.238 and -0.252	0.356 and -0.441	0.185 and -0.183

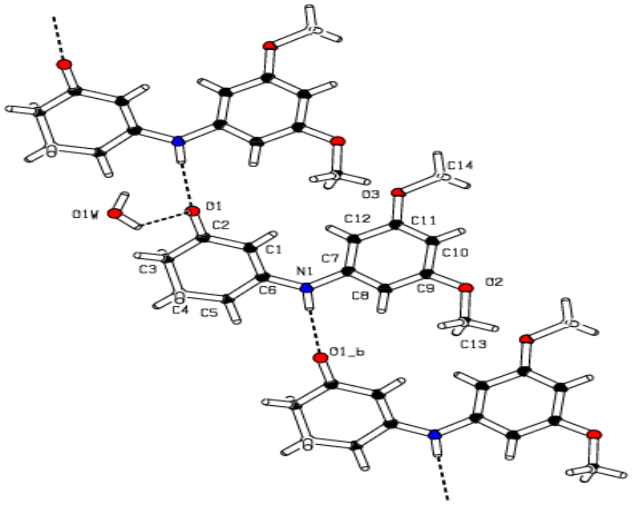


Fig. 3. Hydrogen bonding diagram showing the molcules are linked into one dimensional chain along the [100] direction. The packing is further strengthened by weak O---H...O hydrogen bonding

Crystal structure of 3-(3, 4, 5-trimethoxyphenylamino) cyclohex-2-enone (5): In the asymmetric unit of **5** (Fig. 4), the trimethoxyphenyl ring forms a dihedral angle of 36.35 (16)° with the cyclohex-2-enone unit. The cyclohex-2-enone unit adopts the envelope conformation with the puckering parameters Q = 0.145(5) Å θ = 57.7(16)° and [ϕ = 190.8(18)°. The molcules are linked together by symmetry related N-H…O hydrogen bonds [symmetry code: 1 + x, y] to form infinite one dimensional chain along the [100] direction. The N-H…O hydrogen bonding is shown in (Fig. 5). There is no significant π-π stacking interaction, as the shortest centroid to centroid separation involving aromatic rings being longer than 4.2 Å.

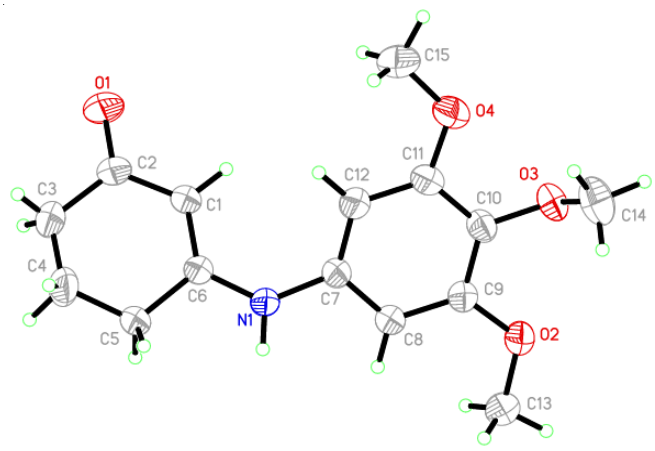


Fig. 4. ORTEP diagram of compound **5** drawn at 50 % ellipsoids for non-hydrogen atoms

Crystal structure of 3-(3, 4, 5-trimethoxyphenylamino)-5,5-dimethylcyclohex-2-enone (7): In the crystal structure **7** (Fig. 6), the trimethoxyphenyl unit and the cyclohex-2-enone are twisted to each other by an angle of 35.65(8)°. The cyclohex-2-enone unit adopts the envelope conformation with the puckering parameters Q = 0.4589 (18) Å; θ = 128.9 (2)° and ϕ = 354.8(3)°. The molcules are linked together by symmetry related N-H…O hydrogen bonds [symmetry code: 1 + x, y, z] to form infinite one dimensional chain along the [100] direction

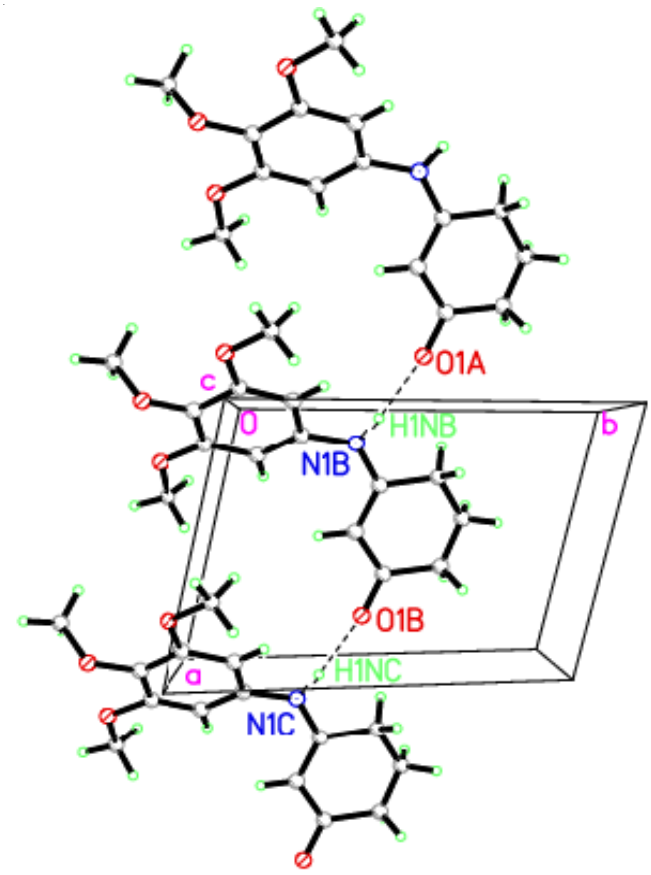


Fig. 5. N---H...O hydrogen bonding linking the symmetry related molcules into one dimensional chain along the [100] direction

(Fig. 7). There is no significant π-π stacking interaction, as the centroid to centroid distance is longer than 5.48(10) Å. ^{1}H-NMR spectrum in (DMSO-d_6) indicated the presence of a singlet at 8.7 ppm which could be assigned to NH of enaminone.

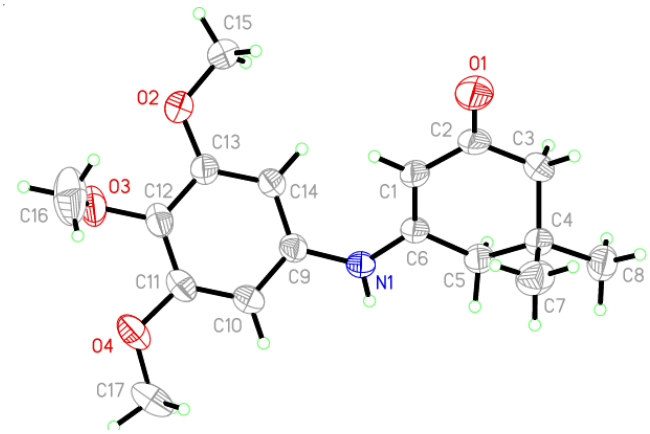


Fig. 6. Molcular structure of compound **7**, ellipsoids drawn at the 40 % probability level

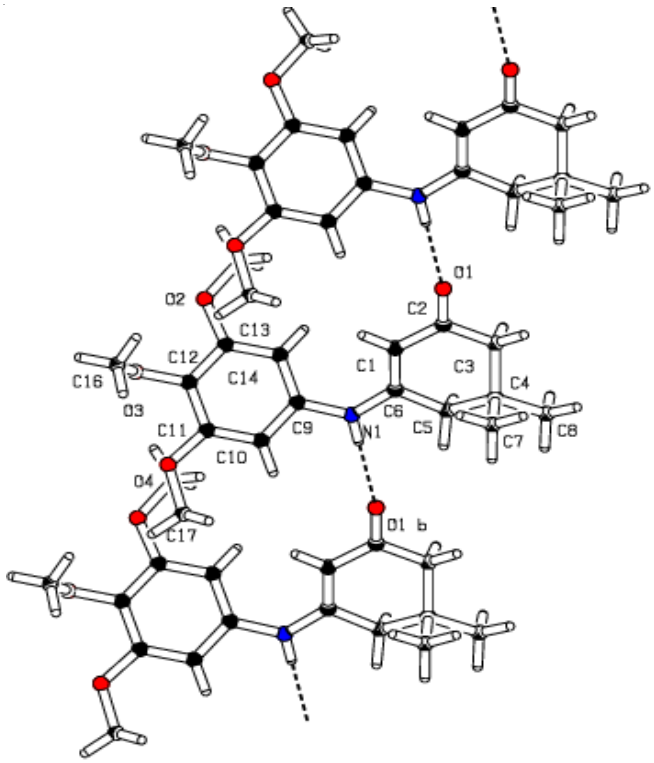


Fig. 7. Molcular packing of compound **7** showing N---H...O hydrogen bonds

***in vitro* Cytotoxic activity:** The enaminone derivatives **3**, **5** and **7** were evaluated for their *in vitro* cytotoxic activity against human breast cancer cell line (MCF7). Doxorubicin which is one of the most effective anticancer agents was used as the reference drug in this study. The relationship between surviving fraction and drug concentration was plotted to obtain the survival curve of breast cancer cell line (MCF7). The response parameter calculated was the IC_{50} value, which corresponds to the concentration required for 50 % inhibition of cell viability. Table-1 shows the *in vitro* cytotoxic activity of the tested compounds compared to the reference drug. It was found that, in the negative control, solvent has no effect on the cells as the surviving fraction is 1.0, the most potent compounds were the 3-(3,4,5-trimethoxyphenylamino)-5,5-dimethyl-cyclohex-2-enone **7** having 3,4,5-trimethoxyphenyl group at 3-position (IC_{50} = 50.49 µM) and the 3-(3,5-dimethoxy-phenylamino) cyclohex-2-enone **3** carrying 3,5-dimethoxy-phenyl moiety (IC_{50} = 55.20 µM) which were found to be more potent than the doxorubicin as positive control (IC_{50} = 71.8 µM), also, 3-(3,4,5-trimethoxyphenylamino) cyclohex-2-enone **5** (IC_{50} = 79.06 µM) which was found to be nearly as potent as the reference drug. Isosteric replacement of the 1,3-cyclohexanone ring with 5,5-dimethylcyclohexane-1,3-dione led to a drop in the activity as in compound **3** (IC_{50} = 55.20

TABLE-3
GEOMETRY OF THE HYDROGEN BONDS (Å, °)

Compound	D-H…A	d(D-H)	d(H-A)	d(D-A)	<(D-H…A)
3	N1-H1B…O1[(a)]	0.86	2.14	2.941(2)	156
3	O1W-H1W1...O1[(b)]	0.86	2.45	2.931(3)	116
5	N1-H1N...O1[(c)]	0.91(4)	1.92(4)	2.819(4)	174(3)
7	N-H...O[d]	0.81(2)	2.00(2)	2.809(2)	178(2)

Symmetry code: [a][1-x, 2-y, 1-z]; [b] [1-x, 2-y, 1-z] [c][1 + x,y,z]; [d][1 + x,y,z]

TABLE-4
SELECTED BOND LENGTHS [Å] AND BOND ANGLES [DEG] FOR COMPOUNDS **3**, **5** AND **7**

3		**5**		**7**	
O1- C2	1.252(3)	O2- C9	1.364(3)	O1- C2	1.235(2)
O2- C9	1.359(3)	O2- C13	1.422(3)	O2- C13	1.360(2)
O3- C11	1.372(3)	O3- C14	1.407(4)	O3- C12	1.376(2)
O3- C14	1.429(3)	O4- C11	1.362(3)	O3- C16	1.403(3)
N1- C6	1.351(3)	O4- C15	1.415(4)	O4- C11	1.363(2)
N1- C7	1.417(3)	N1- C6	1.350(3)	O4- C17	1.411(2)
C1- C6	1.364(3)	N1- C7	1.411(3)	N1- C6	1.352(2)
C1- C2	1.419(3)	N1- H1N	0.91(3)	N1- C9	1.409(2)
C2- C3	1.499(4)	C1- C6	1.359(3)	C1- C6	1.363(2)
C3- C4	1.507(4)	C1- C2	1.411(4)	C1- C2	1.422(2)
C4- C5	1.514(3)	C2- C3	1.490(4)	N1- H1N	0.81(2)
C5- C6	1.500(3)	C3- C4	1.362(5)	C2- C3	1.502(2)
C7- C12	1.377(3)	C4- C5	1.450(4)	C3- C4	1.521(2)
C7- C8	1.395(3)	C5- C6	1.500(3)	C4- C7	1.518(2)
C8- C9	1.376(3)	C7- C12	1.384(4)	C4- C5	1.527(2)
C9- C10	1.399(3)	C7 C8	1.389(3)	C4- C8	1.530(2)
C10- C11	1.371(3)	C8 C9	1.387(3)	C9- C14	1.386(2)
C11- C12	1.386(3)	C9- C10	1.393(3)	C5- C6	1.506(2)
O1W- H1W1	0.8600	C10- C11	1.391(4)	C9- C10	1.389(2)
O1W- H2W1	0.8440	C11- C12	1.390(3)	C10- C11	1.387(2)
Bond Angles					
C9- O2- C13	118.4(2)	C6- N1- C7	130.2(2)	C13- O2- C15	117.3(2)
C11- O3- C14	117.0(2)	C9- O2- C13	117.6(2)	C6- N1- C9	129.0(2)
C6- N1- C7	128.7(2)	C6- C1- C2	122.5(2)	N1- C6- C1	125.8(2)
C6- C1- C2	122.7(2)	O1- C2- C1	121.9(3)	C12- O3- C16	114.4(2)
O1- C2- C1	121.4(2)	N1- C6- C1	126.5(2)	C11- O4- C17	118.1(2)
O1- C2- C3	120.3(2)	C1- C6- C5	120.7(2)	C6 -C1- C2	121.7(2)
C1- C2- C3	118.3(2)	C3- C4- C5	122.7(3)	O1- C2- C1	121.5(2)
C2- C3- C4	112.3(2)	C10- O3-C14	114.9(2)	O1- C2- C3	119.6(2)
C3- C4- C5	111.3(2)	C11- O4- C15	118.3(2)	C1- C2- C3	118.7(2)
C6- C5- C4	111.9(2)	O1- C2- C3	119.8(3)	C2- C3- C4	113.2(2)
N1- C6- C1	125.0(2)	C1- C2- C3	118.3(2)	C7- C4- C3	110.4(2)
N1- C6- C5	114.1(2)	C4- C3- C2	118.5(3)	C7- C4-C5	110.4(2)
C1- C6- C5	120.8(2)	C4- C5- C6	114.8(2)	C3- C4-C5	107.7(2)
C12- C7- C8	120.8(2)	N1- C6- C5	112.8(2)	C7- C4-C8	108.5(2)
C12- C7- N1	121.8(2)	C12- C7- C8	120.6(2)	C3- C4-C8	110.3(2)
C8- C7- N1	117.2(2)	C12- C7- N	123.1(2)	C5- C4-C8	109.2(2)
C9- C8- C7	118.8(2)	C8- C7- N1	116.2(2)	C6- C5-C4	113.8(2)
O2- C9- C8	124.9(2)	O2- C9- C8	124.4(2)	N1- C6-C5	113.7(2)
O2- C9- C10	114.2(2)	O2- C9- C10	115.4(2)	C1- C6-C5	120.4(2)
C8- C9- C10	120.9(2)	C8- C9- C10	120.2(2)	C14- C9-C10	120.7(2)
C11- C10- C9	119.1(2)	O4- C11- C12	124.2(2)	C14- C9-N1	121.6(2)
C10- C11- C12	120.9(2)	O3- C10- C11	121.4(2)	O4- C11-C10	124.1(2)

μM) and also a drop in the activity was shown by replacing the 1,3-cyclohexanone ring with 5,5-dimethylcyc-lohexane-1,3-dione moiety as in compound **5** (IC_{50} = 79.06 μM), an improvement in the activity was observed upon incorporation of 3,4,5-trimethoxyanilino combined with dimedone moiety in compound **7** (IC_{50} = 50.49 μM).

Conclusion

The synthesized enaminone derivatives have been spectroscopically characterized by FT IR, ^{1}H, ^{13}C-NMR and single crystal X-ray diffraction studies. The structural elucidation is used to explore the conformation features of the enaminone derivatives. All the synthesized compounds adopt the envelope conformation. In the crystal packing, the molcules are linked together by N-H…O hydrogen bonds to form one dimensional chain. From the above results, it is concluded that administration of the tested compounds on human breast (MCF7) cell lines showed promising cytotoxic activity, the most potent compounds are 3-(3,4,5-trimethoxyphenylamino)-5,5-dimethylcyclohex-2-enone (**7**) (IC_{50} = 50.49 μM) and 3-(3,5-dimethoxyphenylamino) cyclohex-2-enone (**3**) (IC_{50} = 55.20 μM) which were found to be more potent than doxorubicin (IC_{50} = 71.8 μM) and 3-(3,4,5-trimethoxyphenylamino) cyclohex-2-enone **5** carrying 3,4,5-trimethoxyphenyl moiety at 3-position (IC_{50} = 79.06 μM) which is nearly as active as the reference drug.

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REFERENCES

1. A.G. Cook, In: Enaminas: Synthesis, Structure and Reaction, Marcel Dekker; New York, NY, USA (1969).
2. A.D. Fraser, *Clin. Biochem*., **29**, 97 (1996).
3. R.J. Porter, J.J. Cereghino, G.D. Gladding, B. Hessie, H.J. Kupferberg, B. Scoville and B.G. White, *Clin. Q*, **51**, 293 (1984).
4. D.S. Cox and H. Gao, *Eur. J. Pharm. Biopharm*., **52**, 145 (2001).
5. D.S. Cox, K.R. Scott, H. Gao, S. Raje and N.D. Eddington, *J. Pharm. Sci*., **90**, 1540 (2001).
6. J. Vamecq, D. Lambert, J.H. Poupaert, B. Masereel and J.P. Stables, *Eur. J. Med. Chem*., **38**, 49 (2003).
7. N.D. Eddington, D.S. Cox, M. Khurana, N.N. Salama, J.P. Stables, S.J. Harrison, A. Negussie, R. Taylor, U.Q. Tran, J.A. Moore, J.C. Barrow and K.R Scott, *Eur. J. Med. Chem*., **38**, 49 (2003).
8. N.D. Eddington, D.S. Cox, R.R. Roberts, R.J. Butcher, I.O. Edafiogho, J.P. Stables, N. Cooke, A.M. Goodwin, C.A. Smith and K.R. Scott, *Eur. J. Med. Chem*., **37**, 635 (2002).
9. I.O. Edafiogho, K.V. Ananthalakshmi and S.B. Kombian, *Bioorg. Med. Chem*., **14**, 5266 (2006).
10. K.V. Ananthalakshmi, I.O. Edafiogho and S.B. Kombian, *Neuroscience*, **141**, 345 (2006).
11. C.L. Arthurs, G.A. Morris, M. Piacenti, R.G. Pritchard, I.J. Stratford, T. Tatic, R.C. Whitehead, K.F. Williams and N.S. Wind, *Tetrahedron*, **66**, 9049 (2010).
12. M. Kandhavelu, L. Paturu, A. Mizar, K. Mahmudov, M. Kopylovich, M. Karp, O. Yli-Harja, A. Pombeiro and A. Ribeiro, *Pharm. Chem. J*., **46**, 157 (2012).
13. S.M. Alsaid, M.S. Bashandy and M.M. Ghorab, *Arzneimittelforschung*, **61**, 527 (2011).
14. S.I. Alqasoumi, A.M. Al-Taweel, A.M. Alafeefy, M.M. Hamed, E. Noaman and M.M. Ghorab, *Bioorg. Med. Chem. Lett*., **19**, 6939 (2009).
15. M.M. Ghorab, F.A. Ragab, E. Noaman, H.I. Heiba and E.M. El-Hossary, *Arzneimittelforschung*, **58**, 35 (2008).
16. M.M. Ghorab, F.A. Ragab, E. Noaman, H.I. Heiba and E.M. El-Hossary, *Arzneimittelforschung*, **57**, 795 (2007).
17. M.M. Ghorab, F.A. Ragab and M.M. Hamed, *Eur. J. Med. Chem*., **44**, 4211 (2009).
18. S.I. Alqasoumi, A.M. Al-Taweel, A.M. Alafeefy, E. Noaman and M.M. Ghorab, *Eur. J. Med. Chem*., **45**, 738 (2010).
19. M.M. Ghorab, M.S. Alsaid and E.M. El-Hossary, *J. Heterocycl. Chem*., **48**, 563 (2011).
20. M.M. Ghorab, F.A. Ragab and M.M. Hamed, *Arzneimittelforschung*, **60**, 141 (2010).
21. V.V. Kouznetsov, F.A. Rojas Ruiz, L.Y. Vargas Mendez and M.P. Gupta, *Lett. Drug Des. Discov*., **9**, 680 (2012).
22. S. Eswaran, A.V. Adhikari, I.H. Chowdhury, N.K. Pal and K.D. Thomas, *Eur. J. Med. Chem*., **45**, 3374 (2010).
23. E.W. Chia, A.N. Pearce, M.V. Berridge, L. Larsen, N.B. Perry, C.E. Sansom, C.A. Godfrey, L.R. Hanton, G.L. Lu, M. Walton, W.A. Denny, V.L. Webb, B.R. Copp and J.L. Harper, *Bioorg. Med. Chem*., **16**, 9432 (2008).
24. M.M.F. Ismail, M.M. Ghorab, E. Noaman, Y.A. Ammar, H.I. Heiba and M.Y. Sayed, *Arzneimittelforschung*, **56**, 301 (2006).
25. M.M. Ghorab, F.A. Ragab, S.I. Alqasoumi, A.M. Alafeefy and S.A. Aboulmagd, *Eur. J. Med. Chem*., **45**, 171 (2010).
26. M. Sankaran, C. Kumarasamy, U. Chokkalingam and P.S. Mohan, *Bioorg. Med. Chem. Lett*., **20**, 7147 (2010).
27. S.M. Abdel- Gawad, M.A. El- Gaby, H.I. Heiba, H.M. Aly and M.M. Ghorab, *J. Chin. Chem. Soc*., **52**, 1227 (2005).
28. M.M. Ghorab, M.S. Alsaid and Y.M. Nissan, *Chem. Pharm. Bull. (Tokyo)*, **60**, 1019 (2012).
29. M.S. Alsaid, M.M. Ghorab and Y.M. Nissan, *Chem. Cent. J*., **6**, 64 (2012).
30. M.M. Ghorab, M.S. Alsaid and Y.M. Nissan, *Arzneimittelforschung*, **62**, 497 (2012).
31. M.M. Ghorab and M.S. Alsaid, *Arch. Pharm. Res*., **35**, 965 (2012).
32. M.M. Ghorab and M.S. Alsaid, *Arch. Pharm. Res*., **35**, 987 (2012).
33. E.M. Kassem, E.R. El-Sawy, H.I. Abd-Alla, A.H. Mandour, D. Abdel-Mogeed and M.M. El-Safty, *Arch. Pharm. Res*., **35**, 955 (2012).
34. Bruker, APEX2, SAINT & SADABS. Bruker AXS Inc., Madison, Wisconsin, USA (2009).
35. G.M. Sheldrick, *Acta Crystallogr*., **A64**, 112 (2008).
36. A.L. Spek, *Acta Crystallogr.*, **D65**, 148 (2009).
37. D. Cremer and J.A. Pople, *J. Am. Chem. Soc*., **97**, 1354 (1975).