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Oxidative Products of Cyclohexene-β-keto Esters or Anilidesas Active DNA-Cleaving Agents

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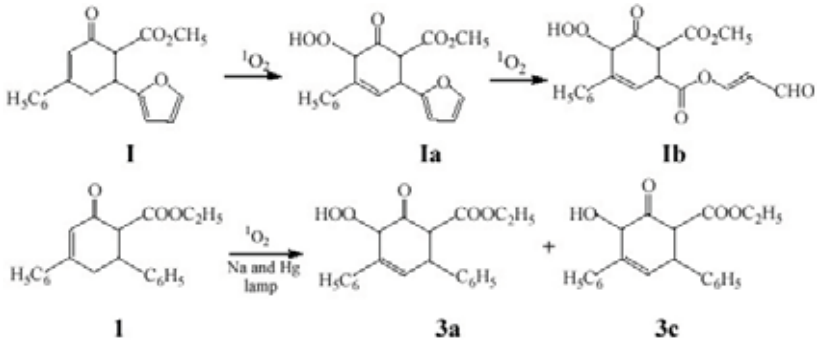
Abstract: Ethyl 3-(phenyl & styryl-), 5-diphenyl-2-cyclohexen-1-one-6-carboxylates (1a,b) were photooxygenated in presence of tetraphenyl porphin (TPP) to give the corresponding hydroperoxides 3a,b. Similarly, photooxygenation of N-(4-methoxyphenyl)-3-(phenyl- & styryl-),5-phenyl-2-cyclohexen- 1 -one-6-carboxanilides (2a,b) gave the hydroperoxides 4a,b. On the other hand, β-keto-esters 1a,b were epoxidized using m - chloroperbenzoic acid (mcpba) to give the corresponding epoxides 5a,b. Similarly, β-keto-anilide 2a gave the epoxide 5c.

Keywords: Photooxygenation, Epoxidation, Ethyl 3,5-diaryl-2-cyclohexenone-6-(carboxylate or carboxanilide), Tetraphenyl porphin, m-Chloroperbenzoic acid.

INTRODUCTION

The ready availability of cyclic β-keto-esters and the enhanced reactivity of their electrophilic sites have made them the starting materialsof choice for a great variety of syntheses (El-Mehwally, *et al.,* 1992).

Much work has appeared on the photo-reaction of cyclohexenone derivatives (Dauben, W.G., *et al.,* 1968). However, little is known about their photooxygenation reactions of 2-cyclohexen- 1 -one-6-carboxylic ester derivative. Previous work (Abou-Elzhab, *et al.,* 1994) showed that the photooxygenation reaction of cyclohexenone (I) gave the hydroperoxide derivatives Ia and Ib. Otherwise, Ibrahim (Ibrahim, M.E.M., 2003) reported that the photooxygenation reaction of β-keto-ester (1) using Sodium and Mercury lamps both together gave hydroperoxide derivative (3a) in very poor yield, beside the hydroxy-keto-ester derivative (3c).



In view of the photobiological interest in the activity of hydroperoxy group as DNA cleaving agent (Epe, B., *et al.,* 1993; Elgendy, E.M., and Abou-Elzahad, M.M., 1997; Schulte-Elte, K.H. and Rautenstrouch, V., 1980). It has been interested to investigat in detail the photooxygenation reactions of cyclohexenone derivatives (1a,b and 2a,b) to obtain the corresponding hydroperoxide derivatives as a powerful oxidizine agents for DNA.

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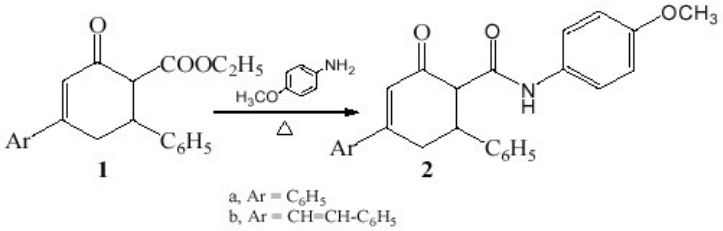
RESULTS AND DISCUSSIONS

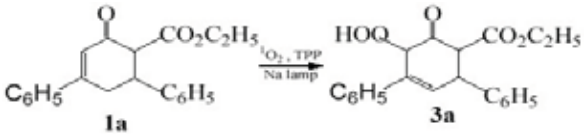
Ethyl 3 -(phenyl & styryl-),5-diphenyl-2-cyclohexen- 1 -one-6-carboxylate (1a,b) were condensed with p-anizidine to give N-(4-methoxyphenyl)-3 -(phenyl- & styryl-),5-phenyl-2-cyclohexen- 1 -one-6-carboxanilide (2a,b) respectively.



1 2

a, Ar = C_6H_5
b, Ar = CH=CH-C_6H_5

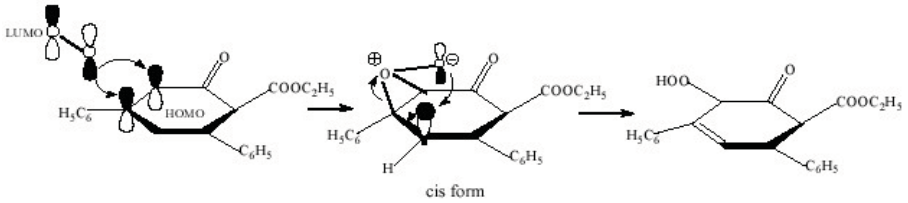
Under carefully controlled photooxygenation conditions it has been possible to isolate the postulated ethyl 2-hydroperoxy-3 ,5-diphenyl-3 - cyclohexen-1-one-6-carboxylate (3a) as a sole photo-product from ethyl 3 ,5-diphenyl-2-cyclohexen- 1 -one-6-carboxylate (1a) through the photo-irradiation using sodium light, and in the presence of tetraphenyl porphin (TPP) as singlet oxygen ($^1O2$) sensitizer.



1a 3a

The chemical structure of the hydroperoxide derivative (3a) was supported by spectral means. The IR spectrum revealed the presence of hydroperoxide group at 3473 cm^{-1}, whereas, the absorption bands at 1735 and 1664 cm^{-1} characteristic for carbonyl groups. Examination of ^1H-NMR spectrum showed AMX system of positions 4, 5, 6, which showed doublet doublet at δ 3.10 with coupling constants 5, 18 Hz for H-6, doublet doublet at 3.64 with coupling constants 5, 16 Hz for H-4, and doublet doublet at 3.83 with coupling constants 16, 18 Hz for H5. The spectrum showed singlet at 11.4 for OOH group. In addition the MS spectrum showed peak at m/z 336 characteristic for molecular ion minus one oxygen atom and corresponding to molecular formula C21H25O4.

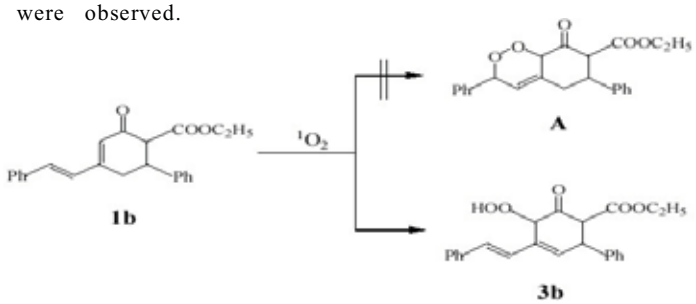
Frontier orbital analysis show that singlet oxygen 1O2 in lowest unoccupied molecular orbital (LUMO) (excited transition state) was combined with the double bond in the cyclohexenone ring in highest occupied molecular orbital (HOMO) (ground electronic state) as peroxirane intermediate followed by hydrogen transfer from α position (cis from) (Schulte-Elte, K.H. and Raurutenstrouch, V., 1980) to obtain the hydroperoxide derivative (3a). (Scheme 1).



cis form

Scheme 1:

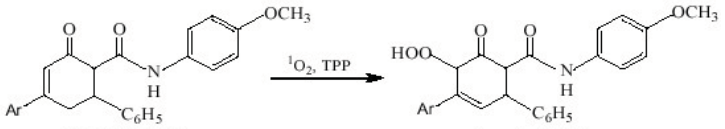
On the other hand, attempts to obtain the dioxane derivative (A) through the photooxygenation reaction of ethyl 5-phenyl-3-styryl-2-cyclohexen-1-one-6-carboxylate (1b) in presence of TPP, failed even the reaction time was prolonged in different temperatures, whereas, the isolated product was ethyl 2-hydroperoxy-5 -phenyl-3 -styryl-3 - cyclohexen-1-one-6-carboxylate (3b) in poor yield, no other photo-products were observed.



1b A 3b

Formulation of chemical structure of 3b was based on spectral measurements. ^{1}H-NMR spectrum showed triplet at δ 1.07 for CH3, AMXsystem: [A at δ 3.05 as complex pattern for proton at position 5, M at 3.77 as complex pattern for proton at position 6 and X at 4.23 as doublet doublet for proton at position 4, which was shifted toward down field], quartet at 4.05 for CH2O,singlet at 6.2 for proton at position 2 and singletat 8.84 for OOH proton. MS spectrum of 7 showed molecular ion M+minus oxygen at m/z 346.

Interesting, N-(4-methoxyphenyl)-3 -(phenyl- & styryl-),5-phenyl-2-cyclohexen- 1 -one-6-carboxanilides (2a,b), were photooxygenated in the presence of TPP as singlet oxygen sensitizer using sodium lamp to give successfully N-(4-mehtoxyphenyl-2-hydroperoxy-3 -(phenyl- & styryl-),5-phenyl-3 -cyclohexen- 1 -one-6-carboxanilide (4a,b).



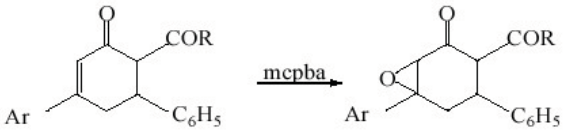
2, a, Ar= C_6H_5
b, Ar= CH=CH-C_6H_5

4, a, Ar=C_6H_5
b, Ar=CH=CH-C_6H_5

Formulation of chemical structure 4a was based on spectral measurements. IR spectrum showed characteristic bands at 3386, 1660 cm^{-1}for hydroperoxide and carbonyl groups respectively. ^{1}H-NMR spectrum showed AMX system of the positions 4, 5, 6, which showed doublet doublets at δ 3.10, 3.57 and 4.04 for the protons at positions 6, 4 and 5 respectively. The spectrum showed two singlets at δ 8.27 and 12.20 for amide NH and hydroperoxide groups respectively. The MS spectrum showed molecular ion at m/z 429 corresponding to molecular formula C26H23NO5. Chemical structure of 4b was based on spectral measurements. IR spectrum showed characteristic bands at 3442, 1734, 1663 and 1600 cm^{-1}for NH, CO, CONH and C=C groups respectively.

^{1}H-NMR spectrum showed singlet at δ 11.28 characteristic for hydroperoxide group and singlet at 8.3 for NH amidic proton. The MS spectrum showed molecular ion minus one at m/z 454 corresponding tomolecular formula minus one $^{C}28^{H}24^{NO}5$.

In view of the well known that the epoxides can be efficient DNA-alkylating agents (Abou-Elzahad, M.M., *et al.,* 1991.), little is known about such activity for cyclohexenone derivatives. To make such novel cyclohexenone epoxide available for genotoxic testing, it have been investigated the epoxidation of ethyl 3-(phenyl- & styryl-)-5-phenyl-2-cyclohexen- 1 -one-6-carboxylate (1 a,b) and N-(4-methoxyphenyl)-3 ,5-diphenyl-2-cyclohexen- 1 -one-6-carboxanilides (2a) using m-chloroperbenzoic acid (mcpba) to obtain the corresponding ethyl 3-(phenyl- & styryl-)-5-phenyl-2,3 -epoxycyclohexan- 1 -one-6-carboxylate and N-(4-methoxyphenyl)-3 ,5-diphenyl-2,3 -epoxycyclohexan- 1 -one-6-carboxamide (5a,b,c) respectively.



1 a, Ar =C_6H_5, R = OC_2H_5
b, Ar =$CH^{+}CH$-C_6H_5, R = OC_2H_5
2 a, Ar = C_6H_5, $NHC_6H_4OCH_3$-p

5 a, Ar =C_6H_5, R = OC_2H_5
b, Ar =$CH^{+}CH$-C_6H_5, R = OC_2H_5
c, Ar = C_6H_5, $NHC_6H_4OCH_3$-p

The chemical structure of 5a was established through the spectral measurements. IR spectrum showed characteristic bands at 1732, 1661, 1221 and 1133 cm^{-1} for carbonyl ester, carbonyl and C–O groups.^{1}HNMR spectrum showed triplet at δ 1.2 for CH3, quarter at 4.2 for CH2,singlet at 6.67 for proton at position 2 which was shifted from 6.30 of the start 66a, and ABX system (A: doublet doublet at 3.15 with the coupling constant of 16 and 5 Hz for proton at position H-5, B: doublet doublet at 3.66 with J = 12 and 5 Hz for proton at position H-4, X: doublet doublet at 3.86 with J = 16, 12 Hz for proton at position H-4). MS spectrum showed molecular ion at 336 for the molecular formula $_{C21H20O4.}$ The chemical structure of 5b was established through the spectral measurements. ^{1}H-NMR spectrum showed doublet doublet at δ 3.07 for proton at position 5 and complex pattern at 3.58 for methylene group, which was shifted from δ 3.82 of same protons at start (1b). MS spectrum showed molecular ion at m/z 362 corresponding to molecular formula C23H22O4. The chemical structure of epoxide 5c was elucidated through the spectral measurements. ^{1}H-NMR spectrum showed singlet

at δ 4.79 for proton at position 2, which was shifted from 6.59 of its starting material 2a, and showed ABX system at δ 3.1 as doublet doublet for proton A of position 4, doublet doublet at 3.57 for other proton B of position 4 and doublet doublet at 3.9 for proton X at position 5. MS spectrum showed molecular ion at m/z 413 characristic for molecular formula $C26^{H}23^{N}O4$.The probable mechanism[9]for production of epoxide derivatives (5 a-c) is believed to be through the formation of the oxirane intermediates *via* elimination m-chlorobenzoic acid. (Scheme 2).

Scheme 2:

DNA Damaging Properties of Photooxygenated Products 3a,b and4a:

It is known that some photochemical hydroperoxide compounds cause photochemical DNA damage (Epe, B., *et al.,* 1993; Elgency, E.M., 2000). Therefore, a sample of DNA was mixed in a solution of product (3a,b or 4a), which were irradiated using a sodium lamp at 0°C. The experimental data clearly indicates that compounds (3a,b) gave a positive results showing high degree of DNA damage, when the irradiation time was prolonged for 10 hours. Whereas, hydroperoxide 4a showed high degradation after 40 hours of irradiation time.

Table 1: Degree of DNA degradation by using hydroperoxides 3a,b and 4a

Lanes	Sample	Time (hour)	Degree of DNA degradation
0	Degradated DNA	---	high
1	3a + DNA	0	0
2	3a + DNA	5	medium
3	3a + DNA	10	high
4	3a + DNA	20	high
5	3a + DNA	30	high
6	3b + DNA	0	0
7	3b + DNA	10	high
8	3b + DNA	20	high
9	3b + DNA	30	high
10	3b + DNA	40	high
11	4a + DNA	0	0
12	4a + DNA	10	poor
13	4a + DNA	20	poor
14	4a + DNA	30	medium
15	4a + DNA	40	high

In Conclusion:

In the Present Study, Confirm Successfully the Following Lines:

- Presented the new hydroperoxide derivatives (3a,b and 4a,b), which were known as mean precursors for hydroxyl radical formations. These hydroperoxide showed strong DNA cleaving activities.
- Preparing the epoxide derivatives (5a-c), as potentially DNA-alkylating agents.

Hydroperoxides and epoxides are presented to biologists to get more biological investigations.

Experimental:

Ethyl 3 -(phenyl- & styryl-),5 -phenyl-2-cyclohexen- 1 -one-6-carboxylate (1a,b) were prepared according literature procedure[11]. Melting points (ºC) were measured on Fisher electric melting point apparatus. IR spectra were performed on a Perkin–Elmer 16 FPC FT–IR spectrophotometer.^{1}H-NMR spectra were obtained in CDCl3or DMSO solutions with a Brucker AVANCE D.P.X 400 MHz apparatus. MS were determined by Shimadzu QP1000 EX spectrometer. A sodium lamp (Phillips G/5812 SON) was used for photo-irradiation reactions. Thin layer chromatography (TLC) and proparative layer chromatography (PLC): Polygram SIL G/W 254, Mecherey–Nagel. The purity of the synthesized compounds was tested by thin layer chromatography and no by–products were noticed in most cases.

N-(4-Methoxyphenyl)-3,5-diphenyl-2-cyclohexen-1-one-6-carboxanilide (2a):

A mixture of ketoester 1a (3,2 g, 0.01 mol) and p-anizidine (1.5 g.,0.012 mol) was fused for one hour at 140 ºC in an oil bath. After cooling the solid material was recrystallized from ethanol to give carboxanilide **3** (3.56 g) as colorless crystals m.p. 200 ºC. 89 % yield. [TLC solvent: pet. ether: ethyl acetate, (8:2), Rf= 0.11].

- IR (KBr, disk), ν~~~, cm^{-1}: 3256 (NHCO), 3137, 3062 (aromatic CH), 2927, 2735 (aliphatic, CH), 1671 (CONH), 1660 (COCH=C), 1601 (C=C), 1030 (C–O).
- ^{1}H-NMR (CDCL3), δ, ppm: A2BX system [A2: 3.1 (dd, 1H, J = 16, 8 Hz, H-4), 3.40 (dd, 1H, J = 16, 6 Hz, H-4), B: 3.66 (d, 2H, J = 8 Hz, H-6),X: 4.2 (comp. pat., 1H, H-5)], 3.76 (s, 3H, OCH3), 6.59 (s, 1H, H-2), 6.8 1-7.60 (comp. pat., 14H, aromatic), 7.77 (s, 1H, CONH).
- MS, m/z: 397 (M^+, C26H23NO3) (85%), 275 (M+- C7H8NO) (8%), 248 (M+- C8H7NO2) (100%), 247 (M+- C8H8NO2) (86%), 220 (M+- C9H7NO4) (2%), 219 (M+- C9H8NO4) (6%), 122 (C7H8NO) (9%), 95 (C6H7O) (5%),77 (C6H5) (13%).

N-(4-Methoxyphenyl)-5-phenyl-3-styryl-2-cyclohexen-1-one-6-carboxanilide (2b):

A mixture of ketoester 1b (3.46 g, 0.01 mol) and p-anizidine (1.5 g,0.012 mol) was fused, to give carboxanilide 2b (3.8 g) as colorless crystals m.p. 199 ºC. 90 % yield. [TLC solvent: pet. ether : ethyl acetate, (8:2), Rf= 0.11].

- IR (KBr, disk), ν~~~, cm^{-1}: 3281 (NHCO), 3023 (aromatic, CH), 2928 (aliphatic CH), 1658 (carbonyl group), 1600 (C=C), 1031 (C–O).
- ^{1}H-NMR (CDCl3), δ, ppm: A2BX system [A2: 2.95 (dd, 1H, J = 16.7 Hz, H-4), 3.26 (dd, 1H, J = 16, 6 Hz, H-4), B: 3.68 (d, 1H, J = 7 Hz, H-6),X: 4.2 (comp. pat., 1H, H-5)], 3.79 (s, 3H, OCH3), 6.27 (s, 1H, H-2), AB system [A: 6.97 (d, 1H, J = 12 Hz, H-2'), B: 7.11 (d, 1H, J = 12 Hz, H-1'), 7.2-7.6 (comp. pat., 14H, aromatic), 7.83 (s, 1H, NHCO).
- MS, m/z: 423 (M^+, C28H25NO3) (30%), 300 (M+- C7H9NO) (15%), 274 (M+- C8H7NO2) (75%), 273 (M+- C8H8NO2) (45%), 195 (C14H11O) (20%), 183 (C13H11O) (80%), 123 (C7H9NO) (100%), 91 (C7H7) (25%), 77 (C6H5) (3 0%).

Ethyl 2-hydroperoxy-3,5-diphenyl-3-cyclohexen-1-one-6-carboxylate (3a):

A solution of 1a (0.32 g., 1 mmol) and tetraphenyl porphin (TPP) (2 mg) in chloroform (25 ml) was irradiated using sodium lamp at -20 ºC for 50 hours, during the irradiation time, a continuous stream of dry oxygen gas was allowed to pass through the reaction mixture at a very slow rate to avoid solvent evaporization and oxygen consumption. Then the solvent was evaporated at 20 ºC/15 torr to give a gummy material which was purified by proparative layer chromatography (PLC) on silica gel adsorbent. Elution of the PLC with the solvent mixture of pet.ether 60-80 ºC and ether (8:2) gave the hydroperoxy derivative (6) (0.14 g) as colorless crystals m.p. 60 ºC, 55 % yield. [TLC solvent pet. ether : ethyl acetate, (8:2), Rf= 0.27].IR (KBr disk), ν^-, cm^{-1}: 3473 (OH), 3035 (CH, aromatic), 2985 (CH, aliphatic), 1734 (COOEt), 1664 (CO), 1610 (C=C), 1135 (C–O), 1023 (C–C).

- ^{1}H-NMR (CDCl3), δ, ppm: 1.18 (t, 3H, CH3), 3.10 (dd, 1H, J = 18, 5 Hz, H-6), 3.64 (dd, 1H, J = 16, 5 Hz, H-4), 3.83 (dd, 1H, J = 18, 16 Hz, H-5), 4.15 (q, 2H, OCH2), 6.64 (s, 1H, H-2), 7.1-7.7 (comp. pat., 10H, aromatic), 11.4 (s, 1H, OOH).
- MS, m/z: 336 (M+- O) (1%), 318 (M+- H2O2) (6.5%), 303 (M+-CH5O2) (1%), 273 (C19H14O2) (4%), 244 (C18H13O) (6%), 218 (C17H13) (2%), 140 (C11H8) (1%), 91 (C7H7) (20%), 77 (C6H5) (19%), 63 (C5H3)(4%).

Ethyl 2-hydroperoxy-5-phenyl-3-styryl-3-cyclohexen-1-one-6-carboxylate (3b):

A solution of 1b (0.35 g, 1.0 mmol) and TPP (2 mg) in chloroform (25 ml) was irradiated to give colorless crystals m.p. 80 ºC of hydroperoxide derivative (3b) (0.2 g), 50 % yield. [TLC solvent: pet. ether : ethyl acetate, (8:2), Rf= 0.125].

- IR (Thin film), ν^-, cm^{-1}: 3446 (OOH), 2933 (CH, str.), 1730 (CO ester), 1662 (CO), 1600 (C=C), 1220 (C–O), 1134 (C–O).
- ^{1}H-NMR (CDCl3), δ, ppm: 1.07 (t, 3H, CH3), 3.05 (comp. pat., 1H, H-5), 3.77 (comp. pat., 1H, H-6), 4.23 (dd, 1H, H-4), 4.05 (q, 2H, CH2– CH3), 6.2 (s, 1H, H-2), 7.0 (d, 1H, H'-1'), 7.13 (d, 1H, H-2'), 7.2-7.8 (comp. pat., 10H, aromatic), 8.84 (s, 1H, OOH).
- MS. m/z: 378 (M^+, C23H22O5) (3.5%), 346 (M+- O2, C23H22O3) (6%), 273 (C20H17O) (7%), 245 $_{(C19H17)}$ (1%), 170 (C12H10O), (37%), 142 (C11H10) (30%), 103 (C8H7) (17%), 77 (C6H5) (13%), 76 (C6H4) (33%), 65 (C5H5) (8%).

N-(4-Methoxyphenyl)-2-hydroperoxy-3,5-diphenyl-3-cyclohexen-1-one-6-carboxanilide (4a):

A solution of 2a (0.32 g., 1.0 mmol) and TPP (2 mg) in chloroform (25 ml) was irradiated to give colorless crystals m.p. 160 ºC. of hydroperoxide derivative (4a) (0.09 g), 35 % yield. [TLC solvent pet. ether : ethyl acetate, (8:2), Rf= 0.29].

- IR (KBr disk), ν^{-}, cm^{-1}: 3386 (OOH), 3060 (CH, aromatic), 2941 (CH, aliphatic), 1660 (CO), 1520 (C=C), 1244 (C–O), 1128 (C–O).
- ^{1}H-NMR (CDCl3), δ, ppm: AMX system [A: 3.1 (dd, 1H, J = 20, 6 Hz, H-6), M: 3.57 (dd, 1H, J = 8, 6 Hz, H-4), X: 4.04 (dd, 1H, J = 8, 20 Hz, H-5)], 3.76 (s, 3H, OCH3), 4.81 (bs, 1H, OH), 6.8 (s, 1H, H-2), 7.0-7.7 (comp. pat., 14H, aromatic), 8.27 (s, 1H, NH), 12.2 (s, 1H, OOH).
- MS. m/z, 429 (M^{+}, C29H23NO5) (1%), 413 (M+- O) (16%), 395 (M+- H2O2) (11%), 382 (M+- CH3O2) (1%), 379 (M+- CH7O2) (1%), 306 (C19H16NO3) (1%), 273 (C19H13O2) (1%), 245 (C18H13O) (17%), 217 (C17H13) (7%), 140 (C11H8) (3%), 123 (C7H9NO) (100%), 91 (C7H7) (24%), 77 (C6H5) (23%), 65 (C5H5) (8%).

N-(4-Methoxyphenyl)-2-hydroperoxy-3-styryl-5-phenyl-3-cyclohexen-1-one-6-carboxanilide (4b):

A solution of 2b (0.35 g., 0.001 mol) and TPP (2 mg) in chloroform (25 ml) was irradiated to give (0.15 g) of the colourless crystals of 4b, m.p. 115 ºC, 40 % yield. [TLC solvent pet. ether : ethyl acetate, (8:2), Rf= 0.29].

- IR (Thin film), ν^{-}, cm^{-1}: 3442 (OOH), 3380 (NH), 3033 (CH, str.), 2960 (CH, str.), 1734, 1663 (CO), 1600 (C=C), 1223 (C–O).
- ^{1}H-NMR (CDCl3), δ, ppm: AMX system [A: 3.1 (dd, 1H, J = 17, 12 Hz, H-5), M: 3.6 (dd, 1H, J = 17, 5 Hz, H-6), 3.93 (dd, 1h, J = 12, 5 Hz, H - 4)], 3.7 (s, 3H, OCH3), 6.8 (s, 1H, H-2), 7.11 (d, 1H, J = 16 Hz, H-1'), 7.25 (d, 1H, J = 16 Hz, H-2'), 7.3-7.6 (comp. pat., 14H, aromatic), 8.28 (s, 1H, NHCO), 11.3 (s, 1H, OOH).
- MS, m/z: 454 (M+- 1, C28H24NO5) (4%), 439 (M+- O, C28H25NO4) (17%), 271 (C20H15O) (16%), 242 (C19H14) (4%), 165 (C13H9) (13%), 123 (C7H9NO) (100%), 103 (C8H7) (26%), 77 (C6H5) (42%), 76 (C6H4) (7%).

Ethyl 3,5-diphenyl-2,3-epoxy-cyclohexan-1 -one-6-carboxylate (5a):

A solution of m-chloroperbezoic acid (10 mmol, 80%) was added cautiously drop wise over 15 min. to a stirr solution of 1a (3.2 g., 1 mmol.) in chloroform (25 ml) at 0 ºC. The mixture was continue stirred in an atmospheric air at room temperature (TLC, peroxide test by KI, 10%), after which it was carefully washed with saturated aqueous solution of NaHCO3(3 × 10 ml), then with distilled water (3 × 10 ml). The organic layer was separated, dried over an hydrous Na2SO4and evaporated under reduced pressure at room temperature. The crude residue product was purified by PLC chromatography on silica gel adsorbent. Elution of the PLC with the solvent mixture of pet. Ether 60-80 ºC and ether (8:2) gave the epoxide derivative (5a)(1 .34 g) as semisolid materials, 40 % yield. [TLC solvent pet. ether : ethyl acetate, (8:2), Rf= 0.35].

- IR (Thin film), ν^{-}, cm^{-1}: br 3450 (enolic OH), 1732 (COOEt), 1661 (CO), 1600 (C=C), 1133 (C–O), 1020 (C–C).
- ^{1}H-NMR (CDCl3), δ, ppm: 1.21 (t, 3H, CH3), ABX system [A: 3.15 (dd, 1H, J = 16, 5 Hz, H-5), B: 3.66 (dd, 1H, J = 12, 5 Hz, H-4), X: 3.86 (dd, 1H, J = 16, 12 Hz, H-4)], 4.16 (comp. pat., 1H, H-6), 4.21 (q, 2H, CH2–O), 6.67 (s, 1H, H-2), 7.3-7.7 (comp. pat., 10H, aromatic).
- MS, m/z: 336 (M^{+}, $_{C21H20O4)}$ (1%), 320 (M+- O, $_{C21H20O3)}$ (1%), 291 (C19H15O3) (0.5%), 247 $_{(C18H15O)}$ (1%), 219 $_{(C17H15)}$ (1%), 142 (C11H10) (1%), 118 $_{(C9H10)}$ (100%), 104 (C8H8) (6%), 77 (C6H5) (11%), 76 (C6H4) (20%), 65 (C5H5) (5.5%).

Ethyl 5-phenyl-3-styryl-2,3-epoxy-cyclohexan-1-one-6-carboxylate(5b):

A solution of m-chloroperbenzoic acid (10 mmol, 80%) was added to a stirred solution of keto-ester 1b (3.46 g, 0.01 mol) to give (1.44 g) of a semisolid materials of 5b, 40 % yield. [TLC solvent pet. ether : ethyl acetate, (8:2), Rf= 0.272].

- IR (Thin film), ν^{-}, cm^{-1}: br 3459 (enolic OH), 3033 (CH, aromatic), 2928 (CH, aliphatic), 1734 (COOEt), 1661 (CO), 1619 (C=C), 1130 (C– O), 1023 (C–C).

- ^{1}H-NMR (CDCl3), δ, ppm: 1.22 (t, 3H, CH3), 3.07 (dd, 1H, J = 10, 16 Hz, H-5), 3.58 (comp. pat., 2H, H-4), 4.17 (comp. pat., 3H, OCH2, H-6), 6.30 (s, 1H, H-2), 7.01 (d, 1H, H-2'), 7.12 (d, 1H, H-1'), 7.2-7.6 (comp. pat., 10H, aromatic).
- MS, m/z: 362 (M^{+}, $_{C23H22O4)}$ (3%), 346 (M+- O) (4%), 332 (M+- CH2) (3%), 316 (M+- C2H6O) (5%), 302 (M+- C2H4O2) (3%), 273 (M+-C3H5O3) (6%), 245 $_{(C19H17)}$ (5%), 169 $_{(C13H13)}$ (25%), 142 (C11H10) (59%), 141 $_{(C11H9)}$ (100%), 91 (C7H7) (76%), 90 (C7H6) (24), 77 (C6H5) (62%), 65 (C5H5) (12%).

N-(4-Methoxyphenyl)-3,5-diphenyl-2,3-epoxy-cyclohexan-1-one-6-carboxanilide (5c):

A solution of m-chloroperbenzoic acid (10 mmol, 80%) was added to a stirred solution of carboxanilide 2a (0.32 g, 0.001 mol) to give (o.12 g) of a semisolid materials of 5c, 35 % yield. [TLC solvent pet. ether : ethyl acetate, (8:2), Rf= 0.26].

- IR (Thin film), ν^{-},cm^{-1}: 3020 (CH str.), 2960 (CH str.), 1662 (CO), 1602 (C=C), 1244 (C–O), 1130 (C–O), 1033 (C–C).
- ^{1}H-NMR (CDCl3), δ, ppm: ABX system [A: 3.10 (dd, 1H, J = 18, 5 Hz, H-4), B: 3.57 (dd, 1H, J = 12.5 Hz, H-4), X: 3.90 (dd, 1H, J = 18, 12 Hz, H-5)], 3.75 (s, 3H, OCH3), 4.30 (s, 1H, enolic OH), 4.79 (s, 1H, H-2), 6.79 (d, 2H, J = 9 Hz, H, aromatic 2',6'), 7.17 (d, 2H, J = 9 Hz, H aromatic 3',5'), 7.2-7.65 (comp. pat., 10H, aromatic), 8.25 (s, 1H, NHCO).
- MS, m/z: 413 (M^{+}, $_{C26H23NO4)}$ (11.6%), 288 $_{(C19H13NO2)}$ (2%), 247 (C18H15O) (10%), 219 $_{(C17H15)}$ (2%), 123 (C7H9NO) (100%), 116 (C9H8) (28%), 103 (C8H7) (35%), 77 (C6H5) (61%), 76 (C6H4) (9%).

Method of DNA damage by the hydroperoxides 3a,b and 4a:

To a solution of 3a,b or 4a in ethanol (1mg/5 ml) was added 1 ml. of DNA in saline solution. The reaction mixture was irradiated at 0°C, using a sodium lamp, for 50 hours. Samples were taken at different times to determine the damaging effects of these products on DNA using the gel electrophoresis technique (Kochevar, I.E. and Dunm, D.A., 1990). The photographs of the gel were taken under U.V. light (365nm).

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