Lebda Medical Journal
Vol. 4, Jun. 2018, p.154-167

Klefah A.K. Musa
Received 13 Mar. 2018; accepted in revised form 25 May. 2018

Mechanism of photoinduced photodegradation of piroxicam, the way to design new drugs of piroxicam derivatives

Klefah A.K. Musa[1,*], Salama Omar[2] and L. A. Eriksson[3]

[1]Department of Medicinal Chemistry, Pharmacy College, Al-khoms, Libya.
[2]Department of Applied Physical Chemistry, Chemical Engineering Section, Faculty of Sciences, Autonoma University-Madrid, 28 049, Spain
[3]Department of Chemistry and Molecular Biology, Göteborg University, SE 405 30 Göteborg, Göteborg, Sweden.

Abstract

Initiation of photodegradation mechanism and subsequently pathways of non steroidal anti inflammatory drugs (NSAID), piroxicam are studied by means of computational quantum chemistry at the DFT-B3LYP/6-311++G(d, p) level. The results show that the drug readily absorbs radiation from the UV region of the spectrum. The proposed initiation process of piroxicam photodegradation, in which the reaction between molecular oxygen and piroxicam in presence of light occurs, required passing an energy barrier computed to be ~21 kcal/mol in solvent. The formed intermediate species from the former reaction undergoes spontaneously intramolecular rearrangements (mainly dioxetane ring opening) once it is optimized in its triplet state. The net of these rearrangements is carboxylic acid compound which also has a capability to absorb radiation from UV region and forms various photoproducts depending upon the site of cleavage. Moreover, energetic of these species, the computed UV spectra by time-dependent density functional theory (TD-DFT) and possible reactive singlet oxygen species formation in different pathways are explained in detail in this manuscript.

Key words: phototoxicity, photodegradation, NSAID, piroxicam, DFT.

Corresponding author: k_20102002@yahoo.com

Introduction

Photostability of drug molecules and drug formulations is of great of interest in research because of the need to provide important information on handling, packaging, labeling, therapeutic, and adverse aspects of the drug and its administration. Loss of drug potency and development of adverse effects may arise due to drug photodecomposition. The photodegraded drug products are initially mostly in form of radicals, oxygenated compounds, or compounds that have the capability to produce reactive oxygen species such as singlet oxygen and superoxide radical anions. Most (if not all) of these species may react with vital biomolecules in the cell such as proteins, lipids and DNA, leading to photoallergy, lipid peroxidation and photogenotoxicity, respectively [1].

The non-steroidal anti-inflammatory drug (NSAID) Piroxicam (4-hydroxy-2-methyl-2H-1,2-benzothiazine-1-(N-(2-pyridyl)carboxamide)-1,1-doxide; PX, Figure 1), chemically belongs to the 'oxicam class' of compounds which contain a N-heterocyclic benzothiazine carboxamide group. The molecule has fast tautomeric equilibria and hence exists in various isomeric forms. In recent studies, the PX enol conformer was indicated to have the largest potency towards involvement in photo-toxicity mechanisms [2].

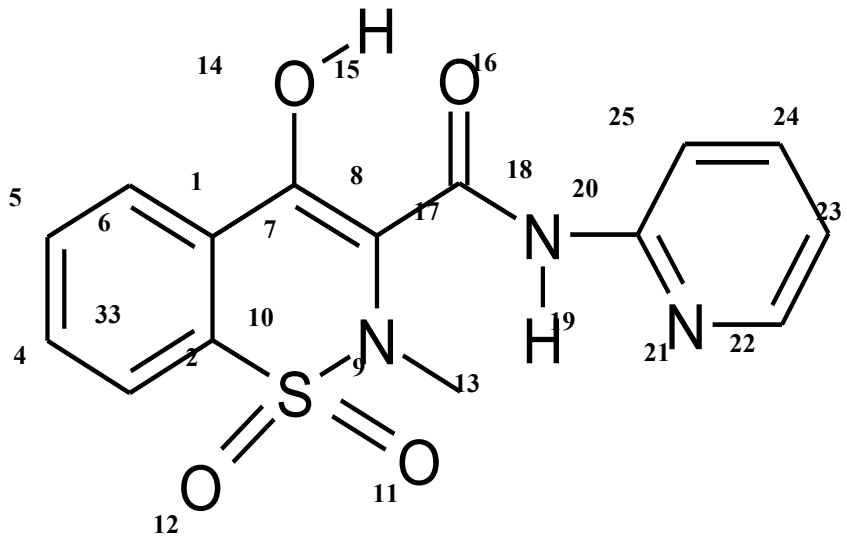

Figure 1. Piroxicam. Atomic numbering used throughout this study.

PX is a well-known drug used in the treatment of rheumatoid arthritis, osteoarthritis and other anti-inflammatory diseases, as well as in relation to fractures, post-operative, and acute pains in the muscular skeletal system [3, 4]. PX acts on the cyclooxygenase enzyme (COX) as a non-selective inhibitor giving analgesic and antipyretic properties. PX is quickly absorbed and its anti-inflammatory activity surpasses that of naproxen, indomethacine and phenylbutazone [2]. The drug is readily absorbed after oral or rectal administration given in doses of 20 mg daily, and displays long plasma half-time (35-60 h) [5].

Corresponding author: k_20102002@yahoo.com

UV-VIS absorption spectra of PX were previously recorded experimentally in ethanol solution. Four maximum absorption bands were observed, at 360, 291, 256 and 205 nm, respectively. The maximum band at 360 nm was also noted in aqueous solution [2,6,7].

Similar to the 'profen' (2-arylpropoinic acid derivatives) subgroup of non-steroidal anti-inflammatory drugs [e.g., 8-11], PX is well-known to display phototoxic side effects which requires either reduced use or use with precautions, during the period of treatment. PX photodegradation plays an important role and renders several photoproducts. Previous studies have shown that the drugs phototoxicity is due to a metabolite of piroxicam [1-3, 12]. Our aim herein is to study the initiation of the PX photodegradation mechanism, which is proposed to occur due to incorporation of oxygen thereby forming a PX-dioxetane intermediate. Upon excitation, the dioxetane ring is degraded to give a carboxylic acid species. Subsequent conversion of the resultant carboxylic acid species will then take place to yield several photoproducts. The proposed initiation of the PX photodegradation mechanism is shown in Figure 2.

In the current work, we investigate the most important step in the PX photodegradation mechanism. This is an initiating step, in which the reaction between a PX molecule and molecular oxygen takes place in presence of light, forming a dioxetane intermediate. The formed intermediate (CP1) is unstable and leads upon excitation to formation of the main carboxylic acid derivative (CP2) by way of dioxetane ring opening. Depending on site of subsequent cleavage of CP2, different compounds are formed, as shown in Figure 2. Information provided herein can assist in improving the level of knowledge about the main critical step in PX-photodegradation and assist towards the development of new derivatives with less or no phototoxic side effect

Corresponding author: k_20102002@yahoo.com

Lebda Medical Journal
Vol. 4, Jun. 2018, p.154-167

Klefah A.K. Musa
Received 13 Mar. 2018; accepted in revised form 25 May. 2018

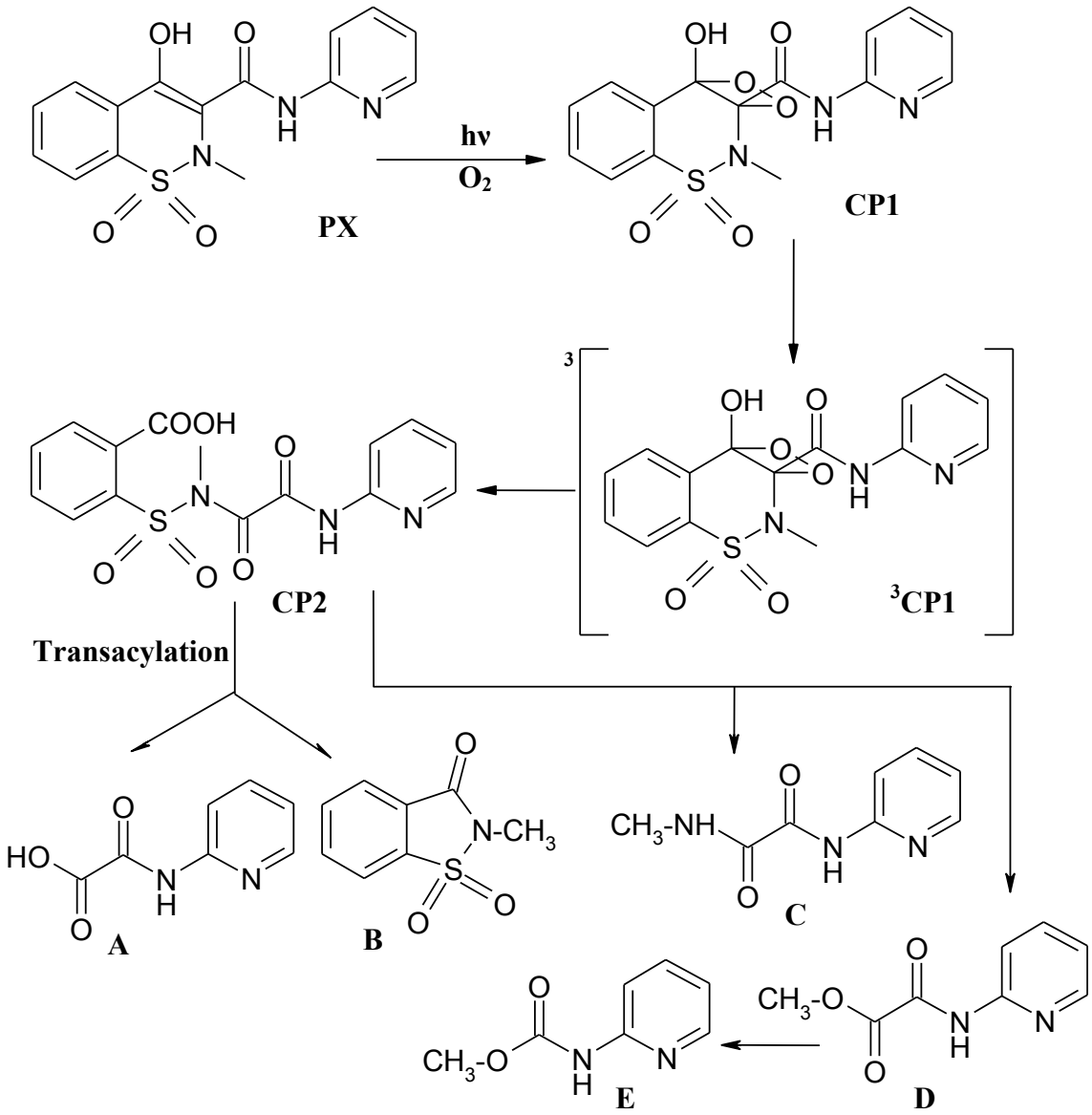

Figure 2. Proposed PX photodegradation mechanism [ref. 1, 12].

Methodology

The geometries of PX and its main photoproducts shown in Figure 2 were optimized in both their singlet and triplet states at the hybrid Hartree-Fock – Density Functional Theory level B3LYP [13-15] with the 6-311++G(d,p) basis set. The radical anion and cation of PX were also optimized at the same level of theory. Solvent effects were taken into consideration implicitly, through single point calculations on the optimized geometries at the same level of theory, including the integral equation formulation of the polarized continuum model (IEFPCM) [16-18]. Water was used as solvent, through the value 78.39

Corresponding author: k_20102002@yahoo.com

for the dielectric constant in the IEFPCM calculations. Frequency calculations were performed on the optimized geometries at the same level of theory, to ensure the systems to be local minima (no imaginary vibration frequencies) and to extract zero-point vibrational energies (ZPE) and thermal corrections to the Gibbs free energies at 298 K. Excitation spectra of PX and CP2 molecules were calculated in gas and solvent phase using the time-dependent formalism (TD-DFT [19-21]), at the same level of theory. The addition of an oxygen molecule to PX forming dioxetane in CP1 was investigated by scanning the O-C_7 and O-C_8 bonds from their optimized bond lengths in steps of 0.1 Å, until the bonds had completely dissociated (see results and discussion). The numbering scheme of the atoms used throughout the study is given in Figure 1. The calculations were performed with the Gaussian 03 programme package [22].

Results and discussion

As mentioned in the introduction, the key step in the PX photodegradation pathways is the incorporation of oxygen, forming the unstable dioxetane intermediate (CP1) which upon excitation produces the carboxylic acid derivative CP2. The latter compound may form various photoproducts depending on the site of subsequent cleavage. For instance, under the effect of transacylation compounds A and B will form. Literature shows that the photoproducts formed depends on the oxygen content of different solutions which, in turn, depends upon the nature of the solvent used, the temperature, and a variety of other experimental conditions. Moreover, previous studies have shown that the quantum yield and lifetime of singlet oxygen formation (which is low in water [23]) is influenced by changes in the reaction parameters. This explains why there is lack of agreement between the different reports on PX photostability [12, 23-25]. Interestingly, if irradiation of the drug is preformed under inert atmosphere using dry argon, no photodegradation is observed even after 60 h irradiation [12].

PX redox-chemistry: In order to shed more light on the initiation of PX photodegradation pathways, we start by investigating the redox-chemistry of PX. In Table 1, we list the absolute and relative ZPE-corrected energies in gas phase and in bulk solution, along with dipole moments obtained in aqueous solution. The computed electron affinity (EA) of PX in gas phase is 36.1 kcal/mol. However, under the influence of bulk solvation through the IEFPCM method, the EA increases to 71.9 kcal/mol, due to solvent stabilization of the charged species. The solvent stabilization of about 35.8 kcal/mol is similar to our previous results for the NSAID ibuprofen for which solvent stabilization of the reduced species was 40.6 kcal/mol [9]. In vacuo, the adiabatic ionization potential (IP) is 177.2 kcal/mol whereas aqueous solution stabilizes the cation by 36.1 kcal/mol, thereby reducing the Gibbs free energy to 141 kcal/mol. This is similar to the data found in our previous studies on, *e.g.,* ketoprofen, ibuprofen, naproxen, and flurbiprofen [8-11].

Corresponding author: k_20102002@yahoo.com

Table 1. B3LYP/6-311++G(d,p) ZPE-corrected Electronic Energies in Gas Phase, and IEFPCM- B3LYP/6-311++G(d,p) Gibbs Free Energies in aqueous solution. Absolute energies in a.u., relative energies in kcal/mol

System	$E_{(ZPE)}$	$\Delta E_{(ZPE)}$	ΔG_{aq}^{298}	$\Delta\Delta G_{aq}^{298}$
^{1}**PX**	-1442.760213	0	-1442.830047	0
PX $^{\cdot-}$	-1442.817749	-36.10	-1442.944632	-71.90
PX $^{\cdot+}$	-1442.477817	177.21	-1442.605183	141.10
^{3}**PX**	-1442.68177	49.22	-1442.753287	48.17
^{1}**CP1**	-1593.106162	0	-1593.180542	0
^{3}**CP1**	-1592.854893	157.67	-1592.936575	153.09
^{1}**CP2**	-1593.251836	0	-1593.340947	0
^{3}**CP2**	-1593.148751	64.68	-1593.248765	57.85

PX Excitation: In order to get more insight on the absorption region and features of the excitations, the UV spectra were computed and are displayed in Figure 3. The spectra show that PX has capability to absorb irradiation in the UV region. There is a strong similarity between the two spectra computed in gas phase and aqueous solvent, as seen in Figure 3A. The main absorption peak in gas phase is found at λ = 339 nm with an oscillator strength *f* = 0.501. Other absorption peaks are found at shorter wavelengths (higher energies) at λ = 261, 248, 205 and 203 nm with oscillator strengths *f* = 0.108, 0.132, 0.123 and 0.166, respectively. In the computed spectrum in solvent phase, the main peak is blue-shifted by a few nanometers to λ = 342 nm, and has higher oscillator strength higher than that in the gas phase spectrum; *f* = 0.640. The absorption peaks found at shorter wavelengths (higher energies) now occur at λ = 261, 249, 202 nm with oscillator strengths *f* = 0.175, 0.108 and 0.233, respectively.

The spectra of PX investigated experimentally in various studies have shown that the maximum absorption (and emission) wavelengths strongly depend on the hydrogen bonding ability of solvent. In addition, the presence of intra-molecular hydrogen bonding between the ortho carbonyl (C_{17}-O_{16}) group of the parent benzothiazine ring and the hydroxyl (O_{14}-H_{15}) group plays an important role in the solvent-dependence of the properties [26]. This is manifested through the differences between the spectra obtained in various solvents used. In early studies, the main absorption peaks of PX were noted at 320, 290, and 250 nm [26]. It was noted that the first band at 320 nm was significantly red-shifted with increased polarity and hydrogen bonding ability of the solvent. This indicates that the first band at 320 nm originates from a strong $S_0 \rightarrow {}^1(\pi, \pi^*)$ transition. In addition, in nonpolar solvents, the $S_0 \rightarrow {}^1(n,\pi^*)$ absorption of the carbonyl of heterocyclic or aromatic compounds commonly noted in the 340-370 nm range is blue-shifted, presumably due to intra-molecular hydrogen bonding and partially hidden by the stronger $S_0 \rightarrow {}^1(\pi,\pi^*)$ absorption such as in case of o-hydroxyl benzaldehyde [27]. This explains why the long and weak tail of the first absorption bands (presumed to be $S_0 \rightarrow {}^1(n, \pi^*)$) found at around 370-380 nm in nonpolar solvents, disappears in the highly polar solvents such as water. In the latter case, the low lying $^1(n,\pi^*)$ state is further blue-shifted by

Corresponding author: k_20102002@yahoo.com

intra-molecular hydrogen binding whereas the $^{1}(\pi,\pi^{*})$ state is red-shifted under the effect of high solvent polarity. Hence, the latter becomes the lowest excited state in water. For more details about experimental findings see ref. [26, 28]. This is in good agreement with the computed UV-spectra in solvent phase, taking into consideration that the methodology currently used is well-known to give excitation energies that are overestimated by 3-5 kcal/mol (~0.2 eV). This means that the computed absorption peaks will be blue-shifted relative to experiment, by approximately 10 nm at λ = 250 nm and by 15 nm at λ = 300 nm. We also note the blue-shift in the lowest-lying absorption, when moving from the polar vacuum conditions to the aqueous environment, in agreement with experiment.

Corresponding author: k_20102002@yahoo.com

Lebda Medical Journal
Vol. 4, Jun. 2018, p.154-167

Klefah A.K. Musa
Received 13 Mar. 2018; accepted in revised form 25 May. 2018

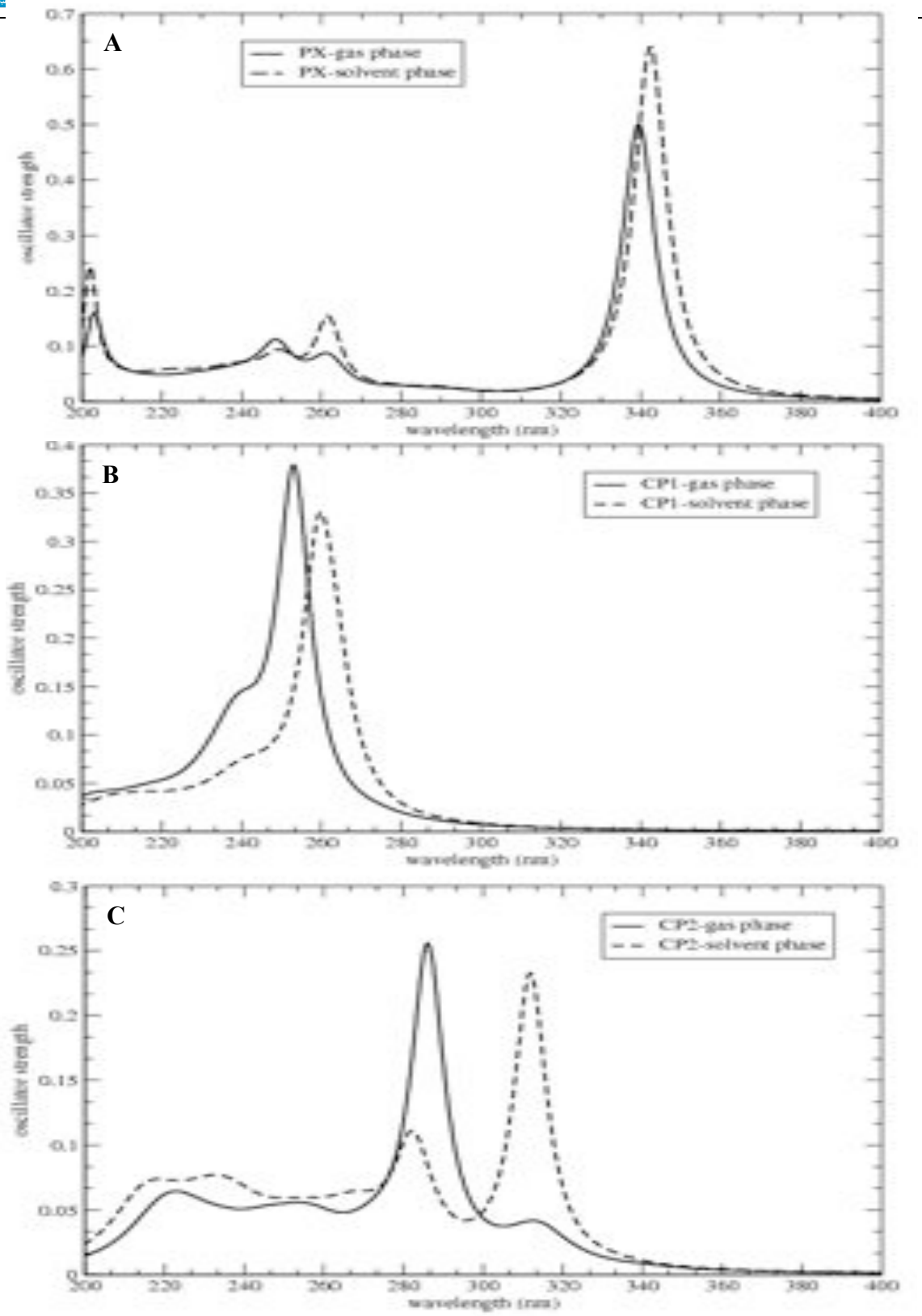

Figure 3. TD-B3LYP/6-311++G(d,p) computed spectra of PX, CP1 and CP2 molecules

Corresponding author: k_20102002@yahoo.com

in vacuum and aqueous solvent.

Overall the PX UV-spectra computed herein or obtained from experimental work show that the drug has the capability to absorb radiation in the UV region of the spectrum. PX becomes excited to a singlet state and is by intersystem crossing (ISC) transferred to the first excited triplet state. The triplet state of PX is computed to lie 49.2 kcal/mol above the ground singlet state in the gas phase, whereas under the effect of bulk solvation the value is 48.2 kcal/mol. Given that the energy required to form singlet oxygen from ground state triplet molecular oxygen is estimated to be ~23 kcal/mol [29], singlet oxygen formation is expected to occur once PX is in its triplet state. However, as noted in the introduction, experimental studies show that the quantum yield of the reactive oxygen species strongly depends on the solvent properties.

The formation of the proposed intermediate species CP1 through the addition of molecular oxygen to PX was investigated by scanning the C_7-O and C_8-O bonds 'backwards' in stepwise increments of 0.1 Å from the optimized bond lengths (1.48 Å) of the product, to a point where the bonds will be completely dissociated; ~3.08 Å. The scan was performed in gas phase and single point calculations were then performed for each point using the PCM method. The scan is hence performed on the singlet PX + singlet O_2 surface. The results in both gas and solvent phase are displayed in Figure 4. The energy of the reactants where molecular oxygen and PX are completely separated, is considered as 'zero'. The energy barrier of the transitions state is computed to be 28.1 kcal/mol in the gas phase with a transition distance equal to 2.08 Å, and 21.0 kcal/mol in solvent phase, with a similar transition state distance. The energy of the optimized dioxetane product is -29.6 and -34.0 kcal/mol in gas and solvent phase, respectively. It is essential to overcome on the transition barrier (~21 kcal/mol in solvent phase) in order to initiate the photodegradation of PX, as the formation of the CP1 intermediate is a critical step in order to form photoproduct CP2 which then degrades and forms the other photoproducts shown in Figure 2.

Corresponding author: k_20102002@yahoo.com

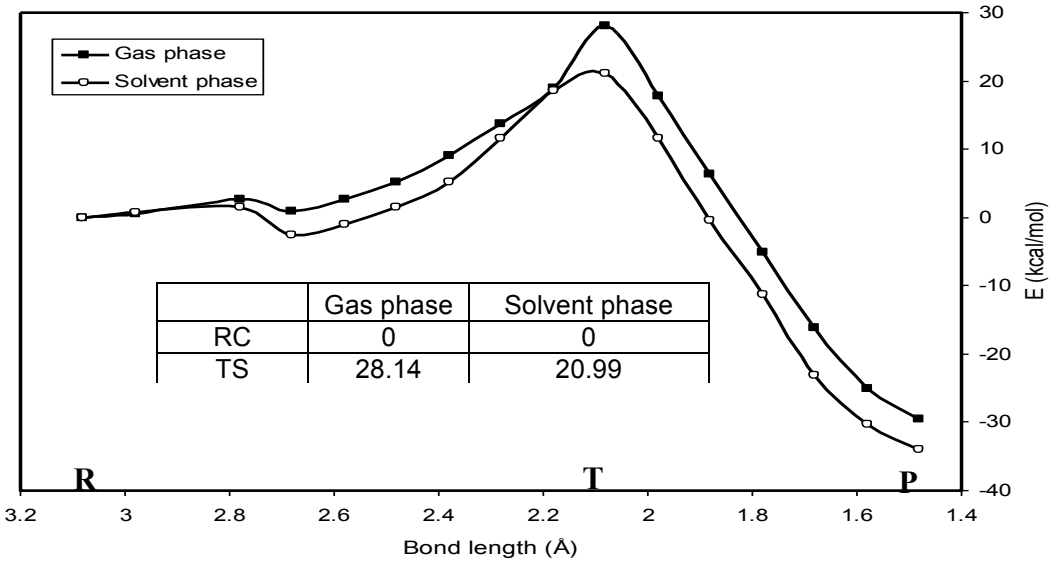

Figure 4. Potential energy surface for the reaction between singlet molecular oxygen and PX in vacuo and in aqueous phase.

Once the unstable CP1 dioxetane species is formed, this is assumed to absorb a foton and convert to the triplet state via intersystem crossing. The computed spectrum of CP1 is shown in Figure 3B, implies that the capability of this species to absorb radiation from UV region of spectrum and could be excited. The main absorption peak of CP1 in the gas phase is noted at 253 nm with oscillator strength equals 0.379, with shoulder at λ = 239 nm with f = 0.138. In the solvent phase, the main absorption peak is shifted by few nanometers and found at λ = 259 nm with oscillator strength equals 0.330, with similar shoulder at the same wavelength but with low probability (0.097). The optimized dioxetane triplet undergoes spontaneous intra-molecular rearrangement in which the dioxetane ring breaks through dissociation of the oxyxen-oxygen bond followed by another bond C_7-C_7 dissociation of the same ring. This leads to formation of the carboxylic acid species CP2. The UV spectra of CP2 are shown in Figure 3C. It also has capability to absorb radiation in the UV region of the spectrum. The main absorption peak is in gas phase found at 285 nm, with oscillator strength f = 0.256. Weaker absorptions are found at λ = 313, 244 and 220 nm with low probabilities as seen in Figure 3C. In solvent phase, the main absorption peak is computed to be at λ = 311 nm with oscillator strength f = 0.233, whereas the weaker absorptions are found at λ = 282, 216 and 213 nm with oscillator strengths f = 0.133, 0.089 and 0.096. Comparing the two spectra (in gas and solvent phases) we note the same absorption pattern but with a shift in probabilities of the two lowest-lying peaks. Comparison of the two spectra of PX with the dioxetane CP1 and the photoproduct CP2 shows that the absorption peaks of CP1 and CP2 are shifted to shorter wavelengths (higher energy), although also with reduced probabilities. The main absorption peaks wavelengths of CP2 (in gas and solvent phases) found in the middle between the main absorption peaks wavelengths of PX itself and CP1 species, with lowest

Corresponding author: k_20102002@yahoo.com

probabilities among these studied species, as seen in Figure 3.

Various photoproducts are produced from the photodegradation of CP2 because of i) CP2 has capability to absorb irradiation in UV region, and excites to single excited state and by ISC to its first excited triplet state. The triplet state of this species is located about 64.68 kcal/mol once it is optimized in vacuo. Whereas, including bulk solvation effect the value is 57.85 kcal/mol. Due to this species has several polar groups capable to be more stabilized by applying bulk solvation. In this connection, the triplet energy level of CP2 species (if it doesn't undergo to fast photodegradation process) also can give its energy to molecular oxygen forming singlet oxygen species since the energy required for forming singlet oxygen from molecular oxygen is only ~23 kcal/mol [29]. ii) CP2 has a different possible cleavage sites inherited in its molecular structure hence, several photoproducts are identified. Such as what is formed in oxygenated media N-(2-pyridyl) oxamic acid (A) and N-methylsaccharin (B) by transacylation. The species D is also formed through transacylation and nucleophilic attack by the oxygen lone pair of electrons of methanol. Upon subsequently decarbonylation of species D lead to formation of E molecule. The latter species is identified as N-(2-pyridyl)-methoxy-amide. The formation of C molecule (N-methyl-N´-(2-pyridyl) ethane-diamide) is postulated to occur as a result of cleavage of the sulphur-nitrogen bond in the carboxylic acid [1,12].

Conclusion

The initiation processes of non steroidal anti-inflammatory drug (NSAID), piroxicam photodegradation is studied herein by means of a computational quantum chemical methodology in the DFT framework. Piroxicam in previous studies show that its capability to cause phototoxicity disorders. The UV spectra computed by TD-DFT calculations show that this drug has capability to absorb radiation in the UV region of the spectrum. The two spectra obtained in vacuo and solvent phases have a similar absorption pattern, however, with different oscillator strengths. In solvent phase, the main peak with f = 0.64 found at 342 nm and other peaks found at lower wavelengths (high energy), this is in line with experimental findings. The spectra of this drug strongly depends upon the polarity of solvent used since this drug inherits in its molecular structure groups able to form intra-molecular hydrogen bonds which may lead to proton transfer once it is excited.

The PX photodegradation is proposed to be initiated by the reaction between the drug molecule and molecular oxygen. Using scan process, the barrier of this reaction is estimated to be ~28 kcal/mol in vacuo phase, instead it is reduced to ~21 kcal/mol by applying bulk solvation effect. The formed unstable molecule CP1 is spontaneously undergone to intra-molecular rearrangements in which the CP1 dioxetane ring dissociation/opening occurred once this species optimized in triplet state. The net of this process is formation of CP2 compound which also has a capability to absorb radiation from UV region of spectrum. Depending upon the site of cleavage several molecules are formed. For instance, CP2 degradation leads to formation of compounds B and D via

Corresponding author: k_20102002@yahoo.com

transacylation and nucleophilic attack. Energetically, the triplet state of CP2 molecule is located 65 kcal/mol above its ground singlet state. This species may transfer its energy to molecular oxygen forming a reactive singlet oxygen species since the energy required for the formation of latter species from molecular oxygen is only ~22.5 kcal/mol. These findings in general provide outline for initiation of PX photodegradation and subsequently pathways, and provides valuable insights into key factors required to be taken in consideration while designing new drugs or improving the old with similar high biological activities yet reduced photochemical adverse effects.

Acknowledgements

Department of Medicinal chemistry, Pharmacy College, Alkhoms, Libya (KAKM), the Swedish Science Research Council and Department of Chemistry and Molecular Biology, Göteborg University, Göteborg, Sweden (LAE) and Faculty of Science at Auotonoma University-Madrid (SO) are gratefully acknowledged for financial support.

References

1. Glass, B. D.; Brown, M. E.; Daya, S.; Worthington, M. S.; Drummond, P.; Antunes, E.; Lebete, M.; Anoopkumar-Dukie, S.; Maharaj, D., Influence of cyclodextrins on the photostability of selected drug molecules in solution and the solid-state. *International Journal of Photoenergy* **2001,** 3, (4), 205-211.
2. de Souza, K. F.; Martins, J. A.; Pessine, F. B. T.; Custodio, R., A theoretical and spectroscopic study of conformational structures of piroxicam. *Spectrochimica Acta Part a-Molecular and Biomolecular Spectroscopy* **2010,** 75, (2), 901-907.
3. Pal, S.; Bindu, P.; DubeyB, P. K.; Chakraborty, S.; Mukherjee, A. K., Synthesis and structure analysis of cyclodehydration product of piroxicam: A metabolite detected in dogs and monkeys. *European Journal of Medicinal Chemistry* **2009,** 44, (8), 3368-3371.
4. Schiantarelli, P.; Acerbi, D.; Bovis, G., Some pharmacokinetic properties and bioavailability by oral and rectal route of piroxicam in rodents and in man. *Arzneimittel-Forschung/Drug Research* **1981,** 31-1, (1), 92-97.
5. Michotte, Y.; Vanklaveren, H. P.; Detaevernier, M. R.; Gusdorf, C. F.; Vanhaelst, L., Bioequivalence of 2 formulations of piroxicam. *Arzneimittel-Forschung/Drug Research* **1991,** 41-1, (3), 244-246.
6. Gehad, G. M.; Nadia, E. A. E., Preparation and spectroscopic characterisation of metal complexes of piroxicam. *Vibrational Spectroscopy* **2004,** 36, (1), 97-104.
7. Banerjee, R.; Sarkar, M., Spectroscopic studies of micro environment dictated structural forms of piroxicam and meloxicam. *Journal of Luminescence* **2002,** 99, (3), 255-263.
8. Musa, K. A. K.; Matxain, J. M.; Eriksson, L. A., Mechanism of photoinduced decomposition of ketoprofen. *Journal of Medicinal Chemistry* **2007,** 50, (8), 1735-1743.
9. Musa, K. A. K.; Eriksson, L. A., Theoretical study of ibuprofen phototoxicity. *Journal of Physical Chemistry B* **2007,** 111, (46), 13345-13352.
10. Musa, K. A. K.; Eriksson, L. A., Theoretical Study of the Phototoxicity of Naproxen and the Active Form of Nabumetone. *Journal of Physical*

Corresponding author: k_20102002@yahoo.com

Chemistry A **2008,** 112, (43), 10921-10930.
11. Musa, K. A. K.; Eriksson, L. A., Photochemical and photophysical properties, and photodegradation mechanism, of the non-steroid anti-inflammatory drug Flurbiprofen. *Journal of Photochemistry and Photobiology a-Chemistry* **2009,** 202, (1), 48-56.
12. Miranda, M. A.; Vargas, F.; Serrano, G., Photodegradation of piroxicam under aerobic conditions - the photochemical keys of the piroxicam enigma. *Journal of Photochemistry and Photobiology B-Biology* **1991,** 8, (2), 199-202.
13. Becke, A. D., Density-functional thermochemistry. 3. The role of exact exchange. *Journal of Chemical Physics* **1993,** 98, (7), 5648-5652.
14. Stephens, P. J.; Devlin, F. J.; Chabalowski, C. F.; Frisch, M. J., Ab-initio calculation of vibrational absorption and circular-dichroism spectra using density-functional force-fields. *Journal of Physical Chemistry* **1994,** 98, (45), 11623-11627.
15. Lee, C. T.; Yang, W. T.; Parr, R. G., Development of the colle-salvetti correlation-energy formula into a functional of the electron-density. *Physical Review B* **1988,** 37, (2), 785-789.
16. Cances, E.; Mennucci, B.; Tomasi, J., A new integral equation formalism for the polarizable continuum model: Theoretical background and applications to isotropic and anisotropic dielectrics. *Journal of Chemical Physics* **1997,** 107, (8), 3032-3041.
17. Mennucci, B.; Tomasi, J., Continuum solvation models: A new approach to the problem of solute's charge distribution and cavity boundaries. *Journal of Chemical Physics* **1997,** 106, (12), 5151-5158.
18. Cossi, M.; Scalmani, G.; Rega, N.; Barone, V., New developments in the polarizable continuum model for quantum mechanical and classical calculations on molecules in solution. *Journal of Chemical Physics* **2002,** 117, (1), 43-54.
19. Casida, M. E., *Time-dependent density functional response theory for molecules. In Recent advances in density functional methods, Part 1*. Ed: Chong, D.P. ed.; World Scientific: Singapore, 1995; p 155-192.
20. Stratmann, R. E.; Scuseria, G. E.; Frisch, M. J., An efficient implementation of time-dependent density-functional theory for the calculation of excitation energies of large molecules. *Journal of Chemical Physics* **1998,** 109, (19), 8218-8224.
21. Casida, M. E.; Jamorski, C.; Casida, K. C.; Salahub, D. R., Molecular excitation energies to high-lying bound states from time-dependent density-functional response theory: Characterization and correction of the time-dependent local density approximation ionization threshold. *Journal of Chemical Physics* **1998,** 108, (11), 4439-4449.
22. Gaussian 03, R. B., Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.;

Corresponding author: k_20102002@yahoo.com

Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. Gaussian, Inc., Wallingford CT, **2004**.

23. Western, A.; Vancamp, J. R.; Bensasson, R.; Land, E. J.; Kochevar, I. E., Involvement of singlet oxygen in the phototoxicity mechanism for a metabolite of piroxicam. *Photochemistry and Photobiology* **1987,** 46, (4), 469-475.

24. Becker, R. S.; Chakravorti, S.; Yoon, M. J., Photochemical and Photophysical properties of piroxicam and benoxaprofen in various solvents. *Photochemistry and Photobiology* **1990,** 51, (2), 151-154.

25. Figueiredo, A.; Ribeiro, C. A. F.; Goncalo, S.; Caldeira, M. M.; Poiaresbaptista, A.; Teixeira, F., Piroxicam-induced photosensitivity. *Contact Dermatitis* **1987,** 17, (2), 73-79.

26. Yoon, M. J.; Choi, H. N.; Kwon, H. W.; Park, K. H., Solvent dependence of absorption and fluorescence-spectra of piroxicam – a possible intramolecular proton-transfer in the excited-state. *Bulletin of the Korean Chemical Society* **1988,** 9, (3), 171-175.

27. Nagaoka, S.; Hirota, N.; Sumitani, M.; Yoshihara, K., Investigation of the dynamic processed of the excited-states of ortho-hydroxybenzaldehyde and ortho-hydroxyacetophenone by emission and picosecond spectroscopy. *Journal of the American Chemical Society* **1983,** 105, (13), 4220-4226.

28. Andrade, S. M.; Costa, S. M. B., Hydrogen bonding effects in the photophysics of a drug, Piroxicam, in homogeneous media and dioxane-water mixtures. *Physical Chemistry Chemical Physics* **1999,** 1, (18), 4213-4218.

29. Lissi, E. A.; Encinas, M. V.; Lemp, E.; Rubio, M. A., Singlet oxygen $O_2(\Delta g)$ bimolecular processes – solvent and comparatmentalization effects. *Chemical Reviews* **1993,** 93, (2), 699-723.

Corresponding author: k_20102002@yahoo.com