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A DFT study for paracetamol and 3,5-disubstituted analogues

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Abstract

Quantum chemistry calculations at the B3LYP theory level, together with the 6-31G* basis set were employed to obtain energy (*E*), ionization potential (IP), bond dissociation energies (BDE), and spin-density distribution for paracetamol (PAR) and 3,5-disubstituted analogues of PAR. Calculations of spin densities were performed for radical formed by hydrogen abstraction from the phenolic hydroxyl group. The unpaired electron remains is localized on the O_7 phenolic oxygen (31–40%), C_3 and C_5 carbon atoms at the *ortho* (17–27 and 21–27%) and C_1 carbon atom at the *para* (25–33%) positions. The correlation between analgesic activity, cytotoxicity, and electronic properties was obtained by using correlation matrix. The IP, and BDE_{O-H} are significant related with the in vitro inhibition of cyclooxygenase, while BDE_{O-H}, BDE_{N-H} and IP are significant related with the cytotoxicity (LDH).

Keywords: Paracetamol analogues; Analgesic activity; Cytotoxicity; DFT; Spin density

1. Introduction

Acetaminophen or paracetamol **1** (PAR) is used extensively as an analgesic and antipyretic and appears to be safe if used in normal therapeutic doses. However, large doses of PAR produce hepatic and/or renal injury in humans and in experimental animals [1,2]. The analgesic activity is due to the anticyclooxygenase properties of PAR. The cyclooxygenase (COX)-inhibiting activity of PAR was suggested to be related to its capacity quench the tyrosyl radical present in prostaglandin endoperoxide synthase (PGES) [3]. In contrast, the peroxidase enzymes probably exhibit only one-electron oxidation activity toward PAR under physiological conditions [4]. Moreover, the hepatotoxicity of PAR is generally accepted to be primed by the formation of *N*-acetyl-*p*-benzoquinone imine (NAPQI), a metabolite formed during cytochrome P450 catalyzed oxidation of PAR [5], although *N*-acetyl-*p*-benzosemiquinone imine (NAPSQI) has also been proposed [6].

Taking the presumed molecular mechanism of analgesic activity as well as that the hepatotoxicity of PAR into consideration, there have been several efforts to improve its analgesic activity while preventing its toxicity by modifying its structure [7–9]. Recently, seven 3,5-disubstituted analogues of paracetamol were synthesized in order to compare their physicochemical, pharmacological and toxicological properties (Fig. 1). The variation on substitution of the positions *ortho* to the phenolic hydroxyl group, disubstitution with the electron-donating substituents ($-CH_3$, $-OCH_3$, and $-SCH_3$) resulted in better cyclooxygenase inhibiting properties (an in vitro test for analgesic activity) than PAR, while electron-withdrawing halogen substituents (–F, –Cl, –Br) decreased the cyclooxygenase inhibiting capacity when compared to PAR. Electron-donating substituents ($-CH_3$, $-OCH_3$, and $-SCH_3$) decreased the cytotoxicity of analogues. Most 3,5-dihalogen substituents (–F, –Cl, –Br) diminished it slightly [10].

Bessems et al. using Hartree Fock method to rationalize the observed ESR spectra, hydrogen atom abstraction of PAR in four of the 3,5-disubstituted analogues of the PAR. These calculations indicated that for all compounds studied an initial hydrogen atom abstraction from the phenolic hydroxyl group is favored by approximately 125 kJ mol^{-1} over an initial hydrogen abstraction from the acetylamine nitrogen atom, and the unpaired electron remains predominantly localized at the phenoxy oxygen atom (±85%) [11–12]. However, it is known that the HF method does not give a good description of the electronic structure of radical. The spin

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contamination is a significant factor affecting the accuracy of HF method.

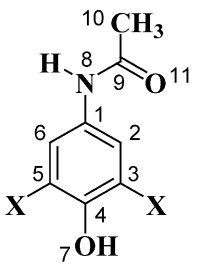
The density functional theory (DFT) using the B3LYP hybrid functional have been shown to give a good description of the electronic structure of radical and least a semi-quantitative picture of the spin-density distribution when compared with the polarized neutron diffraction data [13–14]. The UB3LYP exchange correlation functional gives good results, while the dependence on the basis set was found to be of minor importance [15]. Thus, in the present work DFT calculations were performed to obtained energy (E), ionization potential (IP), bond dissociation energies (BDE), and spin-density distribution for PAR and six 3,5-disubstituted analogues of PAR (Fig. 1). Calculation of correlation matrix was realized with the aim to obtain a correlation between the properties calculated and the analgesic activity and cytotoxicity (LDH).

2. Calculation

Prior to any DFT [16] calculations all structures were submitted to PM3 [17] geometry conformational search. All PM3 geometries were fulfill optimized at B3LYP hybrid DFT [18–19] by using a 6-31G(d) basis [20]. Only those conformations, which are most stable for a given compound, have been used.

Among many descriptors (variables) of physical and electronic properties of potential relevance to the hypothetical mechanism were examined for PAR and 3,5 analogues of PAR: octanol/water partition coefficients, superficial area (A), molecular volume, ionization potential (IP), bond dissociation energies (BDE), spin densities and partial atomic charges (Q_N) derived from the electrostatic potential.

The calculated bond dissociation energies (BDE), and ionization potentials (IP) were not corrected for zero-point energy, assuming a negligible error and thus saving computer-time. HF [11] and DFT [21] calculations indicated that for PAR an initial hydrogen atom abstraction from the phenolic hydroxyl group is favored by approximately 125 kJ mol^{-1} and 30.6 kcal mol^{-1}, respectively, over an initial hydrogen abstraction from the acetylamino nitrogen atom. Therefore, in this work an initial hydrogen abstraction of phenolic hydroxyl group was considered. The O–H bond dissociation energies (BDE_{O-H}) for homolytic O–H bond cleavage was calculated as the energy of the radical *N*-acetyl-*p*-benzosemiquinone imine (NAPSQI) resulting from the hydrogen atom abstraction minus the energy of the neutral molecules. The N–H bond dissociation energies (BDE_{N-H}) for homolytic N–H bond cleavage was calculated as the energy of the *N*-acetyl-*p*-benzoquinone imine (NAPQI) resulting from the secondary hydrogen atom abstraction from the NAPSQI minus the energy of the NAPSQI (Fig. 2). The IP was calculated as the energy differences between a neutral molecule and the respective radical cation.



Compounds	X
1	H
2	CH_3
3	OCH_3
4	SCH_3
5	F
6	Cl
7	Br

Fig. 1. Structure of paracetamol and 3,5-disubstituted analogues.

Table 1
Ionization potential (IP in kcal mol^{-1}), O–H bond dissociation energies (BDE_{O-H} in kcal mol^{-1}), N–H bond dissociation energies (BDE_{N-H} in kcal mol^{-1}), charge net (in u.a.), Log(1/IC_{50}) (mM), and LDH (%) for the PAR and 3,5 analogues

Compound	IP	BDE_{O-H}	BDE_{N-H}	O_{11}	Log(1/IC_{50})	LDH
1	171.31	394.70	788.15	−0.551	−0.456	50.60
2	158.13	391.57	779.99	−0.566	−0.796	15.00
3	149.35	390.31	780.62	−0.564	−0.699	12.40
4	156.25	391.57	782.50	−0.568	−0.854	14.50
5	178.21	394.08	786.27	−0.538	0.057	42.30
6	178.84	395.33	790.03	−0.528	−0.252	36.00
7	170.68	396.59	788.78	–	−0.319	26.10

The biological evaluation of the 3,5-disubstituted analogues of paracetamol was done by using the Log(1/IC_{50}) of the numerical indicator for activity, IC_{50}, that indicates pharmacological potency (concentration which inhibits cyclooxygenase by 50%) [10]. The respective Log(1/IC_{50}) values for all the seven compounds studied are shown in Fig. 1 and Table 1. The LDH value showed in Table 1 describes the cytotoxicity in hepatocytes. In comparison to PAR, two groups of analogues could be distinguished with regard to their property to induce cytotoxicity in freshly isolated hepatocytes [10].

3. Results and discussion

We obtained the correlation matrix between biological values and the respective calculated properties. Properties found to have significant correlation coefficients with anti-cyclooxygenase activity (an in vitro test for analgesic activity) and cytotoxicity are presented in Table 1. The matrix correlation is presented in Table 2; from it we can see that some variables are correlated to each other (we considered correlated variables as those that possess correlation coefficients above 0.70). According to these results IP, and charge on the O_{11} oxygen atom are correlated with the analgesic activity, while that BDE_{O-H}, IP, and BDE_{N-H} are correlated with the cytotoxicity.

The charge on the O_{11} oxygen atom and IP gave the highest correlation coefficient with analgesic activity value

Table 2
Correlation matrix

	$Log(1/IC_{50})$	REDOX	LDH	O11	PI	BDE_{O-H}
$Log(1/IC_{50})$	1					
REDOX	0.85	1				
LDH	0.77	0.84	1			
O11	0.89	0.91	0.73	1		
PI	0.88	0.97	0.87	0.92	1	
BDE_{O-H}	0.75	0.93	0.90	0.87	0.96	1
BDE_{N-H}	0.72	0.83	0.86	0.88	0.90	0.96

of 0.89 and 0.88, respectively, while BDE_{O-H} and BDE_{N-H} had correlation coefficients of 0.75 and 0.72, respectively. The IP representing the ease of electron donation of 3,5 analogues of PAR. Electron-donating substituents at the 3,5-position ($-CH_3$, $-OCH_3$, and $-SCH_3$) decreased the IP resulted in better cyclooxygenase inhibiting properties than PAR, while electron-withdrawing halogen substituents (−F, −Cl, and −Br) increased the IP resulted decreased the COX inhibiting capacity when compared to PAR. The analgesic potency has been suggested to be related to oxidisability, suggesting a link between oxdisability and COX inhibition suggest that for inhibition of PGES, one- or two-electron oxidation is a prerequisite [5]. Moreover, the redox potential is highest correlated with the IP values of 0.97, demonstrating the relation with outlet of the electron of PAR and analogues. On the charge at atom 11 we would like to pay attention to the fact that for the active compounds it is important to have atoms with high negative charge at position 11, i.e. more electron-donor atoms at 3,5-positions of the aromatic ring are required for the more active compound, as they would react with an electro-acceptor biological receptor. In accordance with these results we can suggest an interaction of O_{11} with ferric complex positive charge, while phenolic group promoted reduction in the tyrosyl (Tyr^{385}) radical of the COX [3].

The BDE_{O-H}, IP, and BDE_{N-H}, gave the highest correlation coefficient with cytotoxicity values of 0.90, 0.87 and 0.86, respectively. Electron-donating substituents at the 3,5-position ($-CH_3$, $-OCH_3$, and $-SCH_3$) decreased both BDE_{O-H} and BDE_{N-H} resulted in a decrease of cytotoxicity than PAR, while electron-withdrawing halogen substituents (−F, −Cl, and −Br) increased both BDE_{O-H} and BDE_{N-H}, which resulted in an increase in the cytotoxicity when compared to electron-donating substituents. The BDE_{O-H} and BDE_{N-H} representing the oxidation mechanism of PAR to NAPQI via the free radical species NAPSQI. The toxicity due to a large dose of PAR is mainly dependent on P450-catalyzed oxidative biotransformation to NAPQI [5]. These results demonstrate a relation between hepatotoxicity, chemical stability, and reactivity due oxidation of PAR to NAPQI. Electron-donor substituents in the 3,5-positions stabilize the semiquinone (NAPSQI), making it less reactive, consequently less toxic. Electron-acceptor substituents in the 3,5-positions produce an unstable semiquinone (NAPSQI), making it more reactive, consequently more toxic. Much evidence has accumulated for the involvement of a free-radical intermediate in the conversion of PAR to NAPQI for the hepatotoxicity end point causing liver necrosis [6].

A hypothetical radical pathways for the oxidation of 3,5-disubstituted analogues of paracetamol is show in Figs. 2 and 3, in which as the initial set a hydrogen atom abstraction

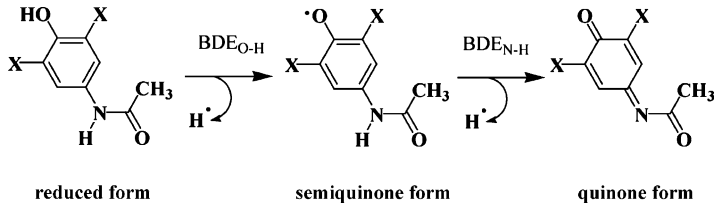


Fig. 2. Abstraction of a hydrogen of the phenolic or acetylamine groups.

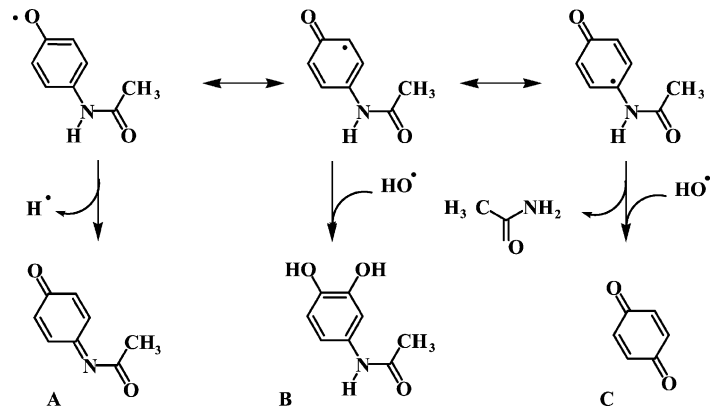


Fig. 3. A hypothetical radical pathway for the oxidation of PAR.

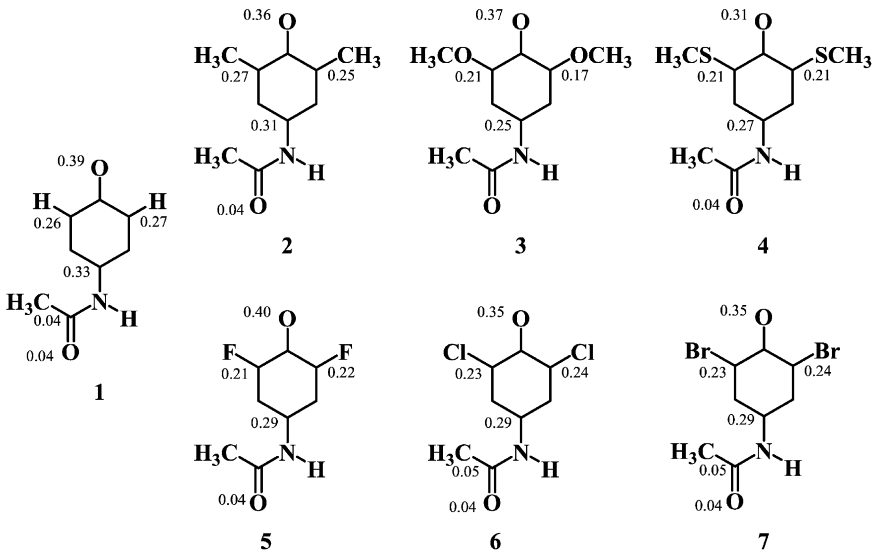


Fig. 4. Calculated spin densities to hydrogen atom abstraction of the PAR and 3,5-disubstituted analogues.

is assumed to take place either at the phenolic hydroxyl group lead to NAPQI-analogues and water. HF and DFT calculations indicated that for PAR an initial hydrogen atom abstraction from the phenolic hydroxyl group is favored by approximately 125 kJ mol^{-1} and 30.6 kcal mol^{-1}, respectively, over an initial hydrogen abstraction from the acetylamine nitrogen atom [11–12,21]. Therefore, in this work an initial hydrogen abstraction of phenolic hydroxyl group was considered.

The calculated spin density to initial hydrogen atom abstraction at the phenolic hydroxyl group (Fig. 4) shows mainly the contribution from the O_7 phenolic oxygen (31–40%), C_3 and C_5 carbon atoms at the *ortho* (17–27 and 21–27%) and C_1 carbon atom at the *para* (25–33%) positions. The contribution from the N_8, and O_{11} atoms is almost an order of magnitude smaller. The localization of the unpaired electron on the C_1, C_3 and C_5 positions explain the formation of the minor metabolites 3-hydroxy-PAR (**B**) and *p*-benzoquinone plus acetamide (**C**) (Fig. 3), while the localization of the unpaired electron on the O_7 phenolic oxygen lead the formation of NAPQI (**A**) via a second hydrogen abstraction from the acetylamino nitrogen atom. These results explained the occurrence of the NAPQI, 3-hydroxy-PAR, and *p*-benzoquinone as phase I metabolites of PAR [12,22]. These results are in contrast with the previous HF results obtained by Bessems et al. [12], who showed that the unpaired electron is primarily localized at the phenolic oxygen atom (±85). The spin contamination is a significant factor affecting the accuracy of HF method.

4. Conclusions

Descriptors (variables) of physical and electronic properties of potential relevance to the analgesic activity and cytotoxicity were examined for PAR and 3,5 analogues of PAR using quantum mechanical calculations at the B3LYP theory level. The IP gave the highest correlation with analgesic activity. Electron-donating substituents at the 3,5-position ($-CH_3$, $-OCH_3$, and $-SCH_3$) decreased the IP resulted in better cyclooxygenase inhibiting properties than PAR, while electron-withdrawing halogen substituents (−F, −Cl, and −Br) increased the IP resulted decreased the cyclooxygenase inhibiting capacity when compared to PAR. The BDE_{O-H}, IP, and BDE_{N-H}, gave the highest correlation with the cytotoxicity, this indicates that both stability of NAPSQI and formation of NAPQI are related to the cytotoxicity. The unpaired electron of the phenoxy radical remains is localized on the O_7 phenolic oxygen, C_3 and C_5 carbon atoms at the *ortho* and C_1 carbon atom at the *para* positions. The localization of the unpaired electron on the C_1, C_3 and C_5 positions explain the formation of the minor metabolites 3-hydroxy-PAR and *p*-benzoquinone plus acetamide, while the localization of the unpaired electron on the O_7 phenolic oxygen lead the formation of NAPQI via a second hydrogen abstraction from the acetylamino nitrogen atom. These results explained the occurrence of the NAPQI, 3-hydroxy-PAR, and *p*-benzoquinone as phase I metabolites of PAR.

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