Regioisomerism and 5-substitution as promising approaches for molecular design of safer acetaminophen derivatives

Kayo Silva Gustavo
Universidade Federal do Pará: Universidade Federal do Para
Roberto Barbosa de Morais
Universidade Federal do Pará: Universidade Federal do Para
Luan Oliveira Ferreira
Universidade Federal do Pará: Universidade Federal do Para
Valéria Bittencourt Pessoa Mourão
Universidade Federal do Pará: Universidade Federal do Para
Gerson Maciel Coelho
Universidade Federal do Pará: Universidade Federal do Para
Tamires Ferreira da Silva
Universidade Federal do Pará: Universidade Federal do Para
Laine Celestino Pinto
Universidade Federal do Pará: Universidade Federal do Para
Dulcidéia da Conceição Palheta
Universidade Federal Rural da Amazonia
Flávia Cristina Matos Oliveira
Universidade Federal Rural da Amazonia
Natália Lopes Soares
Universidade Federal Rural da Amazonia
Arnaldo Jorge Martins-Filho
Evandro Chagas Institute: Instituto Evandro Chagas
Elizabeth Sumi Yamada
Universidade Federal Rural da Amazonia
Dielly Catrina Favacho Lopes
Universidade Federal do Pará: Universidade Federal do Para
Rosivaldo dos Santos Borges (rosborg@ufpa.br)
Universidade Federal do Pará https://orcid.org/0000-0003-4072-7573

Research Article

Keywords: Acetaminophen derivatives, regioisomerism, molecular mechanism, structure-toxicity, drug design, acute toxicity oral test

Posted Date: March 30th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-327926/v1

Abstract

Acetaminophen is a widely used over-the-counter analgesic and antipyretic drug, but in large doses can produce hepatic and/or renal injury. Based on its human toxicity by *N*-acetyl-*p*- benzoquinone imine formation as reactive toxic intermediate, an acetaminophen regioisomer substituted on 5-position, named chloraminophen, was proposed. The electron and hydrogen transfers were related to the ionization potential and bond dissociation energies on phenol and acetamide moieties. These calculations were performed by using quantum chemical calculations at the density functional theory (DFT/B3LYP) with the 6-311 + + G(d,p) basis set. For acute toxicity study was performed using a single dose test of 300 or 2000 mg/kg. No differences were found in the biochemical parameters except for aspartate aminotransferase levels, probably due to corn oil used as dilution solvent. The second energy of hydrogen abstraction may have great impact on amino-phenols toxicity. The regioisomerism can be a useful strategy in drug design for safer acetaminophen derivatives.

Introduction

Acetaminophen (ACP) or paracetamol is a widely used over-the-counter analgesic and antipyretic drug and appears to be safe if used in normal therapeutic doses. However, large doses of ACP produce hepatic and/or renal injury in humans and in experimental animals [1,2]. Its pharmacological effects are generally considered to be based on inhibition of prostaglandin synthesis [3-5].

On the other hand, it is metabolized primarily by glucuronidation and sulfation to form major conjugates ACP-glucuronide and ACP-sulfate, respectively. Similarly, a small amount is probably modified via a third metabolic pathway, that is, oxidation by the microsomal cytochrome P450-containing mixed-function oxidase system to *N*-acetyl-*p*-benzosemiquinone-imine (NAPSQI) and N-acetyl-p-benzoquinone-imine (NAPQI). A glutathione 1,4-Michael adduct of NAPQI and the corresponding cysteine conjugate and mercapturic acid breakdown products were found in human urine after ingestion of ACP [6-8]. Nevertheless, several unsuccessful reports describe investigations into structural modification of acetaminophen in order to improve its analgesic and safety properties [9-14].

Its hepatotoxic effect appears to be confined in compounds that can to form quinoid structures, which are susceptible to direct reaction by irreversible covalent bond and depletion of nonsoluble thiols. The oxidation mechanism of ACP to NAPSQI and NAPQI [15-17] was related to bond dissociation energies of phenol (BDE_{OH}) and acetamide (BDE_{NH}) moieties, respectively, using calculations at B3LYP level, where the BDE_{OH} is energetically favored over BDE_{NH} and the molecules with high BDE_{NH} values are less hepatotoxic (Fig. 1) [18,19].

In this work, a molecular strategy was used for drug design of acetaminophen derivatives using calculations of bond dissociation energies in the selection for synthesis and toxicological evaluation. Eight new derivatives were assembled by a rational drug design by using regioisomerism followed by metabolism blocker at 5 position using electron donating (EDGs) or electron withdrawing groups (EWGs)

looking for the role played by the structural features of these molecules and in obtaining new compounds endowed with less hepatotoxicity by changes on BDE_{OH} and BDE_{NH} values for the semiquinone and quinone-imine formations (Fig. 2). An acetaminophen regioisomer substituted at the 5-position by chloro, named chloramidaphen (CAP), was selected for toxicity evaluation.

Results And Discussion

Design of acetaminophen regioisomers

Our design and development strategy adopted by us in this study is the regioisomerism, changed the phenol moiety of *para*- for *ortho*-position. In Fig. 3, the differences on BDE_{OH} and BDE_{NH} between acetaminophen and chloramidaphen are clearly showed. Both compounds have these electronics properties completely different between *para* (NAPSQI and NAPQI) and *ortho* of *N*-acetyl-*o*-benzosemiquinone imine and *N*-acetyl-*o*-benzoquinone imine (NAOSQI and NAOQI).

The BDE_{OH} values for these compounds were 85.64 and 81.66 kcal mol^{-1} and a difference of 3.97 kcal mol^{-1} was observed between them [18]. Therefore, the regioisomer derivative has a lowest quenching energy than on a first hydrogen transfer. These results indicate that the regioisomerism increased the quenching capacity, which could be related to better biological activity [20]. In addition, the BDE_{NH} values for these two molecules were 77.30 and 90.72 kcal mol^{-1}. This second hydrogen abstraction shows a higher difference of 13.41 kcal mol^{-1} for *ortho*-regioisomer.

From Table 1, electron-donating groups (EDGs) at the 5-position ($-CH_3$, $-N(CH_3)_2$, $-OCH_3$, and $-SCH_3$) show the lowest BDE_{OH} values of 80.01 (**3**), 71.80 (**4**), 76.91 (**5**), and 76.89 kcal/mol (**6**), while electron-withdrawing groups (EWGs) at the same position (–F, –Cl, and $-NO_2$) decreased the BDE_{OH} values for 80.13 (**7**), 80.64 (**8**), and 84.58 kcal/mol (**9**). These results show that all regioisomers would be more reactive than acetaminophen, however, $-N(CH_3)_2$, $-OCH_3$, and $-SCH_3$ would be more effective due to high difference when compared to acetaminophen of 13.83, 8.72, and 8.74 kcal/mol, respectively. In addition, EDGs at the 5-position ($-CH_3$, $-N(CH_3)_2$, $-OCH_3$, and $-SCH_3$) show higher BDE_{NH} values of 89.93 (**3**), 92.67 (**4**), 93.48 (**5**), and 93.29 kcal/mol (**6**). In the same way, EWGs (–F, –Cl, and $-NO_2$) increased BDE_{NH} values for 91.52 (**7**), 91.69 (**8**), and 92.81 kcal/mol (**9**). These results show that all regioisomers would be less toxic than acetaminophen [21].

These results are in agreement to nucleophilicity (HOMO), electrophilicity (LUMO), and chemical stability (GAP) [22]. In addition, due to the high chemical stability of halogen on drug metabolism process [23], we choice the chlorinated derivative for synthesis and toxic evaluation. This compound shows a difference of -5.00 and -14.38 kcal/mol for BDE_{OH} and BDE_{NH} values, respectively. Therefore, our methodology shows that new acetaminophen derivative can be a good strategy in drug design for the safer compounds because of the highest values for quinone production as compared to acetaminophen [18]. In fact, ACP toxicity is related to the level of toxic intermediate metabolites such as *N*-acetyl-*p*-

benzoquinone-imine (NAPQI) as main chemical mechanism [9,14,16]. So, the ability to predict the toxicity profile of CAP is critical to rational pharmaceutical drug design and development. In addition, a similar electronic mechanism is involved for other phenol derivatives [24].

Synthesis of chloramidaphen

After that, CAP was synthesized from 5-chloro-2-aminophenol using acetic acid, sodium carbonate, and water, as acylating group, alkaline catalytic, and solvent medium, respectively. After recrystallization on methanol, a mp 182.6-184^{o}C [183-184^{o}C] [25] was observed. It is soluble in methanol, ethanol, dimethylformamide, acetone, and ethyl acetate and practically insoluble in water, petroleum ether, and pentane. ^{1}H-NMR (300 MHz; $CDCl_3$) d (ppm) 8.43 (1H, s, H-O), 7.43 (1H, s, H-N), 7.09 (2H, dd, 6.0 and 3.0 Hz, H-4 and H-6), 6.95 (1H, d, 6.0 Hz, H-3), and 2.29 (3H, s, CH_3). ^{13}C NMR (75 MHz; $CDCl_3$) d (ppm) 170.52 (C=O), 147.29 (C-2), 126.91 (C-6), 126.46 (C-1), 125.03 (C-5), 121.74 (C-4), 120.82 (C-3), and 23.81 (CH_3). This compound was used on toxicological evaluations.

Biochemical changes upon chloramidaphen exposure

Analyzing the results of AST, ALT, creatinine and urea levels of rats treated with 300 mg/kg of CAP (Table 2), our findings shows that all AST levels measure were increased on days 8 and 15 both treated and control groups and were significant different of day 0 measure ($p < 0.05$) and samples of treated group were different of control group on day 15 ($p < 0.05$). Besides that, there was no significant difference in the ALT, urea and creatinine levels of both treated and control rats throughout the experiment.

The biochemistry parameters for 2000 mg/kg dose were showed on Table 3. The results show that AST levels measure were increased on days 8 and 15 both treated and control groups and were significant different of day 0 measure ($p < 0.05$). On the other hand, ALT level was decreased to control group on day 15 ($p < 0.05$ vs day 0). In addition, no more significant difference in the ALT, urea and creatinine levels were found in both treated and control groups.

The knowledge of normal range or expected values for hematologic and serum chemistry can be a useful tool for interpreting toxicology and safety studies [26]. It is important to notice that several factors can modify these values, like age, diet, handling and environment [27].

Analysis for biochemistry parameters, the toxicological effects were evident to aspartate transaminase (AST, formerly referred to as serum glutamic-oxaloacetic transaminase, SGOT). Here, we showed that high AST levels on both treated groups (300 mg/kg and 2000 mg/kg) and controls groups. These results were different of the values already found in others studies [26-28]. We hypothesized that the corn oil and other vegetable oil may be altering liver physiology by alteration of triglycerides synthesis and hepatic lipid peroxidation in some conditions [29-33]. Thus, these findings showed that the CAP presented neither hepatotoxic effect nor renal toxicity, since that there is no significant change in urea and creatinine.

To be one more way to study the hepatotoxic effect of the drug, we also calculated the alterations in the relation AST/ALT levels to additionally suggest liver toxicity, but these levels are not significant. Our results for 300 and 2000 mg/kg show low levels of AST and ALT. In both doses, there were few differences among control and treated with chloramidaphen or 300 and 2000 mg/kg. None animal died for 15 days. However, an increase of ALT levels on Swiss-Webster mice treated with 400 and 750 mg/kg acetaminophen was showed of 1552 ± 501 to 4030 ± 829 mU/L. The survivors at 24 hours after acetaminophen treatment was 8 (n = 10) for 400 mg/kg and 4 (n = 10) for 750 mg/kg [34]. In fact, chloramidaphen seems to have a much safer therapeutic index when compared to its relative drug acetaminophen, which have oral LD_{50} of 338 mg/kg (oral, mouse) [35].

In addition, both acetaminophen regioisomers, i.e. 2-hydroxyacetanilide (**2**) and 3-hydroxyacetanilide (**10**) were appointed as nonhepatotoxic, however reactive metabolites as arylate hepatic proteins for hydroxilated derivatives of 3-hydroxy-acetanilide (**11**) was detected (36-41). Their oxidation by rat liver P450 leads to the formations of two main products of *para*-aromatic hydroxylation that subsequently can be further oxidized to their respective *ortho* (**12**) and *para*-benzoquinone (**13**) derivatives and form glutathione adduct (42-44), as showed in Fig. 4. Likewise, 2-hydroxyacetanilide (**2**) that is metabolized to 2',5'-dihydroxyacetanilide (**11**) was low hepatotoxic in mice (37,38,45), maybe due to the changes on chemical reactivity of phenol and acetamide moieties. Thus, the chlorinated derivative (**8**) in position 5 can reduce the formation of hydroxylated derivatives and the formation of quinones, reducing toxicity.

In accordance to our biological study, some evidence was observed that *ortho*-regioisomers may be an alternative approach for design and development of low toxic derivative of acetaminophen. The substantial protection afforded by chloramidaphen can be related to decrease of electrophilic mechanism involved for metabolism and toxicity of quinone-imine species due to its high BDE_{NH} values. These mechanisms need to be further investigated.

Conclusion

In this work, a molecular strategy was successful used for understanding of the mechanistic features and drug design of acetaminophen regioisomers using calculations of bond dissociation of phenol (BDE_{OH}) and acetamide (BDE_{NH}) moieties. The less toxicity was more related to changes on BDE_{NH} than BDE_{OH} values. Another strategy used was a metabolism blocker at 5 position. The chloramidaphen does not present toxic characteristics in the acute test. The lethal oral toxicity of this compound was estimated to be higher than 2000 mg/kg, classifying as category 5 according to OECD Guide 420, indicating a certain safety margin associated with corn oil. Although the results obtained in this research, it is known that more studies should be performed in order to evaluate the total safety on this compound. Nevertheless, the low toxicity can be related to BDE_{NH} values and NAPQI formation capacity. Furthermore, these results can be used for drug design for safe analgesic compounds of acetaminophen.

Methodology

Molecular design of chloramidaphen

Geometry optimizations of acetaminophen derivatives have been carried out using density functional theory (DFT) [46]. The calculations were performed with the Gaussian 03 molecular package [47]. All structures were submitted to PM3 [48] geometry conformational search. One hybrid functional of the DFT/B3LYP-31G (d,p) basis set [46,49,50] for the geometry optimizations and hydrogen donations.

The bond dissociation energies of the phenol group or *N*-acetyl-*p*-benzosemiquinone imine (NAPSQI) formation were calculated as the energy differences between a neutral molecule and the respective semiquinone plus hydrogen radical as defined in Equation 1.

$BDE_{OH} = (E CH_3CONHC_6H_4O^{\bullet} + EH^{\bullet}) - E CH_3CONHC_6H_4OH$ (Equation 1)

The bond dissociation energies of the acylamide group or *N*-acetyl-*p*-benzoquinone imine (NAPQI) formation were calculated as the energy differences between a NAPSQI molecule and the respective NAPQI plus hydrogen radical which can be written as Equation 2.

$BDE_{NH} = (E CH_3CON{=}C_6H_4{=}O + EH^{\bullet}) - E CH_3CONHC_6H_4O^{\bullet}$ (Equation 2)

Synthesis of chloramidaphen

Our compound was obtained by soft and ecofriendly chemo-selective acylation of amines moiety in aqueous medium by using sodium carbonate and acetic anhydride as alkaline catalytic and acylation reagent, respectively [51], as showed in Fig. 5.

Acute toxicity oral test

Young adults Wistar male rats (*n* = 12) weighing 220-260g and 8-10 weeks old were used in this study. All animals were housed in a controlled environment (22 ± 2°C; 12/12 h light/dark cycle) with access to food and water ad libitum. All animal experiments complied with the ARRIVE guidelines [52] and were carried out in accordance with the EU Directive 2010/63/EU for animal experiments [53].

The acute toxicity oral test was performed according to the Organization for Economic Cooperation and Development (OECD) guideline 420 [54] and Brazilian Health Regulatory Agency [55], with initial dose at 300 mg/kg. The animals were divided into four groups: control 300 mg/kg (*n* = 2), treated 300 mg/kg (*n* = 4), control 2000 mg/kg (*n* = 2), treated 2000 mg/kg (*n* = 4). After fasting overnight (8 h), the chloramidaphen was administered in a single dose dissolved in corn oil by oral gavage (day 1). Control animals received corn oil proportional of treated dose.

After dosing, all animals were observed individually for mortality and to monitor physical and behavioral alterations for 14 days, giving special attention to the first 30 min, then at 1, 2, 4 and 24 h following

treatment. After that, the animal observation was performed once per day for the post-dosing period. Symptoms of toxicity such as hypo-activity (lethargy), piloerection, salivation, breathing difficulty, diarrhea, tremors, ptosis, hypnosis, muscle tone changes, tail grip response, convulsion and general appearance were evaluated after administration.

Biochemical parameters

The blood samples collected by cardiac puncture underwent evaluation of biochemical and hematological parameters. Regarding the biochemical analysis, tubes without anticoagulant were used and centrifuged, in order to obtain the serum. Serum samples were analyzed to clinical biochemistry parameters included alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea (UREA) and creatinine (CREA).

Data analysis

The results were presented as mean ± standard derivation (SD) and analyzed using one-way analysis of variance (ANOVA). The differences between the means were tested using post hoc Tukey and values of $p < 0.05$ were considered statistically significant. GraphPad Prism for windows version 8.0 was used for the statistical analysis.

Declarations

Acknowledgements

This research was supported by grants from the Brazilian agencies CNPq, CAPES and UFPA.

Compliance with ethical standards

All experimental procedures were approved by the Ethic Committee on Animal Use of the Federal University of Pará (CEUA/UFPA number 3702221117) and followed the guidelines suggested by the NIH Guide for the Care and Use of Laboratory Animals and the National Council for Control of Animal Experimentation (CONCEA).

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Hinson JA, Roberts DW, James LP, Mechanisms of acetaminophen-induced liver necrosis, Handb. Exp Pharmacol. 2010;196:369-405. https://doi.org/10.1007/978-3-642-00663-0_12.
2. Black M, Acetaminophen Hepatotoxicity, Annu Rev Med. 35 (1984) 577–593. https://doi.org/10.1146/annurev.me.35.020184.003045.
3. Flower RJ, Vane JR, Inhibition of prostaglandin synthetase in brain explains the anti-pyretic activity of paracetamol (4-acetamidophenol), Nature. 1972;240:410–411. https://doi.org/10.1038/240410a0.
4. Malmberg A, Yaksh T, Hyperalgesia mediated by spinal glutamate or substance P receptor blocked by spinal cyclooxygenase inhibition, Science. 1992;257:1276–1278. https://doi.org/10.1126/science.1381521.
5. Graham G, Scott KF, Mechanism of action of paracetamol, Am J Ther. 2005;12:46-55. https://doi.org/10.1097/00045391-200501000-00008.
6. Howie D, Adriaenssens PI, Prescott LF, Paracetamol metabolism following overdosage: application of high-performance liquid chromatography, J Pharm Pharmacol. 1977;29:235-237. https://doi.org/10.1111/j.2042-7158.1977.tb11295.x.
7. Knox JH, Jurand J, Determination of paracetamol and its metabolites in urine by high-performance liquid chromatography using reversed-phase bonded supports, J Chromatogr. A. 1977;142:651-670, https://doi.org/10.1016/S0021-9673(01)92075-8.
8. Prescott LF, Paracetamol overdosage: pharmacological considerations and clinical management, Drugs. 1983;25:290-314. https://doi.org/10.2165/00003495-198325030-00002.
9. Van De Straat R, De Vries J, Kulkens T, Debets AJJ, Vermeulen NPE. Paracetamol, 3-monoalkyl- and 3,5-dialkyl derivatives. Comparison of their microsomal cytochrome P-450 dependent oxidation and toxicity in freshly isolated hepatocytes, Biochem Pharmacol. 1986;35:3693-3699. https://doi.org/10.1016/0006-2952(86)90653-2.
10. Van de Straat R, De Vries J, Groot EJ, Zijl R, Vermeulen NPE, Paracetamol, 3-monoalkyl- and 3,5-dialkyl derivates: comparison of their hepatotoxicity in mice, Toxicol Appl Pharmacol. 1987;189:183–189. https://doi.org/10.1016/0041-008x(87)90039-1.
11. Holme JA, Hongslo JK, Bjørge C, Nelson SD, Comparative cytotoxic effects of acetaminophen (N-acetyl-p-aminophenol), a non-hepatotoxic regioisomer acetyl-m-aminophenol and their postulated reactive hydroquinone and quinone metabolites in monolayer cultures of mouse hepatocytes, Biochem Pharmacol. 1991;42:1137-1142. https://doi.org/10.1016/0006-2952(91)90299-K.
12. Barnard S, Kelly DF, Storr RC, Park BK, The effect of fluorine substitution on the hepatotoxicity and metabolism of paracetamol in the mouse, Biochem Pharmacol. 1993;46:841-849. https://doi.org/10.1016/0006-2952(93)90493-G.
13. Bessems JGM, Te Koppele JM, Van Dijk PA, Van Stee LLP, Commandeur JNM, Vermeulen NPE. Rat liver microsomal cytochrome P450-dependent oxidation of 3,5-disubstituted analogues of paracetamol, Xenobiotica. 1996;26:855-875. https://doi.org/10.3109/00498259609046740.

14. Bessems JGM, Van Stee LLP, Commandeur JNM, Groot EJ, Vermeulen NPE, Cytotoxicity of paracetamol and 3,5-dihalogenated analogues: Role of cytochrome P-450 and formation of GSH conjugates and protein adducts, Toxicol in Vitro. 1997;11:9-19. https://doi.org/10.1016/S0887-2333(96)00066-5.
15. De Vries J, Hepatotoxic metabolic activation of paracetamol and its derivatives phenacetin and benorilete: Oxygenation or electron transfer? Biochem Pharmacol. 1981;30:399-402. https://doi.org/10.1016/0006-2952(81)90622-5.
16. Rogers LK, Moorthy B, Smith CV, Acetaminophen binds to mouse hepatic and renal DNA at human therapeutic doses, Chem Res Toxicol. 1997;10:470-476. https://doi.org/10.1021/tx960159i.
17. Bessems JGM, De Groot MJ, Baede EJ, Koppele JM, Vermeulen NPE, Hydrogen atom abstraction of 3,5-disubstituted analogues of paracetamol by horseradish peroxidase and cytochrome P450, Xenobiotica. 1981;28:855-875. https://doi.org/10.1080/004982598239100.
18. Diniz JEM, Borges RS, Alves CN, A DFT study for paracetamol and 3,5-disubstituted analogues, J. Mol. Struct. THEOCHEM. 2004;673:93-97. https://doi.org/10.1016/j.theochem.2003.12.002.
19. Alves CN, Borges RS, Da Silva ABF, Density Functional Theory Study of Metabolic Derivatives of the Oxidation of Paracetamol, Int J Quant Chem. 2006;106:2617-2623. https://doi.org/10.1002/qua.20992.
20. Wu G, Tsai AL, Kulmacz RJ, Cyclooxygenase competitive inhibitors alter tyrosyl radical dynamics in prostaglandin H synthase-2. Biochem. 2009;48:11902–11911. https://doi:10.1021/bi901600f.
21. Queiroz LMD, Rocha JR, Leitão A, Montanari CA, da Silva ABF, Sousa PJC, Borges RS, A combined study using ligand-based design, synthesis, and pharmacological evaluation of analogues of the acetaminophen ortho-regioisomer with potent analgesic activity. Chem Biol Drug Des. 2012;80:100–106. https://doi:10.1111/j.1747-0285.2012.01372.x.
22. Huang Y, Rong C, Zhang R, Liu S, Zhang R, Liu S, Evaluating frontier orbital energy and HOMO/LUMO gap with descriptors from density functional reactivity theory. J Mol Model. 2017;23:3. https://doi:10.1007/s00894-016-3175-x.
23. Stepan AF, Mascitti V, Beaumont K, Kalgutkar AS, Metabolism-guided drug design. Med. Chem. Commun. 2013;4:631-652. https://doi:10.1039/C2MD20317K.
24. Hassan ZK, Elobeid MA, Virk P, Omer SA, ElAmin M, Daghestani MH, AlOlayan EM, Bisphenol A induces hepatotoxicity through oxidative stress in rat model, Oxid Med Cell Longev. 2012;2012:6. https://doi.org/10.1155/2012/194829.
25. Siqueira FA, Taylor JG, Correia CRD, The first intramolecular Heck-Matsuda reaction and its application in the syntheses of benzofurans and indoles, Tet Lett. 2010;51:2102-2105, https://doi:10.1016/j.tetlet.2010.02.011.
26. Kim SJ, Lee MY, Kwon DY, Kim SY, Kim YC, Alteration in metabolism and toxicity of acetaminophen upon repeated administration in rats, J. Pharmacol. Sci. 2009;111:175-181. https://doi:10.1254/jphs.09151fp.

27. Hodgman MJ, Garrard AR, A review of acetaminophen poisoning, Crit. Care Clin. 2012;28:499-516. https://doi:10.1016/j.ccc.2012.07.006.
28. Ganey PE, Luyendyk JP, Newport SW, Eagle TM, Maddox JF, Mackman N, Roth RA, Role of the coagulation system in acetaminophen-induced hepatotoxicity in mice, Hepatology. 2007;46:1177-1186. https://doi:10.1002/hep.21779.
29. Bleeker JS, Hogan WJ, Thrombocytosis: diagnostic evaluation, thrombotic risk stratification, and risk-based management strategies, Thrombosis. 2011;2011:1–16. https://doi:10.1155/2011/536062.
30. Iritani N, Fukuda E, Kitamura Y, Effect of oxidized oil on lipogenic enzymes, Lipids. 1980;15:371-374. https://doi:10.1007/BF02533553.
31. Arunachalam K, Balogun SO, Pavan E, Almeida G, Oliveira RG, Wagner T, et al. Chemical characterization, toxicology and mechanism of gastric antiulcer action of essential oil from Gallesia integrifolia (Spreng.) Harms in the in vitro and in vivo experimental models, Biomed Pharmacother. 2017;94:292–306. https://doi:10.1016/j.biopha.2017.07.064.
32. Mollahosseini M, Daneshzad E, Rahimi MH, Yekaninejad MS, Maghbooli Z, Mirzaei K, The Association between Fruit and Vegetable Intake and Liver Enzymes (Aspartate and Alanine Transaminases) in Tehran, Iran Ethiop J Health Sci. 2017; 27: 401–410. https://doi:10.4314/ejhs.v27i4.11
33. Shidfar F, Bahrololumi SS, Doaei S, Mohammadzadeh A, Gholamalizadeh M, Mohammadimanesh A, The effects of extra virgin olive oil on alanine aminotransferase, aspartate aminotransferase, and ultrasonographic indices of hepatic steatosis in nonalcoholic fatty liver disease patients undergoing low calorie diet, Can J Gastroenterol. Hepatol. 2018;2018:7. https://doi: 10.1155/2018/1053710.
34. Harvison PJ, Forte A J, Nelson SD, Comparative toxicities and analgesic activities of three monomethylated analogs of acetaminophen, J Med Chem. 1986;29:1737–1743. https://doi.org/10.1021/jm00159a029
35. Milne GWA, Drugs: Synonyms and Properties – an international guide to 10.000 drugs, 2nd Edition, Wiley, 2002. https://www.wiley.com/en-us/exportProduct/pdf/9780566084911
36. Roberts SA, Price VF and Jollow DJ, Acetaminophen structure-toxicity studies: in vivo covalent binding of a nonhepatotoxic analog, 3–hydroxyacetanilide. Tox Appl Pharm. 1990;105:195–208. https://doi:10.1016/0041-008x(90)90181-s
37. Tirmenstein MA, and Nelson SD, Hepatotoxicity after 3'-hydroxyacetanilide administration to buthionine sulfoximine pretreated mice. Chem Res Tox. 1991;4:214–217. https://doi:10.1021/tx00020a014.
38. Tirmenstein MA, and Nelson SD, Subcellular binding and effects on calcium homeostasis produced by acetaminophen and a nonhepatotoxic regioisomer, 3'-hydroxyacetanilide, in mouse liver. J Biol Chem. 1989;264:9814–9819. https://doi:10.1021 / tx00020a014.
39. Rashed MS, Myers TG, and Nelson SD, Hepatic protein arylation, glutathione depletion, and metabolite profiles of acetaminophen and a non-hepatotoxic regioisomer, 3'-hydroxyacetanilide, in the mouse. Drug Met Disp. 1990;18:765–770.https://pubmed.ncbi.nlm.nih.gov/1981734/

40. Rashed MS, and Nelson SD, Characterization of glutathione conjugates of reactive metabolites of 3'-hydroxyacetanilide, a nonhepatotoxic positional isomer of acetaminophen. Chem Res Tox. 1989;2:41–45. https://doi: 10.1021/tx00007a007.
41. Holme JA, Hongslo JK, Bjorge C, and Nelson SD, Comparative cytotoxic effects of acetaminophen (Nacetyl-p-aminophenol), a non-hepatotoxic regioisomer acetyl-m-aminophenol and their postulated reactive hydroquinone and quinone metabolites in monolayer cultures of mouse hepatocytes. Biochem Pharm. 1991;42:1137–1142. https://doi: 10.1016/0006-2952(91)90299-k.
42. Hadi M, Dragovic S, van Swelm R, Herpers B, van de Water B, Russel FG, Commandeur JN, Groothuis GM. AMAP, the alleged non-toxic isomer of acetaminophen, is toxic in rat and human liver. Arch Toxicol. 2013;87(1):155-65. https://doi.org/10.1007/s00204-012-0924-1
43. Matthews AM, Hinson JA, Roberts DW, Pumford NR. Comparison of covalent binding of acetaminophen and the regioisomer 3'-hydroxyacetanilide to mouse liver protein. Toxicol Lett. 1997;90(1):77-82. https://doi.org/10.1016/s0378-4274(96)03831-3
44. Halmes NC, Samokyszyn VM, Hinton TW, Hinson JA, Pumford NR. The acetaminophen regioisomer 3'-hydroxyacetanilide inhibits and covalently binds to cytochrome P450 2E1. Toxicol Lett. 1998;94(1):65-71. https://doi.org/10.1016/s0378-4274(97)00100-8
45. Kyriakides M, Maitre L, Stamper BD, Mohar I, Kavanagh TJ, Foster J, Wilson ID, Holmes E, Nelson SD, Coen M. Comparative metabonomic analysis of hepatotoxicity induced by acetaminophen and its less toxic meta-isomer. Arch Toxicol. 2016;90(12):3073-3085. https://doi.org/10.1007/s00204-015-1655-x
46. Lee C, Yang W, Parr RG, Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density, Phys Rev B. 1988;37:785-789. https://doi.org/10.1103/PhysRevB.37.785.
47. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, et al. Gaussian 09, revision D.01. Gaussian Inc., Wallingford 2009.
48. Stewart JJP, Optimization of parameters for semiempirical methods I. Method, J Comput Chem. 1989;10:209-220. https://doi.org/10.1002/jcc.540100208.
49. Hehre WJ, Ab Initio Molecular Orbital Theory, Acc Chem Res. 1976;9:399–406. https://doi.org/10.1021/ar50107a003.
50. Becke AD, Density-functional exchange-energy approximation with correct asymptotic behavior, Phys. Rev. A. 1988;38:3098-3100. https://doi.org/10.1103/PhysRevA.38.3098.
51. Naik S, Mild and eco-friendly chemoselective acylation of amines in aqueous medium, Arkivoc. 2004;1:55-63. https://doi.org/10.3998/ark.5550190.0005.104.
52. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG, Improving bioscience research reporting: The arrive guidelines for reporting animal research, Animals. 2013;4:35–44. https://doi.org/10.3390/ani4010035.
53. Comission E, Directive 2010/63/EU of the European parliament and of council of 22 September 2010 on the protection of animals used for scientific purposes. Off J Eur Union. 2010.

https://data.europa.eu/eli/dir/2010/63/oj.

54. OECD Acute Oral Toxicity – Fixed Dose Procedure (chptr). Oecd Guidel Test Chem. 2001:1–14. https://doi:10.1787/9789264070943-en.

55. Li J, Zhu J, Hu H, Harnett JE, Lei CI, Chau KY, Chan G, Ung COL, Internationalization of traditional/complementary medicine products: market entry as medicine, Chin Med. 2018;13:50. https://doi.org/10.1186/s13020-018-0209-6.

Tables

Table 1. Theoretical properties among acetaminophen (1) and its regioisomers (2-9).

Compound	HOMO (eV)	LUMO (eV)	GAP (eV)	IP (kcal/mol)	BDE_{OH} (kcal/mol)	BDE_{NH} (kcal/mol)
1	-5.89	-0.66	5.22	172.37	85.64	77.30
2	-6.11	-0.75	5.35	177.74	81.66	90.72
3	-5.97	-0.70	5.26	173.52	80.01	89.93
4	-4.97	-0.54	4.42	149.72	71.80	92.67
5	-5.75	-0.72	5.03	167.62	76.91	93.48
6	-5.57	-0.80	4.77	164.55	76.89	93.29
7	-6.24	-1.03	5.20	180.57	80.13	91.52
8	-6.26	-1.05	5.20	179.65	80.64	91.69
9	-6.82	-2.63	4.18	192.25	84.58	92.81

Table 2. Biochemical parameters after acute administration of chloramidaphen 300 mg/kg in male rats. [a] $p < 0.05$ vs Day 0 group. [b] $p < 0.001$ vs Day 0 group. [c] $p < 0.05$ vs Day 15 control group.

Parameter (unit)	Day 0 (n = 12)	Control (n = 2)		Treated (n = 4)	
		Day 8	Day 15	Day 8	Day 15
AST (U/L)	81.1 ± 15.6	145.6 ± 15.1[b]	171.5 ± 72.8[b]	110.6 ± 11.7[a]	128.5 ± 47.1[bc]
ALT (U/L)	114.1 ± 22.2	116.6 ± 6.8	96.0 ± 9.9	108.8 ± 15.5	101.8 ± 30.7
Creatinine (mg/dL)	0.40 ± 0.06	0.54 ± 0.10	0.65 ± 0.07	0.47 ± 0.03	0.53 ± 0.12
Urea (mg/dL)	51.8 ± 4.7	73.0 ± 6.5	72.2 ± 11.7	78.1 ± 14.0	69.4 ± 9.0

Table 3. Biochemical parameters after acute administration of chloramidaphen 2000 mg/kg in male rats. [a] $p < 0.05$ vs Day 0 group. [b] $p < 0.001$ vs Day 0 group.

Parameter (unit)	Day 0 (n = 12)	Control (n = 2)		Treated (n = 4)	
		Day 8	Day 15	Day 8	Day 15
AST (U/L)	81.1 ± 15.6	190.3 ± 0.6[b]	124.5 ± 6.4[a]	149.6 ± 29.8[b]	109.0 ± 35.0[b]
ALT (U/L)	114.1 ± 22.2	140.1 ± 48.9	73.5 ± 23.3[a]	122.7 ± 40.3	64.0 ± 19.7
Creatinine (mg/dL)	0.40 ± 0.06	0.50 ± 0.00	0.71 ± 0.13	0.42 ± 0.03	0.44 ± 0.06
Urea (mg/dL)	51.8 ± 4.7	53.9 ± 2.8	46.6 ± 7.1	65.6 ± 8.7	32.9 ± 8.9

Figures

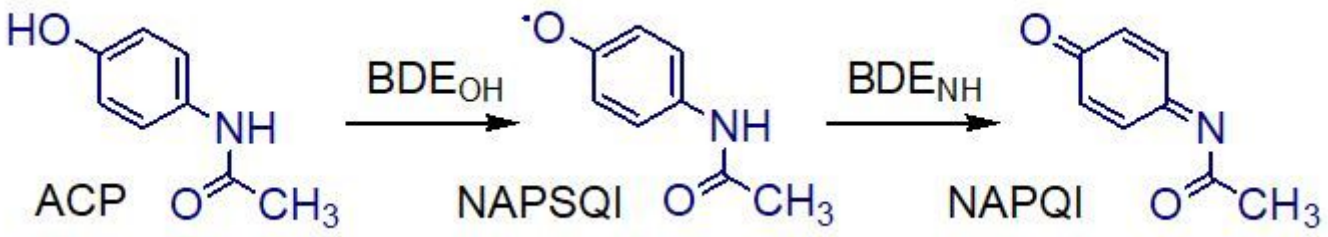

Figure 1

Energies to formation of NAPSQI and NAPQI from acetaminophen oxidation

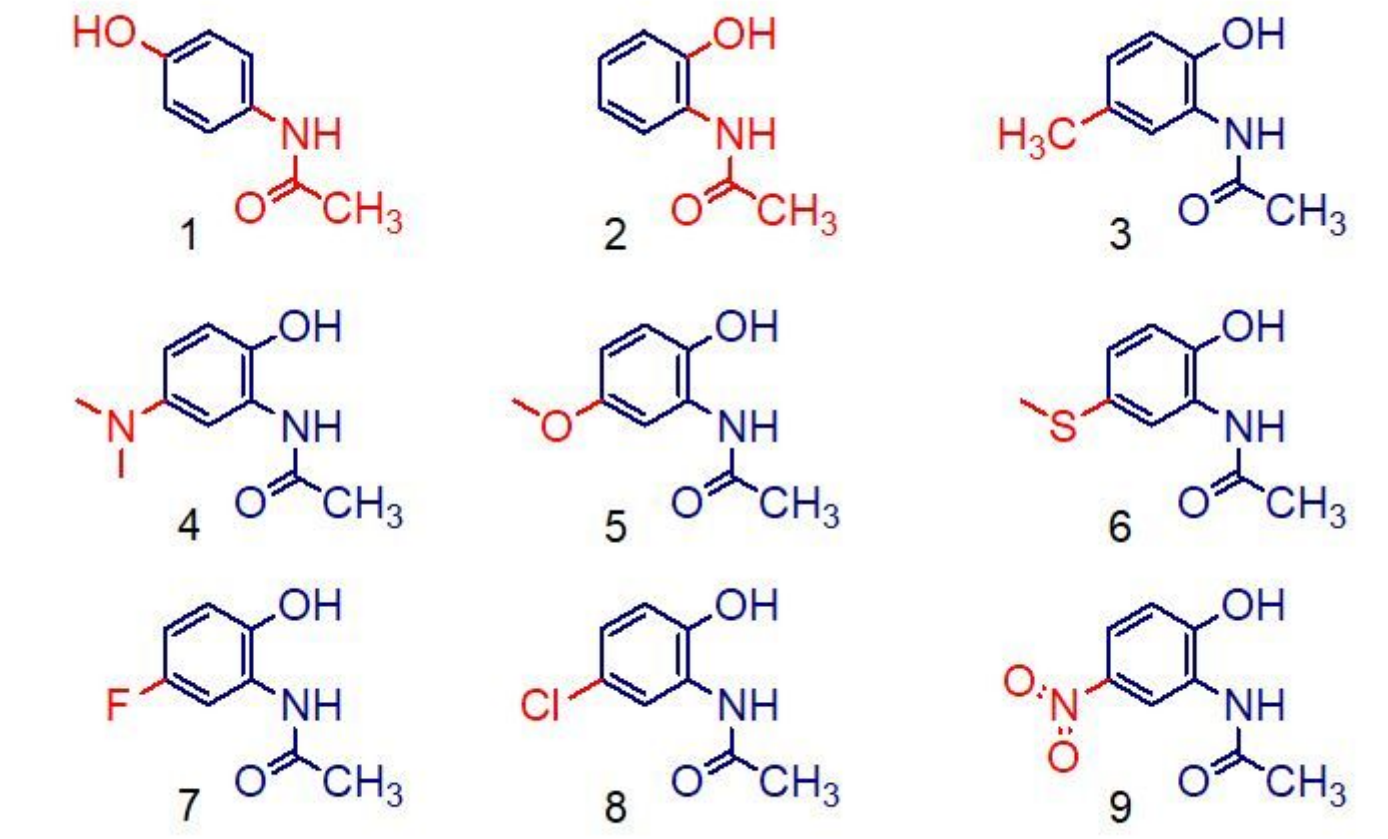

Figure 2

Molecular modification approach on acetaminophen

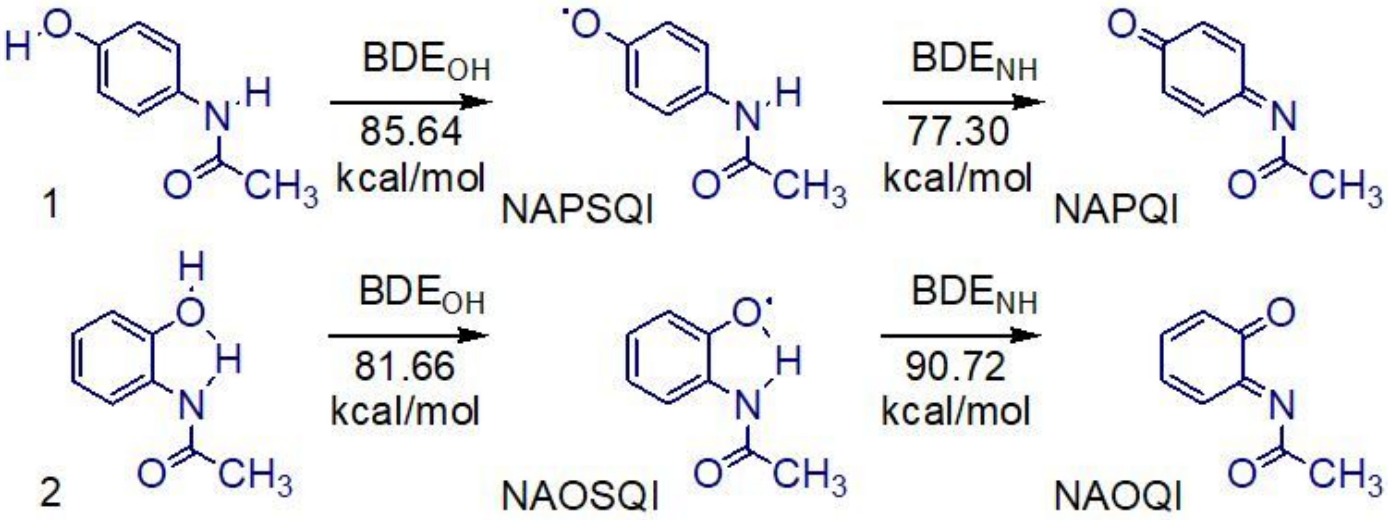

Figure 3

Differences on BDEOH and BDENH between acetaminophen (1) and its regioisomer (2)

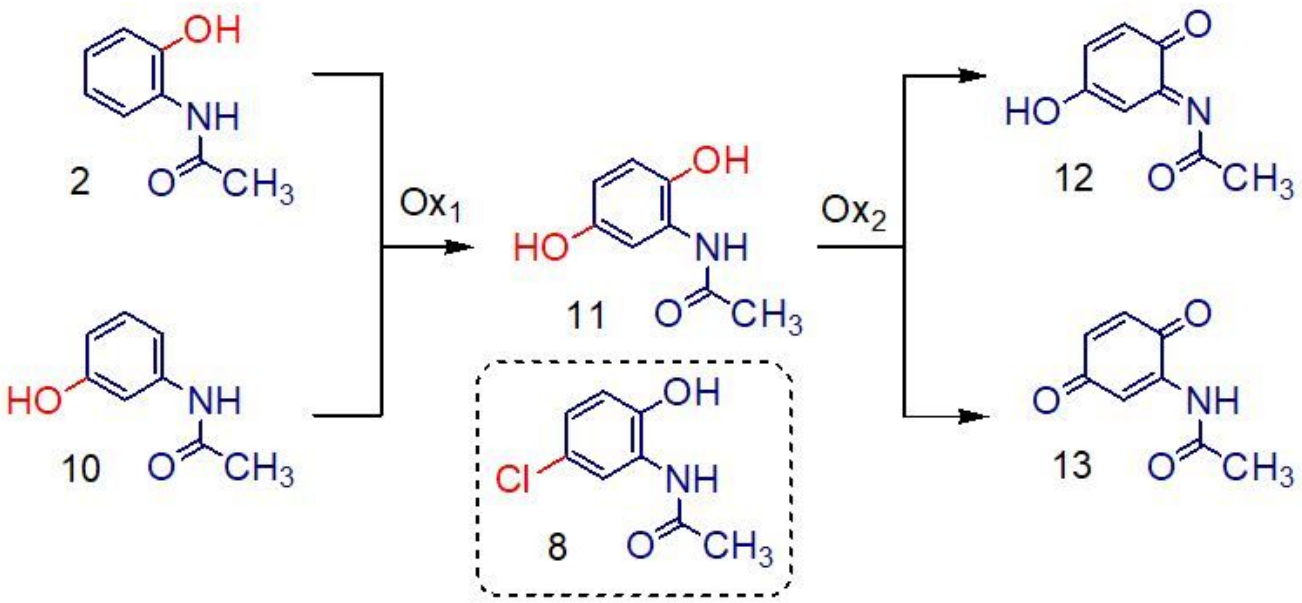

Figure 4

Molecular block of hydroxylation on acetaminophen regioisomers and quinone metabolism.

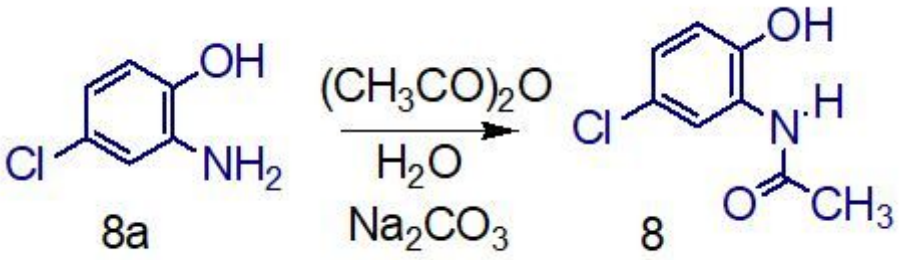

Figure 5

Synthesis reaction of chloramidaphen or 5-chloro-2-hydroxy-acetamide (8)