Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters 16 (2006) 3241–3244
Bioorganic & Medicinal Chemistry Letters

An exploratory theoretical elucidation on the peroxyl-radical-scavenging mechanism and structure–activity relationship of nonsteroidal anti-inflammatory drugs

Lan-fen Wang, Yu-guang Song, Xiu-feng Zhang and Yang Liu*

State Key Lab for Structural Chemistry of Unstable and Stable Species, Center for Molecular Sciences, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, China
Graduate School, Chinese Academy of Sciences, Beijing 100080, China

Received 8 December 2005; revised 6 March 2006; accepted 14 March 2006
Available online 4 April 2006

Abstract—The peroxyl-radical-scavenging mechanism of some nonsteroidal anti-inflammatory drugs (NSAIDs), namely tolmetin, ketorolac, indomethacin, acemetacin, and oxaprozin, is clarified by combined density functional theory (DFT) calculations. It is revealed that H-atom-abstraction rather than electron transfer reaction is involved in the radical-scavenging process of these NSAIDs in polar aqueous solution. This seems contrary to the common viewpoint that the latter is predominant in polar media. The calculated results also show that H-atom at C(β) or C(γ) position is readily to be abstracted, and the lowest C–H bond dissociation enthalpy (BDE) can qualitatively account for the activity difference for the five NSAIDs.

Peroxyl radical (ROO•) is one of reactive oxygen species (ROS) that are usually produced in inflammatory process and implicated in its pathophysiology.[1] Thus, a putative scavenging activity against this damaging species by anti-inflammatory drugs may be of great therapeutical value. Indomethacin is one of the widely studied indoleacetic nonsteroidal anti-inflammatory drugs (NSAIDs) and has been experimentally testified to efficiently scavenge trichloromethyl peroxyl-radical ($CCl_3O_2^{\bullet}$).[2] The possible scavenging mechanism is deduced as one electron transfer reaction according to the energy of the highest occupied molecular orbital (HOMO) of indomethacin calculated by RHF//AM1.[3] Recently, Fernandes et al. pointed out that several NSAIDs, including indomethacin, tolmetin, ketorolac, acemetacin and oxaprozin (see Scheme 1), can effectively inhibit the peroxyl-radical (AOO•, Scheme 1) derived from 2,2′-azobis(2-amidinopropane)dihydrochloride (AAPH).[1c] The order of the scavenging activity is tolmetin > ketorolac > indomethacin > oxaprozin ∼ acemetacin. Further observations exhibited that the five NSAIDs possess no pro-oxidant activity at all,[4] and therefore they are very good candidates in scavenging peroxyl-radical and worth paying attention to. However, how do these compounds scavenge AOO•? Whether they react through donating one electron to reduce AOO•, like the reaction between indomethacin and $CCl_3O_2^{\bullet}$, or via other reaction type?

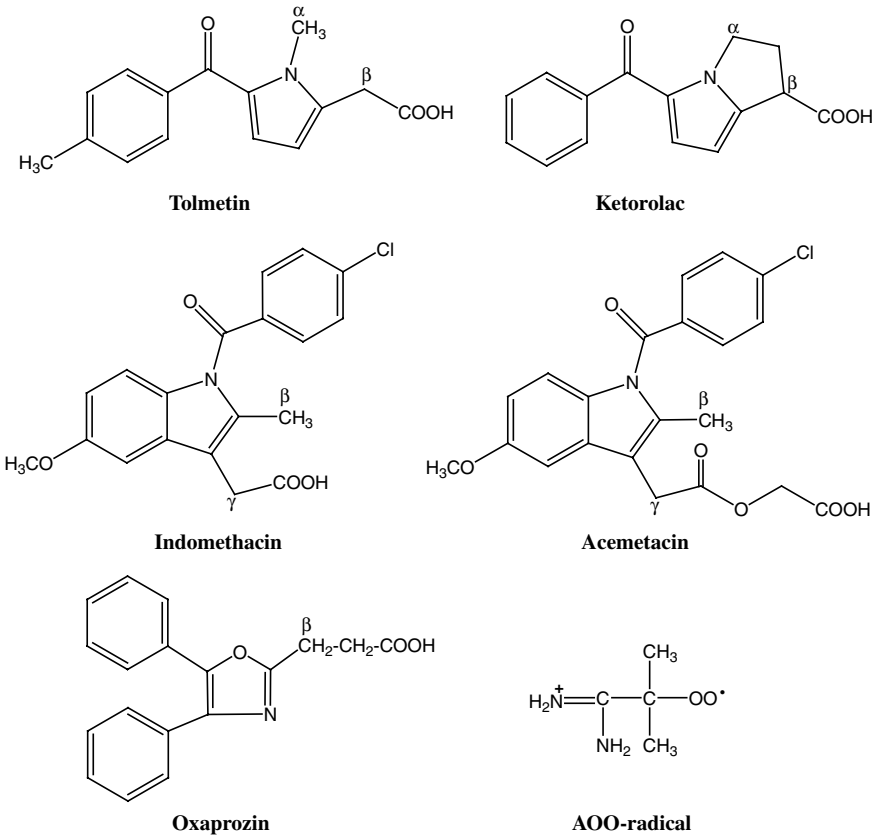
The study undertaken is going to answer the two questions above and to elucidate the structure–activity relationships (SAR) for the antioxidants that will be beneficial to design some new NSAIDs-derived antioxidants with better pharmacological effects. In view of the successful use of theoretical calculations in elucidating radical-scavenging mechanisms and SAR for various antioxidants,[5] we herein attempt to achieve the goal by means of density functional theory (DFT) calculations.

To characterize the radical-scavenging pathways, proper theoretical parameters have to be selected. Up to now, there exist two different routes for antioxidants to scavenge peroxide radical. One is a direct H-atom-abstraction process (Eq. 1)[6] and the other is stepwise electron transfer/proton transfer process (Eq. 2).[7]

$$ROO^{\bullet} + R'XH \rightarrow ROOH + R'X^{\bullet} \quad (1)$$

Keywords: Antioxidants; NSAIDs; DFT; Free radicals.
* Corresponding author. Tel.: +86 10 62571074; fax: +86 10 62559373; e-mail: yliu@iccas.ac.cn

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.03.054



Scheme 1. The structures of NSAIDs and AOO-radical.

$$\begin{aligned} \mathrm{ROO^{\bullet}} + \mathrm{R'XH} &\rightarrow \mathrm{ROO^{-}} + \mathrm{R'XH^{\bullet +}} \\ &\rightarrow \mathrm{ROOH} + \mathrm{R'X^{\bullet}} \end{aligned} \qquad (2)$$

In which, X represents O, N, S, or C atom. H-atom abstraction is preferred in nonpolar solvents,[6] and the reaction is governed to a great extent by the homolytic bond dissociation enthalpies (BDEs) of R′XH and ROOH.[5d,8] Only if the BDE value of the former is lower than that of the latter, the reaction is permitted. While the electron-transfer process predominates in polar solvents,[5d,7] the corresponding theoretical parameter is ionization potentials (IPs) for R′XH and ROO^{-}.[5d,9] The prerequisite for the reaction is that the IP value of R′XH is lower than that of ROO^{-}.

In this paper, taking advantage of accuracy and economy, BDEs were calculated by a combined density functional theory (DFT) proposed by Wright et al.,[10] labeled as (RO)B3LYP/6-311+G(2d,2p)//AM1/AM1. The elaborate calculation procedures are described as follows. The molecular geometries were optimized firstly, by molecular mechanic method MMX, and then, by semi-empirical quantum chemical method AM1.[11] Finally, (RO)B3LYP functional on the 6-311+G(2d,2p) level was used to gain the single-point electronic energies (SPEs). The AM1-calculated zero point vibrational energy (ZPVE) and thermal correction to energy (TCE) were scaled by a factor of 0.973.[12] Whereas, adiabatic IPs were calculated by (U)B3LYP/6-31G(d)//AM1/AM1. Using SPEs, the scaled ZPVE, and the scaled TCE, BDE and IP were obtained: $\mathrm{BDE} = [(\mathrm{SPE_R} + \mathrm{TCE_R} \times 0.973) + H_{\mathrm{H}} - (\mathrm{SPE_P} + \mathrm{TCE_P} \times 0.973)] \times 627.5095$; $\mathrm{IP} = [(\mathrm{SPE_C} + \mathrm{ZPVE_C} \times 0.973) - (\mathrm{SPE_P} + \mathrm{ZPVE_P} \times 0.973)] \times 627.5095$; where H_{H} is the enthalpy for hydrogen atom, −0.49765 hartree; the terms R, C and P in these equations stand for the radical generated after one H-atom-donating, the cation radical generated after electron-donating and the parent molecular, respectively. The solvent effect was also considered by employing the self-consistent reaction field (SCRF) method with polarizable continuum model (PCM).[9] All of the quantum chemical calculations were accomplished by Gaussian 03 program.[13]

Since the possible mechanism for the reaction of indomethacin with $CCl_3O_2^{\bullet}$ was proposed as one electron reduction,[3] a similar electron-transfer mechanism seems to be involved in the $AOO^{\bullet}$-scavenging process, and the IP is an appropriate parameter to evaluate the radical-scavenging ability of NSAIDs. However, the IPs[14] of NSAIDs parent molecules are actually higher than that of AOO^{-} (89.58 kcal/mol), indicating that the electron-transfer reaction between NSAIDs parent molecules and $AOO^{\bullet}$ should be forbidden thermodynamically. In the reported experimental procedure,[1c] all five NSAIDs were dissolved in water as the weak acids, so NSAIDs anions generated after proton dissociation from the carboxyl group should be taken into consideration. Considering their pK_a values are between 3 and 5,[15] all five NSAIDs predominately exist as the corresponding anions (>99%) under the

Table 1. C–H BDEs (in kcal/mol), IPs (in kcal/mol) and $ORACs_{ROO\cdot}$ (in μM trolox equivalents/μM compound) for NSAIDs ($T = 298.15$ K)

	C–H BDE[b]	IP[b]	$ORACs_{ROO\cdot} \pm SE$
Tolmetin-COO^- [a]	78.87 (β)	125.53	0.30 ± 0.06
	91.43 (α)		
Ketorolac-COO^-	77.48 (β)	123.34	0.24 ± 0.09
	89.91 (α)		
Indomethacin-COO^-	82.37 (γ)	114.43	0.17 ± 0.06
	81.97 (β)		
Acemetacin-COO^-	84.38 (γ)	133.59	0.06 ± 0.01
	85.78 (β)		
Oxaprozin-COO^-	83.00 (β)	129.76	0.09 ± 0.01
AOOH	100.00		
AOO^-		89.58	

[a] The anion of NSAIDs.
[b] Data in solvent condition.

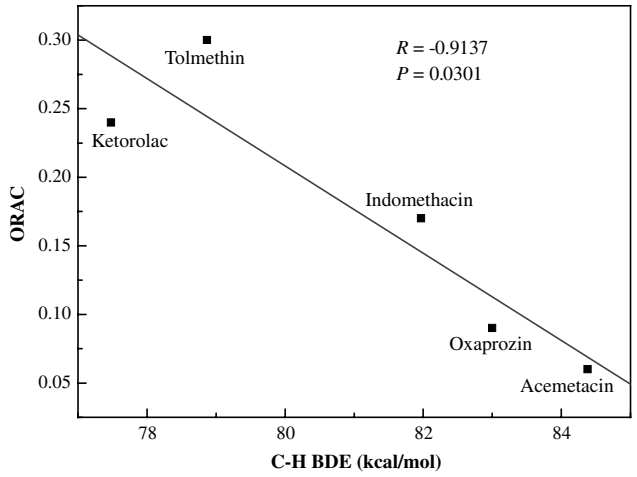


Figure 1. Correlation between the AOO·-scavenging activity ORAC and the lowest C–H BDE.

experimental condition (pH 7.0). However, the IPs for the anions are also obviously higher than that for AOO^- (Table 1), showing that the electron-transfer reaction between the anion molecules and AOO· is not permitted in a thermodynamic view. This implies that the real process for these compounds to scavenge AOO· is more complicated than a simple one-step electron-transfer mechanism, although it is contrary to the common idea that electron-transfer reaction dominates in polar solvents.

On the other hand, according to the molecular structures of the five NSAIDs (Scheme 1), one can find that they all possess potential reactive C–H bonds at α, β and γ sites that are easy to be dissociated by H-atom abstraction, because the formed carbon-center radical can be stabilized by *ortho* *p*–π conjugation effect arising from the pyrrolic-ring and/or the carboxyl group. Consequently, it can be speculated that AOO·-scavenging mechanism is probably a one-step direct H-atom donation. As indicated in Table 1, all C–H BDE calculated for the NSAIDs anions are evidently lower than that for AOOH (100.00 kcal/mol), indicating that the H-atom abstractions from all the anions are thermodynamically permitted in the AOO·-scavenging process. Table 1 shows that all the lowest C–H BDEs values appear at C(β) or C(γ) position, but not at C(α) position. Sequence of the lowest C–H BDE values is acemetacin > oxaprozin > indomethacin > tolmetin > ketorolac, which is basically in accord with the experimental order. Linear correlation analysis was carried out between C–H BDE and experimentally assayed activity-oxygen radical absorbance capability[2] (ORAC) that represents the AOO·-scavenging potency (Fig. 1, $R = -0.9137$, $P = 0.0301$). This correlation strongly supports the above assumption that the AOO·-scavenging mechanism is a direct H-atom abstraction of the anion molecules, and the active site is assigned to C(β) or C(γ) position. Actually, ORAC analysis is just one of the standard assays based on hydrogen atom transfer (HAT).[16]

On the basis of the H-atom abstraction mechanism, we attempt to shed light on the SAR for NSAIDs. Besides a central pyrrolic ring, a common structural feature exhibited by these drugs is an acetic acid group. According to structural optimization, after one hydrogen at C(β) is abstracted, the carboxyl group is nearly coplanar with the pyrrole-ring for two pyrrole derivatives, tolmetin and ketorolac, by which the corresponding radicals at C(β) can be obviously stabilized through strong *ortho* *p*–π conjugation effect of both pyrrolic ring and the carboxyl group. On the other hand, C(α)-central radical can only be stabilized by *p*–π conjugation effect from pyrrolic ring. Thus, C(β)-H BDE is much lower than C(α)-H BDE, and H-atom at C(β)-H is much more readily to be abstracted than that at C(α)-H for two pyrrole derivatives. However, for indomethacin and acemetacin (two indole derivatives), there exists a certain angle between indole-ring and the corresponding carboxyl group for the radical derived at C(γ), and thereby their conjugation stabilizing effect decreases a lot.[17] Consequently, as shown in Table 1, the C(γ)-H BDEs for indole derivatives averagely increase 5-6 kcal/mol, relative to the C(β)-H BDE of the similar sites for pyrrole derivatives, which may result in that indomethacin and acemetacin exhibit lower AOO·-scavenging ability than tolmetin and ketorolac. Beside the C(γ)–H potential active site, the hydrogen atom at C(β) is another proper candidate because of a similar *p*–π conjugation effect from indole-ring to stabilize the C(β)-central radical. Therefore, the calculated C(β)–H BDEs are almost equal to C(γ)–H BDEs. Regarding oxaprozin, except for the *p*–π conjugation effect from the central-ring, there is no *ortho* *p*–π conjugation effect resulting from the carboxyl group to stabilize the radical structure, since the carboxyl group is in the *meta*-position to the produced C(β)-central radical. Despite of this, two phenyl groups can, to a certain extent, stabilize the C(β)-central radical. The ultimate result is that oxaprozin possesses a comparable AOO·-scavenging potency with acemetacin. However, in vivo, acemetacin will be rapidly converted to indomethacin,[18] and therefore, is actually expected to exhibit better peroxyl-radical-scavenging capacity than oxaprozin.

In brief, H-atom-abstraction mechanism can be used to interpret the AOO·-scavenging potency of the five

NSAIDs, in which cleavage of C(β) or C(γ)–H bonds occurs. Meanwhile, the lowest C–H bond dissociation enthalpy (BDE) can qualitatively account for the activity difference.

Acknowledgments

The investigation was supported by the National Natural Science Foundation of China (No. 30570446, No. 30128003 and No. 20473098) and the Outstanding Overseas Chinese Scholars Fund of Chinese Academy of Sciences (2005-1-12).

References and notes

1. (a) Musser, J. H.; Kreft, A. F. *J. Med. Chem.* **1992**, *35*, 2501; (b) Nikolic, D.; Breemen, R. B. *Chem. Res. Toxicol.* **2001**, *14*, 351; (c) Fernandes, E.; Costa, D.; Toste, S. A.; Lima, J. L. F. C.; Reis, S. *Free Radic. Biol. Med.* **2004**, *37*, 1895.
2. Hiller, K.-O.; Hodd, P. L.; Willson, R. L. *Chem. Biol. Interact.* **1983**, *47*, 293.
3. Zoete, V.; Bailly, F.; Maglia, F.; Rougee, M.; Bensasson, R. V. *Free Radic. Biol. Med.* **1999**, *26*, 1261.
4. Parij, N.; Nagy, A. M.; Neve, J. *Free Radic. Res.* **1995**, *23*, 571.
5. (a) de Heer, M. I.; Korth, H.-G.; Mulder, P. *J. Org. Chem.* **1999**, *64*, 6969; (b) Zhang, H. Y. *J. Am. Oil Chem. Soc.* **1999**, *76*, 745; (c) Zhang, H. Y. *Quant. Struct.-Act. Relat.* **2000**, *19*, 50; (d) Wright, J. S.; Johnson, E. R.; DiLabio, G. A. *J. Am. Chem. Soc.* **2001**, *123*, 1173; (e) Pratt, D. A.; DiLabio, G. A.; Brigati, G.; Pedulli, G. F.; Valgimigli, L. *J. Am. Chem. Soc.* **2001**, *123*, 4625; (f) Bakalbassis, E. G.; Chatzopoulou, A.; Melissas, V. S.; Tsimidou, M.; Tsolaki, M.; Vafiadis, A. *Lipids* **2001**, *36*, 181; (g) Zhang, H. Y.; Wang, L. F. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 225; (h) Zhang, H. Y.; Wang, L. F.; Sun, Y. M. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 909; (i) Wang, L. F.; Zhang, H. Y. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3789; (j) Zhang, H. Y. *Curr. Comput.-Aided Drug Des.* **2005**, *1*, 257.
6. (a) Burton, G. W.; Le Page, Y.; Gabe, E. J.; Ingold, K. U. *J. Am. Chem. Soc.* **1980**, *102*, 7791; (b) Burton, G. W.; Doba, T.; Gabe, E. J.; Hughes, L.; Lee, F. L.; Prasad, L.; Ingold, K. U. *J. Am. Chem. Soc.* **1985**, *107*, 7053; (c) Foti, M. C.; Johnson, E. R.; Vinqvist, M. R.; Wright, J. S.; Barclay, L. R. C.; Ingold, K. U. *J. Org. Chem.* **2002**, *67*, 5190; (d) Zhang, H. Y.; Wang, L. F. *J. Phys. Chem. A* **2003**, *107*, 11258.
7. (a) Jovanovic, S. V.; Steenken, S.; Tosic, M.; Marjanovic, B.; Simic, M. G. *J. Am. Chem. Soc.* **1994**, *16*, 4846; (b) Jovanovic, S. V.; Steenken, S.; Hara, Y.; Simic, M. G. *J. Chem. Soc. Perkin Trans. 2* **1996**, *11*, 2497; (c) Litwinienko, G.; Ingold, K. U. *J. Org. Chem.* **2003**, *68*, 3433; (d) Nakanishi, I.; Miyazaki, K.; Shimada, T.; Ohkubo, K.; Urano, S.; Ikota, N.; Ozawa, T.; Fukuzumi, S.; Fukuhara, K. *J. Phys. Chem. A* **2002**, *106*, 11123; (e) Mayer, J. M.; Rhile, I. J. *Biochim. Biophys. Acta* **2004**, *1655*, 51.
8. (a) Van Acker, S. A. B. E.; Koymans, L. M. H.; Bast, A. *Free Radic. Biol. Med.* **1993**, *15*, 311; (b) Migliavacca, E.; Carrupt, P. A.; Te Sta, B. *Helv. Chim. Acta* **1997**, *80*, 1613; (c) Zhang, H. Y. *J. Am. Oil Chem. Soc.* **1998**, *75*, 1705; (d) Zhang, H. Y.; Sun, Y. M.; Zhang, G. Q.; Chen, D. Z. *Quant. Struct.-Act. Relat.* **2000**, *19*, 375.
9. (a) Kong, L.; Sun, Z. L.; Wang, L. F.; Zhang, H. Y.; Yao, S. D. *Helv. Chim. Acta* **2004**, *87*, 511; (b) Nenadis, N.; Wang, L. F.; Tsimidou, M.; Zhang, H. Y. *J. Agric. Food Chem.* **2004**, *52*, 4669; (c) Wang, L. F.; Zhang, H. Y. *Bioorg. Chem.* **2005**, *33*, 108.
10. Wright, J. S.; Carpenter, D. J.; McKay, D. J.; Ingold, K. U. *J. Am. Chem. Soc.* **1997**, *119*, 4245.
11. Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.
12. DiLabio, G. A.; Pratt, D. A.; LoFaro, A. D.; Wright, J. S. *J. Phys. Chem. A* **1999**, *103*, 1653.
13. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B. et al. *Gaussian 03, Revision A. 1*, Gaussian, Inc.: Pittsburgh, PA, **2003**.
14. The IP values of the parent molecules of tolmetin, ketorolac, indomethacin, acemetacin and oxaprozin are in turn 128.12 kcal/mol, 132.52 kcal/mol, 122.19 kcal/mol, 123.45 kcal/mol and 126.00 kcal/mol.
15. Bosek, V.; Cox, C. E. *Ann. Surg. Oncol.* **1996**, *3*, 62.
16. Huang, D.; Ou, B.; Prior, R. L. *J. Agric. Food Chem.* **2005**, *53*, 1841.
17. For indomethacin and acemetacin, the angles for the anion radicals after one hydrogen being abstracted are 140° and 58.3°, respectively.
18. Jones, R. W.; Collins, A. J.; Notarianni, L. J.; Sedman, E. *Br. J. Clin. Pharmacol.* **1991**, *31*, 543.