Bioorganic & Medicinal Chemistry Letters xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

The antioxidant properties of salicylate derivatives: A possible new mechanism of anti-inflammatory activity

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ARTICLE INFO

Article history:
Received 1 May 2015
Revised 29 June 2015
Accepted 1 July 2015
Available online xxxx

Keywords:
Salicylate derivatives
Anti-inflammatory
Antioxidant mechanism
DFT
DPPH·

ABSTRACT

The synthesis and antioxidant evaluation by DPPH· scavenging of a series of salicylic acid derivatives is described. Gentisic acid and its ester, amide, and amino analogs possess more radical scavenging capacity than salicylic acid and other salicylate derivatives. This property can possibly provide an additional pathway for anti-inflammatory activity through either single electron or hydrogen atom transfer, leading to a new strategy for the design of anti-inflammatory agents.

Acetylsalicylic acid (ASA) or aspirin is the most widely used drug in the world.[1] It reduces the risk of many diseases associated with aging,[2] acting via dose-dependent inhibition of arachidonic acid oxidation and prostaglandin production.[3] The enzymatic inhibition of cyclooxygenase (COX) or prostaglandin endoperoxide synthase (PGES)[4] occurs through selective and irreversible acetylation of a single serine residue (Ser 530), an event that inhibits the binding of arachidonic acid.[5] However, at high concentrations and over long periods of time, aspirin will also nonspecifically acetylate a variety of proteins and nucleic acids.[6]

It has been suggested that the biological activity of aspirin extends beyond its ability to acetylate Ser-530. Specifically, the interaction between its carboxylate group and Arg-120 is proposed to contribute to its COX inhibitory properties.[7] Moreover, the ability of ASA to acetylate PGES does not depend on the relative positions of its acetyl and carboxylate groups. Thus, the fact that only acetylsalicylic acid with its *o*-substitution pattern and not the *m*- and *p*-isomers can irreversibly inhibit PGES demonstrates that processes other than acetylation must also be involved.[8] In fact, PGES has two activities that are required for the production of PGH_2, the essential precursor of prostaglandins, thromboxane, and prostacyclin. The first activity catalyzes the oxygenation and cyclization of arachidonic acid within the COX active site to produce PGG_2.[9] In addition to the key Ser-530 residue, a tyrosyl radical present in this active site is critical to the oxidation process.[10] Thus, it has been suggested that the capacity of NSAIDs to quench this radical may play a role in PGES inhibition,[11] but this hypothesis has not been fully explored for salicylates. The second activity involves two-electron reduction of PGG_2, yielding PGH_2 at the peroxidase active site.[12] Clearly, the complexity of this enzyme provides multiple pathways for its inhibition.

Reactive species such as the hydroxyl radical (HO·) and the superoxide radical anion ($O_2^{\cdot-}$) cause oxidative stress, which is important to the pathogenesis and progression of many diseases.[13] For example, inflammatory tissue injuries are mediated by reactive oxygen metabolites from phagocytic leukocytes.[14] While the connection between the antioxidant properties of a given substance and its antiproliferative activity is well-known,[15] in general the correlation between antioxidant ability and anti-inflammatory activity[16] is not as widely recognized.

The low chemical and metabolic stability due to hydrolysis and oxidation is an important aspect related to the use of salicylates. For example, ASA is hydrolyzed to furnish salicylic acid (**1**) and acetic acid (Scheme 1).[17] Salicylic acid is very sensitive to oxidation, as its hydroxylation by liver microsomal hydroxylases forms 2,5-dihydroxybenzoic acid or gentisic acid (**2**).[18] This compound can react further to produce other hydroxylated derivatives by HO· attack.[19] Consequently, it is likely that the in vivo concentration of aspirin and related salicylates decreases as a result of hydrolysis and aromatic hydroxylation. Hydroxylation of benzoic

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http://dx.doi.org/10.1016/j.bmcl.2015.07.001
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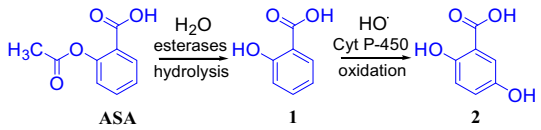

Scheme 1. Enzymatic and non-enzymatic metabolism of salicylate derivatives.

acid derivatives has been shown to affect the ability of these compounds to function as antioxidants.[20]

Surprisingly, the antioxidant properties of hydroxylated salicylate derivatives and their potential roles in the inflammation process have not been examined thoroughly.[21] Herein, we report a theoretical and experimental study of the antioxidant properties of salicylate derivatives including hydroxylated metabolites. As part of this work, ester and amide derivatives of gentisic acid were synthesized and evaluated for their antioxidant activity using the DPPH· method. Based on the concept of bioisosteric substitution, an acetamide analog of gentisic acid was also synthesized and evaluated.

First, the capacities of ASA, salicylic acid (**1**), and gentisic acid (**2**) to function as antioxidants via single electron transfer were studied computationally using density functional calculations at the B3LYP level of theory with the 6-31+G(d,p) basis set.[22] The results are summarized in Table 1. As expected, gentisic acid showed the highest HOMO energy value (−6.11 eV) due to the fact that it is the most electron-rich of these three compounds. Since the differences in LUMO energy values were relatively small, gentisic acid also possessed the smallest HOMO–LUMO gap (4.14 eV). Accordingly, the ionization potential (IP)[23] of gentisic acid was smaller than that of salicylic acid and ASA, rendering it a stronger single electron donor than either of these two compounds. Consequently, it is possible that gentisic acid could inhibit PGES via quenching its tyrosyl radical.[10] Under this scenario, acetylsalicylic and salicylic acids would function as prodrugs that are converted in vivo into a more potent antioxidant.[24]

In order to gain a better understanding of the antioxidant capacity of gentisic acid and search for more potent derivatives, a structure–activity relationship (SAR) study was performed. Initially, the properties of gentisic acid analogs with various substituents including electron-donating groups (EDG) and electron-withdrawing groups (EWG) at C-5 of the benzene ring were calculated and compared to those of gentisic acid. The structures of the compounds studied are shown in Figure 1. The fact that each of these compounds is commercially available simplified the subsequent experimental study. In addition to single electron transfer, the ability of compounds **1**–**7** to effect hydrogen atom transfer was

Table 1
Theoretical properties of salicylate derivatives

Compound	HOMO (eV)	LUMO (eV)	GAP[a] (eV)	IP[b] (kcal/mol)	BDE_{OH}[c] (kcal/mol)	BDE_{XH}[d] (kcal/mol)
ASA	−7.08	−1.89	5.18	198.31	—	—
1	−6.63	−1.91	4.71	194.07	109.91	—
2	−6.11	−1.97	4.14	180.15	104.79	93.54
3	−6.37	−1.85	4.52	186.00	100.85	—
4	−5.98	−1.88	4.09	174.98	97.41	—
5	−5.66	−1.81	3.84	165.87	92.60	—
6	−6.67	−2.20	4.46	192.13	101.92	—
7	−7.50	−2.94	4.56	211.78	114.09	—
8	−5.98	−1.78	4.20	183.04	107.68	93.07
9	−5.96	−1.70	4.26	182.81	92.22	92.30
10	−6.12	−1.98	4.14	175.71	95.94	104.45

[a] *E*LUMO − *E*HOMO.
[b] Ionization potential.
[c] Bond dissociation energy of OH (C-2).
[d] Bond dissociation energy of XH (C-5).

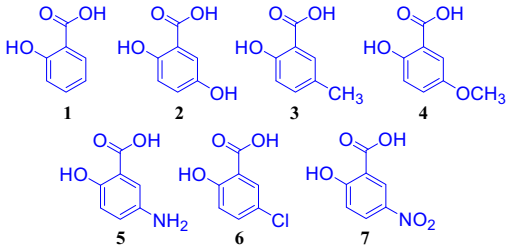

Figure 1. Analogs of gentisic acid.

considered by calculating the bond dissociation energy of the *o*-hydroxyl group (BDE_{OH}).[25] We were interested in determining if any of the compounds **3**–**7** would show greater potential as an antioxidant than gentisic acid (**2**).

The results of the theoretical study are shown in Table 1. 5-Aminosalicylic acid (**5**), which possesses the strongest EDG at C-5, showed both the highest HOMO energy value (−5.66 eV) and the smallest HOMO–LUMO gap (3.84 eV). Furthermore, **5** exhibited the smallest IP and BDE_{OH} values (165.87 and 92.60 kcal/mol, respectively), demonstrating its strong potential as an antioxidant. On the other hand, the compound with the strongest EWG, 5-nitrosalicylic acid (**7**), showed the lowest HOMO and LUMO energy values (−7.50 eV and −2.20 eV, respectively) and the second-largest HOMO–LUMO gap (4.46 eV, second only to salicylic acid). Consequently, the IP and BDE_{OH} values of **7** were very high (211.78 and 114.09 kcal/mol, respectively), indicating that attachment of an EWG to the salicylate skeleton reduces the antioxidant capacity.

In general, the antioxidant capacity by single electron transfer increased in the following order: 5-nitrosalicylic acid (**7**) < salicylic acid (**1**) < 5-chlorosalicylic acid (**6**) < 5-methylsalicylic acid (**3**) < gentisic acid (**2**) < 5-methoxysalicylic acid (**4**) < 5-aminosalicylic acid (**5**). However, there were some variations to the order in relation to hydrogen atom transfer (**7** < **1** < **2** < **6** < **3** < **4** < **5**). Clearly, the compounds with an EDG have better antioxidant capacity than those possessing an EWG. Furthermore, 5-aminosalicylic acid (**5**) had the most potent antioxidant capacity with respect to either single electron or hydrogen atom transfers.

In an effort to compare theory with experiment, the radical scavenging activity of compounds **1**–**7** was evaluated with the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH·).[26] The DPPH· inhibition by gentisic acid and other salicylate derivatives was evaluated at doses of 0 mM, 0.01 mM, 0.1 mM, 1 mM, and 10 mM (Fig. 2). Salicylic acid (**1**) displayed low radical scavenging capacity at all tested concentrations. Interestingly, the radical scavenging activity of gentisic acid (**2**) was comparable to that of 5-aminosalicylic acid (**5**) at higher concentrations, but **5** was more active at 0.01 mM. 5-Methoxysalicylic acid (**4**) showed moderate activity at 10 mM and 1 mM, and the remaining compounds showed only mild activity at the highest concentration studied.

The observed order of radical scavenging activity (**1** < **3** < **7** < **6** < **4** < **2** < **5**) is likely affected by several properties including the presence of an EDG or an EWG, polarizability, resonance effects, inductive effects, and solubility. Nonetheless, the results are in general agreement with the calculations of IP and BDE_{OH} shown in Table 1. They are in also in accordance with biological studies that related the effects of cyclooxygenase inhibitors such as nimesulide, flurbiprofen, and diclofenac to their ability to transfer an electron to the aforementioned tyrosyl radical in the active site of the enzyme.[24]

In previous studies, 5-aminosalicylic acid (**5**), which is also known as mesalazine, exhibited potent antioxidant[27] and anti-inflammatory[28] activities. However, it was also shown to possess