European Journal of Medicinal Chemistry 44 (2009) 4841–4847

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Captopril and 6-mercaptopurine: Whose SH possesses higher antioxidant ability?

Guo-Xiang Li, Zai-Qun Liu*

Department of Organic Chemistry, College of Chemistry, Jilin University, No. 2519 Jiefang Road, Changchun 130021, China

ARTICLE INFO

Article history:
Received 20 April 2009
Received in revised form
11 July 2009
Accepted 23 July 2009
Available online 30 July 2009

Keywords:
Captopril
6-Mercaptopurine
Free radical
Antioxidant

ABSTRACT

Antioxidant capacities of captopril (CAP), 6-mercaptopurine (6-MP) and 9-(β-D-ribofuranosyl)-6-mercaptopurine (6-MPR) were investigated by interacting them with 2,2′-diphenyl-1-picrylhydrazyl (DPPH), galvinoxyl radical, and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) cation radical ($ABTS^{+\bullet}$), and by protecting DNA and erythrocyte against 2,2′-azobis(2-amidinopropane hydrochloride) (AAPH) induced oxidation. It was found that CAP possessed the highest ability to donate the hydrogen atom in –SH to DPPH and galvinoxyl, while 6-MPR had the strongest ability to reduce $ABTS^{+\bullet}$. In the process of protecting DNA and erythrocytes against AAPH-induced oxidation, CAP can trap 0.5 and 1.3 radicals, 6-MP can trap 0.6 and 2.2, and 6-MPR can trap 1.0 and 3.0 radicals, respectively. CAP can also protect erythrocytes against hemin-induced hemolysis.

1. Introduction

Captopril (CAP, D-3-mercapto-2-methylpropanoyl-L-proline), an angiotensin converting enzyme (ACE) inhibitor, is widely used in the treatment of hypertension and congestive heart failure, and in the inhibition of proliferation of a variety of cell lines [1]. Nowadays, researches have been focused on the evaluation of the toxicity of CAP derivative *in vivo* [2], and on the design of novel drugs derived from CAP [3]. 6-Mercaptopurine (6-MP) is an effective drug in the treatment of inflammatory bowel disease, in which some allergic symptoms are found clinically [4]. The investigation of 6-MP on human embryonic kidney cell line reveals that it is able to inhibit the methylation of global DNA [5]. It is also proved that 6-MP may induce apoptotic cell death in neural cells *in vivo* [6], and its inosine monophosphate dehydrogenase may affect the *de novo* biosynthesis of guanine nucleotides and thiopurines metabolism [7]. The mechanism of 6-MP together with 9-(β-D-ribofuranosyl)-6-mercaptopurine (6-MPR) to prevent cancer is clarified recently [8], and 6-MP and 6-MPR are also found to repair the damage of DNA induced by the irradiation of ultraviolet [9].

2. Chemistry

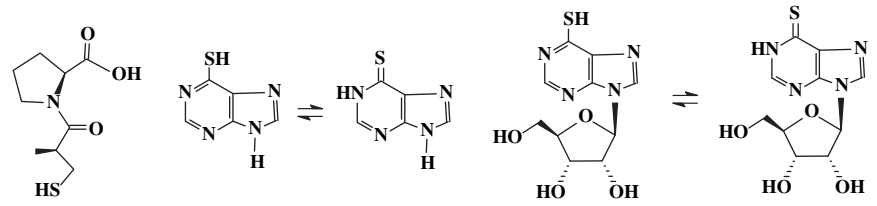
In addition to the aforementioned cytotoxic studies on various cell lines, it is necessary to compare the free-radical-scavenging properties of CAP, 6-MP and 6-MPR chemically since SH group in CAP attaches to an aliphatic chain while SH group in 6-MP attaches to an aromatic ring. Hence, CAP, 6-MP and 6-MPR react with galvinoxyl [10], 2,2′-diphenyl-1-picrylhydrazyl (DPPH) [11], and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) cationic radical ($ABTS^{+\bullet}$) [12] to screen the abilities of SH in CAP, 6-MP and 6-MPR to donate its hydrogen atom to O-centered, N-centered and oxidant-type radical, respectively. The aim of this test is to give direct evidence for CAP, 6-MP and 6-MPR to trap radicals (Scheme 1).

3. Pharmacology

Chemical kinetic deduction gives the relationship between the concentration of an antioxidant and the inhibition period generated by the antioxidant to protect linoleic acid against the oxidation induced by 2,2′-azobis(2-amidinopropane hydrochloride) (AAPH) as shown as Eq. (3) (*vide post*) [13]. We have applied this equation to treat the obtained data from AAPH-induced oxidation of DNA [14] and erythrocytes [15] in the presence of antioxidants. The application of chemical kinetic equation to treat the obtained data obtained from DNA and erythrocytes is beneficial to give the number of radicals trapped by SH group in CAP, 6-MP and 6-MPR. Moreover, AAPH-induced oxidation of DNA and hemolysis of erythrocytes are *in vitro* experimental systems to mimic DNA and erythrocytes undergoing radical-induced oxidative stress. So, another aim of this work is to introduce this *in vitro* experimental method to be employed in the research of pharmacology. Finally, the stabilization effects of these thiols on erythrocyte membrane are evaluated in hemin-induced hemolysis of erythrocytes. The structures of radicals and hemin used in this work are illustrated in Scheme 2.

* Corresponding author. Tel.: +86 431 88499175; fax: +86 431 88499159.
E-mail address: zaiqun-liu@jlu.edu.cn (Z.-Q. Liu).

0223-5234/$ – see front matter © 2009 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2009.07.020



captopril, **CAP** 6-mercaptopurine, **6-MP** 9-(*beta*-D-ribofuranosyl)-6-mercaptopurine, **6-MPR**

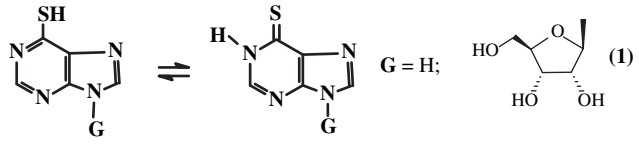
Scheme 1. Structures of captopril, 6-mercaptopurine, and 9-(β-D-ribofuranosyl)-6-mercaptopurine.

4. Results and discussion

4.1. CAP, 6-MP, and 6-MPR scavenge radicals

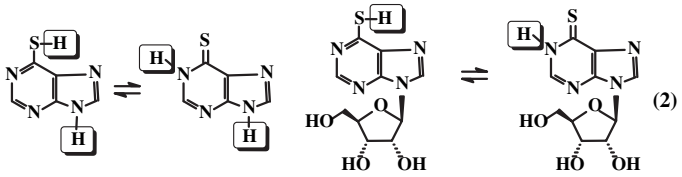
Fig. 1 outlines the percentages of DPPH, galvinoxyl, and $ABTS^{+\bullet}$ scavenged by various concentrations of CAP, 6-MP, and 6-MPR, and the concentrations when 50% radicals are trapped (IC_{50}) are listed in Table 1.

The sequences of IC_{50} are found as CAP < MP < 6-MPR when they react with DPPH, and CAP < 6-MPR < 6-MP when they react with galvinoxyl. Low values of IC_{50} of CAP indicate that CAP donates it hydrogen atom in SH group to either N-centered or O-centered radicals more readily than 6-MP and 6-MPR. This is because the hydrogen atom in SH attaching to an aliphatic carbon chain can donate to radicals directly, whereas, as shown in Eq. (1), the hydrogen atom in SH attaching to an aromatic ring may tautomerize to form a N–H [16], whose hydrogen atom cannot be abstracted by DPPH and galvinoxyl as easy as the hydrogen atom in SH.



G = H; (1)

Furthermore, IC_{50} of 6-MP reacting with DPPH is lower than that of 6-MPR, indicating that 6-MP donates its hydrogen atom more easily than its riboside does. This is because 6-MP does not only donate its hydrogen atom from SH group, but also from two N–H bonds [17]. However, 6-MPR donates its hydrogen atom only from SH group and one N–H bond as shown in Eq. (2).



(2)

On the contrary, when 6-MP and 6-MPR react with galvinoxyl, the IC_{50} of 6-MPR is lower than that of 6-MP, indicating that 6-MPR donates its hydrogen atom more readily to O-centered radical in galvinoxyl. This may be due to more hydroxyl groups contained in riboside that have high ability to exchange its hydrogen in O–H with O-centered radicals [18]. A converse sequence of IC_{50}, 6-MPR < 6-MP < CAP, is found when these thiols react with $ABTS^{+\bullet}$. Low value of IC_{50} of 6-MPR reveals that 6-MPR possesses the highest ability to reduce $ABTS^{+\bullet}$ since the voltammetric determination of 6-MPR indicated that the reduction peak of 6-MPR is as low as −1.6 V [19]. Therefore, SH attaching to aliphatic carbon chain possesses relatively high ability to donate its hydrogen atom, whereas, SH attaching to aromatic ring has high ability to reduce radical. Whether this conclusion is still available in biological experimental system is another concern in this work. So, radical-induced oxidations of erythrocyte and DNA act as *in vitro* models of biological samples under oxidative stress to compare the antioxidant capacities of these thiols.

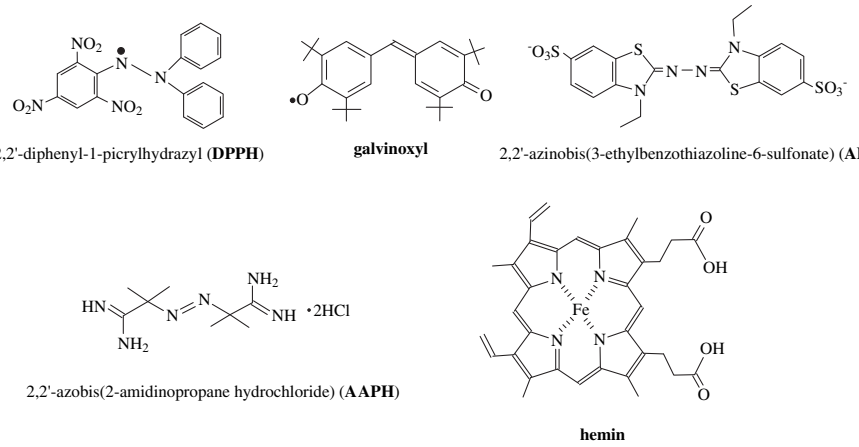
4.2. CAP, 6-MP, and 6-MPR protect DNA against AAPH-induced oxidation

The decomposition of AAPH at 37 °C forms a C-centered radical that converts into peroxyl radical ($ROO^{\bullet}$) by combining with oxygen. $ROO^{\bullet}$ can abstract H atom from the C-4′ atom of DNA and cause strand breaks [20]. The further oxidation of linear DNA generates more than 20 carbonyl species, which can react with thiobarbituric acid (TBA) to form colorful thiobarbituric acid reactive substance (TBARS, $\lambda_{max} = 535$ nm) [21]. As can be seen in Fig. 2, in the blank experiment the increase of the absorbance of TBARS indicates that more carbonyl species are generated with the reaction period increasing. The slope of the line can be regarded as the formation rate of the carbonyl species during the oxidation of DNA. However, the additions of thiols bend the increase line of TBARS. With the concentrations of thiols increasing, the absorbance at the first point rises remarkably. This may be due to the reaction between thiols and TBA to form colorful product measured at 535 nm. With the reaction time increasing, the colorful product formed from the interaction between thiols and TBA decreases to a minimum value. Then, TBARS generated from the oxidation of DNA increases with the same rate as blank experiment. As shown in Fig. 2, the time corresponding to the cross point of two tangents (dot lines) can be regarded as the inhibition period (t_{inh}) derived from the addition of thiols. Fig. 3 illustrates that t_{inh} increases with the concentration of thiols employed. The quantitative equation between t_{inh} and the concentration of thiols can be obtained by linear regression analysis, and listed in Table 2.

In order to understand the meaning of the equation of $t_{inh} \sim [thiols]$, as shown as Eq. (3), chemical kinetic equation related to the concentration for antioxidant (AH) [22] is applied to treat the results from the oxidation of DNA inhibited by thiols.

$$t_{inh} = (n/R_i)[AH] \quad (3)$$

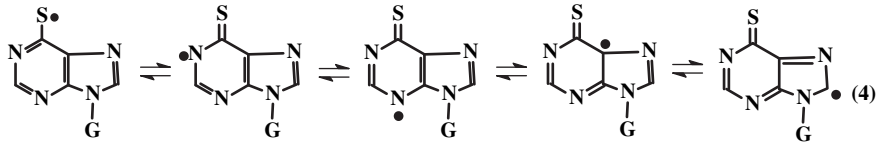
The stoichiometric factor (n) can be regarded as the radicals trapped by one molecule of antioxidant, and therefore, used as



Scheme 2. Structures of radicals and hemin used in this work.

a quantitative index to express the ability of an antioxidant to inhibit the radical-induced oxidation. R_i is the initiation rate of a radical-induced reaction. If R_i is known, n can be obtained by the product of R_i multiplying the coefficient in Eq. (3). However, it is difficult to measure R_i directly. So, in order to apply Eq. (3) to calculate n of an antioxidant, trolox (6-hydroxyl-2,5,7,8-tetramethylchroman-2-carboxylic acid) is assigned to be the reference antioxidant, and various t_{inh} generated by different concentrations of trolox are first measured. Then, R_i results from the equation of $t_{inh} \sim$ trolox in the case of taking n of trolox as 2 [23]. Accordingly, trolox is applied in our experimental system. But the addition of trolox to AAPH-induced oxidation of DNA as a reference antioxidant does not generate t_{inh} (data not shown) although trolox was reported to protect DNA against radical-induced oxidation [24]. In this report, electrophoresis was used to observe that trolox protects super coiled DNA not to transform to open circular or linear chains. And we herein measure the formation of TBARS to follow the oxidation of DNA. So, different determination method employed may provide different observation.

In order to apply Eq. (3) to calculate n of thiols in this case, R_i should be obtained firstly. According to our previous report [25], R_i is equal to the rate of the radical generated (R_g) from the decomposition of AAPH ($R_g = (1.4 \pm 0.2) \times 10^{-6}$[AAPH] s^{-1} [26]) when Eq. (3) is utilized to treat the results from biological samples. This is because both DNA and AAPH are dissolved in PBSo, radicals generated from the decomposition of AAPH can attack DNA at the same phase. It is safe to assume that R_i is equal to R_g in AAPH-induced oxidation of DNA [25]. As listed in Table 2, the values of n of thiols are the products of coefficients in the equation of $t_{inh} \sim$ [thiols] multiplying $R_i = R_g = 1.4 \times 10^{-6} \times 40$ mM $s^{-1} = 3.36$ μM min^{-1}. Therefore, n is a relative value to compare the antioxidant abilities among these thiols. It can be found in Table 2 that 6-MPR can trap ~1 radical, 6-MP can trap 0.6 radical, and CAP can trap 0.5 radical in protecting DNA. 6-MPR possesses the highest ability to protect DNA just due to a riboside contained. This is because the riboside benefits for 6-MPR to incorporate into DNA [27]. Although CAP was reported to be a prooxidant in the media of iron- and copper-induced oxidative injury by using deoxyribose as the oxidizable substrate in the presence of physiological phosphate concentrations [28], we herein still find that CAP can protect DNA against AAPH-induced oxidation of DNA even though the value of n of CAP is as low as 0.5. The antioxidant property of SH group attaching to aromatic ring like 6-MP and 6-MPR is higher than SH attaching to aliphatic carbon chain because the S-centered radical can be stabilized by aromatic ring *via* resonance structures as shown in Eq. (4).



4.3. CAP, 6-MP, and 6-MPR protect erythrocytes against AAPH-induced hemolysis

When erythrocyte encounters an overwhelming external oxidative stress initiated by radicals, the plasma membrane is the initial site of damage, and the ensuing peroxidation of membrane lipids contributes to progressive hemolysis. So, AAPH-mediated hemolysis is a convenient *in vitro* experimental system to mimic erythrocytes undergoing oxidative stress [29]. Fig. 4 outlines the hemolysis process in the presence of thiols.

As the blank experiment shows, hemolysis does not occur at the beginning of the reaction because the endogenous antioxidants defense the attack from radicals. When the endogenous antioxidants are exhausted completely, hemolysis takes place, generating a lag time (t_{lag}). The t_{lag} is prolonged by the addition of exogenous antioxidant. Fig. 5 illustrates the relationship between t_{lag} and the concentrations of thiols, and the quantitative equations of $t_{inh} \sim$ concentration of thiols are involved in Table 2 as well.

We have demonstrated that $R_i = R_g$ in AAPH-induced hemolysis, so, $R_i = R_g = 1.68$ μM min^{-1} when 20 mM AAPH is employed to

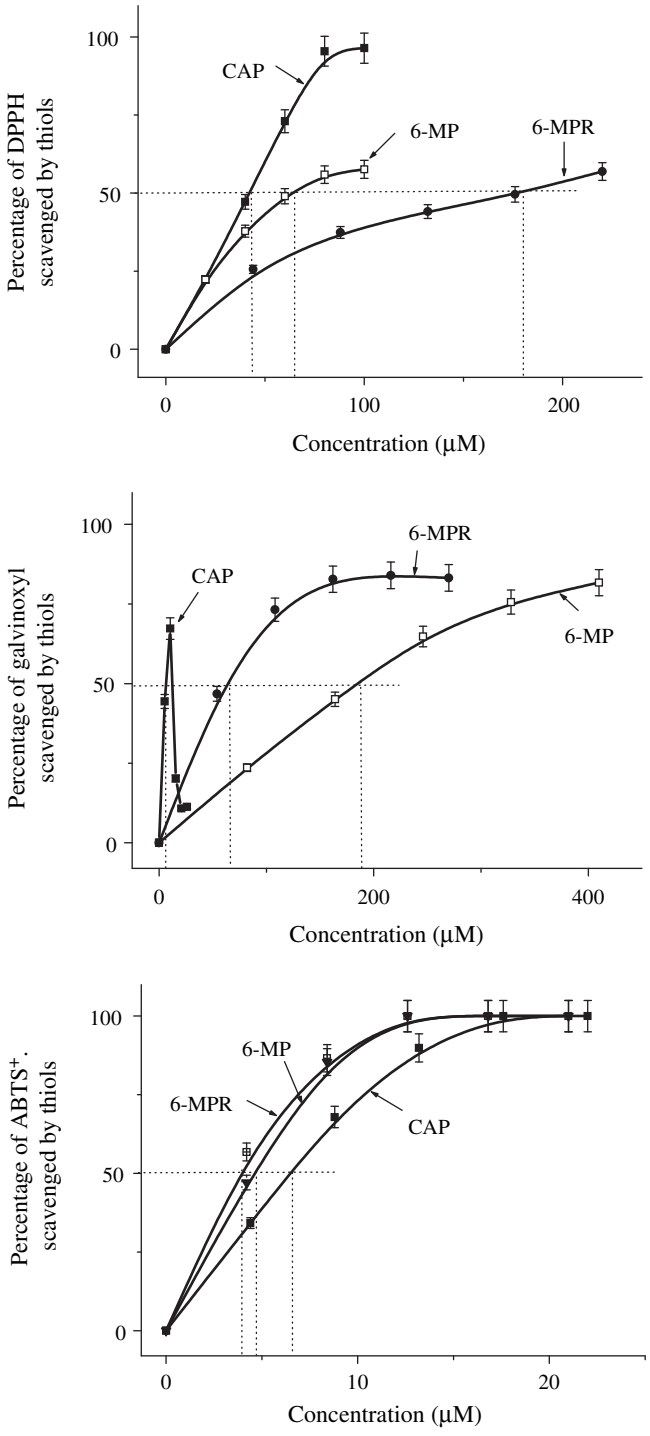


Fig. 1. The percentage of DPPH, galvinoxyl, and $ABTS^{+\bullet}$ scavenged by various concentrations of thiols.

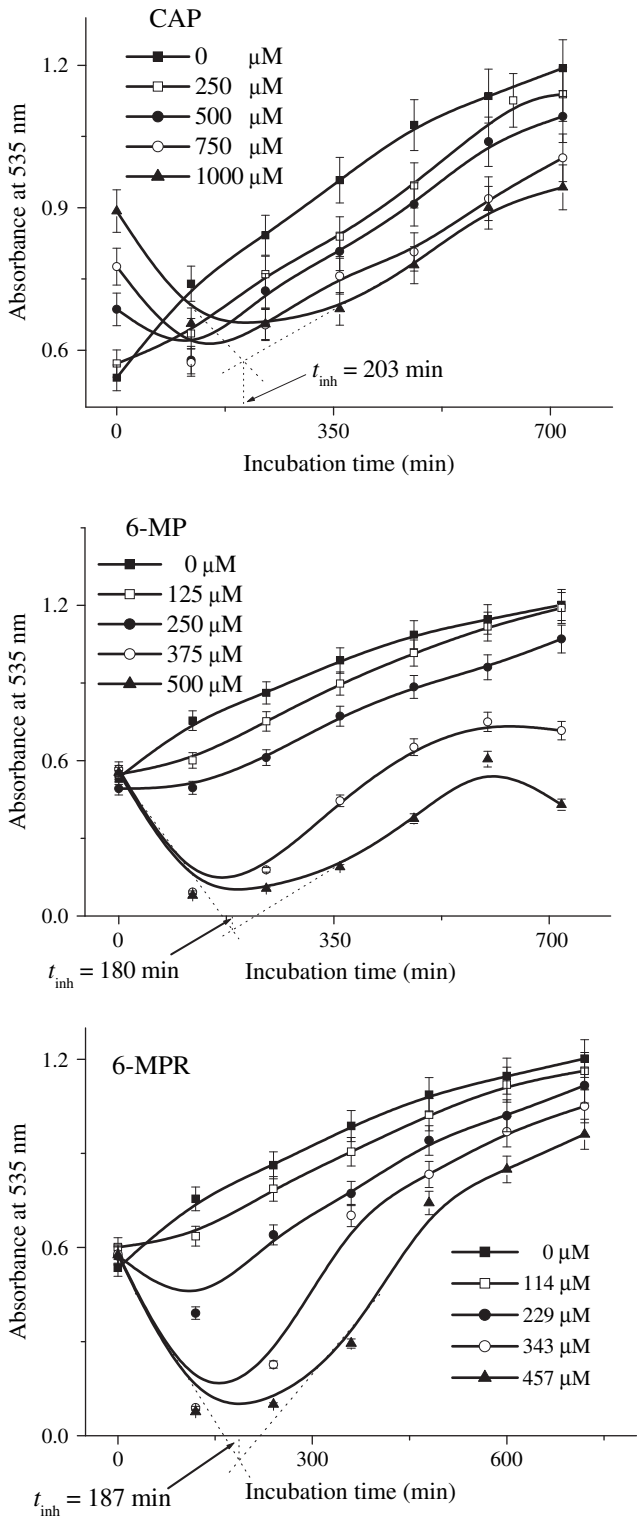


Fig. 2. The increase of the absorbance at 535 nm in the absence and presence of various concentrations (as shown in the panels) of thiols when 2.0 mg/ml DNA is oxidized by 40 mM AAPH at 37 °C.

Table 1
IC_{50} of CAP, 6-MP, and 6-MPR reacting with DPPH, galvinoxyl, and $ABTS^{+\bullet}$.

	IC_{50} (μM)		
	React with DPPH	React with galvinoxyl	React with $ABTS^{+\bullet}$
CAP	43	7.1	6.5
6-MP	65	188	4.7
6-MPR	180	65	3.9
Trolox[a]	22.8		11.6

[a] Cited from Ref. [34].

haemolyze erythrocytes by using trolox as standard antioxidant [30]. The *n* values of thiols can be calculated by multiplying the coefficients in the equation of $t_{inh} \sim$ [thiols] and $R_i = R_g = 1.68\ \mu M\ min^{-1}$. The results are involved in Table 2 as well. It can be found in Table 2 that 6-MPR can trap ~3 radical, 6-MP can trap 2.2 radical, and CAP can trap

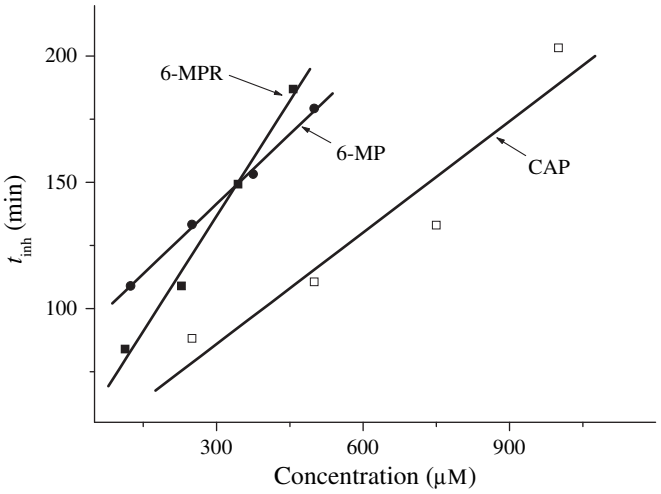


Fig. 3. The relationships between t_{inh} and the concentrations of thiols in AAPH-induced oxidation of DNA.

1.3 radical in protecting erythrocytes against AAPH-induced oxidation. Riboside in 6-MPR also enhances the ability of 6-MPR to protect erythrocytes because riboside improves the transport of 6-MPR to erythrocyte membrane [31]. CAP can also protect erythrocytes against AAPH-induced hemolysis, which is in agreement with previous report [3]. The obtained result still demonstrates that the antioxidant property of SH group attaching to aromatic ring is higher than SH attaching to aliphatic carbon chain.

4.4. CAP, 6-MP, and 6-MPR protect erythrocytes against hemin-induced hemolysis

The hydrophobic hemin is able to intercalate into lipid part of membranes, to accelerate the potassium leakage, to dissociate skeletal proteins in membrane, and to prohibit some erythrocyte enzymes, leading to hemolysis eventually [32]. Therefore, hemin-induced hemolysis can be an *in vitro* experimental system to detect whether a compound can stabilize erythrocyte membrane. Fig. 6 illustrates the percentage of hemolysis of erythrocytes inhibited by various concentrations of thiols.

It can be found in Fig. 6 that hemin-induced hemolysis is prohibited efficiently with the concentration of CAP increasing. The hemolysis is inhibited ~80% by 0.6 μM CAP, demonstrating that CAP possesses the highest efficiency to stabilize erythrocyte membrane. On the other hand, with the concentration of 6-MP increasing, 6-MP promotes the hemin-induced hemolysis slightly. 6-MPR exhibits slight protective effect on erythrocytes still owing to a riboside involved.

Table 2
The equations of t_{inh} ~ [thiol], and n of thiols in protecting DNA and erythrocytes.

	Protect DNA[a]		Protect erythrocytes[b]	
Thiol	t_{inh} (min) = (n/R_i) [thiol (μM)] + C[c]	n	t_{lag} (min) = (n/R_i) [thiol (μM)] + C[c]	n
CAP	t_{inh} = 0.15 [CAP] + 41.8	0.50	t_{lag} = 0.79 [CAP] − 151.8	1.33
6-MP	t_{inh} = 0.18 [6-MP] − 86.2	0.61	t_{lag} = 1.33 [6-MP] − 192.2	2.23
6-MPR	t_{inh} = 0.29 [6-MPR] − 48.4	0.97	t_{lag} = 1.82 [6-MPR] − 197.4	3.07

[a] $R_i = R_g = 1.4 \times 10^{-6}$[AAPH] s^{-1} = 3.36 μM min^{-1} when [AAPH] = 40 mM in protecting DNA, thus, n = coefficient × 3.36 μM min^{-1}.

[b] $R_i = R_g = 1.4 \times 10^{-6}$[AAPH] s^{-1} = 1.68 μM min^{-1} when [AAPH] = 20 mM in protecting erythrocytes, thus, n = coefficient × 1.68 μM min^{-1}.

[c] C is the constant generated from the linear regression analysis.

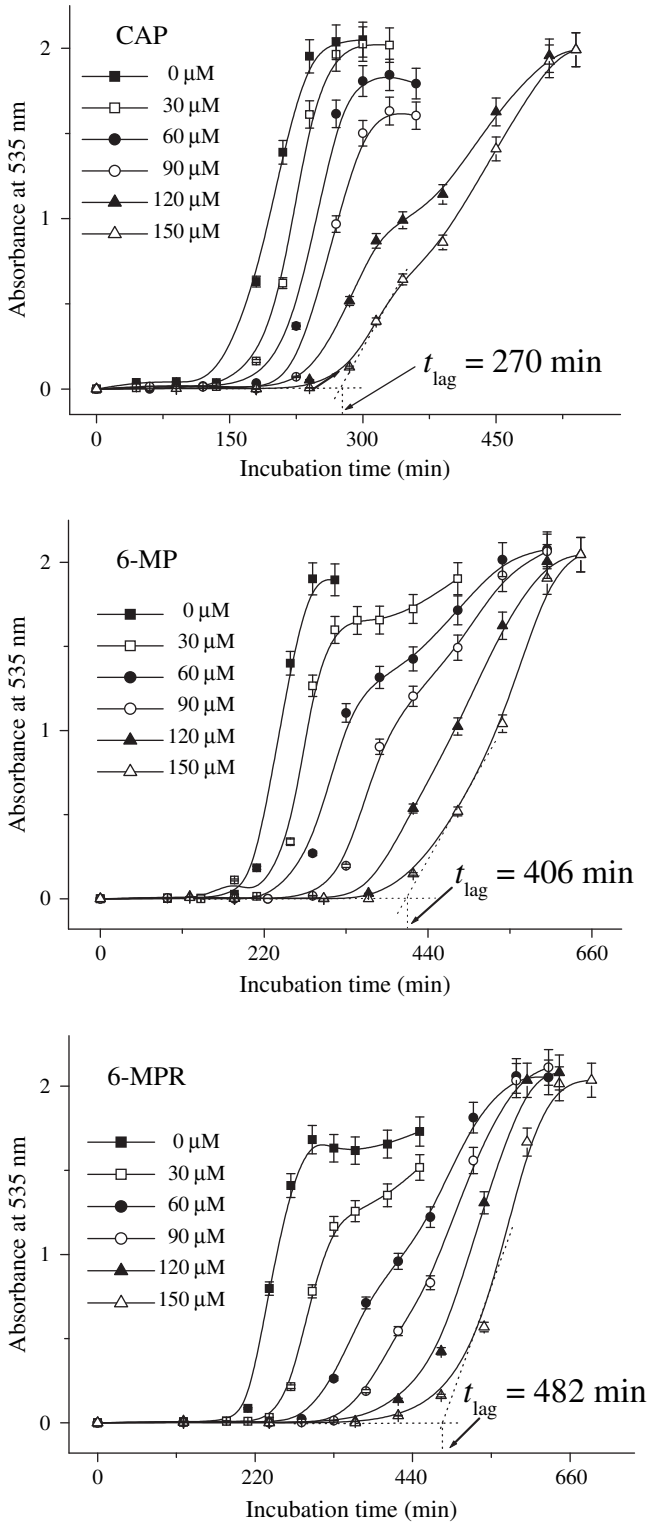


Fig. 4. The process of hemolysis in the absence and presence of various concentrations of thiols when erythrocytes (3.0% suspension in PBSa) are haemolyzed by 20 mM AAPH at 37 °C.

5. Conclusion

The ability of SH group attaching to aliphatic carbon chain to trap radicals is higher than that attaching to aromatic ring. On the contrary, SH group attaching to aromatic ring has higher

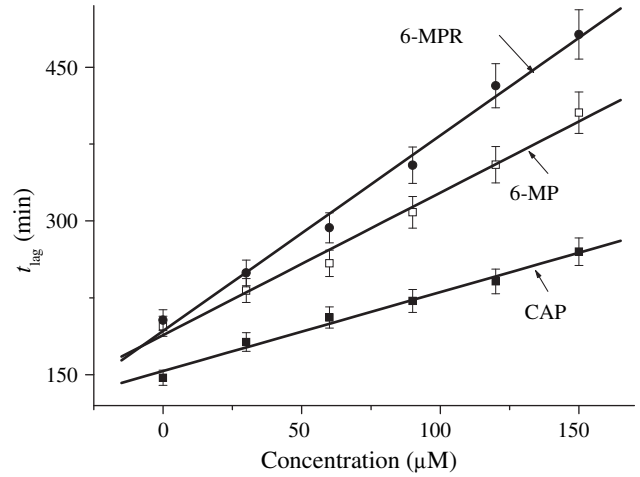


Fig. 5. The relationships between t_{inh} and the concentrations of thiols in AAPH-induced hemolysis of erythrocytes.

ability to protect DNA and erythrocytes against radical-induced oxidations. Especially, riboside enhances the antioxidant ability of 6-MPR in biological samples. SH group attaching to aliphatic carbon chain improves the ability of CAP to stabilize erythrocyte membrane.

6. Experimental protocols

6.1. Materials

AAPH, ABTS, DPPH, galvinoxyl, hemin, naked DNA sodium salt, CAP, 6-MP, and 6-MPR were purchased from ACROS Co. (Switzerland) and used as received, and other reagents with analytical grade were purchased from Beijing Chemical Reagent Co. (China) and used without further purification. Erythrocytes were provided by Haoyue Cattle Co. Ltd, Changchun, China, and washed by phosphate-buffered saline (PBSa: 150 mM NaCl, 8.1 mM Na_2HPO_4, 1.9 mM NaH_2PO_4, 10 μM EDTA) to remove plasma. At the last washing, erythrocytes were centrifugated at 1700 × *g* for exact 10 min to obtain compacted volume for the usage in the experiment [33].

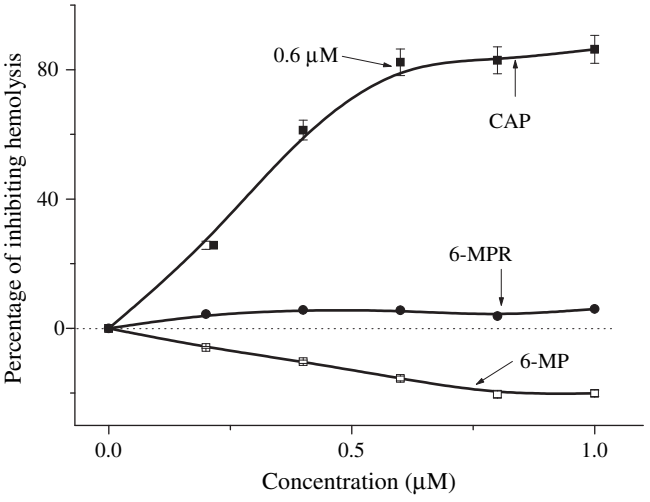


Fig. 6. The relationships between t_{inh} and the concentrations of thiols in hemin-induced hemolysis of erythrocytes.

6.2. CAP, 6-MP, and 6-MPR scavenge DPPH, galvinoxyl, and $ABTS^{+\bullet}$

The experiments of thiols to trap galvinoxyl, DPPH, and $ABTS^{+\bullet}$ followed previous reports [34]. DPPH and galvinoxyl were dissolved in ethanol to make the absorbance (Abs_{ref}) ~ 1.00 at 517 nm and 428 nm, respectively. Two milliliters of 4.0 mM ABTS aqueous solution was oxidized by 1.41 mM $K_2S_2O_8$ for 16 h, then 100 ml of ethanol was added to make the absorbance of $ABTS^{+\bullet}$ (Abs_{ref}) ~ 0.70 at 734 nm. Various concentrations of thiols (dissolved in ethanol) were added to the solutions of DPPH, galvinoxyl, and $ABTS^{+\bullet}$. The absorbances of DPPH, galvinoxyl, and $ABTS^{+\bullet}$ solutions decreased to a stable value (Abs_{detect}). The percentages of DPPH, galvinoxyl, and $ABTS^{+\bullet}$ scavenged by thiols were calculated by $(1 - Abs_{detect}/Abs_{ref}) \times 100$, and plotted *vs* the concentrations of thiols to obtain 50% inhibition concentration (IC_{50}).

6.3. CAP, 6-MP, and 6-MPR protect DNA against AAPH-induced oxidation

The oxidation of DNA induced by AAPH was performed as described in the literature [21] with a little modification [25]. DNA and AAPH were dissolved in phosphate-buffered solution (PBSo: 8.1 mM Na_2HPO_4, 1.9 mM NaH_2PO_4, 10.0 μM EDTA) to a final concentration at 2.0 mg/ml and 40 mM, respectively, to which various concentrations of CAP (dissolved in PBSo), 6-MP, and 6-MPR (dissolved in dimethyl sulfoxide, DMSO) were added. Then, the above solution was dispensed into test tubes to a final volume of 2.0 ml. All the tubes were incubated in a water bath at 37 °C to initiate the reaction. Three tubes were taken out at appropriate interval and cooled immediately, to which 1.0 ml thiobarbituric acid (TBA) solution (1.00 g TBA and 0.40 g NaOH dissolved in 100 ml PBSo) and 1.0 ml trichloroacetic acid (3.0% aqueous solution) were added. The tubes were heated in a boiling water bath for 15 min. After cooling, 1.5 ml *n*-butanol was added and shaken vigorously to extract thiobarbituric acid reactive substance (TBARS, $\lambda_{max} = 535$ nm). The absorbance of TBARS was plotted *vs* incubation time to indicate the amount of DNA oxidation.

6.4. CAP, 6-MP, and 6-MPR protect erythrocytes against AAPH- and hemin-induced hemolysis

Erythrocytes were suspended in PBSa. AAPH and CAP were dissolved in PBSa, and 6-MP and 6-MPR were dissolved in DMSO. AAPH and various concentrations of PBSa solution of CAP, and DMSO solutions of 6-MP and 6-MPR were added to erythrocyte suspension to a final concentration of erythrocyte and AAPH at 3.0% (v:v) and 20 mM, respectively. The above mixture was shaken gently to form homogenous suspension, and then incubated at 37 °C to initiate the hemolysis. Aliquots (1.5 ml) were taken out at appropriate intervals and centrifuged at 1700 × *g* to obtain the supernatant, in which hemoglobin leaked out of erythrocytes was dissolved after hemolysis took place. The absorbance of the supernatant ($\lambda_{max} = 535$ nm) was measured and plotted *vs* incubation time to express the hemolysis process.

The experiment of hemin-induced hemolysis of erythrocytes was performed as described as our previous report [35]. Briefly, hemin was dissolved in 5 mM NaOH to reach 1.0 mM before use. Erythrocytes were suspended in phosphate-buffered saline without EDTA (PBSe, 150 mM NaCl, 8.1 mM Na_2HPO_4, 1.9 mM NaH_2PO_4, pH 7.4), to which various concentrations of PBSe solution of CAP, and DMSO solutions of 6-MP and 6-MPR were added. The mixture was shaken gently to form homogenous suspension, and incubated at 37 °C for 0.5 h, and hemin was added finally. The final concentrations of erythrocytes and hemin were 1.0% (v:v) and 10.0 μM, respectively. After 0.5 h, the mixture was centrifuged at 1700 × *g* to

obtain the supernatant. The absorbance of the supernatant ($\lambda_{max} = 535$ nm) was measured. The absorbance in the blank experiment was designed as Abs_0, and the absorbance in the presence of various concentrations of thiols was designed as Abs. The percentage of thiols to inhibit hemolysis was calculated by $(1 - Abs/Abs_0) \times 100$. It was worthy to note that the same volume of DMSO (less than 1.0% to the total volume) was contained in all the experiment to eliminate its influence on the experiment [36].

6.5. Statistical analysis

All the data were the average value from at least three independent measurements with the experimental error within 10%. The equations were analyzed by one-way ANOVA using Origin 6.0 professional Software, and $p < 0.001$ indicated a significance difference.

Acknowledgment

We thank the National Natural Science Foundation, China, for the financial support.

References

[1] W. Small Jr., A. Molteni, Y.T. Kim, J.M. Taylor, C.-H. Ts'ao, W.F. Ward, Breast Cancer Res. Treat. 55 (1999) 223–229.
[2] L. Jia, R. Pei, M. Lin, X. Yang, Food Chem. Toxicol. 39 (2001) 1135–1143.
[3] C.R. Santos, R. Capela, C.S.G.P. Pereira, E. Valente, L. Gouveia, C. Pannecouque, E.D. Clercq, R. Moreira, P. Gomes, Eur. J. Med. Chem. 44 (2009) 2339–2346.
[4] R.B. Gearry, M.L. Barclay, M.J. Burt, J.A. Collett, B.A. Chapman, Pharmacoepidem. Drug Safety 13 (2004) 563–567.
[5] L.A. Hogarth, C.P.F. Redfern, J.M. Teodoridis, A.G. Hall, H. Anderson, M.C. Case, S.A. Coulthard, Biochem. Pharmacol. 76 (2008) 1024–1035.
[6] H. Kanemitsu, H. Yamauchi, M. Komatsu, S. Yamamoto, S. Okazaki, H. Nakayama, Histol. Histopathol. 24 (2009) 317–324.
[7] E. Petit, S. Langouet, H. Akhdar, C. Nicolas-Nicolaz, A. Guillouzo, F. Morel, Toxicol. In Vitro 22 (2008) 632–642.
[8] S. Rodríguez-Enríquez, A. Marín-Hernández, J.C. Gallardo-Pérez, L. Carreño-Fuentes, R. Moreno-Sánchez, Mol. Nutr. Food Res. 53 (2009) 29–48.
[9] X. Zhang, G. Jeffs, X. Ren, P. O'Donovan, B. Montaner, C.M. Perrett, P. Karran, Y.Z. Xu, DNA Repair 6 (2007) 344–354.
[10] H. Shi, E. Niki, Lipids 33 (1998) 365–370.
[11] A. Jiménez-Escrig, I. Jiménez-Jiménez, C. Sánchez-Moreno, F. Saura-Calixto, J. Sci. Food Agric. 80 (2000) 1686–1690.
[12] M.J.T.J. Arts, G.R.M.M. Haenen, H.P. Voss, A. Bast, Food Chem. Toxicol. 42 (2004) 45–49.
[13] C.E. Boozer, G.S. Hammond, C.E. Hamilton, J.N. Sen, J. Am. Chem. Soc. 77 (1955) 3233–3237.
[14] G.-X. Li, Z.-Q. Liu, J. Agric. Food Chem. 57 (2009) 3943–3948.
[15] Y.-Z. Tang, Z.-Q. Liu, D. Wu, J. Biochem. Mol. Toxicol. 23 (2009) 81–86.
[16] A.V. Szeghalmi, L. Leopold, S. Pînzaru, V. Chis, I. Silaghi-Dumitrescu, M. Schmitt, J. Popp, W. Kiefer, J. Mol. Struct. 735–736 (2005) 103–113.
[17] M. Lucarini, P. Pedrielli, G.F. Pedulli, L. Valgimigli, D. Gigmes, P. Tordo, J. Am. Chem. Soc. 121 (1999) 11546–11553.
[18] O. Martínez, A. Vivoni, Z. Qiao, U. Udeochu, C.M. Hosten, Surf. Sci. 600 (2006) 1787–1792.
[19] A. Ion, F.G. Bănică, C. Luca, J. Electroanal. Chem. 444 (1998) 11–18.
[20] P. Zhang, S.T. Omaye, Food Chem. Toxicol. 39 (2001) 239–246.
[21] R. Aeschbach, J. Loliger, B.C. Scott, A. Murcia, J. Butler, B. Halliwell, O.I. Aruoma, Food Chem. Toxicol. 32 (1994) 31–36.
[22] L. Zennaro, M. Rossetto, P. Vanzani, V.D. Marco, M. Scarpa, L. Battistin, A. Rigo, Arch. Biochem. Biophys. 462 (2007) 38–46.
[23] M. Antolovich, P.D. Prenzler, E. Patsalides, S. McDonald, K. Robards, Analyst 127 (2002) 183–198.
[24] Q.-Y. Wei, B. Zhou, Y.-J. Cai, L. Yang, Z.-L. Liu, Food Chem. 96 (2006) 90–95.
[25] F. Zhao, Z.-Q. Liu, D. Wu, Chem. Phys. Lipids 151 (2008) 77–84.
[26] V.W. Bowry, R. Stocker, J. Am. Chem. Soc. 115 (1993) 6029–6044.
[27] T. Platzek, R. Schwabe, Teratogen. Carcin. Mut. 19 (1999) 223–232.
[28] D. Lapenna, S. de Gioia, A. Mezzttti, G. Ciofani, C. di Ilio, F. Cuccurullo, Biochem. Pharmacol. 50 (1995) 27–32.
[29] J.M. May, Z.-C. Qu, S. Mendiratta, Arch. Biochem. Biophys. 349 (1998) 281–289.
[30] Y.-Z. Tang, Z.-Q. Liu, J. Agric. Food Chem. 56 (2008) 11025–11029.
[31] K. Iseki, M. Sugawara, T. Fujiwara, I. Naasani, M. Kobayashi, K. Miyazaki, Biochim. Biophys. Acta 1278 (1996) 105–110.
[32] F. Wang, T. Wang, J. Lai, M. Li, C. Zou, Biochem. Pharmacol. 71 (2006) 799–805.
[33] S.-L. Deng, W.-F. Chen, B. Zhou, L. Yang, Z.-L. Liu, Food Chem. 98 (2006) 112–119.
[34] Y.Z. Tang, Z.Q. Liu, J. Am. Oil Chem. Soc. 84 (2007) 1095–1100.
[35] G.-X. Li, Z.-Q. Liu, Food Chem. Toxicol. 46 (2008) 886–892.
[36] Y.-Z. Tang, Z.-Q. Liu, Cell Biochem. Funct. 26 (2008) 185–191.