European Journal of Medicinal Chemistry 45 (2010) 3005–3010

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Original article

Design, synthesis, and pharmacological evaluation of the aqueous prodrugs of desmethyl anethole trithione with hepatoprotective activity

Pei Chen, Yu Luo, Li Hai, Shan Qian, Yong Wu*

West China School of Pharmacy, Sichuan University, Chengdu 610041, China

ARTICLE INFO

Article history:
Received 18 October 2009
Received in revised form
25 February 2010
Accepted 19 March 2010
Available online 25 March 2010

Keywords:
Design
Synthesis
Prodrugs
Metabolite
Desmethyl anethole trithione
Hepatoprotective activity

ABSTRACT

A metabolite-based prodrug strategy to increase the solubility of anethole trithione was reported to facilitate the clinical application of this hepatoprotective agent. Water-soluble analogs of anethole trithione were synthesized via substituting the methyl group of anethole trithione with the simple hydrophilic alkylamino group, and subjected to physiochemical, pharmacological and metabolic studies. The prodrugs displayed increased solubility as well as other physiochemical properties favorable for parenteral use. Among the analogs synthesized, the compound **5a** exhibited best hepatoprotective activity at the dose of 2.0 mg/kg in mice equal to that of anethole trithione. The in vivo metabolic investigation demonstrated that the straight-side chain prodrug **5a** could convert to desmethyl anethole trithione in vivo, while the ring-side chain prodrug **5d** could not. The hepatoprotective activity of the prodrugs might result from the active metabolite desmethyl anethole trithione.

1. Introduction

The dithiolthione compound [1] anethol trithione (ATT), [5-(*p*-methoxy-phenyl)-1,2-dithiol-3-thione] (Fig. 1) is considered to protect the liver against various hepatotoxic substances via increasing the activity of phase II enzymatic detoxification systems such as glutathione *S*-transferase, glutathione reductase, UDP-glucuronosyltransferase, and quinone reductase [2–4]. It has been widely used in clinic to treat the symptoms associated with hepatobiliary dysfunctions without any major adverse reactions noted [5,6]. Despite the high potency and low toxicity, the poor water solubility of ATT has long been a concern as it results in low dissolution and limited dosage forms, and has hindered the clinical use of ATT. Most studies [7–10] seeking ATT derivatives with improved water solubility employed the strategy of introducing hydrophilic groups to either 1,2-dithiole-3-thione or the benzene ring of ATT, however few candidates were found to possess hepatoprotective activity superior or equal to that of ATT. Von R. Gmelin and F. Lagler [11] reported that ATT was rapidly broken down in animal and human with the formation of desmethyl ATT (ATX). We hypothesize that the metabolite ATX or the course of conversion to it is attributable to the pharmacodynamic activity of ATT, and seek to develop of a novel prodrug strategy based on this hypothesis. Expected to undergo the similar metabolism pathways as ATT in vivo, a series of prodrugs was synthesized via coupling a simple hydrophilic alkylamine hydrochloride salt group or quaternary ammonium group with metabolite ATX, and was screened in physiochemical studies in vitro and the pharmacological evaluations in mice model.

2. Chemistry

In order to efficiently improve the hydrophilicity of **1**, the methyl group was substituted with either ethylamine hydrochloride or quaternary ammonium groups. These prodrugs were prepared using the procedures illustrated in Schemes 1 and 2. According to the variation in the amino groups, two methods (A and B) were developed to obtain the final water-soluble products starting from **2**, which were obtained by demethylation of **1**. The attempt to demethylate **1**, however, failed due to the high stability of phenol ether to concentrated sulfuric acid, concentrated Hydrochloride, or Lewis acid. The attempt finally succeeded by treatment of anthol-trithione with pyridine hydrochloride under N_2 atmosphere at 215°C through which a satisfactory yield was obtained [11]. Method A (Scheme 1) was found suitable for the efficient preparation of the amine salt derivatives of anethol trithione. Compound **3**, which is commercially available, coupled directly with **2** in the presence of potassium carbonate to yield the amine derivatives, which were

* Corresponding author. Tel./fax: +86 028 85503666.
E-mail address: wyong@scu.edu.cn (Y. Wu).

0223-5234/$ – see front matter © 2010 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2010.03.029

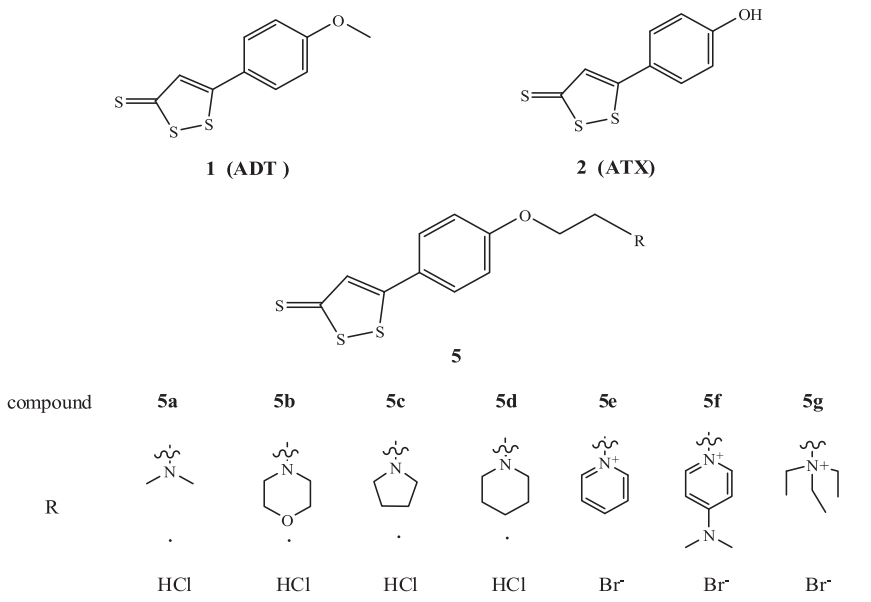


Fig. 1. ATT, **ATX** and target compounds **5**(**a**–**g**).

then quickly converted to the corresponding hydrochlorides **5** (**a**–**d**) with chloride hydrogen–THF solution.

However, Method A was not suitable in the preparation of quaternary ammonium salt prodrugs. Therefore, Method B (Scheme 2) was alternatively employed. O-alklation of **2** with 1,2-dibromoethane was first afforded 5-(bromoethoxyl phenyl)-1,2-dithiol-3-thione **4**, then **4** was quaterisated with triethylamine, pyridine, or N,N-dimethylpyridine to yield the desired compounds.

3. Pharmacology

To characterize the prodrugs, studies in vitro were carried out in two aspects: aqueous solubility and stability.

The aqueous solubility studies were designed to determine whether the prodrugs dissolved in aqueous medium and if so, to what extent.

The stability of the prodrugs was studied using the HPLC method in mice blood plasma and phosphate buffer at pH 1.10, 4.02, 6.84, 9.14, and 12.02, respectively, under 37°C and 60°C.

In addition to **5e** as a representative of quaternary ammonium salt prodrugs, **5a**, **5b**, **5c**, and **5d** were also selected for pharmacological evaluation owing to their good aqueous solubility. The evaluation was carried out by intraperitoneally injecting mice with the synthesized prodrugs at three dose levels separately (1, 2, 4 mg/kg body weight) and 0.5 h later with CCl_4 (5 mL/kg as a 0.2% peanut oil solution, body weight), a model hepatotoxin to induce experimental liver injury [12–14]. ATT was employed as the reference drug. The hepatoprotective effect of these prodrugs was studied by assaying the activities of the serum marker enzymes AST and ALT in experimental mice plasma.

4. Results and discussion

4.1. Aqueous solubility and stability study in vitro

4.1.1. Aqueous solubility

The solubility of these compounds in water is shown in Table 1. As expected, the aqueous solubility of the synthetic prodrugs was significantly higher than that of **1** and **2**. Moreover, amine salt prodrugs obviously had more solubility than quaternary ammonium salt prodrugs.

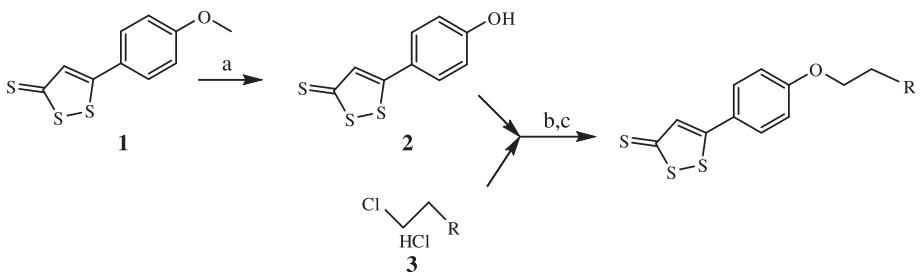
4.1.2. Stability in mice plasma and phosphate buffer solution

At 37°C, the investigated compounds were stable, and few decomposed under the experimental pH except at 12.02. At 60°C, the compounds were stable under the following pH values: 1.10, 4.02, and 6.84. Moreover, the investigated compounds were stable in mice plasma.

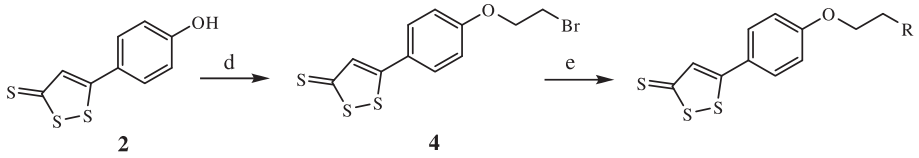
The above studies in vitro demonstrated that these prodrugs possessed preliminary and favorable physicochemical properties for parenteral use.

4.2. Pharmacology

All prodrugs used in the study decreased the alanine transaminase (ALT) and aspartate transaminase (AST) levels in serum



Scheme 1. Method A condition and reagent (a) pyridine hydrochloride 215 °C 3 h (b) compound **5a**: 40% NaOH tetrabutylammonium bromide r.t 48 h, compound: **5b 5c 5d** K_2CO_3 DMSO 60 °C 3–6 h (c) dry hydrochloride THF 0–5 °C 5 h.



Scheme 2. Method B condition and reagent (d) 1,2-dibromoethane K_2CO_3 DMSO 60 °C 6 h (e) pyridine 110 °C 10 min.

compared with the CCl_4 model group, but the values of ALT and AST varied in a large range. Compound **5a** was the most active, and its effect on decreasing the level of ALT and AST was equal to that of **1** at the dose of 2.0 mg/kg and 4.0 mg/kg, while **5b**, **5c**, **5d**, and **5e** were much less effective than **1** in all dose levels (Table 2).

We found an interested finding that **5a** and **1**, which both possessed a straight chain at the O position, were more effective than the other prodrugs possessing a ring chain at the same position. This seemed to indicate that the structure of the side chains had some potential relationship with the efficacy of the prodrugs. However, if the hepatoprotective activity of **1** was associated with its metabolism process or metabolite **2** itself as we postulated, the structure of the side chain would be of little concern to the activity of the prodrugs. Thus the structure-activity relationships above mentioned seem not to support our postulate when these prodrugs undergo the similar metabolism pathways as **1**.

In order to find out whether the major metabolites of these prodrugs were in accordance with that of **1** in mice, **5a** and **5d**, as representatives of straight-chain prodrugs and ring-chain prodrugs respectively, were selected for metabolism investigation. Meanwhile, an HPLC determination method for **5a**, **5d**, and **2** in mice plasma was established.

The results, as illustrated in Figs. 2 and 3, demonstrated that **2** could be detected in mice plasma after injection of **5a**, but not in mice injected with **5d**. This finding proved that different activities between the straight-side chain prodrug **5a** and the ring-side chain prodrug **5d** were due to the fact that the former could convert to **2** in vivo, while the latter could not. In summary, the hepatoprotective activity of the prodrugs might result from their capability of converting to **2** in vivo.

5. Conclusions

In the present work, a metabolite-based prodrug approach for water-soluble hepatoprotective analogs of **1** was described on the hypothesis that **2** played an essential role in hepatoprotective activity during or after **1** was metabolized into it in vivo according to Von R. Gmelin and F. Lagler's study.

The trials in vitro showed that these prodrugs were more soluble than **1** or **2** in water, and were stable in mice plasma and acidic buffer solution. These properties render the prodrugs servable by injection.

In vivo, prodrug **5a** exhibited good hepatoprotective activity equal to that of **1** in the screening dose of 2 mg/kg and 4 mg/kg. It was a potential prodrug candidate for further pharmacokinetics study.

The metabolism study of **5a**, indicating that the good hepatoprotective activity of the prodrugs might result from its capability of converting to **2**, supported directly our prodrug strategy. Furthermore, the fact that no metabolite **2** was found in mice administered with **5d**, which had low hepatoprotective activity, indicated indirectly the rationality of our strategy.

Moreover, the relationship between the structure of the side chain and the metabolite gave us a valuable implication for future research on more potential prodrugs of **2**. Extensive side chain structure–metabolite relationship studies and the further pharmacokinetics of **5a** are currently in progress.

Table 1
The Aqueous Solubility of the Compounds at 25 ± 1 °C.

Drug	**1**	**2**	**5a**	**5b**	**5c**	**5d**	**5e**	**5f**	**5g**
mg/mL	<0.01	<0.13	>20	>20	>20	>20	2.3	2.8	3.5

6. Experimental protocols

6.1. Chemistry

TLC was performed using precoated silica gel GF254 (0.2 mm), while column chromatography was performed using silica gel (100–200 mesh). The melting point was measured on a YRT-3 melting point apparatus (Shantou Keyi instrument & Equipment Co. Ltd, Shantou, China). IR spectra were obtained on a Perkin–Elmer 983 (Perkin Elmer, Norwalk, CT, USA). Elemental analyses were performed by Atlantic Microlab, Atlanta, GA, USA. NMR spectra were taken on a Varian INOVA400 (Varian, Palo Alto, CA, USA) using $CDCl_3$, d_6-DMSO and D_2O as solvent. Chemical shifts are

Table 2
Effect of **1**, **5a**, **5b**, **5c**, **5d**, **5e** on the Levels of ALT, AST in Serum of Mice (mean ± S.D).

Compound	Group	Quantity	Doses (mg/kg)	ALT(IU/L)	AST(IU/L)
	Normal[a]	10	0	65.3 ± 13.5*	89.8 ± 22.1*
	Model[b]	10	0	788.6 ± 336.6	964.3 ± 419.8
1	Low[c]	10	1 mg/kg	150.7 ± 69.0	269.6 ± 90.4*
	Middle[d]	10	2 mg/kg	112.4 ± 64.2*	265.9 ± 159.4*
	High[e]	10	4 mg/kg	177.2 ± 94.2*	407.1 ± 234.0*
5a	Low	10	1 mg/kg	216.5 ± 170.3*	310.9 ± 150.4*
	Middle	10	2 mg/kg	109.7 ± 79.8*	215.7 ± 38.4*
	High	10	4 mg/kg	171.6 ± 118.3*	284.1 ± 167.7*
5b	Low	10	1 mg/kg	595.4 ± 342.1	851.7 ± 534.5
	Middle	10	2 mg/kg	468.0 ± 183.1	552.5 ± 187.2
	High	10	4 mg/kg	457.7 ± 219.8	824.9 ± 585.5
5c	Low	10	1 mg/kg	416.6 ± 124.4	501.2 ± 308.8
	Middle	10	2 mg/kg	535.9 ± 155.9	582.9 ± 169.2
	High	10	4 mg/kg	561.5 ± 252.5	607.8 ± 191.7
5d	Low	10	1 mg/kg	169.3 ± 70.9*	920.6 ± 519.6
	Middle	10	2 mg/kg	259.6 ± 86.7*	416.8 ± 242.9*
	High	10	4 mg/kg	382.9 ± 174.6*	423.9 ± 103.8*
5e	Low	10	1 mg/kg	426.1 ± 265.4	559.2 ± 328.5
	Middle	10	2 mg/kg	630.1 ± 506.1	617.8 ± 448.9
	High	10	4 mg/kg	685.0 ± 248.6	784.3 ± 323.8

*Compared with model group $P < 0.01$.

[a] The mice of this group were intraperitoneally injected with saline solution alone.

[b] The mice of this group were intraperitoneally injected with CCl_4 at a dose of 5 mL/kg as a 0.2% peanut oil solution.

[c] The mice were intraperitoneally injected with the investigated compounds at a dose of 1 mg/kg, and 0.5 h later with CCl_4 at a dose of 5 mL/kg as a 0.2% peanut oil solution.

[d] The mice were intraperitoneally injected with the investigated compounds at a dose of 2 mg/kg, and 0.5 h later with CCl_4 at a dose of 5 mL/kg as a 0.2% peanut oil solution.

[e] The mice were intraperitoneally injected with the investigated compounds at a dose of 4 mg/kg, and 0.5 h later with CCl_4 at a dose of 5 mL/kg as a 0.2% peanut oil solution.

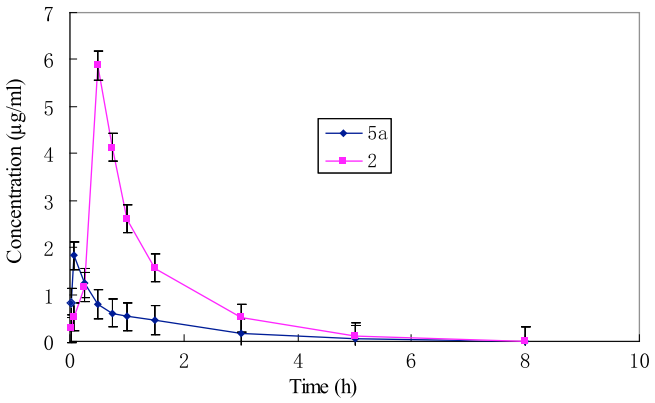


Fig. 2. The Concentration of **5a**, **2** in Mice Plasma after the Injection of **5a**.

expressed in δ(ppm), with tetramethylsilane (TMS) functioning as the internal reference. The following abbreviations were used: s = singlet, d = doublet, t = triplet, and m = multiplet; coupling constants (J) were expressed in Hz. Mass spectra were recorded on an Agilent 1946B ESI-MS instrument (Agilent, Palo Alto, CA, USA).

Anethol trithione (ATT) was purchased from ChengDu GuoJia United Pharmaceutical Ltd. (Chengdu, P. R. China), while **3**(**a**–**d**) was purchased from J&K Scientific Ltd. (Beijing China).

*6.1.1. 5-(4-hydroxyphenyl)-3H-1,2-dithiole-3-thione (**2**,ATX)*

Pyridine hydrochloride (68.0 g, 590 mmol) was added to **1** (25.0 g, 100 mmol) in a dry flask, mixed and then heated to melt at 215°C under argon protection for 20 min. After being cooled to 100°C, warmed water (200 mL) was added and hot-filtered. The cake was placed in a beaker, and 10%NaOH (300 mL) was added. This was stirred for 4 h, filtered, the cake dissolved in water (3 L), then adjusted to pH 2 with concentrated hydrochloride. The red precipitation was filtered and washed to neutral using water, then dried in a vacuum desiccator to yield a yellow solid (18.0 g, 76.5%), m.p. 172–174 (found: C, 47.78; H, 2.67; O, 7.08; S, 42.53. Calcd for $C_9H_6OS_3$: C, 47.76; H, 2.67; O, 7.07; S, 42.50%); ^{1}H NMR d_6-DMSO: 10.47 (br, 1H, -O**H**), 7.84 (d, 2H, *J* = 8.8 Hz, Ar–**H**), 7.70 (s, 1H, = C**H**), 6.88 (d, 2H, *J* = 8.8 Hz, Ar–**H**).

*6.1.2. 5-(4-(2-(dimethylamino)ethoxy)phenyl)-3H-1,2-dithiole-3-thione hydrochloride (**5a**)*

40%NaOH (20 mL) was added to **2** (2.3 g, 10 mmol) in a flask and mixed for 30 min; tetrabutylammonium bromide (0.65 g, 2 mmol), 2-chloro- N,N-dimethyl –ethanamine hydrochloride (2.16 g, 15 mmol), and dichloromethane (20 mL) were then added and stirred for 48 h at room temperature. The reaction mixture was poured into water (100 mL), and the aqueous layer was extracted with dichloromethane (3 × 50 mL). The organic layers were combined and washed with water (50 mL) and brine (50 mL), and then dried (Na_2SO_4). After filtration, the filtrate was evaporated, and the remaining residue was subjected to column chromatography using petroleum–acetone (10:1) as eluent. The base of **5a** was obtained as brown oil (2.0 g, 66.2%).

Dry hydrochloride gas was continually added to the brown oil in anhydrous THF (20 mL) under constant stirring until the precipitation stopped increasing. The reaction mixture was filtered, and the cake was dried in a vacuum desiccator to yield **5a** (2.1 g, 93.6%), m.p. 190–192°C (found: C, 46.79; H, 4.83; Cl, 10.63; N, 4.19; O, 4.79; S, 28.83. Calcd for $C_{13}H_{16}ClNOS_3$: C, 46.76; H, 4.83; Cl, 10.62; N, 4.19; O, 4.79; S, 28.81%) ^{1}H NMR D_2O: 7.63 (d, 2H, *J* = 8.4 Hz, Ar–**H**), 7.36 (s, 1H, = C**H**), 7.07 (d, 2H, *J* = 8.4 Hz, Ar–**H**), 4.48 (*t*, 2H, *J* = 4.4 Hz, OC**H**$_2$CH$_2$), 3.69 (*t*, 2H, *J* = 4.4 Hz, OCH$_2$C**H**$_2$), 3.06 (s, 6H, -C**H**$_3$×2). IR (KBr pellets cm^{-1}) υ 3039, 3009, 2952, 2690, 2518, 2481, 1604, 1575, 1481, 1424, 1294, 1259, 1202, 1060, 1024; ESI–MS: 298 (M^++1).

*6.1.3. General procedure for the synthesis of **5b**, **5c**, and **5d***

To the mixture of **2** (0.1 g, 0.44 mmol), **3** (0.66 mmol), and K_2CO_3 (0.304 g, 2.2 mmol), DMSO (3 mL) was added under stirring and argon protection, which was heated to 60°C and reacted for 2 h. After completion of the reaction (monitored by TLC), the reaction mixture was poured into cold water (25 mL) and extracted with dichloromethane (3 × 15 mL). The combined organic layers were washed with water (1 × 5 mL) and brine (1 × 5 mL), and then dried (Na_2SO_4). After filtration, the filtrate was evaporated under reduced pressure. The residue was subjected to column chromatography using petroleum–acetone (10:1) as eluent. The bases of **5b**, **5c**, or **5d** were obtained as brown oil. Compound **5b**: (0.096 g, 64%), **5c**: (0.122 g, 85.4%), **5d**: (0.102 g, 68.4%).

Dry hydrochloride gas was added to brown oil in anhydrous THF (20 mL) and constantly stirred until the precipitation stopped increasing. The reaction mixture was filtered, and the cake was dried in a vacuum desiccator to yield the corresponding hydrochloride salt.

*6.1.3.1. 5-(4-(2-morpholinoethoxy)phenyl)-3H-1,2-dithiole-3-thione hydrochloride(**5b**).* Obtained from **2** (0.1 g, 0.44 mmol) and **3b** (0.122 g, 0.66 mmol) as yellow solid. (0.090 g, 57.8%), m.p.210–212°C (found: C, 47.93; H, 4.83; Cl, 9.44; N, 3.73; O, 8.52; S, 25.62. Calcd for $C_{15}H_{18}ClNO_2S_3$: C, 47.92; H, 4.83; Cl, 9.43; N, 3.73; O, 8.51; S, 25.59%) 5b in base form: ^{1}H NMR $CDCl_3$: 7.61 (d, 2H, *J* = 8.8 Hz, Ar–**H**), 7.40 (s, 1H, = C**H**), 6.98 (d, 2H, *J* = 8.8 Hz, Ar–**H**), 4.18 (*t*, 2H, *J* = 5.6 Hz, ArOC**H**$_2$CH$_2$), 2.85 (*t*, 2H, *J* = 5.6 Hz, ArOCH$_2$C**H**$_2$), 3.75 (*t*, 4H, *J* = 4.8 Hz, C**H**$_2$O–C**H**$_2$), 2.59–2.62 (m, 4H, NC**H**$_2$CH$_2$OCH$_2$C**H**$_2$); IR (KBr pellets cm^{-1}) υ 3033, 2976, 2930, 2861, 2654, 2578, 2459, 1603, 1479, 1422, 1288, 1250, 1195, 1080, 1027; ESI–MS: 340 (M^++1).

*6.1.3.2. 5-(4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)-3H-1,2-dithiole-3-thione hydrochloride (**5c**).* Obtained from **2** (0.1 g, 0.44 mmol) and **3c** (0.112 g, 0.66 mmol) as yellow solid. (0.118 g, 74.1%), m.p.216–218°C (found: C, 50.09; H, 5.04; Cl, 9.86; N, 3.89; O, 4.44; S, 26.73. Calcd for $C_{15}H_{18}ClNOS_3$: C, 50.05; H, 5.04; Cl, 9.85; N, 3.89; O, 4.44; S, 26.72%); ^{1}H NMR D_2O: 7.76(d, 2H, *J* = 8.4 Hz, Ar–**H**), 7.53 (s, 1H, = C**H**), 7.14(d, 2H, *J* = 8.4 Hz, Ar–**H**), 4.50(*t*, 2H, *J* = 4.4 Hz, OC**H**$_2$CH$_2$), 3.76(*t*, 2H, *J* = 4.4 Hz, OCH$_2$C**H**$_2$), 3.84–3.80(m, 2H, CH$_2$NC**H**$_2$), 3.27–3.33(m, 2H, C**H**$_2$NCH$_2$), 2.10–2.30(m, 4H, NCH$_2$C**H**$_2$C**H**$_2$); IR (KBr pellets cm^{-1}) υ 3063, 2946, 2877, 2668, 2598, 2478, 1598, 1489, 1420, 1282, 1261, 1187, 1056, 1023. ESI–MS: 324 (M^++1).

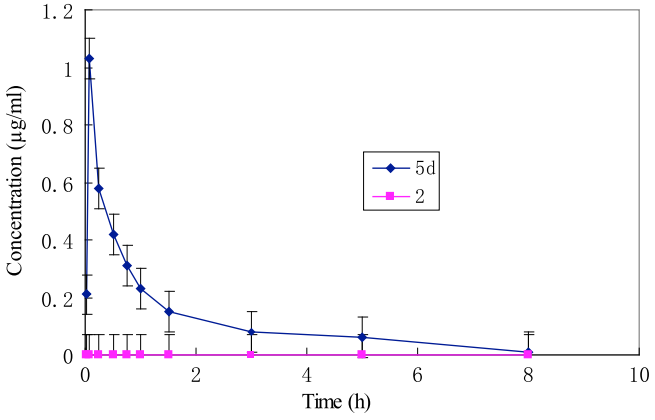


Fig. 3. The Concentration of **5d**, **2** in Mice Plasma after the Injection of **5d.**

*6.1.3.3. 5-(4-(2-(piperidin-1-yl)ethoxy)phenyl)-3H-1,2-dithiole-3-thione hydrochloride (**5d**).* Obtained from (0.1g, 0.44 mmol) and **3d** (0.121g, 0.66 mmol) as yellow solid. (0.105 g, 63.4%), m.p.215–217°C (found: C, 51.41; H, 5.39; Cl, 9.49; N, 3.75; O, 4.28; S, 25.74. Calcd for $C_{16}H_{20}ClNOS_3$: C, 51.38; H, 5.39; Cl, 9.48; N, 3.75; O, 4.28; S, 25.72%); ^{1}H NMR D_2O: 7.66 (d, 2H, J = 8.8 Hz, Ar–**H**), 7.39 (s, 1H, = C**H**), 7.08 (d, 2H, J = 8.0 Hz, Ar–**H**), 4.48 (s, 2H, OC**H**$_2$CH$_2$), 3.14 (*t*, 2H, J = 12.4 Hz, OCH$_2$C**H**$_2$), 3.63–3.70 (m, 4H, C**H**$_2$NC**H**$_2$), 1.80–2.05 (m, 6H, NCH$_2$C**H**$_2$C**H**$_2$C**H**$_2$); IR (KBr pellets cm^{-1}) υ 3063, 2952, 2875, 2730, 2489, 1602,1489, 1420, 1288, 1260, 1191, 1063, 1021; ESI-MS: 338 (M^++1)

*6.1.4. 5-(4-(2-bromoethoxy)phenyl)-3H-1,2-dithiole-3-thione (**4**)*

To the mixture of **2** (0.226 g, 1.0 mmol), 1,2-dibromoethane (0.544 g, 3.0 mmol), and K_2CO_3 (0.690 g, 5.0 mmol), DMSO (3 mL) was added under constant stirring and argon protection. It was then heated to 60°C and reacted for 2 h. After completion of the reaction, (monitored by TLC), the reaction mixture was poured into cold water (25 mL) and extracted with dicholoroethane (3 × 20 mL). The combined organic layers were washed with water (1 × 15 mL) and brine (1 × 15 mL), then dried (Na_2SO_4). After filtration, the filtrate was concentrated under reduced pressure, and the residue was subjected to column chromatography using petroleum–acetone (10:1) as eluent. Compound **4** was obtained as brown solid. (0.256 g, 76.9%), m.p.134–136 °C (found: C, 39.65; H, 2.72; Br, 23.98; O, 4.80; S, 28.87. Calcd for $C_{11}H_9BrOS_3$: C, 39.64; H, 2.72; Br, 23.97; O, 4.80; S, 28.86%); ^{1}H NMR $CDCl_3$: 7.63(d, 2H, J = 8.8 Hz, Ar–**H**), 7.40(s, 1H, = C**H**), 7.00(d, 2H, J = 8.8 Hz, Ar–**H**), 4.36(*t*, 2H, J = 6.0 Hz, ArOC**H**$_2$CH$_2$), 3.68(*t*, 2H, J = 6.0 Hz, BrC**H**$_2$CH$_2$).

*6.1.5. General procedure for the synthesis of **5e**, **5f**, and **5g***

The mixture of **4** (0.333 g, 1.0 mmol) and excess pyridine, DMAP, or Et_3N was heated to 110°C then stirred for 10 min or 6 h. After being cooled to room temperature, the precipitation was filtered and dried in the vacuum desiccator to yield yellow **5e**, **5f**, and **5g**, respectively.

*6.1.5.1. 1-(2-(4-(3-Thioxo-3H-1,2-dithiol-5-yl)phenoxy)ethyl)pyridinium bromide(**5e**).* Obtained from **4** (0.333 g, 1.0 mmol) and pyridine (20 mL) as yellow solid. (0.312 g, 75.5%), m.p. 160°C (found: C, 46.63; H, 3.42; Br, 19.39; N, 3.40; O, 3.88; S, 23.34 Calcd for $C_{16}H_{14}BrNOS_3$: C, 46.60; H, 3.42; Br, 19.38; N, 3.40; O, 3.88; S, 23.33%) ^{1}H NMR d_6-DMSO: 9.15 (d, 2H, J = 6.8 Hz, N=C**H**CH), 8.66 (*t*, 1H, J = 8.0 Hz, N=CHCHC-**H**), 8.20 (dd, 2H, J_1 = 6.8 Hz, J_2 = 7.6 Hz, N=CHC**H**), 7.89 (d, 2H, J = 8.8 Hz, Ar–**H**), 7.78 (s, 1H, = C**H**), 7.08 (d, 2H, J = 8.8 Hz, Ar–**H**), 4.63 (*t*, 2H, J = 5.2 Hz, ArOC**H**$_2$CH$_2$), 5.08 (*t*, 2H, J = 4.8 Hz, ArOCH$_2$C**H**$_2$); IR (KBr pellets cm^{-1}) υ 3055, 2926,2880, 1633, 1603, 1486, 1421, 1289, 1263, 1201, 1025. ESI–MS: 332 (M^+).

*6.1.5.2. 4-(dimethylamino)-1-(2-(4-(3-thioxo-3H-1,2-dithiol-5-yl) phenoxy)ethyl) pyri -dinium bromide(**5f**).* The mixture of **4** (0.333 g, 1.0 mmol) DMAP (0.244 g, 2.0 mmol) and DMF (10 mL) was heated to 100°C and stirred for 6h. DMF was evaporated under reduced pressure, then 10 mL petroleum was added to the residue and stirred overnight in order to precipitate. The mixture was filtered, and the cake was dried in a vacuum desiccator to yield yellow solid. (0.320 g, 70%), m.p. 152–154°C (found: C, 47.50; H, 4.20; Br, 17.55; N, 6.16; O, 3.51; S, 21.13 Calcd for $C_{18}H_{19}BrN_2OS_3$ C, 47.47; H, 4.20; Br, 17.54; N, 6.15; O, 3.51; S, 21.12%); ^{1}H NMR d_6-DMSO: 8.34 (d, 2H, J = 7.6 Hz, C**H**-N=C**H**), 7.89 (d, 2H, J = 8.8 Hz, C**H** = CH-N=CH–C**H**), 7.76 (s, 1H, = C**H**), 7.05–7.07 (m, 4H, Ar–**H**), 4.61 (*t*, 2H, J = 4.4 Hz, ArOC**H**$_2$), 4.47 (*t*, 2H, J = 4.4 Hz, ArOCH$_2$C**H**$_2$); IR (KBr pellets cm^{-1}) υ 3045, 1651, 1602, 1482, 1423, 1284, 1250, 1180, 1025. MS: 375 (M^+).

*6.1.5.3. N,N,N-triethyl-2-(4-(3-thioxo-3H-1,2-dithiol-5-yl)phenoxy) ethanaminium bromide(**5g**).* Obtained from **4** (0.333 g, 1.0 mmol) and Et_3N (20 mL) as yellow solid. (0.305 g, 70.1%), m.p. 226–228°C (found: C, 47.01; H, 5.58; Br, 18.41; N, 3.22; O, 3.68; S, 22.16. Calcd for $C_{17}H_{24}BrNOS_3$: C, 46.99; H, 5.57; Br, 18.39; N, 3.22; O, 3.68; S, 22.14%); ^{1}H NMR d_6-DMSO: 7.94 (d, 2H, J = 8.8 Hz, Ar–**H**), 7.81 (s, 1H, = C**H**), 7.14 (d, 2H, J = 8.8 Hz, Ar–**H**), 4.50 (*t*, 2H, J = 4.4 Hz, ArOC**H**$_2$CH$_2$), 3.71 (*t*, 2H, J = 4.4 Hz, ArOCH$_2$C**H**$_2$), 3.30–3.40 (m, 6H, C**H**$_2$CH$_3$×3), 1.23 (m, 9H, CH$_2$C**H**$_3$×3); IR (KBr pellets cm^{-1}) υ 3043, 2986, 1603, 1484, 1421, 1291, 1264, 1202, 1024; ESI-MS: 354 (M^+).

6.2. Aqueous solubility and stability

6.2.1. Aqueous solubility

Each prodrug measuring 200.0 mg in accurate weight was placed in a 50 mL beaker. Then 10 mL water was added and exposed to ultrasonic oscillation for 5min and stirred for 24 h in a 25°C constant temperature water bath. It was centrifuged at 10 000 rpm for 10 min, and the supernatant was filtered through a microporous membrane and injected to the HPLC system for determination.

6.2.2. Stability

The stability in phosphate buffer solution was investigated at five pH values: 1.10, 4.02, 6.84, 9.14, and 12.02 with two temperature degrees: 37°C and 60°C. The concentration was determined by HPLC in the following intervals: 0, 1, 2, 4, 8, 16, and 24 h.

The stability in mice plasma was investigated using the following steps: precisely 1.0 mL water solution (50 μg/mL) of the target compound was added into 5 mL mice plasma at 37°C. After mixing, it was kept in a 37 ± 1°C constant water bath, and the 200 μL sample was withdrawn at the following time points: 0, 1, 2, 4, 8, and 12 h. These were added to 400 μL acetonitrile, mixed for 5min, then centrifuged at 10 000 rpm for 5 min. The 20 μL supernatant was then injected into the HPLC system for determination.

6.3. Pharmacology

6.3.1. Drug and chemical agents

Compounds **5a**, **5b**, **5c**, **5d**, and **5e** were synthesized and identified in our laboratory. ATT was purchased from ChengDu GuoJia United Pharmaceutical Ltd. (Chengdu, P. R. China). CCl_4 and peanut oil were purchased from J&K Scientific Ltd. (Beijing China).

ALT and AST were investigated by the West China Medical Centre of Sichuan University. Data were expressed as mean ± standard deviation and were analyzed using SPSS version 11.5 software.

6.3.2. Test animals

Adult male Kunming mice weighing 20–22 g were obtained from the animal center of Sichuan University. The animals were left for two days to acclimatize to animal room conditions and were maintained on standard pellet diet and water ad libitum. Food was withdrawn on the day before the experiment, but free access to water was allowed. Since the experiment could be completed within 48 h, there was no significant change in the mices' body weight during the experiment. All animals received human care, and the study protocols complied with the guidelines of the animal center of Sichuan University. Throughout the experiments, the animals were handled according to the international ethical guidelines for the care of laboratory animals.

6.3.3. CCl_4-induced acute liver damage model in mice and investigated compounds treatment

The mice were randomly divided into five groups of ten. In the normal control group, the mice were intraperitoneally injected with saline solution alone. In the model group, the mice were intraperitoneally injected with CCl_4 at a dose of 5 mL/kg as a 0.2% peanut oil solution. The low dose group of mice was intraperitoneally injected with the investigated compounds at a dose of 1 mg/kg, while the middle dose group was intraperitoneally injected with the investigated compounds at a dose of 2 mg/kg. The high dose group was intraperitoneally injected with the investigated compounds at a dose of 4 mg/kg.

The mice in the low, middle, and high dose groups were subsequently injected intraperitoneally with CCl_4 at a dose of 5 mL/kg as a 0.2% peanut oil solution 0.5 h after pretreatment with the investigated compounds.

Sixteen hours after the introduction of CCl_4, all the mice were sacrificed, and blood samples withdrawn from the orbital sinus were collected and allowed to clot; they were then centrifuged at 3 500 rpm for 10 min. The serum was separated and used for the assay of ALT and AST.

6.4. Metabolism study

6.4.1. Chemical agents and animal

Compounds **5a**, **5d**, and **2** were synthesized and identified in our laboratory.

The animals were handled in accordance with the procedures detailed in Section 6.3.2.

6.4.2. Animal test

The mice were randomly divided into ten groups of four and were intraperitoneally injected with the investigated compounds respectively at a dose of 10.00 mg/kg body weight. The blood samples (0.5 mL) withdrawn from the orbital sinus were collected into heparinized tubes at the following post-dose intervals: 0.02, 0.08, 0.25, 0.5, 0.75, 1, 1.5, 3, 5, and 8 h. Plasma was separated immediately through centrifugation (3 000 rpm, 5 min) and then processed in accordance with the procedures detailed in Section 6.4.5.

6.4.3. Instrumentation and chromatography

The Waters liquid chromatographic system employed was an LC-10A liquid chromatographic system (Shimadzu Japan). The analysis was carried out on a SinoChrom ODS-C18 column (200 mm × 4.6 mm, 5 μm), thermostated at 25°C, 0.05 mol $L^{-1}KH_2PO_4$ (adjust pH to 3 with H_3PO_4): methanol: acetonitrile (200:200:150 (v/v)) was used as the mobile phase at a flow rate of 1.0 mL/min. The detection was performed at a wavelength of 354 nm after an injection volume of 20 μL.

6.4.4. Preparation of calibration standards and quality control samples

The calibration standards of **5a**, **5d**, and **2** consisting of six points ranging from 0.05 to 31.11 μg/mL were prepared by adding appropriate amounts of the standard solution into blank plasma. Quality control (QC) samples were prepared by adding the proper **5a**, **5d**, and **2** into blank plasma in order to create concentrations in three levels.

6.4.5. Sample preparation

Precisely 200 μL of mice plasma was added into 400 μL acetonitrile. After being laid down in the ultrasonic instrument for 5 min and being centrifuged at 10 000 rpm for 5 min, 20 μL aliquots of the supernatant were injected into HPLC for the assay.

6.4.6. Method validation

The selectivity of the method was checked for interference from plasma. The standard curve consisting of six points ranging from 0.05 to 31.11 μg/mL was developed. Quality control samples as LQC, MQC, and HQC were used to determine the intra- and inter-day precision and accuracy of the assay. Drug concentrations in the plasma samples along with the same day-standard curve samples were calculated using a linear equation.

References

[1] L. Jirousek, J. Starka, Nature 45 (1958) 386.
[2] S.S. Ansher, P. Dolan, E. Bueding, Hepatology 3 (1983) 932–935.
[3] J.-M. Warnet, M.-O. Christen, M. Thevenin, D. Briard, A. Jaqueson, J.-R. Claude, Arch. Toxicol. 13 (Suppl.) (1989) 322–325.
[4] S.S. Ansher, P. Dolan, E. Bueding, Fund. Chem. Toxicol. 24 (1986) 405–415.
[5] Marie-Odile Christen, Meth. Enzymol. 252 (1995) 316–323.
[6] Hu fulian, Xu xiaoyuan, Ji kaiyu, et al., Chin. J. Med. Guide 5 (2003) 85–87.
[7] M.G. Voronkov, T.V. Lanina, A.S. Saratikov, M.I. Solov'eva, L.I. Dubro, Pharm. Chem. J. 1 (1967) 621–625.
[8] Mita Shigeyoshi, Ando Tomoyuki, Kanematsu Akihito, JP 08026992 (1996).
[9] Mita Shigeyoshi, Ando Tomoyuki, Kanematsu Akihito, JP 07118262 (1995).
[10] Mita, Naruyoshi, Ando Tomoyuki, Kanematsu Akihito, EP 0641792 (1995).
[11] Von R. Gmelin, F. Lagler, Arzneim. Forsch. 13 (1963) 130–133.
[12] S.A. Sheweit, M. Abd El-Gabar, M. Bastawy, Toxicology 165 (2001) 217–224.
[13] Xingshen Ye, Yibin Feng, Yao Tong, Kwan-Ming Ng, SaiWah Tsao, George K. K. Lau, Chowing Sze, Yanbo Zhang, Jun Tang, Jiangang Shen, Seiichi Kobayashi, J. Ethnopharmacol 124 (2009) 130–136.
[14] Chun-Kwan Wong, Vincent E.C. Ooi, Put O. Ang Jr., Hydrobiologia 512 (2004) 267–270.