Design, Synthesis and Biological Evaluation of Lipophilic Analogs of Anethol Trithione

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Abstract: 16 ADT carboxylate esters were prepared and tested for their chemical characteristics, stability, bioavailability, potency and toxicity. Considering the good bioavailability, **3a** was selected for the pharmacology test, and the result showed that the potency of **3a** was remarkably higher than that of ATT. Additionally, **3a** was also chosen for the acute toxicity test. The result indicated that the prodrug **3a** was more safer than ATT. The study suggested the feasibility to improve the bioavailability of ATT by using prodrug strategy.

Keywords: Synthesis, Anethol trithione, Lipophilicity, Bioavailability, Prodrugs.

INTRODUCTION

Anethol trithione (ATT), a sulfur heterocyclic compound first discovered in cruciferous vegetables, is a relatively new chologogue. ATT and its analogues have been studied as sialagogue, antischistosomial, choleretic and hepatic protectant [1, 2], which are widely used in clinic for treating symptoms associated with hepatobiliary dysfunctions without any major adverse reactions being noted [3]. ATT can increase human cholinergic, adrenergic responsiveness and prevent the up-regulation in muscarinic acethylcholine receptor density. Also it is a potent inducer of phase II enzymatic detoxification systems and can increase the activity of glutathione reductase and glutathione peroxidase, improve the level of glutamyl cysteine synthetase and glutathione (GSH) which can deactivate many chemicals by conjugation, resulting in the enhancement of hepatic cell activity and bilifaction [4, 5]. Besides the above effects, Zhang and co-works found that ATT analogues could prevent cellular superoxidant damages and carcinogenesis produced [6].

Previous research described that ATT was firstly metabolized into 4-hydroxy-anethole trithione (ADT) in plasma and then ADT was conjugated with sulfuric acid or glycuronic acid to excrete. The primary pharmacodynamic active constituent was ADT, the demethyl metabolite of ATT, since ATT lose methyl group swiftly in liver after administration [7]. However, the marketed dose form of ATT was tabella which caused the bad absorption of the drug in intestinal tract. Besides this, it must be noted that the rate of metabolism of ATT was comparatively low which limited the pharmacodynamic effect of ADT. Lots of efforts had been done to improve the bioavailability of ATT such as changing the dosage form [8], but no report has been published reporting the structural modification of ATT to increase the bioavailbiity *in vivo*. Inspired by those previous research results and considering the structural characteristics of the active constituent ADT, 16 ADT carboxylate esters prodrugs (Fig. (**1**)) were prepared by means of esterification with ADT in order to improve the biological availability of this active drug. The purpose of this study was to investigate the stability, bioavailability and toxicity of the designed and synthesized ADT analogs.

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RESULT AND DISCUSSION

Chemistry

As prodrugs of ADT, the designed compounds should simultaneously achieve the following features: 1. great lipophilicity, 2. high conversion efficiency of the prodrug to the active molecule ADT, 3. good biological availability of the active drug. In order to improve the fat-solubility of ADT, the methyl group of ATT was removed to afford active form ADT and then coupled with various aromatic or aliphatic acid *via* ester linkage at -*O* position of ADT. We hoped that the lipophilic group of these acids were able to enhance the lipophilicity of the prodrug and caused high uptake rate in intestinal tract. The ester linkage of these lipophilic groups would make sure that these compounds could be swiftly hydrolyzed by the blood esterase and release active drug ADT. These analogs were prepared by the procedures illustrated in Scheme **1**. We performed the first step by treatment the ATT with pyridine hydrochloride under N_2 atmosphere at 215 ^oC with satisfied yield (84.5%) to afford ADT (**2**). Then compound **2** was coupled with different acid groups in the presence of DCC and DMAP to afford **3a**–**3p** with yields 43.8%-96.0%. In total, 16 new compounds **3a**–**3p** were prepared, their structures were confirmed by IR, ^{1}HNMR, ESI-MS. In general, IR spectra showed the ArC=C peak at 1448–1608 cm^{-1}, the C=S stretching vibrations at 1164–1189 cm^{-1}, and the C-O stretching vibrations at 1012–1049 cm^{-1}. In the nuclear magnetic resonance spectra (^{1}H-NMR), the signals of the respective protons of the synthesized compounds were verified on the basis of their chemical shifts, mutiplicities, and coupling constants. The spectra showed the alkene (C=CHCS) proton as a singlet at7.25–7.44 ppm.

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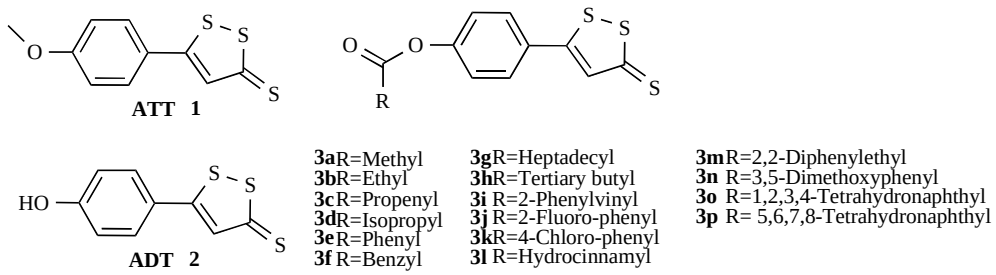
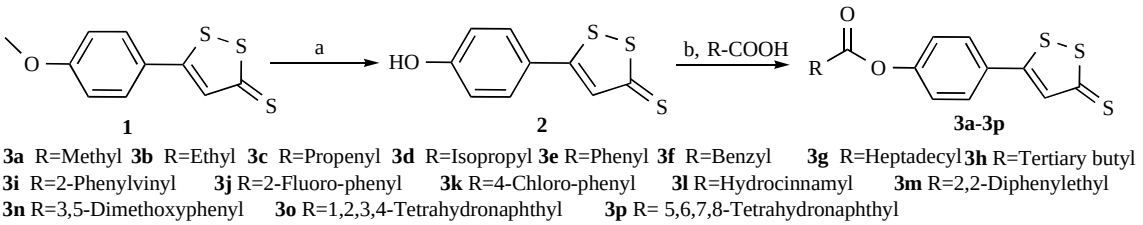


Fig. (1). ATT, ADT and ADT carboxylate esters **3a**-**3p**.



Scheme 1. The synthetic route of **3**a-p. Regents and condition: (a) pyridine hydrochloride, 215 °C, 3h; (b) DCC, DMAP, CH_2Cl_2, 25 °C, overnight.

pH Stability in Aqueous Buffers

In order to achieve hepatic protectant effects, the ADT moiety of these analogs should be released from those forms in plasma by hydrolysis of esterase. Therefore, **3a**, **3d**, **3e**, **3f** and **3i** were selected to the stability test in solutions under different pH and plasma at 37 °C *in vitro*.

The results of the test in aqueous buffer solutions was listed in Fig. (**2**), which showed that these compounds are moderately stable in physiological conditions. These compounds were partially hydrolyzed to parent drug at pH 7.4, indicating that the compounds will undergo hydrolysis in the physiological environment. The compounds appeared to be unstable in pH 1.0, and comparatively stable in pH 6.0 and 9.5 buffer. Generally, the stability of these compounds was determined by the pH deviation from neutral value, the more deviation from neutral value the pH had, the more unstable these compounds were.

Plasma Stability *In Vitro*

To determine the possibility of esterase hydrolysis, the analogs synthesized were incubated with mice plasma at 37 °C (Table **1**). The result showed that all the test compounds were unstable in plasma, as was expected, so as to swift release the active drug ADT after absorbed in intestinal tract. From the five compounds, **3a** and **3e** could be totally hydrolyzed by the esterase in plasma in 180 minutes, how-

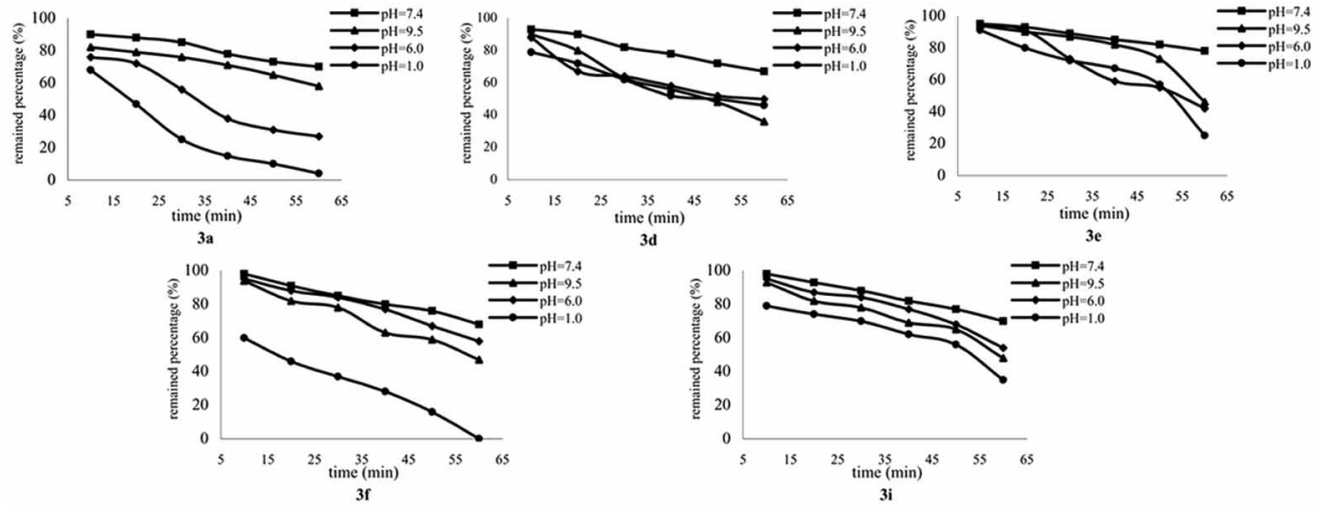


Fig. (2). Stability of ADT analog **3a**, **3d**, **3e**, **3f**, **3i** under different pH at 37 °C (remained concentration%).

Table 1. Stability of ADT Analog 3a, 3d, 3e, 3f, 3i in Mice Plasma at 37 °C (Remained Concentration%)

Compound	Time (min)				
	0	**30**	**60**	**120**	**180**
3a	100.0	13.2	7.5	1.8	nd[a]
3d	100.0	17.4	9.6	4.2	1.8
3e	100.0	14.2	8.7	2.7	nd
3f	100.0	14.5	10.1	4.8	1.23
3i	100.0	16.1	9.1	5.4	2.3

[a]nd: not detected.

ever, we found that the hydrolytic process of **3d**,**3f** and **3i** were slower compared with **3a** and **3e**, and these analogs could not get complete hydrolysis in test time. This might contribute to the steric hinderance of coupled acid in the analogs that the esterase could not reach the substrate and hydrolyzed the compounds effectively.

In Vivo Evaluation

To understand the *in vivo* behavior of the designed analogs, we assessed the plasma pharmacokinetics of the synthesized compounds and the control (ATT) in mice. Compound **3a**, **3d**, **3e**, **3f**, **3i** and ATT were subjected to bioavailability evaluation as these compounds were expected to possess better lipophilicity than ATT. Freshly prepared solutions of these compounds in peanut oil were administrated to mice by intragastric injection in a single dose equivalent to 250mg/kg of ATT. At specific time intervals the animals were sacrificed, and then blood samples were collected for analysis by HPLC method which was established and applied to investigate the major metabolite (ADT) of these compounds and the results was outlined in Table **2**.

In this assay, we found that all the compounds could not be detected at the first time interval (5 minute), which may be due to the high metabolic rate of them in plasma. The results showed that all compounds except **3f** could reach the peak values of blood drug concentration of ADT more swift than ATT (120 min). This could be caused by the better lipid-solubility of the compounds than ATT, which could enhance the uptake rate in mice intestine. From the *in vitro* stability test, decomposition of **3f** was quit fast in pH 1.0, and this could explain the low bioavailability of **3f** *in vivo*. Compound **3f** might be quickly hydrolyzed to ADT in stomach (pH 1-3), of which the structure possessed large polarity and could not be effectively absorbed. Additionally, the ADT concentrations of the compounds **3a**, **3d**, **3f**, **3i** in plasma were higher than that of ADT during 180 as expect. The C_{max} of **3a**, **3d**, **3f** and **3i** were 660.6μg/ml, 236.1μg/ml, 212.1mg/ml and 453.3μg/ml, which were much higher than that of ATT (48.4μg/ml). Pharmacokinetic parameters of ADT in mice after i.g. administration of the prodrugs and ATT were reported in Table **3**. The results in Table **3** showed that in the case of orally administrating **3a**, **3d**, **3f**, **3i**, AUC_{0-t} in plasma were significantly higher than that after the administration of ATT. The REs were enhanced to 4.65, 1.82, 3.22, and 1.57 times that of ATT for compounds **3a**, **3d**, **3f**, and **3i**, and the CEs were increased to 13.65, 4.88, 4.38, and 9.77 times that of ATT, respectively. It was notable that the AUC_{0-t} and C_{max} of **3a** was much higher than that of other test compounds. These data indicated that more prodrug **3a** were transferred to plasma and could be easily hydrolysed in plasma to the active ingredient ADT and render better bioavailability.

Pharmacology Investigation

3a was selected for pharmacology evaluation in view of the good bioavailability. Acute liver lesion induced by CCl_4 was the most classic experimental model of liver lesion [9]. The hepatoprotective effects of these prodrugs were investigated by the treatment with mice at three dose levels respectively, and the mice were sequentially induced liver damage by CCl_4. The results showed that **3a** could decreased the alanine transaminases (ALT) and aspartate transaminases (AST) level in serum compared with the CCl_4 control group, and its effect on decreasing the level of ALT and AST was

Table 2. The ADT Concentration after Intragastric Administration of the Compounds (ng.ml^{-1})

Compound	Time (min)							
	5	**10**	**15**	**30**	**45**	**60**	**120**	**180**
3a	65.3	101.2	178.2	145.8	243.2	660.6	41.0	nd[a]
3d	64.5	120.5	236.1	145.7	156.7	63.4	57.6	35.2
3e	nd	2.7	10.0	17.2	17.0	22.5	21.1	19.6
3f	5.6	9.9	21.8	14.1	77.6	156.2	212.1	120.6
3i	14.9	159.7	453.3	245.1	156.3	49.7	9.1	4.3
ATT	16.0	21.4	29.1	33.4	37.2	46.7	48.4	25.0

[a]nd: not detected.

Table 3. Pharmacokinetic Parameters of ADT after Intragastric Administration of the Compounds and ATT

Parameters	Compounds and ATT					
	3a	**3d**	**3e**	**3f**	**3i**	**ATT**
CL/F(L/min/kg)[a]	5.49	14.47	12.09	58.80	21.14	12.96
AUC_{0-t}(ng.min/mL)[b]	34452.00	13447.87	3263.16	23854.51	11617.08	7401.13
MRT_{0-t}(min)[c]	81.24	67.77	99.01	108.54	35.35	98.45
t1/2(min)[d]	83.76	72.67	69.32	143.61	25.84	174.37
T_{max}(min)[e]	60	15	60	120	15	120
C_{max}(ng/mL)[f]	660.60	236.1	22.5	212.1	453.3	48.4
RE[g]	4.65	1.82	0.46	3.22	1.57	-
CE[h]	13.65	4.88	0.46	4.38	9.37	-

[a]CL/F: clearance.
[b]AUC_{0-t}: area under the concentration time curve.
[c]MRT_{0-t}: the mean residence time.
[d]t1/2: biological half life.
[e]T_{max}: time of maximum concentration.
[f]C_{max}: maximum concentration.
[g]relative uptake efficiency.
[h]concentration efficiency.

superior to ATT at all the three doses as expected (Table **4**). Similar to ATT, **3a** exhibited the highest hepatoprotective ability at the middle dose (2mg/kg) that decreased the level of ALT and AST to 98.2±15.4 IU/L and 132.7±34.8 IU/L respectively. All mentioned above indicated that **3a** could be used as a promising prodrug of ADT, which possessed better bioavailability and potency than ATT.

Acute Toxicity Test

Because of the good bioavailability and potency, the safety of this compound **3a** is a non-negligible factor. To study the acute toxicity of **3a**, we used the method of maximum dose. ICR mice were administered saturated solution of **3a** in peanut oil at a constant volume of 40ml/kg. As a result, no mouse died within the whole 14 days, which indicated that the maximum tolerated dose (MTD) of ADT was no less than 3.25 g/kg (saturated concentration of **3a**: 0. 0813 g/ml). It is known that the LD_{50} of ATT is 1.480 g/kg [10], so the result suggested that compound **3a** was more safe than ATT.

EXPERIMENTAL SECTION

Materials and Apparatus

TLC was performed using precoated silica gel GF_{254} (0.2mm), and column chromatography was performed using silica gel (100-200 mesh). Melting point was measured on an YRT-3 melting point apparatus. IR spectra were obtained on a Perkin-Elmer983. NMR spectra were taken on a Varian INOVA400 and on a Bruker AC-E200. Chemical shifts are expressed in δ (ppm) with tetramethylsilane (TMS) as internal reference and coupling constants (J) are expressed in Hz. Mass spectra were recorded on an Agilent 1946B ESI-MS instrument.

The synthesis route of the ATT analogs (**3a-p**), starting from a commercially available ATT, is outlined in Scheme **1**. As a representative example, the general synthetic procedure of **3a** is provided below. The synthetic procedure of compounds **3b-3p** is similar to that of compound **3a**.

Chemistry

Synthesis of 5-(4-hydroxyphenyl)-3H-1,2-dithiole-3-thione (2)

A mixture of ATT **1** (10.05g, 40mmol) and anhydrous pyridine hydrochloride (34.07g, 290 mmol) was heated to 220°C for 20 min under argon atmosphere. The system was cooled to room temperature, and then 100ml water was poured in and stirred for 5 min. The suspension was filtered, and the filter cake was collected. The solid was dissolved in 100ml of 10% NaOH aqueous solution and stirred for10 min,

Table 4. Effect of ATT and Compounds 3a on the Levels of ALT, AST in Serum of Model Mice

Compound	Group	n	Doses (mg/kg)	ALT(*X*±SD, IU/L)	AST(*X*±SD, IU/L)
	Normal	10	0	65.3±14	89.8±18
	Model	10	0	789±37	964±90
	Low	10	1	151±29	270±90
ATT	Middle	10	2	112±14	266±59
	High	10	4	177±94	407±34
	Low	10	1	111±12	174±31
3a	Middle	10	2	98.2±15	133±35
	High	10	4	87.7±26	288±52

then filtered, the filtrate was acidified to pH 1 by conc HCl to form massive solid, then filtered and the filtter cake was dried in vacuo to afford **2** as orange solid (7.65g, 84.5%). MP: 172-174°C. ^{1}HNMR ($CDCl_3$): δ 7.67 (dt, 3 H, J_1=8.8Hz, J_2=2.0Hz), 7.38 (s, 1 H), 7.24 (dt, 2 H, J_1 = 8.8 Hz, J_2 = 2.0 Hz). IR (KBr): 3179, 3050, 1608, 1474, 1197, 1023cm^{-1}. MS m/z: 227 ($M+H^+$).

Synthesis of 4-(3-thioxo-3H-1,2-dithiol-5-yl)phenyl acetate (3a)

To a solution of DCC (271mg, 1.20mmol) and DMAP (15mg, 0.12mmol) in CH_2Cl_2, acetic acid (66mg, 1.10mmol) was added and the reaction mixture was stirred for 30 min, and then compound **2** (226mg, 1.00mmol) was added to this slurry. The mixture was stirred at room temperature over night, then the white precipitate was filtered off and CH_2Cl_2 was removed in vacuo, the residue was purified on a silica-gel chromatography column to get compound **3a** (239mg, 89%) as a red solid. MP: 140-142°C. ^{1}HNMR ($CDCl_3$): δ 7.69 (dt, 3 H, J_1=8.8Hz, J_2=2.0Hz), 7.40 (s, 1 H), 7.24 (dt, 2 H, J_1 = 8.6 Hz, J_2 = 2.4 Hz), 2.34 (s, 3 H). IR (KBr): 3028, 2922, 1763, 1594, 1516, 1363, 1185, 1023 cm^{-1}. MS m/z: 269 ($M+H^+$).

4-(3-thioxo-3H-1,2-dithiol-5-yl)phenyl propionate (3b)

Yield: 80.4%. MP: 110-111°C. ^{1}HNMR ($CDCl_3$): δ 7.68 (dt, 2H, J_1=8.4Hz, J_2=2.0H), 7.41 (s, 1H), 7.23 (dt, 2H, J_1=8.8Hz, J_2=2.0Hz), 2.63 (q, 2H, J=7.6Hz), 1.29 (t, 3H, J=7.6Hz). IR (KBr): 3107, 2959, 2935, 1759, 1597, 1452, 1228, 1174, 1025cm^{-1}. MS m/z: 283 ($M+H^+$).

(E)-4-(3-thioxo-3H-1,2-dithiol-5-yl)phenyl but-2-enoate (3c)

Yield: 94.4%. MP: 126-127°C. ^{1}HNMR ($CDCl_3$): δ 7.62 (dt, 2H, , 7.36 (s, 1H), 7.20 (dt, 2H, J_1=8.8Hz, J_2=2.0Hz), 7.15 (dq, 1H, J_1=15.6Hz, J_2=1.6Hz), 5.99 (dq, 1H, J_1=15.6Hz, J_2=1.6Hz), 1.93 (dd, 3H, J_1=6.8Hz, J_2=1.6Hz). IR (KBr): 3063, 1753, 2935, 1654, 1596, 1520, 1314, 1174, 1026, 978 cm^{-1}. MS m/z: 295 ($M+H^+$).

4-(3-thioxo-3H-1,2-dithiol-5-yl)phenyl isobutyrate (3d)

Yield: 51.9%. MP: 99–100°C. ^{1}HNMR ($CDCl_3$, 400 MHz): δ 7.67 (dt, 2H, J_1=8.8Hz, J_2=2.0Hz), 7.41 (s, 1H), 7.22 (dt, 2H, J_1=8.8Hz, J_2=2.0Hz), 2.84 (m, 1H), 1.33 (d, 6H, J=7.2Hz). IR (KBr): 3046, 2977, 2932, 1748, 1386, 1164, 1027 cm^{-1}. MS m/z: 297 ($M+H^+$)

4-(3-thioxo-3H-1,2-dithiol-5-yl)phenyl benzoate (3e)

Yield: 97.5%. MP: 161–162°C. ^{1}HNMR ($CDCl_3$, 400 MHz): δ 8.22 (dt, 2H, J_1=8.4Hz, J_2=2.0Hz), 7.75 (dt, 2H, J_1=8.8Hz, J_2=2.0Hz), 7.68(tt, 1H, J_1=7.6Hz, J_2=1.2Hz), 7.54 (t, 2H, J=8.4Hz), 7.44(s, 1H), 7.38(dt, 2H, J_1=8.8Hz, J_2=2.0Hz). IR (KBr): 3061, 1736, 1598, 1451, 1267, 1180, 1012 cm^{-1}. MS m/z: 331 ($M+H^+$)

4-(3-thioxo-3H-1,2-dithiol-5-yl)phenyl 2-phenylacetate (3f)

Yield: 85.9%. MP: 134–136°C. ^{1}HNMR ($CDCl_3$, 400 MHz): δ7.65 (d, 2H, J=8.4Hz), 7.38 (d, 5H, J=4Hz), 7.25 (s, 1H), 7.20 (d, 2H, J=8.4Hz), 3.88 (s, 2H). IR (KBr): 3028, 2914, 1755, 1601, 1453 1353, 1231, 1169, 1026 cm^{-1}. MS m/z: 345 ($M+H^+$)

4-(3-thioxo-3H-1,2-dithiol-5-yl)phenyl stearate (3g)

Yield: 79.3%. MP: 85–86°C. ^{1}HNMR ($CDCl_3$, 400 MHz): δ 7.68 (dt, 2H, J_1=8.4Hz, J_2=2.1H), 7.41 (s,1H), 7.23 (dt, 2H, J_1=8.8Hz, J_2=2.0Hz), 2.59 (t, 2H, J=7.2Hz), 1.76 (q, 2H, J=7.2Hz), 1.40 (br,28H). IR (KBr): 3031, 2953, 2920, 1747, 1599, 1516, 1460, 1379, 1221, 1188, 1018cm^{-1}. MS m/z: 493 ($M+H^+$)

4-(3-thioxo-3H-1,2-dithiol-5-yl)phenyl pivalate (3h)

Yield: 43.8%. MP: 112–113°C. ^{1}HNMR ($CDCl_3$, 400 MHz): δ 77.79 (d, 2H, J=8.8Hz), 7.43 (s, 1H), 7.21 (d, 2H, J=8.4Hz), 1.39 (s, 9H). IR (KBr): 2961, 2930, 1741, 1598, 1516, 1395, 1368,1231, 1168, 1020cm^{-1}. MS m/z: 311 ($M+H^+$)

4-(3-thioxo-3H-1,2-dithiol-5-yl)phenyl cinnamate (3i)

Yield: 91.2%. MP: 161–162°C. ^{1}HNMR ($CDCl_3$, 400 MHz): δ 7.90 (d, 1H, J=16Hz), 7.71 (dt, 2H, J_1=8.8Hz, J_2=2.0Hz), 7.60 (m,2H), 7.44 (m,3H), 7.42 (s,1H), 7.33 (dt, 2H, J_1=8.8Hz, J_2=2.0Hz), 6.62 (d, 1H, J=16Hz). IR (KBr): 3059, 1729, 1630, 1597, 1448, 1309, 1189, 1025cm^{-1}. MS m/z: 357 ($M+H^+$)

4-(3-thioxo-3H-1,2-dithiol-5-yl)phenyl 2-fluorobenzoate (3j)

Yield: 78.9%. MP: 170–171°C. ^{1}HNMR ($CDCl_3$, 400 MHz): δ 8.12 (td, 1H, J_1=7.6Hz, J_2=2.0Hz), 7.75 (d, 2H, J=8.4Hz), 7.66 (m, 1H), 7.45 (s, 1H), 7.40 (dt, 2H, J=8.8Hz), 7.32 (m, 1H), 7.24 (m, 1H). IR (KBr): 3058, 1745, 1721, 1601, 1455, 1292, 1249, 1171, 1037cm^{-1}. MS m/z: 349 ($M+H^+$).

4-(3-thioxo-3H-1,2-dithiol-5-yl)phenyl 4-chlorobenzoate (3k)

Yield: 91.1%. MP: 188–189°C. ^{1}HNMR ($CDCl_3$, 400 MHz): δ 8.14 (dt, 2H, J_1=8.8Hz, J_2=2.0Hz), 7.74 (dt, 2H, J_1=8.8Hz, J_2=2.0Hz), 7.53 (dt, 2H, J_1=9.2Hz, J_2=2.4Hz), 7.44 (s, 1H), 7.37 (dt, 2H, J_1=8.8Hz, J_2=2.0Hz). IR (KBr): 2924, 1736, 1593, 1520, 1229, 1173, 1091, 1029cm^{-1}. MS m/z: 365 ($M+H^+$).

4-(3-thioxo-3H-1,2-dithiol-5-yl)phenyl 3-phenylpropanoate (3l)

Yield: 76.8%. MP: 192–194°C. ^{1}HNMR ($CDCl_3$, 400 MHz): δ 7.67 (dt, 2H, J_1=8.4Hz, J_2=2.0Hz), 7.45 (m, 1H), 7.40 (s, 1H), 7.32-7.36 (m, 4H), 7.15 (dt, 2H, J_1=8.8Hz, J_2=2.0Hz), 3.10 (t, 2H, J=7.6Hz), 2.94 (t, 2H, J=8.0Hz). IR (KBr): 3062, 1736, 1598, 1524, 1480, 1228, 1188, 1028cm^{-1}. MS m/z: 359 ($M+H^+$).

4-(3-thioxo-3H-1,2-dithiol-5-yl)phenyl 3,3-diphenylpropanoate (3m)

Yield: 89.4%. MP: 156–158°C. ^{1}HNMR ($CDCl_3$, 400 MHz): δ 7.58 (d, 2H, J=8.8Hz), 7.35 (s, 1H), 7.30 (s, 10H), 6.88 (d, 2H, J=8.8Hz), 4.64 (t, 1H, J=8.0Hz), 3.33 (d, 2H, J=8.4Hz). IR (KBr): 2924, 1759, 1597, 1523, 1489, 1221, 1171, 1020cm^{-1}. MS m/z: 435 ($M+H^+$).

4-(3-thioxo-3H-1,2-dithiol-5-yl)phenyl 3,5-dimethoxybenzoate (3n)

Yield: 86.1%. MP: 122–123°C. ^{1}HNMR ($CDCl_3$, 400 MHz): δ 7.75 (d, 2H, J=8.4Hz), 7.45 (s, 1H), 7.37 (d, 2H,

J=8.0Hz), 7.34 (d, 2H, *J*=2.4Hz), 6.76 (t, 1H, *J*=2.4Hz), 3.88(s,6H). IR (KBr): 2836, 1733, 1591, 1519, 1230, 1166, 1042cm^{-1}. MS m/z: 391 (M+H$^+$).

4-(3-thioxo-3H-1,2-dithiol-5-yl)phenyl 1,2,3,4-tetrahydronaphthalene-1-carboxylate (3o):

Yield: 96.0%. MP: 25–26°C. ^{1}HNMR ($CDCl_3$, 400 MHz): δ 7.67 (d, 2H, *J*=8.4Hz), 7.41 (s, 1H), 7.23 (d, 2H, *J*=8.8Hz), 7.33-7.16 (m, 4H), 4.10 (t, 1H, *J*=6.0Hz), 2.87(m, 2H, J=6.4Hz), 2.4-1.8 (m, 4H). IR (KBr): 3026, 2935, 1753, 1741, 1593, 1452, 1221, 1176, 1029cm^{-1}. MS m/z: 385 (M+H$^+$).

4-(3-thioxo-3H-1,2-dithiol-5-yl)phenyl 5,6,7,8-tetrahydronaphthalene-1-carboxylate (3p):

Yield: 96.0%. MP: 25–26°C. ^{1}HNMR ($CDCl_3$, 400 MHz): δ 7.96 (d, 1H, *J*=8.0Hz), 7.74 (dt, 2H, J_1=8.8Hz, J_2=2.0Hz), 7.44(s, 1H), 7.36 (dt, 2H, J_1=8.8Hz, J_2=2.0Hz), 7.34 (br, 1H), 7.23 (d, 1H, *J*=7.6Hz), 3.15 (t, 2H, *J*=4.8Hz), 2.87 (t, 2H, *J*=6.0Hz), 1.8-1.9 (br, 4H). IR (KBr): 3026, 2930, 1757, 1741, 1598, 1450, 1220, 1174, 1028 cm^{-1}. MS m/z: 385 (M+H$^+$).

Pharmacological Evaluation

General Method

All chemicals and reagents were analytical grade, and obtained commercially. All liquid reagents were distilled before use. ICR mice weighing 18-22g were received from Sichuan Industrial Institute of Antibiotics (P.R. China) and were maintained empty stomach for 24h. Since the experiment could be finished within 48 h, there was no significant change in mice's body weights during the experiment.

The HPLC system consisted of an SPD-10A variable UV-VIS detector and a set of Model LC-10AT liquid chromatography including a manometric module as well as a dynamic mixer from Shimadzu. The mobile phase consists of pure water and methanol, which was filtered through 0.45mm membrane filter before use. A phenomenex ODS column (4.6×200mm, 5μm) was eluted with the mobile phase (methanol/water: 10/3, v/v) at a flow rate of 1.0ml/min. The eluate was monitored by measuring the absorption at 355nm with a sensitivity of AUFS 0.01 at 25°C.

pH Stability Test In Vitro

An aqueous solution of the compounds (50 ug/mL) at a volume of 1.0 ml was incubated in 10ml buffer under different pH (1, 6, 7.4, 9.5) at 37°C under the protection of nitrogen. At a predetermined time interval, a 20 μL portion of the solution was removed, and the concentration of the compounds was measured by HPLC as described.

Plasma Stability Test In Vitro

Aliquots of the compounds aqueous solution at a volume of 0.1 ml was incubated in 0.4 ml mice plasma at 37°C under the protection of nitrogen. 100μl of the samples were withdrawn at predetermined time points and mixed with 200 μL acetonitrile. After centrifugation at 8000 rmp/min for 7 min, 20 μl of the supernatant was collected to monitor the disappearance of the compounds by HPLC analysis.

Bioavailability Evaluation in Mice In Vivo

According to the requirements of the National Act on the usage of experimental animals (P. R. China), the Sichuan University Animal Ethical Experimentation Committee, approved all procedures of our *in vivo* studies. The ICR mice were separated into 6 groups (8 in each) and intragastric administration of a solution of ATT and five analogs in peanut oil at a dose of compound equivalent to 250mg per kg body weight respectively was done. Mice from each group were sequentially sacrificed at 5, 10, 15, 30, 45, 60, 120 and 180min after administration and blood samples were collected from the ocular artery directly after removing eyeball, and were treated following the same procedure described for *in vitro* test to obtain plasma samples. These samples were collected to analysis the concentration of ADT in each time by HPLC method.

CCl_4-Induced Acute Liver Damage Model in Mice and Treatment of Investigated Compounds

The ICR mice (20-22g) were randomly divided into five groups, and each contained 10. In normal group and model group, the mice were injected intraperitoneally, 5ml/kg saline solution and CCl_4 at a dose of 5ml/kg as a 0.2% peanut oil solution, respectively. In low, middle and high dose group, the mice were intraperitoneally injected with the investigated compound at a dose of 1mg/kg, 2mg/kg and 4mg/kg, respectively. The low, middle and high dose groups were followed by intraperitoneal injection with CCl_4 at a dose of 5ml/kg as a 0.2% peanut oil solution 0.5 hour after pretreatment with tested compound. According to the previously reported methods with some modification[9], 16 hours after introduction of CCl_4, all mice were sacrificed and blood sample withdrawn from orbital sinus was collected and allowed to clot, then centrifuged at 3500rpm for 10min. The serum was separated and used for assay of alanine transaminases (ALT) and aspartate transaminases.

Acute Toxicity Test

Sixty ICR mice were divided into three groups: test group, control group and vehicle group. Every group consisted of 10 males and 10 females, and all drugs were intragastricly administered in a constant volume of 40ml/kg. The three groups were treated once with following drugs respectively: **3a** saturated solution of peanut oil, normal saline and peanut oil. Animals were observed closely during the period 30 min to 1 hour after being administered. The number of dead animals was counted at two weeks, body weights were noted once per week. The body weight of ADT group had no statistics difference compared with control group and vehicle group (On Day 0, ADT group: 20.1 ± 1.2 g vs. control group: 20.0 ± 1.2 g and vehicle group: 20.2 ± 1.3 g; On Day 7, ADT group: 27.3 ± 0.9 g vs. control group: 27.7 ± 0.9 g and vehicle group: 27.6 ± 0.9 g; On Day 14, ADT group: 34.8 ± 1.3 g vs. control group: 35.1 ± 1.1 g and vehicle group: 34.8 ± 1.1 g; all of the $P > 0.05$).

Statistical Analysis

The clearance(CL/F), area under the concentration time curve($AUC_{0\text{-}t}$), the mean residence time($MRT_{0\text{-}t}$), biological half life(t1/2) and maximal concentration(T_{max}) were calculated by Data and max Statistics (DAS, Shanghai, China). The RE and CE were calculated to evaluate the delivery

property of the prodrugs. The value of RE and CE were defined as following:

RE=(AUC0-t)s /(AUC0-t)c

CE=(Cmax)s/(Cmax)c

Where s and c represented sample (the test prodrugs) and control (ATT) respectively.

CONCLUSION

In summary, we have designed and developed a method of synthesis for different ATT analogs. Five of the synthesized compounds were subjected to *in vitro* stability test and *in vivo* biological evaluation. The experimental data highlighted that after oral administration of ATT analogs **3a**, **3d**, **3e**, **3f** and **3i**, concentrations and AUC_{0-t} of ADT in plasma were significantly higher than that of ATT at the same dose. This verified that the prodrugs has the ability to enhance the bioavailability of ADT. Besides this, toxicity test results indicated compound **3a** was safer than ATT. In conclusion, these compounds could be used as potential candidates for the prodrug of ADT in clinical trial for the treatment of hepatobiliary dysfunctions.

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