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Lead detoxification activities and ADMET hepatotoxicities of a class of novel 5-(1-carbonyl-L-amino-acid)-2,2-dimethyl-[1,3]dithiolane-4-carboxylic acids

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ABSTRACT

By linking the mercapto groups with isopropyl and introducing L-amino acid into the 5-carboxyl of DMSA a class of novel 5-(1-carbonyl-L-amino-acid)-2,2- dimethyl-[1,3]dithiolane-4-carboxylic acids were prepared. Their in vivo activities were evaluated on lead loaded mice at the dose of 0.4 mmol/kg. The results showed that the lead levels of the livers, kidneys, femurs and brains in particular could be efficiently decreased by 0.4 mmol/kg of 5-(1-carbonyl-L-amino-acid)-2,2-dimethyl-[1,3]dithiolane-4-carboxylic acids. The benefit of 5-(1-carbonyl-L-amino-acid)-2,2-dimethyl-[1,3]dithiolane-4-carboxylic acids to the detoxification of the brain lead was attributed to their transmembrane ability. Compared with the lead detoxification efficacy, they did not affect the essential metals such as Fe, Cu, Zn, and Ca of the treated mice. Silico molecular modeling predicted that 5-(1-carbonyl-L-amino-acid)-2,2-dimethyl-[1,3]dithiolane-4-carboxylic acids had no hepatotoxicity.

Owing to rapid industrialization and agriculture modernization in both developed and developing countries, during the past two decades the levels of lead (Pb) in air, water, and soils have increased.[1–5] In terms of a widespread toxic metal, lead has a half-life time of 6–10 years.[4,6,7] In addition to hypertension, hyperthyroidism, oste-oporosis and skeletal disorders,[3–5,8] Pb accrued from environmental exposure has been associated with the developmental, neuropsychological and behavioral deficits.[9] Lead exposure will continue to be a major public health issue around the world for the fore-seeable future and there is an urgent need to find novel therapy for the treatment of lead poisoning.[10–13] It has been well documented that the chelation therapy is one of the most common methods in the treatment of Pb intoxication, and meso-2,3-dimer-captosuccinic acid (DMSA) has been known to be the most acceptable antidote in lowering the Pb levels of bone and brain without causing major redistributions of Pb to other organs, especially to the brain.[9,11,14–18,] To increase the efficacy of DMSA in lowering the Pb levels of the organs, especially in lowering the Pb level of the brain in this Letter DMSA was modified and a class of novel 5-(1-carbonyl-L-amino-acid)-2,2-dimethyl-[1,3]dithiolane-4-carboxylic acids were investigated.

In appropriate conditions via three-step-reaction procedure, namely the addition of DMSA and acetone, the dehydration of the thioketal-acid (**1**) and the amidation of the thioketal-anhydride (**2**) and amino acids, which were mentioned in Scheme 1 and 5-(1-carbonyl-L-amino-acid)-2,2-dimethyl-[1,3]dithiolane-4-carboxylic acids (**3a–l**) were prepared. The yields of **1**, **2** and **3a–l** were 78%, 91% and 22–56%, respectively. The synthetic and chemical physical data of all compounds are given in the file of Supplementary data. The data imply that using this three-step-reaction procedure **3a–l** can be smoothly obtained.

In the lead detoxification assay, the lead-loaded mice were treated with normal saline (NS, negative control), DL-penicillamine (positive control), sodium meso-2,3-dimercaptosuccinate (NaDMS, positive control), 2,2-dimethyl-[1,3]dithiolane-4,5-dicarboxylic acid (**1**) and 5-(1-carbonyl-L-amino-acid)-2,2-dimethyl-[1,3]-dithio-lane-4-carboxylic acids (**3a–l**), the lead levels of the livers, the kidneys, the brains and the femurs of the mice were measured and the data are shown in Table 1. The results illustrate that the lead levels of the livers, the kidneys, the left femurs and the brains of the mice receiving 0.4 mmol/kg of DL-penicillamine, NaDMS, **1** and **3a–l** are significantly lower than that of the corresponding organs of the mice receiving NS. This suggests that DL-penicillamine, NaDMS, **1** and **3a–l** are effective in lowering the organs' lead level of the mice. On the other hand, however, the lead level of the brains of the mice receiving 0.4 mmol/kg of **1** is significantly lower than that of the brains of the mice receiving 0.4 mmol/kg of NaDMS. This suggests that the modification of the mercapto groups of meso-2,3-dimercaptosuccinate benefits lowering the lead level of the brains. While the lead level of the brains of the mice receiving

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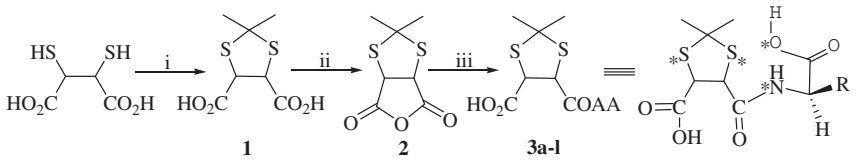

Scheme 1. Synthetic route of 5-(1-carbonyl-L-amino-acid)-2,2-dimethyl-[1,3]dithiolane-4-carboxylic acids. Reagents and conditions: (i) hydrogen chloride and acetone, 9 h, room temperature; (ii) acetyl chloride, 2.5 h, reflex; (iii) amino acid, 48 h, room temperature. In **3a** AA = Gly, **3b** AA = L-Ser, **3c** AA = L-Val, **3d** AA = L-Leu, **3e** AA = L-Ile, **3f** AA = L-Asn, **3g** AA = L-Asp, **3h** AA = L-Gln, **3i** AA = L-Glu, **3j** AA = L-Met, **3k** AA = L-Phe, **3l** AA = L-Trp. *Represents possible binding sites of Pb.

Table 1
Lead in organs of the treated mice[a]

Compd	lead in following organs			Lead in feces/urine		
	Femurs	Brain	Kidney	Liver	Feces	Urine
NS	23.70 ± 3.08	2.04 ± 0.40	8.92 ± 1.46	7.22 ± 1.64	1.27 ± 1.15	0.93 ± 0.11
DL-PA	14.99 ± 2.45[b]	1.61 ± 0.42[b]	7.24 ± 2.08[b]	3.52 ± 0.86[b]	3.26 ± 1.40[b]	1.94 ± 0.67[b]
1	15.47 ± 2.06[b]	1.42 ± 0.32[f]	7.12 ± 2.05[b]	4.02 ± 1.26[b]	4.57 ± 0.89[c]	1.93 ± 0.81[b]
NaDMS	14.31 ± 2.07[b]	1.72 ± 0.34[b]	6.23 ± 1.89[b]	4.75 ± 1.35[b]	4.59 ± 0.84[c]	1.79 ± 1.46[b]
3a	20.82 ± 2.90[c]	1.39 ± 0.28[e]	6.68 ± 2.53[b]	3.29 ± 1.56[b]	4.53 ± 0.56[c]	2.07 ± 0.47[b]
3b	14.70 ± 2.93[b]	1.05 ± 0.51[c]	6.43 ± 2.54[b]	3.21 ± 0.69[b]	4.41 ± 0.74[c]	2.83 ± 1.41[b]
3c	14.93 ± 2.58[b]	1.49 ± 0.47[b]	6.16 ± 1.35[b]	3.19 ± 0.74[b]	4.76 ± 0.84[c]	2.65 ± 1.08[b]
3d	16.33 ± 4.70[b]	1.44 ± 0.55[b]	7.32 ± 2.02[b]	3.64 ± 1.74[b]	3.99 ± 1.71[b]	3.44 ± 1.16[c]
3e	14.32 ± 3.14[b]	1.42 ± 0.61[b]	6.49 ± 1.16[b]	5.00 ± 1.41[b]	4.10 ± 1.84[b]	2.42 ± 0.93[b]
3f	16.62 ± 4.33[b]	0.58 ± 0.42[c]	7.06 ± 1.77[b]	3.99 ± 1.02[b]	4.71 ± 0.35[c]	2.12 ± 0.81[b]
3g	11.10 ± 1.30[d]	1.22 ± 0.46[e]	7.09 ± 1.36[b]	3.82 ± 1.36[b]	4.80 ± 1.04[c]	2.83 ± 0.48[c]
3h	14.59 ± 2.62[b]	1.02 ± 0.50[c]	6.86 ± 1.75[b]	3.07 ± 1.03[b]	4.50 ± 1.41[b]	2.89 ± 0.23[c]
3i	13.69 ± 1.34[b]	1.22 ± 0.48[e]	7.21 ± 2.64[b]	5.64 ± 1.32[c]	4.49 ± 1.61[b]	2.65 ± 0.85[c]
3j	13.71 ± 1.50[b]	0.91 ± 0.40[c]	6.36 ± 1.89[b]	3.51 ± 1.79[b]	4.35 ± 1.12[c]	2.26 ± 1.40[b]
3k	16.75 ± 2.92[b]	1.11 ± 0.27[c]	6.61 ± 1.02[b]	4.60 ± 1.95[b]	4.85 ± 1.38[c]	1.72 ± 0.26[b]
3l	17.66 ± 2.15[b]	1.21 ± 0.80[b]	7.50 ± 1.58[b]	5.59 ± 1.50[c]	4.14 ± 1.05[b]	2.84 ± 1.40[b]

For femurs lead: (b) compared to NS p <0.01; (c) compared to NS p <0.05; (d) compared to NS, DL-PA, **1** and NaDMS p <0.01. *For brain lead*: (b) compared to NS p <0.01; (c) compared to NS, DL-PA, NaDMS and **1** p <0.01; (d) compared to NS and NaDMS p <0.01, to DL-PA p <0.05; (e) compared to NS p <0.01, to DL-PA and NaDMS p <0.05; (f) compared to NS p <0.01, to NaDMS p <0.05. *For kidney lead*: (b) compared to NS p <0.05. *For liver lead*: (b) compared to NS p <0.01; (c) compared to NS p <0.05. *For fecal lead*: (b) compared to NS p <0.01; (c) compared to NS and p <0.01, to DL-PA p <0.05. *For urinary lead*: (b) compared to NS p <0.01; (c) compared to NS p <0.01, to NaDMS, DL-PA and **1** p <0.05.

[a] Date is represented with mean ± SD μg of Pb/g of organ or excrement, NS = normal saline = vehicle, NaDMS = sodium meso-2,3-dimercaptosuccinate, DL-PA = DL-penicillamine, dose of DL-PA, **1**, NaDMS and **3a–l** = 0.4 mmol/kg, n = 12.

0.4 mmol/kg of **3b,f,h,j,k** is significantly lower than that of the brains of the mice receiving 0.4 mmol/kg of **1**. This suggests that the coupling of 5-carboxyl and L-Ser, L-Asn, L-Gln, L-Met and L-Phe may lead to the increase of lead detoxification activity. Besides, no any obvious adverse behavioral reaction in mice following injection of **3a–l** was observed.

To evaluate the effect of **1** and **3a–l** on the lead excretion, the lead levels of the feces and the urine of the mice receiving NS (negative control), DL-penicillamine (positive control), NaDMS (positive control), **1** and **3a–l** were measured and the results are also shown in Table 1. The data demonstrate that the lead level of the feces of the mice receiving 0.4 mmol/kg of **3d,e,h,i,l** is significantly higher than that of the feces of the mice receiving NS, while the lead level of the feces of the mice receiving 0.4 mmol/kg of NaDMS, **1** and **3a,b,c,f,g,j,k** is significantly higher than that of the feces of the mice receiving NS and DL-penicillamine. These suggest that NaDMS, **1** and **3a–l** are able to effectively increase the lead excretion via the feces. The data also demonstrate that the lead level of the urine of the mice receiving 0.4 mmol/kg of DL-penicillamine, NaDMS, **1** and **3a–l** is significantly higher than that of the urine of the mice receiving NS. This suggests that DL-penicillamine, NaDMS, **1** and **3a–l** are able to effectively increase the lead excretion via the urine.

To explore the effect of the treatment on the essential metals, the Fe, Cu, Zn, Mn and Ca in the kidneys, livers, and brains of the mice receiving NS, and 0.4 mmol/kg of **1** as well as **3a–l** were measured and the data are given in the file of Supplementary data (Tables 1–3). The data indicate that these essential metals of the livers, kidneys and brains of the mice receiving 0.4 mmol/kg of **1** and **3a–l** are at the same level as that of the mice receiving NS. This suggests that the treatment of 0.4 mmol/kg of **1** and **3a–l** does not lower the essential metal level of the treated mice.

To evaluate the effect of the treatment on the growth of the treated mice the body weights of the mice receiving NS, 0.4 mmol/kg of **1** and **3a–l** were measured and the data are given in the file of Supplementary data (Table 4). The data indicate that the body weights of the mice receiving 0.4 mmol/kg of **1** and **3a–l** are at the same level as that of the mice receiving NS. This suggests that the treatment of 0.4 mmol/kg of **1** and **3a–l** does not affect on the growth of the treated mice.

To examine the dose-dependent response of the mice to the treatment, **3g** was selected as a representative of **3a–l** and three doses, that is, 0.2, 0.4 and 0.6 mmol/kg, were used. The lead levels of the livers, kidneys, left femurs and brains of the treated mice were measured and the data are listed in Table 2. The data indicate that the lead levels are progressively decreased with the increase of the dose. Therefore, **3g** dose-dependently moves the lead accumulated in the tissues.

The relationship of the membrane permeability and the lead detoxification activity of **1** and **3a–l** were examined with the Caco-2 cell monolayer experiment. To correlate the membrane permeability with the lead detoxification **3e,f,j** were selected as the representatives of **3a–l**, the membrane permeability's of DL-PA, NaDMS, **1** and **3e,f,j** were measured with the experiments of Caco-2 cell monolayer, and the data are listed in Table 3.

The experiments gave DL-PA, NaDMS, **1** and **3e,f,j** the 11.01×10^{-6} cm/s to 16.65×10^{-6} cm/s of P_{app} values from the apical side to the basolateral side and the 4.68×10^{-6} cm/s to

Table 2
Lead in organs of three doses of 3 g treated mice[a]

Compd	Dose (mmol/kg)	Lead in following organs			
		Femurs	Brain	Kidney	Liver
NS	0.2 ml	23.70 ± 3.08	2.04 ± 0.40	8.92 ± 1.46	7.22 ± 1.64
3g	0.6	9.64 ± 1.02[b]	0.84 ± 0.36[b]	5.90 ± 1.30[b]	2.77 ± 1.03[b]
	0.4	11.10 ± 1.30[c]	1.22 ± 0.46[c]	7.09 ± 1.36[c]	3.82 ± 1.36[c]
	0.2	17.21 ± 2.11[d]	1.69 ± 0.38[d]	7.68 ± 1.40[c]	5.81 ± 1.52[d]

For femurs lead: (b) compared to NS and 0.2 mmol/kg of **3g** p <0.01, to 0.4 mmol/kg of **3g** p <0.05; (c) compared to NS and 0.2 mmol/kg of **3g** p <0.01; (d) compared to NS p <0.01. *For brain lead*: (b) compared to NS and 0.2 mmol/kg of **3g** p <0.01, to 0.4 mmol/kg of **3g** p <0.05; (c) compared to NS p <0.01, to 0.2 mmol/kg of **3g** p <0.05; (d) compared to NS p <0.05. *For kidney lead*: (b) compared to NS and 0.2 mmol/kg of **3g** p <0.01, to 0.4 mmol/kg of **3g** p <0.05; (c) compared to NS p <0.01, to 0.2 mmol/kg of **3g** p <0.05; (d) compared to NS p <0.01. *For liver lead*: (b) compared to NS and 0.2 mmol/kg of **3g** p <0.01, to 0.4 mmol/kg of **3g** p <0.05; (c) compared to NS p <0.01, to 0.2 mmol/kg of **3g** p <0.05; (d) compared to NS p <0.01.

[a] Date is represented with mean ± SD μg of Pb/g of organ, NS = normal saline = vehicle, n = 12.

Table 3
The apparent permeability coefficients of **1** and **3e**,**f**,**j**

Compd	$P_{app} \times 10^{-6}$ (cm/s)		
	A→B	B→A	A→B/B→A
DL-PA	13.12	11.50	1.14
NaDMS	11.01	10.00	1.10
1	13.68	4.68	2.91
3e	14.25	4.91	2.90
3f	16.65	5.15	3.23
3j	15.70	5.05	3.11

The standard deviations were generally less than 10% (n = 4); A→B, From apical side to basolateral side; B→A, From basolateral side to apical side.

Table 4
ADMET hepatotoxicity probability of **3a–l**

Compd	ADMET score	Compd	ADMET score
NaDMS	0.033	**3f**	0.092
1	0.019	**3g**	0.059
3a	0.079	**3h**	0.112
3b	0.086	**3i**	0.099
3c	0.072	**3j**	0.105
3d	0.099	**3k**	0.317
3e	0.086	**3l**	0.264

11.50×10^{-6} cm/s of P_{app} values from the basolateral side to the apical side, and defined the P_{app} values from the apical side to the basolateral side to be 1.10-fold to 3.23-fold higher than the P_{app} value from the basolateral side to the apical side. It is generally accepted that an actively absorbed compound shows much faster transport from the apical to the basolateral direction and its P_{app} value from the apical side to the basolateral side should be more than 10×10^{-6} cm/s.[19] According to the P_{app} values from the apical side to the basolateral side, DL-PA, NaDMS, **1** and **3e,f,j** are transported actively across the Caco-2 cell monolayer. For **1** and **3e,f,j**, the P_{app} values from the apical side to the basolateral side are 2.90-fold to 3.23-fold higher than the P_{app} values from the basolateral side to the apical side, while for NaDMS the P_{app} values from the apical side to the basolateral side is only 1.10-fold higher than the P_{app} values from the basolateral side to the apical side. The comparison suggests that the present modification significantly improves the membrane permeability and should be responsible for the higher brain-lead detoxification activities of **1** and **3e,f,j**.

The hepatotoxicities of **1** and **3e,f,j** were calculated by using the absorption, distribution, metabolism, elimination and toxicity (ADMET) program,[20,21] which was developed from available literature data of 382 compounds known to exhibit liver toxicity or trigger dose-related elevated aminotransferase levels in more than 10% of the human population. To predict hepatotoxicity two scores (0 represents non-hepatotoxin and 1 represents hepatotoxin) are defined. The predicted scores (0.019–0.317) of NaDMS, **1** and **3a–l** are listed in Table 4. The data are much less than 1. This suggests that NaDMS, **1** and **3a–l** possess no hepatotoxicity.

In conclusion, linking the mercapto groups with isopropyl and introducing L-amino acid into the 5-carboxyl is a desirable structural modification of DMSA. This modification significantly increases the brain-lead detoxification activity without increasing the lead levels of other organs. This modification significantly increases the membrane permeability and possibly increases the capacity of blood–brain barrier permeability. These properties with the non-hepatotoxin prediction together provide a promising design for brain-lead detoxification agents.

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Supplementary data

Supplementary data (experimental procedures, biological evaluation methods, synthetic data, analytical data, physical chemical constants and spectral data) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.01.070.

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