Chelation in Metal Intoxication XLVI: Synthesis of Some α-Mercapto-β-Substituted Aryl Acrylic Acids and Their *In vitro* Cadmium Chelating Ability[1]

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Objective To synthesize some new α-mercapto-β-substituted aryl acrylic acids, characterize them and investigate their *in vitro* cadmium chelating ability. **Methods** Six α-mercapto-β-substituted aryl acrylic acids were prepared by the alkaline hydrolysis of 5- (aryl methylene) rhodanines, obtained from the condensation of substituted aldehydes and rhodanine following the reported procedure. The new compounds were characterized by elemental analysis, infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy. The liver and kidney from cadmium chloride pre-administered rats were homogenized and their nuclear mitochondrial fraction (NMF) and supernatant cytosol fraction (SCF) were separated. A measured volume of each fraction was dialyzed separately using "dialysis sack" against buffered-KCl medium containing a compound in the final concentration of 1×10^{-3} mol/L for 3 h at 37℃. The whole content of "sack" was subjected to cadmium estimation following digestion with conc. Nitric acid was detected using flame atomic absorption spectrometer. **Results** The *in vitro* screening showed that α-mercapto-β-(p-methoxyphenyl) acrylic acid (compound 2) and α-mercapto-β-(m-methoxy, p-hydroxyphenyl) acrylic acid (compound 4) were more effective than α-mercapto-β-thienyl acrylic acid (compound 1) and α-mercapto-β-(p-dimethylaminophenyl) acrylic acid (compound 3) in mobilizing cadmium as their dialyzable chelates. The presence of a methoxy group on the phenyl moiety (compounds 2 and 4) increases the metal chelating ability of mercapto acrylic acids. **Conclusions** Compounds 2 and 4 seem to have accessibility to the cellular system and capability of chelating-out the intracellularly bound cadmium.

Key words: Acrylic acid; Cadmium; *In vitro* chelation; IR /NMR spectra; Rat; Synthesis

INTRODUCTION

Health effects of low-level cadmium exposure are a still a problem in cadmium based industries and areas with severe environmental cadmium pollution, requiring removal of cadmium from its deposits in the body[1,2]. The problem in chelation of cadmium lies in its special toxicokinetics as this metal after exposure, is rapidly localized in cells bound to metallothionein (MT), mainly in the liver and then cadmium-thionein is gradually transferred via blood to the proximal tubular cells of the kidney where it is deposited[3-5].

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The success of *in vivo* cadmium chelation therefore, depends on the ability of the chelating agent to pass through the cellular membrane and its potential to chelate-out the intracellularly bound cadmium. Mercapto acrylic acids have been found to be useful antidotes for heavy metal poisoning and α-mercapto-β-furyl acrylic acid (MFA) particularly has been shown to protect against cadmium intoxication in rats[6]. An earlier investigation has shown that post-cadmium exposure treatment with certain α-mercapto-β-aryl acrylic acids is effective in decreasing liver and kidney cadmium burden in rats[7]. The reduction in liver cadmium content accompanied by a lower level of cadmium induced MT upon treatment with MFA has demonstrated an ability of MFA to mobilize intracellularly bound cadmium[8].

In an effort to develop a more effective treatment of cadmium poisoning, some mercapto acrylic acids have been recently synthesized according to the scheme and their ability to remove cadmium from the sub-cellular fractions of liver and kidney was tested in cadmium pre-exposed rats. Whether the substitution on the aryl ring at β-position in α-mercapto-β-aryl acrylic acids has any influence on their cadmium mobilizing ability was also investigated.

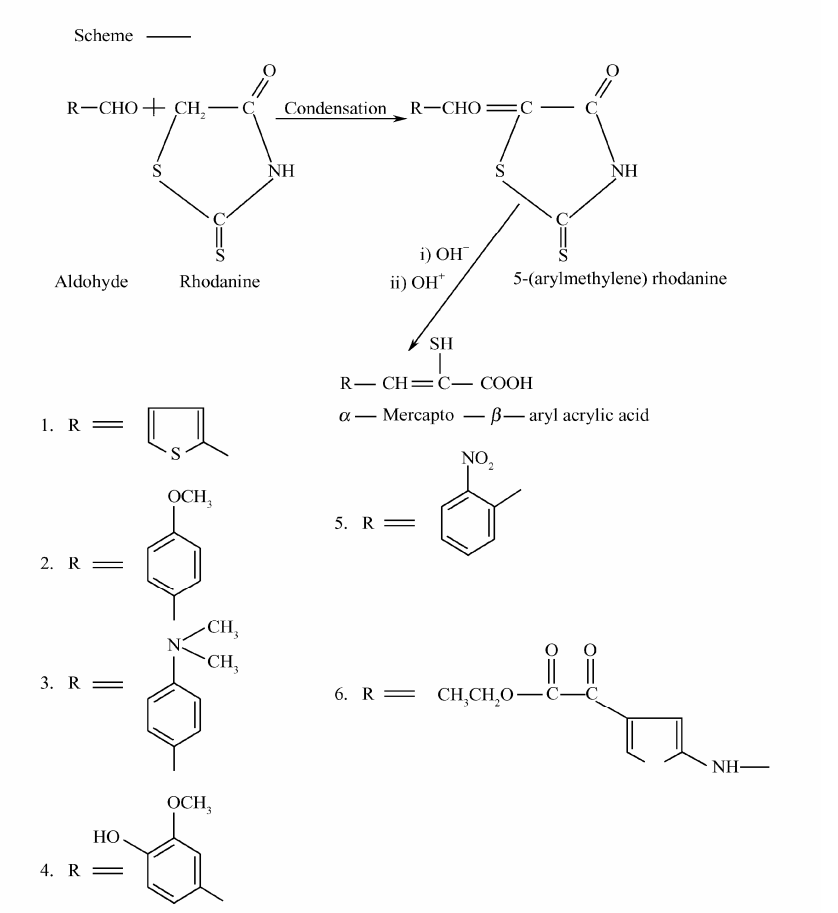

FIG. 1. Synthesis of some α-mercapto-β-substitated aryl acrylic acid and their *in vitro* cadmium chelating ability.

MATERIALS AND METHODS

Chemicals

Various chemical compounds used as starting materials for the synthesis of new compounds were purchased from Aldrich Chemicals Company, USA. All other chemicals and salts employed were obtained from Standard Chemical Companies, Mumbai.

Synthesis and Characterization of the Compounds

The α-mercapto-β-aryl acrylic acids shown in the scheme were prepared by the alkaline hydrolysis of the corresponding 5-(arylmethylene) rhodanines obtained from the condensation of appropriate aldehyde with rhodanine under nitrogen atmosphere following the reported procedure[9]. Melting points were determined on a M.P. apparatus JSGW, India and were uncorrected. Elemental analyses were carried on a Perkin-Elmer 240 CHN analyzer. The IR spectra were recorded on a Perkin-Elmer 783 spectro photometer, (as KBr pellet). The NMR spectra were recorded on a Bruker DPX-200 MHz. spectrometer (in DMSO-D6 or CDCl3) at room temperature using TMS as an internal standard. Chemical shifts were given in ppm (δ) and coupling constants, J in Hz. Abbreviations s, d, dd, t, q, br referred to singlet, doublet, double doublet, triplet, quartet and broad, respectively (Table 1).

In a typical reaction for the preparation of α-mercapto-β-thienyl acrylic acid (1), the solution of 2-thenaldehyde (0.78 g, 6.93 mmole) was warmed and rhodanine (0.92 g, 6.93 mmole) in 20 mL of glacial acetic acid was added into freshly fused sodium acetate (1.70 g, 20.8 mmole). A thick orange mass of 5- (2-thenal) rhodanine was refluxed for half an hour with occasional stirring, and then poured into 30 mL of water. After filtered and washed with water, alcohol and ether, recrystallized from acetone-water 95% mp 231-232℃ was yielded. Twenty mL of 10% NaOH was added to 5- (2-thenal) rhodanine. The resulting deep reddish brown solution was maintained at 75℃ for 40 min. with occasional stirring. It was then cooled to 0℃ and 50 mL of 10% cold HCl was added with stirring. A yellow compound was formed and washed thoroughly with water and dried over calcium chloride $CaCl_2$. It was recrystallized from acetic acid-water to yield pure crystalline α-mercapto-β-thienyl acrylic acid. The experimental and spectral data of the compounds are given in Table 1.

In-vitro Removal of Cadmium by the Compounds

Ten male albino rats (nearly 250 g) were injected intraperitoneally 1.0 mg Cd / Kg, as $CdCl_2$ was dissolved in 4 mL of normal saline (0.9% NaCl) daily for ten days. The animals were sacrificed 48 h after the last injection. The liver and kidney were removed and washed off extraneous materials and weighed. The homogenates of these tissues (15%, w/v) were prepared in phosphate buffer-KCl solution (0.15 mol/L KCl in 0.05 mol/L Na_2HPO_4-NaH_2PO_4 buffer, pH 6.8), and then centrifuged at 18 000 × g for 20 min at 4℃. Nuclear-mitochondrial pellet equivalent to 1 g of tissue was suspended in 3 mL of buffered saline and labeled as nuclear mitochondrial fraction (NMF), whereas the supernatant was labeled as supernatant cytosole fraction (CSF). A measured volume of each fraction was dialyzed (in duplicate) using "dialysis sack" (Sigma, USA) against buffered-KCl medium (ten times the volume of the fraction) containing a α-marcapto-β-aryl acrylic acid in he final concentration of 1×10^{-3} mol/L for 3 h at 37℃ in a water bath with shaker. The pH of the aqueous solution of the compound was adjusted to neutral with $NaHCO_3$ before taking-up in buffered-KCl solution. The dialysis solution was changed after 1.5 h. An equal volume of fraction was

TABLE 1
Experimental and Spectral Data of the Synthesized α-Mercapto-β-Substituted Aryl Acrylic Acids

	Compound	Reacting Aldehyde	Yield (%)	M.P. (oC)	Elemental Analysis (%) (cald. / Found) C H N	IR Absorp. (Cm-1)	NMR pks. (δ ppm)	Compound	Reacting Aldehyde
1	α-Mercapto-β-thienyl acrylic acid ($CH_6O_2S_2$)	2-Thenaldehyde	81	136	45.16 45.10	3.23 2.97	- -	3085 (C-H, Al.), 3038 (C-H, Ar.), 2561 (S-H), 1660 (C=O), 1583 & 1408 (O –C=O), 1059 (S –H), 713 (C-S)	7.17 (dd, 1H, 4.5Hz, C4-H) 7.68 (d, 1H, 3.6Hz, C3–H) 7.89 (d, 1H, 5.1Hz C5–H) 8.19 (s, 1H, Olefinic–H) 13.90 (br.s, 1H, carboxylic-H)
2.	α-Mercapto-β-(p-methoxyphenyl) acrylic acid($C_{10}H_{10}O_3S$)	p-Methoxy-benzaldehyde	73	>300	57.14 57.18	4.76 3.58	- -	3132 (C–H, Al.), 3011 (C–H, Ar.), 2400 (S–H), 1686 (C=0), 1586 & 1446 (O-C=O), 1015 (S- H), 681 (C-S)	3.83 (s, 3H, methoxy- H), 7.10 (d, 2H, 8.7Hz, C2 & C6-H), 7.56 (d, 2H, 8.7Hz, C3 & C5-H), 7.60 (s, 1H, olefinic–H), 13.74 (br.s, 1H, carboxylic-H)
3.	α-Mercapto-β-(p-dimethyl amino phenyl) acrylic acid ($C_{11}H_{13}O_2SN$)	p-imethylamino-benzaldehyde	77	170 (dec)	59.19 59.38	5.83 4.88	6.28 6.69	3136 (C-H, Al.), 3040 (C-H, Ar.), 1682 (C=O), 1574 & 1435 (O-C=O), 1379 & 1215 (C-N), 1070 (S-H), 677 (C-S)	3.02 (s, 6H, two N- substt. Methyl-H), 6.81 (d, 2H, 8.7Hz, C2 & C6-H), 7.41 (d, 2H, 8.7Hz, C3 & C5-H), 7.50 (s, 1H, olefinic-H), 13.78 (br.s, 1H, carboxylic-H)
4.	α- Mercapto-β-m-methoxy, (p-hydroxy phenyl) acrylic acid ($C_{10}H_{10}O_4S$)	m-Methoxy, p-hydroxy-benzaldehyde	80	210	53.10 53.17	4.42 4.04	- -	3340 (O-H), 3265 (C-H, Al.), 3007 (C-H, Ar.), 1715 (C=O), 1591 & 1446 (O-C=O), 1427 & 1211 (O-H, C-O, phenolic), 1026 (S-H), 682 (C-S)	3.83 (s, 3H, methoxy-H), 6.93 (d, 1H, 8.1Hz, C3-H), 7.07 (d, 1H, 8.4Hz, C4-H), 7.13 (s, 1H, C6-H), 7.55 (s, 1H, olefinic-H), 13.70 (br.s, 1H, carboxylic-H)
5.	α-Mercapto-β-(o-nitrophenyl) acrylic acid ($C_9H_7O_4SN$)	o-Nitro-benzaldehyde	72	>260	48.00 47.55	3.11 3.29	6.22 6.54	3098 (C-H, Al.), 3032 (C-H, Ar.), 1734 (C=O), 1608 & 1454 (O-C=O), 1533 & 1354 (C-NO2), 1063 (S-H) 683 (C-S)	7.70 (d, 1H, 7.2Hz, C3-H), 7.73 (dd, 1H, 7.8Hz, C5-H), 7.86 (s, 1H, olefinic-H), 7.89 (dd, 1H, 7.5Hz, C4-H), 8.20 (d, 1H, 8.1Hz, C6-H), 13.90 (br.s, 1H carboxylic-H)
6.	α-Mercapto-β-[ethyl-2-(amino)-4-thiazole glyoxylate] acrylic acid ($C_{10}H_{10}O_5S_2N_2$)	Ethyl-2-(formylamino)-4-thiazole glyoxylate	67	>260	39.73 39.88	3.31 3.47	9.27 9.83	3290 & 3200 (N-H), 3101 (C-H, Al.), 3011 (C-H, Ar.), 1653 (C=O), 1636 (C=N), 1593 & 1431 (O-C=O), 1522 (N-H), 1101 (S-H), 694 (C-S)	1.40 (t, 3H, 10.7Hz, methyl-H), 4.44 (q, 2H, 10.7Hz, methylene-H), 7.35 (s, 1H, C5-H), 8.54 (s, 1H, olefinic-H), 13.75 (br.s, 1H carboxylic-H)

dialyzed similarly without the compound, which served as control. After dialysis, the fraction whole material of the 'sack' was subjected to cadmium estimation following digestion with conc. nitric acid. The digested samples were made-up to 5.0 mL with double glass distilled water and read on a flame atomic absorption spectrometer (Perkin-Elmer 5000) for cadmium content (228.8 nm) using suitable standards.

RESULTS

The results showed that α-mercapto-β-(p-methoxyphenyl) acrylic acid (compound 2) and α-mercapto-β-(m-methoxy, p-hydroxyphenyl) acrylic acid (compound 4) were more effective than α-mercapto-β-thienyl acrylic acid (compound 1) and α-mercapto-β-(p-dimethylaminophenyl) acrylic acid (compound 3) in mobilizing cadmium as there dialyzable chelates, while the remaining compounds (5 and 6) were less effective in removing cadmium (Table 2).

TABLE 2

Removal of Cadmium From Sub-Cellular Fractions of Tissues From Cadmium Pre-treated Rats by Various Compounds

Compound	Removal of Cadmium (%)			
	Liver		Kidney	
	NMF	SCF	NMF	SCF
1.α-Mercapto-β-thienyl Acrylic Acid	21	Nil	15	20
2.α-Mercapto-β-(p-methoxyphenyl) acrylic Acid	24	9	29	30
3.α-Mercapto-β-(p-dimethyl Amino phenyl) Acrylic Acid	22	Nil	29	18
4.α-Mercapto-β-m-methoxy, (p-hydroxy phenyl) Acrylic Acid	31	62	Nil	14
5.α-Mercapto-β-(o-nitrophenyl)Acrylic Acid	Nil	Nil	4	4
6.α-Mercapto-β-[ethyl-2-(amino)-4-thiazole glyoxylate] Acrylic Acid	34	Nil	7	4

Note. Each figure represented mean of values from two sets of fractions and was based on control value taken as 0% remova.

DISCUSSION

The toxic metal removed from tissues by a compound greatly depends on its accessibility to the cellular system and the nature of linkage between the metal and its biomolecules. The present *in vitro* screening showed that compounds 1 to 4 having simple aryl substituted groups in mercapto acrylic acids with lower molecular weights were capable of accessing to the site of cadmium binding and chelating-out MT bound cadmium (Cd – thionein) or similar complexes in tissues. The results indicate that the presence of a methoxy group on the phenyl moiety (compounds 2 and 4) increases the metal chelating ability of mercapto acrylic acids. The compounds (5 and 6) may not have accessibility to the site of

cadmium binding or a capability of forming chelates of adequate stability with cadmium. The investigations suggest that compounds 2 and 4 may be very useful in treatment of cadmium poisoning.

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