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CORRELATION OF OXIDATION POTENTIAL AND TOXICITY IN THIOBENZAMIDES

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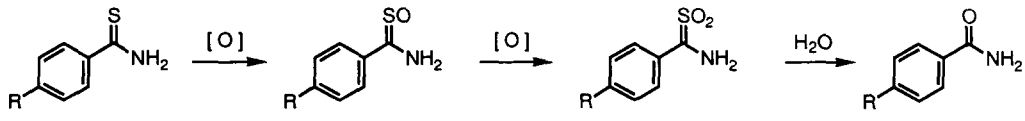
Abstract: The oxidation potentials of a series of 4-substituted thiobenzamides (**1**) were measured by linear sweep voltammetry. When compared with the known toxicological values for these compounds it was found that there is a direct correlation between oxidation potential and toxicity.

Thiocarbonyl-containing compounds produce a wide variety of toxic effects in mammals. Bone marrow depression, liver and lung damage, cancer, and inhibition of cytochrome P-450 dependent monooxygenases are some of the potential adverse side effects which can be observed with these kinds of compounds.[1]

The underlying toxicological mechanism common to virtually all of these types of compounds begins with an enzymatic oxidation of the sulfur atom to form highly reactive S-oxidized species[2-5] which are then capable of covalent binding to cellular nucleophiles.[6]

Thiobenzamide derivatives serve as a useful model for investigating the toxicological properties of compounds containing the thiocarbonyl group. It is hypothesized that *in vivo* they are biotransformed to the S-oxide (**2**) and S,S-dioxide (**3**) which can then react with water to give a benzamide **4** or covalently bind with tissue macromolecules. In fact, S-oxide **2** and benzamide **4** have been detected as metabolites of 4-methyl-thiobenzamide (**1**, R = CH_3) in rat plasma.[6,7]



1 **2** **3** **4**

Several groups have carefully investigated the relationship between the electronic nature of the thiobenzamide and hepatotoxicity[8,9] and have found that in a series of 4-substituted thiobenzamides toxicity is directly linked to the electronic character of the R group. Thiobenzamides containing electron donating groups such as methoxy or methyl are lethal whereas those containing electron withdrawing groups such as trifluoromethyl do not exhibit toxicity in the measured parameters.

The involvement of our laboratory with biologically active thiocarbonyl-containing molecules necessitated the development of a physical method of measuring the electronic properties of such molecules and correlate them with their corresponding toxicological parameters.

A method we felt would be ideally suited for this purpose is cyclic voltammetry and since a plethora of toxicological data is known for thiobenzamides we chose this series as a model for these initial experiments. With the exception of the 4-methyl derivative, all thiobenzamides used in this study were commercially available. The 4-methylthiobenzamide was synthesized from 4-methylbenzonitrile and sodium trimethylsilyl sulfide according to the procedure of Shiao.[10]

The oxidation potential of thiobenzamides were measured on an EG&G Galvanostat Model 263 with a glassy carbon working electrode against a Ag/AgCl reference electrode using a scan rate of 100 mV/second in a positive direction. The samples were prepared as 0.01M solutions in acetonitrile using 0.1M tetrabutylammonium perchlorate in acetonitrile as the supporting electrolyte.

Initial experiments with thiobenzamide (**1**, R = H) were performed in cyclic voltammetry mode and, as shown in Figure 1, it exhibits two voltammetric oxidation peaks, the first (E_1) at 1.147 V presumably[11] attributable to the S-oxide **2** and the second (E_2) at 1.598 V for the S,S-dioxide **3**. Upon scan reversal, the return segment exhibits no voltammetric reduction peaks indicating that the reaction is not reversible under these conditions. Consequently, the remainder of the experiments were performed in linear sweep mode and are shown in Figure 2. The oxidation potentials for each thiobenzamide derivative listed in Table 1 are the mean values of five determinations using a freshly polished carbon electrode for each run.

Figure 1. Cyclic Voltammogram of Thiobenzamide

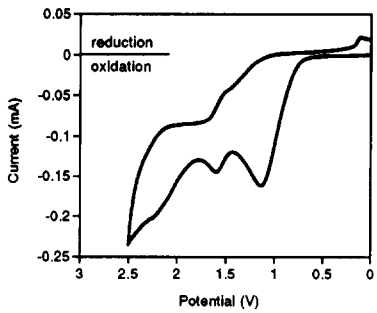
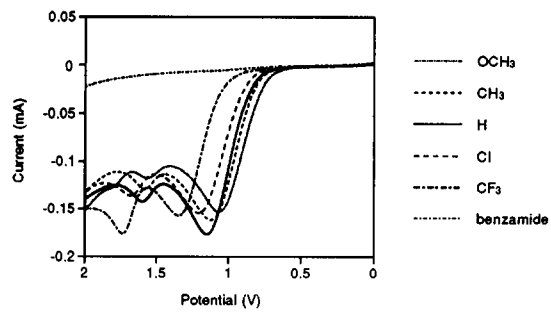


Figure 2. Linear Sweep Voltammogram of 4-Substituted Thiobenzamides



In order to insure that the oxidation peaks are attributed to the sulfur atom on the thiobenzamide, a parallel analysis was conducted on benzamide (**4**, R = H) and as seen in Figure 2 absolutely no oxidation occurs within the observed region.

Table 1. Oxidation potentials of 4-substituted thiobenzamides (**1**)

R	E_1 (volts)	E_2 (volts)
OCH_3	1.061 ± 0.004	1.560 ± 0.003
CH_3	1.117 ± 0.004	1.576 ± 0.002
H	1.147 ± 0.005	1.598 ± 0.002
Cl	1.214 ± 0.006	1.677 ± 0.003
CF_3	1.335 ± 0.007	1.734 ± 0.002

In the rat, the toxicological parameters chosen as the most indicative signs of liver damage were serum bilirubin and transaminases GPT, AST and ALT.[8,9] According to the literature,[8] two doses were used with different thiobenzamides because the more toxic OCH_3 and CH_3 derivatives were lethal at 2.0 mmol/kg whereas a dosage of 0.8 mmol/kg of the less toxic compounds did not produce responses significantly above control values.

When the toxicological parameters were plotted against the first oxidation potential (E_1) of **1** it can be clearly seen that as the oxidation potential increases, the levels of the toxic responses decreases (Figure 3). More importantly, when compared to each other, toxicity and oxidation potential are highly correlated with a coefficient of determination (r^2) exceeding 0.9 for all cases (Table 2). Although not shown graphically, the same correlation can be made between toxicity and the second oxidation potential (E_2).

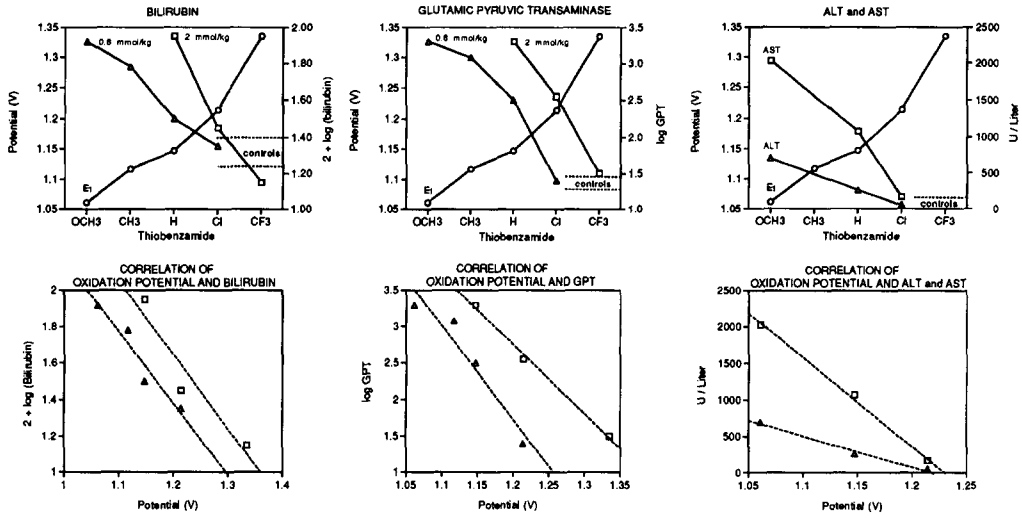
In summary, we have measured the oxidation potentials of a series of electronically diverse 4-substituted thiobenzamides and have shown that there is a correlation between their oxidation potential and toxicity.

Table 2. Coefficient of Determination for Oxidation Potentials vs. Toxicity

Toxic Parameter	r^2 (E_1)	r^2 (E_2)
bilirubin (a)	0.931	0.821
bilirubin (b)	0.908	0.997
GPT (a)	0.927	0.986
GPT (b)	0.995	0.965
ALT	0.984	0.851
AST	0.997	0.955

(a) dose = 0.8 mmol/kg (b) dose = 2.0 mmol/kg

Figure 3. Comparison of Toxic Response and Oxidation Potential of 4-Substituted Thiobenzamides



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11. The first oxidation peak may also be due to the one electron oxidation species ($C=S^+$).

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