ms was used in the NOESY spectrum. Spectra were acquired using 2048 × 512 real data points. Prior to Fourier transformation in the t_2 dimension, the data matrix was multiplied by a sine-bell function and phase-shifted by 22.5° and 45° for the NOESY and COSY spectra, respectively. The data in the t_1 dimension were multiplied by a sine-bell function, phase-shifted by 45° and 90°, respectively, and zero-filled to 1024 complex data points.

^1H T_1's were measured using the fast inversion recovery FT technique (FIRFT),[20] with a recovery delay of 1.5 s between 180°–τ–90° pulse sequences. Ten τ values were used, ranging from 0.02 to 6 s. Raw data were analyzed using a three-parameter exponential fitting program on the Aspect 3000 computer supplied with the spectrometer. T_1 values cited are an average from at least two experiments. The experimental error is less than ±10%.

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Acknowledgment. This work was supported in part by a grant from the Australian Research Council.

Registry No. 1, 51152-91-1; butaclamol hydrochloride, 36504-94-6.

Electron-Deficient Isoalloxazines: Model Systems for Disulfide Prodrug Formation

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Drugs which contain a thiol functionality may be enzymatically or nonenzymatically oxidized to reactive metabolites, some of which cause adverse reactions. The synthesis of disulfide prodrugs to obviate unwanted drug side effects requires the development of novel catalysts. Herein, we describe the synthesis, structure–activity relationship, and mechanism investigations of the oxidation of model thiophenols with isoalloxazine disulfide formation catalysts. *m*-Nitrothiophenol (**31**) reacts with the electron-deficient 8-cyano-*N*-3-(mercaptoalkyl)-10-phenylisoalloxazines (**5–8**) and non-electron-deficient *N*-3-(mercaptoalkyl)-10-methylisoalloxazines (**1–4**) to produce *m*-nitrothiophenol disulfide. *m*-Nitrothiophenol (**31**) reacts with electron-deficient 8-cyano-10-phenyl-3-isoalloxazinepentanoic acid (**10**) or 10-methyl-3-isoalloxazinepentanoic acid (**9**) to form *m*-nitrothiophenol disulfide at a reduced rate, or not at all, respectively. Of the substituted isoalloxazines studied, electron-deficient isoalloxazines containing an *N*-3-mercaptoalkyl side chain were most efficient at catalyzing *m*-nitrothiophenol disulfide formation. Non-electron-deficient isoalloxazines without an *N*-3-alkyl mercaptan side chain (**9**) did not catalyze *m*-nitrothiophenol oxidation. Electron-deficient isoalloxazines without *N*-3-alkyl mercaptan side chains catalyzed *m*-nitrothiophenol oxidation at $^1/_{20}$ the rate for isoalloxazine **5**. From kinetic and product studies, the differences in catalytic activity of **1–10** were judged to be due to changes in the chemical properties of the isoalloxazines and the ability to stabilize intramolecular thiol attack on the C(4a)–N(5) bond of the isoalloxazine. Electron-deficient isoalloxazines may be useful catalysts for the syntheses of disulfide prodrugs.

Introduction

The therapeutic and toxic effects of many thiol-containing drugs including captopril (antihypertensive), penicillamine (antirheumatoid agent), 6-mercaptopurine and 6-thioguanine (cytotoxic agents), *N*-acetylcysteine (mucolytic agent), methimazole, carbimazole, and propylthiouracil (antithyroid agents), and spironolactonethiol (antihypertensive) are dependent upon the chemical reactivity of the mercaptan functional group.[1] Metabolism of thiol-containing drugs is generally extensive, and in many cases, the major metabolite is the disulfide or drug–cysteine disulfide. Extensive covalent binding and reversible disulfide bond formation with proteins in tissue has been observed and in some cases drug side effects may stem from disruption of disulfide bonds in enzymes or by formation of immunogenic drug–protein conjugates. Although a clear relation among covalent binding, disulfide bond formation, and adverse drug reactions has yet to be established, thiol-containing-drug action and toxicity may be influenced by factors which influence the metabolic formation and degradation of disulfides including NADPH levels, the glutathione:oxidized glutathione ratio, and the prooxidant stress status of the cell.[2]

One general approach to the delivery of thiol-containing drugs is to administer a disulfide prodrug. Disulfide prodrugs have been shown to be effectively taken up into cells and reduced to active parent drug within the cell.[3] Development of catalytic agents designed to produce disulfide-containing chemicals and drugs constitutes an important goal of bioorganic chemistry. There are few examples of enzymes that catalyze disulfide synthesis.[2] Glutathione, the predominant cellular thiol/disulfide couple is modulated by glutathione reductase, an enzyme which regulates the glutathione:glutathione disulfide ratio to greatly favor reduced glutathione.[2] Disulfide reductases including glutathione reductase,[4] lipoamide dehydrogenase,[3] and thioredoxin reductase[6] each contain a flavin (FAD) at the active site. Unlike disulfide reduction, the reduction of flavoenzymes by mercaptans (to produce disulfides) has been established in only a few cases.[7] For the few enzymes studied, the mechanism appears to involve nucleophilic attack of a thiol at the C-4a position of the flavin followed by breakdown of the C-4a adduct by displacement of the flavin by thiol[8] or other active site ligands.[9] A number of isoalloxazines have been developed

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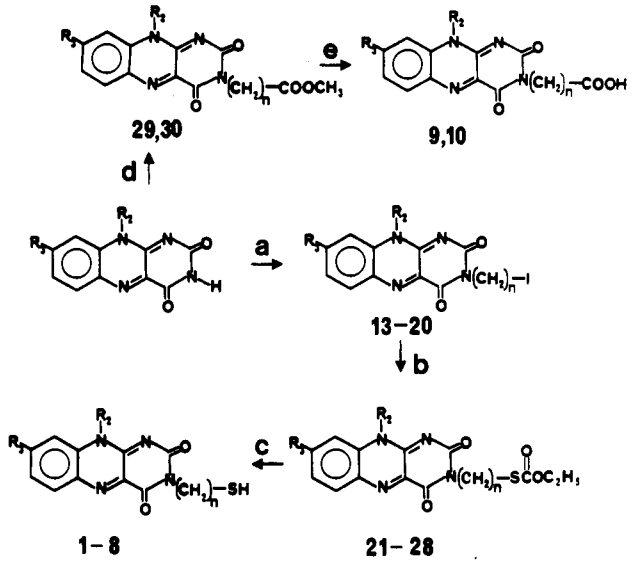
Scheme I. Overall Chemical Synthesis of Isoalloxazine Derivatives 1–10[a]

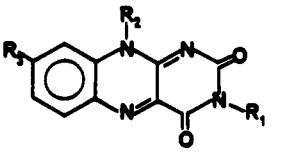


[a] The reagents required for the syntheses include (a) NaH, DMF; diiodoalkane; (b) $EtOC(S)S^-K^+$; EtOH; NH_4Cl; (c) ethylenediamine, THF, 5% H_2SO_4; (d) NaH, DMF, $Br(CH_2)_5COOCH_3$; (e) THF, H_2SO_4.

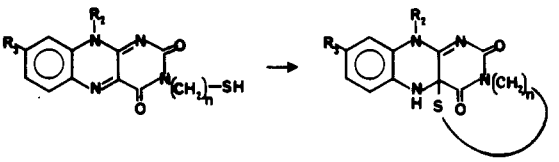
as enzyme models of this process.[10] Gascoigne and Radda studied reduction of flavins by dithiols and showed buffer catalysis to be first order in dithiol and flavin.[11] Loechler and Hollocher showed that a change in rate-determining step occurs when going from a monothiol (rate-determining nucleophilic addition to the flavin) to a dithiol (rate-determining breakdown of the adduct).[12] Nucleophilic addition to isoalloxazines, and by analogy to flavin-containing enzymes, by sulfite,[13] phosphines,[14] hydroxide,[15] and carbanions[16] is well-established. In a few cases, stable adducts to electrophilic isoalloxazines or other model enzyme systems have been demonstrated.[17,18] Additional support for nucleophilic attack of thiols to the C-4a position of flavins has been obtained from studies of sterically hindered isoalloxazine molecules.[13,19] Bruice and co-workers have studied electron-deficient 8-cyanoisoalloxazines as a model system for covalently linked 8α-flavins of various flavoenzymes.[20] Although flavins are reduced by aliphatic mercaptans to disulfides, normal flavins are not reduced by thiophenol.[21] However, electron-deficient 8-cyanoisoalloxazines react with thiophenol nonenzymatically to produce diphenyl disulfide.[21]

Chart I. Composition of the Isoalloxazine Structures

compd no.	R_1	R_3	R_3
1	$(CH_2)_8$ SH	C_6H_5	H
2	$(CH_2)_6$ SH	C_6H_5	H
3	$(CH_2)_5$ SH	C_6H_5	H
4	$(CH_2)_3$ SH	C_6H_5	H
5	$(CH_2)_8$ SH	CH_3	CN
6	$(CH_2)_6$ SH	CH_3	CN
7	$(CH_2)_5$ SH	CH_3	CN
8	$(CH_2)_3$ SH	CH_3	CN
9	$(CH_2)_4$ COOH	C_6H_5	H
10	$(CH_2)_4$ COOH	CH_3	CN



Scheme II. Intramolecular Nucleophilic Attack of the Side-Chain Thiol of 1–8 To Form the *N,S*-Acetal



The purpose of this study was to develop isoalloxazine enzyme models for the oxidation of *m*-nitrothiophenol (**31**) to the disulfide. It was anticipated that electron-withdrawing substituents at the 8-position of an isoalloxazine nucleus would facilitate nucleophilic attack of thiophenol to give the disulfide. In addition, by comparing a series of N-3 substituted isoalloxazines capable of intramolecular thiol addition to the C-4a position we demonstrate a number of important aspects concerning mechanism of disulfide bond formation catalyzed by isoalloxazines. Herein we report the spectrophotometric, product, and kinetic analysis of *N*-3-(mercaptoalkyl)-8-cyanoisoalloxazines and compare the results with non-electron-deficient *N*-3-(mercaptoalkyl)isoalloxazines in the oxidation of *m*-nitrothiophenol (**31**) to the disulfide. Finally, the reaction of *m*-nitrothiophenol (**31**) with isoalloxazines which do not contain an *N*-3-alkyl mercaptan side chain was studied. The *N*-3-(mercaptoalkyl)isoalloxazines are efficient catalysts for disulfide bond formation and provide significant insight as models for FAD-containing thiol oxidase enzymes.

Results

Synthesis of Isoalloxazines. The syntheses of isoalloxazines **1–30** is outlined in Scheme I. The basic isoalloxazine nucleus for the non-electron-deficient isoalloxazines was synthesized following the general procedure of Yoneda et al.[22] For the electron-deficient isoalloxazines the basic isoalloxazine nucleus was synthesized according to the method of Bruice et al.[20] For the synthesis of **1–8**, the isoalloxazine was first alkylated at the N-3 position with a diiodoalkane to produce **13–20**. Iodoalkanes **13–20** were next converted to xanthates **21–28** and then hydrolyzed to target compounds **1–8**. For the synthesis of **9** and **10**, the isoalloxazine was first alkylated at the N-3 position with methyl bromopentanoate to produce **29** and **30**. Methyl esters **29** and **30** were next hydrolyzed in the presence of acid to produce the desired carboxylic acid **9** and **10**.

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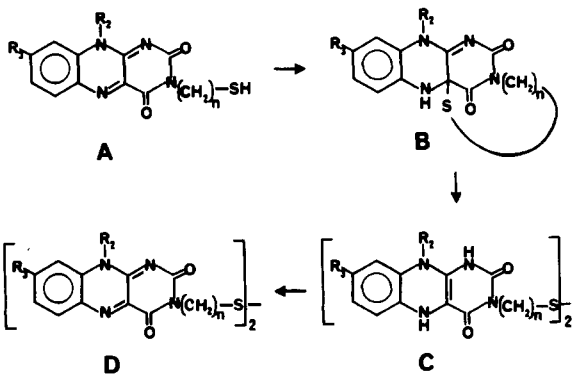

Figure 1. Overall transformation of isoalloxazines 1–8 (A) to the C(4a)–N(5) adduct (B) and isoalloxazine disulfides (reduced, C, and oxidized, D).

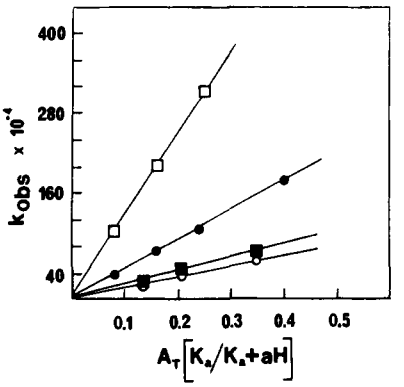

Figure 2. Plot of the observed rate constants (min^{-1}) for isoalloxazine reduction vs $[A]_T K_a/(K_a+a_H)$ as a function of pH (see text). The reaction of isoalloxazine 1 (pH 9, □, and pH 8, ■) and 5 (pH 9, ●, and pH 8, ○) was monitored spectrophotometrically.

Reactions of 1–8 with Weakly Acidic Buffers. The reaction of isoalloxazine derivatives 1–8 (Chart I) with excess aqueous base (e.g. in the pH range 7–10) was followed by monitoring the decrease in absorbance for UV–vis scans observed spectrophotometrically at constant pH and ionic strength. Repetitive scanning revealed a time-dependent loss of the isoalloxazine chromophore. In the early stages of the reaction, the presence of an isosbestic point suggested that only two species were present in solution at the pH values examined. The decrease in absorbance in the visible region (i.e. near 440 nm) was consistent with the loss of the oxidized isoalloxazine chromophore. These results suggested the possibility of the intramolecular reaction shown in Scheme II. After the absorbance change was complete, the product of the reaction of each isoalloxazine 1–8 in buffer (pH 8 and 9) was isolated from large-scale reactions by extraction with dichloromethane followed by preparative TLC on silica gel. The chromatographic properties of the products were distinct from the starting material and the isoalloxazine product was identified by liquid secondary ion mass spectrometry (LSIMS). In each case, the major product gave a molecular ion identical with that of the parent isoalloxazine. In addition, a minor, more polar product gave a molecular ion and fragments consistent with a dimer of the parent isoalloxazine (Figure 1).

Kinetics of 1–8 with Weakly Acidic Buffers. In the pH regions examined (i.e. pH 7–10), preliminary studies with various buffers and buffer concentrations revealed a dependence of isoalloxazine chromophore loss on the concentration of buffer at constant pH and ionic strength.

$$-d[\text{isoalloxazine}]/dt = k_{obsd}[\text{isoalloxazine}] \quad (1)$$

The loss of the isoalloxazine chromophore with increasing phosphate (pH 7–9) and hydroxide (pH 10) buffer concentrations was proportional to the buffer concentration and increased more rapidly at higher pH, which indicated that the reaction was catalyzed by phosphate and hydroxide ion. This suggested that buffer anion was active as a general-base catalyst and that the reaction followed the general rate law of eq 2, where k_o is the spontaneous

$$k_{obsd} = k_o + k_{a_H}[H_3O^+] + k_{HA}[HA] + k_{A^-}[A^-] + k_{OH}K_w/a_H \quad (2)$$

rate constant, k_{a_H} is the specific-acid rate constant, k_{OH} is the specific base rate constant, and K_W is the water product. At low hydronium or hydroxide ion concentration, eq 2 simplifies to eq 3. A plot of k_{obsd} vs $[A_T](K_a/K_a$

$$k_{obsd} = k'_o + k_{HA}[HA] + k_{A^-}[A^-] \quad (3)$$

$$k_{obsd} = k'_o + k_{HA}\left(\frac{[A^-]a_H}{K_a}\right) + k_{A^-}[A^-] \quad (4)$$

$$k_{obsd} = k'_o + \left[k_{HA}\left(\frac{[HA]}{[A^-]}\right) + k_{A^-}\right][A_T]\left(\frac{K_a}{K_a + a_H}\right) \quad (5)$$

$+ a_H$) at fixed pH should be linear, with a slope equal to $k_{HA}[HA]/[A^-] + k_{A^-}$ and with an intercept of k'_o. The results for phosphate buffer are shown in Figure 2 for isoalloxazines 1 and 5. By plotting the slope of these plots vs the buffer ratio $[HA]/[A^-]$, k_{HA}, and k_{A^-} were evaluated as the slope and intercept, respectively (eq 6), where k_{HA}

$$\text{slope} = k_{HA}[HA]/[A^-] + k_{A^-} \quad (6)$$

and k_{A^-} are the general-acid and general-base rate constants, respectively, for the thiol addition reaction (Figure 1). Such secondary plots were linear and the slopes (values of k_o) were obtained by subtracting $k_{OH}(K_W/a_H)$ from the intercept values of eq 5 (k_{HA}). Intercept values (k_{A^-}) were determined (shown in Table I). The specific base hydrolysis of *N*-3-alkyl-substituted isoalloxazines does not demonstrate buffer catalysis[15] and base-catalyzed hydrolysis was not observed at the pH of the reactions examined. As seen from inspection of Table I, the spontaneous or water-catalyzed rate constants (k_o) are very small (i.e. for 1, 6.5×10^{-6} M^{-1} s^{-1}) while the general-base-catalyzed rate constants k_{A^-} are much larger (i.e. for 1, 2.7×10^{-4} M^{-1} s^{-1}). The general-acid-catalyzed rate constant k_{AH} is detectable but significantly less than the general-base-catalyzed rate constant.

Table I. Rate Constants (M^{-1} s^{-1}) for Reduction of Isoalloxazines[a]

compd	$10^7 k_{AH}$	$10^4 k_{A^-}$	$10^6 k'_o$
1	4.7	2.7	6.5
2	3.7	1.8	6.1
3	2.0	1.1	4.6
4	1.2	0.8	3.1
5	17.5	4.2	3.8
6	9.3	2.7	3.6
7	3.7	1.3	3.5
8	2.5	1.0	3.3

[a] Kinetics for the reduction of isoalloxazines were determined spectrophotometrically at 30 °C (μ = 0.4 M) as described in the Experimental Section. The values are the mean of two determinations.

Reaction of 1 and 5 with *m*-Nitrothiophenol. The reaction of 1 and 5 with *m*-nitrothiophenol (31) was monitored with HPLC to determine the products formed and the kinetics of the reaction. The reaction of 1 and 5 with excess 31 (in the pH range 7–10) followed pseudo-first-order kinetics for 3–4 half-lives. The only product

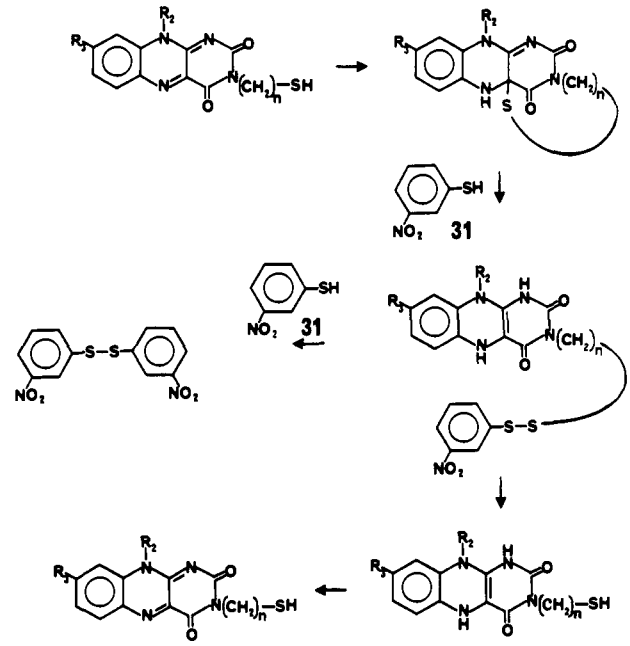

Figure 3. Overall transformation of *m*-nitrothiophenol (31) by isoalloxazines (1–8) to *m*-nitrothiophenol disulfide (*m*NTPD).

detected was *m*-nitrothiophenol disulfide (Figure 3). In the pH region in which the oxidation of 31 was examined (pH 7–10), modest phosphate buffer catalysis was detected. Thus, disulfide formation increased with increasing buffer concentration and increased more rapidly at higher pH, indicating that disulfide formation was catalyzed by phosphate ion. Plots of k_{obsd} vs concentration of buffer (0.08–0.4 M) were linear in the pH range 7–9. Compared with isoalloxazine 1, electron-deficient isoalloxazine 5 was a more efficient catalyst for *m*-nitrothiophenol disulfide formation. Thus, the rate constant for disulfide formation catalyzed by 5 was larger than the rate constant for disulfide formation catalyzed by 1. At constant pH, the plots of pseudo-first-order rate constant (k_{obsd}) vs excess total thiol (*m*-nitrothiophenol and *m*-nitrothiophenolate anion) were linear, establishing the reaction to be first order in total 31. This result suggested that buffer anion was active as a general-base catalyst. The k_{obsd} of eq 7 was rewritten as shown in eq 8:

$$d[m\text{NTPDS}]/dt = k_2[m\text{NTP}][\text{isoalloxazine}] \quad (7)$$

$$k_{obsd} = k_2[m\text{NTP}] \quad (8)$$

$$k_2 = k_o + k_{A^-}[A^-] + k_{AH}[AH] + k_{OH}K_W/a_H \quad (9)$$

where *m*NTPDS is an abbreviation for *m*-nitrothiophenol disulfide, mNTP is an abbreviation for *m*-nitrothiophenol, k_o is the spontaneous rate constant, k_{OH} is the specific-base-rate constant, and k_{A^-} and k_{AH} are the general-base and general-acid rate constants, respectively. At the pH of these studies (e.g. pH 7–9) the spontaneous (k_o) and specific base (k_{OH}) rate constants made a negligible rate contribution to k_{obsd}. Equations 7 and 9 were simplified and rewritten as eq 10. Further rearrangement and simplification transformed eq 10 into eq 11, a form that was more readily evaluated. A plot of $k_{obsd}/[m\text{NTP}]$ vs $[A]_T(K_a/K_a+a_H)$ at fixed pH should be linear with a slope $= k_{AH}[AH]/[A^-] + k_{A^-}$ and intercept $= k'_o$ (from eq 11). By plotting the slope of these plots vs the buffer ratio $[AH]/[A^-]$, k_{AH} and k_{A^-} were evaluated as the slope and intercept, respectively (eq 12). Such secondary plots were

$$k_{obsd}/[\text{isoalloxazine}] = k'_o + k_{A^-}[A^-][m\text{NTP}] + k_{AH}[AH][m\text{NTP}] \quad (10)$$

$$\frac{k_{obsd}}{[m\text{NTP}]} = k'_o + \left[k_{AH}\left(\frac{[AH]}{[A^-]}\right) + k_{A^-}\right][A]_T\left(\frac{K_a}{K_a + a_H}\right) \quad (11)$$

$$\text{slope} = k_{AH}[AH]/[A^-] + k_{A^-} \quad (12)$$

linear and the slopes (k_{AH}) and intercepts (k_{A^-}) were listed in Table II. Data of Table II showed that the spontaneous (k'_o) and general-acid-catalyzed (k_{AH}) rate constants were very small, while the general-base-catalyzed rate constants (k_{A^-}) were much larger.

Table II. Rate Constants (M^{-1} s^{-1}) for *m*-Nitrothiophenol Disulfide Formation[a]

catalyst	10^5k_{AH}	$10^3k_{A^-}$	$10^6k_o'$
1	6.8	0.1	3.0
5	131.7	2.3	4.6

[a] Kinetics for the formation of *m*-nitrothiophenol disulfide was determined by HPLC as described in the Experimental Section. The values are the mean of two determinations.

Reaction of 9 and 10 with *m*-Nitrothiophenol. The reaction of 9 and 10 with 31 was investigated by monitoring the reaction by HPLC. Isoalloxazine 9 did not react with 31 to produce detectable amounts of *m*-nitrothiophenol disulfide. In contrast, isoalloxazine 10 reacted with 31 to produce the disulfide. Buffer-dilution studies suggested that general-acid or general-base catalysis was not occurring. The reaction of 10 with 31 was extremely slow. The observed pseudo-first-order rate constant (k_{obsd}) for the oxidation of 31 by 10 was approximately 20-fold smaller than the rate constant for the oxidation of 31 by 5 (e.g. 1.3×10^{-4} s^{-1} vs 6.5×10^{-6} s^{-1}).

Discussion

Thiol-containing drugs are extensively metabolized in mammalian systems to sulfenic, sulfinic, or sulfonic acids, disulfides, and in some cases to *S*-methyl metabolites.[1] Many of these metabolites undergo extensive covalent yet reversible disulfide bond formation with proteins or other thiols in tissue. For example, 45% of a dose of captopril is metabolized to captopril cysteine disulfide,[3] and 42–56% of the urinary metabolites of penicillamine is the penicillamine cysteine disulfide.[23] Formation of disulfides with plasma proteins following administration of thiol-containing drugs may contribute to many adverse drug reactions observed.[1] To provide insight into new catalysts useful for the syntheses of disulfide prodrugs, we investigated the synthesis and chemical characterization of N-3-substituted isoalloxazine derivatives capable of catalyzing the oxidation of the model thiol *m*-nitrothiophenol (31) to *m*-nitrothiophenol disulfide. Several chemical features of these isoalloxazine catalysts are notable. First, introduction of a strongly electron-withdrawing substitutent at the C-8 position of the isoalloxazine nucleus influences the chemical properties of the substituted isoalloxazine catalyst toward *m*-nitrothiophenol oxidation. Second, appending an *N*-3-alkyl mercaptan side chain markedly increases the efficiency of the isoalloxazine derivatives as catalysts for *m*-nitrothiophenol disulfide formation. Third, the existence of an isolable intermediate that was putatively identified as the intramolecular thiol addition product of the *N*-3-alkyl mercaptan side chain to the C(4a)–N(5) bond of the isoalloxazine suggests that the results obtained for the reaction of 1–8 with thiols are

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in agreement with the mechanisms proposed by others for reactions of flavins with thiols.[10-12,18]

As shown in Table I, the rate constants for reduction of the isoalloxazine derivatives 1–8 were dependent upon the nature of the isoalloxazine C-8 substituent as well as the length of the N-3-alkyl mercaptan side chain. Thus, electron-deficient 8-cyano-*N*-3-(mercaptoalkyl)isoalloxazines 5–8 were more effective catalysts for disulfide formation than the corresponding non-electron-deficient isoalloxazine counterparts 1–4. That the optimal length of the *N*-3-alkyl mercaptan side chain is apparently an eight-carbon linkage is consistent with intramolecular attack of the mercaptan at isoalloxazine C(4a) to form an *N,S*-acetal. Scheme II shows the proposed reaction. The kinetic data support a role for general-acid and, to a larger degree, general-base catalysis in this process, although the range of pH values examined was admittedly small. Thus, a mechanism involving general-base-catalyzed formation of thiolate anion is supported by the increase rate of reaction in the presence of greater concentrations of buffer base anionic species. Presumably, reactions performed in acidic pH ranges would provide a greater role for general acid catalysis via isoalloxazine N(5) protonation. Attack of side chain thiolate at the isoalloxazine C(4a) position in a rate-determining step is the preferred mechanism. Although the kinetic data alone do not permit an unambiguous choice of mechanism, product studies suggest the existence of an isolable intermediate with mass spectral characteristics consistent with the addition of the side-chain mercaptan to the C(4a)–N(5) bond of the isoalloxazine derivatives. That mass spectral evidence for only small amounts of isoalloxazine dimer formation was observed also tends to support the intramolecular reaction mechanism. The results show that the intramolecular reaction is heavily favored over an analogous intermolecular process and illustrate the participation of the electrophilic nature of the C(4a)–N(5) C=N bond in the overall catalytic mechanism leading to disulfide bond formation. We conclude that attack of *m*-nitrothiophenolate on the *N,S*-acetal as well as disulfide exchange is rapid (Figure 3).

Studies with either non-electron-deficient or electron-deficient isoalloxazine derivatives **9** and **10**, respectively, showed that isoalloxazine derivatives without N-3-alkyl mercaptan side chains were not highly effective *m*-nitrothiophenol disulfide formation catalysts. Thus, the rate of *m*-nitrothiophenol disulfide formation with **9** was not detectable and the rate of disulfide formation with **10** was 20-fold smaller than the analogous isoalloxazine **5** containing an *N*-3-alkyl mercaptan side chain. Studies with isoalloxazines **5** and **10** illustrate the catalytic advantage of converting a bimolecular process to a unimolecular process.

It is now recognized that flavin-containing proteins[24] and semisynthetic flavoproteins[25,26] participate in reactions involving transport of electrons, activation of molecular oxygen, and dehydrogenation. As discussed above, only a few examples of enzymes capable of catalyzing thiol oxidation have been described.[7] Thiophenol oxidation is very slow at physiological pH in the absence of metals[27] and enzymatic or nonenzymatic catalysts designed to facilitate thiol-containing drug or thiophenol oxidation could be quite useful.

Table III. Mass Spectrometric Properties of Isoalloxazines

isoalloxazine derivatives	high-resolution mass spectral data		
	calcd	obsd	±ppm
	Mercaptans		
1	434.1776	434.1781	+1.2
2	406.1464	406.1472	+2.1
3	392.1307	392.1321	+3.5
4	364.0994	364.0992	−0.5
5		NP[a]	
6	371.1416	371.1397	−5.1
7		NP	
8		NP	
	Carboxylic Acids		
9	390.1328	390.1321	−2.0
10	353.1124	353.1131	+1.7
	Alkyl Iodides		
13	458.0241	458.0259	+3.9
14	486.0555	486.0564	+1.9
15	500.0711	500.0726	+3.0
16	528.1024	528.1037	+2.5
17		NP	
18		NP	
19	464.0585	464.0601	+3.3
20		NP	
	Xanthates		
21			NA[b]
22			NA
23			NA
24			NA
25			NA
26			NA
27			NA
28			NA
	Methyl Esters		
29			NA
30			NA

[a] NP, not possible; the molecular ion was not strong enough to accurately determine a high-resolution mass spectra. Satisfactory low-resolution electron-impact or liquid secondary ion mass spectrometry spectra were obtained for these compounds. (See supplementary materials). [b] NA, not available; no attempt was made to obtain high-resolution mass spectrometry data.

Results described in this report suggest that significant rate acceleration for oxidation of thiol-containing drugs, in principle, could occur in the presence of electron-deficient isoalloxazine catalysts. This is based on the observation that the rate of *m*-nitrothiophenol oxidation is markedly increased when intramolecular thiol attack can occur to produce C(4a)–N(5) thiol adducts. Electron-deficient isoalloxazines are especially efficient at thiol addition reactions, and enzymes[28] as well as semisynthetic flavoproteins[29] containing substituents which decrease the electron density of the isoalloxazine ring system may be particularly effective as thiol oxidation catalysts.

Experimental Section

Chemicals and Materials. All chemicals used were obtained from Aldrich Chemical Co., Milwaukee, WI, in the highest purity available. Other reagents, solvents, and buffers were the purest commercially available products. *m*-Nitrothiophenol (**31**) was synthesized as previously described.[30] The synthesis of the isoalloxazines followed the general procedures of Yoneda et al.[22] and the electron-deficient isoalloxazines were synthesized by the method of Bruice et al.[20] All of the compounds listed in Table III were fully characterized by IR, UV–vis, NMR, and mass spectrometry. The isoalloxazines were transformed into the re-

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Table IV. HPLC Properties of Isoalloxazine Derivatives

compd	retention[a] volume, mL	retention[b] volume mL	% purity	wavelength monitored, nm
1	34.3	4.31	≥96.7	420
2	31.3	4.38	≥98.7	420
3	29.8	4.35	≥99.4	420
4	26.7	4.38	≥99.4	420
5	31.3	4.39	≥99.5	420
6	27.7	4.30	≥98.4	420
7	26.6	4.30	≥94.3	420
8	25.6	4.46	≥99.2	420
9	17.4	4.30	≥94.3	420
10	18.6	4.28	≥98.3	420

[a] Gradient solvent system: 100% from 0–10 min, 0–45% B from 10–60 min, 45–100% B from 60–65 min, using the reversed phase HPLC method described in the Experimental Section, where solvent A was 0.1% trifluoroacetic acid in water and solvent B was 0.08% trifluoroacetic acid in CH_3CN. [b] Solvent system: 2-propanol/hexane (20:80 v:v) using the straight-phase HPLC method described in the Experimental Section.

quired *N*-3-alkyl mercaptans or carboxylic acids by the following synthetic procedures (Scheme I). Chromatography was done on Silica Woelm (70–150 mesh) (Fisher Scientific). Preparative TLC was done with 20 × 20 cm Analtech Uniplate (500-μm thickness).

To confirm the purity of the isoalloxazine derivatives HPLC methods were used to quantify compounds 1–10. HPLC was performed with a Rainin instrument (Emeryville, CA), fitted with a Vydac C-4 column (Western Analytical, 4.6 mm × 25 cm). The chromatogram was developed at a flow rate of 1.0 mL/min by a 90 min linear gradient from 0 to 45% of solvent B, where solvent A was 0.1% trifluoroacetic acid in water and solvent B was 0.08% trifluoroacetic acid in CH_3CN. The absorbance of the HPLC eluates was monitored at 420 nm and the results are shown in Table IV.

Chromatography was also performed on a IBM (Palo Alto, CA) instrument Model 5399 with UV detection and a Hewlett-Packard Model 13390 integrator (Santa Clara, CA). A 5-μm AXXIOM silica column (Richard Scientific, Novato, CA; 4.5 mm × 25 cm) was used for the straight-phase separations. The HPLC mobile phase consisted of a mixture of 2-propanol/hexane (20:80, v:v) at a flow rate of 1.0 mL/min. The absorbance of the eluates was monitored at 420 nm, and the results are shown in Table IV.

Preparation of Alkyl Iodides 13–20. To a stirred solution isoalloxazine (25 mg, 0.1 mmol) in 30 mL of DMF at 0 °C was added NaH (0.012 mg, 0.3 mmol). After evolution of hydrogen gas had subsided, the diiodoalkane (150 mg, 0.3 mmol) was added. The reaction was stirred for 3 days at room temperature, after which the DMF was evaporated and the reaction mixture was extracted with CH_2Cl_2. The crude material was chromatographed directly on silica gel to afford the *N*-3-(iodoalkyl)isoalloxazine derivatives.

Compound 13: 28% yield (7.0 mg); R_f = 0.36, CH_2Cl_2/EtOAc (9:1, v:v); ^1H NMR ($CDCl_3$) δ 8.34–8.19 (d, 1 H, J = 7.9 Hz), 7.7–7.45 (m, 5 H), 7.3–7.2 (m, 2 H), 6.9–6.8 (d, 1 H, J = 8.4 Hz), 4.15–3.95 (t, 2 H, J = 7.0 Hz), 3.2–3.0 (t, 2 H, J = 7.4 Hz), 2.2–2.1 (m, 2 H); IR (KBr) 2930, 1707, 1653, 1588, 1553 cm^{-1}.

Compound 14: 62% yield (15.5 mg); R_f = 0.44, CH_2Cl_2/EtOAc (9:1, v:v); ^1H NMR ($CDCl_3$) δ 8.70–8.3 (d, 1 H, J = 7.9 Hz), 7.85–7.6 (m, 5 H), 7.39–7.35 (m, 2 H), 6.93–6.88 (d, 1 H, J = 8.4 Hz), 4.17–4.09 (t, 2 H, J = 7.3 Hz), 3.29–3.2 (t, 2 H, J = 7.3 Hz), 1.9–1.45 (m, 6 H); IR (KBr) 2930, 2852, 1705, 1656, 1588, 1550 cm^{-1}.

Compound 15: 42% yield (10.5 mg); R_f = 0.44, CH_2Cl_2/EtOAc (9:1, v:v); ^1H NMR ($CDCl_3$) δ 8.3–8.25 (d, 1 H, J = 7.9 Hz), 7.85–7.66 (m, 5 H), 7.5–7.42 (m, 2 H), 6.87–6.8 (d, 1 H, J = 8.4 Hz), 3.93–3.85 (t, 2 H, J = 7.3), 3.4–3.3 (t, 2 H, 7.3 Hz), 1.85–1.25 (m, 8 H). IR (KBr) 2934, 2852, 1704, 1655, 1588, 1550 cm^{-1}.

Compound 16: 45% yield (11.2 mg); R_f = 0.52, CH_2Cl_2/EtOAc (9:1, v:v); ^1H NMR ($CDCl_3$) δ 8.3–8.25 (d, 1 H, J = 7.9 Hz), 7.65–7.54 (m, 5 H), 7.31–7.22 (m, 2 H), 6.89–6.86 (d, 1 H, J = 8.4 Hz), 3.95–3.86 (t, 2 H, 7.3 Hz), 3.35–3.25 (t, 2 H, J = 7.3 Hz), 1.85–1.2 (m, 12 H); IR (KBr) 2930, 2850, 1704, 1655, 1588, 1550 cm^{-1}.

Compound 17: 26% yield (8.1 mg); R_f = 0.21, CH_2Cl_2/EtOAc (95:5, v:v); ^1H NMR ($CDCl_3$) δ 8.6 (s, 1 H), 8.45–8.35 (d, 1 H, J = 8.4 Hz), 8.15–8.05 (d, 1 H, J = 8.4 Hz), 4.15–3.95 (m, 5 H), 3.5–3.25 (m, 2 H), 2.25–2.15 (m, 2 H); IR (KBr) 2228, 1715, 1659, 1581, 1560, 1500 cm^{-1}.

Compound 18: 36% yield (9.0 mg); R_f = 0.27, CH_2Cl_2/EtOAc (95:5, v:v); ^1H NMR ($CDCl_3$) δ 8.6 (s, 1 H), 8.45–8.35 (d, 1 H, J = 8.4 Hz), 8.05–8.0 (d, 1 H, J = 8.4), 4.0 (s, 3 H), 3.9 (t, 2 H, J = 7.3 Hz), 3.4–3.3 (m, 2 H), 1.85–1.35 (m, 8 H); IR (KBr) 2930, 2853, 2228, 1707, 1658, 1588, 1560 cm^{-1}.

Compound 19: 36% yield (9.0 mg); R_f = 0.32, CH_2Cl_2/EtOAc (95:5, v:v); ^1H NMR ($CDCl_3$) δ 8.7 (s, 1 H), 8.45–8.35 (d, 1 H, J = 8.4 Hz), 8.05–8.0 (d, 1 H, J = 8.4 Hz), 8.05–8.0 (d, 1 H, J = 8.4 Hz), 4.0 (s, 3 H), 4.0–3.9 (t, 2 H, J = 7.3 Hz), 3.4–3.3 (m, 2 H), 1.85–1.35 (m, 8 H); IR (KBr) 2930, 2853, 2228, 1707, 1658, 1588, 1560 cm^{-1}.

Compound 20: 44% yield (11.0 mg); R_f = 0.41, CH_2Cl_2/EtOAc (95:5, v:v); ^1H NMR ($CDCl_3$) δ 8.6 (s, 1 H), 8.35–8.3 (d, 1 H, J = 8.4 Hz), 8.05–8.0 (d, 1 H, J = 8.4 Hz), 4.0–3.97 (s, 3 H), 3.95–3.85 (t, 2 H, J = 7.3 Hz), 3.40–3.3 (m, 2 H), 1.8–1.22 (m, 12 H); IR (KBr) 3040, 2926, 2853, 2228, 1707, 1560, 1500 cm^{-1}.

Preparation of Xanthate Esters 21–28. To a stirred solution of (iodoalkyl)isoalloxazine (20 mg, 0.05 mmol) in 4 mL of CH_2Cl_2 under an atmosphere of argon at 0 °C was added an ethanolic solution of ethyl xanthate potassium salt (24 mg 0.15 mmol). The reaction was stirred for 24 h at room temperature. At the end of the reaction, saturated ammonium chloride was added and the mixture was chromatographed directly on silica gel to afford the *N*-3-alkyl xanthate ester isoalloxazine derivative (Scheme I).

Compound 21: 95% yield (19.0 mg); R_f = 0.32, CH_2Cl_2/hexane/CH_3OH (97:1:2, v:v:v); ^1H NMR ($CDCl_3$) δ 8.36–8.32 (d, 1 H, J = 7.9 Hz), 7.71–7.58 (m, 5 H), 6.92–6.88 (d, 1 H, J = 8.4 Hz), 4.66–4.57 (q, 2 H, J = 7.1 Hz), 4.22–4.16 (t, 2 H, J = 6.9 Hz), 3.2–3.14 (t, 2 H, J = 7.5 Hz), 2.16–2.1 (m, 2 H), 1.44–1.38 (t, 3 H, J = 7.1 Hz); IR (KBr) 3050, 2951, 1707, 1656, 1589, 1556, 1210 cm^{-1}.

Compound 22: 92% yield (18.4 mg); R_f = 0.34, CH_2Cl_2/hexane/CH_3OH (97:1:2, v:v:v); ^1H NMR ($CDCl_3$) δ 8.38–8.31 (d, 1 H, J = 7.9 Hz), 7.72–7.55 (m, 5 H), 7.34–7.24 (m, 1 H), 6.92–6.88 (d, 1 H, J = 8.4 Hz), 4.67–4.59 (q, 2 H, J = 7.1 Hz), 4.12–4.05 (t, 2 H, J = 7.3 Hz), 3.13–3.07 (t, 2 H, J = 7.3 Hz), 1.77–1.37 (m, 6 H), 1.44–1.38 (t, 3 H, J = 7.1 Hz); IR (KBr) 3055, 2931, 2858, 1705, 1651, 1589, 1556, 1217 cm^{-1}.

Compound 23: 92% yield (18.4 mg); R_f = 0.35, CH_2Cl_2/hexane/CH_3OH (97:1:2, v:v:v); ^1H NMR ($CDCl_3$) δ 8.35–8.31 (d, 1 H, J = 7.9 Hz), 7.7–7.54 (m, 5 H), 7.32–7.28 (m, 2 H), 6.9–6.87 (d, 1 H, J = 8.4 Hz), 4.66–4.57 (q, 2 H, J = 7.1 Hz), 4.09–4.03 (t, 2 H, J = 7.3 Hz), 3.11–3.05 (t, 2 H, J = 7.3 Hz), 1.7–1.3 (m, 1 H); IR (KBr) 3050, 2931, 2851, 1705, 1655, 1585, 1556, 1237 cm^{-1}.

Compound 24: 81% yield (16.2 mg); R_f = 0.36, CH_2Cl_2/hexane/CH_3OH (97:1:2, v:v:v); ^1H NMR ($CDCl_3$) δ 8.34–8.31 (d, 1 H, J = 7.9 Hz), 7.65–7.54 (m, 5 H), 7.31–7.22 (m, 2 H), 6.89–6.86 (d, 1 H, J = 8.4 Hz), 4.66–4.56 (q, 2 H, J = 7.1 Hz), 4.09–4.01 (t, 2 H, J = 7.3 Hz), 3.1–3.0 (t, 2 H, J = 7.3 Hz), 1.73–1.25 (m, 15 H); IR (KBr) 2926, 2855, 1703, 1651, 1585, 1555, 1219, 1068 cm^{-1}.

Compound 25: 51% yield (10.1 mg); R_f = 0.33, CH_2Cl_2/hexane/CH_3OH (94:3:3, v:v:v); ^1H NMR ($CDCl_3$) δ 8.43–8.39 (d, 1 H, J = 8.4 Hz), 7.93 (s, 1 H), 7.84–7.81 (d, 1 H, J = 8.4), 4.67–4.61 (q, 2 H, J = 7.1 Hz), 4.26–4.2 (t, 2 H, J = 6.9 Hz), 4.12 (s, 3 H), 3.23–3.17 (t, 2 H, J = 7.4 H), 2.19–2.13 (m, 2 H), 1.45–1.39 (t, 3 H, J = 7 Hz); IR (KBr) 2931, 2853, 2234, 1715, 1659, 1588, 1560 cm^{-1}.

Compound 26: 61% yield (12.2 mg); R_f = 0.37, CH_2Cl_2/hexane/CH_3OH (94:3:3, v:v:v); ^1H NMR ($CDCl_3$) δ 8.43–8.40 (d, 1 H, J = 8.4 Hz), 7.92 (s, 1 H), 7.84–7.80 (d, 1 H, J = 8.4 Hz), 4.69–4.6 (q, 2 H, J = 7.1 Hz), 4.15–4.09 (t, 2 H, J = 7.3 Hz), 4.12 (s, 3 H), 3.15–3.09 (t, 2 H, J = 7.3 Hz), 1.8–1.51 (m, 6 H), 1.45–1.39 (t, 3 H, J = 7.3 Hz), 1.8–1.51 (m, 6 H) 1.45–1.39 (t, 3 H, J = 7.1 Hz); IR (KBr) 2931, 2853, 2233, 1720, 1658, 1588, 1560 cm^{-1}.

Compound 27: 56% yield (11.2 mg); R_f = 0.38, CH_2Cl_2/hexane/CH_3OH (94:3:3, v:v:v); ^1H NMR ($CDCl_3$) δ 8.43–8.39 (d, 1 H, J = 8.4 Hz), 7.91 (s, 1 H), 7.84–7.80 (d, 1 H, J = 8.4 Hz), 4.65–4.59 (q, 2 H, J = 7.1 Hz), 4.14–4.08 (t, 2 H, J = 7.3 Hz), 4.11 (s, 3 H), 3.14–3.08 (t, 2 H, J = 7.3 Hz), 1.76–1.37 (m, 8 H), 1.45–1.39 (t, 3 H, J = 7.1 Hz); IR (KBr) 2950, 2233, 1722, 1656, 1589, 1562 cm^{-1}.

Compound 28: 69% yield (13.8 mg); R_f = 0.45, CH_2Cl_2/hexane/CH_3OH (94:3:3, v:v:v); ^1H NMR ($CDCl_3$) δ 8.38–8.27 (d, 1 H, J = 8.3 Hz), 7.82 (s, 1 H), 7.79–7.68 (d, 1 H, J = 8.3 Hz), 4.7–4.43

(q, 2 H, J = 7.1 Hz), 4.11–3.93 (m, 5 H), 3.11–2.94 (t, 2 H, J = 7.3 Hz), 1.48–1.25 (m, 15 H); IR (KBr) 2924, 2854, 2233, 1722, 1665, 1588, 1560 cm^{-1}.

Preparation of *N*-3-(Mercaptoalkyl)isoalloxazines (1–8). To a stirred solution of xanthate ester (10 mg, 0.025 mmol), in 1.5 mL of dry THF under an atmosphere of argon, was added ethylenediamine (3 mg, 0.05 mmol) in 0.5 mL dry THF for 10 min at 0 °C. The reaction was stirred for 2 h at room temperature. At the end of the reaction, dichloromethane was added to the reaction, washed with cold 5% H_2SO_4, and then cold water. The organic fraction was evaporated to dryness and the crude material was chromatographed directly on silica gel to afford the *N*-3-(mercaptoalkyl)isoalloxazine derivatives.

Compound 1: 39% yield (3.9 mg); R_f = 0.23, CH_2Cl_2/hexane/CH_3OH (97:1:2, v:v:v); ^{1}H NMR ($CDCl_3$) δ 8.34–8.31 (d, 1 H, J = 7.9 Hz), 7.65–7.54 (m, 5 H), 7.3–7.23 (m, 2 H), 6.9–6.86 (d, 1 H, J = 8.4 Hz), 4.1–4.03 (t, 2 H, J = 7.3 Hz), 2.76–2.66 (t, 2 H, J = 7.3 Hz), 1.7–1.4 (m, 12 H), 1.27 (s, 1 H); IR (KBr) 3050, 2931 2851, 1707, 1656, 1589, 1556, 1264 cm^{-1}.

Compound 2: 85% yield (8.5 mg); R_f = 0.22, CH_2Cl_2/hexane/CH_3OH (97:1:2, v:v:v); ^{1}H NMR ($CDCl_3$) δ 8.35–8.31 (d, 1 H, J = 7.9 Hz), 7.7–7.54 (m, 5 H), 7.32–7.28 (m, 2 H), 6.92–6.86 (d, 1 H, J = 8.4 Hz), 4.09–4.03 (t, 2 H, J = 7.3 Hz), 2.67–2.63 (t, 2 H, J = 7.3 Hz), 1.7–1.4 (m, 8 H), 1.27 (s, 1 H); IR (KBr) 2930, 2857, 1709, 1656, 1589, 1556, 1264 cm^{-1}.

Compound 3: 86% yield (8.6 mg); R_f = 0.21, CH_2Cl_2/hexane/CH_3OH (97:1:2, v:v:v); ^{1}H NMR ($CDCl_3$) δ 8.35–8.31 (d, 1 H, J = 7.9 Hz), 7.72–7.55 (m, 5 H), 7.34–7.27 (m, 2 H), 6.92–6.88 (d, 1 H, J = 8.4 Hz), 4.09–4.03 (t, 2 H, J = 7.3 Hz), 2.67–2.63 (t, 2 H, J = 7.3 Hz), 1.7–1.41 (m, 6 H), 1.27 (s, 1 H); IR (KBr) 2930, 2857, 1709, 1656, 1589, 1556, 1264 cm^{-1}.

Compound 4: 83% yield (8.3 mg); R_f = 0.20, CH_2Cl_2/hexane/CH_3OH (97:1:2, v:v:v); ^{1}H NMR ($CDCl_3$) δ 8.36–8.32 (d, 1 H, J = 7.9 Hz), 7.69–7.58 (m, 5 H), 7.35–7.31 (m, 2 H), 6.92–6.88 (d, 1 H, J = 8.4 Hz), 4.22–4.16 (t, 2 H, J = 7.0 Hz), 2.82–2.76 (t, 2 H, J = 7.4 Hz), 2.19–2.13 (m, 2 H), 1.27 (s, 1 H); IR (KBr) 3057, 2924, 1709, 1660, 1589, 1264 cm^{-1}.

Compound 5: 51% yield (5.1 mg); R_f = 0.40, CH_2Cl_2/CH_3OH (96: 4, v:v); ^{1}H NMR ($CDCl_3$) δ 8.42–8.38 (d, 1 H, J = 8.4 Hz), 7.92 (s, 1 H), 7.82–7.79 (d, 1 H, J = 8.4 Hz), 4.1–4.06 (m, 5 H), 2.68–2.62 (t, 2 H, J = 7.3 Hz), 1.72–1.34 (m, 12 H), 1.25 (s, 1 H); IR (KBr) 2924, 2854, 2228, 1715, 1665, 1588, 1560 cm^{-1}.

Compound 6: 34% yield (3.4 mg); R_f = 0.38, CH_2Cl_2/CH_3OH (96:4, v:v); ^{1}H NMR ($CDCl_3$) δ 8.44–8.34 (d, 1 H, J = 8.2 Hz), 7.9 (s, 1 H), 7.86–7.75 (d, 1 H, J = 8.3 Hz), 4.3–4.0 (m, 5 H), 2.74–2.56 (t, 2 H, J = 7.3 Hz), 1.77–1.26 (m, 9 H); IR (KBr) 2931, 2854, 2228, 1659, 1588, 1553 cm^{-1}.

Compound 7: 52% yield (5.2 mg); R_f = 0.37, CH_2Cl_2/CH_3OH (96:4, v:v); ^{1}H NMR ($CDCl_3$) δ 8.42–8.38 (d, 1 H, J = 8.4 Hz), 7.91 (s, 1 H), 7.83–7.80 (d, 1 H, J = 8.4 Hz), 4.13–4.07 (m, 5 H), 2.7–2.64 (t, 2 H, J = 7.2 Hz), 1.76–1.48 (m, 6 H), 1.26 (s, 1 H); IR (KBr) 2931, 2853, 2228, 1715, 1658, 1588, 1560 cm^{-1}.

Compound 8: 51% yield (5.1 mg); R_f = 0.34, CH_2Cl_2/CH_3OH (96:4, v:v); ^{1}H NMR ($CDCl_3$) δ 8.41–8.38 (d, 1 H, J = 8.3 Hz), 7.96 (s, 1 H), 7.9–7.83 (d, 1 H, J = 8.3 Hz), 4.23–4.17 (t, 2 H, J = 7.0 Hz), 4.10 (s, 3 H), 2.82–2.76 (t, 2 H, J = 7.3 Hz), 2.18–2.10 (m, 2 H), 1.26 (s, 1 H); IR (KBr) 2931, 2853, 2234, 1715, 1659, 1588, 1560 cm^{-1}.

Preparation of *N*-3-[(Methoxycarbonyl)alkyl]isoalloxazine Derivatives (29 and 30). To a stirred solution of isoalloxazine (200 mg, 0.7 mmol) in 25 mL of DMF at room temperature was added NaH (50 mg, 2.1 mmol) in DMF. After a few minutes methyl bromopentanoate (136 mg, 0.7 mmol) in DMF was added. The reaction was stirred for 3 days at room temperature after which the DMF was evaporated and the reaction mixture was extracted between CH_2Cl_2 and water and the organic fraction was evaporated to dryness. The crude reaction mixture was chromatographed directly on silica gel to afford the *N*-3-[(methoxycarbonyl)alkyl]isoalloxazine derivatives.

Compound 29: 53% yield (106 mg); R_f = 0.43, CH_2Cl_2/EtOAc (50:50, v:v); ^{1}H NMR ($CDCl_3$) δ 7.7–7.5 (m, 5 H), 7.4–7.2 (m, 2 H), 6.9 (m, 1 H), 4.1 (m, 2 H), 3.6 (s, 3 H), 2.4 (s, 2 H), 1.9–1.6 (m, 4 H); IR (KBr) 3500, 1720, 1638, 1600, 1550 cm^{-1}.

Compound 30: 69% yield (138 mg); R_f = 0.31, CH_2Cl_2/EtOAc (50:50, v:v); ^{1}H NMR (DMSO) δ 8.6 (d, 1 H, J = 8.2 Hz), 8.3 (d, 1 H, J = 8.3 Hz), 8.0 (d, 1 H, J = 8.3 Hz), 4.0 (s, 3 H), 3.9 (m, 2 H), 3.6 (s, 3 H), 2.4 (m, 2 H), 1.6 (m, 4 H); IR (KBr) 3550, 2229, 1720, 1671, 1638, 1196 cm^{-1}.

Preparation of *N*-3-(Carboxyalkyl)isoalloxazine Derivatives (9 and 10). To a cold stirred solution of *N*-3-[(methoxycarbonyl)alkyl]isoalloxazine (25 mg, 0.06 mmol) in 1 mL of THF was added H_2SO_4 (0.07 mmol in 0.5 mL of water). The reaction was stirred for 3 days at room temperature after which the THF was evaporated, the reaction neutralized and extracted with ethyl acetate. The crude reaction mixture was chromatographed directly on silica gel to afford the *N*-3-(carboxyalkyl)isoalloxazine derivatives.

Compound 9: 76% yield (19.0 mg); R_f = 0.32, CH_2Cl_2/CH_3OH (92:4, v:v); ^{1}H NMR (DMSO) δ 7.8 (m, 5 H), 7.5 (d, 2 H, J = 7.5 Hz), 6.8 (d, 2 H, J = 7.4 Hz), 3.9 (m, 2 H), 2.0 (t, 2 H, J = 8.3 Hz), 1.7–1.4 (m, 4 H); IR (KBr) 3500, 1704, 1655, 1600 1556 cm^{-1}.

Compound 10: 58% yield (14.5 mg); R_f = 0.3, CH_2Cl_2/CH_3OH (91:9, v:v); ^{1}H NMR (DMSO) δ 8.5 (s, 1 H), 8.2 (m, 1 H), 8.0 (m, 1 H), 4.1 (m, 2 H), 4.0 (s, 3 H), 2.0 (s, 2 H), 1.65–1.4 (m, 4 H); IR (KBr) 3490, 2228, 1710, 1658, 1623, 1558, 1550 cm^{-1}.

Measurements of Rate Constants. All rate measurements were made at 30 ± 0.2 °C. Rate measurements of *m*-nitrothiophenol disulfide formation were made with a Rainin high-performance liquid chromatography (HPLC) system. Analysis was performed on a 5-μm Zorbax C-8 (7.7 mm × 25 cm) analytical reverse-phase column as described previously.[30] The buffers employed were phosphate (pH 6-8), glycine hydroxide (pH 9), and sodium hydroxide (pH 10); ionic strength was maintained at 0.4 M with KCl. Stock solutions of isoalloxazines were prepared in dry methanol/dichloromethane and aliquots were diluted into aqueous–methanolic reaction solutions. Quantitation of amounts of products were performed by comparison with standard curves of authentic material under the same analysis conditions. Rate constants (k_{obsd}) were measured under pseudo-first-order reaction conditions by monitoring the formation of *m*-nitrothiophenol disulfide for at least 4 half-lives, and second-order rate constants were obtained from plots of k_{obsd} versus the concentration of the reactant present in large excess.

Product Analysis. The major products of the reaction of each isoalloxazine in buffer was isolated from large-scale reactions by dichloromethane extraction followed by preparative thin-layer chromatography on silica gel. The product was identified by an examination of the liquid secondary ion mass spectra (LSIMS) molecular ions and fragmentation pattern.[31,32] For the products of the reaction of *m*-nitrothiophenol with the various isoalloxazines, the disulfide product was identified by a comparison to an authentic sample[30] by normal spectroscopic techniques.

Acknowledgment. This work was financially supported by a grant from NIH (Grant No. GM 36398). We acknowledge the generous help of David Maltby and Katalin Medzihradsky of the UCSF Bioorganic Biomedical Mass Spectrometry Resource (A. L. Burlingame, Director, supported by NIH Division of Research Resources Grant RR016614). We thank Gloria Dela Cruz for her expert typing.

Registry No. 1, 133965-90-9; 2, 133965-91-0; 3, 133965-92-1; 4, 133965-93-2; 5, 133965-94-3; 6, 133965-95-4; 7, 133965-96-5; 8, 133965-97-6; 9, 133965-98-7; 10, 133965-99-8; 13, 133966-00-4; 14, 133966-01-5; 15, 133966-02-6; 16, 133966-03-7; 17, 133966-04-8; 18, 133966-05-9; 19, 133966-06-0; 20, 133966-07-1; 21, 133966-08-2; 22, 133966-09-3; 23, 133966-10-6; 24, 133966-11-7; 25, 133966-12-8; 26, 133966-13-9; 27, 133966-14-0; 28, 133966-15-1; 29, 133966-16-2; 30, 133966-17-3; 31, 3814-18-4; *m*-$O_2NC_6H_4SSC_6H_4NO_2$-*m*, 537-91-7; $Br(CH_2)_4CO_2Me$, 5454-83-1; 10-phenylisoalloxazine, 6851-14-5; 8-cyano-10-methylisoalloxazine, 64910-50-5; ethyl xanthate potassium salt, 140-89-6.

Supplementary Material Available: Low-resolution mass spectrometric data for isoalloxazines 1–10 and 13–30 (2 pages). Ordering information is given on any current masthead page.

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