International Journal of Scientific Research and Modern Technology (IJSRMT) ijsrmt.com
Volume 2, Issue 5, 2023
DOI: https://doi.org/10.38124/ijsrmt.v2i5.1783

Chemical Oxidation of Thiobenzamide and Its Relevance to the Metabolism of Antituberculosis Drugs

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Publication Date: 2023/05/28

Abstract

Tuberculosis (TB) remains a leading cause of death from infectious disease worldwide. The most effective antituberculosis drugs — ethionamide (ETA), protionamide (PTA), and isoniazid (INH) — share thioamide or carboxamide structural fragments whose metabolic activation proceeds via sulfur oxygenation. This study investigates the chemical oxidation of thiobenzamide as a model thioamide compound using chromium trioxide (CrO_3) and hydrogen peroxide (H_2O_2) under varying pH conditions (pH 1–13), monitored by UV–Vis spectrophotometry and thin-layer chromatography. The results demonstrate that thiobenzamide-S-oxide is rapidly formed as the primary oxidation product regardless of medium acidity when equimolar oxidant is used. With excess oxidant, the product distribution is pH-dependent: weakly acidic media yield 3,5-diphenyl-1,2,4-thiadiazole and benzamide, while neutral to mildly alkaline conditions produce benzonitrile and benzamide through desulfurization. These chemical oxidation pathways parallel the enzymatic S-oxygenation observed during the metabolic activation of thioamide prodrugs by flavin monooxygenases (FMOs) and monooxygenase systems in Mycobacterium tuberculosis. The findings support the hypothesis that sulfur oxidation is the initial step in thioamide drug metabolism and provide chemical evidence relevant to understanding the generation of cytotoxic metabolites responsible for bactericidal activity.

Keywords: *Thiobenzamide, Thioamide Oxidation, Thiobenzamide-S-Oxide, Chromium Trioxide, UV–Vis Spectrophotometry, Tuberculosis Drug Metabolism, Ethionamide, Mycobacterium tuberculosis.*

I. INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis*, discovered by Robert Koch in 1882 [1]. Approximately one-third of the global population is infected with the pathogen, with 75% belonging to the most economically productive age group (15–50 years). In 2016, more than 10 million active TB cases and approximately 1.3 million deaths were reported worldwide, with over 95% of fatalities occurring in developing countries. The situation has become more alarming at the beginning of the twenty-first century due to demographic changes, insufficient TB control measures, and the HIV/AIDS epidemic, which have resulted in vast numbers of undiagnosed or inadequately treated cases [1, 2].

Among the principal drugs used for TB treatment are ethionamide (ETA), protionamide (PTA), and isoniazid (INH), which share a common thioamide or carboxamide structural fragment [2,3,4]. The metabolic activation of these drugs proceeds via oxidation of the sulfur atom in the thioamide group, generating reactive intermediates with bactericidal activity. Understanding the oxidative transformations of thioamides is essential for optimizing drug efficacy and minimizing toxic side effects.

The present study uses thiobenzamide as a model compound to investigate the chemical oxidation of the thioamide group with CrO_3 and H_2O_2 under varying pH conditions [2]. The results are discussed in the context of biological S-oxygenation pathways relevant to antituberculosis drug metabolism.

II. LITERATURE REVIEW

- *Pulmonary Tuberculosis: Clinical Features and Treatment*

Tuberculosis is caused by the strictly aerobic bacterium *Mycobacterium tuberculosis*, which reproduces

Samet, A. (2023). Chemical Oxidation of Thiobenzamide and Its Relevance to the Metabolism of Antituberculosis Drugs. *International Journal of Scientific Research and Modern Technology*, *2*(5), 15–20. https://doi.org/10.38124/ijsrmt.v2i5.1783

very slowly with binary fission occurring every 18–24 hours [3]. The most common source of infection is the sputum of TB patients, with transmission depending on the concentration of bacilli in the air and the duration of exposure [3, 4].

Clinical symptoms are classified as general (fatigue, low-grade fever, night sweats, weight loss, pallor) and functional (cough, hemoptysis, chest pain, dyspnea) [4]. Treatment must be initiated early and typically lasts six months in two phases: an initial two-month intensive phase with four drugs, followed by a four-month continuation phase with rifampicin and isoniazid [5–7]. The principal first-line tuberculostatics include isoniazid, rifampicin, pyrazinamide, ethambutol, and streptomycin.

➢ *Chemical Structure of Antituberculosis Thioamide Drugs*

The most successful medications for treating TB infections are ethionamide (ETA), protionamide (PTA), and isoniazid (INH) [8]. These drugs share thioamide or carboxamide functional groups in their structures.

Using various bacterial substrates, the minimal inhibitory concentrations of thioamide- and carboxamide-containing substances have been determined [9], revealing significant differences in antibacterial activity attributable to the pronounced nucleophilicity of the S-atom relative to the amide oxygen. Thioamides have confirmed antimycobacterial, antibacterial, antifungal, antiviral, anticancer, and anti-inflammatory activities [10–12], making them subjects of continued research in numerous laboratories worldwide.

Thioamides are sulfur analogs of amides with the general formula $RCSNH_2$. X-ray diffraction studies show that the atoms of the thioamide group lie in a plane with trigonal hybridization (120° valence angles), indicating partial double-bond character of the C–N bond due to resonance [13].

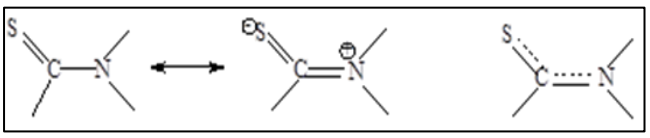


Fig 1 Resonance Structures of the Thioamide Group.

Thioamides participate in numerous reactions — hydrolysis, alkylation, acylation, reduction, oxidation, reactions with metal ions — largely attributable to their ability to react in two tautomeric forms [14]:

➢ *Oxidation Chemistry of Thioamides*

Thioamides and their oxygen analogs (amides) differ markedly in oxidation behavior. While amides are difficult to oxidize with moderate oxidizing agents, thioamides undergo immediate electrophilic attack at the S-atom, producing a variety of products: heterocyclic compounds, thioamide-S-oxides, sulfides, disulfides, and sulfur-free products [13,15]. Numerous oxidizing agents have been studied including H_2O_2, CrO_3, H_2SO_4, $KMnO_4$, SeO_2, HgO, and others.

Oxidation with H_2O_2 in basic and neutral media yields thiobenzamide-S-oxide as the primary product. Kinetic studies at pH 7.4 with excess H_2O_2 demonstrated that the S-oxide successively converts to benzonitrile and benzamide [17]. The electrophilic oxidation mechanism involves deprotonation to form a more reactive conjugate base [15–18].

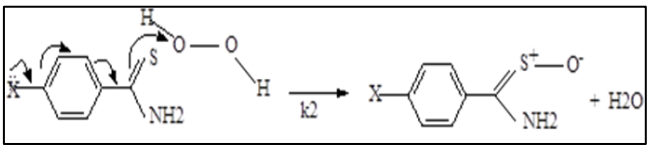


Fig 2 Electrophilic Oxidation Mechanism of Thiobenzamide by H_2O_2.

➢ *Metabolism of Thioamide Drugs*

The exact manner in which bacteria metabolically degrade thioamides remains under investigation. Two biological mechanisms have been proposed: (1) ammonia elimination via direct C–N bond hydrolysis, and (2) oxygenation of the sulfur atom producing intermediates that hydrolyze or eliminate to form amides and nitriles [10, 13].

Studies by Dodge et al. on the transformation of thioacetamide and thiobenzamide by oxygenases of *Ralstonia pickettii* under aerobic conditions demonstrated complete substrate conversion via S-atom oxygenation, followed by elimination of sulfur as sulfite anion [19]. At least one cytotoxic metabolite is generated that inhibits growth of *M. tuberculosis* [11].

According to Cashman and Hanzlik, oxidation of thioamides with MFMO (monoflavin monooxygenase) proceeds toward rapid formation of thioamide-S-oxide as the first oxidation step under both *in vivo* and *in vitro* conditions [18, 20]. The metabolites from enzymatic S-oxidation exhibit greater chemical reactivity than the substrates, enabling spontaneous covalent binding to cellular nucleophiles — these are responsible for the toxic behavior of thioamides toward bacteria [20–22].

The thioamide drugs ETA and PTA are inhibitors of mycolic acid biosynthesis [23]. The mechanism of their physiological activity against *M. tuberculosis* and *M. leprae* has been elucidated by Wang Feng and coworkers [23]. The drugs are prodrugs requiring enzymatic activation by the monooxygenase EthA [23-26].

III. MATERIALS AND METHODS

➢ *Reagents and Solutions*

Thiobenzamide ($PhCSNH_2$, ≥98% purity) was used as the model thioamide substrate. Stock solutions of chromium trioxide (CrO_3) and thiobenzamide were prepared in appropriate solvents for spectrophotometric experiments. Reference compounds — benzamide ($PhCONH_2$), benzonitrile (PhCN), 3,5-diphenyl-1,2,4-thiadiazole, and thiobenzamide-S-oxide — were used for product identification [2,14].

➢ *Reaction Conditions*

The oxidation reactions were carried out in buffer systems covering the pH range from 1 to 13. Strongly acidic conditions (pH 1–2) employed KCl/HCl buffers; weakly acidic (pH 3–6), neutral (pH 7–8), and mildly alkaline (pH 9–11) conditions used phosphate and borate buffers. The oxidant-to-substrate molar ratios investigated were 1:1 (equimolar), 5:1, and 10:1 [2,14].

➢ *UV–Vis Spectrophotometric Monitoring*

Reaction progress was monitored using a Varian Cary 50 UV–Vis spectrophotometer over the wavelength range 200–600 nm. Spectra were recorded at regular time intervals during the reactions. The characteristic absorption maxima of the substrate and products served as diagnostic markers: thiobenzamide (λmax ≈ 270 and 305 nm), thiobenzamide-S-oxide (λmax ≈ 290 nm at neutral pH, 347 nm protonated), benzamide (λmax ≈ 225 nm), and benzonitrile (λmax ≈ 230 nm) [14, 17].

➢ *Thin-Layer Chromatography*

Thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} plates using benzene/ethanol as the mobile phase. Reaction mixtures were spotted alongside reference standards to confirm product identities through R^f comparison [2,14].

IV. RESULTS AND DISCUSSION

➢ *Spectral Characteristics of Thiobenzamide and Reference Compounds*

The UV–Vis spectrum of thiobenzamide (Figure 3) exhibited characteristic absorption maxima at approximately 270 nm and 305 nm, consistent with the $\pi\rightarrow\pi$ *and* $n\rightarrow\pi$ transitions of the thioamide chromophore [14, 17]. These spectral features provided the baseline for monitoring oxidation reactions and identifying products.

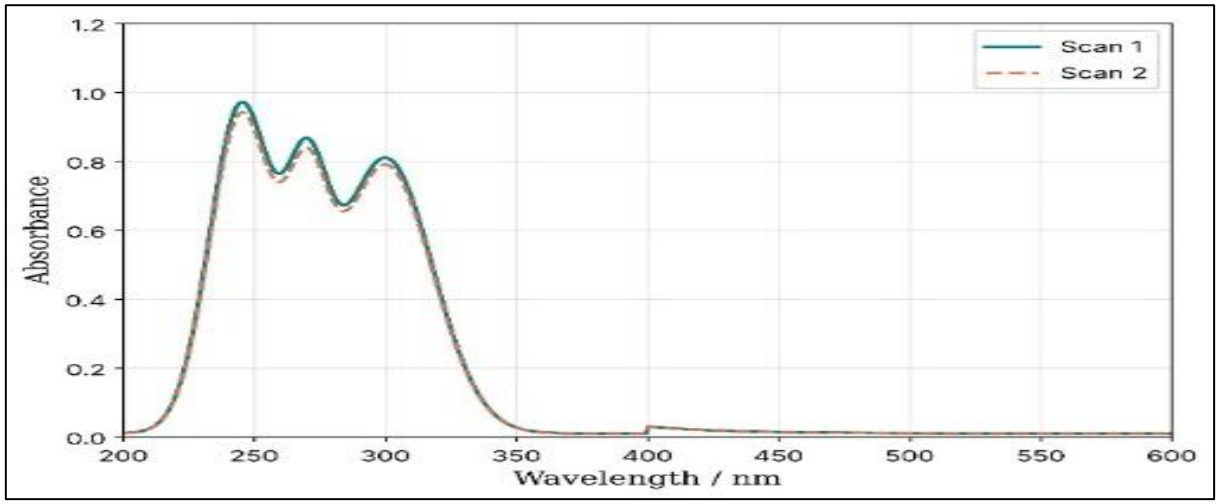


Fig 3 UV–Vis Absorption Spectrum of Thiobenzamide in the Range 200–600 nm.

➢ *Oxidation of Thiobenzamide with H_2O_2: Reaction Profile*

The reaction profile for thiobenzamide oxidation with excess H_2O_2 at neutral pH (7.4) is shown in Figure 4. Thiobenzamide (TB) was rapidly consumed and converted to thiobenzamide-S-oxide (TBSO) as the initial product. The S-oxide subsequently underwent slower transformation to benzonitrile (PhCN) and benzamide ($PhCONH_2$), confirming that H_2O_2 acts as a typical electrophilic oxidant toward the thiobenzamide sulfur atom [15–18].

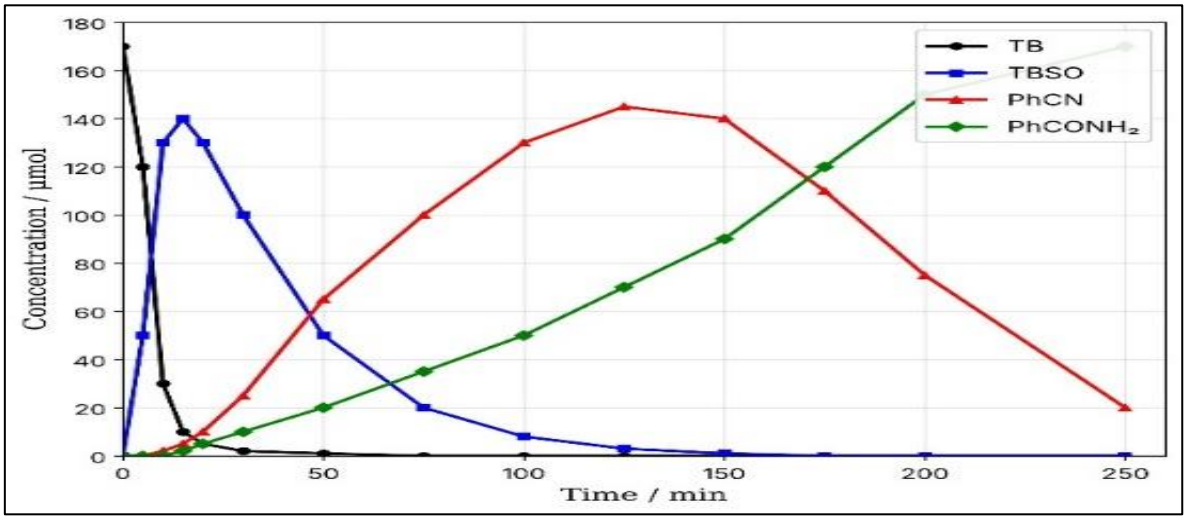


Fig 4 Reaction Profile for Thiobenzamide Oxidation with Excess H_2O_2: Time Course of Substrate (TB) Consumption and Product (TBSO, PhCN, $PhCONH_2$) Formation [6].

Chromatographic monitoring confirmed the presence of thiobenzamide-S-oxide as the main product alongside secondary products benzamide and 3,5-diphenyl-1,2,4-thiadiazole. The proportion of secondary products varied depending on the pH of the reaction medium.

➢ *Oxidation of Thiobenzamide with CrO_3: Effect of pH*

The oxidation of thiobenzamide with equimolar CrO_3 proceeded instantaneously to form thiobenzamide-S-oxide as the primary product regardless of medium acidity [2]. In the pH range of 3–10, no changes were observed in the UV spectrum of unreacted thiobenzamide, indicating that it does not ionize under these conditions [14, 18].

In strongly acidic media (pH 1–2, KCl/HCl buffer), thiobenzamide was protonated with a shift in the UV absorption maximum toward shorter wavelengths. Oxidation with equimolar CrO_3 proceeded instantaneously, producing thiobenzamide-S-oxide as the sole product. The protonated S-oxide exhibited an absorption maximum at 347 nm. With prolonged reaction time, no appreciable changes in concentration were observed, indicating the stability of the product under strongly acidic conditions [14].

➢ *Effect of Oxidant-to-Substrate Ratio*

The amount of oxidant significantly influenced whether the reaction was limited to initial S-oxidation or proceeded to subsequent transformations. At 1:1 ratio, thiobenzamide-S-oxide was the main product under all pH conditions. With 5-fold and 10-fold excess CrO_3, the product distribution became pH-dependent [14]:

In weakly acidic media, excess CrO_3 promoted formation of the heterocyclic compound 3,5-diphenyl-1,2,4-thiadiazole and benzamide alongside thiobenzamide-S-oxide. In neutral and mildly alkaline media (pH 7–11), excess CrO_3 led to the formation of benzonitrile and benzamide through desulfurization reactions.

➢ *UV Spectral Analysis at Neutral and Alkaline pH*

Figure 5 presents the UV–Vis spectra recorded during thiobenzamide oxidation with five-fold excess CrO_3 at three different pH values (7, 8, and 11). The spectral changes illustrate the pH-dependent product formation pathways.

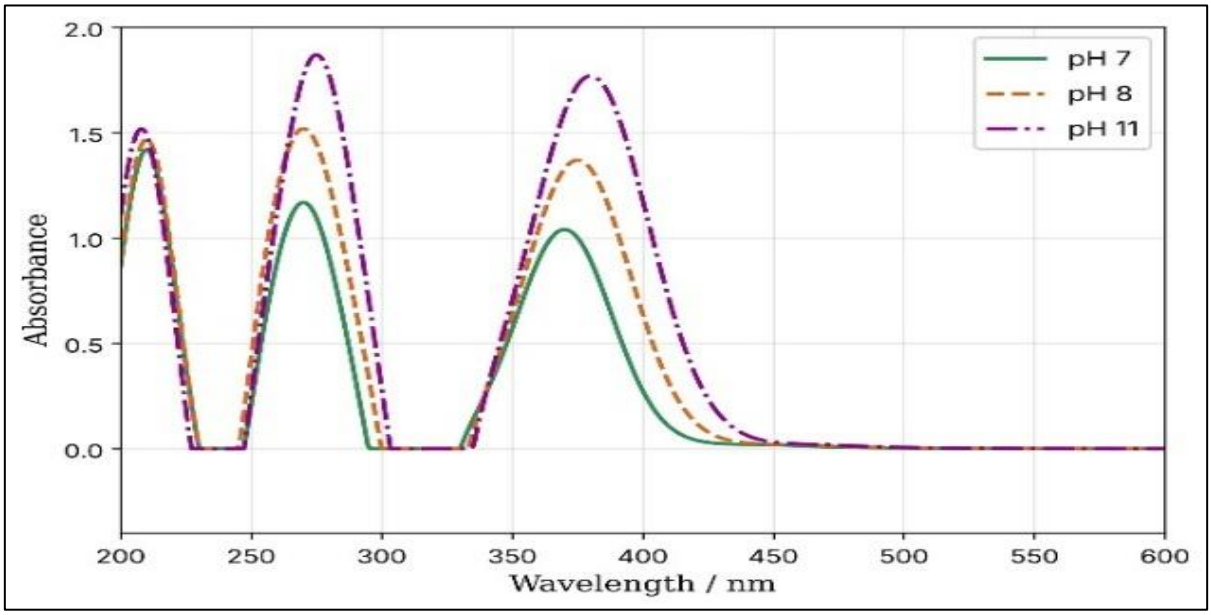


Fig 5 UV–Vis Spectra of Thiobenzamide Oxidation with Five-Fold Excess CrO_3 at pH 7 (Blue), pH 8 (Red), and pH 11 (Purple).

At all three pH values, thiobenzamide-S-oxide formed rapidly as the initial product. Under prolonged influence of excess Cr(VI) at pH 7–11, the S-oxide concentration increased with higher pH values. The formed thiobenzamide-S-oxide was then transformed into benzonitrile, consistent with the literature showing that treatment of thiobenzamide-S-oxide with base slowly produces benzonitrile in a reaction that is first-order with respect to hydroxide [18]. The detected presence of benzamide in neutral and mildly alkaline media is attributed to a desulfurization reaction proceeding through thiobenzamide-S,S-dioxide as an unstable intermediate. This intermediate transforms instantaneously, which is why it has not been directly detected in the reaction mixture [14, 18].

➢ *Relationship Between Chemical Oxidation and Biological Metabolism*

The chemical oxidation results parallel the biological S-oxygenation pathways described for thioamide drug metabolism. Figure 6 presents the UV–Vis spectra from the metabolic transformation of thioacetamide (A) and thiobenzamide (B) by oxygenases of *Ralstonia pickettii* TA under aerobic and anaerobic conditions [8].

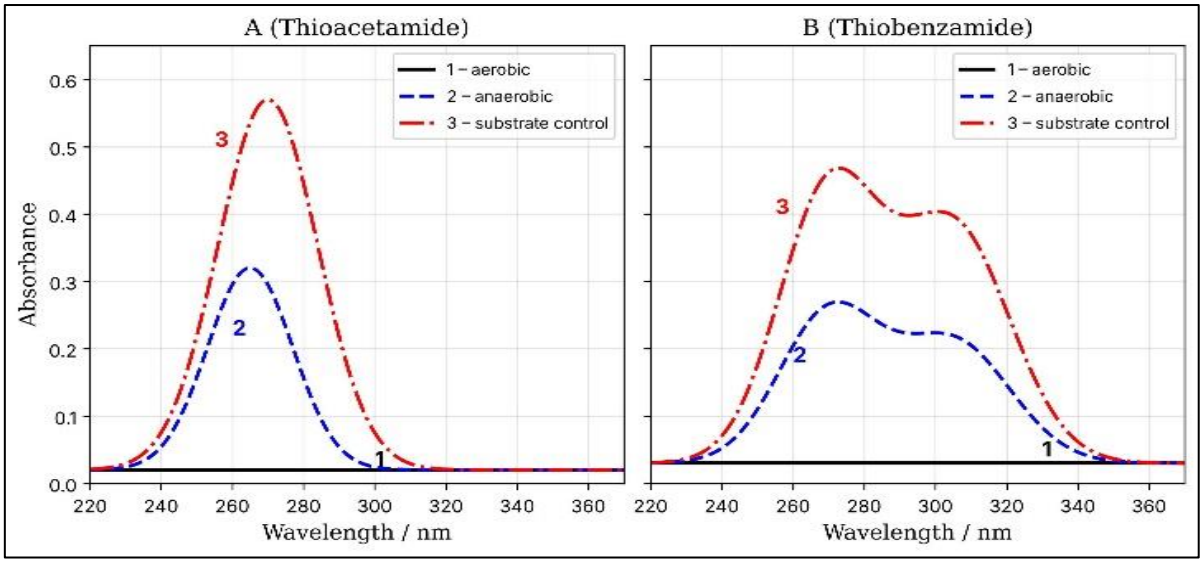


Fig 6 UV–Vis Spectra of Supernatant from R. Pickettii TA Cell Suspension with 0.5 mM Thioacetamide (A) and Thiobenzamide (B). 1 – Aerobic; 2 – Anaerobic; 3 – Substrate Control [8].

Under anaerobic conditions (curve 2), no spectral changes were observed, while aerobic conditions (curve 1) resulted in complete transformation of both thioamide substrates. The metabolic products detected confirmed that oxygenase-catalyzed reactions occur at the nucleophilic S-atom of the thioamide group, followed by sulfur elimination as sulfite anion [8,19]. The absence of elemental sulfur, sulfate, and sulfide excludes direct thioamide hydrolysis as the metabolic pathway. These biological findings corroborate the chemical oxidation results of the present study: in both systems, the sulfur atom of the thioamide group is the primary site of oxidative attack, with thiobenzamide-S-oxide as the initial product. The subsequent chemical transformations (desulfurization to benzamide, dehydration to benzonitrile) mirror the metabolic degradation products observed in bacterial systems.

Of particular significance is that the enzymatic S-oxygenation of antituberculosis drugs ETA and PTA by monooxygenases (including flavin monooxygenase and cytochrome P-450) generates reactive intermediates — thioamide-S-oxides and S,S-dioxides — whose covalent binding to cellular nucleophiles produces the cytotoxic metabolites responsible for bactericidal activity [20, 22–25, 28]. The optimal chemical oxidation conditions identified in this study (equimolar oxidant at neutral pH yielding maximum S-oxide) thus reflect the biologically relevant oxidation pathway.

V. CONCLUSION

This study demonstrates that the chemical oxidation of thiobenzamide with CrO_3 and H_2O_2 provides a useful model for understanding the metabolic activation of antituberculosis thioamide drugs. The principal findings are: (1)Thiobenzamide-S-oxide is formed rapidly as the primary oxidation product under all pH conditions when equimolar oxidant is used, confirming that the sulfur atom of the thioamide group is the preferred site of electrophilic oxidative attack.(2)The subsequent transformation of thiobenzamide-S-oxide is pH-dependent: in weakly acidic media, the heterocyclic compound 3,5-diphenyl-1,2,4-thiadiazole and benzamide are the major secondary products; in neutral and mildly alkaline media, benzonitrile and benzamide are formed through desulfurization. (3) Optimal formation of thiobenzamide-S-oxide (the biologically active intermediate) occurs at approximately pH 7.4 with equimolar oxidant, conditions that closely approximate physiological pH. (4)The chemical oxidation pathways parallel the enzymatic S-oxygenation observed in the metabolic activation of ethionamide and protionamide by flavin monooxygenases in *M. tuberculosis*, supporting the biological relevance of the model system.

These results contribute to the understanding of thioamide drug metabolism and may inform future research on optimizing prodrug activation and mitigating off-target toxicity in tuberculosis chemotherapy.

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