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Structural analysis of photo-degradation in thiazole-containing compounds by LC–MS/MS and NMR

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Abstract

The photo-degradation behavior of a pharmaceutical compound previously under development for treatment of overactive bladder was studied. Samples of {4-(4-chloro-3-fluorophenyl)-2-[4-(methyloxy)phenyl]-1,3-thiazol-5-yl} acetic acid were stressed with visible light and were observed to degrade into a single primary photo-degradation product. This unknown product was analyzed by liquid chromatography tandem mass spectrometry (LC–MS/MS) with accurate mass measurement and hydrogen/deuterium exchange to determine its molecular weight and formula, isotope distribution patterns and exchangeable protons, and product ion structures. By comparison of the fragmentation pathways of the protonated and sodiated species, the charge was found to locate in the electron-rich part of the molecule after fragmentation. MS-derived structural information combined with stopped-flow ^{1}H LC-nuclear magnetic resonance (NMR) analysis suggested that the degradation product was 4-chloro-*N*-(4-methoxybenzoyl)-3-fluorobenzamide. This unique photo-degradation product was subsequently isolated using preparative-scale chromatography, and its structure was confirmed using 1D and 2D NMR techniques involving the ^{1}H, ^{13}C, ^{15}N and ^{19}F nuclei. The structure of this product suggests that {4-(4-chloro-3-fluorophenyl)-2-[4-(methyloxy)phenyl]-1,3-thiazol-5-yl} acetic acid has reacted with singlet oxygen ($^{1}\Delta_{g}$) via a [4 + 2] Diels-Alder cycloaddition upon photo-irradiation to cause photo-oxygenation in the solid-state (as is common in solution phase), resulting in an unstable endoperoxide that rearranges to the final degradation product structure. Photo-degradation of a structurally related thiazole, 4-(4-Chlorophenyl)thiazol-2-amine, proceeded via a similar process but in a less reactive manner. However, when exposed to the same conditions, sulfathiazole did not degrade, indicating that this photo-degradation process may only occur for thiazole-containing compounds with specific substituents, such as aryl rings.

Keywords: Stress testing of pharmaceuticals; Photo-oxygenation; Singlet oxygen; [4 + 2] Diels-Alder cycloaddition; Thiazoles; Accurate mass; LC–MS/MS; NMR

1. Introduction

It is well known that drug products may undergo physicochemical degradation during manufacturing and storage. The testing of both drug substance and the final dosage form to understand inherent stability characteristics of a product is an essential part of drug development [1]. Part of this process involves determining the chemical structures of compounds produced during stability or forced degradation testing, to ensure that safety is not compromised by the presence of toxic impurities [2]. Currently, tandem mass spectrometry (MS/MS), in conjunction with high-performance liquid chromatography (HPLC), plays a key role in the rapid, on-line structural elucidation of pharmaceutical degradation products, due to its unparalleled speed, intrinsic sensitivity, and molecular specificity [3–5]. Nuclear magnetic resonance (NMR) spectroscopy also plays a major role in degradation product analysis, particularly when the structure must be confirmed for inclusion in regulatory documents, when response factors are needed to assess safety coverage, or when potentially toxic or mutagenic impurities or degradation products are encountered. Two approaches are usually taken when NMR data is required; in the first, compounds of interested are isolated by preparative LC and analyzed by conventional solution-state NMR using a microprobe. In the second approach, hyphenated techniques such as LC–NMR and solid-phase extraction (LC–SPE–NMR) are used in conjunction with flow-probe analysis [6–8].

Thiazoles are an important class of compounds that possess a wide range of pharmacological activity [9,10]. This importance is reflected by the large number of marketed drugs containing

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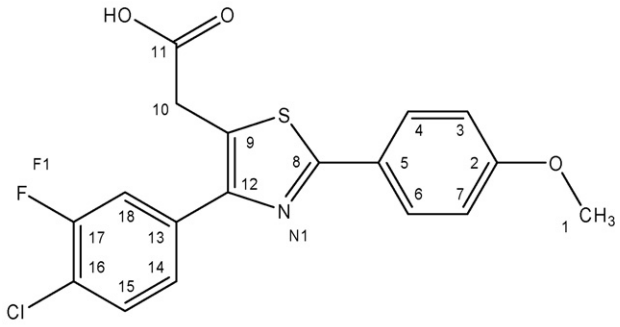
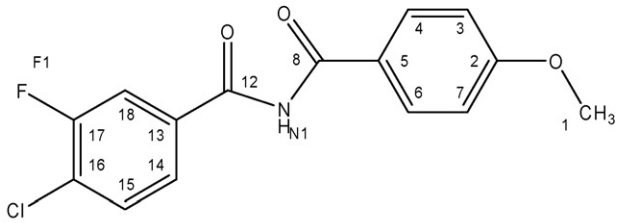
the thiazole group, such as the anticonvulsant riluzole, the antiparkinsonian talipexole, the antibacterial sulfathiazole, the antiviral ritonavir [9], and the novel drug substances being developed as the large conductance calcium-activated potassium channel openers for overactive bladder indications [11]. In addition, the thiazole ring has also been widely used in the synthesis of natural products [12], polymers [13], fluorescent dyes [14], and insecticides [15]. Like other conjugated five-membered heterocycles (such as furans, thiophenes, pyrroles, indoles, oxazoles, and imidazoles), thiazoles can undergo photo-oxygenation involving singlet oxygen ($^1\Delta_g$) under photo-irradiation in solution [16] and in the solid state [17]. Although several modes for singlet oxygen reactions have been proposed [18–20], the singlet oxygenation of these systems occurs mainly via [4 + 2] Diels-Alder cycloaddition, leading to unstable endoperoxides which, in addition to the classical transformations of peroxides (reduction, hydrolysis, and deoxygenation), afford characteristic rearranged products depending on the heteroatoms, substitution patterns and experimental conditions.

In this communication, we report the photo-oxygenation behavior of solid {4-(4-chloro-3-fluorophenyl)-2-[4-(methyloxy) phenyl]-1,3-thiazol-5-yl} acetic acid upon photo-irradiation (structure **I** in Fig. 1). This molecule is a pharmaceutically active compound previously under development for the treatment of overactive bladder [11]. LC–MS/MS analysis and accurate mass determinations are used to rapidly identify the major photo-degradation product as 4-chloro-*N*-(4-methoxybenzoyl)-3-fluorobenzamide (**II**), which is most likely formed via a [4 + 2] Diels-Alder cycloaddition as described above. Preparative chromatography was used to isolate the degradation product of interest for 1D and 2D NMR experiments (including ^{15}N NMR) to confirm its structure as **II**. The generality of this photo-degradation mechanism in thiazole-containing drugs is explored by LC–MS analysis of 4-(4-Chlorophenyl)thiazol-2-amine (**III**) and the antibacterial drug sulfathiazole (**IV**) after exposure to similar photo-degradation conditions. The ability to understand the structure of significant photo-degradation products of thiazoles allows for better decision-making and control during the drug development process.

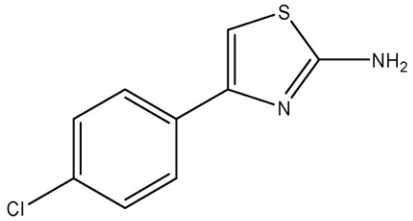
I: $C_{18}H_{13}ClFNO_3S$ (Exact Mass: 377.0289)



II: $C_{15}H_{11}ClFNO_3$ (Exact mass: 307.0412)



III: $C_9H_7ClN_2S$ (Exact Mass: 210.0018)



IV: $C_9H_9N_3O_2S_2$ (Exact Mass: 255.0136)

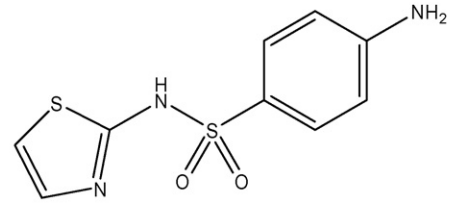


Fig. 1. Structures of the thiazoles examined in this study with their molecular formulae and exact masses. **I**: 4-(4-Chloro-3-fluorophenyl)-2-(4-ethoxyphenyl)-5-thiazole acetic acid (shown with its numbering scheme). **II**: The photo-degradation product of **I**. **III**: 4-(4-Chlorophenyl)thiazol-2-amine. **IV**: Sulfathiazole.

2. Experimental

2.1. Chemicals and reagents

{4-(4-Chloro-3-fluorophenyl)-2-[4-(methyloxy) phenyl]-1,3-thiazol-5-yl} acetic acid was synthesized by GSK. Further discussion of the synthetic preparation of this compound may be found in literature [11]. Samples of 4-(4-Chlorophenyl)thiazol-2-amine and sulfathiazole were purchased from Sigma–Aldrich (Milwaukee, WI, USA).

2.2. LC–MS and LC–MS/MS analysis

Samples were separated on an Agilent 1100 HPLC system (Agilent Technologies, Wilmington, DE, USA). Reversed-phase chromatographic separation was achieved using an Agilent Zorbax SB-Phenyl column (150 mm × 4.6 mm, 3.5 μm particle size) with a mobile phase A of 0.1% formic acid (FA) in water and mobile phase B of 0.1% FA in acetonitrile. The column temperature was kept at 35 °C. The mobile phase composition was linearly ramped to 95% B from 35% B over 10 min with a flow rate of 1 ml/min. UV detection was performed using an Agilent 1100 diode-array detector in the wavelength range of 190–400 nm. For H/D exchange experiments, D_2O was substituted for H_2O in the mobile phase.

The exact mass measurements and MS/MS experiments were performed on a Q-TOF Premier quadrupole orthogonal accel-