Journal of Photochemistry and Photobiology A: Chemistry 298 (2015) 55–61

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

journal homepage: www.elsevier.com/locate/jphotochem

Comparison of photoreactions of flutamide in acetonitrile and 2-propanol solvents in the absence of cage-forming compounds

Yurie Watanabe [a], Shuichi Fukuyoshi [a], Akifumi Oda [a,b,*]

[a] Faculty of Pharmacy, Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University, Kakuma-machi, Kanazawa, Ishikawa 920-1192, Japan
[b] Institute for Protein Research, Osaka University, 3-2 Yamadaoka, Suita, Osaka, Japan

ARTICLE INFO

Article history:
Received 13 July 2014
Received in revised form 2 October 2014
Accepted 10 October 2014
Available online 18 October 2014

ABSTRACT

Flutamide(2-methyl-*N*-[4-nitro-3-(trifluoromethylphenyl)]propanamide) is a widely used anti-cancer drug. It has been reported that photodermatosis is occasionally induced when an individual taking flutamide is exposed to sunlight. In this study, we found that flutamide undergoes different photoreactions in two different solvents: acetonitrile and 2-propanol. The photo-induced nitro–nitrite rearrangement was the predominant reaction when a flutamide solution in acetonitrile was irradiated with UV light, and phenoxy radicals and nitrogen monoxide were generated. The nitrogen monoxide recombined with the phenoxy radical at the *ortho* position and was oxidized by the oxygen dissolved in the acetonitrile. The final product was *o*-nitrophenol derivative. However, the photoreduction of the nitro group followed by solvolysis of the trifluoromethyl group was observed when a flutamide solution in 2-propanol was irradiated with UV light. The three fluorine atoms in the trifluoromethyl group were eliminated by being nucleophilically attacked by a solvent molecule, resulting in an ester bond with 2-propanol being formed.

1. Introduction

Aromatic nitro compounds are known to undergo various photoreactions, such as photosubstitution, photoredox reactions, photodissociation and photo-induced nitro–nitrite rearrangement [1–17]. Photosubstitution and photoredox reactions have been well investigated [1–11], but photodissociation and nitro–nitrite rearrangement have not yet been fully elucidated [10–17]. Nitro–nitrite rearrangement and photodissociation have low reaction efficiencies, and the short-lived intermediates of these reactions are difficult to observe. Chapman et al. proposed a nitro–nitrite rearrangement reaction mechanism [13] in which nitro–nitrite rearrangement arises from an $n\pi^*$ excited state provided by a specific configuration of the nitro group and aromatic ring. This configuration is the nitro group being held almost perpendicular to the plane of the aromatic ring, forming an oxaziridine ring. This three-membered ring immediately collapses and generates a nitrite group, which is converted into a hydroxyl group by the cleavage of the O—N bond.

Flutamide is an aromatic nitro compound that is widely used as a medical treatment [18–20]. Flutamide has been reported to undergo nitro–nitrite rearrangement [21–25]. It is used as an endocrine-system therapy for advanced prostate cancer, and it is converted into the more pharmacologically active metabolite 2-hydroxyflutamide by first pass metabolism in the human liver. The most deleterious side effect of flutamide is its hepatotoxicity, and the pathogenic mechanism related to the enzyme responsible for metabolizing flutamide has been investigated [26,27]. However, it has been reported that when a patient is exposed to sunlight, photodermatosis is a rare side effect of flutamide treatment, with cutaneous symptoms such as erythema, pruritus, and vitiligo appearing [28,29]. This side effect indicates that flutamide is potentially photoreactive within a living organism. Therefore, studying the photochemistry of flutamide is expected to be useful for elucidating the mechanism involved in the pathogenesis of photodermatosis. From a photochemical and photophysical viewpoint, it has been shown in some studies that flutamide photodecomposes at the nitro group when exposed to UV irradiation under various experimental conditions [21–25]. Sortino et al. conducted irradiation experiments in inhomogeneous aqueous solutions using cage-forming products such as β-cyclodextrin. Udagawa et al. also investigated the photochemistry of flutamide using the magnetic field effect and cage-forming compounds [25]. In their studies, irradiation experiments were

* Corresponding author at: Faculty of Pharmacy, Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University, Kakuma-machi, Kanazawa, Ishikawa 920-1192, Japan. Tel.: +81 762344485.
E-mail address: oda@p.kanazawa-u.ac.jp (A. Oda).

http://dx.doi.org/10.1016/j.jphotochem.2014.10.009
1010-6030/

conducted in aqueous solutions, such as a phosphate buffer, meaning that cage-forming compounds were required to increase the solubility of flutamide in the water. Both Sortino et al. and Udagawa et al. suggested that photoreduction reactions will occur only in inhomogeneous solutions, that is, photoreduction is a bimolecular reaction between flutamide and the cage-forming compound *via* a hydrogen abstraction reaction [23–25]. Moreover, Udagawa et al. found that the photoreduction process occurred in the excited triplet state of flutamide and suggested that only a nitro–nitrite rearrangement product was found when flutamide was irradiated in a phosphate buffer in the absence of a cage-forming compound. Likewise, Sortino et al. suggested that the nitro–nitrite rearrangement of flutamide is the only photoreaction that will occur in a homogeneous solution, such as in methanol or 2-propanol [21]. It is surprising that nitro–nitrite rearrangement occurred as a single photoreaction in a homogeneous solution because there are a wide variety of photoreactions of aromatic nitro compounds, including photoredox reactions, photosubstitution, and photodissociation [1–4]. The occurrences of these reactions of flutamide in different media have been insufficiently investigated; hence, the photoreactions of flutamide need to be further studied to obtain more experimental data. This is especially true for unimolecular photoreactions of flutamide in homogeneous solutions. Therefore, we investigated the photoreactions of flutamide under different experimental conditions than those used in previous studies, which used homogeneous solutions in organic solvents. We attempted to determine if various photoreactions could occur by conducting long hour irradiation experiments in highly reactive solutions.

Some medicines are known to be photoreactive, and some lose their pharmacological effect when exposed to light [30]. The photodecomposition of a photolabile drug can cause, as well as the loss of its pharmacological effect, the *in vivo* generation of free radicals. If a photoreactive drug can generate free radicals, the drug can possibly act as a hapten, causing immunogenicity, inducing photoallergic reactions, when bound to a carrier protein. As well as the nitro group, the trifluoromethyl group can photodecompose. In previous studies, it has been shown that some benzotrifluoride derivatives are photoreactive and that the fluorine atoms can easily be eliminated *via* solvolysis [31–33]. Furthermore, it has been found that fluorobenzene derivatives can undergo photodissociation to release fluorine atoms [34]. This is remarkable because the fluorine atom is generally unreactive in thermal dissociation reactions. The trifluoromethyl group is included in many pharmaceuticals, such as lansoprazole, efavirenz, and bicartamide because of its electron-withdrawing properties. These medicines have not yet been reported to induce photoallergies, but new medicines having benzotrifluoride structure or fluorobenzene structure that may be developed in the future might induce photoallergies. It is, therefore, important to study the photoreactivity of the trifluoromethyl group of flutamide. It would be difficult to study the induction of photodermatosis by flutamide *in vivo* because photodermatosis is a rare side effect, with a very low frequency of onset. Therefore, we attempted to clarify the photosensitivity of flutamide to UV *in vitro* before designing *in vivo* trials. The photoreaction of flutamide was examined in homogeneous solutions, such as methanol, 2-propanol and phosphate buffer, in previous studies. Those studies showed that nitro–nitrite rearrangement was the only route to the formation of photoproducts of flutamide in a homogeneous solution. In this study, we irradiated UV to flutamide in a homogeneous organic solvent, acetonitrile or 2-propanol, in which flutamide was more soluble than in water. The irradiation experiments were conducted under more reactive conditions compared with the preceding studies, the solutions being irradiated with UV for long periods in highly reactive solvents. To investigate the photoreactions of flutamide will allow us to predict the photoreactivity of flutamide *in vivo*.

2. Materials and methods

2.1. Chemicals

Flutamide was purchased from Tokyoukasei and used as-received. Flutamide solutions (at 1.0×10^{-3} M) were prepared in acetonitrile (HPLC grade; Nacalai Tesque) and 2-propanol (HPLC grade; Kantou Chemical). A 1.0×10^{-4} M solution in 2-propanol was also prepared. Deuterated acetonitrile and 2-propanol were prepared so that the origins of hydrogen atoms in the reaction products could be identified. A solution of product 4 (at 1×10^{-3} M) in 2-propanol was prepared so that the mechanism involved in the formation of product 5 could be investigated. Dissolved oxygen was removed from aerated sample solutions using the freeze–thaw method, in which a solution was frozen and thawed three times and then sealed from the atmosphere.

2.2. Irradiation experiments

Flutamide and product 4 were irradiated with UV produced using a super-high pressure mercury lamp (500 W, Ushio Inc.) and filtered through UV-29 (Toshiba) and cylindrical cell filter filled with distilled water to remove infrared light. Irradiation experiments using deuterated solvents were conducted using the same apparatus.

2.2.1. Analytical methods and identification of the photoproducts

The photoproducts were identified by comparing their gas chromatography–mass spectrometry (GC–MS) retention times and fragmentation patterns with those of authentic compounds. The authentic compounds matching products 3–6 were synthesized, and their structures was determinate by GC–MS and NMR spectroscopy. Product 2 was isolated and its structure identified by X-ray crystal structural analysis, ^{1}H NMR spectroscopy, ^{13}C NMR spectroscopy, IR spectroscopy, and GC–MS [35]. IR spectroscopy was used to identify the final compounds that were synthesized. ^{13}C NMR spectroscopy of products 2–4 showed that the carbon atom was coupled with the fluorine atom in each of them, and the coupling constants and chemical shifts were recorded. The IR spectra were obtained using a JASCO FT/IR-400 instrument. The GC–MS analyses were performed using a Hewlett-Packard HP6890/5793MSD instrument, the ^{1}H NMR and ^{13}C NMR spectra were obtained using a JOEL ECS-400 instrument, and the absorption spectra were recorded using a Hitachi U-2310 spectrophotometer.

2.3. Synthesis and characterization of the photoproducts

Photoproducts were produced in the sample solutions during the irradiation experiments. The chemical structures of the photoproducts were confirmed by synthesizing authentic matching compounds using the methods shown below.

2.3.1. Synthesis of 2-methyl-N-[4-hydroxy(3-trifluoromethyl) phenyl] propaneamide (product 1)

Product 1 was synthesized by following a previously published procedure [25].

2.3.2. Synthesis of 2-methyl-N-[3-(trifluoromethyl) phenyl] propaneamide (product 3)

2-Methylpropane chloride (0.08 mL, 0.75 mmol) was added dropwise into 3-aminobenzotrifluoride (0.06 mL, 0.5 mmol) in pyridine and the mixture was stirred for 1 h. 2-Methylpropane chloride (0.08 mL, 0.75 mmol) was then added dropwise to the