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Fluorescence derivatization method for sensitive chromatographic determination of zidovudine based on the Huisgen reaction

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ABSTRACT

A novel pre-column fluorescence derivatization method for chromatographic analysis of azide compounds was developed based on the Huisgen reaction, which is a specific cycloaddition reaction between an alkyne and an azide. We designed and synthesized a fluorescent alkyne, 2-(4-ethynylphenyl)-4,5-diphenyl-1*H*-imidazole (DIB-ET) as a reagent. DIB-ET has a lophine skeleton carrying an alkyne acting as fluorophore and reactive center, respectively. In order to evaluate the practicality of DIB-ET, a high-performance liquid chromatography with fluorescence detection method was developed for the determination of zidovudine as a model of azide compound. Zidovudine could be reacted with DIB-ET in the presence of copper(II) sulfate and L-ascorbic acid as catalysts, and the formed derivative was detected fluorometrically. The proposed method allows sensitive and selective determination of zidovudine in plasma samples with the detection limit of 0.28 ng mL^{-1} at a $S/N = 3$. Finally, the proposed method could be applied to determine the zidovudine concentration in rat plasma after administration of zidovudine without interference from biological components.

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1. Introduction

It is important to understand the pharmacokinetics of drugs that can cause beneficial or adverse side effects even in small amounts. Therefore, a sensitive analytical method is necessary to determine small amounts of drugs in biological samples. High-performance liquid chromatography (HPLC) with fluorescence detection is a powerful analytical technique to determine drugs owing to its high sensitivity and selectivity [1,2]. But, since most drugs do not possess native fluorescence, derivatization reagents have been frequently adopted in order to convert non-fluorescent drugs to strongly fluorescent derivatives. Up to now, various types of fluorescence derivatization reagents have been developed and applied for the determination of small amounts of drugs in biological samples [3–7]. However, fluorescence derivatization reagents can easily react with co-existing biological components and the resultant products can interfere with the detection of target drugs. In order to overcome this problem, we have been attempted to develop a novel fluorescence reagent that can react with target drug selectively even in the presence of biological components, based on specific chemical reactions. We so far developed a fluorescent aryl boronic acid, 4-(4,5-diphenyl-1*H*-imidazol-2-yl)phenylboronic acid (DPA, a lophine derivative) as a fluorescence derivatization reagent for aryl halides based on the Suzuki coupling reaction, which is a cross-coupling reaction between aryl halides and aryl boronic acids [8–11]. It was found that DPA could react selectively with aryl halide drugs such as haloperidol in the presence of biological components. Also, we recently reported that a lophine based fluorescent aryl iodide, 4-(4,5-diphenyl-1*H*-imidazol-2-yl)iodobenzene, could be used as a specific fluorescence derivatization reagent for alprenolol that has a terminal double bond moiety based on the Mizoroki–Heck coupling reaction, which is a reaction between aryl halides and terminal double bonds [12]. As an expansion of the analytical strategy, we attempted to develop a new type of specific fluorescence derivatization reagent for azide group based on the Huisgen reaction.

The Huisgen reaction [13–16] is a specific cycloaddition reaction between an alkyne and an azide in the presence of copper(I) as a catalyst to form stable five-membered triazole ring [17]. The Huisgen reaction is known as the most representative reaction of click chemistry and is frequently used to build functional molecules in various research fields. Owing to the high specificity of the Huisgen reaction, we considered that fluorescent alkyne and fluorescent azide could be used as specific derivatization

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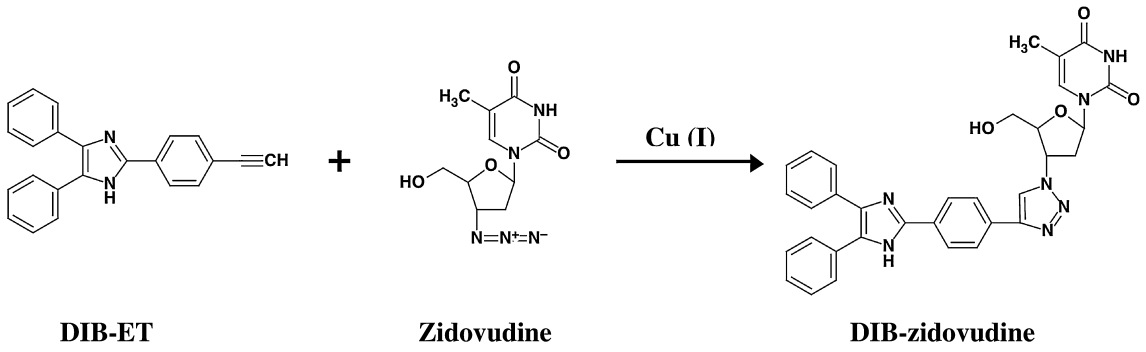

Fig. 1. Fluorescence derivatization reaction of zidovudine with DIB-ET based on the Huisgen reaction.

reagent for azide group and alkyne moiety, respectively. The Huisgen reaction proceeds under mild conditions even in the presence of water, while many general derivatization reactions often require hard conditions such as high temperature and long reaction time, and are likely inhibited by water. Therefore, fluorescence derivatization reaction based on the Huisgen reaction should be suitable for biomedical analysis. From these aspects, we designed and synthesized a fluorescent alkyne, 2-(4-ethynylphenyl)-4,5-diphenyl-1*H*-imidazole (DIB-ET) as a specific fluorescence derivatization reagent for azide group (Fig. 1). DIB-ET has a lophine skeleton carrying an alkyne acting as fluorophore and reactive center, respectively. In the present study, we applied DIB-ET to the development of determination method for zidovudine as a model of azide compound. Zidovudine is an antiretroviral drug used for the treatment of human immunodeficiency virus (HIV) infection [18,19]. Since the adverse side effects of zidovudine, such as lactic acidosis or myelosuppression were reported [20], the sensitive and selective determination method for zidovudine should be useful to facilitate the safe dosing of zidovudine. We confirmed that zidovudine could be converted to fluorescent derivative (DIB-zidovudine) after reaction with DIB-ET (Fig. 1), and DIB-zidovudine could be sensitively determined by HPLC with fluorescence detection. Furthermore, the developed method was successfully applied to the determination of zidovudine in rat plasma after administration without any interference from biological components.

2. Experimental

2.1. Materials and reagents

Zidovudine was purchased from Tokyo Chemical Industries (Tokyo, Japan). Copper(II) sulfate, L-ascorbic acid, benzil and ammonium acetate were obtained from Nacalai Tesque (Osaka, Japan). 4-Ethynylbenzaldehyde was from Sigma (St. Louis, MO, USA). Cellulose acetate membrane filter (0.45 μM) was from Advantec (Tokyo). Wistar male rats were obtained from Kyudo Experimental Animal Laboratory (Saga, Japan). DIB-ET and DIB-zidovudine were synthesized as described in later sections. Water was distilled and passed through a Pure Line WL21P system (Yamato, Tokyo, Japan). All other chemicals were of the highest purity and quality available. Stock solution of zidovudine was prepared in methanol and stored at 4 °C. DIB-ET, copper(II) sulfate and L-ascorbic acid were dissolved in methanol just before use.

2.2. Equipment

The HPLC system consisted of two Shimadzu LC-10AT pumps (Kyoto, Japan), a Shimadzu RF-20Axs fluorescence detector, a Rheodyne (Cotati, CA, USA) 7125 injector with a 20-μL loop and Chromato-Pro chromatography data acquisition system (Run Time Corporation, Kanagawa, Japan).

Fluorescence spectra were measured with a Shimadzu RF-1500 spectrofluorophotometer. Mass spectral data were obtained with a JEOL JMS-700N spectrometer (Tokyo, Japan). Elemental analyses were performed on a Perkin Elmer 2400II (Norwalk, CT, USA). Melting points were measured with a Yanagimoto MP-53 melting point apparatus (Kyoto, Japan).

2.3. HPLC conditions

Chromatographic separation was performed on a Cosmosil 5C18-AR-II (250 × 4.6 mm i.d., Nacalai Tesque, Osaka, Japan) column with a gradient elution program using solvent A (acetonitrile-5 mM Tris–HCl buffer (pH 7.4) (50:50, v/v%)) and solvent B (acetonitrile). The gradient program was programed as follows: 0% B (0–9.5 min), 0% B to 100% B linearly (9.5–10.0 min), and 100% B (10.0–19.0 min). The flow-rate was set at 1.0 mL min^{-1} at ambient temperature. The excitation and emission wavelength were set at 310 nm and 400 nm, respectively.

2.4. Synthesis of DIB-ET

DIB-ET was synthesized according to the previous papers [12,21]. Benzil (157.5 mg, 0.75 mmol), ammonium acetate (500 mg, 6.5 mmol) and 4-ethynylbenzaldehyde (97.5 mg, 0.81 mmol) were dissolved in 1.5 mL of acetic acid. This mixture was heated at 90 °C for 8 h. After cooling to room temperature, the mixture was poured into cold water. The resultant precipitate was obtained as yellow crystals; yield: 191 mg, 78%, mp: 264 °C. Elemental analysis; calculated for $C_{23}H_{16}N_2$: C, 86.22%; H, 5.03%, N, 8.74%, found: C, 86.17%, H, 5.00%, N, 8.82%. FAB-MS (*m*/*z*) calculated: 321 $[M+H]^+$, found 321.

2.5. Synthesis of authentic DIB-zidovudine

DIB-ET (30.0 mg, 94 μmol), zidovudine (24.9 mg, 94 μmol), copper(II) sulfate (224.7 mg, 900 μmol) and L-ascorbic acid (159 mg, 900 μmol) were dissolved in 10 mL of tetrahydrofuran–water (50:50, v/v%). After heating at 50 °C for 2 h, the solution was evaporated and filtered. The resulting precipitate was washed with 10 mL of acetonitrile and water to give DIB-zidovudine as yellow crystals; yield: 28 mg, 51%, mp: 248–250 °C. Elemental analysis; calculated for $C_{33}H_{29}N_7O_4$: C, 67.45%; H, 4.97%, N, 16.68%, found: C, 67.57%, H, 5.22%, N, 16.89%. FAB-MS (*m*/*z*) calculated: 588 $[M+H]^+$, found 588.

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