Antibacterial and Antifungal Activities of Tryptanthrin Derivatives

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Tryptanthrin (T) and 13 of its derivatives (T2NH₂, T2Cl, T2Br, T2NO₂, T8OMe, T8Me, T8F, T8Br, T8NO₂, T2NH₂8OMe, T2NH₂8NO₂, T2Br8Br, and T2NO₂8NO₂) were synthesized, and their antimicrobial activities against a gram-positive bacterium (methicillin-resistant *Staphylococcus aureus*, MRSA) and a fungus (*Malassezia furfur*) were investigated. The antibacterial and antifungal activities were influenced by the substituents on tryptanthrin, with halogen-substituted tryptanthrin derivatives (T2Cl, T2Br, T8F, T8Br, and T2Br8Br) showing the highest potency against MRSA and *M. furfur*.

Key words: Antibacterial activity, Tryptanthrin, MRSA, *Malassezia furfur*

1. INTRODUCTION

Tryptanthrin (T, Fig. 1) is a weakly basic alkaloid found in a number of plant species [1].

Figure 1. Structure of tryptanthrin (T).

This compound possesses antibacterial and antifungal activities against various pathogenic bacteria and fungi [2]. In particular, the antifungal activity against *Malassezia furfur*, which is a causative fungus of atopic dermatitis, is important [3]. Tryptanthrin is also effective for the treatment of contact dermatitis (delayed-type allergy) [4]. Therefore, its use as a therapeutic drug for conditions such as atopic dermatitis or in cosmetics is anticipated. However, only a few reports describe the properties of chemosynthetic tryptanthrin derivatives that are not found in nature. In particular, there are few studies regarding the antifungal activities of tryptanthrin derivatives against *M. furfur* [3]. Therefore, we synthesized 13 different tryptanthrin derivatives (T2NH₂, T2Cl, T2Br, T2NO₂, T8OMe, T8Me, T8F, T8Br, T8NO₂, T2NH₂8OMe, T2NH₂8NO₂, T2Br8Br, and T2NO₂8NO₂) and investigated their antibacterial and antifungal properties against a gram-positive bacterium (methicillin-resistant *Staphylococcus aureus*, MRSA) and a fungus (*M. furfur*). The structures are shown in Fig. 2.

Here we report the results of a detailed study of the influence of various substituents on the antibacterial and antifungal activities.

2. EXPERIMENTAL

2.1 Synthesis

Tryptanthrin (T) and its 13 derivatives (T2NH₂,

Figure 2. Structures of the 13 tryptanthrin derivatives.

T2Cl, T2Br, T2NO₂, T8OMe, T8Me, T8F, T8Br, T8NO₂, T2NH₂8OMe, T2NH₂8NO₂, T2Br8Br, and T2NO₂8NO₂) were synthesized by the reaction of their corresponding isatin and isatoic anhydride derivatives (Scheme 1) [2].

Scheme 1

T: $R_1 = H$, $R_2 = H$; **T2NH₂:** $R_1 = H$, $R_2 = NH_2$; **T2Cl:** $R_1 = H$, $R_2 = Cl$; **T2Br:** $R_1 = H$, $R_2 = Br$; **T2NO₂:** $R_1 = H$, $R_2 = NO_2$; **T8OMe:** $R_1 = OCH_3$, $R_2 = H$; **T8Me:** $R_1 = CH_3$, $R_2 = H$; **T8F:** $R_1 = F$, $R_2 = H$; **T8Br:** $R_1 = Br$, $R_2 = H$; **T8NO₂:** $R_1 = NO_2$, $R_2 = H$; **T2NH₂8OMe:** $R_1 = OCH_3$, $R_2 = NH_2$; **T2NH₂8NO₂:** $R_1 = NO_2$, $R_2 = NH_2$; **T2Br8Br:** $R_1 = R_2 = Br$; **T2NO₂8NO₂:** $R_1 = R_2 = NO_2$.

We describe the preparation of **T2NH₂**. Other tryptanthrin derivatives were similarly prepared. Under a flow of dry nitrogen, 1.0 g (6.8 mmol) of isatin in 15 mL of dry dimethylformamide (DMF) was added over a 15-min period to 0.16 g (6.7 mmol) of NaH with stirring. To the resulting deep purple liquid, 1.3 g (7.3 mmol) of 5-aminoisatin anhydride in 15 mL of dry DMF was added with ice cooling over a 30-min period. The reaction mixture was stirred overnight at room temperature and then quenched with 15 mL of methanol. The resulting mixture was diluted with 60 mL of chloroform and washed once with water. The aqueous layer was extracted three times with chloroform, and the combined organic layers were dried (anhydrous sodium sulfate) and concentrated. Crystallization from acetone afforded the pure compound **T2NH₂** (1.5 g) in 84% yield.

T2NH₂: 1H NMR (400 MHz, $CDCl_3$, Me_4Si) δ 4.34 (2H, s), 7.08–7.92 (6H, m), 8.62 (1H, d, $J = 7.6$ Hz); FAB-MS: m/z 264 ($[M+H]^+$).

The other analogs were prepared in the same manner. The mass spectra, fast atom bombardment mass analysis (FAB-MS), or electrospray ionization mass analysis (ESI-MS) showed the corresponding molecular ion peaks, and the chemical shifts and the integral ratios of protons were appropriate in the nuclear magnetic resonance analysis (1H NMR). The physical data for **T**, **T2Cl**, **T8Me**, **T8Br**, and **T8NO₂** were identical with the literature values [2, 5, 6, 7].

T2Br: 1H NMR (500 MHz, $CDCl_3$, Me_4Si) δ 7.45 (1H, ddd, $J = 7.5$ Hz, $J = 7.5$ Hz, $J = 1.0$ Hz), 7.81 (1H, ddd, $J = 8.0$ Hz, $J = 7.5$ Hz, $J = 1.0$ Hz), 7.85–8.00 (3H, m), 8.57 (1H, d, $J = 2.3$ Hz), 8.62 (1H, d, $J = 8.0$ Hz); ESI-MS: m/z 327 ($[M+H]^+$).

T2NO₂: 1H NMR (500 MHz, $CDCl_3$, Me_4Si) δ 7.50 (1H, t, $J = 7.0$ Hz), 7.85 (1H, ddd, $J = 8.0$ Hz, $J = 7.0$ Hz, $J = 1.0$ Hz), 7.96 (1H, d, $J = 7.0$ Hz), 8.60–8.70 (2H, m), 9.29 (1H, d, $J = 2.6$ Hz); ESI-MS: m/z 294 ($[M+H]^+$).

T8OMe: 1H NMR (400 MHz, $CDCl_3$, Me_4Si) δ 3.90 (3H, s), 7.20–7.40 (3H, m), 7.66 (1H, t, $J = 7.5$ Hz), 7.84 (1H, t, $J = 7.5$ Hz), 8.02 (1H, d, $J = 8.5$ Hz), 8.43 (1H, d, $J = 7.5$ Hz), 8.51 (1H, d, $J = 8.5$ Hz); FAB-MS: m/z 279 ($[M+H]^+$).

T8F: 1H NMR (400 MHz, $CDCl_3$, Me_4Si) δ 7.45–7.92 (4H, m), 8.02 (1H, d, $J = 8.0$ Hz), 8.42 (1H, dd, $J = 8.0$, 1.5 Hz), 8.63 (1H, dd, $J = 8.5$, 4.0 Hz); FAB-MS: m/z 267 ($[M+H]^+$).

T2NH₂8OMe: 1H NMR (500 MHz, $CDCl_3$, Me_4Si) δ 3.89 (3H, s), 7.09 (1H, dd, $J = 8.5$ Hz, $J = 2.6$ Hz), 7.28 (1H, d, $J = 2.6$ Hz), 7.36 (1H, d, $J = 2.6$ Hz), 7.56 (1H, d,

$J = 2.6$ Hz), 7.81 (1H, d, $J = 8.5$ Hz), 8.5 (1H, d, $J = 8.5$ Hz); FAB-MS: m/z 294 ($[M+H]^+$).

T2NH₂8NO₂: 1H NMR (500 MHz, dimethyl sulfoxide ($DMSO$)- d_6 , Me_4Si) δ 6.51 (2H, s), 7.14 (1H, dd, $J = 8.5$ Hz, $J = 2.6$ Hz), 7.43 (1H, d, $J = 2.6$ Hz), 7.67 (1H, d, $J = 8.5$ Hz), 8.48–8.50 (1H, m), 8.67–8.69 (2H, m); FAB-MS: m/z 309 ($[M+H]^+$).

T2Br8Br: 1H NMR (500 MHz, $CDCl_3$, Me_4Si) δ 7.85–8.05 (4H, m), 8.52 (1H, d, $J = 8.5$ Hz), 8.56 (1H, d, $J = 2.3$ Hz); ESI-MS: m/z 403 ($[M+H]^+$).

T2NO₂8NO₂: 1H NMR (500 MHz, $CDCl_3$, Me_4Si) δ 8.24 (1H, d, $J = 8.5$ Hz), 8.65–8.82 (3H, m), 8.88 (1H, d, $J = 8.5$ Hz), 8.30 (1H, d, $J = 2.5$ Hz); ESI-MS: m/z 338 ($[M]^+$).

2.2 Antibacterial and antifungal activity test

Antibacterial and antifungal activities were investigated in vitro on a gram-positive bacterium (MRSA) and a fungus (*M. furfur*) with the agar plate dilution method described by the Japanese Society of Chemotherapy [8, 9, 10, 11]. A bacterial culture (developed overnight for MRSA or over 2–3 days for *M. furfur*) was diluted with the Mueller–Hinton broth (21 g/L in distilled water; Becton, Dickinson and Company) for MRSA or malt extract broth (36 g/L in distilled water; Wako Pure Chemical Industries, Ltd.) containing Tween-40 (10 g/L in distilled water; Wako Pure Chemical Industries, Ltd.), monoolein (2 g/L in distilled water; Tokyo Chemical Industry Co., Ltd.), oxgall (20 g/L in distilled water; Wako Pure Chemical Industries, Ltd.), peptone (6 g/L in distilled water; Wako Pure Chemical Industries, Ltd.), and glycerol (2 g/L in distilled water; Wako Pure Chemical Industries, Ltd.) for *M. furfur* to a density of 1.0×10^6 colony-forming units (CFU)/mL. The compounds for testing (**T**, **T2NH₂**, **T2Cl**, **T2Br**, **T2NO₂**, **T8OMe**, **T8Me**, **T8F**, **T8Br**, **T8NO₂**, **T2NH₂8OMe**, **T2NH₂8NO₂**, **T2Br8Br**, and **T2NO₂8NO₂**) were dissolved in DMSO (Wako Pure Chemical Industries, Ltd.) and diluted with the Mueller–Hinton broth to a concentration of 0.1–100 μ g/mL for MRSA or with the malt extract broth containing Tween-40, monoolein, oxgall, peptone, and glycerol to a concentration of 4–160 μ g/mL for *M. furfur*. Then, each Petri dish was inoculated with the bacterial or fungal suspension and incubated at 37°C for 24 h (for MRSA) or 2–4 days (for *M. furfur*). The lowest concentration at which there was no visible growth was taken as the minimum inhibitory concentration (MIC).

3. RESULTS AND DISCUSSION

The antibacterial activities of tryptanthrin (**T**) and its 13 derivatives (**T2NH₂**, **T2Cl**, **T2Br**, **T2NO₂**, **T8OMe**, **T8Me**, **T8F**, **T8Br**, **T8NO₂**, **T2NH₂8OMe**, **T2NH₂8NO₂**, **T2Br8Br**, and **T2NO₂8NO₂**) were investigated on a gram-positive bacterium (MRSA) and a fungus (*M. furfur*) in culture. MICs for MRSA and *M. furfur* are shown in Table 1. If the MIC value is small, antibacterial and antifungal activities are enhanced. The MIC at which a molecule exerted antimicrobial activity was defined as less than 800 μ g/mL in accordance with the criterion set forth by the Japanese Society of Chemotherapy [9, 10, 11]. Most tryptanthrin derivatives showed very high antibacterial

activities for MRSA and antifungal activities for *M. furfur*. Table 1. Antibacterial activity (MIC) of tryptanthrin (T) and its 13 derivatives (T2NH₂, T2Cl, T2Br, T2NO₂, T8OMe, T8Me, T8F, T8Br, T8NO₂, T2NH₂8OMe, T2NH₂8NO₂, T2Br8Br, and T2NO₂8NO₂) against MRSA and *M. furfur*.

	MIC/ $\mu\text{g mL}^{-1}$	
	MRSA	<i>M. furfur</i>
T	0.5	4
T2NH ₂	2.0	20
T2Cl	0.1	4
T2Br	0.3	4
T2NO ₂	>100	>160
T8OMe	2.5	>120
T8Me	0.5	>120
T8F	0.1	1
T8Br	0.1	2.5
T8NO ₂	0.5	4
T2NH ₂ 8OMe	2.0	20
T2NH ₂ 8NO ₂	0.5	>160
T2Br8Br	0.6	4
T2NO ₂ 8NO ₂	>100	>160

MIC = minimum inhibitory concentration in $\mu\text{g/mL}$.

furfur. The antibacterial activity for MRSA was higher than the antifungal activity for *M. furfur*. The reason for the greater potency against the gram-positive bacterium (MRSA) compared with that of the fungus (*M. furfur*) can be attributed to structural differences between the two species. The MIC order for MRSA was T2Cl = T8F = T8Br < T2Br < T = T8Me = T8NO₂ = T2NH₂8NO₂ < T2Br8Br < T2NH₂ = T2NH₂8NO₂ < T8OMe < T2NO₂, T2NO₂8NO₂, and for *M. furfur*, it was T8F < T2Cl = T2Br = T8NO₂ = T2Br8Br < T < T2NH₂ = T2NH₂8OMe < T8OMe, T8Me, T2NO₂, T8Br, T2NH₂8NO₂, T2NO₂8NO₂. For both MRSA and *M. furfur*, antibacterial and antifungal activities of halogen-substituted tryptanthrin derivatives were higher than those of the other tryptanthrin derivatives. Considering the overall efficacy, T8F was the most potent of all the tested compounds with an MIC of 0.1 mg/mL for MRSA and 1 mg/mL for *M. furfur*. In contrast, antibacterial and antifungal activities of T2NO₂ and T2NO₂8NO₂, which have a nitro group at position 2 of tryptanthrin, were the lowest. MICs of T2NO₂ and T2NO₂8NO₂ were >100 $\mu\text{g/mL}$ for MRSA and >160 $\mu\text{g/mL}$ for *M. furfur*. Antibacterial and antifungal

activities could be related to electrophilicity of carbonyl carbon of five-membered ring by results of theoretical calculations [12]. Further studies of mechanism for antibacterial and antifungal activities are now in progress. Miconazole nitrate is currently used as a therapeutic drug for treating atopic dermatitis. Its MIC for *M. furfur*, which is a causative fungus of atopic dermatitis, is ca. 25 $\mu\text{g/mL}$. MICs of T and halogen-substituted tryptanthrin derivatives T2Cl, T2Br, T8F, and T2Br8Br for *M. furfur* ranged from 1 to 4 $\mu\text{g/mL}$, and their antifungal activities were more than six times that of miconazole nitrate. T8F was found to be particularly effective. These results suggest the potential of halogen-substituted tryptanthrin derivatives as therapeutic agents for the treatment of atopic dermatitis.

4. CONCLUSIONS

Tryptanthrin (T) and its 13 derivatives (T2NH₂, T2Cl, T2Br, T2NO₂, T8OMe, T8Me, T8F, T8Br, T8NO₂, T2NH₂8OMe, T2NH₂8NO₂, T2Br8Br, and T2NO₂8NO₂) were synthesized, and their antibacterial properties for a gram-positive bacterium (MRSA) and antifungal properties for a fungus (*M. furfur*) were investigated. Antibacterial and antifungal activities of halogen-substituted tryptanthrin derivatives against MRSA and *M. furfur* were exceptional. The application of these compounds as antibacterial and antifungal agents or antibacterial and antifungal materials will be further investigated.

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