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Synthesis of hydroxylated tryptanthrins as possible metabolites and characterization

Abstract: A series of dihydroxytryptanthrins was prepared by the reaction of a suitably substituted isatoic anhydride and isatin as possible metabolites of tryptanthrin. A high-performance liquid chromatography analysis of the synthetic compounds confirmed that 8,9-dihydroxytryptanthrin is the metabolite isolated from rat liver cytosolic metabolites. Although tryptanthrin shows strong cytotoxicity against human cancer cell lines, none of the hydroxylated tryptanthrins exhibit any significant cytotoxicity against selected human cancer cell lines up to 50 μM.

Keywords: cytochrome P450; hydroxytryptanthrin; metabolite; tryptanthrin.

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Introduction

Tryptanthrin (**1** in Scheme 1, 6,12-dihydro-6,12-dioxoindolo[2,1-*b*]quinazoline) is an indoloquinazolinone alkaloid first obtained by the sublimation of powdered natural indigo under reduced pressure [1]. Friedländer and Roschdestwensky [2] prepared tryptanthrin chemically from isatin chloride and anthranilic acid and proposed its structure. Tryptanthrin was also isolated from the culture of yeast *Candida lipolytica* [3] and later from higher plant sources, *Couroupita guianensis* [4, 5], as well as from fungus, *Schizophyllum commune* [6].

Tryptanthrin shows a variety of biological properties including antimicrobial [3, 7–10] and antifungal activity [7]. The inhibitory activities of tryptanthrin against COX-2 [11], 5-LOX [12], and prostaglandin E(2) expressions at the cellular level [13] led a new vista for the development of new anti-inflammatory agents. Inhibitory activities on hepatocyte growth factor in human fibroblasts [14] and the multidrug resistance gene MDR1 in breast cancer cells [15] and cytotoxicity against selected human cancer cell lines (IC_{50}=10 μM for HT-1376) [6, 16] and antitumor activity [17] of tryptanthrin have also been reported. Such intriguing properties have led to continuous efforts not only to search for similar compounds in other plant sources but also to trigger the development of new methods for total synthesis of tryptanthrin, as recently reviewed [18].

Our early studies on the two identifiable metabolites from the culture of cytosolic cytochrome P_{450} (CYP)-mediated metabolism of tryptanthrin have revealed their masses of 264 and 280. The MS^2 spectra of protonated tryptanthrin and metabolite (M1) have led us to deduce the position of monohydroxylation as being on the aromatic ring of the indole moiety, and the structure was identified as 8-hydroxytryptanthrin by comparison with a chemically synthesized authentic compound [19]. However, the metabolite (M2) with a mass of 280 has not been characterized as yet. In this study, we synthesized a series of dihydroxytryptanthrins to identify cytosolic metabolite(s) with a mass of 280 and evaluated their biological properties, especially the cytotoxicity against selected human cancer cell lines.

Results and discussion

On the basis of the first metabolite, 8-hydroxytryptanthrin (m/z 264), the isolated but as yet unidentified metabolite with a mass of 280 could be a dihydroxytryptanthrin. Investigation of the first and second metabolites of the related quinazolin-4(3*H*)-one alkaloid rutaecarpine [20–22] and electronic aspects of indole system versus quinazolin-4(3*H*)-one moiety indicates that the benzene ring of the indole moiety would be more susceptible to the cytochrome P_{450} (CYP)-mediated oxidative metabolic process. Thus, the first metabolites of tryptanthrin would be 8-hydroxytryptanthrin and 8,9-dihydroxytryptanthrin (**2a** in Scheme 1). The subsequent CYP-mediated oxidative metabolism of the first metabolite, 8-hydroxytryptanthrin, would lead to the formation of 2,8-dihydroxytryptanthrin (**2b**) as the second metabolite (M2).

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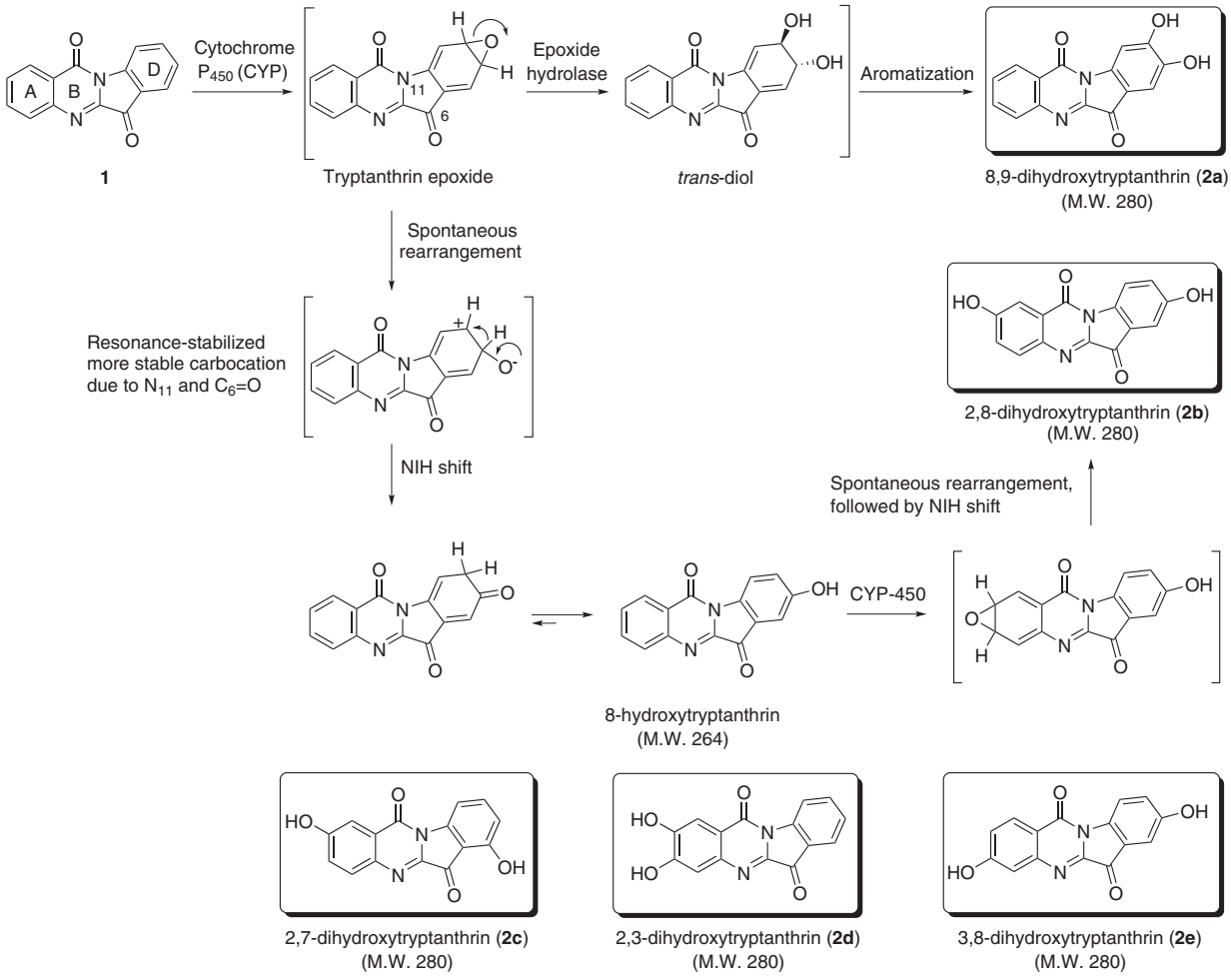
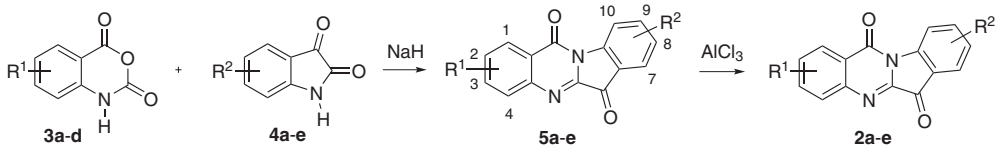


Scheme 1: Proposed mechanisms for metabolites of tryptanthrin.

The isolation of 7-hydroxytryptanthrin (known as phaitanthrin C) [16] from natural sources would point out to 2,7-dihydroxytrptanthrin (**2c**) as an additionally possible candidate for M2. However, the same metabolic process on the less reactive quinazolin-4(3*H*)-one ring (ring A) could not be excluded to lead to 2,3-dihydroxytryptanthrin (**2d**) and less favored 3,8-dihydroxytryptanthrin (**2e**) as possible metabolites. We thus reasoned that the dihydroxytryptanthrins, **2a**–**2e**, could be possible candidates for the as yet unidentified metabolite with a mass of 280 isolated from rat liver microsomes incubated in the presence of an NADPH-generating system.

Syntheses of hydroxylated tryptanthrins are straightforward by using methods previously described in the literature [23], as shown in Scheme 2. Either reactions of suitable methoxyisatins and isatoic anhydride or reactions



3a: R^1 = H
3b: R^1 = 6-OMe
3c: R^1 = 7-OMe
3d: R^1 = 6,7-diOMe

4a: R^1 = 5,6-di-OMe
4b: R^1 = 5-OMe
4c: R^1 = 6-OMe
4d: R^1 = 4-OMe
4e: R^1 = H

5a: R^1 = H; R^2 = 8,9-diOMe
5b: R^1 = 2-OMe; R^2 = 8-OMe
5c: R^1 = 2-OMe; R^2 = 7-OMe
5d: R^1 = 2,3-diOMe; R^2 = H
5e: R^1 = 3-OMe; R^2 = 8-OMe

2a: R^1 = H; R^2 = 8,9-diOH
2b: R^1 = 2-OH; R^2 = 8-OH
2c: R^1 = 2-OH; R^2 = 7-OH
2d: R^1 = 2,3-diOH; R^2 = H
2e: R^1 = 3-OH; R^2 = 8-OH

Scheme 2: Synthesis of hydroxylated tryptanthrins.

of isatin and suitable methoxyisatoic anhydrides afford tryptanthrins with hydroxyl group(s) on only one of the two benzene rings, whereas the use of methoxyisatins and methoxyisatoic anhydrides provide hydroxytryptanthrins with hydroxyl groups on the two benzene rings.

Reactions of isatoic anhydride (**3a**) with 5,6-dimethoxyisatin (**4a**) afforded 8,9-dimethoxytryptanthrin (**5a**) in 35% yield, whereas reactions of 6-methoxyisatoic anhydride (**3b**) with 5-methoxyisatin (**4b**) and 4-methoxyisatin (**4d**) afforded 2,8-dimethoxytryptanthrin (**5b**) and 2,7-dimethoxytryptanthrin (**5c**) in 16% and 51% yields, respectively. Similarly, the reactions of 7-methoxyisatoic anhydride (**3c**) with **4b** and 6,7-dimethoxyisatoic anhydride (**3d**) with isatin (**4e**) afforded 3,8-dimethoxytryptanthrin (**5e**) and 2,3-dimethoxytryptanthrin (**5d**) in 52% and 56% yields, respectively. The $AlCl_3$-mediated *O*-demethylation of **5** afforded the corresponding hydroxytryptanthrins (**2a–e**) in 80%–86% yield. The structures of these products were confirmed by spectroscopic methods. Although most of the hydroxytryptanthrins obtained show poor solubility in common organic solvents, the analytically pure samples were easily obtained by using preparative TLC eluting with methanol.

To identify the metabolite, the natural metabolites and the synthetic compounds **2** were analyzed using high-performance liquid chromatography (HPLC). The retention time of metabolite M2 perfectly matches that of 8,9-dihydroxytryptanthrin (**2a**) at 6.82 min, whereas the retention times of 2,7-, 2,8-, and 3,8-dihydroxytryptanthrin are quite different at 7.38, 7.38, and 7.63 min, respectively. In addition, the mass spectral fragmentation pattern of the metabolite is identical to that of the synthetic compound: m/z 281.1 (M+H), 253.1 [(M+H)-CO], 178.2, and 162.1.

In vitro cytotoxicities of tryptanthrin (**7**) against selected human cancer lines, including ductal breast epithelial tumor cell line (T47D), colon rectal adrenocarcinoma tumor (HCT15), prostate tumor (DU145), and embryonic kidney 293 cells (HEK293), have been reported [24], and the activities are comparable with those of camptothecin. In this work, cytotoxicities of the hydroxytryptanthrins against the previously selected cancer cell lines were evaluated. However, none of the compounds showed any significant cytotoxic activity up to 50 μM.

Conclusions

A series of dihydroxytryptanthrins was prepared by the reaction of suitably substituted isatoic anhydride and isatin as possible second metabolites of tryptanthrin. HPLC analyses of the synthetic compounds confirm 8,9-dihydroxytryptanthrin as the second metabolite isolated from rat liver cytosolic metabolites. The hydroxylated tryptanthrins did not show any significant cytotoxicity against selected human cancer cell lines up to 50 μM.

Experimental

Melting points were determined using a Fischer-Jones melting points apparatus and are not corrected. NMR spectra were measured using a Bruker-250 spectrometer operating at 250 MHz for ^{1}H NMR and 62.5 MHz for ^{13}C NMR. Chemicals and solvents were commercial reagent grade and used without further purification. Electrospray ionization (ESI) mass spectrometry (MS) experiments were performed on an LCQ advantage-trap mass spectrometer (Thermo Finnigan, San Jose, CA, USA). The prerequisite isatoic anhydrides (**3**) were prepared as previously reported in the literature by either oxidation of isatin with CrO_3 [25] or reaction of suitably substituted anthranilic acid with triphosgene [26]. 4-Methoxyisatin (**4b**) and 6-methoxyisatin (**4d**) were prepared by Friedel-Crafts acylation of 2-(hydroxyimino)-*N*-(3-methoxyphenyl)acetamide [27]. In the HPLC separations, an Intertsil® ODS-3 column (5 μm, 4.6×150 mm, GL Sciences Inc., Tokyo, Japan) with a Phenomenex® SecurityGuard™ cartridge C18 (3.0×4.0 mm, Torrance, CA, USA) was used, eluting with a pH 4.0 buffer (90% acetonitrile with 10% 20 mM ammonium formate; the flow rate was 1.0 mL/min.

8,9-Dimethoxytryptanthrin (5a) NaH (170 mg, 7.08 mmol) was added to a mixture of isatoic anhydride (**3a**, 1.16 g, 7.11 mmol) and 5,6-dimethoxyisatin (**4a**, 1.45 g, 7.00 mmol) in DMF (20 mL). The mixture was heated at 65°C for 2 h then poured into ice water and extracted with CH_2Cl_2 (3×30 mL). The solvent was evaporated, and the resulting solid was washed with CH_3OH to afford the desired 2,3-dimethoxytryptanthrin (**5a**, 750 mg, 35%) as a brown solid; mp 283–285°C; ^{1}H NMR ($CDCl_3$): δ 8.37 (1H, dd, *J* = 7.8, 1.4 Hz, H1), 8.18 (1H, s, H10), 8.00 (1H, dd, *J* = 8.0, 0.8 Hz, H4), 7.82 (1H, 2t, *J* = 7.8, 1.5 Hz, H2), 7.64 (1H, 2t, *J* = 7.8, 1.2 Hz, H3), 7.31 (1H, s, H7), 4.09 (3H, s), 3.93 (3H, s); ^{13}C NMR ($CDCl_3$): δ 180.8, 158.1, 157.8, 148.6, 146.9, 143.8, 135.3, 132.0, 130.8, 130.3, 127.5, 123.8, 114.6, 106.3, 101.3, 57.3, 56.6; MS (ESI): m/z 309 $[M + H]^+$. Anal. Calcd for $C_{17}H_{12}N_2O_4$: C, 66.23; H, 3.92; N, 9.09. Found: C, 66.15; H, 3.91; N, 9.11.

2,8-Dimethoxytryptanthrin (5b) CrO_3 (2 g) was added to a mixture of 5-methoxyisatin (**4c**, 690 mg, 3.90 mmol) in glacial HOAc (10 mL) and acetic anhydride (10 mL) in portions at 80°C. The mixture was heated for10 min then cooled to room temperature, and the resultant solid was filtered. The ^{1}H NMR spectrum of the solid showed a mixture of starting 5-methoxyisatin (**4c**) and desired 6-methoxyisatoic anhydride (**3b**) in a 1:1.5 ratio. This mixture was subjected to the reaction described previously for **5a** to afford the desired 2,8-dimethoxytryptanthrin (**5c**, 58 mg, 16%) as a brown solid; mp 230°C (sublimed); ^{1}H NMR ($CDCl_3$): δ 8.48 (1H, d, *J* = 9.0 Hz, H10), 7.90 (1H, d, *J* = 9.0 Hz, H4), 7.78 (1H, d, *J* = 2.8 Hz, H1), 7.36 (1H, dd, *J* = 8.8, 2.5 Hz, H3), 7.34 (1H, d, *J* = 2.5 Hz, H7), 7.30 (1H, dd, *J* = 8.8, 2.5 Hz, H9), 3.96 (3H, s), 3.87 (3H, s); ^{13}C NMR ($CDCl_3$): δ 185.1, 163.8, 161.2, 160.0, 145.5, 143.3, 142.7, 134.9, 127.8, 127.3, 126.7, 125.9, 121.7, 110.8, 110.6, 58.6, 58.5; MS (ESI): m/z 309 $[M + H]^+$. Anal. Calcd for $C_{17}H_{12}N_2O_4 \cdot H_2O$: C, 62.57; H, 4.32; N, 8.59. Found: C, 62.63; H, 4.33; N, 8.61.

2,7-Dimethoxytryptanthrin (5c) The procedure described previously for **4a** was applied to 6-methoxyisatoic anhydride (**3b**, 750 mg, 3.89 mmol) and 4-methoxyisatin (**4b**, 680 mg, 3.84 mmol) to give the desired 2,7-dimethoxytryptanthrin (**5b**, 603 mg, 51%) as a brown solid; mp 270°C (dec); ^{1}H NMR ($CDCl_3$): δ 8.24 (1H, d, *J* = 8.5 Hz, H10), 7.67 (1H, td, *J* = 8.5, 1.2 Hz, H9), 7.49 (1H, d, *J* = 1.5 Hz, H1), 7.33 (1H, dd, *J* = 8.8, 1.3 Hz, H4), 7.18 (1H, dd, *J* = 8.5, 1.2 Hz, H3), 7.06 (1H, d, *J* = 8.5 Hz, H8), 3.93 (3H, s), 3.78 (3H, s); MS (ESI): m/z 309 $[M + H]^+$. Anal. Calcd for $C_{17}H_{12}N_2O_4$: C, 66.23; H, 3.92; N, 9.09. Found: C, 66.13; H, 3.93; N, 9.10.

2,3-Dimethoxytryptanthrin (5d) The procedure described previously for **5a** was applied to 6,7-dimethoxyisatoic anhydride (**3d**, 450 mg, 2.08 mmol) and isatin (**4e**, 290 mg, 1.97 mmol) to give of the desired 2,3-dimethoxytryptanthrin (**5d,** 316 mg, 52%) as a brown solid; mp >300°C. Spectral data are virtually identical to those reported in the literature [28].

3,8-Dimethoxytryptanthrin (5e) The procedure described previously for **5a** was applied to 7-methoxyisatoic anhydride (**3c**, 80 mg, 0.42 mmol) and 5-methoxyisatin (**4b**, 70 mg, 0.40 mmol) to afford the desired 3,8-dimethoxytryptanthrin (**5e,** 69 mg, 56%) as a brown solid; mp 250°C (dec); ^{1}H NMR ($CDCl_3$): δ 8.48 (1H, d, *J* = 9.0 Hz), 8.29 (1H, d, *J* = 9.0 Hz), 7.40 (1H, d, *J* = 1.2 Hz), 7.34 (1H, d, *J* = 1.2 Hz), 7.28 (1H, dd, *J* = 8.8, 1.2 Hz), 7.18 (1H, dd, *J* = 8.8, 1.2 Hz), 4.08 (3H, s), 3.89 (3H, s); MS (ESI): m/z 309 $[M + H]^+$. Anal. Calcd for $C_{17}H_{12}N_2O_4$: C, 66.23; H, 3.92; N, 9.09. Found: C, 66.16; H, 3.91; N, 9.12.

8,9-Dihydroxytryptanthrin (2a) A mixture of 8,9-dimethoxytryptanthrin (0.6 g, 1.96 mmol) in freshly distilled CH_2Cl_2 (100 mL) was treated slowly with $AlCl_3$ (1.50 g, 22.43 mmol). This mixture was heated under reflux for 18 h then cooled to room temperature and carefully treated with water (approximately 200 mL). The resultant precipitate (450 mg) was washed with CH_2Cl_2 (50 mL, three times) to yield 8,9-dihydroxytryptanthrin (**2a**, 450 mg, 82%) as a brown solid; mp >300°C; ^{1}H NMR ($CDCl_3$): δ 8.21 (1H, dd, *J* = 7.5, 0.8 Hz, H1), 7.82 (2H, m, H2 & H3), 7.64 (1H, dd, *J* = 8.0, 0.8 Hz, H4), 7.46 (1H, s, H7/H10), 7.28 (1H, s, H10/H7), 6.56 (1H, phenolic OH, D_2O exchangeable), 6.34 (1H, phenolic OH, D_2O exchangeable); MS (ESI): m/z 281 $[M + H]^+$. Anal. Calcd for $C_{15}H_8N_2O_4 \cdot H_2O$: C, 60.41; H, 3.38; N, 9.39. Found: C, 60.57; H, 3.37; N, 9.38.

2,8-Dihydroxytryptanthrin (2b) The procedure described previously for **2a** was used for **5b** (9 mg, 0.03 mmol) to afford **2b** as a brown solid (6.5 mg, 80%); mp >275°C (sublimed); ^{1}H NMR (DMSO-d_6): δ 10.69 (s, 1H, OH), 10.18 (1H, s, OH), 8.28 (1H, d, *J* = 8.8 Hz, H10), 7.78 (1H, d, *J* = 8.5 Hz, H4), 7.57 (1H, d, *J* = 1.5 Hz, H1), 7.31 (1H, dd, *J* = 8.5, 1.5 Hz, H3), 7.20 (1H, dd, *J* = 8.8, 1.5 Hz, H9), 7.11 (d, 1H, *J* = 1.5 Hz, H7); MS (ESI): m/z 281 $[M + H]^+$. Anal. Calcd for $C_{15}H_8N_2O_4$: C, 64.29; H, 2.88; N, 10.00. Found: C, 64.06; H, 2.87; N, 10.03.

2,7-Dihydroxytryptanthrin (2c) The procedure described previously for **2a** was used for **5c** (31 mg, 0.10 mmol) to afford 2,7-dihydroxytryptanthrin (**2c**, 24 mg, 85%) as a brown solid; mp >275°C; ^{1}H NMR (DMSO-d_6): δ 8.48 (1H, d, *J* = 9.0 Hz), 8.29 (1H, d, *J* = 9.0 Hz), 7.40 (1H, d, *J* = 1.2 Hz), 7.34 (1H, d, *J* = 1.2 Hz), 7.28 (1H, dd, *J* = 8.8, 1.2 Hz), 7.18 (1H, dd, *J* = 8.8, 1.2 Hz); MS (ESI): m/z 281 $[M + H]^+$. Anal. Calcd for $C_{15}H_8N_2O_4$: C, 64.29; H, 2.88; N, 10.00. Found: C, 64.36, H, 2.87; N, 10.01.

2,3-Dihydroxytryptanthrin (2d) The procedure described previously for **2a** was used for **5d** (25 mg, 0.08 mmol) to afford **2d** as a brown solid (20 mg, 86%); mp >275°C; MS (ESI): m/z 281 $[M + H]^+$ [29].

3,8-Dihydroxytryptanthrin (2e) The procedure described previously for **2a** was used for **5e** (16 mg, 0.05 mmol) to afford **2e** as a brown solid (11 mg, 81%); mp >275°C (sublimed); ^{1}H NMR (DMSO-d_6): δ 10.85 (s, 1H, OH), 10.16 (1H, s, OH), 8.24 (1H, dd, *J* = 8.0, 1.3 Hz, H10), 7.78 (1H, d, *J* = 8.0 Hz, H1), 7.20-7.11 (4H, m, H2, H4, H7, H9);^{1}H NMR (CD_3OD): δ 8.29 (1H, dd, *J* = 8.3, 1.3 Hz, H10), 8.14 (1H, d, *J* = 8.3 Hz, H1), 7.14 (2H, overlapped d, *J* = 2.5 Hz, H4 & H7), 7.13 (1H, dd, *J* = 8.3, 1.2 Hz, H2), 7.07 (1H, dd, *J* = 8.3, 2.5 Hz, H9); MS (ESI): m/z 281 $[M + H]^+$. Anal. Calcd for $C_{15}H_8N_2O_4$: C, 64.29; H, 2.88; N, 10.00. Found: C, 64.36; H, 2.89; N, 9.96.

Biotransformation of tryptanthrin Metabolism of tryptanthrin (100 μM, final concentration) was determined with 1 mg/mL of microsomal protein, obtained from rat live using previously reported method [19], in 0.1 M K_2PO_4 buffer, pH 7.4, at 37°C for 2 h, in a final incubation volume of 500 μL. The reactions were initiated by the addition of an NADPH-generating system containing 0.8 mM NADPH, 10 mM glucose 6-phosphate, and 1 U glucose 6-phosphate dehydrogenase to the reaction mixture. The reaction was stopped by the addition of EtOAc (1 mL). After mixing and centrifugation, the organic layer (850 μL) was separated and concentrated under a stream of nitrogen gas to give a solid material, which was analyzed using liquid chromatography–mass spectrometry.

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