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Chemical synthesis and structural elucidation of a new serotonin metabolite: (4*R*)-2-[(5′-hydroxy-1′*H*-indol-3′-yl)-methyl]thiazolidine-4-carboxylic acid

Chunyang Jin,* Jason P. Burgess, Madathil B. Gopinathan and George A. Brine

Organic and Medicinal Chemistry, Science and Engineering Group, Research Triangle Institute, PO Box 12194, Research Triangle Park, NC 27709, USA

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Abstract—A new serotonin (5-hydroxytryptamine) metabolite, (4*R*)-2-[(5′-hydroxy-1′*H*-indol-3′-yl)methyl]thiazolidine-4-carboxylic acid (5′-HITCA), was synthesized in 30% overall yield. The key step involved oxidation of protected 5-hydroxytryptophol using IBX followed by spontaneous cyclization with L-cysteine. The stereochemistry of condensation product thiazolidine-4-carboxylic acid was studied using NMR spectroscopy techniques.

Serotonin (5-hydroxytryptamine, 5-HT) is a biogenic amine that functions as both a neurotransmitter and a hormone in the mammalian central nervous system (CNS) and in the periphery. Serotonin plays an important role in modulating mood, social behavior, appetite, sexual behavior and pain.[1] It appears to be generally accepted that the major route of serotonin catabolism in CNS involves oxidative deamination by monoamine oxidase (MAO) producing 5-hydroxyindole-3-acetaldehyde (5-HIAAL, **2**), an unstable intermediate that is further oxidized by aldehyde dehydrogenase (ALDH) to 5-hydroxyindole-3-acetic acid (5-HIAA, **3**) (Scheme 1).[2] A minor pathway involves reduction of **2** by aldehyde reductase (ADR) to 5-hydroxytryptophol **4**. The biogenic aldehyde **2** has an electrophilic center that can be attacked by cellular nucleophiles. Recently, Dryhurst et al.[3] reported that incubation of serotonin and L-cysteine with mammalian microsomes or synaptosomes (pig and bovine) resulted in the facile formation of the (2*R*,4*R*)- and (2*S*,4*R*)-epimers of 2-[(5′-hydroxy-1′*H*-indol-3′-yl)methyl]thiazolidine-4-carboxylic acid (5′-HITCA, **5**). This compound was thought to be formed as a result of the cyclization between aldehyde **2**, the oxidative deamination product from serotonin, and L-cysteine. Susilo et al.[4] reported similar results using tryptamine incubated with brain homogenates (pig, bovine, or rat) to give (4*R*)-2-(3′-indolylmethyl)-1,3-thiazolidine-4-carboxylic acid (ITCA).

ITCA is a biologically active compound that can act as a weak inhibitor of MAO and of γ-aminobutyric acid uptake in vitro.[4] It was suggested that 5′-HITCA may exist in higher concentration and is more stable against enzymatic degradation than ITCA. However, the biological activity of 5′-HITCA has not yet been studied. In order to investigate its pharmacological effect, 5′-HITCA will be needed in gram quantities. In this letter, we would like to report on a first chemical synthesis of (2*R*,4*R*)- and (2*S*,4*R*)-epimers of 2-[(5′-hydroxy-1′*H*-indol-3′-yl)-methyl]thiazolidine-4-carboxylic acid **5**. A complete structural elucidation was carried out using NMR spectroscopy techniques.

The sequence illustrated in Scheme 1 serves as the basis of a biochemical synthesis of 5′-HITCA. Although this biochemical approach is straightforward, it has only been performed on a milligram scale.[3] In order to obtain gram quantities of 5′-HITCA, the direct oxidation of 5-hydroxytryptophol **4** to the corresponding aldehyde **2** was first investigated. It was expected that aldehyde **2** would undergo cyclization reaction with L-cysteine to give 5′-HITCA. Frigerio et al.[5] reported that treatment of tryptophol with 1.1 equiv of *o*-iodobenzoic acid

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* Corresponding author. Tel.: +1 919 541 6328; fax: +1 919 541 6499; e-mail: cjin@rti.org

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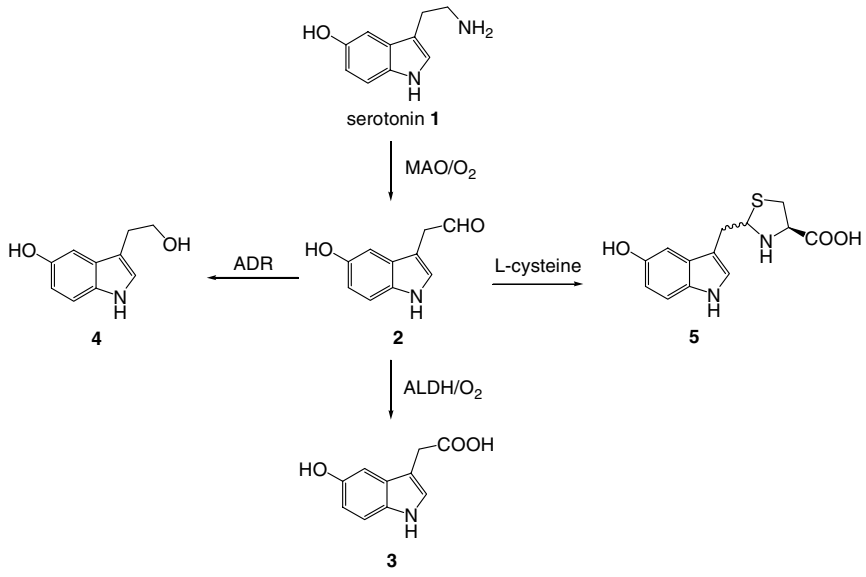
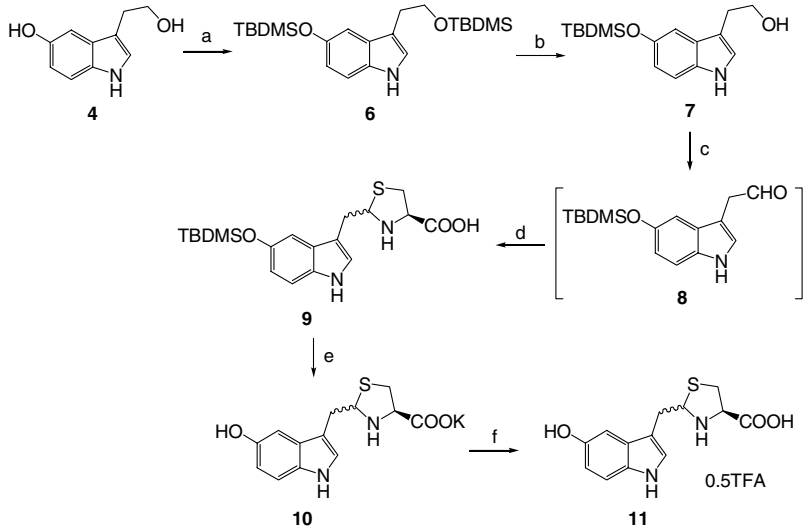


Scheme 1.

(IBX) in dimethylsulfoxide (DMSO) gave the corresponding aldehyde in 79% yield. In our hands, the oxidation reaction of 5-hydroxytryptophol **4** using reported IBX conditions gave a messy result. The desired aldehyde **2** was not isolated. This oxidation reaction was repeated in the presence of L-cysteine that was expected to trap the unstable aldehyde **2**. However, no condensation product **5** was detected in the reaction mixture. The Swern oxidation was also tried with similar results. Indoles, in particular those with an unsubstituted NH group, are known to be unstable in the presence of oxidizing reagents.[6] We suspected that the hydroxy group on the indole ring might be causing more problems in the oxidation reactions. Therefore, we decided to protect the phenolic hydroxy group of compound **4** as its *tert*-butyldimethylsilyl (TBDMS) ether (Scheme 2). Thus, treatment of 5-hydroxytryptophol **4** with excess *tert*-butyldimethylsilyl chloride (TBDMSCl) produced the *O*,*O*′-bis-*tert*-butyldimethylsilyl derivative **6** in essentially quantitative yield.[7] The selective deprotection of the alkyl silyl ether in the presence of the phenolic silyl ether was accomplished in 91% yield using cerium(III) chloride in refluxing acetonitrile to obtain 5-*O*-*tert*-butyldimethylsilytryptophol **7**.[8] Subsequent oxidation of alcohol **7** utilizing IBX in DMSO then produced 5-*O*-*tert*-butyldimethylsilylindole-3-acetaldehyde **8**. The protected aldehyde **8** was not isolated and characterized due to its instability. Instead, it was treated at once with 2 equiv of L-cysteine in ethanol–water (1:1). Subsequent workup and purification afforded 2-[(5′-*O*-*tert*-butyldimethylsilyl-1′*H*-indol-3′-yl)methyl]-thiazolidine-4-carboxylic acid **9** in 46% yield from **7**.[9]



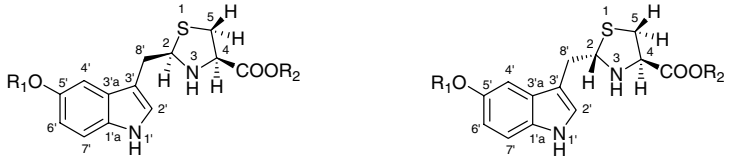
Scheme 2. Reagents and conditions: (a) TBDMSCl, imidazole, THF, rt, 100%; (b) $CeCl_3{\cdot}7H_2O$, CH_3CN, reflux, 91%; (c) IBX, DMSO, rt; (d) L-cysteine, 1:1 EtOH–H_2O, rt, 46%; (e) $KF{\cdot}2H_2O$, MeOH, rt, 76%; (f) TFA, 93%.

The ^{1}H NMR spectrum of compound **9** in DMSO-d_6 displayed two distinct sets of signals in a 1.4:1 ratio. Similarly, the ^{13}C NMR spectrum of this compound also showed the presence of several closely spaced peaks of approximately equal intensity. Based on NMR studies, particularly with the aid of 2D proton–proton (COSY) and proton–carbon (gHSQC and gHMBC) correlation spectroscopy techniques, it was concluded that condensation product **9** was a mixture of two diastereoisomers (Fig. 1, **9a** and **9b**). While these two diastereoisomers could not be physically separated, the ^{1}H NMR resonances corresponding to **9a** and **9b** could easily be assigned.[10] The C(2) and C(4) protons of each diastereoisomer of **9** were well resolved. The C(2) protons presented two sets of signals centered at δ 4.86 and δ 4.71 while C(4) protons displayed two sets of signals centered at δ 4.15 and δ 3.72. Molecular models of **9** revealed that in isomer **9a** the distance between C(2)–H and C(4)–H was approximately 2.53 Å.[11] Thus, a nuclear Overhauser effect (NOE) between these two protons was expected. A ROESY experiment on the diastereoisomeric mixture of **9** showed a strong correlation between the sets of signals centered at δ 4.71 [C(2)–H] and δ 3.72 [C(4)–H]. The spatial proximity of these two signals gave evidence for cis configuration of the indole residue and the carboxylic acid group in the major isomer **9a**. ROESY experiments showed no NOE correlation between C(2)–H and C(5)–H in either cis or trans isomer. Therefore, for the cis isomer **9a**, the absolute configuration at C(2) was *R*, and for the trans isomer **9b**, the absolute configuration at C(2) was *S*. At C(4) in both isomers, the absolute configuration was *R* as derived from the L-cysteine. These absolute configurations are shown in Figure 1.

The equilibrium exchange of diastereoisomers **9a** and **9b** was examined in different deuterated solvents (CD_3OD, CD_3CN, and DMSO-d_6). After several days at room temperature, the cis/trans ratio was equilibrated to approximately 1:1. After complete equilibration, the ratio was not dependent on the temperature or the nature of solvent. It has been suggested that this kind of tautomeric equilibrium proceeds through a Schiff base intermediate as shown in Scheme 3.[12]

Deprotection of the phenolic silyl group utilizing potassium fluoride dihydrate gave, after chromatography purification, (4*R*)-2-[(5′-hydroxy-1′*H*-indol-3′-yl)methyl]thiazolidine-4-carboxylic acid potassium salt **10** in 93% yield.[13] Compound **10** was found to be a 2.5:1 mixture of diastereoisomers differing in configuration at C(2), a finding that suggested that some base-catalyzed opening of the thiazolidine ring has occurred during the potassium fluoride deprotection reaction (the reaction pH was approximately 8). Subsequent careful treatment of compound **10** with trifluoroacetic acid (TFA) in water provided (4*R*)-2-[(5′-hydroxy-1′*H*-indol-3′-yl)methyl]thiazolidine-4-carboxylic acid hemitrifluoroacetate **11** in 93% yield as an approximately 1:1 mixture of diastereoisomers **11a** and **11b**. The stereochemistry of **10** and **11** as shown in Figure 1 was confirmed by ROESY experiments. In both compounds, an upfield shift of the C(2) and C(4) resonances in the cis isomers as compared to the trans isomers was observed.[3,14]

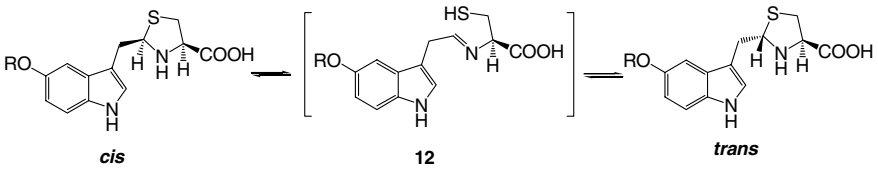
The stability of compounds **10** and **11** was examined in DMSO-d_6 and D_2O. The potassium salt **10** exhibited no change in its ^{1}H NMR spectrum even after several days. In the case of hemi-trifluoroacetate salt **11**, the ^{1}H NMR showed a slow decomposition in D_2O. It was noted that 2-substituted thiazolidine-4-carboxylic acid will undergo solvolysis with ring opening in aqueous solution to uncover aldehyde and amino acid components.[15] It is interesting to observe that when a solution of compound **11** in D_2O containing one drop of trifluoroacetic acid was examined by ^{1}H NMR, the signals for the protons *ortho* [C(4′) and C(6′)] to the phenolic hydroxy group gradually disappeared overtime due to deuterium exchange.



2*R*, 4*R* isomers (*cis*)
9a R_1=TBDMS, R_2=H
10a R_1=H, R_2=K
11a R_1=H, R_2=H

2*S*, 4*R* isomers (*trans*)
9b R_1=TBDMS, R_2=H
10b R_1=H, R_2=K
11b R_1=H, R_2=H

Figure 1.



cis **12** ***trans***

Scheme 3.

In conclusion, we have developed a short synthetic route to access (4*R*)-2-[(5′-hydroxy-1′*H*-indol-3′-yl)methyl]-thiazolidine-4-carboxylic acid **5** (5′-HITCA) in 30% overall yield. The key step involved IBX oxidation of 5-*O*-*tert*-butyldimethylsilyl protected 5-hydroxytryptophol **4** to the corresponding aldehyde followed by cyclization with L-cysteine. The 5′-HITCA hemi-trifluoroacetic acid salt was obtained as a diastereoisomeric mixture in 1:1 ratio.

Acknowledgements

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9. 2-[(5′-*O*-*tert*-Butyldimethylsilyl-1′*H*-indol-3′-yl)methyl]-thiazolidine-4-carboxylic acid (**9**). To a solution of **7** (6.20 g, 21.31 mmol) in DMSO (80 mL) at 0 °C under N_2 was added IBX (6.56 g, 23.44 mmol) and the reaction mixture was stirred 4 h at room temperature. H_2O (50 mL) was then added and the mixture was extracted with Et_2O (3×50 mL). The combined Et_2O extracts were washed with brine (3×30 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue (crude aldehyde **8**) was dissolved in degassed EtOH (200 mL). This solution was added to a solution of L-cysteine (5.16 g, 42.62 mmol) in degassed H_2O (200 mL) at 0 °C. The resultant reaction mixture was stirred 1 h at 0 °C and then 16 h at room temperature. After this time, most of the EtOH was removed in vacuo. The remaining aqueous solution was extracted with EtOAc (3×50 mL). The combined EtOAc extracts were dried (Na_2SO_4) and concentrated in vacuo. Subsequent purification of the crude product by flash column chromatography over silica gel using MeOH/EtOAc as eluent provided **9** as a pale brown solid (3.85 g, 46%).
10. **9a**: ^{1}H NMR (500 MHz; DMSO-d_6) δ 10.71 (1H, br s, 1′-H), 7.18 (1H, d, $J = 8.6$ Hz, 7′-H), 7.15 (1H, d, $J = 2.0$ Hz, 2′-H), 6.95 (1H, d, $J = 2.6$ Hz, 4′-H), 6.61 (1H, dd, $J = 8.6$, 2.6 Hz, 6′-H), 4.71 (1H, dd, $J = 7.4$, 5.2 Hz, 2-H), 3.72 (1H, dd, $J = 9.4$, 6.8 Hz, 4-H), 3.26 (1H, dd, $J = 14.5$, 5.2 Hz, 8′-H), 3.17 (1H, dd, $J = 9.9$, 6.8 Hz, 5-H), 3.08 (1H, dd, $J = 14.5$, 7.4 Hz, 8′-H), 2.74 (1H, dd, $J = 9.9$, 9.4 Hz, 5-H), 0.96 (9H, s), 0.15 (6H, s); **9b**: ^{1}H NMR (500 MHz; DMSO-d_6) δ 10.68 (1H, br s, 1′-H), 7.17 (1H, d, $J = 8.5$ Hz, 7′-H), 7.13 (1H, d, $J = 2.0$ Hz, 2′-H), 6.93 (1H, d, $J = 2.2$ Hz, 4′-H), 6.60 (1H, dd, $J = 8.5$, 2.2 Hz, 6′-H), 4.86 (1H, dd, $J = 8.3$, 5.7 Hz, 2-H), 4.15 (1H, dd, $J = 6.9$, 5.3 Hz, 4-H), 3.14 (1H, dd, $J = 14.4$, 5.7 Hz, 8′-H), 3.12 (1H, dd, $J = 9.9$, 6.9 Hz, 5-H), 2.92 (1H, dd, $J = 9.9$, 5.3 Hz, 5-H), 2.90 (1H, dd, $J = 14.4$, 8.3 Hz, 8′-H), 0.96 (9H, s), 0.15 (6H, s); Resolution of the signals of ^{13}C NMR from each isomer of compound **9** was not good enough for the assignment. ^{13}C NMR (125 MHz; DMSO-d_6) δ 172.9, 172.3, 147.7, 131.7, 127.8, 124.3, 114.9, 114.8, 111.7, 111.2, 110.6, 107.7, 71.8, 71.2, 65.3, 64.3, 37.3, 37.0, 32.8, 30.4, 25.7, 17.9, −4.5; HRMS calcd for $C_{19}H_{28}N_2O_3SSi$ $(M+Na)^+$: 415.1488. Found: 415.1492.
11. Molecular modeling was carried out employing Spartan 2004 V1.0.3 (Wavefunction, Inc.). The minimum energy structures were obtained from a total three computational methods: MMFF molecular mechanics, AM1 semi-empirical quantum mechanics, and B3LYP (6-31+G**) density functional method.
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14. We noted that in our work the resonances assigned to the cis isomer and the trans isomer of compound **11** were reversed from that of Dryhurst et al. for the C(2) and C(4) protons. **11a**: ^{1}H NMR (500 MHz; DMSO-d_6) δ 10.59 (1H, d, $J = 2.0$ Hz, 1′-H), 8.62 (1H, br s, OH), 7.13 (1H, d, $J = 8.3$ Hz, 7′-H), 7.10 (1H, d, $J = 2.0$ Hz, 2′-H), 6.84 (1H, d, $J = 1.8$ Hz, 4′-H), 6.60 (1H, dd, $J = 8.3$, 1.8 Hz, 6′-H), 4.80 (1H, dd, $J = 8.0$, 5.0 Hz, 2-H), 3.96 (1H, dd, $J = 8.9$, 7.5 Hz, 4-H), 3.29 (1H, dd, $J = 14.5$, 5.0 Hz, 8′-H), 3.22 (1H, dd, $J = 9.9$, 7.5 Hz, 5-H), 3.06 (1H, dd, $J = 14.5$, 8.0 Hz, 8′-H), 2.85 (1H, dd, $J = 9.9$, 8.9 Hz, 5-H); **11b**: ^{1}H NMR (500 MHz; DMSO-d_6) δ 10.57 (1H, d, $J = 2.0$ Hz, 1′-H), 8.62 (1H, br s, OH), 7.13 (1H, d, $J = 8.5$ Hz, 7′-H), 7.09 (1H, d, $J = 2.0$ Hz, 2′-H), 6.84 (1H, d, $J = 1.5$ Hz, 4′-H), 6.60 (1H, dd, $J = 8.5$, 1.5, 6′-H), 4.91 (1H, dd, $J = 8.8$, 5.5 Hz, 2-H), 4.35 (1H, dd, $J = 6.4$, 5.7 Hz, 4-H), 3.19 (1H, dd, $J = 14.5$, 5.5 Hz, 8′-H), 3.18 (1H, dd, $J = 10.7$, 6.4 Hz, 5-H), 3.02 (1H, dd, $J = 10.7$, 5.7 Hz, 5-H), 2.91 (1H, dd, $J = 14.5$, 8.8 Hz, 8′-H); Resolution of the signals of ^{13}C NMR from each isomer of compound **11** was not good enough for the assignment. ^{13}C NMR (125 MHz; DMSO-d_6) δ 172.0, 171.5, 150.3, 130.6, 127.7, 123.9, 111.7, 111.4, 111.3, 109.9, 109.6, 102.2, 70.3, 69.7, 64.7, 63.8, 35.8, 35.6, 32.1, 30.2; Anal. Calcd for $C_{13}H_{14}N_2O_3S{\cdot}0.5CF_3COOH$: C, 50.14; H, 4.36; N, 8.35. Found: C, 50.35; H, 4.68; N, 8.56.
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