Active Metabolite of Aeruginascin (4-Hydroxy-\textit{N},\textit{N},\textit{N}-trimethyltryptamine): Synthesis, Structure, and Serotonergic Binding Affinity

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ABSTRACT: The putative active metabolite of aeruginascin, a naturally occurring tryptamine of “magic mushrooms,” has been synthesized and structurally characterized. Competitive radioligand binding assays demonstrate that it has a high affinity at human serotonin receptors 5-HT$_{1A}$, 5-HT$_{2A}$, and 5-HT$_{2B}$, though it does not bind at the 5-HT$_{3}$ receptor, where activity was previously predicted.

INTRODUCTION

More than 200 species of fungi, collectively known as “magic mushrooms,” including those from the genus \textit{Psilocybe}, are known to contain psychoactive tryptamine compounds. Components of “magic mushrooms” (i.e., psilocybin/psilocin) have incredible potential for treating intractable mental and physical conditions. These drugs show promise in the treatment of disorders, including addiction, anxiety, depression, and post-traumatic stress disorder. Of note, psilocybin was granted the “breakthrough therapy” designation by the US Food and Drug Administration (FDA). This FDA designation has cleared the way for clinical trials of psilocybin for the treatment of disorders, including addiction, anxiety, depression, and post-traumatic stress disorder. In the case of psilocybin, its phosphate group is hydrolyzed during metabolism to generate psilocin in the body, which functions as the active psychedelic (Figure 1). A well-known functional analogue of psilocybin is psilacetin, or 4-acetoxy-\textit{N},\textit{N},\textit{N}-trimethyltryptamine (4-AcO-DMT), the 4-acetoxy derivative of psilocybin, which is similarly hydrolyzed to generate psilocin. To synthesize the active metabolite of aeruginascin, we set out to synthesize its putative active metabolite and examine its binding affinity at human serotonin receptors.

RESULTS AND DISCUSSION

In the case of psilocybin, its phosphate group is hydrolyzed during metabolism to generate psilocin in the body, which functions as the active psychedelic (Figure 1). A well-known functional analogue of psilocybin is psilacetin, or 4-acetoxy-\textit{N},\textit{N},\textit{N}-dimethyltryptamine (4-AcO-DMT), the 4-acetoxy derivative of psilocybin, which is similarly hydrolyzed to generate psilocin. To synthesize the active metabolite of aeruginascin,
psilocin (4-AcO-DMT) fumarate was used as the starting material and methylated in the presence of excess iodo-methane. The resulting compound, 4-acetoxy-N,N,N-trimethyltryptammonium (4-AcO-TMT) iodide, was generated in a good yield (53%). In an analogy to psilocin, 4-AcO-TMT would be expected to serve as a convenient source of 4-HO-TMT, which is consistent with our experimental observations.

In aqueous acetic acid, the 4-AcO-TMT iodide is hydrolyzed to generate 4-HO-TMT iodide (Scheme 1). The material was purified by recrystallization from a methanolic solution (60% yield).

The compounds were both recrystallized from water to obtain them in a single-crystalline form. The molecular structures for both compounds are shown in Figure 2. These are the first two quaternary tryptammonium salts ever characterized by single-crystal diffraction. The presence of such structural data is helpful for modeling studies to probe their activity at receptors. NMR data and elemental analyses further demonstrate the high purity of these compounds as synthesized.

The two compounds were screened for binding at the orthosteric sites of human serotonin receptors 5-HT1A, 5-HT2A, 5-HT2B, and 5-HT3.20 Competitive radioligand binding assays were used to assess the affinity of the compounds for the receptors. Binding is reported as the \( K_i \) for the inhibition of binding well-characterized orthosteric ligands (Table 1 and Supporting Information). The aeruginascin active metabolite, 4-HO-TMT, shows binding at 5-HT1A, 5-HT2A, and 5-HT2B receptors. Counter to the prevailing theory that aeruginascin should function as a powerful 5-HT3 agonist, there is no binding \( (K_i > 10,000 \text{ nM}) \) observed at this receptor. The aeruginascin functional analogue, 4-AcO-TMT, shows no binding affinity at any of the receptors. For comparison, psilocybin, the prodrug of psilocin, shows no activity at 5-HT1A, 5-HT2A, or 5-HT3 but does show itself to bind strongly at 5-HT2B. Psilocin, its active metabolite, shows activity at 5-HT1A and 5-HT2A that has a greater, though comparable, binding affinity to 4-HO-TMT. It is two orders of magnitude more potent than 4-HO-TMT at the 5-HT2B receptor, and in fact, psilocybin is more active at this receptor as well.

Despite its close structural relationship to bufotenidine, 4-HO-TMT does not exhibit binding at the serotonin 5-HT3 receptor. The results of receptor screening show that this metabolite has unexpected binding affinity at the serotonin 5-HT2A receptor which is associated with psychotropic activity. The quaternary ammonium functionality makes it less likely that this charged species will cross the blood–brain barrier. However, quaternary ammonium salts have been known to cross the blood–brain barrier through transporters; therefore, psychotropic activity remains a possibility.21 It has been speculated that an inability to cross the blood–brain barrier might lead to the different observed effects from this compound.14,22 Also of note is that it shows significantly less binding than psilocin at the serotonin 5-HT2B receptor, where activation is tied to valvular heart disease.23

**CONCLUSIONS**

In summary, the putative active metabolite of one of the naturally occurring tryptamines found in at least one species of “magic mushrooms” (aeruginascin) has been synthesized and characterized for the first time. Its binding affinity at serotonergic receptors has been assayed, demonstrating that it is not active at the 5-HT3 receptor, as previously predicted, but shows strong binding at the 5-HT2B receptors which was unexpected. In the last year, over 100 U.S. cities launched initiatives to decriminalize “magic mushrooms” despite having limited scientific information about many of the tryptamines.
contained in the fungi. The study of this and other natural products in “magic mushrooms” will be important to understand their effects and to avoid dangerous peripheral consequences.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c02208.

Synthesis of 4-AcO-TMT iodide and 4-HO-TMT iodide, 1H NMR, 13C NMR, and elemental analysis (PDF)
Crystallographic data of 4-HO-TMT (CIF)
Crystallographic data of 4-AcO-TMT (CIF)

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The authors declare the following competing financial interest(s): Andrew R. Chadeayne and Brian G. Reid report ownership interests in CaaMTech, Inc., which owns U.S. and worldwide patent applications, covering new tryptamine compounds, compositions, formulations, novel crystalline forms, and methods of making and using the same.

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ABBREVIATIONS

FDA, United States Food and Drug Administration; 5-HT, 5-hydroxytryptamine receptor; DMT, N,N-dimethyltryptamine; 4-AcO-DMT, 4-acetoxy-N,N-dimethyltryptamine; 4-AcO-TMT, 4-acetoxy-N,N,N-trimethyltryptammonium; 4-HO-TMT, 4-hydroxy-N,N,N-trimethyltryptammonium

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