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Active Metabolite of Aeruginascin (4-Hydroxy-*N*,*N*,*N*-trimethyltryptamine): Synthesis, Structure, and Serotonergic Binding Affinity

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Cite This: *ACS Omega* 2020, 5, 16940−16943

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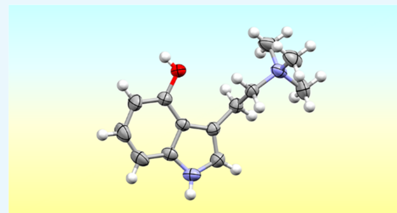
ABSTRACT: The putative active metabolite of aeruginascin, a naturally occurring tryptamine of "magic mushrooms," has been synthesized and structurally characterized. Competitive radioligand binding assays demonstrate that it has a high affinity at human serotonin receptors 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2B}, 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 *Psilocybe*, are known to contain psychoactive tryptamine compounds.[1] Components of "magic mushrooms" (i.e., psilocybin/psilocin) have incredible potential for treating intractable mental and physical conditions.[2] These drugs show promise in the treatment of disorders, including addiction,[3,4] anxiety,[5] depression,[6] and post-traumatic stress disorder.[7] Of note, psilocybin was granted the "breakthrough therapy" designation by the US Food and Drug Administration (FDA).[8] This FDA designation has cleared the way for clinical trials of psilocybin to treat major depressive disorders and treatment-resistant depression.

One of the biggest concerns in using these compounds as pharmaceuticals for humans is the potential for a "bad trip" or dysphoric experience.[9] The extracts of "magic mushrooms" demonstrate the same clinical effects as pure psilocybin at dosages that are an order of magnitude smaller,[10] suggesting important activity by other psychoactive molecules or the presence of an entourage effect.[11] To have a better understanding of how "magic mushroom" extracts function, it is important to understand the properties of the minor active components of "magic mushrooms," alone and in combination with psilocybin.

New psychoactive tryptamines have been identified in "magic mushrooms" as recently as 2017.[12] Until this year, there was no general synthetic method for producing useful amounts of the minor psychoactive tryptamines.[13] One of these minor components is aeruginascin,[14] the *N*-trimethyl analogue of psilocybin. Documented accounts of human exposure to *Inocybe aeruginascens*, a species of mushroom containing aeruginascin, describe hallucinations that exhibited only euphoric experiences.[15] These limited reports about aeruginascin are interesting, given that dysphoria or a "bad trip" is a significant concern associated with consuming psilocybin or mushrooms containing it. Despite these observations, the pharmacological activity of aeruginascin has remained unexplored.[14]

The active metabolite of psilocybin is its hydrolysis product psilocin; it is likely that aeruginascin undergoes similar hydrolysis to generate its active metabolite, 4-hydroxy-*N*,*N*,*N*-trimethyltryptamine (4-HO-TMT).[13,14] This compound is closely related to bufotenidine, the *N*-trimethyl analogue of serotonin found naturally in toad venom. Bufotenidine is a selective 5-HT_3 agonist,[16] with strong binding to that receptor (K_i = 17 nM).[17] The leading hypothesis for aeruginascin has been that it is similarly active at 5-HT_3 and inactive at 5-HT_2 receptors. To assess this leading hypothesis on 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).[18] A well-known functional analogue of psilocybin is psilacetin, or 4-acetoxy-*N*,*N*-dimethyltryptamine (4-AcO-DMT), the 4-acetoxy derivative of psilocybin, which is similarly hydrolyzed to generate psilocin.[19] To synthesize the active metabolite of aeruginascin,

Received: May 12, 2020
Accepted: June 19, 2020
Published: July 2, 2020

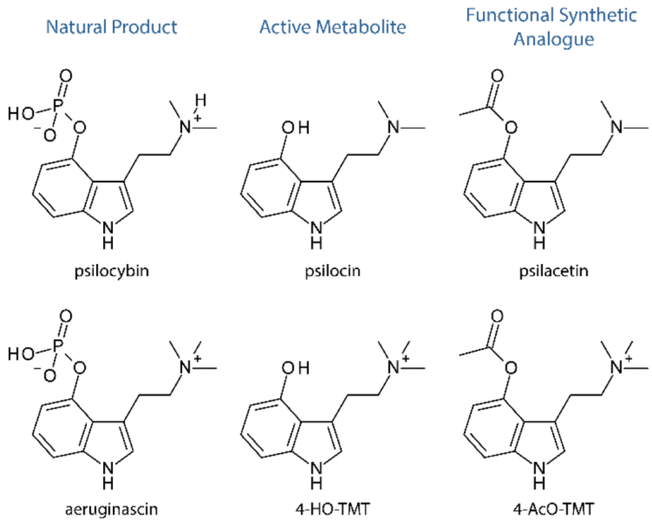
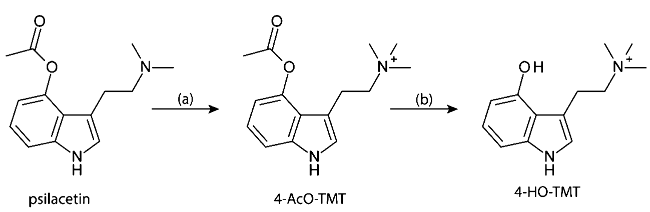


Figure 1. Analogy of psilocybin and aeruginascin derivatives.

psilacetin (4-AcO-DMT) fumarate was used as the starting material and methylated in the presence of excess iodomethane. The resulting compound, 4-acetoxy-*N*,*N*,*N*-trimethyltryptammonium (4-AcO-TMT) iodide, was generated in a good yield (53%). In an analogy to psilacetin, 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).

Scheme 1. Synthesis of the Active Metabolite of Aeruginascin Where (a) MeI/MeOH and (b) AcOH/H_2O



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-HT_{1A}, 5-HT_{2A}, 5-HT_{2B}, and 5-HT_3.[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-HT_{1A}, 5-HT_{2A}, and 5-HT_{2B} receptors. Counter to the prevailing theory that aeruginascin should function as a powerful 5-HT_3 agonist, there is no binding (K_i > 10,000 nM) observed at this receptor. The

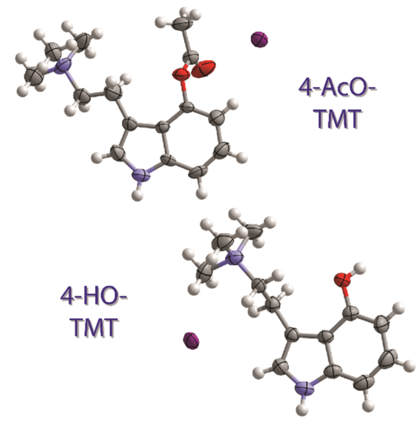


Figure 2. Crystal structures of the iodide salts of 4-AcO-TMT (top) and 4-HO-TMT (bottom) with thermal ellipsoids shown at the 50% probability level.

Table 1. Inhibition Constants (K_i) in nM Units

compound	5-HT_{1A}	5-HT_{2A}	5-HT_{2B}	5-HT_3
4-HO-TMT	4400	670	120	>10,000
4-AcO-TMT	>10,000	>10,000	>10,000	>10,000
psilocin[20]	567.4	107.2	4.6	>10,000
psilocybin[20]	>10,000	>10,000	98.7	>10,000

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-HT_{1A}, 5-HT_{2A}, or 5-HT_3 but does show itself to bind strongly at 5-HT_{2B}. Psilocin, its active metabolite, shows activity at 5-HT_{1A} and 5-HT_{2A} 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-HT_{2B} 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-HT_3 receptor. The results of receptor screening show that this metabolite has unexpected binding affinity at the serotonin 5-HT_{2A} 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-HT_{2B} 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-HT_3 receptor, as previously predicted, but shows strong binding at the 5-HT_2 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

https://dx.doi.org/10.1021/acsomega.0c02208
ACS Omega 2020, 5, 16940−16943

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, ^{1}H NMR, ^{13}C NMR, and elemental analysis (PDF)

Crystallographic data of 4-HO-TMT (CIF)

Crystallographic data of 4-AcO-TMT (CIF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding

CaaMTech, Inc. National Science Foundation (CHE-1429086).

Notes

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.

■ ACKNOWLEDGMENTS

K_i determinations for psilocybin and psilocin were generously provided by the National Institute of Mental Health's Psychoactive Drug Screening Program [Contract #HHSN-271-2018-00023-C (NIMH PDSP)]. The NIMH PDSP is Directed by Bryan L. Roth MD, PhD at the University of North Carolina at Chapel Hill and Project Officer Jamie Driscoll at NIMH, Bethesda MD, USA. The crystal data were collected on a diffractometer funded by a National Science Foundation instrumentation grant (CHE-1429086). Funding for this research was provided by CaaMTech, Inc., Issaquah, WA, USA.

■ 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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https://dx.doi.org/10.1021/acsomega.0c02208
ACS Omega 2020, 5, 16940−16943