Efforts Towards the Synthesis of Epibatidine

By: Marianne Hanna (Junior-Biochemistry major)

Faculty Advisor: Dr. Thomas Montgomery

Duquesne University- Bayer School of Natural Sciences
Abstract:

Epibatidine is a naturally toxic chemical found in the secretion of poison dart frogs. Given its structural similarities to the compound nicotine epibatidine binds strongly to nicotine receptors in the central nervous system (CTS). Epibatidine’s bioactivity arises from its unique geometry which allows it to bind to the α4β2 subunit in the nicotinic receptor. This triggers an analgesic effect without a release of dopamine, differentiating its activity from opioids. Prior efforts towards synthesizing epibatidine and its analogs have involved many steps making them untenable for pharmaceutical use. By using computational and experimental methods to study the mechanism for an interrupted Polonovski [3+2] cycloaddition which will give direct access to the epibatidine core motif. By synthesizing strategic derivatives of epibatidine through this route we will investigate opioid alternatives which lack many of their addictive qualities.

Background:

Epibatidine is a toxic alkaloid that is isolated from the skin of poison dart tree frog *Epipedobates tricolor*, secretions from the frog are used by indigenous tribes in darts for hunting.¹ The chemical structure was established in 1992 using NMR spectroscopy (proton NMR).² Epibatidine possesses significant medicinal properties, it functions as an analgesic agent by binding to the nicotinic acetylcholine receptors (nAChRs) instead of the opioid receptors.³ Moreover it displays an affinity for said receptors that is 100- to 200-fold higher than nicotine. The alkaloid interacts with Nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels and are expressed central and peripherally. The muscle nAChRs consists of four classes of subunits and forms a transmembrane aqueous pore. In mammalian central nervous system (CNS) the heterodimeric nAChR subtypes α4β2, and the monomeric subtypes α7 are the most expressed. Epibatidine has high affinity to nAChRs for the α4β2 subtype, being a potent, but
non-selective nAChR agonist. Its broad-spectrum of activity induces several off-targets effects in several districts, such as the CNS as well as in respiratory, gastrointestinal, and cardiovascular functions, precluding any therapeutic development. These central receptors are involved in various neurological conditions, such as schizophrenia, Parkinson’s, and Alzheimer’s disease (AD), but also in other important physiological functions, including neuroprotection, memory and learning, and pain control. For this reason, new ligands of these receptors have been investigated as upcoming pharmacological research tools or as possible drugs.

The biological targets and mechanisms of action are well established for epibatidine. The information on the metabolism of epibatidine is surprisingly limited, preliminary observations were published in 2000 by Watt et al. where in vitro metabolic routes for both enantiomers were investigated. The results showed the formation of diastereoisomeric N-oxides for (+) epibatidine and hydroxylation of the azabicycle for (−) epibatidine. Studies on epibatidine metabolism and metabolite excretion have undergone a gradual decrease in number over the years due to Epibatidine’s severe side effects. More recently, efforts have been made to address the pharmacokinetics of new derivatives of epibatidine with reduced toxicity. Heugebaert et al. presented five epibatidine analogs containing a substituent on the azabicyclo[2.2.1] heptane bridgehead, namely a ketone linker, an OH linker, an aminomethyl linker, and two ethyl linkers containing an OH and a NH₂ group. They focused on an aminomethyl- and two ethyl linkers containing an OH and a NH₂ group and studied the in vitro pharmacokinetics of the analogs. Both compounds showed a low binding affinity to plasma proteins, suggesting that they are available to be metabolized.

Epibatidine toxicity may be related to its ability to activate not only central neuronal α2β2, but also ganglionic α3β4 nAchR. The research community switched to modifying the
epibatidine structure to obtain analogues with a better pharmacological activity/toxicity ratio and selectivity for different nAchR subtypes. ABT-594 did not show sedative-like effects on electroencephalography typically induced by opioids, but additional studies have highlighted a partial involvement of opioid receptors through indirect activation.\(^7\)

Epibatidine and its analogues have shown promising results in diseases and pain treatment. Because of severe gastrointestinal side effects, the first analogue of epibatidine, ABT-594, is not included in current pain therapies in humans. New synthetic derivative of epibatidine ABT-418 are currently used in treatment plans for moderate ADHD. ABT-418 is well-tolerated by patients with only minor side effects. Epibatidine’s pharmacological effects open new perspectives in drug therapies and also represent an important research tool to investigate nAChR activity.\(^8\)

Results/Discussion:

My hypothesis is that if the mechanism of a [3+2] cycloaddition using a base or electrophile and an oxidized pyrrolidine derivative, then this can be leveraged towards developing a high yielding cycloaddition reaction; a critical step towards epibatidine and its analogs.

![Figure 1: Hypothesis](image)

My first specific aim was to synthesis the analog of epibatidine using trans stilbene and an oxidized pyrrolidine that has a different R-groups. We are using a t-butyl on the pyrrolidine. This prevents possible side reactions arising from deprotonation exo to the ring system.
When trans stilbene and the tert-butyl N-oxo compound were combine the expected product was formed (Figure 2). As expected of a concerted reaction only a single diastereomer formed, greatly simplifying both purification and analysis. The proton NMR, which was used during analysis, does have some error in it because the integrations don’t line up exactly with the number of protons calculated. That is due to the fact there are residual impurities still in the sample. Another factor would the standard that is used. The d-chloroform peaks overlap with several product peaks and will lead to unreliable integration values. A mass spectrum was taken of this product as well using electron spray ionization. The peak at 306.1 is (M+H) is the product’s mass plus one proton confirming the product was formed.

My second specific aim was to synthesis different pyrrolidine derivates and oxidize them. The synthesis for the derivates of pyrrolidine and the oxidation in large quantities and with the best yields. We believe that if you can have a more electron R-group on the pyrrolidine that the trans-stilbene will coordinate better. Figure 3 was what I was working on. The starting material has more electrons on the R-group that could help the trans-stilbene coordinate better.
Within Figure 3 the starting material has a two-step synthesis that I completed this semester. Figure 4 is the first step of the synthesis. It is a known product and I had completed the synthesis with 98% yield.

![Figure 4: Formation of 2-((isopropyl(dimethylsilyl)oxy)aniline](image)

In Figure 5 is the second step of the synthesis that I troubleshooted. The workup was changed slightly to increase the yield. In the workup in requires washing it in acid until it is acidic then concentrate it. I found that when I don’t concentrate it and continue the workup that the yield increased. I speculate the product is being removed in that process. I achieved about 65% yield consistently.

![Figure 5: Formation of 1-(2-((tert-butyldimethylsilyl)oxy)phenyl)pyrrolidine](image)

The oxidation was unsuccessful using peroxide (Figure 3). In the future I plan to find a procedure that successfully oxidizes the 1-((tert-butyldimethylsilyl)oxy)phenyl)pyrrolidine. As well as fully characterize the product using mass spectrometry, IR, carbon NMR, NOSY, and COSY. The product will be used in the [3+2] cycloaddition as the starting material in the hypothesis (Figure 1).
Works Cited

1. Opioid addiction - Genetics Home Reference - NIH.


   https://www.drugabuse.gov/publications/drugfacts/understanding-drug-use-addiction
   (accessed Sep 13, 2019).

4. National Center for Biotechnology Information. PubChem Database. Dopamine,


7. https://www.painphysicianjournal.com/current/pdf?article=OTg3&journal=42

