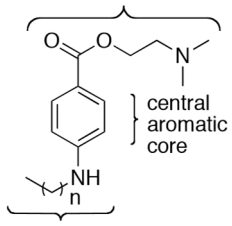
Title of Proposed Research:
The design and synthesis of tetracaine derivatives with enhanced acidity as potent CNG ion channel blockers.

Abstract: Retinitis pigmentosa, a disease that leads to blindness, is caused by excess CNG ion channel activity, which allows an overabundance of calcium ions to enter rod cells. Tetracaine, a local anesthetic, blocks CNG channels with moderate affinity. This proposal seeks to develop more potent blockers of the ion channels that may help to return cells to a healthy equilibrium. This work is focused on two specific goals: understanding the importance of acidity of the aniline in tetracaine and improving the lifetime of tetracaine derivatives. We request funding to support the synthesis, characterization, and binding assay evaluation of several tetracaine derivatives.

Introduction: Cyclic nucleotide-gated (CNG) ion channels play a central role in vision and olfaction, initiating electrical responses to light- and odorant-induced changes in cGMP and cAMP concentration.[1] Several forms of retinitis pigmentosa (RP), a degenerative retinal disorder, affect phototransduction and cGMP metabolism.[2] A genetic mutation in the cGMP phosphodiesterase of rods results in an overabundance of cGMP in the cell causing CNG channels to remain open. Eventually this process results in cell death due to a massive buildup of calcium ions in the cell.[3,4]



1: n= 3, K_d = 4.9 μM
2: n= 7, K_d = 0.8 μM
Figure 1. Tetracaine (**1**) and octylcaine (**2**).

CNG ion channels are located throughout the body. The development of CNG ion channel blockers that can be administered locally and bind specifically in the eye provide an excellent opportunity for the design of treatments for this condition. In *Pde6b*^{rd1} (mouse model of RP) retinal explants,[5] the neuroprotective efficacies of several channel blockers were correlated with their inhibition of cGMP-gated channels. Tetracaine (**1**, Figure 1) is a local anesthetic that has been shown to block a variety of cation channels; however, it blocks with only moderate binding affinity (K_d) and specificity making it unsuitable as a treatment option.[6] The proposed research focuses on the synthesis and evaluation of novel derivatives of tetracaine as potent and specific blockers of CNG channels, and to better understand the functional architecture of the pore and gating machinery of CNG channels. Modifications to the aromatic core, tail, and head regions of **1** will afford derivatives that possess improved pharmacodynamic (binding) and pharmacokinetic (metabolism) profiles relative to **1**.

Significance: An attractive treatment for RP involving high cGMP levels is the design of specific and slowly reversible blockers of CNG channels.[4,7] *L-cis*-diltiazem, which blocks L-type Ca^{2+} channels and CNG channels equally well, and *D-cis*-diltiazem, also fairly nonspecific, are the only channel blockers seriously evaluated so far in the literature for their protective effects.[8] Given the limitations of those compounds, it is evident that the synthesis of a series of more potent and more specific tetracaine derivatives is very desirable. We hypothesize that several of the proposed derivatives will have a neuroprotective effect on retinas in which mutations in photoreceptor proteins have caused too many CNG channels to open. Furthermore, tetracaine provides a platform that is readily accessible. Derivatives can be synthesized in relatively few steps from simple aromatic starting materials using well-established chemical reactions, making this approach viable. Long-term goals include the development of eye drops containing drugs that can be administered locally.

Previous work: Based on extensive studies we have carried out on tetracaine derivatives since 2007, *we hypothesize that enhancing the acidity of the aniline of **1** contributes to its ability to hydrogen bond, an essential element in binding.* Our studies fall into three major categories:

(1) tail length;

(2) *ortho*-substitution of the aromatic core;

(3) heteroatom substitution within the tail.

Our laboratory has found that increasing the hydrophobicity of the tail region of **1** (Figure 1) by substituting an octyl tail (**2**) resulted in a five-fold increase in apparent affinity for the CNG ion channel over native tetracaine (**1**) in an *in vitro* assay. This observed increase in binding affinity was hypothesized to be due to a different binding location in the CNG pore.[9] In contrast, introduction of a polar tail was found to be deleterious to binding.[10] Building on the enhanced binding ability of the elongated tail, an octylcaine series was generated with modifications to the central aromatic core. Binding appears to be impacted by both steric and electronic perturbations of the aromatic ring. Our studies have shown that there is a much greater impact of aromatic substituents on the octylcaine series, perhaps due to an alternate binding site. Attaching strongly σ-electron withdrawing, but π-donating substituents (F, Cl, Br) to the aromatic ring of octylcaine increases binding affinity ten-fold (**4**, **5**, **6**, Figure 2). Electron donating groups (e.g., **3**); however, diminish the affinity, despite being similar in size to the chloro derivative. A correlation is observed between binding affinity (K_d) and pK_a values for *ortho*-substituted aniline derivatives.[11] Those groups that enhance the acidity of the amine lead to higher affinity binding, while those that decrease acidity result in molecules with lower binding affinities for the CNG channels (Table 1). In contrast to this trend, attaching a strongly σ- and π-electron-withdrawing nitro substituent to the aromatic ring (**7**) results in a fifty-fold channel binding decrease.[12] The source of diminished channel binding is intriguing, and may be due to the nitro group's large size, high charge density, or its ability to form an intramolecular hydrogen bond with the adjacent amine. To further investigate this anomaly, we synthesized a cyano derivative (**8**), which is also strongly σ- and π-electron-withdrawing, but removes the aforementioned factors affecting the nitro group. In support of our hypothesis, preliminary binding studies show this derivative to have the lowest pK_a[11] and to bind with the strongest affinity of those measured to date.

To better elucidate the role of the amine, our laboratory replaced the amine with an ether linkage (**9**, Figure 2) and straight alkyl tail (**10**). The oxygen of the ether linkage donates electron density into the ring through the π system, similar to the amine, but eliminates hydrogen bond donation while the alkyl tail removes both properties. Preliminary results show that both of these changes substantially reduce the affinity, confirming the importance of the aniline function on **1**.

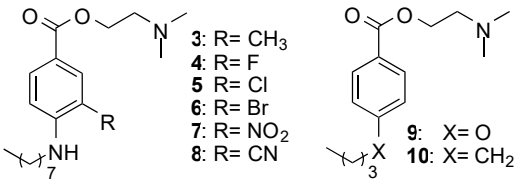


Figure 2. Aromatic-substituted and tail modified derivatives.

Table 1. Dissociation constants for aromatic-substituted compounds[12] and associated pK_a values for ortho-substituted anilines.[11]

Compound	Substituent	K_d (μM)	pK_a
3	CH_3	0.5	4.38
4	F	0.2	2.96
5	Cl	0.14	2.62
6	Br	0.14	2.60
7	NO_2	8.0	0.28
8	CN		0.95

Objective: Synthesize derivatives with increased potency.

(1) *Modify the tail region to elucidate the role of the aniline as well as optimize binding.*

(2) *Modify the aromatic core with electron-withdrawing substituents that enhance affinity, specificity, and solubility.*

Objective: Synthesize hydrolysis resistant derivatives.

(2) Modify the head group attachment to enhance stability of the compounds in vivo.

A complete application that has the following sections:

A. Your name, preferred pronoun, year in school, and your major and minor interests.

Note: Though having taken a year of organic chemistry helps, it is not necessarily required. We have had students of different training levels and backgrounds successfully carry out research in our lab. Everyone is welcome!

B. A name of a faculty member who I can talk to about your abilities in the laboratory and/or classroom.

C. A one-page statement explaining why you want to be part of the program, and what your qualifications are (e.g., interest in biomedical research or medicinal chemistry, relevant course work, previous research experience, and or special skills). Please indicate your preference for studying at Willamette University doing organic synthesis, or at Washington State University doing patch-clamp assays with Prof. Lane Brown. If you are unable or unwilling to participate in one of those two options, please indicate that as well.

D. An unofficial transcript.

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