ACS Medicinal Chemistry Letters

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Novel Modulator of $Na_V1.1$ and $Na_V1.2$ Na^+ Channels in Rat Neuronal Cells

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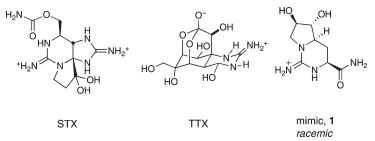
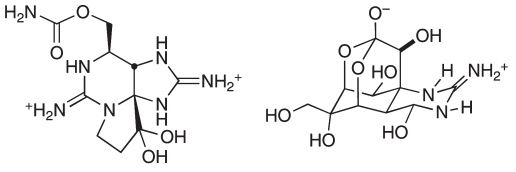
ABSTRACT A novel modulator of sodium ion currents was synthesized in 6 steps from a protected dihydroxypyrrolidine nitrone, *via* 1,3-dipolar cycloaddition reaction with acrylamide. Sodium ion currents in B50 cells were evaluated in comparison to saxitoxin and tetrodotoxin and revealed an IC_{50} of 15.7 μM. The new compound shows no evidence of binding to the C-lobe of the saxitoxin-binding protein saxiphilin.

KEYWORDS Nitrone, 1,3-dipolar cycloaddition, saxitoxin (STX), tetrodotoxin (TTX), sodium channel blocker, B50 cells

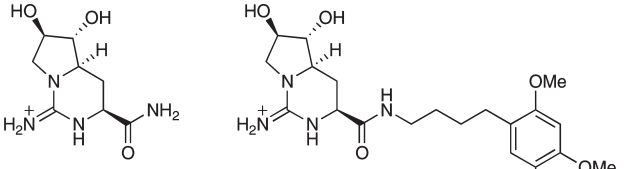


Voltage gated sodium channels are targets for anticonvulsant, antiarrhythmic, and analgesic drugs.[1−5] Sodium channels are responsible for excitatory transmission, and there are more than ten closely related isoforms of mammalian sodium channels known, which primarily affect tissues of the central and peripheral nervous systems, and skeletal muscle.[6] There are a number of drug types that target various sodium channels, and some very potent toxins also target sodium channels. Two such toxins, saxitoxin (STX) and tetrodotoxin (TTX), bind competitively to the same site on voltage-gated sodium channels,[7−10] and are the only two small molecules on the US government's list of select agents.[11] Saxitoxin is a diabolically complex molecule. It has more heteroatoms than carbons, two positive charges, and an unusually low pK_a of 8.24 for the five-membered guanidinium ion (guanidinium itself has a pK_a of ~12.5−13.2). STX and its two-dozen structural analogues are collectively responsible for the symptom known as paralytic shellfish poisoning.[10,12] Tetrodotoxin is similarly complex, and is the causative agent of fugu poisoning: eating of tainted Japanese puffer fish.[13,14]



STX TTX

For several years, we have been interested in new methods of detection of saxitoxin and its analogues,[15] and one of the avenues we have pursued is a displacement assay in a microfluidic channel incorporating an electrochemical detector[16] using the c-lobe of the saxitoxin binding protein, saxiphilin.[17,18] As part of this effort, we sought a new ligand for saxiphilin, a saxitoxin mimic, that could be displaced by saxitoxin and detected electrochemically. We therefore designed the mimic, **1**, and an electrochemically active derivative, **2**, and developed the syntheses outlined in this letter. Although no binding to saxiphilin was detected by surface plasmon resonance spectroscopy, sodium channel blockage analogous to STX and TTX was found in B50 rat neuronal cells[19] that express type I and II sodium channels ($Na_V1.1$ and $Na_V1.2$ in the new terminology[20]), so we hereby disclose our results as a novel scaffold and potential lead for further development.



STX mimic, **1** *racemic*

Electrochemically active STX mimic, **2** *racemic*

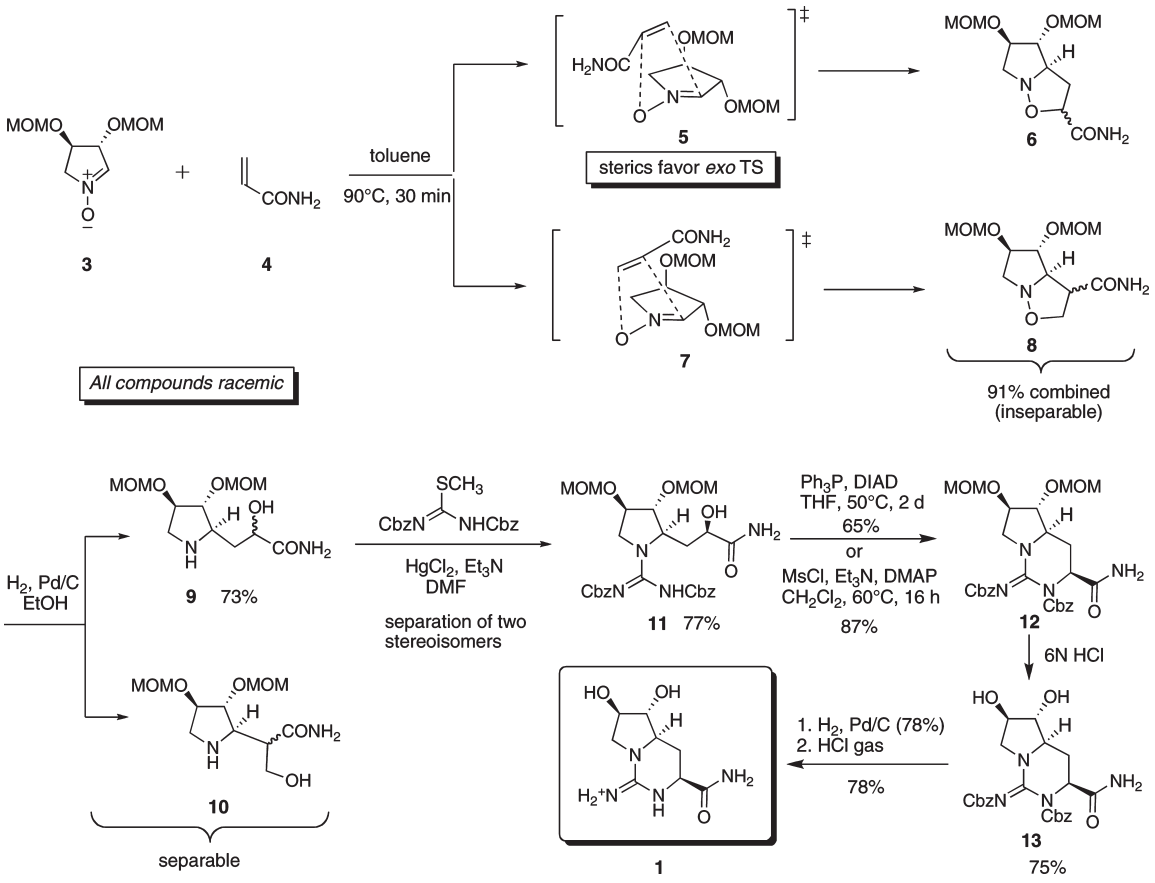
Common features of the structures of STX and TTX are 5- and/or 6-membered rings, multiple heteroatoms, and guanidinium ions. Our STX mimic includes two hydroxyls, one guanidinium, one carboxamide, and two rings. These compounds were prepared using a convergent 1,3-dipolar cycloaddition strategy. To maximize the possibility of observing binding (if binding is enantioselective), the synthesis was executed using racemic tartaric acid. Regiochemical and stereochemical features of the 1,3-dipolar cycloaddition of pyrrolidine nitrones have been carefully studied.[21−23]

The key starting material, (±)-bis(methoxymethoxy)pyrrolidine nitrone **3**, was prepared from *rac*-tartaric acid, according to the literature procedure.[24] Scheme 1 details

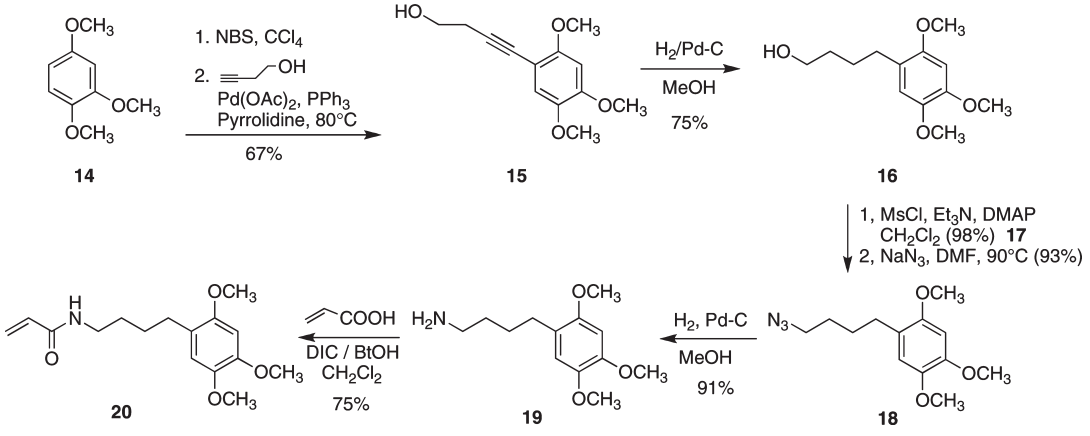
Received Date: February 17, 2010
Accepted Date: March 24, 2010
Published on Web Date: March 31, 2010

DOI: 10.1021/ml100035t | *ACS Med. Chem. Lett.* **2010**, *1*, 135–138

Scheme 1



Scheme 2



the synthesis of the mimic (*rac*-**1**), via 1,3-dipolar cycloaddition of nitrone **3** to acrylamide **4**. Cycloaddition of an unsubstituted nitrone with methyl acrylate affords two regioisomers, with little or no *endo*/*exo* selectivity. In the illustrated case, the two competing transition states, **5** and **7**, provided the two regioisomers **6** and **8**, each as a mixture of two stereoisomers, which were inseparable. Hydrogenolysis gave a separable mixture of constitutional isomers **9** and **10**. Guanidinylation[25] of **9** (mixture of diastereomers) and chromatographic removal of the minor epimer gave **11** in 77 % yield. Cyclization was achieved either under Mitsunobu conditions or, more efficiently, by mesylation of the free hydroxyl group, which resulted in the spontaneous cyclization to give MOM and Cbz protected bicyclic compound **12**. The relative configuration was established by NMR analysis of **12**. After removal of the MOM and Cbz protecting groups, mimic **1** was isolated as its hydrochloride salt.

DOI: 10.1021/ml100035f | *ACS Med. Chem. Lett.* **2010**, *1*, 135–138

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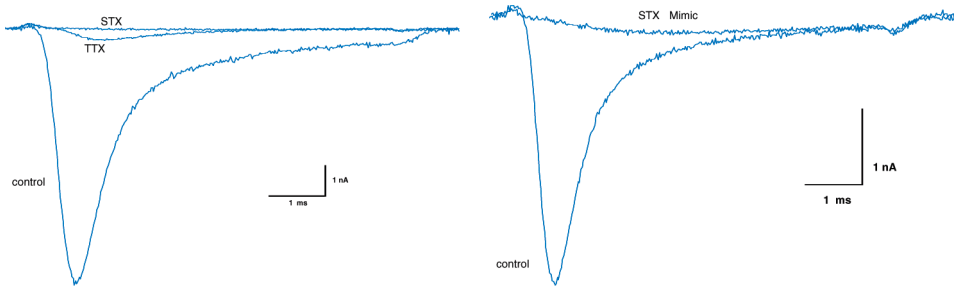
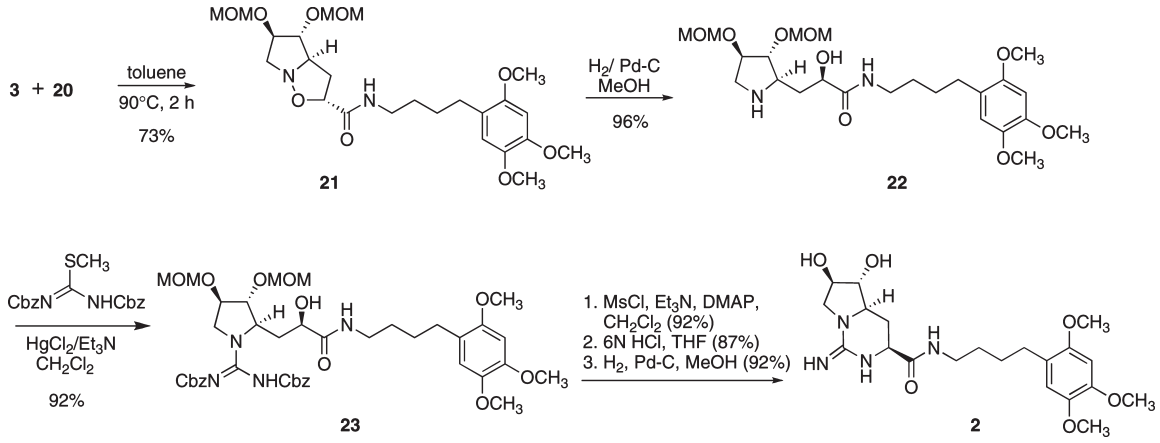


Figure 1. B50 Na^+ currents at −20 mV (V_h = −120 mV) in the extracellular solution (control) and upon exposure to bath-applied tetrodotoxin (TTX; 100 nM), saxitoxin (STX, 12 μM), or **1** (24 μM).

Scheme 3



A similar 1,3-dipolar cycloaddition, employing substituted acrylamide **20**, was used to prepare **2**. The synthesis of the substituted acrylamide was prepared as illustrated in Scheme 2. Bromination of trimethoxybenzene **14** and coupling[26] afforded alkyne **15** in 67% overall yield. Reduction of the alkyne to **16**, mesylation of the hydroxyl to give **17** and azide displacement gave **18** in 68% overall yield for the 3 steps. Reduction to amine **19** and carbodiimide coupling with acrylic acid gave acetamide **20** in 68% yield for the two steps (31% for the seven steps overall).

Cycloaddition of nitrone **3** with acrylamide **20** gave pyrrolidinoisoxazole **21** as the major isomer, and reduction afforded pyrrolidine **22** in 70% yield for the two steps (Scheme 3). Guanidinylation, mesylation/cyclization, and deprotection as before afforded the electrochemically active mimic **2** in a total of 6 steps and 47% overall yield. Full experimental details for the three synthetic schemes are in the Supporting Information.

The STX mimic **1** was tested for sodium channel activity in B50 rat neuronal cells.[19] This cell line originates from the rat CNS[19] and expresses types I and II Na channel mRNAs ($Na_V1.1$ and $Na_V1.2$).[20] Specifically, whole cell Na currents were recorded in B50 cells in extracellular solution (ECS) containing (mM): 170 NaCl, 3 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 10 HEPES−NaOH, pH 7.25. The intracellular solution contained (mM) 150 KCl, 1 ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 1 $CaCl_2$, 5 $MgCl_2$, 5 Na_2ATP and 40 *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid] (HEPES)−KOH, pH 7.0. Drugs were applied to cells during a series of depolarizing episodes of 7 ms separated by 4 s intervals at the holding potential (V_h) of −120 mV. Tetrodotoxin (100 nM in ECS) was applied as a bolus volume of 50 μL in a 1.5 mL bath. STX and our prototype mimic were continuously bath-applied to cells via a 1 μL pipet. Both STX and 1 mimic blocked the same currents blocked by TTX, as shown in Figure 1. (STX and TTX bind competitively to site 1 on voltage gated sodium channels.)

Figure 2 shows the dose−response relationship for the inhibition of Na current by mimic **1** in B50 neuronal cells. The curve was fit to the equation

$$I/I_{max} = I_{max} - (I_{max}/[1 + (\exp(-(B - IC_{50})/n))])$$

where I is the current amplitude, B is the concentration of the **1**, n is the Hill coefficient, and IC_{50} is the half-inhibitory concentration to the electrophysiological responses to bath-applied **1**. The IC_{50} value derived from Figure 2 is 15.7 μM; the Hill coefficient of 2.95 implies some allosteric cooperativity in the binding of the mimic. In comparison, saxitoxin shows an IC_{50} value of 0.38 μM, with a Hill coefficient of 0.99, in these same cells (data not shown).

DOI: 10.1021/ml100035t | ACS Med. Chem. Lett. 2010, 1, 135–138

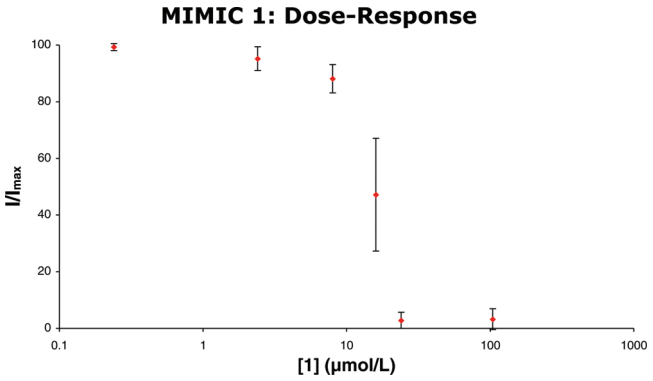


Figure 2

CONCLUSION In summary, the synthetic STX mimic **1** and its electroactive mimic **2** were successfully obtained via the 1,3-dipolar cycloaddition reaction. Either acrylamides **4** or **20** are suitable dipolarophiles toward the nitrone, which led to the final STX mimic and its corresponding electrochemically active derivative. It is very encouraging that the STX mimic exerts a similar effect on the sodium channel of neuronal rat cells, probably by binding to the same site as STX and TTX (site 1).

SUPPORTING INFORMATION AVAILABLE Synthesis and characterization of all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions: ‖ All synthetic work was done by H.M., and the electrophysiology by L.A.F.

Funding Sources: Core facilities for this work were funded by the Arkansas Biosciences Institute and the National Institutes of Health (RR15569).

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DOI: 10.1021/ml100035t | *ACS Med. Chem. Lett.* **2010,** *1*, 135–138