Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 2, pp. 181-186, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(96)00596-3

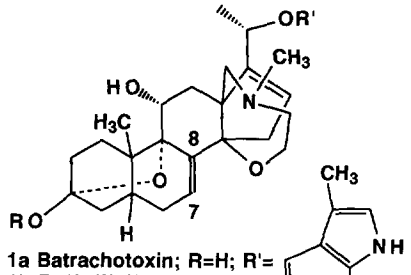
BATRACHOTOXIN BINDING SITE ANTAGONISTS

S. R. Schow,*[1a] D. P. Rossignol,[1b] A. E. Lund, and M. E. Schnee
Biological Chemistry Group, APD, E.I. duPont de Nemours and Company, Experimental Station, Wilmington, DE 19898-0400

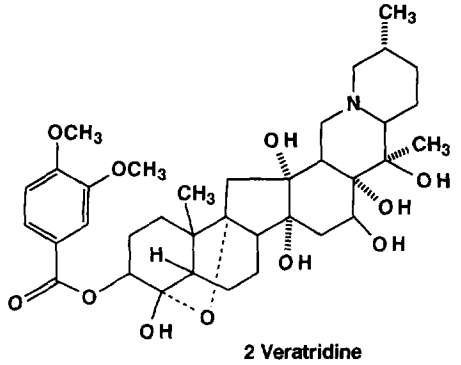
Abstract: The preparation and SAR of a series of batrachotoxin partial structures that antagonize binding and activity of batrachotoxin are reported in this communication.

Propagation of an action potential along the neuronal axon is accomplished by modulating influx of sodium ions through voltage dependent sodium ion channels in the axonal membrane.[2,3] The primary voltage-dependent sodium ion channel is a 260 kDa transmembrane glycoprotein that can sense and respond to membrane potentials by altering its conformation between a number of distinct states.[4] As measured electrophysiologically, the simplest model for this channel function describes it as fluctuating between three conformational states: resting, open, and inactivated. Biochemically, the characterization of this channel and analysis of its state changes has been facilitated by studies on the mechanism of activity of potent, specific, sodium ion channel neurotoxins. These include sodium channel blockers, such as tetrodotoxin and saxitoxin, as well as compounds that induce or increase sodium ion influx, such as specific groups of lipophilic toxins and a class of protein toxins isolated from scorpion venoms.[5]

We have investigated the class of sodium ion channel neurotoxins collectively known as class II lipophilic toxins.[6] This class of neurotoxins consists of four structurally unrelated compounds: grayanotoxin I (a terpene), and three alkaloids; aconitine, batrachotoxin **1a** and veratridine **2**.[6] Catterall demonstrated that these compounds exert their neurotoxic effects by binding to a common site on the ion channel and inhibiting the channel's ability to close (inactivate).[7] Delaying inactivation allows uncontrolled influx of sodium ions into the neuron that collapses the nerve membrane potential and triggers uncontrolled neurotransmitter release at the synapse. This results in neuronal hyperexcitation, convulsions, and death.



1a Batrachotoxin; R=H; R'=
1b R=H; R'=Benzoyl
1c R=H; R'=H
1d R=CH_3; R' as in 1a

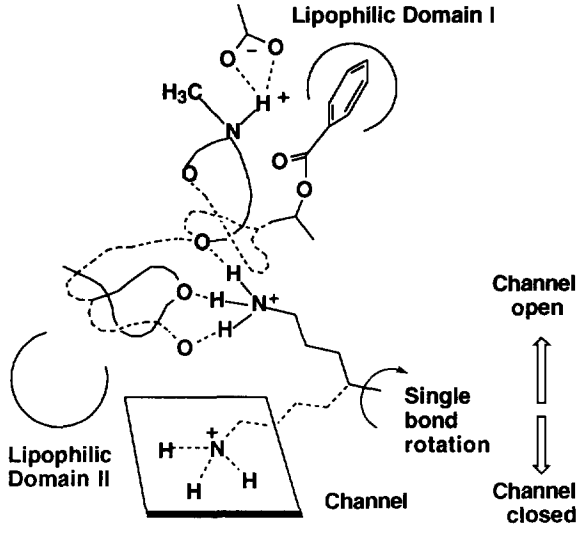


2 Veratridine

Early investigators have speculated on which of the features common to these four toxins may be responsible for their competitive binding properties, and their effects on channel dynamics.[8] Preliminary model building studies suggest that each of the four molecules contains a triad of oxygen atoms approximately 3X 2.5X 5 angstroms, as well as a nitrogen atom (in the three alkaloids) or an oxygen atom (in the terpene grayanotoxin), 5-6 angstroms from the center of the oxygen triad. Codding performed numerous molecular modeling studies using the X-ray crystal structures of the toxins, specifically confirming the atomic overlay of the oxygen triad and remote heteroatom in each structure.[9]

Kosower[10] subsequently proposed that a protonated lysine group near the channel pore opening was responsible for modulating the gating of the channel. In the closed state, the ammonium moiety of a lysine residue would thwart entry of sodium ions into the channel. Movement of the ammonium group into a different conformation, away from the pore opening, would convert the channel to the open state. If the toxin binding site were in the vicinity of this lysine, the triad of oxygens on the toxin could act as a crown ether, capturing the ammonium moiety when shifted away from the channel opening, thus hindering its ability to close the pore opening. The remote heteroatom of the toxin was hypothesized to be a hydrogen acceptor for facilitating high affinity binding of the toxin to the channel.[11] Figure 1 is a graphic representation of this concept.[10]

Figure 1. Channel Opening Via Lysine Capture By Batrachotoxin.



The original model was modified, because examination of the SAR for batrachotoxin **1a** and veratridine **2** suggested that large ancillary lipophilic pockets were required to accommodate both of these steroidal alkaloids.[9,12] Studies on the SAR for batrachotoxin toxicity by Daly and Torgov[11,13,14] demonstrated that an aromatic ester at C20 (**1a** and **1b**) was required for neurotoxic potency. Benzoate **1b** was equipotent to the natural product **1a**,[11,13] whereas the simple alcohol at C-20 (**1c**) was a thousandfold less active than either ester.[11,13]

For this study, we explored the validity of the Kosower hypothesis. We examined the necessity of the oxygen triad, heteroatom and lipophilic moiety[10,11] by preparing and evaluating a number of potential pharmacophore representatives of these toxins. We focused on batrachotoxin **1a**, because it had the least complex structure in the group and the existing SAR suggested a relatively straightforward series of target compounds. To this end, the batrachotoxin

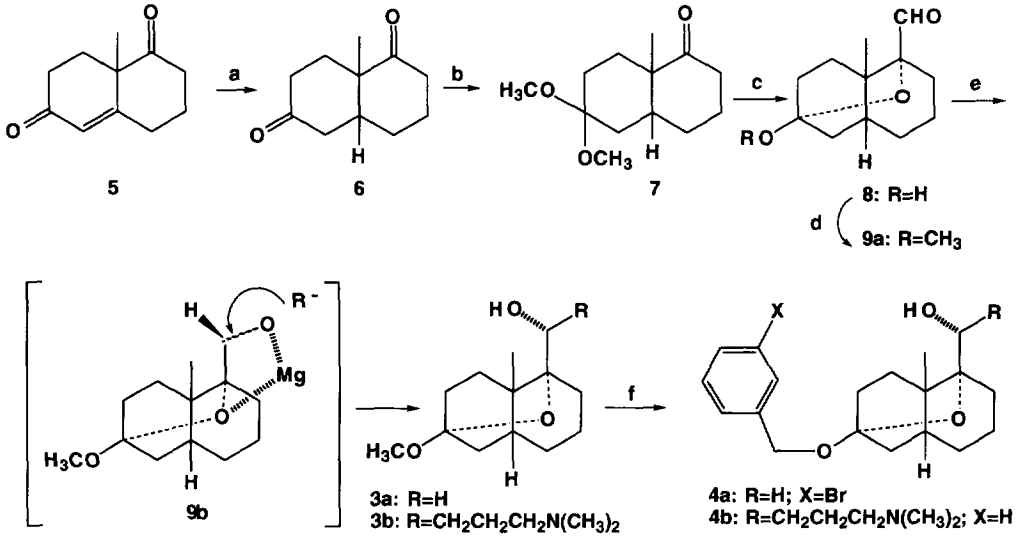
partial structures **3a-d** and **4a-b** which display the key molecular features of the class II channel toxins, were prepared as pharmacophore targets to probe for potential batrachotoxin activity.

In order to simplify our synthetic efforts, we took two other SAR observations into consideration in designing our probes. Daly had shown that the mixed ketal **1d** was only fivefold less potent than the corresponding hemiketal **1a**, thus demonstrating that it was not necessary to preserve the hemiketal functionality at C-3 in order to have high levels of neurotoxicity.[13] Finally, Yelin and coworkers prepared 7,8-dihydrobatrachotoxin and reported it equipotent to the parent natural product **1a**, suggesting that the 7,8-double bond was not crucial for activity.[14]

Chemistry

The synthesis of targets **3a-b** and **4a-b** is presented in Scheme 1.

Scheme 1.



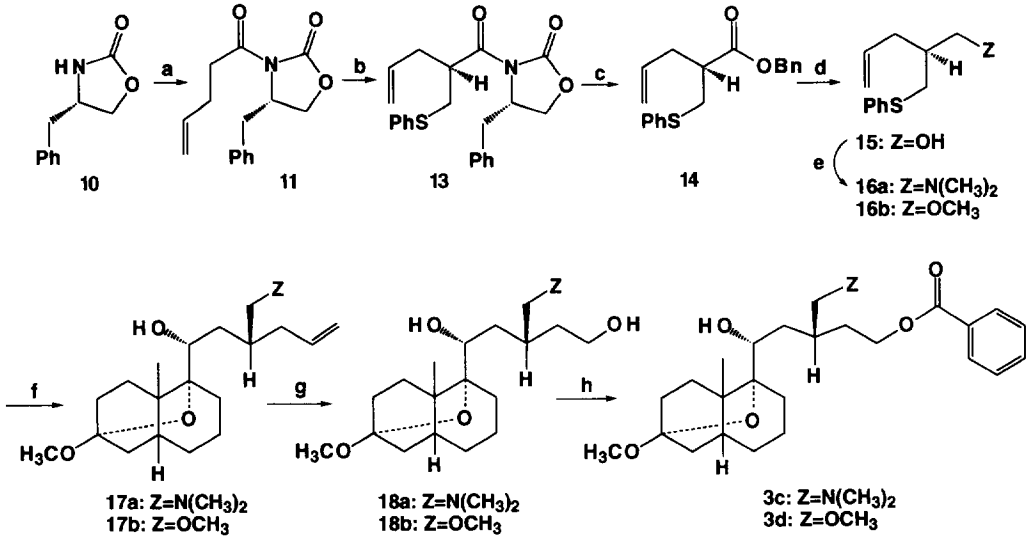
Reagents: (a) H_2/Pd/C/ethanol/HCl, 98%; (b) CH_3OH/TsOH, 81%; (c) i. 2-lithio-1,3-dithiane, ii. $HgCl_2/H_2O$; (d) $CeCl_3/CH_3OH$, 44% for combined steps c and d; (e) $NaBH_4$ to give **3a**, 83% or $ClMg(CH_2)_3N(CH_3)_2$/THF/-78 °C to give **3b**, 79%; (f) TsOH/ *m*-bromobenzyl alcohol to give **4a**, 86% or TsOH/ benzyl alcohol to give **4b**, 79%.

Optically pure Wieland-Miescher ketone **5**[15] was reduced to the previously reported *cis*-decalin **6**.[16] This diketone was monoprotected in acidic methanol to yield ketal **7**. Treatment of **7** with lithiodithiane, followed by mercuric chloride hydrolysis of the thioketal adduct, gave unstable aldehyde **8**. The NMR spectrum of **8** suggested that the compound existed as an equilibrium mixture of keto and hemiketal forms of the molecule. Subsequent reaction of **8** with methanol and cerium(III) chloride yielded aldehyde **9a**.[17]

Compound **9a** was a key intermediate for the preparation of all of the targets listed above. Reduction of **9a** with sodium borohydride gave **3a**. Ketal exchange in the presence of acidic benzyl alcohol solution resulted in the preparation of target **4a**. The addition of Grignard reagent to aldehyde **9a** gave adduct **3b**. The stereochemistry at C-11 in **3b** reflected the addition of the anion to the less hindered face of the magnesium chelated aldehyde (away from the quaternary center, e.g., **9b**). The addition of the less chelating organolithium reagents gave mixtures of stereoisomers. Ketal exchange as outlined above converted **3b** to **4b**.

As outlined in Scheme 2, the preparation of targets **3c-d** required more extensive synthesis than the initial targets.

Scheme 2.



Reagents: (a) i. *n*-BuLi, ii. 4-pentenoyl chloride, 100%; (b) i. LiHMDS/THF/-78 °C, ii. $PhSCH_2I$(**12**)/-20 °C/120h, 74%; (c) LiOBn/THF, 87%; (d) DIBAL/THF; (e) TsCl/TEA, i. $(CH_3)_2NH$ /DMF to give **16a**, 61% from **14**; ii. CH_3ONa/THF to give **16b**, 55% from **14**; (f) i. LDBB/THF/-78 °C, ii. $MgBr_2$/THF, iii. **9a**, 100%(**17a**), 97%(**17b**); g) i. H^+ for **17a** only, ii. $O_3/CHCl_3$/-78 °C, iii. $NaBH_4$/THF; (h) PhCOCl/TEA/-78 °C, 77% (**3c** from **17a**) or 83% (**3d** from **17b**).

The key chiral sidechain intermediates **16a-b** were prepared utilizing chemistry developed by Evans and Baker.[18,19] Acylation of Evans chiral auxiliary **10** with 4-pentenoyl chloride under standard conditions gave oxazolilone **11**.[18] Alkylation of the lithium salt of **11** with iodide **12** under the unusual conditions reported by Baker gave adduct **13**.[19] Chromatography of impure **13** yielded material that appeared to be single diasteromer by NMR.[20] Treatment of **13** with lithium benzyloxide resulted in the formation of ester **14**, which was subsequently reduced with DIBAL to give alcohol **15**. This alcohol was converted to the tosylate in situ and then treated with either DMF saturated with dimethylamine or sodium methoxide in THF to give sidechain intermediates **16a** and **16b**, respectively.

The conversion of intermediates **16a** and **16b** to the corresponding organomagnesium compounds was carried out in a two-step procedure. The reductive removal of the thiophenol moiety to generate the individual alkyl lithium derivatives was facilitated by lithium 4,4'-*tert*-butylbiphenylide (LDBB).[21] Upon the addition of one equivalent magnesium bromide in THF, the alkyllithium derivatives were converted to the respective Grignard agents. As outlined in Scheme 1, these anions were allowed to react with aldehyde **9a** to give the corresponding adducts **17a** and **17b**. The stereochemistry for the newly generated C-11 alcohol is as described above for compound **3b**.

Compound **17a** was converted to target **3c** by a three-step procedure. The amino group was protected by protonation with HCl in methanol and then the salt treated with ozone at -78 °C. The solution of crude ozonide was reduced with excess sodium borohydride to yield alcohol **18a** after workup. Benzyolation gave target **3c**. Except for the protonation protection step, the procedure for conversion of **17b** to **3d** via alcohol **18b** was identical to that described above for the preparation of **3c**.

Biological Evaluation

The compounds prepared for this study were examined in four assay systems designed to identify batrachotoxin-like agonist activity and antagonism of batrachotoxin binding and activity.[22] The initial two assays were used to evaluate our compounds for activity mimicking that of batrachotoxin (i.e., delaying inactivation of voltage-dependent sodium channels). First, in electrophysiological recordings of nerve preparations,[22] batrachotoxin causes repetitive firing with subsequent nerve depolarization. Even at high concentrations, none of the compounds prepared in this study provoked an electrophysiological signature remotely resembling that generated by batrachotoxin. Another assay involved measuring the uptake of ^{22}Na into cultured neuroblastoma cells possessing voltage-dependent sodium channels. When batrachotoxin is added to these cells along with α-scorpion polypeptide toxin(Leiurus quinquestriatus), a significantly enhanced uptake of radioactivity is observed over background.[22] Again, the molecules reported here failed to display any activity in this assay that could be attributable to a batrachotoxin-like effect.

In the second series of tests, the target molecules were examined for their ability to antagonize the above-described batrachotoxin-mediated ^{22}Na uptake into cells, as well as antagonism of [^{3}H]batrachotoxin binding to rat brain neuronal membranes. As shown in Table 1, several of these compounds displayed potent antagonism of batrachotoxin binding and activity. Furthermore, a structure-activity pattern emerges from the results of these assays. The antagonistic potencies in both assay systems coincide for each compound, suggesting that antagonism of batrachotoxin activity is likely to be the result of antagonism of batrachotoxin binding.

Table 1. Inhibition Batrachotoxin Binding And Activity By Batrachotoxin Analogs(IC_{50} in μM).

COMPOUND	SODIUM ION UPTAKE	BATRACHOTOXIN BINDING
3a	1000	2000
3b	200	130
3c	0.5	3.6
3d	5.0	10
4a	80	10
4b	4.0	4.5
tetracaine	0.4	6
veratridine	--	22

The data for compounds **3a** and **3b** suggests that for these semi-rigid batrachotoxin analogs, the triad of oxygens, with or without the nitrogen, is insufficient functionality to compete for the batrachotoxin binding. However, the addition of a lipophilic structural moiety to both **3a** and **3b** to yield **4a** and **4b**, respectively, gave compounds that demonstrate modest to very good antagonism of batrachotoxin activity and binding, indicating that these analogs posses determinants necessary for interacting with the batrachotoxin binding site. The presence of all three hypothesized pharmacophore groups (**4b**) appears to significantly enhance antagonism.

The analog which most resembles batrachotoxin, **3c**, demonstrates the highest levels of antagonist activity. Taken together, these results suggest that the Codding/Kosower pharmacophore does indeed model batrachotoxin binding functionality. However, the necessary structural requirements for exact batrachotoxin mimicry remained to be defined. Clearly, structural rigidity and perhaps additional lipophilic interactions may be important for capturing the full range of batrachotoxin activity.

While the structure-activity relationships outlined above seemed to be consistent with our data, Daly[23] and Catterall[24] subsequently reported that a broad class of lipophilic amines and local anesthetics displayed effects on batrachotoxin binding and batrachotoxin stimulated sodium ion uptake similar to those that we had observed for our batrachotoxin analogs. These effects were ascribed to an allosteric binding phenomena.[23] The results of these studies suggested that our lipophilic amines, **4b** and **3c**, may be nonspecific antagonists. Off-rate studies performed in the presence of **3c** demonstrated that **3c** significantly enhanced the batrachotoxin off rate (approximately 4-fold at a concentration four times its IC_{50} of 16 μM as compared to the allosteric antagonist tetracaine which showed a 8.4-fold enhanced off rate at four times its IC_{50}), implying that **3c** was an allosteric antagonist.[24]

The data on compound **4a** suggested that compounds without a basic nitrogen would antagonize batrachotoxin binding and batrachotoxin driven sodium ion uptake, so the oxygen analog (**3d**) of **3c** was prepared as outlined in Scheme 2. As can be seen in Table 1, target **3d** is only slightly less potent than **3c** in both antagonist assay systems. In an off-rate study to determine if **3d** was an allosteric modifier of BTX binding, it was found that at concentrations up to 100 μM of **3d** (10 times its IC_{50}) no significant enhancement of the off rate of batrachotoxin was observed, thus confirming that at least for analog **3d**, and perhaps in part for **3c**, key structural requirements for batrachotoxin binding had been identified.[25]

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25. Authors wish to acknowledge fruitful discussions with Professor Edward Kosower and Dr. John Daly; and for the technical assistance provided by J. Krwyko, P. Pipenberg, and C. Lynch.

(*Received in USA* 25 *September* 1996; *accepted* 6 *December* 1996)