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3-Amino-1,5-benzodiazepinones: Potent, state-dependent sodium channel blockers with anti-epileptic activity

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Abstract—A series of 3-amino-1,5-benzodiazepinones were synthesized and evaluated as potential sodium channel blockers in a functional, membrane potential-based assay. One member of this series displayed subnanomolar, state-dependent sodium channel block, and was orally efficacious in a mouse model of epilepsy.

Approximately two dozen drugs are currently marketed for the treatment of epilepsy, a common CNS disorder that afflicts nearly 2% of the world's population.[1] Some act by sodium or calcium channel blockade, while others function at the $GABA_A$ receptor, or by mixed or unknown mechanisms of action. Though a broad range of treatment options is available, an estimated 30% of patients do not respond to any current therapy.[2] Additionally, in patients who do respond, existing drugs often elicit adverse effects such as sedation and neuronal impairment.

Epileptic seizures begin with the aberrant firing of action potential bursts in the brain. The initiation and propagation of these action potentials typically require the opening of voltage-gated sodium channels ($Na_v1.x$). Because they can inhibit action potential firing, sodium channel blockers have been investigated as anti-epileptic treatments. Weak blockers such as carbamazepine and lamotrigine have demonstrated clinical anticonvulsant activity, thereby providing validation for this approach (Fig. 1).[3,4]

Sodium channel blockers destined for the clinic must inhibit aberrant neuronal signaling while leaving normal nerve functions intact. We believe that this can be achieved, in large part, via state-dependent channel block. Sodium channels are thought to exist in three main conformational states: resting, open, and inactivated. In healthy nerve and cardiac tissue, these channels exist predominantly in the resting state. During seizure, on the other hand, the aberrant firing of high-frequency action potential bursts causes sodium channels to accumulate in the inactivated state. Compounds that selectively bind and stabilize that inactivated state should inhibit aberrant signaling preferentially, thus minimizing the potential for mechanism-based adverse effects.

We recently reported the discovery of a structurally novel series of benzazepinone sodium channel blockers.

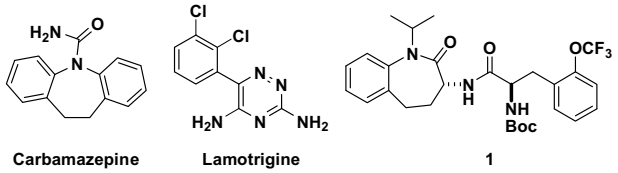

Figure 1. Sodium channel blockers.

Keywords: Sodium channel blocker; Na_v1; Epilepsy.
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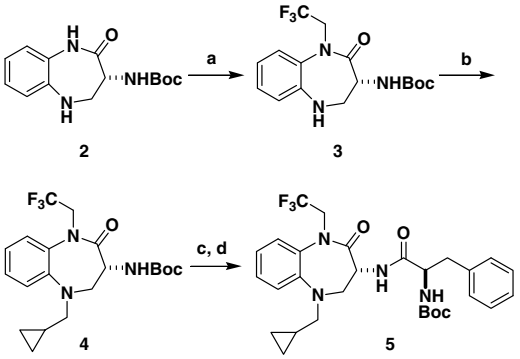


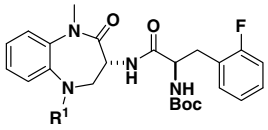
Scheme 1. Reagents and conditions: (a) KHMDS, CF_3CH_2OTf, THF, 0 °C (67%); (b) cyclopropylmethyl bromide, K_2CO_3, Bu_4NI, THF, 100 °C (53%); (c) TFA, CH_2Cl_2; (d)*N*-Boc-D-phenylalanine, BOP, *i*-Pr_2NEt, CH_2Cl_2.

A benchmark in this class displayed potent, state-dependent block of $hNa_v1.7$ in vitro (IC_{50} = 35 nM), blocked spontaneous neuronal firing in vivo, and was orally efficacious in a rat model of neuropathic pain (compound **1**, Fig. 1).[5] Explorations in this general class led us to examine SAR in a related series of 3-amino-1,5-benzodiazepinones. These efforts culminated in the discovery of a highly potent, state-dependent sodium channel blocker that shows good oral efficacy in a rat model of epilepsy.

Target compounds were synthesized as shown in Scheme 1. Benzodiazepinone **2**, the enantiomer of a known compound, was prepared in three steps from commercially available materials via an established procedure.[6] Treatment of **2** with two equivalents of KHMDS and an alkyl halide or triflate such as 2,2,2-trifluoroethane trifluorosulfonate resulted in alkylation to yield **3**. Heating of **3** in the presence of K_2CO_3 and an electrophile such as cyclopropylmethyl bromide then effected alkylation of the anilinic nitrogen to afford **4**. Finally, exposure of **4** to standard conditions for *N*-Boc removal (TFA, CH_2Cl_2) provided a crude TFA salt that could be coupled with amino acids such as *N*-Boc-D-pheynylalanine to afford target compound **5**. Compounds listed in Tables 1–3 were synthesized according to these procedures using the appropriate commercially available starting materials. In several instances (**19** and **27**), the final amide coupling step was performed using a non-commercially available amino acid that had been synthesized via the method of Schollkopf.[7]

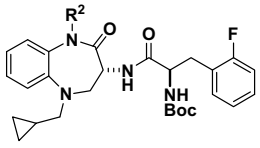
Once synthesized, compounds were then tested for their ability to block $hNa_v1.7$ sodium channels that had been stably expressed in a HEK-293 cell line. The extent of channel block was determined in a functional, membrane potential-based assay that measures the fluorescence resonance energy transfer (FRET) between two membrane-associated dyes. Specific details of the experimental protocols employed have recently been described.[8] Compounds were also counterscreened at several other ion channels targets. Because block of hERG K^+ channels has been associated with potentially lethal ventricular arrhythmias, compounds were screened in a binding assay that measures the displace-

Table 1. Effect of the R^1 group on potency and off-target activity



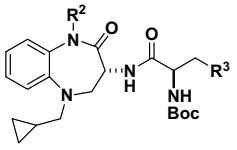
Compound	R^1	$hNa_v1.7$ (IC_{50}, nM)	MK-0499 (% inhibition at 1, 10 μM)
6	H	409	0%, 25%
7	Me	142	0%, 7%
8	F_3C	47	36%, 62%
9		49	1%, 45%
10		81	20%, 65%
11		188	17%, 43%
12		25	14%, 84%

Table 2. Effect of the R^2 group on potency and off-target activity



Compound	R^2	$hNa_v1.7$ (IC_{50}, nM)	MK-0499 (% inhibition at 1, 10 μM)
9	Me	49	1%, 45%
13	CH_2CF_3	101	7%, 20%
14	*i*-Pr	102	7%, 33%

Table 3. Combined effects of R_1 and R_2 on potency and off-target activity



Compound	R^2	R^3	$hNa_v1.7$ (IC_{50}, nM)	MK-0499 (% inhibition at 10 μM)
5	CH_2CF_3	Ph	30	24%
13	CH_2CF_3	2-F–Ph	101	19%
15	CH_2CF_3	3-F–Ph	142	17%
16	CH_2CF_3	4-F–Ph	219	18%
17	CH_2CF_3	2,6-di-F–Ph	185	12%
18	CH_2CF_3	2-CF_3–Ph	131	11%
19	CH_2CF_3	2-OCF_3–Ph	73	13%
20	CH_2CF_3	2,5-di-CF_3–Ph	120	6%
21	*i*-Pr	Ph	52	42%
22	*i*-Pr	2-F–Ph	102	32%
23	*i*-Pr	3-F–Ph	96	17%
24	*i*-Pr	4-F–Ph	196	29%
25	*i*-Pr	2,6-di-F–Ph	97	25%
26	*i*-Pr	2-CF_3–Ph	149	21%
27	*i*-Pr	2-OCF_3–Ph	75	35%

ment of ^{35}S-labeled MK-0499, a known hERG K^+ channel blocker.[9]

As shown in Table 1, a variety of *N*-methyl benzodiazepinones bearing a 2-fluorophenylalanine sidechain were prepared. In this series, the impact of the R^1 group on potency was examined. Analogs with small R^1 substituents such as **6** and **7** (R^1 = H and Me) displayed only moderate $hNa_v1.7$ block. As the size of R^1 increased, potency improved, reaching an optimum level in the trifluoroethyl and methylenecyclopropyl analogs **8** and **9**. Compounds such as **10** and **11** that incorporated larger R^1 groups were correspondingly less potent. In a notable exception to this trend, *N*-benzyl derivative **12** exhibited best-in-series $hNa_v1.7$ block. Unfortunately, **12** was also one of the most active compounds in the MK-0499 counterscreen. On balance, the methylenecyclopropyl group seemed to provide the best combination of potency and low MK-0499 activity, and was thus featured in further studies.

With an optimized R^1 group in hand, we next conducted a limited scan at the lactam R^2 position. To keep final target molecular weights as low as possible, we focused on smaller alkyl groups. As shown in Table 2, the *N*-methyl, *N*-trifluoroethyl and *N*-isopropyl analogs **9**, **13**, and **14** exhibited comparable potencies in both the $hNa_v1.7$ functional assay and the MK-0499 binding assay. Because they can hinder metabolic *N*-dealkylation, the trifluoroethyl and isopropyl groups were thought to offer potential pharmacokinetic advantages, and were thus incorporated in subsequent designs.

A final series of derivatives was prepared wherein the R^3 group was varied (Table 3). Prior work had shown that a lipophilic aromatic substituent was required at R^3 for potent $hNa_v1.7$ block. In the *N*-trifluoroethyl series, a number of mono- and difluorophenyl derivatives (compounds **13** and **15**–**17**) were examined; all were found to be less potent than simple phenyl analog **5**. Because substitution seemed best tolerated at the 2-position, the 2-CF_3–Ph and 2-OCF_3–Ph variants **18** and **19** were prepared; these too were less potent than **5**. Similar trends were observed in the *N*-isopropyl series (compounds **21**–**27**). In this set, substituted derivatives **22**–**27** were typically 2- to 4-fold less potent than the simple phenyl analog **21**.

Maximally potent blocker **5** was selected for further profiling. For reasons outlined above, it was important to ascertain whether **5** blocked $hNa_v1.7$ channels in a state-dependent manner. Block of $hNa_v1.7$ channels by compound **5** was therefore examined by whole cell electrophysiology in stably transfected HEK-293 cells.[10] Figure 2A shows the peak $hNa_v1.7$ current evoked by 20 ms depolarizations from a membrane potential of −70 mV. The solid bar indicates bath application of 3 nM compound **5**. The dose–response for inhibition of $hNa_v1.7$ current by compound **5**, applied at −70 mV, is shown in Figure 2B (n = 2 for each concentration). Fitting the data to the Hill equation yielded an IC_{50} of 1.54 nM. In contrast, 1 μM compound **5** blocked less than 10% of $hNa_v1.7$ currents when applied at a membrane potential of −120 mV. The dependence of

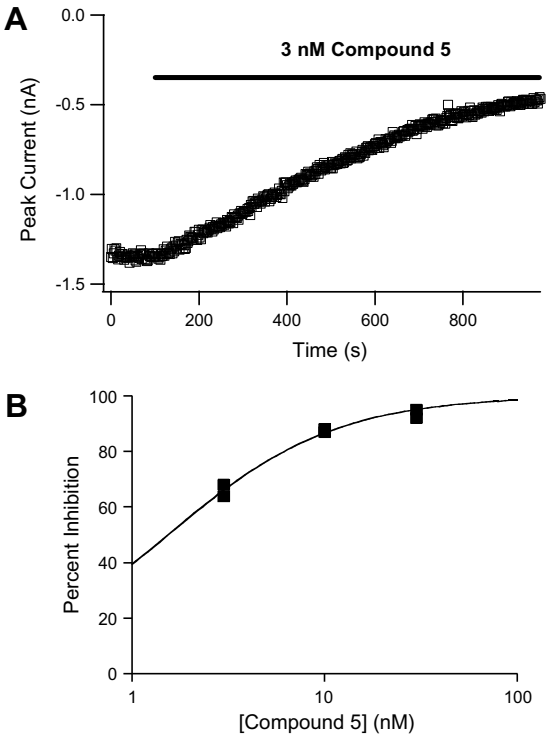
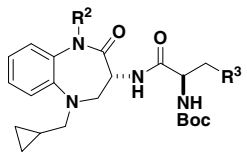


Figure 2. Whole cell electrophysiology data for compound **5**.

block on membrane potential is consistent with preferential block of channels in the inactivated state. At −120 mV, essentially all $hNa_v1.7$ channels reside in the resting state, suggesting that compound **5** affords little binding to resting channels (K_r > 5 μM). At −70 mV, an average of 41% of $hNa_v1.7$ channels reside in the inactivated state (n = 107). Based on the fraction of inactivated channels and the IC_{50} at −70 mV, the affinity of compound **5** for inactivated channels (K_i) was calculated to be 0.63 nM. By this measure, compound **5** is one of the most potent small-molecule sodium channel blockers described in the literature to date.

Compound **5** and several of its analogs were submitted for rat pharmacokinetic (PK) determination.[11] As shown in Table 4, **5** exhibited a modest PK profile,

Table 4. Rat pharmacokinetic data for selected compounds



Compound	R^2	R^3	F%	AUC_N[a]	C_{max}[b]	Cl_P[c]	$t_{1/2}$[d]
5	CH_2CF_3	Ph	5%	0.03	0.03	48	3.1
18	CH_2CF_3	2-CF_3–Ph	10%	0.05	0.06	51	2.4
26	*i*-Pr	2-CF_3–Ph	9%	0.11	0.15	23	2.5
27	*i*-Pr	2-OCF_3–Ph	4%	0.04	0.04	30	2.4

[a] (po, μM h/mpk).
[b] (μM).
[c] (mL/min/kg).
[d] (hours).

and suffered both low bioavailability and a high clearance rate ($F = 5\%$, $Cl_p = 48$ mL/min/kg). Of the other analogs studied, **26** displayed the best profile, offering a moderate improvement over **5** ($F = 9\%$, $Cl_p = 23$ mL/min/kg, $C_{max} = 150$ nM).

Compound **5** proved highly efficacious in the mouse maximum electroshock (MES) assay, a widely used protocol for assessing anticonvulsant activity.[12] When dosed orally at 3 mg/kg, **5** prevented shock induced tonic–clonic seizures in 90% of subjects ($n = 10$) at 30 min post-dosing. These results are broadly comparable to those obtained with clinical standards such as carbamazepine (MES $ED_{50} = 3.4$ mg/kg) and lamotrigine (MES $ED_{50} = 2.2$ mg/kg).[13] Though brain levels of **5** were not determined, these initial results are promising, and provide a basis for further investigation.

In summary, we have identified a series of 3-amino-1,5-benzodiazepinones that are potent blockers of voltage-gated sodium channels. A benchmark compound from this class exhibited state-dependent, subnanomolar block of $hNa_v1.7$, and was orally efficacious in a mouse model of epilepsy. Future work will focus on improving pharmacokinetics in this series, and will be reported in due course.

Acknowledgments

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10. Hamill, O. P.; Marty, A.; Neher, E.; Sakmann, B.; Sigworth, F. J. *Pflugers Arch.* **1981**, *391*, 85. *Procedure.* Sodium currents were examined by whole cell voltage clamp using an EPC-9 amplifier and Pulse software (HEKA Electronics, Lamprecht, Germany). Experiments were performed at room temperature. Electrodes were fire-polished to resistances of 1.5–4 MΩ. Voltage errors were minimized by series resistance compensation (75–85%), and the capacitance artifact was canceled using the amplifier's built-in circuitry. Data were acquired at 50 kHz and filtered at 10 kHz. The bath solution consisted of 40 mM NaCl, 120 mM NMDG–Cl, 1 mM KCl, 2.7 mM $CaCl_2$, 0.5 mM $MgCl_2$, 10 mM NMDG–Hepes, pH 7.4, and the internal (pipet) solution contained 110 mM Cs–methanesulfonate, 5 mM NaCl, 20 mM CsCl, 10 mM CsF, 10 mM BAPTA (tetra Cs salt), 10 mM Cs–Hepes, pH 7.4. Liquid junction potentials were less than 4 mV and were not corrected for. Because whole cell voltage clamp experiments are comparatively labor-intensive, compound **5** is the only analog from this series to be profiled using this technique.
11. Rat PK experiments were conducted as follows: test compounds were typically formulated as 1.5 mg/mL solutions in mixtures of PEG300/water or DMSO/PEG300/water. Fasted male Sprague–Dawley rats were given either a 1.0 mg/kg iv dose of test compound solution via a cannula implanted in the femoral vein ($n = 3$) or a 3.0 mg/kg po dose by gavage ($n = 3$). Serial blood samples were collected at 5 (iv only), 15, and 30 min, and at 1, 2, 4, 6, and 8 h post-dose. Plasma was collected by centrifugation, and plasma concentrations of test compound were determined by LC–MS/MS following protein precipitation with acetonitrile.
12. The threshold for maximal electroshock seizures was determined in male mice (C57BL6J mice from The Jackson Laboratory; 14 weeks of age) using auricular electrodes connected to an electroconvulsive device (Basile 57800) designed for inducing tonic–clonic activity in mice and rats. For anticonvulsant testing, the following parameters were used: frequency = 100 Hz; pulse width = 0.7 ms; shock duration = 0.5 s; current = 18 mA. Compound **5** was prepared in a vehicle consisting of 5% EtOH + 10% Tween 80 + 85% water, sonicated to aid in solubilization, then administered by gavage in a volume of 2 mL/kg. Compound **5** was administered at 3 mg/kg po, and was evaluated for anticonvulsant properties 30 min post-dosage.
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