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Initial SAR studies on apamin-displacing 2-aminothiazole blockers of calcium-activated small conductance potassium channels

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ABSTRACT

An initial SAR study on a series of apamin-displacing 2-aminothiazole $K_{Ca}2$ channel blockers is described. Potent inhibitors such as *N*-(4-methylpyridin-2-yl)-4-(pyridin-2-yl)thiazol-2-amine (**13**) are disclosed, and for select members of the series, the relationship between the observed activity in a thallium flux, a binding and a whole-cell electrophysiology assay is presented.

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Small conductance calcium-activated potassium (K_{Ca}) channels are expressed predominately in neuronal cells.[1] They are voltage insensitive and open in response to elevated concentrations of intracellular calcium. Calcium ions bind to calmodulin, a protein that is constitutively associated with the C-terminal domain of the K_{Ca} channel. This protein modulates the state of the channel in response to its calcium occupancy, whereby higher occupancy is associated with opening of the channel.[2–6] K_{Ca} channel opening results in hyperpolarization of the plasma membrane[7] with a concomitant reduction in neuronal excitability. The hyperpolarization phenomenon (termed an afterhyperpolarization or AHP) can persist for several hundreds of milliseconds,[8] and significantly affects the rate and pattern of neuronal firing.[9,10]

Three isoforms of K_{Ca} channels have been identified and cloned: $K_{Ca}2.1$, $K_{Ca}2.2$ and $K_{Ca}2.3$. In situ hybridization, Northern blot, and RT-PCR studies have demonstrated differential regional expression levels of the isoforms in both rat and human brain.[1,9,11,12]

Correspondingly, there exists the possibility for site selective modulation of neuronal excitability if suitable pan-K_{Ca} or sub-type selective K_{Ca} channel modulators can be identified. Such agents may have utility in the treatment of a number of pathological conditions[13] including epilepsy, depression, and Parkinson's disease,[14] as well as schizophrenia.[15] They may also prove helpful in treating certain cognitive disorders.[16,17]

A limited number of compounds have been reported to be active at K_{Ca} channels. The cyclic octapeptide apamin is a highly selective and potent blocker with reported IC_{50}'s between 30 pM and 20 nM.[1,18] The larger peptide Scyllatoxin is exceptionally potent (K_i = 75 pM)[19,20] and related derivatives display moderate selectivity among the K_{Ca} channel subtypes. As shown in Figure 1 above, dequalinium (IC_{50} = 1.0 μM) was the first non-peptidic K_{Ca} selective blocker identified.[21] Subsequent development of this chemotype led to the discovery of the cyclophane derivative, UCL 1684, a compound that displayed similar potency to apamin (IC_{50} = 3.0 nM).[22] More recently, quinoline appended diazepines[23] (K_i = 140 nM) and isoquinoline analogs related to bicuculline and *N*-methyl laudanosine[24–28] have been reported, as well as the non-apamin displacing 2-aminobenzimidazoles, such as NS8593 (IC_{50} = ~500 nM).[29] In this Letter, we describe a preliminary SAR study on a series of aminothiazoles that display significant K_{Ca} channel activity. In addition, we confirm the previously reported activities of some of these analogs[30] in alternative assay systems, and significantly expand on reported selectivity and mechanism of inhibition studies.

The thiazole chemotype discussed here was identified from a high-throughput screen that employed a thallium flux assay,[33] in which compounds were tested against a HEK 293 cell line recombinantly expressing specific K_{Ca} channel isoforms. Several chemotypes were identified with one of the more interesting being the 2-aminothiazole derivative **1**, as shown in Figure 2.

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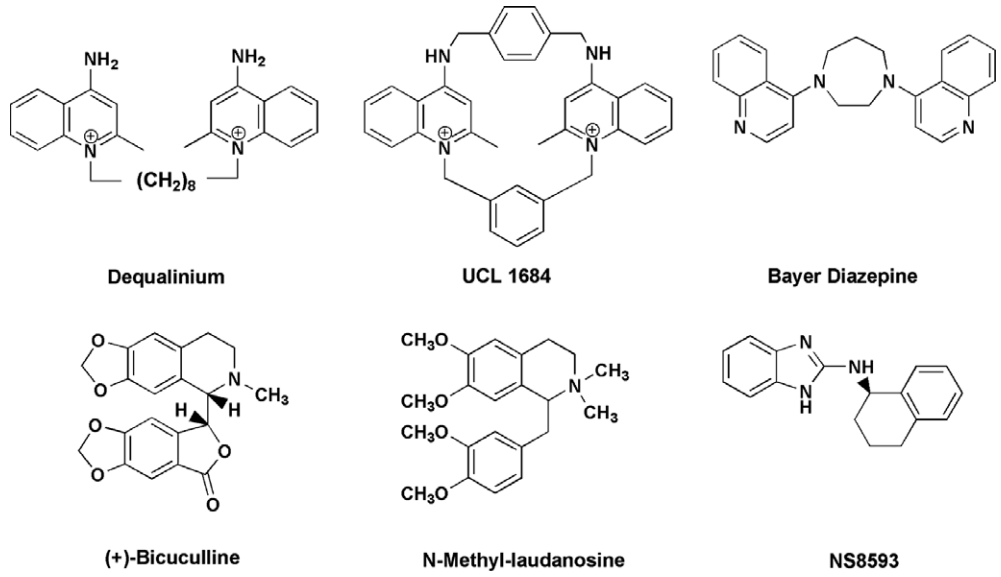

Figure 1. Examples of reported, non-peptidic blockers of K_{Ca} channels.

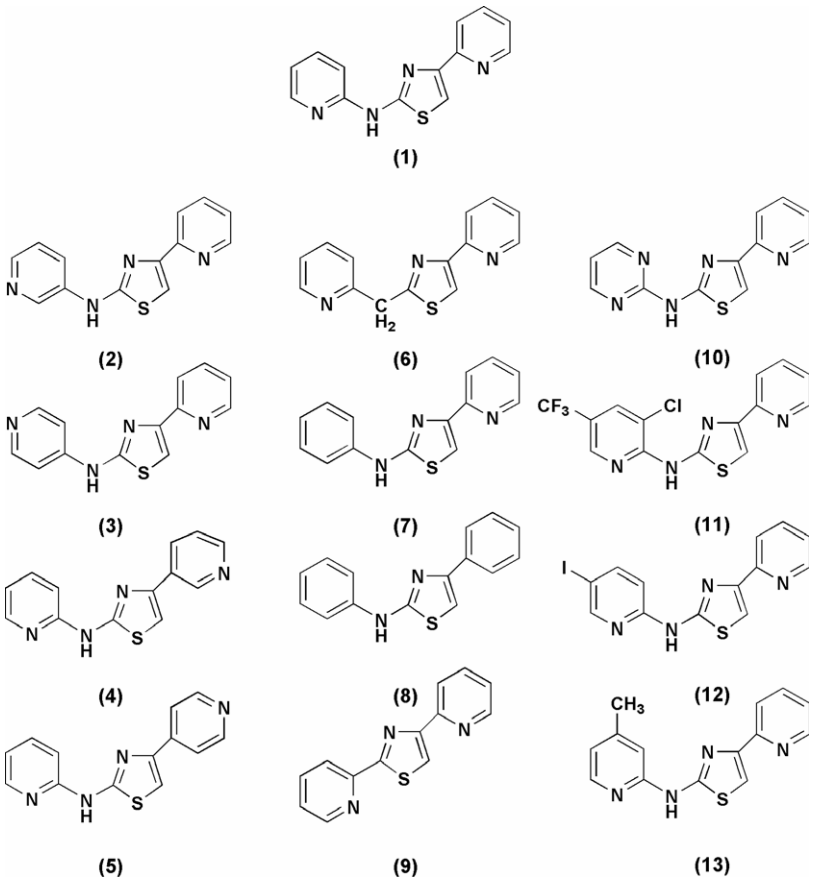

Figure 2. Aminothiazoles evaluated for activity at K_{Ca} channels.

This compound displayed significant potency (89% inhibition at 30 μM) in the original screen. It was resynthesized, and its IC_{50} in the thallium assay was determined to be 0.5 μM. In a subsequent whole-cell electrophysiology experiment, the IC_{50} of **1** was found to be in close agreement with the value obtained in the thallium assay, as shown in Table 1. In addition to the compound's impressive activity, we anticipated that its physicochemical properties [M_W = 254, *C*Log*P* = 2.71, HBD = 1, HBA = 3, ACD pK_a (conjugate acid) = 3.8] held excellent promise for its advancement and utility in investigating CNS effects of K_{Ca} channel block.

Subsequently, we investigated the activities of the series of related aminothiazoles shown in Figure 2. These compounds are either commercially available, or can be synthesized from readily accessible starting materials using the methodology depicted in

Table 1
Activities of compounds **1**–**13** at the $K_{Ca}2.3$ channel in thallium flux and electrophysiology assays

Compound	KCa2.3 thallium flux % inhibition at 30 μM[a]	$K_{Ca}2.3$ thallium flux IC_{50}[a] (μM)	$K_{Ca}2.3$ EP IC_{50}[b] (μM)
1	89	0.543 (±0.05)	0.29 (±0.05)
2	45	ND	
3	14	>30	
4	14	>30	
5	ND	>30	
6	15	>30	
7	9	>30	
8	18	>30	
9	16	>30	
10	24	>30	
11	8	>30	
12	29	>30	
13	98	0.059 (±0.017)	0.056 (±0.015)
14	Apamin	0.000168 (±0.000136)	0.000064 (±0.000006)
15	Dequalinium	1.3 (±0.2)	2.0 (±0.6)

[a] See note 38. ND, not determined.
[b] Values are means of three experiments; standard deviation is given in parentheses.

Figure 3. The majority of the analogs were prepared using the modified Hantzsch[31] procedure shown in reaction **A**. The 2,4-di-(pyridin-2-yl)thiazole analog **9** was synthesized using the same methodology employing a thioamide, as shown in **B**. Lastly, the 4-(pyridin-2-yl)-2-(pyridin-2-ylmethyl)thiazole derivative **6** was synthesized using the two-step procedure shown in reactions **C**[32] and **D**.

Initially, we sought to investigate the importance of the relative disposition of the pyridine nitrogens in **1**. Thus, retaining the connectivity of the 4-(pyridin-2-yl)-*N*-2-aminothiazole moiety, the nitrogen atom of the *N*-pyridinyl group was transposed as shown in structures **2** and **3** above.

As can be seen from Table 1, both analogs lost significant activity at the $K_{Ca}2.3$ channel. A similar exercise was conducted, in which the heteroatom of the pyridinyl group at the 4-position of the thiazole ring was varied in the context of the *N*-(pyridin-2-yl) moiety, as shown in compounds **4** and **5**. Again, a significant loss of activity was observed. The importance of the nitrogens in the two terminal pyridinyl groups was further demonstrated by their progressive replacement, as shown in analogs **7** and **8**. Both compounds displayed very limited activity in the thallium assay. In additional studies, the importance of the pendant amino functionality in **1** was explored in analogs **6** and **9**, in which the amino group was either replaced by a methylene group or simply eliminated. Again, both substitutions were associated with a significant loss of activity at the K_{Ca} channel.

The above observations raised the possibility that the activity of **1** was due to the formation of a chelate. 2-Aminothiazoles are known ligands in a number of chelate complexes,[30,34] and the idea that the chelate may be the active species is partly supported by reports[30] showing that thiazoles and the related iron or zinc chelates have similar activity in a $K_{Ca}2.2$ rubidium flux assay. In relation to the assay systems reported here, the most likely ion that would participate in chelate formation is Mg^{2+}, and several complexes of this type have been reported.[35] Based on the SAR presented above, the most probable structure of such a complex would be **I**, as shown in Figure 4, although **II** and **III** cannot be excluded.

With the key pharmacophoric elements now established, we next attempted to introduce additional functionality into the *N*-(pyridin-2-yl) group while retaining the pyridin-2-yl group at the 4-position of the aminothiazole. The introduction of a nitrogen atom as shown in the pyrimidin-2-yl analog **10** was again associated with a loss of activity, and we tentatively attributed this to a reduction in the basicity of the heterocycle (ACD pK_a's: 3.8 vs 2.4). Additional attempts to introduce steric probes at either C3 or C3 in combination with C5 as in analogs **11** and **12**, resulted in a significant loss of activity.

In a related study focused on the effects of substitution at the C4 vector of the *N*-pyridinyl moiety, we found that the *N*-(4-methylpyridin-2-yl) derivative **13** displayed significantly improved potency relative to **1**. Rather than modulating the basicity of the heterocycle, we envisage that the methyl group interacts directly with the channel. The activity of this compound was also determined in EP experiments, and good agreement between the assays was observed (54 nM vs 56 nM). This level of activity at the $K_{Ca}2.3$ channel is unprecedented in a neutral small molecule, and **13** should prove useful for $K_{Ca}2$ channel functional studies.

To determine the site of action of **1** and **13**, we employed a Scintillation Proximity Assay (SPA) to assess their ability to displace

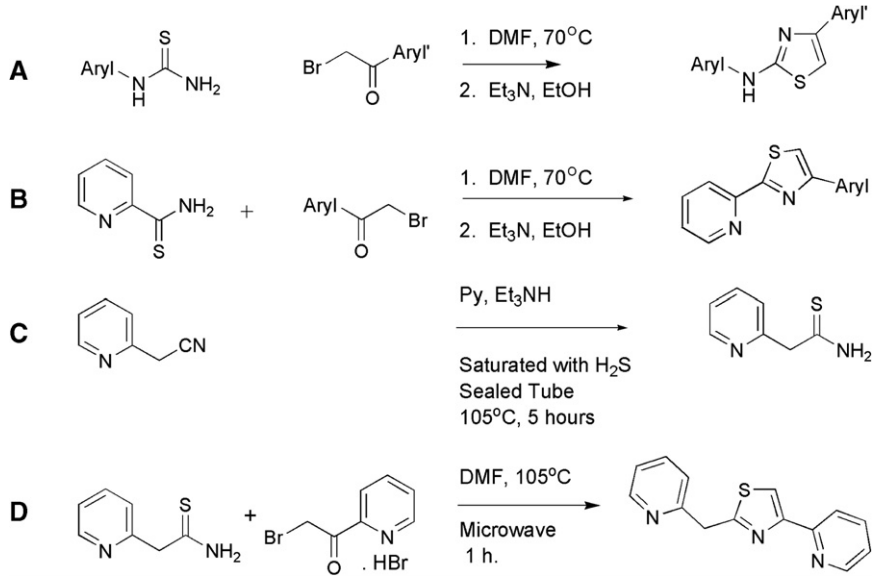

Figure 3. Synthetic methods used for the synthesis of the 2-aminothiazoles listed in Table 1.

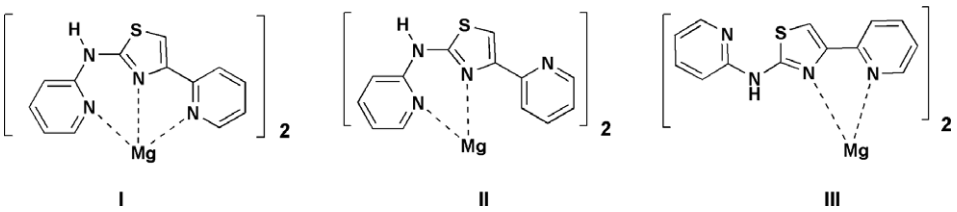

Figure 4. Potential structures of magnesium thiazole chelates.

Table 2
[^{125}I]-apamin displacement assay

Compound	K_{Ca}2.2 thallium flux IC_{50} (μM) [a]	K_{Ca}2.3 [^{125}I]-apamin displacement IC_{50} (μM)[a]
1	0.543 (±0.05)	0.025 (±0.008)
13	0.059 (±0.017)	0.004 (±0.002)

[a] Values are the means of three experiments; standard deviation is given in parentheses. See note 38.

Table 3
Selectivity of compounds **1** and **13** against the K_{Ca} channel isoforms, K_{Ca}2.1, K_{Ca}2.2, and K_{Ca}3.1

Compound	K_{Ca}2.1 thallium flux IC_{50} (μM)[a]	K_{Ca}2.2 thallium flux IC_{50} (μM)[a]	K_{Ca}3.1 thallium flux IC_{50} (μM)[a]
1	0.043	0.123	na
13	0.004	0.011	10% Inh. at 30 μM

[a] Values are the means of three experiments; see note 38.

radio-labeled [^{125}I]-apamin from the K_{Ca}2.3 channel.[36,37] Both compounds competed off the peptide with IC_{50}'s as shown in Table 2. These results suggest that both may function by blocking the pore of the channel, as is observed with apamin (see Table 3).

To assess the K_{Ca} channel selectivity of the thiazole chemotype, selected analogs were assessed in the thallium flux assay against cell lines recombinantly expressing the K_{Ca}2.1, K_{Ca}2.2, and K_{Ca}3.1 channels. No significant selectivity was observed, although it was noted that both **1** and **13** displayed essentially no activity at the K_{Ca}3.1 channel.

In conclusion, we present a series of *N*-(pyridine-2-yl)-4-(pyridine-2-yl)-2-aminothiazoles that display excellent potency as K_{Ca}2 blockers. In binding studies, these compounds appear to interact with the channel at the apamin binding site, and presumably exert their effect by mechanically blocking the pore of the channel. We speculate that the active species may be the thiazole itself, or a metal chelate in which the thiazole functions as a ligand.

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38. Each concentration of a given sample was tested in triplicate. The mean % inhibitions of the triplicates for each concentration were used to determine an IC_{50} value based on a single-site logistic fit (Microsoft XLFit). For selected compounds, IC_{50} determinations were repeated on three separate occasions, and the mean and the standard deviation is reported. Overall, the variability of the IC_{50} determination was observed to be within 1/2 log unit as seen with the standard deviations reported.