A Synthetic Search for AMPA Receptor Up-Modulators, "Memory Enhancing Drugs"

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Biological Sciences and Chemistry

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Peter Krutzik began to do research as a member of the Chamberlin Group because the project was a way of incorporating his two passions of chemistry and biology. Working on this project was exciting and challenging for Peter, giving him the "confidence to pursue a future career in research." He feels that his participation in research as an undergraduate has enhanced his educational experience by allowing him to apply the knowledge he has gained through his academic career. Peter advises students considering research to ask "one simple question…How much do you really want to learn? If the answer is 'everything,' undergraduate research is the place to begin."

Key Terms:

- AMPA receptors
- Benzothiadiazines (BZTDs)
- Desensitization
- Excitatory post-synaptic potentials (EPSPs)
- Up-modulation / Potentiation

Abstract

Due to their prevalence at brain synapses and their role in eliciting excitatory responses upon binding glutamate, glutamate receptors (GluRs) have recently become the focus of much neurological research. Of particular interest is the AMPA subclass of glutamate receptors present largely in the hippocampus, the putative memory center of the human brain. It has been found that by potentiating, or up-modulating, the response of AMPA receptors, learning and memory processes can be enhanced. In this study, various analogs of an AMPA desensitization-inhibiting compound, benzothiadiazine DP-60, were synthesized in an attempt to find a better drug candidate. Of the drugs synthesized, 2-hydroxyethyl-DP-60 (**18b**) and 2-acetonitrile-DP-60 (**16**) were found to be active inhibitors of desensitization. The various synthetic routes employed also expand current understanding of the reactivity of the benzothiadiazine ring.

Faculty Mentor

Peter Krutzik began his research in my laboratory working on a project designed to discover new compounds that might improve memory. For many older people, including some of us "absent minded professors," such a drug could make a significant difference in our daily lives. A key element in the search for any drug is stringing together entirely new molecules, as Peter has done on this project. A typical characteristic of this type of research, whether in an academic laboratory or at a large pharmaceutical company, is that it requires a joint effort between chemists and biologists working as a team. It is hard work, and no one knows what is just around the next corner. But that is part of the excitement, along with the possibility that something we dream up could truly improve the quality of life for many people.

~ Richard Chamberlin
School of Physical Sciences

Introduction

The Role of AMPA Receptors in Neurotransmission

Present at approximately half of all brain neural synapses, ionotropic glutamate receptors (GluRs) are divided into three major subclasses, named after their respective agonists: NMDA (N-methyl-D-aspartate), KA (kainate), and AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid). AMPA receptors are of particular interest because of their great numbers in the hippocampus, which is believed to be devoted to memory functions.

When the neurotransmitter glutamate binds to GluRs, it causes fast excitatory responses known as excitatory post-synaptic potentials (EPSPs). Since over-excitation (i.e., excitotoxicity) may result from prolonged exposure to glutamate in the synapse, GluRs have a natural defense mechanism known as desensitization by which they down-regulate the current evoked by glutamate present in the synapse (see Figure 1). Desensitization occurs when the receptor, although still bound by glutamate, closes its ion channel and thereby halts the current produced in the post-synaptic neuron.

It has been shown that by potentiating the EPSPs elicited by glutamate, long-term potentiation, a process crucial to learning and memory, can be enhanced (Bliss and Collingridge, 1993; Staubli et al., 1994; Staubli et al., 1994). Such up-modulation may serve as a memory-enhancing agent, thereby lessening the effects of such neurodegenerative diseases as Alzheimer's and Parkinson's.

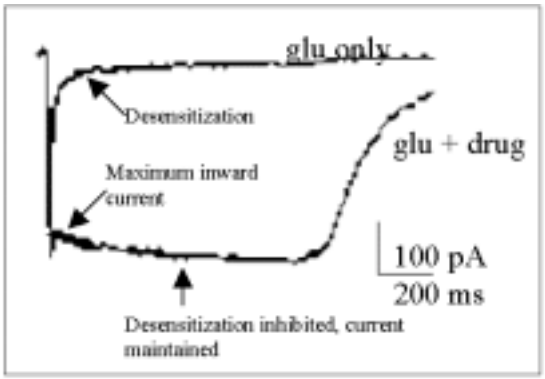


Figure 1

Typical patch-clamp electrophysiogram showing the current invoked at AMPA receptors by glutamate alone and by glutamate with a BZTD drug. Notice the prolonged activity resulting from the application with the drug.

The Benzothiadiazines – AMPA Modulating Drugs

The most notable drugs found to inhibit desensitization of AMPA receptors come from the benzothiadiazine (BZTD) class of compounds (see Figure 2) (Yamada, 1998).

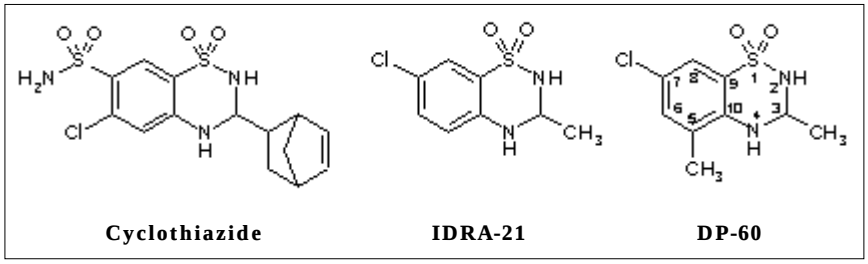


Figure 2

Known inhibitors of AMPA receptor desensitization. Note atom numbers of DP-60.

Cyclothiazide is particularly effective at inhibiting desensitization, but it suffers from two drawbacks. First, only one of its eight stereoisomers is active, requiring difficult enantiospecific syntheses to be carried out (Cordi et al., 1994). Second, the free sulfonamide group causes cyclothiazide to be highly polar and therefore not readily diffusable through the lipophilic, or nonpolar, blood-brain barrier. IDRA-21 was synthesized by Zivkovic and colleagues (1995) and was found to be more lipophilic, but much less active than cyclothiazide (Zivkovic et al., 1995; Arai et al., 1996). In order to arrive at a compound with the desired combination of lipophilicity and activity, the effects of each portion of its structure must be probed. To find a more effective drug, the Chamberlin group screened a library of compounds with similar core structures but with variations in the substituents, or groups, on the core. After creating this combinatorial matrix and performing a few pilot reactions to analyze various substituents on the aryl ring, our group synthesized DP-60. Although DP-60 differs from IDRA-21 only by the methyl group at the 5' position, its effect on AMPA receptor desensitization is dramatic: it increases the steady state current almost five-fold compared to IDRA-21.

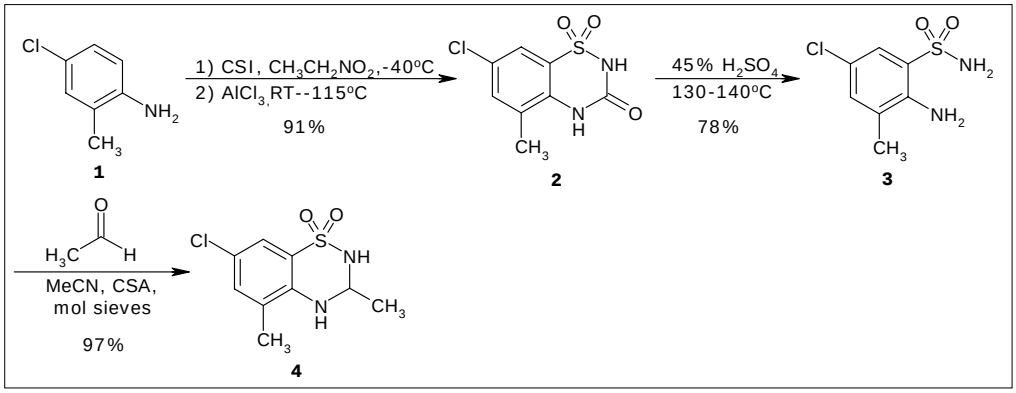
The large effect elicited by this minor alteration drove us to search for analogs of DP-60 that might be even more active, yet maintain the lipophilicity of DP-60. Much of the pioneering work of the Chamberlin group has involved changing the substituents on the aryl ring and the 3 position of the BZTD ring. In order to find drugs that would better inhibit the desensitization of AMPA receptors, we decided to alter the groups attached to the sulfonamide nitrogen (2 position), which had always been left as a hydrogen. The Lynch group at the University of California at Irvine then assayed the drugs that were developed for their effects on AMPA receptors. The goal of the various syntheses and subsequent biological testing was to understand the structure-activity relationships (SAR) of the BZTDs and their effects on AMPA receptor desensitization.

Results and Discussion

Three synthetic routes were followed in order to obtain substituents at the sulfonamide position of DP-60: 1) a deprotonation/S_N2 reaction with sec-BuLi, 2) a regioselective reduction method, and 3) another deprotonation/S_N2 route, this time with potassium carbonate. Prior to discussion of these routes, the synthesis of DP-60, the starting substrate for these studies, will be discussed.

Synthesis of DP-60

Following the work of Girard and colleagues (1979), DP-60 was synthesized in three steps (see Scheme 1). An overall yield of 68% makes this route especially useful as a source of starting material for the preparation of analogs (Girard et al., 1979). The initial reaction involves the nucleophilic attack by the nitrogen of aniline **1** on the isocyanate moiety of the chlorosulfonylisocyanate (CSI). Due to the high reactivity of CSI, this reaction is performed at -40 °C to ensure proper regioselectivity of the reaction (i.e., to avoid attack at the sulfonyl position of CSI). The addition of $AlCl_3$ and reflux at 115 °C drives the Friedel-Crafts-like acylation of the benzene ring with the sulfonyl chloride group, producing the benzothiadiazine ring of **2**. It is important to note that the reflux temperature of 115 °C is critical for complete reaction; lowering this to even 110 °C reduces the yield and product purity. The second reaction involves the hydrolysis of **2** with 45% sulfuric acid at high temperatures (130-140 °C). As with the first step, minor alterations to these reaction conditions have dramatic effects upon the yield; at temperatures below 130 °C or with sulfuric acid concentrations of 40%, yields usually range from 45-55%, a drop of over 20%. Finally, condensation of sulfonamide **3** with an excess of acetaldehyde produces DP-60 with yields consistently between 90% and 100%.

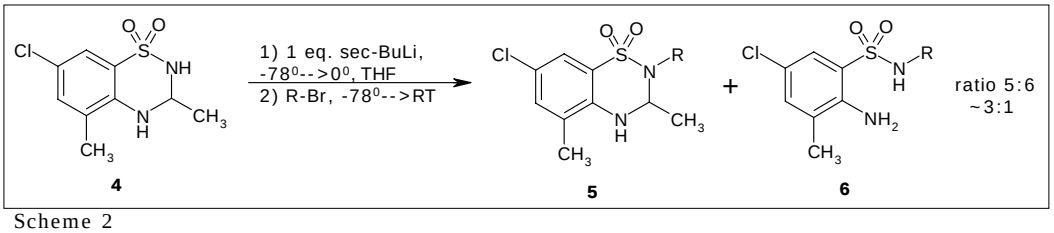


Scheme 1

Synthesis of DP-60

Route 1: Deprotonation by sec-BuLi, an S_N2 Route

The first route employed to obtain analogs of DP-60 involves a simple deprotonation utilizing the strong base, sec-butyl lithium. The nitrogen anion produced attacks the alkyl (R) group of the alkyl bromide, liberating the bromine in a bimolecular nucleophilic substitution (S_N2). The driving force behind this reaction (as in Route 3 below) is the relative acidity of the sulfonamide nitrogen versus the aniline nitrogen. Due to the electron-withdrawing effects of the sulfonyl group (and the subsequent stabilization of the conjugate base), the sulfonamide nitrogen consistently shows a more acidic character than the aniline nitrogen. Therefore, with bases of intermediate strength or steric hindrance, the sulfonamide nitrogen can be selectively deprotonated to become highly nucleophilic; this nucleophile is then allowed to react with the alkyl bromide to give the S_N2 product (see Scheme 2).



Scheme 2

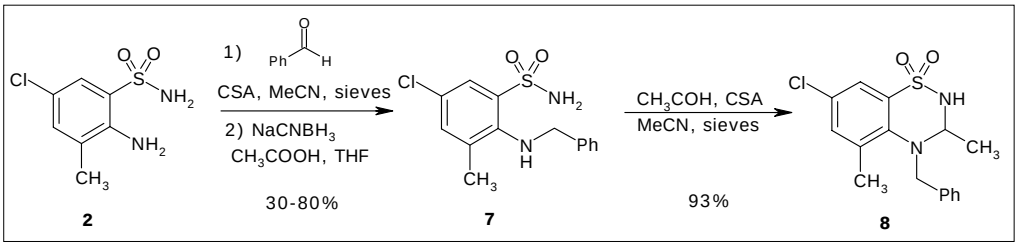
General Reaction for Route 1

Three difficulties arise during the synthesis of DP-60 via Route 1, which prompt a search for other routes. First, the reaction yields only one analog of DP-60 through the reaction of **4** with methyl bromoacetate, and this is in a poor yield of 45-55%. The addition of bromoethyl methyl ether gives no reaction. This indicates that although deprotonation at the sulfonamide nitrogen occurs, the resulting nucleophile is not strong enough to perform the S_N2.

The second drawback of this route is the nature of the reaction conditions themselves. Due to the pyrophoric nature of sec-BuLi, which is flammable upon exposure to air, this reagent must be added very slowly in a dropwise fashion to DP-60 (**4**) at -78 °C. The reaction is then warmed slowly to 0 °C to allow complete reaction of sec-BuLi and cooled to -78 °C for the addition of the electrophile. Finally, it is warmed to room temperature for another hour or left overnight. These laborious conditions make the reaction unsuitable for the production of multiple DP-60 analogs.

Finally, as can be seen in Scheme 2, the reaction breaks open the BZTD ring giving by-product **6**. The ratio of **5**:**6** is approximately 3:1 after purification, which indicates a 25% loss of product due to the side reaction. Since this by-product is produced in significant amounts, it is necessary to understand the mechanism behind this side reaction. Although, as mentioned above, the sulfonamide nitrogen is more acidic than the aniline nitrogen, deprotonation at the aniline position may occur if the base is added too quickly. Therefore, the side reaction most likely occurs due to over-reaction by sec-BuLi and deprotonation at the aniline nitrogen to form the aniline imine. This imine is then hydrolyzed on aqueous work-up to give by-product **6**.

Because this putative mechanism requires the presence of a free hydrogen at the aniline nitrogen, we decided to protect the position with a benzyl group that could later be removed by hydrogenation. As there is no longer a proton at the aniline position for sec-BuLi to deprotonate, the side reaction is blocked. The first method of protection involves the addition of the benzyl group to the starting 4-chloro-2-methyl-aniline, followed by the synthesis of the benzyl-protected DP-60 as in Scheme 1. While it is possible to produce the benzylated aniline in a good yield of 72%, the subsequent reaction with CSI and $AlCl_3$ does not give the desired product. This is possibly due to steric interaction by the benzyl group. Thus, this protection route is no longer utilized.

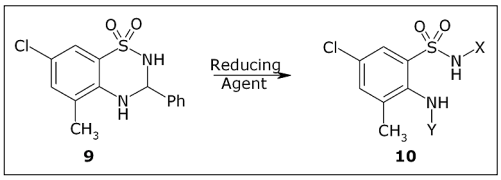


Scheme 3

Aniline Protection

Another method of protecting the aniline nitrogen was concurrently discovered in the Chamberlin lab. This method involves reacting sulfonamide **2** with benzaldehyde to produce the 3-phenyl BZTD ring, which is then regioselectively reduced with sodium cyanoborohydride ($NaCNBH_3$) in acetic acid/THF (see Scheme 3).

Although the mechanism of reduction by $NaCNBH_3$ is not well understood, it shows great utility in various functional group conversions (Gribble and Nutaitis, 1985). The initial condensation with benzaldehyde goes to completion, but the reduction step is highly variable in its yield (30-80%). The order of reagent addition during the reductive step appears to be crucial: $NaCNBH_3$ must first be reacted with acetic acid, most likely to form an alkoxyborohydride *in situ*, prior to addition to the 3-phenyl DP-60 analog. This leads us to believe that the alkoxyborohydride is the reactive reducing agent. Once formed, sulfonamide **7** is smoothly condensed with acetaldehyde to produce aniline-protected DP-60, **8**. This product is then reacted via the sec-BuLi route to produce further analogs. Unfortunately, the reaction does not proceed more completely or with fewer by-products than the initial reaction of the unprotected DP-60. Reaction with methylbromoacetate only yields 45% of the desired analog in this case. This prompted further studies to find another general synthetic route. Prior to further synthesis, however, a small case study on the regioselective reduction with $NaCNBH_3$ was performed to better understand the mechanism of reaction and to optimize the procedure.



Scheme 4

Case Study on Regioselective Reduction

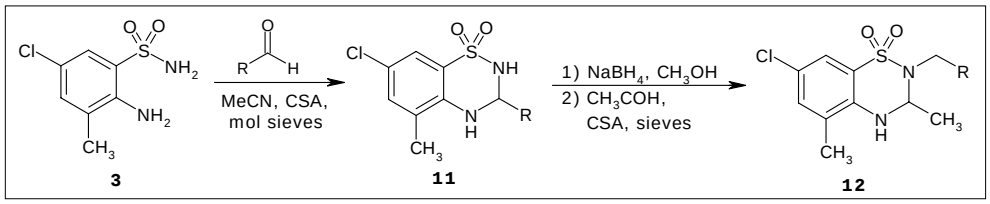
The case study performed is by no means exhaustive, but affords very useful results pertaining to the reactivity of the BZTD ring. In the study, $NaBH_4$, $NaCNBH_3$, and lithium aluminum hydride ($LiAlH_4$) are added to 3-phenyl DP-60, **9**, in various solvents to observe their relative reactivities (see Scheme 4). The two possible products from the reductions are illustrated in **10**: a regioselective ring opening to place the benzyl group at either the sulfonamide nitrogen (labeled "X") or at the aniline nitrogen (labeled "Y"). The results of this study are summarized in Table 1.

Table 1

Results of regioselective reduction case study (X or Y= benzyl).

Reducing agent, conditions	Result, yield
$NaCNBH_3$, AcOH, THF, 0°	Y, varying
$LiA1H_4$, THF, 0°	Y, 75-90%
$NaBH_4$, MeOH	X, 91%
$NaBH_4$, MeOH, AcOH	No rxn
$NaBH_4$, AcOH, THF, 0°	No rxn
$NaBH_4$, MeCN	Reduction of MeCN

As utilized in the protection reaction pathway described above, $NaCNBH_3$ in acetic acid and THF results in regioselective benzylation of the aniline position Y. However, the yield of this reaction varies greatly (30-80%). The reduction with $LiAlH_4$ was found serendipitously from work following Route 2 (below), but the result is mentioned here. Essentially, $LiAlH_4$ gives the same result as $NaCNBH_3$ but with much more consistency and higher yields. Therefore, this serves as the reducing agent of choice for regioselective reaction to the aniline nitrogen.



Scheme 5

General Reactions for Route 2

Due to the hazardous nature of $NaCNBH_3$, which liberates cyanide gas as a by-product, use of $NaBH_4$ is preferable. Because $NaBH_4$ is not soluble in THF, no reaction occurs when it is used under the AcOH and THF conditions used for $NaCNBH_3$. Even when MeOH is used to better solvate the $NaBH_4$, there is still no reaction. Use of acetonitrile (MeCN) only causes cross-reaction and results in the reduction of the nitrile group of the solvent. The addition of $NaBH_4$ to **9**, stirring in methanol without the acid catalyst, affords a remarkable 91% regioselectivity at the sulfonamide nitrogen. This leads to another possible route to analogs of DP-60 by the regioselective reduction of various condensation products: Route 2.

Route 2: Regioselective Reduction via $NaBH_4$

Using the valuable result of the case study (i.e., regioselective reduction of the sulfonamide nitrogen with $NaBH_4$ in MeOH), we envisioned a new synthetic route to obtain analogs of DP-60 (see Scheme 5). In this theoretical route, an aldehyde (or a ketone) is condensed with the sulfonamide **3** to produce substituted DP-60 (**11**). This can then be regioselectively reduced with $NaBH_4$ in MeOH and recondensed with acetaldehyde to produce the desired final analog of DP-60 (**12**). No reaction occurs when aldehyde 4-thiopentanal is added to sulfonamide **3**. In fact, in an attempt to "push" the reduction with a stronger hydride source, it was found that the $LiAlH_4$ reduction gives a product with the thioalkyl chain on the aniline nitrogen.

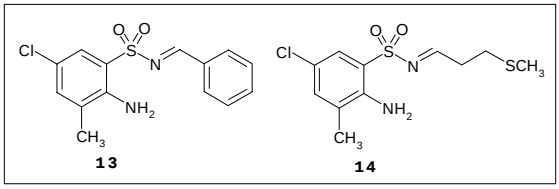


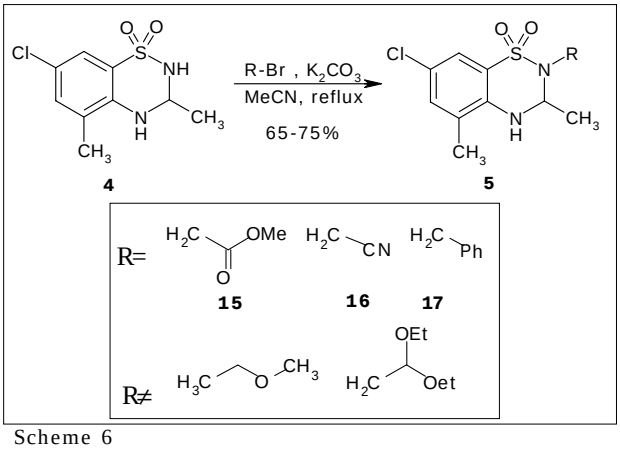
Figure 3

Imines formed when sulfonamide nitrogen is deprotonated. Notice the conjugation produced with the benzyl substituent (13), which is not present in 14.

This result indicates that the weaker hydride source, $NaBH_4$, requires a stabilized imine intermediate (see Figure 3) whereas the more potent $LiAlH_4$ reacts rapidly with any imine that is spontaneously formed. In the case of the slightly basic conditions of the $NaBH_4$ in MeOH reaction, the more acidic sulfonamide nitrogen is deprotonated to give an imine. This imine is stabilized by the conjugating effects of groups such as phenyl rings (**13**), but is not stabilized by alkyl-like chains, as with the thioalkyl chain mentioned above (**14**). Without a prolonged presence of the reducible imine intermediate, the soft hydride source, $NaBH_4$, does not react. Under acidic or neutral conditions, as in the reactions with $LiAlH_4$ or $NaCNBH_3$, the more basic aniline nitrogen most likely donates its electron pair to the C-N bond to give an imine at the aniline position. This imine is subsequently reduced by the strong reducing agents. Therefore, Route 2 is not a viable synthetic route to produce analogs.

Route 3: Potassium Carbonate Induced S_N2 Reaction

Route 3 utilizes the same reactive sequence as Route 1 except that potassium carbonate is used to deprotonate the sulfonamide nitrogen (see Scheme 6). There are three factors that make this route much more amenable to obtaining DP-60 analogs than Route 1. First, the yield is high and consistent, ranging from 65-75%. Second, as compared to the ring opening seen in Route 1, no side reactions are observed. Finally, the reaction itself is very simple to run: 1) DP-60 is dissolved in acetonitrile, 2) potassium carbonate and the electrophile are added, and 3) the reaction is heated at reflux overnight. The next day, after



Scheme 6

General Reaction for Route 3

simple aqueous work-up and purification by column chromatography, the desired product is obtained. Obviously, when repeating reactions, it is optimal to have short set-up times and simple work-up/quenching conditions; both are achieved with this route.

Utilizing this reaction, three crucial functional groups can be placed at the sulfonamide nitrogen: an ester (**15**), a nitrile (**16**), and a benzyl (**17**) group. However, as is the case in the sec-BuLi reaction, there are limits to the electrophile that can be used. The ester, nitrile, and benzyl moieties all have strong electron-withdrawing properties, making the halide carbon more electrophilic and prone to attack by the deprotonated sulfonamide nitrogen. On the other hand, the ether and acetal groups are electron-donating, making their respective halide compounds weakly electrophilic (see Scheme 6). Therefore, it appears that the potassium carbonate route is limited, as is Route 1, to substitution at the desired position by strongly electrophilic halide compounds.

Scheme 7

Further Reactions

Two more analogs were created with ester and nitrile groups at the sulfonamide nitrogen (see Scheme 7, **18a, 18b**). The carboxylic acid (**18a**) is obtained by simple hydrolysis of **15** with aqueous sodium hydroxide while the alcohol is synthesized by reduction of the ester with $NaBH_4$ in MeOH. Although the reduction of nitrile-substituted product **16** with $LiAlH_4$ has not been performed, the conversion of this particular group to amine **19** should proceed smoothly. If $LiAlH_4$ does not work, then $NaBH_4$ or another reducing agent can be used. The ring opening should not occur as long as only one equivalent of $LiAlH_4$ is used because the nitrile is more easily reduced than the BZTD ring.

Summary of Routes 1-3

Of the three routes employed, Route 3 is the most effective, both in yield and in ease of reaction. By using Route 1, analogs at the sulfonamide can be synthesized, but the reaction itself is tedious and produces a ring-opened by-product. Route 2, which involves regioselective reduction with $NaBH_4$, only works with condensed groups that can stabilize the imine intermediate.

The case study on the various reducing agents illustrates the delicate nature of the BZTD ring. Strong bases cannot be employed during the syntheses because such aqueous work-ups often cause ring opening and various other side reactions. Therefore, so-called "soft" conditions must be employed in all cases where a direct reaction with DP-60 is to take place. In addition, the reduction with $NaBH_4$ in MeOH under slightly basic conditions verifies the assumption that the sulfonamide nitrogen is indeed more acidic than the aniline nitrogen.

Throughout the various synthetic routes, the reactivity of the BZTD ring was explored, and valuable reactions were found to produce analogs of DP-60. Additional electrophiles may be employed to obtain more analogs via Route 3. Furthermore, the regioselective reductions by $LiAlH_4$ and $NaCNBH_3$ may be employed to yield variants at the aniline position for further study even though Route 2 did not prove useful for creating analogs at the sulfonamide position.

Biological Effects of DP-60 Analogs Synthesized

After synthesis, drugs were tested by the Lynch group of the University of California at Irvine to characterize their effect upon AMPA receptors. Three general *in vitro* methods were employed in these activity studies: 1) measurement of synaptic responses in whole hippocampal slices, 2) AMPA currents in excised outside-out membrane patches (patch-clamp), and 3) binding tests with the antagonist ^{3}H-fluorowillardiine (FW*). Although these *in vitro* methods are not a direct indicator of drug activity on AMPA receptors, it has been found that drugs which inhibit desensitization also up-modulate the binding affinity of FW* (Lynch, unpublished data). Therefore, the binding assay was used to screen drug candidates prior to pursuing further time-consuming patch clamp data. Inhibitors of desensitization, such as DP-60 and IDRA-21, show a marked increase in the binding of FW*. EC_{50} values given as a percent of control binding can be obtained from the binding curves, along with the maximal effect of the drug on FW* binding. These results are summarized in Table 2 (D-1 and DP-60 are included as references).

Table 2

Results of fluorowillardiine binding assays performed with synthesized analogs

Drug tested (as numbered in the article)	EC_{50} value, % increase in FW* binding
8	NE
11	NE
17	NE
16	90 μM, 210%
18a	NE
18b	15 μM, 120%
D-1	22 μM, 270%
DP-60	50 μM, 190%

NE indicates no effect on FW* binding

It is evident from Table 2 that two of the analogs synthesized are candidates for further study, alcohol-substituted **18b** and nitrile-substituted **16**. In fact, their effect on FW* binding is similar to DP-60, indicating a possible electron-poor pocket in the receptor binding site that interacts with these electron-rich groups. Therefore, analogs **16** and **18b** may serve as AMPA receptor desensitization inhibitors. Further analogs with slight variations to these groups can be synthesized to alter their reactivity. As was the case with the development of DP-60 and its enhancement of reactivity compared to IDRA-21, minor changes to the two analogs may yield dramatically more effective drugs.

Conclusion

This synthetic search for AMPA receptor desensitization inhibitors manifested two primary results. First, the reactivity of the BZTD ring was characterized and better understood. Selective reductions of this ring may provide further analogs of DP-60 at the aniline nitrogen or, through the enhancement of the $NaBH_4$ method, more analogs at the sulfonamide nitrogen. Second, two more drug candidates, with either an alcohol or nitrile group at the sulfonamide position, were found that show drug-potential activity in the FW* binding assay. The reactivity of these analogs provides insight into the SAR of the benzothiadiazines because they indicate a possible electron-poor pocket in the AMPA receptor into which the electron-rich groups enter. The relatively unreactive nature of the benzyl-substituted analog also suggests that there may be a limit to the size of the substituent at the sulfonamide position.

Presently, our group has nearly 70 variants of DP-60 synthesized, and we have only begun to interpret the complex results to produce a sound SAR paradigm. Likewise, further study of the BZTD drugs *in vivo* is necessary to observe their effect upon AMPA potentiation. The potency of the drugs synthesized to date indicates the potential of BZTDs to be effective memory enhancing drugs which can diminish the effects of dementia associated with such neurodegenerative diseases as Alzheimer's and Parkinson's.

Experimental

Reagents were used as purchased from Aldrich, Acros, or Fischer Scientific. Solvents were obtained from a solvent dispensing system (dry) or distilled. ^{1}H-NMRs were obtained on a Bruker DRX400 at 400 MHz. Full spectral characterization is available upon request (mass spec, FT-IR, ^{13}C-NMR).

7-Chloro-5-methyl-3,4-dihydro-3-oxide-1,2,4-benzothiadiazine S,S-dioxide (2). To a cooled solution (-78 °C) of chlorosulfonyl isocyanate (6.0 mL, 69 mmol) in nitroethane (80 mL) was added 4-chloro-2-methyl aniline (**1**) (6.8 g, 48 mmol) dissolved in nitroethane (50 mL). Upon precipitation of the intermediate urea, $AlCl_3$ (8 g, 60 mmol) was added slowly, and the reaction was heated to reflux at 115 °C for 1 hr. The reaction mixture was cooled and the ensuing precipitate was filtered *in vacuo* to obtain **2** as a purple solid (11.5 g, 97%). ^{1}H-NMR (d_6-DMSO) δ 10.55 (s, 1 H), 7.66 (d, J = 2 Hz, 1 H), 7.62 (d, J = 2 Hz, 1 H), 2.37 (s, 3 H).

2-amino, 3-methyl, 5-chloro-benzensulfonamide (3). **2** (6.4 g, 26mmol) was suspended in 200 mL 45% H_2SO_4, heated to reflux at 140 °C for 3-6 hr, and cooled to RT. 320 mL 10 M NaOH were then added to precipitate the product which was collected by vacuum filtration to obtain **3** as a tan solid (4.3 g, 80%). ^{1}H-NMR (d_6-DMSO) δ 7.44 (t, J = 2 Hz, 3 H), 7.25 (d, J = 2 Hz, 1 H), 5.77 (s, 2 H), 3.34 (s, 3 H), 2.11 (s, 3 H).

7-Chloro-3,5-methyl-3,4-dihydro-1,2,4-benzothiadiazine S,S-dioxide, DP-60 (4). 3 Å molecular sieves were activated with heat (open flame) in a 100 mL roundbottom to which 1.1 g of **3** (5 mmol) were added and dissolved in acetonitrile (25 mL). Acetaldehyde (2.5 mL, 45 mmol) and camphorsulfonic acid (10 mol %) were added, and the reaction was stirred for 2 hr until completion as monitored by TLC. The resulting mixture was filtered through Celite with several 50 mL EtOAc washings to ensure all of the product passed through the Celite. The filtrate was then washed with $NaHCO_3$ (2x50 mL), dried ($MgSO_4$), and concentrated *in vacuo* to obtain **4** as a white powder (1.18 g, 95%). ^{1}H- NMR (d_6-DMSO) δ 7.70 (d, J = 11 Hz, 1 H), 7.36 (s, 1 H), 7.30 (s, 1 H), 6.21 (s, 1 H), 4.81 (m, 1 H), 2.16 (s, 3 H), 1.51 (d, J = 6 Hz, 3 H).

Sample reaction for sulfonamide substituted product (5). To **4** (245 mg, 1 mmol) stirring in THF (5 mL) at -78 °C, was added sec-BuLi (1.05 eq) dropwise over approximately 10 min. Note, drops were added upon disappearance of intermediate yellow color. The solution was warmed to 0 °C for 1 hour and then cooled again to -78 °C. Methyl bromoacetate (0.1 mL, 1.05 mmol) was added dropwise over 5 min, the solution was stirred cold for 1 hr, and then warmed to room temperature overnight. The mixture was neutralized with 1N HCl and extracted with EtOAc (3x25 mL) and sat. $NaHCO_3$ (2x25 mL). The organic layers were then dried ($MgSO_4$) and concentrated *in vacuo*. The crude material was redissolved in EtOAc, silica gel was added, and the suspension was concentrated *in vacuo* to dry-load for column chromatography. Chromatography (40% v/v EtOAc/Hex) yielded product **5** as a faint yellow solid (180 mg, 56%). ^{1}H NMR (d_6-DMSO) *see **15** below*.

2-benzylamino,3-methyl,5-chloro-benzensulfonamide (7). See preparation of **9** below. To the condensation product, **9** (307 mg, 1 mmol) in THF (6 mL) was added a mixture of $NaCNBH_3$ (4 eq) in acetic acid (6 mL) dropwise. The solution was stirred overnight, neutralized with 10M NaOH, and extracted with EtOAc (3x25 mL) and sat. $NaHCO_3$ (50 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to obtain an oily residue. The residue was dissolved in hexanes and loaded onto a column (silica gel, eluent 40% v/v EtOAc:hexanes) to obtain **7** as a white solid (280 mg, 90%). ^{1}H NMR (d_6-DMSO) δ 7.72 (s, 2 H), 7.61 (d, *J* = 3 Hz, 1 H), 7.44 (d, *J* = 8 Hz, 3 H), 7.36 (t, *J* = 19 Hz, 2 H), 7.26 (t, *J* = 10 Hz, 1 H) 5.32 (t, J = 7 Hz, 1 H), 4.25 (d, *J* = 7 Hz, 2 H), 2.33 (s, 3H).

7-Chloro-3,5-methyl-4-benzyl-3,4-dihydro-1,2,4-benzothiadiazine S,S-dioxide (8). As in preparation of **4**, using **7** as starting material (1.03 g, 3 mmol) and 1.35 mL acetaldehyde (24 mmol), **8** was obtained as a white powder (1 g, 90%). ^{1}H NMR (d_6-DMSO) δ 8.58 (d, *J* = 6 Hz, 1 H), 7.62 (d, *J* = 2 Hz,1 H), 7.56 (d, *J* = 2 Hz, 2 H), 7.50 (d, *J* = 2 Hz, 2 H), 7.40 (d, *J* = 6 Hz, 2 H), 7.29 (d, *J* = 7 Hz, 1 H), 4.49 (m, 1 H), 4.28 (dd, *J* = 6, 16 Hz, 2 H), 2.34 (s, 3 H), 1.30 (d, *J* = 7 Hz, 3 H).

7-Chloro-3-phenyl,5-methyl-3,4-dihydro-1,2,4-benzothiadiazine S,S-dioxide (9). To activated 3 Å molecular sieves were added 3.08 g of **3** (14 mmol), 1.5 mL benzaldehyde (14.5 mmol), 1 mL acetic acid (catalytic), 1 g camphorsulfonic acid, and 50 mL acetonitrile. The mixture was stirred overnight, filtered through Celite, and extracted with EtOAc/hexanes. The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo* to obtain **9** as a white solid (4 g, 93%). ^{1}H NMR (d_6-DMSO) δ: 8.13 (d, *J* = 12 Hz, 1 H), 7.50 (dd, *J* = 2, 6 Hz, 2 H), 7.44 (m, 4 H), 7.36 (q, *J* = 1 Hz, 1 H), 6.48 (s, 1H), 5.78 (d, *J* = 12 Hz,1 H), 2.18 (s, 3H).

7-Chloro-3,5-methyl-3,4-dihydro-2-methylacetate-1,2,4-benzothiadiazine S,S dioxide (15). To a solution of **4** (490 mg, 2 mmol) in acetonitrile was added methyl bromoacetate (0.28 mL, 3 mmol) and potassium carbonate (620 mg, 4.5 mmol). The suspension was heated to reflux overnight, cooled, and extracted with EtOAc/$NaHCO_3$. The organic layers were combined, dried ($MgSO_4$), condensed *in vacuo*, and the desired product, **15**, was obtained by column chromatography (460 mg, 72%) with 40% v/v EtOAc:hexanes as the eluent. ^{1}H NMR (d_6-DMSO) δ 7.43 (d, *J* = 2 Hz, 1 H), 7.37 (d, *J* = 2 Hz, 1 H), 6.31 (s, 1 H), 5.31 (m, 1 H), 3.80 (d, *J* = 18 Hz, 1 H), 3.67 (s, 3 H), 3.58 (d, *J* = 18 Hz, 1 H), 2.19 (s, 3 H).

7-Chloro-3,5-methyl-3,4-dihydro-2-acetonitrile-1,2,4-benzothiadiazine S,S dioxide (16). Refer to preparation of **15**, except bromoacetonitrile was used as the electrophile (65% yield). ^{1}H NMR (d_6-DMSO) δ 7.49 (d, *J* = 2 Hz, 1 H), 7.40 (s, 1 H), 6.48 (s, 1 H), 5.32 (m, 1 H), 4.18 (q, *J* = 20 Hz, 2 H), 2.19 (s, 3 H), 1.64 (d, *J* = 7 Hz, 3 H).

7-Chloro-3,5-methyl-3,4-dihydro-2-benzyl-1,2,4-benzothiadiazine S,S-dioxide (17). Refer to preparation of **15**, except benzyl bromide was used as the electrophile (68% yield). ^{1}H NMR (d_6-DMSO) δ 7.45 (s, 1 H), 7.36 (m, 4 H), 7.30 (m, 2 H), 6.40 (d, *J* = 3 Hz, 1 H), 5.39 (m, 1 H), 4.05 (q, *J* = 16 Hz, 2 H), 2.19 (s, 3 H), 1.41 (d, *J* = 5 Hz, 3 H).

7-Chloro-3,5-methyl-3,4-dihydro-2-acetate-1,2,4-benzothiadiazine S,S dioxide (18a). To **15** (44 mg, 0.14 mmol), was added 10M NaOH and stirred for 1 hr. The reaction was neutralized with 45% H_2SO_4 and extracted with EtOAc (2x5 mL). The product was obtained by concentration of the organic layers *in vacuo* (25 mg, 60%). ^{1}H NMR (d_6-DMSO) δ 7.42 (s, 1 H), 7.37 (s, 1 H), 6.38 (d, *J* = 3 Hz, 1 H), 5.30 (m, 1 H), 3.69 (d, *J* = 18 Hz, 1 H), 3.57 (d, *J* = 6 Hz, 2 H), 3.48 (d, *J* = 18 Hz, 1 H), 2.12 (s, 3 H), 1.48 (d, *J* = 7 Hz, 3 H).

7-Chloro-3,5-methyl-3,4-dihydro-2-hydroxyethyl-1,2,4-benzothiadiazine S,S dioxide (18b). To **15** (100 mg, 0.31 mmol) in THF (5 mL) was added 100 mg (3mmol) $NaCNBH_3$ in 4 mL MeOH. The reaction was stirred overnight, neutralized with 1 N HCl, and extracted with EtOAc/sat. $NaHCO_3$. The organic layers were combined, dried ($MgSO_4$), and concentrated *in vacuo*. The crude product was dissolved in EtOAc, silica gel was added, and the mixture was again concentrated *in vacuo* for dry-loading onto a silica gel column. Column chromatography, with 40% v/v EtOAc:hexanes as the eluent, yielded **18b** as a white solid (80 mg, 87%). ^{1}H NMR (d_6-DMSO) δ 7.40 (d, *J* = 3 Hz, 1 H), 7.34 (d, *J* = 3 Hz, 1 H), 6.39 (d, *J* = 3 Hz, 1 H), 5.22 (m, 1 H), 4.88 (t, *J* = 6 Hz, 1 H), 3.47 (m, 2 H), 2.98 (m, 1 H), 2.75 (m, 1 H), 2.19 (s, 3 H), 1.54 (d, *J* = 7 Hz, 3 H).

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