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Perspective

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The 2011 E. B. Hershberg Award for Important Discoveries in Medicinally Active Substances: (1*S*,3*S*)-3-Amino-4-difluoromethylenyl-1-cyclopentanoic Acid (CPP-115), a GABA Aminotransferase Inactivator and New Treatment for Drug Addiction and Infantile Spasms†

Richard B. Silverman*

Department of Chemistry and Department of Molecular Biosciences, Chemistry of Life Processes Institute, Center for Molecular Innovation and Drug Discovery, Northwestern University, Evanston, Illinois 60208-3113, United States

Enzymes are excellent targets for drug design because many diseases, or at least the symptoms of disease, can arise from a deficiency of one specific molecule, an excess of one molecule, infestation of a foreign organism, or aberrant cell growth; all of these etiologies can be modulated by specific enzyme inhibition. Inhibition of an enzyme prevents the conversion of substrate to product, thereby increasing the concentration of the substrate and decreasing the concentration of the product, thereby normalizing a deficiency or excess, respectively, of those particular molecules. By targeting an enzyme essential for the life of a foreign organism or tumor cell, it is possible to destroy that organism or cell or, at least, prevent it from replicating. This Award Lecture Perspective takes advantage of an approach to increase the concentration of a single molecule, namely, γ-aminobutyric acid (GABA), for the treatment of seizure disorders and drug addiction.

The two principal neurotransmitters involved in the regulation of brain neuronal activity are GABA, one of the most widely distributed inhibitory neurotransmitters, and L-glutamic acid, an excitatory neurotransmitter.[1] The concentration of GABA is regulated by two pyridoxal 5′-phosphate (PLP) dependent enzymes, L-glutamic acid decarboxylase (GAD), which catalyzes the conversion of L-glutamate to GABA, and GABA aminotransferase (GABA-AT), which degrades GABA to succinic semialdehyde (SSA) and converts α-ketoglutarate to L-glutamic acid (Figure 1).[2] When the concentration of GABA diminishes below a threshold level in the brain, convulsions result;[3] raising the brain GABA levels terminates the seizure and is an effective approach for the treatment of epilepsy.[4] Unfortunately, it is futile to take GABA pills to raise the brain GABA levels because GABA is transported across the blood–brain barrier very poorly[5] and is effluxed from the brain readily.[6]

Seizures can arise from numerous etiologies; therefore, epilepsy is not a single disease, and the incidence of seizure activity is very prevalent in the world. In fact, when epilepsy is defined broadly as any disease characterized by recurring convulsive seizures, then 1–2% of the world population can be classified as having epilepsy.[7] Consequently, anticonvulsant agents have been sought for centuries. Not until diphenylhydantoin (Dilantin) was introduced onto the drug market almost 60 years ago was any particular anticonvulsant drug widely used.[8] However, this drug is not generally applicable. In fact, more than one-quarter of epileptic patients worldwide (about 12 million people) do not respond to any marketed anticonvulsant drug.[5] Therefore, the need for new anticonvulsant drugs is great.[9]

One cause for epilepsy is an imbalance in the GABA/L-glutamate brain levels. A reduction in the concentrations of GABA and/or of the enzyme GAD,[10] which produces GABA, has been implicated not only in the symptoms associated with epilepsy[11] but also with several other neurological diseases such as Huntington's chorea,[12,13] Parkinson's disease,[14,15] Alzheimer's disease,[16] and tardive dyskinesia.[17] Several approaches have been taken to increase the brain concentrations of GABA. One approach has been to make prodrugs of GABA,[18,19] but except for progabide, this has not been highly successful. Another approach taken to increase brain GABA levels is the use of a compound that crosses the blood–brain barrier and then inhibits or inactivates GABA-AT, the enzyme that degrades GABA. Inhibition of this enzyme causes a buildup of GABA, assuming that inhibition of GAD is minimal. This effectively dampens excessive neural activity without affecting basal neuronal activity, thereby controlling seizures.[20]

Numerous competitive inhibitors of GABA-AT, particularly compounds having a similar backbone structure to GABA,[21] show anticonvulsant activity. A variety of mechanism-based inactivators[22] of GABA-AT[23] have been shown to be effective anticonvulsant agents. The most effective of these mechanism-based inactivators as an anticonvulsant agent is 4-aminohex-5-enoic acid (**1**, γ-vinyl-GABA),[24] which has the generic name vigabatrin and trade name Sabril. Vigabatrin is currently marketed as a monotherapy for pediatric patients 1 month to 2 years of age with infantile spasms (West's syndrome) and as an adjunctive therapy for adults with refractory complex partial seizures in 65 countries, including the United States.[25] However, 1–3 g of this drug has to be taken daily to be effective. As with all psychotropic drugs, there are a variety of side effects that arise from the use of this drug (see below), which is sold as a racemic mixture. It is not known how many of these side effects arise from the administration of the inactive enantiomer (*R*-isomer).

About 13 years ago it was found that vigabatrin (**1**) possesses another remarkable activity; namely, it prevents cocaine addiction in rats and baboons.[26] Self-administration of cocaine by rats decreased or was prevented by vigabatrin administration in a dose-dependent fashion, without affecting the craving for food.[27] The current findings indicate that the neurochemical

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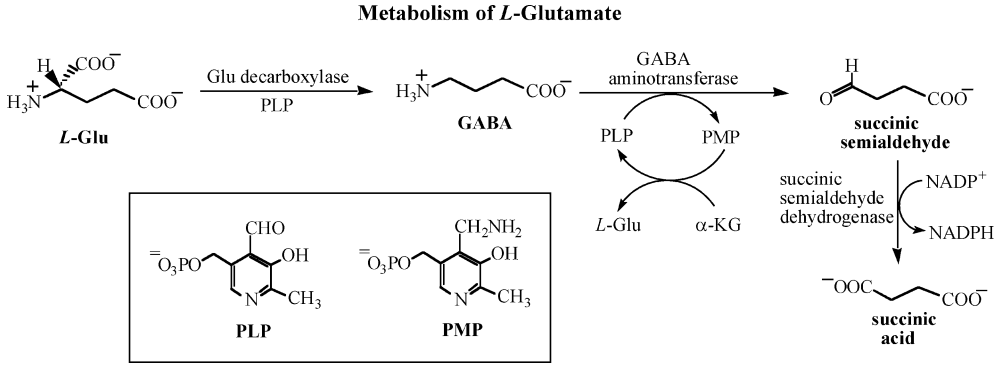
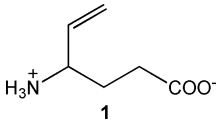


Figure 1. Regulation of brain GABA concentration.



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response to cocaine and other drugs of abuse is a sharp increase in dopamine levels in the nucleus accumbens (NAcc),[28] which activates the neurons responsible for pleasure and rewards responses. The connection between addiction and GABA is that the rise in dopamine and associated addictive behaviors can be antagonized by an increase in the concentration of GABA. The mechanism of vigabatrin responsible for the prevention of drug addiction would be the same as that for epilepsy; namely, it inactivates GABA-AT, thereby increasing the GABA concentration in the brain (by blocking the degradation of brain GABA). It was shown by positron emission tomography (PET) in primates that vigabatrin inhibits these cocaine-induced dopamine increases.[25] Vigabatrin also has been found to have utility in the treatment of a variety of other addictions,[29] specifically in animal models for nicotine,[30] methamphetamine, heroin, ethanol,[31] and combination addictions.[32] Vigabatrin treatment also is effective for addiction in humans,[33] including a randomized, double-blind, placebo-controlled trial of 103 subjects,[34] in which 28.0% of subjects treated with vigabatrin achieved abstinence compared to 7.5% of subjects treated with placebo.

The acceptance of vigabatrin for the treatment of epilepsy and as a potential treatment for addiction has been hampered primarily by concerns about abnormalities of the peripheral visual field (visual field defects or VFDs), resulting from retinal damage, in 25−50% of patients following chronic administration of vigabatrin.[35] The development of VFDs seems to result from prolonged exposure to high doses of vigabatrin.[36] The mechanism leading to the VFDs is not known, but it remains an active area of research. VFDs might occur as a direct toxic effect of vigabatrin, from the inactive enantiomer (*R*-isomer), from elevated GABA levels as a result of inactivation of GABA-AT, as a consequence of an enzymatically produced byproduct (a metabolite) from one of the enzyme inactivation mechanisms, from off-target activities, or from some combination of these potential mechanisms. Although it has been hypothesized that elevated GABA levels might be involved in vigabatrin-elicited retinal damage,[37] it has been demonstrated that in albino rats acute vigabatrin exposure damages the outer retina by a GABA-*independent* and vigabatrin-specific mechanism, resulting in sensitization of photoreceptors to light-induced damage.[38] In this case, it is probable that reactive oxygen species are involved, since they participate in light-mediated retinal toxicity.[39] If the prevailing belief that VFDs arise from prolonged exposure to large doses of vigabatrin is correct, and if much lower doses of an alternative drug can be used, this could dramatically diminish, if not eliminate, the resulting VFDs.

To design an alternative to vigabatrin, a mechanism-based inactivator,[40] it is important to understand how that compound inhibits GABA-AT. A mechanism-based inactivator is an unreactive compound whose structure is related to that of the substrate for the target enzyme, which converts the inactivator into a species that leads to inactivation of that enzyme, prior to escape of that species from the active site. Therefore, the design of a mechanism-based inactivator requires knowledge of the substrate(s) for and mechanism of the target enzyme.

GABA-AT is a dimeric enzyme, each subunit containing an active site pyridoxal 5′-phosphate (PLP) coenzyme; however, the two PLP binding sites are nonequivalent.[41] Following inactivation with 1 equiv of inactivator, it is possible to incorporate a second coenzyme molecule and regain activity. This may explain why some inactivators become incorporated at the level of 1 equiv per enzyme dimer and others incorporate 2 equiv per dimer. The primary sequence of GABA-AT has been deduced from the cDNA of pig brain[42] and from peptide fragments of the pig liver enzyme.[43] In 1999 the X-ray crystal structure of pig liver GABA-AT was reported to 3.0 Å resolution by the Schirmer group in Basel, Switzerland.[44] A 1.9 Å resolution crystal structure with one of our inactivators bound also has been reported.[45]

The mechanism of GABA aminotransferase is shown in Scheme 1. Following Schiff base formation (**2**), tautomerization gives the aldimine (**3**), which is hydrolyzed to succinic semialdehyde (SSA) and pyridoxamine 5′-phosphate (PMP). At this point the enzyme is inactive, as the coenzyme is in the wrong oxidation state. α-Ketoglutarate (α-KG) then converts the PMP back to PLP with concomitant formation of L-glutamate. On the basis of the substrate mechanism, we investigated the inactivation mechanism of vigabatrin and found that it inactivates GABA-AT by at least two mechanisms: a Michael addition mechanism following tautomerization to ketimine **4** (Scheme 2, pathway a, leading to **5**) and an enamine mechanism following tautomerization through the vinyl double bond and release of enamine **6** (pathway b, leading to **7**).[46] These two pathways were shown to occur in about a 70:30 ratio, respectively. A crystal structure of inactivated GABA-AT to 2.3 Å resolution confirmed the Michael addition adduct structure (**5**)[47] (Figure 2).

As noted above, a serious drawback to the use of vigabatrin is retinal toxicity produced in a large percentage of patients using it chronically. One suggested hypothetical cause for the retinal damage might be the formation of a metabolite of vigabatrin.

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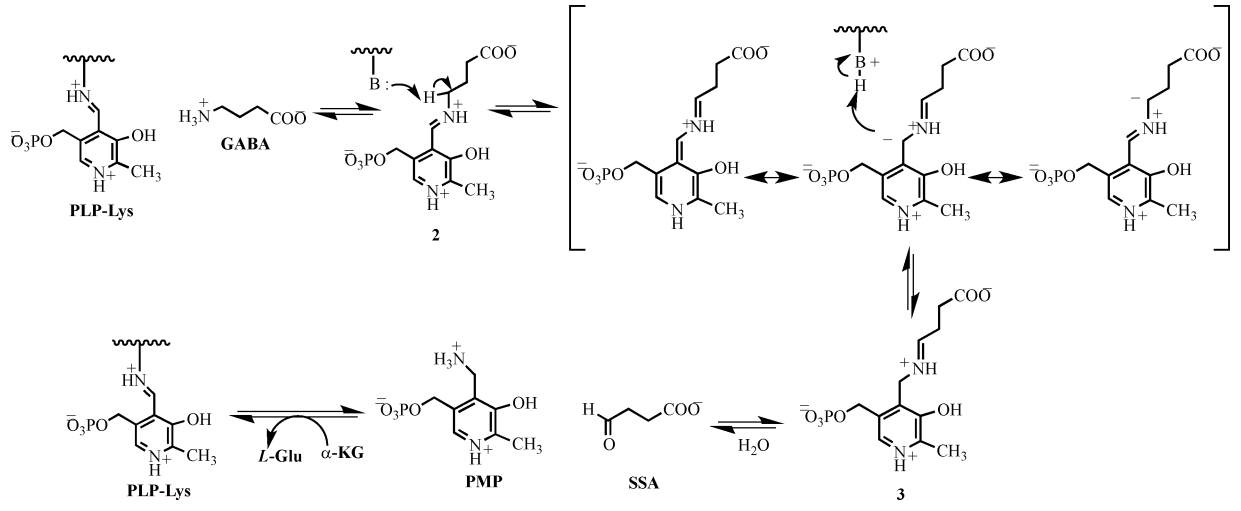
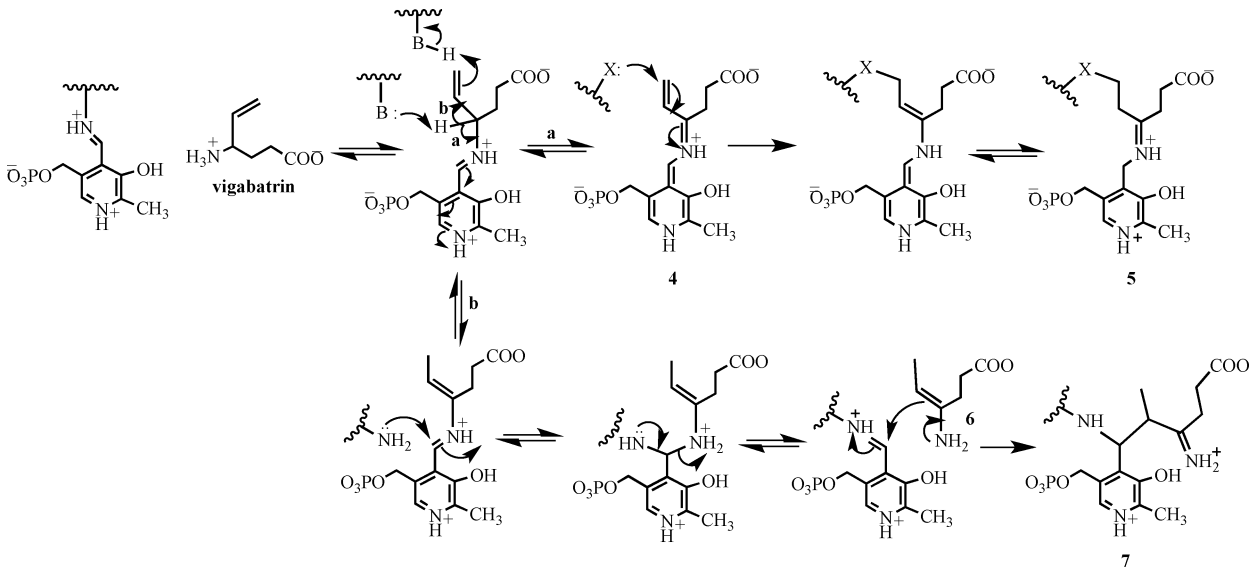
Scheme 1. Mechanism of GABA-AT



Scheme 2. Mechanism of Inactivation of GABA-AT by Vigabatrin



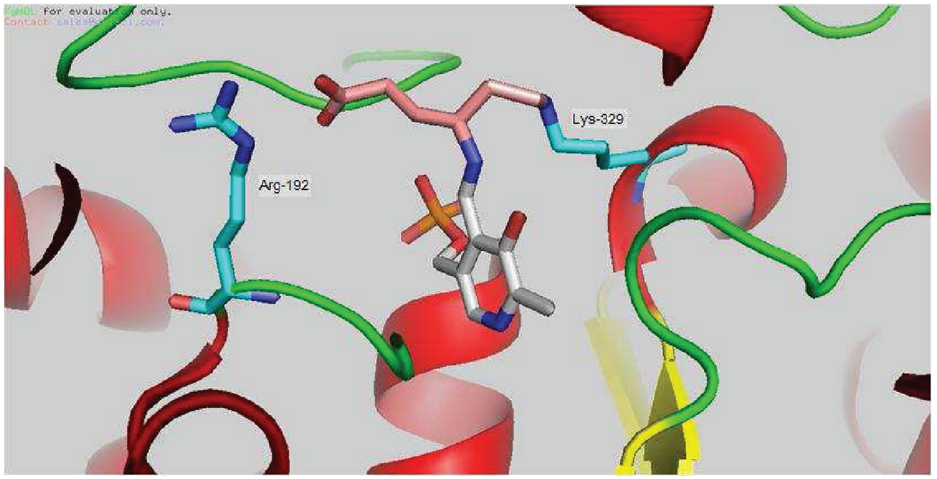


Figure 2. Crystal structure of GABA-AT inactivated by vigabatrin (brown).

During our studies on the mechanism of inactivation of GABA-AT by vigabatrin, it was found that ketimine **4** underwent partial hydrolysis to the corresponding α,β-unsaturated ketone (**8**), a reactive electrophile, and PMP. Possibly that electrophile, released in the GABA-rich regions of the retina, could cause retinal toxicity. Hydrolysis of enamine **6**, however, would give

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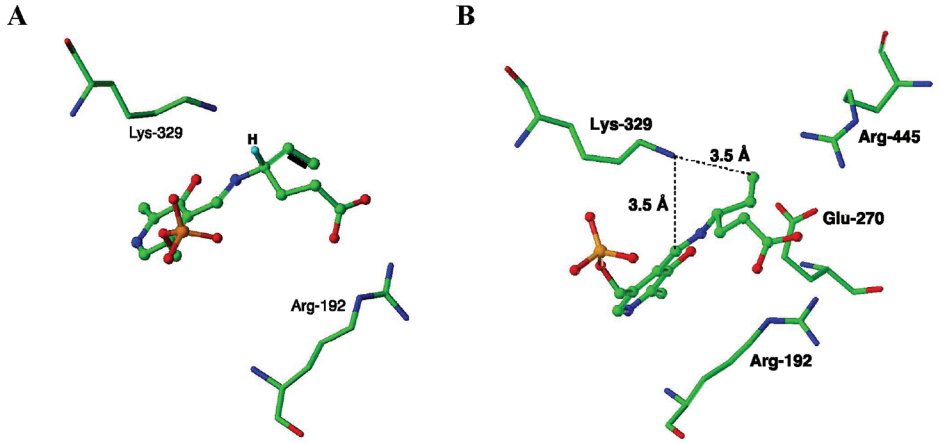
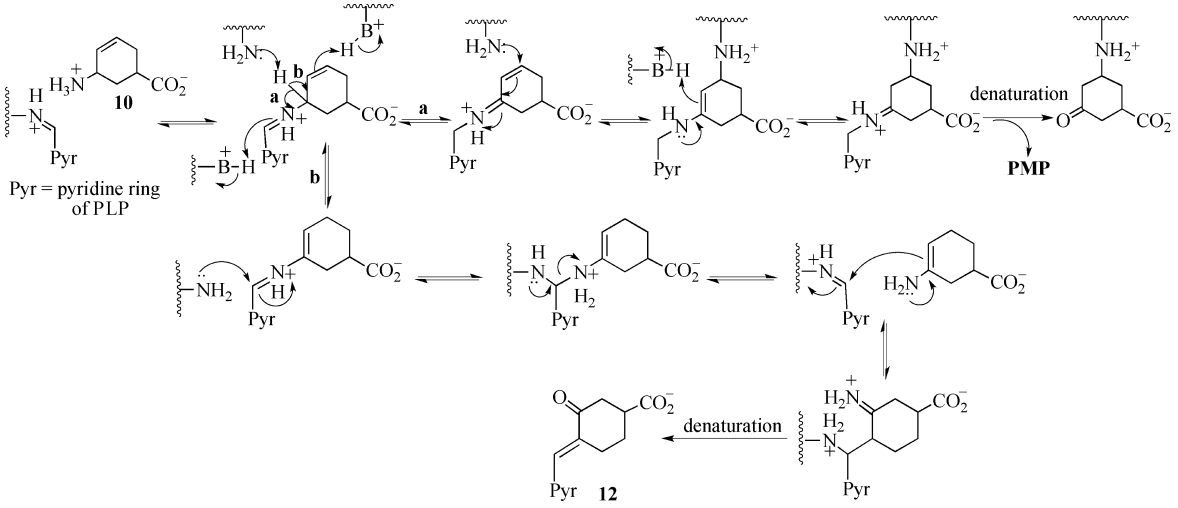
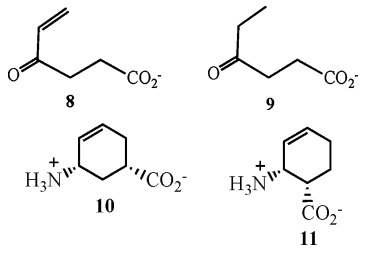


Figure 3. (A) Energy minimized molecular model of vigabatrin bound to PLP in GABA-AT. (B) Model showing necessary bond rotation for proper alignment to allow Michael addition to occur. Lys-329 is set up for both Michael addition and enamine formation.

Scheme 3. Michael Addition Pathway (a) and Enamine Pathway (b) for 10





saturated ketone **9**, which would not be highly electrophilic. Therefore, if the Michael addition pathway could be bypassed in favor of the minor enamine pathway, electrophile **8** could be avoided. An energy minimized molecular model of vigabatrin bound to PLP within GABA-AT (Figure 3A) showed that it is set up for Lys-329 deprotonation, but following tautomerization, the vinyl double bond is in the wrong orientation for Michael addition to occur. Bond rotation is necessary to permit Michael addition (Figure 3B). Therefore, prevention of bond rotation should block the Michael addition pathway but not the enamine pathway. This is readily accomplished with conformationally restricted analogues, such as **10** and **11**.[48] Both of these compounds were found to be irreversible inactivators of GABA-AT; the k_{inact}/K_I for **10** was twice that of **11**, so mechanistic studies were focused on **10**. If the Michael addition pathway occurred with **10**, then, after denaturation, the coenzyme would be in the PMP form (Scheme 3, pathway a). If the enamine mechanism occurred, then, after denaturation, modified coenzyme **12** would result (Scheme 3, pathway b). Modified coenzyme **12** was synthesized as a chromatography standard. The PLP in GABA-AT was removed and replaced with [^{3}H]PLP. Then the tritiated enzyme was inactivated with **10** and denatured. HPLC of the supernatant revealed only **12**, no PMP, indicating that the Michael addition pathway was blocked and only the enamine pathway had occurred. Unfortunately, **10** was not very potent, so an alternative way of preventing the formation of an electrophilic product was sought.

An alternative approach could be to allow the Michael addition pathway to proceed but to design the molecule so that the intermediate ketimine (**4**, Scheme 2) undergoes covalent reaction with Lys-329 much faster than hydrolysis of the ketimine can occur. To accomplish that, a compound was designed having a double bond that was oriented toward Lys-329 after Schiff base formation with the PLP[49] (**13**, Figure 4). Compound **13** was a time-dependent inactivator of GABA-AT but only after a lag in time, suggesting that the compound was converted to a product (**14**), which escaped from the active site, built up in solution, then returned to cause inactivation. When a good nucleophile (β-mercaptoethanol) was added to the incubation mixture to trap any electrophiles released from the enzyme, there was no

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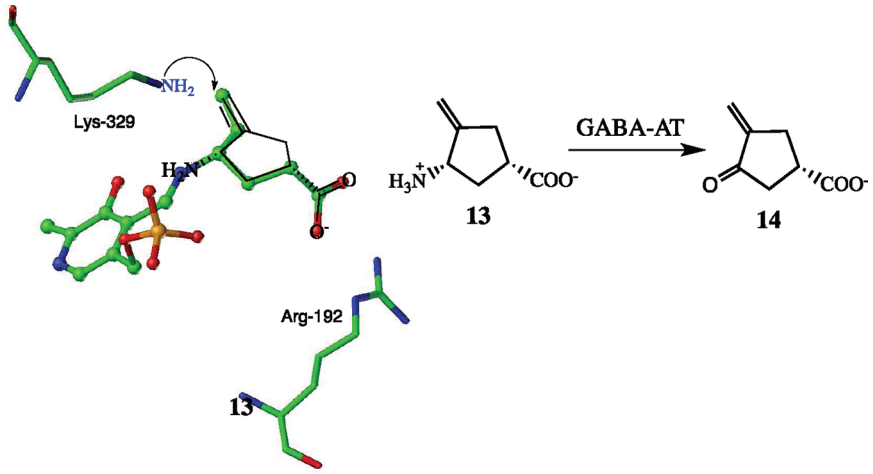
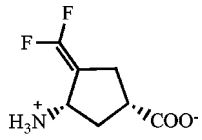


Figure 4. Conformationally rigid vigabatrin analog with the methylene group oriented toward Lys-329 for rapid reaction.

inactivation, supporting this hypothesis. Therefore, a more reactive electrophile was sought. Michael acceptors become more reactive when electron-withdrawing groups are added to the terminus of the double bond. Because fluorine is strongly electron withdrawing, yet relatively small, **13** was modified by the substitution of two fluorine atoms at the alkene terminus to give **15**.[49] Compound **15** (now referred to as CPP-115)[50] was found to inactivate GABA-AT instantaneously, even in the presence of β-mercaptoethanol, with a K_I of 9.7 μM, whereas vigabatrin had a K_I of 850 μM under the same conditions. The k_{inact} values for **15** and vigabatrin were 0.50 and 0.24 min^{-1}, respectively. Therefore, **15** was 187 times more efficient an inactivator of GABA-AT than vigabatrin (k_{inact}/K_I of 52 $mM \cdot min^{-1}$ for **15** and k_{inact}/K_I of 0.28 $mM \cdot min^{-1}$ for vigabatrin).

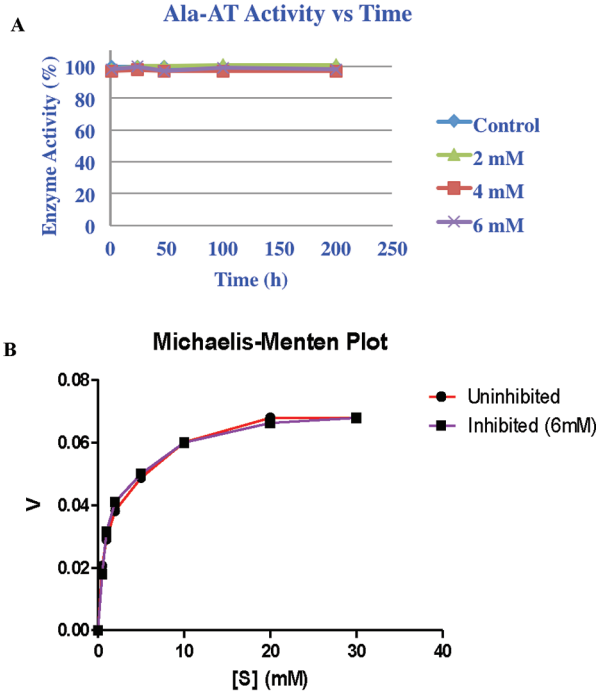


15
CPP-115

Some off-target activities of **15** were investigated. Vigabatrin is known to inhibit alanine aminotransferase in vitro[51] and in vivo,[52] which might account for some of its side effects. It also inactivates aspartate aminotransferase.[53] We have found that **15** neither inactivates (Figure 5A) nor inhibits (Figure 5B) alanine aminotransferase and aspartate aminotransferase (data not shown) up to 6 mM (Figure 5).[50]

To determine if **15** has GABAergic properties, its binding to a variety of GABA binding proteins was investigated.[50] Compound **15** does not bind to human GABA transporters hGAT-1, hBGAT-1, hGAT-2, and hGAT-3, to mouse GABA transporters mGAT-1, mGAT-2, mGAT-3, mGAT-4, or to neurons or astrocytes up to 1 mM. At concentrations up to 100 μM, **15** does not displace GABA (IC_{50} in low nanomolar range) from $GABA_A$ or $GABA_B$ receptors nor is it an agonist or antagonist of the $GABA_C$ receptor.[50] Therefore, it appears that **15** does not exhibit GABAergic activities.

As noted above, drug addiction results from the release of dopamine when an addictive substance is ingested. The effects of vigabatrin and **15** on the release of dopamine in the nucleus accumbens (NAcc) in freely moving rats were determined. As shown in Figure 6, the dopamine concentration increased by



	Uninhibited	Inhibited (6mM)
Vmax	0.07026	0.06934
Km	1.557	1.401
Std. Error		
Vmax	0.002173	0.001631
Km	0.2028	0.1422

Figure 5. (A) Time-dependent effect of **15** on alanine aminotransferase. (B) Concentration-dependent effect of **15** on alanine aminotransferase.

550% (relative to control) in the NAcc after 20 mg/kg cocaine was administered. Treatment with 300 mg/kg vigabatrin reduced the dopamine concentration to about 330% of basal level, but only 1 mg/kg **15** was needed to reduce the dopamine levels to 250% of basal level.[50] Therefore, **15** is >300 times more potent than vigabatrin for reducing dopamine levels in the NAcc.

Another measure of dopamine release can be made by in vivo micropositron emission tomography (micro-PET) imaging.[54]

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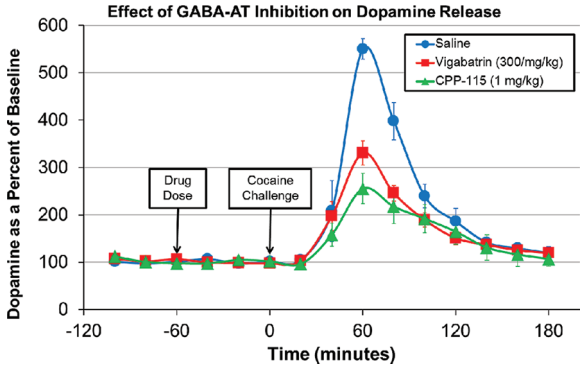

Figure 6. Effect of vigabatrin and **15** (CPP-115) on dopamine release by cocaine in freely moving rats. Cocaine was administered at 20 mg/kg.

Dopamine can release [^{11}C]raclopride from NAcc, so treatment of rats with cocaine shows the displacement of [^{11}C]raclopride as a result of the released dopamine (Figure 7, cocaine panel).

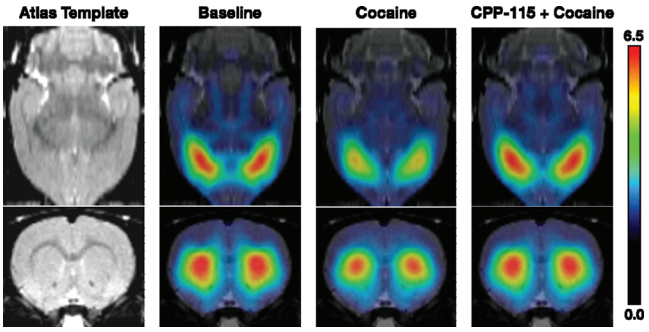

Figure 7. In vivo micro-PET imaging of [^{11}C]raclopride (red color, baseline panel). Administration of 20 mg/kg cocaine leads to the release of dopamine, which displaces the [^{11}C]raclopride (middle panel). Administration of 20 mg/kg cocaine and 0.5 mg/kg **15** (CPP-115) does not release [^{11}C]raclopride (right panel).

The same experiment with either vigabatrin at 300 mg/kg or **15** at 0.5 mg/kg does not cause release of [^{11}C]raclopride, indicating that insufficient dopamine was released to displace the [^{11}C]raclopride. Therefore, **15** is 600 times more potent than vigabatrin in this experiment.

The standard paradigm for measurement of addiction potential of drugs in animals is conditioned place preference.[55] There are two different colored chambers. Some animals are placed into one and given an injection of an addictive substance. Other animals are placed in the other chamber and are given an injection of saline. The next day the animals are reversed. This process continues for 10 days, so they are trained to associate one chamber with the addictive substance and the other with saline. On the test day all animals have free access, in a drug-free state, to both chambers. What generally happens is they exhibit a conditioned place preference for the addictive-substance-paired chamber and just sit in there. The beauty of this paradigm is that animals are tested in a drug-free state and, therefore, make a choice in the absence of any acute drug effects. For **15** and vigabatrin the animals were first trained with cocaine. Then when **15** at 1 mg/kg (or vigabatrin at 300 mg/kg) was administered with cocaine, the animals reverted to no place preference (Table 1), indicating that they no longer were addicted. Once again, **15** was 300 times more potent than vigabatrin. A serious problem with drug addiction

Table 1[a]

		time in chamber (min)	
treatment group	no. of animals	paired with cocaine	unpaired with cocaine
saline/saline	9	7.3 ± 0.5	7.7 ± 0.6
saline/cocaine	9	11.8 ± 0.4	3.2 ± 0.4
saline/**15**	8	8.1 ± 0.2	6.9 ± 0.9
15/cocaine	8	7.8 ± 0.5	7.2 ± 0.9
vigabatrin/cocaine	10	7.9 ± 0.5	7.1 ± 0.6

[a]Compound **15** alone is not rewarding and blocks the expression of place preference to cocaine (20 mg/kg). **15** (1 mg/kg) and vigabatrin (300 mg/kg) produced comparable inhibition of cocaine-induced place preference: $p < 0.001$, Student's *t*-test.

is that following a treatment program the former addict may be subjected to sensory reminders of the addiction, which can lead to a return to the substance abuse. The above experiment demonstrates that, even when faced with a sensory reminder, the animals do not revert to their addictive desire.

The effect of **15** on infantile spasms in rats also was investigated.[56] Infantile spasms are a severe form of epilepsy, leading to cognitive deterioration, mental retardation, other serious seizures, and mortality.[57] The incidence is about 1 in 2000–4000 live births, which accounts for about 30% of childhood epilepsies. There are two drugs that have been used to treat infantile spasms, adrenocorticotropic hormone (ACTH) and vigabatrin, but both cause serious side effects.[55] Because of the ineffectiveness of ACTH in about half of the diagnosed cases, vigabatrin was approved in the United States in 2009 for infantile spasms, despite the associated visual field defect side effect.[58] In a multiple-hit rat model for infantile spasms,[59] which is refractory to ACTH, **15** suppressed spasms at doses of 0.1–1 mg/kg, 100 times lower than doses of vigabatrin, with more sustained effect and better tolerance than vigabatrin. Therefore, **15** has a much larger margin of safety than vigabatrin. Compound **15** was granted orphan drug designation by the FDA for the treatment of infantile spasms.

Oral gavage administration of **15** to rats at a dose of 2, 6, and 20 mg kg^{-1} day^{-1} for 28 days was well tolerated at 2 and 6 mg kg^{-1} day^{-1} and resulted in no clinical observations or changes in body weight or food consumption. Oral gavage administration of **15** to beagle dogs at dose levels of 0.7, 2.3, and 7 mg kg^{-1} day^{-1} for 28 days was well tolerated at all dose levels.

Finally, the effect of **15** and vigabatrin on retinal damage was determined. The effective dose for infantile spasms in rats using vigabatrin is 300 mg kg^{-1} day^{-1}. At 200 mg/kg, roughly the effective dose, after 45 days of treatment there was a 30–60% electroretinographic loss. The effective dose in rats for **15** is 0.5–1 mg kg^{-1} day^{-1}. At 20 mg/kg after 45 days there was only a 5–30% electroretinographic loss. But this is 20–40 times the effective dose, so at the effective dose, there should be an insignificant retinal loss.

In conclusion, on the basis of our early studies of the mechanism of inactivation of GABA-AT by vigabatrin,[46] we found that a byproduct of inactivation was a reactive electrophile, which we wanted to prevent from being produced. A conformationally rigid analogue of vigabatrin was synthesized that avoided the Michael addition inactivation pathway (Scheme 2, pathway a), which was responsible for the generation of the electrophile.[48] Although this was successful, the inactivator had low potency. An alternative approach was investigated by which the inactivator was designed to proceed via the Michael addition pathway, but the intermediate would encourage rapid covalent reaction by the

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enzyme without release of an electrophilic species. This approach looked promising, but the intermediate was not reactive enough and an electrophile was still released. The compound was modified to increase its reactivity, leading to **15**, which was 187 times more potent than vigabatrin in vitro and 300−600 times more potent in vivo in a rat model for drug addiction and >100 times more effective in the treatment of infantile spasms in a rat model. At 20−40 times greater dose of **15** than was needed to treat drug addiction and infantile spasms, there was much less retinal damage than administration of vigabatrin at its effective dose. Compound **15** has been licensed to Catalyst Pharmaceutical Partners, Inc., which has begun phase I clinical trials.

AUTHOR INFORMATION

Corresponding Author

*Phone: 847-491-5653. Fax: 847-467-1566. E-mail: Agman@chem.northwestern.edu.

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ABBREVIATIONS USED

ACTH, adrenocorticotropic hormone; GABA, γ-aminobutyric acid; GABA-AT, γ-aminobutyric acid aminotransferase; GAD, L-glutamic acid decarboxylase; α-KG, α-ketoglutarate; NAcc, nucleus accumbens; PET, positron emission tomography; PLP, pyridoxal 5′-phosphate; PMP, pyridoxamine 5′-phosphate; SSA, succinic semialdehyde; VFD, visual field defect

ADDITIONAL NOTE

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