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Design and Synthesis of Anticonvulsive Agents as γ-Vinyl GABA-Based Potential Dual Acting Prodrugs and their Biological Activities

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Abstract—For the development of new anticonvulsive agents, γ-vinyl GABA (vigabatrin) and GABA mimetics derivatives were covalently coupled as potential dual acting prodrugs and evaluated for their anticonvulsive activities. Among the prepared compounds, **11** showed the most potent anticonvulsive activity, a shorter onset time and a broader spectrum compared to vigabatrin.

Introduction

Approximately 1% of the population is reported to be affected by some form of epilepsy and 20–40% of epileptic patients have experienced failure to control seizures, and 40–60% of patients have suffered from drug resistance to currently available anticonvulsant drugs.[1] Since epilepsy consists of various forms of seizure,[2] combinations of drugs and repeated therapy is required to control such complex convulsions,[3] which increases the toxicity and adverse side effects of clinically using anticonvulsant drugs.

Recently, many studies have been carried out for the development of new types of anticonvulsant agents, including γ-aminobutyric acid (GABA) related compounds,[4] derivatives of amino acids,[5] and structurally modified compounds of currently used drugs.[6] However, these compounds cannot provide complete control of complex seizures. Many GABA mimetic substances such as GABA receptor agonists, GABA reuptake inhibitors and GABA metabolism inhibitors have been reported to be potent anticonvulsant agents, and some of these agents have been clinically used for the treatment of epilepsy.[4,7] However, these compounds do not readily enter into the central nervous system in pharmacologically effective amounts following peripheral administration, since these compounds can not readily pass the blood–brain barrier (BBB), presumably due to their lower lipophilicity.[4,8,9]

In connection with the studies for the development of new anticonvulsants having a broader spectrum with potent anticonvulsant activity and lower toxicity, our previous results have been published.[10]

In this study, we designed and synthesized potential dual acting prodrugs which were covalently coupled with an amide bond of vigabatrin (**1**)[11] and GABA mimetic substances such as isonipecotic acid (**2**)[4], nipecotic acid (**3**)[12] and 2-pyrrolidinone (**4**)[10] (Fig. 1). Vigabatrin (γ-vinyl GABA) was recently developed as an anticonvulsive agent which was reported to be a mechanism based inhibitor of GABA aminotransferase, and has been widely used clinically for the treatment of seizure along with valproic acid. But vigabatrin has a relatively narrow clinical spectrum and long onset time (18 h) due to its hydrophilic character, and a relatively large amount (500–1000 mg) should be administered. 2-Pyrrolidinone was reported as a prodrug of GABA, isonipecotic acid as a GABA receptor agonist, nipecotic

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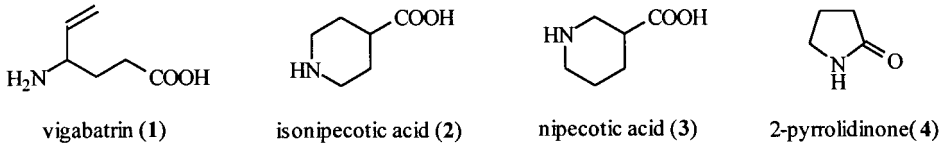


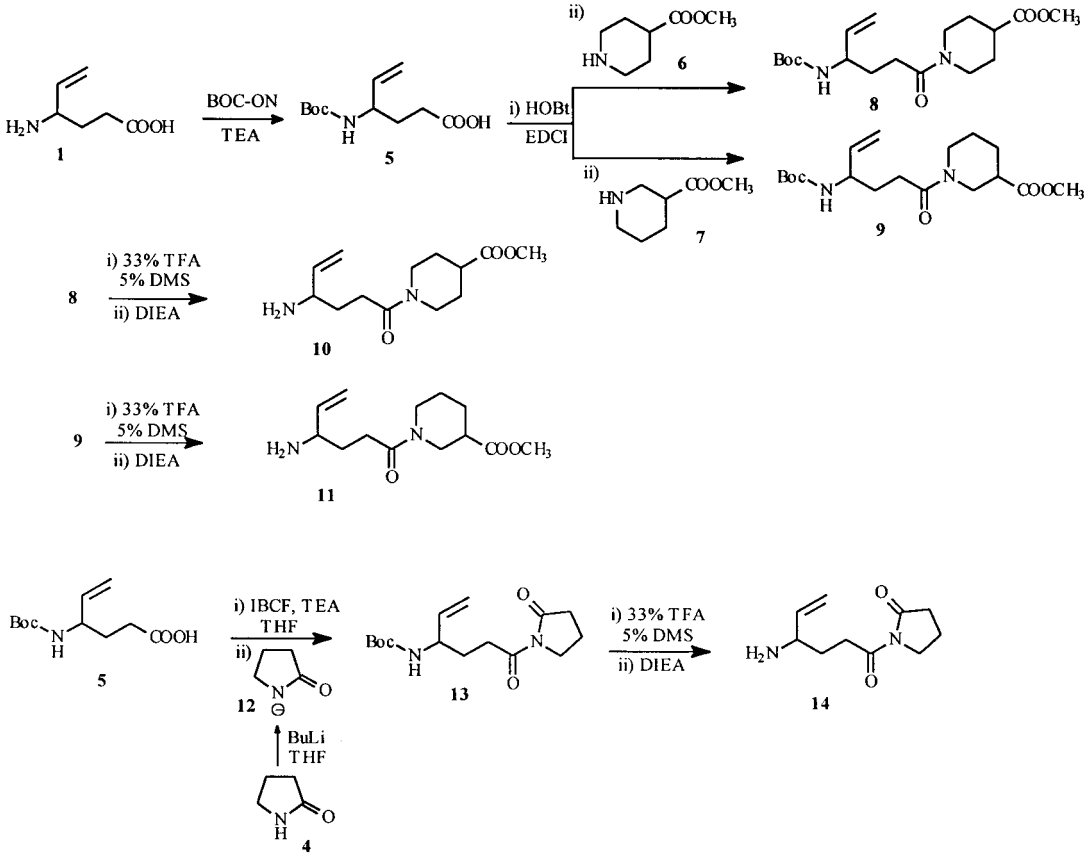
Figure 1.

acid as a GABA uptake inhibitor. They show potent anticonvulsive activities in vitro, but are barely potent in vivo due to their hydrophilic properties.[4,8,12] The biological activity of the prepared prodrugs were evaluated for the development of potent anticonvulsive agents having a broader clinical spectrum and higher hydrophobic properties, which might exhibit dual acting anticonvulsive activities with different mechanisms.[13] We considered that these prodrugs had higher hydrophobic properties sufficient to pass through the BBB, and expected them to enter readily into the CNS and transform into active compounds after metabolic enzymatic hydrolysis in the CNS. Then the hydrolyzed compounds could display their anticonvulsive activity with different mechanisms. Therefore, these prodrugs are expected to have increased potency through a synergistic effect and a broader spectrum. Also we expected the hydrolyzed compounds to stay longer in CNS due to their hydrophilic properties, which would increase the duration of their anticonvulsant effects.

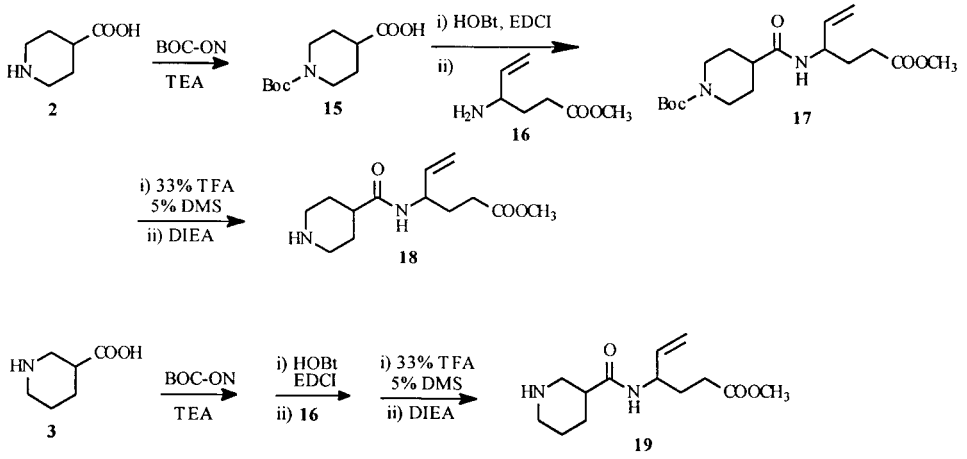
We designed and prepared 5 compounds based on the vigabatrin moiety as follows. First, acid moiety of vigabatrin was coupled by an amide bond with the amine moiety of isonipecotic acid methyl ester, nipecotic acid methyl ester and 2-pyrrolidinone (compounds **10**, **11**, **14**, respectively) as shown in Scheme 1. Second, the amine moiety of vigabatrin methyl ester was coupled by an amide bond with the acid moiety of isonipecotic acid and nipecotic acid (compounds **18**, **19** respectively) as shown in Scheme 2. The prepared compounds were evaluated for their anticonvulsive activity.

Chemistry

Compounds **10** and **11** could be prepared from protected vigabatrin **5** with isonipecotic acid methyl ester (**6**) and nipecotic acid methyl ester (**7**), respectively, in moderate yield by known general reactions as outlined in Scheme 1.



Scheme 1.



Scheme 2.

Boc protected vigabatrin (**5**) was prepared from vigabatrin (**1**) by treatment with BOC-ON and sodium bicarbonate in water/dioxane in 92% yield as a colorless crystal. Intermediates **8** and **9** were prepared from **5** and isonipecotic acid methyl ester (**6**) and nipecotic acid methyl ester (**7**), respectively, with addition of EDCI and HOBT in 68 and 73% yield as colorless oils. Intermediates **8** and **9** were treated with 33% TFA and 5% DMS in CH_2Cl_2 to yield oils as TFA salt, which was treated with diisopropyl ethyl amine (DIEA) to give final products **10** and **11**, respectively in 96 and 94% yield as a colorless oil.

Intermediate **13** was prepared with Boc vigabatrin (**5**) and isobutyl chloroformate in the presence of TEA to form a mixed anhydride, followed by the addition of an anion of 2-pyrrolidinone (**12**) which was prepared from 2-pyrrolidinone (**4**) and *n*-butyllithium in 50% yield as a colorless oil. Intermediate **13** was treated with 33% TFA and 5% DMS in methylene chloride to yield a colorless oil as TFA salt, followed by treatment with DIEA to afford product **14** in 95% yield as a colorless oil.

Boc protected isonipecotic acid **15** was prepared from isonipecotic acid (**2**) by treatment with BOC-ON and sodium bicarbonate in water/dioxane in 90% yield as a colorless crystal (Scheme 2). Intermediate **17** was prepared from the methyl ester of vigabatrin **16** with the addition of EDCI and HOBT in 64% yield as a colorless oil. Intermediate **17** was treated with 33% TFA and 5% DMS in CH_2Cl_2 to yield an oil as TFA salt, which was treated with DIEA to give the final product **18** in 96% yield as a colorless oil. Final product **19** was prepared from nipecotic acid (**3**) in total 48% yield as a colorless oil by the same method as described above. Since both vigabatrin and nipecotic acid are available as its racemic form, the compounds **11** and **19** were synthesized as its diastereomeric mixture.

Pharmacological Results and Discussion

The anticonvulsant activity of the prepared compounds in maximal electroshock seizure (MES), pentylenetetrazole (PTZ), bicuculline (BIC) and picrotoxin (PCR) induced seizure tests were carried out according to the protocol of the Antiepileptic Drug Development Program of the National Institute of Neurological Disorders and Stroke.[11,14] It has been reported that the MES test correlates to both generalized tonic-clonic and psychomotor seizure, and the PTZ test to generalized absence seizure.[14] BIC and PCR were reported to be selective to $GABA_A$ receptor, and vigabatrin was effective only against BIC and PCR induced seizure.[11] These seizure tests are meaningful for clinical prediction of anticonvulsant agents and for recognition of broader clinical spectrum and dual action for prepared compounds. The anticonvulsant activities of the compounds are shown in Table 1, in comparison with the parent compounds.

Compound **11** showed the most potent anticonvulsive activity, and the other compounds showed moderate activity against the PTZ, BIC and PCR induced seizure tests, compared to those of the parent compounds. Especially, compound **11** showed the twice potency of vigabatrin in the BIC and PCR tests and had potent activity in the PTZ test, whereas the parent compound, nipecotic acid, showed no activity. This indicates three conclusions. First, the potent activity in the PTZ test of compound **11** would be derived from the parent compound, nipecotic acid, which was reported to be effective in vitro[12] but not effective in vivo due to its

Table 1. The anticonvulsive activities of prepared compounds

Compound	ED_{50} (mmol/kg)			
	MES	PTZ	BIC	PCR
10	>0.54	0.32	0.15	0.26
11	>0.54	0.15	0.14	0.10
14	>0.54	>0.54	0.20	0.26
18	>0.54	>0.54	0.46	0.39
19	>0.54	0.41	0.31	0.26
Vigabatrin	n[a]	n	0.22	0.21
Nipecotic acid	n	n	n	n
Isonipecotic acid	n	n	n	n
2-Pyrrolidinone	n	0.54	n	n

[a]n: Not effective (>1.0 mmol/kg).

Table 2. Anticonvulsive activities over time

Compound	Administered amount[a]	Time (h)	Convulsive[b]/tested mice
10	100 mg/kg (0.27 mmol/kg)	3	2/4
		6	2/4
		9	0/4
		18	0/4
11	100 mg/kg (0.27 mmol/kg)	3	2/4
		6	0/4
		9	0/4
		18	0/4
Vigabatrin	100 mg/kg	3	4/4
		6	4/4
		9	3/4
		18	0/4

[a]Each mouse was administered a dose of 100 mg/kg (0.27 mmol/kg) of each tested compound, and protection from convulsion was observed over time.
[b]Picrotoxin induced seizure test was performed.

hydrophilicity, since vigabatrin was reported not to be effective in PTZ test. That indicated that compound **11** readily entered into the CNS and was transformed back into its parent compounds by metabolizing enzymes. Second, the twice potent activity in the BIC and PCR test of compound **11** compared to vigabatrin indicated that compound **11** easily entered into the brain, was readily hydrolyzed into its parent compounds and stayed long enough in the brain to display better activity. Finally, the hydrolyzed compounds exhibited dual activity with different mechanisms, to provide increased anticonvulsive potency through a synergistic effect and broader clinical spectrum as expected.

The anticonvulsant activities over time of compounds **10** and **11** in the PCR induced seizure test is shown in Table 2. While vigabatrin exhibited maximum activity 18 h after administration, compound **11** displayed moderate activity 3 h and maximum activity 6 h after administration. The shorter onset time also indicated that compound **11** more readily passed BBB compared to vigabatrin, and easily hydrolyzed to its parent compounds.

In conclusion, we have designed and prepared five potential anticonvulsant agents as dual acting prodrugs to increase potency and to broaden the clinical spectrum. Compound **11**[15] showed the most potent anticonvulsant activity in the PTZ, BIC and PCR tests, and displayed shorter onset time, which are believed to be a promising drug candidate having higher potency and a broader clinical spectrum. Further studies are in progress to evaluate and determine the rate of penetration into the brain and the amount of transformation of compound **11** into parent compounds in the brain.

Acknowledgements

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seizure, a single episode of chronic spasms that persisted for at least 5 sec. Quantitative evaluation of the anticonvulsant activity of ED_{50} was estimated from dose-response data.

15. The spectral data of compound **11**: ^{1}H NMR (300 MHz, $CDCl_3$) ppm 5.82 (m, 1H), 5.38 (dd, 1H), 5.34 (dd, 1H), 3.87 (m, 1H), 3.71 (dt, 1H), 3.69 (s, 3H), 3.45 (dt, 1H), 3.11 (m, 1H), 2.95 (dt, 1H), 2.51 (m, 1H), 2.43 (m, 2H), 2.11–1.47 (m, 6H) ^{13}C NMR (75 MHz, $CDCl_3$) ppm 173.2, 171.7, 132.8, 120.9, 54.3, 52.0, 47.2, 46.3, 44.0, 42.7, 29.39, 27.9, 26.7 FABMS (MH^+) 255 TLC: $R_f = 0.63$ (10%MeOH/CH_2Cl_2 in NH_3 vapor).