Pergamon

SCIENCE DIRECT

Bioorganic & Medicinal Chemistry 11 (2003) 113–121

BIOORGANIC & MEDICINAL CHEMISTRY

N-, α-, and β-Substituted 3-Aminopropionic Acids: Design, Syntheses and Antiseizure Activities

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Received 24 September 2001; accepted 28 June 2002

Abstract—A treatment for epilepsy is proposed based on analogues of 3-aminopropionic acid (β-alanine), a putative neurotransmitter in the central nervous system (CNS). A model three point pharmacophore was proposed based on modelling data obtained from the study of antagonists for both the glial γ-aminobutyric acid (GABA)-uptake site and the glycine co-agonist site of *N*-methyl-D-aspartate (NMDA) receptor. Three series of 3-aminopropionic acids containing, *N*-, α-, and β-substituents, were designed and synthesized to probe the position and the size of a lipophilic binding pocket within the proposed pharmacophore. These analogues were tested in vivo for both their antiseizure activities and their neurologic toxicities. Among the fourteen novel 3-aminopropionic acids synthesized, eight were found to have promising antiseizure activity. This study shows that substitution on the N-terminus confers the greatest antiseizure activity, particularly against pilocarpine-induced seizures.
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Introduction

Epilepsy is a medical disorder characterized by spontaneous, recurrent seizures arising from excessive electrical activity in a portion of the brain. Epilepsy affects 1.5–2.0% of the population in developing countries, and 0.8–1.3% in developed countries; four million people are affected by this condition in North America and Europe alone. Moreover, since epilepsy typically afflicts people during their youth, its socioeconomic impact is disproportionately high. Currently available drugs such as phenytoin, carbamazepine, valproic acid, lamotrigine, and topiramate (Fig. 1) provide symptomatic seizure suppression in only 60–70% of those receiving treatment.[1,2] These drugs are also associated with undesirable side-effects, ranging from cosmetic (gingival hyperplasia) to life threatening (hepatotoxicity, megaloblastic anemia).[3–7] Consequently, a need exists for the development of new antiseizure drugs with improved efficacy and tolerability.

Traditionally, drug design in epilepsy has targeted the neuronal voltage gated Na^+ channel protein, resulting in the majority of currently available antiseizure drugs. More recent approaches to the design of antiseizure drugs have involved (i) antagonizing excitatory neurotransmitters, (ii) mimicking inhibitory neurotransmitters, or (iii) influencing postsynaptic receptors. Our rational design concept in this study was to simultaneously target both (i) and (ii) by designing compounds that could potentially decrease excitation while concomitantly increasing inhibition. This unique approach would theoretically be of greater utility in controlling epilepsy.

After extensive background design research, β-alanine was selected as the prototype lead compound around which to design new molecules. Various studies have suggested that β-alanine may act as an inhibitory neurotransmitter: β-alanine occurs naturally in the central nervous system (CNS), is released by electrical stimulation, can inhibit neuronal excitability, and has binding sites.[8–13] The existence of specific β-alanine receptors is controversial: some investigators propose the existence of specific receptors.[14] Others, however, have clearly shown that β-alanine binds to both glycine and γ-aminobutyric acid (GABA) binding sites.[15] In addition, numerous studies have verified the dual pharmacological action of β-alanine on both glutamatergic and GABAergic processes.[16,17] β-Alanine increases GABAergic inhibition by blocking the glial GABA uptake site,[18,19] and decreases glutamatergic excitation

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PII: S0968-0896(02)00330-9

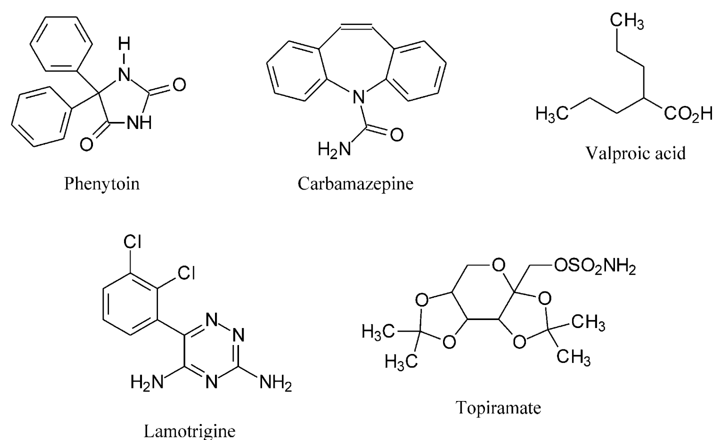


Figure 1. Structures of some current clinically available antiseizure drugs.

by binding to the glycine co-agonist site on the *N*-methyl-D-aspartate (NMDA) receptor.[20,21] In addition, β-amino acid active transport shuttles, capable of transporting β-alanine and related analogues across the blood–brain barrier, have also been identified.[22–24] It is this unique dual property of β-alanine to simultaneously decrease excitation and increase inhibition, as well as its ability to cross the blood–brain barrier via an active transport shuttle, which supports our proposal of β-amino acid analogues as potential new antiseizure agents.

In this paper, we report a novel approach to the treatment of epilepsy through analogues of β-alanine. A model three-point β-amino acid pharmacophore based on molecular modelling data obtained from studies by N'Goka[25] and Leeson[26] is hypothesized. Fourteen 3-aminopropionic acid analogues have been synthesized and tested for both their antiseizure activities as well as their neurologic toxicities. In vivo antiseizure animal models used in this study include pilocarpine, maximal electroshock (MES) and pentylenetetrazole (PTZ) assays.

The study was divided into two parts. First, two homologues for each of *N*-, β-, and α-substituted 3-aminopropionic acids were synthesized to probe the position and the depth of the corresponding lipophilic binding pocket for the proposed pharmacophore. Second, the size of this lipophilic binding pocket was probed using bulkier lipophilic side chains than used in the initial analogues. The structure–activity relationship of these analogues will be discussed.

Results and Discussion

In designing β-alanine analogues capable of interacting in silico with both the GABA glial uptake site and the glycine co-agonist site on the NMDA receptor, we exploited previously proposed computational pharmacophore models for these receptor sites. N'Goka[25] proposed that the binding site for the glial GABA-uptake receptor (Fig. 2) has the following parameters: (i) an amine functional group (preferably a secondary amine), (ii) a lipophilic binding region (preferably aromatic), (iii) a carboxylic acid functional group and (iv) an electron rich functionality (double bond or an oxygen) located between the amine and the lipophilic region. Leeson,[26] in studying antagonists of the glycine co-agonist site on the NMDA receptor, suggested that a pharmacophore (Fig. 2) for this receptor has the following parameters: (i) a secondary amine functional group, (ii) a carboxylic acid functional group, (iii) two small lipophilic groups as well as a larger lipophilic group. Using these pharmacophore models, a hybrid three point pharmacophore (Fig. 2) incorporating the β-amino acid backbone and a lipophilic moiety was proposed to interact computationally with models of both the glial GABA uptake site and the glycine subsite on the NMDA receptor.

We designed and synthesized three series of analogues having lipophilic moieties in the *N*-, α- and β-positions to probe the location of the lipophilic binding pocket. C-terminus substitution in the form of an ester group was not considered because the ester linkage is susceptible to hydrolytic cleavage by various esterases. We examined the location and the depth of the proposed lipophilic pocket by extending the alkyl chain linking the lipophilic moiety to the β-amino acid backbone. Specifically, 2-phenylethyl and 3-phenylpropyl groups were incorporated at the *N*-, α- and β-positions. Once the positions and the depth responsible for the biological activity were determined, the size of the lipophilic pocket was probed using bulkier 2,2-diphenylethyl and 3,3-diphenylpropyl substituents. The structures and yields of these 3-aminopropionic acids are summarized in Table 1.

The desired *N*-substituted 3-aminopropionic acids were prepared by a Lewis acid catalyzed Michael addition of methyl acrylate and the appropriately substituted amine to give their corresponding *N*-substituted-3-aminopropionates. Subsequent saponification followed by acidification yielded the respective *N*-substituted-3-aminopropionic acids as hydrochloride salts (Scheme 1). The corresponding amide derivatives of these *N*-substituted-3-aminopropionic acids were also synthesized to investigate whether C-terminal substitutions would affect the biological

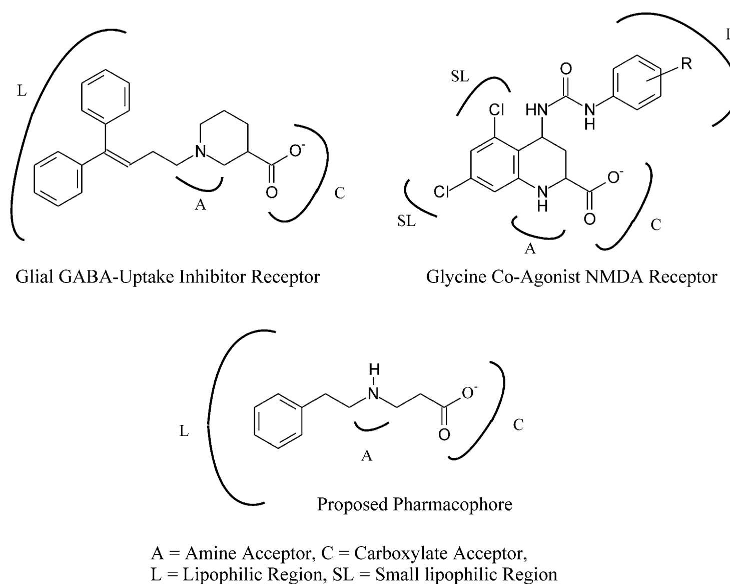
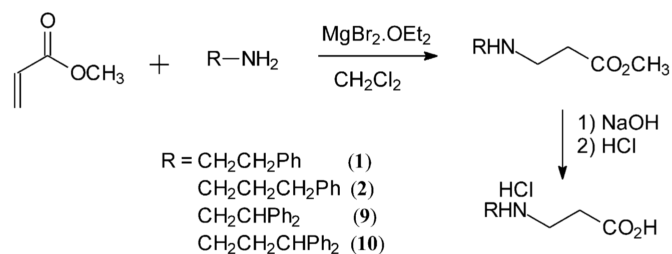


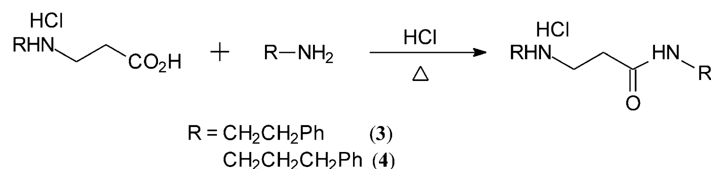
Figure 2. Pharmacophore models for GABA-uptake and glycine NMDA receptor sites[25,26] and proposed common pharmacophore.

Table 1. Structures and yields (overall yield) of 3-aminopropionic acid analogues

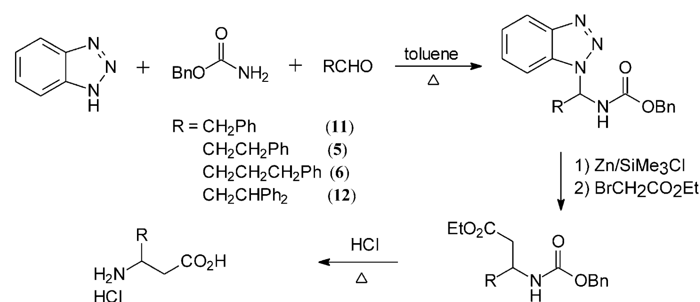
N-substituted	Yield (%)	β-substituted	Yield (%)	α-substituted	Yield (%)
(1)	86.2	**(5)**	50.1	**(7)**	56.7
(2)	84.5	**(6)**	43.2	**(8)**	51.4
(3)	69.6	**(11)**	51.2	**(13)**	38.9
(4)	67.1	**(12)**	32.8	**(14)**	45.7
(9)	78.2				
(10)	80.3				



Scheme 1. Synthesis of *N*-substituted-3-aminopropionic acid hydrochloride salt.



Scheme 2. Synthesis of *N*-substituted-3-aminopropionoamide hydrochloride salt.



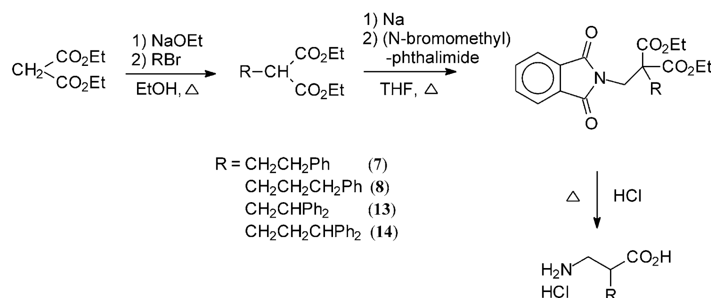
Scheme 3. Synthesis of β-substituted 3-aminopropionic acid hydrochloride salt.

activity of these analogues. These analogues were produced by refluxing the hydrochloride salts with the corresponding amine in 2 N HCl (Scheme 2).

The β-substituted 3-aminopropionic acids were prepared as described by Katritzky.[27] The condensation of benzotriazole with benzyl carbamate and an appropriately functionalized aldehyde gave the 1-benyloxycarbonylamino-1-(benzotriazolyl)alkane intermediate. Subsequent reaction with ethyl bromoacetate under Reformatsky type conditions afforded the *N*-protected 3-aminoalkanoic ester. This intermediate was then hydrolyzed with concentrated hydrochloric acid to give the desired β-substituted 3-aminopropionic acid hydrochloride salt (Scheme 3).

The α-substituted 3-aminopropionic acids were synthesized using a modified procedure first described by Bohme.[28] Starting with diethyl malonate, sequential substitution reactions using an appropriately functionalized alkyl bromide followed by *N*- (bromomethyl)phthalimide gave a disubstituted diethyl malonate. Hydrolysis of this intermediate afforded the corresponding α-substituted 3-aminopropionic acid as the hydrochloride salt (Scheme 4).

All analogues synthesized were tested in vivo for both their antiseizure activities and their neurological toxicities. Seizures are divided into two major groups: partial (focal) and primary generalized. Partial seizures, accounting for approximately 60% of all reported human cases of epilepsy, arise from a localized area of the brain. They are subdivided into simple partial, complex partial, and secondarily generalized seizures. Primary generalized seizures account for 40% of all reported cases of epilepsy and onset with no apparent focality. Primary generalized seizures may further be



Scheme 4. Synthesis of α-substituted 3-aminopropionic acid hydrochloride salt.

Table 2. Antiseizure and neurotoxicity activities of the 3-aminopropionic acid analogues[a]

Compound	Pilocarpine	MES		scPTZ		Toxicity	
		30 min	4 h	30 min	4 h	30 min	4 h
1	Active	—	—	—	—	—	—
2	Active	—	—	—	—	—	—
3	Active	—	—	—	—	+ +	NT
4	—	—	—	—	—	—	—
5	Active	—	—	—	—	—	—
6	—	—	—	—	—	—	—
7	—	—	—	—	—	—	—
8	Active	—	—	—	—	—	—
9	—	—	—	+	—	—	—
10	Active	—	—	+	—	+	—
11	Active	—	—	—	—	—	—
12	—	—	—	—	—	—	—
13	Active	—	—	—	—	+	—
14	—	—	—	—	—	—	—
Carbamazepine[b]	Active	8.8		NE		71.6	
Valproic acid[b]	Active	271.7		148.6		425.8	

Abbreviations: MES, maximal electroshock-induced seizures; scPTZ; subcutaneous pentylenetetrazole; s, seizure; NT, not tested; NE, not effective.

[a]Pilocarpine test was carried out at a dose of 100 mg/kg; each analogue was tested on 4 rats; a compound is active if 3 or 4 of the rats were fully prevented from having seizures. The antiseizure (MES and scPTZ) and neurotoxicity activities were determined 30 min and 4 h after the administration of compounds. The symbols + + +, + +, and + signify activity (seizures prevented in (3 or 4)/4 rats) at 30, 100, and 300 mg/kg, respectively; —denotes no activity observed at 300 mg/kg. Toxicity was determined by the rotorod test.

[b]Commercially available antiseizure drugs. Both MES and scPTZ are reported as the median effective dose (ED_{50}), and toxicity is reported as the median toxic dose (TD_{50}) in mg/kg.[31]

divided into absence (petit mal), myoclonic, clonic, tonic, tonic-clonic (grand mal) or atonic seizures. To reflect this diversity of seizure types, all 3-aminopropionic acid analogues were evaluated in three separate antiseizure assays: pilocarpine, MES (maximal electroshock) and scPTZ (subcutaneous pentylenetetrazole). Each assay models a different seizure type. Pilocarpine-induced seizures are a model for complex partial seizures;[29] MES-induced seizures model tonic-clonic seizures;[30] PTZ-induced seizures model absence seizures.[31] Rotorod neurotoxicity testing for each analogue was also performed. Results are summarized in Table 2.

In probing the location and the depth of the lipophilic pocket, analogues containing either 2-phenylethyl or 3-phenylpropyl substituents in one of the *N*-, α- or β-positions were tested for their antiseizure activity. Both the *N*-substituted 2-phenylethyl- (**1**) and the 3-phenylpropyl-3-aminopropionic acids (**2**) were found to have antiseizure activity against pilocarpine-induced seizures. Of the β-substituted analogues, only the 2-phenylethyl analogue (**5**) was active against pilocarpine-induced seizures, whereas the 3-phenylpropyl analogue (**6**) was not. In contrast, only the α-substituted 3-phenylpropyl analogue (**8**) showed activity against pilocarpine-induced seizures. All six analogues were inactive in the MES and PTZ models. These analogues exhibit selectivity towards pilocarpine-induced seizures and therefore may be of interest in the treatment of complex partial seizures. Two possible structural hypotheses were proposed to explain these biological data: (i) there is only one lipophilic pocket located in close proximity to both the β-carbon and the N-terminus, or (ii) there is more than one lipophilic pocket with one being in close proximity to both the β-carbon and the N-terminus.

To investigate these two hypotheses, analogues containing a benzyl substituent in the β-position (11) as well as the 2,2-diphenylethyl substituent in the *N*-, α- and β-positions, (**9**), (**13**), (**12**) respectively, were synthesized and evaluated for their antiseizure activity. The results showed that the benzyl β-substituted analogue (**11**) was active against pilocarpine-induced seizures while its bulkier β-2,2-diphenylpropyl analogue (**12**) was not. This suggested that there is indeed a lipophilic pocket close to the β-carbon, in agreement with both hypothesis (i) and (ii). However, this lipophilic pocket is relatively small as demonstrated by the lack of activity upon the incorporation of a bulkier substituent. Of the two 2,2-diphenylethyl substituted analogues, (**9**) and (**13**), only the α-substituted analogue (**13**) was active. In contrast, the longer 3,3-diphenylpropyl homologues resulted in a reversal of activity such that the *N*-substituted analogue (**10**) was active against pilocarpine-induced seizures while the α-substituted analogue was not. These results suggested that two separate lipophilic pockets are responsible for the different activities for both the *N*- and the α-substituted series, supporting the second hypothesis. Of these six analogues (**9**, **10**, **11**, **12**, **13** and **14**), only the *N*-substituted analogues (**9**) and (**10**) were found to have activity against MES and PTZ-induced seizures at higher doses. Why only bulky lipophilic groups in the *N*-positions on the 3-aminopropionic acid backbone influence MES and PTZ activity is not readily apparent. Overall, the results suggest that there may be more than one lipophilic pocket, and that a small lipophilic pocket is located in close proximity to both the β-carbon and the N-terminus of 3-aminopropionic acid. In addition, substitution on the N-terminus is the most desirable position for antiseizure activity, particularly against pilocarpine-induced seizures.

Most of the 14 analogues displayed no neurological toxicity as determined by the rotorod test, except for analogues (**3**), (**10**) and (**13**). However, this neurotoxicity was comparable to the commercially available antiseizure drugs such as carbamazepine and valproic acid (Table 2). Analogue (**3**) is an amide of the corresponding *N*-substituted analogue (**2**) whereas analogue (**10**) and (**13**) are diphenyl substituted. The addition of the amide group (analogues **3** and **4**) on the C-terminus either depleted antiseizure activity or augmented the neurotoxicity to the *N*-substituted series.

In conclusion, we have proposed a unique approach to the treatment of epilepsy based on β-alanine, a putative neurotransmitter in the CNS. Interestingly, these compounds had activity against pilocarpine induced seizures (a model of complex partial seizures) but less activity against MES or PTZ induced seizures. This observation is noteworthy given the need for drugs specific for complex partial seizures and may reflect the high density of GABA and NMDA receptor populations in the limbic

regions of the brain from which complex partial seizures preferentially originate. As a backbone upon which to design anticonvulsants, β-alanine is active at both GABA and NMDA receptor populations. Future binding studies will evaluate the structure–activity relationships of the binding of these β-amino acid analogues to GABA-A and NMDA-gly receptor sites.

Experimental

Chemistry

Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 300 (300 MHz) spectrometer with CD_3OD as the solvent unless otherwise stated. Infrared (IR) spectra were recorded on a Bomem MB-120 spectrophotometer using KBr disks. Melting points (mp) were determined using a Mel-Temp II capillary apparatus and are uncorrected. Elemental analyses were performed by G-C-L Laboratories (Guelph, Canada). Solvents were purified using standard methods.

General procedure for *N*-substituted-3-aminopropionic acid hydrochloride salt. Under N_2, a solution of methyl acrylate (1.0 equiv) in 50 mL dry CH_2Cl_2 was added via a dropping funnel to a stirred mixture of amine (1.0 equiv) and $MgBr_2.OEt_2$ (0.3 equiv) in 100 mL dry CH_2Cl_2. The reaction mixture was allowed to stir at room temperature until completion, as determined by TLC. The reaction mixture was then quenched with H_2O. The organic layer was separated and concentrated under reduced pressure to give a colourless oil. It was dissolved in minimal amount of MeOH and saponified with aqueous NaOH (1.0 equiv). The reaction mixture was washed 3 times with Et_2O and acidified with 1.0 N aqueous HCl (pH = 1). The reaction mixture was evaporated to dryness under reduced pressure to give a white solid (containing the product and NaCl). NaCl was removed by repeatedly washing and drying with absolute EtOH (3×50 mL). The final product was recrystallised with EtOH/EtOAc to give white crystals.

Hydrochloride salt of *N*-(2-phenylethyl)-3-aminopropionic acid (1). White crystals of **1** were obtained, mp: 193–194 °C; IR: 3462, 1741, 1604, 1577 cm^{-1}; ^{1}H NMR: δ 7.21–7.17 (m, 5H), 3.25–3.16 (m, 4H), 2.94–2.89 (m, 2H), 2.72–2.67 (m, 2H); ^{13}C NMR: 171.30, 136.71, 129.00, 128.87, 127.30, 49.30, 43.46, 32.22, 30.22; Anal. calcd for $C_{11}H_{16}NO_2Cl$: C, 57.52; H, 7.02; N, 6.10. Found: C, 57.12; H, 7.40; N, 5.72.

Hydrochloride salt of *N*-(3-phenylpropyl)-3-aminopropionic acid (2). **2** as white crystals was obtained, mp: 141–142 °C; IR: 2954, 1729, 1604, 1573 cm^{-1}; ^{1}H NMR: δ 7.12–6.96 (m, 5H), 3.06 (t, 2H, J = 6.72 Hz), 2.85 (t, 2H, J = 7.92 Hz), 2.66–2.56 (m, 4H), 1.89–1.78 (m, 2H); ^{13}C NMR: 172.53, 140.68, 128.67, 128.46, 126.42, 47.71, 43.48, 32.53, 30.13, 27.93. Anal. calcd for $C_{12}H_{18}NO_2Cl$: C, 59.14; H, 7.44; N, 5.75. Found: C, 59.30; H, 7.84; N, 5.49.

Hydrochloride salt of *N*-(2,2-diphenylethyl)-3-aminopropionic acid (9). White crystals (**9**) were obtained, mp: 197–198 °C; IR: 3421, 1718, 1600, 1562 cm^{-1}; ^{1}H NMR: δ 7.29–7.24 (m, 8H), 7.21–7.12 (m, 2H), 4.34 (t, 1H, J = 8.03 Hz), 3.69 (d, 2H, J = 8.03 Hz), 3.24 (t, 2H, J = 6.79 Hz), 2.64 (t, 2H, J = 6.92 Hz); ^{13}C NMR: 172.71, 140.27, 129.15, 127.84, 127.61, 51.66, 48.46, 44.18, 29.52; Anal. calcd for $C_{17}H_{20}NO_2Cl$: C, 66.77; H, 6.59; N, 4.58. Found: C, 66.40; H, 6.60; N, 4.59.

Hydrochloride salt of *N*-(3,3-diphenylpropyl)-3-aminopropionic acid (10). **10** as white crystals was obtained, mp 179–180 °C; IR: 3447, 1742, 1589 cm^{-1}; ^{1}H NMR: δ 7.44–7.42 (m, 8H), 7.14–7.00 (m, 2H), 3.92 (t, 1H, J = 7.95 Hz), 3.05 (t, 2H, J = 6.45 Hz), 2.83–2.78 (m, 2H), 2.60 (t, 2H, J = 6.45 Hz), 2.38–2.30 (m, 2H); ^{13}C NMR: 172.92, 143.77, 128.88, 127.75, 126.85, 48.75, 47.24, 43.46, 31.62, 30.25; Anal. calcd for $C_{18}H_{22}NO_2Cl$: C, 67.60; H, 6.93; N, 4.38. Found: C, 67.54; H, 7.08; N, 4.31.

General procedure for *N*-substituted-3-aminopropionamide hydrochloride salt. The corresponding amine (1.0 equiv) was added to the *N*-substituted-3-aminopropionic acid hydrochloride salt in 150 mL of 2 N HCl aqueous. The reaction mixture was allowed to reflux overnight and was evaporated to dryness under reduced pressure to give a white solid. Subsequent recrystallization from EtOAc then afforded the final product.

Hydrochloride salt of *N*′-(2′-phenylethyl)-*N*-(2-phenylethyl)-3-aminopropionoamide (3). White crystals of **3** were obtained, mp: 242–243 °C; IR: 3314, 1649, 1454 cm^{-1}; ^{1}H NMR: δ 7.31–7.06 (m, 10H), 3.35 (t, 4H, J = 6.48 Hz), 3.18–3.14 (m, 4H), 2.96–2.89 (m, 2H), 2.71 (t, 2H, J = 7.10 Hz), 2.53 (t, 2H, J = 6.50 Hz); ^{13}C NMR: 170.88, 139.20, 136.65, 129.04, 128.78, 128.53, 127.35, 126.43, 44.11, 40.97, 37.98, 35.42, 33.72, 30.67; Anal. calcd for $C_{19}H_{25}N_2OCl$: C, 68.56; H, 7.57; N, 8.42. Found: C, 68.90; H, 7.87; N, 8.54.

Hydrochloride salt of *N*′-(3′-phenylpropyl)-*N*-(3-phenylpropyl)-3-aminopropionoamide (4). **4** as white crystals was obtained, mp 173–174 °C; IR: 3318, 1642, 1578 cm^{-1}; ^{1}H NMR: δ 7.26–7.07 (m, 10H), 3.20–3.14 (m, 4H), 2.99–2.95 (m, 2H), 2.67 (t, 2H, J = 5.75 Hz), 2.61–2.56 (m, 4H), 2.01–1.93 (m, 2H), 1.80–1.73 (m, 2H); ^{13}C NMR: 170.75, 141.83, 140.52, 128.58, 128.34, 126.34, 125.86, 47.32, 43.94, 39.04, 33.10, 32.41, 31.00, 30.74, 27.98; Anal. calcd for $C_{21}H_{29}N_2OCl$: C, 69.88; H, 8.10; N, 7.76. Found: C, 69.77; H, 8.09; N, 7.73.

General procedure for β-substituted-3-aminopropionic acid hydrochloride salt. (1) Benzotriazole (1.0 equiv), benzyl carbamate (1.0 equiv), an aldehyde (1.0 equiv), and a catalytic amount of *p*-toluenesulfonic acid monohydrate (100 mg) were refluxed in toluene (150 mL) using a Dean-Stark trap until the theoretical amount of water was produced. The reaction mixture was concentrated under reduced pressure to produce 1-benzyloxycarbonylamino-1-(1-benzotriazolyl)alkane as an oily residue which was used directly in the next step. (2) Under N_2, chloromethylsilane (0.3 equiv) was added

into a solution of dried THF (100 mL) with Zn powder (1.5 equiv). The reaction mixture was stirred at room temperature for 15 min then 1-benzyloxycarbonylamino-1-(1-benzotriazolyl)alkane in dry THF (30 mL) was slowly added via a dropping funnel. The reaction mixture was heated briefly to reflux and allowed to cool before ethyl bromoacetate (1.0 equiv) was added. The suspension was then heated to reflux and the nitrogen inlet was removed. The grey powder soon turned into thin white suspension, and the reaction was then allowed to reflux until completion, as determined by TLC. The reaction mixture was poured into concentrated NH_4OH (40 mL) containing ice (20 g), and stirred until all solids dissolved. The organic layer was extracted with Et_2O (3×70 mL), washed with H_2O (3×70 mL) and dried over $MgSO_4$ before the solvent was removed under reduced pressure to give 3-dialkylaminoalkanoic ester adduct. (3) The ester adduct was refluxed in 1:1:1 ($HCl/H_2O/HCO_2H$) until the hydrolysis was completed, as determined by TLC. The reaction mixture was then evaporated to dryness under reduced pressure and was recrystallized from EtOH/EtOAc to give a β-substituted-3-aminopropionic acid hydrochloride salt as white solids.

Hydrochloride salt of 3-amino-3-(2-phenylethyl)propionic acid (5). White crystals of **5** were obtained, mp 183–184 °C; IR: 3010, 1598 cm^{-1}; ^1H NMR: δ 7.06–6.89 (m, 5H), 3.41–3.20 (m, 1H), 2.62–2.42 (m, 4H), 1.83–1.69 (m, 2H); ^{13}C NMR: 173.93, 141.90, 129.91, 129.65, 127.65, 50.15, 37.04, 35.80, 32.62; Anal. calcd for $C_{11}H_{16}NO_2Cl$: C, 57.52; H, 7.02; N, 6.10. Found: C, 57.33; H, 7.31; N, 6.02.

Hydrochloride salt of 3-amino-3-(3-phenylpropyl)propionic acid (6). Compound **6** as white crystals was obtained, mp 230–231 °C; IR: 3004, 1615 cm^{-1}; ^1H NMR: δ 7.06–6.89 (m, 5H), 3.41–3.20 (m, 1H), 2.62–2.42 (m, 4H), 1.83–1.69 (m, 2H); ^{13}C NMR: 173.93, 141.90, 129.91, 129.65, 127.65, 50.15, 37.04, 35.80, 32.62. Anal. Calcd. for $C_{12}H_{18}NO_2Cl$: C, 59.14; H, 7.44; N, 5.75. Found: C, 59.31; H, 7.49; N, 5.65.

Hydrochloride salt of 3-amino-3-benzylpropionic acid (11). White crystals (**11**) were obtained, mp 172–174 °C; IR: 3007, 1610 cm^{-1}; ^1H NMR: δ 7.24–7.15 (m, 5H), 3.70–3.62 (m, 1H), 2.99–2.80 (m, 2H), 2.53–2.49 (m, 2H); ^{13}C NMR: 173.50, 136.70, 130.48, 130.10, 128.59, 50.95, 39.35, 36.07; Anal. calcd for $C_{10}H_{14}NO_2Cl$: C, 55.69; H, 6.54; N, 6.49. Found: C, 55.81; H, 6.69; N, 6.52.

Hydrochloride salt of 3-amino-3-(2,2-diphenylethyl)propionic acid (12). White crystals of **12** were obtained, mp 207–209 °C; IR: 3002, 1594 cm^{-1}; ^1H NMR: δ 7.27–7.00 (m, 10H), 4.04 (t, 1H, J = 8.10 Hz), 3.23–3.14 (m, 1H), 2.71–2.49 (m, 2H), 2.44–2.29 (m, 2H); ^{13}C NMR: 173.58, 144.46, 129.81, 128.81, 127.80, 48.35, 48.20, 39.03, 36.60; Anal. calcd for $C_{17}H_{20}NO_2Cl$: C, 66.77; H, 6.59; N, 4.58. Found: C, 66.47; H, 6.72; N, 4.65.

General procedure for α-substituted-3-aminopropionic acid hydrochloride salt. (1) Under N_2, Na (1.0 equiv) in dry absolute EtOH (100 mL) was allowed to stir at room temperature until all the Na was dissolved. Diethylmalonate (1.1 equiv) was added and the reaction mixture was heated to 40 °C. An alkyl bromide (1.0 equiv) was then added via a dropping funnel. The reaction mixture was then refluxed until completion, as determined by TLC. The NaBr was filtered off and the filtrate was concentrated under reduced pressure to give a pale yellow oil. Excess diethylmalonate was distilled off by high vacuum distillation to yield the monosubstituted-diethylmalonate. (2) Under N_2, Na (1.0 equiv) was added to dry THF (150 mL) containing the monosubstituted-diethylmalonate and the reaction mixture was warmed to 40 °C and stirred until the Na was completely dissolved. *N*-(bromomethyl)phthalimide (1.0 equiv) dissolved in dry THF (50 mL) was added via a dropping funnel. Once the *N*-(bromomethyl)phthalimide was added, the reaction mixture was refluxed until completion, as determined by TLC. NaBr was filtered off and the filtrate was concentrated under reduced pressure to give a yellow oil. This yellowish oil was further dissolved in 100 mL of EtOAc and washed with H_2O (3×50 mL) before the EtOAc was dried over $MgSO_4$ and concentrated under reduced pressure to give the disubstituted diethylmalonate as a pale yellow solid. It was then recrystallized from $EtOH/H_2O$ to yield a white solid. (3) The disubstituted-diethylmalonate was hydrolysed to yield the α-substituted-3-aminopropionic acid hydrochloride salt either by mineral acid or by hydrazine hydrate followed by acids.

With mineral acid, the disubstituted-diethylmalonate was refluxed in a mixture of 1:1:3 (H_2O/HCO_2H/concentrated HCl) until the hydrolysis was complete, as determined by TLC. The reaction mixture was then evaporated to dryness under reduced pressure to give white solids (α-substituted-3-aminopropionic acid hydrochloride salt and phthalic acid). The α-substituted-3-aminopropionic acid hydrochloride salt was isolated by strong acid ion exchange column (Amberlite IR-120 plus), where it was eluted by 4 N NH_4OH, evaporated to dryness and acidified with 1 N HCl. α-Substituted-3-aminopropionic acid hydrochloride salt was than recrystallized from EtOH/EtOAc.

With hydrazine hydrate, the disubstituted-diethylmalonate was dissolved in warm EtOH. Hydrazine hydrate (1.0 equiv) was added into the mixture and stirred for 15 min before 15 mL of 1 N HCl was added. The reaction mixture was warmed to 40°C before it was evaporated to dryness to yield a white solid. The white solid was then taken up with MeOH and the undissolved solid was filtered off. The filtrate was evaporated to dryness to give a white solid. The white solid was refluxed in a mixture of 1:1:3 (H_2O/HCO_2H/concentrated HCl) until the hydrolysis was completed, as determined by TLC. The reaction mixture was evaporated to dryness and the white solid was recrystallized with EtOH/EtOAc to yield the α-substituted-3-aminopropionic acid as a hydrochloride salt.

2-Phenylethyl-diethylmalonate (I). ^1H NMR ($CDCl_3$): δ 7.32–7.20 (m, 5H), 4.23 (q, 4H, J = 7.20 Hz), 3.36 (t, 1H, J = 7.50 Hz), 2.68 (t, 2H, J = 7.50 Hz), 2.28–2.20 (m,

2H), 1.30 (t, 6H, $J=7.20$ Hz); ^{13}C NMR: 170.39, 133.21, 128.54, 128.46, 126.19, 61.37, 51.29, 33.32, 30.34, 14.09.

3-Phenylpropyl-phthalimidomethyl-diethylmalonate (II). **II** as white crystals was obtained, mp 74–76 °C; ^{1}H NMR ($CDCl_3$): δ 7.88–7.85 (m, 2H), 7.75–7.72 (m, 2H), 7.31–7.19 (m, 5H), 4.32–4.24 (q, 4H, $J=7.20$ Hz), 2.79–2.73 (m, 2H), 2.14–2.08 (m, 2H), 1.33 (t, 6H, $J=7.20$ Hz); ^{13}C NMR: 171.90, 170.23, 143.32, 136.13, 133.80, 130.57, 130.32, 127.95, 125.15, 63.77, 59.38, 41.36, 34.79, 32.19, 15.98.

Hydrochloride salt of 3-amino-2-(2-phenylethyl)propionic acid (7). White crystals of **7** were obtained, mp 217–218 °C; IR: 3013, 1608 cm^{-1}; ^{1}H NMR: δ 7.17–7.01 (m, 5H), 3.11–2.91 (m, 2H), 2.65–2.54 (m, 3H), 1.94–1.79 (m, 2H); ^{13}C NMR: 175.98, 142.23, 129.56, 129.50, 127.25, 43.38, 41.39, 33.81, 32.78; Anal. calcd for $C_{11}H_{16}NO_2Cl$: C, 57.52; H, 7.02; N, 6.10. Found: C, 57.44; H, 7.40; N, 6.07.

Hydrochloride salt of 3-amino-2-(3-phenylpropyl)propionic acid (8). **8** as white crystals was obtained, mp 166–168 °C; IR: 3009, 1605 cm^{-1}; ^{1}H NMR: δ 7.15–6.99 (m, 5H), 3.04–2.86 (m, 2H), 2.64–2.49 (m, 43H), 1.61–1.52 (m, 4H); ^{13}C NMR: 176.12, 142.99, 129.44, 129.40, 126.94, 43.72, 41.35, 36.47, 30.46, 29.68; Anal. calcd for $C_{12}H_{18}NO_2Cl$: C, 59.14; H, 7.44; N, 5.75. Found: C, 58.98; H, 7.59; N, 5.69.

Hydrochloride salt of 3-amino-2-(2,2-diphenylethyl)propionic acid (13). White crystals of **13** were obtained, mp: 199–200 °C; IR: 3011, 1603 cm^{-1}; ^{1}H NMR: δ 7.23–7.17 (m, 8H), 7.11–7.07 (m, 2H), 4.04 (t, 1H, $J=7.20$ Hz), 3.05–2.98 (m, 2H), 2.48–2.40 (m, 2H), 2.15–2.14 (m, 1H); ^{13}C NMR: 174.97, 144.21, 128.73, 128.06, 126.65, 48.86, 41.31, 40.62, 35.72; Anal. calcd for $C_{17}H_{20}NO_2Cl$: C, 66.77; H, 6.59; N, 4.58. Found: C, 66.94; H, 6.99; N, 4.52.

Hydrochloride salt of 3-amino-2-(3,3-diphenylpropyl)propionic acid (14). White crystals (**14**) were obtained, mp 193–194 °C; IR: 3001, 1593 cm^{-1}; ^{1}H NMR: δ 7.17–7.03 (m, 10H), 3.84 (t, 1H, $J=7.80$ Hz), 3.09–3.02 (m, 1H), 2.92–2.86 (m, 1H), 2.66–2.61 (m, 1H), 2.11–1.99 (m, 2H), 1.60–1.57 (m, 2H); ^{13}C NMR: 176.06, 145.84, 129.49, 128.80, 127.26, 52.33, 43.75, 41.39, 33.61, 29.55; Anal. calcd for $C_{18}H_{22}NO_2Cl$: C, 67.60; H, 6.93; N, 4.38. Found: C, 67.26; H, 7.05; N, 4.12.

Pharmacological methods

Maximal electroshock (MES), subcutaneous pentylenetetrazole (scPTZ), and rotorod neurotoxicity testing were carried out by the Antiepileptic Drug Development (ADD) Program, Epilepsy Branch, National Institutes of Health, Bethesda, MD.[32] All compounds were evaluated in either male Carworth Farms #1 mice or male Sprague–Dawley rats. Each compound was administrated intraperitoneally (ip) at three different dose levels 30, 100, and 300 mg/kg.

MES seizures were induced 30 min after drug treatment by the application of a 60 Hz current (50 mA in mice and 150 mA in rat) for 0.2 s via corneal electrodes primed with an electrode solution containing an anesthetic agent. The protection was defined as the abolition of hind-leg tonic maximal extension component of the seizure.

The scPTZ seizure threshold assay was carried out by an ip administration of PTZ (85 mg/kg in mice and 70 mg/kg in rats). Animals were observed over a 30 min period and the absence of clonic spasms in the observed time period were defined as protect.

Minimal neurotoxicity was measured by the rotorod test. Mice were placed on a 1-in diameter knurled plastic rod rotating at 6 rpm after the administration of the drug, and their ability to maintain their balance was tested. Neurological deficit was indicated by the inability of the animal to maintain its equilibrium for 1 min on the rotating rod in each of three trials.

The pilocarpine assay was performed in the Division of Neurology, Queen's University. All compounds were evaluated in male Sprague–Dawley rats. The tested compound (dissolved in 10% dimethylsulfoxide saline solution) was injected by ip (100 mg/kg). The rat was then observed for 20 min before pilocarpine (350 mg/kg) was administrated. The rat was further observed for another 30 min and the absence of clonic spasms were defined as protection.

Acknowledgements

C.Y.K.T. acknowledges a Natural Sciences and Engineering Research Council (NSERC) of Canada Scholarship. D.F.W. acknowledges operating grants from the Medical Research Council of Canada, NSERC and Neurochem Inc. We would also like to thank the ADD of NIH for carrying out MES, scPTZ and rotorod neurotoxicity testing.

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