DOI 10.1002/ardp.200400958 Arch. Pharm. Chem. Life Sci. 2005, 338, 358–364

Design, Synthesis, and Preliminary Evaluation of Gabapentin-Pregabalin Mutual Prodrugs in Relieving Neuropathic Pain

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As a part of a program for the development of specific analgesics in relieving neuropathic pain, the purpose of the present study was to investigate a new concept that involves the conjugation of two drugs, gabapentin and pregabalin, as mutual prodrugs using a chemical modification approach. A series of gabapentin-pregabalin diester compounds were synthesized using linear or branch bis-hydroxyl linkers. Their pharmacological properties for treating neuropathic pain were investigated in a rat model of chronic sciatic nerve constriction injury (CCI). *In-vivo* evaluation demonstrated that **1a** and **1b** composed of two gabapentin molecules as well as **3a** composed of gabapentin and pregabalin with the short linear linker, were effective in reversing tactile allodynia in CCI rats. Compounds with longer or side-branched linkers showed lower efficiencies and severe adverse effects.

Keywords: Neuropathic pain; Mutual prodrug; CCI; Gabapentin; Pregabalin

Received: November 29, 2004; Accepted: June 1, 2005

Introduction

Neuropathic pain results from disease or injury to the peripheral or central nervous system, including diabetic neuropathy, trigeminal neuralgia, post-herpetic neuralgia, and spinal cord injury. As a common symptom of a heterogeneous group of conditions, neuropathic pain frequently runs a chronic course and will be severe and difficult to treat [1]. The characteristic symptoms of neuropathic pain are usually expressed as allodynia, hyperalgesia, and spontaneous pain. Different pain mechanisms may be involved in the neuropathic pain, for one mechanism could be responsible for many different symptoms, and the same symptom can be caused by different mechanisms. As a result, more than one mechanism can operate in a single patient, and these mechanisms may change with time [2]. Many classical analgesics such as opioids and NSAIDs (nonsteroid anti-inflammation drugs) have a limited effect in treatment of neuropathic pain, while opiates carry long-term risks of habituation with chronic use [3].

Due to the intricate mechanisms of the neuropathic pain, complete symptom reduction or complete functional restoration is seldom achieved and the existing drugs focused on one specific target could only alleviate pain symptoms to some extent. Moreover, some of the drugs show severe adverse effects, for the most common adverse effects of anticonvulsants are sedation and cerebella symptoms (nystagmus, tremor, and incoordination). In order to develop safe and more effective treatments for neuropathic pain a new strategy of drugs has to be considered. The direction for new drug development is to use the combination of different kinds of drugs based on mechanism classification in order to block the pain symptom at different levels. Combination is a potentially effective way to increase efficiency of drugs and to reduce the adverse effect. On the other hand, the conjugation of two drugs having same or different pharmacological activities, which is termed a mutual prodrug, has been synthesized to improve the therapeutic index and reduce adverse effect [4].

It has recently been reported that antidepressants, anticonvulsants, antiarrhythmic drugs, local anaesthetic, or NMDA receptor antagonists become an alternative way for the neuropathic pain treatment [5]. Gabapentin is an anticonvulsive drug that has been found to be effective for the treatment of neuropathic pain. It is a structural analogue of the inhibitory neurotransmitter gamma amino butyric acid (GABA). *S*-(+)-Pregabalin is another GABA analog that inhibit the α2δ-subunit of the voltage-sensitive Ca^{2+}-channel, it is launched by Pfizer for the potential treatment of central nervous system disorders, including neuropathic pain. But gabapentin and pregabalin (Figure 1) exhibit some drawbacks such as higher dosage (900 to 3600 mg/d and 300 mg/d for gabapentin and pregabalin, respectively) resulting from the difficulty to diffuse across the blood-brain barrier, which causes severe adverse effects (dizziness, drowsiness, headache, and somnolence), and a shorter

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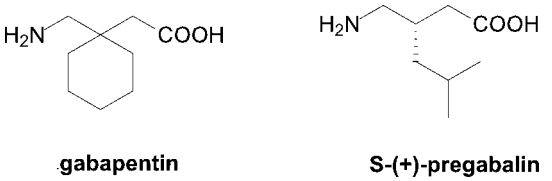


Figure 1. Chemical structure of gabapentin and S-(+)-pregabalin.

duration of action with the need to take the medication orally three or four times daily [6–9].

In our work the objective is to get new potent anti-neuropathic pain drugs with fewer side effects and better pharmacokinetic properties. Gabapentin and pregabalin were conjugated through their carboxyl groups to different bis-hydroxyl linkers. A series of diester compounds were designed and synthesized as a mutual prodrug approach. In the present study, the anti-allodynic effects of systemic administration of mutual prodrugs were investigated in chronic sciatic nerve constriction injury (CCI) rats.

Results

Chemistry

Synthesis of mutual prodrug diester compounds **1a**–**c**, **2a**–**c**, and **3a**–**i** is shown in Scheme 1. The amino groups of gabapentin and pregabalin were protected by *t*-butoxycarbonyl(Boc) groups. The bis-gabapentin diester compounds **1a**–**c** were synthesized by the protected gabapentin with bis-hydroxyl compounds such as glycol or 1,3-propandiol using dicyclohexyl carbodiimide (DCC) and 4-(*N,N*-dimethylamino)pyridine (DMAP) as carboxyl activator and nucleophilic catalyst, respectively. The bis-pregabalin diester compounds **2a**–**c** were synthesized by the protected pregabalin in the same procedure. Synthesis of the gabapentin monoester compounds was achieved by the protected gabapentin with more excessive bis-hydroxyl compounds. The unsymmetrically gabapentin-pregabalin diester compounds **3a**–**i** were synthesized by the gabapentin monoester with the protected pregabalin accordingly. The amino-protecting group was removed by the HCl-EtOAc solution, at the same time the diester compounds hydrochloride were obtained.

Biological studies

The anti-allodynic effects studies were implemented in chronic sciatic nerve constriction injury (CCI) rats. The CCI surgery was conducted according to a procedure previously reported by Bennett [10]. Paw withdrawal threshold was measured by a series of von-Frey filaments, the strength of a filament, which caused 4–6 responses stimulating 10 times, was designated as 50% response threshold. The force of 26 g was designated as the cut-off point. All sample compounds screened were solved in 0.9% (w/v) saline. Two weeks after surgery, the mechanical paw withdrawal threshold was measured at 1 h after mutual prodrug administration (60 mg/kg, i.p.). Broad screen found that compound **1a**, **1b**, and **3a** exhibited anti-allodynic effects in CCI rats by increasing the paw withdrawal threshold significantly when compared with that of the preinjection (Table 1). The diester compounds with longer or branch linker showed lower effectiveness and severe adverse effects (**1c**, **3d**, **3g**, and **3i**, symptoms such as ataxia, inertia, and logy), especially the gabapentin-pregabalin 1,6-hexandiol diester **3e** showed anti-allodynic efficiency, but was more toxic than the references (three rats died in this group).

Discussion

In this report, we describe the synthesis of a series of diester mutual prodrug of gabapentin and *S*-(+)-pregabalin. Their pharmacological properties to treat neuropathic pain were investigated in a rat model of chronic sciatic nerve constriction injury (CCI). Compounds **1a**, **1b**, and **3a** were effective in reversing tactile allodynia *in vivo* in CCI rats. From comparing the structures within the same system compounds **1a**–**c**, **2a**–**c**, and **3a**–**I**, respectively, the length and side-branch of the linker are of vital influence for the biological activity. The bis-gabapentin and gabapentin-pregabalin diester compounds with short linear linkers showed more effective. The bis-pregabalin and other diester compounds with longer or branch linker showed low efficiencies, and the diester compounds with side-branched linkers showed severe adverse effects. There was only one exception: the gabapentin-pregabalin diester **3e** with the long linear 1,6-hexandiol linker was highly potent but rather toxic.

As a result, there three compound **1a**, **1b**, and **3a** were novel potent anti-neuropathic pain compounds in the designed diester mutual prodrug approach. The anti-allodynic effect, combined with its partly non-toxic effect on nerve functions, suggest that the prodrugs may be possible candidates for further development. The pharmacokinetic profiles of these compounds need further to be investigated.

Acknowledgments

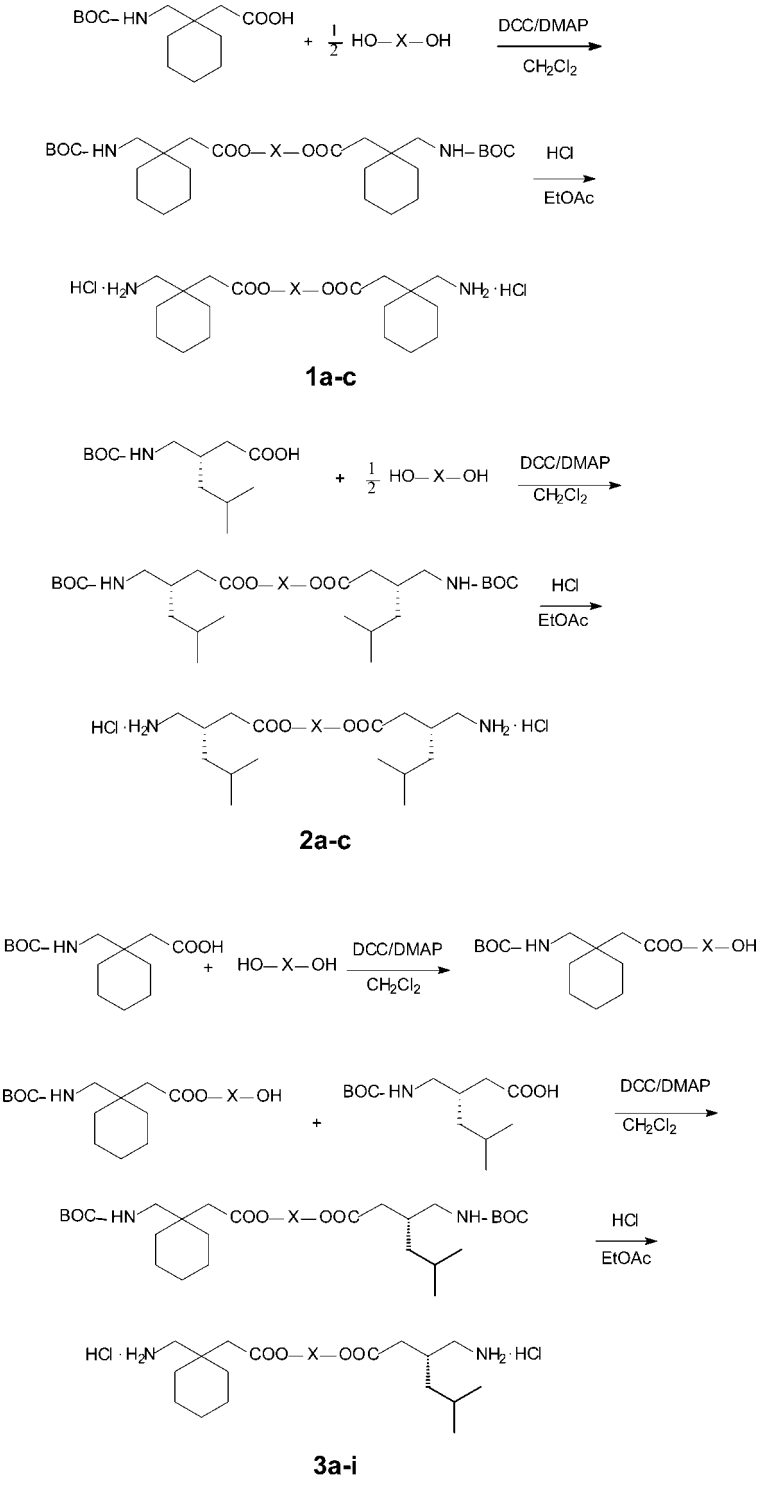
This work was supported by the National Natural Science Foundation of China (20302015).

Experimental

Chemistry

All reagents and solvent were of reagent grade and used without further purification unless indicated otherwise. ^{1}H-NMR spectra were recorded using a Bruker JNM-ECA-400C spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany). Chemical shifts

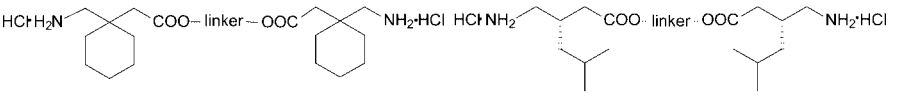
Full Paper



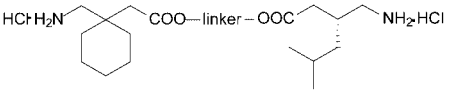
Scheme 1. Synthesis routes to diester compounds.

are reported in parts per million (ppm) relative to TMS as internal standard. All melting points are uncorrected and were determined using a RY-1 type apparatus (Tianjin Analytical Instrument Factory, Tianjin, China). Column chromatography was performed on Merck silica gel 60 (200−400mesh) (Merck, Darmstadt, Germany). Elemental analyses were performed on a Perkin-Elmer model 240 instrument (Perkin Elmer, Norwalk, CT, USA). Mass spectra were recorded on an API 3000 system instrument (Applied Biosystems, Foster City, CA, USA). Gabapentin was obtained from commercial products. *S*-(+)-Pregabalin was synthesized as described in the

Table 1. Effect of diester mutual prodrugs on allodynia response in CCI rats.



1a-c **2a-c**



3a-i

Compound	linker	PWT [g] pre-injection	PWT [g] post-injection	M.P.E [%]
Gabapentin		2.23 ± 0.36	13.50 ± 4.35†	47.41
Pregabalin		3.43 ± 0.39	18.86 ± 3.64‡	68.36
Gabapentin-Pregabalin	combination	3.12 ± 0.51	15.73 ± 3.25	55.11
1a	$-CH_2CH_2-$	3.40 ± 0.77	18.67 ± 4.64†	67.56
1b	$-CH_2CH_2CH_2-$	4.80 ± 0.44	19.40 ± 2.45‡	68.86
1c§	$-CH_2(CH_2)_2CH_2-$	4.00 ± 0.57	5.60 ± 0.89	7.72
2a	$-CH_2CH_2-$	2.90 ± 0.49	7.90 ± 2.45	21.64
2b	$-CH_2CH_2CH_2-$	6.00 ± 0.00	10.33 ± 1.72	21.65
2c	$-CH_2(CH_2)_2CH_2-$	4.50 ± 0.78	8.50 ± 0.78	18.60
3a	$-CH_2CH_2-$	3.33 ± 0.42	19.00 ± 3.40‡	69.12
3b	$-CH_2CH_2CH_2-$	3.60 ± 0.36	7.80 ± 4.92	18.75
3c	$-CH_2(CH_2)_2CH_2-$	4.17 ± 0.91	8.56 ± 3.75	20.11
3d§	$-CH_2(CH_2)_3CH_2-$	2.80 ± 0.73	13.80 ± 2.98‡	47.41
3e§	$-CH_2(CH_2)_4CH_2-$	3.33 ± 0.47	20.67 ± 3.77‡	73.53
3f	$-CH_2\underset{\displaystyle CH_3}{\underset{\vert}{C}}H-$	4.00 ± 0.82	6.80 ± 1.09	12.72
3g§	$-CH_2CH_2\underset{\displaystyle CH_3}{\underset{\vert}{C}}H-$	4.00 ± 0.82	11.40 ± 3.76	33.63
3h	$-CH_2\underset{\displaystyle CH_2}{\underset{\vert}{C}}H-$ (CH3 above)	4.40 ± 0.68	6.80 ± 0.73	11.11
3i§	$-CH_2\underset{\displaystyle CH_3}{\underset{\vert}{C}}C_2H-$	4.40 ± 036	7.20 ± 0.73	12.96

All compounds were administrated at (60 mg/kg, i.p.) 1 h before testing. Data were expressed as mean ± S.E.M, † $P < 0.05$, ‡ $P < 0.01$ *VS* pre-injection, Wilcoxon 2-Sample Test and Kruskal-Wallis Test. § represents compounds with severe adverse effects.

literature [11]. All reactions were monitored by TLC on 15 × 75 mm plastic sheets precoated with silica gel (GF-254) to a thickness of 0.20 mm and viewed at 254 nm UV-light.

*General synthetic method for gabapentin and S-(+)-pregabalin diesters hydrochlorides (**1a–c** and **2a–c**)*

2.5 equivalent gabapentin-Boc or *S*-(+)-pregabalin-Boc and bis-hydroxyl compound were dissolved in anhydrous dichloromethane and the mixture was stirred in an ice bath. DCC in anhydrous dichloromethane was dropped in slowly and maintained at 0 °C for 1h, DMAP was added. Then the mixture was reacted at room temperature. After the reaction was completed (4-8 h), the solution was evaporated *in vacuo*. The residue (solid product) was dissolved in EtOAc, washed with 5% $NaHCO_3$ and water, respectively. The organic phase was dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The colorless oil obtained was purified by silica column chromatography with petroleum ether:EtOAc:MeOH (5:1.5:0.5). The diester compounds were dissolved in EtOAc, 4N HCl/EtOAc was slowly added dropwise. The mixture was stirred at room temperature for 1h. The precipitation was filtered and washed with cooled EtOAc and ether, respectively and dried under vacuum. The gabapentin and *S*-(+)-pregabalin diester hydrochloride was obtained as a white precipitate.

*Gabapentin glycol diester hydrochloride (**1a**)*

Compound **1a** was obtained as a white precipitate (yield 81%), mp. 176–178 °C. ^{1}H-NMR (CD_3OD) δ: 1.41–1.56 (m, 20H, -CH_2-, cyclohexyl), 2.57 (s, 4H, -OOC-CH_2-), 3.07 (s, 4H, -CH_2-NH_2), 4.34 (s, 4H, -COOCH_2CH_2OOC-); MS, *m/z*: 368.9 (M+1). Anal. calcd. for $C_{20}H_{38}N_2O_4Cl_2$: C, 54.42; H, 8.62; N, 6.35. Found: C, 54.21; H, 8.75; N, 6.30.

*Gabapentin 1,3-propandiol diester hydrochloride (**1b**)*

Compound **1b** was obtained as a white solid (yield 73%), mp. 179–181 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.46–1.59 (m, 20H, -CH_2-, cyclohexyl), 2.00 (m, 2H, -COOCH$_2$CH_2CH$_2$OOC-), 2.67 (s, 4H, -OOC-CH_2-), 3.09 (s, 4H, -CH_2-NH_2), 4.27 (m, 4H, -COOCH_2CH$_2$-CH_2OOC-), 8.33 (s, 6H, -NH_2HCl); Anal. calcd. for $C_{21}H_{40}N_2O_4Cl_2$: C, 55.38; H, 8.79; N, 6.15. Found: C, 55.25; H, 8.88; N, 6.06.

*Gabapentin 1,4-butandiol diester hydrochloride (**1c**)*

Compound **1c** was obtained as a white solid (yield 82%), mp. 171–173 °C. ^{1}H-NMR ($CDCl_3$) δ: δ 1.44–1.56 (m, 20H, -CH_2-, cyclohexyl), 1.81 (m, 4H, -COOCH$_2$CH_2CH$_2$CH$_2$OOC-), 2.65 (s, 4H, -OOC-CH_2-), 3.02 (s, 4H, -CH_2-NH_2), 4.15 (t, 4H, -COOCH_2CH$_2$CH$_2$CH_2OOC-), 8.44 (s, 6H, -NH_2HCl). Anal. calcd. for $C_{22}H_{42}N_2O_4Cl_2$: C, 56.29; H, 8.95; N, 5.97. Found: C, 56.40; H, 8.83; N, 5.82.

*S-(+)-Pregabalin glycol diester hydrochloride (**2a**)*

Compound **2a** was obtained as a white solid (yield 77%), mp. 154–155 °C. ^{1}H-NMR (CD_3OD) δ: 0.92–0.95 (m, 12H, -CH_3), 1.27 (m, 4H, -CHR$_1$-CH_2-CHR$_2$-), 1.66 (m, 2H, -CH$_2$-CHR-CH$_2$-), 2.24 (m, 2H, -(CH$_3$)$_2$-CH-CH$_2$-), 2.48 (d, 4H, J=6.6Hz, -OOC-CH_2-), 2.98 (m, 4H, -CH_2-NH_2), 4.33 (s, 4H, -COOCH_2CH_2OOC-). MS *m/z* 345.2 (M+1). Anal. calcd. for $C_{18}H_{38}N_2O_4Cl_2$: C, 51.80; H, 9.11; N, 6.71. Found: C, 51.65; H, 9.26; N, 6.65.

*S-(+)-Pregabalin 1,3-propandiol diester hydrochloride (**2b**)*

Compound **2a** was obtained as a white solid (yield 81%), mp. 132–134 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.91–0.94 (m, 12H, -CH_3), 1.20–1.37 (m, 4H, -CHR$_1$-CH_2- CHR$_2$-), 1.66 (m, 2H, -CH$_2$-CHR-CH$_2$-), 2.01 (s, 2H, -(CH$_3$)$_2$-CH-CH$_2$-), 2.39 (s, 2H, -COOCH$_2$CH_2-CH$_2$OOC-), 2.56 (m, 4H, -OOC-CH_2-), 3.05 (d, 4H, -CH_2-NH_2), 4.23 (m, 4H, -COOCH_2CH$_2$CH$_2$OOC-), 8.31 (s, 6H, -NH_2HCl); Anal. calcd. for $C_{19}H_{40}N_2O_4Cl_2$: C, 52.90; H, 9.28; N, 6.50. Found: C, 52.90; H, 9.54; N, 6.28.

*S-(+)-Pregabalin 1,4-butandiol diester hydrochloride (**2c**)*

Compound **2c** was obtained as a white solid (yield 85%), mp. 114–116 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.91–0.94 (m, 12H, -CH_3), 1.20–1.37 (m, 4H, -CHR$_1$-CH_2-CHR$_2$-), 1.66 (m, 2H, -CH$_2$-CHR-CH$_2$-), 2.05 (s, 2H, -(CH$_3$)$_2$-CH-CH$_2$-), 2.36 (s, 4H, -COOCH$_2$CH_2CH_2CH$_2$OOC-), 2.51–2.62 (m, 4H, -OOC-CH_2-), 3.05 (m, 4H, -CH_2-NH_2), 4.20 (m, 4H, -COOCH_2CH$_2$CH$_2$CH_2OOC-), 8.31 (s, 6H, -NH_2HCl). Anal. calcd. for $C_{20}H_{42}N_2O_4Cl_2$: C, 53.93; H, 9.44; N, 6.29. Found: C, 53.40; H, 9.48; N, 6.22.

*General synthetic method for gabapentin-pregabalin diester hydrochloride (**3a–i**)*

Gabapentin-Boc (10mmol) and bis-hydroxyl compound (50mmol) were dissolved in 20mL anhydrous dichloromethane. The mixture was stirred in an ice bath. DCC (2.0g, 9.7mmol) in 5mL anhydrous dichloromethane was slowly added droppwise. The mixture was maintained in an ice bath for1h, 0.25g DMAP was added. Then the mixture was stirredg at room temperature. After the reaction was completed, the solution was evaporated under reduced pressure. The solid product was dissolved in EtOAc, washed with 5% $NaHCO_3$ and water, respectively. The organic phase was dried over anhydrous $MgSO_4$, then filtered and concentrated under reduced pressure. The gabapentin-Boc monoester compounds were purified by silica column chromatography with petroleum ether:EtOAc:MeOH (5:2:0.5) as a colorless liquid.

Gabapentin-pregabalin diester compounds were synthesized by the gabapentin-Boc monoester with the pregabalin-Boc in the same procedure. The gabapentin-pregabalin diester hydrochloride was obtained as a white precipitate from the protected gabapentin-pregabalin diester by treatment with 4N HCl/EtOAc.

*Gabapentin-Pregabalin glycol diester hydrochloride (**3a**)*

Compound **3a** was obtained as a white solid (yield 63%), mp. 101–103 °C. ^{1}H-NMR (CD_3OD) δ: 0.92–0.95 (m, 6H, -CH_3), 1.27 (m, 2H, -CHR$_1$-CH_2-CHR$_2$-), 1.41–1.56 (m, 10H, -CH_2-,cyclohexyl), 1.66 (m, 1H, -CH$_2$-CHR-CH$_2$-), 2.24 (m, 1H, -(CH$_3$)$_2$-CH-CH$_2$-), 2.48 (m, 2H, -OOC-CH_2-), 2.57 (s, 2H, -OOC-CH_2-), 2.98 (m, 2H, -CH_2-NH_2), 3.07 (s, 2H, -CH_2-NH_2), 4.33 (s, 4H, -COOCH_2CH_2 OOC-); MS *m/z*: 357.4(M+1). Anal. calcd. for $C_{19}H_{38}N_2O_4Cl_2$: C, 53.14; H, 8.86; N, 6.53. Found: C, 53.22; H, 8.83; N, 6.44.

*Gabapentin-Pregabalin 1,3-propandiol diester hydrochloride (**3b**)*

Compound **3b** was obtained as a white solid (yield 60%), mp. 116–118 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.90–0.93 (m, 6H, -CH_3), δ 1.22–1.65 (m, 13H, -CHR$_1$-CH_2-CHR$_2$-, -CH_2-, cyclohexyl, and -CH$_2$-CHR-CH$_2$-), 1.99 (m, 2H, -COOCH$_2$CH_2CH$_2$OOC-), 2.56 (m, 1H, -(CH$_3$)$_2$-CH-CH$_2$-), 2.56–2.65 (m, 4H, -OOC-CH_2-), 3.12–3.13 (m, 4H, -CH_2-NH_2), 4.24 (t, 4H, -COOCH_2CH_2-CH$_2$OOC-), 8.30 (s, 6H, -NH_2HCl); Anal. calcd. for $C_{20}H_{40}N_2O_4Cl_2$: C, 54.17; H, 9.03; N, 6.32. Found: C, 54.15; H, 9.03; N, 6.39.

*Gabapentin-Pregabalin 1,4-butandiol diester hydrochloride (**3c**)*

Compound **3c** was obtained as a white solid (yield 65%), mp. 105−107 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.91-0.93 (m, 6H, -CH_3), 1.21−1.77 (m, 17H, -CHR$_1$-CH_2-CHR$_2$-, -CH_2-, cyclohexyl, -CH$_2$-C*H*R-CH$_2$- and -COOCH$_2$CH_2CH_2CH$_2$OOC-), 2.35−2.67 (m, 5H, -(CH$_3$)$_2$-C*H*-CH_2- and -OOC-CH_2-), 3.06 (s, 4H, -CH_2-NH$_2$), 4.15 (s, 4H, -COOCH_2CH$_2$CH$_2$CH_2OOC-), 8.36 (m, 6H, -NH_2*H*Cl). Anal. calcd. for $C_{21}H_{42}N_2O_4Cl_2$: C, 55.14;, H 9.19; N, 6.12. Found: C, 54.99; H, 9.30; N, 6.18.

*Gabapentin-Pregabalin 1,5-pentandiol diester hydrochloride (**3d**)*

Compound **3d** was obtained as a white solid (yield 68%), mp. 109−111 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.91−0.93 (m, 6H, -CH_3), 1.19−1.78 (m, 19H, -CHR$_1$-CH_2-CHR$_2$-, -CH_2-, cyclohexyl, -CH$_2$-CHR-CH$_2$- and -COOCH$_2$CH_2CH_2CH_2CH$_2$OOC-), 2.33 (m, 1H, -(CH$_3$)$_2$-C*H*-CH_2-), 2.55−2.61 (m, 4H, -OOC-CH_2-), 3.05−3.12 (m, 4H, -CH_2-NH$_2$), 4.15 (s, 4H, -COOCH_2CH$_2$-CH$_2$CH$_2$-CH_2OOC-), 8.34−8.48 (m, 6H, -NH_2*H*Cl); Anal. calcd. for $C_{22}H_{44}N_2O_4Cl_2$: C, 56.06; H, 9.34; N, 5.94. Found: C, 56.09; H, 9.49; N, 6.04.

*Gabapentin-Pregabalin 1,6-hexandiol diester hydrochloride (**3e**)*

Compound **3e** was obtained as a white solid (yield 66%), mp. 88−90 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.91−0.93 (m, 6H, -CH_3), 1.18−1.68 (m, 21H, -CHR$_1$-CH_2-CHR$_2$-, -CH_2-, cyclohexyl, -CH$_2$-C*H*R-CH$_2$- and -COOCH$_2$CH_2CH_2CH_2CH_2CH$_2$OOC-), 2.35 (m, 1H, -(CH$_3$)$_2$-C*H*-CH$_2$-), 2.53−2.58 (m, 4H, -OOC-CH_2-), 3.05−3.12 (d, 4H, -CH_2-NH$_2$), 4.12 (s, 4H, -COOCH_2CH$_2$CH$_2$-CH$_2$ CH$_2$CH_2OOC-), 8.34−8.50 (d, 6H, -NH_2*H*Cl); Anal. calcd. for $C_{23}H_{46}N_2O_4Cl_2$: C, 56.91; H, 9.48; N, 5.77. Found: C, 56.95; H, 9.63; N, 5.87.

*Gabapentin-Pregabalin 1,2-propandiol diester hydrochloride (**3f**)*

Compound **3f** was obtained as a white solid (yield 63%), mp. 87−88 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.91-0.93 (m, 6H, -CH_3), 1.19−1.65 (m, 16H, -CHR$_1$-CH_2-CHR$_2$-, -CH_2-, cyclohexyl, -CH$_2$-C*H*R-CH$_2$-, and −CH_3), 2.38−3.19 (m, 9H, -(CH$_3$)$_2$-C*H*-CH$_2$-, -OOC-CH_2-, -CH_2-NH$_2$), 3.96−4.40 (m, 2H, -COOCH_2CH(CH$_3$)OOC-), 5.18−5.23 (m, 1H, -COOCH$_2$C*H*(CH$_3$)OOC-), 8.30 (s, 6H, -NH_2*H*Cl); Anal. calcd. for $C_{20}H_{40}N_2O_4Cl_2$: C, 54.17; H, 9.03; N, 6.32. Found: C, 53.76; H, 9.02; N, 6.27.

*Gabapentin-Pregabalin 1,3-butandiol diester hydrochloride (**3g**)*

Compound **3g** was obtained as a white solid (yield 61%), mp. 86−88 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.86−0.93 (s, 6H, -CH_3), 1.17−1.68 (m, 18H, -CHR$_1$-CH_2-CHR$_2$-, -CH_2-, cyclohexyl, -CH$_2$-C*H*R-CH$_2$-, and −COOCH$_2$CH_2CH(CH_3)OOC-), 1.90−1.95 (m, 1H, -(CH$_3$)$_2$-C*H*-CH$_2$-), 2.39−2.68 (m, 4H, -OOC-CH_2-), 3.02−3.17 (m, 4H, -CH_2-NH$_2$), 4.11−4.39 (m, 2H, -COOCH_2CH$_2$- CH(CH$_3$)OOC-), 5.04−5.07 (m, 1H, -COOCH$_2$C*H*(CH_2CH$_3$)OOC-), 8.26−8.38 (m, 6H, -NH_2*H*Cl). Anal. calcd. for $C_{21}H_{42}N_2O_4Cl_2$: C, 55.14; H, 9.19; N, 6.12. Found: C, 55.21; H, 9.06; N, 6.26.

*Gabapentin-Pregabalin 1,2-butandiol diester hydrochloride (**3h**)*

Compound **3h** was obtained as a white solid (yield 68%), mp. 88−90 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.91−0.96 (s, 9H, -CH_3), 1.19−1.68 (m, 15H, -CHR$_1$-CH_2- CHR$_2$-, -CH_2-, cyclohexyl, -CH$_2$-C*H*R-CH$_2$-, and −CH_2CH$_3$), 2.08 (m, 1H, -(CH$_3$)$_2$-C H-CH$_2$-), 2.35−3.20 (m, 8H, -OOC-CH_2-, -CH_2-NH$_2$),4.06−4.49 (m, 2H, -COOCH_2CH(CH$_2$CH$_3$)OOC-), 5.01−5.05 (m, 1H, -CO-OCH$_2$C*H*(CH$_2$CH$_3$) OOC-), 8.29−8.40(d, 6H, -NH_2*H*Cl). Anal. calcd. for $C_{21}H_{42}N_2O_4Cl_2$: C ,55.14; H, 9.19; N, 6.12. Found: C, 55.01; H, 9.15; N, 6.31.

*Gabapentin-Pregabalin 2,2-dimethyl-1,3-propandiol diester hydrochloride (**3i**)*

Compound **3i** was obtained as a white solid (yield 69%), mp. 148−149 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.90−0.98 (m, 12H, -CH$_3$), 1.01−1.67 (m, 13H, -CHR$_1$-CH_2-CHR$_2$-, -CH_2-, cyclohexyl, -CH$_2$-C*H*R-CH$_2$-), 2.50 (s, 1H, -(CH$_3$)$_2$-C*H*-CH_2-), 2.53−2.75 (m, 4H,-OOC-CH_2-), 3.02−3.16 (m, 4H, -CH_2-NH$_2$), 3.95 (s, 4H, -CO-OCH_2C(CH$_3$)$_2$CH_2OOC-), 8.29 (s, 6H, -NH_2*H*Cl). Anal. calcd. for $C_{22}H_{44}N_2O_4Cl_2$: C, 56.06; H, 9.34; N, 5.94. Found: C, 56.04; H, 9.41; N, 5.98.

Preliminary biological evaluation

All procedures were conducted in accordance with the IASP guidelines on use of animals for investigation of experimental pain [12]. Surgical preparations and experimental protocols were approved by the Animal Care and Use Committee of Beijing Institute of Pharmacology and Toxicology (BIPT). Young adult male Sprague-Dawley rats, weighting 160-180 g at entry were housed in clear plastic cages at the Experimental Animal Center of BIPT. Rats housed in groups of five per cage have been used to test the analgesic properties of one drug in a controlled 12-h light/12-h dark environment and had free access to food and water. The mechanical allodynia was determined by the paw withdrawal in response to a series of filaments (Von Frey Hair, North Coast Company, Bellevue, WA, USA) and was expressed in grams. A peripheral mono neuropathy was produced in rats by placing loosely constrictive ligatures around the common sciatic nerve according to the method described by Bennett. Rats were anaesthetized with chloral hydrate. The right common sciatic nerve was exposed at the level of the middle thigh by blunt dissection through the biceps femoris. Proximal to the nerve's trifurcation, a 5-7 mm of nerve was freed of adhering tissue and 4 ligature (4.0 chromic gut) were tied loosely around it with about 1 mm spacing. Great care was taken to tie the ligatures such that the diameter of the nerve was seen to be just barely constricted. In every animal, an identical dissection was performed on the opposite side except that the sciatic nerve was not ligated. The left paw was untouched. Data were expressed as mean ± S.E.M and analyzed by SAS data process soft. The raw data of mechanical threshold were converted to a percentage of the maximal possible effect (% M.P.E) for the ED_{50} calculation. % M.P.E was defined according to the following formula in the CCI model: M.P.E% = postdrug threshold−predrug threshold)/(Max threshold−predrug threshold)%. The max thresholds are designed as 26 g.

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