Design, Synthesis, and Potent Antiepileptic Activity with Latent Nerve Rehabilitation of Novel γ-Aminobutyric Acid Derivatives

Dian He,*[a,b,#] Jing Ma,[a,#] Xiuxiao Shi,[a] Chunyan Zhao,[a] Meng Hou,[a] Qingxin Guo,[a] Shangxian Ma,[a] Xiaojun Li,[a] Peicheng Zhao,[a] Wenhu Liu,[a] Zhuqing Yang,[a] Jianping Mou,[a] Pengfei Song,[a] Yang Zhang,[a] and Jing Li[a]

[a] *Institute of Medicinal Chemistry, School of Pharmaceutical Science, Lanzhou University; Lanzhou 730000, Gansu Province, P. R. China: and* [b] *State Key Laboratory of Applied Organic Chemistry, Lanzhou University; Lanzhou Gansu 730000, P. R. China.*

Received April 28, 2014; accepted June 30, 2014; advance publication released online July 18, 2014

We aimed to design and synthesize novel γ-aminobutyric acid (GABA) derivatives with the combination of aspirin (ASA) of nerve rehabilitative pharmacophores so as to develop multifunctional drugs useful in the treatment of neurological disorders. Twenty-four novel esters and amides of 1a were synthesized, biologically evaluated for antiepileptic activity with the model of 4-aminopyridine (4-AP), and tested for their capacity of penetrating the blood–brain barrier (BBB) with HPLC. The distribution of 8a, ASA freed by 8a, 7c, and ASA freed by 7c within 24 h in brain tissue was measured. The structure–activity relationship (SAR) was established and the data of Computer Aided Drug Design (CADD) showed good results. With ED_{50} values of 0.3684–0.5199 mmol/kg, LD_{50} 1.1487–1.3944 mmol/kg, and therapeutic index (TI) 2.65–3.15, compounds 8a, 3b, 4b, 6c, and 7c exhibited better antiepileptic activities in multiples of 0.3 to 2.2 against the control sodium valproate (VPA). Most importantly, 8a and 7c exhibited excellent antiepileptic activities with TI values of 3.15 and 3.12, respectively.

Key words γ-aminobutyric acid (GABA); aspirin (ASA); antiepilepsy; neuroprotection

Currently available antiepileptic drugs (AEDs) are mainly seizure suppressing, while they do not affect the underlying pathology or the progression of the disease.[1,2] Therefore, a medical need exists to develop alternative therapeutics that not only alleviate the symptoms, but also inhibit the process of epileptogenesis.[3,4] Recent experimental and clinical evidence highlights that the activation of inflammatory pathways may contribute to the development of epilepsy.[4–7] Furthermore, neuroprotective agents have received considerable attention for the treatment of neurological disorders, especially Alzheimer's, Parkinson's, ischemia/stroke and epilepsy. Many neuroprotective pathways may have a broad applicability across different neurodegenerative conditions. The underlying molecular mechanisms of neuroprogression are thought to include neurotrophins and regulation of neurogenesis and apoptosis, neurotransmitters, inflammatory, oxidative and nitrosative stress, mitochondrial dysfunction, cortisol and the hypothalamic-pituitary-adrenal axis, and epigenetic influences. Knowledge of the involvement of each of these pathways implies that specific agents that act on some or multiple of these pathways may thus block this cascade and have neuroprotective properties.[8] A recent study[9] has demonstrated that chronic treatment with celecoxib, a selective cyclooxygenase-2 (COX-2) inhibitor, resulted in a remarkable decrease in the frequency of spontaneous recurrent seizures (SRS) after pilocarpine-induced prolonged seizures.

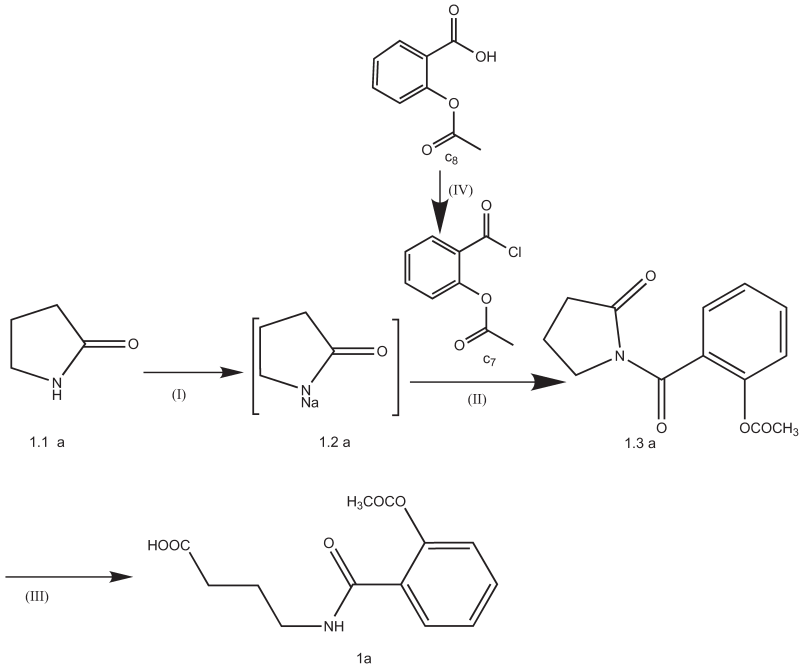
Aspirin, the most widely used medications in the world, also represents one of the non-selective classical COX inhibitors.[10] Apart from its preventive actions against stroke due to its antithrombotic properties, recent data in the literature[11] suggests that high concentrations of aspirin (ASA) also exert direct neuroprotective effects. ASA moderates the inflammatory response in lipopolysaccharide activated microglia by triggering lipoxins, inhibiting activation of nitric oxide (NO), interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), nuclear factor kappaB (NF kappaB),[12,13] extracellular regulated protein kinases (ERK) and mitogen-activated protein (MAP) kinase.[14] And also these effects correlate with the inhibition of excitatory amino acid release and inducible nitric oxide synthase (iNOS) expression caused by ASA.[15,16] A neuroprotective effect of ASA has been found to recover the fall of ATP levels by the inhibition of oxygen glucose deprivation-induced (OGD-induced) glutamate release.[17] Other studies also indicate that the prevention of the loss in cyclin-dependent kinase-5 (CDK-5) activity during reoxygenation is crucial for ASA-induced protection.[18,19] ASA combined with sulfasalazine has neuroprotective effects in two rodent models of ischemia.[20] Previous studies have demonstrated that aspirin itself or in combination with AEDs dose-dependently decreased the incidence of seizures.[21–24] Although a large number of patients with epilepsy take aspirin while they are affected by a broad spectrum of other conditions, the effects of aspirin on the development of epileptogenesis have not been specifically addressed yet.[25] In contrast with the antiplatelet effects of ASA, the neuronal actions require higher doses of ASA, therefore increasing undesirable adverse effects of this drug such as gastrointestinal and bleeding complications. Simultaneously, ASA does not cross the blood–brain barrier (BBB), suggesting that efficacy of combination therapy in mental health treatment is likely to be a downstream effect.

γ-Aminobutyric acid (GABA) is a dominant inhibitory transmitter in the mammalian brain.[26,27] It has been well documented that the reduction of GABAergic neuronal activity plays an important role in a number of neurological disorders, including epilepsy, anxiety, and pain.[28] Accordingly, various antiepileptics related to GABA can be developed based on direct interaction with GABA receptor, or enhancement

The authors declare no conflict of interest.

[#] These authors contributed equally to this work.

* To whom correspondence should be addressed. e-mail: hed@lzu.edu.cn



Reagents and conditions: (I) CH_3ONa, PhMe. (II) TEBA, PhMe, C_7. (III) HCl, 60°C, 8 h. (IV) $SOCl_2$.

Chart 1. Synthetic Route of 4-(2-Acetoxybenzoylamino)butyric Acid

of GABA accumulation mainly by stimulating the activity of glutamic acid decarboxylase, inhibiting GABA transaminase, or inhibiting GABA-transport protein.[29–35] These observations raise the possibility that "replacement" procedures that increase GABA level of central nervous system (CNS) may be useful in the treatment of such neuropsychiatric disorders.[36] Nevertheless, most of antiepileptic drugs including GABA analogues have proven to be discontented as they have little effect on the prognosis of patients with intractable epilepsy due to the lack of potential capacity to rehabilitate the nerve nidus or prevent seizure from injuring neuronal cells. Simultaneously, clinical treatment of epilepsia with GABA is impractical because the present results illustrate that blood–brain barrier (BBB) in adult animals is impermeable to both blood-borne GABA and endogenous cerebral GABA owing to the low lipophilicity ($\log P = -0.82$) (P: partition coefficient).[37] Therefore, it is necessary to develop a suitable carrier for GABA to improve the lipophilicity and the permeability through BBB in order to inhibit seizures. For instance, conjugation of GABA with fatty amino acids or peptides facilitates its passage across BBB.[38]

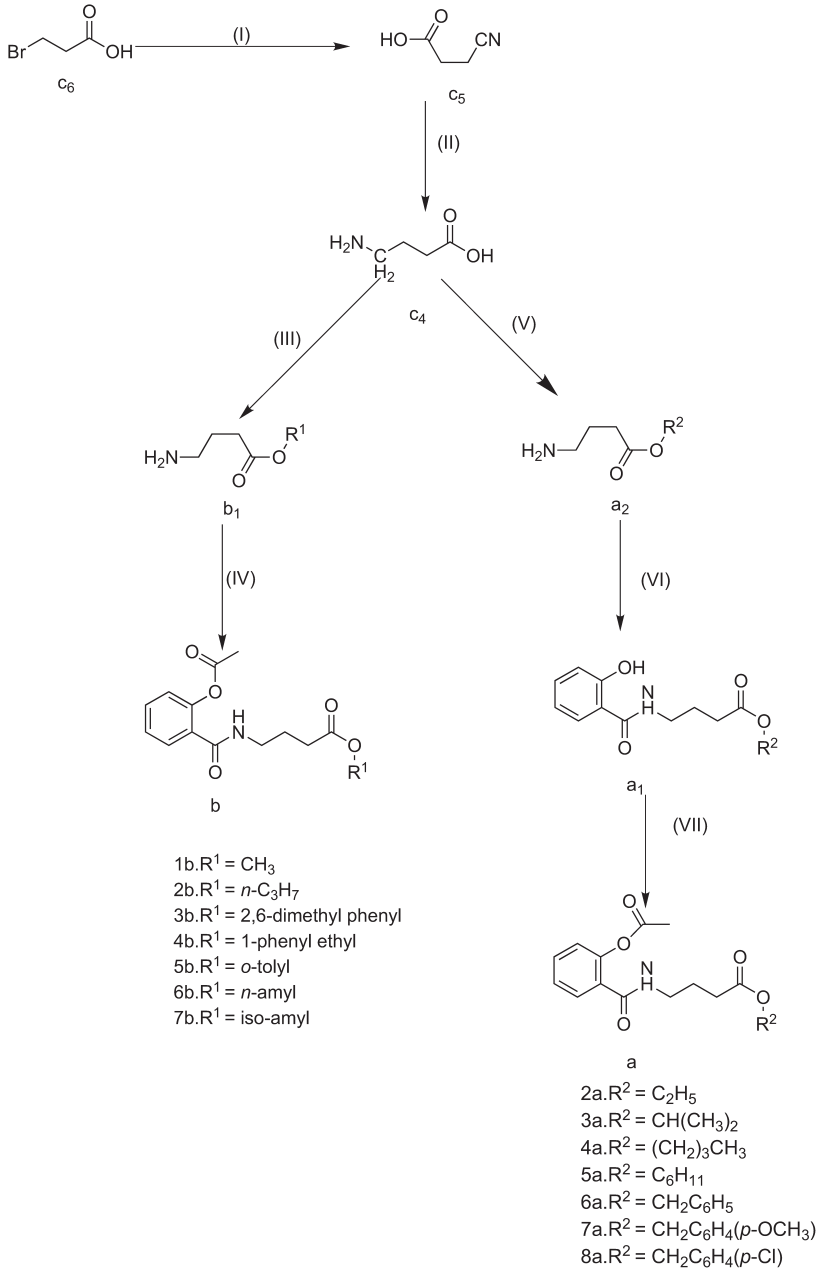
Based on critical modeling clues, twenty-four antiepileptic agents were designed to develop a multifunctional drug useful in the treatment of neurological disorders, **8a** and **7c** of which were identified as the hits to inhibit seizures in our screening campaign. Aspirin was as the key role of preventing seizures from injuring neuronal cells and the lipophilicity was improved.

Results

Chemistry Charts 1–3, describe the synthesis of all target compounds. As was presented in Chart 1, employment of phase transfer catalyst benzyltriethylammonium chloride (TEBA) gave 4-(2-acetoxy-benzoylamino) butyric acid with an increasing yield of 85% based on the earlier reported procedures,[38–42] in which acylation reaction of the starting material GABA handled with trimethylchlorosilane (TMSCl) and 2-(acetyloxy)benzoyl chloride led a yield of 39.9% in dichloromethane, or a yield of 33.7% after crystallization with ethanol-water in presence of aqueous medium sodium hydroxide.

Even if the activity of compound **1a** was preferred, however, the direct esterification is not conducive to work up. During the synthesis of **2a**–**8a** (Chart 2), the key intermediate $\mathbf{a_2}$, was generated by catalytic effect of the commercially available *p*-toluenesulfonate (*p*-TsOH) attempting to surmount the acidic medium incompletion of esterification.[43,44] During the synthesis of **1b**–**7b** (Chart 2), dicyclohexylcarbodiimide (DCC) at low temperature and weak alkaline conditions selectively increased the yield. Finally, the required derivatives **1c**–**9c** (Chart 3) were obtained by reaction of GABA treated with thionyl chloride in present of HCl in methanol solution. The spectra of all target compounds and their key intermediates were consistent with their proposed structures. All target compounds were characterized by their spectral data.

Biological Activities The Initial Anticonvulsant Evaluation of the Synthesized Compounds The aim of this drug discovery program was to prepare novel GABA derivatives with multiple pharmacological actions effective in the treatment of epilepsy. The synthesized compounds (**1a**–**8a**, **1b**–**7b**, **1c**–**9c**) were evaluated at dose levels of 80, 160, and 240 mg/kg intraperitoneally in mice for anticonvulsant activity by following the standard anticonvulsant drug development (ADD) program protocols. Table 1 lists the results obtained from the initial anticonvulsant evaluation of the synthesized compounds compared to the clinically proven antiepileptics sodium valproate (VPA) which was also tested at the same dose levels. The test applied 4-aminopyridine (4-AP) model which induced pattern of abnormal behavior in a dose-dependent manner, characterized by convulsive clonus-like motor patterns with periods of behavioral arrest.



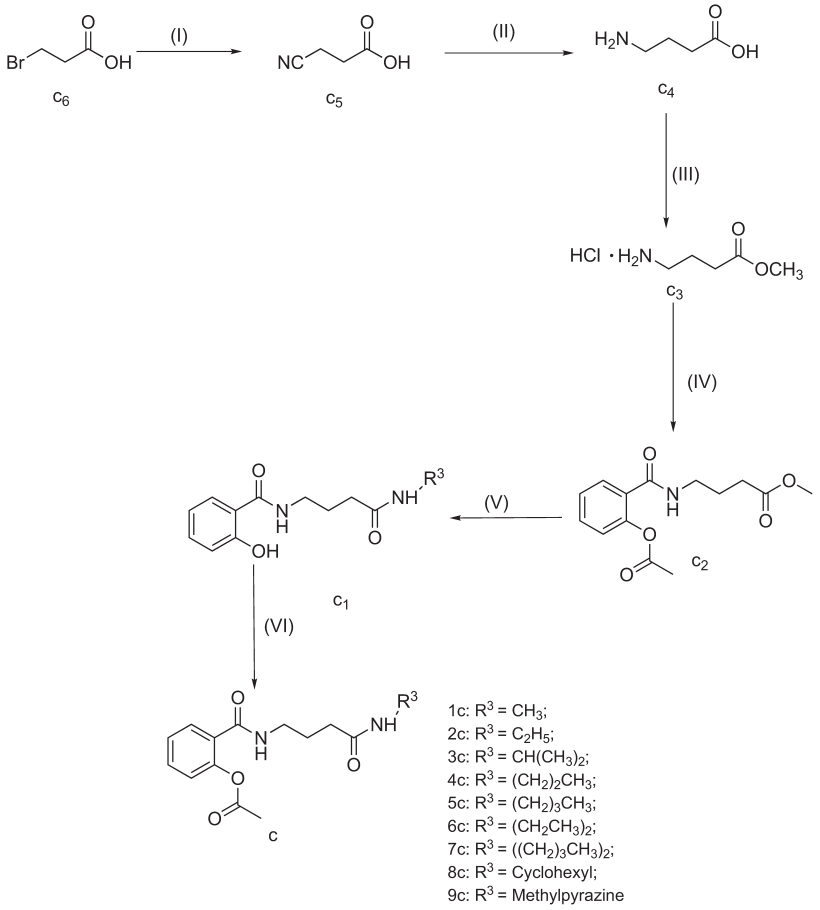
Reagents and conditions: (I) NaCN, yield of 70%. (II) $H_2/PtO_2/H$, yield of 80%. (III) R^1OH, $SOCl_2$, TEA. (IV) DMAP, DCC, CH_2Cl_2, C_7. (V) R^2OH, 4-methylbenzenesulfonic acid. (VI) $NaHCO_3$, Et_3N, CH_2Cl_2, 50°C, 18 h. (VII) Et_3N, AcCl, CH_2Cl_2, 0°C, rt.

Chart 2. Synthetic Route of Esters of 4-(2-Acetoxybenzoylamino)butyric Acid

The initial pharmacological experimental test showed that these compounds have antiepileptic activity, thirteen of which (**1a**, **5a**–**8a**, **3b**–**6b** and **5c**–**8c**) showed conspicuous activity in the initial anticonvulsant evaluation indicative of their ability to prevent seizure spread, showing the effective ratio more than 60% in the dose of 240 mg/kg which implied a significant difference ($p<0.05$ or $p<0.01$). Compounds **5a**–**6a**, **8a**, **3b**–**5b**, and **6c**–**8c** were the most active compounds with effective ratio 83% or above at 240 mg/kg, among which four compounds (**5a**, **8a**, **5b**, **7c**) showed the most protection at 160 mg/kg. Of the four compounds (**5a**, **8a**, **5b**, **7c**), only **8a** and **7c** showed protection at 80 mg/kg. The ED_{50}, LD_{50} and therapeutic index (TI) were determined for those with a better biological activity in the initial anticonvulsant evaluation.

The Further Tests of Pharmacological Activity Based on the results of the initial anticonvulsant evaluation, the further tests of pharmacological activity to the compounds **1a**, **5a**–**8a**, **3b**–**7b**, and **5c**–**8c** were done (Fig. 1). ED_{50} and LD_{50} were determined by Karber method and the relationship of ED_{50}, LD_{50}, TI, and log*P* was shown in Fig. 4. Of these compounds, compounds **8a**, **3b**, **4b**, **6c**, and **7c**, exhibited excellent antiepileptic activities with a higher TI. Compound **8a** with ED_{50} 0.4189 mmol/kg, LD_{50} 1.321 mmol/kg, the more 1.6 times antiepileptic activity and the equivalent safety index compared with sodium valproate can be considered as prospective antiepileptic substances. Compound **7c** with 0.3684 mmol/kg of ED_{50} and 1.1487 mmol/kg of LD_{50} is also worth being further developed.

Determination of 8a, 7c and ASA Concentration in the Brain Tissue by HPLC Regression Equation of ASA, **8a**,



Reagents and conditions: (I) NaCN. (II) H_2, PtO_2, H^+. (III) CH_3OH, $SOCl_2$. (IV) NH_3, CH_2Cl_2, C_7. (V) R^3NH_2, CH_3OH. (VI) CH_2Cl_2, CH_3COCl.

Chart 3. Synthetic Route of Amides of 4-(2-Acetoxybenzoylamino)butyric Acid

and **7c** We chose **8a** and **7c** to measure the concentration of the original drug and ASA in the brain tissue of mice. The results demonstrated that ASA, **8a**, and **7c** had a good linear relationship in the concentration range of 1–80 μg/mL. The regression equation and the values of r were as follows: ASA (Y=2.574×10^4C+2.426×10^4, r=0.9990), **8a** (Y=3.543×10^4C+3.406×10^4, r=0.9960), **7c** (Y=3.191×10^4C+2.297×10^4, r=0.9990). The values of Y and C stand for the peak area of the reference substance and the concentration separately.

Precision and Accuracy The accuracy and precision for ASA, **8a**, and **7c** were determined at concentration of 2, 20, and 80 μg/mL, respectively. Table 2 showed a summary of accuracy and precision for ASA, **8a**, and **7c**. Accuracy was the percentage of the concentration compared with the theoretical concentration. Precision was based on calculation of the R.S.D. (relative standard deviation). The accuracy (R.E., %) ranged from 85.2% to 107.8% and the precision ranged from 2.4% to 6.3% throughout the three concentrations studied. The solution was measured five times for 3d. The accuracy and precision were complied with the Food and Drug Administration (FDA)[45] acceptance criteria (≤15%).

Analyte Stability Stock solutions stability was analyzed at 0, 1, 3, 5, and 8 h. Blank brain tissue homogenate was added to the reference substance with the high, medium and low concentration separately and the corresponding concentration of ASA, **8a** and **7c** was 2 μg/mL, 20 μg/mL and 80 μg/mL. R.S.D. of ASA, **8a** and **7c** less than 1% indicated that the solution was stable within 8h.

Distribution of **8a**, ASA Freed by **8a**, **7c**, and ASA Freed by **7c** Less Than 24 h in the Brain Tissue Mice were randomly divided into 24 groups (n=6). **8a** and **7c** (0.6 mmol/kg) were administrated to 11 groups respectively with the method of intraperitoneal injection. Brain tissue homogenate which was stored at the temperature of −20°C was handled before administration (0 h) and after 0.2, 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 24 h and then detected by HPLC (Fig. 2).

The data indicated that the concentration of **8a** and **7c** was up to the highest after about 1h, respectively 8.960 μg/g, 7.395 μg/g, ASA hydrolyzed by **8a** and **7c** about 2 h, respectively 1.247 μg/g, 1.065 μg/g.

Assay Selectivity Blank brain tissue homogenate, **8a**, and **7c** were tested for presence of endogenous components which might interfere with the internal standard. These samples were prepared in accordance with the sample-preparation procedure. Chromatograms of blank brain tissue homogenate, standard solutions of ASA (20 μg/mL), **8a** (20 μg/mL), and **7c** (20 μg/mL) and brain tissue collected from the mice administered **8a** and **7c** after 0.5 h respectively were compared to analyze the specificity of the procedure (Fig. 3). The retention time for ASA, **8a**, and **7c** were 7.8, 5.0, and 5.4 min, respectively. No interfering peaks were observed. The representative HPLC chromatograms in Fig. 3 indicated that no endogenous peaks were present at the retention times of ASA, **8a**, and **7c**.

Experimental and Calculated (ED_{50} and LD_{50}) by Multilinear Regression (MLR) Model In this paper, the activities of 19 compounds were expressed as ED_{50} and LD_{50} listed

Table 1. The Effective Ratio of the Target Compounds at Different Doses

Compd.	Positive rate[a] (%)		
	80 mg/kg	160 mg/kg	240 mg/kg
1a	0.0	25.0	67.0*
2a	0.0	12.5	25.0
3a	0.0	12.5	25.0
4a	0.0	12.5	25.0
5a	0.0	50.0*	87.0**
6a	0.0	37.5	87.0**
7a	0.0	25.0	67.0*
8a	12.5	50.0*	87.0**
1b	0.0	12.5	25.0
2b	0.0	12.5	25.0
3b	12.5	40.0	87.0**
4b	0.0	40.0	87.0**
5b	0.0	50.0*	87.0**
6b	0.0	25.0	67.0*
7b	0.0	33.0	50.0*
1c	0.0	12.5	33.0
2c	0.0	12.5	33.0
3c	0.0	17.0	40.0
4c	0.0	17.0	40.0
5c	0.0	50.0*	67.0*
6c	0.0	50.0*	83.0**
7c	12.5	50.0*	87.0**
8c	0.0	40.0	83.0**
9c	0.0	33.0	40.0
Sodium VPA	12.5	50.0*	87.0**

a) Number of animal used (n=6). *p<0.05, **p<0.01 *vs.* model group. Significant difference was calculated for by one-way ANOVA.

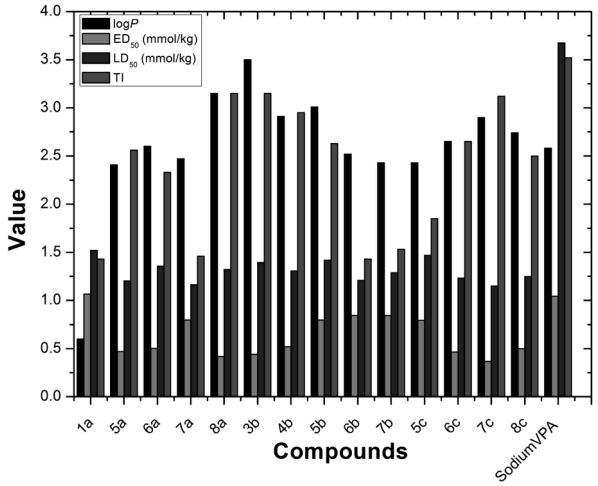


Fig. 1. Structure–Activity Relationship of Target Compounds (**1a**–**8a**, **1b**–**7b**, **1c**–**9c**)

in Table 3. All 19 compounds were presented by molecular descriptors, calculated using CODESSA software, mainly including constitutional, topological, geometrical, electrostatic, and quantum chemical descriptors. In this paper, 423 descriptors for each compound were obtained and heuristic linear regression procedures available in the framework of the CODESSA program were used to perform a complete search. It allowed us to find the most 3 significant descriptor to build quantitative structure–activity relationships (QSAR) models

Table 2. Precision (R.S.D., %) and Accuracy (R.E., %) of HPLC Assay for ASA, **8a** and **7c** (n=5)

Reference substance	Nominal conc. (μg/mL)	Measured conc. (μg/mL)	Accuracy (%)	R.S.D. (%)
ASA	2	1.8±0.1	87.5	4.5
	20	21.6±0.5	107.8	2.4
	80	72.4±4.2	90.4	5.1
8a	2	1.8±0.1	87.5	4.5
	20	19.8±0.6	89.9	3.1
	80	73.5±4.6	91.6	5.7
7c	2	1.7±0.1	85.2	6.3
	20	19.2±0.6	89.6	3.4
	80	73.4±4.5	91.7	5.6

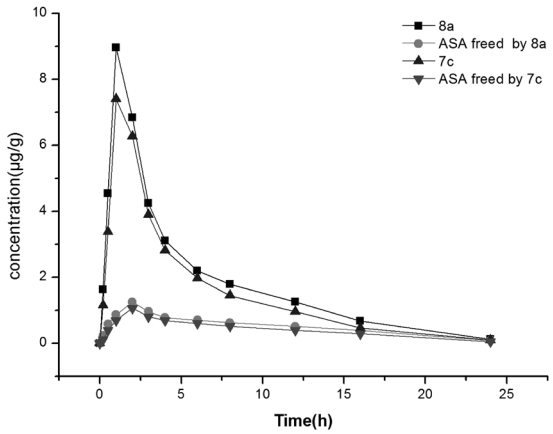


Fig. 2. The Concentration Trend of **8a**, ASA Freed by **8a**, **7c**, and ASA Freed by **7c** within 24 h

shown in Table 3.

As shown, the predicted activities (ED_{50} and LD_{50}) values calculated by MLR model are given in Table 4 and the linear function was built as Functions 1 and 2. For the MLR model, the LOO cross-validated coefficient R^2 was 0.73 and 0.86.

$$ED_{50} = 6.67 - 0.39 \cdot ACI2 - 9.28 \cdot RNCl - 5.16 \cdot MAOE \quad (1)$$

$$LD_{50} = 3.94 + 0.12 \cdot HOMO + 0.042 \cdot NCl - 0.027 \cdot FPSA2 \quad (2)$$

Discussion and Conclusion

The present work focused on the synthesis of a series of analogues to **1a** and their potency and efficacy in inhibiting epilepsia. Appropriate substitution of GABA resulted in antiepileptic agents with latent nerve rehabilitation. A structure–activity relationships (SAR) study demonstrated that the property of lipophilicity modeled by the calculated octanol-water partition coefficient may favor the affinity of GABA congeners to the receptor site.

It appeared that, in the 4-AP model, compounds showed promising results, including **1a**, **5a**–**8a**, **3b**–**7b**, and **5c**–**8c**, and the most active compounds that had been found to be effective in biological activities, would be compounds **8a** and **7c**. Brain tissue homogenate by HPLC before administration (0 h) and after 0.2, 0.5, 1, 2, 3, 4, 6, 8, 12, 16, and 24 h proved

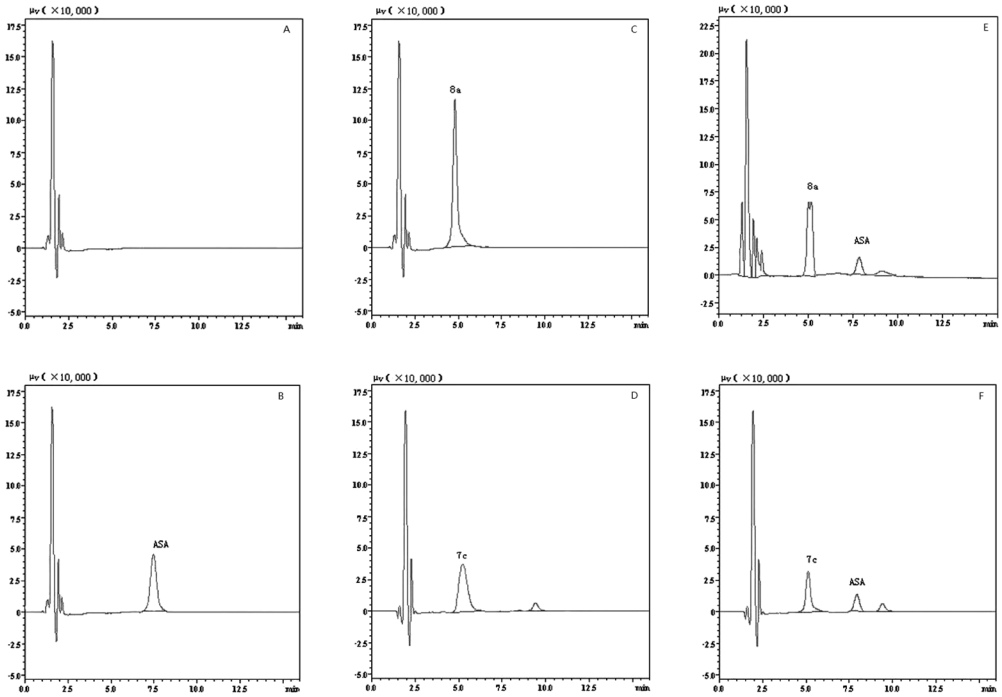


Fig. 3. Representative Chromatograms for **8a**, ASA Freed by **8a**, **7c** and ASA Freed by **7c**

Representative chromatograms resulting from analysis of (A) Blank brain tissue homogenate, (B) standard curve sample of ASA (20 μg/mL), (C) **8a** solution (20 μg/mL), (D) **7c** solution (20 μg/mL), (E) **8a** obtained at 0.5 h post-dose and (F) **7c** obtained at 0.5 h post-dose.

Table 3. Descriptors and the Chemical Meanings

QSAR model for ED_{50}	ACI2	Average complementary information content 2
	RNCl	Relative number of Cl atoms
	MAOE	Min atomic orbital electronic population
QSAR models for LD_{50}	HOMO	HOMO-1 energy
	NCl	Number of Cl atoms
	FPSA2	FPSA-2 fractional PPSA (PPSA-2/TMSA)

Table 4. Experimental and Calculated (ED_{50} and LD_{50}) by MLR Model

No. of compd.	Experimental		Predicted	
	ED_{50} (mmol/kg)	LD_{50} (mmol/kg)	ED_{50} (mmol/kg)	LD_{50} (mmol/kg)
1a	2.4511	2.6052	2.5195	2.6254
5a	2.2123	2.6202	2.1836	2.6176
6a	2.2534	2.6822	2.2711	2.6573
7a	2.4867	2.6507	2.3799	2.6562
8a	2.4867	2.6507	2.4809	2.6589
3b	2.4867	2.6507	2.4483	2.6623
4b	2.2126	2.7114	2.2898	2.7009
5b	2.2126	2.7114	2.1756	2.7195
6b	2.2126	2.7114	2.1723	2.7138
7b	2.2126	2.7114	2.2547	2.6946
1c	2.1956	2.6829	2.2913	2.6691
2c	2.2830	2.7022	2.2955	2.6886
3c	2.4511	2.6056	2.3592	2.6239
4c	2.4511	2.6354	2.4051	2.6303
5c	2.1734	2.5960	2.2872	2.6407
6c	2.4044	2.6721	2.3434	2.6297
7c	2.1414	2.6354	2.2448	2.6459
8c	2.2380	2.6354	2.2211	2.6265
9c	2.1872	2.8032	2.1287	2.8123

that the concentration of **8a** and **7c** was up to the highest after about 1 h, respectively 8.960 μg/g, 7.395 μg/g, ASA hydrolyzed by **8a** and **7c** about 2 h, respectively 1.247 μg/g, 1.065 μg/g. These measurements suggested that **8a** and **7c** can enter CNS and provided a source for a steady supply of GABA and ASA, possibly by hydrolysis. Simultaneously, the data also indicated that **8a** was superior to **7c**, which demonstrated that the novel GABA derivatives substituted by esters were easier to pass through BBB and release ASA.

It was crucial that our study also demonstrated that **8a** and **7c** were antiepileptic agents with latent nerve rehabilitation owing to the released ASA. The novel GABA derivatives was transported and distributed in the brain tissue, after having been hydrolyzed by enzymes to enclose in the CNS and produce a continuous physiological activity. Further research is required to confirm the molecular mechanisms of action of the reported compounds. *In vivo* metabolism activation of esters and amides of 4-(2-acetoxybenzamido)butanoic acid is as in Fig. 4.

SAR of the Target Compounds

The activity evaluation showed that different structures of the target compounds (**1a**–**8a**, **1b**–**7b**, **1c**–**9c**) displayed great influences on the activity. When their ends connected with esters with a larger steric hindrance, such as the cyclohexyl ester (**5a**), benzyl ester (**6a**), benzyl ester substituted by methoxy (**7a**) or chlorine (**8a**), 2,6-dimethyl phenyl ester (**3b**), 1-phenyl ethyl ester (**4b**), and *o*-methyl phenyl ester (**5b**), the compounds showed a better activity. On the contrary, with a smaller steric hindrance such as the ethyl (**2a**), *iso*-propyl (**3a**), *n*-butyl (**4a**), methyl (**1b**) and *n*-propyl (**2b**), a worse activity and an increasing toxicity were displayed. SAR of targeted

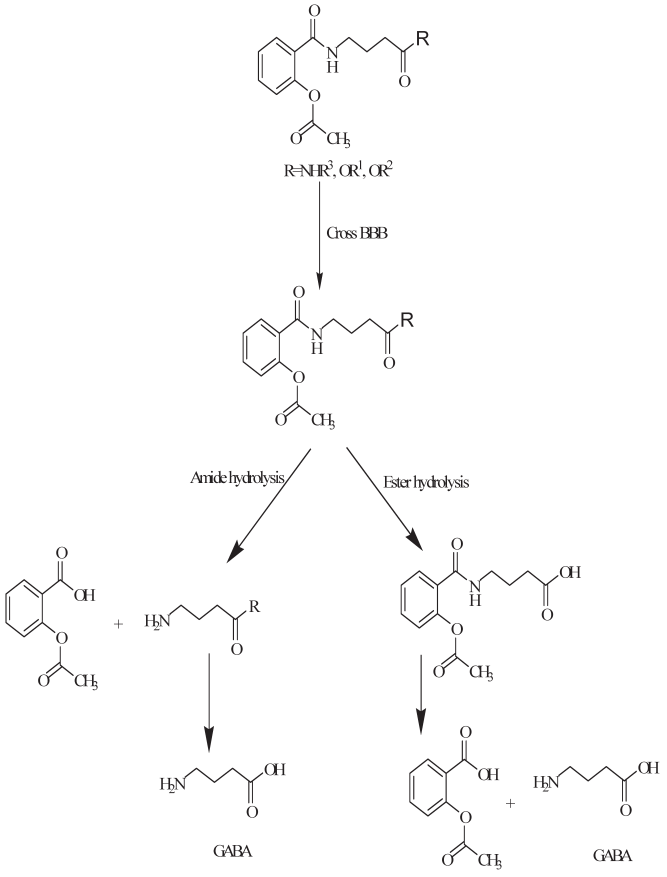


Fig. 4. *In Vivo* Metabolism Activation of Target Compounds (**1a**–**8a**, **1b**–**7b**, **1c**–**9c**)

compounds is as in Fig. 1.

Simultaneously, compounds with electron-donating group substituents on the phenyl ring showed the decreases of the antiepileptic effect and the increases of toxicity, which was opposite to the electron-withdrawing group (**8a**). The results also showed that log*P* had obvious correlation with ED_{50}, LD_{50} and TI, especially TI displaying a obvious parallel relationship with the log*P* (Fig. 1).

Experimental

Chemistry Melting points were determined in capillary tubes on an electrothermal PIF YRT-3 apparatus and without correction. IR spectra were determined on NEXUS 670 FT-IR (Nicolet). ^{1}H-NMR and ^{13}C-NMR (δ ppm) spectra were recorded on a Varian Mercury (400 MHz) using tetramethylsilane (TMS) as the internal standard. Mass spectra were recorded on a VGZAB-HS (70 eV) spectrometer with electrospray ionization (ESI) source as ionization. All reactions were monitored by analytical TLC and silica gel F_{254} was used in TLC. All chemicals were purchased from commercial suppliers and were dried and purified when necessary.

General Synthetic Procedure for the Key Intermediates 2-Acetoxy Benzoyl Chloride (**c**$_7$)[46] Under anhydrous conditions, pyridine as the catalyst,[47] higher yield of product can be obtained without any solvent. Thionyl chloride (4.00 mL) was added dropwise to a solution of ASA (0.05 mol) in the pyridine (0.02 mL) below 50°C. The suspension was gradually heated to 90°C, stirred and refluxed for 3h. The excess thionyl chloride was removed at 40°C under reduced pressure. The white viscous substance **c**$_7$ (9.43g, 90%) was sealed in the desiccator.

General Procedure for the Synthesis of **1a**[38–40] A: Preparation of 2-Acetoxy Benzoyl Pyrrolidone (**1.3a**)[48] Benzyltriethylaminium chloride (TEBA) (0.005 mol) was added to the pyrrolidone sodium salt (**1.2a**) (0.05 mol) dissolved in the anhydrous toluene solution below 10°C and **c**$_7$ (0.05 mol) in anhydrous toluene was added dropwise. Then reacted at room temperature for 0.5 h and at 50°C for 3 h. Water (100 mL) was added and saturated sodium bicarbonate was mixed to wash the toluene layer with 20 mL, 20 mL, 10 mL, respectively, and then the suspension was washed until neutral with water (50 mL), dried over anhydrous magnesium sulfate overnight and crystallized with ethanol to precipitate the product 10.1 g, 81.7%.

B: Perparation of **1a** PtO_2 (12.5 mmol) was added to 2-acetoxy benzoyl pyrrolidone (25 mmol) dissolved in HCl (80 mL) and then the suspension was refluxed 12 h at 60°C with a yield of 72%. mp: 101.2–102.2°C. *Rf* 0.38 (eluent : petroleum : ethyl acetate : glacial acetic acid=3 : 2 : 0.02) IR (KBr) *v* (cm^{-1}): 3381, 3087, 1738, 1582–1445, 1241–1180; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.95 (m, 2H, *J*=6.6 Hz, *J*=6.9 Hz), 2.15 (s, 3H), 2.29 (t, 2H, *J*=5.4 Hz), 3.48 (m, 2H, *J*=6.0 Hz, *J*=6.6 Hz), 6.77–6.96 (m, 2H, *J*=7.5 Hz, *J*=8.1 Hz), 7.21–7.38 (m, 2H, *J*=3.9 Hz, *J*=9.0 Hz), 9.92 (br s, 1H); EI-MS (*m*/*z*): 222 [M^+-43], 220 [M^+-45], 178 [M^+-87], 120 [M^+-145]

General Synthetic Procedure for the Key Intermediates γ-Aminobutyrate *p*-Toluenesulfonate Salt (**a**$_2$)[43,44] γ-Aminobutyrate *p*-toluenesulfonate salt was prepared in a manner similar to that of Shields, McGregor, and Carpenter. GABA (20 mmol), benzenesulfonic acid monohydrate (22.88 mmol), benzyl alcohol (25 mmol) and benzene (25 mL) were refluxed overnight using a Dean–Stark apparatus for the removal of water. The solvent was then reduced to half its original volume under reduced pressure. Anhydrous ether was added after which precipitation occurred.

General Procedure for the Synthesis of **1a**–**8a**[43,44] The prepared **c**$_7$ (22 mmol) dissolved in CH_2Cl_2 (8 mL) was added to **a**$_2$ (20 mmol) mixed with CH_2Cl_2 60 mL dropwise and triethylamine (15 mL) was added to the suspension, which was placed in a reflux bath 60°C for 18–24 h to generate **a**$_1$.

Triethylamine (5 mL) was added to **a**$_1$ (10.6 mmol) dropwise dissolved in CH_2Cl_2 (50 mL) by stirring in ice-water bath for 10 min. Acetyl chloride (21.2 mmol) dissolved in CH_2Cl_2 (5 mL) was added to the suspension dropwise. The reaction was gradually restored to the room temperature after removing the ice-water bath. After completion of the reaction, water (20 mL) was added to quench reactions and the organic layer was washed with water (2×30 mL), dried with anhydrous sodium sulfate overnight. Removing the CH_2Cl_2 under reduced pressure, we can get **1a**–**8a**.

General Procedure for the Synthesis of **1b**–**7b**[43,44] A: The Esterification of GABA To an ice-cold solution of GABA (1 equiv) and alcohol (1 equiv) in tetrahydrofuran (THF) (60 mL), $SOCl_2$ (1.5 equiv) was added dropwise, and then the mixture was stirred at room temperature for 12h. The resulting slurry was evaporated and EtOAc was added. The mixture was stirred at room temperature and triethylamine (TEA) was added dropwise, filtered and evaporated to give a residual yellowish oil. The crude product was chromatographed (*n*-hexane : EtOAc=5 : 1) to give intermediates **b**$_1$.

B: Procedure B Using Dicyclohexylcarbodiimide (DCC) An ice-cold solution of $\mathbf{b}_1$ (1 equiv), ASA (1.1 equiv) and 4-dimethyl aminopyridine (DMAP) (0.3 equiv) in CH_2Cl_2 (60 mL) was added with DCC (1.1 equiv) portionwise and the mixture was stirred at room temperature for 16 h. The resulting slurry (precipitated dicyclohexyl urea, DCU) was ice-cooled for 2 h and filtered. The collected solid was washed with ice-cold CH_2Cl_2, the filtrate was evaporated, and the residue was redissolved in EtOAc (70 mL), cooled, and filtered again (to precipitate any remaining DCU). The EtOAc solution was then washed with 5% citric acid solution, $NaHCO_3$ solution, and finally brine. The solution was then concentrated under vacuum at 40°C to yield the final product as viscous orange oil.

General Procedure for the Synthesis of **1c**–**9c** During the ammonolysis of chloride, carboxyl group in GABA tended to cause cyclization and formation of the mixed anhydride. Generally a procedure easy to be stripped off were needed for its protection. Referred to the literature,[44–49] the following synthetic route was designed.

A: Synthesis of $\mathbf{c}_3$[50–51] To an ice-cold solution of GABA (1 equiv) in methanol (50 mL), $SOCl_2$ (1.2 equiv) was added dropwise and then the mixture was heated to reflux for 1h to yield the white solid in vacuum at 40°C. The product was recrystallized in ethanol–ether (1 : 2) solution. Yield 94%, mp 118.3–120°C.

B: Synthesis of $\mathbf{c}_2$[52,53] A mixture of $\mathbf{c}_3$ (23.00 mmol) and TEA (5 mL) in CH_2Cl_2 was added to $\mathbf{c}_7$, dissolved in CH_2Cl_2 by dropwise and then the mixture was heated to reflux for 2h and partitioned between CH_2Cl_2 and water. The aqueous phase was extracted with CH_2Cl_2. The combined organic layer was washed with $NaHCO_3$ and water, dried, filtered, and evaporated to give yellowish oil (6.10g). Yield 85.3%.

C: Synthesis of **1c**–**9c**[54–56] A mixture of $\mathbf{c}_2$ (6.1 g) and amine (10 mL) in methanol (50 mL) was stirred at room temperature for 36 h, and then the mixture was vacuumed at 40°C and partitioned between CH_2Cl_2 and water. The organic layer was washed with $NaHCO_3$ and water, dried ($MgSO_4$), filtered, and evaporated to give yellowish oil with the yield of 80% ($\mathbf{c}_1$). Then $\mathbf{c}_1$ and TEA was dissolved in CH_2Cl_2 which was cooled to 0°C and chloride (3.0 mL), dissolved in CH_2Cl_2, was added by dropwise. The reaction was monitored by TLC (*n*-hexane : EtOAc=1 : 2). The water was added, partitioned between CH_2Cl_2 and water. The organic layer was washed with water, dried ($MgSO_4$), filtered, and evaporated to give yellowish oil.

4-(2-Acetoxybenzoylamino)butyric Acid Ethyl Ester (**2a**) 1.693 g, Yield, 54.5%; IR (KBr) ν (cm^{-1}): 3326, 1735, 1645; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.22 (t, 3H, J=6.9 Hz), 1.92 (m, 2H, J=6.6 Hz, J=6.9 Hz), 2.13 (s, 3H), 2.25 (s, 3H), 2.42 (t, 2H, J=6.9 Hz), 3.47 (m, 2H, J=6.6 Hz), 4.09 (q, 2H, J=6.9 Hz), 6.77–7.53 (m, 4H, J=1.8 Hz, J=6.9 Hz, J=8.4 Hz), 7.15 (br s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 13.8 (–CH_2–$\underline{C}H_3$), 21.6 (–$\underline{C}H_3$), 23.9 (–CH_2–$\underline{C}H_2$–CH_2–), 31.6 (–CH_2–CH_2–$\underline{C}H_2$–), 39.0 (–$\underline{C}H_2$–CH_2–CH_2–), 60.4 (–$\underline{C}H_2$–CH_3), 114.2, 117.9, 118.4, 119.5, 125.7, 133.7 (Ar), 160.9 (–NH–$\underline{C}$=O), 169.9 (CH_3–$\underline{C}$=O), 173.6 (–CH_2–$\underline{C}$(=O)–O); EI-MS (*m*/*z*): 293[M]$^+$, 294 [M+1]$^+$, 121[M$^+$−172].

4-(2-Acetoxybenzoylamino)butyric Acid Iospropyl Ester (**3a**) 1.562 g, Yield 47.9%; IR (KBr) ν (cm^{-1}): 3376, 1703, 1643; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.23 (d, 6H, J=6.4 Hz), 1.96 (t, 2H, J=6.4 Hz), 2.17 (s, 3H), 2.44 (m, 2H, J=6.4 Hz, J=6.8 Hz), 3.50 (t, 2H, J=6.8 Hz), 5.01 (m, 1H, J=6.4 Hz), 6.84–7.44 (m, 4H, J=1.2 Hz, J=2.0 Hz, J=5.2 Hz, J=7.2 Hz), 7.35 (br s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 21.7 (–$\underline{C}H_3$), 23.8 (–CH–($\underline{C}H_3$)$_2$, 28.4 (–CH_2–$\underline{C}H_2$–CH_2–), 32.5 (–CH_2–CH_2–$\underline{C}H_2$–C=O), 39.6 (–NH–$\underline{C}H_2$–CH_2–CH_2–), 68.3 (–$\underline{C}$H–(CH_3)$_2$), 114.2, 118.4, 118.6, 125.5, 132.3, 134.0 (Ar), 161.5 (–NH–$\underline{C}$=O), 170.1 (CH_3–$\underline{C}$=O), 173.7 (–CH_2–C(=O)); EI-MS (*m*/*z*): 307[M]$^+$, 308[M+1]$^+$, 121 [M$^+$−172].

4-(2-Acetoxybenzoylamino)butanoic Acid *n*-Butyl Ester (**4a**) 1.627 g, Yield 47.8%; IR (KBr) ν (cm^{-1}): 3386, 1713, 1659; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.90 (t, 3H, J=7.2 Hz), 1.33 (m, 2H, J=5.2 Hz, J=7.2 Hz), 1.58 (m, 2H, J=5.2 Hz, J=6.4 Hz), 1.95 (q, 2H, J=6.4 Hz, J=6.8 Hz), 2.11 (s, 3H), 2.45 (t, 2H, J=6.8 Hz), 3.49 (q, 2H, J=6.4 Hz), 4.06 (t, 1H, J=6.4 Hz), 6.80–7.48 (m, 4H, J=0.8 Hz, J=1.6 Hz, J=6.8 Hz, J=8.4 Hz), 7.40 (br s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 13.0 (–(CH_2)$_3$–$\underline{C}H_3$), 20.4 (–CH_2–CH_2–$\underline{C}H_2$–CH_3), 22.7 (O=C–$\underline{C}H_3$), 23.8 (–CH_2–$\underline{C}H_2$–CH_2–C=O), 31.4 (–CH_2–$\underline{C}H_2$–CH_2–CH_3), 33.9 (–CH_2–CH_2–$\underline{C}H_2$–C=O), 40.8 (–NH–$\underline{C}H_2$–CH_2–CH_2–), 63.6 (–O–$\underline{C}H_2$–CH_2–CH_2–CH_3), 114.1, 118.5, 118.8, 126.8, 133.6, 137.3 (Ar), 167.9 (–NH–$\underline{C}$=O), 170.3 (CH_3–$\underline{C}$=O), 173.6 (–CH_2–C(=O)–O); EI-MS (*m*/*z*): 321 [M]$^+$, 294 [M+1]$^+$, 121 [M$^+$−172].

4-(2-Acetoxybenzoylamino)butyric Acid Cyclohexyl Ester (**5a**) 1.635 g, Yield 44.4%; IR (KBr) ν (cm^{-1}): 3378, 1709, 1641; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.26–1.52 (m), 1.70 (t), 1.80 (m, 2H), 2.23 (s, 3H), 2.42 (t, 2H), 3.28 (t, 2H), 4.73 (t), 6.84–7.42 (m, 4H, J=7.2 Hz), 7.27 (br s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 23.7 (–O=C–$\underline{C}H_3$), 23.8 (C3, C5 of cyclohexanyl), 25.2 (C4 of cyclohexanyl), 29.7 (–CH_2–$\underline{C}H_2$–CH_2–), 31.5 (C2, C6 of cyclohexanyl), 32.6 (O=C–$\underline{C}H_2$–CH_2–CH_2–), 39.6 (–NH–$\underline{C}H_2$–CH_2–CH_2–), 73.3 (C1 of cyclohexanyl), 114.2, 118.5, 118.6, 125.5, 133.3, 134.0 (Ar), 161.6 (–NH–$\underline{C}$=O), 170.1 (CH_3–$\underline{C}$=O), 173.7 (–$\underline{C}$(=O)-O); EI-MS (*m*/*z*): 347 [M]$^+$, 348 [M+1]$^+$, 121 [M$^+$−172].

4-(2-Acetoxybenzoylamino)butyric Acid Benzyl Ester (**6a**) 1.524 g, Yield 40.4%; IR (KBr) ν (cm^{-1}): 3316, 1741, 1635; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.97 (m, 2H, J=6.4 Hz, J=6.8 Hz), 2.17 (s, 3H), 2.46 (t, 2H, J=6.8 Hz), 3.50 (q, 2H, J=6.4 Hz), 5.34 (s, 1H), 6.82–6.97 (m, 4H, J=7.2 Hz, J=8.0 Hz), 7.18–7.44 (m, 4H, J=7.6 Hz, J=8.0 Hz), 7.18 (br s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 20.1 (–$\underline{C}H_3$), 23.9 (–CH_2–$\underline{C}H_2$–CH_2–), 32.2 (–CH_2–CH_2– $\underline{C}H_2$–NH–), 38.9 (O=C–$\underline{C}H_2$–CH_2– CH_2–), 68.8 (–$\underline{C}H_2$–ph), 128.9 (C2, C6 of ph), 129.8 (C3, C5 of ph), 118.6, 120.4, 120.6, 122.4, 125.7, 127.7, 133.9, 135.4 (Ar and C4 of ph), 161.6 (–NH–$\underline{C}$=O), 169.9 (CH_3–$\underline{C}$=O), 173.0 (–CH_2–$\underline{C}$(=O)–O); EI-MS (*m*/*z*): 355 [M]$^+$, 356 [M+1]$^+$, 121 [M$^+$−172].

4-(2-Acetoxybenzoylamino)butyric Acid *p*-Methoxy Benzyl Ester (**7a**) 1.952 g, Yield 47.8%; IR (KBr) ν (cm^{-1}): 3314, 1734, 1642; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.97 (m, 2H, J=6.0 Hz, J=7.2 Hz), 2.18 (s, 3H), 2.51 (t, 2H, J=7.2 Hz), 3.49 (q, 2H, J=6.0 Hz), 3.81, 5.12 (s, 2H), 6.81–7.04 (m, 4H, J=7.6 Hz, J=8.8 Hz), 7.26–7.40 (m, 4H, J=3.2 Hz, J=4.8 Hz, J=8.8 Hz), 7.30 (br s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 22.3 (O=C–$\underline{C}H_3$), 23.9 (–CH_2–$\underline{C}H_2$–CH_2–), 31.7 (–CH_2–CH_2–$\underline{C}H_2$–C=O), 39.1 (–NH–$\underline{C}H_2$–CH_2–CH_2–), 54.9 (–O$\underline{C}H_3$), 60.5 (–$\underline{C}H_2$–Ar), 113.5, 113.6, 113.7, 114.2, 114.2, 117.9, 118.4, 125.8, 129.4, 133.7 (2Ar), 161.0 (–NH–$\underline{C}$=O), 169.9 (CH_3–$\underline{C}$=O), 173.7 (–CH_2–$\underline{C}$(=O)); EI-MS (*m*/*z*): 385 [M]$^+$, 386 [M+1]$^+$, 121 [M$^+$−172].

4-(2-Acetoxybenzoylamino)butyric Acid *p*-Chloro Benzyl Ester (**8a**) 2.121 g, Yield 51.3%; IR (KBr) ν (cm^{-1}): 3378, 1702, 1644; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.01 (m, 2H, J=6.0 Hz, J=6.4 Hz), 2.18 (s, 3H), 2.50 (q, 2H, J=6.4 Hz), 3.50 (q, 2H, J=6.0 Hz), 5.07 (s, 2H), 6.81–7.27 (m, 4H, J=8.0 Hz, J=8.8 Hz), 7.30–7.40 (m, 4H, J=1.2 Hz, J=2.4 Hz, J=7.2 Hz, J=7.6 Hz), 7.30 (brs, 1H); ^{13}C-NMR(100 MHz, $CDCl_3$) δ: 23.7 (–$\underline{C}H_3$), 29.6 (–CH_2–$\underline{C}H_2$–CH_2–), 32.1 (–CH_2–CH_2–$\underline{C}H_2$–C=O), 39.3 (–NH–$\underline{C}H_2$–CH_2–CH_2–), 65.8 (–$\underline{C}H_2$–Ar), 118.5, 119.6, 125.4, 126.1, 128.1, 128.5, 129.8, 134.1, 135.4, 138.4 (2Ar), 163.5 (–NH–$\underline{C}$=O), 171.6 (CH_3–$\underline{C}$=O), 173.1 (–CH_2–$\underline{C}$(=O)–O); EI-MS (*m/z*): 390 $[M]^+$, 391 $[M+1]^+$, 121 $[M^+-172]$.

4–(2-Acetoxybenzoylamino)butyric Acid Methyl Ester (**1b**) 6.2 g, Yield, 87%; IR (KBr) ν (cm^{-1}): 3328, 1748, 1653; ^{1}H-NMR (400 MHz,$CDCl_3$) δ: 1.87 (m, 2H, J=6.6 Hz, J=7.2 Hz), 2.33 (s, 3H), 2.41 (t, 2H, J=7.2 Hz), 3.44 (m, 2H, J=6.6 Hz, J=7.2 Hz), 3.68 (s), 7.08 (d, 1H, $J_{4'-5'}$=7.1 Hz), 7.25 (m, 1H, $J_{3'-4'}$=7.3 Hz, $J_{2'-3'}$=6.4 Hz), 7.45 (m, 1H, $J_{2'-4'}$=1.3 Hz, $J_{3'-4'}$=7.3 Hz, $J_{4'-5'}$=7.1 Hz), 7.66 (dd, 1H, $J_{2'-4'}$=1.3 Hz, $J_{2'-3'}$=6.4 Hz), 7.89 (brs, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 20.7 ($\underline{C}H_3$–C=O), 24.5 (–CH_2–$\underline{C}H_2$–CH_2–), 31.3 (–CH_2–CH_2–$\underline{C}H_2$–), 38.7 (–$\underline{C}H_2$–CH_2–CH_2–), 51.8 (–C(=O)–O–$\underline{C}H_3$), 122.8, 125.9, 128.3, 129.2, 131.6, 147.7 (6C, Ar), 165.7 (–NH–$\underline{C}$=O), 169.5 (CH_3–$\underline{C}$=O), 173.7 (–CH_2–$\underline{C}$(=O)–O); RMS (ESI): $(M+H^+)$ 280.1189 (calculated 279.1107), error=2.3 ppm.

4-(2-Acetoxybenzoylamino)butyric Acid *n*-Propyl Ester (**2b**) 5.8 g, Yield 84%; IR (KBr) ν (cm^{-1}): 3338, 1757, 1648; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.86 (m, 2H, J=6.5 Hz, J=7.1 Hz), 2.38 (t, 2H, J=7.2 Hz), 3.44 (t, 2H, J=6.6 Hz, J=7.2 Hz,), 0.89 (t, J=6.2 Hz), 1.34 (q, J=6.2 Hz, J=6.8 Hz), 3.87 (t, J=6.8 Hz), 2.31 (s, 3H), 7.12 (d, 1H, $J_{4'-5'}$=7.5 Hz), 7.24 (m, 1H, $J_{3'-4'}$=7.8 Hz, $J_{2'-3'}$=6.8 Hz), 7.43 (m, 1H, $J_{2'-4'}$=1.2 Hz, $J_{3'-4'}$=7.8 Hz, $J_{4'-5'}$=7.5 Hz), 7.66 (dd, 1H, $J_{2'-4'}$=1.2 Hz, $J_{2'-3'}$=6.8 Hz), 7.86 (brs, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 21.2 ($\underline{C}H_3$–C=O), 24.8 (–CH_2–$\underline{C}H_2$–CH_2–C=O), 31.5 (–CH_2–CH_2–$\underline{C}H_2$–C=O), 37.9 (–$\underline{C}H_2$–CH_2–CH_2–C=O), 57.8 (–O–$\underline{C}H_2$–CH_2CH_3), 23.6 (–O–CH_2–$\underline{C}H_2$–CH_3), 11.5 (–O–CH_2–CH_2–$\underline{C}H_3$), 122.6, 125.7, 128.3, 129.1, 131.6, 147.9 (6C,Ar), 165.8 (–NH–$\underline{C}$=O), 168.9 (CH_3–$\underline{C}$=O), 173.7 (–CH_2–$\underline{C}$(=O)–O); RMS(ESI): $(M+NH_4^+)$ 325.1417 (calculated 307.1420), error=2.1 ppm.

4-(2-Acetoxybenzoylamino)butyric Acid 2,6-Dimethyl Phenyl Ester (**3b**) 4.6 g, Yield 56%; IR (KBr) ν (cm^{-1}): 3418, 1727, 1687; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.85 (m, 2H, J=6.1 Hz, J=7.6 Hz), 2.33 (t, 2H, J=7.6 Hz), 3.34 (t, 2H, J=6.1 Hz), 2.35 (s, 6H), 2.09 (s, 3H), 6.98–7.08 (m, 3H, J=5.4 Hz, J=7.2 Hz), 7.24–7.52 (m, 4H, J=2.2 Hz, J=5.6 Hz, J=6.8 Hz), 8.01 (s); ^{13}C-NMR (100 MHz,$CDCl_3$) δ: 21.6 ($\underline{C}H_3$–C=O), 23.8 (–CH_2–$\underline{C}H_2$–CH_2–C=O), 32.3 (–CH_2–CH_2–$\underline{C}H_2$–C=O), 39.1 (–$\underline{C}H_2$–CH_2–CH_2–C=O), 24.5 (2C, Ar–$\underline{C}H_3$), 119.3, 122.3, 123.6, 126.4, 127.1, 127.8, 128.5, 129.2, 131.5, 132.5, 145.8 (12C, 2Ar), 161.5 (–NH–$\underline{C}$=O), 168.7 (CH_3–$\underline{C}$=O), 171.8 (–CH_2–$\underline{C}$=O); RMS(ESI): $(M+NH_4^+)$ 387.1924 (calculated 369.1576), error=2.6 ppm.

4-(2-Acetoxybenzoylamino)butyric Acid 1-Phenyl Ethyl Ester (**4b**) 4.6 g, Yield 56%; IR (KBr) ν (cm^{-1}): 3363, 1682, 1716; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.88 (m, 2H, J=6.4 Hz, J=7.2 Hz), 2.33 (t, 2H, J=7.2 Hz), 3.34 (t, 2H, J=6.4 Hz), 2.13 (s, 3H), 1.67 (d, 3H, J=6.4 Hz), 4.84 (q, 1H, J=6.4 Hz), 6.78–7.22 (m, 5H, J=1.2 Hz, J=5.6 Hz, J=7.6 Hz) 7.24–7.51 (m, 4H, J=1.5 Hz, J=6.4 Hz, J=7.2 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 19.2 (-CH–$\underline{C}H_3$), 73.8 (–$\underline{C}$H–CH_3), 21.8 ($\underline{C}H_3$–C=O), 23.6 (–CH_2–$\underline{C}H_2$–CH_2–C=O), 32.2 (–CH_2–CH_2–$\underline{C}H_2$–C=O), 38.9 (–$\underline{C}H_2$–CH_2–CH_2–C=O), 24.3 (2C,Ar–$\underline{C}H_3$), 121.2, 122.0, 123.1, 126.1, 128.3, 128.5, 129.2, 131.5, 131.9, 147.9 (12C, 2Ar), 164.0 (–NH–$\underline{C}$=O), 169.4 (CH_3–$\underline{C}$=O), 171.3 (–CH_2–$\underline{C}$=O); RMS(ESI): $(M+NH_4^+)$ 387.2244 (calculated 369.1576).

4-(2-Acetoxybenzoylamino)butyric Acid *o*-Tolyl Ester (**5b**) 4.9 g, Yield 60%; IR (KBr) ν (cm^{-1}): 3313, 1719, 1646; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.85 (m, 2H, J=6.3 Hz, J=7.2 Hz), 2.33 (t, 2H, J=7.2 Hz), 3.37 (t, 2H, J=6.3 Hz), 2.36 (s, 3H), 2.18 (s, 3H), 6.83–7.05 (m, 4H, J=7.2 Hz, J=7.8 Hz), 7.26–7.40 (m, 4H, J=2.5 Hz, J=6.1 Hz, J=7.6 Hz), 7.91 (brs, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 21.3 ($\underline{C}H_3$–C=O), 25.8 (–CH_2–$\underline{C}H_2$–CH_2–C=O), 30.5 (–CH_2–CH_2–$\underline{C}H_2$–C=O), 41.4 (–$\underline{C}H_2$–CH_2–CH_2–C=O), 15.3 (Ar–$\underline{C}H_3$), 120.9, 122.0, 122.6, 126.1, 128.3, 129.2, 131.8, 132.3, 147.8 (2Ar), 164.1 (–NH–$\underline{C}$=O), 168.6 (CH_3–$\underline{C}$=O), 172.1 (–CH_2–$\underline{C}$=O); RMS(ESI): $(M+NH_4^+)$ 373.1913 (calculated 355.1420), error=2.1 ppm.

4-(2-Acetoxybenzoylamino)butyric Acid *n*-Amyl Ester (**6b**) 4.7 g, Yield 62%; IR (KBr) ν (cm^{-1}): 3411, 1736, 1640; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.82 (m, 2H, J=6.6 Hz, J=7.5 Hz), 2.38 (t, 2H, J=7.5 Hz), 3.44 (t, 2H, J=6.6 Hz), 0.87 (t, 3H, J=6.2 Hz), 1.21 (m, 2H, J=6.2 Hz, J=6.4 Hz), 1.37 (m, 2H, J=6.4 Hz, J=6.7 Hz), 1.24 (m, 3H, J=6.5 Hz, J=6.7 Hz), 3.95 (t, 3H, J=6.5 Hz), 2.28 (s, 3H), 7.12 (d, 1H, $J_{4'-5'}$=7.2 Hz), 7.21 (m, 1H, $J_{3'-4'}$=7.4 Hz, $J_{2'-3'}$=6.6 Hz), 7.38 (m, 1H, $J_{2'-4'}$=1.3 Hz, $J_{3'-4'}$=7.4 Hz, $J_{4'-5'}$=7.2 Hz), 7.51 (dd, 1H, $J_{2'-4'}$=1.3 Hz, $J_{2'-3'}$=6.6 Hz), 7.91 (brs, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 21.7 ($\underline{C}H_3$–C=O), 23.9 (–CH_2–$\underline{C}H_2$–CH_2–C=O), 32.3 (–CH_2–CH_2–$\underline{C}H_2$–C=O), 38.7 (–$\underline{C}H_2$–CH_2–CH_2–C=O), 12.4 (–O–CH_2–CH_2– CH_2–$CH_2\underline{C}H_3$), 22.3 (–O–CH_2–CH_2–CH_2–$\underline{C}H_2CH_3$), 25.9 (–O–CH_2–CH_2–$\underline{C}H_2$–CH_2CH_3), 29.2 (–O–CH_2–$\underline{C}H_2$–CH_2–CH_2–CH_3), 64.1 (–O–$\underline{C}H_2$–CH_2– CH_2–CH_2CH_3), 122.6, 126.3, 128.3, 129.2, 131.2, 147.7 (6C, Ar), 165.6 (–NH–$\underline{C}$=O), 169.2 (CH_3–$\underline{C}$=O), 172.3 (–CH_2–$\underline{C}$(=O)–O); RMS(ESI): $(M+NH_4^+)$ 353.1917 (calculated 335.1733), error=1.9 ppm.

4-(2-Acetoxybenzoylamino)butyric Acid *iso*-Amyl Ester (**7b**) 4.9 g, Yield 63%; IR (KBr) ν (cm^{-1}): 3342, 1737, 1671; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.85 (m, 2H, J=6.3 Hz, J=7.2 Hz), 2.33 (t, 2H, J=7.2 Hz), 3.37 (t, 2H, J=6.3 Hz), δ: 0.89 (d, 6H, J=6.2 Hz), 1.73 (m, 1H, J=6.2 Hz, J=6.8 Hz), 1.46 (m, 2H, J=6.8 Hz, J=6.3 Hz), 3.93 (t, 2H, J=6.3 Hz), 2.09 (s, 3H), 7.05 (d, 1H, $J_{4'-5'}$=6.8 Hz), 7.19 (m, 1H, $J_{3'-4'}$=7.2 Hz, $J_{2'-3'}$=6.5 Hz), 7.35 (m, 1H, $J_{2'-4'}$=1.5 Hz, $J_{3'-4'}$=7.2 Hz, $J_{4'-5'}$=7.2 Hz), 7.63 (dd, 1H, $J_{2'-4'}$=1.5 Hz, $J_{2'-3'}$=6.5 Hz), 7.93 (brs, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 22.3 ($\underline{C}H_3$–C=O), 24.6 (–CH_2–$\underline{C}H_2$–CH_2–C=O), 32.1 (–CH_2–CH_2–$\underline{C}H_2$–C=O), 38.6 (–$\underline{C}H_2$–CH_2–CH_2–C=O), 21.7 (2C,–CH($\underline{C}H_3$)$_2$), 23.7 (–$\underline{C}$H(CH_3)$_2$), 33.7 (–O–CH_2–$\underline{C}H_2$–), 61.9 (–O–$\underline{C}H_2$–CH_2–), 122.2, 124.8, 127.7, 127.9, 132.5, 149.3 (6C, Ar), 164.3 (–NH–$\underline{C}$=O), 168.3 (CH_3–$\underline{C}$=O), 175.5 (–CH_2–$\underline{C}$(=O)–O); RMS(ESI): $(M+NH_4^+)$ 353.1712 (calculated 335.1733), error=2.1 ppm.

2-(3-(Methylcarbamoyl)propylcarbamoyl)phenyl Acetate (**1c**) 4.74 g, Yield 63.5%. IR (KBr) ν (cm^{-1}): 3281, 1721, 1648; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.87 (m, 2H, J=6.8 Hz, J=7.4 Hz), 2.30 (s, 3H), 2.39 (t, 2H, J=7.4 Hz), 2.76 (d, 3H, J=2.8 Hz), 3.40 (m, 2H, J=6.8 Hz), 7.07 (d, 1H, $J_{4'-5'}$=6.6 Hz),

7.25 (m, 1H, $J_{2'-3'}$=6.4 Hz, $J_{3'-4'}$=6.2 Hz), 7.43 (m, 1H, $J_{2'-4'}$=1.8 Hz, $J_{3'-4'}$=6.4 Hz, $J_{4'-5'}$=6.6 Hz), 7.65 (dd, 1H, $J_{2'-4'}$=1.8 Hz, $J_{2'-3'}$=6.2 Hz), 7.96–7.98 (brs, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 20.9 (1C, $\underline{C}H_3$–C=O), 24.5 (1C, –CH_2–$\underline{C}H_2$–CH_2–), 26.2 (1C, –$\underline{C}H_3$), 31.3 (1C, –CH_2–CH_2–$\underline{C}H_2$–), 39.5 (1C, –$\underline{C}H_2$–CH_2–CH_2–), 122.9, 126.2, 128.4, 129.2, 131.5, 147.9 (6C, Ar), 165.9 (1C, –NH–$\underline{C}$=O), 169.2 (1C, CH_3–$\underline{C}$=O), 173.6 (1C, –CH_2–$\underline{C}$(=O)–O); HR-MS(ESI): ($M+H^+$) 279.1391 (calculated 279.138), error=2.1 ppm.

2-(3-(Ethylcarbamoyl)propylcarbamoyl)phenyl Acetate (**2c**) 4.86 g, Yield 67.2%; IR (KBr) ν (cm^{-1}): 3357, 1768, 1734, 1659; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.25 (t, 3H, J=7.2 Hz), 1.87 (m, 2H, J=6.8 Hz, J=7.4 Hz), 2.30 (s, 3H), 2.39 (t, 2H, J=7.4 Hz), 3.39 (m, 2H, J=6.8 Hz), 3.53 (m, 2H, J=7.2 Hz), 7.17 (d, 1H, $J_{4'-5'}$=6.6 Hz), 7.25 (m, 1H, $J_{2'-3'}$=6.2 Hz, $J_{3'-4'}$=6.4 Hz), 7.43 (m, 1H, $J_{2'-4'}$=2.0 Hz, $J_{3'-4'}$=6.4 Hz, $J_{4'-5'}$=6.6 Hz), 7.65 (dd, 1H, $J_{2'-4'}$=2.0 Hz, $J_{2'-3'}$=6.2 Hz), 7.86–7.98 (brs, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 15.5 (1C, –CH_2–$\underline{C}H_3$), 20.7 (1C, $\underline{C}H_3$–C=O) 24.2 (1C, –CH_2–$\underline{C}H_2$–CH_2–), 31.2 (1C, –CH_2–CH_2–$\underline{C}H_2$–), 34.2 (1C, –CH_2–$C\underline{H}_3$), 39.5 (1C, –$\underline{C}H_2$–CH_2–CH_2–), 123.6, 126.6, 128.6, 129.5, 131.9, 145.8 (6C, Ar), 167.8 (1C, –NH–$\underline{C}$=O), 169.1 (1C, CH_3–$\underline{C}$=O, 172.3 (1C,–CH_2–$\underline{C}$(=O)–O); HR-MS(ESI): ($M+H^+$) 293.1523 (calculated 293.1517), error=2.0 ppm.

2-(3-(Isopropylcarbamoyl)propylcarbamoyl)phenyl Acetate (**3c**) 5.13 g, Yield 61.4%; IR (KBr) ν (cm^{-1}): 3311, 1767, 1734, 1646; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.28 (d, 6H, J=6.8 Hz), 1.89 (m, 2H, J=6.8 Hz, J=7.4 Hz), 2.33 (s, 3H), 2.43 (t, 2H, J=7.4 Hz), 3.33 (m, 2H, J=6.8 Hz), 3.88 (m, 1H, J=6.8 Hz), 7.01 (d, 1H, $J_{4'-5'}$=6.8 Hz), 7.25 (m, 1H, $J_{2'-3'}$=5.2 Hz, $J_{3'-4'}$=6.4 Hz), 7.45 (m, 1H, $J_{2'-4'}$=1.2 Hz, $J_{3'-4'}$=6.4 Hz, $J_{4'-5'}$=6.8 Hz), 7.64 (dd, 1H, $J_{2'-4'}$=1.2 Hz, $J_{2'-3'}$=5.2 Hz), 7.87–7.98 (brs, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 20.2 (1C, $\underline{C}H_3$–C=O), 23.7 (2C, –CH–($\underline{C}H_3$)$_2$), 26.3 (1C, –CH_2–$\underline{C}H_2$–CH_2–), 32.5 (1C, –CH_2–CH_2–$\underline{C}H_2$–), 39.8 (1C, –$\underline{C}H_2$–CH_2–CH_2–), 42.1 (1C, –$\underline{C}$H–(CH_3)$_2$), 121.1, 124.2, 126.0, 127.2, 129.5, 146.1 (6C, Ar), 164.2 (1C, –NH–$\underline{C}$=O), 168.2 (1C, CH_3–$\underline{C}$=O), 172.1 (1C, –CH_2–$\underline{C}$(=O)–O); HR-MS(ESI): $M+H^+$) 307.1655 (calculated 307.1652), error=1.0 ppm.

2-(3-(Propylcarbamoyl)propylcarbamoyl)phenyl Acetate (**4c**) 5.02 g, Yield 59.3%; IR (KBr) ν (cm^{-1}): 3348, 1773, 1734, 1647; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.96 (t, 3H, J=7.2 Hz), 1.61 (m, 2H, J=7.2 Hz), 1.91 (m, 2H, J=6.8 Hz, J=7.2 Hz), 2.23 (s, 3H), 2.40 (t, 2H, J=6.8 Hz), 3.40 (m, 2H, J=7.2 Hz), 3.45 (m, 2H, J=7.2 Hz), 6.98 (d, 1H, $J_{4'-5'}$=6.8 Hz), 7.25 (m, 1H, $J_{2'-3'}$=6.4 Hz, $J_{3'-4'}$=6.8 Hz), 7.43 (m, 1H, $J_{2'-4'}$=2.0 Hz, $J_{3'-4'}$=6.8 Hz, $J_{4'-5'}$=6.8 Hz), 7.69 (dd, 1H, $J_{2'-4'}$=2.0 Hz, $J_{2'-3'}$=6.4 Hz), 7.96–8.02 (brs, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 11.2 (1C, –CH_2–CH_2–$C\underline{H}_3$), 20.6 (1C, $\underline{C}H_3$–C=O), 23.9 (1C, –CH_2–$C\underline{H}_2$–CH_2–), 25.9 (1C, –CH_2–$\underline{C}H_2$–CH_2–), 31.6 (1C, –CH_2–CH_2–$\underline{C}H_2$–), 39.8 (1C, –$\underline{C}H_2$–CH_2–CH_2–), 41.5 (1C, –$C\underline{H}_2$–CH_2–CH_3), 122.9, 126.3, 128.2 129.9, 131.5147.5 (6C, Ar), 165.6 (1C, –NH–$\underline{C}$=O), 169.1 (1C, CH_3–$\underline{C}$=O), 173.1 (1C, –CH_2–$\underline{C}$(=O)–O); HR-MS(ESI): $M+H^+$) 307.16 (calculated 307.1652), error=1.6 ppm.

2-(3-(Butylcarbamoyl)propylcarbamoyl)phenyl Acetate (**5c**) 5.28 g, Yield 61.2%; IR (KBr) ν (cm^{-1}): 3281, 1765, 1648; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.92 (t, 3H, J=6.0 Hz), 1.32 (m, 2H, J=6.0 Hz, J=6.2 Hz), 1.64 (m, 2H, J=5.8 Hz, J=6.2 Hz), 1.93 (q, 2H, J=6.4 Hz, J=7.2 Hz), 2.28 (s, 3H), 2.41 (t, 2H, J=6.4 Hz), 3.39 (q, 2H, J=7.2 Hz), 3.41 (t, 1H, J=5.8 Hz), 7.08 (d, 1H, $J_{4'-5'}$=7.2 Hz), 7.26 (m, 1H, $J_{2'-3'}$=6.4 Hz, $J_{3'-4'}$=6.8 Hz), 7.44 (m, 1H, $J_{2'-4'}$=1.6 Hz, $J_{3'-4'}$=6.8 Hz, $J_{4'-5'}$=7.2 Hz), 7.67 (dd, 1H, $J_{2'-4'}$=1.6 Hz, $J_{2'-3'}$=6.4 Hz), 7.86–7.92 (brs, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 13.5 (1C, –(CH_2)$_3$–$\underline{C}H_3$), 18.7 (1C, –CH_2–CH_2–$\underline{C}H_2$–CH_3), 20.9 (1C, O=C–$\underline{C}H_3$), 26.5 (1C, –CH_2–$\underline{C}H_2$–CH_2–C=O), 31.1 (1C, –CH_2–$\underline{C}H_2$–CH_2–CH_3), 33.6 (1C, –CH_2–CH_2–$\underline{C}H_2$–C=O), 39.5 (1C, –NH–$\underline{C}H_2$–CH_2–CH_2–), 41.5 (1C, –$\underline{C}H_2$–CH_2–CH_2–CH_3), 122.5, 125.9, 127.6, 128.9, 131.3, 146.8 (6C, Ar), 166.1 (1C, –NH–$\underline{C}$=O), 168.9 (1C, CH_3–$\underline{C}$=O), 173.1 (1C, –CH_2–C(=O)–O); HR-MS(ESI): ($M+H^+$) 321.1802 (calculated 321.1809), error=2.2 ppm.

2-(3-(Diethylcarbamoyl)propylcarbamoyl)phenyl Acetate (**6c**) 4.95 g, Yield 58.3%. IR (KBr) ν (cm^{-1}): 3356, 1768, 1733, 1660; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.11 (m, 6H, J=7.6 Hz), 1.81 (m, 2H, J=6.8 Hz), 2.16 (s, 3H), 2.40 (t, 2H, J=6.8 Hz), 3.39 (q, 2H, J=6.8 Hz), 3.42 (m, 4H, J=7.6 Hz), 7.10 (d, 1H, $J_{4'-5'}$=6.4 Hz), 7.26 (m, 1H, $J_{2'-3'}$=6.0 Hz, $J_{3'-4'}$=6.4 Hz), 7.44 (m, 1H, $J_{2'-4'}$=1.2 Hz, $J_{3'-4'}$=6.4 Hz, $J_{4'-5'}$=6.4 Hz), 7.67 (dd, 1H, $J_{2'-4'}$=1.2 Hz, $J_{2'-3'}$=6.0 Hz), 7.92 (brs, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 13.8 (2C, –CH_2–$\underline{C}H_3$), 20.5 (1C,O=C–$\underline{C}H_3$), 26.9 (1C, –CH_2–$\underline{C}H_2$–CH_2–C=O), 31.3 (1C, –CH_2–CH_2–$\underline{C}H_2$–C=O), 39.2 (1C, –NH–$\underline{C}H_2$–CH_2–CH_2–), 42.0 (2C, –CH_2–$C\underline{H}_3$), 122.3, 125.4, 126.1, 127.8, 131.4, 151.1 (6C, Ar), 167.5 (1C, –NH–$\underline{C}$=O), 168.7 (1C, CH_3–C=O), 171.5 (1C, –CH_2–C(=O)–O); HR-MS(ESI): ($M+H^+$) 321.1819 (calculated 321.1809), error=3.1 ppm.

2-(3-(Dibutylcarbamoyl)propylcarbamoyl)phenyl Acetate (**7c**) 5.26 g, Yield 55.6%; IR (KBr) ν (cm^{-1}): 3319, 1767, 1738, 1640; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.95 (t, 6H, J=7.2 Hz, J=7.6 Hz), 1.32 (m, 4H, J=7.2 Hz, J=7.6 Hz), 1.52 (m, 4H, J=4.6 Hz, J=7.6 Hz), 1.99 (m, 2H, J=6.4 Hz, J=7.2 Hz), 2.14 (s, 3H), 2.47 (t, 2H, J=6.4 Hz), 3.39 (q, 2H, J=7.2 Hz), 3.42 (t, 4H, J=4.6 Hz), 6.82 (d, 1H, $J_{4'-5'}$=7.2 Hz), 6.87 (m, 1H, $J_{2'-3'}$=6.4 Hz, $J_{3'-4'}$=6.8 Hz), 7.47 (m, 1H, $J_{2'-4'}$=1.0 Hz, $J_{3'-4'}$=6.8 Hz, $J_{4'-5'}$=7.2 Hz), 7.47 (dd, 1H, $J_{2'-4'}$=1.0 Hz, $J_{2'-3'}$=6.4 Hz), 7.83 (brs, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 13.7, 13.8 (2C, –CH_2–CH_2–CH_2–$C\underline{H}_3$), 20.0, 20.1 (2C, –CH_2–CH_2–$C\underline{H}_2$–CH_3), 21.4 (1C, O=C–$\underline{C}H_3$), 23.9 (1C, –CH_2–$\underline{C}H_2$–CH_2–C=O), 30.4, 30.9 (2C, –CH_2–$C\underline{H}_2$–CH_2–CH_3), 31.7 (1C, –CH_2–CH_2–$C\underline{H}_2$–C=O), 39.3 (1C, –NH–$C\underline{H}_2$–CH_2–CH_2–), 48.0–48.1 (2C, –$C\underline{H}_2$–CH_2–CH_2–CH_3), 114.3, 118.5, 125.8, 127.2, 129.7, 133.9 (6C, Ar), 161.4 (1C, –NH–$\underline{C}$=O), 170.0 (1C, CH_3–$\underline{C}$=O), 174.4 (1C, –CH_2–C(=O)–O); HR-MS(ESI): ($M+H^+$) 377.2378 (calculated 377.2373), error=1.3 ppm.

2-(3-(Cyclohexylcarbamoyl)propylcarbamoyl)phenyl Acetate (**8c**) 4.86 g, Yield 53.7%; IR (KBr) ν (cm^{-1}): 3310, 1767, 1734, 1646; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.47 (m, 6H, J=6.8 Hz), 1.82 (m, 2H, J=6.4 Hz, J=7.2 Hz), 2.08 (s, 3H), 2.41 (t, 2H, J=6.4 Hz), 3.34 (q, 2H, J=7.2 Hz), 3.67 (t, 4H, J=6.8 Hz), 6.88 (d, 1H, $J_{4'-5'}$=6.8 Hz), 7.05 (m, 1H, $J_{2'-3'}$=6.4 Hz, $J_{3'-4'}$=6.8 Hz), 7.20 (m, 1H, $J_{2'-4'}$=1.6 Hz, $J_{3'-4'}$=6.8 Hz, $J_{4'-5'}$=6.8 Hz), 7.43 (dd, 1H, $J_{2'-4'}$=1.6 Hz, $J_{2'-3'}$=6.4 Hz), 7.78 (brs, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 20.1 (1CO=C–$\underline{C}H_3$), 25.2 (1C, –(CH_2)$_2$–$\underline{C}H_2$–(CH_2)$_2$–), 25.9 (1C, –CH_2–$\underline{C}H_2$–CH_2–C=O), 28.2–28.3 (2C, –CH_2–$\underline{C}H_2$–CH_2–$\underline{C}H_2$–CH_2–), 31.7 (1C, –CH_2–CH_2–$C\underline{H}_2$–C=O), 6.9 (1C, –NH–$C\underline{H}_2$–CH_2–CH_2–), 41.8–41.9 (2C, –$\underline{C}H_2$–(CH_2)$_3$–$\underline{C}H_2$–), 114.8, 119.3, 125.8, 127.3, 129.6, 133.9 (6C, Ar), 161.3 (1C, –NH–$\underline{C}$=O), 170.2 (1C, CH_3–$\underline{C}$=O), 173.9 (1C, –CH_2–C(=O)–O). HR-MS(ESI): ($M+H^+$) 333.1798 (calculated 333.1792), error=1.8 ppm.

2-(3-(Methylpyrazinecarbamoyl)propylcarbamoyl)phenyl

Acetate (**9c**) 4.57 g, Yield 51.6%; IR (KBr) ν (cm^{-1}): 3318, 1770, 1737, 1638; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.85 (m, 2H, J=6.8 Hz, J=7.2 Hz), 2.09 (s, 3H), 2.35 (t, 2H, J=7.2 Hz), 2.53 (s, 3H), 2.74 (t, 4H, J=6.4 Hz), 3.25 (q, 2H, J=6.8 Hz), 3.51 (t, 4H, J=6.4 Hz), 6.97 (d, 1H, $J_{4'-5}$=7.2 Hz), 7.05 (m, 1H, $J_{2'-3'}$=6.6 Hz, $J_{3'-4'}$=6.8 Hz), 7.25 (m, 1H, $J_{2'-4'}$=1.2 Hz, $J_{3'-4'}$=6.8 Hz, $J_{4'-5'}$=7.2 Hz), 7.57 (dd, 1H, $J_{2'-4'}$=1.2 Hz, $J_{2'-3'}$=6.6 Hz), 7.92 (br s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 20.1 (1C, O=C–$\underline{C}H_3$), 25.0 (1C, –CH_2–$\underline{C}H_2$–CH_2–C=O), 31.6 (1C, –CH_2–CH_2–$C\underline{H}_2$–C=O), 37.9 (1C, –NH–$C\underline{H}_2$–CH_2–CH_2–), 45.6 (1C, –N–$C\underline{H}_3$), 48.3 (2C, –O=C–N–$\underline{C}H_2$–CH_2–N–), 53.9 (2C, –O=C–N–CH_2–$\underline{C}H_2$–N–), 115.3, 118.7, 124.3, 127.3, 129.8 and 138.3 (6C, Ar), 162.5 (1C, –NH–$\underline{C}$=O), 167.5 (1C, CH_3–$\underline{C}$=O), 173.2 (1C, –CH_2–C(=O)–O); HR-MS(ESI): ($M+H^+$) 348.2073 (calculated 348.2068), error=1.4 ppm.

Antiepileptic Activity Assay Pentylenetetrazol (PTZ), kainic acid, bicuculline, picrotoxin, 4-AP, and pilocarpine have been tested for their capacity to induce behavioral seizures in freely swimming tadpoles when bath applied. All six chemoconvulsants consistently induced similar patterns of abnormal behavior in a dose-dependent manner, characterized by convulsive clonus-like motor patterns with periods of behavioral arrest.[57] Dose–response studies found 4-AP to be particularly well suitable for an experimental chemo convulsant for mice due to the rapid onset and long duration of action that reliably elicited severe seizure behavior without toxicity or lethality.

Epilepsy Model The 4-AP seizure test was carried out as previously described using a 7 mg/kg dose, which as confirmed in the present study produced tonic seizures in 95% of control mice. Mice were pretreated subcutaneously with either saline (control group) or compounds 30 min before receiving 4-AP. Mice that failed to exhibit tonic seizures (tonic extension of hind limbs) within 45 min after 4-AP administration were considered to be protected. The behavioral seizure that resulted from each stimulus train was graded by the following standardized scale: (behavioral seizure score: 0=no seizure, 1=seizure with mouth and facial movement, 2=seizure with head nodding, 3=seizure with forelimb clonus, 4=seizure with rearing up on hindlimbs, and 5=seizure with rearing and falling).

The Initial Anticonvulsant Evaluation of the Synthesized Compounds Four hundreds and eighty-six mice (bisexual each half) which were ignited by 4-AP were randomly assigned to 81 experimental groups (n=6). Each of compounds was provided by intraperitoneal injection with the dosage of 80 mg/kg, 160 mg/kg, 240 mg/kg, and 4-AP was provided by intraperitoneal injection after 20 min. Control group was provided with isometric the physiological saline, model group isometric the solution of 4-AP and positive control group isometric the solution of Sodium VPA.

The Test of Median Lethal Dose (LD_{50}) Six hundreds mice were randomly divided into 75 groups and were injected intraperitoneal injection with different doses of biologically active compounds as specified (300 mg/kg, 360 mg/kg, 432 mg/kg, 518 mg/kg, 622 mg/kg). The number of mice that died within 3 d after administration was counted. The formula of LD_{50} was as follows:

$$LD_{50}=\lg^{-1}\left[X_m-i\left(\sum P-0.5\right)\right]$$

X_m: logarithms of maximum doses, i: logarithms of the ratio of adjacent groups (lgr), P: mortality of the animals in each group

The Test of Median Effective Dose (ED_{50}) Six hundreds sixteen model mice which were ignited by 4-AP (7 mg/kg) were randomly divided into 77 groups and injected intraperitoneal injection with different doses of biologically active compounds as specified (100 mg/kg, 120 mg/kg, 144 mg/kg, 173 mg/kg, 207 mg/kg). The formula of ED_{50} was as follows:

$$ED_{50}=\lg^{-1}\left[X_m-i\left(\sum P-0.5\right)\right]$$

X_m: logarithms of maximum doses, i: logarithms of the ratio of adjacent groups (lgr), P: mortality of the animals in each group

Therapeutic Index (TI) TI for each compound which was tested in ED_{50} and LD_{50}, was calculated by dividing a given LD_{50} value evaluated in the LD_{50} test, by the respective ED_{50} value determined in the ED_{50} test. The TI is considered as an index of the safety and tolerability between anticonvulsant doses and toxicity.

1-Octanol/Water Partition Coefficients of the Active Compounds Log$P$ were determined with ChemDraw 12.0 software.

Assay of Compounds **8a**, **7c** and ASA Concentration in Brain Tissue The control mice were administered with ASA and a respective vehicle, and meanwhile the tested mice with **8a**/**7c**. Mice were killed by decapitation at times chosen to coincide with those scheduled for the seizure test and brains were removed from skulls, weighed and homogenized using original Abbott buffer (2 : 1, v/w) in an Ultra-Turrax homogenizer. The concentrations of ASA, **8a** and **7c** were determined by HPLC using an automated Gilson (Anachem) HPLC system. The mobile phase comprised of methanol (20 mmol), acetonitrile and citric buffer (pH=3) in a ratio of 25 : 30 : 40 (v/v/v). Chromatographic separation was achieved using a Hypersil BDS-2-C18 5 μm column (Agilent Technologies). Brain homogenate samples were prepared for analysis as follows. Brain homogenate (20 μL) was pipetted into a C18 column conditioned with methanol (2×1 mL) and water (2×1 mL) and eluted with methanol (2×0.2 mL) and water (0.4 mL). Subsequently samples were centrifuged for 5 min, and the supernatant (20 μL) was transferred into the HPLC column. The limit detection of the HPLC method was 0.1 μg/mL and the within-batch and between-batch coefficients of variation were below 6%. Total compounds concentrations were expressed in μg/mL of brain supernatants as mean±S.D. of at least eight separate brain preparations.

Acknowledgment This work was supported by the Fundamental Research Funds for the Central Universities (lzujbky-2010–137).

References

1) Pitkänen A., Sutula T. P., *Lancet Neurol.*, **1**, 173–181 (2002).
2) Rogawski M. A., Loscher W., *Nat. Rev. Neurosci.*, **5**, 553–564 (2004).
3) Perucca E., French J., Bialer M., *Lancet Neurol.*, **6**, 793–804 (2007).
4) Vezzani A., French J., Bartfai T., Baram T. Z., *Nat. Rev. Neurol.*, **7**, 31–40 (2011).
5) Aarli J. A., *Arch. Neurol.*, **57**, 1689–1692 (2000).
6) Ravizza T., Gagliardi B., Noe F., Boer K., Aronica E., Vezzani A.,

Neurobiol. Dis., **29**, 142–160 (2008).
7) Vezzani A., Granata T., *Epilepsia*, **46**, 1724–1743 (2005).
8) Dodd S., Maes M., Anderson G., Dean O. M., Moylan S., Berk M., *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **42**, 135–145 (2013).
9) Jung K. H., Chu K., Lee S. T., Kim J., Sinn D. I., Kim J. M., Park D. K., Lee J. J., Kim S. U., Kim M., Lee S. K., Roh J. K., *Neurobiol. Dis.*, **23**, 237–246 (2006).
10) Vane J. R., Botting R. M., *Thromb. Res.*, **110**, 255–258 (2003).
11) Moro M. A., De Alba J., Cárdenas A., De Cristóbal J., Leza J. C., Lizasoain I., Díaz-Guerra M. J., Boscá L., Lorenzo P., *Neuropharmacology*, **39**, 1309–1318 (2000).
12) Kuhn W., Müller T., Buttner T., Gerlach M., *Stroke*, **26**, 1959–1960 (1995).
13) Thomas T., Nadackal T. G., Thomas K., *Neuroreport*, **12**, 3263–3267 (2001).
14) Wang Y. P., Wu Y., Li L. Y., Zheng J., Liu R. G., Zhou J. P., Yuan S. Y., Shang Y., Yao S. L., *J. Neuroinflammation*, **8**, 95 (2011).
15) Xu T., Qu F., *J. Clin. Neurosci.*, **12**, 220–222 (2004).
16) Tang Z. Y., *Foreign Med. Sci.*, **29**, 100–102 (2002).
17) De Cristóbal J., Moro M. A., Dávalos A., Castillo J., Leza J. C., Camarero J., Colado M. I., Lorenzo P., Lizasoain I., *J. Neurochem.*, **79**, 456–459 (2001).
18) Nguyen M. D., Lariviere R. C., Julien J. P., *Neuron*, **30**, 135–147 (2001).
19) Vartiainen N., Keksa-Goldsteine V., Goldsteins G., Koistinaho J., *J. Neurochem.*, **82**, 329–335 (2002).
20) Gwag B. J., Lee Y. A., Ko S. Y., Lee M. J., Im D. S., Yun B. S., Lim H. R., Park S. M., Byun H. Y., Son S. J., Kwon H. J., Lee J. Y., Cho J. Y., Won S. J., Kim K. W., Ahn Y. M., Moon H. S., Lee H. U., Yoon S. H., Noh J. H., Chung J. M., Cho S. I., *J. Cereb. Blood Flow Metab.*, **27**, 1142–1151 (2007).
21) Dhir A., Naidu P. S., Kulkarni S. K., *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **30**, 1478–1485 (2006).
22) Srivastava A. K., Gupta Y. K., *Indian J. Physiol. Pharmacol.*, **45**, 475–480 (2001).
23) Tandon M., Anuradha K., Pandhi P., *Methods Find. Exp. Clin. Pharmacol.*, **25**, 607–610 (2003).
24) Wali R. S., Patil P. A., *Indian J. Physiol. Pharmacol.*, **39**, 77–79 (1995).
25) Ma L., Cui X. L., Wang Y., Li X. W., Yang F., Wei D., Jiang W., *Brain Res.*, **1469**, 103–113 (2012).
26) Rogawski M. A., *Epilepsy Res.*, **69**, 273–294 (2006).
27) Kardos J., *Neurochem. Int.*, **34**, 353–358 (1999).
28) Lewis D. A., Volk D. W., Hashimoto T., *Psychopharmacology* (Berl.), **174**, 143–150 (2004).
29) Borden L. A., Murali Dhar T. G., Smith K. E., Weinshank R. L., Branchek T. A., Gluchowski C., *Eur. J. Pharmacol.*, **269**, 219–224 (1994).
30) Fueta Y., Kunugita N., Schwarz W., *Neuroscience*, **132**, 335–345 (2005).
31) Czuczwar S. J., Patsalos P. N., *CNS Drugs*, **15**, 339–350 (2001).
32) Zhang J., Zhang P., Liu X., Fang K., Lin G., *Bioorg. Med. Chem. Lett.*, **17**, 3769–3773 (2007).
33) Fuelep G. H., Hoesl C. E., Hoefner G., Wanner K. T., *Eur. J. Med. Chem.*, **41**, 809–824 (2006).
34) Wang Z., Silverman R. B., *J. Enzyme Inhib. Med. Chem.*, **19**, 293–301 (2004).
35) Waniewski R. A., Martin D. L., *J. Neurochem.*, **65**, 355–362 (1995).
36) Shashoua V. E., Jacob J. N., Ridge R., Campbell A., Baldessarini R. J., *J. Med. Chem.*, **27**, 659–664 (1984).
37) Kuriyama K., Sze P. Y., *Neuropharmacology*, **10**, 103–108 (1971).
38) Leone-Bay A., Freeman J., O'Toole D., Rosado-Gray C., Salo-Kostmayer S., Tai M., Mercogliano F., Baughman R. A., *J. Med. Chem.*, **43**, 3573–3576 (2000).
39) Leone-Bay A., Ho K. K., Agarwal R., Baughman R. A., Chaudhary K., DeMorin F., Genoble L., McInnes C., Lercara C., Milstein S., O'Toole D., Sarubbi D., Variano B., Paton D. R., *J. Med. Chem.*, **39**, 2571–2578 (1996).
40) Leone-Bay A., Paton D. R., Freeman J., Lercara C., O'Toole D., Gschneidner D., Wang E., Harris E., Rosado C., Rivera T., DeVincent A., Tai M., Mercogliano F., Agarwal R., Leipold H., Baughman R. A., *J. Med. Chem.*, **41**, 1163–1171 (1998).
41) Leone-Bay A., Paton D. R., Variano B., Leipold H., Rivera T., Miura-Fraboni J., Baughman R. A., Santiago N., *J. Control. Release*, **50**, 41–49 (1998).
42) Haas S., Miura-Fraboni J., Zavala F., Murata K., Leone-Bay A., Santiago N., *Vaccine*, **14**, 785–791 (1996).
43) Poras H., Kunesch G., Barrière J. C., Berthaud N., Andremont A., *J. Antibiot.*, **51**, 786–794 (1998).
44) Dahlgren M. K., Kauppi A. M., Olsson I. M., Linusson A., Elofsson M., *J. Med. Chem.*, **50**, 6177–6188 (2007).
45) U.S. Food and Drug Administration, "Guidance for Industry, Bioanalytical Method Validation," Centre for Drug Evaluation and Research, CDER, Rockville, 2001.
46) Carelli V., Liberatore F., Scipione L., Giorgioni G., Di Stefano A., Impicciatore M., Ballabeni V., Calcina F., Magnanini F., Barocelli E., *Bioorg. Med. Chem. Lett.*, **13**, 3765–3769 (2003).
47) Paris G. Y., Garmaise D. L., Cimon D. G., Swett L., Carter G. W., Young P., *J. Med. Chem.*, **22**, 683–687 (1979).
48) Wegner M. M., Rapoport H., *J. Org. Chem.*, **43**, 3840–3844 (1978).
49) Holzapfel C. M., Pettit G. R., *J. Org. Chem.*, **50**, 2323–2327 (1985).
50) Mühlman A., Lindberg J., Classon B., Unge T., Hallberg A., Samuelsson B., *J. Med. Chem.*, **44**, 3407–3416 (2001).
51) Dobashi A., Kurata K., Senoo M., *Anal. Sci.*, **16**, 829–835 (2000).
52) Rossignol F.R., Cavier, Raymond F. R., U.S. Patent, 950351 (1976).
53) Ge Y., Wu P., *J. Org. Chem.*, **26**, 1124–1127 (2006).
54) Jung J. C., Kim J. C., Park O. S., *Synth. Commun.*, **29**, 3587–3595 (1999).
55) Chamberlin J. W., *J. Org. Chem.*, **31**, 1658–1660 (1966).
56) Pothukanuri S., Winssinger N., *Org. Lett.*, **9**, 2223–2225 (2007).
57) Hewapathirane D. S., Dunfield D., Yen W., Chen S., Haas K., *Exp. Neurol.*, **211**, 480–488 (2008).