New phenyl-glycinamide derivatives with hybrid structure as new effective anticonvulsants candidates

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Introduction

Epilepsy is recognized as one of the most common neurological disorders. Notably, despite huge advances in epilepsy studies and approval ofseveral new antiseizure drugs (ASDs), in **nearly 30%** of patients pharmacotherapy does not produce expected improvement and they suffer from drug resistant epilepsy (DRE). In recent years development of new drugs for the treatment of multifactorial diseases such as Alzheimer's disease, epilepsy and pain of various origin but also diseases with high risk of drug resistance is focused on the multifunctional compounds which possess predominantly hybrid structures. Hybrid molecules are compounds that contain several pharmacophores merged on one chemical scaffold which gives the possibility of interaction with more than one molecular target through the use of one substance.[1,2]

Aim of studies

Following the concept of multi-targeted strategy in drug discovery, compounds reported in the current studies were designed as hybrids that integrate structural fragments of chemical prototype - **KA-104** (pyrrolidine-2,5-dione derivative)[3] characterized by broad-spectrum anticonvulsant properties and acyclic selective TRPV1 antagonist **BCTC** with proven analgesic activity in the preclinical studies[4] (**Figure 1**). Moreover, compounds disclosed herein may be also recognized as close analogs of **KA-104** with degraded succinimide moiety and at the same time structurally closer to **lacosamide** (model ASD, potent in electrically induced seizures) in the aim of significantly enhancing the protection in the 6 Hz (44 mA) model of DRE and additionally increasing activity in other seizure models, namely maximal electroshock seizures (MES) test and 6 Hz (32 mA).

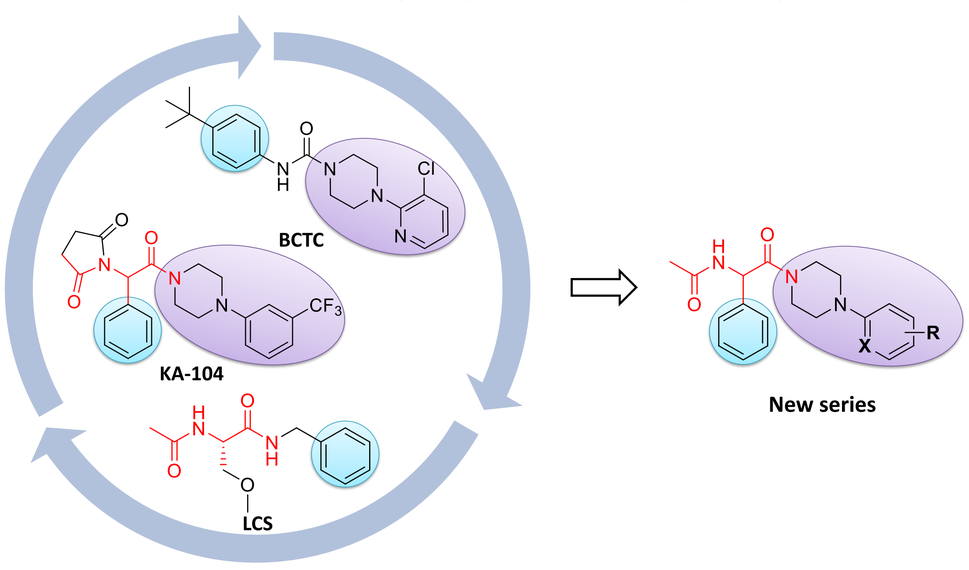
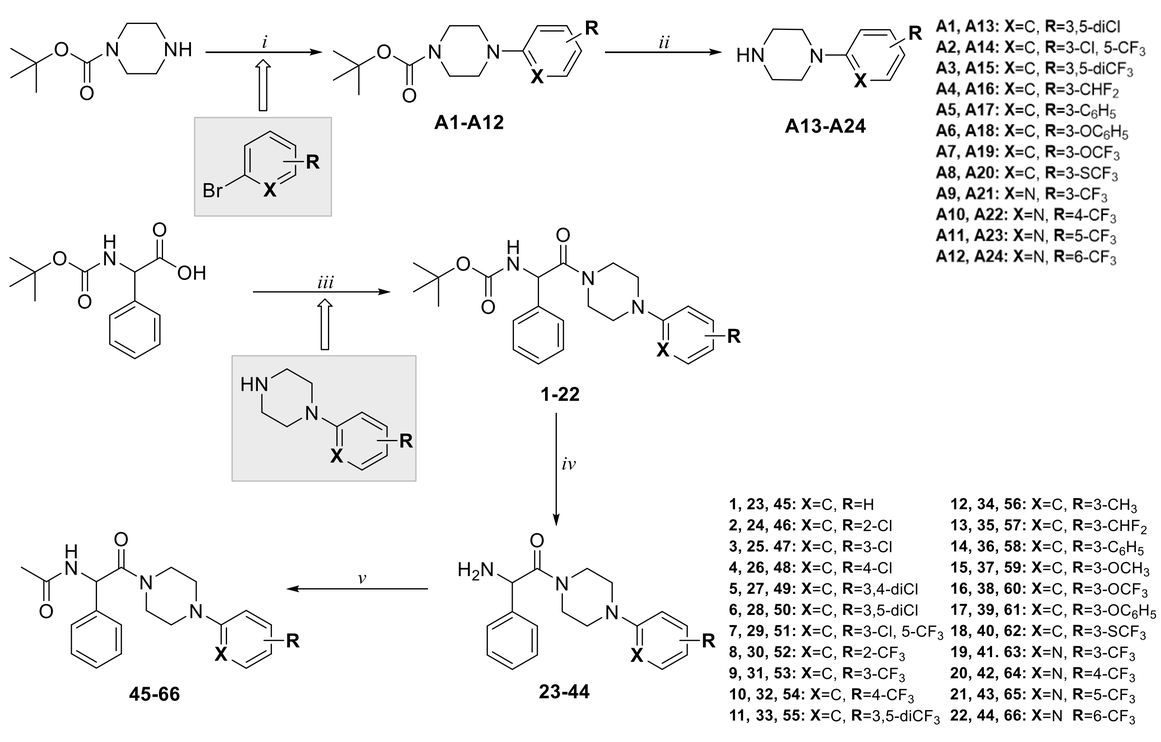


Figure 1. Design strategy and general structure of new hybrid molecules.

Chemistry



Reagents and conditions: (*i*) TRIS, BINAP, Sodium tert-butoxide, Toluene, 118°C, 12 h; (*ii*) TFA, DCM, r.t. 3 h; (*iii*) CDI, DCM, r.t., 12 h; (*iv*) TFA, DCM, r.t. 3 h; (*v*) CH_3COCl, DCM, 0°C, 2 h

Anticonvulsant activity

The anticonvulsant activity of compounds obtained was assessed using the MES test, the 6 Hz seizure model (32 mA) and the 6 Hz (44 mA) seizure model of DRE. The neurotoxicity was determined in chimney test in mice after intraperitoneal injection. The most effective was **53** and **60** with pharmacological parameters (ED_{50} and TD_{50}) depicted in **Figure 2** (pretreatment time of 30 min).

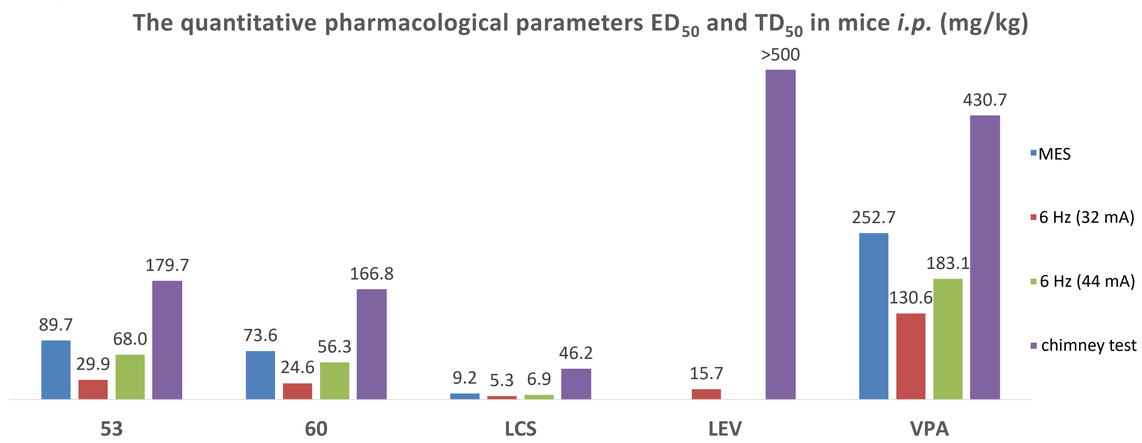


Figure 2. Pharmacological parameters of compound **53, 60**, and reference ASDs: Levetiracetam (LEV), Lacosamide (LCS), and Valproic acid (VPA) tested in the same conditions.

Seizure threshold in the *iv*PTZ test

The timed *iv*PTZ seizure test[5] was employed to further evaluate the acute effects of compounds **53** and **60** on seizure susceptibility in mice (**Figure 3**). The obtained results showed that **53** at the dose of 50 mg/kg significantly increased the threshold for the first myoclonic twitch and generalized clonus but did not produce any significant effect on the threshold for the forelimb tonus. Compound **60** at the same dose raised the threshold for the first myoclonic twitch but was devoid of any significant effects on the PTZ-induced seizure susceptibility for both generalized clonic seizure and forelimb tonic extension.

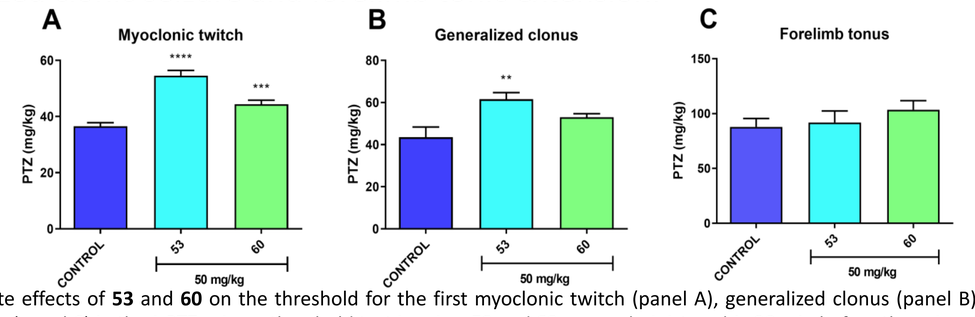


Figure 3. Acute effects of **53** and **60** on the threshold for the first myoclonic twitch (panel A), generalized clonus (panel B), and forelimb tonus (panel C) in the *iv*PTZ seizure threshold test in mice. **53** and **60** were administered *ip* 30 min before the seizure test. Control animals received vehicle only. Each experimental group consisted of 9–12 animals. Each bar represents the mean (mg/kg PTZ) + SEM. **p<0.01, ***p<0.001, ****p<0.0001 vs. the control group (Student's *t* test).

In vitro studies

Due to several structural similarities (e.g., arylpiperazine fragment, aromatic ring position and amine fragment) of compounds obtained in the current studies to selected TRPV1 antagonists we determined the antagonist activity of the most active anticonvulsants against the TRPV1 receptor. The results of functional assays confirmed the TRPV1 channel antagonist activity for compound **53** (IC_{50} = 13 μM, K_B = 1.7 μM) and **60** (IC_{50} = 11 μM, K_B = 1.5 μM). Moreover, the potent activity of i.a. **53** in the electrically induced seizure models (e.g. MES and 6 Hz [32 mA and 44 mA]), as well as the results of our binding studies (sodium channel, data not shown) suggest its influence on neuronal sodium currents. Thus, we determined the influence of **53** on fast voltage-gated sodium channels in rat prefrontal cortex pyramidal neurons (at a concentration of 10 μM) using the patch-clamp technique.[6] The inhibitory effect was not strong but statistically significant (1.0 in control and 0.83±0.03 after application of **53**, p<0,01, **Figure 4**).

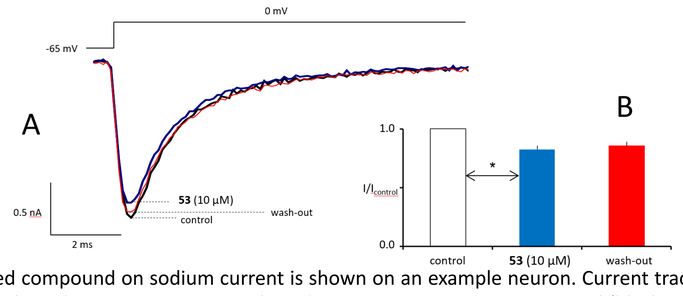


Figure 4. The influence of tested compound on sodium current is shown on an example neuron. Current traces were evoked once every ten seconds by a rectangular voltage-step. A – example sodium current recordings in control (black trace), after application of tested compound (blue trace) *p<0.01, ANOVA with Tukey test and after wash-out (red trace). B – averaged normalized maximal current amplitudes in control, in the presence of **53** and after wash-out.

References

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