Supporting Information

Synthesis and Evaluation of Antiallodynic and Anticonvulsant Activity of Novel Amide and Urea Derivatives of Valproic Acid Analogues

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Elemental Analysis Data

Purity determination of the new amide, and N-methylamide derivatives of VPA not previously published (compounds **6-27**) by combustion analysis:

Compound **6** ($C_7H_{15}NO$): found (calculated) C 65.11 (65.1), H 11.82 (11.6), N 10.93 (10.85).

Compound **7** ($C_6H_{13}NO$): found (calculated) C 62.75 (62.62), H 11.48 (11.38), N 12.40 (12.17).

Compound **8** ($C_7H_{15}NO$): found (calculated) C 65.28 (65.13), H 11.91 (11.62), N 10.99 (10.85).

Compound **9** ($C_6H_{13}NO$): found (calculated) C 62.62 (62.6), H 11.59 (11.38), N 12.34 (12.1).

Compound **10** ($C_6H_{13}NO$): found (calculated) C 62.81 (62.62), H 11.54 (11.29), N 12.24 (12.17).

Compound **11** ($C_5H_{11}NO$): found (calculated) C 59.50 (59.42), H 11.12 (10.88), N 13.9 (13.85).

Compound **12** ($C_6H_{13}NO$): found (calculated) C 62.79 (62.62), H 11.58 (11.29), N 12.3 (12.17).

Compound **13** ($C_5H_{11}NO$): found (calculated) C 59.32 (59.42), H 11.14 (10.90), N 13.78 (13.85).

Compound **14** ($C_8H_{17}NO$): found (calculated) C 67.18 (67.14), H 11.95 (11.88), N 9.83 (9.78).

Compound **15** ($C_8H_{17}NO$): found (calculated) C 67.23 (67.15), H 12.11 (11.88), N 9.70 (9.78).

Compound **16** ($C_8H_{17}NO$): found (calculated) C 66.89 (67.15), H 11.81 (11.88), N 10.04 (9.78).

Compound **17** ($C_9H_{19}NO$): found (calculated) C 68.54 (68.8), H 12.15 (12.09), N 8.87 (8.91).

Compound **18** ($C_9H_{19}NO$): found (calculated) C 68.92 (68.74), H 12.46 (12.18), N 9.05 (8.91).

Compound **19** ($C_9H_{19}NO$): found (calculated) C 68.67 (68.74), H 12.48 (12.18), N 8.94 (8.91).

Compound **20** ($C_9H_{19}NO$): found (calculated) C 68.70 (68.80), H 12.25 (12.09), N 8.98 (8.91).

Compound **21** ($C_9H_{19}NO$): found (calculated) C 69.00 (68.74), H 12.48 (12.18), N 8.95 (8.91).

Compound **22** ($C_9H_{19}NO$): found (calculated) C 68.5 (68.8), H 12.1 (12.09), N 8.93 (8.91).

Compound **23** ($C_9H_{19}NO$): found (calculated) C 68.5 (68.8), H 12.28 (12.1), N 8.69 (8.91).

Compound **24** ($C_9H_{19}NO$): found (calculated) C 68.76 (68.84), H 12.38 (12.1), N 8.99 (8.92).

Compound **25** ($C_9H_{19}NO$): found (calculated) C 68.63 (68.8), H 12.09 (12.1), N 8.86 (8.92).

Compound **26** ($C_9H_{19}NO$): found (calculated) C 69.00 (68.8), H 12.38 (12.09), N 9.2 (8.92).

Compound **27** ($C_7H_{16}NO$): found (calculated) C 65.18 (65.14), H 11.9 (11.62), N 11.02

(10.85).

Description of Animal models used for screening of investigational Anticonvulsant and antiallodynic compounds

The following protocols are used by the Anticonvulsant Drug Development (ADD) Program of the NIH. Methods are described according to White, H. S. et al " Discovery and Preclinical Development of Antiepileptic Drugs", in Antiepileptic Drugs, 5th edition, Levy, R. H., Mattson, R. H., Meldrum, B. S., Perucca, E., Eds.; Lippincott Williams & Wilkins Publishers: New York 2002, 36-48.

Anticonvulsant activity is initially identified for every substance in a combination of three different tests: The Maximal Electroshock Seizure Test (MES), and the Subcutaneous Metrazole Seizure Threshold Test (scMet). The acute toxicity is tested by the Rotorod Test, the Positional Sense Test, the Gait and Stance Test and the Muscle Tone Test. After the initial screening, the ED_{50}s of the active compounds are quantified and the compounds are further tested in other models.

Maximal Electroshock Seizure Test (MES)

In this test an alternating current of 50mA for adult male CF No. 1 albino mice is delivered for a time period of 0.2 second thorough a corneal electrode: A drop of 0.5% tetracaine solution in saline is applied to the subject animal's eyes immediately after the test substance is administered. Saline is placed in each eye and immediately the corneal electrodes are placed. After the stimulation the animals are released and observed throughout the seizure. The end point of the MES test is the abolition of the hind limb

tonic extensor component. When the hind limbs are not fully extended at 180° with the body plane the tonic extension is considered abolished. If such extension does not occur, than the tested substance is able to prevent the spread of seizure discharge through neural tissues.

Subcutaneous Metrazole Seizure Threshold Test (scMet)

In this test a Metrazole convulsive dose is injected subcutaneously to CF No. 1 albino mice (85 mg/kg). The animals are isolated and are observed for the present and absence of clonic spasms that persist for at least 5 seconds. When clonic seizures are not observed than the test compound is considered effective in raising the seizure threshold.

Pilcarpine Induced Status Model

In order to determine if a test substance can prevent acute pilocarpine-induced status the test compound is given i.p. to male albino Sprague Dawley rats (150-180 g). Then a challenge dose of pilocarpine is administered and the treatment-effects were observed. The outcome measures are “protection” or “no protection”. The seizure severity is determined by using the Racine scale. If a compound possesses significant protection at time zero (time from the first stage III seizure) further evaluation in sustained seizure model where the candidate drug is given 30 min after pilocarpine status induction (or post first stage III seizure) is performed.

Determination of Acute Toxicity

Rotorod Test (in mice exclusively)

Mice are placed on a 1 inch diamter rod. The rod rotates at 6 rpm. The inability of a mouse to maintain its equilibrium for a whole minute in three different tests indicates minimal motor impairment.

Positional Sense Test

The hind limb of a mouse is gently lowered over the edge of a table. If the animal is unable to rapidly correct this abnormal position than the animal is neurologically deficit.

Gait and Stance Test

Neurologically deficit animals will demonstrate circular zigzag gait, ataxia, abnormal spread of the legs, abnormal body posture, tremor, hyperactivity, lack of exploratory behavior, somnolence, stupor, catalepsy and the like

Muscle Tone Test

Loss of skeletal muscle tone, which is characterized by hypotonia or flaccidity, is considered neurological deficiency.

SNL model for neuropathic pain

The animal model used for evaluation of antiallodynia was the SNL model for neuropathic pain previously published by Kim, S. H.; Chung, J. M., Pain 1992, 50, (3), 355-63.

Briefly, in order to quantify "pain threshold" we used the rat's foot withdrawal in response to tactile stimulus produced by a set of 9 nylon von-Frey monofilaments (VFF, Semmes-Weinstein monofilaments, Stoelting, Wood Dale, IL, USA). The VFF used

produced an initial bending force of (in mN): 5.8, 13.7, 19.6, 39.2, 58.7, 78.3, 97.9, 146.9, and 254.5, equivalent to a mass of (in grams): 0.6, 1.4, 2, 4, 6, 8, 10, 15, and 26. The same set was calibrated and used in all experiments. Rats were placed on a high wire mesh floor, confined in transparent plastic cages and allowed to acclimate for 30 minutes. Tactile allodynia was assessed before and 30, 60, 120 180, and 240 minutes after injection of a test substance. Each monofilament was applied 5 times with a 1 sec interval, perpendicularly to the mid plantar skin of the operated (ipsilateral) and contralateral hind paw until just bending the monofilament, starting with the weakest VFF (0.6g). If the rat failed to respond to at least 3 out of 5 stimuli, the next monofilament was used following an ascending staircase protocol. A score of 26g was recorded if the rat failed to respond to the highest VFF. The pain threshold was recorded as a mean of two trials, each consisting of 5 stimuli, as previously described.

Tactile allodynia was assessed prior to the surgical procedure and 5 and 6 days post operatively. Inclusion criteria for the experiment was a pain threshold of 15 g or higher pre-operatively and a pain threshold of less than 5 g post-operatively. Rats generally failed to respond to the 15g VFF or higher on the contralateral side. Approximately 10% of rats failed to meet one of the inclusion criteria and were excluded from the experiment. Test compounds were suspended in 0.5% methylcellulose (MC) solution in double-distilled water (DDW) at 80 mg/kg, the highest dose shown previously to completely reverse tactile allodynia in compounds **2-5**. A suspension volume of 4ml/kg was i.p. administered to rats. The experiment protocol allowed a maximum of two compounds and a control (consisting of only MC solution) to be administered at the same study. The compounds were administered three times to the same group of rats 7, 14, and 21 days

after surgery, allowing a wash-out period of 7 days between tests. Base line was recorded before administration of test substance in each experiment. The compounds were administered in a double blind crossover manner using a Latin square design protocol, where the experimenter who performed the sensory tests was unaware of the drug given to each rat at the time of testing.