Acta Poloniae Pharmaceutica – Drug Research, Vol. 71 No. 2 pp. 261–264, 2014 ISSN 0001-6837
Polish Pharmaceutical Society

STABILITY OF NEW ANTICONVULSANT DERIVATIVES OF PICOLINIC, NICOTINIC, CYCLOCARBOXYLIC ACIDS IN BODY FLUIDS AND TISSUES

DOROTA MARSZAŁEK*, ANNA GOLDNIK, ALEKSANDER P. MAZUREK, MAŁGORZATA BALICKA, AGNIESZKA KOZIOROWSKA, AGNIESZKA HERMANOWICZ, MONIKA ŁUKA, MILENA PIĄTKOWSKA, SYLWIA GRODZKA and SYLWIA KUMOROWSKA

Department of Drug Chemistry, Medical University of Warsaw, 1 Banacha St., 02-097 Warszawa, Poland

Abstract: The stability of new compounds with established anticonvulsant activity: picolinic acid 4-pyridylmethylamide (Pic-4-PMA), cyclopentanecarboxylic acid benzylamide (Cpc-BZA), cycloheptanecarboxylic acid benzylamide (Chc-BZA), picolinic acid 2-fluoro-3-trifluoromethylbenzylamide (Pic-2F-3TFM-BZA), 2-chloronicotinic acid benzylamide (2-Cl-Na-BZA), 6-chloronicotinic acid benzylamide (6-Cl-Na-BZA) and 6-trifluoromethylnicotinic acid benzylamide (6-TFM-Na-BZA) in homogenates of body organs and in body fluids was determined after incubation. It was found that three compounds were stable against enzymes present in body fluids and organs and two were found to decompose in liver and kidney homogenates and two decomposed only in liver homogenate.

Keywords: picolinic, nicotinic and cyclocarboxylic acid derivatives, anticonvulsant activity

In the search for new anticonvulsants, picolinic acid benzylamide (Pic-BZA) was previously synthesized. It is a strong antagonist of excitatory amino acid receptor but of short action time (1). Searching for equally effective anticonvulsant but more stable, the basic structure Pic-BZA was modified and the new amide derivatives of some heterocyclic and cyclic acids were synthesized (2, 3). Their stability (Pic-BZA, Na-BZA) was tested. In our previous papers it was reported that Pic-BZA decomposed in liver and kidney homogenates and it was a first-order reaction relative to substance concentration (the relationship ln c *versus* time was linear). The half-time was 1.25 h and 5.73 h for the liver and kidney, respectively (4). Several derivatives with modified structure of Pic-BZA were synthesized (5). Picolinic acid benzylamide was substituted with CH_3 and F in various positions of the ring (Pic-2-F-BZA, Pic-3-F-BZA, Pic-4-F-BZA, Pic-2-Me-BZA) to prolong the stability. Also these compounds decomposed in liver and kidney homogenates. The half-life in liver for Pic-BZA was 0.5 h, for Pic-2-F-BZA 1.5 h and over 3 h for Pic-3-F-BZA and Pic-4-F-BZA. The decomposition in kidney homogenate was much slower. Na-BZA described in paper (4) was stable in all body fluids and organ homogenates but the time of anticonvulsant action was not so long, thus some new derivatives were synthesized. Those were derivatives of Na-BZA (2-Cl-Na-BZA and 6-Cl-Na-BZA) and some benzylamides of acids containing 5- or 7-member rings in the structure (Cpc-BZA and Chc-BZA). Also benzylamide of picolinic acid was substituted with pyridylmethylamide (Pic-4-PMA) (6). All newly synthesized compounds were evaluated in the Anticonvulsant Screening Program (ASP) of Antiepileptic Drug Development Program (ADDP) of NIH, USA.

The stability of all compounds was examined in body fluids and organ homogenates. The concentrations of derivatives were measured at different time points during incubation. The HPLC method was developed and used to determine the concentration of all derivatives isolated from biological material by liquid-liquid extraction.

EXPERIMENTAL

Apparatus and chromatographic conditions

A Shimadzu HPLC apparatus that consisted of an LC-10AT pump and SPD-10A spectrophotometer was used with Chroma computer recorder (POL-

* Corresponding author: e-mail: dorota.marszalek@wum.edu.pl, phone: 48 22 5720630, fax 48 22 5720697

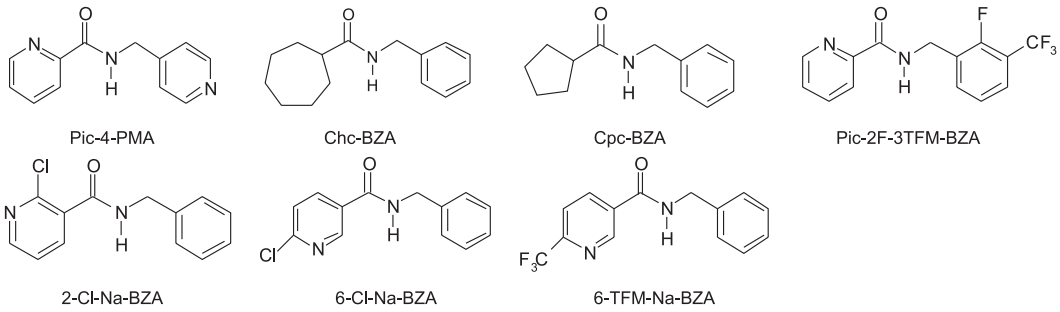

Figure 1. Chemical structures of new synthesized compounds

Table 1. Parameters of chromatographic determination and validation procedure of determined compounds.

Compound	Mobile phase (v/v)	λ nm	Internal standard %	Recovery %	CV	r
Pic-4-PMA	MeOH–H_2O –triethylamine 70 : 130 : 0.02	262	Na-BZA	98.11 ± 5.41	5.45 ± 1.83	0.9958
Chc-BZA	MeOH–H_2O 40 : 60	210	Pic-2-Me-BZA	99.13 ± 3.23	6.28 ± 1.09	0.9996
Cpc-BZA	MeOH-H_2O 60 : 40	210	–	97.84 ± 3.79	6.40 ± 1.34	1.0000
Pic-2F-3TFM-BZA	MeOH–H_2O 62.5:32.5	265	Pic-3F-BZA	98.30 ± 4.38	3.88 ± 3.78	0.9996
2-Cl-Na-BZA	MeOH–H_2O 50 : 50	265	2-Cl-iNa-BZA	100.88 ± 4.04	5.70 ± 2.56	0.9990
6-Cl-Na-BZA	MeOH–H_2O 60 : 40	262	Chlordiazepoxide	100.29 ± 1.51	3.66 ± 3.02	0.9998
6-TFM-Na-BZA	MeOH–H_2O 60 : 40	262	2-Me-Pic-BZA	98.76 ± 3.34	4.54 ± 2.91	0.9990

LAB, Poland) and the Chromax 2001 software (POL-LAB, Poland).

The separation was carried out in the reversed phase system with a Beckman Ultrasphere ODS column (150 mm × 4.6 mm). The flow rate was 1 mL/min. The mobile phases, wavelengths and internal standards are presented in Table 1.

Compounds

Cpc-BZA, Chc-BZA, Pic-2F-3TFM-BZA, Pic-4-PMA, 2-Cl-Na-BZA, 6-Cl-Na-BZA and 6-TFM-Na-BZA and internal standards were synthesised in the Department of Drug Chemistry, Warsaw Medical University. Chemical structures are presented in Figure 1.

Preparation of solution of compounds 1–7

Stock standard solutions of determined compound and internal standards were prepared by dissolving each compound in methanol (0.1 mg/mL). The final working concentration for the examined substances and internal standards was 10 µg/mL. The liquid-liquid extraction method was used for all biological material.

Validation of analytical procedures

The method of Cpc-BZA, Chc-BZA, Pic-2F-3TFM-BZA, Pic-4-PMA, 2-Cl-Na-BZA, 6-Cl-Na-BZA and 6-TFM-Na-BZA determination was validated. Standard deviations, recovery, accuracy and linearity of the analytical procedure are presented in Table 1.

Table 2. Stability of Cpc-BZA, Chc-BZA, Pic-2F-3TFM-BZA, Pic-4-PMA, 2-Cl-Na-BZA, 6-Cl-Na-BZA and 6-TFM-Na-BZA.

Compound	Stability	
	Kidney homogenate	Liver homogenate
Pic-4-PMA	stable	stable
Chc-BZA	a first-order reaction relative to substance concentration $t_{0.5}$ = 142.5 min	a first-order reaction relative to substance concentration $t_{0.5}$ = 96.6 min
Cpc-BZA	a first-order reaction relative to substance concentration $t_{0.5}$ = 36.5 h	a first-order reaction relative to substance concentration $t_{0.5}$ = 20.4 h
Pic-2F-3TFM-BZA	stable	a first-order reaction relative to substance concentration $t_{0.5}$ = 231 min
2-Cl-Na-BZA	stable	stable
6-Cl-Na-BZA	stable	stable
6-TFM-Na-BZA	stable	stable

Determination of Cpc-BZA, Chc-BZA, Pic-2F-3TFM-BZA, Pic-4-PMA, 2-Cl-Na-BZA, 6-Cl-Na-BZA and 6-TFM-Na-BZA in gastric and intestinal juice

Five milligrams of each compound was dissolved in 1 mL of ethanol and 49 mL of freshly prepared gastric or intestinal juice (USP). The solutions were incubated at 37°C and the samples were collected at the time points 0, 30, 60, 90, 120, 150, 180 min. The so-obtained results are presented in Table 2.

Determination of Cpc-BZA, Chc-BZA, Pic-2F-3TFM-BZA, Pic-4-PMA, 2-Cl-Na-BZA, 6-Cl-Na-BZA and 6-TFM-Na-BZA in pork liver, kidney, brain and lung homogenates

The homogenates of body organs (40%) in 0.1 mole/L TRIS solution (pH = 8.4) were prepared. Two milliliters of the homogenate was spiked with 20 µg of each compound and incubated at 37°C. The samples were collected at the time points 0, 30, 60, 90, 120, 150, 180 min. Results are presented in Table 2.

RESULTS AND DISCUSSION

The stability study of seven amide derivatives in body fluids and organs homogenates showed that Chc-BZA and Cpc-BZA only decomposed in liver and kidney homogenates and Pic-2F-3TFM-BZA decomposed only in liver homogenate, while nicotinic acid benzylamides and Pic-4-PMA were resistant to enzymes present in all tested tissues. Decomposition in liver and kidney homogenates is a first-order reaction relative to substance concentration because the relationship ln c *versus* time is linear. For Pic-BZA, the basic structure represented class I of ASP (Anticonvulsant Screening Program). All modification of its structure decreased the anticonvulsant activity to II or III class but significantly prolonged time of action. Even for Chc-BZA and 1-Cpc-BZA, which decomposed in liver and kidney homogenates and Pic-2F-3TFM-BZA, which decomposed in liver homogenates, the half-life time was relatively long (e.g., more than 36 h for 1-Cpc-BZA in kidney homogenates). Introduction of nicotinic acid instead of picolinic acid (2-Cl-Na-BZA, Chc-BZA, Cpc-BZA) and replacement of picolinic acid benzylamide to picolinic acid pyridylmethylamide decreased anticonvulsant activity mostly to III class but made these compounds resistant to enzymes presented in all tested tissues. Based on obtained data we conclude that duration of action was prolonged but the activity was reduced.

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Received: 28. 05. 2013