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METABOLIC STABILITY OF NEW ANTICONVULSANTS IN BODY FLUIDS AND ORGAN HOMOGENATES

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Abstract: The stability as a function of time of compounds with established anticonvulsant activity: picolinic acid benzylamide (Pic-BZA), picolinic acid 2-fluorobenzylamide (Pic-2-F-BZA), picolinic acid 3-fluorobenzylamide (Pic-3-F-BZA), picolinic acid 4-fluorobenzylamide (Pic-4-F-BZA) and picolinic acid 2-methylbenzylamide (Pic-2-Me-BZA) in body fluids and homogenates of body organs were determined after incubation. It was found that they decompose relatively rapidly in liver and kidney and are stable against enzymes present in body fluids and some organs. These results are consistent with the bond strength expressed as total energy of amide bonds (calculated by quantum chemical methods) in the studied anticonvulsants. The calculated values of the amide bond energy are: 199.4 kcal/mol, 200.2 kcal/mol, 207.5 kcal/mol, 208.4 kcal/mol and 198.2 kcal/mol, respectively. The strength of the amide bonds in the studied anticonvulsants correctly reflects their stability in liver or kidney.

Keywords: picolinic acid benzylamide, anticonvulsant, amide bond energy

In the search for new anticonvulsants, a picolinic acid benzylamide (Pic-BZA) (Fig. 1a) was synthesized, which turned out to be the strong acting antagonist for the excitatory amino acid (EAA) receptors.

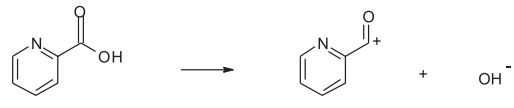
The time of its action is up to several minutes after administration, therefore in a search for similar strong anticonvulsants, but with prolonged time of action, the basic picolinic acid benzylamide structure was further modified. The derivatives of Pic-BZA substituted with fluoro or methyl groups at various positions of the ring were synthesized (1–4) (Fig. 1). These changes, however, failed to prolong the time of action. To explain such short time of action of Pic-BZA derivatives, their stability in some organs (liver, kidney, lung and brain homogenates) and body fluids (blood serum, intestinal juice and gastric juice) was examined (5). The concentrations of Pic-BZA derivatives were measured at different time points during incubation. The HPLC method was developed and used to determine the concentration of Pic-BZA derivatives, isolated from biological material by liquid-liquid extraction.

Searching for new, more effective substituents in the phenyl ring of newly developed anticonvulsants it is important to know some molecular parameters describing how the particular substituents affect the stability of the potential drugs. The theoretically determined total energy of an amide bond seems to be the suitable stability criterion of the studied compounds.

To verify this hypothesis the amide bond energies were calculated for all studied picolinic acid benzylamides: Pic-BZA, Pic-2-F-BZA, Pic-3-F-BZA, Pic-4-F-BZA and Pic-2-Me-BZA. The values of amide bond energies E_{CN} were derived from the following equation:

$$E_r = E_{COH} + E_{NH} - E_{OH} - E_{CN}$$

Symbols in above equation stand for: E_{COH} –C–OH bond energy in the carboxyl group, E_{NH} – N–H bond energy in an amine group, E_{OH} – O–H bond energy in a hydroxyl group and E_R – energy of reaction between picolinic acid and a respective amine. For example, the E_{COH} was calculated according to the Hess' law as the energy of the following reaction:



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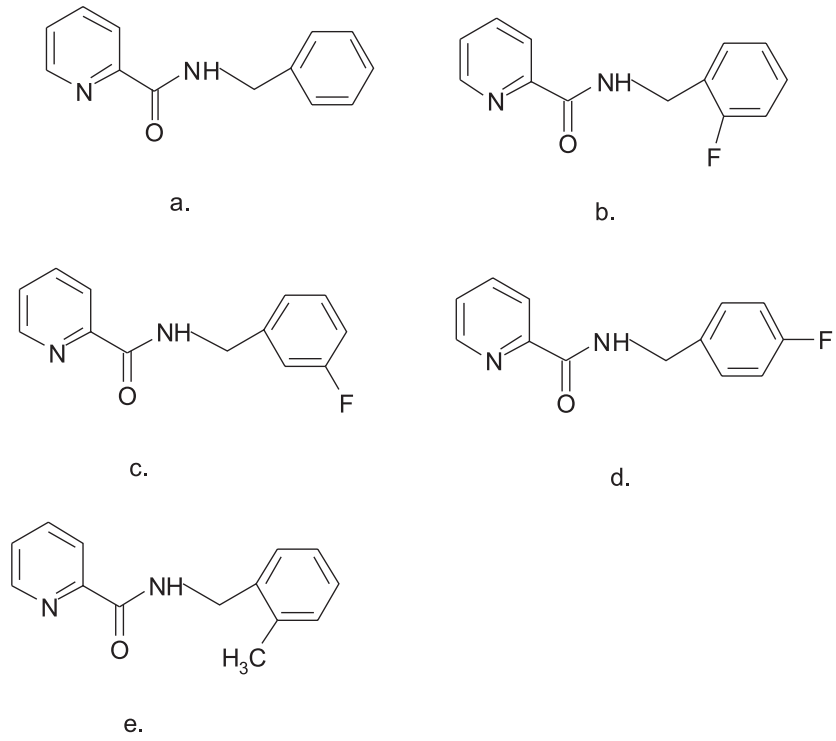

Figure 1. a. picolinic acid benzylamide (Pic-BZA); b. picolinic acid 2-fluorobenzylamide (Pic-2-F-BZA); c. picolinic acid 3-fluorobenzylamide (Pic-3-F-BZA); d. picolinic acid 4-fluorobenylamide (Pic-4-F-BZA); e. picolinic acid 2-methylbenzylamide (Pic-2-Me-BZA)

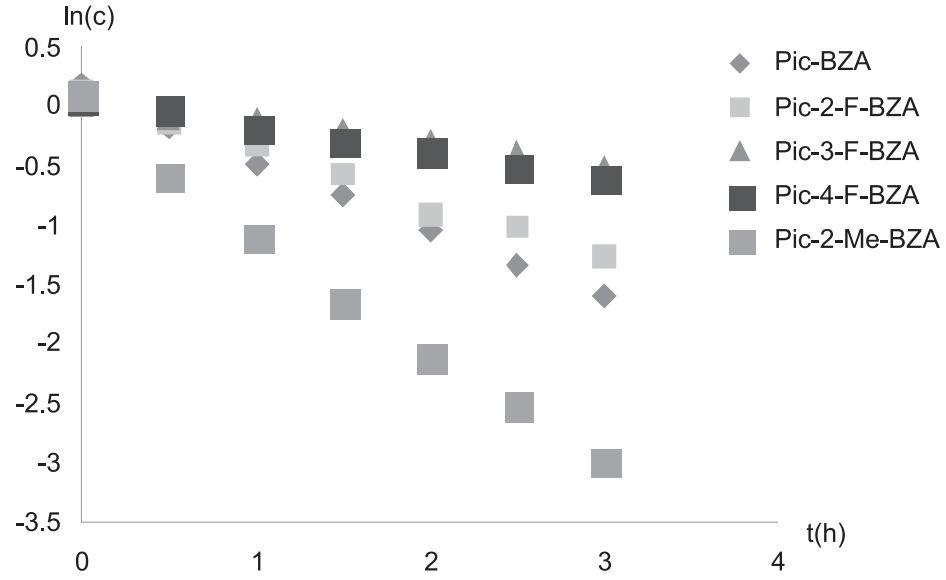

Figure 2. The plots ln(c) = f(t) for liver homogenates for Pic-BZA derivatives

The other values of energy that appear in above equation were calculated in the same manner. All these values of energies were calculated at HF (6-31G*) level of theory (6).

EXPERIMENTAL

Apparatus and chromatographic conditions

A Shimadzu HPLC apparatus that consisted of an LC-10AT pump and an SPD-10A UV spec-

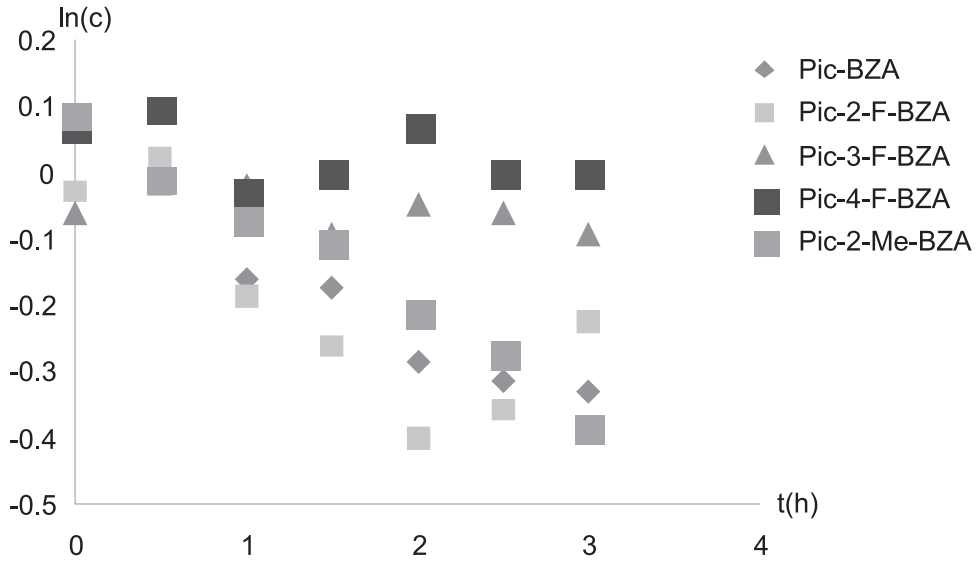

Figure 3. The plots ln(c) = f(t) for kidney homogenates for Pic-BZA derivatives

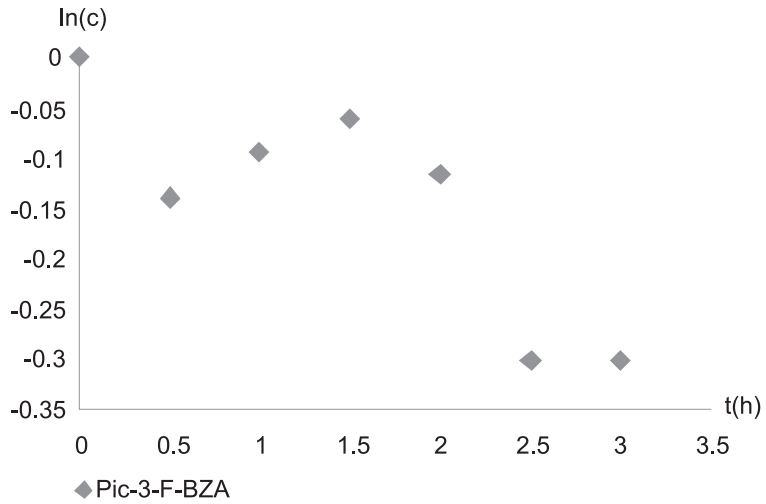

Figure 4. The plot ln(c) = f(t) for lung homogenate for Pic-3-F-BZA

trophotometer was used with a Chroma computer recorder (POL-LAB, Poland) and the Chromax 2001 software (POL-LAB). The detection was at 262 nm for all derivatives The separation was carried out in the reverse phase system with a Beckmann Ultrasphere ODS column (150 × 4 mm) and methanol-water (60:40, v/v) as a mobile phase for all compounds. The flow rate was 1 mL/min.

Standards

Pic-BZA, Pic-2-F-BZA, Pic-3-F-BZA, Pic-4-F-BZA and Pic-2-Me-BZA were synthesized in the Department of Drug Chemistry, Medical University of Warsaw. Chlordiazepoxide (internal standard) was provided by Polfa Tarchomin.

Preparation of standard solution

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

Table 1. Parameters of validation procedure for Pic-BZA derivatives.

Compound	Pic-2-F-BZA	Pic-3-F-BZA	Pic-4-F-BZA	Pic-2-Me-BZA
Recovery [%]	98.55 ± 5.12	100.1 ± 3.87	100.45 ± 1.42	98.41 ± 3.96
CV [%]	7.18 ± 2.58	4.32 ± 1.21	4.94 ± 2.21	2.55 ± 0.93
r	0.9987	0.9989	0.9998	0.9998

Table 2.Concentration of Pic-BZA derivatives in liver homogenates (n = 4).

Time	Concentration μg/mL				
(h)	Pic-BZA	Pic-2-F-BZA	Pic-3-F-BZA	Pic-4-F-BZA	Pic-2-Me-BZA
0	1.19 ± 0.05	1.14 ± 0.06	1.01 ± 0.03	1.06 ± 0.04	1.09 ± 0.08
0.5	0.82 ± 0.05	0.85 ± 0.07	0.91 ± 0.10	0.97 ± 0.11	0.55 ± 0.05
1	0.61 ± 0.06	0.72 ± 0.04	0.88 ± 0.08	0.82 ± 0.09	0.33 ± 0.04
1.5	0.47 ± 0.05	0.56 ± 0.07	0.81 ± 0.06	0.74 ± 0.11	0.19 ± 0.03
2	0.35 ± 0.04	0.40 ± 0.04	0.73 ± 0.05	0.68 ± 0.12	0.12 ± 0.03
2.5	0.26 ± 0.03	0.36 ± 0.03	0.67 ± 0.06	0.59 ± 0.14	0.08 ± 0.05
3	0.20 ± 0.03	0.28 ± 0.04	0.59 ± 0.06	0.54 ± 0.09	0.05 ± 0.01

Table 3. Concentration of Pic-BZA derivatives in kidney homogenates (n = 4).

Time	Concentration μg/mL				
(h)	Pic-BZA	Pic-2-F-BZA	Pic-3-F-BZA	Pic-4-F-BZA	Pic-2-Me-BZA
0	1.07 – 0.15	0.97 – 0.07	0.94 – 0.02	1.07 – 0.06	1.09 – 0.60
0.5	0.98 – 0.14	1.02 – 0.06	0.98 – 0.02	1.10 – 0.06	0.99 – 0.05
1	0.85 – 0.12	0.83 – 0.08	0.98 – 0.02	1.03 – 0.07	0.93 – 0.05
1.5	0.84 – 0.12	0.77 – 0.14	0.91 – 0.07	1.00 – 0.01	0.90 – 0.07
2	0.75 – 0.09	0.67 – 0.13	0.95 – 0.03	1.07 – 0.01	0.81 – 0.06
2.5	0.73 – 0.10	0.70 – 0.05	0.94 – 0.05	1.00 – 0.04	0.76 – 0.05
3	0.72 – 0.12	0.80 – 0.08	0.91 – 0.05	1.00 – 0.03	0.68 – 0.21

Table 4. Concentration of Pic-BZA derivatives in lung hogenates (n = 4).

Time (h)	Concentration μg/mL Pic-3-F-BZA
0	1.00 ± 0.09
0.5	0.87± 0.05
1	0.91± 0.01
1.5	0.94± 0.08
2	0.89 ± 0.01
2.5	0.74 ± 0.14
3	0.74 ± 0.01

Validation of analytical procedure

The method of Pic-BZA determination was validated [5]. The methods of Pic-2-F-BZA, Pic-3-F-BZA, Pic-4-F-BZA and Pic-2-Me-BZA determination were validated and recovery, accuracy and linearity are presented in Table 1.

Determination of Pic-BZA derivatives in gastric and intestinal juice

Five mg of Pic-BZA derivatives was dissolved in 1 mL of ethanol and 49 mL of freshly prepared gastric or intestinal juice (Ritschel and Ort). The solutions were incubated at 37°C and the samples

were collected at the time points 0, 30, 60, 90, 120, 150 and 180 min.

Determination of Pic-BZA derivatives in pork liver, kidney 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 Pic-2-F-BZA, Pic-3-F-BZA, Pic-4-F-BZA and Pic-2-Me-BZA and incubated at 37°C. The samples were collected at the time points 0, 30, 60, 90, 150, 180 min. (Tables 2–4).

RESULTS AND DISCUSSION

The stability study of five Pic-BZA derivatives in body fluids and organs unambiguously proved that all compounds were decomposed in liver and kidney homogenates (Figs. 2, 3). After incubation, the content in the liver homogenate was reduced to about 50% at 0.5 h for Pic-2-Me-BZA and 1.5 h for Pic-BZA and Pic-2-F-BZA and over 3 h for Pic-3-F-BZA and Pic-4-F-BZA.

The decomposition in kidney homogenates was slower, after 3 h the content was reduced to 70–79% for Pic-BZA, Pic-2-F-BZA and Pic-2-Me-BZA, for two others decomposition was noticeable after 24 h.

All compounds (except Pic-3-F-BZA, Fig. 4) were resistant to lung enzymes.

The decomposition of Pic-BZA derivatives takes place mostly in the liver. The interaction of renal enzymes is not so significant since they act on the compounds which have been already excreted. The decomposition of Pic-BZA derivatives in liver homogenates is the first-order reaction since the correlation ln(c) *versus* time is linear. The half-life time for Pic-BZA in the liver is 71 min., for Pic-2-F-BZA 1.5 h, for Pic-3-F-BZA 4 h, for Pic-4-F-BZA 3 h and for Pic-2-Me-BZA 41 min. The half-life time for Pic-BZA in kidney is 5 h. All presented modifications of the basic structure of Pic-BZA (introduction of methyl group or fluorine atom) do not impact on time of anticonvulsant action. However, the stability of these compounds is still not satisfactory.

The calculated amide bond energy for: Pic-BZA, Pic-2-F-BZA, Pic-3-F-BZA, Pic-4-F-BZA and Pic-2-Me-BZA are: 199.4 kcal/mol, 200.2 kcal/mol, 207.5 kcal/mol, 208.4 kcal/mol and 198.2 kcal/mol, respectively, and properly reflect the resistance of studied compounds to liver and kidney hydrolytic enzymes. Generally, based on obtained data it is justified to conclude, that amide bond energy is a suitable parameter to determinate a propensity of amide bonds to undergo enzyme hydrolysis. It is worthy to note, that the calculations of amide bond energy enable estimation of the stability of newly developed amide compounds at the early stages of their computational modeling.

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