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ANTICONVULSANT ACTIVITY OF SUBSTITUTED BENZALDEHYDE SALICYLOYL HYDRAZONES AGAINST PTZ AND MES INDUCED SEIZURES

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Abstract

Anticonvulsant activity of salicyloyl hydrazones of *para*/*meta*-substituted benzaldehydes was estimated after oral administration (100 mg/kg) against maximal electroshock (MES) and pentylenetetrazole (PTZ)-induced seizures in mice. According to the obtained data, hydrazones (1-7) afforded seizure protection both at short (2 h) and long (24 h) time period by blocking electroshock- and chemical-induced convulsions. Compound 3 containing bromine atom into *para* position of aryl ring was found to be the most effective compound with prolonged anticonvulsive action. Structure-activity relationships of substituted benzaldehyde salicyloyl hydrazones are discussed.

Keywords: *salicyloyl hydrazones, anticonvulsant activity, PTZ-induced seizures, MES-induced convulsions*

Introduction

The search and design of novel ameliorated molecules preventing the onset and seizures spreading without substantial side effects persists a major challenge for modern chemistry and pharmacology. According to structure-activity relationship, most anticonvulsants contain three crucial parts in their structure: aryl unit, electron donor atoms and/or NH group [1]. In this context, hydrazide/hydrazone derivatives are promising agents for drug design with anticonvulsant potential since their structure includes all the aforementioned pharmacophore groups. A series of hydrazones derived from pyridyl aldehydes/ketones, camphor, menthone, substituted benzaldehydes and isatin have been synthesized and screed for anticonvulsive action both on chemical and electrical models of seizure induction [2-6].

On the other hand, the potential of nonsteroidal anti-inflammatory drugs (NSAIDs) as anticonvulsant agent is being extensively explored nowadays [7]. Acetylsalicylic acid (aspirin), for example, was found to exhibit anticonvulsant action by decreasing the incidence of seizures in the PTZ-model [8]. Bearing in mind that in organism acetylsalicylic acid is metabolized by carboxylesterases to salicylic acid [9], precisely the latter might be responsible for the pharmacological effect.

Taking into account the above, hydrazones based on salicyloyl hydrazide and *para*/*meta*-substituted benzaldehydes were evaluated for their anticonvulsive effect in pentylenetetrazole (PTZ) and maximal electroshock (MES)-induced seizures test.

Methods

Compounds

Salicyloyl hydrazones 1-7 (Table 1) have been synthesized by standard method – condensation of appropriate *para*/*meta* substituted benzaldehydes with equimolar amount of salicylic acid hydrazide; physico-chemical properties were described previously [10].

Animals

Anticonvulsant activity of salicyloyl hydrazones 1–7 was studied using outbreed male white mice (18–22 g) as experimental animals. All animals were kept under 12 h light regime and in a standard animal facility with free access to water and food, in compliance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Specific Purposes (Strasbourg, 1986) and the principles of the National Ukrainian Bioethics Congress (Kyiv, 2003). All the animals were purchased from Odessa National Medical University, Ukraine. The Animal Ethics Committee (agreement No. 12/2018) of Odessa National University (Ukraine) approved the study.

Drug Administration

Antiseizure action of salicyloyl hydrazones 1–7 was evaluated at 2 h and 24 h after administration. The compounds were administered orally to mice in Tween 80/water emulsion at a dose of 100 mg/kg and Tween 80/water emulsion has been used as a vehicle control.

Pentylenetetrazole (PTZ) induced convulsion

The anticonvulsant effect of compounds 1–7 was evaluated upon intravenous infusion of 1% aqueous PTZ solution into a tail vein as described in [11]. Pentylenetetrazole doses for inducing clonic-tonic convulsions (DCTC) and tonic extension (DTE) were calculated relative to control. The antiseizure activity of compounds was assessed at certain time points (1 and 24 h) from the increase of PTZ minimum effective dose (MED) compared with a control group. MED in percent was calculated using the formula:

$$MED = V/m \times 10^4$$

where MED – minimum effective dose of PTZ inducing DCTC or DTE; V – volume of PTZ solution, ml; m – animal weight, g.

Maximal electroshock induced seizures

Maximal electroshock seizures were induced by the application of corneal electrodes with a current strength of 50 mA (50Hz) for 0.2 sec to mice pre-treated with compounds 1–7 or Tween 80/water emulsion. After electric stimulation, duration of various phases of epileptic attacks along with mortality have been determined.

Statistical analysis

All results are expressed as mean ± standard error mean (SEM). Unpaired Student's *t*-test was used to determine the statistical significance of the results. $p < 0.05$ was considered as significant.

Results

Pentylenetetrazole induced seizures

Anticonvulsant activity of salicyloyl hydrazones 1-7 was estimated after single oral administration (100 mg/kg) at short (2 h) and long (24 h) time periods. According to the obtained data, all salicyloyl hydrazones reveal antiseizure effect throughout the whole period of time as evidenced by increasing of DCTC and DTE values. In the PTZ-induced convulsion model, all synthesized compounds have shown similar antiseizure potency at 2 h after administration with an average DCTC and DTE values 170% and 172%, respectively, compared to 100% control (Figure 1).

It is noteworthy that all investigated compounds demonstrate prolonged anticonvulsant activity at 24 h after administration that is indicated as DCTC and DTE increase in 2 times compared with control group. However, as illustrated in Figure 2, salicyloyl hydrazone 3 with bromine at the 4th position of benzaldehyde moiety (R_1) was found to possess the highest anticonvulsant activity – 242% for DCTC and DTE (compared to control 100%). Thus, the introduction of bromine atom into *para* position of salicyloyl hydrazone molecule results in the inhibition of central nervous system reactions leading to suppression of the excitation process.

Maximal electroshock induced seizures

In the MES test, electrical stimuli induced the rigid extension of the hind limbs in 100% of control animals (Table 2). Two major phases of epileptic attacks were recorded for animals pre-treated with salicyloyl hydrazones 1-7: stupor and clonic convulsions. As seen, compounds 3 and 4 significantly prevented the animals' mortality at 2 h after oral administration and demonstrated 100% and 80% protection, accordingly.

Moreover, the effect of aforementioned hydrazones is exhibited at long time period (24 h) with 60% of mortality protection for each compound. In contrast, compounds 1 and 2 administered 2 h or 24 h prior to the test produced no (or insignificant) action in mice. Interestingly, mice seizures have been characterized by Straub tail when hydrazones 4, 6 and 7 were orally administered.

Discussion

Recently, the main pharmacophores have been proposed for anticonvulsants including hydrophobic binding site, electron donor groups, hydrogen bonding domain and additional electron donor/acceptor substituents that might enhance the pharmacological effect [1]. Considering the structure-activity relationship models suggested for various hydrazones with anticonvulsant activity, we may conclude that the main contribution belongs to hydrazone scaffold whereas additional substituents might enhance or reduce the effect. For example, among menthone aryl acid hydrazones, 4-chlorophenyl derivative was found to be the most active in maximal electroshock (MES) test [4] while hydrazones of 5-chloro-2-(3*H*)-benzoxazolinone-3-acetyl hydrazide with *p*-Me, *p*-NO_2 and *p*-$N(CH_3)_2$ exhibited the higher activity [12]. Along with the substituent nature, its position in aryl ring may also have substantial impact on the antiseizure effect of hydrazones. N′-[(5-chloro-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)methylene] 2/4-substituted hydrazides with an electron withdrawing group at the *para* position were thus more potent and active both in MES and scPTZ tests. Interestingly, all compounds afforded seizure prevention at short time period (0.5 h) except those with 4-chloro and 4-fluoro substituents which had longer duration of action [13].

The structures of hydrazones 1-7 correspond to the aforementioned model since comprise the following elements: hydrophobic aryl rings, electron pair donors –CO–NH–N=, hydrogen bonding site –OH and auxiliary substituents – R_1, R_2 (Figure 3).

Despite the fact that all synthesized derivatives possess antiseizure action, structure-activity relationship depending on the nature of R_1, R_2 substituents (electron donor or acceptor) has not been revealed in PTZ test. However, in PTZ infusion model the greatest anticonvulsant potency was observed for 4-bromo derivative (3) indicating that the presence of a lipophilic bromine atom may

improve the anticonvulsant properties of benzaldehyde salicyloyl hydrazone.

Similarly, the highest antiseizure action and 100% protection against death (2 h after administration) were observed in MES model for mice pretreated with compound 3. At the same time, it is worth emphasizing that hydrazone 4 (with 4-dimethylamino substituent) also significantly abolished the MES-induced tonic seizures while the structurally similar derivative 2 (containing isopropyl moiety) exhibited the lowest potency against electrical stimulation.

Summarizing the results of anticonvulsant activity, we may identify salicyloyl hydrazone 3 as compound that blockade both electroshock- and chemical-induced convulsive seizures. Bearing in mind that MES test is considered as model of generalized clonic-tonic seizures and PTZ test – generalized absence seizures [14], the dual action against these seizures types might be suggested for compound 3.

Conclusion

Based on our experimental data, we may conclude that salicyloyl hydrazones of *para*/*meta*-substituted benzaldehydes possess anticonvulsant activity both in PTZ and MES test at short and long time period (2 h and 24 h, accordingly). However, the introduction of bromine atom into *para* position of aryl ring leads to the enhanced seizure protection – compound 3 showed high potency against generalized clonic-tonic and absence seizures.

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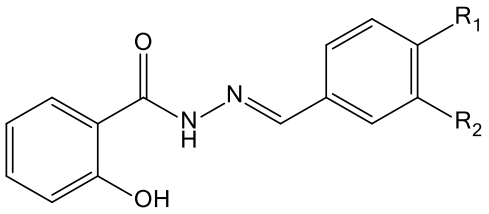
subcutaneous pentylenetetrazol seizure models. Ital J Neurol Sci 1995; 16: 73-77.

Table 1: Chemical structures of substituted benzaldehyde salicyloyl hydrazones (1-7)



1-7

Compound	R_1	R_2
1	H	H
2	*i*-Pr	H
3	Br	H
4	$N(CH_3)_2$	H
5	NO_2	H
6	H	OCH_3
7	OH	OCH_3

Table 2: Anticonvulsant effect of compounds 1-7 against maximal electroshock (MES)-induced seizures in mice

Compound	**2h**			**24 h**		
	Stupor, duration in sec	Clonic convulsions, duration in sec	% Mortality protection	Stupor, duration in sec	Clonic convulsions, duration in sec	% Mortality protection
Control	–	–	0	–	–	0
1	48[a]	–	20	–	–	0
2	60[a]	–	20	–	–	0
3	25±6.9	–	100	11±6.5	40[b]	60
4	27±7.8	–	80	55±5.0	25[b]	60
5	19±8.7	–	60	28±3.3	–	40
6	44±15.5	–	60	21±6.6	–	40
7	23±9.0	–	40	51[a]	–	20

[a]Only for one animal from the whole group (n = 5) stupor was recorded.
[b]Only for one animal from the whole group (n = 5) clonic convulsions were recorded.

Figure 1: Anticonvulsant activity of compounds 1–7 at 2 h after oral administration. Values are given as mean ± SEM, n = 5 mice; for all groups $p < 0.05$ compared with control.

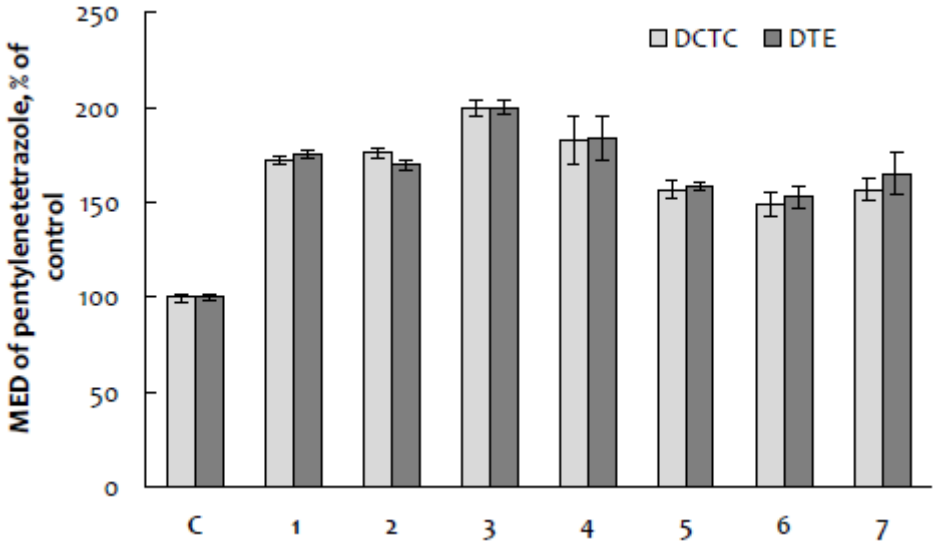


Figure 2: Anticonvulsant activity of compounds 1–7 at 24 h after oral administration. Values are given as mean ± SEM, n = 5 mice; for all groups $p < 0.05$ compared with control.

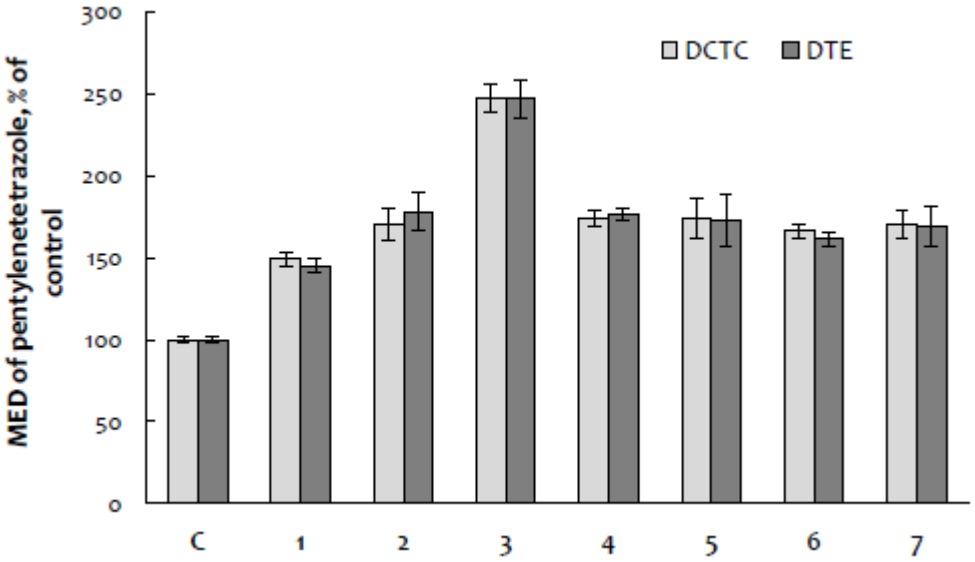


Figure 3: Proposed pharmacophores for salicyloyl hydrazones (1-7).