Bulgarian Chemical Communications, Special Issue E, (pp. 46 – 49) 2017

Pharmacological and toxicological investigations of newly synthesized benzazepine derivatives comprising peptide fragment

I.I. Kostadinova[1]*, N.D. Danchev[1], I.N. Nikolova[1], L.T. Vezenkov[2], Tch.B. Ivanov[2], M.G. Georgieva[2]

[1]*Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University - Sofia, 2 Dunav Str, 1000 Sofia (Bulgaria)*

[2]*University of Chemical Technology and Metallurgy - Sofia, 8 Kl. Ohridski Blvd, 1756 Sofia (Bulgaria)*

Received October 25, 2016 ; Revised February 20, 2017

In the present study, we investigated the toxicological and pharmacological effects of four benzazepine derivatives comprising modified dipeptides holding the residue of the N-(3, 4-dichlorophenyl)-D,L-Ala-OH. The newly synthesized compounds were tested for acute intraperitoneal toxicity (LD_{50}), potential antidepressant activity and for improvement of cognitive function in mice. The results indicated that one of the newly synthesized compounds showed lower toxicity (3 times) and antidepressant activity in forced swim test in comparison with the reference substance Mirtazapine. The compounds didn't reverse scopolamine-induced memory deficit in mice and this result indicated lack of cholinergic activity.

Key words: benzazepine derivatives, Alzheimer's disease, depression, mirtazapine, mice.

INTRODUCTION

Psychiatric disturbances affect as many as 90% of patients with Alzheimer's disease (AD) and are a major focus of treatment. Depression is one of the most frequent psychiatric complications of AD, affecting as many as 50% of patients. In this context, depression is a significant public health problem that has a serious consequences for patients and their caregivers [1].

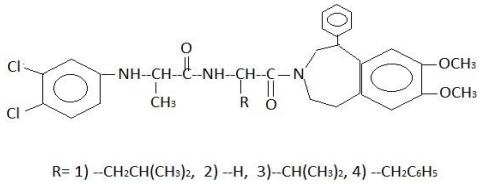
Identifying depression in someone with Alzheimer's can be difficult, since dementia can cause some of the same symptoms. Examples of symptoms common to both depression and dementia include: apathy, loss of interest in activities and hobbies, social withdrawal, isolation, trouble concentrating, impaired thinking [2].

The present study is devoted to the pharmacological and toxicological investigations of newly synthesized benzazepine derivatives including dipeptide residue. In order to evaluate the most active compounds we use standard tests for antidepressant activity and memory-induced deficit in animals.

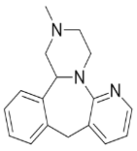
MATERIALS AND METHODS

Compounds

The investigated compounds (synthesized in University of Chemical Technology and Metallurgy-Sofia) comprise benzazepine heterocycle, connected with N-(3,4-dichlorophenyl)-D,L-Ala-OH [3] Scheme 1.



R= 1) --$CH_2CH(CH_3)_2$, 2) --H, 3)--$CH(CH_3)_2$, 4) --$CH_2C_6H_5$



Mirtazapine

Scheme 1. Chemical structure of new compounds and reference compound mirtazapine.

Treatment solutions

Test compounds were homogenized with 1-2 drops of Tween 80, dissolved in distilled water and administered intraperitoneally (i.p.) in dose 1/20 part of LD_{50} in forced swim and passive avoidance tests – compound 1 in dose 54 mg/kg, compound 2 in dose 48 mg/kg, compound 3 in dose 71 mg/kg, compound 4 in dose 82,5 mg/kg. The reference compound mirtazapine was also dissolved in distilled water and administered intraperitoneally in dose of 5 mg/kg. Compounds were administered in a volume of 10 ml/kg body weight. The newly synthesized compounds are with similar molecular weights (between 556 and 645 g/mol).

* To whom all correspondence should be sent:
E-mail: vanq_don25@abv.bg

Animals

Male albino mice line H, 25-32 g body weight. The animals were housed according GLP instruction of animal care, water and food being supplied ad libitum; animal room temperature 22 ± 3 °C; humidity 30 %; lighting schedule 12 h light/dark cycle. Animals were trained and tested during light part of the cycle. The studies were approved by the Institutional Animal Care Committee at the Medical University of Sofia, Bulgaria.

Toxicity test

The newly synthesized compounds were tested for acute intraperitoneal toxicity (LD_{50}). The acute toxicity (LD_{50}) was estimated by OECD-425 FDA method on male mice (12-15 mice per studied compound), line H, weight 25-30 g and the data are presented as mg/kg body weight [4].

Forced swim test

In a single session, mice (8 per group) are forced to swim in a narrow cylinder from which they cannot escape - 13 cm in diameter and 24 cm high, containing water (22°C) to 10 cm. Thirty minutes after intraperitoneal administration of the tested compound, the animals are placed in water containing cylinders and their behavior was observed for 6 minutes [5].

From the second minute onward, immobility of each mouse is recorded.

Scopolamine-induced amnesia in mice

The scopolamine test is performed in groups of 6 male mice weighing 25-32 g in a one-trial, passive avoidance paradigm. Thirty minutes after i.p. administration of 3 mg/kg scopolamine hydrobromide, each mouse is individually placed in the bright part of a two-chambered apparatus for training (Gemini Avoidance System, San Diego, California). After a brief orientation period, the mouse enters the second darker chamber. Once inside the second chamber, the door is closed which prevents the mouse from escaping, and a 1 mA, 3-s foot shock is applied through the grid floor. The mouse is then returned to the home cage. Twenty-four hours later, testing is performed by placing the animal in the bright chamber. The latency time in seconds for entering the second darker chamber within a 5 min test session is measured electronically. The test compounds were administered 90 min before training. A prolonged latency indicates that the animal remembers that it has been punished and, therefore, does avoid the darker chamber [6].

STATISTICAL ANALYSES

The data were processed on personal computer using standard Student t-test.

RESULTS AND DISCUSSION

The newly synthesized compounds are less toxic after i.p. administration than reference substance mirtazapine with LD_{50} values between 958 and 1650 mg/kg body weight (Figure 1).

The results showed that compounds 1, 2 and 3 decrease the duration of immobility in forced swim test in mice. There is significant difference between mirtazapine group treated with 5mg/kg and group of compound 2 with dose 48 mg/kg (Figure 2, $p < 0.05$).

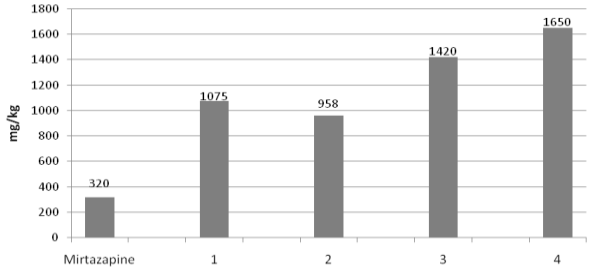


Fig. 1. Comparison between LD_{50} values of mirtazapine and newly synthesized compounds

The results showed that single administration of scopolamine (3 mg/kg, i.p.) impaired memory processes which is demonstrated with decrease in latency time. Compounds 1-4 didn't reverse scopolamine-induced memory impairment and didn't cause statistically significant increase in step-trough latency in comparison with the control group.

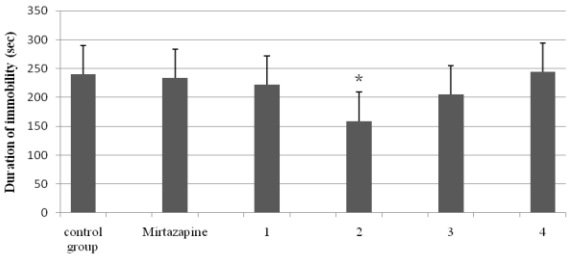


Fig. 2. The effects of compounds on the duration of immobility in the forced swim test in mice (n=8). * p≤ 0,05 (two tailed Student t-test)

Compounds 2, 3 and 4 prolonged latency times in comparison with mirtazapine (Figure 3). Acquisition trial is the first trial before administration of scopolamine and compounds. On

the second day the trial is conducted 30 minutes after scopolamine administration in dose 3 mg/kg i.p. and the retention trial is on the third day 24 h after scopolamine and 90 min after i.p. administration of compounds. For control group were performed only acquisition trial on the first day and retention trial on the second day.

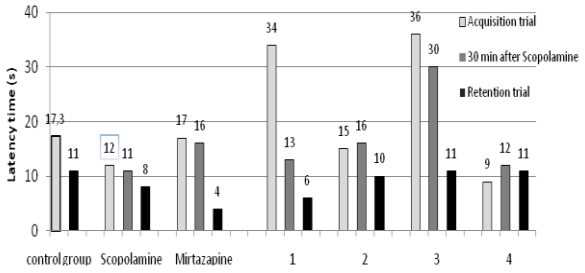


Fig. 3. Effects of investigated compounds and mirtazapine on scopolamine-induced memory deficit on passive avoidance task (n=6)

Mirtazapine is an atypical antidepressant, centrally active presynaptic alpha 2-antagonist, which increases central noradrenergic and serotonergic neurotransmission. The enhancement of serotonergic neurotransmission is specifically mediated via 5-HT_1 receptors, because 5-HT_2 and 5-HT_3 receptors are blocked by mirtazapine. Both enantiomers of mirtazapine are presumed to contribute to the antidepressant activity, the S(+) enantiomer by blocking α2 and 5-HT_2 receptors and the R(-) enantiomer by blocking 5-HT_3 receptors.

The histamine H_1-antagonistic activity of mirtazapine is responsible for its sedative properties. Mirtazapine is generally well tolerated. It has practically no anticholinergic activity and, at therapeutic doses, has practically no effect on the cardiovascular system [7].

Mirtazapine was used as a reference substance in acute toxicity, forced swim and passive avoidance experiments because of benzazepine ring, which is reason for chemical similarity to newly synthesized compounds.

Many of the primary symptoms of depression (depressed mood, low self-esteem, guilt, difficulty in concentration, suicidal ideation, thoughts of death) are by their nature difficult to model in animals. This problem is further confounded by their unknown etiology. Most theories of depression concur in suggesting that stressful life events play an important role [8].

The first tests for screening antidepressants were based on pharmacological interactions. The most common of these was reserpine antagonism. Not only had reserpine sometimes been associated with the induction of depression in humans, it also was known to cause depletion of neuronal stores of monoamines. It was effective in detecting antidepressants with mechanisms of action similar to those already in clinical use (inhibitors of monoamine uptake and metabolism), thereby demonstrating the predictive validity of reserpine antagonism.

The advent of atypical antidepressants, such as iprindole and mianserin, that showed little or no activity in these tests, necessitated the search for new screens that were not based on pharmacological interactions. One of the earliest was the forced swim or "behavioral despair" test, in which rodents become immobile when forced to swim in a restricted space from which there is no exit [5]. During the past 20 years, the behavioral despair test, in both rats and mice, has become one of the most widely used antidepressant screens in experimental pharmacology [9].

The forced swim and tail suspension procedures are best viewed as simple tests for antidepressants rather than as models of depression, because the dependent variable (immobility) is a direct reaction to the test itself and does not persist outside the test situation. There is no obvious induction of a "depressive state," although there are elements of construct validity (stressful inducing conditions, decreased behavioral output). Rodents forced to swim in a narrow space from which there is no escape adopt, after aninitial period of vigorous activity become a characteristic immobile posture, moving only when necessary to keep their heads above the water. The animals' immobility was hypothesized to show they had learned that escape was impossible and had given up hope. Immobility was therefore given the name "behavioral despair." It was subsequently found that immobility could be reduced by a wide range of clinically active antidepressant drugs. This simple behavioral procedure has since become a useful test for screening novel antidepressants [5].

There is a data that in Porsolt test mirtazapine showed decreased immobility time, but only after a 14 days treatment [10].

The administration of antimuscarinic agent to young human volunteers produces transient memory deficits [11]. Analogously, scopolamine has been shown to impair memory retention when given to mice shortly before training in a dark avoidance task [12]. The ability of a range of different cholinergic agonists to reverse the amnesic effects of scopolamine are well documented [13].

Disruption of cognitive performance by antidepressants has been documented for the

tricyclics, and occasionally observed with other compounds such as fluoxetine. The antidepressant mirtazapine has been reported to induce less disruption in tests of psychomotor performance than classical antidepressant drugs in both man and rats. The potential advantage of mirtazapine over other antidepressants in sparing cognitive function was investigated using an autoshaping task. The effects of mirtazapine were compared with a number of clinically available antidepressant drugs: imipramine, amitriptyline, desipramine and fluoxetine. The results showed that, in rats, mirtazapine is less likely to interfere with some aspects of cognition, in this case the ability to acquire and use new information [14].

Acute LD_{50} of mirtazapine is 600-720 mg/kg in mice after oral administration [15] and 320 mg/kg after i.p. administration which indicate that this compound is slight toxic. New compounds are 3 to 5 times less toxic than standard after intraperitoneal administration.

CONCLUSION

Our results indicates that the most perspective is compound 2, which possess low toxicity (958 mg/kg) after i.p. administration in mice, reduce immobility in forced swim test in comparison with control group, mirtazapine and other compounds. Compound 2 and other newly synthesized compounds didn't reverse scopolamine induced memory impairment and this result indicates that compounds didn't possess cholinergic activity.

REFERENCES

1. C.G. Lyketsos, J. Olin, *Biol. Psych.*, **52**, 243 (2002).
2. https://www.alz.org/care/alzheimers-dementia-depression.asp
3. M. Georgieva, L. Vezenkov, Tch. Ivanov, G. Ivanova, *Proc. Bulg. Acad. Sci.*, **57**, № 9, 13-18, (2004).
4. https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/acute-oral-toxicity-and-down-procedure
5. V. Castagne, P. Moser, S. Roux, R. Porsolt, *Curr. Protoc. Pharmacol.*, **49**, 5.8.1 (2010).
6. H. G. Vogel (Ed.), Drug Discovery and Evaluation, Second edition, Springer-Verlag, Berlin Heidelberg, 623, (2002).
7. Mirtazapine 30 mg Tablets, SPC, Public Assessment Report, Medicines and Healthcare products Regulatory Agency, UK (2006)
8. O.Berton, E.J. Nestler, *Nat. Rev. Neurosci*, **7**, 137-151, (2006).
9. F. Borsini, A. Meli, *Psychopharmacology*, **94**, 147 (1988).
10. E. Nowakowska, A. Chodera, K. Kus, A. Massakowska, *Pol J Pharmacol.*, **51**(6), 463, (1999).
11. D.A. Drachman, J. Leavitt, *Arch. Neurol.*, **30**, 113, (1974).
12. S.L. Dilts, C.A. Berry, *J Pharmcol. Exp. Ther.*, **158**, 279, (1976).
13. S. D. Iversen, Behavioural *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **358**(l2), R 371 (1998).
14. J.S. Andrews, S. Bloks, J.H.M. Jansen, *Biol. Psych.*,**42**, 238 (1997).
15. http://www.drugbank.ca/drugs/DB00370

ФАРМАКОЛОГИЧНИ И ТОКСИКОЛОГИЧНИ ИЗСЛЕДВАНИЯ НА НОВОСИНТЕЗИРАНИ БЕНЗАЗЕПИНОВИ ПРОИЗВОДНИ С ПЕПТИДЕН ФРАГМЕНТ

И.И. Костадинова[1]*, Н.Д. Данчев[1], И.Н. Николова[1], Л.Т. Везенков[2], Ч. Иванов, М.Г. Георгиева[2]

[1] Катедра по фармакология, фармакотерапия и токсикология, Фармацевтичен факултет, ул. „Дунав“ 2, 1000 София (България)

[2] Химикотехнологичен и металургичен университет, бул.”Климент Охридски” 8, 1756 София (България)

Постъпила на 25 октомври, 2016 г.; Коригирана на 20 февруари, 2017 г.

(Резюме)

В настоящото експериментално проучване, представяме токсикологичните и фармакологични ефекти на четири бензазепинови производни, съдържащи модифицирани дипептиди с N-(3,4-дихлорофенил)-D,L-Ala-OH в N-края. На съединенията беше определена остра интраперитонеална токсичност (LD_{50}), потенциалната антидепресивна активност и влиянието върху когнитивните функции на мишки. Резултатите показват, че едно от новосинтезираните съединения показва по-ниска токсичност (3 пъти) и антидепресивна активност в доза 48 мг/кг телесно тегло при теста за принудително плуване (forced swim test) спрямо референтното вещество Миртазапин. Съединенията не премахват скополамин-индуцирания паметов дефицит при мишки, което говори за липса на холинергична активност.