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EVALUATION OF NOOTROPIC POTENTIAL OF NOVEL OXAZOLONE DERIVATIVES USING VARIOUS SCREENING MODELS

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ABSTRACT

Memory, one of the most complex functions of the brain comprises of multiple components such as perception, registration, consolidation, storage, retrieval and decay. One of the key concerns of older adults is the experience of memory loss, especially as it is one of the hallmark symptoms of Alzheimer's disease. However, memory loss is qualitatively different in normal aging from the kind of memory loss associated with a diagnosis of Alzheimer's. Certain drugs like diazepam, barbiturates and alcohol disrupts learning and memory of animals and man. However a new class of drugs known as nootropic agents is now used in situations where there are organic disorders in learning abilities. In the present study some novel oxazolone derivatives viz: OXZ1, OXZ2, OXZ3 were evaluated for their nootropic potential. Piracetum was used as a standard drug. Elevated plus maze apparatus serves as the exteroceptive behaviour model and scopolamine induced amnesia in mice using Hebb's William maze serves as the interoceptive behaviour model. The oxazolone derivatives decreased the transfer latency in case of Hebb's William maze and showed a significant increase in inflexion ratio in case of elevated plus maze. The results obtained proved OXZ-2 to be most significant memory enhancing agents as compared to the other two derivatives. Besides OXZ-1 and OXZ-3 also proved to be effective agents in improving memory functions.

Keywords *Oxazolone derivatives, Nootropics, scopolamine, Elevated plus maze, Hebb's William maze.*

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INTRODUCTION

Memory, one of the most complex functions of the brain comprises of multiple components such as perception, registration, consolidation, storage, retrieval and decay. Memory has been classified into several types depending upon the duration for

which the information can be recalled. We have sensory memory lasting for few seconds, short-term memory, lasting for few hours. Alzheimer's disease is a neurodegenerative disorder associated with a decline in cognitive abilities. Patients also frequently have noncognitive symptoms, such as depression, apathy and psychosis that impair daily living. It is the most common form of onset of adult dementia and attention deficit disorders [1]. Centrally acting antimuscarinic drugs (like scopolamine) impaired learning and memory of rats and human beings. Benzodiazepine receptor agonists such as diazepam and alprazolam have been shown to produce anterograde amnesia in rodents and human beings. Nootropics represents a new class of psychotropic agents with selective facilitatory effect on integrative functions of the central nervous system, particularly on intellectual performance, learning capability and memory. Nootropic agents such as piracetum, aniracetum and choline esterase inhibitors like donepezil are being used for improving memory, mood and behavior but the resulting side effects associated with these agents have their applicability limited[2].

During the past few decades many results have been published in the area of the synthesis and the study of physicochemical properties of heterocyclic compounds containing a furan and anthracene rings connected with different heterocyclic systems, which have screened for various pharmacological activities and have exhibited promising effects. Oxazolone has a pivotal role in the manufacturing of various biologically active drugs with analgesic, anti-inflammatory, anti-depressant, anti-cancer, anti-microbial, anti-diabetic and antiobesity properties [3, 4]. The derivatives were shown to have antibacterial activity against an ample number of microorganisms. Their *invitro* fungicidal activity was assessed against *Fuscarium calmorum*, *Phythuim debarian*, *Rhizoctonia solanni* [5]. A large variety of oxazolone derivatives with structural variation at C-2 and C-4 positions were synthesized and evaluated as Chymotrypsin inhibitors. 2-Phenyl-5(4H)-oxazolones are important intermediates in the synthesis of several molecules including amino acids, peptides, antimicrobial or antitumor compounds and heterocyclic precursors, as well as in biosensor coupling and photosensitive composition devices for proteins [6]. It was also found that certain oxazolone derivatives selectively inhibit COX-2 in preference to COX-1. These derivatives have efficacy and good tolerance in the treatment of COX-2 mediated diseases, such as inflammation, pain, fever and asthma with fewer side effects, such as ulcerogenic activity. In the present study some novel synthesized oxazolone derivatives were evaluated for their nootropic activity.

OBJECTIVE

As it is mentioned earlier, many available nootropic agents are associated with side effects which limit their uses. Oxazolone is a novel compound which has a crucial role in the manufacturing of many biological active agents having negligible side effects. The objective of the present study is to assess the nootropic potential of some novel oxazolone derivatives.

MATERIALS AND METHODS

Test substance: Three powdered test substances OXZ-1, OXZ-2, OXZ-3 were provided by the chemistry laboratory of Himalayan Pharmacy Institute.

Name	IUPAC name
OXZ-1	4-[benzylidene]-2-(4-methoxy-phenyl) oxazole-5-one
OXZ-2	4-[methoxy benzylidene]-2-(4-methoxy-phenyl) oxazole-5-one
OXZ-3	4-[furan-3-ylmethylene]-2-(4-methoxy-phenyl) oxazole-5-(4H)-one

Determination of ld_{50}

LD_{50} (median lethal dose) is a statistically derived by administering single dose of a substance that can be expected to cause death in 50% of animals by the oral route. The LD_{50} value is expressed in terms of weight of the test substance per unit weight of the test animal (mg/kg). Acute toxicity studies were conducted for the synthesized

oxazolone derivatives in order to select a suitable dose for the evaluation of nootropic activity. The LD_{50} values of all the given oxazolone derivatives were calculated using the methods described by Organisation for Economic Cooperation and Development (OECD) guidelines, and it was found to be above 5000mg/kg [7].

Animals

8 - 12 weeks old mice were employed in the present study. The animals were provided by Himalayan pharmacy institute. They were acclimatized for the period of three days at room temperature and were housed in a standard cage under standard environmental conditions in the proportion of 12:12 hr light: dark cycle. They had free access to food and water *ad libitum*. All the procedures were performed in accordance to the Institutional Animal Ethics Committee.

Evaluation of nootropic activity

Elevated plus maze apparatus

Elevated plus-maze was used as exteroceptive behavioural model to evaluate memory in rodents. The procedure, technique and end point for testing memory was followed as per the parameters described by investigators working in the area of psychopharmacology. Briefly, the elevated plus maze apparatus consisted of a central platform connected to two open arms and two covered (enclosed) arms and the maze was elevated to a height of 50cm from the floor. In order to record transfer latency (TL), each rat was placed at the end of an open arm facing away from the central platform. The dimensions of this apparatus are Arm width (10cm), arm length (50cm), wall height (30 cm). Transfer latency was defined as the time in seconds taken by the animal to move into one of the enclosed arms with all its four legs. A fall in TL on subsequent, plus- maze exposure [8].

Five groups of mice each having 6 animals, weighing between 18-22 g, were used. Group I was maintained as control which was given distilled water(10ml/kg, p.o) once daily for 7 days, group II with Piracetum(200mg/kg,po) once daily for 7 days, group III, IV and V were treated orally with OXZ1,OXZ2,OXZ3 (400mg/kg) each respectively once daily for 7 days.

On the 7th day, 90 mins after the last dose each mouse was placed at the end of open arm, facing away from the central platform. Transfer latency (TL) was taken as the time taken by mouse to move into one of the covered arm with all its four legs. TL was recorded on the first day. If the animal did not enter into one of the covered arms within 90 sec., it was gently pushed into one of the two covered arms and the TL was assigned as 90 sec. The mouse was allowed to explore the maze for 10 sec and then returned to its home cage. Memory retention was examined 24 h after the 7th day trial. The inflexion ratio was calculated by the formula as follows [9].

$IR = (L_o – L_1)/ L_o$

Where L_o is the initial TL on the first day and L_1 is the TL on the second day.

Hebb-Williams Maze

It is an incentive based exteroceptive behavioural model useful for measuring spatial and working memory of rodents [9]. It consists of mainly three components. Animal chamber (Start Box) which is attached to the middle chamber (Exploratory area) and a reward chamber at the other end of the maze in which the reward (Food) is kept. All the three components are provided with guillotine removable doors. 12 h fasted mice were employed in the study. Each mouse was placed in animal chamber (Start Box) and door was opened to facilitate the entry of the animal into the next chamber. The door of start box was closed immediately after the animal moved into the next chamber so as to prevent its back entry. Time taken in seconds by the animal to reach reward chamber (TRC) from start box was noted for each animal [10].

Experimental protocol

Mice of about 20 to 25mg were taken and divided into five groups (n=6).

Group 1: 0.5% CMC was injected intraperitoneally for 8 days(vehicle control)

Group 2: standard group (piracetam 200mg/kg i.p) for 8 days (positive control)

Group3: scopolamine (0.4 mg/kg) was injected intraperitoneally on 8th day.

Group 4: OXZ-1 (400mg/kg) was injected intraperitoneally for 8 days
Group 5: OXZ-2 (400mg/kg) was injected intraperitoneally for 8 days
Group 6: OXZ-3 (400mg/kg) was injected intraperitoneally for 8 days
Group 7: piracetum (200mg/kg) was injected intraperitoneallly for 8 days. After 60 mins of last injection scopolamine (0.4 mg/kg) was injected.
Group 8: OXZ-1 (400mg/kg) was injected intraperitoneally for 8 days. After 60 mins of last injection scopolamine (0.4 mg/kg) was injected.
Group 9: OXZ-2 (400mg/kg) was injected intraperitoneally for 8 days. After 60 mins of last injection scopolamine (0.4 mg/kg) was injected.
Group 10: OXZ-3 (400mg/kg) was injected intraperitoneally for 8 days. After 60 mins of last injection scopolamine (0.4 mg/kg) was injected.

Transfer latency was noted at 45 min after the injection on the 8th day and after 24 h (i.e.) on the 9th day in all the treated animal.

On the first day all the mice were familiarized with Hebb's William Maze for a period of 10 min. This is known as training session. On 2nd day i.e. on 9th day the mouse was placed in the entry chamber and the timer was activated as soon as the mouse left the entry chamber. The time taken for the mouse to reach the reward chamber was taken as the transfer latency. For each animal four readings were noted, the average was taken as learning score (transfer latency) for that animal. Lower score of assessment indicates efficient learning while higher score indicates poor learning in animals. During learning assessment, the animals were exposed to food and water only after 1 hour of maze exposure [11].

RESULTS

Table 1. Nootropic potential of oxazolone derivatives in mice with EPM apparatus.

Group	Treatment	Dose	Inflexion ratio
Group I	Distilled water	10ml/kg, po	0.865±0.0243
Group II	Piracetum	200mg/kg	2.743±0.592***
Group III	OXZ-1	400mg/kg	1.978±1.743**
Group IV	OXZ-2	400mg/kg	2.112±1.985***
Group V	OXZ-3	400mg/kg	1.322±1.876*

Data expressed as mean ±SEM. Evaluation by one way ANOVA followed by Dunnett's multiple comparison tests. Significant at **P<0.01 and ***P<0.001 insignificant at P<0.01* as compared to control.

Table 2. Nootropic potential of oxazolone derivatives in mice with Hebbs william maze

Groups	Treatment Dose	Transfer latency in secs	
		8^{th} Day	9^{th} day
Group I	0.5% CMC	74±1.63	67±1.36
Group II	Piracetam (200mg/kg i.p)	47±0.87*	40±1.63**
Group III	Scopolamine	89±1.91	95±3.73
Group IV	OXZ-1 (400mg/kg)	59±1.56*	46±1.63**
Group V	OXZ-2 (400mg/kg)	47±1.65*	32±1.97***
Group VI	OXZ-3 (400mg/kg)	53±1.39*	44±3.65**
Group VII	Piracetum(200mg/kg + scopolamine (0.4mg/kg)	79±3.55	72±3.86**
Group VIII	OXZ-1(400mg/kg)+ scopolamine (0.4 mg/kg)	83±2.5	80±1.79
Group IX	OXZ-2 (400mg/kg) + scopolamine (0.4 mg/kg)	83±0.66	65±1.58***
Group X	OXZ-3 (400mg/kg) + scopolamine (0.4 mg/kg)	64±2.94	61±1.86

Data expressed as mean ±SEM. Evaluation by one way ANOVA followed by Dunnett's multiple comparison tests. Significant at **P<0.01 and ***P<0.001 in as compared to control.

DISCUSSION

The nootropic evaluation of the oxazolone derivatives (OXZ-1,OXZ-2,OXZ-3) were performed using elevated plus maze apparatus and Hebb William maze. When elevated plus maze apparatus was used, OXZ-2 showed increased inflexion ratio in compared to normal control which may be due to improved oxygen supply to brain and increased nerve transmission. OXZ-1 and OXZ-3 also showed increased inflexion ratio but less than OXZ-2.

In case of Hebb William apparatus, only OXZ-2 showed significant decrease in transfer latency on day 9 as compared to the normal control group. Repeated injection of Oxazolone derivatives has improved learning abilities and memory capacities in mice, whereas OXZ-1 and OXZ-3 revealed the average effects in improving memory functions. OXZ-2 further reversed the scopolamine induced memory impairment which indicates that they are acting through Ach receptors because they had reversed the amnesic effect of scopolamine which is a muscarinic receptors antagonist[11]. The results obtained indicate that the OXZ-2 possess promising memory enhancement property and be used as nootropics after proper evaluation and clinical trials.

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