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Methods for Assessing the Anxiolytic Activity of Derivatives of the Synthetic Active Substance 1,3,4-Oxadiazole

S. A. Sultanov [1]

Z. I. Sanoev [2]

Abstract: We created a class of 1,2,4-oxadiazole derivatives with mGlu4 receptor positive allosteric modulatory activity in consideration of the allosteric modulation of mGlu receptors for possible therapeutic uses. The elevated plus-maze (EPM) and the light-dark box test, which gauge an animal's preference for open vs closed areas, are two in vivo animal models used to evaluate the anxiolytic effect of 1,3,4-oxadiazole derivatives. Increased time spent in the light compartment of the light-dark box or entries into the open arms of the plus-maze are indicators of anxiolytic activity in these tests. Other popular techniques include using in vitro binding assays to look for interactions with receptors like the GABA-A receptor and assessing the effect on anticonvulsant models like the pentylenetetrazole (PTZ) and maximal electroshock (MES) tests, since anxiolytic and anticonvulsant effects can be related. The present study synthesized novel 1,3,4-thiadiazole derivatives and examined their antinociceptive effects on nervous system nociceptive pathways. Tail-clip, hot-plate, and acetic acid-induced writhing tests were used to assess these compounds' responses to mechanical, thermal, and chemical stimuli, respectively. Additionally, an activity cage was used to measure the animals' locomotor activity. Compounds 3b, 3c, 3d, 3e, 3g, and 3h were shown to have centrally mediated antinociceptive activity by increasing mice's reaction times in both the hot-plate and tail-clip tests. Furthermore, the treatment of compounds 3a, 3c, 3e, and 3f significantly reduced the amount of writhing behaviors, indicating that these four compounds elicited peripherally mediated antinociceptive activity. Compounds 3a, 3c, and 3f considerably reduced the mice's horizontal and vertical locomotor activity, according to the activity cage tests. These three substances may exhibit non-specific antinociceptive behavior due to potential sedative effects or motor limitations.

Keywords: Anxiolytic, Climbing plus maze test, Open space test, ladder (ladder) test, Social isolation test, Kick test, 3R, In Silico, in vitro, in vivo..

[1] Basic doctoral student at the Institute of Chemistry of Plant Substances named after Academician S.Y. Yunusov, Assistant Professor, Department of Pharmacology, and Tashkent State Medical University,
E-mail: sardorsultanov274@gmail.com

[2]PhD, Associate Professor, Department of Pharmacology, Tashkent State Medical University

Introduction. There is a lot of pharmaceutical interest in substituted 1,3,4-oxadiazoles. The -N = C-O- grouping of 2,5-substituted diphenyl-1,3,4-oxadiazoles is linked to a variety of biological functions. We have created substituted derivatives of 1,3,4-oxadiazole (XIII-XXII), a versatile hydrophobic molecule with preliminary CNS properties, in light of the wide range of biological properties associated with this compound. The goal is to enhance the biological activities with minimal or no toxicities. The most frequent clinical cause for seeking medical attention is pain, an unpleasant feeling that can range in intensity from mild to severe to indescribable. The development of analgesic molecules for the treatment of pain is still a topic of continuous research, despite the abundance of analgesic drugs already on the market. Today, mental health problems, especially anxiety disorders (anxiety states), are widespread all over the world, and their treatment with effective and safe medicines is one of the urgent problems. According to the World Health Organization (WHO)As of September 2, 2025, more than 1 billion people are living with mental illnesses, with conditions such as anxiety and depression causing significant damage to human and economic development.Anxiety is an emotional state that includes various symptoms of anxiety, fear, and emotional arousal [1,2,3]. There are 2 types of anxiety: the first is normal anxiety, which occurs at a certain time and with a certain intensity in relation to an event that does not depend on a person, and is an expected reaction in physiological conditions, and the second is pathological anxiety, which can develop as an inadequate reaction in terms of duration or severity.and it is unpredictable in physiological conditions. As a result, it becomes defensive and offensive against the threat directed at it. There are 2 types of anxiety: the first is a normal anxiety that occurs at a certain time and with. While many countries have tightened their mental health policies and programs, more investment and efforts are needed worldwide to expand services to protect and promote people's mental health. Most of the existing drugs with anxiolytic effects have sedative properties that are addictive or negatively affect cognitive functions. While many countries have tightened their mental health policies and programs, more investment and efforts are needed worldwide to expand services to protect and promote people's mental health. Most of the existing drugs with anxiolytic effects have sedative properties that are addictive or negatively affect cognitive functions [4,5,6]. Therefore, the search for new synthetic compounds and the assessment of their pharmacological activity is an important area of modern psychopharmacology. Structural compounds of 1,3,4 — oxadiazole are heterocyclic compounds with high biological activity, and positive data are available in the scientific literature on their anxiolytic, antidepressant, anticonvulsant, and neuroprotective effects. The assessment of the anxiolytic activity of new derivatives belonging to this class lays the foundation for the development of promising therapeutic agents by identifying the relationship between their molecular structure and pharmacodynamic properties. These research methods serve to form innovative approaches in psychopharmacology by evaluating the pharmacological activity of new derivatives. Against the background of technology development, in order not to cause suffering to animals from a bioethical point of view, 3R (replace, reduce, refine), *in vitro*, organoids and computer models are also used, which currently occupy a central place in preclinical research [7,8,9].

The purpose of the study. Providing detailed information on the assessment of the anxiolytic activity of 1,3,4-oxadiazole derivatives and other new substances in experimental animal models and methods for determining their pharmacodynamic properties.

Discussion of research methods. The main experimental methods used to evaluate anxiolytic activity are the following; initially, new compounds are being studied In-Silico (docking, ADMET prognosis) and *in-vitro* (metabolic stability, binding to plasma proteins, CYP inhibition) The effects of anxiolytic drugs or new chemical compounds (including 1,3,4-oxadiazole derivatives) are mainly evaluated on various behavioral models in laboratory experimental animals (mice, rats, guinea pigs, rabbits). Below is information about the methods that affect the behavior [1,4,5]**.**

1. The Ascending Plus Maze test, described by File and his collaborators in 1985, is an experimental model based on research. This test is used to study the behavioral, physiological, and pharmacological effects of medications by evaluating emotional activity. The ascending plus maze test, described by File

and his collaborators in 1985, is an experimental model based on research. This test is used to study the behavioral, physiological, and pharmacological effects of medications by evaluating emotional activity. During the experiment, the animal is placed in a closed corridor and the time elapsed before it entered the open corridor, as well as the duration of its stay in the open corridor, is estimated. The aim of the study is for anxiolytic (anxiety-reducing) substances to prolong both of these time periods, while anxiogenic (anxiety-causing) substances to shorten them [11,12,13].

2. Outdoor test. This is one of the most commonly used tests to determine the emotional state of an experimental animal before any practice, as well as the changes that may occur after practice. This test is also used to determine emotional states, locomotor activity, and sedation levels associated with anxiety (anxiety). The test is outdoors. This is one of the most commonly used tests to determine the emotional state of an experimental animal before any practice, as well as the changes that may occur after practice. This test is also used to determine emotional states, locomotor activity, and sedation levels associated with anxiety (anxiety). There are many test options in the form of a circle, square, rectangle, and in a lighted environment enriched with tunnels, platforms, and columns. The circle-shaped model was originally described by Hall and his collaborators in 1934 to assess the emotional state of animals. Later, in 1971, Soubry developed a square-shaped version [14,15,16].

When an animal is placed in an open space, its disturbing behavior is influenced by the following two factors:

1. Being isolated from your surroundings and placed in an unfamiliar place alone
2. Fear of wide-open spaces, called agoraphobia.

Because an open and spacious environment is a stressful state for rodents. The duration of the animal's stay outdoors during the test can range from 2 to 20 minutes, but is usually set to 5 minutes. During this time, the following behavioral indicators are identified:

Horizontal movement: moving from one square to another.

Vertical movements: standing on its hind legs.

Itching and the number of bowel movements.

Motor activity-by the number of crossings over the line,

Environmental learning behavior is assessed by the number of standing on its hind legs.

On the other hand, itching and defecation are considered as indicators of vegetative functions [15,16,17].

3. **Ladder (ladder) test.** This test is a fast and practical method for assessing anxiolytic activity and is widely used in the laboratory [5,6].
4. **Social isolation test.** It is a widely used, objective and sensitive experimental method for evaluating the effectiveness of drugs for anxiety and stress in animals [1,2].
5. **A test for depletion of nutrition in a new environment.** In this test, the animal is starved for 48 hours, and then placed in a cage in a new environment different from the cage in which it was studied (its own). After that, the amount of food consumed by the animal is determined. The new environment causes stress and anxiety in the animal. As a result, he eats less or postpones meals than usual.. A power depletion test in a new environment. In this test, the animal is starved for 48 hours and then placed in a cage [1,5,6].
6. **Conditional resistance test.** This is an experimental model aimed at assessing the level of anxiety in an animal by artificially creating a motivational contrast. The animal strives for a reward (water or food) based on a conditioned reflex, but the act of achieving a reward carries the risk of punishment by electric shock. Anxiolytic substances increase the animal's desire for reward, despite the risk of punishment [7,9,11].

7. **Passive retention test.** This is a classic method used to evaluate an animal's memory based on previous experiences and anxiety-based avoidance behavior. At the first stage of the experiment, the animal moves from the light part to the dark part, since rodents naturally prefer a dark environment. An electric current is applied at the entrance to the dark section. At the next stage, the animal is placed back in the light compartment and it is ensured that it does not fall into the dark compartment. Anxiolytic substances reduce the access and time spent by an animal in a dark compartment – this indicates a decrease in anxiety levels [3-7].
8. **The kicking test.** It is used to evaluate the emotional and behavioral changes that occur in response to stress in experimental animals. The animal is placed in a special cage, in the lower part of which there are grids that allow electric current to pass through.Psycholytic substances reduce the access and time spent by an animal in a dark compartment – this indicates a decrease in anxiety levels [6-11].

Discussion. Some commonly used medications, such as carbamazepine, phenobarbital, phenytoin, diazepam, etc., are available on the market to manage seizure attacks in order to improve patient quality of life (QOL) and lessen the burden of disease. However, using these medications is also associated with gastrointestinal issues, dizziness, somnolence, and addictive potential. Furthermore, 30% of the population has been found to be ineffective with these drugs. Therefore, in order to enhance patient quality of life, the health system must create safe and efficient treatments for epilepsy [1,7,8,9]. One of the pertinent areas of contemporary psychopharmacology is the evaluation of the anxiolytic potential of 1,3,4-oxadiazole derivatives, which have a high biological activity. A thorough examination of the behavioral and neuropharmacological effects of recently synthesized compounds is made possible by the models "ascending plus maze," "open space", "ladder", "social isolation", "conditional confrontation," and others used to evaluate the anxiolytic effect. The pharmacodynamic characteristics, effects on neurotransmitter systems, and potential therapeutic benefits of 1,3,4-oxadiazole derivatives are determined through the integration of *In Silico, in vitro* and *in vivo* approaches into preclinical research, along with compliance with bioethical criteria based on 3R principles. The results will serve as the basis for the development of new, effective, and less side-effect anxiolytic agents for future clinical use. In general, the methods studied have significant scientific and practical significance for assessing new chemical compounds as anxiolytics, as well as for clinical trials [12-17].

Conclusions. 1,3,4-oxadiazole derivatives have high biological activity, and the assessment of their anxiolytic potential is one of the relevant areas of modern psychopharmacology.1,3,4-oxadiazole derivatives have high biological activity, and the assessment of their anxiolytic potential is one of the relevant areas of modern psychopharmacology. The models "ascending plus maze", "open space", "ladder", "social isolation", "conditional confrontation" and others used to assess the anxiolytic effect allow for a deep study of the behavioral and neuropharmacological effects of newly synthesized compounds.

The integration of *In Silico, in vitro* and *in vivo* approaches into preclinical research, as well as compliance with bioethical criteria based on 3R principles, ensure that new substances are safe and effective. These methods are used to determine the pharmacodynamic properties, effects on neurotransmitter systems, and potential therapeutic benefits of 1,3,4-oxadiazole derivatives. The results will serve as the basis for the development of new, effective and less side-effect anxiolytic agents for future clinical use. In general, the methods studied have important scientific and practical significance for evaluating new chemical compounds as anxiolytics, determining their safety profile, and selecting promising candidates for clinical trials.

The results revealed that B3 is a promising candidate possesses good binding affinity with GABA and form stable complex as confirmed by both molecular docking and molecular dynamic simulation studies. In addition to its role in correction of the behavioral deficits induced by PTZ, It increased the latency of seizures, elevated the level of GSH and GST, and meanwhile decreased the level of LPO and iNOS. It can reverse the PTZ induced pathological changes as established by H & E and IHC staining of brain.

Therefore, the neuroprotective property of B3 may be arbitrated to the down regulation of TNF-α, which is found to be up regulated in PTZ-induced seizures. Hence, the available results explain the potential of B3 for attenuating neurodegeneration and neuroinflammation caused by PTZ.

References.

1. Faheem M, Althobaiti YS, Khan AW, Ullah A, Ali SH, Ilyas U. Investigation of 1, 3, 4 Oxadiazole Derivative in PTZ-Induced Neurodegeneration: A Simulation and Molecular Approach. J Inflamm Res. 2021;14:5659-5679 https://doi.org/10.2147/JIR.S328609
2. Varvel NH, Jiang J, Dingledine R. Candidate drug targets for prevention or modification of epilepsy. Annu Rev Pharmacol Toxicol. 2015; 55:229–247. doi:10.1146/annurev-pharmtox-010814-124607
3. Rosenow F, Strzelczyk A. Behavior. Individualized epilepsy management: medicines, surgery, and beyond. Epilepsy Behav. 2019;91:1–3. doi:10.1016/j.yebeh.2018.09.045
4. Rosenow F, Strzelczyk A. Behavior. Individualized epilepsy management: medicines, surgery, and beyond. Epilepsy Behav. 2019;91:1–3. doi:10.1016/j.yebeh.2018.09.045
5. Ali A, Shah FA, Zeb A, et al. NF-κB inhibitors attenuate MCAO induced neurodegeneration and oxidative stress—a reprofiling approach. Front Mol Neurosci. 2020;13:33. doi:10.3389/fnmol.2020.00033
6. Alvi AM, Al Kury LT, Alattar A, et al. Carveol attenuates seizure severity and neuroinflammation in pentylenetetrazole-kindled epileptic rats by regulating the Nrf2 signaling pathway. Oxid Med Cell Longev. 2021;2021:452.
7. Mohammadi-Khanaposhtani M, Shabani M, Faizi M, et al. Design, synthesis, pharmacological evaluation, and docking study of new acridone-based 1, 2, 4-oxadiazoles as potential anticonvulsant agents. Eur J Med Chem. 2016;112:91–98. doi:10.1016/j.ejmech.2016.01.054
8. Biernacki K, Daśko M, Ciupak O, Kubiński K, Rachon J, Demkowicz S. Novel 1, 2, 4-oxadiazole derivatives in drug discovery. Pharmaceuticals. 2020;13(6):111. doi:10.3390/ph13060111
9. El-Missiry MA, Othman AI, Amer MA, Sedki M, Ali SM, El-Sherbiny IM. Nanoformulated ellagic acid ameliorates pentylenetetrazol-induced experimental epileptic seizures by modulating oxidative stress, inflammatory cytokines and apoptosis in the brains of male mice. Metab Brain Dis. 2020;35(2):385–399. doi:10.1007/s11011-019-00502-4
10. McCorvy JD, Butler KV, Kelly B, et al. Structure-inspired design of β-arrestin-biased ligands for aminergic GPCRs. Nat Chem Biol. 2018;14(2):126–134. doi:10.1038/nchembio.2527
11. Fabjan J, Koniuszewski F, Schaar B, Ernst M. Structure-guided computational methods predict multiple distinct binding modes for pyrazoloquinolinones in GABAA receptors. Front Neurosci. 2021;14:1452. doi:10.3389/fnins.2020.611953
12. Van Erum J, Van Dam D, De Deyn PP. PTZ-induced seizures in mice require a revised Racine scale. Epilepsy Behav. 2019;95:51–55. doi:10.1016/j.yebeh.2019.02.029
13. Imran M, Al Kury LT, Nadeem H, et al. Benzimidazole containing acetamide derivatives attenuate neuroinflammation and oxidative stress in ethanol-induced neurodegeneration. Biomolecules. 2020;10(1):108. doi:10.3390/biom10010108
14. Singh P, Sharma PK, Sharma JK, Upadhyay A, Kumar N. Synthesis and evaluation of substituted diphenyl-1,3,4-oxadiazole derivatives for central nervous system depressant activity. Org Med Chem Lett. 2012 Mar 1;2(1):8. doi: 10.1186/2191-2858-2-8.
15. Ahmad S, Khan AU, Faheem M, et al. Computational and pharmacological evaluation of stevioside derivatives for antinociceptive and antiinflammatory potential. Trop J Pharm Res. 2020;19(8):1677–1684. doi:10.4314/tjpr.v19i8.16

16. Faheem M, Ali SH, Khan AW, et al. 1, 3, 4-Oxadiazole derivative attenuates chronic constriction injury induced neuropathic pain: a computational, behavioral, and molecular approach. Brain Sci. 2020;10(10):731. doi:10.3390/brainsci10100731
17. Altıntop MD, Can ÖD, Demir Özkay Ü, Kaplancıklı ZA. Synthesis and Evaluation of New 1,3,4-Thiadiazole Derivatives as Antinociceptive Agents. Molecules. 2016 Aug 1;21(8):1004. doi: 10.3390/molecules21081004.