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ADVANCED SCREENING PLATFORMS FOR THE DEVELOPMENT OF NEXT-GENERATION ANXIOLYTIC AND ANTIDEPRESSANT COMPOUNDS – A REVIEW ARTICLE

Mrs.Naina Sara Sabu[1*], Ms. Neha Robinson[2], Ms. Rameeza Shanavas[3], Mr. Akash Ben[4]

[1*] Assistant Professor, Department of Pharmacology, Chemists College of Pharmaceutical Sciences and Research, Varikoli, Ernakulam affiliated to Kerala University of Health and Sciences, Thrissur, Kerala, 680596, India

[2,3,4] Students, Chemists College of Pharmaceutical Sciences and Research, Varikoli Ernakulam affiliated to Kerala university of Health and Sciences, Thrissur, Kerala, 680596, India

Abstract:

Anxiety and depression are complex neuropsychiatric disorders with multiple causes involving neurotransmitter imbalances, neuroendocrine dysregulation, impaired neuroplasticity. The development of effective therapeutic agents requires an integrated approach combining neurobiological understanding with advanced screening methods. This review highlights the neurobiological basis of anxiety and depression, focusing on neurotransmitter systems, brain regions involved, HPA axis dysfunction, neuroinflammation, BDNF mediated neuroplasticity. Further it talks about the role of in silico approaches, including target selection, molecular docking, QSAR studies and ADMET prediction in accelerating drug discovery. A comprehensive examination of various in vitro and in vivo screening strategies for evaluating anxiolytic and antidepressant activity is also provided. Using computational, biological, and behavioural methods is an effective strategy to the discovery and development of new therapeutic medicines.

Keywords: *Anxiety, ADMET, Depression, Drug Discovery, In Silico Screening, In Vitro Assays, In Vivo Models, Molecular Docking, Neurobiology, QSAR.*

Corresponding author:

Mrs.Naina Sara Sabu,
Assistant Professor, Department of Pharmacology,
Chemists College of Pharmaceutical Sciences and Research,
Varikoli, Ernakulam affiliated to Kerala University of Health and Sciences,
Thrissur, Kerala, 680596, India
Email id : zarasabu1@mail.com

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1. INTRODUCTION:

Some of the main factors responsible for the severity of worldwide health issues are mental health disorders. The Global Burden of Diseases, Injuries, says anxiety and depression are the two most prevalent mental health conditions. Over 300 million people worldwide suffer from depression, which is the main cause of disability. Important features of the cellular and molecular cause of depression remain mostly unknown. Major depressive episodes affect 17% of men and 25% of women at some point in their lives. Recurrence is very common, with up to 60% of people having more than one major depressive incident in their lifetime. Novel therapeutic agents must be produced regardless of the availability of various pharmacological treatments due to limitations such treatment resistance, adverse effects, and delayed onset of action. So as to recognize promising compounds more quickly, modern drug discovery combines statistical and experimental screening techniques with neurobiological understanding. An extended overview of these integrated methods, is provided in this article.

2. Neurobiological basis of anxiety and depression

The complex relationships between neurochemical, structural, and molecular pathways within the central nervous system comprise the neurobiological bases of anxiety and depression. Major neurotransmitter systems, such as serotonin, dopamine, and norepinephrine, which control mood, thought processes, and emotional reactions, are mostly related to such diseases. Regardless of neurotransmitter dysregulation, excessive stress responses and poor emotional processing are also caused by changes in certain brain regions, that include the hippocampus, prefrontal cortex, and amygdala. These conditions are made worse by dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, which induces prolonged stress and high cortisol levels. Neuro inflammation and reduced levels of brain-derived neurotrophic factor (BDNF), which both affect neuroplasticity and neuronal survival, are also highlighted by this research.

2.1 Neurotransmitter system

[28] A neurotransmitter system is a functional network of neurons that synthesize, release, and respond to a particular neurotransmitter to transmit information across synapses, thereby modulating neural activity and controlling physiological and behavioural processes. Neurotransmitter serve as chemical messengers playing a crucial role in information processing throughout the nervous system, and are essential for healthy physiological and behavioural functions in the body. Neurotransmitter systems are classified as cholinergic, glutamatergic, GABAergic, dopaminergic, serotonergic, histaminergic, or aminergic systems, depending on the type of neurotransmitter secreted by the neuron, allowing effector organs to carry out specific functions by sending nerve impulses. There are more than 40 neurotransmitters in the human nervous system; some of the most important are acetylcholine, norepinephrine, dopamine, gamma-aminobutyric acid (GABA), glutamate, serotonin, and histamine.

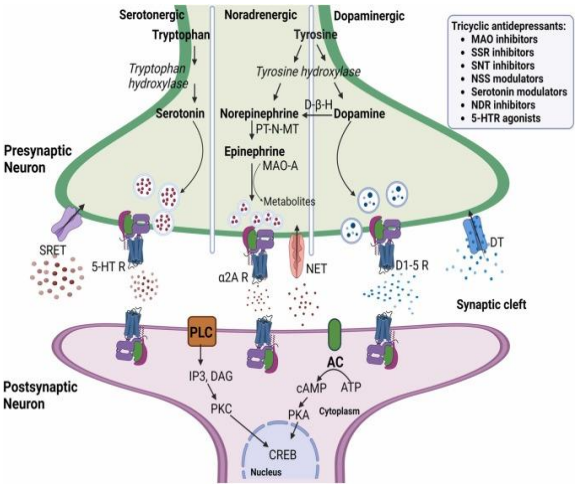


Fig.1 Neurotransmitter systems

2.2 Brain Regions Involved

[26] [27] Brain regions consistently associated with depression include the amygdala, hippocampus, orbitofrontal cortex, prefrontal cortex, amygdala, insula and temporal lobe. Amygdala is part of a larger network in your brain called the limbic system. When it comes to your survival, your amygdala and limbic system are extremely important. These are parts of your brain that automatically detect danger. They also play a role in behaviour, emotional control and learning. Fear is the main emotion that the amygdala is known to control. Structural brain abnormalities have been linked to numerous anxiety disorders. Rodents exposed to stressful experimental conditions showed impaired hippocampal neurogenesis, while human research into anxiety disorders and brain morphology discovered an association between hippocampal volume and generalized anxiety disorder (GAD), though this was not always consistent. when compared to healthy controls, while larger prefrontal volumes were observed in anxious adults compared to controls, but not in children or older adults.

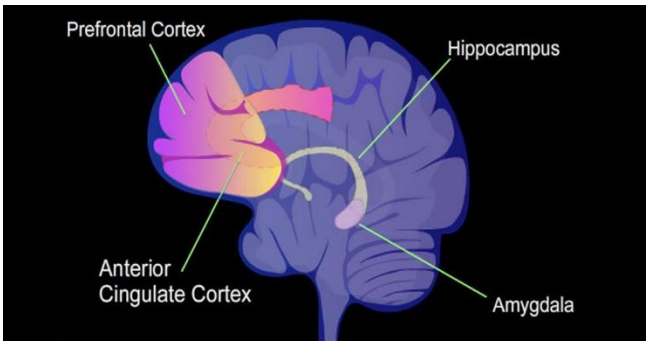


Fig.2 Brain Regions Involved

2.3 HPA Axis Dysfunction

[25] The hypothalamic-pituitary-adrenal (HPA) axis plays a pivotal role in the body's response to stress, regulating the release of glucocorticoids. In chronic exposure to these glucocorticoids contribute to various neurological disorders, including generalised anxiety disorders (GAD), Alzheimer's disease (AD), and depression. Chronic stress or disease that interferes with the brain-adrenal feedback loop can cause HPA axis dysfunction, which can result in abnormal cortisol rhythms (high or low), weakness, anxiety, insomnia, and issues with metabolism. The main function of your HPA axis is to release cortisol (a glucocorticoid, or steroid hormone). This kicks off short-term bodily changes that allow you to respond to stress. The stress response is an automatic and instinctual process.

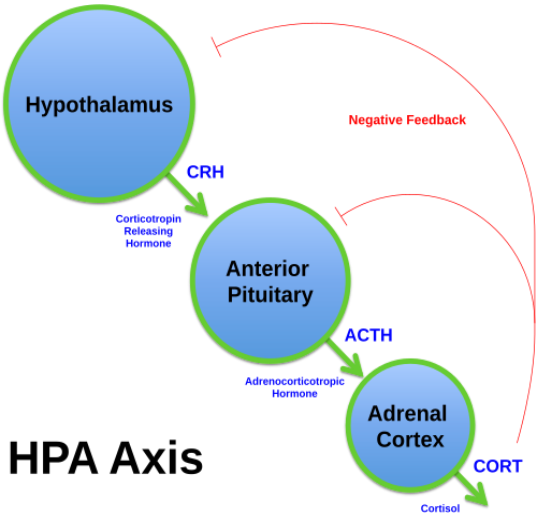


Fig.3 HPA axis dysfunction

3. In silico and Computational Screening

[11] [13] [15] In-silico or computational screening describes the use of computer-based technologies to identify and evaluate possible drug candidates before conducting laboratory or clinical testing. In Major Depressive Disorder and Anxiety Disorder research, these methods are increasingly being employed to accelerate up the development of new therapeutic compounds by predicting how molecules interact with biological targets involved in mood regulation. Instead of testing thousands of chemicals experimentally, researchers use computer programs to mimic molecular interactions, saving time, money, and reducing the amount of compounds evaluated in laboratory studies. Several computational screening methods are employed in drug discovery to discover possible compounds that could be beneficial in treating diseases like Major Depressive Disorder and Anxiety Disorder. These techniques use computer simulations, databases, and algorithms to figure out how chemicals interact with biological targets involved in mood regulation and stress responses. Various computational techniques utilized involve virtual screening, QSAR modelling, molecular dynamic simulations, and pharmacophore modelling. Virtual Screening is another popular computer tool for evaluating immense chemical libraries. Thousands to millions of chemicals stored in digital database are screened using computer methods to select those with the highest possibility of binding to a certain target.

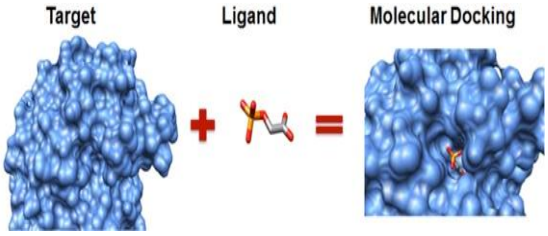
3.1 Target selection

[24] Target selection is an important early stage in the drug discovery process, especially in the development of medicines for neurological and psychiatric illnesses including major depressive disorder and anxiety disorder. This stage includes identifying and validating a particular biological molecule such as a receptor, enzyme, transporter, or signalling protein, that plays a critical part in the disease process and may be affected by a drug. In the case of mental health diseases such as major depressive disorder and anxiety disorder, researchers first identify the molecular targets involved in the cause of the problem. These targets could include proteins involved in neurotransmitter signalling or stress response systems, such as the Serotonin Transporter, which regulates serotonin levels in the brain. Once a suitable target has been found and confirmed, computational screening techniques are utilized to look for chemical compounds that interact well with that target**.** Targets in mood disorders are often linked with neurotransmitter systems, stress response mechanisms, or inflammatory pathways that affect brain function and emotional regulation.

3.2 Molecular Docking

[1] [12] Molecular docking is a powerful computational tool used in drug development to predict the interaction of a tiny molecule (ligand) with a biological target, such as a receptor or

enzyme. Molecular docking is used in research on psychiatric diseases such as Major Depressive Disorder and Anxiety Disorder to identify. prospective medication candidates that can attach to proteins involved in mood regulation and brain signalling The method uses computer algorithms to model how a molecule fits into the active site of a target protein and to quantify the strength and stability of this interaction using binding affinity scores. Docking studies examine the interactions with proteins involved in neurotransmitter systems or signalling pathways associated to stress and neural plasticity, like mTOR in the mTOR signalling pathway.



Molecular docking generally comprises multiple phases. First, the three-dimensional structure of the target protein is determined using structural databases or experimental studies. Potential ligands, which are generally obtained from chemical libraries or natural compound databases, are then generated and optimized for docking study. The docking software then puts each ligand into the protein's binding site and determines potential binding orientations. A scoring function ranks these orientations according to projected binding strength, allowing researchers to find drugs with strong interactions with the target. One of the most significant advantages of molecular docking is that it enables researchers to screen a large number of compounds quickly and cost-effectively prior to doing laboratory trials. This decreases the amount of molecules that must be manufactured or examined in biological experiments. Docking studies are employed in anxiety and depression research to find molecules that may regulate mood modulation, neuronal plasticity, and stress responses. As a result, molecular docking is crucial in the early stages of computational drug development since it directs scientists towards potential candidate molecules that can eventually be developed into novel therapeutic drugs.

3.3 QSAR Studies

[23] QSAR analysis involves developing mathematical and statistical models that correlate specific physicochemical properties of molecules, such as hydrophobicity, electronic distribution, molecular size, and functional groups with their observed pharmacological activity. By analysing these relationships, researchers can predict how modifications in chemical structure may influence the biological effectiveness of a compound. In psychiatric illnesses including major depressive disorder and anxiety disorder, QSAR models are used to find and optimize compounds that may interact with targets engaged in neurotransmission or signalling pathways that regulate mood and stress responses. This strategy enables researchers to screen and create prospective antidepressant or anxiolytic chemicals before they are produced or tested experimentally. The significance of QSAR stems from its capacity to save time, money, and experimental effort by directing researchers to the most promising drug candidates, hence accelerating the development of new therapeutic treatments for anxiety and depression.

QSAR studies help researchers design and optimize compounds that interact with proteins involved in mood regulation, neurotransmitter signalling, and stress-response pathways. By identifying structural features that enhance interaction with targets such as neurotransmitter transporters or signalling proteins, QSAR models can guide the development of new antidepressant or anxiolytic molecules. These models also assist in predicting whether chemical modifications will improve the compound's ability to reach the brain, cross the Blood Brain Barrier, and maintain stable activity in the central nervous system.

3.4 ADMET Prediction

[12] ADMET prediction is a significant step in computational drug development because it analyses the pharmacokinetic and toxicological features of potential drug candidates prior to experimental testing. ADMET stands for absorption, distribution, metabolism, excretion, and toxicity, which together dictate how a substance acts in the human body. In studies connected to mental health problems such as Major Depressive Disorder and Anxiety Disorder, ADMET prediction plays a critical role in discovering compounds that not only demonstrate great biological activity but also possess adequate safety and pharmacological properties. In anxiety and depression research, ADMET prediction ensures that the compounds discovered by computational screening have the necessary properties for central nervous system activation. This approach enables researchers to exclude compounds with low bioavailability, insufficient brain penetration, or a significant toxicity risk. ADMET prediction saves money, time, and effort in laboratory and clinical testing by screening out inappropriate compounds early in the drug discovery pipeline. As a result, it contributes significantly to the creation of safer and more effective therapeutic medicines for anxiety, depression, and other neurological disorders. The ADMET prediction is highly important in modern drug discovery because it

helps researchers evaluate the pharmacokinetic behaviour and safety profile of potential drug candidates before conducting laboratory and clinical experiments. ADMET represents absorption, distribution, metabolism, excretion, and toxicity, which are critical factors that determine whether a compound can function effectively as a therapeutic drug.

4. Experimental Screening Strategies

4.1 In Vitro Screening Methods

4.1.1 Receptor Binding Assay

[2] Receptor binding assays are fundamental experimental techniques in pharmacology and drug discovery that quantitatively evaluate the interaction between a ligand (such as a drug, neurotransmitter, or biomolecule) and its specific receptor under controlled conditions. These assays typically involve the use of labelled ligands, most commonly radio labelled (e.g., tritium or iodine isotopes) or fluorescent probes, to detect and measure receptor-ligand binding in biological systems such as tissue homogenates, membrane preparations, or cultured cells expressing the receptor of interest. The primary objective is to determine key pharmaco-dynamic parameters including binding affinity $(K(d))$, inhibitory constant $(K(i))$, and receptor density $B\{max\}$, which are essential for understanding drug potency and selectivity.

Receptor binding assays can be broadly divided into two categories: competitive binding assays, in which an unlabelled test compound competes with a known ligand to estimate its affinity, and saturation binding assays, which measure the binding of increasing concentrations of a labelled ligand to determine equilibrium constants. By measuring the rates of association and dissociation, kinetic assays shed light on the dynamics of binding. Because they are quick, affordable, and enable screening of several compounds before moving on to in vivo investigations, these in vitro techniques are frequently utilized, greatly cutting down on time and resources in the drug development process.

4.1.2 Radio ligand binding studies

[22] Radio ligand binding assays are a specialized type of receptor binding assay that use radioactively labelled ligands to study receptor–ligand interactions with high sensitivity and precision. In this method, a ligand is tagged with a radioactive isotope such as tritium ($[\hat{}3H]$) or iodine ($[\hat{}125I]$), allowing researchers to track its binding to specific receptor sites in biological samples like membrane preparations or cultured cells. When the radio ligand binds to its receptor binding site, the amount of radioactivity associated with the receptor can be measured using instruments like scintillation counters, providing quantitative data about receptor characteristics. This technique is widely used to determine important pharmacological parameters such as binding affinity $(K(d)), receptor\ density\ (B(\{max\}))$, and inhibitory constant $(K(i))$ in competitive studies.

Radio ligand binding focuses specifically on the binding site of the receptor, which is the region where endogenous ligands (like neurotransmitters) or drugs interact. These binding sites are highly specific and can differ between receptor subtypes, allowing radio ligand assays to help identify and characterize receptor subpopulations. The assay distinguishes between specific binding (radio ligand bound to the intended receptor site) and non-specific binding (binding to unrelated sites), which is an important step for accurate data interpretation. Despite being highly sensitive and reliable, radio ligand binding assays have some limitations, including safety concerns related to radioactive materials, disposal issues, and the requirement for specialized laboratory infrastructure. However, they remain a gold standard technique in pharmacological research due to their accuracy and ability to detect even very low levels of receptor binding.

4.1.3 Cell Viability (MTT Assay)

[21] The MTT assay is a widely utilized colorimetric technique for evaluating cell viability, proliferation, and cytotoxicity in in vitro biological and pharmacological studies. It is based on the principle that metabolically active cells possess functional mitochondrial enzymes, particularly succinate dehydrogenase, which can reduce the yellow tetrazolium salt MTT into insoluble purple-coloured formazan crystals. This conversion occurs only in living cells, whereas dead or damaged cells lack the metabolic activity required for this reduction, making the assay a reliable indicator of cell viability. In drug discovery, the MTT assay serves as an essential early-stage screening tool to evaluate the safety and efficacy of new compounds before progressing to animal or clinical studies. It allows rapid identification of cytotoxic effects and helps in selecting compounds with favourable biological activity and minimal toxicity.

The MTT assay is highly valued due to its simplicity, sensitivity, reproducibility, and compatibility with high-throughput screening, making it a standard method in drug discovery, cancer research, toxicology, and neuropharmacology. However, despite its widespread use, the assay has certain limitations, such as its dependence on cellular metabolic activity rather than direct cell counting, potential interference from coloured or reducing compounds,

and variability due to differences in cell types and experimental conditions. Therefore, careful optimization and, in some cases, validation with complementary assays are necessary to ensure accurate and reliable results.

4.1.4 Neuroprotection Assays

[20] Neuroprotection assays are essential experimental approaches used to evaluate the ability of compounds to prevent, reduce, or reverse neuronal damage under conditions that mimic the pathological features of neurological and psychiatric disorders. These assays are typically conducted in in vitro systems using neuronal cell lines (such as PC12 or SH-SY5Y cells) or primary neuronal cultures, where cells are exposed to various stressors including oxidative stress (e.g., hydrogen peroxide), excite-toxicity (e.g., glutamate), neuro-inflammation (e.g., lipopolysaccharide), or glucocorticoid-induced toxicity, all of which are known to contribute to neuronal dysfunction in mood disorders.

The effectiveness of a test compound is assessed by its ability to improve cell survival, maintain neuronal morphology, and preserve cellular functions compared to untreated or stressed control groups. Common endpoints measured in neuroprotection assays include cell viability (using assays like MTT or resazurin reduction), levels of reactive oxygen species (ROS), mitochondrial membrane potential, apoptosis markers (such as caspase activation), and expression of neurotrophic factors like brain-derived neurotrophic factor (BDNF).

These assays provide insight into the mechanisms of action of potential therapeutic agents.

Despite their advantages, these assays have limitations, including differences between in vitro models and in vivo brain environments, which necessitate further validation. Nonetheless, neuroprotection assays remain a cornerstone in the development of novel treatments aimed at preserving brain function and improving mental health outcomes.

4.1.5 Mono Amine Oxidase (MAO) Inhibition Assay

[18] [19] Monoamine oxidase (MAO) inhibition assays are widely used in vitro screening methods in neuropharmacology and drug discovery, especially for identifying potential therapeutic agents targeting mood disorders such as depression and anxiety disorders. Monoamine oxidase is a mitochondrial enzyme present in neuronal and non-neuronal tissues that catalyses the oxidative deamination of monoamine neurotransmitters, including serotonin, dopamine, and norepinephrine. The fundamental principle of MAO inhibition assays is based on measuring the enzymatic activity of MAO in the presence and absence of test compounds. Typically, these assays employ specific substrates such as tyramine, kynuramine, or benzylamine, which are metabolized by MAO enzymes to produce detectable products.

There are two isoforms of this enzyme, MAO-A and MAO-B, which differ in substrate specificity, tissue distribution, and inhibitor sensitivity. MAO-A preferentially metabolizes serotonin and norepinephrine and is primarily associated with emotional regulation, while MAO-B mainly metabolizes phenyl-ethylamine and contributes significantly to dopamine metabolism in the brain. The inhibition of these enzymes leads to increased availability of monoamines in synaptic clefts, thereby enhancing neurotransmission and producing antidepressant or anxiolytic effects. Therefore, in vitro MAO inhibition assays play a critical role in early-stage screening of compounds to evaluate their potential as monoaminergic modulators before advancing to in vivo studies.

These assays also help in assessing the selectivity of compounds, which is crucial because non-selective MAO inhibition can lead to adverse effects, resulting from the accumulation of dietary tyramine. Therefore, identifying selective and reversible MAO inhibitors is a key objective in drug development.

4.2 *In Vivo* Screening Methods

4.2.1 Anxiety Models

Elevated Plus Maze (EPM)

[3] Elevated plus maze is a simple method for determining anxiolytic responses from rodents. This is done using a Y-shaped instrument with an elevated open platform and an enclosed platform. The instrument's open arm produced a strong approach-avoidance conflict while the closed arm did not. The instrument was designed to have 4 arms (2open arms and 2 closed arms) arranged in a plus shaped manner. The evaluation of anxiolytic responses from the rodents was estimated by assessing the ratio of time spent on the open arm and closed arm. The elevated plus maze is based on mice' natural fear of heights and open areas (avoidance) and a need for dark, enclosed areas (approach).

Behavioural responses in the elevated plus maze are easily assessed and quantified by an observer. Briefly, rodents are placed in the intersection of the four arms of the elevated plus maze and their behaviour is typically recorded for 5 min. Anti-anxiety behaviour can be determined simultaneously with a measure of spontaneous motor activity (total and/or closed arm entries), however the arm entries made in the maze may not be an optimal measure of motor activity.

Light/Dark Box Test

[4] The light/dark (LD) test is based on an approach-avoidance conflict between exploration of novel environments and avoidance of brightly lit, open spaces. Studies showed that time in the light compartment and distance travelled in the light also reflect anxiety-like behaviour and expanded the use of the LD test to rats. The LD test has been commonly used to evaluate anxiety-like behaviour in adult rodents, and it has also been applied to younger animals in a few studies. Risk taking behaviour peaks during adolescence and contributes to most of the major causes of adolescent injury and mortality. Although the effects of puberty and adolescence on EPM behaviour are less evident, preliminary test injections and other experimental procedures may also have an effect on age differences in behaviour because adults are more sensitive to these effects. Despite the widespread use of the LDB to assess anxiety-like behaviours in rodents, there is high variability in reported protocols. Such differences include apparatus size, testing duration, light intensity in the lit compartment, and even the compartment in which the animal is placed at the beginning of the test.

Open Field Test

[5] [6] The open field test (OFT) has been used since 1934 to analyze behaviour. The basic design is simple, consisting of an open area surrounded by walls that prevent individuals from escaping. The test's framework is based on the conflict between exploring new environments and the fearful avoidance of exposed areas. This emotional stimulation is associated with physiological responses, measurable through defecation and urination, and ultimately influences behaviour. Initially, the test was developed to measure shyness based on the number of droppings produced by the animal. “Emotional animals” would defecate more, when placed in a strange environment. The arena is often divided into central and peripheral zones, allowing researchers to assess anxiety levels based on spatial preference. Animals exhibiting higher anxiety tend to remain close to the walls (a behaviour known as *thigmotaxis*), whereas increased movement into the central area is considered indicative of reduced anxiety and potential anxiolytic effects of test compounds.

Key parameters measured in the OFT include total distance travelled, time spent in the central versus peripheral zones, frequency of rearing (vertical activity), grooming behaviour, and number of crossings. These measurements provide insights into both emotional state and locomotor activity, making the test useful not only for anxiety studies but also for assessing sedation or stimulant effects of drugs.

4.2.2 Depression Models

Forced Swim Test

[17] Forced swim test (FST), which is one of the most commonly used assays for the study of depressive-like behaviour in rodents. The FST is based on the assumption that when placing an animal in a container filled with water, it will first make efforts to escape but eventually will exhibit immobility that may be considered to reflect a measure of behavioural despair. This test has been extensively used because it involves the exposure of the animals to stress, which was shown to have a role in the tendency for major depression. The main advantages of this procedure are that it is relatively easy to perform and that its results are easily and quickly analysed. Moreover, its sensitivity to a broad range of antidepressant drugs that makes it a suitable screening test is one of the most important features leading to its high predictive validity. Despite its appeal, this model has a number of disadvantages. First, the issue of chronic augmentation is problematic in this test because in real life patients need to be treated for at least several weeks before they experience any relief from their symptoms. During the FST an animal is placed in a container filled with water from which it cannot escape. The animal will first try to escape but eventually will exhibit immobility (*i.e.* floating with the absence of any movement except for those necessary for keeping the nose above water). The FST is a very popular model in animal research for a number of reasons. First, it involves the exposure of the animals to stress, which was shown to have a role in the tendency for major depression. Moreover, depression is often viewed as a lack of ability to handle with stress.

Tail Suspension Test

[7] The tail-suspension test is a mouse behavioural test useful in the screening of potential antidepressant drugs, and assessing of other manipulations that are expected to affect depression related behaviours. Mice are suspended by their tails with tape, in such a position that it cannot escape or hold on to nearby surfaces. During this test, typically six minutes in duration, the resulting escape oriented behaviours are quantified. The tail-suspension test is a valuable tool in drug discovery for high-throughput screening of prospective antidepressant compounds.

The tail-suspension test (TST) involves suspending mice above the ground by their tails. At the most basic level, the procedure only requires a suspension bar or shelf ledge, and tape. In order to prevent animals from observing or interacting each

other, each mouse is suspended within its own three-walled rectangular compartment (55 height X 15 width X 11.5 cm depth). The mouse is suspended in the middle of this compartment and the width and depth are sufficiently sized so that the mouse cannot make contact with the walls. In this setting, the approximate distance between the mouse's nose and the apparatus floor is 20-25 cm. There are four such identical compartments in the apparatus allowing us to test four mice at a time.

Chronic Mild Stress Test

[8] [9] The Chronic Mild Stress (CMS) test is a widely used *in vivo* experimental model for studying depression-like behaviour in animals, particularly rodents. It is considered one of the most reliable and clinically relevant models because it mimics the effects of long-term exposure to mild, unpredictable stressors similar to those experienced by humans in daily life. In this model, animals are subjected to a variety of mild stressors over an extended period, typically several weeks. These stressors may include changes in light-dark cycles, food or water deprivation, cage tilting, damp bedding, social isolation, or exposure to cold environments.

The key feature of the CMS model is the unpredictability and chronic nature of these stressors, which prevents the animal from adapting and leads to persistent behavioural and physiological changes. Anhedonia, a key symptom of depression, is one of the signature outcomes examined in the CMS test. Anhedonia is defined as a diminished capacity to perceive pleasure. The sucrose preference test is frequently used to measure this; a decline in taste for a sweet solution is indicative of depressive-like behaviour. Changes in body weight, locomotor activity, and behavioural reactions in other tests are examples of other measures.

5. Integration of *In-Silico, In-Vitro* and *In-Vivo* Approaches

The integration of *in silico*, *in vitro*, and *in vivo* methodologies represents a highly effective and systematic strategy for the discovery and development of novel anxiolytic and antidepressant agents, addressing the limitations associated with traditional drug discovery approaches. This multi-layered framework begins with in silico screening, where computational tools such as molecular docking, virtual screening, pharmacophore modelling, and QSAR analyses are employed to rapidly identify and optimize potential lead compounds based on their predicted affinity toward specific neurobiological targets, including neurotransmitter receptors, transporters, and enzymes implicated in anxiety and depression. Additionally, ADMET prediction plays an important role at this stage by evaluating pharmacokinetic properties such as absorption, blood-brain barrier permeability, metabolic stability and toxicity risk, thereby filtering out unsuitable candidates early and significantly reducing time and cost.

The most promising compounds identified through computational approaches are then subjected to *in vitro* evaluation, which provides understanding of mechanisms of their biological activity under controlled laboratory conditions. Techniques such as receptor binding assays and radio ligand binding studies help determine target specificity and binding kinetics, while cell-based assays like the MTT assay assess cytotoxicity and cell viability. Furthermore, neuroprotection assays evaluate the ability of compounds to safeguard neuronal cells against oxidative stress and inflammatory damage, and monoamine oxidase (MAO) inhibition assays explore their potential to enhance monoaminergic neurotransmission.

Compounds demonstrating favourable *in vitro* profiles are subsequently advanced to in vivo studies, where their pharmacological efficacy and behavioural effects are evaluated in animal models. Established models such as the elevated plus maze, light/dark box, and open field test are used to assess anxiolytic activity, while depression-like behaviours are examined using the forced swim test, tail suspension test, and chronic mild stress model. These models not only validate the therapeutic potential of compounds but also provide critical information on dose-response relationships, side effects, and overall safety in a complex biological system.

Importantly, the integration of these approaches is not strictly linear but rather iterative, allowing feedback from *in vitro* and *in vivo* findings to refine computational models and guide further optimization of lead compounds. This continuous refinement enhances predictive accuracy and increases the likelihood of successful translation from preclinical studies to clinical application. Ultimately, the combined use of *in silico, in vitro*, and *in vivo* strategies offers a comprehensive, efficient, and rational framework for the identification of safer and more effective treatment for anxiety and depression, while minimizing resource expenditure and reducing the high attrition rates typically associated with central nervous system drug development.

6. Challenges and Future Perspectives

Despite significant advancements in the integrated screening of anxiolytic and antidepressant compounds, several challenges continue to limit the efficiency and translational success of drug

discovery in this field. One of the primary concerns is the limited predictive validity of animal models used in in vivo studies, as many behavioural patterns which even though is well established it does not fully replicate the complexity of human anxiety and depression. Disorders such as major depressive disorder are heterogeneous in nature, involving genetic, environmental, and psychosocial factors that are difficult to model accurately in rodents. As a result, compounds that show promising results in preclinical models often fail during clinical trials due to lack of efficacy or unforeseen side effects. Additionally, variability in experimental conditions, differences in animal strains, and inconsistencies in stress protocols can lead to reproducibility issues across laboratories.

From a computational perspective, while *in silico* methods such as molecular docking, QSAR, and ADMET prediction have significantly accelerated early-stage drug discovery, their accuracy is still dependent on the quality of available structural data and the assumptions underlying predictive algorithms. Many models may oversimplify complex biological interactions, leading to false positives or overlooking potentially effective compounds.

Emerging technologies offer promising solutions to overcome these limitations. The incorporation of artificial intelligence (AI) and machine learning (ML) into drug discovery pipelines has the potential to improve predictive accuracy by analyzing large datasets and identifying complex patterns that may not be evident through traditional methods. Advanced techniques such as organ-on-a-chip systems and three-dimensional neuronal cultures are being developed to better mimic human brain physiology, thereby bridging the gap between *in vitro* and *in vivo* studies. Moreover, the integration of systems biology and network pharmacology approaches enables a more holistic understanding of disease mechanisms by considering multiple targets and pathways simultaneously, which is particularly relevant for multifactorial disorders like anxiety and depression.

The current integrated approaches have significantly improved the drug discovery process for anxiolytic and antidepressant agents, addressing the existing challenges through technological innovation and collaborative research will be crucial for future progress. The continued evolution of computational tools, experimental models, and personalized therapeutic strategies holds great promise for the development of more effective, safer, and targeted treatments for anxiety and depression.

7. CONCLUSION:

The discovery of novel anxiolytic and antidepressant agents requires an integrated approach that combines neurobiological understanding with advanced screening techniques. Anxiety and depression are complex disorders involving multiple mechanisms, including neurotransmitter imbalance, neuroendocrine dysregulation, neuro inflammation, and impaired neuroplasticity. A thorough understanding of these processes is essential for identifying effective therapeutic targets. Traditional drug discovery methods alone are often time-consuming and inefficient, particularly for central nervous system disorders, highlighting the need for more streamlined and multidisciplinary strategies.

The incorporation of *in silico* methods, such as molecular docking, QSAR modelling, and ADMET prediction, has significantly improved early-stage drug discovery by enabling rapid screening and optimization of potential compounds. These computational approaches are complemented by *in vitro* assays that evaluate biological activity, safety, and mechanisms of action, followed by in vivo models that assess behavioural efficacy and overall pharmacological effects. The integration of these approaches creates a stepwise and iterative framework that enhances efficiency, reduces costs, and increases the likelihood of identifying promising drug candidates.

Overall, this integrated strategy represents a powerful and forward-looking approach for developing safer and more effective treatments for anxiety and depression. While challenges such as limited translational accuracy and biological complexity remain, ongoing advancements in technology and interdisciplinary research are expected to further improve drug discovery outcomes and address the growing global burden of mental health disorders.

REFERENCES:

[1] Biney RP, et al. Exploring the antidepressant-like effect of stigmasterol using network pharmacology with molecular docking and in vivo experimental validation. Sci Rep. 2025;15:27533.

[2] Auld DS, Farmen MW, Kahl SD, Kriauciunas A, McKnight KL, Montrose C, et al. Receptor binding assays for HTS and drug discovery. Methods Enzymol. 2006;414:54–79.

[3] Walf AA, Frye CA. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nat Protoc. 2007;2(2):322–328.

[4] Arrant AE, Schramm-Sapyta NL, Kuhn CM. Use of the light/dark test for anxiety in adult and adolescent male rats. Behav Brain Res. 2013;256:119–127.

[5] Kraeuter AK, Guest PC, Sarnyai Z. The open field test for measuring locomotor activity and

anxiety-like behavior. In: Methods Mol Biol. 2019;1916:99–103.
[6] Monarca RI, Silva RFB, Gabriel SI, Cerveira AM, von Merten S. The presence of a shelter in an open field test has differential effects on the behavior and stress response of two mouse species. Behav Processes. 2015;116:94–103.
[7] Can A, Dao DT, Terrillion CE, Piantadosi SC, Bhat S, Gould TD. The tail suspension test. In: Mood and Anxiety Related Phenotypes in Mice. Methods Mol Biol. 2012;829:449–460.
[8] Willner P. The chronic mild stress (CMS) model of depression: history, evaluation and usage. Neurobiol Stress. 2017;6:78–93.
[9] Willner P. Chronic mild stress (CMS) revisited: consistency and behavioural–neurobiological concordance in the effects of CMS. Neuropsychobiology. 2005;52(2):90–110.
[10] Zhang Y, et al. Network pharmacology screening, in vitro and in vivo evaluation of antianxiety and antidepressant drug-food analogue. Phytomedicine. 2024;134:155999.
[11] Sharma N, et al. Antidepressant-like effect of dehydrozingerone from Zingiber officinale: in silico and in vivo studies. Pharmacol Rep. 2021; 73(4):1027–1038.
[12] Kaur R, et al. 2-Pyrazoline derivatives in neuropharmacology: synthesis, ADME prediction, molecular docking and in vivo biological evaluation. Eur J Med Chem. 2017;138:102–114.
[13] Wang L, et al. Design, synthesis, and in vivo and in silico evaluation of coumarin derivatives with potential antidepressant effects. Bioorg Chem. 2021; 114:105070.
[14] López-Rodríguez ML, et al. Synthesis, antidepressant evaluation and docking studies of long-chain alkyl nitroquipazines as serotonin transporter inhibitors. Bioorg Med Chem. 2013;21(15):4556–4564.
[15 Zhang Y, Zhang Z, Li Y, et al. Network pharmacology-based analysis and experimental validation of antidepressant effects of natural compounds. Phytomedicine. 2021;85:153541.
[16] Liu MY, et al. Antidepressant-like activity of oroxylin A in mice models of depression: behavioural and neurobiological characterization. Front Pharmacol. 2022; 13:921553.
[17] Slattery DA, Cryan JF. Using the rat forced swim test to assess antidepressant-like activity. Nat Protoc. 2012;7(6):1009–1014.
[18] Youdim MBH, Edmondson D, Tipton KF. The therapeutic potential of monoamine oxidase inhibitors. Nat Rev Neurosci. 2006;7(4):295–309.
[19] Bortolato M, Chen K, Shih JC. Monoamine oxidase inactivation: from pathophysiology to therapeutics. Adv Drug Deliv Rev. 2008;60(13–14):1527–1533.
[20] Castrén E, Monteggia LM. Brain-derived neurotrophic factor signaling in depression and antidepressant action. Biol Psychiatry. 2021;90(2):128–136.
[21] Mosmann T. Rapid colorimetric assay for cellular growth and survival (MTT assay). J Immunol Methods. 1983;65(1–2):55–63.
[22] Maguire JJ, Davenport AP. Radioligand binding assays and their analysis. Methods Mol Biol. 2012;897:31–77.
[23] Winkler DA. The role of quantitative structure–activity relationships (QSAR) in biomolecular discovery. Brief Bioinform. 2002;3(1):73–86.
[24] Plenge RM, Scolnick EM, Altshuler D. Validating therapeutic targets through human genetics. Nat Rev Drug Discov. 2013;12(8):581–594.
[25] Smith SM, Vale WW. The role of the hypothalamic–pituitary–adrenal axis in neuroendocrine responses to stress. Dialogues Clin Neurosci. 2006;8(4):383–395.
[26] Drevets WC, Price JL, Furey ML. Brain structural and functional abnormalities in mood disorders. Brain Struct Funct. 2008;213(1–2):93–118.
[27] Phelps EA, LeDoux JE. Contributions of the amygdala to emotion processing: from animal models to human behavior. Neuron. 2005;48(2):175–187.
[28] versen LL. Neurotransmitter transporters and their impact on the development of psychopharmacology. Br J Pharmacol. 2006;147(S1):S82–S88.