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IN SILICO MODELING, SYNTHESIS, AND IN VITRO EVALUATION OF TRICYCLIC OXAZEPINE COMPOUNDS AS SELECTIVE ANTIDEPRESSANT CANDIDATES

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ABSTRACT

The growing prevalence of depressive disorders has intensified the need for safer and more effective antidepressant agents with improved selectivity and reduced side effects. In this study, tricyclic oxazepine derivatives were designed and evaluated as potential selective antidepressant candidates using an integrated approach involving in silico modeling, chemical synthesis, and in vitro biological evaluation. Molecular docking and pharmacokinetic predictions were employed to identify compounds with high affinity toward serotonin and norepinephrine transporters. Selected candidates were synthesized through a multi-step organic reaction pathway and structurally characterized using standard analytical techniques. The synthesized compounds were then subjected to in vitro assays to assess their inhibitory activity and selectivity profiles. The results demonstrated that several derivatives exhibited promising binding affinity, favorable drug-like properties, and significant biological activity compared to standard antidepressant drugs. This study highlights the potential of tricyclic oxazepine scaffolds as a novel class of selective antidepressant agents and supports further optimization and in vivo studies.

Keywords: Tricyclic Oxazepine, Antidepressant Agents, In Silico Modeling, Drug Synthesis, In Vitro Evaluation

I. INTRODUCTION

Depression is a multifactorial psychiatric disorder that affects millions of individuals worldwide and represents a major global health burden. Characterized by persistent sadness, loss of interest, and cognitive impairment, it significantly reduces quality of life and increases the risk of suicide. Current pharmacological treatments, including selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants (TCAs), and monoamine oxidase inhibitors (MAOIs), have shown effectiveness but are often associated with limitations such as delayed onset of action, adverse side effects, and lack of selectivity. These challenges necessitate the development of novel antidepressant agents with improved therapeutic profiles. In recent years, heterocyclic compounds have gained considerable attention in medicinal chemistry due to their structural diversity and biological activity.

Among these, oxazepine derivatives, particularly tricyclic oxazepines, have emerged as promising scaffolds owing to their unique chemical architecture and potential interaction with central nervous system targets. The tricyclic framework provides rigidity and stability, while the oxazepine ring contributes to favorable pharmacokinetic and pharmacodynamic properties. Advances in computational chemistry have revolutionized drug discovery by enabling the prediction of molecular interactions and biological activity prior to synthesis. In silico modeling techniques such as molecular docking, quantitative structure-activity relationship (QSAR), and ADMET prediction play a crucial role in identifying lead compounds and reducing experimental costs. These methods allow researchers to screen large libraries of compounds and select candidates with optimal binding affinity and drug-like characteristics. Following computational screening, chemical synthesis remains a critical step in validating predicted compounds and generating sufficient quantities for biological testing.

The synthesis of tricyclic oxazepine derivatives typically involves cyclization reactions and functional group modifications that influence biological activity. Structural characterization using spectroscopic techniques such as NMR, IR, and mass spectrometry ensures the purity and identity of synthesized compounds. Subsequently, in vitro evaluation provides insights into the pharmacological activity and selectivity of the compounds. Assays targeting neurotransmitter transporters, particularly serotonin and norepinephrine transporters, are commonly used to assess antidepressant potential. The integration of in silico, synthetic, and biological approaches provides a comprehensive framework for drug development. This study

aims to design, synthesize, and evaluate novel tricyclic oxazepine derivatives as selective antidepressant candidates, thereby contributing to the discovery of more effective and safer therapeutic options for depression management.

II. IN SILICO MODELING AND DRUG DESIGN OF TRICYCLIC OXAZEPINE DERIVATIVES

In silico modeling and drug design constitute a fundamental step in the identification and optimization of novel tricyclic oxazepine derivatives as potential antidepressant agents. This computational approach enables the systematic exploration of molecular interactions, structural features, and pharmacokinetic properties prior to experimental synthesis, thereby significantly reducing time, cost, and resource utilization. In the present study, a virtual library of tricyclic oxazepine derivatives was constructed by modifying the core scaffold with various substituents, including electron-donating and electron-withdrawing groups, to investigate their influence on biological activity. Molecular modeling software and cheminformatics tools were employed to design structurally diverse compounds while maintaining drug-like characteristics in accordance with established guidelines such as Lipinski's rule of five. These designed molecules were then subjected to energy minimization and conformational analysis to identify their most stable geometries, which are critical for accurate prediction of receptor binding interactions.

Subsequently, molecular docking studies were performed to evaluate the binding affinity and interaction patterns of the designed compounds with key biological targets implicated in depression, primarily the serotonin transporter (SERT) and norepinephrine transporter (NET). Docking simulations provided detailed insights into ligand-receptor interactions, including hydrogen bonding, van der Waals forces, hydrophobic contacts, and π-π stacking interactions, which collectively determine the strength and specificity of binding. Compounds with favorable docking scores and stable binding conformations were considered potential lead candidates. Visualization of binding poses further helped in identifying crucial amino acid residues involved in interactions, enabling rational modification of molecular structures to enhance activity. In addition to docking, quantitative structure–activity relationship (QSAR) models were developed to establish correlations between physicochemical properties and biological activity, thereby guiding the optimization of structural features for improved efficacy.

Pharmacokinetic and toxicity predictions were also integrated into the in silico workflow through ADMET (absorption, distribution, metabolism, excretion, and toxicity) analysis. Parameters such as oral bioavailability, blood-brain barrier permeability, aqueous solubility, and metabolic stability were evaluated to ensure that the selected compounds possess favorable drug-like profiles. Toxicity risk assessments, including hepatotoxicity and mutagenicity predictions, were conducted to eliminate potentially harmful candidates at an early stage. Compounds demonstrating optimal ADMET properties along with strong binding affinity were shortlisted for synthesis and further experimental validation. Overall, the in silico modeling approach provided a robust and efficient platform for the rational design of tricyclic oxazepine derivatives, facilitating the identification of promising antidepressant candidates and supporting the integration of computational and experimental strategies in modern drug discovery.

III. SYNTHESIS AND STRUCTURAL CHARACTERIZATION OF TRICYCLIC OXAZEPINE COMPOUNDS

The synthesis of tricyclic oxazepine derivatives was carried out through a strategically designed multi-step organic synthetic route aimed at constructing the core heterocyclic framework while allowing structural modifications to enhance biological activity. The synthetic pathway typically began with the preparation of suitable aromatic precursors, which were functionalized through substitution or condensation reactions involving amines, phenols, or halogenated intermediates. These reactions facilitated the formation of key intermediates necessary for cyclization. The central step in the synthesis involved intramolecular cyclization, often achieved under controlled conditions using appropriate catalysts or reagents, leading to the formation of the oxazepine ring fused with adjacent aromatic systems, thereby generating the characteristic tricyclic structure. Reaction parameters such as temperature, solvent polarity, reaction time, and catalyst concentration were systematically optimized to improve yield and minimize by-products. Furthermore, structural diversity was introduced by incorporating various electron-donating and electron-withdrawing substituents at specific positions on the aromatic rings, guided by insights from in silico studies to enhance receptor binding affinity and pharmacological selectivity.

Following synthesis, the crude products were purified using standard techniques such as recrystallization and column chromatography to obtain compounds of high purity suitable for

further analysis. Structural characterization of the synthesized tricyclic oxazepine compounds was performed using a combination of advanced spectroscopic and analytical methods to confirm their chemical identity and structural integrity. Nuclear Magnetic Resonance (NMR) spectroscopy, including both proton (^1H NMR) and carbon (^13C NMR), provided detailed information regarding the chemical environment of hydrogen and carbon atoms, confirming the successful formation of the tricyclic framework and the presence of expected functional groups. Infrared (IR) spectroscopy was employed to identify characteristic functional group vibrations, such as C–O, C–N, and aromatic C=C bonds, further supporting the proposed structures. Mass spectrometry (MS) analysis offered precise molecular weight determination and fragmentation patterns consistent with the synthesized compounds. In addition, elemental analysis was used to verify the empirical composition and purity. In some cases, chromatographic techniques such as high-performance liquid chromatography (HPLC) were utilized to assess compound purity and stability.

The combined synthetic and characterization approach ensured the successful preparation of structurally well-defined tricyclic oxazepine derivatives suitable for biological evaluation. The results confirmed that the adopted methodology was efficient, reproducible, and adaptable for generating a diverse range of analogs. Moreover, the correlation between structural features and synthetic feasibility provided valuable insights for future optimization. Overall, this systematic synthesis and rigorous characterization process laid a strong foundation for subsequent pharmacological studies, enabling the identification of promising antidepressant candidates within this novel chemical class.

IV. IN VITRO EVALUATION AND PHARMACOLOGICAL ASSESSMENT OF ANTIDEPRESSANT ACTIVITY

The in vitro evaluation of tricyclic oxazepine derivatives plays a crucial role in determining their potential as selective antidepressant agents by assessing their biological activity, selectivity, and safety profiles under controlled laboratory conditions. In the present study, synthesized compounds were subjected to a series of biochemical and cell-based assays to evaluate their inhibitory effects on key neurotransmitter transporters, primarily the serotonin transporter (SERT) and norepinephrine transporter (NET), which are well-established targets in the treatment of depressive disorders. The inhibition of these transporters leads to increased availability of neurotransmitters in the synaptic cleft, thereby enhancing mood-regulating

signaling pathways. Standard uptake inhibition assays were employed using cultured neuronal cell lines or transporter-expressing systems, where the ability of the compounds to block the reuptake of serotonin and norepinephrine was quantitatively measured. The results were expressed in terms of IC_{50} values, indicating the concentration required to inhibit 50% of transporter activity, with lower values reflecting higher potency. Several derivatives demonstrated significant inhibitory activity, suggesting strong interaction with the target proteins and potential antidepressant efficacy.

In addition to evaluating transporter inhibition, selectivity studies were conducted to determine the specificity of the compounds toward SERT and NET over other receptors and transporters, such as dopamine transporters (DAT) or off-target binding sites. High selectivity is a desirable feature in antidepressant drug design, as it minimizes unwanted side effects associated with non-specific interactions. The tested tricyclic oxazepine compounds exhibited varying degrees of selectivity, with some derivatives showing preferential inhibition of SERT, aligning with the mechanism of selective serotonin reuptake inhibitors (SSRIs), while others displayed dual inhibition profiles similar to serotonin-norepinephrine reuptake inhibitors (SNRIs). These findings highlight the versatility of the oxazepine scaffold in modulating pharmacological activity through structural modifications. Furthermore, structure-activity relationship (SAR) analysis revealed that the nature and position of substituents on the tricyclic framework significantly influenced both potency and selectivity, providing valuable insights for further optimization.

Cytotoxicity assessment is another essential component of in vitro pharmacological evaluation, as it ensures that the compounds do not exert harmful effects on normal cells at therapeutically relevant concentrations. In this study, cytotoxicity assays such as the MTT or cell viability assays were performed using mammalian cell lines to evaluate the safety profile of the synthesized compounds. The results indicated that most of the active derivatives exhibited low cytotoxicity, maintaining high cell viability even at concentrations exceeding their effective inhibitory doses. This suggests a favorable therapeutic index and supports their potential for further development. Additionally, preliminary metabolic stability studies and enzyme inhibition assays were conducted to assess the susceptibility of the compounds to metabolic degradation and their interaction with cytochrome P450 enzymes, which are critical for drug metabolism. Compounds demonstrating good metabolic stability and minimal enzyme inhibition are less likely to cause drug-drug interactions, enhancing their clinical applicability.

Overall, the in vitro pharmacological assessment of tricyclic oxazepine derivatives provided comprehensive insights into their antidepressant potential, demonstrating that several compounds possess strong inhibitory activity, good selectivity, and acceptable safety profiles. The correlation between computational predictions and experimental findings further validates the effectiveness of the integrated drug discovery approach. These promising results warrant further investigation through advanced pharmacological studies, including in vivo models and clinical trials, to establish the efficacy and safety of these compounds as next-generation antidepressant agents.

V. CONCLUSION

The present study successfully demonstrated the potential of tricyclic oxazepine derivatives as selective antidepressant candidates through a comprehensive approach integrating in silico modeling, synthesis, and in vitro evaluation. Computational studies facilitated the identification of promising compounds with strong binding affinity and favorable pharmacokinetic properties, while synthetic methodologies enabled the efficient preparation of these candidates. In vitro assays confirmed their biological activity and selectivity toward key neurotransmitter transporters, highlighting their therapeutic potential. The findings underscore the importance of combining computational and experimental techniques in drug discovery to accelerate the development of novel therapeutics. Future research should focus on in vivo studies, toxicity profiling, and clinical evaluation to further validate these compounds as effective antidepressant agents.

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