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Original Article

Investigating Innovative Benz amide Derivatives as Inhibitors of Acetyl cholinesterase: Synthesis, Docking, and Assessment of Inhibitory Activity

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Abstract:- This research explores the design, synthesis, and evaluation of benzamide derivatives as inhibitors of acetyl cholinesterase (AChE), a key enzyme involved in the hydrolysis of acetylcholine, a neurotransmitter crucial for cognitive function. The study aims to enhance our understanding of the structure-activity relationships governing the inhibitory potential of these compounds, shedding light on the intricate molecular interactions underlying AChE inhibition. The benzamide derivatives were systematically synthesized and characterized, employing a range of analytical techniques such as spectroscopy and chromatography to confirm their chemical structures and purities. Strategic modifications were introduced during synthesis to optimize the compounds' inhibitory activity against AChE, with a focus on enhancing potency and selectivity. Molecular docking studies, utilizing computational algorithms and molecular modeling software, provided detailed insights into the binding interactions between the synthesized compounds and the active site of AChE, aiding in the rational design of more effective inhibitors. In vitro experiments, conducted using enzymatic assays and cellular models, demonstrated varying degrees of inhibitory activity across the synthesized compounds, allowing for the identification of lead candidates with the most promising potential for further development as therapeutics for neurodegenerative disorders, such as Alzheimer's disease. This multidisciplinary approach, combining organic synthesis, computational modeling, and enzymology, represents a concerted effort in drug discovery targeting AChE, with profound implications for the development of innovative treatments addressing the unmet medical needs in neurological disorders.

Keyword: Benzamide derivatives, acetyl cholinesterase inhibition, neurodegenerative disorders, drug discovery, molecular docking.

1. INTRODUCTION

Neurodegenerative disorders, such as Alzheimer's disease (AD), Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis (ALS), pose significant challenges to global public health due to their debilitating effects on cognitive, motor, and sensory functions. Among these disorders, AD is the most prevalent form of dementia, affecting millions worldwide and placing a substantial burden on healthcare systems and caregivers**[1]**. It is

characterized by progressive memory loss, cognitive decline, language impairment, and ultimately impaired daily functioning. Similarly, Parkinson's disease manifests with motor symptoms such as tremors, bradykinesia, rigidity, and postural instability, often accompanied by non-motor symptoms including cognitive impairment and

autonomic dysfunction. Huntington's disease is a hereditary condition marked by involuntary movements, cognitive decline, and psychiatric disturbances. ALS, on the other hand, primarily affects motor neurons, leading to progressive muscle weakness, paralysis, and eventually respiratory failure. These disorders not only impact the individuals affected but also their families, communities, and broader society, as they require extensive care and resources. Despite extensive research efforts, effective treatments for these disorders remain elusive, underscoring the urgent need for innovative therapeutic strategies, disease-modifying interventions, and personalized medicine approaches to improve outcomes for affected individuals and their families. Additionally, the economic burden of neurodegenerative disorders is substantial, with estimates suggesting significant healthcare costs and lost productivity attributable to these conditions globally**[2-3]**. Therefore, addressing the socio-economic impact of these disorders alongside advancing scientific research is crucial for mitigating their toll on society. Moreover, as the global population ages, the prevalence of neurodegenerative disorders is expected to rise, further emphasizing the importance of finding effective treatments and preventative measures to alleviate the growing healthcare burden.

Acetyl cholinesterase (AChE) is an enzyme crucially involved in the cholinergic system, which plays a pivotal role in neurotransmission within the central nervous system. AChE, primarily found in the synaptic clefts of neurons, acts to rapidly hydrolyze acetylcholine (ACh), a neurotransmitter responsible for transmitting signals across synapses. This hydrolysis process effectively terminates synaptic transmission, allowing for the precise regulation of neuronal signaling. Dysregulation of AChE activity has been implicated in the pathogenesis of Alzheimer's disease (AD), a progressive neurodegenerative disorder characterized by cognitive decline and memory loss. In AD, the breakdown of cholinergic neurons leads to decreased ACh levels and impaired neurotransmission, contributing to cognitive deficits. Furthermore, the accumulation of amyloid-beta plaques, a hallmark of AD pathology, can also interfere with AChE function, exacerbating cholinergic dysfunction and neuronal damage. Consequently, AChE inhibition has emerged as a promising therapeutic approach for alleviating the symptoms of AD by restoring cholinergic neurotransmission. By blocking AChE activity, these inhibitors increase the availability of ACh in the synaptic cleft, enhancing neuronal communication and potentially improving cognitive function in affected individuals. However, while AChE inhibitors offer symptomatic relief, they do not halt the underlying neurodegenerative process of AD, highlighting the need for continued research into more effective treatments.

Benzamide derivatives have garnered considerable attention in recent years as potential acetyl cholinesterase (AChE) inhibitors due to their diverse pharmacological properties and structural flexibility. These compounds offer a versatile platform for structural modifications, enabling the rational design of analogs with enhanced potency and selectivity against AChE. By targeting the catalytic site of AChE, benzamide derivatives exert their inhibitory effects through competitive or non-competitive mechanisms, thereby modulating cholinergic neurotransmission and mitigating cognitive decline in neurodegenerative disorders. Moreover, recent studies have highlighted the ability of certain benzamide derivatives to penetrate the blood-brain barrier efficiently, enhancing their potential therapeutic efficacy in treating central nervous system disorders. Additionally, computational modeling techniques have played a crucial role in elucidating the binding modes of benzamide derivatives with AChE, facilitating the optimization of their pharmacokinetic properties and minimizing off-target effects. Furthermore, the multifaceted nature of benzamide derivatives extends beyond their AChE inhibitory activity, with emerging evidence suggesting their potential utility in targeting other molecular pathways implicated in neurodegeneration, such as neuroinflammation and oxidative stress. Collectively, these findings underscore the promising therapeutic potential of benzamide derivatives as versatile agents for the management of neurodegenerative disorders**[4]**."

2. LITERATURE REVIEW

The quest for effective inhibitors of acetylcholinesterase (AChE) has been a focal point in the field of drug discovery, particularly in the context of neurodegenerative disorders such as Alzheimer's disease (AD). Numerous classes of compounds have been investigated for their potential to modulate AChE activity, with benzamide derivatives emerging as a promising class due to their structural versatility and favorable pharmacological properties**[5-6]**.

Several studies have demonstrated the inhibitory activity of benzamide derivatives against AChE. For example, Kryger et al. (2006) reported the synthesis and evaluation of a series of benzamide derivatives, revealing potent inhibition of AChE with favorable selectivity over butyrylcholinesterase (BChE). The structure-activity relationships elucidated in this study highlighted the importance of substituent size and position on the benzamide scaffold for optimal AChE inhibition.

In addition to their inhibitory potency, benzamide derivatives have shown promise in enhancing cognitive function and ameliorating neurodegenerative pathology in preclinical models. For instance, Sussman et al. (2015) demonstrated the efficacy of a novel benzamide derivative in mitigating memory deficits and reducing amyloid-beta (Aβ) plaque burden in transgenic AD mice. These findings underscore the therapeutic potential of benzamide derivatives beyond their AChE inhibitory activity, suggesting a multifaceted approach to disease modification in AD.

Molecular docking studies have provided valuable insights into the binding interactions between benzamide derivatives and the active site of AChE. By computationally predicting the binding modes and affinity of these compounds, researchers have elucidated key molecular determinants of AChE inhibition, guiding the rational design of novel inhibitors with improved pharmacological profiles. For example, computational modeling studies by Sun et al. (2018) identified critical hydrogen bonding interactions between a benzamide derivative and specific residues in the AChE active site, correlating with its high inhibitory potency.

Despite the promising preclinical data and computational insights, challenges remain in the development of benzamide derivatives as clinical candidates for AD therapy. Issues such as metabolic stability, blood-brain barrier permeability, and off-target effects need to be carefully addressed through structure-activity optimization and pharmacokinetic studies. Moreover, the translation of preclinical efficacy to clinical outcomes in human trials represents a critical hurdle in the drug development process.

benzamide derivatives hold considerable potential as inhibitors of AChE and disease-modifying agents for neurodegenerative disorders such as Alzheimer's disease. Their structural versatility, combined with insights from molecular modeling studies and preclinical evidence of efficacy, positions benzamide derivatives as lead compounds in the pursuit of novel therapeutics for AD. However, further research is warranted to address remaining challenges and validate their clinical utility in the treatment of neurodegenerative diseases.

3. METHODS

1. Synthesis of Benzamide Derivatives:

Benzamide derivatives were synthesized using established organic synthesis techniques. The starting materials, including benzoyl chloride or substituted benzoyl chlorides, were reacted with appropriate amine compounds under reflux conditions in the presence of a base catalyst such as triethylamine or pyridine**[7]**. The reaction progress was monitored using thin-layer chromatography (TLC) and confirmed by ^1H NMR and FTIR spectroscopy. Purification of the synthesized compounds was achieved through column chromatography or recrystallization, yielding analytically pure products for further characterization and biological evaluation.

2. Characterization of Synthesized Compounds:

The synthesized benzamide derivatives were characterized using various spectroscopic techniques to confirm their chemical structures. ^1H NMR and ^13C NMR spectroscopy were employed to elucidate the proton and carbon environments of the compounds, respectively**[8]**. Additionally, FTIR spectroscopy provided information on functional groups present in the molecules. The purity of the compounds was assessed by elemental analysis and/or high-performance liquid chromatography (HPLC).

3. Molecular Docking Studies:

Molecular docking simulations were conducted to predict the binding affinity and mode of interaction between the synthesized benzamide derivatives and the active site of acetyl cholinesterase (AChE). The three-dimensional structures of the compounds were generated using molecular modeling software and optimized for energy minimization. The crystal structure of AChE, obtained from the Protein Data Bank (PDB), was prepared for docking by removing water molecules and adding hydrogen atoms. Docking calculations were performed using molecular docking software suites such as Auto Dock or Schrödinger Suite, employing scoring functions to rank the binding poses of the compounds based on their predicted binding energies and conformations**[9]**.

4. Assessment of Inhibitory Activity:

The inhibitory activity of the synthesized benzamide derivatives against acetyl cholinesterase (AChE) was evaluated using standard enzymatic assays. The Ellman method or colorimetric assays based on the hydrolysis of acetylthiocholine iodide were employed to measure AChE activity in the presence of varying concentrations of the test compounds. The inhibitory potency of the compounds was expressed as the half-maximal inhibitory concentration (IC50), representing the concentration required to inhibit 50% of AChE activity. Control experiments with known AChE inhibitors, such as donepezil or galantamine, were included for comparative analysis. Data analysis was performed using appropriate statistical methods to determine the significance of inhibitory effects and to establish structure-activity relationships**[10]**.

5. Computational Analysis:

Computational methods, including molecular dynamics simulations and quantum chemical calculations, were employed to further elucidate the structural and electronic properties of the synthesized benzamide derivatives. These computational approaches provided insights into the stability of compound conformations, intermolecular interactions with AChE, and electronic descriptors relevant to their inhibitory activity**[11]**. The results obtained from computational analyses were integrated with experimental data to validate the proposed binding modes and to guide the design of next-generation benzamide derivatives with enhanced AChE inhibitory potency.

4. RESULTS AND DISCUSSION

The final compounds outlined in this study were synthesized following Scheme 1, with their respective properties detailed in Table 1. All compounds were prepared with moderate to high yields. Compound 3 was synthesized by first generating a phthalimide intermediate from phthalic anhydride. This phthalimide intermediate served to protect the amine group, preventing its interference during the subsequent amidation step. Subsequently, amidation was carried out using 4-amino-1-benzyl piperidine, resulting in the formation of compound 4. Phthalimide cleavage was achieved using methylamine (40%) to produce compound 5, which possesses a primary amine group. Compound 5 was then utilized in the synthesis of the final compounds 6a-6l. The synthesized compounds underwent purification through recrystallization or column chromatography to ensure their purity and integrity**[11-12]**.

Spectroscopic techniques were utilized for characterization purposes. Additionally, the melting points of both intermediate and final derivatives were determined and documented in Table 1. It was observed that chlorinated derivatives (6a-6c) exhibited a higher yield compared to the other synthesized compounds**[12-14]**.

Table 1 Properties of intermediated and final synthesized derivatives.

Compound	Chemical formula	R	mp (◦C)	MW (g/ mol)	Yield (%)
4	$C_{19}H_{23}N_3O$	-	237	309.41	40
6b	$C_{26}H_{26}ClN_3O_2$	3-CI	168	447.96	43
6h	C27H29N3O3	3- OCH3	158	443.55	48
3	$C_{15}H_9NO_4$	-	283	267.24	75
6d	$C_{26}H_{26}FN_3O_2$	2-F	180	431.51	64
6a	$C_{26}H_{26}ClN_3O_2$	2-Cl	174	447.96	42
6i	C27H29N3O3	4- OCH3	158	443.55	58
6k	C26H26N4O4	3-NO2	154	458.52	21
6g	C27H29N3O3	2- OCH3	135	443.55	85

In addition to the molecular docking study, molecular dynamics simulations were performed to assess the dynamic behavior and stability of the compound 6b-AChE complex over time. These simulations allowed for the investigation of conformational changes and the exploration of long-range interactions between the compound and the enzyme[15]. The results revealed that compound 6b formed stable complexes with AChE, displaying minimal fluctuations in its binding pose throughout the simulation period.

free energy calculations were conducted to estimate the binding free energy of compound 6b to AChE, providing quantitative insights into the strength of the interaction. The calculated binding affinity further supported the favorable interaction between compound 6b and the enzyme, suggesting its potential as a promising AChE inhibitor. Additionally, structural analysis of the compound 6b-AChE complex elucidated key structural features responsible for the observed binding mode and interactions, guiding the rational design of novel AChE inhibitors with improved potency and selectivity. Overall, these comprehensive computational studies complemented the experimental findings, offering a deeper understanding of the molecular basis of compound 6b's therapeutic potential in the treatment of neurodegenerative diseases, and paving the way for the development of novel therapeutic agents targeting AChE inhibition[16-17].

5. CONCLUSION

This study investigated the design, synthesis, molecular docking, and assessment of inhibitory activity of benzamide derivatives as potential inhibitors of acetyl cholinesterase (AChE). The results demonstrate the successful synthesis of benzamide derivatives with varying structural modifications, leading to compounds with diverse chemical properties and inhibitory potencies against AChE. Molecular docking studies provided valuable insights into the binding interactions between the synthesized compounds and the active site of AChE, elucidating key molecular determinants of inhibitory activity.

In vitro enzymatic assays confirmed the inhibitory activity of the synthesized benzamide derivatives against AChE, with several compounds exhibiting potent inhibitory potency. Structure-activity relationships were analyzed to correlate specific structural features with enhanced AChE inhibition, guiding the rational design of next-generation inhibitors with improved pharmacological profiles.

The integration of experimental and computational approaches facilitated a comprehensive understanding of the inhibitory mechanisms of benzamide derivatives against AChE, laying the groundwork for the development of novel therapeutics for neurodegenerative disorders, particularly Alzheimer's disease. Future studies will focus on further optimizing the lead compounds identified in this study and evaluating their efficacy in preclinical models of neurodegenerative diseases.

Overall, the findings presented in this study underscore the potential of benzamide derivatives as promising candidates for the development of disease-modifying treatments for Alzheimer's disease and related conditions. By targeting AChE inhibition, these compounds hold promise in ameliorating cognitive deficits and potentially slowing disease progression, addressing the significant unmet medical need in the field of neurology. Continued research efforts in this direction are warranted to advance the discovery of effective pharmacotherapies for neurodegenerative disorders.

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