Benzimidazole- Donepezil Molecular Hybrids as a Leading Edge in Drug Discovery: Current Landscape and Future Prespectives

Nancy Mathur[1], Rajiv Yadav[1]*, Anshika Saini[1]
[1] Faculty of Pharmaceutical Sciences, Baba Mastnath university Asthal Bohar, Rohtak, Haryana, India

Corresponding Author**:** **Rajiv Yadav**

Abstract: Benzimidazole derivatives have garnered interest as potential therapeutics for Alzheimer's disease due to their diverse pharmacological properties and ability to interact with biological targets implicated in the disease's pathology. These compounds exhibit antioxidant and anti-inflammatory activities, which are crucial in combating oxidative stress and neuroinflammation, both of which play significant roles in Alzheimer's progression. Molecular hybridization is related to a combination of two or more pharmacophores of bioactive frames which generate a single molecular structure with enhanced activity. Previous studies described the Benzimidazole framework as a central structure in numerous synthetic and natural compounds, showing a wide range of biological activities. Here, we review, highlight and discuss a detailed account of the Anti-Alzheimers applications of some important benzimidazole-containing hybrid heterocycles.

Keywords: Benzimidazole, Alzheimers Disease, Hetrocyclic Chemistry

Introduction:

Heterocyclic chemistry is a branch of organic chemistry that focuses on the study and synthesis of heterocyclic compounds (Hossain, M et al., 2018). These compounds are cyclic in nature and contain at least one heteroatom such as nitrogen (N), oxygen (O), sulfur (S), or others, in addition to carbon (C) atoms in their ring structure (Omar, A. et al., 2020). Heterocycles are widely prevalent in nature and are crucial in various biological processes and industrial applications. Heterocyclic chemistry is a vast and critical field in organic chemistry, with extensive applications in biology, medicine, and industry (Shaikh, A. R et al., 2018). The unique properties of heterocyclic compounds make them indispensable in the development of new drugs, materials, and technologies. Heterocyclic compounds are abundant in nature and play a crucial role in living systems (Martins, P et al., 2015).

Research in heterocyclic chemistry continues to explore new synthetic methodologies and applications. Heterocyclic chemistry plays a pivotal role in organic chemistry and interdisciplinary sciences, impacting fields ranging from medicine to materials science. The diverse properties and applications of heterocyclic compounds underscore their importance in advancing scientific knowledge and technological innovation (Kabir, E et al., 2022). Nitrogen-containing heterocyclic compounds are a fundamental part of organic chemistry with extensive applications in biology, medicine, and industry. Their unique properties and versatility make them indispensable in the development of new drugs, materials, and technologies. These compounds are highly significant in various fields, particularly in medicinal chemistry, due to their wide range of biological activities (Amin, A et al., 2022). Nitrogen-containing compounds hold significant medicinal importance due to their diverse biological activities and therapeutic applications. These compounds often feature heterocyclic structures that incorporate nitrogen atoms, such as pyridines, pyrimidines, and indoles, among others. Nitrogen heterocycles serve as core components in many pharmaceutical drugs, including antibiotics, antivirals, anticancer agents, and cardiovascular medications (Pola, S et al., 2016). For instance, pyrimidine-based nucleoside analogues are crucial in antiviral therapies like HIV treatment. Additionally, nitrogen-containing compounds play roles in neurotransmission regulation (e.g., neurotransmitter receptors) and enzyme inhibition (e.g., acetylcholinesterase inhibitors in Alzheimer's disease treatment). Their versatility in targeting specific biological pathways underscores their importance in drug discovery and development, contributing significantly to advancements in medical treatment and patient care. Many N-containing heterocyclic compounds, such as indole, imidazole, thiazole, indolylimidazole, oxadiazole, triazole, and indazole, benzimidazole etc(Romeo, R., et al., 2019). have been identified to exhibit diverse therapeutic activities, including anticancer, anti-HIV, antibacterial, antifungal, antitubercular, antimicrobial, and antidiabetic effects as exemplified in Figure 1. Benzimidazole and its derivatives are of great interest in medicinal chemistry due to their broad spectrum of biological activities such as anticancer, antibacterial, antifungal, antiviral,cardiovascular agents, proton pump inhibitors (PPIs), antioxidant, and anthelmintic properties (Brishty, S. R et al., 2021). Benzimidazole chemistry is a rich field with significant implications for medicinal chemistry. The versatility of the benzimidazole scaffold allows for the development of a wide range of therapeutically valuable compounds. The ongoing research in this area continues to uncover new applications and enhance the efficacy of existing drugs (Mahurkar, N. D et al., 2023).

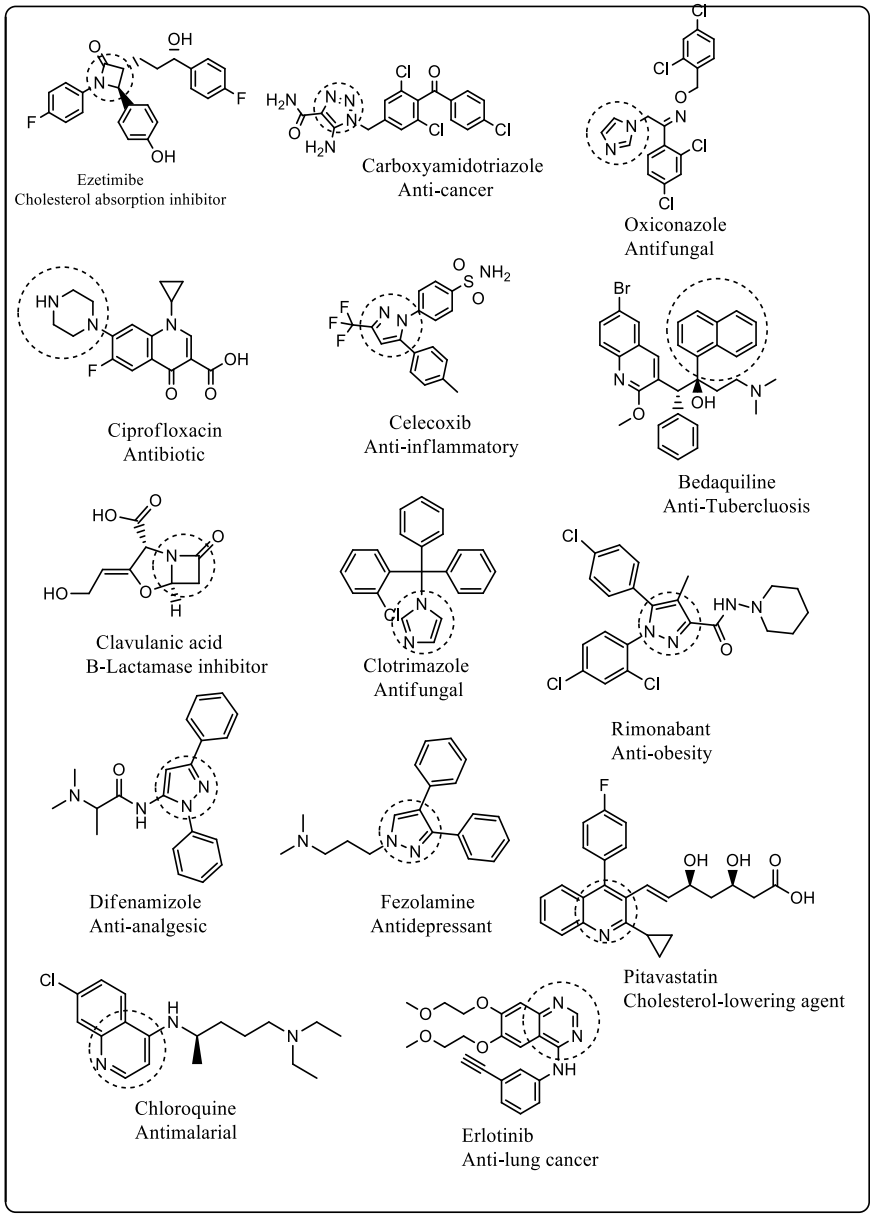
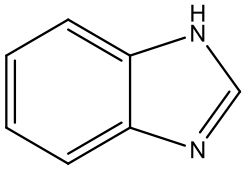


Figure 1: Nitrogen-heterocyclic containing drugs in clinical use

Structure and chemistry of benzimidazole

Benzimidazole, also known as 1H-benzimidazole, is a heterocyclic molecule widely utilized as a fundamental unit in organic synthesis (Manna, S. K et al., 2019) (Figure 2). This bicyclic compound features a benzene ring fused to a five-membered imidazole structure containing two nitrogen atoms. The benzimidazole ring system is formed by the fusion of an imidazole ring to a benzene ring through a 4-bond connection (Pinto, D. C et al., 2007). The two nitrogen atoms in the imidazole ring occupy different positions, imparting distinct properties to the ring system. The nitrogen bonded to a hydrogen atom is in the sp^3 state, referred to as the pyrrolic nitrogen, while the other nitrogen is sp^2 hybridized and known as

the pyridinic nitrogen (Titantah, J. T., et al., 2007). The hydrogen atom attached to the nitrogen of benzimidazole exhibits tautomerism, similar to imidazole and amidine. Positions 4 and 5 are identical to positions 6 and 7 due to this tautomerism. Nitrogen heterocycles are highly significant, and benzimidazole is one of the most important due to its synthetic versatility and extensive pharmaceutical applications (Tolomeu HV et al., 2023) The benzimidazole core, being a large heterocyclic ring, has led to numerous derivatives with medicinal value that are sold as commercial products. Notably, the benzimidazole ring system is a crucial component of vitamin B_{12} in the form of 5,6-dimethyl-1-(-D-ribofuranosyl) benzimidazole (Nardi M, et al., 2023). Benzimidazole and its various derivatives exhibit a range of medicinal properties as exemplified **in figure 2.** Previous research has highlighted that substitutions at positions 1, 2, and 5 of the benzimidazole moiety are essential for the compound's broad pharmacological activity (Lee, Y. T et al., 2023). The chemistry of benzimidazole is rich and diverse, with its unique structure enabling a wide range of chemical modifications and biological activities. Ongoing research continues to explore new synthetic methodologies and therapeutic applications for benzimidazole and its derivatives (Patel M et al., 2023).



Benzimidazole

Chemical formula	$C_7H_6N_2$
Molar mass	118.139 g·mol
Melting point	170 to 172 °C

Figure 2: Chemical structure of benzimidazole

Biological Significance of Benzimidazole:

Benzimidazoles exhibit significant biological importance owing to their versatile pharmacological properties and structural diversity. These heterocyclic compounds are widely utilized in medicinal chemistry for their ability to interact with various biological targets, making them valuable in drug discovery and therapeutic applications. Benzimidazoles are prominent in the treatment of parasitic infections due to their potent anthelmintic activity, effectively targeting parasites by disrupting microtubule formation and function (Kamanna, K et al., 2019). Additionally, benzimidazoles have shown efficacy in treating fungal infections, such as dermatophytosis, through inhibition of fungal microtubules. Their ability to inhibit enzymes like tubulin and helicases underscores their broad spectrum of antimicrobial effects. Beyond antimicrobial uses, benzimidazoles are

also explored for their anticancer properties, acting as inhibitors of kinases involved in cancer cell proliferation and survival pathways. Furthermore, benzimidazoles exhibit potential as antiviral agents and have applications in treating cardiovascular diseases and neurological disorders (Karaburun, A. Ç et al., 2019). Their diverse biological activities highlight benzimidazoles as versatile scaffolds in pharmaceutical research, continuously contributing to advancements in medicine and health care (Figure 3).

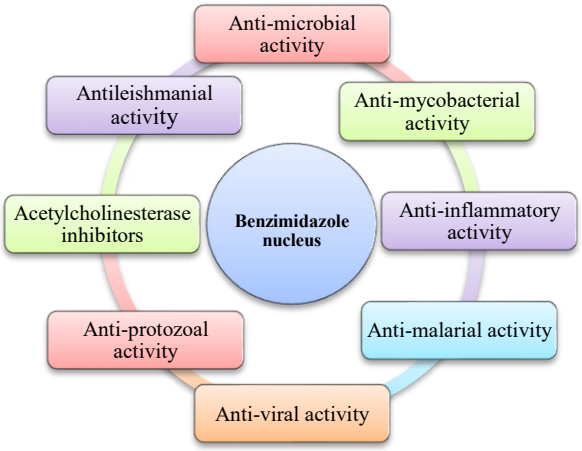
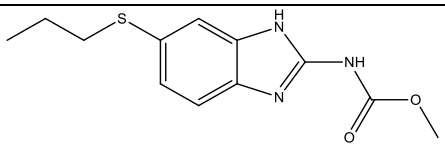
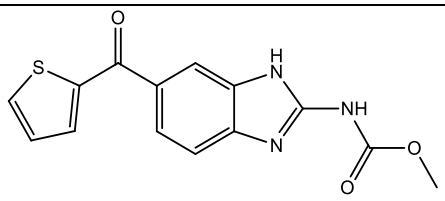
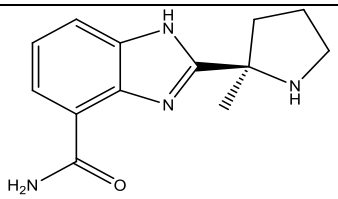
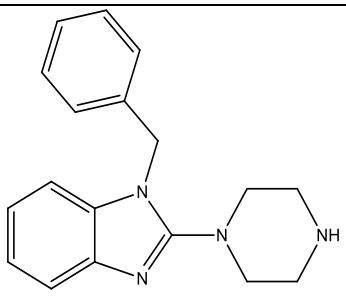
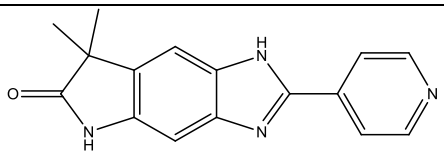
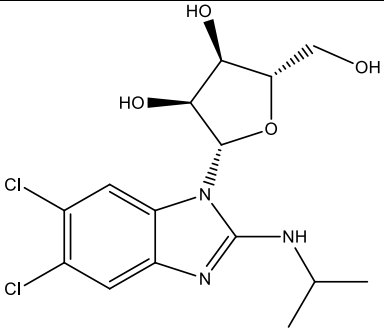
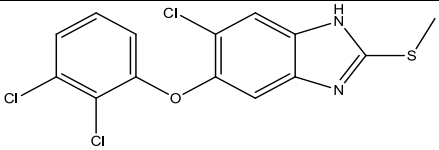
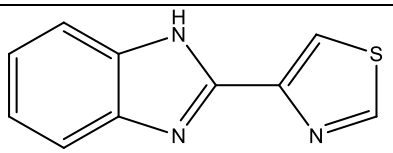
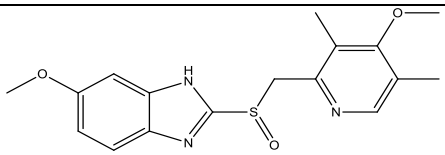
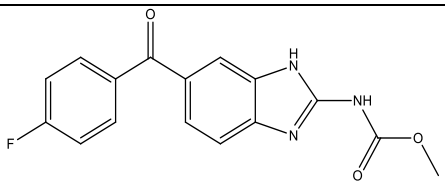
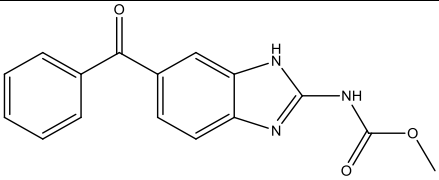
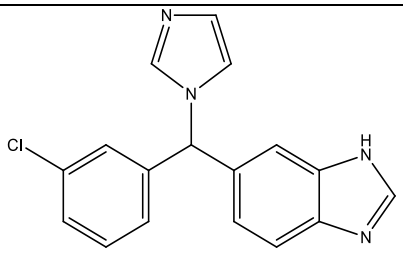
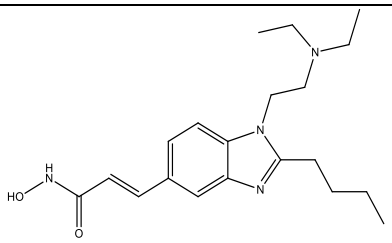
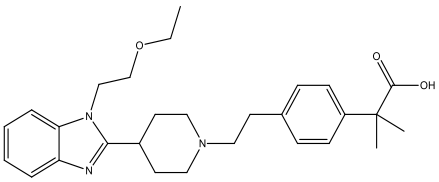
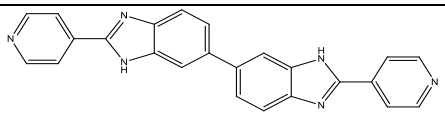
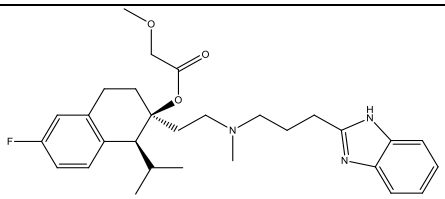
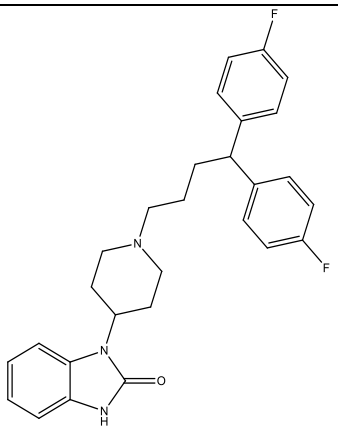
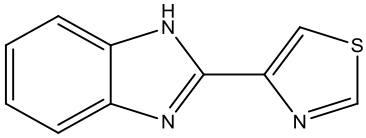
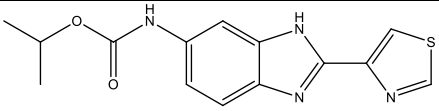


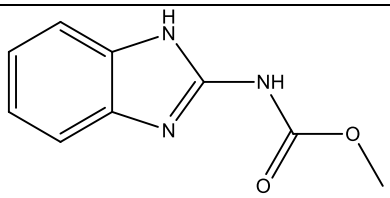
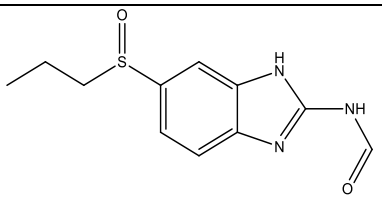
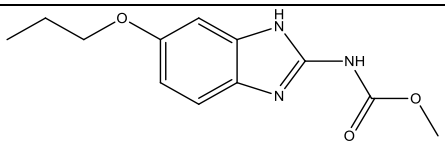
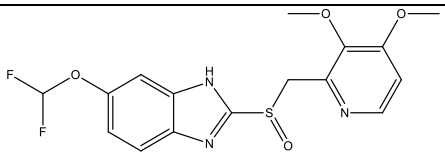
Figure 3: Biological activity spectrum of benzimidazole derivatives.

Table 1: A list of benzimidazole nucleus containing drugs in clinical use

S No	CHEMICAL STRUCTURE	NAME	CLASS/ USE
1		Albendazole	Antiprotozoal
2		Nocodazole	Anticancer
3		Veliparib	Anticancer

4		Lerisetron	Antihistaminic
5		Adibenden	Phosphodiesterase inhibitor
6		Maribavir	Antiviral
7		Triclabendazole	Anthelmintic
8		Tiabendazole	Antifungal and antiparastic
9		Omeprazole	Proton pump inhibitor
10		Flubendazole	Anthelmintic
11		Mebendazole	Anthelmintic

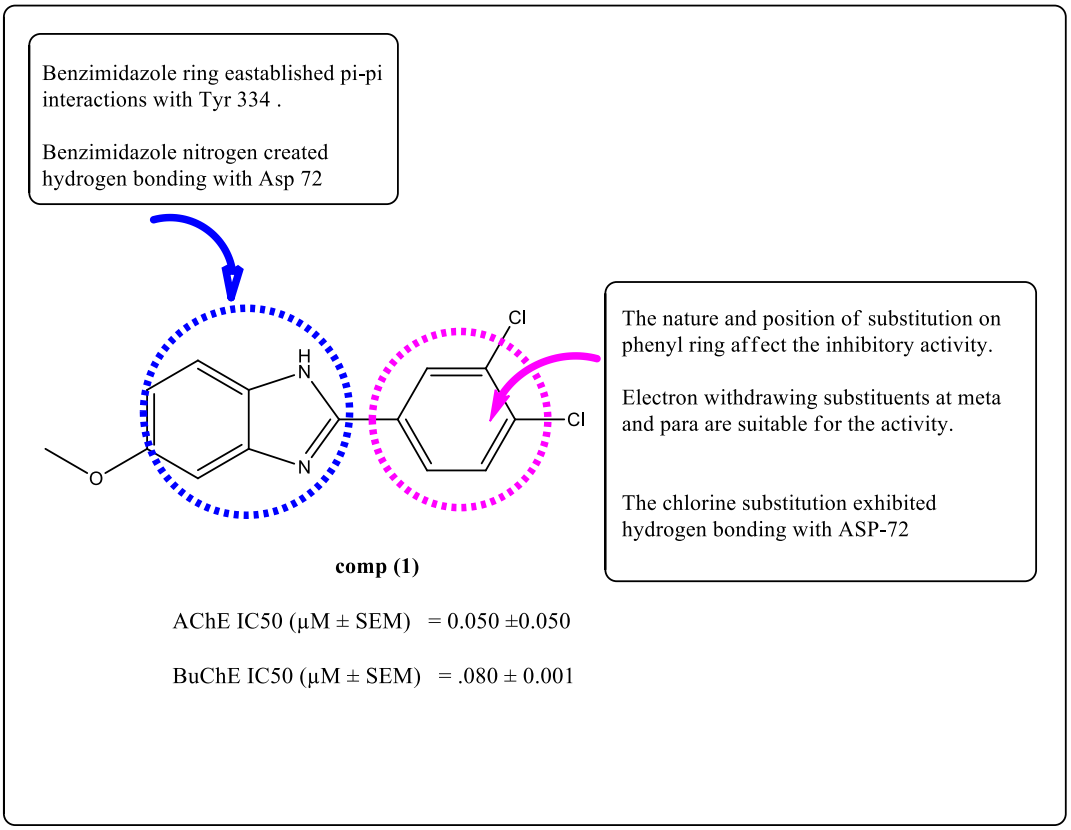
12		Liarozole	Anticancer
13		Pracinostat	Anticancer
14		Bilastine	Antihistaminic
15		Ridinalazole	Antibacterial
16		Mibefradil	Antihypertensive
17		Pimozide	Antisychotic
18		Thiabendazole	Anthelmintic
19		Cambendazole	Anthelmintic

20		carbendazim	Anticancer
21		Ricobendazole	Anthelmintic
22		Oxibendazole	Antiparasitic drugs
23		Pantoprazole	Antiulcer

2. Benzimidazole Heterocycle Based Antialzheimers Derivatives:

Benzimidazole derivatives have garnered interest as potential therapeutics for Alzheimer's disease due to their diverse pharmacological properties and ability to interact with biological targets implicated in the disease's pathology. These compounds exhibit antioxidant and anti-inflammatory activities, which are crucial in combating oxidative stress and neuroinflammation, both of which play significant roles in Alzheimer's progression. Benzimidazoles also demonstrate the ability to inhibit acetylcholinesterase (AChE), an enzyme involved in the breakdown of acetylcholine, a neurotransmitter crucial for memory and cognitive function. By inhibiting AChE, benzimidazoles can potentially enhance cholinergic transmission in the brain, alleviating cognitive deficits associated with Alzheimer's disease (Brishty, S. R et al., 2021). Moreover, some benzimidazole derivatives have shown neuroprotective effects by modulating calcium homeostasis and preventing neuronal apoptosis, further supporting their potential therapeutic utility in combating neurodegeneration. Research into benzimidazole-based compounds continues to explore their efficacy, safety profile, and potential for clinical application in treating Alzheimer's disease (Brishty, S. R et al., 2021). The biological potential of the benzimidazole nucleus has been extensively explored by various research groups across a range of disease conditions, including Alzheimer's disease (Adalat B et al., 2023). Several independent studies have evaluated the anti-Alzheimer's potential of the benzimidazole nucleus. The author had previously investigated the benzimidazole scaffold as an α-glucosidase inhibitor. To further

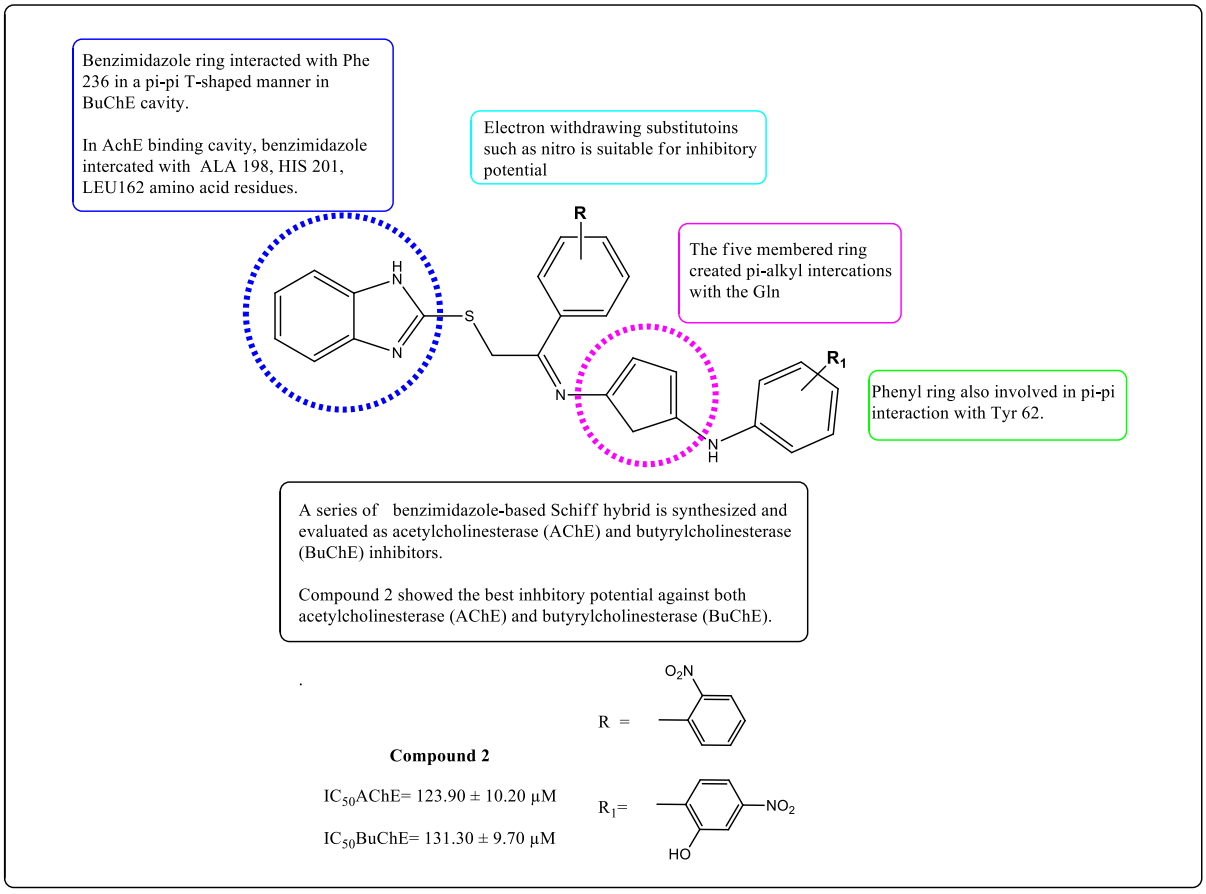
explore its suitability for anti-Alzheimer's drugs, twenty-one new analogs were synthesized by introducing substituted benzaldehyde into benzimidazole, with varying electron-donating and -withdrawing groups on the phenyl ring. All compounds were assessed for their enzyme inhibitory activity, and in silico ADMET studies confirmed their drug-like properties. The synthesized compounds exhibited varying degrees of inhibition, with IC50 values against acetylcholinesterase ranging from 0.050 ± 0.001 µM to 25.30 ± 0.40 µM, and against butyrylcholinesterase from 0.080 ± 0.001 µM to 25.80 ± 0.40 µM. Compound 3 demonstrated the highest potency in both cases, attributed to a 3,4-di-chloro-substituted phenyl ring. Structure-activity relationship (SAR) analysis highlighted the importance of the substituent's nature and position. Molecular docking studies revealed compound 3's excellent inhibitory potential, with Pi-pi stacking interactions with Phe-330 and interactions of the benzimidazole ring with Tyr 334 and Asp72. Additionally, the chlorine substitution exhibited hydrogen bonding with Asp-72, potentially enhancing the compound's effectiveness. The increased potential of these compounds could be attributed to the presence of electron-withdrawing groups at the meta and para positions of the benzene ring, inducing partial positive charges and facilitating Pi-stacking interactions. Molecular dynamics simulations indicated that compound 3 formed the most stable complexes with both enzymes, suggesting that benzimidazole derivativeshold promise as neuroprotective therapeutics.



	AchE IC50 (µM)	BuchE IC50 (µM)
Reported compound	0.60±0.050	-
Reported compound	2.6±0.1	-
Compound 1	0.05 ±0.050	0.80 ±0.001

Figure 4: Synthesis of novel Benzimidazole-Based-Substituted Benzaldehyde Derivatives as Potent Inhibitors for Alzheimer's Disease.

Schiff bases and their structural derivatives are recognized for their diverse and significant biological characteristics owing to their fascinating biological activities. Recognizing the biological significance of both benzimidazole and Schiff base derivatives, (Hussain et al.,2023) synthesized the hybrid structures integrating benzimidazole-based Schiff bases to delve deeper into their ability to inhibit acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), seeking potential lead compounds. All synthesized derivatives exhibited notable inhibition against AChE, with IC50 values ranging from 123.9 ± 10.20 to 342.60 ± 10.60 µM, and BuChE, with IC50 values spanning from 131.30 ± 9.70 to 375.80 ± 12.80 µM. These findings were compared with the standard Donepezil, which showed IC50 values of 243.76 ± 5.70 µM (AChE) and 276.60 ± 6.50 µM (BuChE), respectively. Particularly noteworthy, compound 2 demonstrated significant inhibitory potential, with IC_{50} values of 123.90 ± 10.20 µM (AChE) and 131.30 ± 9.70 µM (BuChE) among the synthesized compounds. Structure-activity relationship (SAR) analysis indicated that benzimidazole-based Schiff base derivatives carrying 2-OH and 4-NO_2 substitutions on the phenyl ring exhibited favorable inhibitory potential. Further investigations involved docking the active compounds with the binding cavity of target enzymes AChE and BuChE. Compound 2, the most potent, exhibited targeted binding with the AChE enzyme, with interactions such as the benzimidazole ring interacting with ALA 198, HIS 201, LEU162, the benzene ring binding with TYR 62, and the five-membered ring forming interactions with pi-alkyl and carbon hydrogen bonds with Glutamine 63. For the BuChE enzyme, the benzimidazole ring bound with PHE 236 in a pi-pi T-shaped manner, alongside interactions with ALA 234 and ASP 232 through conventional hydrogen bonds. Notably, certain analogs, particularly compound 2, displayed significant potency, holding promise as lead compounds in the quest for innovative anti-Alzheimer agents.

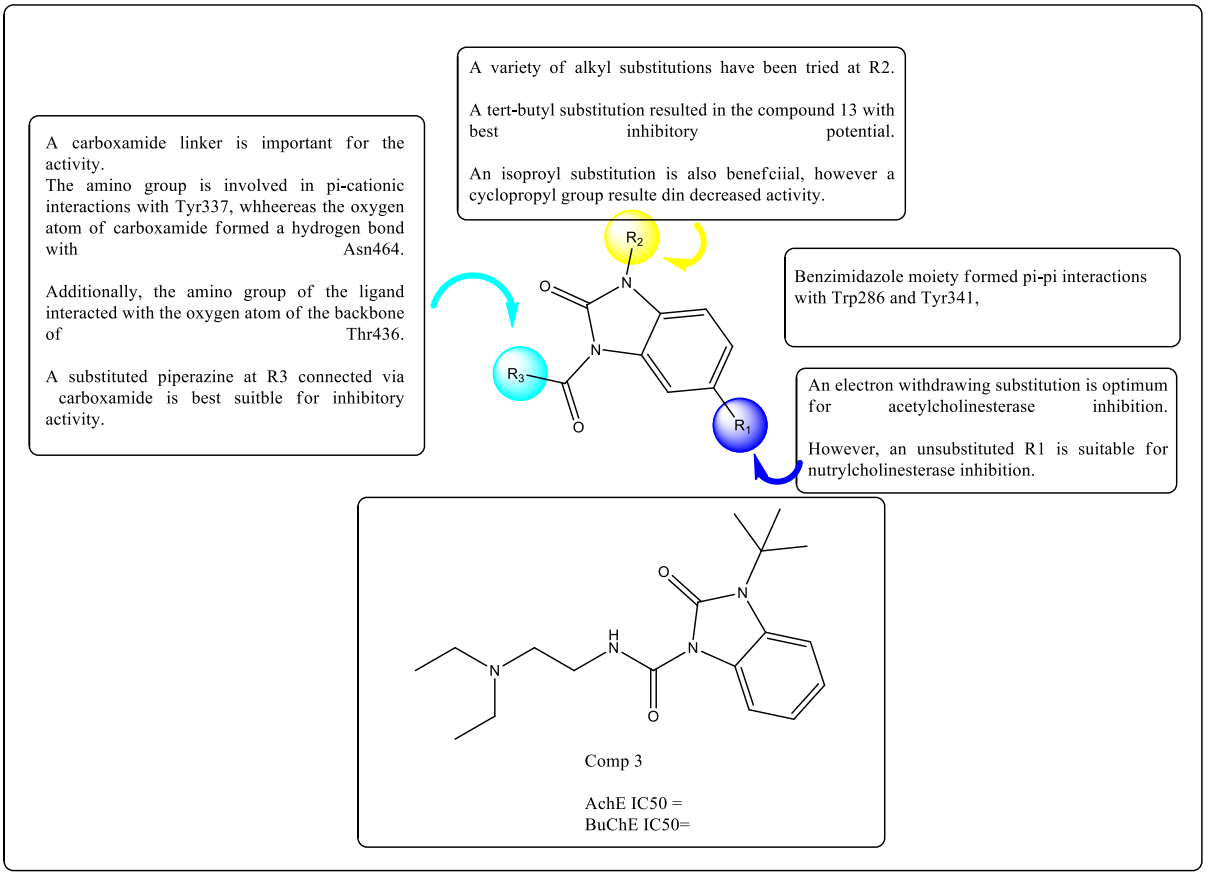


	AchE IC_{50} (µM)	BuchE IC_{50} (µM)
Compound 2	123.90 ±10.20	131.30±9.70

Figure 5: Synthesis of Benzimidazole-Based Schiff Base Hybrid Scaffolds: A Promising Approach to Develop Multi-Target Drugs for Alzheimer's Disease.

(Belinskaia et. al., 2023) also explored the potential of serotonin receptors (5-HTR) along with cholinesterases (ChE) in the development of anti-Alzheimer drugs. Recent evidence suggests that serotonin neurotransmission plays a role in the formation of insoluble aggregates of β-amyloid and tau protein, the primary histopathological markers of Alzheimer's disease (AD). Activation of 5-HT4R has been shown to impede the formation of extracellular amyloid plaques. Compounds such as the 5-HT4R agonist prucalopride and the partial agonist usmarapridehave exhibited promise in enhancing cognitive function and have been beneficial in AD patients. Recent research indicated that 5-HT6R and 5-HT7R receptors, involved in learning and memory processes, could also be viable therapeutic targets for managing neurodegenerative disorders. In line with this, the authors designed a series of derivatives of 1,3-dihydro-2-oxo-1H-benzimidazol-2-ones and evaluated their inhibitory effects on ChE as well as their binding affinity to three types of

Gs-protein-coupled 5-HTR (5-HT4R, 5-HT6R, and 5-HT7R). Various substitutions were explored on the benzimidazole scaffold, denoted as R_1 and R_2. Among all synthesized compounds, N-[2-(diethylamino)ethyl]-2-oxo-3-(tert-butyl)-2,3-dihydro-1H-benzimidazole-1-carboxamide hydrochloride (compound 13) emerged as the most promising for further experimental development. A trifluoromethyl group at R1, a tert-butyl group at R2, and N-(diethylamino)ethyl at R3 resulted in the most potent compound 3. Substituting the isopropyl group with a tert-butyl group in R_2 (compound 4 compared to 3) also enhanced the inhibitory effect without changing the type of inhibition. However, replacing isopropyl with cyclopropyl at R2 decreased the inhibitory constant. A [(4-chlorophenyl)(phenyl)methyl]-piperazin-1-yl substituent (Compound 4) was found to be most suitable at the R3 position for AchE inhibition, but it did not exhibit inhibitory activity against BuchE. An unsubstituted R1 was found to be optimal for BuchE inhibition. To further elucidate the interaction of the synthesized compounds with target receptors, molecular docking and molecular dynamics (MD) simulations were performed. Based on the results of previous studies, Compound 3 was observed to occupy the space between the catalytic active site (CAS) and peripheral anionic site (PAS). The benzimidazole moiety formed pi–pi interactions with Trp286 and Tyr341, while the amino group formed pi-cationic interactions with Tyr337. The oxygen atom of the amide group of compound3 interacted with the hydrogen atom of the side chain of Asn464, and the hydrogen atom of the amino group of the ligand interacted with the oxygen atom of the backbone of Thr436. Additionally, according to molecular modeling data, compound 3 effectively interacts with the serotonin-binding center of 5- HT7R.

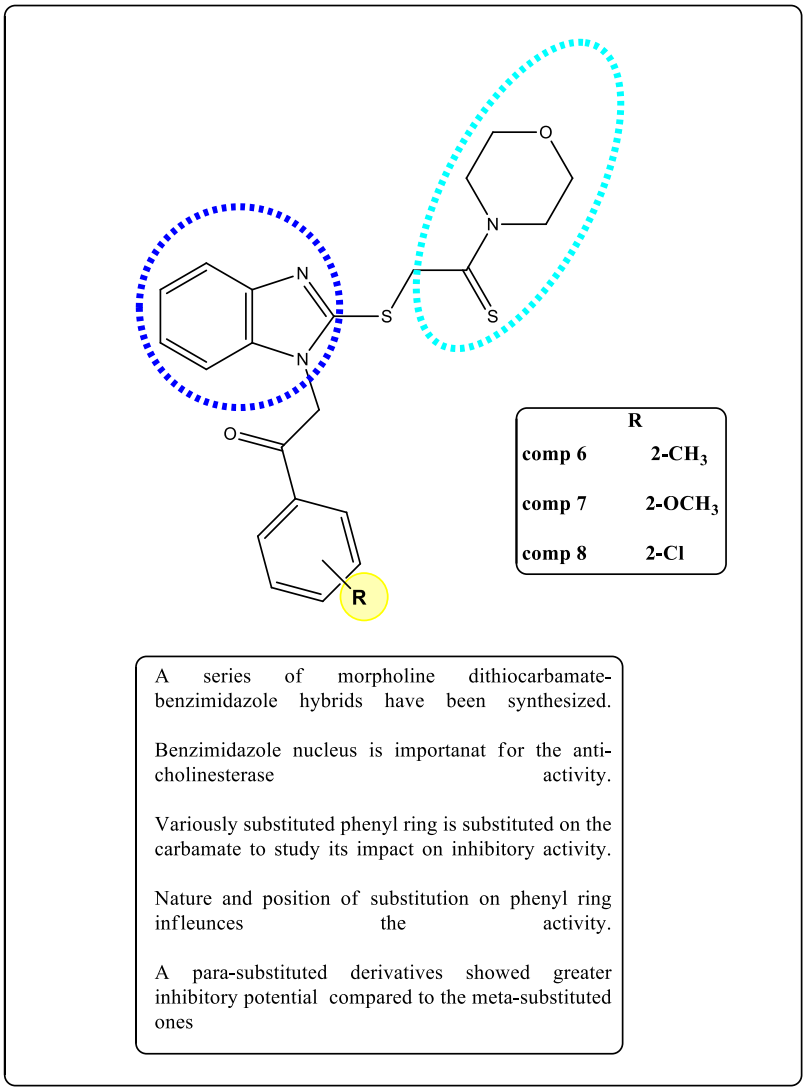


	R1	R2	R3	AchE Ki (μM)	Type of inhibition
Comp 3	H	t-butyl	N-(Diethylamino)ethyl	36.1	Competitive
Comp 4	H	*i-Pr*	N-(Diethylamino)ethyl	97.1	Competitive
Comp 5	H	*c-Pr*	N-(Diethylamino)ethyl	131.8	Mixed

Figure 6 : Synthesis of Benzimidazole–Carboxamides as Potential Multifunctional Agents for the Treatment of Alzheimer's Disease.

(Temel et al.,2023) also presented the synthesis and biological assessment of morpholine dithiocarbamate derivatives incorporating a benzimidazole moiety for their potential as anticholinesterase agents. In this current investigation, novel morpholine dithiocarbamate derivatives (2a-i) containing 1-(2-aryl-2-oxoethyl)-2-substituted benzimidazole motifs were synthesized, and their potential anticholinesterase effects and cytotoxic properties against NIH/3T3 cells were explored. Variously substituted phenyl groups were attached to the carbamate moiety. The evaluation of their ChE inhibitory activities was conducted using a

modified Ellman's assay. The cytotoxic effects of compounds 2a-i on NIH/3T3 cells were determined using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method. Upon examining the structure-activity relationships of the compounds, it was observed that two derivatives containing 4-methyl (6) and 4-chloro (8) substituents exhibited significant inhibition potential against both enzymes. The position of substitution on the phenyl ring influenced the inhibitory potential of the synthesized compounds. Among the compounds, higher anticholinesterase activity was detected in the para-substituted derivatives compared to the meta-substituted ones. Compound 8 emerges as a promising drug candidate either due to its dual inhibitory effect on cholinesterase enzymes or its minimal cytotoxicity towards normal cell lines.



	R	**AChE% inhibition (80 µg/mL)**	**BuChE %inhibition (80 µg/mL)**	**IC50b**
Comp 6	4-CH3	64.69±0.47	65.35±3.44	125.0±22.91
Comp 7	4-OCH3	15.06±2.30	80.51±1.58	125.67±8.14
Comp 8	4-F	78±1.56	70.71±1,53	>200

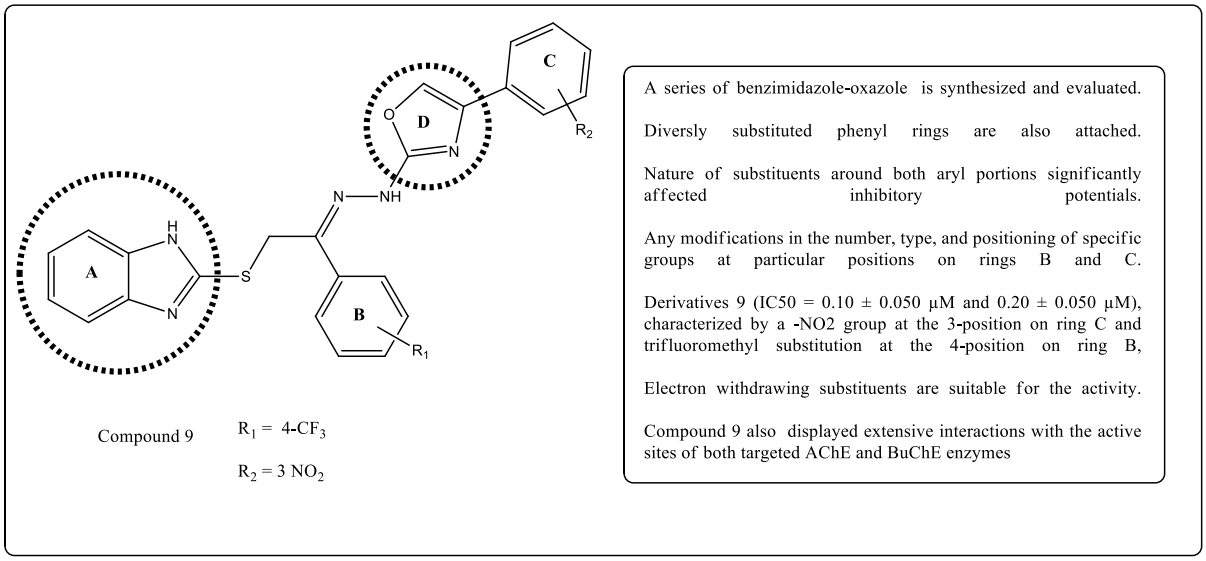
Figure7 : Screening of new morpholine dithiocarbamate derivatives bearing benzimidazole moiety for anticholinesterase potential

(Hussain et al.,2023) also utilized molecular hybridization to merge two biologically significant heterocyclic structures, benzimidazole, and oxazole, to create novel hybrid compounds. Numerous studies emphasize the importance of benzimidazole and oxazole in the treatment of Alzheimer's disease. Consequently, hybrid analogs incorporating these structures were synthesized and then examined for their in vitro inhibition of AChE and BuChE, followed by molecular docking analyses. The newly synthesized benzimidazole-oxazole hybrids displayed a varied spectrum of inhibitory capabilities against the targeted AChE and BuChE enzymes, with IC_{50} values spanning from 0.10 to 12.60 µM against AChE and from 0.20 to 16.30 µM against BuChE, in comparison to the standard donepezil (with IC50 values of 2.16 µM and 4.5 µM,respectively).

Studies on structure-activity relationships (SAR) indicated that variably substituted aryl ring denoted as rings B and C contributed to the distinct inhibitory potentials against the targeted enzymes. It is observed that alterations in either the position or the nature of substituents around both aryl portions significantly affected inhibitory potentials. Overall, it was observed that modifications in the number, type, and positioning of specific groups at particular positions on rings B and C have a notable impact on the inhibitory properties of the synthesized derivatives. Derivatives 9 (IC50 = 0.10 ± 0.050 µM and 0.20 ± 0.050 µM), characterized by a -NO2 group at the 3-position on ring C and trifluoromethyl substitution at the 4-position on ring B, along with derivative **10**, featuring dichloro substitution at the 2- and 3-positions on ring C and a methyl group at the para position on ring B, emerged as the top two most potent inhibitors of both AChE and BuChEenzymes amongthe synthesized derivatives.

A molecular docking analysis was conducted on the most potent benzimidazole-oxazole compound. Compound 9 displayed extensive interactions with the active sites of both targeted AChE and BuChE enzymes, showcasing superior effectiveness, as corroborated by in vitro investigations. It was noted that not only direct incorporation of the electron-withdrawing group (-NO2) led to favorable activity, but also the indirect attachment of the

electron-withdrawing -CF3 moiety, crucial for anchoring the analogue's ring. The heightened interactions, activity, and docking score of compound 9 were predominantly attributed to this factor. Furthermore, the presence of the -NO2 functional group in its meta-position was identified as particularly effective, contributing to the compound's remarkable activity and interactions.



	B-ring	**C-ring**	**AChE** **IC50 ±SEM a (_M)**	**BuChE** **IC50 ±SEM a (_M)**
Comp 9	4-CF_3	3-NO_2	0.10 ± 0.050	0.20 ± 0.050
Comp 10	4-CH_3	2,4-Cl	0.20 ± 0.050	0.30 ± 0.050

Figure 8: synthesis of Benzimidazole-Based Oxazole Analogues: A Promising Acetylcholinesterase and Butyrylcholinesterase Inhibitors.

In addition to targeting cholinesterases, the activation of cannabinoid receptor type 2 (CB2R) has shown potential in alleviating neuroinflammation linked to Alzheimer's disease (AD)[90]. Given that CB2R is predominantly present in immune cells and its expression correlates with AD pathology, a combined approach involving a selective human butyrylcholinesterase (hBChE) inhibitor and a human CB2R agonist was pursued. Recent advancements have unveiled potent CB2R agonists, among which compound 4 exhibited moderate inhibition of equine butyrylcholinesterase (eqBChE), prompting exploration into the fusion of BChE/CB2R functionalities. Various research teams have explored dual inhibition of BChE/CB2R by merging different pharmacophores to target AD. Following a similar trajectory to enhance pharmacokinetic properties, Philipp Spatz et al. introduced a

novel series of hybrid compounds by attaching small carbamate moieties to a 2-benzylbenzimidazole core structure, characterized by low molecular weight and high efficacy against both targets. Through the modification of one of the phenolic positions of the core structure with small, lipophilic carbamates, the resulting compounds were designed to snugly fit into the orthosteric pocket of cannabinoid receptors while interacting with the active center of hBChE. By leveraging smaller carbamate units at various positions of the benzimidazole core, CB2R affinity was incorporated into the benzimidazole-carbamate scaffold of the compounds. A total of fifteen benzimidazole carbamates were synthesized and evaluated for their capacity to inhibit human cholinesterases, with a focus on their pseudoirreversible binding mode and affinity for both cannabinoid receptors via radioligand binding studies.

All test compounds underwent evaluation for their ability to inhibit hBChE and hAChE, aiming to assess the impact of various small carbamate units introduced at the respective phenol moiety. It was observed that small alkyl-substituted carbamates exhibit greater suitability for cholinesterase inhibitory activity. Dimethylated compound (11) showed the significantly more potent cholinesterase inhibitory activity than diethylated compound (15g). However, the direct introduction of the carbamate at the benzimidazole nucleus resulted in a decrease in affinity than at the phenyl moiety as exemplified by dimethylcarbamate 21a which is more than 100-fold less active than its counterpart 11. All the compounds were also evaluated for hCB2R inhibitory activity. It is observed that all monoalkyl carbamates bearing on phenyl ring, exhibited no significant affinity toward hCB2R. However, the compounds carrying the carbamate moiety at the benzimidazole, shows an overall good affinity toward hCB2R. Cyclic carbamates 21c and 12 show an IC50 of 1.5 and 1.0 μM, respectively, making compound 12, which is the only compound modified at the benzimidazole site with a submicromolar inhibition of *h*BChE, a promising hybrid candidate. compounds 11 and 12 were tested for the ability to suppress the production of neurotoxic factors.

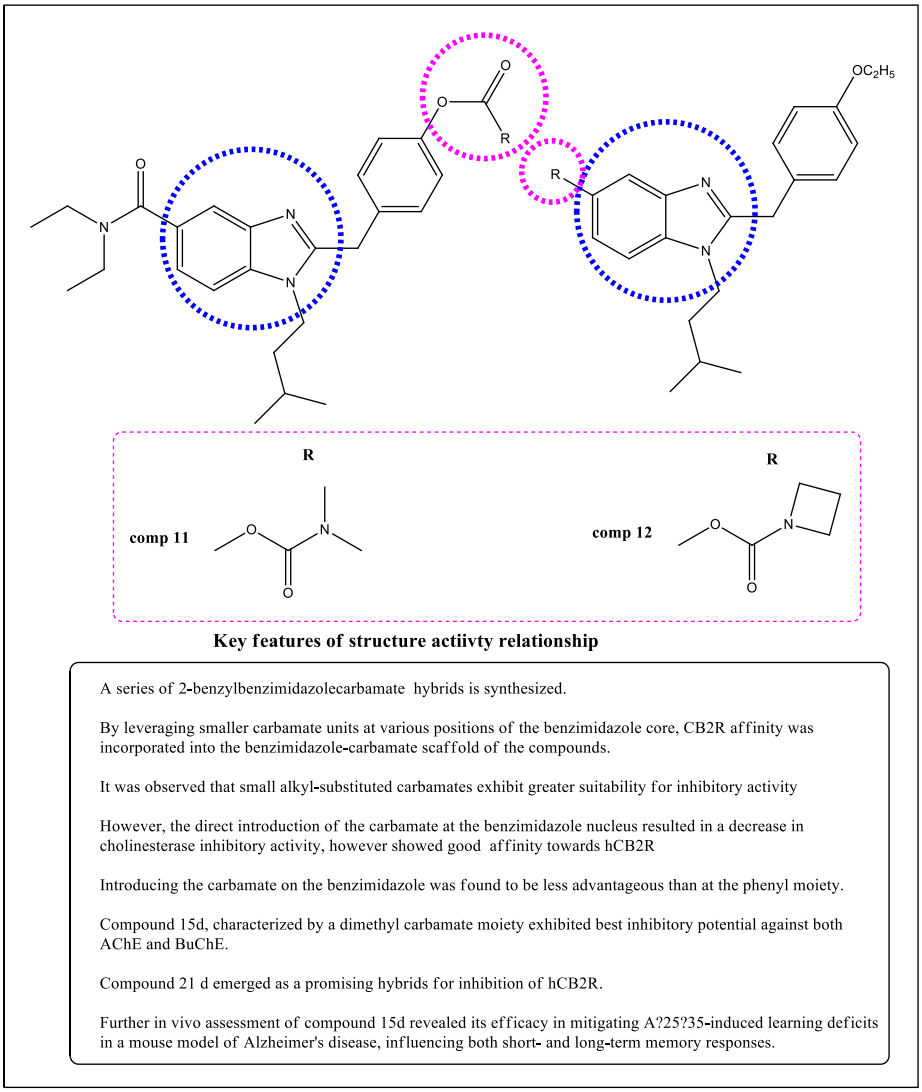
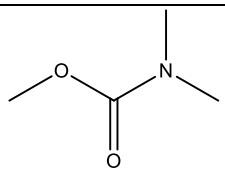
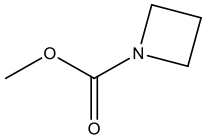
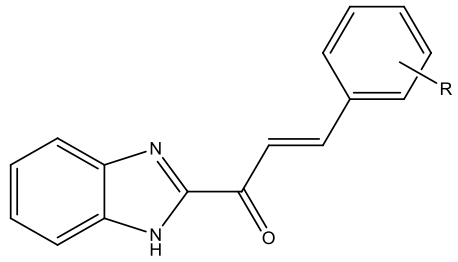


Figure 9: Dual-Acting Small Molecules: Subtype-Selective Cannabinoid Receptor 2 Agonist/Butyrylcholinesterase Inhibitor Hybrids Show Neuroprotection in an Alzheimer's Disease Mouse Model

Compound 11, characterized by a dimethyl carbamate moiety, exhibits a KM value of 7.7 μM, which is 20 times higher than that of compound 12. Additionally, the vmax of compound 11 is 15 times greater. To validate the proposed synergistic effect, a combination study was conducted, confirming the synergistic action of BChE inhibition and CB2R activation in vivo. This underscores the potential of BChE/CB2R hybridization as a therapeutic approach for neurodegeneration. Further in vivo assessment of compound 11 revealed its efficacy in mitigating Aβ25–35-induced learning deficits in a mouse model of Alzheimer's disease, influencing both short- and long-term memory responses.

	R	hBChE IC50 (µM) (IC50 ±SEM)	hAChE IC50 (µM) (IC50 ±SEM)	SI =IC50hAChE/ IC50 hBChE	**hCB2R** IC50 (µM) (IC50 ±SEM)
Comp 11		0.62 (6.2±0.01)	8.66 (5.1±0.06)	14	0.78 (6.1±0.04)
Comp 12		0.15 (6.8±0.06)	>25 (<4.6)	>162	1.02 (6.0±0.10)

Because of its pathological and pharmacological characteristics, MAO-B stands out as a promising target for Alzheimer's disease (AD) therapy. Currently, the market offers three primary MAO-B inhibitors: selegiline, rasagiline, and safinamide. (Athulya et.al.,2023)conducted a study on benzimidazole-based chalcone compounds (BCH), synthesizing them and evaluating their MAO inhibitory properties. Benzimidazole ring was substituted to the chalcone and variety of electron-withdrawing and electron-donating groups were attached on the phenyl ring to assess their impact on the activity profile. Most of the compounds exhibited greater inhibitory activity against MAO-B compared to MAO-A. Specifically, Compound 13 bearing a chloro substituent on the phenyl ring displayed an IC50 value of 0.80 µM, demonstrating the most potent inhibition among all compounds tested. Furthermore, compound 13 alsoshowed the highest MAO-B selectivity index (SI) with an SI value of 44.11 compared to MAO-A. Among all the substituents (methyl, ethyl, methoxy, halogens etc), the halogen group demonstrated the most effective MAO-B inhibition, and the substitution at ortho-position of phenyl exhibited superior inhibitory activity compared to the para-site. Compound 13emerged as a potent, reversible, and selective MAO-B inhibitor, suggesting its potential as a therapeutic candidate for neurological disorders.



Compound 13 R = 2-Cl

A series of benzimidazole-based chalcone compounds (BCH) is synthesized and evaluated for MAO inhibitory properties.

Variosu halogens and small alkyl groups are substituted on the terminal phenyl ring. A chloro substituent at ortho position gave the most favourable results,

All the compounds showerd greater inhibition of MAO-A as compared to MAO-B.

Compound BCH2 displayed an IC50 value of 0.80 µM, demonstrating the most potent MAO-B inhibition and highest selectivity index (SI) with an SI value of 44.11

Comp No	R	Residual activity at 10 µM (%)		IC50 (µM)		**SI**
		MAO-A	MAO-B	MAO-A	MAO-B	
13	2-Cl	76.00 ± 1.89	4.05 ± 1.91	35.29 ± 4.55	0.80 ± 0.0094	44.11

Figure 10: Synthesis of benzimidazole chalcone derivatives

In recent years, there have been notable studies focused on the development of promising selective inhibitors targeting BACE1. Due to the large size and high flexibility of the catalytic active site of BACE1 enzyme, the development of nonpeptide inhibitors with optimal pharmacological properties is still highly demanding. (Quang et.al., 2023) reported the synthesis and evaluation of a novel series of diaryl ether-linked benzimidazole derivatives as potent and selective BACE1 inhibitors. In this work, author discovered 2-aminobenzimidazole-containing ether scaffolds having potent and selective inhibitory potentials against BACE1 enzyme. Benzimidazole derived compounds have previously been reported to exert Anti-Alzheimer's activity. In current study author carried out some structural modification to the previously reported BACE inhibitor compound num OLD. Replacement of ester linkage with an ether linker and replacing the 5-membered pyrrolidine with a phenyl ring resulted in a new and potent derivative 14 which exhibited the highest EC50 = 0.7 µM. Further, molecular docking, molecular dynamics, and DFT studies results further supported the bace 1 inhibitory potential of newly synthesized derivatives.

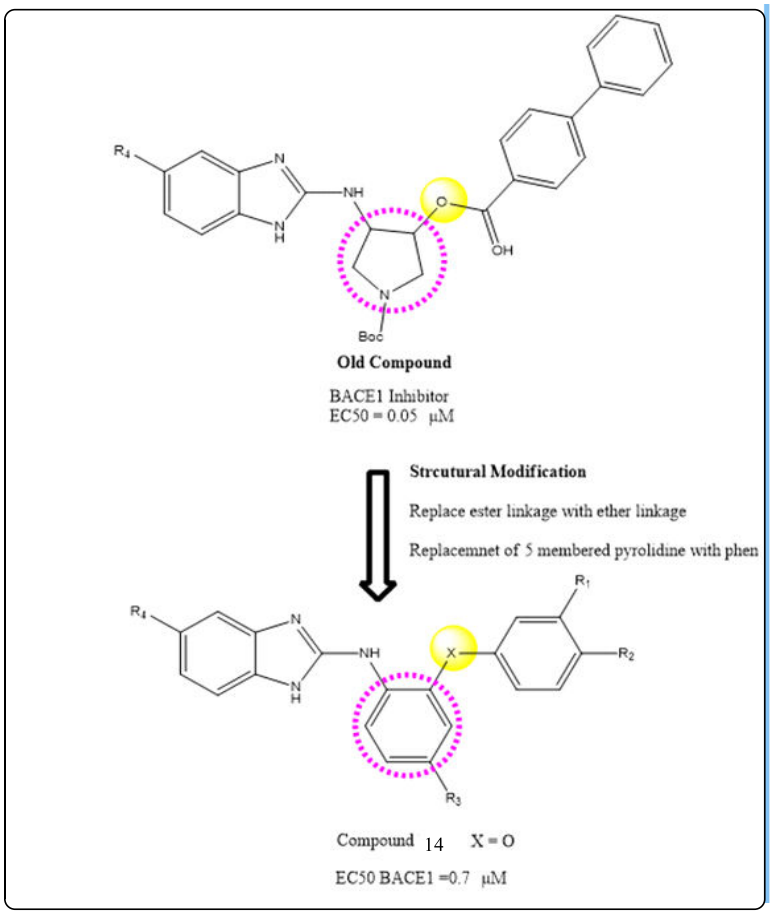
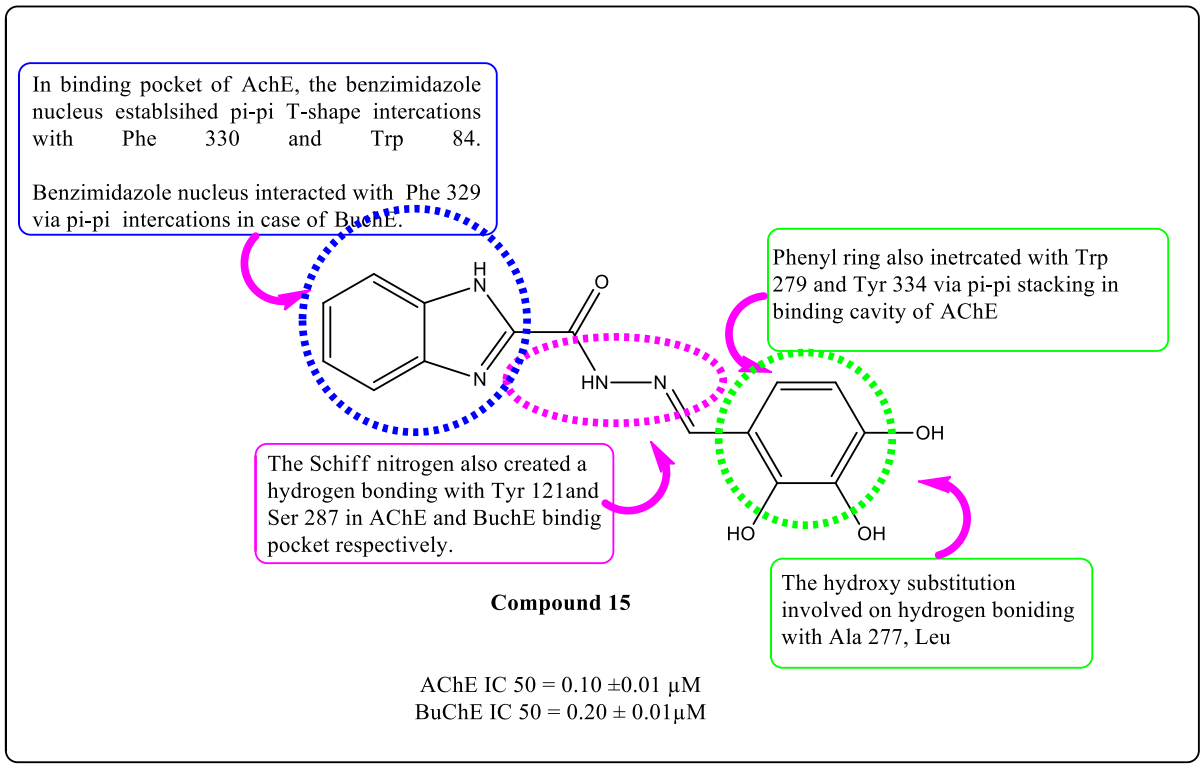


Figure 11: Rational design of novel diaryl ether-linked benzimidazole derivatives as potent and selective BACE1 inhibitors.

(Othman et. al.,2024) reported the synthesis and evaluation of a novel series of benzimidazole based Schiff base derivatives as dual inhibitors for acetylcholinesterase and butyrylcholinesterase enzymes. All analogs showed a variable degree of inhibitory activity with IC_{50} value ranging between 0.10 ± 0.01 to 12.40 ± 0.30 μM for acetylcholinesterase and 0.20 ± 0.01 to 11.10 ± 0.30 μM for butyrylcholinesterase. The most potent analog found among the series was analog 15 bearing trihydroxyphenyl having showed IC50 value 0.10 ± 0.01 and 0.20 ± 0.01 µM for both acetylcholinesterase and butyrylcholinesterase inhibition respectively. To understand the binding interaction of most active derivatives with enzyme active site, molecular docking study were performed. The benzimidazole nucleus established pi-pi T shape interactions with the crucial Phe 330 and Trp 329 amino acid residues. The hydoxy substituents created hydrogen bonding interactions with the Ala 2777 and pi-pi interactions via phenyly ring with Trp 279 and Try 334The toxicity and mutagenicity of compound 8 were predicted using in silico software, namely Derek Nexus® (version 6.3).

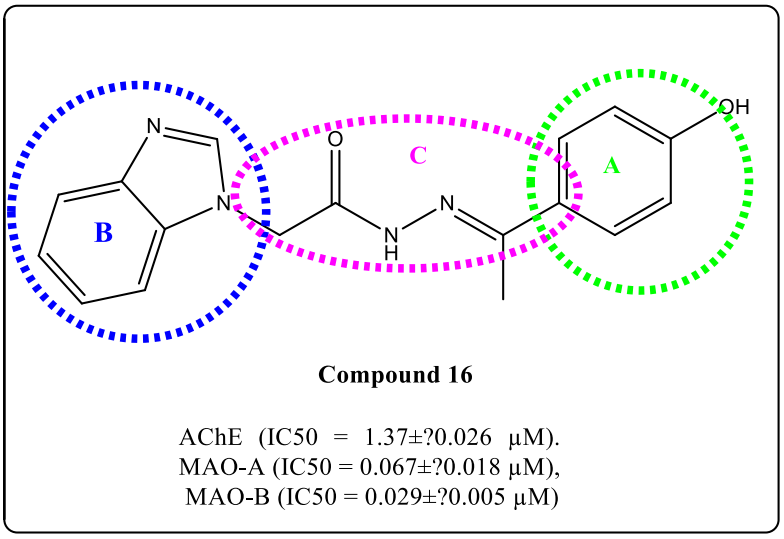


PAPER 19	**AChE** **IC50 ±SEM** (µM)	**BuChE** **IC50 ±SEM** (µM)
Comp 15	0.10 ± 0.01	0.20 ± 0.01

Figure 12 : New benzimidazole based Schiff bases as potent anti-Alzheimer agents: Synthesis, bio-evaluation and molecular docking study

A series of novel benzimidazole-derived carbohydrazones was designed, synthesized and evaluated for their dual inhibition potential against monoamine oxidases (MAOs) and acetylcholinesterase (AChE) using multitarget-directed ligand approach (MTDL)(Othman, M et al., 2024). The investigated compounds have exhibited moderate to excellent *in vitro* MAOs/AChE inhibitory activity at micromolar to nanomolar concentrations. Compound **16**, 2-(1*H*-Benzo[d]imidazol-1-yl)-*N*′-[1-(4-hydroxyphenyl) ethylidene]acetohydrazide emerged as a lead dual MAO-AChE inhibitor by exhibiting superior multi-target activity profile against MAO-A (IC_{50} = 0.067 ± 0.018 µM), MAO-B (IC_{50} = 0.029 ± 0.005 µM) and AChE (IC_{50} = 1.37 ± 0.026 µM). SAR studies suggest that the site A (hydrophobic ring) and site C (semicarbazone linker) modifications attempted on the semicarbazone-based MTDL resulted in a significant enhancement in the MAO-A/B inhibitory potential and a drastic decrease in the AChE inhibitory activity. Further, molecular docking and dynamics simulation experiments disclosed the possible molecular interactions of inhibitors inside the active site of respective enzymes. Also, computational

prediction of drug-likeness and ADME parameters of test compounds revealed their drug-like characteristics.



Compound 16

AChE (IC50 = 1.37±?0.026 µM).
MAO-A (IC50 = 0.067±?0.018 µM),
MAO-B (IC50 = 0.029±?0.005 µM)

	AChE IC50 ±SEM (µM)	**MAO-A IC50 ±SEM** (µM)	**MAO-B IC50 ±SEM** (µM)
Comp 16	1.37 ± 0.026	0.067 ± 0.018	0.029 ± 0.005

Figure 13: Benzimidazole-derived carbohydrazones as dual monoamine oxidases and acetylcholinesterase inhibitors: design, synthesis, and evaluation

A new series of benzimidazole-based thiadiazole hybrids analogs (6a–p) as effective Alzheimer's inhibitors were synthesized and then evaluated for their inhibition profile against acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) enzymes as compared to Donepezil as standard drug(Kumar, S. et al., 2024). All the synthesized benzimidazole-based thiadiazole analogues showed a varied range of inhibitory potentials against targeted AChE & BuChE enzymes with IC_{50} values ranging from 1.32 ± 0.10 µM to 19.26 ± 0.60 µM (for AChE) and 1.94 ± 0.10 µM to 21.33 ± 0.70 µM (for BuChE) when compared to standard Donepezil (IC_{50} = 2.16 ± 0.050 µM for AChE) & 4.50 ± 0.10 µM for BuChE). As structure-activity relationship (SAR) studies revealed that the analogues **17** (bearing *ortho*-hydroxy and *para*-NO_2 on *N*-aryl ring along with *ortho*-NO_2 substitution on another aryl ring) and **18** (that holds *ortho*-hydroxy &*para*-NO_2 substitutions on *N*-aryl ring and 3,4-diCl moieties on another aryl ring) were emerged as the most potent analogues of AChE & BuChE enzymes having IC_{50} values of 1.32 ± 0.10 µM & 1.84 ± 0.20 µM (against AChE) and 1.94 ± 0.10 µM & 2.23 ± 0.20 µM (against BuChE) respectively. Furthermore, the active analogues were subjected to molecular docking studies in order to explore the binding interactions possess by potent analogues with the active sites of amino acids of targeted AChE & BuChE enzymes and result

obtained shows that these active analogues furnished that several key interactions with targeted enzymes active sites. Additionally, all the synthesized analogues were elucidated structurally using variety of spectroscopic (^{1}H NMR & ^{13}C NMR) and spectrometric (HREI-MS) analysis.

Conclusion:

In conclusion, benzimidazole bearing heterocycles proved to be an excellent platform in the field of medicinal chemistry and showed various applications as anticancer agents. Remarkable results obtained for these compounds helped and stimulated researchers to synthesize new benzimidazole derivatives. The recent review described benzimidazole containing heterocycles and showed evidence of their great potential as Anti-Alzheimers agents.

References:

1) Hossain, M., & Nanda, A. K. (2018). A review on heterocyclic: Synthesis and their application in medicinal chemistry of imidazole moiety. Science, 6(5), 83-94.
2) Omar, A. (2020). Review article; anticancer activities of some fused heterocyclic moieties containing nitrogen and/or sulfur heteroatoms. Al-Azhar Journal of Pharmaceutical Sciences, 62(2), 39-54.
3) Shaikh, A. R., Farooqui, M., Satpute, R. H., & Abed, S. (2018). Overview on nitrogen containing compounds and their assessment based on 'International Regulatory Standards'. Journal of Drug Delivery and Therapeutics, 8(6-s), 424-428.
4) Martins, P., Jesus, J., Santos, S., Raposo, L. R., Roma-Rodrigues, C., Baptista, P. V., & Fernandes, A. R. (2015). Heterocyclic anticancer compounds: recent advances and the paradigm shift towards the use of nanomedicine's tool box. Molecules, 20(9), 16852-16891.
5) Kabir, E., &Uzzaman, M. (2022). A review on biological and medicinal impact of heterocyclic compounds. Results in Chemistry, 4, 100606.
6) Amin, A., Qadir, T., Sharma, P. K., Jeelani, I., & Abe, H. (2022). A review on the medicinal and industrial applications of N-containing heterocycles. The Open Medicinal Chemistry Journal, 16(1).
7) Pola, S. (2016). Significance of thiazole-based heterocycles for bioactive systems. Scope of selective heterocycles from organic and pharmaceutical perspective, 1, 13-62.
8) Romeo, R., Iannazzo, D., Veltri, L., Gabriele, B., Macchi, B., Frezza, C., Marino-Merlo, F., &Giofrè, S. V. (2019). Pyrimidine 2,4-Diones in the Design of New HIV RT Inhibitors. Molecules (Basel, Switzerland), 24(9), 1718.
9) Brishty, S. R., Hossain, M. J., Khandaker, M. U., Faruque, M. R. I., Osman, H., & Rahman, S. M. A. (2021). A Comprehensive Account on Recent Progress in Pharmacological Activities of Benzimidazole Derivatives. Frontiers in pharmacology, 12, 762807.

10) Mahurkar, N. D., Gawhale, N. D., Lokhande, M. N., Uke, S. J., &Kodape, M. M. (2023). Benzimidazole: A Versatile Scaffold for Drug Discovery and Beyond-A Comprehensive Review of Synthetic Approaches and Recent Advancements in Medicinal Chemistry. Results in Chemistry, 101139.
11) Manna, S. K., Das, T., &Samanta, S. (2019). Polycyclic benzimidazole: Synthesis and photophysical properties. Chemistry Select, 4(30), 8781-8790.
12) Pinto, D. C. G. A., Santos, C. M., & Silva, A. M. (2007). Advanced NMR techniques for structural characterization of heterocyclic structures. Recent research developments in heterocyclic chemistry, 37(661), 397-475.
13) Titantah, J. T., &Lamoen, D. (2007). Carbon and nitrogen 1s energy levels in amorphous carbon nitride systems: XPS interpretation using first-principles. Diamond and related materials, 16(3), 581-588.
14) Tolomeu HV, Fraga CAM. Imidazole: Synthesis, Functionalization and Physicochemical Properties of a Privileged Structure in Medicinal Chemistry. Molecules. 2023; 28(2):838.
15) Nardi M, Cano NCH, Simeonov S, Bence R, Kurutos A, Scarpelli R, Wunderlin D, Procopio A. A Review on the Green Synthesis of Benzimidazole Derivatives and Their Pharmacological Activities. Catalysts. 2023; 13(2):392.
16) Lee, Y. T., Tan, Y. J., &Oon, C. E. (2023). Benzimidazole and its derivatives as cancer therapeutics: The potential role from traditional to precision medicine. Acta pharmaceuticaSinica. B, 13(2), 478–497.
17) Patel M, Avashthi G, Gacem A, Alqahtani MS, Park H-K, Jeon B-H. A Review of Approaches to the Metallic and Non-Metallic Synthesis of Benzimidazole (BnZ) and Their Derivatives for Biological Efficacy. Molecules. 2023; 28(14):5490.
18) Kamanna, K. (2019). Synthesis and pharmacological profile of benzimidazoles. Chemistry and applications of benzimidazole and its derivatives. London, UK: IntechOpen, 51-69.
19) Karaburun, A. Ç., Kaya Çavuşoğlu, B., AcarÇevik, U., Osmaniye, D., Sağlık, B. N., Levent, S., Özkay, Y., Atlı, Ö., Koparal, A. S., &Kaplancıklı, Z. A. (2019). Synthesis and Antifungal Potential of Some Novel Benzimidazole-1,3,4-Oxadiazole Compounds. Molecules (Basel, Switzerland), 24(1), 191.
20) Brishty, S. R., Hossain, M. J., Khandaker, M. U., Faruque, M. R. I., Osman, H., & Rahman, S. M. A. (2021). A Comprehensive Account on Recent Progress in Pharmacological Activities of Benzimidazole Derivatives. Frontiers in pharmacology, 12, 762807.
21) Brishty, S. R., Hossain, M. J., Khandaker, M. U., Faruque, M. R. I., Osman, H., & Rahman, S. M. A. (2021). A Comprehensive Account on Recent Progress in Pharmacological Activities of Benzimidazole Derivatives. Frontiers in pharmacology, 12, 762807.
22) Adalat B, Rahim F, Rehman W, Ali Z, Rasheed L, Khan Y, Farghaly TA, Shams S, Taha M, Wadood A, et al. Biologically Potent Benzimidazole-Based-Substituted Benzaldehyde Derivatives as Potent Inhibitors for Alzheimer's Disease along with Molecular Docking Study. Pharmaceuticals. 2023; 16(2):208.

23) Hussain R, Khan S, Ullah H, Ali F, Khan Y, Sardar A, Iqbal R, Ataya FS, El-Sabbagh NM, Batiha GE-S. Benzimidazole-Based Schiff Base Hybrid Scaffolds: A Promising Approach to Develop Multi-Target Drugs for Alzheimer's Disease. Pharmaceuticals. 2023; 16(9):1278.
24) Belinskaia, D. A., Voronina, P. A., Krivorotov, D. V., Jenkins, R. O., &Goncharov, N. V. (2023). Anticholinesterase and Serotoninergic Evaluation of Benzimidazole-Carboxamides as Potential Multifunctional Agents for the Treatment of Alzheimer's Disease. Pharmaceutics, 15(8), 2159.
25) Temel, H. E., Çiftçi, G. A., &Yurttaş, L. (2023). Screening of new morpholine dithiocarbamate derivatives bearing benzimidazole moiety for anticholinesterase potential. European Journal of Life Sciences, 2(2), 71-77.
26) Hussain, R., Rahim, F., Ullah, H., Khan, S., Sarfraz, M., Iqbal, R., ... & Al-Sadoon, M. K. (2023). Design, Synthesis, In Vitro Biological Evaluation and In Silico Molecular Docking Study of Benzimidazole-Based Oxazole Analogues: A Promising Acetylcholinesterase and Butyrylcholinesterase Inhibitors. Molecules, 28(20), 7015.
27) Spatz, P., Steinmüller, S. A., Tutov, A., Poeta, E., Morilleau, A., Carles, A., ... & Decker, M. (2023). Dual-acting small molecules: subtype-selective cannabinoid receptor 2 agonist/butyrylcholinesterase inhibitor hybrids show neuroprotection in an Alzheimer's disease mouse model. Journal of Medicinal Chemistry, 66(9), 6414-6435.
28) Krishna, A., Lee, J., Kumar, S., Sudevan, S. T., Uniyal, P., Pappachen, L. K., ... & Mathew, B. (2023). Inhibition of monoamine oxidases by benzimidazole chalcone derivatives. Applied Biological Chemistry, 66(1), 37.
29) Krishna, A., Lee, J., Kumar, S., Sudevan, S. T., Uniyal, P., Pappachen, L. K., ... & Mathew, B. (2023). Inhibition of monoamine oxidases by benzimidazole chalcone derivatives. Applied Biological Chemistry, 66(1), 37.
30) Othman, M., Hayat, S., Rahim, F., Taha, M., Sajid, M., Khan, S., ... & Hafez, M. M. (2024). New benzimidazole based Schiff bases as potent anti-alzheimer agents: Synthesis, bio-evaluation and molecular docking study. Journal of Molecular Structure, 1309, 138058.
31) Kumar, S., Jaiswal, S., Gupta, S. K., &Ayyannan, S. R. (2024). Benzimidazole-derived carbohydrazones as dual monoamine oxidases and acetylcholinesterase inhibitors: design, synthesis, and evaluation. Journal of Biomolecular Structure and Dynamics, 42(9), 4710-4729.