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Review Paper

Benzimidazole Derivatives: A Comprehensive Review Of Synthesis, Biological Evaluation, And Therapeutic Potential in Anticancer and Neuroprotective Applications

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ABSTRACT

The benzimidazole heterocyclic ring system, consisting of a benzene ring fused with an imidazole ring, is a privileged pharmacophore in medicinal chemistry. This review summarizes the biological activities and chemical structures of benzimidazole derivatives reported in two studies with different structural substitutions. Keser et al. (2026) synthesized nine aliphatic-substituted derivatives, identifying compound 4 (oxadiazole-substituted benzimidazole) as the most potent against MCF-7 and MDA-MB-231 breast cancer cells (IC_{50} = 12.8 and 15.7 µM), with selectivity toward normal cells. Its anticancer activity was associated with S-phase cell cycle arrest, apoptosis, and inhibition of Bcl-2, Bcl-xL, and CDK2. Raka et al. (2022) synthesized aromatic-substituted derivatives under environmentally friendly aqueous conditions, where compound 3b showed analgesic activity comparable to morphine and compound 3a exhibited antioxidant activity similar to BHT. Molecular docking and ADMET studies supported favorable drug-like properties and target binding, highlighting the importance of structural modification of the benzimidazole core in achieving diverse pharmacological activities. [1,2]

INTRODUCTION

Benzimidazole is an important heterocyclic scaffold in medicinal chemistry due to its structural similarity to purines, ability to donate and accept hydrogen bonds, and favorable electronic properties that facilitate interactions with various biological targets. Structurally, it consists of a fused benzene and imidazole ring system, enabling strong binding within enzyme active sites and receptor pockets. Owing to these characteristics, benzimidazole derivatives have found extensive pharmaceutical applications. Several clinically important drugs, including

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omeprazole and lansoprazole (proton pump inhibitors), mebendazole and albendazole (anthelmintic), and candesartan (antihypertensive agent), are based on the benzimidazole nucleus. Furthermore, numerous studies have reported anticancer, antimicrobial, antiviral, anti-inflammatory, analgesic, and neuroprotective activities among benzimidazole derivatives. [7,8,18,23,24]

The biological activity of benzimidazole derivatives is significantly influenced by the nature of substituents attached to the core structure. Aliphatic substituents such as methyl, ethyl, and propyl groups can enhance lipophilicity, membrane permeability, and pharmacokinetic properties, whereas aromatic substituents including phenyl, chlorophenyl, hydroxyphenyl, and methoxyphenyl groups improve planarity and electronic conjugation, facilitating interactions with DNA and protein targets. This review highlights findings from two complementary studies that demonstrate the versatility of the benzimidazole scaffold. Keser et al. (2026) investigated aliphatically substituted benzimidazole derivatives as potential anti-breast cancer agents through apoptosis induction and cell cycle arrest mechanisms, while Raka et al. (2022) synthesized aromatic benzimidazole derivatives using a green chemistry approach and evaluated their analgesic, anti-inflammatory, antioxidant, antimicrobial, acetylcholinesterase inhibitory, and computationally predicted biological activities. [7,8,18,23,24]

2. Chemistry: Synthesis and Structural Characterization

2.1 Synthesis of Aliphatic-Substituted Benzimidazole Derivatives

Keser et al. synthesized a series of benzimidazole derivatives starting from a common precursor (Compound 1) containing benzimidazole, methyl, and thienyl groups. Reaction of Compound 1 with phenyl isocyanate and p-chlorophenyl isocyanate produced urea derivatives (2a and 2b), which were further cyclized under basic conditions to form triazole derivatives (3a and 3b). Additionally, treatment of 2a with sulfuric acid resulted in the formation of Compound 4, a 1,3,4-oxadiazole derivative that emerged as an important lead compound. [1]

Further structural diversification was achieved through aldehyde condensation reactions, yielding Schiff base derivatives (5a–5c), while reaction with carbon disulfide and potassium hydroxide produced a thiooxadiazole derivative (6). All synthesized compounds were characterized using ^{1}H-NMR, ^{13}C-NMR, and LC-MS/TOF techniques. The successful preparation of urea, triazole, oxadiazole, Schiff base, and thiooxadiazole derivatives from a single precursor highlights the versatility of the benzimidazole scaffold for developing pharmacologically active molecules. [1]

2.2 Synthesis of Aromatic-Substituted Benzimidazole Derivatives

Raka et al. developed an environmentally friendly method for synthesizing benzimidazole derivatives using solvent-free or aqueous reaction conditions and inexpensive, eco-friendly catalysts. The key reaction involved the condensation of o-phenylenediamine with aromatic aldehydes or glycolic acid. Disubstituted benzimidazole derivatives (3a–3c) were synthesized using iodine/water or SiO_2/$ZnCl_2$ catalytic systems, providing hydroxyphenyl, chlorophenyl, and methoxyphenyl substituted products in good to excellent yields. [2]

A monosubstituted benzimidazole derivative (4) was obtained using boric acid as a selective catalyst, while compound 6 (a benzyl ether derivative) was synthesized through a two-step process involving the formation of 2-

hydroxymethyl-1H-benzimidazole (5) followed by benzylation. The use of water as a reaction medium and mild catalysts demonstrates the principles of green chemistry. All synthesized compounds were purified by column chromatography and characterized using IR, ^{1}H NMR, and ESI-MS spectroscopy. [2]

2.3 Structural Comparison and Chemical Insights

The two series of synthetics vary greatly from each other in their modes of substitution on the core structure benzimidazole. In the case of the aliphatic substituted compounds prepared by Keser et al., the dimethyl groups are introduced on the N-substituted carbons of the benzimidazole ring, leading to spatial restrictions in conformation due to steric hindrance. This is followed by the addition of the oxadiazole, triazole, or Schiff base units to the side-chain moiety, contributing additional pharmacophoric features to the molecule. [1,2]

The structure of compound 4 in the series Keser, the anti-cancer lead, is notably described by the hybridization of the 1,3,4-oxadiazole ring that creates a rigid, electron-withdrawing heteroatom system, allowing for particular interactions with potential biological targets. Indeed, the use of an oxadiazole moiety is widely used as a bioisosteric substitution in medicine chemistry, being a good alternative to both amide and ester linkages, increasing drug metabolic stability while retaining the biological activity of the molecule towards its target. [1,2]

3. Anticancer Biological Evaluation

3.1 Cytotoxicity Assessment — MTT Assay

Cytotoxic activity of all nine synthetic aliphatic substituted benzimidazole compounds was determined using the MTT assay in three different cells: MCF-7 (hormone receptor positive breast carcinoma), MDA-MB-231 (triple negative breast cancer) and MCF-10A (normal breast cells) following exposure for 72 hours. Results of IC50 values are shown in Table 1 below. [1]

Table 1. IC50 values (μM) of synthesized compounds in breast cancer and normal cell lines after 72 h treatment.[1]

Compound	MCF-7 IC50 (μM)	MDA-MB-231 IC50 (μM)	MCF-10A IC50 (μM)	Notes
2a	45.4 ± 0.9	82.3 ± 1.5	64.7 ± 1.3	Low potency
2b	154.3 ± 3.1	114.9 ± 2.0	162.7 ± 3.0	Weak
3a	18.7 ± 1.6	20.4 ± 2.0	14.4 ± 0.8	Notable
3b	52.8 ± 0.8	43.3 ± 1.8	44.7 ± 2.4	Moderate
4 (Lead)	12.8 ± 1.0	15.7 ± 2.1	16.4 ± 2.0	Most potent
5a	91.4 ± 1.6	100.2 ± 3.1	117.6 ± 2.2	Low
5b	115.3 ± 1.7	108.6 ± 1.2	121.9 ± 2.0	Low
5c	59.1 ± 1.3	66.5 ± 1.1	50.8 ± 1.4	Low
6	116.3 ± 2.7	120.0 ± 1.9	125.7 ± 2.3	Low
Cisplatin (Ref.)	14.7 ± 0.8	19.9 ± 0.6	15.4 ± 1.2	Reference

Among the synthesized compounds, Compound 4 showed the strongest anticancer activity against both MCF-7 and MDA-MB-231 breast cancer cell lines, with IC_{50} values comparable to the standard drug cisplatin. Importantly, Compound 4 displayed lower toxicity toward normal MCF-10A cells, indicating a degree of selectivity for cancer cells, which is a desirable characteristic for potential anticancer agents. Compound 3a also demonstrated notable cytotoxic activity but exhibited greater toxicity toward normal cells, suggesting poorer selectivity. [1] In contrast, compounds 2a, 3b, 5a–5c, and 6 showed weak to moderate cytotoxic effects, while Compound 2b was largely inactive. The superior activity of Compound 4 is attributed to the presence of the 1,3,4-oxadiazole ring, which enhances interactions with biological targets, and dimethyl substituents that increase lipophilicity and facilitate cellular uptake. These structural features likely contribute to its enhanced anticancer potential. [1]

3.2 Cell Cycle Analysis

In an effort to determine the mechanism by which compound 4 exhibits antiproliferative properties, a flow cytometric study was carried out through PI staining of cells cultured at IC50 concentration for 72 hours. [1] MCF-7 untreated control cells had a normal proliferation cycle pattern of 55.6% G0/G1 phase, 31.5% S phase, and 9.9% G2/M phase. Likewise, for MDA-MB-231 control cells, the percentages of the phases are 58.1%, 30.7%, and 9.2%, respectively. Significant changes were noted in both cell lines after being exposed to compound 4 for 72 hours. [1] For the case of MCF-7 cells, there was a decrease in the G0/G1 phase from 38% to 40%, an increase in the S-phase from 45% to 47%, and an increase in G2/M from 12% to 14%. MDA-MB-231 cells were observed to have a similar G0/G1 decrease from 40% to 42%, with an increase in S-phase from 43% to 45%, and a G2/M increase from 13% to 15%. This significant increase in the S-phase indicates that 4 affects the processes of DNA synthesis and replication, thus causing DNA replication stress-induced cell cycle arrest. [1]

3.3 Apoptosis Induction

Flow cytometry analysis using Annexin V/PI staining showed that Compound 4 effectively induced apoptosis in both MCF-7 and MDA-MB-231 breast cancer cells after 72 hours of treatment at its IC_{50} concentration. Treatment significantly reduced cell viability and increased both early and late apoptotic cell populations compared with untreated controls. The observed induction of apoptosis, together with S-phase cell cycle arrest, suggests that Compound 4 triggers replication stress-mediated cell death, potentially through activation of the mitochondrial apoptotic pathway, a mechanism commonly associated with benzimidazole derivatives. [1]

4. Molecular Docking and MM-GBSA Analysis

4.1 Docking Studies of Anticancer Compound 4

Docking studies for the interaction between compound 4 and four proteins involved in regulating apoptosis and cell cycles were performed by employing AutoDockVina (v4.2.5.1): Bcl-2 (PDB: 2W3L), Bcl-xL (PDB: 2YXJ), CDK2 (PDB: 2VU3), and Cyclin E (PDB: 2B9R). Preparation of protein and ligand optimization were done with the help of Protein Preparation Wizard and Gaussian 09 W with the OPLS 2005 force field, respectively, at pH of physiological condition. [1]

Table 2. Molecular docking analysis of compound 4 with apoptosis and cell cycle regulatory proteins.[1]

Protein	Binding Energy (kcal/mol)	H-Bond Energy	Key Amino Acid Residues
Bcl-2	-127.644	-13.688	ARG26, ARG65, ARG68, PHE71, VAL118, GLU119, LYS22, SER64
Bcl-xL	-158.828	-0.641	TYR101, TYR195, PHE97, ARG100, GLU92, GLU96, ASN136, TRP137
CDK2	-164.055 (Strongest)	-0.349	LEU134, ASP145, GLN131, LYS33, TYR15, VAL64, ILE35, PHE80
Cyclin E	-140.654	-3.759	MET105, GLU149, SER233, VAL237, TRP95, TYR255, GLN240

Molecular docking studies revealed that Compound 4 exhibited strong binding affinities toward multiple protein targets, suggesting a multitarget mechanism of action. The strongest interaction was observed with CDK2, where Compound 4 formed several hydrogen bonds and hydrophobic interactions within the ATP-binding region. These interactions may inhibit ATP binding and kinase activity, thereby disrupting cell cycle progression and reducing cancer cell proliferation. [1] Compound 4 also showed favorable binding to the anti-apoptotic proteins Bcl-xL and Bcl-2, potentially blocking their ability to suppress apoptosis and promoting cancer cell death. In addition, interactions with Cyclin E at the Cyclin E–CDK2 interface may contribute to G1/S phase cell cycle arrest. Together, these findings support the observed apoptotic and antiproliferative effects of Compound 4 in breast cancer cells. [1]

4.2 MM-GBSA Binding Free Energy Calculations

Energetic validation of the docking results was performed using Molecular Mechanics/Generalized Born Surface Area (MM-GBSA) method to analyze the energetics of binding. The MM-GBSA calculations include interactions due to van der Waals forces, electrostatic interactions, and solvent. [1]

Table 3. MM-GBSA binding free energy analysis of compound 4 with target proteins.[1]

Target Protein	ΔEvdW (kcal/mol)	ΔEele (kcal/mol)	ΔGsolv (kcal/mol)	ΔGbind (kcal/mol)
Bcl-2	-48.7	-21.3	+24.9	-45.1
Bcl-xL	-44.2	-18.6	+23.4	-39.4
CDK2	-51.8	-24.1	+27.6	-48.3
Cyclin E	-42.5	-17.9	+22.8	-37.6

Binding free energies showed favorable binding in the four protein receptors. Highest binding energy was noted for CDK2 with ΔGbind = -48.3 kcal/mol and Bcl-2 with ΔGbind = -45.1 kcal/mol. Van der Waals forces played an important role in the energetics of binding (ΔEvdW from -51.8 to -

42.5 kcal/mol). This is because of the nature of the extended aliphatic chain that effectively occupies hydrophobic regions. This result supports the mechanism of apoptosis induction and cell cycle inhibition at one site. [1]

4.3 Docking of Neuroprotective Benzimidazole Derivatives

In the Raka study, consensus molecular docking was performed against acetylcholinesterase (AChE, PDB: 4pqe) and butyrylcholinesterase (BChE, PDB: 6esy), enzymes relevant to Alzheimer's disease and neurodegeneration. Multiple docking platforms (AutoDock Vina, Chimera, and Achilles docking server) were used for consensus scoring. [2]

Table 4. Consensus docking scores and key interactions of benzimidazole derivatives with AChE and BChE. [11]

Target	Compound	Consensus Score (kcal/mol)	H-bond interactions	Hydrophobic Interactions
AChE	3b	-8.50	None	LEU A:4, ILE A:16 (Pi-Alkyl, Pi-Cation via ARG A:14)
AChE	3a	-8.37	GLU A:181	PRO A:51 (Pi-Alkyl), ARG A:9 (Pi-Cation)
AChE	4	-8.07	None	LEU A:44, ALA A:163, VAL A:260 (Alkyl, Pi-Alkyl)
BChE	4	-9.20	LEU A:123	LEU A:127, HIS A:124, ASN A:94 (Pi-Pi Stacked)
BChE	3a	-8.63	None	LEU A:123 (Pi-Alkyl), HIS A:75 (Pi-Pi Stacked)

With regard to AChE affinity, compound 3b exhibited high docking scores with a docking energy of -8.50 kcal/mol and was bound by hydrophobic interactions (Pi-Alkyl of residues LEU A:4 and ILE A:16) and Pi-Cation interaction with ARG A:14. Meanwhile, compound 3a was found to be involved in hydrogen bonds with residue GLU A:181, Pro-Alkyl interactions with PRO A:51, and a Pi-Cation interaction with ARG A:9 with docking energy of -8.37 kcal/mol. For BChE affinity, compound 4 had the highest docking energy of -9.20 kcal/mol with docking mechanisms involving hydrogen bonding with LEU A:123 and stacking of pi-pi interaction with HIS A:124 and ASN A:94. [2]

5. Analgesic, Anti-inflammatory, and Neuroprotective Activities

5.1 Central Analgesic Activity

Central analgesia was tested using the radiant heat tail flick test on Swiss albino mice, with morphine serving as the standard positive control agent. The compounds were orally administered to the animals in doses of 25 mg/kg and 50 mg/kg, with tail flick latency times determined at 0 minutes, 30 minutes, 60 minutes, and 90 minutes after administration. [2,12,13]

Table 5. Comparison of central anti-nociceptive activity of key benzimidazole derivatives vs. morphine standard (% elongation of tail-flick latency).[11,12,13]

Compound (mg/kg)	% Elongation 0 min	% Elongation 30 min	% Elongation 60 min	% Elongation 90 min	Activity
Morphine (std)	57.14	162.79	82.5	48.08	Reference
3b (25 mg/kg)	42.86	90.70***	25.0	17.31	Promising
3b (50 mg/kg)	108.57	69.63***	35.0	46.15**	Comparable
3a (25 mg/kg)	77.14	52.56**	79.0***	25.10*	Significant
6 (50 mg/kg)	182.86	39.53	87.5***	32.69*	Notable

Evaluation of central analgesic activity showed that compounds 3a, 3b, and 6 produced significant pain-relieving effects. Among them, Compound 3b demonstrated the strongest activity, exhibiting analgesic effects comparable to the standard drug morphine at higher doses. Compound 3a also showed marked analgesic activity, while Compound 6 produced effects similar to morphine at 60 minutes, indicating that these benzimidazole derivatives possess promising central analgesic potential. [2,12,13]

5.2 Peripheral Analgesic Activity

Peripheral analgesic activity was assessed using acetic acid-induced writhing assay, and diclofenac sodium (25 mg/kg) was used as reference drug. Out of all the test drugs studied, compound 6 exhibited highest peripheral analgesic activity (55.60% writhing inhibition) at dose level of 50 mg/kg, followed by compound 3c (54.10% at 25 mg/kg). Compound 3a and compound 3b exhibited moderate peripheral analgesic activity at dose level of 25 mg/kg (49.25%, 26.87%), respectively ($P < 0.001$). Compound 4 (monosubstituted) failed to show any significant peripheral analgesic activity. [2]

5.3 Anti-inflammatory Activity

Anti-inflammatory activity was evaluated using the carrageenan-induced paw edema model, with diclofenac sodium as the reference drug. Among the tested compounds, disubstituted benzimidazoles 3b and 3a exhibited the strongest anti-inflammatory effects, with Compound 3b showing inhibition levels comparable to diclofenac after 4 hours. Compounds 3c and 6 displayed moderate activity, whereas Compound 4 showed only weak anti-inflammatory effects. [11,13] The superior activity of compounds 3a and 3b is likely due to their enhanced interaction with cyclooxygenase (COX) enzymes. Their strong analgesic and anti-inflammatory profiles suggest that these compounds may exert their effects through a mechanism similar to that of diclofenac, involving inhibition of the COX pathway. [2,13]

5.4 Cholinesterase Inhibitory Activity

Cholinesterase inhibitory activity was evaluated using Ellman's assay with donepezil as the reference standard. Among the synthesized compounds, Compound 6 showed the strongest inhibition of AChE, while compounds 3a and 3b exhibited comparatively weaker AChE activity. In contrast, compounds 3a and 3b demonstrated the highest BChE inhibitory effects, with Compound

3a showing notable selectivity toward BChE. These results suggest the potential of these benzimidazole derivatives as lead compounds for developing therapies targeting neurodegenerative disorders. [2,14,15]

5.5 Antioxidant Activity

The antioxidant activities were determined by DPPH free radical scavenging assay using BHT as the reference antioxidant (IC50 value = 14.44 µg/mL). Compound 3a displayed a high antioxidant activity (IC50 value = 16.73 µg/mL), having a similar degree of efficiency compared to BHT. This was due to the phenolic –OH group present on 4-hydroxybenzyl residue, which acts as an important chemical structure responsible for antioxidant activities via donating hydrogen atoms. Moreover, in the lipid peroxidation assay performed using rat brain homogenate as a substrate, compound 3a demonstrated the highest activity among all tested compounds (IC50 value = 75.53 µg/mL versus catechin reference IC50 value = 59.36 µg/mL). The antioxidant activities of compounds 4 and 3b were moderate whereas the antioxidant activities of compounds 6 and 3c were low. [2,16]

5.6 Antimicrobial Activity

Antimicrobial activity was evaluated against several Gram-positive and Gram-negative bacteria as well as fungal strains using the disc diffusion method. Among the tested compounds, only compounds 4 and 6 exhibited weak antimicrobial activity, producing small zones of inhibition against some bacterial strains. In contrast, the disubstituted benzimidazole derivatives (3a, 3b, and 3c) showed no significant antimicrobial effects, suggesting that aromatic substitution may reduce the antibacterial potential of these compounds. [2,21]

5.7 Cytotoxicity (Brine Shrimp Lethality Assay)

The cytotoxicity studies done using the brine shrimp lethality test provided preliminary data for the toxicity of the compounds with vincristine sulfate used as the positive control (LC50 = 1.283 µg/mL). Compound 6 exhibited modest cytotoxicity (LC50 = 16.68 µg/mL) while 3b (LC50 = 21.9 µg/mL) and 3c (LC50 = 35.99 µg/mL) displayed weaker cytotoxicity. Compounds 3a and 4 exhibited minimal cytotoxicity (LC50 = 116.17 and 148.48 µg/mL) in comparison. The cytotoxicity data obtained via brine shrimp test can be supplemented with the findings from MTT test in Keser et al. [2]

6. Comparative ADMET Profiling

Both experiments applied ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) analysis through silico to determine the drug likeness and pharmacokinetics of the compounds. Specifically, the use of AdmetSAR 2.0 web server was made in the Keser study for compound 4, whereas the Raka study used SwissADME and admetSAR to analyze its compounds. These findings are summarized in Table 6 below. [1,2]

Table 6. Comparative ADMET profiles of lead compounds from both studies.

ADMET Parameter	Compound 4 (Anticancer Study)	Compound 3a (Review Study)	Acceptable Range
Molecular Weight	<500 Da (compliant)	316.35 Da	<500 Da
H-bond Donors	Within limit	2	≤5
H-bond Acceptors	Within limit	3	≤10
LogP	Moderate (favorable)	WLOGP 4.16	≤5
GI Absorption	High	High	High preferred
BBB Permeability	Low (advantageous for cancer)	Yes	Low preferred (anticancer)
Ames Mutagenicity	Negative	AMES toxic	Negative preferred
hERG Inhibition Risk	Low	Not reported	Low preferred
Lipinski Violations	0	0	≤1

ADMET analysis identified Compound 4 from the Keser study as the most promising candidate, exhibiting favorable drug-like properties, no Lipinski rule violations, absence of Ames mutagenicity, low risk of hERG inhibition, and minimal blood–brain barrier penetration, which may reduce the likelihood of central nervous system toxicity. Additionally, its moderate plasma protein binding and lack of major CYP450 inhibition suggest a low potential for drug–drug interactions. [1,2] Among the Raka series, Compound 3a showed the best overall pharmacokinetic profile with good oral absorption and BBB permeability, although it was predicted to be Ames toxic. Compound 6 was the only non-mutagenic derivative in this series and demonstrated good solubility and bioavailability. The contrasting Ames toxicity results between the two studies highlight the significant influence of structural modifications on mutagenic potential, with the aliphatic oxadiazole-containing Compound 4 appearing safer than the more aromatic benzimidazole derivatives. [1,2]

7. Structure-Activity Relationships (SAR) and Mechanistic Insights

7.1 Anticancer SAR

Structure–activity relationship (SAR) analysis from the Keser study identified Compound 4 as the most potent anticancer derivative. Its superior activity is attributed to the combined presence of a 1,3,4-oxadiazole ring, which enhances interactions with protein binding sites, aliphatic dimethyl groups that improve lipophilicity and cellular uptake, and an NH group capable of forming important hydrogen bonds with target proteins. [1,3,4] The transformation of triazole derivatives (3a and 3b) into the oxadiazole-containing Compound 4 significantly improved cytotoxic activity, highlighting the importance of the electron-deficient oxadiazole ring in anticancer activity. In contrast, Schiff base derivatives (5a–5c) and the thiooxadiazole derivative (6) showed lower biological activity, suggesting that the oxadiazole moiety is a key pharmacophoric feature responsible for the enhanced anticancer effects. [1,3,4]

7.2 Non-Cancer Pharmacological SAR

Structure–activity relationship (SAR) studies of the Raka series revealed that disubstituted benzimidazole derivatives (3a, 3b, and 3c) exhibited greater analgesic and anti-inflammatory activities than the monosubstituted derivative (4). The presence of an additional aromatic substituent likely enhances receptor binding through favorable electronic and steric effects, leading to improved biological activity. [2,12,13] The phenolic –OH group in Compound 3a plays a key role in its strong antioxidant activity by facilitating hydrogen donation and free radical scavenging. This functional group may also contribute to enhanced interactions with BChE. In contrast, the electron-withdrawing chloro substituent in Compound 3b appears to be responsible for its superior analgesic activity, highlighting the importance of substituent type in determining biological effects. [2,12,13]

7.3 Multi-Target Activity and Drug Resistance Implications

Another significant finding from the synthesis of information is the benefit of multi-target action. For instance, compound 4 as described by Keser acts on Bcl-2, Bcl-xL, CDK2, and Cyclin E – four different molecular targets for cancer cell survival and proliferation. The benefit of having a compound with multi-target actions is its superiority to compounds that have single targets in overcoming the problems of heterogeneity and resistance to treatment in tumors. The resistance of cancers to drugs is caused by mutations in the targets or overexpression of other pathways for survival. [1,6,11] Another significant finding from the synthesis of information is the benefit of multi-target action. For instance, compound 4 as described by Keser acts on Bcl-2, Bcl-xL, CDK2, and Cyclin E – four different molecular targets for cancer cell survival and proliferation. The benefit of having a compound with multi-target actions is its superiority to compounds that have single targets in overcoming the problems of heterogeneity and resistance to treatment in tumors. The resistance of cancers to drugs is caused by mutations in the targets or overexpression of other pathways for survival. [1,6,2]

8. Comparative Analysis and Broader Context

The findings of both studies are consistent with previous benzimidazole research. The potent anticancer activity of Compound 4 supports earlier reports demonstrating that appropriately substituted benzimidazole derivatives can induce apoptosis and cell cycle arrest. In particular, studies on benzimidazole–oxadiazole hybrids have highlighted the importance of the oxadiazole moiety in enhancing anticancer activity, which aligns with the superior performance of Compound 4. Similarly, the analgesic, anti-inflammatory, and cholinesterase inhibitory activities observed in the Raka series are in agreement with earlier studies identifying benzimidazole derivatives as promising agents for pain management, inflammation control, and neurodegenerative disorders. [1,2,3,4,5,12,13,14,15] A key difference between the two studies lies in their synthetic strategies. Keser et al. employed conventional multi-step synthesis using organic solvents and reflux conditions to generate structurally complex derivatives with enhanced anticancer potential. In contrast, Raka et al. adopted a green chemistry approach using aqueous or solvent-free conditions and inexpensive catalysts to produce biologically active compounds in a more sustainable and cost-effective manner. These contrasting approaches highlight the balance between structural complexity, biological activity, and synthetic accessibility in drug development. [1,2,3,4,5,12,13,14,15]

FUTURE DIRECTIONS AND DRUG DEVELOPMENT PERSPECTIVES

The findings from both reviewed studies point to several promising avenues for future research and drug development: [1,2]

• In vivo studies: Evaluate Compound 4 in animal tumor models and further test compounds 3a and 3b to confirm efficacy and safety.

• Mechanistic studies**:** Investigate apoptosis-related biomarkers such as caspases, cytochrome c, ROS generation, mitochondrial membrane potential, and expression of Bcl-2, Bcl-xL, and CDK2.

• Structural optimization: Modify Raka-series compounds to reduce predicted mutagenicity while maintaining biological activity.

• Combination therapy: Assess Compound 4 in combination with existing anticancer drugs to enhance therapeutic efficacy and reduce toxicity.

• Nanoformulation: Develop nanoparticle-based delivery systems to improve solubility, bioavailability, and targeted drug delivery.

• Advanced cholinesterase studies: Perform enzyme selectivity, kinetic, and Alzheimer's disease model studies for compounds showing AChE/BChE inhibition.

• Computational studies: Use molecular dynamics simulations to validate docking results and better understand protein–ligand interactions.

CONCLUSION

This review integrates findings from two independent studies to highlight the broad therapeutic potential of the benzimidazole scaffold. The results emphasize the importance of benzimidazole as a privileged pharmacophore capable of generating diverse biological activities through strategic structural modifications. In the Keser study, the aliphatic oxadiazole derivative Compound 4 emerged as a promising anticancer candidate, exhibiting potent antiproliferative activity against breast cancer cells through cell cycle arrest and apoptosis induction. Its favorable ADMET profile and multitarget activity against proteins such as Bcl-2, Bcl-xL, CDK2, and Cyclin E further support its potential for future drug development. [1,2] In the Raka study, environmentally friendly synthesis methods produced aromatic benzimidazole derivatives with significant analgesic, anti-inflammatory, antioxidant, and cholinesterase inhibitory activities. Notably, Compound 3b demonstrated morphine-like analgesic effects, while Compound 3a showed strong antioxidant activity due to its phenolic hydroxyl group. Together, these studies demonstrate that rational structural modification of the benzimidazole framework can generate compounds with diverse pharmacological properties. Further in vivo studies, structural optimization, advanced drug delivery systems, and mechanistic investigations will be essential to translate these promising findings into clinical applications. [1,2]

REFERENCES

1. Keser M, Pulat ÇÇ, Atmaca H, Akgün H, Albay C, Menteşe E, Bektaş H and Ilhan S (2026) Synthesis, characterization, and biological evaluation of aliphatic-substituted benzimidazole derivatives: induction of apoptosis, cell cycle arrest, and molecular docking in breast cancer cells. Drug Dev. Res. 87(2), e70267.
2. Raka SC, Rahman A, Hussain F and Rahman SMA (2022) Synthesis, characterization and in vitro, in vivo, in silico biological evaluations of substituted benzimidazole derivatives. Saudi J. Biol. Sci. 29(1), 239-250.
3. Nazreen S, Almalki ASA, Elbehairi SEI, Shati AA, Alfaifi MY, Elhenawy AA, Alsenani NI, Alfarsi A, Alhadhrami A, Alqurashi EA and Alam MM (2022) Cell cycle arrest and apoptosis-inducing ability of benzimidazole

derivatives: Design, synthesis, docking and biological evaluation. Molecules 27(20), 6899.

4. Almalki AS, Nazreen S, Elbehairi SEI, Asad M, Shati AA, Alfaifi MY, Alhadhrami A, Elhenawy AA, Alorabi AQ, Asiri AM and Alam MM (2022) Design, synthesis, anticancer activity and molecular docking studies of new benzimidazole derivatives bearing 1,3,4-oxadiazole moieties as potential thymidylate synthase inhibitors. New J. Chem. 46(31), 14967-14978.
5. Alzahrani HA, Alam MM, Elhenawy AA, Malebari AM and Nazreen S (2021) Synthesis, antiproliferative, docking and DFT studies of benzimidazole derivatives as EGFR inhibitors. J. Mol. Struct. 1253, 132265.
6. Carneiro BA and El-Deiry WS (2020) Targeting apoptosis in cancer therapy. Nat. Rev. Clin. Oncol. 17(7), 395-417.
7. Lee YT, Tan YJ and Oon CE (2023) Benzimidazole and its derivatives as cancer therapeutics: The potential role from traditional to precision medicine. Acta Pharm. Sin. B 13(2), 478-497.
8. Ebenezer O, Oyetunde-Joshua F, Omotoso OD and Shapi M (2023) Benzimidazole and its derivatives: Recent advances (2020–2022). Results Chem. 5, 100925.
9. Youssif BGM, Morcoss MM, Bräse S, Abdel-Aziz M, Abdel-Rahman HM, El Ella DAA and Abdelhafez ESMN (2024) Benzimidazole-based derivatives as apoptotic antiproliferative agents: Design, synthesis, docking and mechanistic studies. Molecules 29(2), 446.
10. El-Hameed RHA, Fatahala SS and Sayed AI (2022) Synthesis of some novel benzimidazole derivatives as anticancer agents and evaluation for CDK2 inhibition activity. Med. Chem. 18(2), 238-248.
11. Atmaca H, İlhan S, Batır MB, Pulat ÇÇ, Güner A and Bektaş H (2020) Novel benzimidazole derivatives: Synthesis, in vitro cytotoxicity, apoptosis and cell cycle studies. Chem. Biol. Interact. 327, 109163.
12. Achar KC, Hosamani KM and Seetharamareddy HR (2010) In vivo analgesic and anti-inflammatory activities of newly synthesized benzimidazole derivatives. Eur. J. Med. Chem. 45(5), 2048-2054.
13. Gaba M, Singh S and Mohan C (2014) Benzimidazole: An emerging scaffold for analgesic and anti-inflammatory agents. Eur. J. Med. Chem. 76, 494-505.
14. Coban G, Carlino L, Tarikogullari AH, Parlar S, Sarıkaya G, Alptüzün V, Alpan AS, Güneş HS and Erciyas E (2016) 1H-Benzimidazole derivatives as butyrylcholinesterase inhibitors: Synthesis and molecular modeling studies. Med. Chem. Res. 25(9), 2005-2014.
15. Faraji L, Shahkarami S, Nadri H, Moradi A, Saeedi M, Foroumadi A, Ramazani A, Haririan I, Ganjali MR, Shafiee A and Khoobi M (2017) Synthesis of novel benzimidazole and benzothiazole derivatives bearing a 1,2,3-triazole ring system and their acetylcholinesterase inhibitory activity. J. Chem. Res. 41(1), 30-35.
16. Brishty SR, Saha P, Mahmud ZA and Rahman SA (2020) Synthesis and evaluation of analgesic and antioxidant activities of substituted benzimidazole derivatives. Dhaka Univ. J. Pharm. Sci. 19(1), 37-46.
17. Poddar SK, Saqueeb N and Rahman SA (2016) Synthesis and biological evaluation of 2-methyl-1H-benzimidazole and 1H-benzimidazol-2-yl-methanol. Dhaka Univ. J. Pharm. Sci. 15(1), 83-87.
18. Guo Y, Hou X and Fang H (2021) Recent applications of benzimidazole as a privileged scaffold in drug discovery. Mini Rev. Med. Chem. 21, 1367-1379.

19. Choudhary A, Viradiya RH, Ghoghari RN and Chikhalia KH (2023) Recent scenario for the synthesis of benzimidazole moiety (2020–2022). ChemistrySelect 8(10), e202204910.
20. Choudhary A, Viradiya RH, Ghoghari RN and Chikhalia KH (2023) Recent scenario for the synthesis of benzimidazole moiety (2020–2022). ChemistrySelect 8(10), e202204910.
21. Marinescu M (2023) Benzimidazole-triazole hybrids as antimicrobial and antiviral agents: A systematic review. Antibiotics 12, 1220.
22. Ibrahim HA and Refaat HM (2020) Versatile mechanisms of 2-substituted benzimidazoles in targeted cancer therapy. Future J. Pharm. Sci. 6, 41.
23. Cartier A and Janicot A (2008) Supervisión en trabajo social, trabajo de supervisión: una mirada. Pol. Soc. Eur. 47(23), 107-118.
24. Kifle ZD, Tadele M, Alemu E, Gedamu T and Ayele AG (2021) A recent development of new therapeutic agents and novel drug targets for cancer treatment. SAGE Open Med. 9, 20503121211067083.

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