Molecular docking and dynamics as a tool to study benzimidazole resistance in helminths: A scoping review

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Abstract

Benzimidazole (BZ) resistance remains an emerging grave concern in helminths of public and veterinary health. Resistance against BZ drugs is due to mutations that change the amino acid comprising the β-tubulin protein, which negatively affects its interactions with BZ drug molecules. Several *in silico* modeling studies have been published to decipher the precise mechanism of BZ resistance, but inconsistencies on the resistance consequence mutations confer and the effect of different BZ ligands have led to further confusion regarding the exact mechanism of resistance. Hence, this scoping review was done to unravel the mechanism of BZ resistance based on published research on molecular docking and dynamics. A scoping review was conducted in ScienceDirect, MEDLINE via PubMed and Scopus using the search term "Benzimidazole Resistance AND Beta Tubulin AND Molecular Docking". A total of 37 hits were recovered and from these 6 were included after selection, inclusion, and risk of bias assessment. The six research papers included in this review studied several helminth species: *Haemonchus conturtos, Trichinella spiralis, Ancylostoma duodenale, Ancylostoma caninum, Ancylostoma ceylanicum, Necator americanus, Trichuris trichiura, Trichuris suis, Anisakis simplex, Ascaris suum, Ascaridia galli, Parascaris equorum, Toxocara canis*, and *Fasciola hepatica*. The benzimidazole resistance-associated mutations studied included F167Y (TTC, TTT → TAC, TAT), E198A (GAG, GAA → GCG, GCA), and F200Y (TTC, TTT → TAC, TAT). The results show that the E198A can markedly reduce the binding affinity of BZ ligand-β-tubulin interactions. The F167Y and F200Y also showed a similar effect that could vary based on the helminth species. The F200Y mutation can alter the conformation of the β-tubulin active site, negatively affecting drug binding. While the impact of these mutations can vary depending on the specific helminth species and the BZ drug involved, the overall findings highlight the importance of targeting these residues for the development of novel anthelmintic strategies to address emerging drug resistance.

Keywords: Benzimidazole; Drug resistance; computational biology; docking; helminths

Introduction

Benzimidazole resistance continues to be an emerging grave concern in helminths of public and veterinary health. Benzimidazoles (BZ) are disruptors of microtubule polymerization by binding in the β subunit of the tubulin dimer (Whittaker et al., 2017). The binding of BZ drug molecules in the β-tubulin prevents the polymerization of tubulin subunits into microtubules, disrupting the formation of the cytoskeleton (Furtado et al., 2016). Benzimidazole drugs, like albendazole, mebendazole, and fenbendazole, are used for clinical treatment and preventive chemotherapy in humans and animals (TroCCAP, 2019; World Health Organization, 2011). Resistance against this drug class became a huge concern in the veterinary field as widespread reports of resistant livestock helminths, like *Haemonchus conturtos, Teladorsagia circumcincta,* and *Trichostrongylus colubriformis* (Von Samson-Himmelstjerna et al., 2007). Among helminths of public health concern, soil-transmitted helminth infections that do not respond to conventional BZ treatment have been reported in several areas globally (Ng'etich et al., 2023; Schwenkenbecher et al., 2007). Recently, the emergence of BZ-resistant helminth infections among pets (e.g., canine hookworm) in the United States and Canada raises the zoonotic threat these treatment-irresponsive isolates pose (Jimenez Castro et al., 2021; Tenorio et al., 2024; Venkatesan et al., 2023).

The resistance against BZ drugs is due to mutations that change the amino acid sequence comprising the β-tubulin protein expressed by the helminth (Furtado et al., 2016). These amino acid substitutions are brought about by Single Nucleotide Polymorphisms (SNPs) (Von Samson-Himmelstjerna et al., 2007). These mutations include those that occur in amino acid positions 167 (Phenylalanine, F, TTC, TTT → Tyrosine, Y, TAC, TAT), 198 (Glutamic acid, E, GAG, GAA → Alanine, A, GCG, GCA) and 200 (Phenylalanine, F, TTC, TTT → Tyrosine, Y, TAC, TAT) (Furtado et al., 2016; Tenorio, 2023; Tenorio et al., 2024). These mutations alter the amino acid constitution of the expressed protein negatively affecting the binding of BZ drug molecules structurally or biochemically (Lacey and Gill, 1994). These mutations have been reported in a variety of worms that threaten humans and animals globally (Ng'etich et al., 2023). The atomic underpinnings of BZ resistance in helminths remain understudied, hence its precise mechanism has been put into question (Von Samson-Himmelstjerna et al., 2007).

Several *in silico* modeling studies utilizing advances in computational biology have been undertaken to decipher the precise mechanism of BZ resistance. These research have included modeling the wild-type protein's interaction with BZ drug ligands (Aguayo-Ortiz et al., 2013a) and predicting the effects of BZ resistance mutations (Jones et al., 2022a). However, inconsistency regarding the resistance effects each mutation confers and the consequences of utilizing numerous BZ derivatives as ligands have led to further confusion regarding the exact mechanism of resistance.

This scoping review was done to unravel the mechanism of BZ resistance based on published research that used molecular docking and dynamics**.** The scoping review was done based on the guidelines reported by the PRISMA-ScR (PRISMA Extension for Scoping Reviews) (Tricco et al., 2018) (https://www.prisma-statement.org/scoping). Based on published molecular docking and dynamics studies, this research aims to determine the mechanism of benzimidazole. Specifically, this research answers the following questions:

1. What are the *in silico* underpinnings of benzimidazole resistance based on molecular docking and dynamics study?
2. What are the consequences of these mutations on the measurement of binding efficiency of the β-tubulins-benzimidazole drug complex?
3. What are the consequences of these mutations on the interactions between the β-tubulins and the benzimidazole drug ligand/s?

Materials and Methods

Search Strategy

A systematic search was done in three research databases. Scopus (https://www.scopus.com/search), ScienceDirect (https://www.sciencedirect.com/) and MEDLINE via PubMed (https://pubmed.ncbi.nlm.nih.gov/) were searched using the search term "Benzimidazole Resistance AND Beta Tubulin AND Molecular Docking." The literature search was done on 17 September 2024. The .ris files of the search results were downloaded.

Study Selection, Strategy, and Eligibility

Using the Mendeley citation manager (https://www.mendeley.com), the .ris files were uploaded and utilized for the selection and eligibility assessment. First, duplicates and records with no titles and abstracts (e.g., indexes) were removed. Second, an initial evaluation based on the title and abstract was done. Full-length articles of the studies were accessed for further eligibility appraisal. Figure 1 summarizes the systematic literature search, selection, and eligibility evaluation.

A study was considered eligible for selection if it fulfilled any of the following inclusion criteria:

1. Studies that utilized molecular docking in assessing the *in silico* effects of the BZ resistance mutations; or

2. Studies that utilized molecular dynamics in assessing the *in silico* effects of the BZ resistance mutations

From the included studies, papers that did not meet the following criteria were excluded:

1. Studies that did not report docking scoring functions (i.e., binding affinities) and/or binding free energies (e.g., MM-PBSA or MM-GBSA);
2. Studies that did not report the effects of BZ resistance mutations on the interaction between β-tubulins and benzimidazole drug ligand/s;
3. Studies done using non-helminth β-tubulins as macromolecules;
4. Studies that did not use commercially available benzimidazole drugs as ligands;
5. Studies that utilized newly designed and synthesized benzimidazole derivatives; and
6. Research not in the English language.

Risk of Bias Assessment

Due to the *in silico* and computational nature of the studies being reviewed, traditional checklists for laboratory experiments are not well-suited as the method of bias assessment. Hence, the author developed a simple checklist that is based on the quality of the modeled β-tubulin macromolecule, ligand preparation, docking software, simulation quality, and data analysis utilized. The tool is in the form of a 13-item close-ended questionnaire. All included studies were evaluated using this tool. This risk of bias assessment tool is available at https://assets-eu.researchsquare.com/files/rs-5476123/v1/80004a01623c3bd72b7cd79d.docx.

Data Acquisition and Synthesis

The author's name, year of publication, software used in molecular docking and/or system used in molecular dynamics, helminth species of the β-tubulins used as the macromolecule, benzimidazole ligand used, BZ resistance mutation evaluated, docking scoring functions and/or binding free energies of the complex, description of the changes in interactions, and relative resistance-associated effects were the data acquired from the selected studies. An initial version of this article was deposited as a preprint in Research Square (Tenorio, 2024a).

Results

Characteristics of the studies included

A total of 37 hits were found in the three databases searched (Figure 1). Sixteen of these were removed due to duplication. The full text of one article was not accessed. After the eligibility screening, two were removed for not reporting docking scoring function, eight were removed for not using helminth β-tubulins, three were dropped for using newly designed benzimidazole ligands, and one did not report the effects of the docked complexes. In total, six research papers were included in this scoping review. The six research papers included in this review studied several helminth species: *Haemonchus conturtos, Trichinella spiralis, Ancylostoma duodenale, Ancylostoma caninum, Ancylostoma ceylanicum, Necator americanus, Trichuris trichiura, Trichuris suis, Anisakis simplex, Ascaris suum, Ascaridia galli, Parascaris equorum, Toxocara canis,* and *Fasciola hepatica.* The benzimidazole resistance-associated mutations studied included F167Y (TTC, TTT → TAC, TAT), E198A (GAG, GAA → GCG, GCA), and F200Y (TTC, TTT → TAC, TAT).

Key *in silico* effects of BZ resistance-associated mutations

The included papers noted some key results regarding their findings on the atomic underpinnings of benzimidazole resistance. Aguayo-Ortiz et al. (2013b) reported that the mutated and unsusceptible β-tubulin models suggest that the primary cause of BZ resistance is likely due to an amino acid modification at position 198, resulting in the loss of hydrogen bonding interactions. Conversely, the substitution of phenylalanine for tyrosine at positions 167 and 200 implies that the inhibitory mechanism may occur either during the opening of the binding site or the internalization of the ligand. Aguayo-Ortiz et al. (2013a) study on *Trichinella spirals* showed that the binding site for BZ aligns with previous experimental findings. This site includes amino acids linked to resistance mutations (F167Y, E198A, and F200Y), and overlaps with the colchicine-binding site. Molecular docking and dynamics calculations of BZ drugs reveal that they are stabilized within the binding site primarily through hydrogen bonds with specific amino acids (e.g., Thr165, Glu198, Cys239, and Gln134). Also, Jones et al.'s (2022a) molecular docking studies suggest that BZ drugs can bind to all Ascarid β-tubulin isotypes. Ascarid β-tubulin isotype A was further analyzed using molecular dynamics simulations. These simulations revealed the critical role of amino acid E198 in BZ-β-tubulin interactions. Mutations at E198A and F200Y were found to alter benzimidazole binding, while the F167Y mutation had no significant impact. Another study by Jones et al. (2022b) indicated that BZ acts through similar mechanisms in various helminth species. The amino acid E198 plays a crucial role in BZ binding. However, the Q134–F167Y interaction observed in *Ancylostoma caninum*, along with the presence of the F167Y SNP in susceptible *Ascaris* populations and its reduced frequency after BZ treatment in *Trichuris trichiura* from previous studies, suggests that mutations in F167Y may

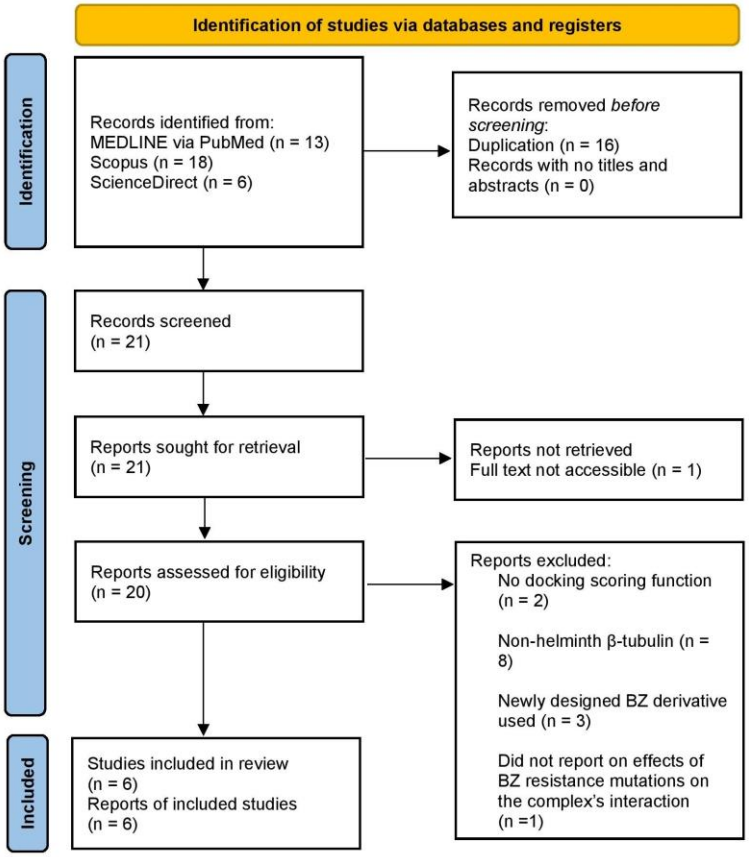

Figure 1. PRISMA-ScR (Preferred Reporting Items for Systematic Reviews and Meta-Analyses-Extension for Scoping Reviews) flowchart of the screening, selection, and eligibility selection in this study.

have a greater impact on strongyle parasites compared to other STHs. Olivares-Ferretti et al. (2023) studied *F. hepatica* β-tubulin and showed that it exhibits a higher affinity for ligands than other known binding sites, such as colchicine, albendazole, the T7 loop, and pβVII. Ligand binding to the polymerization site of β-tubulin can disrupt microtubule formation. Triclabendazole demonstrated significantly higher binding affinity than other ligands across all β-tubulin isotypes. Computational analysis has provided new insights into the mechanism of action of triclabendazole on *F. hepatica* β-tubulin. Lastly, Yashica et al.'s (2024) result showed that mutations at amino acid position 200 can disrupt the conformation of the *H. conturtos* β-tubulin active site and destabilize albendazole binding.

Effects of Mutations on Docking Scoring Function and Binding Free Energy

Table 1 summarizes computational studies investigating the molecular mechanisms underlying benzimidazole (BZ) drug resistance in various helminth species. Based on the results of the included studies, the BZ resistance mutations often lead to a decrease in the docking scoring function and binding free energy, indicating a weaker interaction between the BZ drug and the mutated β-tubulin protein. However, the magnitude of the effect can vary depending on the specific mutation and the BZ drug involved. While F167Y mutations can sometimes decrease binding affinity, their effect is often less pronounced compared to mutations at other residues. Also, the impact of F167Y may vary across different helminth species. Meanwhile, the E198A mutation consistently leads to a marked decrease in binding affinity, suggesting a crucial role of this residue in BZ binding. The E198A mutation likely disrupts hydrogen bonding interactions between the BZ drug and β-tubulin. The F200Y mutations generally result in a moderate decrease in binding affinity. This mutation may alter the conformation of the β-tubulin active site, affecting drug binding. The combined effects of multiple mutations can further reduce binding affinity and enhance resistance. Likewise, the impact of mutations may vary depending on the specific BZ drug being considered. Overall, the results suggest that mutations in these key residues can disrupt the structural and electrostatic interactions between BZ drugs and β-tubulin, leading to reduced binding affinity and ultimately, drug resistance.

Table 1. The key results of the studies included in this scoping review.

Study Code	Authors	Molecular Docking Software	Molecular Dynamics System	Helminth Species	β-tubulin Evaluated	BZ Ligand	Docking Scoring (kcal/mol)	Binding Free Energy (kcal/Mol)
S1	Aguayo-Ortiz et al. (2013b)	Autodock 4.2	GROMACS 4.5.3	*Haemonchus conturtos*	WT	ABZ	-8.10	-68.41
						CBZ	-6.98	-55.08
						OBZ	-8.17	-67.27
						PBZ	-8.09	-65.59
						LBZ	-9.46	-64.77
						MBZ	-9.43	-60.53
						NDZ	-9.36	-64.61
					F167Y	ABZ	-8.07	Not computed
						CBZ	-7.02	
						OBZ	-8.09	
						PBZ	-8.15	
						LBZ	-9.26	
						MBZ	-9.46	
						NDZ	-9.35	
					E198A	ABZ	-7.03	
						CBZ	-6.37	
						OBZ	-6.66	
						PBZ	-7.05	
						LBZ	-8.82	
						MBZ	-8.26	
						NDZ	-7.99	
					F200Y	ABZ	-8.22	
						CBZ	-7.19	
						OBZ	-8.28	
						PBZ	-8.26	
						LBZ	-9.62	
						MBZ	-9.48	
						NDZ	-9.35	
S2	Aguayo-Ortiz et al. (2013a)	Autodock 4.2	GROMACS 4.5.3	*Trichinella spiralis*	WT	ABZ	-7.70	-53.67
						CBZ	-7.05	-21.74
						OBZ	-7.41	-31.92
						PBZ	-7.93	-37.20
						LBZ	-9.41	-44.71
						MBZ	-8.73	-43.04
						NDZ	-8.82	-34.34
S3	Jones et al. (2022a)	Autodock vina v1.1.2	Molecular Operating Environment (MOE) 2020.01	*Ascaris suum*	WT	ABZ	-8.46	Not computed
					F167Y	ABZ	-8.04	
					E198A	ABZ	-8.53	
					F200Y	ABZ	-8.16	
S4	Jones et al. (2022b)	Autodock vina v1.1.2	Molecular Operating Environment (MOE) version 2020.01	*Ancylostoma duodenale*	WT	ABZ	-8.55	Not computed
					F167Y	ABZ	-8.19	
					E198A	ABZ	-8.14	
					F200Y	ABZ	-7.72	
				Trichuris trichiura	WT	ABZ	-8.53	
					F167Y	ABZ	-9.82	
					E198A	ABZ	-7.78	
					F200Y	ABZ	-10.48	
				Anisakis simplex	WT	ABZ	-7.74	
				Ascaridia galli	WT	ABZ	-8.25	
				Parascaris equorum	WT	ABZ	-8.19	
				Toxocara canis	WT	ABZ	-8.82	
				Ancylostoma caninum	WT	ABZ	-7.94	
				Ancylostoma ceylanicum	WT	ABZ	-8.29	
				Necator americanus	WT	ABZ	-7.54	
				Trichuris suis	WT	ABZ	-8.53	
S5	Olivares-Ferretti et al. (2023)	Autodock Vina program in pyrx software	N/A	*Fasciola hepatica*	WT iso 1	TBZ	-6.87	Not computed
					WT iso 2	TBZ	-6.40	
					WT iso 3	TBZ	-6.38	
					WT iso 4	TBZ	-6.33	
					WT iso 5	TBZ	-6.17	
					WT iso 6	TBZ	-6.48	
S6	Yashica et al. (2024)	Autodock Tools in MGL tools 1.5.6	N/A	*Haemonchus contortus*	WT	ABZ	-8.51	Not computed

WT = Wildtype; iso = isotype; ABZ = Albendazole; TBZ = Triclabendazole; CBZ = Carbendazim; OBZ = Oxibendazole; PBZ = Parbendazole; LBZ = Luxabendazole; MBZ = Mebendazole; NDZ = Nocodazole

Discussion

This scoping review was conducted with the aim of unraveling the mechanism of BZ resistance based on published research on molecular docking and dynamics. MEDLINE via PubMed, Scopus, and Science Direct were searched. A total of six eligible studies were included, which encompassed research that utilized β-tubulins from several helminth species and numerous benzimidazole ligands. Of the BZ mutations studied, E198A showed that it can drive down the binding affinity of BZ ligand-β-tubulin interactions. The F167Y and F200Y also showed a similar effect that could vary based on the helminth species. The F200Y mutation can alter the conformation of the β-tubulin active site, negatively affecting drug binding.

The studies included in this scoping review assessed both wild-type and mutated helminth β-tubulins. The three canonical BZ resistance mutations—F167Y, E198A, and F200Y—that are induced by SNPs were all evaluated (Furtado et al., 2016). These mutations have been reported in many helminth species of public and veterinary health concern from many parts of the world (Diawara et al., 2013; George et al., 2022; Jimenez Castro et al., 2021). Hence, their inclusion in computational studies was warranted. However, their actual contribution to conferring resistance has been put into question (Lacey and Gill, 1994). Also, several recent evidence of alternative resistance mechanisms, such as enzymatic biotransformation of drugs using UDP-glycosyltransferases and long non-coding RNA interference (Chen et al., 2024; Dimunová et al., 2022), have been reported. However, laboratory research using gene editing that encoded resistance-associated mutations in *Caenorhabditis elegans* showed their actual potential to confer resistance, as previously mentioned (Dilks et al., 2021, 2020). Particularly, varying mutations that have been reported in amino acid position 198 have been reported to make edited *C. elegans* significantly more resistant to benzimidazole treatment compared to their wild-type counterpart (Dilks et al., 2021). This point has also been substantiated by other recent computational studies in *H. conturtus* and *A. galli* (Borchert et al., 2024; Tenorio, 2024b). These research papers echo the finding in this scoping review that the E198A mutation had a consistent negative effect on the binding of BZ drugs and helminth β-tubulins indicating their important role in conferring resistance.

This scoping review has several limitations. First, only a few accessible databases were searched, hence there might be other research not indexed in these databases that were missed. However, searching only known indexing databases, like MEDLINE via PubMed and Scopus, ensures that only quality research papers from reputable journals are included. Second, the *in silico* nature of the research targeted for this review may lead to conclusions of insufficient evidence for the conclusions that they present due to the lack of laboratory confirmation. However, advances in computational biology have assured that the predictions are of high quality and accuracy, particularly for molecular docking and dynamics studies (Santos et al., 2019; Singh et al., 2022).

Conclusion

The computational studies included in this scoping review provide valuable insights into the molecular mechanisms underlying BZ drug resistance in various helminth species. The results consistently demonstrate that mutations in specific amino acid residues within β-tubulin proteins, particularly F167, E198, and F200, play a critical role in conferring resistance. These mutations disrupt the structural and electrostatic interactions between BZ drugs and helminth β-tubulin, leading to reduced binding affinity and ultimately, drug resistance. While the impact of these mutations can vary depending on the specific helminth species and the BZ drug involved, the overall findings highlight the importance of targeting these residues for the development of novel anthelmintic strategies to address emerging drug resistance. Future studies could explore additional mutations, investigate the interactions between other BZ drugs and other β-tubulin isoforms, or investigate the potential for combination therapies to overcome resistance.

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