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Exploration of interaction zones of β-tubulin colchicine binding domain of helminths and binding mechanism of anthelmintics

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ABSTRACT

Numerous studies postulated the possible modes of anthelmintic activity by targeting alternate or extended regions of colchicine binding domain of helminth β-tubulin. We present three interaction zones (zones vide −1 to −3) in the colchicine binding domain of *Haemonchus contortus* (a helminth) β-tubulin homology model and developed zone-wise structure-based pharmacophore models coupled with molecular docking technique to unveil the binding hypotheses. The resulted ten structure-based hypotheses were then refined to essential three point pharmacophore features that captured recurring and crucial non-covalent receptor contacts and proposed three characteristics necessary for optimal zone-2 binding: a conserved pair of H bond acceptor (HBA to form H bond with Asn226 residue) and an aliphatic moiety of molecule separated by 3.75 ± 0.44 Å. Further, an aliphatic or a heterocyclic group distant (11.75 ± 1.14 Å) to the conserved aliphatic site formed the third feature component in the zone-2 specific anthelmintic pharmacophore model. Alternatively, an additional HBA can be substituted as a third component to establish H bonding with Asn204. We discern that selective zone-2 anthelmintics can be designed effectively by closely adapting the pharmacophore feature patterns and its geometrical constraints.

1. Introduction

The inhibition of microtubule polymerization formed the integral cellular event to arrest helminth infections and numerous anthelmintics were designed to target its microtubule component, the αβ-tubulin heterodimer. Studies suggest that eight isotypes of β-subunit and six isotypes of α subunit have been characterized so far (Massarotti et al., 2012). The β-tubulin monomer is composed of three domains, N-terminal domain (positions 1–201), intermediate domain (positions 202–371) and C-terminal domain (positions 372–427) (Nogales et al., 1996; Joseph and Leslie, 1971). Direct photolabelling technique has identified three major pharmacologically distinct ligand binding sites namely taxol, vinblastine and colchicine (Kiselyov et al., 2007; Lacey, 1988). Colchicine, a highly toxic molecule extracted from *Colchicum autumnale* L. (commonly known as meadow saffron) is the first discovered microtubule destabilizing agent (Massarotti et al., 2012; Peterson and Mitchison, 2002; Weisenberg et al., 1968) which was confirmed by the crystal structure of colchicine structural analog, *N*-deacetyl-*N*-(2-mercaptoacetyl) colchicine (DAMA-colchicine) in complex with tubulin heterodimer (PDB entry: 1SA0; Ravelli et al., 2004) (Fig. 1). The binding of colchicine to tubulin substiochiometrically blocks tubulin assembly and diminishes microtubule disassembly (Susan, 2008). The classification of a microtubule assembly inhibiting substance as colchicine site ligand when introduced with [^{3}H] colchicine with an implicit assumption that colchicine site ligand occupies the same binding pocket of colchicine, formed the major roadblock for identifying the exact binding positions of new leads inside the colchicine binding domain. The pragmatic assumption of competitive inhibition taking place without explicit verification in this case (Susan, 2008; Hastie, 1991) was unraveled by the advent of numerous crystal structures of tubulin in complex with colchicine site molecules along with biochemical characterizations in eukaryotes. However, the photolabelling, site-directed

Abbreviations: GTP, Guanosine triphosphate; RMSD, Root mean square deviation; RCSB, Research Collaboratory for Structural Bioinformatics; HBA, Hydrogen bond acceptor.

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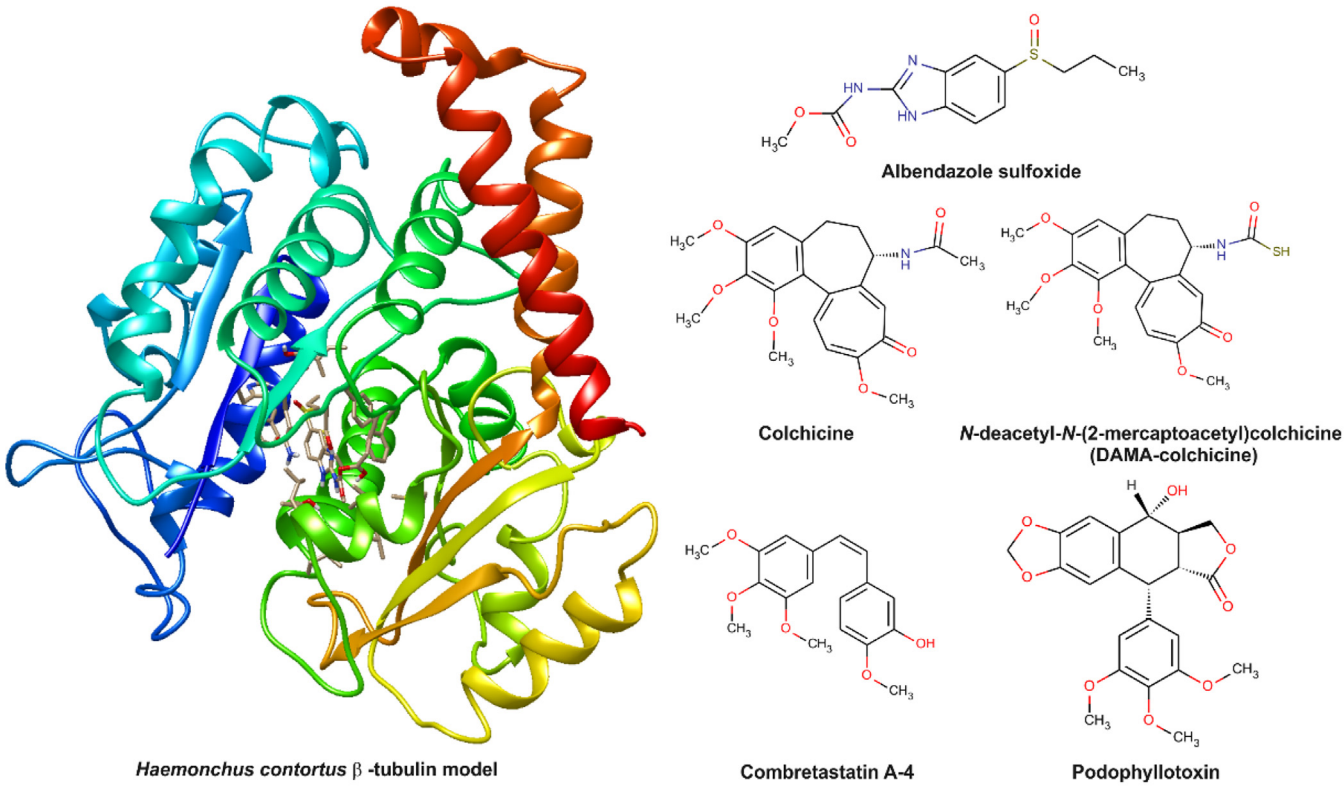

Fig. 1. The three-dimensional structure of *Haemonchus contortus* β-tubulin homology model in complex with albendazole sulfoxide (left) (Robinson et al., 2004). The 2D structure of the some of the widely studied colchicine site molecules is shown on the left side.

mutagenesis and competitive inhibition approaches were the supportive tools to decipher anthelmintics as colchicine site inhibitors in the nematode, trematode and cestode.

Benzimidazoles, the broad class of anthelmintics (or anthelminthics) possess high affinity towards β-tubulin protein and exerts anthelmintic activity by binding to its colchicine binding site (Lubega and Prichard, 1990; Tom, 1984; Lacey, 1988; Kwa et al., 1995). They hamper microtubule-based functions by creating interference in microtubule dynamics in various parasites including nematode, trematode and cestode. A series of amino acid mutations known for susceptibility and resistance against several anthelmintics is well documented in parasites (close homologues of *Haemonchus contortus*) (Silvestre and Cabaret, 2002; Li et al., 1996; Jung and Oakley, 1990; Oakley et al., 1990 Löwe et al., 2001; Robinson et al., 2004; Lacey et al., 1987; Lacey, 1988).The structural relation to resistance mechanism and its corresponding conformational changes have been extensively studied (Lacey and Gill, 1994; Aguayo-Ortiz et al., 2013a; Beech et al., 2011). Supported by the crystal structure of bacterial tubulin homologue FtsZ (Nogales et al., 1998), the cluster of resistance-conferring residues located at the inter-junction of N-terminal and intermediate domains gets accessed during dimer dissociation. It is also hypothesized that accessibility of such residues to ligand binding occurs by a conformational change in β-tubulin leading to microtubule depolymerization, similar to conformational movements induced during GTP hydrolysis by polymerized tubulin (Amos and Lowe, 1999). For example, the mechanism of benzimidazole binding is facilitated by two hypotheses. First, the movement of side chains of certain amino acids (Arg156 and Leu265) enables the accessibility of pocket residues to benzimidazole (Robinson et al., 2004). Second, the speculative observation of cleft gets widened is due to the dissociation of monomers from tubulin heterodimer during microtubule dynamics (Robinson et al., 2004; Lowe and Amos, 1998). Some amino acid stabilizing effects of β-tubulin have also been reported (Detrich et al., 2000) which elevates the activation energy to prohibit the conformational change to undergo polymerization (induced by GTP hydrolysis) and this stabilizing effect can be suppressed by benzimidazole binding which wedges the N- and C-terminal domains in the opposite direction leading to microtubule depolymerization.This inter-domain movement is quite similar to that induced during GTP hydrolysis (Robinson et al., 2004). We selected the homology model of *H. contortus* β-tubulin in complex with albendazole sulfoxide which depicted a ligand binding conformation state during microtubule depolymerization (Robinson et al., 2004), suitable for docking purposes. Robinson et al., 2004 developed this homology model (Fig. 1) using bovine tubulin heterodimer (PDB entry: 1JFF) and performed inter-domain movement which created 'open' conformation suitable for benzimidazole docking in accordance with bacterial homologue FtsZ (RMSD = 2.3 Å) (Robinson et al., 2004; Amos and Lowe, 1999). This 'open' conformation was also supported by crystal structures of tubulin heterodimers in complex with colchicine and stathmin-like domain of RB3 (Ravelli et al., 2004).

Structural and competitive inhibition studies confirmed the presence of alternate or extended colchicine binding site. For instance, the alternations in the colchicine binding site of *Fasciola hepatica (F. hepatica)* β-tubulin provided refractoriness to classical benzimidazoles as evident from colchicine and tubulozole-C competitive binding mechanisms and proposed triclabendazole binding at an alternative site of β-tubulin (Robinson et al., 2001; Fairweather and Boray, 1999). Similarly, the colchicine binding to β-tubulin was averted by tropolone methyl ether (colchicine C-ring analog) whereas the binding of podophyllotoxin was not prevented by this analog (Cortese et al., 1977). These observations collectively suggested the presence of alternate sites other than the colchicine binding site of β-tubulin. Massarotti et al., 2012