

การศึกษาอันตรกิริยาระหว่างอนุพันธ์ของวงแหวน-B ของโคลชิซินกับทูบูลิน เฮเทอโรไดเมอร์ ด้วยวิธีการจำลองทางคอมพิวเตอร์

In silico Study of the Interaction between the Modified B-ring Analogues of Colchicine with Tubulin Heterodimer

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บทคัดย่อ

การศึกษาเชิงทฤษฎีของอันตรกิริยาระหว่างอนุพันธ์ของโคลชิซินที่ได้จากการปรับเปลี่ยนหมู่แทนที่ที่ตำแหน่ง C-5 C-6 และ C-7 ของวงแหวน-B กับทูบูลินเฮเทอโรไดเมอร์ได้ถูกกระทำโดยใช้วิธีจำลองโมเลกุลาร์ดีค็อกกิ้ง ผลลัพธ์จากการดีค็อกกิ้งให้ข้อมูลทางพลังงานและทางโครงสร้าง อาทิ พลังงานยึดเหนี่ยว ความสามารถในการจับ การเกิดพันธะไฮโดรเจน และคอนฟอร์เมชันของลิแกนด์ที่ถูกดีค็อกกิ้งกับเรซิดิวส์ภายในตำแหน่งเข้าจับของโคลชิซิน จากผลลัพธ์ที่ได้แสดงให้เห็นว่าอนุพันธ์ของโคลชิซินที่ปรับเปลี่ยนหมู่แทนที่ที่ตำแหน่ง C-5 (โมเดล A) และที่ตำแหน่ง C-7 (โมเดล C) ของวงแหวน-B มีความสามารถในการจับกับทูบูลินสูงสุด ในขณะที่อนุพันธ์ของโคลชิซินที่มีความสามารถในการจับกับทูบูลินต่ำสุดคือทุกอนุพันธ์ที่ปรับเปลี่ยนหมู่แทนที่ที่ตำแหน่ง C-6 (โมเดล B) ตามที่คาดไว้ลิแกนด์ที่ถูกดีค็อกกิ้งของอนุพันธ์ที่ตำแหน่ง C-5 C-6 และ C-7 ของวงแหวน-B ซึ่งวางตัวอยู่บริเวณผิวหน้า α/β อินทราไดเมอร์ของทูบูลินจะเคลื่อนย้ายเข้าสู่ตำแหน่งเข้าจับของ α -ซับยูนิตเพื่อเกิดสารเชิงซ้อนระหว่างตัวยากับทูบูลิน

คำสำคัญ : โคลชิซิน อนุพันธ์วงแหวน-B ทูบูลิน ความสามารถในการจับ โมเลกุลาร์ดีค็อกกิ้ง

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Abstract

Theoretical investigation of the interaction between the modifications at the C-5, C-6, and C-7 positions of the B-ring of colchicine and tubulin heterodimer has been investigated by using the molecular docking simulation. The docking results provide the energetic and structural information in terms of the binding energy, binding affinity, hydrogen bonding, and conformations of docked ligand poses with residues within colchicine binding site. Overall results show that the modified C-5 (in Model A) and C-7 (in Model C) of B-ring analogues give the highest binding affinities to tubulin, whereas all lowest-affinity isomers belong to the C-6 substituents (in Model B). As expected, the docked ligands of the C-5, C-6, and C-7 of B-ring analogues which are located at the α/β intradimer interface of tubulin were shifting toward the α -subunit binding space to form drug-tubulin complexes.

Keywords : colchicine, B-ring analogues, tubulin, binding affinity, molecular docking

Introduction

Colchicine, an alkaloid extracted from plants of the genus *Colchicum* (*autumn crocus*), has been the prominent target molecule in natural product synthesis, since it is the high potent antimitotic agent, functioning as the microtubule inhibitor (Terkeltaub, 2009; Andrushko *et al.*, 2013; Koehn, 2014). Colchicine inhibits microtubule polymerization by binding to tubulin (the main constituents of microtubules) which results in effective mitotic poisoning (Niel *et al.*, 2006; Leung, 2015). The X-ray structure of tubulin in complex with DAMA-colchicine was firstly reported in 2004, and simultaneously the colchicine binding site was initially identified (Ravelli *et al.*, 2004), in which the colchicine site was found to locate at the α/β intradimer interface, and mostly buried in the intermediate domain of the β -tubulin subunit, whereas the B-ring side chain mainly interacted with residues of the α -subunit. Earlier reviews supported that colchicine and its analogues bind to the β -subunit (of $\alpha\beta$ -tubulin dimer) at the interface with α -subunit of the same tubulin molecule, with little direct interaction with that subunit (Ray *et al.*, 1981; Choudhury *et al.*, 1983; Choudhury *et al.*, 1986; Chaudhuri *et al.*, 2000; Zefirova *et al.*, 2007; Lu *et al.*, 2012). The structure of colchicine consists of three rings, i.e. a trimethoxyphenyl (ring A), a methoxytropone (ring C), and a saturated seven-membered ring (ring B) bearing an acetamido substituent at its C-7 position (Botta *et al.*, 2009), as shown in Fig. 1.

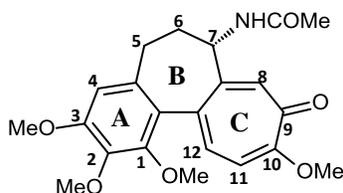


Figure 1 Structure of colchicine: the A-, B-, and C-rings, and the domain carbon positions (C-1 to C-12)

The modifications in the A-, B-, and C-rings of the colchicine and its related analogues were essentially reviewed, owing to understand the effect of these rings on the drug-tubulin interaction (Ray *et al.*, 1981; Choudhury *et al.*, 1983; Choudhury *et al.*, 1986; Chaudhuri *et al.*, 2000; Zefirova *et al.*, 2007; Lu *et al.*, 2012; Maity, S.N. & Bhattacharyya, B., 1987; Hastie *et al.*, 1989; Wolff, J. & Knipling, L., 1995) and also the thermodynamic and kinetic properties. Alterations of rings B and C of colchicine are cumulative in overall binding to tubulin but modify each kinetic step (Dumortier *et al.*, 1996); Thermodynamics of colchicinoid-tubulin interactions: role of B-ring and C-7 substituents (Chakrabarti *et al.*, 1996); Interactions of a bicyclic analog of colchicine with β -tubulin isoforms $\alpha\beta_{II}$, $\alpha\beta_{III}$ and $\alpha\beta_{IV}$ (Banerjee *et al.*, 1997); Mechanism of tubulin-colchicine recognition: a kinetic study of the binding of the colchicine analogues colchicine and isocolchicine (Dumortier *et al.*, (1997); Role of the colchicine ring A and its methoxy groups in the binding to tubulin and microtubule inhibition (Andreu *et al.*, 1998); Mapping the binding site of colchicinoids on β -tubulin (Bai *et al.*, 2000); Insight into tubulin regulation from a complex with colchicine and stathmin-like domain (Ravelli *et al.*, 2004); A common pharmacophore for a diverse set of colchicine site inhibitors using a structure-based approach (Nguyen *et al.*, 2005); Synthesis and biological evaluation of B-ring modified colchicine and isocolchicine analogs (Cifuentes *et al.*, 2006); Antitumor agents 273: design and synthesis of *N*-alkyl-thiocolchicinoids as potential antitumor agents (Kozaka *et al.*, 2010); Exploring the origin of differential binding affinities of human tubulin isotypes $\alpha\beta_{II}$, $\alpha\beta_{III}$ and $\alpha\beta_{IV}$ for DAMA-colchicine using homology modeling, molecular docking and molecular mechanics simulations (Kumbhar *et al.*, 2016).

These previous works on the colchicine (and its analogues) binding to tubulin confirmed that the A- and C-rings of colchicine bind to β -tubulin, while B-ring faces α -tubulin in the $\alpha\beta$ -tubulin dimer. The B-ring of colchicine plays a major role in the stability of tubulin, while the A- and C-rings have little effect on it (Choudhury *et al.*, 1983; Chaudhuri *et al.*, 2000). The colchicine analogues modified in the B-ring (with smaller or no substituents) showed remarkably binding to tubulin faster than colchicine, however, the chemical specificity of the colchicine-binding site of tubulin is less stringent for the presence of the B-ring than the A- and C-rings of colchicine. Absorption spectra of colchicine and its analogues are affected by the presence of the B-ring, although it is not part of chromophore (C-ring). Interaction study of three β -tubulin isoforms with an analogue of colchicine that lacks the B-ring indicated that the B-ring also plays a major role in determining the isoform differences, which is of importance for designing tissue-specific analogues of colchicine for cancer chemotherapy (Banerjee *et al.*, 1997). The selective elimination of the A-ring methoxy groups at the C-1, C-2, and C-3 positions (with the modified C-ring, but lacks of the B-ring) weaken binding of colchicine analogues to tubulin, for which the methoxy group at the C-3 position served as a key attachment point for immobilization of the drugs on the tubulin (Andreu *et al.*, 1998). A well-known analogue of the *structurally related isomer of colchicine* which interchanged the C-9 and C-10 substituents, namely isocolchicine, was confirmed that it binds to the colchicine binding site and inhibits tubulin assembly into microtubules, but its affinity was approximately 500-fold less than that of colchicine (Hastie *et al.*, 1989).

Therein, the thermodynamic and kinetic studies showed that the modifications of B- and C-rings of colchicine are cumulative with respect to overall binding, and mutually influence the intermediate state, suggesting an alteration of the reaction pathway (Dumortier *et al.*, 1996). Another work reported that the role of B-ring and C-7 substituent effectives to the thermodynamics and kinetics of colchicine-tubulin interaction, in which the introduction of the amino groups in the C-7 position of the B-ring lowers the on-rate further with a significant rise in the activation energy, and increases the positive entropy of the binding reaction (Chakrabarti *et al.*, 1996).

As we known, the B-ring substituents of colchicine analogues play an important role in determining the binding properties of colchicine to tubulin, but the modifications in the B-ring at the C-5 and C-6 positions have not been reviewed for their binding to tubulin, except for the abundance of the C-7 substituents in the B-ring analogues. In this work, the theoretical study of the interactions and binding affinities of the modified C-5, C-6, and C-7 of the B-ring analogues of colchicine to tubulin will be investigated. The molecular docking simulation will be performed to provide the energetic and structural information, and discussed in terms of the binding energies, binding affinities, hydrogen bonding, and conformations of docked ligand poses within receptor binding site.

Computational Methods

Softwares

The softwares used for *in silico* study were chosen specifically to match the purpose of each part. The two-dimensional structures of ligands were constructed by using the BIOVIA Draw 2016, while the BIOVIA Discovery Studio Visualizer was used for representing the three-dimensional structures of proteins, ligands, amino acid residues, and hydrogen bonds between these molecules. The AutoDock suite of programs, i.e. AutoDock Vina, was used with a Graphical User Interface called AutoDock Tools (ADT) to perform the docking between ligand (or analogue) and protein (or receptor) (Trott, O. & Olson, A.J., 2010). ADT is available here: mgltools.scripps.edu/downloads. AutoDock Vina along with other helpful information is available here: <http://autodock.scripps.edu>.

Preparation of Ligand models

Three models of colchicine analogues: Model A (R substituted at C-5 position), Model B (R substituted at C-6 position), and Model C (R substituted at C-7 position) were prepared, so-called '*the structurally isomerized colchicine analogues*', as shown in Fig. 2. The substituents at position R of the B-ring are coded as R1, R2, R3, R4, R5, and R6, as illustrated in Table 1.

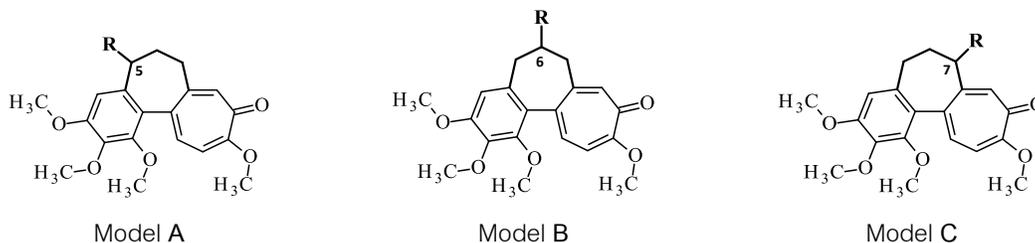


Figure 2 Three models of the structurally isomerized colchicine analogues: R substituted in the B-ring at the C-5 (Model A), C-6 (Model B), and C-7 (Model C) positions

Table 1 The codes and substituents used to indicate the side-chain group, which will be replaced at position R

Code	Substituent	Code	Substituent
R1		R2	
R3		R4	
R5		R6	

To create an individual two-dimensional ligand model, the BIOVIA Draw 2016 was used to draw ligand structure, then 'Chemistry Tools' was chosen with 'Clean' command in order to improve the appearance of the selected structure by giving them uniform bond lengths and angles. For each Model (A, B and C), the 6 ligand models were created: Model A: R1A, R2A, R3A, R4A, R5A, and R6A; Model B: R1B, R2B, R3B, R4B, R5B, and R6B; and Model C: R1C, R2C, R3C, R4C, R5C, and R6C. The three-dimensional structures of these ligands would be generated by using the BIOVIA Discovery Studio Visualizer, whereas the 3-D structural geometry was optimized using the Dreiding-like forcefield (Hahn, M., 1995), as the program default. Therefore, the element, bond orders, number of bonds, and valence were taken into consideration when the terms of the energy equation were calculated.

Preparation of Receptor model

The X-ray crystallographic structure of tubulin heterodimers was taken from the Protein Data Bank (PDB) code: 1SA0, containing of two units of $\alpha\beta$ -tubulin dimer. One unit of the tubulin dimer was deleted, and the binding

drug, namely CN2700 (or DAMA-colchicine), was also deleted from the binding pocket. Hence, the $\alpha\beta$ -tubulin dimer (without binding drug) was assigned as the receptor model.

Molecular Docking Method

The molecular docking method was used to construct binding model for the structurally active site of the $\alpha\beta$ -tubulin dimer, referring to the experimental binding site of DAMA-colchicine as indicated in crystal structure of PDB code 1SA0. In the docking processes, the tubulin heterodimer was held fix as *rigid macromolecular*, while the ligand was *fully flexible* in a grid box dimension parameterized at *center on macromolecule* (to confine the mass center of the docked ligand). The grid center of x/y/z-coordinates was 119.7/92.8/10.8, and the grid box boundary was defined with 64/64/64 number of points which adequately covered every atom of all docked ligands. By using the AutoDock Vina program, the docking calculation was performed using the Simulated Annealing for the optimization algorithm (Trott, O. & Olson, A.J., 2010; Morris *et al.*, 2009), including with Number of runs: 10, Number of cycles: 50, and Accepted or Rejected steps/cycle: 25000. The Lamarckian Generic Algorithm (LGA) (Morris *et al.*, 1998) was chosen to provide the output part, in which the LGA combined local search and genetic algorithm to provide both efficient global space coverage and local search optimization. With the genetic algorithm, a population of ligand poses was generated and optimized iteratively, and the global search space was mainly sampled. Herein, the genetic algorithm search was enabled and 10 independent runs were performed.

The interactions between the tubulin heterodimer (or receptor) and the individual ligand (for all 18 ligands) of the structurally isomerized colchicine analogues in the colchicine binding site were simulated. The docking results were analyzed by incorporating AutoDock Vina and BIOVIA Discovery Studio Visualizer. The receptor-ligand interactions of the modified C-5, C-6, and C-7 of the B-ring analogues of colchicine were discussed in terms of the binding energies, binding affinities, hydrogen bonding, and conformations of docked ligands within receptor binding site.

Results and Discussion

Binding energies and H-bond formations between ligand and tubulin heterodimer

According to the results from molecular docking calculations, the binding energies (in kcal/mol) of all ligands for Models A, B, and C (with substituents R1, R2, R3, R4, R5, and R6) are illustrated in Fig. 3. The intermolecular H-bonds formed between amino acid residues and docked ligands are listed in Table 2.

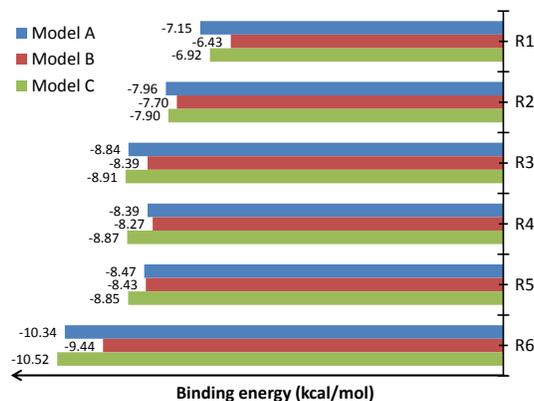


Figure 3 Plot of the binding energies (in kcal/mol) of Models A, B, and C, with substituents: R1 to R6

Table 2 The intermolecular H-bonds formed between amino acid residues and ligands, resulting from the molecular docking calculation

Substituent code	Residue—Ligand (with H-bond distance)					
	Model A		Model B		Model C	
R1	α Thr179:O—H2	(2.1 Å)	β Thr353:O—H1	(2.3 Å)	α Ser178:HG—O22	(2.3 Å)
	α Val181:HN—O32	(1.9 Å)	β Thr353:O—H2	(2.2 Å)		
	β Lys254:HZ2—O8	(1.9 Å)	β Val355:HN—O7	(2.1 Å)		
			β Val355:HN—O8	(2.2 Å)		
R2	α Asn101:OD1—H2	(2.0 Å)	β Thr353:O—H1	(2.1 Å)	α Asn101:HD22—O13	(2.0 Å)
	α Asn101:HD22—O32	(2.1 Å)	β Val355:O—H2	(1.9 Å)	α Thr179:O—H1	(2.1 Å)
	α Ser178:HG—O20	(2.1 Å)	β Val355:HN—O7	(2.0 Å)	α Val181:HN—O8	(2.3 Å)
R3	α Ser178:HG—O3	(1.8 Å)	α Asn101:HD22—O3	(2.1 Å)	α Thr179:O—H1	(1.9 Å)
	β Val355:HN—O7	(2.2 Å)			β Val355:HN—O21	(2.1 Å)
R4	α Thr179:O—H1	(2.0 Å)	α Asn101:HD22—O31	(2.2 Å)	α Thr179:O—H1	(2.0 Å)
	β Ala250:HN—N32	(2.2 Å)	β Thr353:N—H1	(2.7 Å)	β Val355:HN—O21	(2.2 Å)
	β Val355:HN—O8	(2.2 Å)				
R5	No H-bond		α Asn101:HD22—O37	(2.2 Å)	β Thr353:O—H1	(1.8 Å)
			α Gly144:HN—N35	(2.2 Å)	β Val355:O—H2	(2.2 Å)
			α Ser178:O—H1	(2.2 Å)	β Val355:HN2—O5	(2.3 Å)
R6	α Asn101:HD22—O37	(1.9 Å)	α Gln11:HE21—O9	(2.1 Å)	α Gln11:OE1—H10	(1.9 Å)
	α GTP600:O1A—H28	(1.7 Å)	α Asn101:HD22—N35	(1.9 Å)	α Gln11:HE21—N7	(2.2 Å)
	α GTP600:O1G—H29	(1.9 Å)	α Tyr224:OH—H1	(2.0 Å)	α Val181:HN—O35	(2.4 Å)
			α GTP600:O1A—H28	(2.0 Å)	α GTP600:O1A—H9	(1.9 Å)
			α GTP600:O1A—H29	(2.0 Å)		

Note: The symbol, for example, β Ala250:HN—N32 means 'H-bond is formed between H (of —NH group) of β Ala250 with N (no. 32) of docked ligand'

For the calculated values of binding energy, it is obviously found that all ligands of Model B (R1B - R6B) provide the highest binding energies. For the substituents R1 and R2, it is found that the ligands of Model A: R1A and R2A, give lowest binding energies, whereas for the substituents R3, R4, R5 and R6, the ligands of Model C: R3C, R4C, R5C and R6C are all the lowest energies. The sequence of the binding energies can be depicted as follow: R1A < R1C < R1B; R2A < R2C < R2B; R3C < R3A < R3B; R4C < R4A < R4B; R5C < R5A < R5B; and R6C < R6A < R6B, respectively.

Considering to the intermolecular H-bond formation between amino acid residue(s) and docked ligand as indicated in Table 2. Here, the appearance of hydrogen bonding will be represented by using 'Residue—Ligand' symbol, such as, β Ala250:HN—N32 which means '*H-bond is formed between H (of -NH group) of β Ala250 with N (no. 32) of docked ligand*, as represented in Figs. S1, S2, and S3 (Supporting Information). The results of molecular docking calculations suggested that many kinds of hydrogen bonding, as well as a variety of numbers of H-bond (from 0 to 5 HB), can be formed between amino acid residues and docked ligand within colchicine binding pocket. The residues which can be formed H-bonds with these docked ligands are: Gln11, Asn101, Gly144, Ser178, Thr179, Val181, Tyr224 (of α -tubulin); Ala250, Lys254, Thr353, Val355 (of β -tubulin); and also GTP600 (guanosine triphosphate in α -tubulin subunit).

All the appearance of intermolecular hydrogen bonding between individual residue and docked ligands are summarized in Table 3. It is found that α Asn101, as well as β Val355, provides the most probability to form H-bond with 7 docked ligands, suggesting that many of docked ligands are preferably located close to these residues (within binding pocket). Here, residue α Asn101 forms H-bond(s) with R2A [2], R2C [1], R3B [1], R4B [1], R5B [1], R6A [1], and R6B [1], whereas residue β Val355 with R1B [2], R2B [2], R3A [1], R3C [1], R4A [1], R4C [1], and R5C [2]. (Note: The number in a bracket [] declares the number of H-bond for each ligand.) Moreover, it is found that residue α Thr179 forms H-bond with 5 docked ligands; R1A [1], R2C [1], R3C [1], R4A [1], and R4C [1], while residue α Ser178 with 4 docked ligands; R1C [1], R2A [2], R3A [1], and R5B [1], as well as residue β Thr353 with R1B [2], R2B [1], R4B [1], and R5C [1]. For the rest residues, which are α Gln11, α Gly144, α Val181, α Tyr224, β Ala250, and β Lys254, as well as α GTP600, can form H-bond with some docked ligands (see Table 3). More details of position and orientation of hydrogen bonding of all docked ligands will be discussed in the next section.

Table 3 Summary of H-bond formation of individual residue and docked ligands, from R1A to R6C, with the numbers of H-bond

Residue	Docked ligands [with no. of H-bond]
α Asn101	R2A [2], R2C [1], R3B [1], R4B [1], R5B [1], R6A [1], R6B [1]
β Val355	R1B [2], R2B [2], R3A [1], R3C [1], R4A [1], R4C [1], R5C [2]
α Thr179	R1A [1], R2C [1], R3C [1], R4A [1], R4C [1]
α Ser178	R1C [1], R2A [2], R3A [1], R5B [1]
β Thr353	R1B [2], R2B [1], R4B [1], R5C [1]
α Val181	R1A [1], R2C [1], R6C [1]
α GTP600	R6A [2], R6B [2], R6C [1]
α Gln11	R6B [1], R6C [2]
α Gly144	R5B [1]
α Tyr224	R6B [1]
β Lys254	R1A [1]
β Ala250	R4A [1]

Investigation of the ligand-receptor complexes within colchicine binding site

Herein, the conformational orientations of all docked ligands (the structurally isomerized colchicine analogues) from the molecular docking results have been considered. Inside the colchicine binding pocket of receptor model (Fig. 4a), the comparison between DAMA-colchicine in 1SA0, and the most stable conformations of docked ligands for Models A, B, and C are illustrated in Figs. 4b, 4c, and 4d, respectively.

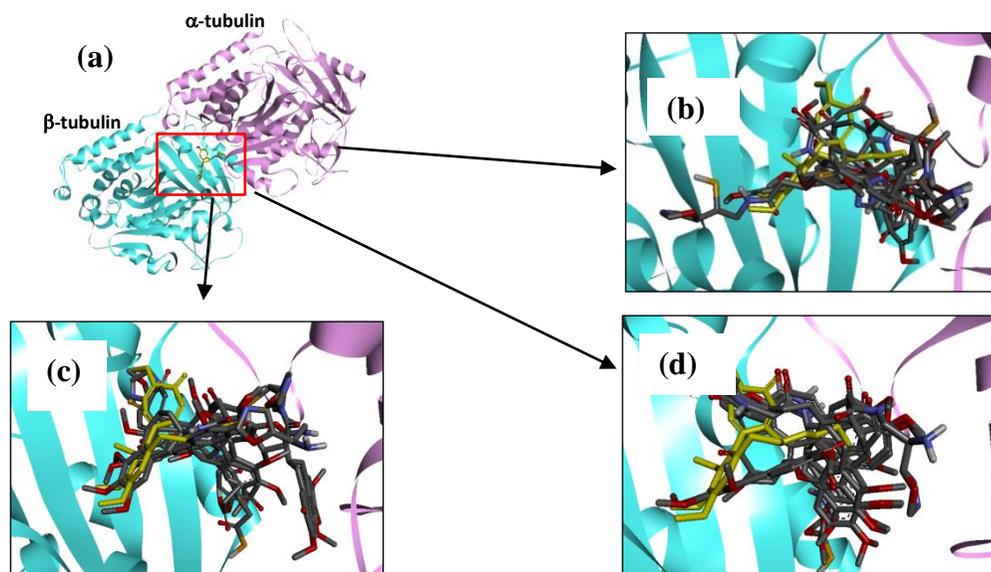


Figure 4 The orientation of DAMA-colchicine (yellow stick) in $\alpha\beta$ -tubulin dimer from PDB code 1SA0, as shown in (a); compared with (b) Model A: R1A to R6A; (c) Model B: R1B to R6B; and (d) Model C: R1C to R6C

By using the molecular docking simulation, therefore, the final pose of docked ligand is of ligand-receptor complex, which provides the lowest-energy configuration at equilibrium state. For Model A (Fig. 4b), overall final poses of docked ligands show that there are moderately distribution of the location of these ligands, with respect to DAMA-colchicine's alignment. Most of docked ligands tend to shift toward α -tubulin binding space, whereas some are located further into β -tubulin region with respect to the position of DAMA-colchicine. For both Models B and C (Figs. 4c and 4d), it is found that the final poses of docked ligands are mostly located further into α -tubulin binding region, while some of docked ligands are lying into β -tubulin binding region as far as DAMA-colchicine. Comparison between all final poses of these three models suggests that the docked ligands for Model A are locally lying about DAMA-colchicine's position rather than those for Models B and C. As seen in Fig. 4, there is not only the distribution of docked ligand positions, but also the variation of molecular distortion (ligand flexibility), supporting the stability of ligand-receptor complex within colchicine binding site.

Comparison of the binding affinities between Models A, B, and C

In the present *in silico* study, overall results suggest that for three different structural isomers, namely Models A, B and C, the higher-affinity isomers (or docked ligands) which would be bound to the receptor binding sites are R1A (for substituent R1), R2A (for substituent R2), R3C (for substituent R3), R4C (for substituent R4), R5C (for substituent R5), and R6C (for substituent R6). These isomers would be predicted as the representatives for each group of structurally isomerized colchicine analogues to form complexes with tubulin heterodimer. Details of the explanation are as follow.

In Fig. 5, the six groups of structural isomers of colchicine analogues, i.e. R1A/R1B/R1C, R2A/R2B/R2C, R3A/R3B/R3C, R4A/R4B/R4C, R5A/R5B/R5C and R6A/R6B/R6C, have been depicted separately. Herein, the five groups of docked ligands (with R1, R2, R3, R4 and R5) would be bound to receptor binding sites in both α - and β -chain regions, while a group of R6A/R6B/R6C (with R6) occurs only in α -chain binding space. Consequently, in Figs. 6, 7 and 8, the insight views of these isomers (docked ligands) have been represented individually by ligand models and three-dimensional structures for visualized comprehension. Moreover, in Figs. 6, 7 and 8, the comparison between each pair of the relatively similar substituents: R1 vs. R2, R3 vs. R4, and R5 vs. R6, are also demonstrated, in that the individual ligand for all structurally isomerized colchicine analogues will be presented by two- and three-dimensional ligands with H-bonded residues, included of the binding energy and number of H-bond.

In Fig. 6, with respect to the lowest binding energies, the binding affinity for substituent R1 is decreased from R1A > R1C > R1B, where R1A forms three H-bonds (to α Val181, α Thr179 and β Lys254); R1C forms only one H-bond (to α Ser178); R1B forms four H-bonds (to β Thr353[2] and β Val355[2]). For substituent R2, it is that R2A > R2C > R2B, where the isomer R2A forms three H-bonds (to α Asn101[2] and α Ser178); R2C forms three H-bonds (to α Asn101, α Val181 and α Thr179); R2B forms three H-bonds (to β Thr353 and β Val355[2]). Comparing between

R1 vs. R2, which differ only one group: -OH (in R1) vs. -SH (in R2), at the end of a long side-chain, it is found for the same Model (A or B or C) that R2 always has higher binding affinity than R1 (ΔBE is ca. -0.8 to -1.3 kcal/mol).

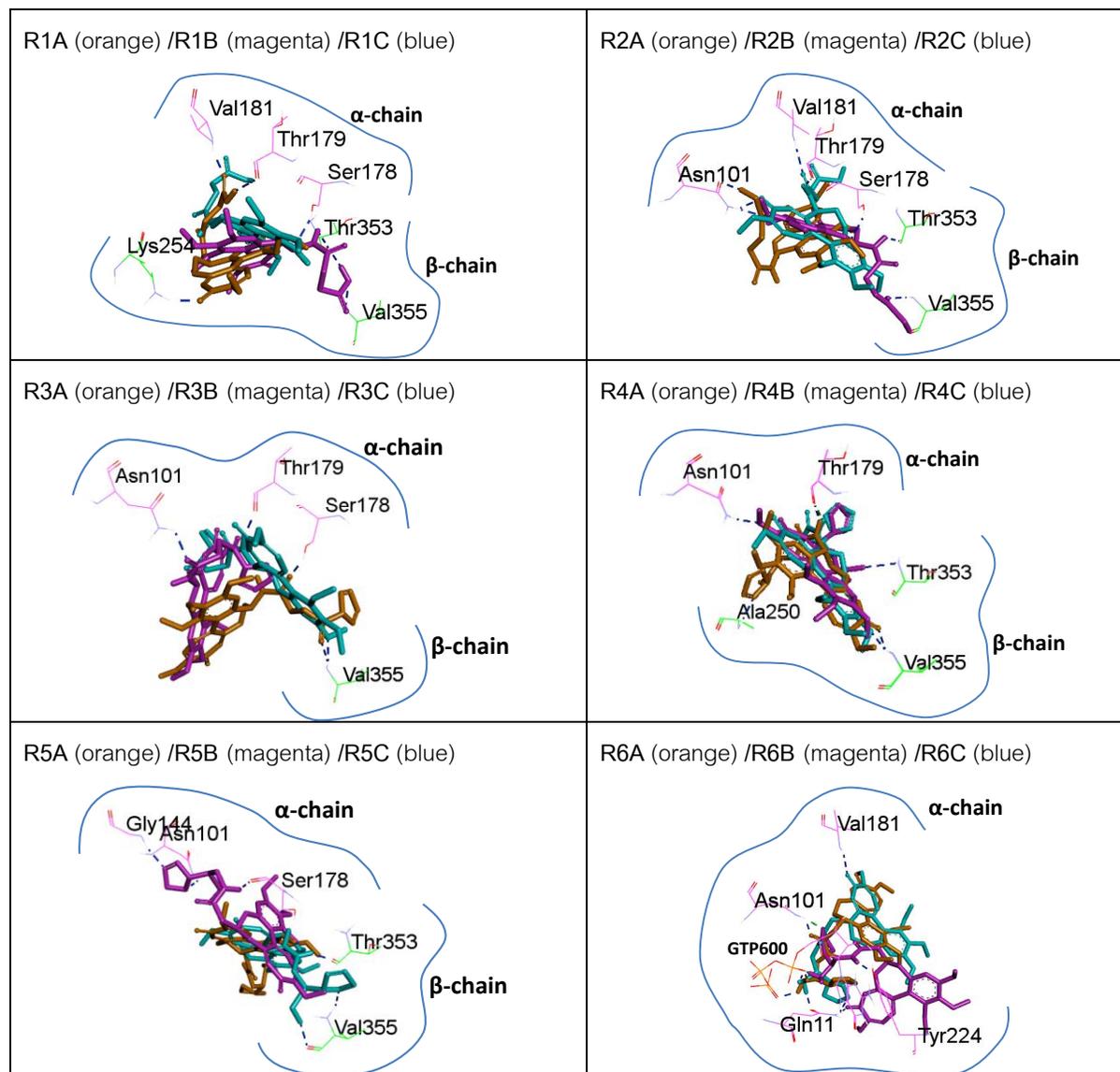


Figure 5 Comparison of docked ligand orientations of three structural isomers of colchicine analogues (Models A, B, and C) for the substituent R1, R2, R3, R4, R5 or R6, together with the H-bonded residues in α - and β -chain regions

In Fig. 7, the binding affinity for substituent R3 is decreased from R3C > R3A > R3B, where the isomer R3C forms two H-bonds (to α Thr179 and β Val355); R3A forms two H-bonds (to α Ser178 and β Val355); R3B forms one H-bond (to α Asn101). For substituent R4, it is that R4C > R4A > R4B, respectively, where R4C forms two H-bonds (to α Thr179 and β Val355); R4A forms three H-bonds (to α Thr179, β Ala250 and β Val355); R4B forms two H-bonds (to α Asn101 and β Thr353). Comparing between R3 vs. R4, which differ only one group: =O (in R3) vs. =S

(in R4), nearby the end of a long side-chain, it is found for the same Model (A or B or C) that R3 always has slightly higher binding affinity than R4 (ΔBE is ca. -0.1 to -0.4 kcal/mol).

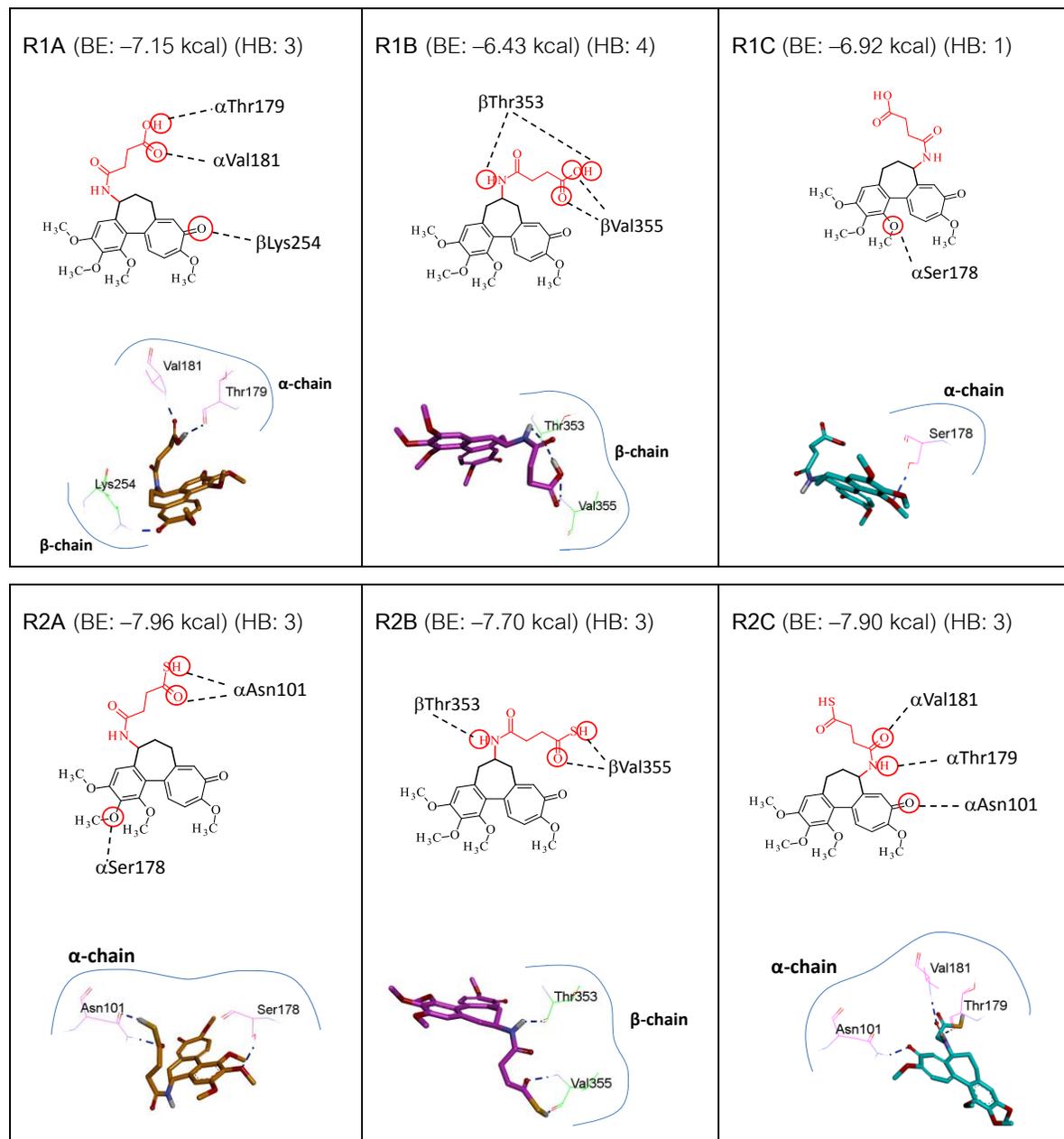


Figure 6 Representation of the docked ligands consisting the substituent R1 vs. R2, for Models A, B, and C, included of the binding energy and number of H-bond (in parenthesis); the ligand model with H-bonded atoms (in circles) and its orientation of docked ligand with H-bonded residues

In Fig. 8, the binding affinity for substituent R5 is decreased from R5C > R5A > R5B, where the isomer R5C forms three H-bonds (to β Thr353 and β Val355[2]); R5A has no H-bond; R5B forms three H-bonds (to α Asn101, α Gly144 and α Ser178). For substituent R6, it is that R6C > R6A > R6B, where R6C forms four H-bonds (to

α Gln11[2], α Val181 and α GTP600); R6A forms three H-bonds (to α Asn101 and α GTP600[2]); R6B forms five H-bonds (to α Gln11, α Asn101, α Tyr224 and α GTP600[2]). Comparing between R5 vs. R6, which differ only one group: $-SH$ (in R5) vs. $-CH_2NH_2$ (in R6), at the end of a long side-chain, it is found for the same Model that R6 always has significantly higher binding affinity than R5 (ΔBE is *ca.* -1.0 to -1.9 kcal/mol).

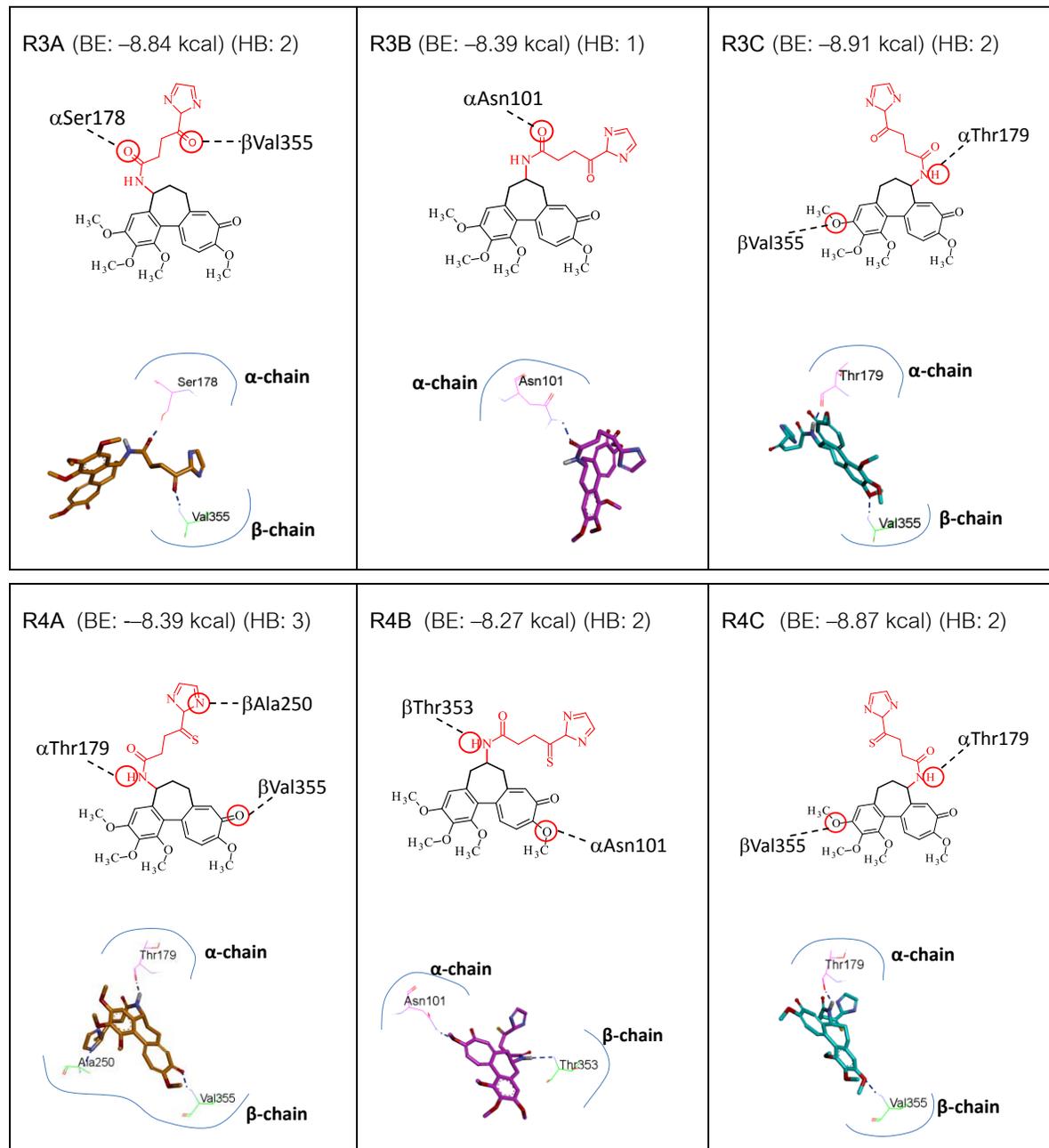


Figure 7 Representation of the docked ligands consisting the substituent R3 vs. R4, for Models A, B, and C, included of the binding energy and number of H-bond (in parenthesis); the ligand model with H-bonded atoms (in circles) and its orientation of docked ligand with H-bonded residues

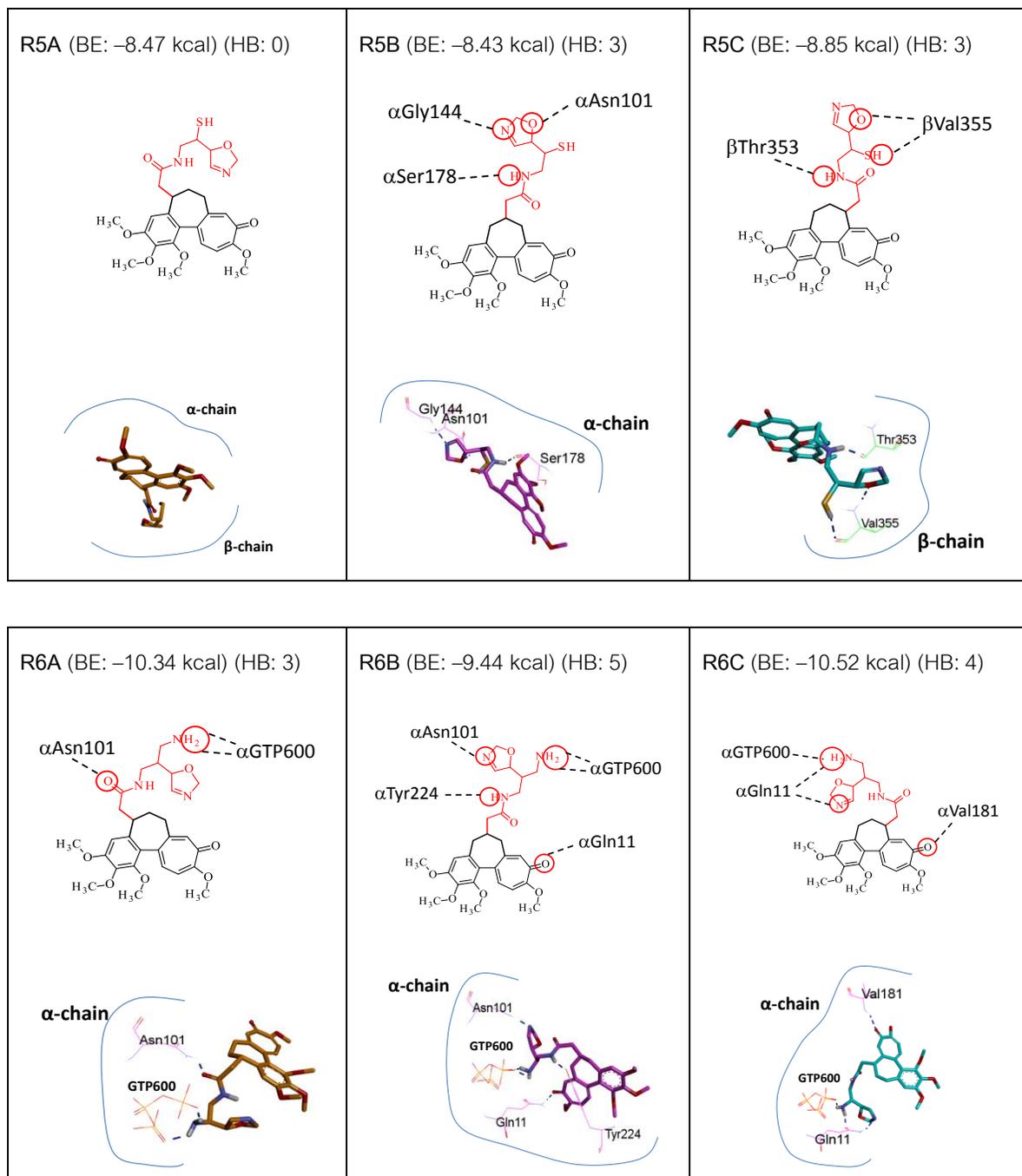


Figure 8 Representation of the docked ligands consisting the substituent R5 vs. R6, for Models A, B, and C, included of the binding energy and number of H-bond (in parenthesis); the ligand model with H-bonded atoms (in circles) and its orientation of docked ligand with H-bonded residues

Conclusions

The present *in silico* study has been made by using the molecular docking simulation to perform over the interactions between the structurally isomerized colchicine analogues with tubulin heterodimer. These analogues are the modified B-ring of colchicine at the C-5, C-6, and C-7 positions, respectively. There have been found that the highest-affinity isomers could be classified as those belonging to Model C (the C-7 substituents in the B-ring) and to Model A (the C-5 substituents in the B-ring), in relevant to the substituents consisting *with* or *without* side-chain (aromatic) ring, respectively. Obviously, all lowest-affinity isomers belong to Model B (the C-6 substituents in the B-ring). The values of binding energy differences predict that all modified isomers (at the C-5, C-6, and C-7 positions) of substituent R1 are higher affinity to bind with tubulin compared to those of substituent R2, whereas the modified isomers of substituent R3 are slightly higher affinity than those of substituent R4, and significantly those of substituent R6 are the higher-affinity isomers compared to substituent R5. It is found that the amino acid residues: α Asn101 and β Val355, provides the most probability to form H-bond with 7 docked ligands (from all 18 ligands), however, in this study there is no directly relevant of these two residues for enhancing the binding energy. Overall results also support an important role of the substituent positions in the B-ring for binding affinity of these colchicine analogues with receptor binding sites. As expected, the docked ligands (of the modified B-ring at the C-5, C-6, and C-7 positions) are located at the α/β intradimer interface of tubulin, but unlike DAMA-colchicine complex which was mostly buried in β -subunit, these analogues shift toward the α -subunit binding space to form drug-tubulin complexes.

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