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ORIGINAL PAPER

Docking studies on kinesin spindle protein inhibitors: an important cooperative 'minor binding pocket' which increases the binding affinity significantly

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Abstract Fifteen KSP inhibitors were docked into the receptor and the binding mode was analyzed for the first time. It was considered that in addition to the main binding pocket all the inhibitors merged in, there exists a cooperative minor binding pocket, which could be explored for significantly increased binding affinity. In addition, a good linear relationship between the biological activities and the lowest binding free energies has also been found. This may help in predicting the binding affinity of newly designed KSP inhibitors.

Keywords KSP inhibitors · Binding pocket · Docking

Introduction

Kinesin spindle protein (KSP), also known as *Hs* Eg5, is a member of the kinesin superfamily of molecular motors that utilize the energy generated from the hydrolysis of ATP to transport vesicles, organelles, and microtubules [1–3]. Inhibition of KSP prevents normal bipolar spindle formation, which leads to mitotic arrest with a characteristic monoastral phenotype and subsequently to apoptosis in transformed cells [4–6]. KSP inhibition represents a novel and specific mechanism to target the mitotic spindle that may be devoid of the neuropathy-associated, mechanism-based side effects common to the taxanes and other natural products that target microtubules.

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In 1999, Mayer et al. reported the first small molecule, monastrol, which targeted KSP and led to mitotic arrest [4]. The crystal structure KSP in complex with Mg^{2+} and ADP has been determined by Turner et al. [7]. The binding mode of monastrol was then reported in detail by Yan et al. [8].

After the finding of monastrol, several classes of compounds have been reported [9–21]. This makes it possible to make a binding mode analysis for KSP inhibitors. Recently, our team has reported the pharmacophore identification of KSP inhibitors based on the reported KSP inhibitors [22].

In this paper, we analyzed the binding mode of the KSP inhibitors for the first time. It was considered that in addition to the main pocket all the inhibitors merged in, there exists a cooperative minor pocket which could be explored for increased binding affinity. In addition, a good linear relationship between the biological activities and the lowest binding free energies were also found for the first time. This may help in predicting the binding affinity of newly designed KSP inhibitors.

Materials and methods

System setup The protein was obtained from the Brookhaven Protein Data Bank (PDB entry code 1Q0B) after the ligand 'nat 604'((*S*)-monastrol) and crystal water were deleted. KSP has two identical subunits (A and B). The study was carried out on only the A subunit of the protein. The complex structures of the 15 compounds were built using 1Q0B as the template. The ligands were drawn, optimized, and saved in a mol2 format with the aid of Sybyl 6.9. The Gasteiger-Hückel charges were assigned to the ligands. The prepared ligands and protein were used as input files for AutoDock 3.0.5 in the next step.

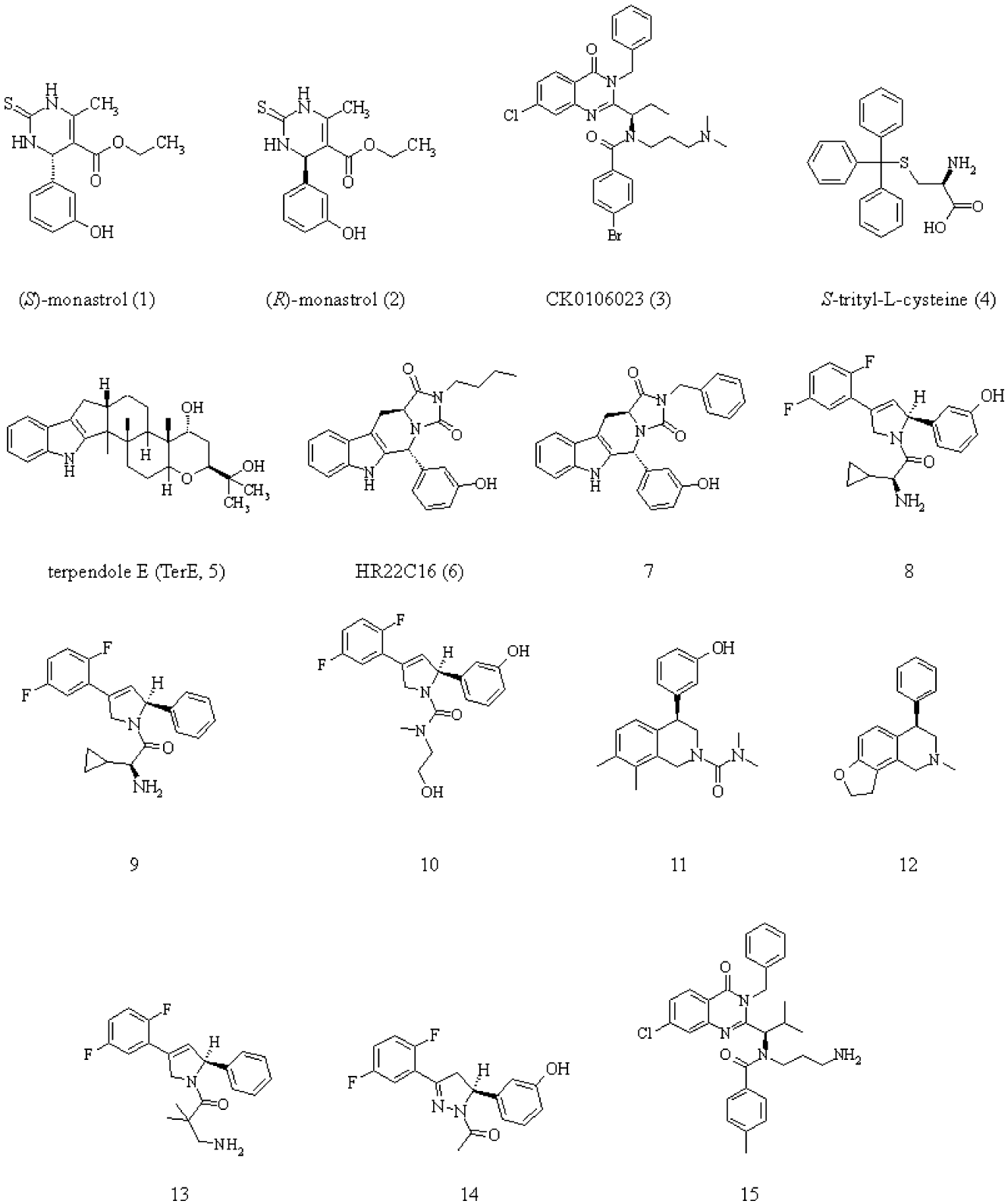

Fig. 1 Structures of the 15 KSP inhibitors tested

Molecular docking Fifteen compounds were selected to analyze the binding mode by considering structural diversity and wide coverage of activity range in terms of IC_{50} ranging from 0.5 nM to 110 μM. The IC_{50} values were all assessed using the same procedure by measuring the release of inorganic phosphate from ATP hydrolysis [23]. The structures of the compounds selected for test were shown in Fig. 1 and the corresponding KSP IC_{50} values reported are shown in Table 1.

Table 1 AutoDock estimated lowest binding free energies (ΔG) and KSP IC_{50} values of the studied inhibitors

No.	ΔG (kcal mol^{-1})	IC_{50} (nM)	pIC50	Reference
1	−9.70	12000	4.92	[4]
2	−8.66	110000	3.96	[4]
3	−10.37	12	7.92	[6]
4	−9.19	1000	6.00	[11]
5	−9.31	23000	4.64	[15]
6	−9.85	4300	5.37	[13]
7	−9.63	650	6.19	[13]
8	−11.64	0. 5	9.30	[17]
9	−10.80	2	8.70	[16]
10	−11.68	4	8.38	[17]
11	−10.70	104	6.98	[20]
12	−9.16	9700	5.01	[20]
13	−10.77	5.2	8.28	[16]
14	−10.75	26	7.59	[12]
15	−10.53	1	9.00	[9]

Docking studies were performed with AutoDock 3.0.5 using a Lamarckian genetic algorithm [24].

The standard docking procedure was used for a rigid protein and a flexible ligand whose torsion angles were identified (for 10 independent runs per ligand). The grid size was set to 60×60×60 points with a grid spacing of 0.375 Å centered on the original ligand in the crystal structural complex. A distance-dependent function of dielectric constant was used for the calculation of the energetic map. The default settings were used for all the other parameters.

The crystal structure of 1Q0B was used to test the validity of the AutoDock 3.0.5 docking program. Ligands with the lowest docking energy were selected as the resultant complex structures after docked into the protein.

All calculations were carried out on a SGI workstation running Linux x86 as the operating system. The resultant structure files were analyzed using DS Viewerpro and LIGPLOT 4.22 program. When docking was finished, the binding free energies would automatically be written into *.dlg* documents. We could open these documents and find the estimated free energies of binding directly.

Table 2 Analysis results using DS Viewerpro and LIGPLOT program after docking

No.	IC_{50}(nM)	A[a]	B[b]	C[c]
1	12000	Glu116, Glu118	Glu116, Glu118, Trp127, Ala133, Pro137, Tyr211	−
2	110000	Glu116	Glu116, Glu118, Ala133, Tyr211	−
3	12	Glu116, Arg221	Glu116, Pro137, Tyr211, Leu214, Glu215, Ala218	+
4	1000	Tyr211, Glu215	Glu116, Gly117, Glu118, Ile136, Pro137, Leu214, Glu215	−
5	23000	Tyr211	Glu116, Gly117, Tyr211, Glu215	−
6	4300	Glu116	Glu116, Gly117, Ala133, Ile136, Pro137, Tyr211, Glu215	−
7	650	Glu116, Glu118	Glu116, Gly117, Ala133, Ile136, Pro137, Tyr211, Glu215	−
8	0.5	Ala133, Glu118, Tyr211	Glu116, Gly117, Glu118, Trp127, Ala133, Pro137, Tyr211, Leu214, Glu215, Phe239	+
9	2	Tyr211	Glu116, Glu118, Trp127, Ala133, Pro137, Tyr211, Leu214, Phe239	+
10	4	Glu118, Arg119, Tyr211	Glu116, Glu118, Ala133, Pro137, Leu214, Phe239	+
11	104	Glu215	Glu116, Gly117, Glu118, Trp127, Ala133, Pro137, Tyr211, Leu214, Glu215	−
12	9700	none	Glu116, Glu118, Trp127, Ala133, Pro137, Leu214	−
13	5.2	none	Glu116, Gly117, Glu118, Ala133, Pro137, Tyr211, Leu214, Phe239	+
14	26	Ala133, Glu118, Tyr211	Glu116, Gly117, Glu118, Ala133, Pro137, Leu214	+
15	1	Gly117	Glu116, Glu118, Pro137, Tyr211, Leu214, Glu215, Ala218	+

[a] Amino acids which form H-bonds with the tested compound.
[b] Amino acids which are mainly contributive in hydrophobic interactions.
[c] Whether the tested compound interact with the cooperative 'minor binding pocket' surrounded mainly by Ala218 and Arg221 or not. '+' represents 'yes' while '−' means 'no'.

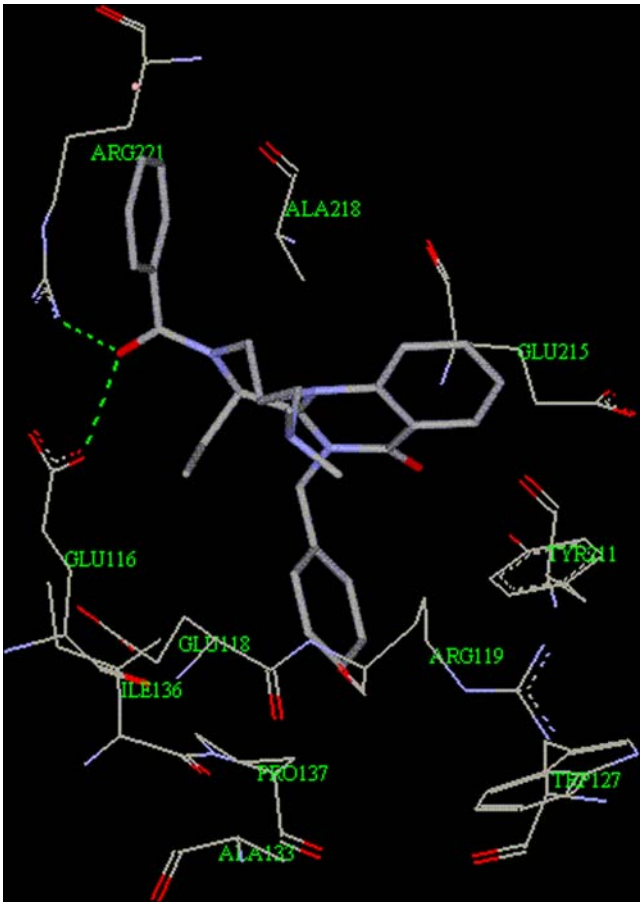

Fig. 2 Docking result of CK0106023 as KSP inhibitors

Results and discussions

The validity of the Autodock3.0.5 docking program used in the docking study of KSP inhibitors was confirmed by the docking of (*S*)-monastrol into the protein.

After docking (*S*)-monastrol into the protein, all ten docked ligand conformations were found in the same cluster, and the RMSDs were all less than 1.14.

Fifteen KSP inhibitors were docked into the protein successfully and the binding free energies were calculated. All the binding mode analyses were processed using the result with lowest docking energies. The analyses of the docking results are shown in Table 2. It could be found that the hydrophobic interactions were essential. All 15 ligands interacted with a cage formed by Glu116, Gly117, Glu118, Trp127, Ala133, Ile136, Pro137, Tyr211, Leu214 and Glu215, as we can see from Table 2. Hydrophobic interactions between the inhibitors and Trp127, Ala133, Tyr211 were especially important. Disruption of such hydrophobic interactions may cause a significant decrease of the activity, such as (*S*)-monastrol vs (*R*)-monastrol. The H-bonds were also determinant, especially the H-bond formed with Glu116, Glu118 and Tyr211, but the losing of H-bond may be compensated by strong hydrophobic interactions.

It could be seen from Table 2 that some inhibitors we tested were more potent than other inhibitors. The IC_{50} values of compound 3, 8, 9, 10, 13, 14, and 15 were less than 30 nM while the values of the other inhibitors were more than 500 nM. These seven more potent inhibitors share a common character when binding to the protein KSP: they all interact with a 'minor pocket' surrounded mainly by Ala218 and Arg221 in addition to the 'main binding pocket' all the other inhibitors merged in.

CK0106023 was one of the most active inhibitors we tested, the IC_{50} value of which was 12 nM. It can be seen from Fig. 2 that the *p*-bromobenzamide group of CK0106023 extends into another small pocket mainly surrounded by Arg221 and Ala218. Two cooperative pockets were considered as the binding site for CK0106023: a main binding pocket mainly composed of Glu116, Gly117, Glu118, Arg119, Trp127, Asp130, Ala133, Ile136, Pro137, Tyr211, Glu215 and a 'minor pocket' surrounded mainly by Ala218 and Arg221. The extending of the binding site is probably the reason for the significantly increased affinity of this compound.

As shown in Fig. 3, the site for KSP inhibitors' interaction was considered to be composed of two cooperative sub-cavities: a main pocket most inhibitors merged in and a minor pocket surrounded mainly by Arg221 and Ala218. The main binding pocket was essential while the interaction with the 'minor binding pocket' may greatly increase the activity (the IC_{50} values decrease from more than 500 nM to less than 30 nM).

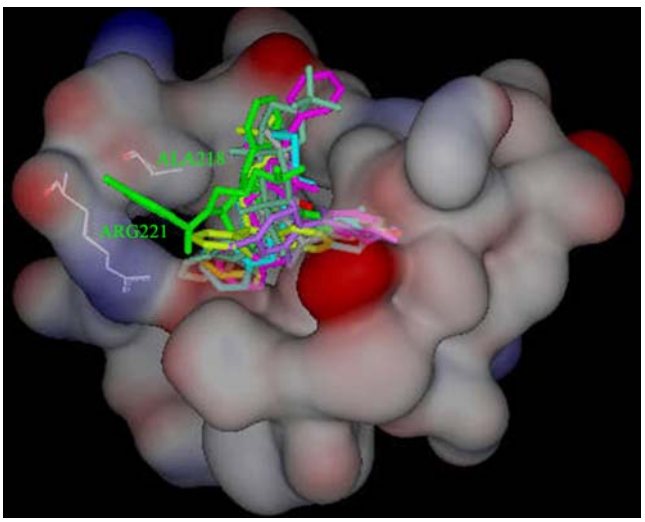

Fig. 3 Two binding pockets considered after the analysis. Seven docked ligands (compound 1–7) were overlapped at the binding site. All inhibitors tested interacted with the main pocket, while CK0106023, interacted also with the cooperative minor pocket mainly surrounded by Arg221 and Ala218. Coloring of the binding site surface are different ends of each amino acid residue: blue represents amino group while red means carboxyl

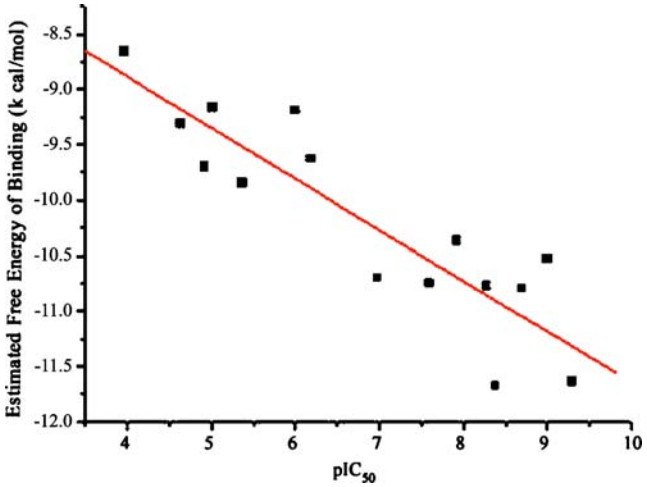

Fig. 4 The linear relationship between the lowest binding free energies and the corresponding pIC_{50} values. (The x axis represents the pIC_{50} values reported in recent literatures and the y axis is the binding free energies)

The lowest binding free energies with the corresponding KSP IC_{50} values of the 15 inhibitors of KSP are listed in Table 1. The good relationship was an indication that the docking calculation produced reasonable binding modes within the binding geometries they researched. Here, according to the two groups of data, a linear relationship can be derived as shown in Fig. 4. The x axis represents the pIC_{50} values reported in recent literatures and the y axis is the lowest binding free energies (shown in Table 1). The equation we gained is $Y=-7.053-0.45916 * X$. And the value of R is 0.89254. This equation may provide us with an important approach for predicting the inhibition constants of newly designed and previously untested KSP inhibitors.

Although there may seem to be some drawbacks in our calculation procedure, this might be the result of simplifications used in the AutoDock program: no explicit water molecules are considered during docking, thus the thiourea group of (*S*)-monastrol interacts via a water molecule with the backbone oxygen of Leu214 was not observed. AutoDock considers desolvation and entropy contributions of the ligands (coefficients derived by regression analysis) in the function which, by this, is a rough approximation of ΔG. Whereas we consider that impacts the results little in detecting the cooperative minor binding pocket or estimating the bio-activities roughly using our equation.

Conclusions

In summary, 15 KSP inhibitors were docked into the protein successfully and the binding free energies were calculated. Binding modes were carefully analyzed using DS Viewerpro and LIGPLOT program. The binding site of the KSP inhibitors was considered formed by two cooperative parts, the 'main pocket' is essential and the interaction of the inhibitor with the 'minor pocket' may greatly increase the bio-activity. A linear relationship was also found between the pIC_{50} values reported in recent literatures and the lowest binding free energies. This may provide us with an important approach for predicting the inhibition constants of newly designed and previously untested Eg5 inhibitors.

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