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Review
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Proposal of Dual Inhibitor Targeting ATPase Domains of Topoisomerase II and Heat Shock Protein 90

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Abstract

There is a conserved ATPase domain in topoisomerase II (topo II) and heat shock protein 90 (Hsp90) which belong to the GHKL (gyrase, Hsp90, histidine kinase, and MutL) family. The inhibitors that target each of topo II and Hsp90 are intensively studied as anti-cancer drugs since they play very important roles in cell proliferation and survival. Therefore the development of dual targeting anti-cancer drugs for topo II and Hsp90 is suggested to be a promising area. The topo II and Hsp90 inhibitors, known to bind to their ATP binding site, were searched. All the inhibitors investigated were docked to both topo II and Hsp90. Four candidate compounds as possible dual inhibitors were selected by analyzing the molecular docking study. The pharmacophore model of dual inhibitors for topo II and Hsp90 were generated and the design of novel dual inhibitor was proposed.

Key Words: Topoisomerase II, Heat shock protein 90, Molecular docking study, Design of dual inhibitor

INTRODUCTION

Topoisomerase II (topo II) and heat shock protein 90 (Hsp90) both contain a conserved ATPase domain and belong to the same family, namely, GHKL (gyrase, Hsp90, histidine kinase, and MutL) domain (Dutta and Inouye, 2000; Chene, 2002). ATPase domain in both of these proteins requires ATP to exert important cellular functions such as cell cycle progression, proliferation and survival. Therefore, inhibitors targeting the ATP binding site of these two proteins through binding in an ATP-competitive manner were searched and characterized in this study. Another important biological implication in topo II and Hsp90 is that they are both overexpressed in proliferating cancer cells and have been attractive targets for the development of anti-cancer drugs (Neckers, 2002; Nitiss, 2009a).

Topo II is very important in cellular processes such as transcription and replication by introducing transient breaks in DNA double strand (Nitiss, 2009b). Topo II requires ATP binding for its conformational change to solve topological problems in DNA. Recently, there are much efforts in developing catalytic inhibitors of topo II in order to overcome the side-effects of topo II poisons such as etoposide (Pogorelcnik *et al*., 2013). Hsp90 is a molecular chaperone which has diverse client proteins involved in tumor growth and survival. Therefore, Hsp90 also has been an attractive target for chemotherapeutic development and phase II clinical trial was conducted for Hsp90 inhibitor, 17-allyaminogeldanamycin (17-AAG) (Sidera and Patsavoudi, 2014). In 2006, Jenkins and coworkers reported that the topo II and Hsp90 form a complex, and co-treatment of 17-AAG showed synergistic efficacy by enhancing the activity of topo II poison (Barker *et al*., 2006; Yao *et al*., 2007). From these findings, development of inhibitors that target both ATPase domains of topo II and Hsp90 can be a promising research area. There are many advantages of multi-target drugs since they can simultaneously inhibit multiple pathways and escape an undesirable drug-drug interaction which may encounter with co-treatment of single-target drugs (Petrelli and Giordano, 2008).

In this review, the topo II and Hsp90 inhibitors that bind to the ATPase domain of each of topo II and Hsp90 are analyzed and the possibility of designing dual inhibitor is explored through molecular modelling studies.

METHODS

Molecular docking studies

The 3D structures of the inhibitors of topo II and Hsp90 were sketched using Sybyl X-2.1.1 (Certara L.P., St. Louis, MO, USA). All the structures were energetically minimized

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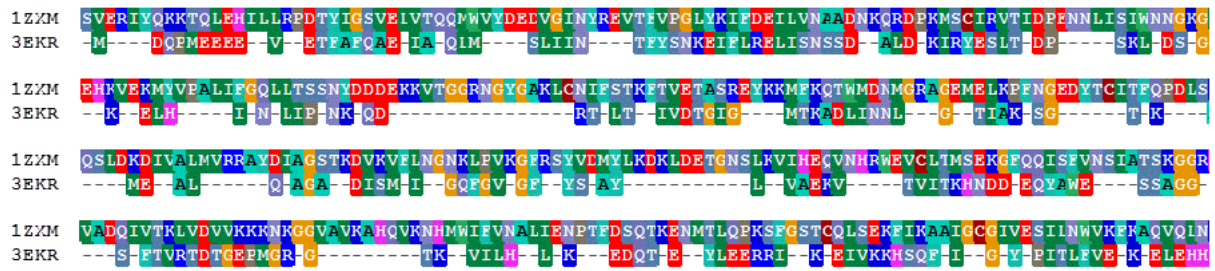


Fig. 1. The sequence alignment of the ATPase domain of topo II (1ZXM) and Hsp90 (3EKR). The alignment were generated using BioEdit.

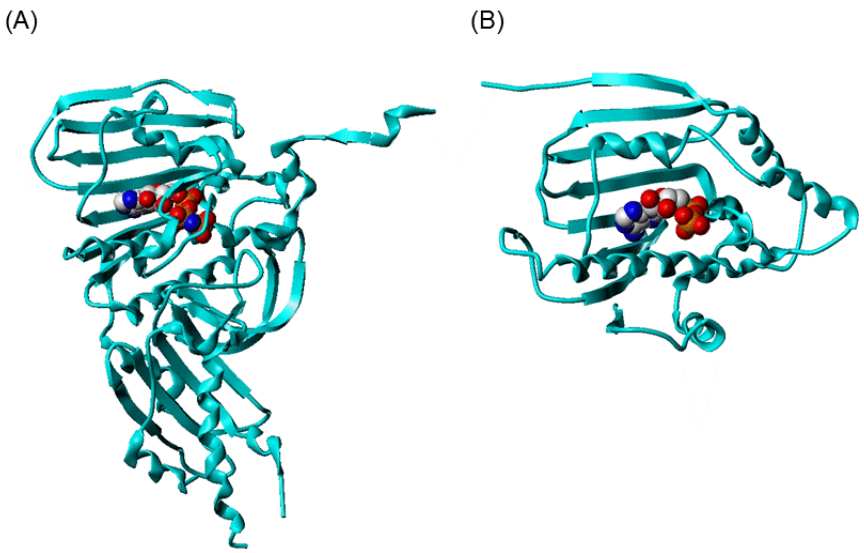


Fig. 2. The structure of ATPase domain of (A) topo II and (B) Hsp90. AMPPNP and ADP bound to topo II and Hsp90, respectively are represented in space-filling model colored by atom type (gray: carbon; red: oxygen; blue: nitrogen; orange: phosphorus). The proteins are represented in ribbon.

using Tripos force field and Gasteiger-Hückel charges. The structures of ATPase domain of topo II and Hsp90 were retrieved from RCSB Protein Data Bank (PDB entry code: 1ZXM and 3EKR) (Wei *et al*., 2005; Kung *et al*., 2008). The ligands were extracted and water molecules were removed from the initial x-ray crystal structure. The docking was carried out for both of the topo II and Hsp90 inhibitors to topo II and Hsp90 using Surflex-Dock (Jain, 2003). The protomol was generated using ligand mode, which used the ligand, extracted from the crystal structure occupying the ATP binding site, to ensure that the inhibitor could bind to the ATP binding site. Then polar hydrogens were added to the structure. After the protomol generation, ligands were docked using Surflex-Dock Geom and GeomX modes using default parameters.

Pharmacophore hypothesis generation

Compounds PU3, 3t, AUY922 and comp. 14 were used as input molecules to generate pharmacophore model using GASP module implemented in Sybyl X-2.1.1. Four molecules selected were used as the data set for the pharmacophore model generations. All the features on each of the molecules were used and the default GA parameters were used. The parameters used for the calculations were as follows; 100 population size, 1.1 selection pressure, 100000 max operations, 6500 operation increment and 0.01 fitness.

RESULTS

Comparison of the structural similarities of ATPase domain of topo II and Hsp90

Among structures of ATPase domain of topo II and Hsp90 deposited in Protein Data Bank (PDB), 1ZXM for topo II and 3EKR and 1BYQ for Hsp90 were chosen for structure comparison and docking. The length of the two proteins is 376 and 217 amino acid residues for topo II and Hsp90, respectively. The similarity of the two proteins were compared by sequence alignment using BioEdit (Fig. 1) (Hall, 1999). Although the sequence identity between the two ATPase domains is 15.8 %, which is rather small value, the overall fold has high similarity where they are superimposable (Fig. 2).

The ATP binding sites of the two proteins can be suggested to have similar environment. The amino acid residues involved in binding with the ligand adenylyl-imidophosphate (AMPPNP) or ADP for topo II and Hsp90, respectively, do not coincide exactly, however the properties of each amino acids are con-

served. For example, in topo II, Asn120 forms hydrogen bond with the N6 amino group of adenine ring, whereas in Hsp90, Asp93 is involved in the hydrogen bond interaction. The hydrophobic residue Ile125 in topo II corresponds to the residue of Met98 in Hsp90 which is also hydrophobic. Additionally, the size of the ATP binding site of each protein was calculated with Computed Atlas of Surface Topography of proteins (CASTp, http://sts.bioe.uic.edu/castp/) (Liang *et al*., 1998). As listed in Table 1 and shown in Fig. 3, the calculated area and volume of topo II ATP binding site were 792.2 Å^2 and 1077.6 Å^3, respectively. The ATP binding site's area and volume of Hsp90 were slightly smaller than topo II, 628.9 Å^2 and 971.0 Å^3, respectively. The mouth opening of the binding pocket was also identified and characterized with CASTp. Although the overall pocket size was slightly larger for topo II, the area and the circumcircle of the mouth opening of Hsp90 were larger than topo II, with the values of 167.6 Å^2, 78.6 Å and 70.9 Å^2, 52.6 Å, respectively.

Topo II inhibitors that bind to the ATPase domain

The topo II inhibitors that bind to the topo II ATPase domain were searched. The inhibitors can be largely divided into two categories, purine analogues and non-purine analogues. Table 2 lists the topo II inhibitors and gives information about their structures and ATPase inhibition activity where applicable.

Table 1. The characterization of the active sites of topo II and Hsp90 by CASTp

Protein	Pocket		Mouth		
	Area (Å^2)	Volume (Å^3)	Number*	Area (Å^2)	Circumcircle (Å)
topo II	792.2	1077.6	2	70.9	52.6
Hsp90	628.9	971.0	1	167.6	78.6

*Number of mouth openings for the pocket. Each has to be large enough to allow the solvent probe to pass through.

Purine analogue inhibitors contain the purine ring and have substitutions on the 2, 6, or 9 positions. In order to develop novel topo II catalytic inhibitors, 1,990 compounds from the National Cancer Institute (NCI) diversity set library was screened and S^6-substituted thioguanine analog, NSC35866 was identified (Jensen *et al*., 2005). This finding was further expanded to discover more potent ATPase inhibitors by screening 40 substituted purine or purine-like compounds in the NCI database and several compounds including NSC348400 were identified from this screening (Jensen *et al*., 2006). Compounds 1 and 2 were searched from the Novartis compound collection to specifically target the ATP binding site of topo II (Furet *et al*., 2009). The hydrogen bond forming residues of topo II were Asn120 and Asn91 which were identical in three topo II complexes with compounds 1, 2 and AMPPNP, however the purine ring of compounds 1 and 2 adopted different orientation compared to that of ATP. Compounds 1 and 2 were further optimized by considering these interactions with the binding site and obtained a purine analogue with substitution of an ethyl group at position C6 and a morpholino-ethoxy group in the quinolone substituted on position N^2 (called quinoline aminopurine, QAP1) (Chene *et al*., 2009). QAP1 showed improvement in topo II ATPase inhibitory activity with the half maximal inhibitory concentration (IC_{50}) of 128 ± 21 nM. 3t has a new scaffold, aloisine moiety, which is similar to purine ring (Li *et al*., 2016). In contrast to compounds 1 and 2, the aloisine ring was aligned with the purine ring of ATP from docking study. 2c is a organoplatinum(II) complex with an attachment of 2-amino-6-chloropurine (Wang *et al*., 2010). 2c inhibited topo II by preventing ATP entering into the ATPase domain. Although 2c is a purine analog, its purine moiety did not occupy the ATP purine ring binding site, but the *tert*-butyl groups of the terpyridine scaffold occupied on it, determined by molecular docking study. 8-chloro-adenosine (8-Cl-Ado) is an anti-cancer agent currently undergoing phase I/II clinical trial. 8-Cl-Ado convert-

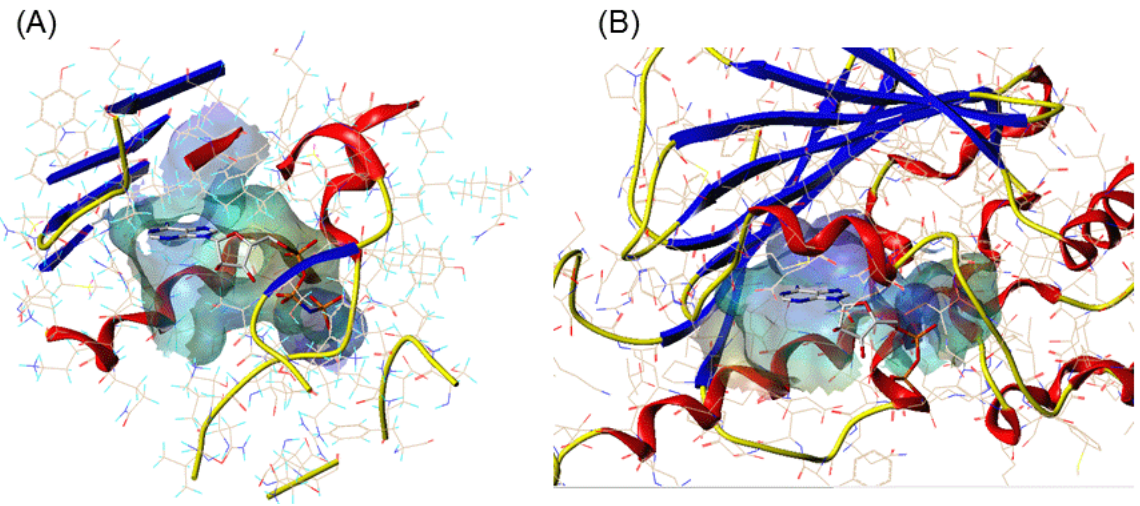


Fig. 3. The comparison of ATP binding site of (A) topo II and (B) Hsp90. The channel was created using MOLCAD implemented in Sybyl, colored by electrostatic potential. The color ramp ranges from red (most positive) to purple (most negative). AMPPNP and ADP bound to topo II and Hsp90, respectively are represented in sticks colored by atom type (gray: carbon; red: oxygen; blue: nitrogen; orange: phosphorus). The proteins are represented in ribbon (blue: β-strand; red: α-helix).

Table 2. Topo II inhibitors that bind to the ATPase domain

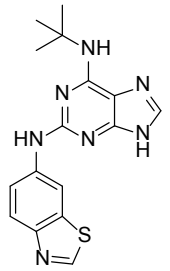
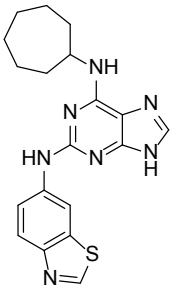
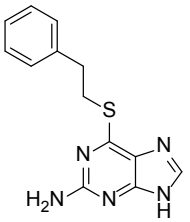
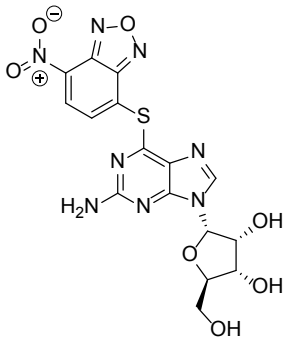
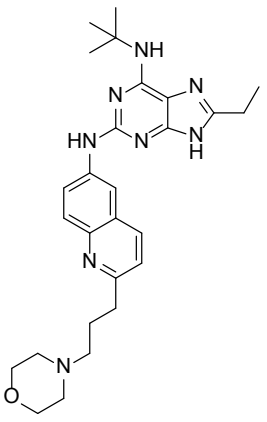
Name	Structure	IC_{50}*	Type	Reference
Comp. 1		1.7 μM	Purine analog	Furet *et al*., 2009
Comp. 2		8.4 μM	Purine analog	Furet *et al*., 2009
NSC35866		50 μM	Purine analog	Jensen *et al*., 2005
NCS348400		0.39 μM	Purine analog	Jensen *et al*., 2006
QAP1		128 nM	Purine analog	Chene *et al*., 2009

Table 2. Continued

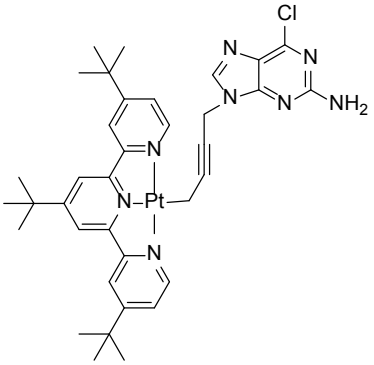
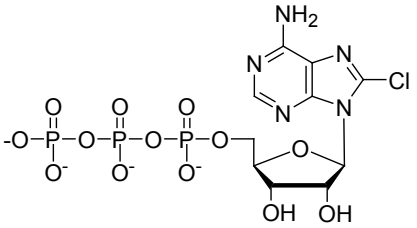
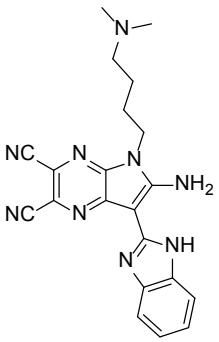
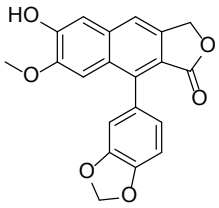
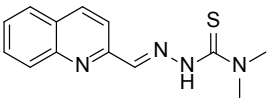
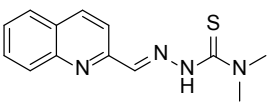
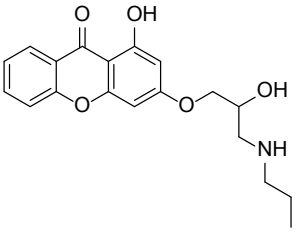
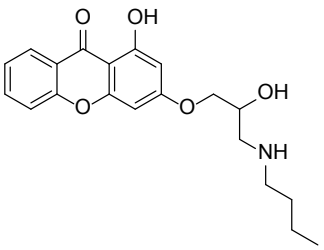
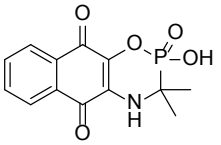
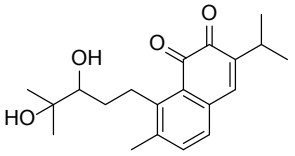
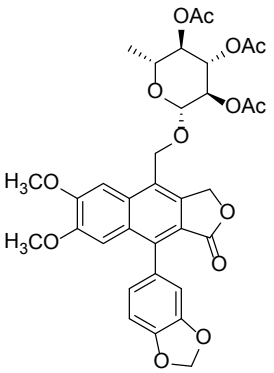
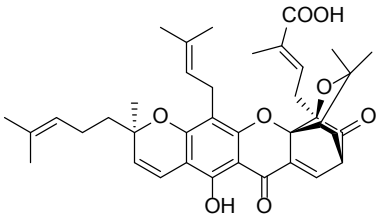
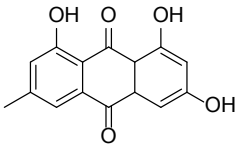
Name	Structure	IC_{50}*	Type	Reference
2c		K_i=1.25 μM in ATP competition assay	Purine analog with platinum	Wang *et al*., 2010
8-Cl-ATP				Yang *et al*., 2009
3t			1,3-benzoazolyl-substituted pyrrolo[2,3-b]pyrazine derivatives	Li *et al*., 2016
Daurinol			Natural product	Wang *et al*., 2010
TSC24		K_d=18.3 μM compared to ATP (615 μM)	Thiosemicarbazone	Huang *et al*., 2010
Comp. 5		52.77 μM, K_i=75 μM	N-fused imidazole	Baviskar *et al*., 2011
Comp. 14			Xanthone	Jun *et al*., 2011

Table 2. Continued

Name	Structure	IC_{50}*	Type	Reference
Comp. 14mod			Xanthone	Park *et al*., 2013
Comp. 18			Naphthoquinone fused cyclic aminoalkyl-phosphonates and aminoalkyl-phosphonic monoester	Wang *et al*., 2008
Salvicine		K_d=74.3 μM	Natural product	Hu *et al*., 2006
D11		K_d=37.7 μM	Diphyllin glycoside	Gui *et al*., 2011
Gambogic acid		K_d=3.32 μM (SPR)	Natural product	Qin *et al*., 2007
Emodin			Anthraquinone analog	Li *et al*., 2010

*IC_{50} values for the compound otherwise noted; inhibition constant (K_i), dissociation constant (K_d).

ed into 8-Cl-ATP in cells and it competed with ATP to inhibit topo II (Yang *et al*., 2009).

There are some topo II inhibitors originated from natural products. Daurinol is a lignan isolated from *Haplophyllum dauricum*, whose structure is similar to etoposide (Kang *et al*., 2014). Etoposide is a well-known cytotoxic anti-cancer drug functioning as a topo II poison. Daurinol occupied the same binding site with AMPPNP, which was shown by molecular docking study, suggesting it inhibited topo II by targeting the ATPase domain. Gambogic acid (GA) is a natural product isolated from *Garcinia hanburi* tree. GA was shown to be a catalytic inhibitor of topo II by binding to the ATPase domain, determined by surface plasmon resonance (SPR) analysis and by molecular docking (Qin *et al*., 2007). Diphyllin was ex-

tracted from *Justicia procumbens*, which showed tumoricidal effects. D11 is a novel acetylated D-quinovose diphyllin analogue exhibiting potent topo II inhibitory activity and binding to the ATPase domain (Gui *et al*., 2011). When the compound binds to the ATP binding site it is not always a topo II catalytic inhibitor, as it is the case with salvicine and emodin. Salvicine is a derivative of diterpenequinone isolated from *Salvia prionitis*, which bound to the ATPase domain validated by SPR and molecular docking, and acted as a topo II poison generating double strand breaks (Hu *et al*., 2006). Emodin is an anthraquinone isolated from *Rheum emodi* and also from molds, lichens and fungi. Emodin also generated DNA double strand breaks and stabilized the topo II-DNA cleavage complex (Li *et al*., 2010).

Last group of topo II inhibitors are synthesized compounds designed from different scaffolds known to be biologically active or potent anti-cancer agents. Thiosemicarbazone (TSC) is one of the scaffolds, and their derivatives including TSC24 showed catalytic inhibition of topo II (Huang *et al*., 2010). TSC24 directly bound to the ATPase domain which was confirmed by competitive inhibition assay, SPR and molecular docking studies. Baviskar and coworkers designed and synthesized bicyclic N-fused aminoimidazole which had similar structure to reported topo II inhibitors and marketed drugs such as zolpidem and zolimidine (Baviskar *et al*., 2011). From the synthesized compounds, comp. 5 is a non-intercalating topo II catalytic inhibitor that bound to the ATP binding site. Compounds 14 and 14mod are xanthone derivatives that bound to the topo II ATPase domain, which was confirmed by ATPase competitive inhibition assay and molecular docking (Jun *et al*., 2011; Park *et al*., 2013). There were several topo II catalytic inhibitors containing quinone moiety that bound to the ATP binding site. Pyranonaphthoquinone, comp. 3a, was shown to be a topo II catalytic inhibitor and suggested to bind to the ATPase domain through docking (Jimenez-Alonso *et al*., 2008). Naphthoquinone fused cyclic aminoalkyl-phosphonates and aminoalkyl-phosphonic monoester were synthesized and tested for their topo II activity (Wang *et al*., 2008). Some of them including comp. 18 were catalytic topo II inhibitors and these compounds were docked into the ATP binding site of topo II (Ma *et al*., 2011).

Hsp90 inhibitors that bind to the N-terminal ATPase domain

Geldanamycin (GDA) and radicicol (RDC), antibiotic isolated from natural product (Roe *et al*., 1999) were the first discovered Hsp90 inhibitors that target the N-terminal ATPase domain. Due to poor solubility and hepatotoxicity of GDA and RDC, GDA and RDC derivatives were designed and synthesized to have good physical properties and stability with improved potency. 17-AAG is a GDA derivative that improved the toxicity and stability of GDA itself (Schulte and Neckers, 1998). The co-crystal structure of 17-DMAG and Hsp90 N-terminal ATPase domain was solved (Jez *et al*., 2003). GDA derivatives were also genetically engineered to produce GDA analogs, such as KOSN1559, showing better binding affinity than GDA (Patel *et al*., 2004).

Another group of Hsp90 inhibitors are RDC analogs. KF25706, KF29163 and KF58333 were chemically synthesized and their biological activities were assessed (Soga *et al*., 1999; Agatsuma *et al*., 2002). Various RDC analogs were further synthesized such as aigialmycin D, c-RDC, pochonin A, pochonin D and O-(piperidinocarbonyl) methyloxime derivative of RDC.

The GDA and RDC analogs are rather big in size and their poor properties in solubility and toxicity led to designing and synthesizing purine analogs for inhibiting Hsp90 by binding to the ATP binding site. PU3 is one of them and it competed with GDA for Hsp90 binding and when treated in cancer cells, HER2 level decreased (Chiosis *et al*., 2001). Other small Hsp90 inhibitors include pyrazole analogs such as CCT018159 and G3130. CCT018159 was searched from high-throughput screening compound collection of more than 56,000 compounds utilizing the ATPase activity assay (Rowlands *et al*., 2004). The crystal structure of G3130 bound to the N-terminal ATP binding domain of Hsp90 was solved and the value of K_d was 280 nM determined by SPR (Kreusch *et al*., 2005). SNX0723 is one of the synthetic compound having a novel scaffold containing benzamide moiety which was discovered to bind to the ATPase domain of Hsp90 by screening a compound library (Putcha *et al*., 2010). Resorcinol moiety was also identified to be an important scaffold for ATPase binding in Hsp90. AUY922, AT-13387 and CPUY201112 are the Hsp90 inhibitors that have resorcinol moiety which plays an important role in hydrogen bonding and hydrophobic interactions with the receptor (Dutta Gupta *et al*., 2014). Hsp90 inhibitors targeting its N-terminal ATP binding site reviewed in the current study are listed in Table 3.

Molecular docking studies

The similar molecular environment in the ATP binding sites of topo II and Hsp90 led us to assess whether reported inhibitors targeting either topo II or Hsp90 could function as a dual inhibitor. The listed topo II and Hsp90 inhibitors mentioned above were subjected for docking against both topo II and Hsp90. Tables 4 and 5 list the docking results of topo II and Hsp90, respectively. Surflex-Dock gives total score, crash and polar values for each of conformers. Generally, the inhibitors targeting their own binding partner scored high total score. Interestingly, the best scoring inhibitor for topo II was PU3, which was reported as an Hsp90 inhibitor with purine ring. The inhibitors showing good docking score for Hsp90 did not perform well with topo II ATP binding site. This may be due to smaller mouth opening in ATP binding pocket of topo II than Hsp90 which was calculated from CASTp. The typical Hsp90 inhibitors are bulkier compared to topo II inhibitors, therefore it would be difficult for bulky Hsp90 inhibitors to enter into the topo II ATP binding pocket.

PU3, 8-Cl-ATP and compound 3t in the docking of topo II, all showed high docking score which are all purine analogs. Fascinatingly, PU3, an Hsp90 inhibitor, scored the highest when docked to topo II. PU3 had hydrogen bonding interactions with Asn91, Asn120, Ala167 and Thr215, where they are key residues that formed hydrogen bonds with ATP (Fig. 4A). Also, PU3 had hydrophobic interactions with the residues comprising the ATP binding pocket, namely, Asn91, Asp94, Arg98, Asn120, Ile125, Ile141, Phe142, Ser149, Asn150, Thr159, Gly161, Arg162, Gly164, Ala167, Lys168 and Thr215. Compound 3t also occupied the ATP binding site and interacted with residues Asn91, Ala92, Asn95, Asn120, Pro126, Ile141, Phe142, Ser149, Gly164, Tyr165, Gly166, Ala167, Lys168, Thr215 and Ile217 (Fig. 4B). However, 3t had only one hydrogen bond interaction with residue Asn91. AUY922 is an Hsp90 inhibitor with isoxazole moiety. There are two

Table 3. Hsp90 inhibitors that bind to the ATPase domain

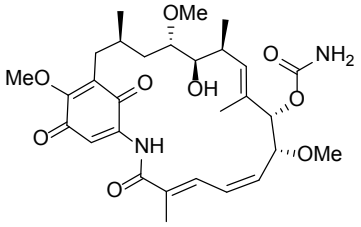
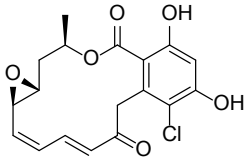
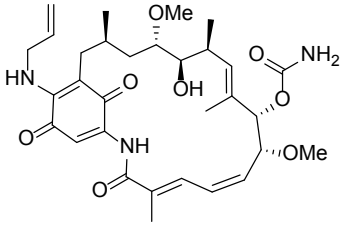
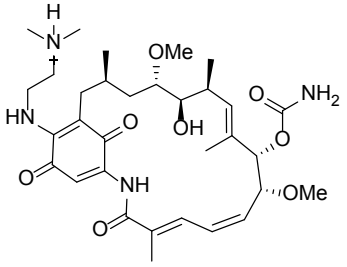
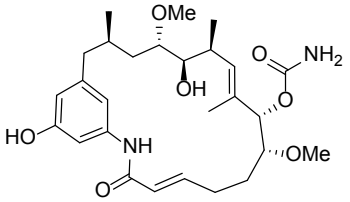
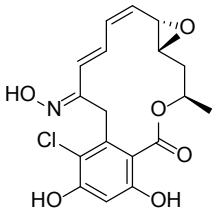
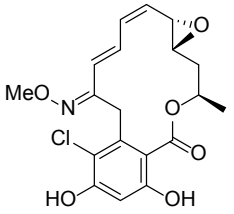
Name	Structure	IC_{50}	Type	Reference
GDA		K_d=1.2 μM (determined from isothermal calorimetry (ITC))		Roe *et al*., 1999
Radicicol		23 nM, K_d=19 nM (ITC)		Roe *et al*., 1999
17-AAG			Geldanamycin derivative	Schulte and Neckers, 1998
17-DMAG			Geldanamycin derivative	Jez *et al*., 2003
KOSN1559		K_d=16 nM	Geldanamycin derivative	Patel *et al*., 2004
KF25706			RDC analog	Soga *et al*., 1999
KF29163			RDC analog	Agatsuma *et al*., 2002

Table 3. Continued

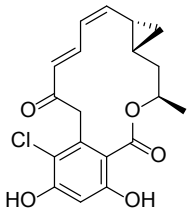
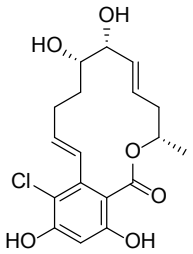
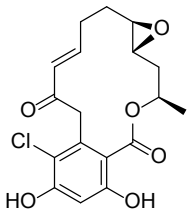
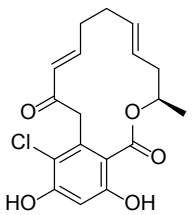
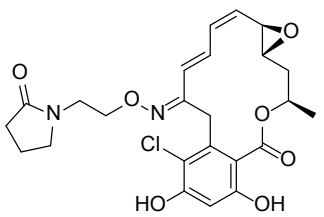
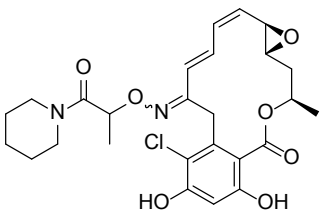
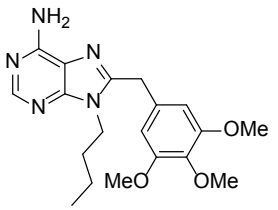
Name	Structure	IC_{50}	Type	Reference
c-RDC			RDC analog	Yang *et al*., 2004
Aigialmycin D			RDC analog	Yang *et al*., 2004
Pochonin A		90 nM	RDC analog	Moulin *et al*., 2005a
Pochonin D			RDC analog	Moulin *et al*., 2005b
KF58333			RDC analog	Soga *et al*., 2001
o-(piperidinocarbonyl) methyloxime derivative of RDC			RDC analog	Ikuina *et al*., 2003
PU3		K_d=15~20 μM	Purine derivative	Chiosis *et al*., 2001

Table 3. Continued

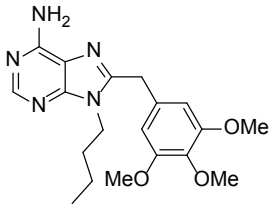
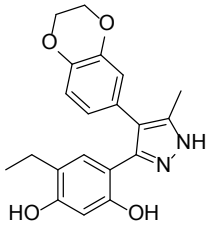
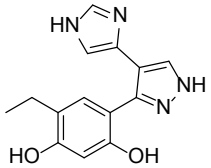
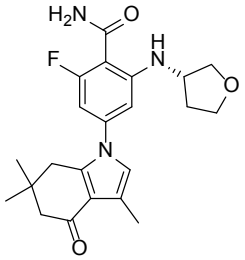
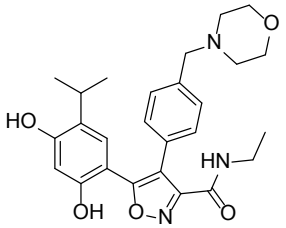
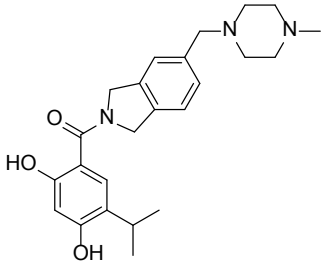
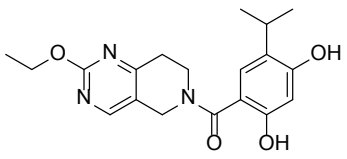
Name	Structure	IC_{50}	Type	Reference
PU3		K_d=15~20 μM	Purine derivative	Chiosis *et al*., 2001
CCT018159		8.9 μM	Pyrazole	Rowlands *et al*., 2004
G3130		K_d=280 nM (SPR)	Pyrazole	Kreusch *et al*., 2005
SNX0723			Benzamide	Putcha *et al*., 2010
AUY922			Resorcinol	Brough *et al*., 2008
AT-13387			Resorcinol	Murray *et al*., 2010
CPUY201112			Resorcinol	Xu *et al*., 2016

Table 4. Topo II docking results of combined inhibitors

Name	Target	Total Score[1]	Crash[2]	Polar[3]	Similarity[4]
PU3	Hsp90	13.1154	-0.9961	4.5667	0.541
8-Cl-ATP	Topo II	11.2764	-2.0944	11.0551	0.527
3t	Topo II	10.4766	-0.6886	1.7276	0.397
Comp. 14mod	Topo II	10.2868	-2.5358	3.0286	0.407
Comp. 14	Topo II	9.927	-2.1249	3.0481	0.427
AUY922	Hsp90	9.8884	-3.5617	3.1905	0.463
Salvicine R	Topo II	8.5495	-2.5071	2.9333	0.333
CCT018159	Hsp90	8.0916	-0.9934	2.739	0.382
SNX0723	Hsp90	8.034	-5.2877	2.8872	0.443
NSC348400	Topo II	7.9712	-3.1936	5.9702	0.568
Comp. 2	Topo II	7.9074	-0.4726	2.1046	0.418
Daurinol	Topo II	7.564	-1.1849	4.962	0.459
Comp. 5	Topo II	7.4758	-1.6766	2.5831	0.404
QAP1	Topo II	7.3683	-2.2725	2.9142	0.372
G3130	Hsp90	7.1866	-0.3941	3.5543	0.336
Comp. 1	Topo II	7.1634	-1.3298	3.7455	0.235
Salvicine S	Topo II	7.0436	-2.892	3.1112	0.461
NSC35866	Topo II	6.9393	-1.0221	2.0358	0.458
KF58333	Hsp90	6.7973	-4.5147	2.4482	0.380
CPUY201112	Hsp90	6.7935	-2.242	1.4817	0.528
AT13387	Hsp90	6.2906	-9.1331	4.0913	0.415
Emodin	Topo II	6.279	-0.6391	3.2784	0.440
2c	Topo II	5.9724	-5.5461	0.0714	0.342
Pochonin D	Hsp90	5.8197	-3.0107	1.6854	0.406
o-RDC	Hsp90	5.743	-6.3103	1.714	0.393
Pochonin A	Hsp90	5.6544	-3.0449	1.7182	0.431
c-RDC	Hsp90	5.571	-3.8203	2.7142	0.353
Comp. 18	Topo II	5.2181	-0.9817	2.1991	0.381
KF25706	Hsp90	4.9578	-3.5256	2.2759	0.467
KF29163	Hsp90	4.8178	-2.5315	2.4504	0.406
RDC	Hsp90	4.2342	-2.6456	1.1539	0.421
Aigialomycin D	Hsp90	4.1481	-4.3331	4.1964	0.433
TSC24	Topo II	4.1158	-0.8441	0.0514	0.392
Gambogic acid	Topo II	3.8992	-7.3216	0.844	0.385
D11	Topo II	-1.4678	-14.195	1.1678	0.361
KOSN1559	Hsp90	-2.5462	-14.8183	2.1768	0.428
GDM	Hsp90	-4.9652	-18.4653	2.1288	0.358
17-AAG	Hsp90	-6.3308	-17.5419	0.7688	0.478
17-DMAG	Hsp90	-8.4678	-21.6041	1.4689	0.508

[1]Total Score represents the total Surflex-Dock score expressed as –log(K_d), [2]Crash is the degree of inappropriate penetration by the ligand into the protein between ligand atoms that are separated by rotatable bonds. Crash scores close to 0 are favorable, [3]Polar values show the contribution of the polar interactions to the total score, [4]Similarity indicates the difference between the top scoring pose and the original ligand (AMPPNP) used as the reference.

hydroxyl substituents from the phenyl ring and amide group that can form hydrogen bonds with residues Asn95, Asn120 and Ser149. Since AUY922 is rather big molecule compared to PU3, 3t, comp. 14 and 8-Cl-ATP, larger number of residues are involved in van der Waals interaction, namely, Ile88, Asn91, Ala92, Asn95, Arg98, Ile118, Asn120, Il2125, Pro126, Ile141, Phe142, Ser149, Asn150, Gly161, Gly164, Tyr165, Gly166, Ala167, Lys168, Thr215 and Ile217. Recently from AstraZeneca, compound with benzisoxazole scaffold, ETX0914, was discovered as a novel DNA gyrase inhibitor undergoing phase II clinical trial for the treatment of uncomplicated gonorrhea (Basarab *et al*., 2015). There is no known topo II inhibitor reported with isoxazole scaffold to the best of our knowledge. Therefore our docking results suggest AUY922 may act as a topo II inhibitor with novel scaffold. Comp. 14 is a small compound with xanthone moiety which competed with ATP. Comp. 14 had hydrogen bond interactions with Asp94 and Thr215 and hydrophobic interactions with Asn51, Ser52, Ala55, Asp93, Ile96, Gly97, Met98, Asn106, Phe138, Val150, Thr184 and Val186.

In the case of docking topo II and Hsp90 inhibitors to Hsp90, the bulky Hsp90 inhibitors were high in rank as mentioned above. However, AUY922, a rather smaller isoxazole derivative compared to classical Hsp90 inhibitors such as

Table 5. Hsp90 docking results of combined inhibitors

Name	Target	Total Score	Crash	Polar	Similarity
AUY922	Hsp90	11.3281	-2.9912	5.8905	0.566
KOSN1559	Hsp90	10.5386	-4.2057	6.0038	0.497
GDM	Hsp90	9.0405	-4.3587	5.7304	0.506
3t	Topo II	8.9981	-1.9006	2.2646	0.451
KF58333	Hsp90	8.8035	-2.216	3.7782	0.475
PU3	Hsp90	8.6779	-1.3914	2.3162	0.465
Comp. 14	Topo II	8.0945	-2.9386	4.3295	0.547
o-RDC	Hsp90	7.4652	-3.5968	3.1916	0.421
CCT018159	Hsp90	7.4339	-0.7895	3.2601	0.521
SNX0723	Hsp90	7.1997	-2.4905	1.3399	0.526
Salvicine S	Topo II	7.1362	-1.4093	1.6745	0.475
AT13387	Hsp90	7.1259	-2.0725	1.7217	0.519
Comp. 14mod	Topo II	7.0102	-2.3685	3.184	0.519
G3130	Hsp90	6.8551	-0.5655	4.089	0.514
QAP1	Topo II	6.7837	-2.2874	0.9711	0.497
CPUY201112	Hsp90	6.7467	-2.5928	2.7711	0.612
NSC348400	Topo II	6.7386	-1.4421	3.7083	0.511
RDC	Hsp90	6.6884	-2.5576	3.4177	0.666
NSC35866	Topo II	6.649	-1.1619	2.6058	0.486
Salvicine R	Topo II	6.4918	-2.4288	2.1968	0.510
Comp. 1	Topo II	6.4641	-1.627	2.2857	0.561
2c	Topo II	6.4036	-3.4089	0.0014	0.329
KF25706	Hsp90	6.185	-2.8315	3.6404	0.647
Gambogic acid	Topo II	6.0092	-2.2055	1.3905	0.297
Comp. 2	Topo II	5.952	-1.2823	1.4835	0.524
Daurinol	Topo II	5.8292	-0.4604	1.3936	0.556
KF29163	Hsp90	5.7498	-2.3134	1.6213	0.530
17-DMAG	Hsp90	5.718	-3.6373	1.0756	0.318
c-RDC	Hsp90	5.6849	-3.5861	3.0525	0.691
Comp. 5	Topo II	5.6657	-1.6574	1.9241	0.430
D11	Topo II	5.6512	-0.8901	2.5246	0.390
Emodin	Topo II	5.6113	-0.429	1.9987	0.398
8-Cl-ATP	Topo II	5.5933	-1.2132	4.7994	0.399
Pochonin D	Hsp90	5.5041	-1.5103	2.1344	0.550
TSC24	Topo II	5.477	-1.2353	0.5795	0.418
Pochonin A	Hsp90	5.4494	-1.3695	2.6113	0.279
17-AAG	Hsp90	5.2911	-2.6657	0.545	0.301
Aigialomycin D	Hsp90	4.8757	-3.236	3.4461	0.549
Comp. 18	Topo II	4.553	-0.4098	0.9114	0.427

GDM or RDC, scored highest. AUY922 also had hydrogen bond interactions with five residues in the ATP binding site of Hsp90, Asn51, Lys58, Asp93, Gly97 and Phe138. The residues involved in hydrophobic interactions are Asn51, Ala55, Lys59, Asp93, Ile96, Gly97, Met98, Asp102, Leu107, Gly135, Val136, Gly137, Phe138, Val150, Thr184 and Val186. PU3, a purine analog Hsp90 inhibitor showed good result in Hsp90 along with 3t, another purine analog topo II inhibitor.

Two purine derivatives of PU3 and 3t and two non-purine compounds of AUY922 and comp. 14 were selected for further comparison in depth since they ranked high in docking study of both topo II and Hsp90. The binding interactions of topo II and Hsp90 with compounds of PU3, 3t, AUY922 and 14 are shown in Fig. 4, 5. The hydrogen bonding residues are labeled and the bonds are displayed as light blue dashed lines. The selected common four compounds and high scoring compounds 8-Cl-ATP and KOSN1599 for topo II and Hsp90, respectively, were analyzed in detail. Table 6 summarizes the residues involved in hydrophobic and hydrogen bond interactions.

Pharmacophore model analysis

Compounds PU3, 3t, AUY922 and comp. 14 were further evaluated for their dual targeting features by generating pharmacophore models. The pharmacophore model was generated using Genetic Algorithm Similarity Program (GASP) module implemented in Sybyl X-2.1.1. From the four compounds, four models were generated by GASP. The fitness score for each model ranged from 2589.82 to 2689.23 and model 2 was chosen as the best model (Table 7). Model 2 consists of two hydrophobic regions (HY, cyan), one acceptor atom (AA, green) and one donor site (DS, green) as shown in Fig. 6 with

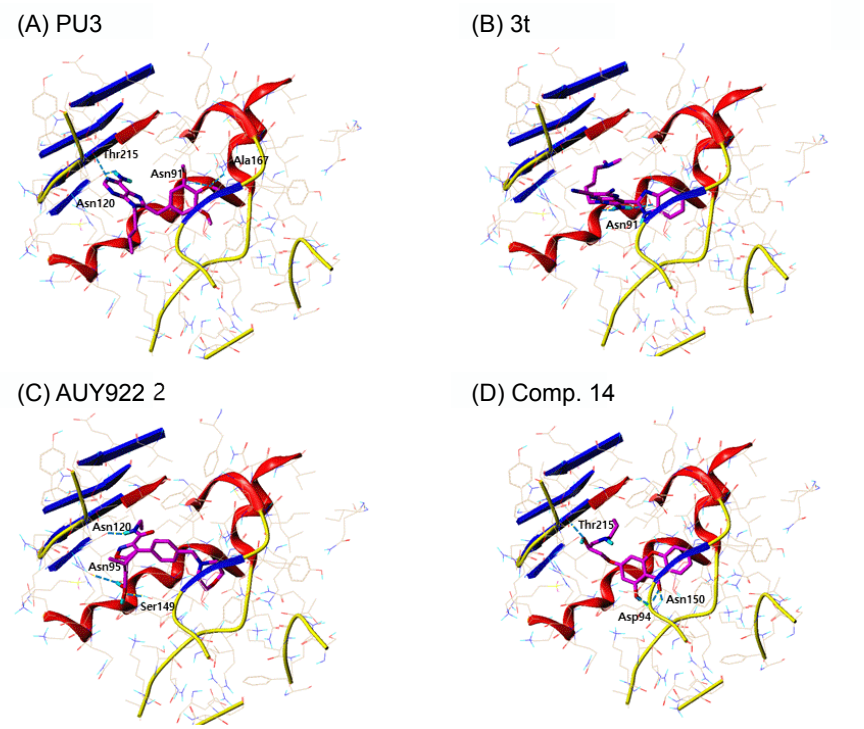


Fig. 4. The docking result of the selected inhibitors against topo II. The ATP binding site of topo II with inhibitors (A) PU3, (B) 3t, (C) AUY922 and (D) Comp. 14. The ligands are represented in sticks colored by atom type (magenta: carbon; red: oxygen; blue: nitrogen; orange: phosphorus) and the residues involved in hydrogen bonds are shown in dotted line colored in cyan.

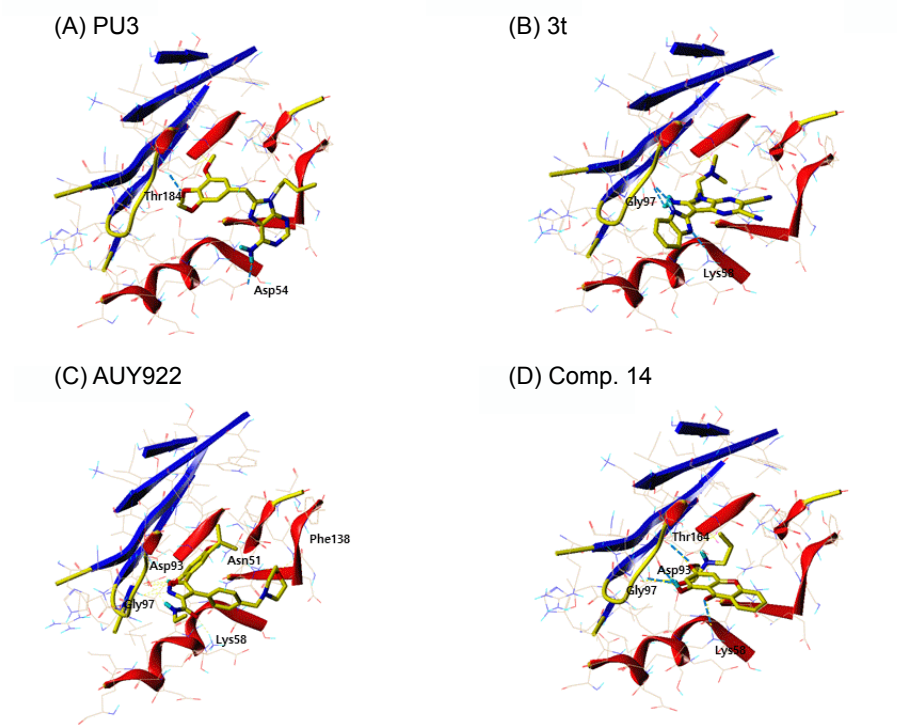


Fig. 5. The docking result of the selected inhibitors against Hsp90. The ATP binding site of topo II with inhibitors (A) PU3, (B) 3t, (C) AUY922, and (D) Comp.14. The ligands are represented in sticks colored by atom type (yellow: carbon; red: oxygen; blue: nitrogen; orange: phosphorus) and the residues involved in hydrogen bonds are shown in dotted line colored in cyan.

Table 6. Docking analysis of selected inhibitors

Topo II			Hsp90		
Name	Hydrophobic	Hydrogen bonding	Name	Hydrophobic	Hydrogen bonding
PU3	Asn91, Asp94, Arg98, Asn120, Ile125, Ile141, Phe142, Ser149, Asn150, Thr159, Gly161, Arg162, Gly164, Ala167, Lys168, Thr215	Asn91, Asn120, Ala167, Thr215	PU3	Asn51, Asp54, Ala55, Asp93, Ile96, Gly97, Met98, Asn106, Leu107, Gly135, Val136, Phe138, Val150, Thr184, Val186	Asp54, Thr184
3t	Asn91, Ala92, Asn95, Asn120, Pro126, Ile141, Phe142, Ser149, Gly164, Tyr165, Gly166, Ala167, Lys168, Thr215, Ile217	Asn91	3t	Asn51, Ala55, Lys58, Ile96, Gly97, Met98, Asn106, Phe138, Val150, His154, Thr184, Val186	Lys58, Gly97
Comp. 14	Ile88, Asn91, Ala92, Asp94, Asn95, Ile118, Asn120, Ile125, Asn150, Gly161, Gly164, Tyr165, Ala167, Lys168, Thr215, Ile217	Asp94, Thr215	Comp. 14	Asn51, Ser52, Ala55, Asp93, Ile96, Gly97, Met98, Asn106, Phe138, Val150, Thr184, Val186	Asp93, Gly97, Thr184
AUY922	Ile88, Asn91, Ala92, Asn95, Arg98, Ile118, Asn120, Ile125, Pro126, Ile141, Phe142, Ser149, Asn150, Gly161, Gly164, Tyr165, Gly166, Ala167, Lys168, Thr215, Ile217	Asn95, Asn120, Ser149	AUY922	Asn51, Ala55, Lys58, Asp93, Ile96, Gly97, Met98, Asp102, Leu107, Gly135, ValL136, Gly137, Phe138, Val150, Thr184, Val186	Asn51, Lys58, Asp93, Gly97, Phe138
8-Cl-ATP	Asn91, Asp94, Asn95, Arg98, Lys123, Gly124, Ile125, Ser149, Asn150, Gly161, Arg162, Asn163, Gly164, Tyr165, Gly166, Ala167, Gln376, Lys378	Asn91, Asp94, Asn150, Arg162, Tyr165, Gly166, Lys378	KOSN1599	Asn51, Ser52, Asp54, Ala55, Lys58, Asp93, Ile96, Met98, ASP102, Asn106, Leu107, Phe138, Thr184, Val186	Ser52, Asp54, Phe138

Table 7. Results of pharmacophore hypothesis generated by GASP

Model	Fitness	Size[a]	Hits[b]	Dmean[c]	Features[d]
1	2676.46	4	4	5.7693	DS, AA, HY1, HY2
2	2689.23	4	4	3.5916	DS, AA, HY1, HY2
3	2589.82	4	4	3.1774	DS, AA, HY1, HY2
4	2663.25	2	4	4.5547	HY1, HY2

[a]Number of features in the model, [b]Number of molecules that matched during the search, [c]Average interpoint distance, [d]Pharmacophore features. DS: donor site, AA: acceptor site, HY: hydrophobic.

PU3 as the template. The two hydrophobic regions are about 5 Å apart and the hydrophobic region 1 and the acceptor atom is 2.5 Å apart. The pharmacophore model suggested here can be used as a template to further optimize the design of the dual inhibitor of topo II and Hsp90.

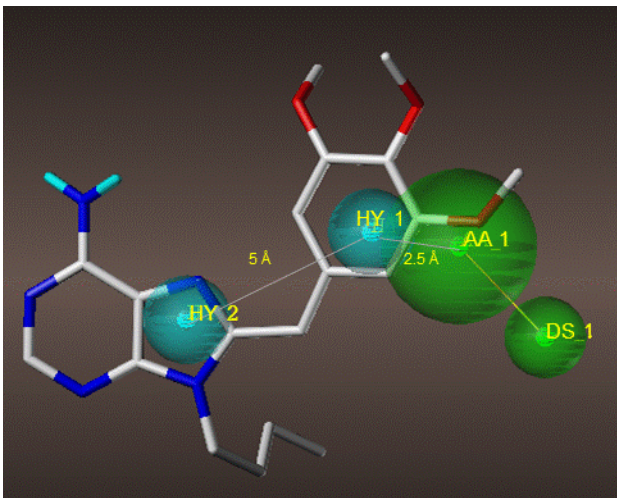


Fig. 6. The pharmacophore model 2 generated from GASP. The pharmacophore features are two hydrophobic regions (HY, cyan), one acceptor atom (AA, green) and one donor site (DS, green) with PU3 as the template represented in sticks colored by atom type (gray: carbon; light blue: hydrogen; red: oxygen; blue: nitrogen; orange: phosphorus).

CONCLUSIONS

In this study, the inhibitors reported to target each ATPase domain of human topo II and Hsp90 were investigated. The structures of ATPase domains of topo II and Hsp90 were compared to evaluate how similar the environment of the receptor sites were. The topo II and Hsp90 inhibitors known to target the ATP binding site were searched and the possibility to function as a dual inhibitor was investigated *in silico*. All the inhibitors searched were docked to both topo II and Hsp90. Through the analysis of docking results, four candidate compounds were selected as possible dual inhibitors. These compounds were used as a template to generate pharmacophore model. This suggested pharmacophore model will be useful in developing dual inhibitor of topo II and Hsp90 by constructing 3D query for virtual screening using publically available database such as ZINC (http://zinc.docking.org/).

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