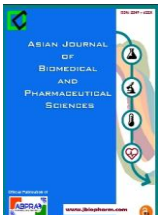
Asian Journal of Biomedical and Pharmaceutical Sciences
ISSN : 2249 - 622X



RESEARCH ARTICLE

Received on: 19-11-2013
Accepted on: 01-02-2014
Published on: 16-02-2014

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Sources of support:
DBT-BIF (Bioinformatics facility)

Conflict of Interest: None Declared !

In Silico Interaction Studies on Inhibitory Action of endophytic fungal Diketopiperazine and its related compounds on Heat-Shock Protein 90 (Hsp90)

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Abstract

In silico interaction studies of twenty diketopiperazines (DKPs) that include the endophytic fungal diketopiperazine; hexahydropyrrollo[1,2-a]pyrazine-1,4-dione and its related compounds were screened against the cancer protein Hsp90 using Autodock Vina. The docking scores indicate that of the compounds screened, 4,4'-(1,2-dimethyl-1,2-ethanediyl)bis-2,6-piperazinedione was most potent with docking score of -7.5 Kcal/mol. This value was better than the standard drugs; Geldanamycin, 17-(Allylamino) Geldanamycin and Alvespimycin (17DMAG). Therefore, this study recommends the consideration of Diketopiperazines for further *in vitro* and *in vivo* studies towards its development as anticancer drug.

Keywords: endophytic fungal, diketopiperazine, Hsp90, docking score

Cite this article as:
Robin Sharma, B. S. Vijaya Kumar. *In Silico* Interaction Studies on Inhibitory Action of endophytic fungal Diketopiperazine and its related compounds on Heat-Shock Protein 90 (Hsp90). Asian Journal of Biomedical and Pharmaceutical Sciences; 04 (28); 2014; 25-29.

INTRODUCTION

Endophytic fungi produce diverse metabolites having tremendous biomedical relevance that include, anti-cancer, anti-fungal, anti-diabetic and immunosuppressant compounds [1]. The low-molecular weight secondary metabolites are produced by organisms in response to environmental abiotic and biotic stress [2]. Of the galore of fungi that produce anti-cancer molecules, fungi belonging to the genus *Penicillium* have also been reported to produce compounds with anti-cancer activity [3]. Heat-shock protein 90 (Hsp90) is an ATP-dependent molecular chaperone exploited by malignant cells to support activated oncoproteins [4]. Thereby, its implications on cancer have led to the emergence of its being considered as a promising target for anti-cancer drugs [5]. Though originally viewed with skepticism, Hsp90 inhibitors are now actively pursued by the pharmaceutical industry, with 17 agents having entered clinical trials [6] with natural products Geldanamycin (GA) and Radicicol leading the pioneering pursuit. Recently, Centenera *et al.,* [7] reported that new generation Hsp90 inhibitors are capable of achieving biological responses in human prostate tumor, with both NVP-AUY922 and NVP-Hsp90 showing potent on-target efficacy. However, many efforts are still needed in understanding the administration of these agents and also the search and synthesis of newer, safer and more potent molecules [8]. Barril *et al.,* [9] and Kim *et al*., [10] in their study reported piperazines as potent Hsp90 inhibitors. Hence, with the results of the GC-MS analysis in our previous study (unpublished data) that highlighted the production of a diketopiperazine by an endophytic fungus *Penicillium* sp., we endeavored to identify the efficacy of the endophytic fungal diketopiperazine (DKP) and its related compounds as inhibitor of human Hsp90 using *in silico* docking methods.

MATERIALS AND METHODS

Retrieval of protein structure:

The target Hsp90-Alpha (resolution - 1.90Å) bound to the ligand 2D7 (PDB ID: 2BYH) was retrieved from the protein data bank (PDB). Bound water molecules and ligand 2D7 were removed; thenafter, charges and H-atoms were added to the receptor molecule using Autodock tools. Standard compounds like; Radicicol, Geldanamycin, 17-(Allylamino) Geldanamycin (17-AAG) and Alvespimycin (17-DMAG) known to have good inhibitory potential against the same protein were also docked to compare the effectiveness of the secondary metabolites. Novobiocin sodium was used as a negative control.

Compounds screened:

A total of twenty diketopiperazines (DKPs) that include the endophytic fungal diketopiperazine; hexahydropyrrollo[1,2-a]pyrazine-1,4-dione and its related compounds were screened against the cancer protein Hsp90. The 3D structures of the standard ligands and the related compounds were collected from the ZINC (http://zinc.docking.org) and the Pubchem (http://pubchem.ncbi.nlm.nih.gov) databases. The ligands were energy minimized using MOE (Molecular Operating Environment). The partial charges on atoms were assigned after adding the polar hydrogens.

Drug likeliness prediction:

Ligand property was predicted by using "Lipinski Drug Filters" (http://www.scfbio-iitd.res.in/utility/LipinskiFilters.jsp). Lipinski rule of five helps in distinguishing drug-like and non-drug-like properties and predicts high probability of success or failure due to drug likeliness for molecules. The Lipsinki filter helps in early preclinical assessment and thereby avoiding costly late-stage preclinical and clinical failures.

Protein ligand interaction using Autodock Vina:

Virtual screening of the Ligand-protein interaction for their binding affinity was carried out using AutoDock Vina [11] and the results that include the understanding of association that involves H-bonding and hydrophobic interactions were analyzed using LIGPLOT+ a programme to generate schematic diagrams of protein ligand interactions [12].

RESULTS AND DISCUSSION

The docking interaction of the protein and the ligand, the predicted ligand binding site residues and binding energies are listed in Table 1 and depicted in Fig. 1 respectively. Of the twenty compounds studied, related ligands, 4,4'-(1,2-dimethyl-1,2-ethanediyl)bis-2,6-piperazinedione and Razoxane were the most potent with least docking score of -7.5 Kcal/mol and -7.4 Kcal/mol respectively. The fungal metabolite hexahydropyrrolo[1,2-a]pyrazine-1,4-dione had the docking score of -6.0 Kcal/mol. Binding energy is associated with the probability of affinity and stable bound between receptor and its ligand, and also predicts the bioactivity value of a ligand with the corresponding receptor [13].

A schematic representation of the interacting residues for 4, 4'-(1, 2-dimethyl-1, 2-ethanediyl) bis-2, 6-piperazinedione, obtained from LIGPLOT+ is depicted in Fig. 2.

As depicted in Table 1 and Fig. 2, Gly 135 is the most favored residue for binding of most of the compounds.

S.No.	Name of the Ligand	ZINC ID	BE*	H-bonding Interaction	Hydrophobic interaction
1	Radicicol	45789132	-8.3	Asn51	Phe134,Gly132,Gly135,Val136,Lys112
2	Geldanamycin	53683707	-5.5	Gly137	Ile131,Phe134,Asp54,Lys112
3	17-(Allylamino) Geldanamycin	49792055	-4.7	Nil	Gly135,Ser113,Lys112
4	Alvespimycin (17DMAG)	53684152	-5.8	Nil	Gly135,Ser113,Lys112
5	novobiocin sodium	14879999	-0.7	Nil	Asn105,Gly97,Met98
6	4,4'-(1,2-dimethyl-1,2-ethanediyl)bis-2,6-piperazinedione	119081†	-7.5	Gly132	Gln133,Phe134,Val136,Lys112
7	Razoxane	30623†	-7.4	Gly132	Phe134,Gln133,Gly135
8	1-[(2S)-2-(4-methylpiperazine-1-carbonyl)pyrrolidin-1-yl]butan-1-one	47975362	-6.5	Nil	Val136,Phe118,Lys116,Ser113,Ala117
9	N-{2-oxo-2-[2-(piperazine-1-carbonyl)pyrrolidin-1-yl]ethyl}acetamide	40508264	-6.5	Nil	Gly135,Gly114,Lys112
10	N-methyl-1-[2-(piperazin-1-yl)acetyl]pyrrolidine-2-carboxamide	37826762	-6.3	Met130	Ser113,Gly114,Ile131,Gly132,Gly135
11	2-[4-[(2S)-1-butanoylpyrrolidine-2-carbonyl]piperazin-1-yl]-N-[(1S)-1-methylpropyl]acetamide	55180397	-6.3	Nil	Val136,Thr115,Gly114,Ile26,Ile110,Thr109,Ser113,Phe134
12	N-cyclopropyl-1-[2-(piperazin-1-yl)acetyl]pyrrolidine-2-carboxamide	37806344	-6.3	Gly135	Gly114,Ser113,Phe134,Gln133,Val136
13	(8aR)-3-butylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione	72233338	-6.2	Gly137	Phe134,Tyr139
14	BLAHdione	12410569	-6.1	Nil	Ser113,Gly114,Gly135,Phe134,Gln133,Lys112
15	BLAHquinone	161231	-6.1	Nil	Gly135
16	1-(2-aminopropanoyl)-N-methylpyrrolidine-2-carboxamide	37826668	-6.0	Nil	Gly135
17	(3S)-3-propylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione	66346808	-6.0	Nil	Gly114
18	Hexahydropyrrolo[1,2-a]pyrazine-1,4-dione	402826	-6.0	Nil	Gly135
19	3-ethylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione	39116552	-6.0	Nil	Ser113
20	N,N-dimethyl-1-[2-(propan-2-ylamino)acetyl]pyrrolidine-2-carboxamide	37609820	-5.9	Nil	Gly135,Asn106
21	1-acetyl-N,N-dimethylpyrrolidine-2-carboxamide	70224924	-5.8	Nil	Gln133,Ser113,Gly114,Phe134
22	N,N-dimethyl-1-[2-(piperazin-1-yl)acetyl]pyrrolidine-2-carboxamide	37631254	-5.8	Nil	Gly135,Phe134,Gln133,Ser113
23	2-methyl-octahydropyrrolo[1,2-a]piperazine-1,4-dione	34235455	-5.7	Nil	Gly135,Lys112,Ser113
24	N,N-dimethyl-1-[2-(methylamino)propanoyl]pyrrolidine-2-carboxamide	54072455	-5.6	Nil	Gly132,Gly135,Phe134
25	N-methyl-1-[2-(methylamino)acetyl]pyrrolidine-2-carboxamide	37826693	-5.6	Nil	Gly135,Phe134,Gly114

Table 1. Binding energy and the amino acid residues participating in the hydrogen bonding interaction and hydrophobic interactions obtained using LIGPLOT+.

BE* represents binding energy (Kcal/mole); †PUBCHEM ID

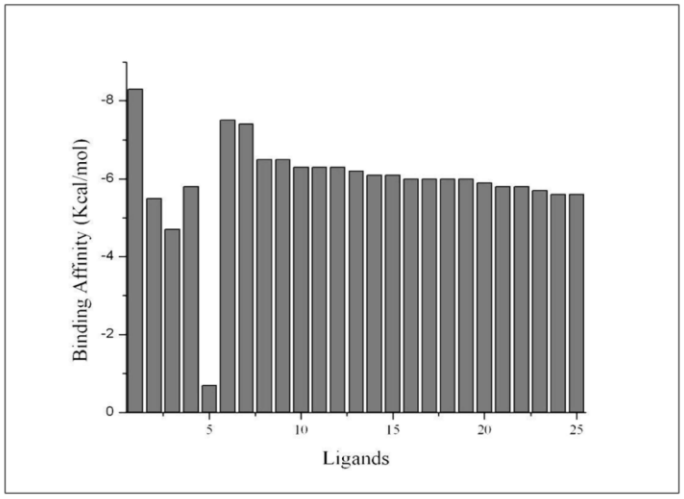

Figure 1. Binding affinity (Kcal/mole) of various ligands studied (1-25)

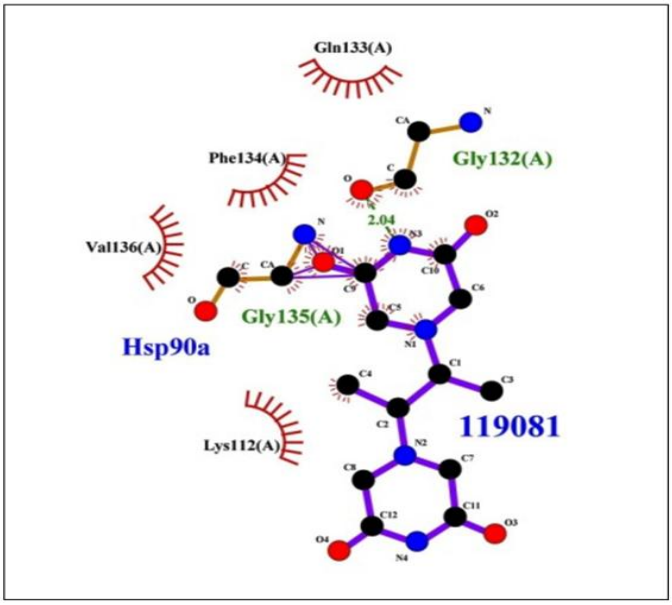

Figure 2. The interaction of amino acid residues in Hsp90 with 4,4'-(1,2-dimethyl-1,2-ethanediyl)bis-2,6-piperazinedione

Though, the binding affinity of potent diketopiperazines; 4,4'-(1,2-dimethyl-1,2-ethanediyl)bis-2,6-piperazinedione, Razoxane and hexahydropyrrolo[1,2-a]pyrazine-1,4-dione in this study were lesser in comparison to Radicicol, they showed stronger binding affinity than potent drugs Geldanamycin and its synthetic derivative 17-(Allylamino) Geldanamycin (17-AAG) and Alvespimycin (17DMAG). Lesser the docking score more is the binding capacity of the ligand [2]. Molecular visualization of interaction between 4,4'-(1,2-dimethyl-1,2-ethanediyl)bis-2,6-piperazinedione with active site amino acid residues in the N-terminal of Hsp90 is depicted in Fig. 3.

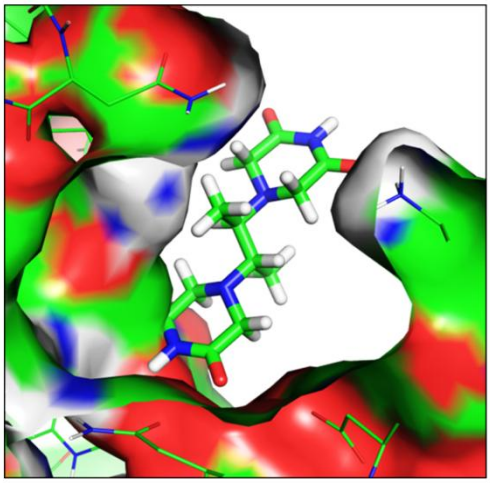

Figure 3. Molecular visualization of interaction between ligand (119081) and the target protein Hsp90

CONCLUSION

The present study on the basis of docking scores indicate that diketopiperazine produced by endophytic fungus *Penicillium* sp., hexahydropyrrolo[1,2-a]pyrazine-1,4-dione and its related compounds: 4,4'-(1,2-dimethyl-1,2-ethanediyl)bis-2,6-piperazinedione and Razoxane could be potent inhibitors against cancer chaperone Hsp90 and therefore can be considered for *in vitro* and *in vivo* analysis towards development of drugs which may act as Hsp90 inhibitors.We anticipate that future studies on functional and mechanistic aspects will further accelerate our understanding on the efficacy of these compounds as inhibitors of Hsp90.

ACKNOWLEDGEMENTS

Authors dedicate this study with gratitude to the founder Chancellor, Sri Sathya Sai Institute of Higher Learning, Bhagawan Sri Sathya Sai Baba. Authors are also grateful to Dr. S. Venketesh, Department of Biosciences, Sri Sathya Sai Institute of Higher Learning for his guidance and DBT-BIF for providing Bioinformatics facility.

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