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Virtual Screening and ADMET studies to identify KSP inhibitors as anticancer therapeutics

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Abstract

To report the virtual screening of several series of Indolo Pyrimidine derivatives for in silico KSP inhibition to arrive at possible potential inhibitors of KSP with acceptable pharmacokinetic or ADMET properties.

In order to identify potential inhibitors we employed various computational approaches. In this work, we computationally screened and analyzed 60 analogs and further tested their ADME/T profiles. Library of the molecules was constructed based upon structural modifications of pyrimidines and indole nucleus. Structural modifications were performed for the series of 4-(3-hydroxyphenyl)-6-methyl-2-oxo-N-substituted[(Z)-(2-oxoindolin-3-ylidene)amino]-3,4-dihydro-1H-pyrimidine-5-carboxamide derivatives in an order to get better binding energies as compared with Ispinseb. The molecules with better (lower) binding energies were subjected to predict ADMET properties.

Ten molecules from the series IP1-IP60 were found acceptable with binding energies and pharmacokinetic properties. On the basis of the binding energies and ADMET properties we have identified compound IP2 and IP4 to be the best interacting molecules. The molecules with acceptable ADMET properties and better binding energies were prioritized for synthesis and anticancer evaluation.

The binding energies and ADMET of the drugs provided suggests the protein ligand binding interactions that can aid in the design and synthesis of more potential inhibitors.

Keywords: KSP inhibitors, virtual screening, ADMET and Indolo Pyrimidines.

Introduction

Kinesin spindle protein (KSP), also known as Hs Eg5 has emerged as a promising target for a new generation antimitotic chemotherapeutic agents, it 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].

Ispinesib has emerged as a potent KSP inhibitor with sub nanomolar Ki having a quinazolinone core is an experimental KSP inhibitor from Cytokinetics which has already entered clinical trials.The “Biginelli reaction” that leads to dihydropyrimidines is one of the most famous Multi Component Reactions (MCRs). It was first reported by the Bignelli in 1893 who obtained the important multifunctionalized heterocycle by refluxing a mixture of an aldehyde, a b-ketoester, and urea in the presence of an acid catalyst. This reaction was named as Biginelli reaction and the product as Biginelli product.

Inhibition of KSP prevents normal bipolar spindle formation, which leads to mitotic arrest with a characteristic monastrol phenotype and subsequently to apoptosis in transformed cells. 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 the microtubules [4-6].

The discovery of monastrol, a small, specific, and cell permeable KSP inhibitor, led to several other small molecules as KSP inhibitors. It was reported by Mayer in 1999, considered to be the first small molecule. It targets KSP and led to mitotic arrest. The crystal structure KSP complex with Mg2+ and ADP has been determined by Turner et al. the binding mode of the monastrol was then reported in detail by Yan et al. after discovering monastrol several classes of compounds have been reported. This makes it to possible to make a binding mode analysis for KSP inhibitors. In the present report we have designed Biginelli compound analogous to Monastrol with different isatins. Only 10 compounds out of 100, which were designed and studied for 2D QSAR and ADMET were identified as potent through virtual screening [7-10].

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Molecular modeling

Designing of the molecules and library design

A library of the molecules was generated by performing structural modifications in descending order on the series of Indolo-Pyrimidine molecules. Structural modifications were carried out on pyrimidines and Indoles and molecules were generated in 3D. This generated set of 60 ligands from series 4-(3-hydroxyphenyl)-6-methyl-2-oxo-N-[(Z)-(2-oxo-substituted indolin-3-ylidene)amino]-3,4-dihydro-1H-pyrimidine-5-carboxamides are utilized to carryout virtual screening.

Methods

Computational methods

Discovery studio visualization program accelrys [11] is utilized to visualize the receptors, ligand structures, hydrogen bonding network, to calculate length of the bonds and to render images. Biovia Draw is used to draw the ligand compounds. Auto dock 4.0.1 [12] is the primary docking program used in this work for the semi-flexible docking studies. Data warrior property explorer program was used to study the ADMET and 2D QSAR properties of the compounds.

Docking by Virtual Screening

PyRx is used to predict binding pose with associated energy prediction of the compounds with drug target KSP domain. Protocol followed for carrying out the docking studies using PyRx virtual tool in order to predict binding pose along with associated binding energies is of default parameters similar to the protocol followed elsewhere. Briefly, the energy scoring grid box was set to 126, 126 and 126 Å (x, y, and z) centered at X = 34.4092; Y = 26.4643 and Z = 11.1914 with 0.375 angstroms grid points spacing assigned with default atomic salvation parameters. The grid box was designed such that the active site of KSP domain was surrounded by the three dimensional grid box centered at its active ligand binding site location. Lamarckian Genetic Algorithm (LGA) [13] was selected as docking engine, with all the docking parameters set to default. After each LGA run, Auto dock reports the best docking solution for each docked complex, and the results are reported based on cluster analysis. Binding Gibbs free energy (ΔG) is calculated as a sum of six energy terms of dispersion/repulsion, electrostatic interactions, hydrogen bonding, deviation from covalent geometry, desolvation effects and internal ligand torsional constraints. From a total of 10 docking modes represented by LGA cluster analysis, the lowest energy docking mode was selected from each docking simulation.

Pharmacological properties of the compounds

Data warrior software [14] was used to check the pharmaceutical fidelity of the drug candidates. Molecular descriptors, such as Log P, the number of hydrogen bond donors, the number of hydrogen bond acceptors, and the molecular mass and toxicity of the compounds were analyzed.

Results and Discussion

Docking of the compounds with KSP domain active site

We have performed the docking studies for the present studied 60 compounds with the KSP domain protein targeting its active binding site in order to know the binding energy involved in this complex formation. Docking results are tabulated in Table 1. In this present work only ten compounds have shown to be successfully docking inside the active site of KSP domain with a binding energy in a range of -7.7 to -7.0 Kcal/mol. We have compared our docking results with Ispinesib (N-(3-aminopropyl)-N-[(1R)-1-(3-benzyl-7-chloro-4-oxoquinazolin-2-yl)-2-methylpropyl]-4-methylbenzamide) under clnical trial for KSP, showing binding energy of -5.2 Kcal/mol. It was identified that all the compounds are showing better binding energies than these control by showing -7.7 to -7.0 Kcal/mol of binding energy respectively. As per the molecular docking results, it was revealed that compounds IP2 and IP4 have the best estimated -7.7 Kcal/mol of binding energy Table 1 for the KSP domain complex formation (Figure.1).

Table 1: Results of ADMET and 2D QSAR studies of new Indolo – Pyrimidine derivatives

S.No	Structure name	Molecular Formula	Total Molwt	cLogP	H-Acceptors	H-Donors	Rotatable Bonds	Polar Surface Area	Druglikeness	Mutagenic	Tumorigenic	Irritant
1. 2	IP1	$C_{20}H_{17}N_5O_4$	391	1.2106	9	5	3	131.92	6.6194	none	none	None
2. 3	IP2	$C_{21}H_{19}N_5O_4$	405	1.5545	9	5	3	131.92	6.6194	none	none	none
3. 4	IP3	$C_{20}H_{16}N_5O_4Cl$	425	1.8166	9	5	3	131.92	6.66	none	none	none
4. 5	IP4	$C_{21}H_{17}N_5O6$	435	0.6957	11	6	4	169.22	6.5259	none	none	none
5. 6	IP5	$C20H_{16}N_5O_4Br$	470	1.9358	9	5	3	131.92	4.8294	none	none	none
6. 7	IP6	$C_{20}H_{16}N_5O_4F$	409	1.3114	9	5	3	131.92	5.2794	none	none	none
7. 8	IP7	$C_{20}H_{18}N_6O_6$	438	0.612	12	7	4	175.62	6.4334	none	none	none
8. 9	IP8	$C_{22}H_{21}N_5O_6$	451	1.0706	11	5	5	150.38	6.6368	none	none	none
9. 10	IP9	$C_{21}H_{19}N_5O_5$	421	1.1406	10	5	4	141.15	6.6368	none	none	none
10. 11	IP10	$C_{20}H_{15}N_5O_4Cl_2$	460	2.4226	9	5	3	131.92	6.66	none	none	none

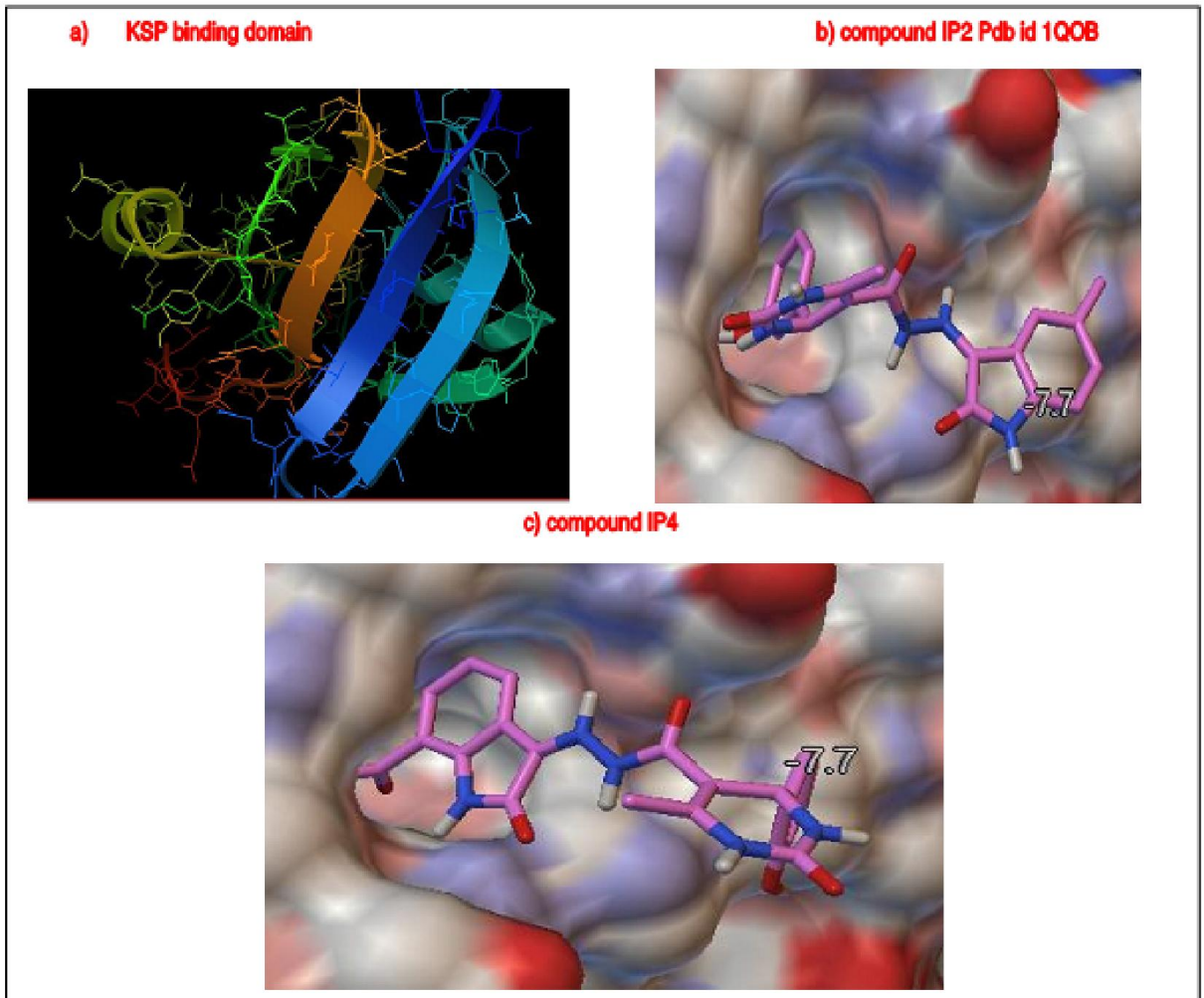


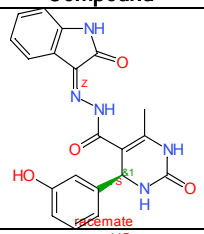
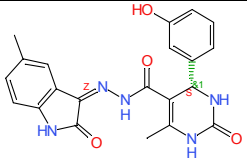
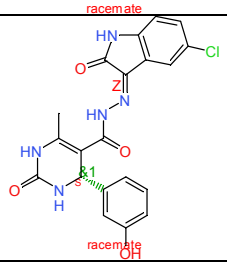
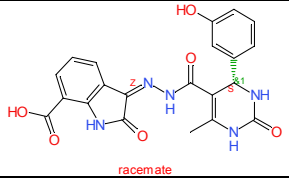
Figure 1 a) Binding domain of KSP (PDB ID- 1QOB)Docking snapshot of compound b) 3D docking snapshot showing compound IP2 (4-(3-hydroxyphenyl)-6-methyl-N-[(Z)-(5-methyl-2-oxo-indolin-3-ylidene)amino]-2-oxo-3,4-dihydro-1H-pyrimidine-5-carboxamide with KSP binding domain c) 3D docking snapshot showing compound IP4 (3Z)-3-[[4-(3-hydroxyphenyl)-6-methyl-2-oxo-3,4-dihydro-1H-pyrimidine-5-carbonyl]hydrazono]-2-oxo-indoline-7-carboxylic acid with KSP binding domain

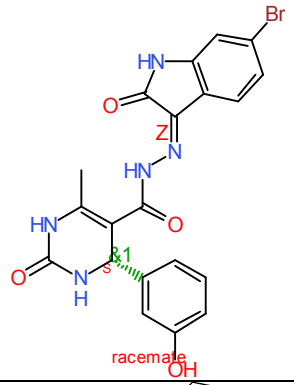
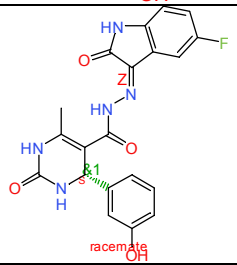
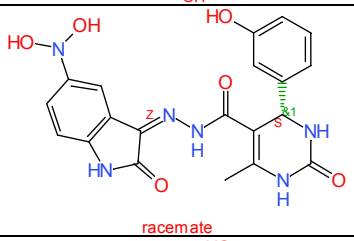
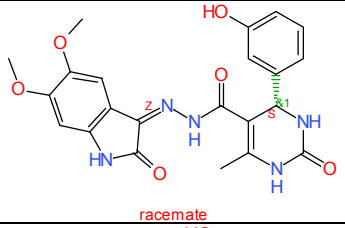
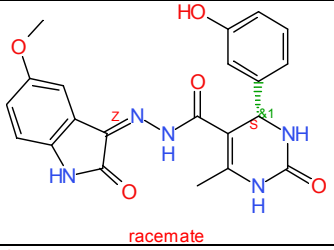
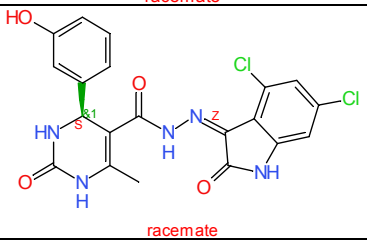
Prediction of pharmacological properties

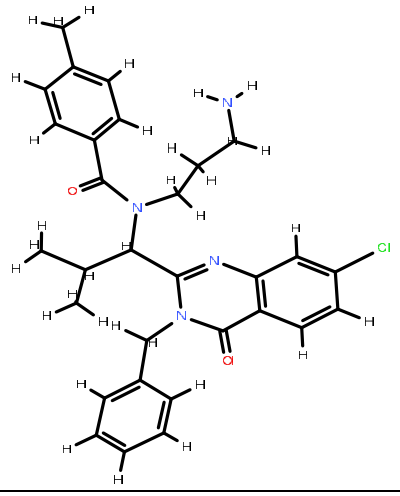
Data warrior was utilized to predict the pharmacological properties of the present studied compounds according to Lipinski's Rule of Five [15]. The pharmacological attributes prediction results are displayed in Table 2.

Based on the experimental values, it was inferred that all the ten compounds successfully satisfied all the parameters of Lipinski's Rule of Five. The parameters of the Lipinski's rule are as follows: the molecular weight must be < 500 Da, Log P < 5, the number of hydrogen donors must be < 5, the number of acceptor hydrogen's must be < 10, and the refractivity molar range must be between 40–130. However, one parameter exception can be given out of above mentioned ones. As per the veber's rule [16] oral bioavailability of drugs could be measured by the molecular weight, number of rotatable bonds (n rotb), number of hydrogen bonds, and the expanse of the drug's polar surface (TPSA). The oral bioavailability was marked by small molecular weight (less than 500 Da); also, the number of rotatable bond must be less than 10, the number of hydrogen bond donors and acceptors must be less than 12, and TPSA values less than 140. Table 2 shows that all the compounds have a promising oral bioavaibility.

Table 2: Docking results of the pyrimidine coumarin series of compounds with KSP domain

S. No	Structure name	Compound	Docking energy (Kcal/mol)
1	IP1		-7.1
2	IP2		-7.7
3	IP3		-7.4
4	IP4		-7.7

5	IP5		-7.3
6	IP6		-7.2
7	IP7		-7.4
8	IP8		-7.3
9	IP9		-7.2
10	IP10		-7

11	Ispenesib		-5.2

Screening for the best compound based on docking and drug likeliness results

Keeping in view of binding energies of the present investigated compounds it was found that only ten compounds has the promising ADMET and 2D QSAR properties. Among ten compounds, compound IP2 i.e 4-(3-hydroxyphenyl)-6-methyl-N-[(Z)-(5-methyl-2-oxo-indolin-3-ylidene)amino]-2-oxo-3,4-dihydro-1H-pyrimidine-5-carboxamide, compound IP4 i.e (3Z)-3-[[4-(3-hydroxyphenyl)-6-methyl-2-oxo-3,4-dihydro-1H-pyrimidine-5-carbonyl]hydrazono]-2-oxo-indoline-7-carboxylic acid has the promising anti cancer drug like properties based on its ΔG binding energy. Based on Pharmacological properties, all the ten compounds showed good pharmacological attributes. These ten compounds were found to comply with Lipinski's rule, Veber's rule and oral bioavailability parameters. Whereas, compound IP2 and IP4 showed good pharmacological attributes, since it satisfied the Lipinski's Rule, Veber's Rule, Log P values with highest binding affinity.

Conclusion

Our *In silico* studies provides a rationalization to the ability of present studied novel ten compounds as a valuable small ligand molecule with strong binding affinity towards KSP domain for plausible anti-cancer activity involving large value of negative binding energy by forming various interactions with the residues. The knowledge gained through this present study could be of high value for computational screening of target specific KSP domain inhibitors by understanding the molecular interaction basis between ligand and receptor. On the other hand, promising ADMET and 2D QSAR drug like profile for the present compounds especially compound IP2 and IP4 substantiates the need of further evaluating this compounds ability to inhibit cancer. The present investigated indolo-pyrimidine scaffold of compounds offers the possibility of expedient additional modifications that could give rise to lead structures with enhanced inhibitory activity and selectivity towards the drug receptor target like KSP.

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