In silico 3D structure modeling and inhibitor binding studies of human male germ cell-associated kinase

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In silico 3D structure modeling and inhibitor binding studies of human male germ cell-associated kinase

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Human male germ cell-associated kinase (hMAK) is an androgen-inducible gene in prostate epithelial cells, and it acts as a coactivator of androgen receptor signaling in prostate cancer. The 3D structure of the hMAK kinase was modeled based on the crystal structure of CDK2 kinase using comparative modeling methods, and the ATP-binding site was characterized. We have collected five inhibitors of hMAK from the literature and docked into the ATP-binding site of the kinase domain. Solvated interaction energies (SIE) of inhibitor binding are calculated from the molecular dynamics simulations trajectories of protein–inhibitor complexes. The contribution from each active site residue in hMAK toward inhibitor binding revealed the nature and extent of interactions between inhibitors and individual residues. The main chain atoms of Met79 invariably form hydrogen bonds with all five inhibitors. The amino acids Leu7, Val15, and Leu129 stabilize the inhibitors via CH–pi interactions. The Asp140 in the active site and Glu77 in hinge region show characteristic hydrogen bonding interactions with inhibitors. From SIE, the residue-wise interactions revealed the nature of non-bonding contacts and modifications required to increase the inhibitor activity. Our work provides 3D model structure of hMAK and molecular basis for the mechanisms of hMAK inhibition at atomic level that aid in designing new potent inhibitors.

Keywords: human male germ cell-associated kinase; prostate cancer; protein structure modeling; molecular docking; molecular dynamics; binding free energies; solvated interaction energies

Introduction

Male germ cell-associated kinase (MAK) is a serine/threonine kinase involved in cell cycle regulation. It was first isolated from a human genomic DNA library using weak cross-hybridization with a tyrosine kinase gene (v-ros) (Matsushima, Jinno, Takagi, & Shibuya, 1990). MAK was identified as a gene highly expressed in testicular germ cells during and after meiosis (Lee, Duan, & Chang, 2000; Matsushima et al., 1990). The homologous protein in rat (Xia et al., 2002) has highly restricted expression pattern in testis and at specific stages of spermatogenesis (Schrantz et al., 2004). MAK was found in different cell types; those that are involved in the sensory transduction, photoreceptors, and olfactory receptors as well as epithelial cells of the respiratory tract and choroid plexus (Bladt & Birchmeier, 1993). MAK is an androgen-inducible gene in LNCaP prostate epithelial cells, and it acts as a coactivator of androgen receptor (AR) signaling in prostate cancer (Ma et al., 2006). Androgen and AR play crucial roles in prostate development as well as in its malignant transformation (Xia et al., 2002).

Prostate cancer cells begin with androgen-dependent growth, but later undergo a transition to an androgen-independent state (Pilat, Kamradt, & Pienta, 1998; Sadar, Hussain, & Bruchovsky, 1999). AR is a ligand-induced transcriptional factor and plays an important role in the normal development of prostate as well as in the progression of prostate cancer. Numerous coactivators associate with AR and function to remodel chromatin, and it recruits the RNA polymerase II to enhance the transcriptional potential of AR (Ma et al., 2006). Some of these coactivators are known as protein kinases, and the studies to identify protein kinases associated with or induced by AR resulted in the discovery of several novel kinases such as androgen receptor-interacting nuclear protein (ANPK) (Moilanen, Karvonen, Poukka, Janne, & Palvimo, 1998), STE20/SPS1-related proline/alanine-rich kinase (SPAK) (Qi et al., 2001), cyclin-dependent kinase 6 (CDK6) (Lim, Mansukhani, & Weinstein, 2005), CDK7/CAK (Lee et al., 2000), and PAK6 (Schrantz et al., 2004; Yang et al., 2001). MAK protein kinase is a direct transcriptional target of AR and also serves as coactivator of AR in propagating the androgen signal (Wang & Kung, 2012). The AR-independent role of MAK in mitosis and its activating mechanisms are also reported (Wang & Kung, 2012).

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The N-terminal region of MAK comprises a kinase domain that shares significant sequence homology with CDK2 and mitogen-activated protein kinase (MAPK) and contains a MAPK-like TDY motif in the activation T-loop. MAK kinase activity requires dual phosphorylation of the conserved TDY motif, and the phosphorylation is dynamic during cell cycle (Wang & Kung, 2012). The phosphorylation levels increased at S phase, peak levels are observed at G2 to early M phase, and decreased levels are observed at late M phase. The high level TDY phosphorylation of MAK at G2/M phase provides the molecular basis for an important role of MAK during the metaphase–anaphase transition. Overexpression of MAK promotes mitotic defects associated with chromosomal aberrations and is often observed in prostate cancer cell lines and patient tissues; the degree of abnormalities positively correlates with tumor staging (Penas, Ramachandran, & Ayad, 2012). These discussions imply that it is essential to design molecules to inhibit activity of human male germ cell–associated kinase (hMAK) that is a target for prostate cancer. Computational methods for rational drug design require the 3D structure of protein and understanding the complementarity of protein–inhibitor interactions. So far, structural studies of this important prostate cancer target are not reported. Therefore, our strategy for in silico drug design is to build the 3D structure of hMAK kinase domain using comparative modeling and identify the interactions with already known inhibitors using molecular docking. This is followed by molecular dynamics (MD) simulations of the protein–inhibitor complexes and binding free energy calculations. The information from docking conformations and binding free energies provides better insights for the design of new potent inhibitors.

Materials and methods

Homology modeling

Comparative protein modeling is a reliable tool for building the 3D structure of a protein based on the experimentally determined structure of another homologous protein. The human MAK protein sequence (NCBI accession ID: AAN16405.1) was taken from the NCBI database (http://www.ncbi.nlm.nih.gov/). BLAST algorithm (Altschul, Gish, Miller, Myers, & Lipman, 1990) against Protein Data Bank (PDB) (Berman et al., 1994) was used to carry out the sequence homology search that identified CDK2 kinase (PDB_ID: 1W8C) (Carbaian et al., 2014) as template for homology modeling. In general, template selection was performed on the basis of sequence similarity, structure completeness, and resolution. The sequence and 3D structure of template protein were extracted from PDB.

The sequences of template and target proteins were aligned using CLUSTALW program (Thompson, Higgins, & Gibson, 1994) with Gonnet weight matrix, gap open penalty 10 and gap extension penalty .1 (Larkin et al., 2007). The protein 3D structure was modeled using Accelrys Discovery Studio 2.5 (DS 2.5; Accelrys Software Inc., San Diego, CA, USA), which implemented MODELLER (Eswar et al., 2006; Sali & Blundell, 1993). The inhibitor structure (6-(cyclohexylmethoxy)-8-isopropyl-9H-purin-2-amine) from CDK2 kinase (PDB ID: 1W8C) was extracted and transferred into the modeled hMAK, in order to use it as a guide for the docking studies.

Validation of predicted homology model

The quality of hMAK 3D model constructed was verified using structure validation methods. VADAR 1.4 program (Volume Area Dihedral Angle Reporter) available at http://vadar.wishartlab.com/ calculates the structural and packing architecture of the modeled protein (Willard et al., 2003). These include accessible surface area, excluded volume, backbone and side chain dihedral angles, secondary structure, hydrogen bonding partners, hydrogen bond energies, steric quality, and solvation free energy as well as local and overall fold quality. These derived parameters can be used to rapidly identify both general and residue-specific problems within newly determined protein structures. PROCHECK analysis (Laskowski, MacArthur, Moss, & Thornton, 1993) calculates the dihedral angles of the model and locates their position in the Ramachandran plot (Ramachandran, Ramakrishnan, & Sasishekaran, 1963). Verify_3D (Lüthy, Bowie, & Eisenberg, 1992) analysis gives the compatibility of hMAK 3D model structure with its 1D amino acid sequence. The PROCHECK and Verify_3D software were accessed from the online SAVES server (http://nihserver.mbi.ucla.edu/SAVES/). The validated protein 3D model was used for the active site identification and docking analysis with the inhibitors.

Molecular docking

Molecular docking determines the probable orientations of inhibitor binding and interactions in the protein active site. Five ATP-competitive hMAK inhibitors collected from the literature (Davis et al., 2011) were docked into the ATP-binding site of hMAK. (i) R547 ([4-Amino-2-(1-methanesulfonyl)piperidin-4-ylamino) pyrimidin-5-yl] (2,3-difluoro-6-methoxyphenyl) methanone), known as a potent and selective CDK inhibitor (Chu et al., 2006), has also shown inhibition toward the hMAK kinase $K_d = 11 \text{nM}$. The crystal structure of R547 bound to CDK2 is available (PDB_ID: 2FVD, $K_d = .53 \text{nM}$) (Chu et al., 2006), (ii) flavopiridol, also known as Alvocidib, HMR 1275, and L86-8275, is a flavonoid derived from an indigenous plant from India. This is a natural substrate, known as a potent and specific in vitro selective
hMAK with CDK4/6 (Sekine et al., 2008) and also inhibits hMAK with \( K_d = 28 \text{nM} \), (iii) AT7519 (N-(4-piperidinyl)-4-(2,6-dichlorobenzoylamino)-1H-pyrazole-3-carboxamide) is a known multi-CDK inhibitor and shows potent anti-multiple myeloma activity (Santo et al., 2010). This pyrazole core-based molecule also inhibits hMAK with \( K_d = 97 \text{nM} \), (iv) CHEMBL162, known as staurosporine (antibiotic AM-2282 or STS), is a natural product (Omura et al., 1977) and prototypical ATP-competitive kinase inhibitor. Staurosporine has high affinity and less selectivity with many kinases (for example, PDB IDs: 2GCD, 2DQ7, and 1YVJ), and it also inhibits hMAK with \( K_d = 2600 \text{nM} \), and (v) CHEMBL603469 is commonly known as lestaurtinib, is a tyrosine kinase inhibitor (Revill, Serradell, Bolos, & Rosa, 2007). This molecule contains the staurosporine glycone core and shows greater activity toward hMAK (\( K_d = 1700 \text{nM} \)) than staurosporine due to substitutions on the core.

The structures of the inhibitors were built using DS 2.5, CHARMM force fields were assigned and energy minimized using the smart minimizer method that performs 1000 steps of steepest descent with a root-mean-square-gradient tolerance of 3, followed by conjugate gradient minimization until a root-mean-square-deviation (RMSD) of .001 kcal/mol was achieved before being used in the study. We performed molecular docking of these ATP-competitive inhibitors into the ATP-binding site of hMAK using GOLD 5.0.1 software (Jones, Willett, & Glen, 1995; Jones, Willett, Glen, Leach, & Taylor, 1997). GOLD is a genetic algorithm for docking flexible ligands into protein-binding sites. During docking, the default algorithm speed was selected, the inhibitor binding site was defined within 15 Å radius around the centroid of the inhibitor (6-(cyclohexylmethoxy)-8-isopropyl-9H-purin-2-amine), and the other docking parameters were used as described in our earlier work (Tanneeru & Guruprasad, 2012). The number of poses for each inhibitor was set to 25, and early termination was allowed if the top 5 bound conformations of an inhibitor were within 1.5 Å RMSD. The best docking pose was selected on the basis of GoldScore and inhibitor orientation. The structures were analyzed using DS 2.5 visualizer to understand the mode of protein–inhibitor binding.

**MD simulations and solvent interaction energy**

The best docked hMAK–inhibitor complexes were used for MD simulations. The protein–inhibitor complex was placed in a 10-Å cubic water box with TIP3P water molecules. In all the complexes, the protein was neutralized with eight chloride ions. All MD simulations were performed using GROMACS 4.5.4 package (Hess, Kutzner, van der Spoel, & Lindahl, 2008; Van Der Spoel et al., 2005) with the AMBER99SB force field (Hornak et al., 2006). The inhibitor parameter files with GAFF force fields using antechamber (Wang, Wang, Kollman, & Case, 2006; Wang, Wolf, Caldwell, Kollman, & Case, 2004) were generated using ACPYPE script (Sousa da Silva & Vranken, 2012). The complexes were subjected to energy minimization for 5000 steps of steepest descent, 5000 steps of conjugate gradient, and 1000 ps position-restrained dynamics to distribute the water molecules throughout the system. Finally, we performed MD simulations of the whole system for 25 ns, using .002 ps time step. The particle-mesh Ewald (PME) summation method (Darden, York, & Pedersen, 1993; Essmann et al., 1995) was employed for the calculation of electrostatics, with a real space cutoff of 10 Å, PME order of 6, and a relative tolerance between long- and short-range energies of \( 10^{-6} \). Short-range interactions were evaluated using a neighbor list of 10 Å updated every 10 steps, and Lennard–Jones (LJ) interactions and the real space electrostatic interactions were truncated at 9 Å. Periodic boundary conditions were used along with the isothermal–isobaric ensemble (NPT) at 1 atmosphere and 298 K. The V-rescale thermostat (Bussi, Donadio, & Parrinello, 2007) was used to maintain the temperature; the Parrinello–Rahman algorithm (Parrinello & Rahman, 1981) was employed to maintain the pressure, and hydrogen bonds were constrained using LINCS algorithm (Hess, Bekker, Berendsen, & Fraaije, 1997). The trajectory file obtained from MD simulations was used for calculation of free energy of binding and other analysis. The RMSD of certain atoms in a molecule with respect to a reference structure can be calculated with the program g_rms of GROMACS by least-square fitting the structure to the reference structure. The binding free energy calculations were performed by the Solvated interaction energies (SIE) method (Cui et al., 2008; Lill & Thompson, 2011; Naïm et al., 2007). Sietraj (http://www2.bri.nrc.ca/ccb/pub/sietraj_main.php) is an alternative to the MM-PBSA software provided by the AMBER distribution. This method was successfully utilized in our earlier work (Tanneeru & Guruprasad, 2013). The binding free energies (\( \Delta G \)) between protein and inhibitors were calculated for snapshot structures taken from the MD trajectory of the system. \( \Delta G \) is the sum of intermolecular van der Waal (vdW) and Coulomb interactions plus the change in reaction-field (RF) energy (determined by solving the Poisson–Boltzmann equation) and nonpolar solvation energy (proportional to the solvent-accessible surface area) (Naïm et al., 2007). Similar to MM-PBSA/GBSA, SIE treats the protein–ligand system in atomistic detail and solvation effects implicitly. The free energy of binding between inhibitor and protein is computed using the equation below:

\[
\Delta G_{\text{bind}}(\rho, D_m, \alpha, \gamma, C) = \alpha [\Delta E_{\text{vdW}}(D_m) + \Delta E_{\text{Coul}}(D_m)] + \Delta G_{\text{RF}}(\rho, D_m) + \gamma \Delta \text{ASA}(\rho) + C
\]
where $\Delta E_{\text{vdW}}$ and $\Delta E_{\text{Coul}}$ are the intermolecular vdW and coulomb interaction energies between protein and inhibitor, $\Delta G_{\text{RF}} (\rho, D_{in})$ is the difference in the reaction-field energy between the bound and free state of the protein–inhibitor complex as calculated by solving the Poisson equation with BRIBEM (Lill & Thompson, 2011; Purisima, 1998; Purisima & Nilar, 1995). The term $\Delta S_A (\rho)$ is the difference in molecular surface area between the bound and free state of the protein. The cavity energy is the change in the molecular surface area $\Delta S_A$, and it is calculated from $\gamma \Delta S_A$ $(\rho)$. The linear scaling factor $\rho$ (1.1) is the vdW radii of the AMBER99 force field, and $D_{in}$ (2.25) is the solute interior dielectric constant. The coefficient $\gamma$ (0.012894) is the molecular surface tension coefficient describing the nonpolar component of solvation free energy, the prefactor $\alpha$ (0.104758) implicitly quantifies the loss of entropy upon binding, also known as entropy–enthalpy compensation, and a constant $C$ (−2.89) includes protein-dependent contributions not explicitly modeled by the SIE methodology, that is, the change in protein internal energy upon inhibitor binding. The scaling can be considered as a crude treatment of entropy–enthalpy compensation containing the caveats of implicit solvation and neglecting the vibrational entropy (Chen, Chang, & Gilson, 2004; Naïme et al., 2007).

Here, we estimated $\Delta G$ of 100 structures from the last 10 ns of selected MD snapshots and averaging over the resulting free energies obtained from each snapshot. We have calculated the contribution of each active site residue to the binding free energy of inhibitor. These results explain the basis for inhibitor binding to hMAK and provide more precise directions in the design of new inhibitors.

**Results and discussion**

**Homology modeling and structural analysis of predicted models**

The hMAK protein has 623 amino acid sequence length, containing a serine/threonine kinase catalytic domain at its N-terminus (4-284), followed by a proline/glutamine-rich domain. The kinase domain of hMAK is highly homologous to the CDK family and to the MAPK family. According to BLAST search results, hMAK homology model was constructed based on CDK2 kinase structure that share 61% sequence homology. The crystal structure of CDK2 kinase bound to 6-(cyclohexylmethoxy)-8-isopropyl-9H-purin-2-amine (PDB_ID: 1W8C) was used as structural template.

Ramachandran plot of the hMAK model showed that 99.2% of residues are located in the allowed regions, only four residues are observed in disallowed regions. In general, a model which has above 90% residues located in the favorable region of Ramachandran plot is considered reliable. From the VADAR analysis, we observed that the mean residue volume in the model was 143.9 Å³ and total packing volume of the model was 40447.4 Å³, which indicated good packing density of the protein structure (Richards, 1977). Validating hMAK model using Verify_3D showed that 86.17% of residues had an average 3D-1D score > .2. Thus, the compatibility of hMAK 3D model structure with its 1D amino acid sequence was confirmed. The homology model of hMAK superimposed onto CDK2 kinase template with RMSD of 0.13 Å, indicating that template and model structures are highly similar. The final model structure of hMAK bound to 6-(cyclohexylmethoxy)-8-isopropyl-9H-purin-2-amine is shown in Figure 1.

The modeled hMAK kinase domain has the characteristic N- and C-terminal lobes, and the substrate ATP is accommodated between the two lobes. The DFG signature sequence motif characteristic of kinases is present in both hMAK and the template proteins. In 3D structures of kinase, the DFG motif exists in two conformations, as DFG-in and DFG-out, which determine whether the kinase is in the active or inactive states, respectively (Aleksandrov & Simonson, 2010; Levinson et al., 2006; Xu, Yu, Wan, Yu, & Huang, 2011). The DFG motif is characteristic of kinases, and we observed that the side chain of Asp140 (amino acid numbering as per the

**Figure 1.** Schematic diagram of the homology model of hMAK 3D structure. The hinge-region residue Met79, DFG motif residues Asp140, Phe141, and Gly142 are indicated. The bound inhibitor 6-(cyclohexylmethoxy)-8-isopropyl-9H-purin-2-amine is indicated in ball and stick.
region of modeled structure) is oriented into the active site, while the side chain of Phe141 is pointing outwards, revealing that the hMAK model is in the active conformation, similar to the conformation observed in CDK2 kinase structure (PDB_ID: 1W8C).

**Molecular docking studies**

The five energy-minimized inhibitors were docked into the hMAK active site. The molecular docking revealed the binding orientations of molecules in the ATP-binding site of the hMAK kinase domain and estimates the affinity of intermolecular interactions. The five molecules with different scaffolds docked into the ATP-binding site revealed the competitive nature of the inhibitor.

**R547:** The docking results of R547 into hMAK active site were analyzed. We obtained a binding orientation in the GOLD docking results (GoldScore = 55.43) akin to the binding mode of R547 in the ATP-binding site of the crystal structure of CDK2 (PDB_ID: 2FVD) (Chu et al., 2006). This best docked hMAK–R547 complex shown in Figure 2(A) was used for further structure analysis and MD simulations. The hinge-region residues Glu77 main chain carbonyl oxygen forms hydrogen bond with the primary amine present on the pyrimidine core (CO…HN7, 2.7 Å), and Met79 main chain carbonyl oxygen forms hydrogen bond with the secondary amine present on the pyrimidine core (CO…HN7, 1.7 Å) of R547. The linker carbonyl oxygen between two aromatic rings of inhibitor forms hydrogen bond with the side chain NH of Lys30 (NH…O2, 2.4 Å). The Lys30 side chain also forms cation–π interactions with the aromatic substituted phenyl ring of R547 in order to stabilize the complex. The Lys30 side chain also forms cation–π interactions with phe76 of the protein.

**Flavopiridol:** From the GOLD docking of flavopiridol into the ATP-binding site of hMAK, we have selected the best docking conformation of the inhibitor with GoldScore of 51.27 and is shown in Figure 2B. The hinge-region residue Met79 main chain NH forms hydrogen bond with the hydroxyl group on the 4-chromenone core of flavopiridol (NH…HO17, 2.6 Å). The side chain NH of Asn82 forms hydrogen bond with the chlorine atom of the inhibitor (NH…Cl2, 3.1 Å). The main chain carbonyl oxygen of Glu126 forms hydrogen bond with the hydroxy group oxygen O28 on piperidine of inhibitor (CO…HO28, 3.0 Å). The side chain NH of Lys30 forms hydrogen bond with the second hydroxyl oxygen O20 on 4-chromenone of flavopiridol (NH…O20, 2.5 Å).

**AT7519:** The best docking conformation of AT7519 into hMAK as shown in Figure 2C had GoldScore 58.5, and the binding orientation is similar to its binding to CDK kinase (PDB_ID: 2VU3). The HN9 of pyrazole core forms hydrogen bond with main chain carbonyl oxygen of Glu77 in the hinge region (CO…HN9, 2.4 Å). The N8 of pyrazole core forms hydrogen bond with main chain NH of Met79 in the hinge region (NH…N8, 3.1 Å). The main chain carbonyl oxygen of Met79 forms hydrogen bond with HN4 of inhibitor (CO…HN4, 2.71 Å). The side chain NH of Lys30 forms cation–π interactions with dichlorophenyl ring of the inhibitor. The Lys30 side chain NH forms cation–π interactions with Phe76 aromatic ring and also forms a salt bridge with Asp140 of DFG motif. The side chain of Val15 has hydrophobic interactions with dichlorophenyl ring.

**CHEMBL162:** The best docking conformation of staurosporine into the hMAK active site as shown in Figure 2D has the GoldScore of 54.82. The molecule forms characteristic hydrogen bond in the hinge region between main chain NH of Met79 and carbonyl oxygen of staurosporine glycone (indolo[2,3-a]pyrrole[3,4-c]carbazol) (NH…O24, 2.9 Å). The side chain NH of Asn82 forms hydrogen bond with methyl substituted secondary amine nitrogen of staurosporine (NH…N3a, 3.1 Å). The hydroxy phenyl ring of Tyr78 forms pi–π interactions with carbazole ring of inhibitor, and the side chain of Leu7 forms CH–π interactions with the aromatic fused ring of staurosporine.

**CHEMBL603469:** The best docked conformation of CHEMBL603469 into the ATP-binding site with a GoldScore of 49.81 is shown in Figure 2E. The main chain NH of Met79 forms hydrogen bond with the carbonyl oxygen of the staurosporine glycone (NH…O24, 2.9 Å) in the hinge region of the kinase domain. The hydroxy methyl OH forms hydrogen bond with side chain carbonyl oxygen of Asn82 (CO…HO4, 2.3 Å). The second OH group on inhibitor forms hydrogen bond with main chain carbonyl oxygen of Glu126 (CO…HO4, 2.4 Å). The side chain of Leu129 in contact with the aromatic ring system of staurosporine forms sigma–π interactions. The side chain of Leu7 also forms hydrophobic interactions with staurosporine glycone core of inhibitor to stabilize the staurosporine conformation.

**MD simulations and SIE calculations**

The RMSD plots mainly explain the extent of deviations of all atom positions in the protein–inhibitor complex during MD simulations. In order to examine the conformational variations of hMAK, in complex with inhibitors, the RMSD of atomic positions with respect to starting conformations was calculated. The Ca atoms of protein RMSD indicated as a function of the simulation time varied in the initial 5 ns, and during the last 10 ns, the structure was stabilized and the RMSD varied...
between 2.6 and 3.7 Å in different complexes (Figure 3). From the MD simulations trajectory (Supplementary Figure 1), we observed that the inhibitors R547, CHEM-BL162, CHEMBL603469, and flavopiridol rapidly converged and stabilized throughout simulations in the protein active site. The AT7519 showed slightly higher fluctuation up to 10 ns and thereafter stabilized throughout simulations. The characteristic hydrogen bond with Met79 was retained with all five inhibitors throughout simulation time, indicating the importance of this interaction in stabilizing the complex and that inhibitor core in the active site of hMAK was stable and only minor fluctuations were observed in the substitutions on the core (Supplementary Figure 2).

The free energy of inhibitor binding to the hMAK active site was calculated using Sietraj program. The SIE free energy and the contribution of its different components are given in the Table 1. The contributions from

![Figure 2](image)

Figure 2. Docking of inhibitors (ball and stick) into hMAK active site (stick) (A) R547, (B) flavopiridol, (C) AT7519, (D) CHEMBL162, and (E) CHEMBL603469. The color representation is as follows: inhibitor carbon (pink), protein carbon (purple), oxygen (red), nitrogen (blue), sulfur (yellow), fluorine (cyan), and chlorine (green). The hydrogen bonds between the donor and acceptor atoms are shown in red broken lines.
amino acid residues to the binding free energies of inhibitors play a key role in the binding and activity of inhibitors. The inhibitor CHEMBL603469 has relatively higher activity ($K_d = 1700$ nM) compared to CHEMBL162 ($K_d = 2600$ nM), while both inhibitors have similar core part, and the free energies of binding were comparable from MD simulations studies. These two inhibitors show similar kind of binding interactions with active site residues during the docking studies, and the free energy of CHEMBL603469 was $-9.82$ kcal/mol, whereas for CHEMBL162, it was $-9.28$ kcal/mol. The residue-wise contribution to the inhibitor binding in the active site is given in Table 2. From the analysis of MD simulations of hMAK complexed with CHEMBL603469 and CHEMBL162, we observed that Phe76 forms sigma–pi interactions with CHEMBL603469 and has high vdW ($-2.55$ kcal/mol), whereas the CHEMBL162 does not have a similar interaction and has relatively less vdW contribution ($-1.77$ kcal/mol). The other active site residue Asn82 has better coulombic interactions with CHEMBL603469 ($-1.61$ kcal/mol) than CHEMBL162 ($-0.75$ kcal/mol). Asp9 has comparatively good vdW and coulomb interactions with CHEMBL603469. The side chain of Glu126 is in contact distance with the hydroxy group of CHEMBL603469 and therefore has higher contribution from coulombic interaction energy. We believe that the free energy calculations provide more information regarding binding interactions than merely obtained from molecular docking.

The other three inhibitors of hMAK are different in the core structure and molecular size. Therefore, binding orientation, activity, and free energies of the inhibitor binding are not exactly comparable to each other. The inhibitor R547 with pyrimidine core has good inhibition values and shows a binding free energy $-7.51$ kcal/mol. Lys30 forms cation–pi interactions with the dichlorophenyl ring and has electrostatic ($-2.80$ kcal/mol) contribution to binding free energy. The hinge-region residues Tyr78 shows high vdW ($-3.25$ kcal/mol), and Met79 also displays good vdW ($-2.99$ kcal/mol) and electrostatic ($-2.68$ kcal/mol) contribution in the free energy of binding. The residues Leu7 and Leu129 stabilize the molecule by contributing favorable vdW ($-4.24$ kcal/mol and $-3.97$ kcal/mol) interactions from either side of the inhibitor. The residue Asn82 shows favorable vdW and unfavorable coulombic interactions with the inhibitor, and Asp140 also shows unfavorable coulombic interactions. Glu81 has favorable vdW and coulombic interactions with the inhibitor.

The pyrazole core-based inhibitor AT7519 binds hMAK with a binding free energy of $-8.35$ kcal/mol. The hinge-region residues Tyr78 and Met79 show good vdW and electrostatic interaction energies. Glu77 forms the characteristic and stable hydrogen bond with pyrazole ring nitrogen of the inhibitor and shows greater electrostatic ($-5.21$ kcal/mol) contribution toward the inhibitor binding.

The flavopiridol showed good inhibition toward hMAK and has reasonable binding free energy ($-8.09$ kcal/mol). The Val15 contributes favorable vdW $-3.86$ kcal/mol, and Glu126 residue contributes favorable vdW and electrostatic interactions ($-2.4$ and $-2.30$ kcal/mol) that favor the binding of flavopiridol to hMAK. From the Table 2, we observed that the residues Glu77, Glu81, Tyr84, and Gln85 display repulsive coulombic interactions that will contribute to reduced binding free energies.

The inhibitor R547 experiences repulsive coulomb interactions with Asp140 ($2.31$ kcal/mol). In R547, 1,2 difluoro groups are located in close proximity to
Table 1. The SIE free energy of inhibitors binding to hMAK with energy components and calculated ΔG. The units of the energies in the table are calculated as kcal/mol.

<table>
<thead>
<tr>
<th>Energy components</th>
<th>R547</th>
<th>Falvopiridol</th>
<th>AT7519</th>
<th>CHEMBL603469</th>
<th>CHEMBL162</th>
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<td>-53.01</td>
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<td>0.104758</td>
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<td>$\gamma$</td>
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<td>0.012894</td>
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<tr>
<td>$\Delta G$</td>
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<td>-8.09</td>
<td>-8.35</td>
<td>-9.82</td>
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</table>

Table 2. Energy contribution of active site residues in SIE free energy calculations. The units of the energies in the table are calculated as kcal/mol.

<table>
<thead>
<tr>
<th>Residue</th>
<th>R547 VdW</th>
<th>Falvopiridol VdW</th>
<th>AT7519 VdW</th>
<th>CHEMBL603469 VdW</th>
<th>CHEMBL162 VdW</th>
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<tr>
<td>Leu7</td>
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</table>

Conclusions

A reliable homology model of hMAK kinase was built, and the inhibitors were docked into the ATP-binding site. Five inhibitors R547, Flavopiridol, AT7519, CHEMBL162, and CHEMBL603469 were docked into hMAK active site. The docking studies revealed key interactions of the inhibitors with hMAK in the ATP-binding site. The MD simulations of the best docked protein–inhibitor complexes and SIE free energy of binding calculations revealed the affinity of inhibitor toward hMAK. Our results provide directions for inhibitor design of specific interactions in the hMAK active site in order to increase the binding affinity and activity of the inhibitors.
Supplementary material
The supplementary material for this paper is available online at http://dx.doi.org/10.1080/07391102.2014.968622.

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References


