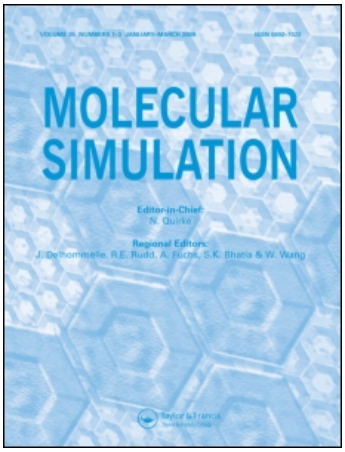

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Pharmacophore identification of PAK4 inhibitors

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Pharmacophore identification of PAK4 inhibitors

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p21-activated kinase 4 (PAK4) is a serine–threonine protein kinase which plays an important role in a wide variety of human diseases including cancer. The inhibition of this kinase is of great interest for the treatment of cancer. In the present study, we report three pharmacophore models of PAK4 based on a small set of PAK4 inhibitors, a crystal structure of PAK4 and a docked complex between PAK4 and a potent inhibitor. These results might provide useful and reliable tools in identifying structurally diverse compounds with desired biological activity.

Keywords: p21-activated kinase 4; pharmacophore model; molecular docking; kinase inhibitor; drug discovery

1. Introduction

The p21-activated kinases (PAKs), a series of key signalling molecules, play an essential role in the control of a wide variety of cellular functions including cell motility, survival, angiogenesis and mitosis [1]. Indeed, many cancers are detected through over-express PAKs, which contribute to the migration, anchorage, independent growth and the metastasis of the cells [1,2]. In humans, PAKs are composed of six isoforms and are classified into group I (PAK1–3) and group II (PAK4–6) based on their regulatory properties [2]. The group I family has already been well studied, and the members of the group II PAK family are now emerging as more interesting targets for cancer therapy [3,4]. It is found that PAK4 is over-expressed in a wide variety of tumour types, including colon carcinoma, oesophageal carcinoma and mammary adenocarcinoma, etc. and that it executes key functions in tumour invasion and metastasis. Therefore, it may be an attractive candidate for cancer therapy [3,5].

In this study, we aimed to obtain pharmacophore models to shed more light on the chemical features which might contribute to the inhibitory activity of PAK4. We selected a total of nine PAK4 inhibitors [6,7]. They only bind in and around the region originally occupied by ATP and are typically ATP competitive inhibitors. Within the binding pocket, residues Ile327, Ala348, Val379, Met395, Phe397 and Leu447 are responsible for forming a hydrophobic region and Leu398 for forming hydrogen bonds with ligands [6]. Furthermore, we generated a pharmacophore model using the Hypogen method based on these molecules. In addition, interactions between PAK4 and its ligands were analysed through molecular docking, and then they were converted into pharmacophores by LigandScout. These two pharmacophore models might be useful for developing potentially novel PAK4 inhibitors (Figure 1).

2. Methods

The pharmacophore in Figure 3 was generated using Catalyst protocols implemented in Discovery Studio 2.1 [8]. Pharmacophores in Figure 6 were generated with the automated pharmacophore generation program LigandScout 2.02 [9]. Docking studies were performed with Fred v2.2 [10].

2.1 Pharmacophore modelling with Discovery Studio

Nine ATP-competitive inhibitors reported were selected to generate pharmacophore models. These compounds came from different studies, and the activity data were determined with different systems. Therefore, The HipHop module within Catalyst was used for constructions of qualitative models. All structures (Figure 2) were built and minimised to the closest local minimum. Diverse conformations for each compound were generated using an energy range of 20 kcal/mol of the calculated potential energy minimum. The maximum number of conformers of each molecule was specified as 250 to ensure maximum coverage of the conformational space. Four criteria were selected to form the pharmacophore hypothesis generation process: hydrogen-bond acceptor (HBA), hydrogen-bond donor (HBD), hydrophobic group (Hp) and ring aromatic (Ar).

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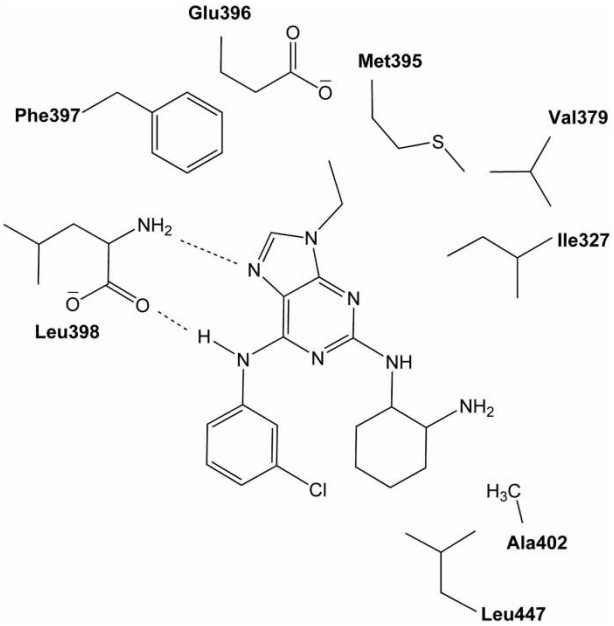

Figure 1. Schematic representation of interactions between PAK4 and the ligand (23D). H-bonding interactions are indicated as dashed lines.

2.2 Molecular docking

The crystal structure of PAK4 in complex with 23D (PDB entry code: 2CDZ) [6] was used. The protein was prepared and the active site box was extended 4.0 Å around the co-crystallised ligand. The co-crystallised ligand (23D, inhibitor 1) and other eight inhibitors were submitted to OMEGA v. 2.1.0 [10] to generate conformers for use in FRED [10]. OMEGA was run as keeping conformations within 5.0 kcal/mol (e_window), having an rmsd cut-off of 0.6 Å and keeping 1000 output conformations in accordance with the recommendation by Bostrom et al. [11]. The docking protocols were set up using the programs' default settings. The generated poses were ranked using the implemented chemscore function. Vida 2.0 [10] was used for visualisation.

2.3 Pharmacophore modelling with LigandScout

The crystal structure of PAK4 in complex with 23D (PDB entry code: 2CDZ) was analysed with LigandScout [9] to identify and visualise the chemical features important for ligand–enzyme interactions to be codified into a structure-based pharmacophoric model (Figure 6(a)).

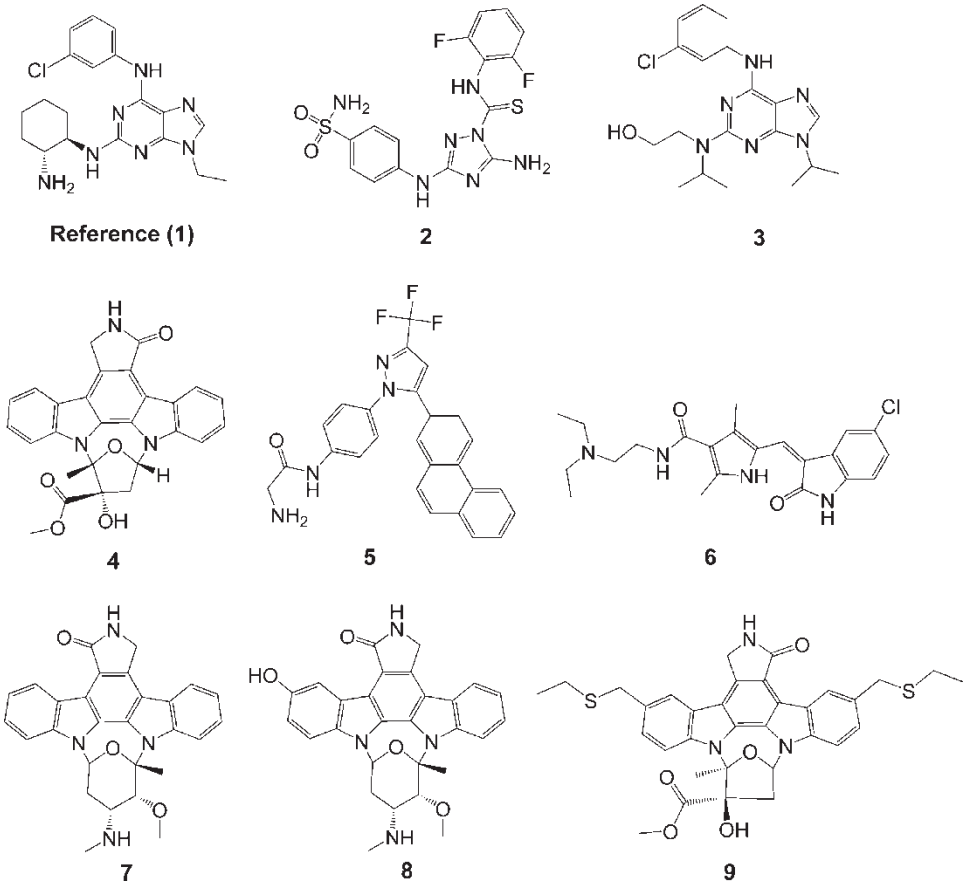

Figure 2. Structures of molecules used in HypoGen studies.

Default parameter settings were used in the calculation. In order to widen the diversity of the pharmacophores of PAK4, the interactions between the docked inhibitor 2 and PAK4 were used in pharmacophore generation with LigandScout again (Figure 6(b)).

3. Results and discussion

Pharmacophore modelling is an approach developed in recent years in an attempt to identify novel scaffolds. Catalyst and LigandScout were used for pharmacophore identification of PAK4 inhibitors. Both model HBD and HBA features as a position for the heavy atom and a projected point representing the position from which the participating hydrogen will extend [12]. These two positions form a vector indicating the direction from the heavy atom to the projected point of the hydrogen bond.

The common Feature Pharmacophore Generation protocol produced 10 hypotheses, and the top-ranked hypothesis consisted of four features: one HBA, two HBDs and one Hp (Figure 3). The reference molecule (23D, inhibitor 1) and potent inhibitors can be well mapped onto the Hypo1 model (Figure 4), indicating that the model provides reasonable pharmacophoric characteristics of the PAK4 inhibitors for the components of their activities. All nine inhibitors were used as a training set. The interactions were analysed through molecular docking (Fred) and LigandScout.

As illustrated, Figure 5 is a docking model of inhibitors in complex with the PAK4, in which both reference and inhibitor 2 bind to PAK4 at the hinge region with its nitrogen atom forming an H-bond interaction with the backbone NH of Leu398. The reference that was re-docked into the binding site reproduced the interaction modes observed in crystal structure (Figure 5(a)). As far as inhibitor 2 is concerned, the phenyl moiety is positioned deep into the ATP-binding pocket and is surrounded by mostly hydrophobic residues. It also forms additional H-bond interaction with the side chain of Glu396, besides the H-bond interaction with the backbone of Leu398. The docked complex between PAK4 and inhibitor 2 was used as an input for LigandScout analysis.

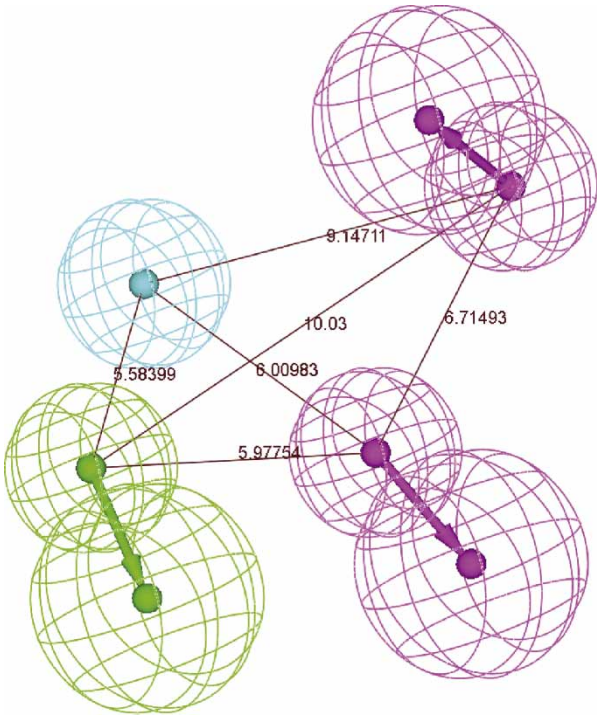

Figure 3. Pharmacophore models produced by Discovery 2.1. HBDs are coloured magenta, HBAs are coloured green, and Hps are coloured cyan.

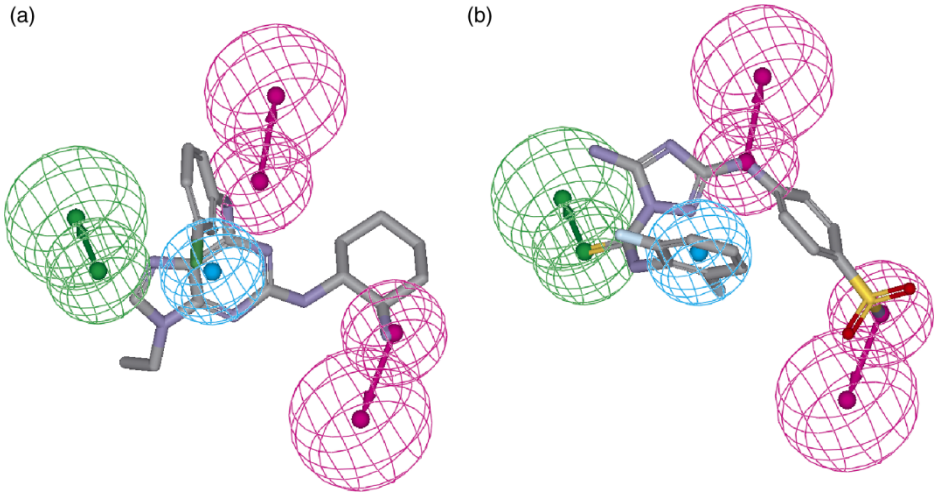

Figure 4. Pharmacophore mapping of reference (a) and inhibitor 2 (b). HBDs are coloured magenta, HBAs are coloured green, and Hps are coloured cyan.

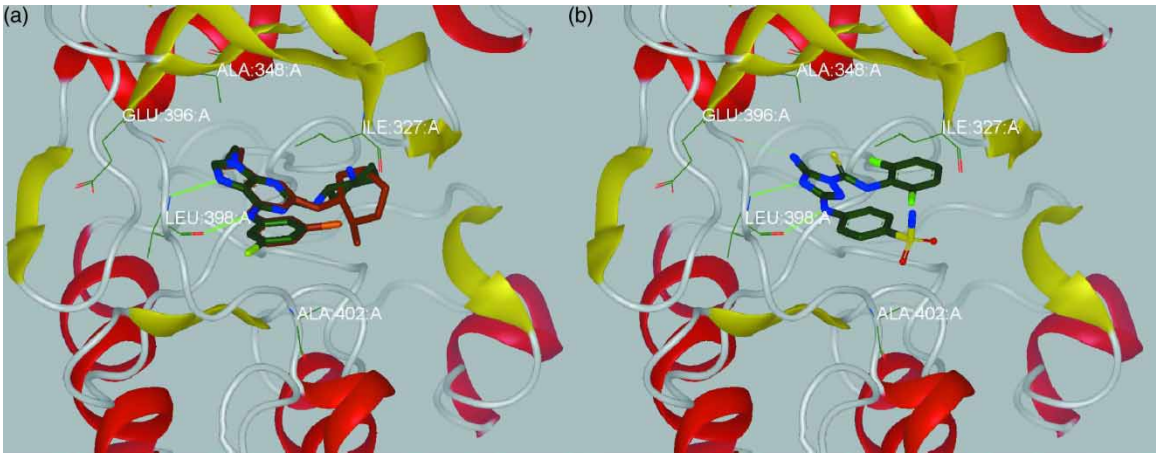

Figure 5. Molecular docking of inhibitors with PAK4. (a) 23D was re-docked into the binding site to reproduce the interaction modes observed in crystal structure. (b) Inhibitor 2 forms hydrogen bonds with Leu398 and Glu396. The PAK4 structure is shown in ribbon form; the small molecules are presented as stick structure, coloured by atom types for poses and green for the reference ligand.

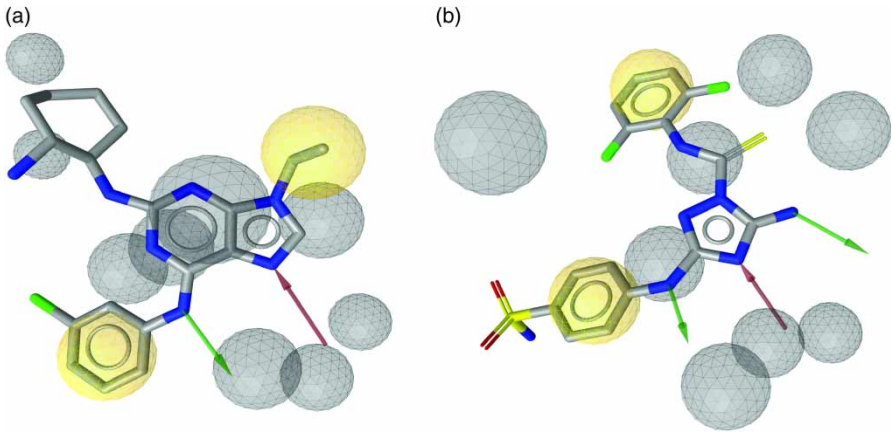

Figure 6. Pharmacophores constructed by LigandScout 2.02. HBDs coloured magenta, HBAs green, Hps yellow and exclusion spheres grey.

LigandScout starts with a macromolecule/ligand complex and automatically derives 3D chemical feature-based pharmacophores [12]. The automated pharmacophore generation for the crystal structure 2CDZ from LigandScout resulted in two HBAs and two hydrophobic features (Figure 6(a)). The acceptors were pointed from the nitrogen atoms of 9H-purine and the donor was pointed from the nitrogen atom on 9H-purine. The two hydrophobes were located at the phenyl and ethyl group in 23D. LigandScout was also used for the pharmacophore generation of the complex between docked inhibitor 2 and PAK4. As a result, two HBD features, one acceptor feature and two hydrophobic features around were generated (Figure 6(b)). Compared to the pharmacophore model based on the crystal structure 2CDZ, LigandScout generated more features, which may be due to the usage of a different ligand.

4. Conclusion

Three pharmacophore models for PAK4 were generated in this study. The models could be useful for identification of potential novel inhibitors.

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