Design of Selective Inhibitors of Tyrosine Kinase 2

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Abstract: Selective inhibitors of tyrosine kinase 2 (Tyk2) were searched for using database screening, *de novo* ligand design and computational docking in Tyk2 and seven other protein kinases. None of the structures in the National Cancer Institute database seem to inhibit Tyk2 selectively, but five of the designed structures seem promising.

Keywords: Tyrosine kinase, Protein Alpha Shape Similarity Analysis (PASSA), Protein Alpha Shape (PAS) Dock, inhibitor design, selectivity, database screening.

INTRODUCTION

Protein kinases contribute to regulation and coordination of e.g. metabolism, gene expression, cell growth, cell motility, cell differentiation and cell division [1]. The Janus kinase (Jak) family of non-receptor tyrosine kinases consists of four known mammalian proteins (Tyk2, Jak1, Jak2 and Jak3) that play a critical role in initiating signalling cascades of a large number of cytokine receptors [2-5]. All Jak family kinases possess a carboxyl-terminal tyrosine kinase catalytic domain, a central kinase-like domain, and a large amino-terminal region, which has been subdivided into five Jak homology regions (JH7 to JH3) based on sequence conservation [5, 6]. In contrast to most other cytoplasmic protein tyrosine kinases, the Janus kinases have no Src homology (SH2 or SH3) domains [2]. The specific and non-covalent association of these kinases to the intracellular region of cytokine receptors governs their activation upon ligand binding [3]. The JH domains have been shown to be the parts of the Janus kinases that are associated with the cytoplasmic domains of cytokine receptors [3, 5, 7]. The activation of the Janus kinases is mediated by ligand-induced receptor oligomerisation [8-10]. The Janus kinases are activated by e.g. the type I interferons (IFNα/β and γ), the interleukins (IL2-7, IL-10 and IL-12), growth hormone (GH), prolactin, erythropoietin (Epo), granulocyte-specific colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), leukaemia inhibitory factor (LIF) and ciliary neurotrophic factor (CNTF) [2, 5, 11].

Activated Janus kinases autophosphorylate [3] and phosphorylate the cytokine receptors with which they are associated, providing binding sites for the Signal Transducers and Activators of Transcription (STAT) family of transcription factors [8]. The Jaks catalyse phosphorylation of the STAT proteins (seven isoforms, STAT1-4, STAT5A-B and STAT6) [12], which occurs by transfer of the γ phosphate of adenosine triphosphate (ATP) to the hydroxyl group of a tyrosine residue in the STAT protein. After phosphorylation on tyrosine residues, the STAT molecules form homo- or heterodimers [9], which are translocated into the nucleus. The STAT proteins then bind to DNA, and activate gene transcription [2]. The Jak-STAT signalling cascade has been shown to contribute to growth and survival of e.g. human multiple myeloma cells [13], acute lymphoblastic leukaemia [14] and a variety of other malignancies [15, 16]. This makes the Janus kinases potential targets for new cancer therapies. One way to interrupt this signalling cascade is to block the binding of ATP to the tyrosine kinases. ATP analogues are generally non-selective, but the development of inhibitors like STI571 [17] shows that ATP binding sites can be used as targets for selective drugs.

At the present time none of the Janus kinases has experimentally determined 3-dimensional (3D) structures [18, 19]. In a recent publication, we predicted the 3D structures of the tyrosine kinase domains of Jak2 and Tyk2 by homology modelling, and suggested functional groups for a selective inhibitor of Tyk2 based on Protein Alpha Shape Similarity Analysis (PASSA) [20]. PASSA is a new method for mapping protein binding sites, and is especially suited for protein structures predicted by homology modelling. In PASSA, several models for the same protein are used together with structures of other, related proteins to single out unique features of the target protein. Hence, this method is developed especially for design of selective drugs. In PASSA, the binding sites of the protein structures are compared using gaussian property distributions. The results are combined with results from Multiple Copy Simultaneous Search (MCSS) [21], to suggest functional groups of a selective inhibitor. The use of gaussian functions to describe the protein binding sites makes PASSA especially suited for use with homology modelled structures, since the functional form of the representation may be more robust than force field based methods against small structural errors typically present in homology models. Homology modelling in drug design has recently been reviewed [22].

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This work utilised previously suggested functional groups for a selective Tyk2 inhibitor [20] in a pharmacophore search of the database of the National Cancer Institute (NCI). The resulting structures were tested for binding to Tyk2 by computational docking in a homology model of Tyk2. Structures having the desired functional groups were also generated by *de novo* ligand design. The most promising drug candidates resulting from this analysis were tested for selectivity towards Tyk2 by computational docking in seven protein kinase structures, in addition to the homology model of Tyk2.

METHODS

Pharmacophore Search

The 3D structure database of the NCI from August 2000 (http://cactus.nci.nih.gov/) (250 241 structures) was searched using the pharmacophore search routines in Molecular Operating Environment (MOE) [23, 24]. Functional groups for a selective Tyk2 inhibitor have been proposed earlier by our group [20], based on MCSS. Selected MCSS fragments [20] defined the pharmacophore, that is, the ligand functional groups that are proposed for a selective Tyk2 inhibitor (Fig. (**1**)). MOE uses "pharmacophore query features" to represent the pharmacophore. A query feature is a point in space with a radius-like tolerance on spatial proximity and an associated expression indicating electrostatic properties. These query features represent volumes in space where ligand functional groups with specified properties (e.g. aromatic) should be found. The MOE pharmacophore search routines search for matches between the functional groups of the ligands in a database and the pharmacophore query features. The query features used correspond to the MCSS fragments found in the previous study [20]. To allow some variation from the MCSS fragments, the following proximity tolerances were used for the different query features: Aromatic (benzene rings): 4.0 Å, hydrophobic (CH_3-groups): 2.0 Å, hydrogen donor or hydrogen acceptor: 1.6 Å. Proximity tolerances of about twice the actual size of the fragments were used as a compensation for potential inaccuracies in the computational methods used. This is important in order to limit the number of false negatives. A match on at least six of the pharmacophore query features (MCSS fragments) was required in the pharmacophore search. The resulting compounds were first filtered according to distance from the ATP binding site. All compounds having atoms within 10 Å of the docked conformation of ATP [20] were kept for further analysis.

Computational Docking Analysis

The hits from the pharmacophore search were docked in a previously reported [20] homology model of Tyk2. Two different docking procedures were used: Docking with MOE-Dock [23, 25], and docking with Protein Alpha Shape (PAS) Dock, a new docking method recently developed by our group [26]. MOE-Dock uses the sum of the electrostatic and the dispersive interaction energy between the ligand and the target and the intramolecular energy of the ligand to rank the structures. The molecular mechanics (MM) force field MMFF94 [27] was chosen for the docking study, as it predicts both intermolecular hydrogen bonding and geometries of small molecules quite well [28]. A smooth non-bonded cut-off of 10-12 Å was used. In the PAS-Dock docking method, a score function based on gaussian property descriptions was used. Both methods use Tabu search [25] for the geometry search.

Docking with MOE-Dock

ATP has previously been docked into the homology model of Tyk2 [20]. The hits from the pharmacophore search having atoms within 10 Å of the docked conformation of ATP were docked into the homology model of Tyk2 as a first screening, using ten MOE-Dock runs of 1000 iterations each. A docking box of 125x125x125 grid points with 0.375 Å spacing between each grid point was used. The docking box was centred on the docked conformation of ATP. All structures from this docking analysis having docking energies <5000 kcal/mol (112 structures) were further docked using ten runs of 25000 iterations each. This threshold of 5000 kcal/mol was chosen based on the distribution of the docking energies.

MOE-Dock uses grid-based potential fields [23] to calculate interaction energies between the ligand and the receptor. This grid-based method calculates the potential energy grids only once, at the beginning of the docking procedure. Hence, protein flexibility is not taken into account in these calculations.

The conformation of each drug candidate having the lowest docking energy was also scored using the gaussian-based score function used in the new docking method PAS-Dock [26].

Docking with PAS-Dock

The structures from the pharmacophore search of the NCI database described above were also docked using the newly developed docking method PAS-Dock [26]. The largest and most flexible compounds (917 out of 1168 structures) were removed from the compound set prior to the docking. The 1168 compounds were sorted according to the Kier flexibility index [29], and a threshold value for the Kier flexibility index was chosen by inspection of the structures. Structures containing several long, un-branched hydrocarbon chains or large ring systems and structures larger in diameter than the ATP-binding pocket of Tyk2 were considered unlikely to fit into the binding pocket.

A docking analysis with 100 Tabu runs of 1000 iterations each was carried out using a docking box with 3 Å padding around the protein structure. MMFF94 with a smooth non-bonded cut-off of 10-12 Å was used. A threshold value of 500 kcal/mol for the ligand Lennard-Jones potential was used in the geometry search [26]. This docking method is independent of hydrogen atoms and partial charges.

De Novo Ligand Design

LigBuilder [30] was used to design new structures having the required functional groups. Structures were built using selected molecular fragments from previous MCSS results [20] as "seed" structures in the "GROW" function of LigBuilder. The binding pocket of Tyk2 was defined by the MCSS fragments defining the Tyk2 pharmacophore. The resulting structures were energy minimised in MOE [23]

(100 iterations with steepest descent, 100 iterations with conjugate gradient and 200 iterations with truncated Newton optimisation) in complex with the homology model of Tyk2 with all receptor atoms fixed. The force field MMFF94 [27] with implicit solvation was used. Following the energy minimisation, the structures were ranked according to binding affinities estimated using the PAS-Dock scoring [26].

The most promising structures from the *de novo* ligand design were tested for similarity to the compounds in the NCI database using the pharmacophore search routines in MOE [23, 24]. To approximate the size of the functional groups of the ligands, the following proximity tolerances were used for the different pharmacophore query features: Aromatic rings: 2.0 Å, hydrophobic groups: 1.8 Å, hydrogen donor or hydrogen acceptor: 0.8 Å.

Testing of Promising Drug Candidates for Selectivity to Tyk2

To test the most promising drug candidates from the pharmacophore search and the *de novo* ligand design for selectivity towards Tyk2, they were docked in the following kinase structures, in addition to the homology models of Tyk2 and Jak2 [20]: RCSB Protein Data Bank (PDB) [18, 19] entries 1ir3 (Insulin-receptor tyrosine kinase in complex with a peptide substrate and an ATP analogue), 1byg (C-terminal Src kinase in complex with an inhibitor), 1fgk (tyrosine kinase domain of Fibroblast growth factor receptor 1), 1fpu (Abelson (Abl) kinase in complex with an inhibitor), 1qcf (Haematopoetic cell kinase (Hck) bound to an inhibitor) and 1qpc (Lymphocyte-specific kinase (Lck) in complex with an inhibitor). The ligand structures were removed from the X-ray structures and the protein structures were aligned to the homology model of Tyk2 in MOE prior to docking. A modified version of the Needleman and Wunsch approach [31] with a structural correction and the Blosum 62 [32] similarity matrix was used for the sequence alignments. The 3D structures were superposed as described by Shapiro *et al.* [33]. The docking analysis was performed with PAS-Dock as described above.

RESULTS AND DISCUSSION

Pharmacophore Search and Docking

The Tyk2 pharmacophore used for searching the NCI database (and for *de novo* ligand design) was based on previously selected molecular fragments from MCSS [20]. These fragments are shown together with the docked conformation of ATP [20] in Fig. (**1**). Pharmacophore searching of the NCI database resulted in 1168 compounds having properties that matched at least six of the specified functionalities, and were placed within 10 Å of the docked conformation of ATP.

Docking with MOE-Dock

The five compounds from the NCI database that were predicted to have the lowest docking energy by MOE-Dock are listed in Table **1**. The docking energies are shown together with the estimated binding affinity to Tyk2 predicted using PAS-Dock scoring. There is good correlation between the docking energies from MOE-Dock and the binding affinity estimated using the PAS-Dock score function for all compounds in Table **1**, except for the compound with NSC number 27773. The correlation coefficient is 0.97 when the data for compound 27773 is kept out of the calculation. Compound 27773 is very hydrophobic compared to the other compounds in Table **1.** The PAS-Dock score function has been shown to predict hydrophobic interactions better than hydrophilic interactions [26]. This might explain the difference between the results obtained with MOE-Dock and PAS-Dock for this compound.

Table 1. Results from Computational Docking of Selected Structures from the NCI Database with MOE-Dock

NSC number	Docking energy from MOE-Dock (kJ/mol)	Estimated binding affinity[a] to Tyk2 (kJ/mol)
40148	-323.0	-2.31
159203	-113.0	-1.20
3766	-37.1	-0.01
29377	-3.31	-0.03
27773	26.3	-4.59

[a] The binding affinity was predicted using the PAS-Dock score function [26].

Comparison of the placement of these ligands in the Tyk2 binding site with the Tyk2 pharmacophore showed that none of the docked conformations of the ligand structures had functional groups completely overlapping with the pharmacophore. The ligand structure with NSC number 40148 is also small, and very flexible. The docked structure of this ligand had groups overlapping with the oxygen-containing sugar ring in the docked conformation of ATP. It is therefore not likely to be Tyk2 selective. The hydrophobic rings of ligand structure 159203 were placed close to benzene rings "5" and "6" from Fig. (**1**), but the ring structures were not overlapping. This may, however, be caused by inaccuracies in the docking analysis. This structure is therefore considered to be a possible drug candidate, in spite of the low estimated binding affinity. The same is true for the ligand structures with NSC numbers 3766 and 29377. The hydrophobic part of ligand structure 27773 was overlapping with benzene ring "5", but in the same way as 40148, this ligand is small and flexible, and therefore not likely to be selective to Tyk2.

The mean experimental binding affinity for the set of structures used to train PAS-Dock [26] was -35 kJ/mol. None of the docked structures from MOE-Dock had estimated binding affinities below -35 kJ/mol. This indicates that even though some of the structures in the NCI database have functional groups that match the Tyk2 pharmacophore, they may not bind very strongly to Tyk2.

For comparison, the binding affinity was also estimated in the same way for six different X-ray structures of protein kinases in complex with known ligands from the PDB [18, 19]. Some of these X-ray structures were used as templates in the homology modelling of Tyk2 [20]. The average estimated binding affinity for these six protein kinase complexes was -18 kJ/mol (Table **2**). None of the compounds from the NCI database shown in Table **1** had estimated binding affinities below -18 kJ/mol. However,

when the 112 docked structures from the last MOE-Dock screening were sorted according to binding affinities to Tyk2 estimated using the PAS-Dock score function (keeping the ligand conformations produced by MOE-Dock), three of the compounds had binding affinities below -18 kJ/mol (Table **3**). The docked conformations of 116725 and 167941 were both placed close to benzene rings "5" and "6", while the docked conformation of 231503 was placed close to fragments "1"-"4" from Fig. (**1**).

Table 2. Estimated Binding Affinities for Protein Kinases in Complex with Known Ligands (from Experimental Structures)

PDB entry	Ligand	Estimated binding affinity[a] (kJ/mol)
1agw	SU4984	-15.9
1fpu	PRC	-29.3
1iep	STI571	-35.3
1ir3	ANP-Mg	-13.0
1k3a	ACP	-9.31
1qpc	ANP	-7.90

[a] The binding affinity was predicted using the PAS-Dock score function [26].

Table 3. The Three Compounds from the NCI Database Having Estimated Binding Affinities to Tyk2 Below -18 kJ/mol

NSC number	Docking energy from MOE-Dock (kJ/mol)	Estimated binding affinity[a] to Tyk2 (kJ/mol)
116725	49.8	-21.7
167941	512.1	-20.5
231503	3073.1	-18.2

[a] The binding affinity was predicted using the PAS-Dock score function [26].

Docking with PAS-Dock

In the same way as for MOE-Dock, docking with PAS-Dock [26] did not identify any compounds from the NCI database with estimated binding affinities to Tyk2 below -35 kJ/mol. However, one of the structures had estimated binding affinity below the average for the six X-ray structures of protein kinase complexes in Table **2** (-18 kJ/mol). The estimated binding affinity to Tyk2 for this compound is given in Table **4**. The docked conformations of this compound from docking with MOE-Dock and PAS-Dock were quite similar (Fig. (**2**)).

Table 4. Results from Computational Docking of Selected Structures from the NCI Database with PAS-Dock [26]

NSC number	Estimated binding affinity to Tyk2 (kJ/mol)
116725	-26.13

Since none of the two docking methods used in this work were able to identify any compounds from the NCI database with estimated binding affinities below the average for the set of X-ray structures used to train PAS-Dock, new structures were generated with *de novo* ligand design, in order to find compounds that bind more strongly to Tyk2.

De Novo Ligand Design

In each LigBuilder run, 200 candidate structures were generated. Benzene rings "1", "5" and "6" (Fig. (**1**)) were used separately as "seed" fragments. Two LigBuilder runs with benzene rings "5" and "6", respectively, and one LigBuilder run with benzene ring "1" were carried out. In total, 1000 structures were generated. Estimation of binding affinities for these structures using the PAS-Dock score function [26] showed that using benzene ring "1" from Fig. (**1**) as "seed" fragment resulted in the most promising drug candidates. In total, 162 of the *de novo* compounds had predicted binding affinities below the mean experimental binding affinity for the set of structures used to train PAS-Dock [26] (-35 kJ/mol). One of these compounds was generated with benzene ring "5" as "seed" fragment (called "5_1"), while all the other compounds were generated with benzene ring "1" as "seed" fragment ("1_1"-"1_161"). Table **5** shows the estimated binding affinities for the structures generated using benzene ring "1" with estimated affinity below -45 kJ/mol (ten structures), together with the one compound generated with benzene ring "5" as "seed" fragment having estimated affinity below -35 kJ/mol. The most promising structures from Table **5** according to Tyk2 selectivity (see Table **6**) are shown in Fig. (**5**).

Table 5. Estimated Binding Affinities to Tyk2 for the Most Promising Drug Candidates Generated by *De Novo* Ligand Design

Ligand structure	Estimated binding affinity[a] to Tyk2 (kJ/mol)
1_1	-47.60
1_2	-46.94
1_3	-46.89
1_4	-46.88
1_5	-46.15
1_6	-45.73
1_7	-45.52
1_8	-45.47
1_9	-45.44
1_10	-45.44
5_1	-35.99

[a] The binding affinity was predicted using the PAS-Dock score function [26].

The results in Table **5** show that the estimated binding affinities for the structures generated with LigBuilder are much stronger than for any of the compounds from the NCI database. Hence, these structures are more likely to be effective as Tyk2 inhibitors. There is, however, no guarantee that they do not bind to other kinases as well. The selectivity of these compounds towards Tyk2 was tested by computational docking.

Table 6. Estimated Binding Affinities (kJ/mol) for the Most Promising Drug Candidates After Docking in Seven Protein Kinase Structures in Addition to the Homology Model of Tyk2

Ligand structure	Estimated binding affinity (kJ/mol)							
	Tyk2	**Jak2**	**1ir3**	**1byg**	**1fgk**	**1fpu**	**1qcf**	**1qpc**
159203	-	-	-	-	-	-	-	-
3766	-	-	-	-	-	-	-	-
29377	-	-	-	-	-	-	-	-
116725	-26.13	-	-33.06	-	-	-	-	-
167941	-	-	-	-	-	-	-	-
231503	-1.73	-	-1.31	-1.30	-	-	-	-1.85
"1_1"	-4.35	-6.50	-5.80	-3.91	-6.19	-1.98	-7.98	-4.79
"1_2"	-49.56	-	-6.61	-	-	-	-6.86	-
"1_3"	-4.46	-6.04	-5.56	-3.81	-5.66	-5.47	-7.93	-
"1_4"	-0.013	-	-	-	-	-	-	-3.60
"1_5"	-5.91	-6.66	-5.59	-3.86	-4.63	-5.24	-7.14	-5.06
"1_6"	-	-	-4.41	-	-	-1.86	-0.93	-
"1_7"	-46.14	-6.27	-5.31	-4.14	-5.87	-5.79	-	-5.66
"1_8"	-22.66	-	-7.22	-	-4.00	-	-	-
"1_9"	-42.56	-5.74	-5.22	-3.76	-4.69	-5.24	-7.08	-4.97
"1_10"	-43.26	-6.89	-5.34	-4.20	-5.0	-0.90	-8.16	-5.47
"5_1"	-4.01	-	-0.0002	-	-0.021	-	-6.89	-

Testing of Promising Drug Candidates for Selectivity to Tyk2

The six most promising structures from the docking analysis with MOE-Dock and the gaussian-based method PAS-Dock (159203, 3766, 29377, 116725, 167941 and 231503), together with the eleven structures in Table **5** were docked in seven protein kinase structures, in addition to Tyk2, using PAS-Dock. The estimated binding affinities are shown in Table **6**. The docking method PAS-Dock was chosen for this study, since it was developed especially for use with homology modelled proteins [26]. The use of gaussian functions gives a representation that may be more robust than force field based methods against small structural errors typically present in homology models. Homology models of Tyk2 and Jak2 were used here.

The entries in Table **6** for which no results are reported indicate ligand placements outside the grid used to estimate the binding affinity. Hence, these ligands are docked outside the binding pocket of the proteins. Ligands binding outside the active site region are less likely to inhibit activity, and therefore not included in this study.

The results in Table **6** indicate that the compound with NSC number 116725 might be a selective inhibitor of Tyk2 and insulin receptor tyrosine kinase. As shown in Fig. (**3 A**), the docked structure of 116725 overlaps with benzene rings "5" and "6" from the Tyk2 pharmacophore shown in Fig. (**1**). Hence, this compound may be a promising drug candidate. The docked conformation of 116725 in the homology model of Tyk2 is shown in Fig. (**3 B**). Fig. (**4**) shows the docked conformation of this compound in insulin receptor tyrosine kinase, together with the ligand in PDB entry 1ir3. As Fig. (**3 B**) and (**4**) indicate, binding of this ligand does not seem to be ATP competitive. The fragments shown in Fig. (**1**) were chosen based on Tyk2 selectivity. Since ATP binds to all protein kinases, choosing a binding site other than the ATP binding site might increase specificity. However, only experimental studies can verify whether binding of these compounds inhibits Tyk2 activity.

Fig. (**3 B**) and Fig. (**4**) show that the orientation of 116725 is different in insulin receptor tyrosine kinase compared to Tyk2, but this compound utilises the same pocket in both structures. Hence, this pocket may not be the best choice in the design of a selective Tyk2 inhibitor. Compounds that bind in the same pocket as fragments "1"-"4" may be more promising. The much stronger estimated binding affinity for the structures generated with LigBuilder using benzene ring "1" as "seed" fragment (Table **5**), and the fact that compound "5_1" is not Tyk2 selective according to the results in Table **6**, support this assumption. Compound 231503 binds in this pocket according to the MOE-Dock study, but the results from docking with PAS-Dock indicated the contrary. According to the results presented in Table **6**, this compound is not Tyk2 selective. None of the other compounds from the NCI database binds in this pocket. Hence, the structures generated with LigBuilder may be more promising as drug candidates. The results from our docking analysis (Table **6**) indicate that five of the structures generated with LigBuilder are selective inhibitors of Tyk2 (Fig. (**5**)).

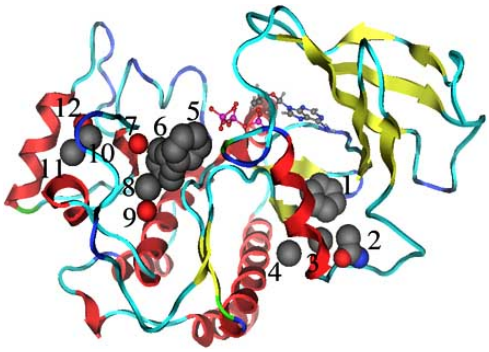


Fig. (1). The Tyk2 pharmacophore used for the database search and *de novo* ligand design. The pharmacophore was defined by fragments from MCSS [20]. The MCSS fragments are numbered from 1 to 12. The docked conformation of ATP [20] is also shown.

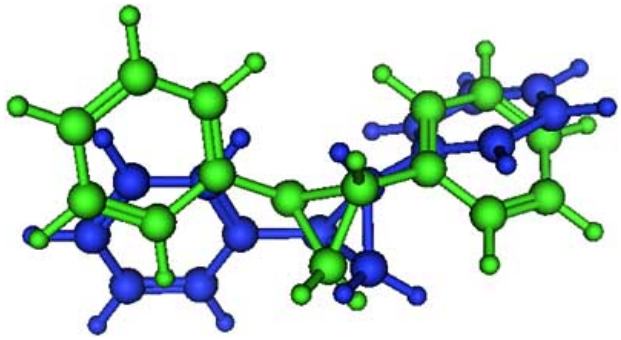
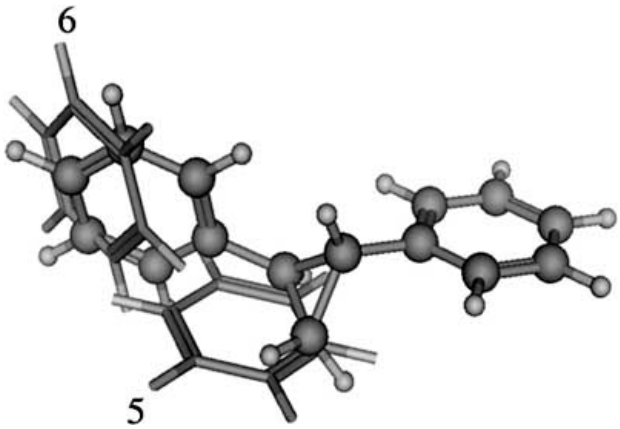


Fig. (2). The docked conformations of 116725 produced by MOE-Dock (green) and PAS-Dock (blue).



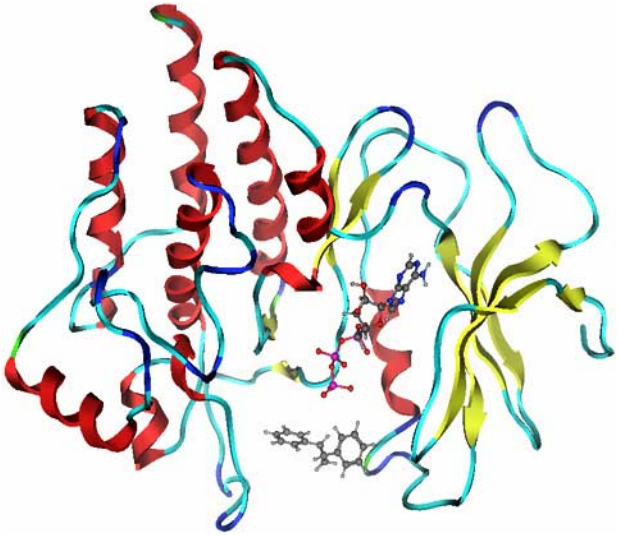


Fig. (3). A: The docked conformation of 116725 in the homology model of Tyk2, together with benzene rings "5" and "6" from the Tyk2 pharmacophore (rotated compared to Fig. (**1**)). The ligand is rendered in "ball and stick", while the fragments are rendered in "stick".

B: The docked conformation of 116725 in the homology model of Tyk2 (rotated compared to Fig. (**1**)), together with the docked conformation of ATP [20]. The docked conformation of ATP is included only for visualisation, and was not included in the docking calculations for compound 116725.

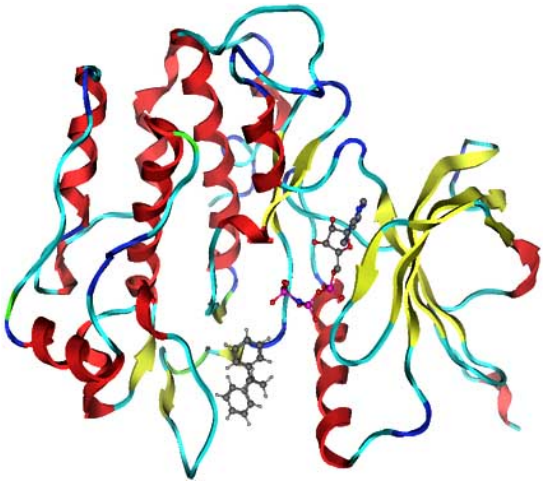


Fig. (4). The docked conformation of 116725 in insulin receptor tyrosine kinase, together with the X-ray structure of insulin receptor tyrosine kinase in complex with ANP-Mg (PDB entry 1ir3). The structure of ANP-Mg is included only for visualisation, and was not included in the docking calculations for compound 116725.

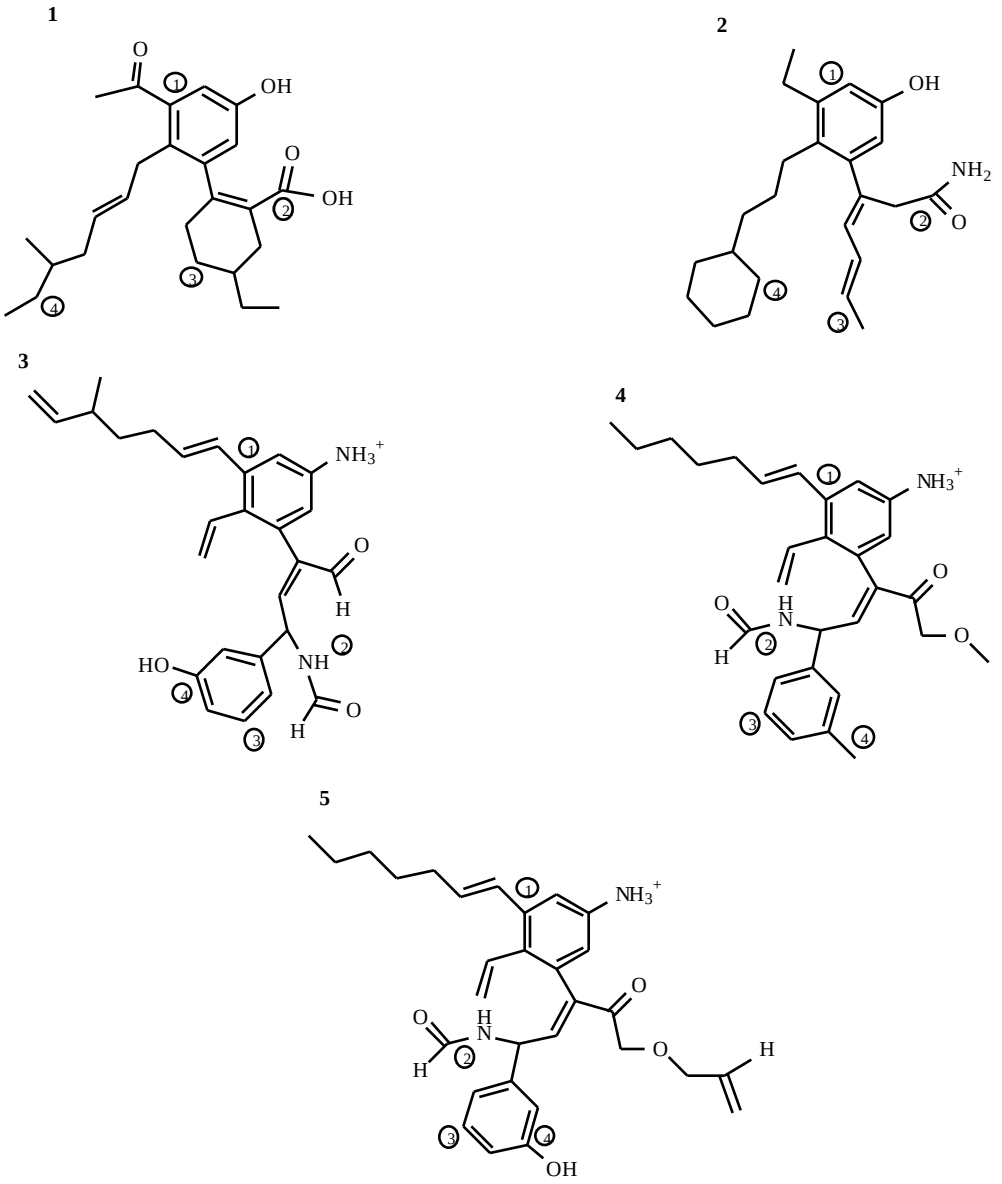


Fig. (5). The structures of the compounds generated by *de novo* ligand design that are Tyk2-selective according to our results ("1_2" (**1**), "1_7" (**2**), "1_8" (**3**), "1_9" (**4**) and "1_10" (**5**), respectively). The fragments from the Tyk2 pharmacophore are indicated by the encircled numbers.

The primary template used in the homology modelling of Tyk2 [20] was the X-ray structure in PDB entry 1qpc (Lck in complex with an inhibitor). A common problem with homology modelling is that the model is more similar to the primary template than to the target protein [34]. The results in Table **6** indicate that this is not the case for the homology model of Tyk2 used here, since there is no strong correlation between the estimated binding affinities for 1qpc and Tyk2. If these protein structures were very similar, one would expect the same compounds to bind to both proteins.

The compounds in the NCI database were searched for similarity to compound (**1**)-(**5**), the most promising structures from the *de novo* ligand design. A match on the pharmacophoric properties of these structures was found for the fourteen structures in Table **7**. These compounds were missed in the original pharmacophore search. In the same way as the compounds in Table **6**, these compounds were docked in the homology model of Tyk2 and seven other protein kinase structures. The results are given in Table **7**.

As the results in Table **7** indicate, none of the compounds in the NCI database found to resemble the most promising structures from the *de novo* ligand design show selectivity towards Tyk2. They all have relatively low Tyk2 activity. Compound 624404 potentially binds to Jak2 and Abl kinase (PDB entry 1fpu). The binding to Jak2 might be an artefact, as the X-ray structure in PDB entry 1fpu (Abl kinase bound to an inhibitor) was the primary template used for the homology modelling of Jak2 [20].

CONCLUSION

The NCI database has been screened for compounds with potential for selective binding to Tyk2, using two different

Table 7. Estimated Binding Affinities (kJ/mol) from Docking of the Compounds in the NCI Database Resembling the Most Promising Structures from *De Novo* Ligand Design

NSC number	Resembling structure	Estimated binding affinity (kJ/mol)							
		Tyk2	Jak2	1ir3	1byg	1fgk	1fpu	1qcf	1qpc
340033	"1_2"	-2.22	-	-3.84	-1.81	-	-4.05	-6.45	-3.53
372408	"1_2"	-4.01	-5.31	-3.72	-2.53	-4.16	-4.17	-	-3.69
372452	"1_2"	-3.71	-5.28	-	-2.34	-1.02	-4.27	-7.21	-3.66
623329	"1_2"	-3.50	-6.61	-6.71	-2.36	-5.71	-5.95	-8.78	-5.39
624404	"1_2"	-3.96	-18.49	-5.95	-9.17	-3.84	-15.77	-5.16	-8.84
627686	"1_2"	-	-	-	-	-	-	-	-
629605	"1_7"	-	-5.69	-5.54	-3.56	-2.72	-	-8.94	-4.90
25585	"1_8"	-2.68	-	-0.27	-	-	-2.67	-	-2.79
119957	"1_8"	-0.32	-	-	-	-	-8.79	-	-
138557	"1_8"	-	-	-	-	-	-	-	-
142574	"1_8"	-	-	-	-	-	-	-	-
157622	"1_8"	-	-	-0.66	-	-	-	-	-
203969	"1_8"	-	-	-	-	-	-	-	-
633715	"1_9"	-	-	-	-	-	-	-	-

docking methods. The results from the docking analysis indicated that none of the structures present in the NCI database can be used to inhibit Tyk2 selectively. Even though the two docking methods did not give the same ranking of the drug candidates, they both produced the same conclusion, namely that there are no promising Tyk2 inhibitors in the NCI database. The main purpose of docking methods is to identify the most active compounds. Most docking methods (as these two) are also trained using X-ray structures of protein-ligand complexes. Hence, internal ranking of inactive compounds is bound to fail, and not interesting for drug design purposes. This may be the reason why the two docking methods ranked the compounds in the NCI database differently. However, this analysis provides useful information about parts of the structures that may be used as functional groups of a selective inhibitor of Tyk2, and one compound was found to be a potential selective inhibitor of Tyk2 and insulin receptor tyrosine kinase. Several promising structures were proposed by *de novo* ligand design. These were tested for selectivity towards Tyk2 by computational docking in seven protein kinase structures, in addition to Tyk2. This study indicated that five of the generated structures might be potential selective inhibitors of Tyk2.

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REFERENCES

[1] Johnson, L.N.; Noble, M.E.M.; Owen, D.J. *Cell* **1996**, *85*, 149.
[2] Ihle, J.N.; Witthuhn, B.A.; Quelle, F.W.; Yamamoto, K.; Silvennoinen, O. *Annu. Rev. Immunol.* **1995**, *13*, 369.
[3] Pellegrini, S.; Dusanter-Fourt, I. *Eur. J. Biochem.* **1997**, *48*, 615.
[4] Van der Geer, P.; Hunter, T.; Lindberg, R.A. *Annu. Rev. Cell Biol.* **1994**, *10*, 251.
[5] Richter, M.F.; Dumenil, G.; Uze, G.; Fellous, M.; Pellegrini, S. *J. Biol. Chem.* **1998**, *273*, 24723.
[6] Harpur, A.G.; Andres, A.C.; Ziemiecki, A.; Aston, R.R.; Wilks, A.F. *Oncogene* **1992**, *7*, 1347.
[7] Yan, H.; Piazza, F.; Krishnan, K.; Pine, R.; Krolewski, J.J. *J. Biol. Chem.* **1998**, *273*, 4046.
[8] Hubbard, S.R.; Till, J.H. *Annu. Rev. Biochem.* **2000**, *69*, 373.
[9] Heldin, C.H. *Cell* **1995**, *80*, 213.
[10] Schlessinger, J.; Ullrich, A. *Neuron* **1992**, *9*, 383.
[11] Carter-Su, C.; Smit, L.S. *Recent. Prog. Horm. Res.* **1998**, *53*, 61.
[12] Xuan, Y.T.; Guo, Y.R.; Han, H.; Zhu, Y.Q.; Bolli, R. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 9050.
[13] Anderson, K. *Semin. Oncol.* **1999**, *26*, 10.
[14] Meydan, N.; Grunberger, T.; Dadi, H.; Shahar, M.; Arpaia, E.; Lapidot, Z.; Leeder, J.S.; Freedman, M.; Cohen, A.; Gazit, A.; Levitzki, A.; Roifman, C.M. *Nature* **1996**, *379*, 645.
[15] Wang, L.H.; Kirken, R.A.; Erwin, R.A.; Yu, C.R.; Farrar, W.L. *J. Immunol.* **1999**, *162*, 3897.
[16] Lindauer, K.; Loerting, T.; Liedl, K.R.; Kroemer, R.T. *Protein Eng.* **2001**, *14*, 27.
[17] Schindler, T.; Bornmann, W.; Pellicena, P.; Miller, W.T.; Clarkson, B.; Kuriyan, J. *Science* **2000**, *289*, 1938.
[18] The RCSB Protein Data Bank, http://www.rcsb.org/pdb/.
[19] Berman, H.M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T.N.; Weissig, H.; Shindyalov, I.N.; Bourne, P.E. *Nucl. Acids Res.* **2000**, *28*, 235.
[20] Tøndel, K.; Anderssen, E.; Drabløs, F. *J. Comput. Aided Mol. Des.* **2002**, *16*, 831.
[21] Miranker, A.; Karplus, M. *Proteins Struct. Func. Genet.* **1991**, *11*, 29.
[22] Wieman, H.; Tøndel, K.; Anderssen, E.; Drabløs, F. *Mini-Rev. Med. Chem.* **2004**, *4*, 793.

[23] Molecular Operating Environment[ä], Version 2002.03, *Chemical Computing Group, Inc.*, **2002**.
[24] Bush, B.L.; Sheridan, R.P. *J. Chem. Inf. Comp. Sci.* **1993**, *33*, 756.
[25] Baxter, C.A.; Murray, C.W.; Clark, D.E.; Westhead, D.R.; Eldridge, M.D. *Proteins Struct. Funct. Genet.* **1998**, *33*, 367.
[26] Tøndel, K.; Anderssen, E.; Drabløs, F. Protein Alpha Shape (PAS) Dock: A new gaussian-based score function suitable for docking in homology modelled protein structures. Unpublished results.
[27] Halgren, T.A. *J. Comp. Chem.* **1996**, *17*, 490.
[28] Halgren, T.A. *J. Comp. Chem.* **1999**, *20*, 730.
[29] Hall, L.H.; Kier, L.B. In *Reviews in Computational Chemistry*; Lipkowitz, K. B.; Boyd, D.B., Eds.; VCH Publishers: New York, **1991**; Vol. *2*, pp. 367-422.
[30] Wang, R.; Gao, Y.; Lai, L. *J. Mol. Model.* **2000**, *6*, 498.
[31] Needleman, S. B.; Wunsch, C. D. *J. Mol. Biol.* **1970**, *48*, 443.
[32] Henikoff, S.; Henikoff, J.G. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 10915.
[33] Shapiro, A.; Botha, J.D.; Pastore, A.; Lesk, A.M. *Acta Cryst.* **1992**, *A48*, 11.
[34] Marti-Renom, M.A.; Stuart, A.C.; Fiser, A.; Sanchez, R.; Melo, F.; Sali, A. *Annu. Rev. Biophys. Biomol. Struct.* **2000**, *29*, 291.