Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medicinal Chemistry 42 (2007) 1102–1108
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Original article

Structure-based discovery of new small molecule inhibitors of low molecular weight protein tyrosine phosphatase

David Vidal [a], Jascha Blobel [a], Yolanda Pérez [a], Michael Thormann [b,1], Miquel Pons [a,c,*]

[a] *Laboratory of Biomolecular NMR, Institute for Research in Biomedicine (IRB), Barcelona Science Park, Josep Samitier 1-5, 08028 Barcelona, Spain*
[b] *Origenis GmbH, Am Klopferspitz 19a, 82152 Martinsried, Germany*
[c] *Departament de Química Orgànica, Universitat de Barcelona, Martí i Franquès 1-11, 08028 Barcelona, Spain*

Received 21 November 2006; received in revised form 11 January 2007; accepted 16 January 2007
Available online 27 January 2007

Abstract

The application of a fully integrated and automated virtual screening method for identifying potential and novel inhibitors of bovine lmwPTP is described. The protocol makes extensive use of our recently introduced LINGO tools, which allow the extraction of the implicit chemical information present in SMILES representations. Nine out of 34 compounds selected from a database of almost 500 000 commercially available compounds were experimentally confirmed to be competitive inhibitors of lmwPTP, two of them showing K_i values around 10 μM. The best inhibitors previously described had K_i values higher than 1 mM. These results constitute an experimental validation of our virtual screening algorithm and provide a basis for the optimization of pharmacologically interesting lmwPTP inhibitors.

Keywords: lmwPTP; Phosphatase inhibitors; Docking; Virtual screening; MPA; LINGO

1. Introduction

Virtual screening methodologies are widely used as a complementary approach to experimental screening (high-throughput screening) in many drug discovery and development programs to enhance the probability of success in the stage of lead identification [1–4]. Computational methodologies can also reduce the high failure rates due to unfavourable properties at the screening stage (e.g. poor solubility) or at later stages (unfavourable ADME properties). Current applications of virtual screening methodologies involve the analysis of very large collections of available compounds to select a reasonable subset for biological testing. For receptors of known structure, massive docking strategies may be used for the identification of novel lead compounds in drug discovery [5–7]. Previous knowledge, such as already known ligands or the identity of key residues in the receptor may be used to focus and increase the success rate of virtual screening algorithms.

Receptor-based virtual screening requires computational fitting of compounds into an active site of a receptor. Therefore, elaborated search algorithms followed by scoring and ranking techniques are used to identify potential leads in a process that tends to be computationally demanding [8]. On the other hand, ligand-based methodologies [9,10] are used when the structure of a therapeutical target has not been solved yet. They tend to be faster than receptor-based methods, as has been shown by molecular similarity methods [11].

Abbreviations: ADME, absorption, distribution, metabolism and excretion; lmwPTP, low molecular weight protein tyrosine phosphatase; HTS, high-throughput screening; MPA, massive processing algorithm; SMILES, Simplified Molecular Input Line Entry System.

* Corresponding author. Departament de Química Orgànica, Universitat de Barcelona, Martí i Franquès 1-11, 08028 Barcelona, Spain. Tel.: +34 93 4034683; fax: +34 93 4039976.
E-mail address: mpons@ub.edu (M. Pons).
[1] Tel.: +49 89 7801676 0; fax: +49 89 7801676 777.

0223-5234/$ - see front matter © 2007 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2007.01.017

Recent developments in combinatorial chemistry and high-throughput screening (HTS) have contributed to the synthesis of a large number of compounds included in databases of molecular structures. In spite of the advances in HTS methods, the cost of exploring the very large databases of available molecules is enormous and the success rates are commonly very low [12], especially in the field of phosphatases [6]. The size of the databases is constantly increasing with the widespread use of combinatorial chemistry approaches. Even receptor-based virtual screening methods using the fastest-working docking algorithms and supercomputers, remain a very time consuming task for databases of millions of compounds. A realistic approach towards high-throughput receptor-based virtual screening requires the restriction of the number of compounds to be actually analyzed to a target-focused small fraction of the database [13]. In this context, faster and more efficient virtual exploration protocols have to be used to allow an evaluation of thousands of compounds in a short period of time [14]. We have recently reported a new virtual screening algorithm that combines exhaustive similarity searches directly in SMILES (Simplified Molecular Input Line Entry System) format using our previously described extremely efficient LINGO tools [15,16] with the docking of flexible ligands, whose 3D structures are generated on the fly from the SMILES representation. This algorithm, named MPA, has been specifically designed to analyze molecular databases containing millions of compounds in a target-dependent manner [17].

In this paper we report the validation of our massive processing algorithm (MPA) virtual screening algorithm in an *in vitro* experimental screening for the identification of inhibitors of bovine low molecular weight protein tyrosine phosphatase (lmwPTP) [18]. Living organisms employ protein phosphorylation, carried out by kinases, and dephosphorylation reactions, carried out by phosphatases, for the regulation of innumerable cellular processes [19]. It is well known that aberrances in protein phosphorylation contribute to the development of many human diseases such as cancer and diabetes [20]. In humans, lmwPTPs are ubiquitously expressed as four different isoforms [21] in a wide range of cells and may be involved in regulating the metastatic nature of epithelial tumors. Recent studies have demonstrated that lmwPTP overexpression is sufficient to transform non-transformed epithelial cells, suggesting that inhibition of lmwPTP could pharmaceutically control their activity in oncogenic cells [22]. Bovine lmwPTP shows a 94% sequence identity with human lmwPTP-B.

Although protein tyrosine phosphatases (PTPs) have been extensively studied and a large amount of inhibitors have been described for many PTPs including PTP1B [23], Cdc25A [24], Cdc25B [25] or CD45 [26], only a few compounds have been described as weak inhibitors of low molecular weight protein tyrosine phosphatases. In this paper, we report the use of an integrated and fast virtual screening protocol useful for the identification of competitive inhibitors of lmwPTP, some of them inhibiting the lmwPTP activity in the low micromolar range.

2. Computational methodology

2.1. Compound database preparation

The initial database, saved in SMILES format, was prepared by selecting all of the 450 212 compounds present in the ChemDiv [27] collection (version 2004) as well as all of the 425 654 compounds from the ChemBridge [28] collection (version 2005), generating thus a virtual database of 875 866 compounds. The initial database was first analyzed in order to detect and remove duplicated molecules and those molecules containing elements other than C, N, O, S, H, P and halides, for which no parameterization exists in Autodock 3.0 [29,30]. The remaining 736 793 compounds were filtered by applying molecular property filters to eliminate compounds with undesired molecular properties using our previously reported LINGO-QSPR models, which allow a throughput of 120 000 molecules per second [15]. The retained database contained 497 157 compounds in SMILES format with molecular weights ≤ 500 Da; $\log P \leq 5$; hydrogen-bond acceptors ≤ 10; hydrogen-bond donors ≤ 5; polar surface areas (PSA) ≤ 150 Å^2; and rotational bonds ≤ 8.

2.2. Protein structure preparation

The bovine-lmwPTP X-ray structure was downloaded from the Protein Data Bank (PDB code: 1DG9 [31]). The nucleophilic residue Cys^{12} side chain, which has a remarkably low $pK_a < 4$, was set to its ionic thiolate form as proposed in several previous publications [32]. The HEPES molecule present in the X-ray structure was removed. Hydrogens were added and partial atomic charges were assigned using the MAB force field as implemented in Moloc [33]. Grids were build up and centered in the active site of the protein, resulting in a three-dimensional box of $20.4 \times 20.1 \times 19.8$ Å^3 surrounding all amino acids of the active site groove. Grid maps for C, N, O, S, H, P and halides were calculated using Autogrid 3.0.

2.3. Virtual screening

Our previously reported MPA [17] was used to computationally analyze the database containing 497 157 compounds. The MPA is a highly efficient virtual search protocol that, with very limited computational resources, is able to handle very large databases using a combination of slow protein-based and very fast ligand-based screening techniques. The protocol makes extensive use of our recently introduced LINGO tools, which allow the extraction of the implicit chemical information present in SMILES [34] representations. A working scheme of MPA is shown in Fig. 1.

The key element of MPA is the selection of the molecules in the database that will be evaluated by docking. Only the molecules to be docked are explicitly converted to their 3D structures. In this study, the maximum number of compounds to be docked was restricted to 50 000 (10% of database).

The complete database is screened at each selection step by similarity to the molecules that showed the best docking

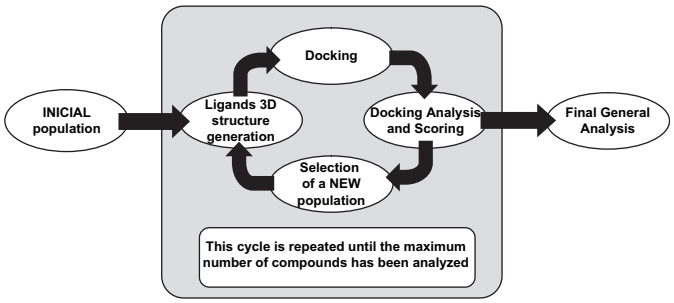


Fig. 1. Massive processing algorithm (MPA) general scheme.

energies in previous steps. Similarities are directly evaluated from SMILES representations by LINGOsim.

As described in the MPA original work [17], the starting population consisted in a simple random selection of molecules from the database. Starting from this population of molecules chosen randomly from the database, binding energies are extracted from the output of a modified Autodock 3.0 docking algorithm. The empirical free energy function implemented in Autodock 3.0 takes into account the ligand–receptor interaction energy, conformational entropy loss and desolvation effects.

The ranked list is used as the input to select the new population from a database, in a way reminiscent of a standard genetic algorithm, whereas no offspring is generated by parents but the new generation is instead *adopted*: candidate molecules are not created using the information contained in a previous population but are directly selected from the initial database according to their similarity. Ligands to be docked are automatically prepared from SMILES. First, the protonation state of ionizable groups is determined by using the *cxcalc* module of *JChem* [35] software, which calculates the major microspecies at a given pH, giving thus the corresponding modified SMILES codes. Next, geometry-optimized 3D coordinates and partial atomic charges are calculated with the MAB force field by using the *Msmab* module of *Moloc* software. The rotatable bonds are identified by using the *autotors* module of *Autodock*. Finally, a home-made program additionally constrains torsions with high barriers, like C_{sp2}–C_{sp2} bonds.

3. Results

3.1. Virtual screening analysis

MPA was used to select 50 000 molecules to be analyzed by Autodock 3.0. from an edited database containing 497 157 compounds. The top scoring 1289 molecules with estimated $\Delta G_{binding} \leq -8.00$ kcal/mol (estimated K_i below 1 μM) were selected for further analysis. Three-dimensional coordinates for the binding conformation of all 1289 molecules were extracted and the corresponding enzyme–inhibitor complexes analyzed by a completely automated process using LIGPLOT [36], and 767 complexes forming hydrogen bonds with the active site residues Gly^{14}, Ile^{16}, la Cys^{17} and Arg^{18} were selected. Solubilities were estimated using LlogS, a program included in our previously reported LINGO toolkit [15] and only compounds with a predicted minimum solubility higher than 100 μM were retained.

The remaining 274 complexes were energetically and structurally analyzed and cross-validated using the MAB force field as implemented in Moloc. This process led to the elimination of undesired compounds, such as adamantane, and molecules having energetically unfavourable binding modes. One hundred and sixty-two out of 274 compounds, all of them exhibiting functional groups such as carboxylic acids, nitro groups, sulfones and/or sulfates with hydrogen-bond capabilities to interact with the specific residues in the active site, passed the validation and visual inspection step. The final 162 virtual screening hits were clustered/joined using a molecular structure-dependent hierarchical clustering algorithm. Cross-similarities were calculated using LINGOsim and analyzed by the program Cluster 3.0 [37], identifying 24 different clusters, ranging from two members to 26 with a median of six.

3.2. First subset selection and experimental screening

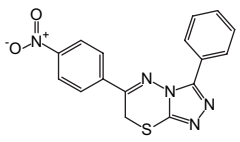
A subset of 21 compounds, belonging to 19 of the 24 clusters, was selected for initial experimental estimation of their K_i values. All of these compounds passed the final validation step and were commercially accessible at the time of the experiment. When different molecules from the same cluster were available, the ones predicted to have better affinity, to be more soluble and structurally simpler, were chosen. All these 21 compounds were experimentally analyzed and four of them were identified to have inhibition constant values in the range of 50–900 μM. Molecular structures for the 21 analyzed compounds, the identification code and the corresponding cluster number are shown in Fig. 2.

3.3. Second subset selection and experimental screening

Following the initial experimental screening, further members of clusters 3, 14, 22 and 24, containing compounds ptp-04, ptp-12, ptp-19 and ptp-21, were selected using molecular similarity criteria. Like the MPA, this follows the Active Analogue Principle, but using experimental, instead of virtual, inhibition constants. These compounds, shown in Fig. 3, were experimentally tested, and five of them were identified to exhibit inhibition constant values in the range of 10–900 μM.

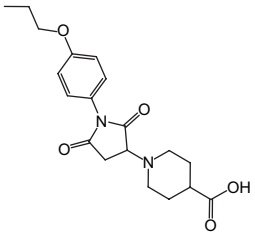
4. Discussion

New small molecule inhibitors of bovine lmwPTP have been discovered using our virtual screening MPA algorithm. The non-phosphorous inhibitors found belong to different structural classes and clearly show competitive inhibition at low micromolar concentration. The analysis of the calculated structures of experimentally validated complexes provides some insight into the structural determinants of lmwPTP inhibitors. The best inhibitor found in this study ptp-194 with an inhibition constant of 9 ± 3 μM, shows a hydrogen-bond



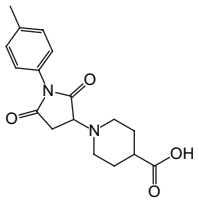
ptp-01 (**1**)

i.n.d.



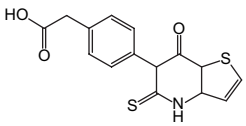
ptp-02 (**2**)

i.n.d.



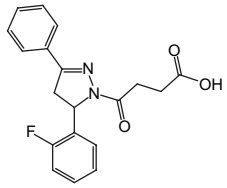
ptp-03 (**2**)

i.n.d.



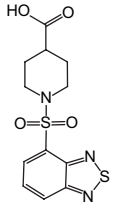
ptp-04 (**3**)

K_i = 880 ± 330 μM



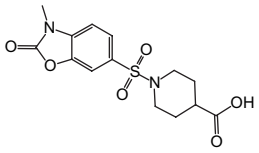
ptp-05 (**4**)

K_i > 1 mM(*)



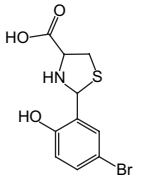
ptp-06 (**5**)

i.n.d.



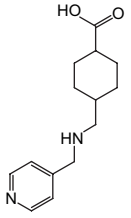
ptp-07 (**7**)

K_i > 1 mM(*)



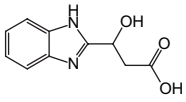
ptp-08 (**8**)

i.n.d.



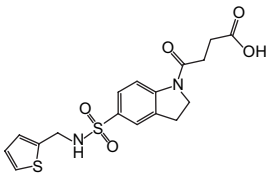
ptp-09 (**8**)

i.n.d.



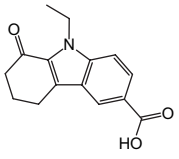
ptp-10 (**10**)

i.n.d.



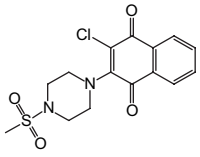
ptp-11 (**11**)

K_i > 1 mM(*)



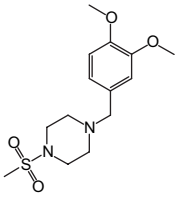
ptp-12 (**14**)

K_i = 610 ± 90 μM



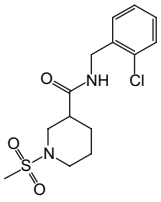
ptp-13 (**15**)

i.n.d.



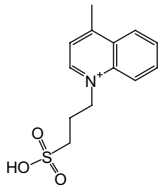
ptp-14 (**16**)

i.n.d.



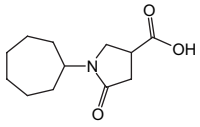
ptp-15 (**17**)

i.n.d.



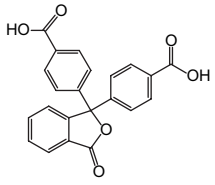
ptp-16 (**19**)

i.n.d.



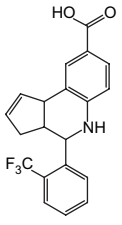
ptp-17 (**20**)

i.n.d.



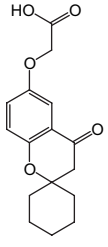
ptp-18 (**9**)

i.n.d.



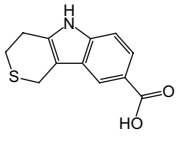
ptp-19 (**22**)

K_i = 50 ± 10 μM



ptp-20 (**23**)

i.n.d.



ptp-21 (**24**)

K_i = 240 ± 70 μM

Fig. 2. Molecular structures and experimental inhibition constants of the 21 compounds obtained in the first selection. (*) Indicates that a low degree of inhibition was detected (i.n.d. = inhibition not detected).

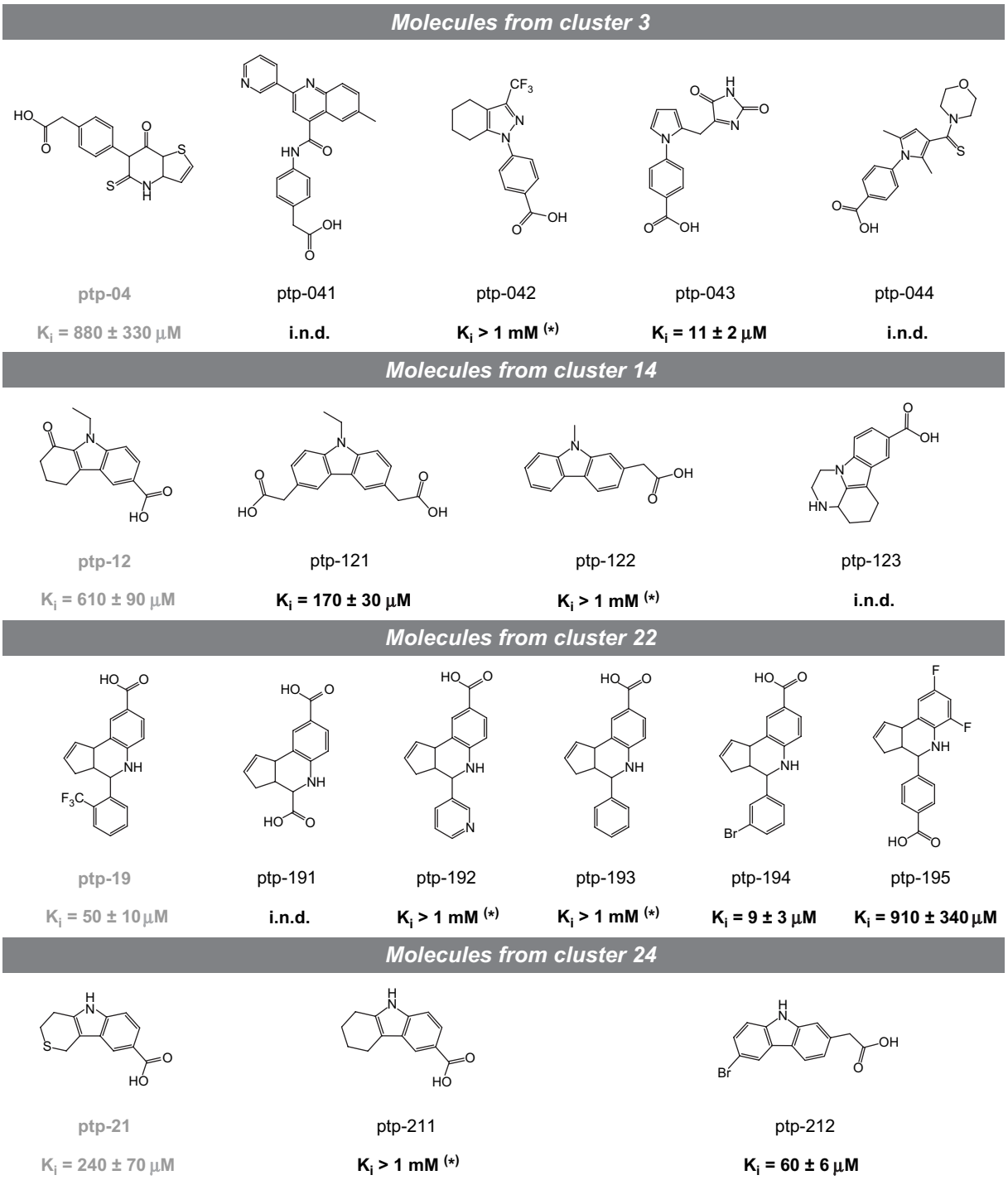


Fig. 3. Molecular structures and inhibition constants of the 14 compounds obtained from the second selection. (*) Indicates that a low degree of inhibition was detected (i.n.d. = inhibition not detected).

network linking the carboxylate group of the ligand with residues in the active site involved in the recognition of the phosphate group of phosphotyrosine, Leu[13], Ile[16], Cys[17] and Arg[18]. Further stability of the complex results from shape complementarity and van der Waals interaction with residues surrounding the catalytic site, as for example, Tyr[131], Gly[14] and Trp[49]. On the other hand, compound ptp-043, which has an inhibition constant of 11 ± 2 μM, presents very different interactions in the same region of the enzyme. Although the carboxylate group of the ligand is crucial for the interaction forming hydrogen bonds with the same residues that participated in hydrogen bonding with compound ptp-194, except Leu[13], ptp-043 interacts with a second region, that presents a good shape complementarity to the ligand, by forming a hydrogen bond with Ser[47](Fig. 4).

Compound ptp-212, with an inhibition constant of 60 ± 6 μM, forms hydrogen bonds through its carboxylate group with Leu[13], Gly[14] and Arg[18]. An additional hydrogen

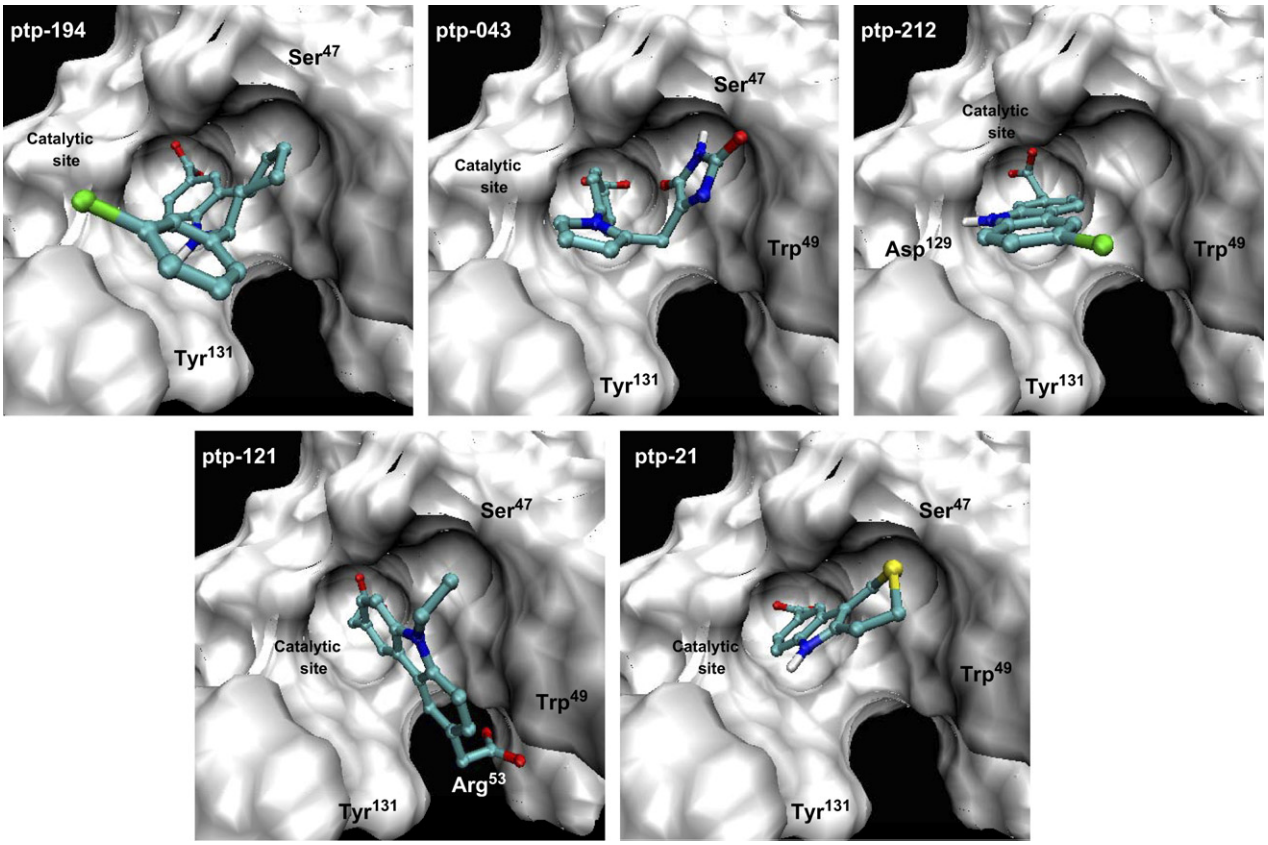


Fig. 4. Binding conformations of compounds ptp-194, ptp-043, ptp-212, ptp-121 and ptp-21 obtained from docking calculations.

bond is formed between the ligand's heterocyclic nitrogen and the side chain of Asp^{129} placed close to the active site. This residue is critical in the dephosphorylation mechanism as it is involved in the correct release of the substrate. Compound ptp-121, exhibiting a $K_i = 170 \pm 30$ μM, has a C_2 symmetry axis in its free form. The two carboxylic acids are separated by a rigid scaffold. While one of them is hydrogen bonded to residues Gly^{14}, Ile^{16}, Cys^{17} and Arg^{18}, the second one forms an electrostatic interaction with the side chain of Arg^{53} and a hydrogen bond with Asn^{50}.

As a last example, compound ptp-21, with an inhibition constant of 240 ± 70 μM, binds through a sulfur atom to the region occupied by Ser^{47}, as shown before by compound ptp-043. This hydrogen bond seems to be important for inhibition as shown by a decrease of K_i to about 1 mM when this sulfur atom is substituted for a methylene group in compound ptp-211.

Very few inhibitors are known for the closely related human lmwPTP isoforms A and B. In the case of the human lmwPTP-B, which has 94% sequence homology to bovine lmwPTP, different inhibitors containing a phosphonic acid have been described but their inhibition constants are higher than 1 mM, ranging from 1.4 to 13 mM [38]. Docking of these compounds shows that they would have been chosen as promising candidates in virtual screening (data not shown).

The results of the screening for active site inhibitors of bovine lmwPTP provide another validation of the MPA algorithm for handling large databases through fully integrated and completely automated virtual screening. In this project, the MPA was efficiently applied to reduce the number of compounds to be tested experimentally from 500 000 to 35 compounds, nine of which showed micromolar inhibition constants. From them, two compounds with different scaffolds showed K_i values around 10 μM and four additional molecules with K_i between 50 and 250 μM. Thus, MPA has successfully identified novel small molecule inhibitors with different scaffolds that were experimentally validated to be competitive inhibitors and could, therefore, provide starting points for the development of pharmacologically optimized inhibitors.

5. Experimental protocols

5.1. Bovine-lmwPTP expression and purification

pET-11d vector harboring the wild type bovine-lmwPTP gene was transformed into *Escherichia coli* BL21(DE3) cells and expressed in LB media as described before [39]. Purification steps included a cation exchange column (SP Sepharose High Performance) followed by size exclusion chromatography (Superdex 75) and the purity was checked after each purification step by SDS PAGE. The final protein concentration was determined by UV absorption at 280 nm by diluting the desired solution 1/40 in a buffer containing 6 M guanidinium and 20 mM sodium phosphate at pH = 6.5. The average of three measurements of buffer only was subtracted from the average of three measurements of protein solution and the

concentration determined by the relationship 1AU (A_{280}) = 0.84(±0.05) g/l [40].

5.2. Enzymatic activity

Kinetic parameters were measured at 37 °C in 40 mM sodium acetate buffer at pH 5.0 containing 150 mM NaCl and 1 mM TCEP (tris[2-carboxyethyl]phosphine) assuring complete reduction of the active site Cys^{12}. *p*-Nitrophenyl phosphate (*p*NPP) was used as the substrate and the reaction rate was determined by monitoring the release of *p*-nitrophenolate by measuring the absorbance at a wavelength of 405 nm as described before [39]. A constant concentration of 10 nM bovine lmwPTP was used at the five final *p*NPP concentrations of 0.4, 0.8, 1.2, 1.6 and 2.0 mM. Each velocity was measured three times and the V_{max} and K_m values were obtained giving $K_m = 0.57 \pm 0.03$ mM and $V_{max} = 21.3 \pm 0.6$ μmol/min, which is in good agreement with previous results [39,41].

5.3. Measurement of inhibition constants K_i

Measurements of the dissociation constants (K_i) of the enzyme–inhibitor complexes were carried out at pH 5.0 and 37 °C using the same reaction buffer as mentioned before. All inhibitors were stored at 100 mM in DMSO. Inhibition constants were determined at constant concentration of 10 nM bovine lmwPTP, five different *p*NPP concentrations (0.4, 0.8, 1.2, 1.6 and 2.0 mM) and four to five suitable inhibitor concentrations. DMSO concentration was kept constant at 1% throughout all measurements. Details, such as Lineweaver–Burk plots and experimental data, for the measurement of K_i can be found in the Supplementary material.

Acknowledgements

This work was partially supported by funds from the Spanish Ministerio de Educación y Ciencia-FEDER (BIO2004-5436, PTR1995-0795-OP, GEN2003-20642-C09-04). D.V. and J.B. acknowledge predoctoral fellowships from the Spanish Ministerio de Educación y Ciencia.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2007.01.017.

References

[1] K.H. Bleicher, H.J. Böhm, K. Müller, A.I. Alanine, Nat. Rev. Drug Discov. 2 (2003) 369–378.
[2] J.C. Alvarez, Curr. Opin. Chem. Biol. 8 (2004) 365–370.
[3] J. Mestres, Biochem. Soc. Trans. 30 (2002) 797–799.
[4] M.H.J. Seifert, K. Wolf, D. Vitt, BIOSILICO 1 (2003) 143–149.
[5] P. Burkhard, U. Hommel, M. Sanner, M.D. Walkinshaw, J. Mol. Biol. 287 (1999) 853–858.
[6] T.N. Doman, S.L. McGovern, B.J. Witherbee, T.P. Kasten, R. Kurumbail, W.C. Stallings, D.T. Connolly, B.K. Shoichet, J. Med. Chem. 45 (2002) 2213–2221.
[7] C.N. Cavasotto, M.A. Ortiz, R.A. Abagyan, F.J. Piedrafita, Bioorg. Med. Chem. Lett. 16 (2006) 1969–1974.
[8] D.B. Kitchen, H. Decornez, J.R. Furr, J. Bajorath, Nat. Rev. Drug Discov. 3 (2004) 935–949.
[9] J.S. Mason, A.C. Good, E.J. Martin, Curr. Pharm. Des. 7 (2001) 567–597.
[10] R.D. Cramer, D.E. Patterson, J.D. Bunce, J. Am. Chem. Soc. 110 (1988) 5959–5967.
[11] P. Willett, J.M. Barnard, G.M. Downs, J. Chem. Inf. Comput. Sci. 38 (1998) 983–996.
[12] R. Macarron, Drug Discov. Today 11 (2006) 277–279.
[13] W.P. Walters, M.T. Stahl, M.A. Murcko, Drug Discov. Today 3 (1998) 160–178.
[14] T. Lengauer, C. Lemmen, M. Rarey, M. Zimmermann, Drug Discov. Today 1 (2004) 27–34.
[15] D. Vidal, M. Thormann, M. Pons, J. Chem. Inf. Model. 45 (2005) 386–393.
[16] J.A. Grant, J.A. Haigh, B.T. Pickup, A. Nicholls, R.A. Sayle, J. Chem. Inf. Model. 46 (2006) 1912–1918.
[17] D. Vidal, M. Thormann, M. Pons, J. Chem. Inf. Model. 46 (2006) 836–843.
[18] M. Zhang, R.L. Van Etten, C.V. Stauffacher, Biochemistry 33 (1994) 11097–11105.
[19] T. Hunter, Cell 100 (2000) 113–127.
[20] L. Bialy, H. Waldmann, Angew. Chem. Int. Ed. 44 (2005) 3814–3839.
[21] P. Tailor, J. Gilman, S. Williams, T. Mustelin, Eur. J. Biochem. 262 (1999) 277–282.
[22] K.D. Kikawa, D.R. Vidale, R.L. Van Etten, M.S. Kinch, J. Biol. Chem. 277 (2002) 39274–39279.
[23] Y.A. Puius, Y. Zhao, M. Sullivan, D.S. Lawrence, S.C. Almo, Z.Y. Zhang, Proc. Natl. Acad. Sci. 94 (1997) 13420–13425.
[24] P.J. Scrivens, M.A. Alaoui-Jamali, G. Giannini, T. Wang, M. Loignon, G. Batist, V.A. Sandor, Mol. Cancer Ther. 2 (2003) 1053–1059.
[25] K.R. Kim, J.L. Kwon, J.S. Kim, Z. No, H.R. Kim, H.G. Cheon, Eur. J. Pharmacol. 528 (2005) 37–42.
[26] R.A. Urbanek, S.J. Suchard, G.B. Steelman, K.S. Knappenberger, L.A. Sygowski, C.A. Veale, M.J. Chapdelaine, J. Med. Chem. 44 (2001) 1777–1793.
[27] ChemDiv Inc. San Diego (CA) USA,<http://www.chemdiv.com>.
[28] ChemBridge Research Laboratories, Inc. San Diego (CA) USA.http://chembridge.com.
[29] G.M. Morris, D.S. Goodsell, R.S. Halliday, R. Huey, W.E. Hart, R.K. Belew, A.J. Olson, J. Comput. Chem. 19 (1998) 1639–1662.
[30] M. Thormann, M. Pons, J. Comput. Chem. 22 (2001) 1971–1982.
[31] M. Zhang, M. Zhou, R.L. Van Etten, C.V. Stauffacher, Biochemistry 36 (1997) 15–23.
[32] B. Evans, P.A. Tishmack, C. Pokalsky, M. Zhang, R.L. Van Etten, Biochemistry 35 (1996) 13609–13617.
[33] P.R. Gerber,<http://www.moloc.ch> (accessed Jan 2006).
[34] D.J. Weininger, Chem. Inf. Comput. Sci. 28 (1988) 31–36.
[35] ChemAxon Ltd., Budapest, Hungary,<http://www.chemaxon.com> (accessed Mar 2005).
[36] A.C. Wallace, R.A. Laskowski, J.M. Thornton, Prot. Eng. 8 (1995) 127–134.
[37] Cluster 3.0, command line version,<http://bonsai.ims.u-tokyo.ac.jp/~mdehoon/software/cluster> (accessed July 2005).
[38] A.P.R. Zabell, S. Corden, P. Helquist, C.V. Stauffacher, O. Wiest, Bioorg. Med. Chem. 12 (2004) 1867–1880.
[39] Y.Y.P. Wo, M.M. Zhou, P. Stevis, J.P. Davis, Z.Y. Zhang, R.L. Van Etten, Biochemistry 31 (1992) 1712–1721.
[40] T. Akerud, E. Thulin, R.L. Van Etten, M. Akke, J. Mol. Biol. 322 (2002) 137–152.
[41] J.P. Davis, M.-M. Zhou, R.L. Van Etten, Biochemistry 33 (1994) 1278–1286.