ACS chemical biology

Letters

pubs.acs.org/acschemicalbiology

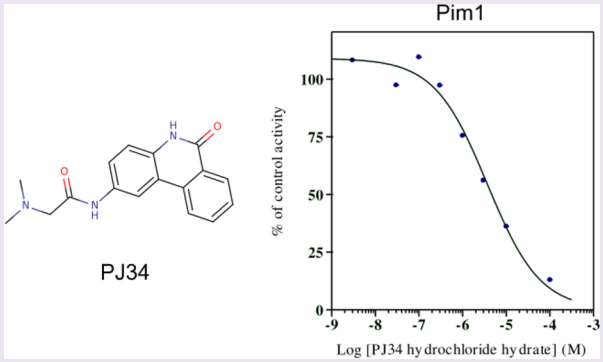
Identification of Pim Kinases as Novel Targets for PJ34 with Confounding Effects in PARP Biology

Albert A. Antolín,[†] Xavier Jalencas,[†] José Yélamos,[‡] and Jordi Mestres*[,†]

[†]Chemogenomics Laboratory, Research Program on Biomedical Informatics and [‡]Department of Immunology, Research Program on Cancer, IMIM Hospital del Mar Research Institute and Universitat Pompeu Fabra, Doctor Aiguader 88, 08003 Barcelona, Catalonia, Spain

Supporting Information

ABSTRACT: Small molecules are widely used in chemical biology without complete knowledge of their target profile, at risk of deriving conclusions that ignore potential confounding effects from unknown off-target interactions. The prediction and further experimental confirmation of novel affinities for PJ34 on Pim1 (IC_{50} = 3.7 μM) and Pim2 (IC_{50} = 16 μM) serine/threonine kinases, together with their involvement in many of the processes relevant to PARP biology, questions the appropriateness of using PJ34 as a chemical tool to probe the biological role of PARP1 and PARP2 at the high micromolar concentrations applied in most studies.



The ability of small molecules to activate, inhibit, or simply perturb signaling events in organisms through their interaction with macromolecules has been largely exploited in chemical biology as a natural noninvasive means to investigate the functional role of proteins in biological processes.[1] However, the use of small molecules as chemical tools to probe biology is not exempt of fundamental risks.[2] Unlike gene knockout strategies, the true specificity of a small molecule for the protein target under investigation is always a big unknown. In this respect, several criteria to guide chemical probe qualification have been suggested, including the widest possible knowledge of its selectivity against potentially confounding targets.[2−5] In spite of these precautions, the risk of getting results that mask the effects of yet undetected off-target affinities is always there.

To alleviate this issue, the affinity of chemical probe candidates can be currently profiled *in vitro* against a panel of over a hundred diverse protein targets,[6] but even such a laborious and costly experimental effort manages to cover only a limited portion of target space. The latest developments of computational methods to predict the target profile of small molecules offer the possibility to extend target space coverage to thousands of proteins in a highly efficient manner.[7] Some of these methods have already lead to the successful identification of previously unknown targets for marketed drugs,[8,9] and they were recently used to discover the targets of organic molecules active in phenotypic screens.[10] Here, we illustrate for the first time their potential application to reveal new targets of chemical probes that may be confounding with the biological role of their established primary target(s).

PJ34 is a widely used chemical tool to probe the biological role of poly(ADP-ribose) polymerases (PARPs). Since its discovery in 2001, it has been used (generally at rather high micromolar concentrations) in more than 150 publications on PARP biology due to its high affinity for PARP1, water solubility, and cellular residence time.[11] Interestingly, the extremely limited knowledge about its target profile was not perceived as an issue in all these studies that usually took for granted that PJ34 was a highly potent and selective PARP1 inhibitor (pIC_{50} = 7.7).[11] In fact, it was not until very recently that extensive profiling of PJ34 across 13 members of the human PARP family was performed.[12] The study confirmed that PJ34 is a high affinity inhibitor of PARP1 (pIC_{50} = 7.8), but it also revealed affinities to PARP2 (pIC_{50} = 7.6), TNKS1 (pIC_{50} = 6.2), PARP3 (pK_d = 6.1), PARP4 (pK_d = 5.7), and TNKS2 (pK_d = 4.7), in addition to residual affinities to PARP14, PARP15, and PARP16. Beyond the PARP family, the only interaction for human targets that has been reported is a weak affinity to the matrix metalloproteinase MMP2 (pIC_{50} = 4.4).[13] For the sake of clarity, a summary of the current knowledge on the affinity profile of PJ34 for human targets is compiled in Figure 1.

Having a more complete knowledge of its target profile may strengthen the confidence level on the use of PJ34 to probe the biological role of PARP1 and PARP2. However, an increasing number of publications highlight that PJ34 seems to have some unique properties relative to other PARP inhibitors and suggest

Received: June 28, 2012
Accepted: October 1, 2012
Published: October 1, 2012

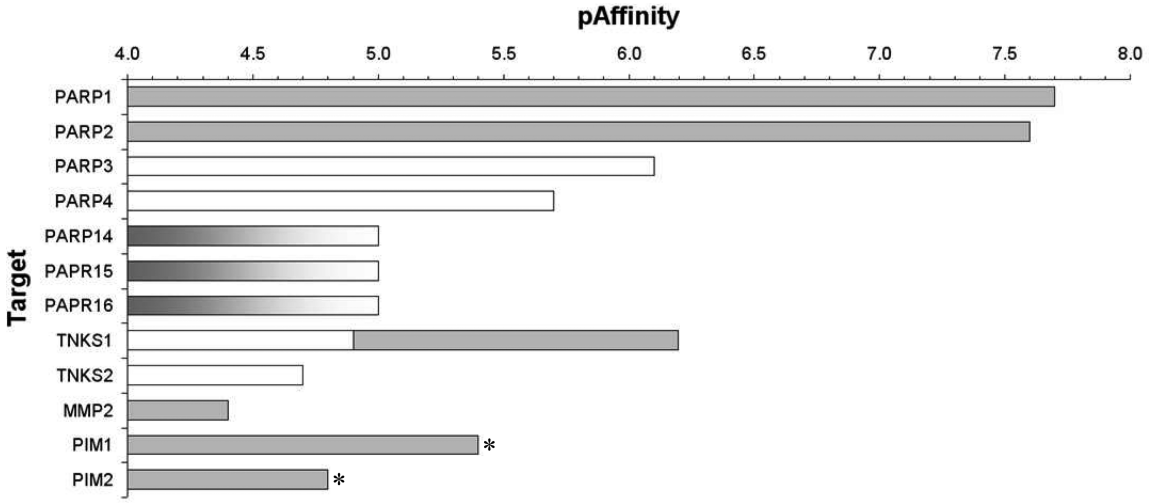


Figure 1. Currently known affinity profile for PJ34 on human targets. The two novel affinities for Pim1 and Pim2 identified in this work are marked with an asterisk. Gray and white bars refer to pIC_{50} and pK_d values, respectively. The three color graded bars refer to qualitative affinities (see text for references).

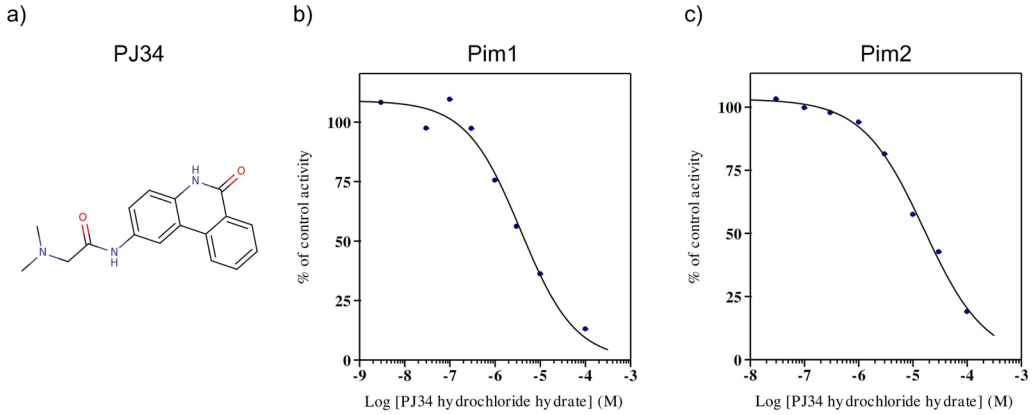


Figure 2. (a) Structure of PJ34 and dose−response curves for (b) Pim1 (IC_{50} = 3.7 μM) and (c) Pim2 (IC_{50} = 16 μM) serine/threonine kinases.

that PARP1-independent factors, yet to be identified, may be responsible for the effects observed when using PJ34.[14−16] This scenario prompted us to explore the target profile of PJ34 beyond current knowledge. To this aim, an *in silico* target profiling of PJ34 was performed using a similarity-based approach that was recently validated both retrospectively, on its ability to predict the entire experimental interaction matrix between 13 antipsychotic drugs and 34 protein targets,[17] and prospectively, on its capacity to anticipate the affinity profile of cyclobenzaprine.[9] The results revealed that, apart from recovering some of the already known interactions for members of the PARP family, additional novel affinities were predicted for two serine/threonine kinases, namely, Pim1 and Pim2 based on the similarity of PJ34 to a high-affinity Pim1/2 compound (CHEMBL572783: pK_i-Pim1 = 8.1, pK_i-Pim2 = 8.5).[18] As illustrated in Figure 2, subsequent *in vitro* testing confirmed that PJ34 indeed interacts with both kinases in a dose dependent manner with IC_{50} values of 3.7 and 16 μM for Pim1 and Pim2, respectively. The identification of these two novel proteins expands by 20% the known target profile of PJ34 (Figure 1).

The confirmation of micromolar affinities for Pim kinases, together with the availability of multiple ligand-bound crystal structures in the Protein Data Bank (PDB),[19] prompted us to generate a plausible binding mode hypothesis of the interaction of PJ34 in the active site of Pim1 kinase. Modeling was performed on the basis of two crystal structures of Pim1 kinase, namely, one containing LXG (PDB entry 3jxw), the ligand structurally most similar to PJ34 among all cocrystallized with Pim1 kinase, and the other containing staurosporine (PDB entry 1yhs), the standard reference compound used in the *in vitro* binding assays. Backbone superposition of the two Pim1 kinase structures produced the experimental relative superposition of the two ligands in the binding site of Pim1, onto which PJ34 was then flexibly superimposed.[20] The best alignment solution produced had a three-dimensional similarity score of 0.61, and the corresponding orientation of PJ34 relative to LXG and staurosporine was then projected back to the active site of Pim1 kinase. Local energy minimization of this best PJ34 pose in the Pim1 binding site resulted in a binding energy of −7.46 kcal/mol,[21] with only a slight deviation from the original orientation generated by flexible superposition (rmsd = 0.80 Å). Figure 3 shows the binding model produced for PJ34 in the active site of Pim1 kinase (PDB entry 3jxw) relative to the known binding orientations of LXG and staurosporine in their respective crystal structures. This binding hypothesis suggests that PJ34 adopts a hybrid binding mode between LXG and staurosporine, with a potential ability to

dx.doi.org/10.1021/cb300317y | *ACS Chem. Biol.* 2012, 7, 1962−1967

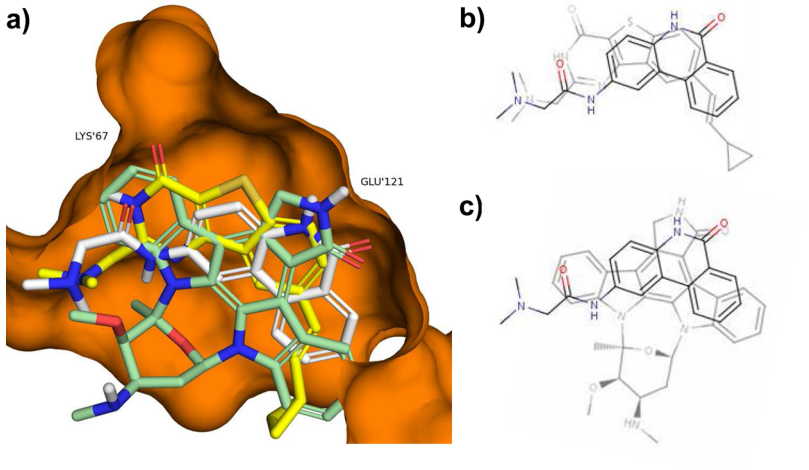


Figure 3. (a) Binding mode hypothesis of PJ34 (white carbon atoms) in the active site of Pim1, superimposed with the structures of LXG (yellow carbon atoms) and staurosporine (green carbon atoms) complemented with two-dimensional schemes of the structural superposition between PJ34 (structure with black carbons and heteroatoms in color) and (b) LXG and (c) staurosporine.

establish hydrogen bond interactions with GLU'121 and LYS'67. In spite of the observation that PJ34 can indeed be accommodated in the active site of Pim1 kinase in an energetically favorable binding mode, the nonoptimal directionality of those hydrogen bonds could partly explain the modest affinity of PJ34 for Pim1 (pIC_{50} = 5.4) compared to LXG (pK_i = 9.0) and staurosporine (pK_d = 8.5).[22]

It is worth stressing that Pim kinases do not even bind to NADH, and so, they have never been remotely considered suspected off-targets for PJ34. Originally identified as oncogenes, they are overexpressed in a wide range of hematopoietic malignancies and solid cancers.[23] Their biological roles include protein transcription and translation, regulation of cell cycle progression, and the regulation of survival signaling, all of them being overlapping functions with PARPs.[23−25] Under these circumstances, the risk of confounding effects when using PJ34 to study the biological role of PARPs ought to be considered and some of the functions attributed in the past to PARP1/2 inhibition may have to be revisited on the grounds of new evidence that Pim1/2 inhibition may take place as well.

However, the new affinities identified between PJ34 and Pim1/2 should not be an issue if PJ34 were administered in all experiments at appropriate concentrations (optimally below 1 μM) to derisk the possibility of confounding effects from interacting with Pim kinases. Accordingly, all publications in PubMed including PJ34 in the title or abstract, published in the years 2010 and 2011 and linked to PARP biology, were identified and carefully inspected. A total of 33 publications were examined (Supporting Information) from which 63 concentrations used in cellular experiments reported in 29 articles were extracted, some articles reporting experiments at various PJ34 concentrations. The results are provided in Figure 4. As can be observed, over 60% of those studies used PJ34 at concentrations above 5 μM unaware of the fact that at this concentration PJ34 may actually interact with at least 6 targets, Pim1 among them (Figure 1). Remarkably, less than 10% of those experiments used PJ34 at concentrations that could be considered safe to avoid any potentially confounding effects from off-target affinities other than PARP1 and PARP2. One may argue that higher concentrations of PJ34 are required in cellular experiments due to nuclear PARP1/2 localization and low compound permeation in the cell and into the nucleus. In this respect, the assignment of the number of enzymes potentially modulated by PJ34 at each concentration (Figure 4) purely on the basis of *in vitro* affinities (Figure 1) may not be an exact representation of the real intracellular situation, but without control of the actual concentration at the target site, it is difficult to strictly rule out confounding effects from Pim1/2 inhibition, particularly since Pim1 is a cytoplasmatic kinase.[23] Some studies contrast the use of PJ34 with siRNA control experiments as a means to substantiate the involvement of PARP1/2 in the effects observed with the chemical probe, but in the light of present findings, even with independent siRNA controls, the possibility of synergistic effects from Pim1/2 inhibition cannot be discarded, and in fact, recent reports highlight that, when siRNA experiments are performed in the presence of PJ34, various effects are still observed.[14−16]

A recent study by Madison et al.[15] reported a PARP1-independent p21-dependent effect in G2/M cell cycle arrest with the use of PJ34 at high micromolar concentrations. In particular, a 10 μM concentration of PJ34 was found to activate p21 and cause a reduction in cdc25c. Pim1 kinase is known to regulate cell cycle progression through several pathways, including p21 phosphorilation, leading to p21 relocalization in the cytoplasm with enhanced protein stability, and it is also believed to regulate G2/M transition by modulating cdc25c activity.[23] This evidence points to the direction that the effects on cell cycle arrest observed upon treatment with high concentrations of PJ34 that have been linked to PARP1/2 inhibition may well be confounded by Pim1/2 inhibition.

Another recent study by Castiel et al.[16] identified unique effects in mitosis with PJ34 at concentrations in the 20−30 μM range when compared with other PARP inhibitors. PJ34 was found to be an extra-centrosomes declustering agent in cancer cells with supernumerary centrosomes, a property observed also mildly in Phen and Tiq-A but not in the rest of PARP inhibitors studied. In this respect, Pim1 kinase is known to be associated with protein complexes necessary for mitosis, and its upregulation leads to genomic instability.[26] Specifically, Pim1 is known to phosphorilate the mitotic apparatus protein (NuMa), a main structural component of the spindle poles, promoting its complex formation with dynein, which in turn has been shown to be key in promoting supernumerary

dx.doi.org/10.1021/cb300317y | *ACS Chem. Biol.* 2012, 7, 1962−1967

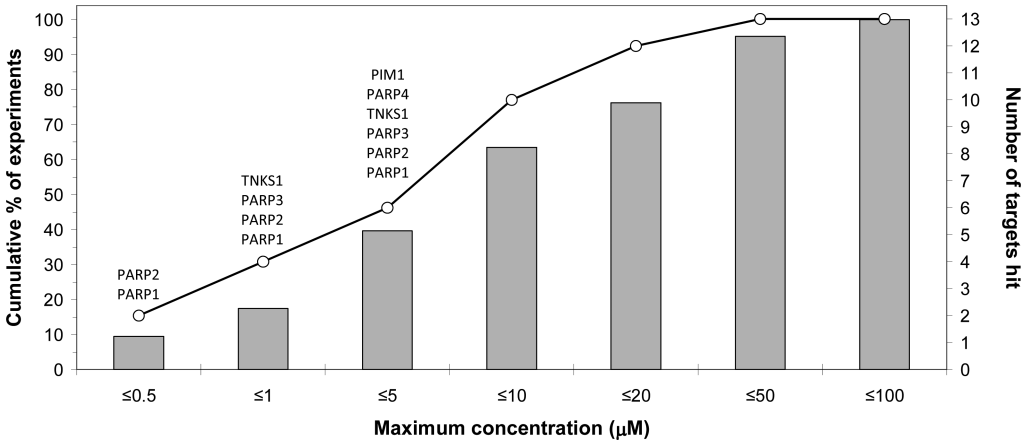


Figure 4. Distribution of the percentage of cellular experiments found in the last two years using PJ34 concentrations below certain ranges. Also plotted as small connected circles is the number and abbreviation of targets potentially hit by PJ34 at increasing concentration levels (based on the *in vitro* potencies reported in Figure 1). Over 60% of all experiments used PJ34 concentrations above 5 μM, and at this concentration, assuming reasonable cellular permeability, PJ34 is likely to interact with at least six targets, Pim1 among them.

centrosome clustering.[27] Among the targets currently known to interact with PJ34 (Figure 1), both TNKS1 and PARP16 could contribute to the peculiar centrosome declustering properties of this compound. However, at the range of concentrations that PJ34 was used in these experiments, Pim1/2 inhibition should be considered as a potential source of confounding effects (Figure 4).

Finally, it is worth stressing that all publications administering PJ34 in the presence of PARP1 knockouts observed PARP1-independent effects. This is a particularly relevant piece of information since it provides clear clues that the effect observed upon using PJ34 goes beyond PARP1/2 inhibition. In particular, Toller et al.[14] observed a PARP1-independent prevention of *Helicobacter pylori* induced preneoplasia using PJ34. *H. pylori* is known to produce an upregulation of Pim1 that is believed to be involved in cancer production,[28] and Pim1 is also known to have a role in T-cell responses and is upregulated by cytokines promoting Th1 but not Th2 T-cell differentiation, as the researchers observed under PJ34 treatment.[29] It cannot be excluded that other PARP family members could very well be responsible for this effect but, at the concentrations of PJ34 used, counfounding effects from Pim kinases should definitely be taken into consideration.

In the past decade, the biological roles of PARP1/2 in numerous diseases have been established using PJ34 as a chemical probe at high concentrations in a wide range of experiments without complete awareness of its target profile.[24] The prediction and *in vitro* confirmation of novel affinities of PJ34 for Pim1 and Pim2 in the low micromolar range, and the realization that these targets share many of their biological roles with PARP1/2, raise some clouds on the validity and precise scope of some of the conclusions derived in previous studies using this compound. In fact, it was recently emphasized that target validation studies reported in the scientific literature were often difficult to reproduce in internal industrial settings.[30] The use of chemical tools at concentrations that may have an action on targets beyond what is known to be their primary selective target could be a potential source for such a disparity. The case of PJ34 exposed here highlights the message that caution should be taken when using chemical tools to probe biology, as the pharmacology of small molecules is always likely to extend beyond current knowledge. In this respect, *in silico* target profiling emerges as an efficient derisking strategy in chemical biology for the identification of potential confounding targets for chemical probes.

METHODS

***In Silico* Target Profiling.** Computational prediction of the affinity of PJ34 across multiple proteins was performed with PredictFX (Chemotargets), a similarity-based approach to target profiling that relies on the availability of data sources that contain chemical structures with information on the binding or functional activity to protein targets (such as ChEMBL[30]) and the use of two-dimensional mathematical descriptors to encode those chemical structures (such as PHRAG, FPD, and SHED).[17] Pharmacophoric fragments (PHRAG) are all possible fixed-length segments of five atom-features that can be extracted from the topology of a molecule. In contrast, feature-pair distributions (FPD) capture the overall spreading of pairs of atom-centered features at different predefined bond lengths. Finally, SHED quantifies the variability within all possible feature-pair distributions using the concept of Shannon entropy. When using PHRAG and FPD, the similarity between two molecules corresponds to the overlapping fraction of their respective profiles, whereas with SHED, Euclidean distances are calculated instead. The biological relevance of the similarity and distance values obtained with these descriptors was assessed on their ability to discriminate active from random compounds for all targets chemically represented in publicly available sources. As a result of this validation analysis, compounds above similarity values of 0.76 and 0.87 for PHRAG and FPD, respectively, and below a distance value of 0.52 for SHED were considered to be within the applicability domain of these descriptors. Then, for any biological target under study, the ensemble of molecular descriptors capturing the structural and pharmacophoric features of all molecules for which affinity data is available[31] represents a mathematical description of this target from a chemical perspective. On this basis, the affinity of a compound for a given target is estimated by inverse distance weighting interpolation of the experimental affinities from all neighboring molecules found within a predetermined applicability domain.[17] If only one neighbor molecule is identified, an active annotation is provided instead. In total, PJ34 was processed against 4643 proteins. The output returned the list of protein targets for which affinity is predicted for PJ34.

***In Vitro* Binding Assays.** The test compound, PJ34, was obtained from Sigma-Aldrich, with guaranteed purity >98%. The Pim1 and Pim2 *in vitro* binding assays were performed at Cerep (catalogue numbers 2919 and 2920). Evaluation of the effects of compounds on the activity of the human Pim1 and Pim2 kinases is quantified by measuring the phosphorylation of the substrate Ulight-CREBtide (CKRREILSRRPSYRK) using either Pim1 or Pim2 human recombi-

dx.doi.org/10.1021/cb300317y | *ACS Chem. Biol.* 2012, 7, 1962–1967

nant enzymes expressed in insect cells and the LANCE detection method. PJ34, reference compound, or water (control) are mixed with either Pim1 (4.08 ng) or Pim2 (6.36 ng) enzymes in a buffer containing 40 mM Hepes/Tris (pH 7.4), 0.8 mM EGTA/Tris, 8 mM MgCl2, 1.6 mM DTT, and 0.008% Tween 20. Thereafter, the reaction is initiated by adding 25 nM of the substrate Ulight-CREBtide (CKRREILSRRPSYRK) and ATP (30 μM for Pim1 and 3 μM for Pim2), and the mixture is incubated for 60 min at RT. For control basal measurements, the enzyme is omitted from the reaction mixture. Following incubation, the reaction is stopped by adding 13 mM EDTA. After 5 min, the antiphospho-CREB antibody labeled with europium chelate is added. After 60 more min, the fluorescence transfer is measured at λ_{ex} = 337 nm, λ_{em} = 620 nm, and λ_{em} = 665 nm using a microplate reader (Envision, Perkin-Elmer). The enzyme activity is determined by dividing the signal measured at λ_{em} = 665 nm by that measured at λ_{em} = 620 nm (ratio). The results are expressed as a percent inhibition of the control enzyme activity. The standard inhibitory reference compound is staurosporine, which is tested in each experiment at several concentrations to obtain an inhibition curve from which its IC_{50} value is calculated. The IC_{50} values (concentration causing a half-maximal inhibition of control specific activity) and Hill coefficients (nH) were determined by nonlinear regression analysis of the inhibition curves generated with mean replicate values using Hill equation curve fitting $(Y = D + [(A - D)/(1 + (C/C50)nH)]$, where Y = specific activity, D = minimum specific activity, A = maximum specific activity, C = compound concentration, C50 = IC_{50}, and nH = slope factor). This analysis was performed using a software developed at Cerep (Hill software) and validated by comparison with data generated by the commercial software SigmaPlot 4.0 for Windows (1997 by SPSS Inc.).

■ ASSOCIATED CONTENT

Supporting Information

List of articles published in 2010 and 2011 using PJ34 to probe PARP biology that were considered to derive Figure 4. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: jmestres@imim.es.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was funded by the Catalan Government grant 2012FIB1-00175 (to A.A.A.), the Spanish Ministerio de Economía y Competitividad grants PTA2009-1865-P (to X.J.), SAF2011-26900 (to J.Y.), and BIO2011-26669 (to J.M.), and the Instituto de Salud Carlos III (to J.M.).

■ REFERENCES

(1) Schreiber, S. L. (2005) Small molecules: the missing link in the central dogma. *Nat. Chem. Biol. 1*, 64−66.

(2) Oprea, T. I., Bologa, C. G., Boyer, S., Curpan, R. F., Glen, R. C., Hopkins, A. L., Lipinski, C. A., Marshall, G. R., Martin, Y. C., Ostopovici-Halip, L., Rishton, G., Ursu, O., Vaz, R. J., Waller, C., Waldmann, H., and Sklar, L. A. (2009) A crowdsourcing evaluation of the NIH chemical probes. *Nat. Chem. Biol. 5*, 441−447.

(3) Frye, S. V. (2010) The art of the chemical probe. *Nat. Chem. Biol. 6*, 159−161.

(4) Workman, P., and Collins, I. (2010) Probing the probes: fitness factors for small molecule tools. *Chem. Biol. 17*, 561−577.

(5) Garcia-Serna, R., and Mestres, J. (2011) Chemical probes for biological systems. *Drug Discovery Today 16*, 99−106.

(6) Entzeroth, M., Chapelain, B., Guilbert, J., and Hamon, V. (2000) High throughput drug profiling. *J. Autom. Methods Manage. Chem. 22*, 171−173.

(7) Koutsoukas, A., Simms, B., Kirchmair, J., Bond, P. J., Whitmore, A. V., Zimmer, S., Young, M. P., Jenkins, J. L., Glick, M., Glen, R. C., and Bender, A. (2011) From in silico target prediction to multi-target drug design: current databases, methods and applications. *J. Proteomics 74*, 2554−2574.

(8) Keiser, M. J., Setola, V., Irwin, J. J., Laggner, C., Abbas, A. I., Hufeisen, S. J., Jensen, N. H., Kuijer, M. B., Matos, R. C., Tran, T. B., Whaley, R., Glennon, R. A., Hert, J., Thomas, K. L., Edwards, D. D., Shoichet, B. K., and Roth, B. L. (2009) Predicting new molecular targets for known drugs. *Nature 462*, 175−181.

(9) Mestres, J., Seifert, S. A., and Oprea, T. I. (2011) Linking pharmacology to clinical reports: cyclobenzaprine and its possible association with serotonin syndrome. *Clin. Pharmacol. Ther. 90*, 662−665.

(10) Laggner, C., Kokel, D., Setola, V., Tolia, A., Lin, H., Irwin, J. J., Keiser, M. J., Cheung, C. Y., Minor, D. L., Jr., Roth, B. L., Peterson, R. T., and Shoichet, B. K. (2011) Chemical informatics and target identification in a zebrafish phenotypic screen. *Nat. Chem. Biol. 8*, 144−146.

(11) Garcia-Soriano, F., Virág, L., Jagtap, P., Szabó, E., Mabley, J. G., Liaudet, L., Marton, A., Hoyt, D. G., Murthy, K. G., Salzman, A. L., Southan, G. J., and Szabó, C. (2001) Diabetic endothelial dysfunction: the role of poly(ADP-ribose) polymerase activation. *Nat. Med. 7*, 108−113.

(12) Wahlberg, E., Karlberg, T., Kouznetsova, E., Markova, N., Macchiarulo, A., Thorsell, A. G., Pol, E., Frostell, Å., Ekblad, T., Öncü, D., Kull, B., Robertson, G. M., Pellicciari, R., Schüler, H., and Weigelt, J. (2012) Family-wide chemical profiling and structural analysis of PARP and tankyrase inhibitors. *Nat. Biotechnol. 30*, 283−288.

(13) Nicolescu, A. C., Holt, A., Kandasamy, A. D., Pacher, P., and Schulz, R. (2009) Inhibition of matrix metalloproteinase-2 by PARP inhibitors. *Biochem. Biophys. Res. Commun. 387*, 646−650.

(14) Toller, I. M., Altmeyer, M., Kohler, E., Hottiger, M. O., and Müller, A. (2010) Inhibition of ADP ribosylation prevents and cures helicobacter-induced gastric preneoplasia. *Cancer Res. 70*, 5912−5922.

(15) Madison, D. L., Stauffer, D., and Lundblad, J. R. (2011) The PARP inhibitor PJ34 causes a PARP1-independent, p21-dependent mitotic arrest. *DNA Repair 10*, 1003−1013.

(16) Castiel, A., Visochek, L., Mittelman, L., Dantzer, F., Izraeli, S., and Cohen-Armon, M. (2011) A phenanthrene derived PARP inhibitor is an extra-centrosomes de-clustering agent exclusively eradicating human cancer cells. *BMC Cancer 11*, 412.

(17) Vidal, D., and Mestres, J. (2010) In silico receptorome screening of antipsychotic drugs. *Mol. Inf. 29*, 543−551.

(18) https://www.ebi.ac.uk/chembldb/compound/inspect/CHEMBL572783 (accessed on Sept 18, 2012).

(19) Rose, P. W., Beran, B., Bi, C., Bluhm, W. F., Dimitropoulos, D., Goodsell, D. S., Prlic, A., Quesada, M., Quinn, G. B., Westbrook, J. D., Young, J., Yukich, B., Zardecki, C., Berman, H. M., and Bourne, P. E. (2011) The RCSB Protein Data Bank: redesigned web site and web services. *Nucleic Acids Res. 39*, D392−D401.

(20) Mestres, J., Rohrer, D. C., and Maggiora, G. M. (1997) MIMIC: a molecular-field matching program. Exploiting applicability of molecular similarity approaches. *J. Comput. Chem. 18*, 934−954.

(21) Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., and Olson, A. J. (2009) AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J. Comput. Chem. 30*, 2785−2791.

(22) Liu, T., Lin, Y., Wen, X., Jorissen, R. N., and Gilson, M. K. (2007) BindingDB: a web-accessible database of experimentally determined protein-ligand binding affinities. *Nucleic Acids Res. 35*, D198−D201.

(23) Nawijn, M. C., Alendar, A., and Berns, A. (2011) For better or for worse: the role of Pim oncogenes in tumorigenesis. *Nat. Rev. Cancer 11*, 23−34.

(24) Jagtap, P., and Szabó, C. (2005) Poly(ADP-ribose) polymerase and the therapeutic effects of its inhibitors. *Nat. Rev. Drug Discovery 4*, 421−440.
(25) Schreiber, V., Dantzer, F., Ame, J.-C., and de Murcia, G. (2006) Poly(ADP-ribose): novel functions for an old molecule. *Nat. Rev. Mol. Cell Biol. 7*, 517−528.
(26) Bhattacharya, N., Wang, Z., Davitt, C., McKenzie, I. F., Xing, P. X., and Magnuson, N. S. (2002) Pim-1 associates with protein complexes necessary for mitosis. *Chromosoma 111*, 80−95.
(27) Quintyne, N. J., Reing, J. E., Hoffelder, D. R., Gollin, S. M., and Saunders, W. S. (2005) Spindle multipolarity is prevented by centrosomal clustering. *Science 307*, 127−129.
(28) Sepulveda, A. R., Tao, H., Carloni, E., Sepulveda, J., Graham, D. Y., and Peterson, L. E. (2002) Screening of gene expression profiles in gastric epithelial cells induced by *Helicobacter pylori* using microarray analysis. *Aliment. Pharmacol. Ther. Suppl. 2*, 145−157.
(29) Aho, T. L., Lund, R. J., Ylikoski, E. K., Matikainen, S., Lahesmaa, R., and Koskinen, P. J. (2005) Expression of human pim family genes is selectively up-regulated by cytokines promoting T helper type 1, but not T helper type 2, cell differentiation. *Immunology 116*, 82−88.
(30) Prinz, F., Schlange, T., and Asadullah, K. (2011) Believe it or not: how much can we rely on published data on potential drug targets? *Nat. Rev. Drug Discovery 10*, 712−713.
(31) Gleeson, M. P., Hersey, A., Montanari, D., and Overington, J. (2011) Probing the links between *in vitro* potency, ADMET and physicochemical parameters. *Nat. Rev. Drug Discovery 10*, 197−208.