

# Protein Phosphatases: A Neglected Target Family for Drug Discovery

Joe Lewis and Gerhard Müller\*

**Abstract:** The gene family of protein phosphatases is a rich but under-exploited source of therapeutically validated drug targets modulating signal transduction pathways. Unlike the kinase family, research and development activities have not yet yielded any approved small-molecule drugs against a phosphatase. Approximately 20 years ago, the phosphatase family was classified as undruggable and intractable. This was primarily due to the spectacular failure of the cumulated industry-wide drug discovery efforts to develop PTP1B inhibitors. Recently, allosteric inhibitors against SHP2, a member of the phosphatase family, have entered clinical trials, which has reawakened industry's interest towards this neglected enzyme family. This contribution reviews the recent R&D trends around small-molecule efforts towards phosphatase modulators over the last years, rather than providing an exhaustive review of the field of allosteric phosphatase inhibitors.

**Keywords:** Allosteric inhibitors · Inhibitors · Kinases · Phosphatases · PTP1B · SHP2 · Target family



**Joe Lewis** has over twenty years of experience in small molecule drug discovery and development against novel targets. From 2001 he was a group leader at Anadys Pharmaceuticals and from 2004 Head of Chemical Biology Core Facility at EMBL (European Molecular Biology Laboratory). During his time at EMBL he was also concurrently a co-founder and CEO of Elara Pharmaceuticals, an oncology company.

He holds a PhD from the University of Vienna for his work at the Institute of Molecular Pathology and a Master of Business Administration from Mannheim Business School. Since 2021, he is SVP Biology at Anavo Therapeutics and Site Head Heidelberg.



**Gerhard Müller** studied chemistry at the University of Frankfurt/Main and Technical University of Munich. He obtained his PhD in organic chemistry under the supervision of Prof. Dr. Horst Kessler in 1993 on anti-adhesive integrin antagonists. After 10 years in the pharmaceutical industry (Glaxo Group Research, Verona, Italy; Bayer AG, Leverkusen, Germany; Organon, Oss, The Netherlands) he joined Axxima

Pharmaceuticals in 2003 as Chief Scientific Officer. He spent a couple of years in Contract Research Organizations (Proteros Fragments, CSO; Mercachem, SVP Medicinal Chemistry), and co-founded Gotham Therapeutics in New York, US (2017) and became their Chief Scientific Officer. In 2021, he co-founded Anavo Therapeutics, a European phosphatase platform company where he holds the position of the Chief Scientific Officer.

## 1. Signal Transduction Therapy: Kinases and Phosphatases

The reversible phosphorylation of polar amino acid sidechains such as those of serine, threonine, or tyrosine on surfaces of intracellular signalling proteins is a post-translational modification

event controlling signal transduction pathways in all human cells. Protein phosphorylation is orchestrated by the opposing activities of protein kinases, catalysing the addition of a phosphate group and protein phosphatases, hydrolysing the respective phosphate esters, and removing the phosphate groups. It has been estimated that at least 70% of intracellular proteins undergo reversible phosphorylation reactions catalysed by kinases and phosphatases, respectively.<sup>[1–3]</sup>

The protein phosphatase family, the phosphatome, was systematically analysed by Gerard Manning and co-workers in a recent bioinformatics analysis that uncovered the phylogenetic and structural details underlying the distinct phosphatase folding families.<sup>[4]</sup> The human protein phosphatome is composed of ‘only’ 189 known and predicted genes encoding for proteins with phosphatase activity. Comparing 518 protein kinases encoded within the human genome<sup>[5]</sup> to the phosphatase family, it seems that with only 189 identified members, the phosphatase family is considerably smaller. In contrast to kinase catalytic domains which follow a highly conserved protein fold topology, the phosphatase family encompasses ten distinct folds with a very imbalanced distribution of distinct proteins over those topological families,<sup>[4]</sup> thus representing an increased structural heterogeneity with diverse options for therapeutic modulation. Importantly, several phosphatase catalytic domains, such as PP1 or PP2, form complexes with unique scaffolding domains and dozens of regulatory domains which control substrate specificity, thereby following the holoenzyme concept (Fig. 1).<sup>[6,7]</sup> Therefore, a single catalytic phosphatase domain can be part of hundreds of substrate-specific phosphatase enzyme complexes. Consequently, the family of functionally distinct active phosphatase enzymes, including holoenzymes, is significantly larger than the protein kinase family.

Despite the multiple options to therapeutically modulate phosphatase signalling, there is a significant imbalance in research activities, especially in the biopharmaceutical industry, between the kinase and the phosphatase families.<sup>[9]</sup> This is illustrated by a quantitative analysis of patent applications claiming inhibitors/modulators of protein targets from both classes (Fig. 2). In the kinase inhibitor field, we have witnessed a steep increase in research activities from the beginning of the 2000's with more than 2000 patent applications published in 2021. For phosphatase-modulating compounds only around 80 applications were published last year.

\*Correspondence: Dr. G. Müller, E-mail: gmueller@anavotx.com, Anavo Therapeutics, J.H. Oortweg 19, 233 CH Leiden, NL

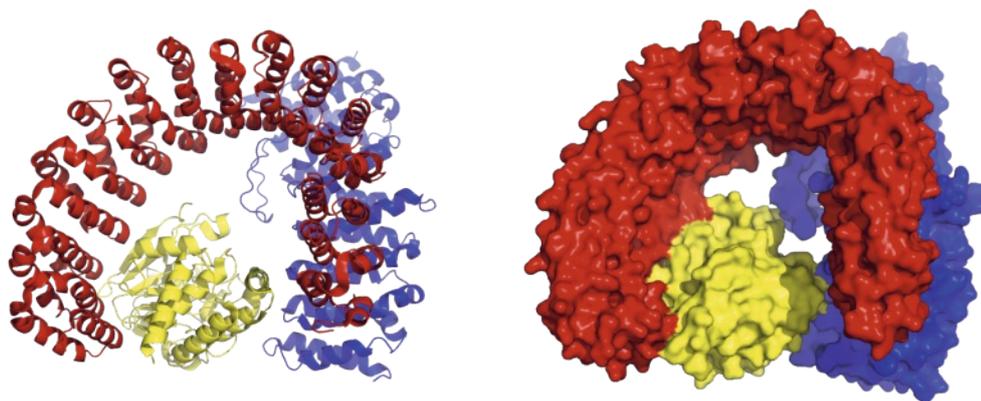


Fig. 1. Cryo-EM structure of a heterotrimeric PP2A holoenzyme complex<sup>[6]</sup> (pdb-code: 6nts) consisting of the catalytic PP2a alpha subunit (yellow), regulatory subunit B56alpha (blue), and the scaffolding domain (red). The protein complex is shown in a cartoon representation (left), and in a Connolly surface mode (right).

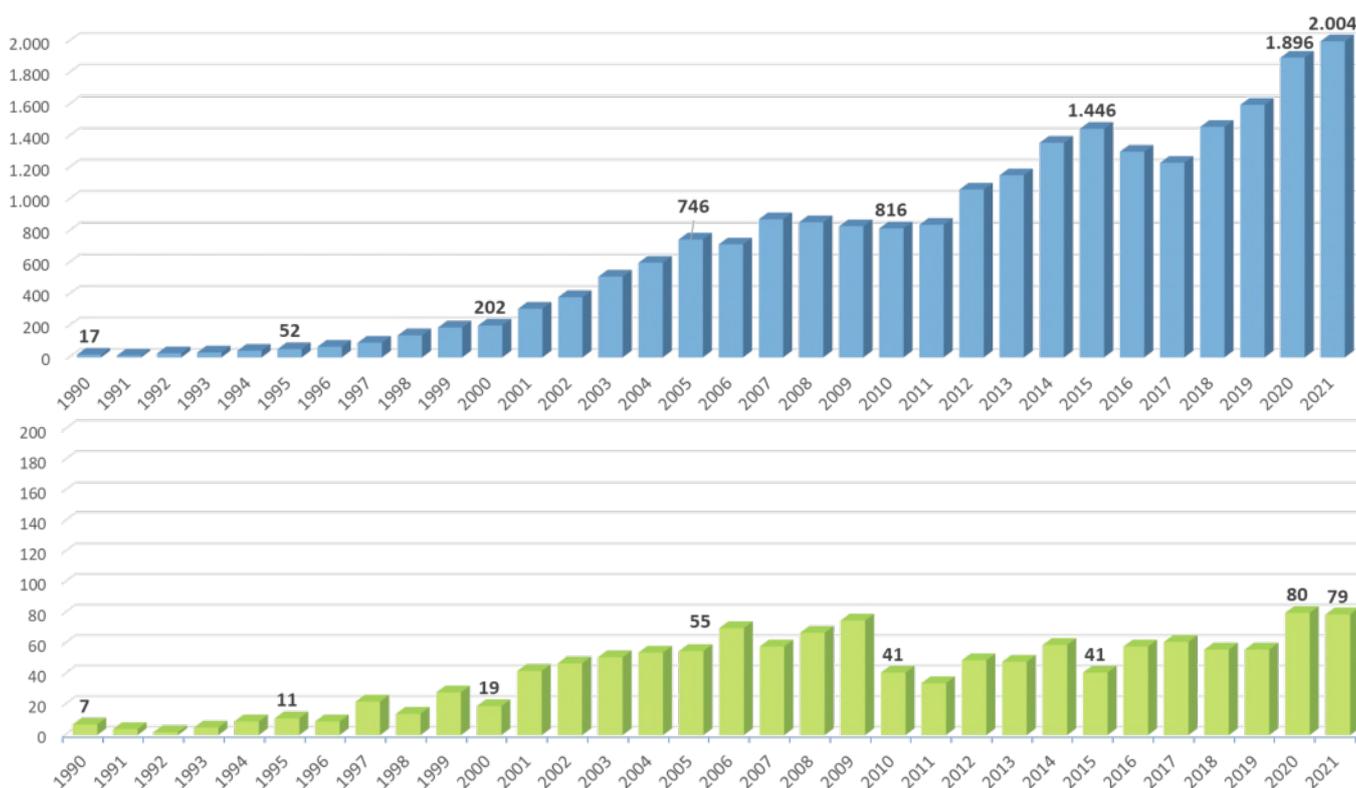


Fig. 2. Results of a SciFinder analysis on published patent applications on kinase inhibitors (top, blue bars) and phosphatase inhibitors (bottom, green bars). Publication year is depicted along the x-axis, number of published patent applications along the y-axis. Note the ten-fold difference in scale along the vertical axis between both families.

These significant research and development activities in the kinase field have yielded approximately 80 approved small molecule drugs targeting kinases<sup>[10]</sup> (Fig. 3). The majority of kinase inhibitors are approved in oncology disease areas. Surprisingly, to date, not a single approved drug was explicitly developed to target a disease-relevant member of the phosphatase family.

In oncology, phosphatases were originally thought to act as an ‘off-switch’ for kinase-activated oncogenic activity. Today it is widely accepted that phosphatase activity can also drive tumour cell proliferation and survival. Phosphatases exert both oncogenic and tumour-suppressive functions, depending on the cellular context. In general, deregulation of phosphatase signalling contributes to cancer development, rendering phosphatase modulator approaches highly relevant for disease treatment and potentially complementary to kinase inhibitors.<sup>[11–13]</sup> However, over the last

decade, members of the phosphatase family have been strongly associated with the class of ‘undruggable’ drug targets<sup>[14,15]</sup> and referred to as the ‘ugly ducklings of cell signalling’.<sup>[16]</sup> The reasons for this stigmatization of the phosphatase target family can be found approximately 20 years ago, coinciding with the beginning of the protein kinase ‘gold-rush’.<sup>[17]</sup>

## 2. PTP1B as Anti-diabetes Target

The origin of the troubled history of phosphatase-targeted drug discovery dates to the end of the 1990s. At this time huge research efforts were being made to find efficacious and selective PTP1B (protein tyrosine phosphatase 1B) inhibitors for oral treatment of type 2 diabetes.<sup>[18–20]</sup>

PTP1B specifically recognizes phospho-tyrosine (pTyr) residues embedded in the corresponding protein substrates and hy-

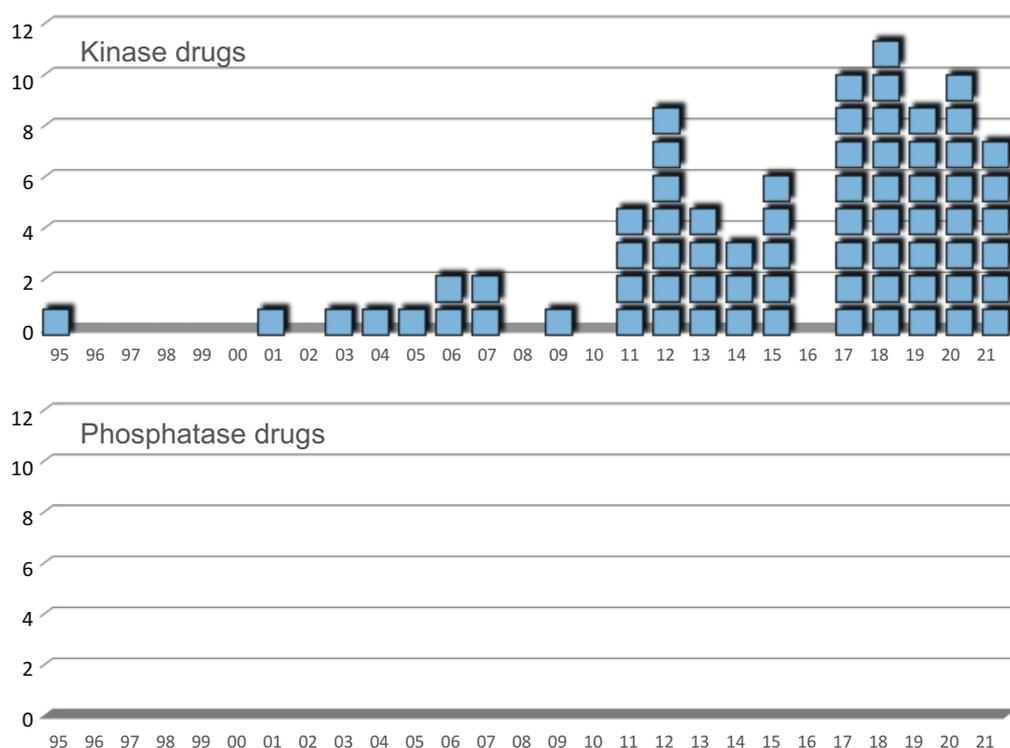


Fig. 3. The number of drugs approved from 1995 to 2021 is depicted for the kinase inhibitor family (top) and the phosphatase family (bottom). Each blue box represents a drug approval within the respective year, shown along the x-axis.

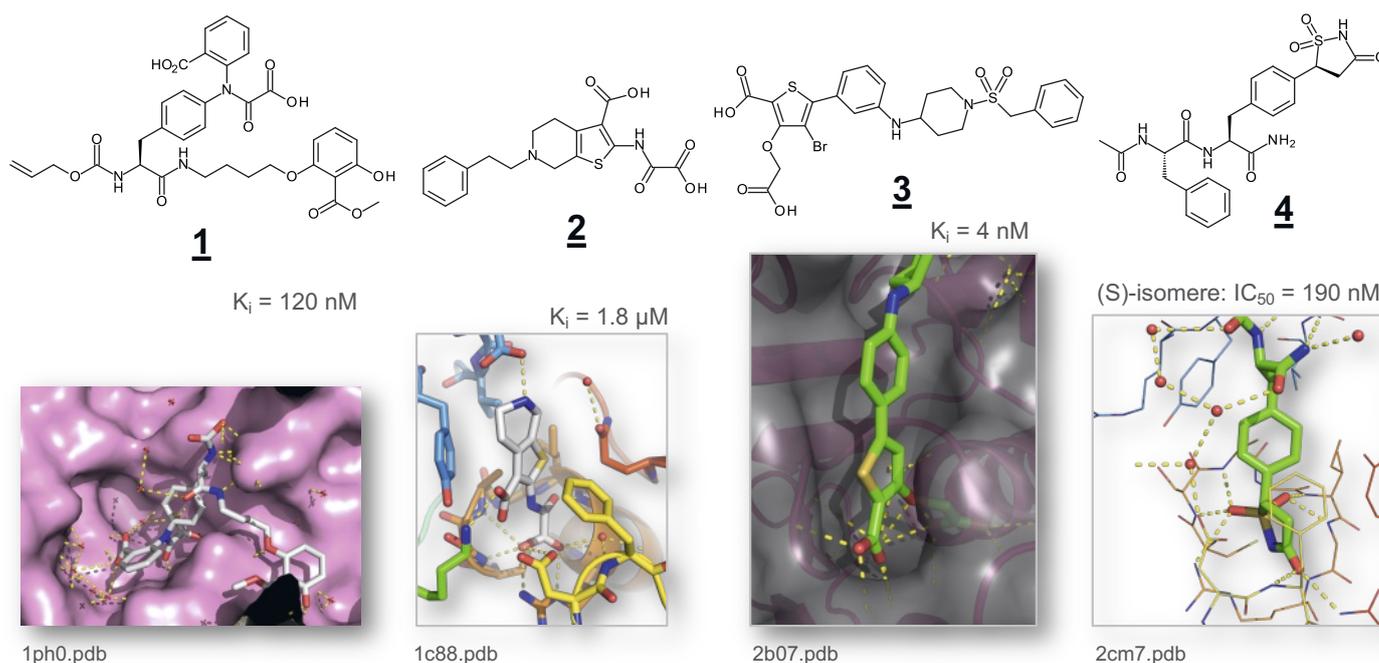


Fig. 4. Selected orthosteric PTP1B inhibitor structures with respective binding affinities or inhibition constants and corresponding binding modes determined by X-ray crystallography. The oxalate moiety in compounds **1** and **2** mimics the pTyr of the substrate structure and is accommodated deep in the PTP1B active site.<sup>[21,22]</sup> Compound **3** features two carboxylic acids,<sup>[23]</sup> and compound **4** carries an isothiazolidinone-ring mimicking the pTyr moiety.<sup>[24]</sup> Polar interactions between inhibitors and protein are depicted as stippled lines.

drolyses the phosphate ester to generate a non-phosphorylated Tyr-containing protein. The medicinal chemistry design attempts towards hit-generation of that era were dominated by substrate-analogue approaches, trying to mimic the pTyr residue flanked by a few additional residues. In Fig. 4 a number of active site-directed PTP1B inhibitors are shown.<sup>[21–24]</sup>

Their substrate-derived nature becomes apparent in that many of them are built on a peptidomimetic backbone (e.g. in **4**), which carries a peripherally exposed group that mimics the phosphate ester of the tyrosine residue. Efforts were made to mimic pTyr with non-hydrolysable phosphonates or di-fluoro-phospho-

nates or, alternatively, with mono- or di-carboxylic acid-based isosteres (as in **1**, **2**, and **3**).<sup>[21–24]</sup> The high local concentration of hydrogen bond acceptors and fractional negative charge is key to meet the pharmacophoric requirements of the PTP1B active site. The catalytic centre is optimised to recognise a double negative charged tetrahedral phosphate group. Consequently, the binding of active substrate-competitive inhibitors accommodated by the enzyme's active site is dominated by polar interactions, i.e. mostly charge-enforced hydrogen bonds to active site residues or metal ions. As exemplified in Fig. 4, numerous research groups developed orthosteric PTP1B inhibitors following the pTyr mimic

approach. In general, such compounds suffer from poor physicochemical properties leading to permeability, selectivity, and metabolic stability issues. Hence, taking this approach, the generation of potent, selective, bioavailable, and safe PTP1B inhibitors failed industry wide. Consequently, only very few compounds were successfully progressed to pre-clinical development and reached advanced phases of clinical development.

One of them, Ertiprotafib **5**, was assumed to bind to the active site of PTP1B and was promoted to advanced phases of clinical development as an anti-diabetic drug candidate. Due to insufficient clinical efficacy and dose-limiting adverse effects, further development of the compound was discontinued. Only after discontinuation in phase II was the precise molecular mechanism-of-action elucidated by a thorough biophysical investigation of the target binding mechanism (Fig. 5). It was demonstrated using heteronuclear 2D NMR spectroscopy that the efficacy of the drug candidate is not due to a specific binding to PTP1B, the compound triggers PTP1B aggregation in a dose-dependent manner.<sup>[25]</sup>

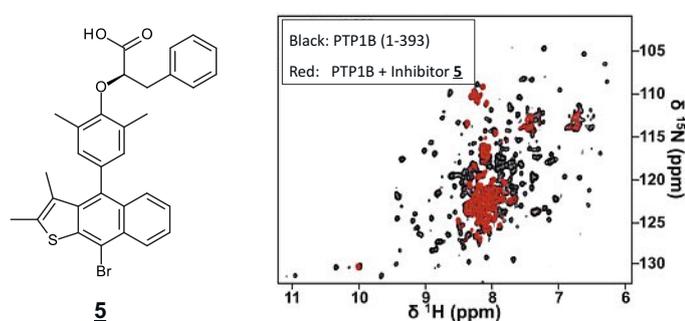


Fig. 5. Chemical structure of Ertiprotafib **5** (left) and an overlay of two heteronuclear 2D NMR [<sup>1</sup>H,<sup>15</sup>N]-TROSY spectrum of PTP1B alone (black peaks) and PTP1B with Ertiprotafib (1:15 molar equivalents), indicating a collapse of the formerly (PTP1B alone) well-dispersed set of signals (modified from ref. [25]).

At the beginning of the 2000s, it was found that parts of the active site of protein kinases, *i.e.* the co-substrate (ATP) binding site, proved to be readily druggable. In contrast the phosphatase active site was recognized to be dominated by pharmacophoric requirements that rendered attempts to obtain safe and selective drug candidates nearly impossible.

Based on this disappointing outcome and the significant investments that had been assigned to this member of the phosphatase target class, the family of phosphatase enzymes was classified as undruggable. This perception has since dominated target selection processes within the pharmaceutical and biotech industry until very recently. In summary, the outcome of this intensive research phase can be classified as the ‘*Waterloo of the phosphatase*’ drug discovery efforts. An entire target class acquired a reputation as being intractable and undruggable.<sup>[26–28]</sup>

Despite the lack of interest from the biopharmaceutical industry for the phosphatase target family, academia has never abandoned that research field. Over the last 20 years, much progress has been made in understanding structural and mechanistic aspects of phosphatases and their role in disease biology.

### 3. Non-orthosteric SHP2 Inhibitors

SHP2 plays a modulatory role in numerous oncogenic cell signalling pathways such as the Ras-Raf-Mek-Erk, PI3K-Akt, or the Jak-Stat pathways, respectively. Novartis pursued drug discovery attempts focussed on inhibiting the SHP2 phosphatase, and the achieved results can be envisioned as a turning point in the history of industrial phosphatase inhibitor research.<sup>[29]</sup>

The SHP2 enzyme is a nonreceptor phosphotyrosine phosphatase containing two N-terminal SH2 (Src homology 2) domains, and a catalytic PTP domain. In its inactive state, the phosphatase adopts a closed, autoinhibited structure in which the N-terminal SH2 domain prevents access to the catalytic centre of the PTP domain. Upon binding to bis-phospho-tyrosine peptides such as IRS-1, both SH2 domains unfold from the catalytic domain of SHP2 and thus activate the phosphatase.

To overcome the limitations of the PTP1B approach, Novartis Pharmaceuticals reported in 2016 on the identification of a potent allosteric SHP2 inhibitor, **SHP099** (Fig. 6)<sup>[29]</sup>. This compound emerged from a tailored differential screening approach aimed at the discovery of novel allosteric inhibitors. For this purpose, a 100,000-compound library was screened against a near full-length version of SHP2 and in parallel against a truncated version, encompassing the catalytic domain only. The results of the screen against the catalytic domain were used to filter out active site-directed compounds from the HTS campaign against the full-length SHP2 protein. The remaining hits from the screen against the full-length enzyme qualify them as non-orthosteric inhibitors.

The piperidinyl-phenyl-pyrazine **SHP099** functionally inhibits the catalytic dephosphorylation activity of SHP2. Extensive X-ray structural studies revealed the precise molecular mechanism of action of the SHP099 class. The compounds bind to a pocket at the interface between the catalytic domain and the N-terminal SH2 domains and thereby function as protein-protein interaction agonists.<sup>[29]</sup> This mechanism generated significant interest in a number of other areas of medicinal chemistry, and the research area of ‘molecular glues’ was born.

In the meantime, Novartis progressed members of this family (**TNO155**) into clinical development. They have been rapidly followed by companies that pursued opportunistic approaches using the same inhibition mechanism for SHP2,<sup>[30]</sup> and the structural similarity of the clinical compounds shown in Fig. 6 is obvious.

Since this first publication of a clinic-ready allosteric SHP2 inhibitor, the focus on allosteric modulation of enzyme activity has increased dramatically and is helping to end the stigma of the phosphatase family.

### 4. Pre-clinical Allosteric Phosphatase Inhibitors

Over the last few years, a number of phosphatase inhibitors have been described in literature that can modulate activity *via* a non-orthosteric (allosteric) molecular mechanism of action (examples in Fig. 7).<sup>[31–33]</sup>

Compounds **6–8** are only a few examples of a variety of published compounds that act according to an allosteric molecular mechanism of action. A review of phosphatase inhibitor compounds which are profiled enzymatically and often by X-ray crystallography as allosteric inhibitors shows that only few molecules score high on a drug-likeness scale. In general, most of the drug discovery efforts of the last decade in the phosphatase inhibitor space suffer from poor compound quality. The target family of the phosphatases can still be characterised as a compound-poor area in which most of the compounds are of poor quality.

Despite these limitations, they clearly show that hit matter can be identified and should give impetus to finding improved hit and lead compounds. These new drug-like molecules will hopefully progress forward towards the clinic and help banish the stigma of the phosphatase family one and for all.

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## Differential screening approach

100,000 compound library  
against full-length SHP2



Hits: ortho- and allosterics  
100,000 compound library  
against catalytic domain



Hits: allosterics



Biophysics, X-ray, MedChem



SHP099

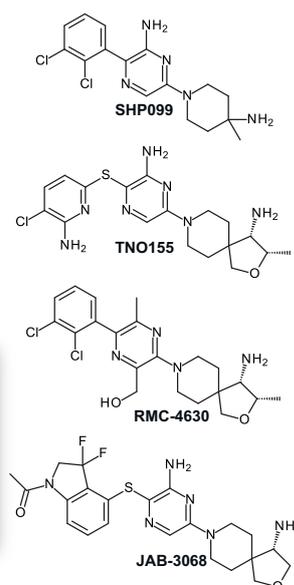
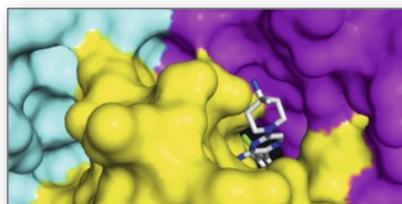
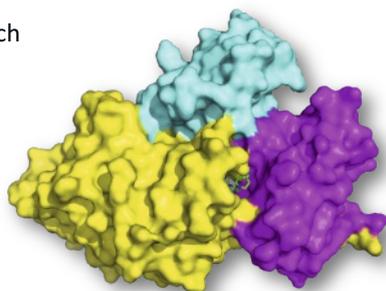


Fig. 6. Schematic representation of the discovery of allosteric SHP2 inhibitors. The differential screening approach pursued by Novartis Pharmaceuticals is shown as flow chart on the left.<sup>[29]</sup> The multidomain topology of the SHP2:inhibitor (**SHP099**) complex (pdb-code: 5ehr) is depicted in a solid surface mode in the middle. The N-terminal SH2 domain is shown in light blue, the C-terminal SH2 domain in magenta, while the catalytic phosphatase domain is depicted in yellow. The binding pocket of **SHP099** is at the interface of the distinct subunits (middle bottom). Chemical structures of allosteric SHP2 inhibitors in clinical development are depicted on the right.

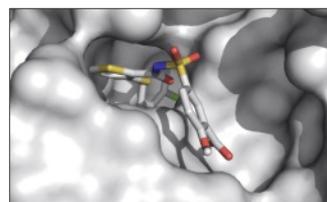
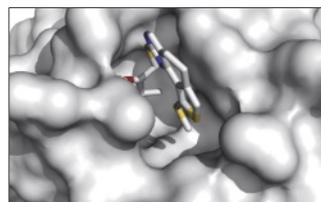
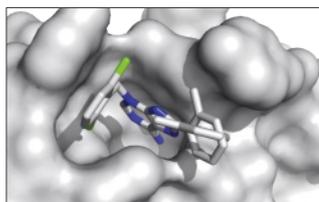
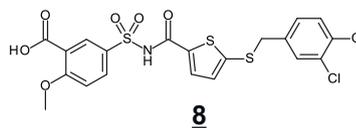
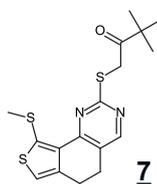
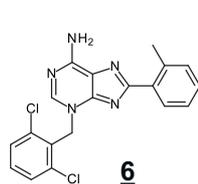


Fig. 7. A selection of allosteric phosphatase inhibitors (top) together with their experimentally determined binding modes (bottom) are depicted. Compound **6** is an allosteric inhibitor of the low-molecular-weight protein tyrosine phosphatase LMPTP (pdb-code: 7kh8) and shows a 20 nM inhibition constant and high selectivity,<sup>[31]</sup> compound **7** was found to be an allosteric mitogen-activated protein kinase phosphatase 5 (MKP5, pdb-code: 6mc1) inhibitor with a 4 μM inhibition constant,<sup>[32]</sup> while compound **8** was found to be an allosteric inhibitor of receptor protein tyrosine phosphatase g (RPTPg; pdb-code: 3qcj) with an inhibition constant around 500 nM.<sup>[33]</sup>

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