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Substrate Selection Influences Molecular Recognition in a Screen for Lymphoid Tyrosine Phosphatase Inhibitors

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Abstract

Assay design is an important variable that influences the outcome of an inhibitor screen. Here, we have investigated the hypothesis that protein tyrosine phosphatase inhibitors with improved biological activity could be identified from a screen using a more biologically relevant peptide substrate rather than traditional phosphotyrosine mimetic substrates. A 2000-member library of drugs and drug-like compounds was screened for inhibitors of the lymphoid tyrosine phosphatase using both a peptide substrate (Ac-ARLIEDNE-pCAP-TAREG-NH₂, peptide 1) and a small molecule, phosphotyrosine mimetic substrate (difluoromethyl umbelliferyl phosphate, DiFMUP). The results demonstrate that compounds that inhibited enzyme activity on the peptide substrate had greater biological activity than compounds that only inhibited enzyme activity on DiFMUP. Finally, epigallocatechin-3,5-digallate was identified as the most potent inhibitor of lymphoid tyrosine phosphatase activity to date, with an IC₅₀ of 50 nM and significant activity in T cells. Molecular docking simulations provide a first model for binding of this potent inhibitor to LYP that will constitute the platform for ongoing lead optimization efforts.

Keywords

Protein tyrosine phosphatase; library screening; computer modeling; molecular docking; peptide substrates; epigallocatechin-3,5-digallate

Introduction

Tyrosine phosphorylation is one of the most important cellular post-translational modifications, generating recognition motifs for protein-protein interactions, modulating enzyme activity and controlling many aspects of cell growth, differentiation, metabolism, migration and survival.^[1, 2] The enzymes responsible for modulating tyrosine phosphorylation are the protein tyrosine phosphatases (PTPs) and protein tyrosine kinases (PTKs). Both PTPs and PTKs have been recognized as relevant therapeutic targets for the

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treatment of diabetes, cancer and autoimmunity, but only PTK inhibitors have made it to the clinic thus far.^[3, 4] Considerable effort has been devoted to the development of potent, selective PTP inhibitors for use as chemical probes and potential therapeutic leads, but there are still several challenges in the field.^[5-8] Specifically, many of the PTP inhibitors identified to date are phosphotyrosine mimetic compounds with poor bioavailability. In addition, due to the highly conserved nature of the PTP active site, inhibitors that target the phosphotyrosine binding pocket tend to inhibit many members of the PTP family of enzymes and selectivity has been difficult to obtain. However, similar challenges have been resolved in the design of therapeutically relevant PTK inhibitors, and it is likely that with the right set of tools for inhibitor screening, PTP-targeted therapeutics can be developed as well.

The human genome contains a total of 107 genes encoding a PTP domain; 81 of these genes are predicted to encode an active PTP domain.^[9] The PTP catalytic domain is highly conserved and contains the PTP signature motif, (H/V)C(X)₅R(S/T), which includes the catalytic cysteine residue and the arginine residue which plays a key role in recognizing the phosphotyrosine (pY) moiety of the substrate.^[10] However, PTPs exhibit diverse surface features outside the pY binding pocket that remain largely unexplored in inhibitor development.^[4, 11] An exception is the secondary substrate binding pocket which has been exploited for the development of selective inhibitors of PTP1B and TCPTP.^[12-14]

Recently, the lymphoid tyrosine phosphatase (LYP or PTPN22) has been recognized as an intriguing therapeutic target because of its association with human autoimmunity.^[15-19] LYP is a negative regulator of T-cell receptor signaling.^[20] A single nucleotide polymorphism (SNP) (C1858T) in the *PTPN22* gene results in a gain of function variant of LYP that is associated with an increased risk of autoimmune disorders including type 1 diabetes, rheumatoid arthritis, systemic lupus erythematosus, Grave's disease and myasthenia gravis.^[21-23] Correspondingly, the G788A SNP in the *PTPN22* gene leads to a loss-of-function variant that is protective against systemic lupus erythematosus.^[24] Furthermore, the mouse ortholog of LYP (PEP) is a positive regulator of anaphylaxis in mice and both genetic and chemical knockdown of LYP (PEP) activity has been shown to alleviate anaphylaxis in mice.^[25] Taken together, these findings reinforce the importance of LYP in the immune response.

A handful of LYP inhibitors with low micromolar to high nanomolar potency have been reported. Several of these inhibitors are pY mimetic salicylic acid derivatives,^[18, 19, 26] including the two most potent LYP inhibitors to date (IC₅₀ = 270 nM and 110 nM).^[26, 27] Our laboratory has demonstrated that analogs of auranofin, a gold-based drug used to treat the autoimmune disorder rheumatoid arthritis, are fairly potent and selective inhibitors of LYP activity (IC₅₀ = 0.75 - 3.5 μ M, >7-fold selective over several other PTPs).^[16, 28] In addition, a series of allosteric LYP inhibitors with IC₅₀ values in the 8-20 μ M range have also been described.^[29] Importantly, several of these inhibitors have activity not only *in vitro* but also in cellular^[16, 19, 26, 28-30] and animal studies.^[25]

Although these LYP inhibitors have proven useful as chemical probes of the biological activity of LYP, greater potency and selectivity must be achieved in order to obtain a widely applicable probe. We^[16, 29] and others^[31] have hypothesized that the choice of substrate in an enzyme inhibitor screen is crucial in guiding the outcome of the screen. In a traditional PTP inhibitor screen, a non-peptidic pY mimetic substrate such as *p*-nitrophenyl phosphate (*p*NPP) or 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP) is used (see Scheme 1).^[32] These pY-mimetic substrates interact only with the pY binding pocket in the active site of the enzymes. It has been suggested that a substrate capable of interacting with sites on the enzyme outside of the pY binding site, such as the secondary substrate-binding site

described earlier, would be superior to a simple small molecule pY mimetic substrate in identifying hits with biological activity and selectivity for the enzyme of interest.^[16, 31]

Our group has developed a phosphorylated coumaryl aminopropionic acid (pCAP, Scheme 1). This pY mimetic amino acid can be readily incorporated into a peptide chain and, upon PTP-mediated dephosphorylation, undergoes a large increase in fluorescence, providing a facile, high-throughput assay for PTP activity.^[16, 33] For example, by incorporating pCAP in the place of pY in a peptide sequence based on the amino acid residues flanking Y394 of Lck, a known physiological substrate of LYP,^[23, 34] we obtained **peptide 1** (Scheme 1), an excellent substrate for LYP.^[16] Results from a small pilot screen using **peptide 1** as a LYP substrate indicated that this substrate might offer advantages in PTP inhibitor screening.^[16]

To test the hypothesis that substrate selection can influence screening results, we designed an experimental screen of the Spectrum collection, a commercially available collection of 2000 compounds with known biological activity, for compounds with the ability to inhibit the activity of LYP using both **peptide 1** and DiFMUP as substrates. Screening of small, focused libraries of chemically diverse, drug-like compounds can help in speeding up the drug discovery process by finding new applications of known compounds.^[35-37] Libraries of this kind have been used to obtain hits for many different applications including cancer, malaria and amebiasis.^[35-38] From this study, we have identified epigallocatechin-3,5-digallate (EGCDG) as the most potent LYP inhibitor reported so far (IC₅₀ = 50 nM). In addition, we have also identified selective LYP inhibitors with biological activity, establishing the utility of our pCAP peptide substrates in high-throughput screens for potent, selective and biologically active PTP inhibitors.

Results & Discussion

Two, parallel, medium-throughput experimental screens for LYP inhibitors were carried out, one using **peptide 1** as the substrate and the second using DiFMUP. The Spectrum collection, a commercially available library of 2000 compounds with a wide range of biological activities and structural diversity, was chosen for our comparative screen. This library consists of approximately 50% clinically available compounds, 20% compounds with known biological activity and 30% natural products with known and unknown biological activity isolated from variety of sources. The screening conditions were optimized for each substrate to achieve acceptable assay performance, sensitivity and robustness. The Z' value of 0.68 for **peptide 1** and 0.81 for DiFMUP indicated excellent performance in the screening assays. The signal to background ratio (S/B) of ~10 and ~128, and the signal to noise ratio (S/N) of ~235 and ~1325, for **peptide 1** and DiFMUP assays, respectively, indicate sensitive and robust assay conditions.

The results of the initial screen using **peptide 1** as the substrate are shown in Figure 1. The most potent hits, along with hits that showed significantly different inhibition of LYP activity depending on whether **peptide 1** or DiFMUP was used as the substrate were selected for follow-up. The top 80 compounds were re-screened at 5 μ M and 50 μ M concentrations. Results are shown in Figure 2 (selected results) and Figure S2 (full data). A few previously known PTP inhibitors including suramin (compound 1), purpurin (compound 5) and aurothioglucose (compound 9) came up as top hits in both of our screens, validating our screening protocol.^[5, 17, 39-41] However, most of the top hits were compounds that had not previously been identified as phosphatase inhibitors. While some of the hits were equally potent inhibitors of LYP activity regardless of substrate used, others inhibited LYP activity on **peptide 1** significantly better than on DiFMUP or vice versa (Figure 2, Table 1). Finally, a more detailed investigation was carried out on several top hits by determining their IC₅₀ values against LYP using both DiFMUP and **peptide 1** in secondary dose-

response screens. The selectivity of these top hits was profiled by determining their IC₅₀ values against PTP-PEST, HePTP and CD45 (Scheme 2, Table 1).

Several of the top hits were rigid aromatic compounds with negative charge, mimicking the pY moiety.

Quite a few of the hits contained a coumarin, flavone or polyphenolic moiety, features that are also known to mimic the pY residue. Metal-containing compounds including aurothioglucose and merbromin were among the most potent hits, likely due to the thiophilicity of gold and mercury, respectively. More unexpected was the result that several cephalosporin derivatives inhibit LYP activity. This may be attributed to the carboxylate functionality present in many of the cephalosporins, including cefotaxime. While some of the top hits bear a resemblance to compounds known to frequent hitting false positives showing promiscuous activity in many assays by virtue of aggregate formation,^[42] it has been shown that non-specific inhibition by aggregates can be eliminated by the addition of a detergent to the assay buffer.^[43, 44] As evidence that our screens were designed to avoid false positives due to aggregation, known aggregate forming compounds in our library (such as quercetin)^[42] were not hits with either substrate.

It is interesting to note that greater activity against one substrate or the other in the initial screen (Figure 1) often translated into differing potencies with the two substrates. For example, suramin (compound 1) appeared slightly more potent against the **peptide 1** as a substrate than DiFMUP in the initial screen and has an IC₅₀ that is 8-fold lower against the peptide *in vitro*. Conversely, sertraline (compound 4) appeared more potent against DiFMUP in the initial screen and has an IC₅₀ that is 3.4-fold lower with DiFMUP. While the IC₅₀ values for these two compounds are less than 2-fold different against the substrate DiFMUP, they are 50-fold different against **peptide 1**. Most importantly, these *in vitro* differences in inhibitory potency seem to translate into differential biological activity. We tested the ability of suramin and sertraline to inhibit LYP activity in Jurkat T cells by monitoring the phosphorylation of LckY394, a known biological substrate of LYP. While suramin showed marked LYP inhibitory activity, manifested as an increase in phosphorylation at LckY394 (Figure 3), sertraline had no activity. It would appear that the ability of a compound to inhibit LYP-mediated hydrolysis of the more biologically relevant substrate, **peptide 1**, is a better predictor of biological activity than ability to inhibit LYP-mediated hydrolysis of a small molecule substrate. Inhibition of a peptide substrate has been used as a secondary screen to narrow down a panel of LYP inhibitors previously, but has not been explicitly demonstrated to improve prediction of biological activity.²⁷

The most potent hit from our screen, epigallocatechin-3,5-digallate (EGCDG, compound 6), a pigment from green tea, is the most potent inhibitor of LYP and PTP-PEST reported so far with IC₅₀ of 50 nM against LYP and 20 nM against PTP-PEST. Notably, EGCDG is significantly less potent against CD45 (IC₅₀ = 500 nM). In order to validate the biological relevance of this hit, we investigated the ability of EGCDG to inhibit LYP activity in T cells. In this case, we monitored the phosphorylation levels of Zap-70 Y319, a direct substrate of Lck and an extremely sensitive read-out of Lck activity. Gratifyingly, we found that treatment of Jurkat T cells with 500 nM EGCDG resulted in a significant increase in phosphorylation of Y319 in Zap-70 as measured by phosphoflow cytometry (Figure 3), indicating inhibition of intracellular LYP activity resulting in an increase in intracellular Lck activity.

Molecular docking of EGCDG in LYP active site was performed to obtain a model for this binding and to gain further insight into this interaction (Figure 4). To estimate the binding score, the GlideXP program was used as it is based on extensive parameterization to

reproduce experimental binding free energy of the protein-ligand complex [45, 46]. A binding score of -9.4 kcal/mol for EGCDG indicated high propensity of binding considering that the average docking score from the top 80 hits was -5.2 kcal/mol. The gallate moiety at 5-position of the EGCDG compound docked into the active site and was stabilized by cation- π interactions provided by R233 in the P-loop (residues 226-233), hydrogen bonding with K138 of the $\beta 3$ - $\beta 4$ loop (residues 131-139) and T-shaped aromatic interaction with the Y60 residue of the pY recognition loop (residues 54-60). The gallate ring at 3-position docked into the hydrophobic pocket adjacent to the WPD-loop (residues 193-204) created by M130 and is stabilized by hydrogen bonding provided by backbone of K191. The pyrogallol-like ring of epigallocatechin preferentially interacted with WPD-loop, making hydrogen bonds with D195 and favorable Van der Waal interaction with W193, while the 7-hydroxy group of the epigallocatechin ring was found to make hydrogen bonds with T275 and E277 in the Q-loop (274-301).

A closer investigation into the activity of compounds closely related to EGCDG supports some of the interactions found in the docking study, and provides insight into the structure-activity relationships of these compounds. EGCDG is a flavonoid belonging to the flavan-3-ol class. There were several closely related flavan-3-ols in our initial screen, as shown in Scheme 3. With the exception of 2',2'-bisepigallocatechin digallate, which showed some LYP inhibition (25% at $10\text{ }\mu\text{M}$ in the initial screen), several related epigallocatechins and catechins showed little to no inhibition of LYP activity. In this series of compounds, EGCDG is the only compound with a gallate moiety at 5-position of EGCDG. These data support the docking pose in which the 5-gallate moiety is involved in key interactions within the pY binding pocket. Gallic acid alone and other gallate esters did not inhibit LYP, indicating that the pY-mimicking ability of the polyphenols is not enough for potent inhibition of LYP. Furthermore, neither the interactions between LYP and the 3-gallate moiety nor the 7-hydroxy moiety are sufficient for potent inhibition, as evidenced by the fact that epigallocatechin-3-gallate had very little inhibitory activity. Finally, the presence of multiple gallate moieties is helpful but not sufficient for potent inhibition, as demonstrated by the moderate inhibitory potency of 2,2'-bisepigallocatechin digallate.

Conclusions

Here we tested the hypothesis that the use of a more biologically relevant, peptide-based substrate in PTP inhibitor screens would result in hits with greater potential for biological activity. While it has previously been postulated that peptide-based substrates could provide more biologically relevant hits in PTP inhibitor screens, our pCAP peptide substrates have provided the opportunity to test this hypothesis for the first time. Our experimental screens for LYP inhibitors using both DiFMUP and **peptide 1** as substrates demonstrated that, in some cases, different hits are identified with the two different substrates and, most importantly, that potency against the peptide substrate correlated more strongly with biological activity. Epigallocatechin-3,5-digallate (EGCDG) was identified as the most potent inhibitor of LYP to date, with an IC_{50} value of 50 nM and significant cellular activity at 500 nM . A molecular model for the interaction of EGCDG with LYP was proposed based on molecular docking, providing the basis for future lead optimization efforts.

Experimental Methods

General Considerations

All the reagents were purchased from commercial sources and used without further purification. The library of compounds, the Spectrum collection, was purchased from Microsource Discovery Systems, Inc. The pCAP residue and **peptide 1** were synthesized as described previously by Mitra *et al.*^[33] Stock solutions and serial dilutions of the substrates

and inhibitors were made in DMSO. The catalytic domain of human recombinant CD45 was obtained from Biomol. The modified pBAD plasmid encoding the catalytic domain of HePTP (aa 44-339) in frame with a noncleavable 6xHis tag was obtained as a generous gift from Lutz Tautz. cDNA fragments encoding the catalytic domains of LYP (aa 2-309) and PTP-PEST (aa 2-323) were cloned between the BamH1 and the Xho1 sites of pET28a plasmid (Novagen) in frame with a cleavable N-terminal 6xHis-tag. Recombinant proteins were purified from lysates of IPTG-induced *Escherichia coli* BL21 cells by affinity chromatography on Ni-nitrilotriacetic acid columns. 6xHisHePTP was eluted using 250 mM imidazole. Untagged LYP and PTP-PEST were eluted by incubating columns with thrombin, followed by removal of thrombin from the protein preparation by a second chromatography step on benzamidine columns. All of the enzyme activity assays were performed at ambient temperature using a buffer containing 50 mM Tris, pH 6.5, 100 mM NaCl, 1 mM dithiothreitol (DTT), 2 mM EDTA, and 0.01% Brij 35. The DMSO concentration in each assay was held constant at 6% by volume. We have previously demonstrated that LYP activity is insensitive to DMSO up to a concentration of 10%.^[29] Fluorescence data were collected on a Molecular Devices Spectramax M5 multimode plate reader with excitation and at 360 and 455 nm, respectively. The increase in the fluorescence due to the hydrolysis of the substrates was measured every 60s for 30 min and the fluorescence values in the linear range of the Michaelis-Menten kinetics curve were used for the data analysis.

Experimental library screen

The Spectrum collection obtained consisted of 25 96-well plates, each of which contained 200 μ M DMSO solutions of 80 compounds in columns 2-11. A 5 μ L aliquot of each of these compounds was transferred to corresponding position in the assay plate. A 5 μ L aliquot of DMSO was transferred to each well of column 12 of the assay plate to serve as a control. A 1 μ L aliquot of a DMSO solution of DiFMUP (150 μ M stock) or **peptide 1** (1 mM stock) was also transferred to the wells of the assay plates. The total amount of DMSO in each reaction well was held constant at 6% of the total reaction volume (100 μ L). A 50 nM solution of enzyme in activity buffer was preincubated with 1 mM DTT for 30 min to ensure complete activation. The preactivated enzyme was further diluted with the activity buffer just prior to addition to the assay plate, so that addition of a 94 μ L of the diluted enzyme to each well would result in a final enzyme concentration of 5 nM. The final DTT concentration in each reaction well was 100 μ M. The final concentrations of the substrates used were 1.5 μ M and 10 μ M for DiFMUP and **peptide 1**, respectively. The final concentration for each compound screened in the library was 10 μ M. Upon addition of the preactivated enzyme, the enzyme activity was monitored at room temperature (21-24°C) in black 96-well plates. The activity of the enzyme was measured by monitoring the increase in fluorescence ($\lambda_{\text{ex}} = 360$ nm, $\lambda_{\text{em}} = 455$ nm) every 60 sec for 30 min as the enzyme dephosphorylated the substrate (either DiFMUP or **peptide 1**). Each source plate in the library was screened in triplicate with each of the substrates and results were averaged. The percent relative activity for each compound was determined by factoring the measured fluorescence values for the compound treated wells over the control wells treated with DMSO only.

Inhibition assays

Stock solutions and serial dilutions of the compounds for determination of IC₅₀ were made in DMSO. All the hits were screened at inhibitor concentrations ranging between 100 nM to 500 μ M. The total DMSO concentration in each reaction was held constant at 6% of the total reaction volume (100 μ L). The concentrations of enzymes, DTT and substrates were the same as those used in the library screening assays. The percent relative activity at different concentrations was determined by factoring the measured fluorescence values for the

compound treated wells over the control wells treated with DMSO only. The resulting plot of inhibitor concentration versus percent enzyme activity provided the IC₅₀ values. The lead compounds were counterscreened against PTP-PEST, HePTP and CD45 to provide an indication of selectivity.

Cell culture

Jurkat T cells expressing the SV-40 large T Antigen (JTA_g)^[47] were kept at logarithmic growth in RPMI 1640 medium (Mediatech, Manassas, VA) supplemented with 10% fetal bovine serum (Omega Scientific, Tarzana, CA), 2 mM glutamine, 1 mM sodium pyruvate, 10 mM HEPES pH 7.3, 2.5 mg/ml D-glucose, 100 units/ml of penicillin and 100 µg/ml streptomycin (Life Technologies, Carlsbad, CA).

Phospho-flow cytometry

JTA_g cells were pretreated with the indicated concentrations of LYP inhibitors or DMSO for 30 minutes in RPMI 1640 at 37°C. Cells were then stimulated with supernatants of C305 hybridoma^[48] for 2 min at 37°C. Cells were fixed immediately with BD Cytotfix buffer, permeabilized using BD Phosflow Perm Buffer III and stained with AlexaFluor-488-conjugated anti-pZAP-70(Y319) antibody or anti-pSrc(Y418) antibody (which is cross-reactive with pLck(Y394, and labeled as such in Figure 4) according to the manufacturer's protocols (BD Biosciences, San Jose, CA). Cell fluorescence was analyzed by FACS using a BD LSR II (BD Biosciences). Data was analyzed using FlowJo software (TreeStar, Ashland, OR).

Docking Simulations

The Schrödinger suite of programs^[49] was used for the docking studies. The crystal structure (PDB entry 2P6X) prepared for the simulation was utilized for docking the library of compounds. A scoring grid was pre-computed by placing an outer cubical box of length 36 Å and an inner box of length 14 Å centered at the P-loop residues (227-231). The hydroxyl groups of all Ser, Thr, and Tyr residues in the binding site were allowed to be flexible during the grid generation process. Docking calculations were performed using Glide (version 5.7)^[45]. The ligand library comprised of hit compounds from the experimental screening and were prepared using LigPrep.^[49] Low energy conformations were generated for all possible protonation and ionization states (pH range 7.0 ± 2.0). All the ligand conformers were docked using Glide extra precision (XP).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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ABBREVIATIONS

PTP	protein tyrosine phosphatase
PTK	protein tyrosine kinase
pY	phosphotyrosine
PTP1B	a PTP involved several key cellular signaling pathways including insulin signaling
TC-PTP	a T cell derived PTP
LYP	the lymphoid tyrosine phosphatase
SNP	single nucleotide polymorphism
pNPP	<i>para</i> -nitrophenyl phosphate
DiFMUP	difluoromethylumbelliferyl phosphate
pCAP	phosphocoumaryl aminopropionic acid
Lck	a Src-family tyrosine kinase
FDA	Food and Drug Administration
EGCDG	epigallocatechin-3,5-digallate
ZAP-70	a tyrosine kinase involved in T cell receptor signaling
IPTG	isopropyl- β -D-1-thiogalactopyranoside
DTT	dithiothreitol
EDTA	ethylenediamine tetraacetic acid
JTA_g	Jurkat T cells
FACS	fluorescence activated cell sorting

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Figure 1. Results from the Spectrum collection screen for LYP inhibitors using peptide 1 as the substrate

Each compound is represented by its position in one of the 25 96-well plates in the collection. Compounds showing significant inhibition at 10 μ M are indicated as white squares, compounds showing no inhibition are indicated as black squares, compounds with intermediate inhibitory properties are shown in grey (gradient from white to black).

Figure 2. Select hits from the rescreening of top 80 compounds from the Spectrum collection at 50 μ M using **peptide 1** (black bars) or DiFMUP (grey bars) as the substrate.

Figure 3. The LYP inhibitors suramin and epigallocatechin-3,5-digallate increase T cell activation

Left panel: suramin increases phosphorylation of Lck Y394 in human T cells. JTA9 cells were pre-incubated with 25 μ M compound (red graph) or DMSO alone (blue graphs) for 30 min at 37°C followed by stimulation with C305 supernatant (solid graphs) or left unstimulated (dashed graphs) for 2 min at 37°C. Graphs show cell fluorescence after staining with an AlexaFluor-488-conjugated anti-phospho-Lck(Y394) antibody. Median fluorescence intensity (MFI) of each sample is shown. The % positive cells in the compound-treated, TCR-stimulated sample compared to the DMSO-treated, TCR-stimulated control was 16.7%. **Right panel:** epigallocatechin-3,5-digallate increases activation of ZAP-70 in human T cells. JTA9 cells were pre-incubated with 500 nM compound (red graphs) or DMSO alone (blue graphs) for 30 min at 37°C, followed by stimulation with C305 supernatant (solid graphs) or left unstimulated (dashed graphs) for 2 min at 37°C. Graphs show cell fluorescence after staining with an AlexaFluor-488-conjugated anti-phospho-ZAP-70 (Y319) antibody. Median fluorescence intensity (MFI) of each sample is shown. The % positive cells in the compound-treated, TCR-stimulated sample compared to the DMSO-treated, TCR-stimulated sample was 22.8%. The % positive cells in the compound-treated unstimulated sample compared to the DMSO control was 3.4%.

Figure 4. EGCDG docked pose

Most representative docked pose of Epigallocatechin-3,5-digallate from computer modeling.

Left panel: LYP surface representation. **Right panel:** key residues interacting with the inhibitor.

Scheme 1.
Colorimetric and fluorogenic PTP substrates.

Scheme 2.

Selected hits from the Spectrum Library Screen with numbering as in Table 1.

Scheme 3.

Structure-activity relationships among the flavanol compounds in this study.

Table 1

Hit validation and counter screening of selected hits from the Spectrum library screen. IC₅₀ values are shown in μ M. NA indicates data not available.

Compound number	Compound name	LYP (DiFMUP)	LYP (peptide 1)	PTP-PEST (DiFMUP)	PTP-PEST (peptide 1)	HePTP (DiFMUP)	CD45 (DiFMUP)
1	Suramin	25.0 $\pm$ 0.3	3.0 $\pm$ 0.1	27 $\pm$ 3	11 $\pm$ 1	30 $\pm$ 3	42 $\pm$ 5
2	Caperatic acid	12 $\pm$ 1	6.5 $\pm$ 0.7	NA	NA	NA	NA
3	Cefotaxime	40 $\pm$ 2	17 $\pm$ 2	70.0 $\pm$ 0.8	NA	NA	NA
4	Sertraline	44 $\pm$ 3	150 $\pm$ 10	55 $\pm$ 5	NA	110 $\pm$ 5	80 $\pm$ 4
5	Purpurin	4.0 $\pm$ 0.2	2.0 $\pm$ 0.1	7.0 $\pm$ 0.6	5.0 $\pm$ 0.6	NA	6 (ref 43)
6	Epigallocatechin 3,5-digallate	0.050 $\pm$ 0.002	0.050 $\pm$ 0.003	0.020 $\pm$ 0.001	0.020 $\pm$ 0.001	NA	0.50 $\pm$ 0.03
7	Harmalol HCl	15 $\pm$ 2	7.0 $\pm$ 0.8	35 $\pm$ 2	35 $\pm$ 2	NA	14 $\pm$ 1
8	Benzanthrone	28 $\pm$ 1	13 $\pm$ 1	> 150	> 150	35 $\pm$ 5	> 150
9	Aurothioglucose	4.0 $\pm$ 0.1	1.5 $\pm$ 0.1	8 $\pm$ 1	5 $\pm$ 0.8	NA	NA
10	Sennoside A	5.0 $\pm$ 0.3	5.0 $\pm$ 0.1	0.40 $\pm$ 0.03	0.20 $\pm$ 0.01	1.0 $\pm$ 0.1	2.0 $\pm$ 0.1