Evaluation of Virtual Screening as a Tool for Chemical Genetic Applications

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ABSTRACT

A collection of over 50,000 functionally annotated drugs, clinical candidates and endogenous ligands was docked in silico against nine binding sites from seven protein targets, representing diverse function and structure, namely the sulfotransferases SULT1A3 and SULT1E1, the histone methyltransferase EHMT1, the histone acetyltransferase MYST3, and the nuclear hormone receptors ERα, PPARγ, and TRβ. For 5 of the 9 virtual screens, compounds that docked best to the receptors clearly recapitulated known biological functions of the genes, or identified novel biology subsequently validated experimentally, in 2 cases, the hit list indicated some relevant, but isolated biological functions which would probably have been ignored a priori, and in 2 cases, selected compounds were completely unrelated to known gene function. This study demonstrates that virtual screening of pharmacologically annotated compound libraries can be used to derive target biology.

INTRODUCTION

Chemical genetics – the systematic use of small molecules to probe biological phenomena – is an approach that has gained momentum in the life sciences. In the last decade, landmark publications have demonstrated that libraries of synthetic or natural chemicals can be used in a systematic way to explore biological functions. For instance, phenotypic screens of compound libraries uncovered monastrol, the first non-tubulin inhibitor to affect mitosis, as a precious molecular tool to study the mitotic mechanism.[1] Similarly, chemical genetics screens successfully identified pumorphamine as a small molecule that could serve as a chemical tool to study the molecular mechanisms of osteogenesis and bone development.[2]

Continuing progresses in diversity oriented organic synthesis,[3] and small molecule microarray technology open a wide array of opportunities for chemical genetics applications (see for instance[4]), and large scale public efforts such as ChemBank[5] (http://chembank.broad.harvard.edu/) should further promote original attempts at probing biological complexity with chemistry. Other initiatives which are making substantial contributions in this area include the NIH Molecular Libraries Screening Center Network (MLSCN), where results of high-throughput screens and chemical probe development projects are made available to the scientific community by depositing such data into PubChem (http://pubchem.ncbi.nlm.nih.gov/).[6] Chemical genetics thinking is also captivating computational biologists and chemists: in silico approaches clustering protein targets based on the chemistry of their ligands have been reported that underpin biological promiscuity between seemingly unrelated genes.[7] Interestingly, it was also shown that clustering of targets in the biological space based on their sequence, and in the chemical space based on the chemistry of their ligands produce diverging results.[8] Indeed, binding site similarity does not imply convergence of ligand chemistry.[9] In other studies, virtual screening of a library of marketed drugs was successfully used for drug-repurposing (leading to the

identification of nonsteroidal antagonists against the human androgen receptor[10]), and in silico docking of putative substrates has permitted the assignment of function for some enzymes.[11-13]

Clearly, virtual screening has been used successfully to probe receptor function, and proof of concept was achieved for this novel technology. However, no systematic effort has been described to date that addresses the reliability of the method. In the current investigation, the World Drug Index (WDI, Thomson Derwent, Alexandria, VA), a compilation of over 50,000 functionally characterized natural and synthetic small molecules, was screened in silico against the nine ligand binding pockets of seven targets covering four functionally and structurally diverse protein families, to assess the success rate of high-throughput docking for functional annotation of the receptor. Results demonstrated that, in most cases, the virtual hit list could recapitulate known biology or selectivity profile of the target.

METHODS

Virtual screening of sulfotransferases. The first sulfotransferase, SULT1E1, specifically binds estrogens (e.g. estrone), while the second, SULT1A3, specifically binds catecholamines (e.g. dopamine, norepinephrine) and simple phenols.[14] In the first case, the crystal structure of human SULT1E1 bound to its coenzyme, 3'-phosphoadenosine-5'-phosphosulfate (PAPS), was used for virtual screening (PDB: 1hy3). The co-crystal structure of estradiol bound to mouse SULT1E1 (PDB: 1aqu) was used to define the location of the substrate binding pocket. For the second protein, the co-crystal structure of human SULT1A3 bound to dopamine was selected (PDB: 2a3r), using the dopamine molecule to define the binding pocket and the SULT1E1 structure (PDB: 1hy3) to determine the proper position of PAPS in the SULT1A3 structure. For both SULTs, a constraint was imposed to reject putative ligands that did not possess a hydroxyl group at the sulfate accepting site in their predicted binding pose, as the goal was not to indentify the sulfotransferase activity but to predict the substrate selectivity profile. The WDI was first docked with Glide SP, and the top scoring compounds were then re-docked and scored with Glide

XP. (Ligprep, which was used to generate different enantiomers and protonation states of the WDI compounds, and Glide, which was used to carry out docking, are both part of the Schrödinger program suite)

Virtual screening of histone modifying enzymes. The structures of histone methyltransferase EHMT1 bound to S-adenosyl L-homocysteine (SAH, the cofactor product), and of histone acetyltransferase MYST3 bound to acetyl-CoA were selected (PDB: 2igq and 2ozu, respectively). Docking was performed either at the cofactor binding site in the absence of substrate or at the substrate lysine binding site in the presence of cofactor. In the latter scenario, a positional constraint was applied to ensure that putative ligands would be docked in proximity of the cofactor, since the question was whether virtual screening would give some indication on the type of substrates methylated or acetylated by these enzymes. The substrate binding site of MYST3 contains an acetylated Lys604 residue in 2ozu, which was changed to an unmodified lysine residue for the purpose of this study. The WDI was docked with Glide HTVS, followed by re-docking of the top 10% of structures with Glide SP, and finally by re-docking the top 10% of SP scored structures with Glide XP. (Ligprep, which was used to generate different enantiomers and protonation states of the WDI compounds, and Glide, which was used to carry out docking, are both part of the Schrödinger program suite)

Virtual screening of nuclear hormone receptors. Three nuclear hormone receptors were selected for screening against the WDI: the estrogen receptor-α (PDB: 3erd), the peroxisome proliferator activated receptor-γ (PDB: 1fm6), and the thyroid hormone receptor-β (PDB: 1bsx). For each of these proteins, ICM (Molsosft LLC) was used to carry out virtual ligand screening.[15]

RESULTS

Virtual screening of sulfotransferases. Sulfotransferases (SULTs) are enzymes that catalyze the sulfonation of various endogenous compounds and xenobiotics, thus playing a key role in their metabolism.[14] Two SULTs with different substrate selectivity profiles were chosen to investigate the ability of virtual screening not only to recapitulate known biology, but also to differentiate between distinct substrate classes for members of a single protein family. The top 12 compounds from the WDI that scored highest against SULT1E1 are shown in Table 1: the second compound is an estrogen, while 3 more compounds (ranks 4, 6 and 12) are estrogen antagonists. Furthermore, a total of 30 compounds among the top 100 hits are listed as estrogens or estrogen antagonists in the WDI (data not shown), which would have provided significant insight into this enzyme's substrate, had it not been known.

In the case of SULT1A3, the results are perhaps even more striking: 10 of the top 12 compounds (and almost half of the top 100 hits – data not shown) are sympathomimetics/dopaminergics or dopamine antagonists (Table 2). It is therefore apparent from these results that virtual screening performed very well in recapitulating known biology for both SULT1E1 and SULT1A3. The result for SULT1A3 should be taken with caution since the structure used for virtual screening was in a conformation co-crystallized with dopamine, but no similar bias was introduced in the SULT1E1 virtual screen. Not only was significant enrichment of estrogenic compounds for SULT1E1 and of dopaminergic compounds for SULT1A3 obtained, but only one dopaminergic was present in the top 100 hits for SULT1E1, while no estrogenics were in the top 100 hits for SULT1A3, demonstrating that virtual screening was able to differentiate between their selectivity profiles. The docking poses for the best scoring estrogen against SULT1E1 (estrynamine) and the best scoring dopaminergic against SULT1A3 (SDZ-GLC-756) are shown in Figure 1, and compared to estradiol and dopamine, respectively.

Virtual screening of histone modifying enzymes. The euchromatic histone methyltransferase 1 (EHMT1/GLP) and the MYST histone acetyltransferase (monocytic leukemia) 3 (MYST3/MOZ) are two enzymes involved in epigenetic modifications of histone tails. The former mono- and di-methylates

the Lys9 residue of histone 3 (H3K9) and requires the methyl-donating cofactor S-adenosyl L-methionine (SAM),[16] while the latter has been shown to acetylate the Lys14 residue of histone 3 (H3K14) using acetyl coenzyme-A (acetyl-CoA).[17]

Virtual screening of the WDI against the cofactor binding pocket of EHMT1 did not yield any cofactor analogues in the top 12 compounds (Table 3). SAM ranked 73rd and three cofactor analogues were also in the top 100 hits: sinefungin (a known methyltransferase inhibitor[18]), diolsinefungin (a close analogue of sinefungin), and A-9145C (another compound reminiscent of the cofactor). Although these biologically relevant compounds were docked accurately, their docking scores were not sufficient to separate them from the noise. This highlights the need for improved scoring functions, as true positives can be missed if only a very small number of top ranking compounds are considered.

In the case of MYST3, compounds 1 and 6 (thioguanosine-diphosphate and aica-adenine-dinucleotide, respectively), and to a lesser extent compound 12 (SR-3745A), are mimetics of the adenosine-diphosphate (ADP) scaffold of acetyl-CoA, and point at the type of chemistry binding at the cofactor site (Table 4). Although this result in itself would not have been sufficient to identify acetyl-CoA as the cofactor for MYST3, it would have provided insight into the type of chemistry capable of binding in this pocket, narrowing down the number of putative endogenous ligands. It should be noted however that the selection of ADP mimetics may have been partially fortuitous: the best scoring pose of these compounds reveals a predicted complex in which one or multiple phosphates occupy the approximate position of the acetyl-CoA diphosphate moiety, but the adenosine scaffold (or its analogue) does not overlap with the adenosine fragment of acetyl-CoA. Considering that several water molecules are involved in bridging hydrogen bonds between MYST3 and acetyl-CoA in the crystal structure, it is not surprising that the correct pose is not retrieved for acetyl-CoA or its analogues when water molecules are removed from the receptor site.

Virtual screening of the WDI against the substrate site of EHMT1 (Table 5), identified several peptides or peptido-mimetics in the top 12 compounds, which would have suggested, had we not known it, that the substrate for EHMT1 is in fact a peptide. The top ranking compound for instance, amastatin (AHMHA-Val-Val-Asp-OH), is a peptide-hydrolase inhibitor.[19] Compound 4, capreomycin, is a cyclic peptido-mimetic, possessing a lysine-like moiety which, according to the docking model (Figure 2), is capable of extending into the narrow channel where the physiologically relevant Lys9 side-chain of histone H3 binds and is subsequently di-methylated by EHMT1. Another hit suggesting that the substrate is a lysine residue is compound 10, Lys4-tuftsin (Thr-Lys-Pro-Lys). In the best scoring pose, the backbone of this tetra-peptide docks into the groove where the backbone of H3 is known to bind (PDB: 2rfi), while the Lys4 side-chain sits in the narrow lysine binding channel.

Unlike the hit list obtained against the peptide binding site of EHMT1, the top 12 compounds that scored best against the substrate peptide site of MYST3 do not appear to indicate that the substrate is a peptide or a lysine residue, and therefore virtual screening was unsuccessful at identifying known biology for this target (Table 6).

Virtual screening of nuclear hormone receptors. Nuclear hormone receptors (NR) are ligand-dependent transcription factors which are activated via binding of small molecules to their ligand-binding domain.[20]

A first observation is that 7 of the top 12 hits predicted to bind ERα have estrogenic activity (Table 7). Additionally, another 3 compounds are agonists for the progesterone and androgen receptors, close ER homologues. Clearly, if the function of the target had not been known, this selection would have strongly suggested that it is involved in estrogen related signaling. This encouraging result should still be taken with caution for two reasons. First, estrogens are overrepresented in the database screened, which increases chances of finding estrogenics in the hit list. Second, the ERα structure screened was

derived from a co-crystal of the receptor complexed to diethylstilbestrol, an ER agonist, which introduces a bias in favor of estrogenic compounds.

Screening against the crystal structure of PPARγ failed to identify known agonists present in the compound library, illustrating the inability of current virtual screening tools to avoid false negatives (Table 8). Relevant information was still extracted from the wealth of pharmacological data contained in the library screened: 4 of the 12 best scoring compounds against PPARγ have anti-aggregant activity (compounds 1, 3, 5 and 8), which would suggest that PPARγ is involved in atherogenesis, a hypothesis that is largely substantiated in the literature (see [21] for review). Additionally, the 1st and 8th compounds docking best to PPARγ are prostaglandin receptor agonists, pointing at a putative promiscuity between the two receptors. Indeed 15-deoxy-delta prostaglandin J2 (15d-PGJ2) is a known endogenous PPARγ agonist.[22] Decaprenoic acid, which ranked 7th, is also a non fortuitous hit, as fatty acids are known PPARγ ligands.[22] However, this is the only fatty acid at the top of the hit list, and though relevant, this putative link would not have been identified a priori.

In the last case, the WDI was screened virtually against the active form of the TRβ ligand binding pocket. As for PPARγ, known TR ligands were not in the top of the list (Table 9), even though they were present in the source library, which illustrates the need to continue improving virtual screening docking algorithms and scoring functions. Quite interestingly, two of the top 12 hits inhibit farnesyl protein transferase (compounds 10 and 12), an enzyme that transfers a farnesyl moiety from farnesyl pyrophosphate (FPP) to target proteins, and another two are inhibitors of squalene synthase (compounds 3 and 11), an enzyme that converts FPP into squalene during cholesterol biosynthesis. Together, one third of the top 12 compounds bind to active sites recognized by FPP. If diverse compounds that comply with the structural chemistry of FPP binding sites dock well to TRβ, FPP might bind to TRβ itself. This hypothesis was tested, confirmed, and extensively documented and discussed elsewhere.[23] Importantly,

it was shown that 1) FPP activates TR as well as other NRs at physiologically relevant concentrations, 2) FPP mediates cross-talk between cholesterol biosynthesis and a variety of NR-related signaling and metabolic pathways and 3) FPP may contribute to the pleitropic effect of statins.

While known ligands present in the chemical library for the three NRs screened were not always hit, well documented function or signaling pathways for two of these targets were mirrored in the hit list. First, the estrogenic activity of ERα was recapitulated by the presence of over 50% estrogenic compounds in the top scoring molecules. Similarly, the presence of 4 anti-aggregant compounds among the 12 compounds docking best to PPARγ pointed at promiscuity between the structural chemistry of the PPARγ binding pocket and receptors involved in blood clotting cascade. Mounting evidence indicate that this promiscuity is not only structural, but functional. The presence of 2 prostaglandin receptor antagonists was also an indicator of the putative binding of prostaglandins to PPARγ, a biological fact. In the third example, the virtual screen suggested a cross-talk between TR and the cholesterol biosynthesic pathway via FPP, a hypothesis so far not documented, but subsequently validated experimentally.[23]

DISCUSSION

Previous reports have shown that virtual screening technology can be used as a chemical genetic tool that links molecular probes to protein targets.[11-13] Here, we show that these successes should not be held as exceptions, but representative of a technology that is reaching maturity. Out of 9 virtual screens, 5 could have been used for a priori predictions, 2 gave some indication, but noise to signal ratio may not have been sufficient, and 2 failed to identify known relevant biology.

We believe that screening functionally annotated chemical libraries such as the WDI used here, the DrugBank,[24] or the human metabolome[25] brings an additional dimension to the probing exercise

typically used in chemical genetics, since the molecular probes are no longer tools that can be used to modulate the activity of the target, but are used directly as functional tags. The pharmacology of compounds selected against PPARγ correctly indicated a functional link between this target and platelet aggregation. The chemistry of compounds selected against EHMT1 accurately pointed at peptides, and more specifically lysine residues as substrate of this methyltransferase. In vitro screens of drug libraries have been described that could reposition old drugs for new applications (see [26] for review), and more recently, virtual screening was used to identify novel nonsteroidal antagonists of the androgen receptor from marketed drugs.[10] Though questioned by some, it is reasonable to assume that in silico screens still suffer from a higher rate of false positives and negatives than in vitro screens. Paradoxically, we would like to argue that this may well be a strength of the application presented in this work: though they all dock well to PPARγ, it is unlikely that all four anti-aggregant molecules selected against this receptor actually bind at μM concentration, considering that virtual screening hit rates typically levitate around 5 to 10% and occasionally rise to 35% (see [27] for review). Some of these putative ligands are actually "close-miss binders", i.e. compounds that do not bind to the receptor, but that have a chemistry that is very close to complying with the pharmacophoric topology of the receptor. As such, these compounds ended-up as false positives in silico, but would have been "accurately missed" by an in vitro screen. Unlike in vitro screens, in silico screens can identify such close-miss binders that point at structural and functional promiscuity. In the TRβ screen, it is highly unlikely that all four farnesyl related ligands do bind to TR, however, they are all close to binding: their ability to dock well in silico to TR revealed subsequently validated promiscuity between the cholesterol biosynthesis and TR signaling pathways that would not have been identified by an in vitro screen.

While these results demonstrate the potential of virtual screening to reveal unknown target biology, this study also highlights some of the limitations of current in silico docking methods. For instance, one of the deficiencies of commonly employed docking protocols is the lack of explicit receptor-flexibility. Indeed, if a ligand induces a significant receptor conformational change upon binding, it is unlikely that

a rigid-receptor/flexible-ligand docking approach would correctly identify this compound as a hit. Inversely, it is perhaps not surprising that we observe such a high rate of dopaminergics in the hit list for SULT1A3 and of estrogenics in the hit list for ERα: the structures used for performing in silico screening against these two proteins were co-crystallized with dopamine and diethylstilbestrol (an ER agonist), respectively. The receptors are in a ligand-bound conformation, and are biased towards dopaminergics and estrogens, respectively. Interestingly, virtual screening against ligand-bound structures can also reveal unknown biology, as was clearly demonstrated with the TRβ screen, conducted against a structure of the receptor co-crystallized with thyroid hormone: known TR ligands were not recovered, but compounds binding to farnesyl protein transferase and squalene synthase were identified, which lead to the discovery that FPP activates TR.

Our results also demonstrate that virtual screening can, in some cases, recapitulate known biology from screening against apo structures, as best exemplified by screening the SULT1E1 apo structure, which resulted in estrogens and estrogen antagonists accounting for one third of the top 12 compounds (Table 1). Similarly, the hit list obtained from screening against apo EHMT1 (PDB: 2igq) was enriched in peptide and peptido-mimetics, even though the peptide binding groove becomes fully ordered only upon complexation to the substrate peptide (PDB: 2rfi). On the other hand, docking results against MYST3 did not suggest that the substrate is the lysine residue of a peptide, and one possible reason for this may have been the lack of receptor flexibility, although this cannot be verified as there are presently no available co-crystal structures of MYST3 with a bound substrate. It is also known that formation of a multi-subunit complex significantly increases the acetyltransferase activity of MYST3, and screening against the MYST domain alone may be doomed from the start.

An unexpected outcome of this work is the emergence of in silico frequent hitters. For instance, xenocoumacin-1 appears in the top 12 hits for both SULT1E1 (Table 1) and the MYST3 substrate site (which also features xenocoumacin-2 and the related amicoumacin-A and -B, Table 6), while diverse

nikkomycins rank in the top 12 best predicted binders for the EHMT1 cofactor (Table 3) and substrate (Table 5) sites, as well as for the MYST3 cofactor site (which also contains related polyoxins, Table 4). This is more likely to reflect an artifact of virtual screening than true ligand promiscuity. First, most of the compounds that scored well against more than one binding site in this study are relatively large molecules, and scoring functions often overestimate binding affinities of larger compounds.[28,29] Additionally, these compounds are comprised of a large number of hydrogen-bond donors and acceptors, making them more likely to dock and score well in a variety of putative binding pockets, and under-estimation of the desolvation penalty in scoring functions may be at the origin of such false positives.

Countless hours were spent over decades in the pharmaceutical industry and academia to annotate the pharmacological space. Mining this goldmine of information by literature or patent search is often the best way to address focused, target- or compound-oriented questions. Recent work illustrated how 2D computational structural chemistry approaches could be used to mine in a systematic way the pharmacological space.[30] The objective of this study was not to compare different docking protocols, but to show that, regardless of the virtual screening software used, high-throughput docking has reached a level of accuracy sufficient to interface the biological and pharmacological spaces and provide key insight into unknown biological function of proteins.

CONCLUSION

The virtual "reverse profiling" application evaluated here, whereby one target is matched against a collection of drugs and other functionally annotated compounds, relies on virtual screening, a technology that still needs to gain in reliability. Nevertheless, it is often successful at assigning new putative functions to extensively or poorly characterized receptors. It can also be used to suggest putative endogenous ligands to orphan receptors, and to propose repositioning strategies for drugs with

satisfactory pharmacokinetic properties, but sub-optimal potency. Finally, it can help uncover endogenous small molecule "missing links" in the biological maze.[31]

ACKNOWLEDGMENTS

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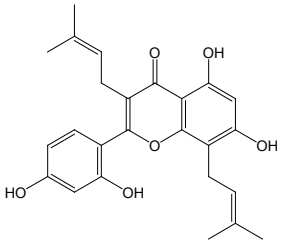
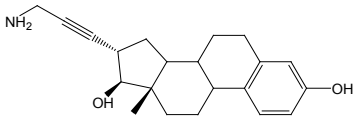
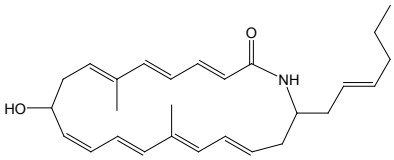
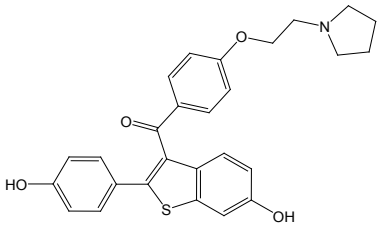
Supporting Information Available. References cited in Tables 1-9. This material is available free of charge via the Internet at http://pubs.acs.org.

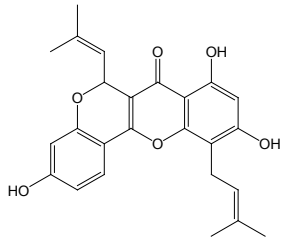
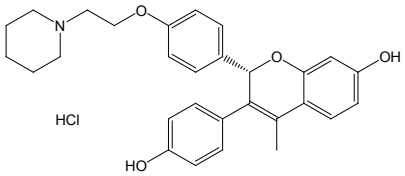
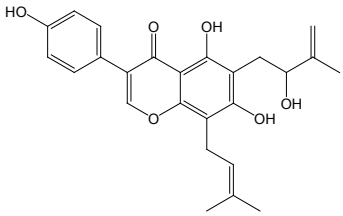
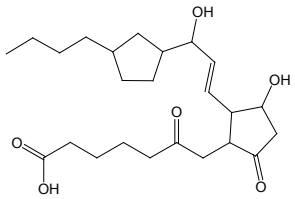
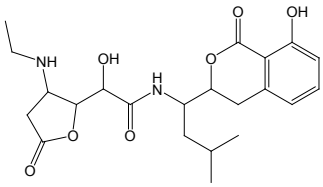
FIGURE CAPTIONS

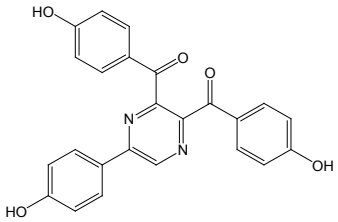
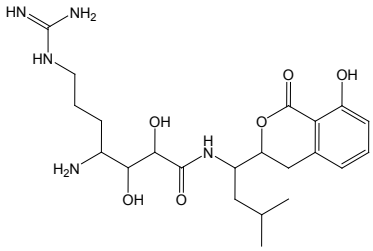
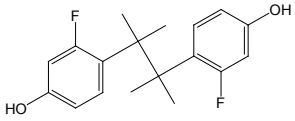
Figure 1. Virtual screening hits recapitulate the estrogenic and dopaminergic selectivity profiles of the metabolic enzymes SULT1E1 and SULT1A3 respectively. a) The estrogen estrynamine (green - Table 1) docked to SULT1E1 (1hy3), with estradiol (cyan) from the mouse SULT1E1 co-crystal structure (1aqu) superimposed. b) The dopaminergic SDZ-GLC-756 (green - Table 2) docked to SULT1A3 (2a3r), with co-crystallized dopamine (cyan) superimposed. 3'-phosphoadenosine-5'-phosphosulfate (PAPS) is located on the left side.

Figure 2. Virtual screening hits mimic the biological substrate of the histone lysine methyltransferase EHMT1. The natural substrate peptide (cyan, H3K9) from the EHMT1 co-crystal structure (PDB: 2rfi) is superimposed on the structure of capreomycin (green - Table 5) which was docked to apo EHMT1 (PDB: 2igq). The cofactor, S-adenosyl L-homocysteine (SAH), is located at the bottom.

Table 1. Virtual screening hit list against SULT1E1. The World drug index of over 50,000 compounds was docked to the SULT1E1 substrate binding pocket using Glide (Schrödinger). For each of the top 12 compounds, the rank, compound name and structure, biological activity and computed score are listed.

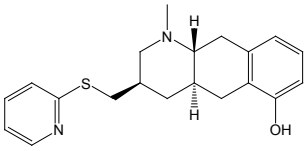
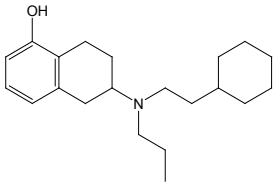
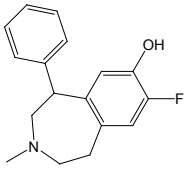
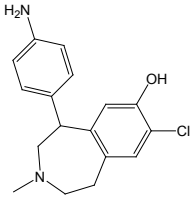
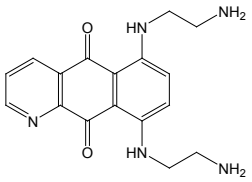
Rank	Compound	Activity	Score	Reference[a]
1	 Kuwanon-C	phosphodiesterase inhibitor, tyrosinase inhibitor	-19.16	Lee 2004
2	 Estrynamine	estrogen	-19.10	Blickenstaff 1986
3	 GT-32-B	antibiotic, cytostatic	-18.58	Takahashi 1997
4	 LY-117018	cytostatic, estrogen-antagonist	-18.54	Black 1980

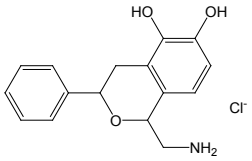
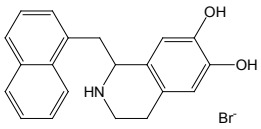
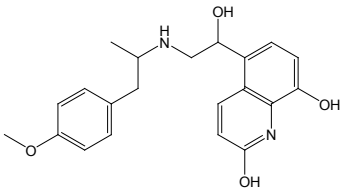
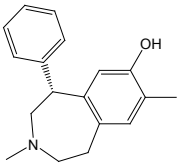
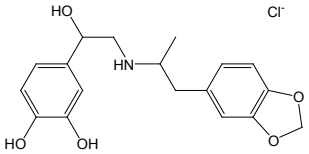
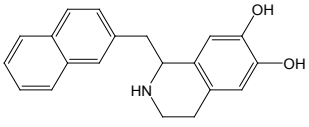
5	 Cyclomulberrin	anti-aggregant	-18.46	Lin 1993
6	 EM-652-hydrochloride salt	cytostatic, synergist, estrogen-antagonist, radiosensitizer	-18.45	Labrie 1999
7	 Lysisteisoflavone	cytostatic	-18.09	Ito 2006
8	 ONO-1579	prostaglandin, anti-aggregant, hypotensive	-18.00	Imaki 1989
9	 AI-77-C-2	antiulcer, gastric-secretion inhibitor, anti-inflammatory	-17.94	Shimojima 1985

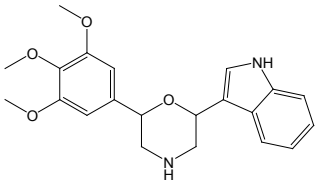
10	 Botryllazine-A	tested for cytotoxicity (inactive)	-17.84	Durán 1999
11	 Xenocoumacin-1	antibiotic, fungicide, antiulcer	-17.63	McInerney 1991
12	 D-18954	cytostatic, estrogen-antagonist	-17.48	Schwarz 1990

[a] Provided as Supporting Online Material.

Table 2. Virtual screening hit list against SULT1A3. The World drug index of over 50,000 compounds was docked to the SULT1A3 substrate binding pocket using Glide (Schrödinger). For each of the top 12 compounds, the rank, compound name and structure, biological activity and computed score are listed.

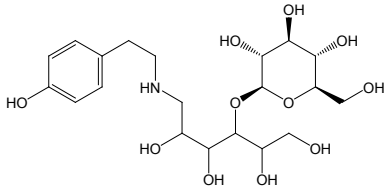
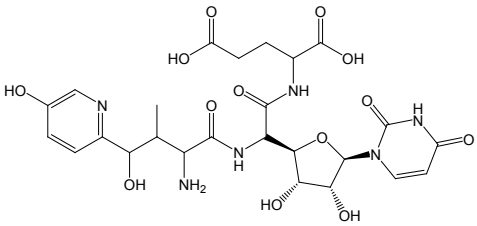
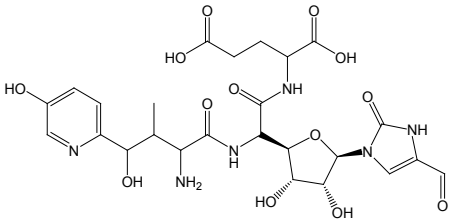
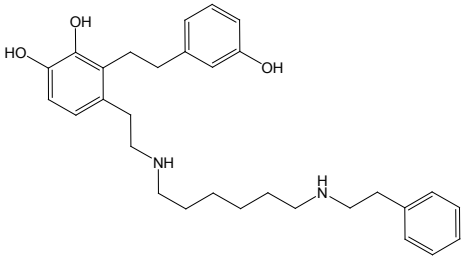
Rank	Compound	Activity	Score	Reference[a]
1	 SDZ-GLC-756	dopaminergic	-18.89	Markstein 1996
2	 ZYY-339	dopaminergic, imaging agent	-18.52	Shi 1999
3	 SCH-25873	dopamine receptor antagonist	-17.76	McQuade 1988
4	 SCH-39111	dopamine receptor antagonist	-17.64	Gingrich 1988
5		cytostatic, antibiotic	-17.63	Hazlehurst 1995

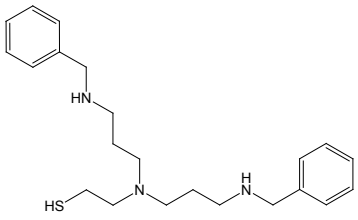
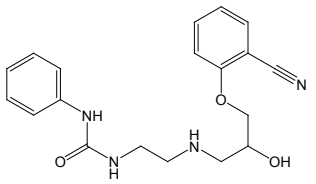
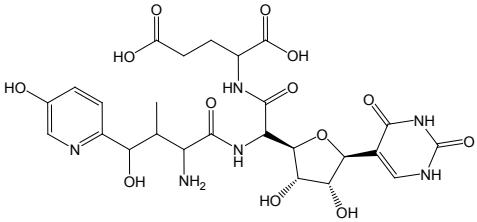
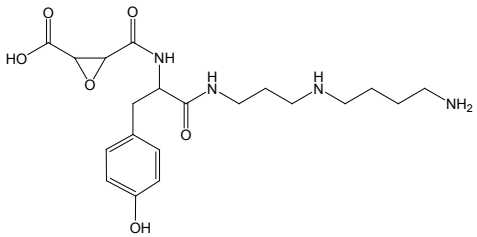
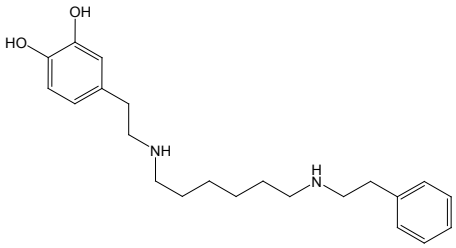
	BBR-2828			
6	 A-68930	dopaminergic	-17.59	Kebabian 1990
7	 YS-49	nitric-oxide-synthase inhibitor, sympathomimetic-beta, vasodilator	-17.55	Lee 1994
8	 TA-2005	bronchodilator, sympathomimetic-beta	-17.35	Hikkawa 1991
9	 SCH-23389	dopamine receptor antagonist	-17.31	McQuade 1988
10	 Protokylol hydrochloride	sympathomimetic-beta, antiasthmatic, bronchodilator	-17.30	Chahl 1972
11	 YS-51	sympathomimetic-beta, vasodilator	-17.28	Avdeeva 1998

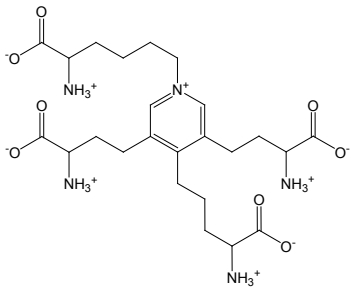
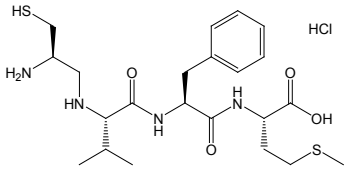
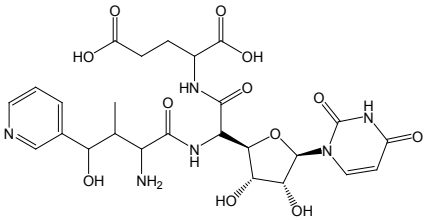
12	 Chelonin-A	antimicrobial, anti-inflammatory	-17.26	Bobzin 1991

[a] Provided as Supporting Online Material.

Table 3. Virtual screening hit list against EHMT1. The World drug index of over 50,000 compounds was docked to the EHMT1 cofactor binding pocket using Glide (Schrödinger). For each of the top 12 compounds, the rank, compound name and structure, biological activity and computed score are listed.

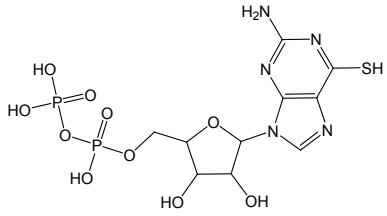
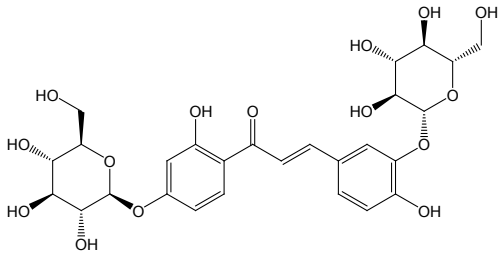
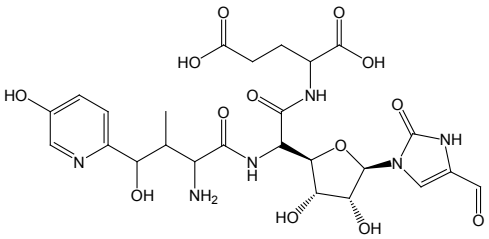
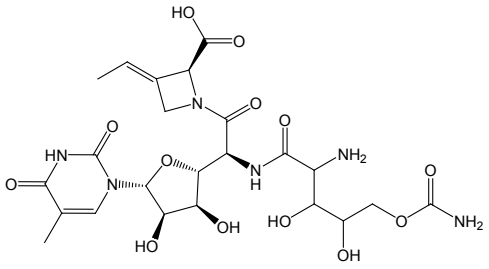
Rank	Compound	Activity	Score	Reference[a]
1	 Tyramine-cellobiose	125I-tyramine-cellobiose is used as a radio-labeled ligand to trace protein accumulation	-15.97	Glass 1983
2	 Nikkomycin-J	fungicide, antibiotic	-15.80	Decker 1989
3	 Nikkomycin-I	antibiotic	-14.62	Dähn 1976
4	 FPL-63012AR	vasodilator, dopaminergic	-14.46	Smith 1990

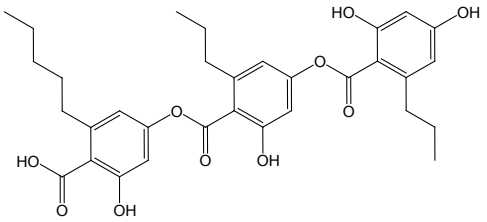
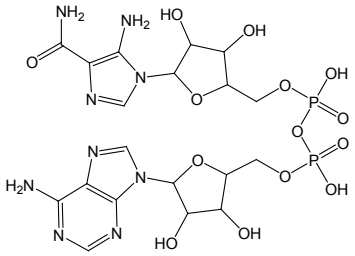
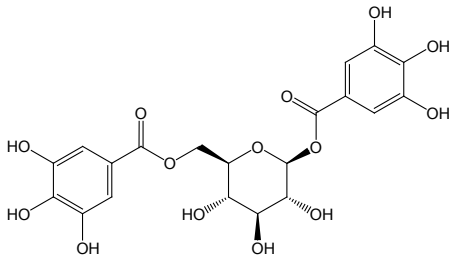
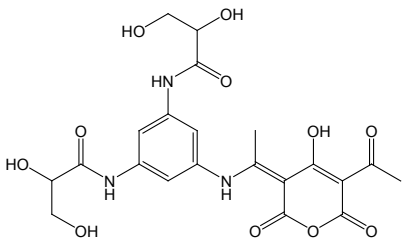
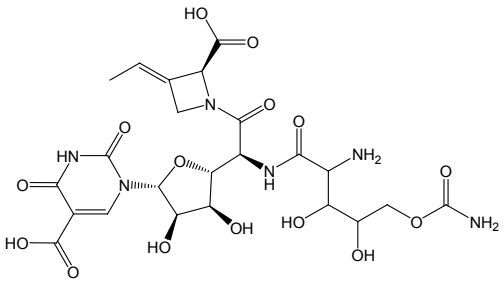
5	 MDL-101895	radioprotective	-13.97	Edwards 1994
6	 ICI-89406	sympathomimetic, beta-adrenoceptor antagonist	-13.83	Majid 1980
7	 Nikkomycin-pseudo-J	fungicide, antibiotic	-13.80	Decker 1989
8	 TMC-52-A	peptide-hydrolase inhibitor	-13.67	Isshiki 1998
9	 Dopexamine	dopaminergic, sympathomimetic-beta	-13.66	Brown 1985

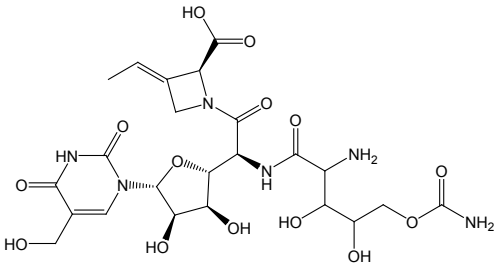
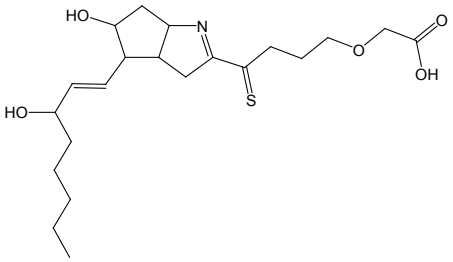
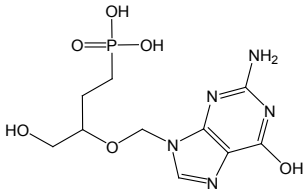
10	 Desmosine	biomarker of elastin degradation	-13.46	Luisetti 2008
11	 B-515	farnesyl-transferase inhibitor	-13.37	Garcia 1993
12	 Nikkomycin-RZ	antibiotic, fungicide	-13.28	Koenig 1986

[a] Provided as Supporting Online Material.

Table 4. Virtual screening hit list against MYST3. The World drug index of over 50,000 compounds was docked to the MYST3 cofactor binding pocket using Glide (Schrödinger). For each of the top 12 compounds, the rank, compound name and structure, biological activity and computed score are listed.

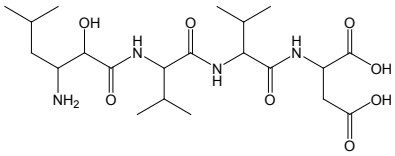
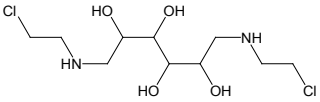
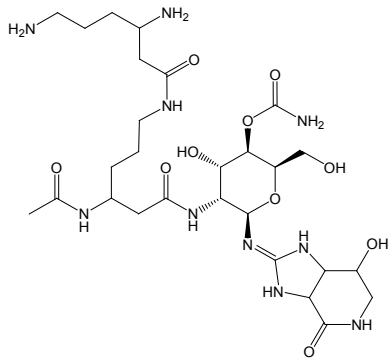
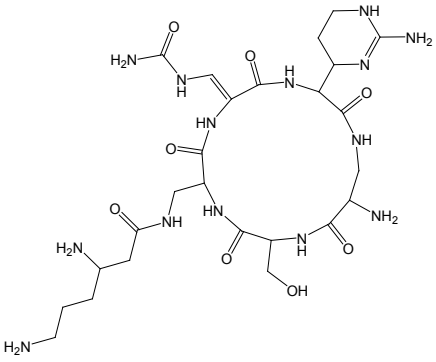
Rank	Compound	Activity	Score	Reference[a]
1	 Thioguanosine-diphosphate	metabolite of thiopurine immuno-suppressant drugs: can be used for monitoring	-15.45	Neurath 2005
2	 Isobutrin	patented for use in treating hepatocellular carcinoma, antihepatotoxic	-15.24	Saxena 2006
3	 Nikkomycin-I	antibiotic	-14.57	Dähn 1976
4	 Polyoxin-H	antibiotic	-14.54	Isono 1967

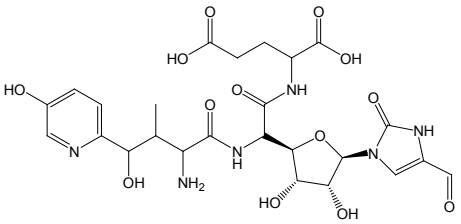
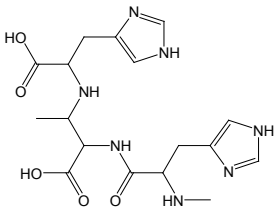
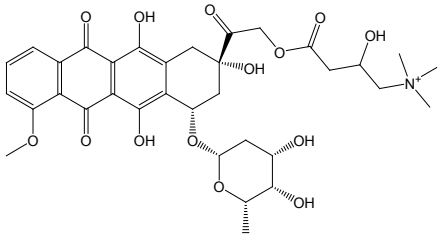
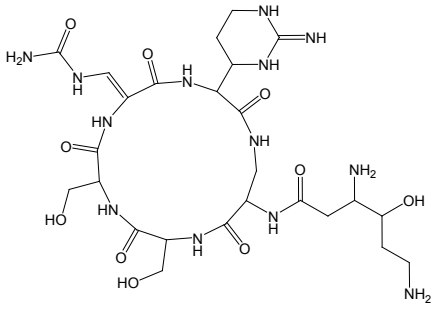
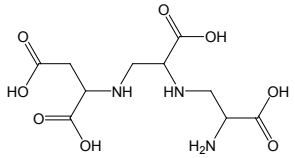
5	 Cytonic acid B	peptide hydrolase inhibitor	-14.48	Guo 2000
6	 Aica-adenine-dinucleotide	tested as an inhibitor for inosine monophosphate dehydrogenase (inactive)	-14.46	Gebeyehu 1985
7	 1,6-di-*O*-Galloylglucose	antiproliferative	-14.33	Kinjo 2001
8	 SKF-84210	antianaphylactic	-14.13	Snader 1979
9		antibiotic	-14.11	Isono 1967

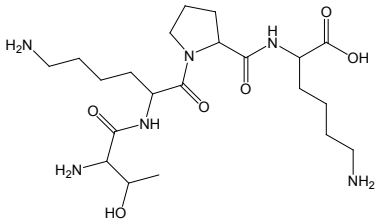
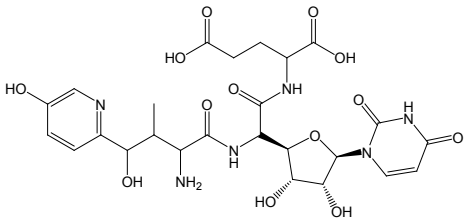
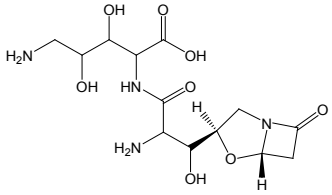
	Polyoxin-F			
10		antibiotic	-14.10	Suzuki 1965
	Polyoxin-A			
11		prostaglandin	-14.06	N/A
	HR-892			
12		virucide	-14.05	Duke 1986
	SR-3745A			

[a] Provided as Supporting Online Material.

Table 5. Virtual screening hit list against EHMT1. The World drug index of over 50,000 compounds was docked to the EHMT1 substrate binding pocket using Glide (Schrödinger). For each of the top 12 compounds, the rank, compound name and structure, biological activity and computed score are listed.

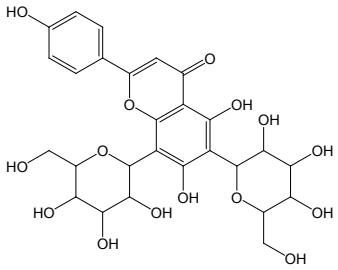
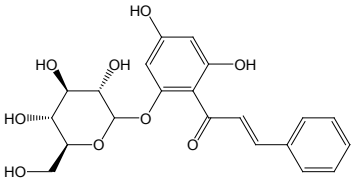
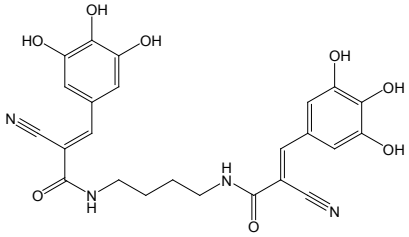
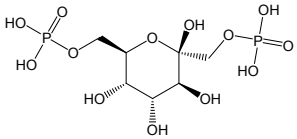
Rank	Compound	Activity	Score	Reference[a]
1	 Amastatin	peptide-hydrolase inhibitor	-14.95	Rich 1984
2	 Mannomustine	cytostatic	-14.34	Barlow 1959
3	 AN-201I	antibiotic, cytostatic	-13.92	Miyashiro 1983
4	 Capreomycin	antibiotic, tuberculostatic	-13.85	Stark 1963

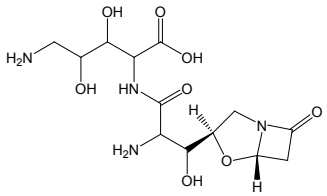
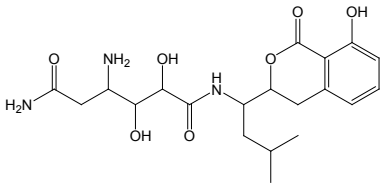
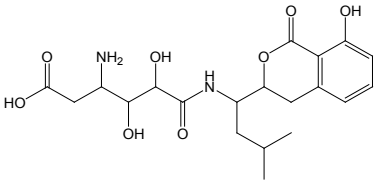
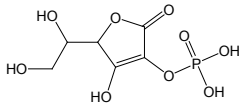
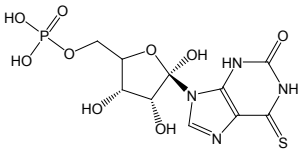
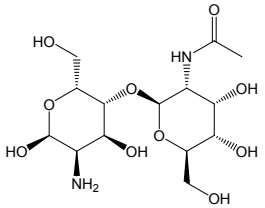
5	 Nikkomycin-I	antibiotic	-13.82	Dähn 1976
6	 Feldamycin	antibiotic	-13.73	Argoudelis 1976
7	 WP-620	antibiotic, cytostatic	-13.65	Gallois 1996
8	 Enviomycin	antibiotic, tuberculostatic	-13.53	Ando 1971
9	 Aspergillomarasmine-A	angiotensin-converting enzyme inhibitor	-13.35	Mikami 1983

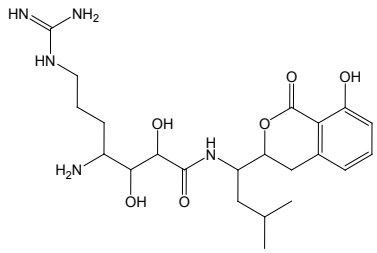
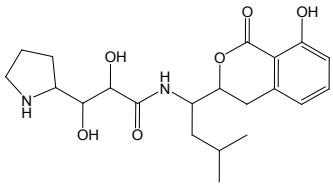
10	 Lys4-tuftsin	stimulates phagocytic activity	-13.31	Hisatsune 1983
11	 Nikkomycin-J	fungicide, antibiotic	-13.19	Decker 1989
12	 Clavamycin-B	antibiotic, fungicide	-13.11	King 1986

[a] Provided as Supporting Online Material.

Table 6. Virtual screening hit list against MYST3. The World drug index of over 50,000 compounds was docked to the MYST3 substrate binding pocket using Glide (Schrödinger). For each of the top 12 compounds, the rank, compound name and structure, biological activity and computed score are listed.

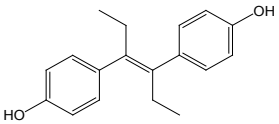
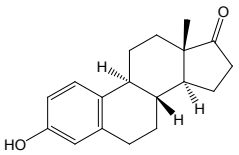
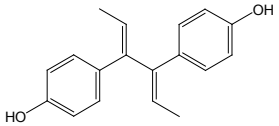
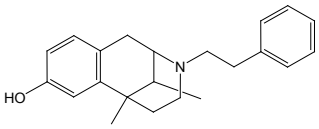
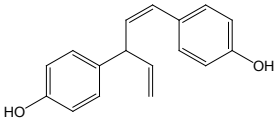
Rank	Compound	Activity	Score	Reference[a]
1	 Vicenin-2	anti-inflammatory (flavonoids mixture)	-11.43	Aquila 2009
2	 Isosalipurposide	antioxidant	-10.35	Agnihotri 2008
3	 AG-575	EGF receptor tyrosine kinase inhibitor	-10.33	Gazit 1996
4	 Sedoheptulose-diphosphate	may protect against hypoxic injury	-10.28	Miller 1996

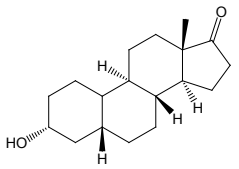
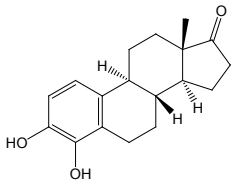
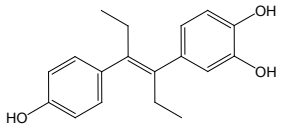
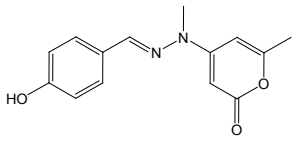
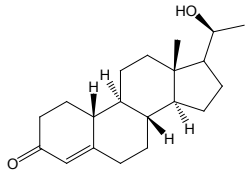
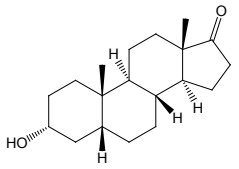
5	 Clavamycin-B	antibiotic, fungicide	-9.96	King 1986
6	 Amicoumacin-A	antibiotic, anti-inflammatory, antiulcer	-9.71	Itoh 1982
7	 Amicoumacin-B	antibiotic	-9.67	Itoh 1982
8	 Ascorbate-2-phosphate	vitamin C derivative	-9.18	Takamizawa 2004
9	 Thioxanthine-ribotide	cytostatic	-9.13	N/A
10	 Chitinosan	excipient	-8.96	Rege 1999

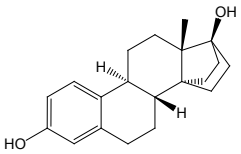
11	 Xenocoumacin-1	antibiotic, fungicide, antiulcer	-8.94	McInerney 1991
12	 Xenocoumacin-2	antibiotic, antiulcer	-8.80	McInerney 1991

[a] Provided as Supporting Online Material.

Table 7. Virtual screening hit list against ERα. The World drug index of over 50,000 compounds was docked to the ERα agonist binding pocket using ICM (Molsoft LLC). For each of the top 12 compounds, the rank, compound name and structure, biological activity and computed score are listed.

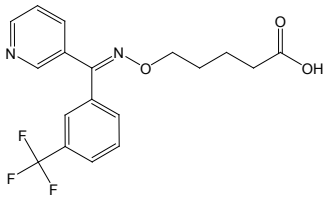
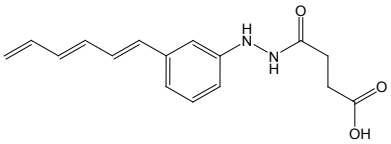
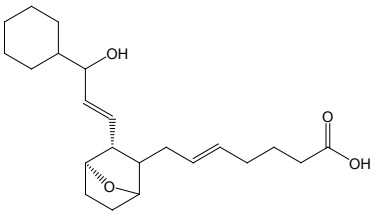
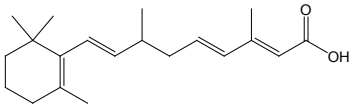
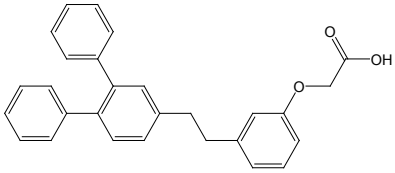
Rank	Compound	Activity	Score	Reference[a]
1	 Diethylstilbestrol	estrogen	-49.4	Williams 1996
2	 Estrone	estrogen	-48.2	Williams 1996
3	 Dienestrol-β	estrogen	-48.1	Summa 1965
4	 Phenazocine	Sigma 1 receptor agonist, analgesic	-47.8	Froimowitz 1986
5	 Nyasol	estrogen	-47.6	Minami 2000

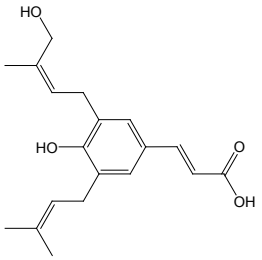
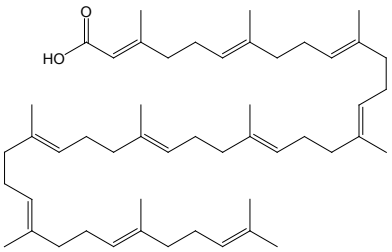
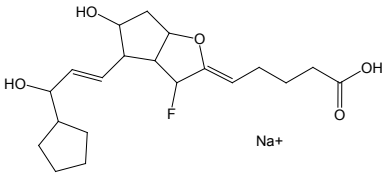
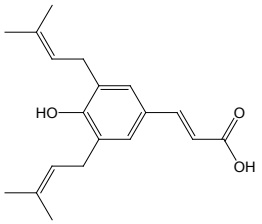
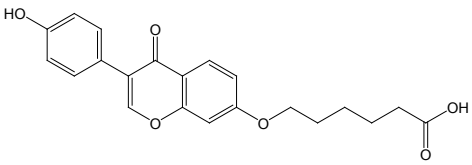
6	 Norethiocholanolone-19	metabolite of nandrolone, an androgen	-47.5	Ozer 1997
7	 4-hydroxyestrone	estrogen	-47.3	Williams 1996
8	 3'-hydroxy diethylstylbetrol	estrogen	-46.9	Williams 1996
9	 CK-134	interleukin-1 antagonist, anti-inflammatory	-46.9	Chiou 2000
10	 Oxogestone	progestogen	-46.7	El-Mahgoub 1980
11	 Etiocholanolone	androgen	-46.6	Williams 1996

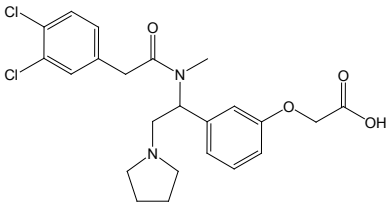
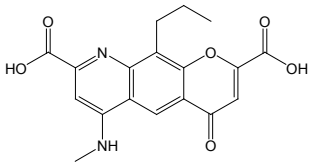
12	 ZK-115194	estrogen	-46.6	Baumann 1996

[a] Provided as Supporting Online Material.

Table 8. Virtual screening hit list against PPARγ. The World drug index of over 50,000 compounds was docked to the PPARγ agonist binding pocket using ICM (Molsoft LLC). For each of the top 12 compounds, the rank, compound name and structure, biological activity and computed score are listed.

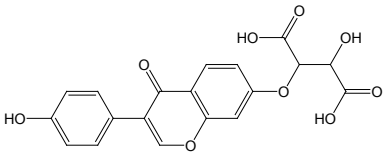
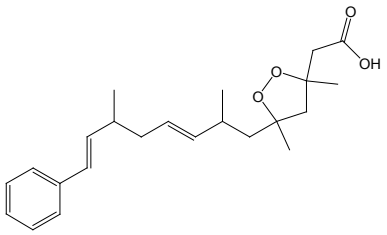
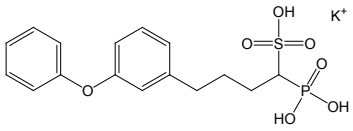
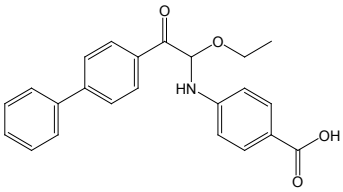
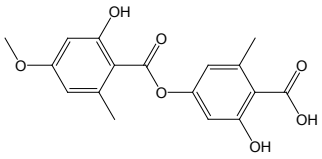
Rank	Compound	Activity	Score	Reference[a]
1	 Ridogrel	dual thromboxane A2 synthase inhibitor / receptor antagonist, anti-aggregant	-54.4	Bourgain 1991
2	 Spinamycin	antibiotic	-51.1	Wang 1966
3	 SQ-27986	prostaglandin receptor agonist, anti-aggregant	-48.6	Seiler 1990
4	 9,10- dihydro retinoic acid	retinoid	-48.7	Willhite 1986
5	 F-1070	anti-aggregant	-48.5	Miyamae 1997

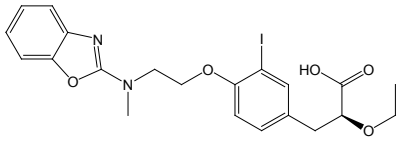
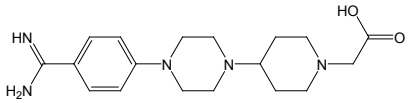
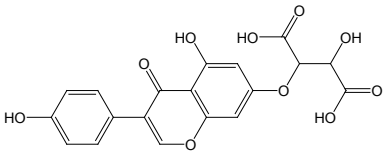
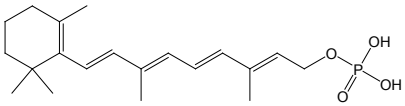
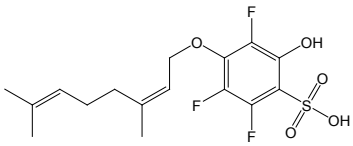
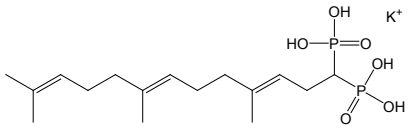
6	 Capillartemisin B	choleretic	-48.3	Kitagawa 1983
7	 Decaprenoic acid	fatty acid, retinoid	-47.9	Muto 1981
8	 TEI-8153	prostaglandin receptor agonist, inhibition of tumor cell induced platelet aggregation	-47.5	Niitsu 1988
9	 Artepillin C	apoptosis inducer	-47.4	Kimoto 2001
10	 Hexzein	5-HT antagonist	-46.8	Keung 1998

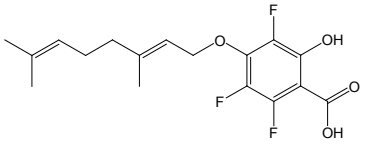
11	 ICI-204448	kappa-opioid receptor agonist	-46.7	Shaw 1989
12	 Minocromil	anti-asthmatic	-45.7	Svendsen 1985

[a] Provided as Supporting Online Material.

Table 9. Virtual screening hit list against TRβ. The World drug index of over 50,000 compounds was docked to the TRβ agonist binding pocket using ICM (Molsoft LLC). For each of the top 12 compounds, the rank, compound name and structure, biological activity and computed score are listed.

Rank	Compound	Activity	Score	Reference[a]
1	 Shoyuflavone A	histidine decarboxylase inhibitor	-72.1	Kinoshita 1998
2	 Plakinic acid A	fungicide	-69.9	Chen 2001
3	 BMS-187745	squalene synthase inhibitor, anti-arteriosclerotic	-68.7	Flint 1997
4	 Xenazoic acid	virucide	-67.3	Lee 2000
5		contact allergens from lichen	-67.3	Thune 1980

	Evernic acid			
6	 SB-236636	PPARγ agonist, anti-diabetic	-66.8	Young 1998
7	 GR-144053	integrin α2β3 antagonist, anti-thrombic	-66.5	Eldred 1994
8	 Shoyuflavone B	histidine decarboxylase inhibitor	-66.5	Kinoshita 1998
9	 Retinol phosphate	mannosyl acceptor/donor	-66.3	Shidoji 1982
10	 CB-7756	farnesyl protein transferase inhibitor	-65.6	Marriott 1999
11	 Farnesyl bisphosphonate	squalene synthase inhibitor	-65.0	Abe 1994

12	 CB-7752	farnesyl protein transferase inhibitor	-64.8	Marriott 1999

[a] Provided as Supporting Online Material.

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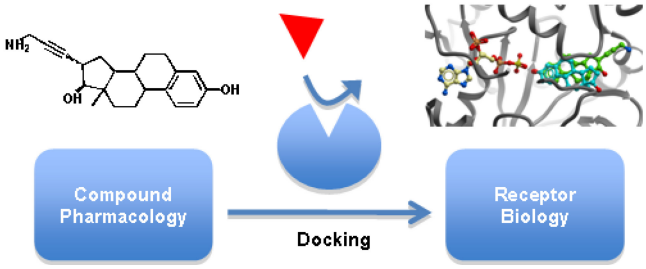
Evaluation of Virtual Screening as a Tool for Chemical Genetic Applications

Valérie Campagna-Slater and Matthieu Schapira



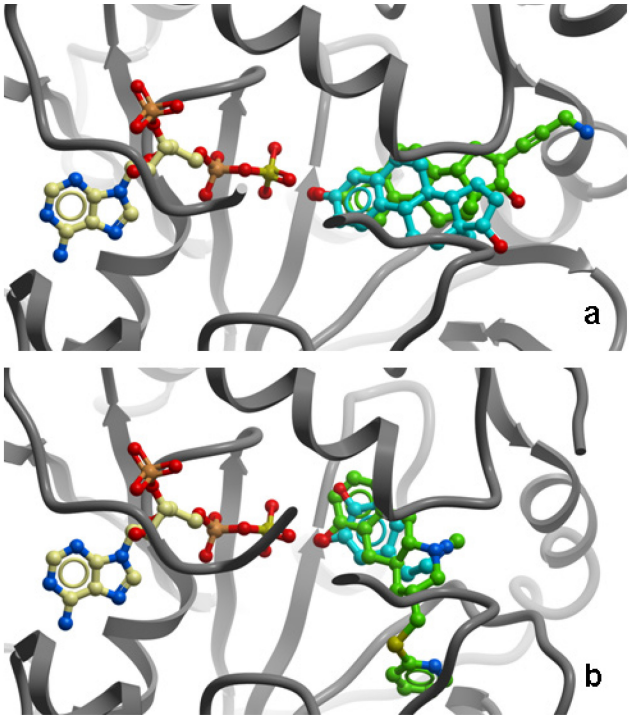


Figure 1.

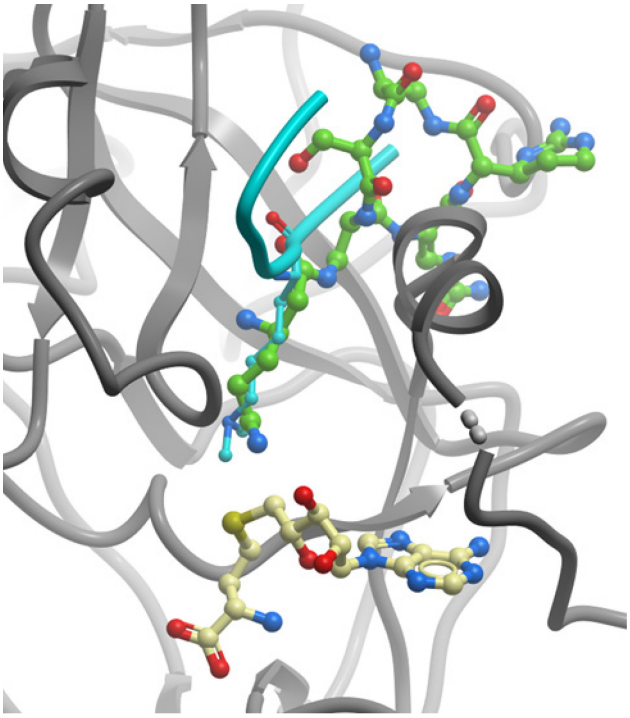


Figure 2.

Supporting Information.

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Valérie Campagna-Slater and Matthieu Schapira

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