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Phosphoinositide-3-kinase (PI3K) inhibitors: Identification of new scaffolds using virtual screening

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ABSTRACT

In the present work, we used virtual screening (VS) of the ZINC database of 2.5 million compounds to seek new PI3K inhibitory scaffolds. The VS flowchart implemented various filters, including a 3D-database screen, and extensive docking studies, to derive 89 derivatives that were experimentally assayed against the four PI3K isoforms. Seven compounds showed inhibitory activities between 1 and 100 μM, with four being sufficiently potent to constitute potential new scaffolds. The binding conformations of these four were analyzed to provide a rationalization of their activity profile.

Phosphoinositide 3-kinases (PI3Ks) are a class of enzymes that catalyze phosphorylation of the 3-hydroxyl position of phosphoinositides (PIs). The resulting second messengers, phosphatidylinositol 3,4-bisphosphate (PIP2) and phosphatidylinositol 3,4,5-trisphosphate (PIP3), can regulate a remarkably diverse array of physiological processes, including glucose homeostasis, cell growth, differentiation, and motility.[1,2] Eight related PI3Ks, possessing unique substrate specificity, localization and mode of regulation, have been identified in vertebrates. These include the class IA PI3Ks (p110α, p110β, p110δ kinase domains), which are activated by receptor tyrosine kinases via a p85 receptor domain, and the class IB PI3K (p110γ), which is activated by heterotrimeric G-proteins.

Recent interest in PI3K signaling has been fueled by evidence that the PI3K pathway is among the most commonly activated signaling pathway in cancer. Different observations have been critical in establishing this relation: (i) the p110α isoform is frequently mutated in a range of primary tumors,[3–5] (ii) the phosphatase PTEN, which antagonized PI3K signaling, is a well characterized tumor suppressor that is inactivated by mutation, gene deletion or epigenetic silencing with a high frequency,[6] and (iii) PI3K is allosterically activated by the oncogene Ras and many tyrosine kinases that activate PI3K are themselves the target of mutation or amplification in cancer.[7] Together, these observations strongly suggest that PI3K activation is likely to be an essential step in tumorigenesis and have thus stimulated intense efforts to develop drugs that target PI3Ks and particularly p110α as anticancer agents.[8–12] The selective inhibition of p110γ/δ and p110β has also been proven valuable in the fields of inflammation,[13] allergy,[14] and thrombosis,[15] respectively.

The early discovery of pan-specific PI3K inhibitors wortmannin and LY294002 (Fig. 1) was a critical event that enabled rapid exploration of PI3K signaling. However, these compounds deprived of any selectivity against the different isoforms could not be used to probe signaling by specific PI3Ks. A lots of effort has therefore been devoted to develop new PI3K inhibitors displaying enhanced potency, selectivity and pharmacological properties. To date, although a wide variety of different chemotypes with inhibitory activity have been reported,[16] only a few compounds displaying substantial isoform selectivity are known. Among them, PIK90 and TGX286 were reported as dual p110α/γ and p110β/δ inhibitors, respectively.[7] The imidazo[1,2-*a*]pyridine PIK75 was described to be a sub-nanomolar p110α inhibitor with >100-fold selectivity over p110β and δ.[17] In addition, PIK75 showed activity in a human cancer xenograft model.[17] PIK39, a quinazolinone purine, inhibited p110δ at mid-nanomolar concentration, p110γ and β at concentration ~100-fold higher and showed no activity against any other PI3K family member including p110α at concentrations up to 100 mM.[7] AS605240 was reported to show low nanomolar inhibition of 110γ with >30-fold selectivity for p110β and δ and 7.5-fold selectivity over p110α.[18] Finally, a number of analogues are now reported to be

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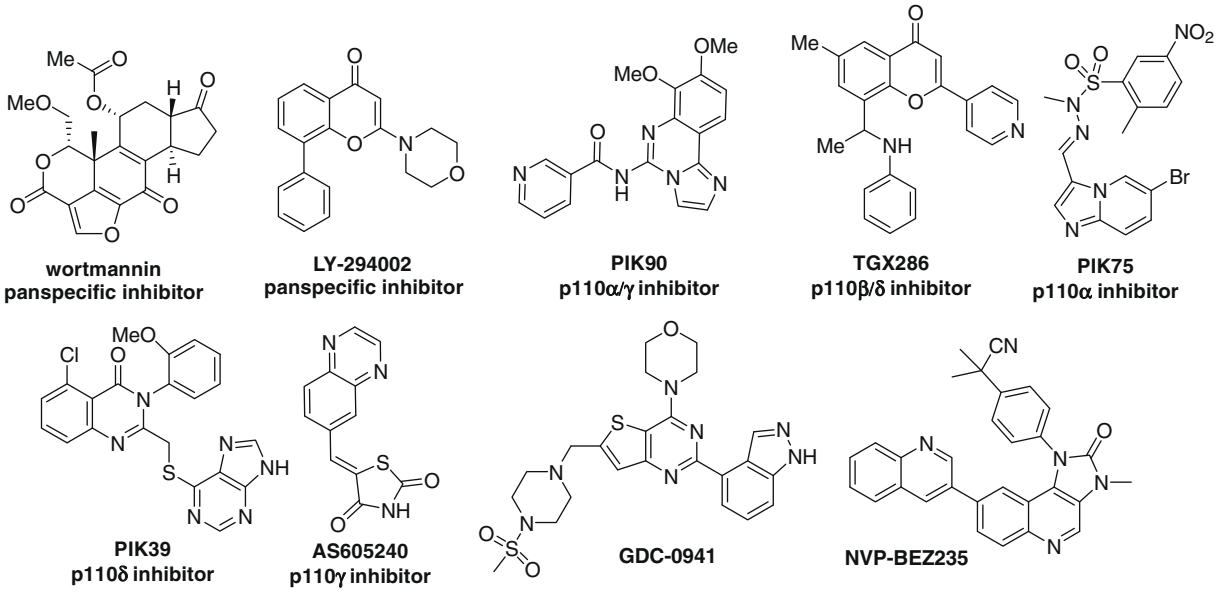


Figure 1. Structures of known PI3K inhibitors.

in early clinical trial, the most advanced being NVP-BEZ235[19] and GDC-0941.[20] The limited number of selective PI3K inhibitors available, and an apparently promiscuous enzyme, suggested that broad screening for new scaffolds that inhibit PI3Ks and which could be further optimized was a worthwhile challenge for virtual screening (VS).

VS involves the computational screening of very large libraries for commercially-available chemicals that complement targets of known structure, followed by the experimental testing of those with the best predicted binding energies.[21] We have applied it in the present work for the discovery of new PI3K inhibitors, and identified four new scaffolds with inhibitory potencies in the low-micromolar range. To the best of our knowledge, this is the first example of successful VS against PI3K.

Before investigating a large library of compounds by VS, we assessed the capabilities of the docking software package GOLD[22] to reproduce the binding conformation of known PI3K inhibitors, and evaluated the enrichment rate obtained by VS for the discovery of new PI3K inhibitors. Although the structure of p110α recently became available,[23,24] the p110γ isoform is the only PI3K that possesses known 3D-coordinates of complexes with inhibitors (LY294002, PIK90, PIK93, and AS605240, for example) bound in the active site.[7,18,25] These derivatives were thus docked inside the p110γ cavity by mean of the automated GOLD program using parameters especially designed for VS experiments (7–8-fold acceleration).[22] For each structure, 20 conformations were generated and ranked according to their affinity (*GOLDSCORE* fitness function). In each case the conformation possessing the best score was in excellent agreement with the binding position determined by X-ray diffraction.

Then, to assess the capability of GOLD to discriminate the 'true' ligands from the non-binders (enrichment rate) when searching for PI3K inhibitors, a database of 1000 structures comprised of 995 derivatives randomly selected from the ZINC library and five known PI3K inhibitors (LY294002, PIK90, PIK93, AS605240, and PIK75) was docked inside the p110γ binding site. For each compound, three conformations were produced and the one possessing the best *GOLDSCORE* value was retained. As a result, three of the five known inhibitors (LY294002, PIK90, and PIK93) were ranked among the top 10 compounds, while the other two derivatives (AS605240 and PIK75) were ranked in the top 100 solutions, thus validating our virtual screening strategy.

Various chemical libraries like the NCI database (National Cancer Institute), the ACD (Available Chemical Directory) or ZINC library[26] (over 2.5 million commercially-available compounds) can be used to perform VS. We chose the latter as it is free, web-accessible, offers the ligands in a ready-to-dock 3D format, and hits are able to be purchased for confirmatory evaluation.

Since careful docking of the entire ZINC library would take a prohibitively long time, filters were used to limit the number of compounds to be considered before the docking experiment. The VS flowchart we employed is depicted in Figure 2.

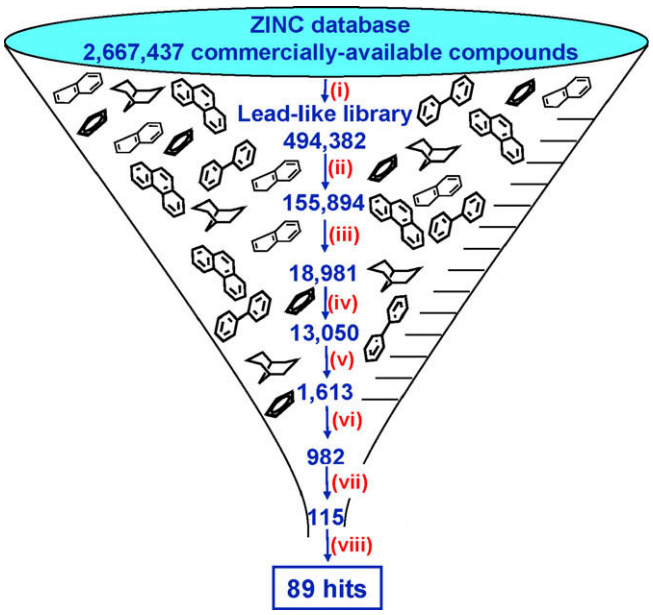


Figure 2. Virtual screening flowchart. (i) Lipinski-style rules; (ii) general PI3K pharmacophore; (iii) docking inside the p110γ cavity; (iv) evaluation of ADME parameters using *VOLSURF*; (v) evaluation of the binding with *CSCORE*; (vi) visual analysis and selection of compounds effectively displaying an H-bond with the Val882 NH backbone; (vii) selection based on structural diversity, synthetic accessibility and 'good looking' conformation; (viii) commercial availability.

(i) Lipinski-style rules were first applied. As our primary aim at this very first step was to select only compounds that could serve as leads for future medicinal chemistry development, we used simple molecular descriptors[27] such as the molecular weight ($150 \leqslant MW \leqslant 350$), the hydrophobicity ($-2 \leqslant \log p \leqslant 3$), the number of H-bond donor ($DH \leqslant 3$) and acceptor atoms ($HA \leqslant 6$) as the first filter. Then, all of the qualifying structures were downloaded from the ZINC website and transformed into a *UNITY* hit list file (*SYBYL* version 7.3) for compatibility with our modeling system. This resulted in a drug-like library of roughly 500,000 structures (Fig. 2, (i)).

(ii) The second filter was a simplified pharmacophore describing PI3K inhibitor interactions that are important for binding, along with additional geometric constraints accounting for the shape of the cavity. This geometric set was elaborated using the co-crystal structure of p110γ with PIK90 (Fig. 1) (PDB code 2CHX). An H-bond acceptor area (Fig. 3, in green) was centered on the nitrogen atom implicated in the H-bond contact with the Val882 NH backbone, as this interaction was shown to be critical for PI3K inhibition.[7] Two hydrophobic regions (Fig. 3, in cyan) were also defined in the region of both the central heteroaromatic ring and the pyridyl lateral side chain. Finally, various cavity constraints (Fig. 3, in white) were defined around the nearest atom defining the cavity, with a view to discarding all compounds that would not fit the active site due to their size. This geometric set (Fig. 3b) was then used to flexibly screen the selected set of 500,000 molecules using the *UNITY-3D* module implemented in *SYBYL*. About 150,000 structures were found to be capable of fitting the pharmacophore in some way (Fig. 2).

(iii) The resulting 150,000 structures were then docked in the p110γ cavity, employing the automated GOLD program as described earlier. For each compound, three docking positions were generated and the one with the best score (*GOLDSCORE*) was retained for further analysis. A *GOLDSCORE* value of 50 was then chosen as a third filter to isolate the top-ranked solutions (best fit compounds). This led to a library of roughly 19,000 lead-like structures that tightly bind p110γ.

(iv) Since it is important to take into account ADME parameters as early as possible in the drug design process[28] we then evaluated a cell penetration index by means of the VOLSURF software for each of the 19,000 compounds. A set of 94 molecular determinants relevant to the process of membrane partitioning were computed and projected onto a predictive model of Caco2 permeation. This model, developed elsewhere,[29] correlates the 94 molecular descriptors with the experimentally obtained permeation of nearly 750 chemically diverse compounds, and compounds with a computed Caco2 cell-permeation index >0.4 predicted to cross the cell membrane readily by passive diffusion. This filter limited the set to about 13,000 structures (Fig. 2).

(v) Concomitantly with the *VOLSURF* calculations, the *CSCORE* module implemented in *SYBYL* was used to evaluate more precisely the binding of the compounds in the PI3K active site. *CSCORE* consists of a combination of five different scoring functions, that is, G-, D-, PMF-, F-, and Chemscore and is used to compute a Cscore value. This value is an integer between 5 (all of the five scoring function have identified the ligand as a good potential binder) and 0 (none of the five scoring functions have identified the ligand as a binder) and helps distinguish the best potential binders from less probable ones. Compounds characterized by a Cscore value between 3 and 5 were retained, leading to a set of 1613 derivatives.

(vi) All these structures were then visually analyzed in the p110γ cavity, with all that did not effectively displayed the critical H-bond interaction with the Val 882 NH backbone (see Fig. 3) being discarded. This led to a final set of 982 structures, from which 115 were chosen based on structural diversity, synthetic accessibility and appropriate conformation. From this last set, 89 structures were in fact commercially available and were obtained from the vendors (Chembridge, ChemDiv, Enamine, Interbioscreen, and Life Chemicals).

Biological evaluation. The biological activity of the compounds was evaluated using an isolated enzyme inhibition assay for PI3Ks.[12,30] All compounds were first screened at a single concentration of 100 μM, and full IC_{50}s were determined for compounds displaying at least 40% of inhibition at this concentration. The results are summarized in Table 1. From the 89 structures identified by VS, seven compounds displayed a p110γ inhibitory potency in the low/medium micromolar range (Table 1). This corresponds to an enrichment rate of about 8%. This is an excellent rate when compared to those usually obtained by HTS (around 0.02% in most case[21]). The present study is therefore another example highlighting the excellent capabilities of VS for the discovery of new hits.

Compound **2** appears to be the most potent p110γ inhibitor of the 89 compounds assessed, with an IC_{50} of 4 μM. It is characterized by a quinolinone scaffold substituted in the 6-position with a methoxy group and in the 2-position with a 5-(3-methoxyphenyl)-1,2,4-oxadiazole side chain. The benzoxazol-2-one **1**, the

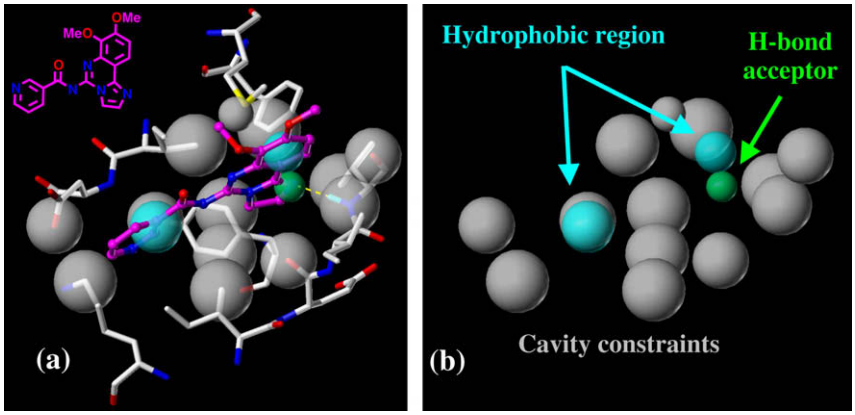
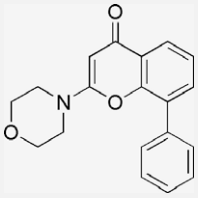
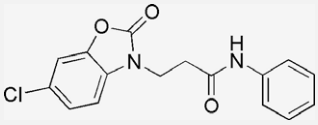
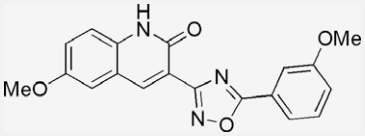
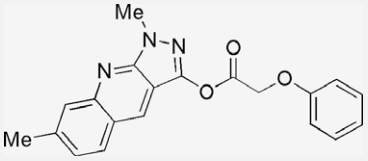
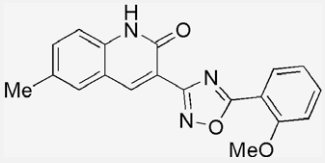
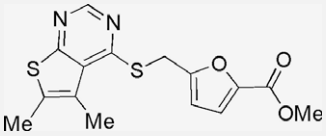
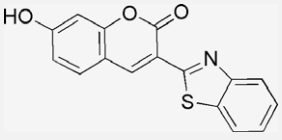
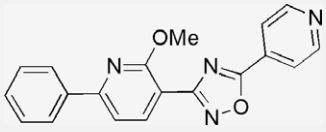


Figure 3. General PI3K pharmacophore built based on the co-crystal structure of p110γ.

Table 1
Inhibition of PI3K isoforms by structures **1**–**7**

Compd	Structure	IC_{50}[a] (μM)			
		p110γ	p110α	p110β	p110δ
LY-294002		7[b]	0.5	0.9	0.5
1		6	19	12	>100
2		4	0.9	3	0.9
3		16	38	>100	47
4		75	>100	ND	ND
5		71	>100	>100	>100
6		12	3	14	4
7		>100	78	>100	ND

[a] Values are means of two experiments, variation between experiments is no more than ±20%. ND: not determined.
[b] Taken from Ref. 33.

pyrazolo[3,4-*b*]quinoline **3** and the 7-hydroxycoumarin **6** also present promising p110γ inhibition with IC_{50}s of 6, 16, and 12 μM, respectively. For the best p110γ inhibitors (**1**–**3** and **6**), the inhibition of the class IA PI3Ks p110α, β, and δ were also determined, to investigate their isoform selectivity profile. Although compound **1** exhibits some degree of selectivity for p110γ (IC_{50} = 6 μM), particularly compared to p110δ (IC_{50} = 100 μM), a pan-specific PI3K inhibition profile is generally observed.

Interestingly, no biological properties have been reported so far for compounds **1**, **3**, and **6**, but analogues of compound **2** have been described as benzodiazepine receptor agonists[31] and as inhibitors of anti-apoptotic Bcl-2 polypeptides such as BFL-1.[32]

To understand how the best inhibitors (**1**–**3** and **6**) achieve their potency, we investigated in more detail their binding conformation inside the p110γ cavity. The compounds were docked inside the p110γ ATP-binding site as previously described. In order to take into account protein flexibility, the conformation with the highest score (*GOLDSCORE*) was further refine using the *MINIMIZE* module as implemented in *SYBYL 8.0* (Tripos force field and Gasteiger-Hückel charges).[34] Key interactions stabilizing the compounds are depicted in Figure 4.

As a general trend, the inhibitors are deeply inserted in the p110γ cavity. As expected, for each compound, one acceptor atom onto the ligand is H-bonded to the Val882 NH backbone located in the p110γ hinge region (ATP-binding site). Interestingly, apart from this critical H-bond, the good potency of these compounds seems mainly the results of close shape complementarities and van der Waals contacts between the inhibitors and the p110γ active site. Although the carbonyl group of the critical Val882 is H-bonded to a donor atom on compound **2** (Fig. 4b) and **6** (Fig. 4d), no other H-bonds to other active site residues appear to stabilize these derivatives. Appraising these results in the light of a recent study[8] suggesting that little but critical differences explain selectivity among PI3K isoforms, and particularly the mutation of two residues: Thr886 and Ala805 (according to the p110γ numbering), we hypothesize in the present case that the lack of specific interactions with these residues might account for the non-selective profile of these compounds.

In conclusion, a virtual screening strategy combining various filters including a 3D-database screen as well as high-throughput docking was used to search for new PI3K inhibitors. From the 89 final compounds identified and assayed, seven compounds exhibited enzyme inhibitory activities at 1-100 μM. Among them, four derivatives were sufficiently potent to constitute new potential scaffolds for pan-PI3K inhibition. The binding modes of these four within p110γ were evaluated, and revealed essential features responsible for the PI3K inhibition potency.

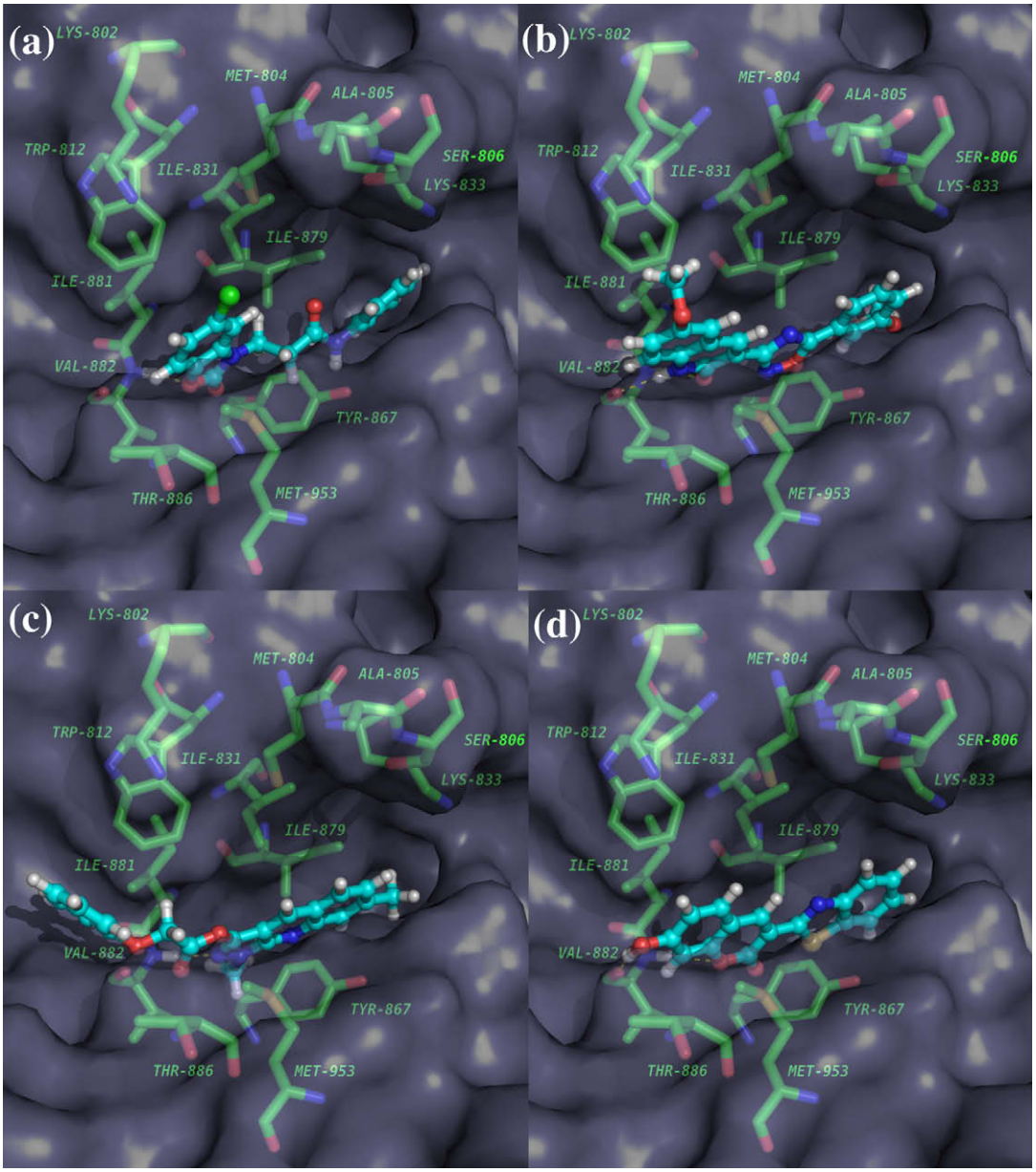


Figure 4. Docking of (a) **1**; (b) **2**; (c) **3**; (d) **6** within the active site of p110γ (pdb code 2CHX). Pictures made using *PYMOL*.[35]

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