

Application of ensemble pharmacophore-based virtual screening to the discovery of novel antimitotic tubulin inhibitors.



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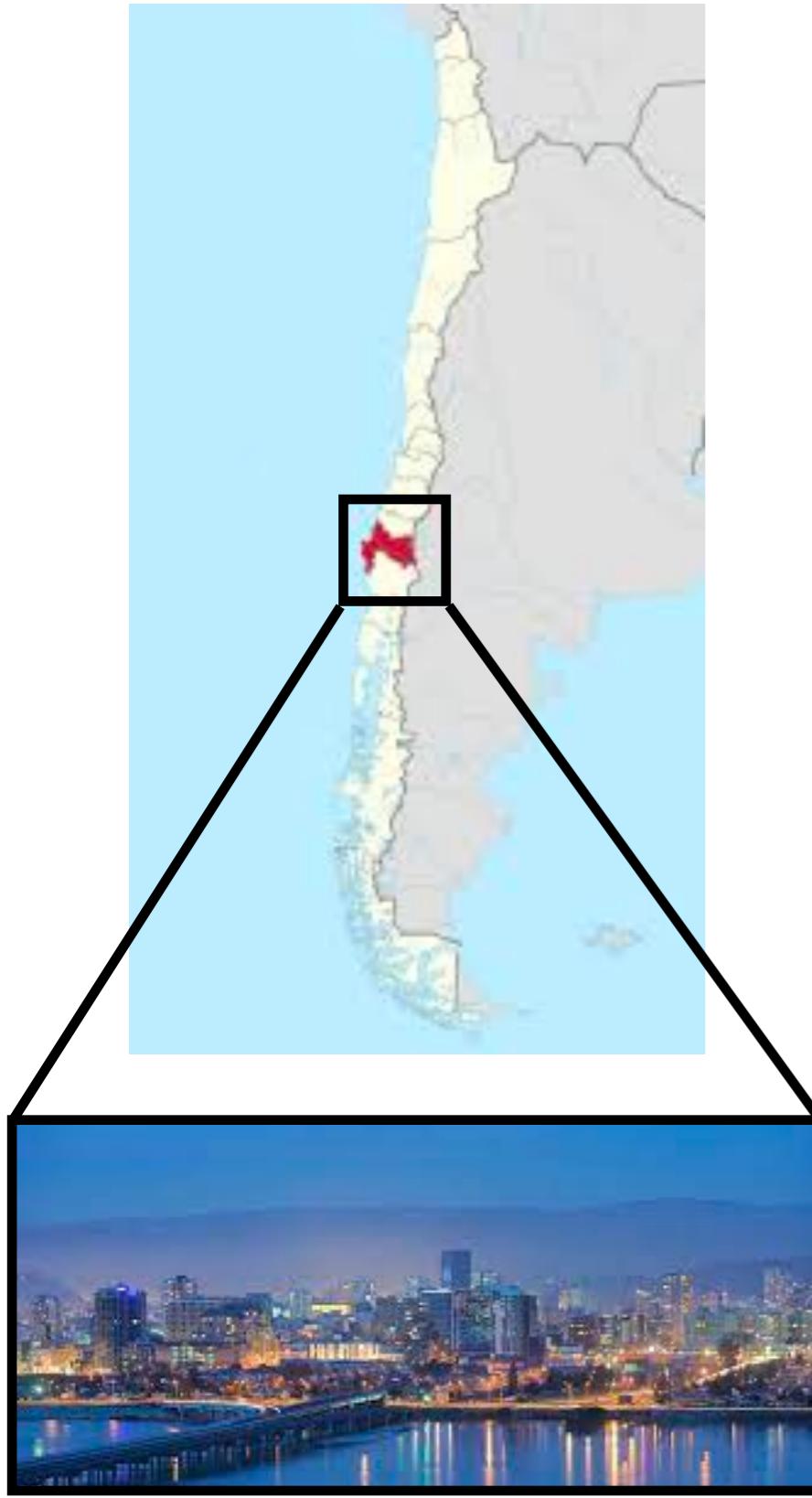


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Application of ensemble pharmacophore-based virtual screening to the discovery of novel antimitotic tubulin inhibitors

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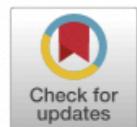
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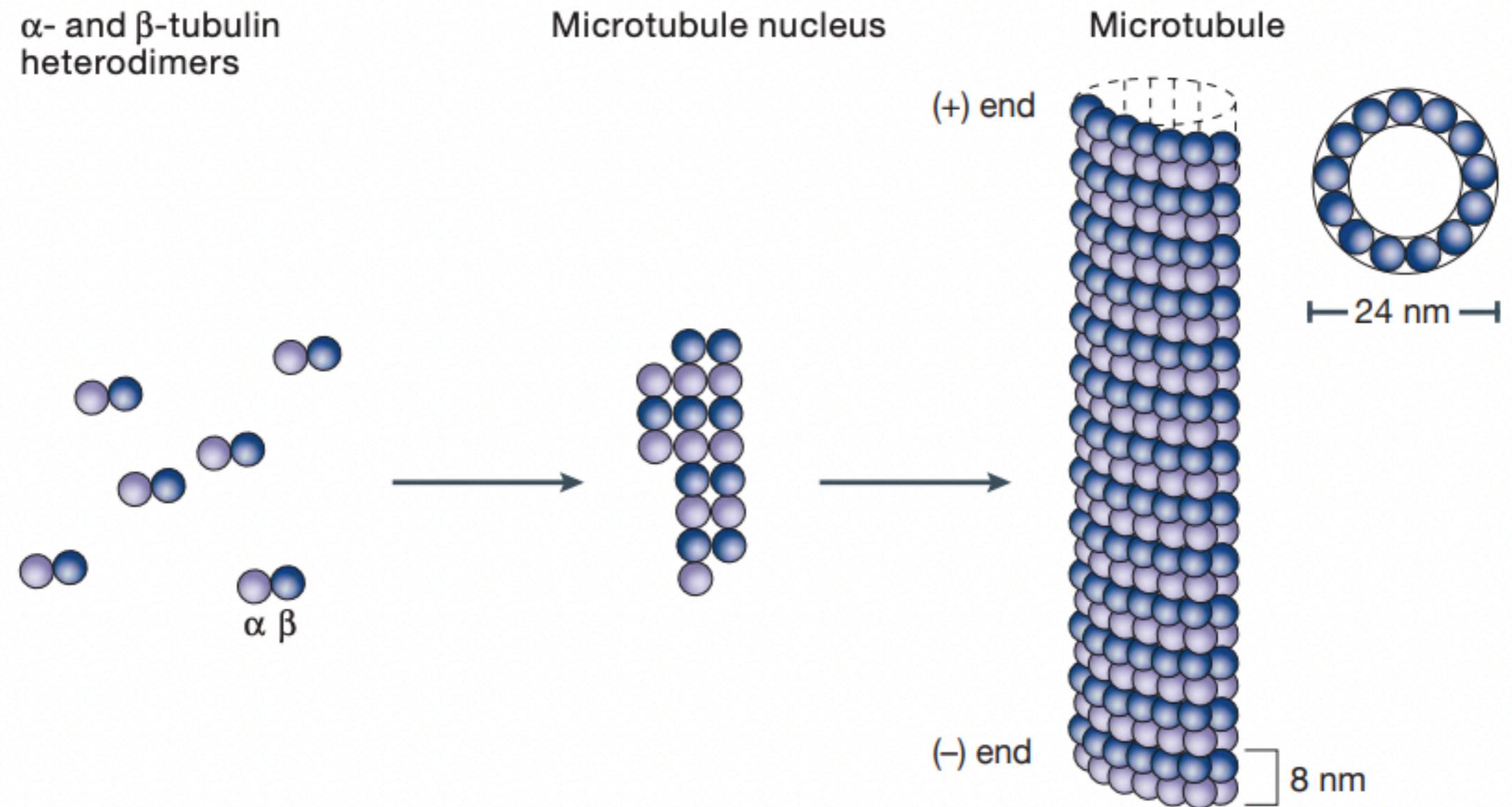
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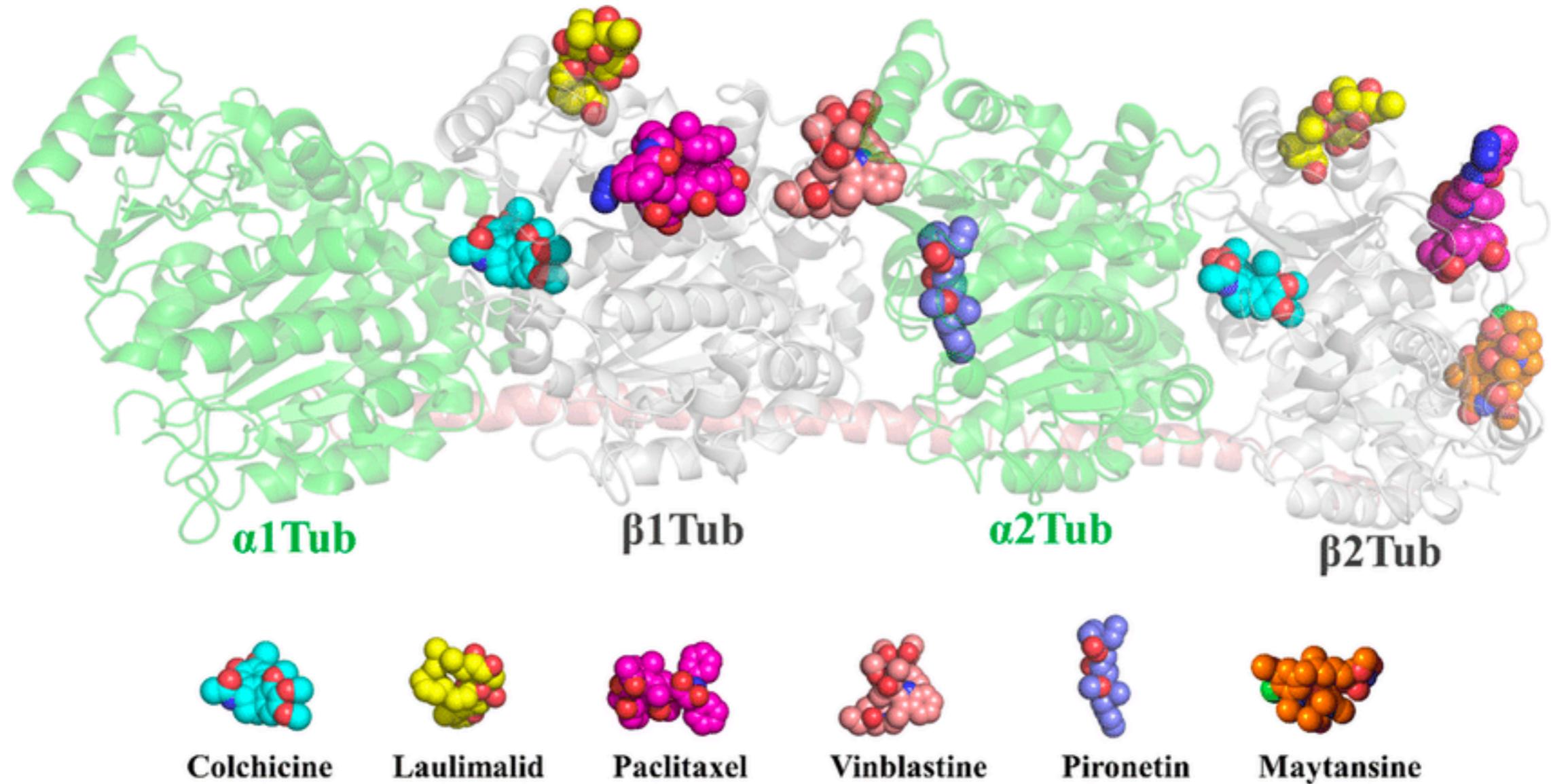
Microtubules as target for anticancer drugs

Microtubule-targeted drugs, including paclitaxel and Vinca alkaloids, were previously considered to work primarily by increasing or decreasing the cellular microtubule mass. Although these effects might have a role in their chemotherapeutic actions, we now know that at lower concentrations, microtubule-targeted drugs can suppress microtubule dynamics without changing microtubule mass; this action leads to mitotic block and apoptosis. In addition to the expanding array of chemically diverse antimitotic agents, some microtubule-targeted drugs can act as vascular-targeting agents, rapidly depolymerizing microtubules of newly formed vasculature to shut down the blood supply to tumours.

Microtubules as target for anticancer drugs



Tubulin binding sites



Six different binding sites are deposited in the PDB

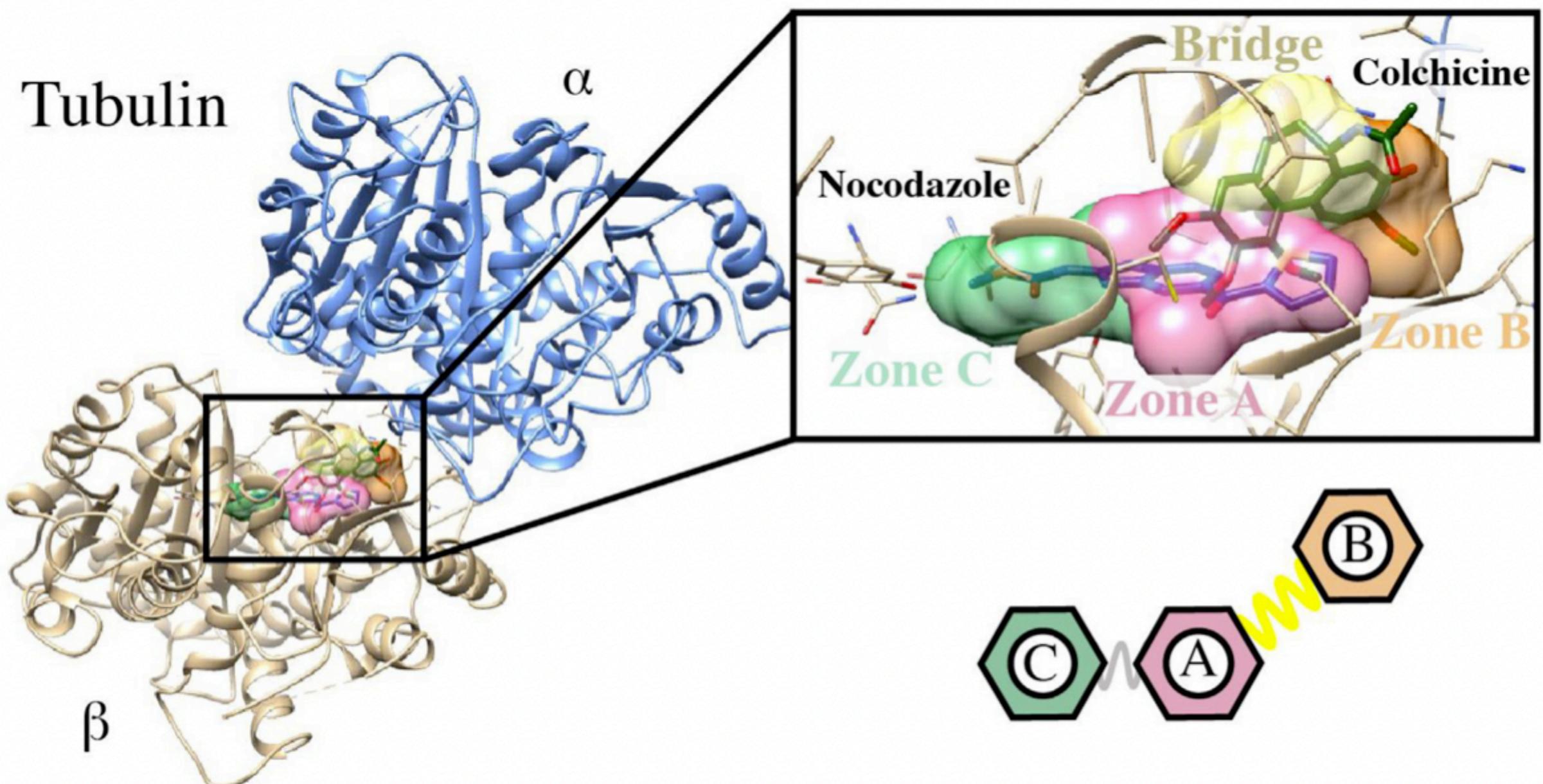
Question:

How to design novel antimitotic tubulin inhibitors?

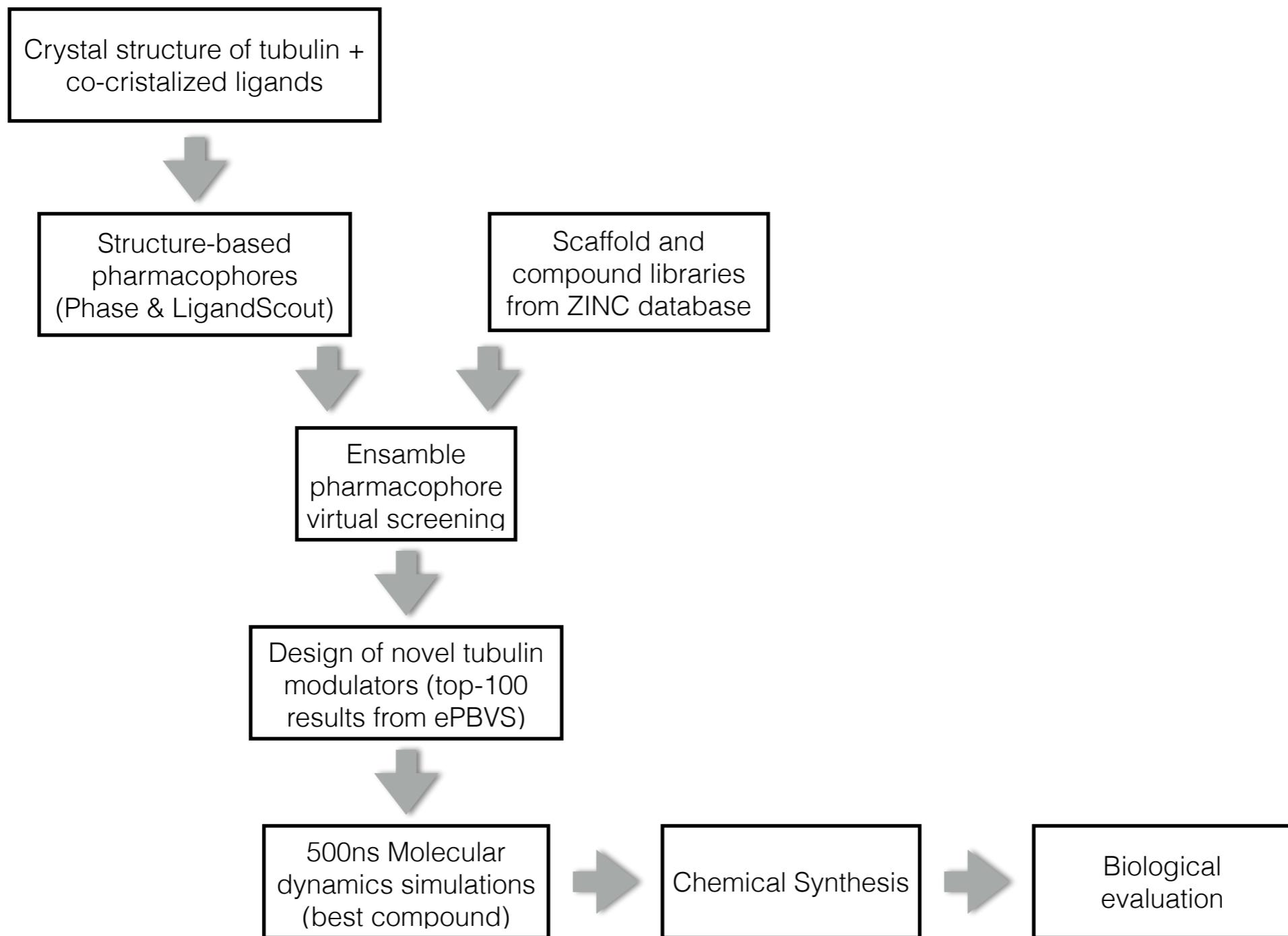
Hypothesis:

Using an ensemble pharmacophore virtual screening is possible to identify new active scaffolds/moieties to design novel antimitotic tubulin inhibitors

Colchicine binding site



Methods



Results

Structural and computational studies

81 crystal structures of tubulin with a co-crystallized ligand at the colchicine site were retrieved from the Protein Data Bank (PDB). Only the tubulin α and β subunits were conserved, as well as the modulators at the colchicine site, waters, ions, and phosphorylated nucleotides. The structures were aligned against the tubulin-colchicine complex (PDB code: 4O2B) using the α -subunit as reference. (Table S1)

Pharmacophores

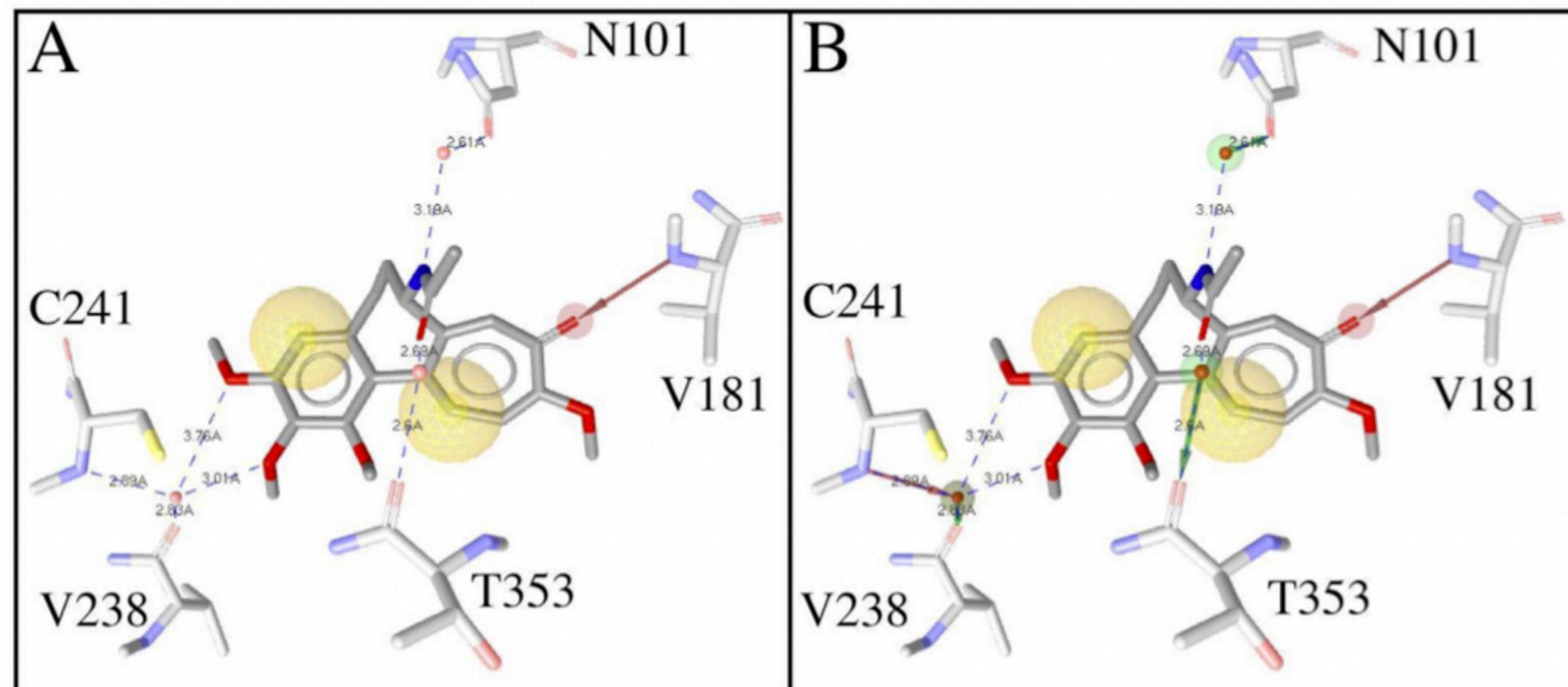


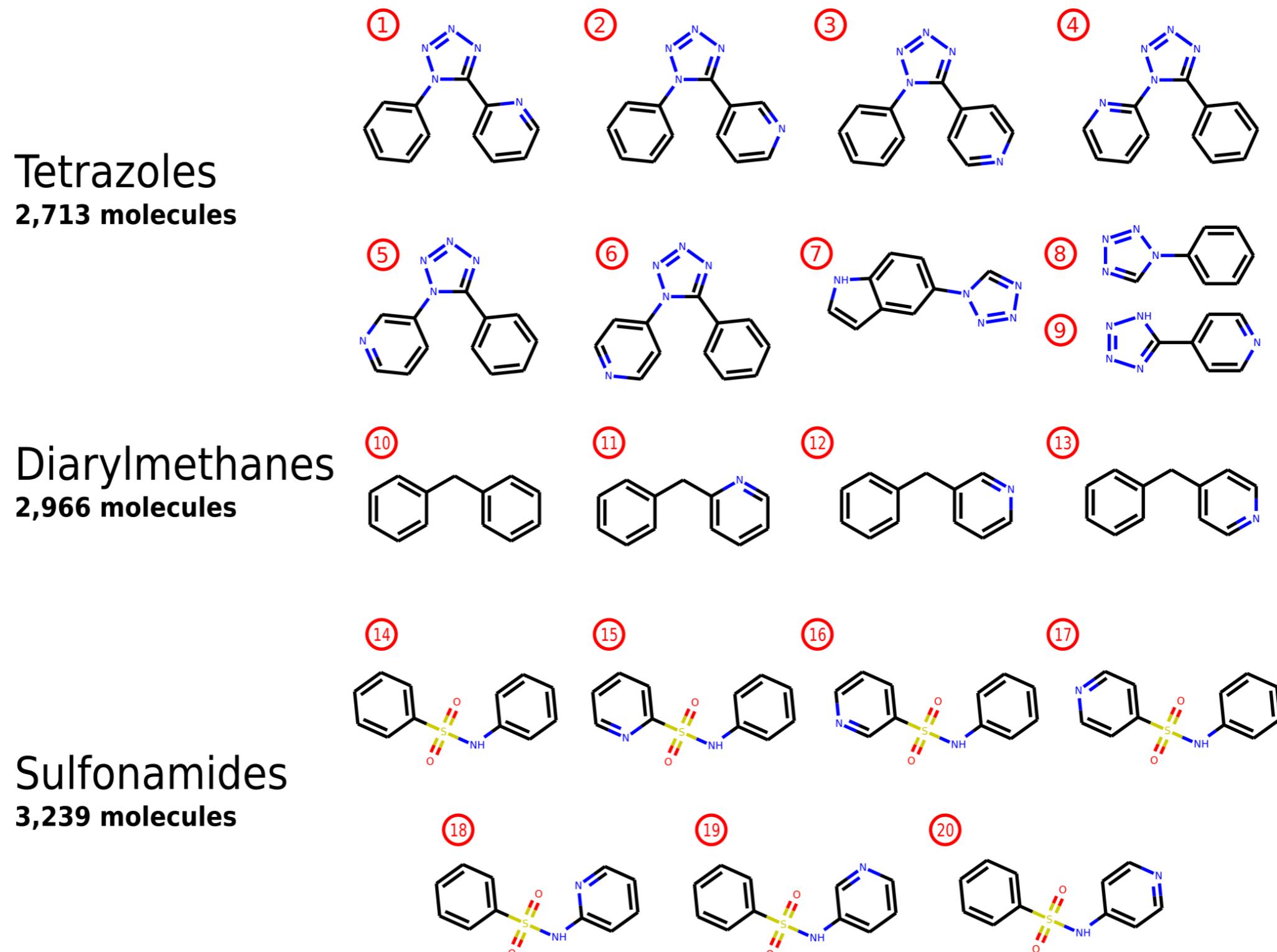
Fig. 2. Structure-based pharmacophores for tubulin-colchicine complex (PDB code: 5ITZ). Pharmacophores without (A) and with (B) water included. Hydrogen bond acceptor (red), hydrogen bond donor (green), and hydrophobic (yellow) pharmacophoric features are displayed to illustrate how colchicine interacts with tubulin. Distances between interacting atoms are shown as dotted lines. Waters are illustrated as red spheres. For better visualization, hydrogens are not displayed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Pharmacophores

Table S1: Tubulin-ligand complexes used in this study.

Protein PDB code	Ligand PDB code	RMSD*	Binding site occupation (ABC)
4O2B	LOC **	0	AB
1SA1	POD	0.889	AB
1Z2B	CN2 **	0.857	AB
3DU7	CN2 **	0.833	AB
3E22	LOC **	0.833	AB
3HCK	E70	0.811	aBc
3HKE	T13	0.797	AB
3N2G	G2N	0.813	AC
3N2K	K2N	0.815	AC
3UT5	LOC **	0.494	AB
4O2A	2RR	0.537	AB
4X1I	LOC **	0.479	AB
4X1K	LOC **	0.542	AB
4X1Y	LOC **	0.52	AB

Selection of scaffolds and library construction



Flexi-pharma virtual-screening using the crystallographic ensemble

Journal of Computer-Aided Molecular Design (2020) 34:1063–1077
<https://doi.org/10.1007/s10822-020-00329-7>

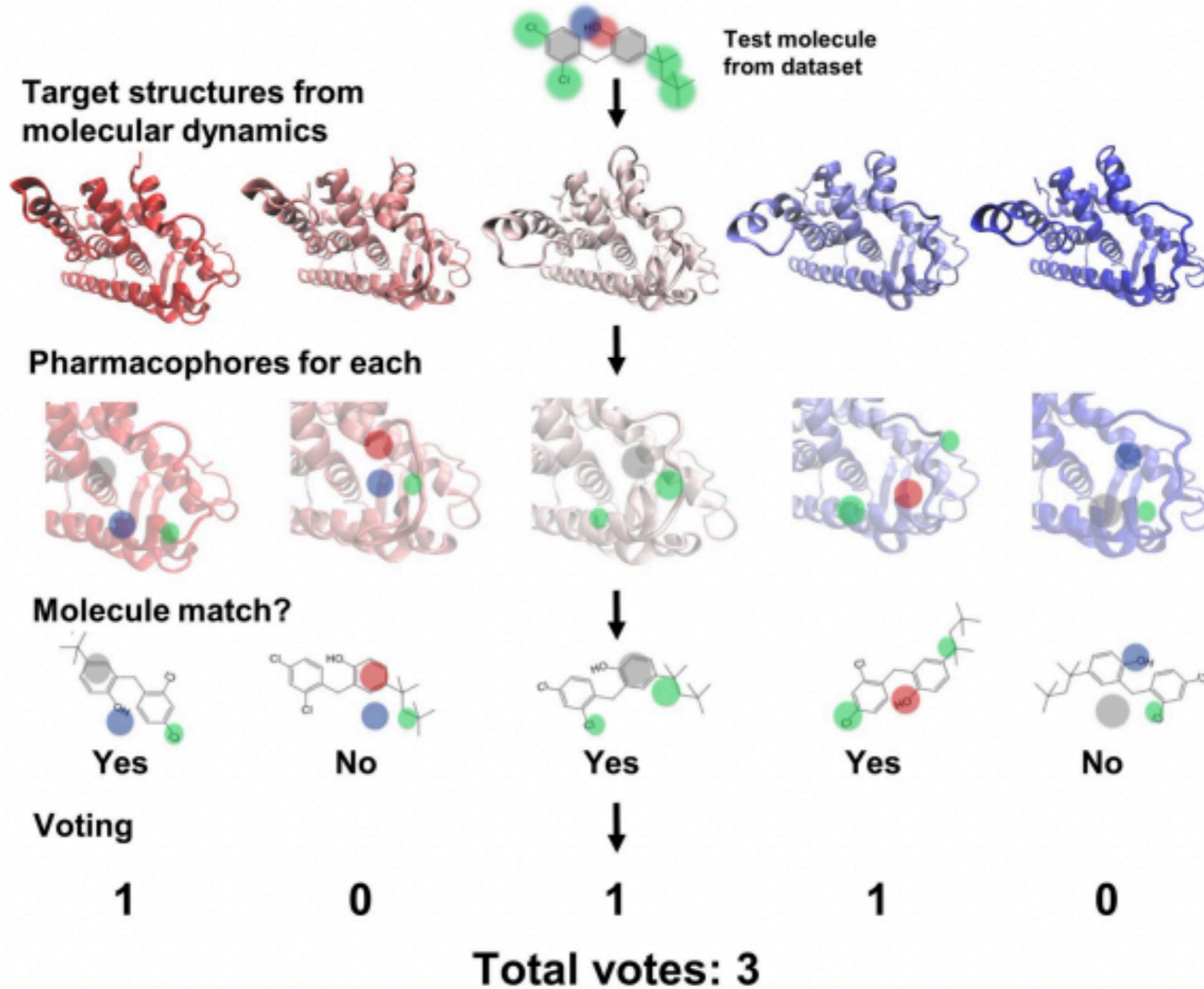


Flexi-pharma: a molecule-ranking strategy for virtual screening using pharmacophores from ligand-free conformational ensembles

Isaias Lans¹ · Karen Palacio-Rodríguez¹ · Claudio N. Cavasotto^{2,3,4}  · Pilar Cossio^{1,5} 

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Flexi-pharma: molecule ranking by vote



Ensamble pharmacophore-based virtual screening (positive control)

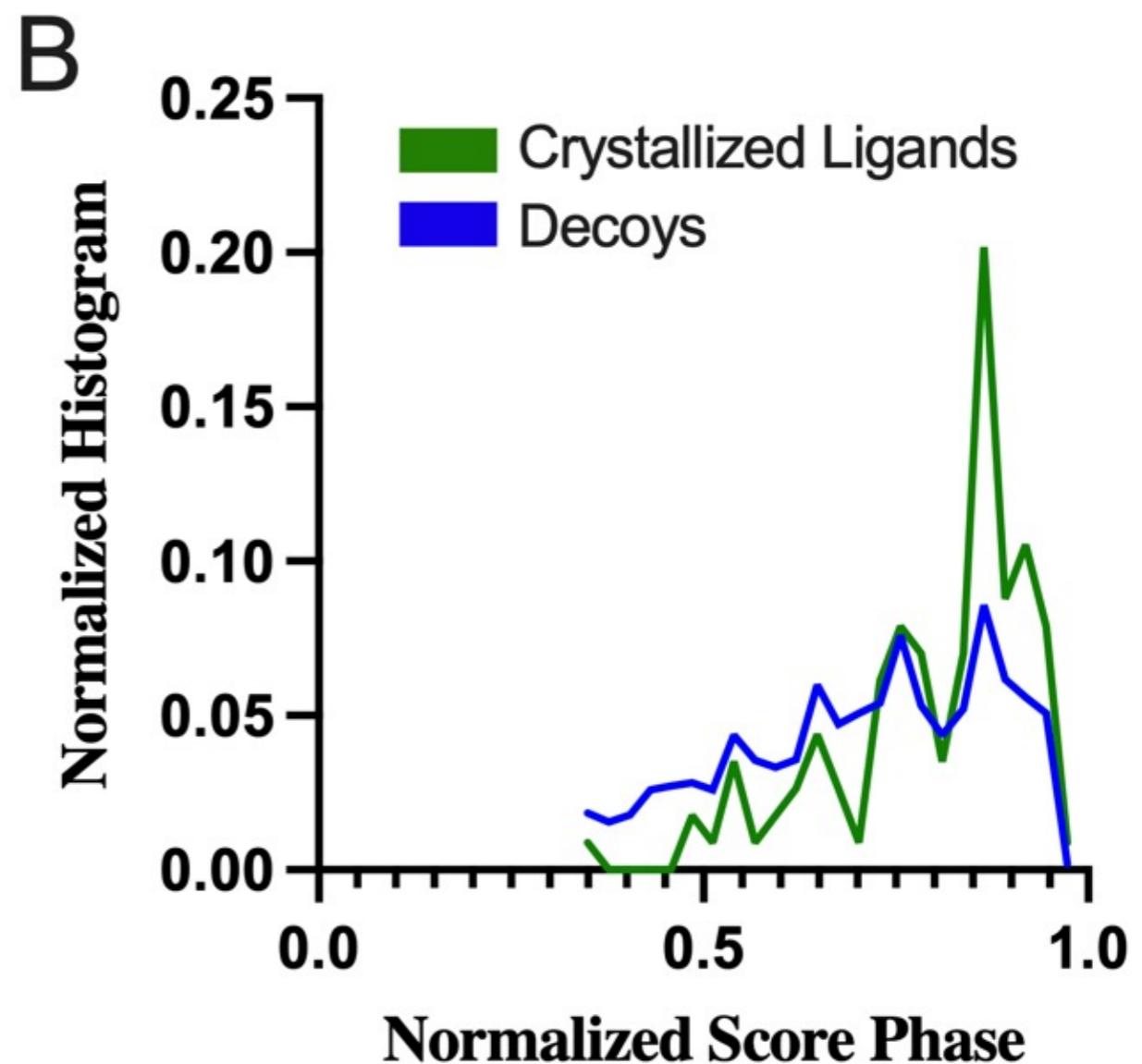
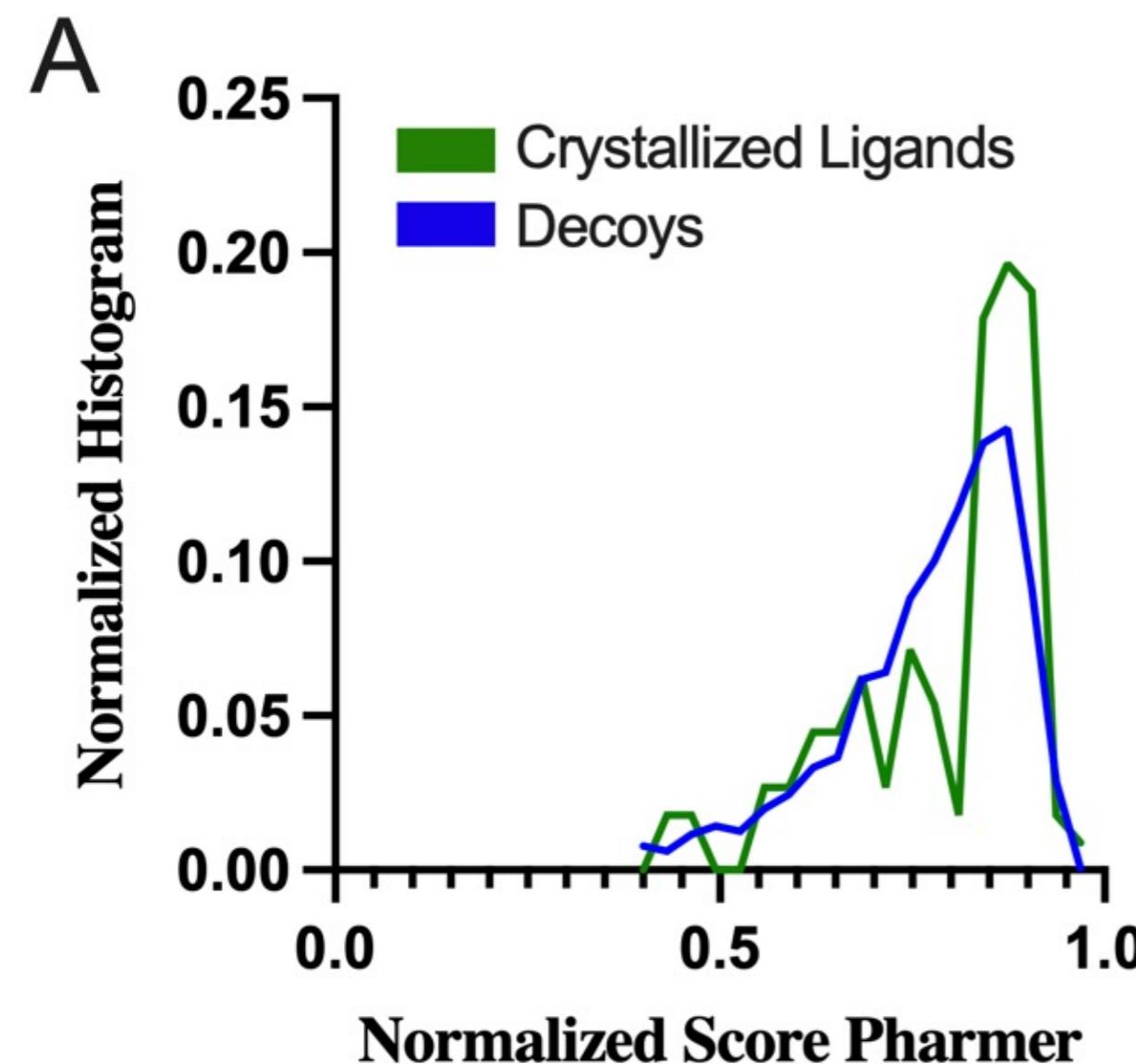
Table S2. Control of mPBVS using co-crystallized ligands as reference.

PDB ligand	No water		Water
	Phase Hits	Pharmer Hits	Pharmer Hits
1SA1	1	x	x
1Z2B	1	0	x
3DU7	1	1	x
3e.22	1	1	x
3HKC	1	1	x
3HKE	1	1	x
3N2G	1	1	x
3N2K	1	1	x
3UT5	1	1	x
4O2A	1	1	x
4O2B	1	x	1
4X1I	1	1	x
4X1K	1	1	x
4X1Y	1	1	x
4X20	1	1	x
4YJ2	1	1	1
4YJ3	1	1	x
5CA0	1	0	x
5CA1	1	1	x
5CB4	1	0	1
5EYP	1	1	1
5GON	1	1	x
5H7O	1	1	x

6O5N	1	1	1
6O61	1	x	1
6PC4	1	1	1
6QQN	1	1	x
Total screened pharmacophores	81	71	37
Match	96.3%	85.92%	86.49%
Mismatch	3.7%	14.08%	13.51%

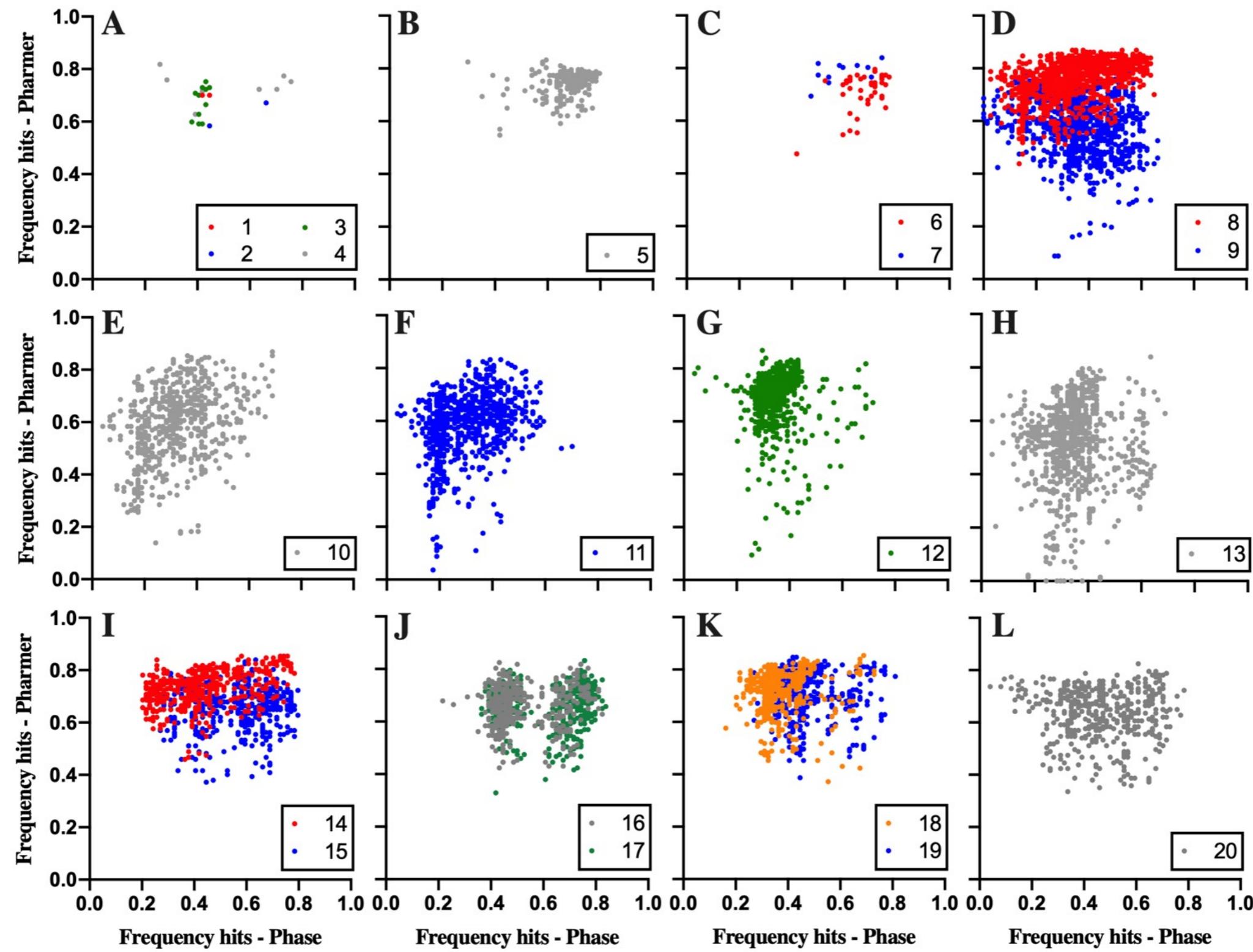
x: The pharmacophore was not screened

Ensamble pharmacophore-based virtual screening (positive control)



Decoys from DUD-E Database -> <http://dude.docking.org/>

Ensamble pharmacophore-based virtual screening



Ensamble pharmacophore-based virtual screening

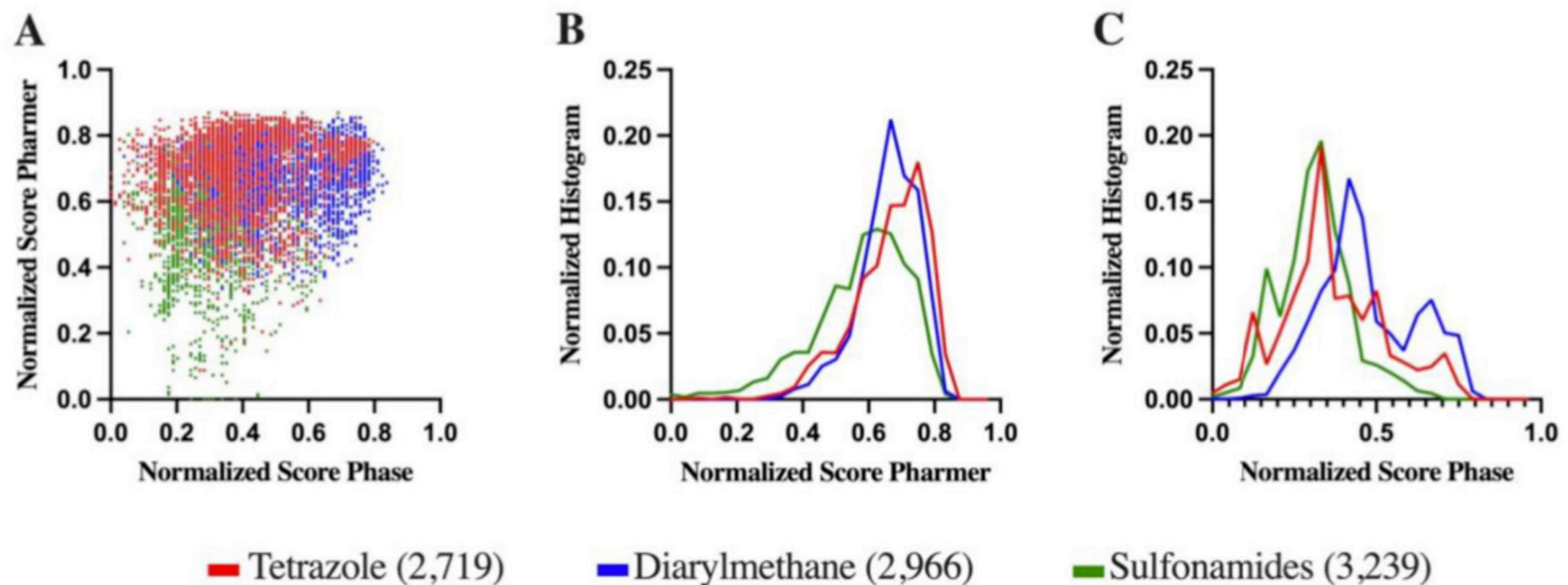
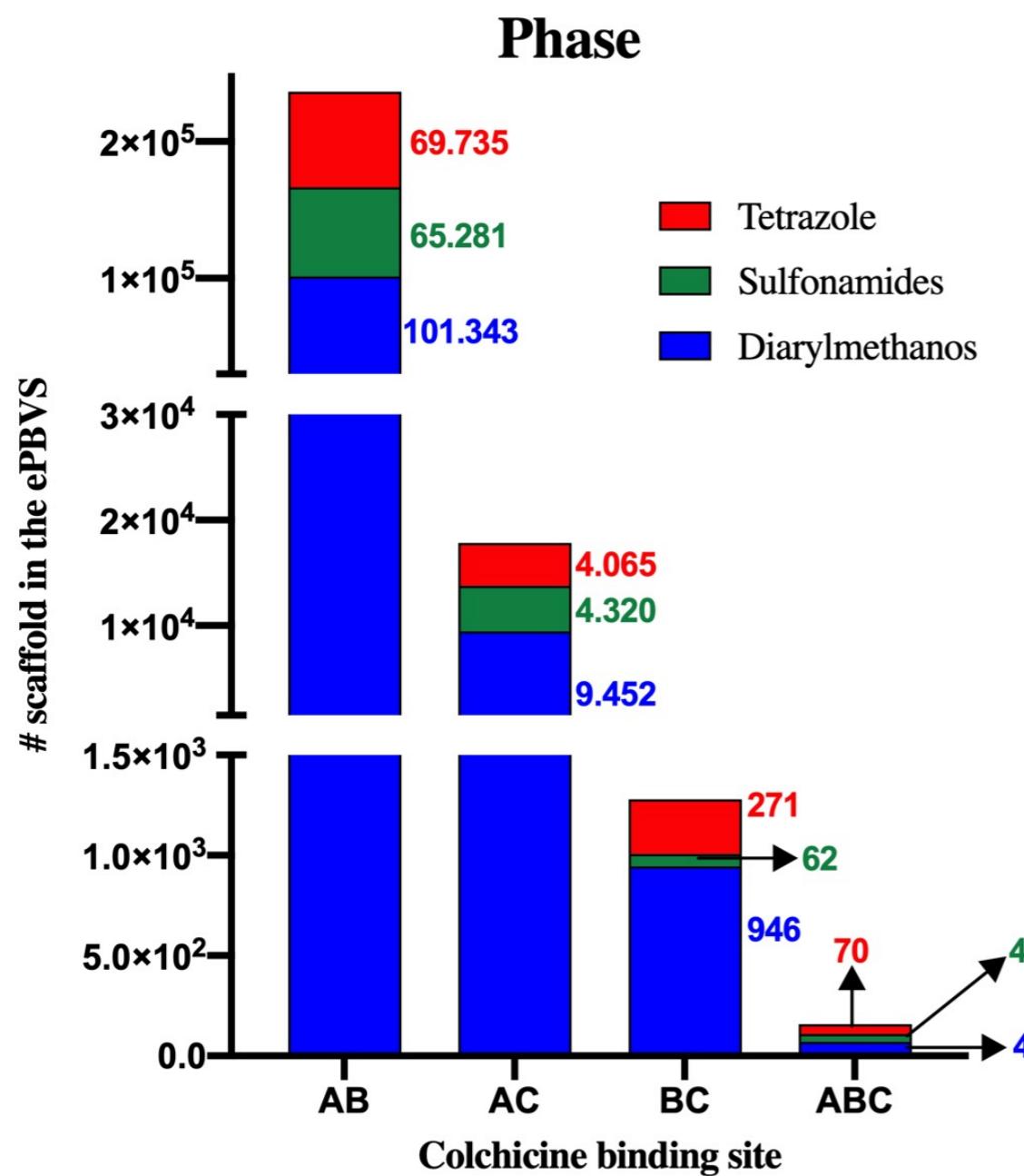


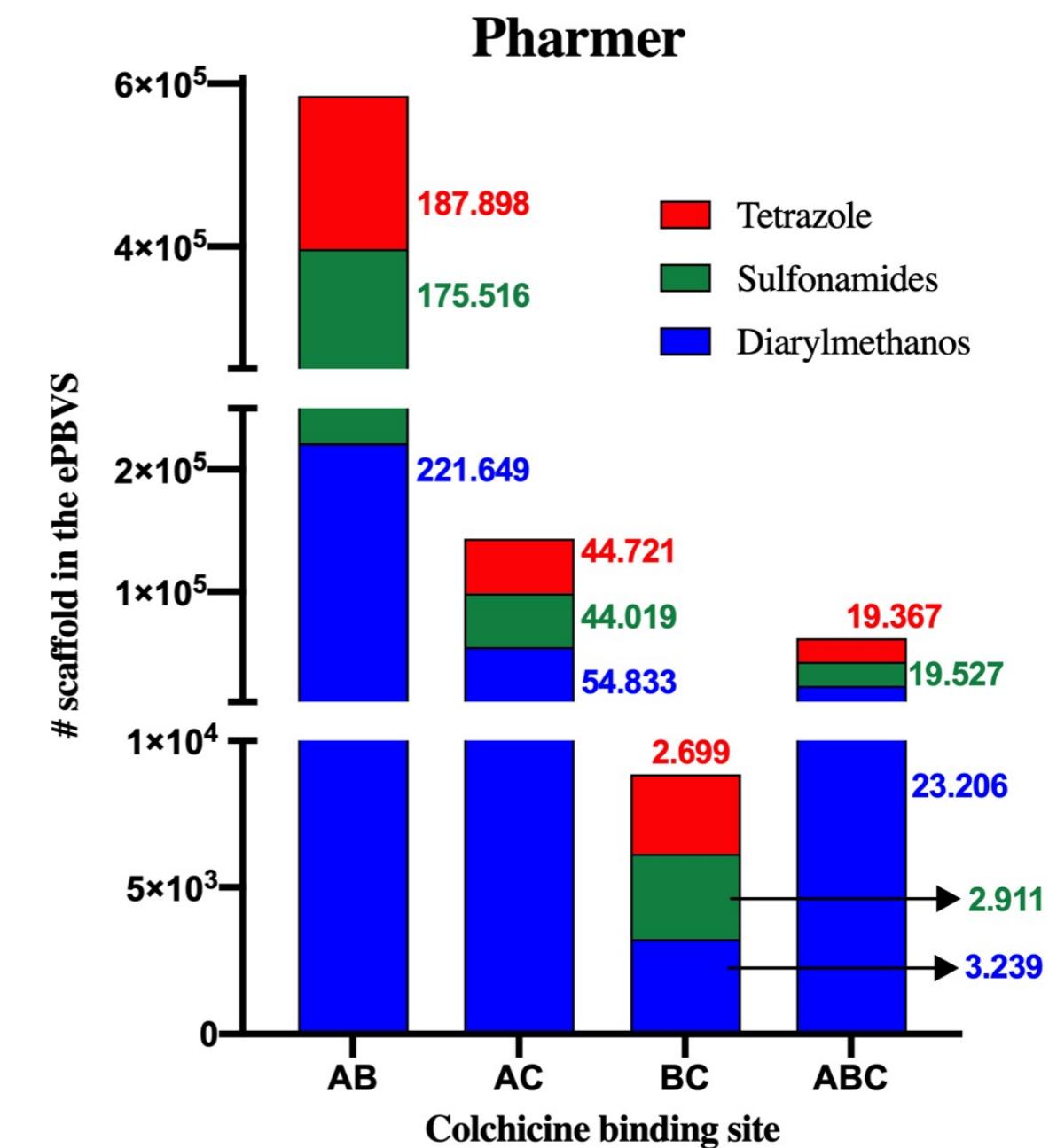
Fig. 3. Frequency hits (normalized number of matches) for Pharmer and Phase: correlation plot (A), and normalized histograms for Pharmer (B) and Phase (C) for the three subgroups of compounds.

Ensamble pharmacophore-based virtual screening

A

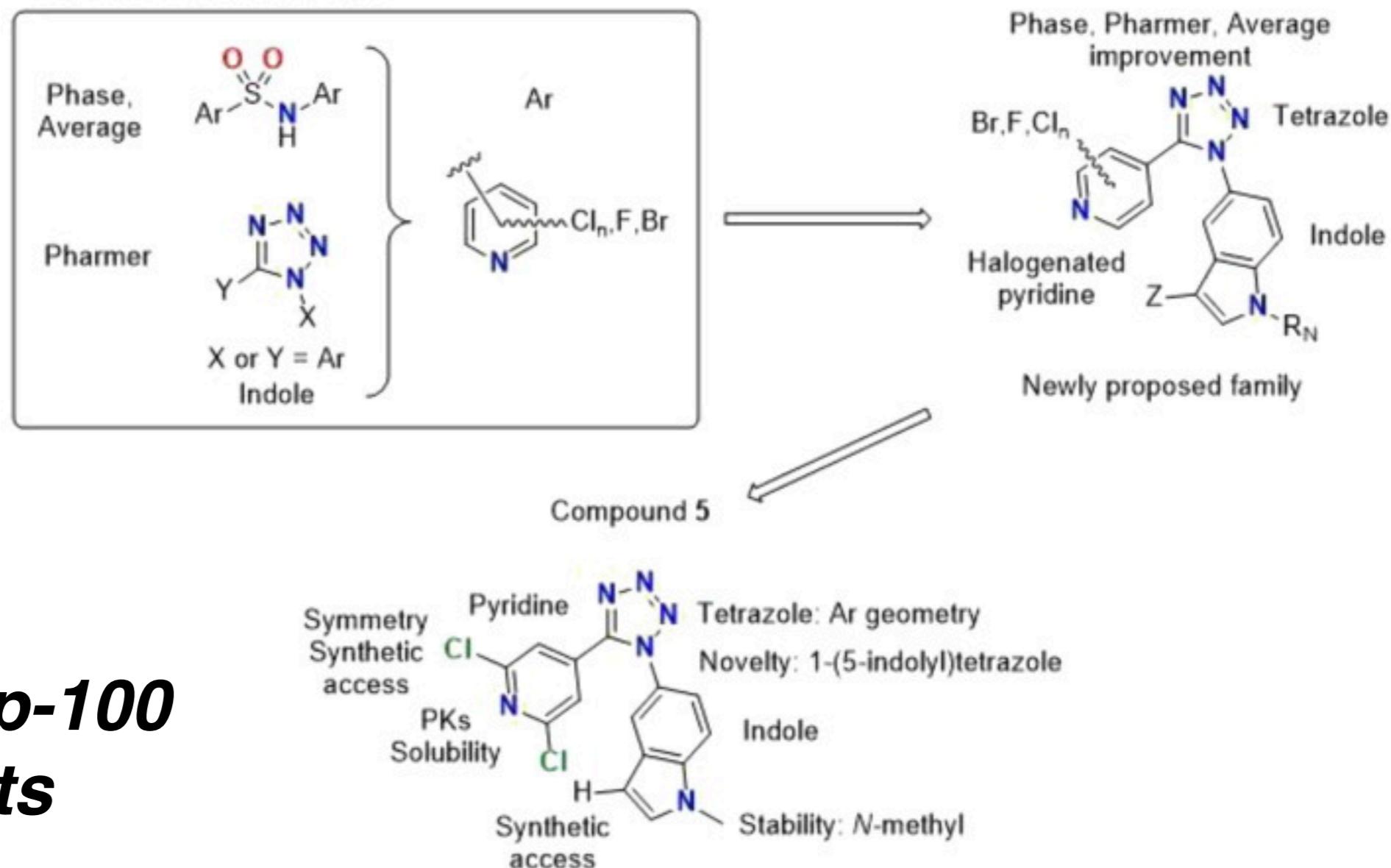


B



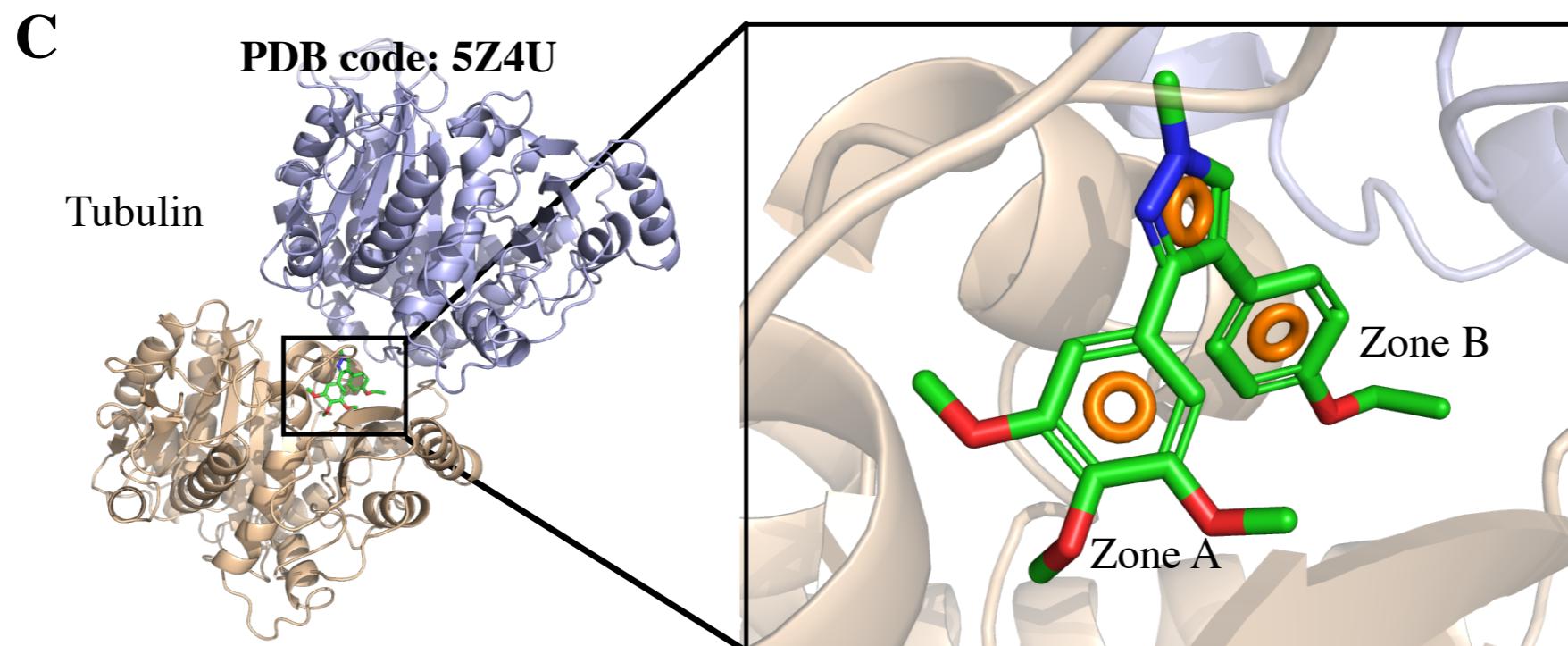
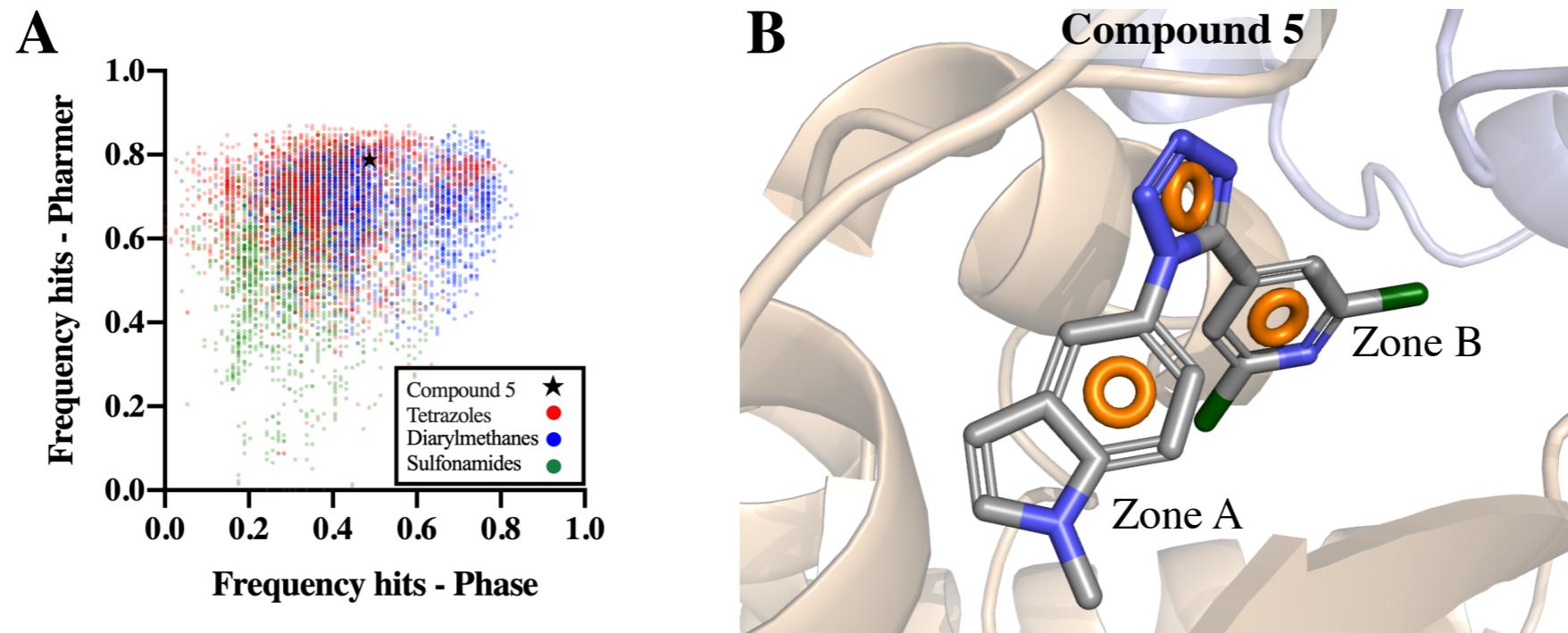
Design of the new family of colchicine site inhibitors based on the results of the Flexi-pharma virtual screening on the focussed ZINC sub-library

Preferred structural features:

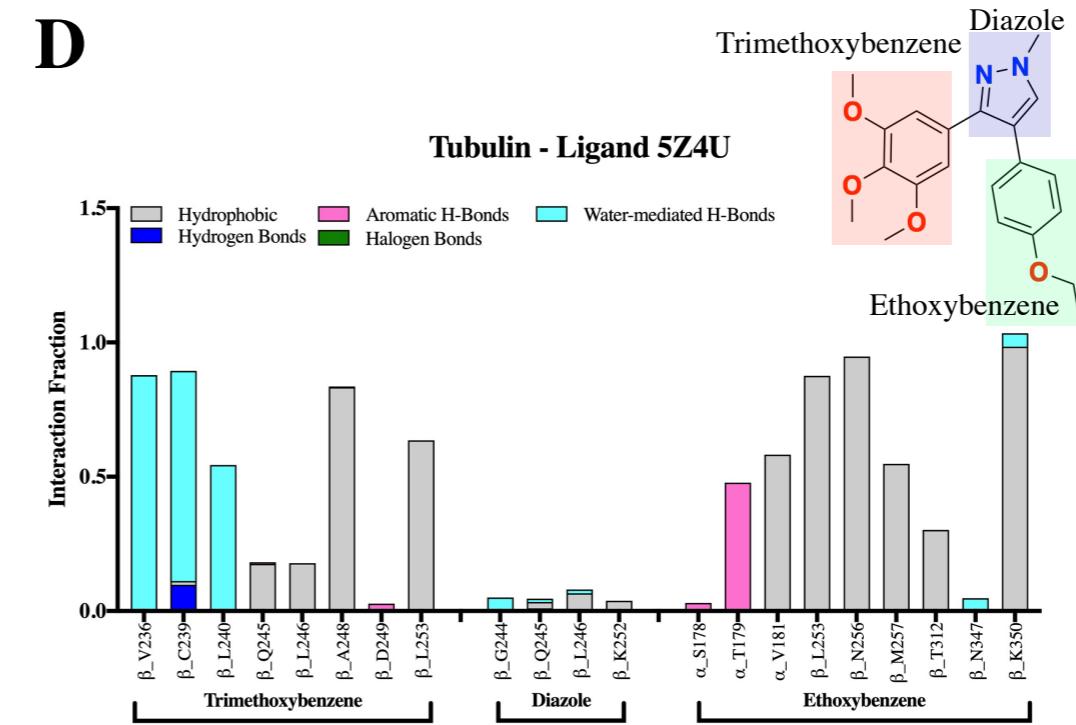
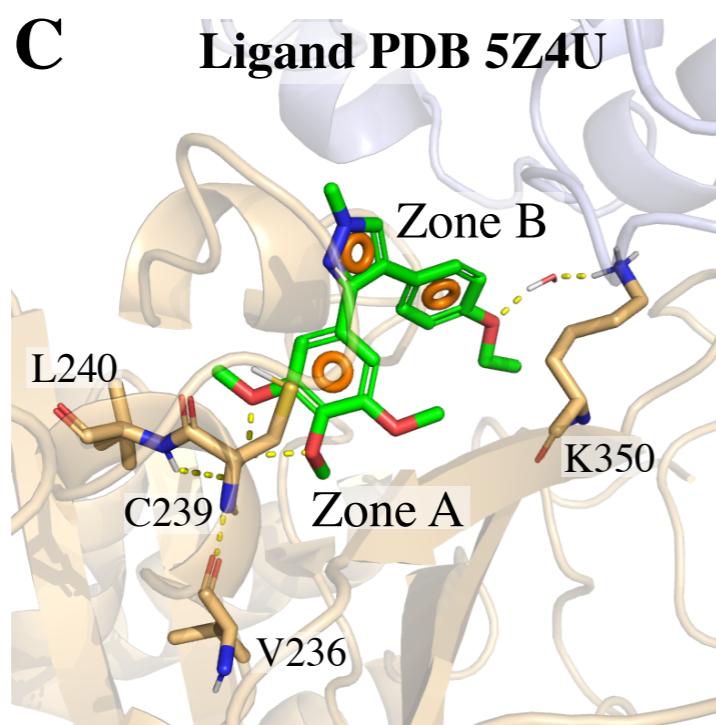
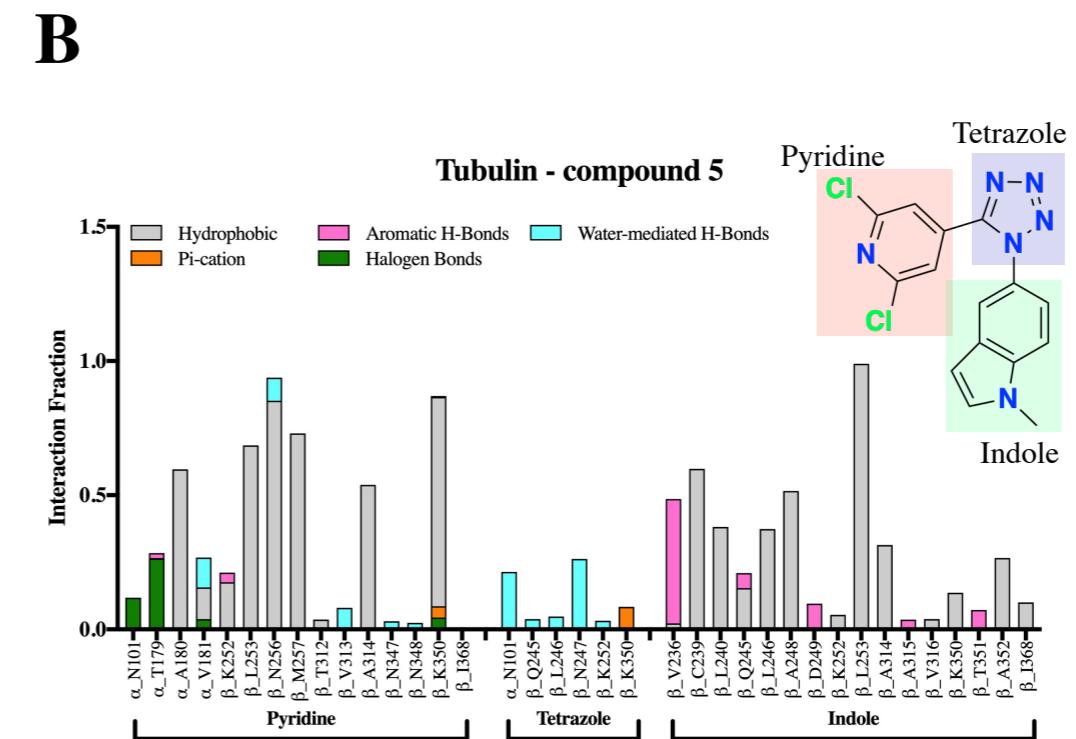
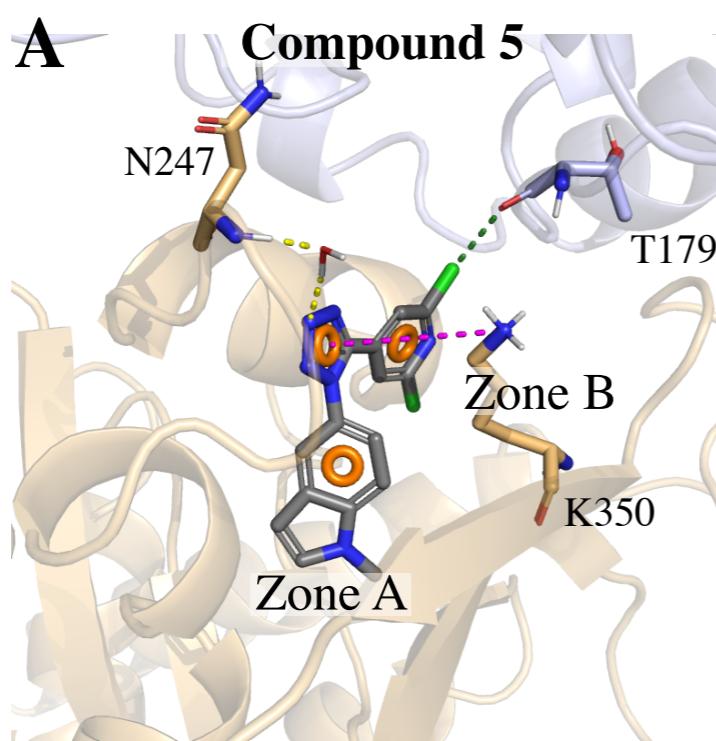


Using top-100 results

Compound 5

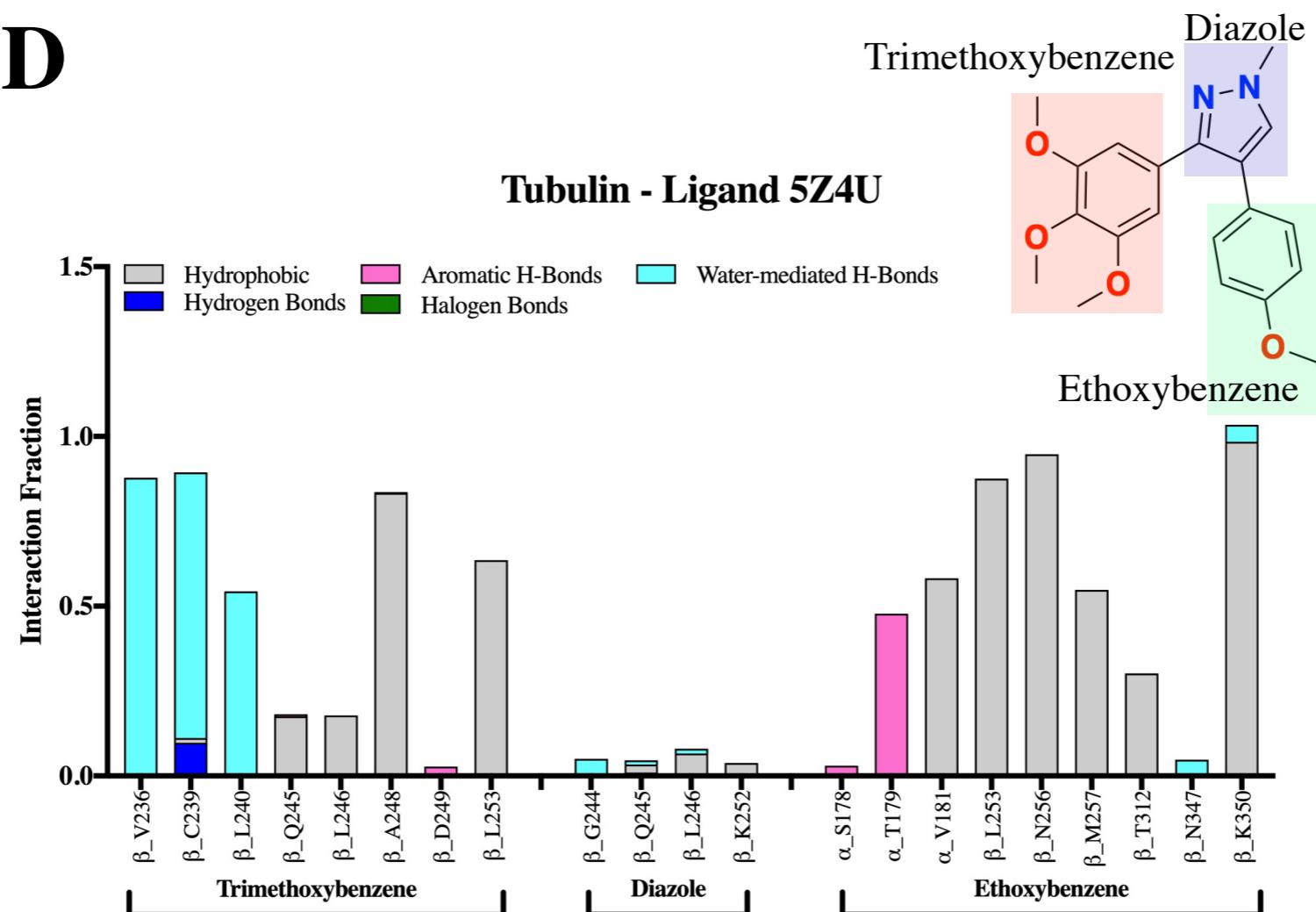


500ns MDs - Compound 5 + Tubulin



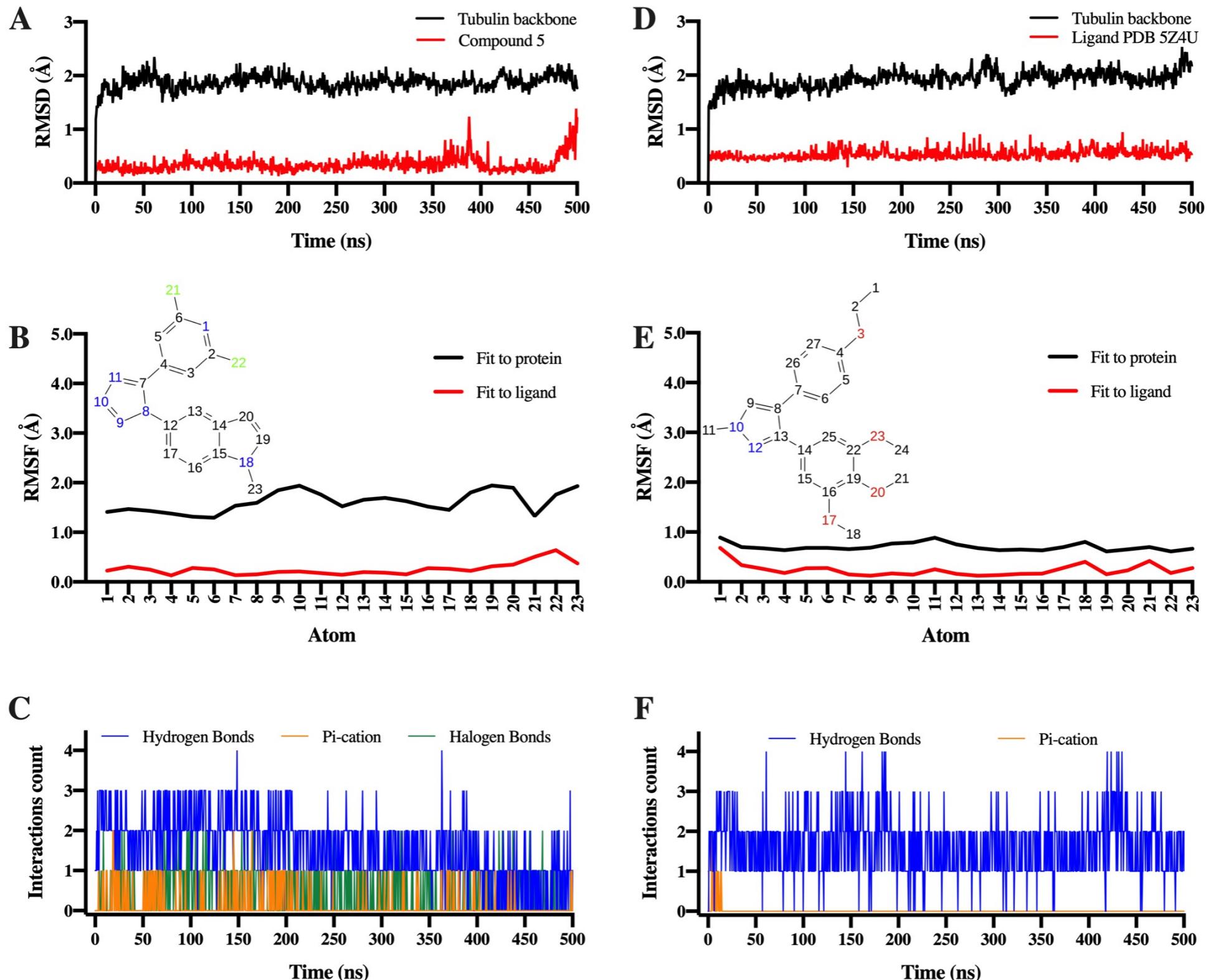
Ligand-Receptor Interaction Diagram

D



https://github.com/ramirezlab/WIKI/tree/master/Docking_and_Virtual_Screening/ligand-receptor_interactions_frequency_multiple_clusters

500ns MDs - Compound 5 + Tubulin



Compound 5

Table 1

Physicochemical and pharmacokinetic descriptors of compound 5 calculated with SwissADME [36].

Physicochemical Properties		Lipophilicity	Water Solubility (mol/L)		Pharmacokinetics	
MW ¹	345.19	Log Po/w (iLOGP)	2.93	ESOL	1.26E-05	GI abs ⁶
RB ²	2	Log Po/w (XLOGP3)	3.82	Ali	1.57E-05	BBB ⁷
HB-A ³	4	Log Po/w (WLOGP)	3.52	SILICOS-IT	1.07E-06	log K _p (cm/s) ⁸
HB-D ⁴	0	Log Po/w (MLOGP)	3.23			-5.69
TPSA ⁵	61.43	Log Po/w (SILICOS-IT)	2.6			
		Average Log Po/w	3.22			

¹ Molecular weight (g/mol).

² Number of rotatable bonds.

³ Number of hydrogen bond acceptors.

⁴ Number of hydrogen bond donors.

⁵ Topological polar surface area (Å) [38].

⁶ Gastrointestinal absorption.

⁷ Blood-brain barrier permeation.

⁸ Skin permeation: QSPR model [39].

Table 2

Drug-likeness properties of the compound 5 calculated with SwissADME [36].

Lipinski # violations ¹	Ghose # violations ²	Veber # violations ³	Egan # violations ⁴	Muegge # violations ⁵	Bioavailability Score ⁶
0	0	0	0	0	0.55

¹ Lipinski (Pfizer) filter [29]: MW ≤ 500; MLOGP ≤ 4.15; N or O ≤ 10; NH or OH ≤ 5.

² Ghose filter [30]: 160 ≤ MW ≤ 480; -0.4 ≤ WLOGP ≤ 5.6; 40 ≤ MR ≤ 130; 20 ≤ atoms ≤ 70.

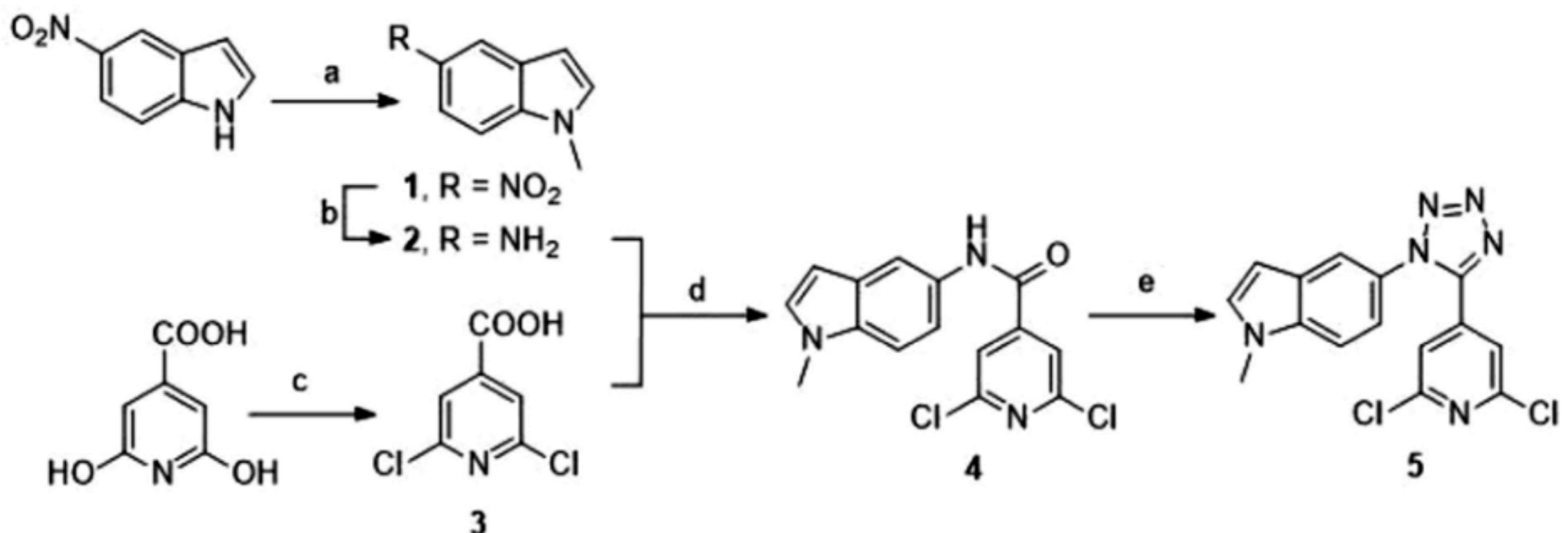
³ Veber (GSK) filter [31]: Rotatable bonds ≤ 10; TPSA ≤ 140.

⁴ Egan (Pharmacia) filter [32]: WLOGP ≤ 5.88; TPSA ≤ 131.6.

⁵ Muegge (Bayer) filter [33]: 200 ≤ MW ≤ 600; -2 ≤ XLOGP ≤ 5; TPSA ≤ 150; Number of rings ≤ 7; Number of carbon atoms > 4; Number of heteroatoms > 1; Number of rotatable bonds ≤ 15.

⁶ Abbott Bioavailability Score [40].

Synthesis of tetrazole derivative 5



Scheme 1. Synthesis of tetrazole derivative **5**. Reagents and conditions: (a) MeI, NaOH, n-Bu₄NHSO₄, dry DCM, rt, 24 h; (b) H₂, Pd/C, MeOH, DCM, rt, 24 h; (c) POCl₃, Me₄NBr, 90–140 °C, 24 h; (d) EDC, DMAP, dry DCM, reflux, 24 h; (e) NaN₃, SiCl₄, MeCN, reflux, 24 h.

Biological activity compound 5

In vitro

Inhibitory activity on the microtubular protein polymerization



$$IC_{50} = 2.8 \mu M$$

HeLa cells from human cervix epithelioid carcinoma

1 mM -> (three independent assays in at least two independent assays) showed more than 50% growth inhibition



$$IC_{50} = 45 nM$$

Effect on the cell cycle progression



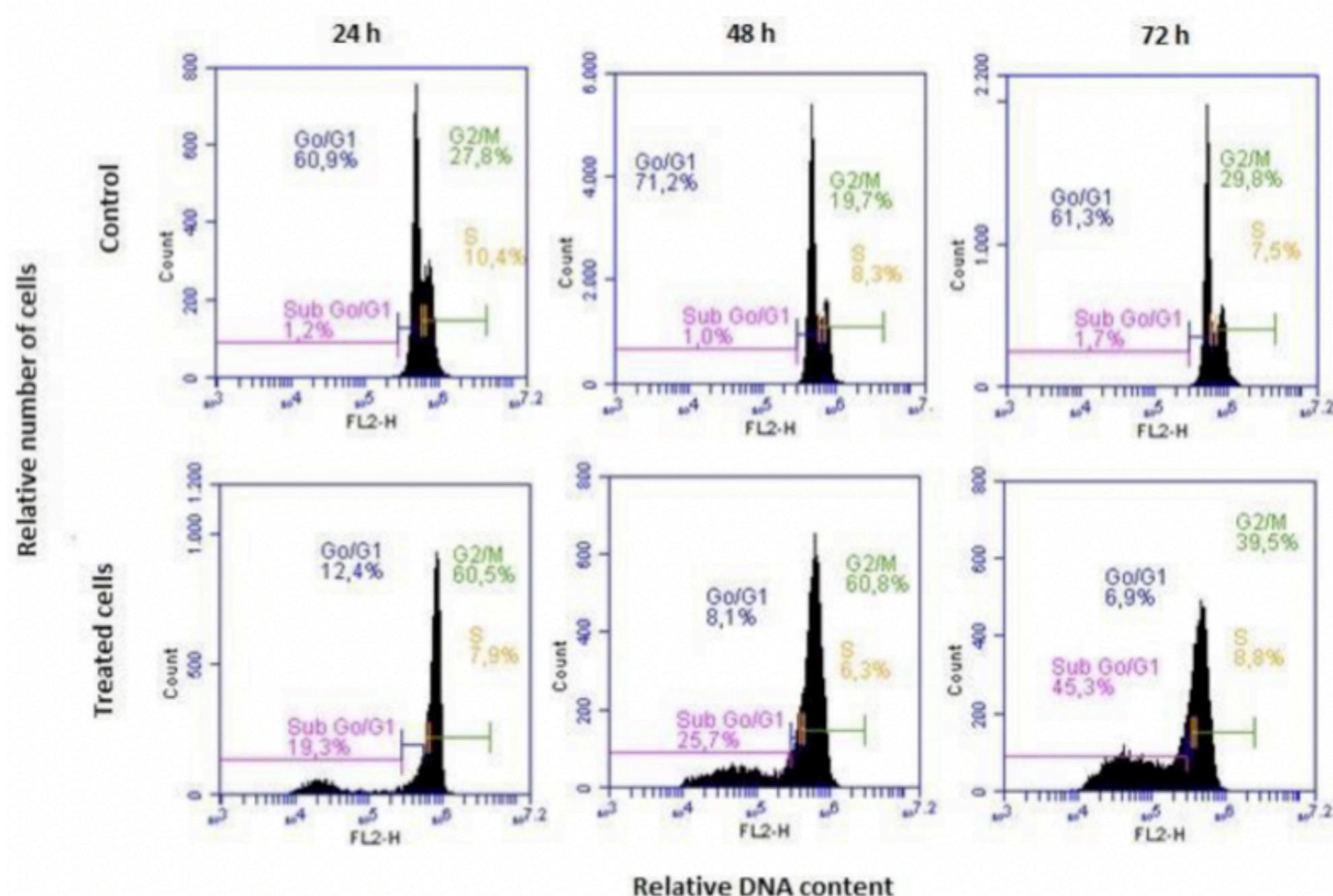
Flow cytometry (100 nM) after 24, 48 and 72h



After 24 h, compound 5 promoted a significant increase in the percentage of cells in the G2/M phase as compared with the untreated sample (60.5% vs. 27.8%, respectively), and the G2/M arrest was maintained 48 h after treatment. This effect was accompanied by the appearance of the Sub G0/G1 fraction, an hypodiploid peak which corresponds to apoptotic cells that undergo DNA condensation and fragmentation. The Sub G0/G1 region represented 19.3% and 25.7 % of cell population after 24 h and 48 h, respectively. At a later time point of 72 h the G2/M peak decreased to 39.5% while more than 45% of the cells were suffering apoptosis.

The observed mitotic arrest followed by the induction of apoptosis is consistent with the mechanism of action of tubulin binding drugs.

Biological activity compound 5

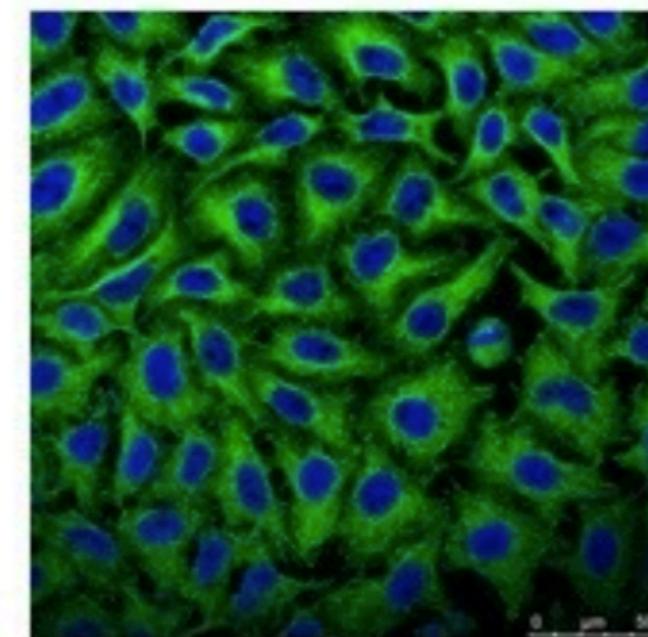


Effects of compound 5 on the microtubule network and cell cycle in HeLa cells. Cells were incubated in the absence (control) or in the presence of 100 nM of compound 5 for 24, 48 or 72 h and analyzed by flow cytometry

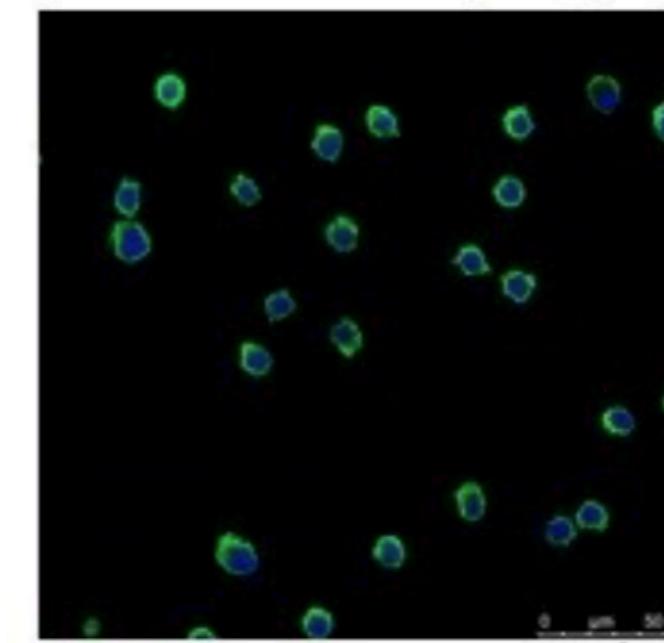
Biological activity compound 5

The alteration of tubulin cytoskeleton in HeLa cells was assessed by confocal immunofluorescence microscopy. The experiments were performed 24 h after cell treatment when the maximum antimitotic effect can be observed having minimal cell death. The compound 5 promoted a microtubule network disruption in HeLa cells whereas in the micrograph for the untreated sample it is possible to distinguish the organized tubulin fibers.

Control (24 h)

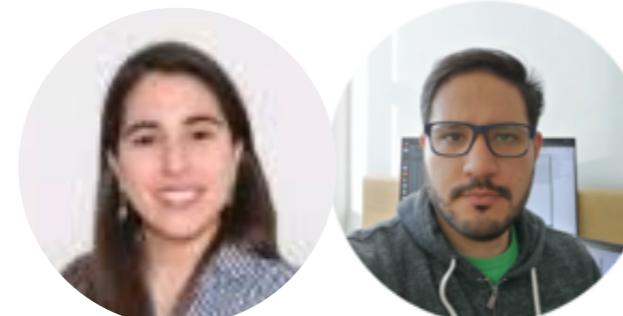


Treated cells (24 h)



Chilean Team

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Dra. Melisa Alegria
Carlos Peña.



Spanish Team

Dr. Rafael Peláez
Dra. Laura Gallego



Colombian Team

Dra. Pilar Cossio
Dr. Rodrigo Ochoa
Dr. Isaías Lans



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