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Computational Polypharmacology Analysis of the Heat Shock Protein 90 Interactome

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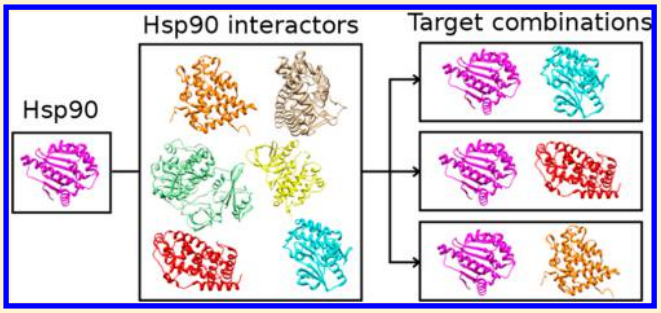
Supporting Information

ABSTRACT: The design of a single drug molecule that is able to simultaneously and specifically interact with multiple biological targets is gaining major consideration in drug discovery. However, the rational design of drugs with a desired polypharmacology profile is still a challenging task, especially when these targets are distantly related or unrelated. In this work, we present a computational approach aimed at the identification of suitable target combinations for multitarget drug design within an ensemble of biologically relevant proteins. The target selection relies on the analysis of activity annotations present in molecular databases and on ligand-based virtual screening. A few target combinations were also inspected with structure-based methods to demonstrate that the identified dual-activity compounds are able to bind target combinations characterized by remote binding site similarities. Our approach was applied to the heat shock protein 90 (Hsp90) interactome, which contains several targets of key importance in cancer. Promising target combinations were identified, providing a basis for the computational design of compounds with dual activity. The approach may be used on any ensemble of proteins of interest for which known inhibitors are available.



INTRODUCTION

Several drugs exert their therapeutic effects by modulating multiple biological targets, a phenomenon known as polypharmacology.[1−5] This is often the case for small molecules used in the treatment of cancer[6] and also other diseases.[7,8] A prime example of polypharmacological agents are kinase inhibitors, which often inhibit several members of the kinome.[6,9] On the one hand, a broad and indiscriminate spectrum of biological activity may result in side effects and adverse reactions as a consequence of binding unwanted off-targets.[10] On the other hand, a desired multitarget activity profile may be beneficial to improve drug efficacy and safety, ultimately resulting in more effective therapeutic agents.[3,5] To this end, an optimal selection of target combinations to be used in polypharmacology approaches is crucial. Methods aimed at the identification of such compounds have recently been proposed,[11−15] and other approaches are currently being explored.[5]

Here we propose a computational strategy aimed at the identification of promising target combinations for a polypharmacology approach. As a test case, the strategy was applied to the interactome of heat shock protein 90 (Hsp90),[16] a molecular chaperone that interacts with several hundreds of proteins. These so-called Hsp90 interactors are mainly cochaperones and client proteins (kinases and transcription factors, among others). Hsp90 assists in the maturation, activation, and stability of its client proteins through ATP hydrolysis cycles. Interestingly, Hsp90 as well as many members of the Hsp90 interactome are validated anticancer drug targets, being involved in signal transduction and malignancy-related pathways. Several classes of Hsp90 inhibitors, including macrocycles (geldanamycin and radicicol derivatives), purines, resorcylic pyrazoles, isoxazoles, pyrimidines, aminopyridines, and azoles, among others, have been developed.[17,18] Seventeen Hsp90 inhibitors are currently undergoing clinical trials, but none of them has been approved to date, mainly because of hepatotoxicity.[19] Hsp90 inhibitors in clinical trials are also evaluated in combination with other anticancer drugs such as taxanes, cisplatin, proteasome inhibitors, death receptor ligands, histone deacetylase inhibitors, and protein kinase inhibitors.[20] Remarkably, many of these ligands bind to biological targets that belong to the Hsp90 interactome. Therefore, the design of a single molecule that is able to modulate the activities of Hsp90 and one or more of its interactors may provide a complementary or alternative approach to combination therapy.[5] For these

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reasons, the Hsp90 interactome represents an interesting network of targets for a polypharmacology approach.

In this work, the suitability of proteins of the Hsp90 interactome for a multitarget approach was systematically evaluated through the analysis of known active compounds deposited in public databases. The workflow of the computational strategy put forward is shown in Figure 1.

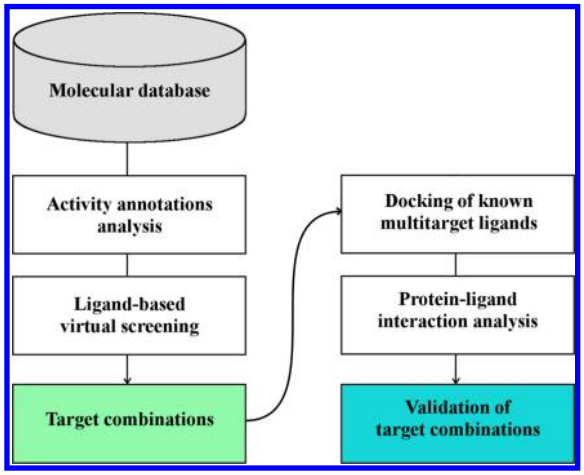

Figure 1. Computational approach for the ligand- and structure-based analysis of target combinations for polypharmacology. The approach integrates molecular database analysis, similarity searching, and docking calculations for the discovery of promising target combinations.

Previous analyses have highlighted that public databases are valuable sources of compounds with promiscuous activity.[21] Therefore, an extensive analysis of the data deposited in ChEMBL[22] and BindingDB[23] was carried out, and a ligand-based virtual screening was performed to identify interesting target combinations. A subset of these combinations was then analyzed with structure-based methods to demonstrate that the identified dual-activity compounds are able to establish key molecular interactions with targets characterized by low binding site similarity. The ligand-based approach resulted in the identification of promising target combinations and compounds with known multitarget activities. The comparative docking of selected multitarget compounds into the Hsp90 and Hsp90 interactor binding sites revealed analogies in the protein−ligand interaction patterns in spite of remote binding site similarities, suggesting that these target combinations are amenable to structure-based design of compounds with dual activity. The results show that a combination of ligand-based and structure-based methods can be used to identify and validate target combinations for polypharmacology approaches in drug design.

METHODS

Known Active Compounds and Activity Annotation Analysis. All compounds with an activity annotation for Hsp90 were extracted from the ChEMBL database[22] (release 13) and BindingDB,[23] yielding 1583 initial candidates. A high-confidence subset of 418 Hsp90 inhibitors was identified by filtering the activity annotations using a KNIME[24] protocol with the following criteria:

(i) inhibitory activity expressed as K_i or IC_{50};
(ii) K_i or IC_{50} values ≤ 50 μM;
(iii) only compounds for which multiple activity values did not differ by more than 1 order of magnitude were retained;
(iv) ChEMBL assay confidence score equal to 9 (the maximum).

The list of known Hsp90 interactors was obtained from the Picard Laboratory Web site.[25] The entry codes of the Hsp90 interactors were manually searched in the UniProt database.[26] Activity annotations for Hsp90 interactors of the high-confidence Hsp90 inhibitors were analyzed by displaying all of the available bioactivities and sorting them by the relative UniProt IDs. A total of 26 Hsp90 inhibitors with activity annotations for 17 Hsp90 interactors were identified. Among these, 11 Hsp90 interactors were selected in consideration of their relevance in cancer, reducing the ensemble of dual-activity compounds to 25. For each target, a reference compound set was generated.

Ligand-Based Virtual Screening. MACCS structural keys[27] were calculated for the ChEMBL database and for the Hsp90 inhibitors with Molecular Operating Environment (MOE).[28] The ChEMBL database was ranked by performing one-nearest-neighbor (1-NN) similarity searching (SS), taking as a reference the 418 Hsp90 actives. Eleven rankings of the database were obtained by taking as references the 11 prioritized subsets of multitarget compounds (Hsp90/Hsp90 interactor dual inhibitors). Moreover, two ChEMBL rankings were generated by performing support vector machine (SVM) calculations.[29] One was obtained by using the 418 Hsp90 inhibitors as positive training examples and the other one by using an ensemble including all 25 multitarget compounds. As negative training instances, 500 and 25 randomly selected compounds from the ZINC database[30] with numbers of heavy atoms comparable to those of the actives were used, respectively. A total of 14 rankings of the database were obtained. The top 100 compounds of each of the 11 rankings resulting from the use of multitarget reference compounds and the top 1000 compounds (i.e., ∼1% of the screening database) of the remaining three rankings were searched for second target annotations, selecting compounds annotated as active against at least one Hsp90 interactor. It should be noted that the 418 known Hsp90 inhibitors used for SVM modeling were not excluded from the fingerprint search rankings (because both low- and high-confidence annotations were considered for the identification of second targets). On the basis of the activity annotations of these compounds, a total of 81 target combinations including Hsp90 and an additional interactor were established. For comparison, all of the virtual screening calculations were repeated with a two-dimensional pharmacophore fingerprint, the Graph π-Donor−Acceptor Polar Hydrophobe Triangle (GpiDAPH3) available in MOE. In GpiDAPH3, each atom is assigned to one of eight types derived from three atomic properties ("in pi system", "is donor", and "is acceptor") and interatomic distances are divided into eight different bond distance ranges. The fingerprint yields 30240 possible pharmacophore features. When GpiDAPH3 was used instead of MACCS, a total of 113 target combinations including Hsp90 and an additional interactor were obtained. The union of these two selections yielded 117 unique interactors and an overlap of 77 targets.

Selection of Targets and Ligands for Structure-Based Analysis. Compounds for which activity annotations expressed as IC_{50} values were available for both Hsp90 and one Hsp90 interactor were selected. These were ranked according to the average of the two IC_{50} values (avg $IC_{50\ \text{Hsp90-2ndTarget}}$). Activity annotations were carefully examined in database records and

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related publications to make sure that reliable data were available. The top-ranked compound was CHEMBL362307 (compound **1**), an estrogen receptor α (ER-α) and estrogen receptor β (ER-β) antagonist (avg $IC_{50\ Hsp90\text{-}ER\text{-}\alpha}$ = 46.5 nM, avg $IC_{50\ Hsp90\text{-}ER\text{-}\beta}$ = 31 nM). Compound **1** was identified as an Hsp90 inhibitor in a high-throughput screening (HTS) reported by Gopalsamy et al.[31] The same compound was reported to be active on ER-α and -β by Malamas et al.[32] The second-ranked compound was CHEMBL53898 (compound **2**), an Hsp90 inhibitor discovered in an HTS deposited in PubChem Bioassays (AID: 429)[33] by the Emory University Molecular Libraries Screening Center. The HTS protocol was published by Du et al.[34] Compound **2** is also a receptor tyrosine-protein kinase ErbB2 inhibitor (avg $IC_{50\ Hsp90\text{-}ErbB2}$ = 27061 nM) reported by Gazit et al.[35] Hence, two compounds and three target combinations (Hsp90/ER-α, Hsp90/ER-β, and Hsp90/ErbB2) were used for the structure-based analyses.

Ligand and Protein Preparation. The structures of compounds **1** and **2** were prepared for docking using LigPrep.[36] The protein structures used for docking were the crystal structure of Hsp90 in complex with 17-DMAG (PDB code 1OSF),[37] Hsp90 in complex with NVP-AUY922 (PDB code 2VCI),[38] ER-α in complex with WAY-244 (PDB code 1X7E),[39] ER-β in complex with CL-272 (PDB code 1U3Q),[32] and ErbB2 in complex with SYR127063 (PDB code 3PP0).[40] All of the structures were prepared for docking with the Protein Preparation Wizard available in Maestro.[36,41] All of the water molecules were removed from the structures, except for the three conserved water molecules in Hsp90 (HOH 402, 407, and 418 in 1OSF and HOH 2099, 2232, and 2233 in 2VCI) that are part of a hydrogen-bonding network at the interface between Hsp90 and the cocrystallized ligand. These water molecules are known to play a crucial role in ligand binding to Hsp90.[42,43]

Docking. Docking calculations were performed with the Induced Fit Docking[36,44] protocol available in the Schrödinger 2014-1 suite[36] with default settings. Complexes were visually inspected (up to 20 top-scoring complexes per ligand), and a representative binding mode was selected from among the top-scoring ones. In particular, complexes were investigated for the presence of key protein−ligand interactions known to be important for activity. In the case of Hsp90, we searched for a hydrogen bond between the ligand and residue Asp93.[45−48] In the case of ER-α (ER-β), we investigated whether hydrogen bonds with Glu353 and Arg394 (Glu305 and Arg346) were formed.[32,49−51] For ErbB2, we searched for a hydrogen bond with the backbone nitrogen of Met801 of the hinge.[40] In all cases, the selected complexes were among the three top-scoring solutions. Finally, the docked complexes were minimized with MacroModel[36] using the OPLS_2005 force-field with a generalized Born/solvent-accessible surface area (GB/SA)[52] continuum solvation model. Ligand interaction diagrams were generated with Maestro.[36] UCSF Chimera[53] was used to superimpose the modeled complexes of compounds **1** and **2** and to compute root-mean-square deviations between bound conformations of ligands.

Selection of Ligands for Structure−Activity Relationship (SAR) Analysis. Benzisoxazole-based compounds **3**−**8** (analogues of compound **1**) were selected from the same articles reporting the activities of compound **1** on Hsp90[31] and ER-α.[32] Besides compound **1**, compounds **3** and **4** were the only ones for which inhibitory activity was determined on both targets. Compounds **5**−**7**, whose activities were reported only for ER-α, were selected because they lack each of the three hydroxyls involved in hydrogen bonds with Hsp90 and ER-α. Compound **8**, whose activity was reported only for Hsp90, was chosen because substitution of the hydroxyl groups with methoxy groups makes the ligand unable to function as hydrogen-bond donor.

RESULTS

Selection of Active Compounds in ChEMBL and BindingDB. As a first step, ChEMBL and BindingDB were extensively analyzed to extract known Hsp90 inhibitors. All types of activity annotations were initially considered. We obtained 1358 ChEMBL and 500 BindingDB compounds annotated as active against Hsp90. As reported in Table 1 a total of 1583 unique compounds were found, with an overlap of 275 compounds between the two databases.

Table 1. Hsp90 Inhibitors from ChEMBL and BindingDB[a]

activity annotation confidence	ChEMBL	BindingDB	overlap	total
low	1358	500	275	1583
high	78	395	55	418

[a]Compounds with low- and high-confidence activity annotations for Hsp90 from ChEMBL and BindingDB are reported. In addition, the overlap between the two databases (number of shared compounds) is given.

Compounds in public repositories report different types of activity values (K_i, IC_{50}, percentage of residual activity or inhibition, etc.). In view of the variety of sources, assay conditions, and standard units, not all of the annotations can be considered equally reliable. Therefore, a high-confidence subset of 418 Hsp90 actives was selected by applying the confidence criteria described in Methods to the pool of initial candidates. High-confidence compounds were taken as a representative sample of publicly available Hsp90 inhibitors.

In the next step, we determined whether these Hsp90 inhibitors also had target annotations for members of the Hsp90 interactome. For this purpose, the high-confidence Hsp90 inhibitors described above were first inspected for any additional target (here termed the "second target") annotations. No confidence filters were applied in this step in order to avoid discarding any possible yet not fully confirmed multitarget activities. A subset of 102 of the 418 Hsp90 inhibitors were found to have one or more activity annotations for a total of 163 other targets. As reported in Table 2, 26 of these inhibitors were found to have activity annotations for 17 Hsp90 interactors. Table 3 reports these targets and the numbers of annotated active compounds shared with Hsp90.

Table 2. Hsp90 Inhibitors with Additional Activity Annotations for Other Targets[a]

number of multitarget compounds	number of second targets	second target class
102	163	any target
26	17	Hsp90 interactor

[a]The number of Hsp90 inhibitors with known multitarget activities and the number of annotated targets are reported.

Intriguingly, no previous reports about the dual activities of these Hsp90 inhibitors were found in the literature, although the data were deposited in publicly available databases. A subset of 11 from among the 17 second targets (highlighted in bold in Table 3) was initially prioritized for further analysis on the basis of their

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Table 3. Targets of the Hsp90 Interactome Sharing Ligands with Hsp90[a]

target name	UniProt ID	active compounds shared with Hsp90
estrogen receptor β	Q92731	12
estrogen receptor α	P03372	6
polo-like kinase 1 (PLK1)	P53350	4
mitogen-activated protein kinase kinase kinase 5 (ASK1)	Q99683	3
tyrosine kinase ErbB2	P04626	3
mitogen-activated protein kinase 6	Q16659	2
vitamin D receptor	P11473	2
serine/threonine-protein kinase PIM1	P11309	2
microtubule-associated protein τ	P10636	2
apoptosis regulator Bcl-2	P10415	2
tyrosine-protein kinase FES	P07332	2
protein kinase C ε	Q02156	1
heat shock factor protein 1	Q00613	1
DNA-dependent protein kinase	P78527	1
FK506 binding protein 12 (mTor)	P42345	1
serine/threonine-protein kinase B-Raf	P15056	1
tyrosine-protein kinase SRC	P12931	1

[a]Seventeen Hsp90 interactors sharing active compounds with Hsp90 are reported. The number of shared compounds is given for each target. Prioritized Hsp90 interactors are shown in bold.

relevance in anticancer drug discovery. The selection of 11 second targets reduced to 25 the number of unique active compounds shared with Hsp90. Some of the second targets shared only one active compound with Hsp90, whereas ER-β shared 12 compounds.

Ligand-Based Virtual Screening. The ChEMBL database was ranked on the basis of SS and SVM calculations using the 418 Hsp90 inhibitors as reference compounds. MACCS keys were calculated as descriptors for both the database and reference compounds. Additional rankings of the database were generated using the subset of 25 multitarget compounds as reference compounds (as detailed in Methods). Because we were interested in multitarget compounds within members of the Hsp90 interactome, the 1000 top-ranking compounds were searched for reported activities against Hsp90 interactors. The numbers of compounds with relevant activity annotations identified by virtual screening are reported in Tables 4 and 5.

In the calculations performed with MACCS fingerprints (Tables 4a and 5a), when all of the 418 Hsp90 actives were used as the reference set, a total of 580 unique compounds with activity against Hsp90 and/or 74 targets of the Hsp90 interactome were identified. On the other hand, when the 25 multitarget compounds were used as the reference set, 672 compounds active against 75 targets were detected. Both rankings were enriched with ligands having known multitarget activity on Hsp90 and one or more interactors. These included 35 and 25 compounds, respectively, of 102 actives present within the top 1000 rank positions (corresponding to ∼1% of the screening database). Moreover, calculations performed with the 25 multitarget reference compounds led to a higher number of compounds active against Hsp90 interactors compared with that obtained using the 418 Hsp90 actives (641 vs 478, respectively), especially for the SVM calculations, where 416 vs 263 compounds active against any Hsp90 interactor were identified. However, there was an overlap between the compound rankings of the SS and SVM calculations, as shown in Figure 2.

Table 4. Computational Identification of ChEMBL Compounds with Activity Annotations for Hsp90 and/or Hsp90 Interactors Using (a) MACCS and (b) GpiDAPH3 Fingerprints[a]

(a) MACCS Fingerprints				
activity annotation	SS	SVM	overlap	total
Reference Set: 418 Hsp90 Actives				
Hsp90 OR Hsp90 interactor(s)	356	286	62	580
Hsp90	131	34	28	137
Hsp90 interactor	256	263	41	478
Hsp90 AND Hsp90 interactor(s)	31	11	7	35
Reference Set: 25 Multitarget Actives				
Hsp90 OR Hsp90 interactor(s)	306	419	53	672
Hsp90	49	17	10	56
Hsp90 interactor	277	416	52	641
Hsp90 AND Hsp90 interactor(s)	20	14	9	25
(b) GpiDAPH3 Fingerprints				
activity annotation	SS	SVM	overlap	total
Reference Set: 418 Hsp90 Actives				
Hsp90 OR Hsp90 interactor(s)	469	469	210	728
Hsp90	144	127	124	147
Hsp90 interactor	377	380	121	636
Hsp90 AND Hsp90 interactor(s)	52	38	35	55
Reference Set: 25 Multitarget Actives				
Hsp90 OR Hsp90 interactor(s)	321	481	189	613
Hsp90	55	53	45	63
Hsp90 interactor	289	457	167	579
Hsp90 AND Hsp90 interactor(s)	23	29	23	29

[a]For SS and SVM calculations using different reference sets, the numbers of prioritized ChEMBL compounds with known Hsp90 activity and/or Hsp90 interactome second-target annotations among the 1000 top-ranked database compounds are reported.

Table 5. Numbers of Hsp90 Interactors Binding to Reference Compounds Identified Using (a) MACCS and (b) GpiDAPH3 Fingerprints[a]

(a) MACCS Fingerprints				
reference set	SS	SVM	overlap	total
418 Hsp90 actives	58	70	54	74
25 multitarget actives	58	69	52	75
(b) GpiDAPH3 Fingerprints				
reference set	SS	SVM	overlap	total
418 Hsp90 actives	74	69	59	84
25 multitarget actives	81	106	76	111

[a]The numbers of Hsp90 interactors for which active compounds were identified via SS and SVM calculations are reported. The prioritized ChEMBL compounds used as reference sets are according to Table 4.

The histograms reveal that in a number of cases a considerable number of compounds were retrieved for the same targets by both SS and SVM calculations. These include ER-α and -β, microtubule-associated protein τ, and DNA polymerase η (Figure 2). The list of 74 and 75 Hsp90 interactors obtained by using the 418 and 25 reference set compounds (Table 5a) yielded a total of 81 unique targets for the MACCS fingerprint, as reported in Table S1a in the Supporting Information along with the number of annotated compounds retrieved in each ranking; a total of 113 unique targets were obtained for the GpiDAPH3 fingerprint, as reported in Table S1b. Therefore, the ligand-based virtual screening performed on ChEMBL further expanded the

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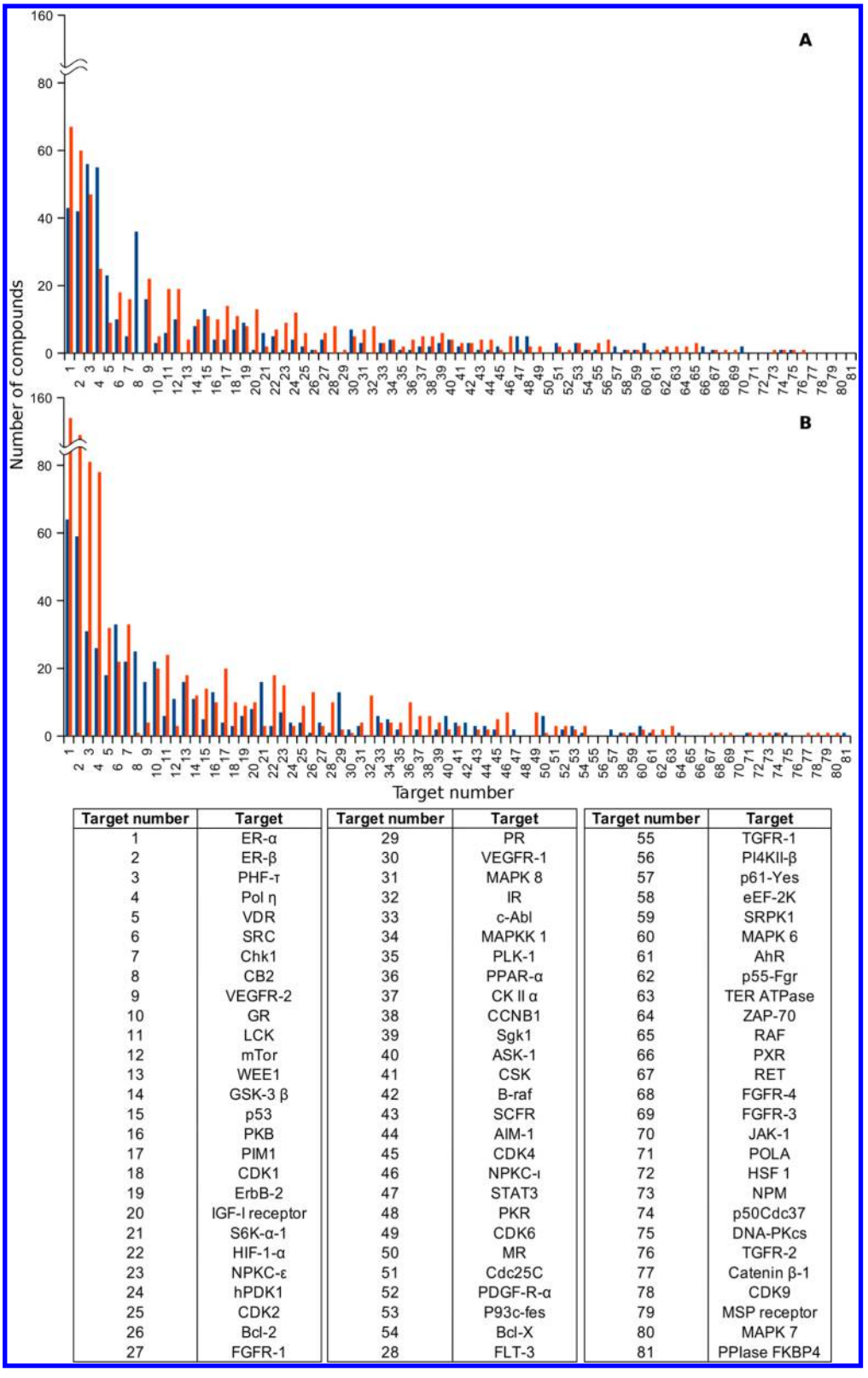

Target number	Target	Target number	Target	Target number	Target
1	ER-α	29	PR	55	TGFR-1
2	ER-β	30	VEGFR-1	56	PI4KII-β
3	PHF-τ	31	MAPK 8	57	p61-Yes
4	Pol η	32	IR	58	eEF-2K
5	VDR	33	c-Abl	59	SRPK1
6	SRC	34	MAPKK 1	60	MAPK 6
7	Chk1	35	PLK-1	61	AhR
8	CB2	36	PPAR-α	62	p55-Fgr
9	VEGFR-2	37	CK II α	63	TER ATPase
10	GR	38	CCNB1	64	ZAP-70
11	LCK	39	Sgk1	65	RAF
12	mTor	40	ASK-1	66	PXR
13	WEE1	41	CSK	67	RET
14	GSK-3 β	42	B-raf	68	FGFR-4
15	p53	43	SCFR	69	FGFR-3
16	PKB	44	AIM-1	70	JAK-1
17	PIM1	45	CDK4	71	POLA
18	CDK1	46	NPKC-ι	72	HSF 1
19	ErbB-2	47	STAT3	73	NPM
20	IGF-I receptor	48	PKR	74	p50Cdc37
21	S6K-α-1	49	CDK6	75	DNA-PKcs
22	HIF-1-α	50	MR	76	TGFR-2
23	NPKC-ε	51	Cdc25C	77	Catenin β-1
24	hPDK1	52	PDGF-R-α	78	CDK9
25	CDK2	53	P93c-fes	79	MSP receptor
26	Bcl-2	54	Bcl-X	80	MAPK 7
27	FGFR-1	28	FLT-3	81	PPIase FKBP4

Figure 2. Ligand-based virtual screening results. The numbers of active compounds identified in ChEMBL for each Hsp90 interactor through similarity searching (blue) and support vector machine ranking (orange) are reported. (A) Results obtained using the 418 Hsp90 inhibitors as reference compounds. (B) Results obtained using the 25 compounds with multitarget activities as reference molecules.

initial list of 17 Hsp90 interactors (Table 3) to 81 (for MACCS) and 113 (for GpiDAPH3) (Table S1), with an overlap of 77 targets. We also note that the ligand-based virtual screening calculations also yielded compounds at high ranks with additional target annotations outside of the Hsp90 interactome. Interestingly, the lists of 81 and 113 interactors include many validated targets for cancer therapy. ER-α, the top-ranked protein based on the number of known inhibitors shared with Hsp90 (for

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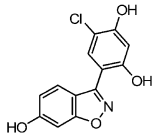
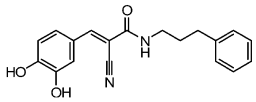
MACCS), is a nuclear hormone receptor involved in breast cancer that acts as a transcription factor.[54] Another transcription factor of interest in breast cancer emerging from our analysis is the glucocorticoid receptor.[55] Moreover, several kinases such as B-raf, SRC, ErbB2, and mTor were identified.[6,9] Notably, drug combinations of Hsp90 and kinase inhibitors are under evaluation in clinical trials with promising results.[56,57]

Docking of Known Multitarget Ligands. One of the interesting features emerging from the ligand-based screening was that the list of Hsp90 interactors sharing known ligands with Hsp90 (Table 3) comprised proteins characterized by low structural similarity with Hsp90. Under these circumstances, the identification and optimization of compounds with dual activity is generally deemed to be difficult.[5] However, local binding site similarities may be more important than global structural similarity to determine dual activity, especially when ligands are able to interact with key residues of both proteins. Indeed, the integration of data from protein−ligand interaction profiles has only recently come into focus, especially in the study of polypharmacology.[58] In this context, recognizing similarities of protein−ligand interaction patterns between compounds and multiple targets may help to validate target combinations and highlight common interaction patterns useful for obtaining multitarget drugs. Therefore, an analysis of the interactions established by known dual inhibitors in different binding sites can help rationalize the observed dual activity and provide useful information to direct multitarget drug design efforts. To investigate this aspect, we performed a comparative docking analysis of compounds with dual activity on two top-scoring target combinations of Table 3. We selected the estrogen receptor (both subtypes α and β) as a member of the nuclear hormone family of intracellular receptors and ErbB2 as a representative example of a kinase. ER-α,[54] ER-β,[59] and ErbB2[60,61] are relevant targets for cancer therapy.

For each target combination, the compound with the best average activities (expressed as IC_{50} values) between Hsp90 and the second target was docked as described in Methods. The structures and activities of the two compounds are reported in Table 6. Docking was performed on two different conformations

Table 6. Dual Hsp90/ER(α and β) and Hsp90/ErbB2 Inhibitors for Docking Analysis[a]

Cmpd	CHEMBL ID	Structure	IC_{50} (nM)	
			Hsp90	Hsp90 interactor
1	CHEMBL362307		60	33 (ER-α) 2 (ER-β)
2	CHEMBL53898		19122	34673 (ErbB2)

[a]Compounds and activity annotations were retrieved from the ChEMBL database. The ChEMBL ID is reported together with IC_{50} values (nM) for each of the two compounds active against Hsp90 and ER-α, ER-β, or ErbB2.

of Hsp90 bound to different cocrystallized ligands in the presence of three conserved buried water molecules that participate in a hydrogen-bonding network with these ligands. The use of two different protein conformations partly accounted for the binding site flexibility,[62] especially in Hsp90, where the binding of different ligands is accompanied by pronounced induced fit effects.[63] The predicted binding modes of compounds **1** and **2** are shown in Figure 3, and protein−ligand interaction diagrams generated from the predicted poses are shown in Figure 4 for comparison.

In the modeled complex, compound **1** binds Hsp90 through an extended network of hydrogen bonds (Figures 3A and 4A): the hydroxyl groups of the resorcinol moiety interact with Asp93 and a conserved water molecule; the nitrogen of the benzisoxazole establishes a water-mediated interaction with Asp93; and the hydroxyl group of the benzisoxazole interacts with Lys58. These interactions resemble those established by most Hsp90 inhibitors in currently available crystal structures of Hsp90 complexes, especially for the interaction with Asp93, which is widely conserved and known to be crucial for inhibitory activity.[37,38,45−48] Furthermore, the predicted binding mode is almost identical to that in the crystal structure of Hsp90 in complex with compound **3** (Table 7; PDB code 3BM9),[31] a close analogue of compound **1**.

In ER-α, the two resorcinol hydroxyls of compound **1** interact with His524 and the backbone carbonyl of Gly521, and the benzisoxazole hydroxyl interacts with Glu353 and Arg394 (Figures 3B and 4B). Hydrogen bonds with Glu353, Arg394, and His524 are reported in the literature to be important for inhibitory activity, as compounds lacking groups able to establish these interactions generally are inactive or poorly active on ER-α.[32,49−51] The binding mode of compound **1** in ER-β (data not shown) is almost identical to the one described for ER-α, in agreement with the high structural similarity of the two isoforms. The predicted binding mode of compound **1** in ER-α and -β is almost identical to that of the close analogue **4** cocrystallized with ER-β (PDB code 1U3Q).[32] Figure 4C summarizes the portions of the ligand that form similar interactions within the two active sites. Three regions of the binding sites are likely to form hydrogen bonds with the three hydroxyl groups of compound **1** in both Hsp90 and ER-α (marked in magenta in Figure 4C). Moreover, an extended hydrophobic region was found in both targets (marked in green). Finally, the docked ligand adopted almost identical conformations in the binding sites of the two targets.

Compound **2** was predicted to bind Hsp90 through hydrogen bonds with Asp93 and a conserved water molecule via the catechol hydroxyls, with Lys58 via the cyano group, and with Asn106 via the amide carbonyl (Figures 3C and 4D). As for the interaction of compound **2** with ErbB2, one of the two hydroxyls of the catechol forms a hydrogen bond with the backbone amino group of Met801 of the hinge (Figures 3D and 4E), which is a conserved interaction known to be of key importance for inhibitory activity on ErbB2[40] and kinases in general. The nitrogen of the amide hydrogen bonds with Thr862. Moreover, both compounds make hydrophobic contacts with a number of apolar residues of the binding sites (Figures 3 and 4). Comparison of the two binding modes (Figure 4F) shows that the catechol hydroxyls and the amide establish important hydrogen bonds with both Hsp90 and ErbB2 and that the phenyl ring is involved in hydrophobic contacts with both targets.

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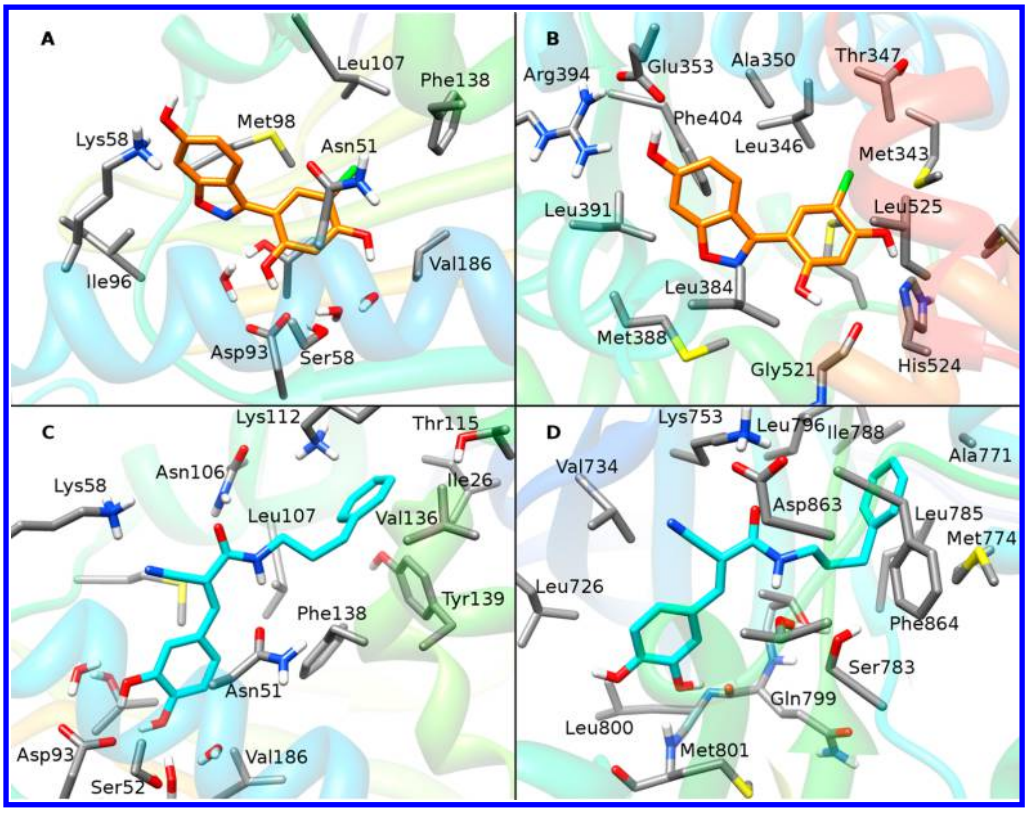

Figure 3. Predicted binding modes of the multitarget compounds **1** and **2**. The figure shows the binding modes of compound **1** (orange) in Hsp90 (panel A) and ER-α (panel B) and those of compound **2** (cyan) in Hsp90 (panel C) and ErbB2 (panel D).

The feasibility of dual-inhibitor design was further analyzed in a comparative SAR analysis carried out on a small series of analogues of compound **1**, focusing on the top-ranking Hsp90 and ER-α target combination. For consistency, analogues were taken from the same articles reporting activities of compound **1** on these targets.[31,32] Six compounds were chosen (as described in Methods) on the basis of the availability of dual-activity annotations or structural modifications of the hydroxyl groups involved in hydrogen bonds with Hsp90 and ER-α. Compounds **1**, **3**, and **4** suggest that a halogen substituent on the resorcinol is important for Hsp90 activity but dispensable for ER-α activity. Compounds **5**, **6**, and **7** (Table 7) alternately lack each of the three hydroxyl groups discussed above. The significantly reduced ER-α inhibitory activities of these compounds with respect to **1** confirm that the hydrogen bonds established with ER-α are indeed important. Unfortunately, activity data are not available for Hsp90. In compound **8**, the three hydroxyls are replaced with methoxy groups. The lack of Hsp90 inhibitory activity of this compound confirms that hydrogen-bond donors provided by the three hydroxyl groups are important for interaction with the target, as predicted in Figures 3 and 4. This finding is consistent with the recognized importance of hydrogen bonds for Hsp90 activity.[37,38,45−48] In general, four cases can be distinguished: the substituent/scaffold is (1) important for both targets, (2) important for one target but dispensable for the other, (3) important for one target but detrimental for the other, or (4) dispensable for both targets. The SAR results described above suggest that the hydroxyl and the halogen substituents belong to cases 1 and 2, respectively, both of which are beneficial for generating ligands with dual activity. Hence, structural features of the ligand that are important for dual activity on the studied targets were identified. This information has the potential to be exploited for dual-activity optimization efforts.

Overall, in both the Hsp90/ER (α and β) and Hsp90/ErbB2 target combinations, despite an inherent difference in the architectures of the two binding sites, common protein−ligand interaction motifs could be identified that may provide a fertile ground for multitarget drug design and dual-activity optimization efforts.

DISCUSSION

The computational polypharmacology approach introduced in this work is aimed at exploring compound activity annotations to discover and prioritize target combinations for multitarget drug design. Target selection is based on the search for protein combinations that share structurally related inhibitors. For Hsp90, from a wide ensemble of potential target combinations (Hsp90 with more than 200 members of the Hsp90 interactome), 11 of these were identified as promising targets for dual-activity approaches. The list was further expanded to 81 targets by including compounds structurally similar to Hsp90 inhibitors but not yet confirmed by biological testing. The similarity ensemble approach (SEA) method introduced by Keiser et al.[12] is based on similar principles but makes use of different techniques. SEA relies on Tanimoto coefficient[64] calculations on ligand pairs normalized by using a statistical model to evaluate the significance of computed similarities. The similarity of sets of known inhibitors was evaluated to discover off-target activities[12] and to assess the "target bias" of a commercial compound library toward a target of interest by evaluating the enrichment of chemical structures similar to known active compounds.[65] Our method, on the other hand, relies on ligand-based virtual screening (in particular SS and

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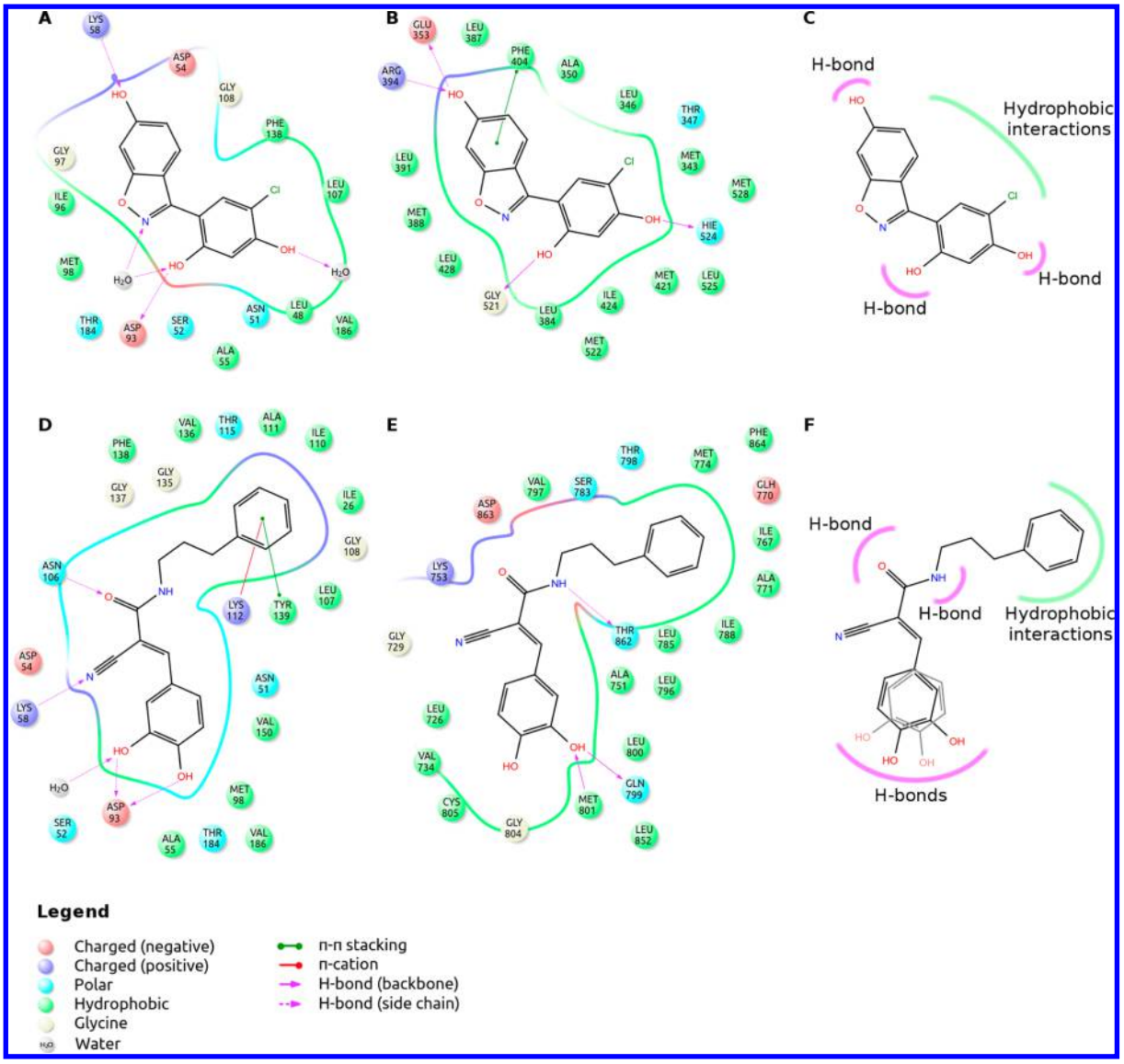

Figure 4. Protein–ligand interaction diagrams generated with Maestro. The sketches illustrate putative hydrogen-bonding and hydrophobic interactions of compound **1** with Hsp90 (panel A) and ER-α (panel B) and of compound **2** with Hsp90 (panel D) and ErbB2 (panel E). Panels C and F summarize the portions of ligands **1** and **2** that form similar interactions with the two active sites. Regions involved in hydrogen-bonding and hydrophobic interactions are colored in magenta and green, respectively. Panel F shows two alternative orientations of the catechol ring of compound **2**, one in complex with Hsp90 and the other with ErbB2. The docked conformation of compound **1** was almost identical in the two targets (Panel C).

SVM) to rank prioritized target combinations from a pool of candidate proteins and on structure-based methods to analyze and validate target combinations that emerge from the ligand-based analyses. Promising target combinations within the Hsp90 interactome and structural features able to make up for similar interaction motifs of known multitarget ligands with different binding sites were thus identified. In this respect, assessment and comparison of protein–ligand interaction maps may help in designing multitarget ligands when the structural similarity of the two targets is not self-evident. We checked for such similarities by docking compounds with known multitarget activity (compounds **1** and **2** in Table 6) in the binding sites of Hsp90 and selected top-ranking Hsp90 interactors, namely, ER-α, ER-β, and ErbB2. Comparison of the predicted binding modes and protein–ligand interaction maps indicated that comparable hydrogen-bonding features and hydrophobic contacts could be established with both targets, suggesting that structure-based multitarget drug design on these target combinations is viable. Furthermore, a comparative SAR analysis was carried out to further probe the importance of the interactions observed between compound **1** and Hsp90/ER-α in the docking study. The results of the analysis confirmed that dual-inhibitor design on this target combination is feasible.

■ CONCLUSIONS

In this work we have described a new computational protocol for the identification of promising target combinations for polypharmacology approaches. The method was applied to the Hsp90 interactome. An extensive analysis of activity annotations available in public molecular databases for a large ensemble of targets was performed. Twenty-six known compounds with an experimentally validated multitarget activity profile were identified, inhibiting Hsp90 and one or more targets within a set of 17 Hsp90 interactors. Approximately 80 target combinations for multitarget inhibitor design were selected through ligand-based analysis of known inhibitors available in

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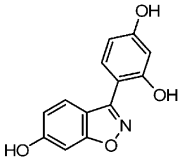
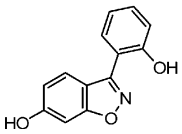
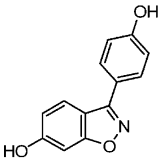
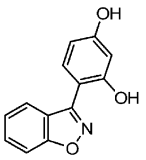
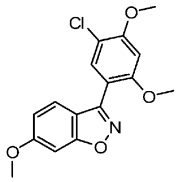
Table 7. ERα and Hsp90 Inhibitors Used for SAR Analysis[a]

Cmpd	CHEMBL ID	Structure	IC_{50} (nM)	
			Hsp90	ER-α
3	CHEMBL361078		190	30
4	CHEMBL360410		15200	24
5	CHEMBL188619			3000
6	CHEMBL188882			815
7	CHEMBL365293			6100
8	CHEMBL254874		>2000	

[a]Compounds and activity annotations were retrieved from the ChEMBL database. The ChEMBL ID is reported together with IC_{50} values (nM).

ChEMBL. Selected target combinations were analyzed with structure-based methods (Hsp90/ER (α and β) and Hsp90/ErbB2). These studies showed that compounds with dual activity were able to establish similar ligand−protein interactions with targets characterized by low binding site similarities. The Hsp90/ER-α combination was further analyzed through a comparative SAR analysis using analogues of compound **1**. Structural features important for activity on both targets were identified, suggesting that dual-inhibitor design is feasible. Overall, the results may provide useful information for polypharmacology approaches within the Hsp90 interactome. This approach can be applied to any ensemble of proteins of interest for which known inhibitors and structural information are available.

■ ASSOCIATED CONTENT

Supporting Information

Table S1 reporting the complete list of Hsp90 interactors resulting from the ligand-based virtual screening of the ChEMBL database and the numbers of compounds with activity annotations. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED:

Hsp90, heat shock protein 90; ER, estrogen receptor; ErbB2, receptor tyrosine-protein kinase ErbB2; SS, similarity searching; SVM, support vector machine; NN, nearest neighbor; PDB, Protein Data Bank; SEA, similarity ensemble approach

■ REFERENCES

(1) Hopkins, A. L. Network Pharmacology: The Next Paradigm in Drug Discovery. *Nat. Chem. Biol.* **2008**, *4*, 682−690.
(2) Mestres, J.; Gregori-Puigjané, E. Conciliating Binding Efficiency and Polypharmacology. *Trends Pharmacol. Sci.* **2009**, *30*, 470−474.
(3) Boran, A. D. W.; Iyengar, R. Systems Approaches to Polypharmacology and Drug Discovery. *Curr. Opin. Drug Discovery Dev.* **2010**, *13*, 297−309.
(4) Peters, J. U. Polypharmacology—Foe or Friend? *J. Med. Chem.* **2013**, *56*, 8955−8971.
(5) Anighoro, A.; Bajorath, J.; Rastelli, G. Polypharmacology: Challenges and Opportunities in Drug Discovery. *J. Med. Chem.* **2014**, *57*, 7874−7887.
(6) Knight, Z. A.; Lin, H.; Shokat, K. M. Targeting the Cancer Kinome through Polypharmacology. *Nat. Rev. Cancer* **2010**, *10*, 130−137.
(7) Roth, B. L.; Sheffler, D. J.; Kroeze, W. K. Magic Shotguns versus Magic Bullets: Selectively Non-Selective Drugs for Mood Disorders and Schizophrenia. *Nat. Rev. Drug Discovery* **2004**, *3*, 353−359.
(8) Youdim, M. B. H.; Buccafusco, J. J. Multi-Functional Drugs for Various CNS Targets in the Treatment of Neurodegenerative Disorders. *Trends Pharmacol. Sci.* **2005**, *26*, 27−35.
(9) Zhang, J.; Yang, P. L.; Gray, N. S. Targeting Cancer with Small Molecule Kinase Inhibitors. *Nat. Rev. Cancer* **2009**, *9*, 28−39.
(10) Giacomini, K. M.; Krauss, R. M.; Roden, D. M.; Eichelbaum, M.; Hayden, M. R.; Nakamura, Y. When Good Drugs Go Bad. *Nature* **2007**, *446*, 975−977.
(11) Morphy, R.; Rankovic, Z. Designed Multiple Ligands. An Emerging Drug Discovery Paradigm. *J. Med. Chem.* **2005**, *48*, 6523−6543.
(12) Keiser, M. J.; Roth, B. L.; Armbruster, B. N.; Ernsberger, P.; Irwin, J. J.; Shoichet, B. K. Relating Protein Pharmacology by Ligand Chemistry. *Nat. Biotechnol.* **2007**, *25*, 197−206.
(13) Apsel, B.; Blair, J. A.; Gonzalez, B.; Nazif, T. M.; Feldman, M. E.; Aizenstein, B.; Hoffman, R.; Williams, R. L.; Shokat, K. M.; Knight, Z. A. Targeted Polypharmacology: Discovery of Dual Inhibitors of Tyrosine and Phosphoinositide Kinases. *Nat. Chem. Biol.* **2008**, *4*, 691−699.
(14) Besnard, J.; Ruda, G. F.; Setola, V.; Abecassis, K.; Rodriguiz, R. M.; Huang, X.-P.; Norval, S.; Sassano, M. F.; Shin, A. I.; Webster, L. A.; Simeons, F. R. C.; Stojanovski, L.; Prat, A.; Seidah, N. G.; Constam, D. B.; Bickerton, G. R.; Read, K. D.; Wetsel, W. C.; Gilbert, I. H.; Roth, B. L.; Hopkins, A. L. Automated Design of Ligands to Polypharmacological Profiles. *Nature* **2012**, *492*, 215−220.
(15) Reker, D.; Rodrigues, T.; Schneider, P.; Schneider, G. Identifying the Macromolecular Targets of de Novo-Designed Chemical Entities through Self-Organizing Map Consensus. *Proc. Natl. Acad. Sci. U.S.A.* **2014**, *111*, 4067−4072.
(16) Echeverría, P. C.; Bernthaler, A.; Dupuis, P.; Mayer, B.; Picard, D. An Interaction Network Predicted from Public Data as a Discovery Tool: Application to the Hsp90 Molecular Chaperone Machine. *PLoS One* **2011**, *6*, No. e26044.
(17) Sgobba, M.; Rastelli, G. Structure-Based and in Silico Design of Hsp90 Inhibitors. *ChemMedChem* **2009**, *4*, 1399−1409.
(18) Neckers, L.; Workman, P. Hsp90 Molecular Chaperone Inhibitors: Are We There Yet? *Clin. Cancer Res.* **2012**, *18*, 64−76.
(19) Samuni, Y.; Ishii, H.; Hyodo, F.; Samuni, U.; Krishna, M. C.; Goldstein, S.; Mitchell, J. B. Reactive Oxygen Species Mediate Hepatotoxicity Induced by the Hsp90 Inhibiting Anti-Cancer Geldanamycin and Its Analogs. *Free Radical Biol. Med.* **2010**, *48*, 1559−1563.
(20) Wright, J. J. Combination Therapy of Bortezomib with Novel Targeted Agents: An Emerging Treatment Strategy. *Clin. Cancer Res.* **2010**, *16*, 4094−4104.
(21) Hu, Y.; Bajorath, J. Growth of Ligand−Target Interaction Data in ChEMBL Is Associated with Increasing and Activity Measurement-Dependent Compound Promiscuity. *J. Chem. Inf. Model.* **2012**, *52*, 2550−2558.
(22) Gaulton, A.; Bellis, L. J.; Bento, A. P.; Chambers, J.; Davies, M.; Hersey, A.; Light, Y.; McGlinchey, S.; Michalovich, D.; Al-Lazikani, B.; Overington, J. P. ChEMBL: A Large-Scale Bioactivity Database for Drug Discovery. *Nucleic Acids Res.* **2012**, *40*, D1100−D1107.
(23) Liu, T.; Lin, Y.; Wen, X.; Jorissen, R. N.; Gilson, M. K. BindingDB: A Web-Accessible Database of Experimentally Determined Protein−Ligand Binding Affinities. *Nucleic Acids Res.* **2007**, *35*, D198−D201 (www.bindingdb.org, accessed April 2012).
(24) Berthold, M. R.; Cebron, N.; Dill, F.; Gabriel, T. R.; Kötter, T.; Meinl, T.; Ohl, P.; Sieb, C.; Thiel, K.; Wiswedel, B. KNIME: The Konstanz Information Miner. In *Data Analysis, Machine Learning and Applications*; Preisach, C., Burkhardt, H., Schmidt-Thieme, L., Decker, R., Eds.; Studies in Classification, Data Analysis, and Knowledge Organization; Springer: Berlin, 2008; pp 319−326.
(25) Picard Lab Downloads Page. http://www.picard.ch/downloads (accessed February 2012).
(26) Magrane, M.; UniProt Consortium. UniProt Knowledgebase: A Hub of Integrated Protein Data. *Database* **2011**, No. bar009.
(27) Durant, J. L.; Leland, B. A.; Henry, D. R.; Nourse, J. G. Reoptimization of MDL Keys for Use in Drug Discovery. *J. Chem. Inf. Comput. Sci.* **2002**, *42*, 1273−1280.
(28) Chemical Computing Group Inc.. Molecular Operating Environment (MOE). *Sci. Comput. Instrum.* **2004**, *22*, 32.
(29) Cortes, C.; Vapnik, V. Support Vector Networks. *Mach. Learn.* **1995**, *20*, 273−297.
(30) Irwin, J. J.; Sterling, T.; Mysinger, M. M.; Bolstad, E. S.; Coleman, R. G. ZINC: A Free Tool To Discover Chemistry for Biology. *J. Chem. Inf. Model.* **2012**, *52*, 1757−1768.
(31) Gopalsamy, A.; Shi, M.; Golas, J.; Vogan, E.; Jacob, J.; Johnson, M.; Lee, F.; Nilakantan, R.; Petersen, R.; Svenson, K.; Chopra, R.; Tam, M. S.; Wen, Y.; Ellingboe, J.; Arndt, K.; Boschelli, F. Discovery of Benzisoxazoles as Potent Inhibitors of Chaperone Heat Shock Protein 90. *J. Med. Chem.* **2008**, *51*, 373−375.
(32) Malamas, M. S.; Manas, E. S.; McDevitt, R. E.; Gunawan, I.; Xu, Z. B.; Collini, M. D.; Miller, C. P.; Dinh, T.; Henderson, R. A.; Keith, J. C.; Harris, H. A. Design and Synthesis of Aryl Diphenolic Azoles as Potent and Selective Estrogen Receptor-β Ligands. *J. Med. Chem.* **2004**, *47*, 5021−5040.
(33) National Center for Biotechnology Information. PubChem BioAssay Database. HTS for Tumor Hsp90 Inhibitors: AID=429, Source=Emory University Molecular Libraries Screening Center. http://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=429 (accessed May 2012).
(34) Du, Y.; Moulick, K.; Rodina, A.; Aguirre, J.; Felts, S.; Dingledine, R.; Fu, H.; Chiosis, G. High-Throughput Screening Fluorescence Polarization Assay for Tumor-Specific Hsp90. *J. Biomol. Screening* **2007**, *12*, 915−924.
(35) Gazit, A.; Osherov, N.; Posner, I.; Yaish, P.; Poradosu, E.; Gilon, C.; Levitzki, A. Tyrphostins. 2. Heterocyclic and α-Substituted Benzylidenemalononitrile Tyrphostins as Potent Inhibitors of EGF Receptor and ErbB2/neu Tyrosine Kinases. *J. Med. Chem.* **1991**, *34*, 1896−1907.
(36) *Schrödinger*, release 2014-1; Schrödinger, LLC: New York, 2014.
(37) Jez, J. M.; Chen, J. C.-H.; Rastelli, G.; Stroud, R. M.; Santi, D. V. Crystal Structure and Molecular Modeling of 17-DMAG in Complex with Human Hsp90. *Chem. Biol.* **2003**, *10*, 361−368.
(38) Brough, P. A.; Aherne, W.; Barril, X.; Borgognoni, J.; Boxall, K.; Cansfield, J. E.; Cheung, K.-M. J.; Collins, I.; Davies, N. G. M.; Drysdale, M. J.; Dymock, B.; Eccles, S. A.; Finch, H.; Fink, A.; Hayes, A.; Howes, R.; Hubbard, R. E.; James, K.; Jordan, A. M.; Lockie, A.; Martins, V.;

DOI: 10.1021/ci5006959
J. Chem. Inf. Model. 2015, 55, 676−686

Massey, A.; Matthews, T. P.; McDonald, E.; Northfield, C. J.; Pearl, L. H.; Prodromou, C.; Ray, S.; Raynaud, F. I.; Roughley, S. D.; Sharp, S. Y.; Surgenor, A.; Walmsley, D. L.; Webb, P.; Wood, M.; Workman, P.; Wright, L. 4,5-Diarylisoxazole Hsp90 Chaperone Inhibitors: Potential Therapeutic Agents for the Treatment of Cancer. *J. Med. Chem.* **2008**, *51*, 196−218.
(39) Manas, E. S.; Unwalla, R. J.; Xu, Z. B.; Malamas, M. S.; Miller, C. P.; Harris, H. A.; Hsiao, C.; Akopian, T.; Hum, W.-T.; Malakian, K.; Wolfrom, S.; Bapat, A.; Bhat, R. A.; Stahl, M. L.; Somers, W. S.; Alvarez, J. C. Structure-Based Design of Estrogen Receptor-β Selective Ligands. *J. Am. Chem. Soc.* **2004**, *126*, 15106−15119.
(40) Aertgeerts, K.; Skene, R.; Yano, J.; Sang, B.-C.; Zou, H.; Snell, G.; Jennings, A.; Iwamoto, K.; Habuka, N.; Hirokawa, A.; Ishikawa, T.; Tanaka, T.; Miki, H.; Ohta, Y.; Sogabe, S. Structural Analysis of the Mechanism of Inhibition and Allosteric Activation of the Kinase Domain of HER2 Protein. *J. Biol. Chem.* **2011**, *286*, 18756−18765.
(41) Sastry, G. M.; Adzhigirey, M.; Day, T.; Annabhimoju, R.; Sherman, W. Protein and Ligand Preparation: Parameters, Protocols, and Influence on Virtual Screening Enrichments. *J. Comput.-Aided Mol. Des.* **2013**, *27*, 221−234.
(42) Rastelli, G.; Tian, Z. Q.; Wang, Z.; Myles, D.; Liu, Y. Structure-Based Design of 7-Carbamate Analogs of Geldanamycin. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5016−5021.
(43) Yan, A.; Grant, G. H.; Richards, W. G. Dynamics of Conserved Waters in Human Hsp90: Implications for Drug Design. *J. R. Soc., Interface* **2008**, *5*, 199−205.
(44) Sherman, W.; Day, T.; Jacobson, M. P.; Friesner, R. A.; Farid, R. Novel Procedure for Modeling Ligand/Receptor Induced Fit Effects. *J. Med. Chem.* **2006**, *49*, 534−553.
(45) Prodromou, C.; Roe, S. M.; O'Brien, R.; Ladbury, J. E.; Piper, P. W.; Pearl, L. H. Identification and Structural Characterization of the ATP/ADP-Binding Site in the Hsp90 Molecular Chaperone. *Cell* **1997**, *90*, 65−75.
(46) Stebbins, C. E.; Russo, A. A.; Schneider, C.; Rosen, N.; Hartl, F. U.; Pavletich, N. P. Crystal Structure of an Hsp90−Geldanamycin Complex: Targeting of a Protein Chaperone by an Antitumor Agent. *Cell* **1997**, *89*, 239−250.
(47) Roe, S. M.; Prodromou, C.; O'Brien, R.; Ladbury, J. E.; Piper, P. W.; Pearl, L. H. Structural Basis for Inhibition of the Hsp90 Molecular Chaperone by the Antitumor Antibiotics Radicicol and Geldanamycin. *J. Med. Chem.* **1999**, *42*, 260−266.
(48) Dymock, B.; Barril, X.; Beswick, M.; Collier, A.; Davies, N.; Drysdale, M.; Fink, A.; Fromont, C.; Hubbard, R. E.; Massey, A.; Surgenor, A.; Wright, L. Adenine Derived Inhibitors of the Molecular Chaperone HSP90-SAR Explained through Multiple X-ray Structures. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 325−328.
(49) Brzozowski, A. M.; Pike, A. C. W.; Dauter, Z.; Hubbard, R. E.; Bonn, T.; Engström, O.; Öhman, L.; Greene, G. L.; Gustafsson, J.-Å.; Carlquist, M. Molecular Basis of Agonism and Antagonism in the Oestrogen Receptor. *Nature* **1997**, *389*, 753−758.
(50) Shiau, A. K.; Barstad, D.; Loria, P. M.; Cheng, L.; Kushner, P. J.; Agard, D. A.; Greene, G. L. The Structural Basis of Estrogen Receptor/Coactivator Recognition and the Antagonism of This Interaction by Tamoxifen. *Cell* **1998**, *95*, 927−937.
(51) Manas, E. S.; Xu, Z. B.; Unwalla, R. J.; Somers, W. S. Understanding the Selectivity of Genistein for Human Estrogen Receptor-β Using X-ray Crystallography and Computational Methods. *Structure* **2004**, *12*, 2197−2207.
(52) Still, W. C.; Tempczyk, A.; Hawley, R. C.; Hendrickson, T. Semianalytical Treatment of Solvation for Molecular Mechanics and Dynamics. *J. Am. Chem. Soc.* **1990**, *112*, 6127−6129.
(53) Pettersen, E. F.; Goddard, T. D.; Huang, C. C.; Couch, G. S.; Greenblatt, D. M.; Meng, E. C.; Ferrin, T. E. UCSF Chimera—A Visualization System for Exploratory Research and Analysis. *J. Comput. Chem.* **2004**, *25*, 1605−1612.
(54) Sommer, S.; Fuqua, S. A. Estrogen Receptor and Breast Cancer. *Semin. Cancer Biol.* **2001**, *11*, 339−352.
(55) Moutsatsou, P.; Papavassiliou, A. G. The Glucocorticoid Receptor Signalling in Breast Cancer. *J. Cell. Mol. Med.* **2008**, *12*, 145−163.
(56) Lu, X.; Xiao, L.; Wang, L.; Ruden, D. M. Hsp90 Inhibitors and Drug Resistance in Cancer: The Potential Benefits of Combination Therapies of Hsp90 Inhibitors and Other Anti-Cancer Drugs. *Biochem. Pharmacol.* **2012**, *83*, 995−1004.
(57) Modi, S.; Stopeck, A.; Linden, H.; Solit, D.; Chandarlapaty, S.; Rosen, N.; D'Andrea, G.; Dickler, M.; Moynahan, M. E.; Sugarman, S.; Ma, W.; Patil, S.; Norton, L.; Hannah, A. L.; Hudis, C. HSP90 Inhibition Is Effective in Breast Cancer: A Phase II Trial of Tanespimycin (17-AAG) Plus Trastuzumab in Patients with HER2-Positive Metastatic Breast Cancer Progressing on Trastuzumab. *Clin. Cancer Res.* **2011**, *17*, 5132−5139.
(58) Salentin, S.; Haupt, V. J.; Daminelli, S.; Schroeder, M. Polypharmacology Rescored: Protein−Ligand Interaction Profiles for Remote Binding Site Similarity Assessment. *Prog. Biophys. Mol. Biol.* **2014**, *116*, 174−186.
(59) Palmieri, C.; Cheng, G. J.; Saji, S.; Zelada-Hedman, M.; Wärri, A.; Weihua, Z.; Van Noorden, S.; Wahlstrom, T.; Coombes, R. C.; Warner, M.; Gustafsson, J.-Å. Estrogen Receptor Beta in Breast Cancer. *Endocr.-Relat. Cancer* **2002**, *9*, 1−13.
(60) Yu, D.; Hung, M. C. Overexpression of ErbB2 in Cancer and ErbB2-Targeting Strategies. *Oncogene* **2000**, *19*, 6115−6121.
(61) Badache, A.; Gonçalves, A. The ErbB2 Signaling Network as a Target for Breast Cancer Therapy. *J. Mammary Gland Biol. Neoplasia* **2006**, *11*, 13−25.
(62) Sgobba, M.; Caporuscio, F.; Anighoro, A.; Portioli, C.; Rastelli, G. Application of a Post-Docking Procedure Based on MM-PBSA and MM-GBSA on Single and Multiple Protein Conformations. *Eur. J. Med. Chem.* **2012**, *58*, 431−440.
(63) Patel, H. J.; Modi, S.; Chiosis, G.; Taldone, T. Advances in the Discovery and Development of Heat-Shock Protein 90 Inhibitors for Cancer Treatment. *Expert Opin. Drug Discovery* **2011**, *6*, 559−587.
(64) Willett, P. Searching Techniques for Databases of Two- and Three-Dimensional Chemical Structures. *J. Med. Chem.* **2005**, *48*, 4183−4199.
(65) Carlsson, J.; Yoo, L.; Gao, Z.-G.; Irwin, J. J.; Shoichet, B. K.; Jacobson, K. A. Structure-Based Discovery of A2A Adenosine Receptor Ligands. *J. Med. Chem.* **2010**, *53*, 3748−3755.

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