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*Commentary*

# *In-Silico* Approaches to Multi-target Drug Discovery

## Computer Aided Multi-target Drug Design, Multi-target Virtual Screening

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***Abstract.*** Multi-target drugs against selective multiple targets improve therapeutic efficacy, safety and resistance profiles by collective regulations of a primary therapeutic target together with compensatory elements and resistance activities. Efforts have been made to employ *in-silico* methods for facilitating the search and design of selective multi-target agents. These methods have shown promising potential in facilitating drug discovery directed at selective multiple targets.

**KEY WORDS:** computer aided dug design; multiple ligands; multi-target; multi-target drug discovery; virtual screening.

### MULTI-TARGET THERAPEUTICS

Therapeutic agents directed at an individual target frequently show reduced efficacies, undesired safety profiles and drug resistances due to network robustness (1), redundancy (2), crosstalk (3), compensatory and neutralizing actions (4), anti-target and counter-target activities (5), and on-target and off-target toxicities (6). Multi-target agents directed at selected multiple targets have been increasingly explored (1,7) for achieving enhanced therapeutic efficacies, improved safety profiles, and reduced resistance activities by simultaneously modulating the activity of a primary therapeutic target and the counteractive elements and resistance activities (8) while limiting unwanted cross-reactivities via optimization of target selectivity (9).

Examples of clinically successful multi-target drugs are anticancer kinase inhibitors sunitinib against PDGFR and VEGFR, dasatinib against Abl and Src, and lapatinib against EGFR and HER2 (10,11). These multi-target anticancer agents inhibit a primary therapeutic target that promotes tumor growth in a specific cancer patient group and block the alternative signalling or escape mechanism (4,12,13). Fig. 1 illustrates an example of alternative signalling in response to EGFR inhibition. EGFR inhibition in some cases leads to enhanced HER2-HER4 and HER2-HER3 heterodimerization to alternatively activate MAPK and AKT signalling for promoting proliferation and survival independent of EGFR (14). This alternative signalling route cannot be blocked by an EGFR inhibitor alone, but may be blocked by an EGFR-HER2 dual-inhibitor such as lapatinib.

Multi-target antidepressant drugs achieve enhanced efficacies by at least two mechanisms. One mechanism, represented by clomipramine, duloxetine and imipramine, involves inhibition of multiple monoamine reuptakes (15). Simultaneous blockade of complementary monoamine reuptake routes synergistically enhances the overall therapeutic efficacy (16). Monoamines in CNS are reduced via monoamine reuptake (17) and COMT- and MAO-mediated catabolism (18). Inhibition of one mechanism may elevate the compensatory activity of another. For instance, COMT inhibition shifts levodopa metabolism toward the MAO-B-dependent oxidative pathway (19). Therefore, inhibition of one monoamine reduction route is complemented by the inhibition of the other routes to reduce their compensatory activities, which leads to therapeutic synergy. The second mechanism involves collective monoamine reuptake inhibition and receptor antagonism. For instance, A-80426 both inhibits serotonin reuptake and antagonizes α2-adrenoceptor (20). Blockade of α2-adrenoceptor leads to increased serotonin levels (21) to complement the inhibition of serotonin reuptakes, which is a typical mode of synergistic therapeutic action (22).

Table I summarises 17 multi-target drugs approved or in advanced development stages together with information about their targeted diseases, potencies against individual targets and cell-lines, and multi-target mode of action. These drugs target members of the same protein family that regulate the same signalling process at different upstream points, act as alternative signalling molecules, or complement each other in conducting similar functions. The potencies of these drugs against the

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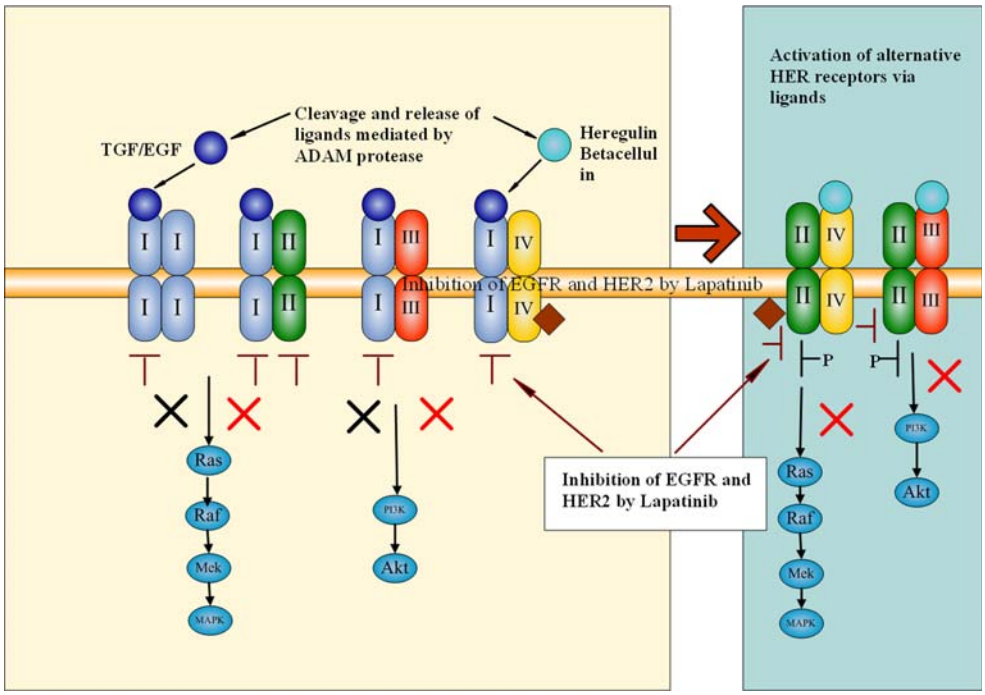


**Fig. 1.** One of the alternative signalling paths in response to EGFR inhibition. EGFR inhibition may lead to the activation of alternative HER receptors via EGFR-HER2, EGFR-HER3, EGFR-HER4, HER2-HER4, and HER2-HER3 heterodimerization. EGFR inhibitors can only block the first four (*pink background*), while proliferation and survival signalling can still proceed via the last two (*blue background*). The use of an EGFR-HER2 dual inhibitor, such as Laptinib, blocks both EGFR and this alternative path.

corresponding multiple targets are mostly in the 1 nM–700 nM range. Multi-target agents directed at proteins of different families have also been reported. Examples are Curoumin against HIV integrase (30 μM) and Tat (<30 μM) and Suramin against HIV gp120 (7.7 μM), integrase (2.4 μM), and reverse transcriptase (1.4 μM) (23). But these are yet to reach advanced development stages. The clinical success of multi-target drugs seems to be partly linked to the ability to achieve high potencies against all of the selected multiple targets. It is possible that most of the multi-target drugs approved or in advanced development stages target members of the same protein family partly because it is relatively easier to design, search and optimize agents of high potencies against multiple proteins of the same family than against proteins of different families.

Two multi-target drugs show better potency against specific cell-lines than against their intended multiple targets. These are JNJ-7925476 against hSERT (0.9 nM), hNET (16 nM), and hDAT (5 nM) (24,25), and CHIR-265/RAF-265 against VEGFR2 (1.3 μM) and BRAF (1.2 μM)(26). A recent study has shown that VEGFR2 recruits and activates c-Src (27); c-Src subsequently associates directly with BRAF and regulates activation of CRAF in some cells to activate MAPK pathway in a Ras-independent manner (28,29). Therefore, VEGFR2-BRAF dual-inhibitors, such as CHIR-265/RAF-265, are expected to show enhanced activity against cell-lines of sufficiently expressed c-Src by partly blocking this Ras-independent signalling route. Moreover, five drugs show comparable potency against specific cell-line(s) with respect to the potencies against their intended multiple targets (less than ten fold difference). These include ABT-869 against VEGFR2 (8.1 nM), FLT3 (0.63 nM), and CSF1R (3.4 nM), AMG-706 against VEGFR (2:26 nM), FLT1 (12 nM), FLT4 (9.7 nM), and KIT (3.7 nM), AST-487 against FLT3 (0.79 nM) and KIT (5.4 nM), Dasatinib against ABL1 (0.53 nM) and Src (0.21 nM), and GW-786034 against VEGFR2 (14 nM), FLT1 (14 nM), FLT4 (27 nM)(26). The therapeutic efficacy of ABL-SRC dual-inhibitor dasatinib is partly due to its additional capability in inhibiting Src-mediated BCR-ABL—independent pathways that are active in imatinib resistant patients (30). FLT1 is frequently co-expressed with VEGFR2 and plays key roles in survival (31,32).

## *IN-SILICO* METHODS FOR SEARCHING AND DESIGNING MULTI-TARGET DRUGS

*In-silico* methods have been widely explored for facilitating lead discovery against individual targets (33,34). In particular, molecular docking (35), pharmacophore (36), structure-activity relationship (SAR) and quantitative structure activity relationship (QSAR) (37), machine learning (38), and combination methods (39) have been extensively used for searching and designing active compounds against individual targets. Some of these methods have recently been explored for searching and designing multi-target agents. Figs. 2, 3, 4, and 5 outline the strategies of using molecular docking, combined molecular docking and pharmacophore, framework combination, and fragment-based approaches for multi-target drug discovery using dual-inhibitor discovery as examples. These methods are classified into combinatorial approaches and fragment–based approaches. Combinatorial approaches (Figs. 2 and 3) straightforwardly conduct parallel searches against each individual target to find virtual hits that simultaneously interact with multiple targets. Combinatorial approaches are practically useful if the retrieval rates against

**Table I.** Reported Multi-target Drugs, Targeted Diseases, Potencies Against Individual Targets and Cell-lines, and Multi-target Mode of Action

| Drug | Targeted disease | Multi-targets and potency against each individual target (IC50, Ki, EC50) | Potency against specific cell line | Multi-target mode of action | Notes |
|---|---|---|---|---|---|
| JNJ-7925476 | Major depression (58) | hSERT: 0.9 nM<br>hNET: 16 nM<br>hDAT: 5 nM (24,25) | JAR cells: 0.3 nM (24) | Inhibiting monoamine transporter family members that perform complementary and compensatory actions on neural activities in synapse | Potency against specific cell-line better than those against individual targets |
| ABT-869 | Solid malignancies (59), acute myeloid leukemia (60) | VEGFR2: 8.1 nM<br>FLT3: 0.63 nM<br>CSF1R: 3.4 nM (26) | CSF-1R cellular: 4 nM (61) | Inhibiting tyrosine kinase receptors that regulate angiogenesis (VEGFR2) and proliferation (Flt3) | Potency against specific cell-line comparable to those against individual targets |
| AMG-706 | Thyroid cancers (62), non-small-cell lung cancer (63) | VEGFR2: 26 nM<br>FLT1: 12 nM<br>FLT4: 9.7 nM<br>KIT: 3.7 nM (26) | HUVEC: 10 nM<br>NHDF: 207 nM<br>MO7e cells: 37 nM | Inhibiting tyrosine kinase receptors that regulate angiogenesis at different upstream points (VEGFR2, FLT1), sequester upstream signal (FLT1), and regulate kinase expression (KIT) | Potency against specific cell-line comparable to those against individual targets |
| AST-487 | Acute myelocytic leukemia (64), thyroid cancer (65) | FLT3: 0.79 nM<br>KIT: 5.4 nM (26) | FLT-ITD-Ba/F3 and D835Y-Ba/F3: <5 nM | Inhibiting tyrosine kinase receptors that regulate survival and growth at different upstream points (FLT3, KIT) | Potency against specific cell-line comprable to those against individual targets |
| CHIR-258/TKI-258 | Multiple myeloma (66), acute myelogenous leukemia (67), colon cancer (68) | FLT3: 0.64 nM<br>FGFR3: 230 nM (26) | WT: 25 nM<br>B9 cells: 25 nM<br>Y373C-expressing KMS11 cell line: 90 nM<br>OPM2 cell line: 90 nM (66) | Inhibiting tyrosine kinase receptors that regulate survival and growth (FLT3), and angiogenesis and tumor progression (FGFR3) | |
| CHIR-265/RAF-265 | Melanoma (69) | VEGFR2: 1300 nM<br>BRAF: 1200 nM (26) | SK-MEL-28, Malme-3 M, and A375M cell lines: 140-300 nM | Inhibiting kinases that regulate angiogenesis (VEGFR2) and proliferation (BRAF), and Src-mediated alternative signalling (BRAF) | Potency against specific cell-lines better than those against individual targets |
| Dasatinib | Myeloid leukemia (70), castration-resistant prostate cancer (71), Ovarian cancer (72) | ABL1: 0.53 nM<br>SRC: 0.21 nM (26) | K562: 1.5 nM<br>BV173: 10.9 nM<br>KU812: 3.5 nM<br>KT-1: 4.2 nM<br>MEG01: 6.3 nM<br>MYL: 6.5 nM<br>K562-IMR: 63.8 nM<br>MYL-R1: 6.4 nM (73)<br>MSTO-211H: 25 nM (74)<br>TF-1: 0.75 nM<br>K562: 1 nM (75) | Inhibiting tyrosine kinases that regulate proliferation at different upstream points and crosstalk to each other that may act as back-up alternative for each other | Potency against specific cell-line comparable to those against individual targets |
| Flavopiridol | Colorectal cancer (76), liver tumour (77), chronic lymphocytic leukemia (78), pancreatic cancer (79) | CDK2: 550 nM<br>CDK9: 6.4 nM<br>CDK5: 110 nM<br>CDK7: 23 nM (26) | SUDHL4 : 120 nM (80)<br>NCI 60 cell line: 66 nM (81) | Inhibiting kinase CDK family members that regulate cell cycle (CDK2), activate CDKs and initiate transcription and | |

**Table I.** (continued)

| Drug | Targeted disease | Multi-targets and potency against each individual target (IC50, Ki, EC50) | Potency against specific cell line | Multi-target mode of action | Notes |
|---|---|---|---|---|---|
| | | | | repair (CDK7) , and act as elongation factor of transcription (CDK9) | |
| GW-786034 | Renal cell carcinoma (82), breast cancer (83) | VEGFR2: 14 nM<br>FLT1: 14 nM<br>FLT4: 27 nM (26) | HUVEC: 21.3 nM (84) | Inhibiting tyrosine kinase receptors that regulate angiogenesis at different upstream points (VEGFR2, FLT1), and sequester upstream signal (FLT1) | Potency against specific cell-line comparable to those against individual targets |
| Imatinib | Chronic myelogenous leukemia (85), gastrointestinal stromal tumor (86) | ABL1: 12 nM<br>KIT: 14 nM<br>PDGFRB: 14 nM (26) | EM3: 100 nM (87) | Inhibiting tyrosine kinases that regulate proliferation at different upstream points | |
| JNJ-7706621 | Melanoma tumour (88) | CDK2: 23 nM<br>AURKB: 160 nM (26) | PC3: 112 nM<br>HCT-116: 189 nM (88) | Inhibiting kinases that regulate cell cycle (CDK2) and mitosis (Aurora) | |
| Lapatinib | Breast cancer (89) | EGFR: 2.4 nM<br>ERBB2: 7 nM (26) | RT112: 1100 nM<br>J82: 1220 nM (90) | Inhibiting tyrosine kinase receptor ERBB family members that regulate proliferation and survival at different upstream points, and act as back-up alternative for each other | |
| MLN-518 | Acute myelogenous leukemia (91) | FLT3: 3 nM<br>KIT: 2.7 nM (26) | D816V-expressing cell lines: 160 nM<br>D814Y-expressing cell lines: 180 nM (92) | Inhibiting tyrosine kinase receptors that regulate proliferation at different upstream points | |
| Roscovitine/CYC-202 | NSCLC | CDK1: 700 nM<br>CDK2: 650 nM<br>CDK5: 160 nM<br>CDK7: 500 nM | A375 cells: <20 μM/L<br>888-Mel: <15 μM/L (93) | Inhibiting kinase CDK family members that regulate cell cycle (CDK2, CDK1), activate CDKs and initiate transcription and repair (CDK7) | |
| Sorafenib | Renal cell carcinoma (26), hepatocellular carcinoma (94) | VEGFR2: 59 nM<br>BRAF: 540 nM (26) | ATC cell lines: 2-6 μM/L (95) | Inhibiting kinases that regulate angiogenesis (VEGFR2) and proliferation (BRAF), and Src-mediated alternative signalling (BRAF) | |
| Sunitinib | Gastrointestinal stromal tumor (96), renal cell carcinoma (97) | VEGFR2: 1.5 nM<br>KIT: 0.37 nM<br>FLT3: 0.47 nM (26) | Kasumi-1: 75.7 nM (35) | Inhibiting tyrosine kinase receptors that regulate angiogenesis (VEGFR2), proliferation (FLT3), and kinase level (KIT) | |
| ZD-6474 | Non-small-cell lung cancer, small-cell lung cancer, myeloma (98) | EGFR: 9.5 nM<br>VEGFR2: 820 nM<br>RET: 34 nM (26) | Lung ECs: 1.25 μM<br>Brain ECs: 2.56 μM<br>MDA-MB-435 cells: 0.89 μM | Inhibiting tyrosine kinase receptors that regulate proliferation at different upstream points (EGFR, RET), and regulate angiogenesis (VEGFR2) | |

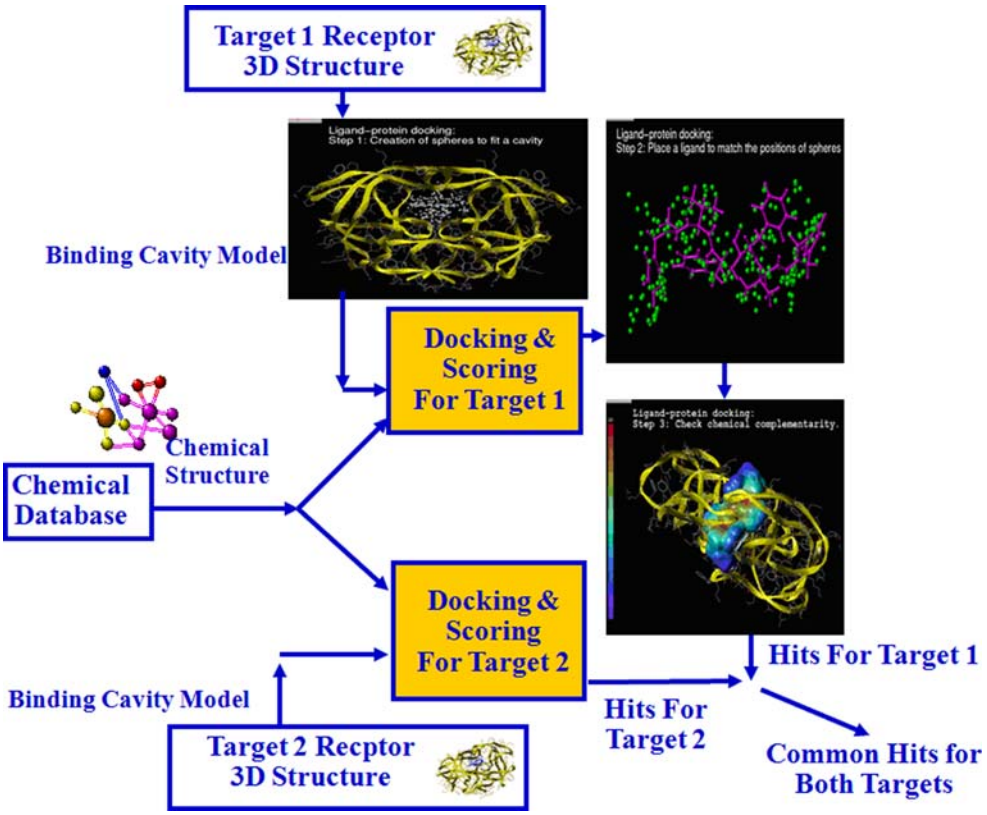


**Fig. 2.** Molecular docking strategy for multi-target inhibitor discovery.

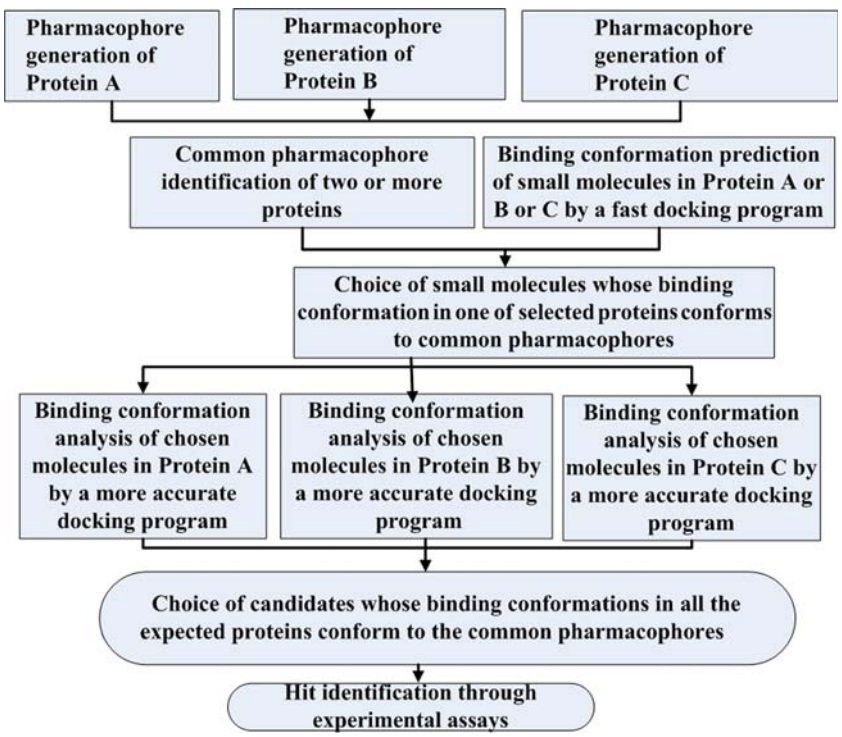


**Fig. 3.** Combined pharmacophore and molecular docking strategy of multi-target inhibitor discovery.

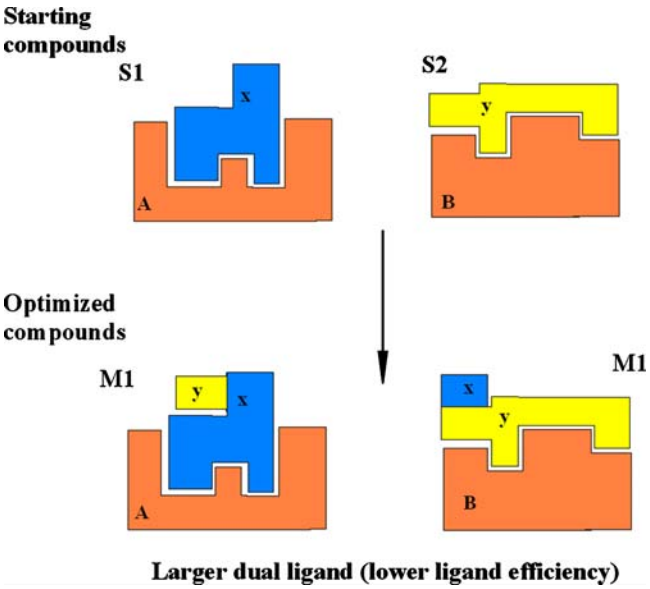


**Fig. 4.** Illustration of framework combination approach to multi-target drug discovery.

individual targets are sufficiently high and the false-hit rates are sufficiently low. High retrieval rates compensate for the reduced collective retrieval rates (if the retrieval rate against individual target is 50~70%, the collective retrieval rate for multi-target agents against two targets may be statistically reduced to 25~49%). Low false-hit rates are needed for high enrichment in searching multi-target agents that are significantly fewer in numbers and more sparsely distributed in the chemical space than agents against an individual target.

Molecular docking is a widely used virtual screening method that uses geometrical matching to dock small molecules to the target site 3D structure followed by the analysis of binding feasibility by consideration of chemical complementary and molecular interaction energies (35). This method does not require knowledge about known active compounds and their structural features or frameworks, but in some cases may have limited capability on account of target structural flexibility and specific chemical features of drug binding. To improve virtual screening performance, molecular dynamics-enhanced molecular docking method has been used in virtual screening against the individual targets in HIV and its associated opportunistic pathogens to find multi-target agents, such as KNI-764, that inhibit both HIV-1 protease and malarial plasmepsin II enzyme (40). Molecular docking and pharmacophore matching methods have been used for identifying dual-inhibitors of two anti-inflammatory targets, PLA2 and LTA4H-h, in the arachidonic acid metabolic network (41).

Fragment-based approaches (Figs. 4 and 5) combine multiple elements of structural frameworks or multiple fragments that bind to each individual target to design compounds that bind to multiple targets, which have been introduced as tools for the design of multi-target agents (42). In one approach, the structure-activity relationships against individual targets are analyzed to find molecular fragments and essential binding features which are either combined or incorporated into active agents against selected multiple targets (42). Fragment combination often results in larger and more complex non-drug-like molecules. Drug-like features may be retained if the degree of framework overlap is maximized and the size of the selected fragments is minimized. In another approach, molecular fragment libraries are searched to find the fragments with certain levels of activity against selected multiple targets, and the identified fragments are further optimized into more potent, bigger-sized multi-target active agents (42). Optimizing fragments with weak multiple activities into potent multi-target, drug-like agents can be more easily achieved for targets sharing a conserved binding site (43). As binding sites become more dissimilar, it is increasingly difficult to improve and adequately balance the high binding affinities needed to achieve acceptable *in-vivo* efficacy and safety. One way to reduce this difficulty is to explore synergistic targets, such that multi-target agents with modest activity at one or more of the relevant targets may still produce similar or better *in-vivo* effects compared with higher-affinity, target-selective compounds (22).

Moreover, multi-target QSAR models for identification of multi-target agents (44) and active agents against multiple bacterial (45), fungal (46,47) and viral (45) species have been developed by incorporating multi-target or species variations of binding-site features into the multi-target dependent molecular descriptors or species-dependent molecular descriptors, and stochastic Markov drug-binding process models. These multi-target QSAR models achieve high retrieval rates of 72~85% and moderately low false-hit rates of 15~28%. Development of multi-target QSAR models may be limited by the inadequate number of drug data for some of the targets or species. Moreover, the molecular size of the testing drugs needs to be in a certain range for accurate computation of multi-target-dependent or species-dependent molecular descriptors, which in some cases may also affect one's capability for developing multi-target QSAR models (47).

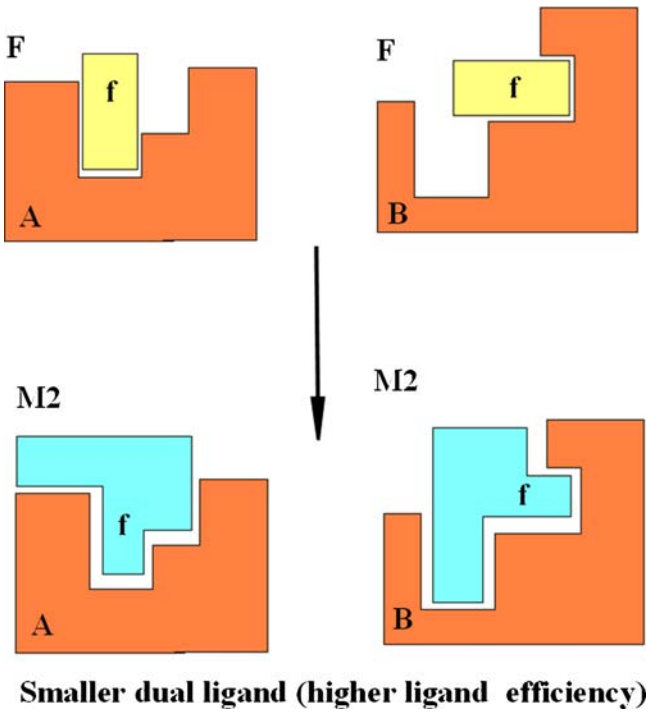


**Fig. 5.** Illustration of fragment-based approach to multi-target drug discovery.

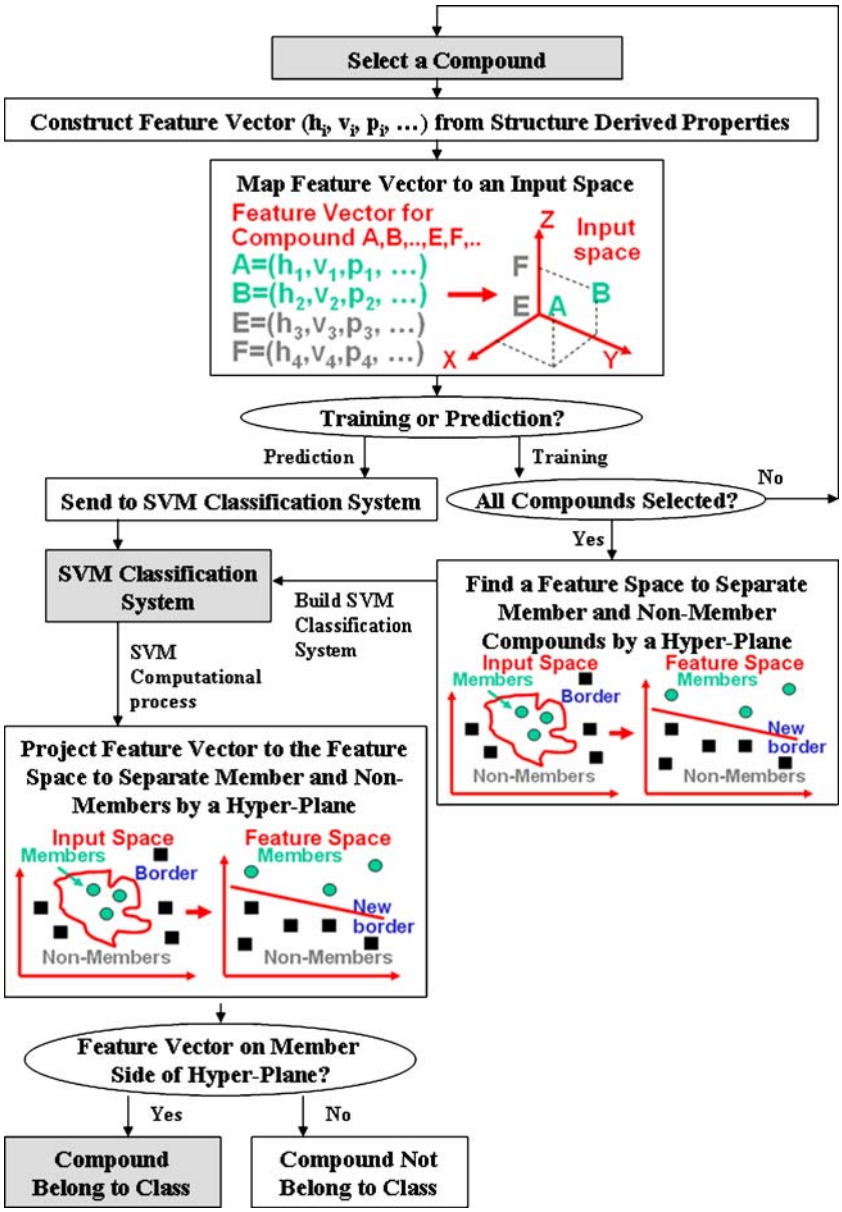


**Fig. 6.** Illustration of training a support vector machine virtual screening model and using it for searching inhibitors of an individual target.

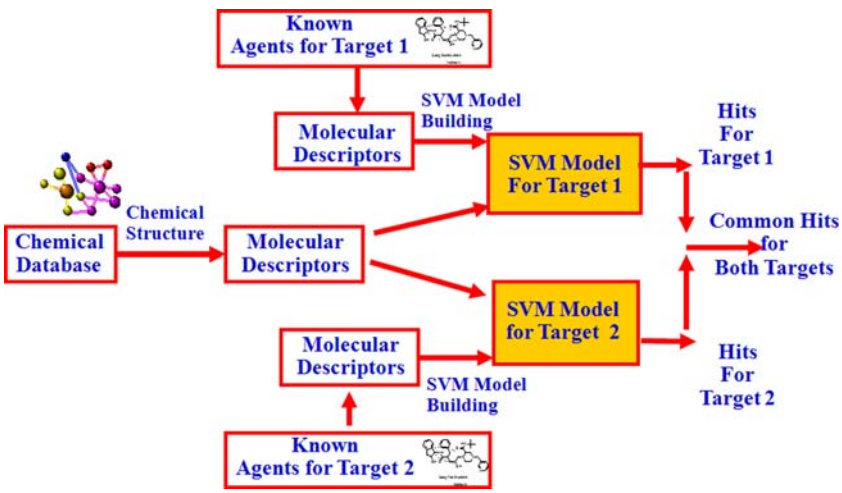


**Fig. 7.** Illustration of using support vector machines method for searching multi-target inhibitors.

**Table II.** Virtual Screening Performance of Combinatorial SVMs

| Kinase pair | Inhibitors in SVM training sets | | Virtual screening performance against known inhibitors of kinase pair | | | Virtual screening performance against other compounds | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Kinase A–Kinase B | Training set for kinase A | Training set for kinase B | Known dual-inhibitors of kinase pair | | | Known non-dual inhibitors of the same kinase pair | | Known inhibitors of other three kinase pairs | MDDR compounds similar to the known dual inhibitors of kinase pair | All 168 K MDDR compounds | 13.56 M PubChem compounds |
| | No. of inhibitors of A that are non-inhibitor of B (No. of families) | No. of inhibitors of B that are non-inhibitor of A (No. of families) | Retrieval rate | No. (%) of identified true hits outside the common training active families of both kinases | False hit rate for inhibitors of kinase A | False hit rate for inhibitors of kinase B | False hit rate | Virtual hit rate (No. of virtual hits) | Virtual hit rate (No. of virtual hits) | Virtual hit rate (No. of virtual hits) |
| EGFR-FGFR | 1,303 (388) | 392 (131) | 40.9% | 6 (8.5%) | 10.1% | 8.7% | 0.2~3.4% | 6.5% (65) | 0.07% (126) | 0.016% (2,200) | |
| VEGFR-Lck | 1,232 (427) | 445 (171) | 52.6% | 8 (13.1%) | 6.6% | 29.2% | 2.0~12.7% | 5.1% (21) | 0.10% (170) | 0.036% (4,817) | |
| PDGFR-Src | 492 (174) | 672 (213) | 38.3% | 30 (16.0%) | 25.8% | 11.6% | 0.7~7.7% | 2.9% (84) | 0.104% (175) | 0.021% (2,799) | |
| Src-Lck | 804 (236) | 450 (175) | 48.2% | 9 (16.1%) | 15.8% | 18.7% | 1.0~2.3% | 9.4% (26) | 0.078% (131) | 0.020% (2,674) | |

SVM for each individual kinase was trained by using published inhibitors of that kinase excluding dual-inhibitors of the related kinase pair. SVMs for pairs of kinases were combinatorially used for identifying published dual-inhibitors of four kinase pairs of EGFR-FGFR, VEGFR-Lck, PDGFR-Src, and Src-Lck. Non-dual inhibitors of a kinase pair refer to compounds known to inhibit one of the kinases but not both kinases. Dual-inhibitors of a kinase pair refer to compounds known to inhibit both kinases of a kinase pair.

## VIRTUAL SCREENING PERFORMANCE FOR SEARCHING MULTI-TARGET AGENTS FROM LARGE COMPOUND LIBRARIES

The capability of virtual screening methods in searching active compounds against individual targets from large compound libraries has been extensively evaluated (48), but their capability for searching multi-target agents from large compound libraries has not been tested. Nonetheless, some of the virtual screening methods can be readily evaluated by large library screening tests. We specifically evaluated one virtual screening method, support vector machines (SVMs), for its performance in searching dual-inhibitors of specific kinase pairs from large libraries of 13.56 M compounds in PubChem database and 168 K active agents from MDDR database. SVMs of each of the six individual anticancer kinase targets, EGFR, FGFR, VEGFR, PDGFR, Src, and Lck, were trained by using published non-dual inhibitors of each kinase excluding dual-inhibitors of related kinase pairs. Non-dual inhibitors of a kinase pair refer to compounds known to inhibit one of the kinases but not both kinases. Dual-inhibitors of a kinase pair refer to compounds known to inhibit both kinases of a kinase pair. These SVMs were combinatorially used for searching published dual-inhibitors of the four kinase pairs EGFR-FGFR, VEGFR-Lck, PDGFR-Src, and Src-Lck. We only evaluated dual-inhibitor search performance because of the availability of sufficient number of dual-inhibitors for conducting the tests and the relatively lower computational load for developing virtual screening models. SVMs were tested because of their good performance and high speed in screening large compound libraries (49) as well as our own experiences in developing SVM virtual screening tools (50,51). SVM for searching individual target and multi-target inhibitors is illustrated in Figs. 6 and 7 respectively. The four selected kinase pairs are frequently co-expressed or co-activated in various cancers (32,52), and targeted by multi-target drugs with good anticancer efficacies (10,11).

We used a rigorous testing method that assumes no explicit knowledge of known multi-target agents. SVM of each individual kinase was developed by using 392~1,303 known non-dual inhibitors published in the literature and 63,846~66,214 putative non-inhibitors of EGFR, VEGFR, PDGFR, FGFR, Src and Lck respectively (representative compounds in PubChem and MDDR databases not known to inhibit each of these kinases respectively) by using the algorithm and procedure described in our earlier publications (50,51). The collective retrieval rate for each kinase pair was estimated by using 56~188 known dual-inhibitors of EGFR-FGFR, VEGFR-Lck, PDGFR-Src, and Src-Lck published in the literature, respectively. Target selectivity with respect to a particular kinase pair was assessed by using non-dual inhibitors of the kinase pair and the inhibitors of other kinase pairs. The capability for searching large compound libraries was evaluated by using 13.56 M PubChem, 168 K MDDR, and 276~2,893 MDDR compounds similar in structural and physicochemical properties to the known dual-kinase inhibitors.

Virtual screening performance of combinatorial SVMs in identifying dual-inhibitors of the four kinase pairs is summarised in Table II. The dual-inhibitor retrieval rates are 40.9% for EGFR-FGFR, 52.6% for VEGFR-Lck, 38.3% for

PDGFR-Src, and 48.2% for Src-Lck, respectively. In screening 392~1,303 non-dual inhibitors of each kinase pair, combinatorial SVMs misidentified 10.1% of the non-dual EGFR inhibitor and 8.7% the non-dual FGFR inhibitors for EGFR-FGFR, 6.6% and 29.2% for VEGFR-Lck, 25.8% and 11.6% for PDGFR-Src, and 15.8% and 18.7% for Src-Lck, respectively. Therefore, combinatorial SVMs show reasonably good capability for selectively identifying multi-target agents without requiring explicit knowledge of multi-target agents. There are two possible reasons for the misidentification of a substantial percentage of non-dual inhibitors as dual-inhibitors. First, SVMs were trained by non-dual inhibitors only, which may not fully distinguish dual and non-dual inhibitors. Second, some of the misidentified non-dual inhibitors are probably true dual-inhibitors not yet experimentally tested for specific multi-target activities.

Target selectivity was further tested by using combinatorial SVMs to screen the 2,781~3,323 inhibitors of the four kinases not in a kinase pair, 0.2~3.4% of which were misidentified as dual-inhibitors for EGFR-FGFR, 2.0~12.7% for VEGFR-Lck, 0.7~7.7% for PDGFR-Src, and 1.0~9.8% for Src-Lck, respectively. Combinatorial SVMs appear to be fairly selective in separating inhibitors of a specific kinase pair from those of other kinases. Combinatorial SVMs also showed low false-hit rates in predicting as dual-inhibitors 2,200~4,817 (0.016~0.036%) of the 13.56 M PubChem compounds, 126~175 (0.07~0.104%) of the 168 K MDDR compounds, and 21~84 (2.9~9.4%) of the 276~2,893 MDDR compounds similar to the known dual-inhibitors. It is further noted that 49.6~61.9% of the 65~103 SVM identified MDDR compounds belong to the classes of antineoplastic, tyrosine-specific protein kinase inhibitors, and signal transduction inhibitors.

## CONCLUDING REMARKS

Multi-target-based *in-silico* methods have been increasingly explored and have shown promising potential as virtual screening tools for identifying selective multi-target agents. The capability of these methods may be further enhanced by incorporating knowledge of newly discovered selective multi-target agents from the current and future drug discovery efforts (10,11), and by the improvement of virtual screening methods (50,51,53–57). It is possible to introduce more comprehensive elements of distinguished structural and physicochemical features of selective multi-target agents or multi-target activity and binding site profiles into the development of more effective tools for the identification of selective multi-target agents and active compounds against an individual target.

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