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Predicting Targeted Polypharmacology for Drug Repositioning and Multi-Target Drug Discovery

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Abstract: Prediction of polypharmacology of known drugs and new molecules against selected multiple targets is highly useful for finding new therapeutic applications of existing drugs (drug repositioning) and for discovering multi-target drugs with improved therapeutic efficacies by collective regulations of primary therapeutic targets, compensatory signalling and drug resistance mechanisms. In this review, we describe recent progresses in exploration of *in-silico* methods for predicting polypharmacology of known drugs and new molecules by means of structure-based (molecular docking, binding-site structural similarity, receptor-based pharmacophore searching), expression-based (expression profile/signature similarity disease-drug and drug-drug networks), ligand-based (similarity searching, side-effect similarity, QSAR, machine learning), and fragment-based approaches that have shown promising potential in facilitating drug repositioning and the discovery of multi-target drugs.

Keywords: Computer aided drug design, drug discovery, drug repositioning, gene expression, multi-target, network pharmacology, systems pharmacology, virtual screening.

1. INTRODUCTION

Drugs typically interact with multiple proteins, and those interacting with selected combination of targets have found useful therapeutic applications [1]. Some existing drugs against a specific target of one disease have been found to be active against another target of a different disease, and the additional activities of these drugs have been explored in repositioning them for new therapeutic applications [2]. Table **1** lists 36 approved drugs explored for repositioning against a different target and diseases [2, 3]. Multi-target drugs active against selected multiple targets of the same diseases have been increasingly explored [4, 5] for achieving enhanced therapeutic efficacies and reduced drug resistance activities by simultaneously modulating a primary therapeutic target and drug response and resistance mechanisms [6, 7]. Table **2** lists 32 approved and clinical trial multi-target drugs against the same diseases [8].

There have been increasing interests in identifying additional targets of existing drugs [9] and in discovering multi-target drugs [10] by means of experimental and *in-silico* methods [8, 11]. In particular, a number of *in-silico* methods have been used for predicting multiple targets of known drugs and newly designed molecules [8]. These methods are broadly classified into structure-based, expression-based, ligand-based, and fragment-based methods. Structure-based methods, such as molecular docking [12-14], target-site structural similarity [15] and receptor-based pharmacophore searching [16], explore target site structural features to find binding molecules with structural and energetic complementarity. Expression-based methods exploit similarity in gene expression profiles or signatures of drug-treated samples to predict new targets and therapeutic effects [17-19]. Ligand-based methods use such techniques as similarity searching [9, 20], drug side effect similarity [21], quantitative structure-activity relationships (QSAR) [22-28], and machine learning methods [29, 30] to select molecules with structural and physicochemical profiles matching those of the known active molecules. Fragment-based methods combine multiple structural frameworks of active molecules of individual target into a single molecule that binds to multiple targets [31]. Here we describe recent progresses in exploring these methods for predicting polypharmacology aimed at drug repositioning and multi-target drug discovery.

2. MULTI-TARGET MOLECULAR SCAFFOLDS

Some molecular scaffolds have been found in high percentages of multi-target agents against selected targets. For instance, the six scaffolds in (Fig. **1**) are reportedly contained in high percentages of the published dual inhibitors of tyrosine kinase pairs EGFR-PDGFR, PDGFR-Src, EGFR-Src, EGFR-FGFR, VEGFR-Lck, Src-Lck, and PDGFR-FGFR published before 2010 [29]. The seven scaffolds in (Fig. **2**) are in high percentages of the published dual inhibitors of serotonin reuptake paired with noradrenaline transporter, H3 receptor, 5-HT1a receptor, 5-HT1b receptor, 5-HT2c receptor and Neurokinin 1 (NK1) receptor respectively [30]. Some scaffolds have been found to form multi-target activity

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Table 1. Approved Drugs Explored for Repositioning against a Different Target and Diseases

Drug	Primary target	Original indication	Additional or new indication
Allopurinol	Xanthine dehydrogenase/oxidase	Cancer	Gout
Amantadine	Influenza A virus M2 protein	Antiviral	Parkinson's disease
Amphetamine	N-methyl-D-aspartate (NMDA) receptor	Stimulant	Hyperkinesis in children
Atomoxetine	Noradrenergic reuptake	Depression; Parkinson's disease	Attention deficit hyperactivity disorder
Bupropion	Norepinephrine reuptake; Serotonin reuptake	Depression	Smoking cessation
Celecoxib	Cyclooxygenase-2	Inflammation; Osteoarthritis; Adult rheumatoid arthritis	Familial adenomatous polyposis; Colon and breast cancer
Chlordiazepoxide	Benzodiazepine receptor	Muscle relaxant	Tranquilizer
Chloroquine	Glutamate dehydrogenase	Malaria	Rheumatism
Chlorpromazine	D(2) dopamine receptor	Anthelmintic; Anti-emetic; Antihistamine	Schizophrenia
Dapoxetine	Serotonin reuptake	Analgesia; Depression	Premature ejaculation
Duloxetine	Noradrenergic reuptake; Serotonin reuptake	Depression	Stress urinary incontinence
Eflornithine	Ornithine decarboxylase	Anti-infective	Reduction of unwanted facial hair in women
Finasteride	5-alpha reductase	Benign prostatic hyperplasia	Hair loss
Fluoxetine	Serotonin reuptake	Depression	Premenstrual dysphoria
Galantamine	Acetylcholinesterase	Polio; Paralysis; Anaesthesia	Alzheimer's disease
Imipramine	Norepinephrine reuptake	Sedative	Depression
Lidocaine	Voltage-gated sodium channel	Local anaesthesia	Arrhythmia; Oral corticosteroid-dependent asthma
Mecamylamine	Nicotinic acetylcholine receptor	Moderately severe to severe essential hypertension; Uncomplicated cases of malignant hypertension	Attention deficit hyperactivity disorder
Metronidazole	DNA	Antitrichomonal	Antibacterial (anaerobic organisms)
Mifepristone	Progesterone receptor	Pregnancy termination	Psychotic major depression
Milnacipran	Norepinephrine reuptake	Depression	Fibromyalgia syndrome
Minoxidil	ATP-sensitive potassium channel	Hypertension	Hair loss
Estrogens	Estrogen receptor	Replacement therapy	Contraception
Paclitaxel	Tubulin beta	Cancer	Restenosis
Pemetrexed	Thymidylate synthase	Mesothelioma	Lung cancer
Penicillamine	HIV-1 Tat protein	Copper chelating agent	Rheumatism
Phentolamine	Alpha adrenergic receptor	Hypertension	Impaired night vision
Raloxifene	Estrogen receptor	Contraception; Breast and prostate cancer	Osteoporosis
Ropinirole	D(2) dopamine receptor; D(3) dopamine receptor	Hypertension	Parkinson's disease; Idiopathic restless leg syndrome
Sibutramine	Noradrenergic reuptake; Serotonin reuptake	Depression	Obesity

(Table 1) contd….

Drug	Primary target	Original indication	Additional or new indication
Sildenafil	CGMP-specific 3',5'-cyclic phosphodiesterase	Angina	Male erectile dysfunction
Tadalafil	CGMP-specific 3',5'-cyclic phosphodiesterase	Inflammation; Cardiovascular disease	Male erectile dysfunction
Thalidomide	Tumor necrosis factor receptor	Sedation; Nausea; Insomnia	Cutaneous manifestations of moderate to severe erythema nodosum leprosum in leprosy; Multiple myeloma
Tofisopam	GABA receptor; Peripheral-type benzodiazepine receptor	Anxiety-related conditions	Irritable bowel syndrome
Topiramate	Glutamate receptor, ionotropic kainate 1 (GLUR5)	Epilepsy	Obesity
Zidovudine	HIV reverse transcriptase	Cancer	HIV/AIDS

Table 2. Literature 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 (IC_{50}, K_i, EC_{50})	Potency against specific cell line	Multi-target mode of action
ABT-263	Advanced small cell lung cancer; Relapsed or refractory chronic lymphocytic leukemia; Relapsed or refractory lymphoid malignancies [65]	BCL-2: <1 nM BCL-xL: <0.5 nM BCL-W: <1 nM [66]	CCRF-CEM: 450 nM CHLA-136: 2170 nM CHLA-258: 780 nM CHLA-266: 1140 nM COG-LL-317: 570 nM Kasumi-1: 90 nM MOLT-4: 260 nM NALM-6: 1080 nM NB-1643: 500 nM NB-EBc1: 1910 nM Rh18: 200 nM Rh41: 190 nM RS4;11: 50 nM [67]	Inhibiting Bcl-2 protein family members that regulate apoptosis and impact tumor formation, progression and chemoresistance
Afatinib	NSCLC [65]	EGFR: 0.5 nM HER2: 14 nM [68]	HCC827: <1 nM PC9: <1 nM [69]	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
AT9283	Adult solid tumors; NHL; AML; ALL; CML; MDS; Myelofibrosis [65]	AURKA: 3 nM AURKB: 3 nM [70]	A2780: 7.7 nM A549: 12 nM HCT116: 13 nM HT-29: 11 nM MCF7: 20 nM MIA-Pa-Ca-2: 7.8 nM SW620: 14 nM [71]	Inhibiting Aurora kinases that regulate prophase of mitosis (Aurora A) and the attachment of the mitotic spindle to the centromere (Aurora B)

(Table 2) contd….

Drug	Targeted Disease	Multi-targets and potency against each individual target (IC_{50}, K_i, EC_{50})	Potency against specific cell line	Multi-target mode of action
Axitinib	Metastatic pancreatic cancer; RCC; NSCLC; Breast cancer; Melanoma [57]	CSF-1: 73 nM PDGFR: 1.6-5 nM VEGFR2: 0.2 nM [72]	HUVEC: 573 nM IGR-NB8: 849 nM SH-SY5Y: 274 nM [73]	Inhibiting cytokine and tyrosine kinases receptors that regulate cell proliferation at different upstream points (CSF-1, PDGFR) and angiogenesis (VEGFR2)
AZD0530	Haematological malignancies; Solid tumors [57]	ABL1: 30 nM SRC: 2.7 nM [74]	LS180: 500 nM H508: 500 nM LS174T: 500 nM [75] 1483: 1000 nM UM-22B: 1000 nM PCI-15B: 1300 nM PCI-37B: 1000 nM Cal-33: 600 nM [76]	Inhibiting tyrosine kinases that regulate cell proliferation at different upstream points
Batimastat	Various cancers [65]	MMP-1: 5 nM MMP-2: 4 nM MMP-7: 6 nM [77]	MDA435ILCC6: >5000 nM [78]	Inhibiting MMP proteases that regulate cell invasion and proliferation (MMP-1 and 7), invasion and metastasis (MMP-2)
BMS-599626	Various cancers [57]	EGFR: 22 nM HER2: 32 nM [79]	AU565: 630 nM BT474: 310 nM GEO: 900 nM HCC1419: 750 nM HCC1954: 340 nM HCC202: 940 nM KPL-4: 380 nM MDA-MB-175: 840 nM N87: 450 nM PC9: 340 nM Sal2: 240 nM ZR-75-30: 510 nM [79]	Inhibiting tyrosine kinase receptor ERBB family members that regulate proliferation and survival at different upstream points
Bosutinib	CML; Leukemia; Various cancers [57]	ABL1: 1 nM SRC: 1.2 nM [80]	MDA-MB-435s: 9000 nM Hs578T: 5900 nM [81]	Inhibiting tyrosine kinases that regulate cell proliferation at different upstream points
Bupropion	Depression [65]	NET: 1900 nM [82] SERT: 22000 nM [83]	TE671/RD: 10500 nM SH-SY5Y: 1514 nM [84]	Inhibiting monoamine transporter family members that perform complementary and compensatory actions on neural activities in synapse
HKI-272	NSCL; Breast cancer; Various cancers [57]	EGFR: 92 nM HER2: 59 nM [85]	3T3: 700 nM SK-Br-3: 2 nM BT 474: 2 nM A431: 81 nM MDA-MB-435: 960 nM SW620: 690 nM [85]	Inhibiting tyrosine kinase receptor ERBB family members that regulate proliferation and survival at different upstream points

(Table 2) contd….

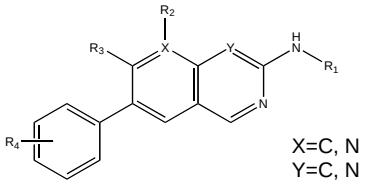
Drug	Targeted Disease	Multi-targets and potency against each individual target (IC_{50}, K_i, EC_{50})	Potency against specific cell line	Multi-target mode of action
Imatinib	CML; GIST; Intestinal cancer; Myeloid leukemia; Glioma; Lung, prostate, solid tumors [57]	ABL1: 38 nM [86] KIT: 100 nM [87] PDGFR: 300 nM [86]	BV173: 240 nM EM3: 100 nM K562: 560 nM LAMA84: 320 nM [88]	Inhibiting tyrosine kinases that regulate proliferation at different upstream points
Lapatinib	Refractory metastatic breast cancer; RCC; Bladder, head & neck, NSCLC, brain cancer [57]	EGFR: 10.8 nM HER2: 9.2 nM [89]	BT474: 100 nM MCF-7: 4000 nM T47D: 3000 nM [89]	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
Midostaurin	Colon, breast, CLL, AML, GIST, solid tumors; Non-Hodgkin's lymphoma [57]	FLT3: 528 nM PKC: 22 nM [90]	MCF-7: 97 nM [91] Canine mastocytoma cell line C2: 157 nM HMC-1.1 (lacking KIT D816V): 191 nM HMC-1.2 (possessing KIT D816V): 196 nM [92] HEL 92.1.7: 500 nM K562: 250 nM [93]	Inhibiting tyrosine kinases that regulate cell proliferation at different upstream points
MK-5108	Various cancers [65]	AURKA: 0.064 nM AURKB: 14.1 nM [94]	AU565: 450 nM CAL85-1: 740 nM Colo205: 500 nM ES-2: 1100 nM HCC1143: 420 nM HCC1806: 560 nM HCC1954: 910 nM HCT116: 270 nM HeLa-S3: 2100 nM MB157: 810 nM MCF-7: 520 nM MIAPaCa-2: 6400 nM SKOV-3: 1100 nM SW48: 160 nM [94]	Inhibiting Aurora kinases that regulate prophase of mitosis (Aurora A) and the attachment of the mitotic spindle to the centromere (Aurora B)
Motesanib	GIST; Metastatic thyroid cancer; NSCLC; Breast, colorectal cancer [57]	KIT: 8 nM PDGFR: 84 nM VEGFR2: 3 nM [95]	MCF-7 : >3000 nM MDA-MB-231: >3000 nM [96]	Inhibiting tyrosine kinase receptors that regulate proliferation (PDGFR), angiogenesis (VEGFR2), and kinase expression (KIT)
Nilotinib	ALL; CML; GIST; Leukemia [57]	ABL1: 20-60 nM KIT: 27 nM PDGFR: 71 nM [97]	Canine mastocytoma cell line C2: 55 nM HMC-1.1 (lacking KIT D816V): 10 nM HMC-1.2 (possessing KIT D816V): 2363 nM [92]	Inhibiting tyrosine kinases that regulate tumor growth and proliferation at different upstream points

(Table 2) contd….

Drug	Targeted Disease	Multi-targets and potency against each individual target (IC_{50}, K_i, EC_{50})	Potency against specific cell line	Multi-target mode of action
OSI-930	Various cancers [65]	KIT: 80 nM VEGFR2: 9 nM [98]	H526: 9.6 nM HMC-1: 9.5 nM HUVEC: 10.1 nM NIH-3T3: 51.5 nM [99]	Inhibiting tyrosine kinase receptors that regulate cell proliferation (KIT) and angiogenesis (VEGFR2)
P276-00	Multiple myeloma; Mantle cell lymphoma; Head & neck cancers; Cyclin D1-positive melanoma [65]	CDK1: 79 nM CDK4: 63 nM CDK9: 20 nM [100]	U266B1: 500 nM RPMI-8226: 900 nM [101]	Inhibiting CDK family members that are involved in cell cycle regulation (CDK1 and 4) and transcription (CDK9)
Pasireotide	Neuroendocrine tumor; Carcinoid tumor; Pancreatic neuroendocrine tumor; Pancreatic cancer [65]	SS1R: 9.3 nM SS2R: 1 nM SS3R: 1.5 nM SS5R: 0.16 nM [102]	HUVEC: 1000-10000 nM [103]	Binding to multiple somatostatin receptor subtypes (i.e. 1, 2, 3, and 5) to mimic the action of natural somatostatin
Pazopanib	Advanced/metastatic renal cancer; Solid tumors; NSCLC [57]	KIT: 74 nM PDGFR: 71-84 nM VEGFR2: 30 nM [104]	HUVEC: 21.3 nM [105]	Inhibiting tyrosine kinase receptors that regulate cell proliferation and angiogenesis at different upstream points
PF-03814735	Advanced solid tumors [65]	AURKA: 5 nM AURKB: 0.8 nM [106]	A549: 90 nM C6: 93 nM H125: 150 nM HCT-116: 70 nM HL60: 110 nM L1210: 140 nM MDCK: 42 nM [106]	Inhibiting Aurora kinases that regulate prophase of mitosis (Aurora A) and the attachment of the mitotic spindle to the centromere (Aurora B)
PHA-739358	CML; MHRPC [65]	AURKA: 13 nM AURKB: 79 nM [107]	DU145: 220 nM K562: 260 nM PC-3: 120 nM [107]	Inhibiting Aurora kinases that regulate prophase of mitosis (Aurora A) and the attachment of the mitotic spindle to the centromere (Aurora B)
SNS-032	B-lymphoid malignancies; Advanced solid tumors [65]	CDK2: 38 nM CDK7: 62 nM CDK9: 4 nM [108]	HCT116: <300 nM [109]	Inhibiting CDK family members that are involved in cell cycle regulation (CDK2), transcription (CDK9) and CDK activating and transcription (CDK7)
Sorafenib	RCC; Hepatocellular carcinoma; NSCLC; Melanoma; Myelodyspalstic syndrome; AML; Head & neck cancer; Breast, colon, ovarian, pancreatic cancer [65]	BRAF: 22 nM [110] RET: 5.9 nM [111] VEGFR: 20-90 nM [110]	HepG2: 4500 nM PLC/PRF/5: 6300 nM [112] EOL-1: 0.033 nM MV4-11: 0.88 nM RS4;11: 12 nM [113]	Inhibiting kinases that regulate angiogenesis (VEGFR2) and proliferation (BRAF), RET lysosomal degradation (RET), and Src-mediated alternative signalling (BRAF)

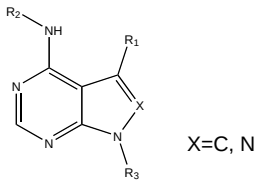
(Table 2) contd….

Drug	Targeted Disease	Multi-targets and potency against each individual target (IC_{50}, K_i, EC_{50})	Potency against specific cell line	Multi-target mode of action
Sotrastaurin	Acute rejection after de novo renal transplantation [65]	PKC-alpha: 0.95 nM PKC-beta: 0.64 nM PKC-theta: 0.22 nM [114]	PBMC: 37 nM [115]	Inhibiting PKC family members that regulate the induction of transcription factors (PKC-alpha and beta) and sustainability of intracellular signals (PKC-theta), and in turn blocking T cell activation
SU-6668	Advanced solid tumors [65]	AURKA: 850 nM AURKB: 47 nM [116] FGFR: 1200 nM PDGFR: 8 nM VEGFR2: 2100 nM [117]	H526: 8500 nM [118] MO7E: 290 nM [119]	Inhibiting Aurora kinases that regulate prophase of mitosis (Aurora A) and the attachment of the mitotic spindle to the centromere (Aurora B), and tyrosine kinase receptors that regulate angiogenesis (FGFR, PDGFR and VEGFR2)
Sunitinib	RCC; GIST; Breast, neuroendocrine tumors [57]	FLT3: 50-250 nM [120] KIT: 1-10 nM [121] PDGFR: 2 nM [122] VEGFR2: 80 nM [122]	Kasumi-1: 75.7 nM [34]	Inhibiting tyrosine kinase receptors that regulate angiogenesis (PDGFR, VEGFR2), proliferation (FLT3), and kinase level (KIT)
TAK165	Various cancers [57]	EGFR: >25000 nM HER2: 6 nM [123]	BT474: 5 nM UMUC-3: 1812 nM T24: 91 nM DU145: 1647 nM PC-3: 4620 nM LN-REC4: 90 nM LNCaP: 53 nM [123]	Inhibiting tyrosine kinase receptor ERBB family members that regulate proliferation and survival at different upstream points
TKI258	RCC [65]	FGFR3: 8 nM PDGFR: 27-210 nM [104]	G384D: 550 nM K650E: 90 nM Y373C: 90 nM [124]	Inhibiting tyrosine kinase receptors that regulate survival and growth (FLT3), and angiogenesis and tumor progression (FGFR3)
VX-680	Colorectal cancer; Hematological malignancies; Various solid tumors; Hematological cancers [57]	AURKA: 0.6 nM AURKB: 18 nM LCK: 520 nM [125]	HL60: 15 nM [125]	Inhibiting Aurora kinases that regulate prophase of mitosis (Aurora A) and the attachment of the mitotic spindle to the centromere (Aurora B)
XL880	Gastric cancer; RCC; Solid tumors [65]	MET: 0.4 nM VEGFR2: 0.86 nM [126]	B16F10: 21 nM MDA-MB-231: 4 nM PC-3: 23 nM [126]	Inhibiting tyrosine kinases that regulate tumor growth (c-MET) and angiogenesis (VEGFR2)
ZK 304709	Advanced solid tumors [65]	CDK1: 50 nM CDK2: 4 nM CDK4: 61 nM CDK7: 85 nM CDK9: 5 nM [127]	BON: 129 nM QGP-1: 79 nM [128]	Inhibiting CDK family members that are involved in cell cycle regulation (CDK1, 2 and 4), transcription (CDK9) and CDK activating and transcription (CDK7)



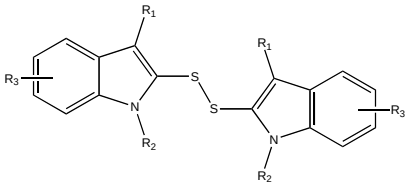
Scaffold A

PDGFR-Src: 76.1%
PDGFR-FGFR: 67.4%
EGFR-PDGFR: 63.8%
EGFR-FGFR: 54.9%
EGFR-Src: 33.9%
VEGFR-Lck: 27.9%



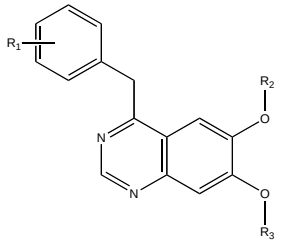
Scaffold B

Src-Lck: 57.1%
VEGFR-Lck: 29.5%
EGFR-Src: 25.9%



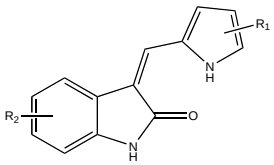
Scaffold C

EGFR-Src: 19.6%



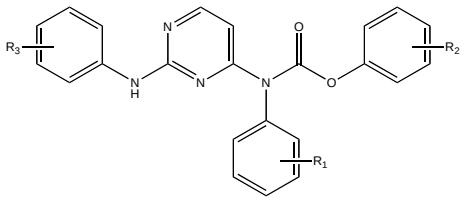
Scaffold D

EGFR-FGFR: 32.4%
EGFR-Src: 4.5%
Src-Lck: 1.8%
EGFR-PDGFR: 1.7%



Scaffold E

PDGFR-FGFR: 17.8%
EGFR-PDGFR: 8.6%
EGFR-FGFR: 7.0%
PDGFR-Src: 6.9%
EGFR-Src: 2.7%
Src-Lck: 1.8%



Scaffold F

Src-Lck: 37.5%
VEGFR-Lck: 34.4%

Fig. (1). Six scaffolds reportedly contained in high percentages of the dual inhibitors of tyrosine kinase pairs EGFR-PDGFR, PDGFR-Src, EGFR-Src, EGFR-FGFR, VEGFR-Lck, Src-Lck, and PDGFR-FGFR published before 2010. The percentage value behind each target-pair indicates the percentage of known dual inhibitors of the target-pair that contain this scaffold.

scaffolds with their structural analogs having significantly different potencies against multiple targets [32]. For instance, the two scaffolds in (Fig. **3**) are in some inhibitors of carbonic anhydrase (CA) I, II and IX and some inhibitors of protein kinase B (PKB) Akt1 and Akt2, mitogen- and stress-activated protein kinase 1 (MSK1) and ribosomal S6 kinase 1 (RSK1) respectively, each with close analogs showing highly different potencies against different targets [32]. In particular, analogs a and b of scaffold A, and analogs b and c of scaffold B show markedly different pIC50 values (activity cliff). These and other multi-target scaffolds appear to be the backbone of multi-target inhibitors of selected targets, and specific variations of side-chain groups of these scaffolds seem to be sufficient to significantly alter multi-target activities. This suggests that structural and physicochemical properties are important for distinguishing multi-target inhibitors, which can be explored for predicting polypharmacology [8, 29].

3. PREDICTING POLYPHARMACOLOGY FOR DRUG REPOSITIONING

Structure-based methods have been explored for identifying targets of existing drugs. The most extensively used structure-based method is the molecular docking method that predicts ligand-protein binding by geometrically docking

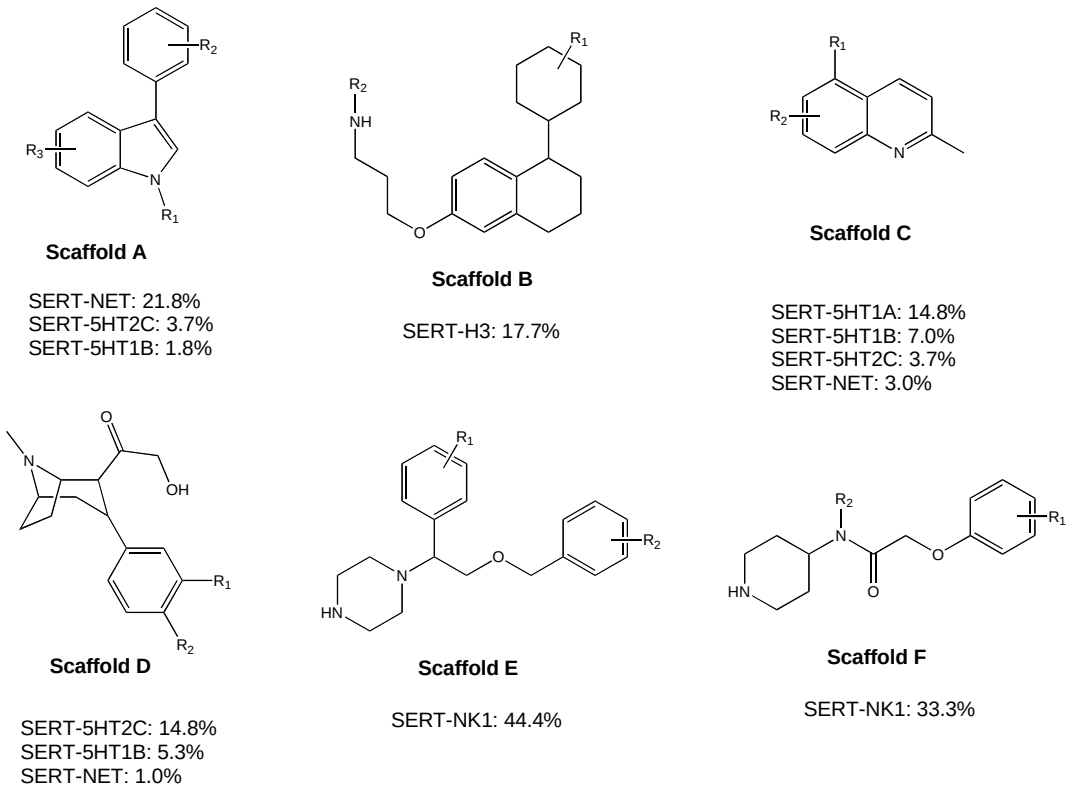


Fig. (2). Seven scaffolds reportedly contained in high percentages of the published dual inhibitors of serotonin reuptake paired with noradrenaline transporter, H3 receptor, 5-HT1a receptor, 5-HT1b receptor, 5-HT2c receptor and NK1 receptor respectively. The percentage value behind each target-pair indicates the percentage of known dual inhibitors of the target-pair that contain this scaffold.

molecules to a pre-selected target site followed by binding configuration optimization and scoring [33, 34]. A ligand-protein inverse docking method has been proposed for identifying potential targets of small molecules, and testing results on two drugs, namely tamoxifen and vitamin E, have shown that this method is capable of predicting additional targets of these drugs implicated or confirmed by experiments [12]. Two web-servers TarFisDock [13] and DRAR-CPI [14] have been developed for facilitating target identification particularly for drug repositioning and side-effect prediction based on this inverse docking strategy and the known protein 3D structures from Protein Data Bank. A second strategy is to predict additional targets of existing drugs by comparative analysis of the binding site structural characteristics of the drug target with respect to those of other targets, which has been used for predicting polypharmacological activities of existing drugs against *Mycobacterium tuberculosis* [15]. The third strategy is to use established receptor-based pharmacophore models, which describe the spatial arrangement of features essential for a molecule to interact with a specific target [35], to predict potential targets of drugs, and such a strategy has been implemented in a web-server PharmMapper for predicting potential drug targets from >7,000 receptor-based pharmacophore models [16]. These structure-based methods are only applicable to those targets with sufficiently high quality 3D structures.

Expression-based methods can be more generally used for predicting polypharmacology of drugs because they do not require any prior information on the targets and compounds being analyzed [17-19]. In these methods, disease-drug networks are constructed based on the gene expression profile-profile similarity [36] or the expression signature-profile similarity [37] of drug-treated samples. These disease-drug networks enable the prediction of new therapeutic applications or side-effects of drugs. For instance, a network extracted from ~24.5 million comparisons of ~7,000 publicly available transcriptomic profiles indicates that drugs against neurological disorders, hypertension/heart diseases, cancer, AIDs, migraine headaches, and inflammation may have effects on Huntington's disease [18]. These are consistent with experimental findings. Huntington's disease is a neurodegenerative disease which can be reduced by stimulating autophagy [38]. The immunosuppressant rapamycin [39] and several anti-migraine and anti-hypertension drugs [40] have been found to stimulate autophagy, and some anti-cancer and anti-HIV drug combinations halt the progress of Huntington's disease [41, 42]. Drug-drug networks may also be derived based on similarity levels of the corresponding drug-treated samples, which have been used to correctly predict the mode of action of nine anticancer compounds and to discover a new therapeutic effect of fasudil, a Rho-kinase inhibitor, as an enhancer of cellular autophagy against neurodegenerative disorders [19].

Ligand-based methods have also been explored for predicting polypharmacology of existing drugs [9, 20, 21]. Similarity searching method, which predicts the activity of compounds based on their structural similarity to the known active compounds [43, 44], have also been used for predicting new targets of existing drugs [9]. In one study, similarity between ligand sets of drug targets has been evaluated to

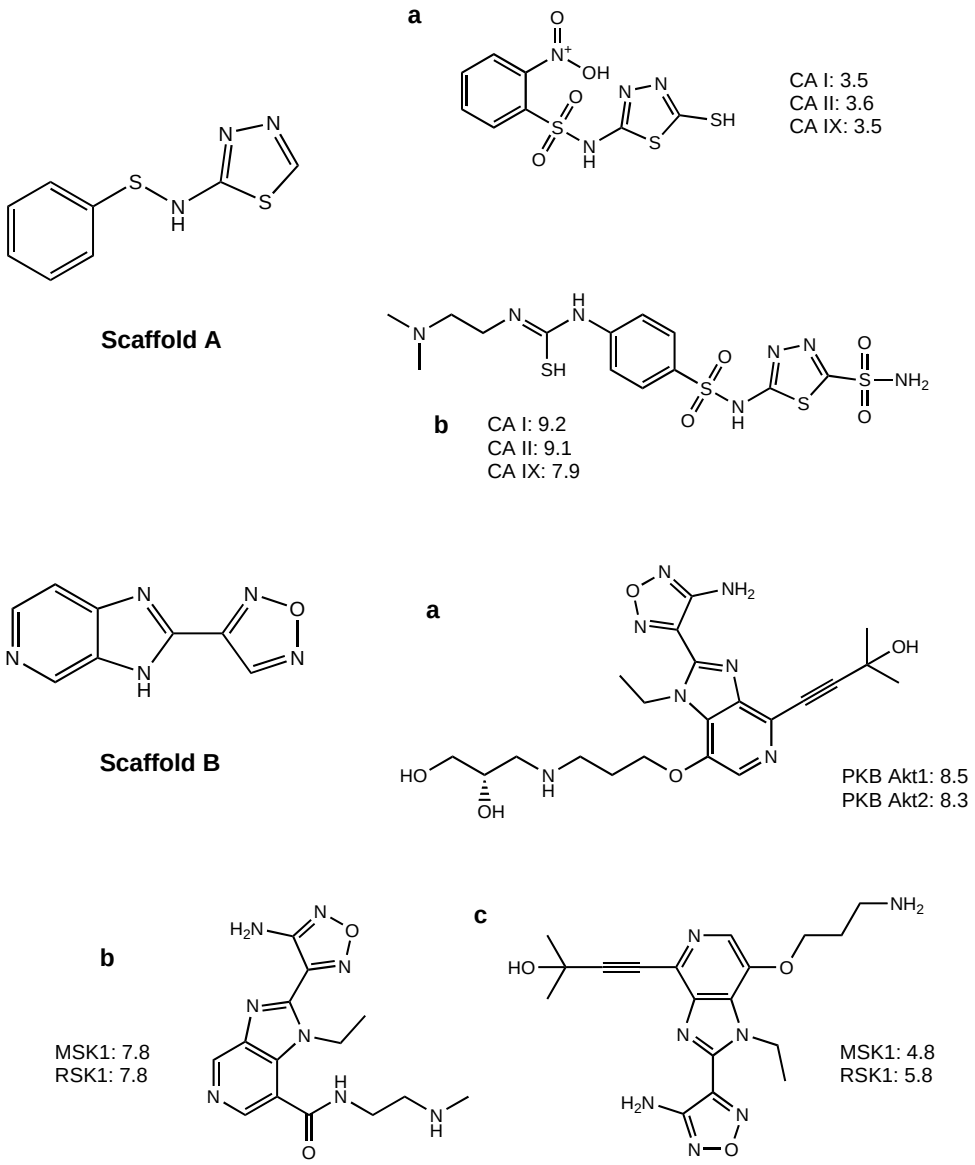


Fig. (3). Two molecular scaffolds in some multi-target inhibitors of CAI, CAII and CAIX and some inhibitors of Akt1, Akt2, MSK1 and RSK1 respectively, each with representative multi-target analogs showing potencies in pIC50 against respective target combinations. In particular, analogs a and b of scaffold A, and analogs b and c of scaffold B show markedly different pIC50 values (activity cliff).

identify unexpected links between drugs and new targets, which led to the prediction of methadone, emetine and loperamide as potential muscarinic M3, α2 adrenergic and neurokinin NK2 receptor antagonists, respectively, that have subsequently been confirmed experimentally [20]. In another study, thousands of unanticipated associations have been predicted based on chemical similarities between existing drugs and ligands of known targets, and 23 of the 30 experimentally tested associations have been confirmed (five of which with <100 nM potency) including the antagonism of the β1 receptor by the transporter inhibitor Prozac and the inhibition of the 5-hydroxytryptamine transporter by the ion channel drug Vadilex [9]. Based on the similarity of phenotypic side effect profiles of 746 marketed drugs, a network of 1018 side effect-driven drug-drug relations have been constructed which include 261 unexpected relations formed by chemically dissimilar drugs from different therapeutic indications, and testing studies of 20 of these unexpected relations have led to the identification of 11 new drug-target interactions at < 10 μM potencies in *in vitro* binding assays [21].

4. PREDICTION OF MULTI-TARGET AGENTS

Two structure-based methods, molecular docking and receptor-based pharmacophore searching, have been extensively used for facilitating the identification of multi-target molecules. In particular, molecular docking method does not require knowledge about known active compounds and their structural features or frameworks, but in some cases may have limited capability in 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 inhibits both HIV-1 protease and malarial plasmepsin II enzyme [45]. Molecular docking and pharmacophore matching methods have been used for identifying dual-inhibitors of two anti-inflammatory targets, phospholipase A2 (PLA2) and leukotriene A(4) hydrolase (LTA4H), in the arachidonic acid metabolic network [46]. Combined receptor-based pharmacophore searching and molecular docking have been used for identifying multi-target Chinese herbal ingredients against four anti-inflammatory targets cyclooxygenases 1 & 2, p38 MAP kinase, c-Jun terminal-NH2 kinase and type 4 cAMP-specific phosphodiesterase [47].

Some ligand-based methods have also been used for identifying multi-target active compounds. In particular, a number of multi-target QSAR models have been developed for identifying multi-target kinase inhibitors [22], dual action anti-Alzheimer and anti-parasitic GSK-3 inhibitors [23, 24], HIV-HCV co-inhibitors [25], and active agents against multiple bacterial [26], fungal [27, 28] and viral [26] 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 have been reported to achieve high retrieval rates of 72%~85% and moderately low false-hit rates of 15%~28% [26-28]. 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 [28].

Another ligand-based method, machine learning method, has also been explored as virtual screening tools for multi-target drug discovery. Combinatorial support vector machine (SVM) models for searching dual inhibitors of 11 kinase pairs have been developed, for which *in-silico* tests have shown reasonably good dual kinase inhibitor yields (12.2%-57.3%), hit rates (0.22%~4.3%), and selectivity against individual kinase inhibitors (individual kinase inhibitor false selection rates 3.7%-48.1% for the same kinase pair and 0.98%-4.77% for other kinases) in screening 13.56 million compounds [30]. Some of the SVM selected virtual hits that passed drug-like filter and molecular docking have been tested in bioassays, which have found that 3 of the 19 selected dual Abl and PI3K inhibitor hits [48], 1 of the 21 selected dual VEGFR2 and Src inhibitor hits [49] and 1 selected dual EGFR and VEGFR inhibitor hit [50] are active. Combinatorial SVM has also been applied for predicting dual target serotonin reuptake inhibitors of 7 target pairs, and *in-silico* tests have shown similar level of dual target inhibitor yields (22.0%~83.3%), hit rates (0.12%~12.6%), and selectivity against individual target inhibitors (individual target inhibitor false selection rates 2.2%-29.8% for the same target pair and 0.58%-7.1% for other similar targets) in screening 17 million compounds [30].

Fragment-based approaches have also been explored for designing multi-target agents [31]. One method, framework combination, incorporates essential binding features into a single lead molecule by linking, fusing or merging the frameworks of two selective molecules [31]. However, this method may in some cases generate large, complex and less druglike molecules [31]. Druglikeness can be retained if the degree of framework overlap is maximized and the size of the selective ligands minimized. Another method, screening-based method, searches chemical (fragment) libraries to find multi-target fragment hits possibly with weak activities, followed by optimization of the fragment into more potent multi-target active agents [31]. 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 [51]. As binding sites become more dissimilar, it remains a challenge to design agents with potent multi-target activities, *in vivo* efficacy and safety profiles. One solution is to explore synergistic targets, such that multi-target agents with modest activity against one or more of these synergetic targets may still produce similar or better *in vivo* effects compared to higher-affinity target-selective compounds [52].

CONCLUSIONS

A number of structure-based, expression-based, ligand-based and fragment-based in-silico methods have been developed, explored and have shown promising potential in predicting polypharmacology for drug repositioning and multi-target drug discovery. With rapid advances in systems pharmacology [53], disease and drug response mechanisms [54], and disease-drug and target-drug networks [36, 55], the capability of these methods may be further improved together with continued efforts in drug discovery [56, 57], and in the development of *in silico* drug discovery methods [58-64]. It is possible to introduce more comprehensive elements of distinguished structural and physicochemical features of selective multi-target activity and binding site profiles into the development of more effective tools for the prediction of polypharmacology of drugs and for the discovery of new multi-target agents.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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ABBREVIATIONS

Ca	=	Carbonic anhydrase
LTA4H	=	Leukotriene A(4) hydrolase
MSK1	=	Mitogen- and stress-activated protein kinase 1
NK1	=	Neurokinin 1
PKB	=	Protein kinase B
PLA2	=	Phospholipase A2
QSAR	=	Quantitative structure-activity relationships
RSK1	=	Ribosomal S6 kinase 1
SVM	=	Support vector machine

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