REVIEW ARTICLE

Multi-targeted anticancer drugs: Design strategies and recent development

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Abstract

Mono-targeted drug medications are less effective in complex multifactorial disorders involving several biochemical pathways and various types of mutation, such as cardiovascular disease, cancer, diabetes, and neurodegenerative syndromes, due to treatment resistance and side effects. Multiple targets drug simultaneously acting on a variety of targets may have several advantages, including enhanced therapeutic effects, less risk of drug interactions complications, improved patient compliance, and more predictable pharmacokinetics and pharmacodynamics. The multi-targeted anticancer agents such as Sunitinib, Sorafenib, Vandetanib, Pazopanib, Axitinib, Rituximab, Trastuzumab, Raltitrexed, Methotrexate, and Pemetrexed, are in clinically used and huge number are under different stages of clinical trials. The muti-targeted drug design strategies can be divided into three various steps: First is the selection of a target combination; the second is identifying the pharmacophore against individual targets; the last step is combining the identified pharmacophore for the development of the multi-targeted drug. The aim of the present review is to elucidate the recent advances in multi-targeted anticancer drugs.

Key words: Anticancer, design strategies, multi-targeted, recent advances

INTRODUCTION

Cancer is a complicated illness that can damage any organ in the body due to the quick spread of abnormal cells that have the ability to invade nearby tissues and kill them as well as invade other organs. Mono-targeted drugs medications are less effective in complex multifactorial disorders involving several biochemical pathways and various types of mutation, such as cardiovascular disease, cancer, diabetes, and neurodegenerative syndromes, due to treatment resistance and side effects. The simultaneous action of the numerous targets by the multi-targeted medicines increases therapeutic effectiveness and overcomes toxicity and resistance issues.[1-4] The drug research and development nowadays are focusing on multi-targeted agents.[1,2] The simultaneous action of cancer targets for the treatment of cancer is superior to mono-target drug therapy or combination of drug therapies. Multiple targets drug therapy may have several advantages, including enhanced therapeutic effects, less risk of drug interaction complications, improved patient compliance, and more predictable pharmacokinetics and pharmacodynamics.[1,5] These features of multi-targeted anticancer agents make them promising prospects for the discovery of the next generation of anticancer drugs.[2-4] The multi-targeted anticancer agents such as Sunitinib, Sorafenib, Vandetanib, Pazopanib, Axitinib, Rituximab, Trastuzumab, Raltitrexed, Methotrexate, and Pemetrexed, are in clinically used and huge number are under different stages of clinical trials.[2-7]

MULTI-TARGETED DRUG DESIGN STRATEGIES

The muti-targeted drug design strategies can be divided into three various steps: First is the selection of a target

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combination; the second is identifying the pharmacophore against individual targets; the last step is combining the identified pharmacophore for the development of the multi-targeted drug [Figure 1]. The selection of target combinations to design multi-targeted anticancer agents is based on organized high-throughput screening (HTS) and network pharmacology analytic techniques. Through the analysis of intricate and multi-layered networks, network pharmacology offers useful knowledge about target/drug combinations that have synergistic effects and probable pathways for multiple substances at the systemic level.[1-3] After the selection of a target combination, identified the pharmacophore against individual targets by rational and computation approaches such as molecular docking, 3D QSAR analysis, shape-based pharmacophore matching, and combinations of these methods.[1-3] The identified pharmacophore can combine with merged, fused, linked with cleavable or non-cleavable linker to design multi-targeted anticancer agents [Figure 1]. The methodology for the combination is based on the characteristics of key pharmacophore elements and scaffold structures, an appropriate strategy should be used to design multi-targeted drugs. The designed compound of multi-targeted anticancer agents should be able to maintain the interaction with the original target while being compatible with the other target. The computational methods such as structure-based drug design (SBDD), ligand-based drug design (LBDD), *de novo* designing (fragment-based drug designing), multi-target virtual ligand screening (VLS), or the combination of these methodologies are used to design dual or multi-targeted anticancer drugs with remarkable

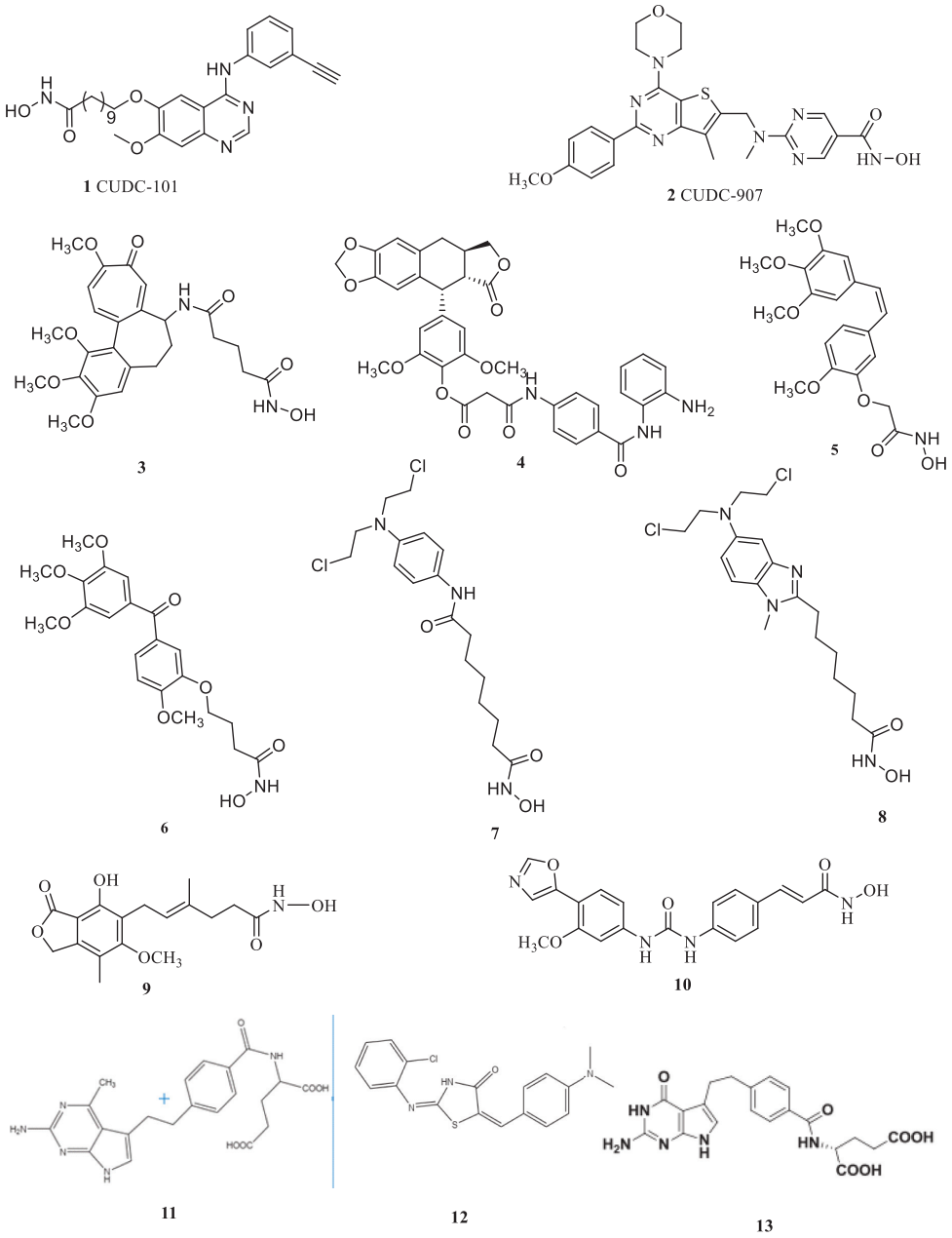

Figure 1: Chemical structure of muti-targeting agents

success to accelerate this process.[1,2,6] The aim of the present review is to elucidate the recent advances in multi-targeted anticancer drugs.

RECENT ADVANCES IN MULTI-TARGETEDANTICANCERAGENTS

In recent years, various multi-targeted anticancer agents have been developed and showed potential biological activity against cancer. Here, we will write over a few examples of multi-targeted agents that have recently been discovered to have anticancer properties.

Multi-Targeted Protein Tyrosine Kinases (PTK) Inhibitors

PTK play a crucial role in cell division, differentiation, and apoptosis.[8] PTKs have approximately 90 members families and are divided into two subfamilies: Receptor tyrosine kinases (RTKs) and non-RTKs. The RTK family is divided into several subfamilies, which include epidermal growth factor receptors (EGFRs), fibroblast growth factor receptors, vascular endothelial growth factor receptors (VEGFRs), insulin and insulin-like growth factor receptors (IR and IGFR), platelet-derived growth factor receptors, hepatocyte growth factor receptors, and proto-oncogene c-KIT.[8,9] The various multi-targeted PTK inhibitors are clinically used for the treatment of various types of cancer such as Imatinib, Dasatinib, Vandetanib, Sunitinib, Sorafenib, Pazopanib, ponatinib, Afatinib, and Lenvatinib. [Table 1]. Multi-target kinase inhibitor Imatinib was the first to be clinically approved by the US Food and Drug Administration in 2001.[9,10]

Histone Deacetylase (HDAC) Inhibitors and RTK

HDAC inhibitors are simple molecular structured compounds and easy to synthesize by multi-step synthesis process. HDAC alters gene expression, affecting angiogenesis, metastasis, and apoptosis, among other processes, by acetylating histones and non-histone proteins.[11-15] The single therapy of HDAC inhibitors leads to therapeutic resistance in cancer treatments due to the stimulation of alternative pathways including AKT and CDK. Therefore, the modification of the chemical structure of HDAC inhibitors in this way can target dual/multi-protein. The RTK and HDAC are involved in a wide range of biological functions. A huge number of drug candidates in this class are designed and synthesized and tested in clinical trials for their anticancer activity. It has been shown that dual targeting RTK and HDAC inhibitors have additive or synergistic effects by downregulating transcriptional or post-transcriptional expression levels of RTKs. Cai *et al*. synthesized compound **1** (CUDC-101) are erlotinib HDAC analogs as EGFR and HDAC inhibitors exhibited the highest activity and is in clinical phase I [Figure 2].[16] The compound **2** CUDC-907 is a first-in-class studied as a dual HDAC and Phosphatidylinositol 3-kinases (PI3Ks) inhibitors reach in various phases of clinical trials for the treatment of several tumors.[17,18]

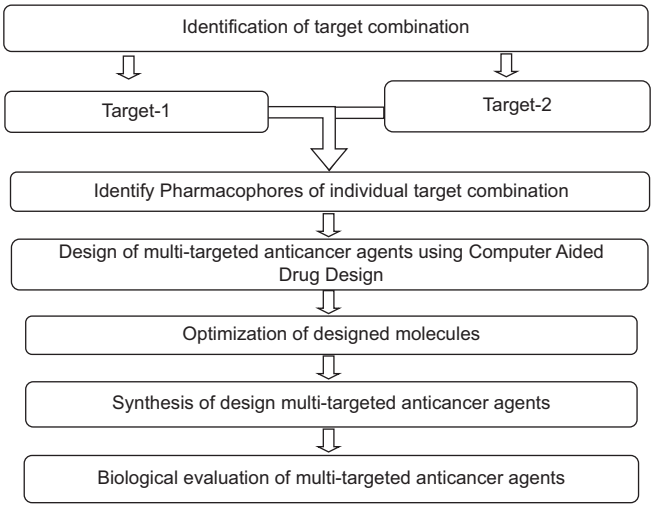

Figure 2: Designing Strategies for the development of multi-targeted anticancer drugs

Table 1: FDA Approved muti-target tyrosine kinase inhibitors used for the treatment of cancer

Inhibitor	First approved	Target	Cancer type
Imatinib	2001	ABL, c-KIT, PDGFR	Ph+ CML, GISTs, ALL
Dasatinib	2006	SFK, ABL	CML, ALL
Vandetanib	2012	EGFR, VEGFR, RET	Medullary thyroid
Crizotinib	2011	ALK, MET, ROS	NSCLC
Sunitinib	2006	VEGFR, PDGFR, c-KIT, FLT-3	GIST, renal
Sorafenib	2005	B-Raf, VEGFR, PDGFR	Renal, hepatocellular, prostate
Pazopanib	2009	VEGFR, c-KIT, PDGFR	Renal; soft tissue sarcoma
Regorafenib	2012	VEGFR, TIE2, PDGFR, RET, c-KIT, RAF	Colorectal
Cabozantinib	2012	VEGFR, RET, MET, TRKB, TIE2	Medullary thyroid

ABL: Abelson kinase, PDGFR: Platelet-derived growth factor receptor, SFK: Src family kinases, HER: Human epidermal growth factor receptor, VEGFR: Vascular endothelial growth factor receptor family, Ph+CML: Philadelphia chromosome+chronic myeloid lymphoma, GIST: Gastrointestinal stromal tumor, NSCLC: Non-small cell lung carcinoma

HDAC and Tubulin Inhibitor

Recent scientific studies suggested that dual HDAC and tubulin inhibitors have potential anticancer activities.[19,20] The combination of SAHA (HDACi) and vincristine (a tubulin inhibitor) achieved synergistic effects in the treatment of leukemia.[19] Most of the tubulin targeting drugs have complex structure, such as vinca alkaloids, taxole, epothilones, and leucinamide while colchicine binding site inhibitors such as Colchicine, Podophyllotoxin.[21,22] Combretastatin A-4 (CA-4) has simple and easy structure to design and development of dual/multi-targeting agents. The trimethoxy phenyl moiety of Colchicine, Podophyllotoxin, CA-4, and its analogs have is forming a hydrogen bond with tubulin protein at colchicine binding site thus inhibit the polymerization of microtubules.[23,24] The pharmacophore of colchicine, 4-deoxypodophyllotoxin, CA-4, and Phenastatin analogs of CA-4 fused linker with ZBG for the designing of dual HDAC and tubulin inhibitors compounds **3, 4, 5,** and **6,** respectively, having the most potent antiproliferative activity [Figure 2].[23-25]

Hybrids Targeting HDAC and DNA Alkylation

One of the earliest anti-cancer treatments to be studied involved DNA-alkylating chemicals, but their clinical application was constrained by resistance, non-selectivity, and considerable side effects. The invention of dual-targeted HDAC and DNA-alkylating compounds, which produce the synergistic effect, may be used to overcome the aforementioned shortcomings.[26,27] Although they have limited potency and poor treatment efficacy when it comes to targeting cellular DNA and affecting DNA damage, DNA-alkylating drugs such as chlorambucil and bendamustine are often utilized in clinical practice around the world. Compounds **7** and **8** are dual-targeting HDAC and DNA alkylating agents with higher anticancer potency and stronger inhibitory activity than the parent compounds as a result of the integration of Bendamustine and Chlorambucil hybrid pharmacophores with linker and ZBG of HDAC inhibitor [Figure 2].[28,29]

HDAC and IMPDH Inhibitor

Inosine monophosphate dehydrogenase (IMPDH) is a nicotinamide adenine dinucleotide (NAD)-dependent enzyme that is important for cell growth and proliferation, making it an appealing target for anticancer drug development.[30,31] The IMPDH inhibitors mycophenolic acid (clinical trials), VX-497 showed potential anticancer activity for cancer treatment. The benzofuranone of **8**, *para*-phenyloxazole of compound **9** derivative bind into the NAD binding site of IMPDH. The opposite terminal group of NAD binding site of IMPDH of the compound was replaced with hydroxamic acid attached with a linker that is perpendicular to the NAD binding site, allowing to insert 11 Å deep narrow tubular channel HDAC proteins and bind to ZBG for the designing of compound **9 and 10** as dual-targeting HDAC and IMPDH Inhibitor. The NAD binding site of IMPDH used as CAP attached with linker and ZBG for HDAC inhibitor showed activity in the micromolar range [Figure 2].[32,33]

Inhibitors of Heat Shock Protein 90 (HSP90) and Tubulin

Recent evidence suggests that having a single molecule that simultaneously inhibits, the combination of HSP90 and tubulin is important for diverse cellular functions, including chromosome segregation during cell division, intracellular transport, development and maintenance of cell shape, cell motility, and distribution of molecules on the cell membranes.[34,35] The simultaneously inhibits of HSP90 and tubulin showed synergist anticancer effects. The compound **11** MDG892 capable to target HSP90 and tubulin protein is designed by combined the pharmacophore of structure and ligand-based virtual screening.[36] Similarly, compound **12** CDBT [2-(2-Chlorophenylimino)-5-(4-dimethylamino-benzylidene) thiazo-lidin-4-one] targeting HSP90 and tubulin were discovered through phenotypic screening including cell proliferation and cell binding assays.[37,38]

Dihydrofolate Reductase (DHFR) and Thymidylate Synthase (TS) Inhibitors

DHFR is an enzyme that reduces dihydrofolic acid to tetrahydrofolic acid and is essential for the *de novo* synthesis of many nucleobases and some certain amino acids. TS is an enzyme that catalyzes the conversion of deoxyuridine monophosphate tothymidine monophosphate using tetrahydrofolic acid as acofactor.[39-41] DHFR and TS are recognized as important targets for cancer chemotherapy due to inhibition of DHFR or TS activity leads to the "thymineless death". The Methotrexate and 5-Fluorouracil are important drugs that inhibit DHFR and TS, respectively, used as anticancer agents. Several antifolates inhibiting TS and DHFR have come into clinical practice as anticancer agents including Raltitrexed, Methotrexate, and Pemetrexed. The combining the pharmacophoric feature of methotrexate and raltitrexed used to develop Pemetrexed **13** as clinically used potent dual inhibitor of DHFR and TS without pharmacokinetic disadvantages.[39-42]

CONCLUSION

Cancer is a complicated condition that involves numerous metabolic processes and different kinds of mutations. A dual-targeted or multi-targeted anticancer drug operates simultaneously on several cancer targets, increasing therapeutic effectiveness and removing toxicity and resistance. Designing dual or multi-target inhibitors are a crucial tactic to combat side effects and medication resistance while improving the effectiveness in managing cancer. The selection of a target combination using methodical

HTS, network pharmacology analysis methodologies, and synergistic effects of the drug combination are the first steps in the design of multi-targeted inhibitors. After identifying the pharmacophore against individual targets using rational or computational methods, the pharmacophore can be merged, fused, and joined using cleavable or non-cleavable linkers to keep the interaction identical to the original target while also being compatible with the second target after being identified against individual targets using rational or computational approaches. To quicken the design of dual or multi-targeted agents, computational methods such as SBDD, LBDD, *de novo* designing (fragment-based drug designing), multi-target VLS, or the mixture of these strategies are being used with success. In conclusion, this review elucidates the recent development of dual/multi-targeted agents as potential anticancer agents.

FUTURE PROSPECT

Designing and finding multi-targeted anticancer drugs that target numerous receptors to trigger the necessary physiological responses is part of a new paradigm for treating complex diseases. A growing number of dual/multi-targeted agents are being designed using the data from internet databases and computer algorithms. The multi-targeted anticancer agents may have an impact on selectivity, membrane permeability, the binding affinity of the receptor, drug pharmacology, therapeutic potency, and drug safety profiles. The multi-targeted anticancer agents such as Sunitinib, Sorafenib, Vandetanib, Pazopanib, Axitinib, Rituximab, Trastuzumab, Raltitrexed, Methotrexate, and Pemetrexed are in clinically used and huge number are under different stages of clinical trials. The exploration of multi-targeted agents would be assisted by biological target network analysis using network pharmacology of target and clinical observations of synergistic drug combinations. The challenge for the successful development of multi-targeted agents is to balance the two activities by combining two pharmacophores with the desired pharmacological properties and safety profiles and concurrently combating anticancer resistance and adverse effects. The cancer target integrated with the pharmacophore of other established drugs has a lot of promise that needs to be explored, and it is anticipated that more such types of hybrid molecules will be under pre-clinical and clinical trials.

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