Strategies on the Development of Small Molecule Anticancer Drugs for Targeted Therapy

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Abstract: The main challenges currently encountered in chemotherapy are the lack of tumor selectivity and drug resistance. The design of novel cytostatic drugs has become the state-of-the-art technology in terms of targeted tumor therapy. This review illustrates the mechanisms and the advantages of representative chemotherapeutic agents, and presents an updated summary of the various drug design strategies developed by modern medicinal chemists during the most recent tumor targeting research which include rational design for overcoming drug resistance, the combi-targeting strategy, the prodrug approach, and tumor specific transporter based drug design. The concept of transporter related tumor targeting strategies for small molecule anticancer drug design discussed in this review may be amenable to predictable drug discovery for targeted therapy.

Keywords: Targeted therapy, anticancer, drug design, drug resistance, prodrug, transporter, conjugate.

INTRODUCTION

Cancer remains among the top three global leading risks for mortality [1]. Tumor diseases arising from the lung, colon, breast, prostate, pancreas, ovary, and testis account for the majority of cancer deaths. Cancer mortality rates for solid tumors have remained largely unchanged in spite of the many advances in cancer research with much better understanding in the biological mechanisms of the diseases, improvements in diagnostic screening, and discovery of an array of new anticancer drugs. Indisputably, there still remains a large gap between effective treatment of cancer and the currently available anticancer drugs.

Traditional approaches to cancer treatment such as surgery, radiation therapy, chemotherapies with various anticancer drugs are still the most effective methods available for treating tumors. Small molecule chemotherapeutics continue to be an important component of cancer therapy due to their efficacy against a broad range of tumor types and their ability to penetrate solid tumors. Protein based therapies are also starting to show promise in treating common cancers, but due to the highly heterogeneous nature of solid tumors which causes poor protein penetration as well as the high cost of the treatment, clinical use of protein drugs is still rather limited.

Due to the non-specific nature, as drugs are usually designed to target the fundamental mechanisms common to all dividing cell populations, toxicity of chemotherapy drugs is the biggest drawback to their clinical use. Toxic side effects not only greatly reduce patients' quality of life, but also limit the chemotherapy dose to below what is required for complete tumor killing. Many chemotherapy drugs not only interfere with cell division, but also impair normal cellular function in non-proliferating cells and cause additional side effects. Some side effects such as immunosuppression, neuropathy, and nausea can in some way be managed with additional drugs. But overall, toxic adverse reactions to chemotherapy drugs have a strong deleterious effect on patients' life. For this reason, an even more serious problem of most existing chemotherapy drugs is that the therapeutic index is so narrow that treatment of solid tumors or metastases has only modest effects on patient survival rates and results rarely in cures [2]. More complete control of tumor growth would require higher drug doses that are too toxic to be clinically useful. It is therefore critical that new chemotherapy drugs must exhibit improved therapeutic indices. Higher tumor selectivity as well as specific activity are two essential properties for the cancer drugs if they could be of effective within the range of tolerated doses.

Targeted tumor therapy delivers toxic payloads selectively into tumors to destroy cancer cells while leaving normal cells unharmed. The past decade has witnessed striking development of improved tumor targeting agents in clinical oncology. These drugs are either evolved from the fundamental knowledge of key pathways involved in oncogenesis which ensures selectivity of the drug, or, alternatively, they were screened from targeting specific biomarkers of cancer cells for specific activity. In particular, discovery of the molecular aspects of angiogenesis has made it possible to test the hypothesis that blocking the angiogenic switch may be able to interfere with initial tumor development, and this has indeed added a new investigational arm to the field of cancer therapy [3-5]. With the two most desired properties of selectivity and specificity, small-molecule anticancer drugs stand out among the main categories of targeted therapy, and are believed to, play an

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increasingly important role in the future of targeted therapy. On the other hand, as solid tumor cancer continues to be recognized as a chronic disease, novel small molecule anticancer drugs with high safety profile and better clinical tumor specificity and selectivity are urgently needed for providing long-term cancer treatment [6].

The present review keeps a special eye on the design strategies of such small molecule anticancer drugs which will be practical for bench-work medicinal chemists and pharmaceutical scientists in drug R&D for targeted therapy.

TARGETED THERAPY DRUGS AND THEIR MECHANISMS

Targeted therapy is about identifying specific differences between cancer cells and normal cells in order to create a tumortargeting drug which exclusively attacks the cancer cells without damaging the normal cells, and thus minimizing undesired side effects. There are different types of targeted therapies, with each type working a little differently but all sharing a common ground of interfering with the cancer cell's ability to grow, divide, repair and/or communicate with other cells. Targeted therapy can generally be divided into the following three broad categories: The first category focuses on the internal components and functions of the cancer cell. This type of targeted therapy usually involves small molecules as they can get inside the cell and directly disrupt the functions of the tumor cells and cause them to die. The second type applies to cases where there is a variety of target receptors on the outside or the surface of the tumor cells. This form of targeted treatment includes monoclonal antibodies, antibody-small molecule conjugates. The last type involves antiangiogenesis inhibitors which target the blood vessels that supply oxygen and nutrients to the cancer cells, ultimately causing the cells to die of starvation.

Successful targeted therapy drugs that have passed the scrutiny of clinical trials and the regulatory standards are mostly developed by implementing one of the strategies mentioned above. To better discuss the design strategies toward novel targeted therapy drugs, we give a brief review, in the following section, of the up to date, currently approved tumor targeting drugs with their manufacturers, mechanisms, and approach rationales. For those small molecule-protein conjugated drugs, we put them into the category of small molecule targeted therapy drugs because the active components responsible for tumor killing in these drugs come from small molecule toxins or radioactive isotopes.

Antibody and Related Drugs

Antibodies are molecules from the immune system that bind selectively to different proteins. The rationale of developing monoclonal antibodies against cancer cells is based on the over expression of tumor specific antigen or other biomarkers. Some surface antigens are present predominantly and sometimes exclusively on malignant cells but not on the surrounding normal cells. These tumor specific antigens therefore become the perfect targets for researchers to develop monoclonal antibodies. When the antibody binds to the tumor surface antigen, it can either trigger a host immune reaction that leads to cell death, or act as a transport vehicle to deliver immunotoxins, radioisotopes, or cytotoxins to harm tumor cells. Several clinically used monoclonal antibody drugs for targeted therapy are used in treating hematologic malignancies.

Small Molecule Targeted Therapy Drugs

Tables **1** and **2** list some important and currently available protein and small molecule anticancer drugs for targeted therapies, with a brief discussion of the proposed mechanisms and a summary of their indications, dosage forms and routes, as well as their main side effects.

DESIGN STRATEGIES AND UNIQUE APPROACHES

Although many of the successful targeted therapy drugs were developed by using the strategies of disrupting those internal and surface targets involved in tumor cell's signaling pathways (e.g. EGFR, HER2, PDGF-Rs, VEGFR etc.), these approaches cannot set a limit to the targeted therapies. Targeted therapy is about differentiation between the cancer cell and the normal cells; and while considering the proven efficacy of many chemotherapeutic drugs, targeted therapy develops new analogs with a similar mechanism of action, but higher tumor-specific accumulation which promises higher tumor selectivity and fewer side-effects. Lower general toxicity will allow higher doses for potentially greater treatment efficacy. In this section, we discuss the concerted effort in anticancer drug research conducted by researchers in medicinal chemistry and describe ideas and strategies developed toward the design and concept of small molecule tumor targeting drugs.

Fighting Drug Resistance

Chemotherapy resistance is generally classified into two categories, namely, intrinsic (de novo) and acquired resistance [39]. De novo resistance is usually associated with endogenous drug efflux and multidrug resistance (MDR) gene expression. For the acquisition of chemotherapy resistance, the most cited mechanisms include expression of energy-dependent transporters that eject anti-cancer drugs from cells, insensitivity to drug-induced apoptosis, and induction of drug-detoxification [40].

Therefore, increasing drug sensitivity is a key step towards improved treatment for cancer patients and it has become a basic criterion in designing targeted therapy drugs. Other than those MDR-related efflux studies on traditional tumor cells, emerging evidence also suggests an intricate role of cancer stem cells (CSCs) and epithelial–mesenchymal transition (EMT)-type cells in anticancer drug resistance [41-43]. Recent studies also demonstrated that micro RNAs (miRNAs) play critical roles in the regulation of drug resistance [44].

Low oxygen levels in tumor hypoxia have also been recognized for several years to be a primary mechanism of tumor resistance to chemo and radiation therapies. Hypoxia is a universal feature of solid tumors. Very recent research on the antitumor agent 3-amino-1,2,4-benzotriazine-1,4-dioxide (tirapazamine, TPZ, **1**) has testified that the activity of the tumor targeting compound is through its ability to selectively damage the DNA in the hypoxic cells found inside the solid tumors. This occurs *via* one-electron

Table 1. Antibody and Protein Related Anticancer Drugs for Targeted Therapy

DRUG	DOSAGE FORM AND ROUTE	INDICATIONS	PROPOSED MECHANISMS	MAIN SIDE EFFECTS
Rituxan (Rituximab) – GENENTECH [7-9] First Approval: November 26, 1997, FDA	Vial; Intravenous	Lymphomas Leukemia Acute myelogenous	The antibody binds to the cluster of differentiation 20 (CD20). CD20 is widely expressed on B cells, from early pre-B cells to later stage during differentiation, but it is absent on terminally differentiated plasma cells. Although the function of CD20 is unknown, it may play a role in Ca^{2+} influx across plasma membranes, maintaining intracellular Ca^{2+}concentration and allowing activation of B cells.	Severe infusion reactions; Cardiac arrest; Acute renal failure.
Herceptin (Trastuzumab) – GENENTECH First Approval: September 1998, FDA	Vial; Intravenous	As a single agent for treatment of HER2-overexpressing breast cancer In combination with paclitaxel for the first-line treatment of HER2-overexpressing metastatic breast cancer	Trastuzumab is a humanized monoclonal antibody that binds to domain IV of the extracellular segment of the HER2/neu receptor. The HER2 gene (also known as HER2/neu and ErbB2 gene) is amplified by 20-30% of early-stage breast cancers, which makes it overexpressed [10]. HER2 extends through the cell membrane, and carries signals from outside the cell to the inside. The signals pass through different biochemical pathways and promote invasion, survival and growth of blood vessels (angiogenesis) of cells [11]. Trastuzumab suppresses angiogenesis of the tumor cells and induces the growth of immune cells to cure cancer. Experiments in laboratory animals indicate that, when trastuzumab is bound to a cell, the cell will be killed by induced immune cells, therefore such antibody-dependent cell-mediated cytotoxicity may also be an important mechanism of the drug [12].	Cardiac dysfunction in 2-7% of the cases [13]; Cardiomyopathy is increased when trastuzumab is combined with anthracycline chemotherapy (which itself is associated with cardiac toxicity).
Campath (Alemtuzumab) – ILEX PHARMACEUTICALS First Approval: May 10, 2001, FDA	Vial; Intravenous	(B-CLL)/B-cell chronic lymphocytic leukemia	Alemtuzumab is a recombinant DNA-derived humanized monoclonal antibody that is directed against the 21–28 kDa cell surface glycoprotein, CD52. Alemtuzumab targets CD52, a protein present on the surface of mature lymphocytes, but not on the stem cells from which these lymphocytes are derived.	Low blood counts; Nausea and vomiting; Infection.
Erbitux (Cetuximab) IMCLONE [14, 15] First Approval: February 12, 2004, FDA	Vial; Intravenous	Colorectal cancer Head and neck cancer	Cetuximab targets and binds to the epidermal growth factor receptors (EGFR) on the surface of the cell. EGFR is found on the surfaces of many normal and cancer cells, but clinical studies indicate that many cancers overexpress this type of receptors. By binding to these receptors, Cetuximab blocks an important pathway that promotes cell division, and this results in inhibition of cell growth and tumor cell apoptosis (cell suicide).	Acne-like Rash; Fevers; Rigors; Urticaria; Pruritis; Hypotension.
Vectibix (Panitumumab) – AMGEN First Approval: September 27, 2006, FDA	Injectable; Intravenous (Infusion)	Colorectal cancer Colon cancer that expresses EGFR	Panitumumab is a targeted therapy that targets and binds to EGFR on the surface of the cell.(See Erbitux)	Skin reactions; Hypomagnesemia.

enzymatic reduction of TPZ to yield an oxygen-sensitive drug radical (**2**) that leads to oxidatively generated DNA damage under hypoxic conditions. Two possible mechanisms have been proposed to account for the oxidatively generated DNA damage by TPZ. First mechanism suggests the homolysis of the N-OH bond in **2** yields the well-known DNA-damaging agent, the hydroxyl radical. The alternative mechanism suggests the elimination of water from **2** which generates a benzotriazinyl radical (**4**) as the ultimate DNA-damaging species. (Fig. **1**) [45]

Platinum-based chemotherapy drugs are among the most investigated drug resistance examples. Ohmichi *et al*. concluded that the reduced platinum accumulation plays a key role in drug resistance rather than the increased drug efflux because the main multidrug resistance efflux pump, P-glycoprotein, is not usually overexpressed in cisplatin-resistant tumors [46].

Many challenging approaches have been taken by medicinal chemists to overcome this issue. Some of the

Table 2. Summary of Currently FDA Approved Small Molecule Anticancer Drugs for Targeted Therapy

DRUG	DOSAGE FORM AND ROUTE	INDICATIONS	PROPOSED MECHANISM	MAIN SIDE EFFECTS
Femara(Anastrozole) – ASTRAZENECA [16] First Approval: December 27, 1995, FDA	Tablet; Oral	Estrogen receptor positive or hormone receptor unknown locally advanced or metastatic (spread) breast cancer.	Anastrozole is a hormone therapy. It is classified as an aromatase (found in the body's muscle, skin, breasts and fat) inhibitor. Anastrozole blocks the enzyme aromatase which is used to convert androgens (hormones produced by the adrenal glands) into estrogen. In the absence of estrogen, tumors dependent on this hormone for growth will shrink.	Vaginal bleeding; Allergic reaction; Difficulty breathing.
Ontak (Denileukin Diftitox)- SERAGEN [17,18] First Approval: February 5, 1999, FDA	Vial; Intravenous	Cutaneous T-cell lymphoma (CTCL)	Denileukin Diftitox is an engineered protein combining Interleukin-2 and Diphtheria toxin. This can bind to Interleukin-2 receptors which some leukemias and lymphomas malignant cells over express this kind of receptor. The drug introduces the diphtheria toxin into the target and kill the tumor cells.	Infusion reactions; Capillary leak syndrome; Loss of vision.
Mylotarg (Gemtuzumab Ozogamicin) – WYETH PHARMS INC [19-21] First Approval: May 17, 2000, FDA	Vial; Intravenous	Acute myelogenous leukemia.	Gemtuzumab Ozogamicin is a humanized monoclonal antibody to CD33 linked to a cytotoxic agent from the class of calicheamicins. CD33 is expressed in most leukemic blast cells, the intensity of which diminishes with the maturation of stem cells.	Myelosuppression; Type III hypersensitivity; Shivering; Fever; Nausea and vomiting.
Gleevec (Imatinib) – NOVARTIS [22-24] First Approval: May 10, 2001, FDA	Capsule; Oral	Philadelphia chromosome positive chronic myeloid leukemia (Ph+ CML) Philadelphia chromosome + acute lymphoblastic leukemia (Ph+ ALL) C-kit positive gastrointestinal stromal tumors	Imatinib belongs to the signal transduction inhibitor category of targeted therapies. It functions as a specific inhibitor of a number of tyrosine kinase enzymes. Imatinib is specific for the *TK* domain in *abl* (the Abelson proto-oncogene), c-kit and PDGF-R (platelet-derived growth factor receptor) which functions to stimulate cell division. It works to prevent the pro-growth signals sent by these enzymes found in cancerous cells.	Low blood counts; Nausea and vomiting; Edema; Muscle cramps and bone pain; Diarrhea; Skin rash.
Zevalin (Ibritumomab Tiuxetan) – SPECTRUM PHARMS [25] First Approval: February 19, 2002, FDA	Vial; Intravenous	Follicular non-Hodgkin's lymphoma	Ibritumomab Tiuxetan is a radioactive small molecule antibody conjugate. The antibody binds to the CD20 antigen found on the surface of normal and malignant B cells, allowing radiation from the attached isotope to kill it and some nearby cells. In addition, the antibody itself may trigger cell death *via* antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and apoptosis.	Cytopenias; Fatigue; Abdominal pain; Nausea; Nasopharyngitis; Asthenia; Diarrhea; Cough and pyrexia.
Iressa (Gefitinib) – ASTRAZENECA [26, 27] First Approval: May 5, 2003, FDA	Tablet; Oral	Locally advanced or metastatic non-small cell lung cancer	Gefitinib is a selective inhibitor of the EGFR tyrosine kinase domain. Thus the function of the EGFR tyrosine kinase in activating the Ras signal transduction cascade is inhibited by this molecule, which leads to the death of the malignant cells.	Acne; Diarrhea; Nausea and vomiting; Anorexia; Stomatitis.

(Table 2). Contd…..

DRUG	DOSAGE FORM AND ROUTE	INDICATIONS	PROPOSED MECHANISM	MAIN SIDE EFFECTS
Velcade (Bortezomib) – MILLENNIUM PHARMS [28-30] First Approval: May 13, 2003, FDA	Injectable; Intravenous	Relapsed multiple myeloma Mantle cell lymphoma	The boron atom in Bortezomib binds to the catalytic site of the 26S proteasome with high affinity and specificity. Proteasome inhibition may prevent degradation of pro-apoptotic factors, permitting the activation of programmed cell death in neoplastic cells which are dependent on the suppression of the pro-apoptotic pathways.	Peripheral neuropathy; Myelosuppression Shingles; Gastro-intestinal (GI) effects; Asthenia.
Tarceva (Erlotinib) – OSI PHARMS [31, 32] First Approval: November 18, 2004, FDA	Tablet; Oral	Locally advanced or metastatic non-small cell lung cancer (NSCLC) Locally advanced, unresectable or metastatic pancreatic cancer	Erlotinib is designed to block tumor cell growth by targeting a protein EGFR. (see Erbitux).	Rashes; Diarrhea; Fatigue; Loss of appetite.
Sutent (Sunitinib) – CPPI CV [33, 34] First Approval: January 26, 2006, FDA	Capsule; Oral	Renal cell carcinoma (RCC) Gastrointestinal stromal tumor (GIST)	Sunitinib is a receptor protein-tyrosine kinase inhibitor. It inhibits cellular signaling by targeting multiple receptor tyrosine kinases (RTKs). These include all receptors for platelet-derived growth factor (PDGF-Rs) and vascular endothelial growth factor receptors (VEGFRs). The simultaneous inhibition of these targets leads to both reduced tumor vascularization and cancer cell death, which ultimately result in tumor shrinkage.	Fatigue; Diarrhea; Nausea; Anorexia; Hypertension.
Tykerb (Lapatinib) – SMITHKLINE BEECHAM[35,36] First Approval: March 13, 2007, FDA	Tablet; Oral	Breast cancer	Lapatinib inhibits the tyrosine kinase activity associated with two oncogenes, EGFR and HER2/neu (Human EGFR type 2). Lapatinib has shown to decrease tumor-causing breast cancer stem cells. Lapatinib inhibits receptor signal processes by binding to the ATP-binding pocket of the EGFR/HER2 protein kinase domain, preventing self-phosphorylation and subsequent activation of the signal mechanism of tumor cells.	Diarrhea; Fatigue; Nausea; Rashes.
Torisel (Temsirolimus) – WYETH PHARMS INC [37, 38] First Approval: May 30, 2007, FDA	Solution; Intravenous	Advanced renal cell cancer	Temsirolimus is an inhibitor of mTOR. It blocks the translation of genes that regulate cancer cell proliferation, and also causes reduced levels of certain cell growth factors involved in the development of new blood vessels, such as vascular endothelial growth factor (VEGF).	Weakness; Low blood counts; Rashes; Mouth sores; Nausea; Swelling.

studies have focused on the solubility of the platinum complexes (Fig. **2**) in aqueous solution [47-49]. However, simply improving the water solubility of the platinum-based complex will although increase its stability but unfortunately in most of the cases it will rather decrease the cellular drug uptake if the complex does not have special functional groups that can target cell membrane drug-uptake transporters.

The rational approach for compounds bearing the phosphonic acid functional group is based on its mineral bone-targeting property as bisphosphonates show a high affinity for bone and other calcified tissues [50], targeting of a cytotoxic moiety with a phosphonate functional group is suitable to bone-related diseases. The therapeutic activity of platinum phosphonate complexes *in vivo* involves reduction of both bone tumor volume and anti-metastatic activities [51, 52].

One strategy used to overcome drug resistance is to design and build specific functionalities into the platinum-based complexes so as to enhance uptake *via* drug targeting or simply inhibit resistance mechanisms. Barnes *et al.* designed an ethacrynic acid (EA) tethered platinum complex, ethacraplatin **5**, to target GST enzymes in human cancer cells (Fig. **3**) [53]. EA belongs to GST inhibitors and has been

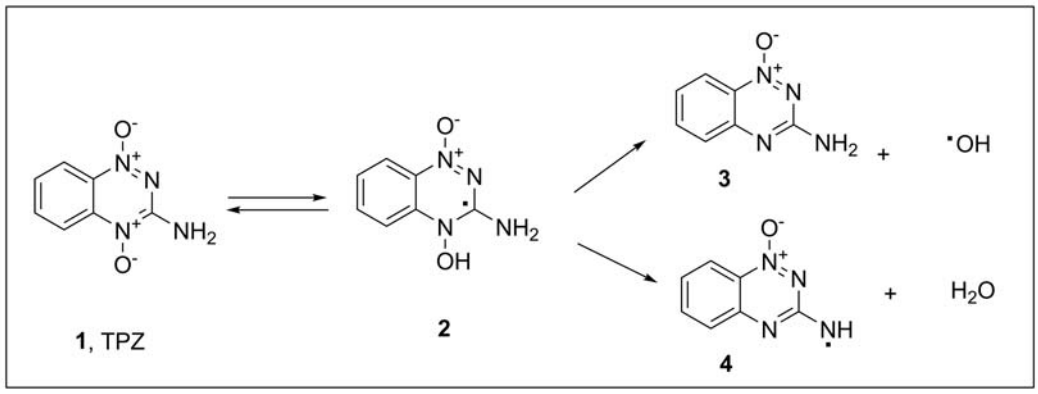

Fig. (1). Hypothetical mechanism of tirapazamine.

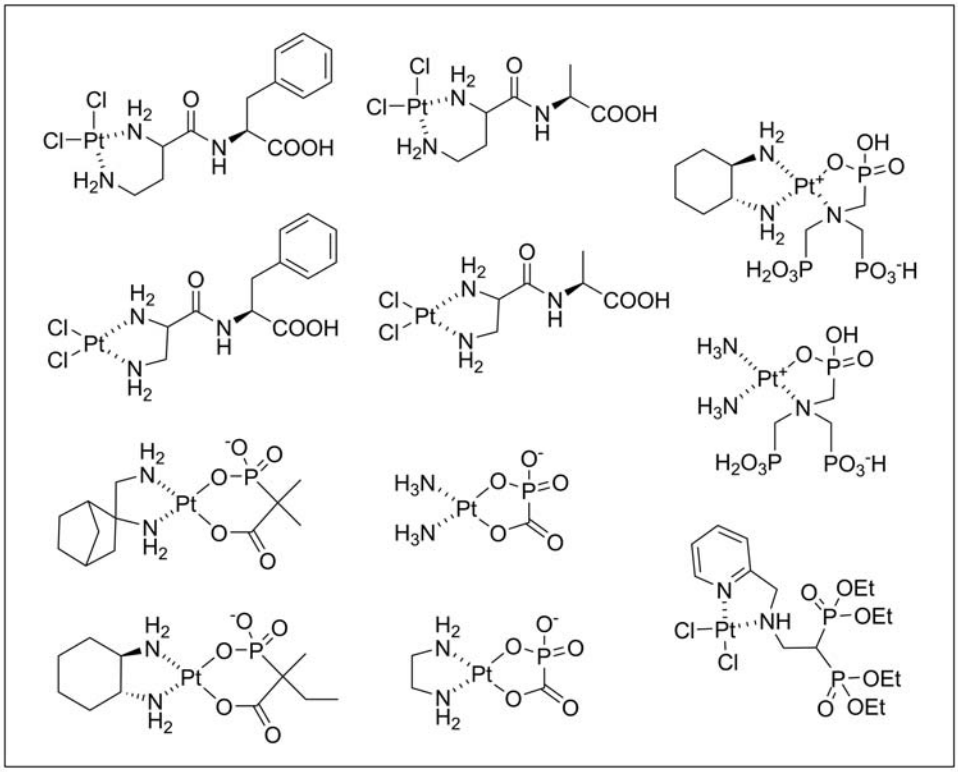

Fig. (2). Water soluble platinum complexes and phosphonate-type of platinum compounds.

tested in combination with a range of alkylating agents against multidrug resistant cancers as an adjuvant [54, 55]. Release of EA after *in vivo* drug uptake can reverse cisplatin-associated drug resistance. The assay result showed that compound **5** is a potent GST inhibitor, more powerful than EA itself, and is capable of reducing the activity of GST enzymes to less than 10% of the original activity. The great experimental results of growth inhibition of this compound against cisplatin-resistant cancer cell lines in comparison with cisplatin suggests a successful strategy for this approach.

Another unique approach conducted by Lippard's group used estrogen-tethered platinum (IV) to selectivily induce overexpression of high-mobility group domain proteins, HMGB1, a protein that shields cisplatin-DNA adducts from nucleotide excision repair (Fig. **4**) [56]. The ability of compound **6** to up-regulate HMGB1 levels was investigated by immunofluorescence microscopy in MCF-7 cells and showed a similar degree of effectiveness as treatment with an equal amount of esdradiol itself, and this implies that ER(+) cells will be more sensitive than ER(-) cells toward the antitumor compounds. Therefore, compound **6** is concluded to be significantly more cytotoxic in the MCF-7 cells. Lippard recently showed a new dichloroacetate (DCA)-based platinum compound that is able to destroy tumor cells more efficiently than cisplatin itself [57]. This study focused on the drug sensitivity issue of the platinum-based complex and indirectly answered the question of how to strategically circumvent drug resistance.

As was mentioned earlier, recent studies have shown that cancer stem cells (CSCs) and epithelial–mesenchymal transition (EMT)-type cells can play critical roles in drug

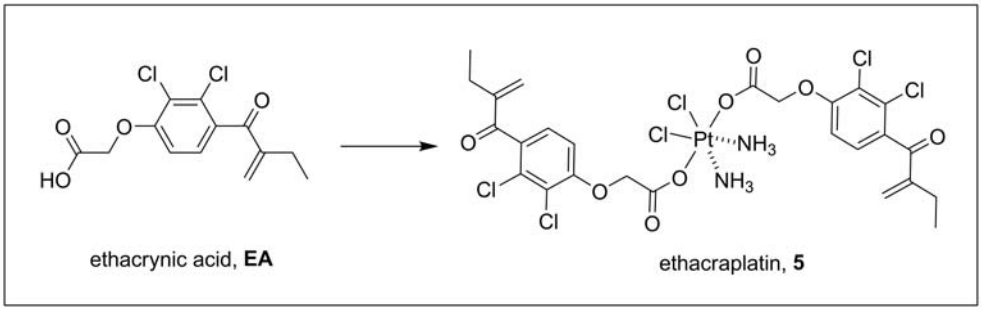

Fig. (3). Anticancer drug designed from combination of GST inhibitor and platinum complex.

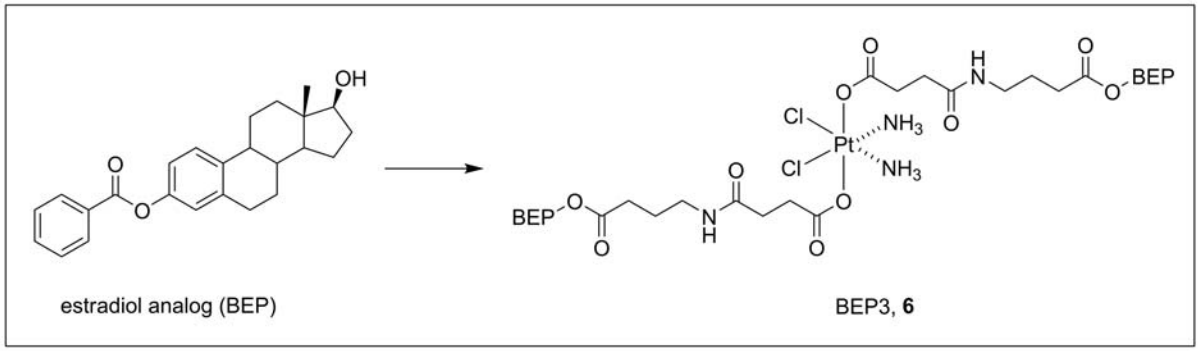

Fig. (4). Anticancer drug designed from combination of estradiol and platinum complex.

resistance. Thus, molecular design strategies to circumvent drug resistance related to CSCs and EMT-type cells is now considered a focal point in targeted therapy [58, 59]. Over the past 20 years, acquired resistance mediated by DNA repair enzymes has often imposed severe limitations to the use of DNA-interactive agents and in many cases useful clinical antitumor activity could only be observed with the administration of multiple antitumor drugs, consisting of different mechanisms of actions. Based on this observation, we summarize below some novel compounds possessing multiple intracellular targets that are expected to be more effective against resistant tumors than their classical counterparts and the structures and properties of which may offer useful insights into design strategies.

The Combi-Targeting Strategy

The combi-targeting concept refers to designing an optimized single molecule which possesses dual targeting properties. In most cases the parent molecule targets a specific tumor related pathway on its own and then further degrades to another species of a different inhibitor or cell killing agent. Domarkas *et al.* synthesized a series of such combi-targeting molecules to target different tumor related pathways (Fig. **5**) [60-62]. Among them, **SMA41** retained the strongest affinity for the ATP binding site of EGFR in an enzyme assay and blocked EGF-induced tyrosine phosphorylation and EGFR autophosphorylation in A431 cells in a dose-dependent manner. With long term drug exposure (3–6 days to sulforhodamine and clonogenic assays), **SMA41** showed more sustained growth inhibitory activity than its degraded molecules. The study has conclusively demonstrated that this one-molecule combination is of superior activity compared to a two-drug combination involving the degraded parts. The increased potency of **SMA41** may be due to a combination of events associated with its binary EGFR TK and DNA targeting properties.

Other combi-targeting designs from this group include **ZRF1**, **ZRCM5** and **AKO4** (Fig. **6**) [63-65]. For example,

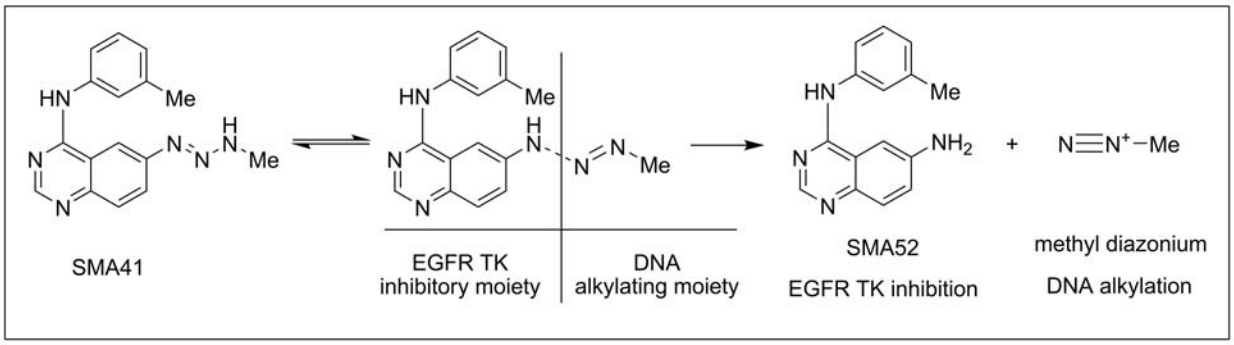

Fig. (5). Anticancer drug SMA41 designed from combi-targeting strategy.

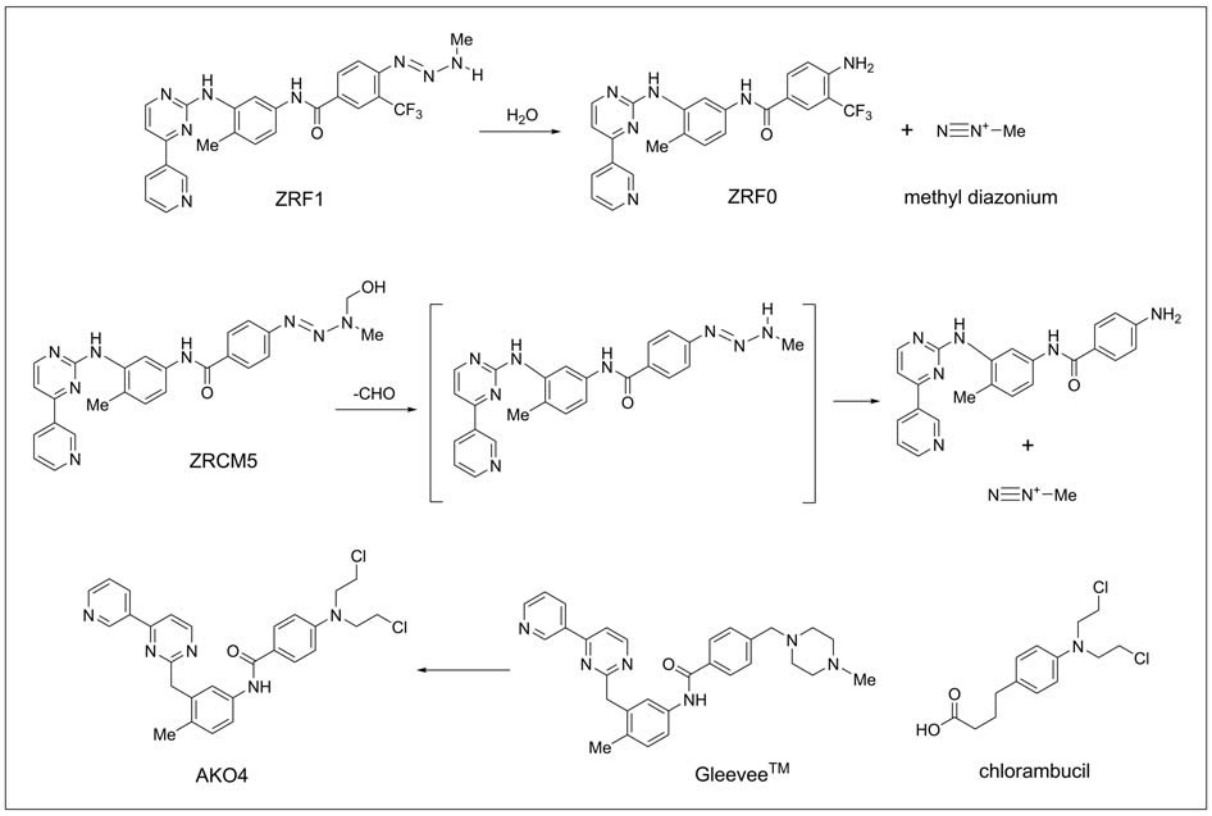

Fig. (6). Anticancer drugs ZRF1, ZRCM5, and AKO4 designed from combi-targeting strategy.

AKO4 was designed to behave as a combination of a bcrabl kinase inhibitor similar to STI571 (Gleevee or Glivec) and a DNA damaging agent close to chlorambucil. The design includes replacing the methylpiperazine ring of STI571 by a nitrogen mustard function fused with a benzamide spacer, leading to the 2-phenylaminopyrimidine aniline mustard conjugate [65].

As another type of combination approach, Pasini and coworkers investigated a platinum complex bearing doxorubicin as the bioactive carrier ligand, (compound **7**, Fig. **7**) to form a multifunctional drug by combining two antitumor agents which are often administrated together in combination chemotherapy [66]. The resulted complex is active against both doxorubicin-resistant P388 leukemia cell and cisplatin-resistant L1210 leukemia cell while maintaining antitumor activity against sensitive parent cell lines.

Prodrug Approaches to Enhance Selectivity

Prodrugs are bioreversible derivates of drug molecules that undergo an enzymatic or chemical cleavage *in vivo* to release the active parent drug. This is done to increase the efficacy of a drug through improved physicochemical, biopharmaceutical or pharmacokinetic properties of the compound. About 5-7% of all the drugs approved worldwide nowadays can be classified as prodrug, while in 2001 and 2002 approximately 15% of the new drugs approved were prodrugs. This shows that prodrug research is a growing trend. Current trends in targeted anticancer prodrug design

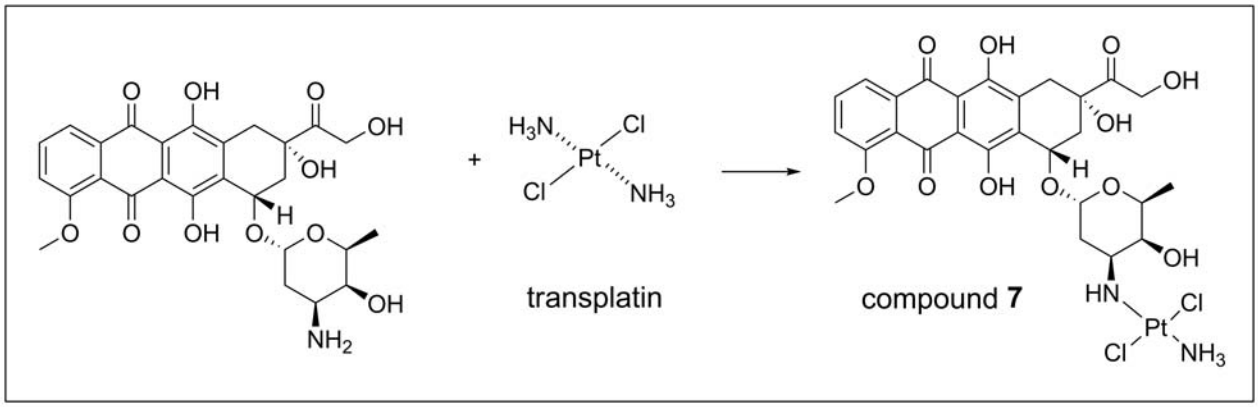

Fig. (7). Multifunctional anticancer drug designed from two antitumor agents.

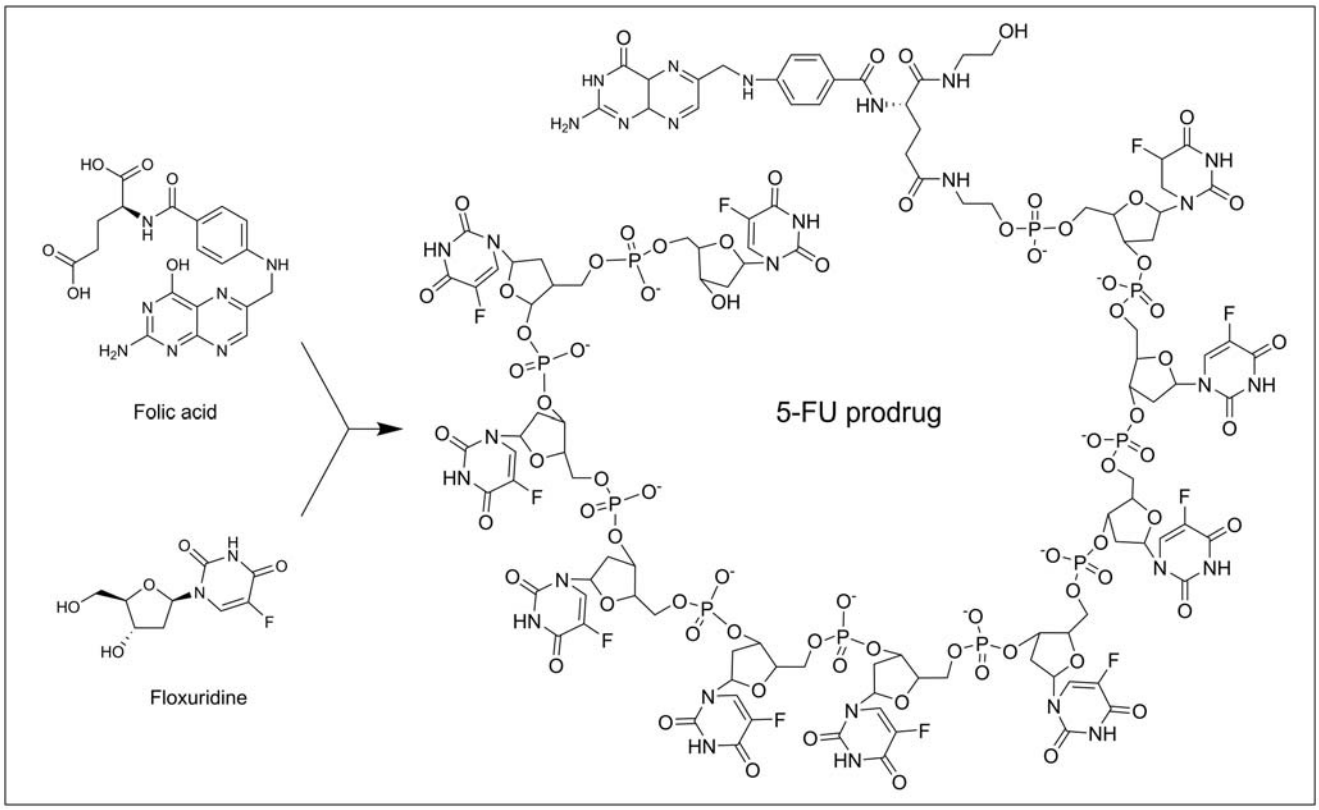

Fig. (8). Prodrug for targeted therapy designed from folic acid and 5-FU precursor.

hinge on selective delivery of anticancer agents to tumor tissues while avoiding their cytotoxic effects on noncancerous cells.

One of the most attractive molecular targets emerge in this area is the folate receptor (FR), for the reasons that, firstly, it is overexpressed on many tumors, including breast, lung, kidney, ovary, and brain cancers, as well as myelogenous cells; and secondly, it is present in low or non-detectable quantities in most normal tissues. Moreover, the vitamin folic acid and its drug conjugates bind to FR with nanomolar affinity and enter cancer cells by receptor-mediated endocytosis. Thus, development of folate-tethered gene therapy vectors, immunogenic haptens, protein toxins, liposomes, imaging agents, and low molecular weight drugs has attracted special attention from researchers [67].

Liu *et al*. synthesized FA-FdUMP prodrug which has proven to be useful for the treatment of 5-FU-resistant malignancies. The synthesized fluorinated pyrimidine prodrug is oligodeoxyribonucleotides (ODN) composed of *N*-5-fluoro-2'-deoxyuridine-5'-O-monophosphate (FdUMP) nucleotides. The *in vitro* assay result showed that FA-FdUMP was about 10-fold more cytotoxic toward H630 cells than FdUMP, and between 10 and approximately 10000-fold more cytotoxic than 5-FU (Fig. **8**) [68].

Liu and coworkers designed and synthesized a folate-mediated prodrug by attaching camptothecin (CPT) to folate through a hydrophilic spacer linked to folate *via* a cleavable disulfide carbonate moiety (Fig. 9) [68]. CPT is a cytotoxic quinoline alkaloid which inhibits the DNA enzyme topoisomerase I. The synthesized CPT conjugate inhibiting cell proliferation in human KB cells and folate receptor mediated uptake were demonstrated.

Steinberg and Borch synthesized and evaluated pteroic acid conjugates of nitroheterocyclic phosphoramidites (alkylating agent) for folate receptor targeting (Fig. **10**) [69]. This nitroheterocyclic bis(haloethyl)phosphoramidate prodrug linked through lysine to a pteroic acid has been designed as a potential alkylating agent to target tumor cells that overexpress the folate receptor.

Tumor Specific Transporter Targeted Strategy

Membrane transporters are integral plasma membrane proteins that mediate the uptake of different substrates including polar nutrients, amino acids, peptides, nucleosides, and sugars. Transporters in general fall into two major families: (1) the ATP-binding cassette (ABC) family, which includes those transporters responsible for drug resistance through efflux transporters like Pgp; and (2) the solute carrier (SLC) transporters, which are capable of influx into the cell [70].

Imaging studies of human tumors using PET provides the strongest support for the hypothesis that tumors exhibit unusually high levels of nutrient transport. The most widely used imaging reagent is fluorodeoxyglucose (FDG). FDG accumulates to high levels in many kinds of solid tumors, and has proven to be very useful for detecting the presence of solid tumors. It is thought that FDG is taken up into tumor cells by sugar transporters that are overexpressed in tumor tissues and quickly trapped in cells by phosphorylation.

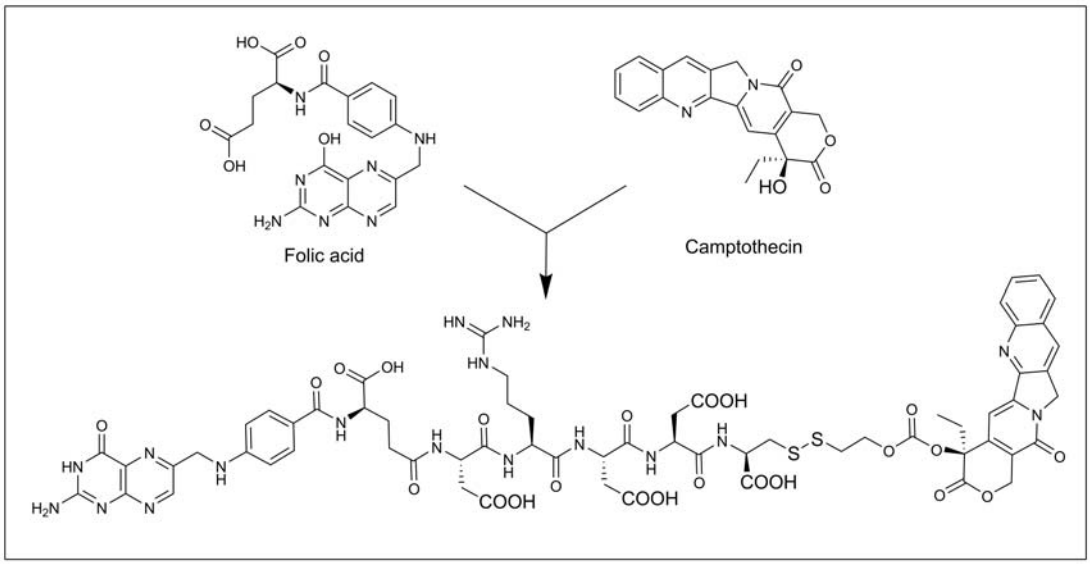

Fig. (9). Folate conjugate anticancer drug designed from folic acid with antitumor agents.

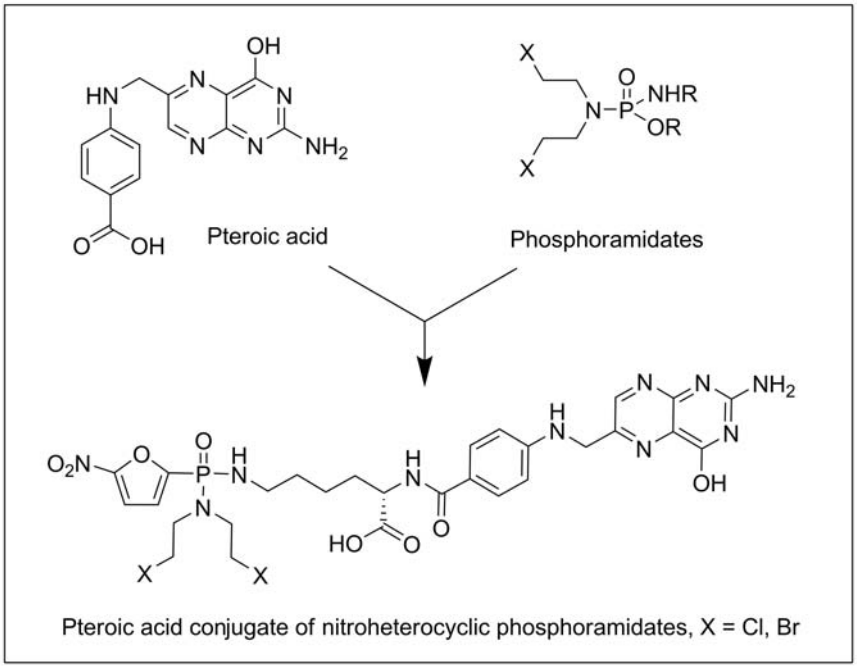

Fig. (10). Anticancer prodrug designed by targeting folate receptor with pteroic acid and phosphoramidate.

An extensive body of literature has demonstrated tumor overexpression of the facilitative glucose transporter GLUT1 [71], and in certain tumors, GLUT3 and GLUT5 [72, 73]. Especially, the presence of GLUT1 appears to be strongly correlated with patient prognosis. High GLUT1 expression is usually indicative of more advanced cancer and reduced life expectancy. More recently, iodomethyltyrosine, a substrate for amino acid transporters has been shown to be useful for imaging solid tumors as well. These studies indicate that tumors overexpress nutrient transporters and exhibit enhanced nutrient uptake relative most normal tissues.

To achieve greater tumor-specific drug accumulation, targeted tumor drug design can be executed in three different ways: 1) design of drug molecules to be substrates of the tumor high express transporters, especially through using DNA-interactive agents that can be actively uptake by the tumors; 2) design of inhibitors to block tumor specific transporters which causes damage to the tumor cells or tumor stem cells; 3) design of prodrugs containing promoities that can act as substrates for the desired tumor transporters to deliver cytotoxics into targeted tumor tissues.

The sodium-dependent multivitamin transporter (SMVT) is another membrane transporter that has been exploited for targeted prodrug design. Studies from Sinko's group showed that human ovarian carcinoma cell line A2780 and its multidrug resistant form A2780/AD expresses high levels of SMVT, which can potentially be exploited for the uptake of anti-cancer prodrug conjugates (Fig. **11**) [74]. This transporter is also responsible for the uptake of several essential nutrients including pantothenate, biotin, and lipoate

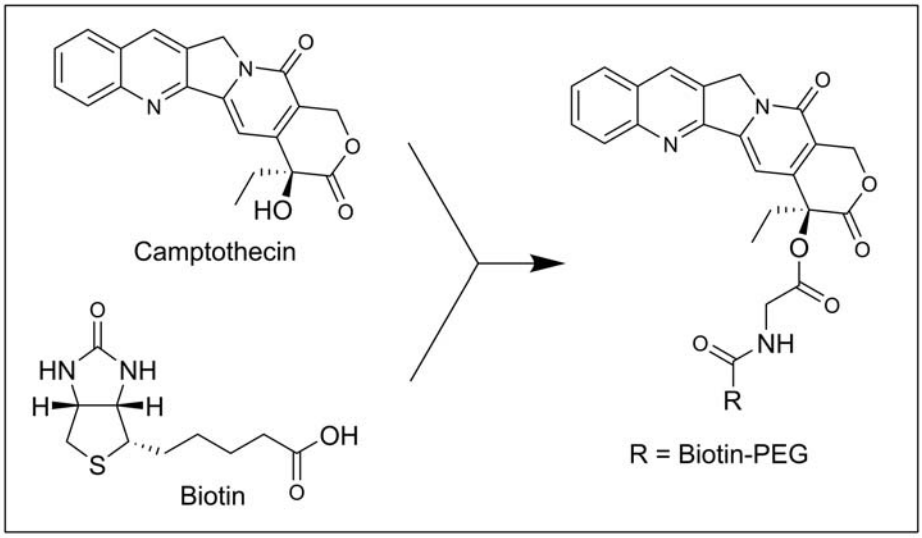

Fig. (11). Anticancer prodrug designed by targeting active transporter with biotin and camptothecin.

into cells [75, 76]. Minko *et al*. synthesized CPT-PEG-biotin prodrug which showed an increased cytotoxicity of CPT by 12- and 60-fold respectively in A2780 cells and by 12- and 30- fold respectively in multidrug resistant A2780/AD cells. The cytotoxicity of the conjugates was attributed to the enhanced apoptotic effects by the activation of the caspase pathway. The research suggested that the targeting approach utilizing transporters such as SMVT may substantially improve the delivery of CPT as well as its antitumor selectivity by enhanced cellular uptake and possible retention of CPT [77].

Studies on malignant brain tumors carried out by Mathupala *et al.* have found another very useful tumor targeting transporter group that MCT 1 and MCT 2 (monocarboxylate transporters) are highly expressed in malignant glioma [78, 79], while MCT 3 (previously known as type 4) is predominant in normal brain tissue [80]. Having trans-membrane transporters exposed to the extracellular milieu, the MCTs are amenable to targeting by systemic application of small-molecule inhibitors. The "classic" inhibitors of monocarboxylate transporters have been derivatives of cinnamic acid, first identified by Halestrap and coworkers for their effect on isolated mitochondrial pyruvate transport [81], and later by Lehninger and co-workers on intact Ehrich ascites tumor [82]. More studies by other groups [83, 84] have indicated that the cinnamic acid derivatives can also be competitive inhibitors of lactate transport in tumors: the α-cyano-4-hydroxy cinnamic acid (ACCA), as one of the most potent inhibitors of lactate transport, with a K*i* of 0.5 mM. ACCA has been used to treat glioma and is highly radiosensitive. Inhibition of lactate efflux *via* ACCA in glioma also resulted in key changes to intracellular metabolites including significant reductions in radio-protective metabolites, including reductions in glutathione and taurine [79]. These results from Mathupala and coworkers have paved the way for a future combined therapeutic strategy, where the tumors are firstly exposed to lactate transport inhibitors to enhance their radiosensitivity immediately prior to radiotherapy. When ACCA was delivered to the tumor bed in an orthotopic nude rat glioma model, the survival rate of the rats was enhanced by approximately 2-fold, indicating that the strategy of targeting malignant tumors *via* inhibition of lactate efflux holds therapeutic applicability without significant side-effects. On the other hand, from our experience and unpublished results, we suggest another unique approach to be achieved through a rational design by using MCT inhibitor or substrates coupled with appropriate anticancer agents and therefore reduced adverse effects as a successful tumor targeting strategy for targeted cancer therapy (Fig. **12**).

CONCLUSIONS

Even the best chemotherapeutic agents are nowadays being slowly supplemented by a new generation of drugs

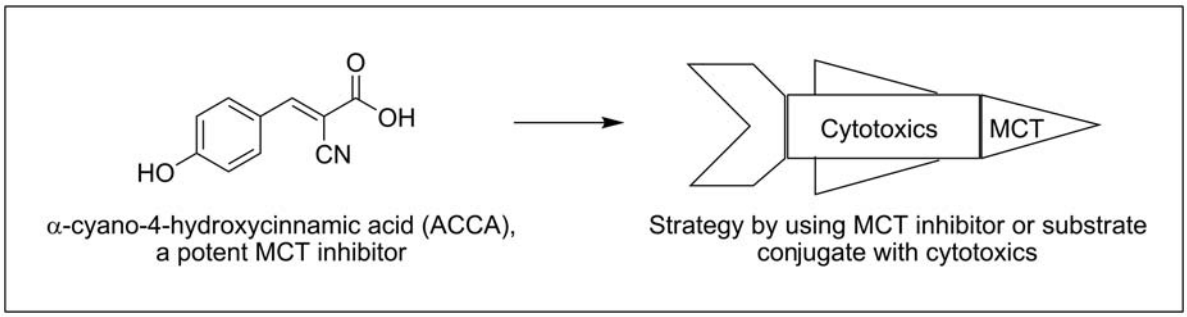

Fig. (12). Design concept of tumor therapy drug by targeting active transporters.

that well designed by medicinal scientists to recognize tumor specific targets. Although these molecular and genetic approaches and strategies are still not perfect for our patients, targeted therapies hold the promise of much higher effectiveness and markedly fewer side effects. However, researchers agree that targeted therapies shouldn't be a simple replacement for traditional therapies, but the best way to cure cancer is to jointly use both therapies. Both the combi-targeting strategy and multifunctional prodrug approach have been conventionally employed to enhance the efficacy and tumor selectivity. With the discovery of a large number of tumor specific membrane transporters and receptors, the design of prodrugs that exclusively target these membrane transporters claims significant therapeutic potential. Both inhibitory transporter targeting and active transported drug delivery strategy can be successfully employed for tumor targeting at the cellular level, and they can be also used for gene delivery to modify the cellular functions toward the tumorspecific receptors.

Clearly, in order to effectively treat cancer, novel therapies and drugs must be developed to eliminate cancer stem cells (CSC). Biomarkers obtained and validated from CSC animal models provide the basic tools for designing such drugs. These targets can be used to identify either small molecule or antibody-based drug candidates, and all strategies and approaches described in this review are also applicable in CSC targeted therapy drug development.

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