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AN OVERVIEW OF OPTIMAL MANAGEMENT OF CANCER BY COMPUTER AIDED DRUG DESIGN

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ABSTRACT

In the developed world, cancer is the leading cause of death whereas in the developing world it is the second leading causes of death and cancer is the most critical obstacle for the increase of global life expectancy. The design and development of anticancer drugs via traditional drug development has been estimated that around 12 years and 2.7 billion USD, on average. To overcome these demerits in new drug development computer- aided drug discovery/design (CADD) methods have been developed. Computational methods can be utilized to design experiments, and elucidate structure-activity relationships for drug discovery and lead optimization methods. Various approaches of CADD are evaluated as promising techniques according to their need, in between all these structure-based drug design and ligand-based drug design approaches are known as very efficient and powerful techniques in drug discovery and development. The two approaches of drug design ligand based by topoisomerase and structure based by peptide deformylase inhibitors.

KEYWORDS: Anticancer, Computer Aided Drug Design, structure-based drug design, ligand-based drug design, Topoisomerase, peptide deformylase inhibitors.

1. INTRODUCTION

1.1 CANCER

Cancer is one of the most dreadful diseases all over the world and 1% of annual increase in cancer incidence and mortality has been estimated, by 2030 there could be 26.4 million new patients with cancer, 1.7 million annual cancer deaths and 80 million people alive with cancer within five years of diagnosis. [Schomall et. Al 2009] Cancer manifests while cells have unusual growth and spread in parts of the body. The main reason of cancer is the cells that

expose to material including ultraviolet lights and radiation. Therefore, chemically harmed cells caused cancer. 22% of deadly cancers are related to the use of tobacco and 10% of deadly cancers depend on to the nutrition. In fact, 90-95% of cancers occurred because of environmental issues like drug abuse, nutrition, stress, lake of physical activity and environmental pollutants. Remaining 5-10% is due to inherited cancer genetics. [Cole et. Al. 2015]

Cancer treatment methods are different and they are related to the size, depth and the stage of tumour. The oncologist is in-charge of choosing the strategy of the treatment by considering the condition of patient and tumour as a discipline juncture of physics and biology. Radiation oncologist addresses the therapeutic uses of ionization radiation alone or in combination with other treatment modalities such as surgery, drugs, oxygen and heat. For final remedy in clinical practice, a multidisciplinary approach (surgery, medical oncology, nuclear medicine, radiotherapy) will be the best choice. [Halperin et. Al 2008, Beyzadeoglu et. Al 2010]

1.1.2 Types of cancer treatment

These cells may spread to other parts of the body and this is called metastasis. Although conventional anticancer therapies, consisting of surgical resection, radiotherapy and chemotherapy, are effective in the management of many patients but for about half of cancer sufferers these are ineffective, so alternative techniques are being developed to target their tumours. [Isaac et. Al 2017] Experimental cancer treatments are medical therapies intended or claimed to treat cancer by improving, supplementing or replacing conventional methods. These include photodynamic therapy, HAMLET (Human Alpha-lactalbumin Made Lethal to Tumour cells), gene therapy, telomerase therapy, hyperthermia therapy, complementary and alternative therapy, diet therapy, insulin potentiating therapy and bacterial treatment. Cancer treatment are following

- Hormone therapy
- Hyperthermia
- Immunotherapy
- Photodynamic therapy
- Radiation therapy
- Stem cell transplant
- Surgery
- Targeted therapy

- Chemotherapy

1.1.3 Defects

Currently available anticancer drugs are either of synthetic or of plant origin that produce side effects or toxic effects, such as myelosuppression, suppression of both cellular and humeral immunity, oral mucosal ulceration and intestinal denudation, pulmonary fibrosis, venoocclusive disease (lead to renal failure), alopecia, mucositis, thrombocytopenia, pneumonitis, hepatic fibrosis, cirrhosis etc, and as such no anticancer drug is free of toxicity. [Bruce et al 2001]

Many of these therapies are controversial due to lack of

- Evidence,
- Efficacy,
- Feasibility,
- Availability,
- Specificity and
- Selectivity.

1.2 Computer aided drug design

Drug discovery and advancement is a complex, lengthy, time expending and exceptionally costly process. It includes the cooperation of different fields, for example, medicinal chemistry, pharmacology, clinical research, drug metabolism, process chemistry…..etc., additionally combinatorial chemistry, high throughput screening and molecular modelling assumes a fundamental part in current drug discovery process It takes around 7-12 years and $ 800 million to $1.8 billion to bring new lead from drug revelation to market. Initially 1, 00,000 applicant compounds, hundreds of preclinical animal testing and clinical trials on thousands of volunteers and patients is conveyed to recognize a solitary showcased drug. The process from the ID of new medication to the promoting is alluded as pipeline which includes following real advances.

- Disease selection
- Target identification
- Lead identification
- Lead optimization
- Preclinical trials
- Clinical trials. [Gao 2016]

Discovery and development of a new drug is generally known as a very complex process which takes a lot of time and resources. So now a day's computer aided drug design approaches are used very widely to increase the efficiency of the drug discovery and development course. Various approaches of CADD are evaluated as promising techniques according to their need, in between all these structure-based drug design and ligand-based drug design approaches are known as very efficient and powerful techniques in drug discovery and development. These both methods can be applied with molecular docking to virtual screening for lead identification and optimization. In the recent times computational tools are widely used in pharmaceutical industries and research areas to improve effectiveness and efficacy of drug discovery and development pipeline.

Computational approaches in drug design, discovery and Development process gaining very rapid exploration, implementation and admiration. Introducing a new drug in a market is a very complex, risky and costly process in terms of time, money and manpower. Generally, it is found that drug discovery and development process take around 10-14 years and more than 1 billion dollars capital in total. [Daina et al 2017] So, for reducing time, cost and risk borne factors computer aided drug design (CADD) method is widely used as a new drug design approach. It has been seen that by the use of CADD approaches we can reduce the cost of drug discovery and development up to 50%. CADD consist use of any software program-based process for establishing a standard to relate activity to structure. [Xiang et. Al 2012]

2. Background of the study

2.1 Approaches in computer aided drug design

2.1.1 Structure-based drug design

Structure- based drug design (or direct Drug design) relies on knowledge of the Three - dimensional structure of the biological target obtained through methods such as x-ray crystallography or NMR spectroscopy. If experimental structure is not available. Within many of the rational drug design projects in the group, computer-aided methods, such as virtual screening and de novo design techniques, play an important role in follows 3-D structure three dimensional structure of compounds can generated and studied using molecular modelling software package such as ChemDraw, then imported into a molecular modelling software package such as Chem3D . the 2-D structure is converted into a 3-D structure is converted into a 3-D structure, which is quite clever, but is not error free, since the structure created is usually distorted (the bond lengths and The bond angles are not ideal).

There once a 3-D structure has been built or generated, it is important to carry out an operation called energy minimization. This involves running a program that modifies the bond angles and lengths in the structure, then calculates the static energy of the new model compared to the previous one. If the energy changes significantly, it means that neither structure is particular stable nor the process is repeated. Changes that decrease the total energy of the structure are retained, while those that increase the energy are not, and this continues until any modifications carried out have little effect energy on the total energy of the molecule. This corresponds to a stable structure or an energy minimum. Obtained, the molecules can study, its dimensions measured (e.g. bond angle, bond length, torsion angles, and atom-atom distances). [Ajay Kumar Shukla 2011]

An overlay operation is carried out where the program attempts to match up each defined pair of atoms. Once the overlay has been carried out, it is possible to measure how closely the corresponding atom in each structure overlap with each other. This sort of operation is crucial when aligning molecules for 3D QSAR studies and for comparing pharmacophores in different molecules. [Ajay Kumar Shukla 2011]

In SBDD, structure of the target protein is known and interaction or bio-affinity for all tested compounds calculate after the process of docking; to design a new drug molecule, which shows better interaction with target protein. [Imam ET. Al 2017]

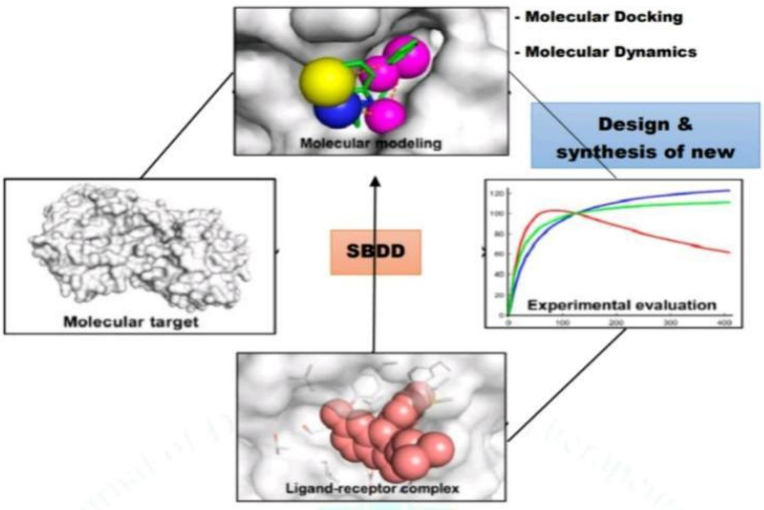


Figure 2.1: Layout of SBDD (Imam et al., 2017).

2.1.1.1 Overview of the process involved in sbdd

SBDD runs through multiple cycles before the optimized lead reached into clinical trials. The first cycle comprises isolation, purification and structure determination of the target protein by one of three key methods: like X-ray crystallography, homology modelling or NMR. Using compounds comes through Virtual screening of different databases are placed into a selected region (active site) of the protein. These compounds are scored and ranked on the bases of steric, hydrophobic, electrostatic interaction of these molecules with the active site of target protein. Top ranked compounds are tested with biochemical assays.

Second cycle comprises structure determination of the protein in complex with the most optimistic lead of the first cycle, the one with minimum micro-molar inhibition in-vitro, and shows sites of the compound which can be optimized for further increment in the potency. After several additional cycles like synthesis of lead, further optimization of lead through complex structure of protein with lead compound, the optimized compounds generally show marked increment in the target specificity and binding affinity. [Ajay Kumar Shukla 2011]

2.1.1.2 Steps involved in sbdd

- Target Identification & validation,
- Analyze structure for potential ligand binding sites,
- Lead identification & molecular docking,
- Lead validation & optimization,
- Clinical trials.

2.1.2 Ligand-based drug design

In LBDD, 3D structure of the target protein is not known but the knowledge of ligands which binds to the desired target site is known. These ligands can be used to develop a pharmacophore model or molecule which possesses all necessary structural features for bind to a target active site. Generally, ligand-based techniques are pharmacophore-based approach and quantitative-structure activity relationships (QSARs). In LBDD it is assumed that compounds which having similarity in their structure also having the same biological action and interaction with the target protein. [Macalino et. al 2015]

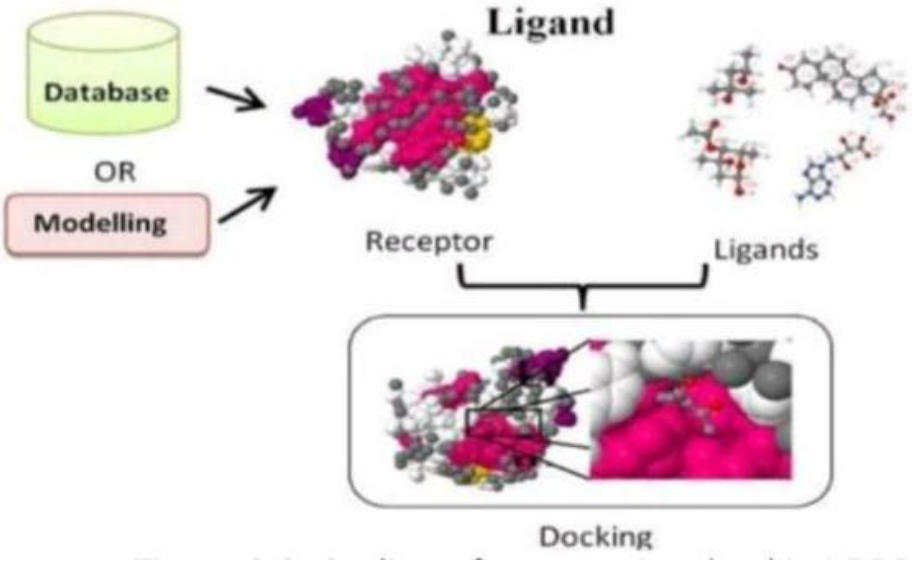


Figure 2.2: Outline of process involved in LBDD.

2.1.3 Virtual screening

Virtual screening has been worked as a most convenient tool now a day to find out the most favourable bioactive compounds with the help of information about the protein target or known active ligands. In the recent time virtual screening is known as a mind -blowing alternative of high-throughput screening mainly in terms of cost effectiveness and probability of finding most appropriate novel hit through filter the large of libraries of compounds. [Lill 2013]

There are generally two types of virtual screening approaches like structure-based virtual screening (SBVS) and ligand-based virtual screening (LBVS), SBVS method rely on the structure of target protein active site and LBVS method is based on estimation of calculated similarity between the known active and compound come from databases.

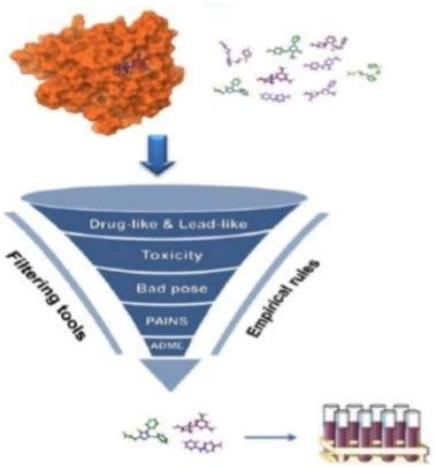


Figure 2.3: Overview of virtual screening process.

2.1.4 Molecular docking

Molecular docking is in-silico method which predicts the placement of small molecules or ligands within the active site of their target protein (receptor). It is mainly used to accurate estimation of most favourable binding modes and bio-affinities of ligands with their receptor, presently it has been broadly applied to virtual screening for the optimization of the lead compounds.

Molecular docking methodology comprises mainly three goals which are interconnected to each other like: prediction of binding pose, bio affinity and virtual screening. In the molecular docking method, the basic tools are search algorithm and scoring functions for creating and analysing conformations of the ligand.

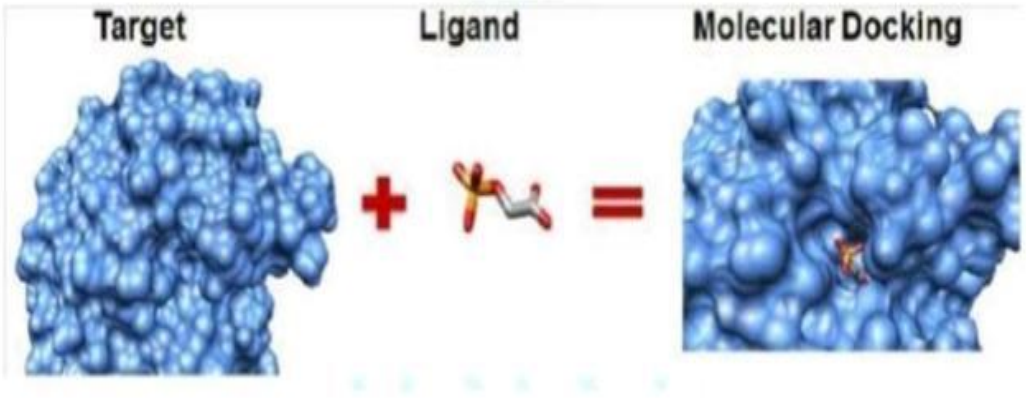


Figure 2.4: Process of docking.

3. Current Status and Future prospects

3.1 Topoisomerase as anticancer target

3.1.1 Structural studies of topoisomerases

3.1.1.1 Type ib topoisomerases

Compared to relaxed DNA that is in the lowest energy conformation, both positively and negatively supercoiled DNA molecules are in higher energy states due to the existence of torsional strains in these structures. In a simple three-step process that does not require additional input of energy, type IB topoisomerases effectively harvest the free energy stored as DNA supercoils to return these energetically strained DNA molecules to their relaxed forms . [Chen et al., 2013] This relaxation reaction starts with the catalytic tyrosine-mediated nucleophilic attack on a scissile phosphodiester bond in a DNA duplex, which results in the

formation of a transient single-strand break composed of a 3′-linked phosphotyrosyl bond and freed 5′-OH. The tension resulting from DNA supercoiling can be dissipated via rotation of the DNA molecule about the break (Fig. 3.1). Finally, the cleaved phosphodiester bond is resealed and the catalytic tyrosine is regenerated, resetting the enzyme for the next relaxation event.

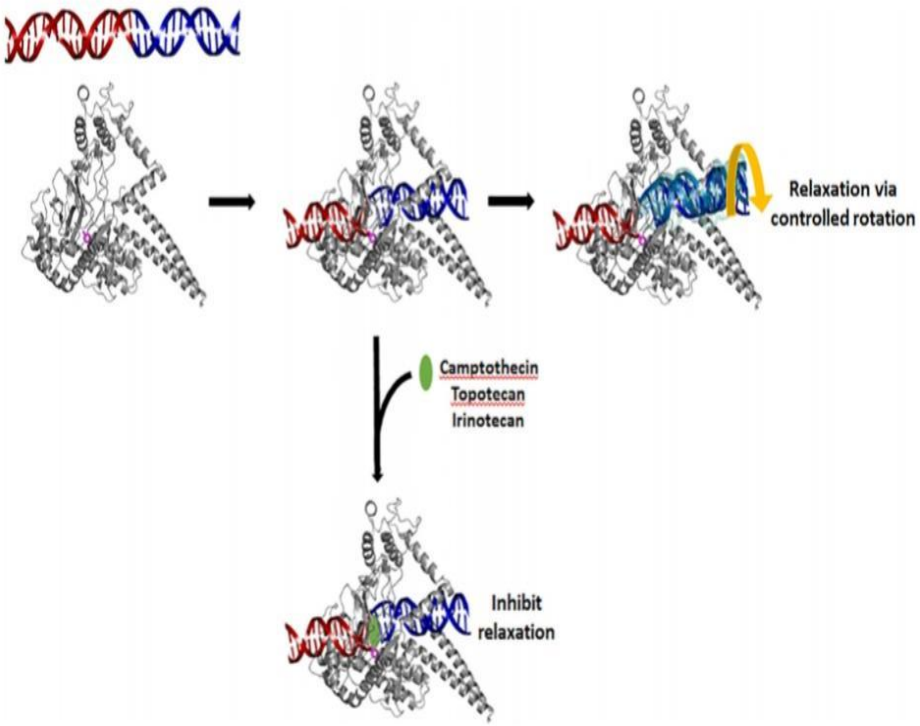


Figure 3.1: Relaxation reaction catalyzed by hTop 1 and The effect of camptothecin class of anticancer agents.

Structural analyses of hTop 1 in covalent and noncovalent complexes with DNA suggested that the postulated DNA rotation during the catalytic cycle is likely achieved via a "controlled rotation mechanism". Briefly, the catalytic core of type IB topoisomerases is composed of a Cap domain and a catalytic (CAT) domain, which was first observed in tyrosine recombinases and these two domains clamp around the DNA duplex upstream to the cleavage site. The DNA upstream of the single-strand break is tightly bound through several protein–DNA interactions between the core of Top 1 and DNA, primarily to the phosphate backbone of the DNA, and is thus unable to rotate. In contrast, the DNA downstream of the break is only loosely held by the nose cone of the Cap domain and linker regions of CAT domain. Conceivably, this weak protein-DNA interface can be easily ruptured by the stored superhelical tension, and the downstream DNA can then rotate about the cleavage site by one

or more turns until all torsional strains are released or the break is resealed. Given the limited number of DNA-interacting residues, it appears to be difficult for the enzyme to capture the downstream DNA in the presence of high superhelical tension. Nevertheless, this interface may be re-established at lower superhelical density, which halts the rotating DNA and places the 5′-OH in a favorable position for engaging in the religation reaction. The rotation and orientation of downstream DNA is 'controlled' by the enzyme, as opposed to random rotation, which would complicate the Religation reaction. [Stewart et al., 1998]

Types IA, IB, and IIA topoisomerases have distinct requirements of Mg2+ for their catalytic activities. Structural analysis of the enzyme-DNA covalent complex revealed why type IB topoisomerases do not require a divalent cation for their activity. The transesterification reaction catalyzed by topoisomerases is known to proceed through a high energy bipyramidal transition state in which two oxyanions are attached to the pentavalent phosphorus. Unlike types IA and IIA topoisomerases, which utilize Mg2+ as an essential cofactor for electrostatic stabilization of the transition state, type IB topoisomerases and members of the tyrosine recombinase family employ positively charged arg and Lys residues for this purpose. [Sherratt et al., 1998]

3.1.1.2 Type iia topoisomerases

Even before the first crystallographic visualization of type IIA topoisomerases was available, a series of elegant biochemical studies had established that type II topoisomerases change the linking number of DNA through a 'two-gate' mechanism. According to this mechanism (Fig. 3.2), the catalytic cycle of type IIA topoisomerases starts from the binding of the G-segment DNA to the enzyme, a process corresponding to the assembly of the so-called 'DNA gate' . The formation of such a topoisomerase-DNA binary complex allows both strands of the G-segment to be cleaved via Mg2+-dependent transesterification reactions occurring between the catalytic tyrosine residues and the DNA backbone's phosphodiester bonds [Pitts et al., 2011] , which introduces a reversible DSB into the G-segment. When a DSB is formed, the DNA backbones are unlocked and the two halves of the G-segment can move apart from each other. This potentiates the DNA gate to enter an open state in which the gap between cleaved DNA ends is spacious enough for the T-segment to be passed through. The incoming T-segment approaches the DNA gate by going through the entry gate located on one end of a type IIA.

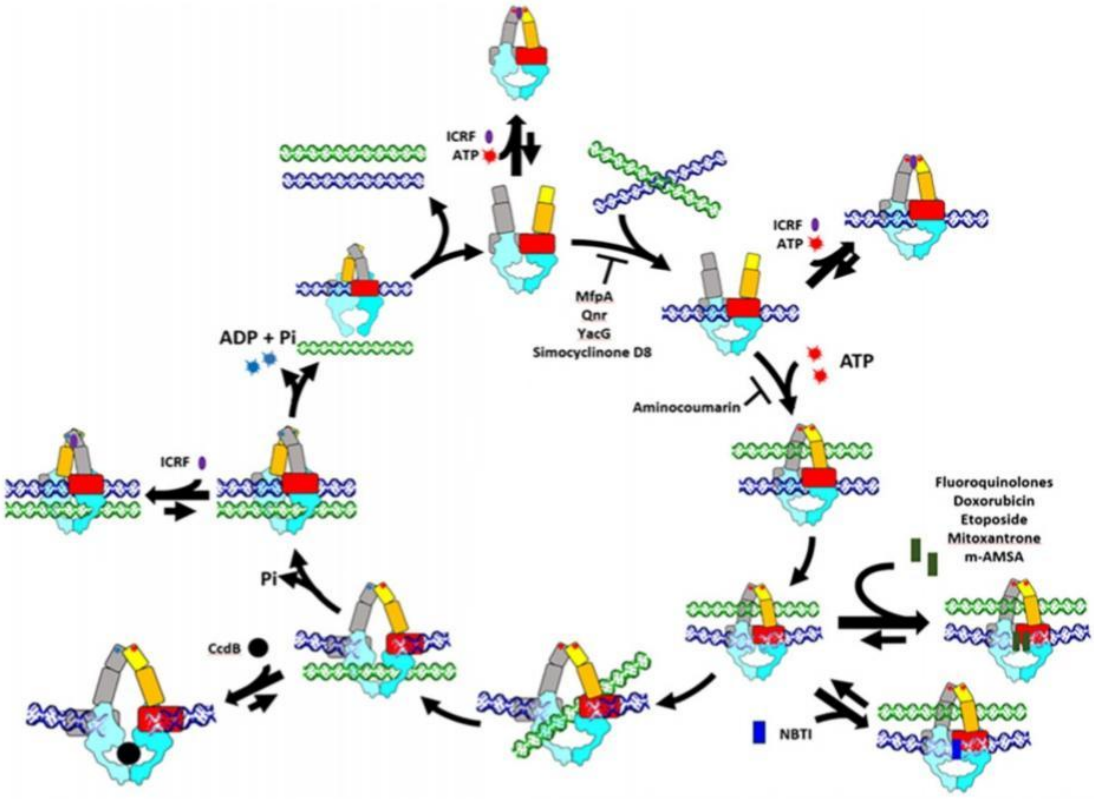


Figure 3.2: Catalytic Cycle and Inhibitors of type IIA topoisomerases.

Opoisomerase (the N-terminal end of eukaryotic Top 2) and moving along the DNA conducting path that is used by the G-segment to reach its binding surface. After passing through the DNA gate, the T-segment is directed toward, and eventually released from, the exit gate at the other end of the topoisomerase (the C-terminal end for eukaryotic Top 2). [Champoux 2011] This unidirectional DNA transport mode is referred to as being 'two-gated' because the entry and exit of T-segment is mediated via two distinct protein gates of type IIA topoisomerases.

A more detailed depiction of the catalytic mechanisms of type IIA topoisomerases would require the following aspects to be addressed in structural terms: the enzyme's overall architecture and spatial domain organization; the assembly of a protein-linked DNA gate; strand breakage of the bound G-segment to unlock the DNA backbones; capture of the T-segment; conformational changes associated with the DNA gate; release of the T-segment and resealing of the cleaved G-segment; and resetting the enzyme conformation for the next catalytic cycle. Thanks to a large number of crystal structures obtained for various fragments of type IIA topoisomerases and two catalytically competent type IIA topoisomerases, which represent structural snapshots of different regions of type IIA topoisomerases at distinct

stages of the catalytic cycle, we have obtained valuable Insights into each of these steps. [Schoeffler et al., 2005]

All type IIA topoisomerases exhibit a two-fold symmetric architecture with the constituting domains appearing in pairs. The entry gate is formed by two GHKL ATPase domains, which are known to dimerize upon ATP-binding, but dissociate in the nucleotide-free state. With gate closure and opening being controlled by ATP, the gate appears to act as a nucleotide-operated protein clamp suitable for T-segment capture. Structural analyses revealed that each nucleotide binding pocket is formed by elements from both ATPase domains; ATP would thus promote dimerization by 'gluing' the two domains together. Although each type IIA topoisomerase possesses two structurally equivalent ATP binding sites, it has been shown that one ATP is sufficient to lock the entry gate in the closed state. This finding suggests that, once closed, the entry gate would not re-open until the end of the catalytic cycle when both bound ATP molecules are hydrolyzed (Fig3.2).

The DNA gate is compositely formed by a type IIA topoisomerase and G-segment DNA. The protein part of the DNA gate is composed of two copies of each of the following domains: the topoisomerase-primase (Toprim) domain, winged-helix domain (WHD), and a so-called 'tower' domain. The Toprim domain adopts a Rossmann-like fold and contains an Mg2+-coordinating DXD motif that is essential for the transesterification reaction. The WHD domain is central to type II topoisomerase functions by harboring a helix- Turn-helix DNA-binding motif and the catalytic tyrosine residue. The tower domain provides additional DNA-binding residues and is an integral part of the DNA gate. Crystal structures of the DNA gate in its closed state revealed that all these domains contribute to the formation of a G-segment binding groove, with the WHD domains at the bottom and the Toprim and tower domains lining the sides. The bound G-segment is primarily anchored by interacting with the helix-turn-helix motifs of the WHD domains and is bent into a U-shape by a pair of conserved isoleucine residues thatintercalate into DNA at sites 12 base pairs apart. Basic residues from the tower domains interact with the DNA backbone, presumably to stabilize the bent conformation of the G-segment. The DNA duplex enclosed within the two intercalating residues is distorted toward the A-form, which likely places the scissile phosphodiester bonds, the active site tyrosine residues, and the catalytic Mg2+ ions at an optimal orientation for engaging in the transesterification reactions. A structural feature unique to the DNA gate is that, to perform strand breakage and religation reactions, the catalytic tyrosine of one

subunit must team up with the Mg2+ presented by the Toprim domain of the opposing subunit. Therefore, closure of the DNA gate is a prerequisite for the phosphotyrosyl bond to be reactive, and the decoupling between these two catalytic modules would protect the covalent tether from being attacked by nucleophiles present in the vicinity.

The exit gate corresponds to the C-terminal dimerization interface that is usually observed in the closed state. It is expected that the opening of exit gate would be a transient event during the catalytic cycle and the conformational equilibrium associated with the gate should be biased toward the closed form, because premature gate opening would significantly alter the overall architecture of a type IIA topoisomerase and may prevent the cleaved G-segment from being religated. Nevertheless, opening of the exit gate has been visualized by crystallography in the absence of a T-segment. [Wendorff et al., 2012]

The structures of a fully active type IIA topoisomerase showed that the DNA gate is sandwiched between the entry and exit gates, an arrangement that agrees fully with the proposed two-gate mechanism. In addition, a cavity is observed on each side of the DNA gate, suggesting that the T-segment may be temporarily held within the enzyme during its transportation. These structures also revealed that the DNA gate is connected to the entry and exit gates via the transducer domains and coiled-coil regions, respectively. These bridging elements are thought to mediate allosteric communications between the gates. For example, the conformational changes induced by ATP hydrolysis may alter the structure of a DNA gate via the rearrangement of the transducer domain. Also it has been shown that conformations of the DNA and exit gate may be coupled by adjusting the kink of the coiled-coil region. However, more conformational states of type IIA topoisomerases, such as the opening of DNA gate, would be required to understand how the opening and closing of these gates are coordinated.

3.1.2 hTop 1-targetting anticancer drugs

Camptothecin (Fig. 3.3) is a plant alkaloid that poisons hTop 1. Clinical development of camptothecin was discontinued because of its intolerable adverse effects and low therapeutic index. However, derivatives of camptothecin, topotecan and and irinotecan (Fig. 3.3), are currently used in the clinic. Unfortunately, their clinical use is limited due to their dose-limiting toxicity, especially neutropenia, myelosuppression, and diarrhea, as well as chemical instability due to the rapid opening of the E-ring. mTop 1 is inhibited by camptothecins and mTop 1 can be targeted by topotecan, but not by camptothecin, in vivo [Loza et al 2009]. It is

not clear, however, if the effect of camptothecins on mTop 1 contribute to the anticancer activities of these drugs.

Camptothecins

Topotecan

Topotecan (Hycamtin) (Fig. 3.3) is a semi-synthetic water-soluble derivative of camptothecin. It was the first hTop 1 inhibitor approved for oral administration. It is often used to treat ovarian and small cell lung cancer. [O'Reilly et al 1999]

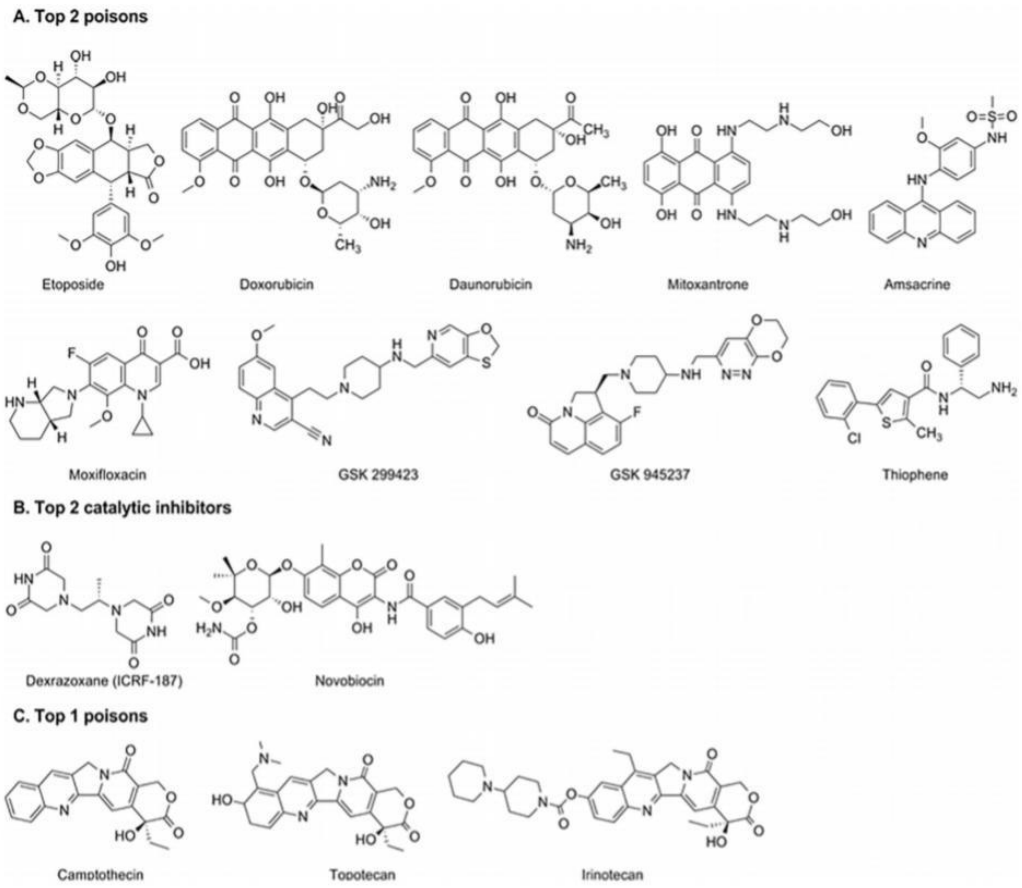


Figure 3.3: Structures of topoisomerase inhibitors.

A. Type IIA topoisomerase (Top 2) poisons. B. Type IIA topoisomerase (Top 2) catalytic Inhibitors. C. Type IB topoisomerase (Top 1) poisons.

Irinotecan

Irinotecan (CPT-11, Campostar) (Fig. 3) is another water-soluble derivative of camptothecin. It is a prodrug and is converted to a biologically active metabolite, ethyl-10-hydroxy-

camptothecin (SN-38), by a carboxylesterase. Irinotecan, together with fluorouracil, is often used for the treatment of advanced colorectal cancer.

Non-camptothecins

Camptothecins

Camptothecins are the only hTop1 inhibitors approved for clinical use. Despite their effectiveness, these drugs have limitations due to their instability and severe side effects, as well as drug resistance caused by P-glycoprotein. To overcome these limitations, non-camptothecin hTop 1 inhibitors have been developed and investigated. Among them, indolocarbazoles (NB-506), indenoisoquinolines, and dibenzonaphthyridinones (ARC-111) are under clinical development.

Indolocarbazoles

Indolocarbozoles are synthetic analogs of antibiotics isolated from several actinomycetes. NB-506 is a DNA intercaltor that can poison hTop 1. NB-506 and camptothecins share the binding site on hTop 1 but hTop 1 is not the only target of NB-506. Edotecarin (J-107088) is a derivative of NB-506 that does not intercalate into DNA. Similar to NB-506, it poisons hTop 1 but its effect on an unidentified target(s) also contributes to its promising anticancer activity.

Indenoisoquinolines

Indenoisoquinolines are synthetic non-camptothecin analogs that poison hTop 1. Since the first indenoisoquinline, NSC 314622, was reported in 1978, many derivatives have been synthesized and tested for anticancer activity. A Phase 1 clinical study of indotecan (LMP400) and indimitecan (LMP776) in adults with relapsed solid tumors and lymphomas was recently completed although study results have not been published.

Dibenzonaphthyridinones

ARC-111 was selected among dibenzonaphthyridinones for preclinical studies of its anticancer activity. More recently, Genz-644282 has been the subject of both preclinical and clinical studies [Kurtzberg et al 2011]

3.1.3 hTop 2-Targetting anticancer drugs

As listed below, hTop 2 poisons are successful anticancer drugs used in the treatments of various cancers. However, two serious side effects, therapy-related cancer and cardiotoxicity,

associated with these drugs limit their use. Etoposide (Fig. 3) and other hTop 2 poisons cause the development of secondary malignancies, especially therapy-related acute myeloid leukemia (t-AML) and therapy-related acute promyelocytic leukemia (t-APL). t-AML is caused by hTop 2-mediated, and more specifically hTop 2β-mediated, DSBs and chromosome rearrangements in the mixed lineage leukemia (MLL) gene, and t-APL is caused by the translocation between the promyelocytic leukemia (PML) gene and the retinoic receptor α gene.

Doxorubicin (Fig. 3) is a potent anticancer drug that can be used to treat many cancers. However, its full potential has not been realized due to the cardiotoxicity associated with its use. The primary mechanism of doxorubicin-associated cardiotoxicity appears to be oxidative stress, due to increased levels of reactive oxygen species (ROS) and lipid peroxidation. The main pathway for ROS formation by anthracyclines is the formation of semiquinone radicals resulting in redox cycling [283,285]. Another source of ROS generated by anthracyclines results from formation of doxorubicin-iron complexes that catalyze the Fenton reaction. Cardiomyocytes are particularly sensitive to oxidative stress. HTop 2β is the only topoisomerase expressed in myocytes and it's poisoning by doxorubicin, in addition to increased ROS, may contribute to the cardiotoxicity of doxorubicin. Further studies are required to determine the exact mechanism(s) of the cardiotoxicity of doxorubicin but the current strategy to prevent cardiotoxicity is to limit the cumulative dose of doxorubicin.

In addition to these side effects, P-glycoprotein (multidrug resistance protein 1) confers resistance to epipodophyllotoxins and anthracyclines [Hoffmeyer et al., 2000]. Despite the clinical issues described above, hTop 2 poisons are among the most widely prescribed anticancer drugs. Some Top 2 poisons currently used in the clinic are described below.

hTop 2 Poisons

Epipodophyllotoxins

Etoposide and Teniposide—Podophyllotoxin was o riginally isolated from the podophyllum plants, American Podophyllum peltatum and podophyllum emodi. Easily accessible podophyllotoxin is used in the synthesis of etoposide (Etopophos, VePesid, and VP-16) and teniposide (Vumon, VM-26). Etoposide (Fig. 3) is widely used in the treatments of solid tumors, such as testicular cancer and small cell lung cancer. It is also used to treat lymphomas and nonlymphocytic leukemia. To improve its water solubility, a prodrug etoposide phosphate (Etophos) was developed for intravenous use. Teniposide is an analog of etoposide

and is approved for the treatment of acute lymphoblastic leukemia (ALL) in children. It is also used to treat other cancers, including Hodgkin's lymphoma and some brain cancers. Teniposide.

Anthracyclines

Doxorubicin, Daunorubicin, and Epirubicin—Doxorubicin (Adriamycin, Doxil) (Fig. 3) is an anthracycline antibiotic originally isolated from streptomyces peucetius. It is used to treat a wide variety of cancers, including aLL, acute myeloblastic leukemia (AML), breast carcinoma, ovarian carcinoma, Kaposi's sarcoma, Wilms' tumor, thyroid carcinoma, gastric carcinoma, and Hodgkin's disease. Doxil is a form of doxorubicin contained inside a liposome (liposomal doxorubicin) for a slow release to treat AIDS-related Kaposi's sarcoma, multiple myeloma and ovarian cancer. Doxorubicin is one of the most potent anticancer drugs but its use is limited by the cumulative dose-dependent cardiotoxicity Daunorubicin (Cerubidine, DaunoXome) (Fig. 3) has similar therapeutic effects to doxorubicin. It is used to treat ALL, AML, chronic myelogenous leukemia (CML), and Kaposi's sarcoma. DaunoXome is liposomal daunorubicin that is used for the treatment of AIDS-related Kaposi's sarcoma.

Epirubicin (Ellence) is an active isomer of doxorubicin although epirubicin has similar therapeutic effect to doxorubicin, epirubicin is preferred over doxorubicin in certain chemotherapy regimens because it has fewer side effects. Epirubicin is eliminated faster and has reduced toxicity compared to doxorubicin. The spatial orientation of the hydroxyl group at the 4' carbon of the sugar moiety differs in doxorubicin and epirubicin. Epirubicin's 4' hydroxyl is in an equatorial position allowing for rapid conjugation of epirubicin with glucuronic acid, which could account for its faster elimination and lower toxicity. Liposomal epirubicin has shown to be an effective agent to treat brain glioma. Anthracyclines act as Top 2 poisons. In addition, they can intercalate into DNA [326] and may influence the functions of various proteins besides hTop 2. As previously described, anthracyclines also generate ROS. [Xu et al., 2015]

Mitoxantrone

Mitoxantrone (Novantrone) is a synthetic anthracenedione (Fig. 3) developed as an alternative to anthracyclines to reduce cardiotoxicity while retaining antineoplastic activity. It is used for the treatment of multiple sclerosis and AML. While mitoxantrone has higher

tolerability than the anthracyclines, cardiomyopathy is still a concern in long-term treatment [Damiani et al., 2013]

hTop 2 Catalytic inhibitor

Dexrazoxane

Dexrazoxane (Zinecard, Cardioxane, and ICRF-187) is a bisdioxopiperazine (Fig. 3) and is a derivative of ethylenediaminetetraacetic acid. Dexrazoxane is the only drug approved to treat cardiotoxicity caused by anthracyclines. Its cardioprotective mechanism is attributed to the chelation of iron by dexrazoxane, thus decreasing the production of ROS during anthracycline treatment. Dexrazoxane is orally active as a prodrug that is hydrolyzed, by sequential ring openings, into the active metabolite ADR-925. Recent studies have indicated that dexrazoxane's cardioprotective effect may be associated with catalytic inhibition of hTop 2 [Vavrova et al., 2013]

4. Structure-based drug design of small molecule peptide deformylase inhibitors to treat cancer

Human peptide deformylase (HsPDF) is an important target for anticancer drug discovery. In view of the limited HsPDF, inhibitors were reported, and high-throughput virtual screening (HTVS) Studies based on HsPDF for developing new PDF inhibitors remain to be reported. We reported here on diverse small molecule inhibitors with excellent anticancer activities designed based on HTVS and molecular docking studies using the crystal structure of HsPDF. The compound M7594_0037 Exhibited potent anticancer activities against HeLa, A549 and MCF-7 cell lines with IC50s of 35.26, 29.63 and 24.63 μM, respectively. Molecular docking studies suggested that M7594_0037 and its three derivatives could interact with HsPDF by several conserved hydrogen bonds. Moreover, the pharmacokinetic and toxicity properties of M7594_0037 and its derivatives were predicted using the OSIRIS property explorer. Thus, M7594_0037 and its derivatives might represent a promising scaffold for the further development of novel anticancer drugs.

Peptide deformylase (PDF), a metalloenzyme containing Fe2+, is responsible for the removal of the N-formyl group from the terminal methionine residue in nascent proteins, which is essential to the synthesis of proteins. [Leeds et Al 2006]In the past few decades, peptide deformylase of pathogenic microorganisms has been considered as a potential target for antibacterial drug discovery, and plenty Of PDF inhibitors have been widely reported. [Meinnel ET. Al 1999] However, recent studies have demonstrated that peptide deformylase

also can be found in most eukaryotes, including parasites, plants, and mammals. [Giglione et Al 2000] Human genes of peptide deformylase (def) have been cloned and expressed and the resulting human peptide deformylase (HsPDF) has been studied extensively. HsPDF distributes over the mitochondrion in human cells, and also functions. [Serero et Al 2003] similarly to the PDF in pathogenic microorganisms, which catalyzes the deformylation of polypeptides. [Spencer et Al 2004] Inhibition of HsPDF results in mitochondrial membrane depolarization and promotes cell death. [Nguyen 2003] HsPDF is over-expressed in breast cancer, colon cancer and lung cancer, and the inhibition of HsPDF would significantly reduce the proliferation of these cancer cells. [Randhawa et Al 2013] Actinonin (Figure 4.3), the first found naturally occurring bacterial PDF inhibitor, also shows strong HsPDF inhibitory activity. [Han et Al 2010] As suggested above, HsPDF is a promising novel target for the development of anticancer drugs. [Sangshetti et al 2015]

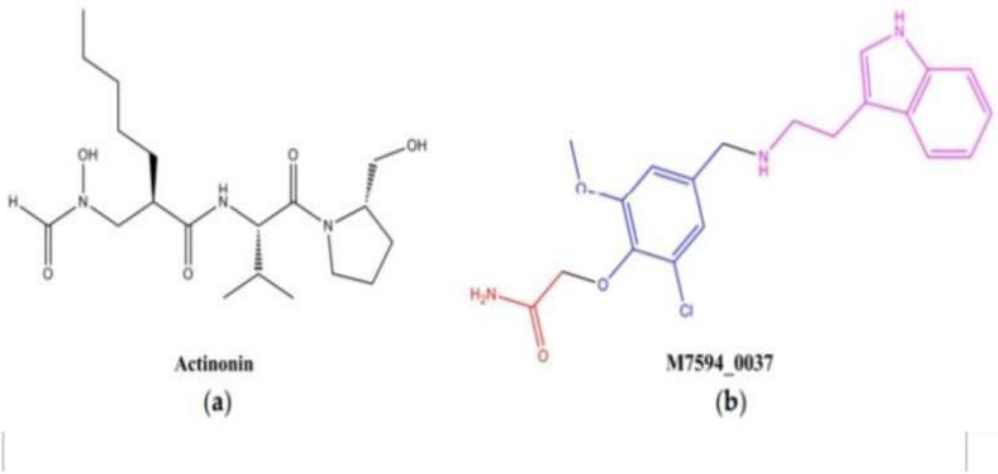


Figure 4.3: Structures of PDF inhibitor actinonin (a) and The shortlisted compound M7594_0037 (b).

Include a class of peptide inhibitors based on actinonin reported by Mona's research group and two series of non-peptide inhibitors reported by Christophe's research group. In addition, in view of the fact that the crystal structure of HsPDF has been reported, high-throughput virtual screening (HTVS) based on HsPDF remains to be reported. Hence, this study was intended to discover novel small molecule inhibitors with novel structural class based on our drug design strategies involving high-throughput virtual screening and molecular docking techniques. The resulting new structure was named M7594_0037, and the bioactivity

evaluation substantiated its superior anticancer activity. To optimize the structure of M7594_0037, our research group designed three derivatives of vanillin N-hydroxy acetamide. One of them, NA-2, was observed to have better anticancer activities against MCF7 and HeLa cell lines compared to M7594_0037. Molecular docking studies also indicated that the derivatives of vanillin N-hydroxy acetamide could have a stable interaction with HsPDF, which was similar to the binding mode of actinonin. In summary, the derivatives of vanillin N-hydroxy acetamide designed in this study have the potential to become a new class of anticancer drugs. [Antczak et al 2011]

4.1 High-throughput virtual screening

As described in the materials and methods section, the crystal structure (3G5K) was utilized to screen the HsPDF inhibitors from the Topscience Chem Div, database (commercially available). The top-ranked 100 compounds were shortlisted based on the docking score and hydrogen bond. Finally, six commercially available compounds were selected from the top-ranked list of 100 by clustering analysis and were tested experimentally. Among them, M7594_0037 (Figure 1) exhibited dramatic anticancer activities against HeLa, A549 and MCF-7 human cancer cell lines, and was selected as a lead compound for further study. The structure of M7594_0037 differed from the common peptidic Inhibitors, and it can be classified as a non-peptidic inhibitor. Non-peptidic inhibitors of HsPDF are superior to peptidic inhibitors in terms of pharmacokinetics. [Sangshetti et al 2015]

4.2 Design and Synthesis of derivatives of M7594_0037

M7594_0037 consisted of the amide at its terminal, the methyl vanillin at its middle section and the indole at its other terminal. The amide group was responsible for binding with Fe2+ as a metal chelating group, while the known strong metal chelating groups reported in previous papers are N-formyl-N-hydroxylamine and hydroxamic acid . In other words, the amide functioning as the metal chelating group at the terminal of M7594_0037 seemed to be a weaker one, so alteration of this group will be focused on in this article. The terminal indole ring of M7594_0037 interacted with the hydrophobic S3 region (described in the section on molecular docking studies) where it can provide additional binding energy, selectivity, and favorable pharmacokinetic and toxicity properties. [Sangshetti et al 2015] Moreover, the S3 regions of the binding sites of HsPDF are generally solvent accessible, and especially large in size to accommodate various substituent groups. For the purpose of improving the anticancer activity, three derivatives of M7594_0037, which were named 3AP-2, NA-2, M-2, were

designed. The structures of all the derivatives were characterized by 1H-NMR and mass spectra. The synthetic routes are shown in Scheme 1. Molecules 2016, 21, 396 3 of 10 anticancer activity, three derivatives of M7594_0037, which were named 3AP-2, NA-2, M-2, were designed. The structures of all the derivatives were characterized by 1H-NMR and mass spectra.

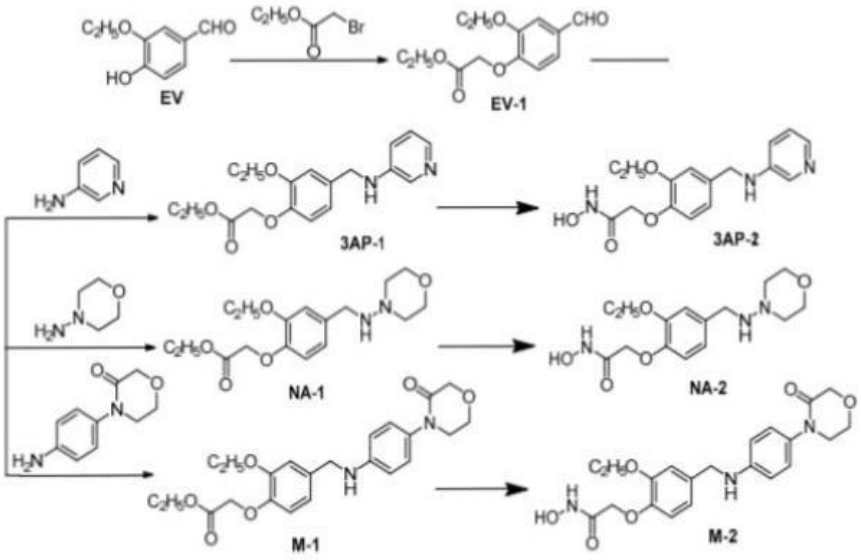


Figure 4.4: Synthesis of M7594_0037 derivatives.

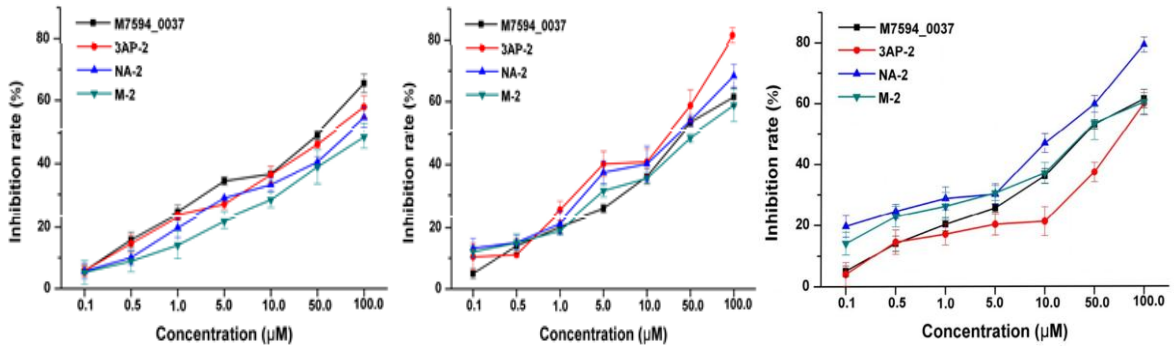
4.3 Anticancer activity OF M7594_0037 and ITS Derivatives

As we know that HsPDF has been reported to be over-expressed in many cancers, it was proven that HsPDF inhibitors could specifically be used in cancer treatment. Hence, the anticancer activities Of M7594_0037 and its derivatives were studied on HeLa, A549 and MCF-7 human cancer cell lines in a dose-dependent manner (Figure 4.5). The cytotoxic activity was expressed as the mean IC50 of three independent experiments, and the results were represented table 4.3. It is clearly shown that M7594_0037 had inhibitory activity against HeLa, A549 and MCF-7, whose IC50 values are 35.26 ˘ 3.17, 29.49 ˘ 2.09 and 24.63 ˘ 2.19 µM, respectively. Its inhibitory activity against HeLa was slightly smaller than that of actinonin [22] (IC50 = 27.40 µM). For further optimization of M7594_0037, the amide at its terminal was replaced with N-formyl-N-hydroxylamine to give three derivatives. Each of them exhibited.

Table 4.3: Effect (IC50) of M7594_0037 and Analogs on human cancer cell lines.

Compounds	A549 (μM)	MCF7(μM)	HeLa(μM)
M7594-0037	29.49 ±2.09	24.63±2.19	35.26±3.17
3AP-2	45.37±2.08	13.62±3.42	96.57±3.73
NA-2	67.85±2.22	22.89±3.41	9.60±3.04
M-2	119.95±3.71	48.02±2.32	36.39±3.90

Obvious anticancer activities. The compound 3AP-2 showed better inhibitory activity against MCF-7 than M7594_0037 (IC50 = 13.62 ˇ 3.42 μM), while NA-2 was even superior to M7594_0037 in Inhibition against both HeLa and MCF-7 cell lines (IC50 = 9.60 ˇ 3.04 and 22.89 ˇ 3.41 μM, respectively).Thus, M7594_0037 and its derivatives have potential to become lead compounds of a class of novel anticancer drugs.



a **b** **c**

Figure 4.5: Inhibition rate of M7594_0037 and Analogs against A549 (a); MCF7 (b) and HeLa (c) in Dose-dependenc.

4.4 Binding modes of M7594_0037 and ITS derivatives

M7594_0037 and its derivatives were docked into the binding site where the ligand actinonin was placed by the surf Lex module in SYBYL 7.1. The binding modes of M7594_0037 and its derivatives with HsPDF were obtained from the results of surf lex molecular docking. As is vividly shown in Figure 4.6, many hydrogen bonds were formed between M7594_0037 and HsPDF, and most of them were allowed by the amide group at the terminal of M7594_0037. Three hydrogen bonds were networked with side-chains of Gln-57 and Glu-157, and another three hydrogen bonds were networked with backbones of Gly-52, Glu-115. The methoxyl group at the vanillin section allowed two hydrogen bonds networked with Gly-52 and Val-51 as well, and the NH group on the indole ring allowed the hydrogen bond networked with Glu-76 to be formed. These hydrogen bonds played an important role in the binding interaction between M7594_0037 and HsPDF. The terminal indole ring was located

in the hydrophobic pocket constituted by Trp-149, Leu-73, Met-87, Arg-85, and Cys-77. Analogous to the binding mode of M7594_0037, NA-2 also formed stabilized hydrogen bonds with Gln-57, Gly-52, Glu-115, and Glu-157, and its terminal morpholine was also placed in a similar hydrophobic pocket. In the interaction between stabilized hydrogen bonds and M7594_0037, NA-2 coincided with the conservative hydrogen bond interactions which has been reported in the past.[21] For example, actinonin would emerge from a conservative interaction between Val-51 and Gly-52, and it would be located in the parallel hydrophobic pocket as well. [Escobar et al 2009]

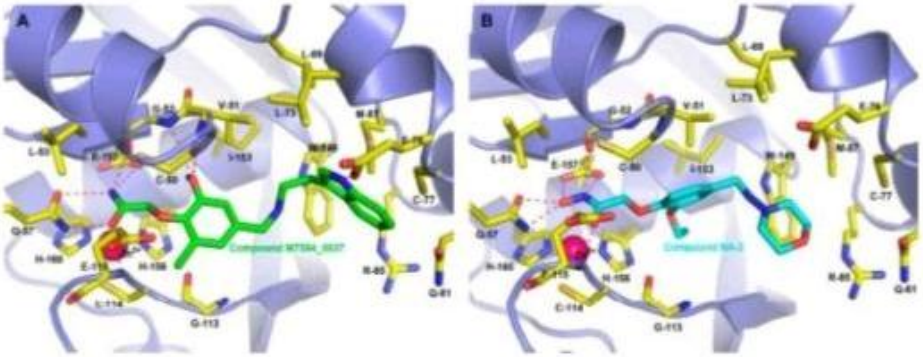


Figure 4.6: Depicting the binding pose of inhibitors in the HsPDF binding packet M7594_0037 (a) and NA 2(b). The red colure bonds indicate the hydrogen bonds between inhibitors and amino acid (coloured by yellow).

4.5 In silico Pharmacokinetic and Toxicity predictions of M7594_0037 and ITS derivatives

The in silico pharmacokinetic and toxicity predictions of M7594_0037 and its derivatives were predicted using the OSIRIS property explorer. Results of the toxicity prediction study (Table 4.4) suggested that M7594_0037 and 3AP-2 have no risk of toxicity, while NA-2 has a low risk of tumorigenicity and irritant effects, and M-2 has a low risk of irritant and reproductive effects. Results of the pharmacokinetic prediction (Table 4.4) revealed that all compounds are good with the properties of solubility (logS), partition coefficient (clogP), drug likeness and molecular weight. The drug score was calculated in view of the contribution of logS, clogP, drug likeness, molecular weight and toxicity risk prediction parameters (Table 4.5). The drug score showed that M7594_0037 and 3AP-2 have a higher drug score than NA-2 and M-2. Nevertheless M7594_0037 can be regarded as a lead compound for further designing new molecules with better pharmacokinetic properties.

Table 4.4: In silico Pharmacokinetic and Toxicity prediction of M7594_0037 and its derivatives.

Parameters	M7594_0037	3AP-2	NA-2	M-2
Toxicity risk management				
a) Mutagenicity [a]	No	No	No	Yes
b) Tumorigenicity [b]	No	No	Yes	Yes
c) Irritant [c]	No	No	Yes	No
d) Reproductive effective [d]	No	No	No	No
Solubility [e]	´4.08	´2.59	´1.68	´3.47
cLogP	2.1	0.61	´1.29	0.71
Drug likeness [f]	3.69	0.3	1.07	´1.2
Molecular weight	387.0	317.0	325.0	415.0
Drug score [g]	0.74	0.71	0.39	0.17

A Mutagenicity refers to the induction of permanent transmissible changes in the structure of the genetic material of cells or organisms; b Tumorigenicity refers to the process by which neoplastic cells are grown in tissue culture from tumors;c irritant refers to a stimulus from compound which causes irritation; d reproductive effective refers to adverse effect of compounds that interfere with the reproductive organs of an organism; e the logS value is estimated, and it is a unit-stripped logarithm (base 10) of the solubility measured in mol/liter;f It is a value of the compound's drug-like properties. The drug likeness is calculated by summing up score values of those fragments that are present in the molecule under investigation. The fragment list was created by shredding 3300 traded drugs as well as 15,000 commercially available chemicals (Fluka), yielding a complete list of all available fragments; drug score is composed of drug likeness, cLogP, logS, molecular weight and factors of toxicity risk management, and is used to judge the compound's overall potential to qualify for a drug candidate. The value of the drug score is between 0 and 1, and the larger value means better pharmacokinetic properties.

Table 4.5: Contribution of different parameters to the drug score of M7594_0037 and its.

Parameters	M7594_0037	3AP-2	NA-2	M-2
Score from cLoP	0.947	0.987/1.0	0.998/1.0	0.986/1.0
Score from logS	0.716	0.917/1.0	0.965/1.0	0.822/1.0
Score from molecular weight	0.795/1.0	0.899/1.0	0.89/1.0	0.734/1.0

Score from drug likeness	0.975/1.0	0.575/1.0	0.744/1.0	0.23/1.0
No risk of mutagenicity	1.0/1.0	1.0/1.0	1.0/1.0	0.6/1.0
No risk of tumorigenicity	1.0/1.0	1.0/1.0	0.6/1.0	0.6/1.0
Risk of irritating effect	1.0/1.0	1.0/1.0	0.8/1.0	1.0/1.0
No risk of reproductive effect	1.0/1.0	1.0/1.0	1.0/1.0	1.0/1.0
Average total drug score	0.740	0.712	0.388	0.173

5. CONCLUSION

Both hTop 1 and hTop 2s are targets of current anticancer drugs. New inhibitors of these enzymes are also in the pipeline. Thus, topoisomerases continue to be important therapeutic targets of anticancer drugs. Human cells also contain two type IA topoisomerases, hTop 3α and hTop 3β. Although some small molecules that inhibit bacterial type IA topoisomerases have been identified only in recent years, hTop 3 inhibitors may be discovered and developed as new anticancer drugs in the future.

We take advantage of virtual screening to obtain the small molecular compound M7594_0037 with an all-new structure. It has been validated that it had obvious inhibitory activity against HeLa, A549 and MCF-7 human cell lines. For the optimization of M7594_0037, three vanillin N-hydroxyacetamide analogs were designed and synthesized. Among them, NA-2 had better anticancer activity than M7594_0037. Moreover, we investigated the binding modes of M7594_0037 and its derivatives with HsPDF. In the end, we evaluated the pharmacokinetic and toxicity properties of M7594_0037 and its derivatives. Therefore, the derivatives of vanillin N-hydroxyacetamide have great potential to become a class of novel anticancer compounds with high activity

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