A REVIEW ON RATIONAL DESIGN OF COVALENTLY AND NON-COVALENTLY BINDING ENZYME INHIBITORS

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ABSTRACT:

The design of enzyme inhibitors is one of the most captivating research topics in medicinal chemistry. Covalent inhibitors provide the opportunity of combining concepts of chemical reactivity and mechanisms of organic reactions with the structural features required for optimal molecular recognition in order to obtain the appropriate reactivity and selectivity profiles towards the desired enzyme target. A look at drug approvals in recent years suggests that covalent drugs will continue to make an impact on human health for years to come. The toxicity, high potencies and prolonged effects of covalent drugs result in less-frequent drug dosing. There are several examples of covalent inhibitors that are widely used drugs, including acetylsalicylic acid (an active ingredient of aspirin), orlistat (anti-obesity drug) and ampicillin (antibiotic). Overall, nearly 30% of the enzymes are irreversibly inhibited via covalent modification and highlights the therapeutic potential of covalent inhibitors.

KEYWORDS: Enzyme inhibitors, covalent inhibitors, toxicity, less-frequent drug dosing, acetyl salicylic acid, orlistat, and ampicillin.

INTRODUCTION:

Most small molecules drugs are designed to interact with their biological target and form the desired drug-protein interaction in a rapid and reversible fashion. The ratio of drug-protein complex to the unbound drug and free protein is dependent on the intrinsic affinity of the two partners. This interaction leads to a therapeutic response which is a common focus of modern drug discovery and to maximize the strength of these non-covalent molecular interactions. However, a nonconventional strategy termed 'covalent inhibition' has brought the consciousness in the number of drug hunting teams. In recent years, it has been recognized that distinct strengths of covalent and non-covalent modes of drug action may be brought together by designing compounds that combine their reactivity with specific complementarity to the target. This concept has a long track record in the form of mechanism-based or suicide inhibitors that directly target a catalytic nucleophile within the active site of the enzyme. However, current covalent drug discovery programs take a different approach by targeting a non-catalytic nucleophile. Such compounds are referred to as "targeted covalent inhibitors" (TCIs). [1, 2] These types of drugs possess distinct selectivity profiles compared to reversible inhibitors.

In this review, we briefly examine the clinical utility of covalent drugs and their potential pharmacological advantages compared to conventional agents. We also discuss the potential risks and challenges associated with covalent drugs and how they can be overcome by careful optimization of binding and reactivity using structure-based drug design.

COVALENT INHIBITORS AS AVOIDED LIABILITIES:

A widespread view in drug discovery is that electrophiles should be excluded from the drug candidates for safety [3]. Over the decades, studies on the toxic effects of xenobiotics, revealed strong associations between a variety of simple

chemicals and carcinogenesis (e.g., N, N-dimethyl-4-aminoazobenzene and N-acetyl-2-aminofluorene). The Millers postulated[4,5] that certain inert chemicals are converted to electrophilic metabolites in the body that react with proteins, lipids, DNA and other biomolecules to cause cellular damage. Other observations during the second half of the 20th century also conspired against the use of electrophiles in the drugs. During the 1970s, the acute toxicity resulting from large doses (i.e., several grams) of acetaminophen was traced to N-acetyl-p-benzoquinone imine (NAPBQI) a primary drug metabolite [6]. NABPQI is an electrophile that reacts with circulating nucleophiles such as glutathione and various hepatic proteins. The acute toxicity of NABPQI is so powerful that suicide by acetaminophen overdose is common worldwide [7].

Bromobenzene, although not a medicine is another agent whose primary metabolite is a potent alkylator of macromolecules. The toxic metabolites 2, 3- and 3, 4-bromobenzene epoxide are formed by the processing of bromobenzene by mixed-function oxidases in the liver and are known to cause tissue damage [8]. Urushiol, an oily substance produced by certain plants including poison ivy, is readily oxidized *in vivo* to generate electrophilic ortho-quinones that react with nucleophilic amines and thiols on proteins of the membrane [9]. The haptenization of host proteins by urushiol quinine causes dermatitis at the site of contact, resulting from activation of the immune system. Idiosyncratic toxicity is a concern for all drug development programs, irrespective of whether a drug is covalent or non-covalent by design. Some examples of non-covalent drugs which still cause idiosyncratic toxicity include halothane (anesthetic), sulfamethoxazole (antibiotic), carbamazepine (antiepileptic) and felbamate (antiepileptic) [10].

ADVANTAGES OF COVALENT DRUGS:

These covalent drugs have been found to exhibit uniquely high levels of biochemical efficiency i.e., high efficacy and favorable therapeutic margins [11]. They function under nonequilibrium binding kinetics and their advantage is the mitigation of any potential competition with endogenous substrates for target binding, such as endogenous ATP as in case of kinase inhibitors whereas the conventional non-covalent drugs suffer from decreased potency as endogenous substrates build up during therapy and compete for target binding [11].

Houk *et al.* found that covalent irreversible inhibitors can overcome theoretical limits on potency as a function of molecular size because they are capable of binding to their targets permanently [12]. An additional significant advantage of covalent inhibition is the prolonged duration of action that results from the neutralization of target under nonequilibrium kinetics. In many cases, the pharmacodynamics of covalent inhibition can persist even after a drug is cleared from the body or the target organ. For example, a 3mg dose of rivastigmine, covalent inhibitor of acetylcholinesterase (AChE) for dementia is sufficient to induce target inhibition for >10 hrs despite the plasma half-life of only 1hour [13]. These unique features of covalent inhibitors bring certain practical advantages such as increased scope to advance molecules that have short exposure against a particular target [14]. The prolonged duration of action of covalent drugs results in less-frequent drug dosing, this reduces the idiosyncratic toxicity and potentially improves medication compliance [15].

DRUG RESISTANCE:

A major drawback for the treatment of cancer and infectious diseases is the prevalence of drug resistance due to mutations in the binding site of the target. Irreversible inhibitors maintain against drug-resistant mutations that are acquired after treatment with reversible inhibitors [16]. For example, 50% of patients with non-small cell lung carcinoma(NSCLC) who initially responded to reversible EGFR relapsed due to the emergence of tumor cells that express EGFR with mutations at T790M and L858R in the ATP binding site[16,17]. Screening of a panel of known inhibitors for activity against T790M-L858R double mutant form of EGFR showed that the irreversible inhibitors

were all effective at inhibiting cell proliferation where none of the reversible EGFR inhibitors tested was effective against the mutant cell line [16, 18].

EXAMPLES OF TARGETED COVALENT INHIBITORS:

ONCOLOGY INDICATIONS:

1) Bruton's Tyrosine Kinase, a target to treat B-cell malignancies was discovered in 1993. Ibrutinib, the only approved drug in 2013 by Johnson & Johnson marketed by AbbVie.[19]

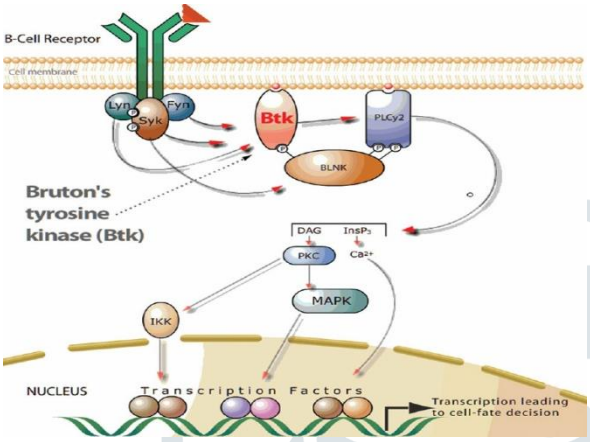
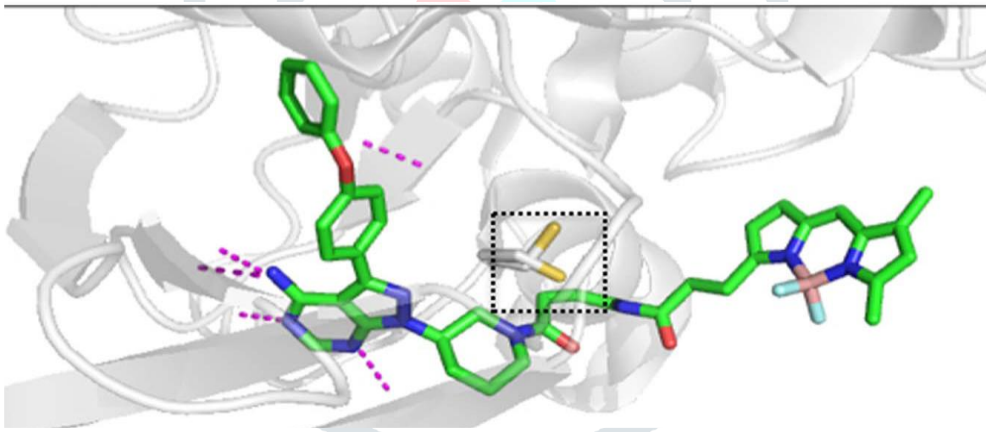


Figure 1: Mechanism of BTK in inhibition.

a)



b)

Figure 2: a. Crystal structure prediction of Ibrutinib binding to BTK. The reactive cysteine is highlighted in yellow inside the box. Hydrogen bonds are shown as purple dotted lines. 3D molecules were rendered using PyMol. b) Ibrutinib binding to the cysteine residue of BTK active binding site.

EGFR:

EGFR is a receptor tyrosine kinase responsible for malignancies including non-small cell lung cancer(NSCLC) and glioblastoma[20]. The EGFR inhibitor Afatinib was approved in 2013, whereas EGFR inhibitors rociletinib, dacomitinib, neratinib, and AZD9291 are currently in clinical trials for several cancer indications[21].

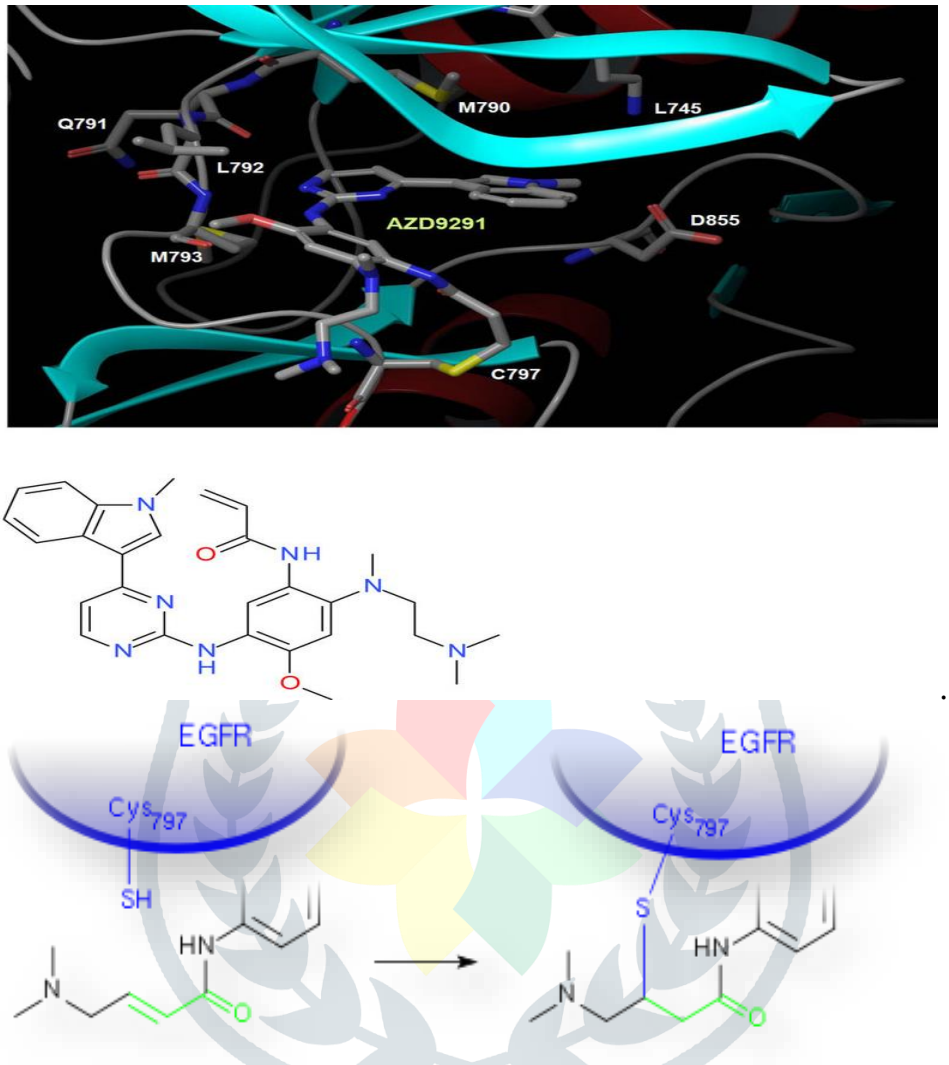


Figure 3: AZD9291 (Osimertinib) binding to EGFR active site.

MEK1:

Mitogen-activated protein kinases 1 and 2 (MEK1 and MEK2) play key roles in signal transduction within the Ras-Raf-MEK-ERK1/2 pathway commonly responsible for carcinogenesis[22]. Although a single noncovalent inhibitor of MEK1/2 has been approved in the past decade i.e. trametinib.

E6201 is a low nanomolar, covalent inhibitor of MEK1 which is currently in clinical trials for solid tumors and also for psoriasis. It exhibits improved plasma stability and is available for intravenous or topical use[23]. The enone embedded within the macrocyclic ring of E6201 accepts a cysteine nucleophile in the active site of the MEK1.

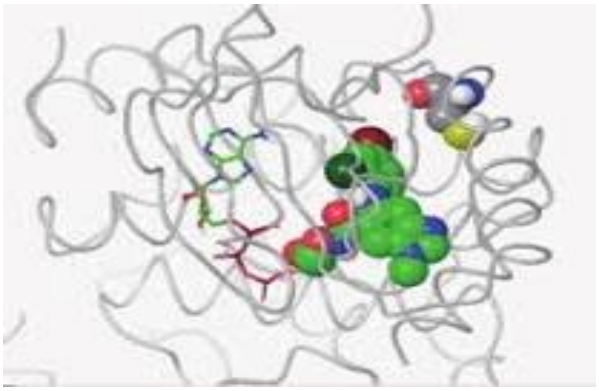
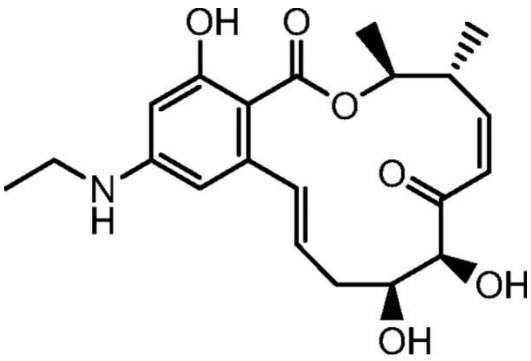
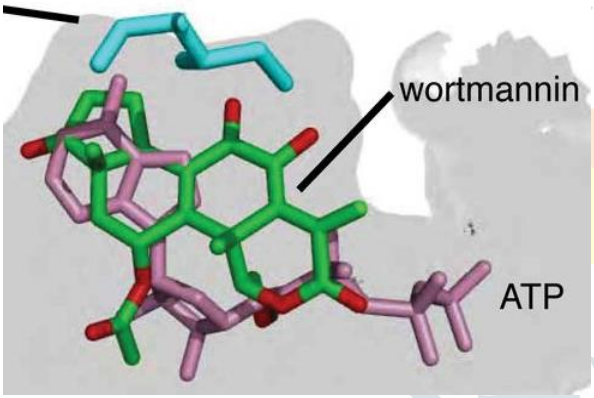



Figure 4: E6201 binding to MEK active site.

P13K:

Phosphatidylinositol 3-kinases (P13Ks) are a family of signal transduction enzymes that phosphorylate the inositol ring of phosphatidylinositol which plays a major role in cell proliferation, apoptosis and other cellular functions. This enzyme is altered in many human cancers and has emerged as a promising target in oncology. Wortmannin is a steroidal natural product that is potent but unselective P13K and binds covalently through an enoate within a furan ring[24]. To overcome this drawback of wortmannin, an analog named PX-866 has been developed as a stable P13K oral inhibitor[25]. PX-866 reacts with the lysine residue in the catalytic site of P13K through vinylogous transamidation reaction which results in irreversible inhibition of the kinase. It is currently in clinical trials against advanced tumors like glioblastoma and castration-resistant prostate cancer.



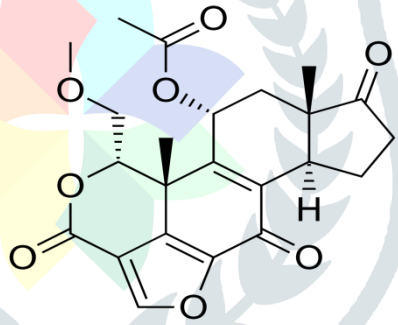


Figure 5: Wortmannin binding to P13K active site.

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NON-ONCOLOGY INDICATIONS:

Although, covalent drugs are often related to oncology applications, there are 80% of approved covalent drugs that are used in therapeutic areas other than cancer. Electrophiles used for non-oncology indications include not only Michael acceptors but also epoxides, nitriles, β-ketocarboxamides, ureas, and carbamates.

Keap1-Nrf2 pathway:

Nrf2 is a transcription factor that has a role in the cellular response to stress by upregulating genes involved in cytoprotection [26]. Under nonstressed conditions, Nrf2 forms a complex with the scaffolding protein Keap1, signaling the degradation of Nrf2 by nuclear export and proteolysis. In 2013, dimethyl fumarate was approved as an inducer of Nrf2 for **multiple sclerosis** [27]. Its metabolite monomethyl fumarate alkylates Cys151 of Keap1 [28, 29].

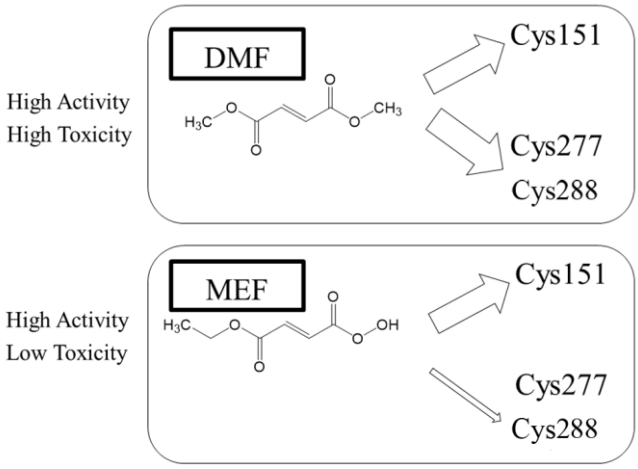

Figure 6: Dimethyl Fumarate binding to the cysteine residue of Keap1-nrf2 active site

Pancreatic lipase:

Pancreatic lipase is an enzyme that hydrolyzes triacylglycerol fatty acids and is a pharmacological target against obesity. The hydrolysis of dietary fat esters is required by the body to absorb low molecular weight fatty acids. Inhibition of pancreatic and gastric lipase activity results in the passage of unhydrolyzed, intact triacylglycerols through the stools [30]. Orlistat is an oral inhibitor of pancreatic lipase derived from the natural product lipstatin. The β-lactone of orlistat covalently reacts with a catalytic active-site serine of pancreatic lipase [31].

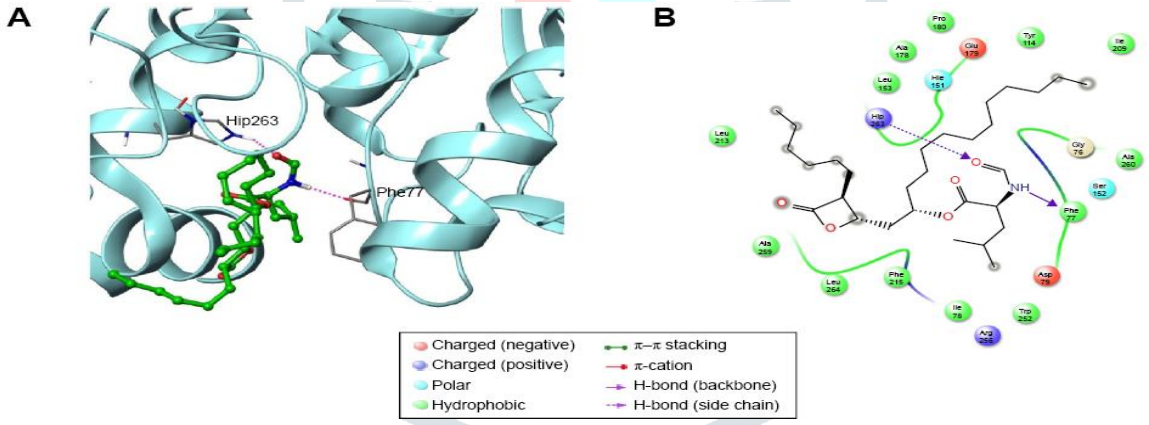

Figure 7: Orlistat binding covalently to Pancreatic Lipase by Serine residues.

FAAH:

Fatty acid amide hydrolase (FAAH) is an integral membrane protein responsible for the hydrolysis of bioactive fatty acid amides, which have a role in pain reception and inflammation. Inhibition of FAAH leads to elevated levels of these fatty acid neurotransmitters. PF-04457845 is an orally covalent inhibitor of FAAH which is clinical trials and is used to treat chronic pain and nervous disorders [32]. PF-04457845 forms a carbamate linkage with the catalytic nucleophile Ser241 of FAAH which ultimately releases 3-aminopyridazine [33].

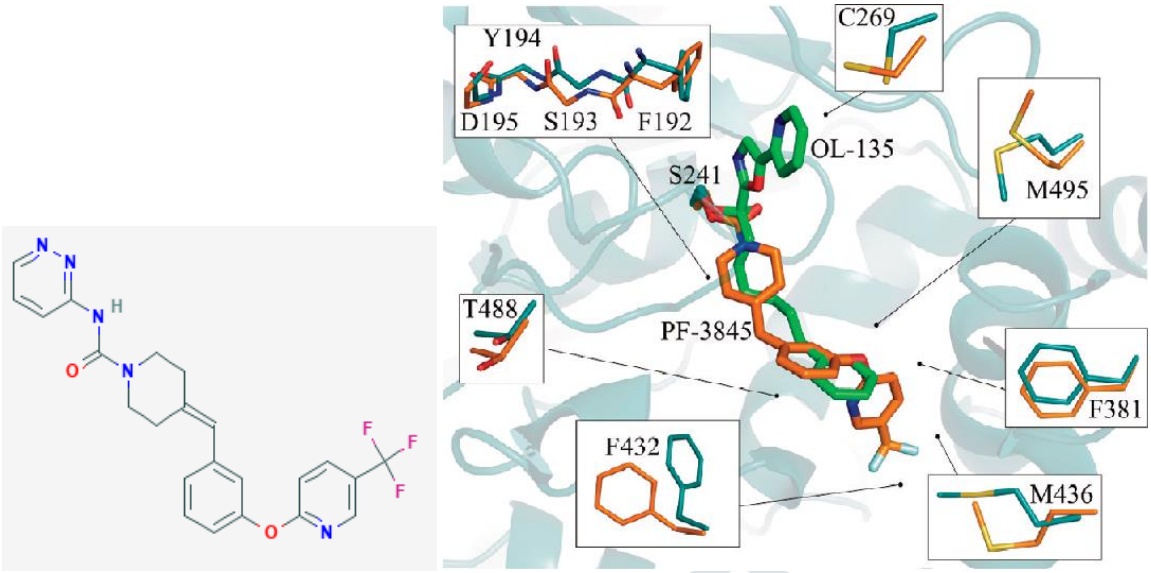

Figure 8: PF-04457845 binding to FAAH by Serine-241 residue.

AChE:

Acetylcholine is a neurotransmitter that stimulates cholinergic receptors at chemical synapses in the central nervous system. Patients with Alzheimer's disease (AD) possess decreased levels of these receptors. Therefore to combat the dementia symptoms of AD acetylcholine levels are increased at these synapses [34]. Rivastigmine is approved covalent inhibitor acetylcholinesterase (AChE), an enzyme that hydrolyzes acetylcholine. When bound to AChE, rivastigmine acylates an active site serine through its phenolic carbamate. Although rivastigmine is cleared quickly, its inhibitory effects on AChE lasts up to 10 hrs [35]. Rivastigmine is an analog of the natural product physostigmine is used as an oral or transdermal agent in the treatment of dementia in AD and Parkinson's disease.

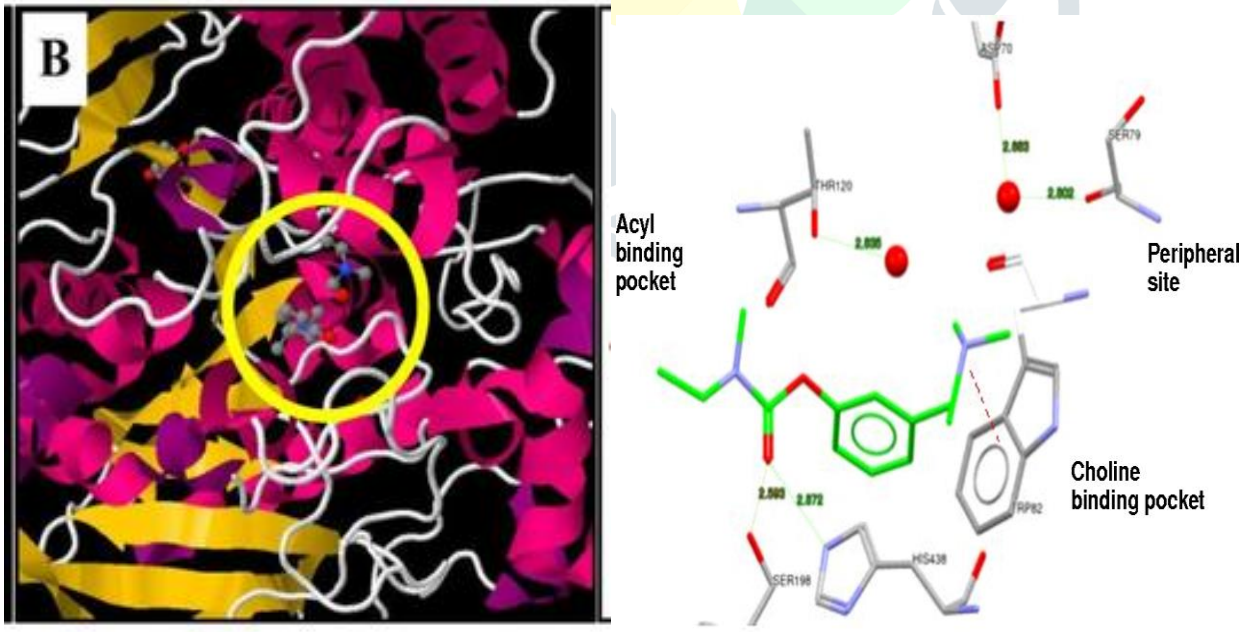

Figure 9: Rivastigmine binding to Acetylcholinesterase by Serine-198 residue.

Cat K:

Cathepsin K (Cat K) is a cysteine protease that degrades collagen, a non-mineral component of bone [36]. Collagen degradation is responsible for mammalian bone resorption and thus Cat K is an attractive target for treating

osteoporosis-related bone loss. Odanacatib is a Cat K inhibitor currently in clinical trials for reducing bone fractures in older women [37]. It is an electrophilic nitrile that acts covalently on Cat K by reacting with the cysteine residue and generates a thioimidate intermediate [38]. Its long half-life and prolonged inhibition is being investigated as a once-weekly oral osteoporosis agent.

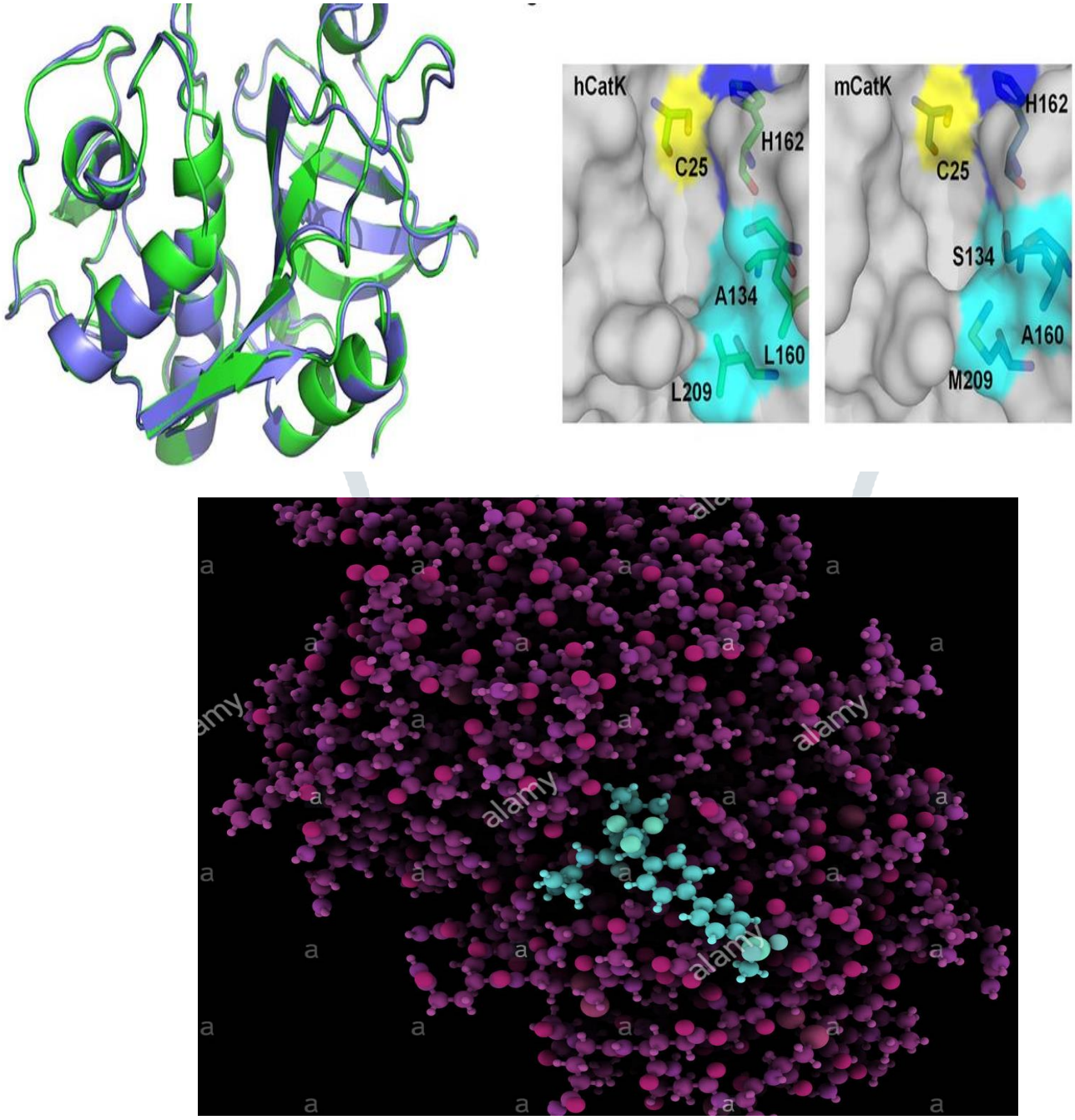

Figure 9: Odanacatib binding to Cathepsin K by cysteine residue.

Conclusion:

Regardless of many examples of prosperous covalent drugs, principles for the rational design of these covalent drugs have just emerged. This manifests that structural bioinformatics approaches coupled with structure-based drug design empower the design of highly selective covalent drugs. A better understanding of the benefit-risk balance of the mechanism of drug action is enabled by better knowledge of the important insights into the safety and efficacy profiles of the advanced TCI clinical development.

The purpose of this review is to encourage the study and search for the advantages and limitations of the covalent approach. It is anticipated that in the next decade we will see a target-directed, structure-guided drug discovery paradigm with the resurgence of great interest in this important class of therapeutics.

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