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Design of Potential Inhibitors of Urokinase in Silico Approach

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Short Communication

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ABSTRACT

Urokinase-type plasminogen activator (uPA) plays an important role in the regulation of diverse physiological and pathological processes. Elevated uPA expression is associated with cancer progression, metastasis, and shortened survival in patients, whereas suppression of proteolytic activity of uPA leads to evident decrease of metastasis. Therefore, uPA has been considered as a promising molecular target for development of anticancer drugs. The aim of this study was to find effective and selective inhibitors of uPA using molecular modeling study. A library of chemical compounds derived from Naphtalin and Theophylline cores was then docked within the active site of the uPA. The binding affinity and the binding positions of the suggested compounds with the active site of the previously prepared uPA enzyme were determined. The study led to three effective and selective inhibitors of the uPA enzyme; one compound derived from the Naphtalin core -Naph 15- and two compounds derived from the Theophylline core -Theo17 and Theo 18-. The study was completed by chemical synthesis of Theo17 molecule.

INTRODUCTION

Proteolytic enzymes (proteases) comprise a family of enzymes which hydrolyse protein or peptide substrates in the generalized process of intracellular protein degradation, a process essential for the normal functioning of all cells. Proteolytic processes are necessary for normal physiological functions in the body; the same enzyme system for these functions is also used by the cancer cells for their growth and spread. These enzymes are produced by the tumor cells or cells surrounding them and can degrade the basement membrane and Extracellular Matrix (ECM). Degradation of the surrounding connective tissue is considered a necessary step to allow malignant cells to locally invade, to enter the lymphatic or blood circulation and to metastasize [1]. Proteases include many groups; one of the most important groups is serine proteases. Urokinase-type plasminogen activator (uPA), the trypsin like serine protease is strongly associated with tumor cells and is implicated in a large number of malignancies, including cancers of the breast, lung, bladder, stomach, cervix, kidney, and brain, and high levels of urokinase have been correlated with poor patient prognosis. Thus, there is great clinical interest in the development of potent and bioavailable inhibitors of urokinase that can serve as therapeutic agents for the treatment of cancer.

The uPA is a 53 kDa multidomain glycoprotein of 411 residues, glycosylated at Asn302, synthesized and secreted as a single-chain enzymogen (pro-uPA or sc-uPA); once released in extracellular environs, the sc-uPA is exposed to the action of proteases which may generate enzymatically active or inactive high-molecular-weight forms of uPA (HMW-uPA). Plasmin, cathepsin B and L, kallikrein, trypsin or thermolysin cleave the sc-uPA peptide bond K158-I159 converting the proenzyme in the active disulfide bridge-linked two-chain form (tc-uPA). Of the two chains, the N-terminal (A-chain) contains the kringle domain and the epidermal growth factor (EGF)-like domain, the latter responsible for the binding to uPAR, whereas the C terminal (B-chain) is composed of two subdomains formed by six -strands folded in an antiparallel manner and connected by twist or helical regions [2]. The active site is located at the interface between the two subdomains, and consists of the catalytic triad of His57, Asp102 and Ser195 residues.

The tc-uPA has restricted substrate specificity with plasminogen as main substrate. Plasmin is the primary activator of sc-uPA and is in turn activated by tc-uPA, thus enhancing its own production. Such phenomenon, referred as “reciprocal zymogen activation”, occurs much more efficiently when the sc-uPA is associated with its receptor uPAR. As a consequence, the active uPA generation is concentrated in the pericellular area, where it represents an effective and rapid source of plasmin during cell migration and invasion under physiological or pathological conditions. There are reports of nonpeptidic, reversible inhibitors of uPA from as far back as the late 1950’s. These small molecule inhibitors include benzamidines, phenylguanidines, acylguanidines and bisbenzamidines. The best of these early uPA inhibitors have μM potencies and poor selectivity. Several novel uPA inhibitors with nM potency and selectivity towards uPA include benzothiopheneamidines (Eisai), 5-thiomethylthiopheneamidine, Naphthylamidines (Abbott), and isoquinolynylguanidines (Pfizer).

In the past few years, a number of novel small molecule uPA inhibitors have been proposed. However, among these inhibitors, only WX-UK1 (WILEX, Munich, Germany) entered clinical development, showing a against human uPA of 0.6 M. Since WX-UK1 is not absorbed orally, more recently, Wilex developed an oral prodrug, WX-671, for the systemic delivery of the active WX-UK1. This prodrug is currently under evaluation in two independent studies of phase II clinical trials in combination with classical cytotoxic treatments to estimate its efficiency. The crystal structure of uPA catalytic domain displays a trypsin-like topology in which the Asp189 is retained, conferring to the S1 site an affinity for positively charged Arg and Lys residues. Therefore, the majority of synthetic uPA inhibitors, conceived so far, share a common structural feature consisting of a mono- or biaromatic moiety substituted with an amidino or guanidino function, acting as arginine mimetic.

However, a strong limitation in the choice of feasible compounds is represented by the necessity to inhibit uPA without affecting the activity of other trypsin-like serine proteases, and especially tPA and plasmin, essential for the fibrinolytic processes. In the development of a clinical agent, selectivity for the target protein is important for reducing the potential for harmful side-effects [3]. This is particularly true for the trypsin-like family of proteases that have been implicated in a number of highly regulated processes. The S1β pocket of bovine trypsin is different from that of urokinase primarily because of an asparagine substitution for Lys143; filling this site could result in a change of the specificity profile. Occuping S1β more fully, results in a loss of potency for trypsin while maintaining high potency for urokinase.

Hence, binding at S1β appears to exploit structural differences between urokinase and trypsin, thus has resulted in potent and specific urokinase inhibitors. Though, both the design and synthesis of a vast number of selective uPA inhibitors, there is still an inability to find critical chemical features of uPA inhibitors. This leads to discover new inhibitors by studying new molecules through molecular modeling and synthesizing them. Montelukast sodium is a leukotriene receptor antagonist (LTRA) used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies. Desloratadine (descarboethoxyloratadine), invented in 2005, is a non sedative metabolite of Loratadine, a second generation long acting antihistaminic drug with selective peripheral H1 receptor antagonistic activity.

According to the importance of the inhibition of uPA enzyme and the crucial role of molecular modeling at this time in designing new inhibitors for various kinds of enzymes, the molecular modeling has been used in this research to design new inhibitors of uPA enzyme using Discovery Studio 2016 (DS) program and protein Data Bank (PDB), a huge bank which contains a big number of crystalline proteins and macromolecules with or without compounds attached to them.

DISCUSSION

When preparing a set of data, all the available crystalline forms of uPA were collected from the PDB. A crystalline form of the enzyme in complex with an inhibitor was chosen for the study (pdb code: 1owd). Different amino acid residues in the active site are important for binding, such as the catalytic triad residues Ser195, Asp102, His57 and the S1 pocket residues like Asp189, Gly218 and Arg 217. Asp189 is located at the bottom of UPA's S1 pocket. The negative charge of Asp189 is important because it stabilizes amino acids with positively charged side chains which found in the substrate Plasminogen .The crystalline form of the uPA protein 1owd was asso ciated with an inhibitor derived from the 2- Naphtamidine core. The shape is highly accurate R=2.3A° and the Root Mean Square Deviation was less than 2. Depending on the nature of the cores commonly used in the design of uPA inhibitors, which showed good IC50 values, two cores were proposed to develop uPA inhibitors. The first core (Naphtalin) provides the appropriate dimensions for the ligand in order to fit within the active site. The second chosen core was Theophylline which is generally available with low price. After drawing and designing the compounds derived from these two cores, the study of the docking process of these compounds was carried out within the active site of the uPA enzyme [4]. The docking process between the protein 1O WD and the compounds drawn from the cores was carried out using the CDocker process in DS program. The validation of the docking method was first investigated by comparing the standard deviation of the basic inhibitor with the deviation placement according to the CDocker method provided, where the standard deviation RMSD must be less than 2A°. This was followed by a docking procedure between 1OWD and the drawn compounds according to the protocol followed in the program.

After the completion of the docking process, the binding mode was studied and the binding affinity, which is expressed as a score function, was measured as the value of -CDocker energy. In other words, the binding of the CDocker method is indicated by the negative value of the CDocker energy and Theophylline core. Many of the chemical substituents, including hydrogen and alkyl groups, were analyzed and their affinity was determined based on two basic principles. The first was the expression of the correlation between position, type of substituents and the binding affinity [5]. The second was the best binding with the most important amino acids such as Ser195, Asp102, His57 and the S1 pocket residues like Asp189, Gly218 and Arg217. The Selection of the best compound was dependent on two parameters, the first was the score function and the second was the ability of binding with the important amino acids in the active site of uPA. In addition to that, the uPA enzyme has an additional extra hydrophobic pocket within the S1 pocket; it is called S1β which can be exploited. This can add an extra feature for the new inhibitor of the uPA enzyme. Regarding the Naphtalin core, many kinds of substituents were added in order to carry out a detailed study of the potential inhibitors within the active pocket based on the principles mentioned above. The results were discussed in detail.

CONCLUSION

Molecular modeling was performed on uPA. Docking study using Naphtalin and theophylline cores led to identify different hits which had high affinity to the uPA active pocket; In addition to that, these compounds formed bonds with important amino acids such as Ser195, Cys191 and Asp189. Theophylline derivatives like Theo17 and Theo18 can be synthesized, first by N-alkylation of the core unit with an alkyl group such as dichloroethane in presence of strong base according to caffeine synthesis process. And then, N-alkylation between urea and the intermediate compound can be achieved to get Theo17, or we can get Theo18 using tartaric acid with the same intermediate compound in the presence of an appropriate base.

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