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Editorial

Special Issue of "Synthesis, Biological Evaluation and Molecular Modeling of Enzyme Inhibitors"

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1. Introduction

Enzymes are essential macromolecules responsible for biochemical processes occurring in living organisms. The action of enzymes as biocatalysts is highly selective and dependent on the structure of substrates. Research on the enzymes' function in relation to the interactions between substrates and the enzyme active site is an endless resource of exciting discoveries. Investigating the structural fitting of ligands to the enzyme binding sites and the role of specific ligand configuration in the enzyme cavity might be the way to learn more about the mechanisms of catalytic action of these biomolecules. Furthermore, knowing the crucial structural determinants, we may design and synthesize new ligands—the substances of better affinity to the enzyme cavity, which in turn may be more efficient therapeutic agents. In other words, it is possible to modulate the enzyme's biological activity and to control substrate metabolism more precisely if the character of enzyme–substrate interactions is known. One of the mechanisms of drug action is to modulate, both activate and inhibit, the activity of enzymes that regulate important life processes [1,2].

In drug design and development, computational methods such as protein structure modeling and structure-based drug design (SBDD) are widely employed. This approach requires knowledge of the structure of the enzyme being the target, as opposed to the ligand-based drug design (LBDD), which does not use the three-dimensional structure of the enzyme [3,4]. In drug design research, molecular docking is used to obtain information about how ligands bind to a macromolecular target and to estimate the affinity of a ligand for a protein, providing insight into the molecular recognition process [5,6]. On the other hand, molecular dynamics (MD) simulations allow us to take into account an induced fit in the formation of ligand–protein complexes, and help to optimize hit molecules found in virtual screenings. MD simulations also enable the study of the phenomena of binding and unbinding of ligands along with the kinetics and energetics of these processes [6,7]. Computational analysis and calculations need to be integrated with experimental evaluation of the biologic activity of the studied compounds (Scheme 1).

In the Special Issue "Synthesis, Biological Evaluation and Molecular Modeling of Enzyme Inhibitors", we aimed to gather the reports within the frame of computational enzymology, the field of research devoted to the substrate–enzyme interactions studied with the use of computational methods.

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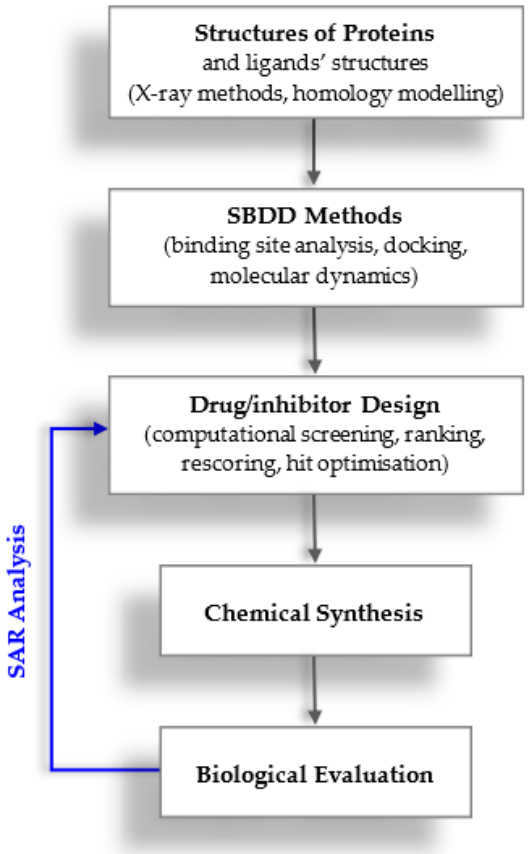

Scheme 1. Application of computational methods in the process of drug discovery and development.

2. An Overview of Published Articles

The articles published in the Special Issue present studies performed in part with SBDD methods, such as homology modeling, docking, and geometry optimization of ligand–protein complexes through energy minimization or short MD simulations to analyze and elucidate structure–activity relationships. A comprehensive review titled "New Perspectives of CYP1B1 Inhibitors in the Light of Molecular Studies" (contribution 1) demonstrates the role of molecular docking and MD simulations in the studies of cytochrome P450 1B1 (CYP1B1) ligands, which could be used to modulate CYP1B1 activity. CYP1B1 is a member of the superfamily of cytochromes P450—the membrane-bound hemoproteins responsible for the metabolism of numerous endo- and exogenic substrates, including substances involved in carcinogenesis [8,9]. Inhibitors of CYP1B1 activity are supposed to be used in chemoprevention and therapy of some diseases, such as glaucoma, metabolic disorders, cardiovascular diseases, and cancer. Studies presented in the review point to the dependence of enzyme–ligand interactions on the structure, flexibility, and shape of a ligand, electrostatic environment, and malleability of the enzyme active site. The article examines computational methods used in the CYP1B1 studies, including MD simulations of inhibitor–enzyme interactions.

In the studies reported in the article titled "Gold(I) Complexes with P-Donor Ligands and Their Biological Evaluation" (contribution 2), three gold(I) complexes were synthesized, and their cytotoxic effect on cancer cells and inhibitory activity against thioredoxin reductase were estimated. Differences in their physicochemical and biological properties were presumably related to the ligand in the metal complex and the mode of the ligand binding with a Au atom. Using crystallographic analysis, the authors tried to find structural determinants that influence the cytotoxicity of the tested compounds differing from the complex ligands bound with Au atoms. Previous studies have shown that the gold(I) complexes with P-donor ligands are compounds exhibiting biological activities that seem

promising in cancer therapy [10]. Although their anticancer activity is well documented, the mechanism of action is still being studied and as such is not fully understood. The Au-containing metallodrug, auranofin, accepted for rheumatoid arthritis treatment exerts an inhibitory effect against thioredoxin reductase, an enzyme engaged in the regulation of cellular redox balance, cell growth, and survival. Therefore, thioredoxin reductase, as a component of the thioredoxin system, is the target of anti-cancer therapy [11]. In this article, the biological evaluation of the studied compounds as potential drugs also involved their lipophilicity, which determines the bioavailability of compounds and can, to a large extent, influence their action in the target cells (contribution 2).

Another subject of the studies presented in the Special Issue, levan, is a carbohydrate homopolymer produced by plants and microorganisms, including bacterial strains, due to the presence of the levansucrase gene (contribution 3). This polysaccharide composed of fructose units has been recently tested for health-promoting properties, including antioxidative, antiobesity, antifungal, antidiabetes, and antitumor effects [12]. The authors of the article entitled "Cloning and Expression of Levansucrase Gene of *Bacillus velezensis* BM-2 and Enzymatic Synthesis of Levan" (contribution 3) obtained the levansucrase with the use of biotechnological methods and purified and optimized the process of levan enzymatic synthesis, finally achieving very good efficiency. By means of molecular docking, it was possible to identify the amino acid residues of levansucrase of *Bacillus velezensis* BM-2, which form catalytic triads (acid-base-nucleophile) responsible for sucrose interaction with the enzyme. It should be noted that this study is of particular importance because of possible applications in industrial levan production.

The article entitled "Synthesis of 2-(4-hydroxyphenyl)ethyl 3,4,5-trihydroxy benzoate and Its Inhibitory Effect on Sucrase and Maltase" (contribution 4) is devoted to the determination of kinetic properties of α-glucosidase inhibition by the compound previously obtained from *Rhodiola crenulate*, in comparison with the inhibitors widely used for the treatment of diabetes. The results show the potential of the new α-glucosidases' inhibitor in hyperglycemia prevention. The authors used molecular docking to identify and characterize inhibitor–enzyme interactions, whereas molecular modeling was used in the studies of the effect of mutations in the binding site of human glutathione transferase P1-1 on the binding of glutathione derivatives as substrates (contribution 5). Xu et al. used SBDD methods to a fairly large extent, generating a protein model and using it for substrate docking [13]. Three of the six recombinant mutant enzymes expressed in *Escherichia coli*, and generated in the studies, showed measurable glutathione transferase activity. Steady-state kinetics comparing glutathione with the alternative thiol substrate glutamylcysteine points to the glycine in the glutathione molecule as an important residue on which, to a large extent, catalytic efficiency depends. In this study, the authors attempt to explain the differences in the binding energies determined for three transferase mutants and six substrates—glutathione derivatives with molecular modeling. By superposition of molecules in the active site, they demonstrate a similar orientation of S-hexylglutathione and TER117, the compound of therapeutic value in the therapy of GST P1-1 expressing cancer cells.

3. Conclusions

To summarize, the articles included in the Special Issue of "Synthesis, Biological Evaluation and Molecular Modeling of Enzyme Inhibitors" present various applications of molecular docking, modeling, and crystallographic analysis in relation to the biological activity of enzymes and structural properties of their ligands: inhibitors or substrates [13–16]. SBDD methods, although known and used for a long time, are constantly being developed and improved, providing increasingly detailed data on the ligand–protein interactions that determine enzyme catalytic activity. Regarding docking, there are new attempts to solve the problems related to the flexibility of the ligand and protein, the role of the conserved structural water molecules in ligand binding, docking of covalent inhibitors, or a ligand's interactions with metal cofactors [5,6]. Scoring functions (SFs) are constantly improving, especially those from the machine-learning-based group [17]. Docking programs using new hardware such

as graphical processing units (GPUs) are also developing [18–20]. New perspectives are created by the improvement of algorithms used in simulations [7,21–24] and force fields (FFs), including FFs that contain an explicit representation of polarizability [22–25]. It remains to be hoped that further development of in silico methods will facilitate a consent search for new bioactive molecules, including drug candidates [26,27]. Consequently, studies of the structure–activity relationship at the molecular level are particularly important due to their use in designing new effective and safe therapeutic agents.

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