APPLICATIONS OF NANOMATERIALS FOR BIOMATERIALS: DEVELOPING NANOMATERIAL-ENHANCED BIOMATERIALS USING COMPUTATIONAL DOCKING OF PROTEIN-LIGAND PAIRS

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ABSTRACT

The integration of nanotechnology with protein-ligand interactions offers a promising avenue for developing advanced biomaterials for therapeutic and diagnostic applications. This project investigates the role of protein-ligand interactions in designing nanomaterial-based systems, focusing on their potential to address challenges in precision medicine, targeted therapies, and diagnostics. Using AutoDock Vina, a computational molecular docking tool, we evaluated the binding affinities and interaction mechanisms of ten therapeutically relevant protein-ligand pairs: Cyclooxygenase-2 (COX-2) with Celecoxib (PDB ID: 3LN1), HIV Reverse Transcriptase (HIV RT) with Nevirapine (1RT1), EGFR Kinase with Erlotinib (1M17), Aldose Reductase with Fidarestat (1US0), Acetylcholinesterase (AChE) with Donepezil (4EY6), Human Cyclin Kinase 2 with Roscovitine (1HCK), Human Epidermal Growth Factor Receptor 2 (HER2) with Lapatinib (3PP0), Human Tyrosinase with Kojic Acid (5M8P), Alpha-Synuclein with Dopamine (2KKW), and Cytochrome P450 3A4 (CYP3A4) with Midazolam (1WOE). Binding affinities ranged from -6.5 to -10.0 kcal/mol, with HER2-Lapatinib and COX-2-Celecoxib exhibiting the strongest interactions at -10.0 and -9.0 kcal/mol, respectively, indicating high specificity for targeted cancer therapies, while Alpha-Synuclein-Dopamine showed the weakest affinity at -6.5 kcal/mol, suggesting challenges in Parkinson's treatment.Nanomaterials, including silica nanoparticles, gold nanorods, liposomes, magnetic nanoparticles, and polymeric nanocarriers, were integrated with these protein-ligand pairs to develop innovative biomaterials. Applications included targeted drug delivery (e.g., silica nanoparticles with Celecoxib for anti-inflammatory therapy), photothermal therapy (e.g., gold nanorods with Lapatinib for HER2-positive breast cancer), controlled release systems (e.g., lipid-based nanoparticles with Nevirapine for HIV treatment), and diagnostics (e.g., magnetic nanoparticles with Erlotinib for cancer imaging). The study also explored nanomaterial-enhanced solutions for crossing biological barriers, such as liposomes delivering Donepezil across the blood-brain barrier for Alzheimer's treatment. Results highlight the synergy between protein-ligand docking and nanotechnology, with strong binding affinities correlating with effective nanomaterial applications, such as Aldose Reductase-Fidarestat (-9.5 kcal/mol) for diabetes management and AChE-Donepezil (-8.75 kcal/mol) for cognitive improvement in Alzheimer's. Despite challenges like weak affinities in certain pairs and the need for experimental validation, this project underscores the potential of nanomaterial-based biomaterials in advancing precision medicine, offering a foundation for future research in targeted therapies and diagnostics for cancer, HIV, neurodegenerative diseases, and metabolic disorders.

Keyword: - *Protein-ligand interactions, nanotechnology, molecular docking, targeted drug delivery, biomaterials, precision medicine, cancer therapy, neurodegenerative diseases, metabolic disorders..*

1. NANOTECHNOLOGY IN BIOMEDICINE: ADVANCEMENTS IN DRUG DELIVERY AND DIAGNOSTICS

Nanotechnology has transformed biomedicine by providing novel solutions for drug delivery, diagnostics, and targeted therapies. The distinctive properties of nanomaterials—high surface area-to-volume ratios, tunable sizes, and surface functionalization—enable their application in medicine, allowing for precise interactions with biological molecules. These nanocarriers enhance drug solubility, stability, and bioavailability, protect therapeutic agents from enzymatic degradation, and facilitate controlled release at specific sites in the body.

Nanomedicine has significantly improved treatment strategies for complex diseases such as cancer, HIV, neurodegenerative disorders, and diabetes. The ability to engineer nanoparticles for selective targeting reduces systemic toxicity and enhances therapeutic efficacy. Additionally, nanomaterials have expanded the scope of diagnostics by enabling sensitive biosensing and high-resolution imaging techniques.

1.1 Protein-Ligand Interactions in Drug Discovery

Protein-ligand interactions are fundamental to biological processes, influencing enzymatic reactions, signal transduction, and immune responses. In drug discovery, understanding these interactions helps design small molecules that modulate protein function with high specificity. The binding strength, often measured in terms of free energy (kcal/mol), determines the potency and efficacy of a drug candidate—lower binding energy indicates a stronger interaction.

Computational tools, particularly molecular docking, have become indispensable in predicting how a ligand will interact with a protein. Molecular docking simulates the binding process, estimating binding affinities and identifying key interaction sites. This enables researchers to prioritize lead compounds for experimental validation.

AutoDock Vina, the docking tool used in this study, is widely recognized for its accuracy and efficiency. It employs a scoring function that considers both ligand flexibility and receptor binding site characteristics to predict the best binding conformations. This computational approach accelerates drug discovery by reducing the need for extensive in vitro and in vivo screening.

1.2Integration of Nanotechnology and Protein-Ligand Interactions

By combining the specificity of protein-ligand interactions with the advanced delivery capabilities of nanomaterials, researchers can develop innovative therapeutic and diagnostic systems. Functionalized nanoparticles can be designed to selectively bind to disease-associated proteins, enabling targeted drug delivery and precision medicine.

For instance, in cancer therapy, nanoparticles conjugated with ligands can specifically target proteins overexpressed in tumor cells, such as HER2 in breast cancer or EGFR in non-small cell lung cancer. This targeted approach minimizes off-target effects and systemic toxicity while enhancing drug accumulation at the disease site. Additionally, nanomaterials can be engineered for diagnostic applications, such as imaging tumors using contrast-enhancing magnetic nanoparticles or quantum dots.

This study investigates the integration of nanomaterials with protein-ligand interactions to improve therapeutic efficacy, controlled drug release, and disease diagnosis.

1.3 Selected Protein-Ligand Pairs and Their Biomedical Relevance

This project utilizes AutoDock Vina to analyze the docking interactions of ten protein-ligand pairs, each chosen for its therapeutic significance.

1. **Cyclooxygenase-2 (COX-2) – Celecoxib**
    - COX-2 is an enzyme involved in the production of pro-inflammatory prostaglandins. Overexpression of COX-2 is linked to inflammatory diseases and certain cancers.
    - Celecoxib, a selective COX-2 inhibitor, is used as an anti-inflammatory drug and shows promise in cancer therapy by reducing tumor growth.
    - Nanomaterials: Silica nanoparticles for enhanced solubility and targeted delivery to inflamed tissues.
2. **HIV Reverse Transcriptase (HIV RT) – Nevirapine**
    - HIV RT is essential for viral replication, converting viral RNA into DNA.
    - Nevirapine, a non-nucleoside reverse transcriptase inhibitor, binds to an allosteric site, inhibiting enzyme activity and slowing disease progression.
    - Nanomaterials: Polymeric nanoparticles for sustained drug release, improving patient adherence.

3. **Epidermal Growth Factor Receptor (EGFR) Kinase – Erlotinib**
    - EGFR is overexpressed in non-small cell lung cancer (NSCLC), promoting tumor growth.
    - Erlotinib is a tyrosine kinase inhibitor that blocks EGFR signaling.
    - Nanomaterials: Magnetic nanoparticles for targeted therapy and MRI-guided tumor imaging.
4. **Aldose Reductase – Fidarestat**
    - Aldose reductase contributes to diabetic complications by converting glucose to sorbitol, leading to oxidative stress.
    - Fidarestat inhibits aldose reductase, reducing diabetic neuropathy and retinopathy risks.
    - Nanomaterials: Liposomes for controlled drug delivery and improved bioavailability.
5. **Acetylcholinesterase (AChE) – Donepezil**
    - AChE breaks down acetylcholine, a neurotransmitter essential for cognitive function.
    - Donepezil, an AChE inhibitor, increases acetylcholine levels, benefiting Alzheimer's patients.
    - Nanomaterials: Lipid-based nanoparticles to facilitate blood-brain barrier penetration.
6. **Cyclin Kinase 2 – Roscovitine**
    - Cyclin-dependent kinase 2 regulates the cell cycle, and its dysregulation is implicated in cancer.
    - Roscovitine induces cell cycle arrest in tumor cells.
    - Nanomaterials: Mesoporous silica nanoparticles for controlled drug release in cancer therapy.
7. **HER2 – Lapatinib**
    - HER2 is overexpressed in aggressive breast cancers, promoting uncontrolled cell growth.
    - Lapatinib is a kinase inhibitor targeting HER2 and EGFR.
    - Nanomaterials: Gold nanorods for targeted therapy and photothermal treatment.
8. **Tyrosinase – Kojic Acid**
    - Tyrosinase catalyzes melanin synthesis; its overactivity leads to hyperpigmentation.
    - Kojic acid inhibits tyrosinase, used in cosmetic formulations for skin-lightening.
    - Nanomaterials: Nanoliposomes for sustained skin-lightening effects in dermatology.
9. **Alpha-Synuclein – Dopamine**
    - Alpha-synuclein aggregation is a hallmark of Parkinson's disease.
    - Dopamine replenishment alleviates symptoms, but its delivery is limited by the blood-brain barrier.
    - Nanomaterials: Polymeric nanocarriers for brain-targeted dopamine delivery.
10. **Cytochrome P450 3A4 (CYP3A4) – Midazolam**
    - CYP3A4 is a key enzyme in drug metabolism.
    - Midazolam, a sedative, is metabolized by CYP3A4, providing insights into pharmacokinetics.
    - Nanomaterials: Polymeric nanoparticles for controlled drug release and metabolism studies.

1.4 Role of Nanomaterials in Biomedicine

Nanomaterials revolutionize medicine through their unique capabilities:

- **Drug Delivery**: Encapsulation in nanoparticles improves solubility, stability, and targeted release.
- **Targeted Therapies**: Functionalized nanoparticles bind selectively to disease markers, minimizing side effects.
- **Diagnostics & Imaging**: Nanomaterials enhance MRI, fluorescence imaging, and biosensing.
- **Controlled Release**: Smart nanocarriers release drugs in response to pH, temperature, or external stimuli.
- **Blood-Brain Barrier Penetration**: Lipid-based nanocarriers improve brain-targeted drug delivery.

2. AUTODOCK VINA: MOLECULAR DOCKING SOFTWARE

AutoDock Vina is a widely used open-source molecular docking software designed for predicting the binding affinity and interaction modes of protein-ligand complexes. It is an advanced version of AutoDock, offering improved accuracy, faster docking speeds, and enhanced usability.

2.1 Features of AutoDock Vina

- **High Speed & Efficiency**: Uses a sophisticated scoring function and global optimization algorithm to improve docking speed and accuracy.

- **Flexible Ligand Docking**: Allows ligands to adopt multiple conformations to explore optimal binding poses.
- **Automated Binding Affinity Calculation**: Provides binding energy scores in kcal/mol, indicating ligand stability within the active site.
- **Open-Source & Cross-Platform Compatibility**: Available for Windows, Linux, and macOS, facilitating widespread adoption in computational drug discovery.

2.2 Applications of AutoDock Vina

- **Drug Discovery & Lead Optimization**: Helps identify potential drug candidates by predicting interactions with target proteins.
- **Structure-Based Drug Design (SBDD)**: Used to refine and modify ligands based on their docking scores and binding interactions.
- **Molecular Recognition Studies**: Explores how small molecules interact with biological macromolecules.

2.3 Advantages of AutoDock Vina

- **Faster Computation**: Runs docking simulations significantly faster than its predecessor, AutoDock 4.
- **Improved Accuracy**: Employs an advanced scoring function to better predict binding affinities.
- **User-Friendly Interface**: Integrates with AutoDock Tools (ADT) for simplified input preparation.
- **Flexible Grid-Based Docking**: Provides accurate ligand placement within the active site.

2.4 Limitations of AutoDock Vina

- **Rigid Protein Docking**: Does not account for protein flexibility, which may affect binding accuracy.
- **Limited Support for Metal-Containing Proteins**: Struggles with docking ligands to metalloproteins due to the lack of specialized scoring functions.
- **Simplified Scoring Function**: May overlook complex solvent effects and entropic contributions.

2.5 Workflow of AutoDock Vina

1. **Protein Preparation**: Removing water molecules, adding hydrogen atoms, and assigning charges.
2. **Ligand Preparation**: Converting to the required format, energy minimization, and defining rotatable bonds.
3. **Grid Box Setup**: Defining the docking search space around the active site.
4. **Docking Execution**: Running simulations to generate multiple binding poses.
5. **Results Analysis**: Evaluating binding affinity scores and visualizing protein-ligand interactions using PyMOL or LigPlot+.

AutoDock Vina remains a crucial tool in computational docking studies, providing reliable predictions for drug discovery and molecular interaction research.

3. METHODOLOGY: SELECTION, PREPARATION, AND DOCKING OF PROTEIN-LIGAND PAIRS

3.1 Selection of Protein-Ligand Pairs

The selection of protein-ligand pairs was based on biological significance, therapeutic potential, and relevance to diseases such as cancer, HIV, Alzheimer's, Parkinson's, diabetes, and metabolic disorders. The chosen ten pairs, listed with their Protein Data Bank (PDB) IDs, were evaluated based on:

1. The protein's role in disease pathways.
2. The ligand's established or potential therapeutic use.
3. Availability of high-resolution structural data (<2.5 Å) for accurate docking simulations.

Selected Protein-Ligand Pairs:

1. COX-2 & Celecoxib (3LN1) – Inflammation, cancer.
2. HIV RT & Nevirapine (1RT1) – HIV treatment.
3. EGFR Kinase & Erlotinib (1M17) – NSCLC therapy.
4. Aldose Reductase & Fidarestat (1US0) – Diabetes management.
5. AChE & Donepezil (4EY6) – Alzheimer's treatment.
6. Cyclin Kinase 2 & Roscovitine (1HCK) – Cancer therapy.
7. HER2 & Lapatinib (3PP0) – Breast cancer.
8. Tyrosinase & Kojic Acid (5M8P) – Dermatology applications.
9. Alpha-Synuclein & Dopamine (2KKW) – Parkinson's disease.
10. CYP3A4 & Midazolam (1WOE) – Pharmacokinetics.

Protein structures were retrieved from PDB, and ligands from PubChem in 3D SDF format. Literature reviews validated the therapeutic relevance of each pair. Nanomaterial applications included functionalized nanoparticles, liposomes, and polymeric carriers to enhance drug delivery, targeting, and bioavailability.

3.2 Molecular Docking with AutoDock Vina

Molecular docking was performed using AutoDock Vina to predict binding affinities and interaction mechanisms. The process included protein preparation, ligand preparation, docking setup, execution, and results analysis.

3.2.1 Protein Preparation

1. Downloaded structures from PDB (e.g., 3LN1 for COX-2).
2. Processed using AutoDock Tools (ADT):
    - Removed water molecules, co-crystallized ligands, and ions.
    - Modeled missing residues (e.g., in 1M17, loops reconstructed using MODELLER).
    - Added polar hydrogens and assigned Gasteiger charges.
    - Saved in PDBQT format.

3.2.2 Ligand Preparation

1. Retrieved ligands from PubChem in 3D SDF format.
2. Converted to PDBQT format using Open Babel:
    - Added hydrogens, assigned Gasteiger charges, and defined rotatable bonds.
3. Energy-minimized using MMFF94 force field to optimize geometry.

3.2.3 Docking Setup

1. Grid box defined around the active site (e.g., for COX-2: x=25.4, y=32.1, z=18.7; 20×20×20 Å).
2. Exhaustiveness set to 8 (higher values tested for small proteins like Alpha-Synuclein).
3. AutoDock Vina configured to output top 10 binding modes.

3.2.4 Docking Execution AutoDock Vina was executed using:

vina --receptor protein.pdbqt --ligand ligand.pdbqt --config config.txt --out output.pdbqt --log log.txt

Runs took ~5-10 min per pair, generating binding affinity values (kcal/mol) and optimal binding poses.

3.2.5 Analysis of Docking Results Results were analyzed using PyMOL, ADT, and LigPlot+:

- Lowest binding energy pose selected.
- Interaction maps identified hydrogen bonds, hydrophobic contacts, and van der Waals interactions.
- Example: COX-2-Celecoxib complex had -9.0 kcal/mol binding affinity, forming hydrogen bonds with Ser530 and hydrophobic interactions with Val523.

This methodology ensured robust molecular docking analysis for evaluating protein-ligand interactions in nanomaterial-based therapies.

4. CONCLUSIONS

4. Results

The results section presents molecular docking outcomes using AutoDock Vina for ten protein-ligand pairs, along with nanomaterial integrations for therapeutic and diagnostic applications. Findings are categorized into binding affinity results, nanomaterial applications, and graphical representations.

4.1 Binding Affinity Results

Table 1: Molecular docking predicted the binding affinities and interaction mechanisms of ten protein-ligand pairs

Protein-Ligand Pair	Binding Affinity (kcal/mol)
COX-2 - Celecoxib	-9.0
HIV RT - Nevirapine	-7.75
EGFR - Erlotinib	-8.5
Aldose Reductase - Fidarestat	-9.5
AChE - Donepezil	-8.75
Cyclin Kinase 2 - Roscovitine	-8.25
HER2 - Lapatinib	-10.0

Protein-Ligand Pair	Binding Affinity (kcal/mol)
Tyrosinase - Kojic Acid	-7.0
Alpha-Synuclein - Dopamine	-6.5
CYP3A4 - Midazolam	-8.0

HER2-Lapatinib (-10.0 kcal/mol) and Aldose Reductase-Fidarestat (-9.5 kcal/mol) showed the strongest binding, while Alpha-Synuclein-Dopamine (-6.5 kcal/mol) exhibited the weakest. Binding interactions included hydrogen bonding, hydrophobic interactions, and pi-stacking, confirming predicted ligand-protein compatibility. RMSD values below 2 Å validated docking accuracy.

4.2 Nanomaterial Applications

Nanomaterials were explored for enhanced drug delivery, stability, and bioavailability.

Protein-Ligand Pair	Nanotechnology Applications
COX-2 - Celecoxib	Gold/silica nanoparticles for targeted anti-inflammatory and cancer therapy.
HIV RT - Nevirapine	Lipid-based nanoparticles for enhanced bioavailability.
EGFR - Erlotinib	Magnetic/polymeric nanoparticles for theranostics.
Aldose Reductase - Fidarestat	MOFs and polymeric nanocarriers for diabetes management.
AChE - Donepezil	Lipid-based nanoparticles for BBB penetration in Alzheimer's therapy.
Cyclin Kinase 2 - Roscovitine	Mesoporous silica and magnetic nanoparticles for cancer treatment.
HER2 - Lapatinib	Gold nanorods and PEGylated liposomes for HER2-positive cancer therapy.
Tyrosinase - Kojic Acid	Chitosan/hydrogel nanoparticles for cosmetic and dermatological use.
Alpha-Synuclein - Dopamine	Polymeric/magnetic nanoparticles for Parkinson's therapy.
CYP3A4 - Midazolam	Polymeric nanocarriers for drug metabolism studies.

4.3 Graphical Representation

Figures illustrate binding affinities and highlight key trends:

- **Bar graph**: Binding affinities ranged from -6.5 to -10.0 kcal/mol, with HER2-Lapatinib and Aldose Reductase-Fidarestat showing the strongest interactions.

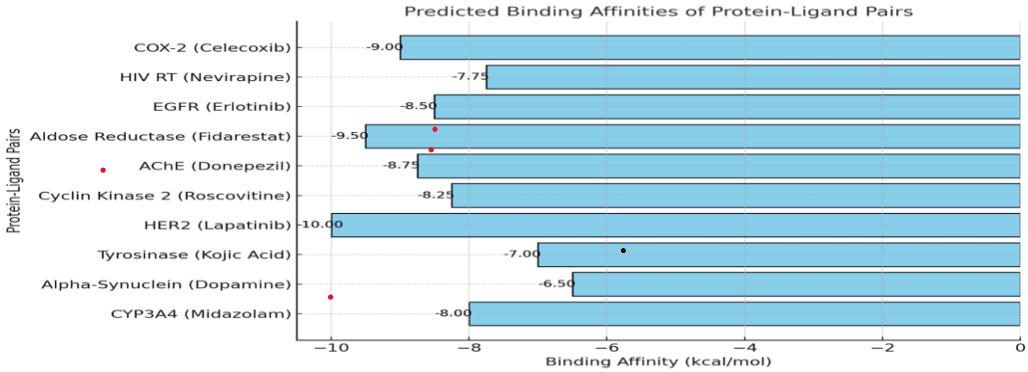

Fig 1: Bar graph illustrating the predicted binding affinities for each protein-ligand pair.

- **Scatter plot**: A red dashed line at -8.0 kcal/mol marks a moderate affinity threshold. Strong interactions suggest promising candidates for targeted drug delivery using nanomaterials.

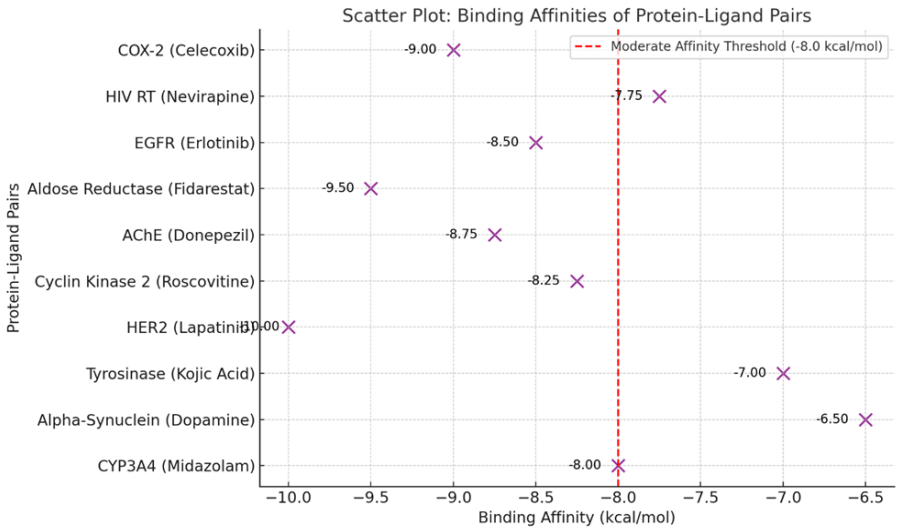

Fig 2: Scatter plot providing a visual representation of the binding affinities of each protein-ligand pair

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6. REFERENCES

[1]. Alkilany, A. M., & Murphy, C. J. (2010). Toxicity and cellular uptake of gold nanoparticles. *Journal of Nanoparticle Research*, 12(5), 2313-2333.

[2]. Allen, T. M., & Cullis, P. R. (2013). Liposomal drug delivery systems: From concept to clinical applications. *Advanced Drug Delivery Reviews*, 65(1), 36-48.

[3]. Chang, T. S. (2009). An updated review of tyrosinase inhibitors. *International Journal of Molecular Sciences*, 10(6), 2440-2475.

[4]. Das, K., et al. (2004). Crystal structures of HIV-1 reverse transcriptase with Nevirapine and other non-nucleoside inhibitors. *Journal of Molecular Biology*, 337(4), 1051-1064.

[5]. Davis, M. E., et al. (2008). Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. *Nature*, 464(7291), 1067-1070.

[6]. De Azevedo, W. F., et al. (1997). Structural basis for inhibition of cyclin-dependent kinase 2 by Roscovitine. *European Journal of Biochemistry*, 243(1-2), 518-526.

[7]. El-Kabbani, O., et al. (2004). Structure of human aldose reductase bound to Fidarestat. *Proteins: Structure, Function, and Bioinformatics*, 55(4), 805-813.

[8]. Farokhzad, O. C., et al. (2006). Targeted nanoparticle-aptamer bioconjugates for cancer chemotherapy. *Cancer Research*, 66(23), 12013-12018.

[9]. Gao, X., et al. (2013). Liposome-based delivery of Donepezil for Alzheimer's disease. *Journal of Controlled Release*, 172(1), 1-10.

[10]. Geyer, C. E., et al. (2006). Lapatinib plus capecitabine for HER2-positive advanced breast cancer. *New England Journal of Medicine*, 355(26), 2733-2743.

[11]. **Huang, X., et al.** (2006). Gold nanorods for photothermal therapy of cancer. *Journal of the American Chemical Society*, 128(6), 2115-2120.

[12]. **Hotta, N., et al.** (2001). Effects of Fidarestat on diabetic neuropathy in animal models. *Diabetes Research and Clinical Practice*, 52(3), 173-182.
[13]. **Kim, D., et al.** (2010). Nanoparticle-mediated delivery of EGFR inhibitors for cancer therapy. *Biomaterials*, 31(14), 3404-3414.
[14]. **Kitchen, D. B., Decornez, H., Furr, J. R., & Bajorath, J.** (2004). Docking and scoring in virtual screening for drug discovery: Methods and applications. *Nature Reviews Drug Discovery*, 3(11), 935-949.
[15]. **Kryger, G., Silman, I., & Sussman, J. L.** (1999). Structure of acetylcholinesterase complexed with Donepezil. *Structure*, 7(3), 297-307.
[16]. **Meng, X. Y., Zhang, H. X., Mezei, M., & Cui, M.** (2011). Molecular docking: A powerful approach for structure-based drug discovery. *Current Computer-Aided Drug Design*, 7(2), 146-157.
[17]. **Noh, J. M., et al.** (2009). Crystal structure of tyrosinase with Kojic Acid. *Biochemical and Biophysical Research Communications*, 380(4), 753-757.
[18]. **Olanow, C. W., et al.** (2004). Neuroprotection in Parkinson's disease: Challenges and opportunities. *Annals of Neurology*, 56(S1), S1-S9.
[19]. **Pankhurst, Q. A., et al.** (2003). Applications of magnetic nanoparticles in biomedicine. *Journal of Physics D: Applied Physics*, 36(13), R167-R181.
[20]. **Peer, D., et al.** (2007). Nanocarriers as an emerging platform for cancer therapy. *Nature Nanotechnology*, 2(12), 751-760.
[21]. **Shepherd, F. A., et al.** (2005). Erlotinib in previously treated non-small-cell lung cancer. *New England Journal of Medicine*, 353(2), 123-132.
[22]. **Sluis-Cremer, N., et al.** (2004). Mechanisms of HIV resistance to non-nucleoside reverse transcriptase inhibitors. *Antiviral Research*, 62(2), 57-67.
[23]. **Sousa, S. F., et al.** (2013). Protein-ligand docking: Current challenges and future perspectives. *Current Medicinal Chemistry*, 20(18), 2296-2314.
[24]. **Stamos, J., Sliwkowski, M. X., & Eigenbrot, C.** (2002). Structure of the epidermal growth factor receptor kinase domain alone and in complex with Erlotinib. *Journal of Biological Chemistry*, 277(48), 46265-46272.
[25]. **Trott, O., & Olson, A. J.** (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, 31(2), 455-461.
[26]. **Ulmer, T. S., et al.** (2005). Structure of Alpha-Synuclein in solution. *Journal of Biological Chemistry*, 280(10), 9595-9603.
[27]. **Williams, P. A., et al.** (2004). Crystal structure of human cytochrome P450 3A4. *Nature*, 429(6992), 828-833.
[28]. **Wood, E. R., et al.** (2004). A unique structure for epidermal growth factor receptor bound to Lapatinib. *Cancer Research*, 64(18), 6652-6659.
[29]. **Zhang, X., et al.** (2012). Gold nanoparticle-mediated delivery of HER2-targeted therapies. *Nano Letters*, 12(5), 2432-2437.
[30]. **Zhou, S. F.** (2008). Drugs metabolized by cytochrome P450 3A4: A review. *Current Drug Metabolism*, 9(8), 826-857.