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Molecular Docking Study of Novel Isatin-Niflumic Acid Derivatives as Possible VEGFR Tyrosine Kinase Inhibitors

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ABSTRACT

Designing and evaluating six novel isatin-niflumic acid derivatives as a tyrosine kinase inhibitor of vascular endothelial growth factor receptor-2 (VEGFR-2). Since there is overexpression of VEGFR-2 in aggressive malignancies, including glioblastoma, hepatocellular carcinoma (HCC), renal cell carcinoma (RCC), and non-small cell lung cancer (NSCLC), this overexpression leads to dysregulated angiogenesis and uncontrolled cellular growth. Inhibiting VEGFR-2 kinase active site (PDB code: 4AG8) is a critical target in developing anticancer medications, as it can aid in the prevention of tumor growth and metastasis through blocking phosphorylation cascades required for endothelial cell proliferation and utilizing the molecular operating environment to assess the binding affinity of novel design compounds against targeting proteins (VEGFR-2) kinase active site. The docking outcomes depend on two important parameters, S-score and RMSD, for investigating molecular interactions and drug discovery. The newly designed compounds (I-VI) exhibit improved binding energy (S.score) ranging from -9.1782 to -9.5120 Kcal/mol and reduced RMSD values ranging from 1.0608 to 1.8914 with the enzyme active site with additional four to five binding interactions, in comparison to Sunitinib binding energy of -8.7276 Kcal/mol and RMSD value of 2.0611 and only exhibit two binding interactions with the active site. These results suggest that the newly designed compounds have the potential to be effective VEGFR-2 tyrosine kinase inhibitors when compared to the reference ligand (Sunitinib).

Keywords: VEGFR-2 Inhibitors, Molecular Docking, Niflumic acid, Isatin derivatives

I. INTRODUCTION

Cancer is a complex disease defined by unregulated cell proliferation, invasion, and metastasis, rendering it among the primary causes of global mortality[1]. Globally, cancer is among the most lethal diseases, with a particular emphasis on Western nations. Approximately 9.9 million individuals passed away in 2020 as a consequence of cancer, as reported by the International Cancer Observatory[2]. Multidrug resistance is a substantial obstacle in cancer treatment and a major barrier to successful outcomes. Consequently, it is essential to develop a new therapeutic target that selectively distinguishes cancer cells from normal ones[3,4]. Physiologically, angiogenesis is necessary to satisfy the oxygen requirements of cells, tissues, and organs and provide systemic and local nutrition. However, pathological conditions are associated with the onset and progression of

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angiogenesis In autoimmune, inflammatory, and malignant disorders, angiogenesis inhibitors have been prominently targeted for therapy[5]. Angiogenesis is a critical stage in the progression of benign tumors to malignant forms, as well as in the management of several other conditions. Consequently, angiogenesis inhibitors continue to be among the most efficacious alternatives for cancer therapy[6]. Angiogenesis is generally governed by a variety of endogenous pro- and antiangiogenic factors[7]. The overexpression of pro-angiogenic factors disturbs this equilibrium, resulting in pathological angiogenesis[8]. When vascular endothelial growth factors (VEGFs) bind to vascular endothelial growth factor receptors (VEGFRs), especially VEGFR-1 to VEGFR-3, autophosphorylation of tyrosine residues in these receptors takes place, resulting in the activation of intracellular elements, including members of the mitogen-activated protein kinase (MAPK) superfamily and phosphatidylinositol 3-kinase (PI3K)/Akt, which regulate cell proliferation, differentiation, and survival. The interaction of VEGF-A with VEGFR-2 has been shown to promote angiogenesis more than other VEGF-VEGFR pairings[9,10]. VEGFR-2 is mainly found in vascular endothelial cells and functions as a signal transducer for angiogenesis, The expression of VEGFR-2 in endothelial cells is carefully regulated, exhibiting low to moderate levels (1,000–10,000 molecules per cell) essential for maintaining vascular homeostasis. VEGFR-2 overexpression in neoplastic disorders, with levels reaching up to tenfold higher, and in some cases, in the cancer cells themselves[11]. VEGFR-2 levels are substantially elevated in aggressive malignancies, including glioblastoma, hepatocellular carcinoma (HCC), renal cell carcinoma (RCC), and non-small cell lung cancer (NSCLC)[12]. The most prevalent subtype of lung cancer is non-small cell lung cancer (NSCLC); frequently, NSCLC is not identified until it has progressed to an advanced stage[13]. Focusing on the angiogenic functions of VEGFR is a novel approach targeting the NSCLC treatment[14]. VEGFR-2 has three different structural domains: an extracellular domain, which is responsible for binding ligands; a transmembrane domain; and the tyrosine kinase domain. VEGFR-2 TK inhibitors that compete with adenosine triphosphate upon binding to ATP-binding site in the receptor's catalytic domain, preventing the receptor from autophosphorylation and eventually inhibiting angiogenesis[15,16]. This study investigates the potential of six novel Isatin-Niflumic Acid derivatives as a possible VEGFR-TK inhibitor using a molecular operating environment (MOE 2015) program. Isatin (1H-indole-2,3-dione) is a unique scaffold in medicinal chemistry. It demonstrates anticancer, and kinase inhibitory properties. Due to its planar structure and capacity to form hydrogen bonds, it is an exceptional candidate for targeting the VEGFR ATP-binding site. Isatin derivatives have been reported to interact with critical residues in the active site, thereby inhibiting a variety of tyrosine kinases[17]. Niflumic Acid is a non-steroidal anti-inflammatory drug that shows anti-angiogenic and anti-proliferative properties[18]. Interactions of niflumic acid with hydrophobic and polar residues in VEGFR TK are facilitated by its aromatic and carboxyl functionalities. Niflumic acid has been associated with the modulation of cancer-related pathways, rendering them promising candidates for kinase inhibition therapy[19].

II. METHODOLOGY

A. Chemical synthesis

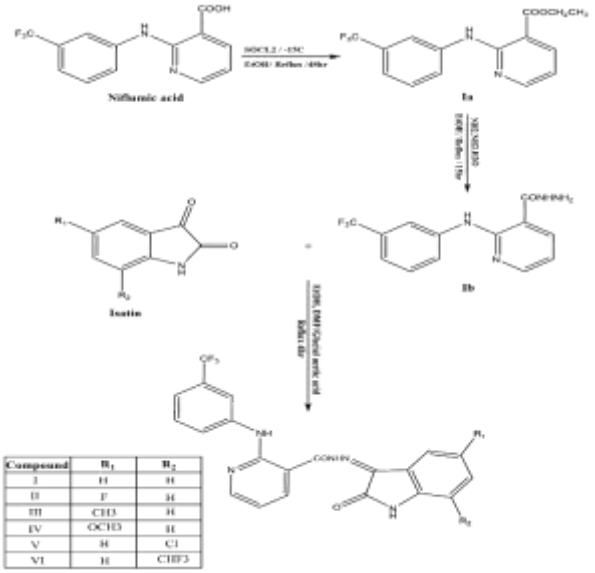
The reaction employs a collection of chemicals to attain the intended changes. Ethanol and DCM function as solvents, establishing an appropriate medium for the process. Thionyl chloride is significant and frequently utilized in numerous synthetic procedures. Niflumic acid and hydrazine are essential organic molecules, acting as

precursors or reagents. Ethyl acetate, chloroform, and petroleum ether, each possessing distinct characteristics, are employed for solvent extraction. The choice of these chemicals is determined by their compatibility with the reaction circumstances and their ability to promote certain transformations. The synthetic methodologies are designed to be as follows:

1- synthesis of niflumic acid ethyl ester (compound Ia) by the reaction of niflumic acid with thionyl chloride utilizing ethanol as solvent, the initial step involve the formation of acid chloride, which reacts immediately with ethanol to form the ethyl ester of carboxylic acid[20,21].

2- synthesis of niflumic acid hydrazide (compound Ib) by reaction compound Ia with hydrazine hydrate 99% under reflux conditions[22].

3- synthesis of the final six acyl hydrazone compounds (I-VI) by the reaction of compound Ib with isatin derivatives utilizing ketone-amine condensation reaction to form the final six imine containing compounds[23,24]. The synthesis procedure is illustrated in scheme1. Below.



Compound	R_1	R_2
I	H	H
II	F	H
III	CH_3	H
IV	OCH_3	H
V	H	Cl
VI	H	CH_3

Scheme 1. The synthesis scheme of the designed compounds.

B. **Computational Approaches and Molecular Docking**

This study employs Chem Draw Professional 15 and Molecular Operating Environment (MOE 2015) software programs for docking criteria. This computational work is performed using laptop-based data entry embedded with 16 GB RAM, Intel Core i7 2.6 GHz CPU, and 265GB solid-state drive (SSD) running on Windows 10 Operating system. These properties provide high-performance computing processes, allowing an accurate docking study. For docking study, two main steps are required:

1. Ligand preparation: Which include drawing the desired derivatives using Chem Draw software followed by residues protonation, partial charge correction and energy minimization utilizing the Molecular Operating Environment (MOE 2015) software[25].

2. Target receptor preparation: which includes importing the crystal structure of VEGFR-2 tyrosine kinase domain (PDB code: 4AG8) from the protein data bank (PDB) website (www.rcsb.org) into the MOE 2015 software program followed by removal of water, un desirable ligands, active site isolation and protonation. After preparation of the desired active site of the receptor docking study is started to assess the values of binding energy (S.score) and root mean square deviation (RMSD), derivatives with high level of S.score and low level of RMSD will show optimum binding and fitting with the target receptor site[26].

III. RESULT

This study investigates the optimal method for a ligand to bind its target site through the simulation technique known as molecular docking. The Molecular Operating Environment (MOE) was employed for its capacity to visualize, describe, and assess protein-ligand interactions. It offers superior graphical representations of results by illustrating the

positions and interactions of the ligand with receptor-binding residues[27]. The molecular operating environment confirmed the binding selectivity of the proposed compounds for the VEGFR-2 tyrosine kinase (PDB code: 4AG8). This demonstrates that the newly synthesized compounds (I-VI) bind selectively to the VEGFR-2 kinase domain like the reference ligand (sunitinib) as shown in Table 1. and Figures 1-7.

Compound	S. score (Kcal/mol)	RMSD	No. of binding sites	Binding amino acids
Sunitinib	-8.7276	2.0611	2	Asp 1046, Leu 840
Compound I	-9.1782	1.5147	5	Asp 1046, Glu 885, Lys 868, Leu 889, Phe 1047
Compound II	-9.2425	1.1745	4	Asp 1046, Glu 885, Lys 868, Leu 889
Compound III	-9.4254	1.0608	4	Asp 1046, Glu 885, Phe 1047, Leu 889
Compound IV	-9.2709	1.8914	4	Asp 1046, Glu 885, Lys 868, Leu 889
Compound V	-9.2731	1.5234	4	Asp 1046, Glu 885, Lys 868, Leu 889
Compound VI	-9.5120	1.3227	4	Asp 1046, Glu 885, Lys 868, Leu 889

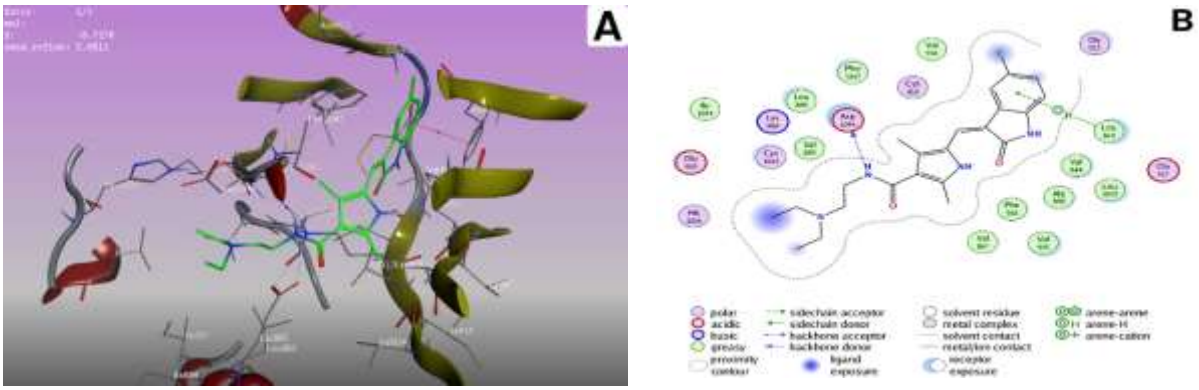


Fig 1: Docking result of Sunitinib with vascular endothelial growth factor (VEGFR-2) PDB Code (4AG8).
Where A) Represents 3D docking, B) Represents 2D docking with active site.

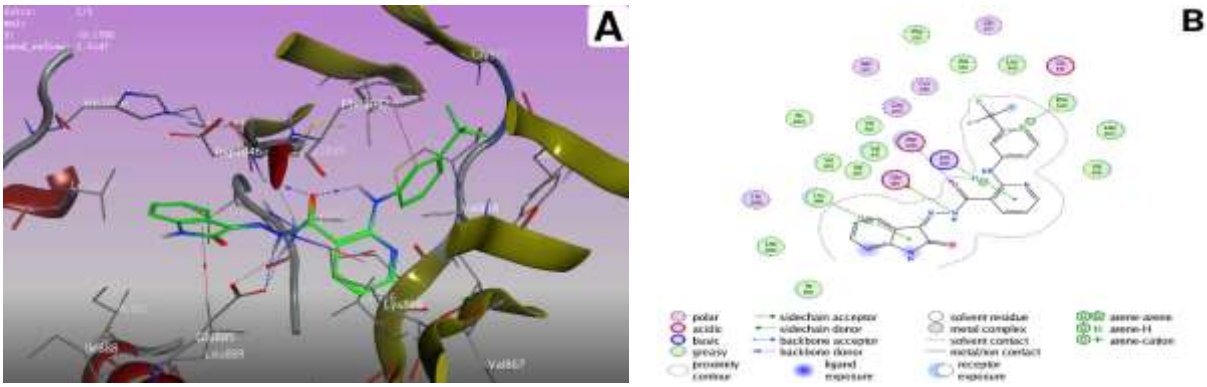


Fig 2: Docking result of compound I with vascular endothelial growth factor (VEGFR-2) PDB Code (4AG8). Where A) Represents 3D docking, B) Represents 2D docking with active site.

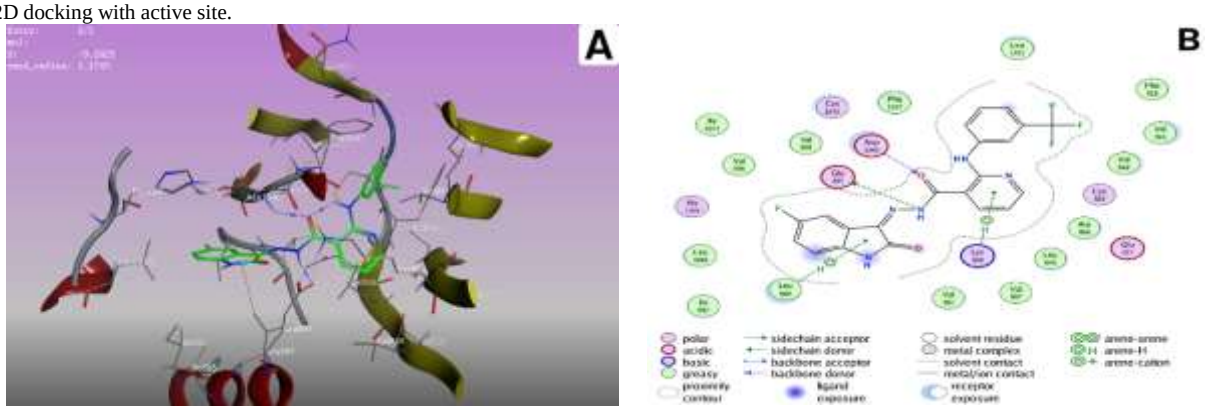


Fig 3: Docking result of compound II with vascular endothelial growth factor (VEGFR-2) PDB Code (4AG8). Where A) Represents 3D docking, B) Represents 2D docking with active site.

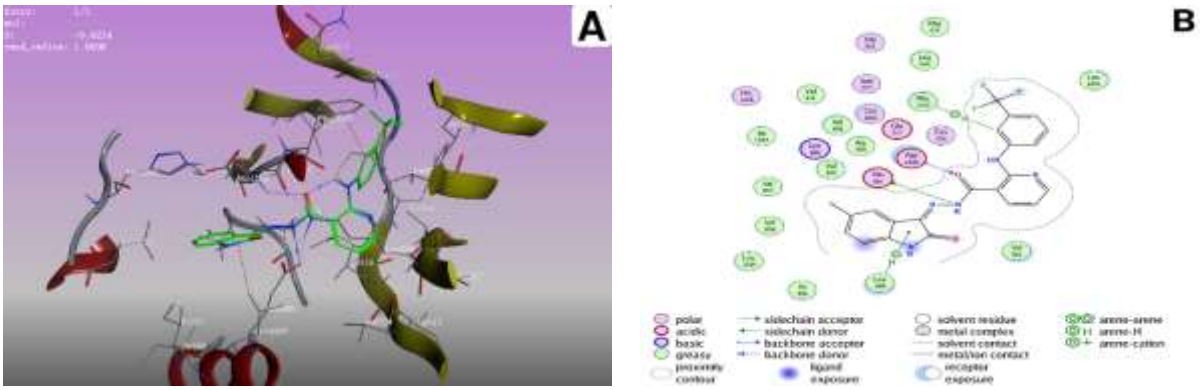


Fig 4: Docking result of compound III with vascular endothelial growth factor (VEGFR-2) PDB Code (4AG8). Where A) Represents 3D docking, B) Represents 2D docking with active site.

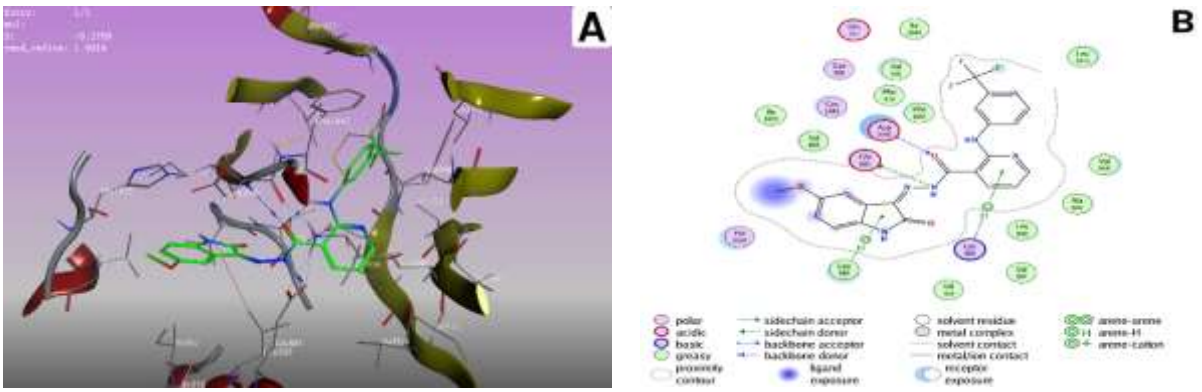


Fig 5: Docking result of compound IV with vascular endothelial growth factor (VEGFR-2) PDB Code (4AG8). Where A) Represents 3D docking, B) Represents 2D docking with active site.

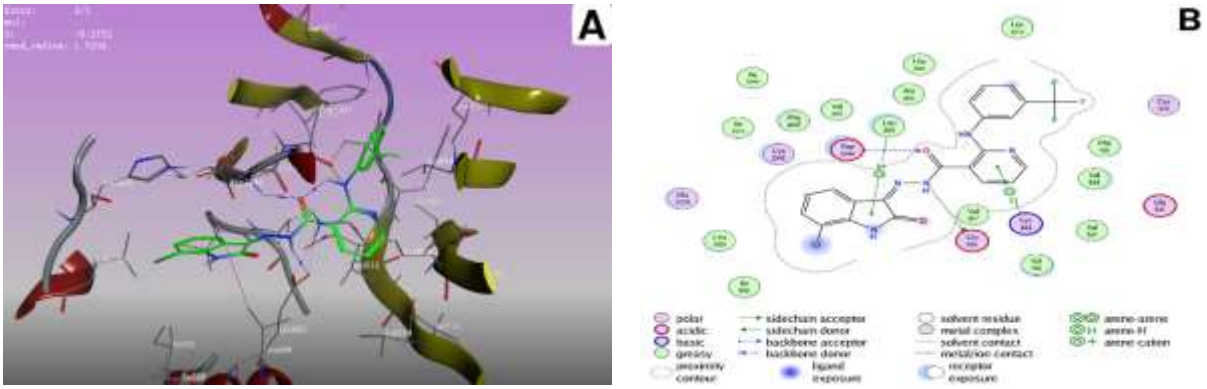


Fig 6: Docking result of compound V with vascular endothelial growth factor (VEGFR-2) PDB Code (4AG8). Where A) Represents 3D docking, B) Represents 2D docking with active site.

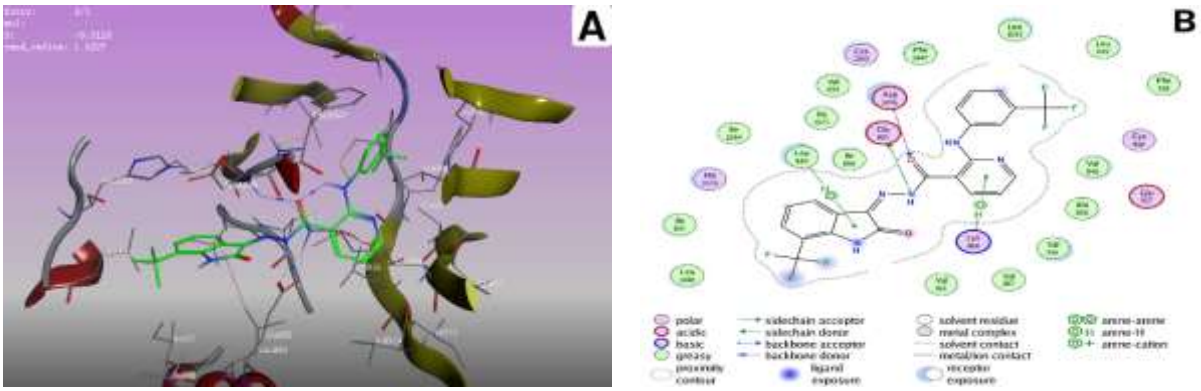


Fig 7: Docking result of compound VI with vascular endothelial growth factor (VEGFR-2) PDB Code (4AG8). Where A) Represents 3D docking, B) Represents 2D docking with the active site.

IV. DISCUSSION

The kinase active site is composed of a substrate-binding region and an ATP-binding area, as adenosine triphosphate (ATP) is the cofactor utilized by all kinases to phosphorylate substrates. Inhibitors that can bind selectively to the cofactor-binding site have been demonstrated to be more effective in inhibiting protein kinase and blocking substrate phosphorylation. An understanding of how ATP is connected to the kinase active site has significantly enhanced the development of specific and potent medications. The main characteristics of the designed compounds that allow them to act as selective VEGFR-2 TK inhibitors are a flat heteroaromatic ring system that contains a nitrogent atom, which binds the gate-keeper region through the binding with Lys 868, A linker region that functions as a pharmacophore participating in the hydrogen bonding with two critical amino acid residues (Glu885 and Asp1046) in the DFG motif (Asp-Phe-Gly) (an essential tripeptide sequence in the active kinase domain), and a terminal hydrophobic moiety that occupies the allosteric hydrophobic pocket. All these characteristics enabled the newly designed compound (I-VI) to attain a high binding affinity and a greater number of interactions within the enzyme's ATP binding site than the reference ligand (Sunitinib).

Utilizing the Molecular Operating Environment (MOE) instrument to determine the efficacy of newly synthesized compounds as VEGFR-2 tyrosine kinase inhibitors. The docking results showed that all designed compounds (I-VI) exhibited higher energy of binding (S-score) and lower root mean square deviation (RMSD) values, suggesting that they have the potential to be effective VEGFR-2 tyrosine kinase inhibitors when compared to the reference ligand (sunitinib).

V. CONCLUSION

Utilizing the Molecular Operating Environment (MOE) instrument to determine the efficacy of newly synthesized compounds as VEGFR-2 tyrosine kinase inhibitors. The docking results

showed that all designed compounds (I-VI) exhibited higher energy of binding (S-score) and effective VEGFR-2 tyrosine kinase inhibitors when compared to the reference ligand (sunitinib). lower root mean square deviation (RMSD) values, suggesting that they have the potential to be

VI. Refrences

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