Pravir Kumar

Tanishka pdf

Apurva

Document Details

Submission ID
trn:oid:::27535:137172891

Submission Date
Apr 30, 2026, 12:20 PM GMT+5:30

Download Date
Apr 30, 2026, 12:29 PM GMT+5:30

File Name
Tanishka pdf.pdf

File Size
898.0 KB

51 Pages

14,018 Words

82,004 Characters

5% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

Filtered from the Report

- Bibliography
- Quoted Text
- Cited Text
- Small Matches (less than 8 words)

Exclusions

- 2 Excluded Matches

Match Groups

63 Not Cited or Quoted 5%
Matches with neither in-text citation nor quotation marks

0 Missing Quotations 0%
Matches that are still very similar to source material

0 Missing Citation 0%
Matches that have quotation marks, but no in-text citation

0 Cited and Quoted 0%
Matches with in-text citation present, but no quotation marks

Top Sources

4% Internet sources
3% Publications
3% Submitted works (Student Papers)

Integrity Flags

0 Integrity Flags for Review

Our system's algorithms look deeply at a document for any inconsistencies that would set it apart from a normal submission. If we notice something strange, we flag it for you to review.

A Flag is not necessarily an indicator of a problem. However, we'd recommend you focus your attention there for further review.

Match Groups

- **63** Not Cited or Quoted 5%
  Matches with neither in-text citation nor quotation marks
- **0** Missing Quotations 0%
  Matches that are still very similar to source material
- **0** Missing Citation 0%
  Matches that have quotation marks, but no in-text citation
- **0** Cited and Quoted 0%
  Matches with in-text citation present, but no quotation marks

Top Sources

- 4% Internet sources
- 3% Publications
- 3% Submitted works (Student Papers)

Top Sources

The sources with the highest number of matches within the submission. Overlapping sources will not be displayed.

1	Internet	
www.frontiersin.org		<1%
2	Internet	
www.guidetopharmacology.org		<1%
3	Internet	
patents.google.com		<1%
4	Internet	
cdn.impactglobalhealth.org		<1%
5	Student papers	
University of Durham on 2023-03-14		<1%
6	Internet	
files01.core.ac.uk		<1%
7	Publication	
Victor Constantinescu, Katja Akgün, Tjalf Ziemssen. "Current status and new deve...		<1%
8	Student papers	
B.S. Abdur Rahman Crescent Institute of Science & Technology on 2026-04-29		<1%
9	Student papers	
Bannari Amman Institute of Technology on 2026-03-24		<1%
10	Publication	
Kakarla Ramakrishna, Sachchida Nand Rai, Santosh Kumar Singh, Veer Singh. "Ne...		<1%

11	Internet	
bmrat.biomedpress.org		<1%
12	Internet	
www.prolekare.cz		<1%
13	Student papers	
Rijksuniversiteit Groningen - Tii on 2026-04-16		<1%
14	Student papers	
University of Bradford on 2025-02-14		<1%
15	Internet	
www.alperbulbul.co		<1%
16	Internet	
aithor.com		<1%
17	Internet	
ijbpas.com		<1%
18	Internet	
www.mdpi.com		<1%
19	Internet	
www.researchgate.net		<1%
20	Internet	
123dok.net		<1%
21	Publication	
John L. Gilmore, Hai-Yun Xiao, T. G. Murali Dhar, Michael Yang et al. " Bicyclic Liga...		<1%
22	Student papers	
Pondicherry University on 2013-02-05		<1%
23	Student papers	
University of Keele on 2023-05-03		<1%
24	Internet	
dspace.dtu.ac.in:8080		<1%

25 Internet

dspace.sctimst.ac.in <1%

26 Student papers

Mepco Schlenk Engineering college on 2025-11-07 <1%

27 Publication

Shamim I. Ahmad. " Aging: Exploring a Complex Phenomenon", CRC Press, ... <1%

28 Student papers

University of St Andrews on 2011-04-20 <1%

29 Internet

jhip.gdpu.edu.cn <1%

30 Internet

medchem.pharmaceuticalconferences.com <1%

31 Student papers

Associatie K.U.Leuven on 2012-01-09 <1%

32 Publication

Gary Álvarez Bravo, René Robles Cedeño, Marc Puig Casadevall, Lluís Ramió-Torre... <1%

33 Publication

Raj Bawa. "Advances in Clinical Immunology, Medical Microbiology, COVID-19, an... <1%

34 Publication

Russell Fritzemeier, Daniel Foster, Ashley Peralta, Michael Payette et al. "Discover... <1%

35 Publication

Yaser Mohammed Al-Worafi. "Handbook Of Evidence-Based Pharmacist Care: Clin... <1%

36 Internet

dipot.ulb.ac.be <1%

37 Publication

Carla Vitorino, Andreia Jorge, Alberto Pais. "Nanoparticles for Brain Drug Delivery... <1%

38 Student papers

Johns Hopkins Unversity on 2025-04-21 <1%

39 Student papers

KTH - The Royal Institute of Technology on 2011-12-11 <1%

40 Student papers

London Metropolitan University on 2025-05-09 <1%

41 Publication

Odom, Laura B.. "The OGF-OGFR Axis in Angiogenesis in Multiple Sclerosis and Ex... <1%

42 Publication

Tiffany Tran, Pedro Paz, Sharlene Velichko, Jill Cifrese et al. "Interferonβ-1b Induc... <1%

43 Internet

en.wikipedia.org <1%

44 Internet

joyceho.github.io <1%

45 Internet

mts.intechopen.com <1%

46 Internet

www.scribd.com <1%

Computational Screening and Binding Analysis of Novel Emerging Small-Molecule S1PR1 Modulators for Multiple Sclerosis

A dissertation submitted to complete a portion of the requirements for the

Master of Science degree

IN

BIOTECHNOLOGY

BY:

Tanishka

24/MSCBIO/59

Under the supervision of

Prof. Pravir Kumar

Department of Biotechnology

DELHI TECHNOLOGICAL UNIVERSITY

(FORMELY DELHI COLLEGE OF ENGINEERING)

Shahbad Daulatpur , Bawana Road , Delhi -110042, India

"Computational Screening and Binding Analysis of Novel Emerging Small-Molecule S1PR1 Modulators for Multiple Sclerosis"

ABSTRACT

Aim: Multiple sclerosis (MS) is a chronic neurological disorder that develops when the body's immune system mistakenly attacks the central nervous system. As a result, the myelin coating that envelops nerve fibers is damaged, which eventually impairs brain-to-body communication. This eventually results in increasing neurological issues like cognitive decline, coordination issues, and muscular weakening. Inflammation, demyelination, and axonal degeneration are the disease's hallmarks, which make it complicated and difficult to treat. Instead of offering a full recovery, current treatment approaches mostly focus on treatments which will modify the disease, which try to slow the course of the illness and regulate immune system activity. Treatments that target sphingosine-1-phosphate receptors (S1PRs), particularly S1PR1, have demonstrated significant potential. These receptors are crucial for controlling lymphocyte migration. In order to lessen inflammatory damage, autoreactive immune cells can be stopped from leaving lymph nodes and entering the CNS by adjusting S1PR1 activity. The first oral medication authorized for the treatment of multiple sclerosis was fingolimod (FTY720), which marked a significant departure from injectable treatments. It has been shown to be successful in lowering relapse rates and functions as a functional modulator of S1PR1. However, there are a number of restrictions on its use. Cardiovascular problems, retinal edema, and an increased vulnerability to infections because of immunosuppression are among the negative effects that patients on fingolimod may encounter. Furthermore, the medication has to be phosphorylated by the body in order to become active, which might have an impact on its overall effectiveness and response consistency among various people. Given these constraints, it is imperative to investigate alternative S1PR1 small-molecule modulators that have better pharmacological and safety characteristics. A computational method was employed in this investigation to find possible compounds that could function as efficient S1PR1 modulators. Molecular docking methods were used to investigate compounds that were selected based on their structural similarity to fingolimod in order to assess their binding interactions with the target protein. Promising possibilities were identified by closely examining the stability and intensity of these interactions. Additionally, significant drug-like characteristics such absorption, distribution, metabolism, and excretion were evaluated by ADME study. The primary goal of this endeavor is to find molecules with excellent pharmacokinetic properties and substantial binding affinity toward S1PR1. These substances might be promising avenues for the creation of safer and more efficient multiple sclerosis medicines. Additionally, this work shows how computational techniques may aid in the initial stages of drug development and assist in reducing the number of potential candidates prior to experimental confirmation.

Conclusion : With the greatest binding affinity and BBB permeability, ligand 6 is the most effective of the six discovered ligands. We advise using in vivo investigations to confirm these results.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

1. MS – Multiple Sclerosis
2. CNS – Central Nervous System
3. BBB – Blood–Brain Barrier
4. S1P – Sphingosine-1-Phosphate
5. S1PR1 – Sphingosine-1-Phosphate Receptor 1
6. S1PR – Sphingosine-1-Phosphate Receptor
7. GPCR – G Protein-Coupled Receptor
8. OPC – Oligodendrocyte Precursor Cell
9. ROS – Reactive Oxygen Species
10. RNS – Reactive Nitrogen Species
11. IFN-γ – Interferon Gamma
12. IL – Interleukin
13. TNF-α – Tumour Necrosis Factor Alpha
14. CD – Cluster of Differentiation
15. Th – T Helper Cell
16. MMP – Matrix Metalloproteinase
17. ICAM-1 – Intercellular Adhesion Molecule-1
18. VCAM-1 – Vascular Cell Adhesion Molecule-1
19. ZO-1 – Zonula Occludens-1
20. DNA – Deoxyribonucleic Acid
21. RNA – Ribonucleic Acid
22. ATP – Adenosine Triphosphate
23. PI3K – Phosphoinositide 3-Kinase
24. Akt – Protein Kinase B
25. MAPK – Mitogen-Activated Protein Kinase
26. ERK – Extracellular Signal-Regulated Kinase
27. RRMS – Relapsing-Remitting Multiple Sclerosis
28. SPMS – Secondary Progressive Multiple Sclerosis
29. PPMS – Primary Progressive Multiple Sclerosis
30. FDA – Food and Drug Administration
31. EMA – European Medicines Agency
32. ADME – Absorption, Distribution, Metabolism, Excretion
33. LogP – Partition Coefficient
34. MW – Molecular Weight
35. HBD – Hydrogen Bond Donor
36. HBA – Hydrogen Bond Acceptor
37. QSAR – Quantitative Structure–Activity Relationship
38. SAR – Structure–Activity Relationship
39. PDB – Protein Data Bank
40. RMSD – Root Mean Square Deviation
41. kcal/mol – Kilocalories per Mole
42. HTS – High Throughput Screening
43. VS – Virtual Screening
44. SBDD – Structure-Based Drug Design
45. LBDD – Ligand-Based Drug Design

46. PK – Pharmacokinetics
47. PD0 – Pharmacodynamics
48. CNSP – Central Nervous System Penetration
49. BBBP – Blood–Brain Barrier Permeability
50. SM – Small Molecule

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

In most cases, the immune system can be misdirected and attack the insulation that surrounds nerves. The central nervous pathway is struck by trouble, which accumulates slowly, causing irritation and wear. Wounded shielding causes messages along nerves to be dragged by tripping. In the beginning the symptoms show themselves in fits, so as to come like flashes. Initially, symptoms arise and disappear, appearing in waves. As time goes by, skills can be lost forever. What starts in fragments becomes constant, and leaves an indelible imprint on motion, and feeling. The harm accumulates, even in cases where there is no apparent external noise. The ways of the mind are losing, not aged but wrongly assailed. MS occurs when the immune system malfunctions as the brain cells break down simultaneously [1–4]. Scattergun, some white blood cells get awakened - T cells and B cells, among others, and slide under the protective wall that surrounds the brain. After entering them, they generate inflammation waves in nerve areas. The injuries appear quickly: myelin is lost, nerves are injured, certain neurons perish. Such alterations accumulate, making a person more disabled with the years [3,4]. The wall to safeguard leaks better now, and letting in more mischief-makers. Whenever it is breached, the damage is just a little more distant [10,11].

In recent years, novel therapies which modify the course of disease progression have emerged, particularly those which modify immune responses to postpone the onset of exacerbations. These are drugs that target the sphingosine-1-phosphate receptor group, which have actual potential to modulate immunity [12-14]. Located on cell surfaces, S1PRs are part of a broader receptor category associated with numerous body processes - immune movement, blood vessel stability, formation of new vessels, even cell life or cell death [15-17]. A single one of these, S1PR1, is of the greatest importance in directing the white blood cells out of the glands such as lymph nodes, and into the bloodstream [18,19]. Trapping of rogue immune cells in lymph node occurs following a change in S1PR1 activity to prevent their journey to the brain and alleviate nerve inflammation [20,21].

Fingolimod (FTY720), the initial pill-based therapy against this pathway, substituted older needle-based treatments to multiple sclerosis patients - a definite turning point [13,14]. Fingolimod is converted into its active form in an enzyme called sphingosine kinase 2 once it is inside the body. That modified molecule mimics natural signals, however, pulls S1PR1 receptors off cell surfaces and flags them to be destroyed, preventing lymphocytes to move about freely [24,25]. The adverse effects of fingolimod include heart issues, swelling of the eye and high vulnerability to infections, although it is effective in treatment [26-28]. Its unknown location of strike in the body - although it must be converted into the body - can obscure its safety of action [29].

Newer options, including Siponimod and ozanimod, fill this empty space: they target their effect more precisely, and are less likely to cause undesirable reactions [30 -32]. S1P signaling is no longer implicated in the control of immune responses, but now also involved in the protection of nerve cells, in the promotion of myelin-generating cells, and also in repair of damaged neural areas - indicating a dual role in immunity and brain repair [33-35]. These outcomes led to the fact that, the idea of S1PR1 as a target in treating multiple sclerosis has taken traction, particularly in the development of specific, simplified drug-like molecules as opposed to generalized agents.

The digital technologies in molecule docking and absorption screening have gained critical importance as a part of the first stage of medicine hunting due to computer-based biology. Instead of trying to test it at the lab at once scientists model the behavior of small fragments binding to proteins. This helps to weed out bad candidates in one way or another, and also bring out the good ones in bodies. The consequence of this change is a decrease in the number of lab tests that are necessary at the beginning - time and energy saving [36-38-55]. In research, multiple sclerosis can be studied using these tools to narrow down the discovery of new compounds to S1PR1 switch. Some of these molecules attach more firmly, target the inappropriate sites and find their ways in tissues easier than the older molecules.

This analysis is reduced to computer-based testing and interaction assays of new tiny molecules in S1PR1. It uses simulated placement of molecules, absorption, and metabolism predictions to hunt compounds that have the potential to substitute existing Multiple Sclerosis therapies. The search is fuelled by safer profiles and better performance. Results can indicate solutions that are more patient-oriented. Exploration speculates on the nature of behaviour of these substances in the body. These are predicted to move. Every action eliminates the potential competitors [55–58].

LITERATURE REVIEW

2.1 Multiple Sclerosis: Pathophysiology and Disease Mechanism

When multiple sclerosis develops, strange changes take place within the body. This is an incurable disease that mainly impacts on the nervous system. Its development differs with individuals, depending on their genetic disposition and exposure to the environment [1]. The immune system instead of defending the body wrongly attacks the body tissues. With the weakening of the myelin, trauma develops, and scar tissue forms and the nerves are harmed. The impairment of functions like movement, sense, and vision occurs in unpredictable ways, over time.

Destruction occurs at sites of lost myelin, leading to plaques and impaired nerve signalling. These transformations progress slowly and there are no obvious symptoms of their occurrence and the development of the disease is different in each person. In its simplest form, multiple sclerosis is the loss of normal immune response towards the myelin sheath around the nerve fibre. This is a sheath generated by oligodendrocytes and promotes the speed at which electrical signals are passed. Inflammation causes demyelination, which slows down or completely stops the conduction of signals. Recurrent rounds of injury and partial repair ultimately lead to the loss of axons and neurodegeneration [2]–[4].

2.1.1 Etiological Factors and Disease Triggers

Multiple sclerosis has no known cause but there are various factors that have been associated with it. A significant role is played by genetic predisposition, especially variations in genes that regulate the immune system like HLA-DRB1 [5]. The environmental factors play an important role as well. Increased risk has been linked to low levels of vitamin D which is usually as a result of insufficient sunlight exposure. Disease onset is strongly connected with viral infections, in particular, Epstein-Bar virus [6].

Other lifestyle diseases like smoking increase the risk of getting MS. Also, geographical distribution patterns indicate that geography affects disease prevalency. All these factors combined modify the behaviour of the immune systems and ultimately lead to autoimmune reactions against

central nervous system. Genetic and environmental factors interact to predispose and modulate disease and symptoms [7].

2.1.2 Immune System Dysregulation

In multiple sclerosis, autoreactive T and B cells (immune tolerance failure) are activated. Th1 and Th17 T helper cells (CD4+) have a central role in the initiation of disease. Th1 cells secrete interferon-gamma (IFN-7) which stimulates macrophages and Th17 cells secrete interleukin-17 (IL-17) which stimulates inflammation and immune cell recruitment into the CNS [8], [9].

Neurons and oligodendrocytes are directly harmed by CD8+ cytotoxic T cells. B cells also contribute to the production of autoantibodies, the release of cytokines, and the presentation of antigens. Oligoclonal bands of the cerebrospinal fluid indicate enduring immune reactions in the CNS. These immune responses result in an inflammatory lesion or plaque, which is characteristic of multiple sclerosis pathology [10], [11].

2.1.3 Demyelination and Lesion Formation

White matter areas are the main sites of demyelination, where the immune mediated assaults destroy the myelin and oligodendrocytes. Grey matter participation has also been found to be significant in recent studies [12]. MS lesions are characterized by:

- Loss of myelin
- Infiltration of immune cells
- Activation of microglia and astrocytes
- Axonal injury

Such plaques interfere with neural communication and are highly associated with clinical symptoms noted in patients [13].

2.1.4 Axonal Damage and Neurodegeneration

The axonal damage is predominant as the disease advances. The combination of inflammatory attacks, metabolic stress and decreased trophic support causes irreversible damage to nerve fibers. After axons have been cut, a functional recovery is not possible, which adds to permanent neurological impairments [14], [15].

Atrophy of the brain is caused by loss of axons as well as degeneration of neurons. This results in mental deterioration, motor coordination and sensory weakness. Long-term disability in MS patients is greatly affected by the progressive neurodegeneration [16].

2.1.5 Mitochondrial Dysfunction and Oxidative Stress

It is also becoming evident that the mitochondrial dysfunction is becoming a significant player in development of MS. The damaged mitochondria produce less ATP, causing neuronal and glial energy deficiencies. This cellular homeostasis and ionic disturbance [17].

Excessive generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) kills lipids, proteins, and DNA. It is an oxidative stress which facilitates neurodegeneration. This is further increased by impaired mitochondria that leads to a cycle of cell injury that further adds to the development of the disease [18], [19].

2.1.6 Clinical Course and Disease Progression

Multiple sclerosis has a heterogeneous clinical course in various individuals. Others experience mild symptoms and others develop rapid disabilities. The most common form is the relapsing-remitting MS (RRMS) that is characterized by intervals of neurological disabilities and recoveries [20].

RRMS is normally succeeded by secondary progressive MS (SPMS) that is marked by progressive degeneration of the nervous system and fewer relapse. Primary progressive MS (PPMS) on the other hand presents itself with a progressive deterioration of the disease since its onset with no distinct relapses [21].

At the increasing phase of disease, neurodegeneration becomes predominant factor, and inflammation subsides. Such transformation demonstrates the importance of early diagnosis and management of the illness to prevent irreparable harm and improve the long-term outcomes [22].

2.2 Blood Brain Barrier Problems and Brain Inflammation

29 The blood-brain barrier (BBB) is a special structure which regulates most of the substances entering the brain. This wall is a discriminative gateway to the central nervous system. It is produced by an intricate system of endothelial cells that envelop the brain capillaries that are not free to permit free passage of undesirable substances. The stability of this structure is supported by astrocytic end-feet which surround the vessels and pericytes. This system is reinforced by a basement membrane. These elements combine to create a very well-coordinated system that only permits necessary molecules to move but blocks harmful agents [23].

37 The BBB is a physical and biochemical barrier under normal conditions, which does not allow the entry of immune cells and ensures the homeostasis of the nervous system. But, in multiple sclerosis, this protective system is early impaired in the initial phases of the illness.The permeability is increased, which permits the entry of harmful substances and immune cells into the CNS triggering inflammation and consequent neural damage [24].

2.2.1 Structural and Functional Alterations

In the case of multiple sclerosis, the BBB integrity is impaired because of the inflammatory signalling, endothelial dysfunction, and molecular changes. These modifications make the barriers more permeable and allow immune cells to enter the CNS.
Key structural changes include:

- **Disruption of tight junction proteins:**
  BBB integrity is ensured by proteins like claudins, occluding and zonula occludes (ZO-1). Their organization is disturbed by the inflammatory cytokines in MS which enhances paracellular permeability [25].
- **Upregulation of adhesion molecules:**
  Endothelial cells express higher levels of adhesion molecules such as ICAM-1 and VCAM-1, promoting leukocyte adhesion and migration into the CNS [26].
- **Enhanced endothelial permeability:**
  The inflammatory mediators can modify the endothelial activity, enhancing transcellular movement and extravasation of plasma elements into the CNS [27].

- **Matrix metalloproteinase (MMP) activation:**
  MMPs destroy extracellular matrix and basement membrane, weakening BBB, and facilitating immune infiltration [28].

All these effects lead to a weakened BBB that allows the penetration of autoreactive immune cells into the CNS to cause inflammatory reactions [29].

2.2.2 Mechanisms of Immune Cell Transmigration

The **migration of immune** cells across the BBB is a highly regulated process with multiple steps that entail sequential interactions between the circulating **leukocytes and endothelial cells**.
First, the leukocytes are rolled through adhesion, which is mediated by selectin molecules on the endothelial surfaces. This is succeeded by the hard sticking, the integrins adhere strongly to ICAM-1 and VCAM-1 and fix the attachment [30].
Then, leukocytes experience transmigration, by passing between cells (paracellular) or across cells (transcellular) across the endothelial layer. The chemokine gradients that steer the immune cells towards the areas of inflammation guide this process [31].
Lastly, the immune cells infiltrate the basement membrane and gain access into the CNS parenchyma where they build up and cause tissue damage. This carefully controlled process is dysregulated in multiple sclerosis, which means that autoimmune cells can gain access to the CNS without any restrictions [32].

2.2.3 Inflammatory Cascade

After BBB impairment, inflammatory cascade within CNS is self-amplifying, triggered by infiltrating immune cells. T cells, B cells and macrophages are activated and release pro-inflammatory cytokines, which increase the intensity of the immune response.
Key cytokines involved include:

- Tumor necrosis factor-alpha (TNF-α)
- Interleukin-1 beta (IL-1β)
- Interferon-gamma (IFN-γ)
- Interleukin-6 (IL-6)

These cytokines contribute to:

- Recruitment of additional immune cells
- Increased permeability of blood brain barrier
- Activation of resident glial cells
- Damage to oligodendrocytes

The chemokines involved include CCL2 and CXCL10 which direct the immune cells to the inflamed area resulting in the formation of lesions typical of MS [33], [34].

This triggers a loop of immune activation in which inflammation is perpetuated by the ongoing activation of the immune system leading to eventual neural damage with time [35].

2.2.4 Role of Glial Cells in Neuroinflammation

The glial cells and especially the microglia and astrocytes are important in maintaining neuroinflammation in multiple sclerosis.
Microglia Activation
Microglia act as resident immune cells of the CNS. In MS, they become chronically activated and contribute to disease progression by:

- Releasing pro-inflammatory cytokines
- Producing reactive oxygen species (ROS) and reactive nitrogen species (RNS)
- Phagocytosing myelin debris and damaged cells

Although it is initially protective, long-term activation results in additional inflammation and neuronal damage [36].

Astrocyte Reactivity

Astrocytes are generally involved in the support of neurons and in BBB integrity. But as the MS progresses, they are reactive and add to the pathology.

Reactive astrocytes:

- Secrete inflammatory mediators
- Promote gliosis and scar formation
- Inhibit axonal regeneration and remyelination

The formation of glial scar produces a physical and biochemical barrier, which inhibits neural repair and recovery[37].

2.2.5 Oxidative Stress and Secondary Damage

CNS inflammation causes excess production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which causes oxidative stress. These reactive molecules cause damage to critical components of the cell, such as:

- Lipids (lipid peroxidation)
- Proteins (oxidative modification)
- DNA (genomic instability)

Such oxidative damage increases neuronal damage and mitochondrial dysfunction, aiding in the continued neurodegeneration and progression of the disease [38], [39].

2.3 Role of Sphingosine-1-Phosphate Receptors (S1PR1) in MS

Sphingosine-1-phosphate (S1P) is a bioactive lipid signalling molecule that controls various physiological functions. It is very important in the immune cell trafficking, vascular integrity, angiogenesis and cell survival. The action of S1P is through binding to a group of five G-protein-coupled receptors, S1PR1-S1PR5, which are tissue-specifically expressed and functionally diverse [40].

S1PR1 is one of these receptors that have been of much interest because it is the center of both immune regulation and vascular stability. It is also expressed strongly on lymphocytes, endothelial cells and some types of neural cells, which is why it is specifically important in autoimmune diseases including multiple sclerosis [41]. The role of S1PR1 is observed when the immune control mechanism is lost and the inflammatory response starts.

The S1P-S1PR1 signalling axis has been shown to be dysregulated in the pathogenesis of multiple sclerosis. The abnormal signalling facilitates the immigration of immune cells across blood-brain barrier, which plays a role in neuroinflammation. Though the exact mechanisms remain to be studied, changes in this pathway are constantly noted in the case of MS patients. S1PR1 has a therapeutic potential that has been shown to respond differently based on the stage of the disease and biological differences [42].

2.3.1 Lymphocyte Trafficking and Immune Regulation

Controlling the lymphocyte's migration from lymphoid organs into the circulation requires S1PR1. An S1P concentration gradient is present under normal conditions, where in the lymphoid tissues, the concentration is low, and in the blood and lymph, the concentration is high. This gradient causes the lymphocytes to be driven towards the circulation due to the movement of the lymphocytes along the S1P concentrations that are increased [43].

The S1PR1 is expressed on the surface of lymphocytes and helps them to sense this gradient and to leave the lymph nodes. This is required in immune surveillance and correct immune functioning. This mechanism is pathogenic in multiple sclerosis. S1PR1-mediated pathway is used by autoreactive lymphocytes to leave lymphoid organs and enter the central nervous system. Once in, they cause inflammatory processes which result in demyelination and neuronal damage [44], [45]. S1PR1 signalling can be modulated to entrap lymphocytes in lymphoid tissues to limit their migration into the CNS. This will lead to reduced inflammation and better disease outcomes.

2.3.2 Intracellular Signalling Pathways of S1PR1

S1PR1 leads to the activation of intracellular signalling cascades upon binding of S1P using Gi-type G proteins. These include:

- PI3K/Akt pathway: Promotes cell survival and anti-apoptotic signalling
- MAPK/ERK pathway: Regulates cell proliferation and differentiation
- Rac/Rho signalling: Controls cytoskeletal dynamics and cell migration

Together, these pathways regulate lymphocyte trafficking, endothelial function, and cellular responses to stress. Disruption of these signalling pathways in MS results in immune imbalance and vascular dysfunction[46].

2.3.3 Vascular Stability and BBB Integrity

S1PR1 plays a very important role in ensuring the structural and functional integrity of the blood-brain barrier. It improves the junction of endothelial cells and tight junction protein like claudins and occluding, which decreases the vascular permeability.

This results in:

- Reduced vascular permeability
- Strengthened endothelial barrier function
- Prevention of excessive immune cell infiltration

In multiple sclerosis, the loss of S1PR1 signalling affects the integrity of the BBB, enabling immune cells to enter the CNS. The effect of inflammatory mediators is also to worsen the breakdown of the barrier by further hindering the functioning of the receptors [47], [48].

Because of its key role in vascular stability, S1PR1 modulation is a promising approach to restoring BBB functions and decreasing neuroinflammation**.**

2.3.4 Neuroprotective and Reparative Functions

S1PR1 signalling contributes significantly to neuroprotection and repair mechanisms within the CNS. Its activation supports several critical processes:

- Oligodendrocyte survival
- Remyelination of damaged nerve fibers
- Neuronal survival through anti-apoptotic signalling
- Maintenance of synaptic stability

Activation of intracellular pathways such as PI3K/Akt enhances cell survival and reduces oxidative damage. These processes aid in reversing neuronal death and metabolic disease.

S1PR1-mediated signalling is anti-inflammatory and neuroprotective in multiple sclerosis where demyelination and neurodegeneration are prominent. This two-fold role shows that it is a therapeutic target [49], [50], [51].

2.3.5 Functional Modulation of S1PR1 in Therapeutics

S1PR1 functional modulators are pharmacological agents like fingolimod. Initially, these drugs stimulate the receptor but with extended exposure, the receptor undergoes internalization and degradation, which essentially suppresses S1PR1 signalling.
This results in:

- Reduced lymphocyte circulation
- Decreased CNS infiltration
- Suppression of inflammatory responses

Targeting of S1PR1 is selective which helps to improve therapeutic effects and reduce adverse effects caused by non-specific receptor modulation. Such specificity renders S1PR1 a central target of the development of novel MS therapies[52].

2.4 Current Therapeutic Strategies Targeting S1PR1

The treatment of multiple sclerosis has witnessed startling transformations in that its treatment is not anymore founded on general immunosuppression but it focuses more on the immune pathways. One of the most important developments is the control of lymphocyte movement by means of S1PR1 signalling. Instead of suppressing the immune system of the whole body, the technique selectively changes the migration of immune cells, therefore reducing the inflammation in the CNS. These specific strategies have enhanced the management of diseases by matching treatment with the biological processes [53].
The approval of fingolimod (FTY720) as the first oral S1PR modulator in the treatment of relapsing multiple sclerosis was a significant change in the paradigm of injectable to oral treatment options [54]. Its launch enhanced patient compliance and made S1PR1 a critical drug target in the management of MS [55].

2.4.1 Mechanism of Action

Fingolimod is a prodrug which is a structural analog of sphingosine. Once administered it is phosphorylated by sphingosine kinase-2 to fingolimod-phosphate, the bioactive molecule [56].
First, fingolimod-phosphate attaches itself to S1PR1 and stimulates the receptor. Prolonged exposure, however, causes the receptors to be internalized and degraded, and this will cause functional antagonism. This decreases the availability of receptors on the cell surface, and eventually inhibits S1PR1-mediated signalling.
As a consequence:
• The capacity of lymphocytes to detect S1P gradients is lost.
• T and B cells are prevented from leaving lymph nodes.

- Circulating autoreactive lymphocytes are significantly reduced

This helps to avoid the immune cells entering the central nervous system and minimize inflammation and demyelination [57], [58].

Further, fingolimod is capable of penetrating the blood-brain barrier and acting on CNS cells including astrocytes and oligodendrocytes, which indicates that it may have neuroprotective properties other than immune modulators [59].

2.4.2 Clinical Benefits

Fingolimod has demonstrated strong efficacy in both clinical trials and real-world studies.

- **Reduction in relapse rate:**
  In relapsing-remitting multiple sclerosis, fingolimod is a powerful inhibitor of the annual relapse rate, indicating the best possible suppression of inflammatory processes.
- **Slowing disease progression:**
  There is a correlation between long-term treatment and delayed disability progression and secondary progressive MS transition..
- **Decrease in MRI lesion activity:**
  Treatment results in fewer gadolinium-enhancing lesions and fewer new or growing T2 lesions, both of which point to less CNS inflammation.
- **Improved patient compliance:**
  Oral administration improves adherence compared to injectable therapies.
- **Potential neuroprotective effects:**
  There is an increasing body of evidence that fingolimod can help preserve the survival of neurons, but this needs to be further investigated to determine its neuroprotective effects in the long-term.

2.4.3 Pharmacokinetic and Pharmacodynamic Considerations

Fingolimod exhibits favourable pharmacokinetic properties, including:

- Elevated oral bioavailability
- Extended half-life permitting once-daily administration
- Long half-life allowing once-daily dosing
- Wide tissue distribution, including CNS penetration

Nevertheless, its reliance on in vivo phosphorylation adds randomness to drug activation, which can affect the response to treatment in individuals.

2.4.4 Limitations and Adverse Effects

Despite its effectiveness, fingolimod is associated with several limitations:

Cardiovascular Complications

- Bradycardia
- Atrioventricular conduction delays
- Hypertension with prolonged use

These effects are linked to non-selective interaction with other S1PR subtypes, particularly S1PR3.

Macular Edema

The retina may be affected by fluid build-up, which may lead to visual impairment, and it is necessary to monitor it with ophthalmologic care on a regular basis.

Increased Risk of Infections

Reduced lymphocyte circulation increases susceptibility to viral and opportunistic infections.

Hepatic and Pulmonary Effects

Some patients have been noted to have elevated liver enzymes and mild changes in lung functions.

Metabolic Activation Requirement

Fingolimod requires phosphorylation for activation, which:

- Introduces variability in efficacy
- Delays onset of action

Lack of Receptor Selectivity

Fingolimod binds multiple S1PR subtypes (S1PR1, 3, 4, 5), contributing to adverse effects and limiting specificity [26]–[29].

2.4.5 Emergence of Second-Generation S1PR Modulators

34 Fingolimod's drawbacks have led to the development of safer and more selective second-generation S1PR modulators.

Examples include:

- **Siponimod:** Targets S1PR1 and S1PR5; effective in secondary progressive MS
- **Ozanimod:** Improved cardiovascular safety profile
- **Ponesimod:** Highly selective S1PR1 modulator with shorter half-life

These newer agents offer:

- Reduced off-target effects
- Improved tolerability
- Better pharmacokinetic control

However, challenges such as long-term safety, cost, and variability in patient response remain.

2.5 Advances in Small-Molecule S1PR1 Modulators

The effectiveness of the first-generation S1PR modulators like fingolimod showed that S1PR1 is a valuable therapeutic approach in multiple sclerosis. Nevertheless, shortcomings such as the inability to selectively bind the receptors, cardiovascular side effects, and the need to activate the drug metabolism resulted in the creation of second-generation agents with a better specificity and safety profile [60].

One goal of designing these newer compounds is to specifically inhibit S1PR1 and not other receptor subtypes including S1PR2 and S1PR3, which have adverse effects. Siponimod, ozanimod, and ponesimod are better receptor selective and have better tolerability profiles [61], [62].

2.5.1 Evolution from First- to Second-Generation Modulators

The first-generation S1PR modulator fingolimod is able to interact with several receptor subtypes (S1PR1, 3, 4 and 5) leading to both therapeutic and undesirable effects. Second-generation modulators on the other hand have been optimally designed to be structurally:

- Improve receptor subtype specificity
- Reduce off-target interactions
- Enhance pharmacokinetic predictability

This evolution reflects a shift toward precision medicine, where therapies are designed to target specific disease pathways while minimizing systemic toxicity.

2.5.2 Improved Selectivity

Drug development has been a major target of S1PR1. Second-generation modulators are highly affinity to S1PR1 and in certain instances, S1PR5, but not S1PR3-related side effects.

- **Siponimod:** Selectively targets S1PR1 and S1PR5
- **Ozanimod:** High selectivity for S1PR1 and S1PR5
- **Ponesimod:** Highly selective for S1PR1

This improved selectivity leads to:

- Reduced cardiovascular complications
- Lower incidence of bradycardia
- Decreased pulmonary and vascular side effects

Although the safety is improved, targeted lymphocyte trafficking modulation is essential to maintain the therapeutic efficacy level.

2.5.3 Pharmacological Advantages

Second-generation S1PR modulators offer several pharmacological improvements:

Enhanced Safety Profiles

Selective receptor targeting reduces off-target effects, improving long-term tolerability.

Reduced Cardiovascular Risk

Limited interaction with S1PR3 minimizes cardiac side effects.

Improved Pharmacokinetics

- Shorter and more predictable half-lives
- Reduced tissue accumulation
- Faster onset and reversibility

No Requirement for Metabolic Activation

Unlike fingolimod, some newer agents do not require phosphorylation, leading to:

- More consistent drug response
- Reduced inter-individual variability
- Faster therapeutic onset

Improved CNS Penetration

Optimized molecular properties improve blood brain barrier permeability which may have better neuroprotective effects.

2.5.4 Comparative Clinical Outcomes

Clinical studies indicate that second-generation modulators:

- Reduce relapse rates
- Decrease MRI lesion activity
- Improve overall disease control

Siponimod was shown to be effective in secondary progressive multiple sclerosis and ozanimod is better tolerated than first-generation agents.

2.5.5 Remaining Challenges

Despite advancements, several challenges persist:

Long-Term Safety Concerns

Chronic immune modulation may increase susceptibility to infections and other complications.

Incomplete Receptor Specificity

Residual off-target effects may still contribute to adverse reactions.

Variable Patient Response

Genetic, metabolic, and disease progression differences impact the results of treatment.

Resistance and Adaptation
Long-term receptor modulation may alter immune responses, reducing drug efficacy.
Cost and Accessibility
High treatment costs limit widespread availability despite therapeutic benefits.

2.5.6 Importance in Drug Development
The development of S1PR modulators highlights key principles in modern pharmacology:
- Target-specific drug design
- Minimization of off-target effects
- Optimization of efficacy–safety balance

These principles are still guiding the future treatment approach to MS and other autoimmune diseases.

2.6 Remyelination and Neuroprotective Mechanisms
In multiple sclerosis, remyelination is a vital process of repair which entails the replacement of the myelin sheaths in damaged axons. Although this is a natural process that happens in the initial stages, it is inefficient in the later stages of the disease causing permanent neurological damage.

2.6.1 Mechanism of Remyelination
Oligodendrocyte precursor cells (OPCs) are the main mediators of remyelination. These cells occur after demyelination:
- Proliferate in response to injury
- Migrate to lesion sites
- Develop into mature oligodendrocytes
- Create fresh myelin sheaths to encase axons.

However, this process is impaired in chronic MS due to:
- Persistent inflammation
- Accumulation of myelin debris
- Inhibitory extracellular matrix molecules
- Reduced differentiation of OPCs

2.6.2 Neuroprotective Mechanisms
Neuroprotection is essential in maintaining neuronal integrity and functioning. The major mechanism of protection is:
- Maintenance of mitochondrial function
- Regulation of intracellular calcium
- Prevention of excitotoxicity
- Reduction of oxidative stress

S1P signalling can help in neuronal survival by promoting intracellular pathways that alleviate cellular stress and apoptosis.

2.6.3 Role in MS Treatment
MS treatments should be effective and target both inflammation and neurodegeneration. Long term management of the disease involves enhancing remyelination and protecting neurons. The main focus of current research is on strategies aimed at survival and differentiation of OPC.

2.6.4 Link to Current Research

New studies indicate that S1PR1 modulation could play a role in immunosuppression and neurorepair. Computational methods of drug discovery have also generated molecules that can act on S1PR1 and which have the potential of having dual action in the treatment of inflammation and regeneration.

2.7 How S1PR1 Binds and Holds Together

Located in the GPCR family, S1PR1 processes signals of sphingosine-1-phosphate. Since it influences cellular reactions, understanding its folding and its binding to molecules is of great importance in constructing targeted medicines. It is small in size but powerful in its impact on drug development - its shape informs its purpose.

2.7.1 How S1PR1 Is Structurally Arranged

S1PR1 exhibits the typical GPCR architecture, consisting of:
• There are seven transmembrane α-helices
• Extracellular loops that aid in the identification of ligands
• G protein coupling intracellular domains
Buried within the structure lies a water-repelling cavity, shaped to fit molecules resembling fats - sphingosine-1-phosphate among them, along with similar compounds.

2.7.2 Ligand–Receptor Interaction Mechanism

Ligand attachment to S1PR1 relies on multiple interaction forms:

- Water-avoiding contacts occur at membrane-spanning amino acids
- Hydrogen bonds that stabilize ligand orientation
- Electrostatic interactions between charged groups

When the receptor undergoes shape shifts as a result of these encounters, activation of intracellular signalling pathways occurs. Although it may not be very noticeable, these structural adaptations play a very crucial role in relaying signals across the cell membrane.

2.7.3 Binding Specificity and Selectivity

To minimize undesirable interactions, it is possible to target S1PR1 in isolation as other subtypes of S1PR (S1PR2 -S1PR5) have side effects. Due to slight variations in the shape of the binding site, scientists exploit these minute differences in the making of precise compounds. Each receptor in spite of its similar structure reacts differently to customized molecules.

2.7.4 Role in Creating Medications

Detailed knowledge of receptor structure enables:

- Identification of key amino acid residues involved in binding
- Optimization of ligand affinity and selectivity
- Rational design of novel therapeutic compounds

2.7.5 Link to Current Research

The binding of the selected compounds to S1PR1 is investigated in this work using molecular docking.By these associations scientists have an opportunity to know about which substances are the best bonding agents. Some of them are more fitting due to their structural fit. Such trends are understood and help in the development of new medicines. What matters is stability, and agreement of parts as they collide.

2.8 Using computers to find new medicines

The beginning with computers has transformed the way new medicines are discovered by scientists it is now cheaper and quicker to view potential treatments. Due to this change, digital tools are now invaluable in addressing challenging diseases such as multiple sclerosis [66].

2.8.1 Molecular Docking and Structure Based Drug Design

First of all, molecular docking - it is a computer technique which determines how small molecules bind to selected proteins. Direction and strength of the connection are indicated in this process. The next step is based on the simulation of interactions at the atomic level [65].

- Placement of ligands into the binding site
- Interaction energy assessment
- Prediction of binding poses

Docking provides insights into:

- Binding strength
- Interaction patterns
- Stability of protein–ligand complexes

Beginning with protein shapes in three-dimensional shapes, scientists design compounds, which are supposed to bind better. Due to the fact that structural details determine decisions, molecular alterations are made in accordance with definite spatial hints [65].

2.8.2 Virtual Screening and Ligand Optimization

Beginning with digital models, virtual screening of huge sets of chemical compounds is done to identify potential medicines. Researchers save time because it involves computer simulations as opposed to using lab-only methods [66].

- Ligand-driven methods rely on information from previously identified active molecules
- Structure-based (based on target protein structure)

Selected compounds are further optimized to improve:

- Binding affinity
- Selectivity
- Pharmacokinetic properties

2.8.3 ADME and Drug Likeness Prediction

For judging how well a drug might work, looking at ADME - how it gets absorbed, spreads through the body, changes chemically, and leaves - is crucial. Important factors to consider are:

- Lipophilicity (LogP)
- Molecular weight

• Hydrogen bond donors/acceptors
• Bioavailability
Often applied in early drug screening, Lipinski's Rule of Five helps identify molecules with potential absorption issues [67].

2.8.4 Limits of Computer Calculations
Even so, these methods come with constraints:
• Approximate scoring functions
• Static representation of proteins
• Lack of biological complexity
For this reason, experiments are needed to confirm what computations show [68].

2.8.5 Present Study Link
The first part of this work starts by describing molecule shape, in which docking simulations are applied, together with absorption predictions, to discover new S1PR1 regulators. The combination of these methods enables scientists to not only establish the strength with which these substances bind but also approximate how the body handles the substances. They are not perfect, but help to filter candidates at an earlier stage [65–67].

2.9 What We Don't Know and Why This Study Matters
Although the treatment of MS has been improved, it still has some issues. As much as the therapeutic interventions including fingolimod and enhanced versions result in better outcomes, they have their share of faults - side effects manifest with a high frequency. Their effect on receptors is imprecise and they lead to various responses in individuals. As a result of such gaps, more differences in the responses emerge than expected among patients [51,52].

2.9.1 Identified Research Gaps
• Lack of highly selective S1PR1 modulators
• Adverse effects associated with existing therapies
• Limited exploration of novel small-molecule candidates
• Need for improved pharmacokinetic and safety profiles

2.9.2 Need for New Treatment Methods
• Safer and more selective drugs
• Compounds with improved bioavailability
• Therapies that address both immune and neurodegenerative aspects

2.9.3 How Computers Help Solve Problems
Computational drug discovery provides an efficient platform for:
• Screening large compound libraries
• Predicting binding interactions
• Evaluating pharmacokinetic properties [65–67]

2.9.4 Study Rationale

One goal of this work is to find new small molecules that affect S1PR1, using computer-based approaches. To reach it, researchers combine molecular docking with ADME evaluation. This path allows a closer look at potential compounds without relying on broad assumptions.

• Identify compounds with high binding affinity
• Evaluate their drug-likeness properties
• Propose potential candidates for further development [65–68]

3. METHODOLOGY

3.1 Retrieval of S1PR1 Protein Structure

From the Protein Data Bank - a huge collection of known biological structures - came the 3D shape of S1PR1, the protein under investigation. Because docking results rely heavily on how well the protein's form is defined, picking a reliable version mattered right at the start. Though many models exist, only one fit the needed precision for these calculations.

A human S1PR1 structure is resolved by either X-ray crystallography or cryo-electron microscopy formed the starting point. Since finer details emerge at higher resolutions, such data allows clearer views of where atoms sit. That clarity matters when mapping out the pocket that binds molecules. With precision in mind, just those entries reaching 3.0 Å resolution - or sharper - moved forward into evaluation. Of importance also was the identification of a ligand embedded right into the shape of the protein. Since it is the binding site, researchers are able to observe precisely the manner in which molecules occupy it. With that molecule in place, scientists make their boundaries around the point where new ones are to be placed. It challenges the consistency of computer guesses with real-world behaviour, so that findings are not rooted in real biology.

- To crown it all, protein shapes were favored that were on display.
- Clearly defined places where molecules are bound, each part is immediately at the point of work.
- Full chain information, with structural integrity.

Only a few small cleavages in the structure, particularly around the molecule insertion point.

A file was downloaded in .pdb format - compressed with atom locations, part names, string labels, inter-part connections. The tools creating small shapes often assume this form, which simply fits into steps preparing proteins.

Simply to be in a position to observe where the molecule fits, it is better to check the structure initially to denote opening it in a viewer. Everything can go wrong since now something looks improper. The timely identification of issues increases predictability of future outcomes. The tying spot should have been tidy, no knots or waste. That way, the subsequent steps are more likely to be comprehensible.

This technique yielded a stable protein shape. That one had become the criterion, in ascertaining the fit of molecules with each other. It also enabled the scientists to explore the places of compounds attachment. Next binding strength was determined. There was specificity in these tests. Form guided everything of the attractions of molecules.

3.2 Preparation of Target Protein

The starting point was the raw protein file, which was refined through each step to the structure to achieve better docking results. The need of these pieces was because the entries of the Protein Data Bank are likely to have extras like water molecules or metal ions. A series of changes helped to create a validated variant of the protein that was able to be used in testing interactions. Only at that time the model could be applied without any unwanted interference.

The protein preparation was done by sending Auto Dock Tools out in the periphery of the working space. In Pyrx, visualization was given and a clear view of the structure was provided. Every cut was by the snipe of a pencil, by purgatory until something began to move. Having such programs running simultaneously kept adjustments accurate. The entire arrangement ensured that nothing passed a close scrutiny before docking.

Removal of Non-Essential Molecules

Out came every part that wasn’t needed from the downloaded setup first. Things like extra scripts, unused files, filler folders - gone

- Crystallographic water molecules
- Bound ions
- Co-crystallized ligands

In the majority of cases, the water molecules are omitted in the course of docking work since they can complicate things. Although such molecules might assist in connections, their elimination facilitates the setup. The same with ligands already in

situ - away they go. Nuclear clearing allows other compounds to enter the active site without complications.

Addition of Hydrogen Atoms

The majority of proteins available in PDB files are not provided with explicit hydrogen information, particularly polar bond - major contributors to hydrogen bonds. So, these missing polar hydrogens were inserted into the model where they were required.
This step is essential because:

- Hydrogen bonds are significant when molecules are sticking together. These connections aid in the formation of the relationships between the substances. In their absence, gluing one piece to another would not be the same. They silently tend to show the way parts their counterparts.
- Correct states of protonation affect accuracy of interaction.
- It enhances accuracy of docking forecasts.

Charge Assignment

Beginning with the protein structure, Kollman partial charges were applied using Auto Dock Tools to determine the electrical interaction of the protein structure with ligands. And the absence of these values would make the simulation of attraction or repulsion imprecise.
Computing binding energies Modelling the electrostatic repulsive and attractive forces Ensuring natural behaviour at the docking point.
energy loss and shape enhancement.

Although experimental data are used in the creation of PDB models, minor problems such as overlapping atoms or strained bonds may still be present. Where necessary, minor modifications were done to enhance geometry. The refinements involved only problematic areas and did not change the overall structure. Each change was guided by precision and was minimally invasive.

This process supports the following outcomes

- Relieve steric hindrance
- Stabilize local geometry
- Improve the overall quality of the receptor structure

Great effort went into limiting changes to the protein, ensuring its natural shape - particularly how the binding site is structured - stayed unchanged.

3.3 Selecting and Finding Ligands

Among early stages in computer-aided drug design, pinpointing proper ligands is at the heart of things - as the choice of compounds determines the extent to which the results of docking can be trusted. The choice of these molecules in this case was driven by rational filtering; structural similarity was used to pick some of them, biological activity and similarity with existing medicines were used to pick others - all in the quest to identify potential agents at the S1PR1 receptor.

The approach using the similarity of the molecules started with the known structure of fingolimod (FTY720), a known S1PR1 modulator. When compounds contained common core structures or functional centers, they rose in priority - structural similarity often portends bioactivity similarity. Owing to shape and group positioning that affects the binding of molecules, the choices were determined by these properties. Close architecture does not necessarily imply similar functionality, but it is frequently the case. Therefore, it focused on parallel pattern of building.

Find Possible Compounds

Beginning with PubChem, scientists identified potential ligand hits using the large repository of molecular data and associated bioactivity information available in PubChem. In order to identify these, queries were based on certain terms typed in the system. In that, similar compounds were identified using structural similarity and reported interactions.

- Fingolimod-related structural resemblance searches
- Keyword-based searches related to S1PR1 modulators
- Filtering options to narrow down relevant small molecules

Out of that first check came several possible compounds, now lined up for closer look.

Selecting Compounds by How They Act Like Drugs

Beginning with simple characteristics associated with absorption, every compound was analyzed by a set of proven standards. Rules such as these may not be the best predictors but they tend to result in the identification of viable candidates at an early stage. In such a system, properties matching standard thresholds - e.g., molecular weight or solubility - were propelled forward. Others got through because of borderline values; some others were exceptional because they passed more than one

marker. Making it this far did not insure success but it lessened apparent dangers associated with behaviour within the body.

- Appropriate molecular weight
- Balanced lipophilicity
- Acceptable number of hydrogen bond donors and acceptors

Compounds with poor absorption were put under early review. By eliminating such early, there is an opportunity to discover viable treatments in later life. Structural Feature.

The occurrence of certain structural features known to mediate S1PR1 binding, such as, was given special consideration:

- **Hydrophobic chains:**
  These aid in interaction with the lipophilic binding pocket of S1PR1, leading to an increase in binding stability.
- **Polar functional groups:**
  Functional groups, including hydroxyl, amine or phosphate-like groups, promote hydrogen bonding and electrostatic interactions with functional groups of key amino acid residues.
- **Amphipathic character:**
  It was found desirable to include molecules with hydrophobic and hydrophilic regions, since they would imitate the structural behaviour of endogenous ligands and known modulators.

Ligand Retrieval and File Format

Among the selected molecules, one of them was downloaded through PubChem in the .sdf format - a format that contains structural information. The given extension contains data on atom positions as well as bond configurations. Each record contained coordinates and molecular properties that can be further analysed. The data package provided the 2D and 3D representations as standard output.

Three-dimensional structural coordinates

- Bond connectivity information
- Chemical property data
- Importantly, the .sdf format is a good starting point in most computational projects since it can be easily converted into the forms needed to be docked. Its wide usage facilitates the easy ligand preparation before simulations.

Preliminary Screening and Selection

Among all the tested substances, some of the molecules were propagated, having satisfied certain conditions. There are those candidates who stood out due to their suitability to certain requirements. Some few were picked after preliminary findings recorded encouraging results. After examination, specific structures gained more ground as a result of favourable interactions. It was only those with appropriate binding behaviour that survived the first stage.

- Connection to S1PR1 modulators based on molecular framework
- Chemical stability
- Processing readiness for subsequent computational tasks

After selection, the ligands were processed and refined by subsequent steps and then applied in molecular docking.

Ligand Choice Matters

Choosing ligands with precision means that:

- Docking results are biologically meaningful
- Interactions that bind matter when it comes to hitting the intended receptor
- Identified compounds have potential for further development

The work done here is used to build up computational work, either in terms of docking, molecular interactions, or drug behaviour. This stage is critical in later stages, in that it unobtrusively drives the outcomes.

3.4 Ligand Preparation

After the selection and collection of candidates, their structures had to be adjusted and docking could only occur. This step is important - errors here can cause changes in the way molecules will fit in the future.

Beginning with molecule setup Open Babel was used to do initial formatting and then structures were passed to Auto Dock Tools. Each program assumes its role as the next step without encroachment. They are independent but when used together they conform to typical patterns found in digital screening

pipelines. Such commonly used tools as these frequently go hand in hand when compounds are being prepared to be studied using simulations.

File Format Conversion

PubChem database ligands were in the form of .sdf files - perfect to store molecular information, but incapable of directly inserting into docking software. This type is not readied to deal with simulation workflows although it was designed to deal with chemistry data.
With Open Babel, conversion into .pdb format became possible

- Compatibility with molecular visualization tools
- Further structural editing and preparation
- Integration into docking workflows

Standardization of every ligand structure happened here, preparing them for what followed next.

Geometry Optimization

Beginning with modified ligand shapes, energy-based refinement ensued to achieve low-stress shapes. In this way, interatomic distances change slightly and angular relations change - decreasing tension throughout the structure.
Energy minimization helps to:

- Remove unfavourable steric interactions
- Stabilize molecular geometry
- Improve the accuracy of docking predictions

As optimized structures are more realistic biological shapes, the evaluation of interactions becomes more reliable. It is these shapes that molecules actually assume in life. Their accuracy increases when the models resemble the natural conditions. The inferences about binding are more powerful when the configurations resemble actual behavior. Improved consistency with biology will imply reduced errors in contacts predictions.

Addition of Hydrogen Atoms

Purposely, some hydrogen atoms - that are normally missing or omitted in structure files - were replaced in every ligand. That includes both polar and non-polar ones.
The addition of hydrogens is important because:

- Atoms of hydrogen take part in essential connections, including those known as hydrogen bonds
- With correct valency and bonding setups confirmed
- Accuracy in electrostatic computations increases due to it

Charge Calculation

To model accurately electrostatic forces between ligands and proteins, partial charges based on Gasteiger were used - each ligand was run through Auto Dock Tools. Automated, the technique is used to ensure uniform distribution of charges which is vital in accurate interaction.
These charges are crucial to:

- Predicting binding affinity
- Simulating intermolecular interactions
- Ensuring realistic docking results

Rotatable Bonds Explained

The ligand should be flexible enough in the process of docking since there can be different shapes when the ligand binds. This is why all the bonds of compounds were found to be rotatable.
This step allows:

- Exploration of different conformations during docking
- Identification of the most stable binding pose
- A tighter fit inside the receptor's binding site improves interaction. Placement is slightly changed and contact is improved. Such a modification contributes to the better alignment. Shape changes help stabilize the connection. Improved positioning follows naturally from these adjustments

Preserving rigid functional groups remained a priority during assignment of rotatable bonds. While setting up molecular connections, correct placement guided each decision. Not every bond allowed rotation - some stayed fixed by design. Attention focused on maintaining structural accuracy throughout the process. Where flexibility mattered, adjustments followed clear chemical rules. Integrity of key components shaped how rotations were defined. Correctness came from balancing movement with stability.

Final Conversion to Docking Format

Once preparations finished, the ligands changed into .pdbqt form - needed only for Auto Dock-style docking runs. This format step came last, following every earlier task. Software of that type cannot process anything else. Conversion made sure everything ran without glitches later on.
The .pdbqt file includes:

- Atomic coordinates
- Assigned partial charges
- Defined atom types
- Information on rotatable bonds

With this version locked in, docking software works without hiccups while modelling how molecules bind to proteins precisely. Though built for stability, its real strength shows when predicting complex biological fits reliably.

3.5 Molecular Docking Procedure

Starting with molecular docking, predictions emerged about how tightly certain molecules might stick to S1PR1 and where they would connect. Since this process unveils information regarding the fit within the working region of the protein, it carries some weight in the analysis. The execution of these tests was based on AutoDock Vina - a software that is characterized by fast execution and does not compromise performance in terms of score estimation.

Docking Setup

Before running, the ready protein and ligand models entered the docking setup. AutoDock Vina input consisted of the receptor, which is saved in the form of.pdbqt, and correspondingly formatted ligand files. Though formatted separately, each piece fit the software's requirements. Each file type - protein and small molecule - arrived prepped for interaction analysis. Running began only once both components were recognized by the system.
28 The location of the grid box is a key factor in docking and determines the location of ligand interaction to be assessed over the protein surface. Located strategically around familiar points of contact, it surrounds the active site of S1PR1 - often guided by prior structural clues such as bound molecules or critical amino acids involved in recognition.
Among the values set were:

- Position of the middle point along x, y, z axes:

- These values are centered around the binding pocket, and they indicate where the search starts. Their location is the result of the features of that particular area.
- The extent of the grid is measured in three perpendicular directions. The elements are aligned in dimensions along each axis. Rules of spacing are applicable irrespective of position. Total volume capacity is based on values obtained. Direction-specific lengths have the effect of affecting structural behavior under load.
- A marginally bigger size was necessary to be sure to include the active site, but omit irrelevant parts. This change reduced the processing power that was wasted due to a concentrated culmination in areas of necessity. Efficiency increased because additional areas remained beyond the border.

The grid is a good place to start as it only creates the best docking and also consumes less computing time as only the areas that are likely to be of interest are visited. This may be a humble gesture, but it can direct the entire process by putting the efforts where they matter.

Docking Parameters

For consistent capture of ligand shapes, docking settings were carefully chosen:

- **Exhaustiveness**:
- Searching more deeply depends on this setting. The greater the number, the better the chance to find the best fit for binding - yet each step adds time to processing. Reaching a balance matters when running these calculations.
- How many poses appear in the result:
- In each case of a ligand, a number of binding shapes were obtained - all of which provide how an orientation could change during interactions. Various configurations were observed, indicating the possible different positions of the molecules during attachment. These different forms allowed the observation of contrasts in positioning. Rather than a single pose, there were various compositions that appeared. Both structures provided an insight into other possibilities of contact.
- **Scoring function:**
- Predictions of binding strengths based on the standard scoring of AutoDock Vina, which takes into account the attractive forces between

molecules, such as hydrogen bonds as well as hydrophobic touchpoints or electrostatic pulls. Although simplistic in nature, the methodology is able to represent important forces that can determine the degree of sticking together of molecules in the normal circumstances.

These settings were built jointly, maintaining speed and accuracy in step in docking runs.

Docking Execution

Docking runs were performed into the given region of S1PR1 using AutoDock Vina, with each ligand being placed individually. The individual simulations tested various positions in the active site, with the scoring functions used to determine fit.
Each ligand produced a result that contained:

- Energy of interaction in kilocalories per mole.
- These numbers manifest themselves in binding strength. The lower they are the more strongly they are attached.

Multiple binding poses:

- Starting from varied angles, multiple shapes emerged, each showing how the molecule might sit inside the pocket. One after another, these arrangements revealed shifts in placement and direction across the space where binding occurs.

Interaction coordinates:

- Position details of the ligand in relation to receptor amino acids are clearly mapped. The layout shows how close each residue sits beside the binding molecule. Where the ligand rests depend on specific contact points along the protein surface. Nearby chemical groups influence placement through directional interactions. Mapping reveals alignment patterns that shift across different conformations.

Selecting the Best Docking Pose

Out of the docking outcomes produced, top-ranked configurations for every ligand emerged through careful evaluation using multiple indicators together. Selection leaned on scoring values alongside spatial fit and interaction patterns observed across runs. Preferred poses showed consistent alignment with favourable energy levels seen during analysis. Each choice reflected a balance between stability signs and repeatable positioning noted in outputs

- Lowest binding energy:
- Favourable energy levels marked the pose showing the lowest binding affinity.

Favourable interaction pattern:

- Of the shapes that could be obtained, ones that could make clear hydrogen bonds or be snugly packed around essential components of the active site were of particular interest. There were those that were paired by water bridges, and others that perfectly matched surface characteristics. Stability was significantly enhanced where molecules were bound in place with amino acids that were important. Fits with non-polar surfaces tended to add strength without charge interactions. When contact points were aligned accurately positioning became the most important thing.
- Placed in the right location within the cavity of binding.
- Proper fitting in the binding pocket is important - when misaligned this creates problems. Form is incompatible or it is placed in strange positions. Avoidance of straining by proper orientation. The positioning should be in accordance with the natural movement patterns. Form is met by space and stability ensues.

Matching established patterns of interaction:

- When possible, researchers matched the chosen pose against established S1PR1 modulators, checking for alignment with real-world activity. Though less obvious at first glance, similarity to documented compounds helped confirm meaningful results. In cases where reference points existed, consistency with prior data shaped confidence in the selection. Only those poses mirroring recognized patterns moved forward into further analysis. Without such comparison, uncertainty would have grown quickly behind the scenes.

3.6 Binding Interaction Analysis

After molecular docking, examination of the protein–ligand complexes revealed specifics about how tightly and in what manner the chosen ligands bind to the S1PR1 receptor. Understanding these details helps clarify which amino acids play a central role during binding, along with the forces that stabilize the interaction.

From start to finish, PyRx paired with Discovery Studio Visualizer guided how structures were viewed and explored. Through these platforms, close attention was paid to where molecules settled, how they connected, and their positioning inside

target regions. What stood out was the clarity in seeing ligand behaviour once docked - revealed step by step through visual mapping.

Analysis of Binding Interactions

From every docked complex, a close look revealed which forces helped hold molecules together. Hydrogen bonds, hydrophobic contacts, electrostatic attractions, van der Waals forces, and pi stacking took part in stabilization. With each structure, patterns emerged showing how these connections varied in strength and position. Though subtle, shifts in distance or angle sometimes changed the dominant force at play. Where one interaction weakened, another often compensated nearby

Hydrogen Bonding

Among key factors shaping how tightly molecules stick, hydrogen bonds stand out. When parts of a molecule offer hydrogen atoms, others nearby can pull them gently into place. The power depends upon the spacing, the amount, and the location. It is not the presence but the proximity and alignment that matters. The measurements of these links are taken to produce patterns.

Hydrophobic Interactions

The forces that avoid water aid in keeping the ligand stationary within the greasy regions of the receptor site. Due to the clustering effects of these segments, the non-soluble parts of the molecule that are not aqueous connect with the corresponding regions in the protein structure.

π–π Stacking Interactions

In the case of aromatic rings of the ligands, their attraction with amino acids such as phenylalanine, tyrosine, or tryptophan was studied. These groups act in the same direction and the binding strength becomes high, and the internal structure is stabilized within the target one.

Electrostatic Interactions

To understand how charged opposites are attracted, it is useful to consider what goes on with parts of a molecule attaching to charged regions on a protein surface due to static forces such as salt bridges or aligned dipoles. To understand these attractions, one is required to follow where positive collides with negative without the need to have covalent bonds to hold things together. The presence of these connections is because of differences in the distribution of the electrons among the atoms involved. What matters most is the spatial match between donor and acceptor sites along with their relative polarity. Forces driven by charge show up clearly when molecules approach one another under stable conditions.

Key Binding Residues Identified

Among those lining the S1PR1 binding site, certain amino acids stand out due to their role in engaging the ligand. Their presence proves crucial for:

- Stabilizing the ligand within the active site
- Determining binding specificity
- Influencing receptor activation or inhibition

Patterns of interaction seen during analysis matched against documented contact points from proven S1PR1 modulators helped confirm the docking outcomes. Though validation relied on prior data, alignment with existing residue information supported accuracy. Where connections formed in simulations, they lined up closely with those previously identified. These overlaps arose not by chance but through consistent spatial and chemical compatibility. Confirmation emerged gradually as each binding site detail fell into place alongside earlier findings.

Interaction Maps Generated

Maps showing interactions in two and three dimensions came from work inside Discovery Studio Visualizer. Built this way, they revealed details clearly about:

- Binding orientation of ligands
- Interaction kinds that emerge
- Residues involved in binding

Visual tools help make sense of docking outcomes, offering a straightforward way to share results. These displays bring clarity while organizing complex data into something easier to follow.

3.7 ADME and Drug Likeness Evaluation

While binding strength matters, how a molecule moves through the body also shapes its potential as a medicine. To explore this, computer-based analysis looked at absorption, distribution, metabolism, and excretion traits. This step helped judge whether the chosen compounds act like realistic drugs. Instead of relying only on interaction data, their overall journey in biological systems was examined.

Using SwissADME, an online tool, the evaluation examined key traits like solubility and absorption through trusted computational methods. Though web-based, its algorithms draw from widely tested frameworks for forecasting drug behaviour. This approach allows insight into how compounds might perform inside the body without lab testing. Performance indicators such as molecular weight or lipophilicity emerge directly from structure inputs. Because predictions rely on pattern recognition, results align closely with observed trends in real molecules.

Evaluation of Physicochemical Properties

Analysis of crucial molecular traits aimed to assess if chosen ligands show features typical of drugs taken by mouth

- **Molecular Weight:**

Often, substances of medium size work best because very big ones tend to struggle getting absorbed or moving through barriers.

- **Lipophilicity (LogP):**

Because lipophilicity affects how well compounds cross membranes and dissolve, its role matters greatly. A balanced LogP helps maintain both water attraction and fat compatibility - key for proper uptake into the body. What happens next depends on that equilibrium staying intact.

- **Hydrogen Bond Donors and Acceptors**:
- Hydrogen bonding ability defines the dissolution or interaction of molecules with the biological systems. The characteristics which characterize such bonds are that such bonds are readily formed - this tunes behaviour in water and target binding strength.
- Calculated surface area of polar atoms (topological).
- TPSA is commonly disregarded and it is a measure of the possibility of a molecule to cross cell membranes. The lower the values, the more flow into cells is likely to be increased - the same can be said about the brain. A shift towards the lesser figures would suggest the diminution of the challenge in surmounting the impediments like that produced by blood vessels.

Pharmacokinetic Property Assessment

For a deeper look at how the drug might perform, researchers checked multiple factors tied to absorption, distribution, metabolism, and excretion

- **Water Solubility:**

For a medicine to be absorbed and reach circulation, it must dissolve well. Poor dissolution limits how much enter the bloodstream. Without sufficient breakdown in fluids, effectiveness drops sharply. How easily it mixes with bodily liquids decides its success. Absorption hinges on this physical property above others.

- **Gastrointestinal (GI) Absorption:**

Oral intake of a substance often leads to quick uptake if its GI absorption is high. What matters most is how rapidly the body pulls it into circulation after ingestion. Efficiency shows up clearly in blood concentration levels soon afterward. When digestion releases it fast, measurable effects follow without delay. Speed of entry becomes obvious through consistent patterns seen across studies.

- Permeability of the Blood–Brain Barrier (BBB)
- Beyond its impact on nerve function, Multiple Sclerosis demands treatments capable of reaching the brain - making passage through the blood-brain barrier essential. What works elsewhere in the body may fail here if it cannot traverse this protective layer. Effectiveness hinges not just on molecular design but also on access. Without entry into the central nervous system, even potent agents fall short. The journey across the BBB shapes whether a compound can actually engage the disease.
- **Metabolic Stability (Predicted):**

These compounds - metabolic changes can happen - these changes affect the length of action of a drug. Their behavior during breakdown would help to achieve overall effectiveness.

Drug-Likeness Evaluation

The possibility of the chosen ligands to be used as orally active agents was quantified by Lipinski Rule of Five - a validated criterion that has been associated with oral absorption.

In case where the substance exceeds some limits, it is likely to show more opportunities provided that the substance is not beyond the limits:

- Weight at the molecular level stays under five hundred daltons
- LogP ≤ 5
- The maximum number of hydrogen atoms that can be donors is five.
- Bonding locations with ten or fewer hydrogen.

The positive drug-like characteristics were associated with the compounds passing the required criteria.

Extra Screening Checks If Needed

Additional filters might apply, including:

- Bioavailability score
- Synthetic accessibility
- PAINS (Pan Assay Interference Compounds) alerts

Other factors played a role in sharpening the pool of possible applicants.

Selecting Possible Drug Candidates

An examination of physical characteristics and the way the body takes them in facilitated the elimination of potential ligands. Individuals with good performance in these aspects were given priority.

- Acceptable ADME profiles

- Compliance with drug-likeness rules
- Favourable balance between solubility and permeability

Some showed potential, making them suitable for deeper study.

Why ADME Analysis Matters

ADME analysis is an important process in computer-based drug discovery in that it assists in:
Get rid of compounds that have bad pharmacokinetic characteristics.
Minimise the chance of late drug failure.
Enhance lead optimization effectiveness.
Both ADME profiling and docking information should be given a starting point in order to have a more complete picture of possible drug candidates. A combination of these techniques provides an insight that each one would not provide individually.

4) RESULTS AND DISCUSSION

4.1 Adme Analysis and Initial Screening

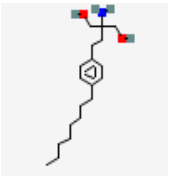
Beginning with structural approaches, scientists sought molecules that were similar to fingolimod in shape. SwissSimilarity was one of the tools that were used in order to identify potential matches between existing compounds. The compounds with scores over 0.85 were regarded as good candidates because of their similarity. There are twenty such substances that have been advanced, exhibiting both structure and activity that could be consistent with S1PR1 effects.
Based on the chosen group, compounds proceeded to the analysis of their behavior within the body - SwissADME was in charge of these tests. Each was required to meet a number of requirements to remain in consideration, including brain access and general similarity to known medicines. Whether a compound could cross into the brain and get absorbed through the gut shaped part of the decision process. Often, success depended on traits like BBB penetration and GI uptake after being taken by mouth. Another checkpoint involved Lipinski's five key markers, which hint at whether an oral dose would work well.
Out of the initial group, some were dropped once screened through PAINS and Brenk rules - structural red flags or toxicity concerns being the reason. Those left behind passed every checkpoint without triggering warning signals worth noting. What remained stood apart by showing steady, promising ADME traits across the

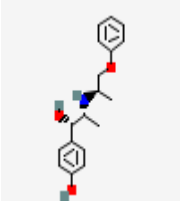
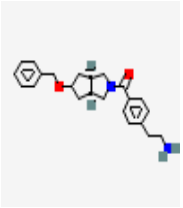
board. These few emerged with properties aligned well toward possible development as drugs.
This method cut down the pool of potential molecules by focusing early on structural fit and absorption traits, so fewer reached the docking phase yet quality stayed high. Only those matching both shape requirements and body-processing needs moved forward, streamlining what followed without losing key candidates.

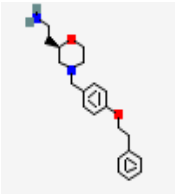
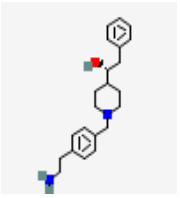
4.2 Visualizing Ligands in Docked Positions

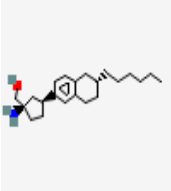
Looking at the way ligands bind to S1PR1, two-dimensional diagrams were built with BIOVIA Discovery Studio. Through these images, the positioning of each molecule inside the receptor's cavity became more apparent. What stood out was how fully each compound filled the region responsible for activation. From there, specific connections between the ligands and parts of the protein came into view - hydrogen bridges, nonpolar associations, and additional bonding influences. Each contact helped explain why certain molecules held their place more firmly than others.
Among the tested compounds, shifts in how they bind revealed distinct interaction styles, hinting at divergent stabilities. These contrasts brought clarity to molecular arrangements, enriching what scores alone could show. Looking closely helped confirm predictions, strengthening confidence in selecting potential leads.

TABLE 1 : TABULAR REPRESENTATION OF IUPAC NAMES ,2D STRUCTURE AND BINDING AFFINITIES OF INTERACTING LIGANDS

PUBCHEM ID	***IUPAC Name***	***2D Structure***	***Binding affinity(kj/mol)***
107970 (REFERENCE Ligand)	2-amino-2-[2-(4-octylphenyl) ethyl] propane-1,3-diol		-7.3

PUBCHEM ID	*IUPAC Name*	*2D Structure*	*Binding affinity(kj/mol)*
11779629 (ligand 2)	4-[(1S,2R)-1-hydroxy-2-[[(2R)-1-phenoxypropan-2-yl] amino] propyl] phenol		-7.4
110145611 (ligand3)	[(3Ar,6As)-5-phenylmethoxy-3,3a,4,5,6,6a-hexahydro-1H-cyclopenta[c]pyrrol-2-yl]-[4-(2-aminoethyl) phenyl] methanone		-7.6

PUBCHEM ID	*IUPAC Name*	*2D Structure*	*Binding affinity(kj/mol)*
126442807 (ligand4)	2-[(2R)-4-[[4-(2-phenylethoxy) phenyl] methyl] morpholin-2-yl] ethanamine		-7.7
95895820 (ligand5)	(1R)-1-[1-[[4-(2-aminoethyl) phenyl] methyl] piperidin-4-yl]-2-phenylethanol		-8.1

PUBCHEM ID	*IUPAC Name*	*2D Structure*	*Binding affinity(kj/mol)*
77050638 (ligand6)	[(1*R*,3*S*)-1-amino-3-[(6*R*)-6-hexyl-5,6,7,8-tetrahydronaphthalen-2-yl]cyclopentyl]methanol		-8.8

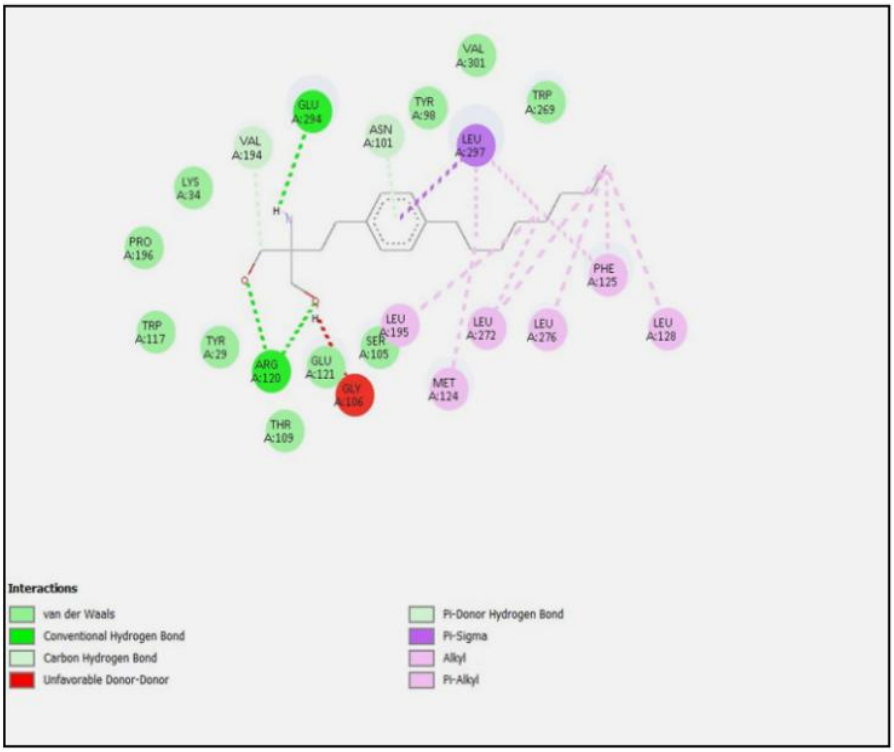


Fig.1. Interaction between ligand no.1 2-amino-2-[2-(4-octylphenyl) ethyl] propane-1,3-diol i.e. Reference compound and target S1PR1

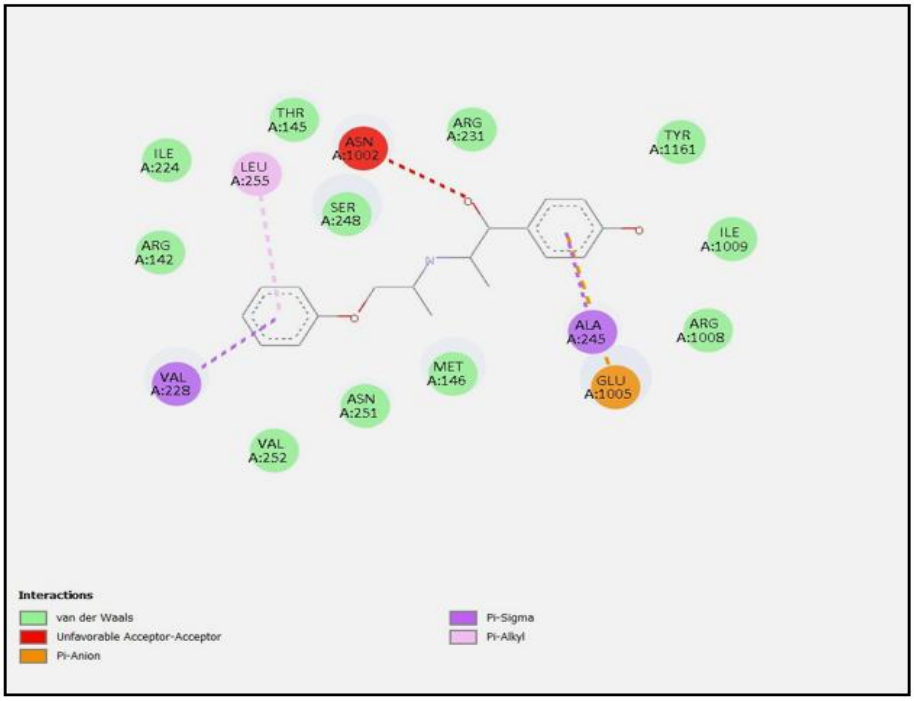


Fig.2.Interaction between ligand no. 2 4-[(1S,2R)-1-hydroxy-2-[[(2R)-1-phenoxypropan-2yl] amino] propyl] phenol and target S1PR1

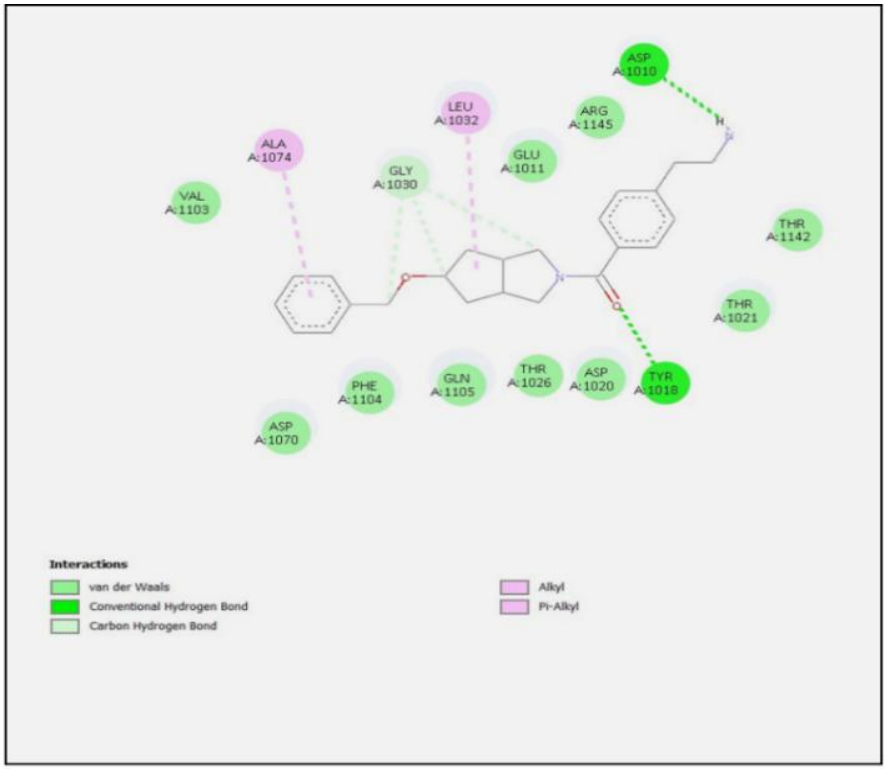


Fig.3. Interaction between ligand no.3 [(3Ar,6As)-5-phenylmethoxy-3,3a,4,5,6,6a-hexahydro-1H-cyclopenta[c]pyrrol-2-yl]-[4-(2-aminoethyl) phenyl] methanone with target S1PR1

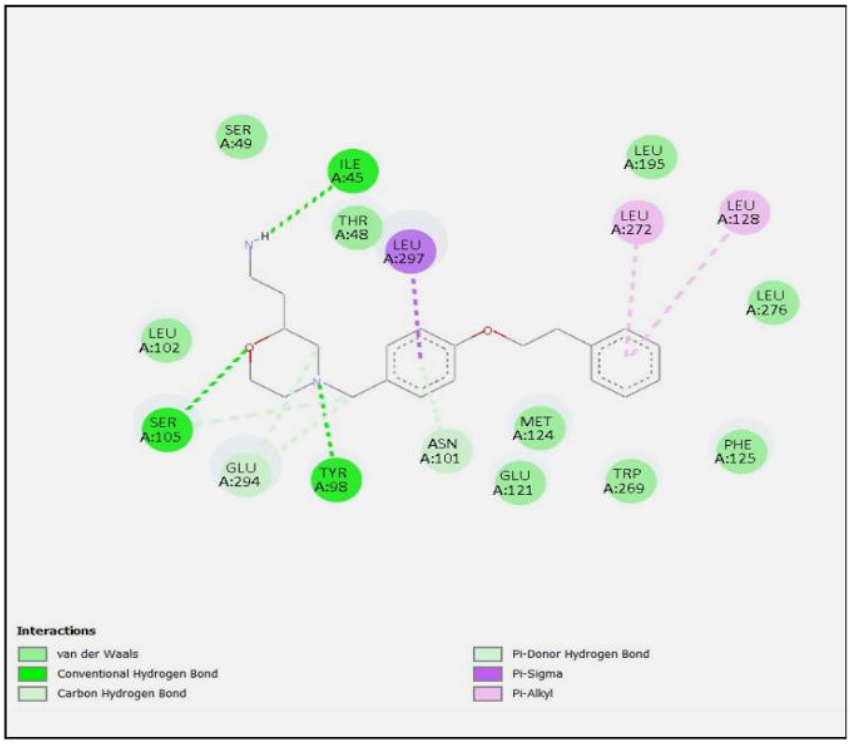


Fig.4. Interaction between ligand no. 4 2-[(2R)-4-[[4-(2-phenylethoxy) phenyl] methyl] morpholin-2-yl] ethanamine with target S1PR1

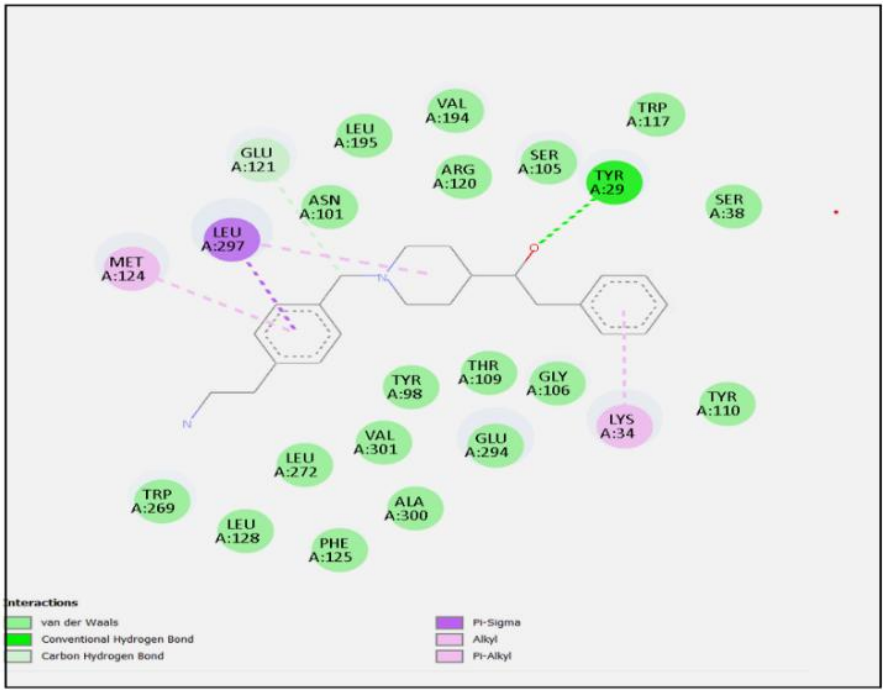


Fig. 5. Interaction between ligand no.5 (1R)-1-[1-[[4-(2-aminoethyl) phenyl] methyl] piperidin-4-yl]-2-phenylethanol and target S1PR1

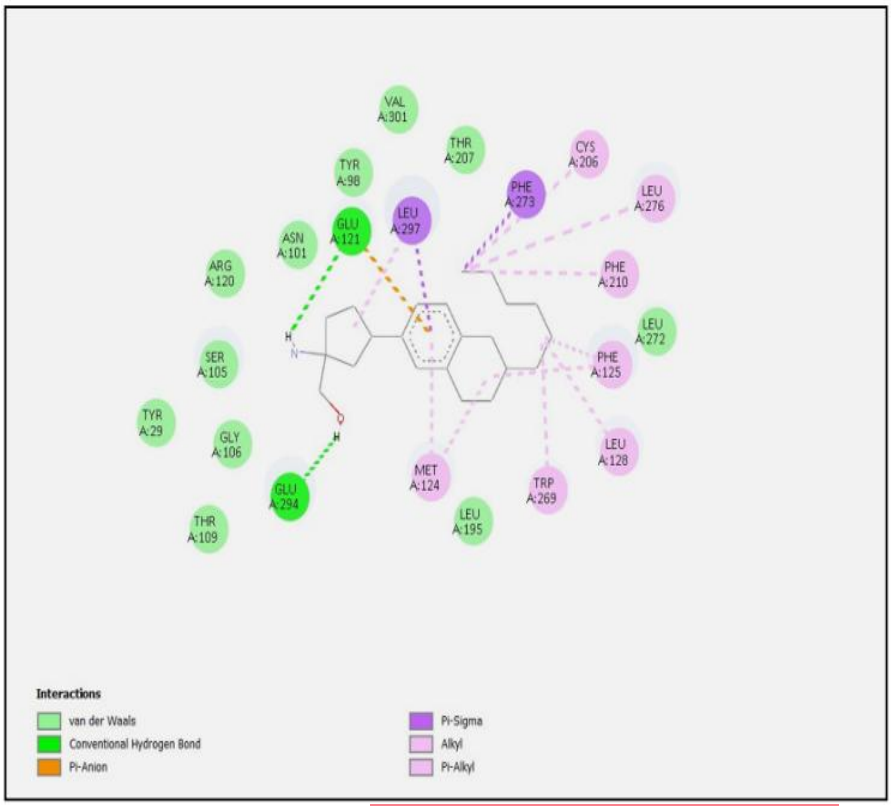


Fig. 6. Interaction between ligand no. 6 [(1*R*,3*S*)-1-amino-3-[(6*R*)-6-hexyl-5,6,7,8-tetrahydronaphthalen-2-yl] cyclopentyl]methanol and target S1PR1

TABLE 2 : TABULAR RESPRESENTATION OF ADME ANALYSIS OF INTERACTING LIGANDS

PUBCHEM ID	*BBB Permeant*	*GI Absorption*	*Lipinski*	*Ghose*	*Log Kp value*
107970	Yes	High	Yes	Yes	-5.22
11779629	Yes	High	Yes	Yes	-6.14
110145611	Yes	High	Yes	Yes	-6.47
126442807	Yes	High	Yes	Yes	-6.40
95895820	Yes	High	Yes	Yes	-6.12
77050638	Yes	High	Yes	Yes	-4.46

5) CONCLUSION

The molecular docking analysis carried out in this study provided important insights into the binding behaviour of fingolimod and a series of structurally related compounds toward the S1PR1 receptor, a well-established therapeutic target in Multiple Sclerosis. The results indicate that all selected ligands were able to successfully occupy the receptor's binding pocket and form energetically favourable complexes, suggesting that the chosen chemical space is relevant for S1PR1 modulation.

Among the screened molecules, **ligand 6** exhibited the most favourable binding profile, showing the lowest binding energy compared to all other compounds included in the study. This indicates a stronger and more stable interaction with the receptor, which may be attributed to optimal alignment within the binding site and the formation of multiple stabilizing interactions such as hydrogen bonds and hydrophobic contacts. Such a higher binding affinity of this ligand suggests that even subtle structural differences present at the chemical scaffold may significantly alter the receptor interaction.

The remainder of the compounds had binding affinities that were comparable or slightly lower than the reference drug fingolimod. This finding points out a significant feature of drug design

based on structure: **structural similarity alone does not guarantee improved biological activity or binding efficiency**. Though the compounds shared a number of similar features with fingolimod, the position shift of functional groups, flexibility of the molecules and geometry of the compounds were likely the causes of the difference in the docking performance.

In any case, the overall binding trends of the entire set of ligands would indicate that the underlying chemical scaffold used in the present study is pre-programmed to bind the S1PR1 receptor. The compounds exhibited some stable binding patterns and interactions patterns that resemble those of known modulators, which can be interpreted as the support of the idea that this scaffold can be a reliable starting point to be further modified.

One of the most interesting discoveries is that there is a similarity in the pattern of interaction between high-performing ligands, and more specifically with key residues being engaged in the binding pocket. This implies that some of the structural features play a key role in effective receptor binding and can be strategically conserved or optimized in subsequent compounds. In this regard, ligand 6 turns out to be a promising lead molecule, as it not only has a higher docking score, but also a favourable interaction profile.

In general, the docking outcomes highlight the power of structure-based computational methods to determine potential drug candidates. Through the integration of binding affinity analysis with interaction analysis, this paper will show how in silico approaches can be used to inform the rational design of novel S1PR1 modulators. Since ligand 6 has good binding properties, it is an interesting candidate to undergo additional experimental validation, such as in vitro and in vivo experiments, to ensure the therapeutic potential of this ligand.

REFERENCES

[1] A. Compston and A. Coles, “Multiple sclerosis,” *The Lancet*, vol. 372, no. 9648, pp. 1502–1517, 2008.
[2] C. Confavreux and S. Vukusic, “Natural history of multiple sclerosis,” *Brain*, vol. 129, pp. 606–616, 2006.
[3] B. Hemmer, J. Kerschensteiner, and T. Korn, “Role of the immune system in MS,” *Nat. Rev. Neurosci.*, vol. 16, pp. 545–558, 2015.
[4] S. L. Hauser and J. R. Oksenberg, “The neurobiology of multiple sclerosis,” *Neuron*, vol. 52, pp. 61–76, 2006.
[5] J. Chun and V. Brinkmann, “Sphingosine-1-phosphate receptors in immune regulation,” *Nat. Rev. Drug Discov.*, vol. 9, pp. 883–897, 2010.
[6] V. Brinkmann et al., “Fingolimod (FTY720): discovery and mechanism,” *Nat. Rev. Drug Discov.*, vol. 9, pp. 883–897, 2010.
[7] H. Rosen and E. J. Goetzl, “S1P and immune cell trafficking,” *Nat. Rev. Immunol.*, vol. 5, pp. 560–570, 2005.
[8] B. D. Trapp and K. A. Nave, “Multiple sclerosis: axonal degeneration,” *Annu. Rev. Neurosci.*, vol. 31, pp. 247–269, 2008.

[9] S. M. Gold et al., “Mechanisms of neurodegeneration in MS,” *Nat. Rev. Neurol.*, vol. 8, pp. 563–572, 2012.
[10] R. M. Ransohoff, “Natalizumab for MS,” *N. Engl. J. Med.*, vol. 356, pp. 2622–2629, 2007.
[11] L. Steinman, “Immunology of MS,” *Annu. Rev. Immunol.*, vol. 32, pp. 257–281, 2014.
[12] B. Bielekova and R. Martin, “Development of MS therapies,” *Nat. Rev. Drug Discov.*, vol. 3, pp. 585–598, 2004.
[13] J. Cohen et al., “Oral fingolimod for relapsing MS,” *N. Engl. J. Med.*, vol. 362, pp. 402–415, 2010.
[14] L. Kappos et al., “Fingolimod vs placebo in MS,” *N. Engl. J. Med.*, vol. 362, pp. 387–401, 2010.
[15] T. Hla and V. Brinkmann, “S1P receptors: signaling and function,” *J. Biol. Chem.*, vol. 286, pp. 9999–10003, 2011.
[16] H. Chun et al., “S1P signaling in disease,” *Annu. Rev. Pharmacol. Toxicol.*, vol. 53, pp. 95–120, 2013.
[17] S. Spiegel and S. Milstien, “Sphingosine-1-phosphate signaling,” *Nat. Rev. Mol. Cell Biol.*, vol. 4, pp. 397–407, 2003.
[18] T. Schwab and M. Cyster, “Finding a way out: lymphocyte egress,” *Nat. Immunol.*, vol. 8, pp. 1295–1301, 2007.
[19] V. Brinkmann, “S1P receptor modulators in MS,” *J. Neuroimmunol.*, vol. 308, pp. 1–8, 2017.
[20] L. Matloubian et al., “Lymphocyte egress controlled by S1P receptor,” *Nature*, vol. 427, pp. 355–360, 2004.
[21] M. Mandala et al., “FTY720 mechanism,” *Science*, vol. 296, pp. 346–349, 2002.
[22] H. Cohen and V. Chun, “Fingolimod therapy overview,” *Nat. Rev. Neurol.*, vol. 7, pp. 495–502, 2011.
[23] FDA, “Fingolimod approval summary,” 2010.
[24] V. Brinkmann, “FTY720 mechanism of action,” *Transplant. Proc.*, vol. 33, pp. 3078–3080, 2001.
[25] A. Paugh et al., “Sphingosine kinase activity,” *J. Biol. Chem.*, vol. 278, pp. 42952–42959, 2003.
[26] D. Calabresi et al., “Safety of fingolimod,” *Lancet Neurol.*, vol. 13, pp. 545–556, 2014.
[27] S. Jain and A. Bhatti, “Adverse effects of S1PR modulators,” *Drug Saf.*, vol. 40, pp. 321–332, 2017.
[28] EMA, “Fingolimod safety profile,” 2012.
[29] M. Scott et al., “Limitations of fingolimod,” *CNS Drugs*, vol. 30, pp. 1–10, 2016.
[30] L. Kappos et al., “Siponimod in MS,” *Lancet*, vol. 391, pp. 1263–1273, 2018.
[31] J. Cohen et al., “Ozanimod trial,” *Lancet Neurol.*, vol. 15, pp. 373–381, 2016.
[32] R. Comi et al., “Selective S1PR modulators,” *Mult. Scler. J.*, vol. 23, pp. 1084–1092, 2017.
[33] S. Groves et al., “Neuroprotective role of S1P,” *J. Neurosci.*, vol. 33, pp. 1878–1891, 2013.
[34] M. Miron et al., “Remyelination and oligodendrocytes,” *Nat. Neurosci.*, vol. 16, pp. 1211–1218, 2013.
[35] R. Chun and H. Hartung, “S1P in CNS repair,” *Lancet Neurol.*, vol. 9, pp. 111–120, 2010.
[36] D. Kitchen et al., “Docking and scoring in drug design,” *Nat. Rev. Drug Discov.*, 2004.
[37] W. Walters et al., “Virtual screening overview,” *J. Med. Chem.*, 1998.
[38] P. Lionta et al., “Structure-based virtual screening,” *Curr. Top. Med. Chem.*, 2014.
[39] B. Lublin et al., “MS disease course,” *Neurology*, 2014.
[40] F. Dendrou et al., “Immunopathology of MS,” *Nat. Rev. Immunol.*, 2015.

[41] J. Goverman, “Autoimmune T cell responses,” *Nat. Rev. Immunol.*, 2009.
[42] C. Bjartmar et al., “Axonal damage in MS,” *Ann. Neurol.*, 2003.
[43] D. Mahad et al., “Mitochondrial dysfunction in MS,” *Brain*, 2015.
[44] H. Lassmann, “Neurodegeneration mechanisms,” *J. Neurol. Sci.*, 2013.
[45] T. Hla, “S1P signaling overview,” *Science*, 2001.
[46] M. Cyster and S. Schwab, “S1P gradients,” *Annu. Rev. Immunol.*, 2012.
[47] M. Camerer et al., “S1P and vascular integrity,” *Nature*, 2009.
[48] S. Sanchez et al., “S1P receptor biology,” *J. Biol. Chem.*, 2003.
[49] J. Pyne and S. Pyne, “S1P signaling pathways,” *Nat. Rev. Cancer*, 2010.
[50] E. Jaillard et al., “Oligodendrocyte survival,” *Glia*, 2005.
[51] J. Pelletier and D. Hafler, “MS therapeutics,” *N. Engl. J. Med.*, 2012.
[52] G. Scott et al., “New S1PR modulators,” *Drugs*, 2016.
[53] R. Pan et al., “Selective S1PR1 targeting,” *J. Pharmacol. Exp. Ther.*, 2013.
[54] Y. Tsukamoto et al., “Next-gen S1PR modulators,” *Bioorg. Med. Chem.*, 2018.
[55] I. Ferreira et al., “Molecular docking review,” *Molecules*, 2015.
[56] J. Bajorath, “Chemoinformatics in drug discovery,” *Nat. Rev. Drug Discov.*, 2002.
[57] A. Daina et al., “SwissADME tool,” *Sci. Rep.*, 2017.
[58] D. Pires et al., “pkCSM tool,” *J. Med. Chem.*, 2015.
[59] J. Warren et al., “Docking limitations,” *J. Med. Chem.*, 2006.
[60] L. Kappos et al., “Siponimod vs placebo in SPMS (EXPAND),” *Lancet*, 2018.
[61] J. Cohen et al., “Ozanimod vs interferon beta-1a (RADIANCE),” *Lancet Neurol.*, 2016.
[62] L. Kappos et al., “Ponesimod vs teriflunomide (OPTIMUM),” *JAMA Neurol.*, 2021.
[63] M. A. Hanson et al., “Crystal structure of S1P1 receptor,” *Science*, 2012.
[64] T. Hla and V. Brinkmann, “S1P receptor biology,” *Nat. Rev. Drug Discov.*, 2011.
[65] I. Ferreira et al., “Molecular docking strategies,” *Molecules*, 2015.
[66] W. L. Jorgensen, “Role of computation in drug discovery,” *Science*, 2004.
[67] C. A. Lipinski et al., “Drug-likeness and Rule of Five,” *Adv. Drug Deliv. Rev.*, 2001.
[68] J. B. Warren et al., “Docking scoring limitations,” *J. Med. Chem.*, 2006.