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Computational approaches to identify novel drug-like immunomodulators against multiple sclerosis

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ABSTRACT

Sphingosine1-Phosphate Receptor1 (S1PR1) a G protein-coupled receptor is critically involved in the trafficking of peripheral T-Lymphocyte into the Central Nervous System (CNS) leading to Remitting type of Multiple Sclerosis (RSMS). In the present scenario, the long-term benefits of the current immunomodulator against RSMS that bind specifically to S1PR1preventing the upward movement of lymphocytes toward CNS are uncertain due to the undesirable side effects. Therefore, in this paper, the author aims to screen derivatives of known immunomodulators used in Multiple Sclerosis (MS) treatment that binds specifically with S1PR1 receptor with better affinity and pharmacological properties than their parental compound. In this context, two promising analogs were screened namely CID_11623444 (L7A) and CID_445354 (RTL) of mitoxantrone and fingolimod, respectively that showed better pharmacokinetic properties, immunomodulatory activity, BBB permeability and affinity for S1PR1 receptors than their corresponding parental immunomodulator compound. Moreover, both the analogs were found to be specific inhibitors of S1PR1receptor by Baell and Holloway method. Therefore, based on the results it can be proposed that chemical analogs CID_11623444 and CID_445354 are useful lead molecules which may slow the progression of Multiple Sclerosis (MS) with greater efficacy and minimum side effects.

1. Introduction

Multiple sclerosis (MS) is a long-lasting demyelinating autoimmune disorder, of the central nervous system (CNS). MS is stated as one of the major causes of disability in adults ranging from 25-30 years of age (Noseworthy et al., 2000; Sawcer et al., 2011) and the male/female ratio in this group is nearly 1:3 and may be increasing (Handel et al., 2010). However, the root cause of MS remains unidentified (Orton et al., 2006; Goodin, 2014; Nylander and Hafler, 2012). The most commonly accepted theory is that MS initiates as an inflammatory autoimmune disorder facilitated by S1PR1 signaling. The S1PR1signaling is crucial in the regulation of maturation, migration, and trafficking of autoreactive lymphocytes from peripheral lymphoid organ and mature thymocytes into the CNS through the Blood-Brain Barrier (BBB). Eventually, the disease is subjugated by the activation of microglial cells and chronic neurodegeneration (Roach, 2004). The reactive microglial cell found in active as well as a chronic inactive lesion of the brain had a manifold increase in the expression of S1PR1 and S1PR3, respectively (Allende et al., 2004). Nearly, 15 % of the patients suffer from a primary progressive type of multiple sclerosis (PPMS), which means gradually progressive and unremitting loss of neurological function for more than 1 year. While, the remaining eighty-five percent of the patients suffer from the relapsing-remitting type of multiple sclerosis (RRMS) (Lublin and Reingold, 1996; Miller and Leary, 2007). Available preventive Drug Modifying Therapies (DMT's) for MS mostly aim at reducing the frequency and severity of relapses, but with many unmet need remain to be fulfilled. Primarily, two most important problems associated with the treatment of MS are: 1) firstly dearth of treatment that credibly slow or cease the progressive nature of MS, and 2) secondly the issue of greater side effects associated with the available DMTs of MS. Immunosuppressants apart from mitoxantrone, however, have not shown any

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significant reduction in the progression of MS or on the frequency of its relapses. Mitoxantrone, on the other hand, has been linked with acute leukemia and cardiotoxicity (Marriott et al., 2010), natalizumab, fingolimod and other oral DMTs of MS have been linked with progressive multifocal leukoencephalopathy (incidence 0.001%), elevated liver transaminases, acute renal failure (1%), lymphopenia, hypertension, diarrhea, nausea, peripheral neuropathy (1%-2%), bradyarrhythmia, macular edema, flushing, alopecia, secondary autoimmunity, gastrointestinal symptoms and gastrointestinal side effects (Ransohoff, 2007; Alcorn et al., 2009).

Thus, keeping in view failure associated with the current treatment schema of MS, the study was focused on finding new drug-like analog from the available repertoire of immunoregulatory medicine against MS, which may slow the progression of the disease with greater efficacy and minimum or no side effects. Consequently, the study led to the screening of two promising lead analogs of known immunomodulators used in MS having better pharmacological properties and lesser side effects.

2. Methodology

2.1. A brief outline of the workflow to identify novel analogs of approved DMTs

The steps involved in the identification of novel analogs in the current study are outlined in Fig. 1.

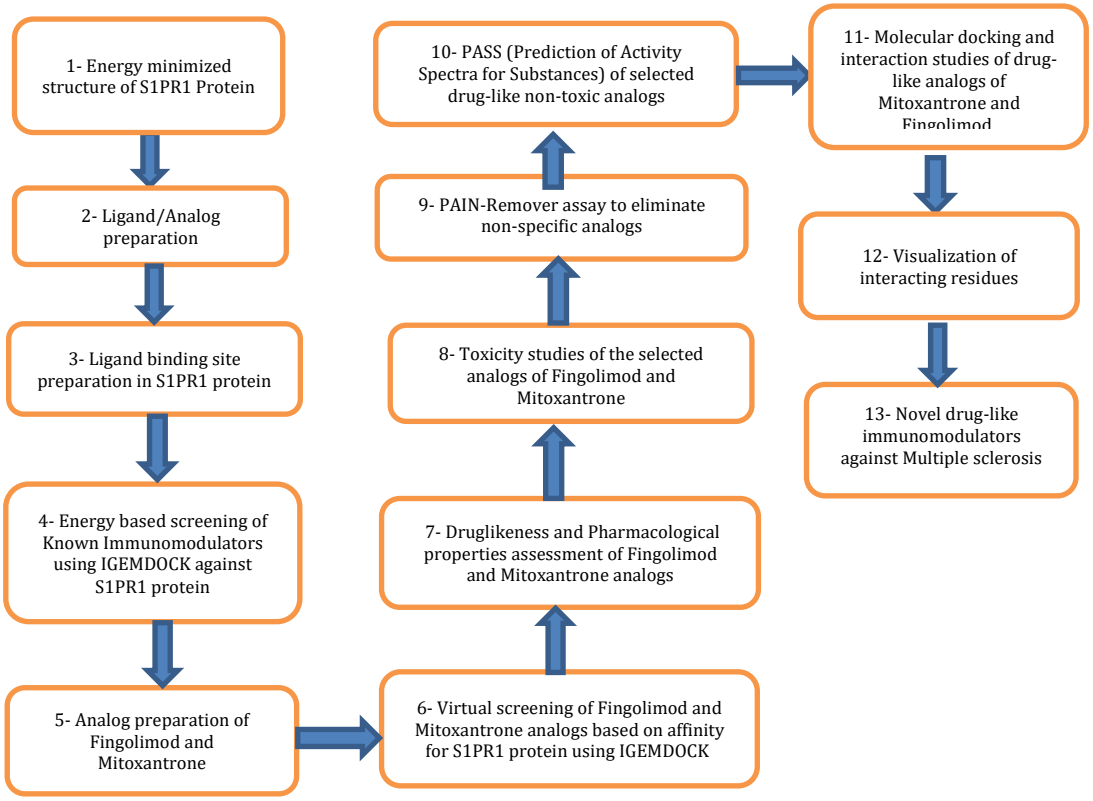

Fig. 1: The Pictorial depiction of the workflow involved in the screening of novel non-toxic drug-like analog of known immunomodulator of MS

2.2. Virtual screening using iGEMDOCK

The docking tool iGEMDOCK v2.0 (Yang and Chen, 2004; Yang, 2004) was used to perform rapid virtual screenings of S1PR1 inhibitors using the crystal structure S1PR1 protein (Hanson et al., 2012). The following four important steps are involved in virtual screening using iGEMDOCK:

(1) retrieval of target protein structure;
(2) preparing compound library;
(3) Preparation of binding site;
(4) protein-ligand docking and
(5) Docked poses/post-screening analysis.

2.2.1. Retrieval and preparation of target protein structure

The crystal structure of S1PR1 PDB ID 3V2Y (resolution 2.80 Å) with an antagonist was retrieved from Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) The ready to dock minimized structure of PDF protein was prepared using the Dock Prep tool of Chimera 1.10.2

2.2.2. Ligand preparation

The 3D structural files of four known immunomodulators namely mitoxantrone (Scott and Figgitt, 2004; Fox, 2004), fingolimod (Antel, 2014; Mehling et al., 2011), teriflunomide (He et al., 2016;

Miller, 2017) and dimethyl fumarate/BG-12 (Motte et al., 2017; Kasarełło et al., 2017) and their corresponding analogs (Similar conformers or 3-D neighbors) were downloaded from PubChem database (https://pubchem.ncbi.nlm.nih.gov/compound/). The similar conformers (analogs) for each of the immunomodulators are generated by PubChem (https://pubchem.ncbi.nlm.nih.gov/search/search.cgi) based on 3-D neighboring's technique. The 3-D neighboring technique is based on PubChem shape overlay-based 3-D similarity method (where the threshold for feature similarity was 50 %, and for the shape, the similarity was 80 %) to determine "neighbor" relationships among chemical compound available in PubChem database (Bolton et al., 2011; 2011a; 2011b, 2011c; Kim et al., 2011a; 2011b; 2012; 2013). A library of analogs (similar conformers) of each immunomodulator under study was created where mitoxantrone had 197 conformers, fingolimod had 49 conformers, teriflunomide had 817 records, and finally, dimethyl fumarate (BG-12) had 2072 records. The 3-D structures of the immunomodulators and their corresponding analogs (similar conformers) downloaded were in *.sdf* format. OpenBabel (http://openbabel.org/wiki/Main_Page) software was used to convert the *.sdf* files to *.mol2* format since iGEMDOCK docking tool needs structural data in *.mol2* format for executing molecular docking calculations. Ready to use dock prep structures of immunomodulator were prepared using Chimera 1.10.

2.2.3. Ligand binding site preparation

The S1PR1 protein in complex with antagonist was downloaded in .pdb format from RCSB PDB (PDB ID 3V2Y). The binding region of the bounded inhibitor was defined as the binding site for virtual screening. The bounded inhibitor was identified as the center of the binding domain, and the size of the binding position was set to a default value of 8 Å.

2.2.4. Ligand-protein docking

Standard docking protocol of iGEMDOCK v2.0 was used to screen immunomodulator compound having a higher affinity for S1PR1 protein. A population size of 200 with 70 generations and two solutions for each generation was set for molecular docking studies. Top two S1PR1 binders were selected based on their affinity for the ligand binding domain of the crystal structure of the S1PR1 protein.

Analog preparation and virtual screening

A library of analogs of top two binders screened based on their affinity for S1PR1 protein was retrieved from PubChem, dock prepared using chimera 1.10.2 and screened against the crystal structure of the S1PR1 protein using the standard docking procedure of iGEMDOCK. The top ten analogs selected based on their affinity for the targeted ligand binding domain of S1PR1 protein were further checked for their pharmacological and drug-likeliness properties.

2.3. Drug-likeness and pharmacological analysis

The physiochemical descriptors of the selected ten ligands were estimated for oral drug availability, drug-likeness and pharmacokinetic properties of the screened ligands using SwissADME (http://www.swissadme.ch/index.php) (Daina et al., 2017).

2.4. Toxicity analysis

All the homologous components of known immunoregulatory agents of MS will be subjected to toxicity prediction through ACD/Labs for all toxicity profiles like mutagenicity, carcinogenicity, irritant effects and reproductive effect. Toxicity analysis was done to know about the probable undesired effects of the drug in the body.

2.5. Blood-brain-barrier studies

BBB penetration studies using ACD/Labs predict the ability of the analogs of known immunoregulatory agents of MS to penetrate the BBB and interact with the S1PR1 receptor sufficient for CNS activity.

2.6. PAIN-remover assay

The PAIN-remover assay is performed using various structural filters proposed by Bell and hallway to screen out the Pan-Assay Interference (PAIN) compounds (False Positives) which nonspecifically interact with many biological targets instead of targeting a specific target (Dahlin et al., 2015). The Assay was employed to test the specificity of the screened drug-like molecules (inhibitors) for S1PR1 protein.

2.7. Molecular docking and post-dock interaction studies of screened ligand and its parental compound

By binding affinity, drug-likeliness, ADMET properties and PAIN-Remover Assay analogs of mitoxantrone and fingolimod were selected for performing very slow (accurate) docking protocol of iGEMDOCK. The screened drug-like lead analog molecule and its parent compound were subjected to the prolonged docking procedure of iGEMDOCK. The docking accuracy settings for accurate docking module of iGEMDOCK are 1) generations: 80, 2) number of solutions (poses per generation):10, and 3) population size (generation x number of solutions (poses)): 800. Once molecular docking was completed, protein-ligand interaction profile consisting of Van der Waal's (V), hydrogen-bonding

(H) and electrostatic (E) was generated. Based on these profiles the compounds are compared using the energy-based scoring function of iGEMDOCK (Yang et al., 2005).

2.8. Visualization of the interacting residues

The 2-D representation of the interacting residues of the docked complexes was generated by Chimera 1.10.2 program. The pictorial representation was helpful in determining the interacting functional groups of the novel drug-like lead molecule with the target protein.

2.9. Prediction of biological activity and toxicity of the screened drug-like molecule and its parental compound

PASS (Prediction of Activity Spectra for Substances) software based on the structure-activity relationship (Borodina et al., 1996) was used to predict the biological activity and potential toxic effects of both the screened drug-like ligand and its parental molecule.

3. Result and discussion

3.1. Virtual screening analysis

Based upon their total binding energy (affinity) for S1PR1 protein top two immunomodulators namely Mitoxantrone (-119.478 kcal/mol) and Fingolimod (-97.2652 kcal/mol) were selected using standard screening protocol of iGEMDOCK and tabulated in Table 1.

Further, ten best derivatives (analogs) derived from parent immunomodulator molecules namely mitoxantrone and fingolimod were chosen based on their affinity (binding energy) for S1PR1 protein and are listed in Table 2.

Table 1: Binding Energies for the known immunomodulators of MS against the crystal structure of S1PR1 protein using the standard docking protocol of iGEMDOCK

Sl. No.	Immunomodulators	Total Energy	VDW	H-Bond
1	Mitoxantrone	-119.478	-103.97	-15.50
2	Fingolimod	-97.265	-77.76	-21.5
3	Terifunomide	-65.695	-59.69	-6
4	Dimethyl Fumarate	-58.949	-41.12	-17.82

Table 2: Binding Energies for the ten best analogs of MS immunomodulators docked against the crystal structure of S1PR1 protein

Sl. No.	Analog of mPDF inhibitors	Parent connectivity (Immunomodulator)	Total Energy	VDW	H-Bond
1	CID_11623444	Mitoxantrone	142.01	-96.17	-45.84
2	CID_445354	Fingolimod	-98.68	-87.55	-11.13
3	CID_49839561	Mitoxantrone	-97.39	-93.89	-3.50
4	CID_3863978	Fingolimod	-90.78	-76.36	-14.42
5	CID_85466	Mitoxantrone	-89.02	-58.99	-30.03
6	CID_24901725	Mitoxantrone	-73.32	-55.32	-18.00
7	CID_843781	Fingolimod	-72.58	-67.92	-4.66
8	CID_53245673	Fingolimod	-72.10	-54.26	-17.84
9	CID_54684141	Fingolimod	-65.01	-57.61	-7.40
10	CID_54714524	Fingolimod	-64.31	-57.31	-7

It can be observed that the chemical derivative CID_11623444 and CID_445354 of standard immunomodulators mitoxantrone and fingolimod as shown in Fig. 2, showed a better binding affinity for S1PR1 protein when compared to other derivatives of fingolimod and mitoxantrone.

3.2. Oral bioavailability and drug-likeness studies

The oral bioavailability and drug-likeness of the ten best-screened ligands based on affinity for S1PR1 protein were evaluated using SwissADME server and are tabulated in Table 3. Oral bioavailability of the ligands was evaluated based on the threshold value of certain physiochemical descriptors namely Lipophilicity (-0.7 <XLOGP3 < +5.0), Size (150 g/mol < MV < 500 g/mol), Polarity (20 Å^2 < TPSA < 130 Å^2), Insolubility (0 < Log S (ESOL) < 6), Instauration (0.25 < Fraction Csp3 < 1) and Flexibility (0 < Num. rotatable bonds < 9) of the SwissADME server. It can be observed from Table 3 that all the chemical analog molecules except CID_24901725, CID_3863978, and CID_53245673 showed no violation for any of the physiochemical descriptors used for describing the oral bioavailability of the analog molecules.

The analog CID_24901725 of mitoxantrone showed two violation namely molecular weight (> 350) and a number of rotatable bonds (> 7). Additionally, the analog CID_53245673 showed a violation in the number of hydrogen bond donor (> 5) and a number of rotatable bonds (> 7). Moreover, the analog molecules CID_3863978 showed a violation of molecular weight (< 250 g/mol). Mitoxantrone, on the other hand, showed violations of Total Polar surface Area (TPSA) (> 130 Å^2) and Number of rotatable bonds (> 7). While Fingolimod showed violation only related to the number of rotatable bonds (> 7). Therefore, considering the violation associated with the parental compounds and some of their derivatives it was found that the chemical analog CID_11623444, CID_49839561, CID_85466 of mitoxantrone and CID_445354,

CID_843781, CID_54684141, and CID_54714524 of fingolimod showed better oral bioavailability properties than their corresponding parental compounds.

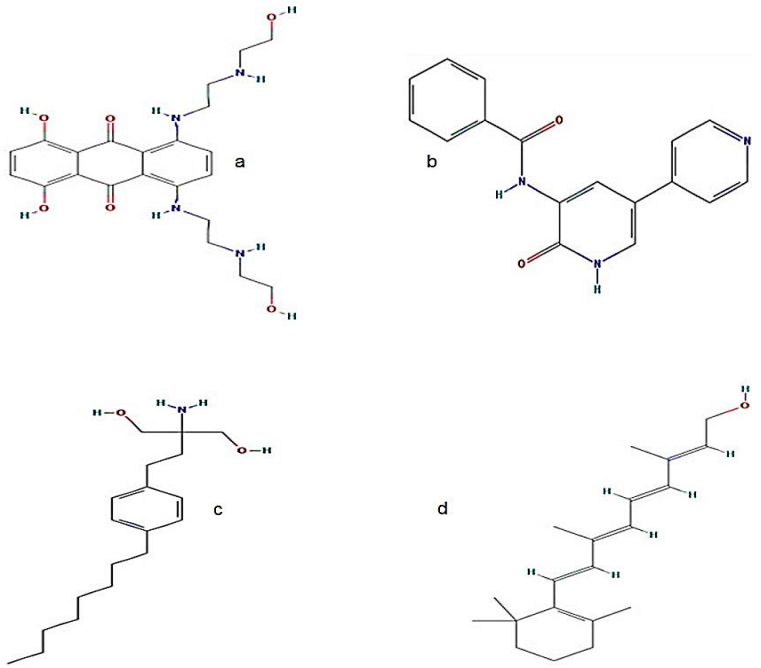

Fig. 2: Chemical structures of (a) Mitoxantrone (b) CID_11623444 (analog of mitoxantrone) (c) Fingolimod (d) CID_445354 (analog of fingolimod)

Table 3: The physiochemical properties depicting the oral bioavailability and drug-likeliness of the ten best-fitted analogs of immunomodulators

Sl. No	Molecule	MW	Fraction Csp3	Rotatable bonds	H-bond acceptors	H-bond donors	TPSA	XLOGP3	ESOL Log S	Lipinski violations	Drug Likeness	Lead likeness
1	Mitoxantrone	444.48	0.36	12	8	8	163.18	1	-2.71	1	Yes	No, 2 Volilations
2	Fingolimod	307.47	0.68	12	3	3	66.48	4.16	-3.78	0	Yes	No, 2 Volilations
3	CID_24901725	582.69	0.53	16	8	4	149.14	2.33	-4.15	1 (MW>500)	Yes	No; 2 violations: MW>350, Rotors>7
4	CID_11623444	291.3	0	4	3	2	74.85	1.36	-2.84	0	Yes	Yes
5	CID_49839561	350	0.19	6	3	2	71.09	2.91	-3.83	0	Yes	Yes
6	CID_3863978	197.23	0.4	4	4	2	64.71	1.74	-0.72	0	Yes	No; 1 violation: MW<250
7	CID_85466	270.24	0	0	4	4	126.64	1.9	-3.16	0	Yes	Yes
8	CID_445354	286.45	0.5	5	1	1	20.23	2.68	-4.86	0	Yes	Yes
9	CID_843781	274.26	0.27	5	5	1	78.19	3.02	-3.36	0	Yes	Yes
10	CID_53245673	336.52	0.56	10	0	6	88.86	0.69	-1.89	1 violation: H-don>5	Yes	No; 1 violation: Rotors>7
11	CID_54684141	270.21	0.17	4	6		73.12	3.27	-3.55	0	Yes	Yes
12	CID_54714524	270.21	0.17	4			73.12	3.27	-3.55	0	Yes	Yes

Additionally, druglikeness and leadlikeness of the chemical analogs were also evaluated using the Lipinski's rule of five (RO5) (Lipinski, 2004). As per RO5, a chemical compound to be orally active in human should follow minimum three criteria of the following: (a) molecular weight ≤ 500, (b) XLOGP3 <3.5, (c) hydrogen bond acceptor ≤ 10 and (d) hydrogen bond donor ≤ 5. Therefore in this context, it was observed that the chemical compound namely CID_11623444, CID_49839561, CID_85466, CID_843781, CID_54684141, and CID_54714524 and fingolimod had zero violation of the RO5. On the contrary analog molecules CID_24901725 and CID_53245673 showed violation in Molecular weight (>500), hydrogen bond donor (> 5), respectively. Moreover, mitoxantrone a standard immunomodulator molecule showed a violation of the number of hydrogen bonds (> 5). Since the parental compounds and their corresponding analog molecules satisfy a minimum of three criteria of RO5, therefore, they were classified as virtual drug-like molecules. Moreover, the leadlikeness property of the analogs was also evaluated. The rule of five has been modified to describe the leadlikeness of the molecule. Therefore a compound to qualify as a lead-like the following criteria are to be met (a) XLOGP3 < 3, (b) molecular mass (< 300), (3) Hydrogen bond donors (≤ 3), hydrogen bond acceptor (≤ 3) and lastly the number of rotatable bonds should not more than 3 (Lipinski, 2004). The analog CID_24901725 of mitoxantrone showed two violations of the criteria mentioned above namely

molecular weight (>350) and a number of rotatable bonds (> 7). While the analog CID_3863978 of fingolimod showed violation in only one criterion namely molecular weight (> 350 g/mol). Similarly, the analog CID_53245673 of fingolimod showed a violation in the number of hydrogen bond donor (> 5) atoms. Moreover, the parental compound mitoxantrone showed a violation of a number of rotatable bonds (> 7), and molecular weight (> 350) and fingolimod showed violation in the number of rotatable bonds (> 7) and lipophilicity (XLOGP3 > 3.5). However, the drug-like analog molecules namely CID_11623444, CID_49839561, CID_85466, CID_445354, CID_843781, CID_54684141, and CID_54714524 showed no violation of RO3 and thus were further classified as lead-like molecules.

3.3. Pharmacokinetics assessment of the analogs

The important pharmacokinetic properties of the analog molecules namely gastrointestinal (GI) absorption, drug metabolism, Blood Brain Barrier (BBB) permeation and permeability glycoprotein activity are tabulated in Table 4. From Table 4, it is observed that except mitoxantrone, CID_24901725 and CID_53245673 other analog molecules showed higher gastrointestinal absorption capability and therefore can easily cross the gastrointestinal mucosa before entering the bloodstream. The potency of the parental compound (mitoxantrone and fingolimod) and their corresponding analogs molecules to cross the BBB were estimated. In SwissADME the potency of the chemical compound to cross the BBB is estimated by BOILED-Egg (Brain or IntestinaL EstimateD) permeation predictive model (Daina and Zoete, 2016). The model is based on Support Vector Machine (SVM) classification algorithm (Cortes and Vapnik, 1995) to predict the permeation capability of the chemical compound based on two attributes, i.e., tPSA and WLOGP. The new instances (chemical molecule) for BBB permeation is either classified as brain-permeable or non-permeable compound. Therefore, in this regard fingolimod and some of its derivatives namely CID_445354 (RTL), CID_54684141 and CID_54714524 showed a tendency to cross the blood-brain barrier for CNS activity. Mitoxantrone showed negative potency to cross the BBB. However, CID_11623444 and CID_49839561 the analogs of mitoxantrone showed potency to cross the BBB an essential feature for a molecule to be considered as a drug for MS. The current discovery of central nervous system lymphatic system (Louveau et al., 2015) greatly enhance the present finding since the current study is targeting S1PR1 receptor which is responsible for the egress of lymphocyte from the lymphoid organ present in our human system. Drug metabolism is a process of detoxification and eventual excretion of the drug from the human system. Therefore, the role of cytochromes P450 (CYPs) drug metabolizing enzymes are essential for the detoxification and later excretion of the drug from the human system (Sigel et al., 2007). The current results regarding the action of the immunomodulators and their corresponding analogs on cytochrome p450 family of detoxifying enzymes namely CYP1A2, CYP2D6, CYP2C19, CYP3A4, and CYP2C9 are based on SVM classifier based predictive model (Daina et al., 2017). The supervised learning based model is used by SwissADME to predict the inhibitory activity of the query drug on detoxifying enzymes. The new or query instances (chemical molecule) are screened through the SVM model into two class, i.e., inhibitor or non-inhibitor. Therefore, in this regard the ligands CID_49839561, CID_11623444 were screened as potent inhibitors of cytochrome p450 family of oxidizing enzymes since the proper functioning of CYPs enzymes is essential for the metabolic clearance of the drug from the body. Therefore the inhibition of these enzymes by the analog molecule might result in increased bioavailability and the strong possibility of overdosing and eventually toxicity. However, mitoxantrone and the analogs of fingolimod namely CID_3863978, CID_53245673 were found be non-inhibitor of cytochrome p450 family of oxidizing enzymes, therefore, have better pharmacokinetic property than the other analog molecules which show inhibition of one or more members of cytochrome p450 family of detoxifying enzymes namely CYP1A2, CYP2D6, CYP2C19, CYP3A4, and CYP2C9.

P-glycoprotein (P-gp) is a transmembrane permeability glycoprotein that functions as a primary active efflux transporter present in our human system. Some essential immunomodulators drugs namely mitoxantrone and fingolimod are substrates to P-gp, and that negatively affect their bioavailability and resistance is induced because of the effluxing nature of the protein. Additionally, the substrate and inhibitor nature of some drugs for drug-metabolizing enzymes may lead to the reason for dangerous drug interactions. Therefore chemical compound which is non-substrate of P-gp protein is expected to overcome the problems relating to poor bioavailability, multi-drug resistance and dangerous drug interactions (Amin, 2013). In SwissADME the analysis of P-gp is based upon the supervised SVM classifier based predictive BOILED-Egg model (Daina and Zoete, 2016). In this context, the new instances (chemical molecule) are predicted either as a substrate or non-substrate for Pgp. Hence, based upon the SVM model the analogs except CID_24901725 and CID_49839561 are non-substrate of for P-gp protein. The non-P-gp substrate nature of these analogs will prevent pre-metabolized exit from the human body. Thereby enhancing their efficacy as a therapeutic agent against MS.

Table 4: The pharmacokinetic properties of the top ten best-fitted analogs of MS immunomodulators

Sl. No	Molecule	GI absorption	BBB permeant	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	CYP2C9 inhibitor	Pgp substrate
1	Mitoxantrone	Low	No	No	No	No	No	No	Yes
2	Fingolimod	High	Yes	No	No	No	Yes	Yes	Yes
3	CID_24901725	Low	No	No	Yes	No	Yes	No	Yes
4	CID_11623444	High	Yes	No	No	No	Yes	No	No
5	CID_49839561	High	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	CID_3863978	High	No	No	No	No	No	No	No
7	CID_85466	High	No	Yes	No	No	Yes	No	No
8	CID_445354	High	Yes	Yes	No	No	No	Yes	No
9	CID_843781	High	No	Yes	Yes	No	No	No	No
10	CID_53245673	Low	No	No	No	No	No	No	No
11	CID_54684141	High	Yes	Yes	No	No	No	No	No
12	CID_54714524	High	Yes	Yes	No	No	No	No	No

3.4. Toxicity studies

The drug-like analog of mitoxantrone and fingolimod were screened further for their toxic nature. Tables 5 and 6 show the results of ACD/I-Lab toxicity analysis of the drug-like analogs of mitoxantrone and fingolimod. It was observed that CID_11623444 an analog of mitoxantrone was identified as a non-toxic chemical entity when compared to CID_49839561, CID_24901725, and CID_85466. Both CID_24901725 and CID_85466 were predicted to be carcinogenic agents since the probability of positive Ames test for CID_24901725 and CID_85466 were 0.99 and 1, respectively. Ames test is formally a biological assay to evaluate the potential of chemical compounds to cause mutation in DNA (Mortelmans and Zeiger, 2000). The chemical analog CID_49839561, on the other hand, was predicted to be an endocrine disruptor since CID_49839561 was predicted to be a binder of Estrogen Receptor alpha (ERα). ERα is a type of estrogen receptor, which get activated by the sex hormone estrogen (Walter et al., 1985; Greene et al., 1986). The probability of toxic effect of CID_24901725, CID_85466 and CID_49839561 on the gastrointestinal system, lungs, blood, kidney, and blood, were comparably higher than CID_11623444. Similarly, the analogs of fingolimod CID_445354 (RTL), CID_54684141 and CID_54714524 were found to be non-toxic when compared to L94, CID_843781, and CID_3863978. The probability of the effect of the analogs CID_53245673, CID_843781 and CID_3863978 on blood, cardiovascular system, Gastrointestinal System, kidney, liver, and lungs were comparatively higher than CID_445354, CID_54684141, and CID_54714524.

It is noteworthy to state that CID_54684141 (Teriflunomide) and CID_54714524 (CHEMBL999) chemical analogs of fingolimod which are currently in use as an immunomodulator for the treatment of MS (Mehling et al., 2011; He et al., 2016; Miller, 2017). Therefore, in the present study, the analogs CID_54684141 (Teriflunomide) and CID_54714524 (CHEMBL999) of fingolimod will not be further considered for docking studies.

3.5. Comparative pharmacological and toxicity studies

After a thorough evaluation of the pharmacological and toxicity properties of the analogs of mitoxantrone and fingolimod, it was observed that the analogs CID_11623444 (L7A) and CID_445354 (RTL) of mitoxantrone and fingolimod, respectively showed better pharmacological and lesser toxic properties than the other analogs of both mitoxantrone and fingolimod. The oral bioavailability and pharmacokinetic property of CID_11623444 and CID_445354 were comparatively better than their parental compound mitoxantrone and fingolimod as shown in Tables 3 and 4, respectively.

Table 5: ACD/I-Lab toxicity properties analysis of the drug-like analogs of Mitoxantrone

Chemical Molecule	AMES Test [Carcinogenic]	Endocrine disruption	Genotoxicity Hazard	Probability of effect on					
				Blood	Cardiovascular system	Gastrointestinal System	Kidney	Liver	Lungs
Mitoxantrone	Positive	Negative	Positive	0.67	0.95	0.84	0.75	0.36	0.83
CID_24901725	Positive	Negative	Positive	0.68	0.29	0.88	0.43	0.32	0.69
CID_11623444	Negative	Negative	Negative	0.64	0.72	0.79	0.26	0.17	0.5
CID_85466	Positive	Negative	Positive	0.23	0.21	0.75	0.3	0.17	0.91
CID_49839561	Negative	Positive	Negative	0.4	0.77	0.88	0.45	0.11	0.35

Table 6: ACD/I-Lab toxicity properties analysis of the drug-like analogs of Fingolimod

Chemical molecule	AMES Test [Carcinogenic]	Genotoxicity Hazard	Endocrine disruption	hERG inhibitor	Probability of effect on					
					Blood	Cardiovascular system	Gastrointestinal system	Kidney	liver	Lungs
Fingolimod	Negative	Negative	Negative	Positive	0.52	0.63	0.09	0.62	0.25	0.87
CID_3863978	Negative	Negative	Negative	Negative	0.87	0.99	1	1	0.72	0.99
CID_53245673	Negative	Negative	Negative	Negative	0.83	0.85	0.89	0.7	0.69	0.77
CID_445354	Negative	Negative	Negative	Negative	0.23	0.21	0.11	0.26	0.32	0.50
CID_843781	Negative	Negative	Negative	Negative	0.51	0.38	0.17	0.46	0.6	0.43

Table 5 and Table 6 depicts the comparative study of the toxic properties of the conventional immunomodulators namely mitoxantrone and fingolimod of MS with their corresponding analogs CID_11623444 and CID_445354. From Table 5 and Table 6, it can be predicted that both CID_11623444 and CID_445354 are comparatively non-toxic than their corresponding parental compound. The analogs CID_11623444 and CID_445354 were further evaluated for their binding affinity for S1PR1 receptors using very slow (accurate) docking module of iGEMDOCK.

3.6. PAIN-remover assay

PAINS-Remover is designed and constructed to remove the Pan Assay Interference Compounds (PAINS) from screening libraries and for their exclusion in bioassays. In this study, it was found that both the drug-like analogs CID_11623444 and CID_445354 derivatives of the standard MS drugs Mitoxantrone and Fingolimod, respectively passed the bell and Holloway filter.

3.7. Comparative docking studies

Molecular docking studies of both the parent compound Mitoxantrone and Fingolimod and the drug-like derivative molecules CID_11623444 and CID_445354 of Mitoxantrone and Fingolimod, respectively was performed against the crystal structure of an S1PR1 protein by selecting the accurate docking (very slow) option available in the docking accuracy setting of iGEMDock2.1. The interaction of a parental compound and its analogs withS1PR1 protein was estimated using total binding, van der Waals interaction (VDW) and Hydrogen bonding interaction energy. The energy profiles along with the hydrogen bond forming residues are tabulated in Tables 7 and 8).

Table 7: Binding energies of mitoxantrone and its drug-like analog docked against the crystal structure of S1PR1 protein using accurate (very slow) docking protocol of iGEMDOCK

Sl. No.	Ligand	Total Energy	VDW	H-Bond
1	Mitoxantrone	-132.478	-105.97	-16.503
2	CID_11623444	-155.558	-130.08	-25.4722

Table 8: Binding energies of Fingolimod and its drug-like analog docked against the crystal structure of S1PR1 protein using accurate (very slow) docking protocol of iGEMDOCK

Sl. No.	Ligand	Total Energy	VDW	H-Bond
1	Fingolimod	-100.265	-87.765	-12.5
2	CID_445354	-118.24	-99.4	-18.84

From the Tables 7 and 8, it is evident that the analogs CID_11623444 (-155.558 kcal/mol) and CID_445354 (118.24 kcal/mol) shows a higher total binding energy (affinity) for S1PR1 protein than their corresponding parent compounds namely mitoxantrone (-132.478 kcal/mol) and fingolimod (100.265 kcal/mol). The van der Waals and hydrogen bond interaction energy of the analogs CID_11623444 and CID_445354 were with S1PR1protein were far better than their parent compounds. Therefore both CID_11623444 and CID_445354 can be considered as better lead molecules as they interact with S1PR1protein with higher affinity and efficacy than their corresponding parent compounds.

A global view of the crystal stricture of S1PR1 protein with the seven transmembrane helical segment with analogs CID_11623444 and CID_445354is illustrated in Fig. 3 and Fig. 4, respectively. There are 7 transmembrane segments 1(44-71), 2(80-104), 3(119-141), 4(157-180), 5(201-222), 6(257-280), 7(292-314) in S1PR1 protein (Hanson et al., 2012).

3.8. Interaction analysis of drug-like analogs with S1PR1protein

The most crucial factor in protein-ligand interaction is the Van der Waals force of interaction and hydrogen bonding as they play an essential role in determining the binding efficacy and orientation of drug-like molecule to its targets receptor or protein. The interaction profile of screened drug-like derivatives of mitoxantrone and fingolimod with S1PR1 is tabulated in Table 9. The hydrogen bond and Van der Waals force of interaction between the drug-like analogs CID_11623444 and CID_445354 of mitoxantrone and fingolimod, respectively with S1PR1 protein is illustrated in Fig. 5 and Fig. 6, respectively.

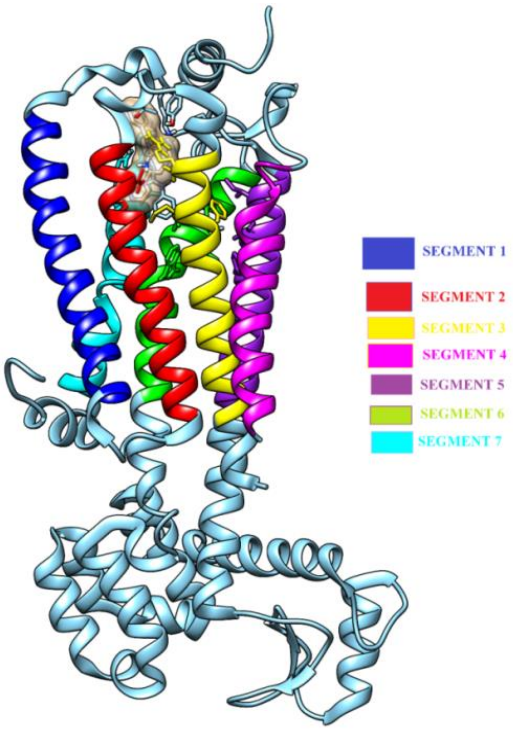

Fig. 3: Illustrate the analogs CID_11623444 bound in the transmembrane helical region of S1PR1 protein

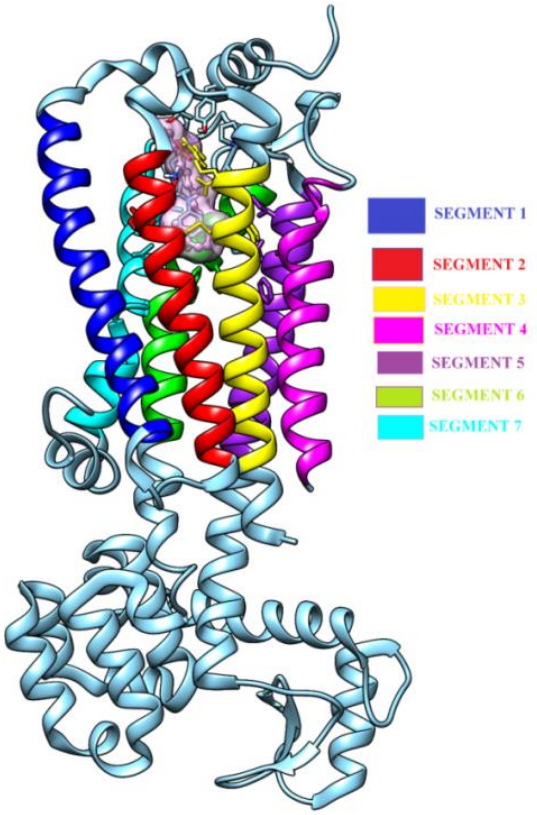

Fig. 4: Illustrate the analogs CID_445354 bound in the transmembrane helical region of S1PR1 protein

As shown in Fig. 5 the residue GLY106 of S1PR1 protein displayed strong hydrogen bonding with an oxygen atom attached to the carbonyl oxygen of CID_11623444 with a corresponding bond length of 4.2 Å. Similarly, in Fig. 6, GLY106 of the target protein forms a hydrogen bond with the hydroxyl oxygen atom of CID_445354 with a bond length of 2.78 Å. Results suggest that the carbonyl group of CID_11623444 and the hydroxyl group of the CID_445354 act as the major functional groups assisting in the hydrogen binding process of the analogs with S1PR1protein. Similar interacting group amine group and the oxygen atom of the hydroxyl group of ML5 (antagonist of S1PR1protein) are found to interact with ASN101and GLY106, respectively to form hydrogen bonding with S1PR1protein. Moreover, both the analogs were found to sit in the hydrophobic pocket of S1PR1 receptor protein with strong van der Waals force of interaction between the ligands and the hydrophobic residues (PHE125, ASN101, LEU297, MET124, LYS34, THR109) of the binding pocket derived from the binding site of ML5 (antagonist molecule) with the crystal structure of S1PR1protein (Hanson et al., 2012). Since the screened analogs show a high binding affinity for the ML5 binding domain, therefore, it can be proposed that the screened analogs will also act as an antagonist molecule and thereby inhibit the activity of the S1PR1 protein. Therefore based on the higher affinity and efficacy of the analogs for the binding domain of ML5 in S1PR1 protein it can be suggested that the drug-like analogs namely CID_11623444 and CID_445354 of mitoxantrone and fingolimod, respectively can be a useful lead antagonist molecule against the targeted S1PR1 protein.

Table 9: The Hydrogen bond and van der Waals Interacting Residues of S1PR1 receptor protein with the putative antagonist drug-like molecules CID_11623444 and CID_445354

Protein Used	Ligand	Interacting Residue (Hydrogen Bond)	Hydrogen bond interaction energy (kcal/mol)	Interacting Residue (Van der Waals)	Vander Waals interaction energy (kcal/mol)
S1PR1	CID_11623444 (Analog of Mitoxantrone)	TYR29	-5.10	TYR29	-9.9
		ARG120	-6.36	TYR98	-9.3
		GLY106	-8.01	SER105	-12.3
				ASN101	-11.5
				GLY106	-10.5
				THR109	-13.3
				TYR110	-10.1
				ARG120	-11.8
				GLU121	-10.9
				MET124	-10.9
				LEU195	-8.0
				GLU294	-7.8
				LEU297	-10.7
	CID_445354 (Analog of Fingolimod)	GLY106	-9.24	TYR29	-5.4
		THR109	-9.6	TYR98	-6.3
				ASN101	-6.4
				SER105	-7.6
				GLY106	-8.7
				THR109	-7.4
				ARG120	-6.1
				GLU121	-7.3
				MET124	-8.2
				MET124	-8.4
				PHE125	-8
				LEU195	-6.2
				TRP269	-7.3
				GLU294	-7.1
				LEU297	-6.3

3.9. In silico biological activity studies of drug-like molecule and its parent compound

PASS (Prediction of Activity Spectra for Substances) software was used to predict the biological activity of the drug-like analog molecules and their corresponding parental compound.

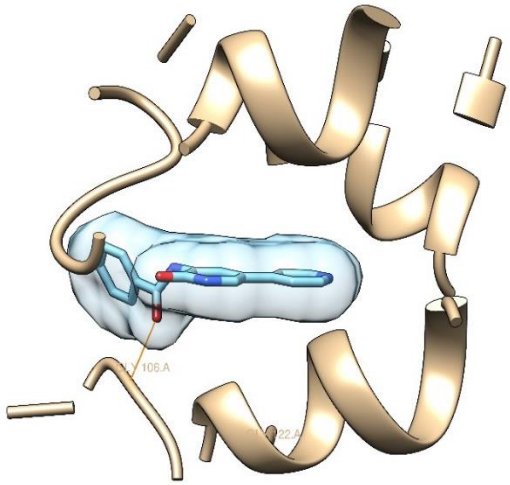

Fig. 5: Pictorial representation of the hydrogen bonded interactions of the putative antagonist molecule CID_11623444 (analog of mitoxantrone) with S1PR1 receptor protein with the blue colored line represents the hydrogen bonded interaction between the ligand and protein

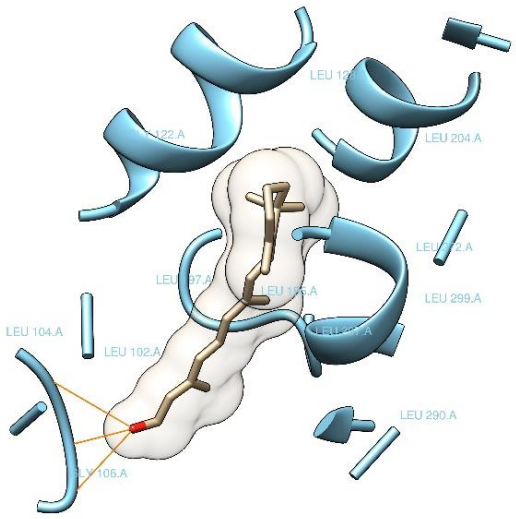

Fig. 6: Pictorial representation of the hydrogen bonded interactions of the putative antagonist molecule CID_445354 (analog of fingolimod) with S1PR1 receptor protein with the blue colored line represents the hydrogen bonded interaction between the ligand and protein

The prediction is based on the structure-activity relationships calculated from the experimental data of known compound and then compared with data of the studied compound. The PASS algorithm also estimates the probability of the studied compound to be active or inactive as depicted by Pa (probability to be active) and Pi (probability to be inactive).

The estimated biological activity of parental compound (mitoxantrone and fingolimod) and their corresponding drug-like analogs namely CID_11623444 and CID_445354 are tabulated in Tables 10 and 11. From Tables 10 and 11, it is observed that both the analogs CID_11623444 and CID_445354 and their corresponding parental structures show similar immunomodulatory activity and the probability for them to be an active inhibitor of S1PR1 protein is also more or less same. Since the analog CID_11623444 and CID_445354 are hypothesized as an inhibitor of an S1PR1 protein, therefore once attached, the ligand will inhibit the egress of activated T-Lymphocyte from the lymph node to the blood circulation and thereby prevent the target organ or tissue from the destructive inflammatory attack of activated T-lymphocytes. Therefore, it can be proposed that autoimmune disorder caused by activated T-lymphocyte namely MS and Crohn's disease (a type of inflammatory bowel disease (IBD) mediated by inflammatory T-cells) will be modulated by the administration of CID_11623444. Hence, it can be proposed that the derivatives CID_11623444 and CID_445354 of mitoxantrone and fingolimod, respectively can be used as lead molecules to inhibit the S1PR1 protein with minimum toxicity and higher specificity resulting in the modulation of autoimmune disorder resulting from the egress of activated T-lymphocyte from a lymph node.

Table 10: Prediction of the biological activity of Mitoxantrone and its derivative CID_11623444 based on the structure-activity relationship function of PASS software

Sl. No.	Ligand	Pa[1]	Pi[2]	Activity
1	CID_11623444	0.698	0.003	Immunomodulator
2	Mitoxantrone	0.384	0.069	Immunomodulator

[1]Pa: Probability of chemical compound to be active
[2]Pi: Probability of the chemical compound to be inactive

Table 11: Prediction of the biological activity of fingolimod and its derivative (CID_445354) based on the structure-activity relationship function of PASS software

Sl. No.	Ligand	Pa[1]	Pi[2]	Activity
1	CID_445354	0.748	0.003	Autoimmune Disorder Treatment
2	Fingolimod	0.719	0.004	Multiple Sclerosis treatment

[1]Pa: Probability of chemical compound to be active
[2]Pi: Probability of the chemical compound to be inactive

4. Conclusion

In our quest for identifying molecules with better therapeutic potential against MS, the current study was able to screen drug-like analogs of mitoxantrone and fingolimod with better pharmacological properties when compared to their corresponding parental compound. The toxicity and BBB studies of the drug-like analogs of mitoxantrone and fingolimod showed that CID_11623444 (L7A) and

CID_445354 (RTL) are comparatively lesser-toxic and also permeable to BBB than their parental compounds. The docking studies of analogs CID_11623444 (L7A) and CID_445354 (RTL) showed that the screened analogs could dock with S1PR1 receptor with high specificity and affinity using accurate (very slow) docking protocol of IGEMDOCK 2.1. The current docking procedure showed that CID_11623444 (L7A) and CID_445354 (RTL) could bind to the binding pocket of ML5 an antagonist of S1PR1 receptor protein of human. The docking of the screened analogs at the ML5 binding site is critical since the putative functional antagonist activity of the screened analogs may interfere with a key S1P mechanism that lymphocytes use to exit lymph nodes. Finally, the structure passed the bell and Holloway filters thereby proving that results obtained are not false positives. Thus based on our current results it can be suggested that the drug-like analogs CID_11623444 (L7A) and CID_445354 (RTL) of mitoxantrone and fingolimod, respectively will aid in interfering the lymphocyte propagation toward CNS thereby preventing the relapses associated with MS with minimum toxicity and higher efficacy. This study reveals for the first time the possible use of analogs CID_11623444 and CID_445354 for the treatment of MS. However further molecular dynamic and experimental studies need to be carried out to corroborate our results and establish the role of CID_11623444 and CID_445354 analogs molecule in the treatment of MS.

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

References

Alcorn N, Saunders S, and Madhok R (2009). Benefit-risk assessment of leflunomide. Drug Safety, 32(12): 1123-1134. https://doi.org/10.2165/11316650-000000000-00000 **PMid:19916579**

Allende ML, Dreier JL, Mandala S, and Proia RL (2004). Expression of the sphingosine 1-phosphate receptor, S1P1, on T-cells controls thymic emigration. Journal of Biological Chemistry, 279(15): 15396-15401. https://doi.org/10.1074/jbc.M314291200 **PMid:14732704**

Amin ML (2013). P-glycoprotein inhibition for optimal drug delivery. Drug Target Insights, 7: 27–34. https://doi.org/10.4137/DTI.S12519 **PMid:24023511 PMCid:PMC3762612**

Antel J (2014). Mechanisms of action of fingolimod in multiple sclerosis. Clinical and Experimental Neuroimmunology, 5(1): 49-54. https://doi.org/10.1111/cen3.12079

Bolton EE, Chen J, Kim S, Han L, He S, Shi W, and Yu B (2011). PubChem3D: A new resource for scientists. Journal of Cheminformatics, 3(1): 32-46. https://doi.org/10.1186/1758-2946-3-32 **PMid:21933373 PMCid:PMC3269824**

Bolton EE, Kim S, and Bryant SH (2011a). PubChem3D: Conformer generation. Journal of Cheminformatics, 3(1): 4-19. https://doi.org/10.1186/1758-2946-3-4 **PMid:21272340 PMCid:PMC3042967**

Bolton EE, Kim S, and Bryant SH (2011b). PubChem3D: diversity of shape. Journal of Cheminformatics, 3(1): 9-22. https://doi.org/10.1186/1758-2946-3-9 **PMid:21418625 PMCid:PMC3072936**

Bolton EE, Kim S, and Bryant SH (2011c). PubChem3D: Similar conformers. Journal of Cheminformatics, 3(1): 13-34. https://doi.org/10.1186/1758-2946-3-13 **PMid:21554721 PMCid:PMC3120778**

Borodina YV, Filimonov DA, and Poroikov VV (1996). Computer-aided prediction of prodrug activity using the PASS system. Pharmaceutical Chemistry Journal, 30(12): 760-763. https://doi.org/10.1007/BF02218831

Cortes C and Vapnik V (1995). Support-vector networks. Machine Learning, 20(3): 273-297. https://doi.org/10.1007/BF00994018

Dahlin JL, Nissink JWM, Strasser JM, Francis S, Higgins L, Zhou H, and Walters MA (2015). PAINS in the assay: Chemical mechanisms of assay interference and promiscuous enzymatic inhibition observed during a sulfhydryl-scavenging HTS. Journal of Medicinal Chemistry, 58(5): 2091-2113. https://doi.org/10.1021/jm5019093 **PMid:25634295 PMCid:PMC4360378**

Daina A and Zoete V (2016). A BOILED-Egg to predict gastrointestinal absorption and brain penetration of small molecules. ChemMedChem, 11(11): 1117-1121. https://doi.org/10.1002/cmdc.201600182 **PMid:27218427 PMCid:PMC5089604**

Daina A, Michielin O, and Zoete V (2017). SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific Reports, 7: 42717. https://doi.org/10.1038/srep42717 **PMid:28256516 PMCid:PMC5335600**

Fox EJ (2004). Mechanism of action of mitoxantrone. Neurology, 63(12 suppl 6): S15-S18. https://doi.org/10.1212/WNL.63.12_suppl_6.S15 **PMid:15623664**

Goodin DS (2014). The epidemiology of multiple sclerosis: Insights to disease pathogenesis. Handbook of Clinical Neurology, 122: 231-266. https://doi.org/10.1016/B978-0-444-52001-2.00010-8 **PMid:24507521**

Greene GL, Gilna P, Waterfield M, Baker A, Hort Y, and Shine J (1986). Sequence and expression of human estrogen receptor complementary DNA. Science, 231(4742): 1150-1154. https://doi.org/10.1126/science.3753802 **PMid:3753802**

Handel AE, Giovannoni G, Ebers GC, and Ramagopalan SV (2010). Environmental factors and their timing in adult-onset multiple sclerosis. Nature Reviews Neurology, 6(3): 156-166. https://doi.org/10.1038/nrneurol.2010.1 **PMid:20157307**

Hanson MA, Roth CB, Jo E, Griffith MT, Scott FL, Reinhart G, and Sanna MG (2012). Crystal structure of a lipid G protein–coupled receptor. Science, 335(6070): 851-855. https://doi.org/10.1126/science.1215904 **PMid:22344443 PMCid:PMC3338336**

He D, Zhang C, Zhao X, Zhang Y, Dai Q, Li Y, and Chu L (2016). Teriflunomide for multiple sclerosis. The Cochrane Library,

John Wiley and Sons, Hoboken, New Jersey, USA.
https://doi.org/10.1002/14651858.CD009882.pub3

Kasarełło K, Cudnoch-Jędrzejewska A, Członkowski A, and Mirowska-Guzel D (2017). Mechanism of action of three newly registered drugs for multiple sclerosis treatment. Pharmacological Reports, 69(4): 702-708.
https://doi.org/10.1016/j.pharep.2017.02.017
PMid:28550802

Kim S, Bolton EE, and Bryant SH (2011a). PubChem3D: Biologically relevant 3-D similarity. Journal of Cheminformatics, 3(1): 26-47.
https://doi.org/10.1186/1758-2946-3-26
PMid:21781288 PMCid:PMC3223603

Kim S, Bolton EE, and Bryant SH (2011b). PubChem3D: Shape compatibility filtering using molecular shape quadrupoles. Journal of Cheminformatics, 3(1): 25-38.
https://doi.org/10.1186/1758-2946-3-25
PMid:21774809 PMCid:PMC3158422

Kim S, Bolton EE, and Bryant SH (2012). Effects of multiple conformers per compound upon 3-D similarity search and bioassay data analysis. Journal of Cheminformatics, 4(1): 28-57.
https://doi.org/10.1186/1758-2946-4-28
PMid:23134593 PMCid:PMC3537644

Kim S, Bolton EE, and Bryant SH (2013). PubChem3D: Conformer ensemble accuracy. Journal of Cheminformatics, 5(1): 1-17.
https://doi.org/10.1186/1758-2946-5-1
PMid:23289532 PMCid:PMC3547714

Lipinski CA (2004). Lead-and drug-like compounds: The rule-of-five revolution. Drug Discovery Today: Technologies, 1(4): 337-341.
https://doi.org/10.1016/j.ddtec.2004.11.007
PMid:24981612

Louveau A, Smirnov I, Keyes TJ, Eccles JD, Rouhani SJ, Peske JD, and Harris TH (2015). Structural and functional features of central nervous system lymphatic vessels. Nature, 523(7560): 337-341.
https://doi.org/10.1038/nature14432
PMid:26030524 PMCid:PMC4506234

Lublin FD and Reingold SC (1996). Defining the clinical course of multiple sclerosis: Results of an international survey. Neurology, 46(4): 907-911.
https://doi.org/10.1212/WNL.46.4.907 **PMid:8780061**

Marriott JJ, Miyasaki JM, Gronseth G, and O'connor PW (2010). Evidence report: The efficacy and safety of mitoxantrone (Novantrone) in the treatment of multiple sclerosis report of the therapeutics and technology assessment subcommittee of the American academy of neurology. Neurology, 74(18): 1463-1470.
https://doi.org/10.1212/WNL.0b013e3181dc1ae0
PMid:20439849 PMCid:PMC2871006

Mehling M, Kappos L, and Derfuss T (2011). Fingolimod for multiple sclerosis: Mechanism of action, clinical outcomes, and future directions. Current Neurology and Neuroscience Reports, 11(5): 492-497.
https://doi.org/10.1007/s11910-011-0216-9
PMid:21789537

Miller AE (2017). Teriflunomide in multiple sclerosis: An update. Neurodegenerative Disease Management, 7(1): 9-29.
https://doi.org/10.2217/nmt-2016-0029 **PMid:27937746**

Miller DH and Leary SM (2007). Primary-progressive multiple sclerosis. The Lancet Neurology, 6(10): 903-912.
https://doi.org/10.1016/S1474-4422(07)70243-0

Mortelmans K and Zeiger E (2000). The Ames Salmonella/microsome mutagenicity assay. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 455(1): 29-60.
https://doi.org/10.1016/S0027-5107(00)00064-6

Motte J, Pitarokoili K, Bachir H, Sgodzai M, Ambrosius B, Linker R, and Gold R (2017). Immunomodulatory effects of oral dimethyl fumarate on intestinal immune regulation during experimental autoimmune neuritis in Lewis rats (P2.357). Neurology, 88(16 Supplement): P2.357.

Noseworthy JH, Lucchinetti C, Rodriguez M, and Weinshenker BG (2000). Multiple sclerosis. The New England Journal of Medicine, 343(13): 938–952.
https://doi.org/10.1056/NEJM200009283431307
PMid:11006371

Nylander A and Hafler DA (2012). Multiple sclerosis. The Journal of Clinical Investigation, 122(4): 1180–1188.
https://doi.org/10.1172/JCI58649
PMid:22466660 PMCid:PMC3314452

Orton SM, Herrera BM, Yee IM, Valdar W, Ramagopalan SV, Sadovnick AD, and Canadian Collaborative Study Group (2006). Sex ratio of multiple sclerosis in Canada: A longitudinal study. The Lancet Neurology, 5(11): 932-936.
https://doi.org/10.1016/S1474-4422(06)70581-6

Ransohoff RM (2007). Natalizumab for multiple sclerosis. New England Journal of Medicine, 356(25): 2622-2629.
https://doi.org/10.1056/NEJMct071462 **PMid:17582072**

Roach ES (2004). Is multiple sclerosis an autoimmune disorder?. Archives of Neurology, 61(10): 1615-1616.
https://doi.org/10.1001/archneur.61.10.1615
PMid:15477522

Sawcer S, Hellenthal G, Pirinen M, Spencer CC, Patsopoulos NA, Moutsianas L, and Edkins S (2011). Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. Nature, 476(7359): 214-219.
https://doi.org/10.1038/nature10251
PMid:21833088 PMCid:PMC3182531

Scott LJ and Figgitt DP (2004). Mitoxantrone. CNS Drugs, 18(6): 379-396.
https://doi.org/10.2165/00023210-200418060-00010
PMid:15089110

Sigel A, Sigel H, and Sigel RK (2007). The ubiquitous roles of cytochrome P450 proteins. Vol. 10, John Wiley and Sons, Hoboken, New Jersey, USA.
https://doi.org/10.1002/9780470028155

Walter P, Green S, Greene G, Krust A, Bornert JM, Jeltsch JM, and Waterfield M (1985). Cloning of the human estrogen receptor cDNA. Proceedings of the National Academy of Sciences, 82(23): 7889-7893.
https://doi.org/10.1073/pnas.82.23.7889 **PMid:3865204**

Yang JM (2004). Development and evaluation of a generic evolutionary method for protein–ligand docking. Journal of Computational Chemistry, 25(6): 843-857.
https://doi.org/10.1002/jcc.20013 **PMid:15011256**

Yang JM and Chen CC (2004). GEMDOCK: A generic evolutionary method for molecular docking. Proteins: Structure, Function, and Bioinformatics, 55(2): 288-304.
https://doi.org/10.1002/prot.20035 **PMid:15048822**

Yang JM, Chen YF, Shen TW, Kristal BS, and Hsu DF (2005). Consensus scoring criteria for improving enrichment in virtual screening. Journal of Chemical Information and Modeling, 45(4): 1134-1146.
https://doi.org/10.1021/ci050034w **PMid:16045308**