MOLECULAR DOCKING OF SELECTED CD22 INHIBITORS TARGETING HUMAN CD22 RECEPTOR ON B CELLS

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ABSTRACT

Background

The CD22 is a B cell restricted receptor with a critical role in the maintenance of B cell inhibition to maintain humoral immunity homeostasis. The inhibitory function of CD22 and its specific expression on B cells makes it an attractive target for B cell depletion in autoimmune diseases and B cell derived malignancies.

Objectives

Determine the potential affinity for binding of fifteen commercially available CD22 inhibitors targeting CD22 protein was investigated using iGemdock software.

Methods

In the present study, the binding affinities of fifteen commercially available CD22 inhibitors have been investigated on CD22 protein using iGemdock software.

Results

The results showed that CD22 inhibitor, Thapsigargin produced greater affinity for the CD22 protein with the first rank. It binds with the CD22 protein with lowest interaction energy (fitness value) of -75.465 kcal/mol.

Conclusion

The interaction confirms that the studied inhibitors interacted with CD22 protein by building hydrogen bonds with active site residues in addition to the hydrophobic interactions. Further *in vitro* studies are required to confirm these results.

Keywords: *CD22 inhibitors; Cell Signalling; Docking; iGemdock software.*

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INTRODUCTION

CD22 or sialic acid-binding immunoglobulin-like lectin 2 (siglec-2) is a cell surface receptor whose expression is restricted specifically to B cells [1]. CD22 is a B cell receptor (BCR) co-receptor that functions to maintain a baseline level of B cell inhibition and therefore has role in the maintenance of B cell homeostasis in humoral immunity [2]. Molecular characterisation has shown that CD22 comprises a single-spanning membrane glycoprotein of ~140 kDa organised in membrane clusters on the surface of B cells [3]. The activation of CD22 is mediated through recruitment of intracellular phosphatases SHP-1 and Grb-2 into the immunoreceptor tyrosine-based inhibitory motifs (ITIMs) within their cytoplasmic tail to bind to phosphorylated tyrosines of its intracellular domain [1]. CD22 constitutively undergoes recycling and internalisation with kinetics ($t½$) of less than one hour through clathrin-mediated endocytosis mechanism [4].

It is well known that B cells have role in the pathogenesis of autoimmune diseases by producing autoantibodies leading to deposition of immune complexes in several organs and also B cell derived malignancies [1]. The restricted pattern of CD22 expression on mature B cells, their rapid endocytosis following engagement by monoclonal antibodies and their ability to maintain a baseline level of B cell inhibition makes CD22 an attractive therapeutic target. Therefore, therapeutic antibodies such as epratuzumab or antibody-based therapeutics can be used to target dysfunctional B cells through CD22 [5]. However, antibodies can lead to unwanted reactions due to activation of the complement proteins or Fc receptors. In addition, these antibodies are usually very expensive therapies [6].

By targeting key signalling pathways, the biology of B cells can be modulated. In the view of the fact that CD22 functions to maintain a baseline level of B cell inhibition, therefore this receptor can be exploited for targeting in pathological setting such as B cell mediated autoimmune diseases and B cell leukaemias and lymphomas. Commercially available CD22 inhibitors targeting CD22 protein were selected. Basic facts about these compounds are emphasised briefly in the following section.

AS 2444697 (*N*-[3-Aminocarbonyl)-1-(tetrahydro-2*H*-pyran-4-yl)-1*H*-pyrazol-4-yl]-2-(2-methyl-4-pyridinyl)-4-oxazolecarboxamide hydrochloride) is a potent and selective interleukin 1 receptor-associated kinase 4 (IRAK4) inhibitor. IRAK4 is a serine/threonine kinase and plays a key role in both inflammation and cancer. It inhibits lipopolysaccharide (LPS)-induced TNF-α and interleukin 6 (IL 6) production in peripheral blood mononuclear cells (PBMCs) *in vitro*. In disease models, it has been shown to be renoprotective and anti-inflammatory especially in a rodent model of chronic kidney disease [7]. Siguazodan (N-Cyano-*N*'-methyl-*N*''-[4-(1,4,5,6-tetrahydro-4-methyl-6-oxo-3-pyridazinyl)phenyl]guanidine) is a selective phosphodiesterase inhibitor with IC_{50} = 117 nM [8]. Cyclapolin 9 (IUPAC name: 7-Nitro-5-(trifluoromethyl)-2-benzothiazolecarboxamide-3-oxide) is a selective, ATP-competitive polo-like kinase 1 (PLK1) inhibitor with IC_{50} = 500 nM [9]. Febuxostat (IUPAC name: 2-[3-Cyano-4-(2-methylpropoxy) phenyl]-4-methyl-5-thiazolecarboxylic acid) is a non-purine inhibitor of xanthine oxidase with Ki = 1.2 nM. It binds to a channel leading to the enzyme active site and hence obstructs substrate binding [10]. Miglitol (IUPAC name: (2*R*,3*R*,4*R*,5*S*)-1-(2-Hydroxyethyl)-2-(2-hydroxymethyl)-3,4,5-piperidinetriol) is an inhibitor of α glucosidase and hence has an antihyperglycaemic effect. It suppresses postprandial hyperglycaemia *in vivo* and reduces plasma glucose concentration in normal rats and in several animal models of diabetes. It has also been shown to inhibit the loss of pancreatic β cells in type 2 diabetic rats. Also inhibits apoptosis and mitochondrial overproduction of reactive oxygen species (ROS) in endothelial cells *in vitro* [11]. Another compound is UNC 3230 (IUPAC name: 5-[(Cyclohexylcarbonyl)amino]-2-(phenylamino)-thiazolecarboxamide), which is a potent and selective PIP5K1C inhibitor with IC_{50} = 41 nM. It exhibits selectivity for PIP5K1C over PIP5K1A, the PI3 kinase family and reduces PIP levels and LPA-induced calcium signalling in dorsal root ganglia (DRG) neurons *in vitro*. It has also been shown to reduce nociception in mouse models of chronic pain [12]. Celecoxib (IUPAC name: 4-[5-(4-Methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]-benzenesulfonamide) is a selective cyclooxygenase 2 (COX2) inhibitor with IC_{50} = 0.04 μM. It has an anti-inflammatory effect and displays chemopreventive activity in *in vivo* tumour models [13]. ML 351 (IUPAC name: 5-(Methylamino)-2-(1-naphthalenyl)-4-oxazolecarbonitrile) is a selective 12/15 LOX inhibitor with IC_{50} = 200 nM. It exhibits selectivity for human COX-2 and protects against oxidative glutamate toxicity in HT-22 mouse neuronal cells and reduces infarct size in a mouse ischaemic stroke model [14]. Leflunomide (IUPAC name: 5-Methyl-*N*-[4-(trifluoromethyl)phenyl]-4-isoxazolecarboxamide) is a

dihydroorotate dehydrogenase inhibitor with IC_{50} = 2.5 µM. It inhibits *de novo* pyrimidine synthesis in human T cells *in vitro* and also inhibits lymphocyte proliferation. Leflunomide has been shown to exhibit efficacy in several animal models of autoimmune disease, arthritis and graft rejection [15]. T 5601640 (IUPAC name: 3-Methyl-*N*-[3-[[[3-(trifluoromethyl)phenyl] amino]carbonyl]phenyl]-5-isoxazolecarboxamide) is a selective LIM kinase 2 (LIMK2) inhibitor and it inhibits cofilin phosphorylation in cells that overexpress LIMK2 but not LIMK1. It attenuates the growth of several cancer cell lines and reduces phospho-cofilin levels and Panc-1 tumour size in a mouse xenograft model [16]. ITE (IUPAC name: 2-(1*H*-Indol-3-ylcarbonyl)-4-thiazolecarboxylic acid methyl ester) is an endogenous aryl hydrocarbon receptor (AhR) agonist with K_i = 3 nM. It decreases Oct4 levels in U87 glioblastoma cells and induces stem-like cancer cell differentiation in U87 tumour spheres and inhibits ovarian cancer cell proliferation *in vitro*. It also suppresses tumour growth in U87 and OVCAR-3 cell xenografts in mice and also inhibits TGF-β-induced human myofibroblast differentiation [17-20]. Miglustat hydrochloride (IUPAC name: (2*R*,3*R*,4*R*,5*S*)-1-Butyl-2-(hydroxymethyl)-3,4,5-piperidinetriol hydrochloride) is an orally active α glucosidase I and II and ceramide-specific glycosyltransferase inhibitor. It rescues trafficking-deficient F508del-CFTR in human airway epithelial cells via inhibition of ER α glucosidases I and II [21, 22]. CP 690550 citrate (IUPAC name: (3*R*,4*R*)-4-Methyl-3-(methyl-7*H*-pyrrolo[2,3-d]pyrimidin-4-ylamino)-β-oxo-1-piperidinepropanenitrile citrate) is a potent JAK inhibitor with IC_{50} values = 1, 20 and 112 nM for JAK3, JAK2 and JAK1 respectively. It is an orally active immunosuppressant and exhibits efficacy in rodent rheumatoid arthritis models [23]. Dasatinib (IUPAC name: *N*-(2-Chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide) is a highly potent pan-Src/Bcr-Abl inhibitor with K_i values = 16 and 30 pM, respectively. It inhibits Bcr-Abl, Src, Lck, Fyn, c-kit and Yes with IC_{50} values in the subnanomolar range. It inhibits proliferation of tumour cells *in vitro* and exhibits anticancer acivity *in vivo* in a mouse chronic myelogenous leukaemia (CML) xenograft model [24]. Thapsigargin (IUPAC name: (3*S*,3a*R*,4*S*,6*S*,6A*R*,7*S*,8*S*,9b*S*)-6- (Acetyloxy)-2,3,3a,4,5,6,6a,7,8,9b-decahydro-3,3a-dihydroxy-3,6,9-trimethyl-8-[[(2*Z*)-2-methyl-1-oxo-2-butenyl] oxy]-2-oxo-4-(1-oxobutoxy)azuleno[4,5-*b*]furan-7-yl octanoate) is a potent inhibitor of sarcoendoplasmic reticulum Ca^{2+}-ATPases and causes ER stress. It can be used to induce autophagy in mammalian cells [25, 26].

In the view of the fact that key signalling pathways can modulate B cell biology in particular by engagement of CD22 receptor, therefore the aim of this study was to investigate the most fitting of commercially available CD22 inhibitors targeting CD22 protein using iGemdock software.

COMPUTATIONAL METHODS

The protein file of the X-ray crystalline structure of human CD22 receptor was obtained from RCSB protein data bank (https://www.rcsb.org/) (PDB Code: 5VKJ) [3]. A total of fifteen CD22 inhibitors (Table 1) were investigated in this study. The three-dimensional (3D) structures of all commercially available CD22 inhibitors (Figure 1) were obtained and downloaded from (https://pubchem.ncbi.nlm.nih.gov/) [27]. Discovery Studio software 4.1 (http://accerys.com) [28] was used to generate the 3D structures in (.pdb) file format.

The computational molecular docking method was employed for screening of human CD22 inhibitors targeting the human CD22 protein active site. The molecular docking was performed by iGemdock v2.1 software [29]. The stable docking of iGemdock was selected for performing the computational docking. The following parameters were selected for docking including Population size: 300, Number of generations: 80 and Number of solutions: 10. The CD22 inhibitors were sorted at the end of docking process based on their interaction energies and fitness values produced by the docking using iGemdock software. The most stable conformation and the best poses of the inhibitors were selected based on the lowest fitness value. Finally, the inhibitors were analysed at the active site of the CD22 protein using Discovery Studio 4.1 software (http://accerys.com) [28].

Analysis with iGemdock software

The docking process involves two basic steps: prediction of the ligand conformation as well as its position and orientation within these sites (usually referred to as *pose*) and assessment of the binding affinity.

iGemdock computes a ligand conformation and orientation relative to the binding site of target protein based on a generic selection method. iGemdcok is a suite of automated docking/screening tools. The

interface of iGemdock has two main tags, docking/screening tag and post-analysing tag. The architecture of iGemdock consists of four major modules. The docking/screening and post-analysing modules contain several components to make the screening/analysing procedure smoothly. The predicted or clustered protein-ligand complexes can be visualised in the visualisation module. The parallel processing module provides the parallel computation of screening jobs.

To start a docking/screening job, two files have to be prepared which are protein structure and ligand files.

The protein structure file can be obtained from Protein Data Bank (http://www.rcsb.org/). The ligand files are available from online compound databases such as ZINC (http://zinc.docking.org/) or PubChem (http://www.ncbi.nlm.nih.gov/sites/entrez?db=pccompound).

Binding site preparation

The acceptable receptor file in iGemdock can be a user defined binding site or a whole protein structure. The protein structure may include a ligand and iGemdock can help define the binding site.

Ligand preparation

Before docking a ligand, a 3D ligand file need to be generated or alternatively a file can be used from set of ligand library has already been prepared

Table 1. List of commercially available human CD 22 Inhibitors.

No.	Product Name with their Molecular Formula (Ref. provided)	Chemical Abstracts Service (CAS) No.	Molecular Weight (g/mol)	Inhibitory Concentration (IC_{50})* or Inhibitory Constant (K_i)**
1	Interleukin 1 receptor-associated kinase 4 (IRAK4) inhibitor AS 2444697 (30) $C_{19}H_{20}N_6O_4.HCl.H_2O$	1287665-60-4	450.88	IC_{50} = 21 nM
2	Selective PDE3 phosphodiesterase inhibitor Siguazodan (31) $C_{14}H_{16}N_6O$	115344-47-3	284.32	IC_{50} = 117 nM
3	Selective, ATP-competitive polo-like kinase 1 (PLK1) inhibitor Cyclapolin 9 (32) $C_9H_4F_3N_3O_4S$	40533-25-3	307.21	IC_{50} = 500 nM
4	Non-purine inhibitor of xanthine oxidase Febuxostat (33) $C_{16}H_{16}N_2O_3S$	144060-53-7	316.37	K_i =1.2 nM
5	Antihyperglycemic α glucosidase inhibitor Miglitol (34) $C_8H_{17}NO_5$	72432-03-2	207.22	IC_{50} = 0.35 – 1.2 μM
6	Selective PIP5K1C inhibitor UNC 3230 (12) $C_{17}H_{20}N_4O_2S$	1031602-63-7	344.43	IC_{50} = 41 nM
7	Selective cyclooxygenase 2 (COX 2) inhibitor Celecoxib (35) $C_{17}H_{14}F_3N_3O_2S$	169590-42-5	381.37	IC_{50} = 0.04 μM
8	Selective 12/15 LOX inhibitor ML 351 (14) $C_{15}H_{11}N_3O$	847163-28-4	249.27	IC_{50} = 200 nM
9	Dihydroorotate dehydrogenase inhibitor Leflunomide (36) $C_{12}H_9F_3N_2O_2$	75706-12-6	270.21	IC_{50} = 2.5 μM
10	Selective LIM kinase 2 (LIMK2) inhibitor T 5601640 (37) $C_{19}H_{14}F_3N_3O_3$	924473-59-6	389.33	IC_{50} = 35.2 μM
11	Aryl hydrocarbon receptor (AhR) agonist ITE (17) $C_{14}H_{10}N_2O_3S$	448906-42-1	286.3	K_i = 3 nM
12	α glucosidase I and II and ceramide-specific glycosyltransferase inhibitor Miglustat hydrochloride (38) $C_{10}H_{21}NO_4.HCl$	210110-90-0	255.74	IC_{50} = 0.14 μM
13	JAK inhibitor CP 690550 citrate (39) $C_{16}H_{20}N_6O.C_6H_8O_7$	540737-29-9	504.49	IC_{50} = 1, 20 and 112 for JAK3, JAK2 and JAK1
14	Pan Src/BCR-ABL inhibitor Dasatinib (40) $C_{22}H_{26}ClN_7O_2S$	302962-49-8	488.01	IC_{50} = subnanomolar range K_i =16 and 30 pM
15	Sarcoendoplasmic reticulum Ca^{2+}-ATPases inhibitor Thapsigargin (41) $C_{34}H_{50}O_{12}$	67526-95-8	650.76	IC_{50} = 0.448 nM

*** The inhibitory Concentration (IC_{50}) is the concentration of drug required for 50% inhibition.**

**** Inhibitory Constant (K_i) is an indication of how potent an inhibitor is, it is concentration required to produce half maximum inhibition.**

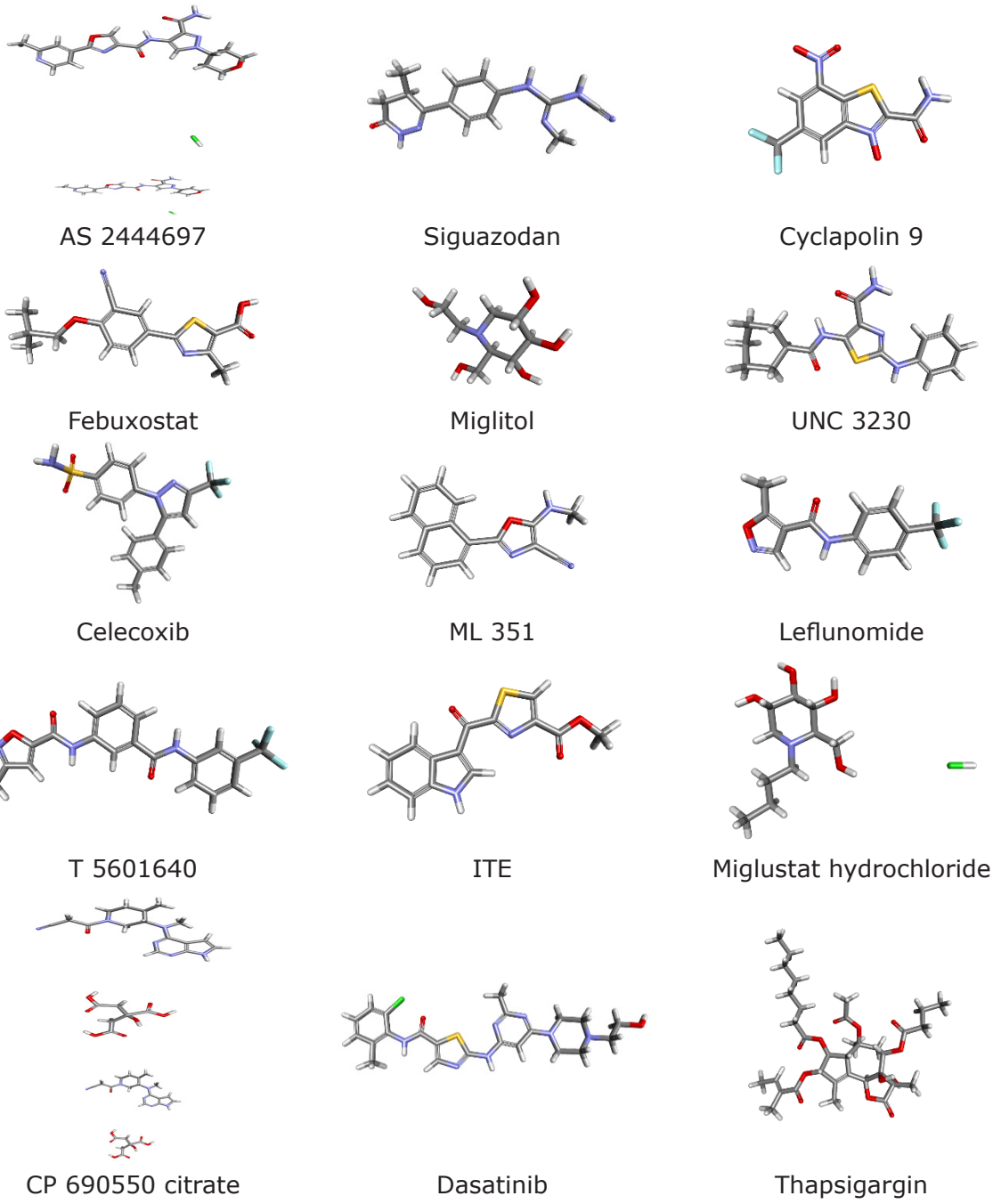

Figure 1. Diagrams showing 3D structures of studied human CD22 inhibitors.

RESULTS

Following generation of a successful docking protocol, all of the CD22 inhibitors included in the present study were well docked into the active site of the human CD22 protein via iGemdock. Docking studies predicted the potency of interactions between studied inhibitors and CD22 protein residues. The requirements of these interactions were a suitable orientation and location of the inhibitors that fitted to the CD 22 binding sites to form protein-ligand complexes. Selectivity of the studied CD22 inhibitors was certified earlier by Tocris Bioscience (www.tocris.com). Therefore, best interactions and the lowest energy fitness scores were used as standards to understand the best conformation among the 15 studied inhibitors generated by iGemdock software.

The results have shown that Thapsigargin had greater affinity for the CD22 protein at the first rank. It binds with the CD22 protein with lowest interaction energy (fitness value) of -75.465 kcal/mol. The distribution of interaction energies (fitness values) produced after docking of the CD22 inhibitors sorted from lowest energy to highest one is shown in Table 2.

Docking results showed that CD22 inhibitors; Thapsigargin, AS 2444697 and Dasatinib give fitness values (lowest energy) scores of -75.465, -69.887 and 66.159 kcal/mol, respectively.

The best fitness value was obtained when Thapsigargin docked into CD22 active site (Figure 2) as it formed hydrogen bond interactions with Asparagine 101 (Asn101), hydrophobic and pi-pi interaction with Tryptophan 41 (Trp41).

The next best fitness was obtained with AS 2444697 when docked into CD22 active site (Figure 3) as it formed hydrogen bond interactions with Asn101 and Phenylalanine 94 (Phe94), hydrophobic interaction with Phe94, Leucine 95 (Leu95), Pro43 and Isoleucine 195 (Ile195).

The third fitness among the studied inhibitors was obtained with Dasatinib when docked into CD22 active site (Figure 4) as it formed hydrogen bond interactions with Asn101 and Phe94, hydrophobic interaction with Phe94, Leu95, Pro43 and Ile195.

Table 2. Fitness value (kcal/mol) for human CD22 inhibitors docked to CD22 protein.

No.	Human CD22 Inhibitors	Fitness value (kcal/mol)
1	**Thapsigargin**	-75.465
2	**AS 2444697**	-69.8879
3	**Dasatinib**	-66.1589
4	**Leflunomide**	-60.6073
5	**ITE**	-58.3017
6	**ML 351**	-56.9783
7	**Siguazodan**	-56.2543
8	**Cyclapolin 9**	-55.7146
9	**Miglustat hydrochloride**	-54.0461
10	**Miglitol**	-47.1042
11	**Celecoxib**	-15.5705
12	**Febuxostat**	-10.1323
13	**UNC 3230**	24.8007
14	**T 5601640**	111.13
15	**CP 690550 citrate**	186.61

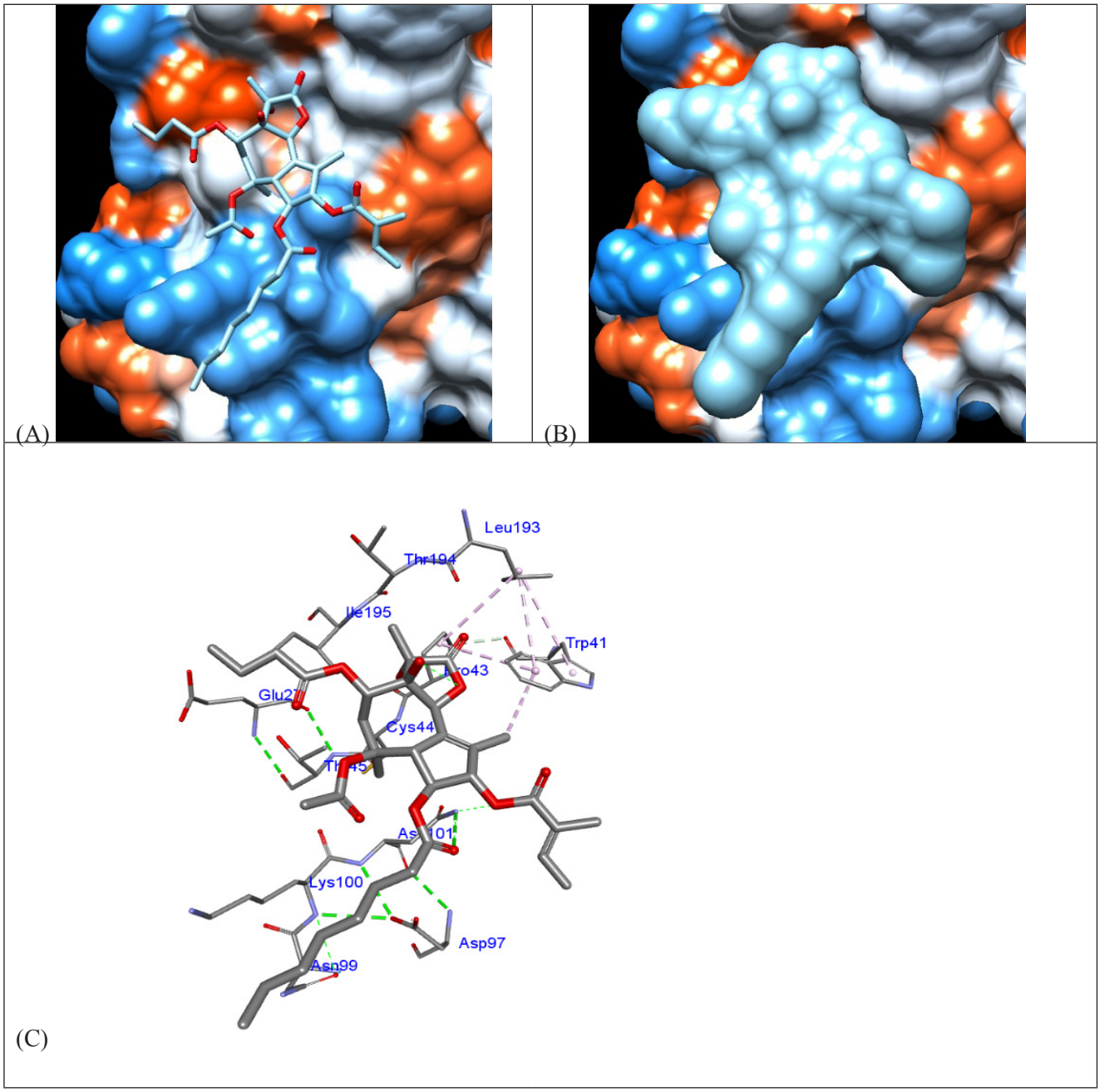

Figure 2. Molecular characteristics of the specific interactions of the inhibitor Thapsigargin within the best predicted conformational model of the active site of CD22 protein (A) and (B); (C) 3D configuration showing interaction between the residues of CD22 protein with Thapsigargin. The compound Thapsigargin has formed H bonds with Asn101, hydrophobic and pi-pi interactions with Trp41 in the active site of CD22 protein.

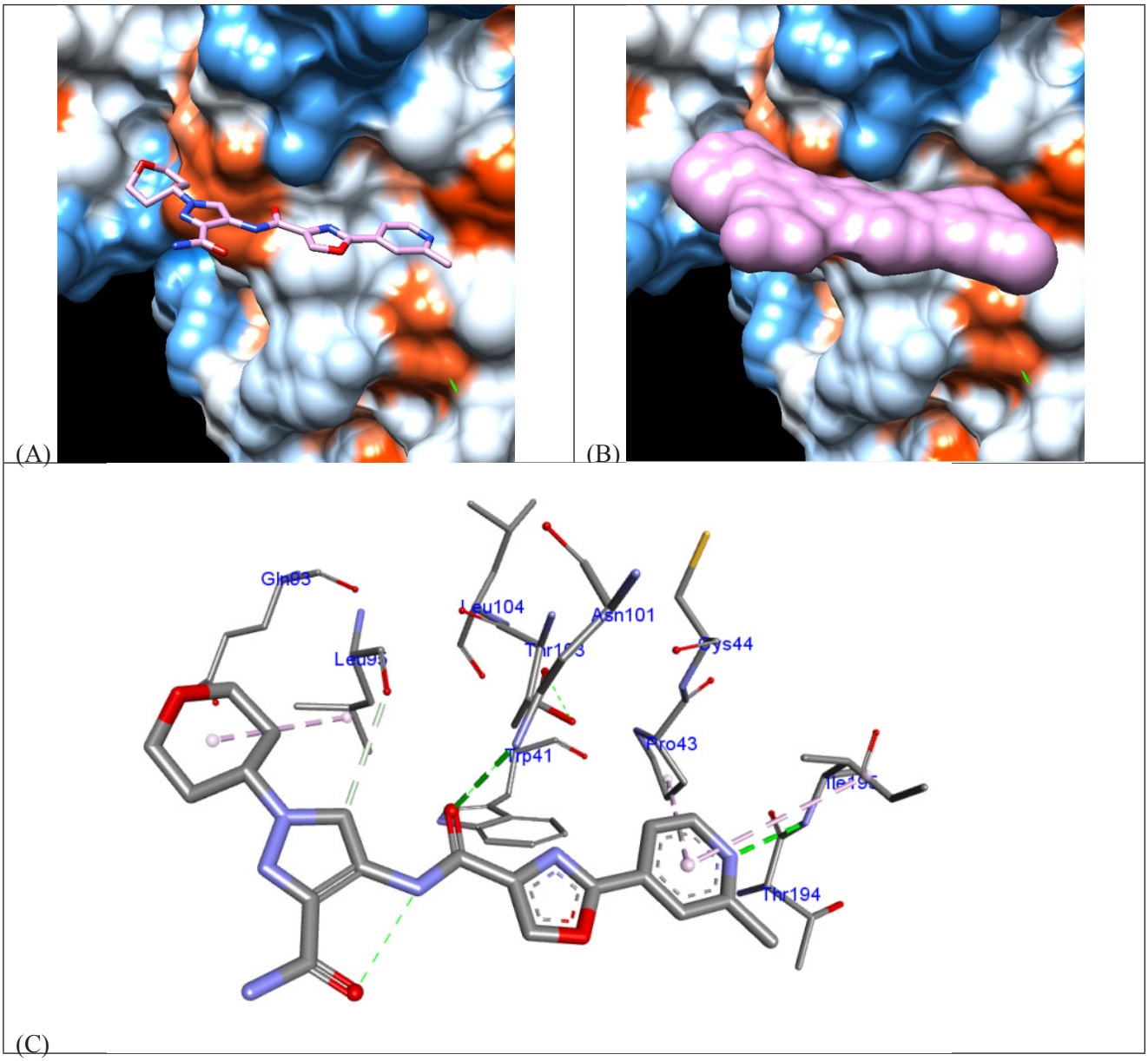

Figure 3. Molecular characteristics of the specific interactions of the inhibitor AS 2444697 within the best predicted conformational model of the active site of CD22 protein (A) and (B); (C) 3D configuration showing interaction between the residues of CD22 protein with AS 2444697. The compound AS 2444697 has formed H bonds with Asn101 and Phe94, hydrophobic interaction with Phe94, Leu95, Pro43 and Ile195.

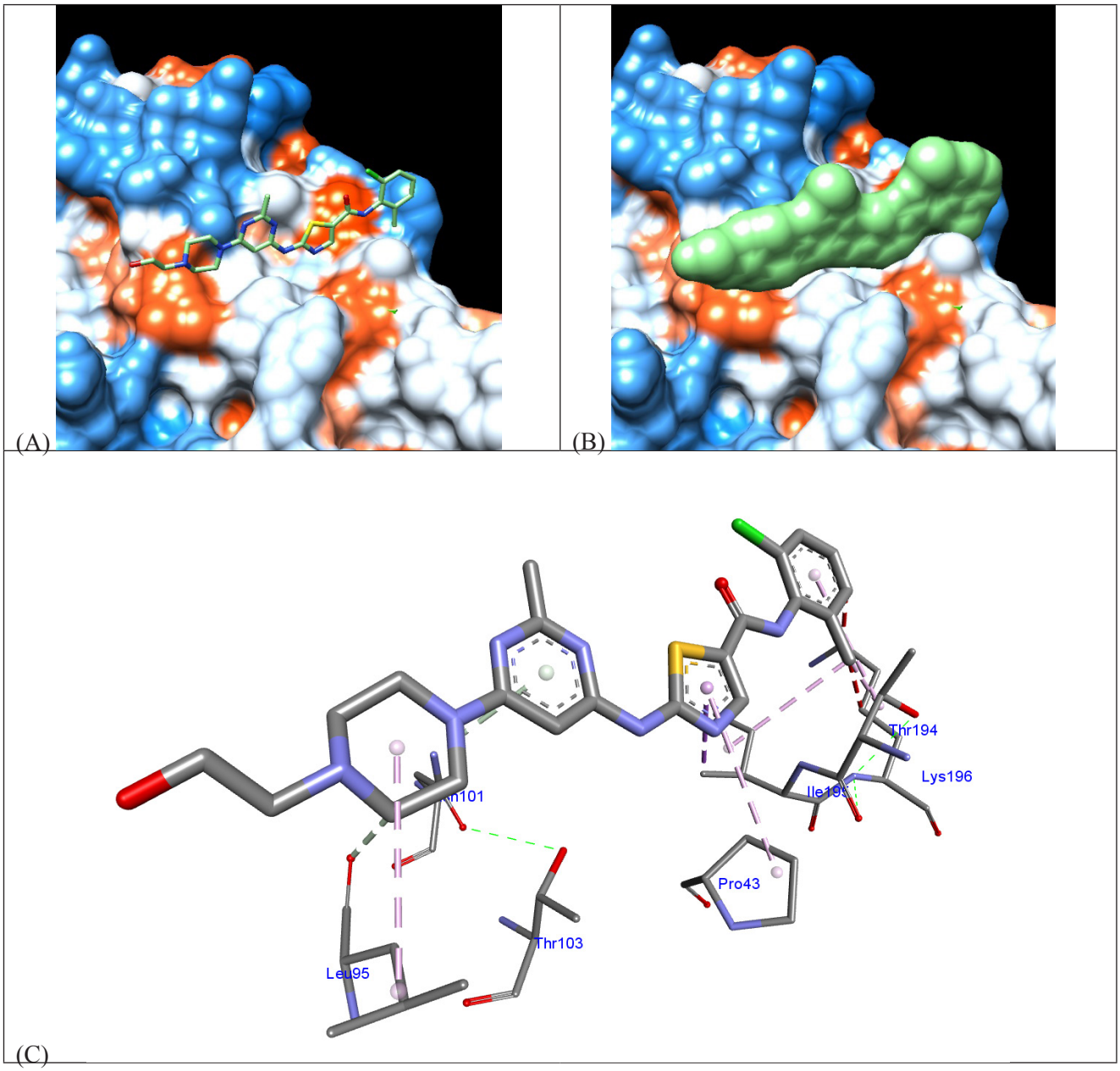

Figure 4: Molecular characteristics of the specific interactions of the inhibitor Dasatinib within the best predicted conformational model of the active site of CD22 protein (A) and (B); (C) 3D configuration showing interaction between the residues of CD22 protein active site with Dasatinib. Dasatinib has formed H bonds with Asn101 and Phe94, hydrophobic interaction with Phe94, Leu95, Pro43 and Ile195.

DISCUSSION

Structure-based virtual screening and post-screening analysis are emergent tasks in computer-based drug discovery. Combining these two methods to effectively reduce the false positives from a large compound database is considered as a key step to finding the lead compounds.

The molecular docking approach can be used to model the interaction between a small molecule and a protein at the atomic level, which allow us to characterise the behavior of small molecules in the binding site of target proteins as well as to elucidate fundamental biochemical processes.

In this study, results obtained from docking simulation have predicted the interactions of CD22 inhibitors examined with CD22 protein residues involved in the complex formation. In these interactions, the most important requirements were a proper orientation and conformation fitting between the extracellular domain of CD22 binding site and the formed protein-ligand complex. Therefore, conducting molecular characterisation is necessary to have a better understanding for dynamic behaviors and stability of predicted complexes. In this regard the iGemdock software was used and helped in understanding possible docking interactions of inhibitors with CD22.

CD22 is a B cell restricted membrane glycoprotein associated with the BCR and negatively regulates BCR signalling by modulation of B cell activation upon BCR cross-linking with an antigen (1). The exclusive pattern of CD22 expression only on B cells and its constitutive internalisation allows for the development of targeted therapeutics and delivery systems based on inhibitors or antibody-drug conjugates to induce tolerance or anergy or to deplete dysregulated B cells to treat autoimmune diseases and B cell derived leukaemias [5].

The possibility to exploit CD22 as a target for the therapy of B cell malignancies has been investigated in clinical trials based on CD22-targeting immunotoxins (BL22 and HA22) [42, 43]. However, maximal efficacy with these therapeutic monoclonal antibodies could be obtained when CD22 has undergone modification so that the epitope of CD22 was positioned in a close proximity to the B cell membrane. Furthermore, there are concerns with regard to the use of antibodies such as the immunogenicity of mouse derived and humanised anti-CD22 antibodies in addition to the high expenses of their production. Therefore, numerous therapeutic approaches in development harness B cell inhibition through CD22 by exploiting small molecule CD22 inhibitors. Thus, I have selected and tested fifteen different CD22 inhibitors to assess the binding efficacy of these inhibitors to the extracellular domain of CD22 receptor.

Among all the 15 inhibitors used in this study, only Thapsigargin, AS 2444697 and Dasatinib where further emphasised because results obtained reveal that these inhibitors are more likely to form a stronger complex with CD22 protein.

According to the docking results obtained for the CD22 inhibitors in the present study, Thapsigargin, AS 2444697 and Dasatinib were among the best studied CD22 inhibitor compounds targeting CD22 protein, respectively. Thapsigargin is the principle active compound extracted from *Thapsia garganic* plant, which has been shown to provokes apoptosis by inhibiting the sarcoendoplasmic reticulum Ca^{2+} ATPase (SERCA) in cells [44].

Results from a previous study has shown that Thapsigargin can block cell proliferation and significantly induced apoptosis in cultured human cells in a time- and dose-dependent manner [45]. Furthermore, another study has shown that Thapsigargin-mediated endoplasmic reticulum (ER) stress effectively sensitises cancer cells to TRAIL-mediated apoptosis [46]. Collectively, these studies support the fact that Thapsigargin could be a potent inhibitor and an ideal inducer of apoptosis in CD22 positive B cell mediated diseases.

Evidence obtained from the molecular docking results in the present study specifically determined that the best fitness value obtained for Thapsigargin interaction with the extracellular domains of CD22 active site is through formation of hydrogen bond with Asn101, hydrophobic and pi-pi interactions with Trp41.

Pre-clinical studies in mice model of disease has shown that administration of AS2444697 resulted in a dose-dependent and significant improvement of inflammatory disease. Additionally, AS2444697 attenuated plasma levels of pro-inflammatory cytokines including IL-6; plasma levels of endothelial dysfunction markers including intercellular adhesion molecule-1 (ICAM-1) and plasma levels of oxidative stress markers [7, 47]. These results suggest that AS2444697 attenuates disease progression mainly via anti-inflammatory mechanisms through inhibition

of IRAK-4 activity and may represent a promising therapeutic option for treatment. IRAK-4 is a pivotal molecule for IL-1 receptor- and Toll-like receptor-induced activation of pro-inflammatory mediators.

From a literature search, few studies have addressed the effect of Dasatinib on normal blood cells especially *in vivo*. Dasatinib is an inhibitor of s B cell receptor Abelson murine leukaemia viral oncogene homologue 1 (ABL1), Src and other tyrosine kinases. Dasatinib was shown to induce the inhibition of the early events of B and T cell receptor signalling. Furthermore, Dasatinib was shown to be a more prominent inhibitor of both basal and B cell receptor-induced activity of Bruton's tyrosine kinase and PLCγ2. Targeting dysregulated kinases involved in B cell antigen receptor (BCR) signalling is an attractive therapeutic approach. The Src/c-Abl tyrosine kinase inhibitor Dasatinib on BCR signal transduction in chronic lymphocytic leukaemia (CLL) cells has been studied and shown to be effective in the treatment of these patients. Furthermore, Dasatinib was shown to induce rapid mobilisation and activation of cytotoxic lymphocytes, which may enhance anti-leukaemia immune responses [48-50].

Therefore, this study provides a clue about the nature of the molecular interaction between selected inhibitors and CD22 receptor and characterisation on where and how small molecule inhibitors could specifically bind with high affinity to the extracellular domains of CD22 receptor.

Future *in vitro* studies are required to confirm these results. It is also important to perform Molecular Dynamic Simulations to have exact information regarding the dynamic behaviors of the complexes.

In conclusion, the results from the present study showed that Thapsigargin inhibitor is the best compound among the studied CD22 inhibitors targeting CD22 protein. The interaction confirmed that the studied inhibitors interacted with CD22 protein by building hydrogen bonds with active site residues in addition to the hydrophobic interactions.

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Conflict of Interest

There is no conflict of interest.

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