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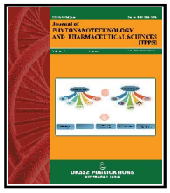

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Discovery of toll-like receptors 7 (TLR7) antagonists to minimise the risk of COVID infection in rheumatoid arthritis *via* virtual screening approaches

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Abstract

Understanding COVID-19 pathophysiology in rheumatoid arthritis is crucial for a better understanding of the disease and the development of more effective treatments. TLR7 recognises pathogenic single-stranded RNAs (ssRNAs) and plays a crucial role in the innate immune response to viral infections and auto-immune disorders. Recent evidence has suggested that selective, specific antagonists for TLR7 might be more beneficial in certain diseases, such as rheumatoid arthritis (RA). Thus, the use of novel small molecule TLR7 inhibitors with a larger safety window and differentiated selectivity may potentially have significant clinical utility in COVID infection with RA condition. Herein, we review efforts to develop novel TLR7 antagonists. According to current findings, repurposing existing available compounds will result in more effective functioning than using newly designed medications. Based on this fact, we used natural compounds, and a computational study has been conducted. This comprises the screening of these drugs' binding affinity with TLR7, which is upregulated in COVID infection as well as the RA condition. The results show that the top six scores achieved by toll-like receptors (PDB ID: 6LW1) are, *viz*., -13.8036, -13.4501, -13.3095, -12.5435, -11.4339, and -10.5996, for ZINC08635349, ZINC12602116, ZINC08635473, ZINC08635326, ZINC08635431, and ZINC20112269, Thus, the compounds discovered through the use of various softwares can possibly be used for the management of rheumatoid arthritis during and after COVID infection. Hence, we can conclude that these molecules show good activity in reducing the activity of TLR7.

1. Introduction

New and pathogenic strains of coronaviruses of the family Coronaviridae (MERS-CoV, SARS-CoV) have emerged with high fatality rates, resulting in acute respiratory distress syndrome, reduced lung function, arrhythmia, and eventually death. The human angiotensin-converting enzyme 2 (ACE2) is identified as the host cell-surface receptor for the envelope spike glycoprotein of SARS-CoV-2, facilitating its entry and infection in the host cell (Hoffmann *et al*., 2020). The ACE2 is a cell membrane receptor expressed on a number of different types of cells, including the cells of the GI tract, blood vessels, lung AT2 alveolar epithelial cells, and others. The interaction of ACE2 with the SARS-CoV-2 spike protein results in ACE2 downregulation, an increased production of angiotensin II, and a subsequent activation of type 1 a angiotensin II receptor (AT1RA) that increases pulmonary vascular permeability, thereby increasing lung damage (Zhao *et al.*, 2020). The COVID-19 is also detrimental to the debilitating population with autoimmune rheumatic diseases that are either at the higher risks of infection leading to disease severity or may suffer due to the induced effects of the immune-suppressive agents like the disease-modifying antirheumatic drugs.

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Rheumatoid arthritis (RA) is one of the most prevalent and debilitating autoimmune diseases, characterised by the inflammation of the synovium (Firestein, 2003). This disease condition is marked by the presence of a dysfunctional immune system that recognises self-antigens as foreign. The preclinical phase is said to be characterised by the generation of autoantibodies, and the clinical phase is attained when the body reacts to these autoantibodies, leading to inflammation. The inflammatory condition is driven by the cytokines that are upregulated during the disease condition (Vukmanovic-Stejic *et al.*, 2000). The similarities in the cytokine profile, lymphocyte population characteristics, and inflammatory mediators interestingly present an intricate relationship between COVID-19 and RA. We, thus have tried to find potential reasons for the coexistence of COVID-19 and RA as well as the consequences of the COVID-19 pandemic on the increase in the population of people with RA (Figure 1) worldwide.

TLR7, an endosomal receptor for ssRNA, is present on the endosomal membrane (Heil *et al.*, 2004). It can differentiate self-RNA from non-self-viral ssRNAs due to the presence of a higher number of modified nucleotide bases in cellular RNA (Karik´o *et al.*, 2005). TLR7 is predominantly expressed by RA synovial macrophages and by RA synovial fibroblast cells. Expression of TLR7 is also upregulated in RA monocytes and shows a strong positive correlation with TNF-α levels (Chamberlain *et al.*, 2013). It has been reported that the SARS-CoV infection induces upregulation of TLR7 in monocytes (THP1 cells) (Hu *et al*., 2012). TLR7 signalling promotes the transcription of cytokines involved

in Th17 cell differentiation as well as inhibits the TGF-β signaling (De Marcken *et al.*, 2019). TGF-β is widely recognized for its role in reducing inflammation and maintaining pulmonary homeostasis by inhibiting both Th2 and Th1 responses (Joetham *et al.*, 2007).

It thus becomes imperative to understand if the increased TLR7 in RA patients can efficiently recognise the ssRNA of SARS-CoV-2 and initiate the TLR7-mediated inflammatory response, further potentiating the disease severity.

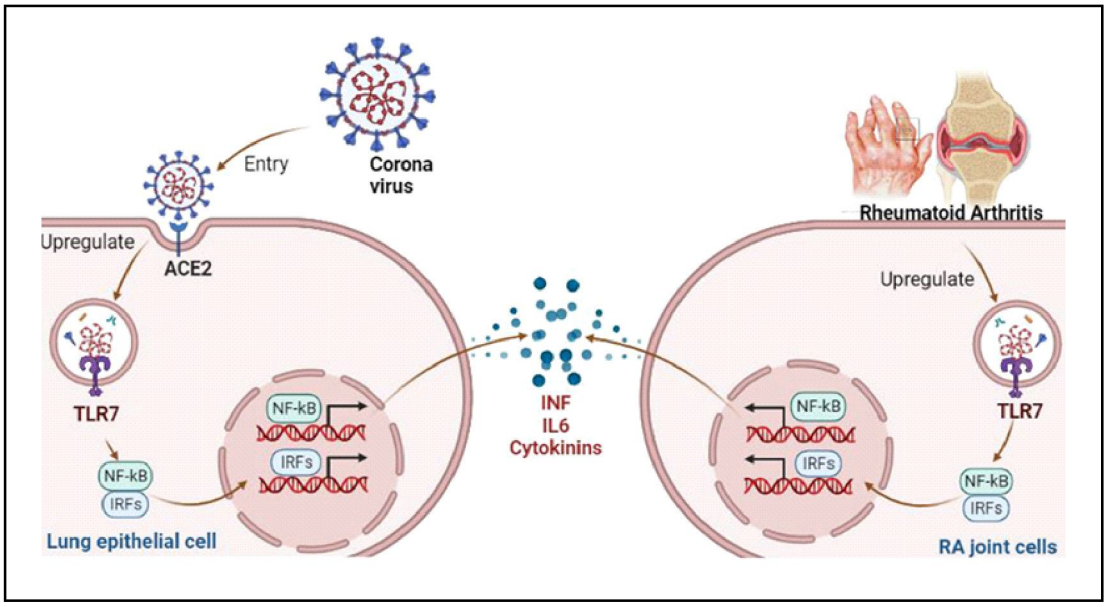


Figure 1: Increased level of cytokines in rheumatoid arthritis affected with COVID infection.

COVID-19 has been confirmed to trigger the activation of innate immune system pathways, the TLR4, mainly expressed on a variety of immune system cells, the first line of defense against viral pathogens. It can interact with damage-associated molecular pattern molecules (DAMPs), including oxidised phospholipids released by lung tissue acutely injured by SARS-CoV-2 infection. This interaction results in downstream activation of nuclear factor-kappa B (NF-κB) and induction of type I IFN (including IFN-α and IFN-β) and other proinflammatory cytokine production, such as IL-6 and TNF-α (Imai *et al.*, 2008). COVID-19 can further activate TLRs through the process of endocytosis. COVID-19 is able to interact with angiotensin-converting enzyme-2 receptors, especially those on the type II pneumocytes of the lungs, resulting in endocytosis and transport of viral nucleotides into endosomes. This enables interaction between viral nucleotides and TLR3, TLR7, and TLR8, which are found within the endosomal membrane (Ni *et al.*, 2020).

Recent studies have implicated rare genetic variants in TLR7 as contributors to differential immune response and recovery from COVID-19 infection (Van der Made *et al.*, 2020). Consideration of these TLR7 variants may be especially important in approaching SARS-CoV-2 relative to other coronaviruses. Comparisons of SARS-CoV-2 whole-genome sequencing, relative to SARS-CoV and MERS-CoV, showed that SARS-CoV-2 contained a greater number of ssRNA motifs that are able to interact with TLR7. TLRs are highly expressed in patients with RA. Increased expression of TLR2 and TLR4 on peripheral blood monocytes from patients with RA has been demonstrated (Sorensen *et al.*, 2008). Utilizing immune histochemistry, both TLR2 and TLR4, as well as endosomal TLR3 and TLR7, were expressed in RA synovial tissues (Radstake *et al.*, 2004).

TLR7 recognises pathogenic single-stranded RNAs (ssRNAs) and plays a crucial role in the innate immune response to viral infections, such as human immunodeficiency virus 1 and influenza virus (Barchet *et al.*, 2005). In fact, excessive activation of TLR7 is considered to be involved in the pathogenesis of several autoimmune diseases, such as systemic lupus erythematosus (SLE) (Pisitkun *et al.*, 2006). Several TLR7 inhibitors, including oligonucleotides and antimalarial agents, have been reported (Robbins *et al.*, 2007). Antimalarial agents such as hydroxychloroquine (HCQ), chloroquine, and quiracrine inhibit TLR7 and also TLR9 *via* indirect mechanisms that do not involve direct binding to TLRs (Wallace *et al.*, 2012).

RA patients prompt to have high level of TLR7, when coronavirus infect to these patients, further the level of TLR7 cloud be upregulated and leads to severe condition of RA. So, our aim is to inhibit the upregulation of TLR7 in RA patients during the infection with CoV-2. Based on the above facts and discussion, we have framed a hypothesis to identify known antagonists to prevent the upregulation of TLR7, followed by the generation of a pharmacophore model using the ligands collected from the literature survey known to inhibit and/or interact with TLR7, which can help us find the novel molecules from available ZINC databases. In addition, ADME and docking studies have been performed to identify the best HIT molecules out of the generated library of compounds through the pharmacophore model.

2. Materials and Methods

2.1 Devices used

The *in silico* research was carried out using a number of online as well as offline bioinformatics tools. The online tools include Pub Chem Database and Zinc Database for obtaining the ligands, Protein Data Bank (PDB) for obtaining the crystal structure of a protein of interest; PharmaGist Webserver and ZINCPharmer for pharmacophore modeling; and PreADMET for ADMET studies. The offline tools that were employed comprise MOE for ligand preparation, protein preparation, and docking.

2.2 Data collection

A dataset of five TLR7 antagonist ligands and structures was generated from the literature (Sun *et al.*, 2007; Tojo *et al.*, 2020). This dataset was generated using a set of search criteria in the Google Scholar database using the keywords "TLR7" and "Antagonist." A result has been obtained in Google Scholar, which was manually filtered. In these selected studies, some compounds displayed good antagonistic activity on TLR7, which was included in the dataset.

2.3 Pharmacophore modelling

A pharmacophore can be defined as a set of electronic, steric, and hydrophobic features that are required for optimal binding of a ligand to the desired target at a molecular level to show favourable biological activity (Khedkar *et al.*, 2007). Pharmacophore modelling is a new field for systematically studying the effect of a set of features, present in a drug like molecule, on the binding capacity to the target of interest. The pharmacophore features that are commonly used for the study are hydrophobicity, aromaticity, hydrogen bond acceptor, hydrogen bond donor, cation, and anion.

If the 3D structural information, such as the X-ray crystallography coordinates of the target, which are associated with an active ligand, is available, then the pharmacophore can be generated by performing structure-based techniques. Binding points can be extracted from the ligand-protein complex along with corresponding interaction features, and if no 3D structure is obtained, homology modelling is used to find the correct structure. Molecular docking combined with mutagenesis will help define the ligand-receptor interaction and binding mode. In the absence of any information regarding the 3D structure of the target, a ligand-based approach is adopted, which suggests possible pharmacophore questions regarding a set of aligned active compounds to create ligand-based pharmacophore models (Yang, 2010).

PharmaGist is a web-based tool that is commonly used to carry out ligand-based pharmacophore modeling. This does not require the structure of the target receptor, but rather an input of drug-like molecules that are known to bind to the receptor. Candidate pharmacophores are then computed by multiple flexible alignments of the ligands. The PharmaGist method handles the flexibility of the input ligands explicitly and in a deterministic manner within the alignment process. On a standard personal computer, a normal run takes about a few seconds to a few minutes. The capability of the detection of pharmacophores that is shared by various subsets of input molecules is an important characteristic of this webserver especially when ligands belong to various other binding modes or when there are outliers in the input. The highest-scoring molecules are then reported from the given set of molecules in an efficacious manner. PharmaGist gives the results very quickly, and it requires only two imperative inputs: the input molecules in mol2 format and the email address (Schneidman-Duhovny *et al.*, 2008).

For our study, we used PharmaGist to carry out ligand-based pharmacophore modelling using a total of 5 drug molecules compiled from a literature survey. The results are then accessed using the link, and the jmol files obtained are prioritised based on the number of molecules aligned.

2.4 Virtual screening

ZINCPharmer is a freely available web-based tool that is a pharmacophore search engine that is used for purchasable chemical space. It addresses two main challenges of pharmacophore hypotheses: identifying a representative pharmacophore for an interaction and identifying the compounds with a relevant chemical library. A database of conformations calculated from the purchasable compounds is searched by ZINC Pharmer. The ZINC Pharmer library is synchronised with the ZINC library monthly, where compounds are added or removed to maintain consistency and ensure that only purchasable compounds are retained. ZINC compounds are converted to 3D, and the generated conformers are converted into an efficient search format using the Pharmer open-source software. Hydrophilic, hydrophobic, hydrogen bond donor/acceptor, positive/negative ions, and aromatic pharmacophoric features are identified using the Pharmer (Zhang *et al.*, 2017).

These molecules are then downloaded as SDF files from ZINC Database which is a public access database and tool set, initially developed to enable ready access to compounds for virtual screening. This is very commonly used for virtual screening, pharmacophore screens, ligand discovery, benchmarking, and force field development (Irwin *et al.*, 2005).

2.5 Molecular docking

Aberrant activation of TLR7 has been implicated in several autoimmune diseases, including rheumatoid arthritis and systemic lupus erythematosus (SLE). Our work provides small-molecule TLR7-specific antagonists and suggests the TLR7-targeting strategy for treating autoimmune diseases like rheumatoid arthritis in COVID condition.

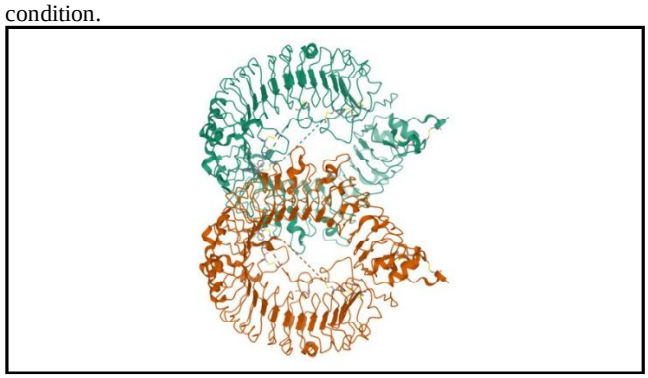


Figure 2: Proteins structure of toll-like receptors 7 (TLR7) (PDB ID: 6LW1).

In this study, the crystal structure of TLR7 (PDB codes: 6LW1) (Tojo *et al.*, 2020). For further evaluation of the hit compounds, all the retrieved compounds from the ZINC pharmer database were docked into the binding site of toll-like receptor 7 (TLR7) (PDB code: 6LW1) (Figure 2). Removal of water molecules, 3D protonation, and energy minimization were carried out using MOE with the following parameters: force field: MMFF94X+ solvation, gradient: 0.05, chiral constraint, and current geometry. This minimised structure was used as the ligand for docking analysis. The active site of the binding pocket was selected with the help of the MOE site finder tool. For docking simulations, the placement was set as a triangular matcher, the number of retaining was set at 10, and the refinement was set as a forcefield on the MOE suite to generate 10 poses of each target ligand confirmation (Prabha *et al.*, 2019). The most appropriate docked ligand target structure was selected on the basis of higher S-score and root mean square deviation (RMSD) values. The S-score is the value calculated by the built-in scoring functions of MOE on the

basis of ligand binding affinity with the receptor protein after docking (Prabha *et al.*, 2018). While RMSD values are generally used to compare the docked conformation with the reference conformation or with other docked conformations, the only compounds that have a higher S-score and a lower RMSD value than their natural substrates can be developed as potential inhibitors (Yang, 2010).

2.6 ADME prediction

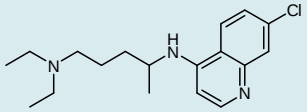
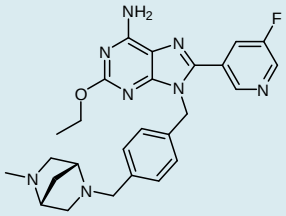
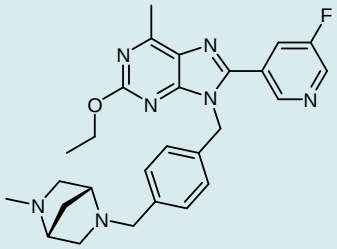
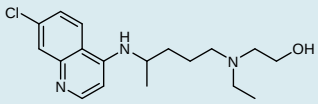
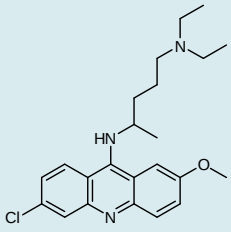
The ADME property predictions were calculated using a web-based tool known as Swiss ADME. Swiss ADME provides free access to a wide range of fast yet robust predictive models for the determination of different physicochemical properties, medicinal chemistry friendliness, drug likeness, and pharmacokinetics. These ADME studies are essential to understanding and discovering pharmacologically relevant parameters that have a significant effect on the binding affinity and bioavailability of any given compound.

3. Results

3.1 Data collection

A dataset of 5 TLR7 antagonist ligands and structures was collected from the literature were listed in Table 1 below.

Table 1: TLR7 antagonist ligands

Name	Structure	IUPAC name
Chloroquine		N4-(7-chloroquinolin-4-yl)-N1,N1-diethylpentane-1,4-diamine
Cpd-6		2-ethoxy-8-(5-fluoropyridin-3-yl)-9-(4-(((1R,4R)-5-methyl-2,5 diazabicyclo[2.2.1]heptan-2-yl)methyl)benzyl)-9H-purin-6-amine
Cpd-7		2-ethoxy-8-(5-fluoropyridin-3-yl)-6-methyl-9-(4-(((1R,4R)-5-methyl -2,5-diazabicyclo[2.2.1]heptan-2-yl)methyl)benzyl)-9H-purine
Hydroxychloroquine		2-((4-((7-chloroquinolin-4-yl)amino)pentyl)(ethyl)amino)ethan-1-ol
Quinacrine		N4-(6-chloro-2-methoxyacridin-9-yl)-N1,N1-diethylpentane-1,4-diamine

3.2 Pharmacophore modelling

A pharmacophore can be defined as the spatial arrangement of attributes or features required for a drug to interact with a certain target receptor. This is extremely essential for developing an optimal pharmacophore model. For this, a web server known as PharmaGist is used. Our current study was divided into two portions, the first being a search carried out with a set of input ligands for pharmacophore candidates, and the second being pharmacophore-based virtual screening carried out using another web-based tool known as ZINCPharmer.

Upon submitting the 5 drug molecules as shown in Table 1, an email is received at the email address provided, containing a link to a web page containing the results. These results are preserved by the PharmaGist server for up to a month. The primary output page is shown in Figure 3. This page contains a number of tables wherein the input molecules are listed in a table along with the number of atoms and their assigned physicochemical properties. The tables at the bottom are a summary of the results obtained upon completing a run. The tables are arranged in descending order of the number of molecules aligned.

An example of a page that explains a single potential pharmacophore is shown in Figure. The page is divided into two sections: At the top, there is a synopsis of the pharmacophore's properties, and on the right, there is a Jmol display. Finally, we need to download the Jmol file with the maximum number of aligned molecules. Parts of the main output page obtained for input with five TLR7 antagonists are shown in Figure 4.

Input Molecules view details: visualization of the detected features

#	Molecule	Atoms	Features	Spatial Features	Aromatic	Hydrophobic	Donors	Acceptors	Negatives	Positives
1	Quinacrine.mol2	58	12	12	3	5	1	3	0	0
2	Cpd6.mol2	65	15	15	4	2	1	7	0	1
3	Cpd7.mol2	66	15	15	4	3	0	7	0	1
4	Hydroxychloroquine.mol2	49	10	9	2	3	2	3	0	0
5	Chloroquine.mol2	48	9	9	2	4	1	2	0	0

Figure 3: Primary output page of PharmaGist.

Sort by score

Number of Aligned Molecules: 5

Score	Jmol	Features	Spatial Features	Aromatic	Hydrophobic	Donors	Acceptors	Negatives	Positives	Molecules
26.304	Jmol	5	5	2	1	0	2	0	0	Chloroquine.mol2 Quinacrine.mol2 Cpd6.mol2 Cpd7.mol2 Hydroxychloroquine.mol2
25.456	Jmol	4	4	2	0	0	2	0	0	Cpd6.mol2 Quinacrine.mol2 Cpd7.mol2 Hydroxychloroquine.mol2 Chloroquine.mol2
23.812	Jmol	4	4	2	0	0	2	0	0	Cpd7.mol2 Cpd6.mol2 Hydroxychloroquine.mol2 Chloroquine.mol2 Quinacrine.mol2
22.062	Jmol	4	4	2	1	0	1	0	0	Chloroquine.mol2 Quinacrine.mol2 Cpd6.mol2 Cpd7.mol2 Hydroxychloroquine.mol2
22.062	Jmol	4	4	2	1	0	1	0	0	Cpd7.mol2 Quinacrine.mol2 Cpd6.mol2 Hydroxychloroquine.mol2 Chloroquine.mol2
21.213	Jmol	3	3	2	0	0	1	0	0	Cpd7.mol2 Quinacrine.mol2 Cpd6.mol2 Hydroxychloroquine.mol2 Chloroquine.mol2
21.213	Jmol	3	3	2	0	0	1	0	0	Cpd7.mol2 Quinacrine.mol2 Cpd6.mol2 Hydroxychloroquine.mol2 Chloroquine.mol2
21.213	Jmol	3	3	2	0	0	1	0	0	Cpd7.mol2 Quinacrine.mol2 Cpd6.mol2 Hydroxychloroquine.mol2 Chloroquine.mol2
17.819	Jmol	4	4	1	1	0	2	0	0	Hydroxychloroquine.mol2 Quinacrine.mol2 Cpd6.mol2 Cpd7.mol2 Chloroquine.mol2
17.819	Jmol	3	3	2	1	0	0	0	0	Chloroquine.mol2 Quinacrine.mol2 Cpd6.mol2 Cpd7.mol2 Hydroxychloroquine.mol2

Figure 4: Parts of the main output page obtained for input with five TLR7 antagonist.

3.3 Virtual screening

The virtual screening was performed using the generated pharmacophore in ZINCPharmer (Figure 5). For our research, we chose the ZINC natural derivatives and the ZINC natural product database. The Pharma Gist's pharmacophore data is used to perform virtual screening. Based on the drug database, ZINCPharmer generated 71 hits. From there, we have chosen 10 compounds based on their RMSD values. These 10 molecules were then subjected to docking studies with our target of interest, TLR7.

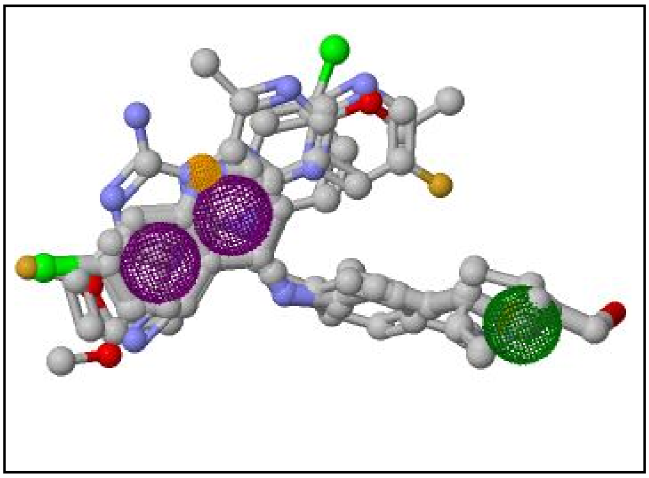


Figure 5: Generated pharmacophore visualised using ZINC Pharmer.

3.4 Molecular docking

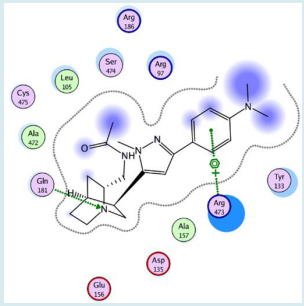
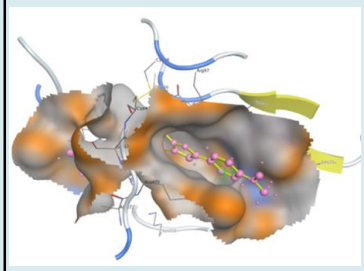
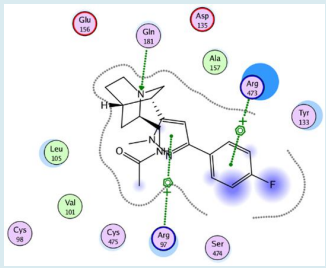
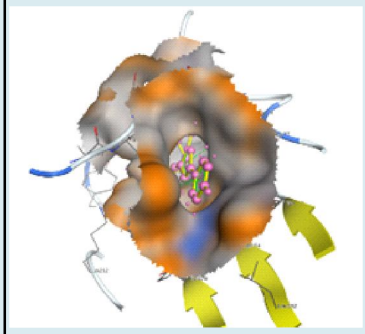
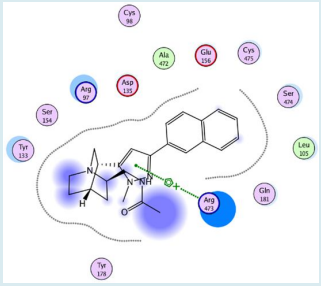
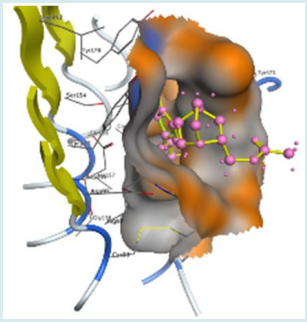
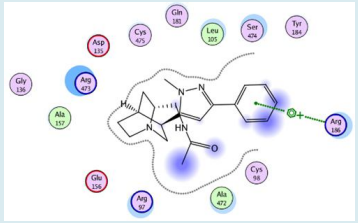
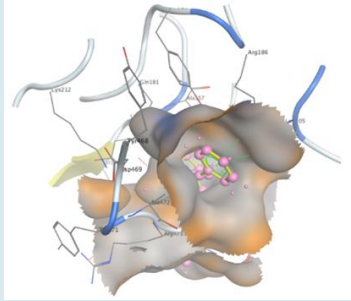
The results of the docking study show that the drug can directly affect the protein TLR7, which is a prime target, and these could be repurposed for the treatment of COVID-19 for rheumatoid arthritis conditions. Binding energy is the energy of the interaction between the protein and the ligand. This value is strongly indicative that the compounds were effectively bound to the active site of TLR7. The highest score achieved by the top six selected ligands for toll-like receptor 7 in activity (PDB ID: 6LW1) showed the binding affinity, *viz.*, -13.8036, -13.4501, -13.3095, -12.5435, -11.4339, and -10.5996, for ZINC08635349, ZINC12602116, ZINC08635473, ZIN C08635326, ZINC08635431, and ZINC20112269, respectively (Table 2).

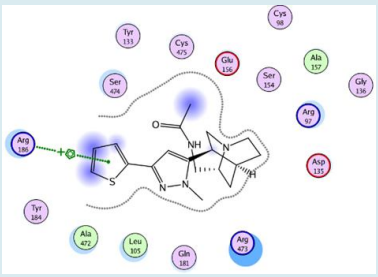
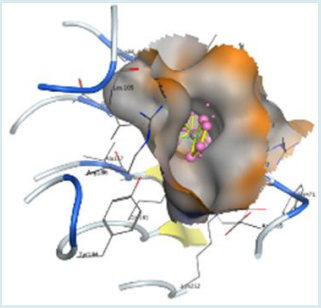
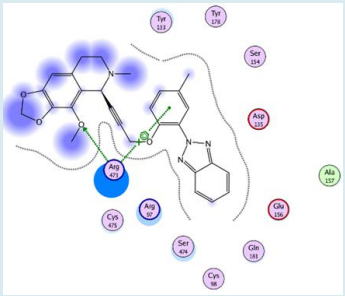
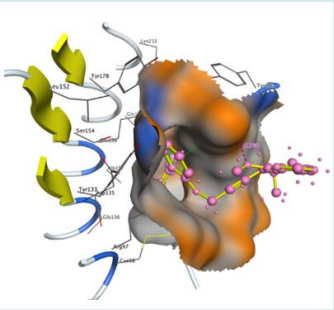
Table 2: Binding affinity score for the selected compounds from the ZINC drug database

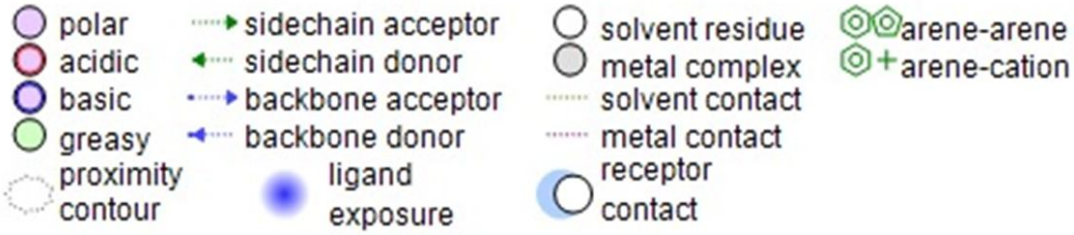
S.No.	Compound's code	Binding affinity (kcal/mol) (PDB ID:6LW1)
1	ZINC08635349	-13.8036
2	ZINC12602116	-13.4501
3	ZINC08635473	-13.3095
4	ZINC08635326	-12.5435
5	ZINC08635431	-11.4339
6	ZINC20112269	-10.5996
7	Co-crystal ligand (Standard)	-13.5837

The active site of TLR7 proteins comprises amino acid residues such as the backbone acceptor, arene-arene interaction, and aryl ring, as well as being surrounded by amino acid residues such as *Trp 194*, *Ile 340*, *Tyr 244*, *Lys 193*, *Phe 252*, *Met 242*, *Thr 203*, and *Lys 290*. Most of the amino acid residues in the active site are hydrophobic, so they are the main contributors to the receptor-ligand interaction. All the selected molecules (ligands) are well surrounded with polar and greasy centers, exposed well, and have receptor contact (Table 3).

Table 3: Binding affinity, 2D, and 3D interactions of the top six HITs from ZINC database compounds

Ligands	Binding affinity (kcal/mol)	2D interactions	3D interactions
ZINC08635349	-13.8036		
ZINC12602116	-13.4501		
ZINC08635473	-13.3095		
ZINC08635326	-12.5435		

ZINC08635431	-11.4339		
ZINC20112269	-10.5996		



3.5 ADME prediction

The following properties from ADME studies are important: Aqueous solubility refers to the ability of the compound to dissolve in body fluids. Blood-Brain Barrier (BBB) penetration refers to the ability of the compound to penetrate the blood-brain barrier and cause any action in the CNS. It is an indication of the highly lipophilic nature of the compounds. The topological polar surface area is the sum of all polar atoms, primarily oxygen and nitrogen, also including their attached hydrogen atoms. The ability of a compound to inhibit cytochrome P450-CYP2D6 refers to its ability to inhibit any enzyme. Human gastrointestinal absorption refers to the ability of the compound to be absorbed by the GI tract. LogP is known as the partition coefficient, which is a direct representation of the hydrophobicity of the compound (Table 4).

Table 4: ADME prediction of top HITs selected from docking study

S. No.	Compound name	Solubility	BBB	Cyp2D6	Synthetic accessibility	Absorption	Wlogp	Tpsa
1	ZINC08635349	Soluble	Yes	Yes	5.06	High	2.09	53.4
2	ZINC12602116	Soluble	Yes	Yes	4.86	High	2.58	50.16
3	ZINC08635473	Soluble	Yes	Yes	5.04	High	3.17	50.16
4	ZINC08635326	Soluble	Yes	Yes	4.81	High	2.02	50.16
5	ZINC08635431	Soluble	No	Yes	5.06	High	2.08	78.4
6	ZINC20112269	Poorly soluble	Yes	Yes	4.52	High	3.45	70.87

4. Discussion

COVID-19 has presented itself as the greatest global crisis of this millennium and has affected almost all the countries in the world. Research on the pathophysiology of COVID-19 has brought to light different aspects of this novel disease. The increased levels of angiotensin II which is one of the key characteristics of the disease has been shown to up regulate the levels of pro-inflammatory cytokines like TNF-α, IL-6 and IL-8 which are significantly increased in case of rheumatoid arthritis (Singhal, 2020). Angiotensin has also been correlated with the production of IFN-γ which is a key cytokine in case of RA. The increase in the level of CRP can be seen as a link to the potential occurrence of RA in a COVID-19 patient (Qin *et al.*, 2020). There have been studies where the relation between other respiratory viral infections and RA has been shown. Thus, we propose that RA patients might be predisposed to disease severity after SARS-CoV-2 infection, suggesting that severe RA and COVID-19 might coexist and that the genetically predisposed individuals to RA may have a high risk of developing RA if they get infected with SARS-CoV-2.

To date, various small-molecule TLR7 agonists have been synthesized, and many of them are under clinical trials for treating viral diseases such as hepatitis B and influenza, as well as several types of cancer (Patinote *et al.*, 2020). In contrast, few small molecules TLR7-specific antagonist has been reported in literature. Since blocking TLR7 is considered a promising strategy for treating autoimmune diseases, including SLE and psoriasis (Kanno *et al.*, 2015), there is an urgent need for the development of a TLR7-specific antagonist.

5. Conclusion

The toll-like receptor 7(TLR7) antagonists have been discovered to minimise the risk of COVID infection in rheumatoid arthritis *via* virtual screening approaches. Among the 71 compounds chosen from the ZINC database, the top six molecules were found to have a higher binding affinity for TLR7 (PDB ID: 6LW1) when compared to their co-crystal ligand. Further, we have found that those drugs, which were not traditionally used for COVID-19 with rheumatoid arthritis condition treatment Moreover, the top six binding affinities show that these selected ligands from the ZINC database are well bound in the protein pockets of proteins. Besides, the ADME study result showed the pharmacologically relevant parameters that have a significant effect on the binding affinity, bioavailability, and toxicity of the selected compounds from the ZINC drug database. As a result, it is possible to conclude that COVID-19 with rheumatoid arthritis and its associated comorbidities can be reduced by decreasing the level of TLR7 and could be repurposed for effective clinical treatment.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Barchet, W.; Krug, A.; Cella, M.; Newby, C.; Fischer, J.A.; Dzionek, A.; Pekosz, A. and Colonna, M. (2005). Dendritic cells respond to influenza virus through TLR7 and PKR independent pathways. European Journal of Immunology, **35**(1):236-242.

Chamberlain, N.D.; Kim, S.J.; Vila, O.M.; Volin, M.V.; Volkov, S.; Pope, R.M.; Arami, S.; Mandelin, A.M. and Shahrara, S. (2013). Ligation of TLR7 by rheumatoid arthritis synovial fluid single strand RNA induces transcription of TNFα in monocytes. Annals of the Rheumatic Diseases, **72**(3):418-426.

deMarcken, M.; Dhaliwal, K.; Danielsen, A.C.; Gautron, A.S. and Dominguez-Villar, M. (2019). TLR7 and TLR8 activate distinct pathways in monocytes during RNA virus infection. Science Signaling, **12**(605):1347.

Firestein, G.S. (2003). Evolving concepts of rheumatoid arthritis. Nature, **423**(6937):356-361.

Heil, F.; Hemmi, H.; Hochrein, H.; Ampenberger, F.; Kirschning, C.; Akira, S.; Lipford, G.; Wagner, H. and Bauer, S. (2004). Species-specific recognition of single-stranded RNA *via* toll-like receptor 7 and 8. Science, **303**(5663):1526-1529.

Hoffmann, M.; Kleine-Weber, H.; Schroeder, S.; Krüger, N.; Herrler, T.; Erichsen, S.; Schiergens, T.S.; Herrler, G.; Wu, N.H.; Nitsche, A. and Müller, M.A. (2020). SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell, **181**(2):271-280.

Hu, W.; Yen, Y.T.; Singh, S.; Kao, C.L. and Wu-Hsieh, B.A. (2012). SARS-CoV regulates immune function-related gene expression in human monocytic cells. Viral Immunology, **25**(4):277-288.

Imai, Y.; Kuba, K.; Neely, G.G.; Yaghubian-Malhami, R.; Perkmann, T.; van Loo, G.; Ermolaeva, M.; Veldhuizen, R.; Leung, Y.C.; Wang, H. and Liu, H. (2008). Identification of oxidative stress and toll-like receptor 4 signaling as a key pathway of acute lung injury. Cell, **133**(2):235-249.

Irwin, J.J. and Shoichet, B.K. (2005). ZINC" a free database of commercially available compounds for virtual screening. Journal of Chemical Information and Modeling, **45**(1):177-182.

Joetham, A.; Takada, K.; Taube, C.; Miyahara, N.; Matsubara, S.; Koya, T.; Rha, Y.H.; Dakhama, A. and Gelfand, E.W. (2007). Naturally occurring lung CD4+ CD25+ T cell regulation of airway allergic responses depends on IL-10 induction of TGF-β. The Journal of Immunology, **178**(3): 1433-1442.

Kanno, A.; Tanimura, N.; Ishizaki, M.; Ohko, K.; Motoi, Y.; Onji, M.; Fukui, R.; Shimozato, T.; Yamamoto, K.; Shibata, T. and Sano, S. (2015). Targeting cell surface TLR7 for therapeutic intervention in autoimmune diseases. Nature Communications, **6**(1):1-12.

Karikó, K.; Buckstein, M.; Ni, H. and Weissman, D. (2005). Suppression of RNA recognition by Toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA. Immunity, **23**(2): 165-175.

Karlsson, L.; Sun, S.; Rao, N.L.; Venable, J. and Thurmond, R. (2007). TLR7/9 antagonists as therapeutics for immune-mediated inflammatory disorders. Inflammation and Allergy-Drug Targets (Formerly Current Drug Targets-Inflammation and Allergy) (Discontinued), **6**(4):223-235.

Khedkar, S.A.; Malde, A.K.; Coutinho, E.C. and Srivastava, S. (2007). Pharmacophore modeling in drug discovery and development: An overview. Medicinal Chemistry, **3**(2):187-197.

Ni, W.; Yang, X.; Yang, D.; Bao, J.; Li, R.; Xiao, Y.; Hou, C.; Wang, H.; Liu, J.; Yang, D. and Xu, Y. (2020). Role of angiotensin-converting enzyme 2 (ACE2) in COVID-19. Critical Care, **24**(1):1-10.

Patinote, C.; Karroum, N.B.; Moarbess, G.; Cirnat, N.; Kassab, I.; Bonnet, P.A. and Deleuze-Masquéfa, C. (2020). Agonist and antagonist ligands of toll-like receptors 7 and 8: Ingenious tools for therapeutic purposes. European Journal of Medicinal Chemistry, **193:**112238.

Pisitkun, P.; Deane, J.A.; Difilippantonio, M.J.; Tarasenko, T.; Satterthwaite, A.B. and Bolland, S. (2006). Autoreactive B cell responses to RNA-related antigens due to TLR7 gene duplication. Science, **312**(5780):1669-1672.

Prabha, T.; Aishwaryah, P.; Manickavalli, E.; Chandru, R.; Arulbharathi, G.; Anu, A. and Sivakumar, T. (2019). A Chalcone Annulated Pyrazoline Conjugates as a Potent Antimycobacterial Agents: Synthesis and in

Silico Molecular Modeling Studies. Research Journal of Pharmacy and Technology, **12**(8):3857-3865.

Qin, C.; Zhou, L.; Hu, Z.; Zhang, S.; Yang, S.; Tao, Y.; Xie, C.; Ma, K.; Shang, K.; Wang, W. and Tian, D. S. (2020). Dysregulation of immune response in patients with coronavirus 2019 (COVID-19) in Wuhan, China. Clinical infectious diseases: An official publication of the Infectious Diseases Society of America, **71**(15):762-768.

Radstake, T.R.; Roelofs, M.F.; Jenniskens, Y.M.; Oppers Walgreen, B.; van Riel, P.L.; Barrera, P.; Joosten, L.A. and van den Berg, W.B. (2004). Expression of Toll like receptors 2 and 4 in rheumatoid synovial tissue and regulation by proinflammatory cytokines interleukin 12 and interleukin 18 *via* interferon γ. Arthritis and Rheumatism, **50**(12):3856-3865.

Robbins, M.; Judge, A.; Liang, L.; McClintock, K.; Yaworski, E. and MacLachlan, I. (2007). 22-O-methyl-modified RNAs act as TLR7 antagonists. Molecular Therapy, **15**(9):1663-1669.

Schneidman-Duhovny, D.; Dror, O.; Inbar, Y.; Nussinov, R. and Wolfson, H.J. (2008). Pharma Gist: A webserver for ligand-based pharmacophore detection. Nucleic Acids Research, **36**(2):W223-W228.

Singhal, T. (2020). A review of coronavirus disease-2019 (COVID-19). The Indian Journal of Pediatrics, **87**(4):281-286.

Sørensen, L.K.; Havemose Poulsen, A.; Sønder, S.U.; Bendtzen, K. and Holmstrup, P. (2008). Blood cell gene expression profiling in subjects with aggressive periodontitis and chronic arthritis. Journal of Periodontology, **79**(3):477-485.

Prabha, T. and Sivakumar, T. (2018). Design, synthesis, and docking of sulfadiazine Schiff base scaffold for their potential claim as Inhaenoyl-(acyl-carrier-protein) reductase inhibitors. Asian Journal of Pharmaceutical and Clinical Research, **11**(10):233.

Tojo, S.; Zhang, Z.; Matsui, H.; Tahara, M.; Ikeguchi, M.; Kochi, M.; Kamada, M.; Shigematsu, H.; Tsutsumi, A.; Adachi, N. and Shibata, T. (2020). Structural analysis reveals TLR7 dynamics underlying antagonism. Nature Communications, **11**(1):1-11.

Tripathy, A.; Swain, N. and Gupta, B. (2021). The COVID-19 pandemic: An increased risk of rheumatoid arthritis. Future Virology, **16**(6):431-442.

Van Der Made, C.I.; Simons, A.; Schuurs-Hoeijmakers, J.; Van Den Heuvel, G.; Mantere, T.; Kersten, S.; Van Deuren, R.C.; Steehouwer, M.; Van Reijmersdal, S.V.; Jaeger, M. and Hofste, T. (2020). Presence of genetic variants among young men with severe COVID-19. Journal of the American Medicinal Association, **324**(7):663-673.

Vukmanovic-Stejic, M.; Vyas, B.; Gorak-Stolinska, P.; Noble, A. and Kemeny, D.M. (2000). Human Tc1 and Tc2/Tc0 CD8 T-cell clones display distinct cell surface and functional phenotypes. Blood, the Journal of the American Society of Hematology, **95**(1):231-240.

Wallace, D.J.; Gudsoorkar, V.S.; Weisman, M.H. and Venuturupalli, S.R. (2012). New insights into mechanisms of therapeutic effects of antimalarial agents in SLE. Nature Reviews Rheumatology, **8**(9):522-533.

Yang, S.Y. (2010). Pharmacophore modeling and applications in drug discovery: Challenges and recent advances. Drug Discovery Today, **15**(11-12):444-450.

Zhang, J. and Luo, Y. (2017). Degree centrality, betweenness centrality, and closeness centrality in social network. In 2017 2nd international conference on modelling, simulation and applied mathematics (MSAM2017). Atlantis Press, pp:300-303.

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