Amodiaquine as COVID-19 M^{pro} Inhibitor: A theoretical study

Badri Narayan Acharya

Synthetic Chemistry Division
Defence R&D Establishment, Jhansi Road, Gwalior-474002

Abstract COVID-19 is caused by severe respiratory syndrome –coronavirus 2 (SARS CoV-2). This has been declared as a global pandemic by World Health Organization (WHO). Currently only supportive care is available for treatment of patients. However availability of direct therapeutic approaches would greatly benefit the patient care and reduce death among COVID-19 patients. Repurposing of approved drugs against COVID-19 would be a faster method to identify direct therapeutics against COVID-19. This study describes screening and identification of *Amodiaquine* a known antimalarial as COVID-19 M^{pro} inhibitor by pharmacophore modeling and molecular docking. *Amodiaquine* may be repurposed as COVID-19 drug after thorough clinical tests.

Keywords Pharmacophore ● COVID-19 ● M^{pro} ● Docking ● Inhibitor ● *Amodiaquine*

✉ Badri Narayan Acharya
bnacharya@yahoo.com

Introduction

A novel corona virus (CoV) identified as COVID-19 has been declared as global pandemic by world health organization (WHO). As of June 22, 2020, there have been more than 8.7 million reported cases and more than 461,000 deaths in more than 200 countries. In India more than 410,000 cases infection and 13,254 deaths are reported in same period of time. COVID-19 virus genome is comprised of ~30,000 nucleotides encodes two overlapping poly proteins required for viral replication and transcription [1, 2]. The functional proteins are released by extensive proteolysis of the polyproteins by a 33.8 kDa main protease (M^{pro}) [3]. The function of viral M^{pro} in the life cycle of the virus and absence of similar protease in humans makes it an automatic choice for antiviral drug target [4]. Recently Jin et al have reported the structure of M^{pro} co-crystallized with an inhibitor N3 by x-ray crystallography [5]. They have also identified six M^{pro} inhibitors with IC_{50} values of enzyme inhibition in the range of 0.67 to 21.4 μM by using a fluorescence resonance energy transfer (FRFT) based high throughput enzyme activity assay. Thus they have revealed both the structures of drug targets and potential drug molecules against COVID-19 and all the information can be used in structure based approach for drug designing (SBDD). No proven effective therapies for the virus currently exist. SBDD can be used to facilitate rapid discovery of antiviral drug compounds with clinical potential by repurposing existing drugs to target COVID-19 virus M^{pro}.

Structure-based drug designing (SBDD) is an effective tool for discovery of potential bioactive molecules. Based on the receptor structures, effective inhibitors can be designed. These crystal structures can be used in structure based design of new inhibitors for COVID-19 viral M^{pro}. Earlier we have demonstrated a pharmacophore based method to design dihydropyrimidione (DHPM) based molecules showing mAChR antagonist activity [6]. The

molecules were designed based on three template molecules screened from *NCI2000* database by pharmacophore modeling. We also used a combination of pharmacophore modeling and structure based receptor-ligand docking to design a mAChR antagonist based on a template molecule screened from *Maybridge* database [7]. In this work, similar approach has been adopted to discover *Amodiaquine* as a potential repurposing drug for COVID-19. With adequate experimental validation *Amodiaquine* can be repurposed as a drug against COVID-19.

Materials and Methods

Pharmacophore modeling

Pharmacophore hypotheses were generated using Catalyst HipHop algorithm as present in DiscoveryStudio^{TM} 2.0 [8]. In brief, training set molecules shown in Fig 1a were drawn and geometry optimized. Input parameters include features hydrogen bond donor (HBD), hydrogen bond acceptor (HBA), positive ionizable (PI), hydrophobe (HY) and ring aromatic (RA), maximum 10 and minimum 1 features, minimum inter feature distance 2.97 Å, maximum conformations 255, energy threshold 20 kcal/mol. Poling algorithm was used to generate conformations for each molecule in 'fast mode'. HipHop conducts an exhaustive search starting with the simplest to more complicated pharmacophores. The process continues until common pharmacophore combinations can no longer be generated. The final HipHop output was top ten unique pharmacophores sorted from highest to lowest scoring. Then the top ranked hypothesis was used to screen *NCI 2000* databases to shortlist potential molecules [9].

Docking Studies

Structures of M^{pro} of COVID-19 virus (pdb id: 6lu7) [5] was downloaded from protein data bank and used for receptor-ligand interaction studies. Structure was cleaned from water and ligand molecules coded as N3. The protein contains many binding pockets in its structure as

identified by inbuilt method of DiscoveryStudio^{TM} 2.0. The pocket closer to catalytic aminoacid CYS 145 was identified having the central coordinates at -12.12, 13.884, 64.03. The cleaned structure of M^{PRO} was imported to MGLTools-1.5.4 [10] for preparation of docking studies. HIS 164 of M^{PRO} was selected as flexible residue. Docking studies were carried out by AutoDock 4.1 [10]. For docking of inhibitors with M^{pro}, grid center was set at -12.12, 13.884, and 64.03. Number of grid points in each direction was 40 and grids were generated for each ligands before docking. Ligands shortlisted from pharmacophore screening were prepared with Gastegier's charges at prescribed torsions. Lamarckian Genetic Algorithm (LGA) was used for conformation generation and determination of best interactions between receptor and ligand. Seed population was 150, number of evaluations was 2,500,000, and number of generation was set at 27000. Rate of mutation and crossover were 0.02 and 0.8 respectively. Top ten conformations were returned after each run. Binding energy (kcal/mol) and predicted inhibition constant (K_i) were recorded.

Results and Discussion

Pharmacophore model and virtual screening

Common feature pharmacophore modeling [11] requires structure of active molecules to identify and enumerate all possible pharmacophore configurations which in common within the training set molecules. Jin et.al [5] screened ~10,000 compounds consisting of approved drugs, drug candidates in clinical trials and natural products by fluorescence resonance energy transfer (FRET) assay. Six compounds namely *Ebselen*, *Tideglusib*, *Shikonin*, *Disulfiram*, *Carmofur*, and *PX-12* were found as hits in the FRET assay and their IC_{50} values were found in between 0.67 to 21.4 μM concentration. These six molecules (Fig 1a) were taken in the training set to generate common feature pharmacophore models as described in the materials and methods. HipHop performs an exhaustive search starting with the simplest two feature configuration followed by

more complex configurations. The process continues till HipHop no longer generates any new more complex pharmacophore configurations. In this process, a three feature pharmacophore model containing one HBA (hydrogen bond acceptor) and two HY (hydrophobe) was obtained (Fig 1b). The distances between HBA-HY1, HBA-HY2 and HY1-HY2 are 7.611, 5.780 and 8.3 Å respectively.

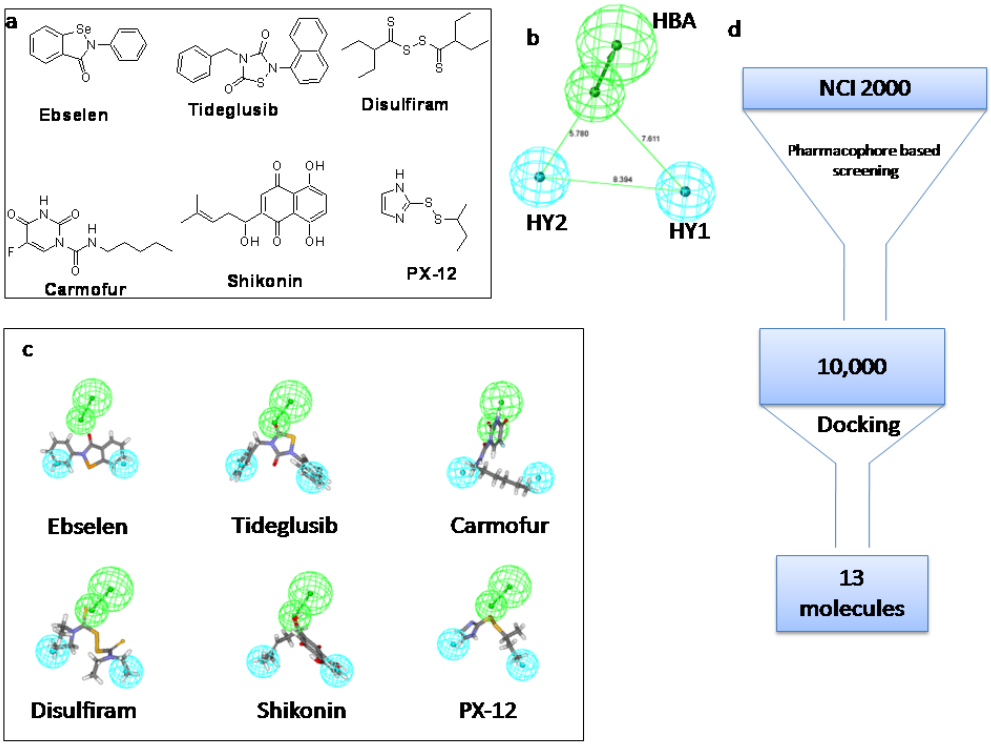

Fig.1 Training set molecules (a); pharmacophore model (b); training set molecules mapped with pharmacophore model; screening of NCI 2000 database (d)

Pharmacophore based virtual screening is a quick and effective screening method to search putative inhibitors from larger databases. The burden of experimentation could be reduced many-fold by receptor-ligand docking in combination with pharmacophore screening. Pharmacophore model was used screening *NCI 2000* database to shortlist top 10,000 molecules (Fig. 1d). In return 9958 molecules were obtained. They were sorted with respect to their fit

values with the the pharmacophore model. Pharmacophore mapping of top six molecules in order of their fit values are shown in Fig 2.

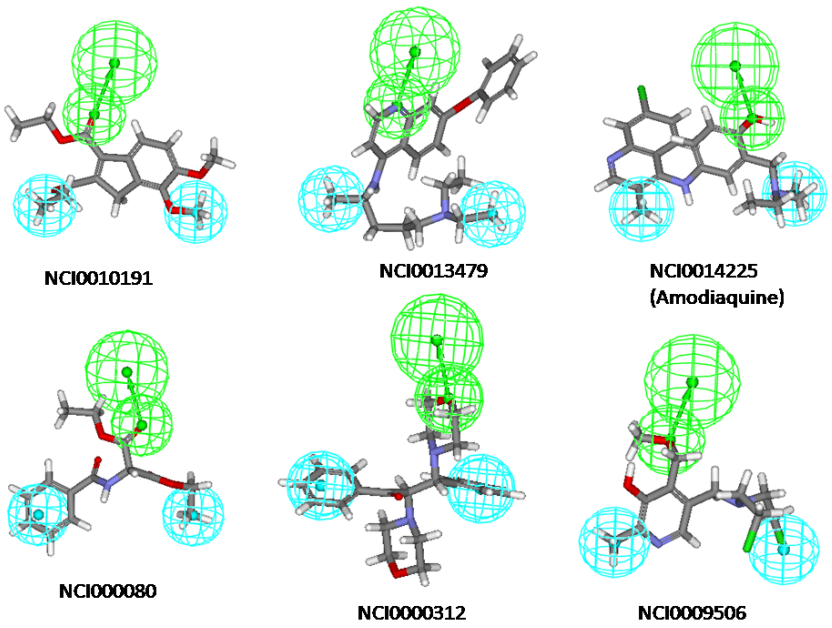

Fig. 2 Mapping of molecules from NCI 2000 database screened by pharmacophore model.

Molecule NCI 0010191 and NCI 0014225 showed Fit values 2.995 and 2.992 respectively. NCI 0014225 draws our attention as it is a clinically proven drug for malaria with a generic name *Amodiaquine*. In order to find the mode of binding of *Amodiaquine* with M^{pro} docking studies between the receptor and the ligand was carried out. Similar studies were also carried out with some other molecules cherry picked from the screened molecules (Fig 3). Their Fit values binding energies and theoretical Ki values are given in Table 1. Hydroxychloroquine (HCQ) was also studied for Receptor-Ligand interaction.

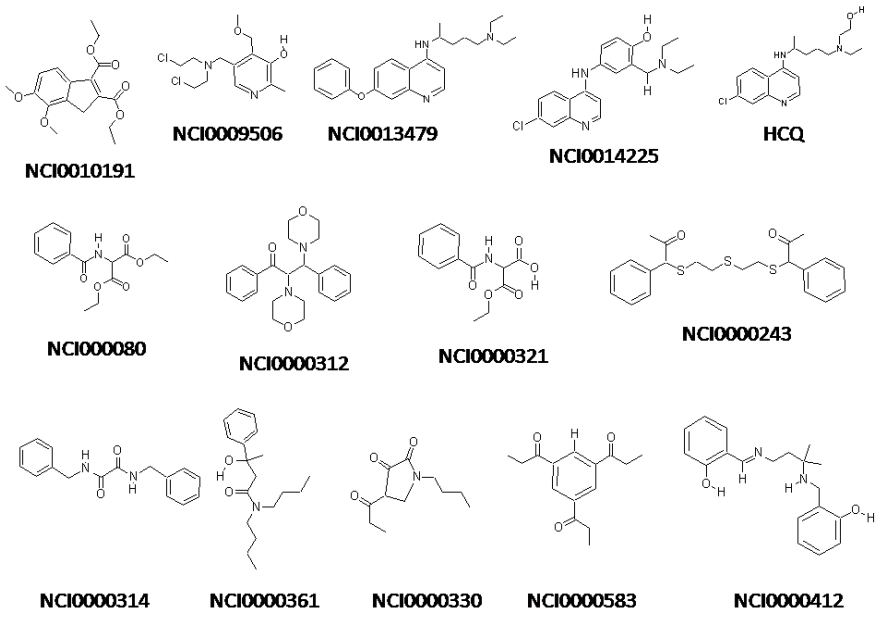

Fig 3. Molecules cherry picked from pharmacophore screening.

When fit values with corresponding binding energies of the molecules were compared, no correlation was observed. Therefore having the pharmacophores are not enough for a molecule to be active until unless it is not binding strongly to the receptor. Molecule NCI0000312 showed maximum binding energy (-10.8 Kcal/mol) followed by NCI0013479 (-10.60 Kcal/mol). NCI0013479 is a quinoline based molecule and structurally similar to chloroquine. *Amodiaquine* and HCQ are quinoline based antimalarials already in clinical use for malaria in several countries including India. *Amodiaquine* (NCI0014225) and HCQ (hydroxyl chloroquine) showed binding energy -9.73 Kcal/mol and -9.29 Kcal/mol respectively. The estimated protease inhibition constant (Ki) value of *Amodiaquine* is 73.44 nM which is very encouraging.

Table 1 Pharmacophore fit values, binding energy and and Ki values of screened molecules.

Sl No	Code	Rank in pharmacophore screening	Fit value[@]	Binding energy (Kcal/mol)[$]	Ki (µM)[$]
1	NCI0010191	1	2.995	- 9.04	0.237
2	NCI0009506	2	2.994	- 7.78	1.97
3	NCI0013479	3	2.993	- 10.60	0.017
4	NCI0014225 (*Amodiaquine*)	4	2.992	-9.73	0.073
5	NCI0000080	53	2.974	- 8.46	0.63
6	NCI0000312	68	2.971	- 10.80	0.012
7	NCI0000321	134	2.962	- 7.04	4.06
8	NCI0000243	220	2.951	- 9.00	0.253
9	NCI0000314	414	2.936	- 8.78	0.369
10	NCI0000361	449	2.934	- 8.79	0.363
11	NCI0000330	662	2.92	- 7.59	2.73
12	NCI0000583	887	2.904	- 8.91	0.295
13	NCI0000412	939	2.901	- 9.47	0.114
14	HCQ	--	--	- 9.29	0.154

[@]Fit value from pharmacophore mapping estimated by Discovery Studio;

[$]Estimated by AutoDock

The theoretical binding poses of *Amodiaquine* molecules are shown in Fig 4. Strong hydrogen bonding was observed between phenolic group of *Amodiaquine* and PHE 140 residue of M^{pro}. Catalytic CYS 145 was also present in close proximity of *Amodiaquine*. From receptor-ligand docking it may be inferred that *Amodiaquine* can effectively bind in the catalytic site of M^{pro} and inhibit the enzyme function. However experimental validation of the Ki value is required to conclude the inhibitory potential of *Amodiaquine* as well as other molecules as M^{pro} inhibitors.

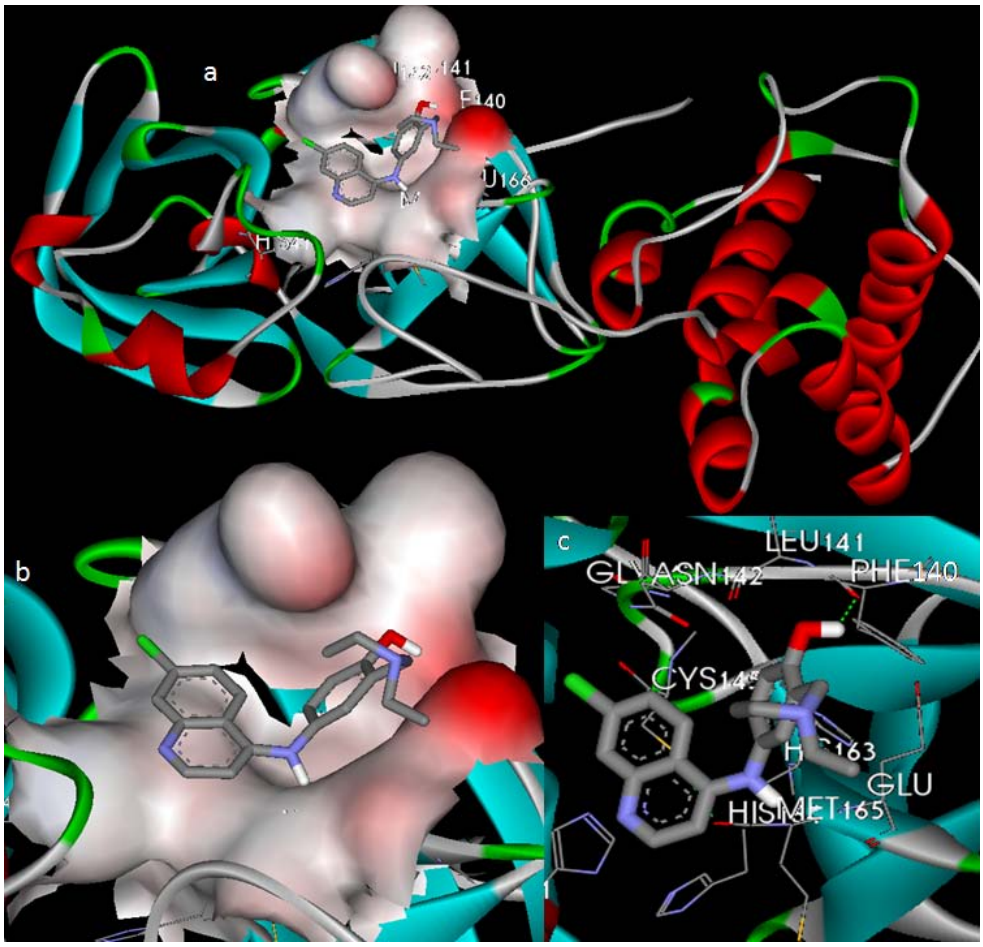

Fig 4 Binding of *Amodiaquine* (NCI0014225) with COVID-19 M^{pro} catalytic site (a); An electrostatic surface of the M^{pro} binding site around *Amodiaquine* (b); Aminoacids of M^{pro} binding site in close proximity of *Amodiaquine* (c).

Conclusion

In the current scenario the world is struggling with COVID-19 pandemic without any vaccine and anti COVID-19 drugs. To facilitate the rapid discovery of antiviral compounds with clinical potential, repurposing of existing drugs will be very helpful and effective to tackle the pandemic. COVID-19 M^{pro} is a potential drug target for antiviral discovery and methods like pharmacophore modeling and receptor-ligand docking are very effective virtual tools to discover potential anti viral molecules targeting M^{pro}. In this work we have demonstrated the application

of both ligand and structure based drug designing tools to screen large molecular database like *NCI 2000* and identify *Amodiaquine* (NCI0014225) a clinically established drug molecule against malaria as a repurposing drug against COVID-19. Although this is a theoretical study, after experimental validation by enzymatic assay and antiviral assay, *Amodiaquine* can be repurposed as a drug for COVID-19 virus.

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Conflict of interests

None

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