Chloroquine and Lopinavir (COVID-19 Drug Candidates) Signal Amplification by Reversible Exchange

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ABSTRACT

To overcome the recent coronavirus pneumonia (COVID-19), several drug candidates are suggested and tested for the latest clinical treatment. Chloroquine and lopinavir are showing definite effects after treatment. To understand more about those roles in molecular level and future application on NMR/MRI, hyperpolarization technique can open new opportunities in the diagnosis and biomedical researches to cope with COVID-19. SABRE-based hyperpolarization studies on those two drug candidates are carried out and we observed hyperpolarized proton signals from the whole structures, which can be possible by unprecedented long-distance polarization transfer by *para*-hydrogen. Base on this result, future work on isotope labeling, and further polarization transfer on long T1 time nuclei including clinical perspectives will open a new door for overcoming this dreadful catastrophe.

Keywords : Chloroquine, Lopinavir, SABRE, Hyperpolarization, NMR, COVID-19

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Introduction

The recent coronavirus pneumonia (COVID-19) is a serious respiratory infectious disease. Patients with this coronavirus infection demonstrate fever with their body temperatures exceeding 38 °C and symptoms such as dry cough, fatigue, dyspnea, difficulty breathing, and frost-glass-like symptoms in the lungs[1]. The disease is known to be highly transmittable without severe symptoms. The number of cases has reached hundreds of thousands throughout the world.

To overcome this catastrophe, recently several drugs have been suggested and tested for the latest clinical treatments. These drugs can inhibit certain functions of the virus. One of them, chloroquine phosphate, has a definite effect on the novel coronavirus pneumonia[2]. Chloroquine phosphate, which has been utilized clinically for the last seventy years, is an antimalarial and an autoimmune disease drug. This organic compound has recently been reported as a potential broad-spectrum antiviral drug for COVID-19.[3,4] In molecular perspective, chloroquine is known to block viral infection by increasing the endosomal pH required for virus and cell fusion and interfering with the glycosylation of cellular receptors of SARS-CoV.[5] Along with its molecular perspective on antiviral activity, chloroquine has an immune-modulating activity, which may synergistically enhance its antiviral effect *in vivo*.[6] Chloroquine is known to be distributed in the whole body, including the lungs, after oral administration. The EC_{90} value of chloroquine against the 2019-nCoV in Vero E6 cells was 6.90 μM, which can be clinically achieved after dosing as much as 500 mg.[7,8] For COVID-19 treatment, mega dosage and/or its dynamic distribution for an *in vivo* study with the specific clinical condition may be inevitable.

Lopinavir is the relatively newly developed medication for the treatment and prevention of HIV. This compound is also suggested as the potent drug candidate against COVID-19 due to its role as the proteinase inhibitor, which can inhibit the polyprotein processing of Coronavirus[9,10]. Further, several evidences with recent reports about lopinavir treatment of COVID-19 support its role as a potential drug, which lessen viral loads and improve clinical symptoms[11,12]. However, its understanding, in terms of molecular perspective, lacks in study compared to the chloroquine.

To understand more about drug interactions with proteins, metabolism, and other activities, nuclear magnetic resonance (NMR) spectroscopy has been widely used under

the name of pharmacokinetics. However, its inherent insensitivity arises because of the small population differences in spin state energy. The hyperpolarization technique, which generates the non-Boltzmann distribution of the spin state population, could be a breakthrough. Furthermore, to understand its spread throughout the body *in vivo* and to study its real-time activity, magnetic resonance imaging (MRI) might be one of the best solutions to determine its distribution and activity in the body. To see the MRI signal in real time, we need to tag the drug with special materials, such as chelating agents with T1/T2 contrast. However, if additional materials are tagged in the drug candidate, it is likely to result in unpredictable effects from the different molecular structures. The only way of doing this is to use the hyperpolarization technique to see the hyperpolarized signal through the MRI. Even though this state-of-the-art technique needs to overcome several hurdles, it could be the key to see the *in vivo* real-time distribution and activity to cope with COVID-19 in the future and to study more deeply the activity of chloroquine and lopinavir in the body.

Of several methods to hyperpolarize drugs, the *para*-hydrogen based signal amplification by reversible exchange (SABRE) method is a promising tool for hyperpolarization of several key structures with nitrogen. Furthermore, its polarization could be transferred in real time from protons to other isotopes such as ^{13}C[13], ^{15}N[14,15], ^{31}P[16], ^{19}F[17], ^{119}Sn[18], and ^{29}Si[19] without chemical changes.

Several great recent studies of producing hyperpolarized drugs or metabolites[16–20], including pharmacokinetics using SABRE[21], confirm that such a study would widen its application in this era, when scientific solutions are the only way to conquer this disaster. Therefore, a hyperpolarization study using SABRE may be a good and key start to applying MRI scanning by using hyperpolarized chloroquine/lopinavir in the future. To the best of our knowledge, this study is the first to study the hyperpolarization of chloroquine and lopinavir in real time using SABRE.

Experimental Section

Chloroquine diphosphate salt (98.5%) and lopinavir (98%) were acquired from Sigma-Aldrich. Methanol-*d*$_4$ (CD_3OD, 99.8 atom % D, Eurisotop) was used as received. ^1H NMR and ^{13}C NMR spectra employed for the characterization of chloroquine and lopinavir were acquired on a

Bruker Avance III NMR spectrometer operating at a ^{1}H resonance frequency of 300 MHz and referenced to the residual CH_3 peak of CD_3OD (δ = 3.31). Hyperpolarization studies were conducted in the same manner. A home-built instrument designed as the *para*-hydrogen generator for hydrogen gas (a mixture of the spin isomers ortho-hydrogen and *para*-hydrogen) was conceived to go through a heat exchanger packed with a FeO(OH)catalyst (Sigma Aldrich).[26] This structure was filled with liquid nitrogen in a Dewar flask and produced ca. 50% *para*-hydrogen. In each experiment, *para*-hydrogen was continuously flowing into the chloroquine sample at a rate of 6 mL/min at 23 °C and 1 atm. To obtain the various magnetic field data, the following system was established and developed: The power supply was GPS-1850D (Bench Power Supply, Linear DC). A shielded coil wound with copper wire and a shielded coil outside the first consisted of a 200 mm diameter and 190 mm height. The magnetic field through the shielded coil was regulated by setting the current, which was in the range 0-5 A.

A sample of the hyperpolarization for chloroquine was prepared by treating a solution of chloroquine diphosphate salt (2 mg, $3.9x10^{-3}$ mmol) and the pre-catalyst ([Ir(IMes)(COD)Cl], 2 mg, $3.1x10^{-3}$ mmol) in CD_3OD (900 μL). In case of the lopinavir (10 mg, $1.6x10^{-2}$ mmol) was added to the CD_3OD (900 μL) solution of pre-catalyst ([Ir(IMes)(COD)Cl], 2 mg, $3.1x10^{-3}$ mmol). The mixture of chloroquine and the pre-catalyst was bubbled by *para*-hydrogen for 20 min for activation on the NMR tube. The mixture was analyzed in the NMR spectrometer, all NMR spectra were acquired with 1 scan in a varying magnetic field (Earth's magnetic field, 30 G, 50 G, 70 G, 90 G, and 110 G, respectively). The experiment of lopinavir was conducted in the same manner as mentioned above.

Results and Discussion

Chloroquine SABRE Chloroquine contains a quinoline structure, which potentially could be hyperpolarized using SABRE. However, chloroquine has a long attachment (Figure 1), which could not be operated because SABRE is dependent on the chemical exchange reaction and it is hard to expect its binding and kinetics to be transferred to the hyperpolarization with enough time from *para*-hydrogen (Figure 2).

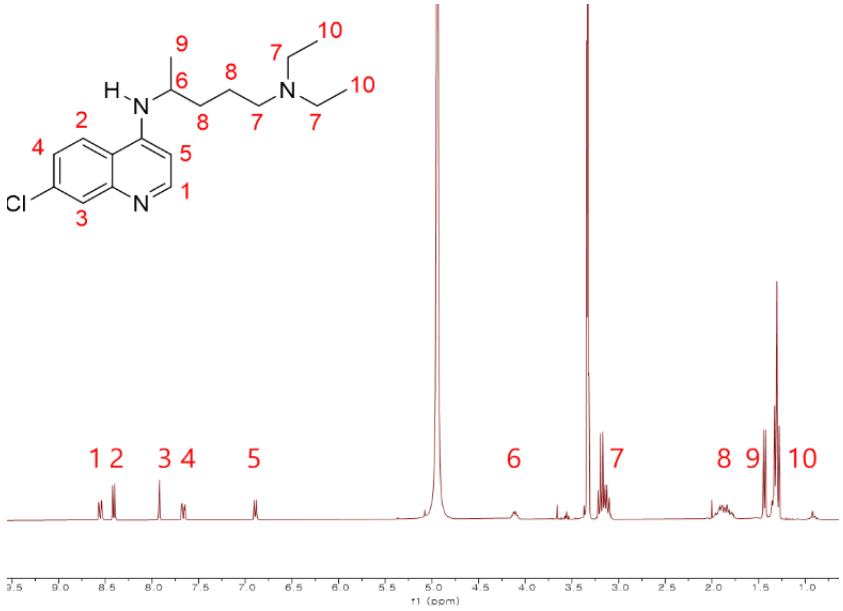

Figure 1. Chloroquine molecular structure and its normal NMR signal.

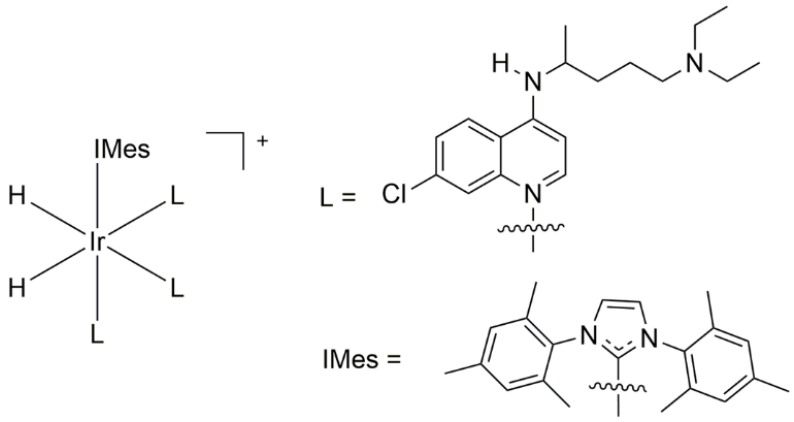

Figure 2. Ir-catalyst and Chloroquine complex structure for SABRE in methanol

Interestingly, polarization transfer from *para*-hydrogen to chloroquine is noteworthy because of the polarization transfer across the long distance of 11 bonds (from nitrogen to hydrogen number 10), which is the first case of observing long-range hyperpolarization via SABRE. (Figure 3)

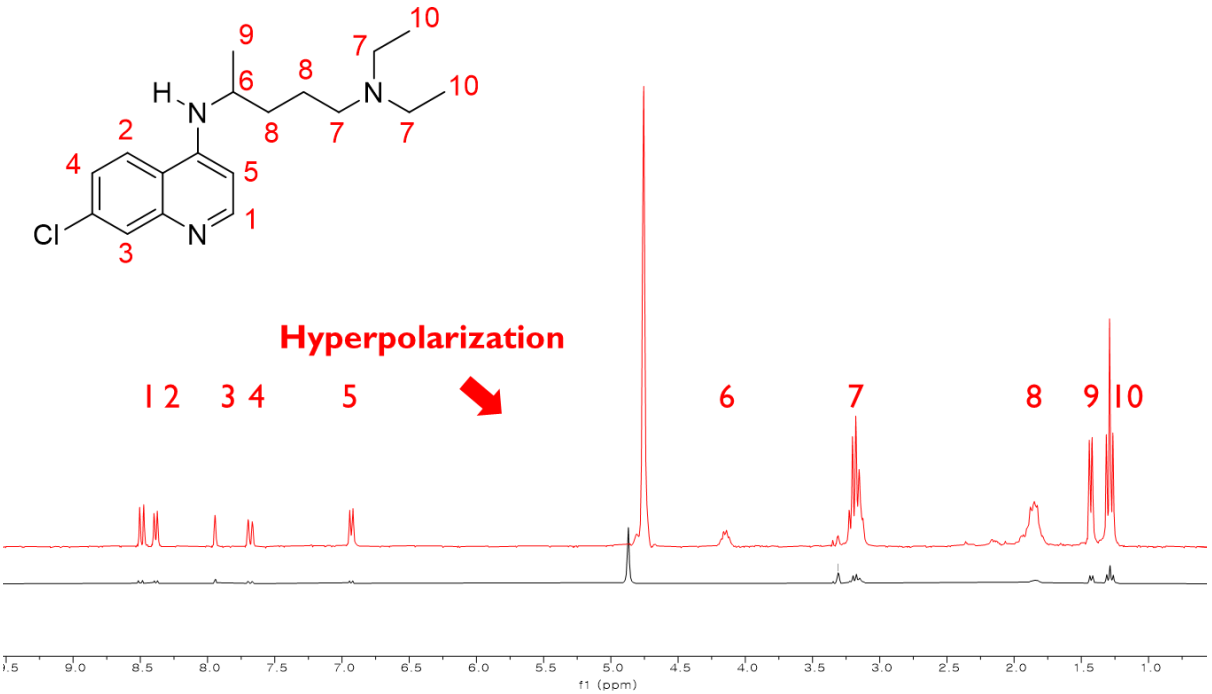

Figure 3. Hyperpolarized signal from chloroquine after SABRE in the presence of a 90 G magnetic field.

To understand its mechanism, the enhancement of chloroquine by hyperpolarization is measured by changing the magnetic field in the polarization transfer period. (Figure 4)

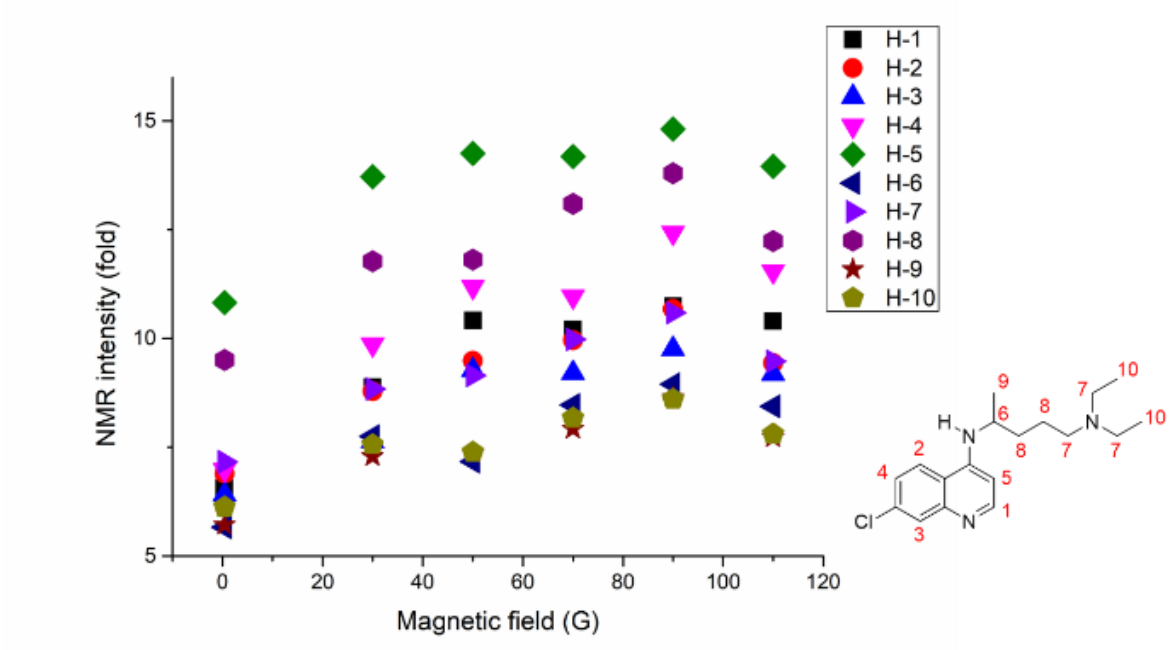

Figure 4. Amplification number of protons from hyperpolarized chloroquine structure.

Despite its relatively small polarization enhancement, it is expected to be increased by using a higher *para*-hydrogen concentration and partial pressure. Therefore, this enhancement could be drastically improved. As Figure 4 indicates, polarization is maximized around 90 G, which is almost the same trend as the previous reports on the polarization transfer mechanisms. Interestingly, H-8 has the second highest polarization enhancement after SABRE. This might stem from the direct polarization transfer from hydride, which is bonded to the Ir-catalyst to H-8 by dipolar coupling or polarization transfer from H-5 to H-8 by dipolar coupling, which needs to be studied in more detail in the future.

Lopinavir SABRE Lopinavir does not contain any sp^2 nitrogen in the structure, which has been widely used for polarization transfer, however, it has the carbonyl group, which can transfer polarization from *para*-hydrogen to carbonyl group with ester group in the neighborhood. Recently, a research paper that carbonyl group can bind to Ir-catalyst and polarization can be transferred to pyruvate is reported [27]. Futher we proved that the Ir-catalyst is binding with carbonyl and phenyl ether by detecting the chemical shift of protons of **1** (figure 5) after forming complex.

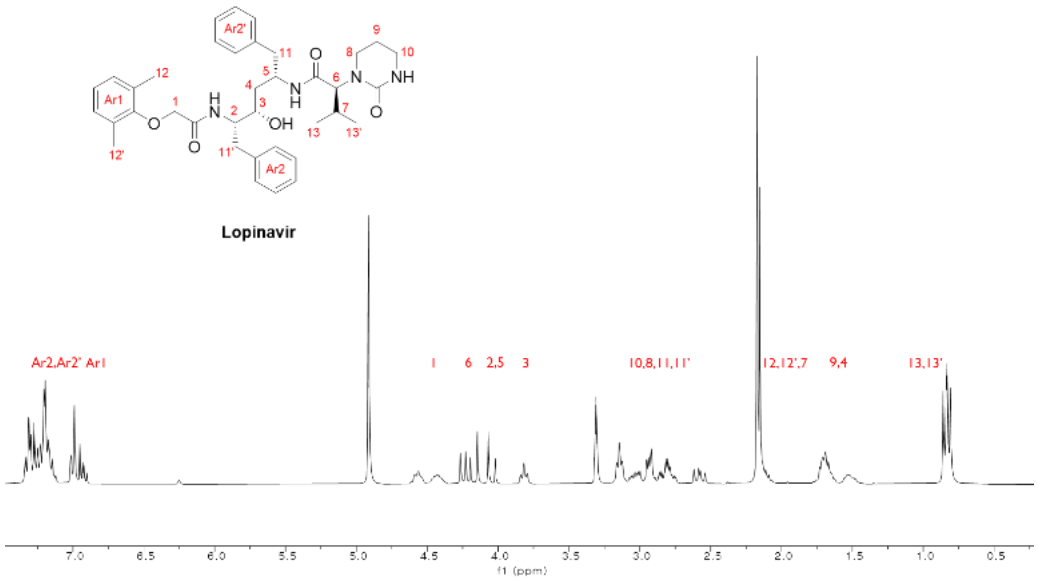

Figure 5. Lopinavir molecular structure and its normal NMR signal. Proton of **1** is shifted after binding with Ir-catalyst (data not shown).

This binding trend is also the first case to be identified and it is interesting to find out that the polarization is transferred to almost all of protons of lopinavir through this binding site.

As polarization transfers from *para*-hydrogen to lopinavir, it is noteworthy because the polarization can transfer across the long distance of more than 12 bonds, which is also the first case of observing extremely long-range hyperpolarization via SABRE after binding with carbonyl group. (Figure 6)

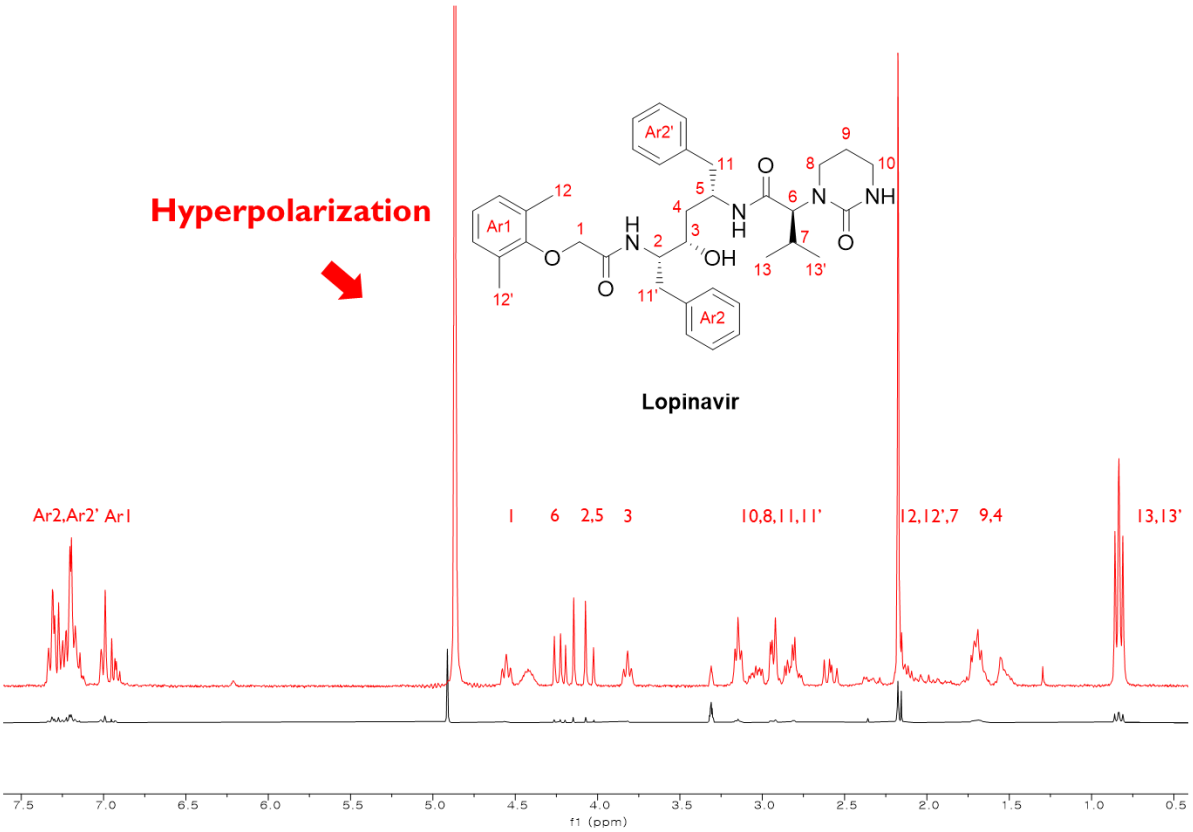

Figure 6. Hyperpolarized signal from lopinavir after SABRE in the presence of a 70 G magnetic field.

The enhancement of proton NMR signal on lopinavir by hyperpolarization is measured by changing the magnetic field in the polarization transfer period to understand SABRE mechanism. (Figure 7)

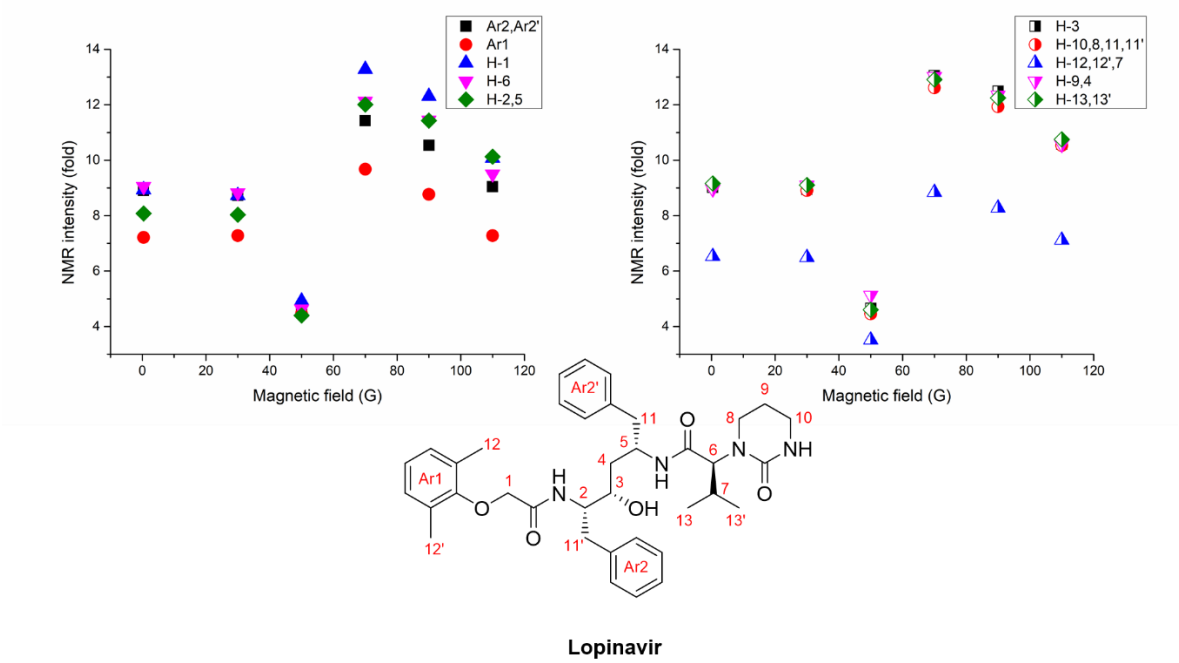

Figure 7. Amplification number of protons from hyperpolarized lopinavir structure.

This enhancement is also relatively small; however, it is expected to be increased by using a higher *para*-hydrogen concentration and partial pressure. As Figure 7 shows, polarization is maximized around 70 G, which is almost the same trend as the previous reports on the polarization transfer mechanisms including chloroquine case. It is impressive to see that H-8 and H-11 has the highest polarization enhancement during SABRE. This might attribute to the dipolar coupling, polarization transfer through the space, and SABRE-RELAY mechanism, which needs to be studied in more detail in the future.

These both long-range polarization transfers imply its potential use with hyperpolarized signals in NMR/MRI. This can provide a better understanding of molecular dynamics with targeted proteins and pharmacokinetics. More importantly, it is expected to be used for tracking the hyperpolarized signal through MRI in future studies. Examples include isotope labeling (such as carbon to ^{13}C isotope and nitrogen into ^{15}N) in the structure of chloroquine and lopinavir, which might have a long T1 time. This is not just because of the lower gyromagnetic ratio, but also because of its smaller relaxation effect from the Ir-catalyst by short binding time due to its relatively unstable complex structure.

Conclusions

Chloroquine and lopinavir are known to be promising substances in responding to the COVID-19 pandemic situation. Spin hyperpolarization on chloroquine and lopinavir could open new opportunities in the diagnosis and biomedical research of COVID-19 via MRI and pharmacodynamics, metabolomics, and binding dynamics with proteins, which can be achieved by using enhanced signal intensity in NMR/MRI. Furthermore, this SABRE-based hyperpolarization study based on these high molecular weight structures, was not performed earlier due to its special polarization transfer mechanism. Herein, high molecular weight chloroquine and lopinavir were successfully tested for its SABRE-based hyperpolarization. Interestingly, polarizations over long distances were detected; this is worth noting for future research, as this technique can be used for other materials. Importantly, this method can be used to monitor the drug's spread and activity *in vivo* by MRI, and it can be harnessed to further investigate about the molecular interaction of drug candidates with key proteins and to unveil its unknown activity on COVID-19, including pharmacokinetics.

Moreover, this polarization of the proton can be transferred to any other isotope nuclei. This leads to the ultimate objective of obtaining hyperpolarized chloroquine and lopinavir to study its activity on COVID-19 with a sufficiently long time of T1 in future studies.

This study could not shed significant light on the real applications of the treatment of COVID-19; however, many related studies have reported applications in drug discovery, detecting tumors *in vivo*, and polarization transfer into many other isotopes along with pulse sequence development. Future work on isotope labeling, and further polarization transfer on long T1 time nuclei including clinical perspectives will open a new door for overcoming this dreadful catastrophe.

ACKNOWLEDGMENT

This work was supported by the National Research Foundation of Korea(NRF) grant

funded by the Korea government(MSIT) (No. 2020R1C1C1007888).

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