**In-silico** Studies of Antimalarial-agent Artemisinin and Derivatives Portray More Potent Binding to Lys353 and Lys31-Binding Hotspots of SARS-CoV-2 Spike Protein than Hydroxychloroquine: Potential Repurposing of Artenimol for COVID-19.

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**Abstract:**
In this paper, we demonstrate how hydroxychloroquine can act as a good inhibitor of SARS-CoV-2 Spike protein receptor-binding-domain using molecular docking studies. We also unveil how hydroxychloroquine can interfere in the prevention of Lys353 in hACE2 from interacting with the corresponding binding hotspot present on the Spike protein. Further screening of artemisinin & derived compounds produced better Vina docking score than hydroxychloroquine (-7.1 kcal mol⁻¹ for the best scoring artemisinin derivative vs. -5.5 kcal mol⁻¹ for hydroxychloroquine). Artesunate, artemisinin and artemimol, showed two mode of interactions with Lys353 and Lys31 binding hotspots of the Spike protein. Given that these molecules are effective antivirals with excellent safety track records in humans against various ailment, we recommend their potential repurposing for the treatment of SARS-CoV-2 patients after successful clinical studies. In addition, an extraction protocol for artemisinin from *Artemesia annua* L. is proposed in order to cope with the potential urgent global supplies.

**Keywords:** SARS-CoV-2; COVID-19; Spike protein; hACE2; Antiviral; Hydroxychloroquine; Artemisinin
Introduction

The spread of COVID-19 pandemic has triggered a race to unveil the secrets of severe SARS-CoV-2 and its underlying acute respiratory syndrome. It has been confirmed that the binding of the viral trimeric surface spike glycoprotein (SP) of SARS-CoV-2 to the human receptor angiotensin-converting enzyme 2 (hACE2) is the first step in host infection.[1] Yan et al. identified the structure of hACE2 as a dimer in complex with membrane protein, they also showed that the two trimeric SP proteins of the receptor binding domain (RBD) of SARS-CoV-2 bind very tightly to the hACE2 dimer.[2]

Recently, remdesivir and chloroquine have been proved to inhibit in vitro Vero E6 cells of emerged novel coronavirus (2019-nCoV).[3] Chloroquine has been envisaged for SARS-CoV-1,[4] and has long been used for malaria therapy but has been replaced with hydroxychloroquine (HCQ) due to the increased Plasmodium falciparum parasite resistance, whereas an overdose of CQ can cause acute poisoning and death.[5] In fact, HCQ has been found to be effective in inhibiting SARS-CoV-2 infection in vitro and attenuate inflammatory response.[6] Organic extracts of Artemisia annua L. have been found to be more effective, faster, and less toxic than CQ and HCQ in treating malaria. A. annua contains a vital compound known as artemisinin, a sesquiterpene lactone with a peroxide linkage exhibiting low toxicity (Table 1), also the parent compound for semisynthetic derivatives chemically modified at the C-10 position to produce artesunate, arteether, arteether, artemimol (dihydroartemisinin), and artelinic acid (Table 1). These compounds, and in some cases their sodium salts, have been formulated as antimalarials for oral, rectal, and parenteral administration.[7]

Several reports proved the efficiency of artemisinin derivatives as potent antivirals for HPV bovine viral diarrhea virus (BVDV) for the treatment of anal and cervical intraepithelial high-grade neoplasia, human herpes virus-6 (HHV-6), human immunodeficiency virus (HIV) and more particularly, artesunate, against human cytomegalovirus (HCMV).[8] This antiviral potency put artemisinin class of compounds as promising candidates for the treatment of patients suffering from SARS-CoV-2 virus. The encouraging results generated from the utilization of HCQ to treat patients suffering from COVID-19 pandemic further raises many questions surrounding its mode of action. It is suggested that the mechanism of action of HCQ follows the same approach to that of CQ; however, the absence of binding assay studies between the SP protein and hACE2 protein in the presence of HCQ opens the door to two main possibilities: the first possibility revolves around HCQ prevention of terminal glycosylation of hACE2 protein which consequently impacts the final attachment between the SP protein and hACE2 protein, whereas the second possibility revolves around HCQ interaction with the receptor binding domain (RBD) of the SP protein, thus preventing its docking on the hACE2 receptor.[9]

To further expand on the second possibility, we elected to perform computational studies based on molecular docking to help us understand more about the mode of interaction between HCQ and the RBD of SARS-CoV-2 SP protein, and ultimately, how such interaction prevents the SP protein from docking on the hACE2. In addition, such mode of interaction is compared with another class of antimalarial and antiviral molecules comprised of artemisinin and artemisinin derived compounds.
Experimental section

The PDB file of SARS-CoV-2 SProtein RBD-hACE2 complex (PDB Ref. 6LZG, version 1.0) was obtained from the Research Collaboratory for Structural Bioinformatics (RCSB) protein data bank (PDB) (http://www.rcsb.org/structure/6LZG). UCSF Chimera1.14 was used to visualise the structure of the ligand and/or protein-complex structure, to perform the various functions associated with ligand and protein preparations, and acting as an interface to enable molecular docking calculations using locally hosted AutoDock Vina software.[10, 11] Prior to molecular docking, the hACE2 protein (part A) was deleted from the PDB file of the complex. In addition, all non-standard residues including that of water were also removed. The structure of each ligand was incorporated into UCSF Chimera using SMILES string followed by structure minimisation.

The PDBQT files of the SProtein RBD and each ligand was generated after adding all hydrogens and charges to each structure. The SProtein docking search box was assigned by varying the receptor search volume in terms of Center and Size to cover the contact area previously observed between the SProtein RBD and hACE2. The number of binding modes was set to 10 with exhaustiveness of search set to 8. The maximum energy difference was set to 3 kcal mol$^{-1}$. The obtained molecular docking results were then aligned with the PDBQT file of the SProtein RBD-hACE2 complex in order to visualise the type of interactions of each docked molecule in the SProtein-hACE2 binding interface.

Results and Discussions

Most explanations associated with HCQ mode of action are based on findings revolving around the mode of action of CQ on SARS-CoV infection.[12, 13] Amongst the cited reasons are: (i) CQ can increase the value of endosomal pH which can reduce the transduction of SARS-CoV pseudo-type viruses, [12, 13] (ii) CQ can raise the possibility of affecting the endosome-mediated fusion if added at the initial stage of the infection,[9] and (iii) once the virus is inside the cell, CQ can inhibit the production of glycoproteins in vesicular stomatitis, thus preventing virus replication.[14]

Previously, Vincent et al. showed that introduction of CQ prevents terminal glycosylation of ACE2 receptor protein of the host cell, thus destabilizing the recognition mode of the SProtein on the surface of the virus, albeit such action did not impact the level of expression of surface hACE2 proteins of the host cell.[9] Despite the validity of the latter point, the authors fell short of performing binding assay studies of SARS-CoV SProtein with hACE2 in the presence and absence of CQ to further validate their rationale.[9] Therefore, we elected to perform computational docking studies of HCQ against SProtein RBD of SARS-CoV-2 to further study the nature of such interaction and explore the inhibition potential of HCQ to the SProtein.

Molecular docking studies

Our molecular docking studies of HCQ on the RBD of SARS-CoV-2 SProtein produced a Vina score of -5.5 kcal mol$^{-1}$. The obtained scoring is relatively good given that Vina score of the best molecule, i.e., Physalin b, previously docked on a homology model of SARS-CoV-2 SProtein RBD was -7.2 kcal mol$^{-1}$.[15] Analysis of our docking results showed that the hydroxyl group (OH) of HCQ molecule formed strong hydrogen bonding with Asn501 side chain residing on one part of the receptor binding motif (RBM) of the SProtein (Figure 1).
In the SProtein-hACE2 complex, Asn501 of SARS-CoV-2 S protein forms favourable hydrogen bonding with Tyr41 of hACE2 while at the same time helps neutralising the charge of Lys353.[16] Therefore, the resulting hydrogen bonding between the OH group of HCQ and Asn501 can play a role in destabilising other interactions with hACE2 residues, e.g., Tyr41, Lys353, Gly354 and Asp355, which were already found to play key roles in the interaction with the SProtein.[2, 16]

Furthermore, when our initial docking results were aligned with the structure of SProtein-hACE2 complex, we successfully observed significant clash between the aminoalkyl side-chain of HCQ and the Lys353 residue side-chain of hACE2. (Figure 2) Equally, Lys353(O) was found to form one hydrogen bonding with Gln502 of the SProtein in the complex;[16] at the same time, Lys353(N) forms important hydrogen bonding with Gln496 while maintaining a salt bridge with Asp38.[2, 15, 16] In hACE2, Lys353 and Lys31 were both found to be important hotspots responsible for binding to the SProtein of SAR-CoV-2.[16] It is believed that the latter specie developed key mutations to help stabilise and/or neutralise both lysine hotspots via introduction of Asn501 (to neutralise Lys353) and Gln 493 & Leu 455 (to neutralise Lys31) of hACE2 protein, thus ensuring tighter incorporation of both hotspots deep into the hydrophobic pockets of the SProtein. Such observations clearly explain the main reason behind the higher affinity of SARS-CoV-2 S protein to hACE2 compared to that of SARS-CoV.[16] Therefore, it is very likely that selective interaction of HCQ with the surface of SARS-CoV-2 SProtein through the formation of an inclined tape over the hydrophobic pocket responsible for hosting the Lys353 hotspot (the OH group in this case is acting like a hook by forming a hydrogen bond with Asn501), can be responsible for the prevention of tighter binding with hACE2 protein via restricting penetration of Lys353 into its finally assigned destination on the SProtein RBD. (Figure 2)

Similar to Asn501 in SARS-CoV-2, Thr487 in SARS-CoV SProtein was previously found to interact with Tyr41, Lys353, Gly354 and Asp355 in hACE2 protein.[17] The existence of such similar hydrogen bond networking system between both types of SProteins, i.e., that of SARS-CoV and SARS-CoV-2, and that of hACE2 may be used to explain the greater efficacy of HCQ in inhibiting SARS-CoV and
SARS-CoV-2 interaction with hACE2. On the other hand, our molecular docking results also showed good interaction between the quinoline aromatic ring in HCQ and His34 in hACE2; in normal circumstances, the latter residue interacts well with Leu455 and Gln493 of the SProtein. By doing that, HCQ can also disturb interaction in the middle region of the binding interface between the SProtein and hACE2 (Figure 2).

With this information on hand, we then elected to perform in-silico screening of other potent antimalarial compounds derived from the core structure of artemisinin; by doing so, we believe we can gain more insight about the potential use of such class of compounds as safer and more potent substitutes to the currently widely used HCQ. In this regard, a total number of 11 compounds were successfully screened against SProtein RBD using the same molecular docking approach previously followed with HCQ. The obtained results are shown in Table 1.

**Figure 2:** Alignment of HCQ top Vina pose with SProtein RBD-hACE2 complex structure. Hydrogen bondings are shown in blue lines. Clashes/contacts are shown in yellow lines. Direct clash is observed between the aminoalkyl chain of HCQ and the Lys353 side-chain. The secondary structure of SProtein RBD is shown in cyan whereas that of hACE2 is shown in green. HCQ is shown in purple.
Table 1: Top Vina score of artemisinin and its derivative compounds against SProtein RBM.
(Highlighted in light-grey are compounds with good clinical records)

| Entry | Name                      | Structure | Vina Score (kcal mol⁻¹) |
|-------|---------------------------|-----------|------------------------|
| 1     | Artelinic acid            | ![Structure](artelinic_acid.png) | -7.1                   |
| 2     | Artesunate                | ![Structure](artesunate.png)   | -6.8                   |
| 3     | Artemisone                | ![Structure](artemisone.png)   | -6.6                   |
| 4     | Artemisinin               | ![Structure](artemisinin.png)  | -6.5                   |
| 5     | Artemiside                | ![Structure](artemiside.png)   | -6.4                   |
| 6     | Artenimol (Dihydroartemisin) | ![Structure](artenimol.png) | -6.4                   |
| 7     | Artemotil                 | ![Structure](artemotil.png)    | -6.3                   |
| 8     | PubChem CID: 90667934     | ![Structure](pubchem_90667934.png) | -6.3                   |
| 9     | PubChem CID: 122185220    | ![Structure](pubchem_122185220.png) | -6.3                   |
| 10    | Artemether                | ![Structure](artemether.png)   | -6.0                   |
| 11    | PubChem CID: 10380074     | ![Structure](pubchem_10380074.png) | -6.0                   |
| 12    | HCQ                       | ![Structure](hcq.png)         | -5.5                   |
Analyses of the data show that artemisinin and its derivative compounds have scored better than HCQ, with compounds in Entry 9, 10 & 11 of Table 1 producing the least and closest Vina score (-6.0 kcal mol⁻¹) to HCQ. On the other hand, artelinic acid (Table 1, Entry 1) gave the best Vina score of -7.1 kcal mol⁻¹, followed by artesunate (Table 1, Entry 2) with Vina score of -6.8 kcal mol⁻¹. Although artemisinin and its derivative compounds resulted in good Vina scoring (-6.0≤Scores≤7.1 kcal mol⁻¹), only three compounds namely, artesunate (Table 1, Entry 2), artemisinin (Table 1, Entry 4) and artemimol (Table 1, Entry 6) were found to possess good clinical records, thus enhancing their potential to be repurposed for the treatments of SARS-CoV-2. Besides its antiviral activity, artemisinin derivatives hold immunoregulatory properties and modulate neutrophils, T-cell and B-cell components of the immune system, but enhancing system immunity and touting themselves as promising candidates to synergistically enhance their antiviral effect in vivo and treat inflammation-associated diseases.[18]

The nature of interaction between the aforementioned three compounds and the RBD of SARS-CoV-2 SProtein was also analysed in order to see which molecule best influence the repulsion of hACE2 Lys353 and Lys31 from binding to the inner hydrophobic pockets of the SProtein. By analysing the top scoring pose of artesunate (Table 1, Entry 2), we observe that the molecule binds far away from the hydrophobic regions of Lys353 and Lys31 hotspot binding sites (Figure 3). Furthermore, upon alignment of artesunate docking results with the structure of SProtein-hACE2 complex, no clashes were observed between artesunate structure and the other side-chains present in the hACE2 protein. However, the carboxylic acid moiety of the artesunate side chain was observed to form hydrogen bonding with Lys353(N), which can further neutralise the overall charge through the formation of a salt bridge, this can adversely lead to tighter interaction between hACE2 and SProtein. Therefore, in spite of the high Vina scoring associated with artesunate, we predict that this molecule is unlikely to act as a good inhibitor, in its current form, to SARS-CoV-2 SProtein (Figure 4).
In the case of artemisinin's (Table 1, Entry 5) top pose, despite no hydrogen bonding is observed with the SPProtein RBD, we notice a lateral incorporation of the six-membered ring cyclohexane group of artemisinin into the Lys353 hotspot binding pocket, with the peroxy bridge facing the peripheral hydrophilic surface of the binding region (Figure 5 (a), region coloured in blue next to the peroxy-bridge). Such mode of interaction could well be used to prevent the penetration of Lys353 side-chain into the hydrophobic pocket (Figure 5).

**Figure 4:** Top Vina scoring pose of artesunate aligned in the binding interface of SPProtein RBD-hACE2 complex. Shown in cyan is the SPProtein RBD whereas that in green show hACE2. Artesunate is shown in purple.

**Figure 5:** Top Vina pose of artemisinin on SPProtein RBM (Lys353 binding hotspot) following molecular docking. (a) Columbic surface characteristics of part of the SPProtein RBM bound to artemisinin. (b) Secondary structure of part of SPProtein RBM bound to artemisinin.

**Figure 6:** Vina pose of artemisinin on SPProtein RBM (Lys31 binding hotspot) following molecular docking. (a) Columbic surface characteristics of part of the SPProtein RBM bound to artemisinin. (b) Secondary structure of part of SPProtein RBM bound to artemisinin.
Artemisinin was also found to interact with Lys31 hotspot binding pocket, although at slightly lower Vina score (-5.6 kcal mol\(^{-1}\)). The average score obtained in this binding region is perhaps attributed to the absence of hydrophilic surfaces close to the binding pocket (Figure 6).

Therefore, the selective interaction of artemisinin with both Lys353 and Lys31 hotspot binding regions raises its possibility to be repurposed for the treatment of SARS-CoV-2 patients following successful clinical trials.

Artemisinol on the other hand showed a similar mode of interaction to that of artemisinin with both Lys353 and Lys31 hotspot binding sites, although at slightly lower Vina scores of 6.4 and 5.4 kcal mol\(^{-1}\), respectively (Figure 7). Both artemisinin and artesunate are susceptible to CYP450 reduction to generate artemisinol once incorporated into the human body, albeit at different conversion rates. We therefore recommend that artemisinol can be prioritised for clinical trials to achieve the repurposing of such class of molecules for COVID-19, however, careful considerations need to be taken into account given the water solubility characteristics of each compound.\[^{[7]}\]

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**Figure 7:** Top Vina pose of artemisinol on SProtein RBM (Lys353 & Lys 31 binding hotspot) following molecular docking. (a) Columbic surface characteristics of part of the SProtein RBM bound to artemisinol in the Lys31 binding hotspot. (b) Secondary structure of part of SProtein RBM bound to artemisinol in the Lys31 binding hotspot. (c) Columbic surface characteristics of part of the SProtein RBM bound to artemisinol in the Lys353 binding hotspot. (d) Secondary structure of part of SProtein RBM bound to artemisinol in the Lys353 binding hotspot.
Proposed Artemisinin Extraction Process

Currently, major production of artemisinin is based on solvent extraction from *A. annua* despite modest but not scalable enough trials to produce it chemically or semi-synthetically via its precursor artemisinic acid in engineered bacteria.[19, 20] Artemisinin is abundant in *A. annua* leaves (0.4-1.4%) and production includes several steps starting with screening and drying before biomass being processed generally via solvent leaching or percolation at 30-50 °C using low polarity solvents like hexane, toluene or petroleum.[21] This operation is not selective for artemisinin, therefore terpenes, fatty acids and some pigments are inevitably co-extracted which calls for secondary refinery steps including adsorption, flash chromatography and sequential crystallization.

Herein, an indicative facile production setup is proposed to enhance worldwide production capacity (Figure 8). However, a fire-proof equipment and facility is a pre-requisite to ensure safety and security measures are met. The plant comprises an extraction step (1) in which biomass is placed in hollow-fibre bags and processed at 40 °C for 60 minutes using a solvent mixture of hexane/ethyl acetate (95:5 v/v) with solvent/biomass ratio of 6 to 1 (6 L for each Kg). This step can be conducted by means of a stirring tank or a percolation type reactor to ensure up to 99% extraction yield is achieved. Then, the mixture passes through a cloth or diaphragm plate and frame filter-press (2) in order to discard fine biomass particles and recover the extract. The latter is submitted to an adsorption bed column (3) filled with activated carbon aiming at the removal of pigments and tannins. Another clarification stage is required to remove other impurities such as free fatty acids and pigments; for instance, an adsorption bed column (4) filled with Celite 545 (Merck) is recommended. Due to some affinity with activated carbon and Celite 545, an artemisinin loss of 3-5% is expected.

After that, the purified extract should be concentrated to at least 1/8th its initial volume using an evaporator. The concentrated extract is submitted into a jacketed crystallization reactor (6) equipped with a stirring shaft set at tip speed in the range of 100-150 rpm. The cooling rate is set to 0.5 °C/min to reach 4 °C and is kept at this temperature for 60 minutes to let artemisinin crystals settle down. The crystals are sent into a spray drying system (7) to recover high purity crystals of 98-99% as a final product. The overall yield of artemisinin is expected to reach 60% from the initial content of artemisinin in *A. annua* leaves. The spent mother liquor is guided for another row of crystallization with longer residence time. The recovered crude crystals are washed with cold ethanol to recover purer artemisinin and increase the final yield.
Figure 8: Simplified extraction plant for high purity Artemisinin

1: Extraction reactor; 2: Frame and plate filter-press (or vibrating-screener/decanter); 3: Adsorption column bed; 4: Clarification column bed; 5: Crystallization stirred reactor; 6: Spray dryer; 7: Distillation column; 8: Solvent storage tank

Conclusion

The inhibition of SARS-CoV-2 SProtein RBD with HCQ was successfully studied using molecular docking techniques. HCQ was found to selectively interact with the Lys353 hotspot binding pocket via the formation of an inclined tape over the binding site with the OH group of HCQ acting like a hook. Artemisinin class of compounds were also found to interact the same binding pocket. In addition, artemisinin & derived molecules showed extra mode of interaction with the Lys31 binding hotspot, although at slightly lower Vina score. These results demonstrate the likelihood of repurposing artemisinin as a less toxic HCQ substitute to block the SProtein RBD of the virus from docking onto hACE2, while at the same time enhancing the immune system of the patient. More focus should be intended to study the in-vivo mode of action of artemimol as most artemisinin derivatives are converted to this compound once incorporated to the body.

Author Contributions

"These authors contributed equally."

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References

[1] P. Zhou, X. Yang, X. Wang, B. Hu, L. Zhang, W. Zhang, H.R. Si, Y. Zhu, B. Li, C.L. Huang, H.D. Chen, J. Chen, Y. Luo, H. Guo, R.D. Jiang, M.Q. Liu, Y. Chen, X.R. Shen, X. Wang, X.S. Zheng, K. Zhao, Q.J. Chen, F. Deng, L.L. Liu, B. Yan, F.X. Zhan, Y.Y. Wang, G.F. Xiao, Z.L. Shi, Nature 2020, 579, 270, https://doi.org/10.1038/s41586-020-2012-7

[2] R. Yan, Zhang Y., Li Y., Xia L., Guo Y., Zhou Q., Science 2020, 367, 1444, https://doi.org/10.1126/science.abb2762
[3] M. Wang, R. Cao, L. Zhang, X. Yang, J. Liu, M. Xu, Z. Shi, Z. Hu, W. Zhong, G. Xiao, *Cell Res.* 2020, 0, 1, https://doi.org/10.1038/s41422-020-0282-0

[4] E. De Clercq, *Expert. Rev. Anti Infect. Ther.* 2006, 4, 291

[5] H. Weniger, *Bull. World Health* 1979, 79, 906

[6] J. Liu, R. Cao, M. Xu, X. Wang, H. Zhang, H. Hu, Y. Li, Z. Hu, W. Zhong, M. Wang, *Cell Discov.* 2020, 6, 16, https://doi.org/10.1038/s41421-020-0156-0

[7] C.J. Woodrow, R.K. Haynes, S. Krishna., *Postgrad Med J* 2005, 81, 71, https://doi.org/10.1136/pgmj.2004.028399

[8] S. D’Alessandro, D. Scaccabarozzi, L. Signorini, F. Perego, D. P. Ilboudo, P. Ferrante, S. Delbue. *Microorganisms* 2020, 8, 85, https://doi.org/10.3390/microorganisms8010085

[9] M.J. Vincent, , E. Bergeron, S. Benjannet, B.R. Erickson, P.E. Rollin, T.G. Ksiazek, N.G. Seidah, S.T. Nichol, *Virol. J.* 2005, 2, 69, https://doi.org/10.1186/1743-422X-2-69

[10] E.F. Pettersen, Goddard T.D., Huang C.C., Couch G.S., Greenblatt D.M., Meng E.C., Ferrin T.E. *J. Comput. Chem.* 2004, 13, 1605

[11] O. Trott, A. J. Olson, *J. Comput. Chem.* 2010, 31, 455

[12] G. Simmons, Reeves J.D., Rennekamp A.J., Amberg S.M., Pipher A.J., Bates P., *Proc Natl Acad Sci USA* 2004, 101, 4240, https://doi.org/10.1073/pnas.0306446101

[13] Z.Y. Yang, Huang Y., Ganesh L., Leung K., Kong W.P., Schwartz O., Subbarao K., Nabel G.J., *J Virol* 2004, 78, 5642, https://doi.org/10.1128/JVI.78.11.5642-5650.2004

[14] B.J. Dille, Johnson T.C., *J Gen Virol* 1982, 62, 91

[15] S. Micholas, Jeremy C., ChemRxiv. 2020, https://doi.org/10.26434/chemrxiv.11871402.v4

[16] J. Lan, J. Ge, J. Yu, S. Shan, H. Zhou, S. Fan, Q. Zhang, X. Shi, Q. Wang, L. Zhang, X. Wang., *Nature 2020*, https://doi.org/10.1038/s41586-020-2180-5

[17] J. Shang, G. Ye, K. Shi, Y. Wan, C. Luo, H. Aihara, Q. Geng, A. Auerbach, F. Li, *Nature 2020*, https://doi.org/10.1038/s41586-020-2179-y

[18] W. Yao, F. Wang, H. Wang. *Sci. Bull.* 2016, 61,1399, https://doi.org/10.1007/s11434-016-1105-z

[19] V. Hale, J.D. Keasling, N. Renninger, T.T. Diagana, *Am. J. Trop. Med. Hyg.* 2007, 77, 198

[20] J.A. Dietrich, Y. Yoshikuni, K.J. Fisher, F.X. Woolard, D. Ockey, D.J. McPhee, N.S. Renninger, M.C.Y. Chang, D. Baker, J.D. Keasling, *ACS Chem. Biol.* 2009, 4, 261

[21] S. Chemat, S. Boudjelal, I. Malki, A. Lapkin, Ind. Crops and Prod. 2019, 137, 521