

Clinical Research Proposal
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Composed by EMSKE Phytochem



(Author)

Abstract

While much clinical research attention has focused on vaccines, mAbs, and certain small-molecule antivirals for COVID-19, by contrast there has been comparatively little attention on plant-derived compounds, especially those that are considered safe through different ingestion routes at common doses. The potential benefit especially to developing countries, but with application to countries such as the UK, the US, and in the EU, is strong: when effective antivirals are determined and verified, it will take months for the manufacturers to ramp up production to the quantities required for a worldwide population of patients, and the expense is likely to be prohibitive for many. Developing countries especially will be even later in line to receive those medications. But the prospect looms that rural countries might be able to grow their way to supply themselves if effective plant-derived compounds can be identified. With this intent, a repurposed off-the-shelf drug candidate is identified for early treatment trialing for COVID-19.

Table of Contents

Introduction

- Primary mechanism
- Further mechanisms

Study Drugs

Pharmacology

- Safety
- Bioavailability & Pharmacokinetics
- Drug-Drug Interactions
- Local authenticity verification

Clinical Trial Strategy

Project Risks

- Assessment of Project's Safety Risks
- Assessment of Project's Efficacy Risks

Clinical Trial Short-form Protocol

Execution Team

- Clinical Trial Execution Team
- Key Vendors
- Advisors
- Partners

Parallel Studies

Appendix

- Appendix A - In silico tool qualification
- Appendix B - Human longitudinal study of plant extract containing flavonoids
- Appendix C - Inventory of in silico assayed compounds

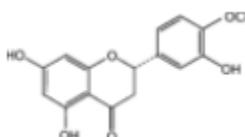
Introduction

Primary mechanism

In February 2020, Yu Wai Chen (Chen WY et al 2020) found in the course of an in silico study on SARS-CoV's (the virus causal to 2002-2004 SARS disease centered in China) main protease enzyme protein 3CLpro that hesperidin and diosmin were very effective at binding to the protein, with the presumption that doing so inhibits its operation to some extent. Considering a cleaving protein such as 3CLpro is effectively a very refined nanomachine the mechanism for doing so is akin to throwing a shoe into a machine - it 'stops up the works'.

(Chen WY et al 2020) noted that 3CLpro shares 95% sequence homology with SARS-CoV-2's own main protease enzyme - technically also a 3CLpro, but more typically referred to in the literature as Mpro for "Main protease".

Fully fifteen years earlier, a metabolite of hesperidin, hesperetin, had in fact been successfully verified on *inhibiting* the action of SARS-CoV's 3CLpro *in vitro* by Lin et al of China Medical University in Taichung, Taiwan, in 2005 (Lin et al 2005)

Compound	Structure	IC ₅₀ ^a of cell-free cleavage (µg/ml)	IC ₅₀ ^a of cell-based cleavage (µg/ml)	CC ₅₀ ^b of cell death (µg/ml)
Hesperetin		18.1 ± 0.6 (60 µM)	2.5 ± 0.8 (8.3 µM)	820 ± 15 (2718 µM)

From (Lin et al 2005)

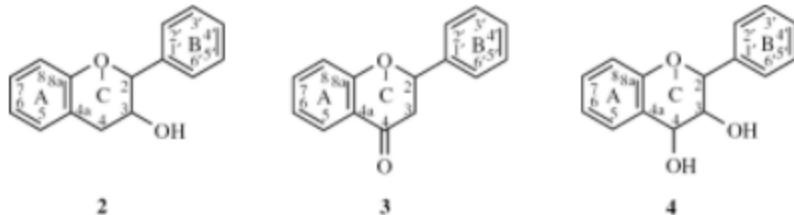
Particularly, that "hesperetin dose-dependently inhibited cleavage activity of the 3CLpro, in which the IC₅₀ was . . . 8.3 µM for hesperetin in the cell-based assay" (being 2.5mcg / mL as Lin et al. notes). Alongside, in the same paper, hesperetin shows 18.1 ug / mL IC₅₀ inhibition of 3CLpro in cell-free cleavage assay.

These literature findings and subsequent engagement with Yu Wai Chen inspired additional research into hesperidin, diosmin, and their analogues and metabolites.

The approach begins with in silico assays of similar compounds as hesperidin and diosmin on SARS-CoV's 3CLpro. Hesperidin and diosmin are flavonoids, and there are on the order of one thousand flavonoids known to exist that mostly share similar forms. More than that, in addition to (Lin et al 2005)'s paper, there are several other papers that document IC₅₀ inhibitory values on 3CLpro *in vitro* for different compounds. (Jo, Seri et al 2019), (Ryu et al 2010), (Nguyen et al, 2012)

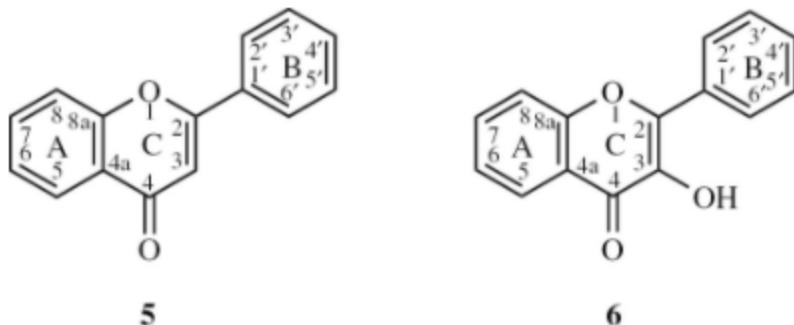
Flv-2.1.1.1 Flavans

Flavans are compounds with a 2-phenyl-3,4-dihydro-2*H*-1-benzopyran skeleton **1**, that may be substituted, and include flavan-3-ols (compounds derived from 2-phenyl-3,4-dihydro-2*H*-1-benzopyran-3-ol skeleton **2**) and flavan-4-ones (compounds derived from 2-phenyl-2,3-dihydro-4*H*-1-benzopyran-4-one skeleton **3**), which are generically designated as flavanols and flavanones, respectively. Compounds derived from 2-phenyl-3,4-dihydro-2*H*-1-benzopyran-3,4-diol skeleton **4** are flavan-3,4-diols, designated leukoanthocyanidins because these colourless compounds have their structure derived from that of an anthocyanidin (see Flv-2.1.1.3), specifically they are dehydronated 3,4-dihydroxy-1,2,3,4-tetrahydroanthocyanidins.



Flv-2.1.1.2 Flavones and 3-hydroxyflavones (flavonols)

Flavones and 3-hydroxyflavones (flavonols) are compounds with a 2-phenyl-4*H*-1-benzopyran-4-one (2-phenyl-4*H*-chromen-4-one) skeleton **5**. The term "flavonol" is used as a class name for compounds with a 3-hydroxy-2-phenyl-4*H*-1-benzopyran-4-one (3-hydroxy-2-phenyl-4*H*-chromen-4-one) skeleton **6**.



From: Nomenclature of flavonoids (IUPAC Recommendations 2017) (Rauter 2018)

Given the broad structural similarity across the flavonoid class, it was undertaken to explore more flavonoids in *in silico* molecular docking assays, along with other compounds encountered in the SARS-CoV *in vitro* literature.

In silico Tooling Verification

(This summary is expanded in detail in Appendix A). With a molecular docking program, one has latitude to specify what region of a protein the simulator should allow for the compound's docking. This is ordinarily accomplished by specifying a 'box size' and 'box center coordinates' which the program will treat as its searchable volume within the limits of its coinciding with the protein's surface binding sites.

One such kind of docking is a 'blind' docking wherein the entire protein is enclosed by the searchable box, so the program is free to locate sites across the protein's bindable surface. Another approach is to target the searchable volume to a known active target site of the protein or enzyme, such as the site an enzyme normally uses to initiate and execute its action.

Both SARS-CoVs 3CLpro and SARS-CoV-2's Mpro proteases are dimers. They are comprised of an A-chain and a B-chain. In SARS-CoV-2's case, the A-chain and B-chain are identical and symmetrical.

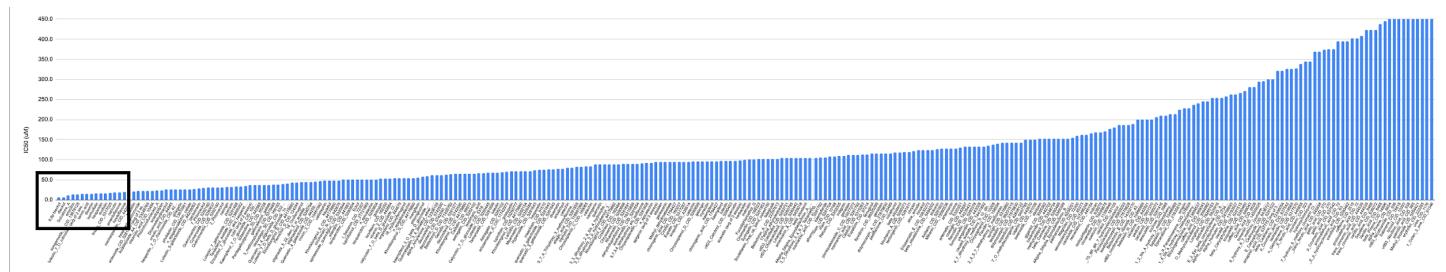
Both the SARS-CoV 3CLpro and SARS-CoV-2's Mpro have a "target active site", characterized by the residue pair "Histidine-41, Cystein-145". These A-chain and B-chain coordinates are then made the center of the search box for active site-targeting searches.

Several permutations of the Autodock Vina (Trott and Olson, 2010) trials, between blind docking and targeted active site docking, were compared against published in vitro inhibitory values of the same compounds in the literature. This calibration and tool qualification process is described in the tooling efficacy section of the Appendix. In finding the permutation that had a high correlation with published values, a model for correlating and mapping in silico to in vitro results was determined

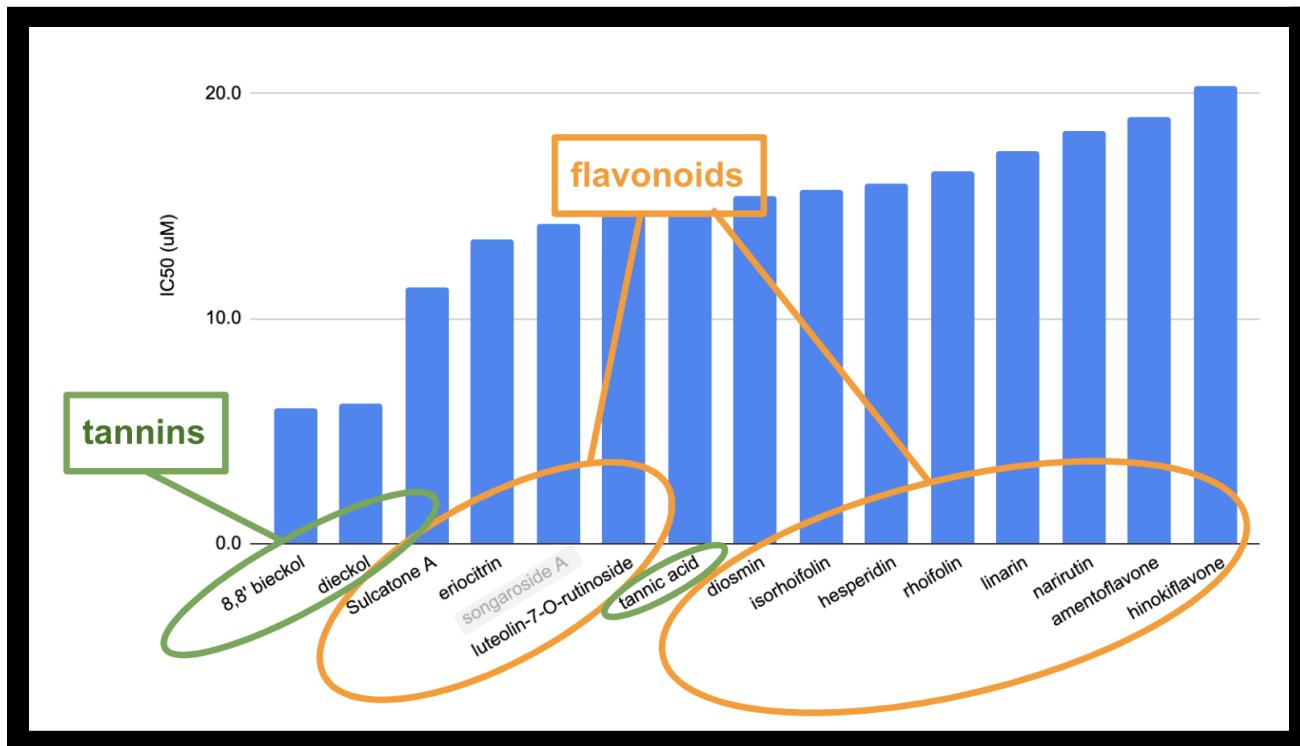
Critically benefiting from this tool verification, autodocking trials of strategically-selected compounds then proceeded in earnest. The protein file used was a variant of a PDB SARS-CoV-2 Main protease protein file 6yb7, adapted for ease of autodocking studies by Wai Yu Chen PhD into pdb format.

In silico assays

Given 1) hesperidin and its metabolites' strong potency results in the SARS-CoV in silico docking and in vitro literature, 2) diosmin and its metabolite's strong potency results in the SARS-CoV in silico docking literature, 3) amentoflavone's strong potency results in the docking in vitro docking literature, and 4) recognition that hesperidin and diosmin are flavonoids alongside that amentoflavone is a biflavonoid (two-flavonoid structure), it was deemed a likely pool of efficacious ligands would be found among other flavonoids, be they the root aglycone form or their glucosides. And so flavonoids became the focus of study.



(The full list of assayed compounds is in Appendix C). Zooming in on the top 5% performing compounds on the far left side:

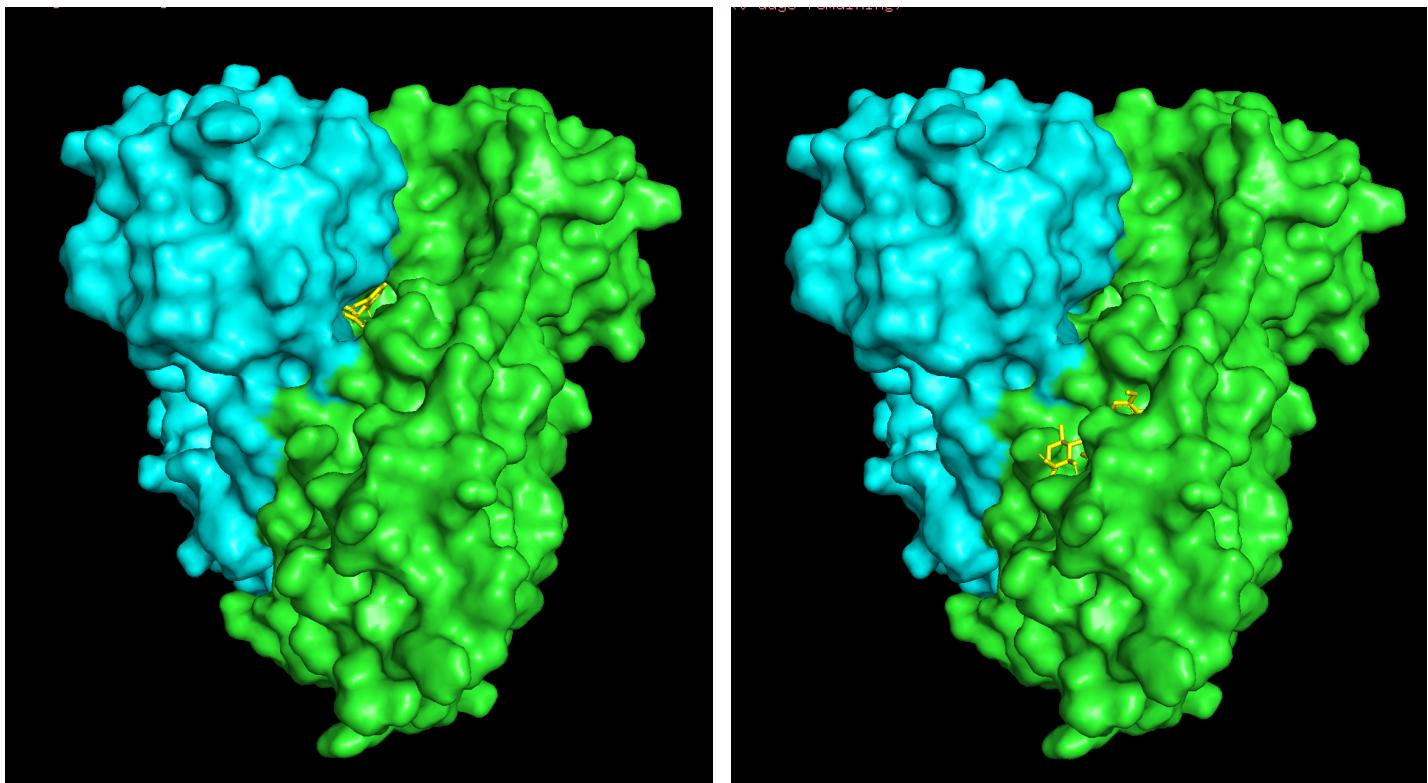


Projected IC₅₀ concentration based on SARS-CoV correlation to SARS-CoV-2 in silico docking results - top inhibiting compounds

The top performing classes of compounds are flavonoid glycosides and phlorotannins. The phlorotannins are common in brown algae / seaweed. Hinokiflavone is cytotoxic. Amentoflavone is common in gingko. The rest of the flavonoids are common in many plants, notably in the rinds of citrus (hesperidin & diosmin), or in the leaves of lemon trees (rhoifolin), or in certain mint varieties (eriocitrin, luteolin-7-O-rutinoside). Mango seed kernel is notable for prevalence of both of the highlighted families, containing both hesperidin and tannic acid. Our study focuses on flavonoids despite phlorotannins' quantitative superiority because flavonoids' pharmacology is better understood and is already available in the widely available off-the-shelf pharmaceutical preparation, Daflon, manufactured by France's Laboratoires Servier.

Inhibitory mechanism binding strategy

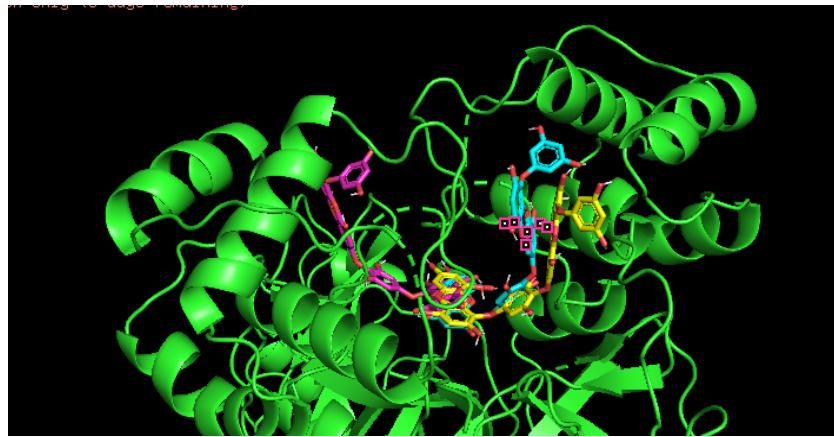
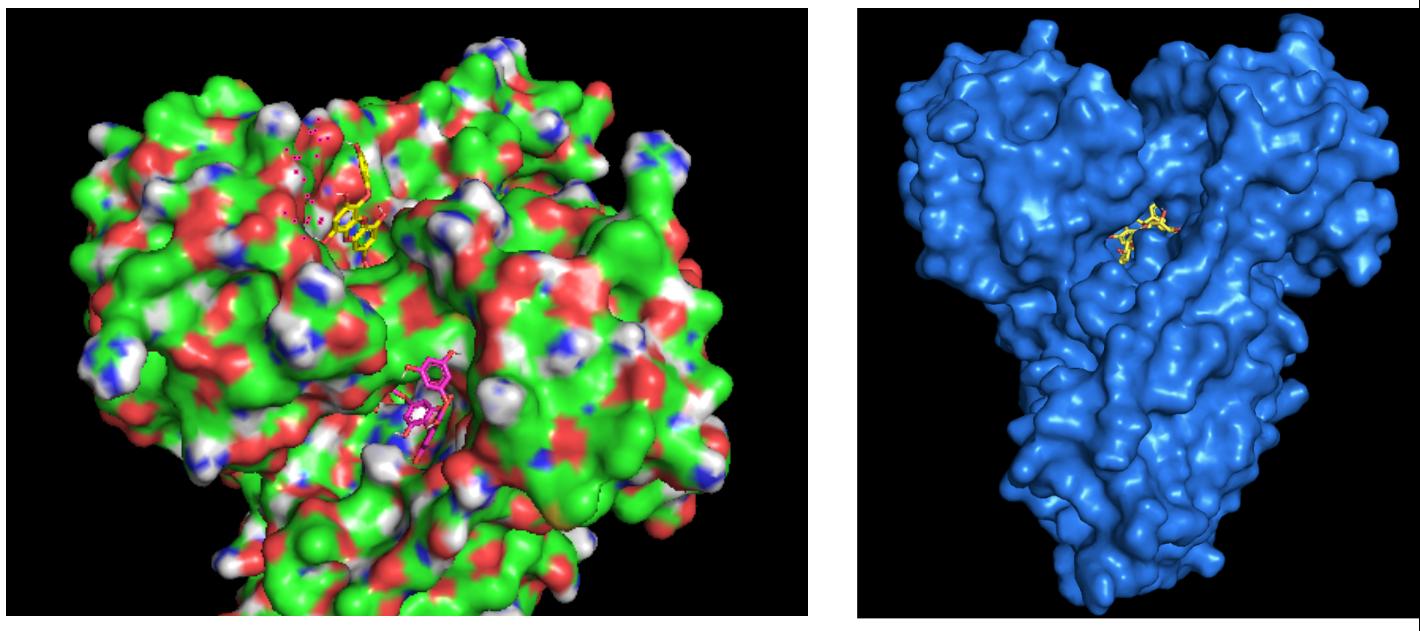
Most SARS-CoV-2 Mpro protease docking studies in the literature focus on the HIS41-CYS145 active site of the protease. Active sites may indeed be antagonized by the study drug ligands. However it should not be assumed that an active site alone is antagonized to the exclusion of other sites to cause the inhibition of the protease. Non-active site binding is well documented in the literature [cit..] While these can be allosteric regulation sites in the formal sense, they can also simply be noncompetitive binding sites.



**Figure: Left side: a ligand (hesperetin, yellow) docking at the dimeric interface;
Right side: the same ligand docking under pre-specified restriction to the active site**

What can be readily visualized in [cit. Fig.] is that when docking boundaries are opened up from being limited to the active site, the cleft or “tunnel” formed by the dimeric interface (blue: A chain against green: B chain) is found as the highest-affinity pose by the docking algorithm. These poses almost always feature higher binding affinity than the active site. While we find there is some affinity of the study’s relevant flavonoid glycosides and flavonoid aglycones at the active site, much greater potency in docking binding energy is found in the cleft formed by the dimeric interface. [cit fig].

The protease is treated like the extraordinary nanomachine that it is, an enormously sophisticated structure for the cleaving of peptides. Different parts of the machine move in three dimensions (similar [to this helpful animation of HIV's protease](#)). However even a sophisticated machine is exposed to inhibition if a ‘wrench’ can be thrown into ‘the works’. In the simplest possible inhibitory mode, the ligand in this case acts like ‘plumber’s tape’, binding otherwise independently self-actuating parts of the machine into a immobile state, and thus degrading and even disabling the function of the machine.



Left: Top view of main protease showing the tunnel's bridge structure with high-affinity ligand poses protruding on either side of it. **Right:** Front view of main protease showing hesperidin inside the tunnel. **Bottom:** Bottom: tunnel elucidated by ligand result poses. The tunnel structure is rendered visible by the position and conformation of the ligands inside of it. (product credit: PyMol)

Cleft / tunnel structures are known to exist in other proteases, including plant proteases and other viruses such as HIV. Indeed, in the case of HIV, one target mechanism of common HIV protease inhibitors is to 'block' this tunnel. Blocking the tunnel keeps other compounds, such as potentially agonists, or proteins that the protease breaks down from correctly allowing the protease to function normally. The tunnel may possess an active site, or it may be a conduit for proteins to pass through for cleaving.

The SARS-CoV-2 Mpro tunnel is characterized by these amino acid residues:

- residues A 282 Leu, A 283 Gly, B 282 Leu, B 283 Gly (making up the 'bridge' of the tunnel)
- A 004 Arg A 005 Lys, B 004 Arg, B 005 Lys (making up the 'bottomed-out' section of the tunnel)
- And A 197 Asp, A 287 Leu, A 199 Thr, A 238 Asn, B197 Asp, B 287 Leu, B 199 Thr, B 238 Asn, making up the ingress and egress surfaces of the tunnel cavity

Other mechanisms

Potential spike protein binding

This proposal allows that there could be additional mechanisms of efficacy of the study compounds. Early in the pandemic, a Chinese group [identified hesperidin as a potent binder in silico of the ACE2 - spike RBD interface](#). Indeed, it is found when running an in silico screen against a detailed model of SARS-CoV-2's spike protein's RBD receptor binding domain site, that many of the same ligands show potent binding efficacy to different sites of the RBD. However a correlation study of ligands, as has been accomplished for the protease, has yet to be performed on the SARS-CoV spike protein. Spike inhibition will probably be worth in vitro investigation, but of course a completed clinical trial obviates the need for that.

Anti-inflammatory focus

While hesperidin is known for anti-inflammatory properties, those properties have not been investigated in detail here. A cursory literature search on the study compounds vis-a-vis the most common markers for severe COVID-19 cytokine storm: IL-8, MCP-3, CRP, IL-6, and IL-19, easily yields four research papers with successful inhibiting results of several of the relevant cytokines:

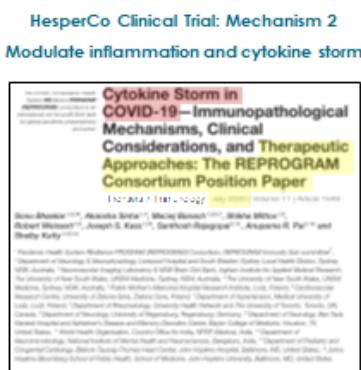
In vitro:	(Xiao et al 2018) , (Zaragoza et al 2020)
In vivo:	(Dokumacioglu et al 2018) , (Xie et al 2020)

And courtesy of a peer study group, ingenew - HesperCo JV (Montreal):

Hesperidin to address inflammation and cytokine storm in COVID-19 finds support in the scientific literature

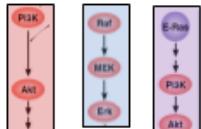
PubMed identified >80 papers related to the benefit of Hesperidin in inflammation



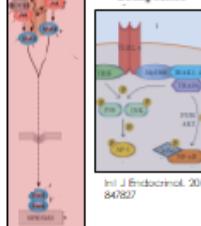


The REPROGRAM Consortium Position Paper		HesperCo Clinical Trial: Hesperidin for 14 day		
	Preferred Regulation			
Interleukin-6 (IL-6)	↓	✓	✓	✓
Interleukin-1 β (IL-1 β)	↓	✓	✓	✓
Tumor Necrosis Factor- α (TNF α)	↓	✓	✓	✓
Reactive Oxygen Species (ROS)	↓		✓	✓
Peroxisome Proliferator-Activated Receptors (PPAR) Agonist	↑	✓	✓	
Nuclear Factor of kappa-light-chain-enhancer in B-cells inhibitor (IkB)	↓		✓	✓
Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF κ B)	↓		✓	✓
Superoxide Dismutase (SOD)	↑	✓	✓	
Monocyte Chemoattractant Protein-1 (MCP-1)	↓	✓		
Signal Transducer and Activator of Transcription 3 (STAT3)	↓	✓		
Intercellular Adhesion Molecule 1 (ICAM-1)	↓	✓		
Macrophage Inflammatory Protein 2 (MIP-2/CXCL2)	↓		✓	
Keratinocyte Chemo-Attractant (KC/CXCL1)	↓		✓	
Mitogen Activated Protein Kinase p38 (p38)	↓		✓	
c-Jun N-terminal kinases (JNK)	↓		✓	

Jak/Stat Signaling: IL-6 Receptor Family



Inflammasome Complexes

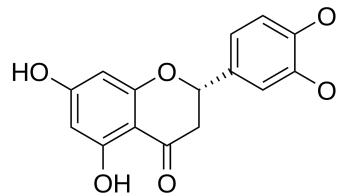
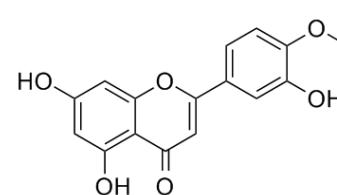


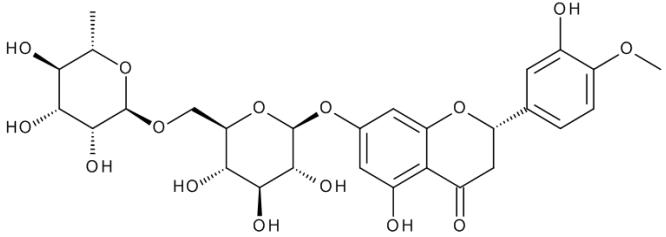
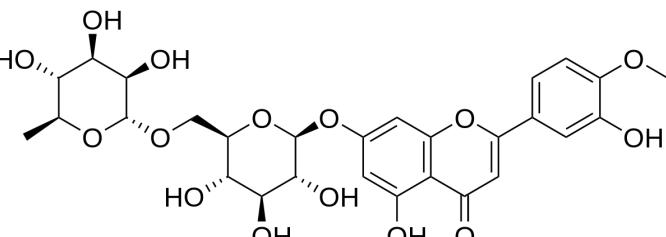
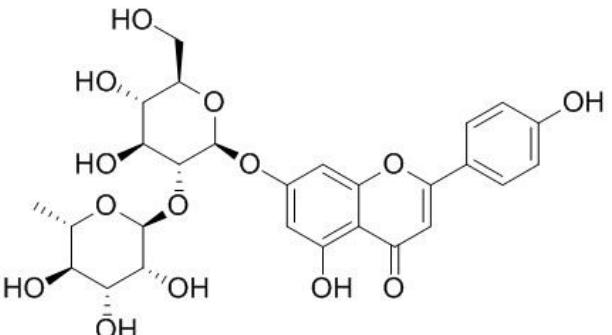
Int J Endocrinol. 2014; 847627

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Study Drugs

The primary study drug is Daflon - 450mg diosmin , 50mg ‘other flavonoids expressed as hesperidin’ (but primarily hesperidin). A 2nd arm is proposed of fluvoxamine.

	Molecule diagram	Target	In vitro* IC50	Cite:
hesperetin		SARS-CoV Mpro (aka 3CLpro)	8.3 µM in cell-based assay (more real-world than cell-free assay) on SARS-CoV main protease in 2005	(Lin et al, 2005): Anti-SARS coronavirus 3C-like protease effects of Isatis indigotica root and plant-derived phenolic compounds China Medical University, Taiwan
diosmetin		---	No assays in literature but essentially identical structure to hesperidin. Assumed to feature similar inhibitory performance.	---

hesperidin	 <p>Hesperidin = hesperetin (a flavanone aglycone) + rutinose (a simple disaccharide)</p>	---	<p>In vitro not taken place - our predicted cell-free IC₅₀ value is 16 uM</p> <p>See correlation study in appendix for justification</p>	---
diosmin	 <p>Diosmin = diosmetin (a flavone aglycone) + rutinose (a simple disaccharide)</p>	---	<p>In vitro not taken place - our predicted cell-free IC₅₀ value is 15.5 uM. (Essentially identical performance to hesperidin).</p> <p>See correlation study in appendix for justification</p>	---
rhoifolin	 <p>Rhoifolin = Apigenin (aglycone) + neohesperidose (a simple disaccharide)</p>	SARS-CoV-2 Mpro	<p>20uM solution yielded an IC₆₅ result. In silico correlation study predicted IC₅₀ is 16uM which is in line with the in vitro findings.</p>	<p>(Loschwitz et al. 2020) Novel inhibitors of the main protease of SARS-CoV-2 identified via amolecular dynamics simulation-guided in vitroassay</p> <p>Forschungszentrum Jülich (Helmholtz Institute), Germany</p>

History of SARS-CoV main protease inhibition by other flavonoids over the last decade:

Cite.	In-vitro verified flavonoids on SARS-CoV main protease:	
Jo, Seri et al. "Inhibition of SARS-CoV 3CL protease by flavonoids", J Enzyme Inhib Med Chem. 2020; 35(1): 145–151. Nov 2019. https://doi.org/10.1080/14756366.2019.1690480	herbacetin rhoifolin pectolinarin	33µM 27µM 38µM
Ryu et al. "Biflavonoids from <i>Torreya nucifera</i> displaying SARS-CoV 3CLpro inhibition", Bioorganic & Medicinal Chemistry Volume 18, Issue 22, 15 November 2010, Pages 7940-7947. https://doi.org/10.1016/j.bmc.2010.09.035	gallocatechin gallate quercetin	47µM 73µM*
Nguyen Thi Thanh Hanh et al. "Flavonoid-mediated inhibition of SARS coronavirus 3C-like protease expressed in <i>Pichia pastoris</i> ", Biotechnol Lett. 2012; 34(5): 831–838. Feb 2012. https://doi.org/10.1007/s10529-011-0845-8	luteolin quercetin amentoflavone gingketin sciadopitysin	20µM 24µM 8µM 32µM 38µM

* repeated

Pharmacology & Pharmacokinetics: Safety, Bioavailability, and Drug-drug interactions

Safety

	In vivo (human or animal model dose)	cite.
diosmin	<p>Up to at least 5000 mg / day remains safe in humans</p> <p>“Many clinical trials have been conducted in adults with various manifestations of CVI to assess the efficacy of orally administered diosmin in doses ranging from 400 to 5,000 mg/day for up to a year. No serious adverse events were reported in any of the studies. Commonly reported adverse events included gastrointestinal disturbances and headaches; these were generally mild in severity and did not usually result in patients discontinuing participation in the study. The following adverse events (and approximate percentages) were reported in clinical trials but their frequency did not differ from placebo: rash (1%), cramping in lower limb (2%), phlebitis (2%), venous thrombosis (4%), and skin changes around existing ulcer, swelling of the extremities and body rash (1.6%). Dyspepsia, or non-specific mild stomach upset, occurred in up to 7% of subjects taking diosmin at various doses and was seen with approximately twice the frequency seen in the placebo groups. Rare adverse events include inguinal pain, cystitis, asthenia, metrorrhagia and menometrorrhagia. In clinical trials, the incidence of adverse events in elderly populations (≥ 70 years of age) was not significantly different from that in younger populations nor were adverse events higher in patients with concomitant hypertension, atherosclerosis, diabetes, neurologic/psychiatric disease or alcoholism.”</p>	(Primus Pharmaceuticals, 2012) archived: https://archive.vn/mlKqw
hesperidin	LD50 (rats) 4840 mg/kg / 6.2 (allometric factor) = 780 mg/kg (human equivalent) = 46,800 mg (60-kg human)	(Li, Y. 2019), & https://www.fda.gov/media/72309/download
Daflon	In animal studies, the safety of Daflon 500 mg is shown by an LD50 (lethal dose so) of more than 3 g/kg, ie, 180 times the daily therapeutic dose, [3g / kg divided by 6.2 allometric factor = 484 mg/kg,	(Meyer 1994)

= 29,040 mg (60-kg human)], or as well as by the absence of any toxic effect after repeated oral dosing for thirteen and twenty-six weeks, using a dose representing 35 times the daily dosage, in the rat and primate. Daflon 500 mg has no mutagenic action nor any significant effect on reproductive function. Gastrointestinal tolerance is good when administered orally in the rat. Transplacental passage and passage into breast milk are minimal. In the rat, 0.003% of the administered dose has been found in each fetus and 1% in breast milk.

Trials as of 1994:

- One multicenter, open trial involving a oneyear treatment period in patients with functional venous insufficiency⁹
- two two-month multicenter open trial 111 2
- two six-month open clinical trials

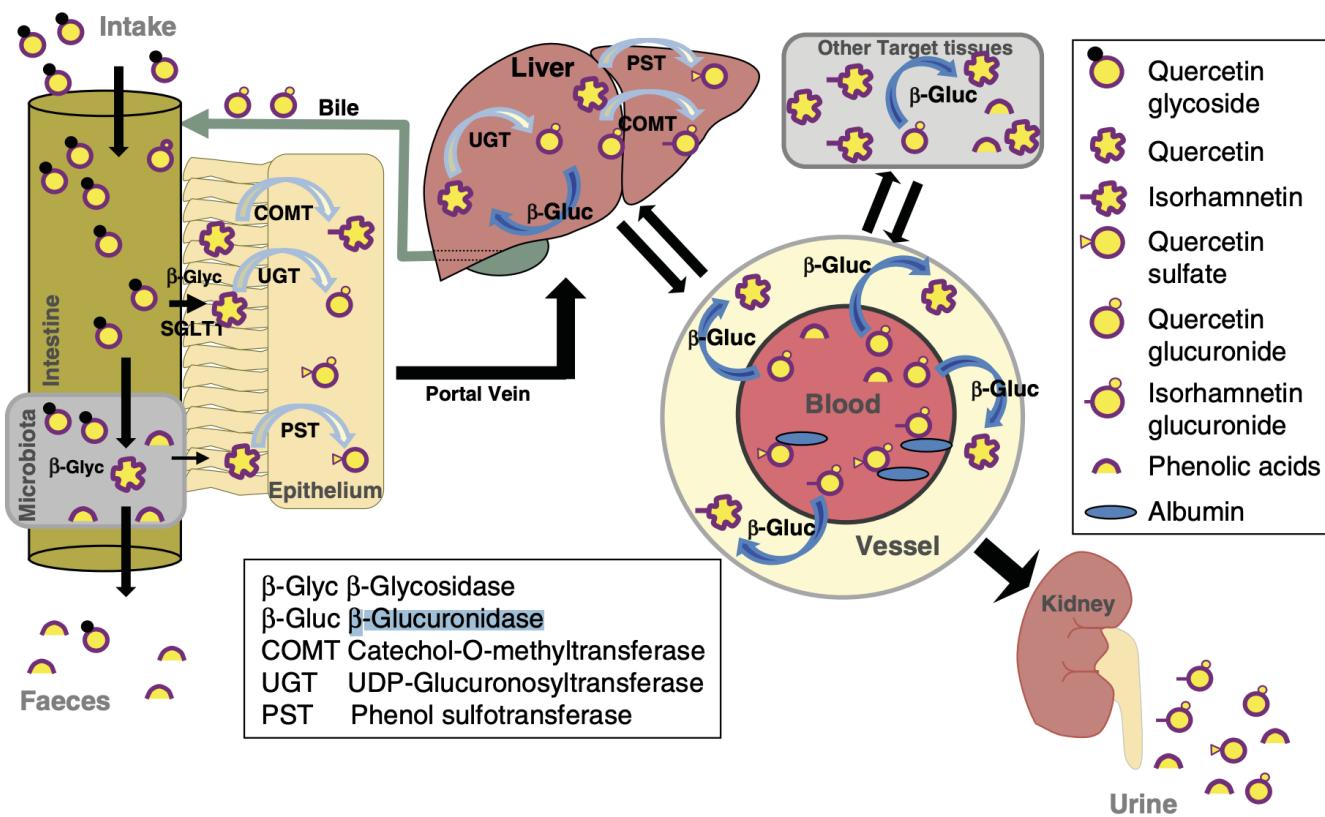
Clinical trials fulfill international scientific requirements and have collected more than 2850 patients treated with Daflon 500 mg at the dosage of two tablets per day for six weeks to one year. The proportion of patients with side effects (10% of those treated), essentially of a gastrointestinal or autonomic nature and leading to a rate of only 1.1% trial dropouts, is less than described in 225 patients given a placebo (13.9%) in controlled trials. Satisfactory clinical acceptability already confirmed in the short term was equally found in long-term treatment.

No contraindications have been found during the therapeutic use of Daflon 500 mg, even in the elderly and in pregnant women. No evidence has been found of any interference with combined drugs. Daflon 500 mg is free of any photosensitizing action. Daflon 500 mg combines thoroughly proven therapeutic efficacy with excellent safety of use confirmed in specific and methodologically reliable toxicologic studies as well as in a large number of clinical trials with patients treated daily for six weeks to one year.

Bioavailability & Pharmacokinetics

The most recent and complete survey paper on diosmin and particularly Daflon pharmacokinetics is ([Bajraktari et al 2020](#)). Diosmin sheds its disaccharide in the GI tract to become diosmetin and pass through the intestinal lining. The liver metabolizes it to diosmetin-3-O-glucuronide, which is further esterified to the diosmetin-3,7-O-diglucuronide. The blood plasma peak concentration value of diosmetin in its glucuronidated forms that are most referenceable to Daflon is 1.3 uM per 450 mg (Campanero et al., 2010, with correction by Bajraktari et al 2020).

In addressing the “flavonoid paradox” (Menendez et al 2012, Perez-Vizcaino et al 2013), the “deconjugation in inflammation hypothesis” was verified over 2012-2019 ((Terao 2017, Yoschichika 2018, Vinson 2019, Galvez et al 2019)). In other words flavonoid glucuronides from plasma are deglucuronidated to their cell-membrane penetrating aglycone form. During inflammation (as happens during infection), phagocytes arrive at the extracellular fluid surrounding the sites of inflammation. The phagocytes exude β -glucuronidase accomplishing deglucuronidation of the flavonoid glucuronide into its aglycone form:



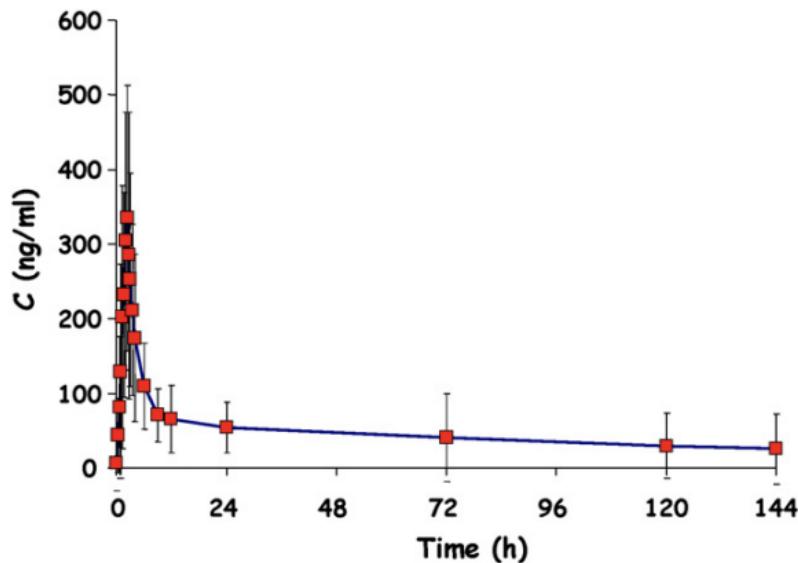
Schematic representation of the pharmacokinetics of quercetin.

Figure: quercetin (as the usual representative flavonoid in literature). Mechanism for tissue cell entry, the site of protease inhibitory action. (Perez-Vizcaino et al 2013). Diosmetin and hesperetin take the place of quercetin in this graphic

Treating hesperetin and diosmetin as bioequivalent, and assuming and applying a linear model, then achieving the (Lin et al 2005) 8.3 uM value requires **six tablets of 500 mg Daflon**, totaling **3000 mg**, which is the maximum precedent for ordinarily prescribed dose (the indication for hemorrhoids).

Even looking at hesperitin independently of diosmetin, we know from ((AIBMR Life Sciences & Ferrer HealthTech, 2018) that 220 mg of imbibed hesperitin leads to 1.28 uM plasma Cmax of hesperitin. Translating to the hesperidin-hesperitin (2:1) ratio , the 50mg of hesperidin in Daflon should metabolize to 25 mg of hesperitin, translating to **0.15 uM** Cmax, assuming linearity. This finding shows that hesperitin plasma bioavailability is essentially similar to diosmetin.

Plasma Cmax is typically achieved in 1-3 hours after consumption, and is cleared from the body at approximately 24 hours from ingestion.



“Mean concentration-time profile of diosmetin after an oral dose of Daflon in healthy volunteers” (Campanero, M. A. et al., 2009)

Drug-Drug Interactions

([Bajraktari et al 2020](#)) also features the most up-to-date data on liver enzyme interactions for prediction of drug-drug interaction. While all possible drug drug interactions for any drug in the pharmacopeia vis-a-vis all others are unknowable, but this study and those it surveys make clear that drugs known to be attenuated by BCRP, OATP1A2, OATP1B1, OATP1B3, OATP2B1, CYP1A2, CYP2C19, and CYP3A4 should be avoided in concert with the study drugs. Due attention is given particularly to CYP3A4, given its prevalence for attenuation in the pharmacopoeia, particularly to the likely covid therapeutic fluvoxamine.

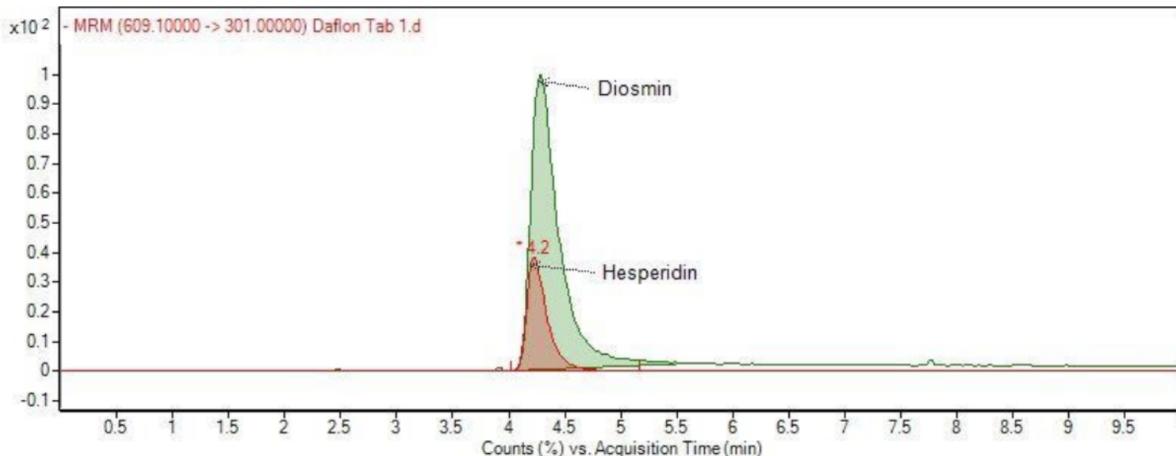
Local source (Nairobi):

Daflon, by Laboratoires Servier, as packaged (local specimen). Purchaseable OTC at GoodLife pharmacies. (Certificate of Analysis and manufacturing site GMP procurable during clinical trial preparation).



Local authenticity verification

The Strathmore University CREATES laboratory engaged with EMKSE Phytochem to provide preliminary verification of constituent compounds locally-sourced off-the-shelf Daflon, producing the LC-MS readout below.



The diosmin - hesperidin relative magnitudes are within expectation based on the specified contents of the pharmaceutical preparation.

Clinical Trial Strategy

This clinical trial, called FLAVOCOV, aims as a primary endpoint to determine whether the study drugs reduce the severity of COVID-positive patients' illness. Secondary endpoint: to determine whether the study drugs are efficacious at clearing viral load in cov+ individuals at a superior rate than natural immunity would allow.

Trial Execution

The trial will be primarily remote in nature. Sample collectors will collect physical nasopharyngeal samples per the FLAVOCOV Protocol sampling schedule from patients who are self-isolating at home following positive COVID test.

Primary (Clinical symptoms) Endpoint

The primary endpoint of clinical symptoms is straightforward and articulated in our included protocol.

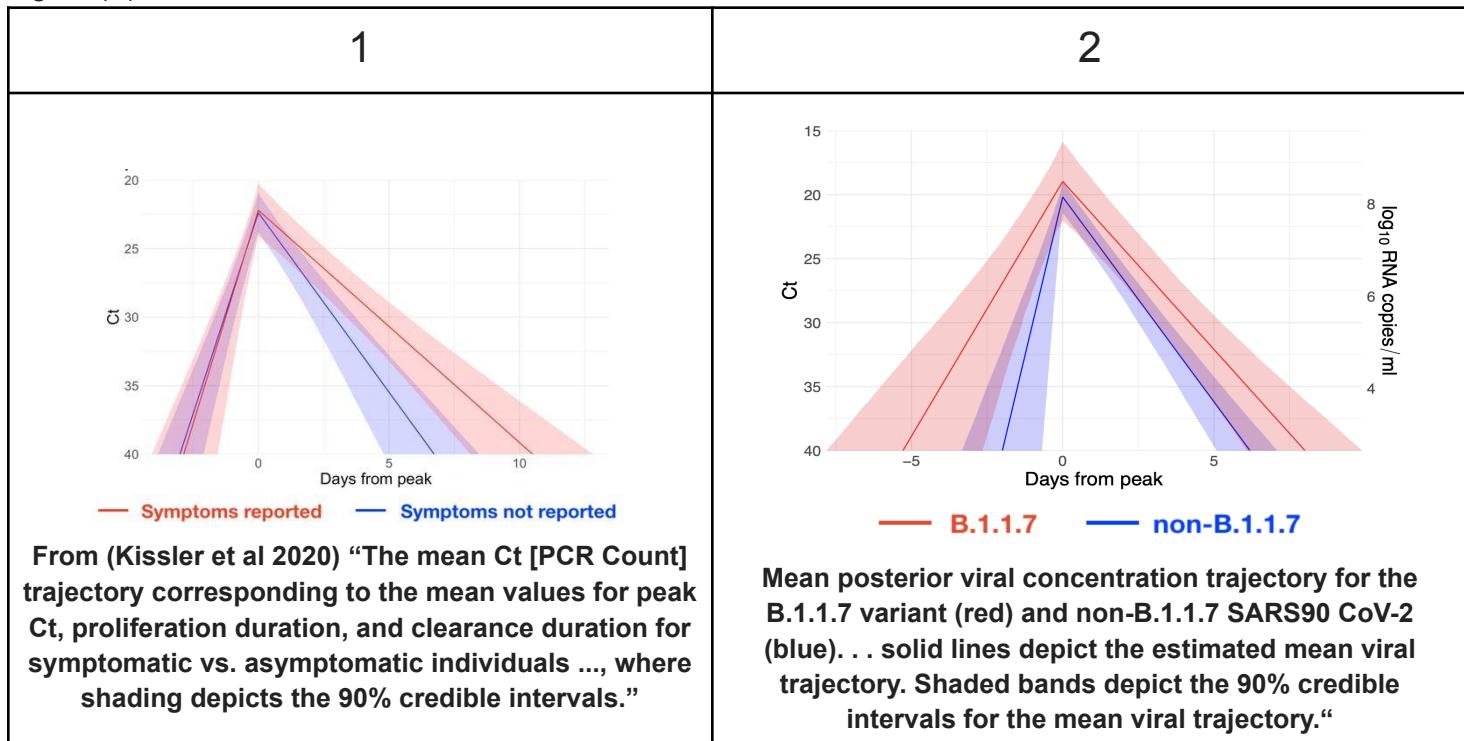
Secondary (PCR) Endpoint

The secondary endpoint provides a quantitative readout that is independent of human interpretation and discretion. Referencing (Jefferson et al 2020):

[A report in June \[2020\]](#) from researchers at Weill Cornell Medicine found that among 678 hospitalized patients, 35% of those with a CT value of 25 or less died, compared with 17.6% with a CT value of 25 to 30 and 6.2% with a CT value above 30. [In August \[2020\]](#), researchers in Brazil found that among 875 patients, those with a CT value of 25 or below were more likely to have severe disease or die.

(Kissler et al 2020) (Figure X-1) demonstrate that showing antiviral effect requires speedy intervention and follow-on assaying, independent of whether the patients are symptomatic.

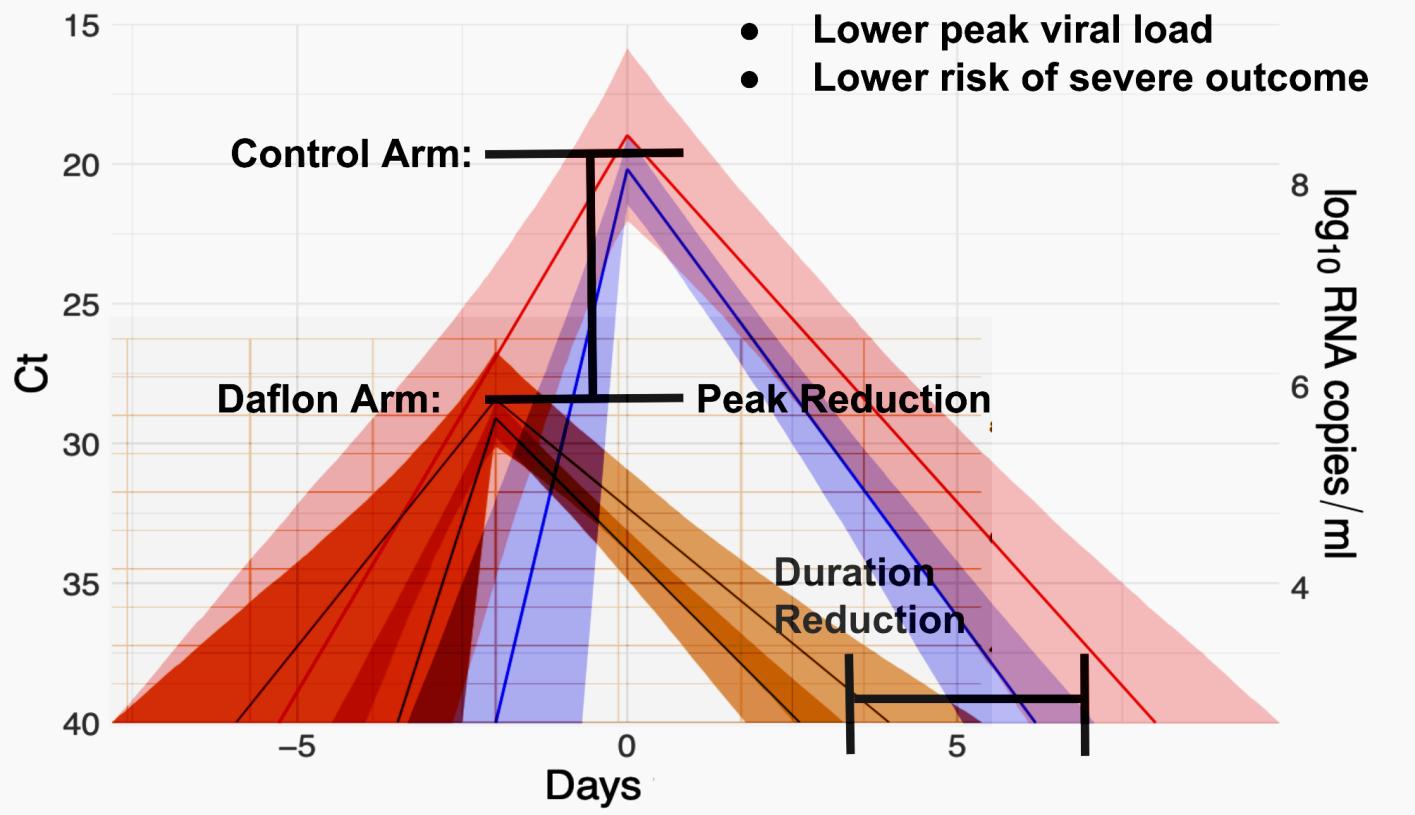
Figure (X)



Subsequently (Kissler et al 2021) (Figure X-2) demonstrated that the increasingly prevalent B.1.1.7 strain results in extended viraemia duration, allowing more time to perform the follow-on PCR assays.

An unaffiliated n=1 case study close to us has already demonstrated that once the peak is reached, that the experimental intervention probably has little measurable effect on the rate of decrease of measurable viral clearance or viral RNA debris clearance in immunocompetent patients.

Figure (Y)\



PCR Endpoint A is viral load Peak Reduction . **PCR Endpoint B** is viral load Duration Reduction

The difference between intervention and placebo for **PCR Endpoint A** and **PCR Endpoint B** across the trial population will be calculated using Fisher's exact test and presented as a relative risk ratio.

As is readily apparent from Fig Y, it is:

- It is of paramount importance to reach patients who are **passively screened** rather than self-selecting for PCR tests based on symptoms, as by that time they are likely at or beyond peak viral load.
- Expediency in collecting patients' first post-enrollment sample is critical.

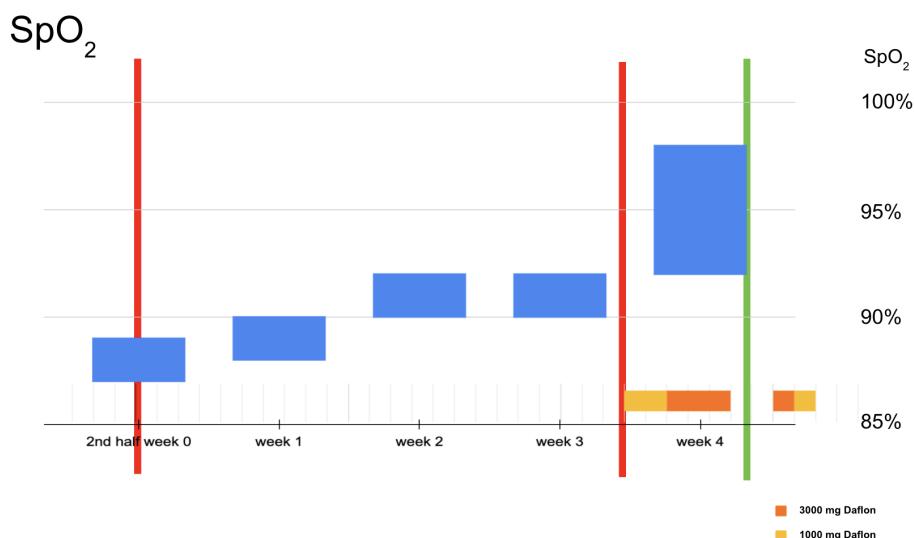
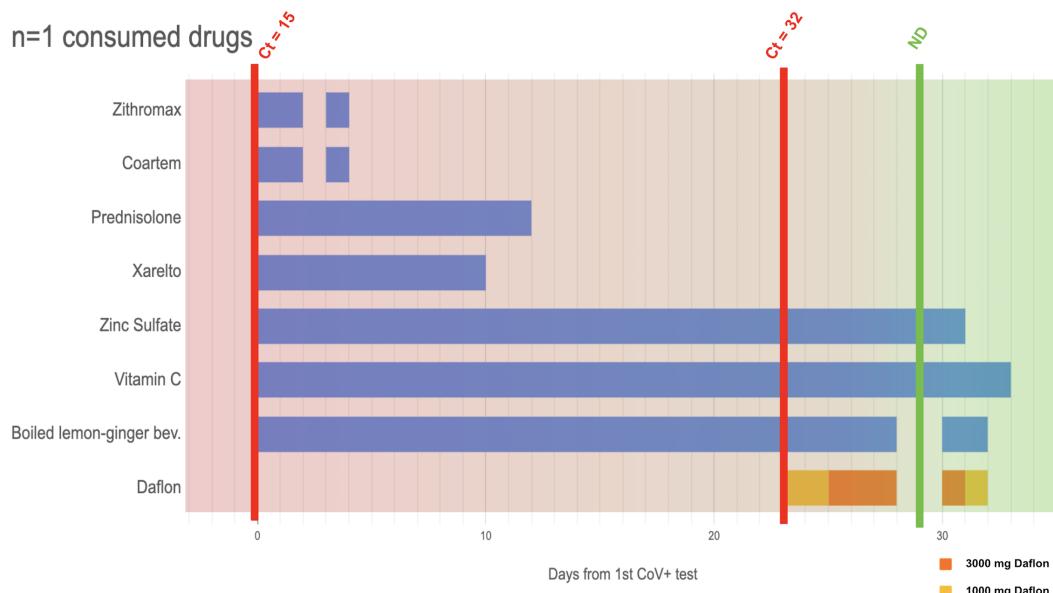
Attention due PCR Inhibitors

As polyphenolic compounds are known inhibitors of polymerase in PCR assays, it is necessary to ensure that the industry-standard practice of adding bovine serum albumin BSA to the assay be available to verify any samples. In such cases, the BSA must be added at a concentration appropriate to neutralize up to 10 μ M of PCR inhibitors. The criteria for such verification is if the participant's viral trajectory is erratic, or if it is known that their PCR took place between 1 and 6 hours after dosage and is yielding high-to-undetectable Ct value. .

Patient Charts

Data collected should be of an accuracy & precision for forming figures as the below (real-world) example:

Figure example 1: PCR test results vs intervention medication consumption (& any allowable drugs consumed). Figure example 2: SpO₂ vs intervention medication consumption:



Project Risks

Assessment of Project's Risks to Patient Safety

- Daflon / diosmin / hesperidin's ingested safety profile through extensive clinical trials with many participants over long durations is well-known.
- Due awareness is to be given to the importance of avoiding drug-drug interactions as outlined in the protocol.
- That said, these compounds' safety profile in their aglycone form following deglucuronidation in the course of an inflammatory event such as during infection (referred to in the literature as "deconjugation in inflammation") should be seen as having less investigatory study to date.
- This risk is mitigated by the fact that two COVID+ cases are documented for taking Daflon over the course of the infection, and no serious side-effects have been reported.

Assessment of Project's Risks to Efficacy

- The primary risk to efficacy is whether the duration during which the IC₅₀ value is achieved for a single dose per day (approximately 2 hours) is sufficient to inhibit protease-mediated viral replication. Uncertainties in this are bi-directional - they could contribute to efficacy or reduce efficacy. Particularly in that, in an inflammation-prevalent patient as in the course of an infection, it is unknown to what quantitative extent flavonoid glucuronides and aglycones may in fact selectively permeate sites of inflammation (in interstitial fluid & inflamed cells). Such permeation could be nearly independent of the plasma concentration of the same compounds.
- A secondary risk to efficacy is that the (Lin et al 2005) study was performed on Vero E6 cells. These are standard kidney cells commonly used for in vitro assays. Being kidney cells they may express more of the cross-membrane transport enzymes for bringing in a polar molecule for excretion than cells of interest for SARS-CoV-2 infection. Therefore they could have created a greater (but unassayable) in-cell concentration of hesperetin in that study, and therefore could underestimate the operative IC₅₀ value for non-kidney cell-based protease inhibition.
- A tertiary risk to efficacy is that plasma concentration of flavonoid metabolites varies significantly for a given dose due to natural human-to-human metabolic variation. So plasma concentrations would be expected to vary significantly across the patient cohort.
- For the viral replication reduction endpoint uniquely, it is critical that patients be identified and enrolled prior to reaching peak viral load. Where patients are primarily found after reaching peak viral load, then the efficacy for achieving the viral load reduction endpoint will be unidentifiable, yielding only the study drug's anti-inflammatory capabilities as the remaining endpoint of interest.

Clinical Trial Short-form Protocol

Protocol summary for protease inhibitor clinical trial for SARS-CoV-2

- **Trial parameters**

- The trial will take place in Nairobi, Kenya.
- The trial will be registered on the Pan-Africa Clinical Trial Registry
- An external data monitoring committee (DMC) will be formed from 3rd party personnel and will review cumulative unblinded data throughout the study
- EMSKE, EMSKE JV partners, and CRO leadership will form an Internal Review Committee.
- The trial will be of type: 1. double-blind (with respect to EMSKE and CRO leadership), 2. randomized, 3. matched.
 - Matching will be on basis of age, within 10-year bands. (20-30 y.o., 30-40, y.o., etc.)
 - Other than these matchings, assignation of patients to each arm will be random
- Immortal time bias will be quantified by identifying the nature of any trial drop-outs between enrollment and first dose.

- **Eligibility criteria**

- Patients must be COVID-19 positive with a positive SARS-CoV-2 PCR scan within the past 96 hours from an accredited laboratory.
- Patients' age will be 18+ years old.
- Patients' SARS-CoV-2 vaccination history will be recorded.
- Patients' SARS-CoV-2 self-reported and documented SARS-CoV-2 prior infection history will be recorded.
- Patients' self-reported durations since earliest symptoms prior to their covid PCR test will be recorded.

- **Interaction with patients**

- Patients will be liaised with over phone voice, Whatsapp-voice. (Excluded are SMS, Whatsapp-text).
- Patients will have intervention and placebo dropped off at their front door in hygienic fashion.

- **Arm breakdown:**

- For baseline numbers of patients of **[50, 100, 150]** patients
 - 1 primary experimental arm with the baseline number of patients.
 - 1 control arm , # patients same as primary experimental arm.
 - ~~1 dose dependent response verification arm containing half of the number of patients in primary experimental arm~~

- **Experimental intervention**

- primary experimental arm 1: **3000 mg Daflon**, (equivalent to 6 tablets) once per day, without food.
- Primary experimental arm 2: **Fluvoxamine** replication trial arm. Fluvoxamine is a promising drug showing strong Ph II results. Likely mechanism is as SIGMAR1 ligand. Fluvoxamine to be administered as dose of 50 mg of fluvoxamine in the evening immediately after the baseline assessment and confirmation of eligibility, then for 2 days at a dose of 100 mg twice daily as tolerated, and then increasing to a dose of 100 mg 3 times daily as tolerated through day 15
- Control arm: a placebo (consisting of 6 tablets) certified for administering in the nation of Kenya

◦ Dose dependent arm: ~~1500 mg Daflon, (equivalent to 3 tablets) once per day~~

- **Exclusion criteria**

- Other than COVID-19, patients will be excluded for a history of comorbidity.
- Patients will be excluded for taking medication that is known to interact (inhibit or augment the activity of) liver enzymes BCRP, OATP1A2, OATP1B1, OATP1B3, OATP2B1, CYP1A2, CYP2C19, and CYP3A4.
 - Including but not limited to:
 - Cholesterol management medication
 - Heart arrhythmia medication
 - Diabetes management medication
 - Kidney management medication
 - Blood thinner medication
 - HIV medication
 - Antihypertensives
 - Capecitabine
 - Fluorouracil
 - Bisoprolol
 - Enoxaparin
 - Antioxidants
 - BP medication
- Patients will further be excluded if they are under any of the following health conditions
 - Pregnancy
 - HIV
 - Kidney disease
 - Diarrhea
 - Sarcoidosis
 - High blood calcium levels
 - Kidney stones
 - Addison's disease
 - Elevated blood potassium
 - Thompson disease
- Patients will be excluded for taking any non standard-of-care medication / supplement that may suppress viral load or otherwise affect patient outcome whether verified or purported.
 - Fluvoxamine (for Daflon or control arm)
 - Ivermectin
 - Vitamin-D supplements
 - Vitamin-C supplements
 - Chloroquine
 - Hydroxychloroquine
 - Colchicine
 - Zinc
 - Selenium

- **Endpoints**

- Patients will undergo PCR swabs from the accredited laboratory's field staff at **Day 2, 4, 6** from enrollment where enrollment is defined as **Day 1**. Nasopharyngeal swabs will be collected at patient's home by accredited nasopharyngeal swab sample collectors.
 - The swabs will be applied into RT-PCR assay at the accredited laboratory

- The accredited laboratory's test results will be recorded as positive or negative per their approved guidelines.
 - The accredited laboratory will further record the **Ct** quantitative value from the RT-PCR assay.
 - Temperature (sample collectors equipped with IR gun thermometer)
 - SpO₂ (sample collectors equipped with pulse oximeters)
- At 3-day intervals until 30 days of enrollment in the study are completed, each patient will be interviewed by CRO administering staff and provide results on a 5-point Likert scale for the following symptoms:
 - Dry cough
 - Fatigue
 - Fever (self-reported)
 - Headache
 - Dizziness
 - Confusion
 - Dyspnea (i.e. shortness of breath)
 - Difficulty with breathing
 - pneumonia
 - Taste insensitivity
 - Smell insensitivity
 - [any other currently recognized / new-variant COVID-19 symptoms]
- If patients self-report requiring hospitalization or in the opinion of the CRO's administering staff that hospitalization of a patient is required, same will be recorded. SpO₂ equal to or less than 90% will be recorded and recommended for hospitalization. Commitment from partner hospital required for hospital's acceptance (i.e. non-chargeability to patient or to trial) of treatment cost shall be required in advance of trial start.
- **Patient compensation**
 - Patients will be awarded with airtime-cum-data bundles direct to the phone through which they will liaise with trial staff for the duration of the study, at a rate of KES 1000 /- per week. At no point shall electronic currency be transferred to the patient's phone.
- **Reporting**
 - The clinical report form (CRF) shall be exclusively electronic in nature. At no time shall data be recorded on paper for subsequent transcribing.
 - 3rd party Data Management (DM) provider will be provided by referral from EMSKE.

Clinical Trial Execution Team

Central Execution Team

logo	Profile photo	Bio / LinkedIn
logo	Profile photo	<ul style="list-style-type: none">• Bio / LinkedIn
logo	Profile photo	Bio / LinkedIn

Technical Advisors

Profile photo	<ul style="list-style-type: none">• Bio / LinkedIn
Profile photo	<ul style="list-style-type: none">• Bio / LinkedIn

Profile photo	<ul style="list-style-type: none"> ● Bio / LinkedIn
Profile photo	<ul style="list-style-type: none"> ● Bio / LinkedIn

Institutional Clinical Research Partner:

logo	<ul style="list-style-type: none"> ● Institutional bio 	
	Logo #1	Logo #3

Key Vendors

PCR Vendo logo	<ul style="list-style-type: none"> ● Bio / LinkedIn
Medical sampling & delivery vendor logo	<ul style="list-style-type: none"> ● Bio / LinkedIn

Governance Advisor

logo	Profile photo	<ul style="list-style-type: none"> ● Bio / LinkedIn
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Commercialization Partner

logo	Profile photo	<input type="radio"/> Bio / LinkedIn
logo	Profile photo	<input checked="" type="radio"/> Bio / LinkedIn

Parallel Studies

Proposals for hesperidin / diosmin efficacy studies have been made and represent the independent motivation for future replication studies.

1. [Hesperidin and Diosmin for Treatment of COVID-19](#)

Prof. Dr. Kamal Mohammed Okasha, Tanta University, Egypt

2. HesperCo (JV Ingenew) Clinical Trial, Quebec, Canada

[Pierre Laurin](#), Ingenew Pharmaceutical, MSc Pharm, Pharmacology, University of Montreal
(Former CEO, Prometic Life Sciences, took public on Toronto Stock Exchange)

Mentions

I have indeed received the package from EMSKE Phytochem, and considering the possible activity of flavonoids against SARS-CoV-2 it may be tempting to have a closer look into their potential. I have therefore passed the relevant information to my colleagues Prof. Johan Neyts and Prof. Dominique Schols.

Kindest regards,
Prof. (em.) Dr. Erik De Clercq

From Dr. Erik De Clercq, famous virologist, wrote “Potential antivirals and antiviral strategies against SARS coronavirus infections” 2006 ; “Therapeutic optiins for the 2019 novel coronavirus (2019-nCoV)” 2020.

Relevant Media

- See folder in data package
 - [American Nutrition Association](#), 2020
 - [Andolu Agency \(Turkey\)](#) 2020
 - [LaPresse \(Montrea\) 2020](#), & [LaPresse \(Montreal\) 2021](#)

Appendix

Appendix A

About EMSKE Autodock Vina Toolchain validation (Online narrative version [available here](#))

This is the training set of compounds applied for our model:

Compound	Reported ex-cell log10(IC50 (uM))	Compound	Reported ex-cell log10(IC50 (uM))
beta-sitosterol	2.06	epigallocatechin gallate	1.86
hesperetin	1.78	gallocatechin gallate	1.67
indican	2.05	herbacetin	1.52
sinigrin	2.08	rhoifolin	1.43
indirubin	2.47	pectolinarin	1.58
indigotin	2.48	Eckol	0.94
daidzein	2.02	dioxynodehydroeckol	2.20
aloemodin	2.12	2-Phloroeckol	1.12
apigenin	2.45	7-Phloroeckol	1.62
luteolin	1.30	fucodiphloroethol	1.35
quercetin	1.38	phlorofucofuroeckol A	1.03
amentoflavone	0.92	hinokiol	2.37
quercetin (again)	1.86	Isopimaric Acid	2.45
daidzein (again)	2.55	ginkgetin	1.51
puerarin	2.58		

Recognizing that daidzein and quercetin had historically been assayed twice in the vitro literature, and the very different values on linear scales that professional researchers ascertain comparing one compound to its counterpart in its alternative study, it was recognized that log10 measures of the compounds' efficacies would be more appropriate than simple linear measures. Indeed as much of biochemistry is logarithmic in nature in terms of concentration efficacies, it is recognized that this data processing approach is appropriate.

With a molecular docking program, one has latitude to specify what region of a protein the simulator should allow for the compound's docking. This is ordinarily accomplished by specifying a 'box size' and 'box center coordinates' which the program will treat as its searchable volume within the limits of its coinciding with the protein's surface binding sites. Another parameter to specify is the exhaustiveness of the search. Finally the target site itself can be targeted. Once a target residue or residue pair is identified, those skilled in the art can easily determine three dimensional coordinates for the geometric center of the residue targeting area by looking up the residues and their coordinates in the protein pdb / pdbqt file. Throughout this disclosure the residue pair HIS41 - CYS145 is targeted during target active site studies for each of the protease chains A and B, and on both SARS-CoV and SARS-CoV-2 proteases. Further for such target active site studies a cubic box size of 30 Angstroms to a side is specified, and exhaustiveness value of 8 is used. In this disclosure, targeted dockings are always run in sets of three trials for each ligand assay with the top binding energy pose results averaged, with remaining poses discarded.

Throughout this disclosure's use of Autodock Vina, the parameters Num_modes and Energy_range are maintained at 10, and 4 respectively.

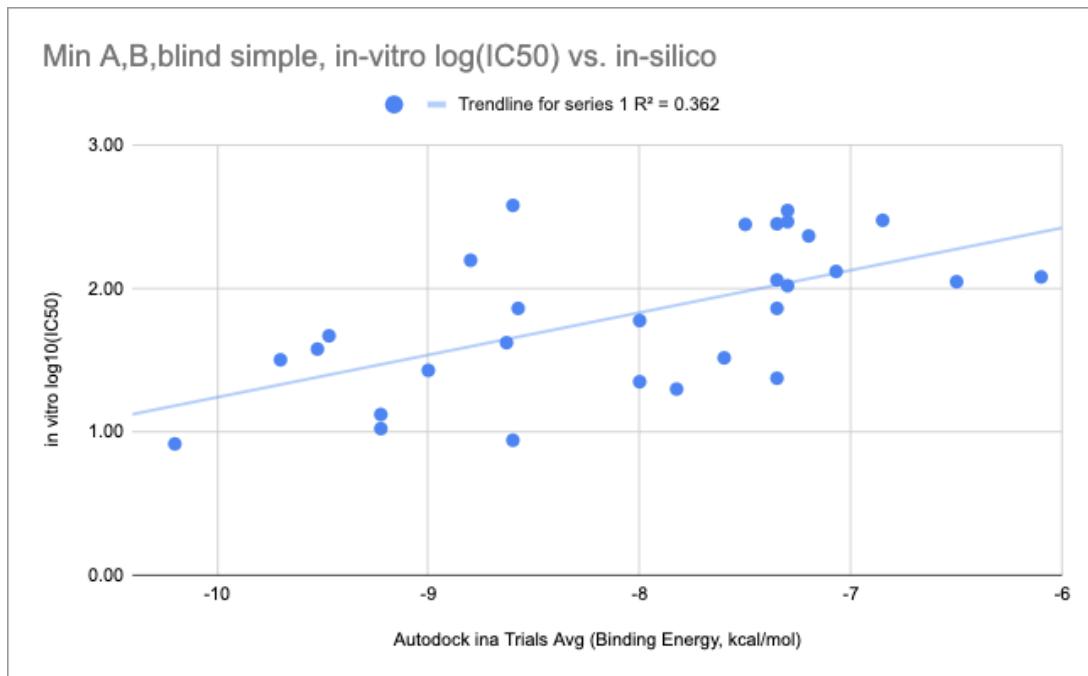
Another such kind of docking is a ‘blind’ docking wherein the entire protein is enclosed by the searchable box, so the program is free to locate sites across the protein’s bindable surface. Another approach is to target the searchable volume to a known active target site of the protein or enzyme, such as the site an enzyme normally uses to initiate and execute its action. Throughout this disclosure as regards SARS-CoV or SARS-CoV-2 proteases, an origin of (0,0,0), a cubic box size of 150 Angstroms to a side, and exhaustiveness value of 10 is used for blind docking. In this disclosure, blind dockings are always run in sets of four trials for each ligand assay with the top binding energy pose results averaged, with remaining poses discarded.

Several permutations of the Autodock Vina trials, between blind docking and targeted active site docking, were compared against published in vitro inhibitory values of the same compounds in the literature. (Lin et al 2005) (Ryu et al 2010), (Nguyen et al 2012), (Jo et al 2020), (Park et al 2013) This was performed in order to ascertain which binding locations could best explain inhibitory effects in vitro. The following studies were performed using the training ligand set:

study	R² correlation value with log10(IC50) in vitro data
Blind (4 trials) averaged	0.354
A-targeted (3 trials) averaged	0.328
B-targeted (3 trials) averaged	0.309
Min A & B	0.328
Min blind A & B	0.362
Min blind A & B (deep pockets removed)	0.365

In finding the permutation that had the highest correlation with published values, the top two models for correlating and mapping in silico to in vitro results were determined, being 1. Min blind A&B (deep pockets included), and 2. Min blind A & B (deep pockets removed)

Deep pockets included:

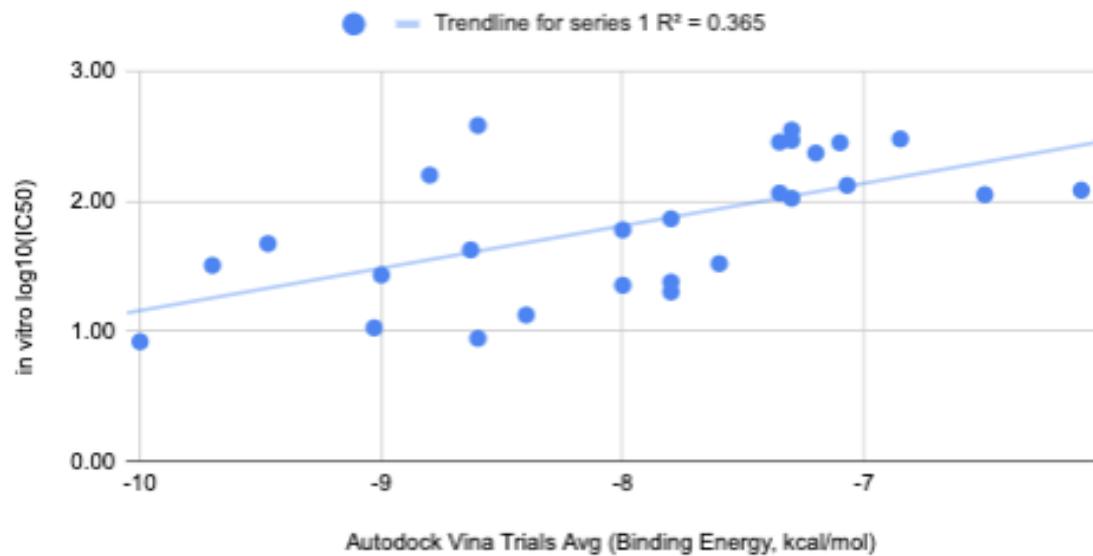


Autodock Vina results (Y-axis), literature-sourced in vitro IC50 values on 3CLpro (X-axis). Deep-pocket poses maintained. best-fit line $m = 0.2963$, $b = 4.1964$

There is some discussion in the literature and the autodocking software development community on whether ligand poses identified inside of deep-pockets are a valid site for ligands. A particularly strong version of a deep-pocket though is a tunnel, and the protein literature recognizes that tunnels are a normal and appropriate binding site for ligands such as the HIV protease's structure and its inhibitors such as Indinavir.

As the science on this specific matter remains unresolved, and the R2 correlation values are very close to each other, we allow for deep-pocket binding results in our studies even while recognizing that a sub-scope of the current disclosure can contemplate the filtering-out of deep-pocket results. As taken into account with deep-pockets removed (where epigallocatechin gallate and pectolinarin had to be removed from the dataset because all of their blind runs were deep pocket results), the resulting fit is:

2duc 3CLpro: Min A,B,blind (deeppockets removed), in-silico vs. in-vitro log(IC50) (x-y flipped)



Autodock Vina results (Y-axis), literature-sourced in vitro IC50 values on 3CLpro (X-axis). Deep-pocket poses removed. best-fit line m = 0.32603, b = 4.4182

Taken into account, the R² value is only minutely increased, and well within the noise of correlation studies of any variety as known to anyone practiced in the statistics / data-science art.

The trendline of best fit without removing any deep pocket poses is found to be:

$$[\text{predicted in vitro log10(IC50)}] = 0.2963 * [\text{ModelBindingEnergymin(blind,A,B)}] + 4.1964$$

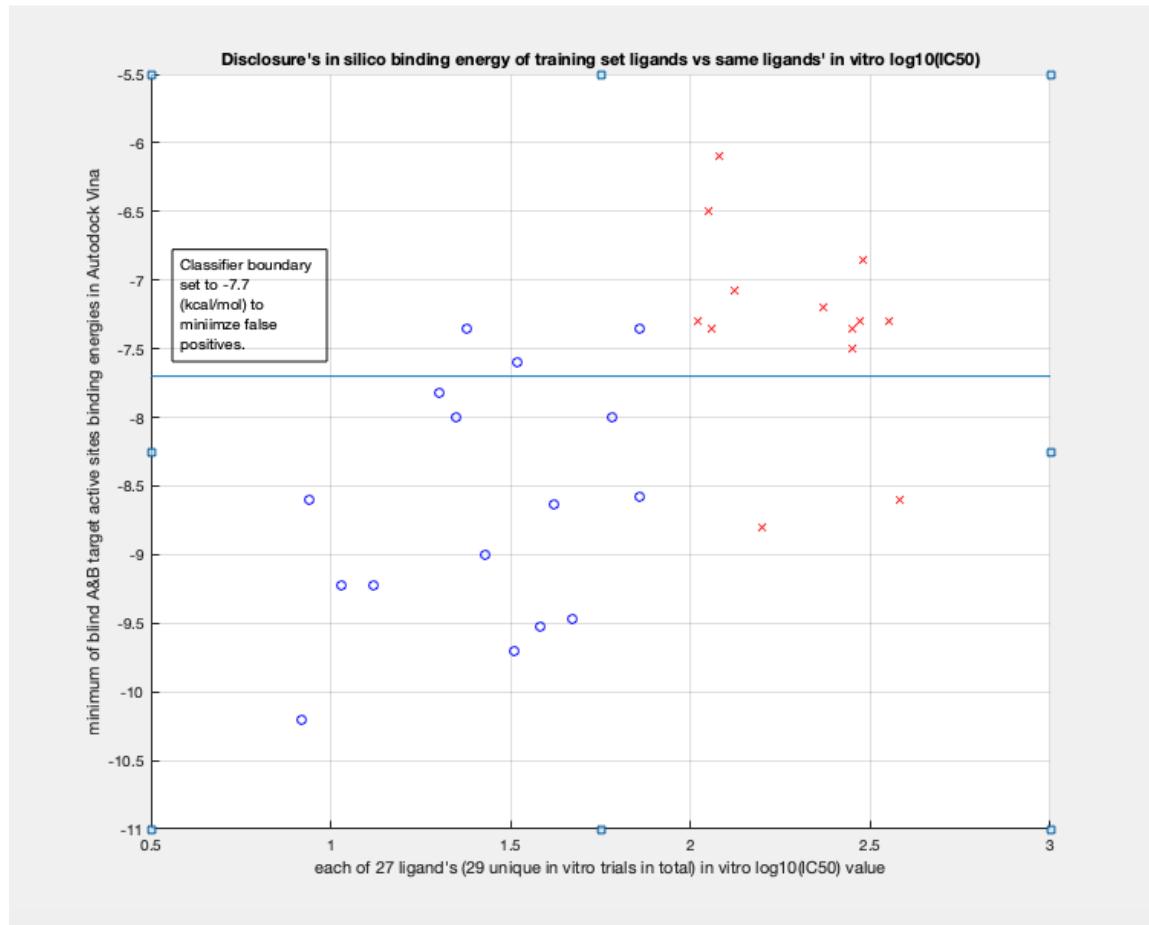
The trendline of best fit with removing deep pockets is found to be:

$$[\text{predicted in vitro log10(IC50)}] = 0.3260 * [\text{ModelBindingEnergymin(blind,A,B)}] + 4.4182$$

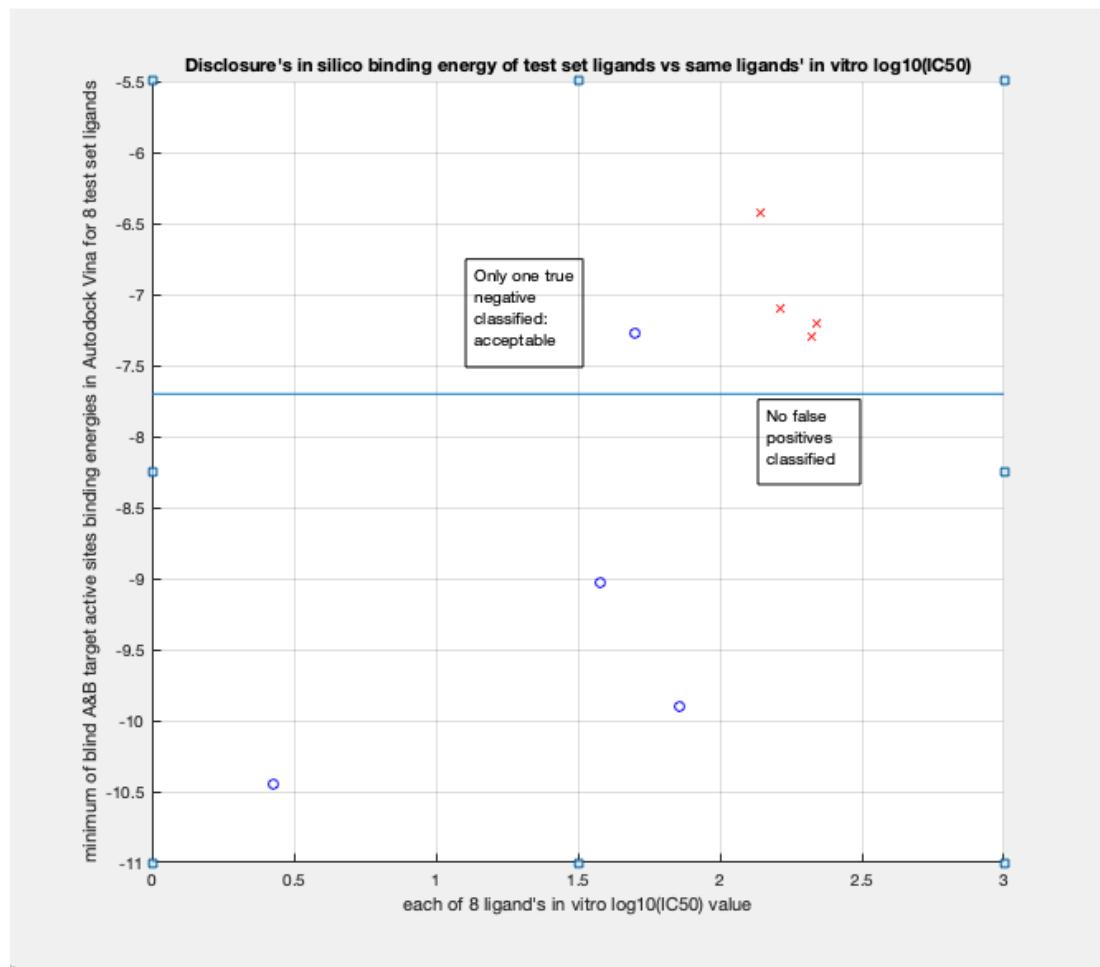
Compound	In silico assayed Min(blind,A,B) kcal/mol (pocket poses maintained)	Compound	In silico assayed Min(blind,A,B) kcal/mol (Pocket poses maintained)()
beta-sitosterol	-7.35	epigallocatechin gallate	-8.58
hesperetin	-8	gallocatechin gallate	-9.47
indican	-6.5	herbacetin	-7.60
sinigrin	-6.1	rhoifolin	-9.00
indirubin	-7.3	pectolinarin	-9.53
indigotin	-6.85	Eckol	-8.6
daidzein	-7.3	dioxynodehydroeckol	-8.8
aloeemodin	-7.07	2-Phloroeckol	-9.23
apigenin	-7.5	7-Phloroeckol	-8.63
luteolin	-7.83	fucodiphloroethol	-8
quercetin	-7.35	phlorofucofuroeckol A	-9.23
amentoflavone	-10.2	hinokiol	-7.2
quercetin (again)	-7.35	Isopimaric Acid	-7.35
daidzein (again)	-7.3	ginkgetin	-9.7
puerarin	-8.6		

Test set's in silico results, taking the minimum of 1. the average of the blind study (4 trials), 2. The average of the Achain target active site study (3 trials) and 3. the average of the Bchain target active site study.

Where an in vitro 'hit' is understood in the art of inhibitory assaying to be an IC₅₀ value of 100uM or less, it is then noted that a classifier in the machine-learning sense can be generated from the dataset. A classifier that asserts distinguishing hits from misses based on a threshold of **-7.7 kcal/mol** as output by Autodock Vina results is generated by graphic inspection. (Some 'cushion' is applied to maintain distance from the cluster of otherwise costly false positives, even at the expense of a true negative):



As per ordinary practice in the machine learning art, we then apply our classifier and classifier to a 'test set' that is independent from the training set used to generate the above model. That test set is disclosed:



SARS-CoV 3CLpro test set (deep pocket poses included)

Compound	Literature in vitro cell-free log10(IC50 uM)	In vitro 'hit' or 'miss'	Discloser's Autodock Vina Binding Energy result:	Disclosed model's inferred 'hit' or 'miss' (-7.7 kcal/mol boundary, inclusive)	Discloser's predicted in vitro cell-free log10 IC50 uM value predicted from linear fit: m = 0.2963 b = 4.1964	Classifier accuracy
18-Hydroxy-ferruginol	2.34	'miss'	-7.20	'miss'	2.07	Correct
Ferruginol	1.70	'hit'	-7.27	'miss'	2.05	Incorrect
18-Oxofer-ruginol	2.21	'miss'	-7.10	'miss'	2.10	Correct
Methyl dehydro-abietate	2.32	'miss'	-7.30	'miss'	2.04	Correct

Kayadiol	2.14	'miss'	-6.43	'miss'	2.29	Correct
Bilobetin	1.86	'hit'	-9.90	'hit'	1.19	Correct
Sciado-pitysin	1.58	'hit'	-9.03	'hit'	1.52	Correct
Dieckol	0.43	'hit'	-10.45	'hit'	1.10	Correct

SARS-CoV 3CLpro test set (deep pocket poses removed)

Compound	Literature in vitro cell-free $\log_{10}(\text{IC50}$ $\mu\text{M})$	In vitro 'hit' or 'miss'	Discloser's Autodock Vina Binding Energy result:	Disclosed model's inference 'hit' or 'miss' (-7.7 kcal/mol boundary, inclusive)	Discloser's predicted in vitro cell-free \log_{10} IC50 μM value predicted from linear fit: $m = 0.32603$ $b = 4.4182$	Classifier accuracy
18-Hydroxy-ferruginol	2.34	'miss'	-7.20	'miss'	2.07	Correct
Ferruginol	1.70	'hit'	-7.27	'miss'	2.05	Incorrect
18-Oxofer-ruginol	2.21	'miss'	-7.10	'miss'	2.10	Correct
Methyl dehydro-abietate	2.32	'miss'	-7.30	'miss'	2.04	Correct
Kayadiol	2.14	'miss'	-6.33	'miss'	2.35	Correct
Bilobetin	1.86	'hit'	-9.90	'hit'	1.19	Correct
Sciado-pitysin	1.58	'hit'	-9.03	'hit'	1.47	Correct
Dieckol	0.43	'hit'	-10.2	'hit'	1.09	Correct

Thusly it is shown that the model's value as a classifier in both the deep pockets maintained and deep pockets removed models, equally assert **87.5% classification accuracy** of compounds inhibitory efficacy identification on SARS-CoV-like proteases. The only incorrect classification in (both) sets is a true-negative, and so there is no evidence that the method will produce false-positives, and so remains a desirable quality for purposes of ligand efficacy disclosures.

Therefore the ligand efficacies identified in this Disclosure do not have deep pocket poses removed from their 'total = min(blind,A,B)' processed values.

Appendix B

Epidemiological results of a plant medicinals context with flavonoid focus in Chad (full version accessible [here](#))

About Covid Organics

Covid Organics was promulgated by Madagascar's Institut Malgache de Recherches Appliquées (IMRA). It is an herbal extract of *artemisia annua* and *ravintsara (cinnamomum camphora)* [cit;] Madagascar has shown competitive case-to-fatality performance data under a regime of universal administering of Covid Organics to its coronavirus patients. However it has not had a sufficient number of cases to be able to demonstrate a controlled study. Madagascar did, however, export Covid Organics across several countries in Africa. In reviewing the literature for countries actually administering of Covid Organics on a national scale to coronavirus patients, Chad alone stood out as having affirmatively done so.

Chad's epidemiological experience with Covid Organics in May 2020

Media reports in Chad attest to Chad's experience with applying Covid Organics in the clinical setting. [Cite article A, B, C, D]

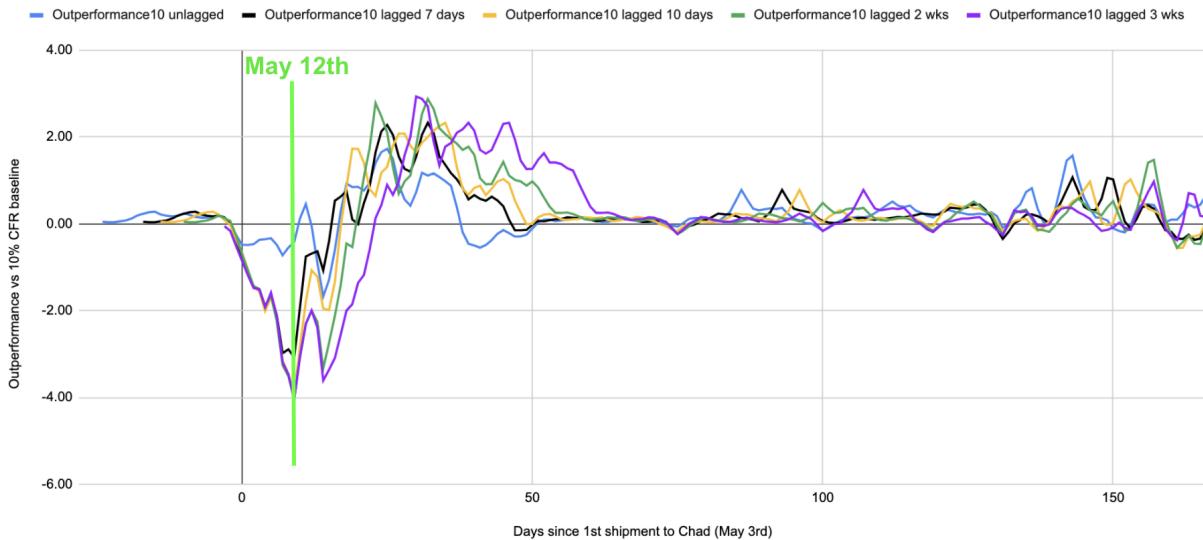
From [article C cit.] we know that 34 'high-risk' covid patients were treated exclusively with Covid-Organics by 4-June. Per the report, all were brought back to health again. Given Chad's high initial fatality rate at the start of their outbreak, this is notable. [Article C cit.] implies that additional patients were treated with covid-organics alongside other medications. However [Article D cit.] goes on to mention that covid-organics should not be used with hydroxychloroquine. This isn't surprising as similar flavonoid glycosides are known to competitively inhibit two liver enzymes known for processing many common chronic medications — and so the possibility of hydroxychloroquine overdoses in patients so treated exists. One of their government ministers is quoted saying (Google translated), "We are pleased today to reiterate what our Minister of Health has already said. Covid-Organics has been a positive experience in Chad"

An Outperformance metric is introduced to examine how Chad's case to fatality ratio varies over the course of the covid organics administering.

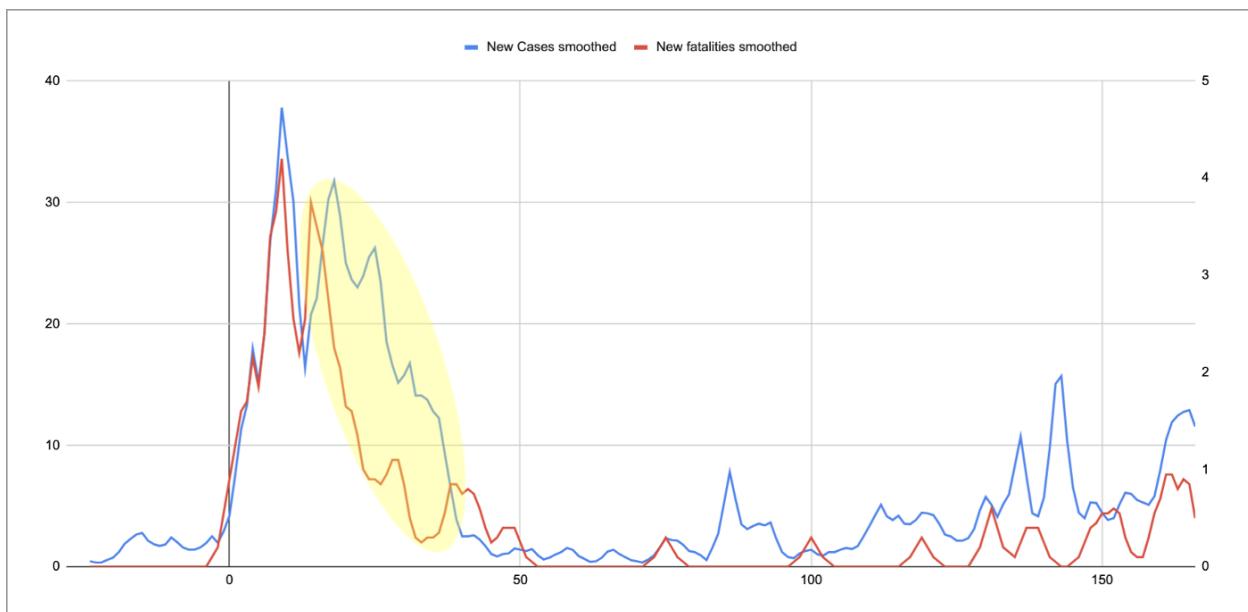
$$(\text{Arbitrary CFR \%}) * (\# \text{ of cases}) - (\# \text{ of fatalities}) = \text{Outperformance}$$

Given that there is typically a lag time between a reported case and any fatality associated with it, the metric is brought out in terms of zero lag, 1-week lag, 10-day lag, 2-wk lag, and 3-wk lag. The outperformance data for Chad accounting for case-fatality lag, (set relative to an arbitrary 10% baseline) is:

Chad Outperformance vs 10% CFR (arbitrary) baseline; Day 0 = May 3rd



What we see is the case fatality rate initially doing poorly; Then from May 12th we start to see an improvement in Chad's case fatality ratio performance (across all lag metrics). This can be compared to the raw Case vs. Fatality charts for Chad from Worldometer [cit.] below — note the gap of the case trace (blue) over the fatality trace (red) in days 20–40. So the above chart is essentially highlighting the gap seen in yellow below:



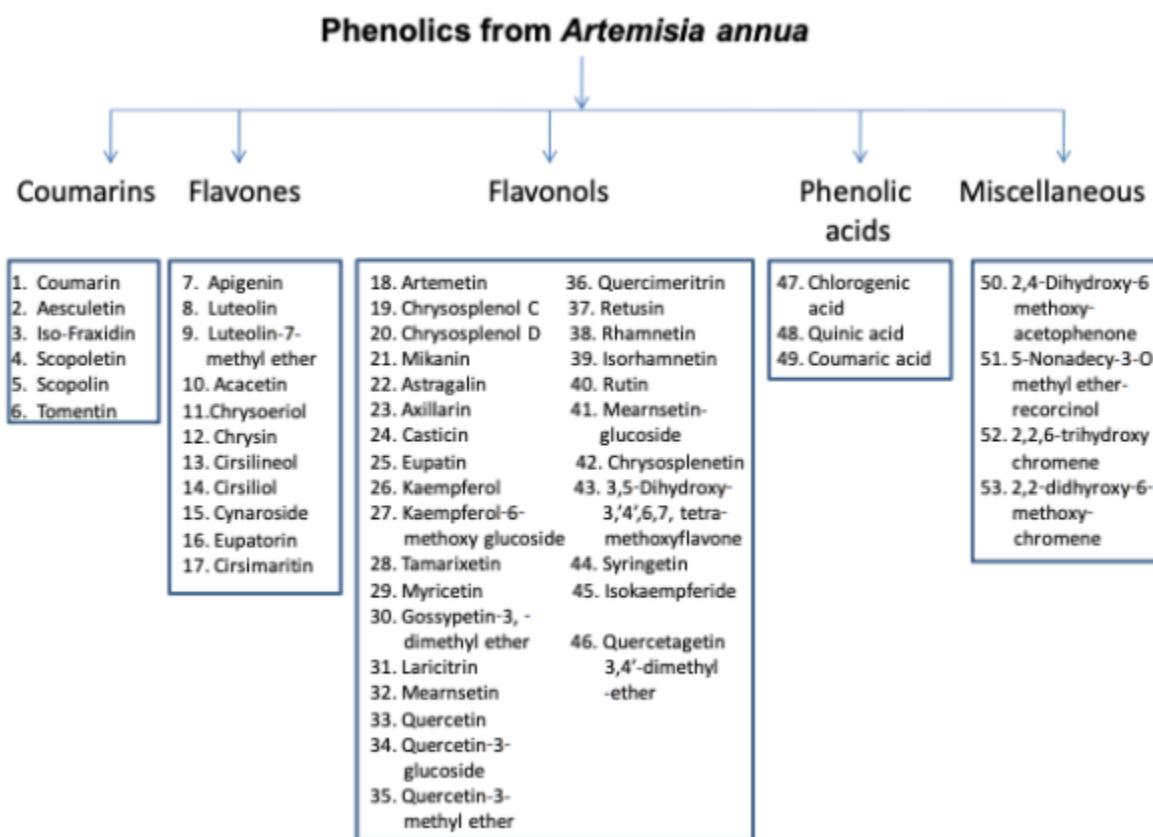
Day 0 = May 3rd. Blue: Chad covid cases (left side scale), Red: Chad covid fatalities (right side scale). Yellow highlighted is anticipated fatalities that (thankfully) never materialized.

Indeed, 12-May (as per Article A) is when the first Covid-Organics (aka Tambavy CVO) shipment is confirmed to have arrived in Chad. It is not known precisely how soon after 12-May they start administering to patients, but we presume soon afterward as by 4-June media reports Chad's health ministry having already administered to patients and seeing encouraging results. The right-side and left-side scales have been proportionally aligned to each other for easy comparison. Reviewing the yellow highlight, it's possible that Chad saved up to 20–25 lives with their CVO intervention.

By contrast, might any lives have actually been lost due to the intervention? It's actually not outside the realm of possibility. Article D seems to acknowledge the possibility when they warn about co-administration of CVO with chloroquine. This drug interaction makes sense, as flavonoids competitively inhibit liver enzymes that are relied upon to process xenobiotics such as chloroquine. (The resulting overdose causes heart arrhythmia which could prove difficult to manage in a low capability health care setting). Net-net of any such complications, it looks like Chad and its patient caseload came out the better for the intervention. In the face of a pandemic outbreak with essentially nil treatment options at the time, then the unique actions that the Chad health ministry took during their May outbreak can be appreciated.

Covid Organics' phytochemistry

While there was speculation early in the pandemic that artemisinin could be serving as an effective inhibitor of viral replication by associating from its experience as an antimalarial, the Max Planck Institute found no inhibitory effect of artemisinin on viral replication. [cit.] And yet they did find that the extract taken in its entirety did inhibit viral replication. This suggests that other compounds in *a. annua* extract may be instead operative. The phenolic compounds of *Artemisia annua* were catalogued by [Ferreira et al]. Of the 53 phenolic compounds identified in *Artemisia annua* identified, 29 of them were flavonols, with a significant representation of flavonoid glycosides among those eighteen.



(from [Ferreira et al 2010](#))

VeroE6 cells	EC50 ($\mu\text{g}/\text{mL}$) ^a		CC50 ($\mu\text{g}/\text{mL}$) ^b	SI ^c
	Pretreatment Assay	Treatment Assay		
Extract				
<i>A. annua</i> ethanolic extract	173	142	1044	6 / 7
<i>A. annua</i> + coffee ethanolic extract	176	128	632	3 / 5
<i>A. annua</i> aqueous extract	390	260	2721	7 / 10
Compound				
Artemisinin	238	151	8216	35 / 54
Artesunate	12	7	41	3 / 6
Artemether	>179	>179	1220	<7 / <7
HuH7.5 cells	EC50 ($\mu\text{g}/\text{mL}$) ^a		CC50 ($\mu\text{g}/\text{mL}$) ^b	SI ^c
	Extract			
	<i>A. annua</i> ethanolic extract	118	483	4
	Compound			
	Artemisinin	>208	5066	<24
Artesunate	11	93	8	
Artemether	135	303	2	

^a EC50, median effective concentration ($\mu\text{g}/\text{mL}$) was determined in VeroE6 cells in pretreatment or treatment antiviral assays or in HuH7.5 cells in treatment antiviral assays as described in Material and Methods. For artemether in VeroE6 cells and for artemisinin in HuH7.5 cells, <50% inhibition was observed at the highest non-cytotoxic concentration where cell viability was >90% of that of non-treated control cultures.

^b CC50, median cytotoxic concentration ($\mu\text{g}/\text{mL}$) was determined as described in Material and Methods.

^c SI, selectivity index, was determined as CC50 divided by EC50 based on results in pretreatment / treatment antiviral assays in VeroE6 cells or based on results in treatment antiviral assays in HuH7.5 cells.

[\(Gilmore et al. 2020\)](#)

(Gilmore et al. 2020) found that ethanolic and water extracts of *A.annua* were inhibitory to viral replication at 142 and 260 $\mu\text{g}/\text{ml}$, respectively.

Appendix C - Inventory of in silico assayed compounds

Total ligands assayed

The totality of ligands assayed for this disclosure is in the table below:

All compound \assayed in silico		
scutellarein tomentin scopolin scopoletin isoquercitrin isofraxidin coumarin artemetin aesculetin Tamarixetin Syringetin Rhamnetin Retusin Quinic_acid Quercimeritin Quercetagetin_3_4_dimethyl-ether Mikanin Mearnsitin Luteolin Laricitrin Eupatorin Eupatin Cirsimarin Cirsiliol Cirsilineol Chrysosplenol_D Chrysosplenol_C Chrysosplenetin Chrysoeriol Casticin Axillarin Astragalin vitD3_Cholecalciferol vitD3_Calcitriol vitD3_Calcifediol vitB3_nicotinamide_riboside vitB3_nicotinamide_adenine_dinucleotide vitB3_Nicotinic_acid vitB3_Nicotinamide isorhamnetin vitD3_Cholecalciferol vitD3_Calcitriol vitD3_Calcifediol vitB3_nicotinamide_riboside vitB3_nicotinamide_adenine_dinucleotide Khonklonginol A Khonklonginol B Khonklonginol C Khonklonginol D Khonklonginol E Khonklonginol F Khonklonginol G Khonklonginol H lupinifolinol (2) dehydrolupinifolinol (10),	vitB3_Nicotinic_acid vitB3_Nicotinamide 5_7_4_Trihydroxy_3_methoxyflavone 3_5_Dihydroxy_3_4_6_7Tetramethoxyflavone Cynaroside kayadiol Methyl_dehydroabietate 18_Oxoferuginol ferruginol 18_Hydroxyferuginol sciadopitysin bilobetin ginkgetin isopimaric_acid hinokiol galocatechin_gallate Dioxinodehydroeckol 2_Phloroekol 7_Phloroekol Fucodiphloroethol_G Phlorofucofuroeckol_A phloroglucinol Eckol puerarin n_epigallocatechin n_epigallocatechin gallate herbacetin rhoifolin pectolinarin Sulcatone_A gossypetin gossypin callistephin artemisinin 17alpha_ethynylestradiol myrecitin amentoflavone genkwanin n_epicatechin rutin catechin tideglusib crocin III, apigenin, acacetin luteolin apigenin syringin (14) methylcatalpol (15 and) buddlejoside A4 songaroside A, echinacoside, alpha-Amyrenone	shikonin ebselen 5-7-3'-4'-tetrahydroxy-2'-(3-3-dimethylallyl) isoflavone sinigrin daidzein aloe_emodin beta_sitosterol indican indirubin indigotin galangin eriocitrin eriodictyol chrysin quercetin genistein kaempferol kaempferide acacetin apigenin luteoli-7-O-rutinoside luteolin narngin naringenin norathyriol mangiferin diosmetin diosmin hesperidin hesperetin Quercetin-3,4'-diglucoside Spiraeoside hesperetin-7-O-rhamnoside quercetin-4'-galactoside luteolin-7-galactoside luteolin-5-galactoside Hyperoside Dieckol 8,8'-bieckol Tannic acid eriodictyol-7-O-glucoside hesperitin-7-O-glucoronide beta-sitosterol daucosterol l-Sesamin quercetin, quercetin-3-galactoside isorhamnetin catechin epicatechin quercetin

flemichin D (11), eriosemaone A (12), lupinifolin (13), yangambin beta-sitosterol stigmasterol 7-O-methyltectorigenin, tectorigenin, genistein, kaempferol, 2',4',5,7-tetrahydroxy-6-methoxyisoflavone kaempferol-7-O-beta-D-glucopyranoside (aka: Kaempferol 7-O-glucoside) genistein-7-O-beta-D-glucopyranoside (aka: Genistin) astragalin cajanol clovanediol Eriosematin E Chrysophanol; rhein ; sweroside picraquassioside C puerarin 3'-methoxypuerarin caffeic acid ; ellagic acid ; gallic acid ; p-Coumaric acid ; protocatechuic acid ; sinapinic acid ; vanillic acid ; catechin ; chrysin ; kaempferol ; myricetin ; naringenin ; quercetin ; resveratrol ; rutin oleanolic acid trans-ferulic acid 3-methoxy-4-hydroxybenzoic acid (aka Vanillic acid) sinapic acid luteolin luteolin-7-O-rutinoside, acteoside, luteolin-7-O-glucoside (aka - Cynaroside) neobudofficide, linarin, germacrene D B-caryophyllene (E)-Caryophyllene delta-Cadinene Beilschmin D Magnolol	cacalol, cacalone, cacalal, cacalol acetate, cacalolide, cacalonol O-Methylcacalodienol ; Peroxycacalonol ; Epicacalone ; acetate taraxasterol (taxasterol acetate) pedicin, isopedicin, pedicinin, pedicillin, pashanone, didymocarpin; isodidymocarpin, didymocarpin A, pediflavone, lignoceric acid, behenic acid, stearic acid, palmitic acid, beta-sitosterol, α- Humulen Cedroxyde (-)-α- Panasinsanene Longifolol 1-Octen-3-one E- Caryophyllene beta-Carotene 1,4-bis(4-hydroxy-3-methoxyphenyl)- 2,3-bis(hydroxymethyl)butane-1,4- diol (-)-silandrin B ((-)-silandrin) (-)-(7S,8R,8'R)-7-hydroxysecoisolaric iresinol (+)-cyclolariciresinol secoisolariciresinol americanin A integracin B 1,2-bis(4-hydroxy-3- methoxyphenyl)propane-1,3-diol cinchonain Ib linarigenin 7-hydroxy-6-methoxy-coumarin 8-hydroxy-6,7-methoxy-coumarin kaempferol kaempferol-3-O-beta-D-glucoside alpha-humulene bicyclogermacrene alpha-Pinene beta-Pinene Sabinene 9-epi-(E)-Caryophyllene 4α,5α-Epoxybeilschmin A	chlorogenic acid rosmarinic acid sinapic acid caffeine, tyrosol apigenin biochanin A maackiain formononetin afromosin genistein calycosin-7-O-beta-D-glucoside vicenin-2 isoliquiritigenin tessalatin, coelogin, flavidin, flaccidin, flavidinin, coelonin, lusianthridin, gigantol, batatasin III, kaempferol, trans-cinnamic acid, vomifoliol, ergosterol, sucrose ursolic acid luteolin urea stigma-5,22-dien-3-ol acetate erythrol, also known as("erythritol" isovitexin daucosterol beta-sitosterol 4, 7-dihydroxy-2-methoxy-9,10-dihydrophenanthrene batatasin III 3', 5-dihydroxy-2-(p-hydroxybenzyl) -3-methoxybibenzyl 3,3'-dihydroxy-2,6-bis(4-hydroxybenzyl)-5-methoxybibenzyl triphyllol pholidotin (E) -p-hydroxycinnamic acid (E)-ferulic acid (E)-ferulic acid hexacosyl ester solasodine 4α,5α-Epoxybeilschmin B Beilschmin A Beilschmin B beta-sitosterol beta-sitostenone squalene a-tocopherylquinone methyl linoleate
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The totality of ligands assayed under in silico trials on SARS-CoV-2's main protease