Oxadiazoles for Covid-19?

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Oxadiazoles for Covid-19?

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Oxadiazoles are a class of heterocyclic aromatic chemical compounds, having different isomeric forms. They appear in a variety of pharmaceutical drugs. Among the drugs based on the 1,3,4-oxadiazole ring, we find for instance an antiviral such as Raltegravir, which is used to treat HIV/AIDS. Here we will discuss Raltegravir and in-silico simulations concerning SARS-COV-2. Besides the compounds based on 1,3,4 rings, there exist those with 1,2,4-oxadiazole rings which are reported as anti-inflammatory and analgesic agents. One of these drugs is Oxolamine. Of this drug we will stress the fact that it is an inhibitor of the formation of N-acetylneuraminic acid, a sialic acid. Let us stress that the main target of SARS-COV-2 is considered the ACE2 receptor. Then, is the sialic acid supporting the virus to enter the cell? A study, 10.1021/acscentsci.0c00855, that used a glyco-nanoparticle platform, reported the discovery that N-acetyl neuraminic acid has affinity toward the SARS-COV-2 spike glycoprotein, that is a glycan-binding function. However, a recent study, 10.1016/j.scib.2021.01.010, which has investigated the binding of the spikes of the virus by means of micro-arrays, tells that no binding with sialic acid residues was detected. On 8 March 2021, a bioRxiv preprint 10.1101/2021.03.08.434228 has been published evidencing a sialic acid-dependent binding and viral entry of SARS-CoV-2. In any case, since the sialic acids have an antiviral protective role, it could be relevant to investigate carefully any ligand/protein docking property of Oxolamine (docking with human enzymes, virus enzymes and proteins). Based on the 1,2,4-oxadiazole scaffold, we have also the antiviral Pleconaril, that has been investigated by means of in-silico simulated interactions with SARS-COV-2 proteins. We will discuss also some news oxadiazole compounds, which are given as selective COX-2 inhibitors, antioxidant agents and inhibitors of proteins of the virus, according to in-silico simulations. Therefore, can these drugs have a possible role in the treatment of Covid-19? Or, why can't they be used for?

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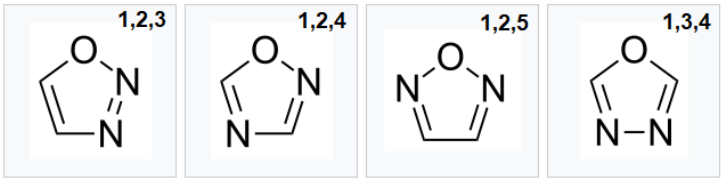
Before reading this work, please consider that I am not a physician. I do not suggest the use of the mentioned drugs. I am not touting these drugs. The analysis of publications is made in the framework of a wider investigation about information concerning drugs used for Covid-19.

Several drugs have been and are under investigation of clinical trials to find the best of them in the treatment of Covid-19 (see for instance [1]). Some have been and are used to reduce the cytokine storms, others are antivirals. In the case of Hydroxychloroquine, an antimalarial, it has been used for its anti-inflammatory effect and also for a possible antiviral effect in patients of Covid-19. Ivermectin is used too [2,3].

However, some drugs exist, based on Oxadiazole compounds, which have antimalarial, anti-inflammatory and antiviral features. Then, here we want to pose a question: can Oxadiazoles have a possible role in the treatment of Covid-19?

Let us remember that Oxadiazoles are a class of heterocyclic aromatic chemical compounds, which are interesting also for their liquid crystalline phases [4].



Oxadiazoles are characterized by five-membered heteroaromatic rings, which are containing two carbon atoms, two nitrogen atoms, and one oxygen atom.

In one of their isomeric forms - that is 1,3,4 -, Oxadiazoles appear in a variety of pharmaceutical drugs. In particular, in [5] it is told that "compounds containing 1,3,4-Oxadiazole ring in their structure are characterised by multidirectional biological activity. Their anti-proliferative effects associated with various mechanisms, such as inhibition of growth factors, enzymes, kinases and others, deserve attention. ... In most publications, the most active derivatives of 1,3,4-Oxadiazole exceeded the effect of reference drugs, so they may become the main new anti-cancer drugs in the future".

In fact, the "Oxadiazole isomers occur in the structure of many drugs, e.g., antitussive Oxolamine, antimicrobial Furamizole, antiviral Raltegravir and others" [5]. Moreover, it is also stressed that there "are numerous literature reports confirming the multidirectional effect of compounds containing the 1,3,4-oxadiazole ring in its structure". We find, among the others, antimalarial [6], anti-inflammatory [7] and antiviral effect [8,9] (see also [10,11]), besides anti-cancer properties.

For what concerns the form as 1,2,4-Oxadiazole, in [12], it is told that this is "one of the important heterocycles used by medicinal chemists in designing a new therapeutic molecule. The compounds containing this nucleus are reported to have a wide range of pharmaceutical and biological applications including anti-inflammatory and analgesic activities. The fused and pendent 1,2,4-oxadiazole moiety has been traced in a number of well established, commercially available drugs". Ref.12 is a review article about the

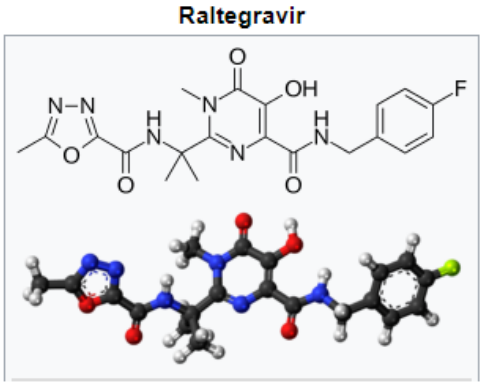
synthesis of 1,2,4-oxadiazoles, and "their therapeutic importance as anti-inflammatory and analgesic agents". For some other drugs, see Refs. 13 and 14.

If we consider the drugs used for Covid-19, we find among them antimalarial, anti-inflammatory and antiviral molecules. Since Hydroxychloroquine is an antimalarial, and an antimalarial Oxadiazole compound exists, it could be interesting to test for this drug a possible effect on Sars-Cov-2, the virus producing Covid-19. The same we can repeat for the Oxadiazoles having anti-inflammatory and antiviral properties.

The posed question (can these drugs have a possible role in the treatment of Covid-19?) is therefore reasonable. Alternatively, why can't Oxadiazoles be used for treatment?

Let us discuss first **Raltegravir, Oxolamine** and **Pleconaril.** These are three well-known drugs.

Then, we will consider new compounds.



Raltegravir (1,3,4-oxadiazole)

Raltegravir is an antiretroviral medication used, together with other medication, to treat HIV/AIDS. According to NIH, currently, there are 339 clinical trials about this drug (no one about Covid-19).

Molecular models from the web page:

https://en.wikipedia.org/wiki/Raltegravir

However, the following study exists, given in Ref. [15]. For the authors of the study entitled "Targeting SARS-CoV-2: a systematic drug repurposing approach to identify promising inhibitors against 3C-like proteinase and 2′-O-ribose methyltransferase", it was imperative "to identify promising inhibitors from pre-existing antiviral drugs". They have studied the two druggable targets mentioned in the title. They have been selected "due to their indispensable nature in the viral life cycle". "The selected drug target proteins were screened against an in-house library of 123 antiviral drugs. Two promising drug molecules were identified for each protein based on their estimated free energy of binding (ΔG), the orientation of drug molecules in the active site and the interacting residues. The selected protein-drug complexes were then subjected to MD simulation, which consists of various structural parameters to equivalently reflect their physiological state. From the virtual screening results, two drug molecules were selected for each drug target protein [Paritaprevir (ΔG = −9.8 kcal/mol) & Raltegravir (ΔG = −7.8 kcal/mol) for 3CLpro and Dolutegravir (ΔG = −9.4 kcal/mol) and

Bictegravir (ΔG = −8.4 kcal/mol) for 2′-OMTase]. After the extensive computational analysis, [the authors] proposed that Raltegravir, Paritaprevir, Bictegravir and Dolutegravir are excellent lead candidates for these crucial proteins and they could become potential therapeutic drugs against SARS-CoV-2". Raltegravir is also considered in [16].

In [17], we find that a "drug screening research team led by The Shanghai Tech University and Shanghai Institute of Materia Medica recently reported 30 agents with potential antiviral activity against SARS-CoV-2.46 These agents include remdesivir, saquinavir, indinavir, ritonavir, lopinavir, darunavir, fosamprenavir, carfilzomib, presatovir, atazanavir, enzaplatovir, tipranavir, maribavir, abacavir, bortezomib, **raltegravir**, elvitegravir, deoxyrhapontin, montelukast, polydatin, disulfiram, chalcone, ebselen, tideglusib, carmofur, PX12, shikonin, TDZD-8, cinanserin, and cyclosporin A" [18]. In [19], it is told that the screening was made "in silico"[1].

Raltegravis is included in the "in silico" study proposed in [20].

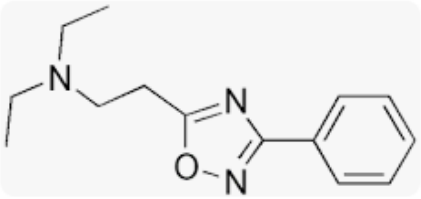
Raltegravir is also mentioned in [21] (published 15 June 2020). "This study elaborates the role of HIV and HCV drug in targeting nsp12 gene of SARS CoV 2 that is responsible for RNA dependent RNA polymerase activity. The work employed homology modelling, structure validation and molecular docking analysis to evaluate the binding affinity and interaction analysis between NSP12 (RdRp) protein and HIV/HCV drugs. Outcome of the study impacted on Nelfinavir (NFV), Raltegravir (RAL) and Delavirdine (DLV) among Anti-HIV and Paritaprevir (PTV), Beclabuvir (BCV) and Ledipasvir (LDV) among Anti-HCV as the most effective inhibitors of SARS CoV-2 RdRp ternary complexes. The drugs have a strong binding affinity with the residues that are present in the active site of RdRp of the virus and essential for its replication. This study [21] establishes significant information in the direction of therapeutic development as we are dealing with the situation where we urgently required medication or vaccine to combat COVID-19. Therefore, this study [21] provides essential molecular information about the FDA approved antiviral drugs that can be used to treat this disease. Importantly, the listed drugs had never prioritized for their effectiveness against COVID-19".

In [22] (December 2020), we find the following. "Out of 65 FDA approved small molecule antiviral drugs screened, Raltegravir showed highest interaction energy value of -9 kcal/mol against Mpro of SARS-CoV-2 and Indinavir, Tipranavir, and Pibrentasvir exhibited a binding energy value of ≥−8 kcal/mol. Similarly Indinavir showed the highest binding energy of -11.5 kcal/mol against the target protein RdRp and Dolutegravir, Elbasvir, Tipranavir, Taltegravir, Grazoprevir, Daclatasvir,

1 "In silico (Pseudo-Latin for "in silicon", alluding to the mass use of silicon for computer chips) is an expression meaning "performed on computer or via computer simulation" in reference to biological experiments. The phrase was coined in 1987 as an allusion to the Latin phrases in vivo, in vitro, and in situ, which are commonly used in biology (see also systems biology) and refer to experiments done in living organisms, outside living organisms, and where they are found in nature, respectively". https://en.wikipedia.org/wiki/In_silico

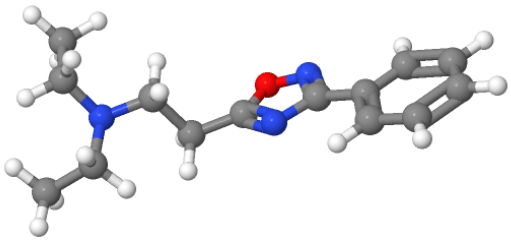
Glecaprevir, Ledipasvir, Pibrentasvir and Velpatasvir showed a binding energy value in range from -8 to -11.2 kcal/mol. The antiviral drugs Raltegravir, Indinavir, Tipranavir, Dolutegravir, and Etravirine also exhibited good bioavailability and drug-likeness properties". The authors conclude that their "study suggests that the screened small molecule antiviral drugs Raltegravir, Indinavir, Tipranavir, Dolutegravir, and Etravirine could serve as potential drugs for the treatment of COVID-19 with further validation studies".

In [23] (October 2020). "Remarkably, analyzing top ten CMap positive connections with BCG-CGS obtained from nine cell lines indicated that two compounds are approved antiviral drugs: raltegravir (top 3rd positive connection, an HIV integrase inhibitor) and lopinavir (top 6th positive connection, an HIV protease inhibitor)".



Oxolamine (1,2,4-oxadiazole)

Formula $C_{14}H_{19}N_3O$. "Oxolamine is a cough suppressant that is available as a generic drug in many jurisdictions"[2]. Can this drug be used to reduce one of Covid-19 symptoms?



3D model at https://chemapps.stolaf.edu/jmol/jmol.php?model=n1c%28onc1c2ccccc2%29CCN%28CC%29CC

In [24], we find that Oxolamine is the 3-phenyl-5β diethylaminoethyl-1,2,4-oxadiazole. In the conclusions of this article dated 1961, it was told that "the antitussive action would depend mainly on the anti-inflammatory and spasmolytic effect of the drug on structures of the lung". In the abstract we find that it is suggested "a predominantly peripheral mechanism of action. Oxolamine also possesses analgesic-anti-inflammatory, local anaesthetic and antispasmodic properties. The acute and chronic toxicities of Oxolamine are low, and the experimental results indicate the absence of side effects".

For the use of any drug, it is necessary to consider side-effects and interactions with other drugs. In [25], its interaction with an anticoagulant was studied. "Oxolamine citrate is an oral anti-inflammatory agent with particular antitussive activity. Oxolamine also has analgesic and antispastic actions. ... ***Oxolamine inhibits the formation of N-***

2 https://www.drugs.com/international/oxolamine.html

acetylneuraminic acid, which is an early and sensitive warning signal of inflammation. It has been used extensively in hospitals ... in Korea; Oxolamine is also being given to patients ... who are stable on Warfarin therapy. Anecdotal reports from practice indicate that Oxolamine potentiates the anticoagulant effects of Warfarin ...". In [25], the authors studied how to adjust Warfarin dosages.

In [26], further investigations have been made. In it we find again that "Oxolamine [3-phenyl-5-(b-diethyloaminoethyl)-1,2,4-oxadiazole] citrate is an antiinflammatory agent with a particular antitussive activity. Therefore, it has been used extensively in hospitals for patients with coughs". In this article, we find a ***gender difference*** in the pharmacokinetic interaction between oral Warfarin and Oxolamine in rats.

Details on this drug in https://pubchem.ncbi.nlm.nih.gov/compound/Oxolamine

Receptors

What is told in [25], that the drug is a *N-acetylneuraminic acid inhibitor* is very important. ***N-Acetylneuraminic acid***[3] *"(Neu5Ac or NANA) is the predominant sialic acid*[4] *found in human cells, and many mammalian cells. Other forms, such as N-Glycolylneuraminic acid, may also occur in cells. This residue is negatively charged at physiological pH and is found in complex glycans on mucins and glycoproteins found at the cell membrane. Neu5Ac residues are also found in glycolipids, known as gangliosides, a crucial component of neuronal membranes found in the brain. Along with involvement in preventing infections (mucus associated with mucous membranes - mouth, nose, GI, respiratory tract),* ***Neu5Ac acts as a receptor for influenza viruses****, allowing attachment to mucous cells via hemagglutinin (an early step in acquiring influenza virus infection)".*

About hemagglutinin and neuraminidase glycoproteins of influenza A virus, which are responsible for the surface interactions of the virion with the host, see Ref. 27. See also "Discovery and development of neuraminidase inhibitors", at the following link en.wikipedia.org/wiki/Discovery_and_development_of_neuraminidase_inhibitors , archived https://archive.is/rfA1K

N-acetyl neuraminic acid and SARS-COV-2 spikes

In [28], September 22, 2020, it is told that "There is an urgent need to understand the behavior of the novel coronavirus (SARS-COV-2), which is the causative agent of COVID-19, and to develop point-of-care diagnostics. Here, *a glyconanoparticle platform is used to discover that N-acetyl neuraminic acid has affinity toward the*

3 https://en.wikipedia.org/wiki/N-Acetylneuraminic_acid archived https://archive.is/jOjIk

4 "Sialic acid is a generic term for a family of derivatives of neuraminic acid, an acidic sugar with a nine-carbon backbone. It is also the name for the most common member of this group, N-acetylneuraminic acid (Neu5Ac or NANA)". https://en.wikipedia.org/wiki/Sialic_acid archived https://archive.is/IR0fJ

SARS-COV-2 spike glycoprotein, demonstrating its glycan-binding function. Optimization of the particle size and coating enabled detection of the spike glycoprotein in lateral flow and showed selectivity over the SARS-COV-1 spike protein. Using a virus-like particle and a pseudotyped lentivirus model, paper-based lateral flow detection was demonstrated in under 30 min, showing the potential of this system as a low-cost detection platform". "The spike-protein from SARS-COV-2 is shown to bind sialic acids, which is exploited to assemble a lateral flow diagnostic tool, using glycans rather than antibodies, as the recognition unit".

In [33], an article entitled "Human Sialome and Coronavirus Disease-2019 (COVID-19) Pandemic: An Understated Correlation", the role of sialic acid is considered too. Also the article [46] entitled "Is Sialic Acid a Gate to the Eye of Cytokine Storm?", is investigating the role of this acid. Here some sentences from the abstract proposed by authors. "The high pathogenic potential of human CoVs, including SARS-CoV, MERS-CoV and SARS-CoV-2, is closely related to the invasion mechanisms underlying the attachment and entry of viral particles to the host cells. There is increasing evidence that sialylated compounds of cellular glycocalyx can serve as an important factor in the mechanism of CoVs infection. Additionally, the sialic acid-mediated cross-reactivity with the host immune lectins is known to exert the immune response of different intensity in selected pathological stages". However, we can find a recent study (2021, in press [47]), a study that has investigated the binding of the spikes of the virus by means of micro-arrays, which is telling that no binding with sialic acid residues was detected. All the tested protein molecules can bind to heparan sulfate glycans [48] in a sulfation-dependent manner. But, an even more recent article, published on March 8, 2021, [49] is claiming a sialic acid-dependent binding and viral entry of SARS-CoV-2.

In any case, since the sialic acids have an antiviral protective role, it could be relevant to investigate carefully any ligand/protein docking property of Oxolamine (docking with human enzymes, virus enzymes and proteins).

A potential target

As we have seen, there are some new different targets for drugs against the virus.

In [29], concerning chloroquine, it was told that "The first step of the viral replication cycle", which is the attachment to the surface of respiratory cells, mediated by the spike viral protein, "offers several potential therapeutic targets. The S protein uses the angiotension-converting enzyme-2 (ACE-2) receptor for entry, but *also sialic acids linked to host cell surface gangliosides*. Using a combination of structural and molecular modelling approaches, this study showed that chloroquine (CLQ), one of the drugs currently under investigation for SARS-CoV-2 treatment, binds sialic acids and gangliosides with high affinity". Also in [30], for gastrointestinal SARS-CoV-2 infections, it is told that the "viral spike protein is prevented from binding gangliosides, which are composed of a glycosphingolipid with one or more sialic acids, in the presence of chloroquine or hydroxychloroquine". However it is also told that "In

gastrointestinal SARS-CoV-2 infection the efficiency of these repositioned drugs is debated".

"Can chloroquine interact with sugar-modifying enzymes?" - This is the question that we find in the caption of a figure[5] in Ref.31 (February 2006). In this article, it was told that "glycosylation inhibition might represent a major mechanism for the antiviral effects of chloroquine, suggesting that specific interactions of chloroquine with sugar-modifying enzymes or glycosyltransferases may occur within human cells (figure). ... *If chloroquine should indeed inhibit the biosynthesis of sialic acid,* this effect could explain not only the effects of chloroquine on HIV and SARS[6] coronavirus (sialic acid moieties are present in HIV-1 glycoproteins and SARS coronavirus receptor ACE2), but also the in-vitro effects on orthomyxoviruses (which use sialic acid moieties as receptors). These effects deserve further investigation, in that they may lead to new strategies controlling the replication of several viruses"[7].

Questions

Is it possible to use an Oxadiazole compound for Covid-19? Oxolamine for instance? Oxolamine possesses analgesic-anti-inflammatory, local anaesthetic and antispasmodic properties. Moreover, is it better to suppress sialic acid or increase it? *(and this question can be the same for the use of hydroxychloroquine).*

Since sialic acids have, at the same time, an antiviral protective role (see the following discussion), it could be relevant to investigate any docking of Oxolamine with human enzymes, virus enzymes and proteins. It is a small drug, which seems being a sialic acid inhibitor, like the hydroxychloroquine is [32].

5 Figure caption tells that it is showing a "computer-assisted simulation of ligand/protein docking by use of the program GOLD12 indicates that chloroquine (red) fits to the active site of UDPN-acetylglucosamine 2-epimerase (grey). This evidence suggests that chloroquine could inhibit the enzyme that catalyses the rate-determining step in the sialic acid biosynthetic pathway".

6 Authors were referring to Sars-Cov virus.

7 From rndsystems.com/resources/articles/ace-2-sars-receptor-identified archived archive.is/qywuf. "Identification and sequencing of the virus responsible for COVID-19 (view SARS-CoV-2 protein sequence) determined that it was a novel CoV that shared 88% sequence identity with two bat-derived SARS-like CoV, suggesting it had originated in bats. Additionally, it was shown that this CoV, which was termed ... SARS-CoV-2, shared 79.5% sequence identity with SARS-CoV. The coronaviral genome encodes four major structural proteins: the spike (S) protein, nucleocapsid (N) protein, membrane (M) protein, and the envelope (E) protein. The spike protein is responsible for facilitating entry of the CoV into the target cell. It is composed of a short intracellular tail, a transmembrane anchor, and a large ectodomain that consists of a receptor binding spike S1 subunit and a membrane-fusing spike S2 subunit. Sequence analysis of the SARS-CoV-2 spike protein genome showed that it was only 75% identical with the SARS-CoV spike protein. However, analysis of the receptor binding motif (RBM) in the spike protein showed that most of the amino acid residues essential for receptor binding were conserved between SARS-CoV and SARS-CoV-2, suggesting that the two CoV strains use the same host receptor for cell entry.The entry receptor utilized by SARS-CoV is Angiotensin-Converting Enzyme 2 (ACE-2)". See references therein.

Human Sialome, Age and Sex

The term "human sialome" is used for the broad variety of sialic acid compounds in the human body. It has been hypothesized that it is "the result of genomic changes occurred under the selective impulse of an alleged pandemic event, roughly 3 million years ago, provoking the so-called sialoquake" [33] (see also [34][8]).

A section in [33] is discussing the antiviral protective role of the Sialome. "Sialic acid viral recognition has been long known to be a virulence factor for various pathogens. However, the sialome exerts also a protective effect against viral infections (see the Figure 1B in [33]). As a host defence mechanism, sialylated O-linked glycans covering mucins on mucosal cell surfaces provide a large layer of sialylated residues that acts as a barrier, preventing pathogens from entering the cell by offering a decoy alternative binding site".

Moreover, it is necessary to note that the "sialome undergoes aging-dependent deleterious processes as well. Sialylation is a modification through which a sialic acid unit is added at the end of an oligosaccharide chain in a glycoprotein. ... Recent studies have identified an age-related accumulation of aglycosylated IgGs, which is linked to a pro-inflammatory status, typical of the elderly. Moreover, elderly patients exhibit a lower sialic acid content in saliva compared to children, confirming that sialylation processes decrease all over the body with aging. Similarly, sialome seems to be affected by the body's hormonal asset, in that estrogens upregulate antibody sialylation, determing an anti-inflammatory effect, whilst a decrease in estrogen levels, as seen in menopause, leads to lower sialylation activity" [33].

Pleconaril

In [35], we find, "after a concise historical introduction", "a comprehensive overview of the recent achievements in the synthesis of *1,2,4-oxadiazole-based* compounds and the major advances in their biological applications in the period of the last five years as well as brief remarks on prospects for further development". In [35], among the commercial drugs having the 1,2,4-oxadiazole scaffold we find Pleconaril.

This drug has been considered in [36]. "The set of antiviral drugs was taken from the

8 From Ref.34 "Whereas most vertebrates, including our closest evolutionary relatives (bonobos and chimps), express significant amounts of NeuGc as well as NeuAc ... , humans only synthesize NeuAc. The inability of humans to synthesize NeuGc is due to an exon deletion in the gene responsible for converting the N-acetyl to the N-glycolyl form, CMP-N-acetylneuraminic acid hydroxylase (CMAH). Molecular clock comparison of the disrupted human and intact chimpanzee genes places the insertion at ~3 million years ago. What caused the species to diverge? Since sialic acid recognition is a virulence factor for some pathogens, it has been speculated that evolutionary selection against expression of functional CMAH in the human lineage was due to a catastrophic pandemic by a pathogen that targeted NeuGc. Varki has termed this theoretical event the “sialoquake.” His hypothesis is consistent with the glycan binding specificities of related human and non-human pathogens, and with evolutionary changes in immune system sialic acid binding proteins that postdate the loss of CMAH".

Influenza Research Database (fludb.org). Potential inhibitors of SARS-CoV-2 protease were identified among all the anti-protease drugs in the mentioned database: Asunaprevir (DB11586), Nelfinavir (DB00220), Simeprevir (DB06290), Faldaprevir (DB11808), Indinavir (DB00224), Ritonavir (DB00503), Amprenavir (DB00701), Tipranavir (DB00932), Atazanavir (DB01072), Saquinavir (DB01232), Darunavir (DB01264), Fosamprenavir (DB01319), Lopinavir (DB01601). Furthermore, three molecules were chosen for the spike protein: Umifenovir (DB13609), Enfuvirtide (DB00109), and Pleconaril (DB05105)".

"The docking of three different inhibitors was performed on the spike protein. As the inhibitors are expected to block the membrane binding, [the researchers] limited the docking search region to the area around the protein head in its pre-fusion “down” configuration. Moreover, due to the large size of the protein and to limit the computational cost, we performed a rigid docking of the inhibitors against the target multimeric protein. Autodock Vina was used to identify the best binding pose of three different drugs: Umifenovir (DB13609), Enfuvirtide (DB00109), and Pleconaril (DB05105)".

Pleconaril is used for the prevention of asthma exacerbations and common cold symptoms in asthmatic subjects exposed to respiratory infections [37]. Moreover " Pleconaril is orally bioavailable and active against Picornaviridae viruses" [36].

In a discussion by Michael Skinner

Pleconaril is mentioned in a discussion about Ref.38, a study based on cryo–electron microscopy. Dr Michael Skinner, Reader in Virology, Imperial College London, is also shortly explaining method and results. He said[9]:

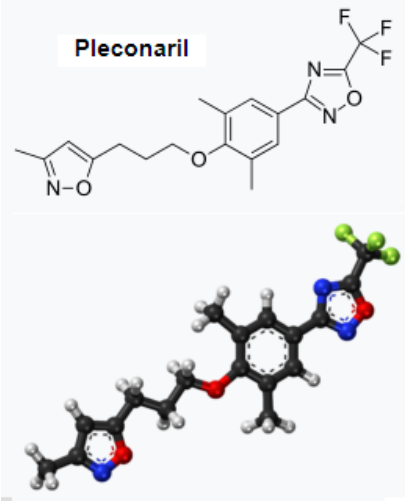
"This study [38] started as an exercise to produce recombinant SARS-COV-2 spike protein in the test tube for studies on how it binds to cells in the respiratory tract ... and to antibodies produced by our immune system in response to infection or vaccination. ... The structure looked correct and in line with results reported by other groups but the authors spotted small patches of unexpected ‘electron density’ embedded at the same place in each of the 3 components of each spike trimer. Comparing the shape of the density with all the structures in public databases revealed about 3 structures that had similar patches of density, caused by known molecules. *This pointed to the linoleic acid, a polyunsaturated omega-6 fatty acid* ... required for formation of prostaglandins ... and in formation of cell membranes".

"As far as [Michael Skinner is] aware, this is the first time that a fatty acid has been found embedded in a virus glycoprotein inserted into its envelope ... . There is however a precedent from a totally different type of virus that also causes common colds, *the rhinovirus*. ... In both of these different viruses, the role of the fatty acid seems to be to

9 https://www.sciencemediacentre.org/expert-reaction-to-study-on-druggable-pocket-in-structure-of-sars-cov-2-virus-spike/

stabilise the protein or the particle before it interacts with receptors on the surface of the cell. Identification of the fatty acid in rhinovirus eventually led to a chemical mimic that acted as an antiviral, Pleconaril. In trials, it shortened the length of the cold by a day and it was licensed for emergency use against related more dangerous picornaviruses. However, it has side effects (for instance, reducing the effectiveness of the contraceptive pill) that precluded wider uptake".

"The linoleic acid-binding pocket in SARS-COV-2 will make an attractive target for possible designer drugs, lead candidates for which already exist from screens for rhinovirus. Drug development and testing is likely to take some time ... The discovery, which is robustly supported by comprehensive study using several different approaches, might also offer insight into COVID-19 pathology, given the various functions of linoleic acid as an essential fatty acid."



The molecule is from
https://en.wikipedia.org/wiki/Pleconaril

"Pleconaril, a specific inhibitor of human picornaviruses, showed therapeutic efficacy against community-acquired colds caused by rhinoviruses in two placebo-controlled trials" [39]. Let us note that Pleconaril has several variants, eleven basic variants have been discussed in [40].

Selective COX-2 inhibition

Let us consider now some recently developed new Oxadiazole compounds.

In [41], it is told that "Based on analyses of available data, [the authors] deduced that the excessive prostaglandins E2 (PGE2) accumulation mediated by cyclooxygenase-2 (COX-2) was the key pathological basis of COVID-19". For this reason, the authors tell that "An experimental study about Celebrex to treat COVID-19 was conducted based on routine treatment". In [41], it is concluded that "Celebrex significantly reduced the PGE2 levels and promoted recovery of ordinary or severe COVID-19". Celebrex is the brand name of Celecobix. This compound contains a pyrazole ring. *Paper [41] has been published in Frontiers in Pharmacology, 06 November 2020, https://doi.org/10.3389/fphar.2020.561674*

The Celecobix is used *as a reference drug* for the study of compounds containing the

1,3,4-ring in [42]. In this study, several compounds have been proposed. It is told that "selective COX-2 inhibitors might provide good anti-inflammatory agents with improved therapeutic potency and reduced side effects associated with the use of conventional nonsteroidal anti-inflammatory drugs (NSAIDs)". A selective COX-2 inhibitor is the celecoxib. However, it can have several cardiovascular adverse effects. For this reasons, the authors of [42], proposed the search for novel COX-2 selective inhibitors with an improved safety profile.

In [43], a new series of compounds comprising of 2,5-diaryl-1,3,4-oxadiazoles was synthesized and evaluated as potential COX-2 inhibitors.

New Antioxidant 2,5-Disubstituted-1,3,4-oxadiazoles

In [44], 12 October 2020, it is told the following.

"Repurposing of known drugs and compounds as anticoronavirus disease 2019 (anti-COVID-19) agents through biological reevaluation of their activities, specially the antisevere acute respiratory syndrome coronavirus 2 (anti-SARS-CoV-2) activities, is a new viable trend in drug discoveries for the pandemic COVID-19 in 2020. Comprehensive inhibition of the coronaviral or coronaviral-2 enzymes and proteins (i.e., multitarget inhibition) can be considered one of the most powerful promising strategies for developing highly potent remedies for COVID-19. However, almost all the reported inhibitors of the different life cycle stages of SARS-CoV-2 lack the extreme potencies against the major and fateful SARS-CoV-2 enzymes (e.g., RNA-dependant RNA polymerase "RdRp", papain-like protease "PLpro", and main protease "Mpro"). Herein, [Amgad M. Rabie] repurposed and introduced two antioxidant polyphenolic 1,3,4-oxadiazole compounds, ... (named CoViTris2020) ... (named ChloViD2020), as the first multitarget SARS-CoV-2 inhibitors with extremely higher potencies ... These two unique 2,5-disubstituted-1,3,4-oxadiazole derivatives were computationally studied (through molecular docking in almost all SARS-CoV-2 proteins and one human protein) and biologically evaluated (through one of the most credible in vitro anti-COVID-19 assays) for their anti-COVID-19 activities. The results of the **computational docking** showed that CoViTris2020 and ChloViD2020 amazingly exhibited very high inhibitory binding affinities with most docked SARS-CoV-2/human proteins (e.g., they exhibited extremely lower binding energies of -12.00 and -9.60 kcal/mol, respectively, with RdRp-RNA). The results of the biological assay showed that CoViTris2020 and ChloViD2020 interestingly exhibited very high and extremely significant anti-COVID-19 activities (anti-SARS-CoV-2 EC50 = 0.31 and 1.01 μM, respectively), and, in addition, they could be also very promising parent lead compounds for the design and synthesis of new anti-COVID-19 agents (through structural modifications and further computational studies). Further investigations for the development of CoViTris2020 and ChloViD2020 as anti-COVID-19 drugs, through in vivo biological evaluations and clinical trials research, are needed".

A2 and A4

In [45], the results of studies on some compounds have been proposed (on line 17 November 2020). "Lack of specific treatments [against Sars-Cov-2] raised an effort to find potential inhibitors for the viral proteins. The recently invented crystal structure of SARS-CoV-2 main protease (Mpro) and its key role in viral replication, non-resemblance to any human protease makes it a perfect target for inhibitor research. This article reports a computer-aided drug design (CADD) approach for the screening of 118 compounds with 16 distinct heterocyclic moieties in comparison with 5 natural products and 7 repurposed drugs. Molecular docking analysis against Mpro protein were performed finding isatin linked with a **oxidiazoles** [oxadiazoles] (A2 and A4) derivatives to have the best docking scores of −11.22 kcal/mol and −11.15 kcal/mol respectively. Structure-activity relationship studies showed a good comparison with a known active Mpro inhibitor and repurposed drug ebselen with an IC50 value of −0.67 μM. Molecular Dynamics (MD) simulations for 50 ns were performed for A2 and A4 supporting the stability of the two compounds within the binding pocket, largely at the S1, S2 and S4 domains with high binding energy suggesting their suitability as potential inhibitors of Mpro for SARS-CoV-2".

"Further Molecular dynamic (MD) analyses of the two best docked compounds A2 and A4, both derivatives of isatin and oxadiazole, showed that the interactions for these compounds are mostly governed by the hydrophobic and electrostatics interactions".

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