




Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

COMPARE Analysis, a Bioinformatic Approach to Accelerate Drug Repurposing against COVID-19 and Other Emerging Epidemics

SLAS Discovery
2021, Vol. 26(3) 345–351
© Society for Laboratory
Automation and Screening 2020
DOI: 10.1177/2472555220975672
journals.sagepub.com/home/jbx


Imad Naasani¹ 

Abstract

A novel bioinformatic approach for drug repurposing against emerging viral epidemics like COVID-19 is described. It exploits the COMPARE algorithm, a public program from the National Cancer Institute (NCI) to sort drugs according to their patterns of growth inhibitory profiles from a diverse panel of human cancer cell lines. The data repository of the NCI includes the growth inhibitory patterns of more than 55,000 molecules. When candidate drug molecules with ostensible anti-SARS-CoV-2 activities were used as seeds (e.g., hydroxychloroquine, ritonavir, and dexamethasone) in COMPARE, the analysis uncovered several molecules with fingerprints similar to the seeded drugs. Interestingly, despite the fact that the uncovered drugs were from various pharmacological classes (antiarrhythmic, nucleosides, antipsychotic, alkaloids, antibiotics, and vitamins), they were all reportedly known from published literature to exert antiviral activities via different modes, confirming that COMPARE analysis is efficient for predicting antiviral activities of drugs from various pharmacological classes. Noticeably, several of the uncovered drugs can be readily tested, like didanosine, methotrexate, vitamin A, nicotinamide, valproic acid, uridine, and flucloxacillin. Unlike pure *in silico* methods, this approach is biologically more relevant and able to pharmacologically correlate compounds regardless of their chemical structures. This is an untapped resource, reliable and readily exploitable for drug repurposing against current and future viral outbreaks.

Keywords

COVID-19, SARS-CoV-2, antiviral, drug discovery, algorithm, bioinformatics, *in silico*, COMPARE analysis, drug repurposing

Introduction

The surprising outbreak of COVID-19 alarmed the world about the need for an agile approach to quickly tackle and mitigate unprecedented pathogenic diseases. Accelerated drug discovery by repurposing existing therapeutics is the most practical strategy because the fast development of totally new drugs and vaccines can be hampered by the requirement of lengthy safety studies and regulatory processes. Most of all, newly developed vaccines and therapeutics can quickly lose their value if the pathogen is progressively mutating over the course of time, making the fresh development of safe and effective vaccines and therapeutics a very difficult goal to attain. This study elaborates on the effectiveness of COMPARE analysis, an algorithm used in anticancer drug screening, for uncovering and repurposing effective compounds against emerging viruses like SARS-CoV-2. The COMPARE algorithm was originally introduced by the Developmental Therapeutic Program (DTP) of the National Cancer Institute (NCI) and is publicly accessible.^{1,2} The DTP and its Japanese counterpart, the Disease Oriented Screening

(DOS),³ were established nearly three decades ago in the hope of identifying the most effective compounds against specific cancers. The concept of these programs is based on relating *in vitro* growth inhibition of tested compounds (GI50, or the concentration needed to induce 50% growth inhibition) on a panel of diverse human cancer cell types to performance against corresponding clinical cancers (**Fig. 1**).

Apart from the original objective of these programs, mining the collected data using bioinformatics can uncover new facts on pharmacological modes, biological traits, and

¹Nanoco Life Sciences, Nanoco Technologies, Ltd., Manchester, UK

Received Aug 4, 2020, and in revised form Oct 29, 2020. Accepted for publication Nov 2, 2020.

Supplemental material is available online with this article.

Corresponding Author:

Imad Naasani, Nanoco Life Sciences, Nanoco Technologies, Ltd., 46 Grafton Street, Manchester M13 9NT, UK.
Email: inaasani@nanocotechnologies.com

entirely new prospects for anticancer drug discovery.⁴ The author previously used the Japanese DOS program to establish molecules with strong inhibitory effects against the enzyme telomerase.^{5,6} In this study, the author demonstrates that COMPARE analysis using the current DTP data repository can be also useful for fast and low-cost antiviral drug discovery. Indeed, by seeding compounds with presumed effects against the SARS-CoV-2 virus in COMPARE, several correlated compounds (preys) with approved status for clinical use were easily identified. The value of this efficient resource seems to have been overlooked during the rush for discovering active antiviral drugs that are urgently needed to combat new epidemics like COVID-19.

Methods

The current version of the DTP data repository comprises an aggregate of more than 88,000 compounds tested on a panel of 59 different human cancer cell lines using the sulforhodamine B (SRB) assay.⁷ Within the repository, GI50 data from ~55,000 compounds are publically accessible. The SRB assay is by design a quantitative reporter on protein mass and is directly correlated with cell growth and division rate. The used cell panel is highly diverse and represents a variety of cancers, such as leukemia, melanoma, lung, colon, brain, ovary, breast, prostate, and kidney malignancies. The full description of the cell panel and the screening method are described in detail at the following DTP link; https://dtp.cancer.gov/discovery_development/nci-60/methodology.htm. Typically, all cell lines are grown on RPMI 1640 growth medium supplemented with 5% fetal bovine serum. Chemosensitivity is performed using a 48 h assay, and then GI50 values are collected for each tested compound on the 59 different cell lines. For each tested compound, the average of the GI50 values from all cell lines in the panel is calculated and converted to its log₁₀ to form the origin of the “mean graph.” Then, each log₁₀ GI50 value is subtracted from the average to give a value called the delta value and presented as a horizontal bar. A pattern mean graph is then created by plotting the positive and negative delta bars along the vertical axis. For each cell line, a bar to the right of the vertical axis (positive) means that the test agent is exerting a growth inhibitory effect that exceeds the average from all cell lines. Equally, a negative value bar to the left represents a growth inhibitory effect that is less than the average value from all cell lines. Thus, a bar with 3 units to the right means that the GI50 for that cell line is at a concentration that is 1000 times less than the average value from all the cell lines in the panel.

Candidate drugs for seeding in COMPARE were identified from current medical reports using medical sources like Medscape, Cure ID, and Medline. Then it was checked whether the gathered drugs with the most promising clinical performance are included in the NCI’s DTP repository. At

the time of writing this paper, only chloroquine, hydroxychloroquine, ritonavir, and dexamethasone were found to be in clinical evaluation against COVID-19 and at the same time included in the DTP repository. Running the COMPARE analysis was performed first by identifying the NSC (National Service Center) number for each drug molecule from the PubChem website (<https://pubchem.ncbi.nlm.nih.gov/>). Data Collection was conducted by accessing the NCI’s DTP link at https://dtp.cancer.gov/databases_tools/compare.htm and then clicking on the “New Technology PUBLIC COMPARE” link. This opens the COMPARE algorithm page: <https://nci60.cancer.gov/publiccompare/>. For seeding a compound in the COMPARE algorithm, the corresponding NSC code is entered in the “NSCs - any delimiter” tab; also, the “GI50 data endpoint” box is checked. With these parameters, “SEARCH AS CONFIGURED BELOW” can be clicked. After the COMPARE algorithm finishes identifying the fingerprint of the seeded compound, it will ask for the option of Pearson minimum correlation to be selected in order to call and present the most correlated compounds. Once the Pearson value is selected (e.g., >0.5), the algorithm will return a list of the correlated compounds ranked in a table with actual structures, CAS numbers (when available), SMILE codes, and NSC codes. In this study, a Pearson correlation higher than 0.5 was adopted based on prior experience, where it was obvious that the higher the correlation, the more meaningful the similarity that is detectable. Although using a correlation of <0.5 can lead to spurious results, lower values can still be used in certain desperate situations where very few choices are available (e.g., an entirely new disease with very limited and ambiguous drug candidates). Finally, the results were converted to Excel and saved (see Supplementary Information). In order to easily transform the collected NSCs of the correlated compounds to useful information about their commercial availability, detailed identity, and source, the list is copied from the Excel sheet and then inserted into the search window of the site: Enhanced NCI Database Browser 2.2 (<https://cactus.nci.nih.gov/ncidb2.2/>). This site can convert a batch of NSC numbers to the corresponding molecules with useful details on identifiers and sources.

Results and Discussion

While the SRB assay is considered simple and straightforward, in effect, it accurately and categorically reports on the numerous events involved in the cellular growth machinery where hundreds of intra- and extracellular events are involved. For example, it is reported that more than 850 genes are operating in only cell cycle regulation activities.⁸ Any exogenous compound that changes the readout from the SRB assay is in effect a compound that interferes with one or more of the numerous events involved in cell growth.

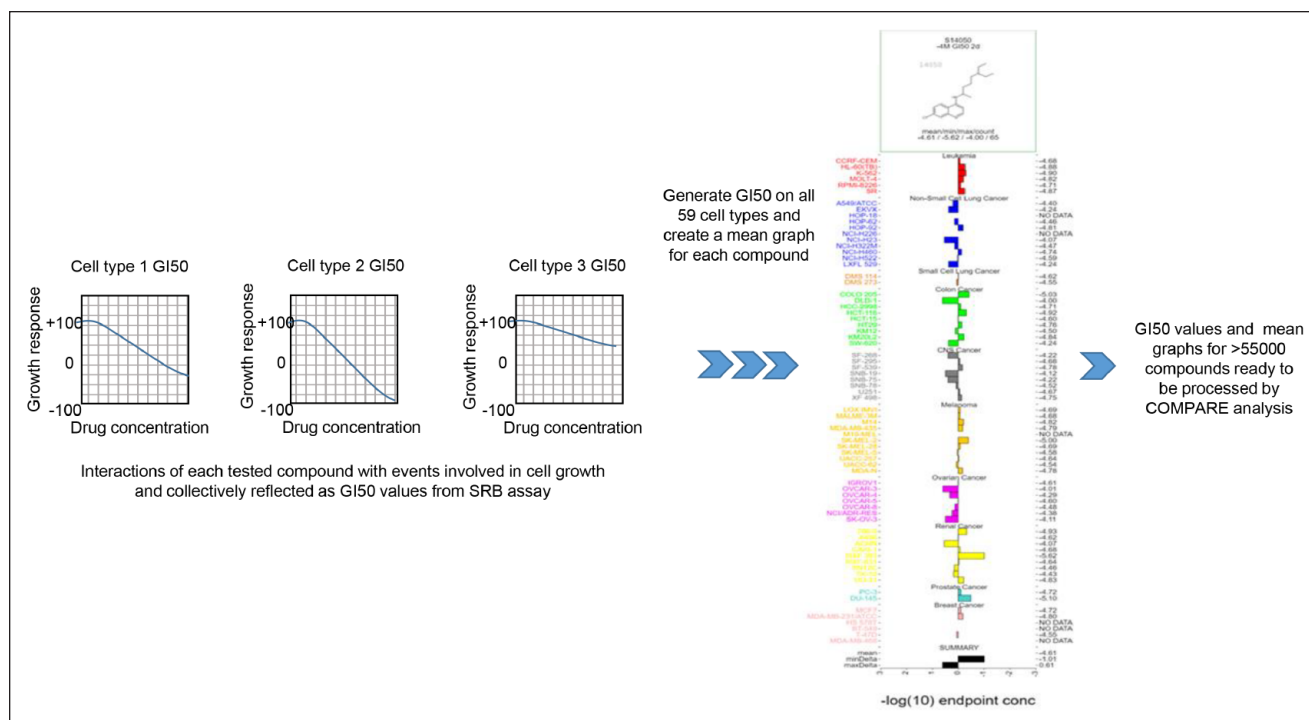


Figure 1. Overview on how chemosensitivity data are assembled to enable COMPARE analysis.

Meanwhile, the use of 59 different cell lines from various tissues warrants sufficient heterogeneity of the modes of interactions between each tested compound and the cell panel. It is well established in pharmacology that the molecular modes of interactions between drug molecules and biological targets are intrinsic properties produced by the various chemical attributes and descriptors of drug molecules, like electron density, polarity, charge, hydrogen bonding, aromaticity, size, and conformation.⁹ When a given compound is tested on the cell panel, its impact on the growth rate of each cell line is governed by a plethora of interactions and events, like binding to membrane receptors and transporters, signaling, checkpoints, suppression or activation of enzymes, nucleic acid synthesis, and the entire machineries involved in cell growth and division. Once the growth inhibitory values are plotted as mean graphs, a signature response (a pharmacological code or fingerprint) unique to each compound is generated. **Figures 1 and 2** are descriptions on how the variable GI50 values from the cell panel encrypt a unique fingerprint for each tested compound. The COMPARE algorithm was developed to enable the identification and stratification of compounds presenting similar mean graph fingerprints. What has been clearly observed is that the similarity of fingerprints is a reflection of the similarities in the pharmacological modes of action. This is because of the unique set of interactions between a given compound and a given type of cancer cells. In other words, for every biological readout (e.g., cell growth rate)

there is a labyrinth of known and unknown multitudes of events, all acts at variable levels in response to interactions with the applied exogenous compound. The DTP's large pool of molecules (~55,000 thus far) and the heterogeneity of the cell panel both form a rich source for identifying drug similarities in pharmacological activities. Therefore, any compound with unknown pharmacological effect can be used as a seed molecule in the COMPARE algorithm to enable the discovery of compounds that share similar fingerprints and hence have similar modes of action. This approach has been used to shed light on the modes of actions of new anticancer drugs.^{4-6,10,11} The current study shows that the same COMPARE-based approach can also be exploited for discerning previously unknown pharmacological activities of existing drugs as long as the screened drug molecules interfere with cell growth processes. Since viruses are known to affect host cells and hijack cellular machineries, particularly those machineries involved in cell cycle and protein synthesis, the author demonstrates here that COMPARE analysis can also be useful for antiviral drug discovery and can be applied to uncover drugs suitable for repurposing against the SARS-CoV-2 virus.

Seed compounds with a presumed effect against COVID-19 were chosen from ongoing clinical experience and reports from professional sites like Medscape and Cure ID. Among these compounds, chloroquine, hydroxychloroquine, ritonavir, and dexamethasone¹² were found enlisted in the publicly accessible ~55,000 chemicals screened by the DTP.

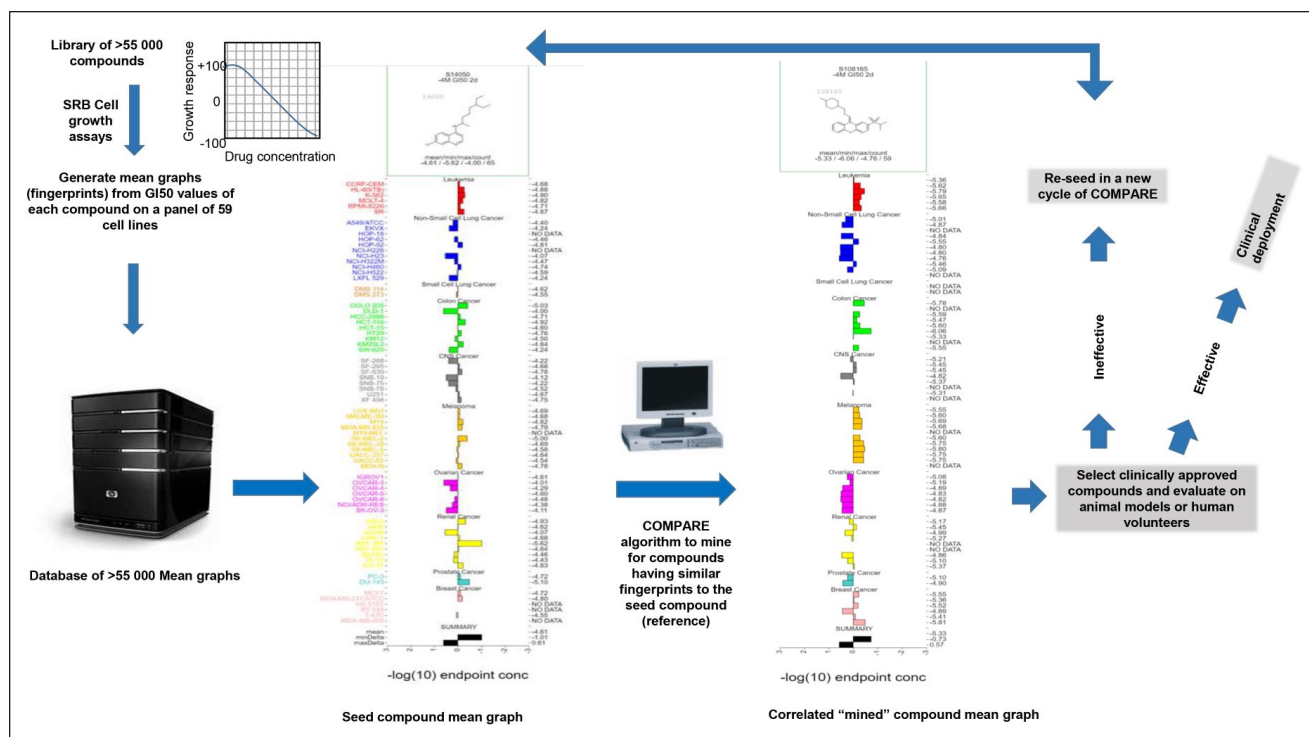
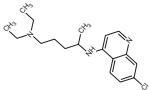
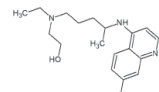
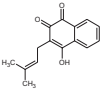
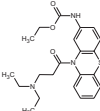
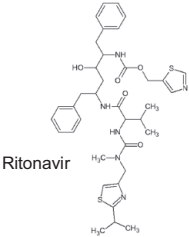
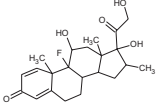
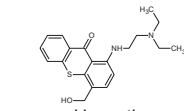


Figure 2. Overview of the data mining concept using COMPARE analysis.

Moreover, based on a recent study by Gorshkov et al.¹³ that used the cytopathic effect of SARS-CoV-2 in Vero-E6 cells to screen for potential drugs, the antiparasitic drug hycanthone was found in the DTP repository and thus was added to the list of seed candidates. Each of the seed candidates was used as a “bait” and a Pearson correlation factor of >0.5 was set for each screening attempt. Using the above criteria, the algorithm typically returned 100–250 compounds per seed. Only compounds that are clinically relevant or considered as an active principle in commonly used medicinal plants are shown in this study. Because hydroxychloroquine as a seed yielded only two clinically relevant compounds (lapachol, an active principle in antimalarial and antiviral medicinal plants,¹⁴ and ethacizine, an antiarrhythmic and psychotropic agent),¹⁵ a second cycle of COMPARE analysis was conducted on each of these two compounds. As shown in **Table 1** and **Figure 3**, several known natural and synthetic compounds were extracted based on mean graph similarities. They all belong to one of the following pharmacological classes: antiviral, antiparasitic, antiarrhythmic, and psychotropic agents. Interestingly, the pharmacological classes of the extracted compounds are the same classes that were described in recent screening studies seeking potentially effective therapeutics for viral infections like SARS-CoV-2.^{13,16–19} In particular, it is striking that the extracted compounds in this study are very similar to the compounds identified by the recent extensive quantitative structure–activity relationship (QSAR)-based anti-COVID-19 screening study conducted by a consortium of 49 research centers,¹⁹ confirming that the COMPARE-based repurposing strategy

presented here is capable of achieving comparable results, but in a way that is easier, faster, and at a far lower cost, as it uses an already established database. The results also validate the concept that the cell type-dependent variability of the GI50 values in the DTP repository is indeed correlated pharmacologically at the molecular level and reflects a unique pharmacological pattern for each compound. It is noteworthy that although the resulting hits share common pharmacological effects, they do not always share common pharmacophore structures, indicating that the conventional view of using dedicated pharmacophores in QSAR in silico drug discovery studies may not always be relevant and the results from COMPARE screening have more biological relevance. Observations on the mined compounds are summarized in **Table 1**. Of particular interest are the currently evaluated compounds for the treatment of COVID-19, like retinol,^{18,20,21} methotrexate,^{22,23} and didanosine.²³ Interesting to also see is that the psychotropic compounds ethacizine (a phenothiazine with antiarrhythmic effects) and thiothixene (a thioxanthene) are in the list. This type of compound was found to be effective in inhibiting virus entry and virus–cell fusion.²⁴ This finding further validates the vision that COMPARE is sophisticated enough to enable the identification of compounds with clinical relevance even when they belong to different pharmacological classes. When dexamethasone was used as a seed, COMPARE produced several related corticosteroids in addition to scopolamine *N*-oxide, 2'-deoxyuridine, succimer, and nicotinamide (vitamin B3); all are potentially good candidates for further clinical evaluation against COVID-19. The fact that several corticosteroids

Table 1. List of Uncovered Compounds with Potential Anti-COVID-19 Activities Following COMPARE Analysis.

Seeded Drug	Correlated Drug "Preys"	Pearson Correlation	Reported Pharmacological Effects
 Chloroquine	Lucanthone ^a	0.55	Antineoplastic, anti-infective, antiparasitic, autophagy inhibitor Antiarrhythmic and vasodilatory An alkaloid from fruits (<i>Berberis</i>) with antimicrobial, hepatoprotective, and immunomodulatory activities ²⁵ An alkaloid from traditional Peruvian medicine with antineoplastic and antiparasitic activities
	Amiodarone ^a	0.59	
	Oxycanthine ^b	0.56	
	Pheanthine ^b	0.60	
 Hydroxychloroquine	Thiothixene ^a	0.56	Antipsychotic Natural naphthoquinone with antimalaria and antiviral activities ¹⁴ A phenothiazine with antiarrhythmic activities
	Lapachol ^b	0.60	
	Ethacizine ^a	0.56	
 Lapachol	Methotrexate ^a	0.67	Antineoplastic and immunosuppressant activities Antineoplastic and antipsoriatic activities
	Azaribine ^a	0.61	
 Ethacizine	Brequinar ^a	0.61	Antineoplastic Vitamin A A penicillin antibiotic A reverse transcriptase inhibitor used to treat HIV A natural nucleoside used as a supplement Adamantine derivative (experimental drug)
	Retinol ^a	0.80	
	Flucloxacillin ^a	0.79	
	Didanosine ^a	0.78	
	Uridine ^a	0.72	
	1-adamantyl 2-methyl-2-propanesulfinate	0.77	
 Ritonavir	Brucine ^b	0.67	An alkaloid found in <i>Strychnos nux-vomica</i> tree with antineoplastic, antiviral, and anti-inflammatory activities ²⁶ A coumestan derivative with estrogen-like activities (phytoestrogens) made by some plants; coumestans may have anticancer effects
	Coumestrol ^b	0.65	
 Dexamethasone	Hydrocortisone ^a	0.97	Corticosteroids Anti-Parkinson and antimuscarinic agent
	Fluocinolone acetonide ^a	0.81	
	Betamethasone ^a	0.80	
	Methylprednisolone ^a	0.73	
	Flumethasone ^a	0.69	
	Triamcinolone ^a	0.66	
	Cortivazol ^a	0.63	
	Scopolamine N-oxide ^a hydrobromide ^a	0.66	
	Nicotinamide ^a	0.66	
	Succimer ^a	0.64	
2'-Deoxyuridine ^a	0.64		
 Hycanthone	Valproic acid ^a	0.52	Antiepileptic and potential antineoplastic and antiangiogenic

^aIndicates existing clinically approved drugs.^bIndicates active principles in medicinal plants.

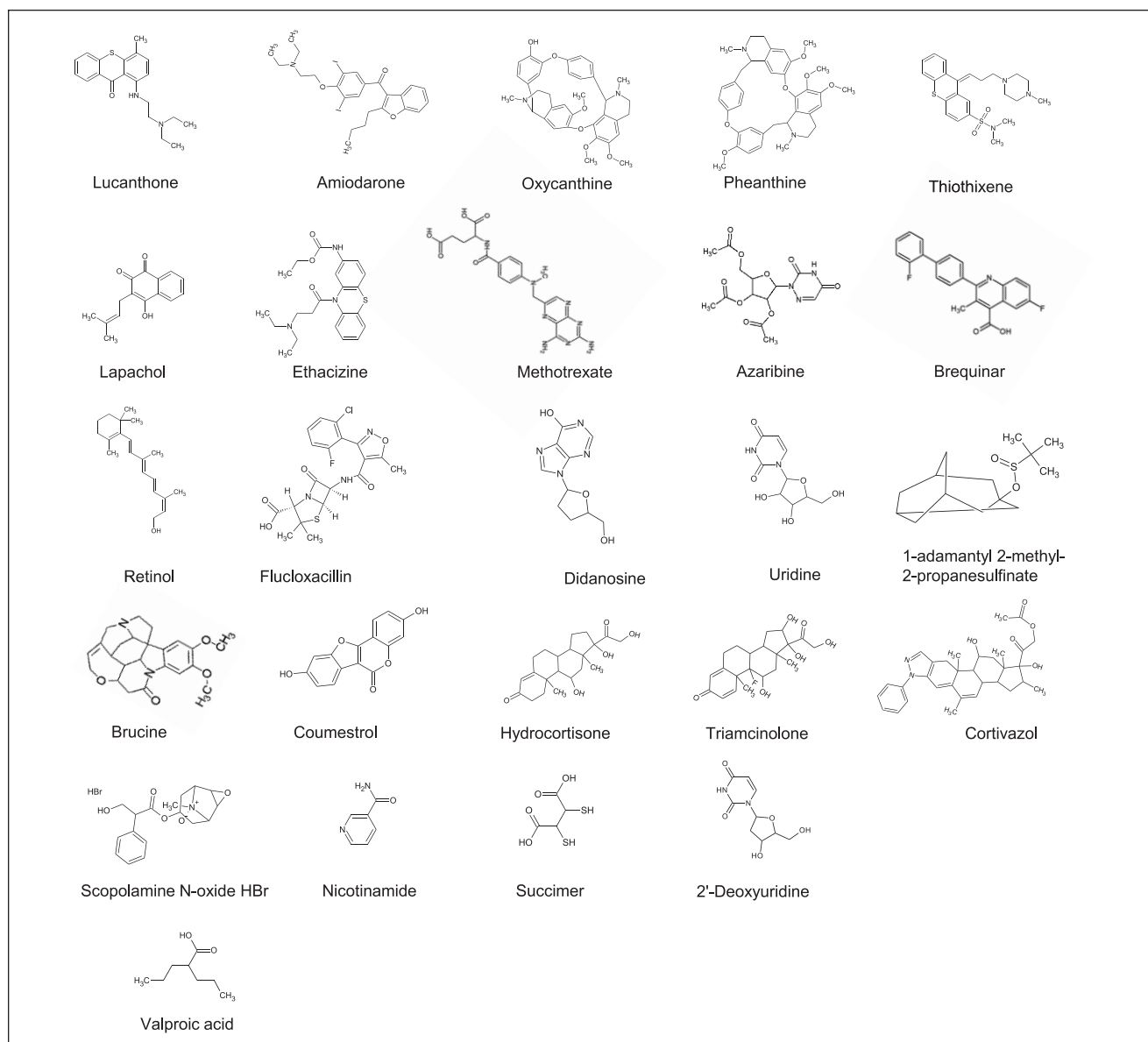


Figure 3. Chemical structures of correlated compounds with potential anti-SARS-CoV-2 activity.

were among the hits from seeding dexamethasone confirms again that COMPARE is reliable and efficient in detecting drugs with similar pharmacological modes. Also interesting to notice is that flucloxacillin, a synthetic penicillin, is among the extracted molecules. This suggests that the currently trialed penicillin antibiotics on COVID-19 patients (e.g., piperacillin and amoxicillin, CURE ID updates) might be assisting the patients via additional mechanisms beyond their antibacterial effects. One more observation is the uncovering of lucanthone from using chloroquine as a seed because this can be related to the study of Gorshkov et al.¹³ that identified both chloroquine and hycanthone, a metabolite of lucanthone, as effective inhibitors of autophagy. This indicates again that the COMPARE approach can give analogous results to actual

benchwork screening. Finally, although this study does not provide direct experimental evidence on the anti-SARS-CoV-2 effect of the compounds in **Table 1**, the fact that most of the identified compounds are reportedly involved in antiviral activities proves that this approach is valid and accurate in guiding drug repurposing efforts.

In summary, although the purpose of the DTP repository and COMPARE analysis is anticancer drug discovery, it is demonstrated here that these unique assets of bioinformatics are also valuable in guiding drug repurposing to combat new viral diseases like SARS-CoV-2. Unlike QSAR-based in silico screening methods, the COMPARE-based approach is biologically more relevant, easier, faster, and more economical. Moreover, this approach is flexible and can continuously evolve to respond to mutational changes or a potential surge

of infections. A variety of compounds were singled out using this approach. Some are existing drugs and can be quickly evaluated on volunteer patients. Others are components of existing medicinal plants already known for antiviral activities. The findings also warrant the need for extending the DTP repository to include a wider range of clinically approved therapeutics so that more possibilities are allowed. Finally, the study urges health response authorities to consider COMPARE analysis and DTP as an additional tool in the fight against current and future viral outbreaks.

Acknowledgments

The author is grateful to Dr. Mark W. Kunkel of the NCI for helpful information on the latest versions of the COMPARE algorithm.

Declaration of Conflicting Interests

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author received no financial support for the research, authorship, and/or publication of this article.

ORCID iD

Imad Naasani  <https://orcid.org/0000-0002-3294-1126>

References

- Paull, K. D.; Shoemaker, R. H.; Hodes, L.; et al. Display and Analysis of Patterns of Differential Activity of Drugs against Human Tumor Cell Lines: Development of Mean Graph and COMPARE Algorithm. *J. Natl. Cancer Inst.* **1989**, *81*, 1088–1092.
- Monks, A.; Scudiero, D.; Skehan, P.; et al. Feasibility of a High-Flux Anticancer Drug Screen Using a Diverse Panel of Cultured Human Tumor Cell Lines. *J. Natl. Cancer Inst.* **1991**, *83*, 757–766.
- Yamori, T. A Human Cell Line Panel for Screening Anti-Cancer Drugs. *Gan To Kagaku Ryoho* **1997**, *24*, 129–135.
- Weinstein, J. N.; Myers, T. G.; O'Connor, P. M.; et al. An Information-Intensive Approach to the Molecular Pharmacology of Cancer. *Science (New York, N.Y.)* **1997**, *275*, 343–349.
- Naasani, I.; Seimiya, H.; Yamori, T.; et al. FJ5002: A Potent Telomerase Inhibitor Identified by Exploiting the Disease-Oriented Screening Program with COMPARE Analysis. *Cancer Res.* **1999**, *59*, 4004–4011.
- Naasani, I.; Yamori, T.; Tsuruo, T. Screening with COMPARE Analysis for Telomerase Inhibitors. *Methods Mol. Biol.* **2002**, *191*, 197–207.
- Vichai, V.; Kirtikara, K. Sulforhodamine B Colorimetric Assay for Cytotoxicity Screening. *Nat. Protoc.* **2006**, *1*, 1112–1116.
- Whitfield, M.; Sherlock, G.; Saldanha, A. J.; et al. Identification of Genes Periodically Expressed in the Human Cell Cycle and Their Expression in Tumors. *Mol. Biol. Cell.* **2002**, *13*, 1977–2000.
- Cherkasov, A.; Muratov, E. N.; Fourches, D.; et al. QSAR Modeling: Where Have You Been? Where Are You Going To? *J. Med. Chem.* **2014**, *57*, 4977–5010.
- Kitajima, Y.; Ishii, T.; Kohda, T.; et al. Mechanistic Study of PpIX Accumulation Using the JFCR39 Cell Panel Revealed a Role for Dynamin 2-Mediated Exocytosis. *Sci. Rep.* **2019**, *9*, 8666.
- Luzina, E. L.; Popov, A. V. Synthesis, Evaluation of Anticancer Activity and COMPARE Analysis of *N*-Bis(trifluoromethyl) alkyl-*N'*-Substituted Ureas with Pharmacophoric Moieties. *Eur. J. Med. Chem.* **2012**, *53*, 364–373.
- Li, H.; Zhou, Y.; Zhang, M.; et al. Updated Approaches against SARS-CoV-2. *Antimicrob. Agents Chemother.* **2020**, *64*, e00483-20.
- Gorshkov, K.; Chen, C. Z.; Bostwick, R.; et al. The SARS-CoV-2 Cytopathic Effect Is Blocked with Autophagy Modulators. *bioRxiv* **2020**. 10.1101/2020.05.16.091520.
- Sacau, E. P.; Estévez-Braun, A.; Ravelo, A. G.; et al. Inhibitory Effects of Lapachol Derivatives on Epstein-Barr Virus Activation. *Bioorg. Med. Chem.* **2003**, *11*, 483–488.
- Kaverina, N. V.; Sokolov, S. F. Pharmacology and Clinical Use of a New Group of Antiarrhythmic Drugs: Derivatives of Tricyclic Nitrogen-Containing Systems. *Pharmacol. Res.* **1992**, *25*, 217–225.
- Bleasel, M. D.; Peterson, G. M. Emetine, Ipecac, Ipecac Alkaloids and Analogues as Potential Antiviral Agents for Coronaviruses. *Pharmaceuticals (Basel)* **2020**, *13*, 51.
- Choy, K. T.; Wong, A. Y.; Kaewpreedee, P.; et al. Remdesivir, Lopinavir, Emetine, and Homoharringtonine Inhibit SARS-CoV-2 Replication In Vitro. *Antiviral Res.* **2020**, *178*, 104786.
- Riva, L.; Yuan, S.; Yin, X.; et al. A Large-Scale Drug Repositioning Survey for SARS-CoV-2 Antivirals. *bioRxiv* **2020**. DOI: 10.1101/2020.04.16.044016.
- Gordon, D. E.; Jang, G. M.; Bouhaddou, M.; et al. A SARS-CoV-2 Protein Interaction Map Reveals Targets for Drug Repurposing. *Nature* **2020**, *583*, 459–468.
- Trasino, S. E. A Role for Retinoids in the Treatment of COVID-19? *Clin. Exp. Pharmacol. Physiol.* **2020**, *47*, 1765–1767.
- Yuan, S.; Chan, J.; Chik, K.; et al. Discovery of the FDA-Approved Drugs Bexarotene, Cetilistat, Diiodohydroxyquinoline, and Abiraterone as Potential COVID-19 Treatments with a Robust Two-Tier Screening System. *Pharmacol. Res.* **2020**, *159*, 104960.
- Beck, S.; Zhu, Z.; Oliveira, M. F.; et al. Mechanism of Action of Methotrexate against Zika Virus. *Viruses* **2019**, *11*, 338.
- Perricone, C.; Triggianese, P.; Bartoloni, E.; et al. The Anti-Viral Facet of Anti-Rheumatic Drugs: Lessons from COVID-19. *J. Autoimmun.* **2020**, *111*, 102468.
- Dyall, J.; Coleman, C. M.; Hart, B. J.; et al. Repurposing of Clinically Developed Drugs for Treatment of Middle East Respiratory Syndrome Coronavirus Infection. *Antimicrob. Agents Chemother.* **2014**, *58*, 4885–4893.
- Chander, V.; Aswal, J. S.; Dobhal, R.; et al. A Review on Pharmacological Potential of Berberine; an Active Component of Himalayan *Berberis aristata*. *J. Phytopharmacol.* **2017**, *6*, 53–58.
- Enkhtaivan, G.; John, K. M. M.; Ayyanar, M.; et al. Anti-influenza (H1N1) potential of leaf and stem bark extracts of selected medicinal plants of South India. *Saudi J. Biol. Sci.* **2015**, *22*, 532–538.