***In silico* characterization of the NiRAN domain of RNA-dependent RNA
polymerase provides insights into a potential therapeutic target against
SARS-CoV2**

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Abstract

Apart from the canonical fingers, palm and thumb domains, the RNA dependent RNA polymerases (RdRp) from the viral order *Nidovirales* possess two additional domains. Of these, the function of the Nidovirus RdRp associated nucleotidyl transferase domain (NiRAN) remains unanswered. The elucidation of the 3D structure of the RdRp from the novel coronavirus – SARS-CoV2, provided the first ever insights into the domain organisation and possible functional characteristics of the NiRAN domain. Using *in silico* tools, this study predicts that the NiRAN domain assumes a kinase or phosphotransferase like fold and binds GTP and UTP at its proposed active site. Additionally, using molecular docking this study predicts the binding of five well characterized anti-microbial compounds at the NiRAN domain active site and their drug-likeness and DFT properties. In line with the current global COVID-19 pandemic urgency, this study provides a new target and potential lead compounds for drug repurposing against SARS-CoV2.

Keywords

COVID-19, SARS-CoV2, Coronavirus, RNA-dependent RNA polymerase, NiRAN, Inhibitors, Docking.

Introduction

RNA dependent RNA polymerases (RdRp) are conserved across all RNA virus superfamilies harbouring a positive or negative sense RNA genome with the exception of retroviruses. RdRp catalyses the replication of RNA genomes from an RNA template, thus plays a key role in the viral life cycle and pathogenesis [1]. The structure of RdRp is broadly divided into three domains- fingers, palm and thumb, the shape resembling that of a right hand. This structural organization is conserved not only in RNA viruses, but also in the DNA polymerases across all kingdoms of life [1,2]. However, the positive-stranded RNA (+RNA) viruses from the order *Nidovirales* present a conspicuous difference in the structural organization of their RdRp molecules [2–4]. The viruses in the order *Nidovirales* are known to infect a broad spectrum of hosts. While members of the families *Arteriviridae* and *Coronaviridae* infect vertebrates, members of *Mesoniviridae* and *Roniviridae* primarily infect invertebrates [2–4]. Despite a significant difference in the sizes of their genomes which ranges from 13 kb to 35 kb, the RdRp molecules encoded by each genome show a common structural organization [2].

Apart from the basic RdRp structural organisation comprised of the canonical palm, thumb and finger domains, the Nidovirus RdRp possesses two additional domains- the Nidovirus RdRp associated nucleotidyl transferase domain (NiRAN) and the interface domain [2–6]. The interface domain primarily serves as a connector between the NiRAN and canonical RdRp regions. However information on the functional aspects of the NiRAN domain remains limited. The first ever study that defines the NiRAN domain from the RdRp/Nsp9 of equine arteritis virus (EAV), demonstrates a manganese-dependent covalent binding of guanosine phosphate and uridine phosphate to an invariant lysine residue from the NiRAN domain [3]. The study proposes three possible molecular functions of the NiRAN domain: as a ligase, as a

54 GTP dependent 5' nucleotidyl transferase and as a UTP dependent protein priming function
55 facilitating the initiation of RNA replication [2,3]. Various studies from other RNA viruses
56 have demonstrated the nucleotidyl transferase activities exhibited by the N-terminal regions
57 of the respective RdRp molecules [7–9]. However these transferase activities have been
58 attributed to both 5'-priming as well terminal ribonucleotide addition functions. In addition,
59 RdRp enzymes are known to exhibit either a primer dependent or a primer independent
60 initiation of RNA replication [1,10]. Interestingly, nidovirus RdRp molecules have been
61 experimentally demonstrated to possess both modes of initiation, thus hinting as a probable
62 UTP mediated priming function of the NiRAN domain [11,12].

63 An earlier study reporting the cryo-EM structure of replicase polyprotein complex from
64 SARS-CoV had significant portions of the NiRAN domain missing, thus failed to provide
65 any functional association to this domain [5]. However, a recent study reporting the cryo-EM
66 structure of the replicase polyprotein complex from SARS-CoV2 provides the complete
67 structural preview of the NiRAN domain [6]. Additionally, these studies together suggest that
68 the interface domain also serves as the binding partner for Nsp8 protein of the polyprotein
69 complex. COVID-19, the disease caused by the nidovirus SARS-CoV2 is an on-going global
70 crisis that necessitates the exploration of viral protein functions in addition to the exploration
71 for novel anti-coronavirus inhibitor scaffolds [13–15]. In this study, utilizing computational
72 and bioinformatics tool, we propose that the NiRAN domain of SARS-CoV2 possess a kinase
73 like fold and is possibly involved in the catalysis of a phosphotransferase reaction. In
74 addition, we also report the structure aided docking of a number of known kinase and
75 nucleotidyl transferase inhibitors.

Results

Analysis of the NiRAN and Interface domains

As described earlier in a recent study [6], the NiRAN and the interface domains span over residues 1- 365 of the SARS-Cov2 RdRp polypeptide sequence. These two domains form an arrow head like structure, which acts as a base for the RdRp region of protein (Fig. 1A). Of these, amino acids 4-28 and 69-249 have been designated as the NiRAN region that comprises of 8 α -helices (H1-H8) and 5- β strands (S1-S5), respectively. Two independent β -strands (B1, B2) form a hairpin like structure (residues 29-68) in the vicinity of NiRAN domain and are not considered integral to the NiRAN domain. The interface domain stretches from residue 250 to residue 365 and is comprised of a 6 α -helix bundle (h1-h6) followed by 3 antiparallel β strands (s1-s3). The overall topology suggests that the two distinct domains are connected by a small linker of 1 residue (Fig. 1B). As proposed in the EAV- NiRAN study, several key residues such as K94, R124, S129, D132, D165 and F166 [3], were found conserved in the SARS-CoV2-NiRAN as revealed in a pairwise alignment of the two sequences. The corresponding residues from SARS-CoV2-NiRAN are K73, R116, T123, D126, D218 and F219. These residues have been predicted to be crucial for the enzymatic function of the NiRAN domain. A multiple sequence alignment of 17 RdRp polypeptide sequences from coronaviruses across multiple host species (human, bat, cow, pigs and rodents) revealed an absolute conservation of these residues (Supplementary Fig. 1). Apart from the aforementioned residues, 78 (total of 84; 45 in NiRAN and 39 in interface domains) other residues were found strictly conserved in the RdRp molecules across all 17 viruses, hinting at a key role of these residues in the enzymatic and/or functional properties of NiRAN and Interface domains. A visualization of the conserved residues of the NiRAN domain shows that a significant majority of these residues are localized between the 4 stranded β -

sheet and the following helix bundle (Fig. 1B). A surface potential representation of the regions reveals the presence of five potential entry pockets suggesting that the active site of the NiRAN domain is possibly flanked by these conserved residues (Supplementary Fig. 2). The 39 residues conserved in the interface domain are however spread all over the domain architecture (Fig. 1B). The conservation of the residues across species suggests an important role of the NiRAN domain in RdRp mediated RNA replication in coronavirus, as suggested by the previous study on EAV-RdRp [3].

Prediction of the plausible functions of the NiRAN domain

The understanding of the overall topology, localization of the conserved residues prompted an investigation on understanding the enzymatic function of the NiRAN domain. For this, two different approaches were considered with the Protein data bank as the target database. The first approach utilized the prediction of Hidden Markov Models (HHPred) [16] while the second approach predicted the presence of similar folds (ORION) [17]. The list of the top 15 hits retrieved for the first and second approaches are listed in Supplementary Tables 1 and 2, respectively. Notably, both approaches predicted a variety of kinase and kinase like transferase (phosphotransferase molecules). A previous report had evidenced at the similarity of the NiRAN domain to that a known pseudokinase molecule SelO [5]; however further characterization was not feasible due to absence of the NiRAN 3D structure. We compared the topologies of the NiRAN domain with that of the canonical kinase fold. Similar to the canonical kinase fold, the NiRAN domain comprises of an antiparallel β -sheet flanked by alpha helices. The canonical kinase domain exhibits a 5 stranded antiparallel β -sheet, which is flanked by two helices running parallel and one helix running perpendicular to the β -sheet (Fig. 2A, middle panel) [18,19]. However, the NiRAN domain shows a 4 stranded antiparallel β -sheet (S1-S4) flanked by one parallel (H2) and two perpendicular helices (H1 and H3) (Fig. 2A, left panel). Further investigation into the available literatures confirmed the

presence of many non-canonical kinase folds, one of which presents a 4 stranded antiparallel β -sheet flanked by three parallel and one perpendicular helix (Fig. 2A, left panel) [20]. Taken together, these results suggest that the NiRAN domain assumes a kinase like fold, possibly functioning either as a pseudokinase or a phosphotransferase.

To further investigate this proposition, a structural alignment of the NiRAN domain was performed against 8 randomly selected kinase molecules retrieved in the HHPred and ORION searches. The molecules Lim 2 kinase domain (PDB ID- 5NXD) (Fig. 2B, Supplementary Fig. 3A and 4A), Syk kinase domain (PDB ID- 4YJR) (Fig. 2C, Supplementary Fig. 3B and 4B), O-mannosyl kinase domain (PDB ID- 5GZ9) (Fig. 2D, Supplementary Fig. 3C and 4C) and IRAK4 kinase domain (PDB ID- 2NRU) (Fig. 2E, Supplementary Fig. 3D and 4D) aligned with the NiRAN domain with rmsd values ranging from 0.6 - 4 Å and with alignment scores ≥ 0.5 , suggesting that the kinase domains in these molecules share nearly similar fold with the NiRAN domain. As a proof of principle, two additional kinase molecules not listed in the aforementioned search results- FGFR2 kinase domain (PDB ID- 2PVF) (Fig. 2F, Supplementary Fig. 3E and 4E); and Insulin receptor kinase domain (PDB ID- 1GAG) (Fig. 2F, Supplementary Fig. 3F and 4F) were aligned with the NiRAN domain. Both these molecules displayed rmsd values ranging from 0.22 - 4 Å and alignment score of ~ 0.5 , further highlighting the presence of a kinase like fold in the NiRAN domain. The results clearly exhibited the alignment of both the C α backbone of the two molecules and the conservation of secondary structural elements. Of note, the alignments of the helix H2, helix H6, helix H8 and the antiparallel β -sheet (S1-S4) are strikingly evident. Of note, the previously mentioned pseudokinase molecule SelO (PDB ID- 6EAC) also aligned with the NiRAN domain. With rmsd values ranging from 2 - 8 Å and an alignment score of 0.42, the alignment suggests that though both the molecules roughly assume similar folds, the amino acid conservation differs significantly. Collectively, results of the alignments further

highlight the presence of a non-canonical kinase/phosphotransferase like fold within the NiRAN domain.

Predicting the NiRAN domain active site

The earlier study with EAV-RdRp experimentally demonstrated the binding of GTP and UTP nucleotides to the NiRAN domain [3]. A docking analysis of these nucleotides with the NiRAN domain was performed to delineate the probable active site. Kinase domains in general possess an active site between the antiparallel β - sheet and the helix bundle that follow [18,21,22]. Both GTP and UTP docked well within the probable active site region with docking scores of -9.84 kcal/mol and -6.59 kcal/mol, respectively (Fig 3 A and C). Besides, end-point binding free energy calculation with MM/GBSA suggest a strong interaction between the nucleotides and the residues in the probable active site (-15.77 kcal/mol and -17.67 kcal/mol for GTP and UTP, respectively). Examination of the molecular interactions suggests that the GTP molecule formed possible H-bonding with R116 and salt bridge interaction with K73. The UTP molecule however only displayed a salt bridge interaction with the K73 residues. It is worth mentioning that mutation of the corresponding K94 residue in EAV-RdRp resulted in complete loss of nucleotidylation activity for both GTP and UTP [3]. Using the above interactions and the critically conserved residues, we computationally predicted the active site of NiRAN domain. The predicted active site pocket is lined with the following residues: F35, D36, I37, Y38, N39, F48, L49, K50, T51, N52, R55, V71, K73, R74, H75, N79, E83, R116, L119, T120, K121, Y122, T123, V204, T206, N208, N209, Y217, D218, G220, D221, F222 and S236; the underlined residues being strictly conserved across coronavirus RdRp molecules. K73 has been predicted as one of the key residues in the NiRAN active site. The corresponding lysine from the EAV-RdRp (K94) has been deemed critical to the function of NiRAN domain, owing to its interactions with GTP and UTP. The study also demonstrated that mutating lysine to alanine results in

complete loss of RdRp function [3]. An overview of the active site pocket depicting the electrostatic surface potential indicates the localization of charged residues at the entry points to the site, while the interior of the pocket remains lined with largely non polar residues (Fig. 3E).

With the above results suggesting a kinase like fold and the active site showing possible GTP and UTP binding function, a motif search was performed using the complete sequence of the NiRAN, β -hairpin and interface domains. The results predicted the presence of kinase like motifs belonging to Protein kinase C family, Protein kinase A family and Src Kinase family [23]. Few sequences motifs belonging to unspecified kinase families were also predicted. Interestingly, no kinase like motif was predicted for the interface domain and the β -hairpin region. Of note, a myristoylation consensus sequence overlapping with an unspecified kinase consensus was also predicted. Host enzymes are known to interact with viral proteins inducing post translational modifications following host or virus mediated signals. In particular, host kinases are known to post translationally modify viral protein often resulting in enhanced virulence or induced of various metabolic pathways [24–26]. A kinase site prediction suggested 6 different kinase phosphorylation sites with specificity for the eIF2 kinase family. A sequence based visualization of these motifs is presented in Supplementary Fig. 5. These predictions hint at possible post translational modifications of viral proteins, mediated by the interplay of host – SARS-CoV2, possibly aimed at regulating the host and viral metabolism.

In order to further explore the kinase like catalytic nature of NiRAN domain, three broad specificity kinase inhibitors- Sunitinib, Sorafenib and SU6656 were randomly selected and docked into the predicted active site of the NiRAN domain. Sunitinib is an inhibitor of the tyrosine family kinases [27]; Sorafenib inhibits activities of both serine/threonine and tyrosine family kinases [28,29]; while SU6656 inhibits the Src family kinases [29,30]. All

three kinase inhibitors show strong binding at the predicted active site as evident from the low free energy of binding (Fig. 4 A, C and E). Interestingly, these inhibitors also demonstrate potential H-bond with residues lining the active site. While Sunitinib and Sorafenib primarily interact with aspartate residues, SU6656 exhibits H-bond interaction with lysine residue 73 (Fig. 4 B, D and F), which is the critical lysine residue predicted to be involved in NiRAN domain's catalytic activity, as mentioned earlier. The results of the docking analysis are presented in Table 1. In a nutshell, the above predictions further suggest that the NiRAN domain of coronavirus RdRp has a kinase/phosphotransferase like catalytic activity.

Prediction of inhibitors targeting the NiRAN domain

As mentioned earlier, this domain might be involved in GTP induced protein phosphorylation, thus enabling a primer independent RNA replication (G-capping) [3]. Also, the domain may be involved in phosphorylation of UTP, thus functioning as a terminal nucleotidyl transferase. A wide range of viruses, both with DNA and RNA genomes are known to possess either multifunctional or dedicated proteins exhibiting the aforementioned activities [7,31]. Interestingly, many pathogenic bacteria also encode for proteins that possess G-capping and/or terminal nucleotidyl transferase activities [32,33]. Years of research have also presented a plethora of inhibitor molecules that target these essential functions in pathogens that cause diseases in human and non-human mammals [31]. After careful examination, a list of 77 compounds with experimentally demonstrated inhibitory potential against members of *Flaviviridae*, *Togaviridae*; Human cytomegalovirus, Herpes simplex virus, and few gram negative bacterial species were selected for docking against the CoV2-RdRp NiRAN domain active site [31,34–40]. The list of the selected compounds is presented in Supplementary Table 3.

Further, this study will discuss the best five inhibitors based on the docking analysis performed on the aforementioned 77 compounds. The five inhibitors with the best docking in scores in a decreasing order are- 65482, 122108, 135659024, 4534, and 23673624 (numbers represent the PubChem IDs). The results of the docking analysis are presented in Table 2. All these five inhibitors occupy varying regions within the active site pocket in a manner that their aromatic rings align with the uncharged/non-polar regions, while the charged moieties fit in the extremities of the active site pocket (Fig. 5 A-E). All the five inhibitors exhibit H-bonding interactions with the active site residues. Interestingly all the five inhibitors present potential salt bridges with active site residues and also exhibit interactions with enzymatically critical residue K73, either via H-bonding or salt bridges. In addition to the above interactions, Compound 65482 also exhibits the pi-pi interactions between its own purine ring and residues F35 and F48, as is evident from its best docking score. The molecular interactions of these inhibitors with the CoV2-RdRp NiRAN domain active site are presented in Fig. 6 A-E. In order to estimate the drug-likeness of the aforementioned inhibitors, an ADME/T analysis was performed. The ADME/T analysis failed to provide any results for the compound 135659024. The ADME/T properties of the other four molecules were within the acceptable range in terms of Lipinski's rule of five [41] and related pharmacokinetic criteria such as H-bonding atoms probability, human oral absorption and the IC₅₀ values for blockage of human Ether-a-go-go-Related Gene (hERG) K⁺. The results of this analysis are listed in Table 3.

Molecular electronic features of predicted inhibitors

The quantum mechanical features of the best five inhibitors were investigated to understand the chemical reactivity of the molecules against the NiRAN active site. The Highest Occupied Molecular Orbital (HOMO) and Lowest Unoccupied Molecular Orbital (LUMO) of five inhibitors describe the electron donate (electron rich) and accepting (low electrons)

regions respectively. Molecular electrostatic potential energy defines the positive and negative charge distribution of the molecule for necessary reactivity. In terms of HOMO and LUMO, it is pertinent to understand the molecular properties of the inhibitors; in order to better predict and comprehend the electron transfer reaction (nucleophilic and electrophilic attack). Localization of HOMO, LUMO and electrostatic potential surface density of five molecules are presented in Fig. 7 A-E.

In the inhibitor 65482, both the HOMO and LUMO occupy regions in the adenosine ring. HOMO occupies significantly more regions, thus suggesting a possible nucleophilic reaction by the inhibitor. In term of electrostatic potential surface, the inhibitor 65482 consists of highly negatively charged surface around the molecule, the only positive charged surface being around the hydroxyl group of the side chain. In the inhibitor 122108, the HOMO region localizes in the phosphate group while the LUMO regions encompasses the triazole-3-carboxamide group. Also, a strong positive charged surface is present around the phosphate group. In the inhibitor 135659024, HOMO and LUMO regions cover the adenosine ring and a highly positive surface charge density is located around the phosphate group, thus indicative of a possible nucleophilic reaction. In case of the inhibitor 4534, HOMO regions localize in both the catechol rings, while LUMO encompasses a single catechol ring of the molecule. With a significantly higher proportion of HOMO regions is indicative a nucleophilic reaction. Also, the negative charge surface potential is localized in small region in one of the two catechol rings, with positive charge surface potential encompassing the remain regions of the inhibitor. For the inhibitor 23673624, the HOMO regions occupy the pyrimidine ring and the LUMO regions occupy the azide anion group. In term of electrostatic surface potential, a high negative charge is spread over the molecule while the positive surface charge covers the phosphate group. The energy values and HOMO-LUMO energy

273 gap of all the five inhibitors are presented in Table 4. Lowering the energy gap between
274 HOMO-LUMO couples is known to improve the chemical reactivity of the inhibitors.

275

Discussions and conclusions

SARS-CoV2, the causative agent of the on-going global pandemic COVID-19, is a recently emerged pathogen that has infected over 2.5 million and caused over 150 thousand deaths as of 20th April, 2019 [42]. With a basic reproduction number ranging from 1.4 to 3.9, the disease quickly spread across the globe within past five months [43]. The situation demands urgent attention from researchers worldwide in order to develop a better understanding of the viral pathogenesis, clinical presentations and biology of the disease in order to develop therapeutics such as vaccines and small molecule inhibitors targeting the viral proteins. Prevalent hypotheses suggest that multiple genome level recombination and zoonotic events between coronaviruses affecting human and bat host have resulted in the evolution of SARS-CoV2 [15]. However it is worth noticing that the RdRp molecule of SARS-CoV2 remains largely unchanged at the protein sequence level when compared to previous human coronaviruses such as SARS-CoV, MERS-CoV, SARS-Hku1 as well as non-human coronaviruses like Bat-CoV-Hku4 and Bat-CoV-ZC45, to name a few [44].

Despite being a well-known pathogen, following the SARS-CoV outbreak in 2002, the functional aspects of the RdRp molecule from coronaviruses remains unclear [5,6,11]. In addition, even fewer information is available on the peculiar NiRAN and interface domains that are specific to the RdRp molecules of viruses of the order *Nidovirales* [2,3]. In this study, we propose that the NiRAN domain harbors an atypical kinase/phosphotransferase like fold, with GTP and UTP binding efficiency. The binding of the wide specificity kinase inhibitors at the predicted active site, further suggest the possible enzymatic action of the NiRAN domain. Interestingly, a recent report has suggested the possibility of kinase inhibitors in management of COVID-19 [45]. In line with the previous study on EAV-RdRp, this study

299 delineates the possible catalytic residues at the NiRAN active site- F35, D36, F48, K73,
300 R116, D218 and D221.

301 Using computational docking and simulation, this study predicts five possible inhibitor
302 compounds against the NiRAN domain. The compound 65482, also known as Sinefungin, is
303 broad specificity microbial nucleotidyl transferase inhibitor, and is known to inhibit RNA
304 replication in flaviviruses and herpes viruses [46,47]. The compound 122108 or Ribavirin 5'-
305 triphosphate, inhibit the formation of g-capping of RNA in a wide range of viruses, such as
306 Dengue virus, Hantaan virus and Hepatitis C virus [8,48,49]. Officially known as m7GTP,
307 the compound with the PubChem Id 135659024, also interferes with the RNA/DNA g-
308 capping activity in many viral pathogens such Rift valley fever virus, influenza virus, Zika
309 virus and dengue virus, to name a few [50–52]. The compound 4534, also named
310 Nordihydroguaiaretic acid has been demonstrated to be an efficient inhibitor of Poxvirus,
311 West Nile virus and Zika virus [53,54]. Officially named as 6-MADTP, the compound
312 23673624 is an inhibitor of RNA capping activity in chikungunya virus and Venezuelan
313 equine encephalitis virus [55,56].

314 Given the unavailability of SARS-CoV2 specific drugs and vaccine therapies, drug
315 repurposing might prove crucial and cost and time effective while combating the on-going
316 epidemic. This study presents five well studied drug scaffolds that might be effective against
317 the catalytic activity of the NiRAN domain of SARS-CoV2-RdRp. As previous studies have
318 indicated the indispensability of NiRAN catalytic function towards a successful RNA
319 replication in Nidoviruses, it is pertinent to explore the *in vitro* and *in vivo* anti-viral
320 efficacies of these compounds against coronaviruses.

Materials and Methods

Characterization of NiRAN and Interface domains of CoV2-RdRp

The coordinates of the 3D structure of SARS-CoV2-RdRp were retrieved from the Protein Data Bank (PDB IDs- 7BTF and 6M71) [6]. The dataset 7BTF was used for subsequent analysis as it provided information on all amino acid residues of CoV2-RdRp. The coordinate data within the file was trimmed to amino acids 1-365 (sequence for NiRAN and Interface domains). This file was used for computational characterization of NiRAN and Interface domains. For sequence analysis, all the coronavirus RdRp sequences were retrieved from the NCBI database and subjected to multiple sequence alignment using the Clustal Omega [57] and Multalin [58] servers. The sequences alignment visualization was generated using the ESPript 3.0 web-tool [59]. Functional analyses were performed using the MPI Bioinformatics Toolkit [16], the DALI server [60] and the DSIMB server [17,61]. The coordinate files for the kinases were retrieved from the Protein Data Bank (PDB IDs- 1CSN, 1GAG, 2NRU, 2PVF, 3Q60 and 6EAC) for structural alignment with the NiRAN and Interface domains of CoV2-RdRp. The alignments were performed using the flexible “jFATCAT” algorithm in the Java application “strucalign” available at the Protein Data Bank [62]. The default alignments parameters were used (RMSD cut off- 3 Å, AFP distance cut off- 5, Maximum number of twists- 5, and Fragment length- 8). All visualization were performed in Pymol [63]. Motif prediction and kinase functional prediction were performed using the web servers- ELM [64], PSIPRED [65], CDD [66], MyHits [67], and PhosphoPredict [68].

***In silico* ligand preparation, active site pocket prediction and molecular docking**

The coordinate files for all the inhibitors were retrieved from the PubChem Database and/or from Protein Data Bank. All the inhibitors were prepared by assigning appropriate geometries, bond orders, tautomers and ionization states using LigPrep module implemented

in the “Schrodinger Suite 2019” [69]. The coordinate file for the NiRAN and Interface domains was used to predict the probable ligand binding pockets using the SiteMap module of the “Schrodinger Suite 2019”. The accuracy of the sites were verified using the Phyre [70] and CASTp servers [71] and the results were compared. These sites were used to generate a Grid for molecular docking of the inhibitors. The prepared inhibitors were docked onto the predicted binding sites using Glide XP (extra Precision) module of the “Schrodinger Suite 2019”.

MMGBSA Calculation

The free energies of binding for the inhibitor-domain complexes (NiRAN and Interface domains with inhibitors) were analysed by MMGBSA (Molecular Mechanics Generalized Born Surface Area) calculation [72]. The calculation was executed using the following equation:

$$G = \Delta E_{MM} + \Delta G_{SGB} + \Delta G_{SA}$$

(ΔE_{MM} represents the minimized molecular energy changes in gas phase; G_{SGB} represents the surface calculation using the GB model; and G_{SA} represents the accessible surface area of the complexes.)

ADME/T properties prediction

The top 5 inhibitors with the highest docking scores and minimum binding energies were analysed for their pharmacokinetic properties such ADME (Absorption, Distribution, Metabolism, Elimination or Toxicity) and drug likeliness [73]. The QikProp module of the “Schrodinger Suite 2019” was utilized to evaluate the drug likeliness by employing the Lipinski’s rule of five [41] and various pharmacokinetic properties such as probable Hydrogen bonding atoms; Human Oral Absorption (<25%: poor; >above 80%: good); QP

LogS (Predicted aqueous solubility of the compounds, acceptable range is -6.0 to -0.5) and the IC₅₀ values for blockage of the human Ether-a-go-go-Related Gene (hERG) K⁺ channels (< -5: satisfactory)[74].

DFT Calculation

In quantum mechanics, DFT (density functional theory) calculation is an estimation of the molecular electronic features such as electron density, frontier molecular orbital (HOMO and LUMO) density and electrostatic molecular map in order to predict the reactivity of proposed inhibitors [75,76]. Geometry optimization of compound was performed using hybrid DFT approach at B3LYP (Becke's three-parameter exchange potential and the Lee–Yang–Parr correlation functional) with 6-31G** basis set [77,78]. The Poisson–Boltzmann solver was utilized to estimate the energy in an aqueous condition that simulates a near physiological state of the target molecule and provides information about the global and local indices of the molecules with respect to their chemical reactivity. All DFT calculations were carried out using Jaguar, version 9.1 [79,80].

383

384 **Figure 1: The organization and conservation of CoV2-RdRp NiRAN and Interface**
385 **domain. (A)** The NiRAN and the interface domains of the CoV2-RdRp present an arrow
386 head like structure (helices in royal blue, strands in peach and loops in green). **(B)** The overall
387 topology of the NiRAN (brown background, helices as marked as H and strand are marked as

388 S) and interface domain (grey background, helices as marked as h and strand are marked as s)
389 along with β -hairpin structure (brick red background, strand are marked as B). (C) While the
390 conserved residues in the interface domain are scattered across the structural elements, the
391 majority of the conserved residues in NiRAN domain lie between the antiparallel β -sheet and
392 the immediately following helix bundle, possibly hinting at the NiRAN domain active site.

393

394 **Figure 2: The CoV2-RdRp NiRAN domain present a kinase/phosphotransferase like**
 395 **structural organisation.** (A) The NiRAN domain possess a topology (left panel) that
 396 borrows elements from the canonical kinase fold (centre panel) as well as the non-canonical
 397 kinase fold of TgBPK1 (right panel). (B-F) The structural superimpositions of the secondary
 398 structural elements of CoV2-RdRp NiRAN with known kinases reveal significant alignment
 399 in the antiparallel β -sheet and in the helices that follow. (B) Lim 2 kinase domain. (C) Syk
 400 kinase domain (D) O-mannosyl kinase domain. (E) IRAK4 kinase domain. (F) FGFR2 kinase

401 domain. **(G)** Insulin receptor kinase domain. (The aforementioned kinases' structural
402 elements are shown in cyan, CoV2-RdRp NiRAN structural elements shown in yellow.)

Figure 3: The active site of CoV2-RdRp NiRAN domain binds GTP and UTP and exhibits kinase like motifs. GTP and UTP bind at the probable active site of the NiRAN domain with notably low free binding energies. (A) GTP within the active site pocket. (B)

407 UTP within the active site pocket. **(C)** GTP binding reveals salt bridge interaction with K73
408 and H-bonding with Asp 116. **(D)** UTP binding exhibits salt bridge interaction with K73. **(E)**
409 The predicted active site of the NiRAN domain possess both charged and uncharged residues,
410 where in the charged residues primarily line the entry points of the pocket and the uncharged
411 residues line in the deeper sections. (Red indicates positively charged regions, blue indicates
412 negatively charged regions and green indicates neutral regions, grey indicates regions beyond
413 GTP-binding pocket).

Figure 4: The active site of CoV2-RdRp NiRAN domain binds kinase inhibitors. (A, C and E) Broad specificity kinase inhibitors bind within the predicted NiRAN active site with

417 significantly low free binding energies. (Red indicates positively charged regions, blue
418 indicates negatively charged regions and green indicates neutral regions, grey indicates
419 regions beyond GTP-binding pocket). **(A)** Sunitinib within the active site pocket. **(B)**
420 Sorafenib within the active site pocket. **(C)** SU6656 within the active site pocket. **(B, D and**
421 **F)** The kinases inhibitors demonstrate H-bond interactions between with the enzymatically
422 critical aspartate and lysine residues lining the active site. **(D)** Sunitinib. **(E)** Sorafenib. **(F)**
423 SU6656.

Figure 5: The binding of the best five predicted nucleotidyl transferase inhibitors at the active site of CoV2-RdRp NiRAN. The computationally directed binding of- (A) 65482; (B)

427 122108; **(C)** 135659024; **(D)** 4534; and **(E)** 23673624; within the active site pocket. (Red
428 indicates positively charged regions, blue indicates negatively charged regions and green
429 indicates neutral regions, grey indicates regions beyond GTP-binding pocket).

430

431 **Figure 6: The molecular interaction of the best five predicted nucleotidyl transferase**
 432 **inhibitors at the active site of CoV2-RdRp NiRAN. The molecular interactions between**

433 the inhibitors and the active site pocket reveal H-bonds, salt bridges and pi-pi interactions.
434 **(A)** 65482. **(B)** 122108. **(C)** 135659024. **(D)** 4534. **(E)** 23673624. Of note, the best predicted
435 inhibitor- 65482 presents all the aforementioned molecular interactions.

437

438 **Figure 7: The DFT predictions of the best five predicted nucleotidyl transferase**
439 **inhibitors. (A) 65482. (B) 122108. (C) 135659024. (D) 4534. (E) 23673624. (Left panels**
440 **represent HOMO; Centre panels represent LUMO; and Right panels represent Electrostatic**
441 **potential surface density.)**

442 **Table 1: Docking analysis of kinase inhibitors at the proposed active site of NiRAN**
 443 **domain.**

No.	Name	PubChem ID	Docking Score (Kcal/Mol)	Binding Energy (ΔG bind)	H-bond Interactions
1	Sunitinib	5329102	-3.50	-28.07	Asp36, Asp218 and Asp221
2	Sorafenib	216239	-3.06	-36.06	Asp36
3	SU6656	5312137	-2.61	-25.07	Lys73

444

445 **Table 2: Docking analysis of best 5 proposed inhibitors at the proposed active site of**
 446 **NiRAN domain.**

No.	PubChem ID	Docking Score (Kcal/Mol)	Binding Energy (ΔG bind)	H-bond Interactions	Other Interactions
1	65482	-10.35	-25.38	Asp36, Asn52, Lys73, Asp218, Asp221	Phe35 (Pi-Pi), Phe48 (Pi-Pi), Glu83 (Salt Bridge)
2	122108	-7.42	-10.03	Lys73, Asp36, Asn52, Asp218, Asp221	Lys73 (Salt Bridge)
3	135659024	-6.94	-24.33	Asn79, Thr76, Arg74, Asn52, Val204, Asn209,	Lys73 (Salt bridge), Asp218 (Salt bridge)
4	4534	-6.84	-32.07	Lys73, Asn52, Asp218	NA
5	23673624	-6.76	-34.18	Arg33, Thr51, Asn52,	Lys73 (Salt bridge), Arg116 (Salt Bridge)

447

448 **Table 3: The predicted ADME/T properties of best 5 proposed inhibitors.**

No.	PubChem Id	Mol.Wt.	H-bond donor	H-bond acceptor	QP LogS	QPLog HERG	Lipinski rule of 5
1	65482	381.3	9	13	-0.11	-4.52	2
2	122108	484.1	4	21	-0.00	2.26	2
3	*135659024	537.2	-	-	-	-	-
4	4534	302.3	4	3	-3.39	-4.55	0
5	23673624	509.3	6	21	-1.62	-2.95	3

449 * The program failed to predict the ADME/T properties

450 **Table 4: The DFT predictions for the best 5 proposed inhibitors.**

No.	Compounds ID	HOMO (eV)	LUMO (eV)	E _{HOMO} -E _{LUMO} (eV)
1	65482	-0.228	-0.034	-0.194
2	122108	-0.196	-0.051	-0.145
3	135659024	-0.211	-0.047	-0.164
4	4534	-0.208	-0.002	-0.206
5	23673624	-0.243	-0.044	-0.199

451

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457 **Conflicts of Interests**

458 The authors declare no conflicts of interests.

459 **Author Contributions**

460 The manuscript was written through contributions of all authors. All authors have given
461 approval to the final version of the manuscript.

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707

708 **Figure 1:** A multiple protein sequence alignment of NiRAN and interface domains from the

709 RdRp molecules of coronaviruses affect various vertebrate hosts.

710

711 **Figure 2:** Organization of the entry pockets at CoV2-RdRp NiRAN domain lined with the
 712 strictly conserved residues.

A 344:A QAIKVTHKA- - TGKVMVMKE L I RCDEE TQKTFLEVKVMRS- LDHPNVLKFI GVL YKDKKL NLLTEYI EG 410:A
31:A VYRAFDI YNDKV AGFAKFLK YFVVKR- TFSNYQHEETI YNLL KDCPAVAKHDFEP- - - - - HI SRQRLTK 121:A

411:A GTLKDFLR- - - - - SM- - - - - DPFPWQQKVRFAKGI AS 437:A
122:A YTMADLVYAL RHFDGNCDTL KEI LVTYNCCDDYFNKKDWYDFVENPDI LRVYANLGERVRQALLKT VQ 191:A

438:A GMAYLHSMCI I HRDLNSHNCLI KL DK T VVVADF G LSRLI V PYWMAPEMLNGKSY DETVDI FSFGI V LCEI 539:A
192:A FCDAMRNAGI VGV- LTLDNQDL- - - - - NGNWDYDF- GDFI QT TP G- - - - - SGVPVVDSSYSLMP I 244:A

540:A I GQVYADPDCLPRTLDFGLNVKLFWEKF VPTDCPPAFF PLAAI CCRLEPESRPAFSKLEDSFEALS L YLG 609:A
245:A LTLTR- - - - - ALTAESHVDT DLT KPY- - - - - I KW 268:A

610:A ELGI PLPAEEELDHTVSMQYGLDLI HGEVLGKGF GQAI KVTHKATGKVMVMKE L I RCDEETQKTFLE 376:B
269:A DLLKYDFTEERLKLFDRIYFKYWDQTYHPNC- - - - - VNCDDRCI LHCANF 313:A

377:B VKVMRSLDHP- NVLKFI GVL YKDKKL NLLTEYI EGGTLKDFLR- - - - - SMDPFPWQQKVRFAKGI ASGMA 440:B
314:A NVLFSTVF PPT SFGPLVRKI FVDGVPFVSTGVHFR ELGVVHNQDVNL HSSRLSEKELLVYAADPAMHAA 383:A

441:B Y L HSMG 446:B
384:A S GNLLI 389:A

PDB ID- 5NXD
Block 1 rmsd 2.28
Block 2 rmsd 4.02
Block 3 rmsd 2.23
Block 4 rmsd 4.27
Block 5 rmsd 4.05
Score- 0.52695

B 384:A TVKKGYYQMKV VKTAVAKI L KNEANDPA LKDELLAEANVMQ- LDNPYI VRM GI CEAESWMLVMEAE 452:A
31:A VYRAFDI YNDKV AGFAKFLK YFVVKR- - - TFSNYQHEETI YNLL KDCPAVAKHDFEP- - - - - HI SRQRLTK 120:A

453:A LGPLNKYLQON- - - - - RHVKDKNII ELVHQVS 479:A
121:A KYTMADLVYAL RHFDGNCDTL KEI LVTYNCCDDYFNKKDWYDFVENPDI LRVYANLGERVRQALLKT V 190:A

480:A MGMKYLEESNFV HRDLAARNVLL VTQH YAKI SDFGLSKALRA DE NKAQTHGKWPVKWYAPECI NYYKF SS 551:A
191:A QFCDAMRNAGI V- GVLTLDNQDL- - - - - NGNWDYDFGDFI QTTP GS- - - - - GV 231:A

552:A KSDVW SFGVLMWEAFSY GQKPYRGMKGSEV T AMLEKGERMGCPAGCPREMYDLMNL CWTYDV ENRPG- - - 613:A
232:A P- - - - - VVDSYYSLLMP I LTLTRALTAESH- VDT DLT KPYI KWDLLKYDFTEERLKLFDRIYFKYWDQTYHP 296:A

619:A - - - - - FAAVELRL 626:A
297:A NCVNCLDDRCI LHC ANFNVLFS 318:A

PDB ID- 4YJR
Block 1 rmsd 1.52
Block 2 rmsd 3.37
Block 3 rmsd 2.72
Block 4 rmsd 2.60
Score- 0.51235

C 93:A RVFLSEWK- - - - - EHKVALSR LTRLE- MKEDFLHGLQMLKSLQS- EHVVTLVGYCEE DGTI L TEYHPLGSL 156:A
31:A VYRAFDI YNDKV AGFAKFLK YFVVKR- TFSNYQHEETI YNLL KDCPAVAKHDFEP- - - - - HI SRQRLTKYTM 124:A

157:A SNLE- - - - - ETLQKYQD- - - - - VNTWOHRLQLAMEYVSI I N 189:A
125:A ADLVYAL RHFDGNCDTL KEI LVTYNGCDDDYFNKKDWYDFVENPDI LRVYANLGERVRQALLKT VQFC D 194:A

190:A YLHHSPL GTRVMCD SNDLPK T L SQYLL TSNF SI VANDLDALP LVDSGVLI KCGHRELHGDFVAPEQL WPY 261:A
195:A AMRN- - - - - AGI VGV L- - - - - TLDNQDL- - - - - NGNWDYDFGDFI QT- - - - - 223:A

262:A GEDTPF QDDLMP SYNEKVDI WKI PDVSSFL LGH- - - - - VEGSDMVRFH LFDI HKA 311:A
226:A - - - - - TPGSGVPVVDSSYSLMP I LTLTRALTAESHVDTDLTKPYI KWDLLK YDFTEERLKLFDRIYFKY 289:A

312:A CKSQI PAER- - - - - PTAQNVLDAYQRVFHSL 337:A
290:A WDQTYHPNCVNC LDDRCI LHCANFNVLFS 318:A

PDB ID- 5GZ9
Block 1 rmsd 1.72
Block 2 rmsd 2.58
Block 3 rmsd 1.57
Block 4 rmsd 0.64
Score- 0.49517

PDB ID- 2NRU

Block 1 rmsd 4.67

Block 2 rmsd 2.00

Block 3 rmsd 1.71

Block 4 rmsd 2.01

Block 5 rmsd 2.38

Score-0.50480

PDB ID- 2PVF

Block 1 rmsd 4.02

Block 2 rmsd 1.70

Block 3 rmsd 1.71

Block 4 rmsd 3.00

Block 5 rmsd 4.09

Score-0.48912

PDB ID- 1GAG

Block 1 rmsd 4.05

Block 2 rmsd 3.53

Block 3 rmsd 1.71

Block 4 rmsd 2.72

Block 5 rmsd 0.22

Score-0.47941

716

717 **Figure 3:** Pairwise alignments corresponding to the alignment of the structural elements of
718 CoV2-RdRp NiRAN with known Kinases. (A) Lim 2 kinase domain. (B) Syk kinase domain
719 (C) O-mannosyl kinase domain. (D) IRAK4 kinase domain. (E) FGFR2 kinase domain. (F)
720 Insulin receptor kinase domain. (Colour codes- Block 1: orange-cyan; Block 2: yellow-
721 turquoise; Block 3: lime- deep blue; Block 4: green- deep blue; Block 5: green- purple)

722

723 **Figure 4:** The structural superimposition of the C α chains of CoV2-RdRp NiRAN with
 724 known Kinases reveal significant alignment of the polypeptide chains. **(A)** Lim 2 kinase
 725 domain. **(B)** Syk kinase domain **(C)** O-mannosyl kinase domain. **(D)** IRAK4 kinase domain.
 726 **(E)** FGFR2 kinase domain. **(F)** Insulin receptor kinase domain. (The aforementioned kinases'
 727 C α chains are shown in cyan, CoV2-RdRp NiRAN C α chains are shown in yellow.)

>RdRp_CoV2_NiRAN_Interface

SADAQSFLNRVCGVSAARLTPCGTGTSTDWYRAFDIYNDKVAGFAKFLKTNCCRFQEKDEDDNLIDSYFVVKR
HTFSNYQHEETIYNLLKDCPAVAKHDFKFRIDGDMVPHISRQRLTKYTMADLVYALRHFDEGNCDTLKEILVTY
NCCDDDYFNKKDWYDFVENPDILRVYANLGERVRQALLKTVQFCDAMRNAGIVGVLTLDNQDLNGNWYDFGD
FIQTTGSGVPVVDSSYSLMPILTLTRALTAESHVDTDLTKPYIKWDLLKYDFTEERLKLFDTRYFKYWDQTYHPN
CVNCLDDRCILHCANFNVLFSTVPPTSFGPLVRKIFVDGVPFVSTGYHFRELGVVHNQDVNLHSSR

728

729 **Figure 5:** The prediction of kinase like motifs reveals the presence of sequence motifs
730 belonging to kinase families PkA, PkC and Src. A kinase consensus site search prediction
731 also predicts multiple phosphorylation sites. (Bold- NiRAN domain; Italics- Interface
732 domain; Underlines: Red- Kinase consensus sequence, Blue- PkC like motif, purple- PkA
733 like motif, Green- Src kinase like motif, Grey- Unspecified Kinase like motifs, Yellow-
734 Myristoylation site consensus sequence.)

735

736

737 **Supplementary Table 1: Top 15 molecules with structurally similar fold(s) predicted by**
738 **HHPred analysis.**

No.	Hit (PDB)	Prob	E-value	P-value	Score	SS	Query HMM	Template HMM
1	6NUR	100.0	4.2E-72	8.4E-77	552.9	19.6	1-190	62-251
2	7BTF	100.0	6E-69	1.2E-73	528.8	20.6	1-190	60-249
3	4YJR	86.2	5.7	0.00011	32.1	7.9	4-161	38-160
4	2H34	86.2	7.4	0.00015	32.8	8.9	8-161	59-179
5	6INY	82.7	2.6	5.1E-05	40.0	5.1	117-161	217-262
6	6EAC	82.7	2.2	4.3E-05	40.6	4.7	117-161	220-265
7	5GZ9	81.4	29	0.00057	29.5	10.6	8-172	57-195
8	5DRB	81.1	11	0.00021	30.8	7.6	8-161	52-178
9	6BXI	81.1	13	0.00026	32.1	8.5	8-161	31-151
10	5NXD	79.1	18	0.00036	30.3	8.6	9-161	28-144
11	6TU9	77.8	32	0.00063	29.0	9.7	4-161	44-183
12	6FEX	76.9	36	0.00072	28.8	9.8	8-161	57-188
13	3M2W	74.7	30	0.00059	28.7	8.6	8-161	43-165
14	6CQE	74.5	42	0.00083	27.4	9.3	9-161	42-158
15	4BTF	74.5	24	0.00048	31.2	8.4	4-161	214-343

739

740 **Supplementary Table 2: Top 15 molecules with structurally similar fold(s) predicted by**
741 **ORION_DSIMB analysis.**

No.	Score	Ungap_ score	Pval_ Query	Pval_ Target	Query_St art_End	Target_Start- end	HIT (PDB)
1	57.323	43.106	1.64E-09	1.76E-112	1-181	1-192	3H93
2	56.250	47.533	7.00E-09	5.15E-112	1-189	1-214	3BWY
3	55.739	46.048	1.35E-08	8.59E-112	3-190	1-223	1G7S
4	55.713	49.929	1.39E-08	8.82E-112	6-188	1-185	1GBS
5	54.632	48.730	5.18E-08	2.60E-111	15-188	1-181	3MGW
6	54.567	47.204	5.59E-08	2.77E-111	1-186	1-189	1U6M
7	53.917	44.721	1.17E-07	5.31E-111	18-173	1-154	1DCQ
8	53.779	41.717	1.37E-07	6.10E-111	1-190	1-209	2FR1
9	53.320	44.212	2.26E-07	9.65E-111	1-188	1-198	3C3P
10	53.242	47.062	2.46E-07	1.04E-110	14-189	1-177	1EB6
11	53.233	44.408	2.48E-07	1.05E-110	2-190	1-247	2YVL
12	53.060	45.677	2.98E-07	1.25E-110	21-189	1-157	3TWR
13	52.739	43.585	4.17E-07	1.73E-110	1-190	1-225	3C3Y
14	52.364	45.722	6.12E-07	2.51E-110	31-187	1-187	2CVB
15	52.343	44.148	6.25E-07	2.56E-110	1-190	1-230	1G8S

742

743 **Supplementary Table 3: The complete list of compounds selected for docking at the**
744 **predicted active site of CoV2-RdRp NiRAN domain.**

	NAME	PUBCHEM ID	PDB ID(s)	STRUCTURE 2D
1	Nootkatin	238797
2	NSC 282885	323364
3	Manicol	5351306	3QLH
4	2,4,6-Trimethyl-7-oxocyclohepta-1,3,5-trien-1-yl 4-chlorobenzoate	873418
5	7-Oxocyclohepta-1,3,5-trien-1-yl 4-methylbenzenesulfonate	562886
6	NSC 79555	254856
7	Labotest 72543251	14027158
8	N-Hydroxy-1,8-naphthalimide	82263

9	(9-(4-Ethoxyphenyl)-2,6,7-trihydroxy-xanthen-3-one)	2931798		
10	9-(4'-Dimethylaminophenyl)-2,6,7-trihydroxyfluorone Sulfate Hydrate	90475469		
11	9-METHYL-2,3,7-TRIHYDROXY-6-FLUORONE	72721		
12	Asinex BAS0223612			
13	Elvitegravir	5277135	3L2U, 3L2W	
14	Raltegravir	54671008	4MDA, 4MDB, 3L2V, 3OYA	
15	7-Chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid	483180		
16	Ethyl 5-({[(5-benzyl-1,3,4-oxadiazol-2-yl)thio]acetyl}amino)-4-cyano-3-methyl-2-thiophenecarboxylate	2222655		
17	Ethyl 4-cyano-3-methyl-5-{{[(4H-1,2,4-triazol-3-ylthio)acetyl]amino}-2-thiophenecarboxylate	2193853		

18	Ethyl 5-[[2-[[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl]acetyl]amino]-4-cyano-3-methylthiophene-2-carboxylate	2224333		
19	Ethyl 4-cyano-3-methyl-5-(((4-methyl-2-pyrimidinyl)thio)acetyl)amino)-2-thiophenecarboxylate	2198264		
20	Nordihydroguaiaretic acid	4534	4PWJ	
21	5,6-Dimethyl-2-(4-nitrophenyl)thieno[2,3-d]pyrimidin-4(3H)-one	5345286		
22	Acyclovir	135398513	3KD1, 3KD5, 1VZV, 1PWY, 5I3C	
23	Ribavirin 5'-triphosphate	122108	1R6A, 2E9R	
24	(Z)-3-Amino-5-(4-chlorobenzylidene)-2-thioxothiazolidin-4-one	1379483		
25	MADTP	23673624		
26	Sinefungin	65482	5YNB, 5YNN, 5YNP, 5MRK, 4R8S	

27	NSC 293161	325174
28	NSC 23217	3246089
29	F3043-0013	16644047
30	NSC 125910	277277
31	NSC 371880	340799
32	NSC 372295	3246679
33	F0922-0796	16193614
34	3-Deazaneplanocin	73087
35	EGCG	65064	4AWM

36	(5Z)-2-[2-(2-oxoindol-3-yl)hydrazinyl]-5-(2-oxo-1H-indol-3-ylidene)-1,3-thiazol-4-one	1554535
37	Flutimide	6443207	1WL8
38	m7GTP	135659024	6QHG, 6EVK, 5FMM, 5EFA, 4NCE, 4EQK
39	VX-787	126970300	5WL0, 6EUV, 4P1U
40	[4-(Aminomethyl)phenyl]methanesulfonamide	9167228
41	9-(4-Hydroxybutyl)-N2-phenylguanine	135415618
42	1-Hydroxy-2(1H)-quinolinone	264295
43	2,4,6-Trimethyl-7-oxocyclohepta-1,3,5-trien-1-yl 4-chlorobenzoate

44	2-Hydroxyisoquinoline-1,3(2H,4H)-dione	514100
45	Ciclopirox	2749	6J10
46	7-Oxocyclohepta-1,3,5-trien-1-YL benzoate	767419
47	beta-Thujaplicinol	72605	3IG1, 3V1R, 5C2F
48	1,6-Dibenzyl-5-ethyl-3-hydroxy-pyrimidine-2,4-dione	52936423
49	6-(3,5-Dimethylbenzoyl)-1-(ethoxymethyl)-5-ethyl-3-hydroxy-pyrimidine-2,4-dione	52918273
50	5-Benzyl-6-ethyl-1-[(4-fluorophenyl)methoxymethyl]-3-hydroxy-pyrimidine-2,4-dione	52936326

51	6-Benzyl-5-ethyl-1-[(4-fluorophenyl)methyl]-3-hydroxy-pyrimidine-2,4-dione	52936424
52	6-(3,5-Dimethylbenzoyl)-5-ethyl-1-[2-(4-fluorophenyl)ethoxymethyl]-3-hydroxy-pyrimidine-2,4-dione	54587147
53	1-(Ethylamino)-3-hydroxypyrimidine-2,4-dione	68725888
54	5-Benzyl-1-(ethoxymethyl)-6-ethyl-3-hydroxypyrimidine-2,4-dione	56959484
55	6-(3,5-Dimethylphenoxy)-1-(ethoxymethyl)-5-ethyl-3-hydroxypyrimidine-2,4-dione	56959332
56	6-Ethyl-1-[(4-fluorophenyl)methoxymethyl]-5-[(4-fluorophenyl)methyl]-3-hydroxypyrimidine-2,4-dione	56959779

57	6-(3,5-Dimethylphenoxy)-5-ethyl-1-[(4-fluorophenyl)methoxymethyl]-3-hydroxypyrimidine-2,4-dione	56959481
58	1-[(2R,3R,4S,5R)-3,4-Dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-3-hydroxypyrimidine-2,4-dione	14330990
59	6-(Cyclohexylmethyl)-1-[(3,4-difluorophenyl)methoxymethyl]-5-ethyl-3-hydroxypyrimidine-2,4-dione	129908680
60	1-[(3-Chloro-2-fluorophenyl)methoxymethyl]-6-(cyclohexylmethyl)-5-ethyl-3-hydroxypyrimidine-2,4-dione	129908679
61	1-[(2S,5S)-3-Hydroperoxy-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl]-3-hydroxypyrimidine-2,4-dione	90111589
62	Sun B8155	135484493

63	Rilopirox	71778	6J10
64	N-[(4-Fluorophenyl)methyl]-2-hydroxy-7-methoxy-1,3-dioxo-4H-isoquinoline-4-carboxamide	57389495
65	7-Benzamido-N-[(4-fluorophenyl)methyl]-2-hydroxy-1,3-dioxo-4H-isoquinoline-4-carboxamide	57389416
66	2,3-dihydroxy-N-[(4-methoxyphenyl)methyl]-7-nitro-1-oxo-isoquinoline-4-carboxamide	163671823
67	7-Chloro-N-[(4-fluorophenyl)methyl]-2-hydroxy-1,3-dioxo-4H-isoquinoline-4-carboxamide	67984418
68	Ethyl 1-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxylate	14027158
69	(5Z)-2-Hydroxy-4-methyl-6-oxo-5-[(5-phenylfuran-2-yl)methylidene]-5,6-dihydropyridine-3-carbonitrile	2275754
70	N-Hydroxyphthalimide	10665

71	2,3-dihydroxyquinoxaline	27491	5U8C (subset)
72	3-(alpha-Hydroxy-4-nitrobenzyl)-2,5-dihydroxy-2,4,6-cycloheptatrien-1-one	13924966
73	3-(alpha-Hydroxy-4-nitrobenzyl)-2,5-dihydroxy-2,4,6-cycloheptatrien-1-one	13924966
74	1-Hydroxy-4H-pyridine-2,3-dione	139594038
75	1,5-Naphthyridin-2(1H)-one	589680	5TVT, 4BW4 (subset)
76	N-Hydroxy-2-[4-[4-(2-hydroxyethylamino)phenyl]phenyl]-N-methyl-1,6-naphthyridine-4-carboxamide	77106704
77	5-Bromo-N-(4-fluorobenzyl)-8-hydroxy-1,6-naphthyridine-7-carboxamide	22346401