Design, Synthesis and In Vitro Evaluation of Spirooxindole-Based Phenylsulfonyl Moiety as a Candidate Anti-SAR-CoV-2 and MERS-CoV-2 with the Implementation of Combination Studies

Assem Barakat 1,* , Ahmed Mostafa 2, M. Ali 3, Abdullah Mohammed Al-Majid 1, Luis R. Domingo 3, Omnia Kutkat 2, Yassmin Moatasim 2, Komal Zia 1, Zaheer Ul-Haq 4,5 and Yaseen A. M. M. Elshaier 6,*

1 Department of Chemistry, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia
2 Center of Scientific Excellence for Influenza Viruses, National Research Centre, Giza 12622, Egypt
3 Department of Organic Chemistry, University of Valencia, Dr. Moliner 50, 46100 Burjassot, Valencia, Spain
4 Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan
5 H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan
6 Department of Organic and Medicinal Chemistry, Faculty of Pharmacy, University of Sadat City, Menoufiya 32958, Egypt

* Correspondence: ambarakat@ksu.edu.sa (A.B.); yaseen.elshaier@fop.usc.edu.eg (Y.A.M.M.E.);
Tel.: +966-11467-5901 (A.B.); Fax: +966-11467-5992 (A.B.)

Abstract: The search for an effective anti-viral to inhibit COVID-19 is a challenge for the specialized scientific research community. This work investigated the anti-coronavirus activity for spiropinoxindole-based phenylsulfone cycloadducts in a single and combination protocols. The newly designed anti-SARS-CoV-2 therapeutics spiropinoxindoles synthesized by [3 + 2] cycloaddition reactions represent an efficient approach. One-pot multicomponent reactions between phenyl vinyl sulfone, substituted isatins, and amines afforded highly stereoselective anti-SARS-CoV-2 therapeutics spiropinoxindoles with three stereogenic centers. Herein, the newly synthesized spiropinoxindoles were assessed individually against the highly pathogenic human coronaviruses and proved to be highly potent and safer. Interestingly, the synergistic effect by combining the potent, tested spiropinoxindoles resulted in an improved antiviral activity as well as better host-cell safety. Compounds 4i and 4d represented the most potent activity against MERS-CoV with IC50 values of 11 and 23 µM, respectively. Both compounds 4c and 4e showed equipotent activity with the best IC50 against SARS-CoV-2 with values of 17 and 18 µM, respectively, then compounds 4d and 4k with IC50 values of 24 and 27 µM, respectively. Then, our attention oriented to perform a combination protocol as anti-SARS-CoV-2 for the best compounds with a different binding mode and accompanied with different pharmacophores. Combination of compound 4k with 4c and combination of compounds 4k with 4i proved to be more active and safer. Compounds 4k with 4i displayed IC50 = 3.275 µM and half maximal cytotoxic-concentration CC50 = 11832 µM. MD simulation of the most potential compounds as well as in silico ADMET properties were investigated. This study highlights the potential drug-like properties of spiropinoxindoles as a cocktail anti-coronavirus protocol.

Keywords: spiropinoxindole; SARS-CoV-2; drug combination protocol; molecular dynamic simulation (MDS); ADMET

1. Introduction

Since 2012, the world has been confronted with two highly pathogenic coronaviruses including the Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1,2]. MERS is a viral respiratory infection that is caused by MERS-CoV, leading to severe disease with high mortality rates (approximately 35%) [1]. More recently, in 2019, a novel coronavirus (CoV) outbreak was
reported in Wuhan, China [3]. This novel coronavirus disease (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), mainly attacks the respiratory system and causes acute respiratory distress syndrome (ARDS) which leads to medical disorder complications including death plus worldwide economic devastation [2]. Due to this exceptional outbreak, a number of pharmaceutical companies and academic researchers have been focused on developing and designing new anti-coronavirus candidate drugs to diminish this disease. So far, many anti-coronavirus vaccines have been developed which are effective, including those of Oxford/Astra-Zeneca, Janssen, Moderna, CoronaVac, and Covaxin. Despite these vaccines showing a high efficiency, it takes a long time to vaccinate all of the population worldwide, particularly in some developing countries. In addition, the vaccines may not meet all of the individuals’ needs due to medical complications [4–6]. The disadvantages, including unknown long-term side effects, occur rarely, but include severe allergic reactions such as anaphylaxis, short term immunization, and the need for booster doses. On similar lines, the trials to discover a new anti-coronavirus drug to reduce the transmission and inhibit viral infection are still ongoing for designed potential drug inhibitors. Due to the emergency pharmacological treatment for one of the most rapidly spread and easily transmissible coronaviruses in the world, SARS-CoV-2, drug repurposing was employed at the beginning of the global outbreak as one of the most common approaches to find a quick solution for the outbreak [6,7].

For example, the antiviral drugs suggested for pharmacological emergency use to block coronavirus (SARS-CoV-2) were umifenovir (arbidol), ruxolitinib, remdesivir, sofosbuvir, and chloroquine as an antimalarial drug [8]. The use of these drugs was accompanied by remarkable side effects. Nevertheless, remdesivir, an RNA-dependent RNA polymerase inhibitor and adenosine nucleotide analog that was initially developed to treat Ebola and Marburg viral infections, demonstrated promising in vitro and in vivo antiviral action against MERS-CoV and SARS-CoV-2 infections in experimental animal models, during clinical trials and in treatment protocols for COVID-19 patients [2].

Recently, another candidate suggested for COVID-19 treatment is a prodrug oral ribonucleoside analog with broad-spectrum anti-viral potential, namely Molnupiravir (EIDD-2801, MK-4482) [9]. The urgent development of a high efficacy and safe anti-viral drug to block or treat SARS-CoV-2 infection remains a challenge.

Due to the wide panel of the pharmacological activities and promising drug candidates for drug discovery, oxindole scaffolds and more specifically indole moieties as a class of heterocycles exhibited several pharmacological features including anti-viral, antimicrobial, anticancer, anti-inflammatory, antioxidant, analgesic, and antidiabetic potential [10,11]. Indeed, some merits of this type of privileged scaffold chemistry are the low cost, high chemical yield, eco-friendly synthesis, and simple workup procedure.

One of the virtual screening and in silico molecular docking studies suggested that the oxindole scaffold as a class of heterocyclic compound has the potential ability to bind with the main protease crystallized protein structure of COVID-19 (PDB ID: 6LU7) [12–14]. Out of the 30 oxindole derivatives computed, only four hits showed a strong binding affinity and low energy; interestingly, one hit of those leads was designed and synthesized by Barakat et al [15]. A recent study concluded about the potential scope of isatin derivatives for SARS-CoV-2 as protease inhibitors [14–21]. Therefore, to discover this spirooxindole scaffold as being anti-coronavirus is quite interesting.

Umifenovir (arbidol, Figure 1) which was developed in Russia as an indole containing an antiviral drug acts as a membrane fusion and an inhibitor for viral replication. Based on this finding, G. Sellitto et al used arbidol as a lead compound for structural derivatizations and reported the synthesis and evolution of new compounds having sulphone functionality. The arbidol derivatives exhibited strong antiviral activity against hepatitis C virus (HCV) with higher selectivity indices. The virus inhibition on entry and replication indicated that the phenylsulfonyl moiety played a crucial role for the anti-HCV activity [22]. Among the anti-viral agents designed, synthesized, and evaluated as protease inhibitors were non-peptidic heteroaromatic-based indole carboxylate small molecules having a phenylsulfone
moiety and reported by Chiummiento et al. [23]. Other researchers have reported and stressed that indolylarylsulfones exhibited high potency as reverse transcriptase inhibitors which are a class of antiretroviral drugs for AIDS or HIV infection treatment [24–33].

![Image of indole-derived moieties](image1)

**Figure 1.** Representative examples of indole-derived moieties with pharmacological applications and our designed compounds as anti-viral drug for SARS-CoV-2.

To explore the phenylsulfone moiety in the spirooxindole framework and then to examine their activity against coronavirus is the source of a lot of attention for us. The goal of a combination protocol in a synergetic action is to combine medications that function through different or the same mechanisms, reducing the chances of side effects and developing resistance.
On the other hand, it is clinically recommended to combine two or more drugs as a cocktail protocol approach for the treatment of viral infections [34–37]. In addition, the use of an effective combinational protocol could reduce the effective concentration of compounds below the therapeutic plasma concentrations, providing better clinical benefits. S. Yuan and H. Sun, as co-workers, demonstrated one representative example for an orally administered cocktail therapy for SARS-CoV-2 viral infection treatment. This preclinical cocktail therapy consisted of N-acetyl-L-cysteine combined with colloidal bismuth subsalicylate (BSS) or bismuth sub-citrate (CBS) assessed in vivo, and exhibited high efficacy against a wide range of coronavirus replications such as the recently circulating SARS-CoV-2 Alpha variant (B.1.1.7), the low pathogenic human coronavirus 229E (HCoV-229E), and the Middle East respiratory syndrome coronavirus (MERS-CoV), blocking or inhibiting several cell-based and viral-based targets including angiotensin-converting enzyme 2 (ACE2), helicase (Hel), main protease (Mpro), and papain-like protease (PLpro) [38].

Based on these findings and continuing with our research program for drug discoveries [39–46], here we report on the design, synthesis, and anti-viral evaluation of a new spirooxindole with a phenylsulfonyl moiety as a promising lead compound with high efficacy and a safer cocktail protocol to block and inhibit the outbreaks of a new coronavirus disease.

2. Results and Discussion

2.1. Synthesis of the Spirooxindole-Based Phenylsulfones 4a–n

The design, construction, and synthesis of new materials with significant antiviral applications towards COVID-19 are a challenge. In this text we employed the one pot–multi component [3+2] cycloaddition (32CA) reaction approach for the synthesis of spirooxindole-based phenylsulfones as new materials [44]. The synthetic route is shown in Scheme 1. The spirooxindole-based phenylsulfone cycloadducts were obtained via 32CA reaction of phenyl vinyl sulfone 1 as the ethylene with the generated azomethine ylide (AYs) by reaction of many substituted isatins 2a–h (Isatin 2a, 5-Chloroisatin 2b, 5-Nitroisatin 2c, 5-Bromoisatin 2d, 5-Methoxyisatin 2e, 1-Methylindoline-2,3-dione 2f, 1-(2-Bromoethyl)indoline-2,3-dione 2g, 6-Chloroisatin 2h) with three secondary amino acids 3a–c (L-proline 3a, L-thioproline 3b, and (2R,4R)-4-hydroxypyrrolidine-2-carboxylic acid 3c) under thermal conditions in MeOH for 8 h. The target spirooxindole-based phenylsulfone cycloadducts were afforded in a high chemical yield in a regioselective and diastereoselective fashion. Initially, the isatin reacted with l-proline to afford the azomethine ylide (AY), then reacted with the sulfone derivatives to afford the final product. The chemical architecture was assigned based on a number of spectrophotometric tools including 1HNMR, and 13CNMR spectral analysis. The stereochemical and regio-specific outcomes of the 32CA reactions were confirmed by HNMR and X-ray single diffraction analysis for the compound 4m which has been published in our reported article [47,48].

2.2. Biological Studies

To test the preliminary antiviral activity of the synthesized spiro compounds against the highly pathogenic coronaviruses, SARS-CoV-2 “NRC-03-nhCoV” and MERS-CoV “NRCE-HKU270”, the half maximal cytotoxic “CC50” and virus-inhibitory (IC50) concentrations were determined using MTT assay and plaque reduction assay, respectively (Table 3). Except for the compounds 4b, 4f, 4g, 4j, and 4i–n, the tested spiro-compounds showed high to moderate antiviral activity against SARS-CoV-2 ranging from 17 to 37 µM against NRC-03-nhCoV and 15 to 74 µM against NRCE-HKU270 (Table 3). Interestingly, 4c, 4i, and 4k showed the best selectivity index (SI > 10) against SARS-CoV-2 and MERS-CoV in VERO E6 cells (Table 3). These results compared with remdesivir as a drug control that the FDA approved as anti-SARS-CoV-2, but it had more cytoxic effects and side effects on patients so there remains an urgent need to present alternatives against anti-SARS-CoV-2 with higher safety and lower side effects.
Proposed mechanism as a model

Scheme 1. Synthesis of the spirooxindole-based phenylsulfone 4a–n.

Table 1. Chemical structures, cytotoxicity, antiviral activities of the tested compounds against SARS-CoV-2 and MERS-CoV as determined by MTT and plaque reduction assay, respectively.

| Cpd | Chemical Structure | Cytotoxicity (CC₅₀, µM) | Antiviral Activities |
|-----|-------------------|-------------------------|---------------------|
|     |                   |                         | SARS-CoV-2 MERS-CoV |
|     |                   | IC₅₀ (µM) SI IC₅₀ (µM) SI |                      |
| 4a  | ![Image](4a.png)  | 326 29 11.24 62 5.25    |                     |
| 4b  | ![Image](4b.png)  | 1045 194 5.38 105 9.95   |                     |
Table 1. Chemical structures, cytotoxicity, and antiviral activities of the tested compounds against SARS-CoV-2 and MERS-CoV as determined by MTT and plaque reduction assay, respectively.

| Cpd | Chemical Structure | Cytotoxicity (CC₅₀, μM) | Antiviral Activities |          |          |          |          |          |
|-----|-------------------|-------------------------|---------------------|----------|----------|----------|----------|----------|
|     |                   |                         |                     | SARS-CoV-2 | MERS-CoV  | SARS-CoV-2 | MERS-CoV  |          |
|     |                   |                         |                     | IC₅₀ (μM) | SI      | IC₅₀ (μM) | SI      |          |
| 4c  | ![Chemical Structure](image) | 1095 | 18 | 60.83 | 65 | 16.84 |
| 4d  | ![Chemical Structure](image) | 206 | 24 | 8.58 | 23 | 8.95 |
| 4e  | ![Chemical Structure](image) | 289 | 17 | 17 | 74 | 3.90 |
| 4f  | ![Chemical Structure](image) | 345 | 2251 | <1 | 88 | 3.92 |
| 4g  | ![Chemical Structure](image) | 405 | 405 | 1 | 101 | 3.97 |
| 4h  | ![Chemical Structure](image) | 571 | 37 | 15.43 | 15 | 38.06 |
Table 3. Chemical structures, cytotoxicity, antiviral activities of the tested compounds against SARS-CoV-2 and MERS-CoV as determined by MTT and plaque reduction assay, respectively.

| Cpd | Chemical Structure | Cytotoxicity (CC₅₀, μM) | Antiviral Activities |  |
|-----|-------------------|-------------------------|---------------------|---|
|     |                   |                         | SARS-CoV-2          | MERS-CoV |
|     |                   |                         | IC₅₀ (μM) | SI | IC₅₀ (μM) | SI |
| 4i  | ![Structure](image) | 1989                    | 34       | 58.5 | 11        | 180.81 |
| 4j  | ![Structure](image) | 200                     | 197      | 1.01 | 376       | <1   |
| 4k  | ![Structure](image) | 883                     | 27       | 32.70| 66        | 13.37 |
| 4l  | ![Structure](image) | 206                     | 107      | 1.92 | 146       | 1.41 |
| 4m  | ![Structure](image) | 225                     | 305      | <1  | 191       | 1.17 |
| 4n  | ![Structure](image) | 222                     | 667      | <1  | 15        | 14.8 |

Remdesivir 473.10 6.72 70.40 2.74 172.66

Abbreviations: “Cpd”, Compound; “CC₅₀”, half maximal cytotoxic concentration; “IC₅₀”, half maximal inhibitory concentration; “SI”, Selectivity index; Remdesivir as a drug control.
2.3. Combination Protocol

A drug combination protocol using anti-viral drugs provides several advantages as it will be more effective than monotherapy due to the synergistic effect of complementary drugs, have lower side effects, and lower toxicity due to reducing the doses. For these reasons, the use of combinations of drugs which may ultimately have a positive impact on alleviating COVID-19 severity have been studied [49,50]. Most drug combinations against COVID-19 have included the in vitro investigation of the recommended doses of FDA-approved drugs in 1:1 or 1:0.5 combinations [49,51]. For newly synthesized compounds, rare studies have considered investigating the synergistic or antagonistic effect of the approved drugs in 1:1 or 1:0.5 combinations [49,51]. For newly synthesized compounds, studies have considered investigating the synergistic or antagonistic effect of the compounds in combination against COVID-19.

To further validate the anti-SARS-CoV-2 activity of the compounds 4c, 4i, and 4k, a colorimetric crystal violet assay was used as previously described [52]. After equal concentrations from each compound were prepared (10 mg/mL), the mixture was prepared with an equal volume from each compound (1:1). Accordingly, the compound 4k showed the best IC50 values. Therefore, we included it in combination with the other two safe/active compounds (4c and 4i). Interestingly, combinations with 4k improved the IC50 of the compounds 4c and 4i. In addition, the mixture’s CC50 values improved when 4k was used in combination with 4c, and 4i compared to each individual compound. Based on these results, the selectivity indices for the combinations 4c/4k and 4i/4k were remarkably improved, to be 698.4 and 3612.8, respectively (Figure 2).

![Graph showing the results of the combination protocol](image)

**Figure 2.** Half maximal cytotoxic concentration (CC50) in Vero-E6 cells and half maximal inhibitory concentration (IC50) of the tested compounds 4c, 4i, and 4k, individually and in combination, against NRC-03-nCoV in Vero-E6 cells, compared with remdesivir as a drug control. The 50% inhibitory concentration (IC50) of each tested compound was calculated using nonlinear regression analysis in triplicate for each concentration used. The best fitting line was drawn between log concentrations and viral inhibition % using Graph Pad Prism software.

Taking into consideration that remdesivir is one of the most promising anti-COVID-19 drugs, as demonstrated by previous in vitro and clinical studies [2,53,54], the inhibitory concentrations for the selected combinations 4c/4k and 4i/4k were lower than that for the control remdesivir drug (IC50 = 6.721 µM). Interestingly, the IC50 values for the selected combinations were also potent compared to the other FDA-approved drugs that are applied in COVID-19 treatment protocols including viral protease inhibitors Lopinavir.
(IC$_{50}$ = 26.63 µM) and Ritonavir (IC$_{50}$ ≥100 µM), RdRp inhibitors such as Favipiravir (IC$_{50}$ = 61.88 µM) and Ribavirin (IC$_{50}$ = 70 µM), and other small-molecule inhibitors including Azithromycin (IC$_{50}$ = 2.12 µM), and Hydroxychloroquine (IC$_{50}$ = 4.51 µM) [53].

2.4. Molecular Docking Study

2.4.1. Docking against SARS-CoV-2 against RNA Polymerase (PDB:ID: 6m71)

The compounds exhibited different binding modes and poses against the target protein. According to the binding mode and site of interaction with the receptor, all compounds are classified in to three categories:

1. Compounds 4n, 4b, 4e, 4m, 4f, 4j, and 4i have the same binding mode and these compounds were originated from secondary amino acids L-proline 3a, and L-thioproline 3b and were docked with complete overlay and with the detection of hydrogen bond (HB) with Arg: 116A through the carbonyl of oxoindoline moiety, Figure 3a (left domain of receptor);

2. Compounds 4a, 4g, and 4k were prepared from secondary amino acid L-proline 3a. These compounds connected with same amino acids cleft with complete overlay and similarity without detection of any hydrogen bonds (HBs), Figure 3a (right domain of receptor);

3. Compounds 4d, 4l, 4h, and 4c exhibited the same binding mode and pose with formation of HB with Arg: 33A through the hydroxyl functionality of pyrrolidine ring, Figure 3b. These compounds were synthesized from the secondary amino acids L-proline 3a and 2R,4R)-4-hydroxypyrrolidine-2-carboxylic acid (3c). Compound 4h formed HB with Thr:120A through NH of oxoindoline moiety, Figure S28. Compound 4l illustrated specific binding pose through hydrophobic–hydrophobic interaction, Figure S29.

![Figure 3](image-url)

Figure 3. Visual representation for compounds docked against (PDBID: 6m71) visualized by vid application (a) shape alignment for synthesized compounds 4i, 4n, 4b, 4e, 4m, 4f, and 4j (left domain of receptor). Compounds 4a, 4g and 4k (right domain of receptor); (b) Compounds 4c, and 4d have the same binding mode and pose with formation of HB with Arg: 33A.

The combination study was designed between compound 4k (second category) with compound 4i (first category); and compound 4k (second category) with compound 4c (third category). Interestingly, combinations with 4k improved the IC$_{50}$ of the compounds 4c and 4i. In addition, the mixture’s CC$_{50}$ values were improved. From Figure 4a, compound 4k (grey color) binds with the amino acid cleft which differs from those interacting with compound 4i (green color). Compound 4i located in the site of the receptor close to the binding region of co-crystalized ligand. They formed HB with Arg: 116A and His:99A, respectively. Concerning the combination (compound 4k and 4c), compound 4c (green color) adopted the same region as shown before from compound 4i but in a different binding mode and pose, Figure 4b. Compound 4c formed weak HB with Arg: 33A through its hydroxyl functionality of the pyrrolidine ring, Figure 4b [55].
but in a different binding mode and pose, Figure 4b. ... pharmacokinetic features of a compound, in silico ADMET analysis provides a valid alternative to earlier stage experiments.

2.4.2. Docking Study with MERS-CoV Viral Proteins nsp5 (PDB:ID: 4ylu)

In order to examine the activity of these compounds against the MERS-CoV virus, the docking protocol was employed here against the main protease of MERS-CoV (PDB ID: 4ylu [56]). Both compound 4h and 4i were the most potent derivatives. Compound 4i docked with the receptor with hydrophobic–hydrophobic interaction in the same amino acids’ clefts interacted with the co-crystalized ligand, Figure 5a, however compound 4h participated in a docking pose in a different domain, Figure 5b.

2.5. ADMET Analysis

Due to adverse pharmacokinetic properties, many of the prospective drug candidates never reach clinical trials. To examine the fundamental pharmacokinetic features of a compound, in silico ADMET analysis provides a valid alternative to earlier stage experiments to increase the success rate of clinical development. The bioavailability, pharmacokinetics, and toxicity of 4c, 4i, and 4k were attained by using ADMETlab and the obtained results are summarized in Table 4. The bioavailability and physiochemical properties were evaluated by plotting a radar representing 13 properties (Figure 6). It is interesting to note that all
of the properties were within their optimal ranges, which showed that 4c, 4i, and 4k had good oral bioavailability and druggability. Similarly, all of the compounds were predicted to follow the Lipinski rule of five with high gastrointestinal absorption. To further evaluate the toxicity and cross-reactivities of 4c, 4i, and 4k, the acute toxicity and PAINS were predicted. All of the three compounds were found to be non-toxic and non-cross-reactive. Taken together, 4c, 4i, and 4k were in significant agreement with the given criteria to be considered as drug-like.

Table 4. In silico predicted ADMET properties of spirooxindole analogs.

| Compounds | Mol. Weight g/mol | nHA | nHD | TPSA | LogP | Lipinski Rule | Acute Toxicity Rule | HIA | PAINS |
|-----------|------------------|-----|-----|------|------|---------------|---------------------|-----|-------|
| 4c        | 429.100          | 9   | 2   | 129.8| 1.80 | Accepted      | 0 alerts            | Yes | 0 alerts |
| 4i        | 386.080          | 5   | 1   | 66.4 | 2.38 | Accepted      | 0 alerts            | Yes | 0 alerts |
| 4k        | 382.140          | 5   | 0   | 57.6 | 2.35 | Accepted      | 0 alerts            | Yes | 0 alerts |

Figure 6. In silico ADMET properties of selected spirooxindole analogs predicted by ADMETlab.

2.6. Molecular Dynamics Simulation

The compounds 4c and 4k with the best selectivity index and significant antiviral activity against SARS-CoV-2 were subjected to molecular dynamics (MD) simulation to evaluate the time-dependent dynamics and stability of protein–ligand complexes. The docked pose of 4c and 4k in complex with RdRp of SARS-CoV-2 were subjected to 100ns of simulation and the Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSF) were calculated (Figure 7). The RMSD plot of 4k-RdRp showed variable fluctuations throughout the 100ns of simulation with the average RMSD of 4.3 ± 0.70 nm. The 4c-RdRp projected the more stable RMSD in comparison with the 4k complex with an average RMSD of 3.8 ± 0.47 nm. The 4c-RdRp complex was converged after 55ns and remained stable till the end of the simulation. To further evaluate the ligand-induced flexibility of SARS-CoV-2 RdRp residues, RMSF was calculated. Consistent with the RMSD results, the RMSF plot of the 4c complex showed a lesser magnitude of fluctuations as compared to the 4k complex. The average RMSF for the 4k-RdRp and 4c-RdRp complexes was found to be 6.04 ± 2.23 nm and 5.05 ± 1.78 nm, respectively. By analyzing the results, it was inferred that the experimental and theoretical results were consistent with one another.
were reacted according to GP1 for 8 h and yielded white solid phenylsulfone spirooxindole was maintained at room temperature overnight, and the solid crystalline product was filtered off without any further purification.

3. Methodology/Experimental Section

3.1. General

The $^1$H-NMR and $^{13}$C-NMR spectra of 4a–n were recorded on a JEOL 400-MHz spectrometer (JEOL, Ltd, Tokyo, Japan) at ambient temperature. DMSO-$d_6$ was used as the solvent; the chemical shifts ($\delta$) are given in ppm.

Synthesis of the Spirooxindole-Based Phenylsulfone 4a–n (GP1)

A mixture of phenyl vinyl sulfone 1 (0.5 mmol), isatin derivatives 2a–h (0.5 mmol), and amino acids 3a–c (0.5 mmol) in methanol (10 mL) was refluxed in an oil bath for the appropriate time of 8 h. After completion of the reaction, as evident from TLC, the reaction was maintained at room temperature overnight, and the solid crystalline product was filtered off without any further purification.

3.1.1. (1′R,3R,7a′R)-1′-(Phenylsulfonyl)-1′,2′,5′,6′,7′,7a′-hexahydrospiro[indoline-3,3′-pyrrolizin]-2-one 4a

1 (84 mg, 0.5 mmol), isatin 2a (73.5 mg, 0.5 mmol) and L-proline 3a (57.5 mg, 0.5 mmol) were reacted according to GP1 for 8 h and yielded white solid phenylsulfone spirooxindole 4a (166 mg, 90%); m.p.298 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.39 (s, 1H), 8.00 (d, $J = 7.8$ Hz, 2H), 7.82 (t, $J = 7.4$ Hz, 1H), 7.71 (d, $J = 7.6$ Hz, 2H), 7.36–7.24 (m, 2H), 7.05 (t, $J = 7.6$ Hz, 1H), 6.83 (d, $J = 7.9$ Hz, 1H), 4.21 (d, $J = 8.9$ Hz, 2H), 4.02 (d, $J = 8.9$ Hz, 2H), 3.94–3.77 (m, 2H), 2.81 (dd, $J = 10.3$, 6.5 Hz, 2H), 2.27 (dd, $J = 13.3$, 6.8 Hz, 1H); $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 179.48, 172.96, 143.19, 139.19, 134.99, 130.29, 128.41, 126.09, 125.84, 122.38, 69.56, 68.82, 65.52, 54.51, 53.15, 36.61, 31.41, 22.52, 14.42; [Anal. Calcd. for C$_{20}$H$_{20}$N$_2$O$_3$S: C, 65.20; H, 5.47; N, 7.60; Found: C, 65.29; H, 5.58; N, 7.65]; LC/MS (ESI, $m/z$): found 369.16 [M+H]$^+$; Exact Mass: 368.12.

3.1.2. (3R,7′R,7a′R)-5-Chloro-7′-(phenylsulfonyl)-1′,6′,7′,7a′-tetrahydro-3′H-spiro[indoline-3,5′-pyrrolo[1,2-c]thiazole]-2-one 4b

1 (84 mg, 0.5 mmol), 5-Cl-isatin 2b (90.5 mg, 0.5 mmol) and L-thioproline 3b (66.5 mg, 0.5 mmol) were reacted according to GP1 for 8 h and yielded white solid phenylsulfone spirooxindole 4b (194 mg, 92%); m.p.282 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.53 (s, 1H), 8.01 (d, $J = 7.9$ Hz, 2H), 7.82 (t, $J = 7.4$ Hz, 1H), 7.71 (t, $J = 7.7$ Hz, 2H), 7.40–7.31 (m, 2H), 6.84 (d, $J = 8.1$ Hz, 1H), 5.01 (dt, $J = 12.2$, 6.5 Hz, 1H), 3.91 (d, $J = 10.0$ Hz, 1H), 3.80 (dt, $J = 9.5$, 6.2 Hz, 1H), 3.35 (d, $J = 17.4$ Hz, 5H), 3.20–3.00 (m, 2H), 2.57 (d, $J = 13.2$ Hz, 1H), 2.29 (dd, $J = 13.3$, 6.8 Hz, 1H); $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 179.55, 144.93, 139.29,
3.1.3. (1'R,3R,6'S,7a'R)-6'-Hydroxy-5-nitro-1'-[phenylsulfonyl]-1',2',5',6',7',7a'-hexahydropyrrolo[1,2-c][indoline]-3,5'-pyrrolo[1,2-c][thiazole]-2-one 4c

1 (84 mg, 0.5 mmol), 5-NO2-isatin 2c (96 mg, 0.5 mmol) and (2R,4R)-4-hydroxyprrolidine-2-carboxylic acid 3c (66.5 mg, 0.5 mmol) were reacted according to GP1 for 8 h and yielded yellow solid phenylsulfone spirooxindole 4c (185 mg, 86%); m.p.141 °C H NMR (400 MHz, DMSO-d6) δ 11.16 (s, 1H), 7.97 (d, J = 7.5 Hz, 1H), 7.82 (d, J = 2.2 Hz, 1H), 7.76–7.66 (m, 2H), 7.56 (d, J = 7.8 Hz, 2H), 7.06–6.98 (m, 2H), 4.79 (s, 2H), 4.35 (dd, J = 13.0, 6.1 Hz, 1H), 4.27 (d, J = 6.2 Hz, 1H), 4.19 (d, J = 7.6 Hz, 1H), 4.09–3.96 (m, 1H), 2.31–2.16 (m, 3H), 1.95–1.85 (m, 1H), 1.61 (dd, J = 13.3, 9.0, 5.0 Hz, 1H); 13C NMR (101 MHz, DMSO-d6) δ 179.08, 178.50, 149.91, 142.73, 142.13, 140.69, 138.50, 129.98, 124.90, 105.12, 70.34, 70.19, 68.71, 64.85, 57.59, 31.13, 23.63; [Anal. Calcd. for C20H19N3O6S: C, 55.94; H, 4.46; N, 9.78; Found: C, 55.90; H, 4.40; N, 9.70]; LC/MS (ESI, m/z): found 430.18 [M+H]+; Exact Mass: 429.10.

3.1.4. (1'R,3R,6'S,7a'R)-5-Bromo-6'-hydroxy-1'-[phenylsulfonyl]-1',2',5',6',7',7a'-hexahydropyrrolo[1,2-c][indoline]-3,5'-pyrrolo[1,2-c][thiazole]-2-one 4d

1 (84 mg, 0.5 mmol), 5-Br-isatin 2d (113 mg, 0.5 mmol) and (2R,4R)-4-hydroxyprrolidine-2-carboxylic acid 3c (66.5 mg, 0.5 mmol) were reacted according to GP1 for 8 h and yielded faint brown solid phenylsulfone spirooxindole 4d (185 mg, 84%); m.p.220 °C; 1H NMR (400 MHz, DMSO-d6) δ 10.55 (s, 1H), 8.01 (d, J = 7.7 Hz, 2H), 7.81 (q, J = 6.9, 6.4 Hz, 1H), 7.70 (p, J = 7.5 Hz, 2H), 7.48 (d, J = 7.3 Hz, 2H), 6.79 (d, J = 8.6 Hz, 1H), 4.60 (dt, J = 11.9, 6.2 Hz, 1H), 4.21 (d, J = 8.9 Hz, 1H), 4.02 (d, J = 8.8 Hz, 1H), 3.91 (d, J = 10.2 Hz, 1H), 3.87–3.74 (m, 2H), 3.36 (d, J = 9.8 Hz, 1H), 3.18–2.99 (m, 3H), 2.81 (dd, J = 10.2, 6.6 Hz, 1H), 2.56 (d, J = 12.9 Hz, 1H), 2.28 (dd, J = 13.3, 7.0 Hz, 1H); 13C NMR (101 MHz, DMSO-d6) δ 179.15, 173.08, 142.72, 139.28, 130.62, 128.61, 114.17, 69.76, 68.93, 65.65, 54.62, 53.36, 36.75, 34.68; [Anal. Calcd. for C20H19BrN3O6S: C, 51.84; H, 4.13; N, 6.05; Found: C, 51.91; H, 4.18; N, 6.09]; LC/MS (ESI, m/z): found 462.18 [M+H]+; Exact Mass: 462.02.

3.1.5. (3'R,7'R,7a'R)-5-Nitro-7'-[phenylsulfonyl]-1',6',7',7a'-tetrahydro-3'H-spiro[indoline-3,5'-pyrrolo[1,2-c][thiazole]-2-one 4e

1 (84 mg, 0.5 mmol), 5-NO2-isatin 2c (96 mg, 0.5 mmol) and L- thioproline 3b (66.5 mg, 0.5 mmol) were reacted according to GP1 for 8 h and yielded yellow solid phenylsulfone spirooxindole 4e (176 mg, 80%); m.p.95 °C; 1H NMR (400 MHz, CDCl3) δ 8.99 (s, 1H), 8.28 (dd, J = 8.8, 2.2 Hz, 1H), 7.64 (dd, J = 8.0, 5.8 Hz, 5H), 7.50 (d, J = 7.6 Hz, 2H), 7.01 (t, J = 8.0 Hz, 2H), 3.83 (d, J = 11.5 Hz, 2H), 3.80 (s, 1H), 3.19 (dd, J = 11.9, 6.9 Hz, 2H), 3.03 (dd, J = 11.7, 2.7 Hz, 1H); 13C NMR (101 MHz, CDCl3) δ 178.45, 147.73, 142.67, 138.52, 138.24, 134.62, 129.56, 129.51, 128.40, 128.05, 128.00, 127.52, 126.15, 110.38, 71.91, 71.04, 67.53, 54.16, 38.14; [Anal. Calcd. for C19H17N3O5S2: C, 52.89; H, 3.97; N, 9.74; Found: C, 52.98; H, 3.98; N, 9.85]; LC/MS (ESI, m/z): found 432.12 [M+H]+; Exact Mass: 431.06.

3.1.6. (3'R,7'R,7a'R)-5-Methoxy-7'-[phenylsulfonyl]-1',6',7',7a'-tetrahydro-3'H-spiro[indoline-3,5'-pyrrolo[1,2-c][thiazole]-2-one 4f

1 (84 mg, 0.5 mmol), 5-MeO-isatin 2e (88.5 mg, 0.5 mmol) and L- thioproline 3b (66.5 mg, 0.5 mmol) were reacted according to GP1 for 8 h and yielded yellow solid phenylsulfone spirooxindole 4f (171 mg, 82%); m.p.105 °C; 1H NMR (400 MHz, DMSO-d6) δ 10.21 (s, 1H), 8.01 (d, J = 7.8 Hz, 2H), 7.82 (t, J = 7.3 Hz, 1H), 7.71 (t, J = 7.7 Hz, 2H), 6.98 (d, J = 7.2 Hz, 1H), 6.87 (dd, J = 8.1, 2.8 Hz, 1H), 6.74 (d, J = 8.6 Hz, 1H), 4.62 (dt, J = 12.4, 6.5 Hz, 1H), 3.91 (d, J = 9.7 Hz, 1H), 3.82–3.71 (m, 1H), 3.75 (s, 2H), 3.38 (d, J = 9.7 Hz, 2H), 3.33 (s, 2H), 3.16–3.00 (m, 2H), 2.28–2.20 (m, 1H); 13C NMR (101 MHz, DMSO-d6) δ 179.58, 155.45, 139.34, 136.38, 130.39, 127.31, 119.34, 113.81, 112.62, 70.14, 58.53, 34.69, 12.34; [Anal. Calcd.
3.1.7. (1'R,3'R,7a'R)-5-Chloro-1'-(phenylsulfonyl)-1',2',5',6',7',7a'-hexahydrospiro[indoline-3',3'-pyrrolozin]-2-one 4g

1 (84 mg, 0.5 mmol), 5-Cl-isatin 2b (90.5 mg, 0.5 mmol) and L-proline 3a (57.5 mg, 0.5 mmol) were reacted according to GP1 for 8 h and yielded yellow solid phenylsulfone spirooxindole 4g (177 mg, 88%); m.p. 254 °C; 1H NMR (400 MHz, DMSO-d6) δ 10.44 (s, 1H), 7.95 (d, J = 7.9 Hz, 2H), 7.80–7.71 (m, 1H), 7.66 (t, J = 7.7 Hz, 2H), 7.53 (s, 1H), 7.34–7.27 (m, 1H), 6.80 (d, J = 8.6 Hz, 1H), 4.47 (dt, J = 10.8, 7.7 Hz, 1H), 4.04 (q, J = 8.2 Hz, 1H), 2.83 (td, J = 9.2, 6.4 Hz, 1H), 2.58 (t, J = 11.8 Hz, 1H), 2.50 (s, 3H), 2.47 (d, J = 7.6 Hz, 0H), 2.34 (t, J = 10.9 Hz, 1H), 2.03 (dd, J = 13.1, 7.3 Hz, 1H), 1.92 (dt, J = 13.9, 7.4 Hz, 2H), 1.64 (q, J = 9.2, 8.3 Hz, 1H); 13C NMR (101 MHz, DMSO-d6) δ 179.37, 142.26, 140.72, 130.17, 129.25, 128.96, 128.42, 126.19, 92.09, 78.91, 69.36, 66.27, 61.69, 52.61, 52.74; [Anal. Calcd. for C20H17ClN3O3S: C, 57.25; H, 4.32; N, 6.95]; LC/MS (ESI, m/z): found 417.17 [M+H]+; Exact Mass: 416.09.

3.1.8. (1'R,3'R,7a'R)-5-Methoxy-1'-(phenylsulfonyl)-1',2',5',6',7',7a'-hexahydrospiro[indoline-3,3'-pyrrolozin]-2-one 4h

1 (84 mg, 0.5 mmol), 5-MeO-isatin 2e (88.5 mg, 0.5 mmol) and L-proline 3b (57.5 mg, 0.5 mmol) were reacted according to GP1 for 8 h and yielded faint brown solid phenylsulfone spirooxindole 4h (172 mg, 86%); m.p. 240 °C; 1H NMR (400 MHz, DMSO-d6) δ 10.13 (s, 1H), 7.94 (d, J = 7.8 Hz, 2H), 7.76 (t, J = 7.3 Hz, 1H), 7.66 (t, J = 7.6 Hz, 2H), 7.03 (d, J = 2.7 Hz, 1H), 6.83 (dd, J = 8.7, 2.7 Hz, 1H), 6.71 (d, J = 8.6 Hz, 1H), 4.49 (dt, J = 11.0, 7.9 Hz, 1H), 4.02 (q, J = 8.2 Hz, 1H), 3.74 (s, 2H), 2.86 (td, J = 9.2, 6.2 Hz, 1H), 2.61–2.52 (m, 1H), 2.40–2.24 (m, 1H), 1.93 (ddt, J = 20.2, 14.4, 7.4 Hz, 3H), 1.69–1.56 (m, 1H); 13C NMR (101 MHz, DMSO-d6) δ 179.63, 155.26, 140.76, 136.49, 133.55, 131.02, 130.21, 128.37, 128.33, 116.18, 112.47, 69.73, 64.69, 57.35, 46.86, 32.48, 27.74; [Anal. Calcd. for C21H18ClN3O3S: C, 63.30; H, 5.57; N, 7.03; Found: C, 63.38; H, 5.69; N, 7.11]; LC/MS (ESI, m/z): found 399.19 [M+H]+; Exact Mass: 398.13.

3.1.9. (3R,7'R,7a'R)-7'-Phenylsulfonyl-1',6',7',7a'-tetrahydro-3'H-spiro[indoline-3,5'-pyrrolo[1,2-c][thiazole]-2-one 4i

1 (84 mg, 0.5 mmol), isatin 2a (73.5 mg, 0.5 mmol) and L-thioproline 3b (66.5 mg, 0.5 mmol) were reacted according to GP1 for 8 h and yielded white solid phenylsulfone spirooxindole 4i (168 mg, 87%); m.p. 260 °C; 1H NMR (400 MHz, DMSO-d6) δ 10.31 (s, 1H), 7.94 (d, J = 7.9 Hz, 2H), 7.76 (t, J = 7.3 Hz, 1H), 7.66 (t, J = 7.5 Hz, 2H), 7.40 (d, J = 7.4 Hz, 1H), 7.25 (t, J = 7.7 Hz, 1H), 7.00 (t, J = 7.4 Hz, 1H), 6.80 (d, J = 7.9 Hz, 1H), 4.48 (dt, J = 10.9, 7.6 Hz, 1H), 4.03 (q, J = 8.0 Hz, 1H), 2.83 (td, J = 9.4, 6.5 Hz, 1H), 2.31 (dd, J = 20.3, 11.8, 8.7 Hz, 1H), 1.94 (ddt, J = 34.9, 14.9, 7.7 Hz, 3H), 1.69–1.56 (m, 1H); 13C NMR (101 MHz, DMSO-d6) δ 179.73, 143.30, 140.71, 134.97, 130.12, 128.18, 127.16, 126.30, 50.64, 26.60; [Anal. Calcd. for C15H16ClN2O3S2: C, 59.05; H, 4.69; N, 7.25; Found: C, 59.15; H, 4.77; N, 7.36]; LC/MS (ESI, m/z): found 387.17 [M+H]+; Exact Mass: 386.08.

3.1.10. (1'R,3'R,7a'R)-5-Bromo-1'-(phenylsulfonyl)-1',2',5',6',7',7a'-hexahydropyrospiro[indoline-3,3'-pyrrolizin]-2-one 4j

1 (84 mg, 0.5 mmol), 5-Br-isatin 2d (113 mg, 0.5 mmol) and L-proline 3a (57.5 mg, 0.5 mmol) were reacted according to GP1 for 8 h and yielded faint grey solid phenylsulfone spirooxindole 4j (199 mg, 89%); m.p. 250 °C; 1H NMR (400 MHz, DMSO-d6) δ 10.45 (s, 1H), 7.95 (d, J = 7.6 Hz, 2H), 7.76 (t, J = 7.4 Hz, 1H), 7.70–7.61 (m, 3H), 7.44 (d, J = 8.2 Hz, 1H), 6.76 (d, J = 8.3 Hz, 1H), 4.45 (t, J = 9.2 Hz, 1H), 4.03 (q, J = 8.1 Hz, 1H), 2.83 (q, J = 8.2, 7.7 Hz, 1H), 2.59 (t, J = 12.0 Hz, 1H), 2.46 (d, J = 9.5 Hz, 1H), 2.32 (q, J = 10.4, 9.8 Hz, 1H), 2.04 (dd, J = 13.1, 7.3 Hz, 1H), 1.92 (dt, J = 13.6, 7.3 Hz, 2H), 1.64 (q, J = 9.9 Hz, 1H); 13C NMR (101 MHz, DMSO-d6) δ 179.25, 142.68, 140.73, 134.58, 133.00, 132.60, 130.23, 129.66, 128.30, 113.82, 110.26, 108.28, 100.23, 69.30, 64.72, 61.14, 49.00, 33.84, 26.62; [Anal. Calcd. int. J. Mol. Sci. 2022, 23, 11861 14 of 19
For C_{20}H_{19}BrN_{2}O_{3}S: C, 53.70; H, 4.28; N, 6.26; Found: C, 53.78; H, 4.33; N, 6.34; LC/MS (ESI, m/z): found 447.15 [M+H]^+; Exact Mass: 446.03.

3.1.11. (1'R,3'R,7'a'R)-1-Methyl-1'-(phenylsulfonyl)-1',2',5',6',7',7'a'-hexahydrospiro[indoline-3,3'-pyrrolizin]-2-one 4k

1 (84 mg, 0.5 mmol), N-Me-isatin 2f (80.5 mg, 0.5 mmol) and L-proline 3a (57.5 mg, 0.5 mmol) were reacted according to GP1 for 8 h and yielded yellow solid phenylsulfonyl spirooxindole 4k (153 mg, 80%); m.p. 71 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 7.95 (s, 1H), 7.65 (s, 1H), 7.49 (dd, J = 19.6, 7.7 Hz, 2H), 7.35 (dq, J = 17.2, 8.6, 7.9 Hz, 2H), 7.08 (t, J = 7.5 Hz, 1H), 7.01 (dd, J = 26.7, 7.7 Hz, 1H), 4.51 (dt, J = 11.5, 7.8 Hz, 1H), 4.06 (q, J = 8.1 Hz, 1H), 3.32 (s, 1H), 3.00 (s, 2H), 2.85 (td, J = 9.3, 6.2 Hz, 1H), 2.80 (s, 1H), 2.55 (d, J = 12.3 Hz, 1H), 2.47 (t, J = 7.8 Hz, 1H), 2.39–2.26 (m, 1H), 2.04–1.87 (m, 3H), 1.72–1.48 (m, 2H), ^13C NMR (101 MHz, DMSO-d_6) δ 177.72, 144.79, 140.73, 138.53, 135.92, 130.39, 129.16, 128.41, 126.41, 123.10, 110.65, 108.12, 71.01, 69.15, 63.90, 62.48, 29.32. [Anal. Calcd. for C_{22}H_{22}O_{2}N_{2}S: C, 65.95; H, 5.80; N, 7.32; Found: C, 66.01; H, 5.85; N, 7.40]; LC/MS (ESI, m/z): found 383.28 [M+H]^+; Exact Mass: 382.14.

3.1.12. (1'R,3'R,7'a'R)-1-(2-Bromoethyl)-1'-(phenylsulfonyl)-1',2',5',6',7',7'a'-hexahydrospiro[indoline-3,3'-pyrrolizin]-2-one 4l

1 (84 mg, 0.5 mmol), N-Br-Et-isatin 2g (126.5 mg, 0.5 mmol) and L-proline 3a (57.5 mg, 0.5 mmol) were reacted according to GP1 for 8 h and yielded yellow solid phenylsulfonyl spirooxindole 4l (216 mg, 91%); m.p. 70 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 7.96 (d, J = 7.9 Hz, 1H), 7.81–7.62 (m, 3H), 7.52 (t, J = 7.7 Hz, 2H), 7.35 (b, J = 6.6, 5.6 Hz, 3H), 7.09 (dq, J = 24.3, 7.6 Hz, 3H), 4.33 (dd, J = 13.1, 6.0 Hz, 1H), 3.99 (q, J = 7.2 Hz, 1H), 3.83 (s, 0H), 3.66 (ddtt, J = 26.4, 19.9, 6.1 Hz, 3H), 2.39–2.25 (m, 1H), 2.25–2.06 (m, 1H), 1.96 (qd, J = 10.9, 10.4, 4.8 Hz, 2H), 1.85–1.68 (m, 1H), 1.65 (d, J = 8.2 Hz, 1H), 1.56 (p, J = 8.5 Hz, 1H); ^13C NMR (101 MHz, DMSO-d_6) δ 143.41, 138.53, 134.72, 130.28, 129.78, 129.36, 129.06, 128.51, 128.36, 127.97, 126.89, 123.31, 122.23, 109.74, 97.23, 72.43, 70.14, 66.67, 65.09, 32.50, 32.30, 29.71, 28.60, 26.59; [Anal. Calcd. for C_{31}H_{23}BrN_{2}O_{2}S: C, 55.58; H, 4.88; N, 5.89; Found: C, 55.64; H, 4.93; N, 5.97]; LC/MS (ESI, m/z): found 475.09 [M+H]^+; Exact Mass: 474.06.

3.1.13. ((1'R,3'R,7'a'R)-6-Chloro-1'-(phenylsulfonyl)-1',2',5',6',7',7'a'-hexahydrospiro[indoline-3,3'-pyrrolizin]-2-one 4m

The spectral data are matched with the reported literature [47,48].

3.1.14. (3'R,7'R,7'a'R)-6-Chloro-7'-(phenylsulfonyl)-1',6',7',7'a'-tetrahydro-3'H-spiro[indoline-3,3'-pyrrolo [1,2-c]thiazole]-2-one 4n

1 (84 mg, 0.5 mmol), 6-Cl-isatin 2h (90.5 mg, 0.5 mmol) and L-thioproline 3b (66.5 mg, 0.5 mmol) were reacted according to GP1 for 8 h and yielded white solid phenylsulfonyl spirooxindole 4n (189 mg, 90%); m.p. 271 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 10.54 (s, 1H), 8.00 (d, J = 7.7 Hz, 2H), 7.82 (t, J = 7.5 Hz, 1H), 7.70 (t, J = 7.8 Hz, 2H), 7.35 (d, J = 8.1 Hz, 1H), 7.10 (d, J = 8.1 Hz, 1H), 6.84 (s, 1H), 4.62 (dt, J = 12.4, 6.5 Hz, 1H), 3.90 (d, J = 10.0 Hz, 1H), 3.78 (dt, J = 11.0, 6.2 Hz, 1H), 3.34 (s, 2H), 3.14–2.96 (m, 2H), 2.29 (dd, J = 13.4, 7.0 Hz, 1H); ^13C NMR (101 MHz, DMSO-d_6) δ 179.55, 144.93, 139.29, 135.14, 134.96, 130.44, 128.52, 127.64, 125.12, 122.17, 110.52, 69.86, 62.52, 53.28, 34.56, 32.64; [Anal. Calcd. for C_{10}H_{12}ClN_{2}O_{2}S_{2}: C, 54.22; H, 4.07; Cl, 8.42; N, 6.66; Found: C, 54.33; H, 4.10; N, 6.76]; LC/MS (ESI, m/z): found 421.12 [M+H]^+; Exact Mass: 420.04.

3.2. Biological Activity Assays

The protocol for the biological activity assay is provided in the Supplementary Materials.

3.3. Molecular Docking

The protocol for the molecular docking study is provided in the Supplementary Materials.
3.4. ADMET Analysis

The protocol for the ADMET analysis is provided in the Supplementary Materials.

3.5. Molecular Dynamic Simulation

The protocol for the molecular dynamics simulation is provided in the Supplementary Materials.

4. Conclusions

Here, a detailed design and synthesis of spirooxindole-based phenylsulfonyl moiety compounds are provided and their in vitro antiviral activity was evaluated against pandemic SARS-CoV-2 and MERS-CoV with multiple sporadic human infections. Based on the preliminary screening of the anti-coronaviral activity of the tested compounds, compounds 4k, 4c, and 4i showed high safety and high-to-moderate anti-coronavirus activities. Synergistic combinations of the three compounds against SARS-CoV-2 displayed a more potent inhibitory activity. These promising combinations with high selectivity indices are recommended for further in vitro and in vivo preclinical studies. The determination of dosage form will be decided through pharmacodynamics and pharmacokinetic studies in the preclinical phases. However, based on the current physicochemical parameters virtually, these bioactive candidates could be administered in an oral dosage form, for example as capsules or tablets.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijms231911861/s1, Protocols for the in vitro biological activity assays, molecular docking, ADMET Analysis, and Molecular Dynamic Simulation. Figures S1–S24: NMR spectra (1H and 13C), Figures S25–S27: Cytotoxicity, Antiviral activities against SARS-CoV-2 and MERS-CoV. Figures S28 and S29: Molecular docking optimization.

Author Contributions: The strategy was designed by A.B., L.R.D. and Y.A.M.E.; Experimental work was performed by M.A.; A.M.A.-M.; biological studies were performed by A.M., O.K. and Y.M.; ADMET Analysis and Molecular Dynamic Simulation were carried out by K.Z. and Z.U.-H. All of the authors discussed the results and prepared the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: The authors would like to extend their sincere appreciation to the Researchers Supporting Project (RSP-2021/64), King Saud University, Riyadh, Saudi Arabia. This research has been also funded by the Egyptian Academy of Scientific Research and Technology (ASRT) within the “Ideation Fund” program to AM under contract #7303.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to extend their sincere appreciation to the Researchers Supporting Project (RSP-2021/64), King Saud University, Riyadh, Saudi Arabia. This research has been also funded by the Egyptian Academy of Scientific Research and Technology (ASRT) within the “Ideation Fund” program to AM under contract #7303.

Conflicts of Interest: There are no conflict to declare.

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