Research Article

Antimicrobial Alkaloids from Marine-Derived Fungi as Drug Leads versus COVID-19 Infection: A Computational Approach to Explore their Anti-COVID-19 Activity and ADMET Properties

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Received 16 February 2022; Accepted 11 June 2022; Published 8 July 2022

Academic Editor: Sakineh Kazemi Noureini

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Therapeutic strategies based upon enzyme inhibition have recently gained higher attention in treating hazardous ailments. Herein, the potential use of seventy-two antimicrobial alkaloids isolated from marine-derived fungi to fight COVID-19 infection via inhibition of SARS-CoV-2 lethal virus was performed using in silico analyses. Molecular modelling was performed to assess their enzyme inhibitory potential on the main protease SARS-CoV-2 MPro, 3-chymotrypsin-like protease SARS-CoV-2 3CLpro, and papain-like protease SARS-CoV-2 PLpro using Discovery Studio 4.5. Validation of the docking experiments was done by determination of RMSD (root mean square deviation) after redocking the superimposition of the cocrystalized ligands. Results showed that gymnastatin Z (72) showed the best fitting score in SARS-CoV-2 MPro and SARS-CoV-2 3CLpro active sites with ΔG equal −34.15 and −34.28 Kcal/mol, respectively. Meanwhile, scalusamide C (62) displayed the highest fitting within SARS-CoV-2 PLpro active sites (ΔG = −26.91 Kcal/mol) followed by eutypellazine M (57). ADMET/TOPKAT prediction displayed that eutypellazine M and scalusamide C showed better pharmacokinetic and pharmacodynamic properties. Gymnastatin Z is safer showing better toxicity criteria and higher rat oral LD50 and rat chronic LOAEL (lowest observed adverse effect level). Chemometric analysis using principle component analysis (PCA) based on the binding energies observed for the compounds with respect to the three tested enzymes revealed the clustering of the compounds into different clusters. Eutypellazine M, scalusamide C, and gymnastatin Z appear in one cluster due to their closeness in activity. Thus, these compounds could serve as promising SARS-CoV-2 enzymes inhibitors that could help in alleviation of COVID-19 infection. Further investigations are recommended to confirm the results of molecular modelling.

1. Introduction

The oceans constitute unique mines that offer a wide array of naturally occurring compounds derived mainly from marine organisms and their accompanied microorganisms [1]. As a result of evolution of infectious diseases and the subsequent appearance of resistance microbes versus the currently used anti-infectious agents, the marine resources may afford novel antimicrobial entities [2]. They could serve as promising leads combating viral, bacterial, fungal, and parasitic infections as well [3, 4]. Many naturally occurring marine products have successfully proceeded to the final stage of clinical trials; meanwhile others act as promising candidates for preclinical assessment [5]. Marine-derived fungal metabolites are characterized by favorable physicochemical behavior, oral bioavailability, and relative safety with respect to synthetic agents. This greatly highlights the considerable importance of these metabolites in the formulation of promising efficient pharmaceutical dosage forms that encourages us to carry out this study [6, 7].
The presence and dissemination of viral infections currently produce great distress as viral infections cause major health hazards. It is noteworthy to highlight that many antiviral leads versus *Herpes simplex* virus (HSV), influenza A (H1N1), and human immunodeficiency viruses (HIV) were isolated from marine sources [8]. Spongouridine as well as spongouridin nucleosides isolated from *Tethya crypta* marine sponges led to Ara-A synthesis, an FDA approved antiviral drug that effectively prohibited viral DNA synthesis. Ara-A is converted into adenine arabinoside triphosphate that prohibits DNA polymerase and inhibits DNA synthesis of *Varicella zoster*, *Herpes*, and *Vaccinia* viruses. The clinical utilization of this compound has been used for the curing *Herpes* virus infection but was replaced by acyclovir (Zovirax) [9].

At the end of December 2019, patients possessing pneumonia of unidentified cause had spread in Wuhan, China. This was proved to be a novel coronavirus strain that triggered this fatal respiratory disease [10, 11]. The virus was termed severe acute respiratory syndrome-related coronavirus and abbreviated by SARS-CoV-2. On 12th March 2020, coronavirus disease 2019 (COVID-19) has been ascertained in 125,048 patients all over the globe displaying about 3.7% mortality rate in comparison to influenza that showed less than 1% mortality rate [12]. Meanwhile, on 16 April, this lethal virus rapidly invaded 185 different countries and seriously infected more than 2,000,000 patients resulting in 130,000 deaths [10, 11, 13]. Besides, patients who suffer from severe COVID-19 also experienced cytokine storm syndrome. This is characterized by hyperinflammation, secondary hemophagocytic lymphohistiocytosis, continuous fever, cytopenias, hyperferritinemia, and pulmonary involvement that resulted in acute respiratory distress syndrome [10, 11]. Thus, searching for effective treatments to combat this pandemic is felt mandatory worldwide. This required the discovery of novel therapeutics comprising mainly vaccines and antivirals which target different viral proteins.

It is noteworthy to highlight that many researches have recently tried to use active constituents derived from natural products to combat COVID-19 infection guided by screening their activities using in silico studies [14–21].

As therapeutic strategies based upon enzyme inhibition have recently gained higher attention in treating hazardous ailments, thus in this study alkaloids from marine-derived fungal strains with antimicrobial potential reported in literature were collected based upon the authors’ previous comprehensive literature survey was performed by the authors focusing on the biological activities and chemical properties of alkaloids isolated from marine associated twenty-seven fungal genera. These fungal genera comprise *Acrostalagmus*, *Aspergillus*, *Arthrinium*, *Curvularia*, *Chaetomium*, *Coniothyrium*, *Cladosporium*, *Eurotium*, *Dichotomomyces*, *Exophiala*, *Hypocrea*, *Microsphaeropsis*, *Neosartorya*, *Microsorum*, *Nigrospora*, *Paeclimonoryces*, *Pleosporales*, *Penicillus*, *Scedosporium*, *Pseudallescheria*, *Stagonosporopsis*, *Scopulariopsis*, *Westedykella*, *Xylariaeaceae*, and *Thielavia* [22, 23]. Around 514 alkaloid metabolites were isolated and identified via various chromatographic and spectroscopic techniques. They were additionally ascertained using High Resolution Mass Spectrometry together with Mosher reactions for further confirmation of the stereochemistry. Around 72 alkaloid metabolites were reported to possess antiviral, antibacterial, and antifungal activities which were selected to be the target of our study. Data were collected from PubMed (https://www.ncbi.nlm.nih.gov/pubmed/), sciFinder (https://scifinder.cas.org/scifinder/login), and Web of Knowledge (https://www.webofknowledge.com).

### 2. Materials and Methods

#### 2.1. Selection of Compounds Used in This Study

A comprehensive literature survey was performed by the authors focusing on the biological activities and chemical properties of alkaloids isolated from marine associated twenty-seven fungal genera. These fungal genera comprise *Acrostalagmus*, *Aspergillus*, *Arthrinium*, *Curvularia*, *Chaetomium*, *Coniothyrium*, *Cladosporium*, *Eurotium*, *Dichotomomyces*, *Exophiala*, *Hypocrea*, *Microsphaeropsis*, *Neosartorya*, *Microsorum*, *Nigrospora*, *Paeclimonoryces*, *Pleosporales*, *Penicillus*, *Scedosporium*, *Pseudallescheria*, *Stagonosporopsis*, *Scopulariopsis*, *Westedykella*, *Xylariaeaceae*, and *Thielavia* [22, 23]. Around 514 alkaloid metabolites were isolated and identified via various chromatographic and spectroscopic techniques. They were additionally ascertained using High Resolution Mass Spectrometry together with Mosher reactions for further confirmation of the stereochemistry. Around 72 alkaloid metabolites were reported to possess antiviral, antibacterial, and antifungal activities which were selected to be the target of our study. Data were collected from PubMed (https://www.ncbi.nlm.nih.gov/pubmed/), sciFinder (https://scifinder.cas.org/scifinder/login), and Web of Knowledge (https://www.webofknowledge.com).

#### 2.2. In silico Studies

**Molecular modelling** was performed on SARS-CoV-2 3CLpro (PDB ID: 6L2E; 1.50 Å), SARS-CoV-2 3CLpro (PDB ID: 6M2N; 2.20 Å), SARS-CoV-2 PLpro (PDB ID: 4OW0; 2.10 Å), and ACE2 (PDB ID: 1R4L; 3.00 Å) using Discovery Studio 4.5 (Accelrys Inc., San Diego, CA, USA) applying C-Docker protocol as previously discussed [24–26]. The X-ray crystal structures of the examined proteins were obtained from protein data bank (https://www.pdb.org) in PDB format. This was done for seventy-two compounds of the identified alkaloids that previously showed to exhibit significant antimicrobial and antiviral activities.

#### 2.2.1. Validation of Molecular Docking Studies Using Redocking and Superimposition

Molecular docking validation was done for all the performed docking experiments through performing a comparison between the alignments of the most stable docking poses of the lead compound together with the lead conformer cocrystallized with the respective enzyme from PDB. The value of RMSD (root mean square deviation) is utilized to ascertain the docking
experiment validity and gives an indication to the ability of prediction of the binding affinity of new ligands.

2.2.2. Performing Molecular Docking Studies. The structure for each enzyme was prepared via adopting the default protocol of Discovery Studio 4.5 (Accelrys Inc., San Diego, CA, USA) [24, 27, 28]. This occurs in several steps starting with removal of water molecules, adding of hydrogen atoms accompanied by cleansing the structure of the protein from undesired interactions. CHARMM was employed as the forcefield; however MMFF94 was adopted for partial charge calculation that subsequently was followed by minimizing the added hydrogen in 2000 steps. Determination of the binding center was accomplished depending upon the data reported approaching the catalytic domain of the targeted protein. ChemDraw 13.0 was used to construct the 2D structures of the compounds for docking experiments. Docking was then performed for the prepared structure within the active centers of the energy-minimized protein employing default parameters in C-Docker protocol. CHARMM force field was chosen and the binding energy (ΔG) was computed using distance dependent dielectric implicit solvation model for the chosen docking poses. Calculation of (ΔG) in Kcal/mol was performed using the following equation:

\[
\Delta G_{\text{binding}} = E_{\text{complex}} - (E_{\text{protein}} + E_{\text{ligand}}),
\]

where \( \Delta G_{\text{binding}} \) is the ligand-protein interaction binding energy, \( E_{\text{complex}} \) is the potential energy for the complex of protein bound with the ligand, \( E_{\text{protein}} \) is the protein potential energy alone, and \( E_{\text{ligand}} \) is the ligand potential energy alone.

2.2.3. ADMET/TOPKAT Prediction. ADMET/TOPKAT (absorption, distribution, metabolism, excretion, and toxicity) prediction was done on the compounds that showed the highest activity towards the examined enzymes together with the cocrystallized ligands using Discovery Studio 4.5 (Accelrys Inc., San Diego, CA, USA). Human intestinal absorption, blood brain barrier penetration (BBB), aqueous solubility, hepatotoxicity level, plasma protein binding prediction (PPB), and cytochrome P450 (2D6) were selected as ADMET descriptors. However, Ames mutagenicity, carcinogenic effect female and male rat FDA, rat oral LD_{50}, and rat chronic LOAEL in addition to eye and dermal irritancy were selected as toxicity parameters [29].

2.3. Chemometric Analysis. Chemometric analysis using principle component analysis (PCA) as unsupervised pattern recognition technique was done based on the binding energies observed for the compounds with respect to the three tested proteins using CAMO’s Unscrambler® X 10.4 software (Computer-Aided Modeling, As, Norway) [26].

3. Results and Discussion

3.1. Diverse Alkaloids Identified from Marine-Derived Fungal Strains Showing Antimicrobial Activities. Around 72 alkaloids isolated from marine associated fungal strains belonging to Arthrinium, Aspergillus, Eurotium, Curvularia, Eutypella, Dichotomomyces, Fusarium, Neosartorya, Penicillium, Paecilomyces, Stagonosporopsis, Thielavia, Pseudallescheria, and Westerdykella were reported to possess antimicrobial activities [22, 23]. These include arthropyrines F-I (1–4) and apioporsamide (5) isolated from *Arthrinium* [30]. Additionally, *Aspergillus* species act as a rich source of alkaloids with anti-infective properties such as fumitremorgin C (6), fumiquinazoline C (7), 12,13-dihydroxy fumitremorgin C (8), fumiquinazoline G (9), fumigatioside E (10), fumigatioside F (11), api-azsonalenin A (12), versicoloïds A and B (13-14), aspergicin (15), stephacidin A (16), 7α,14-dihydroxy-6β-p-nitrobenzyloxycoumefertifolin (17), 9α,14-dihydroxy-6β-p-nitrobenzoylaminomolide (18), 3-((1-hydroxy-3-(2-methylbut-3-en-2-yl)-2-oxoindolin-3-yl) methyl)-1-methyl-3,4-dihydrobenzo[e][1,4] diazepine-2,5-dione (19), cytocchalasin Z17 (20), and gliotoxin (21) [30–37]. Moreover, curvulamine (22) obtained from *Curvularia* species as well as scequinadoline A (23) isolated from *Dichotomomyces* revealed potent antimicrobial activity [38].

Meanwhile, *Eurotium* isolated from marine sources contains alkaloids that showed antimicrobial activity including neoecchinulin B (24), crystalustin A (25), rubramazines A-C (26–28), compounds (29-40), isoechinulin A (41), rubrumline D (42), variacerceine O (43), and neoecchinulin C (44) [39, 40]. New compounds, eutypellazines A-M (45–57), obtained from *Eutypella*, are potent antimicrobial fungal metabolites [41]. Oxysporizoline (58), a new polycyclic quinazoline alkaloid isolated from *Fusarium*, [42] and varioxepine A (59) isolated from the marine associated fungi *Paecilomyces*, exhibited a potent antimicrobial activity [43]. *Penicillium* is a popular source of marine associated fungi rich in bioactive alkaloids particularly showing anti-microbial properties. These are scalusamides A-C (60–62), penipanoid A (63) and C (64), raistrickindole A (65), raistrickin (66), breviamide F (67), α-cyclopiazonic acid (68), and terretrione A (69) [44, 45]. Didymellamide A (70) from Stagonosporopsis [46] and thielavialazine (71) from *Thielavia*, a marine associated fungus, are potent antimicrobial agents [47]. Gymnastatin Z (72), a new tyrosine-derived alkaloid, was isolated from *Westerdykella dispersa* and displayed moderate antimicrobial effect [48]. The structures of all the antimicrobial alkaloids isolated from the previously mentioned fungi are depicted in Figures 1–3.

3.2. In silico Studies. SARS-CoV-2 reveals a genome greatly similar to *beta*-coronaviruses comprising a replicate region containing a replicate complex (orf1ab) that produces nonstructural proteins (nsps), a 5’-untranslated region (UTR), a structural region composed of an envelope protein (E) gene, a spike protein (S) gene, a nucleocapsid protein (N) gene, and a membrane protein (M) gene in addition to 3’-UTR and multiple unidentified non-structural open
Although SARS-CoV-2 was classified as beta-coronaviruses, it shows certain similarities and other differences from MERS-CoV and SARS-CoV genome. Besides, during genome transcription, beta-coronaviruses generate a polypeptide (about 800 kDa) that undergoes proteolytic cleavage performed by main protease (M^pro), papain-like protease (PL^pro), and 3-chymotrypsin-like protease (3CL^pro) to produce different proteins at 11 specific sites.
which are crucial for viral replication. Thus, targeting these enzymes may act as important therapeutic strategies to inhibit SARS-CoV-2 [11]. Thus, herein shedding the light on the potential use of diverse antimicrobial alkaloids derived from marine fungi as SARS-CoV-2 MPro, papain-like protease (PLpro), and SARS-CoV-2 3CLpro inhibitors was adopted. This was performed via molecular modelling study as future perspectives in an effort to inhibit this virus and

**Figure 2:** Diverse alkaloids identified from marine-derived fungal strains showing antimicrobial activities (cont’d).
combat COVID-19 infection. Molecular modelling was performed on SARS-CoV-2 M\textsuperscript{Pro} (PDB ID: 6LZE; 1.50 Å), SARS-CoV-2 3CL\textsuperscript{pro} (PDB ID: 6M2N; 2.20 Å), and SARS-CoV-2 PL\textsuperscript{pro} (PDB ID: 7LOS; 2.9 Å) using Discovery Studio 4.5 (Accelrys Inc., San Diego, CA, USA) employing C-Docker protocol as previously discussed [24–26]. This was done for seventy-two compounds of previously identified alkaloids that previously showed significant antimicrobial and antiviral activities. The structures of the three targeted proteins were illustrated in Figure 4.

Figure 3: Diverse alkaloids identified from marine-derived fungal strains showing antimicrobial activities (cont’d).
3.2.1. Validation of the Molecular Docking Experiments. Validation experiments showed a perfect alignment between the docking pose of the lead compounds that showed the highest fitting and the lead conformers that are co-crystallized with their respective enzymes. They displayed RMSD values of 1.30, 1.75, and 2.02 Å for SARS-CoV-2 PL<sub>Pro</sub>, SARS-CoV-2 M<sub>Pro</sub>, and SARS-CoV-2 3CL<sub>Pro</sub>, respectively. Thus, it perfectly ascertained the validity of the docking experiment as shown in Figure 5 [49].

3.2.2. Molecular Modelling Studies. Molecular modelling of the seventy two selected compounds revealed that gymnastatin Z (72) showed the best fitting score in SARS-CoV-2 M<sub>Pro</sub> and SARS-CoV-2 3CL<sub>Pro</sub> active sites followed by scalusamide C (62) and eutypellazine M (57); meanwhile scalusamide C (62) displayed the highest fitting within SARS-CoV-2 PL<sub>Pro</sub> active sites followed by gymnastatin Z (72) and eutypellazine M (57). They showed binding energies (\(\Delta G\)) equal to −34.15, −29.34, and −25.44 Kcal/mol, respectively, for SARS-CoV-2 M<sub>Pro</sub>, −24.29, −26.91, and −22.21 Kcal/mol, respectively, for SARS-CoV-2 PL<sub>Pro</sub>, whereas they showed \(\Delta G\) of −34.28, −32.73, and −31.52 Kcal/mol, respectively, for SARS-CoV-2 3CL<sub>Pro</sub>. Thus, in this aspect, they showed superior activity in comparison to the main SARS-CoV-2 M<sub>Pro</sub> ligand (FHR/PRD_002347 (~N)-[(2~S)-3-cyclohexyl-1-oxidanylidene-1-[(2~S)-1-oxidanylidene-3-[(3~S)-2-oxidanylidene-pyrrolidin-3-yl] propan-2-yl]amino]propan-2-yl-1~{H}-indole-2-carboxamide) (\(\Delta G = -4.60\) Kcal/mol) and that of SARS-CoV-2 PL<sub>Pro</sub> ligand: Y975-((azetidin-3-ylamino)-2-methyl-~{N}-[(1~{R})-1-[[3-[[3~{R}]-oxolan-3-yl] amino]methyl]thiophen-2-yl]phenyl]ethyl]benzamide (\(\Delta G = -4.08\) Kcal/mol) downloaded with the protein from PDB. Meanwhile, they approach that of the main SARS-CoV-
| Compound                  | Genus                  | SARS-CoV-2 M\textsuperscript{Pro} | SARS-CoV-2 Pf\textsuperscript{Pro} | SARS-CoV-2 3CL\textsuperscript{Pr} |
|---------------------------|------------------------|-----------------------------------|------------------------------------|----------------------------------|
| **Arthpyrone F (1)**      | Aspergillus            | 20.01                             | 26.33                              | 12.49                            |
| **Arthpyrone G (2)**      | Aspergillus            | 47.11                             | 47.43                              | 44.89                            |
| **Arthpyrine H (3)**      | Aspergillus            | 21.01                             | 29.33                              | 14.49                            |
| **Arthpyrine I (4)**      | Aspergillus            | 23.28                             | 39.35                              | 23.92                            |
| **Apiosporamide (5)**     | Aspergillus            | 7.29                              | 23.30                              | 6.18                             |
| **Fumitremorgin C (6)**   | Aspergillus, P. aurea | 5.12                              | 12.48                              | 4.36                             |
| **Fumiquinazoline C (7)** | Aspergillus            | 11.92                             | 43.37                              | 12.07                            |
| 12,13-Dihydroxy fumitremorgin C (8) | P. aurea | −5.12                             | −12.48                             | −4.36                            |
| **Fumiquinazoline G (9)** | Aspergillus            | 19.91                             | 29.28                              | 17.30                            |
| **Fumigatoside E (10)**  | Aspergillus            | 2.57                              | 4.77                               | 6.28                             |
| **Fumigatoside F (11)**  | Aspergillus            | −1.57                             | 2.77                               | −5.28                            |
| **epi-Assonalenalin A (12)** | Aspergillus          | 27.74                             | 233.99                             | 27.2962                           |
| **Versicoldoid A (13)**  | Aspergillus            | −14.40                            | −6.18                              | −15.17                           |
| **Versicoldoid B (14)**  | Aspergillus            | −10.39                            | −4.22                              | −12.12                           |
| **Aspergicin (15)**       | Aspergillus            | −12.43                            | −5.21                              | −14.19                           |
| **Stephacidin A (16)**   | Aspergillus            | 23.86                             | 29.94                              | 23.69                            |
| **Compound (17)**         | Aspergillus            | 18.25                             | 20.25                              | 20.25                            |
| **Compound (18)**         | Aspergillus            | 24.49                             | 22.05                              | 20.66                            |
| **Compound (19)**         | Aspergillus            | 0.12                              | 21.08                              | 0.95                             |
| **Cytochalasin Z17(20)**  | Aspergillus            | 39.52                             | 128.81                             | 41.66                            |
| **Gliotxin (21)**         | Aspergillus            | 24.35                             | 32.94                              | 26.95                            |
| **Curvulamine (22)**      | Curvularia             | 15.23                             | 17.72                              | 9.62                             |
| **Scequinolamine A (23)** | Dichotomomyces         | −5.32                             | 31.30                              | 10.14                            |
| **Neoehchinulin B (24)**  | Eurotium               | −12.62                            | −12.99                             | −17.19                           |
| **Cristatum A (25)**      | Eurotium               | −17.28                            | −14.32                             | −21.48                           |
| **Rubrumazine A (26)**    | Eurotium               | −20.945                           | 10.63                              | −23.22                           |
| **Rubrumazine B(27)**     | Eurotium               | −21.11                            | 10.37                              | −23.02                           |
| **Rubrumazine C (28)**    | Eurotium               | −20.334                           | −12.64                             | −22.96                           |
| **Compound (29)**         | Eurotium               | −13.83                            | −17.65                             | −18.81                           |
| **Compound (30)**         | Eurotium               | −2.41                             | 6.93                               | −3.06                            |
| **Compound (31)**         | Eurotium               | −6.73                             | 17.15                              | −3.05                            |
| **Compound (32)**         | Eurotium               | 15.00                             | 54.20                              | 6.22                             |
| **Compound (33)**         | Eurotium               | −26.77                            | −9.58                              | −27.69                           |
| **Compound (34)**         | Eurotium               | −19.50                            | −10.22                             | −24.53                           |
| **Compound (35)**         | Eurotium               | 18.16                             | 67.10                              | 22.15                            |
| **Compound (36)**         | Eurotium               | 33.22                             | FD                                 | 30.89                            |
| **Compound (37)**         | Eurotium               | −19.24                            | −25.36                             | −21.94                           |
| **Compound (38)**         | Eurotium               | −15.36                            | −12.32                             | −21.52                           |
| **Compound (39)**         | Eurotium               | −6.69                             | 26.60                              | −8.54                            |
| **Compound (40)**         | Eurotium               | −13.83                            | 15.93                              | −1.76                            |
| **Isoehchinulin A (41)**  | Eurotium               | −1.17                             | −0.52                              | −6.21                            |
| **Rubrumline D (42)**     | Eurotium               | −22.96                            | −3.98                              | −21.88                           |
| **Variecolorine O (43)**  | Eurotium               | −20.22                            | −21.52                             | −24.26                           |
| **Neoehchinulin C (44)**  | Eurotium               | −2.17                             | 3.17                               | 15.50                            |
| **Eutypellazine A (45)**  | Eutypella              | 34.07                             | 59.98                              | 36.55                            |
| **Eutypellazine B (46)**  | Eutypella              | 41.54                             | 52.74                              | 42.17                            |
| **Eutypellazine C (47)**  | Eutypella              | 30.37                             | 59.19                              | 31.74                            |
| **Eutypellazine D (48)**  | Eutypella              | 17.54                             | 63.22                              | 15.50                            |
| **Eutypellazine E (49)**  | Eutypella              | 27.24                             | 12.27                              | 23.06                            |
| **Eutypellazine F (50)**  | Eutypella              | 17.83                             | 12.58                              | 17.47                            |
| **Eutypellazine G (51)**  | Eutypella              | 21.80                             | 9.57                               | 24.47                            |
| **Eutypellazine H (52)**  | Eutypella              | 30.28                             | 7.70                               | 30.27                            |
| **Eutypellazine I (53)**  | Eutypella              | −19.44                            | 6.62                               | −23.38                           |
| **Eutypellazine J (54)**  | Eutypella              | −22.19                            | −6.84                              | −23.75                           |
| **Eutypellazine K (55)**  | Eutypella              | −24.46                            | −3.43                              | −28.53                           |
| **Eutypellazine L (56)**  | Eutypella              | −24.24                            | −6.01                              | −26.82                           |
interactions with Tyr 268 and Pro 248 together with one alkyl interaction with Pro 248 and Van der Waals interaction (Figure 6(b)). However, scalusamide C (62) showed the highest fitting score evidenced by the presence of one alkyl interaction with Pro 248, four C-H interactions with Asp 164, Pro 247, and Pro248, and Van der Waals interaction (Figure 7(b)). Additionally, eutypellazine M (57) forms one conventional H-bond with Arg 166, π-π stacked bond with Tyr 268, and C-H bond with Pro 248, Asp164, and Arg 166 with many Van der Waals bonds (Figure 8(b)). Meanwhile, the main SARS-CoV-2 PLpro ligand forms four H-bonds with Glu 167, Asp 164, Gln 269, and Gly 266, one π-π bond with Tyr 268, two π-alkyl interactions with Pro 248 and Tyr 264, 2 alkyl interactions with Pro 248 and Leu 162, and four C-H bonds with Glu 167, Gly 266, and Pro 248 and π-sulfur bond with Tyr 264 (Figure 9(b)).

Regarding SARS-CoV-2 3CL<sup>P</sup>, gymnastatin Z (72) also exerted the highest fitting that relied upon the presence of three H-bonds with His 41, His 164, and Glu 166 and alkyl-alkyl interactions with Met 49, Cys 44, Pro 52, and His 41, whereas the π-δ bond is formed between Asn 142 and the aromatic moiety of the phenyl ring (Figure 6(c)). Scalusamide C (62) is also tightly bound to the active site due to the presence of one H-bond with Glu 166, one C-H bond with Met 165, and alkyl interactions with Cys 145, His 41, and Asn 142 in addition to Van der Waals interaction with the amino acid residues present in the active site (Figure 9(a)).

Concerning SARS-CoV-2 PL<sup>pro</sup>, gymnastatin Z (72) forms one H-bond with Gly 266 in addition to two π-alkyl interactions with Tyr 268 and Pro 248 together with one alkyl interaction with Pro 248 and Van der Waals interaction (Figure 6(b)). However, scalusamide C (62) showed the highest fitting score evidenced by the presence of one alkyl interaction with Pro 248, four C-H interactions with Asp 164, Pro 247, and Pro248, and Van der Waals interaction (Figure 7(b)). Additionally, eutypellazine M (57) forms one conventional H-bond with Arg 166, π-π stacked bond with Tyr 268, and C-H bond with Pro 248, Asp164, and Arg 166 with many Van der Waals bonds (Figure 8(b)). Meanwhile, the main SARS-CoV-2 PLpro ligand forms four H-bonds with Glu 167, Asp 164, Gln 269, and Gly 266, one π-π bond with Tyr 268, two π-alkyl interactions with Pro 248 and Tyr 264, 2 alkyl interactions with Pro 248 and Leu 162, and four C-H bonds with Glu 167, Gly 266, and Pro 248 and π-sulfur bond with Tyr 264 (Figure 9(b)).

Regarding SARS-CoV-2 3CL<sup>P</sup>, gymnastatin Z (72) also exerted the highest fitting that relied upon the presence of three H-bonds with His 41, His 164, and Glu 166 and alkyl-alkyl interactions with Met 49, Cys 44, Pro 52, and His 41, whereas the π-δ bond is formed between Asn 142 and the aromatic moiety of the phenyl ring (Figure 6(c)). Scalusamide C (62) is also tightly bound to the active site due to the presence of one H-bond with Glu 166, one C-H bond with Met 165, and alkyl interactions with Cys 145, His 41, Met 49, and Pro 168 (Figure 7(c)). Eutypellazine M (57) also revealed the best fitting score that is manifested by its firm binding at the active site via the formation of several bonds with the amino acid residues present at the active sites. They are 2 conventional H-bonds with His 164 and His 41, π-sulfur bond with Cys 44, π-π T-shaped bond with His 41, and Van der Waals interaction with most of the amino acid groups at the active center, π-alkyl bond with Met 49 and...
Figure 6: 2D and 3D binding modes of the gymnastatin Z (72) in SARS-CoV-2 M\textsuperscript{PrO} (a), SARS-CoV-2 PLpro (b), and SARS-CoV-2 3CL\textsuperscript{Pr} (c) active sites.
Figure 7: 2D and 3D binding modes of the scalusamide C (62) in SARS-CoV-2 M^\text{Pro} (a), SARS-CoV-PL^\text{Pr} (b), and SARS-CoV-2 3CL^\text{Pr} (c) active sites.
Figure 8: 2D and 3D binding modes of the eutypellazine M (57) in SARS-CoV-2 \( \text{M}^\text{Pro} \) (a), SARS-CoV-2 \( \text{PL}^\text{Pro} \) (b), and SARS-CoV-2 \( \text{3CL}^\text{Pr} \) (c) active sites.
Cys145 (Figure 8(c)). However, main SARS-CoV-2 3CLpr ligand: 3WL formed 4 H-bonds with Leu 141, Ser 144, Gly 143, and Cys 145, \(\pi\)-sulfur bond with Cys 145, \(\pi\)-alkyl bond with Met 49, \(\pi\)-\(\pi\) stacked bond with His 41, and C-H bond with His 163 together with Van der Waals interaction with most of the amino acid groups at the active center (Figure 9(c)). The presence of gymnastatin Z in the active pocket of SARS-CoV-2 M\(^{\text{Pro}}\) (A), SARS-CoV-2 PL\(^{\text{pro}}\) (B), and SARS-CoV-2 3CL\(^{\text{pr}}\) (C) active sites showing regions of hydrogen bond formation, hydrophobicity regions, and

**Figure 9:** 2D and 3D binding modes of the respective ligands in SARS-CoV-2 M\(^{\text{Pro}}\) (a), SARS-CoV-2 PL\(^{\text{pro}}\) (b), and SARS-CoV-2 3CL\(^{\text{pr}}\) (c) active sites.
The presence of gymnastatin Z in the active pocket of SARS-CoV-2 MPro (a), SARS-CoV-2 PLpro (b), and SARS-CoV-2 3CLpro (c) active sites showing regions of hydrogen bond formation, hydrophobicity regions, and ionizable regions.

Figure 10: The presence of gymnastatin Z in the active pocket of SARS-CoV-2 MPro (a), SARS-CoV-2 PLpro (b), and SARS-CoV-2 3CLpro (c) active sites showing regions of hydrogen bond formation, hydrophobicity regions, and ionizable regions.

ionizable regions is illustrated in Figure 10. Additionally, the compounds that showed the highest fitting within the previously mentioned enzymes, namely, gymnastatin Z (72), scalusamide C (62), and eutypellazine M (57), were docked within the active sites of angiotensin-converting enzyme 2 ACE2 where they also showed a pronounced fitting with \( \Delta G \) of \(-40.90\), \(-37.74\), and \(-52.28\) kcal/mol, respectively. Eutypellazine M (57) that showed the best fitting with the active site forms different bonds evidenced by the formation of four H-bonds with Arg273, Glu402, His378, and Tyr515, one \( \pi \)-alkyl bond with Pro346 in addition to one C-H bond with Ala348, and many Van der Waals interactions.

3.2.3. ADMET/TOPKAT Prediction. ADMET/TOPKAT prediction was evaluated for eutypellazine M (57), scalusamide C (62), and gymnastatin Z (72), as well as the cocrystallized ligands with the examined proteins in order to determine their pharmacokinetic and pharmacodynamic characters (Table 2). Both eutypellazine M and scalusamide C revealed good human intestinal absorption level, good solubility level, and certain BBB level and thus they lie within the 99% absorption ellipse as well as the 99% BBB confidence ellipse as shown in ADMET plot (Figure 11). Meanwhile, gymnastatin Z showed low human intestinal absorption level with possible solubility and undefined BBB level and thus in ADMET plot it is found outside the 99% BBB confidence ellipse. Regarding plasma protein binding level that is crucial in the determination of pharmaceutical activity as it controls the concentration of free drug, gymnastatin Z displayed less than 90% PPB whereas both eutypellazine M and scalusamide C showed more than 90% binding. Regarding the toxicity parameters, eutypellazine M, scalusamide C, and gymnastatin Z are nonhepatotoxic, nonmutagen, and noncarcinogen to both male and female rat FDA. They also caused no cytochrome P450 2D6 (CYP2D6) inhibition that is involved in the metabolism of many xenobiotics so its inhibition may be the cause of uncontrolled drug-drug interactions. Rat oral LD\(_{50}\) was 0.28, 1.35, and 1.16 g/kg.bw for eutypellazine M, scalusamide C, and gymnastatin Z, respectively; meanwhile 0.03, 0.08 and 0.18 g/kg.bw were the values for rat chronic LOAEL (lowest-
observed-adverse-effect level) for them, respectively. Regarding ocular and dermal irritation, gymnastatin Z showed mild skin irritation with no eye irritation in contrast to eutypellazine M that showed severe eye irritation and no dermal irritancy. From ADMET/TOPKAT prediction, it can be concluded that eutypellazine M (57) and scalusamide C (62) showed better pharmacokinetic and pharmacodynamic properties when compared to gymnastatin Z; however gymnastatin Z is safer showing better toxicity criteria and properties when compared to gymnastatin Z; however showed better pharmacokinetic and pharmacodynamic properties to be suitable for certain semisynthetic treatment to improve its pharmacokinetic and pharmacodynamic properties to be suitable for incorporation in pharmaceutical dosage forms without affecting its activity or safety.

3.3. Chemometric Analysis. Chemometric analysis using principle component analysis (PCA) as an unsupervised pattern recognition technique was done based on the binding energies observed for the compounds with respect to the three tested proteins. Compounds are clustered into four main clusters taking different colors and symbols as revealed in Figure 12 with compounds similar in activity for the three tested proteins gathered in one cluster. Moreover, eutypellazine M (57), scalusamide C (62), and gymnastatin Z (72) are gathered together in a subcluster lying in the left lower quadrant near to main axis of PC2 due to their closeness in activity. In contrast, epi-azonalenalin A (12) and cytochalasin Z17 (20) are outliers that mainly relied upon their completely different values particularly towards SARS-CoV-PLpro. This in turn reflects their ineffectiveness towards the examined enzymes. Chemometric analysis acts as a simple tool that makes it easier to recognize the variation and closeness in activities among the tested chemical entities. The clustering of the different compounds based upon their ΔG towards the three tested proteins is illustrated in Figure 12.

4. Discussion

Molecular modelling has been adopted by many researchers to screen the possibility of using the active constituents derived from natural products to combat COVID-19 infection. Many viral enzymes serve as promising targets for the discovery of new drug leads combating COVID-19 infection. This includes main protease (MPro), papain-like protease (PLpro), 3-chymotrypsin-like protease (3CLpro), angiotensin-converting enzyme 2 (ACE2), and RNA-dependent RNA polymerase (RdRp) [14–16]. The structural protein, the viral S protein, and angiotensin-converting enzyme 2 receptor (host receptor) may particularly act as an important therapeutic target in the virus eradication. S protein allows viral infection through angiotensin-converting enzyme 2 (ACE2) and membrane fusion [50]. Components that affect SARS-CoV-2 serve as a perfect strategy for the discovery of lead drug entities combating COVID-19 infection.

Like MERS and SARS, the genome of SARS CoV-2 possesses two open reading frames which are ORF1a and ORF1ab that perfectly help in the translation of two viral polyproteins pp1a and pp1ab. They are overlapped together and crucial in the replication and transcription of the virus. Consequently, functional polypeptides are obtained from two viral polyproteins via proteolytic processing performed
bioactive constituents derived from Moroccan and Indian medicinal plants such as digitoxigenin, arjunglucoside-I, crocin, carnosol, β-eudesmol, and rosmanol are prospected to be promising SARS CoV-2 M\textsuperscript{pro} inhibitors [51].

Regarding marine-derived compounds, in silico studies revealed the effectiveness of many drug entities versus SARS CoV-2 various enzymes. Callophysis A isolated from red alga Callophycus oppositifolius displayed a potent SARS-CoV-2 3CL\textsuperscript{P} inhibitory potential. This was achieved via the formation of hydrogen bonds, salt bridge, and hydrophobic interactions with the amino acid moieties at the catalytic sites (Cys145 and His41) [53]. Additionally, in silico study performed on marine compounds possessing antiviral potential revealed that pyrrole carboxamide, acyl indole, and compounds having a flavonoid nucleus displayed the highest docking scores while binding to spike glycoprotein, RNA polymerase, and main protease of SARS-CoV-2 active sites. Besides, debromosceptrin and sceptrin revealed complete alignment with the ligand co-crystallized with main protease. Meanwhile, thalassolin (A-B) displayed the best fitting with all the tested proteins [54].

Besides, a study carried out by Ibrahim et al. revealed that bis([1, 3]dioxolo)pyran-5-carboxamide derivatives showed significant activity against SARS-CoV-2 M\textsuperscript{pro} and are ready to be examined for in vitro inhibition against SARS-CoV-2 [55]. Meanwhile, canthin-6-one 9-O-beta-glucopyranoside showed the highest binding affinity and less binding energy with both PL\textsuperscript{pro} and M\textsuperscript{pro}/3CL\textsuperscript{P} proteases and thus it can be used as a potential natural drug against COVID-19 protease [56]. Furthermore, phytochemicals of the plant Boerhavia diffusa such as β-ecdysone, bioquercetin, biorbin, boeravinone J, boerhavisterol, kaempferol, liriodendrin, quercetin, and trans-caftaric acid were docked to SARS-CoV-2 main protease. Biorbin, bioquercetin, and boerhavisterol showed the best binding energies and were found to be favorable for an efficient docking and resultant inhibition of the viral main protease [57].

Herein, molecular modelling of 72 antimicrobial alkaloids derived from fungi performed on the main protease SARS-CoV-2 M\textsuperscript{pro}, 3-chymotrypsin-like protease SARS-CoV-2 3CL\textsuperscript{P}, and papain-like protease SARS-CoV-PL\textsuperscript{pro} showed that gymnastatin Z exhibited the best fitting on SARS-CoV-2 M\textsuperscript{pro}, SARS-CoV-2 3CL\textsuperscript{P}, and SARS-CoV-PL\textsuperscript{pro} followed by scalusamide C and eutypellazine M. The significant fitting of these compounds can be interpreted by the means of formation of several bonds with the amino acid moieties existing in the active pocket as revealed by 2D and 3D binding modes. The evident variation among the compounds with respect to the presence or absence of functional moieties as well as the size of the molecule even the length of the substituted alkyl chains effectively contributes to the activity of the tested metabolites. Increasing the complexity of the structure as in eutypellazine series reduces the activity that is evidenced by the high activity of eutypellazine M compared to eutypellazine A. ADMET/TOPKAT prediction displayed that eutypellazine M and scalusamide C possess better pharmacokinetic and pharmacodynamic properties. However, gymnastatin Z is safer showing better toxicity criteria and higher Rat oral LD\textsubscript{50} as

![Figure 11: ADMET Plot for bioactive compounds, eutypellazine M (57), scalusamide C (62), and gymnastatin Z (72) and the co-crystallized ligands with the examined proteins showing the 95% and 99% confidence limit ellipses corresponding to the blood brain barrier (BBB) and the human intestinal absorption models; eutypellazine M (57), (triangle); scalusamide C (62) (filled square); and gymnastatin Z (72), (star) in ADMET_AlogP98.](image)
well as rat chronic LOAEL in addition to better inhibitory activity on the tested enzymes as revealed from the docking scores. Thus, gymnastatin Z should be subjected to certain treatment to improve its pharmacokinetic and pharmacodynamic properties to be suitable for incorporation in pharmaceutical dosage forms without affecting its activity or safety. Thus, these compounds could serve as promising lead entities with high enzyme inhibitory potential that could inhibit SARS-CoV-2. Further investigations are required to confirm the results of molecular modelling. Thus, computational studies performed on natural occurring compounds could help in searching for new lead entities to solve the crisis of COVID-19 with low cost in comparison to in vitro and in vivo assays. However, that revealed activity should be subjected to further in depth in vitro and in vivo studies followed by preclinical trials to confirm the obtained results.

5. Conclusions

Marine-derived fungal strains constitute a promising source of bioactive alkaloids. Seventy-two alkaloid metabolites showed antimicrobial activities as recently collected from literature. Molecular modelling of these compounds performed after validation of the docking experiments revealed that gymnastatin Z showed the best fitting score on SARS-CoV-2 M\textsuperscript{Pro}, SARS-CoV-PL\textsuperscript{pro}, and SARS-CoV-2 3CL\textsuperscript{pr}. This is followed by scalusamide C and eutypellazine M. ADMET/TOPKAT prediction displayed that eutypellazine M and scalusamide C showed better pharmacokinetic and pharmacodynamic properties. However, gymnastatin Z is safer showing better toxicity criteria; thus, it could be subjected to certain treatment to improve its pharmacokinetic and pharmacodynamic properties to be suitable for incorporation in pharmaceutical dosage forms without affecting its activity or safety. Additionally, they were clustered close to each other as manifested by the PCA analysis. Thus, these compounds could serve as promising enzyme inhibitors for drug discovery of successful pharmaceutical products that could inhibit SARS-CoV-2 and overcome COVID-19 pandemic. However, further investigations via in vitro and in vivo studies are required to be conducted to confirm the results of molecular modelling.

Data Availability

Data are available in the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This publication was supported by the Deanship of Scientific Research at Prince Sattam Bin Abdulaziz University, Alkhairj, Saudi Arabia.

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