processes

MDPI

Article

Characterization of *Streptomyces* Species and Validation of Antimicrobial Activity of Their Metabolites through Molecular Docking

Sobika Bhandari [1,2,†], Bibek Raj Bhattarai [1,†], Ashma Adhikari [1], Babita Aryal [1], Asmita Shrestha [1], Niraj Aryal [3], Uttam Lamichhane [1], Ranjita Thapa [1], Bijaya B. Thapa [1], Ram Pramodh Yadav [1], Karan Khadayat [1], Achyut Adhikari [1], Bishnu P. Regmi [4,*] and Niranjan Parajuli [1,*]

1 Central Department of Chemistry, Tribhuvan University, Kirtipur 44618, Kathmandu, Nepal
2 Department of Chemistry, University of Virginia, Charlottesville, VA 22903, USA
3 Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, WI 53706, USA
4 Department of Chemistry, Florida Agricultural and Mechanical University, Tallahassee, FL 32307, USA
* Correspondence: bishnu.regmi@famu.edu (B.P.R.); niranjan.parajuli@cdc.tu.edu.np (N.P.)
† These authors contributed equally to this work.

Abstract: Finding new antibacterial agents from natural products is urgently necessary to address the growing cases of antibiotic-resistant pathogens. Actinomycetes are regarded as an excellent source of therapeutically important secondary metabolites including antibiotics. However, they have not yet been characterized and explored in great detail for their utility in developing countries such as Nepal. In silico molecular docking in addition to antimicrobial assays have been used to examine the efficacy of chemical scaffolds biosynthesized by actinomycetes. This paper depicts the characterization of actinomycetes based on their morphology, biochemical tests, and partial molecular sequencing. Furthermore, antimicrobial assays and mass spectrometry-based metabolic profiling of isolates were studied. Seventeen actinomycete-like colonies were isolated from ten soil samples, of which three isolates showed significant antimicrobial activities. Those isolates were subsequently identified to be *Streptomyces* species by partial 16S rRNA gene sequencing. The most potent *Streptomyces* species_SB10 has exhibited an MIC and MBC of 1.22 µg/mL and 2.44 µg/mL, respectively, against each *Staphylococcus aureus* and *Shigella sonnei*. The extract of *S.* species_SB10 showed the presence of important metabolites such as albumycin. Ten annotated bioactive metabolites (essramycin, maculosin, brevianamide F, cyclo (L-Phe-L-Ala), cyclo (L-Val-L-Phe), cyclo (L-Leu-L-Pro), cyclo (D-Ala-L-Pro), N6, N6-dimethyladenosine, albumycin, and cyclo (L-Tyr-L-Leu)) were molecularly docked against seven antimicrobial target proteins. Studies on binding energy, docking viability, and protein-ligand molecular interactions showed that those metabolites are responsible for conferring antimicrobial properties. These findings indicate that continuous research on the isolation of the *Streptomyces* species from Nepal could lead to the discovery of novel and therapeutically relevant antimicrobial agents in the future.

Keywords: *Streptomyces*; antimicrobial; molecular docking

Citation: Bhandari, S.; Bhattarai, B.R.; Adhikari, A.; Aryal, B.; Shrestha, A.; Aryal, N.; Lamichhane, U.; Thapa, R.; Thapa, B.B.; Yadav, R.P.; et al. Characterization of *Streptomyces* Species and Validation of Antimicrobial Activity of Their Metabolites through Molecular Docking. *Processes* **2022**, *10*, 2149. https://doi.org/10.3390/pr10102149

Academic Editor: Olivier Sire

Received: 23 August 2022
Accepted: 19 October 2022
Published: 21 October 2022

1. Introduction

Antibiotics have been considered powerful tools to fight against microbial infections and have revolutionized the healthcare system globally. However, the misuse and overuse of antibiotics have accelerated the development of antibiotic resistance. Such resistance is one of the most serious problems confronting healthcare facilities in the twenty-first century [1]. Several bacterial resistance mechanisms, such as the multidrug efflux system [2], the production of β-lactamases [3], and aminoglycoside-modifying enzymes [4] have emerged in the last decades. The microbial world has shown incredible resilience in

prevalent antibiotics, and the development of resistance is virtually impossible to avoid. The relentless development of resistance can render valuable antibiotics useless. This scenario demands an urgent need for the development of new antibiotics that have effective therapeutic activities against infectious diseases [5,6].

Actinomycetes have proven particularly beneficial to the pharmaceutical industry due to their seemingly limitless ability to make secondary metabolites with diverse chemical structures and biological functions. Among actinomycetes, *Streptomyces* is the most prolific genus used in therapeutic applications and pharmaceutical industries [7,8]. Approximately 60% of antibiotics used today are derived from the genus *Streptomyces* [9]. *Streptomyces* are filamentous, Gram-positive, and sporulating bacteria with high GC content in a range of 55–75% [10]. *Streptomyces* species are saprophytic organisms that spend the majority of their life cycles as dormant spores [11]. Though *Streptomyces* are valuable prokaryotes of clinical and medicinal importance, novel antibiotics have not been discovered in the last few decades. This is because of the repeated rediscovery of similar secondary metabolites from bacteria of similar ecological niches [12]. We believe the continuous screening of actinomycetes from different habitats could lead to the discovery of alternative antibiotics.

The process of new drug discovery is an arduous task; however, the use of computer-aided tools in drug discovery is now gaining more popularity and appreciation [13]. To find medications from natural sources, a computational platform has emerged as a key area of research. The computer-aided drug discovery (CADD) approach entails the discovery of potential drug targets, high-throughput screening, optimization of lead compounds, and examination of potential side effects and toxicity [14]. Molecular docking can be used to model the interaction between a small molecule and a target protein, thereby allowing the characterization of the behavior of small molecules at the binding site of target proteins [15].

Nepal, being a country with a wide range of geographies, altitudes, and vegetation along with many unexplored and untouched ecosystems, has a high probability of isolating actinomycetes producing new and potent secondary metabolites. Soil is the habitat for a large number of *Streptomyces* species, and a majority of soils have 10^4 to 10^7 *Streptomyces* colony-forming units per gram of soil, accounting for 1 to 20% or even more of the total viable counts [16]. Nevertheless, Nepalese ecosystems have not been extensively explored in the search for actinomycetes.

In the present paper, an attempt was made to isolate, characterize, and validate the antimicrobial properties of *Streptomyces* species isolated from soil collected from untouched habitats of Nepal covering higher altitudes to lowlands. The focus was on characterizing the *Streptomyces* species based on morphology, biochemical tests, molecular sequencing, and molecular annotation of secondary metabolites in some potent isolates. Finally, those metabolites were used to dock against some target proteins of several bacteria to understand the mechanism of action of these metabolites for the exploration and validation of antimicrobial properties.

2. Materials and Methods

2.1. Collection of Samples and Isolation of Streptomyces

Ten soil samples (SB1 to SB10) were collected from different geographical locations in Nepal covering different altitudes and untouched habitats, as shown in Table 1.

2.2. Isolation of Streptomyces

One gram of soil was taken and suspended in 10 mL of autoclaved water by vortexing. The vegetative cells in the soil sample were killed by heat treatment at 80 °C for 30 min. The suspension was further diluted up to 1000-fold in sterilized water and 100 µL of suspension was spread into individual ISP4 (International Streptomyces Project 4) plates and incubated at 28 °C for 7 to 14 days. The actinomycetes were isolated on the ISP4 medium with the addition of nalidixic acid (20 mg/mL) and cycloheximide (50 mg/mL). The growth of Gram-negative bacteria was inhibited by nalidixic acid, and the growth of the fungus was controlled by cycloheximide. Based on morphological characterization, the colonies were

sub-cultured and the glycerol stocks were prepared for further use. The details of the soil sample collection and isolation of *Streptomyces* species were carried out as described in the literature [17,18].

Table 1. Description of the soil samples collected from untouched habitats in Nepal.

Soil Samples	Location	Habitats	Altitude (m)	Geographical Coordinates
SB1	Halesi, Khotang	Bare land	3100	27.1846° N, 86.5938° E
SB2	Muchchok, Gorkha	Forest	1300	28.1371° N, 84.6584° E
SB3	Shigash, Baitadi	Forest	2800	29.5174° N, 80.5938° E
SB4	Pame, Kaski	Agriculture land	822	28.2256° N, 83.9466° E
SB5	Nagarkot, Bhaktapur	Forest	2175	27.7107° N, 85.5023° E
SB6	Simbhanjyang, Makawanpur	Rhizosphere	2310	27.5921° N, 85.0855° E
SB7	Tatopani, Myagdi	Hot spring	2180	28.4949° N, 83.6194° E
SB8	Swargadawari, Pyuthan	Rhizosphere	2100	28.1214° N, 82.6744° E
SB9	Betini, Okhaldhunga	Forest	1500	27.2866° N, 86.4733° E
SB10	Muktinath, Mustang	Bare land	3710	28.8190° N, 83.8716° E

2.3. Morphological Characterization

Macroscopic and microscopic studies were implemented for the morphological characterization of the isolated colonies. The isolates were preliminarily identified by macroscopic characterization in which aerial and substrate mycelium, color, shape, pigmentation, and appearance of the colonies were observed [19]. The appearance of the colonies was noted based on dryness, roughness, toughness, and elevation. Microscopic identification was done by Gram staining and observing under 100× magnification [20]. Likewise, several biochemical tests including lipase, gelatin, amylase, sulfur test, indole, nitrate reduction, motility, urease, catalase, and MRVP tests were performed (Tables S3 and S4) [21].

2.4. Molecular Characterization

The isolates were cultured in a tryptic soy broth (TSB) medium in an Erlenmeyer flask at 28 °C and 180 rpm for 4–5 days with glass beads. Then, 15 mL of the culture was taken in a falcon tube and the cells were separated by centrifugation at 8000 rpm for 10 min. Genomic DNA was isolated using the phenol-chloroform method [22]. Universal primers 27F: AGAGTTTGATCCTGGCTCAG and 1492R: GGTTACCTTGTTACGACTT were used for the amplification of 16S rDNA. The PCR amplification was performed in 29 cycles in a 50 µL reaction mixture containing 10 µM oligonucleotides, 5× premix having *Taq* polymerase, and 2 µL (100 ng) genomic DNA. A cycle was run at 95 °C initial denaturation, 51.4 °C annealing, and 95 °C extensions. The resulting PCR products were then purified using Monarch® PCR and a DNA Cleanup kit (New England Biolabs Inc., Ipswich, MA, USA). The unidirectional sequencing of 16S rDNA was carried out in Macrogen, Seoul, South Korea. The BLAST search tool was used for the annotation of 16S rDNA partial sequences in the database [23].

2.5. Shake Flask Fermentation

Bacterial isolates were cultured in TSB broth (30 g/L) for 3 to 4 days at 140 rpm, 28 °C in a shaking incubator. After the full growth of the bacteria, its 1 mL suspension was transferred to freshly prepared TSB broth (100 mL) for fermentation and kept for 5 to 7 days (until bacterial growth meets stationary phase) at 28 °C with constant shaking at 140 rpm [24]. The suspension was mixed with an equal volume of ethyl acetate for the extraction of secondary metabolites. Finally, the organic phase was further used for antimicrobial assays.

2.6. Antimicrobial Assays

The antimicrobial activity of 17 *Streptomyces* isolates was initially tested by the primary screening method. The pathogens examined were *Staphylococcus aureus* ATCC 43300, *Escherichia coli* ATCC 2591, *Kleibsella pneumonia* ATCC 700603, *Salmonella typhi* ATCC 14028, *Shigella sonnei* ATCC 25931, and *Acinetobacter baumanii* ATCC 19606. The antimicrobial assays of potent bacterial isolates in perpendicular screenings were performed by using the agar well diffusion method. The assays were carried out in triplicate with 5 µg of *Streptomyces* extract in each well along with 1 mg/mL neomycin and 50% DMSO as a positive and negative control, respectively. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the most potent bacterial isolates were determined using broth microdilution [25].

2.7. Mass Spectrometry

The mass spectrometry of the ethyl acetate extracts of *S*. species_SB1 and *S*. species_SB3 was performed in the Sophisticated Analytical Instrument Facility (SAIF), Lucknow, India using an Agilent 6520 Q-TOF mass spectrometer. The data were acquired using positive electron spray ionization (ESI) mode. The mobile phase consisted of 5 to 80% gradient acetonitrile and 5 mM acetate buffer [25]. For SB10, low resolution (LR)-LCMS was obtained by using an HPLC system (1100 Series, Agilent Technologies, Waldbronn, Germany) coupled with an MS (ABSCIEx 3200 Q Trap, Darmstadt, Germany). Likewise, the extract was also subjected to HR-LC-ESI-MS/MS (Bruker TOF-MS MaXis impact ESI-HR-MS) profiling [18]. The positive mode ESI spectrum was deposited in the Global Natural Products Social (GNPS) Molecular Networking Platform (https://gnps.ucsd.edu) (accessed on 1 October 2022).

2.8. Molecular Annotation

The molecular profiling of the extracts from isolates *S*. species_SB1 and *S*. species_SB3 was executed by the previously described procedure [26]. The raw data of LC-HRMS obtained from extracts of isolates *S*. species_SB1 and *S*. species_SB3 were analyzed using MestreNova 12.0 software (Santiago de Compostela, SPAIN) for peak detection, alignment, and annotation. They were then compared to the database library, literature, dictionary of natural products, and METLIN metabolite searching cloud. The molecular formula for *m/z* and MS/MS fragmentation of isolate *S*. species_SB10 was read using Bruker Compass Data Analysis 4.4 and the molecule annotation was carried out in SIRIUS 4.9.12 through CSI: FingerID user interface by exporting a .mgf file [18].

2.9. Molecular Docking for the Connection with Antimicrobial Assays

2.9.1. Binding Site Prediction

The binding site residues of the target proteins were chosen in this investigation based on previous findings as well as through the co-crystallized ligand in the retrieved protein [27,28]. After discovering binding sites, the coordinates of the binding pocket for each target protein were generated using the define and edit binding site tool of BIOVIA Discovery Studio.

2.9.2. Ligand Preparation

The annotated compounds were downloaded in .sdf format through PubChem [29,30] and translated to .pdb format via Pymol [31,32]. For docking purposes, polar hydrogens and Gasteiger charges were added, and the compounds were saved in PDBQT format using the AutoDock application.

2.9.3. Receptor Preparation

The protein targets, 1JIJ (crystal structure of *S. aureus* TyrRS in complex with SB-239629); 6J33 (crystal structure of ligand-free PulA from *Klebsiella pneumoniae*); 3TTZ (crystal structure of a topoisomerase ATPase inhibitor); 3SRW (*S.aureus* Dihydrofolate Reductase

complexed with novel 7-aryl-2,4-diaminoquinazolines); 4UMW (crystal structure of zinc-transporting PIB-type ATPase in E2.PI state); 3UDI (crystal structure of *Acinetobacter baumannii* PBP1a in complex with penicillin G); and 7KRK (putative FabG from *Acinetobacter baumannii*) were attained from the RCSB PDB database. The proteins were prepared in BIOVIA Discovery Studio by detaching water components and other associated ligands before the docking experiments. The structures were saved in PDBQT format after optimizing with Kollmann charges and adding polar hydrogen in AutoDock tools [33]. A three-dimensional affinity grid box was generated around the binding sites of the target proteins with the size of 40 × 40 × 40 Angstrom and centered on the important residues of all target proteins where potent metabolites would bind.

2.9.4. Molecular Docking and Validation

AutoDock tools version 1.5.6 was used to dock the annotated compounds into the binding site of the target proteins. The outcome is a list of poses that are arranged according to ΔG, the predicted binding energy in kcal/mol. Using the superimposition technique, the root-mean-square deviation (RMSD) values of the first and second docked low-energy protein-ligand complex postures were determined. If the RMSD values are under 2 Å, the validation is said to have been successful. The poses of compounds in target proteins were analyzed using BIOVIA Discovery studio, and the best pose was chosen based on hydrogen bonding, hydrophobic, and π–π interactions with the binding residues.

3. Results

3.1. Morphological Characterization

A total of 17 isolates showing the characteristics of *Streptomyces* were isolated from 10 different soil samples. The detail and morphological features of all the isolates are presented in Supplementary Tables S1 and S2. The aerial and substrate mycelium of *S*. species_SB10 were grayish and white, respectively, along with no diffusible pigments (Figure 1).

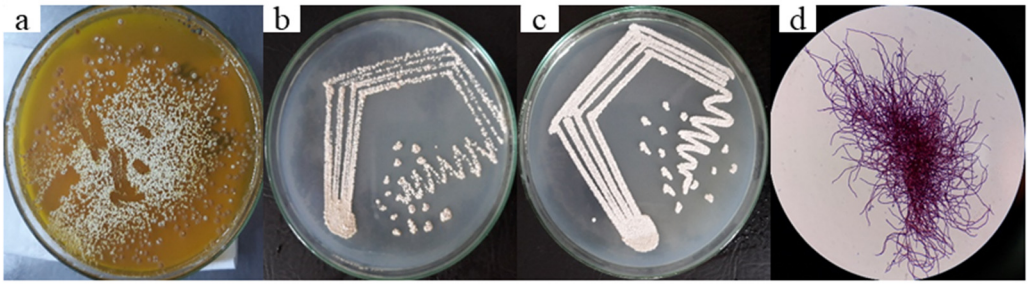


Figure 1. Morphological characterization; (**a**) Isolation plate; (**b**) Substrate mycelium; (**c**) Aerial mycelium; and (**d**) Gram staining of *Streptomyces* species SB10.

3.2. Molecular Characterization of Isolates

Universal primers were used to amplify the 16S rRNA. The 16S rRNA gene sequencing of SB1, SB3, and SB10 showed that the isolates resembled the genus *Streptomyces* on the BLAST sequence search generated from NCBI. A DNA sequence analysis showed that SB1, SB3, and SB10 were closely related to *Streptomyces bungoensis*, *Streptomyces aureus*, and *Streptomyces griseoplanus* with percentage similarities of 84.31, 95.79, and 95.92, respectively. Thus, according to morphological and molecular characterization, it was concluded that isolates from the soil samples SB1, SB3, and SB10 were members of the genus *Streptomyces*.

3.3. Antimicrobial Assays

Out of 17 isolates, *S*. species_SB1, *S*. species_SB3, and *S*. species_SB10 showed potential antimicrobial activities in the primary screening and hence were considered for further studies. The antimicrobial assays performed on the three *Streptomyces* isolates

showed moderate to strong activity against Gram-positive (*S. aureus*) and Gram-negative (*S. typhi*, *K. pneumoniae*, *S. sonnei*, *A. baumannii*, and *E. coli*) bacteria. The zones of inhibition (ZoIs) measured were recorded as shown in Table 2 and Supplementary Figure S1. The antibacterial activity of *S.* species_SB10 was the highest against *S. aureus* and *S. sonnei* with a ZoI of 30 mm for each bacterium. The results showed that the extract of *S.* species_SB10 was more potent than the extracts of *S.* species_SB1 and *S.* species_SB3. Therefore, only the MIC and MBC of the *S.* species_SB10 were determined. The MIC of *S.* species_SB10 against *S. aureus* and *S. sonnei* was found to be 1.22 µg/mL, while the MBC was 2.44 µg/mL (Figure S2). On the other hand, the MIC of neomycin (positive control) against *S. aureus* and *S. sonnei* was 1.56 µg/mL, and the MBC was 12.5 and 6.25 µg/mL, respectively.

Table 2. Zones of inhibition exhibited by *Streptomyces* isolates.

Organisms	Zone of Inhibition (mm)			
	***Streptomyces* Species_SB1**	***Streptomyces* Species_SB3**	***Streptomyces* Species_SB10**	**Neomycin (Control)**
S. aureus	11	19	30	22
E. coli	-	-	18	18
K. pneumoniae	-	10	-	16
S. typhi	-	-	-	15
S. sonnei	10	17	30	23
A. baumannii	10	10	18	20

3.4. Liquid Chromatography–Mass Spectrometry Analysis

Streptomyces species showing good antimicrobial properties (*S.* species_SB1, *S.* species_SB3, and *S.* species_SB10) were further processed for mass analysis in ethyl acetate extracts. The annotated metabolites are shown in Tables 3 and 4. The metabolites were found to contain multiple chemical scaffolds. Extracts of *S.* species_SB1 and *S.* species_SB3 primarily consisted of diketopiperazines (DKPs). The extract of *S.* species_SB10 showed the presence of the valuable metabolite albumycin and aminocoumarin antibiotics. At least 20 different secondary metabolites were identified from these three isolates.

Table 3. Some annotated compounds from the ethyl acetate extracts of *S.* species SB1 and *S.* species SB3.

S.N.	Annotated Compounds	Calculated Mass	Observed Mass	Molecular Formula	Double Bond Equivalence	Absolute Error (ppm)	Retention Time (min)	Source	Reference
1.	Cyclo-(L-Pro-4-OH-L-Leu)	227.13	226.13	$C_{11}H_{18}N_2O_3$	4.0	4.87	5.63	SB1/SB3	[34]
2.	cyclo-(L-Pro-L-Val)	196.12	197.12	$C_{10}H_{16}N_2O_2$	4	3.76	5.39	SB1/SB3	[35]
3.	cyclo (Tyr-Pro)/Maculosin	260.11	261.12	$C_{14}H_{16}N_2O_3$	8	1.17	6.09	SB1/SB3	[36]
4.	Cyclo-(L-Leu-L-Pro)	210.13	211.14	$C_{11}H_{18}N_2O_2$	4	1.92	6.71	SB1/SB3	[37]
5.	(3R,8aS)-3-Methyl-1,2,3,4,6,7,8, 8a-octahydropyrrolo [1,2-a]pyrazine1,4-dione	168.08	169.09	$C_8H_{12}N_2O_2$	4	3.79	3.24	SB3	[38]
6.	Essramycin	268.09	268.103	$C_{14}H_{12}N_4O_2$	11	3.38	4.82	SB3	[39]
7.	2-Piperidinone	99.06	100.07	C_5H_9NO	2	0.47	3.66	SB1/SB3	[40]
8.	cyclo-[Pro-Phe]	244.12	245.12	$C_{14}H_{16}N_2O_2$	8	0.62	7.65	SB1/SB3	[41]
9.	cyclo-(L-Pro-L-Val)	196.12	197.12	$C_{10}H_{16}N_2O_2$	4	3.76	5.42	SB1/SB3	[35]

3.5. Molecular Docking Analysis

The major ten annotated compounds in mass spectrometry were docked through AutoDock tools with the target proteins mentioned earlier. The binding energies of ligands (docked compounds) with proteins are displayed in Table S5. The results of the docking analysis suggested that brevianamide F, essramycin, cyclo (L-Phe-L-Ala), and cyclo (L-Val-L-Phe) were the potential candidates that could inhibit the target proteins of various antimicrobials. These compounds exhibited suitable binding affinities with acceptable binding interactions along with one or more hydrogen-bonding interactions with binding residues of proteins. Most of the interacting residues fell under binding sites

of co-crystallized ligands on target proteins, which are in bold in Table 5. The binding interactions of essramycin and Brevianamide F complexed with target proteins, *S. aureus* TyrRS (PDB ID: 1JIJ*); S. aureus* dihydrofolate reductase (3SRW); and *S. sonnei* Zinc transporting PIB-type ATPase in E2.PI state (4UMW), respectively, are shown in Figure 2 and the other interactions of potent complexes were displayed in supplementary Figures S3–S6.

Table 4. Annotated compounds from the ethyl acetate extract of *S.* species SB10.

S.N.	Annotated Compound	Calculated Mass	Observed Mass	Adduct Type	Molecular Formula	Retention Time (Min)	RDB	Error ppm	Spectral Match (Sirius Score)	Reference
1.	(3S,6S)-3-benzyl-6-isopropylpiperazine-2,5-dione [Cyclo (L-Val-L-Phe)]	246.14	247.14	$[M + H]^+$	$C_{14}H_{18}N_2O_2$	16.0	7.0	−1.1	32.59%	[42]
2.	Nonactic acid-trihomononactic acid dilactone	410.27	411.27	$[M + H]^+$	$C_{23}H_{38}O_6$	35.1	5.0	3.1	50.26%	[43]
3.	Albaflavenol	220.18	221.18	$[M + H]^+$	$C_{15}H_{24}O$	16.5	4.0	−2.4	56.10%	[44]
4.	Succinilene D	338.25	339.25	$[M + H]^+$	$C_{20}H_{34}O_4$	33.7	4.0	4.1	45.32%	[45]
5.	Benzyl acetate	150.07	151.07	$[M + H]^+$	$C_9H_{10}O_2$	13.2	5.0	−1.2	27.38%	[46]
6.	(6S,3S)-6-benzyl-3-methyl-2,5-diketopiperazine [Cyclo(L-Phe-L-Ala)]	218.11	219.11	$[M + H]^+$	$C_{12}H_{14}N_2O_2$	12.7	7.0	−1.5	66.22%	[47]
7.	P-hydroxyphenylacetaldoxime	151.06	152.06	$[M + H]^+$	$C_8H_9NO_2$	10.1	5.0	−1.0	46.36%	[48]
8.	N6,N6-dimethyladenosine	295.23	296.23	$[M + H]^+$	$C_{12}H_{17}N_5O_4$	9.3	7.0	−1.2	75.56%	[49]
9.	Cyclo(leucylprolyl)	210.14	211.14	$[M + H]^+$	$C_{11}H_{18}N_2O_2$	13.0	4.0	−3.3	49.77%	[37]
10	Albumycin	190.07	191.07	$[M + H]^+$	$C_{10}H_{10}N_2O_2$	8.8	7.0	4.1	18.59%	[50]
11.	Cyclo (L-Tyr-L-Leu)	276.15	277.15	$[M + H]^+$	$C_{15}H_{20}N_2O_3$	12.8	7.0	−1.9	69.25%	[51]
12.	Brevianamide F[Cyclo (L-Trp-L-Leu)]	283.13	284.13	$[M + H]^+$	$C_{16}H_{17}N_3O_2$	15.6	10.0	−1.2	81.80%	[52]
13.	Maculosin[cyclo (Tyr-Pro)]	260.11	261.12	$[M + H]^+$	$C_{14}H_{16}N_2O_3$	14..4	8.0	−2.5	-	[40]
14.	Cyclo-(L-Pro-4-OH-L-Leu)	226.13	227.13	$[M + H]^+$	$C_{11}H_{18}N_2O_3$	5.6	4.0	4.87	-	[34]
15.	cyclo-(L-Pro-L-Val)	196.12	197.12	$[M + H]^+$	$C_{10}H_{16}N_2O_2$	5.3	4.0	3.76	-	[35]
16.	Cyclo-(L-Leu-L-Pro)	210.13	211.14	$[M + H]^+$	$C_{11}H_{18}N_2O_2$	6.0	4.0	1.92	-	[37]
17.	cyclo-[Pro-Phe]	244.12	245.12	$[M + H]^+$	$C_{14}H_{16}N_2O_2$	7.6	8.0	0.6	-	[41]

Note: Metabolites 13–17 are common DKPs detected in *S.* species SB1, SB3, and SB10.

Table 5. The binding energies and interacting residues (important residues are represented in bold) of target proteins with potent compounds.

Target Proteins (PDB ID)	Binding Energy (kcal/mol)				Interacting Residues			
	Brevianamide F	Essramycin	Cyclo(L-Phe-L-Ala)	Cyclo (L-Val-L-Phe)	Brevianamide F	Essramycin	Cyclo(L-Phe-L-Ala)	Cyclo (L-Val-L-Phe)
S. aureus TyrRS (1JIJ)	−9.0	−9.1	−8.0	−7.8	**Gly 38** **Asp 40** **Tyr 170** **Gly 193** Gln 196 **His 50** Pro 53	**Asp 40** **Tyr 170** **Gln 174** Gln 196 Ala 39 **His 50** Leu 70 **Asp 195**	**Gly 38** **Gln 174** Leu 70	**Gly 38** **Asp 80** **Tyr 170,** **Gln 174** Gln 196 Lys 84
K. pneumonia PulA (6J33)	−8.7	−7.6	−7.3	−7.0	His 607 Leu 678 Asp 560 Tyr 892	Asp 834 Glu 706 Asn 835 Arg 675 Tyr 559 Trp 557 Trp 708 Pro 745	Glu 706 His 833 Arg 675 Tyr 559 Cys 643	Glu 706 His 833 Arg 675 Asp 834 Tyr 559 Cys 643 Trp 708 Leu 678
S. aureus topoisomerase ATPase inhibitor (3TTZ)	−8.7	−8.5	−6.2	−6.3	**Asp 81** Ser 55 Glu 58 **Ile 86** **Ile 51** **Ile 175** Arg 84 Pro 87 Leu 103	**Asp 81** Ser 55 Gly 85 Asn 54 **Ile 86** **Pro 87** **Ile 51** **Ile 175** Leu 103	**Asp 81** Ser 55 Glu 58	Gln 66 His143 Lys 170
S. aureus dihydrofolate reductase (3SRW)	−9.1	−8.7	−7.5	−8.0	**Leu 21** Ile 15 Thr 47 Lys 46	Gly 95 Thr 47 Lys 46 Leu63	**Leu 21** Phe 93 Gly 95	Ala 8 Ile 15 Thr 47 Phe 93 **Leu 21** **Val 32**

Table 5. *Cont.*

Target Proteins (PDB ID)	Binding Energy (kcal/mol)				Interacting Residues			
	Brevianamide F	Essramycin	Cyclo(L-Phe-L-Ala)	Cyclo (L-Val-L-Phe)	Brevianamide F	Essramycin	Cyclo(L-Phe-L-Ala)	Cyclo (L-Val-L-Phe)
*S. sonnei*Zinc transporting p-type ATPase (4UMW)	−8.1	−7.0	−7.3	−5.5	Arg 345 Glu 184 **Phe 210** Leu211 Pro 166 Glu214 Val397	Arg 345 Leu 396 Ile 347 Pro 401 Val 397 Glu 184	Arg 345 Glu 184 **Phe 210** Ile 167 Pro 166 Glu 214	Gly 694 **Lys 693** Leu 69 Tyr 354 Phe 350 Leu 701 Val 361 Leu 697
A. baumannii PBP1a (3UDI)	−7.5	−7.7	−6.2	−6.6	**Ser 434** Gly 708, **Tyr 707** **Thr 672**	**Ser 434** Gly 708 **Tyr 707**	**Ser 434** **Thr 672** Leu 486 **Asn 489**	**Ser 434** Thr 672 Leu 48 **Asn 489** **Tyr 707**
A. baumannii FabG (7KRK)	−7.1	−7.4	−6.6	−6.5	Ile 19 Asn 88 Gly 90 Ala 89 Gly18 Ser 15 Leu 39 Val 111	Asp 38 Gly 90 Ala 89 Leu 39 Val 111 Val 62	Gly 12 Ile 19 Gly 20 Asn 88 Gly 90 Gly 18 Ala 89	Ile 19 Gly 20 Asn 88 Gly 90 Ala 89

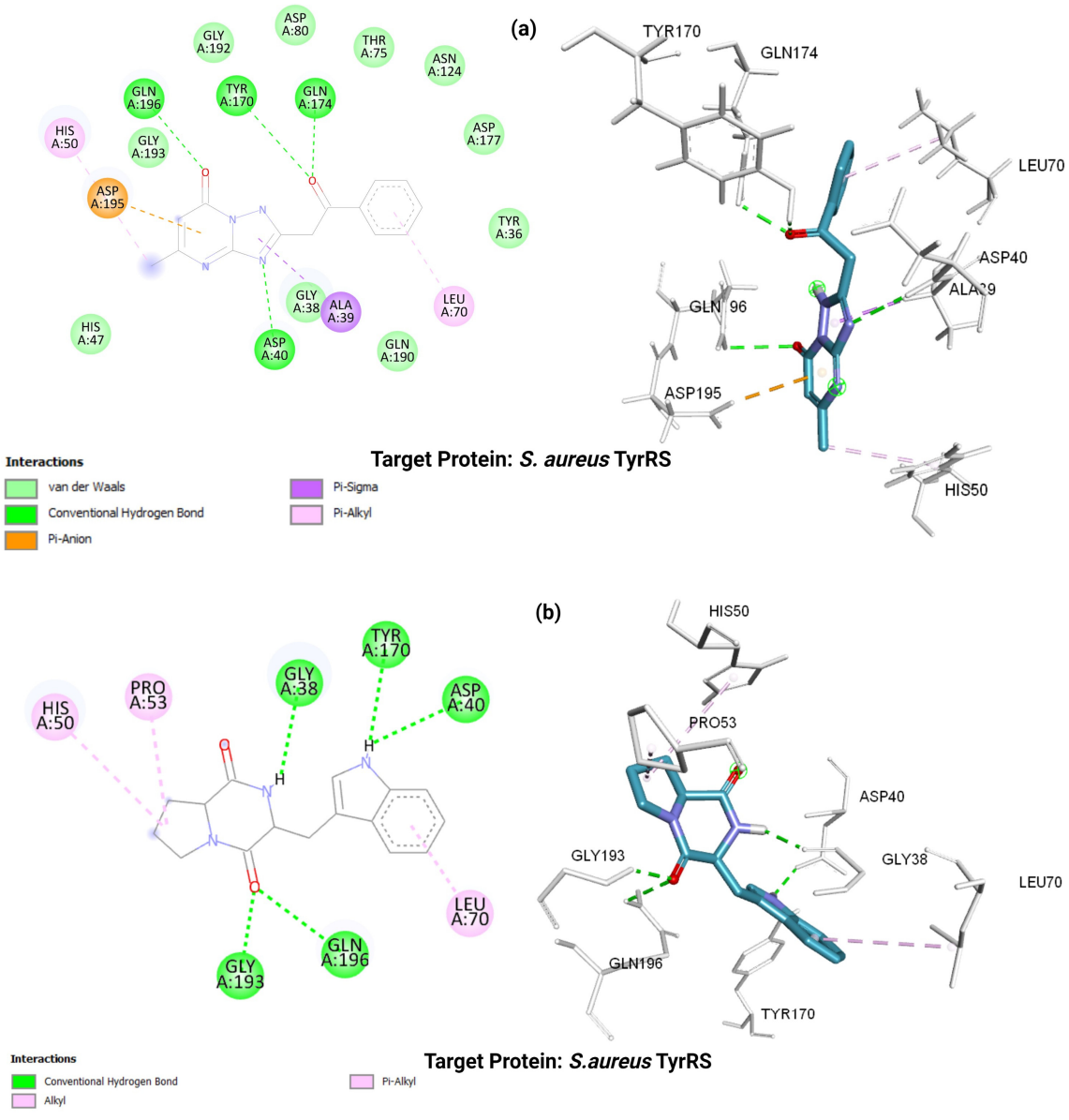
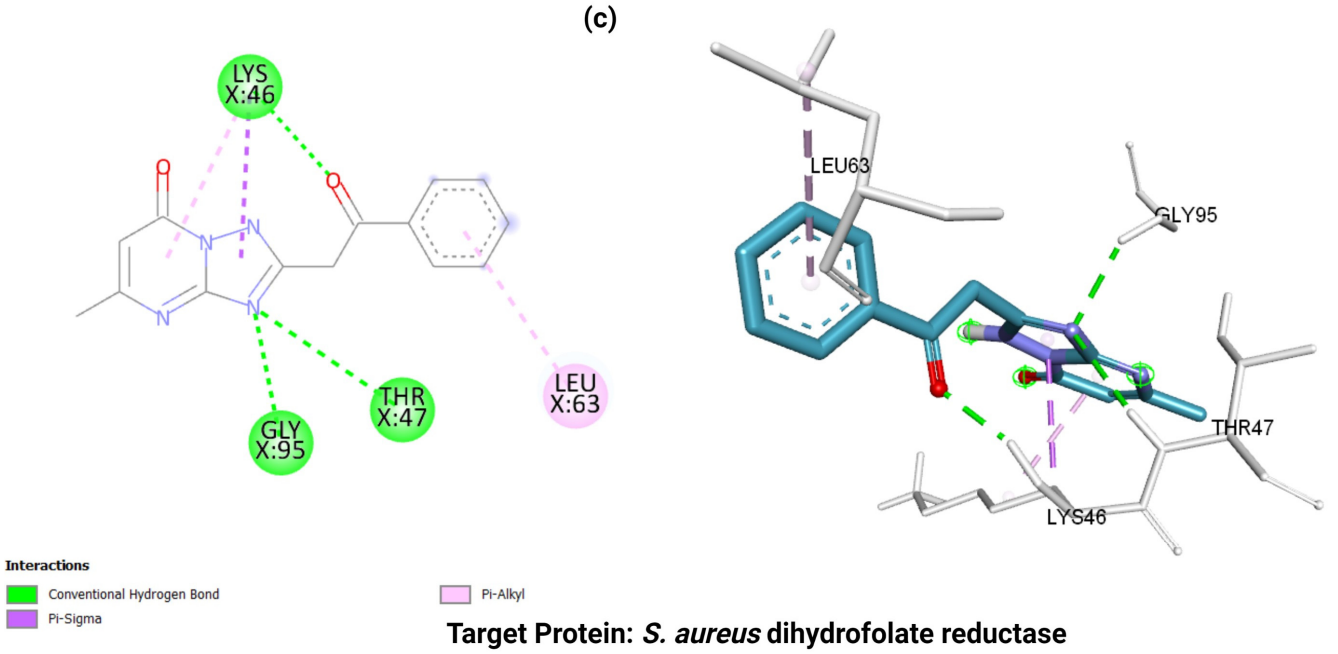


Figure 2. *Cont.*



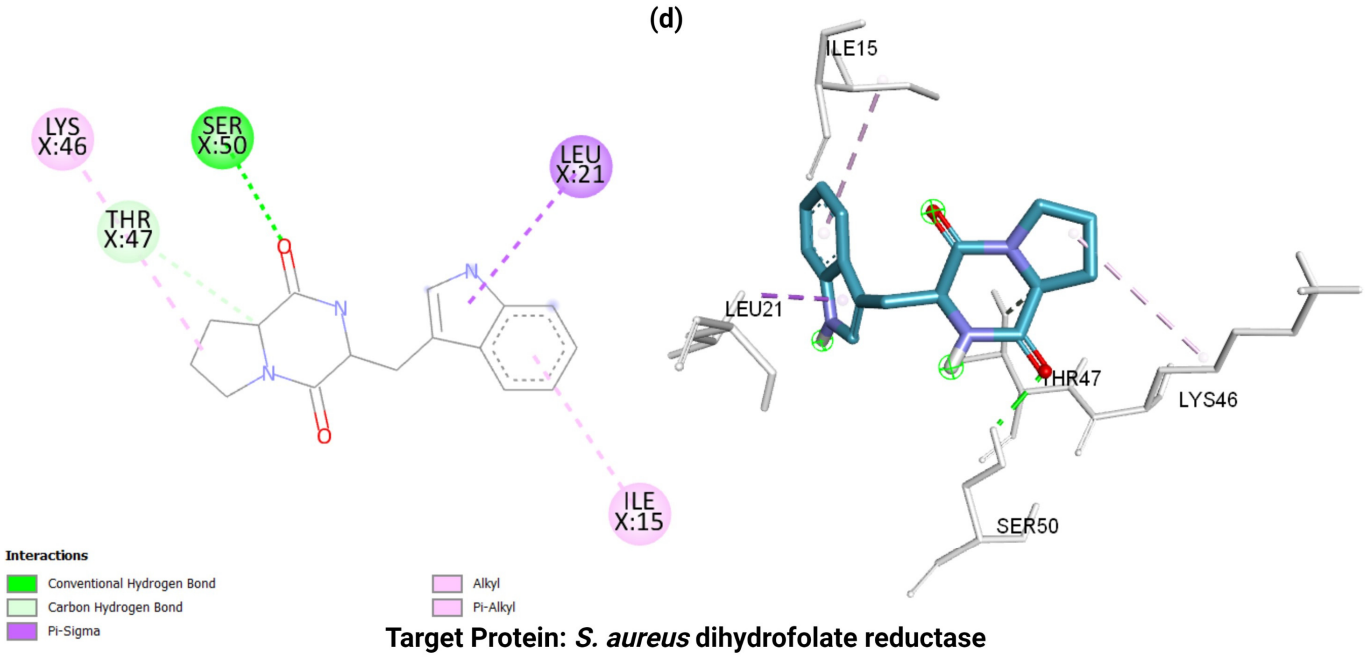


Figure 2. *Cont.*

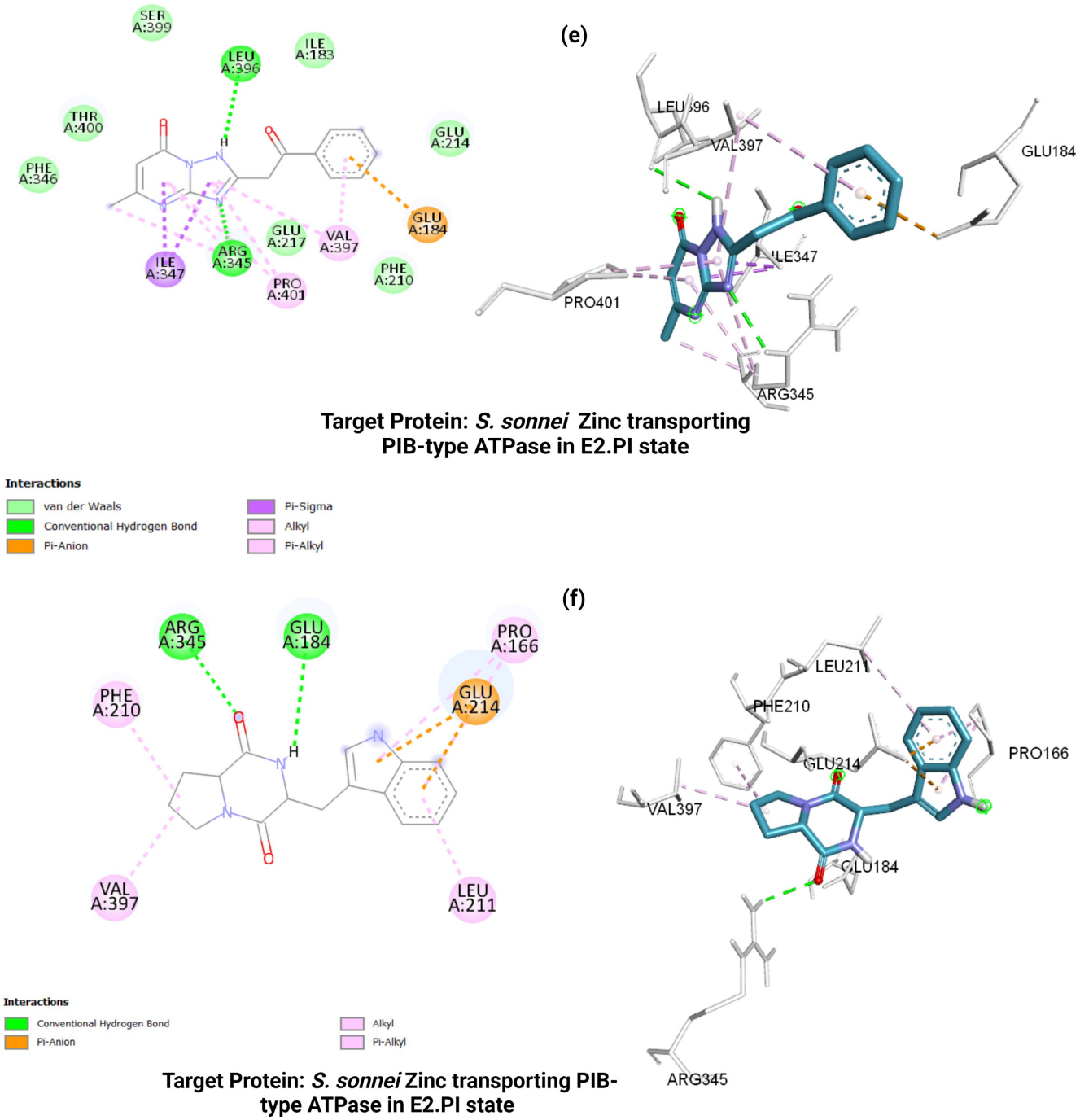


Figure 2. (**a**,**b**) represent binding interactions of essramycin and brevianamide F complexes with target protein *S. aureus* TyrRS, respectively; (**c**,**d**) represent binding interactions of essramycin and brevianamide F complexes with the target protein *S. aureus* dihydrofolate reductase; (**e**,**f**) represent binding interactions of essramycin and brevianamide F complexes with target protein *S. sonnei* Zinc-transporting PIB-type ATPase in E2.PI state.

4. Discussion

Microbial species are the source of secondary metabolites with undeniable biological activities. Most importantly, actinomycetes are given particular attention for their role in the production of various bioactive secondary metabolites. *Streptomyces* species are continually used to discover novel compounds with various biological functions [53]. Antibiotics are the primary and most crucial output of *Streptomyces* species [54]. So, in this work, we

have investigated the antibiotic-producing *Streptomyces* species from untouched habitats in Nepal.

Despite the development of antibiotics and the success of their use, infectious diseases continue to be the second biggest cause of death globally. Bacterial infections account for over 17 million fatalities each year, primarily affecting the elderly and young [54]. In addition to this, multi-drug resistance (MDR) has posed a serious issue in treating infectious diseases. This situation highlights the urgent need to investigate new antimicrobial agents to tackle MDR. The main contender for antibiotic discovery is still soil microorganisms [55]. Hence, this study is focused on the isolation and characterization of *Streptomyces* species and the validation of their antimicrobial activities using the metabolites present in their extracts through a computational approach.

We have isolated 17 different strains of actinomycetes from varying locations. Among these, three *Streptomyces* species, namely, *S*. species_SB1, *S*. species_SB3, and *S*. species_SB10 were determined to possess potential antibacterial properties. *S*. species_SB10 showed an interesting antibacterial effect against *S. aureus* and *S. sonnei* with ZoIs even higher than neomycin (1 mg/mL). This isolate showed an MIC and MBC of 1.22 µg/mL and 2.44 µg/mL, respectively, against *S. aureus* and *S. sonnei*. The potential of *S*. species_SB10 as well as *S*. species_SB1 and *S*. species_SB3 to inhibit the pathogens establishes them as effective candidates for antibiotic production that can combat MDR.

These three potent *Streptomyces* strains were found to contain DKPs such as maculosin, cyclo (L-Phe-L-Ala), cyclo (L-Val-L-Phe), cyclo (L-Leu-L-Pro), cyclo (D-Ala-L-Pro), cyclo (L-Tyr-L-Leu), and brevianamide F. DKPs are known for their rich source of new biologically active compounds. They show a wide spectrum of biological activities, suggesting them as potential therapeutic candidates [56]. They are known to function as antibacterial [57], antiviral [58], antifungal [59], anti-hyperglycemic [60], and antitumor agents [61]. These 2,5 DKPs are also involved as enzyme modulators and biochemical mediators [62]. The availability of donor and acceptor atoms for hydrogen bonding, rigid conformation, and resistance towards proteolysis favor interaction with biological targets [62]. Likewise, Brevianamide F is used as a medicament for cardiovascular dysfunction and cognitive enhancement [62]. The presence of these chemical scaffolds might be the reason for the effective antibacterial activity shown by the extracts. Furthermore, the isolation and characterization of these compounds are needed for other bioactivity-related studies. Moreover, to obtain the full potential of isolated strains, different media cultivation might be required in the future.

Albumycin was previously noted to have antibacterial activities [50]. Similarly, aminocoumarins were also described to show potential antibacterial effects against *Staphylococci*, including a methicillin-resistant *Staphylococcus aureu*s strain [63]. Reports show that 3-aminocoumarin has been used for the synthesis of metal complexes possessing antimicrobial and antioxidant activities [64]. The presence of these chemical scaffolds increases the likelihood of *S*. species_SB1, SB3, and SB10 being employed as therapeutic drugs, particularly as antimicrobial agents. For further validation of antimicrobial activity, molecular docking was performed.

Molecular docking helps minimize the binding energy by optimizing the conformation of both the receptor and ligand and the orientation between the ligand and receptor. Although activity assays are still required to verify the activities of the ligands, molecular docking is a theoretical and reliable approach to anticipating the interactions between the receptor and ligand [65]. To find possible inhibitors for target proteins, we employed molecular docking analysis to predict binding sites and the potential activity of the top-scoring compounds [66]. The results were evaluated according to the docking poses and the protein-ligand interactions [65]. Furthermore, the lower the binding energy, the higher the stability of the complex [67]. The present study suggests brevianamide F, essramycin, cyclo (L-Phe-L-Ala), and cyclo (L-Val-L-Phe), as potent inhibitors for target proteins with significant binding energy and appropriate interactions.

The compounds brevianamide F and essramycin have comparatively lower binding energies with the target proteins, and most of the interacted residues fall into binding sites on comparison with the interacted residues of co-crystallized ligands of retrieved protein from RCSB and also with the important residues of target proteins, according to the literature [68,69]. The orientation of ligands in the target proteins within the binding site was analyzed through hydrogen-bonding, hydrophobic, and π–π interactions. According to earlier in silico and in vitro studies, natural compounds such as acacetin and chrysin can prevent the growth of *S. aureus* by inhibiting tyrosyl-tRNA synthetases (TyrRSs) [70]. In addition, through the molecular docking method too, ciprofloxacin analogs were identified as potential candidates against *S. aureus* DNA gyrase [66]. Furthermore, a molecular docking study revealed acetylated abietane quinone as a suitable inhibitor of *S. aureus* clumping factor A (ClfA) with good binding interactions and binding energy [71]. Hence, molecular docking methods can be taken into account to corroborate the potent metabolites.

The secondary metabolites from *Streptomyces* are significant products that can be utilized to combat various MDR pathogens. The production and commercial viability of these secondary metabolites are not yet fully established, but shortly, their application may accelerate, specifically in the manufacturing of antibiotics. It has been estimated that only 10% of discovered antibiotics are from screened bacterial strains and only 1% from all microbes [72]. Actinomycetes found in terrestrial soil comprise around two-thirds of all natural antibiotics. According to recent estimates, to find the next new antibiotics class, 107 actinomycetes strains would need to be screened [73]. Hence, for the utilization of the soil actinomycetes for antibiotics production, the pace of screening and validation should be intensified. Considering this fact, the use of computational techniques may be extended into validating newer antibiotic-potent products showing a broad spectrum of activity.

5. Conclusions

The present study depicts that based on the variations of altitude and soil types and their contents, there is a huge probability of obtaining a varying diversity of antimicrobial-producing *Streptomyces*. In this study, a total of 17 distinct strains were isolated based on the morphological characteristics of 10 different soil samples collected from different habitats. Three samples were selected for further study based on the antimicrobial screening test. The three *Streptomyces* species SB1, SB3, and SB10 exhibited significant antibacterial activity against *S. aureus*, *S. sonnei*, and *A. baumanii*. At least 24 different secondary metabolites were identified from these three isolates, and these metabolites included different chemical scaffolds such as DKPs and aminocoumarin antibiotics. These metabolites were further subjected to docking to validate their antibacterial properties. Docking positively signified brevianamide F, essramycin, cyclo(L-Phe-L-Ala), and cyclo(L-Val-L-Phe) as potent inhibitors for target proteins, thereby showing the potential of isolated *Streptomyces* species for use in the production of antibiotics.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr10102149/s1, Table S1: Soil profile, total isolated actinomycetes and characterization method of 10 samples; Table S2: Culture characteristic and Gram staining of samples SB1–SB10; Table S3: Biochemical test of microbial strains; Table S4: Cultural and physiological characteristics of microbial strains; Table S5: The binding energies of target proteins with potent compounds; Figure S1: Zone of inhibition exhibited by SB1, SB3, and SB10 against *S. aureus* and *S. sonnei*; Figure S2: MIC and MBC of the extract SB10 against *S. sonnei* and *S. aureus*; Figure S3: (A–D) represents 2D structures of essramycin with target proteins: PulA from *Klebsiella pneumonia* (6J33), Topoisomerase ATPase inhibitor (3TTZ), *A. baumannii* PBP1a (3UDI), and Putative FabG from *A. baumannii* (7KRK), respectively; Figure S4: (A–D) represent 2D structures of brevianamide F with target proteins: PulA from *Klebsiella pneumonia* (6J33), Topoisomerase ATPase inhibitor (3TTZ), *A. baumannii* PBP1a (3UDI), and Putative FabG from *A. baumannii* (7KRK), respectively; Figure S5: (A–G) represent 2D structures of cyclo(L-Val-L-Phe) with target proteins: *S. aureus* TyrRS (1JIJ), PulA from *Klebsiella pneumonia* (6J33), Topoisomerase ATPase inhibitor (3TTZ), *S.aureus* Dihydrofolate Reductase (3SRW), Zinc transporting PIB-type ATPase in E2.PI state (4UMW), *A. baumannii* PBP1a

(3UDI), and Putative FabG from *A. baumannii* (7KRK), respectively; Figure S6: (A–E) represent 2D structures of cyclo(L-Phe-L-Ala) with target proteins: PulA from Klebsiella pneumonia (6J33), Topoisomerase ATPase inhibitor (3TTZ), *S.aureus* Dihydrofolate Reductase (3SRW), *A. baumannii* PBP1a (3UDI), and Putative FabG from *A. baumannii* (7KRK), respectively.

Author Contributions: Conceptualization, N.P., B.R.B. and S.B.; methodology, B.R.B., S.B. and K.K.; validation, B.R.B., U.L. and A.A. (Ashma Adhikari); formal analysis, B.R.B., B.A., A.S., N.A. and R.T.; investigation, S.B., B.R.B., K.K. and U.L.; resources, N.P. and B.R.B.; data curation, N.A. and B.R.B.; writing—original draft preparation, S.B. and B.R.B.; writing—review and editing, N.P., N.A., B.P.R., A.A. (Achyut Adhikari), R.P.Y. and B.B.T.; visualization, B.R.B. and B.P.R.; supervision, N.P.; project administration, N.P.; funding acquisition, N.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the University Grants Commission, Nepal under grant number CRG-75/76-S&T-1 to Niranjan Parajuli.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: Authors would like to acknowledge Ishwor Pathak, Shreesti Shrestha, Ganesh BK, and Sajan Shakya for their help. We are very thankful to Gross lab, University of Tübingen, Germany, and SAIF, India for the acquisition of MS data.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Uddin, T.M.; Chakraborty, A.J.; Khusro, A.; Zidan, B.R.M.; Mitra, S.; Emran, T.B.; Dhama, K.; Ripon, M.K.H.; Gajdács, M.; Sahibzada, M.U.K.; et al. Antibiotic Resistance in Microbes: History, Mechanisms, Therapeutic Strategies and Future Prospects. *J. Infect. Public Health* **2021**, *14*, 1750–1766. [CrossRef]
2. Baucheron, S.; Monchaux, I.; Le Hello, S.; Weill, F.-X.; Cloeckaert, A. Lack of Efflux Mediated Quinolone Resistance in Salmonella Enterica Serovars Typhi and Paratyphi A. *Front. Microbiol.* **2014**, *5*, 12. [CrossRef]
3. Chuma, T.; Miyasako, D.; Hesham, D.; Takayama, T.; Nakamoto, Y.; Shahada, F.; Akiba, M.; Okamoto, K. Chronological Change of Resistance to β-Lactams in Salmonella Enterica Serovar Infantis Isolated from Broilers in Japan. *Front. Microbiol.* **2013**, *4*, 113. [CrossRef] [PubMed]
4. Frontiers | Prospects for Circumventing Aminoglycoside Kinase Mediated Antibiotic Resistance. Available online: https://www.frontiersin.org/articles/10.3389/fcimb.2013.00022/full (accessed on 5 August 2022).
5. Bassetti, M.; Vena, A.; Castaldo, N.; Righi, E.; Peghin, M. New Antibiotics for Ventilator-Associated Pneumonia. *Curr. Opin. Infect. Dis.* **2018**, *31*, 177–186. [CrossRef] [PubMed]
6. Terreni, M.; Taccani, M.; Pregnolato, M. New Antibiotics for Multidrug-Resistant Bacterial Strains: Latest Research Developments and Future Perspectives. *Molecules* **2021**, *26*, 2671. [CrossRef]
7. Al-shaibani, M.M.; Radin Mohamed, R.M.S.; Sidik, N.M.; Enshasy, H.A.E.; Al-Gheethi, A.; Noman, E.; Al-Mekhlafi, N.A.; Zin, N.M. Biodiversity of Secondary Metabolites Compounds Isolated from Phylum Actinobacteria and Its Therapeutic Applications. *Molecules* **2021**, *26*, 4504. [CrossRef] [PubMed]
8. Rajivgandhi, G.N.; Vimala, R.T.V.; Ramachandran, G.; Kanisha, C.C.; Manoharan, N.; Li, W.-J. An Overview on Natural Product from Endophytic Actinomycetes. In *Natural Products from Actinomycetes: Diversity, Ecology and Drug Discovery*; Rai, R.V., Bai, J.A., Eds.; Springer: Singapore, 2022; pp. 151–165. ISBN 9789811661327.
9. Arefa, N.; Sarker, A.K.; Rahman, M.A. Resistance-guided Isolation and Characterization of Antibiotic-producing Bacteria from River Sediments. *BMC Microbiol.* **2021**, *21*, 116. [CrossRef]
10. Connell, N.D. Expression Systems for Use in Actinomycetes and Related Organisms. *Curr. Opin. Biotechnol.* **2001**, *12*, 446–449. [CrossRef]
11. Vikineswary, S.; Nadaraj, P.; Wong, W.H.; Balabaskaran, S. Actinomycetes from a Tropical Mangrove Ecosystem- Antifungal Activity of Selected Strains. *Asia-Pac. J. Mol. Biol. Biotechnol.* **1997**, *5*, 81–86.
12. Belknap, K.C.; Park, C.J.; Barth, B.M.; Andam, C.P. Genome Mining of Biosynthetic and Chemotherapeutic Gene Clusters in *Streptomyces* Bacteria. *Sci. Rep.* **2020**, *10*, 2003. [CrossRef]
13. Chaudhary, K.K.; Mishra, N. A Review on Molecular Docking: Novel Tool for Drug Discovery. *Databases* **2016**, *3*, 1029.
14. Segall, M.D.; Barber, C. Addressing Toxicity Risk When Designing and Selecting Compounds in Early Drug Discovery. *Drug Discov. Today* **2014**, *19*, 688–693. [CrossRef]
15. Pagadala, N.S.; Syed, K.; Tuszynski, J. Software for Molecular Docking: A Review. *Biophys. Rev.* **2017**, *9*, 91–102. [CrossRef]
16. Kurtböke, İ. *Bacteriophages*; BoD—Books on Demand; InTech: Rijeka, Croatia, 2012; ISBN 978-953-51-0272-4.

17. Khadayat, K.; Sherpa, D.D.; Malla, K.P.; Shrestha, S.; Rana, N.; Marasini, B.P.; Khanal, S.; Rayamajhee, B.; Bhattarai, B.R.; Parajuli, N. Molecular Identification and Antimicrobial Potential of *Streptomyces* Species from Nepalese Soil. *Int. J. Microbiol.* **2020**, *2020*, 8817467. [CrossRef]
18. Bhattarai, B.R.; Khadayat, K.; Aryal, N.; Aryal, B.; Lamichhane, U.; Bhattarai, K.; Rana, N.; Regmi, B.P.; Adhikari, A.; Thapa, S.; et al. Untargeted Metabolomics of *Streptomyces* Species Isolated from Soils of Nepal. *Processes* **2022**, *10*, 1173. [CrossRef]
19. *Bergey's Manual® of Systematic Bacteriology*; Goodfellow, M.; Kämpfer, P.; Busse, H.-J.; Trujillo, M.E.; Suzuki, K.; Ludwig, W.; Whitman, W.B. (Eds.) Springer: New York, NY, USA, 2012; ISBN 978-0-387-95043-3.
20. Sitachitta, N.; Gadepalli, M.; Davidson, B.S. New α-Pyrone-Containing Metabolites from a Marine-Derived Actinomycete. *Tetrahedron* **1996**, *52*, 8073–8080. [CrossRef]
21. Maheshwari, D.K. *Practical Microbiology*; S. Chand Publishing: New Delhi, India, 2002; ISBN 978-81-219-2153-4.
22. Nybo, S.E.; Shepherd, M.D.; Bosserman, M.A.; Rohr, J. Genetic Manipulation of *Streptomyces* Species. *Curr. Protoc. Microbiol.* **2010**, *19*, 10E.3.1–10E.3.26. [CrossRef]
23. Tamura, K.; Peterson, D.; Peterson, N.; Stecher, G.; Nei, M.; Kumar, S. MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Mol. Biol. Evol.* **2011**, *28*, 2731–2739. [CrossRef]
24. Klausen, C.; Nicolaisen, M.H.; Strobel, B.W.; Warnecke, F.; Nielsen, J.L.; Jørgensen, N.O.G. Abundance of Actinobacteria and Production of Geosmin and 2-Methylisoborneol in Danish Streams and Fish Ponds. *FEMS Microbiol. Ecol.* **2005**, *52*, 265–278. [CrossRef]
25. Aryal, B.; Adhikari, B.; Aryal, N.; Bhattarai, B.R.; Khadayat, K.; Parajuli, N. LC-HRMS Profiling and Antidiabetic, Antioxidant, and Antibacterial Activities of *Acacia Catechu* (L.f.) Willd. *BioMed Res. Int.* **2021**, *2021*, e7588711. [CrossRef]
26. Aryal, B.; Niraula, P.; Khadayat, K.; Adhikari, B.; Khatri Chhetri, D.; Sapkota, B.K.; Bhattarai, B.R.; Aryal, N.; Parajuli, N. Antidiabetic, Antimicrobial, and Molecular Profiling of Selected Medicinal Plants. *Evid.-Based Complement. Altern. Med.* **2021**, *2021*, e5510099. [CrossRef]
27. Egbert, M.; Porter, K.A.; Ghani, U.; Kotelnikov, S.; Nguyen, T.; Ashizawa, R.; Kozakov, D.; Vajda, S. Conservation of Binding Properties in Protein Models. *Comput. Struct. Biotechnol. J.* **2021**, *19*, 2549–2566. [CrossRef]
28. Belkin, S.; Kundrotas, P.J.; Vakser, I.A. Inhibition of Protein Interactions: Co-Crystalized Protein-Protein Interfaces Are Nearly as Good as Holo Proteins in Rigid-Body Ligand Docking. *J. Comput. Aided Mol. Des.* **2018**, *32*, 769–779. [CrossRef]
29. PubChem. Available online: https://pubchem.ncbi.nlm.nih.gov/ (accessed on 23 July 2022).
30. Kim, S. Exploring Chemical Information in PubChem. *Curr. Protoc.* **2021**, *1*, e217. [CrossRef]
31. PyMOL | Pymol.Org. Available online: https://pymol.org/2/ (accessed on 14 August 2022).
32. Yuan, S.; Chan, H.C.S.; Hu, Z. Using PyMOL as a Platform for Computational Drug Design. *WIREs Comput. Mol. Sci.* **2017**, *7*, e1298. [CrossRef]
33. Morris, G.M.; Huey, R.; Lindstrom, W.; Sanner, M.F.; Belew, R.K.; Goodsell, D.S.; Olson, A.J. AutoDock4 and AutoDockTools4: Automated Docking with Selective Receptor Flexibility. *J. Comput. Chem.* **2009**, *30*, 2785–2791. [CrossRef]
34. Li, B.; Chen, G.; Bai, J.; Jing, Y.-K.; Pei, Y.-H. A Bisamide and Four Diketopiperazines from a Marine-Derived *Streptomyces* Sp. *J. Asian Nat. Prod. Res.* **2011**, *13*, 1146–1150. [CrossRef]
35. Fang, Q.; Maglangit, F.; Wu, L.; Ebel, R.; Kyeremeh, K.; Andersen, J.H.; Annang, F.; Pérez-Moreno, G.; Reyes, F.; Deng, H. Signalling and Bioactive Metabolites from *Streptomyces* Sp. RK44. *Molecules* **2020**, *25*, 460. [CrossRef]
36. Sunaryanto, R.; Marwoto, B.; Irawadi, T.T.; Mas'ud, Z.A.; Hartoto, L. Antibiotic Compound from Marine Actinomycetes (*Streptomyces* Sp. A11): Isolation and Structure Elucidaton. *Indones. J. Chem.* **2010**, *10*, 219–225. [CrossRef]
37. Alshaibani, M.M.; MohamadZin, N.; Jalil, J.; Sidik, N.M.; Ahmad, S.J.; Kamal, N.; Edrada-Ebel, R. Isolation, Purification, and Characterization of Five Active Diketopiperazine Derivatives from Endophytic *Streptomyces* SUK 25 with Antimicrobial and Cytotoxic Activities. *J. Microbiol. Biotechnol.* **2017**, *27*, 1249–1256. [CrossRef]
38. Tan, L.T.-H.; Chan, K.-G.; Pusparajah, P.; Yin, W.-F.; Khan, T.M.; Lee, L.-H.; Goh, B.-H. Mangrove Derived *Streptomyces* Sp. MUM265 as a Potential Source of Antioxidant and Anticolon-Cancer Agents. *BMC Microbiol* **2019**, *19*, 38. [CrossRef] [PubMed]
39. El-Gendy, M.M.A.; Shaaban, M.; Shaaban, K.A.; El-Bondkly, A.M.; Laatsch, H. Essramycin: A First Triazolopyrimidine Antibiotic Isolated from Nature†. *J. Antibiot.* **2008**, *61*, 149–157. [CrossRef] [PubMed]
40. Qiu, L.; Li, J.-J.; Li, Z.; Wang, J.-J. Production and Characterization of Biocontrol Fertilizer from Brewer's Spent Grain via Solid-State Fermentation. *Sci. Rep.* **2019**, *9*, 480. [CrossRef]
41. Wang, G.; Dai, S.; Chen, M.; Wu, H.; Xie, L.; Luo, X.; Li, X. Two Diketopiperazine Cyclo(pro-Phe) Isomers from Marine Bacteria Bacillus Subtilis Sp. 13-2. *Chem. Nat. Compd.* **2010**, *46*, 583–585. [CrossRef]
42. Pérez-Picaso, L.; Olivo, H.F.; Argotte-Ramos, R.; Rodríguez-Gutiérrez, M.; Rios, M.Y. Linear and Cyclic Dipeptides with Antimalarial Activity. *Bioorganic Med. Chem. Lett.* **2012**, *22*, 7048–7051. [CrossRef]
43. Řezanka, T.; Spížek, J.; Přikrylová, V.; Dembitsky, V.M. Four New Derivatives of Trihomononactic Acids from *Streptomyces* Globisporus. *Eur. J. Org. Chem.* **2004**, *2004*, 4239–4244. [CrossRef]
44. Takamatsu, S.; Lin, X.; Nara, A.; Komatsu, M.; Cane, D.E.; Ikeda, H. Characterization of a Silent Sesquiterpenoid Biosynthetic Pathway in *Streptomyces Avermitilis* Controlling *Epi* -Isozizaene Albaflavenone Biosynthesis and Isolation of a New Oxidized *Epi* -Isozizaene Metabolite: Characterization of a Silent Sesquiterpenoid Biosynthetic Pathway. *Microb. Biotechnol.* **2011**, *4*, 184–191. [CrossRef] [PubMed]

45. Bae, M.; Park, S.; Kwon, Y.; Lee, S.; Shin, J.; Nam, J.-W.; Oh, D.-C. QM-HiFSA-Aided Structure Determination of Succinilenes A–D, New Triene Polyols from a Marine-Derived *Streptomyces* Sp. *Mar. Drugs* **2017**, *15*, 38. [CrossRef]
46. Dickschat, J.S.; Martens, T.; Brinkhoff, T.; Simon, M.; Schulz, S. Volatiles Released by a *Streptomyces* Species Isolated from the North Sea. *CB* **2005**, *2*, 837–865. [CrossRef]
47. Cho, J.Y.; Kang, J.Y.; Hong, Y.K.; Baek, H.H.; Shin, H.W.; Kim, M.S. Isolation and Structural Determination of the Antifouling Diketopiperazines from Marine-Derived *Streptomyces Praecox* 291-11. *Biosci. Biotechnol. Biochem.* **2012**, *76*, 1116–1121. [CrossRef]
48. Hazato, T.; Kumagai, M.; Naganawa, H.; Aoyagi, T.; Umezawa, H. *p*-Hydroxyphenylacetaldoxime, an Inhibitor of β-Galactosidase, Produced by Actinomycetes. *J. Antibiot.* **1979**, *32*, 91–93. [CrossRef] [PubMed]
49. Vaden, R.; Oswald, N.; Potts, M.; MacMillan, J.; White, M. FUSION-Guided Hypothesis Development Leads to the Identification of N6,N6-Dimethyladenosine, a Marine-Derived AKT Pathway Inhibitor. *Mar. Drugs* **2017**, *15*, 75. [CrossRef]
50. Huang, C.; Yang, C.; Zhang, W.; Zhu, Y.; Ma, L.; Fang, Z.; Zhang, C. Albumycin, a New Isoindolequinone from *Streptomyces* Albus J1074 Harboring the Fluostatin Biosynthetic Gene Cluster. *J. Antibiot.* **2019**, *72*, 311–315. [CrossRef]
51. Thissera, B.; Alhadrami, H.A.; Hassan, M.H.A.; Hassan, H.M.; Behery, F.A.; Bawazeer, M.; Yaseen, M.; Belbahri, L.; Rateb, M.E. Induction of Cryptic Antifungal Pulicatin Derivatives from Pantoea Agglomerans by Microbial Co-Culture. *Biomolecules* **2020**, *10*, 268. [CrossRef]
52. Elleuch, L.; Shaaban, M.; Smaoui, S.; Mellouli, L.; Karray-Rebai, I.; Fourati-Ben Fguira, L.; Shaaban, K.A.; Laatsch, H. Bioactive Secondary Metabolites from a New Terrestrial *Streptomyces* Sp. TN262. *Appl. Biochem. Biotechnol.* **2010**, *162*, 579–593. [CrossRef]
53. Lee, L.-H.; Chan, K.-G.; Stach, J.; Wellington, E.M.H.; Goh, B.-H. Editorial: The Search for Biological Active Agent(s) From Actinobacteria. *Front. Microbiol.* **2018**, *9*, 824. [CrossRef]
54. De Lima Procópio, R.E.; da Silva, I.R.; Martins, M.K.; de Azevedo, J.L.; de Araújo, J.M. Antibiotics Produced by *Streptomyces*. *Braz. J. Infect. Dis.* **2012**, *16*, 466–471. [CrossRef] [PubMed]
55. Chandra, N.; Kumar, S. Antibiotics Producing Soil Microorganisms. In *Antibiotics and Antibiotics Resistance Genes in Soils*; Hashmi, M.Z., Strezov, V., Varma, A., Eds.; Soil Biology; Springer International Publishing: Cham, Switzerland, 2017; Volume 51, pp. 1–18. ISBN 978-3-319-66259-6.
56. Martins, M.B.; Carvalho, I. Diketopiperazines: Biological Activity and Synthesis. *Tetrahedron* **2007**, *63*, 9923–9932. [CrossRef]
57. Fdhila, F.; Vázquez, V.; Sánchez, J.L.; Riguera, R. DD -Diketopiperazines: Antibiotics Active against *Vibrio a Nguillarum* Isolated from Marine Bacteria Associated with Cultures of *Pecten m Aximus*. *J. Nat. Prod.* **2003**, *66*, 1299–1301. [CrossRef] [PubMed]
58. Sinha, S.; Srivastava, R.; De Clercq, E.; Singh, R.K. Synthesis and Antiviral Properties of Arabino and Ribonucleosides of 1,3-Dideazaadenine, 4-Nitro-1, 3-dideazaadenine and Diketopiperazine. *Nucleosides Nucleotides Nucleic Acids* **2004**, *23*, 1815–1824. [CrossRef]
59. Byun, H.-G.; Zhang, H.; Mochizuki, M.; Adachi, K.; Shizuri, Y.; Lee, W.-J.; Kim, S.-K. Novel Antifungal Diketopiperazine from Marine Fungus. *J. Antibiot.* **2003**, *56*, 102–106. [CrossRef] [PubMed]
60. Kwon, O.S.; Park, S.H.; Yun, B.-S.; Pyun, Y.R.; Kim, C.-J. cyclo(Dehydroala-L-Leu), an α-Glucosidase Inhibitor from Penicillium Sp. F70614. *J. Antibiot.* **2000**, *53*, 954–958. [CrossRef]
61. Nicholson, B.; Lloyd, G.K.; Miller, B.R.; Palladino, M.A.; Kiso, Y.; Hayashi, Y.; Neuteboom, S.T.C. NPI-2358 Is a Tubulin-Depolymerizing Agent: In-Vitro Evidence for Activity as a Tumor Vascular-Disrupting Agent. *Anti-Cancer Drugs* **2006**, *17*, 25–31. [CrossRef] [PubMed]
62. Preciado, S.; Mendive-Tapia, L.; Torres-García, C.; Zamudio-Vázquez, R.; Soto-Cerrato, V.; Pérez-Tomás, R.; Albericio, F.; Nicolás, E.; Lavilla, R. Synthesis and Biological Evaluation of a Post-Synthetically Modified Trp-Based Diketopiperazine. *Med. Chem. Commun.* **2013**, *4*, 1171–1174. [CrossRef]
63. Anderle, C.; Stieger, M.; Burrell, M.; Reinelt, S.; Maxwell, A.; Page, M.; Heide, L. Biological Activities of Novel Gyrase Inhibitors of the Aminocoumarin Class. *Antimicrob. Agents Chemother.* **2008**, *52*, 1982–1990. [CrossRef] [PubMed]
64. Kadhum, A.A.H.; Mohamad, A.B.; Al-Amiery, A.A.; Takriff, M.S. Antimicrobial and Antioxidant Activities of New Metal Complexes Derived from 3-Aminocoumarin. *Molecules* **2011**, *16*, 6969–6984. [CrossRef] [PubMed]
65. Fan, J.; Fu, A.; Zhang, L. Progress in Molecular Docking. *Quant. Biol.* **2019**, *7*, 83–89. [CrossRef]
66. Hasan, M.R.; Chowdhury, S.M.; Aziz, M.A.; Shahriar, A.; Ahmed, H.; Khan, M.A.; Mahmud, S.; Emran, T.B. In Silico Analysis of Ciprofloxacin Analogs as Inhibitors of DNA Gyrase of Staphylococcus Aureus. *Inform. Med. Unlocked* **2021**, *26*, 100748. [CrossRef]
67. Dolgonosov, A.M. The Universal Relationship between the Energy and Length of a Covalent Bond Derived from the Theory of Generalized Charges. *Russ. J. Inorg. Chem.* **2017**, *62*, 344–350. [CrossRef]
68. Han, S.; Caspers, N.; Zaniewski, R.P.; Lacey, B.M.; Tomaras, A.P.; Feng, X.; Geoghegan, K.F.; Shanmugasundaram, V. Distinctive Attributes of β-Lactam Target Proteins in *Acinetobacter Baumannii* Relevant to Development of New Antibiotics. *J. Am. Chem. Soc.* **2011**, *133*, 20536–20545. [CrossRef]
69. Wang, K.; Sitsel, O.; Meloni, G.; Autzen, H.E.; Andersson, M.; Klymchuk, T.; Nielsen, A.M.; Rees, D.C.; Nissen, P.; Gourdon, P. Structure and Mechanism of Zn^{2+}-Transporting P-Type ATPases. *Nature* **2014**, *514*, 518–522. [CrossRef] [PubMed]
70. Skupińska, M.; Stępniak, P.; Łętowska, I.; Rychlewski, L.; Barciszewska, M.; Barciszewski, J.; Giel-Pietraszuk, M. Natural Compounds as Inhibitors of Tyrosyl-TRNA Synthetase. *Microb. Drug Resist.* **2017**, *23*, 308–320. [CrossRef] [PubMed]
71. Wadapurkar, R.M.; Shilpa, M.D.; Katti, A.K.S.; Sulochana, M.B. In Silico Drug Design for Staphylococcus Aureus and Development of Host-Pathogen Interaction Network. *Inform. Med. Unlocked* **2018**, *10*, 58–70. [CrossRef]

72. Watve, M.; Tickoo, R.; Jog, M.; Bhole, B. How Many Antibiotics Are Produced by the Genus *Streptomyces*? *Arch. Microbiol.* **2001**, *176*, 386–390. [CrossRef] [PubMed]
73. Antibiotic Discovery from Actinomycetes: Will a Renaissance Follow the Decline and Fall? | CiNii Research. Available online: https://cir.nii.ac.jp/crid/1570291226223159168 (accessed on 14 August 2022).