

Article

Design of Marine Cyclodepsipeptide Analogues Targeting *Candida albicans* Efflux Pump CaCdr1p

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Abstract: Fungal infections are a significant threat to human health and the environment. The emergence of multidrug-resistant strains of fungi and the growing prevalence of azole resistance in invasive fungal infections exacerbate the problem, with efflux pumps being a major cause of antifungal resistance and a prime target for several counteractive strategies. In *Candida albicans*, the ATP-binding cassette superfamily transporter CaCdr1p is the predominant efflux pump involved in azole resistance. Marine organisms have unique phenotypic characteristics to survive in challenging environments, resulting in biologically active compounds. The cyclodepsipeptides unnarmicin A and C have shown promising results as inhibitors of rhodamine 6G efflux in cells expressing CaCdr1p. Herein, a series of unnarmicin analogues were designed and docked against a CaCdr1p efflux pump based on the cryogenic electron microscopy structure available to select the most promising compounds. Analogue **33** was predicted to be the best considering its high affinity for the efflux pump and pharmacokinetic profile. These results pave the way for further synthesis and in vitro biological studies of novel unnarmicins seeking a synergistic effect with fluconazole.

Keywords: antifungal activity; CaCdr1p; cyclodepsipeptides; design; marine organisms; molecular docking; multidrug resistant; unnarmicins



Citation: Ribeiro, R.; Fortes, S.; Costa, L.; Palmeira, A.; Pinto, E.; Sousa, E.; Fernandes, C. Design of Marine Cyclodepsipeptide Analogues Targeting *Candida albicans* Efflux Pump CaCdr1p. *Drugs Drug Candidates* **2024**, *3*, 537–549. <https://doi.org/10.3390/ddc3030031>

Academic Editor: George Mihai Nitulescu

Received: 13 June 2024

Revised: 24 July 2024

Accepted: 26 July 2024

Published: 1 August 2024



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1. Introduction

Microbial agents, including bacteria, fungi, and viruses, have always been a great concern within the scientific and medical communities. Contrary to previous expectations of a multidrug-resistant bacterial pandemic, the emergence of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2 virus) brought to light the potential problem of fungal infections [1]. Fungi are part of the normal human gastrointestinal tract, but they can cause deadly infections if the immune system is weakened. It is estimated that fungal infections are responsible for 1.5 million deaths each year, a number that is particularly relevant among the increasing population at risk, including immunocompromised individuals, organ transplant recipients, cancer patients, elderly people, and premature newborns [2,3]. Fungal pathogens also have a significant impact on the environment, with potential disruptions to ecosystems and food security, and they can even contribute to species extinction [4].

Candida, *Aspergillus*, and *Cryptococcus* genera are the most common causes of invasive fungal infections [2,5], with candidemia being a particular concern due to its high mortality

rate and economic burden associated with longer hospitalizations [2,5,6]. *Candida* spp. are commensal yeasts of the human gastrointestinal tract, mucous membranes, and skin. However, when the body's normal balance is disrupted, *Candida* can become an opportunistic pathogen. Different types of *Candida* have different invasive potential, virulence, and susceptibility to antifungal drugs [3,5]. Interestingly, when *Candida* opportunistic pathogens are examined on a phylogenetic tree, they are found to be part of different lineages that are mixed with non-pathogenic relatives. This suggests that the ability to infect humans has independently arisen, multiple times throughout evolution. Therefore, different *Candida* species may use different mechanisms to evade the host's immune system, displaying a variety of virulence phenotypes [6].

C. albicans is the most common pathogen associated with nosocomial invasive candidosis worldwide [7]. However, in recent years, an increasing number of infections by non-*albicans* *Candida* species have been reported, including *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, and, more recently, *C. auris* [8]. This change in the epidemiology of *Candida* infection has been partly attributed to the selection of less-sensitive *Candida* strains by the widespread use of the azole fluconazole (FLC) [7].

Currently, there are two main challenges that limit the effectiveness of candidosis treatment: first, it can be difficult to quickly and accurately diagnose the specific agent causing the infection and, second, the number of available treatment options is limited [6]. There are only three major classes of antifungal drugs available to treat candidosis: azoles, which inhibit the production of ergosterol, a fungal cell membrane component; polyenes, which bind to ergosterol and cause cell death by damaging the cell membrane; and echinocandins, that block the biosynthesis of a key cell wall component, β -1,3-D-glucan [2,3,5–7]. The emergence of multidrug-resistant (MDR) strains of fungi and increasing azole-resistant invasive fungal infections (IFIs) to antifungal treatments poses a serious threat to patient outcomes, especially in developing countries where antifungal agents are limited [2].

Azole resistance can occur through different mechanisms. One mechanism involves the overexpression of efflux pumps, which reduces drug concentrations at the target site. In *Candida* species, efflux pumps are encoded by the CDR genes of the ATP-binding cassette (ABC) superfamily and the MDR genes of the major facilitator superfamily (MFS). ABC pumps use ATP hydrolysis as an energy source and can accept a wide range of compounds as substrates, while the MFS class utilizes the proton motive force of the membrane as an energy source [5,8,9]. While CDR pumps can confer resistance to most azoles, MDR pumps appear to be selective for FLC resistance.

Many ABC and MFS transporter proteins have been identified in *Candida* species; however, only a few are significant in clinical drug resistance. Among the 19 ABC transporter proteins, only CaCdr1p and CaCdr2p, and among the 95 MFS superfamily members, only CaMdr1p, have been directly linked to the emergence of antifungal resistance in clinical isolates of *C. albicans*. The MDR1 gene is involved specifically in resistance to FLC rather than to other azoles. Although CaCDR1 and CaCDR2 genes are upregulated, CaCdr1p is the predominant protein involved in azole resistance [8]. CaCdr1p is a transporter with two transmembrane domains (TMDs) and two nucleotide-binding domains (NBDs) arranged in a reverse topology, with the NBDs preceding the TMDs (NBD–TMD). The NBDs are the energy source of these proteins [10]. Each TMD of CaCdr1p is made up of six transmembrane helices (TMH1–12) which are connected to each other and with the NBDs via the intracellular loops (ICL1–4) and the extracellular loops (ECL1–6) (Figure 1) [5,8,11].

Since efflux pumps are a major cause of antifungal resistance, they are a prime target for several counteractive strategies. One approach is to use antifungal drugs that are not substrates for efflux pumps to maintain a high drug concentration at the site of action, inside the fungal cell. Another strategy is to develop efflux inhibitors or chemosensitizers that impair the activity of the efflux pump, either by blocking access to the binding site or by inhibiting the efflux pump transcription. Additional strategies include designing inhibitors that de-energize ATP or H^+ -dependent transporters to promote antifungal uptake. However, these strategies could have unintended effects on other cellular metabolic activities,

and the use of immunosuppressive drugs could be problematic for immunosuppressed patients [12–14].

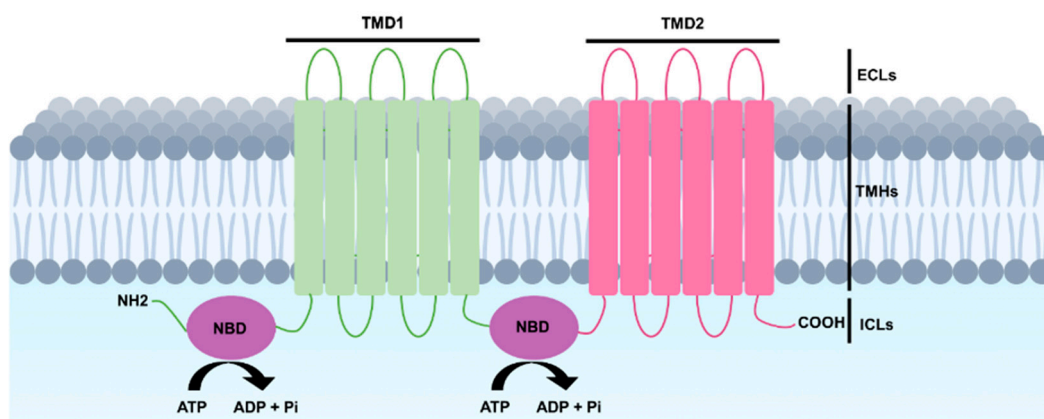


Figure 1. Schematic view of CaCdr1p showing its two transmembrane domains (TMDs), two nucleotide-binding domains (NBDs), six extracellular loops (ECLs), four intracellular loops (ICLs), and twelve transmembrane helices (TMHs).

The ocean is a vast and yet largely unexplored source of potentially valuable bioactive compounds [15,16]. The challenging marine environments characterized by extreme variations in temperature, pressure, light, salinity, and nutrient availability drives marine organisms to develop unique metabolic and physical capabilities to adapt and survive in these everlasting, changing, demanding habitats [17,18]. Through a process of natural selection, this taxonomic diversity leads to a range of metabolites that greatly differ in structure and function from those produced by terrestrial organisms, forming the foundation of chemical diversity in the marine environment [19,20].

Among the vast number of antimicrobial marine-derived compounds [21–23], the cyclodepsipeptides unnarmicin A (1) and C (2) (Figure 2), isolated from a marine gammaproteobacterium, have shown high in vitro antimicrobial potency and promising in vivo results against fungal infections, especially those caused by MDR fungi [24,25]. These marine cyclodepsipeptides have proven to be potent inhibitors of rhodamine 6G efflux in CaCdr1p-expressing cells, with half-maximal inhibitory concentration (IC₅₀) values of 2.29 and 3.75 mg/mL, respectively [24]. Additionally, both were able to inhibit a broader range of fungal multidrug efflux pumps, including *C. glabrata* CgCdr1p and CgPdh1p, *C. albicans* CaCdr1p, and *S. cerevisiae* ScPdr5p. It appears that the inhibitory activity of these compounds is selective for CaCdr1p in *C. albicans*, as they did not affect the growth of CaCdr2p and CaMdr1p overexpressing cells. Although the length of the alkyl side chain may affect the different efficacies of these two compounds in different biochemical assays, it seems that both unnarmicins have similar affinities to the target proteins and, therefore, similar inhibitory activities [24].

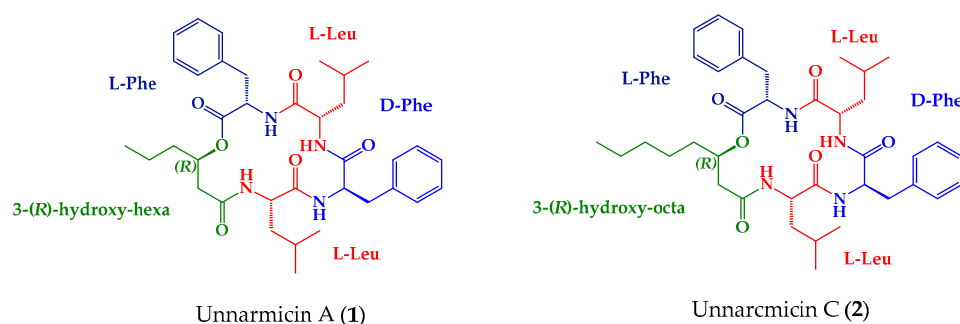


Figure 2. Structures of unnarmicin A (1) and unnarmicin C (2).

In this work, a library of unnarmicin analogues was designed and their affinity to the *C. albicans* efflux pump CaCdrp1p was evaluated through a molecular docking approach.

2. Results and Discussion

2.1. Structure-Based Virtual Screening

Drug discovery and development is a time- and resource-intensive process. The combination of computational tools with traditional chemical and biological assays is proving to be a powerful approach to streamline this process by improving the success rate of drug screening and reducing the blindness of research. In silico design can expedite and facilitate the various stages of drug discovery, including hit identification [26], with molecular docking among the most popular and successful structure-based computational methods [27,28].

Structurally, the cyclodepsipeptides unnarmicin A (1) and C (2) comprise four amino acid residues, L-Phe, D-Phe, and two L-Leu, and a 3-hydroxy fatty acid, with the main difference between them being the length of the alkyl chain (Figure 2).

The library of unnarmicin analogues designed includes 33 compounds (3–35) whose structures are illustrated in Figure 3. The unnarmicin analogues vary in terms of the tetrapeptide composition, amino acid residues sequence and/or stereochemistry, or alkyl chain.

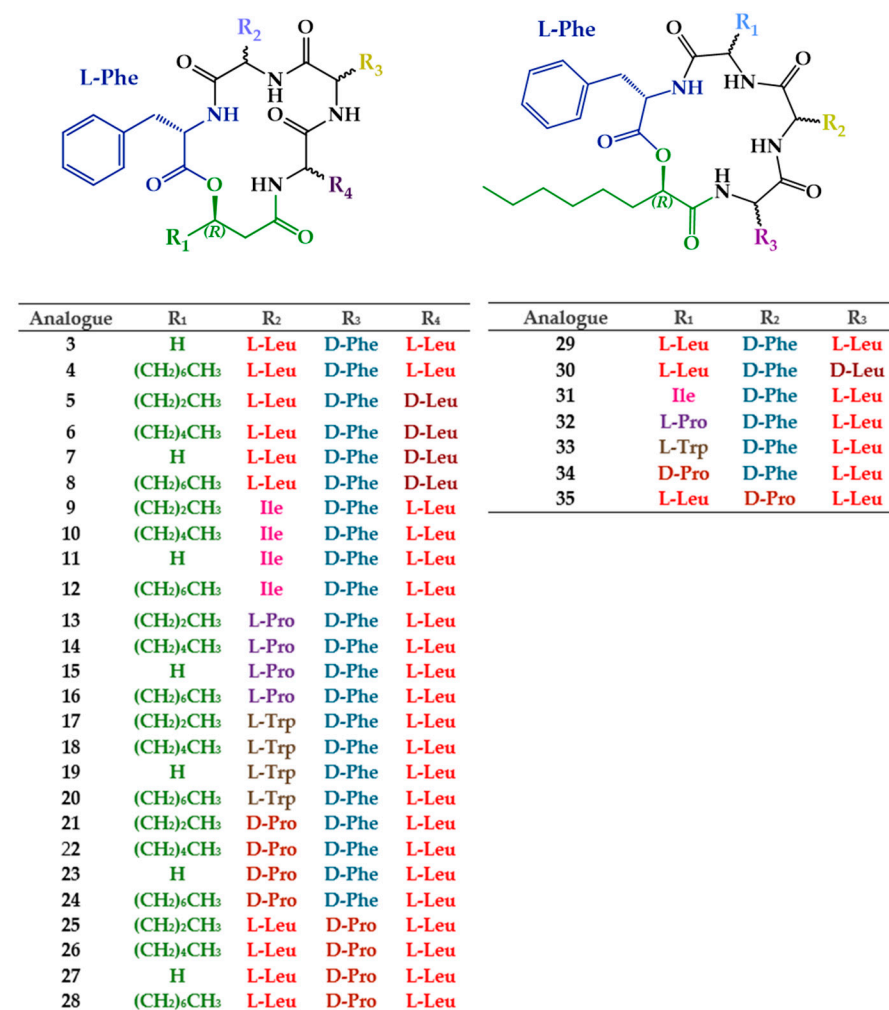


Figure 3. Structures of unnarmicin analogues 3–35. The diverse structural moieties are highlighted at different color.

Regarding the peptide's composition, in addition to Phe and Leu amino acids present in both unnarmicins **1** and **2**, other amino acid residues were selected. As shown in Figure 3, Ile, Pro, and Trp were included in the structure of some analogues, as these amino acids are pharmacophoric moieties in many bioactive compounds for various biological activities [29].

The planning of the unnarmicin analogues **3–35** also took into consideration the commercial availability of building blocks, predicting that their further synthesis is feasible. Hence, two alkyl side chains, with different length and stereogenic center positions, and an intramolecular alkyl chain were included considering the commercial building blocks 2-hydroxy-*n*-octanoic acid (**36**), 3-hydroxy-*n*-decanoic acid (**37**), and 3-hydroxy-*n*-propanoic acid (**38**), respectively. The last hydroxy acid was selected for comparison purposes and subsequent evaluation of the relevance of the alkyl side chain for target interaction and, therefore, for biological effect. Considering that the chiral alkyl moiety of both marine cyclodepsipeptides **1** and **2** has (*R*)-configuration, only this configuration was selected for the design of the alkyl moiety stereogenic center of unnarmicin analogues (**3–35**).

The binding affinity between the Cdr1p and the small molecules was evaluated by the binding free energy approximation (ΔG , kcal/mol), using AutoDock Vina (Table 1). The more negative the score, the greater the affinity of the compound for the target protein [30].

Table 1. Docking results of unnarmicin A (**1**), unnarmicin C (**2**), and unnarmicin analogues **3–35**.

Compound	ΔG (kcal/mol)	Hydrogen Bonds between the Compound and Cdr1p			Hydrophobic Interactions
		Functional Group of Compound	Amino Acid	Distance (Å)	
1	−7.7	CO (L-Leu)	Thr1351	2.4	Leu562, Leu563, Met657, Ala660
		CH (Alkyl chain)	Phe559	3.7	
2	−8.7	CO (L-Phe)	Gln1244	3.2	Ile565, Ala656, Met657, Ala660, Leu664, Ile1237
3	−9.4	CO (Alkyl chain)	Thr661	3.3	Phe559, Leu562
		CH (D-Phe)	Thr1351	3.4	
4	−8.8	NH (L-Leu)	Phe559	2.9	Phe556, Phe643, Ala660
		CO (L-Leu)	Leu562	3.1	
		CO (L-Leu)	Thr1355	3.1	
		CO (D-Phe)	Thr1355	2.9	
5	−9.7	CO (L-Leu)	Thr1351	2.6	Phe556, Leu562, Leu563, Ala660
6	−9.9	CO (D-Phe)	Thr1351	2.6	Leu562, Leu563, Ala660
7	−10.1	CO (L-Leu)	Thr1351	2.6	Phe556, Leu562, Ala660
8	−9.5	CO (D-Phe)	Thr1351	2.6	Phe556, Leu562, Leu563, Ala660
9	−8.7	CO (Alkyl chain)	Leu562	3.1	Phe556, Leu563, Leu1352
		CO (L-Ile)	Asn1240	3.0	
		CO (L-Ile)	Thr1355	3.2	
		NH (L-Ile)	Thr1355	1.7	
10	−8.8	CO (L-Leu)	Leu562	3.1	Phe556, Leu563, Ile565, Leu1352
		CO (L-Ile)	Asn1240	3.1	
		CO(L-Ile)	Thr1355	3.1	
		NH (L-Ile)	Thr1355	1.8	

Table 1. Cont.

Compound	ΔG (kcal/mol)	Hydrogen Bonds between the Compound and Cdr1p			Hydrophobic Interactions
		Functional Group of Compound	Amino Acid	Distance (Å)	
11	−8.6	CH (L-Phe)	Asn1240	4.1	Leu1352
		CO (L-Ile)	Asn1240	3.2	
		CO (D-Phe)	Thr1355	3.0	
		NH (L-Ile)	Thr1355	2.8	
12	−9.0	CO (alkyl chain)	Leu562	3.8	Leu563, Phe556, Phe643, Ala660, Leu1352
		CO (L-Ile)	Asn1240	3.0	
		NH (L-Leu)	Thr1355	3.0	
		CO (L-Ile)	Thr1355	3.2	
13	−11.2	---	---	---	Leu555, Leu563, Leu664, Leu1352
14	−10.7	CH (L-Pro)	Thr1355	3.1	Met525, Phe556, Ala660, Leu664
15	−11.1	CO (Alkyl chain)	Gln1244	3.2	Leu555, Leu562, Leu563, Leu564, Val668
		CH (Alkyl chain)	Asn1348	3.5	
16	−10.3	CH (L-Pro)	Thr1355	3.1	Met525, Phe556, Leu562, Phe643, Ala660, Leu664
17	−10.3	CO (L-Trp)	Thr1355	3.0	Leu563, Leu664, Leu1352
		NH (L-Trp)	Thr1355	2.3	
		CH (L-Trp)	Thr1355	3.3	
18	−10.2	NH (L-Trp)	Thr1355	2.3	Leu563, Leu664, Leu1352
		CO (L-Trp)	Thr1355	3.0	
		CH (L-Trp)	Thr1355	3.3	
19	−11.1	CH (L-Trp)	Asn1240	4.1	Phe556, Leu562, Ala660
		CO (L-Trp)	Thr1351	2.7	
20	−10.4	CO (L-Trp)	Thr1355	3.1	Leu563, Phe643, Ala660, Leu664, Val668, Leu1352
		NH (L-Trp)	Thr1355	2.3	
		CO (D-Phe)	Thr1355	3.0	
		CH (L-Trp)	Thr1355	3.3	
21	−10.4	---	---	---	Leu555, Phe556, Leu563, Met566, Val668
22	−10.1	---	---	---	Leu555, Leu563, Val668
23	−10.5	CH (Alkyl chain)	Thr1355	3.6	Phe517, Ala660, Phe1354
24	−10.3	---	---	---	Leu555, Leu563, Met566, Leu664, Val668
25	−10.3	CO (L-Phe)	Asn1240	3.3	Met525, Leu555, Phe559, Leu562, Ala660, Leu664
		CO (L-Leu)	Thr1355	2.9	
26	−9.8	O (Ester)	Asn1240	3.3	Leu664
		NH (L-Phe)	Thr1355	2.5	
27	−10.6	CO (L-Phe)	Gln1244	3.3	Leu555, Leu562, Ala660, Val668
		CO (L-Leu)	Thr1355	3.2	
		CH (L-Pro)	Thr1355	3.6	
28	−9.8	O (Ester)	Asn1240	3.3	Leu564, Ile1237
		NH (L-Phe)	Thr1355	2.6	

Table 1. Cont.

Compound	ΔG (kcal/mol)	Hydrogen Bonds between the Compound and Cdr1p			Hydrophobic Interactions
		Functional Group of Compound	Amino Acid	Distance (Å)	
29	−9.8	CO (Alkyl chain)	Phe556	3.5	Phe559, Leu664, Val668, Ile1237, Leu1358
		CH (L-Phe)	Thr1355	3.5	
30	−10.2	CO (L-Phe)	Asn1240	3.3	Phe517, Leu555, Leu562, Ala660, Val668, Leu1352, Phe1354
31	−10.3	NH (L-Ile)	Phe559	2.3	Met525, Leu555, Val668, Leu1358, Phe1354
32	−9.3	---	---	---	Met525, Phe556, Ile1237, Leu1352
33	−11.3	CO (L-Leu)	Asn1240	3.3	Leu555, Leu563, Phe1233, Ile1237, Leu1352, Leu1358
		CH (L-Trp)	Gln522	3.5	
34	−7.8	---	---	---	Ile646, Val649, Ala666, Ile690
35	−10.0	CO (L-Leu)	Thr1355	3.1	Met525, Leu562, Leu563, Leu664, Val668, Leu1352

To perform the structure-based virtual screening, several reported inhibitors of CaCdr1p (39–46, Figure S1, Supplementary Materials) [24,31] and known substrates (47–48, Figure S2, Supplementary Materials) [24,31–33] were used as positive controls. The docking analysis of the positive controls supported those reported in the in vitro studies, that is, the compounds with the best performance in vitro were also the ones with the greatest results in silico (Table 2).

Table 2. Docking results of known inhibitors (39–46) and substrates (47–48) of CaCdr1p.

Compound	ΔG_b (kcal/mol)
FK506 (39)	−7.1
FK520 (40)	−6.5
Enniatin_B (41)	−6.9
Dissulfiram (42)	−4.8
Ibuprofen (43)	−6.8
Milbemycin α_{11} (44)	−11.5
Milbemycin α_{20} (45)	−11.6
Milbemycin β_{11} (46)	−11.7
FLC (47)	−7.2
Rhodamine 6G (48)	−8.9

FLC: Fluconazole.

Rhodamine 6G (48) is a fluorescent compound widely used in the in vitro evaluation tests for these inhibitors [24,31]. As for FLC (47), it is a drug widely used in therapy but it has been associated with an increasing number of cases of resistance, namely, due to the overexpression of efflux pumps, specifically, CaCdr1p [24,31]. This justifies the interest in this specific drug for the docking analysis. Furthermore, the unnarmicin analogues (3–35) were designed aiming to obtain competitive inhibitors with similar binding sites in the protein to FLC (47) and, therefore, to promote a steric hindrance to avoid its efflux. Non-competitive inhibition, for example, at the ATP-binding site of this pump, was not pursued to avoid potential interactions with other ATP-dependent efflux pumps. Such interactions could result in a loss of selectivity of the molecule and adverse effects on the host.

As shown in Table 1, several analogues, namely, compounds 7, 13–25, 27, 30, 31, 33, and 35, revealed promising results having ΔG values in the range of −10.0 to −11.3 kcal/mol. It was found that, for the designed new unnarmicin analogues (3–35), the proposed aliphatic moiety (3-hydroxypropionic, 2-hydroxy-*n*-octanoic, and 3-hydroxydecanoic moieties, in compounds 3, 29, and 4, respectively) improved the affinity for Cdr1p and there was a slight preference towards the smallest alkyl chains (ΔG 29 > 3 > 4). The carbon making

the connection between the alkyl chain and the tetrapeptide played an important role in the affinity, as seen by the increased affinity for the 2-hydroxy-*n*-octanoic aliphatic (**29**). The stereochemistry was also important, as shown by the increased affinity resulting from substituting the L-Leu with the D-Leu (**1–4** and **29** versus **5–8** and **30**, respectively).

The Ile analogues (**9–12** and **31**) provided insight into the effects of a simple change in the position of a methyl group, which differentiates Leu from Ile, on the interaction with the protein. This change was sufficient to disrupt the interaction (increase the ΔG) but the extent of the disruption was also dependent on the specific carbon atom that was involved in the connection to the tetrapeptide, as evidenced by the significant difference observed between compound **31** and the others (**9–12**).

The position of the hydroxyl group and the volume of the residues are also correlated: the 2-hydroxyoctanoic aliphatic interacted better with a more linear residue like an Ile (**31** had a ΔG of -10.3 kcal/mol, the best of the Ile analogues (**9–12** and **31**)), while the L-Pro, a larger amino acid, worked better with the 3-hydroxy position (compounds **13–16** had a lower ΔG than analogue **32**), with the smaller the alkyl chain the better, even though **15** had a slight decrease in the affinity (**13** > **15** > **14** > **16**).

The substitution of L-Leu by L-Trp (**17–20** and **33**) was indeed the one that ensures the best compound conformation for the interaction with CaCdr1p, since all analogues comprising L-Trp (without exception) had ΔG values in the range of -10.2 to -11.3 kcal/mol.

In comparison to other molecules, a visual inspection revealed that analogue **33** exhibited a unique conformation that remarkably fills the active site, resembling a cannon-like structure (Figure 4A). Interaction details are shown in Figure S3 (Supplementary Materials). This exceptional fitting is primarily attributed to the indole ring of Trp, allowing it to occupy the space with nearly perfect occupancy (it is the only Trp analogue that had its ring facing downward, as shown in Figure 4B). While other molecules also occupy the active site, they leave unfilled gaps, which is in accordance with the reduced affinity (Figure 4C,D). This distinctive conformation suggests that a possible synergistic effect with FLC (to prevent its efflux) may be more effective with this analogue (**33**) than with the others, possibly leading to a significantly enhanced in vitro effect. These findings further highlight the potential of **33** as a promising candidate for future research and development efforts.

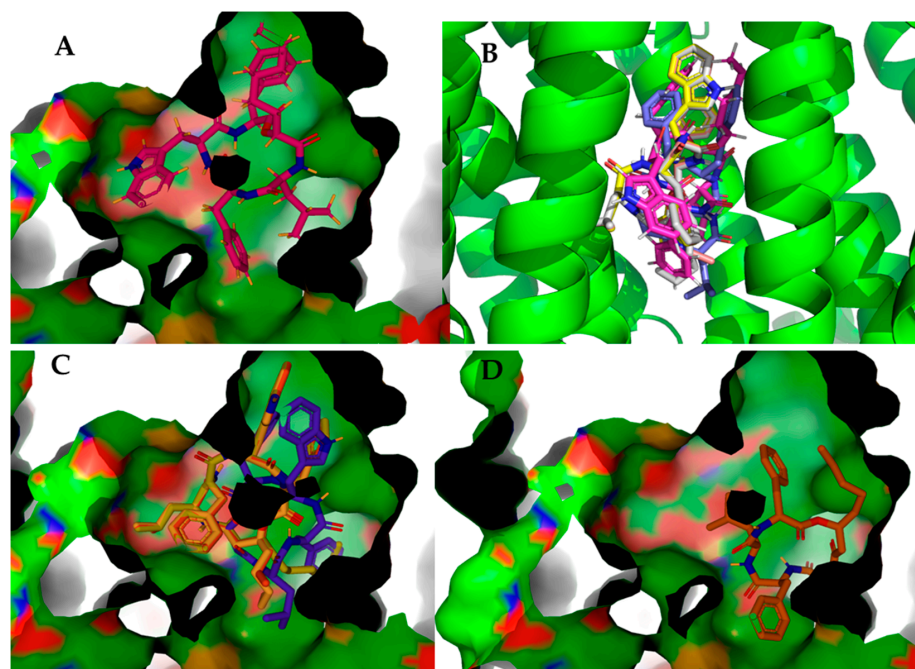


Figure 4. (A) Unique conformation of compound **33** filling the active site, resembling a cannon-like structure. (B) The CaCdr1p active site showing all the Trp analogues (**17–20** and **33**). Notice the indole ring downward conformation of **33** highlighted in pink. (C) Conformations of the other Trp analogues (**17–20**) in the active site. (D) Conformation of unnarmicin C (**2**) in the active site.

Analogue 33 interacts in the same place as FLC (47) (Figure 5A,B) but with greater affinity (Table 1), which means that it is indeed the optimal choice for an inhibitor of CaCdr1p, with a potential synergistic effect being hypothesized when combined with FLC (47). Therefore, it is also the choice for further development and synthesis. The other analogues also bind to the same binding site but the blockage is not so successful (Figure 5C,D).

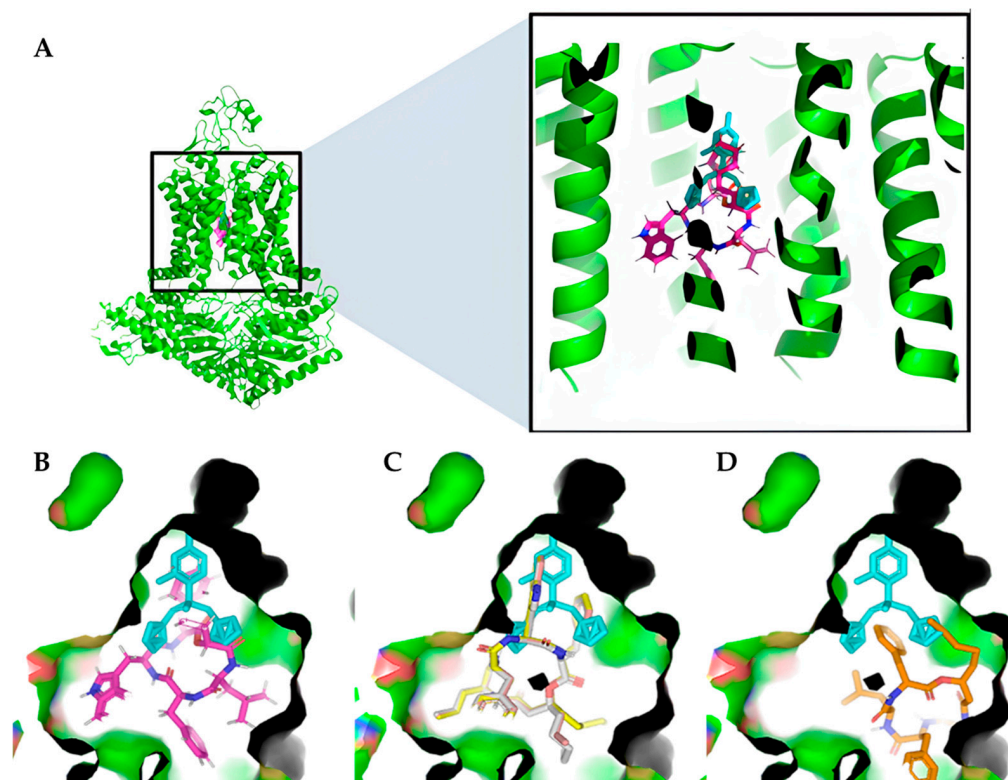


Figure 5. (A) Interaction of FLC (blue) (47) and analogue 33 (pink) in the same binding site of CaCdr1p efflux pump. Notice the perfect blockage of the FLC (47) by 33. (B) View of the interaction of FLC (blue) (47) and 33 (pink) in the cannon-like structure of the active site. (C) View of the interaction of FLC (blue) (47) and other Trp analogues (17–20) in the cannon-like structure of the active site. (D) View of the interaction of FLC (blue) (47) and the unnarmicin C (orange) (2) in the cannon-like structure of the active site.

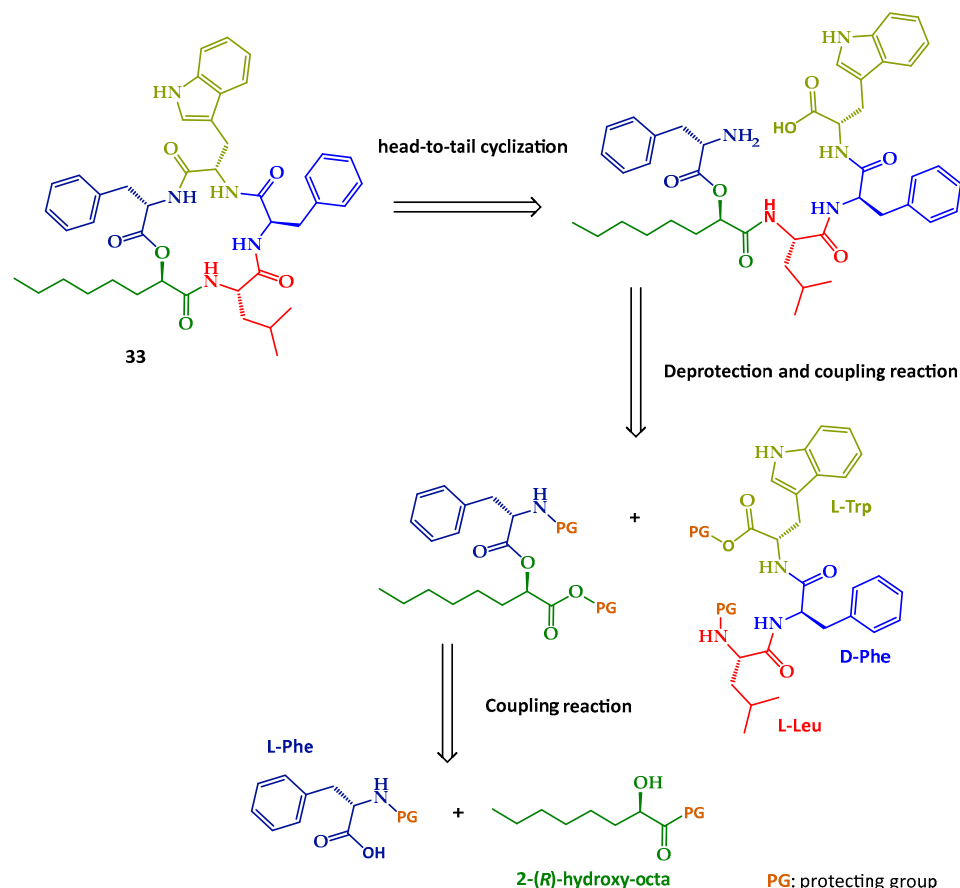
2.2. In Silico Pharmacokinetics Prediction

The physicochemical descriptors, as well as ADME parameters, pharmacokinetic properties, druglike nature, and medicinal chemistry friendliness, were predicted for the unnarmicins (1–2, Figure 2) and their analogues (3–35, Figure 3) to support drug discovery using the SwissADME web tool, available at <http://www.swissadme.ch/index.php> (accessed on 22 May 2024) [34]. Some of the most relevant data are summarized in Table S1 (Supplementary Materials).

The topological polar surface area (TPSA) and logarithm of *n*-octanol/water partition coefficient ($\text{Log } P_{o/w}$) values for all the cyclodepsipeptides (1–35) ranged from 133.91 to 158.49 and from 2.69 to 6.20, respectively. The predicted bioavailability score for the cyclodepsipeptides was 0.55, except for five analogues (21–25), whose bioavailability score was lower (0.17). None of the compounds were predicted to be blood–brain barrier (BBB) permeants. Regarding the gastrointestinal (GI) absorption, the scenario is diverse, with the majority with one violation to Lipinski’s rule of five, considering the molecular weight (MW) > 500.

Comparing the pharmacokinetics prediction data of the most promising analogue (**33**) with the natural unnarmicins (**1–2**), it is important to highlight some improvements in the physicochemical descriptors, ADME parameters, and pharmacokinetic properties.

Analogue **33** has a MW in the range of 500, while unnarmicin A (**1**) and C (**2**) have a MW higher than 634. The Log $P_{o/w}$ value of **33** is the lowest of all compounds and it complies with the parameters of Veber's rule. Unlike **1–2**, high GI absorption is expected for **33**. Analogue **33** was identified to have relatively optimal drug-likeness and medicinal chemistry characteristics. Hence, the synthesis and in vitro biological evaluation of analogue **33** will be further carried out. A proposal for the retrosynthesis of analogue **33** is presented in Scheme 1.



Scheme 1. Retrosynthesis proposal for analogue **33**.

3. Materials and Methods

3.1. Computational

3.1.1. Preparation of Unnarmicins A (**1**) and C (**2**), Unnarmicin Analogues (**3–35**), and Known Controls (**39–48**)

The unnarmicin A (**1**, Figure 2), unnarmicin C (**2**, Figure 2), 33 unnarmicin analogues (**3–35**, Figure 2), reported inhibitors (**39–46**, Figure S1), and known substrates (**47–48**, Figure S2) were drawn using ChemDraw Professional 16.0 and minimized using the Chem3D 16.0 Molecular Mechanics 2 minimization tool.

3.1.2. Preparation of CaCdr1p Efflux Pump

The protein X-ray crystallographic structure is the experimental basis of docking simulations [35,36]. However, until this moment, the experimental X-ray crystallographic structure for CaCdr1p has not been available. This way, the predicted model of CaCdr1p (Swiss-Model Repository, Q5ANA3) based on the cryogenic electron microscopy structure

from the Pdr5 efflux pump of *Saccharomyces cerevisiae* (ScCdr1p) was used [37]. The protein was prepared by removing both the water molecules and the ligands.

3.1.3. Docking Studies

Docking simulations between all compounds and CaCdr1p were undertaken in AutoDock Vina 1.5.7. [38,39], a virtual screening tool software. Vina was run using an exhaustiveness of 100 and a grid box with the dimensions of X: 44, Y: 44, Z: 42 engulfing the translocation pathway. PyMol Molecular Graphics System, Version 2.0 Schrödinger, LLC, and BIOVIA Discovery Studio Visualizer, Version 21.1.0.20298, Dassault Systèmes, were used for a visual inspection of the results and graphical representations.

3.1.4. Pharmacokinetics Prediction

The physicochemical descriptors, ADME parameters, pharmacokinetic properties, druglike nature, and medicinal chemistry friendliness were predicted using the SwissADME web tool, available at <http://www.swissadme.ch/index.php> (accessed on 22 May 2024) [34].

4. Conclusions

Drug discovery is a time- and resource-consuming process but in silico design can expedite and facilitate the various stages of this process, improving the success rate of drug screening. Marine organisms have shown to be a resourceful font of interesting bioactive compounds. This work is an example of that, as it started with the docking of a series of unnarmicin analogues followed by pharmacokinetics prediction, allowing the selection of the most promising analogue, compound **33**, in addition to other interesting compounds, such as **13**, **15**, and **19**, considering both their ΔG values and pharmacokinetic profiles. These first steps are expected to proceed with the successful synthesis and in vitro biological evaluation of analogue **33** and other promising analogues to further validate this in silico approach.

In conclusion, marine natural products analogues were predicted to overcome azole-resistant *C. albicans* strains.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/ddc3030031/s1>: Figure S1: Structures of known inhibitors of CaCdr1p. FK506 (**39**), FK520 (**40**), Enniatin B (**41**), Dissulfiram (**42**), Ibuprofen (**43**), Milbemycin α 11 (**44**), Milbemycin α 20 (**45**), and Milbemycin β 11 (**46**). Figure S2: Structures of known substrates of CaCdr1p. Fluconazole (FLC) (**47**) and Rhodamine 6G (**48**). Figure S3: Specific interactions between analogue **33** and CaCdr1p efflux pump. Distances expressed in angstroms (Å). Table S1: Some physicochemical descriptors and predicted ADME parameters, pharmacokinetic properties, and druglike nature for unnarmicins (**1–2**) and analogues (**3–35**). Available in <http://www.swissadme.ch/index.php> (accessed: 22/05/2024).

Author Contributions: Conceptualization: C.F. and E.S.; Methodology: C.F. and E.S.; Software: S.F., R.R. and A.P.; Formal analysis: E.S., A.P., E.P. and C.F.; Investigation: R.R., L.C. and S.F.; Data curation: E.S., A.P. and C.F.; Writing—original draft preparation: S.F., L.C. and R.R.; Writing—review and editing: E.S., E.P., A.P. and C.F.; Supervision: E.S., A.P. and C.F.; Funding acquisition: E.S., E.P. and C.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by national funds through the FCT (Foundation for Science and Technology) within the scope of UIDB/04423/2020, UIDP/04423/2020 (Group of Marine Products and Medicinal Chemistry—CIIMAR).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article and Supplementary Materials.

Acknowledgments: R.R. thanks the BYTPhD program by CIIMAR and the grant provided by the FCT UI/BD/150912/2021.

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

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