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The Kelch/Nrf2 Antioxidant System as a Target for Some Marine Fungal Metabolites

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Abstract: Marine fungal metabolites often exhibit antioxidant activity, but their effects on the Keap1/Nrf2 cellular system are rarely studied, possibly due to insufficient isolated amounts. In this work, we used a bioinformatics approach to evaluate the ability of some promising cytoprotective compounds to bind Kelch domain of Keap1 protein, and thus inhibit its interaction with Nrf2. The molecular docking data suggested that gliorosein, niveoglaucin A, 6-hydroxy-N-acetyl-β-oxotryptamine, 4-hydroxyscytalone and 4-hydroxy-6-dehydroxyscytalone can form the hydrogen building with Arg415 or Arg483 amino acid residues of P1-P2 sub-pockets in the Nrf2 binding site of Keap1's Kelch domain. These positions of the small molecules in the Kelch domain of Keap1 can inhibit the interaction of Keap1 with Nrf2 and enhance the nuclear translocation of Nrf2 from cytosol that can result in overexpression of relative genes. This assumption, based on virtual screening of a number of low molecular weight metabolites of marine fungi, makes them promising for further studies.

Keywords: antioxidant defense system; marine fungal metabolites; Nrf2; cytoprotection

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1. Introduction

The reactive oxygen species (ROSs) are a number of oxygen-based molecules which play a significant signal role in the living cell, but overexpression of ROSs resulted in oxidative stress and cell damage [1]. The nuclear factor erythroid 2–related factor 2 (Nrf2) is an emerging regulator of cellular resistance to intracellular ROS and xenobiotics [2].

Keap1 belongs to the BTB-Kelch family of proteins, and contains three parts: the N-terminal BTB domain (which mediates the homodimerization of Keap1, and contributes additionally to its interaction with Cul3), the IVR domain (the central 3-box motif, which provides Cul3 interaction), and the C-terminal Kelch domain, which is required for substrate capture and can bind separately to the ETGE or DLG motifs of Nrf2 [3]. The cytosol 16-residue AFFAQLQLDEETGEFL domain (named Neh2) of Nrf2 interacts with Kelch domain of Keap1 [4]. After dissociation from the cytosolic protein Keap1, Nrf2 rapidly accumulates in the nucleus and transactivates the antioxidant response element (ARE) in the promoter region of many antioxidant genes [5]. Nrf2-dependent genes regulate the expression of antioxidant enzymes, phase 2 detoxifying enzymes, biosynthesis of glutathione, chaperone proteins and others [6].

So, the Keap1/Nrf2 pathway is the target for various small molecules and peptides shown antioxidant cytoprotective effects [7]. The mainly known plant Keap1/Nrf2 modulators are polyphenols (resveratrol, caffeic acid) and flavonoids (baicalein, quercetin) [8]. The well-known natural antioxidant polyphenols resveratrol, curcumin, and genistein

interact with cysteine residues of Keap1, while quercetin interacts with Tyr527, Arg415 and Gly364 residues, and all of these induce the release of Nrf2 and its translocation in the cell nucleus [9]. Astaxanthin from red basidiomycetous yeast *Phaffia rhodozyma* is present in the cytoplasmic membrane with a rigid cell wall, and is one of the most studied Keap1/Nrf2 natural modulators [10].

Modulation of Keap/Nrf2 can be divided into 2 types: (a) irreversible, through modification of Keap1 thiols followed by destabilization of the dimer or cleavage of Cul3 ubiquitin ligase from Keap1, and (b) reversible, through competitive displacement of Nrf2 from the complex with Keap1 using Nrf2 peptides or small molecules specifically targeting the Kelch domain Keap1.

Covalent activators could irreversibly inhibit the ubiquitination of Nrf2 via reacting with the critical thiols (such as Cys151, Cys273, and Cys288) in Keap1 to induce the expression of Nrf2-regulated cytoprotective factors. Dimethyl fumarate modulates the Nrf2 pathway by reacting with Cys151 in Keap1 in vitro and in vivo, and was approved as Tecfidera in 2014 by Biogen for the treatment of patients with relapsing multiplate sclerosis. However, its undesirable side effects are known, and companies are actively working to obtain safer analogues [11]. Sulforaphane is electrophilic Nrf2 activator, and more than 20 ongoing clinical trials, mainly about its neuroprotective effects in various neurologic disease, were in progress in 2020 [12]. Bardoxolone methyl and its analogues with α-β unsaturated scaffold act as Michael acceptors by interacting with Cys151 of Keap1 so that they obstruct its mutual effects with Cul3, then activate Nrf2 [13].

Non-covalent activators could directly disrupt Keap1-Nrf2 interaction but do not require a covalent group for their mode of action, which may result in a better safety profile [14]. Most polar residues, including Arg483, Ser508, Ser363, Arg380, Asn382, and Arg415, and nonpolar residues, including Tyr525, Gln530, Tyr572, Tyr334, and Phe577, were found as the molecular determinants of Keap1-Nrf2 protein–protein interactions and key points for its non-covalent inhibitors. Direct experiments using FITC-labeled 16-mer Nrf2 16-mer peptide show that two antioxidant small peptides from eggs can block Keap1–Nrf2 binding via interaction with Arg380 and Asn382 amino acid residues (DKK peptide) or Arg380, Asn382, Arg415, Arg483, and Ser508 amino acid residues (DDW peptide) [15]. More than 25 compounds with 1,4-diaminonaphthalene skeleton, 3-phenylpropanoic core, 1-phenylpyrazole ring, and 1,2,4-oxadiazole core were patented from 2017 to 2020 by various companies as potent inhibitors of Keap1/Nrf2 interactions [11].

Marine fungal metabolites show various biological activities, including direct and indirect antioxidant effects [16]. Most marine fungal antioxidants were investigated as direct antioxidants, but their effects on the Keap1/Nrf2 pathway are insufficiently studied. It was reported that terrein from *Penicillium* sp. fungus induces translocation of Nrf2 from cytosol to nucleus in glial BV2 and primary microglial cells [17]. Alkaloid N-Me-trichodermamide B from *Penicillium janthinellum* induced the nuclear localization of Nrf2 in HaCaT human keratinocytes [18].

Our investigation from last year of marine fungal metabolites with various biological activity resulted in discovery of a number of polyketides and alkaloids with cytoprotective properties (Figure 1). Polyketide gliorosein (**1**) protected the cardiomyocytes H9c2 from rotenone and cobalt (II) chloride induced damage [19]. *p*-Terphenyl polyketides terphenyllin (**4**), 3″-hydroxyterphenyllin (**5**), and 3′-hydroxyterphenyllin (**6**) significantly increased the viability of paraquat- and rotenone-treated Neuro-2a cells [20]. Another polyketide niveoglaucin A (**3**) increased the viability of 6-hydroxydopamine-treated Neuro-2a cells [21]. 4-Hydroxyscytalone (**8**) and 4-hydroxy-6-dehydroxyscytalone (**9**) have shown significant increasing of paraquat- and rotenone-treated Neuro-2a cell viability [22]. Finally, a new melatonin analogue 6-hydroxy-N-acetyl-β-oxotryptamine (**7**) and other *p*-terphenyl polyketide 4″-dehydroxycandidusin A (**2**) demonstrated neuroprotective effects [23]. In all experiments, the cytoprotective influence of the compounds was accompanied with the decrease in ROS level mediated by neurotoxins in treated cells that indicates their likely effect on the intracellular antioxidant system.

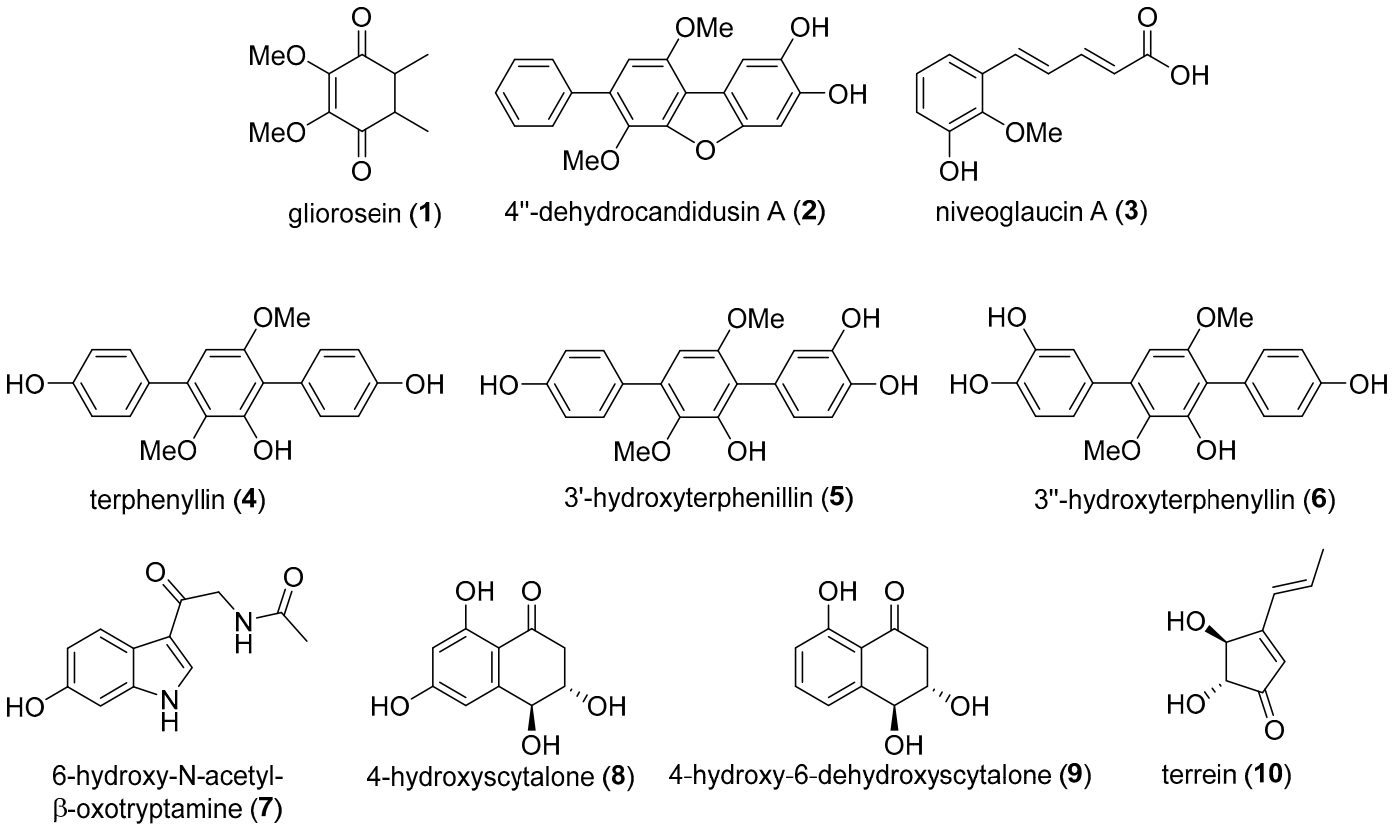


Figure 1. The chemical structures of investigated marine fungal metabolites.

To detect the possible influence of the fungal metabolites **1**–**9** on Nrf2/Keap1/ARE antioxidant cellular machinery, we investigated their interactions with the Kelch domain of Keap1 by bioinformatic methods using molecular docking. Moreover, terrein (**10**) was used as a known fungal modifier of the Nrf2/Keap1 interaction.

2. Materials and Methods

The pdb file of the Kelch domain (PDB ID 6I0M) was obtained from the RCSB Protein Data Bank (https://www.rcsb.org accessed on 28 September 2023) and prepared for docking by PrepDock package of UCFS Chimera 1.16 software. The pdb file of the X-ray-determined structure of the Kelch/Nrf2 complex (PDB ID 2FLU) was obtained from RCSB Protein Data Bank (https://www.rcsb.org accessed on 28 September 2023), and the Nrf2 structure was exported in mol2 format for docking. The chemical structures of ligands were prepared for docking by ChemOffice and checking by PrepDock package of UCFS Chimera 1.16 software.

The docking was made by SwissDock online server (http://www.swissdock.ch accessed on 28 September 2023) based on the docking software EADock DSS [24]. The algorithm implies the generation of many binding modes in the vicinity of all target cavities (blind docking) and estimation of their CHARMM energies via the Chemistry at Harvard Macromolecular Mechanics (CHARMM) algorithm [25] for evaluation of the binding modes with the most favorable energies from Fast Analytical Continuum Treatment of Solvation (FACTS) [26] and, finally, clustering of these binding modes [27]. First, the ligand/protein complexes are ranked according to the total energy of the system calculated with the CHARMM22 molecular mechanics force field. Second, clusters of complexes are formed and confronted to the FullFitness function. The most favorably ranked complex is chosen as the center for the first cluster. Its neighboring complexes in the population are assigned to this first cluster. The next most favorably ranked complex is chosen as a center for

the second cluster, and its neighbors are assigned to this second cluster. This procedure continues until all complexes of the population have been assigned to a cluster [28].

The FullFitness of a cluster in EADock family of algorithms is calculated by averaging the 30% most favorable effective energies of its elements, and the effective energy (Geff) is written as the sum of the total energy of the system and a solvation term. Neglecting the solute entropic contribution, it can be written as:

$$Geff = E^{lig}{}_{intra} + E^{rec}{}_{intra} + E_{intra} + \Delta G_{elec,solv} + \sigma \times SASA,$$

where $E^{lig}{}_{intra}$ and $E^{rec}{}_{intra}$ are the internal energy of the ligand and the receptor, respectively. They are equal to the sum of the internal bonded (bonds, angles, etc.) and nonbonded (electrostatic and van der Waals interactions) terms. E_{intra} is the interaction energy between the ligand and the receptor, and is equal to the sum of the van der Waals and electrostatic interaction energies. $\Delta G_{elec,solv}$ is the electrostatic solvation energy. σ is the nonpolar contributions and can be considered as the sum of a cavity term, and a solute–solvent van der Waals term SASA is the solvent accessible surface area [28].

The FullFitness scoring function used for clustering and evaluation of predicted binding modes (BMs) occurring between a small molecule and a target protein as a third step after the generation and rough scoring of BMs and their minimization [27].

The obtained predicted building models for each compound/Keap1 pair were visualized and analyzed by UCFS Chimera 1.16 software. The docking parameters Gibb's free energy (ΔG, kcal/mol), FullFitness score (FF, kcal/mol), and hydrogen bonds (H-binding amino acid residue and distance) were used for analysis of target/ligand complexes.

3. Results

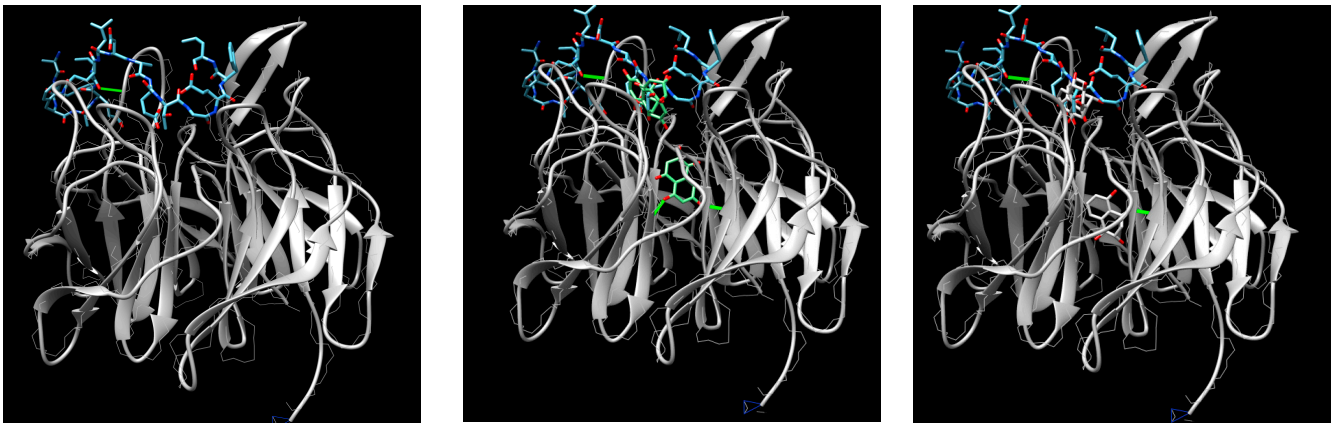
First, the molecular docking of the Neh2 domain of the Nrf2 peptide (69–84 a.o.), having an EGTE motif with A and B chains of the Kelch domain (PDB ID 6I0M), was carried out. The obtained clusters were with well-known X-ray data of Kelch–Nrf2 complex (PDB ID 2FLU) structures, and one cluster was used for next step of our investigation. The molecular docking data of this complex are presented in Table 1 and visualized in Figure 2a.

So, the calculated building site of the Nrf2 peptide with the Kelch domain includes amino acid residues such as Arg415 and Arg483, as well as Ser439, Arg459, Hsd436, and Thr481. The free Gibb's energy of this complex was calculated as −14.760137 kcal/mol, and the FF score as −900.57 kcal/mol. The hydrophobic interactions between the Nrf2 and Kelch domains in this complex were detected with Tyr525, Ala556, Tyr572, Tyr334, Cys434, ILe435, ILe461, Gly462, Phe478, Gly480, and Thr481 of the Kelch domain chain.

Then, the structures of compounds **1–9** were prepared for molecular docking with the Kelch domain. The obtained clusters were analyzed and compared with the Kelch/Nrf2 docked building site. The molecular docking data of two complexes with minimal ΔG value and/or maximal amount of H-bond in Kelch/Nrf2 binding site are presented in Table 2.

Table 1. The molecular docking data of Keap1 Kelch domain/Nrf2 complex.

ΔG (kcal/mol)	Full Fitness (kcal/mol)	H-Binding Residue/ H-Bonding Distance, A°
−14.760137	−900.57	Arg 415 ⟷ O23/2.674 O19 ⟷ Arg 415/2.682 Arg 415 ⟷ O23/2.068 Arg 415 ⟷ O19/2.072 Arg 483 ⟷ O14/2.386 O5 ⟷ Ser 439/2.445 Arg 459 ⟷ O3/1.835 O6 ⟷ Hsd 436/2.105 O8 ⟷ Thr 481/2.021



(**a**) (**b**) (**c**)

Figure 2. The molecular docking of the Kelch (white) and Nrf2 (blue) complex (**a**). The superpositions of complex of the Kelch domain (white) with Nrf2 (blue) and complexes of the investigated compounds (**b**) 4-hydroxyscytalone (green) and (**c**) 4-hydroxy-6-dehydroxyscytalone (white) with Kelch. The red color indicates the oxygen atoms in compound's structures. The green lines show predicted hydrogen bonds between the compounds and Keap1.

Table 2. The molecular docking data of predicted complexes between the Kelch domain of Keap1 and the investigated marine fungal metabolites.

Compound	Cluster	ΔG (kcal/mol)	Full Fitness (kcal/mol)	H-Binding Residue/ H-Bonding Distance, A°	Location in Nrf2-Binding Site of Keap1
Gliorosein (**1**)	2	−6.909368	−1111.3994	Val606 ⟷ O/2.049 Val418 ⟷ O1/2.309 Val465 ⟷ O1/2.543	no
	1	−6.7755146	−1112.5961	Arg415 ⟷ O/2.004 Arg415 ⟷ O/2.497	yes
4″-Hydroxycandidusine A (**2**)	26	−8.116934	−1084.1508	Val606 ⟷ O4/2.123 H8 ⟷ Val463/2.382 Val465 ⟷ O3/2.215	no
	2	−7.9192557	−1090.6539	Val606 ⟷ O4/2.486 Val465 ⟷ O3/2.202	no
Niveoglaucin A (**3**)	4	−7.22126	−1138.7821	H3 ⟷ Ala510/1.975 H8 ⟷ Val418/1.894 Val418 ⟷ O2/2.080	no
	3	−6.7057815	−1139.0631	Arg415 ⟷ O2/2.704 H3 ⟷ Leu365/2.164	yes
	13	−6.941605	−6.941605	H3 ⟷ Ser363/2.201	yes
Terphenyllin (**4**)	18	−6.979565	−1051.2461	Val418 ⟷ O3/1.976 Gly367 ⟷ O2/1.940 H11 ⟷ Gly367/2.021 Val606 ⟷ O2/2.176 Val606 ⟷ O4/2.621	no
	0	−7.871525	−1079.2283	Val465 ⟷ O2/2.322 H11 ⟷ Val465/1.927	no

Table 2. *Cont.*

Compound	Cluster	ΔG (kcal/mol)	Full Fitness (kcal/mol)	H-Binding Residue/ H-Bonding Distance, A°	Location in Nrf2-Binding Site of Keap1
3′-Hydroxyterphenyllin (**5**)	6	−8.2964945	−1072.0981	H10 ⟷ Val418/1.719 Val606 ⟷ O4/2.171 Val465 ⟷ O3/2.433	no
	0	−7.9774666	−1076.5175	H11 ⟷ Val606/1.803 Val465 ⟷ O4/2.394 H10 ⟷ Leu365/2.110 Val606 ⟷ O3/2.478	no
3″-Hydroxyterphenyllin (**6**)	0	−7.9407578	−1075.1831	H8 ⟷ Val463/2.042 H10 ⟷ Ile559/2.486 Thr560 ⟷O2/2.469	no
	26	−7.0460024	−1068.861	Thr560 ⟷ O1/2.321 H9 ⟷ Val606/1.950 Val369 ⟷ O2/2.526 Gly423 ⟷ O3/2.232 Asp422 ⟷ O/2.324	no
6-Hydroxy-N-acetyl-β-oxotryptamin (**7**)	1	−7.4022284	−1135.5000	H5 ⟷ Val606/1.901 H3 ⟷ Val512/1.949	no
	23	−6.5958056	−1131.7983	Arg483 ⟷ O2/2.564 Arg415 ⟷ O1/2.703	Yes
4-Hydroxyscytalone (**8**)	1	−7.197147	−1133.7689	Val606 ⟷ O3/2.464 Val512 ⟷ O2/2.414 H9 ⟷ Val604/2.132	no
	5	−6.555147	−1132.1863	Arg483 ⟷ O3/2.294 H6 ⟷ Ser555/2.154	yes
	12	−6.406861	−1133.5903	Arg483 ⟷ O1/2.400 Arg483 ⟷ O2/2.801 H9 ⟷ Ser555/2.364 Gln530 ⟷ O4/2.445	yes
4-Hydroxy-6-dehydroxyscytalone (**9**)	15	−7.199379	−1114.5966	H7 ⟷ Gly367/2.664 Gly367 ⟷ O1/2.155 Val606 ⟷ O1/2.084 H9 ⟷ Val465/2.201 H7 ⟷ Val606/1.114	no
	7	−6.592193	−1121.9755	no	yes
	21	−6.27284	−1117.4307	no	yes
Terrein (**10**)	19	−5.9071436	−1112.7003	Arg483 ⟷ O1/2.084 Arg483 ⟷ O2/2.215	yes
	12	−5.8997755	−1106.2816	Ser508 ⟷ H4/2.276	yes

All investigated *p*-terphenyl polyketides terphenyllin (**4**), 3′-hydroxyterphenyllin (**5**) and 3″-hydroxyterphenyllin (**6**) did not dock with the Kelch domain in its site for binding with Nrf2. The complex of terphenyllin (**4**) was calculated with a ΔG of −7.871525 kcal/mol, two H-bonds with Val465 or a ΔG of −6.979565 kcal/mol and five H-bonds with Val418, Gly367, and Val606. FF scores were calculated as −1079.2283 kcal/mol and −1051.2461 kcal/mol, respectively.

The complex of 3′-hydroxyterphenyllin (**5**) was calculated with ΔG of −8.2964945 kcal/mol and three H-bond (Val418, Val606 and Val465) or with ΔG of −7.9774666 kcal/mol and four H-bond (Leu365, Val606 and Val465). FF scores were calculated as −1072.0981 kcal/mol and −1076.5175 kcal/mol, respectively.

The complex of 3″-hydroxyterphenyllin (**6**) was calculated with ΔG of −7.9407578 kcal/mol and three H-bonds (Ile559, Val463, Thr560), or with ΔG of −7.0460024 kcal/mol and

five H-bond (Thr560, Val606, Val369, Gly423 and Asp422). FF scores were calculated as −1075.1831 kcal/mol and −1068.861 kcal/mol, respectively.

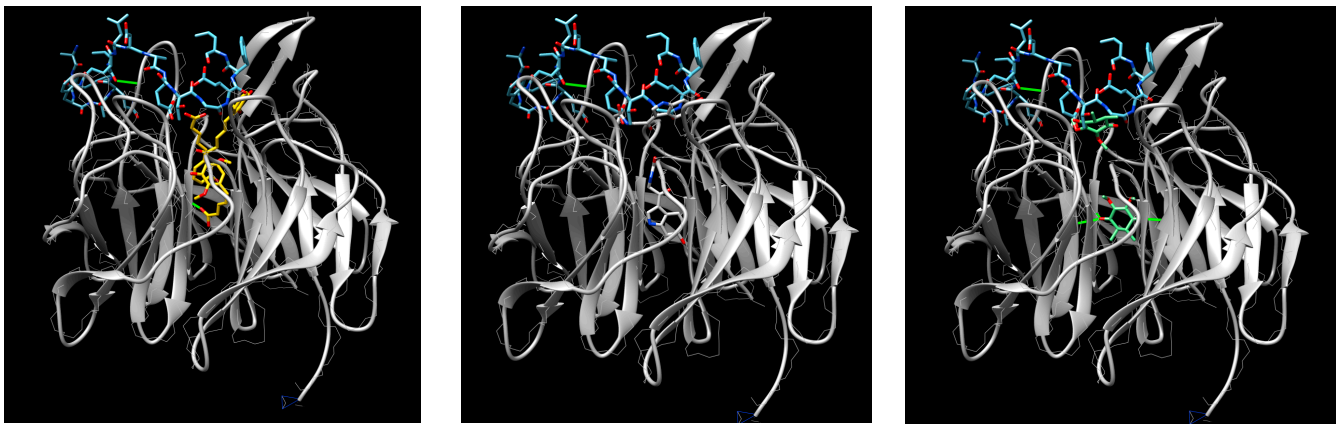
Polyketide 4″-hydroxycandidusine A (**2**) also did not dock in the Kelch/Nrf2 binding site of the Kelch domain. The complex of 4″-hydroxycandidusine A was calculated with a ΔG of −8.116934 kcal/mol and three H-bonds (Val463, Val606 and Val465), or with a ΔG of −7.9192557 kcal/mol and two H-bonds (Val606 and Val465). FF scores were calculated as −1084.1508 kcal/mol and −1090.6539 kcal/mol, respectively.

Other polyketides niveoglaucin A (**3**), 4-hydroxyscytalone (**8**), and 4-hydroxy-6-dehydroxyscytalone (**9**) were docked with similar sites of the Kelch domain to the Kelch/Nrf2 binding site.

Two complexes of 4-hydroxyscytalone (**8**) were docked in the Kelch/Nrf2 binding site (Figure 2b). The first has a ΔG of −6.555147 kcal/mol, two H-bonds with Arg483 (similar to Nrf2), and Ser555 amino acid residues. And the second model has a ΔG of −6.406861 kcal/mol, two H-bonds with Arg483 (similar to Nrf2), and two H-bonds with Ser555 and Gln530 a.a. residues. The FF scores of these both were calculated as −1132.1863 kcal/mol and −1133.5903 kcal/mol, respectively. The hydrophobic interactions of 4-hydroxyscytalone (**8**) were calculated with Gly509 and ILe461, Gly462, Phe 478, and Tyr525 of the Kelch/Nrf2 binding site for the first model, and Gly509 and ILe461, Gly462, PHE 478, Tyr525, and Ala556 for the second model.

Two complexes of 4-hydroxy-6-dehydroxyscytalone (**9**) were docked in the Kelch/Nrf2 binding site, similarly to 4-hydroxyscytalone (**8**) (Figure 2c). The first has a ΔG of −6.555147 kcal/mol and an FF score of −1121.9755 kcal/mol, and the second has a ΔG of −6.27284 kcal/mol and an FF score of −1117.4307 kcal/mol. H-bonds for both of these were not calculated. The hydrophobic interactions of 4-hydroxy-6-dehydroxyscytalone (**9**) were calculated with Gly509 and ILe461, Gly462 and Tyr525 of the Kelch/Nrf2 binding site for both models.

The complex of niveoglaucin A (**3**)/Kelch with a ΔG of −6.7057815 kcal/mol and an FF score of −1139.0631 kcal/mol bonded with Leu365 and Arg415 from Kelch/Nrf2 binding site (Figure 3a). This calculated model includes hydrophobic interactions of niveoglaucin A (**3**) with Gly364, Leu365, ILe416, Val463, Gly509, Ala510, Gly603, Val604, and Tyr334, Gly462, and Ala556 from the Kelch/Nrf2 binding site.



(**a**) (**b**) (**c**)

Figure 3. The superpositions of complex of Kelch domain (white) with Nrf2 (blue) and the complexes of the investigated compounds niveoglaucin A (yellow) (**a**), 6-hydroxy-N-acetyl-β-oxotryptamin (white) (**b**), and gliorosein (green) (**c**) with the Kelch domain. The red color indicates the oxygen atoms in the compound's structures. The green lines show a predicted hydrogen bonds between compounds and Keap1.

Alkaloid 6-hydroxy-N-acetyl-β-oxotryptamin (**7**) were docked in Kelch/Nrf2 binding site with a ΔG of −6.5958056 kcal/mol, an FF score of −1131.7983 kcal/mol, and

two H-bonds with Arg483 and Arg415 (Figure 3b). The hydrophobic interactions of **7** were calculated with Gly509, Phe577 and ILe461, Gly462 and Tyr525 of the Kelch/Nrf2 binding site.

Polyketide gliorosein (**1**) was also docked in the Kelch/Nrf2 binding site with a ΔG of −6.7755146 kcal/mol, an FF score of −1112.5961 kcal/mol, and two H-bonds with Arg415 (Figure 3c). The hydrophobic interactions of gliorosein (**1**) were calculated with Gly509 and Tyr525 and Ala556 of the Kelch/Nrf2 binding site.

Terrein (**10**) was docked in Kelch/Nrf2 binding site with a ΔG of −5.9071436 kcal/mol, an FF score of −1112.7003 kcal/mol, and two H-bonds with Arg483. The hydrophobic interactions of terrain (**10**) were calculated with Phe478, Gly509, and Tyr525. Another pose of terrain (**10**) was formed with a ΔG of −5.8997755 kcal/mol, an FF score of −1106.2816 kcal/mol and one H-bond with Ser508. The hydrophobic interactions of terrein (**10**) in this pose were calculated with Ala556, Tyr525, and Gly509.

4. Discussion

The binding between Keap1 and Nrf2 is limited by interactions between Keap1′s Kelch domain and two low-affinity (DLG) and high-affinity (ETGE) Nrf2 motifs. Based on the co-crystal structure of the Keap1–Nrf2 ETGE complex, the Keap1 binding pocket can be artificially divided into five sub-pockets, namely P1-P5: P1 and P2 contain most of the polar residues (Arg483, Ser508, Ser363, Arg380, and Arg415); P4 and P5 contain mostly non-polar residues including Tyr525, Gln530, Tyr572, Tyr334, and Phe577; the P3 sub-pocket consists of small polar and non-polar residues (Gly509, Ala556, Ser602, and Ser555). The small molecules interacting with one of these sub-pockets prevents the binding between Keap1 and Nrf2 and, thus, contributes to the nuclear translocation of Nrf2, resulting in ARE activation and overexpression of antioxidant effectors (Figure 4).

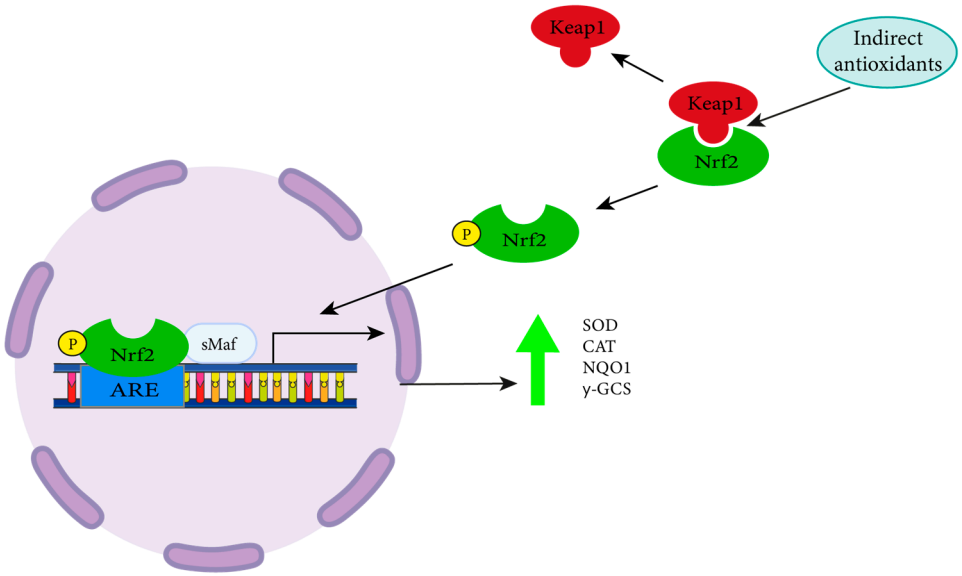


Figure 4. Schematization of indirect induction of cytoprotective proteins through Keap1/Nrf2/ARE system by antioxidant compounds. The picture adapted from [29]. The indirect antioxidants inhibit the interaction between Kelch-like ECH-associated protein 1 (Keap1) and nuclear factor erythroid 2-related factor 2 (Nrf2) that resulted in the release of Nrf2, its phosphorylation, and translocation in nucleus when Nrf2 combines with one of the small Maf proteins (sMaf) and binds to the antioxidant response element (ARE) in the upstream promoter region of many antioxidative genes, and initiates their transcription resulted in overexpression of superoxide dismutase (SOD), catalase (CAT), NAD(P)H dehydrogenase (quinone) 1 (NQO1), γ-glutamylcysteine synthetase (GCS), and others.

The X-ray spectroscopy of crystal of 16-mer peptide of Nrf2 with Keap1 found the specific electrostatic interactions that are made by both glutamate residues in the ETGE

motif of Nrf2. Glu79 in Nrf2 forms hydrogen bonds with Keap1 residues Arg415, Arg483, and Ser508, whereas Glu82 hydrogen bonds with Keap1 residues Ser363, Asn382, and Arg380 [30]. Moreover, electrostatic contacts mediated through water or the peptide backbone are supplemented by additional van der Waals interactions [3]. SwissDock calculations correspond to these data. Moreover, the calculated interactions of terrein (**10**) with Kelch in its Nrf2 binding site agree with the ability of this one caused nuclear translocation of Nrf2 [17].

In this work, marine fungal metabolites of two different chemical classes, such as melanin-related alkaloid 6-hydroxy-N-acetyl-β-oxotryptamine (**7**) and polyketides glliorosein (**1**), 4″-hydroxycandidusine A (**2**), niveoglaucin A (**3**), terphenyllin (**4**), 3′-hydroxyterphenyllin (**5**) and 3″-hydroxyterphenyllin (**6**), 4-hydroxyscytalone (**8**), and 4-hydroxy-6-dehydroxyscytalone (**9**), were evaluated as probable inhibitors of Nrf2/Keap1 interactions. As a result, the building of compounds **1**, **3**, **7**–**9** with Arg415 and/or Arg483 residues in Nrf2 binding site of Keap1 were computed. It should be noted that the binding energy predicted for complexes of **1**–**3** and **7**–**9** was low, and like the binding energy predicted for terrein (**10**). This may be due to the small number of bonds predicted for complexes, which, in turn, depends on the size of the molecule and the number of functional groups.

Our experimental data show that melatonin derivative 6-hydroxy-N-acetyl-β-oxotryptamine (**7**) acts as antioxidant in paraquat-treated Neuro-2a cells. Other melatonin derivatives were studied in surface plasmon resonance experiments, and their bonding with the Kelch domain of Keap1 was found [31]. The molecular dynamics simulations on the Keap1 Kelch domain showed that 5-[(E)-2-(5-methoxy-1H-indol-3-yl)ethenyl]-1,3,4-oxadiazol-2(3H)-one and 5-[(E)-2-(5-methoxy-1H-indol-3-yl)ethenyl]-3-(prop-2-yn-1-yl)-1,3,4-oxadiazol-2(3H)-one interact with several essential residues involved in the binding of Keap1/Nrf2. Both of these ones show luciferase activation in Nrf2-dependent luciferase reporter gene assay in the AREc32 cell line, which confirms its influence on the Keap1/Nrf2 pathway in in vitro experiments [31]. Our molecular docking data showed that a neuroprotective effect of a rare natural melatonin derivative 6-hydroxy-N-acetyl-β-oxotryptamine may be caused its interaction with the Kelch domain of Keap1, similarly to other melatonin-related alkaloids.

Earlier, we found polyketide niveoglaucin A (**3**) as a neuroprotective compound against 6-OHDA-induced toxicity and ROS overexpression [21]. Recently it was published that the *Aspergillus chevalieri* TM2-S6 extract containing another auroglaucine-related tetrahydroauroglaucin and flavoglaucin as main compounds protects human fibroblasts under oxidative stress via induction of Nrf2-dependent antioxidant pathway. Our data predict the interaction of niveoglaucin A (**3**) with Kelch domain Keap1 that can cause translocation Nrf2 and activation of antioxidant defense.

Polyketides with *p*-terphenyl moiety **4**–**6** were found as cytoprotective compounds against paraquat and rotenone toxicity and ROS overexpression [20]. Our predicted models show that the planar structure of p-terphenyl polyketides apparently does not allow them to stably form complexes with the Kelch domain of Keap1 at the site of binding to Nrf2, and other data about influence of *p*-terphenyl-derived polyketides on Keap1/Nrf2 interaction in in vitro experiments are absent in scientific databases. Nevertheless, the cytoprotective properties of *p*-terphenyl-derived polyketides were reported, and it may be provided by direct radical scavenging activity of these compounds.

In previously published experiments, scytalone derivatives **8** and **9** were effective against paraquat and rotenone toxicity and ROS overexpression [22]. Now, the predicted complexes of polyketide 4-hydroxyscytalone (**8**) and 4-hydroxy-6-dehydroxyscytalone (**9**) with the Kelch domain confirmed that these may prevent Keap1/Nrf2 interactions and thereby facilitate translocation of Nrf2 into the nucleus. The molecular docking data show that the presence of the OH group at C-6 in the structure of 4-hydroxyscytalone (**8**) may ensure the formation of hydrogen bonds in the complex of this polyketide with the Kelch domain and lower ΔG value in contrast with 4-hydroxy-6-dehydroxyscytalone (**9**). The experimental data on the effect of naphthalenone derivatives **8** and **9** on Keap1/Nrf2 interaction were not published yet. It was reported only that naphthalene-naphthalenone dimers

from the traditional Chinese medicinal fungus *Xylaria nigripes* decreased the levels of malondialdehyde, and increased the levels of superoxide dismutase and glutathione peroxidase in PC12 cells by oxygen and glucose deprivation, which can indicate these influences on the Keap1/Nrf2 pathway, although this does not indicate it unequivocally [32].

Finally, gliorosein (**1**) has shown cardioprotective activities against rotenone and cobalt chloride (II) toxicity, and its weak DPPH radical scavenging activity cannot fully explain its significant effect in in vitro experiments [19]. Experimental data about effect of gliorosein (**1**) or similar tetraketides on Keap1/Nrf2 interaction was not published.

Thus, the molecular docking of several marine fungal metabolites with Keap1 predicted that gliorosein (**1**), 4″-hydroxycandidusine A (**2**), niveoglaucin A (**3**), 6-hydroxy-N-acetyl-β-oxotryptamine (**7**), 4-hydroxyscytalone (**8**), and 4-hydroxy-6-dehydroxyscytalone (**9**) can form the hydrogen binding with P1-P2 sub-pockets of Kelch domain of Keap1. The effect of these compounds on Keap1/Nrf2 interaction in living systems should be confirmed for future study.

At the present time, a number of approach can be used for cell-free and cell-based investigation of compounds' influence on Nrf2/Keap1 interaction. For example, to determine the effect of the inhibitor on the formation of Keap1/Nrf2 complexes, the Keap1 protein and the fluorescent Nrf2 peptide are incubated with or without the test inhibitor, and changes in rotational mobility of the Nrf2 peptide are measured using a plate reader capable of measuring fluorescence polarization [33]. The binding affinities of small molecules to the Kelch domain of Keap1 in solution can be directly experimented using a surface plasmon resonance-based competition assay [34]. The cell lines contains a stably integrated firefly luciferase gene under the control of ARE, which is recognized by Nrf2 [35], or cell lines, transfected with ARE luciferase reporter vector, which is an Nrf2 pathway-responsive reporter [36], can be used for in vitro cell-based experiments. These actual approaches can be used in future investigations of the influence of promising marine fungal metabolites on Nrf2/Keap1 interaction. Moreover, the off-target effects should be studied before any practical conclusions can be made, because small molecules are multi-targeted due to their small size. Nevertheless, the absence of toxicity in compounds **1**–**3** and **7**–**9** toward Neuro-2a or H9c2 cells in earlier tests is encouraging.

5. Conclusions

Earlier, several marine fungal metabolites were reported as antioxidants in cell-based experiments. Now, the virtual screening of these compounds has been carried out in computational modeling of their interactions with Keap1. It was calculated that gliorosein (**1**), 4″-hydroxycandidusine A (**2**), niveoglaucin A (**3**), 6-hydroxy-N-acetyl-β-oxotryptamine (**7**), 4-hydroxyscytalone (**8**), and 4-hydroxy-6-dehydroxyscytalone (**9**) may interact with Arg415 or Arg483 residue in Nrf2 binding site of Keap1 and, so, inhibit of Keap1/Nrf2 interaction. This may induce the activation of intracellular antioxidant defense system and a decrease in ROS level which was reported earlier. Therefore, these marine fungal compounds may be promising as modulators of the Keap1/Nrf2-dependent antioxidant system in future experiments.

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