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Interaction of Asparagusic Acid, Asparaptines and Related Dithiolane Derivatives With Angiotensin-Converting Enzyme-2 (ACE-2): A Molecular Docking Study

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ABSTRACT

A variety of sulfur-containing small molecules can be found in the spears of asparagus (*Asparagus Officinalis* L.) including compounds derived from asparagusic acid such as the amino acid derivatives asparaptines A, B, and C. The previous characterization of asparaptine A as an inhibitor of angiotensin-converting enzyme (ACE) prompted us to compare the binding of the three asparaptines to ACE2 using molecular modeling. The lysine conjugate asparaptine B was found to bind better to the enzyme than the arginine (asparaptine A) and histidine (asparaptine C) conjugates. The stability of ACE2-asparaptine B complexes was only a little inferior to that observed with the reference ACE2 inhibitor MLN-4760. On this basis, 20 additional compounds bearing a thiol group or a dithiolane motif were evaluated as potential binders to ACE2 using the same docking methodology. Three compounds emerged as robust ACE2 binders: the natural products isovalthine and N-acetyl-felinine, and the drug candidate CMX-2043. The empirical energy of interaction (ΔE) of N-acetyl-felinine with ACE2 was comparable to that measured with asparaptine B, and a little higher with the thiol metabolite isovalthine. Remarkably, CMX-2043 revealed a high capacity to form stable complexes with ACE2, superior to that of the reference MLN-4760. Both the L-Glu-L-Ala dipeptide motif and the α-lipoic acid moiety of CMX-2043 are implicated in the protein interaction. Our observations pave the way to the design of novel ligands of ACE2 equipped with a dithiolane motif.

1 | Introduction

Asparagus is one of the most common plants exploited for its nutritional and medicinal benefits. The *Asparagus* family (order Asparagaceae) comprises about 300 species, including the major species *A. officinalis* L. which is widely cultivated as a vegetable crop (also known as sparrow grass). This perennial flowering plant is well-known for its nutritional and pro-health properties, notably its marked diuretic effect. Asparagus spears contain a variety of bioactive and healthy compounds, including saponins, alkaloids, carotenoids, phenolic compounds, as well as oligo- and polysaccharides, dietary fibers, vitamins and minerals [1]. Fresh, cooked and processed asparagus spears are largely consumed worldwide. In addition, asparagus root powder or extracts are used as additives to prepare different food products, such as beverages and bakery products [2]. Many processing technologies have been developed to exploit the plant while preserving the product quality and to enhance the yields of bioactive substances [3]. The nutraceutical compounds found in *Asparagus* display antioxidant, anti-inflammatory,

Abbreviation: ACE, angiotensin-converting enzyme.

neuroprotective effects of interest in the management of neurological conditions such as anxiety, depression, epilepsy, Parkinson's, and Alzheimer's disease [4].

Sulfur-containing compounds are largely present in the spears of asparagus. A recent study inventoried 80 S-containing metabolites in spears of green and white *Asparagus* varieties, contributing to their peculiar taste and odorous properties [5]. They contribute importantly to the antioxidant and anti-inflammatory effects of *A. officinalis* extracts [6]. Asparagus contains unusual sulfhydryl compounds such as asparagusic acid, and amino acid derivatives such as asparaptines A-B-C, and a few other compounds with a dithiolane motif [7] (Figure 1). Together with other sulfur-containing alkyl compounds, asparagusic acid contributes importantly to the pungent urinary odor produced by certain individuals within a few hours of eating asparagus [8, 9]. Despite this olfactory characteristic, asparagusic acid is a nontoxic compound very useful as a cellular delivery system via the transferrin receptor-mediated uptake pathway. Liposomes functionalized with asparagusic acid can be used to enhance cellular drug delivery [10]. The appendage of a single asparagusic acid molecule is sufficient to ensure efficient cellular uptake and intracellular distribution of peptides via the transferrin receptor [11].

Asparaptine A is a conjugate compound derived from asparagusic acid and arginine (Figure 1). The compound was initially discovered in the spears of *A. officinalis* and characterized as an inhibitor of angiotensin-converting enzyme (ACE) [12, 13]. It contributes importantly to the blood pressure-lowering action of asparagus via inhibition of ACE and β-antagonistic effects [14]. Asparaptine A accumulates at high levels in developing lateral shoot of *A. officinalis* tissues but its endogenous functions are not known at present [15]. Ņikitjuka and Žalubovskis mentioned the isolation of 61 mg of asparaptine A from 57.2 g of lyophilized *Asparagus officinalis* L. powder [7]. In nature, the production of the compound is subject to seasonal variations depending on the harvesting period [16, 17] but it can be controlled via the preparation of callus and suspension cell lines from asparagus [18].

Two structural analogs of asparaptine A have been identified in a metabolomic study using asparagus calluses. Asparaptines B and C correspond to the lysine and histidine conjugates of asparagusic acid [14] (Figure 1). Nothing is known at present about their biological functions in the plant and their potential medicinal interest. Here we have analyzed and compared the interaction of the three asparaptine derivatives with ACE using a molecular docking approach. Asparaptine B was identified as a potentially tighter binder to ACE compared to asparaptines A and C. In a second time, a search for related sulfur-containing compounds capable of interacting with ACE led to the identification of the branched-chain sulfur amino acid isovalthine and the drug candidate CMX-2043 as other potential ACE binders, among a series of 20 sulfur-containing compounds. The study will help the identification and rational design of other compounds targeting ACE.

2 | Materials and Methods

2.1 | Molecular Structures and Software

The three-dimensional structure of human angiotensin converting enzyme-related carboxypeptidase (ACE2) was retrieved from the protein data bank (www.rcsb.org) under the pdb code 1R4L. The crystallographic protein structure (with a resolution of 3.00 Å) comprises 805 amino acids and includes a zinc-binding consensus sequence. It includes also two glycosylation sites at Asn90 and Asn103 [19]. Docking experiments were performed using the GOLD software (GOLD 5.3 release, Cambridge Crystallographic Data Centre, Cambridge, UK). Before the docking operations, the structure of each ligand was optimized using a classical Monte Carlo conformational searching procedure via the BOSS software [20]. Molecular graphics and analysis were performed using Discovery Studio Visualizer, Biovia 2020 (Dassault Systèmes BIOVIA Discovery Studio Visualizer 2020, San Diego, Dassault Systèmes, 2020). Potential drug-binding sites for the different molecules were searched using the web server Computed Atlas of Surface Topography of proteins (CASTp) 3.0 and visualized with the molecular modeling software Chimera 1.15 [21]. Compounds with a dithiolane motif were screened essentially from the PubChem database and selected based on their physicochemical properties.

2.2 | In Silico Molecular Docking Procedure

The process used includes the following five steps:

1. Monte Carlo (MC) conformational search of the ligand using the BOSS (Biochemical and Organic Simulation System) software, freely available to academic users. The structure of the ligand was optimized using a classical MC conformational search procedure, as described in BOSS [22]. The protein preparation scheme includes a determination of the protonation states of the amino acids, treatment of water molecules in the binding cavity and the assignment of hydrogen atoms to allow formation of a protein hydrogen bond network. A conformational

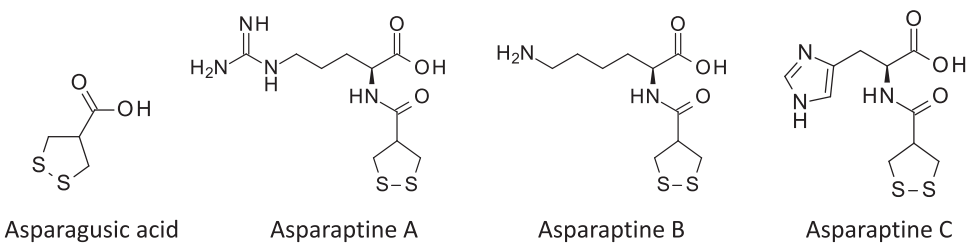

FIGURE 1 | Structures of the studied compounds.

analysis has been performed to define the best starting geometries for each compound. An energy minimization was carried out to identify all minimum-energy conformers, leading to the identification of a unique conformer for the free ligand. Within BOSS, MC simulations were performed in the constant-temperature and constant-pressure ensemble (NPT).

2. Evaluation of the free energy of hydration for the chosen structure of the ligand. The molecular mechanics/generalized Born surface area (MM/GBSA) procedure was used to evaluate the free energies of hydration (ΔG) [20]. MC search and computation of ΔG were performed within BOSS using the xMCGB script according to procedures given in references [20, 23]. The best ligand structure is then used in the docking procedure.

3. Definition of the ACE2 site of interaction. In the crystallographic structure, the ACE2 active site is occupied with the potent inhibitor MLN-4760 ((S,S)-2-[1-carboxy-2-[3-(3,5-dichlorobenzyl)-3H-imidazol4-yl]-ethylamino]-4-methylpentanoic acid). The same binding site has been considered as the potential binding site in our study for the studied compounds. During the process, the side chains of the following amino acids within the binding site were rendered fully flexible: Tyr127, Trp271, His345, Glu375, Phe504, His505, Tyr510, Phe512, Arg514, and Tyr515. Shape complementarity and geometry considerations favor a docking grid centered in the volume defined by the central amino acid. The side-chain protonation state of ionizable residues (Asp, Glu, His, Tyr, Lys) and the tautomeric forms of histidine (His) are controlled.

4. Docking procedure using GOLD. In our typical docking process, 100 energetically reasonable poses (according to the ChemPLP scoring function) are retained while searching for the correct binding mode of the ligand. The decision to maintain a trial pose is based on ranked poses, using the PLP fitness scoring function (which is the default in GOLD version 5.3 used here) [24]. Six poses are kept. The empirical potential energy of the interaction ΔE for the ranked complexes was evaluated using the simple expression ΔE(interaction) = E(complex) − [E(protein) + E(ligand)]. Calculations of the final energy are performed on the basis of the SPASIBA spectroscopic force field. SPASIBA reproduces vibrational frequencies (with a higher accuracy than molecular mechanics potentials), and potential energy distributions of normal modes. It reproduces crystal-phase infrared data. The parameters are derived from vibrational wavenumbers obtained in the infrared and Raman spectra of a large series of compounds including organic molecules, amino acids, saccharides, nucleic acids and lipids [25].

5. Validation using the SPASIBA force field. This last step is considered essential to define the best protein-ligand structure. The spectroscopic SPASIBA (Spectroscopic Potential Algorithm for Simulating Biomolecular conformational Adaptability) force field has been specifically developed to provide refined empirical molecular mechanics force field parameters [26]. SPASIBA empirical energies of interaction are calculated as described by Lagant and coworkers [27, 28]. SPASIBA (integrated into CHARMM) [29] has been shown to be excellent to reproduce crystal phase infrared data. Using this specific force field for, Monte Carlo (MC) simulations achieve the same level of convergence as Molecular Dynamics (MD), with less computer time [22]. The same procedure was used to establish molecular models for the various drug-protein complexes.

3 | Results

3.1 | Molecular Docking of Asparaptines A-C to ACE2

The high-resolution crystal structure of human angiotensin converting enzyme-related carboxypeptidase 2 (ACE2, pdb: 1R4L) was used to model the interaction of the asparaptines A, B and C with the protein via a molecular docking approach. The protein presents a well-defined drug binding site, occupied with the inhibitor MLN-4760 in the crystal structure. This site offers a large and deep cavity in which different types of ligands can bind. Asparaptines were docked into this cavity which is the only accessible site inside the protein. A binding site analysis using the web server CASTp 3.0, which is a convenient tool to analyze the topography of proteins and to locate potential drug binding sites [21], indicated that this is the only possible drug binding site. This drug binding site could easily accommodate bulky ligand. The other surrounding cavities are considerably smaller, too small to accommodate a molecule of asparaptine (Figure 2a). A comparison of the 3D structure of MLN-4760-bound ACE2 (pdb: 1R4L) with that of the ligand-free protein (pdb: 1R42) showed that the binding of drug MLN-4760 has not significantly distorted the protein structure, as estimated notably from the distance between the chlorine and zinc atoms included in the protein structure: 24.1 Å for 1R4L versus 22.2 Å for 1R42. The presence of the ligand MLN-4760 tends to tighten locally the protein structure. For examples, the distances between residues T129 and N53 changes from 24.6 to 11.0 Å in the presence of the ligand, and the distances between residues T121 and E57 varies from 23.5 to 13.6 Å when the small molecule binds into the protein cavity.

The methodology used to investigate the binding of asparaptines to ACE2 was the same as that utilized in different molecular docking studies with other proteins [30–32]. We started with a modeling of MLN-4760 to the protein so as to evaluate the empirical energy of interaction (ΔE) and free energy of hydration (ΔG) for these isomeric molecules. The same analysis was then performed with asparagusic acid and asparaptines A-C. The calculated ΔE and ΔG values are reported in Table 1. The ΔE values were more favorable (more negative) with the three asparaptines compared to asparagusic acid. The amino acid moiety participates to the protein interaction. The best ΔE value was obtained with asparaptine B, providing a much better protein interaction than asparaptines A and C. A molecular model of the ACE2-asparaptine B complex is shown in Figure 3. The compound fits well into the large protein cavity and establishes several contacts with key amino acid residues. Notably, the dithiolane motif engages in two π-sulfur interactions with Phe504 and Tyr510, whereas the lysine moiety establishes several H-bonds with Glu402, Tyr515, Thr571 and a

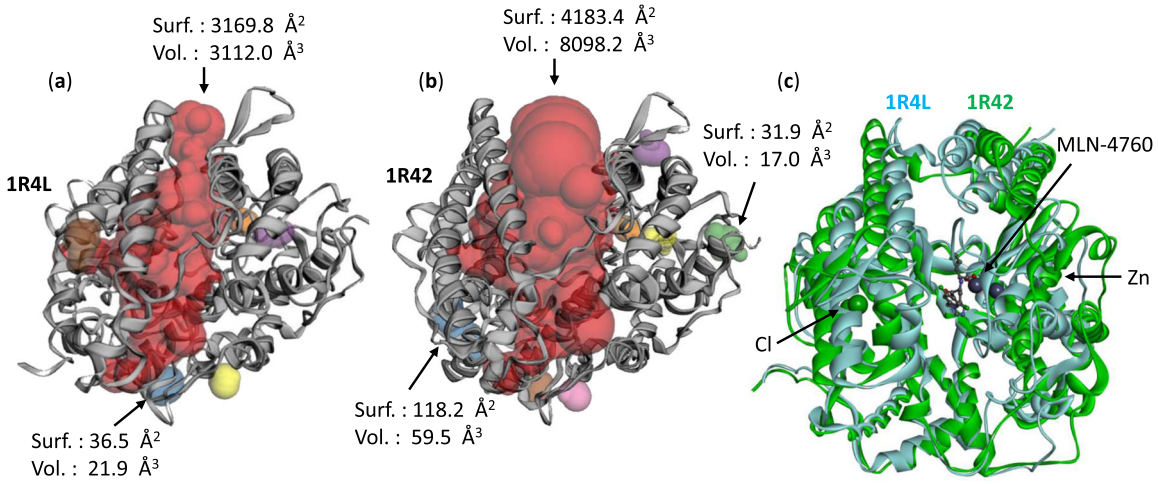

FIGURE 2 | (a) Binding site analysis of ACE2 using web server CASTp 3.0. The analysis of the full-length protein (1R4L) reveals the presence of a large central cavity (in red), and several small cavities (colored) surrounding the main sites. The sizes of the main site (in red) and two minor areas (in green and blue) are indicated (surface and volume) for comparison. (b) Superimposed models of ACE2 bound with MLN-4760 (PDB: 1R4L) and the ligand-free protein (PDB: 1R42).

TABLE 1 | Calculated potential energy of interaction (ΔE) and free energy of hydration (ΔG) for the interaction of the indicated products with ACE2.

Compounds	ΔE (kcal/mol)	ΔG (kcal/mol)
MLN-4760	−165.20	−34.40
Asparagusic acid	−102.00	−31.70
Asparaptine A	−128.70	−31.85
Asparaptine B	−146.25	−34.50
Asparaptine C	−122.70	−35.40

few other residues. The central carbonylamino motif plays a key role in the protein binding via interactions with His345, Glu375, Glu402, Arg514. Asparaptines A and C can also form complexes with ACE2 at the same site but they are a little less stable than those obtained with asparaptine B. The calculated free energies of hydration (ΔG) are similar for asparaptines B and C but the imidazole group (histidine) of asparaptine C is less adapted to bind the protein than the lysine side chain of asparaptine B. The terminal ε-amino group of lysine participates to the interaction via residue Thr371 (Figure 3c). Over 15 molecular contacts stabilize the ACE2-asparaptine B, including interactions between the drug and the Zn^{2+} ion. ACE2 is a metalloprotease that requires Zn^{2+} for activity. The Zn^{2+} ion contributes to the structural stability of the ACE2 protein. It is bound in the catalytic site and modulates the protein activity, notably its interaction with protein partners, such as the spike protein (S) of SARS-CoV-2 [33, 34]. All three asparaptines were found to interact with the Zn^{2+} domain of the ACE2 catalytic site. A similar arrangement has been reported with bisartan-type angiotensin receptor blockers [35]. Asparaptine B is the best ligand in the series, placing its lysine side chain deep into the protein cavity and the dithiolane-carboxyl moiety at the top of the protein cavity. The molecule remains relatively well accessible to the solvent. The drug-protein complex is stabilized by an array of contacts.

3.2 | Screening of Compounds With a Dithiolane Motif

The discovery that asparaptine B can bind well to ACE2 prompted us to search for other thiol-containing compounds as potential ACE2 binders. Twenty compounds equipped with a dithiolane motif or a thiol function were selected (Figure 4) and evaluated for binding to ACE2 using the same methodology as used with the asparaptines. Molecular models were constructed and the ΔE and ΔG values were calculated for each compound (Table 2). The molecules can be categorized into three groups: (i) 10 compounds with a $\Delta E < 100$ kcal/mol, representing a weak binding, (ii) 5 compounds with a ΔE in the range 100–130 kcal/mol, as obtained with asparaptines A and C, and (iii) 5 compounds with a $\Delta E > 130$ kcal/mol, corresponding to the best binders.

The binding of asparagusic acid is considerably weakened when the acid function is replaced with a dimethylamino group, as in nereistoxin. This cationic group, also present in thiocyclam and bisultap (a metabolite of nereistoxin), is not well adapted to ACE2 binding. The trithiane unit of thiocyclam (also known as sultamine) or a trithiolane unit (as in diethyltrithiolane) is apparently not a good option to promote drug binding. The introduction of an amino group, as in 4-amino-1,2-dithiolane-4-carboxylic acid (Adt, also known as NSC212561) reduces the ACE2 binding capacity. Adt is a cysteine-related achiral residue used in peptides to constrain conformation [36]. Adt is a precursor to the synthesis of the natural product kottamide E isolated from ascidian *Pycnoclavella kottae* [37]. This dibrominated alkaloid showed a poor affinity for ACE2. In contrast, the substitution of the dithiolane unit of asparagusic acid to afford a S-oxide derivative reinforces significantly the protein interaction. A comparable improved binding can be observed when comparing the reduced form of asparagusic acid (dihydroasparagusic acid) with its S-acetyl derivative. This later compound bound much better to ACE2 than the corresponding dithiol derivative. Dihydroasparagusic acid is a naturally-occurring dimercaptanic product capable of inhibiting

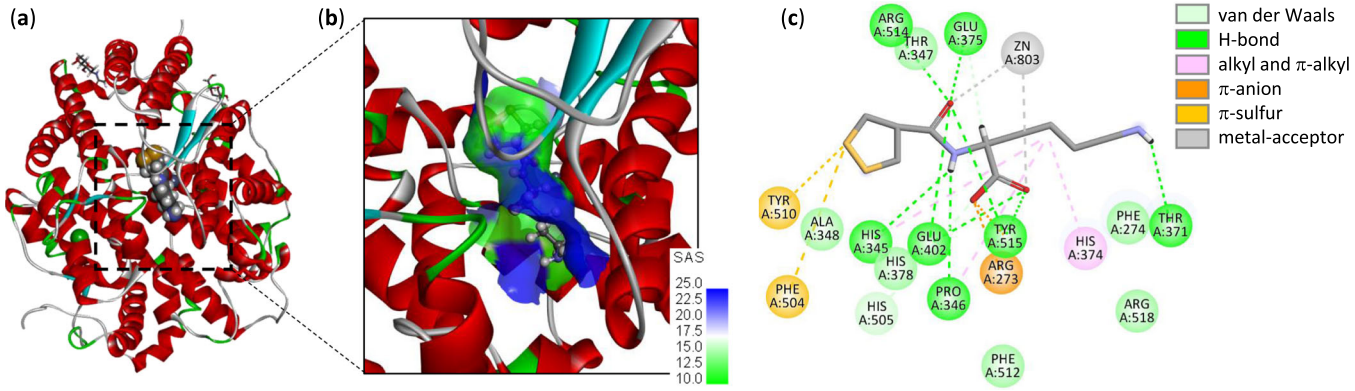

FIGURE 3 | (a) Molecular model of asparaptine B bound to the ACE2 protein (PDB: 1R4L). (a) The natural product bound to the entire protein (α-helices in red and β-sheets in blue). (b) A close-up view of the asparaptine B-bound ligand with the solvent-accessible surface (SAS) surrounding the drug binding zone (color code indicated). (c) Binding map contacts for asparaptine B bound to ACE2 (color code indicated).

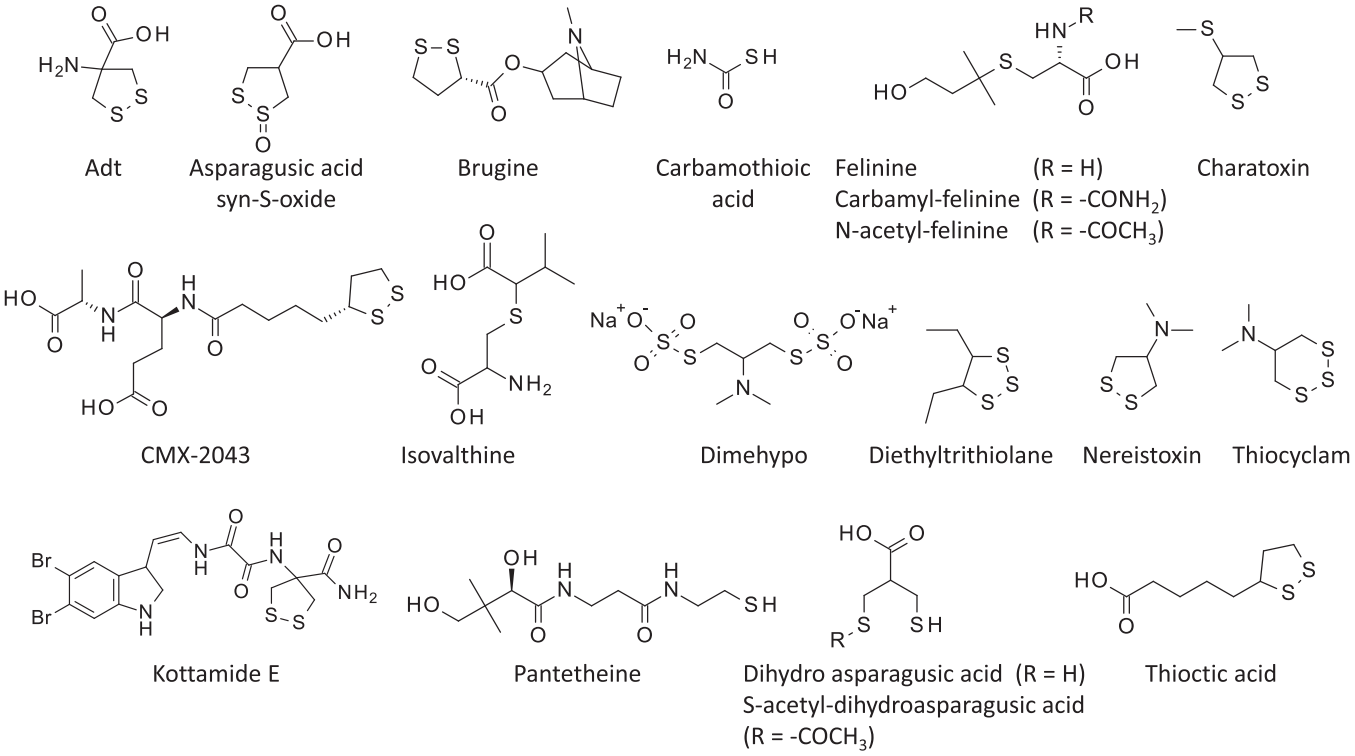

FIGURE 4 | Structures of the studied compounds.

inflammatory and oxidative processes [38, 39]. S-acetyl dihydroasparagusic acid is also a natural product, found in *A. officinalis* [40] but its pharmacological effects are not documented. This little-known compound deserves further studies as a potential ACE2 inhibitor.

The relative affinity of asparagusic acid for ACE2 is enhanced when the acid group is moved away from the dithiolane moiety, as in thioctic acid (also known as α-lipoic acid). In contrast, the replacement of the acid group with a S-methyl (as in charatoxin) has a profound negative impact on the protein binding. The marine compound brugine, also bearing a dithiolane motif, revealed a poor binding affinity to ACE2. This anticancer alkaloid, isolated from *Bruguiera* species, has been suggested recently to interact with protein kinase A [41]. The case of felinine is interesting because the substitution of the amino group with an acetyl or a carbamyl group reinforces the interaction with the target protein. N-acetyl-felinine is almost equivalent to asparaptine B in term of ACE2 binding. The N-acetyl group plays a marked role, via interaction with the Arg273 residue. The drug establishes multiple H-bonds with ACE2, notably via its terminal hydroxyl group and the acid function (Figure 5). N-acetyl-felinine binds well to ACE2 but a related compound in the series, pantetheine, was found to be considerably less adapted to interact with the protein. The later compound is the functional subunit of coenzyme A, but apparently not a good ACE2 binder, unlike felinine.

TABLE 2 | Calculated potential energy of interaction (ΔE) and free energy of hydration (ΔG) for the interaction of the indicated products with ACE2.

Compounds	CID[a]	ΔE (kcal/mol)	ΔG (kcal/mol)
Adt[b]	269217	−78.30	−30.90
Asparagusic acid	16682	−102.00	−31.70
Asparagusic acid syn-S-oxide	6451512	−128.00	−28.85
Brugine	442998	−44.60	−21.25
Carbamothioic acid (Cartap)	27159	−54.50	−15.30
Carbamyl-felinine	129709672	−138.50	−34.70
Charatoxin	156360	−24.70	−10.20
CMX-2043[c]	49802864	−182.10	−41.15
Diethyltrithiolane	129632532	−30.70	−10.75
Dihydroasparagusic acid	440312	−108.40	−27.00
Dimehypo (Thiosultap)	162117	−48.50	−22.80
(-)-Felinine	164607	−122.85	−29.70
Isovalthine	273527367	−159.70	−26.20
Kottamide E		−69.50	−18.70
N-acetyl-felinine	87631712	−142.10	−32.70
Nereistoxin	15402	−35.90	−7.60
Pantetheine	439322	−73.85	−23.00
S-acetyl dihydroasparagusic	85798858	−139.70	−35.30
Thioctic acid[d]	864	−119.80	−34.10
Thiocyclam (sultamine)	35970	−27.75	−11.95

[a]Compound Identity number, as defined in PubChem (https://pubchem.ncbi.nlm.nih.gov).
[b]Adt: 4-amino-1,2-dithiolane-4-carboxylic acid (NSC212561).
[c]CMX-2043: N-[(R)-1,2-dithiolane-3-pentanoyl]-L-glutamyl-L-alanine, or (R)-Lip-L-glu-L-ala-OH.
[d]Thioctic acid: α-lipoic acid.

The diacid compound isovalthine appeared to bind well to ACE2, notably via interaction with two arginine residues (Arg514 and Arg518) (Figure 5). This branched-chain sulfur amino acid has been little studied thus far. It has been essentially described in animals, notably cats [42]. Isovalthine turned out to be a good ACE2 binder, even better than asparaptine B. This atypical amino acid, probably acting a as pheromones in cats [43], warrants further investigation.

Finally, the best compound among the molecules bearing a dithiolane motif is the drug CMX-2043 bearing a L-Glu-L-Ala dipeptide motif coupled to a thioctic acid residue ((R)-Lip). The compound revealed a remarkable affinity for ACE2. Both the calculated potential energy of interaction (ΔE = −182.10 kcal/mol) and free energy of hydration (ΔG = −41.15 kcal/mol) were remarkably high, surpassing all the other compounds. The CMX-2043/ACE2 drug-protein complex is stabilized by an array of molecular contacts, involving both the peptide portion of the molecule and the dithiolane motif. This later motif engaged a π-sulfur interaction with Cys361, which represents a very favorable noncovalent interaction in protein complexes [44]. On the other side of the molecule, three π-anion interactions (with Arg273, Arg518 and His374) contributed to the stability of the complex. Altogether, the CMX-2043 molecule can solidly anchor to the ACE2 protein occupying the ACE2 binding pocket (Figure 6). The discovery is potentially important because this synthetic derivative is currently evaluated in human for the treatment and prevention of ischemic complications of percutaneous coronary intervention (PCI). It is a well-tolerated cytoprotective agent capable of reducing myonecrosis in patients with ischemia-reperfusion injury [45–47]. ACE2 plays a role in the pathophysiology of various fatal cardiovascular diseases, including ischemic heart disease, and ACE2 binders are considered for the treatment of myocardial ischemic reperfusion injury [48]. Altogether, our docking study identified a few dithiolane-containing compounds capable of interacting with ACE2. The best three compounds are asparaptine B, isovalthine and CMX-2043.

4 | Discussion

The identification of sulfur-containing metabolites with inhibitory activity against the angiotensin-converting enzyme in *A. officinalis* [13] has prompted us to investigate further the interaction of asparaptines with ACE using molecular docking. These compounds have been essentially ignored in the past, it is useful to investigate their potential biological function and pharmacological effects. Here we compared the ACE2 binding capacity of the three asparaptines A-B-C and a panel of structurally related compounds [12, 13].

Asparaptine B is a conjugate of lysine and asparagusic acid. This compound was identified as a better binder to ACE2 compared

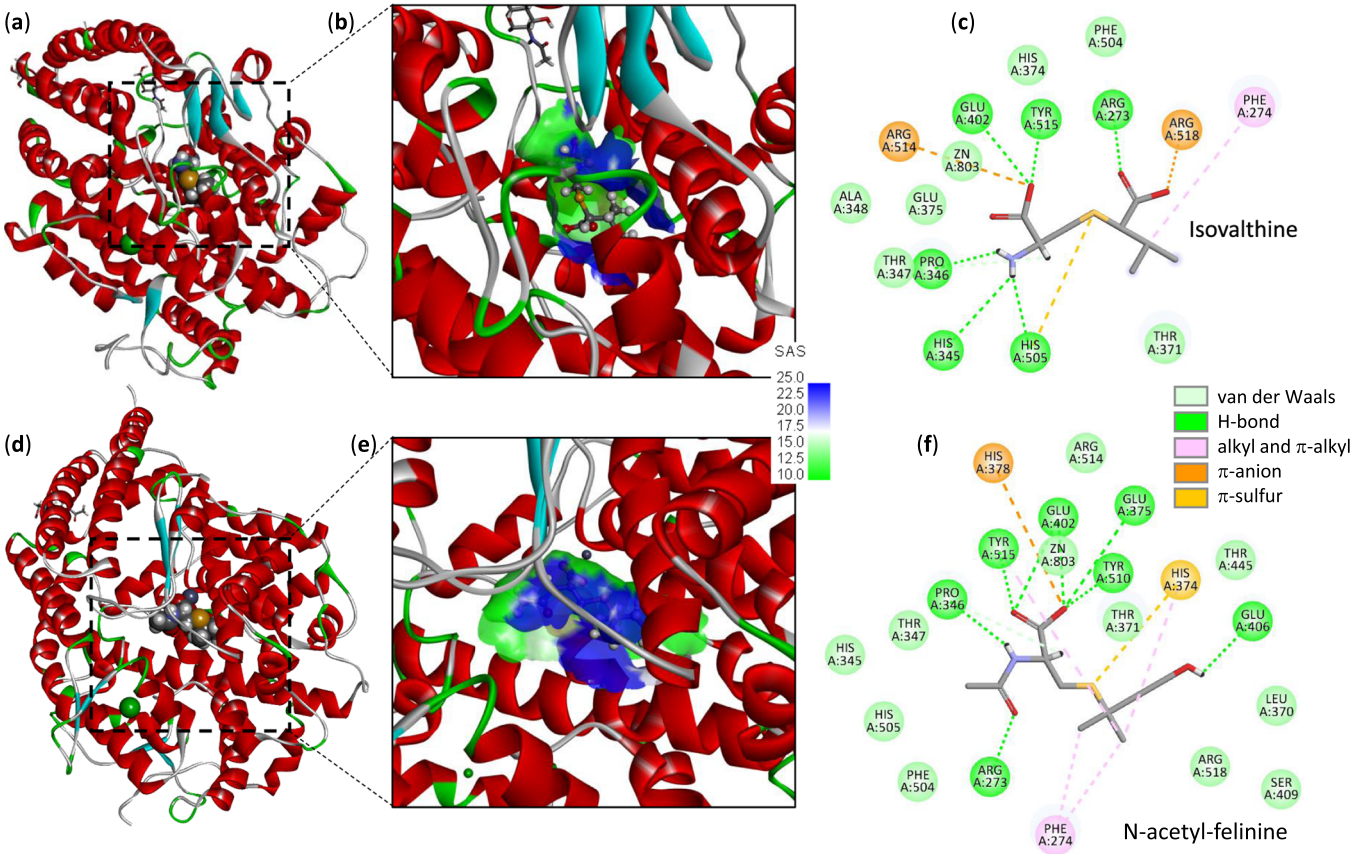

FIGURE 5 | Binding of isovalthine and N-acetyl-felinine to ACE2. (a, d) Molecular model of the drug bound to the ACE2 protein. (b, e) A close-up view of the drug-bound ligand with the solvent-accessible surface (SAS) surrounding the drug binding zone. (c, f) Binding map contacts for the drug bound to ACE2. Other details as for Figure 3. Top (a–c) isovalthine. Bottom (d–f) N-acety-l-felinine.

to its two analogs asparaptines A and C. Asparaptine B has been isolated from *A. officinalis* (green asparagus) [14, 15] but it is likely present in other *Asparagus* species as it is the case for asparaptine A, detected in callus from the species *A. pastorianus*, *A. persicus*, and *A. plocamoides* [18]. Green and white asparagus do not significantly differ with regard to their content in asparaptine A [17, 49, 50]. These compounds contribute to the prohealth properties of *A. officinalis* [1]. Like other vegetables, asparagus presents a good potential in preventing cardiovascular diseases [51, 52]. This pharmacological activity may well be linked to inhibition of ACE2, at least in part. In addition, the lysine moiety of asparaptine B may play a role in the drug delivery process, notably to facilitate a crossing of the blood-brain barrier, as it has been observed with a lysine-flavone conjugate [53].

From the asparaptines, we then searched for related compounds susceptible to interact with ACE2 and identified two other molecules of interest: the atypical amino acid isovalthine and the drug candidate CMX-2043. The former is a little-known metabolite from S-(isopropylcarboxy-methyl) glutathione in cat and guinea pig [54, 55]. But a long time ago, this compound has been isolated also from the urine of patients with hypercholesterolemia [56]. Its biological function, if any, is not known. This compound should be reinvestigated as a potential ACE2 binder. The second compound CMX-2043 is more interesting because it is an injectable drug candidate (developed by Ischemix Inc., Massachusetts, USA) currently evaluated for the treatment of traumatic brain injury (TBI) and ischemia-reperfusion injury after myocardial infarction [45, 57]. A phase 2 trial (NCT02103959) has shown that CMX-2043 was well tolerated and efficacious to reduce acute kidney injury in patients undergoing percutaneous coronary intervention [58]. This multi-modal cytoprotective compound is evaluated in different pathologies. It looks promising for the treatment of patients who have suffered a moderate-to-severe TBI. Its mechanism of action is not well defined at the molecular level. The drug has demonstrated a significant increase in mitochondrial bioenergetics, associated to a decreased production of mitochondrial reactive oxygen species and oxidative injury. This analog of α-lipoic acid has been shown to function via activation of Akt in addition to an antioxidant action. The drug stimulates Akt phosphorylation and reduces calcium overload [57]. Binding to ACE2 could play a role in this signaling pathway. But the drug has also been shown to bind to human manganese superoxide dismutase (MnSOD) which is a member of the oxidative metabolic pathway [59].

ACE2 is a key regulator of the renin-angiotensin-aldosterone system and a key receptor used by SARS-CoV-2 and SARS-CoV viruses for entry into host cells. CMX-2043 has been predicted to bind covalently to the main protease of SARS-CoV-2 (M^{pro}) via opening of the 1,2-dithiolane ring upon nucleophilic attack by the Cys145 residue of the protein [60]. Our calculation proposes an alternative pathway, via noncovalent binding of the drug to the ACE2 partner protein. The hypothesis is conceivable because α-lipoic acid has been shown to regulate the expression

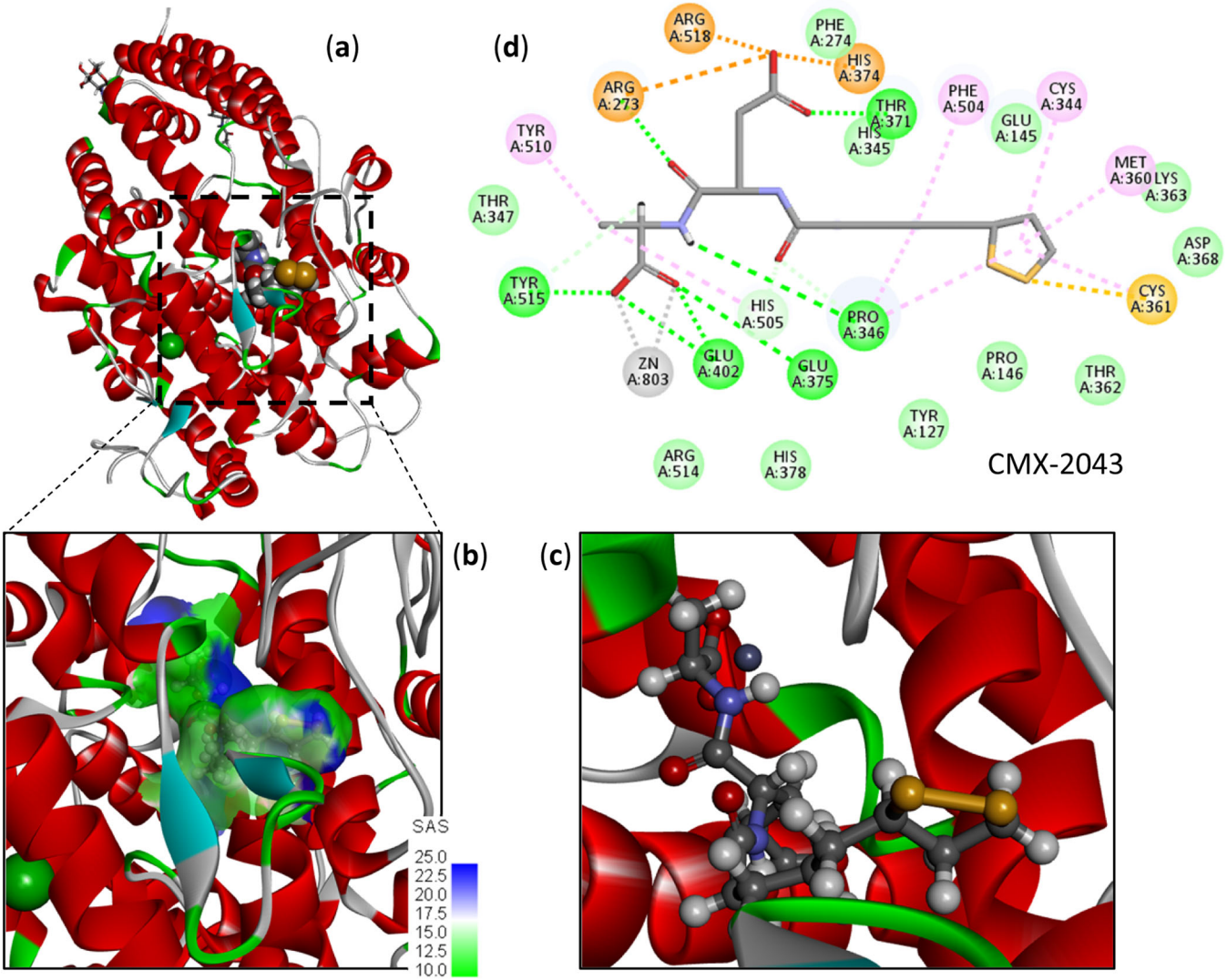

FIGURE 6 | Binding of CMX-2043 to ACE2. (a) Molecular model of the drug bound to the ACE2 protein. (b) A close-up view of the drug-bound ligand with the solvent-accessible surface (SAS) surrounding the drug binding zone. (c) A detailed view of the drug binding site. (d) Binding map contacts for the drug bound to ACE2. Other details as for Figure 3.

of ACE2 [61]. This antioxidant compound can interfere with several signaling pathways related to SARS-CoV-2 infection [62]. It is therefore not unrealistic to consider also CMX-2043 as a candidate to address COVID-19.

The dithiolane motif is not often considered in drug design strategies. However, this motif can be found occasionally in synthetic molecules of biological interest, including iNOS regulators and tyrosinase inhibitors, and in natural products [63–65]. We shall recommend the use of the dithiolane motif to target ACE2, via the design of conjugates of α-lipoic acid, similar to CMX-2043 and asparaptine B. α-Lipoic acid is a multipotent small molecule with pleiotropic effects [66, 67]. The design of additional conjugates of α-lipoic acid to target ACE2 is anticipated.

Author Contributions

Gérard Vergoten: Software, methodology, formal analysis, visualization. **Christian Bailly:** conceptualization, investigation, writing – original draft, writing – review and editing, formal analysis, supervision, data curation, visualization.

Conflicts of Interest

The authors declare no conflict of interest associated with this publication and there has been no significant financial support for this study that could have influenced its outcome. This study did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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