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Review

# Beauvericin: the journey of a pesticide into a humanized drug

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**Abstract:** Drug discovery has been initially attributed to coincidence or trial and error where the traditional approach was complex, lengthy, and expensive. Conventional drug discovery methods require the costly random screening of synthesized compounds or natural products. Another downside for this approach is the wide dependency on the experimental use of animals for in vivo testing. Currently, in silico modeling has become a vital tool for drug discovery and repurposing, and molecular docking is being used to find the best matching between a ligand and a molecule. Practical application of in silico docking will predict the biomolecular interactions between the drug and the target host. Beauvericin (BEA) is an emerging mycotoxin produced by the entomopathogenic fungus *Beauveria bassiana*. Originally investigated for its pesticide capability, BEA is now considered as a molecule of interest for its potentially diverse biotechnological applications in the pharmacological industry and the field of medicine. In this manuscript we will provide an overview on the repurposing of BEA into a potentially superior therapeutic molecule in a broad range of diseases. Furthermore, a considerable attention has been given to the fundamental role of in silico techniques to i) further investigate the spectrum of this secondary metabolite and ii) elucidate the pathways of BEA for its promising therapeutic action.

**Keywords:** Drug repositioning, Molecular modelling, Beauvericin

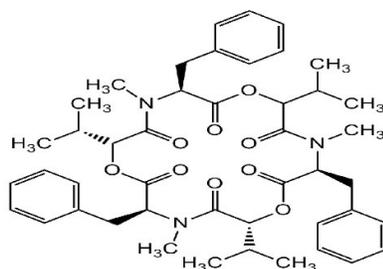
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## 1. BEAUVERICIN: A FUNGAL METABOLITE

Ages ago, before pure chemicals were synthesized, people used natural compounds, for their beneficial effect in traditional medicine and pest management within a harmonized ecosystem. In particular, the secondary metabolites of such natural products are synthesized mainly by bacteria, fungi, and plants [1]. They are molecules of low molecular weight with diverse chemical structures and biological activities. Although they are not required for the development of organisms, they are produced to confer a selective advantage for the organism's survival in nature [2]. Paradoxically, these compounds (or their derivatives) constitute approximately 35% of the FDA-approved drugs list used by humans during the last two decades to control/cure diseases and/or combat pathogens including bacteria/fungi/viruses [3]. Specifically, recent interest has been noticed in the assessment of fungal metabolites for their wide spam of bioactivities [4]. *Beauveria*, a cosmopolitan anamorphic genus of soilborne necrotrophic entomopathogenic fungi, is the most studied biological control agent. The global distribution in addition to its broad range of target pests have made *Beauveria* the most famous entomopathogenic fungi [5]. Its ability to cause diseases in insects was first reported more than 60 years ago. Since that time, *Beauveria* is the most studied alternative control agent, and currently, its conidia form the basis of several commercial insecticides available in the world trade [6]. The first

taxonomical characteristic for identifying *Beauveria* genus was based on the morphology of conidiogenous cells and the conidiogenesis process itself [7]. This traditional approach was however always controversial, but in 2011 the first molecular approach was adopted instead, using a multi-locus phylogeny of *Beauveria* based on partial sequences of protein-coding genes RPB1, RPB2, TEF, and the nuclear intergenic region, Bloc [8]. Twelve ex-type species of *Beauveria* genus were described. Among these, *B. bassiana* was found in different isolates infecting a wide variety of insects and mites including but not limited to Acari, Lepidoptera, Coleoptera, Hymenoptera, Homoptera, Diptera, Hemiptera, Orthoptera [9,10]. Despite being considered a generalist fungus with no host preference, *B. bassiana* virulence on its hosts varies considerably among its different strains [11]. Additionally, and in sharp contrast to other entomopathogenic agents, *B. bassiana* can infect its host by ingestion or by simple contact. The most familiar mode of infection is penetration through the host tegument, however, infections caused by conidial ingestion and penetration through natural orifices were also reported [12]. After cuticle penetration, the host will initiate cellular and/or humoral immunity reactions. The success of fungal infection usually depends on the capability of entomopathogenic fungi to overcome epicuticular fungistatic compounds, cuticle thickness, mineralization, sclerotization, melanization, protease inhibitors, antimicrobial peptides, and cellular/humoral responses [13]. Interestingly, it was also reported that some hypervirulent strains of *B. bassiana* are able to overcome encapsulation and nodule formation by producing secondary metabolites that are able to dampen the host cellular response [14]. BEA is the most famous secondary metabolite produced by many fungi, including but not limited to *Beauveria* spp. The cyclic hexadepsipeptide molecule belongs to the enniatin antibiotic family [15]. It contains three D-hydroxyisovaleryl and three N-methyl phenylalanyl residues in an alternating sequence (Figure 1). Nevertheless, BEA lacks resemblance with enniatins produced by *Fusarium* spp. with respect to biological activities. This distinction could be accounted to the nature of the N-methylamino acid [16].

**Molecular Formula:** C<sub>46</sub>H<sub>59</sub>N<sub>3</sub>O<sub>9</sub>  
**Molecular Weight:** 797.99 g/mol



**Figure 1.** Chemical structure of beauvericin

#### Biochemical properties

Numerous entomopathogenic fungi are known to produce BEA after reaching the hemocoel of their host. It was shown that mutant strains of *B. bassiana* with a knock-out of the *bbBeas* gene, responsible for the expression of beauvericin, will cause significantly lower virulence against different insect hosts [17]. It was suggested that this metabolite is able to overpower insect cell defenses including, but not limited to, the antimicrobial lipids and phenols, enzyme inhibitors, and proteins [18]. In addition, BEA was found to inhibit the growth of the insect's microbial flora as well as other organisms that may compete for the limited nutrition [19]. Initially, the insecticidal activity of BEA was confirmed against *Aedes aegypti*, a mosquito that can spread dengue fever, and *Calliphora erythrocephala*, flies are important in the field of forensic entomology [20]. Since then,

much more information on the insecticidal activity of BEA has become available. It was demonstrated that BEA can efficiently control the growth of the Colorado potato beetle, *Leptinotarsa decemlineata* [21]. BEA also significantly reduced the fitness of *Schizaphis graminum* by increasing the number of abortive embryos produced by treated females [22]. Among 16 fungal metabolites, BEA showed the highest cytotoxicity to lepidopteran (*Spodoptera frugiperda*) cell line (SF-9) [23]. In addition, studies on the plant bugs *Lygus* spp. provided insight into a potential role for BEA in controlling them [24]. Concomitantly, the acaricidal activity of BEA has been explored and solid evidence was put forward to support the potential usefulness of BEA as a promising pest management strategy [25]. Under greenhouse conditions, applications of BEA significantly reduced the populations of *Tetranychus urticae*, and increased strawberry yields while showing no phytotoxicity and ecotoxicological risk. Furthermore, the efficacy of BEA against all developmental stages of the mite *Sarcoptes scabiei* was recently confirmed paving the way for its usage as a treatment for sarcoptic infections [26].

The above observations are linked directly to the mechanisms by which BEA exerts its effect on the host, which are tightly associated to the molecular characteristics of the molecule. The first hint to these characteristics is the ionophoric nature of BEA which was first demonstrated in a study back in the 1990's where the authors provided evidence that beauvericin is able to transport small ions across cytoplasmic membranes [27]. This observation was further supported by demonstrating that BEA could establish a cation-selective channel after incorporating in the membrane of mammalian cells [28]. The increased intracellular bioavailability of  $Ca^{2+}$  decreases the transmembrane potential of mitochondria, releases cytochrome c, and activates caspase 3, a critical executioner of apoptosis [29]. Consequently, BEA can initiate cell death via calcium-dependent pathways [30]. Furthermore, an increased concentration of BEA could breach the nucleus and alter the DNA [31]. It was proposed that the secondary metabolite can randomly bind to DNA sequences and that the damaged areas could be fragmented by nucleases.

#### *BEA contamination in food commodities*

Associated with its potential use as a beneficial pesticide for humans, BEA was classified as an emerging mycotoxin due to its high occurrence in food commodities including a broad range of food crops such as wheat, rye, oats, barley, and rice [32]. Moreover, the mycotoxin is linked to intoxication of eggs [33], dried fruits [34], coffee [35], medicinal herbs [36], and feed [37]. The amount of BEA present in food has become an important issue for its risk assessment to human and animal health. Strikingly, the presence of BEA was recorded in 90% of the examined wheat-based products (pasta, cereals, biscuits, and baby formulas) [38, 39]. The concentration of the mycotoxin within consumed food may range from negligible traces to multiple mg/kg [18]. A review on the toxicological relevance of BEA in food considered an acute exposure to the mycotoxin will unlikely cause any concern for human and livestock health despite the potential ionophoric-related effects it has on mammalian cell walls in general [40]. So far and in the absence of adequate toxicological data, risk assessment on the chronic exposure of BEA is still lacking.

## **2. BEA IN HUMAN HEALTH AND DISEASES**

In the last few years, BEA has been exploited in the search for new therapeutic options to be used against human pathogens and diseases. Accumulated data from different angles further showed that BEA is being considered a potential candidate for medicinal research due to its broad range of biological properties. The antibacterial activity of BEA has been investigated against gram-negative and positive bacteria of human, animal, and plant interest [16,17,41]. The cytotoxic activity against different cancer cell lines was further explored mainly towards the PC-3M (prostate) and MDA-MB-231 (breast) cells [42], and the secondary metabolite was able to significantly reduce CT-26 and KB-3-1-grafted tumors in a murine model [43]. The first antiviral attributes for BEA were examined on

the human immunodeficiency virus type 1 (HIV-1) where the specificity of the inhibitory effect of the secondary metabolite was mapped to the enzyme (integrase) involved in the integration of the virus into the host genome [44]. Remarkably, this inhibitory concentration was low relative to the cytotoxic concentrations against all tested human cell lines [45]. The inhibitory potential of BEA was comparable to those of baicalein and robinetin which are previously demonstrated as effective antiviral compounds [46]. Furthermore, and along the anti-inflammatory effects of BEA, it was shown to significantly inhibit the nuclear translocation of the NF- $\kappa$ B pathway subunits p65 and p50 within the RAW264.7 cells without inducing cell toxicity [47]. The inhibition of the NF- $\kappa$ B pathway involved in the upregulation of genes encoding cytokines such as interleukin (IL)-1 and tumor necrosis factor (TNF)- $\alpha$ , chemokines, and inflammatory mediators such as inducible nitric oxide (NO) synthase (iNOS) (involved in NO production) and cyclooxygenase (involved in prostaglandin E2 secretion) could alleviate the cytokine storm, a dangerous overreaction of the immune system.

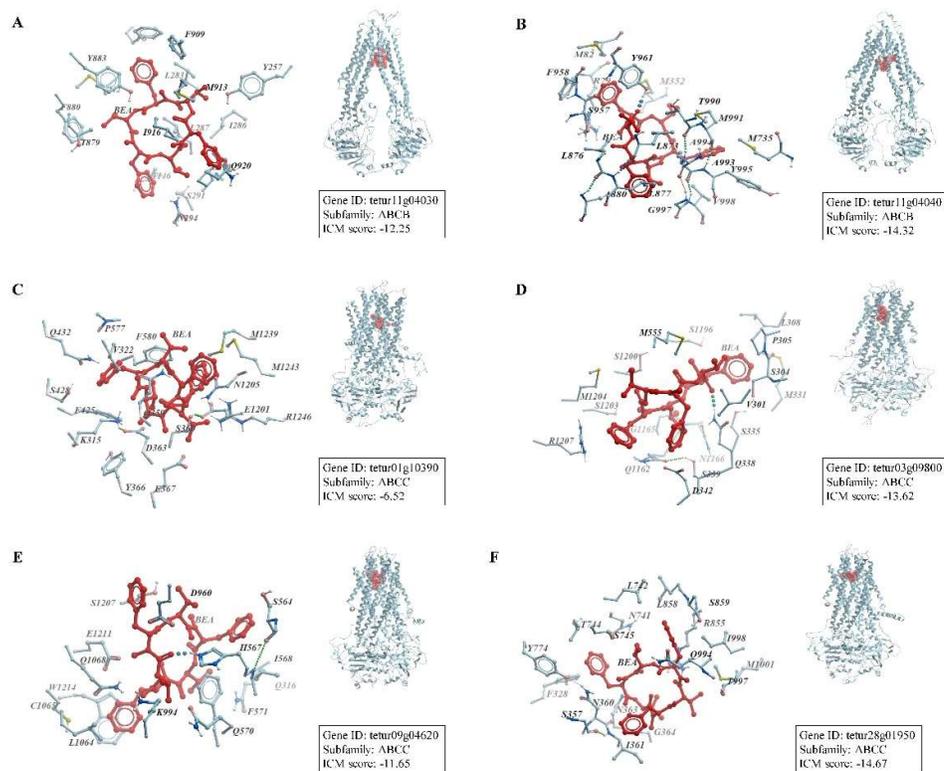
A growing body of literature has revealed the synergetic effect of BEA in combination with other compounds. A study conducted by [48] demonstrated the activity of BEA against the yeast *Candida albicans*. The authors proved that the secondary metabolite could synergize the effect of a low dosage of ketoconazole and could be used in combination to develop new anti-fungal therapeutics. BEA has a significantly higher toxicological effect when combined with deoxynivalenol in mammalian kidney cells [49]. Furthermore, mixed doses of BEA and pesticides (cyflumetofen, bifenazate, or abamectin) significantly suppressed populations of *Tetranychus urticae* Koch when compared to single-pesticide treatment [50].

#### *In silico modeling and in vitro confirmation for the role of BEA in drug resistance*

In light of accumulated observations in the field and few published reports, BEA could potentiate the activity of drugs. Most importantly, secondary metabolites could restore the sensitivity of certain molecules against multi-drug resistant (MDR) organisms. It was up to the modern in silico techniques to elucidate the pathway of this interesting activity of BEA. Multi-drug resistance is generating considerable interest since it's the major hurdle against treating a wide variety of diseases [51]. The development of resistance is mainly driven by the misuse and the overuse of antimicrobial agents [52]. The emergence and spread of antimicrobial resistance reduces the effectiveness of the available therapeutic arsenal against a wide range of human, animal, and plant pathogens [53]. According to the World Health Organization (2020), the development of resistance is threatening global health and development thus understanding the underlying resistance mechanisms is important to mitigate the growth and dissemination of resistant populations. In addition to chromosomal mutations, development of resistance was mainly attributed to the xenobiotic efflux pumps from different families [54]. The most common group of transmembrane pumps is the ATP-binding cassettes (ABC) transporters that are ubiquitously present in prokaryotes, archaea, and eukaryotes [55]. The inward-facing conformation of ABC transporters, formed from two bundles of six transmembrane helices, results in a large internal cavity that binds xenobiotics and chemotherapeutic drugs [56]. The switch from the inward- to the outward-facing conformation will transport the substrate to the extracellular environment [55], reducing as a result its bioavailability inside the cell.

To counteract the multi-drug resistant phenotype of *Candida albicans*, an opportunistic pathogenic yeast, researchers relied on structural base virtual screening to identify modulators of the fungal ABC transporters [57]. The Alignment Mode algorithm of Swiss-Model was used to build homology models for the ABC transporters Cdr1 and Cdr2, and virtual screening using docking of ZINC15 database was implemented to find structures that could bind to the internal cavity [58]. Interestingly, among 230 million compounds, BEA and its analogues had the best binding affinity to *C. albicans'* ABC transporters. We have recently corroborated these findings using in silico techniques and demonstrated the

ability of BEA to neutralize two and four ABC transporters belonging to ABCB and ABCC subfamilies, respectively [50]. The position and conformation of BEA, docked to the binding cavity of the efflux pumps, were compared to those linked to pesticides. Relatively to all controls, BEA recorded the lowest binding affinity [50] (Figure 2). Using computational techniques, it was speculated that BEA was a competitive inhibitor of membrane-bound proteins and prevents xenobiotic efflux by inhibiting substrate binding, consequently restoring sensitivity. Thus, combinational therapy using BEA and other drugs can be used to overcome multidrug resistance.



**Figure 2.** Docking conformations of BEA in ABC transporters (A: tetur11g04030; B: tetur11g04040) and C (C: tetur01g10390; D: tetur03g09800; E: tetur09g04620; F: tetur28g01950).

#### *Beauvericin as a potential anti-viral agent: SARS-CoV-2*

In December 2019, a rapidly spreading contagious pneumonia outbreak was first recorded in Wuhan, Hubei province, China. This respiratory disease quickly evolved into a pandemic affecting millions worldwide and referred to as COVID-19 caused by SARS-CoV-2 [59]. Multiple studies have confirmed that SARS-CoV-2 is able to attach to host cells via the enzyme angiotensin-converting enzyme 2 (ACE2), which shows enhanced expression in type II alveolar cells of the lungs [60]. In addition, the “cytokine storm” elicited by the virus has channeled the interest of scientists towards reducing the inflammatory response concomitantly with developing antiviral agents for prevention and post-infection therapy. At present, however, the clinical trial registry (ClinicalTrials.gov) encompasses only ongoing clinical trials for existing repurposed FDA-approved drugs. In contrast, researchers in basic laboratory settings are actively testing novel small molecules for their potential therapeutical value. It is therefore imperative in the wake of the challenges caused by the virus to fetch out for such molecules, some of which are actively synthesized in other species or kingdoms. Examples of such potential drugs are secondary metabolites produced by fungi which have been used in agriculture as pesticides and are being

considered in the medical field as potential therapeutics for different diseases. We thus used the same *in silico* approach [50] to assess the potential effect of BEA on the different proteins making up the virus (see supplementary material).

#### *Selective and specific docking to viral proteins*

In Numerous drugs as potential inhibitors of the 3CL protease of SARS-CoV-2 were used as positive controls i) chloroquine, an antimalarial drug found to be efficacious in treating COVID-19 infections [61,62] ii) remdesivir effective in preventing replication of SARS-CoV-2 [63] (iii) luteolin, the main flavonoid in honeysuckle [64] that docks perfectly with the main protease of the virus [65] vi) N3 (5-Methylisoxazole-3-carboxylic acid) the inhibitor in complex with the protein crystal structure [66] v) boceprevir a clinically approved anti-hepatitis C drug vi) GC376 the inhibitor of the virus linked to feline infectious peritonitis [67] and the principle components of Kaletra (vii) ritonavir and viii) lopinavir an inhibitor of the SARS-CoV 3CL<sup>pro</sup> [68]. The most druggable pocket (pocket A: Supplementary Table S1) found in the crystal structure consisted of the inhibitor N3 binding site which demonstrated the high accuracy of the software in predicting “ligandable” orthosteric and allosteric interaction sites. Interestingly, the docking score for BEA against pocket A of 3CL protease was the highest (ICM score= -7.24 kcal/mol) when compared to the positive control ligands (Table 1). Hydrophobic interactions were formed between BEA and H41, M49, F140, L141, H163, M165, L167, P168, D187, R188, Q189, T190, A191. In addition, 1-H bond was established between BEA and E166 (Figure 3). In parallel, BEA recorded the best docking conformation against Pocket B (Supplementary Table S1) relative to the drugs that are in common use. The BEA docked strongly (ICM score: -12.53 kcal/mol) forming 1 H-bond with N72, 13 hydrophobic interactions (E14, G15, M17, Q19, A70, G71, V73, P96, K97, N119, G120, S121, and P122), and 5 Van der Waals forces (C16, V18, N28, N95, and Y118) (Figure 3). The ligands used as positive controls presented good docking positions against the main protease of the virus (Pocket A and Pocket B).

In addition to remdesivir and luteolin, ivermectin was docked against the Spike protein of the SARS-CoV-2. Ivermectin showed antiviral activity against SARS-CoV-2-infected Vero-hSLAM cells [69]. Furthermore, data has emerged about the beneficial administration of ivermectin amid COVID-19 infection in hospitalized patients which could account for lower mortality rates [70]. The docking results (-9.88 kcal/mol) for pocket A, the most “druggable binding site (Supplementary Table S1), revealed that BEA mainly anchors itself to 6VXX through hydrophobic interactions (P225, L226, V227, D228, A520, P521, T523, N544 R557, P579, P561, F562, L582) and one hydrogen bond (K41) (Figure 3). Among all ligands, only luteolin recorded a lower binding affinity when compared to BEA. The BEA docked poorly (Table 1) to the second pocket (Pocket B Supplementary Table S1) identified by the software. We further explored the pocket responsible for the binding of the spike protein to the cell receptor ACE2 [71]. The ICM scores for BEA, remdesivir, luteolin, and ivermectin when docked against the receptor-binding domain were -1.8, -3.42, -11.42, and 7.8, respectively.

MLS001181552, the inhibitor of helicase, as well as astilbin (NPC270578), a plant flavonol, were used as a reference for their demonstrated ability to dock strongly against helicase of SARS-CoV-2 [72]. The SARS-CoV-2 helicase protein recorded a docking score of -4.82 kcal/mol with BEA. The anchoring includes i) hydrophobic residues P406, P408, R409, L412, T413, K414, L417, T552, A553, and H554 and ii) a hydrogen bond Y515 to maintain optimal binding (Figure 3). The BEA docked effectively against Pocket A (Supplementary Table S1) however, it docked poorly against the remaining pocket of the SARS-CoV-2 helicase (Table 1). Finally, the binding affinity was the highest amongst all tested ligands (Table 1).

RO-7 and arzanol were used as positive controls because of their abilities to neutralize endoribonucleases [73]. Two different receptor sites were selected for ligand docking (Pocket A and B Supplementary Table S1). The BEA docked poorly against all selected receptors. The best conformation (-0.07 kcal/mol, Table 1) was recorded against pocket A.

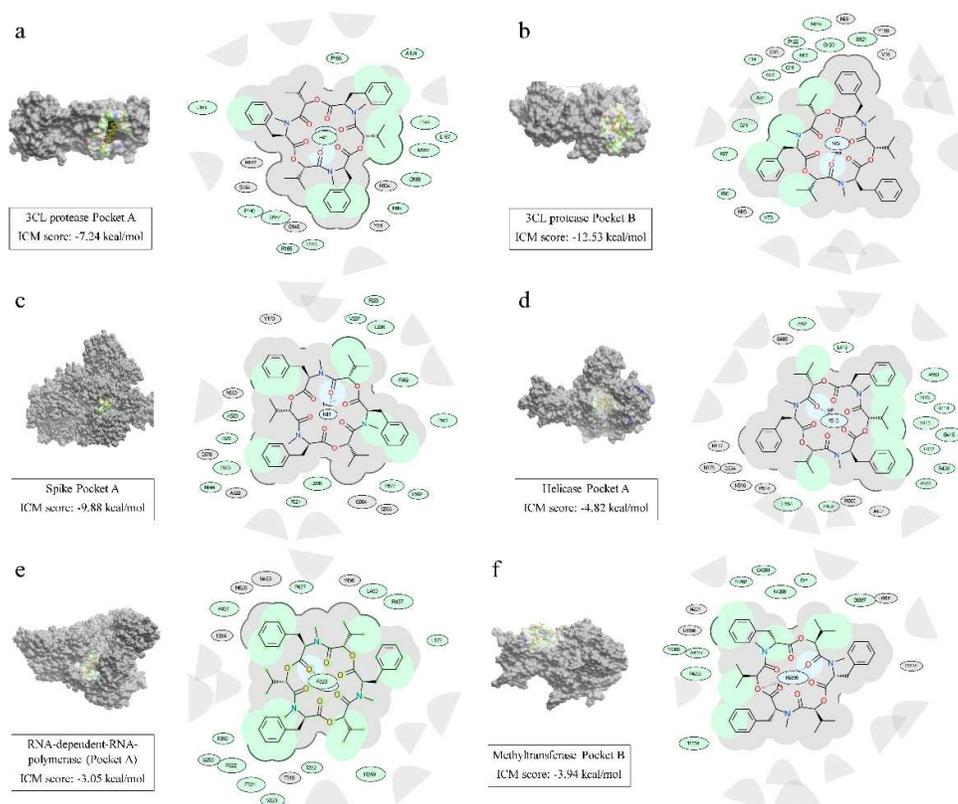
Based on these findings, BEA will probably not interact with the SARS-CoV-2 endonuclease.

The SARS-CoV-2 *RNA-dependent-RNA-polymerase* (RdRp) enzyme was also used for docking against BEA, remdesivir, and dATP [72,74]. BEA docked to the active site (Pocket A) of the protein, while showing a relatively poor docking against pocket B (the second most druggable pocket, supplementary table S1). BEA recorded a -3.05 kcal/mol score against 7BTF recorded through i) hydrophobic interactions on L172, R249, T252, F321, P322, S255, F396, P461 ii) hydrogen bond on R349 (Figure 3). Despite its relatively low score as compared to M<sup>pro</sup> and spike, the binding energy was the highest when compared to the controls.

As for the viral methyltransferase enzyme (PDB:6W4H), the following controls: i) S-adenosyl-L-methionine, the natural ligand of the protein already bound to the 6W4H crystal structure ii) sinefungin, a natural nucleoside related to S-adenosyl-L-methionine with demonstrated antiviral activity [75], and iii) 2-(3,4-dihydroxyphenyl)-5,6-dihydroxy-7-[(2S,3R,4R,5R,6S)-3,4,5-trihydroxy-6-ethylloxan-2-yl]oxochromen-4-one (NPC226294) [72] were used. The binding receptor site (Pocket A) with the highest “druggability” score (DLID) was the cleft bounding the natural ligand S-adenosyl-L-methionine. BEA docked poorly against this active site of the protein and the binding energy was the highest among all docked ligands. In parallel, BEA docked nicely against pocket B (with the second highest DLID score) (Supplementary Table S1). However, the binding energy was still the lowest when compared to the controls (Table 1). The methyltransferase (6W4H) demonstrated a docking score of -3.94 kcal/mol. BEA bound to the protein mainly across 7 hydrophobic interactions and 2 hydrogen bonds involving G6837, Q4289, P4290, T4292, T4354, T4355, A4357, and N4358 as well as K6836 respectively (Figure 3).

Finally, IcmPocketFinder identified two different druggable pockets in *Exoribonuclease* (PDB: 5C8T) protein (Supplementary Table S1). 2-(N-Morpholino)-ethanesulfonic acid was earlier identified as an inhibitor of exonucleases [76], and in addition, the natural compound 1-O-(2-Methoxy-4-Acetylphenyl)-6-O-(E-Cinnamoyl)-Beta-D-Glucopyranoside (NPC137813) was recently found as a promising drug to be used against the SARS-CoV-2 exoribonuclease [72]. BEA docked poorly to all identified pockets and the recorded binding energy was the highest when compared to both aforementioned controls (Table 1).

Awaiting in vitro and pre-clinical experiments, the tests revealed that BEA could bind to different proteins. However, the most striking results that emerged from the data is the low binding energy recorded against the main protease and the spike proteins of the virus. Taken together, BEA may neutralize proteins involved in key developmental stages of SARS-CoV-2. Due to its potential activity on two key proteins, different BEA molecules could have a synergistic effect and thus higher efficacy over existing drugs. Even within one protein, BEA could strongly bind to the orthosteric and allosteric sites. Previous studies showed that BEA has anti-inflammatory activity and it could significantly inhibit the nuclear translocation of the NF- $\kappa$ B pathway subunits p65 and p50 in RAW264.7 cells without inducing cellular toxicity [47]. The inhibition of the NF- $\kappa$ B pathway was linked to the cytokine storm in severe cases of SARS-CoV-2 [77]. This cellular protection against toxicity when using BEA in vitro was also corroborated in vivo in mice. At this stage of understanding, we believe that BEA may also enhance the therapeutic index of drugs and therefore BEA could be used alone or in combination with other drugs for treating COVID-19 infection.



**Figure 3.** Docking conformations of BEA in SARS-CoV-2. a) 3CL protease (Pocket A) b) 3CL protease (Pocket B) c) Spike Glycoprotein (Pocket A) d) Helicase (Pocket A) e) RNA-dependent-RNA-polymerase (Pocket B) f) Methyltransferase (Pocket B). The ligand 2D representation is showing the contacts between BEA and the different proteins of SARS-CoV-2 (Green shading represents hydrophobic region, blue shading represents hydrogen bond acceptor, white dashed arrows represent hydrogen bonds, grey parabolas represent accessible surface for large areas, grey residues represent "generic" van der Waals contact (non-hydrophobic, non Hbond), size of residue ellipse represents the strength of the contact, 2D distance between residue label and ligand represents proximity). The 3D protein representation is showing the ligand binding pocket on the proteins surface in which these interactions occur (Green: hydrophobic, Red: hydrogen bond acceptor, Blue: hydrogen bond donor).

**Table 1.** ICM score of beavericin and different known inhibitors against viral proteins implicated in vital life stages of SARS-CoV-2

Protein ID	Pocket ID	Drug	ICM Score (kcal/mol)
6LU7 3CL protease	A	Beavericin	-7.24
		Chloroquine	-11.24
		Remdesivir	-14.37
		Luteolin	-13.57
		N3	-20.46
	B	Boceprevir	-10.4
		GC376	-20.16
		Ritonavir	-14.52
		Lopinavir	-14.35
		Beavericin	-12.53
		Chloroquine	-8.04
		Remdesivir	-7.9
		Luteolin	-15.15

		N3	-2.26
		Boceprevir	-3.5
		GC376	-4.7
		Ritonavir	2.53
		Lopinavir	2.09
6VXX Spike Glyco- protein	A	Beauvericin	-9.88
		Remdesivir	-2.1
		Luteolin	-20.94
		Ivermectin	20.7
	B	Beauvericin	2.2
		Remdesivir	10.58
		Luteolin	-23.42
		Ivermectin	26.03
	Receptor bind- ing domain spike-ACE2	Beauvericin	-1.8
		Remdesivir	-3.42
		Luteolin	-11.42
		Ivermectin	7.8
6ZSL Helicase	A	Beauvericin	-4.82
		MLS001181552	-12.24
		NPC270578	-7.7
	B	Beauvericin	-1.25
		MLS001181552	-0.5
		NPC270578	-10.5
6VWW Endoribonu- clease	A	Beauvericin	-0.07
		Arzanol	-3.5
		RO-7	-1.18
		NPC169474	-10.35
	B	Beauvericin	9.35
		Arzanol	-8.211
		RO-7	-1.5
		NPC169474	-6.64
7BTF RNA-de- pendent- RNA-poly- merase	A	Beauvericin	-3.05
		Remdesivir	-5.515
		NPC161224	-14.92
	B	Beauvericin	0.5
		Remdesivir	-1.3
		NPC161224	-15.82
6W4H Methyltrans- ferase	A	Beauvericin	13.64
		S-adenosylmethionine	-7.28
		Sinefungin	-6.37
		NPC226294	-12
	B	Beauvericin	-3.94
		S-adenosylmethionine	-2.1
		Sinefungin	-0.7
		NPC226294	-12.04
5C8T Exoribonu- clease	A	Beauvericin	2.01
		MES	-15.27
		NPC137813	-6.745
	B	Beauvericin	1.39

		MES	-14.63
		NPC137813	-11.04

### 3. Conclusion

In this review, we described how the advanced in silico strategies guided the drug repositioning of a pesticide to a molecule that inhibits multidrug efflux, providing a powerful strategy to abrogate drug resistance and enhance the efficacy of existing drugs. In addition, we used the most reliable in silico receptor-ligand docking software to determine the likelihood of using a naturally synthesized molecule as an anti-SARS-CoV-2 drug. We thus confirm that modern computational methods have a tremendous impact on the pharmaceutical industry and should be tailored to uncover novel therapeutic options.

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

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