



Article

Hidden Activities of Tyrosine Phenol-Lyase and Tryptophan Indole-Lyase: Recombinant PLP-Dependent C–C Lyases as New Biocatalysts for Antimicrobial Thiosulfinate Generation

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Abstract

Background: Lyases are used in a wide scope of applications, making them invaluable tools in both industrial biotechnology and molecular biology. Many examples of lyases belong to the extensive family of pyridoxal 5'-phosphate (PLP)-dependent enzymes, which catalyze numerous reactions involved in amino acid metabolism, like tryptophan indole-lyase (Trpase or Tnase), tyrosine phenol-lyase (TPL), and methionine γ -lyase (MGL). Beyond their role in physiological processes, these lyases can also facilitate the synthesis of other biologically active products from non-canonical substrates. **Objectives:** Up till now there were only two C–S lyases known for the thiosulfates' biosynthesis from S-substituted L-cysteine sulfoxides—alliinase and MGL. Our study reveals for the first time that C–C lyases are capable of C–S lyase activity in reactions with S-alkyl, S-allyl and S-benzyl cysteine sulfoxides. **Methods:** We have compared the kinetic profiles of S-substituted L-cysteine sulfoxide degradation mediated by carbon–sulfur lyase MGL versus carbon–carbon lyases TPL and Trpase. **Results:** Among other S-alkyl-L-cysteine sulfoxides, petiveriin (S-benzyl-L-cysteine sulfoxide) was proven to be a substrate for all three enzymes. The potential utility of these enzymes in thiosulfate production was supported by in vitro testing of enzyme-generated thiosulfates against clinically relevant pathogens such as *Candida albicans*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. **Conclusions:** Both C–S and C–C lyases—MGL, TPL, and Trpase—can be implemented for practical application in thiosulfate synthesis.

Keywords: tyrosine phenol-lyase; tryptophan indole-lyase; methionine γ -lyase; thiosulfate generation system; S-substituted L-cysteine sulfoxides; antimicrobial thiosulfates



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1. Introduction

Biocatalysis has been regarded as an environmentally friendly and highly effective alternative to organic synthesis. In recent years biocatalytic transformations have been employed in medicine for the development of new synthetic routes to pharmaceutical ingredients and biologically active molecules [1,2]. Enzymes traditionally serve as green catalysts in the pharmaceutical industry, originating from natural sources that were for centuries used for in vivo production of remedies and antimicrobial agents.

Special importance is given to plant-derived enzymes that facilitate biologically active sulfur-containing molecules. For example, myrosinase (EC 3.2.1.147) converts various glucosinolates to isothiocyanates, thiocyanates, cyanates, indoles, and related products [3].

It has been biotechnologically adapted as a recombinant enzyme in anticancer enzyme therapy or as a possible biofumigant pesticide [4]. Another example is the C–S lyase alliinase (EC 4.4.1.4) from *Allium*, *Brassica* and other genera of plants, where this enzyme catalyzes the elimination of some aliphatic S-substituted L-cysteine sulfoxides [5–7] into thiosulfinates with antimicrobial and anticancer properties [8]. Aromatics-containing S-benzyl-L-cysteine sulfoxide (petiveriin) as a substrate for alliinase has been identified in different parts of the American plant *Petiveria alliacea* [9]. Combining purified alliinase or myrosinase with their substrates is effective for the rapid and efficient destruction of microorganisms and nematodes by the products of their enzymatic reactions, while neither the enzymes nor the substrates alone possess significant activity [10]. Plant-derived thiosulfinates were found to effectively inhibit key enzymes in pathogens through modification of the SH groups [11]. The alliinase-based system was reported to be used for biogenesis of thiosulfinates in vitro [12]; however, plant enzymes were found to be prone to rapid inactivation by their own products, and they remained inaccessible as the recombinant enzymes [13].

Microbial enzymes are engineered and developed for the needs of biotechnology, offering greater stability under a wide range of environmental conditions, including high temperatures and variable pH levels, compared to plant enzymes [14]. Additionally, microorganisms serve as an attractive resource of enzymes due to economic reasons, ability to be rapidly cultivated on a large scale through fermentation, and predictability and control over enzyme yields. In our previous works we revealed that thiosulfinates can be obtained using bacterial pyridoxal 5'-phosphate (PLP)-dependent methionine γ -lyase (MGL, EC 4.4.1.11). MGL usually catalyzes the breakdown of L-methionine by an α,γ -elimination reaction to yield α -ketobutyrate, ammonia, and methanethiol (Figure 1) [15]. In addition to this physiological reaction, it catalyzes the α,β -elimination reaction of S-substituted analogs of L-cysteine, including S-alk(en)yl L-cysteine sulfoxides [16,17], and can be isolated as a recombinant enzyme in high yields, outperforming plant alliinase in stability. Thiosulfinates produced by the enzymatic cleavage of S-alk(en)yl-L-cysteine sulfoxides mediated by MGL from *Clostridia species* and *Citrobacter freundii* demonstrate notable cytotoxic effects in cancer cells [18] and inhibit the growth of Gram-positive and Gram-negative bacteria, including pathogenic *Pseudomonas aeruginosa*, *Achromobacter ruhlandii*, *Burkholderia cenocepacia*, *Staphylococcus aureus*, and *Salmonella typhimurium* [17,19,20]. The efficiency of the thiosulfinate generation system MGL/S-alk(en)yl-L-cysteine sulfoxides in enzyme prodrug therapy has been shown in vitro [18] and in vivo [21]. Despite the progress in unraveling MGL potential for thiosulfinate generation, other enzymes with such activity still remain underdeveloped.

Our current research is focused on studying new relevant activities of PLP-dependent C–C lyases, in particular, their capability of α,β -elimination of S-alkylcysteine sulfoxides into biologically active thiosulfinates. Carbon–carbon lyases tryptophan indole-lyase (Trpase, EC 4.1.99.1) and tyrosine phenol-lyase (TPL, EC 4.1.99.2) catalyze the reversible hydrolytic cleavage of L-tryptophan and L-tyrosine, respectively (Figure 2) [22,23]. Both enzymes are multifunctional, capable of catalyzing α,β -elimination and β -replacement reactions involving several other β -substituted amino acids in vitro, such as L-serine, O-alkyl and O-acetyl-L-serine, L-cysteine, and S-alkyl-L-cysteines [24,25]. TPL is used in industrial biocatalysis for the production of valuable amino acids, such as L-DOPA, by reversing its natural role of cleaving L-tyrosine in a β -replacement reaction [26].

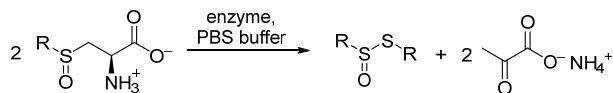
We have evaluated the kinetics of S-substituted L-cysteine sulfoxide cleavage by both TPL and Trpase, comparing this process with our previously established results for MGL. Additionally, we examined the cleavage of the aromatic sulfoxide compound petiveriin by these three enzymes. Finally, we assessed the antibacterial and anticandidal

activities of two-component systems composed of MGL, TPL or Trpase enzymes and their sulfoxide substrates.

Previous works:

C–S lyases:

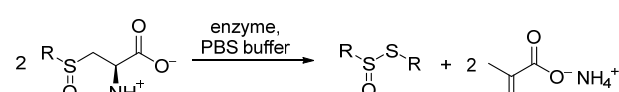
Alliinase / EC 4.4.1.4 (from the plants of *Allium* and *Brassica* genera)



Alliin and other alkyl C4-substrates:

R = CH₃, C₂H₅, C₃H₇, CH-CH=CH₂

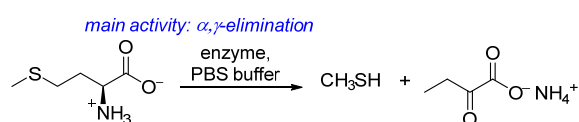
Alliinase / EC 4.4.1.4 (from the plants of *Petiveria* genus)



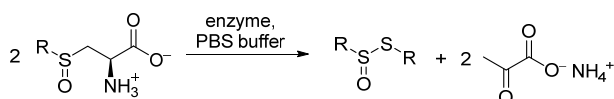
Petiveriin and other substrates:

R = Bn, S-2-hydroxyethyl

Methionine γ-lyase / MGL / EC 4.4.1.11 (from bacterial sources)



secondary activity: α,β-elimination



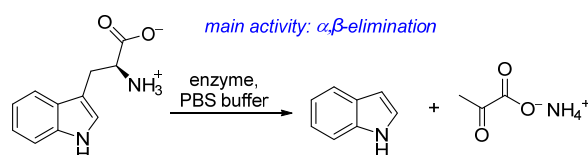
Alliin and other alkyl C4-substrates:

R = CH₃, C₂H₅, C₃H₇, CH-CH=CH₂

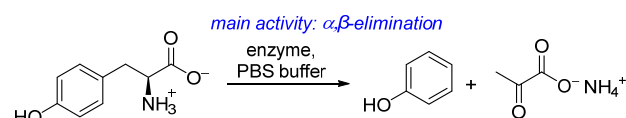
This work:

First examples of C–C lyase specificity for thiosulfinate generation:

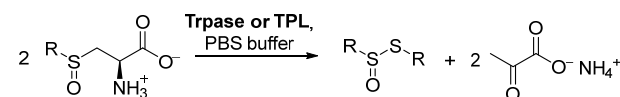
Tryptophan indole-lyase / Trpase / EC 4.1.99.1 (from bacterial sources)



Tyrosine phenol-lyase / TPL / EC 4.1.99.2 (from bacterial sources)



secondary activity: α,β-elimination of the plant-origin substrates



Alliin and other alkyl C4-substrates:

R = CH₃, C₂H₅, C₃H₇, CH-CH=CH₂, Bn

Figure 1. C–S and C–C lyases, their physiologically catalyzed reactions (blue) and newly found biocatalytic transformations (red) for thiosulfinate generation.

2. Results and Discussion

2.1. Enzymatic Thiosulfinate Generation

Extracts from plants of the *Alliaceae* and *Petiveriaceae* families rich in thiosulfates have been historically employed to combat bacterial and fungal infections owing to their well-established antimicrobial properties [27]. Thiosulfate precursors—S-alkylcysteine sulfoxides—originate in plants from glutathione via S-alkylation, and they are also readily available for organic synthesis in the lab [28]. The key plant enzyme found in garlic and broccoli cabbage responsible for thiosulfate production—alliinase—remains inaccessible as a recombinant one due to its instability in the glycolyzed form [29].

The only other recombinant enzyme described for thiosulfate generation as a secondary activity is MGL. This enzyme alone possesses anticancer properties, promoting Met-starvation and apoptosis in tumors; complemented with plant-origin substrates, MGL acts in a binary system “enzyme + prodrug”, capable of β-elimination of S-alk(en)yl L-cysteine sulfoxides into sulfenic acids, which experience direct self-condensation into antimicrobial thiosulfates [17,30,31].

Although the mechanism of the PLP catalysis is general for the amino acid part of the molecule condensed with PLP, the outcome of sulfoxide elimination into sulfenic acid is still speculated. Due to the high reactivity of the intermediate complexes, it is impossible to collect X-ray data. The formal oxidation state of sulfur in the products, sulfenic acids, is 0. This results in a unique ability to function as both a nucleophile and an electrophile, highly

unstable and prone to coupling with all kinds of neighboring groups and to be further oxidized or reduced [32].

The classical mechanism is described as typical for PLP-catalyzed reaction postulations, with the elimination step being assisted by the neighboring amino acids in the active site (Figure 2, top, pathway I) [7]. Initial α -proton cleavage is necessary (typically assisted by the Lys amino group of the spatial environment), and the elimination of a highly reactive sulfenic acid has been postulated to be governed by the ω -amino or phenolic group of the neighboring amino acid (usually, Tyr). This mechanism relies on the internal proton recycling between Lys and Tyr residues and explains the elimination of low-reactive good leaving groups/neutral molecules like H_2S or acetate in the PLP catalysis. However, from the chemical reactivity perspective, sulfenic acids are very prone to oxidizing *N*- and *O*-nucleophiles during this coordination while being reduced to the starting sulfides themselves [33,34]. This also restricts the variety of the substituents at the cysteine sulfoxide part, given that *N*- and *O*-nucleophiles would self-condensate with the R-S-OH product [35]. In organic reactions, a typical pathway of the S=O coordination to an electrophile leads to the Pummerer fragmentation, resulting in the nucleophilic substitution alongside the reduction into the sulfide [36]; the oxygen atom is typically not preserved at the sulfur as a result of these transformations (Figure 2, bottom, Example I). At the same time, alliinase, methionine- γ -lyase, tryptophanase and tyrosine phenol-lyase all have different special environments in the active site to uniformly assist R-S-OH elimination by the similar universal residues. That is why we additionally consider two other mechanisms: intermolecular retro-Michael addition and phosphate-assisted sulfoxide coordination with proton transfer (Figure 2, top, pathways II and III).

A self-driven pre-equilibrium proton transfer can be assumed based on the chemical reactivity [37] in the retro-Michael reaction catalyzed by the enolate formation susceptible to fragmentation (Figure 2, bottom, Example II).

Phosphate assistance is quite often encountered in enzymatic catalysis due to the unique phosphate ability to accept nucleophiles, forming a pentacoordinate intermediate [38]. This mechanism is hypothesized based on the uncertainty of the phosphate role. Braunstein [39] and Floss [40] considered a possibility of rotation of the C5–C5' bond due to the pKa shift between catalytic steps, which includes phosphate repositioning and its assistance for deprotonation during crucial steps. In the early 90s, the C4–C4' rotation became a predominant assumption based on several (but incomplete) linear dichroism data interpretations [41]. At the same time in the 90s, according to the published NMR data, Torchinsky and Kawata postulated that quinoid intermediate formation was accompanied by loosening of contacts between the phosphate group of PLP and the enzyme [42]. These observations correlate with recent structural insights that cysteine is capable of coordination to the phosphate in other PLP-dependent C–S lyases, like cysteine desulfhydrases (Figure 2, bottom, Example III) [43].

In either of these mechanisms, the outcome of the reaction is the same, which makes this reaction plausible to be catalyzed by enzymes other than C–S lyases. To date, there have been no reports regarding the production of thiosulfinates mediated by C–C lyases, which is covered in our research.

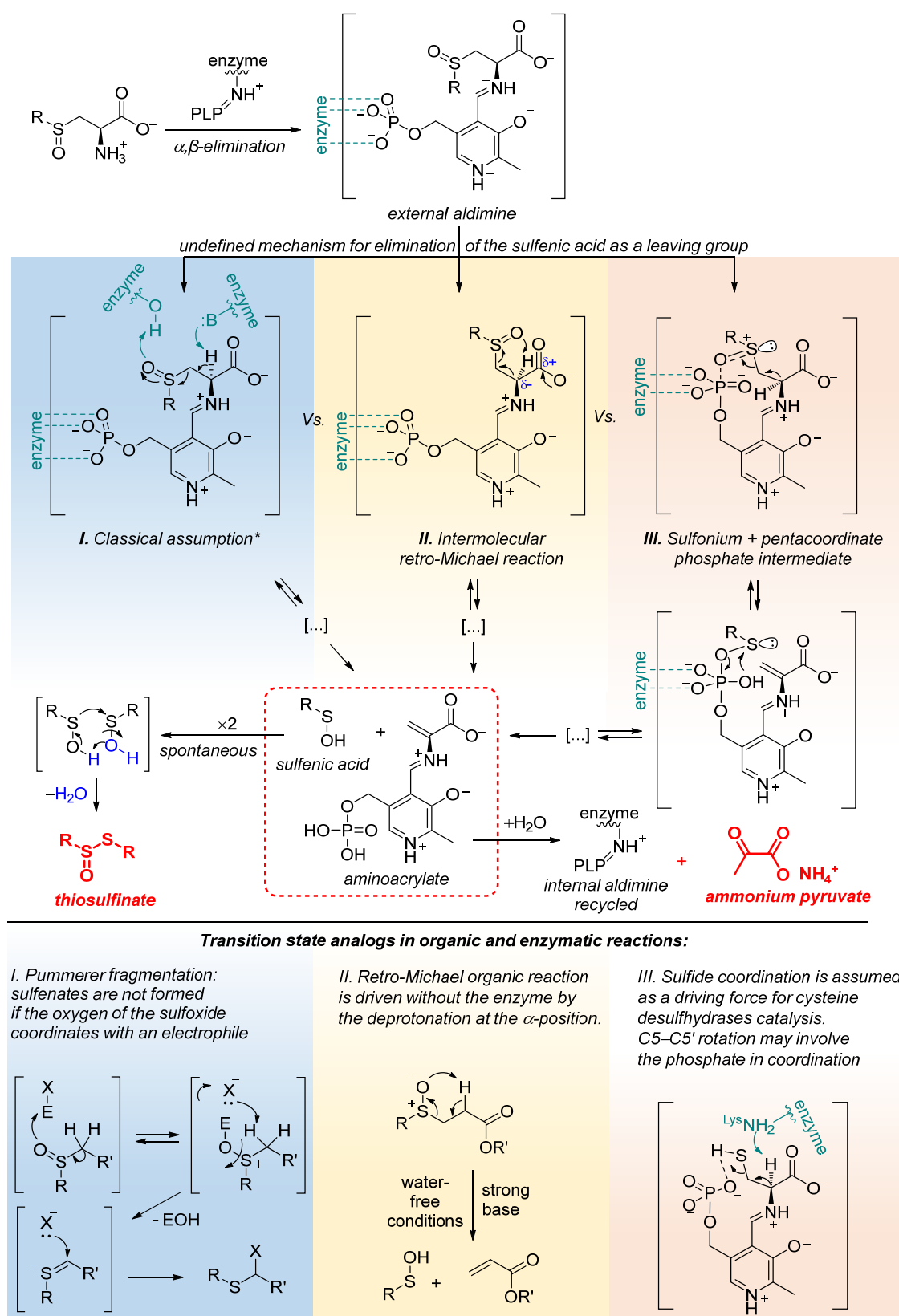


Figure 2. Mechanistic insights for PLP-assisted enzymatic generation of sulfenic acids.

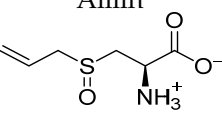
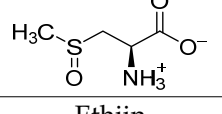
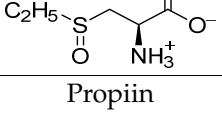
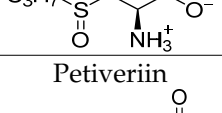
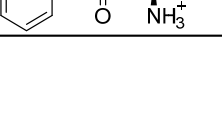
The native substrate of MGL is an unbranched aliphatic amino acid, but our investigation also confirms the transformation of an aromatic natural sulfoxide—petiveriin. Among the known C–C β -lyases, we have chosen TPL and Trpase based on their capacity to catalyze the cleavage of aromatic amino acids. These enzymes belong to the 4.1.99

subclass (“Other C–C Lyases”), neighboring the class of C–S lyases. TPL derived from *C. freundii* and Trpase from *P. vulgaris* are both well-studied, display robust stability profiles, and feature accessible crystal structures. To assess whether TPL and Trpase could accommodate *S*-substituted L-cysteine sulfoxides within their active sites, we conducted molecular docking studies using PLP bound to either alliin or petiveriin as ligands. Molecular docking showed that the active sites of TPL and Trpase are capable of binding both sulfoxides. Moreover, the orientation of PLP aligns with previously reported structural data, and the sulfoxides adopt conformations similar to those observed for typical ligands (Figures S2 and S3). Thus, MGL from *C. novyi*, TPL from *C. freundii*, and Trpase from *P. vulgaris* were selected as key objects of our study.

2.2. Kinetics of Decomposition of *S*-substituted L-cysteine Sulfoxides Catalyzed by TPL, Trpase and MGL

In this work, we report the kinetics of α,β -elimination reactions of *S*-substituted L-cysteine sulfoxides catalyzed by three different lyases—TPL, Trpase, and MGL. The enzymes demonstrated broad substrate specificity, reacting with L-cysteine sulfoxides bearing aliphatic, alkenyl, or aromatic substituents (Table 1). Among the compounds tested, no clear trends in catalytic efficiency were observed. However, a substantial difference was observed for petiveriin: specifically, both TPL and Trpase showed approximately tenfold higher k_{cat} values and about 3–4 times higher catalytic efficiency (k_{cat}/K_m) relative to MGL. Interestingly, although Trpase and TPL generally processed alliin, methiin, ethiin, and propiin at 2–4 times faster rates than MGL, their catalytic efficiencies remained comparable to those of MGL, primarily due to higher K_m values for these substrates.

Table 1. Steady-state kinetic parameters of the α,β -elimination reactions catalyzed by PLP-dependent lyases (37 °C).

Substrate	Kinetic Parameters *	Enzymes		
		<i>C. freundii</i> TPL	<i>P. vulgaris</i> Trpase	<i>C. novyi</i> MGL
 Alliin	k_{cat} , s ^{−1}	15.4 ± 0.07	10.4 ± 0.06	4.5 ± 0.06 **
	K_m , mM	3.42 ± 0.91	8.6 ± 0.5	1.4 ± 0.2 **
	k_{cat}/K_m , M ^{−1} s ^{−1}	4.5 × 10 ³	1.2 × 10 ³	3.2 × 10 ³ **
 Methiin	k_{cat} , s ^{−1}	3.9 ± 0.03	4.1 ± 0.06	0.75 ± 0.01 **
	K_m , mM	3.8 ± 1.26	3.9 ± 0.5	0.7 ± 0.08 **
	k_{cat}/K_m , M ^{−1} s ^{−1}	1.0 × 10 ³	1.1 × 10 ²	1.1 × 10 ³ **
 Ethiin	k_{cat} , s ^{−1}	7.9 ± 0.07	8.9 ± 0.07	2.5 ± 0.04 **
	K_m , mM	3.8 ± 0.7	5.1 ± 1.5	1.0 ± 0.2 **
	k_{cat}/K_m , M ^{−1} s ^{−1}	2.1 × 10 ³	1.7 × 10 ³	2.5 × 10 ³ **
 Propiin	k_{cat} , s ^{−1}	15.8 ± 0.06	11.4 ± 0.06	3.8 ± 0.06 **
	K_m , mM	3.7 ± 0.24	3.1 ± 0.5	1.6 ± 0.3 **
	k_{cat}/K_m , M ^{−1} s ^{−1}	4.3 × 10 ³	3.7 × 10 ³	2.3 × 10 ³ **
 Petiveriin	k_{cat} , s ^{−1}	19.0 ± 0.06	21.1 ± 0.03	2.3 ± 0.03
	K_m , mM	2.4 ± 0.4	2.3 ± 0.6	1.1 ± 0.2
	k_{cat}/K_m , M ^{−1} s ^{−1}	7.9 × 10 ³	9.2 × 10 ³	2.1 × 10 ³

* Each data point represents the average of triplicate measurements. ** Published data [44].

The enzymes were then examined for their stability and activity over a temperature range of 25 to 80 °C. Upon heating for 60 min, they retained full stability up to 55–60 °C

when incubated with propiin in 0.1 M potassium phosphate buffer (pH 8.0) (Figure 3A). Maximum activity for the α,β -elimination of propiin was observed at 55 °C for MGL and 60 °C for TPL and Trpase (Figure 3B). The results were consistent across other sulfoxides as well.

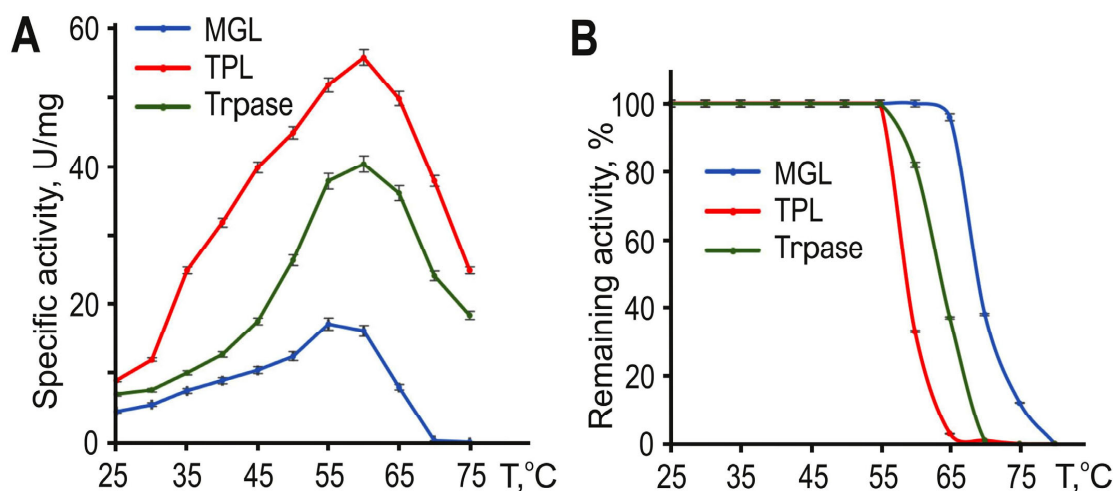


Figure 3. Effect of temperature on activity (A) and stability (B) of MGL, TPL and Trpase. The activity with propiin as a substrate was measured in the standard reaction mixture for 60 min at different temperatures by the standard activity assay. Enzyme stability was assessed based on the residual activity following incubation in 0.1 M potassium phosphate buffer (pH 8.0) at indicated temperatures. Error bars represent the standard deviation of a triplicate of experimental measurements.

2.3. Analysis of Thiosulfinate Synthesis Efficiency

Evaluation of thiosulfinate formation in enzyme-mediated reactions with sulfoxides revealed that the highest conversion occurred in the reactions of MGL, TPL and Trpase with alliin, as well as in the reactions of TPL and Trpase with petiveriin (Table 2). During the reaction process, enzyme activity may decline due to modifications of critical SH groups in the active site caused by highly active thiosulfates. It is worth noting that MGLs from diverse sources contain surface-exposed cysteine residues susceptible to oxidation [16,30]. Interestingly, neither TPL nor Trpase demonstrated significant loss of activity over the experimental 1h period, in contrast to MGL, which experienced partial inactivation (Figure 4). After the reaction completed, TPL and Trpase were separated from the products by dialysis and then reused, achieving the same yields. This demonstrates their superior suitability for repeated enzymatic cycles in practical applications compared to MGL.

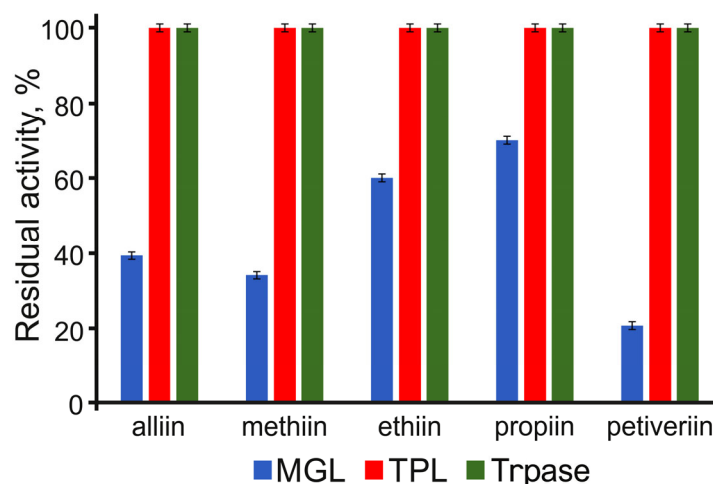
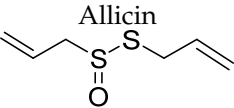
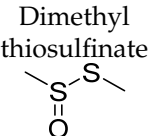
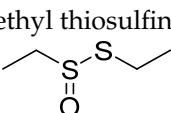
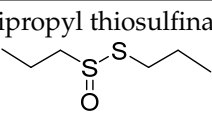
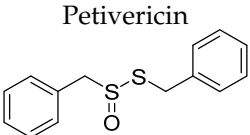


Figure 4. Inactivation of the enzymes following 1h reaction with S-substituted L-cysteine sulfoxides.

Table 2. Thiosulfinate production by TPL, Trpase and MGL.

Substrate, 3 mg	Product	Theoretical Product Yield, mg	Actual Yield of the Product, mg (%) *		
			MGL	TPL	Trpase
Alliin	 Allicin	1.40	1.40 ± 0.01 (100)	1.40 ± 0.01 (100)	1.40 ± 0.01 (100)
Methiin	 Dimethyl thiosulfinate	1.09	1.02 ± 0.01 (94)	0.80 ± 0.02 (73)	0.81 ± 0.01 (74)
Ethiin	 Diethyl thiosulfinate	1.25	0.97 ± 0.02 (78)	0.90 ± 0.01 (72)	0.90 ± 0.02 (72)
Propiin	 Dipropyl thiosulfinate	1.40	1.10 ± 0.01 (79)	1.20 ± 0.01 (86)	1.30 ± 0.08 (93)
Petiveriin	 Petivericin	1.70	1.10 ± 0.01 (65)	1.70 ± 0.01 (100)	1.70 ± 0.01 (100)

* Actual yield of the products was calculated based on the determined concentration of thiosulfates in the reaction mixture, as described in Section 2.4. The values presented are the result of the average of the three definitions.

One of the main advantages of an enzyme-based thiosulfinate generation system compared to chemical oxidation is its ability to generate thiosulfates in situ through separate administration of the enzyme and sulfoxide directly into the organism [31] while being non-toxic in the separate prodrug + enzyme formulation [45].

2.4. Product Characterization

NMR monitoring of the enzymatic conversion of *S*-alk(en)yl-L-cysteine sulfoxides confirmed that the products of biotransformation were consistent within all three enzymes and in accordance with the previous data on natural thiosulfates [31], taking into consideration an overlap with the substrate signals (Figure 5). The reaction was set in the standard conditions in the PPB buffer (400 µL) with 5 mg of the substrates; after 1 h, the reaction mixture was transferred into an NMR ampule, and 50 µL of D₂O was added for the signal lock. In the case of *S*-benzyl cysteine sulfoxide, the product was insoluble in the buffer solution and required separation from the reaction mixture using flash chromatography. Petivericin was further analyzed in ¹H NMR in C₆D₆ as a solvent; the spectrum was reproducible for all three enzymes (Figure S3). A good proportionality was found in the intensities of the signals for each of the four aliphatic products. It was found that the rate of conversion is slower for C–C lyases, while MGL yielded almost pure products in just 1 h. At the same time, both C–C lyases retained their activity after the reaction completion.

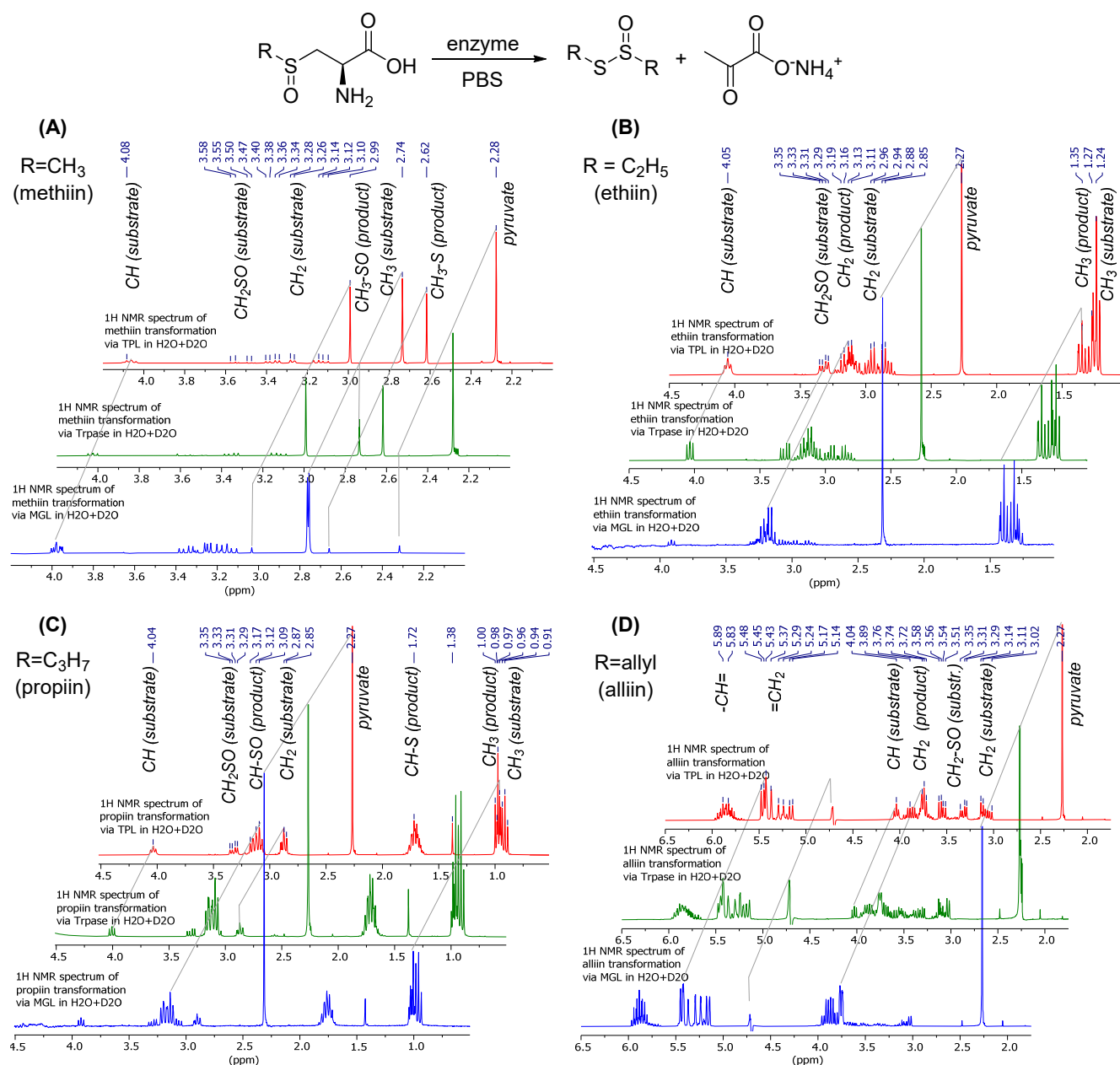


Figure 5. ¹H NMR spectra of the biotransformation products obtained from natural S-substituted cysteine sulfoxides with different enzymes (reaction mixtures).

2.5. Evaluation of Antimicrobial Activity of Thiosulfinates Produced Through Enzyme-Mediated Reactions with Sulfoxides

Both synthetically derived and enzymatically obtained thiosulfinates are known to exhibit antitumor and antimicrobial activities [11,46]. Recent findings showed that fungi are more sensitive to thiosulfinates when compared to bacteria [11,31]. Our data on anti-candidal activity support these previous observations. Thiosulfinates generated via binary systems were evaluated against a diverse panel of the most common human pathogens: fungi (*Candida albicans*) and Gram-positive and Gram-negative bacteria from the ESKAPE group (*S. aureus* and *P. aeruginosa*) (Table 3). According to literature reports, the naturally occurring thiosulfinates exhibit little or negligible activity against *P. aeruginosa* when administered at therapeutically relevant concentrations [47,48]; however, they demonstrate potential utility in the treatment of multi-drug-resistant bacterial infections in cystic fibrosis patients [20,21]. In our study, all thiosulfinates inhibited *P. aeruginosa* growth at relatively

high concentrations above 32 µg/mL (Table 3). Regarding *S. aureus*, DMTS, DETS, DPTS and DATS exhibited perceptible antimicrobial activity only at high concentrations. This is consistent with our previously obtained data for synthetic thiosulfates [49]. Importantly, petivericin demonstrated fivefold greater efficacy against *S. aureus* relative to its aliphatic counterparts; however, it is markedly less effective compared to gentamicin (Table 3).

Table 3. Antimicrobial activity of thiosulfates produced through enzyme-mediated reactions in the presence of the enzyme and after product separation from the enzymatic component by dialysis.

Thiosulfate	Enzyme	MIC ± CI, µg/mL (µM)		
		<i>Candida albicans</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>
DMTS	TPL	1 ± 0 (9.1)	≥32 (290)	≥32 (290)
	Trpase	1.92 ± 0.18 (17.4)	>32 (290)	>32 (290)
	MGL	1.67 ± 0.31 (15.2)	≥32 (290)	32 (290)
	average	1.53 ± 0.17 (13.9)	≥32 (290)	≥32 (290)
	after dialysis	1.44 ± 0.17 (13.1)	≥32 (290)	≥32 (290)
DETS	TPL	0.63 ± 0.14 (4.6)	>32 (231)	≥32 (231)
	Trpase	1.08 ± 0.18 (7.8)	>32 (231)	>32 (231)
	MGL	1 ± 0 (7.2)	>32 (231)	32 (231)
	average	0.9 ± 0.1 (6.5)	>32 (231)	≥32 (231)
	after dialysis	0.83 ± 0.11 (6)	≥32 (231)	≥32 (231)
DPTS	TPL	0.44 ± 0.07 (2.6)	>32 (192)	32 (192)
	Trpase	0.63 ± 0.14 (3.8)	>32 (192)	>32 (192)
	MGL	0.42 ± 0.08 (2.5)	>32 (192)	32 (192)
	average	0.49 ± 0.06 (2.9)	>32 (192)	≥32 (192)
	after dialysis	0.56 ± 0.08 (3.4)	>32 (192)	32 (192)
DATS (allicin)	TPL	0.46 ± 0.06 (2.8)	32 ± 0 (197)	>32 (197)
	Trpase	0.31 ± 0.07 (1.9)	21.33 ± 8.67 (131)	32 (197)
	MGL	0.5 ± 0.12 (3.1)	26.67 ± 8.67 (164)	>32 (197)
	average	0.42 ± 0.05 (2.6)	26.67 ± 3.86 (164)	≥32 (197)
	after dialysis	0.58 ± 0.07 (3.6)	32 ± 0 (197)	≥32 (197)
DBTS (petivericin)	TPL	0.33 ± 0.07 (1.3)	13 ± 3.46 (50)	>32 (122)
	Trpase	0.23 ± 0.02 (0.9)	9.71 ± 5.6 (37)	>32 (122)
	MGL	0.24 ± 0.05(0.9)	4 ± 0 (15)	>32 (122)
	average	0.26 ± 0.03 (1)	9.90 ± 2.68 (38)	>32 (122)
	after dialysis	0.19 ± 0.04 (0.7)	7.29 ± 1.16 (28)	>32 (122)
Amphotericin B	-	0.4 ± 0 (0.4)	not determined	not determined
Gentamicin	-	not determined	0.6 ± 0.1 (1.3)	2.0 ± 0.3 (4.2)

Latest research findings showed the fungi *C. albicans* to be more sensitive to thiosulfates when compared to bacteria [31,50,51]. The antifungal potency of DMTS, DETS, DPTS, and DPTS produced by synthetic and enzymatic methods has been analyzed in our previous work [31]. In the present study, we compared the potency of products derived from binary “enzyme + substrate” systems, additionally evaluating the impact of petivericin

on this particular pathogen. The potency of thiosulfinates produced via biocatalysis was found to be independent of the enzymatic component utilized. With the sole exception of petivericin, the antimicrobial activity remained consistent between the crude reaction mixture and the separated final product. Thus, the additional extraction of thiosulfinates from the reaction mixture is not strictly necessary. MIC differences for petivericin in the presence of the enzyme and the extracted product from the reaction mixture (26% for both *C. albicans* and *S. aureus*) can be attributed to the low solubility of the substance and its partial incorporation into the internal cavities of the enzyme.

In 2006 Kim et al. [47] reported that petivericin has no significant antifungal activity. However, Leontiev et al. demonstrated the better potency of petivericin in yeast-like fungus, *Saccharomyces cerevisiae*, where it found its activity among other thiosulfinates in the following order: DMTS < DETS < allicin < DPTS < petivericin [11]. Due to this data inconsistency and low solubility of the substance, we have doubled the number of biological replicates in the MIC assay for petivericin. The results for the DBTS with *C. albicans* showed that the petivericin potency is comparable to that of amphotericin B, with a MIC of 0.7 μM and 0.4 μM , respectively (Table 3). Similar to *S. aureus*, petivericin proved to be roughly five times more potent than aliphatic thiosulfinates, which makes it a good candidate for antifungal treatment of crops in soil. The structure of aliphatic thiosulfinates is known to affect their potential in altering the permeability of plant cell membranes and the tonoplast of the algae *Chara corallina* [52]. The presence of the benzyl moiety in the petivericin molecule likely enables it to penetrate the peptidoglycan layer of Gram-positive bacteria and fungal membranes more easily than aliphatic thiosulfinates do.

The antifungal activity of the studied thiosulfinates turned out to be significantly higher than their antibacterial activity, comparable to the action of the commercial drug (Table 3). Thiosulfinates are also known to have cytotoxic activity against human cells [11,46,53]. At the same time, some cancer cells are more sensitive to the action of thiosulfates than healthy human cells, with the CC_{50} in human dermal fibroblasts ranging from 34 μM to 223 μM [54,55]. These values are an order of magnitude higher than the concentrations required to suppress the growth of *C. albicans*. It has also been shown that the intravenous injections of encapsulated C115H MGL/methiin system in a $\times 10$ therapeutic dose do not cause significant abnormalities in mice [45]. Individual components of the system are completely non-toxic to mouse embryos [56].

2.6. Stability of Enzymatic Systems

To verify the stability of the studied enzymatic systems, we measured the kinetic parameters and antimicrobial activity of the drugs after 1, 2 and 7 months when the tested samples were stored at $-20\text{ }^{\circ}\text{C}$. With an acceptable margin of error, the results (10% for kinetic parameters and a range of 0–1 dilution of adjacent MIC values for the method of double serial dilutions) did not differ from the data presented in Tables 1 and 3.

Thus, TPL and Trpase both show an advantage over MGL in the binary enzyme/S-substituted L-cysteine sulfoxide systems without losing activity, rendering them highly effective for repeated cycles in practical enzymatic processes. Future efforts should focus on optimizing these systems for industrial-scale production and medical applications.

3. Materials and Methods

3.1. Materials

L-Methionine, L-tyrosine, L-tryptophan, nicotinamide adenine dinucleotide reduced form (NADH), lactate dehydrogenase (LDH) from rabbit muscle, pyridoxal 5'-phosphate (PLP), D,L-dithiothreitol (DTT), potassium phosphate, and S-allyl-L-cysteine sulfoxide (alliin) were purchased from Sigma-Aldrich (Burlington, MA, USA). 2-Nitro-5-thiobenzoate

(NTB) was prepared according to Miron et al. [57]. The plasmid with the gene of D-2-hydroxyisocaproate dehydrogenase was a kind gift of Dr. K. Muratore (University of California, Department of Molecular and Cell Biology, Berkeley, CA, USA). The RPMI-1640 medium, 3-(*N*-morpholinyl)propanesulfonic acid (MOPS, >99.5%), and gentamicin were purchased from PanEco (Moscow, Russia). Agar and Mueller-Hinton broth were produced by HiMedia Laboratories Pvt. Limited (Mumbai, Maharashtra, India); YPD agar and the pharmaceutical secondary standard of amphotericin B were purchased from Sigma-Aldrich. *S*-Methyl-L-cysteine sulfoxide (methiin), *S*-ethyl-L-cysteine sulfoxide (ethiin), *S*-propyl-L-cysteine sulfoxide (propiin), and *S*-benzyl-L-cysteine sulfoxide (petiveriin) were synthesized according to the published procedures [58–60]. Compound structures were verified by ^1H and ^{13}C NMR spectra, and their purity exceeded 99%.

3.2. Enzyme Preparation and Assay

Recombinant MGL from *C. novyi*, TPL from *C. freundii*, and Trpase from *P. vulgaris* were expressed and purified following the previously described protocols [30,61,62]. Activities of TPL and Trpase were assayed with L-tyrosine and L-tryptophan (Figure 2), respectively, under the same conditions for both proteins. A 1 mL assay contained 0.1 M potassium phosphate buffer (pH 8.0), 0.1 mM PLP, 0.2 mM NADH, 8 units of LDH, and either 2 mM L-tyrosine or 2.8 mM L-tryptophan. The reactions were initiated by the addition of 1.0–5.0 μg of each enzyme. The appearance of pyruvic acid was monitored at 37 °C in a coupled system with LDH by observing the reduction in absorbance of NADH at 340 nm ($\epsilon = 6220 \text{ M}^{-1}\text{cm}^{-1}$) [63] using a Cary-50 spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). MGL activity was monitored using L-methionine as a substrate. A 1 mL assay solution consisted of 0.1 M potassium phosphate buffer (pH 8.0), 0.1 mM PLP, 0.2 mM NADH, 8 units of LDH, and 30 mM L-methionine. The reaction was initiated by adding 0.5–1.0 μg of the enzyme. Formation of α -ketobutyrate was followed at 37 °C in a coupled system with D-2-hydroxyisocaproate dehydrogenase by monitoring the decline in NADH absorbance at 340 nm [64]. One unit of enzyme activity corresponds to the quantity of enzyme required to catalyze the formation of 1 μmol of product per minute.

3.3. Kinetic Studies

The kinetic parameters of the α,β -elimination reactions of *S*-substituted L-cysteine sulfoxides catalyzed by MGL, TPL, and Trpase were determined according to Section 2.2 by varying substrate concentrations. Steady-state kinetic parameters were calculated using the Michaelis–Menten equation implemented in the EnzFitter 1.05 software package (BioSoft, Cambridge, UK).

3.4. Evaluation of Thiosulfinates Formation in Reaction Mixtures of Enzymes with Sulfoxides

Alliin, methiin, ethiin, propiin and petiveriin (each 3 mg) were individually dissolved in 1 mL of the potassium phosphate buffer (50 mM, pH 8.0) and then incubated with TPL, Trpase, or MGL (all at a final concentration of 2 mg/mL) for 1 h at room temperature. The products (DMTS, DETS, DPTS, and allicin) were separated from the enzyme by dialysis using centrifugal concentrators 30,000 MWCO (Sigma-Aldrich) and analyzed. Petivericin was separated from the enzyme by centrifugation followed by three consecutive washings with water; the resulting solid precipitate was dissolved in C_6D_6 and analyzed by ^1H NMR.

The concentrations of both individual and total thiosulfinates in the reaction mixtures were quantified using the NTB assay method described by Miron et al. [57].

3.5. Analysis of Enzyme Inactivation During Incubation with *S*-substituted-L-cysteine Sulfoxides

MGL, TPL, and Trpase (each at a concentration of 5 μM in 0.1 M potassium phosphate buffer, pH 8.0) were separately incubated with 50 μM of corresponding *S*-substituted

L-cysteine sulfoxides at 37 °C for 1 h. Samples of the reaction mixtures were periodically withdrawn and tested for residual enzyme activity using the procedure outlined in Section 2.2.

3.6. Cell Cultures

S. aureus ATCC 29213, *P. aeruginosa* ATCC 27853, and *C. albicans* ATCC 10231 strains were obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA). Subculturing and inoculum preparation of bacteria were prepared according to the Clinical and Laboratory Standards Institute (CLSI)-recommended method, M7-A9 [65], using MHB. Subculturing and inoculum preparation of *C. albicans* were carried out according to the CLSI-recommended method M27-A3 [66], using RPMI-1640. The stock suspension of *C. albicans* and bacteria were 1×10^6 and 1×10^8 cells per mL, respectively.

3.7. Antibiotic Activity Evaluation

Minimum inhibitory concentrations (MICs) were determined via the broth microdilution method according to CLSI guidelines M7-A9 and M100-A30 [67] for bacteria and M27-A3 for *C. albicans* [66] in three independent experiments with three or four replicates each. For poorly soluble petivericin, six independent experiments were performed with four replicates each. Stock solutions of the tested thiosulfinate were prepared as described in Section 2.4. The thiosulfinate solutions were diluted to final concentrations in the test medium and added to the wells of 96-well plates in a volume of 100 µL. Then 100 µL of inoculum was added to each well (see Section 3.6). Final tested concentration ranges were as follows: DMTS, DETS, DTPS and allicin, 0.125 to 32 µg/mL; petivericin, 0.016 to 32 µg/mL.

An inoculum with a broth medium without drugs was used as a negative control, and a broth medium was used as a sterility control. Amphotericin B was used as a positive control for the effect of the test substances on *C. albicans*, and gentamycin was used for bacteria. MIC values of positive control drugs were consistent with CLSI guidelines. MGL, TPL and Trpase were tested at a concentration of 2 mg/mL. Enzymes had no effect on the growth of microbial cultures.

MIC endpoints were determined following 24 h of incubation using an iMark microplate absorbance reader (Bio-Rad, Hercules, CA, USA).

3.8. Structure Elucidation of the Biotransformation Products

^1H and ^{13}C NMR spectra (δ , ppm; J , Hz) were registered on an AMX III 300 spectrometer (Bruker BioSpin GmbH, Karlsruhe, Germany) with a working frequency of 300 MHz for ^1H NMR (Me_4Si as an internal standard for organic solvents) and 75 MHz for ^{13}C NMR (with carbon–proton interaction decoupling) and on an Avance II 600 spectrometer (Bruker BioSpin GmbH, Karlsruhe, Germany) with a working frequency of 600 MHz for ^1H NMR (Me_4Si as an internal standard for organic solvents) and 151 MHz for ^{13}C NMR (with carbon–proton interaction decoupling). High-resolution mass spectra (HRMSs) were registered on a micrOTOF-Q II hybrid quadrupole time-of-flight mass spectrometer (Bruker Daltonics GmbH, Bremen, Germany) using electrospray ionization (ESI); the measurements were carried out in positive ion mode. The samples were injected into the mass spectrometer chamber using a syringe injection or from the Agilent 1260 HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with an Agilent Poroshell 120 EC-C18 column (3.0×50 mm; $2.7 \mu\text{m}$) and an identically packed security guard using an autosampler. The samples were in 50% methanol in water (MilliQ ultrapure water; Merck Millipore KGaA, Darmstadt, Germany). The column was eluted with a gradient of methanol (A) concentrations in water (B) with a flow rate of 400 µL/min in the following

gradient parameters: 0–15% A for 6 min, 15–85% A for 1.5 min, 85–0% A for 0.1 min, and 0% A for 2.4 min.

NMR and HRMS data for the compounds were in accordance with the previous reports, as described below. Dimethylthiosulfinate: ^1H NMR ($\text{D}_2\text{O} + \text{H}_2\text{O}$, 300 MHz): 2.62 (s, 3H), 2.99 (s, 3H); HRMS: m/z : calcd for $[\text{M} - \text{H}]^-$ 108.9776, found: 108.9782. Diethylthiosulfinate: ^1H NMR ($\text{D}_2\text{O} + \text{H}_2\text{O}$, 300 MHz): 1.27 (t, J 7.46 Hz, 3H), 1.35 (t, J 7.4 Hz, 3H), 3.12 (q, J 7.4 Hz, 2H), 3.18 (q, J 7.3 Hz, 2H); HRMS (ESI) of $\text{C}_4\text{H}_{10}\text{OS}_2$, m/z : calcd for $[\text{M} - \text{H}]^-$ 137.0089, found: 137.0098. Dipropylthiosulfinate: ^1H NMR ($\text{D}_2\text{O} + \text{H}_2\text{O}$, 300 MHz): 0.91 (t, J 7.3 Hz, 3H), 0.96 (t, J 7.4 Hz, 3H), 1.72 (m, 4H), 3.12 (m, 4H); HRMS (ESI) of $\text{C}_6\text{H}_{14}\text{OS}_2$, m/z : calcd for $[\text{M} - \text{H}]^-$ 165.0402, found: 165.0410. Diallylthiosulfinate: ^1H NMR ($\text{D}_2\text{O} + \text{H}_2\text{O}$, 300 MHz): 3.67–3.79 (m, 4H), 5.15–5.45 (m, 4H), 5.66–5.89 (m, 2H); HRMS (ESI) of $\text{C}_6\text{H}_{10}\text{OS}_2$, m/z : calcd for $[\text{M} - \text{H}]^-$ 161.0089, found: 161.0098. Dibenzylthiosulfinate: 6.98–7.10 (m, 6H), 3.72 (m, 2H), 3.34 (m, 2H); HRMS (ESI) of $\text{C}_6\text{H}_{10}\text{OS}_2$, m/z : calcd for $[\text{M} - \text{H}]^-$ 263.0559, found: 263.0567.

3.9. Molecular Docking

Molecular docking was performed using AutoDock Vina 1.1.2 (author: Dr. Oleg Trott, open-source software) with default parameters [68]. The structures of PDB IDs 8v6p (*P. vulgaris* Trpase) and 6mpd (*C. freundii* TPL), from which the water molecules and ligands were removed, were used as models. Three-dimensional structures of external aldimines of PLP with alliin and PLP with petiveriin were prepared in the BIOVIA Discovery Studio 25.1.0.24284 software package (Dassault Systèmes BIOVIA, Paris, France). Molecular docking of the compounds was calculated for their positions within the active sites of *P. vulgaris* Trpase and *C. freundii* TPL with a docking box of $18 \times 12 \times 22 \text{ \AA}$. The resulting docked structures were ranked according to their predicted binding energies and validated by comparing them with the known positions of intermediates within the corresponding three-dimensional protein structures (8v6p and 6mpd). The interactions of the positions of the model ligands with the amino acid residues of the enzymes were calculated using the Schrödinger Maestro 12.4 program (Schrödinger Inc., New York, NY, USA) at a distance of 4 Å.

3.10. Testing the Stability of Enzymatic Systems

To check the stability of the enzyme systems, samples of individual components in a 0.1 M potassium phosphate buffer solution (pH 8.0) were stored at -20°C . After 1, 2 and 7 months, experiments were carried out with those components according to Sections 3.3 and 3.7. All measurements were carried out in triplicate.

4. Conclusions

In our study we have demonstrated for the first time the capabilities of the two C–C lyases—tryptophan indole-lyase (Trpase, EC 4.1.99.1) and tyrosine phenol-lyase (TPL, EC 4.1.99.2)—to exhibit auxiliary activity beyond their traditional roles. These enzymes can act as C–S lyases, enabling the biosynthesis of thiosulfinates from *S*-substituted L-cysteine sulfoxides. Using comparative analysis of kinetic profiles, we have revealed that these enzymes display remarkable substrate diversity for typical plant-origin substrates, efficiently processing both aliphatic and aromatic *S*-substituted cysteine sulfoxides. Notably, TPL and Trpase display superior performance compared to MGL in the biotransformation of specific substrates like petiveriin, making them alternative biocatalysts for thiosulfinate production.

This study confirms the antimicrobial efficacy of thiosulfinates produced by these enzymes, particularly against clinically relevant pathogens *C. albicans*, *P. aeruginosa* and

S. aureus. Enzymatically obtained petivericin is significantly more effective against *S. aureus* compared to other thiosulfinates, proving it to be a highly potent antibacterial agent against Gram-positive organisms. Furthermore, petivericin demonstrates exceptional antifungal activity against *C. albicans*, achieving an MIC similar to that of amphotericin B. The advantages of TPL and Trpase compared to MGL were found in terms of catalytic efficiency and stability, making them viable candidates for industrial-scale biocatalysis.

Overall, our research expands the scope of biocatalytic strategies for generating biologically active compounds, highlighting the unexplored potential of C–C lyases in the production of antimicrobial agents for combating infectious diseases and advancing sustainable chemical synthesis methodologies.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ph19020291/s1>, Figure S1: ¹H NMR spectrum of dibenzylthiosulfinate; Figure S2: Molecular docking of the position of the external aldimine with petiveriin and alliin in the active site of *P. vulgaris* Trpase (pdb 8v6p); Figure S3: Molecular docking of the position of the external aldimine with petiveriin and alliin in the active site of *C. freundii* TPL (pdb 6mpd).

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References

1. Zhang, N.; de María, P.D.; Kara, S. Biocatalysis for the Synthesis of Active Pharmaceutical Ingredients in Deep Eutectic Solvents: State-of-the-Art and Prospects. *Catalysts* **2024**, *14*, 84. [\[CrossRef\]](#)
2. Cosgrove, S.C.; Miller, G.J. Advances in biocatalytic and chemoenzymatic synthesis of nucleoside analogues. *Expert Opin. Drug Discov.* **2022**, *17*, 355–364. [\[CrossRef\]](#)
3. Bones, A.M.; Rossiter, J.T. The myrosinase-glucosinolate system, its organisation and biochemistry. *Physiol. Plant.* **1996**, *97*, 194–208. [\[CrossRef\]](#)
4. Rosenbergová, Z.; Hegyi, Z.; Ferko, M.; Andelová, N.; Martin Rebroš, M. Improved Production of Recombinant Myrosinase in *Pichia pastoris*. *Int. J. Mol. Sci.* **2021**, *22*, 11889. [\[CrossRef\]](#)
5. Stoll, A.; Seebeck, E. Über die Spezifität der Alliinase und die Synthese mehrerer dem Alliin verwandter Verbindungen. *Helv. Chim. Acta* **1949**, *32*, 866–876. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Hamamoto, A.; Mazelis, M. The C-S lyases of higher plants: Isolation and properties of homogeneous cystine lyase from broccoli (*Brassica oleracea* var *botrytis*) buds. *Plant. Physiol.* **1986**, *80*, 702–706. [\[CrossRef\]](#)
7. Block, E. The organosulfur chemistry of the genus *Allium* implications for the organic chemistry of sulfur. *Angew. Chem.* **1992**, *31*, 1135–1178. [\[CrossRef\]](#)
8. Rose, P.; Whiteman, M.; Moore, P.K.; Zhu, Y.Z. Bioactive S-alk(en)yl cysteine sulfoxide metabolites in the genus *Allium*: The chemistry of potential therapeutic agents. *Nat. Prod. Rep.* **2005**, *22*, 351–368. [\[CrossRef\]](#)
9. Kubec, R.; Musah, R.A. Cysteine sulfoxide derivatives in *Petiveria alliacea*. *Phytochemistry* **2001**, *58*, 981–985. [\[CrossRef\]](#)
10. Estevam, E.C.; Griffin, S.; Jasim, N.M.; Zielinski, D.; Aszyk, J.; Osowicka, M.; Dawidowska, N.; Idroes, R.; Bartoszek, A.; Jacob, C. Inspired by nature: The use of plant-derived substrate/enzyme combinations to generate antimicrobial activity in situ. *Nat. Prod. Commun.* **2015**, *10*, 1733–1738. [\[CrossRef\]](#)

11. Leontiev, R.; Hohaus, N.; Jacob, C.; Martin, C.H.; Gruhlke, M.C.H.; Slusarenko, A.J. A comparison of the antibacterial and antifungal activities of thiosulfinate analogues of alliin. *Sci. Rep.* **2018**, *8*, 6763. [\[CrossRef\]](#)
12. Shen, C.; Parkin, K.L. In vitro biogenesis of pure thiosulfonates and propanethial-S-oxide. *J. Agric. Food Chem.* **2000**, *48*, 6254–6260. [\[CrossRef\]](#)
13. Krest, I.; Keusgen, M. Quality of herbal remedies from *Allium sativum*: Differences between alliinase from garlic powder and fresh garlic. *Planta Med.* **1999**, *65*, 139–143. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Makino, T.; Skretas, G.; Georgiou, G. Strain engineering for improved expression of recombinant proteins in bacteria. *Microb. Cell Fact.* **2011**, *10*, 32. [\[CrossRef\]](#)
15. Tanaka, H.; Esaki, N.; Soda, K. Properties of L-methionine gamma-lyase from *Pseudomonas ovalis*. *Biochemistry* **1977**, *16*, 100–106. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Morozova, E.A.; Revtovich, S.V.; Anufrieva, N.V.; Kulikova, V.V.; Nikulin, A.D.; Demidkina, T.V. Alliin is a suicide substrate of *Citrobacter freundii* methionine γ -lyase: Structural bases of inactivation of the enzyme. *Acta Crystallogr. D Biol. Crystallogr.* **2014**, *70*, 3034–3042. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Anufrieva, N.V.; A Morozova, E.; Kulikova, V.V.; Bazhulina, N.P.; Manukhov, I.V.; I Degtev, D.; Gnuchikh, E.Y.; Rodionov, A.N.; Zavilgelsky, G.B.; Demidkina, T.V. Sulfoxides, analogues of L-methionine and L-cysteine as pro-drugs against Gram-positive and Gram-negative bacteria. *Acta Naturae* **2015**, *7*, 128–135. [\[CrossRef\]](#)
18. Morozova, E.; Anufrieva, N.; Koval, V.; Lesnova, E.; Kushch, A.; Timofeeva, V.; Solovieva, A.; Kulikova, V.; Revtovich, S.; Demidkina, T. Conjugates of methionine γ -lyase with polysialic acid: Two approaches to antitumor therapy. *Int. J. Biol. Macromol.* **2021**, *182*, 394–401. [\[CrossRef\]](#)
19. Kulikova, V.; Morozova, E.; Rodionov, A.; Koval, V.; Anufrieva, N.; Revtovich, S.; Demidkina, T. Non-stereoselective decomposition of ($\pm$)-S-alk(en)yl-L-cysteine sulfoxides to antibacterial thiosulfonates catalyzed by C115H mutant methionine γ -lyase from *Citrobacter freundii*. *Biochimie* **2018**, *151*, 42–44. [\[CrossRef\]](#)
20. Kulikova, V.V.; Chernukha, M.Y.; Morozova, E.A.; Revtovich, S.V.; Rodionov, A.N.; Koval, V.S.; Avetisyan, L.R.; Kuliastova, D.G.; Shaginyan, I.A.; Demidkina, T.V. Antibacterial effect of thiosulfonates on multiresistant strains of bacteria isolated from patients with cystic fibrosis. *Acta Naturae* **2018**, *10*, 77–80. [\[CrossRef\]](#)
21. Morozova, E.; Kulikova, V.; Koval, V.; Anufrieva, N.; Chernukha, M.; Avetisyan, L.; Lebedeva, L.; Medvedeva, O.; Burmistrov, E.; Shaginyan, I.; et al. Encapsulated methionine γ -lyase: Application in enzyme prodrug therapy of *Pseudomonas aeruginosa* infection. *ACS Omega* **2020**, *5*, 7782–7786. [\[CrossRef\]](#)
22. Snell, E.E. Tryptophanase: Structure, catalytic activities, and mechanism of action. *Adv. Enzymol.* **1975**, *42*, 287–333. [\[CrossRef\]](#)
23. Kumagai, H.; Yamada, H.; Matsui, H.; Ohkishi, H.; Ogata, K. Tyrosine phenol lyase. I. Purification, crystallization, and properties. *J. Biol. Chem.* **1970**, *245*, 1767–1772. [\[CrossRef\]](#)
24. Phillips, R.S.; Demidkina, T.V.; Faleev, N.G. Structure and mechanism of tryptophan indole-lyase and tyrosine phenol-lyase. *Biochim. Biophys. Acta* **2003**, *1647*, 167–172. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Phillips, R.S.; Demidkina, T.V.; Faleev, N.G. The role of substrate strain in the mechanism of the carbon-carbon lyases. *Bioorg Chem.* **2014**, *57*, 198–205. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Zheng, R.-C.; Tang, X.-L.; Suo, H.; Feng, L.-L.; Liu, X.; Yang, J.; Zheng, Y.-G. Biochemical characterization of a novel tyrosine phenol-lyase from *Fusobacterium nucleatum* for highly efficient biosynthesis of L-DOPA. *Enzyme Microb. Technol.* **2018**, *112*, 88–93. [\[CrossRef\]](#)
27. Musah, R.A.; He, Q.; Kubec, R.; Jadhav, A. Studies of a novel cysteine sulfoxide lyase from *Petiveria alliacea*: The first heteromeric alliinase. *Plant Physiol.* **2009**, *151*, 1304–1316. [\[CrossRef\]](#)
28. Yoshimoto, N.; Yabe, A.; Sugino, Y.; Murakami, S.; Sai-Ngam, N.; Sumi, S.-I.; Tsuneyoshi, T.; Saito, K. Garlic γ -glutamyl transpeptidases that catalyze deglutamylation of biosynthetic intermediate of alliin. *Front. Plant Sci.* **2015**, *5*, 758. [\[CrossRef\]](#)
29. Weik, R.; Francky, A.; Striedner, G.; Raspor, P.; Bayer, K.; Mattanovich, D. Recombinant expression of alliin lyase from garlic (*Allium sativum*) in bacteria and yeasts. *Planta Med.* **1998**, *64*, 387–388. [\[CrossRef\]](#)
30. Kulikova, V.V.; Morozova, E.A.; Revtovich, S.V.; Kotlov, M.I.; Anufrieva, N.V.; Bazhulina, N.P.; Raboni, S.; Faggiano, S.; Gabellieri, E.; Cioni, P.; et al. Gene cloning, characterization, and cytotoxic activity of methionine γ -lyase from *Clostridium novyi*. *IUBMB Life* **2017**, *69*, 668–676. [\[CrossRef\]](#) [\[PubMed\]](#)
31. Revtovich, S.; Lyfenko, A.; Tkachev, Y.; Kulikova, V.; Koval, V.; Puchkov, V.; Anufrieva, N.; Solyev, P.; Morozova, E. Anticandidal activity of in situ methionine γ -lyase-based thiosulfonate generation system vs. synthetic thiosulfonates. *Pharmaceuticals* **2023**, *16*, 1695. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Kharasch, N.; Potempa, S.J.; Wehrmeister, H.L. The sulfenic acids and their derivatives. *Chem. Rev.* **1947**, *39*, 2269–2332. [\[CrossRef\]](#)
33. Gangemi, M.A.; D’Agostino, E.; Aversa, M.C.; Barattucci, A.; Bonaccorsi, P.M. Sulfoxides and disulfides from sulfenic acids: Synthesis and applications. *Tetrahedron* **2023**, *143*, 133550. [\[CrossRef\]](#)
34. Tanner, J.J.; Parsons, Z.D.; Cummings, A.H.; Zhou, H.; Gates, K.S. Redox regulation of protein tyrosine phosphatases: Structural and chemical aspects. *Antioxid. Redox Signal.* **2011**, *15*, 77–97. [\[CrossRef\]](#)

35. Karabulut, S.; Wijerathne, D.V.; Gauld, J.W. Computational insights into the formation and structure of S-N containing cyclic peptides. *ACS Omega* **2023**, *8*, 18234–18244. [\[CrossRef\]](#)
36. Laleu, B.; Machado, M.S.; Lacour, J. Pummerer fragmentation vs. Pummerer rearrangement: A mechanistic analysis. *Chem. Commun.* **2006**, *26*, 2786–2788. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Gupta, V.; Carroll, K.S. Sulfenic acid chemistry, detection and cellular lifetime. *Biochim. Biophys. Acta* **2014**, *1840*, 847–875. [\[CrossRef\]](#)
38. Swamy, K.C.K.; Kumar, N.S. New features in pentacoordinate phosphorus chemistry. *Acc. Chem. Res.* **2006**, *39*, 324–333. [\[CrossRef\]](#) [\[PubMed\]](#)
39. Braunstein, A.E. Amino group transfer. *The Enzymes* **1973**, *9*, 379–481. [\[CrossRef\]](#)
40. Vederas, J.C.; Floss, H.G. Stereochemistry of pyridoxal phosphate catalyzed enzyme reactions. *Acc. Chem. Res.* **1980**, *13*, 455–463. [\[CrossRef\]](#)
41. Zakomirdina, L.N.; Sakharova, I.S.; Torchinsky, Y.M. Linear dichroism studies of tryptophanase and its quasisubstrate complexes oriented in polyacrylamide gel. *Eur. J. Biochem.* **1990**, *193*, 243–247. [\[CrossRef\]](#)
42. Torchinsky, Y.M.; Kawata, Y. Tryptophanase: Structural, Spectral, and Catalytic Properties. In *Molecular Aspects of Enzyme Catalysis*, 1st ed.; Fukui, T., Soda, K., Eds.; Kodansha Ltd.: Tokyo, Japan, 1994; pp. 165–190. [\[CrossRef\]](#)
43. Muñoz-Vargas, M.A.; López-Jaramillo, J.; González-Gordo, S.; Paradela, A.; Palma, J.M.; Corpas, F.J. H₂S-Generating cytosolic L-cysteine desulphydrase and mitochondrial D-cysteine desulphydrase from sweet pepper (*Capsicum annuum* L.) are regulated during fruit ripening and by nitric oxide. *Antioxid. Redox Signal.* **2023**, *39*, 2–18. [\[CrossRef\]](#) [\[PubMed\]](#)
44. Kulikova, V.V.; Anufrieva, N.V.; Morozova, E.A.; Revtovich, S.V.; Solyev, P.N. Redesigning methionine γ -lyase for improved stability and catalytic activity in the β -elimination reaction for the synthesis of thiosulfinates. *Biochimie* **2025**, *232*, 35–42. [\[CrossRef\]](#)
45. Revtovich, S.V.; Kulikova, V.V.; Koval, V.S.; Lyfenko, A.D.; Kazakov, V.A.; Chernov, A.S.; Telegin, G.B.; Zemskaya, A.S.; Anufrieva, N.V.; Morozova, E.A.; et al. Toxicity Study of the Pharmacological Pair of Encapsulated *Citrobacter freundii* C115H Methionine γ -Lyase/Methiin. *Mol. Biol.* **2024**, *58*, 1063–1073. [\[CrossRef\]](#)
46. He, W.; Fu, Y.; Zheng, Y.; Wang, X.; Liu, B.; Zeng, J. Diallyl thiosulfinate enhanced the anti-cancer activity of dexamethasone in the side population cells of multiple myeloma by promoting miR-127-3p and deactivating the PI3K/AKT signaling pathway. *BMC Cancer* **2021**, *21*, 125. [\[CrossRef\]](#)
47. Kim, S.; Kubec, R.; Musah, R. Antibacterial and antifungal activity of sulfur-containing compounds from *Petiveria alliacea* L. *J. Ethnopharmacol.* **2006**, *104*, 188–192. [\[CrossRef\]](#)
48. Yoshida, H.; Katsuzaki, H.; Ohta, R.; Ishikawa, K.; Fukuda, H.; Fujino, T.; Suzuki, A. Antimicrobial activity of the thiosulfinates isolated from oil-macerated garlic extract. *Biosci. Biotechnol. Biochem.* **1999**, *63*, 591–594. [\[CrossRef\]](#)
49. Puchkov, V.M.; Lyfenko, A.D.; Koval, V.S.; Revtovich, S.V.; Kulikova, V.V.; Anufrieva, N.V.; Zemskaya, A.S.; Morozova, E.A.; Solyev, P.N. Synthesis and Antimicrobial Activity of Thiosulfinates and Allicin Analogues. *Mol. Biol.* **2024**, *58*, 966–974. [\[CrossRef\]](#)
50. Yamada, Y.; Azuma, K. Evaluation of the in vitro antifungal activity of allicin. *Antimicrob. Agents Chemother.* **1977**, *11*, 743–749. [\[CrossRef\]](#) [\[PubMed\]](#)
51. Khodavandi, A.; Alizadeh, F.; Harmal, N.S.; Sidik, S.M.; Othman, F.; Sekawi, Z.; Jahromi, M.A.F.; Ng, K.P.; Chong, P.P. Comparison between efficacy of allicin and fluconazole against *Candida albicans* in vitro and in a systemic candidiasis mouse model. *FEMS Microbiol. Lett.* **2011**, *315*, 87–93. [\[CrossRef\]](#) [\[PubMed\]](#)
52. Gruhlke, M.C.H.; Hemmis, B.; Noll, U.; Wagner, R.; Lühring, H.; Slusarenko, A.J. The defense substance allicin from garlic permeabilizes membranes of *Beta vulgaris*, *Rhoeo discolor*, *Chara corallina* and artificial lipid bilayers. *Biochim. Biophys. Acta* **2015**, *1850*, 602–611. [\[CrossRef\]](#)
53. Kulikova, V.V.; Morozova, E.A.; Koval, V.S.; Solyev, P.N.; Demidkina, T.V.; Revtovich, S.V. Thiosulfinates: Cytotoxic and Antitumor Activity. *Biochemistry* **2023**, *88*, 912–923. [\[CrossRef\]](#) [\[PubMed\]](#)
54. Abo Qoura, L.; Karshieva, S.S.; Borisova, J.A.; Pokrovsky, V.S. Cytotoxic and Anticancer Activity of Pharmacological Pairs of C115H Methionine–Gamma-lyase and S-Propyl-L-Cysteine Sulfoxide. *Russ. J. Oncol.* **2023**, *28*, 5–14. [\[CrossRef\]](#)
55. Chhabria, S.; Desai, K. Purification and characterisation of alliinase produced by *Cupriavidus necator* and its application for generation of cytotoxic agent: Allicin. *Saudi J. Biol. Sci.* **2018**, *25*, 1429–1438. [\[CrossRef\]](#)
56. Morozova, E.; Koval, V.; Revtovich, S.; Lyfenko, A.; Minakov, A.; Chernov, A.; Telegin, G.; Kirilenko, D.; Chobanian, A.; Anufrieva, N.; et al. Phytoestrogens decorated nanocapsules for therapeutic methionine γ -lyase targeted delivery. *Biochimie* **2023**, *209*, 1–9. [\[CrossRef\]](#) [\[PubMed\]](#)
57. Miron, T.; Rabinkov, A.; Mirelman, D.; Weiner, L.; Wilchek, M. A spectrophotometric assay for allicin and alliinase (alliin lyase) activity: Reaction of 2-nitro-5-thiobenzoate with thiosulfinates. *Anal. Biochem.* **1998**, *265*, 317–325. [\[CrossRef\]](#) [\[PubMed\]](#)
58. Frankel, M.; Gertner, D.; Jacobson, H.; Zilkha, A. Synthesis of poly-S-alkyl-L-cysteines. *J. Chem. Soc.* **1960**, 1390–1393. [\[CrossRef\]](#)
59. Sausen, P.J.; Elfarrar, A.A. Cysteine conjugate S-oxidase. Characterization of a novel enzymatic activity in rat hepatic and renal microsomes. *J. Biol. Chem.* **1990**, *265*, 6139–6145. [\[CrossRef\]](#)
60. Briggs, W.H.; Xiao, H.; Parkin, K.L.; Shen, C.; Goldman, I.L. Differential inhibition of human platelet aggregation by selected Allium thiosulfinates. *J. Agric. Food Chem.* **2000**, *48*, 5731–5735. [\[CrossRef\]](#)

61. Chen, H.; Gollnick, P.; Phillips, R.S. Site-directed mutagenesis of His343Ala in *Citrobacter freundii* tyrosine phenol-lyase. Effects on the kinetic mechanism and rate-determining step. *Eur. J. Biochem.* **1995**, *229*, 540–549. [[CrossRef](#)]
62. Zakomirdina, L.N.; Kulikova, V.V.; Gogoleva, O.I.; Dementieva, I.S.; Faleev, N.G.; Demidkina, T.V. Tryptophan indole-lyase from *Proteus vulgaris*: Kinetic and spectral properties. *Biochemistry* **2002**, *67*, 1437–1445. [[CrossRef](#)] [[PubMed](#)]
63. Morino, Y.; Snell, E.E. Tryptophanase (*Escherichia coli* B). *Meth. Enzymol.* **1970**, *17 Part A*, 439–446. [[CrossRef](#)]
64. Aitken, S.M.; Kim, D.H.; Kirsch, J.F. *Escherichia coli* cystathionine γ -synthase does not obey ping-pong kinetics. Novel continuous assays for the elimination and substitution reactions. *Biochemistry* **2003**, *42*, 11297–11306. [[CrossRef](#)] [[PubMed](#)]
65. *CLSI Document M7-A9*; Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically (9th ed.). Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2012.
66. *CLSI Document M27-A3*; Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeast. National Committee for Clinical and Laboratory Standards: Wayne, PA, USA, 2008; p. 14.
67. *CLSI Document M100-A30*; Performance Standards for Antimicrobial Susceptibility Testing (30th ed.). Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2020.
68. Trott, O.A.; Olson, J. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* **2010**, *31*, 455–461. [[CrossRef](#)]

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