Study of antimicrobial, biochemical and nanotechnological aspects of novel sulfur, selenium and tellurium compounds

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Dedicated to my loving family

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Abstract

Chalcogen chemistry is widely interdisciplinary, especially in the field of biology, material sciences and supra-molecular chemistry and biology. Whilst oxygen and sulfur are fairly omnipresent in biology selenium has its nutritional role in animals and humans; however, for tellurium, this is yet to be discovered. The common behaviour of all three elements is related to their antioxidant properties and their therapeutic effects. Most drugs for present day diseases are either natural or derivatives of compounds found in natural products. *Allium* plant species have a wide range of effective therapeutics benefits. Garlic and its components, namely allicin, polysulfanes, thiosulfinates, thiosulfonates and dithienes, form an important class of natural compounds and represent a field where tremendous research has been ongoing for the past decades.

As part of this thesis, a series of unsymmetrical tailor-made tetrasulfanes have been synthesised. They were tested along with some other symmetrical tetrasulfanes, selenium and tellurium compounds for their physico-chemical properties and therapeutic benefits, in both microorganisms and primary cell lines. Biological activities were investigated by testing the tetrasulfanes in nematodes, bacteria, yeast, fungi and bacteriophages. The effects on red blood cells and neuronal cell lines of rats were also studied. A cytotoxicity assay and ELISA were performed on human synovial fibroblast cell lines.

Kurzfassung

Die Chemie der Chalkogene ist sehr interdisziplinär, besonders, wenn Biologie, Medizin, Materialwissenschaften und supramolekulare Wissenschaften miteinbezogen werden. Schwefel und Selen tragen bei Mensch und Tier zur Bedarfsdeckung bei; ob dies auch auf Tellur zutrifft muss hingegen noch bewiesen werden. Diesen drei Elementen sind ihre antioxidativen Eigenschaften und therapeutischen Effekte gemeinsam. Die meisten Medikamente für aktuelle Krankheiten sind entweder auf Naturstoffen basierend oder Derivate dieser Stoffe. Alle *Allium*-Arten weisen eine große Anzahl an potenten Wirkstoffen mit therapeutischen Wirkungen auf. Knoblauch und seine Bestandteile, nämlich Allicin, Polysulfane, Thiosulfinate, Thiosulfate und Dithiene, formen wichtige Gruppen natürlicher Stoffe, zu denen in den letzten Jahrzenten enorme Forschungsarbeiten geführt worden sind.

Ein Teil dieser Arbeit beschäftigt sich mit der Synthese einer Reihe unsymmetrischer und maßgeschneiderter Tetrasulfane. Sie wurden gemeinsam mit anderen symmetrischen Tetra- und Polysulfanen, Selen- und Tellur-Gemischen auf Grund ihrer physikalisch-chemischen Eigenschaften und ihres möglichen therapeutischen Nutzens in Mikroorganismen und Primärzelllinien getestet. Die biologische Aktivitäten der Tetrasulfane wurden in Nematoden, Bakterien, Pilzen (v.a. Hefe) und Bakteriophagen untersucht. Die Auswirkungen auf rote Blutzellen wurden mit Hilfe von Hämoglobinassays erforscht. Studien zur Zytotoxizität, sowie weiterführende Experimente und Analysemethoden (z.B. ELISA) wurden an menschlichen synovialen Fibroblasten durchgeführt.

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List of Abbreviations

AChE	acetylcholine esterase
Ag/AgCl	silver/silver chloride electrode
BnTTSPh	1-benzyl-4-phenyltetrasulfane
BnTTSPs	3-(benzyltetrasulfanyl)-propanoic acid
BSA	bovine serum albumin
$CDCl_3$	deuterochloroform
CH_2Cl_2	dicholoromethane
$CHCl_3$	chloroform
CMC	critical micelle concentration
DADS	diallyldisulfide
DAS	diallylsulfide
DATS	diallyltrisulfane[1]
DATTS	diallyltetrasulfane
DBTTS	1, 4-dibenzyltetrasulfane
DEETTS	1, 4-bis(2-ethoxyethyl)tetrasulfane
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DPhTTS	1, 4-diphenyltetrasulfane
DPSEETTS	diethyl 3, 3'-tetrasulfanediyldipropanoate
DPSTTS	3, 3'-tetrasulfanediyldipropanoic acid
DPTTS	dipropyltetrasulfane
DU145	human prostate cancer cell line

[1] The compounds with chemical formula RS_xR, where $R \neq H$ and $x \geq 3$ are termed as polysulfanes. These compounds are sometimes incorrectly referred to as 'polysulfides' in bio-chemical publications.

$E_{1/2}$	half wave potential
*E*pa	anodic oxidation potential
*E*pc	cathodic reduction potential
ESI	electrospray ionisation
GO	garlic oil
GPx	glutathione peroxidase
GSH	glutathione
GSSG	glutathione disulfide
h	hours
H_2O_2	dihydrogen peroxide
HRMS	high-resolution mass spectroscopy
IC_{50}	half-maximal inhibitory concentration
*K*sv	Stern Volmer constant
LD_{50}	lethal dose (50 %)
m/z	mass by charge ratio
MDA-MB-231	human mammary gland carcinoma cell lines
$MgSO_4$	magnesium sulfate
MIC	minimum inhibitory concentration
NMR	nuclear magnetic resonance
OSCs	organosulfur compounds
PC3	prostate cancer cell line 3
PEG	polyethylene glycol
PhTTSPs	3-(phenyltetrasulfanyl)-propanoic acid
p*K*a	logarithmic acid dissociation constant
PrTTSBn	1-benzyl-4-propyltetrasulfane
PrTTSPh	1-phenyl-4- propyltetrasulfane

PrTTSPs	3-(propyltetrasulfanyl)-propanoic acid
RBCs	red blood cells
R_f	retention factor
S_2Cl_2	sulfur monochloride
SDS	sodium dodecyl sulfate
S-S	sulfur-sulfur bond
ST	symmetrical tetrasulfanes
TLC	thin layer chromatography
UT	unsymmetrical tetrasulfanes

PART I

Study of tetrasulfanes

1. Introduction

The elements in group 16 (formerly group 6) of the Periodic Table are called chalcogens. This group contains Oxygen (O), Sulfur (S), Selenium (Se), Tellurium (Te) and the radioactive element Polonium (Po).

1.1 Sulfur and its biological significance

Sulfur is an important constituent of various amino acids, the most important being cysteine and methionine. Sulfur is also part of cellular enzymes and proteins, which take part in substantial biochemical and cellular signalling pathways; hence, small changes in their amount and activity may cure many diseases [1]. It is therefore important to keep an eye on the optimum intake of sulfur. Sulfur is found in a large number of natural products, with *Allium* species being the most significant, as well as in other micro- and marine organisms in the form of various organo sulfur compounds (OSCs) [2] [3] [4]. The *Allium* species with enormous amounts of OSCs play an importunate role as dietary supplements for individuals [5].

1.2 A brief history of *Allium* species

One of the oldest cultivated vegetables, *Allium cepa,* commonly known as onion, has been reported for over 5000 years by now. In India, reports of onions are found in writings from the 6th century onwards [6]. Onion was cultivated and became widespread as a crop in Europe, only during the Middle ages. The second most important vegetable of *Allium* species is *Allium sativum,* commonly known as garlic, has been cultivated for over 5000 years [7]. One of the most accepted history is that garlic originated from Central Asia and then became widespread from there to different parts of the world. Garlic has been grown by village people for both culinary and medicinal purposes [6] [8] .

1.3 Garlic based therapeutics and therapies

Before the invention of antibiotics, preparations of garlic were used to treat diseases [9] [10] [11] such as cholera, tuberculosis, dysentery, diphtheria *etc* [12] [13]. To some extent, Dengue fever was also treated with the same techniques [13].

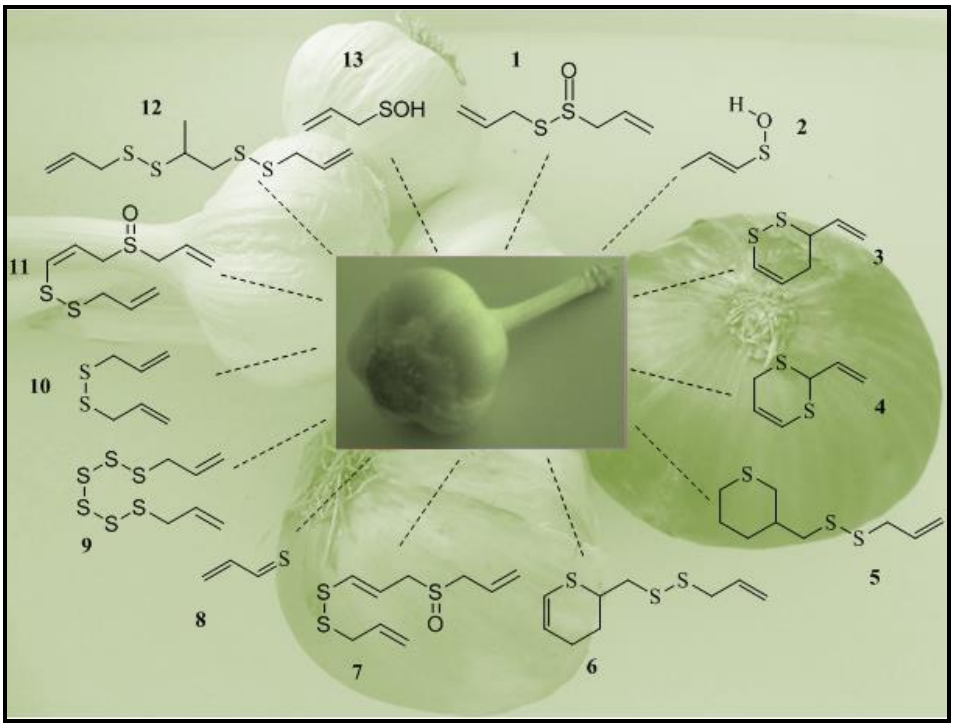


Figure 1 : Collection of organo sulfur compounds and their intermediates present in Garlic. Allicin (1), 1 propene sulfenic acid (2), 3-Vinyl-3,4-dihydro-1,3-dithiin (3), 2-Vinyl-2,4-dihydro-1,3-dithiin (4), ((allyldisulfanyl)methyl)tetrahydro-2*H*-thiopyran (5), 2-((allyldisulfanyl)methyl)-3,4-dihydro-2*H*-thiopyran (6), *E*-ajoene (7), thioacrolein (8), 1,6diallylhexasulfane (9), diallyldisulfide (10), *Z*-ajoene (11), 2,2'-(propane-1,2-diyl)bis(1-allyldisulfane) (12), 2 propenesulfenic acid (13). Figure modified in reference to [4].

The OSCs found in garlic (Figure 1) have properties such as cancer prevention, antimicrobial activity, insect and animal attractive/repulsive activity, olfactory-gustatory-lachrymatory properties, effects on lipid metabolism, and platelet aggregation [4] [14] [15] [16] [17]. All of these different properties of sulfur compounds are due to their ability to undergo diverse chemical reactions such as reduction, oxidation, reactions involving sulfur radicals, pericyclic and re-arrangement reactions [18] [19].

1.3.1 Antimicrobial activity

Anti-microbial effects probably represent the most prominent activity of garlic and its components [20] [21] [22]. Garlic shows a growth inhibitory effect against both Gram-positive and Gram-negative bacteria. Allicin, even at 1:100,000 dilutions, has been proven to exhibit inhibitory effects against microbes [21] [23]. Methyl methanethiosulfinate [24], propyl propane-thiosulfinate [25] and thiosulfinates of onion also exhibit activity against the bacteria, but this is definitely weaker than that of allicin [26] [27] [28] [29]. Other than the anti-bacterial activity, garlic has also shown activities against fungi and yeast. The pathogenic strains of *Candida albicans* and *Cryptococcus neoformans* for instance were also affected by garlic extracts [26] [30] [31] [32].

Table 1: Different constituents of garlic oil and their respective concentrations in mg/g of dry weight of garlic oil. Table adapted from [33].

Analysis of undiluted GO	
GO component	**Concentration (mg/g)* of dry weight**
Diallyl monosulfide	106 ± 7 (10.6)
Diallyl disulfide	530 ± 7 (53.0)
Diallyl trisulfide	115 ± 4 (11.5)
Diallyl tetrasulfide	43 ± 2 (4.3)
Diallyl pentasulfide	10.5 ± 0.4 (1.1)
Diallyl hexasulfide	0.14 ± 0.01 (0.01)
Methyl allyl disulfide	44.1 ± 2 (4.4)
Methyl allyl trisulfide	69.9 ± 2.2 (7)
Methyl allyl tetrasulfide	24.6 ± 2.0 (2.5)
Methyl allyl pentasulfide	6.3 ± 0.6 (0.6)
Methyl allyl hexasulfide	1.5 ± 0.1 (0.2)
Dimethyl trisulfide	12.0 ± 1.3 (1.2)
Dimethyl tetrasulfide	4.3 ± 0.6 (0.2)
Dimethyl pentasulfide	2.0 ± 0.4 (0.2)
*Values in parentheses are percentages	

Garlic oil (GO) has been tested frequently against microbes [34] [35]. This preparation contains the maximum number of variable polysulfanes [36]. Table 1 indicates that GO contains 53 % of diallylsulfide, DATS is found at 11.5 % and all other polysulfanes are present in trace amounts. In Thailand, GO and chive oil have been used for the treatment of food borne infections for many years. In China, the DATS constituent of garlic oil was used to treat viral infections and cryptococcal meningitis. GO also exerts antifungal properties against various species of *Aspergillus*, *Candida* and *Fusarium* [31] [37].

Polysulfanes in garlic have been reported to exhibit an identical activity as that of raw garlic extract, garlic oil and allicin. As DATTS is more stable than its higher derivatives, it has an extraordinary effect against *Helicobacter pylori* (MIC range, 3 to 6 mg/ml) [33]. It is also evident from various studies that the activities of polysulfanes are directly proportional to the length of S-S chains in them [18].

The results from various studies are compiled in Table 2, which shows a comparison of the activities of various sulfur containing compounds DAS, DADS, DATS, and DATTS along with allicin, against different strains of microbes. Although it is not an appropriate method, this gives some hint about the activities of these compounds against the different microbial strains. It is evident from the comparative study that, DAS and DADS are required in larger amounts to visualise an effective activity against the microorganisms [18]. DATS shows a similar activity as that of allicin. Surprisingly, DATTS exhibited MIC values at very low concentrations starting from 0.5 g/ml.

Table 2: This table compiles the values of minimum inhibitory concentrations in g/ml exhibited by allicin, diallylsulfide, diallyldisulfide, diallyltrisulfane and diallyltetrasulfane on various species of bacteria and fungi. The values also prove that diallyltrisulfane and diallyltetrasulfane are the most active amongst all of the compounds. Table adapted from [18].

Organism	Allicin	DAS	DADS	DATS	DATTS
Helicobacter pylori	6-12	2100-4100	100	13-25	3-6
Klebsiella pneumonia	-	96-104	72-80	40-48	20-24
Pseudomonas aeruginosa	15	80-88	64-72	32-36	12-16
Staphylococcus aureus	15	20	4	2	0.5
MRSA	28	32	12	8	2
Candida albicans	0.8	32	4	1	0.5
Aspergillus niger	8-32	40	8	2	1

Polysulfanes were used as treatment against numerous food borne bacterial pathogens namely *Bacillus cereus, Campylobacter jejuni, Escherichia coli, Listeria monocytogenes, Salmonella sp., Shigella sp., Staphylococcus aureus, Vibrio cholera* and *Yersinia enterocolytica* [32]. The MIC values of DATS and DATTS were 2 µg/ml and 1 µg/ml against *C. botulinum,* respectively, 4 µg/ml and 1 µg/ml against *C. jejuni,* respectively, and 12 µg/ml and 4 µg/ml against *V. cholera,* respectively [32].

1.3.2 Anticancer effect

Garlic and its derivatives exhibit antiprolierative activities in human cancer cell lines [38] [39]. An international study involving men and women from various countries has been carried out and this study is termed as 'The European Prospective Investigation into Cancer and Nutrition' (EPIC) [40]. This study involves an investigation of the influence of diet on cancer. This study revealed that the risk of intestinal cancer was reduced with a more extensive intake of garlic and onion [41].

The studies by *Pinto et al* state that *Allium* derivatives inhibit the proliferation of the human prostate cancer cell line (LNCaP) and human breast cancer cell line (MCF-7) [14]. Further studies, however, are required to reveal the mechanism of action of these *Allium* derivatives [42]. DATS and DATTS have been reported as strong inducers of early mitotic arrest and for subsequent apoptosis of cancer cells [43]. One of the studies by *Kelkel et al* states that DATTS acts independently of ROS and one of the major cellular targets of this compound has been identified as *tubulin* [44] [45] [46].

1.4 Chemistry/Reactivity of Polysulfanes

In general, polysulfanes such as DATS and DATTS are oxidants that modify cellular signalling processes and hinder protein functions. For instance, they have the ability to react with cellular thiols and modify them to mixed disulfides. This is one of the explanations of the biological activity of tri- and tetrasulfanes. The reaction of trisulfanes with thiols results in the formation of mixed disulfides and persulfides. In the case of the reaction of tetrasulfanes with thiols, mixed trisulfanes and hydropersulfide are formed. Alternatively, the formation of mixed disulfides and hydrogen trisulfanes also probably occurs [18] [47].

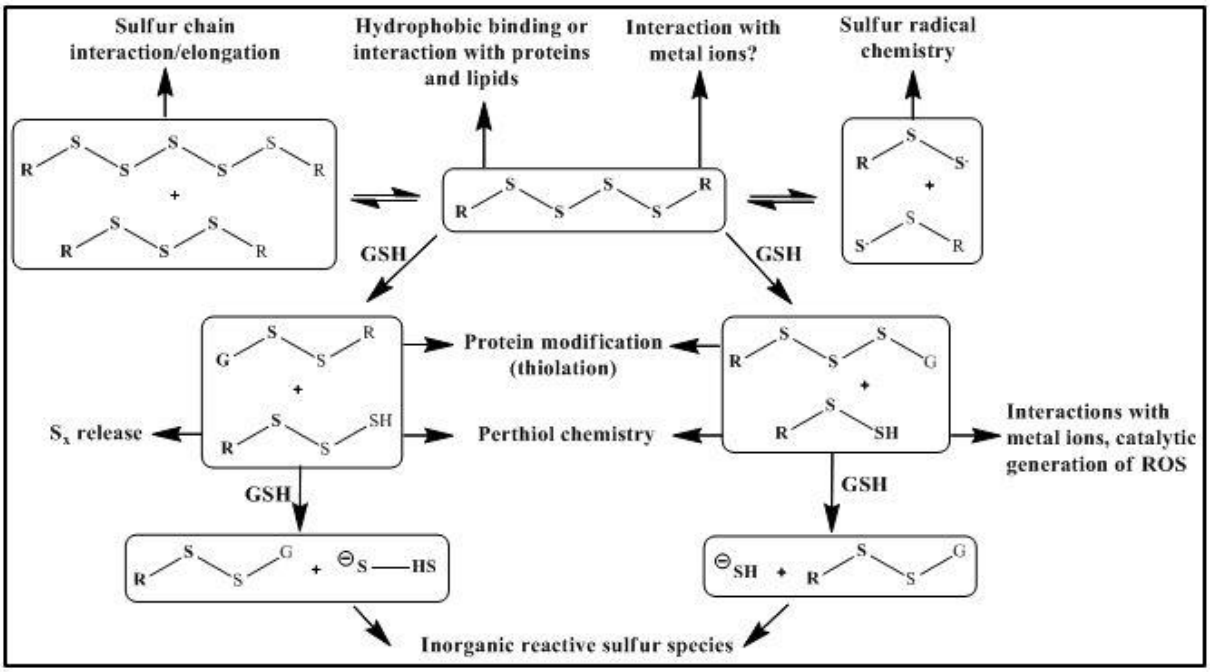


Figure 2: Polysulfanes undergo various chemical and biochemical reactions. These include hydrophobic bonding and various other interactions with proteins and lipids, interactions with metal ions and the catalytic generation of ROS. Here, the schematic overview of such reactions characteristic for a given tetrasulfane is shown [18].

Disulfides react with cysteine residues in proteins to undergo thiol/disulfide reactions. One of the best examples is the thiolation behaviour of disulfides such as glutathione disulphide (GSSG) [18].

Nonetheless, it will not be sufficient to explain the diversified biological activities of polysulfanes such as antibacterial, antifungal and anti-carcinogenic effects, by just considering thiolation. In 2006, Jacob *et al.* proposed several possibilities for the reactions characteristic of polysulfanes based on their known chemical properties. Chemical reactions of polysulfanes endow them with unique biological activities. The foremost important reaction, the thiolation reaction has been discussed above (Figure 2) [18] [48] [49].

Other than the thiolation reactions, polysulfanes may undergo homolytic S-S bond cleavage. This bond cleavage results in the formation of perthiyl radicals. Polysulfanes also undergo sulfur-transfer reactions. This reaction involves the transfer of S_2 or S_3 subunits to molecules containing one or two conjugated double bonds. Another important reaction of polysulfanes is their interaction with metal ions. Polysulfanes can probably form metal complexes due to their ability to coordinate with several sulfur atoms at a time. Other aspects of polysulfane chemistry are the polysulfane-perthiol-perthiyl radical chemistry and hydrogen sulfide release mechanisms [50] [51] [52] [53] [52].

1.5 From simple polysulfanes to pharmaceutically interesting molecules

Lissoclinotoxin is isolated from the ascidian, *Lissoclinum* (*Didemnidae*). In order to improve the availability of the compound (Figure 3), the natural benzo-polysulfane has been modified with a polyethylene glycol (PEG) group. Comparatively, the solubility of these compounds was 50-fold higher than that of natural benzo-polysulfanes and they displayed higher anti-tumour activity against human tumour cell lines PC3 (prostate), DU145 (prostate), MDA-MB-231 (breast), and Jurkat (T-cell leukaemia) [54].

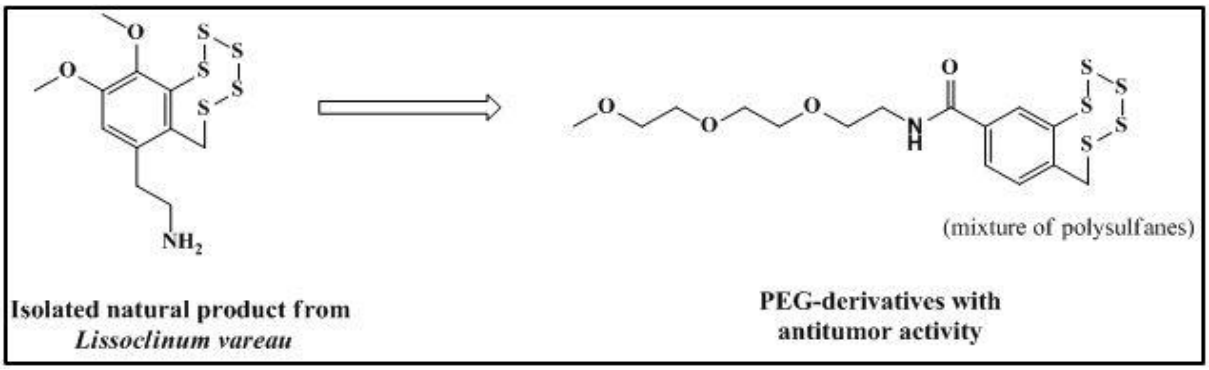


Figure 3: Natural pentapolysulfide isolated from *Lissoclinum vareau*; has been modified with polyethylene glycol to exhibit comparatively greater anti-tumour activity. Figure adapted and modified from [54]].

The improved idea obtained here is that the enahanced solubility of the polysulfanes caused this activity. Sun and Guo isolated a macro-polysulfide named Gymnorrhizol from Chinese Mangrove and this compound exhibited an IC_{50} value of 14.5 µM against protein tyrosine phosphatase 1B, which is a potential drug target for the treatment of Type II diabetes and obesity [55]. This macro-polysulfide was synthesised under laboratory conditions. [56].

Garlic research has inspired various organic chemists to synthesise polysulfanes that are present in natural products [57] [58] [59] [60]. Modifications have been carried out to the structure of natural polysulfanes and their derivatives have been synthesised [61].

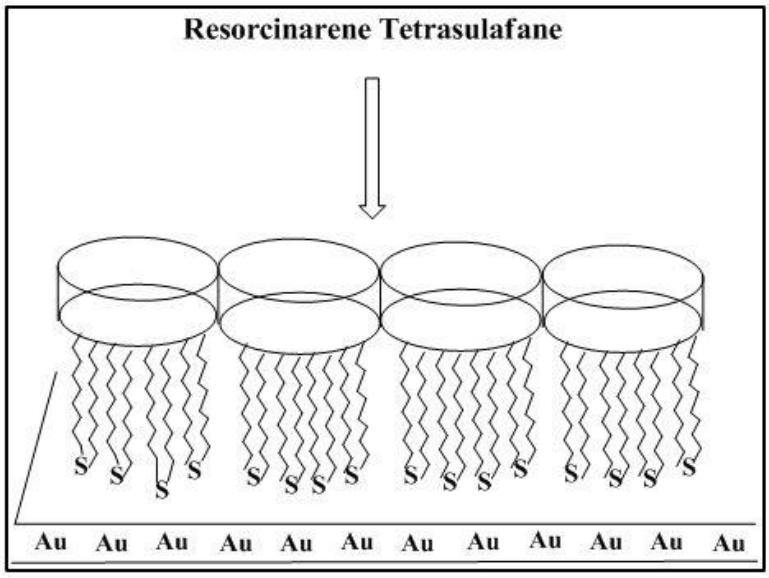


Figure 4: Pictorial representation of self-assembly, a unique property of resorcinarene tetrasulfane adsorbates on gold monolayers. Figure adapted from [62].

The resorcinarene tetrasulfanes have been synthesised such that the resorcinarene acts as a head group and tetrasulfanes as the tail group, which can easily self-assemble [63] [64] [65] on gold substrate (Figure 4) [55].

2 Objectives

Diallyltetrasulfanes and dipropyltetrasulfanes from the *Allium* species of garlic and onion, respectively, possesses high biological activity. These naturally occurring tetrasulfanes have been therefore chosen and modified.

In the present project, a series of unsymmetrical tetrasulfanes have been synthesised, with a decent stability and solubility. The novel unsymmetrical tetrasulfanes have been found to be odourless. These tetrasulfanes exhibit amphiphilic properties that improve the permeability and absorption through biological membranes. These compounds were analysed for various physico-chemical properties using methods such as electrochemistry, sita/ring tensiometry and densitometry. In-depth biological studies have been performed using different antimicrobial assays and in different cell culture assays.

3. Results and Discussion

3.1 Synthesis of unsymmetrical tetrasulfanes

For many years, trisulfanes and tetrasulfanes have been under exploration to identify their effective physico-chemical and biological activities. Diallyltetrasulfane (DATTS) is one such active compound that is present in garlic. With regard to DATTS, attempts have been undertaken to synthesise its derivatives. The research has been mainly concentrated on aliphatic symmetrical tri- and tetrasulfanes. For the first time in Prof Jacob's group, and as part of this thesis, studies have been carried out to synthesise tailor-made unsymmetrical tetrasulfanes.

The chemical compounds such as, 3-thiopropionic acid, 1-propanethiol, benzylthiol and thiophenol have been used in the modified Derbesy and Harpp method [66]. Different combinations of these compounds have been used to design the novel unsymmetrical tetrasulfanes. Novel compounds such as DPhTTS (**7**) BnTTSPs (**8**), PrTTSPs (**9**) BnTTSPh (**10**) and PhTTSPs (**11**) PrTTSBn (**12**) PrTTSPh (**13**) have been synthesised. Diploma students, Sher Ali and Ahsan Raza, from the group of Prof. Jacob have been also a part of this project.

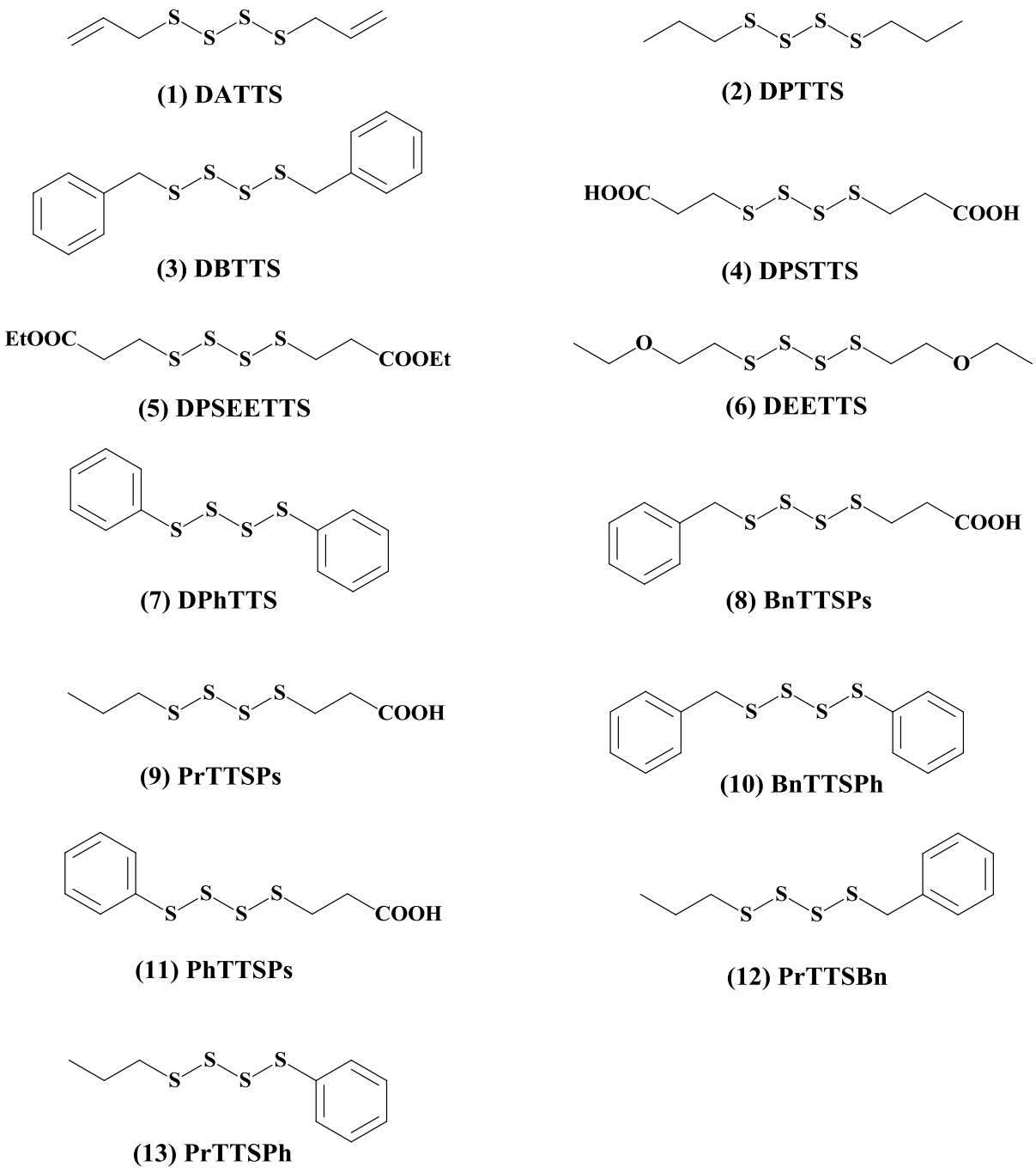


Figure 5: Overview of symmetrical (1-7) and unsymmetrical tetrasulfanes (8-13) Compounds 7-13 were synthesised and purified as part of this thesis.

Simple modifications to the Derbesy and Harpp method have been carried out (Figure 6). Two different thiol-containing compounds (reactants) have been used in our reaction. The time-period for the reaction has also been increased based on the completion of the reactions, to include approximately one hour of stirring after each addition of thiol moieties. A high yield of unsymmetrical tetrasulfanes (UT) has not been observed here due to the formation of symmetrical tetrasulfane analogues as the side products (Table 3). UT have been found to be odourless, which is an advantage for testing these compounds in biological systems when compared to DATTS. The chemical structure of UT has been characterised using the techniques such as NMR (^{1}H and ^{13}C) spectroscopy and mass spectroscopy.

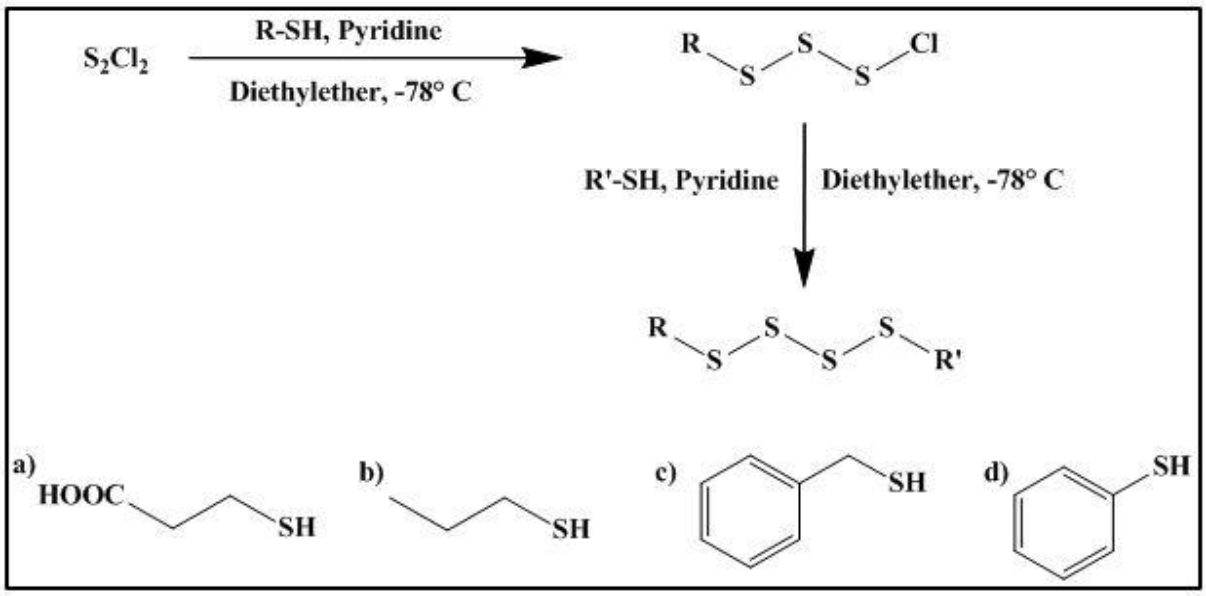


Figure 6: Overview of the modified method of Derbesy and Harpp for the synthesis of unsymmetrical tetrasulfanes; a) 3-thiopropionic acid, b) 1-propanethiol, c) benzylthiol and d) thiophenol were chosen as the side chains and different combinations of structures containing these side chains were synthesised as a part of this thesis [66].

Figure 5 shows the overview of different tetrasulfanes. Compounds **3-6** shown in Figure 5 have been synthesised and purified by Brigitte Czepukojc. Compounds **7-13** have been synthesised and purified as part of this thesis. The tetrasulfanes shown in Figure 5 are divided

into two groups such as symmetrical (**1-7**) and unsymmetrical tetrasulfanes (**8-13**), for the sake of comparative physico-chemical and biological studies.

Table 3: The tetrasulfanes synthesised as part of this thesis with their clog*P* and respective yields.

Compounds	clog*P*	Yield (%)
DPhTTS (7)	5.04	90
BnTTSPs (8)	2.76	75
PrTTSPs (9)	2.74	19
BnTTSPh (10)	5.37	49
PhTTSPs (11)	3.12	72
PrTTSBn (12)	5.00	59
PrTTSPh (13)	4.66	62

3.2 Physico-chemical properties

Physico-chemical properties of tetrasulfanes have been analysed. Surface tension analysis of the compounds has been performed using the techniques such as ring tensiometry and sita tensiometry. The surface tension activity has also been confirmed with densitometry experiments. This activity of the compounds in turn reveals their amphiphilic nature. The redox properties of the compounds have been studied using Cyclic Voltammetry. The results and discussion of each experiment is discussed individually under different sections. Figure 7 illustrates the overview of the experiments performed with the compounds and their aims.

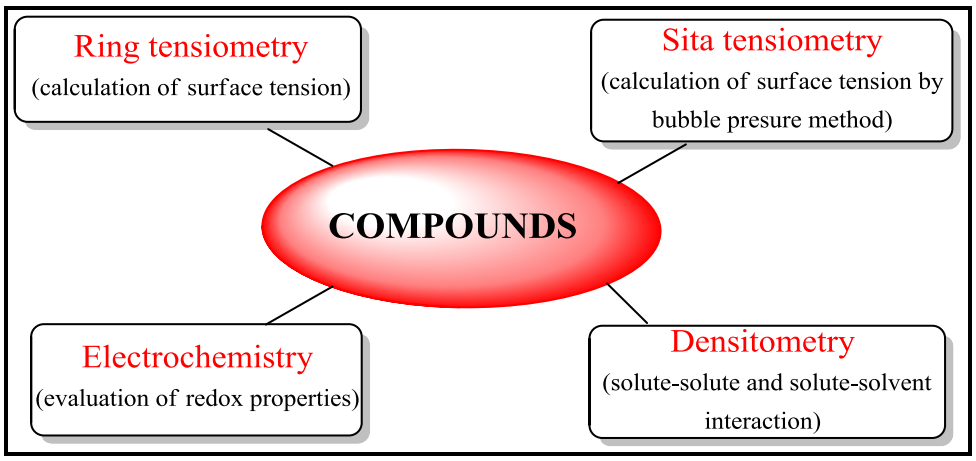


Figure 7: Overview of the physico-chemical measurements performed with tetrasulfanes and Se- and Te-containing compounds

3.2.1 Electrochemistry

In the case of polysulfanes redox activity is considered to be key to their biological activity [67] [68]. Hence, electrochemical analysis of compounds DATTS (**1**), DBTTS (**3**), DPSTTS (**4**), DPSEETTS (**5**), DEETTS (**6**), DPhTTS (**7**), BnTTSPs (**8**), PrTTSPs (**9**), PhTTSBn (**10**) and PhTTSPs (**11**) has been performed using Cyclic Voltammetry in conjunction with a dropping mercury electrode [69] [70] [71]. The cathodic reduction potentials (*E*pc) and the anodic oxidation potentials (*E*pa) of the compounds have been compared with the ones of DATTS (**1**) (Table 4). The ST and UT were found to be redox active and the obtained $E_{1/2}$ values of these compounds were similar to the value of DATTS. Cyclic voltammogram of BnTTSPs (**9**) and PrTTSPs (**8**) in a dropping mercury electrode experiment is shown in Figure 8.

Table 4: Overview of *E*pc, *E*pa and $E_{1/2}$ of tetrasulfanes.

Compound	*E*pa (mV)	*E*pc (mV)	$E_{1/2}$ (mV)
DATTS (**1**)	-603	-680	-642
DPTTS (**2**)	-597	-682	-640
DBTTS (**3**)	-594	-645	-619
DPSTTS (**4**)	-614	-688	-651
DPSEETTS (**5**)	-602	-679	-641
BnTTSPs (**8**)	-533	-840	-687
PrTTSPs (**9**)	-560	-786	-674
PhTTSBn (**10**)	-559	-652	-606
PhTTSPs (**11**)	-566	-748	-658

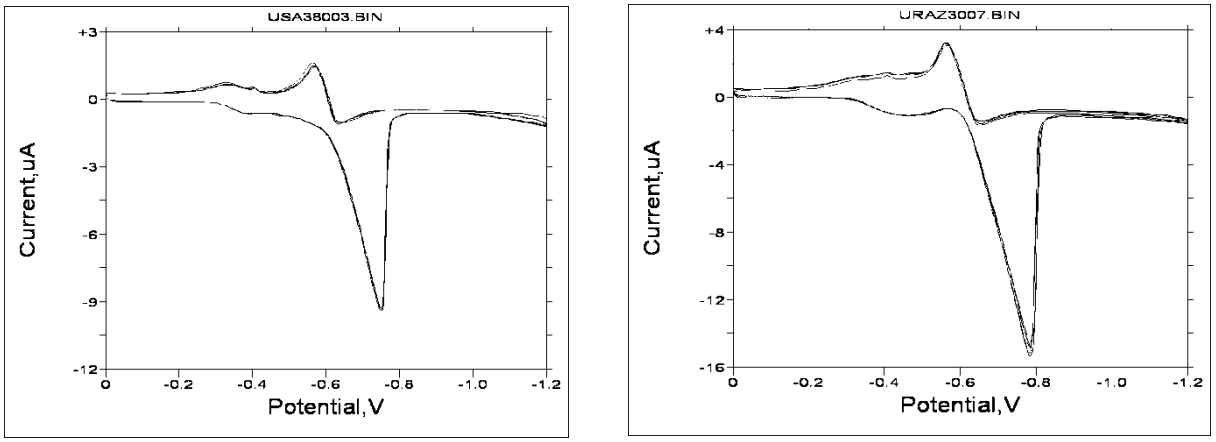


Figure 8: Cyclic voltammogram of BnTTSPs (9) and PrTTSPs (8) in a dropping mercury electrode experiment.

3.2.2 Sita tensiometry measurements

The unsymmetrical tetrasulfanes with propionic acid side chains have been expected to exhibit amphiphilic properties. This experiment has been performed to measure the changes in surface tension of the compounds with the change in their concentration using the bubble pressure method [72] [73] [74]. The identification of micelle formation could be carried out by calculating the CMC of the compounds [75] [76]. SDS (**14**), a surface-active molecule, has been used as a control. These compounds have been analysed along with DPSTTS (**4**) at different concentrations from 1 µM to 3 mM in distilled water with 1 % DMSO. Surprisingly, PrTTSPs (**9**) exhibited a CMC of 1.2 mM (Figure 9). This initial experiment supports the idea of micelle formation of tetrasulfanes. PhTTSPs (**11**) behaved differently in this experiment. The surface tension of the compound dropped at 2.6 mM (data not shown). This concentration could be the CMC of the compound, but it could also be due to the high ionic concentration of the liquid medium. The CMC also varies with temperature and the solvent used in the experiment. The other tetrasulfanes tested did not show any surface activity under the experimental conditions used.

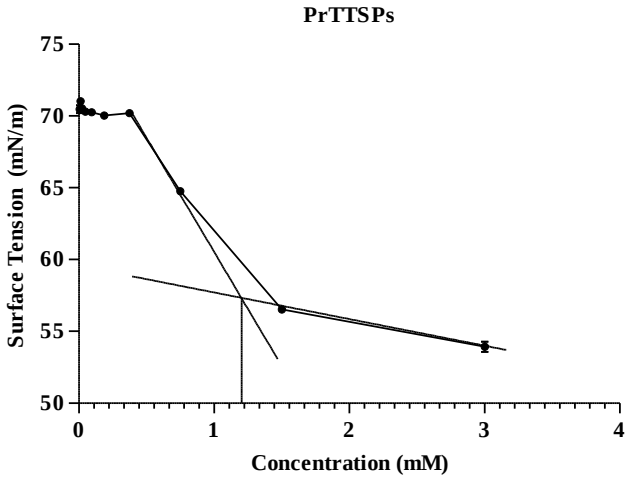


Figure 9: Change in surface tension with change in concentration of PrTTSPs in distilled water with 1 % DMSO. The CMC of this compound has been calculated as 1.2 mM. Each measurement was repeated three times and one representative measurement is shown for each compound

3.2.3 Densitometric measurements

In the case of drugs, physical properties of drugs such as density, apparent molar volume, and viscosity help to study the drug, its dissolution and availability in body fluids [77] [78]. Densitometric experiments have been performed to analyse the change in the density of the compounds with the change in concentration at room temperature. The apparent molar volume has been calculated from the obtained densities of the compounds [79]. This kind of study is important for determination of the drug dissolution rate, availability and possibly also degradation [78].

This experiment has been performed as an alternative method to confirm the results obtained from sita tensiometry. It was found that the apparent molar volume is inversely proportional to the concentration. It is very high at low concentrations and first decreases and then stays constant at higher concentrations, such as at 1 mM to 3 mM (Figure 10). It is also evident from the literature that the CMC of the surface-active compounds could be obtained from the graph of apparent molar volume against the square root of concentration (√C). The CMC value of PrTTSPs in these studies has been found to be 1.6 mM, which couples well with the value obtained by sita tensiometry. On the other hand, temperature-dependence studies have

to be performed to understand the various cellular-drug interactions and biochemical reactions occurring in the body.

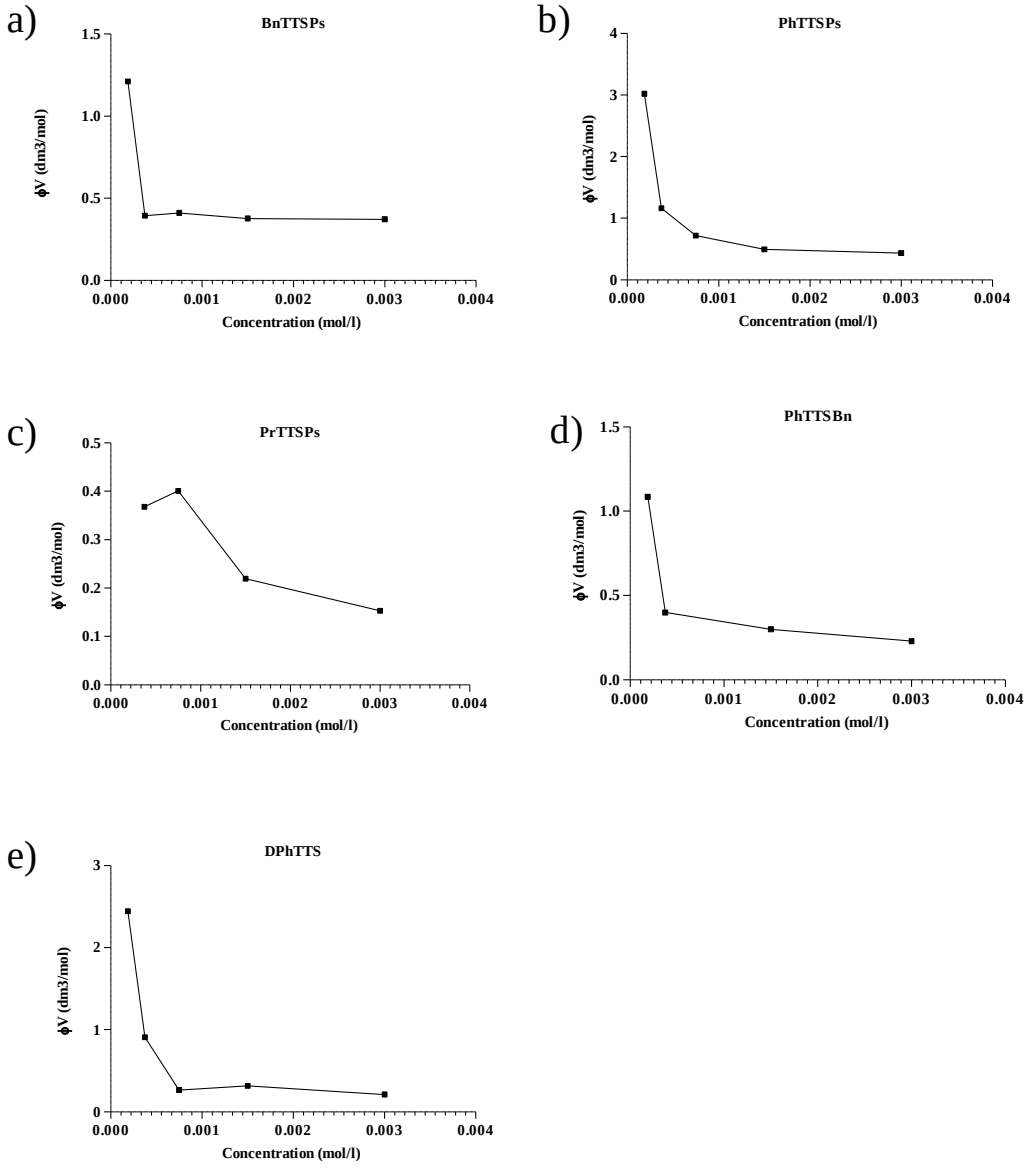


Figure 10: Apparent molar volume (ϕ_V) vs. concentration of a) BnTTSPs (8), b) PhTTSPs (11), c) PrTTSPs (9), d) BnTTSPh (10) and e) DPhTTS (7). Each measurement was repeated three times and one representative measurement is shown for each compound

3.3 Molecular Non-Covalent Interaction Studies

3.3.1 Haemolytic assay

This assay has been performed to measure the non-covalent molecular interactions of the tetrasulfanes with Red Blood Cells (RBCs) [49]. In the following study, the results of all the compounds have been compared to DPTTS (**2**). PrTTSPs (**9**) exhibited nearly 60 % of haemolysis and an EC_{50} value of 264.2 µM, when compared to DPTTS (**2**), which exhibited nearly 42 % of haemolysis and an EC_{50} value of 724.2 µM (Figure 11). The compounds DPhTTS (**7**) and BnTTSPh (**10**), however, presented an EC_{50} value of 385.6 µM and 266.2 µM, respectively. These compounds also indicated the haemolysis almost equal to that of DPTTS (**2**). BnTTSPs (**8**) also exhibited haemolysis of nearly 46 % (data not shown), which is in close accordance with DATTS (**1**). On the other hand, PhTTSPs (**11**) did not show significant haemolysis of RBCs.

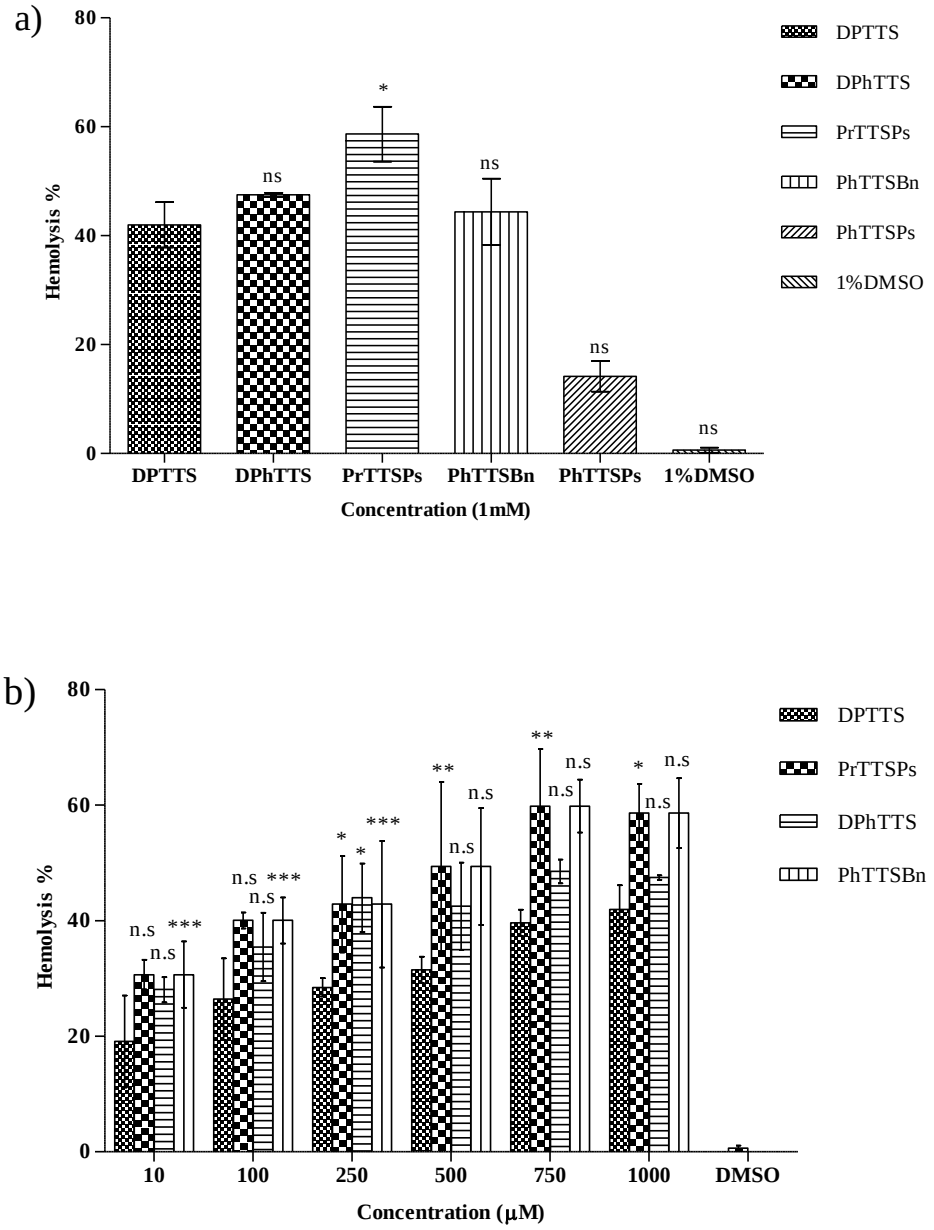


Figure 11: a) Haemolysis of RBCs incubated with the compounds at 1 mM concentration. b) Concentration-dependence studies of DPTTS (2), PrTTSPs (9), DPhTTS (7), and PhTTSBn (10) on haemolysis of RBCs. Absorbance was recorded in UV-visible spectrophotometer at 540nm. Data presented as haemolysis percentage ± SEM. ANOVA test: $p < 0.05$ (*), $p < 0.01$ (**) or $p < 0.001$ (***) in relation to the relevant values for the particular control DPTTS.

3.3.2 FRET measurements

The Fluorescence Resonance Energy Transfer (FRET) mechanism involves two chemical species, a fluorophore, that when excited emits fluorescence, and a quencher that reduces the fluorescence of the fluorophore [80]. When the FRET mechanism is applied to optical microscopy the spatial approach between two molecules, within several nanometers, can be studied [81]. The combination of FRET and optical microscopy also reveals information about the binding to and interaction with proteins, lipids, enzymes, DNA and RNA [82] [83].

The fluorescence quenching can be measured quantitatively and analysed using the Stern-Volmer equation.

$$F_o / F_{[Q]} = 1 + Ksv\,[Q] \quad \text{or} \quad Ksv = (F_o / F_{[Q]} - 1) / [Q]$$

Equation 2: The Ster-Volmer equation.

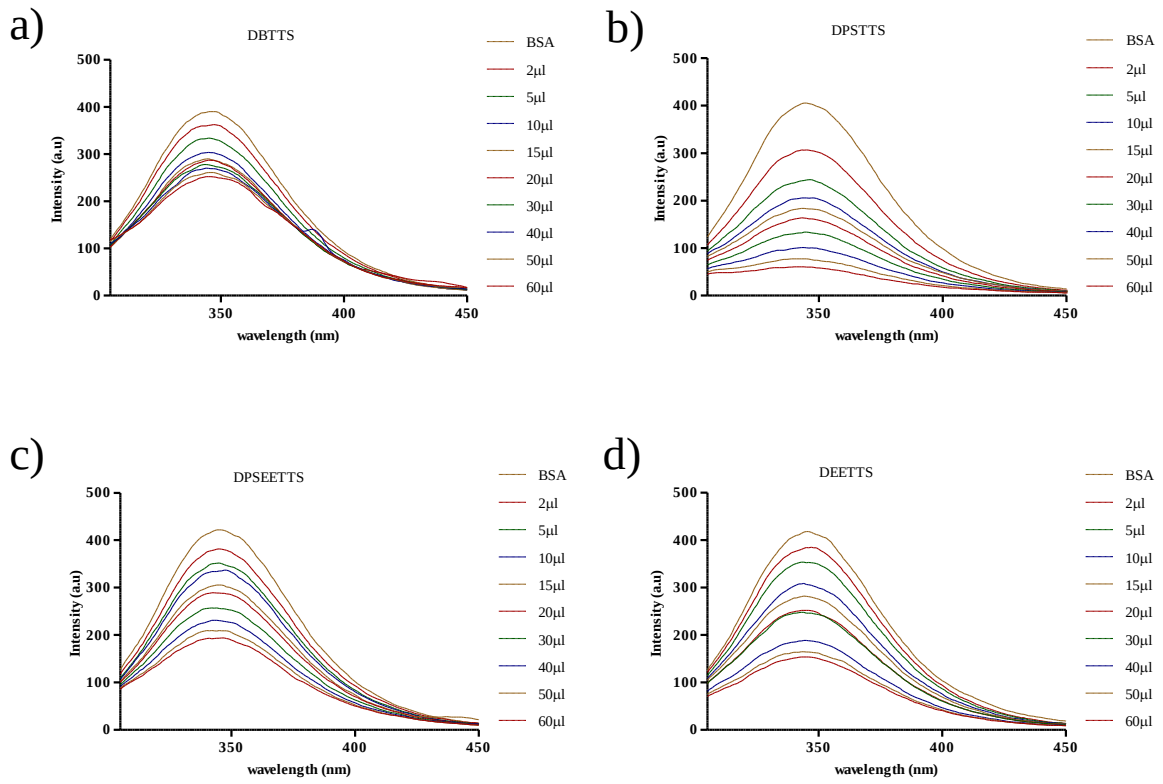
In equation 2, [Q] represents the concentration of the quencher; F_o represents the fluorescence intensity measured in the absence of a quencher, $F_{[Q]}$ represents the fluorescence intensity measured in the presence of a quencher, and *K*sv represents the Stern-Volmer constant. A slope can be obtained from the plot of $F_o / F_{[Q]}$ against [Q]. This slope value represents the Stern-Volmer constant. In general, a more sensitive system results in a steeper slope value, which in turn represents a higher *K*sv.

The FRET measurements of compounds **3-11** have been performed with BSA. A shift in the intensity of the fluorescence of BSA has been observed in the presence of tetrasulfanes. This effect is also visible in Figure 12. SDS (**14**) was used as a control and showed a *K*sv value of $2.20 \cdot 10^4$ l/mol. UT exhibited higher values of *K*sv when compared to the ones of ST. PhTTSPs (**11**) and BnTTSPs (**8**) exhibit the greatest fluorescence quenching effect among all the tetrasulfanes. PrTTSPs (**9**) (UT) exhibited a similar effect to that of DPSTTS (**4**) (ST). The *K*sv values of tetrasulfanes are summarised in Table 5.

Table 5: Overview of Stern Volmer constants obtained from the fluorescence quenching of BSA in the presence of tetrasulfanes.

Compounds	*Ksv* .10^4 (l/mol)	Compounds	*Ksv* .10^4 (l/mol)
SDS **(14)**	2.20	BnTTSPs **(8)**	18.67
DBTTS **(3)**	2.27	PrTTSPs **(9)**	7.40
DPSTTS **(4)**	7.00	PhTTSPs **(10)**	11.75
DPSEETTS **(5)**	2.17	PhTTSBn **(11)**	3.88
DEETTS **(6)**	3.21	DPhTTS **(12)**	5.11

Unfortunately, it is difficult to explain the nature of fluorescence quenching of BSA with these preliminary results. A temperature-dependence study needs to be carried out in order to explain the nature of quenching. These studies could probably help to calculate the binding constant and would explain the nature of binding. The preliminary fluorescence quenching effect proved that UT exhibit a more effective interaction with BSA when compared to ST.



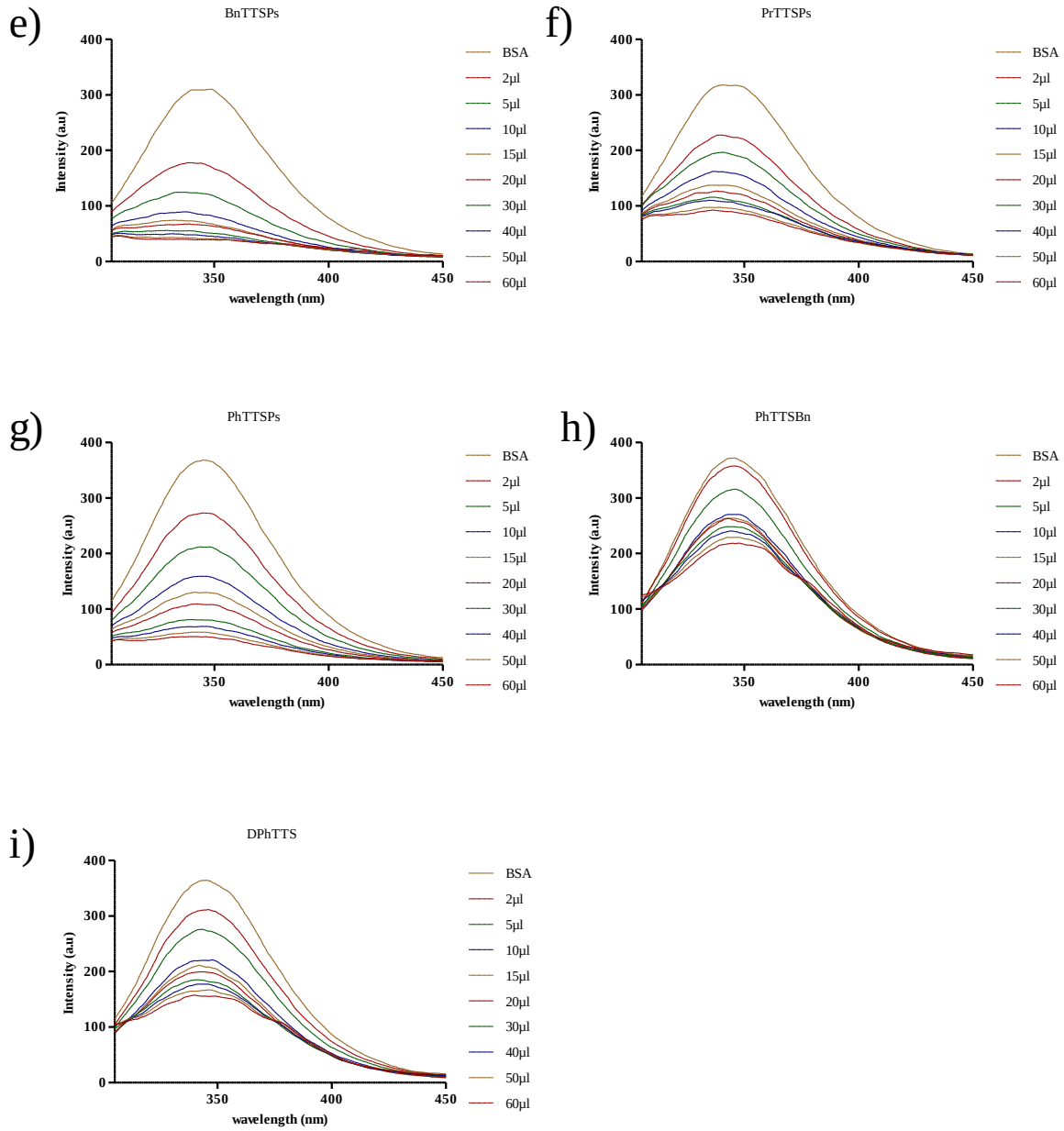


Figure 12: The quenching of the fluorescence bovine serum albumin with different concentrations of a) DBTTS (3), b) DPSTTS (4), c) DPSEETTS (5), d) DEETTS (6), e) BnTTSPs (8), f) PrTTSPs (9), g) PhTTSPs (11), h) PhTTSBn (10) and i) DPhTTS (7) at an excitation wavelength of 295 nm. Each measurement was repeated three times and one representative measurement is shown for each compound.

3.3.3 Circular dichroism measurements

This experiment has been performed to study the interaction of compound PrTTSPs (9) with the protein, haemoglobin. This experiment was an alternative method to study the non-covalent membrane interactions of tetrasulfanes. PrTTSPs (**9**) showed a maximum haemolysis in our experiments and therefore was chosen for circular dichroism (CD) [84] [85] measurements, at various concentrations of 50 µM, 100 µM and 200 µM. A significant change in the shift of the wavelength of absorption of haemoglobin was not observed. It could be also possible that 200 µM of PrTTSPs (**9**) was a very low concentration to produce a significant shift in the wavelength. In the future, CD experiment needs to be performed with higher concentrations of tetrasulfanes.

3.4 Biological activities of the compounds

The biological activities of compounds **1-11** have been studied. First of all, detailed antimicrobial analyses have been carried out. Experiments have been performed with eukaryotic cell lines such as MH7A and neuronal cell lines of rats. An overview of the biological assays performed with the compounds is shown in Figure 13

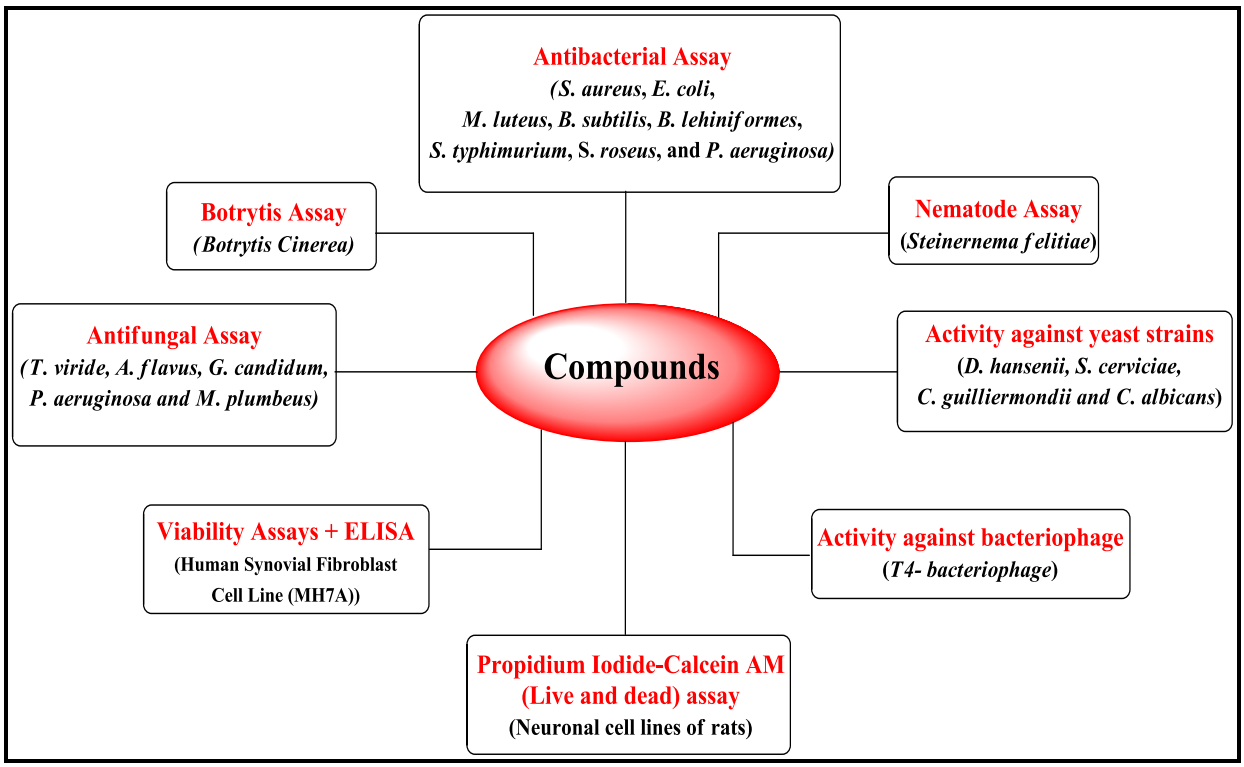


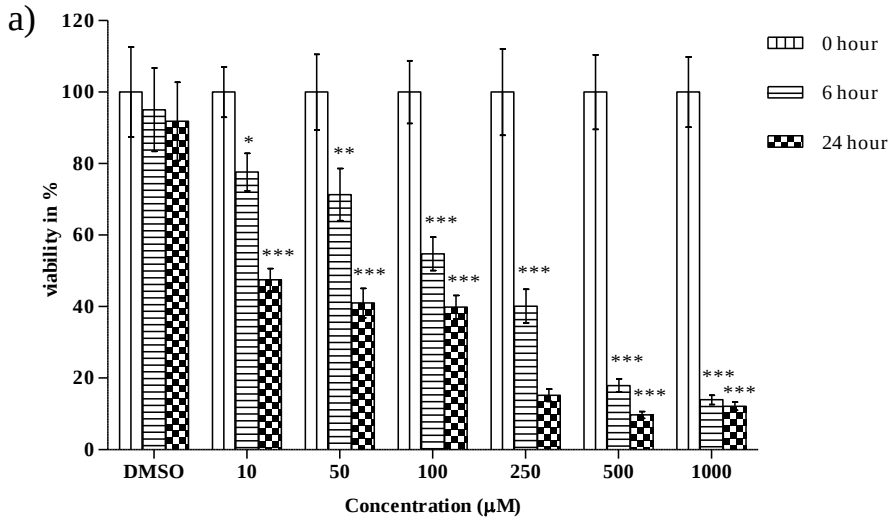
Figure 13: Overview of the biological assays performed with various tetrasulfanes and Se- and Te-containing compounds against different microorganisms and in different cell lines.

3.4.1 Activity against microbes and small organisms

3.4.1.1 Nematode assay

As a pre-screening of drugs or to study the important metabolites and their actions, model organisms such as the common yeast strain, *Saccharomyces cerevisiae*, fruit flies or nematodes are being widely used by scientists primarily due to ethical considerations [48]. These organisms also show easy reproduction, enabling repeated studies.

Nematodes are genetically tractable hermaphrodites, transparent organisms visible under the microscope. The co-ordination, reproduction and chemotaxis could be studied in these organisms; thus, they are ideal for the pre-screening of our compounds before any complex analysis [86]. They may also reveal certain possible agricultural applications of our compounds.



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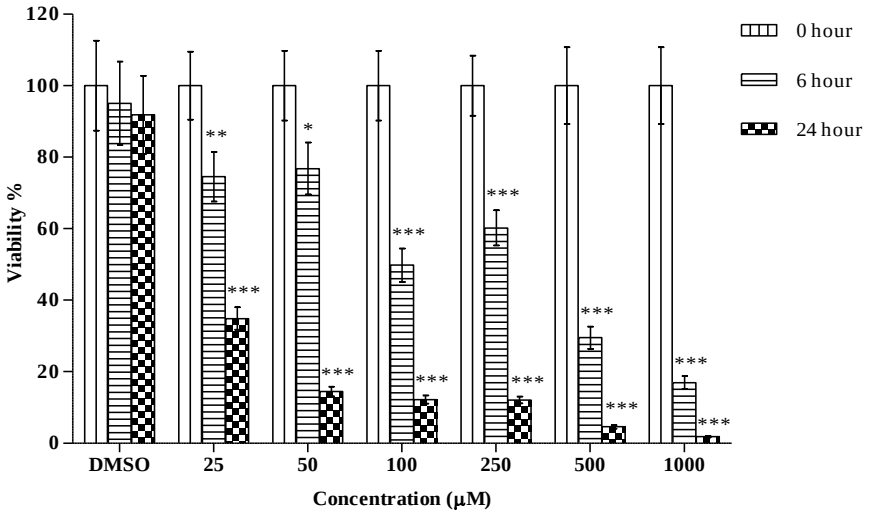
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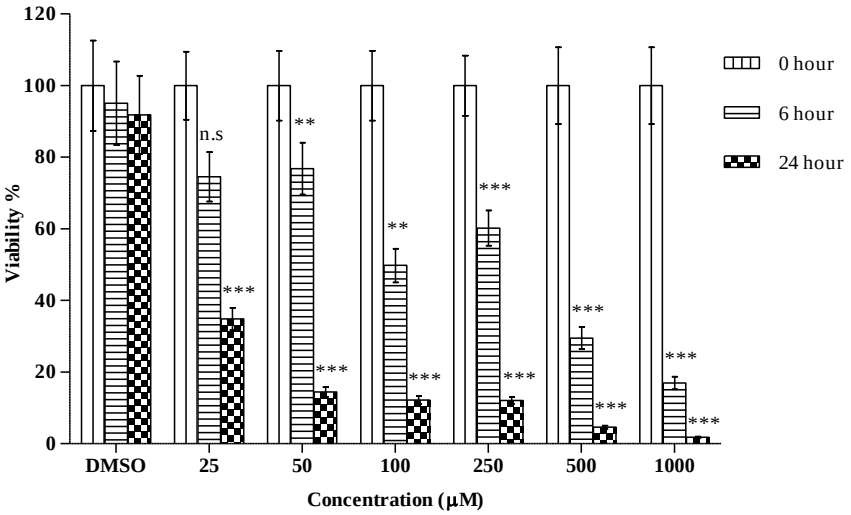
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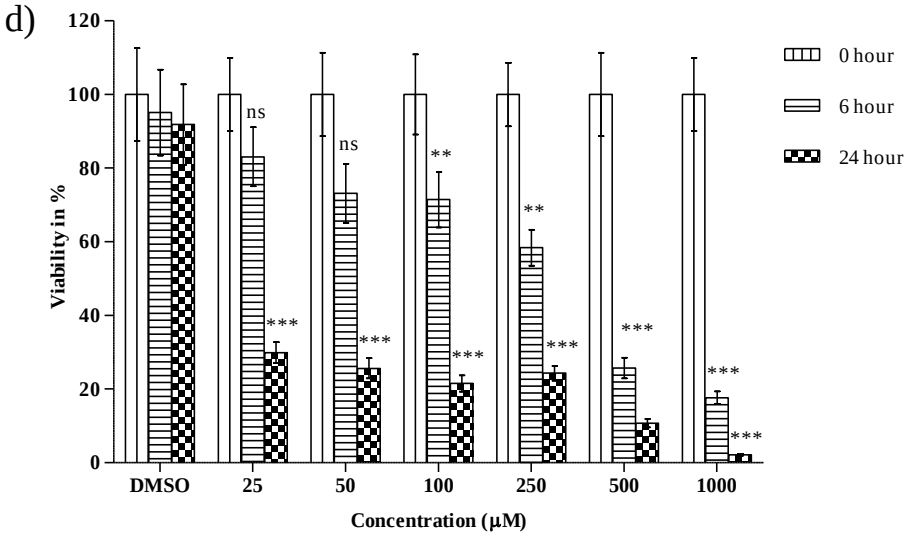


Figure 14: Viability of nematodes *S. feltiae* in the presence of a) BnTTSPs (8) and b) PrTTSPs (9) c) PhTTSPs (11) and d) PhTTSBn (10) after 0, 6 and 24 h with 1 % DMSO as a control. Data presented as viability percentage ± SEM. Significances: *n.s.* = $p \geq 0.05$, * = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$ in relation to the relevant values for the particular control DMSO.

Compounds **7-11** have been tested against *Steinernema feltiae* in triplicate and at different time points such as 0, 6 and 24 h (Figure 14). PrTTSPs (**9**), BnTTSPh (**10**) and DPhTTS (**7**) exhibited a LD_{50} value of 42.7 µM, 48.5 µM and 55.3 µM respectively, and were the most active compounds. Whereas, BnTTSPs (**8**) and PhTTSPs (**11**) exhibited a LD_{50} value of 170.5 µM and 294.8 µM respectively (Table 6). Growth viability curve of nematode *S. feltiae* after 24 h of co-incubation with BnTTSPs (8) is shown in Figure 15.

Table 6: Overview of the compounds tested against nematode *S. feltiae* and the LD_{50} values

Compounds	LD_{50} (µM)
DATTS (**1**)	150.0
DPhTTS (**7**)	55.3
BnTTSPs (**8**)	170.5
PrTTSPs (**9**)	42.7
BnTTSPh (**10**)	48.5
PhTTSPs (**11**)	294.8

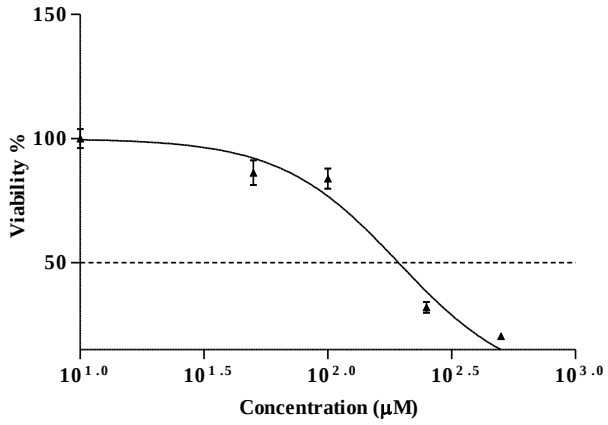


Figure 15: Growth viability curve of nematode *S. feltiae* after 24 h of co-incubation with BnTTSPs (8). Data presented as viability percentage ± SEM.

3.4.1.2 Toxicity against different yeast strains

Agar well-diffusion method has been used to study the activity of tetrasulfanes against different strains of yeast. Here, four different strains of yeast have been used, namely *Debariomyces hansenii*, *Saccharomyces cerevisiae*, *Candida guilliermondii* and *Candida albicans* 174.

The tetrasulfanes DBTTS (**3**), DPSTTS (**4**), DPSEETTS (**5**), DEETTS (**6**), DPhTTS (**7**), BnTTSPs (**8**), PrTTSPs (**9**), BnTTSPh (**10**) and PhTTSPs (**11**) have been pre-screened against the different strains of yeast (Table 7). ST did not exhibit any significant activity against the strains of yeast except DEETTS (**6**). Table 7 shows that the compounds DBTTS (**3**) and DPSTTS (**4**) did not exhibit any activity against *D. hansenii*, but these compounds presented a minimal inhibitory zone against the other three strains of yeast. DPSEETTS (**5**) exhibits a moderate activity against all the four different strains of yeast. DEETTS (**6**) is the most active compound among the ST. It shows the zonal diameter of 17.5 ± 4 mm, 19 ± 1 mm, 17.5 ± 4 mm and 23 ± 1 mm against *D. hansenii*, *S. cerevisiae*, *C. guilliermondii*, and *C. albicans*, respectively. Thus, DEETTS (**6**) has been chosen for further concentration-dependence studies.

Table 7: Pre-screening of compounds 3-11 and 14 at a concentration of 3 mM against four different strains of yeast, namely *D. hansenii, S. cerevisiae, C. guilliermondii and C. albicans* 174. A diameter of 12 mm is considered as the minimum inhibitory zone. 100 % of DMSO has been used as control. The values represent the mean ± SEM of three independent experiments.

Compounds	***D. hansenii***	***S. cerevisiae***	***C. guilliermondii***	***C. albicans* 174**
DBTTS (**3**)	-	11.5 ± 0	12.0 ± 0	14.5 ± 2
DPSTTS (**4**)	-	14.5 ± 0	12.0 ± 1	14.5 ± 1
DPSEETTS (**5**)	12.0 ± 0	15.5 ± 0	13.5 ± 2	17.5 ± 1
DEETTS (**6**)	17.5 ± 4	19.0 ± 1	17.5 ± 4	23.0 ± 1
DPhTTS (**7**)	-	13.3 ± 1	17.5 ± 1	15.5 ± 4
BnTTSPs (**8**)	-	14.5 ± 2	16.0 ± 0	14.5 ± 4
PrTTSPs (**9**)	12.5 ± 1	19.5 ± 7	14.3 ± 0	17.0 ± 4
BnTTSPh (**10**)	-	12.5 ± 1	-	15.0 ± 3
PhTTSPs (**11**)	-	20.0 ± 4	16.0 ± 1	16.3 ± 1
SDS (**14**)	16.3 ± 0	11.5 ± 1	14.3 ± 0	14.5 ± 1

The activities of all of the compounds have been compared to the activity of SDS (**14**). SDS exhibited a minimum inhibitory zone of 16.25 ± 0 mm, 11.5 ± 1 mm, 14.25 ± 0 mm and 14.5 ± 1 mm against *D. hansenii, S. cerevisiae, C. guilliermondii and C. albicans* 174 respectively. DEETTS (**6**) did not exhibit a similar activity of SDS (**14**) against *D. hansenii*, whereas a

significant difference was found against the other three yeast strains (Figure 16). DPSEETTS (**5**) also exhibited a significant difference in the activity from that of SDS (**14**), but the activity was very low against *D. hansenii* (**14**), whereas the activity against *S. cerevisiae* and *C. albicans* was considerably higher. The concentration-dependence studies of DEETTS (**6**) also exhibited a similar activity as that of SDS (**14**), against *D. hansenii.* The respective percentage inhibition curves are illustrated in Figure 16.

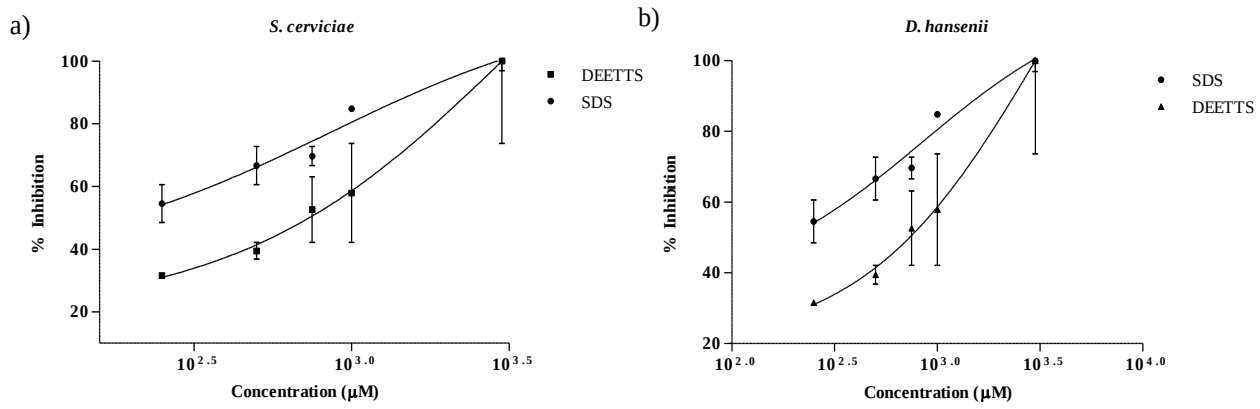


Figure 16: Growth inhibition curve of yeast strains treated with compounds, a) DEETTS against *S. cerevisiae* and b) DEETTS against *D. hansenii*. SDS is used as a particular control. Data presented as percentage inhibition ± SEM.

As evident from the pre-screening test, tetrasulfanes did not show any significant effect against *S. cerevisiae* and SDS did not exhibit a concentration-dependent activity either. The activities among the compounds themselves were compared to each other. It was found that PrTTSPs (**9**) and PhTTSPs (**11**) at a concentration of 3 mM were considerably more active against *S. cerevisiae*, while other compounds failed to show any significant activity.

The percentage inhibition of PhTTSPs and SDS against *C. guilliermondii* was analysed. Compounds BnTTSPs (**8**), PrTTSPs (**9**), PhTTSPs (**11**) and DPhTTS (**7**) presented significantly higher activities against *C. guilliermondii* than the activity of SDS (**14**) (Figure 17). BnTTSPs (**8**) exhibited a minimum inhibition at a concentration of 1 µM and PrTTSPs (**9**) at a concentration of 10 µM, whereas SDS exhibited a minimum inhibition only at a

concentration of 500 µM. DPhTTS (**7**), which did not show any activity against *D. hansenii*, was effective against *C. guilliermondii* at very low concentrations (1 µM).

C. albicans 174 is a semi-pathogenic strain. Compounds DPhTTS (**7**), BnTTSPs (**8**), PrTTSPs (**9**), BnTTSPh (**10**) and PhTTSPs (**11**) exhibited an activity against *C. albicans* 174. Nevertheless, the activity of DPhTTS (**7**), BnTTSPs (**8**) and BnTTSPh (**10**) could be probably due to solvent (DMSO) influence but, importantly, PhTTSPs (**11**) exhibited a complete inhibitory activity against *C. albicans* 174 with a MIC of 16.1 µM (Figure 18) (Table 8). DMSO exhibited a static effect against *C. albicans*, thus, it is important to note that the activity of some of the compounds tested is probably due to the influence of DMSO.

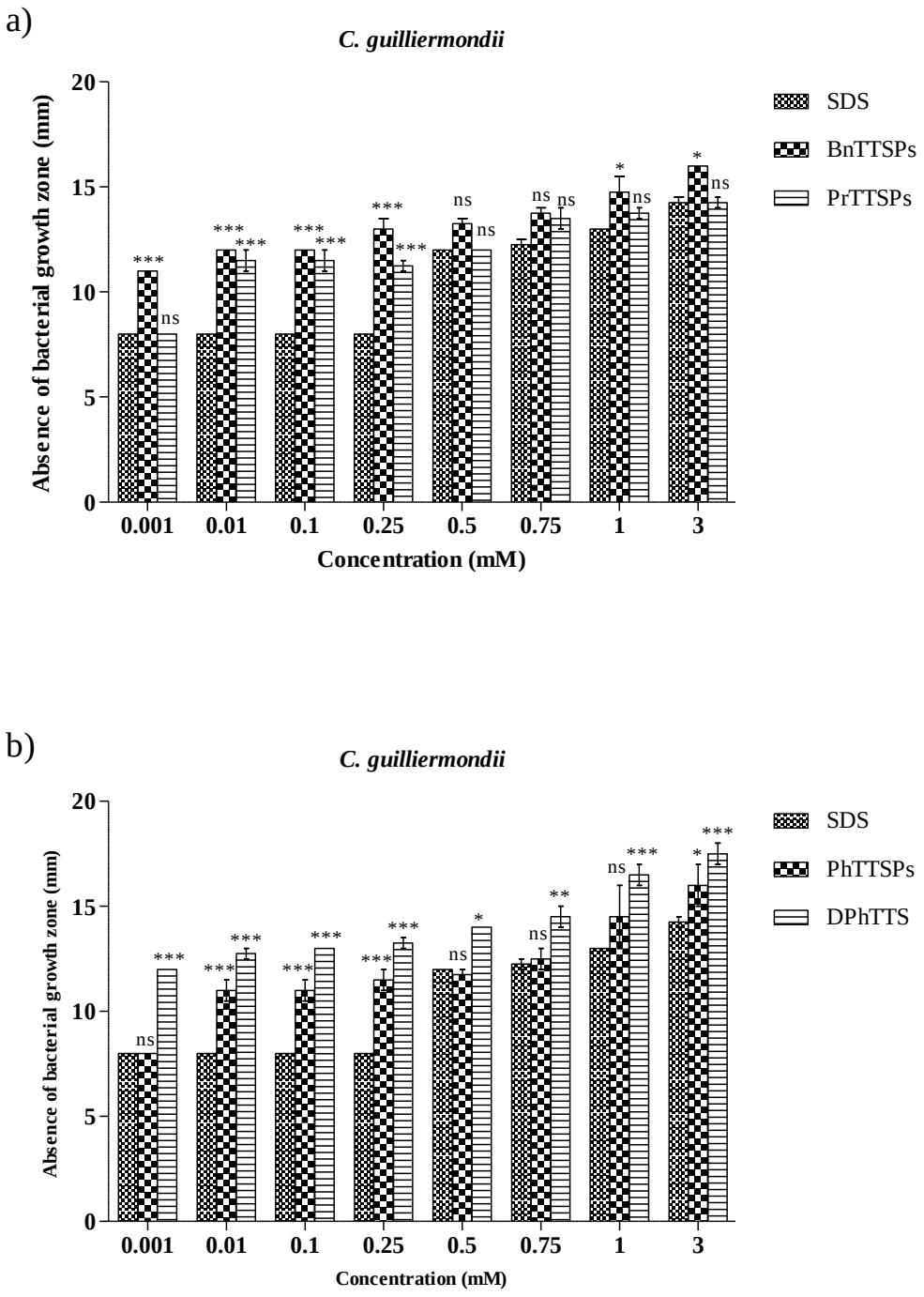


Figure 17: Activity of a) BnTTSPs (8) and PrTTSPs (9) b) PhTTSPs (11) and DPhTTS (7) against *C. guilliermondii*. SDS (14) has been used as a control. Data presented as absence of the yeast growth zone in mm ± SEM. ANOVA test: n.s. = $p \geq 0.05$, $p < 0.05$ (*), $p < 0.01$ (**) or $p < 0.001$ (***) in relation to the relevant values for the particular control SDS.

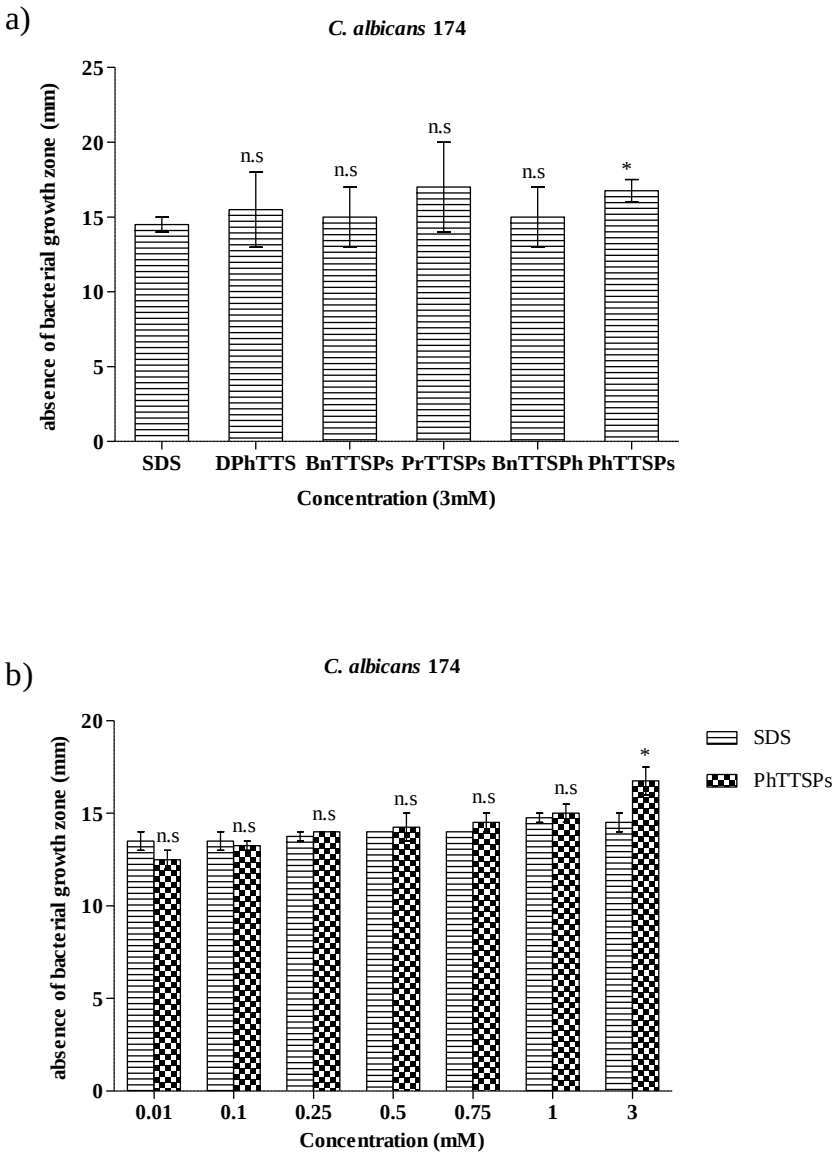


Figure 18: a) Pre-screening of DPhTTS (7), BnTTSPs (8), PrTTSPs (9), and PhTTSBn (10) at 3 mM against *C. albicans* and b) activity of PhTTSPs (11) against *C. albicans* at various concentrations. SDS (14) has been used as a control. Data presented as absence of the bacterial growth zone in mm ± SEM. ANOVA test: n.s. = $p \geq 0.05$ and $p < 0.05$ (*) in relation to the relevant values for the particular control SDS.

Table 8: Overview of the tetrasulfanes tested against corresponding yeast strains with relevant EC_{50} values.

Compounds	Fungi			
	D. hansenii	***C. albicans174***	***C.guillirmondii***	***S. cerviciae***
DEETTS (**6**)	612.5	n.a	n.a	860.6
DPhTTS (**7**)	n.a	n.a	12.9	n.a
BnTTSPs (**8**)	n.a	n.a	15.8	n.a
PrTTSPs (**9**)	n.a	n.a	41.1	625.2
PhTTSPs (**11**)	n.a	16.1	334.7	836.5
SDS (**14**)	230.0	671.9	501.8	n.a

It should be noted here that the commercially available sulfur-containing antibiotic, sulfadimethoxin was also tested against the different strains of yeast. It is essential to note that at a concentration of 3 mM, this antimicrobial agent did not show any activity within 24 hours. However, after 48 hours, it exhibited an average inhibitory zone of 18 mm against *C. guilliermondii*. The mode of action of Sulfadimethoxin is by inhibiting the formation of folic acid and this antibiotic is used as a treatment against infections in the respiratory tract, skin or soft tissue. Nevertheless, compounds DPhTTS, BnTTSPs and PrTTSPs have been considerably more active against *C. guilliermondii,* even at very low concentration and within 24 hours.

3.4.1.3 Activity against different filamentous fungi

This assay has been performed to study the activity of the tetrasulfanes against filamentous fungi such as *Trichoderma viride, Aspergillus flavus, Geotrichum candidum, Penicillium aeruginosa* and *Mucor plumbeus,* using the agar dilution method. The growth of fungi was monitored for 8-10 days. Initially compounds **3-11,** at a concentration of 3 mM were pre-screened against the fungal strains. The activity of the compounds is tabularised below (Table

9). Among ST, only DPSEETTS (**5**) was considerably active against four different strains of fungi and statically on *T. viride.* DPSTTS (**4**) and DEETTS (**6**) exhibited only a temporary effect against *P. aeruginosa* and *A. flavus*, respectively, and failed to stop the complete growth of the fungi.

Table 9: Overview of the activity of compounds 3-11, against *T. viride, A. flavus, G. candidum, M. plumbeus* and *P. aeruginosa*, with DMSO as a control. + indicates the complete inhibition of the growth of fungus, - indicates the absence of any activity of compounds on the fungus and ± indicates the static effect of the compounds.

Compounds	*Trichoderma viride*	*Aspergillus flavus*	*Mucor plumbeus*	*Geotrichum candidum*	*Penicillium aeruginosa*
DBTTS (**3**)	-	-	n.a	-	-
DPSTTS (**4**)	-	-	n.a	-	Prolonged the growth until 8 days
DPSEETTS (**5**)	±	-	n.a	-	-
DEETTS (**6**)	±	Prolonged the growth until 6 days	n.a	+	+
BnTTSPs (**8**)	+	±	-	+	+
PrTTSPs (**9**)	+	+	+	+	+
PhTTSBn (**10**)	-	-	-	-	-
PhTTSPs (**11**)	+	+	+	+	+
DMSO	-	-	-	-	-

UT were the most active compounds against the fungal strains in this experiment. BnTTSPs (**8**), PrTTSPs (**9**), and PhTTSPs (**11**) were active against all five strains of the filamentous fungi tested, except for BnTTSPs (**8**) against *M. plumbeus.* These results clearly show that the ST did not exhibit any activity against these strains. The UT (PrTTSPs (**9**), and PhTTSPs (**11**)) stood out among all the other compounds in this experiment. BnTTSPs (**8**), however, exhibited only a static activity against *A. flavus*. PhTTSBn (**10**) did not exhibit any activity against the fungal strains. There was also no solvent (DMSO) effect against these strains.

The concentration-dependence studies have also been carried out in the presence of UT against *T. viride*. The MIC of compounds BnTTSPs (**8**), PrTTSPs (**9**) and PhTTSPs (**11**) was determined as $\leq$ 1 mM, $\leq$ 2 mM and $\leq$ 2 mM, respectively. Further in-depth studies have to be conducted to reveal the mode of action of tetrasulfanes against the different strains of fungi.

3.4.1.4 *Botrytis* assay

The experiments have been performed by Brigitte Czepukojc in the group of Prof. Dr. Matthias Hahn, Department of Biology, Technical University of Kaiserslautern. The compounds DPhTTS (**7**), BnTTSPs (**8**) and PrTTSPs (**9**) have been synthesised as a part of this thesis and have been used in this experiment. The polysulfanes have been tested against the grey mould *Botrytis cinerea* [87] [88], a plant pathogen that is often seen on grapes and strawberries. The *B. cinerea* strains used in these experiments were wild type (MDR) and artificially grown strains (MDR1) termed multidrug resistance (MDR) strains [89]. The symmetrical and unsymmetrical compounds tested here are compared with the naturally occuring tetrasulfane DATTS, DPTTS and the trisulfane analogue DPTS.

The trisulfane analogues DATS and DPTS exhibited a MIC value of 1.4 mM and 2.74 mM, respectively whereas the tetrasulfane analogues DATTS (1) and DPTTS (2) presented a MIC value of 48 µM and 23-47 µM, respectively against WT and MDR1 strains (Table 10) [90]. The compounds DPhTTS (**7**), PrTTSPs (**9**) and BnTTSPs (**8**) also showed considerable activity against these strains [90]. The respective MIC values are summarised in Table 10.

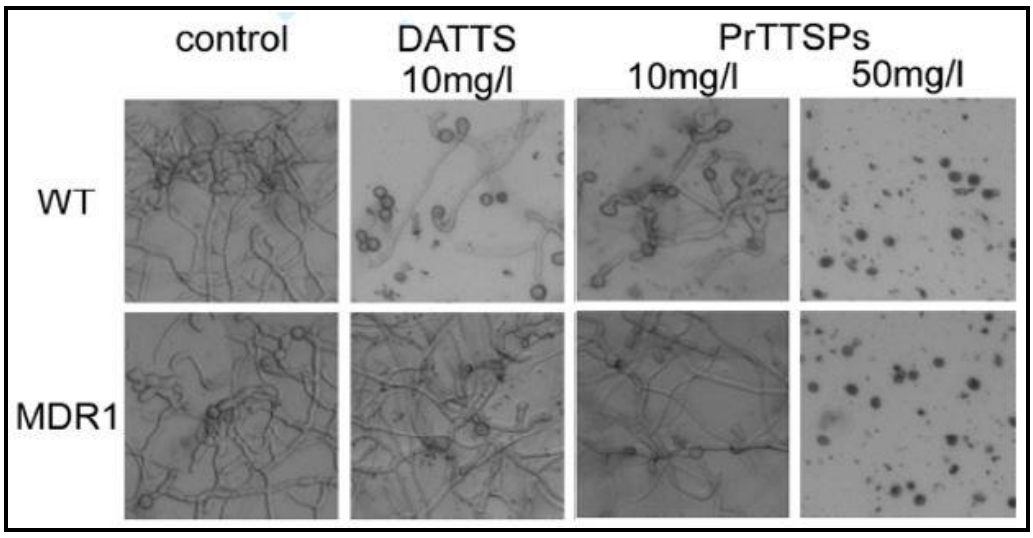


Figure 19: Microscopic images of mycelium growth of wild type and MDR1 strains of *B. cinerea* when treated with DATTS (1), and PrTTSPs (9). The fungal spore appears to be shrunken at 50 mg/l of PrTTSPs. Figure adapted from [90].

Table 10: Overview of the polysulfanes tested against the wild type and MDR1 *B. cinerea* strains. The MIC values in WT strains, resistance factor of MDR1 strains and the strength of AtrB induction are tabulated below. Table adapted from [90].

Compounds	WT MIC [mg/L]	[µM]	Resistance factor of MDR1
DATTS (**1**)	10	48	>100x*
DPTTS (**2**)	5-10	23-47	>100x*
DPhTTS (**7**)	50	177	2x
BnTTSPs (**8**)	50	171	1x
PrTTSPs (**9**)	50	205	1x
DPTS	500	2740	> 2x*

0 no induction or only slight induction at 100 mg/1; + Strong induction only at 100 mg/1 (no induction at 1 mg/1); ++ Medium induction already at 1 mg/1. *the compounds had less solubility at higher concentrations and hence were not analysed at this concentration.

The compounds have been treated against the mycelium of *B. cinerea* and their microscopic images were captured. The fungal spore appears to be shrunken at 50 mg/l of PrTTSPs (**9**) and confirmed the apoptosis of the cells (Figure 19). DATTS (**1**) was found to be considerably active against the strain of WT compared to the MDR1 strain. Compounds BnTTSPs (**8**), PrTTSPs (**9**) and DPhTTS (**7**) exhibited effective activity against both the strains of WT type and MDR1. These compounds could therefore be toxic against the strain of *B. cinerea.* This strain, however, may induce resistance mechanism.

3.4.1.5 Antibacterial assay

The inhibitory activity against bacteria has been established by the agar well-diffusion method as stated in [91]. This assay was performed in order to evaluate the growth inhibitory effect (bactericide/bacteriostatic) of the compounds implemented. The compounds have been investigated using a series of concentrations and the EC_{50} values have been calculated accordingly.

Compounds **3-11** have been tested against eight different strains of bacteria, namely *Staphylococcus aureus* WDCM 5233, *Escherichia coli* VKPM-M17, *Micrococcus luteus*, *Bacillus subtilis* A1 WT, *Bacillus licheniformis* WT, *Salmonella typhimurium* 1754, *Staphylococcus roseus,* and *Pseudomonas aeruginosa.* A pre-screening of the compounds at a concentration of 3 mM is shown in Table 11, which represents the inhibitory zone diameters as the mean ± SEM [22].

Table 11: Pre-screening of compounds 4-6, 8, 9, 11 and 14 at a concentration of 3 mM against five different bacterial strains: *S. aureus, M. luteus, B. subtilis, B. licheniformis* and *S. roseus.* 12 mm of diameter is considered as the minimum inhibitory zone. 100 % DMSO has been used as a control. The values represent the mean ± SEM of three independent experiments.

Compounds	*S. aureus*	*M. luteus*	*B. subtilis*	*B. licheniformis*	*S. roseus*
DPSTTS (**4**)	-	-	-	-	12.0 ± 1
DPSEETTS (**5**)	19.0 ± 1	15.5 ± 1	14.0 ± 1	-	14.8 ± 0
DEETTS (**6**)	19.5 ± 1	13.0 ± 1	-	-	-
BnTTSPs (**8**)	19.7 ± 1	19.0 ± 1	-	25.8 ± 0	28.5 ± 2
PrTTSPs (**9**)	15.0 ± 2	23.3± 0	20.5 ± 1	25.3 ± 1	25.0 ± 1
PhTTSPs (**11**)	17.5 ± 1	18.5 ± 2	20.0 ± 1	24.5± 2	23.0 ± 1
SDS (**14**)	22.5 ± 1	n.a	-	n.a	n.a

Compounds **3-7**, being the ST, were active only against four different strains of bacteria. DPSEETTS (**5**) is one of those compounds, which showed an inhibitory diameter of 19 ± 1 mm against *S. aureus*; this is comparable to BnTTSPs (**8**), which shows a similar inhibitory zone. In addition, DPSEETTS (**5**) exhibited an activity against *B. subtilis*, *M. luteus* and *S. roseus*. DBTTS (**3**), DPhTTS (**7**) and BnTTSPh (**10**) did not show any activity against any of the strains of bacteria. None of the compounds exhibited an activity against *E. coli*, *S. typhimurium* or *P. aeruginosa*.

Compounds BnTTSPs (**8**), PrTTSPs (**9**) and PhTTSPs (**11**) exhibited a very high activity against *S. aureus, M. luteus, B. licheniformis* and *S. roseus.* Surprisingly, only PhTTSPs (**11**) showed an activity against the sporulating *B. subtilis* strain, with an inhibitory zone of 17.5 ± 1 mm. These results indicates the effectiveness of the activity of the compounds with a propionic acid as a side chain, which is expected to be amphiphilic in nature. Nevertheless, none of the compounds was active against the Gram-negative bacteria, *E. coli.* In other words, this could be a simple hint of the mode of action of tetrasulfanes, as Gram-positive and Gram-negative bacteria have different structures of cell walls. From the above results, it is evident that UT exhibited greater activity against the strains of bacteria when compared to ST.

In order to perform the concentration-dependence studies with ST and UT *S. aureus* strain has been selected among the eight different strains of bacteria. *S. aureus* is a Gram-positive

bacterium, that is found in the human respiratory system and on the skin. This species can cause serious skin infections, respiratory diseases and food poisoning. It also has the ability to rapidly gain resistance against the antibiotics. Thus, for the concentration-dependence studies, *S. aureus* was the primary choice and most of the tetrasulfanes were active against this strain during the pre-screening (Table 11). A series of concentrations of the compounds from 1 µM to 3 mM was prepared in DMSO and the relevant EC_{50} values were calculated. DMSO and SDS (**14**) have been used as negative and positive controls, respectively.

None of the tetrasulfanes exhibited an activity against *E. coli* or *S. typhimurium*, which are Gram-negative bacteria. SDS (**14**), however, exhibited an EC_{50} value of 773.1 µM. against *E. coli*. The *B. subtilis* strain used in this experiment was a unique one that possess spore-forming ability. Only compounds DPSEETTS (**5**) and PhTTSPs (**11**) exhibited activity against *B. subtilis* in this experiment.

Among ST, the concentration dependence studies were only performed with DEETTS (**6**) against *S. aureus.* This compound exhibited a significant activity at a concentration of 0.25 mM when compared to SDS. The EC_{50} value of DEETTS against *S. aureus* was calculated as 667.6 µM and that of SDS as 772.9 µM (Table 12). Only DPSEETTS (**5**) was active against *B. subtilis*, whilst SDS (**14**) did not show any inhibitory effect against this strain even at a concentration of 3 mM. DPSEETTS was also active against *S. aureus,* but did not exhibit a concentration-dependent activity. The bar graphs without the standard deviation show that the same zonal diameters were obtained in three independent experiments.

BnTTSPs (**8**), PrTTSPs (**9**) and PhTTSPs (**11**) were found to show inhibitory zones with diameters of 19.66 ± 1 mm, 15 ± 2 mm and 17.5 ± 1 mm, respectively, against *S. aureus* at a concentration of 3mM. Concentration-dependence studies have been carried out (Figure 20). BnTTSPs (**8**) exhibited a significant activity at very low concentration of 0.1 mM, against *S. aureus*, when compared to SDS. PrTTSPs (**9**), PhTTSPs (**11**) and SDS (**14**) did not show any activity against *S. aureus* at low concentrations (below 0.5 mM). PhTTSPs (**11**) was the only compound that exhibited a significant effect against *B. subtilis* with an inhibitory percentage of 85-90 % at a concentration of 3 mM and an EC_{50} value of 646.6 µM. This compound did not show any effect against Gram-negative bacteria (*E. coli* and *S. typhimurium*).

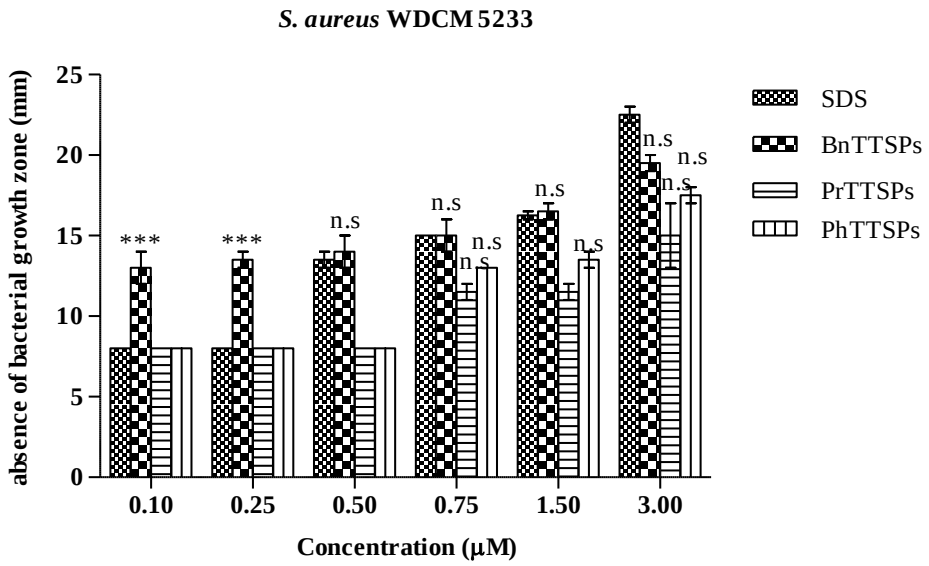


Figure 20: Activity of BnTTSPs (8), PrTTSPs (9) and PhTTSPs (11) against *S. aureus*. SDS (14) has been used as a control. Data presented as absence of the bacterial growth zone in mm ± SEM. ANOVA test: n.s. = $p \geq 0.05$ and $p < 0.001$ (***) in relation to the relevant values for the particular control SDS.

After the treatment of bacteria with the tetrasulfanes and the formation of growth inhibitory zones, a small portion of the growth media from the inhibitory zone was incubated in 5 ml of LB broth. This broth was incubated for 24 hours in a temperature controlled shaker. Further, a recovery of growth of bacteria was evaluated. A recovery of growth of *S. aureus*, when tested in the presence of BnTTSPs (**8**) was analysed (Figure 21). The IC_{50} value was obtained as 656.4 μM. This proves that BnTTSPs (**8**) exhibited a *bacteriostatic* effect against *S. aureus*.

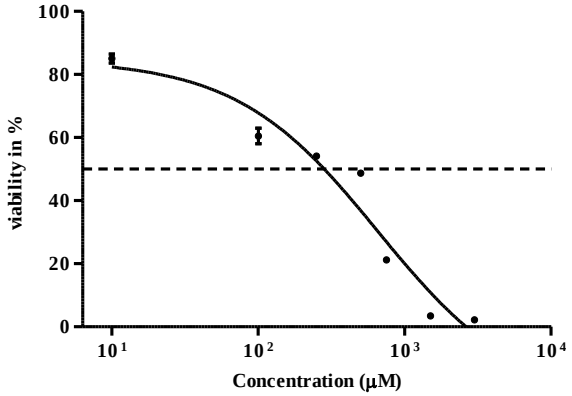


Figure 21: a) Recovery of viability curve of *S. aureus*, after 24 h of treatment with BnTTSPs (8). Data provided as viability percentage ± SEM. DMSO has been used as a control

Table 12: Overview of the tetrasulfanes tested against different bacterial strains with relevant EC_{50} values of growth inhibition.

Compounds	**EC_{50} of Inhibition of *S. aureus* (µM)**
DEETTS (**6**)	667.6
BnTTSPs (**8**)	259.4
PrTTSPs (**9**)	747.0
PhTTSPs (**11**)	775.9
SDS (**14**)	772.9

Different sulfur-containing compounds and their MIC values against different microbial strains, collected from different experiments are tabulated in Table 13. Although it is not appropriate method, a small comparative study has been performed. It is evident that DATTS is more active than ST. UT, however exhibit greater activity than DATTS. The MIC value of DATTS against *S. aureus* has been obtained as 0.5 mg/ml, whereas the EC_{50} value of UT is in

the micromolar range. Importantly, DATTS shows an effect against *P. aeruginosa*, which is not found for any of the tetrasulfanes in our experiments [13].

Table 13: The minimum inhibitory concentrations of sulfur-containing compounds against different microbial strains are tabulated. These are the results from different publications, in order to compare the activity of different compounds. The MIC value is provided in g/ml.

Microorganism	Allicin	DAS	DADS	DATS	DATTS	Reference
S. aureus	15	20	4	2	0.5	[13]
P. aeruginosa	15	88	72	36	16	
C. albicans	0.8	32	4	1	0.5	
A. niger	32	40	8	2	1	

3.4.1.6 Anti-bacteriophage assay

The anti-bacteriophage assay was performed using the classical agar overlay method [92]. The structure of T4 phage consists of a head part, a tail and a base plate. The head part of the phage contains the DNA. The base plate is attached to both long and short tail fibres. Using the long and short tail fibres the phage binds with the host cell organism and bacteria respectively. Followed by the binding, the phage transfers its genome into the host cell organism. [93]. The T-4 phage was bound with the strain of *E. coli* C-T4 in our experiments. Tetrasulfanes, **3-11,** have been screened at specific concentrations of 0.2 mM, 0.5 mM, 0.75 mM and 1.5 mM and with different dilutions of phage solutions (T4 phage in 0.96 % NaCl). DMSO has been used as a solvent control and the values of viability obtained for DMSO have been normalised to 100 % of viability.

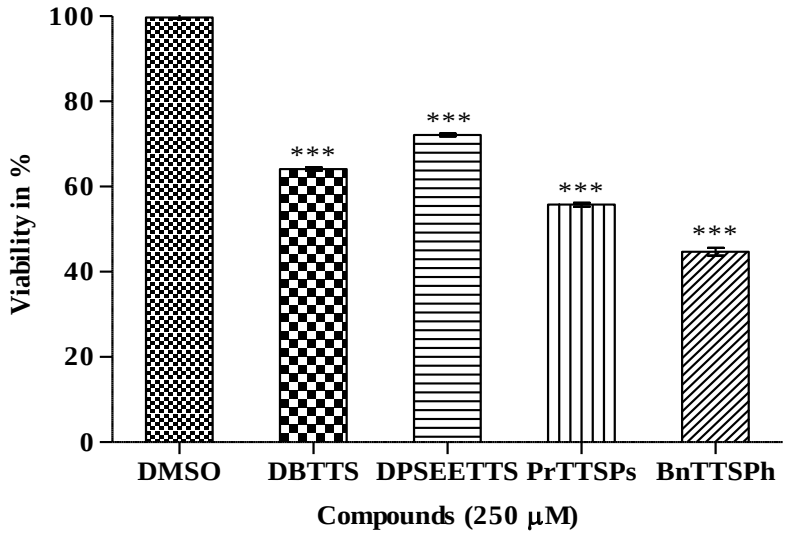


Figure 22: Activity against T-4 bacteriophage by DBTTS (8), DPSEETTS (5), PrTTSPs (9) and BnTTSPh (10) at 0.25 mM. ANOVA test: $p < 0.001$ (***) in relation to the relevant values for the particular control DMSO.

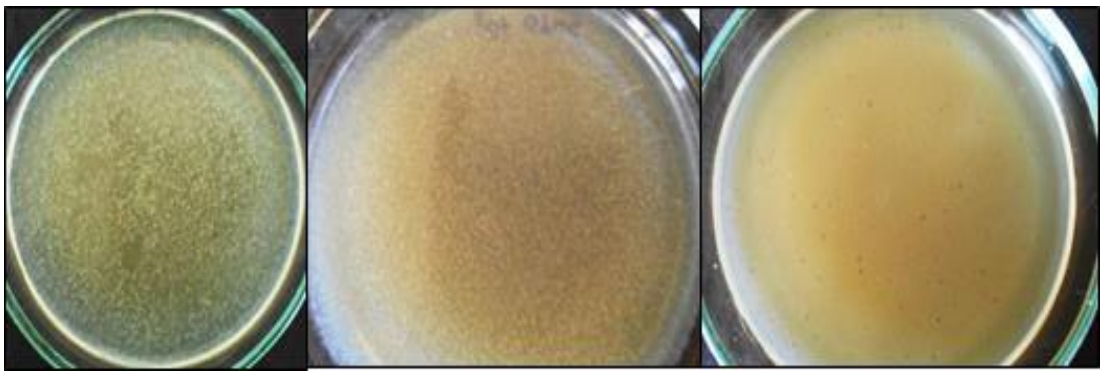


Figure 23: Classical agar overlay method used to measure the activity of different compounds against T-4 bacteriophage. From left to right: 0.96 % NaCl, DMSO and BnTTSPs (8) exhibiting anti-bacteriophage activity.

Surprisingly, all of the tetrasulfanes studied were highly active against T-4 phage at the concentrations employed. The ST and UT were equally active against T-4 bacteriophage. DBTTS (**8**), DPSEETTS (**5**), PrTTSPs (**9**) and BnTTSPh (**10**) were most active and showed a reduced viability percentage ranging from 60-80 % when used at a concentration of 0.25 mM (Figure 22).

Table 14: IC_{50} values of compounds DBTTS (8), DPSTTS (4), DPSEETTS (5), DEETTS (6), and PrTTSPs (9) with respect to T4- bacteriophage after 24 h of incubation.

Compounds	IC_{50} (mM)
DBTTS (**3**)	≤ 0.25
DPSTTS (**4**)	≤ 0.75
DPSEETTS (**5**)	≤ 0.30
DEETTS (**6**)	≤ 0.75
PrTTSPs (**9**)	≤ 0.25

Application of BnTTSPs (**8**), PhTTSPs (**11**) and DPhTTS (**7**) resulted in 100 % inhibition of growth of T-4 phage at a concentration of 0.25 mM of tetrasulfanes. Further concentration-dependence studies are now required for compounds **8**, **11** and **7** in order to determine their respective MIC values. Figure 23 shows the reduced number of bacteriophage when treated with BnTTSPs (**8**) in comparison to the number of bacteriophages in 0.96 % NaCl physiological solution and DMSO as solvent control. From these preliminary studies, the IC_{50} values of the tetrasulfanes were identified (Table 14). Further in-depth analyses must be conducted to reveal the mode of action and specific interactions of the compounds.

3.5 Activity in Primary Cell lines

3.5.1 Activity in human synovial fibroblast cell lines

Several natural and ancient therapies involve natural products that contain sulfur or sulfur-containing compounds. Inflammatory or allergic reactions, arthritis and other joint disorders, as well as control of tumour growth are some of the cases where these compounds have shown a considerable impact. Therefore, a comparative study of the known compound DBTTS (**3**), together with its structural analogues DPhTTS (**7**) and BnTTSPh (**10**) (synthesised as part of this thesis) on human synovial fibroblast cell lines (MH7A) has been performed. These experiments have been performed in the group of our co-operation partner Dr Burkhard Kloesch, at the Ludwig Boltzmann Institute for Rheumatology and Balneology, Vienna, Austria.

The apoptotic and anti-inflammatory properties have been studied with tetrasulfanes containing aromatic benzyl and phenyl side chains. The cytotoxic effects of these cell lines have been investigated by measuring the lactate dehydrogenase (LDH activity). The cell lines have been co-incubated with the compounds at different concentrations for 24 h and the increase in LDH activity in the supernatants of the cell culture was determined using a commercially available kit (Roche). DBTTS (**3**) considerably increased LDH activity, which confirms the high cytotoxic effect that interestingly disappeared when the compound has been co-incubated with GSH. BnTTSPh (**10**) had a reduced effect and DPhTTS (**7**) did not show any cytotoxic effect as well.

The pro-apoptotic impact of the compounds in these cell lines has been studied by Annexin-V/7-AAD. In comparison to other compounds, DBTTS (**3**) triggered an increase in apoptosis, when applied in concentrations of less than 2 µM. The increase in caspase 3/7 activity has been measured with a commercially available kit.

The inhibition of IL-1β induced secretion of IL-6 of the MH7A cell lines have been studied. The compounds have been incubated with MH7A cell lines at different concentrations. These studies were performed with the enzyme-linked immunosorbent assay (ELISA). DPhTTS (**7**)

and BnTTSPh (**10**) did not show significant inhibition of IL-6 secretion, whereas DBTTS (**3**) exhibited a dose-dependent inhibition of IL-6 secretion additionally changed the morphology of the cells.

The adhesiveness of the MH7A cell lines was studied in the presence of tetrasulfanes. The experiments were performed by trypsinising the cells at different time points and counting the cells using a cell analyser. The studies were confirmed by Crystal Violet staining techniques. All three compounds inhibited the adherence of the cells, but at different concentrations. DPhTTS (**7**) showed the inhibition of adherence only when employed at higher concentrations, such as 5 and 10 µM. DBTTS (**3**) and BnTTSPh (**10**), in contrast, inhibited the adherence of the cells in a concentration-dependent manner and DBTTS (**3**) in the most effective way at very low concentrations, starting at 0.5 µM.

These results are, however, preliminary. Further studies are ongoing to confirm these preliminary results and potential activity of tetrasulfanes in MH7A cell lines needs to be understood completely.

3.5.2 Activity in neuronal cell lines of rats

The propidium iodide-calcein AM (live and dead) assay has been performed to analyse the cytotoxic effect of the tetrasulfanes, in the neuronal 2A cell lines of rats. Dr Khairan Khairan performed the experiments in the group of Prof. Dr. med. Karl-Herbert Schaefer (at the University of Applied Sciences, Zweibruecken). A suitable control with water has been used and the results have been normalised to 100 %. Calcein AM and propidium iodide were chosen according to their nature of staining in cells. The former is highly lipophilic and permeable through the cell membrane and differentiates it with a green fluorescence, unlike the latter, which intercalates with the DNA of the nucleus to give red fluorescence. Thus, live and dead cells could be determined by this assay [94].

PrTTSPs (**9**), PhTTSPs (**11**), BnTTSPs (**8**) and BnTTSPh (**10**) did not show any considerable cytotoxic effects. DPhTTS (**7**) exhibited a significant activity by decreasing the viability of the cells by 28 % at a concentration of 100 µM.

4. Summary and Outlook

The aim of the present study was to synthesise and characterise novel unsymmetrical redox-modulating tetrasulfanes. The activity of these amphiphilic tetrasulfanes was investigated in various biological systems.

Novel amphiphilic unsymmetrical tetrasulfanes were the foremost focus and the compounds DPhTTS (**7**), BnTTSPs (**8**), PrTTSPs (**9**), BnTTSPh (**10**), PhTTSPs (**11),** PrTTSBn (**12**) and PrTTSPh (**13**) were synthesised with simple modifications to the method of Derbesy and Harpp. The characterisation of the compounds was performed by NMR spectroscopy and HRMS analysis. These compounds were studied in different assays, together with the symmetrical counterparts DBTTS (**3**), DPSTTS (**4**), DPSEETTS (**5**) and DEETTS (**6**), which were synthesised and purified by a colleague, Brigitte Czepukojc.

Physico-chemical and biological studies were performed and comparative correlations and the significance of symmetrical (ST) and unsymmetrical (UT) tetrasulfanes was elucidated. Redox activity of ST and UT was confirmed and found to be similar to the one of DATTS.

In order to analyse the amphiphilic nature of the tailor-made UT, surface tension analysis was performed with a ring and sita tensiometer, using the compounds BnTTSPs (**8**), PrTTSPs (**9**) and PhTTSPs (**11**). Remarkable results were obtained for PrTTSPs (**9**), at a CMC of 1.2 mM. Densitometry was performed with UT and the apparent molar volume was calculated using the change in density of the compounds with respect to the concentrations. This experiment was used as an alternative method to confirm the CMC of PrTTSPs (**9**).

The haemolysis assay was performed in order to study possible membrane/protein interactions. The haemolysis percentage was calculated for tetrasulfanes treated with RBCs and was compared to DPTTS (**2**). Here PrTTSPs (**9**) had a significantly higher activity than DPTTS (**2**). In order to investigate possible interactions with proteins circular dichroism measurements were performed with haemoglobin and FRET measurements with BSA. Overall BnTTSPs (**8**), PrTTSPs (**9**) and PhTTSPs (**11**) seemed to bind stronger with the proteins when compared to their analogues.

In the nematode assay, BnTTSPs and PrTTSPs (UT) and DPhTTS (ST) were effective. These compounds could be potential candidates for agricultural applications in future.

Detailed microbiological studies were performed with tetrasulfanes **3-11,** in eight different bacterial strains, four different yeast strains, and five different fungal spores and with the T-4 bacteriophage. ST were not widely active against these strains when compared to UT. BnTTSPs (**8**), PrTTSPs (**9**) and PhTTSPs (**11**) were active against strains such as *S. aureus*, *M. luteus* and *B. subtilis.* In contrast, both ST and UT were inactive against Gram-negative bacterial strains (*E. coli* and *S. typhimurium).* This implies that the mode of action of these compounds might be related to differences in the cell wall structures of the Gram-positive and Gram-negative bacteria. Similar results were obtained in the case of yeasts. DPhTTS (**7**) and DEETTS (**6**) from ST were active. DPhTTS (**7**) had an outstanding activity against *C. guilliermondii*, whereas DEETTS had greater effect against *S. cerevisiae* and *D. hansenii*. BnTTSPs (**8**), PrTTSPs (**9**) and PhTTSPs (**11**) (UT) however, showed significant activities against yeasts. The activities of UT are in good agreement with the results in bacteria. BnTTSPh (**10**), however, did not show any effective activity against bacteria or yeast strains. This, in turn, underlines the structural importance of the propionic acid side chain and possibly benefits of an amphiphilic nature.

The activity of ST and UT was tested against filamentous fungal spores like *Trichoderma viride, Aspergillus flavus, Geotrichum candidum, Penicillium aeruginosa and Mucor plumbeus*. DPSEETTS (**5**) and DEETTS (**6**) exhibited only fungistatic activity against *T. viride*. Apart from these effects, ST (**3**-**6**) did not show any specific activity against filamentous fungi. BnTTSPs (**8**), PrTTSPs (**9**) and PhTTSPs (**11**), however, were able to completely inhibit the growth of all five types of spores. The compounds were also tested against the specific wild type and multi-drug resistant fungal strains of *Botrytis cinerea*. DPhTTS (**7**), BnTTSPs (**8**) and PrTTSPs (**9**) achieved MIC values higher than the one of DATTS (**1**). PrTTSPs (**9**) displayed an apoptotic effect against these fungal spores. Activity against the T-4 bacteriophage shows that UT can be extremely harmful in very low concentrations and ST were also active. This is in accordance with the results obtained against bacteria and fungi. Antimicrobial activities reveal the diverse actions of these compounds and their mode of action is indeed an interesting topic for future study.

Overall, BnTTSPs (**8**), PrTTSPs (**9**) and PhTTSPs (**11**) were found to be positive in various experiments. Among these compounds, PrTTSPs (**9**) is generally the best. This compound is amphiphilic, has a CMC (hence forms micelles) and seems to interact strongly with biological molecules *in vitro*. PrTTSPs (**9**) stood out in all the assays performed in this present study.

Further analyses need to be performed to study the structure-activity relationships of both ST and UT. Attempts are currently underway to study the biochemical pathways and dose-dependent absorption of such polysulfane compounds *in vivo* and *in vitro*. In the future, it would also be interesting to study the impact of these compounds in tumour-encoding genes.

PART II

From sulfur to selenium and tellurium containing compounds

1. Introduction

The compound PrTTSPs (**9**) turned out to be amphiphilic and exhibited interesting redox properties. This compound also showed considerable biological activities among all the tetrasulfanes tested, in the first part of this thesis. The results obtained for compound PrTTSPs (**9**), therefore, provided some hints about the possible physico-chemical properties and biological activities and stimulated our interest in more potent amphiphilic redox modulators.

The most obvious idea was therefore to move down in Group 16 of the Periodic Table from sulfur to selenium and on to tellurium [95] [96]. According to our previous studies, Se- and Te-containing compounds should exhibit enhanced biological activity and also more stability [97] [98] [99]. The amphiphilicity of the compounds could possibly enable them to cross the cell membranes easily and to interact with cellular components even stronger than PrTTSPs (**9**) [48] [100]. The Se- and Te-containing compounds should also strongly interact with membrane proteins.

Remarkably, only a few redox modulating amphiphilic agents have been reported so far [101]. Extensive biological studies with such amphiphilic agents are also not found in the literature. Therefore, in the group of Prof. Jacob, a series of amphiphilic Se- and Te-containing compounds with an anionic head group and hydrocarbon tails of different tail lengths was synthesised. A PhD student of the group at the time, Peng Du., performed the synthesis and purification of the compounds. The Se- and Te-containing compounds have been found to be fairly soluble in water, more stable than most polysulfanes, odourless and easy to handle. These compounds are also expected to show a selective biological activity against different cancer cell lines [75] [76].

2 Objectives

In the second part of this thesis, studies have therefore been performed with novel amphiphilic selenium- and tellurium-containing compounds. The physico-chemical analyses have been carried out as described previously for the polysulfanes, including ring tensiometry and sita tensiometry. A study of the non-covalent interactions of the compounds with the protein BSA has been performed using FRET measurements.

Subsequently, the novel Se- and Te-containing compounds have been tested in various biological systems. The activity of these compounds against different types of yeast has been studied. An investigation of the activities of the compounds against various strains of bacteria has also been performed. Figure 24 provides an overview of the structure of Se- and Te-containing compounds **15-27** which have been employed as a part of this study and have been synthesised and purified by Peng Du.

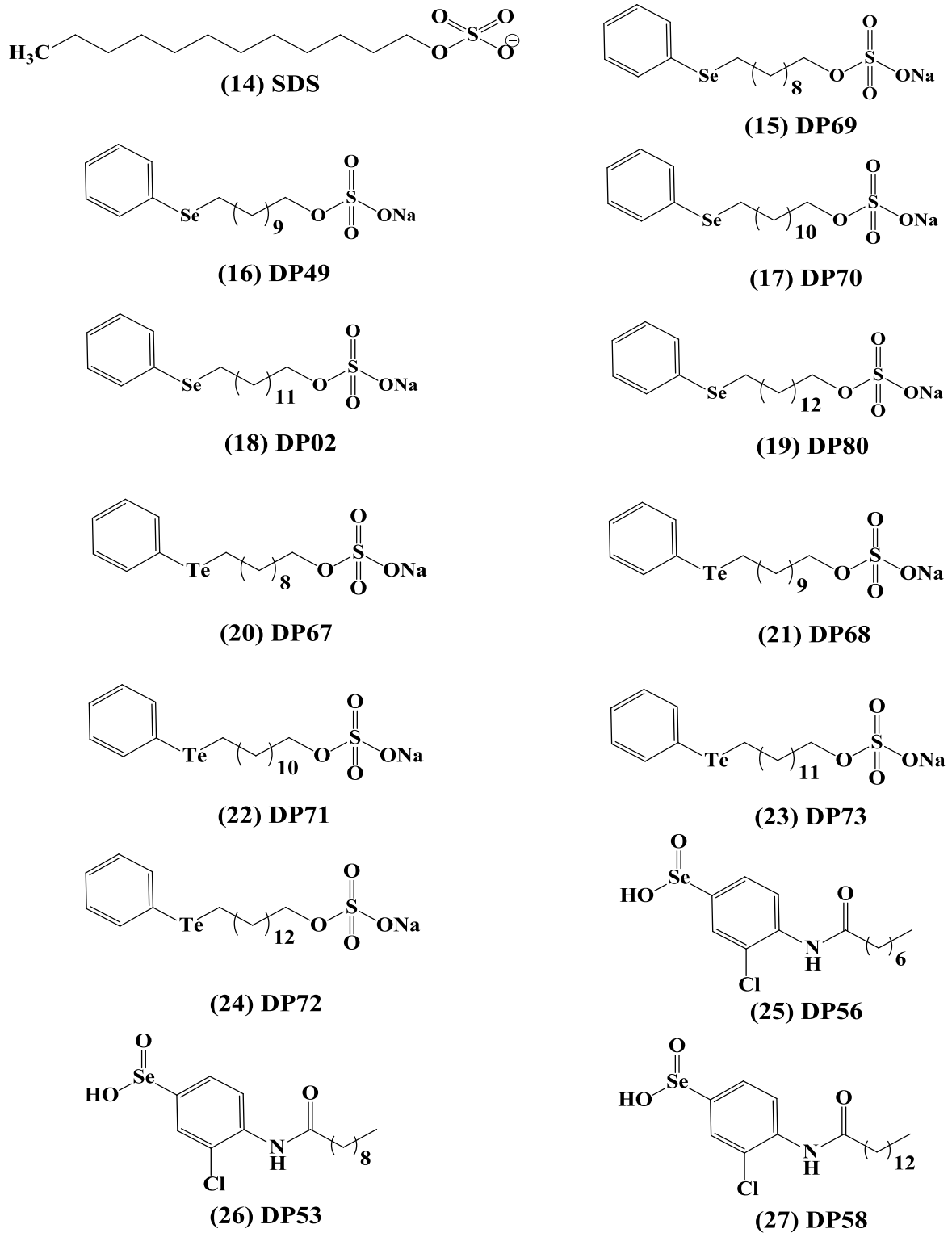


Figure 24: Overview of novel selenium and tellurium compounds synthesised and purified by Peng Du; these were analysed in various systems. The chemical names of compounds are mentioned in page 124.

3. Results and Discussion of Se- and Te-containing compounds

3.1 Physico- Chemical Properties

3.1.1 Ring and Sita Tensiometry

Ring tensiometric and sita tensiometric experiments have been used to calculate the CMC of the Se- and Te-containing compounds [72] [73] [74]. The experiments were performed with compounds **14-24**. In essence, the results illustrated in Figure 25 and table 15 confirm the amphiphilic properties of the compounds tested. These compounds exhibit a CMC in water in the millimolar range. The CMC of the compounds generally decreases with the increasing hydrocarbon chain length. SDS (**14**) was used as a control and showed a CMC of 0.9 mM. DP71 (**22**) exhibited the same CMC as the one of SDS (**14**). DP80 (**19**), DP73 (**23**) and DP72 (**24**), however, showed the lowest CMC values of 0.7 mM, 0.5 mM and 0.3 mM, respectively (Table 15). These results prove that the novel Se- and Te-containing compounds are surface-active amphiphilic molecules as may have been expected from their molecular structure.

Table 15: Overview of CMC values of selenium- and tellurium-containing compounds. As measured by ring tensiometry, 'n' represents the carbon chain length.

Compound	Element present	n	CMC (mM)
SDS (**14**)	-	-	0.9
DP69 (**15**)	Se	8	2.6
DP 49 (**16**)	Se	9	2.5
DP 70 (**17**)	Se	10	1.2
DP 02 (**18**)	Se	11	1.1
DP 80 (**19**)	Se	12	0.7
DP 67 (**20**)	Te	8	5.0
DP 68 (**21**)	Te	9	4.8
DP 71 (**22**)	Te	10	0.9
DP 73 (**23**)	Te	11	0.5
DP 72 (**24**)	Te	12	0.3

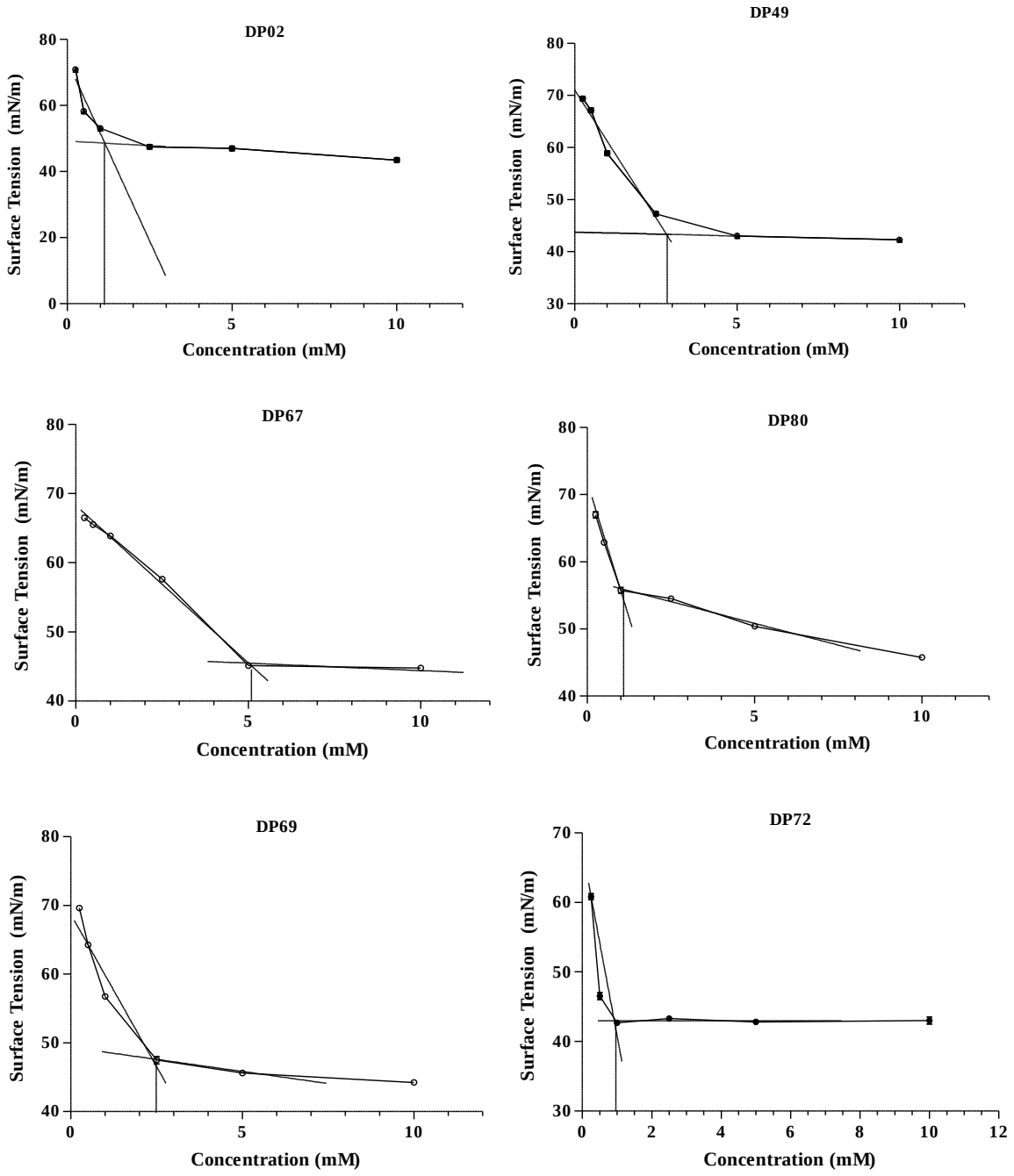


Figure 25: Concentration-dependent changes in surface tension for compounds of DP02 (18), DP49 (16), DP67 (20), DP80 (19), DP69 (15), DP72 (24), in distilled water as measured by ring tensiometry. Each measurement was repeated three times and one representative measurement is shown for each compound.

Table 16: Comparison of CMC values calculated by Sita tensiometric (bubble pressure method) and ring tensiometric method for the compounds with the shortest and longest, hydrocarbon tails, respectively.

Compound	CMC (mM) from Sita tensiometer	CMC (mM) from Ring tensiometer
DP80 (**19**)	0.4	0.7
DP67 (**20**)	0.5	5.0
DP72 (**24**)	0.5	0.3

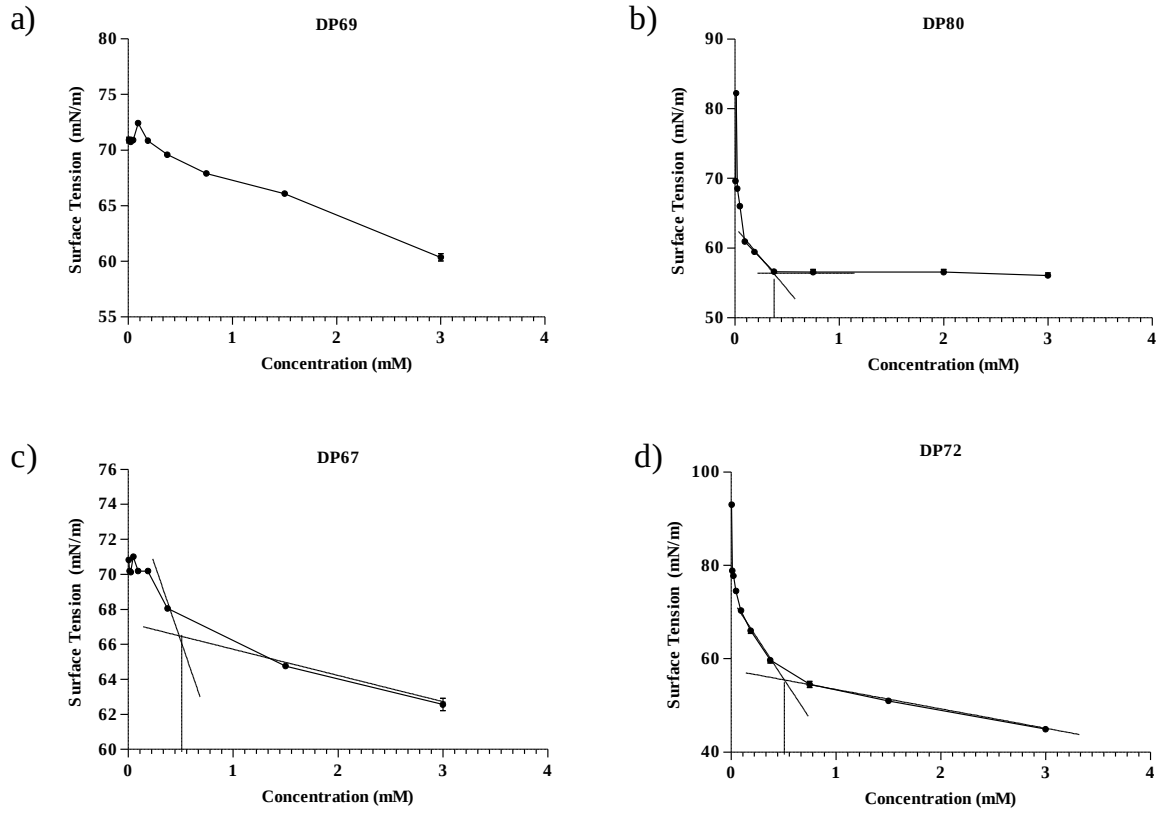


Figure 26: Concentration-dependent changes in surface tension for compounds as measured by the bubble pressure method. a) DP69 (15) b) DP80 (19) c) DP67 (20) d) DP72 (24) in distilled water with 1 % DMSO. Each measurement was repeated three times and one representative measurement is shown for each compound.

A comparison of the CMC values obtained by both ring tensiometric and sita tensiometric experiments (Figure 26) is shown in Table 16. The difference in the values of CMC obtained is probably due to the different solvent systems used in both the experiments.

3.2 Non-Covalent Membrane Interaction Studies

3.2.1 FRET Measurements

Quenching of the fluorescence of BSA [80] [82] [83] was performed with the compounds SDS (14), DP69 (15), DP80 (19), DP67 (20), DP70 (24), DP56 (25) and DP53 (26). SDS (14) was used as a control and showed a Stern-Volmer constant of 2.20 .104 l/mol (Table 17). The Se- and Te-containing compounds analysed point towards a certain correlation between the hydrocarbon chain length of the compounds and their intensity of quenching. The compounds with the shortest carbon chain lengths showed the lowest Stern-Volmer constants, whereas the longest carbon chain lengths compound resulted in the highest values. Te-containing compound DP72 (24), with the largest carbon chain length (12), showed the maximum Stern Volmer value of 56.76 .104 l/mol. Thus, the longest carbon-chain containing compounds have the maximum quenching of fluorescence of BSA (Figure 27). This is not entirely unexpected as a longer hydrocarbon chain also implies a stronger hydrophobic interaction with-parts of-the protein.

Table 17: Stern Volmer constants calculated from the quenching effect of bovine serum albumin fluorescence with various selenium- and tellurium-containing compounds.

Compounds	n	K_{sv} .10^4 (l/mol)
SDS (**14**)	-	2.20
DP69 (**15**)	8	6.99
DP80 (**19**)	12	28.70
DP67 (**20**)	8	2.10
DP72 (**24**)	12	56.76
DP56 (**25**)	8	1.90
DP53 (**26**)	10	4.53

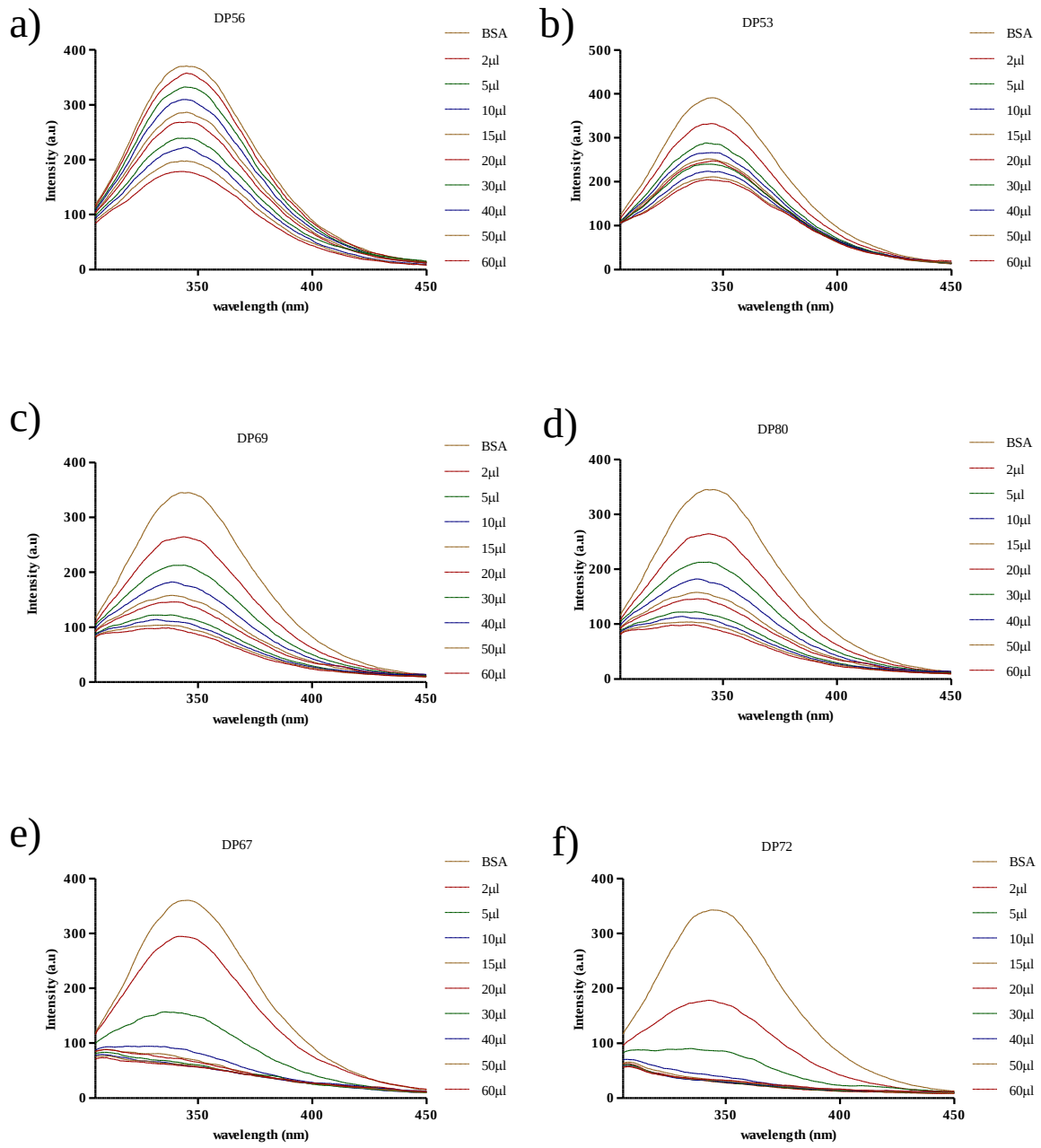


Figure 27: Quenching of bovine serum albumin fluorescence in the presence of different concentrations of a) DP56 (25), b) DP53 (26), c) DP69 (15), d) DP80 (19), e) DP67 (20) and f) DP72 (24). Spectra were obtained using UV-light with an excitation wavelength of 295 nm.

3.3 Biological activities of the selenium and tellurium compounds

3.3.1 Toxicity against yeast strains

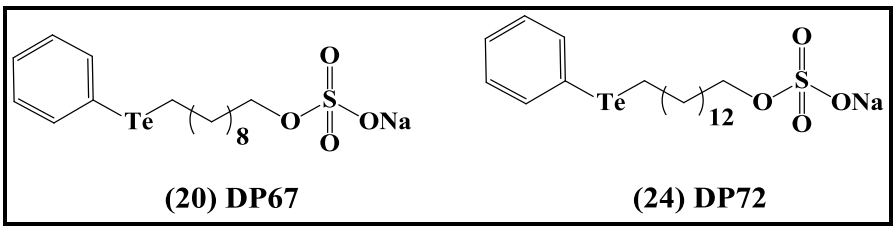
As already mentioned a change from sulfur to selenium and especially also to tellurium is expected to increase the biological-cytotoxic-activity considerably. In order to test this hypothesis, the rather simple, fast and straight forward yeast assay has been performed first.

The Se- and Te-containing compounds were tested against *D. hansenii, S. cerevisiae, C. guilliermondii* and *C. albicans*. A Pre-screening of compounds **14**, **15**, **19**, **20** and **24**-**27** at a concentration of 3 mM was carried out against the different strains of yeast. Furthermore, concentration-dependence studies were also performed and the relevant EC_{50} value was calculated. The results of the pre-screening experiments reveal that only the Te-containing compounds DP67 (**20**) and DP72 (**24**) were active against *D. hansenii*. Both the Se- and Te-containing compounds, however, were active against *S. cerevisiae, C. guilliermondii* and *C. albicans* 174.

Remarkably, DP72 (**24**) exhibited an inhibitory zone diameter of 21 ± 1 mm against *S. cerevisiae* when compared to SDS (**14**), which showed a diameter of just 11.25 ± 0 mm (Table 18). DP72 (**24**) also exhibited greater activity against the strain of *C. albicans* than SDS. Unfortunately, DMSO also showed a minimum inhibition against this strain. The inhibition caused by this particular solvent was however, only a static effect. DP72 (**24**) was the most active compound against the various strains of yeast when compared to its short tail analogue DP67 (**20**).

Table 18: Pre-screening of compounds 14, 15, 19, 20 and 24-27 at a concentration of 3 mM against four different strains of yeast, namely *D. hansenii, S. cerevisiae, C. guilliermondii and C. albicans* 174. A diameter of 12 mm is considered as the minimum inhibitory zone. DMSO (100 %) has been used as a control. The values represent the mean ± SEM of three measurements.

Compounds	*D. hansenii*	*S. cerevisiae*	*C. guilliermondii*	*C. albicans* 174
SDS (**14**)	16.3 ± 0	11.3 ± 0	14.5 ± 1	14.5 ± 1
DP69 (**15**)	-	12.0 ± 0	12.5 ± 1	14.5 ± 2
DP80 (**19**)	-	11.5 ± 1	15.5 ± 1	14.5 ± 2
DP67 (**20**)	25.0 ± 7	12.0 ± 0	15.0 ± 1	15.5 ± 2
DP72 (**24**)	14.5 ± 2	21.0 ± 1	15.5 ± 1	17.0 ± 3
DP56 (**25**)	-	12.5 ± 2	12.0 ± 1	13.5 ± 2
DP53 (**26**)	-	-	-	14.0 ± 3
DP58 (**27**)	-	-	16.0 ± 1	11.5 ± 1



(20) DP67 **(24) DP72**

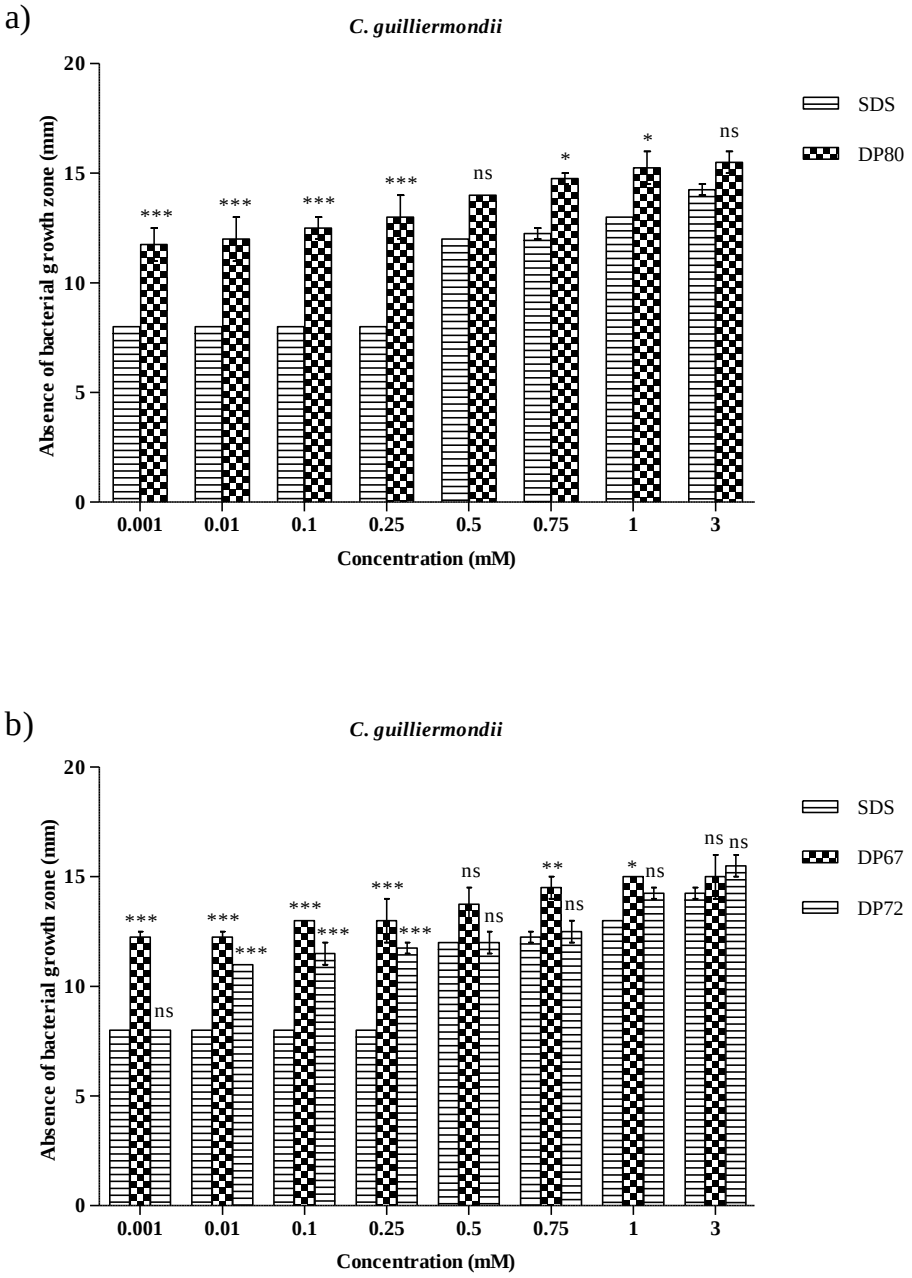


Figure 28: Activity of a) DP80 (19), b) DP67 (20) and DP72 (24) against *C. guilliermondii*. SDS (14) has been used as a control. Data presented as absence of the bacterial growth zone in mm ± SEM. ANOVA test: n.s. = $p \geq 0.05$, $p < 0.05$ (*), $p < 0.01$ (**) or $p < 0.001$ (***) in relation to the relevant values for the particular control SDS.

Compounds DP80 (**19**), DP67 (**20**) and DP72 (**24**) exhibited significantly higher activity against *C. guilliermondii;* hence, concentration-dependence studies of these compounds were performed and compared with the results obtained for SDS (**14**) (which also serve as 'archive' for the statistical evaluation). DP80 (**19**) and DP67 (**20**) showed an inhibitory effect at very low concentrations (1 µM) (Figure 28). Surprisingly, DP72 (**24**), exhibited a minimum inhibitory effect at a concentration of 10 µM against *C. guilliermondii*. SDS (**14**) exhibited a minimum inhibition against this strain only at a concentration of 500 µM. This confirms once more that the presence of tellurium, in addition to amphiphilicity, is important for biological activity.

As evident from the pre-screening experiments, all the compounds including SDS (**14**) showed an inhibitory effect against *S. cerevisiae*. Interestingly, DP72 (**24**) was the most active compound against this strain. All of the compounds exhibited an inhibitory activity against *C. albicans*. Yet again, DP72 (**24**) was the most active compound against *C. albicans* and the EC_{50} of growth inhibition was calculated as just 33.17 µM (Figure 29). DP72 (**24**) was clearly the best compound employed in this assay, that exhibited an inhibitory effect against all the strains of yeast studied. Various compounds and the EC_{50} of growth inhibition against different strains of yeast are summarised in Table 19.

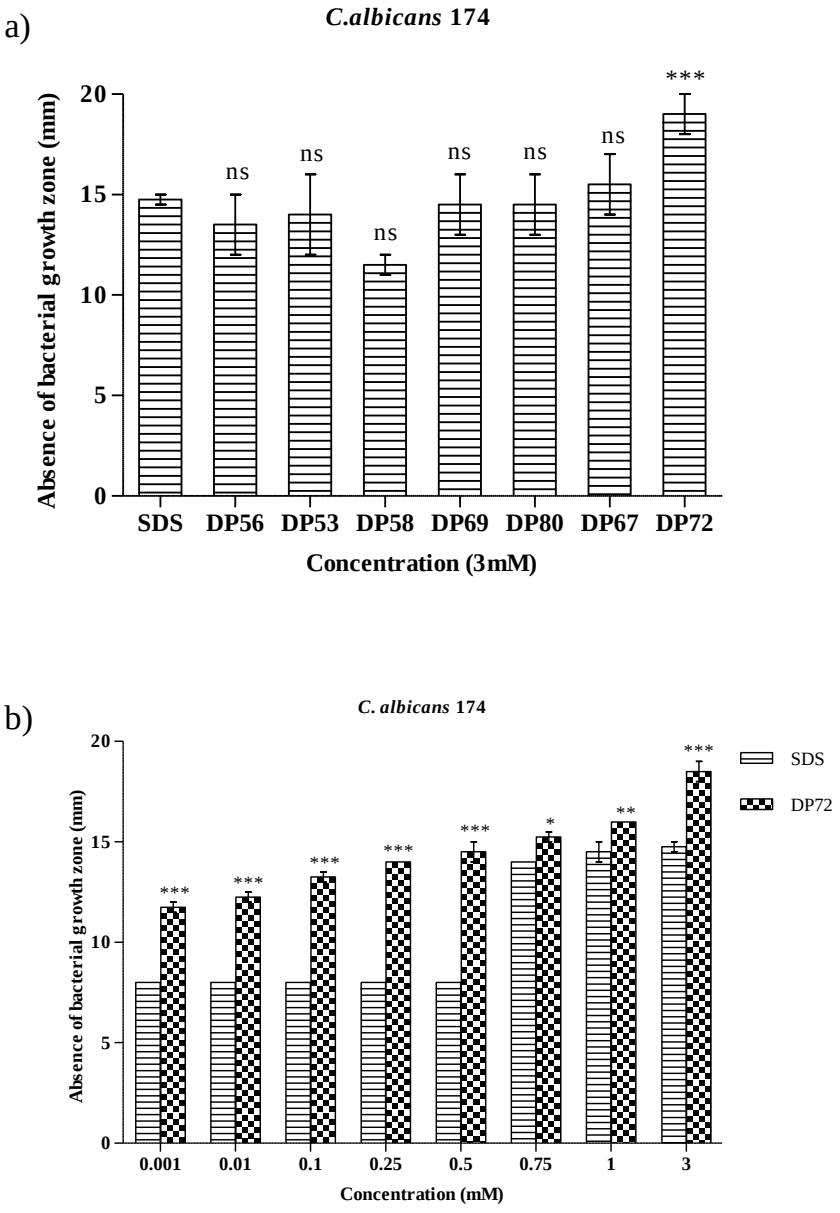


Figure 29: a) Pre-screening of DP56 (25), DP53 (26), DP58 (27), DP69 (15), DP80 (19), DP67 (20), DP72 (24) and SDS (14) against *C. albicans* 174 at a concentration of 3 mM, b) Activity of DP72 (24) against *C. albicans* 174. SDS (14) has been used as a control. Data presented as absence of the bacterial growth zone in mm ± SEM. ANOVA test: n.s. = $p \geq 0.05$, $p < 0.05$ (*), $p < 0.01$ (**) or $p < 0.001$ (***) in relation to the relevant values for the particular control SDS

Table 19: Overview of the Se- and Te-containing compounds tested against corresponding yeast strains and their respective EC_{50} values indicative of inhibition of growth.

Compounds	*D. hansenii*	*C. albicans174*	*C. guilliermondii*	*S. cerevisiae*
SDS (**14**)	230.0	671.9	501.8	n.a
DP80 (**19**)	n.a	n.a	n.a	24.5
DP 67 (**20**)	n.a	n.a	10.8	n.a
DP72 (**24**)	n.a	33.2	125.9	27.6

3.3.2 Antibacterial Assay

A pre-screening of compounds **14**, **15**, **19 20** and **24-27** was performed against eight different strains of bacteria at a compound concentration of 3 mM. All of the compounds were dissolved in DMSO, as some of them showed a poor solubility in water.

Table 20: The pre-screening of compounds 14, 15, 19, 20 and 24-27 at a concentration of 3 mM against six different bacterial strains: *S. aureus, E. coli, M. luteus, B. subtilis, B. licheniformis,* and *S. roseus.* A 12 mm of diameter is considered as the minimum inhibitory zone. DMSO (100 %) has been used as a control. The values represent the mean ± SEM of three independent measurements.

Comp.	*S. aureus*	*E. coli*	*M. luteus*	*B. subtilis*	*B. licheniformis*	*S. roseus*
DP56 (25)	22.5 ± 2	-	20.8 ± 0	22.5 ± 2	26.0 ± 0	23.0 ± 1
DP53 (26)	20.0 ± 1	-	20.5 ± 1	14.3 ± 1	13.0 ± 1	19.5 ± 2
DP58 (27)	-	-	16.5 ± 2	14.5 ± 2	15.5 ± 2	18.5 ± 4
DP69 (15)	-	-	-	-	-	-
DP80 (19)	17.5 ± 1	-	20.5 ± 2	13.5 ± 1	13.3 ± 0	23.0 ± 1
DP67 (20)	25.0 ± 1	20.5 ± 1	20.8 ± 1	16.3 ± 0	13.0 ± 1	30.5 ± 2
DP72 (24)	22.5 ± 1	19.5 ± 1	20.5 ± 1	18.0 ± 3	14.0 ± 0	31.3 ± 1
SDS (14)	22.5 ± 1	12.5 ± 1	28.5 ± 1	20.0 ± 1	17.3 ± 1	20.5 ± 2

The Se- and Te-containing compounds showed an activity against most of the strains tested, except *P. aeruginosa*. DP56 (**25**) and DP53 (**26**) exhibited inhibitory zone diameters of 22.5 ± 2 mm and 20 ± 1 mm, respectively, against *S. aureus* (Table 20). DP58 (**27**), with the longest hydrocarbon chain, when compared to DP56 (**25**), did not show any major toxicity effect against *S. aureus*. DP58 (**27**), however, exhibited a minimum inhibitory activity against the strains such as *M. luteus*, *B. subtilis*, *B. licheniformis* and *S. roseus.* DP80 (**19**), with the longest hydrocarbon chain length showed significant activity against more than five different strains when compared to its 'short tail analogue' DP69 (**15**).

The Te-containing compounds DP67 (**20**) and DP72 (**24**) exhibited the best results and showed activity against both Gram-positive (*S. aureus*) and Gram-negative bacteria (*E. coli* and *S. typhimurium*) (Figure 30). DP67 (**20**), DP72 (**24**) and SDS (**14**) exhibited a respective diameter of the inhibitory zone of 14 ± 1 mm, 13 ± 0 mm and 12.5 ± 1 mm against *S. typhimurium*. None of the other compounds in our studies exhibited an activity against this bacterium. The concentration-dependence studies of the active compounds were performed against *S. aureus* and *E. coli*. The EC_{50} values of growth inhibition for the various compounds were calculated and are tabularised in Table 21.

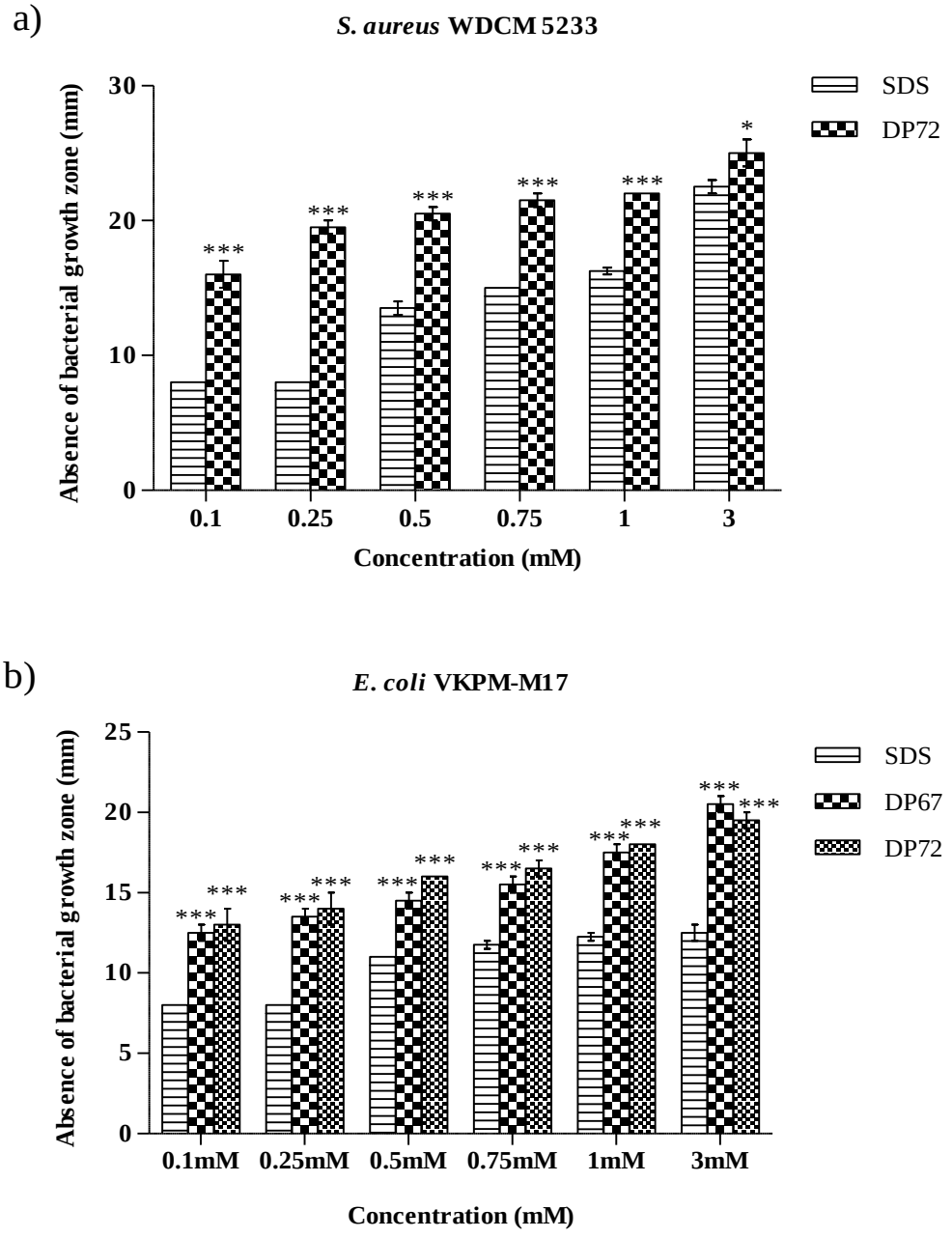
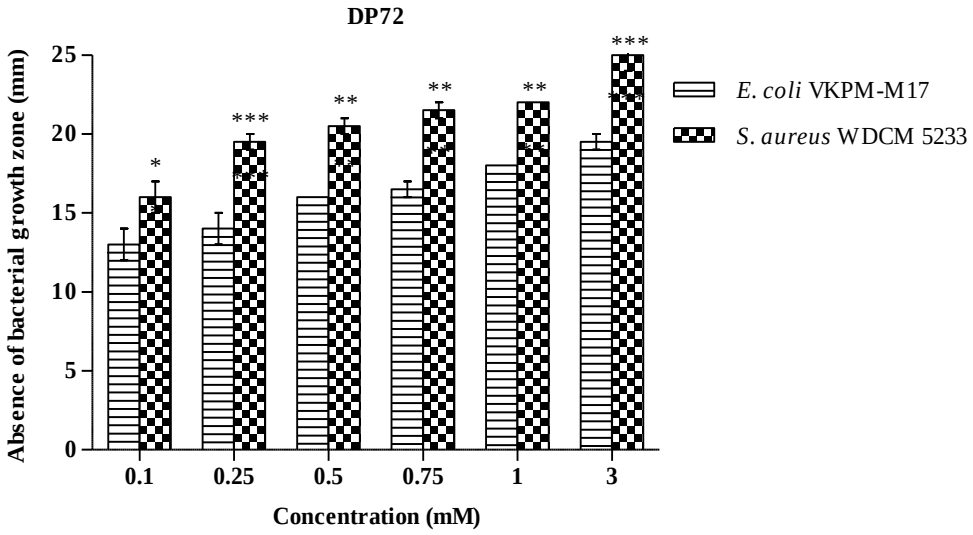


Figure 30: Activity of a) DP72 (24) against *S. aureus* and b) DP67 (20) and DP72 (24) against *E. coli*. SDS (14) has been used as a control. Data presented as absence of the bacterial growth zone in mm ± SEM. ANOVA test: $p < 0.05$ (*) or $p < 0.001$ (***) in relation to the relevant values for the particular control SDS.

a)



b)

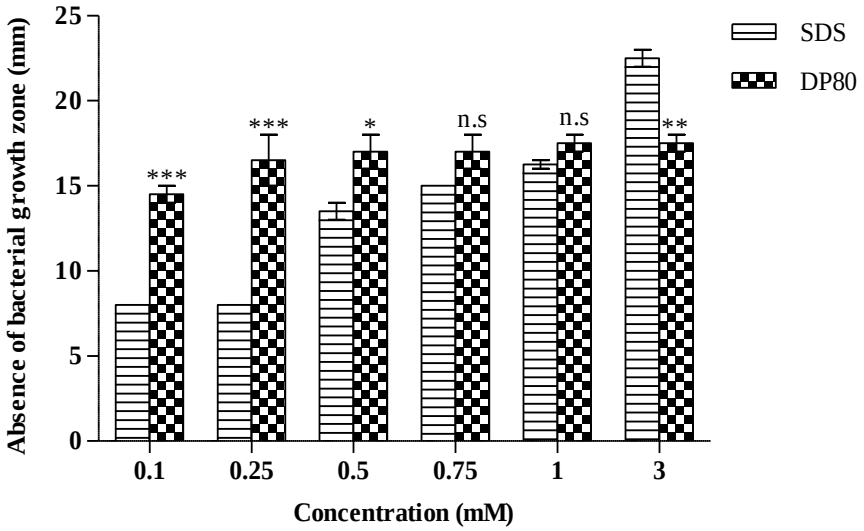


Figure 31: a) Comparative study of the activity of DP72 (24) against *S. aureus* and *E. coli* and b) DP80 (19) against *S. aureus*. The absence of growth zone is represented as mm ± SEM. SDS (14) has been used as control. ANOVA test: n.s. = $p \geq 0.05$, $p < 0.05$ (*), $p < 0.01$ (**) or $p < 0.001$ (***) in relation to the relevant values for the particular control SDS

The study concentration-dependence of activity for compound DP72 (**24**) was performed against *S. aureus* and *E. coli*. The results of all of the concentration dependence studies were compared with the one for SDS (**14**). DP72 (**24**) exhibited the most significant activity against *S. aureus* at a concentration of 100 µM, when compared with that of SDS (**14**) (Figure 30). The EC_{50} values of growth inhibition of DP72 (**24**) and SDS (**14**) against *S. aureus* were calculated as 116.2 µM and 772.9 µM, respectively (Table 21).

Only the Te-containing compounds DP67 (**20**) and DP72 **(24**) exhibited activity against the Gram-negative bacterium, *E. coli.* (Figure 30). Both of the compounds exhibited a significant activity at a concentration of 100 µM when compared to SDS (**14**). DP72 (**24**) exhibited a more significant activity against *S. aureus* when compared to its activity against *E. coli* (Figure 31). The Se- and Te-containing compounds tested against different bacterial strains, their respective EC_{50} values of growth inhibition and IC_{50} values of recovery of viability is summarised in Table 21.

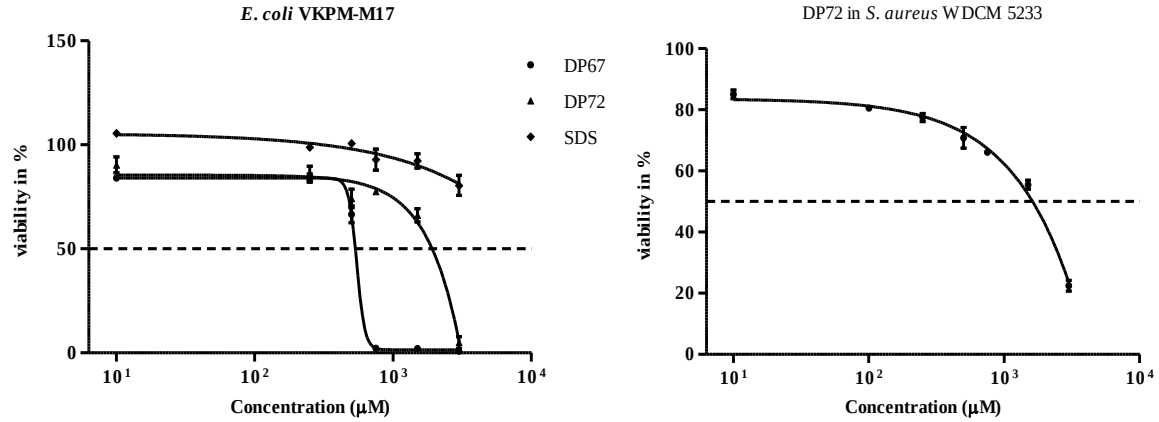


Figure 32: The recovery of viability curve of bacterial strains after 24 h of treatment with various compounds. Figure a) Activity of DP67 (20) and DP72 (24) against *E. coli*. Figure b) DP72 (24) against *S. aureus*. Data presented as viability percentage ± SEM.

After the treatment of bacteria with the Se- and Te-containing compounds and the formation of growth inhibitory zones, a small portion of the growth media from the inhibitory zone was incubated in 5 ml of LB broth. This broth was incubated for 24 hours in a temperature-controlled shaker. The recovery of growth of the bacteria was subsequently evaluated (Figure 32).

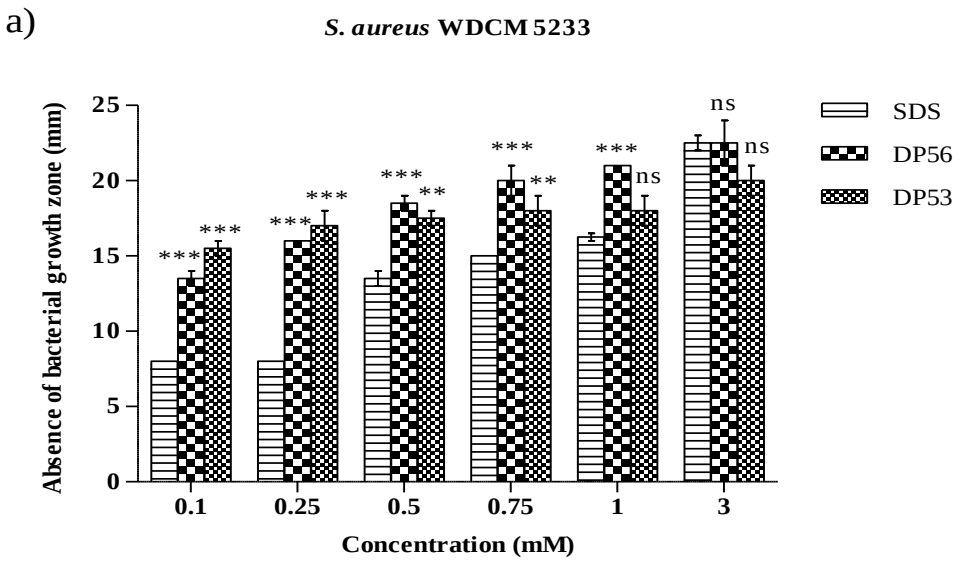


Figure 33: a) The activity of compounds DP56 (25) and DP53 (26) against *S. aureus*. SDS (14) has been used as a control. The absence of the bacterial growth zone is represented in mm ± SEM. ANOVA test: n.s. = $p \geq 0.05$, $p < 0.01$ (**) or $p < 0.001$ (***) in relation to the relevant values for the particular control SDS.

Figure 32 shows the recovery of viability curve for *E. coli* in the case of DP67 (**20**) and DP72 (**24**) and *S. aureus* in the case of DP72 (**24**). Subsequently, the IC_{50} of the recovery of growth of the strains of bacteria was calculated. DP56 (**25**) and DP53 (**26**) exhibited a significant activity against *S. aureus* at a concentration of 100 µM when compared to the activity of SDS (**14**) (Figure 33). Unlike the Te-containing compounds, DP56 (**25**) and DP53 (**26**) did not show the typical structure-activity relationship against *S. aureus*. For instance, DP56 (**25**), with a shorter hydrocarbon chain length, exhibited a higher activity than DP53 (**26**). Compounds DP56 (**25**) and DP53 (**26**) exhibited an IC_{50} of recovery of viability as 787.3 µM and 334.3 µM respectively, against *S. aureus*. The recovery of viability of *S. aureus* in the presence of SDS was unable to be calculated due to the effective growth of this bacterium even at very low concentrations starting from 10 µM.

Overall, Te-containing compounds DP 67 (**20**) and its 'long-tail analogue', DP72 (**24**) were the most active compounds tested against the bacteria. The Se-containing compound, DP53 (**26**) was also highly active against *S. aureus*.

Table 21: Se- and Te-containing compounds tested against various strains of bacteria. EC_{50} values of inhibition and IC_{50} value of recovery of viability are summarised.

Compounds	Bacterial Strains	EC_{50} of Inhibition (µM)	IC_{50} of Viability (µM)
SDS (**14**)	*S. aureus*	772.9	n.a
	E. coli	773.1	n.a
DP80 (**19**)	*S. aureus*	39.6	n.a
DP67 (**20**)	*E. coli*	336.7	546.4
DP72 (**24**)	*E. coli*	174.8	ambiguous
	S. aureus	116.2	ambiguous
DP56 (**25**)	*S. aureus*	172.5	787.3
DP53 (**26**)	*S. aureus*	83.3	334.3

A commercially available antibiotic, sulfadimethoxin, was also tested against *S. aureus*, *B. subtilis*, *E. coli* and *S. typhimurium* as a particular control. This antibiotic exhibits a growth inhibitory zone of 20 mm, on an average, against *S. aureus* and *E. coli*, but only at a concentration of 100 mM, which is extremely high when compared to the concentrations considered for the Se- and Te-containing compounds. Sulfadimethoxin also shows a static effect against *B. subtilis*.

4. Summary and Outlook

In the second part of this thesis, more sophisticated compounds have been derived based on the idea of amphiphilicity and chalcogen (bio-) chemistry. Se- and Te-containing amphiphilic compounds were produced and subsequently analysed physico-chemically and biologically. The surface tension analysis was performed by ring tensiometric and sita tensiometric experiments. The CMC value decreases as the length of the hydrocarbon chain increases in the compounds. FRET experiments were performed to study the non-covalent interactions of the compounds with BSA. The quenching of the fluorescence of BSA was studied and the Stern-Volmer constants were calculated. As may be expected the value of the Stern-Volmer constants increases with the increase in the hydrocarbon chain length in the compounds.

The anti-microbial activities of the Se- and Te-containing compounds were studied. The compounds were tested against four different strains of yeast and eight different strains of bacteria. All the compounds tested exhibited activity against strains such as *S. aureus, M. luteus, B. subtilis, B. licheniformis* and *S. roseus,* except for DP69 (**15**). DP67 (**20**) and DP72 (**24**) exhibited activity against both Gram-positive and Gram-negative bacteria. These compounds were even more active than SDS. The compounds containing the longest hydrocarbon chains exhibited lower EC_{50} values of growth inhibition. Similar results were obtained for the Se- and Te-containing compounds against different strains of yeast. Se-containing compounds exhibited an effective inhibitory activity against *C. guilliermondii* and the semi-pathogenic strain *C. albicans*. Undoubtedly, the Te-containing compounds, DP67 (**20**) and DP72 (**24**), were the most active ones against all the four strains of yeast. DP72 (**24**) also showed a complete inhibitory activity against *C. albicans.*

As expected, the idea to move from the tetrasulfane PrTTSPs (**9**) to other amphiphilic agents, but this time with the more stable yet also more reactive Se- and Te-containing compounds has worked rather well. Se- and Te-containing compounds were highly active in all our biological assays when compared to the activity of PrTTSPs (**9**). DP67 (**20**) and DP72 (**24**) should be considered in the future for potential applications as fungicides or bactericides.

5. Experimental Part

5.1 Materials and Methods

All of the chemical reagents used for the synthesis of tetrasulfanes were purchased from Sigma-Aldrich, including thionylchloride, pyridine, thiophenol, mercaptobenzene, thiopropionic acid and used without further purification unless stated. All of the reactions were carried out under an Argon (4.6) atmosphere, at room temperature and with deionised MilliQ water (resistance ≥18 MP.cm). The completion of the reactions were monitored by thin layer chromatography technique with Merck aluminium-backed plates (Kieselgel 60 F254, Darmstadt, Germany) and visualised under ultraviolet light. Column Chromatography technique was used to purify the compounds with Silica gel 60 (40-63 Lm, Fluka) and under mild nitrogen pressure.

5.2 NMR Spectroscopy

All NMR spectra were recorded on Bruker (Rheinstetten) type Avance 500, at the Institute of Pharmaceutical Chemistry. ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively. Chemical shifts recorded in $CDCl_3$ solvent were expressed in δ (ppm) relative to the signal at 7.26 ppm for ^{1}H NMR and at 77.0 ppm for ^{13}C NMR.

5.3 Mass spectroscopy

Spectra were recorded in QTRAPR LC/MS/MS System by AB Sciex Del Optiplex 960. HPLC grade methanol was used as the solvent.

5.4 Calculation of clog*P*

The calculation of the clog*P* was performed using the ALOGPS 2.1 Software and ChemBioDraw Ultra 12.0.

5.5 Synthesis of unsymmetrical tetrasulfanes

The chemicals required for the synthesis of unsymmetrical tetrasulfanes were thionyl chloride, pyridine, and other thiol compounds such as propanethiol, mercaptopropionic acid, thiophenol, and mercaptobenzene.

5.5.1 General procedure for the synthesis of tetrasulfanes (I)

To a solution of S_2Cl_2 (10 mmol) in diethyl ether (25 ml), an equimolar volume of respective thiol-containing compound (10 mmol) and pyridine (10 mmol) in diethyl ether (10 ml) was added drop wise for 30 minutes at a temperature of -78°C and was stirred for another 30 minutes at the same temperature. Then, another equimolar volume of the same thiol-containing compound (10 mmol) and pyridine (10 mmol) in diethyl ether (10 ml) was added drop wise for another 30 minutes and again stirred at the same temperature for an additional 30 minutes before the reaction mixture was allowed to return to room temperature. After the completion of the reaction, the organic layer of the reaction mixture was washed with distilled water (3 times). The organic layer was dried over $MgSO_4$. The solvent was evaporated and the product was purified by column chromatography.

5.5.2 General procedure for the synthesis of UT (II)

The general procedure I was modified in the following way to obtain the unsymmetrical polysulfanes. To a solution of S_2Cl_2 (2.70 g, 20 mmol) in diethyl ether (50 ml), an equimolar volume of the first respective thiol-containing compound (20 mmol) and pyridine (1.58 g, 10 mmol) in diethyl ether (25 ml) was added drop wise for 1 hour at -78 °C and stirred for another 45 minutes at the same temperature. Then, another equimolar volume of the respective second thiol-containing compound (20 mmol) and pyridine (20 mmol) in diethyl

ether (25 ml) was added drop wise for 1 hour and continued to stir at the same temperature for another 1 hour, before the reaction mixture was allowed to come to room temperature. Subsequently, the organic layer was washed with distilled water. This layer was dried over $MgSO_4$. The solvent was evaporated and the crude compound obtained was further purified by column chromatography.

5.5.2.1 1, 4-diphenyltetrasulfane (DPhTTS)

DPhTTS (**7**) was synthesised by the general procedure for the synthesis of tetrasulfanes (I) (refer section 5.5.1). Thiophenol was used as the respective thiol-containing compound. DPhTTS (**7**) was purified by column chromatography using the solvent petroleum benzene. The compound was obtained as yellow oil with a yield of 90 %. TLC: R_f: 0.36, (Petroleumbenzene). clog*P*: 5.04.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.283- 7.345 (complex, 3H), 7.546- 7.571 (m, 2H), ^{13}C NMR ($CDCl_3$): 128.341, 129.135, 130.165, 136.194. HRMS (ESI) *m/z* = 281.9673

5.5.2.2 3-(propyltetrasulfanyl)-propanoic acid (PrTTSPs)

PrTTSPs (**9**) was synthesised by the general procedure for the synthesis of unsymmetrical polysulfanes (II) (refer section 5.5.2). The first thiol-containing compound used was mercaptopropane and the second thiol-containing compound used was mercaptopropionic acid. PrTTSPs (**9**) was purified by column chromatography using the solvent dichloromethane. The compound was obtained as pale yellow oil with a yield of 71 %. TLC: R_f: 0.20, (CH_2Cl_2). clog*P*: 2.74.

^{1}H NMR (500 MHz, $CDCl_3$): δ 1.010- 1.040 (t, 3H), 1.763-1.836 (m, 2H), 2.905-2.948 (q, 4H), 3.175- 3.204 (t, 2H), ^{13}C NMR ($CDCl_3$): δ 13.048, 22.359, 33.294, 33.833, 41.452, 176.883. *m/z* = 266.9614

5.5.2.3 3-(benzyltetrasulfanyl)-propanoic acid (BnTTSPs)

BnTTSPs (**8**) was synthesised by the general procedure for the synthesis of unsymmetrical polysulfanes (II) (refer section 5.5.2). The first thiol-containing compound used was benzylmercaptan and the second thiol-containing compound used was mercaptopropionic acid. BnTTSPs (**8**) was purified by column chromatography using the solvents dichloromethane:methanol (9:1). The compound was obtained as yellow oil with a yield of 75 %. TLC: R_f: 0.27 (CH_2Cl_2: CH_3OH-9:1). clog*P*: 2.76.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.230-1.258 (t, 1H), 2.171 (s, 3H), 2.852-2.881 (t, 2H), 3.142-3.171 (t, 1H), 7.307- 7.345 (m, 4H), ^{13}C NMR ($CDCl_3$): δ 30.910, 33.272, 33.582, 43.748, 53.409, 127.799, 128.703, 129.497, 129.544, 136.082, 175.650, *m/z* = 314.961

5.5.2.4 3-(phenyltetrasulfanyl)-propanoic acid (PhTTSPs)

PhTTSPs (**11**) was synthesised by the general procedure for the synthesis of unsymmetrical polysulfanes (II) (refer section 5.5.2). The first thiol-containing compound used was thiophenol and the second thiol-containing compound used was mercaptopropionic acid. PhTTSPs (**11**) was purified by column chromatography using the solvents dichloromethane:methanol (9.5:0.5). The compound was obtained as light yellow powder with a yield of 84 %. TLC: R_f: 0.32 (CH_2Cl_2: CH_3OH-9.5:0.5). clog*P*: 3.12.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.731-2.760 (t, 2H), 3.115-3.144 (t, 2H), 7.233-7.293 (m, 3H), 7.482-7.501 (m, 2H), ^{13}C NMR ($CDCl_3$): 33.634, 33.715, 128.038, 128.805, 129.714, 135.051, 172.943, *m/z* = 277.9

5.5.2.5 1-benzyl-4-phenyltetrasulfane (BnTTSPh)

BnTTSPh (**10**) was synthesised by the general procedure for the synthesis of unsymmetrical polysulfanes (II) (refer section 5.5.2). The first thiol-containing compound used was thiophenol and the second thiol-containing compound used was benzylmercaptan. BnTTSPh

(**10**) was purified by column chromatography using the solvent n-Hexane. The compound was obtained as yellow powder with a yield of 49 %. TLC: R_f: 0.23 (n-Hexane). clog*P*: 5.37.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 4.068 (s, 2H), 7.202-7.251 (complex, 5H), ^{13}C NMR ($CDCl_3$): 33.634, 33.71, 128.038, 128.805, 129.71, 135.65, 172.94, *m/z* = 296.8

5.5.2.6 1-propyl-4-benzyltetrasulfane (PrTTSBn)

PrTTSBn (**12**) was synthesised by the general procedure for the synthesis of unsymmetrical polysulfanes (II) (refer section 5.5.2). The first thiol-containing compound used was benzylmercaptan and the second thiol-containing compound was thiopropane. PrTTSBn (**12**) was purified by column chromatography using the solvent n-Hexane. The compound was obtained as yellow powder, with a yield of 59 %. TLC: R_f: 0.465 (n-Hexane). clog*P*: 5.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.577-3.594 (m, 5H), 5.214-5.276 (complex, 5H), 5.847-5.930 (m, 3H), ^{13}C NMR ($CDCl_3$):42.053, 119.569, 132.478, *m/z* = 261.2

5.5.2.7 1-propyl-4-phenyltetrasulfane (PrTTSPh)

PrTTSPh (**13**) was synthesised by the general procedure for the synthesis of unsymmetrical polysulfanes (II) (refer section 5.5.2). The first thiol-containing compound used was thiophenol and the second thiol-containing compound used was thiopropane. PrTTSPh (**13**) was purified by column chromatography using the solvents dichloromethane:methanol (9:1). The compound obtained was obtained as pale yellow oil with a yield of 62 %. TLC: R_f: 0.340 (CH_2Cl_2: CH_3OH-9:1).clog*P*: 4.66.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.967-0.997 (t, 2H), 1.150-1.178 (t, 1H), 1.664-1.796 (m, 2H), 2.730-2.901 (t, 2H), 3.413-3.455 (q, 1H), 7.211-7.319 (complex, 3H), 7.502- 7.521 (m, 1H), ^{13}C NMR ($CDCl_3$): 13.054, 15.252, 22.358, 41.365, 65.821, 128.314, 129.111, 130.136, 136.169, *m/z* = 248.6

5.6 Physico-Chemical Measurements

5.6.1 Electrochemistry

The redox behaviour of the symmetrical, unsymmetrical tetrasulfanes and other selenium and tellurium containing compounds was studied at a BAS 100 W electrochemical workstation by recording their cyclic voltammograms. During the analysis of tetrasulfanes, a dropping mercury electrode was used as the working electrode and a glassy carbon electrode for the analysis of Se and Te containing compounds. In both the experiments, the Ag/AgCl electrode (SSE) was used as the reference electrode and a platinum spiral was used as the counter electrode. Four full cycles were recorded, the first of which was used to record the peak potentials. The parameters were set and the potential range varied between -1200 to 0 mV for tetrasulfanes and -1200 to +1200 mV for Se and Te containing compounds; the scan rate was fixed as 250 mV/s. The stock solutions of the compounds were prepared in methanol. The compounds were dissolved in a cuvette containing 33 % methanol in 50 mM potassium phosphate buffer at pH 7.4 to obtain the final concentration of the compound as 250 µM. Before each measurement, the electrolysis cell was purged with nitrogen and stirred well. All of the measurements were performed in triplicate at room temperature.

5.6.2 Ring tensiometry

The instrument KRÜSS Tensiometer K6 (KRÜSS GmbH, Hamburg) was used at the Institute of Pharmaceutical Nanotechnology, Department of Pharmacy, University of Saarland. All the compounds were dissolved in distilled water at different concentrations, such as 1, 3, 5, 10, 15 and 30 mM and all of the measurements were performed at room temperature. 10 ml of each concentration of compound was prepared in a glass vial. The vial was placed on the sample holder, which was levelled using the set screw and the micrometre screw. The ring was immersed into the vial such that it was very close to the surface of the liquid. The balance beam, on which the ring was hanging, was set at the zero reference mark. With the help of a wheel, the tension was increased slowly from 0 mN/m until the ring came out of the liquid.

The tension at which the ring came out of the liquid was recorded as the surface tension of the liquid. The experiment was repeated five times with each concentration of a compound. A control experiment was performed with SDS (**14**). The critical micelle concentration of the compounds was obtained by plotting a graph of different concentrations of the compounds against their values of surface tension.

5.6.3 Sita tensiometry

The surface tension analysis of the compounds was performed with SITA science line t60 using the bubble pressure method. The range of the measurement of surface tension was 10-100 mN/m and the resolution was 0.1 mN. The measuring range of bubble frequency was 30 ms-60 s and the resolution was 1 ms. All the compounds were dissolved in 2 ml of distilled water, containing less than 1 % DMSO, at various concentrations such as 5 µM to 3 mM. The capillary was inserted and air was pumped into the solutions of the compounds. The bubble pressure developed in the solution is proportional to its surface tension. The surface tension in mN/m was recorded electronically by the computer. All of the experiments were performed in triplicate at room temperature. SDS (**14**) was used as a control. In order to obtain the CMC value of a compound, a graph was plotted with different concentrations of the compound against the values of surface tension.

5.6.4 Densitometry

All of the compounds were dissolved in ethanol containing less than 1 % of DMSO. Different concentrations of compounds were prepared such as, from 188.7 µM to 3 mM. The density measurements were performed using the instrument, Anton Paar DMA 4500, Density meter. Before measuring the densities of different concentrations of the compounds, the instrument was calibrated with distilled water. The densities were measured for each concentration of the compounds and their corresponding apparent molar volumes were calculated. Graphs were plotted with apparent molar volumes of the compounds against their concentrations.

5.7 Molecular Non-Covalent Interaction Studies

5.7.1 Haemolysis assay

5.7.1.1 Preparation of buffer

HPS buffer A was prepared by mixing 145 mM NaCl, 7.5 mM KCl, 10 mM glucose and 10 mM HEPES at a pH 7.4. This buffer was used for washing human blood.

5.7.1.2 Preparation of human blood

All of the blood samples were obtained from the Institute of Clinical Haematology and Transfusion Medicine, Saarland University hospital (Homburg). Human venous blood was used for the experiments and was collected from healthy donors in Na-EDTA anticoagulant-coated test tubes, which were stored at 4 °C for a maximum of two days. Fresh blood samples were used before each experiment.

5.7.1.3 Preparation of red blood cells

The whole blood from the anticoagulant-coated test tubes was aliquot into eppendorfs. These aliquots were centrifuged at 2000 g for 5 minutes at room temperature, and the supernatant was removed by aspiration. Then, the blood cells were suspended in HPS buffer A and vortexed well. Similarly, the washing steps were repeated three times under the same conditions. After washing, the cells were re-suspended again in HPS buffer A. Cells were centrifuged at 16000 g for 10 s in order to compact them. These compact cells were used for further experiments.

5.7.1.4 Procedure

Drabkin's reagent, purchased from Sigma Aldrich was used for this assay. This reagent determines the haemoglobin content at an absorption of 540 nm. Different stock solutions of the compounds, such as 1, 5, 25, 50, 75 and 100 mM, were prepared in DMSO. The RBCs were suspended in 990 µl of HPS buffer A, to make 2.5 % haematocrit. Then, 10 µl of the

stock of each concentration, of every compound was added to the RBC suspension to make the final concentrations of 10, 50, 250, 500, 750 and 1000 µM of the compounds with 1 % of DMSO in each of them. A control experiment was performed in a similar way with only 10 µl of DMSO in 990 µl of RBC suspension. All of the above mixtures were prepared in triplicate and were incubated in a water bath at 37 °C for 30 minutes. Meanwhile, 2 ml of Drabkin's reagent was prepared in 3 ml cuvettes. After incubation, all of the samples along with the DMSO control were centrifuged at 12,000 g for 30 seconds. Then, 500 µl of supernatant was transferred to the 2 ml of Drabkin's reagent that was prepared in the cuvettes. A control for the completely haemolysed RBCs was prepared by adding 2.5 % haematocrit in 500 µl of a detergent solution in 2 ml of Drabkin's reagent. All of the samples with Drabkin's reagent were incubated in the dark for 30 minutes. After incubation, the absorption was measured at 540 nm in a UV-Vis spectrophotometer (UV mini 1240, UV-Vis Spectrophotometer, Shimadzu), with a blank of 500 µl HPS buffer A in 2 ml Drabkin's reagent.

5.7.2 FRET measurements

All the experiments were performed in a Variance Cary Eclipse fluorescent spectrometer and the obtained results were analysed using Cary Eclipse software. BSA was purchased from Sigma Aldrich and a stock solution was prepared at a concentration of $1 .10^{-6}$ M, which was later diluted to $1 .10^{-7}$ M in 3ml of sodium citrate buffer solution. The stock solution of the compounds was prepared in ethanol at 3 mM with less than 1 % of DMSO. The excitation wavelength was set at 280 nm and the fluorescence intensity was recorded between 300 and 500 nm. The excitation slit value was set at 5 and the emission slit value was set at 10. The graphs were smoothed by setting the smoothing factor at 11. Initially the fluorescence intensity of BSA was recorded. Then, 2 µl of the compound was added to 3 ml of $1 .10^{-7}$ M BSA solution and quenching of the fluorescence of BSA was recorded. The spectra were then recorded with 5 µl of compound, and then for each 5 µl addition of the compound until a total volume of 60 µl of compound had been added to BSA solution. Fluorescence intensity of the BSA solution, in the presence of different concentrations of the compounds was measured. A control experiment was performed with only the solvent ethanol and 1 % DMSO.

5.8 Biological Assays

5.8.1 Nematode assay

Steinernema feltiae nematode sample, in the form of a soft cake, was purchased from Sautter & Stepper GmbH (Ammerbruch, Germany) and stored in the dark at 4 ºC. *S. feltiae* is the commercially available non-pathogenic model organism for agricultural crops and hence the best for pre-screening of the compounds. Fresh samples were ordered before each experiment and not used for more than a week. A 1.5 g sample of the nematode soft cake was dissolved in 250 ml of distilled water at 27 °C. This solution was allowed to stay at room temperature and in moderate light for nearly 30 minutes by stirring at regular intervals in order to resuscitate the nematodes. The viability of the suspension of nematodes was calculated under the microscope (TR 200, VWR International, Belgium) at a four-fold magnification. The viability of more than 80 % was considered optimal and the samples were then subjected to the experiment. Stock solutions of all of the compounds were prepared in DMSO and further diluted in distilled water at concentrations ranging from 10 µM to 1 mM, such that final concentration of DMSO was less than 1 % in each case. Then, 100 µl of each concentration of the compound and 100 µl of the nematode suspension were added to 96-well plates in triplicate. A control experiment was performed with 1 % DMSO. The live and dead nematodes were counted under the microscope immediately after 30 minutes, 6 hours and 24 hours of incubation. LD_{50} value was calculated for each compound and the whole experiment was repeated three times.

5.8.2 Anti-yeast experiment

5.8.2.1 Preparation of Saburo broth and media

Saburo broth was prepared by adding glucose (40 g/l) peptone (10 g/l), and yeast extract (5 g/l) in distilled water. Saburo media was prepared using the same constituents along with 0.9 % agar.

5.8.2.2 Re-cultivation of yeast

Using a microbial inoculation needle, a small inoculum of strain of yeast, from the yeast culture deposits (YSU), was removed and dispersed well in 5 ml of broth of Saburo in a test tube. The test tube was stored overnight in a temperature-controlled shaker. After the incubation period the culture of yeast measuring an optical density of 0.5 a.u turbidity in a UV-spectrometer, was used for the study of the activity of compounds against yeast.

5.8.2.3 Procedure

The procedure of the yeast assay was followed according to [91]. The experiments were performed in Yerevan State University, Armenia. Different strains of yeast namely; *Debariomyces hansenii, Candida guilliermondii, Saccharomyces cerevisiae and Candida albicans* 174 were used for the experiments. All of the chemical compounds were dissolved in DMSO (100 %) to prepare the following concentrations: 0.001, 0.01, 0.1, 0.25, 0.5, 0.75, 1 and 3 mM. Concentration-dependence studies of all the compounds were performed against the strains of yeast using the agar well-diffusion method. DMSO (100 %) was used as a control. The test-organisms were added on sterile petridishes. Then, approximately 20 ml of the growth media (Saburo) for yeast, was added to the petridishes with the microorganisms. The petridishes were shaken well such that the microorganisms had spread throughout. Small wells of 8 mm in diameter were punched in the agar plates (petridishes) using a sterile stainless steel borer. Then, 100 µl of each concentration of a compound was transferred to the wells. The plates were incubated micro-aerophilically at a temperature of 28 °C for 24 hours. After the incubation, the diameters of the inhibitory zones were measured in mm.

5.8.3 Antifungal experiment

5.8.3.1 Preparation of Wort broth and media

A 50 g wort extract was mixed in 200 ml of distilled water at a temperature of 45°C and this temperature was maintained for 30 minutes. The temperature of the water bath was slowly increased such as 1 °C every minute and continued for 25 minutes up to a final temperature of 70 °C. Then, 100 ml of distilled water (70 °C) was added to the mixture. The temperature of the solution was maintained at 70 °C for 55 minutes, before the solution was cooled down to room temperature. Then, the solution was weighed and made up to 450 g by adding the required amount of distilled water. The broth was filtered and the required amount of agar was added. The media was sterilised for further use.

5.8.3.2 Spore collection

0.96 % NaCl physiological solution was prepared and sterilised. The fungi were grown in petridishes for 10 days. Using 2-3 ml of the 0.96 % NaCl solution, the spores were collected into eppendorfs. The spores were counted on square grid under the microscope and spore count was adjusted to 1 $.10^7$ spores in the Petri dish for each experiment. Spores were collected from *Trichoderma viride, Aspergillus flavus, Penicillium aeruginosa, Fusarium CB 1853, Mucor plumbeus* and *Geotrichum candidum*.

5.8.3.3 Procedure

Sterile plastic petridishes with a diameter of 35 mm were used for all of the experiments. All of the compounds were dissolved in DMSO (100 %). Compounds were added to the petri dishes and then wort media was added to make a final concentration of 3 mM for each compound. Subsequently, spores were added to the plates to make a final number of 1 $.10^7$ spores. The plates were incubated at 27 °C for 1 week.

5.8.4 Antibacterial experiment

5.8.4.1 Preparation of LB broth and media

LB broth was prepared by adding peptone (10 g/l), yeast extract (5 g/l), NaCl (10 g/l), sucrose (5 g/l) and $MgSO_4$ (0.5 g/l) in distilled water. LB media was prepared with the same constituents using 0.9 % agar.

4.8.4.2 Re-cultivation of bacteria

Using a microbial inoculation needle, a small inoculum of strain of bacteria, from the bacterial-culture-deposits (YSU), was removed and dispersed well in 5 ml of broth of LB in a test tube. The test tube was incubated in a temperature (37 °C) controlled shaker (100 rpm) overnight. The culture measuring an optical density of 0.5 a.u turbidity was used for the experiment.

4.8.4.3 Procedure

The antibacterial experiments were performed under the Chair of Microbiology, Plants and Microbes Biotechnology, Department of Biology in Yerevan State University. The procedure of the experiment was followed according to the [91]. *Staphylococcus aureus* WDCM 5233 and *S. typhimurium* 1754 were obtained from the Microbiological Depository Centre, Armenia, and *E. coli* M-17 VKPM-B8208 from the Russian National Collection of Industrial Microorganisms. *M. luteus*, *B. subtilis* A1, *B. licheniformis* WT, *S. roseus*, *P. aeruginosa* were the wild isolates. All of the compounds were dissolved in DMSO (100 %) to prepare the following concentrations: 0.001, 0.01, 0.1, 0.25, 0.5, 0.75, 1 and 3mM. A concentration-dependence study of all of the compounds was performed on *S. aureus* WDCM 5233 and *E. coli* M17 using the agar well-diffusion method. DMSO (100 %) was used as a control. The test organism was placed on the agar plate. Wells of 8 mm in diameter were punched in the agar plates using a sterile stainless steel borer. Then, 100 µl of each concentration of a compound was transferred to the wells. The plates were incubated in a thermostat micro-

aerophilically at 37 °C for 24 hours. The diameters of the inhibitory zones were measured in mm.

5.8.5 Anti-bacteriophage experiment

5.8.5.1 Preparation of LB broth and media

The sterile LB broth was prepared with the same constituents as described above in the section 4.7.1. Sterile LB media was prepared with 1.8 % and 0.8 % agar.

5.8.5.2 Re-cultivation of *E. coli* C-T4

Using a microbial inoculation needle, a small inoculum of strain of *E. coli* C-T4, from the bacterial-culture deposits (YSU), was removed and cultivated on a nutrient slant agar media (commercially available).

5.8.5.3 Isolation of T4-bacteriophage

A petridish with LB media (1.8 % agar) was prepared. 1 $.10^8$ colonies of T4 bacteriophage and 1 $.10^8$ cells of *E. coli* C-T4 were mixed in a falcon tube. This solution was further diluted with 7 ml of 0.8 % LB agar and was poured onto the 1.8 % agar plate. The plate was incubated overnight in an incubator at 37 °C. Then, 0.96 % NaCl physiological solution was used to isolate the T4 phage from the agar plates. The phage solution was centrifuged at 12000 g for 5 minutes. The supernatant was collected and filtered through a bacterial filter of diameter of 0.2 nm. The purified T4 phage was stored at -30 °C for further use.

5.8.5.4 Procedure

The procedure of the experiment was followed according to [92]. All of the experiments were performed in the Faculty of Biology, YSU, Armenia. All of the chemicals were purchased from Sigma-Aldrich. A stock solution of T4-bacteriophage with 10^8 cells was used. Here, 30 µl of T4 bacteriophage and 30 µl of different concentrations of compounds such as 0.25, 0.75, 1.5, and 3mM were incubated for 90 minutes. Then, the petridishes containing LB media with

1.8 % agar were prepared and dried under UV for 20 minutes for sterilisation. 10^2, 10^4, 10^6 and 10^8 dilutions of T4 phage along with the compounds were prepared using 5 ml of sterile LB broth. The test tubes were shaken well for 5 minutes, to allow the phage to completely diffuse. 10^8 cells of *E. coli* C-T4 was prepared using the *E. coli* C-T4 slant agar culture. The 10^8 cells of *E. coli* C-T4 culture was prepared in 5 ml LB broth with an optical density of 0.4/0.5 a.u. 1ml of each dilution of the compounds with 500 µl of *E. coli* C-T4 culture were mixed with 7 ml of LB media (0.8 % agar) and poured on to the petridish containing LB media with1.8 % of agar. The petridishes were incubated in a thermostat at 37 °C for 24 hours. The viable colonies of bacteriophage were counted by naked eye.

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P. Du, **U.M. Viswanathan**, Z. Xu, H. Ebrahimnejad, B. Hanf, T. Burkholz, M. Schneider, I. Bernhardt, G. Kirsch, M. Montenarh, C. Jacob, *Synthesis and biological activity of amphiphilic seleninic acid derivatives with considerable activity against membranes, cancer cells and certain pathogenic microbes*, Journal of Hazardous Materials, 269, 74-82 (2014)

P. Du, **U.M. Viswanathan**, K. Khairan, T. Buric, N.E.B. Saidu, Z. Xu, B. Hanf, I. Bazukyan, A. Trchounian, F. Hannemann, I. Bernhardt, T. Burkholz, B. Diesel, A.K. Kiemer, K.-H. Schaefer, M. Montenarh, G. Kirsch, C. Jacob, *Synthesis of amphiphilic, chalcogen-based redox modulators with in vitro cytotoxic activity against cancer cells, macrophages and microbes*, Med. Chem. Commun., 5, 25-31 (2014).

B. Czepukojc, A.K. Baltes, M. Kelkel, C. Cerella, **U.M. Viswanathan**, F. Salm, T. Burkholz, C. Schneider, M. Montenarh, M. Diederich, C. Jacob, *Towards pharmaceutically useful, redox-modulating polysulfanes based on naturally occurring garlic compounds*, Food and Chemical Toxicology, 64, 249–257 (2014).

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M. Doering, B. Diesel, M.C.H. Gruhlke, **Uma M. Viswanathan**, T. Burkholz, A. Slusarenko, A.K. Kiemer and C. Jacob, *Selenium- and Tellurium-Containing Redox Modulators with Distinct Activity against Macrophages: Possible Implications for the Treatment of Inflammatory Diseases*, Tetrahedron, 68, 10577-10585 (2012)

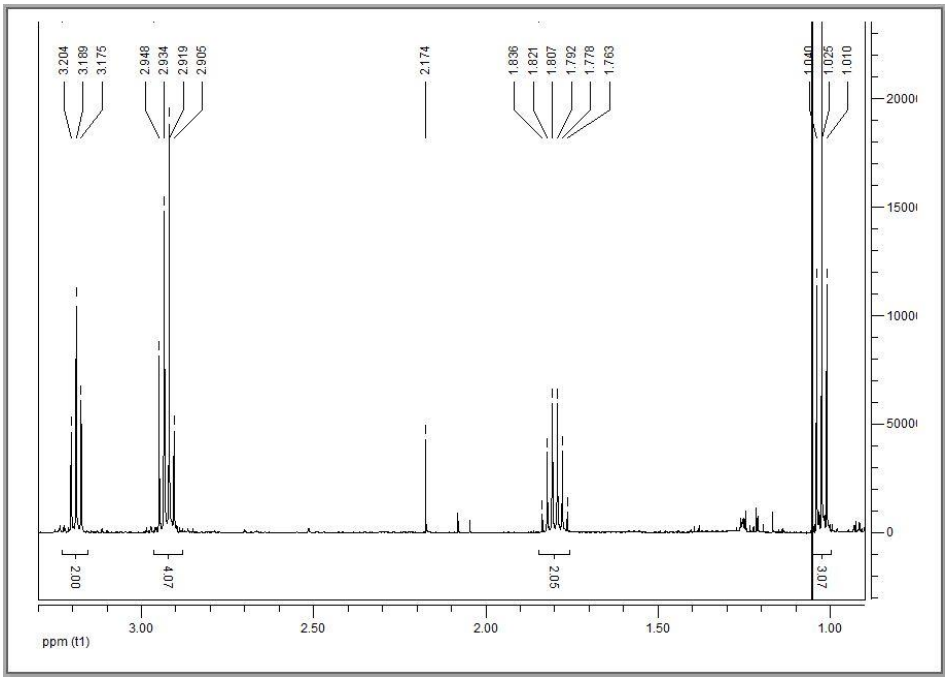
P. Du, **U.M. Viswanathan**, Z. Xu, H. Ebrahimnejad, B. Hanf, T. Burkholz, M. Schneider, I. Bernhardt, G. Kirsch, M. Montenarh, C. Jacob, *Emerging Biological Applications of Chalcogens: Amphiphilic Selenic Acids with Multiple Impact on Living Cells*. in E.R. Rene, P. Kijjanapanich, P.N.L. Lens (eds.), Proceedings of the 3rd International Conference on Research Frontiers in Chalcogen Cycle Science & Technology, Delft, The Netherlands, May 27-28, 59-68 (2013).

Poster Presentation

Uma M. Viswanathan, Brigitte Czepukojc, Thomas Schneider, Torsten Burkholz, Claus Jacob, Shiraz Markarian, Reactive Sulfur Species: *From natural product research to applications in Medicine and Agriculture*, International conference on Frontiers in Chemistry,Armenia, 25-29 August 2013.

Uma M. Viswanathan, Lisa Faulstich, Torsten Burkholz, Claus Jacob: *From Intracellular Redox Signalling to the Development of Sensor / Effector Agents and Green Pesticides*, International Conference in Metabolism 2014 - Alterations of metabolic pathways as therapeutic targets Luxemberg, 29-30 January 2014.

Appendix



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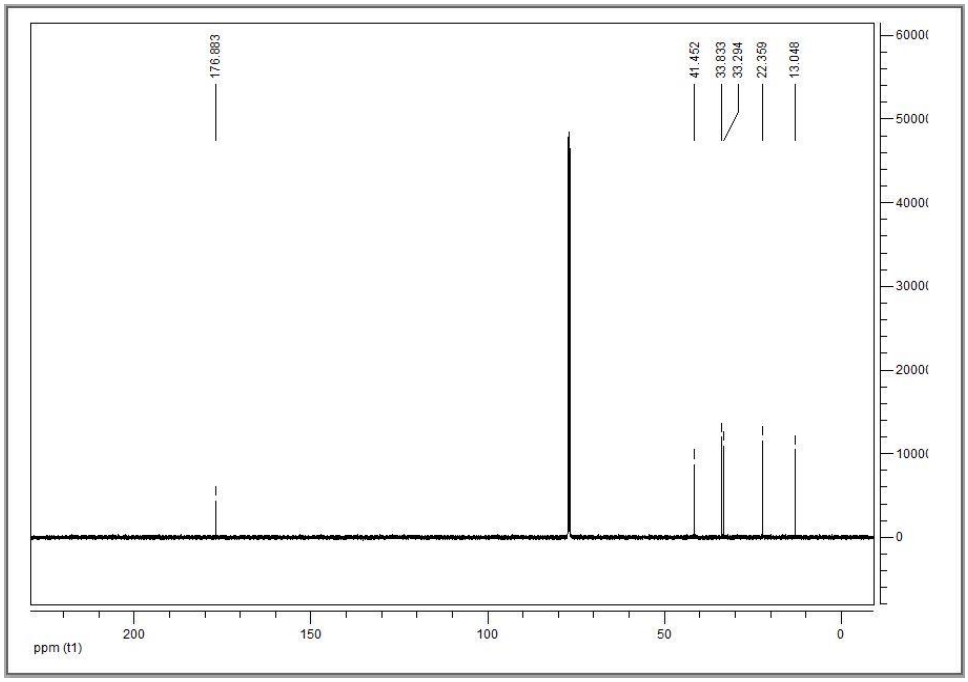

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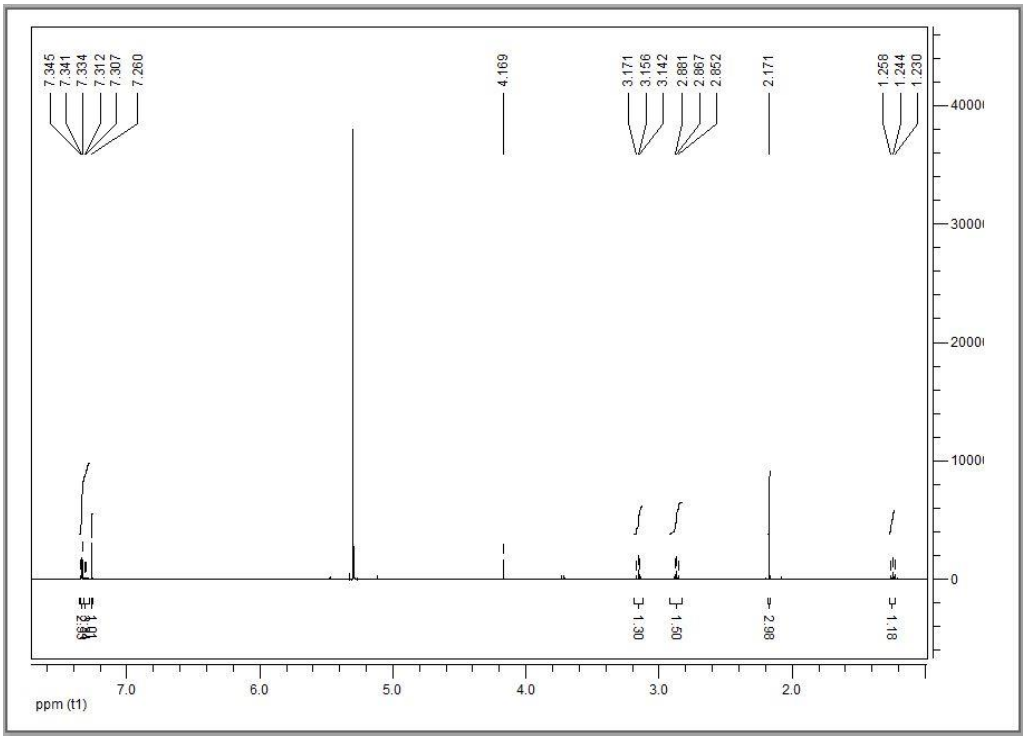

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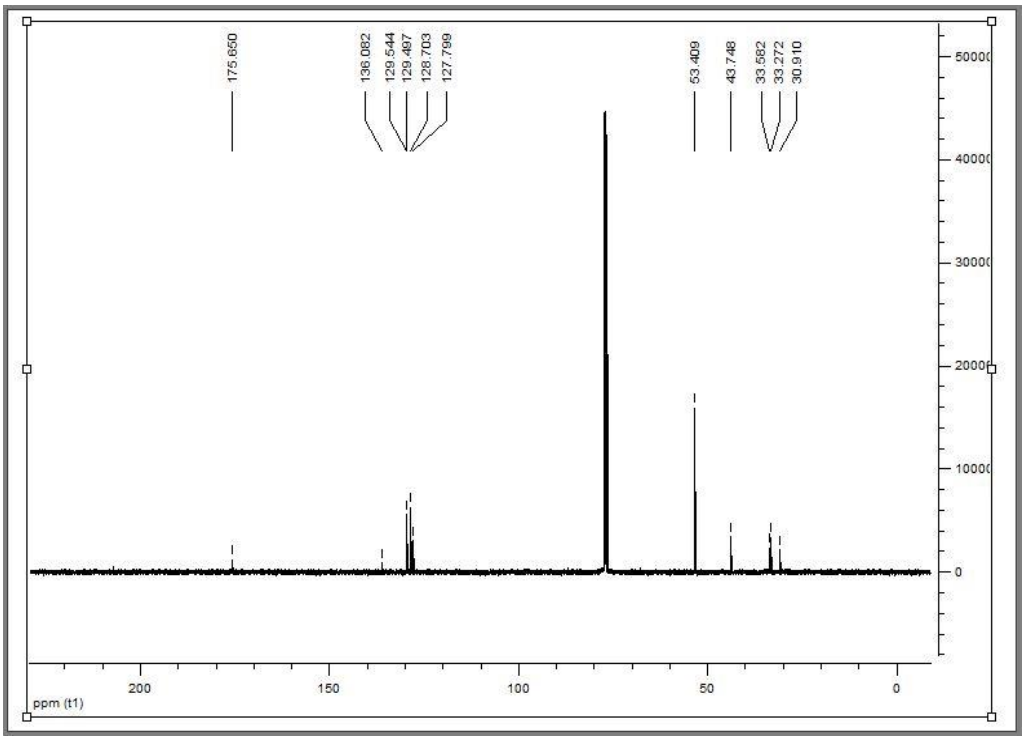

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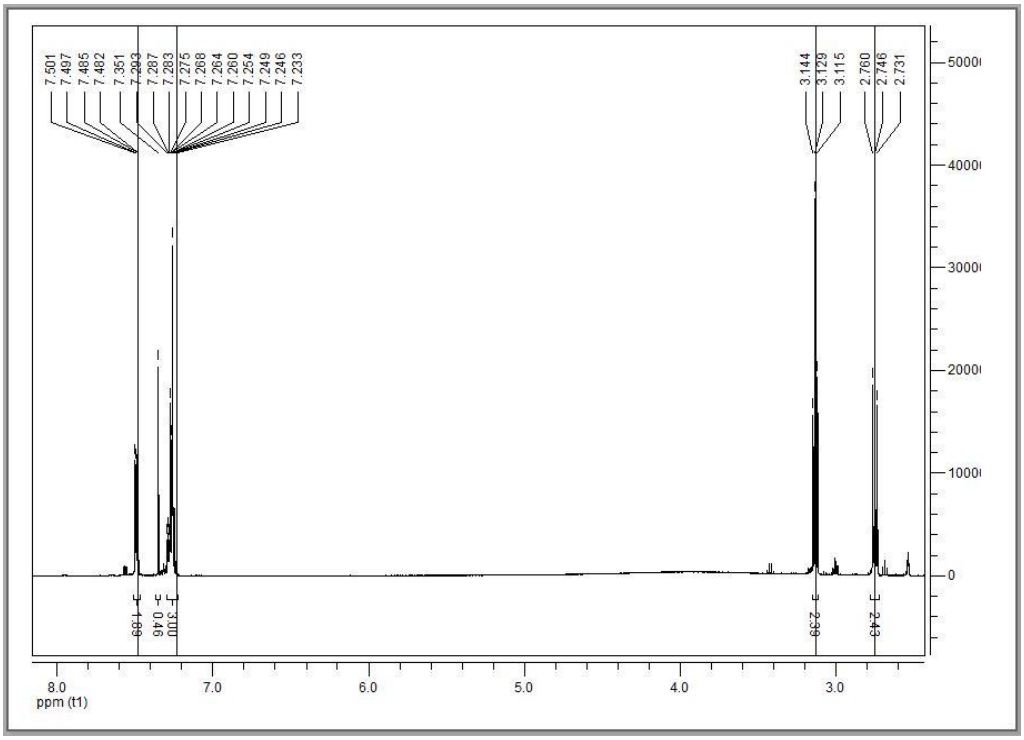

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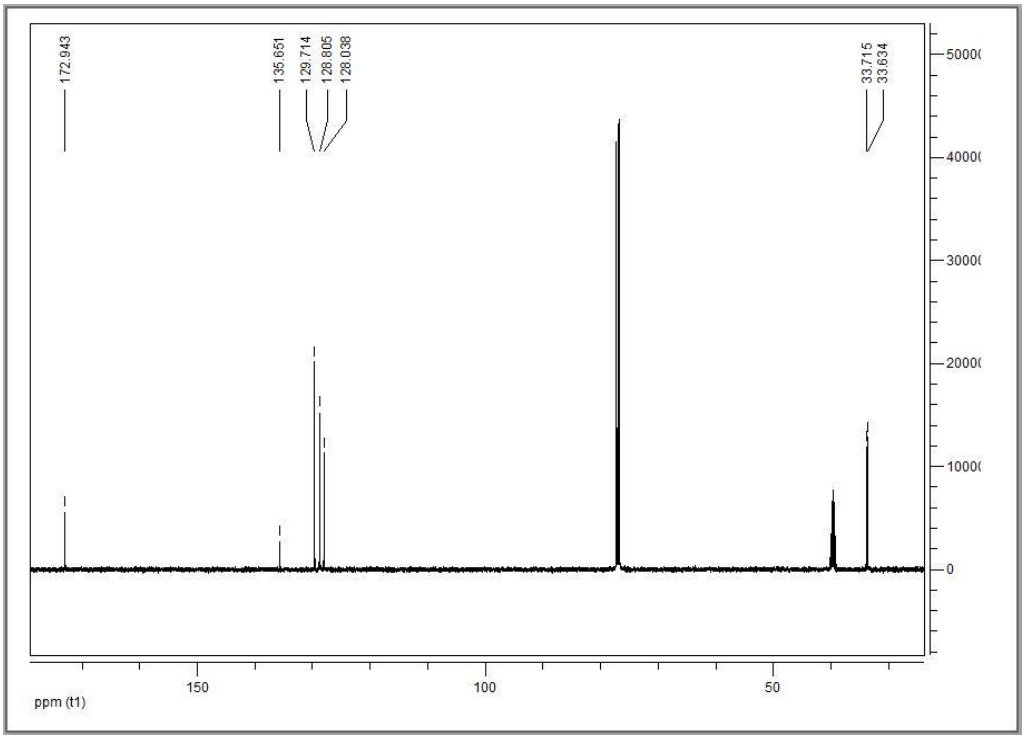

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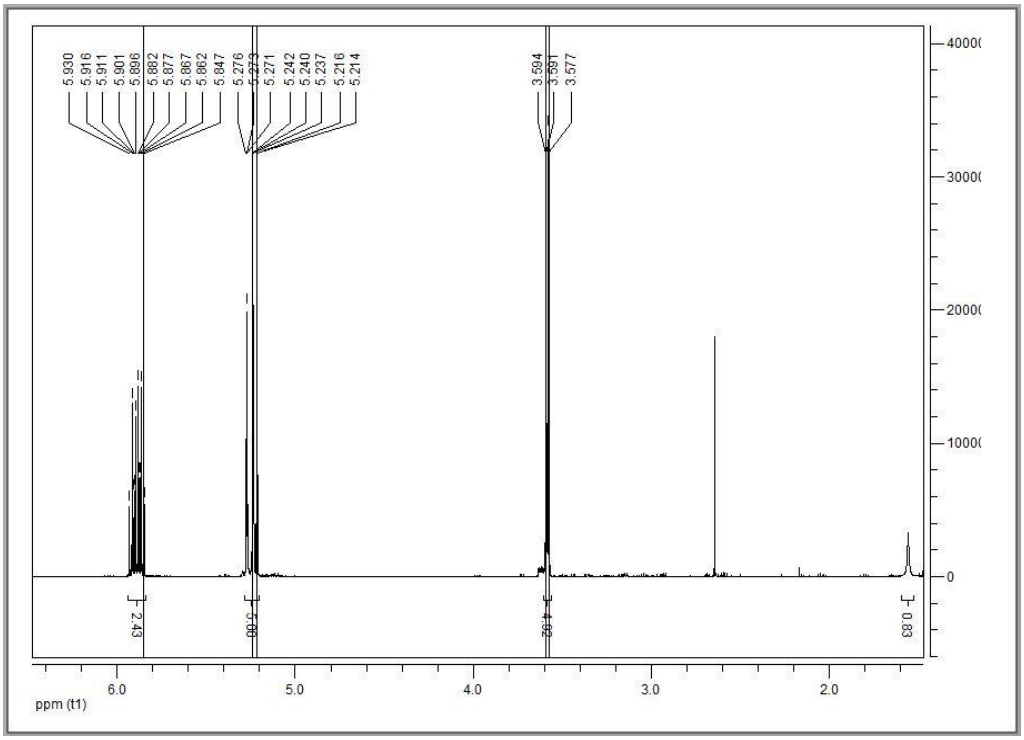

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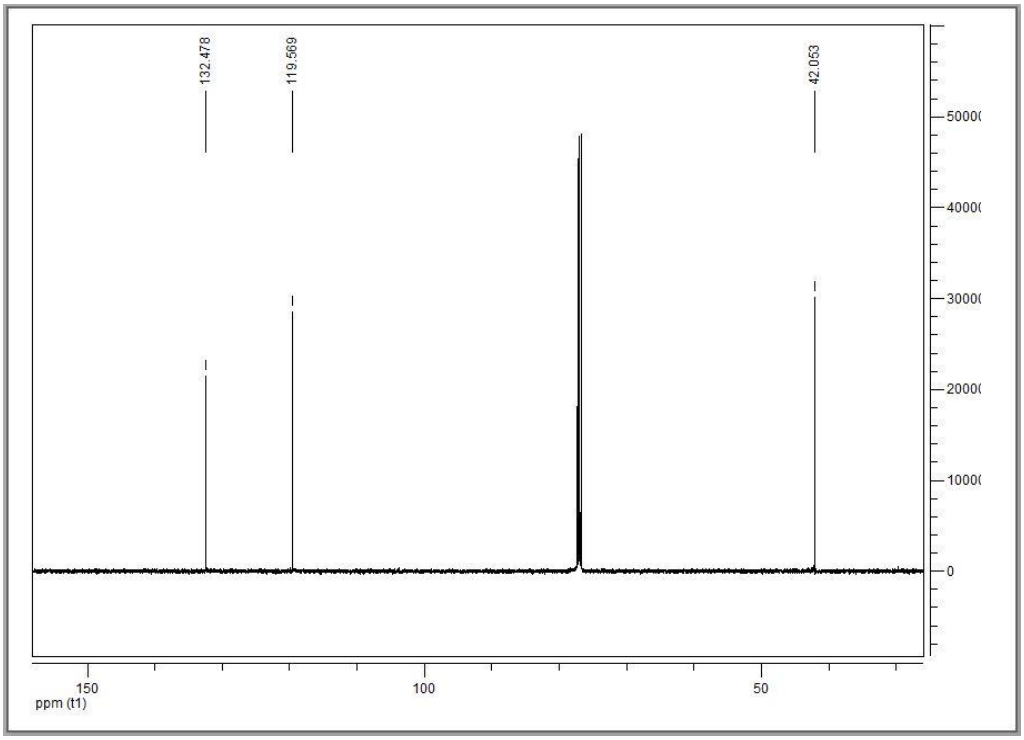

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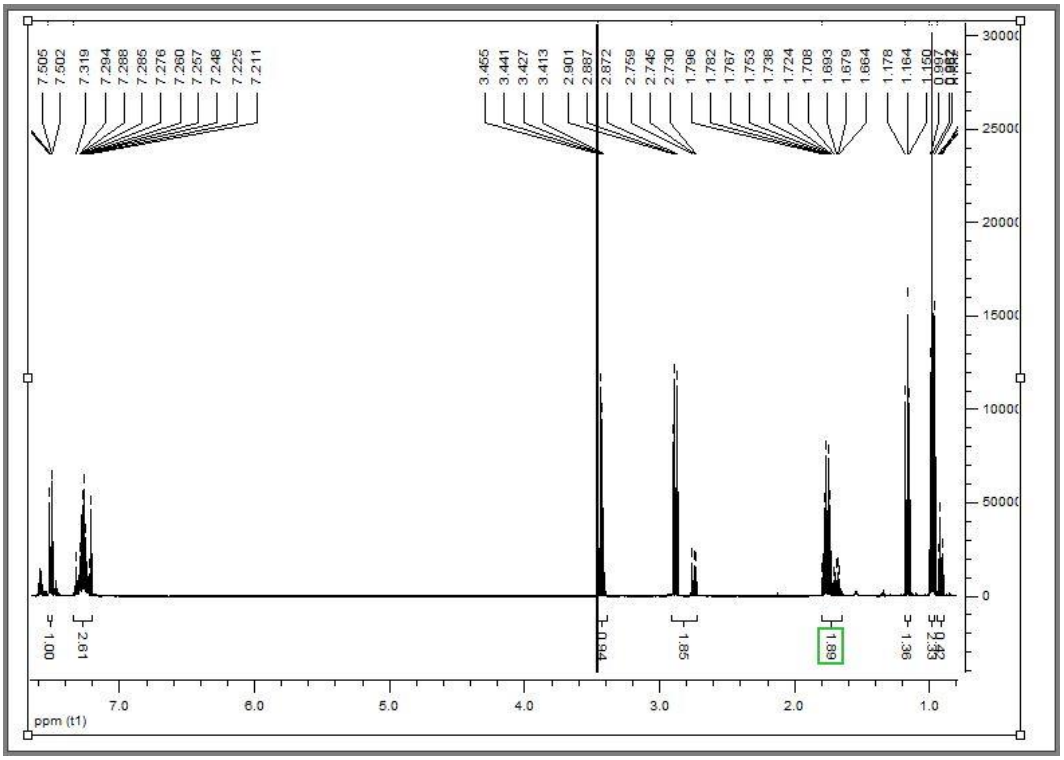

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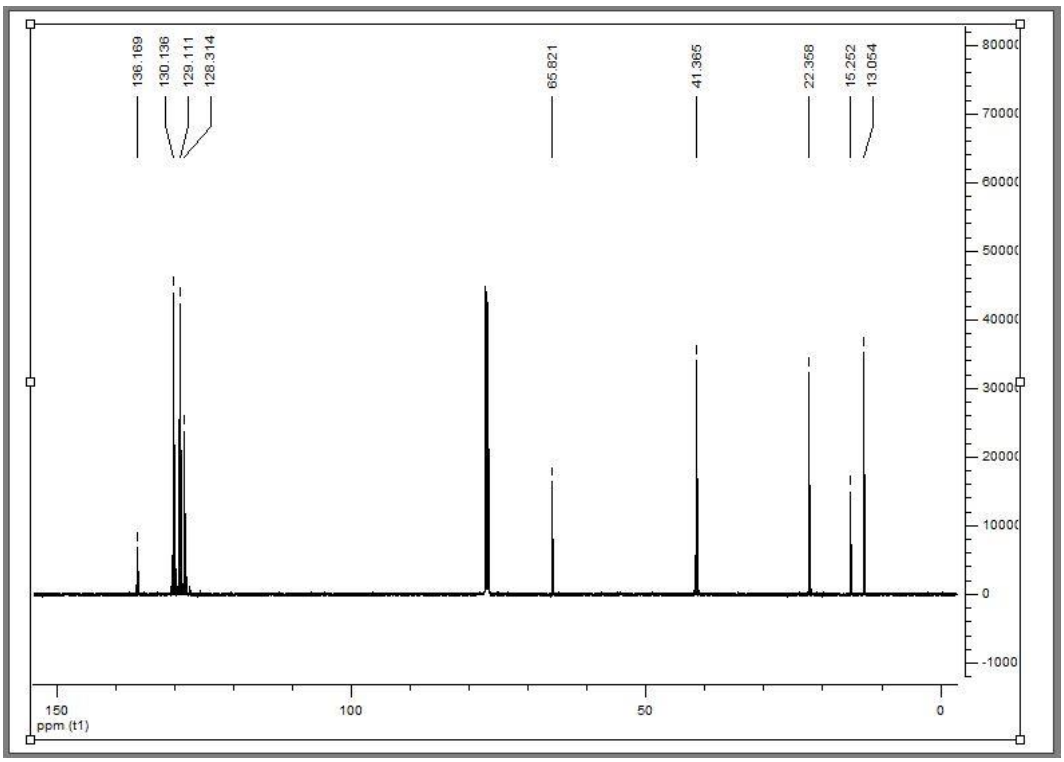

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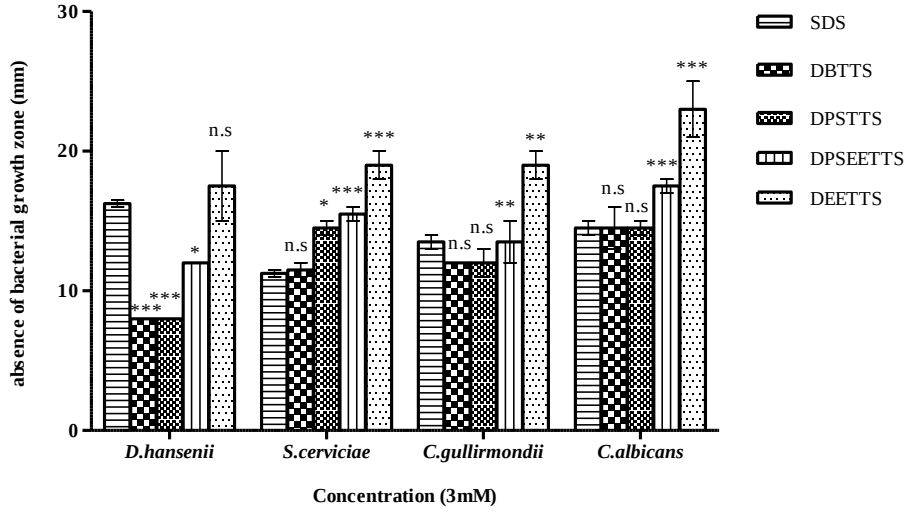
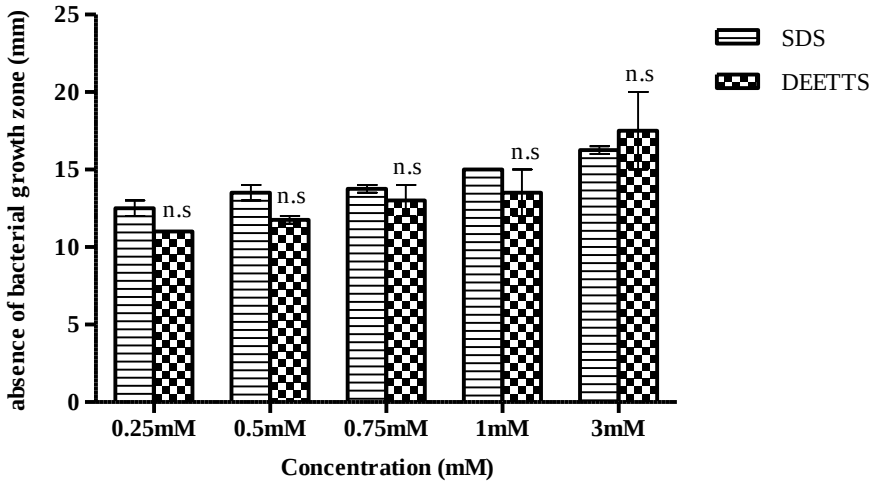
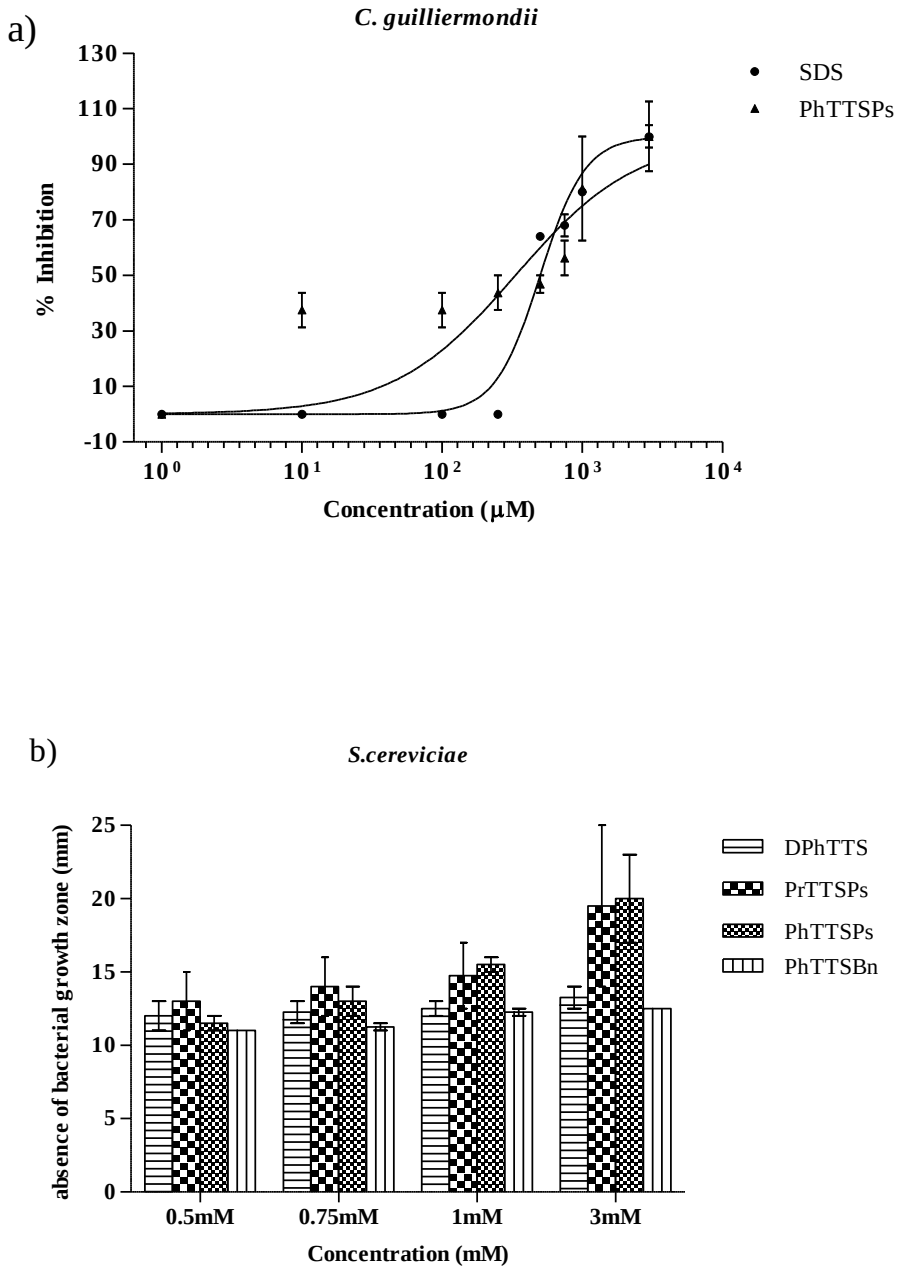


D. hansenii

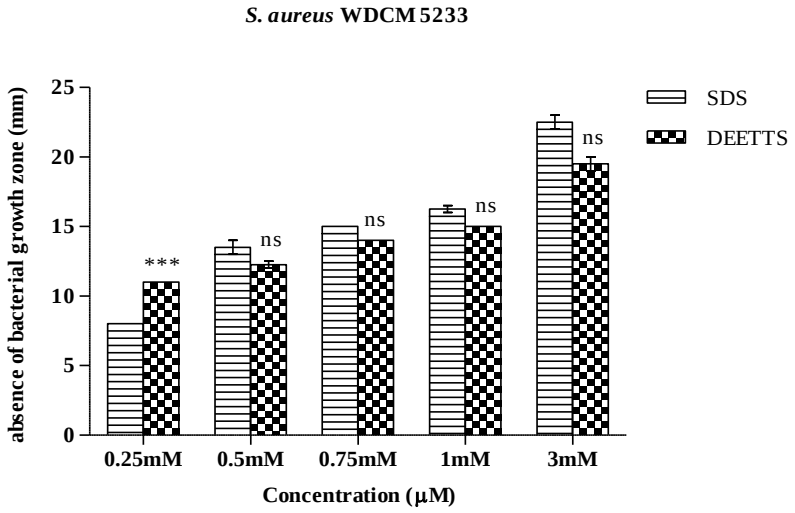


Pre-screening of DBTTS (3), DPSTTS (4), DPSEETTS (5) and DEETTS (6) at a concentration of 3 mM against four different strains of yeast: *D. hansenii, S. cerevisiae, C. guilliermondii and C. albicans*. SDS (14) has been used as a control. Data presented as absence of the bacterial growth zone in mm ± SEM of three independent experiments. ANOVA test: $p < 0.05$ (*), $p < 0.01$ (**) or $p < 0.001$ (***) in relation to the relevant values for the particular control SDS.

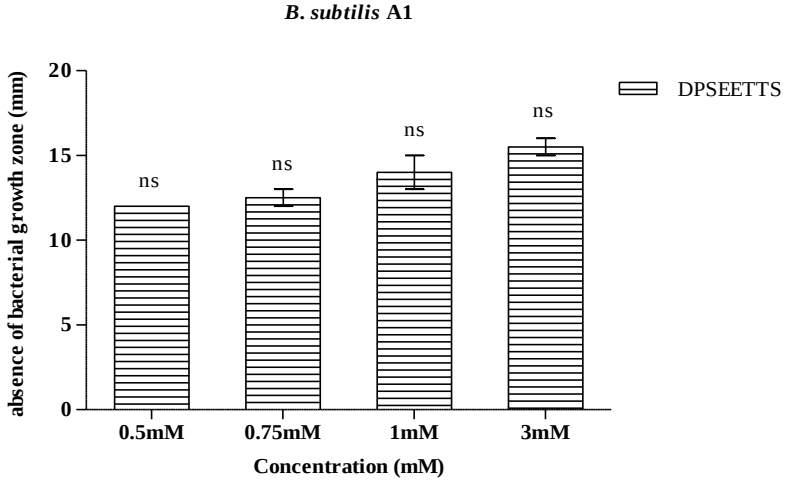


a) Growth inhibition curve for *C. guilliermondii* in the presence of PhTTSPs (11) and SDS (14). Data presented as percentage inhibition ± SEM. b) activity of a) DPhTTS (7), PrTTSPs (9), PhTTSPs (11) and PhTTSBn (10) against *S. cerevisiae*. SDS (14) has been used as a control (no effect on *S. cerevisiae*). Data presented as absence of the yeast-growth zone in mm ± SEM of three independent experiments.

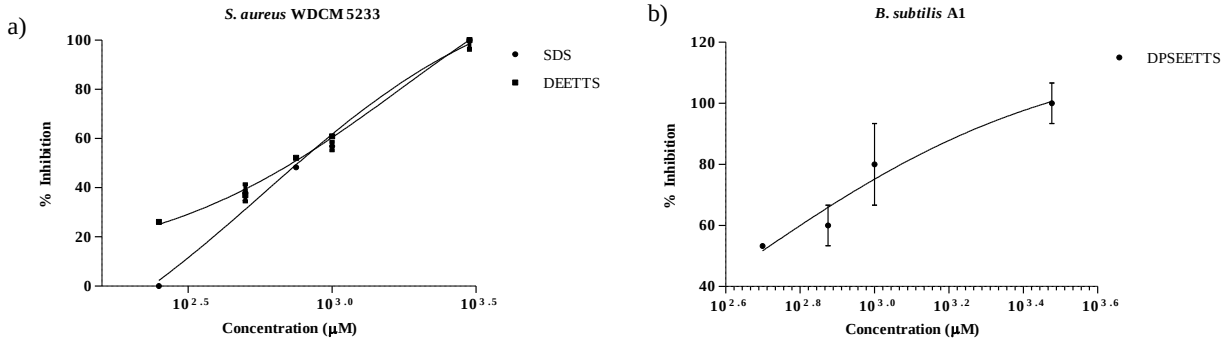
a)



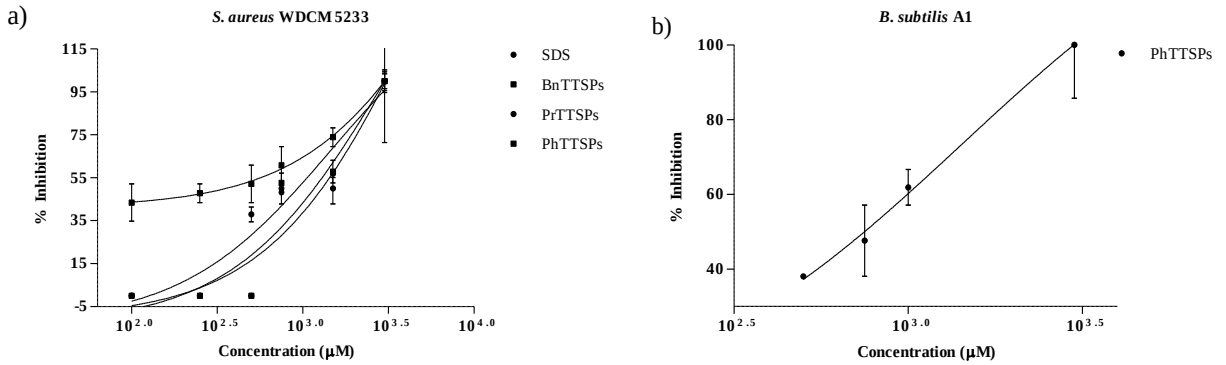
b)



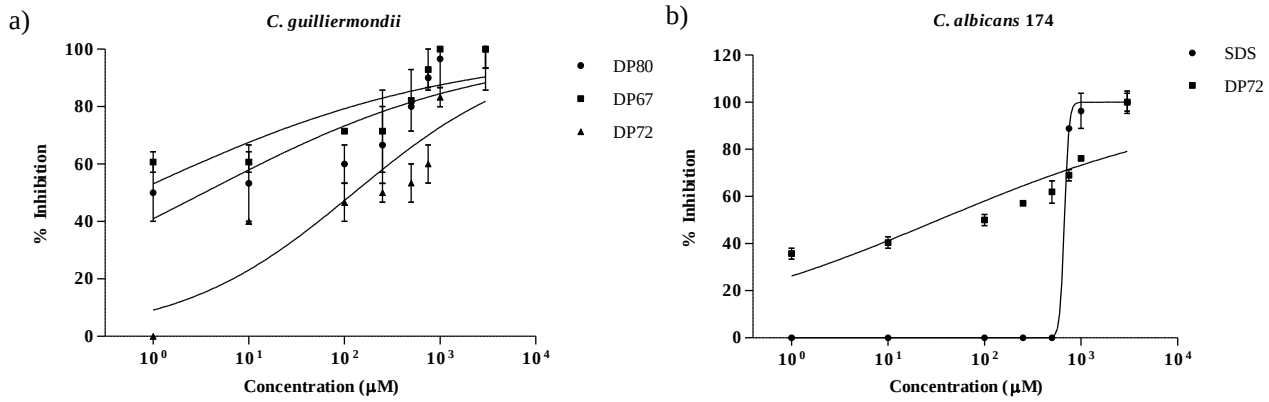
Activity of a) DEETTS (6) against *S. aureus* and b) DPSEETTS (5) against *B. subtilis*. SDS (14) has been used as a control. SDS (14) did not show any activity against *B. subtilis*. Data presented as absence of the bacterial growth zone in mm ± SEM of three independent experiments. ANOVA test: n.s. = $p \geq 0.05$ or $p < 0.001$ (***) in relation to the relevant values for the particular control SDS.



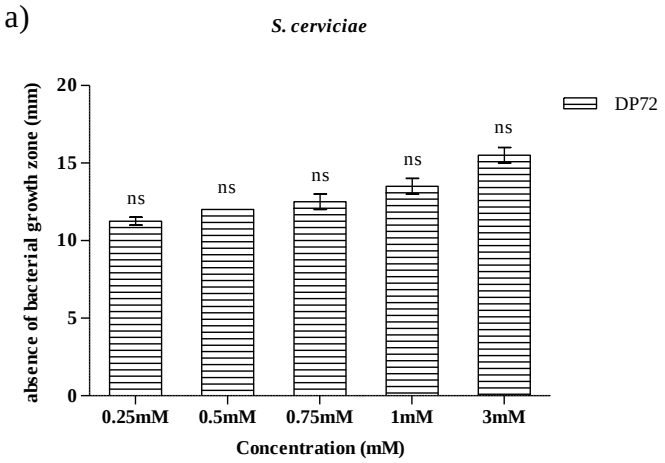
Growth inhibition curve for bacterial strains treated in the presence of compounds. a) DEETTS (6) against *S. aureus* and b) DPSEETTS against *B. subtilis*. Data presented as percentage inhibition ± SEM.



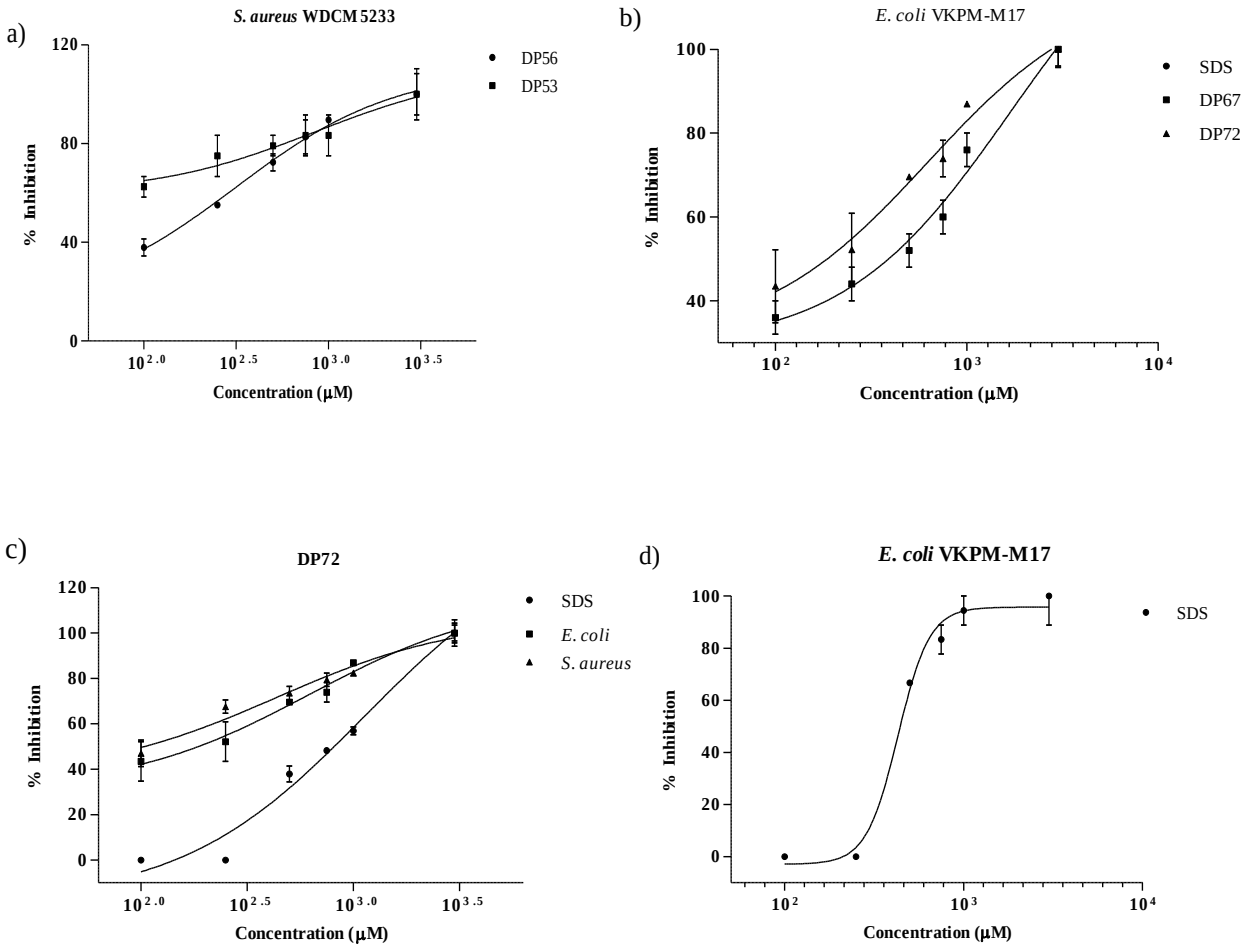
Growth inhibition curve for different strains of bacteria treated with: a) BnTTSPs (8), PrTTSPs (9), PhTTSPs (11) and SDS (14) against *S. aureus* and b) PhTTSPs (11) against *B. subtilis*. Data presented as percentage inhibition ± SEM.



Growth inhibition curve for different strains of yeast treated in the presence of compounds: a) DP80 (19), DP67 (20) and DP72 (24) against *C. guilliermondii* and b) DP72 against *C. albicans*. Data presented as percentage inhibition ± SEM.

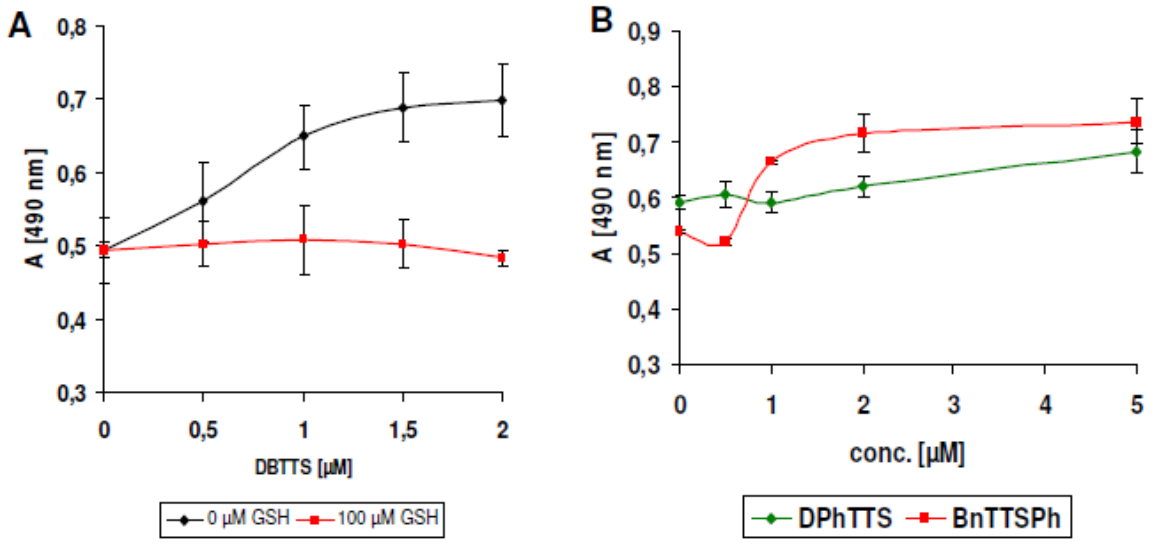


Activity of a) DP72 (24) against *S. cerevisiae*. Data presented as absence of the bacterial growth zone in mm ± SEM of three independent experiments. SDS (14) did not show any effect against *S. cerevisiae*. ANOVA test: n.s. = $p \geq 0.05$ in relation to the relevant values for the particular control SDS.

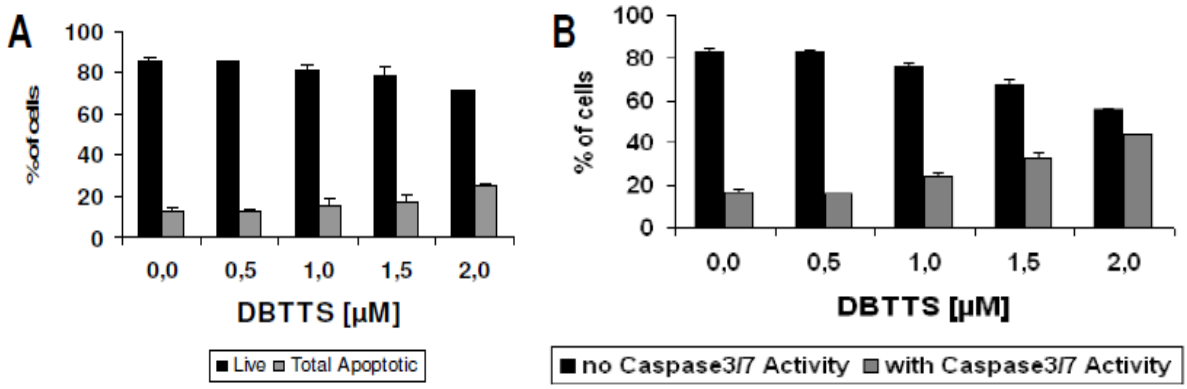


Growth inhibition curve for different bacterial strains treated in the presence of compounds: a) DP56 (25) and DP53 (26) against *S. aureus*, and b) SDS (14), DP67 (20) and DP72 (24) against *E. coli*; c) a comparison of DP72 (24) against *S. aureus* and *E. coli* and d) SDS (14) against *E. coli* control is shown above. Data presented as percentage inhibition ± SEM.

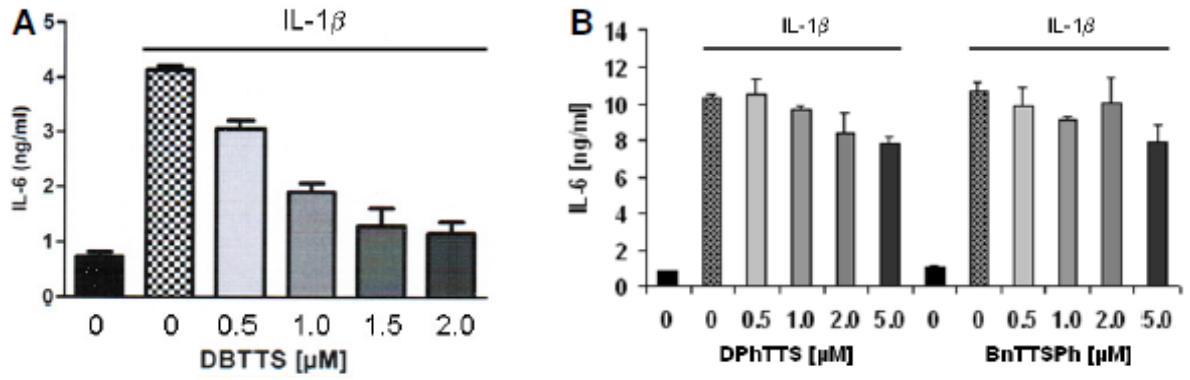
Compound	Chemical Name
SDS (**14**)	Sodium dodecyl sulfate
DP69 (**15**)	Sodium 8-(phenylselanyl)octyl sulfate
DP 49 (**16**)	Sodium 9-(phenylselanyl)nonyl sulfate
DP 70 (**17**)	Sodium 10-(phenylselanyl)decyl sulfate
DP 02 (**18**)	Sodium 11-(phenylselanyl)undecyl sulfate
DP 80 (**19**)	Sodium 12-(phenylselanyl)dodecyl sulfate
DP 67 (**20**)	Sodium 8-(phenyltellanyl)octyl sulfate
DP 68 (**21**)	Sodium 9-(phenyltellanyl)nonyl sulfate
DP 71 (**22**)	Sodium 10-(phenyltellanyl)decyl sulfate
DP 73 (**23**)	Sodium 11-(phenyltellanyl)undecyl sulfate
DP 72 (**24**)	Sodium 12-(phenylselanyl)dodecyl sulfate
DP 56 (**25**)	3-Chloro-4-octanamidobenzeneseleninic acid
DP 53 (**26**)	3-Chloro-4-decanamidobenzeneseleninic acid
DP 58 (**27**)	3-Chloro-4-tetradecanamidobenzeneseleninic acid



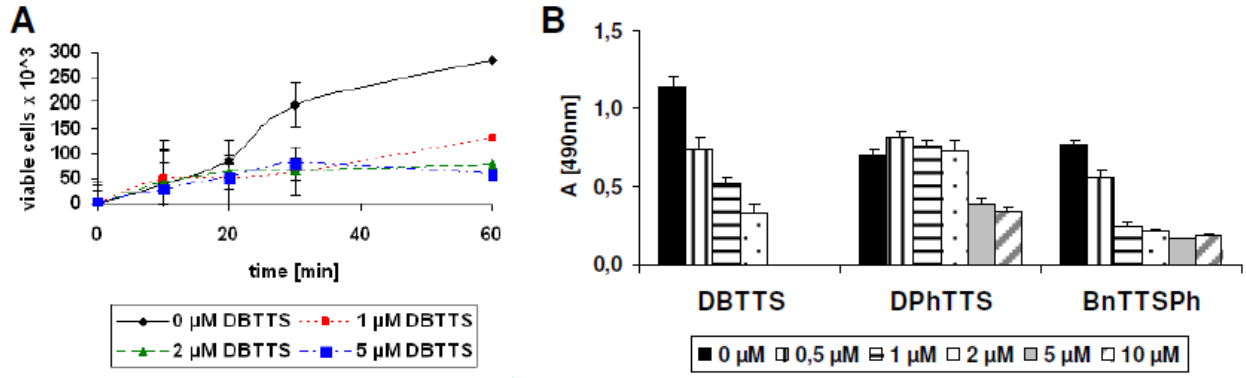
Cytotoxic effects of the MH7A cells when incubated at different concentrations compounds for 24 hours. a) DBTTS (3), b) DPhTTS (7) and BnTTSPh (10). Data represented as the mean +/- SD.



a) Concentration dependent induction of apoptosis by DBTTS as defined by Annexin-V/7-AAD staining technique and b) measurement of caspase 3/7 activity in the presence of different concentrations of DBTTS (3). Data represented as the mean +/- SD.



The inhibition of IL-1β induced secretion of IL-6 of MH7A cells lines, by the technique, ELISA, when incubated with the compounds a) DBTTS (3) and b) DPhTTS (7) and BnTTSPh (10) for 24 hours. Data represented as the mean +/- SD.



Study of the tetrasulfane-mediated influence of the adhesive characteristics of synovial fibroblast cell lines. a) Concentration-dependent inhibition of the adhesion of cells by DBTTS (3) at different time points. b) Comparison of the same effect by DBTTS (3), DPhTTS (7) and BnTTSPh (10) at different concentrations. Data represented as the mean +/- SD.

Curriculum Vitae

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Academic Qualifications

July 2011- August 2014	Doctor of Natural Science (Dr. rer. nat.), Bioorganic Chemistry, School of Pharmacy, University of Saarland, Saarbruecken, Germany
July 2007- April 2009	Master of Science (M. Sc.) in Nanoscience and Technology Department of Nanoscience and Technology, Bharathiar University, Coimbatore, INDIA, Grade 5.97 / 6 (CGPA out of 6).
August 2004- May 2007	Bachelor of Science (B. Sc.) in Bio-Chemistry, Pondicherry University, INDIA, 80 %
June 2002- April 2004	Higher Secondary Examinations, Kendriya Vidyalaya Palakkad Central Board of Secondary Education, India, 76.20 %
June 2000- April 2002	Secondary School Leaving Certificate, Kendriya Vidyalaya, Palakkad Central Board of Secondary Education, India, 78.80 %

Research Experience

July 2011- August 2014	Doctorate student in the group of Prof. Dr. Claus Jacob, Bioorganic Chemistry, School of Pharmacy, University of Saarland, Saarbruecken, Germany. "**Study of metabolic, biochemical, nanotechnological and electrochemical behaviour of novel sulfur, selenium and tellurium compounds**"

September 2012-November 2013	Visiting Doctorate student in Yerevan State University, Armenia. **"Physico-chemical analysis and Antimicrobial activities of Sulfur, Selenium and Tellurium compounds"**
March 2011- July 2011	Visiting Project Assistant, in the group of Dr. Anja Philippi, Cellular Immunotherapy Team, KIST Europe, Saarbruecken, Germany **"T-Cells culturing and Immuno mediated targeted drug delivery"**
January 2011- February 2011	Visiting Project Assistant, in the group of Dr. Kristina Riehemann, R & D in Nanomedicine, CeNTech, Muenster, Germany. **"Inflammatory studies on CD-DOX Nanoperticles"**
August 2010- January 2011	Researcher in the group of Prof. Dr. Kurt Geckeler, department of Nano Biomaterials and Electronics, Gwangju Institute of Science and Technology, South Korea. **"Synthesis and Characterization of complex Nanoparticles**
May 2009- June 2009	Research Assistant in the group of Associate Prof. Dr. N Ponpandian, Department of Nanoscience and Technology, Bharathiar University, TamilNadu, India. "**Amino Acid mediated synthesis of Iron Oxide Nanoparticles**"
January 2009- April 2009	Project Assistant (MSc Thesis work) in the group of Dr. Vetrichelvan, Department of Chemistry, Syngene International Limited, A Biocon Company, Bangalore, India. "**Amino Acid Mediated Synthesis and Characterization of Silver Nanoparticles and Thin Film**