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Synthesis and redox-enzyme modulation by amino-1,4-dihydro-benzo[*d*][1,2]dithiine derivatives

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ABSTRACT

A convenient method to prepare a series of benzodithiine derivatives was developed, via the synthesis of cyclic disulfide building blocks containing an amino-group linker. Some of the novel cyclic disulfide compounds are shown to modulate the activity of the redox-enzyme glutathione reductase.

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The redox activities of cyclic disulfides and their reactivity with thiols and relative redox potentials have been the subject of detailed physico-chemical studies.[1] More recently, the utility of the cyclic disulfide group has found interest in a variety of applications. For example, cyclic disulfides have been used to serve as a bridging molecule between a gold layer and a single-walled carbon nanotube through a thioalkylthiol linkage[2], to attach an oligonucleotide to a gold nanoparticle providing increased stability[3], or as a linker to connect folic acid to gold nanoparticles for potential use as drug delivery vehicles.[4] Although the role of the dithiolane α-lipoic acid in biological processes is well established, the study of synthetic cyclic disulfides remains largely unexplored. Recent examples include the unsubstituted benzodithiine **4** (NO_2 replaced by H), which inhibits Respiratory Syncytial Virus replication[5] and oxidized dithiothreitol, which interferes with HIV-replication by ejecting zinc from Zinc-finger protein via interaction with active-site sulfhydryl groups.[6]

In view of the above, a systematic exploration of a broader range of compounds containing cyclic disulfide building blocks could prove interesting. Although various non-cyclic disulfide compounds have been demonstrated to interact with active-site cysteine residues of a number of proteins[7], they are prone to intracellular reductive inactivation. The 1,4-dihydro-benzo[*d*][1,2]dithiines have a much more negative reduction potential than linear disulfides[1], which provides increased stability. Therefore, we selected the benzodithiines for further exploration. The most convenient way to potentially synthesize a wide variety of cyclic disulfide derivatives would be via a benzodithiine core that has a handle for further derivatization. We now present the synthesis of substituted 1,4-dihydro-benzo[*d*][1,2]dithiines that contain an amino group as a linking unit. These novel building blocks have been reacted with various electrophilic compounds and preliminary studies of their potential biological activity have been carried out via investigation of their potential for modulation of the redox-enzyme glutathione reductase.

A general synthetic scheme is provided in Figure 1. Starting with either 3- or 4-nitro substituted *ortho*-xylene, both 5-amino- and 6-amino substituted benzodithiine derivatives **6** were synthesized, respectively. In the first step, the nitroxylene **1** is brominated with bromine in a biphasic methylene chloride/water mixture, from which the α,α′-dibromide **2** can be isolated and purified via a single crystallization. Nucleophilic substitution with potassium thioacetate in methanol provides bis-thioacetate **3**. Direct hydrolysis of **3** with sodium hydroxide in different solvents led to insoluble products, most likely polymeric polysulfides. However, when the hydrolysis was carried out with ammonium hydroxide in high dilution (0.01 M) in methanol, concomitant air oxidation provided the cyclic disulfide **4** in good yields.[8,9] A last challenge was to reduce the nitro-group of compound **4** to an amino group, while maintaining the reduction-prone cyclic disulfide group intact. Reducing agents such as Fe/HCl, Zn/H_2NNH_2, Ni/HCOOH, or Pd/C/H_2, either did not react, or provided a mixture of unidentified or polymeric products. However, reduction with sodium dithionite successfully provided the desired amino-substituted dihydrobenzodithiins **5**.[12] Subsequently, the amino group could be reacted

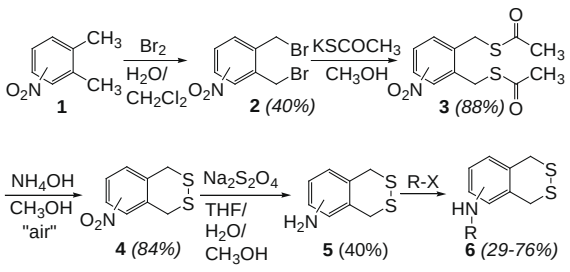

Figure 1. Synthesis of cyclic disulfides depicted in Table 1. In brackets: yields for 6-substituted derivatives.

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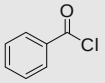
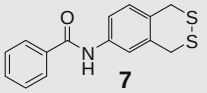
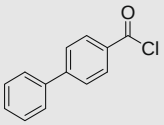
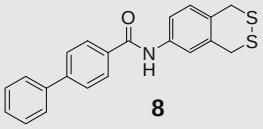
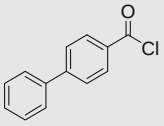
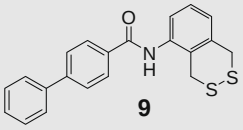
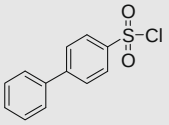
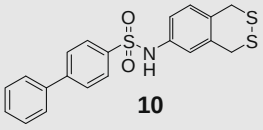
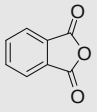
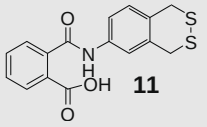
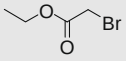
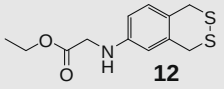
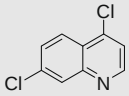
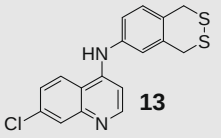
Table 1
Preparation of benzodithiine derivatives and their effects on glutathione reductase activity

Entry	Electrophile	Product[a]	Yield (%)	% Enzyme activity at 50 μM[c]	% Enzyme activity at 25 μM[c]
1		 **7**	76	144.5 ± 13.3	90.5 ± 6.5
2		 **8**	67	18.5 ± 1.9	46.9 ± 5.2
3		 **9**	45	59.4 ± 14.7	72.7 ± 9.8
4		 **10**	55	66.0 ± 2.5	91.0 ± 4.4
5[17]		 **11**	93	90.5 ± 6.9	91.3 ± 15.1
6		 **12**	35	100.0 ± 11.9	101.8 ± 6.2
7[b]		 **13**	29	90.4 ± 7.7	76.2 ± 4.2

[a] The products were formed by adding the electrophile to a solution of 5- or 6-amino-benzodithiine at 0 °C in THF in the presence of 1.2 equiv triethyl amine.
[b] The product was obtained via CuI/*N*,*N*-dimethylglycine/Cs_2CO_3 catalyzed amination reaction in DMSO[15] with microwave heating to 120 °C for 1 h.
[c] Activity of yeast glutathione reductase compared with vehicle [activity = 100%] (for method used see Ref. 16).

with a number of electrophilic compounds to provide derivatives **6**. The products synthesized are summarized in Table 1.

In order to provide a preliminary investigation of their biological potential, the interaction of the new cyclic disulfide compounds **7**–**13** with commercially available yeast glutathione reductase was investigated. The redox-enzyme glutathione reductase is responsible for the reduction of oxidized glutathione (GSSG) to two molecules of reduced glutathione (GSH), with NADPH as the co-reductant (Fig. 2). The enzyme maintains GSH concentrations, and possible inhibitors of this enzyme have been indicated as potential anti-malarial[13] or anti-cancer agents.[14] A reversible

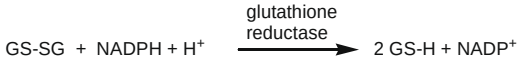


Figure 2. Reaction catalyzed by glutathione reductase.

dithiol-disulfide couple, formed by two active-site cysteine residues provides the key-functionality in the oxidation–reduction process. Based on the reactivity of thiols with disulfides, our novel benzodithiine derivatives seem especially suitable for enzyme modulation, and are intended to be able to interact with the active-site sulfhydryl groups of the enzyme (Fig. 3). Depending on enzyme kinetics, the benzodithiines could prove to be reversible covalent inhibitors, or alternatively function as competitive substrates, leading to dithiol products. Although an extensive pharmacological study is beyond the scope of the present Letter, a proof-of-principle for the structure-dependent activity of benzodithiine derivatives to serve as modulators of cysteine-containing redox enzymes was evidenced by their effect on yeast glutathione reductase. The interference with enzymatic activity of the novel benzodithiine products is summarized in Table 1.

As can be seen from Table 1, at a concentration of 50 μM, three compounds (**8**–**10**) reduce the activity of yeast glutathione reduc-

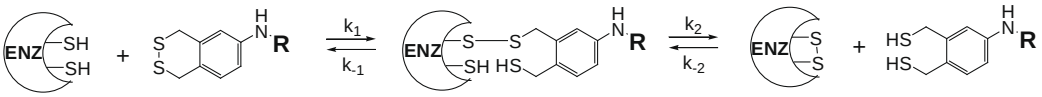


Figure 3. Possible interaction of benzodithiine derivatives with the active-site cysteine residues of glutathione reductase.

tase to 19%, 59%, and 66%, respectively, compared with the baseline enzymatic activity. Thus, at this concentration, compound **8** inhibits more than 80% of enzyme activity, as measured by NADPH consumption. At a concentration of 25 μM, as can be expected, the effect of all three compounds on enzyme activity is much reduced. Nevertheless, even at this reduced concentration, compound **8** shows an enzyme inhibitory activity of about 50%. For comparison, a bis-dithiocarbamate[18] was recently revealed as a covalent irreversible inhibitor of yeast glutathione reductase with $K_i = 56$ μM and $k_{inact} = 0.1\ min^{-1}$.

In contrast to the above compounds, at both 25 and 50 μM concentrations, the modulatory effect of compounds **11** and **12** on enzyme activity is only moderate at most. These data indicate that the observed effects are not only the result of interaction with the cyclic disulfide group, but are also indeed dependent on the complete molecular architecture of the modulating compounds. Interestingly, at a concentration of 50 μM, compound **7** seems to exhibit an increase in activity of glutathione reductase, as measured by an increased rate of NADPH consumption.[19] A similar increase in NADPH consumption was observed in the interaction of *human* glutathione reductase with either ajoene (50% inhibition within 15 min. at 200 μM)[21] or with fluoro-M5 ($IC_{50} = 4.1$ μM).[22] These compounds, respectively, thioalkylate or alkylate an active-site cysteine residue of the enzyme, thereby inhibit the reduction of GSSG to GSH. Nevertheless, the covalently inhibited enzyme shows an increased NADPH-oxidase activity, with a faster turnover of NADPH than in the non-inhibited enzyme. In this case the substrate is not GSSG, but rather, either molecular oxygen or a naphthoquinone derivative, that presumably binds to a second, unidentified binding site. We suggest that the observed increased activity of compound **7**, when compared to enzyme activity in the absence of **7**, is due to a similar increase in oxidase activity, leading to the observed NADPH consumption.[23]

In summary, we have developed a procedure for the easy generation of a library of cyclic disulfides, via the preparation of aminobenzodithiine building blocks. These can easily be connected to a variety of electrophiles, some examples of which have been presented. Potential applications of the presented, and of other benzodithiine derivatives, could be in the fields of nanotechnology, as well as in the modulation of redox enzymes. Some of the examples prepared have been shown to interact with glutathione reductase. Possible interactions of other compounds with this novel pharmacophoric group, targeted to other enzymes with active-site cysteine residues will be investigated.

Acknowledgments

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8. Synthesis of 6-nitro-1,4-dihydro-benzo[*d*][1,2]dithiine **4** (*m*-nitrobenzodithiine): In a 2 L erlenmeyer flask, 2.99 g (10.0 mmol) **3** was dissolved in 1000 mL methanol. To this solution was added 5.8 mL NH_4OH and, while maintaining the vessel at open air, the solution was stirred vigorously for 16 h at room temperature, to obtain a dark-brown solution. The methanol was removed in vacuo on the rotavap (can be recycled for a subsequent batch of the reaction) and the remaining solid was extracted with water and ethyl acetate (2×) (THF may be added to aid in dissolution of the solids). Extraction of the combined organic phases with brine, drying on Na_2SO_4 and removal of the solvents gave a dark-brown solid that was purified by silica gel column chromatography (hexanes/ethyl acetate, 6:1) to provide 1.79 g (8.4 mmol) of a yellowish solid which by TLC (hexanes/ethyl acetate = 6:1: $R_f = 0.78$) contained a minor impurity ($R_f = 0.72$) (probably a dimeric tetrasulfide product, as more of it is observed when the reaction is carried out at higher concentrations). Although further purification by column chromatography is possible, the product can be utilized as obtained for the next step. ^1H NMR (DMSO-d_6) δ 4.28 (2H, s), 4.30 (2H, s), 7.47 (1H, d, $J = 8.4$ Hz), 8.06 (1H, dd, $J = 8.6$ Hz, $J = 2.4$ Hz), 8.11 (1H, d, $J = 2.4$ Hz); ^{13}C NMR δ 34.1, 34.7, 114.0, 116.1, 122.8, 131.0, 133.5, 144.9; HRMS *m/z* calcd for $[C_8H_7O_2N^{32}S_2+H]^+$ 213.9991, found 213.9992.
9. A possible alternative method could be to convert the α,α′-dibromide **2** directly into cyclic disulfide **4** via reaction with Na_2S_2[10], if needed in the presence of a phase transfer catalyst.[10b] However, as aqueous polysulfide S_n^{2-} consists of various homologous anions in equilibrium ($n = 1, 2, 3, \ldots$), the resulting product mixture might contain products with different amounts of S_n which are difficult to separate at a preparative scale.[11] As our further investigations required cyclic disulfides without the possibility of contamination with mono-, tri-, or polysulfides, we preferred the above-described multi-step procedure (Fig. 1).
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12. Synthesis of 6-amino-1,4-dihydro-benzo[*d*][1,2]dithiine **5**. A solution of 1.65 g (7.7 mmol) **4** in methanol/THF/water (60 mL/20 mL/20 mL) was heated in an oil bath of 60 °C. To this solution was added in one portion 5.53 g of (27.0 mmol) 85% $Na_2S_2O_4$ (should be fresh). After 30 min, the reaction was shown to be complete by TLC, and after cooling to room temperature, CH_2Cl_2 and 10% K_2CO_3 were added. The precipitated solids were removed by vacuum filtration, and the aqueous phase was extracted 2× with CH_2Cl_2. The combined organic phases were extracted with water, dried on Na_2SO_4 and after removing the solvent, the obtained solid was purified by silica gel column chromatography (hexanes/ ethyl acetate 3:1) to obtain 0.60 g (3.3 mmol) of an off-white solid. ^1H NMR ($CDCl_3$) δ 3.59 (2H, br s), 3.95 (2H, s), 3.96 (2H, s), 6.40 (d, $J = 2.4$ Hz), 6.52 (1H, dd, $J = 8.4$ Hz, $J = 2.4$ Hz), 6.86 (1H, d, $J = 8.4$ Hz); ^{13}C NMR δ 34.1, 34.7, 114.0, 116.1, 122.8, 131.0, 133.5, 144.9; HRMS *m/z* calcd for $[C_8H_9N^{32}S_2+H]^+$ 184.0249, found 184.0250.
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GSSG was added and the rate of consumption of NADPH was measured for 3 min (scan every 5 s) via its absorption at 340 nm. Each compound concentration was measured in triplicate, and the rate of NADPH consumption was compared with baseline enzyme activity.

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19. The dithiol derivative of compound **7** was prepared independently via reduction with tributylphosphine/H_2O.[20] Separate measurements indicated very low absorption of both **7** and its dithiol reduction product at 340 nm, demonstrating that the observed increase in absorption is not due to substrate interference in the assay procedure, but confirms an increase in NADPH consumption.
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23. The observed effect of increased NADPH consumption observed at the lower concentration of compound **13** could be suggested to occur via a similar mechanism.