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FULL-LENGTH PAPER

Discovering *Echinococcus granulosus* thioredoxin glutathione reductase inhibitors through site-specific dynamic combinatorial chemistry

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Abstract In this study, we report a strategy using dynamic combinatorial chemistry for targeting the thioredoxin (Trx)-reductase catalytic site on Trx glutathione reductase (TGR), a pyridine nucleotide thiol-disulfide oxido-reductase. We chose *Echinococcus granulosus* TGR since it is a bottleneck enzyme of platyhelminth parasites and a validated pharmacological target. A dynamic combinatorial library (DCL) was constructed based on thiol-disulfide reversible exchange. We demonstrate the use of 5-thio-2-nitrobenzoic acid (TNB) as a non-covalent anchor fragment in a DCL templated by *E. granulosus* TGR. The heterodimer of TNB and bisthiazolidine (**2af**) was identified, upon library analysis by HPLC ($IC_{50} = 24\,\mu M$). Furthermore, 14 analogs were synthetically prepared and evaluated against TGR. This allowed the study of a structure–activity relationship and the identification of a disulfide TNB-tricyclic bisthiazolidine (**2aj**) as the best enzyme inhibitor in these series, with an $IC_{50} = 14\,\mu M$. Thus, our results validate the use of DCL for targeting thiol-disulfide oxido-reductases.

Keywords Thioredoxin glutathione reductase · Dynamic combinatorial chemistry · Bisthiazolidines · Sulfur-heterodimers · Drug discovery

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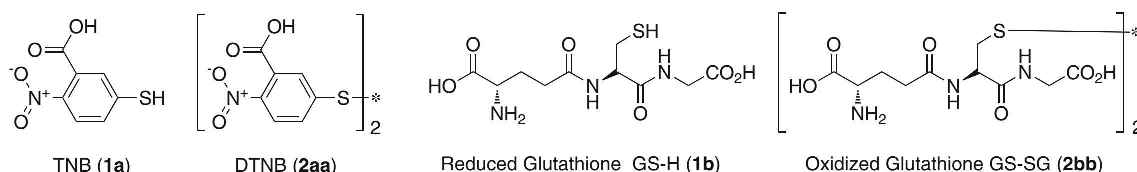
Introduction

Chemical Biology and Drug Discovery disciplines are continuously looking for new small-molecule ligands. The preferred methods used for the generation of lead compounds are high throughput screening [1], rational design [2], and fragment-based approaches [3]. The latter has become increasingly widespread both in academia and industry [4,5].

During the last decade, a fragment-based methodology was developed combining thermodynamic control with combinatorial libraries: dynamic combinatorial chemistry (DCC; [6–8]). This strategy merges the chemical synthesis with biological tests in one single pot. The use of proteins as templates in dynamic combinatorial libraries (DCLs) has been reported for ligand identification more than a decade ago. The template could bind and stabilize the best compound (hit) from a discrete library, through a library redistribution, via reversible bonds. This stabilization can result on an amplification of the best binder. Recent advances include the discovery of glutathione-*S*-transferase inhibitors [9,10], the study of adenosine-binding to the *M. tuberculosis* pantothenate synthetase [11], the discovery of an Aurora kinase inhibitor through site-specific DCC [12], and the identification of a selective small-molecule ligand for a vital RNA regulator of the HIV-1 life cycle [13].

Parasitic flatworms are responsible for serious infectious diseases that affect humans as well as livestock animals in many countries [14]. The emergent drug resistance is a health threat that requires the development of new antiparasitic



Scheme 1 Synthetic and natural substrates of TGR

drugs [15]. *Echinococcus granulosus* is a flatworm parasite responsible for cystic echinococcosis or cystic hydatid disease; the adult worm parasitizes the small intestine of *canids*, whereas the larval stage (hydatid cyst) parasitizes livestock and humans.

In most living organisms, two major enzymatic systems are responsible for providing reducing equivalents through thiol-disulfide exchange: the thioredoxin (Trx) and the glutathione (GSH) pathways. In contrast, platyhelminth parasites lack typical Trx and GSH systems. In these parasites conventional thioredoxin (TR) and glutathione (GR) reductase enzymes are absent and reduction of oxidized Trx and GSH (GS-SG) are carried out by the selenoenzyme thioredoxin glutathione reductase (TGR) [16,17], a fusion of GSH and TR domains. Therefore, the thiol redox homeostasis is dependent on this essential single enzyme, which is a chockpoint of flatworm metabolism. The lack of alternative redox systems makes TGR a key drug target for flatworm infections [18,19]. Recent studies support this idea: inhibition of TGR expression by RNA-interference caused death of the platyhelminth parasite *Schistosoma mansoni*; Auranofin, a potent TGR inhibitor, causes partial cure in experimental *Schistosoma* infection [20]. There are also reported TGR inhibitors that efficiently killed, in vitro, *E. granulosus* larval worms and *Fasciola hepatica* newly excysted juveniles [21].

In this study, we describe a general DCC approach to probe the *E. granulosus* TGR binding sites as a model system. We use thiol-disulfide exchange DCL, templated by TGR, a robust enzyme compatible with the required experimental conditions. We identified novel TGR inhibitors using 5-thio-2-nitrobenzoic acid (TNB) as fragment anchor.

Results and discussion

Library design and synthesis

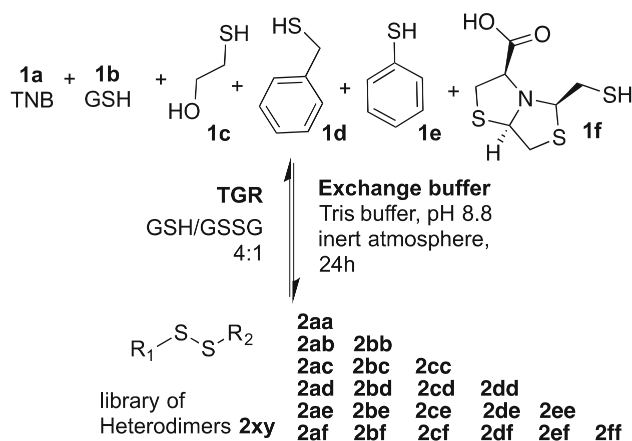
It has been well established that thiol-disulfide exchange can occur under mild conditions, usually compatible with biological templates [12,13,22]. In this work, we describe the generation of a thiol-disulfide DCL using TGR as a template.

We designed a biased library based on two anchor thiols that bind to different sites of TGR: TNB acid (**1a**), and reduced GSH (**1b**), see Scheme 1. Ellman's reagent (5,5′-dithiobis 2-dinitrobenzoic acid [DTNB] **2aa**) is a synthetic substrate of TGR and it is reduced to TNB by the selenocysteine-containing carboxy terminal redox center of TGR (TR domains). Oxidized GSH (GS-SG, **2bb**) is a natural substrate of TGR and is reduced to GSH by the N-terminal redox center of the enzyme (Grx domain). Both DTNB and GS-SG are reduced in presence of NADPH, an essential cofactor needed for the activity [23]. Our approach was to use these substrates as binding recognition sites and to explore further structural motifs in order to find new lead compounds.

The library was prepared using TNB (**1a**), GSH (**1b**), commercially available thiols **1c–e** and the synthetic thiol **1f**, all in one molar ratio at 200 μM, except for GSH that was used in excess. The redox buffer GSH/GS-SG (4:1) was used to favor the thiol-disulfide exchange as Ladame et al. reported [24]. The selected scaffold was bisthiazolidine **1f**, as we envisioned a possible interaction with the binding sites of TGR due to its structural similarity with the amino acid L-cysteine. The thiols can exchange disulfide bonds and could theoretically produce a total of 21 different disulfide compounds **2xy** at basic pH 8.8, see Scheme 2.

It is important to notice that the template experiment was performed in the absence of NADPH, so formally the TGR enzyme was inactive.

The dynamic library was constructed in aqueous Tris (50 mM) buffer at pH 8.8, which increases the R-S$^-$/R-SH ratio, and favors exchange reactions, since the thiolate is the reactive species. The enzyme is still active at pH 8.8 (80 %



Scheme 2 Virtual dynamic combinatorial library of thiols **1a–f** and disulfides **2xy**

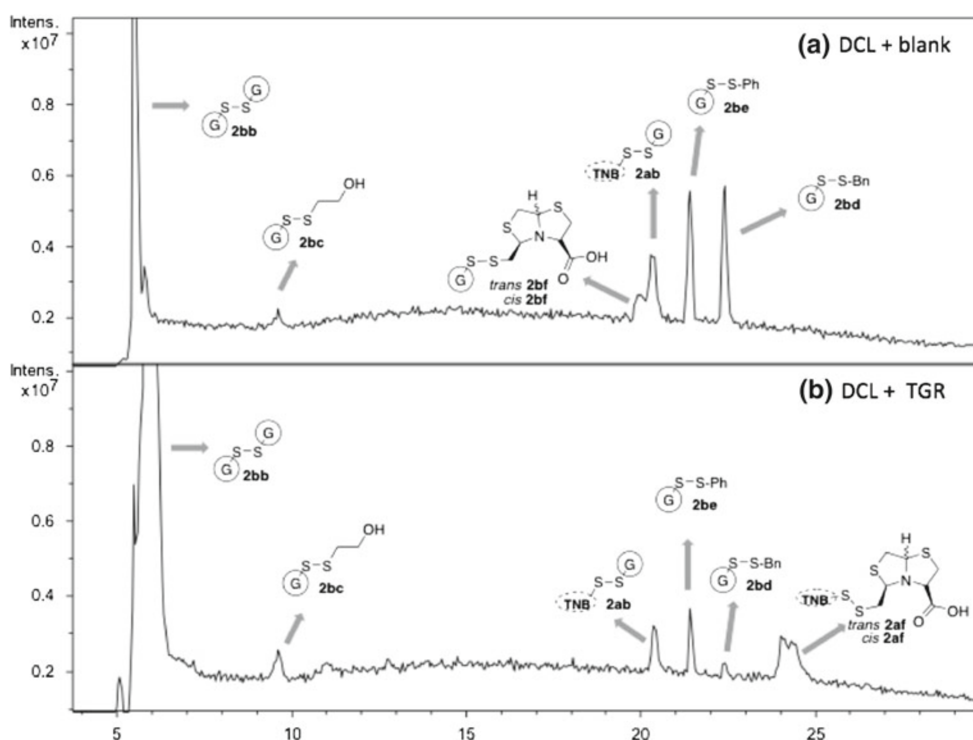


Fig. 1 LC–MS analysis of the libraries (full scan negative ion-MS). **a** Library distribution in absence of TGR template, **b** library distribution in presence of TGR template

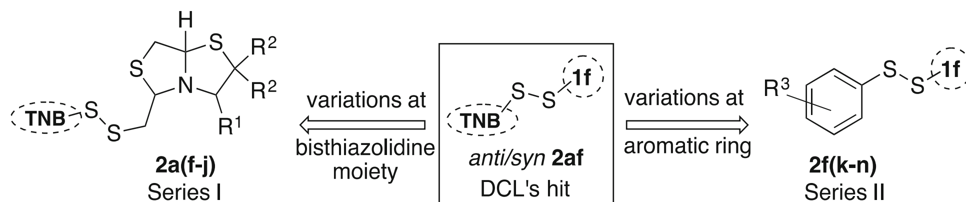


Fig. 2 Structural variation proposed for Series I and II

compared to standard conditions at pH 7.0) and therefore most of the protein is in its native conformation during the template selection/amplification process. Thiols **1a**–**f** were added under the mentioned conditions, and once the equilibrium was achieved (24 h) the mixture was split into two vials: template (TGR) was added to one vial and a buffer solution was added to the other one as a control. The libraries were re-equilibrated in absence of O_2 for another 24 h. The inter-conversion was stopped by addition of trichloroacetic acid (50 % aq.) to both DCLs. The acidic media produced also denaturation of the enzyme, allowing protein filtration to analyze the library composition [11]. In order to compare the libraries' distribution pattern, both systems were analyzed by HPLC-MS in negative ion mode. As it is shown in Fig. 1a, in the absence of protein only a mixture of all the possible thiol-GSH disulfides was observed. This library distribution is expected due to the large excess of GSH (1.5 mM) used in the system. When the enzyme is present, the chromatogram shows a different distribution pattern, see Fig. 1b. Two new signals were identified corresponding to the diastereomeric mixture of heterodimers *cis*/*trans*-**2af**, the conjugated compound that combines TNB (**1a**) with the bisthiazolidines *cis*/*trans*-**1f**. The amplification of *cis*/*trans*-**2af** was observed with the correlative disappearance of adduct *cis*/*trans*-**2bf**, a shift in the equilibrium towards the new stabilized species.

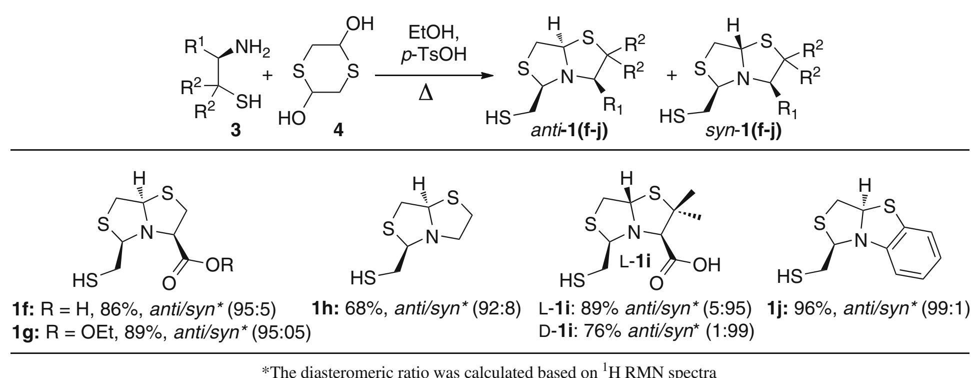
Synthesis of bisthiazolidines heterodimers

Building upon these promising results, we started an independent synthesis of the library hit **2af** and the analogs **2a(g–j)**, **2(k–n)f**, see Fig. 2. The variations were performed at the bisthiazolidine moiety (Series I) and at the aromatic ring of TNB (Series II), see Fig. 2.

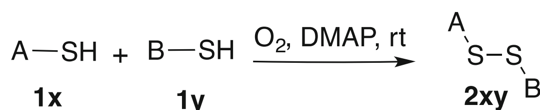
Synthesis of bisthiazolidine scaffolds

Bisthiazolidines **1f–j** were prepared by double cyclization of aminothiols and mercaptoacetaldehyde following the

Scheme 3 Synthesis of bisthiazolidines **1f–j**



*The diasteromeric ratio was calculated based on ^{1}H RMN spectra



Scheme 4 General preparation of **A**–S–S–**B** heterodimer

methodology recently developed by our group, see Scheme 3 [25].

We obtained five bisthiazolidines from readily available starting materials, in high diastereomeric ratios (*trans*/*cis*, dr from 98:2 to 99:1), see Scheme 3. The *trans*-diastereomer was mainly found when using α,α unsubstituted aminothiols like cysteine, cysteamine or *o*-amino-mercaptobenzene, compounds **1f–h** and **j**. Compound *cis*-**1i** was only observed when the α, α disubstituted penicillamine was used as starting aminothiol, see Scheme 3. Isolation and characterization of *trans*-**1i** was possible by ^{1}H NMR and ^{13}C NMR but re-equilibration to the *cis*-**1i** was observed after two days in solution. The relative configuration of the diastereomers **1f–h** and **j** was confirmed and elucidated by NOESY correlations, ^{1}H and ^{13}C NMR.

Heterodimer preparation

All the heterodimers **A**–S–S–**B** were prepared using O_2 by simple exposure to air of thiols **A**–SH and **B**–SH in the presence of DMAP as the catalytic base, where **A**–SH represents bisthiazolidine rings and **B**-SH aromatic thiols, see Scheme 4.

Synthesis and evaluation of heterodimers with variation at bisthiazolidine moiety: Series I

The oxidative coupling between different bisthiazolidines **1f–j** and TNB (**1a**), produced the heterodisulfides **2xy**, the yields ranged from moderate to good (35–62 %), see Table 1.

It is noteworthy that the coupling of bisthiazolidine **1f** with TNB produced a diastereomeric mixture of the heterodimer **2af** in a 3:1 ratio. As previously assessed by our group, thiazolidines can re-equilibrate *via* iminium ion into the most stable product [25,26]. This effect was only observed for the heterodimers **2af** and **g**, affording an inseparable diastereomeric mixture 3:1 and 4:1, respectively. The other heterodimers maintained the configuration of the bisthiazolidine used for its formation.

The ability of the prepared heterodimers **2a(f–j)** to inhibit TGR was tested at 30 μM, as it is shown in Table 1. The library hit **2af**, synthetically obtained as a diastereomeric mixture (74/26), exhibits an inhibition of 62 %, see entry 1, Table 1. The ethyl ester of the carboxylic acid present in the bisthiazolidine (**2ag**) led to a complete loss of the inhibitory activity, see entry 2 Table 1. Elimination of the carboxylic acid present in the heterocycle, compound ±**2ah**, maintains the activity at 63 %, see entry 3, Table 1. α, α-Dimethyl bisthiazolidine **L-2ai** and **D-2ai** showed inhibition values of 18 and 58 %, respectively (see entries 4 and 5, Table 1). These results evidenced some stereo-preference in the recognition site of the enzyme, since compound **D-2ai** prepared from D-penicillamine is three times more active than **L-2ai**, the inhibition value for **D-2ai** is also quite similar to the reference **2af**. The most active compound for this series is the tricyclic compound **2aj** showing an inhibition value of 85 %, see entry 6, Table 1. This derivative contains a phenyl group instead of the carboxylic acid at the bisthiazolidine.

The theoretical lipophilicity (log*P*) for all the Series I was calculated (Table 1) [27]. This parameter partly correlates with the inhibitory activity, since the most lipophilic compounds **2ah** and **j**, are also the most active (Table 1). However, the acid **2af** and ethyl ester **2ah** do not follow this trend, since **2af** is less lipophilic than the ester. The activity in these compounds could be related to an electrostatic or H-bond interaction between the carboxylic acid at the bisthiazolidine heterocycle and the protein.

Synthesis and evaluation of heterodimers with variations at the aromatic ring: Series II

Compounds **2(k–n)f** from Series II were designed by changing the substituents present in the aromatic ring of TNB but keeping the bisthiazolidine heterocycle. Compounds were obtained following the general Scheme 4, with yields ranging

Table 1 Series I, synthesis and TGR inhibition at [Inh] = 30 μM of compounds **2a(f–j)**

Entries	A-	Products	Yield % (dr)[a]	TGR inhibition (%) ± SD[b]	Log*P*[c]
1	*trans*-**1f**	**2af**	62 (3:1)	62 ± 2	1.38
2	*trans*-**1g**	**2ag**	48 (4:1)	10 ± 2	3.83
3	(±) *trans*-**1h**	(±)**2ah**	41 (92:8)	63 ± 2	3.60
4	*(2R,5S,8R) cis-* **1i**	L-**2ai**	35 (95:5)	18 ± 1	2.19
5	*(2S,5R, 8S) trans-* **1i**	D-**2ai**	44 (99:1)	58 ± 1	2.19
6	(±) *trans*-**1j**	(±)**2aj**	43 (95:5)	85 ± 1	5.16

[a]Diastereomeric ratio; [b]Values are means of three independent experiments; [c]Log *P* calculated logarithm of compound partition coefficient between *n*-octanol and water

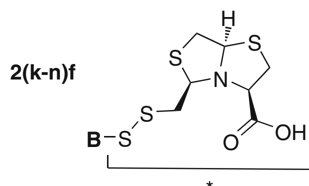
from 19 to 54 %, see Table 2. Isomerization of the bisthiazolidine moiety for these compound series was not observed. Finally, two homo-dimers were also prepared according to the general reaction shown in Scheme 4, *trans*-**2ff** (75 % yield), and *trans*-**2jj** (67 % yield) see entries 5 and 6, Table 2.

The prepared disulfides were screened for TGR inhibition at 30 μM, as shown in Table 2. Compounds bearing electron withdrawing groups (EWGs) on the aromatic ring **2lf** and **2mf**, present 10 and 0 % activity, see entries 2 and 3, Table 2; both being less active than the reference **2af** (*I*% = 62). Compounds with electron-donating group (EDG), like **2kf**, or no substituents (**2nf**), present 25 and 12 % inhibitory activities, respectively, see entries 1 and 4, Table 2. The inhibition values do not show a clear correlation between the structures since EWG and EDG are mainly inactive. The disulfides **2ff**, **2jj** are also less active than **2af**, see entries 5 and 6, Table 2. All together the results suggest that the aromatic ring conjugated with both a *p*-NO_2 group and a *m*-COOH group (e.g., TNB) has a major contribution to the inhibition activity, and this region cannot be easily modified without loss of activity.

IC_{50} determination

Firstly, when compound **2af** was tested as an enzyme substrate; in absence of DTNB, no NADPH oxidation was observed, indicating that **2af** is not a mixed-disulfide substrate of TGR. Compounds with the highest *I*% were selected for IC_{50} determination: **2af**, **2ah** and **2aj**; values are shown in Fig. 3. Compound **2aj** presents the highest activity with an IC_{50} value of 14 μM, 1.7-fold more active than the library hit **2af** (IC_{50} = 24 μM). Also **2aj** is easier to prepare and purify than **2af**. Furthermore, similar to **2af**, bisthiazolidine **1f** was not a TGR substrate. All these results evidenced that

Table 2 Series II, synthesis and TGR inhibition at [Inh] = 30 μM of compound **2(k–n)f**



Entries	B-*	Products	Yield (%)	TGR inhibition (%) ± SD
1	*	**2kf**	54	25 ± 1
2	Br *	**2lf**	49	10 ± 1
3	HO_2C *	**2mf**	19	0
4	*	**2nf**	25	12 ± 1
5	HO O N S S H *	**2ff**	75	0
6	N S S H (±) *	**2jj**	67	10 ± 2
7	H S S N HS O OH **1f**	**1f**	–	0

I% % of TGR Inhibition, values are means of three independent experiments

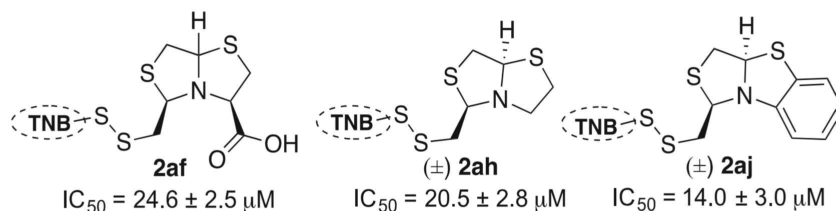


Fig. 3 IC_{50} ± SD values of compounds **2af**, **h**, **j**

the presence of a TNB-mixed disulfide is an essential factor to achieve enzyme inhibition.

Conclusions

We have demonstrated the use of thiols as useful building blocks in a DCL using *E. granulosus* TGR as a template. The disulfide **2af** was identified by amplification as the best binding compound, a heterodimer combining TNB (**1a**) and bisthiazolidine **1f**. Independent synthesis of **2af** and 14 analogs were carried out for biological evaluation on TGR. This confirmed **2af** as an inhibitor and allowed the identification of a novel disulfide, **2aj**, approximately twice more active than **2af**.

It is important to note that TGR inhibition assays use the non-natural DTNB substrate and the exact nature of the binding site to the enzyme is not known, since a high-resolution structure of the complex has not been determined. Our findings show that TNB moiety is an essential part of the inhibitor molecule that could be recognized by the enzyme through a similar interaction used for the substrate DTNB. On the other hand, the bisthiazolidine portion could be broadly modified while maintaining the inhibition profile.

We have provided a proof-of-concept for a screening platform for TGR and were able to identify low-affinity fragment hits *via* formation of reversible covalent bond thiol/disulfide. This report should encourage medicinal chemists to consider protein-directed synthesis for exploring the conformational space of a ligand-binding pocket and the ability of the protein to guide its inhibitor.

Experimental part

Expression and purification of recombinant TGR

Expression and purification of *E. granulosus* TGR was carried out as previously described [28]. In brief, transformed *E. coli* cells were pre-cultured O/N at 37 °C. Then they were diluted (1:100) and cultured at 37 °C in Luria–Bertani broth (LB) or "modified-rich LB", supplemented with 0.1 g/L cysteine and 0.37 g/L methionine, in the presence of kanamycin (50 µg/mL), and chloramphenicol (33 µg/mL). Induction of recombinant proteins was carried out with 100 µM isopropyl 1-thio-β-D-galactopyranoside at late exponential phase (A_{600} 2.4), during 24 h at 24 °C. At the time of induction the culture was supplemented with 5 µM sodium selenite, 20 µg/mL riboflavin, 20 µg/mL pyridoxine, and 20 µg/mL niacin according to a previous study. The bacterial cultures were centrifuged, and the pellets were resuspended in modified nickel–nitrilotriacetic acid lysis buffer (300 mM NaCl, 50 mM sodium phosphate, 20 mM imidazole, pH 7.2) containing 1 mM phenylmethylsulfonyl fluoride and 1 mg/mL lysozyme, and sonicated (10 pulses of 1 min with 1-min pauses). The lysates were centrifuged for 1 h at 30,000 × *g*, and supernatants were applied to a nickel–nitrilotriacetic acid column (Qiagen), washed with 300 mM NaCl, 50 mM sodium phosphate, 30 mM imidazole, pH 7.2, and eluted with 250 mM imidazole. The protein-containing fractions were applied to PD10 desalting columns (GE Healthcare) using phosphate-buffered saline, 150 mM potassium chloride, 50 mM sodium phosphate, pH 7.2. Fractions containing the recombinant proteins were stored at −70 °C before use. Total protein concentration and FAD content were determined spectrophotometrically at 280 (ε = 54.24/mM/cm) and 460 nm (ε = 11.3/mM/cm), respectively. The selenium content of selenoproteins was determined by atomic absorption using a Plasma Emission Spectrometer (Jarrell-Ash 965 ICP) in Chemical Analysis Laboratory, University of Georgia. The purity of the recombinant proteins was analyzed by running 10 % SDSPAGE gels, under reducing conditions, and by size-exclusion chromatography on a Superose 12 column (GE Healthcare). Selenium incorporation was 10 % and FAD incorporation was 14 % in the batch used in the present work. The total protein content was 680 µM in the assay. The concentration of active protein was 10 µM considering the amount of Sec and FAD incorporated [680 µM (total protein) × 14/100 (FAD) × 10/100 (Sec)].

DCL preparation

All buffered stock solutions were prepared in Tris buffer (50 mM, pH 8.8). Stock solutions of thiols were freshly prepared in dimethylsulfoxide (DMSO) at 20 mM. To a stirred solution of Tris buffer (9.4 mL) were added: 5 thiols solutions (5 × 100 µL), 100 µL of ethanol, GSH (4.6 mg) and GS-SG (2.3 mg). Oxygen was removed by three cycles of vacuum/N_2 and protected from light. After 24 h *E. granulosus* TGR (50 µL) was added to 50 µL of the equilibrated mixture in an eppendorf tube; the final concentration of protein was 340 µM. A blank was performed in parallel by adding 50 µL of buffer (instead of TGR solution) to 50 µL of the equilibrated mixture. The final volume of the DCL was 100 µL. After 24 more hours in absence of O_2, the exchange was quenched by the addition of an aqueous solution of trichloroacetic acid 50 % (20 µL). Denatured protein was removed by centrifugation, spinning at 14,000 rpm for 5 min (×3). The supernatant was filtered and injected into the HPLC-MS.

Library Amplification

HPLC-MS analyses were performed on a HPLC Agilent 1200 equipped with a diode array detector, binary pump and a thermostated column at 40 °C, coupled to an ion-trap Mass spectrometer Esquire 6000 (Bruker Daltonik GmbH). The samples were analyzed with a reverse 5 µm Luna-C18 column (Phenomenex) 150 mm × 4.6 mm. The mobile phases were formic acid (10 mM in ultra-pure H_2O; A) and acetonitrile (B). Injection volume: 20 µL, the samples were in the buffer solution used to prepare the DCLs. Eluent B was held at 2 % for 5 min, increased to 85 % over 45 min. Then it was decreased to 2 % over 5 min and finally with an isocratic period of 2 % of B over 5 min. The total run time is 60 min and the flow rate 0.8 mL/min. The flow is split in two before going into de MS. The analysis uses a trap-ion with electrospray ionization, alternating positive–negative ions. Electrospray conditions: endplate off set, voltage −500 V, capillary voltage −4,000 V, N_2 nebulizer (40 psi), N_2 drying flow of 9.0 L/min and a temperature of 350 °C.

DTNB reduction assay for the TR activity of TGR

The TR activity was measured using the DTNB reduction assay [23]. The NADPH-dependent reduction of DTNB was followed by the increase in absorbance at 412 nm due to the formation of 5-thionitrobenzoic acid at 25 °C (ε = 13.6/mM/cm). The absorbance was recorded using a PG-T70+ spectrophotometer (PG Instruments, UK) connected to a temperature control device. The reaction mixtures (1 mL) contained 100 µM NADPH, 10 mM EDTA, 5 µL DMSO and 1 nM *E. granulosus* TGR in 100 mM potassium phosphate buffer (pH 7). The reaction was initiated by the addition of 50 µM DTNB. All experiments were performed in triplicate.

TGR inhibition studies

Inhibitor stock solutions were prepared at a final concentration of 10 mM in DMSO. The screening was performed using the DTNB assay for TR activity.

TGR in 50 mM potassium phosphate buffer pH 7, NADPH and 5 μL of inhibitor stock solution was preincubated for 4 min. The reaction started by addition of DTNB and Abs_{412} was recorded for 3 min. In every case, a control progress curve without enzyme was performed to control for non-catalyzed reactions between substrates and inhibitors. % TGR inhibition was calculated: $v_1/v_0 \times 100$, with v_1 and v_0 corresponding to the initial velocities of TNB formation (mM)/t(s) with and without inhibitor, respectively.

Synthesis of bisthiazolidines **1f–j**

*(2R,3S,8R)-8-carboxylate-2-mercaptomethyl-1-aza-3, 6-dithiobicyclo[3.3.0] octane (trans-**1f**)*

To a stirred suspension of L-cysteine (0.5 g, 4.1 mmol) in EtOH (16 mL), was added 1,4-dithiane-2,5-dithiol **4** (0.8 g, 5.0 mmol) and *p*-TsOH ac (0.030 g, 0.17 mmol). The mixture was heated to reflux for 2 h. Then it was cooled down and poured into brine, extracted with CH_2Cl_2 (5 × 30 mL), dried (Na_2SO_4) and filtered. The solvent was removed under reduced pressure and the residue was purified by chromatography on SiO_2 (EtOAc/hexanes/AcOH, 1:3:0.1) to led compound **1f** (0.830 g, 86 %, *trans*/*cis* 95/05) as a white solid: mp 103–104 °C; ^{1}H NMR ($CDCl_3$) δ 1.86 (t, *J* = 8.5 Hz, $1H_{SH}$), 2.81 (dd, *J* = 8.5, 6.9 Hz, 2H), 3.11 (dd, *J* = 12.0, 4.2 Hz, 1H), 3.33 (dd, *J* = 11.4, 7.1 Hz, 1H), 3.43 (dd, *J* = 11.4, 3.3 Hz, 1H), 3.55 (dd, *J* = 12.0, 5.8 Hz, 1H), 4.25 (dd, *J* = 7.1, 3.3 Hz, 1H), 4.32 (t, *J* = 6.9 Hz, 1H), 5.05 (dd, *J* = 5.8, 4.2 Hz, 1H); ^{13}C NMR ($(CD_3)_2CO$) δ 34.0, 34.3, 39.2, 71.5, 74.5, 75.6, 172.1; HRMS calculated for $C_7H_{11}NO_2S_3$, $[M+H]^+$ 238.0025, found: 238.0033; $\alpha_D = -57.7°$ (20 °C, AcCN, *c* = 0.6).

*(3S,5R,7aR)-ethyl 5-(thiomethyl)tetrahydro-2H-thiazolo [4,3-b]thiazole-3-carboxylate (trans-**1g**)*

Prepared in analogous route as described for *trans*-**1f**, starting from L-cysteine ethyl ester HCl. Purification by chromatography on SiO_2 (1:3, EtOAc:hexanes) led to compound **1g** (89 %, *trans*/*cis* 95/05) as an oil: *trans*-**1g**: ^{1}H NMR ($CDCl_3$) δ 1.30 (t, *J* = 7.1 Hz, 3H), 1.98 (dd, *J* = 9.5, 7.5 Hz, 1H), 2.64 (ddd, *J* = 13.6, 9.5, 6.1 Hz, 1H), 2.89 (ddd, *J* = 13.6, 7.5, 7.5 Hz, 1H), 3.09 (dd, *J* = 11.8, 3.9 Hz, 1H), 3.28 (dd, *J* = 10.9, 6.6 Hz, 1H), 3.32 (dd, *J* = 10.9, 5.1 Hz, 1H), 3.54 (dd, *J* = 11.8, 5.5 Hz, 1H), 4.21 (dd, *J* = 6.6, 5.1 Hz, 1H), 4.23 (q, *J* = 7.1 Hz, 2H), 4.30 (dd, *J* = 7.5, 6.1 Hz, 1H), 5.09 (dd, *J* = 5.3, 3.9 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 14.1, 33.7, 34.2, 39.1, 61.7, 70.3, 73.3, 74.9, 170.4; HRMS calculated for $C_9H_{16}NO_2S_3$, $[M+H]^+$ 266.0343, found 266.0329; $[\alpha]_D = -256°$ (20 °C, MeOH, *c* = 0.6).

*±(5RS,7aRS)-tetrahydro-2H-thiazolo[4,3-b]thiazol-5-yl)methanethiol (trans-**1h**)*

Prepared in an analogous route as described for *trans*-**1f**, starting from cysteamine. Purification by chromatography on SiO_2 (1:6, EtOAc:hexanes) led to compound **1h** (68 %, *trans*/*cis* 92:08) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.84 (dd, *J* = 9.1, 7.6 Hz, 1H), 2.65 (ddd, *J* = 13.6, 9.1, 6.0 Hz, 1H), 2.82 (ddd, *J* = 13.6, 7.6, 7.0 Hz, 1H), 3.12 (m, 1H), 3.08 (m, 2H), 3.21 (ddd, *J* = 11.5, 7.2, 6.6 Hz, 1H), 3.48 (ddd, *J* = 11.5, 5.7, 4.8 Hz, 1H), 3.54 (ddd, *J* = 11.6, 5.2, 0.5 Hz, 1H), 4.24 (dd, *J* = 7.0, 6.0 Hz, 1H), 4.97 (dd, *J* = 5.2, 3.7 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 31.8, 33.5, 38.4, 57.1, 73.2, 74.5; HRMS calculated for $C_6H_{12}NS_3$, $[M]^+$ 194.0132, found: 194.0156.

*(2R,5S,8R)-2-mercaptomethyl-7-dimethyl-8-carboxylate-1-aza-3, 6-dithiobicyclo [3.3.0]octane cis-L-**1i**)*

Prepared in analogous route as described for **1f**, starting from L-penicillamine. Purification by chromatography on SiO_2 (1:3, EtOAc:hexanes) led to compound **1i** (89 %, *cis*/*trans*: 95:05).

Cis-L-**1i** white solid, mp 89–97 °C; ^{1}H NMR ($CDCl_3$) δ 1.52 (s, 3H), 1.62 (s, 3H), 1.89 (t, *J* = 8.7 Hz, $1H_{SH}$), 2.81 (m, 2H), 3.06 (dd, *J* = 11.7, 5.4 Hz, 1H), 3.43 (dd, *J* = 11.7, 6.6 Hz, 1H), 3.80 (s, 1H), 4.31 (t, *J* = 7.3 Hz, 1H), 4.98 (dd, *J* = 6.6, 5.4 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 28.0, 28.1, 32.0, 40.5, 55.1, 68.7, 75.7, 78.5, 170.4; HRMS calculated for $C_9H_{16}NO_2S_3$, $[M+H]^+$ 266.0343, found 266.0330; $[\alpha]_D = -45.2°$ (20 °C, MeOH, *c* = 1.0).

Trans-L-**1i** foamy oil, ^{1}H NMR ($CDCl_3$) δ 1.49 (s, 3H), 1.66 (dd, *J* = 9.0, 8.3 Hz, $1H_{SH}$), 1.77 (s, 3H), 2.66 (m, 1H), 2.87 (m, 1H), 3.21 (dd, *J* = 10.1, 5.2 Hz, 1H), 3.38 (dd, *J* = 10.1, 8.1 Hz, 1H), 3.97 (s, 1H), 4.55 (dd, *J* = 7.3, 5.7 Hz, 1H), 5.07 (dd, *J* = 8.1, 5.2 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 25.6, 32.9, 33.9, 40.5, 58.6, 70.1, 70.5, 77.0, 174.6.

*(2S,5R,8S)-2-mercaptomethyl-7-dimethyl-8-carboxylate-1-aza-3,6-dithiobicyclo [3.3.0]octane cis-D-**1i***

Prepared in an analogous route as described for **1f**, starting from D-penicillamine. The residue was purified by chromatography on SiO_2 (1:3, EtOAc:hexanes) led to compound *cis*-D-**1j** (76 %, *trans*/*cis*: 01:99). The spectroscopic properties were identical to those obtained for *cis*-**L-1i**, $[\alpha]_D = 40.0°$ (20 °C, MeOH, *c* = 1.0).

*±(2RS,5RS) 1-thiomethyl-3,3a-dihydro-benzo[d]thiazolo [4,3-b]thiazole (trans-**1j**)*

Prepared in analogous route as described for *trans*-**1f**, starting from *o*-amino-mercaptobenzene, the residue was purified by chromatography on SiO_2 (1:3, EtOAc:hexanes) led to compound **1j** (96 %, *trans*/*cis*: 99:01) as an oil; ^{1}H NMR ($CDCl_3$) δ 1.91 (dd, J= 10.8, 6.3 Hz, 1H), 2.72 (ddd, J= 13.8, 10.8, 4.8 Hz, 1H), 2.93 (dd, J= 11.8, 8.7 Hz, 1H), 2.99 (ddd, J= 13.8, 9.0, 6.3 Hz, 1H), 3.23 (dd, J= 11.8, 5.3 Hz, 1H), 5.11 (dd, J= 8.7, 5.3 Hz, 1H), 5.19 (dd, J= 9.0, 4.8 Hz, 1H), 6.88 (m, 2H), 7.11 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 33.5, 40.0, 70.2, 70.5, 110.9, 122.2, 123.0, 124.5, 126.2, 145.2; HRMS calculated for $C_{10}H_{12}NS_3$ [M+H]$^+$ 242.0132, found 242.0119.

General procedure for the preparation of Series I heterodimers

*(3R,5R,8R) 5-(((3-carboxy-4-nitrophenyl)disulfanyl)methyl) tetrahydro-2H-thiazolo[4,3-b]thiazole-3-carboxylic acid (**2af**)*

A stirred solution of DTNB (**2aa**; 0.2 g, 0.5 mmol) in MeOH (10 mL) was cooled down to 0 °C and $NaBH_4$ (0.04 g, 1 mmol) was added portion-wise. After 3 h, a solution of bicycle **1f** (0.24 g, 1.0 mmol) in MeOH (3 mL) and DMAP (0.010 g) was added with stirring. The mixture was stirred at room temperature overnight opened to air. The solvent was then removed under reduced pressure and the crude was poured into water and the pH was adjusted to 4 using HCl (5 % aqueous solution). The aqueous layer was extracted with EtOAc (5 × 50 mL), dried (Na_2SO_4) and filtered. The solvent was removed under reduced pressure and the residue was purified by chromatography on SiO_2 (1:2, EtOAc/hexanes and 0.5 % AcOH) led to compound **2af** (0.270 g, 62 %, *trans*/*cis* dr 74/26) as a yellow oil: ^{1}H NMR ($(CD_3)_2CO$) δ 3.13 (dd, J= 11.9, 3.9 Hz, 1H), 3.17 (dd, J= 13.5, 6.5 Hz, 1H), 3.32 (m, 2H), 3.40 (dd, J= 13.5, 6.5 Hz, 1H), 3.62 (dd, J= 11.9, 5.4 Hz, 1H), 4.40 (dd, J= 6.6, 4.4 Hz, 1H), 4.72 (t, J= 6.5 Hz, 1H), 5.19 (dd, J= 5.4, 3.9 Hz, 1H), 8.00 (m, 3H); ^{1}3C NMR ($(CD_3)_2CO$) δ 34.2*, 34.3, 39.3*, 39.5, 48.6, 70.6, 70.8*, 71.7, 71.9*, 71.8, 74.3, 74.4*, 125.6, 127.2, 129.3, 130.0, 142.8, 145.6, 166.0 (*appreciable signals of the minor diastereomer); HRMS calculated for $C_{14}H_{15}N_2O_6S_4$ [M+H]$^+$ 434.9813, found 434.9800.

*(3R,5R,8R) 5-(((3-carboxy-4-nitrophenyl)disulfanyl)methyl) tetrahydro-2H-thiazolo[4,3-b]thiazole-3-ethyl ester (**2ag**)*

Prepared in analogous route as described for **2af**, purification by chromatography on SiO_2 (1:3, EtOAc:hexanes) led to compound **2ag** (48 % yield, *trans*/*cis* dr 80/20) as a yellow oil; ^{1}H NMR ($(CD_3)_2CO$) δ 1.25 (t, J= 7.1 Hz, 3H), 3.11 (dd, J= 11.7, 4.2, 1H), 3.17 (m, 1H), 3.27 (dd, J= 10.9, 5.2 Hz, 1H), 3.36 (m, 2H), 3.59 (m, 1H), 4.19 (q, J= 7.1 Hz, 2H), 4.29 (t, J= 6.0 Hz, 1H), 4.70 (t, J= 6.6 Hz, 1H), 5.16 (t, J= 4.8 Hz, 1H), 8.01 (m, 3H); ^{1}3C NMR ($CDCl_3$) δ 14.2, 34.2, 34.3*, 38.9*, 39.3, 47.8*, 48.1, 61.8*, 61.9, 69.6, 70.0*, 71.0*, 71.1, 73.2, 73.7*, 124.8, 126.5, 128.0, 128.5, 145.3, 145.8, 168.3, 170.3 (*appreciable signals of the minor diastereomer); HRMS calculated for $C_{16}H_{18}N_2O_6S_4$ [M+Na]$^+$ 484.9940, found 484.9964. $[\alpha]_D = +202°$ (20 °C, MeOH, c= 0.1).

*±5-(((3-Carboxy-4-nitrophenyl)disulfanyl)methyl) tetrahydro-2H-thiazolo[4,3-b]thiazole (**2ah**)*

Prepared in analogous route as described for **2af**, purification by chromatography on SiO_2 (1:2, EtOAc:hexanes, 0.5 % AcOH) led to compound **2ah** (41 % yield, *trans*/*cis* dr 92/08) as a pale yellow oil: ^{1}H NMR ($(CD_3)_2CO$) δ 3.06 (m, 5H), 3.32 (dd, J= 13.6, 7.0 Hz, 1H), 3.41 (dd, J= 10.9, 5.3 Hz, 1H), 3.56 (dd, J= 11.4, 5.5 Hz, 1H), 4.55 (dd, J= 7.0, 5.3 Hz, 1H), 5.00 (dd, J= 5.5, 4.0 Hz, 1H), 7.95 (dd, J= 8.5, 2.2 Hz, 1H), 8.02 (d, J= 2.2 Hz, 1H), 8.02 (d, J= 8.5 Hz, 1H); ^{1}3C NMR ($(CD_3)_2CO$) δ 31.9, 39.1, 49.1, 57.0, 70.3, 74.9, 125.6, 165.5, 126.0, 128.1, 130.1, 142.8, 146.0; HRMS calculated for $C_{13}H_{14}N_2O_4S_4$ [M+H]$^+$ 390.99092, found 390.99257.

*(3R,5R,8S) 5-(((3-carboxy-4-nitrophenyl)disulfanyl)methyl) tetrahydro-2H-thiazolo[4,3-b]thiazole-2-dimethyl-3-carboxylic acid (cis-**L-2ai**)*

Prepared in an analogous route as described for **2af**, purification by chromatography on SiO_2 (1:2, EtOAc:hexanes, 0.5 % AcOH) led to compound L-**2ai** (35 % yield, *cis*/*trans* dr 95/05) as a pale yellow oil; ^{1}H NMR ($(CD_3)_2CO$) δ 1.45 (s, 3H), 1.60 (s, 3H), 3.06 (dd, J= 11.0, 7.5 Hz, 1H), 3.15 (dd, J= 13.3, 8.0 Hz, 1H), 3.42 (m, 2H), 3.70 (s, 1H), 4.76 (dd, J= 8.0, 6.4 Hz, 1H), 4.98 (t, J= 10.3 Hz, 1H), 7.97 (m, 3H); HRMS calculated for $C_{16}H_{17}N_2O_6S_4$ [M−H]$^-$ 460.99749, found 460.99570; $[\alpha]_D = +38.5°$ (20 °C, MeOH, c= 0.4).

*(3S,5S,8R) 5-(((3-carboxy-4-nitrophenyl)disulfanyl)methyl) tetrahydro-2H-thiazolo[4,3-b]thiazole-2-dimethyl-3-carboxylic acid (cis-D-**2ai**)*

Prepared in analogous route as described for L-**2ai**, starting from D-**1i**, purification by chromatography on SiO_2 (1:2, EtOAc:hexanes, 0.5 % AcOH) led to compound D-**2ai**(44 % yield, *cis*/*trans* dr 99/01) as a pale yellow oil. The spectroscopic properties were identical to those obtained for L-**2ai**, $[\alpha]_D = -35°$ (20 °C, MeOH, c= 0.3).

*±5−(((3-carboxy-4-nitrophenyl)disulfanyl)methyl) (2RS, 5RS)-3,3a-dihydro-benzo[d]thiazolo[4,3-b]thiazole (**2aj**)*

Prepared in analogous route as described for **2af**, purification by chromatography on SiO_2 (1:3, EtOAc:hexanes, 0.5 % AcOH) led to compound **2aj** (43 % yield, *trans*/*cis* dr 95/05) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 2.89 (t, $J=9.5$ Hz, 1H), 3.12 (dd, $J=14.0$, 4.6 Hz, 1H), 3.23 (dd, $J=10.3$, 5.7 Hz, 1H), 3.44 (dd, $J=14.0$, 9.7 Hz, 1H), 5.06 (dd, $J=9.5$, 5.7 Hz, 1H), 5.30 (dd, $J=9.7$, 4.6 Hz, 1H), 6.63 (d, $J=7.4$ Hz, 1H), 6.87 (d, $J=7.4$ Hz, 1H), 7.06 (m, 2H), 7.74 (s, 2H), 7.89 (s, 1H); ^{13}C NMR ($(CD_3)_2CO$) δ 40.3, 48.3, 66.9, 70.7, 110.8, 122.6, 123.4, 124.7, 126.4, 127.3, 129.4, 144.5, 145.0; HRMS calculated for $C_{17}H_{13}N_2O_4S_4$ $[M-H]^-$ 436.97636, found 436.97422.

General procedure for the preparation of Series II heterodimers

*(3R,5R,7aR)-5-((p-tolyldisulfanyl)methyl)tetrahydro-2H-thiazolo[4,3-b]thiazole-3-carboxylic acid (**2kf**)*

To a stirred solution of bicycle **1f** (150 mg, 0.63 mmol) in CH_2Cl_2 (3 mL), were added a solution of 4-methyl mercaptobenzene (170 mg, 1.37 mmol) in MeOH (6 mL), Et_3N (0.058 mL, 0.42 mmol) and DMAP (10 mg, 0.081 mmol). The mixture was stirred at room temperature overnight opened to air. The solvent was then removed under reduced pressure and to the crude was added water and the pH was adjusted to 4, using HCl (5 %, aqueous solution). The aqueous layer was extracted with EtOAc (5×50 mL), dried (Na_2SO_4) and filtered. The solvent was removed under reduced pressure and the residue was purified by chromatography on SiO_2 (1:4, EtOAc/hexanes and 0.1 % AcOH) to afford **2kf** (0.082 g, 54 %) as a pale oil: ^{1}H NMR ($CDCl_3$) δ 2.35 (s, 3H), 2.96 (dd, $J=14.1$, 9.4 Hz, 1H), 3.03 (dd, $J=14.1$, 4.7 Hz, 1H), 3.12 (dd, $J=12.2$, 3.7 Hz, 1H), 3.20 (dd, $J=11.3$, 7.0 Hz, 1H), 3.38 (dd, $J=11.3$, 2.4 Hz, 1H), 3.54 (dd, $J=12.2$, 5.8 Hz, 1H), 3.92 (dd, $J=7.0$, 2.4 Hz, 1H), 4.52 (dd, $J=9.4$, 4.7 Hz, 1H), 4.99 (dd, $J=5.8$, 3.7 Hz, 1H), 7.16 (d, $J=8.1$ Hz, 2H), 7.44 (d, $J=8.1$ Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 21.3., 33.5, 40.0, 47.6, 71.3, 72.4, 73.2, 130.2, 130.3, 133.0, 138.7, 172.2; HRMS calculated for $C_{14}H_{16}NO_2S_4$ $[M-H]^-$ 358.00694 found 358.00607; $[\alpha]_D = -8.9°$ (20 °C, MeOH/AcCN 1:1, $c=0.3$).

*(7aS)-5-(((4-bromophenyl)disulfanyl)methyl)tetrahydro-2H-thiazolo[4,3-b]thiazole-3-carboxylic acid (**2lf**)*

Prepared in analogous route as described for **2kf**, starting with a solution of 4-bromo mercaptobenzene in MeOH. Purification by chromatography on SiO_2 (1:4, EtOAc: hexanes, 0.1 % AcOH) led to compound **2lf** (49 % yield) as a pale oil: ^{1}H NMR ($CDCl_3$) δ 2.96 (dd, $J=13.9$, 5.4 Hz, 1H), 3.03 (dd, $J=13.9$, 8.5 Hz, 1H), 3.12 (dd, $J=12.1$, 4.0 Hz, 1H), 3.29 (dd, $J=11.3$, 7.0 Hz, 1H), 3.40 (dd, $J=11.3$, 3.2 Hz, 1H), 3.54 (dd, $J=12.1$, 5.8 Hz, 1H), 4.13 (dd, $J=7.0$, 3.2 Hz, 1H), 4.53 (dd, $J=8.5$, 5.4 Hz, 1H), 5.04 (dd, $J=5.8$, 4.0 Hz, 1H), 7.41 (m, 2H), 7.47 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 33.7, 39.7, 47.3, 71.3, 71.6, 73.3, 121.8, 130.2, 132.5, 135.6, 173.3; HRMS calculated for $C_{13}H_{13}BrNO_2S_4$ $[M-H]^-$ 421.90180 found 421.90092; $[\alpha]_D = -50.6°$ (20 °C, MeOH/AcCN 1:1, $c=0.3$).

*(7aS)-5-(((4-carboxyphenyl)disulfanyl)methyl)tetrahydro-2H-thiazolo[4,3-b]thiazole-3-carboxylic acid (**2mf**)*

Prepared in analogous route as described for **2kf**, starting with a solution of 4-carboxyl mercaptobenzene in MeOH. Purification by chromatography on SiO_2 (1:2, EtOAc:hexanes, 0.5 % AcOH) led to compound **2mf** (19 % yield) as a solid: mp = 80.9–81.2 °C; ^{1}H NMR ($(CD_3)_2CO$) δ 3.09 (dd, $J=13.5$, 5.3 Hz, 1H), 3.12 (dd, $J=11.9$, 5.2 Hz, 1H), 3.31 (m, 2H), 3.35 (dd, $J=13.5$, 6.8 Hz, 1H), 3.60 (dd, $J=11.9$, 5.3 Hz, 1H), 4.36 (dd, $J=6.4$, 4.4 Hz, 1H), 4.69 (t, $J=6.7$ Hz, 1H), 5.18 (dd, $J=5.3$, 3.9 Hz, 1H), 7.70 (d, $J=8.8$ Hz, 2H), 8.02 (d, $J=8.8$ Hz, 2H), 11.23 (s, 1H); ^{13}C NMR ($(CD_3)_2CO$) δ 34.3, 39.5, 48.8, 70.9, 71.8, 74.4, 126.9, 127.0, 129.6, 131.2, 131.4, 144.1, 167.1, 171.8; HRMS calculated for $C_{14}H_{14}NO_4S_4$ $[M-H]^-$ 387.98111 found 387.98068; $[\alpha]_D = -27.5°$ (20 °C, MeOH/AcCN 1:1, $c=0.3$).

*(3R,5R,7aR)-5-((phenyldisulfanyl)methyl)tetrahydro-2H-thiazolo[4,3-b]thiazole-3-carboxylic acid (**2nf**)*

Prepared in analogous route as described for **2kf**, starting with a solution of mercaptobenzene in MeOH. Purification by chromatography on SiO_2 (1:9, EtOAc:hexanes, 0.5 % AcOH) led to compound **2nf** (25 % yield) as a pale oil: ^{1}H NMR ($CDCl_3$) δ 2.97 (dd, $J=14.1$, 9.4 Hz, 1H), 3.05 (dd, $J=14.1$, 4.7 Hz, 1H), 3.12 (dd, $J=12.2$, 3.8 Hz, 1H), 3.21 (dd, $J=11.4$, 7.0 Hz, 1H), 3.40 (dd, $J=11.4$, 2.4 Hz, 1H), 3.54 (dd, $J=12.2$, 5.9 Hz, 1H), 3.94 (dd, $J=7.0$, 2.4 Hz, 1H), 4.53 (dd, $J=9.4$, 4.7 Hz, 1H), 5.00 (dd, $J=5.9$, 3.8 Hz, 1H), 7.30 (m, 1H), 7.36 (m, 2H), 7.55 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 33.5, 40.0, 47.6, 71.4, 72.4, 73.1, 128.1, 129.2, 129.5, 136.4, 171.9; HRMS calculated for $C_{13}H_{15}NO_2S_4Na$ $[M+Na]^+$ 367.98879 found 387.99218; $[\alpha]_D = +15.5°$ (20 °C, MeOH, $c=0.2$).

General procedure for the preparation of homodimers

*(2R,5R,8R)-8-carboxylate-1-aza-3,6-dithiobicyclo[3.3.0] octane 2-methyldisulfide (**2ff**)*

To a stirred solution of thiol **1f** (100 mg, 0.42 mmol) in MeOH (5 mL) was added K_2CO_3 (120 mg, 0.84 mmol). The mix-

ture was exposed to air and stirred at room temperature for 48 h. The solvent was removed under reduced pressure, the residue was poured into water and the pH was adjusted to 4 with HCl (5 %, aqueous solution). The aqueous layer was extracted with CH_2Cl_2 (4×40 mL), dried (Na_2SO_4) and filtered. The solvent was removed under reduced pressure and the residue was purified by chromatography on SiO_2 (1:5, EtOAc/hexanes) to afford **2ff** (75 % yield) as a pale oil: ^{1}H NMR ($CDCl_3$) δ 3.03 (m, 3H), 3.35 (dd, $J=$ 11.3, 6.9 Hz, 1H), 3.42 (dd, $J=$11.3, 4.4 Hz, 1H), 3.53 (dd, $J=$12.0, 5.8 Hz, 1H), 4.22 (dd, $J=$6.9, 4.4 Hz, 1H), 4.64 (dd, $J=$8.5, 5.3 Hz, 1H), 5.04 (dd, $J=$5.8, 4.9 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 33.8, 39.8, 47.5, 70.8, 71.3, 73.0, 173.9; HRMS calculated for $C_{14}H_{20}N_2NaO_4S_6$ $[M+Na]^+$ 494.96450, found 494.96726 $[\alpha]_D = -69.9°$ (20 °C, MeOH, c=0.4).

*± (2RS,5RS) 3,3a-dihydro-benzo[d]thiazolo[4,3-b]thiazole 1-methyldisulfide (**2jj**)*

Prepared in analogous route as described for **2ff**. Purification by chromatography on SiO_2 (1:9, EtOAc:hexanes, 0.5 % AcOH) led to compound **2nf** (67 % yield) as a pale oil: ^{1}H NMR ($CDCl_3$) δ 2.91 (m, 2H), 3.06 (dd, $J=$13.9, 5.6 Hz, 1H), 3.12 (d, $J=$6.9 Hz, 2H), 3.21 (dd, $J=$10.2, 5.6 Hz, 2H), 3.28 (dd, $J=$13.9, 8.4 Hz, 1H), 5.10 (dt, $J=$10.2, 5.3 Hz, 2H), 5.36 (dt, $J=$13.9, 6.4 Hz, 2H), 6.77 (d, $J=$7.9 Hz, 2H), 6.88 (t, $J=$7.5 Hz, 2H), 7.09 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 40.2, 40.3, 48.3, 48.5, 66.8, 67.4, 70.8, 70.9, 110.9, 122.4, 123.3, 124.6, 124.7, 126.4, 126.5, 145.1, 145.2.

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