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A New Class of Anti-HIV-1 Agents Targeted Toward the Nucleocapsid Protein NCp7: The 2,2′-Dithiobisbenzamides

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Abstract—As part of the National Cancer Institute's Drug Screening Program, a new class of antiretrovirals active against the human immunodeficiency virus HIV-1 has been identified, and the HIV-1 nucleocapsid protein NCp7 was proposed as the target of antiviral action. The 2,2′-dithiobis-[4′-(sulfamoyl)benzanilide] (**3x**) and the 2,2′-dithiobis(5-acetylamino)benzamide (**10**) represented the prototypic lead structures. A wide variety of 2,2′-dithiobisbenzamides were prepared and tested for anti-HIV-1 activity, cytotoxicity, and their ability to extrude zinc from the zinc fingers for NCp7. The structure–activity relationships demonstrated that the ability to extrude zinc from NCp7 resided in the 2,2′-dithiobisbenzamide core structure. The 3,3′ and the 4,4′ isomers were inactive. While many analogs based upon the core structure retained the zinc extrusion activity, the best overall anti-HIV-1 activity was only found in a narrow set of derivatives possessing carboxylic acid, carboxamide, or phenylsulfonamide functional groups. These functional groups were more important for reducing cytotoxicity than improving antiviral potency or activity vs NCp7. All of the compounds with antiviral activity also extruded zinc from NCp7. From this study several classes of low μM anti-HIV agents with simple chemical structures were identified as possible chemotherapeutic agents for the treatment of AIDS.

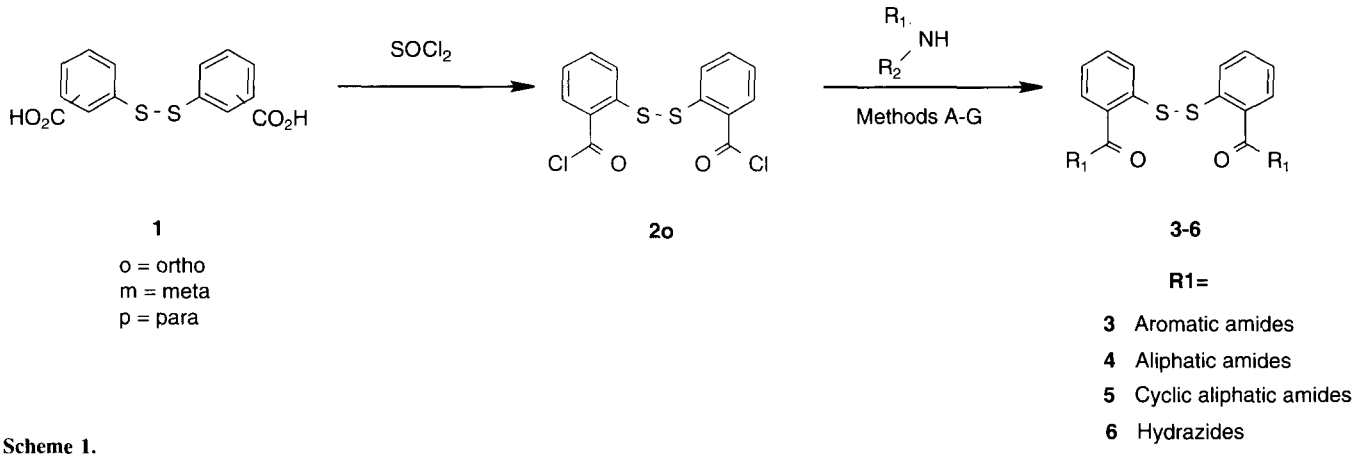
Introduction

The spread of the human immunodeficiency virus (HIV) continues to grow at an alarming rate with an estimated 30 million individuals infected by the year 2000.[1] A large number of chemotherapeutics have been delineated, but only the inhibitors of reverse transcriptase and protease have met with clinical success.[2,3] It is also apparent that monotherapy against HIV has little hope for success, since the viral dynamics indicate massive viral replication ($>10^9$ virions produced daily), promising very high selective pressure toward resistance development.[4,5] Thus, there is a rapidly increasing need for new agents directed at new targets, which do not show cross-resistance to the current drugs.[6] Such new agents will have a valuable place in the combination therapy armamentarium. However, as the number of drugs needed for effective therapy increases, so will the costs. As this issue is receiving national attention, there will be great potential advantages to agents which are efficiently prepared.[7]

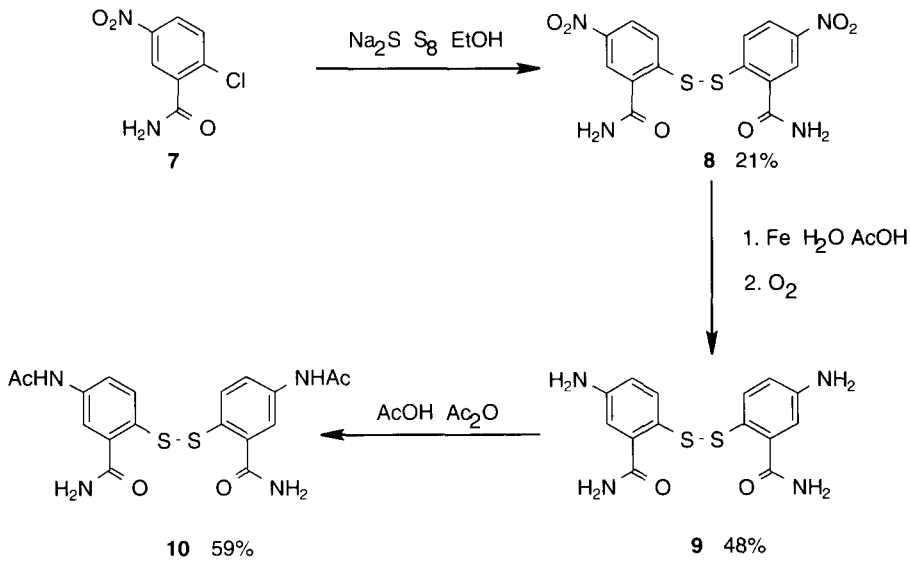
Recently, the anti-HIV activity of a new, chemically simple group of 2,2′-dithiobisbenzamides **I** was communicated (Figure 1).[8] These agents were originally identified as part of the National Cancer Institute's Drug Screening Program, and were active against multiple HIV isolates in several cell lines. Rice and co-workers demonstrated that these HIV active agents caused the extrusion of zinc from the zinc fingers of the HIV-1 nucleocapsid protein (NCp7), and suggested the inhibition of NCp7 function as a possible mechanism of action.

The nucleocapsid proteins of all retroviruses select viral RNA from cellular RNA, for dimerization[9] and packaging.[10–12] NC proteins also promote the binding of the essential *t*RNA primer to the primer site,[13] stimulate reverse transcription,[14,15] protect the viral RNA from nucleases,[16] and are essential in the viral life-cycle.[10,11] The HIV-1 NCp7 is a 55-amino-acid protein which contains two zinc fingers of a unique $C(X)_2C(X)_4H(X)_4C$ motif flanked by basic amino acids on each side. The solution structure of the 55AA[17] and the 72AA subtype[18] of NCp7 has been solved by NMR.

Tummino et al. recently reported[19] that the kinetics of Zn finger ejection by the 2,2′-dithiobisbenzamides is biexponential and nonsaturable, implying different rates of zinc ejection from each finger and little or no direct drug binding to NCp7. Using gel shift assays, he also demonstrated a direct inhibition of NCp7's binding to viral RNA.



Scheme 1.



Scheme 2.

All of the previously reported results with the 2,2′-dithiobisbenzamides have been based on a very limited number of active agents. In this paper, we wish to reveal the synthesis and detailed structure–activity relationships of the 2,2′-dithiobisbenzamides revealing the molecular features required for Zn extrusion, antiviral activity, and reduced cytotoxicity.

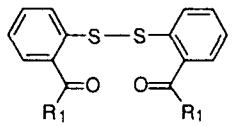
Chemistry

All of the target compounds **3–6** (Table 1) were prepared from the commercially available 2,2′-dithiobisbenzoic acid **1o** via the acid chloride **2o** (Scheme 1), which could be prepared in bulk and stored dry. The general method paralleled that of Okachi, who prepared a similar compound set for testing vs Mycobacteria.[20] In each case excess amine (2–10 equiv) was employed in inert solvents (CH_2Cl_2, Method A; pyridine, Method C; CH_2Cl_2:pyridine, Method B; and THF or benzene, Method F), with the addition of cobase such as pyridine (Methods A–C) or triethylamine (Method F). For amines with reactive functional groups or poor solubility, silylation with *N*-methyl-*N*-(trimethylsilyl) acetamide was employed prior to addition of the acid chloride (Method D). When the amine was part of an amino acid, the sodium salt was preformed using sodium in ethanol (Method G), or alternatively, the *t*-butyl ester of the amino acid was employed followed by deprotection with TFA (Method E). The disulfides were generally unstable to base. For easier reference, the target compounds are grouped according to the nature of the amine, i.e. aromatic amines (**3**), aliphatic amines (**4**), secondary amines (**5**), and hydrazines (**6**).

The only compound containing substitution of the parent phenyl rings was the 5,5′-*N*-diacetyldiamide **10**, which was synthesized according to Scheme 2 from the 2-chloro-5-nitrobenzamide **7**. The key step is the reduction of the nitro groups with iron followed by an oxidative work up to regenerate the disulfide **9**.

The isomeric 3,3′- (**12** and **14**) and 4,4′-disulfides (**13**) were readily prepared as in Scheme 1 using the corresponding acids **1m**[21,22] and **1p**,[22] respectively, and are shown in Table 2. Most of the amines used in this study were commercially available. The aminophenylsulfonamides were prepared according to the method of Bell and Roblin.[23]

Table 1. Physical and biological properties of the 2, 2′-dithiobisbenzamides **3–6**



3-6

Compd	R1	Method of preparation	Method of purification	mp °C	Yield[a] %	Formula	Elements analyzed	Biological activity: Antiviral EC_{50}/TC_{50} μM	Biological activity: Zn extrusion A or NA[J]
3a	C_6H_5-NH	C[b]	recrys. Me cellosolve	238–239	37	$C_{26}H_{20}N_2O_2S_2$	C, H, N	3.7/7.0	NA
3b	3-Me-C_6H_4-NH	A	recrys. Et_2O:EtOAc	193–195	44	$C_{28}H_{24}N_2O_2S_2$	C, H, N	20/44	–
3c	4-Me-C_6H_4-NH	C	recrys. Me cellosolve	230–231	66	$C_{28}H_{24}N_2O_2S_2$	C, H	9.1/28	–
3d	4-*t*-Bu-C_6H_4-NH	A	recrys. Et_2O:EtOH	136–138	12	$C_{34}H_{36}N_2O_2S_2$	C, H, N	10/75	–
3e	3-OH-C_6H_4-NH	C	recrys. Me cellosolve	243–246	83	$C_{26}H_{20}N_2O_4S_2+0.5H_2O$	C, H	15/22	NA
3f	2-OH-C_6H_4-NH	C	trit. 5% HCl	208–210	55	$C_{26}H_{20}N_2O_4S_2+0.75H_2O$	C, H	>10/10	–
3g	4-OH-C_6H_4-NH	C	recrys. Me cellosolve:H_2O	186–189	69	$C_{26}H_{20}N_2O_4S_2+1.0H_2O$	C, H	>33/33	NA
3h	4-OMe-C_6H_4-NH	C	recrys. DMF:H_2O	240–242	55	$C_{28}H_{24}N_2O_4S_2$	C, H	5.0/22	M
3i	4-O-i-Pr-C_6H_4-NH	C	recrys. DMF:H_2O	228–230	60	$C_{32}H_{32}N_2O_4S_2$	C, H	12/67	–
3j	4-O-i-Bu-C_6H_4-NH	C	recrys. Me cellosolve	239–241	77	$C_{34}H_{36}N_2O_4S_2$	C, H	30/>120	M
3k	2, 5-di-OMe-C_6H_3-NH	C	recrys. DMF:H_2O:EtOH	142–143	47	$C_{30}H_{28}N_2O_6S_2$	C, H	19/53	M
3l	4-CN-C_6H_4-NH	A	recrys. EtOH:DMF:H_2O	239–241	13	$C_{28}H_{18}N_4O_2S_2+0.2H_2O$	C, H, N	15/30	NA
3m	4-F-C_6H_4-NH	A	recrys. EtOH:DMF	242–244	10	$C_{26}H_{18}F_2N_2O_2S_2$	C, H, N	4.5/14	NA
3n	4-Cl-C_6H_4-NH	C	trit. hot Me cellosolve	253–255	85	$C_{26}H_{18}Cl_2N_2O_2S_2$	C, H	>8.0/8.0	NA
3o	4-Br-C_6H_4-NH	C	recrys. DMF	257–258	48	$C_{26}H_{18}Br_2N_2O_2S_2$	C, H	17/40	NA
3p	4-$COCH_3$-C_6H_4-NH	C	recrys. AcOH	249–251	36	$C_{30}H_{24}N_2O_4S_2$	C, H	19/54	M
3q	4-COEt-C_6H_4-NH	C	recrys. DMF:H_2O	238–240	76	$C_{32}H_{28}N_2O_4S_2+0.25H_2O$	C, H	16/105	M
3r	4-CO_2Et-C_6H_4-NH	C[b]	recrys. EtOAc	213–215	39	$C_{32}H_{28}N_2O_6S_2$	C, H	9.0/64	M
3s	4-CO_2H-C_6H_4-NH	D	recrys. CH_3CN	>285	24	$C_{28}H_{20}N_2O_6S_2$	C, H, N,	3.0/28	A
3t	4-$CONH_2$-C_6H_4-NH	A	recrys. DMF:EtOH:H_2O	>270	9	$C_{28}H_{22}N_4O_4S_2+0.2H_2O$	C, H, N	2.3/>100	A
3u	4-NO_2-C_6H_4-NH	C	recrys. Me cellosolve	224–226	35	$C_{26}H_{18}N_4O_6S_2$	C, H	4.0/14	–
3v	4-NH_2-C_6H_4-NH	exp[c]	trit. CH_3CN:MeOH	>260	39	$C_{26}H_{22}N_4O_2S_2+2HCl$	C, H, N	7.0/8.0	–
3w	4-$N(Et)_2$-C_6H_4-NH	C	recrys. EtOH	198–200	44	$C_{34}H_{38}N_4O_2S_2+0.5H_2O$	C, H	10/20	–
3x	4-SO_2NH_2-C_6H_4-NH	B[c]	suspend DMF:EtOH, filter, prec w 8% $NaHCO_3$	311–312	50	$C_{26}H_{22}N_4O_6S_4$	C, H, N	3.4/>120	M
3y	4-SO_2NH_2-C_6H_4-CH_3N	F[d, e]	trit. H_2CCl_2	243–245	60	$C_{28}H_{26}N_4O_6S_4+0.5H_2O$	C, H, N	22/>100	A
3z	4-$SO_2N(CH_3)$-C_6H_4-NH	B	prec. DMF:EtOH:H_2O w 8% $NaHCO_3$	245–247	46	$C_{28}H_{26}N_4O_6S_4$	C, H, N	1.7/12	–

Table 1. Continued

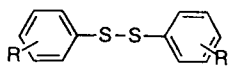
Compd	R1	Method of preparation	Method of purification	mp °C	Yield[a] %	Formula	Elements analyzed	Biological activity Antiviral EC_{50}/TC_{50} μM	Biological activity Zn extrusion A or NA[J]
3aa	4-SO_2N(iPr)-C_6H_4-NH	B	prec. DMF:EtOH:H_2O w 8% $NaHCO_3$	146–148	29	$C_{32}H_{34}N_4O_6S_4+0.1H_2O$	C, H, N	16/38	A
3bb	4-$SO_2N(COCH_3)$-C_6H_4-NH	B	recrys. EtOH:H_2O	180–182	9	$C_{30}H_{26}N_4O_8S_4+1.4H_2O$	C, H, N	16/42	A
3cc	4-SO_2NH-(2-pyrimidine)-C_6H_4-NH	B	prec. DMF:EtOH:H_2O w 8% $NaHCO_3$	>300	58	$C_{34}H_{26}N_8O_6S_4+0.3H_2O$	C, H, N	12/30	–
3dd	2-SO_2NH_2-C_6H_4-NH	A	recrys.DMF	150–151	20	$C_{26}H_{22}N_4O_6S_4$	C, H, N	2.9/67	M
3ee	4-SO_2CH_3-C_6H_4-NH	A	recrys. CH_3CN:DMF	236–238	56	$C_{28}H_{24}N_2O_6S_4+0.4H_2O$	C, H, N	14/22	M
3ff	3, 5-di-CF_3-C_6H_4-NH	A	recrys. EtOAc:Hexane	213–214	9	$C_{30}H_{16}F_{12}N_2O_2S_2$	C, H, N	>3.6/3.6	M
3gg	2-pyrimidine-NH	C	recrys. Me cellosolve	236–238	22	$C_{22}H_{16}N_6O_2S_2$	C, H, N	>6.2/6.2	A
3hh	2-thiazole-NH	C	trit. hot EtOH, solids recryst. Me cellosolve	228–230	15	$C_{20}H_{14}N_4O_2S_4$	C, H, N	12/18	A
4a	NH_2-	F[f]	prec. DMF:EtOH:H_2O w 8% $NaHCO_3$	243–245	74	$C_{14}H_{12}N_2O_2S_2+0.75H_2O$	C, H, N, S	>21/21	A
4b	MeNH-	commmercial[f]				$C_{16}H_{16}N_2O_2S_2$		>11/11	A
4c	EtNH-	F[b]	trit. Et_2O	197–203	67	$C_{18}H_{20}N_2O_2S_2$	C, H, N, S	>5.1/5.1	–
4d	PrNH-	C	recrys. Me cellosolve	195–197	32	$C_{20}H_{24}N_2O_2S_2$	C, H, N	>19/19	–
4e	c-PrNH-	F	extract w 5% HCl, conc to product	257–259	51	$C_{20}H_{20}N_2O_2S_2+0.5H_2O$	C, H, N, S	24/90	M
4f	$CH_3CH_2CH(CH_3)NH$-	C	recrys. Me Cellosolve	232–234	47	$C_{22}H_{28}N_2O_2S_2$	C, H	>15/15	–
4g	$(CH_3)_2CHCH_2NH$-	C	recrys. Me Cellosolve	201–203	12	$C_{22}H_{28}N_2O_2S_2$	C, H,	1.8/4.6	M
4h	*t*-BuNH-	F	trit. EtOAc	180–181	58	$C_{22}H_{28}N_2O_2S_2$	C, H, N	58/>100	–
4i	$CH_3(CH_2)_{11}NH$-	C	recrys. EtOAc	168–170	26	$C_{38}H_{60}N_2O_2S_2$	C, H, N	16/20	–
4j	$PhCH_2NH$-	C[b]	recrys. AcOH	205–206	19	$C_{28}H_{24}N_2O_2S_2$	C, H, N	5.0/10	M
4k	$HOCH_2CH_2NH$	F	recrys. iso-PrOH	177–-179	24	$C_{18}H_{20}N_2O_4S_2$	C, H, N	4.5/16	–
4l	$HOCH_2CH(CH_2CH(CH_3)_2)NH$	D	recrys. DMF:H_2O	195–196	29	$C_{26}H_{36}N_2O_4S_2$	C, H, N	>12/12	M
4m	HO_2CCH_2NH	G	diss. in $NaHCO_3$, prec 1 N HCl	210–214	40	$C_{18}H_{16}N_2O_6S_2$	C, H	51/>200	A
4n	$HO_2CCH(CH_3)NH$	G	recrys. AcOH	226–227	69	$C_{20}H_{20}N_2O_6S_2$	C, H, N	38/>200	–
4o	$HO_2CCH_2CH_2NH$	G	recrys. EtOH	201–203	53	$C_{20}H_{20}N_2O_6S_2$	C, H, N	7.8/>120	A
4p	$HO_2CCH(CH(CH_3)_2)NH$	G	recrys. Acetone:H_2O	231–233	23	$C_{24}H_{28}N_2O_6S_2$	C, H, N	17/>200	A
4q	$HO_2CCH(CH(CH_3)$-$CH_2CH_3)NH$-(d, l)	G	recrys. 60% EtOH	217–218	5	$C_{26}H_{32}N_2O_6S_2$	C, H, N	9.0/>120	A
4r	$HO_2CCH(CH_2CH(CH_3)_2NH$	G	diss. in $NaHCO_3$, prec. 1 N HCl	204–206	56	$C_{26}H_{32}N_2O_6S_2$	C, H, N	8.9/130	M
4s	$HO_2CCH(CH(CH_3)CH_2CH_3)$ NH(R)	E	trit. Et_2O	194–196	54	$C_{26}H_{32}N_2O_6S_2+0.6H_2O$	C, H, N	6.3/113	A

Table 1. Continued

Compd	R1	Method of preparation	Method of purification	mp °C	Yield[a] %	Formula	Elements analyzed	Biological activity Antiviral EC_{50}/TC_{50} μM	Biological activity Zn extrusion A or NA[J]
4t	$HO_2CCH(CH(CH_3)CH_2CH_3)NH(S)$	E	wash w EtOAc:$CHCl_3$, conc filtrate	195–197	42	$C_{26}H_{32}N_2O_6S_2+0.7H_2O$	C, H, N	5.0/140	A
4u	$HO_2CCH(Ph)NH$	E	trit. hexane:Et_2O	231-232	10	$C_{30}H_{24}N_2O_6S_2+1.1H_2O$	C, H, N	25/>100	A
4v	$HO_2CCH(CH_3)NHCOCH(CH_2CH(CH_3)_2)NH$	D	recrys. EtOH:DMF:H_2O	234–235	37	$C_{32}H_{42}N_4O_8S_2+0.7H_2O$	C, H, N	71/157	–
4w	EtO_2CCH_2NH	F[b, h]	recrys. EtOH:H_2O	147–149	76	$C_{22}H_{24}N_2O_6S_2$	C, H	15/25	–
4x	$EtO_2CCH_2CH_2NH$	C	trit. Et_2O	154–156	10	$C_{24}H_{28}N_2O_6S_2$	C, H	>40/40	–
4y	$(Me)_2NCH_2CH_2CH_2NH$	Fd	recrys. Toluene	134–136	47	$C_{24}H_{34}N_2O_2S_2$	C, H, N, S	6.5/29	A
4z	4-SO_2NH_2-C_6H_4-CH_2NH-	D	prec. DMF:EtOH:H_2O w 8% $NaNCO_3$	267–269	43	$C_{28}H_{26}N_4O_6S_4$	C, H, N	1.4/30	M
5a	1-pyrrolidinyl	F	extract w 5% HCl, conc to product	62–63	67	$C_{22}H_{24}N_2O_2S_2$	C, H, N	>25/25	M
5b	1-morpholinyl-	F[b]	extract w 5% HCl, conc to product	107–110	84	$C_{22}H_{24}N_2O_4S_2$	C, H, N	>50/50	M
5c	1-3-OH-pyrrolidinyl	F	chromatog. $CHCl_3$:MeOH 95:5	168–172	68	$C_{22}H_{24}N_2O_4S_2$	C, H, N, S	65/>200	M
5d	1-piperidinyl-	C	recrys. EtOAc	141–143	51	$C_{24}H_{28}N_2O_2S_2+0.4H_2O$	C, H	21/27	M
5e	1-thiomorpholinyl-	F	extract w 5% HCl, conc to product	90–92	75	$C_{22}H_{24}N_2O_2S_4$	C, H, N	>42/42	A
6a	NH_2NH-	F[i]	conc., mix oil w MeOH/EtOH(1:1)	219–220	54	$C_{14}H_{14}N_4O_2S_2+0.4H_2O$	C, H, N	>16/16	M
6b	4-pyridinyl-CONHNH-	C	recrys. DMF:H_2O	237–239	55	$C_{26}H_{20}N_6O_4S_2+3.0H_2O$	C, H	8.2/67	M
6c	Ph-NHNH-	C	recrys. DMF:H_2O	189–191	65	$C_{26}H_{22}N_4O_2S_2$	C, H	>5.2/5.2	–

[a]Represents the % yield for the last step of the sequence except for method E where it represents the overall yield.
[b]Previously described, see refs 20 and 24.
[c]See experimental section for full procedure
[d]Solvent employed was THF.
[e]Base employed was N-methylmorpholine.
[f]Previously described, see ref 25.
[g]Previously described, see ref 26.
[h]Solvent employed was benzene.
[i]Excess hydrazine was employed neat with solid acid chloride.
[J]Zn extrusion activity: A = Active M = Moderate NA = Not Active. See Biological section in text for details.

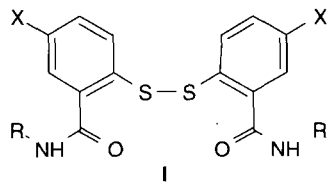
Table 2. Physical and biological properties of select compounds from this study



10-14

Compd	R	Method of preparation	Method of purification	mp °C	Yield[a] %	Formula	Elements analyzed	Biological activity Antiviral EC_{50}/TC_{50} μM	Biological activity Zn extrusion A or NA[J]
1o	2-CO_2H	commercial				$C_{14}H_{10}O_4S_2$		>100/>100	M
11	2-CO_2Me	F[b]	cryst. MeOH	130–131	100	$C_{16}H_{14}O_4S_2$	C, H	>9.3/9.3	M
10	2-$CONH_2$- 4-NHAc	exp[c]	recrys. DMF:DMSO:H_2O	301–303	59	$C_{18}H_{18}N_4O_4S_2$ +0.3H_2O	C, H	5.0/>120	A
12	3-$CONH_2$	F[d]	recrys. AcOH	223–225	65	$C_{14}H_{12}N_2O_2S_2$	C, H, N	61/101	NA
13	4-CONH-C_6H_4- p-SO_2NH_2	exp[c]	prec. DMF:EtOH:H_2O 8% $NaHCO_3$	>300	55	$C_{26}H_{22}N_4O_6S_4$	C, H, N	>100/>100	NA
14	3-CONH-C_6H_4- p-SO_2NH_2	exp[c]	prec. DMF:EtOH:H_2O 8% $NaHCO_3$	295–296	28	$C_{26}H_{22}N_4O_6S_4$	C, H, N	>100/>100	NA

[a]Represents yield for the last step of the sequence.
[b]Methanol was used as solvent and reagent.
[c]See Experimental for full procedure.
[d]Compound previously prepared, see ref 26.
[e]Zn extrusion activity: A = Active, M = Moderate, NA = Not Active. See Biological section in text for details.



I

		R	X
DIBA-1, PD022551 [a]	3x	SO_2NH_2	H
DIBA-2, PD156202 [a]	3bb	SO_2NHAc	H
DIBA-3, PD024886 [a]	10	H	—NHAc

[a] Nomenclature used in References 8 and 19

Figure 1. Prototype 2,2′-dithiobisbenzamides.

Biology

All of the compounds were tested for anti-HIV activity in lymphocyte-derived CEM cells using the XTT cytopathic procedure performed at the National Cancer Institute[27]/Southern Research Institute.[28] The assay quantitates drug-induced protection from the cytopathic effects of HIV-1. The results are expressed as a 50% effective concentration (EC_{50}) and a 50% cytotoxic concentration in the absence of HIV-1 (TC_{50}). Multiple runs of 2–10 were averaged and the results displayed in Tables 1 and 2. Certain compounds of the set have undergone extensive antiviral testing confirming the results of the XTT assays.[8] Selected compounds were also tested for their ability to extrude zinc from NCp7 using a fluorescence-based assay, which measures the Zn^{+2} ions in solution through a coordination complex with the Zn^{+2} fluorophore TSQ (*N*-(6-methoxy-8-quinolyl)-*p*-toluenesulfonamide).[19] Fluorescence was measured at 460 nM (excitation 355 nM). Background fluorescence of TSQ and NCp7 was 10 and maximal fluorescence (100% zinc extrusion) was 40–45. Compounds were ranked as active (75–100% maximal fluorescence), moderate (25–75% maximal), or inactive (<25% maximal). Compounds were tested at 10 μM, 40 μM TSQ, and 2.8 μM NCp7 for 90 min. The fluorescence of each compound with zinc-free NCp7 was also measured and subtracted from that of the test mixture. The expression and purification of NCp7 were also reported.[19]

Results and Discussion

The *N*-aryl benzamides (**3**), from which the prototypic lead **3x** was derived, generally show moderate to no activity in the zinc extrusion assay, except for the 4-carboxyl (**3s**), the 4-carboxamide (**3t**), and the 4-sulfonamide analogs **3x**–**3bb**. Even the lead structure **3hh** displays only moderate Zn extrusion activity. Similarly, most of the compounds of series **3** exhibit little antiviral activity and are generally cytotoxic ($TC_{50} \leq 50$ nM); compounds whose antiviral activity occurs near the cytotoxic endpoints (one- to fourfold) are generally not considered as active in these discussions since the antiviral activity may be due to nonspecific cellular effects. The cytotoxicity generally diminishes as the 4-substituent increases in size (compare **3a**:**3c**:**3d** or **3h**:**3i**:**3j**). The SAR clearly points to the acid (**3s**) and amide substituents (**3t**, **3x**, **3z**, and **3dd**) conferring the bulk of the antiviral activity (≤ 5 μM). Mild activity was observed for the ethylketone **3q** (EC_{50}/TC_{50} 16/105 μM), the ethyl ester **3r** (9/64 μM), the 4-*t*-butyl compound **3d** (10/75 μM), and the isopropyl ether **3i** (12/67 μM). The original sulfonamide lead, **3x**, displays the best antiviral activity and therapeutic index within the *N*-aryl series. The *N*-substituted sulfonamides **3y**–**3cc** show a fall-off in potency after *N*-methyl, with a concomitant increase in cytotoxicity. The ortho sulfonamide **3dd** retained respectable activity (2.9/67 μM). The biological activity was essentially unaffected by the position of the substituent on the phenyl ring. Replacement of the sulfonamide NH_2 with methyl to form the 4-methyl sulfone **3ee** caused increased cytotoxicity (14/22 μM). Alkylation of the benzamide nitrogen (**3y**) caused a sixfold loss of antiviral activity even though the Zn extrusion value remained unchanged. The majority of the compounds are quite cytotoxic which masks the true antiviral activity associated with several substituent changes.

The simple unsubstituted or *N*-alkyl 2,2′-dithiobenzamides (**4**), unlike the *N*-aryl series **3**, do show generally good zinc extrusion from NCp7. Indeed compounds **4a** and **4b**, along with **10**, present the core structure required for zinc extrusion among the benzamides, with the *N*-aryls of series **3** generally losing activity except for the few derivatives noted above. Within the *N*-alkyl series many of the compounds with zinc extrusion activity such as **4a**, **4b**, **4u**, and **4y** do not show good antiviral efficacy, again due to increased cytotoxicity. As above, antiviral activity increased and cytotoxicity decreased significantly with the presence of the carboxylic acid moiety. Thus, the benzamides derived from the amino acids **4m**–**4u** are the most active (5.4–51 μM) of all the *N*-alkyl series (**4**) peaking with the isoleucine analogues **4q**, **4s**, and **4t**. The general inhibitory activity of the acids and amides might be due to an interaction with the basic amino acids flanking the zinc fingers/to improved transport and penetration. The activity is not limited only to benzamides derived from the α-amino acids, since the β-acid analogue **4o** (7.8/120 nM) was also quite active. The alcohols (**4k** and **4l**) and the esters (**4w** and **4x**) derived from active acids were cytotoxic. Chirality of the amino acids was not an important determinant as seen from comparing the optically pure isomeric isoleucine analogues **4s** and **4t** (6.3 and 5.0 μM EC_{50}s). A final curiosity is compound **4z** containing a methylene between the phenylsulfonamide and the benzamide

nitrogen. Good antiviral activity is retained with increased cytotoxicity (1.4/30 μM).

Cyclic amides **5a–5e** retained the core-related Zn extrusion activity but displayed unremarkable antiviral potency. The hydrazides also retained Zn extrusion ability with a glimmer of antiviral activity (**6b**, 8.2/67 μM). The core activity required for the extrusion of Zn does not require a benzamide as the simple acid **1o** and the ester **11** show moderate extrusion activity. However, the *ortho* arrangement of substituents is clearly required, as the other positional isomers, **12–14**, show no significant biological activity.

Our results, presented here, are consistent with the biochemical results of Tummino[19] and the solution NMR results recently presented by Reily et al.[29] All three studies strongly suggest that the 2,2′-dithiobisbenzamides do not form a drug:NCp7 complex in any significant manner. The disulfide acts as a strict electrophile reacting with a cysteine of the NCp7 zinc fingers in a typical sulfur exchange mechanism without appreciable prebinding. Clearly there is some molecular recognition in that ortho derivatives are exclusively preferred, and this may be attributed to a possible coordination with the Zn as part of the extrusion process.

Thus, our interpretation of the SAR is that there is a core structure, the 2,2′-dithiocarboxyl moiety, with an intrinsic activity toward the NCp7 zinc fingers, where most analogs have some ability to extrude zinc. The ultimate function of the substituents is to modulate cellular cytotoxicity, maybe by modulating activities toward mammalian zinc fingers or other cysteine targets. Certain substituents like the amino acid analogs and the phenyl amides are less cytotoxic and, therefore, have high therapeutic indices. They are not significantly more active in the viral assays, just less toxic. Unfortunately, the SAR does not suggest groups that improve zinc extrusion or antiviral potency, but instead leads to groups which cause reduced cellular toxicity. It should be recognized, however, that the SAR does not include optimization of the parent phenyl disulfide rings. Comparing **4a** to **10** suggests that substitution of these rings may be fruitful.

The very nature of the chemistry of disulfides and the proposed Zn extrusion mechanism for the 2,2′-dithiobisbenzamides raise a multitude of questions regarding selectivity. From the results presented here and those of several recent studies with this class of agents,[8,19,29,31] the link between the zinc extrusion activity of the 2,2′-dithiobisbenzamides and their anti-HIV activity appears to be strong. The most convincing evidence to date was reported by Turpin et al.[31] in which several of the direct effects expected from NCp7 zinc extrusion were indeed observed in treated HIV-infected cells in culture. This is not to say that other mechanisms involving sulfide reactivity may not be operating in some capacity, such as a possible interaction with protein-disulfide-isomerase PDI[32] or other proteins/enzymes with critical-exposed cysteines. Only that the data implicating NCp7 is abundant and consistent with all of the structure–activity relationships.

Even if HIV-NCp7 is the accepted target, nagging questions about selectivity remain. A few of these questions may be addressed within the scope of this paper.

Since all that is required is an electrophilic sulfur, then what is so special about the 2,2′-dithiobisbenzamides?

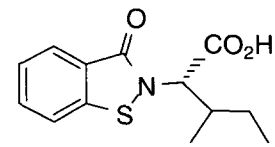
In this paper and previous work[8] we clearly show that only the 2,2′-substituted disulfides possess the ability to extrude Zn from NCp7 even though the electrophilic nature of the S-S bond should be very similar when comparing the para isomer **13** with the ortho isomer **3x**. More convincing was the recent report by Rice and coworkers on their evaluation of a vast library of disulfide compounds.[33] Despite great diversity, the most active compounds were still ortho substituted and in fact one of the best was a direct derivative of the hydrazides **6b** and **6c**. Clearly only a small subset of disulfides have the proper orientation and electronic configuration to effectively extrude zinc from NCp7.

Why wouldn't these agents extrude zinc from all zinc finger proteins?

It seems reasonable that not all cysteines in all zinc fingers are equally exposed or nucleophilic. Even within the NCp7 protein itself, the C-terminal finger reacts faster with the 2,2′-dithiobisbenzamides than the N-terminal finger. This has now been confirmed both biochemically[19] and spectroscopically by two methods.[29] Gel-shift studies[19] in our own laboratories (to be published later) using various other zinc finger proteins such as SP1 (CCHH) and T4 (CHCC) have shown high selectivity (no binding) for certain 2,2′-dithiobisbenzamides such as **4t**, and reactivity with others, all suggesting that selectivity for NCp7 is possible.

Won't the 2,2′-dithiobisbenzamides undergo exchange with other sulfhydryl proteins?

We have reported our preliminary data[34] on the mixed disulfide formed between **3x** and *N*-acetyl cysteine. This mixed disulfide was fully active at extruding Zn from NCp7 and displayed potent HIV activity in culture. Thus small molecular-weight mixed disulfides possibly formed with glutathione or other cysteine moieties are expected to be active. Furthermore, Reily et al. have



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reported an equilibrium between the disulfide **4t** and the isothiazolone **15**. It has also been shown that **15** is active in the Zn extrusion and HIV assays.[29,34] Such an equilibrium would enable the mixed disulfides formed with various proteins to be reversible via isothiazolone formation causing reduced toxicity and freeing an active 'sulfide' for Zn extrusion at NCp7.

Won't glutathione ultimately reduce the 2,2′-dithiobisbenzamides to the free sulfhydryls?

Because the mechanism of antiviral action involves an electrophilic disulfide, clearly the fully reduced 2,2′-dithiobisbenzamides should not be active. Rice et al. have shown that the free sulfhydryl derivative of **3x** was indeed active in cell culture but not at extruding Zn.[8] Thus the question regarding glutathione demands knowledge of the final equilibrium in the plasma and the various in vivo compartments. Only if all of the active disulfides in their various forms are reduced and are kept reduced should the activity be negated. At this point, no data on the in vivo equilibrium exist.

Conclusion

In this paper, we have presented structure–activity relationships for the 2,2′-dithiobisbenzamide with regard to zinc extrusion, antiviral, and cytotoxic activities. The best compounds to emerge are optimized for reduced cytotoxicity and have high therapeutic indices. Several derivatives, such as **3t**, **3x**, and **4o–4t**, emerge as possible drug candidates. The SAR also points to other areas of profitable analog work, such as in β or γ-amino acids. The compounds within provide a new mechanism of action and should be relatively easy to prepare, offering new potential opportunities in AIDS chemotherapy.

Experimental

Melting points were determined in open capillary tubes on a Hoover melting-point apparatus and are uncorrected. Infrared (IR) spectra were determined in KBr pellet on a Mattson FT IR Cygnus 100 spectrophotometer. Proton magnetic resonance (NMR) spectra were recorded on a 300 or 400 MHz Varian Unity 300/400 spectrometer. Chemical shifts are reported in δ values relative to TMS. Mass spectra were recorded on a VG TRIO 2 or VG TRIO 2000 spectrometer. Elemental analyses were performed on a Lehman Labs 440 elemental analyser or at Robertson Microlit Laboratories, Madison, NJ. All concentrations were carried out on a rotoevaporator at 2–30 mmHg at temperatures between 25 and 37 °C. Solutions were dried with $MgSO_4$. Flash or medium pressure chromatography were performed using silica gel 230–400 mesh. All starting materials were commercially available unless otherwise noted.

2,2′-Dithiobisbenzoyl chloride (2o). To 50 g (160 mmol) of 2,2′-dithiobisbenzoic acid[3] was added 600 mL of $SOCl_2$ and the mixture was refluxed for 24 h. The mixture was concentrated, chased with CH_2Cl_2, and the residue triturated with hexane. The solids were filtered, washed with hexane and dried over P_2O_5 to give 47.26 g (84%) of **2o**: mp 150–152 °C; IR (KBr) 1719, 1583, 1554 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.02 (d, J = 8.5 Hz, 2H), 7.62 (d, J = 8.5 Hz, 2H), 7.56 (*t*, J = 8.5 Hz, 2H), 7.34 (*t*, J = 8.5 Hz, 2H). Anal. ($C_{14}H_8Cl_2O_2S_2$) C, H, N, Cl.

General method A: 2,2′-dithiobis[*N*-[4-(1,1-dimethylethyl)phenyl]benzamide] (3d). To a solution of 1.04 g (6.99 mmol) 4-*tert*-butylaniline in 8 mL of pyridine at room temperature was added 1.20 g (3.50 mmol) of 2,2′-dithiobisbenzoyl chloride in 25 mL of CH_2Cl_2. After 18 h, the mixture was concentrated and the residue triturated with 5% HCl. The resulting solids were collected, washed with H_2O, and recrystallized from ethyl ether:ethanol to yield 0.24 g (13%) of **3d**: mp 136–138 °C; IR (KBr) 3294, 1651, 1597, 1520 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.48 (s, 2H, N*H*), 7.78 (m, 4H), 7.68 (d, J = 9Hz, 4H), 7.50 (m, 2H), 7.40 (m, 6H), 1.25 (s, 18H, C(CH_3)$_3$); MS *m/z* 569 (21%, m+1) 284 (100%, 1/2 m). Anal. ($C_{34}H_{36}N_2O_2S_2$) C, H, N.

General method B: 2,2′-dithiobis-[4′-(sulfamoyl)-benzanilide] (3x). To 6.20 g (36.0 mmol) of 4-(aminosulfonyl)-aniline in 125 mL of pyridine at 0–5 °C was added dropwise 5.00 g (14.0 mmol) of 2,2′-dithiobisbenzoyl chloride in 50 mL of CH_2Cl_2. The mixture was stirred at 0–23 °C for 18 h and the solids were collected, washed with 1 N HCl, H_2O, and dried to give 7.6 g of crude product. The material was suspended in 50 mL DMF and 60 mL EtOH, filtered and precipitated from the filtrate with 10 mL of 5% $NaHCO_3$. The product was collected by filtration, washed with H_2O and then EtOH to give 5.0 g (58%) of **3x**: mp 311–312 °C; IR (KBr) 3361, 1662, 1649, 1520 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.9 (s, 2H, N*H*), 8.0–7.7 (m, 12H), 7.5 (m, 2H), 7.4 (m, 2H), 7.3 (s, 4H). Anal. ($C_{26}H_{22}N_4O_6S_4$) C, H, N.

General method C: 2,2′-dithiobis-[*N*-(3-methylphenyl)-benzamide] (3b). To 21.4 g (200.0 mmol) of 3-methylaniline in 100 mL of pyridine at 10 °C was added 17.4 g (50.0 mmol) of 2,2′-dithiobisbenzoyl chloride in portions. After 8 h the mixture was poured into 5% HCl and the solids collected, washed with H_2O, and dried. The crude material was recrystallized from methyl cellosolve to give 15.3 g (63%) of **3b**: mp 183–185 °C; IR (KBr) 3239, 1647, 1612, 1580, 1543 cm^{-1}; 1H NMR (DMSO-d_6) δ 10.46 (s, 2H, N*H*), 7.88 (m, 4H), 7.63 (s, 2H), 7.54 (m, 4H), 7.40 (*t*, J = 7.5 Hz, 2H), 7.25 (*t*, J = 8 Hz, 2H), 6.96 (d, J = 7.5 Hz, 2H), 2.32 (s, 6H, CH_3). Anal. ($C_{28}H_{24}N_2O_2S_2$) C, H, N.

General method D: 2-[2-(2-[2-[1(1-carboxy-ethylcarbamoyl)-3-methyl-butylcarbamoyl]-phenyldisulfanyl]-benzoylamino)-4-methyl-pentanoylamino]-propionic acid (N-[2-[[2-[[1-[[(1-carboxyethyl)amino]carbonyl]-3-methylbutyl]amino]carbonyl]-phenyl]dithio]benzoyl])

L-Leu-L-Ala (4v). To a slurry of 1.0 g (4.9 mmol) of l-leucyl-L-alanine hydrate in 50 mL of CH_2Cl_2 was added 3.4 mL (21 mmol) of *N*-methyl-*N*(trimethylsilyl)-acetamide and the mixture stirred until homogenous. To this solution was added 0.5 g (2.0 mmol) of 2,2′-dithiobisbenzoyl chloride in 20 mL of CH_2Cl_2 dropwise over 10 min. After 4 h, the reaction was quenched with 50% aq AcOH. The crude solids were collected, washed with H_2O, dried, and recrystallized from DMF:EtOH:H_2O to give 0.5 g (37%) of **4v**: mp 234–235 °C; ^{1}H NMR (DMSO-d_6) δ 12.5 (s, 2H, CO_2H), 8.66 (d, J = 8 Hz, 2H, N*H*), 8.29 (d, J = 7 Hz, 2H, N*H*), 7.70 (d, J = 6 Hz, 2H), 7.63 (d, J = 8 Hz, 2H), 7.43 (*t*, J = 7 Hz, 2H), 7.30 (*t*, J = 8 Hz, 2H), 4.59 (m, 2H, C*H*), 4.22 (m, 2H, C*H*), 1.8–1.5 (m, 6H, CH_2 and (CH_3) C*H*), 1.31 (d, 7 Hz, 6H, CH_3), 0.93 (d, 7 Hz, 12 H, CH(CH_3)$_2$. Anal. ($C_{32}H_{42}N_4O_8S_2$.0.7H_2O) C, H, N.

General method E: [S-(R*,R*)2-[[2-(1-carboxy-2-methylbutylcarbamoyl)phenyldisulfanyl]-benzoyl]-amino]-3-methylpentanoic acid (4t). To 5.70 g (30.4 mmol) of isoleucine *t*-butyl ester in 50 mL of pyridine at 0–10 °C was added dropwise, 4.80 g (14.0 mmol) of 2,2′-dithiobisbenzoyl chloride in 70 mL of CH_2Cl_2. After 18 h at room temperature, the mixture was concentrated, suspended in H_2O and filtered. The solids were dissolved in $CHCl_3$, which was extracted three times with H_2O, then dried and concentrated. The solids were triturated with ether:hexane (1:1) to give 3.4 g (38%) of crude *t*-butyl ester. This material was dissolved in 50 mL CH_2Cl_2 and reacted with 50 mL trifluoroacetic acid at 35 °C. After 2 h, the mixture was concentrated and the residue triturated with ether. The solids were suspended in EtOAc:$CHCl_3$ and filtered. The filtrate was concentrated to give 1.1 g (42%) of **4t**: mp 195–197 °C; IR (KBr) 1722, 1641 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 12.7 (bs, 2H, N*H*), 8.73 (d, J = 8 Hz, 2H), 7.65 (d, J = 8.8 Hz, 4H), 7.45 (m, 2H), 7.31 (t, J = 7.5 Hz, 2H), 4.35 (t, J = 8.0 Hz, 2H, NHC*H*), 1.96 (m, 2H), 1.53 (m, 2H), 1.33 (m, 2H), 0.96 (d, J = 6.7 Hz, 6H, CHCH_3), 0.89 (t, J = 7.8 Hz, 6H, CH_2CH_3); MS *m/z* 266 (100%, 1/2 m). Anal. ($C_{26}H_{32}N_2O_6S_2$.0.7H_2O) C, H, N.

General method F: 2,2′-dithiobis-(*N*-*t*-butyl)benzamide (4h). To 3.0 g (8.75 mmol) of the 2,2′-dithiobisbenzoyl chloride in 100 mL of CH_2Cl_2 was added dropwise a mixture of 6.39 g (87.5 mmol) of *tert*-butylamine and 8.85 g (87.5 mmol) of triethylamine in 20 mL of CH_2Cl_2. After 18 h the mixture was concentrated and the residue suspended in 100 mL of EtOAc. The solids were filtered to give 2.1 g (58%) of **4h**: mp 180–181 °C; IR (KBr) 3251, 1652, 1633 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.88 (s, 2H, N*H*), 7.58 (d, J = 7.5 Hz, 2H); 7.39 (m, 2H), 7.31 (d, J = 7.8 Hz, 2H), 7.25 (m, 2H), 1.32 (s, 18H, C(CH_3)$_3$), MS (CI *m/z*), 431 (5% m+CH_3), 222 (100% 1/2 m-1+CH_3). Anal. ($C_{22}H_{28}N_2O_2S_2$) C, H, N.

General procedure G: 2-[[2-[(1-carboxy-2-methyl butyl carbamoyl)phenyl disulfanyl]-benzoyl]-amino]-3-methylpentanoic acid (4q). Racemic isoleucine (26.2 g, 200 mmol) was slurried in 100 mL of absolute ethanol, and treated with a solution of 4.6 g (200 mmol) of sodium in 100 mL of ethanol, keeping the temperature between −30 and −50 °C. After 2 h, solution was nearly complete, and 17.2 g (50 mmol) of 2,2′-dithiobisbenzoyl chloride was added portionwise over 1 h. After 18 h at room temperature, the mixture was concentrated, the residue suspended in H_2O and the insoluble material filtered. The filtrate was adjusted to pH 3.0 with 1 N HCl and the solids collected, washed with H_2O, redissolved in $NaHCO_3$ and the procedure repeated. After drying, 8.9 g of crude material was obtained. Recrystallization from 60% aq EtOH gave 1.3 g (5%) of **4q** as a 3:2 mixture of diastereomers: mp 217–218 °C; ^{1}H NMR (DMSO-d_6) δ 12.7 (s, 2H), 8.8–8.6 (m, 2H), 7.6 (m, 4H), 7.4 (m, 2H), 7.3 (m, 2H), 4.6–4.3 (m, 2H), 2.0 (m, 2H), 1.5 (m, 2H), 1.3 (m, 2H), 0.9 (m, 12H). Anal. ($C_{26}H_{32}N_2O_6S_2$) C, H, N.

2,2′-Dithiobis-5-nitrobenzamide (8). To 6.8 g (33 mmol) of 2-chloro-5-nitrobenzamide (**7**) in 90 mL of ethanol at reflux was added a mixture of 2.6 g (20.5 mmol) of $Na_2S.9H_2O$ and 0.7 g (20.5 mmol) of sulfur in several portions. After 1 h at reflux, the mixture was cooled and the solids filtered to give 2.6 g (21%) of **8**: mp 266–269 ° ^{1}H NMR (DMSO-d_6) δ 8.70 (s, 2H), 8.69 (s, 2H), 8.3 (m, 2H), 8.0 (s, 2H), 7.8 (m, 2H).

2,2′-Dithiobis-5-aminobenzamide (9). To a slurry of 8.7 g of reduced iron in 65 mL of H_2O and 0.1 mL AcOH at reflux, was added in small portions, 2.6 g (7.0 mmol) of **8**. After 2 h of reflux, the mixture was cooled to room temperature, made basic (pH 10) with 1 N NaOH and filtered. The filtrate was neutralized to pH 6–7 with AcOH while bubbling O_2 through the solution via a gas dispersion tube. A solid gradually formed and was filtered, washed with water and dried to give 1.1 g (48%) of **9**: mp 188–190 °C; ^{1}H NMR (DMSO-d_6) δ 7.7 (s, 2H), 7.3–7.2 (m, 4H), 6.6–6.5 (m, 4H), 5.3 (s, 4H).

2,2′-Dithiobis(5-acetylamino)benzamide (10). To 1.1 g (3.4 mmol) of **9** was added 10 mL AcOH and 0.8 mL (8.2 mmol) of Ac_2O. The mixture was heated at 100 °C for 4 h, cooled, and the solids collected. Recrystallization from DMF:DMSO:H_2O (30:30:40) gave 0.8 g (59%) of **10**: mp 301–303 °C; ^{1}H NMR (DMSO-d_6) δ 10.1 (s, 2H, CH_3CON*H*), 8.0 (m, 2H), 7.85 (d, J = 3 Hz, 2H), 7.60 (m, 6H, NH_2, Ph-*H*), 2.05 (s, 6H, $COCH_3$). Anal. ($C_{18}H_{18}N_4O_4S_2$.0.3H_2O) C, H, N.

4,4′-Dithiobis-[4′-(sulfamoyl)benzanilide] (13). To 200 mL of thionyl chloride was added 5.0 g (16.3 mmol) of 4,4′-dithiobisbenzoic acid,[1] and the mixture was refluxed until dissolution was complete. The mixture was concentrated, chased with CH_2Cl_2 twice and then dissolved in 30 mL of CH_2Cl_2. This solution was added dropwise to 7.0 g (40.7 mmol) of 4-(aminosulfonyl)-aniline in 60 mL of pyridine at 0–10 °C. After 18 h, the solids were filtered, washed with 1 N HCl and dissolved in 60 mL of DMF. To this solution was added 60 mL of EtOH, 5 mL of 8% $NaHCO_3$ and 50 mL of H_2O in that order. The solids were collected and dried to give 5.5 g (55%) of **13**: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 10.6 (s, 2H, N*H*), 7.98 (d, J = 8.7 Hz, 4H), 7.92 (d, J = 8.7

Hz, 4H), 7.79 (d, J = 8.7 Hz, 4H), 7.72 (d, J = 8.6 Hz, 4H), 7.28 (s, 4H, SO_2NH_2). Anal. ($C_{26}H_{22}N_4O_6S_4$) C, H, N.

3,3′-Dithiobis-[4-(sulfamoyl)benzanilide] (14). This material was prepared from 3,3′-dithiobisbenzoic acid,[2] according to the procedure used for **13** to give 0.78 g (28%) of **14**: mp 295–296 °C; ^{1}H NMR (DMSO-d_6) δ 10.6 (bs, 2H, N*H*), 8.16 (s, 2H), 7.92 (m, 6H), 7.83 (m, 6H), 7.60 (*t*, J = 8.0 Hz, 2H), 7.30 (bs, 4H, SO_2NH_2). Anal. ($C_{26}H_{22}N_4O_6S_4 \cdot 0.2H_2O$) C, H, N.

2,2′-Dithiobis-[*N*-(4-aminophenyl)benzamide] dihydrochloride (3v). To a solution of 2,2′-dithiobis[*N*-(4-nitrophenyl)benzamide **3u** (0.309 g, 0.565 mmol) in 75 mL of CH_3OH, was added 0.3 g of Ra-Ni. The mixture was stirred for 30 h at room temperature under H_2 atmosphere. The mixture was filtered and the filtrate mixed with 10 mL of 6 N HCl, and concentrated. The solids were triturated with CH_3CN:MeOH to give 0.124 g (39%) of **3v**: mp >260 °C; IR (KBr), 3408 (br), 3292 (br), 2853 (br), 2850 (br), 1645, 1526, 1513 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 11.5 (s, 2H, N*H*), 7.8 (m, 8H), 7.76 (d, J = 8.5 Hz, 4H), 7.55 (t, J = 7 Hz, 2H), 7.41 (t, J = 7 Hz, 2H), 7.33 (m, 4H); MS *m/z* 243 (60%, 1/2 m). Anal. ($C_{26}H_{22}N_4O_2S_2 \cdot 2HCl$) C, H, N.

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