Tyrosine Kinase Inhibitors. 4. Structure–Activity Relationships among *N*- and 3-Substituted 2,2′-Dithiobis(1*H*-indoles) for *in vitro* Inhibition of Receptor and Nonreceptor Protein Tyrosine Kinases

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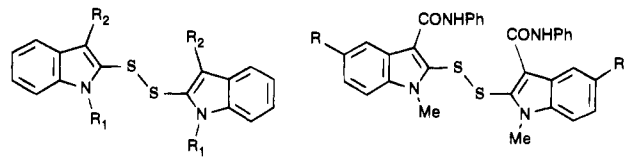
A series of 3-substituted 2,2′-dithiobis(1*H*-indoles) were synthesized and evaluated for their ability to inhibit the tyrosine kinase activity of both the epidermal growth factor receptor (EGFR) and the nonreceptor pp60$^{v\text{-}src}$ tyrosine kinase, to extend the available structure–activity relationships for this series. The majority of the compounds were prepared either by reaction of 2-chloro-1-methylindole-3-carbonyl chloride with amines, followed by thiomethylation, demethylation, and oxidative dimerization, or by reaction of isocyanates with the anion of 1-methyl-2-indolinethione followed by dimerization. Overall, inhibitory activity is retained by analogues having a wide variety of side chains. A series of 3-carboxamide analogues had moderate to good activity against isolated EGFR (IC_{50}s 1–20 μM), with monoalkyl substitution of the carboxamide being optimal. Polar side chains were generally less effective than lipophilic ones, with benzyl being particularly effective. However, *N*,*N*-disubstitution was the most effective pattern for inhibition of pp60$^{v\text{-}src}$. A variety of substituted *N*-phenylcarboxamides had lower activity against EGFR than the parent derivative, and a *N*-thienylcarboxamide also had low activity. A series of 3-ketones, including methyl, phenyl, and furyl derivatives, showed moderate activity against the pp60$^{v\text{-}src}$ kinase, but were less effective against EGFR. The mechanism of inhibition of both kinases by these drugs was shown to be noncompetitive with respect to both ATP and peptide substrate. Selected compounds inhibited the growth of Swiss 3T3 cells with IC_{50}s in the low micromolar range and inhibited bFGF-mediated intracellular tyrosine phosphorylation in the same cell line. Thiol inhibits the effects of the compounds, suggesting that one possible mechanism of inhibition is thiol–disulfide exchange with thiol-containing residues in the catalytic sites. Crystal structures of two representative compounds show a folded, V-shaped structure, with the disulfide bridge exposed, consistent with this hypothesis.

An outline of one of the pathways by which signals from external growth factors are transmitted to the nucleus is now available.[1,2] Many of the enzymes (primarily protein tyrosine kinases) involved in this pathway are encoded by proto-oncogenes, the transformation or overexpression of which is considered in many cases to result in malignancy.[3–5] Despite a high degree of homology among the kinase domains of different protein tyrosine kinases,[6] a variety of small-molecule inhibitors of phosphorylating activity are known, some of which show considerable selectivity between different enzymes.[7] Therefore, selective interruption of signal transduction in tumor cells by specific inhibitors of tyrosine kinase (TK) activity has recently emerged as a major new approach for the design of tumor-specific drugs.[8–10]

We have previously reported that 2,2′-dithiobis(1*H*-indole-3-alkanoic acids)[11] (e.g., **1**) and amides[12] (e.g., **2**), but not the related esters[11] (e.g. **3**), are potent inhibitors of both EGFR and pp60$^{v\text{-}src}$ tyrosine kinases, probably by noncompetitive inhibition at the tyrosine substrate binding site.[13] Structure–activity relationship studies on this class of compounds show that changes in the 3-alkyl side chain markedly affect biological activity.[11,12]



1: R_1 = H; R_2 = CH_2CH_2COOH
2: R_1 = H; R_2 = $CH_2CH(NH_2)CONHCH_2Ph$
3: R_1 = Me; R_2 = CH_2CH_2COOMe

4: R = Cl
5: R = CF_3

In a later study we also showed that a phenylcarboxamide side chain was permissible.[14] In this series of 2,2′-dithiobis(1-methyl-*N*-phenyl-1*H*-indole-3-carboxamides), no clear relationships were seen between indole ring substitution and inhibitory potency, although significant specificity between EGFR and pp60$^{v\text{-}src}$ kinases were found for some analogues. For example, the 5-Cl derivative (**4**) preferentially inhibited pp60$^{v\text{-}src}$, while the 5-CF_3 compound (**5**) preferentially inhibited EGFR.[14]

To complete our evaluation of the novel class of 2,2′-dithiobis(1*H*-indoles) as tyrosine kinase inhibitors, we now present data on a further series of compounds which bear a wider range of 3-substituents of differing electronic properties (*N*-methyl disulfides **7a-bb**), together with some corresponding NH analogues (**6**) and

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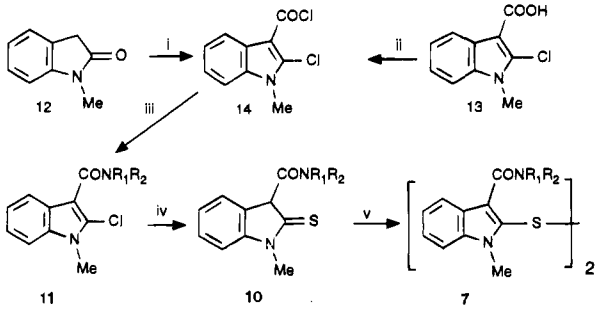
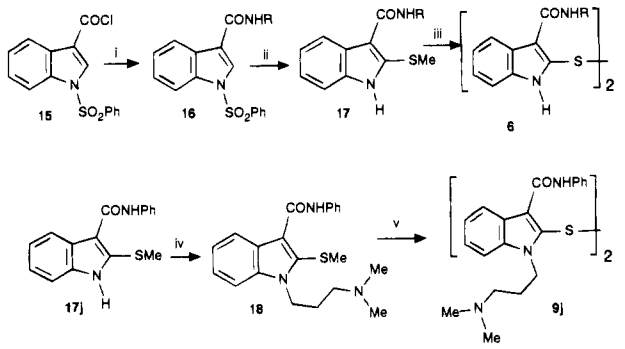
Scheme 1. Method A[a]



[a] (i) $COCl_2$/80 °C/1 h; (ii) $SOCl_2/ClCH_2CH_2Cl$/reflux/2 h; (iii) HNR_1R_2; (iv) MeSLi/DMA/60 °C/7 h; (v) $NaBO_3/AcOH/H_2O$/20 °C/30 min.

Scheme 2. Method B[a]



[a] (i) RNH_2/CH_2Cl_2; (ii) *n*-BuLi/THF/−78 °C, then MeSSMe/−78 °C, then K_2CO_3/MeOH/H_2O/reflux/2 h; (iii) MeSLi/DMA/80 °C/6 h, then H_2O_2/MeOH/20 °C; (iv) $Cl(CH_2)_3NMe_2$·HCl/EtOH/K_2CO_3/reflux/8 h; (v) MeSLi/DMA/80 °C/8 h, then I_2/MeOH/CH_2Cl_2/20 °C.

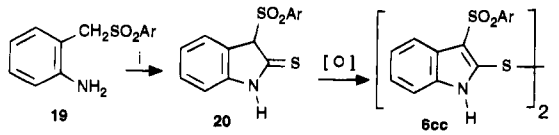
other *N*-alkyl derivatives (**8j**, **9j**). We also report X-ray crystallographic data for two representative compounds (**3** and **5**).

Chemistry

Many of the 3-substituted 2,2′-dithiobis(1-methyl-1*H*-indoles) (**7**) were prepared from the known[15] 2-chloro-1-methylindole-3-carbonyl chloride (**14**) (method A; Scheme 1), which was prepared either from 1,3-dihydro-1-methyl-2*H*-indol-2-one (**12**) by treatment with phosgene or from 2-chloro-1-methylindole-3-carboxylic acid (**13**).[15,16] Treatment of **14** with appropriate amines gave carboxamides **11**, which were reacted with an excess of MeSLi in DMA[17] at 60−80 °C. Under these conditions, the initially-formed 2-(thiomethyl)indole was demethylated to give the 2-thioindoles (2-indolinethiones) **10**, which underwent clean oxidative dimerization on treatment with H_2O_2 or sodium perborate to give the required disulfides **7**. Under the *S*-demethylation conditions used, 3-carboxamide side chains containing ester functionality were cleaved to the corresponding carboxylic acids (**7d**,**g**). The 3-acetyl disulfide **7v** was prepared by this method from the known 3-acetyl-2-chloro-1-methylindole.[18]

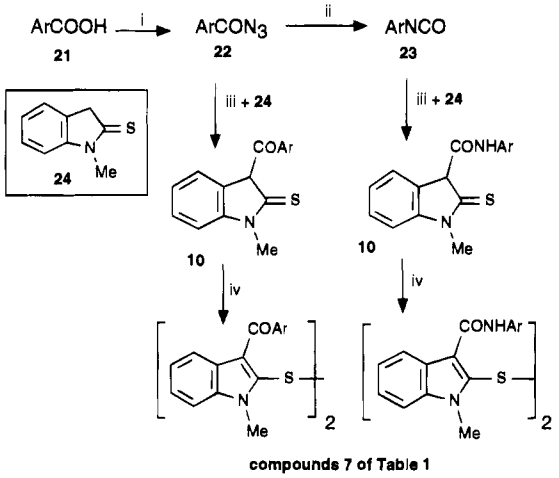
Disulfides unsubstituted at N1 were obtained from the analogous 2-methylthio compounds (**17**) (method B; Scheme 2). These were prepared from the known 3-(chlorocarbonyl)-1-(phenylsulfonyl)-1*H*-indole[19] (**15**) by condensation with amines, followed by lithiation of the resulting 1-(phenylsulfonyl) 3-carboxamides (**16**) and quenching with dimethyl disulfide.[20] *S*-Demethylation of the 2-methylthio compounds **17** with MeSLi as above, followed by oxidation, gave the required disulfides **6**.

Scheme 3. Method C[a]



[a] (i) *n*-BuLi/THF/−78 °C, then CS_2/−78 °C, then H^+.

Scheme 4. Method D[a]



[a] (i) $SOCl_2$/DMF/reflux, then NaN_3/Me_2CO/20 min; (ii) PhMe/reflux/2 h; (iii) NaH/THF/20 °C/1 h; (iv) H_2O_2/MeOH.

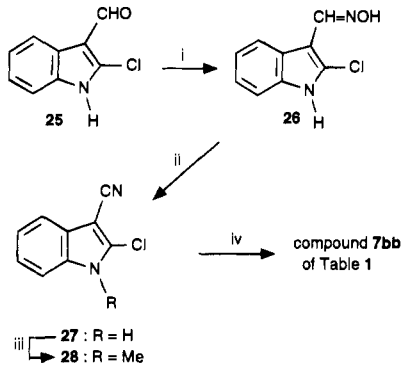
Reaction of the 3-(phenylcarboxamide) analogue (**17j**) with 3-(dimethylamino)propyl chloride gave the *N*-substituted analogue **18**, which was *S*-demethylated as above to give **9j**.

The 3-phenylsulfonyl derivative **6cc** was prepared from 2-(tosylmethyl)aniline (**19**) by lithiation and quenching with CS_2 to give the thione **20**, which spontaneously oxidized to the disulfide **6cc** (method C; Scheme 3). The 3-phenyl disulfide **6dd** was prepared by reaction of 3-phenylindole with S_2Cl_2 as reported.[21]

An alternative route to the disulfides **7** was from reaction of isocyanates **23** with the anion formed from 1-methyl-2-indolinethione (**24**) and NaH in THF, followed by dimerization, as described previously[14] (Method D; Scheme 4). The *N*-ethyl derivative (**8k**) was prepared similarly from 1-ethylindoline-2-thione.[22] This method gave moderate yields with aromatic isocyanates, but very poor yields with aliphatic ones. The isocyanates required for method D were generated by Curtius rearrangement of the acyl azides (**22**), which were obtained from the appropriate carboxylic acids (**21**). Reaction of deprotonated 1-methylindoline-2-thione (**24**) with the acyl azides themselves gave 3-keto derivatives (**10w**,**y**,**z**) which could be oxidatively dimerized with I_2 or H_2O_2 to give the corresponding disulfides (**7w**,**y**,**z**).

The 3-carbonitrile **7bb** was prepared from 2-chloro-1*H*-indole-3-carboxaldehyde (**25**)[23] (method E; Scheme 5). Treatment with hydroxylamine gave the oxime **26**, which was dehydrated in refluxing Ac_2O to the carbonitrile **27**. This was *N*-alkylated with MeI to give **28**, which was converted to **7bb** following method C. The 3-methyl disulfide **7aa** was prepared by $FeCl_3$ oxidation of 1,3-dihydro-1,3-dimethyl-2*H*-indole-2-thione,[24] which in turn was prepared by thiation of 1,3-dihydro-1,3-dimethyl-2*H*-indol-2-one.[25] The acids **7l-n**,**x** and **10x** were obtained from the corresponding methyl esters by hydrolysis with aqueous KOH, followed by reoxidation with H_2O_2.

Scheme 5. Method E[a]



[a] (i) NH_2OH·HCl/pyridine/EtOH/reflux/2 h; (ii) Ac_2O/reflux/1 h; (iii) MeI/K_2CO_3/Me_2CO/reflux/1 h; (iv) MeSLi/DMA/80 °C/6 h, then H_2O_2/MeOH/20 °C.

Results and Discussion

Table 1 gives IC_{50} values for inhibition of the tyrosine kinase activity of both the native EGFR complex contained in plasma membrane vesicles shed from cultured A431 epidermoid carcinoma cells[26] and the pp60$^{v\text{-}src}$ protein obtained from v-*src* baculovirus-infected insect cells which was coupled to 0.65 μm diameter latex beads via a monoclonal antibody.[27] IC_{50} values are defined as the concentration of drug necessary to reduce by 50% incorporation of ^{32}P (from added [γ-^{32}P]ATP) into a substrate (a 6:3:1 random copolymer of glutamate, alanine and tyrosine for EGFR, and poly[$(glu)_4(tyr)_1$] for v-*src*).

Previous studies have shown that 2,2′-dithiobis[1*H*-indoles] with alkylcarboxylic acid[11] (e.g., **1**), alkylcarboxamide[12] (e.g., **2**) and *N*-phenylcarboxamide[14] (e.g., **4**, **5**, **7j**) side chains show potent inhibitory activity against both receptor and nonreceptor tyrosine kinase enzymes. The results of Table 1 greatly extend the range of active 3-substituents. Compounds **7a-i** are a variety of unsubstituted and *N*-alkyl substituted 3-carboxamides which have moderate to good activity against EGFR. As shown by compounds **7a-c**, monoalkyl substitution of the carboxamide is optimal. Within this substitution pattern (compounds **7d-i**), polar side chains, whether anionic (**7d**), neutral (**7e**), or cationic (**7f**), were less effective than more lipophilic ones, particularly benzyl (**7g**). However, a quite different pattern was seen for inhibition of pp60$^{v\text{-}src}$, where *N*,*N*-disubstitution was the most effective pattern (compound **7c**), and the anionic derivatives (**7d** and **7h**) were preferred side chains.

As noted previously,[14] the *N*-phenylcarboxamide (**7j**) is an effective inhibitor. Disubstitution of the carboxamide (compound **7k**) abolished activity against EGFR (but not against pp60$^{v\text{-}src}$). The substituted *N*-phenylcarboxamides (**7l-t**) were either inactive or had much lower activity against EGFR than did **7j** (the best was the 4-COOH analogue **7n**), and their activity against pp60$^{v\text{-}src}$, while better, was not as good as that of the *N*-alkylcarboxamides. The *N*-thienylcarboxamide **7u** also had low activity.

A small number of analogous 3-ketones were also evaluated. The methyl ketone **7v** showed moderate activity against both EGFR and pp60$^{v\text{-}src}$, whereas the phenyl ketone **7w** was inactive against EGFR but quite effective against pp60$^{v\text{-}src}$. However, both the 4-COOH and 4-COOMe derivatives (**7x** and **7y**) were moderately active in both assays. The 2-furyl analogue **7z** also had moderate activity against both enzymes. Finally, the 3-Me derivative **7aa** was inactive, whereas the 3-nitrile **7bb** was a moderately potent inhibitor of EGFR but less active against pp60$^{v\text{-}src}$.

Because previous work (in the 3-alkyl carboxylic acid series)[11] had shown that alkylation on the indole nitrogen reduced activity, the NH analogues (**6b**, **6g**, and **6j**) of three representative compounds were prepared. However, the results suggest that, in the present series, the NH compounds are less effective than their corresponding NMe derivatives (**7b**, **7g**, and **7j**). In this regard, the moderate activity of the 3-tosyl derivative **6cc** is of interest, suggesting that the corresponding NMe analogue might be even more effective. To further study the effects of *N*-substitution, the NEt and $N(CH_2)_3NMe_2$ analogues of **7j** were prepared. Surprisingly, while the NEt analogue (**8j**) was inactive, the latter cationic derivative (**9j**) showed good inhibitory activity, particularly against pp60$^{v\text{-}src}$ (IC_{50} 5.6 μM).

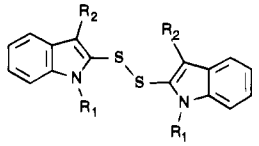
Table 1 also records data for a number of thiones (**10**) corresponding to several of the disulfides (**7**) in the table. Previous studies have shown the thiones to be in general much less effective inhibitors than the disulfides,[11,12,14] and because 3-alkyl substituted thiones undergo facile oxidative dimerization to the disulfides,[11,28] the activity seen with some of these thiones was considered due to adventitious generation of disulfide. However, the 3-(phenylcarboxamide) analogues undergo much slower conversion; the half-life for oxidative dimerization of **10j** (to **7j**) is ca. 5 h at pH 7,[14] compared with 10–20 min for 3-alkyl analogues under similar conditions (unpublished work, this laboratory). Thus the activity of **10j** and **10x** is interesting, since it appears likely to be due to the thione itself.

The mechanism of enzyme inhibition by compounds of this class has been studied previously,[13] and the 3-CONHPh derivative **7j** has been shown to be noncompetitive with respect to both ATP and peptide substrate.[29] In the present study, the inhibition of the v-*src* enzyme by the 3-CONHMe derivative **7b** was determined with respect to varying concentrations of both ATP and peptide polymer substrate (Figure 1). The data used to generate the Michaelis–Menton saturation curves (panel A) were fit to Cleland's enzyme inhibition equations using a commercial software package (Grafit, Erithacus Software, Ltd.) and a K_i value was obtained from the curve-fit data. The Lineweaver–Burk plots (panel B) indicate noncompetitive inhibition with respect to both ATP and peptide polymer substrate, with K_i values of 8 μM with respect to varying ATP concentration and 1.4 μM with respect to varying peptide substrate.

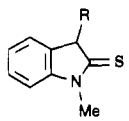
Selected compounds were shown to inhibit the growth of Swiss 3T3 cells with IC_{50}s in the low micromolar range (Table 3). Both the 3-Ph and 3-CONHMe compounds (**6dd** and **7b**) were considerably more potent than the 3-CONHPh analogue (**7j**) previously studied,[29] indicating the degree of tolerance available at this position. However, the cationic derivative **7f**, with a 3-$CONH(CH_2)_2NMe_2$ side chain, was much less effective.

To assess the effect of this class of compounds on intracellular tyrosine phosphorylation, Swiss 3T3 fibroblasts were pretreated with varying concentrations of the 3-CONHMe derivative **7b** for 2 h and then exposed

Table 1. Physicochemical and Biological Properties of 3-Substituted 2,2′-Dithiobis[1*H*-indoles] and 1,3-Dihydro-2*H*-indole-2-thiones



						tyrosine kinase inhibition (IC_{50})[b]		
no.	X	R	method[a]	mp (°C)	formula	analyses	EFGF-R	src
1			–		ref 11		4.2	5.1
2			–		ref 11		3.3	0.6
3			–		ref 11		>100	
4			–		ref 14		4.3	>100
5			–		ref 14		>100	5.8
6b	H	CONHMe	B	232–236	$C_{20}H_{18}N_4O_2S_2$	C,H,N,S	6.1	7.9
6g	H	$CONHCH_2Ph$	B	203–205	$C_{32}H_{26}N_4O_2S_2$	C,H,N,S	ca. 15	16.3
6j	H	CONHPh	B	220–223	$C_{30}H_{22}N_4O_2S_2$	C,H,N,S	ca. 100	7.6
6cc	H	$SO_2Ph(4\text{-}Me)$	C	230–233	$C_{30}H_{24}N_2O_4S_4 \cdot 0.2C_6H_6$[c]	C,H,N,S	28.7	5.5
6dd	H	Ph	–	196–197.5	ref 21		ca. 100	>100
7a	Me	$CONH_2$	A	186–188	$C_{20}H_{18}N_4O_2S_2 \cdot 0.5H_2O$	C,H,N,S	4.7	0.8
7b	Me	CONHMe	A	186–187	$C_{22}H_{22}N_4O_2S_2 \cdot 0.5H_2O$	C,H,N,S	1.8	6.7
7c	Me	$CONMe_2$	A	96–102	$C_{24}H_{26}N_4O_2S_2 \cdot 0.5H_2O$	C;H;N[d]	21.2	0.5
7d	Me	$CONHCH_2COOH$	A	197 (dec)	$C_{24}H_{22}N_4O_6S_2 \cdot H_2O$	C,H,N;S[e]	10.0	1.0
7e	Me	$CONHCH_2CH(OH)CH_2OH$	A	198	$C_{26}H_{30}N_4O_6S_2$	H,N,S;C[f]	43[g]	22.5[g]
7f	Me	$CONH(CH_2)_2NMe_2$	A	163.5–165	$C_{28}H_{36}N_6O_2S_2$	C,H,N,S	17.5	15.2[g]
7g	Me	$CONHCH_2Ph$	A	147–148	$C_{34}H_{30}N_4O_2S_2$	H,N,S;C[f]	1.7	8.0
7h	Me	$CONHCH_2Ph(4\text{-}COOH)$	F	168–170	$C_{36}H_{30}N_4O_6S_2 \cdot 1.5H_2O$	C,H,N,S	5.3	0.8
7i	Me	$CONHCH_2Ph(4\text{-}COOMe)$	D	178–180	$C_{38}H_{34}N_4O_6S_2 \cdot 0.5H_2O$	C,H,N,S	13	2.5
7j	Me	CONHPh	–		ref 14		10.9	3.2
7k	Me	CON(Me)Ph	A	160–163	$C_{34}H_{30}N_4S_2O_8$	C,H,N,S	>100	5.5
7l	Me	CONHPh(2-COOH)	F	184–186	$C_{34}H_{26}N_4O_6S_2 \cdot 2H_2O$	C,H,N,S	47	3.5
7m	Me	CONHPh(3-COOH)	F	219–220	$C_{34}H_{26}N_4O_6S_2 \cdot 0.5H_2O$	C,H,N,S	12	5.3
7n	Me	CONHPh(4-COOH)	F	221 (dec)	$C_{34}H_{26}N_4O_6S_2 \cdot H_2O$	C,H,N,S	16.9	4.3
7o	Me	CONHPh(2-COOMe)	D	179–181	$C_{36}H_{30}N_4O_6S_2 \cdot 0.5H_2O$	C,H,N,S	>100 (insol.)	15.6
7p	Me	CONHPh(3-COOMe)	D	193–195	$C_{36}H_{30}N_4O_6S_2$	C,H,N,S	>100	5.4
7q	Me	CONHPh(4-COOMe)	D	184–186	$C_{36}H_{30}N_4O_6S_2 \cdot H_2O$	C,H,N,S	>100	4.2
7r	Me	CONH(2-pyridyl)	A	270–272	$C_{30}H_{34}N_6O_2S_2 \cdot 0.25H_2O$	C,H,N,S	47	47
7s	Me	CONH(3-pyridyl)	A	257–260	$C_{30}H_{24}N_6O_2S_2$	C,H,N,S	>100	>50
7t	Me	CONH(4-pyridyl)	A	226–229	$C_{30}H_{24}N_6O_2S_2 \cdot 0.5H_2O$	C,H,N,S	>100	>50
7u	Me	CONH(2-thienyl)	D	183 (dec)	$C_{28}H_{22}N_4O_4S_2$	HRMS[h]	>100	>50
7v	Me	COMe	A	178.5–179.5	$C_{22}H_{20}N_2O_2S_2 \cdot 0.5H_2O$	C,H,N	5.6	11.5
7w	Me	COPh	D	199–202	$C_{32}H_{24}N_2O_2S_2 \cdot H_2O$	H,N,S;C[f]	>100	2.6
7x	Me	COPh(4-COOH)	F	246	$C_{34}H_{24}N_2S_2O_6 \cdot 2H_2O$	C,H,N	5.5	4.0
7y	Me	COPh(4-COOMe)	D	200–203	$C_{36}H_{28}N_2O_6S_2$	C,H,N,S	6.1	7.1
7z	Me	CO(2-furyl)	D	175–176.5	$C_{28}H_{30}N_2O_4S_2 \cdot 0.5H_2O$	C,H,N,S	7.7	8.3[g]
7aa	Me	Me	–	121–122	ref 21		>100	>50
7bb	Me	CN	E	205–207	$C_{20}H_{14}N_4S_2$	C,H,N;S[e]	6.9	28
8j	Et	CONHPh	D	200–202	$C_{34}H_{30}N_4O_2S_2$	C,H,N,S	>100	>100
9j	$(CH_2)_3NMe_2$	CONHPh	B	nc[i]	$C_{40}H_{45}N_6O_2S_2$	HRMS[h]	26	5.6



						tyrosine kinase inhibition (FC_{50})[b]	
no.	R	method[a]	mp (°C)	formula	analyses	EFGF-R	src
10j	CONHPh	–	149–151	ref 14		1.0	10.0
10v	COMe	A	180	$C_{11}H_{11}NOS$	C,H,N,S	>100	>50
10w	COPh	D	132–134	$C_{16}H_{13}NOS$	C,H,N,S	>100	69
10x	COPh(4-COOH)	F	282 (dec)	$C_{17}H_{13}NO_3S \cdot 0.25H_2O$	C,H,N:S[e]	8.7	2.1
10y	COPh(4-COOMe)	D	164–166	$C_{18}H_{15}NO_3S$	C,H,N,S	>100	>100
10z	CO(2-furyl)	D	113.5	$C_{14}H_{13}NO_2S$	C,H,N,S	>100	10.8[g]
10aa	Me	–	49–51	ref 26		>100	>100

[a] Method A, Scheme 1; method B, Scheme 2; method C, Scheme 3; method D, Scheme 4; method E, Scheme 5; method F, by hydrolysis of corresponding methyl ester with KOH. See text for details. [b] Drug concentrations (μM) to cause 50% inhibition of tyrosine phosphorylation activities of the EGF receptor and $pp60^{v\text{-}src}$ tyrosine kinases; see the text. [c] Benzene solvate, confirmed by NMR. [d] N off by 0.5%. [e] S off by 0.5%. [f] C off by 0.5%. [g] Duplicate determinations in a single test; the remainder of the values are means of duplicate determinations from at least two separate tests. [h] High-resolution FAB mass spectrum molecular ion. [i] Noncrystalline.

to different growth factors. Figure 2 is an antiphosphotyrosine Western blot, showing the effect of **7b** on bFGF-mediated tyrosine phosphorylation. Typically, when Swiss 3T3 fibroblasts are exposed to bFGF, a protein of approximately 85 kDa is phosphorylated on tyrosine, and **7b** inhibited the phosphorylation of this protein in a concentration-dependent manner with an IC_{50} value of approximately 12 μM. No effects on EGF

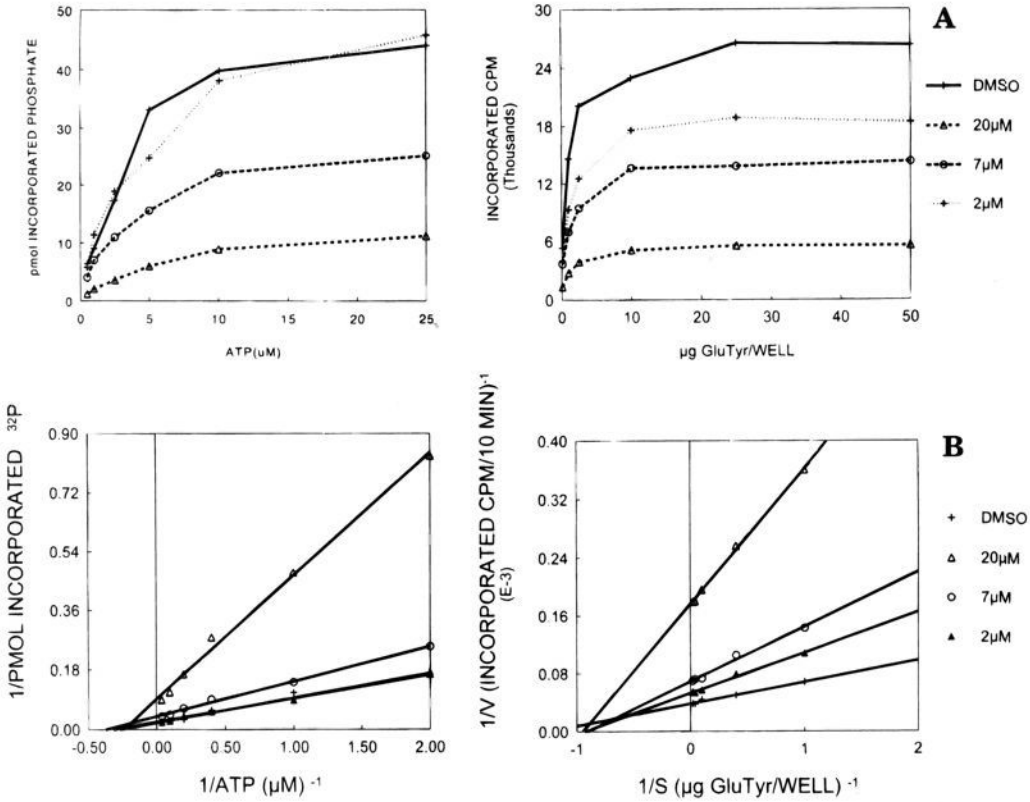
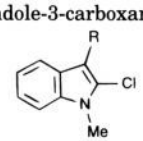


Figure 1. (Panel A) Michaelis–Menten saturation curves for the activity of v-*src* kinase in the presence of different concentrations of **7b**, with respect to varying ATP (left-hand) and peptide polymer substrate (right-hand). (Panel B) Lineweaver–Burke transformations of the data in panel A. These describe noncompetitive inhibition with respect to both ATP and peptide polymer substrate, with K_i values of 8 μM with respect to varying ATP concentrations and 1.4 μM with respect to varying the peptide substrate concentration.

Table 2. *N*-Substituted 2-Chloro-1-methyl-1*H*-indole-3-carboxamides



no.	R	mp (°C)	formula	analyses
11a	$CONH_2$	–	ref 39	
11b	CONHMe	148–151	$C_{11}H_{11}ClN_2O$	C,H,N
11c	$CONMe_2$	–	ref 15	
11d	$CONHCH_2COOMe$	102–104	$C_{13}H_{13}ClN_2O_3$	C,H,N
11e	$CONHCH_2CH(OH)CH_2OH$	nc[a]	$C_{13}H_{15}ClN_2O_3$	HRMS[b]
11f	$CONH(CH_2)_2NMe_2$	nc[a]	$C_{14}H_{18}ClN_3O$	HRMS[b]
11i	$CONHCH_2Ph(4\text{-}COOMe)$	173–175	$C_{19}H_{17}ClN_2O_3$	C,H,N,Cl
11k	CON(Me)Ph	163	$C_{17}H_{15}ClN_2O$	C,H,N,Cl
11r	CONH(2-pyridyl)	123	$C_{15}H_{12}ClN_3O$	C,H;N[c]
11s	CONH(3-pyridyl)	175–177	$C_{15}H_{12}ClN_3O$	C,H,N,Cl
11t	CONH(4-pyridyl)	220–223	$C_{15}H_{12}ClN_3O$	C,H;N[d]

[a] Noncrystalline. [b] High-resolution mass spectrum. [c] N off by 0.6%. [d] N off by 0.5%.

Table 3. Ability of 2,2′-Dithiobis(1*H*-indoles) To Inhibit the Proliferation of Swiss 3T3 Mouse Fibroblasts

no.	IC_{50} (μM)[a]	no.	IC_{50} (μM)[a]
6dd	2.8	**7g**	8.5
7b	1.8	**7j**	12
7f	>50		

[a] Concentration of compound necessary to inhibit cell growth rate by 50%. Values are the mean of two separate duplicate determinations.

or PDGF-mediated tyrosine phosphorylation were observed at concentrations up to 50 μM (data not shown).

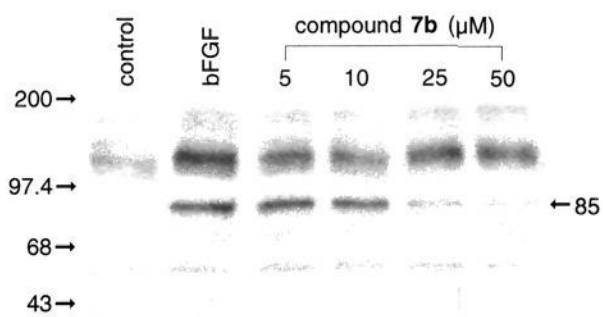


Figure 2. Effect of **7b** on bFGF-mediated tyrosine phosphorylation in Swiss 3T3 cells. Cells were grown to confluence in 6-well plates and growth arrested in serum-free medium for 18 h. The cells were exposed to varying concentrations of **7b** for 2 h and then to 20 ng/mL of bFGF for 15 min. Whole cell extracts and Western blots with antiphosphotyrosine antibodies were performed as described in the Experimental Section.

X-ray crystal structures were determined for two representative disulfides, the 3-alkyl derivative **3** and the 3-(*N*-phenylcarboxamide) **5** (Figure 3). The results show that the two molecules adopt almost identical conformations, with the different side chains having little effect on the overall structure of the molecule. When viewed along the S–S bond, in each case the indole rings form a twisted V-shape, with the side chains lying on the outer sides of the V and the sulfur atoms at the apex. The accessibility of the disulfide linkage in these molecules is compatible with disulfide exchange processes occurring during the drug–protein interactions.

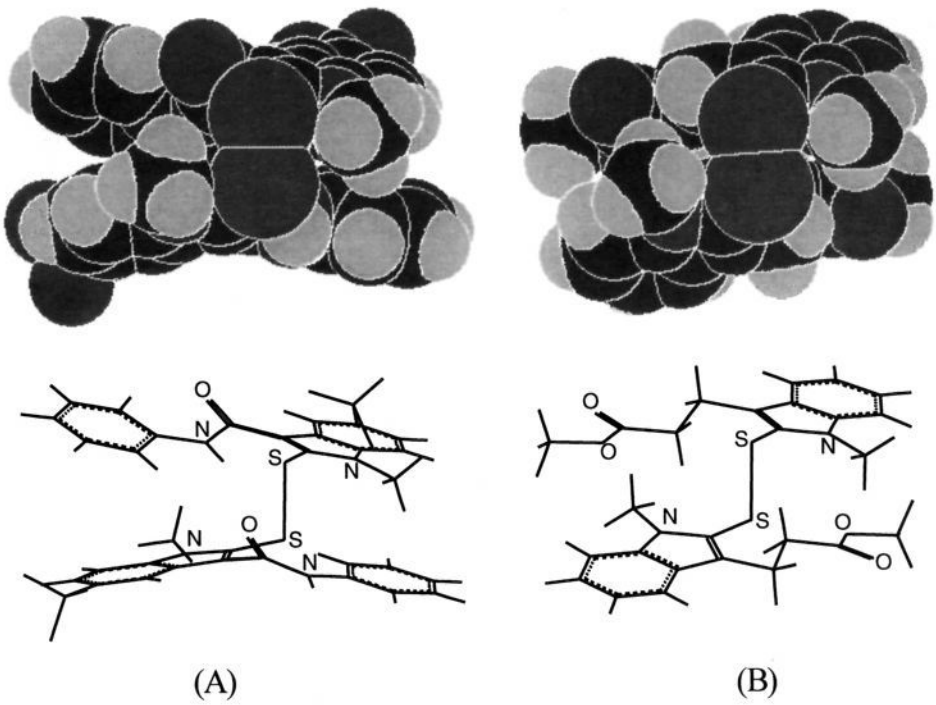


Figure 3. Crystal structures of compounds **5** (A) and **3** (B) shown in space-filling and line forms. The structures are aligned in analogous orientations to reveal their similarities, with the disulfide linkages placed at the front of the diagrams and the indole rings projecting into the page.

Conclusions

This study concludes our initial exploration[11,12,14] of the 2,2′-dithiobis(1*H*-indoles), which have proved to be novel inhibitors of EGFR and pp60$^{v\text{-}src}$ tyrosine kinases. Overall, wide variation in the nature of the side chain is tolerated, with alkyl acids, alkylamides, *N*-phenylcarboxamides, ketones, sulfones and nitrile groups all providing active examples. However within a particular class, further modifications of the side chain have marked effects on biological activity. As an example, the provision of hydrogen-bonding capability and a further lipophilic region is beneficial for EGFR activity, so that the preferred side chain is probably an *N*-benzylpropanamide.[12] However, 3-(*N*-phenylcarboxamide) side chains also provide compounds with good inhibitory potency against the isolated enzyme.[14] In this series, no clear relationships could be seen between nuclear substitution and inhibitory potency.[14] Finally, varying effects were noted for *N*-substitution on the indole. Among 3-(alkylcarboxamides), *N*-alkylation of the indole lowered activity, but this had little effect in the 3-(*N*-phenylcarboxamide) subclass.

The mechanism by which compounds of this class inhibit the tyrosine kinase activity of enzymes in the signal transduction pathway has not been determined. Recent kinetic studies indicate noncompetitive inhibition at both the tyrosine substrate and ATP binding sites.[13] To date, crystal structure data have been reported for three protein kinase enzymes[30] (but not EGFR), all of which show a bilobed structure, with the substrate binding site close to the protein surface and the ATP site deep in the cleft. The inhibitory effect of the 2,2′-dithiobis(1*H*-indoles) on kinases is itself sensitive to thiol,[31] suggesting that one possible mechanism of enzyme inhibition is thiol−disulfide exchange with thiol-containing residues in the catalytic site. These reactions are known to be facile,[32,33] and the crystal structures of the disulfides reported here show the availability of the sulfur atoms in these molecules. Modifications to the link group which might lower this sensitivity to thiol while an ability to exchange with protein thiol residues is retained would be interesting, and one such approach (diselenide analogues) has recently been reported.[31]

Experimental Section

Where analyses are indicated by symbols of the elements, results were within ±0.4% of the theoretical, and were performed by the Microchemical Laboratory, University of Otago, Dunedin, New Zealand. Melting points were determined using an Electrothermal Model 9200 digital melting point apparatus and are as read. ^{1}H NMR spectra were measured on Bruker AM-400 or AC-200 spectrometers and are referenced to Me_4Si; ^{13}C NMR spectra were recorded in the same solvent stated for the ^{1}H spectra. Mass spectra were recorded on a Varian VG 7070 spectrometer at nominal 5000 resolution. High-resolution FAB mass spectra were obtained for the disulfides from a 3-nitrobenzyl alcohol matrix.

Preparation of 2,2′-Dithiobis(*N*,1-dimethyl-1*H*-indole-3-carboxamide) (7b): Example of General Method A. A mixture of 1,3-dihydro-1-methyl-2*H*-indol-2-one (**12**) (20 g, 136 mmol) and dichloroethane (250 mL) in a 500 mL stainless steel autoclave was cooled to less than −10 °C, and phosgene (60 g, 0.6 mol) was distilled into the vessel. The reactor was then sealed, heated to 80 °C with rocking for 1 h, and then cooled to 20 °C, vented, and purged with N_2. Evaporation of solvent gave crude 2-chloro-1-methyl-1*H*-indole-3-carbonyl chloride (**14**), which was dissolved in CH_2Cl_2 (300 mL). The solution was cooled in an ice bath and treated over 50 min with anhydrous gaseous $MeNH_2$ and then washed with water (2 × 300 mL) and brine, dried, and concentrated to 150 mL. This solution was chromatographed on silica gel, eluting with CH_2Cl_2 followed by CH_2Cl_2/Me_2CO (49:1, then 19:1). Fractions were pooled (with recrystallization from EtOH/light petroleum where necessary) to give 2-chloro-*N*,1-dimethyl-1*H*-indole-3-carboxamide (**11b**) (19.45 g, 64%), mp 148−151 °C. ^{1}H NMR ($CDCl_3$) δ 8.26 (d, J = 6.5 Hz, 1 H, H-4), 7.26−7.21 (m, 3 H, H-5, 6, 7), 6.36 (br s, 1 H, NH), 3.77 (s, 3 H, 1-CH_3), 3.06 (d, J = 4.5 Hz, 3 H, NHCH_3). Anal. ($C_{11}H_{11}ClN_2O$) C, H, N.

The above carboxamide (**11b**) (9.30 g, 41.8 mmol) was treated with MeSLi (7.0 g, 129.5 mmol) in DMA (36 mL), and the mixture was heated at 60 °C for 7 h and then cooled in an ice bath and treated slowly with aqueous HCl (150 mL of 5%). The resultant suspension was diluted with CH_2Cl_2 (150 mL) and stirred for 1 h. The organic phase was separated, the aqueous phase was extracted twice more, and the combined organic extracts were washed with water (3 × 200 mL) and then brine, dried, and concentrated (finally under high vacuum) to give an orange solid (12.5 g). This was suspended in a mixture of AcOH/water (2:1; 150 mL) and treated with sodium perborate (12.85 g, 150 mmol) with vigorous stirring for 30 min. The resulting thick suspension was filtered, and the residue was washed successively with MeOH/water (1:9), water, and Et_2O and air-dried to give **7b** (6.38 g, 70%), mp 186–187 °C. ^{1}H NMR ($CDCl_3$) δ 8.06 (d, J = 8.0 Hz, 1 H, H-4), 7.40–7.21 (m, 3 H, ArH), 6.43 (br s, 1 H, NH), 3.83 (s, 3 H, 1-CH_3), 2.12 (d, J = 3.8 Hz, 3 H, NHCH_3). Anal. ($C_{22}H_{22}N_4O_2S_2$·0.5 H_2O) C, H, N, S.

2,2′-Dithiobis[*N*-[(4-carboxyphenyl)methyl]-1-methyl-1*H*-indole-3-carboxamide] (7i). A solution of 2-chloro-1-methyl-1*H*-indole-3-carboxylic acid (**13**)[16] (0.95 g, 4.52 mmol) and $SOCl_2$ (0.99 mL, 13 mmol) in 1,2-dichloroethane (100 mL) containing DMF (1 drop) was heated under reflux under N_2 for 2 h and then concentrated to dryness. The residue of crude 2-chloro-1-methyl-1*H*-indole-3-carbonyl chloride (**14**) was dissolved in CH_2Cl_2 (50 mL) and treated with a slurry of [[4-(methoxycarbonyl)phenyl]methyl]amine hydrochloride[34] (1.00 g, 4.98 mmol) and Et_3N (1.58 mL, 11 mmol) in CH_2Cl_2 (50 mL). After vigorous stirring at 20 °C for 24 h the mixture was washed with water, and the organic portion was worked up to give a solid which was chromatographed on silica. Elution with EtOAc/petroleum ether (1:1) gave 2-chloro-*N*-[[(4-methoxycarbonyl)phenyl]methyl]-1-methyl-1*H*-indole-3-carboxamide (**11i**) (1.40 g, 86%), mp (aqueous Me_2CO) 173–175 °C. ^{1}H NMR [$(CD_3)_2SO$] δ 8.38 (t, J = 5.8 Hz, 1 H, CON*H*CH_2), 7.95 (d, J = 7.9 Hz, 2 H, H-2′,6′), 7.91 (d, J = 7.8 Hz, 1 H, H-4), 7.56 (d, J = 7.9 Hz, 1 H, H-7), 7.52 (d, J = 7.9 Hz, 2 H, H-3′,5′), 7.29 (dd, J = 7.9, 7.1 Hz, 1 H, H-6), 7.19 (dd, J = 7.8, 7.1 Hz, 1 H, H-5), 4.60 (d, J = 5.8 Hz, 2 H, CONHCH_2), 3.84 (s, 3 H, COOH), 3.79 (s, 3 H, NCH_3). ^{13}C NMR δ 166.09 (*C*OOCH$_3$), 162.77 (CONH), 145.65, 135.00 (2 s), 129.18, 129.14 (2 d), 127.94 (s), 127.34, 127.25 (2 d), 126.34, 124.77 (2 s), 122.57, 121.19, 119.97 (3 d), 110.21 (s), 107.11 (d), 51.95 (COO*C*H$_3$), 42.15 (CH_2), 29.97 (NCH_3). Anal. ($C_{19}H_{17}ClN_2O_3$) C, H, N, Cl.

A solution of **11i** (1.00 g, 2.80 mmol) in DMA (10 mL) was added under N_2 to a stirred suspension of MeSLi (1.06 g, 19 mmol) in DMA (25 mL). After warming at 80 °C for 6 h, the mixture was acidified with 3 N HCl, extracted with CH_2Cl_2, and worked up to give a yellow oil. Traces of DMA were removed under high vacuum, and the residue was dissolved in MeOH (20 mL) and treated dropwise with H_2O_2 (0.60 mL of 30% solution). After chilling at −30 °C overnight, the precipitate was filtered off, washed well with MeOH, and dried to give 2,2′-dithiobis[*N*-[(4-carboxyphenyl)methyl]-1-methyl-1*H*-indole-3-carboxamide] (**7i**) (0.68 g, 72%), mp 168–170 °C. ^{1}H NMR [$(CD_3)_2SO$] δ 12.86 (br, 1 H, COOH), 8.13 (t, J = 5.8 Hz, 1 H, CON*H*CH_2), 7.92–7.80 (m, 3 H, H-4, 2′,6′), 7.56 (d, J = 8.3 Hz, 1 H, H-7), 7.37 (t, J = 8.3, 7.8 Hz, 1 H, H-6), 7.27 (d, J = 8.3 Hz, 2 H, H-3′,5′), 7.20 (dd, J = 8.1, 7.8 Hz, 1 H, H-5), 4.02 (d, J = 5.8 Hz, 2 H, CONHCH_2), 3.62 (s, 3 H, N-CH_3). ^{13}C NMR δ 167.08 (COOH), 163.08 (CONH), 144.51, 137.64, 130.35 (3xs), 129.25 (d), 129.04 (s), 126.85 (d), 125.25 (s), 124.44, 121.23, 121.10 (3 d), 118.33 (s), 110.87 (d), 41.92 (CH_2), 29.94 (NCH_3). Anal. ($C_{36}H_{30}N_4O_6S_2$·1.5H_2O) C, H, N, S.

Similar reaction of **14** with other amines or amine hydrochlorides gave the *N*-substituted 2-chloro-1-methyl-1*H*-indole-3-carboxamides (**11**) of Table 2. These were then treated with MeSLi as above to give compounds **7a-g,k,r-t**. Details of yields and NMR spectra are available as supplementary material.

3-Acetyl-1,3-dihydro-1-methyl-2*H*-indole-2-thione (10v) and 2,2′-Dithiobis(3-acetyl-1-methyl-1*H*-indole) (7v). 3-Acetyl-2-chloro-1-methylindole[18] was reacted with MeSLi as above to give 3-acetyl-1,3-dihydro-1-methyl-2*H*-indole-2-thione (**10v**) (66% yield), mp 180 °C. ^{1}H NMR [$(CD_3)_2SO$] δ 15.60 (br, 1 H, SH), 7.64 (d, J = 6.5 Hz, 1 H, H-4), 7.39 (d, J = 7.6 Hz, 1 H, H-7), 7.32 (dd, J = 7.6, 7.3 Hz, 1 H, H-6), 7.24 (dd, J = 7.3, 6.5 Hz, 1 H, H-5), 3.65 (s, 3 H, NCH_3), 2.66 (s, 3 H, $COCH_3$). ^{13}C NMR δ 178.29 (*C*OCH$_3$), 140.56 (s), 125.21 (d), 124.67 (s), 123.27, 120.60 (2 d), 111.31 (s), 109.99 (d), 29.31 (NCH_3), 22.44 (CO*C*H$_3$). Anal. ($C_{11}H_{11}NOS$) C, H, N, S.

A solution of **10v** (0.10 g, 0.49 mmol) in MeOH/EtOAc (1:9) (10 mL) was stirred vigorously with 30% H_2O_2 (0.20 mL), for 4 h. The solution was concentrated to a volume of 0.5 mL, and the orange precipitate was filtered off and washed well with MeOH to give 2,2′-dithiobis(3-acetyl-1-methyl-1*H*-indole) (**7v**) (100% yield), mp 178.5–179.5 °C. ^{1}H NMR [$(CD_3)_2SO$] δ 8.14 (d, J = 8.1 Hz, 1 H, H-4), 7.62 (d, J = 8.3 Hz, 1 H, H-7), 7.39 (dd, J = 8.3, 7.3 Hz, 1 H, H-6), 7.27 (dd, J = 8.1, 7.3 Hz, 1 H, H-5), 3.75 (s, 3 H, NCH_3), 2.00 (s, 3 H, $COCH_3$). ^{13}C NMR δ 192.56 (*C*OCH$_3$), 137.65, 133.73, 125.41 (3 s), 124.79, 122.73, 121.95 (3 d), 121.43 (s), 110.92 (d), 30.34 (CO*C*H$_3$), 29.43 (NCH_3). Anal. ($C_{22}H_{20}N_2O_2S_2$·0.5H_2O) C, H, N.

Preparation of 2,2′-Dithiobis[*N*-(phenylmethyl)-1*H*-indole-3-carboxamide] (6g): Example of General Method B. *N*-(Phenylmethyl)-1-(phenylsulfonyl)-1*H*-indole-3-carboxamide (**16g**) was prepared by the method of Ketcha and Gribble[19] [reaction of an excess of the appropriate amine with 3-(chlorocarbonyl)-1-(phenylsulfonyl)-1*H*-indole (**15**) in CH_2Cl_2], mp (MeOH) 188–189 °C. ^{1}H NMR ($CDCl_3$) δ 8.05 (s, 1 H, H-2), 8.03–7.86 (m, 4 H, ArH), 7.56–7.26 (m, 10 H, ArH), 6.43 (m, 1 H, NH), 4.64 (d, J = 5.7 Hz, 2 H, CH_2). Anal. ($C_{22}H_{18}N_2O_3S$) C, H, N, S.

Similar reactions gave the following compounds.

***N*-Methyl-1-(phenylsulfonyl)-1*H*-indole-3-carboxamide (16b)**, mp (MeOH) 192.5–195 °C. ^{1}H NMR ($CDCl_3$) δ 8.06 (s, 1 H, H-2), 8.03–7.84 (m, 4 H, ArH) 7.53–7.26 (m, 5 H, ArH), 6.37 (m, 1 H, NH), 2.99 (d, J = 4.9 Hz, CH_3). Anal. ($C_{16}H_{14}N_2O_3S$) C, H, N, S.

***N*-Phenyl-1-(phenylsulfonyl)-1*H*-indole-3-carboxamide (16j)**, mp (MeOH) 220–222.5 °C. ^{1}H NMR δ ($CDCl_3$) 8.18 (s, 1 H, H-2), 8.12 (d, 1 H, J = 7.8 Hz, H-4), 7.99 (d, J = 8.3 Hz, 1 H, H-7), 7.91 (d, J = 7.9 Hz, 2 H, ArH), 7.90 (m, 1 H, NH), 7.65 (d, J = 8.4 Hz, 2 H, ArH), 7.57 (t, J = 7.8 Hz, 1 H, ArH), 7.45 (t, J = 7.8 Hz, 2 H, ArH), 7.41–7.33 (m, 4 H, ArH), 7.15 (t, J = 7.4 Hz, 1 H, H-5). Anal. ($C_{21}H_{16}N_2O_3S$) C, H, N, S.

A solution of *N*-(phenylmethyl)-1-(phenylsulfonyl)-1*H*-indole-3-carboxamide (**16g**) (4.2 g, 11 mmol) in dry THF (200 mL) was treated at −78 °C with a solution of 2.5 M *n*-BuLi in hexanes (9.1 mL, 23 mmol), and the stirred mixture was allowed to warm to −20 °C for 15 min, before being recooled to −78 °C, when it was treated with dimethyl disulfide (2.5 mL, 28 mmol). The mixture was allowed to warm to 20 °C and then quenched with water (25 mL). Volatiles were removed under reduced pressure, and the residue was extracted with EtOAc. Workup of the organic layer gave a crude product. This was dissolved in MeOH (300 mL), mixed with a solution of K_2CO_3 (6.9 g, 50 mmol) in water (125 mL), and heated under gentle reflux under N_2 for 2 h to ensure complete hydrolysis of the phenylsulfonyl group.[25] MeOH was removed under reduced pressure, and the residue was diluted with water and extracted with CH_2Cl_2. Chromatography of the resulting oil on Al_2O_3 (eluting with CH_2Cl_2) gave 2-(methylthio)-*N*-(phenylmethyl)-1*H*-indole-3-carboxamide (**17g**) (2.8 g, 88% yield) as an oil. ^{1}H NMR ($CDCl_3$) δ 10.65 (s, 1 H, H-1), 8.29 (d, J = 5.1 Hz, H-4), 7.87 (t, J = 5.6 Hz, 1 H, CONH), 7.34–7.08 (m, 8 H, ArH), 4.73 (d, J = 5.6 Hz, 2 H, CH_2), 2.33 (s, 3 H, SMe). ^{13}C NMR ($CDCl_3$) δ 165.6 (CO), 138.5, 136.4, 133.1, 110.8 (4 s), 128.5, 127.2, 127.1, 122.9, 121.4, 126.8, 111.2 (7 d), 43.2 (CH_2), 18.5 (CH_3). HREIMS Calcd for $C_{17}H_{16}N_2OS$: 296.0983; Found 296.0985.

Similar treatment of the corresponding phenylsulfonyl derivatives (**16b** and **16j**) respectively gave the following compounds.

***N*-Methyl-2-(methylthio)-1*H*-indole-3-carboxamide (17b)** (95%), mp (hexane/CH_2Cl_2) 138.5–139.5 °C. ^{1}H NMR ($CDCl_3$) δ 10.31 (s, 1 H, H-1), 8.35–8.26 (m, 1 H, H-4), 7.44 (t, J = 4.8 Hz, 1 H, NH), 7.38–7.30 (m, 1 H, ArH), 7.19–7.11 (m, 2 H ArH), 3.06 (d, J = 4.8 Hz, 3 H, CH_3), 2.49 (s, 3 H, SCH_3). ^{13}C NMR ($CDCl_3$) δ 166.4 (CO), 136.4, 132.4, 127.4, 111.7 (4 s),

123.1, 121.5, 121.2, 111.1 (4 d), 26.3, 18.9 (2 CH_3). Anal. ($C_{11}H_{12}N_2OS$) C, H, N, S.

2-(Methylthio)-*N*-phenyl-1*H*-indole-3-carboxamide (17j) (81%, as an oil). ^1H NMR ($CDCl_3$) δ 10.19 (s, 1 H, H-1), 9.59 (s, 1 H, CONH), 8.47 (d, J = 6.8 Hz, 1 H, H-4), 7.80 (d, J = 8.5 Hz, 2 H, ArH), 7.43–7.35 (m, 3 H, ArH), 7.28–7.16 (m, 3 H, ArH) and 2.51 (s, 3 H, SCH_3). ^{13}C NMR ($CDCl_3$) δ 163.5 (CO), 138.2, 136.1, 132.5, 127.3, 111.2 (5 d), 19.1 (CH_3). HREIMS Calcd for $C_{16}H_{14}N_2OS$ 282.0827; Found 282.0827.

A solution of 2-(methylthio)-*N*-(phenylmethyl)-1*H*-indole-3-carboxamide (**17g**) (0.85 g, 2.87 mmol) in DMA (5 mL) was added under N_2 to a stirred suspension of MeSLi (0.93 g, 17.2 mmol) in DMA (10 mL). After warming at 80 °C for 6 h, the mixture was acidified with 3 N HCl, extracted with CH_2Cl_2, and worked up. Traces of of DMA were removed under high vacuum, and the residue was dissolved in MeOH (15 mL) and treated dropwise with H_2O_2 (0.50 mL of 30% solution). After chilling at −30 °C overnight, the precipitate was filtered off and washed with MeOH to give 2,2′-dithiobis[*N*-(phenylmethyl)-1*H*-indole-3-carboxamide] (**6g**) (74%), mp 203–205 °C. ^1H NMR [$(CD_3)_2SO$] δ 12.97 (s, 1 H, NH), 8.48 (t, J = 5.7 Hz, 1 H, CON*H*CH_2), 7.86 (d, J = 8.2 Hz, 1 H, H-4), 7.40 (d, J = 8.3 Hz, 2 H, H-2′,6′), 7.34 (dd, J = 8.3, 8.2 Hz, 3 H, H-7,3′,5′), 7.25 (t, J = 8.2 Hz, 1 H, H-4′), 7.20–7.10 (m, 2 H, H-5,6), 4.56 (d, J = 5.7 Hz, 2 H, CONH*C*H_2). ^{13}C NMR δ 164.71 (CONH), 139.77, 136.69, 135.30 (3 s), 128.16, 127.15, 126.56 (3 d), 124.44 (s), 122.63, 120.78, 119.25, 111.60 (4 d), 110.54 (s), 42.62 (CONH*C*H_2). Anal. ($C_{32}H_{26}N_4O_2S_2$) C, H, N, S.

The following were similarly prepared.

2,2′-Dithiobis(*N*-methyl-1*H*-indole-3-carboxamide) (6b) (57% yield), mp 232–236 °C (dec). ^1H NMR [$(CD_3)_2SO$] δ 12.94 (s, 1 H, NH), 7.85 (br, 1 H, CONH), 7.81 (d, J = 8.0 Hz, 1 H, H-4), 7.46 (d, J = 8.0 Hz, 1 H, H-7), 7.20 (dd, J = 8.0, 7.7 Hz, 1 H, H-6), 7.14 (dd, J = 8.0, 7.7 Hz, 1 H, H-5), 2.88 (d, J = 4.5 Hz, 3 H, CONHCH_3). ^{13}C NMR δ 165.20 (CONH), 136.70, 134.76, 124.47 (3 s), 122.61, 120.71, 119.26, 111.55 (4 d), 111.02 (s), 26.22 (CONH*C*H_3). Anal. ($C_{20}H_{18}N_4O_2S_2$) C, H, N, S.

2,2′-Dithiobis(*N*-phenyl-1*H*-indole-3-carboxamide) (6j) (67%), mp 220–223 °C. ^1H NMR [$(CD_3)_2SO$] δ 12.73 (s, 1 H, NH), 9.88 (s, 1 H, CONH), 7.81 (d, J = 7.9 Hz, 1 H, H-4), 7.69 (d, J = 8.4 Hz, 2 H, H-2′,6′), 7.46 (d, J = 7.7 Hz, 1 H, H-7), 7.34 (dd, J = 8.4,8.3 hz, 2 H, H-3′,5′), 7.24 (dd, J = 7.7, 7.7 Hz, 1 H, H-6), 7.17 (dd, J = 7.9, 7.7 Hz, 1 H, H-5), 7.10 (dd, J = 8.3 Hz, 1 H, H-4′). ^{13}C NMR δ 163.27 (CONH), 138.89, 136.73, 133.94 (3 s), 128.53 (d), 125.12 (s), 123.49, 123.17, 120.99, 120.32. 119.97 (5 d), 112.89 (s), 111.67 (d). Anal. ($C_{30}H_{22}N_4O_2S_2$) C, H, N, S.

2,2′-Dithiobis[1-[3-(dimethylamino)propyl]-*N*-phenyl-1*H*-indole-3-carboxamide] (9j). A solution of 2-(methylthio)-*N*-phenyl-1*H*-indole-3-carboxamide (**17j**) (1.8 g, 6.4 mmol) in EtOH (400 mL) was treated with 3-(dimethylamino)-propyl chloride hydrochloride (10.0 g, 64 mmol) and K_2CO_3 (13 g, 96 mmol), and heated under reflux for 8 h. A further 10 equiv of the reagents were then added, and the mixture was heated under reflux for a further 48 h. EtOH was removed under reduced pressure, and the residue was diluted with water to give crude product. This was chromatographed on alumina, eluting with CH_2Cl_2 containing 0.2% MeOH, to give 1-[3-(dimethylamino)propyl]-2-(methylthio)-*N*-phenyl-1*H*-indole-3-carboxamide (**18**) (0.49 g, 21%) as an oil. ^1H NMR ($CDCl_3$) δ 9.93 (s, 1 H, NH), 8.54 (d, J = 7.8 Hz, 1 H, H-4), 7.74 (d, J = 8.6 Hz, 2 H, H-2′,6′), 7.42–7.24 (m, 5 H, ArH), 7.11 (t, J = 7.4 Hz, 1 H, ArH), 4.46 (t, J = 7.4 Hz, 2 H, 1−CH_2), 2.47 (s, 3 H, SCH_3), 2.37 (t, J = 6.9 Hz, 2 H, CH_2N), 2.27 (s, 6 H, $N(CH_3)_2$), 1.97 (dt, J = 7.4, 6.9 Hz, 2 H, $CH_2CH_2CH_2$). ^{13}C NMR δ 162.6 (CO), 138.8, 136.7, 131.4, 127.5, 114.1 (5 s), 129.0, 124.1, 123.7, 122.8, 122.1, 119.8, 110.0 (7 d), 56.5, 42.0, 28.3 (3 CH_2), 45.3 ($N(CH_3)_2$), 21.1 (SCH_3). HRFABMS Found: [M + H]$^+$ = 368.1812. $C_{21}H_{25}N_3OS$ requires [M + H]$^+$ = 368.1797.

The methylthio derivative (**18**) was treated with MeSLi at 80 °C for 8 h as above. Water was added, the mixture was washed with CH_2Cl_2, and the aqueous portion was carefully neutralized with 3 N HCl and extracted with CH_2Cl_2. This extract was worked up to give an oil which was dissolved in MeOH and treated dropwise at room temperature with a saturated solution of I_2 in CH_2Cl_2 until no starting material was evident on TLC analysis. The reaction mixture was adsorbed directly onto silica gel by concentration, and chromatographed. MeOH/EtOAc (1:9) eluted foreruns, while MeOH/EtOAc (1:9) containing a trace of concentrated NH_4OH gave 2,2′-dithiobis[1-[3-(dimethylamino)propyl]-*N*-phenyl-1*H*-indole-3-carboxamide] (**9j**) (10% yield) as a yellow foam. ^1H NMR (CD_3OD) δ 8.19 (d, J = 7.3 Hz, 1 H, H-4), 7.64 (d, J = 7.5 Hz, 1 H, H-7), 7.30–7.20 (m, 3 H, ArH), 7.10–6.95 (m, 4 H, ArH), 4.41 (t, J = 6.2 Hz, 2 H, CH_2N), 2.74 (t, J = 6.7 Hz, 2 H, CH_2NMe_2), 2.64 (s, 6 H, $N(CH_3)_2$), 2.09 (m, 2 H, $CH_2CH_2CH_2$). HRFABMS Found: [M + H]$^+$ = 705.3035. $C_{40}H_{45}N_6O_2S_2$ requires [M + H]$^+$ = 705.3045.

2,2′-Dithiobis[3-[(4-methylphenyl)sulfonyl]-1*H*-indole] (6cc): Method C. A solution of 2-[[(4-methylphenyl)-sulfonyl]methyl]aniline (**19**)[35] (2.47 g, 10 mmol) in dry THF (60 mL), under N_2, was cooled to −78 °C, and *n*-butyllithium (9.6 mL, 2.5 M solution in hexanes) was added dropwise. The mixture was allowed to warm to −10 °C to give a deep red solution, which was recooled to −78 °C after 30 min. CS_2 (3 mL, 5 mmol) was added rapidly, and the mixture was allowed to warm slowly to 20 °C. The solvent was removed under vacuum and the residue was diluted with water and acidified with 2 M HCl. After stirring at 20 °C for 3 h, the solution was extracted with EtOAc and dried (Na_2SO_4). The solvent was removed, and chromatography of the residue on silica gel, eluting with CH_2Cl/EtOAc (9:1), gave 2,2′-dithiobis[3-[(4-methylphenyl)sulfonyl]-1*H*-indole] (**6cc**) (0.2 g, 7%), mp (benzene) 230–233 °C. ^1H NMR ($CDCl_3$) δ 8.06 (m, 1 H, NH), 7.91 (m, 3 H, H-4, H-2′, H-4′), 7.45 (m, 1 H, H-6), 7.21 (m, 4 H, H-5,7,3′,5′), 2.33 (s, 3 H, CH_3). ^{13}C NMR δ 144.1, 140.0, 136.6, 134.0 (4 s), 129.9, 126.4 (2 d), 125.4 (s), 124.5, 122.8, 119.1 (3 d), 115.1 (s), 112.2 (d), 21.5 (CH_3). Anal. ($C_{30}H_{24}N_2O_4S_4$·0.2-(C_6H_6) C, H, N (benzene detected by NMR).

Preparation of 2,2′-Dithiobis[*N*-[4-(methoxycarbonyl)-phenyl]-1-methyl-1*H*-indole-3-carboxamide] (7q) and 2,2′-Dithiobis[*N*-(4-carboxyphenyl)-1-methyl-1*H*-indole-3-carboxamide] (7n): Example of General Method D. A solution of monomethyl terephthalate (**21**; Ar = 4-COOMe) (1.32 g, 7.33 mmol) and DMF (1 drop) in $SOCl_2$ (30 mL) was heated under reflux for 45 min, before concentration to dryness under reduced pressure. The residue was dissolved in benzene and evaporated to dryness again. The resulting crude acid chloride was dissolved in dry Me_2CO (20 mL), cooled to 0 °C, and treated with a solution of NaN_3 (0.52 g, 8.00 mmol) in water (3 mL). After 20 min, the solution was diluted with water, extracted with CH_2Cl_2, and worked up to give the crude acyl azide (**22**; Ar = 4-COOMe) which was immediately dissolved in dry toluene (25 mL) and heated under reflux under N_2 for 2 h. Concentration to dryness under reduced pressure afforded the crude isocyanate (**23**; Ar = 4-COOMe) which was used directly.

A solution of 1,3-dihydro-1-methyl-2*H*-indole-2-thione (**24**) (1.00 g, 6.13 mmol) in THF (2 mL) was added under N_2 to a suspension of NaH (0.26 g of 60% w/w dispersion in mineral oil, 6.50 mmol) in THF (15 mL). After gas evolution had ceased (5 min), a solution of the crude isocyanate (**23**; Ar = 4-COOMe) (prepared above) in THF (10 mL) was added, and the solution was stirred at 20 °C for a further 1 h. The mixture was acidified with 3 N HCl, extracted with EtOAc and worked up to give an oily solid. Chromatography on silica gel, eluting with EtOAc, afforded crude 1,3-dihydro-*N*-[4-(methoxycarbonyl)phenyl]-1-methyl-2*H*-indole-2-thione (**10r**) as a greenish solid. This was not characterized, but was dissolved in MeOH and treated with 30% H_2O_2 (0.20 mL). The yellow precipitate was filtered off and washed well with MeOH to give 2,2′-dithiobis[*N*-[4-(methoxycarbonyl)phenyl]-1-methyl-1*H*-indole-3-carboxamide] (**7q**) (0.74 g, 35%), mp 184–186 °C. ^1H NMR [$(CD_3)_2SO$] δ 9.87 (br, 1 H, CONH), 7.80 (d, J = 8.0 Hz, 1 H, H-4), 7.74 (d, J = 8.7 Hz, 2 H, H-2′,6′), 7.37 (d, J = 8.3 Hz, 1 H, H-7), 7.32 (d, J = 8.7 Hz, 2 H, H-3′,5′), 7.26 (dd, J = 8.3, 7.6 Hz, 1 H, H-6), 7.15 (dd, J = 8.0, 7.6 Hz, 1 H, H-5), 3.84 (s, 3 H, CO_2CH_3), 3.66 (s, 3 H, NCH_3). ^{13}C NMR δ 165.79 (*C*$OOCH_3$), 161.56 (CONH), 143.01, 137.68 (2 s), 129.79 (d), 125.41 (s), 124.35 (d), 123.37 (s), 121.40, 120.82 (2 d), 119.90

(s), 118.33 (d), 117.93 (s), 110.74 (d), 51.74 ($COOCH_3$), 30.04 (NCH_3). Anal. ($C_{36}H_{30}N_4O_6S_2 \cdot H_2O$) C, H, N, S.

A suspension of **7q** (0.23 g, 0.34 mmol) in MeOH (40 mL) was treated with 3 N KOH (15 mL) and stirred at 20 °C for 90 min. The resulting solution was filtered and acidified, and the resulting precipitate was collected, redissolved in CH_2Cl_2 (10 mL) containing MeOH (1 mL), and treated with H_2O_2 (0.20 mL of 30%) for 1 h. Solvents were then removed under reduced pressure, and the residue was triturated with MeOH to give 2,2′-dithiobis[*N*-(4-carboxyphenyl)-1-methyl-1*H*-indole-3-carboxamide] (**7n**) (100% yield), mp 221 °C (dec). ^{1}H NMR [$(CD_3)_2SO$] δ 12.63 (br, 1 H, COOH), 9.78 (s, 1 H, CONH), 7.80 (d, J = 8.0 Hz, 1 H, H-4), 7.72 (d, J = 8.7 Hz, 2 H, H-3′,5′), 7.39 (d, J = 8.4 Hz, 1 H, H-7), 7.30 (d, J = 8.7 Hz, 2 H, H-2′,6′), 7.28 (t, J = 8.4, 7.7 Hz, H-6), 7.16 (t, J = 8.0, 7.7 Hz, 1 H, H-5), 3.66 (s, 3 H, NCH_3). ^{13}C NMR δ 166.95 (COOH), 161.58 (CONH), 142.67, 137.78 (2 s), 129.99 (d), 129.81, 125.41, 124.72 (3 s), 124.54, 121.50, 120.93, 118.39, 110.89 (5 d), 30.12 (NCH_3). Anal. ($C_{34}H_{26}N_4O_6S_2 \cdot 0.5H_2O$) C, H, N, S.

Similar reaction of 1-methyl-2-indolinethione (**24**) with appropriate isocyanates (**23**) prepared as above gave compounds **7j**, **7l**, **7m**, **7o**, **7p**, and **7u** of Table 1. Details of yields and NMR spectra are available in the supplementary material.

Similar treatment of the anion of **24** with the acyl azide (**22**; Ar = 2-furyl) derived from 2-furoic acid gave 1,3-dihydro-3-(2-furoyl)-1-methyl-2*H*-indole-2-thione (**10z**) (85% yield), mp 113.5 °C. ^{1}H NMR [$(CD_3)_2SO$] δ 15.90 (br, 1 H, SH), 8.28 (d, J = 1.6 Hz, 1 H, H-5′), 7.97 (d, J = 8.0 Hz, 1 H, H-4), 7.56 (d, J = 3.6 Hz, 1 H, H-3′), 7.46 (d, J = 8.0 Hz, 1 H, H-7), 7.37 (dd, J = 8.0, 7.4 Hz, 1 H, H-6), 7.21 (dd, J = 8.0, 7.4 Hz, 1 H, H-5), 6.94 (dd, J = 3.6, 1.6 Hz, 1 H, H-4′), 3.72 (s, 3 H, NCH_3). ^{13}C NMR δ 180.09 (CS), 160.65 (CO), 147.95 (d), 147.27, 140.92 (2 s), 126.05 (d), 123.26 (s), 123.12 (d), 121.04, 119.19, 113.22, 110.11 (4 d), 109.64 (s), 29.79 (NCH_3). Anal. ($C_{14}H_{11}NO_2S$) C, H, N, S.

Oxidation of **10z** with I_2 in CH_2Cl_2 gave 2,2′-dithiobis[3-(2-furoyl)-1-methyl-1*H*-indole] (**7z**) (85% yield), mp (EtOAc/petroleum ether) 175–176.5 °C. ^{1}H NMR ($CDCl_3$) δ 7.47 (d, J = 8.1 Hz, 1 H, H-4), 7.33 (dd, J = 1.6, 0.7 Hz, 1 H, H-5′), 7.23 (dd, J = 8.1, 7.8 Hz, 1 H, H-6), 7.21 (d, J = 8.1 Hz, 1 H, H-7), 7.09 (dd, J = 8.1, 7.9 Hz, 1 H, H-5), 6.63 (dd, J = 3.6, 0.7 Hz, 1 H, H-3′), 6.23 (dd, J = 3.6, 1.6 Hz, 1 H, H-4′), 3.73 (s, 3 H, NCH_3). ^{13}C NMR δ 177.09 (CO), 152.55 (s), 145.91 (d), 138.18, 131.32, 125.80 (3 s), 124.72 (d), 123.60 (s), 121.73, 121.12, 119.16, 111.91, 110.06 (5 d), 30.54 (NCH_3). Anal. ($C_{28}H_{30}N_2O_4S_2 \cdot 0.5H_2O$) C, H, N, S.

Similar reactions with the acyl azides (**22**) derived from benzoic acid and monomethyl terephthalate gave compounds **7w-y** of Table 1, via compounds **10w-y** of Table 1.

2,2′-Dithiobis(2-chloro-1-methyl-1*H*-indole-3-carbonitrile) (7bb): Method E. A mixture of 2-chloro-1*H*-indole-3-carboxaldehyde (**25**)[23] (7.0 g, 36 mmol) was reacted with a slight excess of hydroxylamine hydrochloride and pyridine in refluxing EtOH for 1 h, to give the crude oxime (**26**).[36] A solution of this in Ac_2O (100 mL) was heated under reflux for 1 h, cooled, and stirred with water (700 mL). The precipitated solid was collected, washed with water, and crystallized from aqueous MeOH to give 2-chloro-1*H*-indole-3-carbonitrile (**27**) (3.7 g, 58%), mp 177–180 °C. ^{1}H NMR [$(CD_3)_2SO$] δ 13.23 (s, 1 H, NH), 7.60 (d, J = 7.5 Hz, 1 H, ArH), 7.50 (d, J = 7.9 Hz, 1 H, ArH), 7.34 (t, J = 7.5 Hz, 1 H, ArH), 7.29 (t, J = 7.3 Hz, 1 H, ArH); ^{13}C NMR δ 134.0, 131.5, 126.2, 114.1 (4 s), 123.8, 112.23, 117.9, 112.3 (4 d), 83.8 (CN). Anal. ($C_9H_5ClN_2$) C, H, N.

A solution of **27** (2.5 g, 14 mmol) in Me_2CO was treated with a slight excess of MeI and K_2CO_3 under reflux for 1 h. Crystallization of the crude product from hexane gave 2-chloro-1-methyl-1*H*-indole-3-carbonitrile (**28**) (1.88 g, 70%), mp 112–114 °C. ^{1}H NMR ($CDCl_3$) δ 7.61–7.55 (m, 1 H, ArH), 7.34–7.21 (m, 3 H, ArH), 3.74 (s, 3 H, CH_3). ^{13}C NMR δ 135.0, 133.4, 126.0, 114.1 (4 s), 123.9, 122.7, 118.8, 110.1 (4 d), 85.2 (CN). Anal. ($C_{10}H_7ClN_2$) C, H, N.

Treatment of **28** with MeSLi as in method C gave 2,2′-dithiobis(2-chloro-1-methyl-1*H*-indole-3-carbonitrile) (**7bb**) (53% yield), mp 205–207 °C. ^{1}H NMR [$(CD_3)_2SO$] δ 7.69 (d, J = 8.3 Hz, 1 H, H-4), 7.51 (d, J = 8.0 Hz, 1 H, H-7), 7.42 (dd, J = 8.0, 7.3 Hz, 1 H, H-6), 7.28 (dd, J = 8.3, 7.3 Hz, 1 H, H-5), 3.82 (s, 3 H, NCH_3). Anal. ($C_{20}H_{14}N_4S_2$) C, H, N, S.

Crystallographic Determination of Disulfides 3 and 5. Dimethyl 2,2′-dithiobis(1-methyl-1*H*-indole-3-propanoate) (**3**)[11] crystallized from CH_2Cl_2/MeOH as yellow crystals: mp 139–141.5 °C; space group *Fdd*2; cell constants, a = 14.282(2) Å, b = 44.520(6) Å, c = 7.820(2) Å; z = 8; V = 4972.5(9) Å^3. Lattice constants and intensity data were measured using graphite-monochromated Mo Kα radiation, λ = 0.71069 Å, on a Nonius CAD-4 diffractometer. The data set consisted of 2071 unique reflections, of which 2020 were considered observed ($I > 3\sigma(I)$). The structure was refined using SHELX-76.[37] All atoms including hydrogens were found from successive difference maps and allowed to refine freely. Anisotropic temperature factors for atoms other than H and isotropic temperature factors for H atoms were allowed. R and R_w were 0.0308 and 0.0327. The largest shift/esd values during the final refinement were less than 0.05, and maximum and minimum peaks in the final difference map were 0.23 and 0.39 e Å^{-3}, respectively.

Dithiobis(1-methyl-*N*-phenyl-5-(trifluoromethyl)-1*H*-indole-3-carboxamide) (**5**)[14] crystallized from MeOH as yellow crystals: mp 214–216 °C; space group *C*2/*c*; cell constants, a = 33.155(8) Å, b = 12.430(1) Å, c = 22.652(4) Å; z = 8; V = 7370(3) Å^3. The data set consisted of 4092 unique reflections, of which 1264 were considered observed ($I > 3\sigma(I)$). The structure was solved as above, with all atoms except hydrogens being found from successive difference maps and allowed to refine freely. Hydrogen atoms were placed in calculated positions and allowed to ride on the atom to which they were bonded. Because of the poor quality of the crystal, and disorder in the CF_3 atoms, further refinement was undertaken with SHELX-93,[38] to give the final structure shown. Anisotropic temperature factors were used for atoms other than H and the disordered CF_3 groups, which were allowed to be isotropic. R and R_w were 0.0308 and 0.0327. The largest shift/esd values during the final refinement were less than 0.05, and maximum and minimum peaks in the final difference map were 0.23 and 0.39 e Å^{-3}, respectively.

Cell Culture and Growth Inhibition Assays. Swiss 3T3 mouse fibroblasts were obtained from the American Type Culture Collection, Bethesda, MD. The cells were maintained as monolayers in dMEM/F12 (50:50), Gibco, Grand Island NY, supplemented with 10% fetal bovine serum and 50 μg/mL gentamicin. For growth inhibition assays, dilutions of compounds in 10 μL of DMSO were placed in 24-well Linbro plates (1.7 × 1.6 cm, flat bottom) followed by the addition of cells (2 x 10^4) in 2 mL of media. The plates were incubated for 72 h at 37° C in a humidified atmosphere containing 5% CO_2 in air. Cell growth was determined by cell count with a Coulter Model AM electronic cell counter (Coulter Electronics, Inc., Hialeah, FL).

Antiphosphotyrosine Western Blotting. Cells were grown to 100% confluency in 6-well plates (35 mm wells). After treatment with compound and growth factor, the cells were scraped into 0.2 mL of boiling Laemmli buffer, transferred to a microfuge tube and heated to 100 °C for 5 min. Aliquots (35 μL) of the whole cell extract were electrophoresed on a 4–20% polyacrylamide gel. Proteins in the gel were electrophoretically transferred to nitrocellulose and the membrane was washed once in 10 mM Tris, pH 7.2, 150 mM NaCl, 0.01% azide (TNA) and blocked overnight in TNA containing 5% bovine serum albumin and 1% ovalbumin. The membrane was blotted for 2 h with antiphosphotyrosine antibody (UBI, Lake Placid, NY, 1 μg/mL in blocking buffer) and then washed two times sequentially in TNA, TNA containing 0.05% Tween-20, and 0.05% nonidet P-40 and TNA. The membranes were then incubated for 2 h in blocking buffer containing 0.1 μCi/mL of [^{125}I]protein A and washed again as above. The dried blots were loaded into a film cassette and exposed to X-AR X-ray film for 1–7 days. Bands were quantified by scanning densitometry.

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Supplementary Material Available: ^{1}H and ^{13}C NMR data for the compounds of Tables 1 and 2, and X-ray bond angles and bond lengths (24 pages) and structure factors for compounds **3** and **5** (16 pages). Ordering information is given on any current masthead page.

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