Tyrosine Kinase Inhibitors. 1. Structure–Activity Relationships for Inhibition of Epidermal Growth Factor Receptor Tyrosine Kinase Activity by 2,3-Dihydro-2-thioxo-1*H*-indole-3-alkanoic Acids and 2,2′-Dithiobis(1*H*-indole-3-alkanoic acids)

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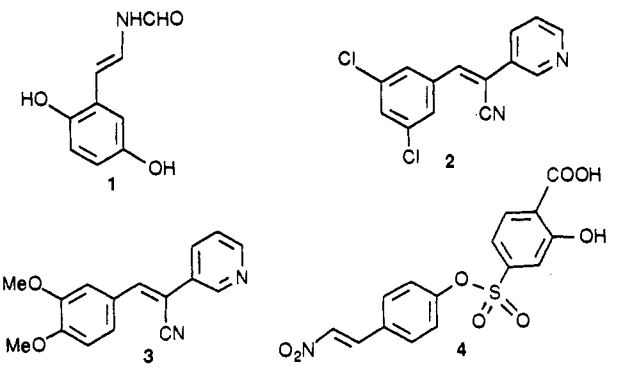
A series of 2,3-dihydro-2-thioxo-1*H*-indole-3-alkanoic acids, and their methyl esters were prepared, the majority by oxidation of 1*H*-indole-3-alkanoic acids (DMSO/HCl), followed by thiation of the corresponding 2,3-dihydro-2-oxo-1*H*-indole-3-alkanoic acid esters. The monomeric thiones undergo facile and reversible oxidation to the corresponding 2,2′-dithiobis(1*H*-indole-3-alkanoic acids). The compounds were evaluated for their abilities to inhibit the tyrosine kinase activity of the epidermal growth factor receptor using a native complex contained in plasma membrane vesicles shed from cultured A431 cells, and to inhibit the growth of Swiss 3T3 mouse fibroblast in culture. Enzyme inhibitory activity is dependent on the length of the side chain, with propanoic acid derivatives showing the highest activity. The acids are generally significantly more potent than the corresponding esters, and the disulfides more active than the corresponding monomers. An ability to undergo the thione–thiol tautomerism necessary for dimerization is essential, with 3,3-disubstituted compounds being inactive. Overall, the data suggest that the disulfide is the more active form, with much of the activity of the monomeric thiones being due to varying degrees of conversion to the disulfide during the assay. In the growth inhibition assay, the methyl esters are more potent than their corresponding carboxylic acids, and the dimers are generally more potent than the monomers. The data show these compounds to be a novel and potent class of inhibitors of epidermal growth factor receptor tyrosine kinase activity.

Protein phosphorylation is a critical mechanism for regulating protein function in the signal transduction pathway in normal and transformed cells.[1] Protein tyrosine kinases (PTKs) catalyze the transfer of the terminal phosphate from ATP to the phenolic OH group of tyrosine in substrate proteins. Many transmembrane growth factor receptors possess intracellular PTK activity, with initiation of this activity following external binding of a growth factor being the first step in the cellular signal transduction pathway which controls mitogenesis and cell proliferation.[2,3] The over-expression or inappropriate expression of normal or mutant PTK activity in these receptors can thus result in loss of growth control and the unregulated cell proliferation associated with malignancy.[4] Small molecules (capable of efficient cellular uptake) which can selectively inhibit such enzyme activity are therefore of therapeutic interest, as potential mediators of cell growth and as antitumor agents.[5,6]

Several classes of such molecules have been identified, with particular emphasis on inhibitors of the epidermal growth factor receptor tyrosine kinase (EGFR-TK), and these have been shown to possess antitumor activity both *in vitro* and *in vivo*. Thus, erbstatin (1) is reported to inhibit the growth of human epidermoid carcinoma A431 cells with an IC_{50} = 3.6 μg/mL[7] and to inhibit growth of the human mammary carcinoma MCF-7 and some esophageal tumors in nude mice in a dose dependent manner.[8] Compounds of the tyrphostin class of PTK inhibitors also potently inhibit the EGF-dependent growth of A431 cells *in vitro*.[9,10] The tyrphostins (2 and 3) have been reported to be active against human squamous cell carcinoma MH-85 xenografts *in vivo* in nude mice.[11] *In vitro* and *in vivo* antitumor activity against A431 tumors has also been reported for a series of sulfonylbenzoyl–nitrostyrene PTK inhibitors (e.g., 4).[12]



We now report the synthesis and evaluation of a new class of compounds, the 2,3-dihydro-2-thioxo-1*H*-indole-3-alkanoic acids and their closely-related oxidation products, the 2,2′-dithiobis(1*H*-indole-3-alkanoic acids), which are novel and potent inhibitors of EGFR tyrosine kinase.

Chemistry

The 2,3-dihydro-2-thioxo-1*H*-indole-3-alkanoic acids **5**, **9**, and **13** and their esters and related disulfides were prepared by the synthetic methods outlined in Schemes I–V. The majority of the compounds were prepared by

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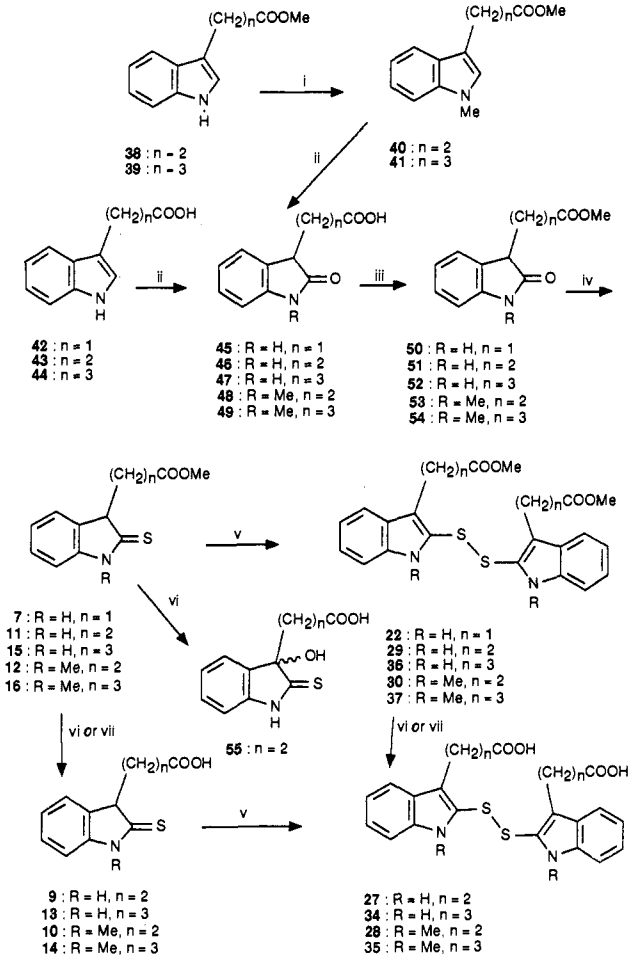
Scheme I[a]



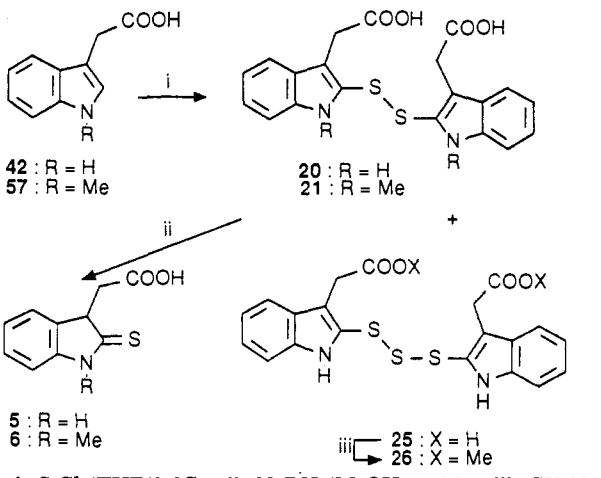
[a] (i) 18-crown-6/KOtBu/MeI/C_6H_6. (ii) DMSO/HCl. (iii) CH_2N_2/Et_2O. (iv) P_2S_5/$NaHCO_3$/dioxane. (v) O_2 or $FeCl_3$. (vi) NaOH/aqueous EtOH/20 °C. (vii) K_2CO_3/aqueous MeOH/20 °C.

the method outlined in Scheme I, by oxidation of 1*H*-indole-3-alkanoic acids with DMSO and concentrated HCl, followed by thiation of the corresponding 2,3-dihydro-2-oxo-1*H*-indole-3-alkanoic acid esters. In this manner Takase[13] obtained methyl 2,3-dihydro-2-thioxo-1*H*-indole-3-acetate (**7**) from 1*H*-indole-3-acetic acid (**42**) (via **45** and **50**), as an intermediate in the synthesis of a marine alkaloid derivative, debromo-8,8a-dihydroflustramine C.

Methyl 1*H*-indole-3-alkanoates were converted into their 1-methyl derivatives by phase-transfer alkylation with potassium *tert*-butoxide, methyl iodide, and 18-crown-6 in benzene[14] (Scheme I). These were then oxidized with DMSO/HCl as above, although ester hydrolysis occurred concurrently, requiring reesterification.

Some of the intermediate oxindole alkanoic esters obtained by these methods were found to be unstable to storage and were not fully purified, but were thiated directly to give the methyl 2,3-dihydro-2-thioxo-1*H*-indole-3-alkanoates. The thiation procedure of Scheeren,[15] as employed by Takada[16] (using a 0.55:2:1 mol ratio of P_2S_5/$NaHCO_3$/substrate), was further modified by the use of dioxane rather than THF as solvent. The higher-boiling solvent enabled the conversion of both NH and NMe oxindole ester substrates into their 2-thioxo analogues in high yield (Scheme I). As noted in a previous study,[17] the major byproducts of extended thiation reactions in the presence of excess P_2S_5 were indolealkanoic esters. The NH oxindole esters **50–52** also gave significant amounts of the related disulfides, by facile air oxidation of the

Scheme II[a]



[a] (i) S_2Cl_2/THF/0 °C. (ii) $NaBH_4$/MeOH/20 °C. (iii) CH_2N_2/Et_2O.

resulting 2-indolinethiones.[18,19] These disulfides were not easily separable from the 2-indolinethione derivatives by chromatography, but reduction of the crude mixture with $NaBH_4$ in alcohol converted them readily to the latter.[19]

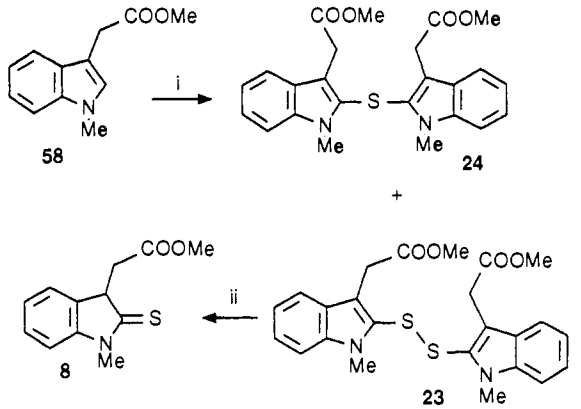
Alternatively, solutions of the crude mixtures could be readily oxidized to the disulfides by exposure to air, or by treatment with a mild oxidizing agent such as $FeCl_3$. In several cases fractional crystallization was used to obtain the purified products directly from the mixture in non-optimized yields. An alternative thiation procedure (employing a 2.4:1.2:1 mol ratio of P_2S_5/Na_2CO_3/substrate at room temperature)[20] was also found to be moderately effective for the oxindoles **51** and **65–67**.

The NMe 2,3-dihydro-2-thioxo-1*H*-indole-3-alkanoic acids were obtained by saponification of the esters with NaOH/aqueous EtOH, followed by reduction with $NaBH_4$ to reconvert adventitiously-oxidized material, and crystallization (Scheme I). The corresponding disulfides were obtained similarly by saponification and direct crystallization.

The NH 2,3-dihydro-2-thioxo-1*H*-indole-3-alkanoic acids were found to be particularly unstable to hydroxide ion. For example, treatment of methyl 2,3-dihydro-2-thioxo-1*H*-indole-3-propanoate (**11**) with NaOH in aqueous EtOH gave the 3-hydroxy compound **55** and the indole acid **43**. However, these were minor products when K_2CO_3 in aqueous MeOH[21] was employed for the hydrolysis. Subsequent chromatography of the crude products, reduction with $NaBH_4$, and crystallization as above gave 2,3-dihydro-2-thioxo-1*H*-indole-3-propanoic acid (**9**) in moderate yield.

This synthetic methodology (Scheme I) failed completely for the preparation of the corresponding NH and NMe 2-indolinethione acetic acids and their disulfides, due to the extreme instability of the desired products toward base, and an alternative synthesis was employed (Schemes II and III). Treatment of 1*H*-indole-3-acetic acid (**42**) with freshly purified[22] S_2Cl_2 in THF as described by Wieland[23] gave, instead of the reported disulfide **20**, a mixture including mono-, di-, and trisulfides, which could be separated only by multiple recrystallizations (Scheme II). This is more consistent with results reported by Palmisano,[24] who found that methyl 1*H*-indole-3-acetate (**38**) reacted with S_2Cl_2 in CH_2Cl_2 to give a mixture of dimethyl 2,2′-dithiobis(1*H*-indole-3-acetate) (**22**) and dim-

Scheme III[a]



[a] (i) S_2Cl_2/THF/0 °C. (ii) $NaBH_4$/MeOH/20 °C.

ethyl 2,2′-trithiobis(1*H*-indole-3-acetate) (**26**), and with the reported reactions with S_2Cl_2 of other 3-alkylindoles.[19,22]

Both di- and trisulfides **20** and **25** were reduced by $NaBH_4$ in alcohol[19] to yield the desired 2,3-dihydro-2-thioxo-1*H*-indole-3-acetic acid (**5**). Therefore, the optimal synthesis devised for the acetic acid disulfides consisted of $NaBH_4$ reduction of the crude product mixture from the S_2Cl_2 reaction and reoxidation with $FeCl_3$. Similar treatment of methyl 1-methyl-1*H*-indole-3-acetate (**58**) (Scheme III) and the corresponding acid **57** (Scheme II, obtained by hydrolysis of **58**) gave the desired disulfides **23** and **21** and 2-indolinethiones **8** and **6**.

The 5-, 6-, and 7-methyl derivatives of ethyl 2,3-dihydro-2-oxo-1*H*-indole-3-propanoate were prepared by the condensation of methyl-substituted oxindoles with diethyl malonate to give methyl-substituted ethyl isatylidenehydroxyacetates, which undergo catalytic hydrogenation over palladium-on-carbon catalyst in the presence of concentrated H_2SO_4 to the desired products (Scheme IV), as described by Julian.[25] The 5-, 6-, and 7-methyl oxindoles were prepared by the base-catalyzed reduction of the appropriate isatin-3-hydrazones.[26]

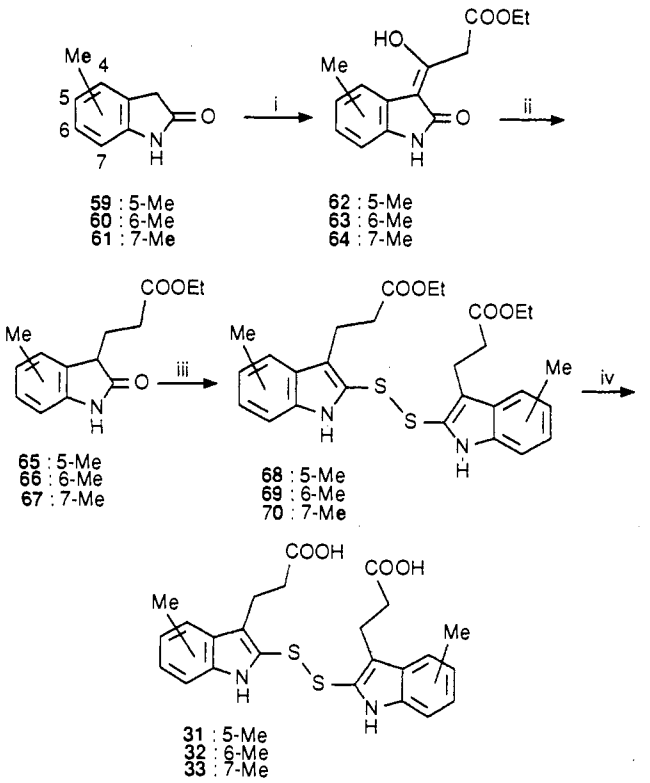
Finally, the condensation of oxindole with ethyl acrylate in refluxing sodium ethoxide solution[27] gave, after hydrolysis and methylation, a mixture of the di- and trimethyl oxindolepropanoates (**72** and **71**), which were separable by chromatography. These compounds were thiated with P_2S_5, and the diester **18** hydrolyzed as above to give the diacid **17** for comparison with **9** (Scheme V).

Results

The ability of the compounds to inhibit EGF-stimulated EGFR tyrosine kinase activity was measured using a native complex contained in plasma membrane vesicles shed from cultured A431 cells.[28] A random copolymer of glutamate, alanine, and tyrosine was used as the substrate, and IC_{50} values are defined as the concentration of drug necessary to reduce incorporation of ^{32}P (from added [γ-^{32}P]ATP) by 50% and are recorded in Tables I and II. Analysis of the kinetics of inhibition suggests these compounds are noncompetitive inhibitors with respect to peptide substrate,[29] and are also noncompetitive with ATP (data not shown).

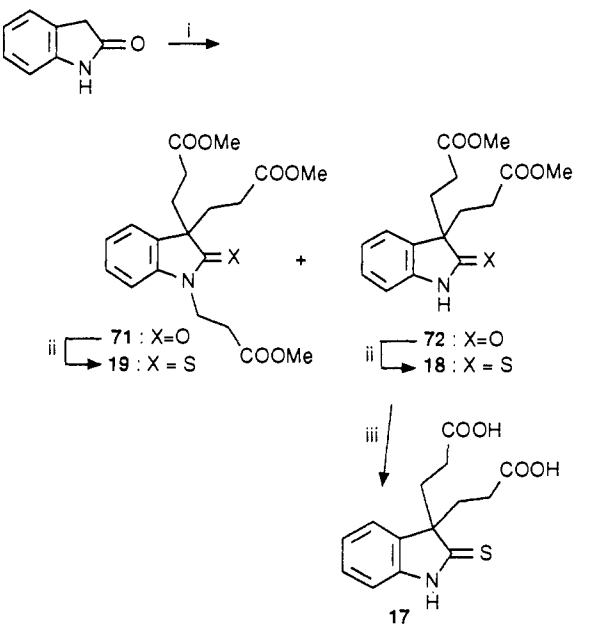
The compounds prepared were designed to answer a number of questions concerning structure–activity relationships for EGFR-TK inhibitory activity among the 2,3-dihydro-2-thioxo-1*H*-indole-3-alkanoic acids. Three series of compounds were studied: the 3-acetic, 3-propanoic, and

Scheme IV[a]



[a] (i) $EtOOCCH_2COOEt/EtONa/EtOH$. (ii) H_2-Pd/C in AcOH/H_2SO_4. (iii) P_2S_5/Na_2CO_3/THF; $FeCl_3$/MeOH. (iv) LiOH/aqueous EtOH.

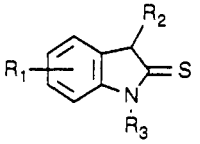
Scheme V[a]



[a] (i) Ethyl acrylate/EtONa/EtOH/reflux/16 h; K_2CO_3/water/20 °C/2 days; HCl/MeOH. (ii) $P_2S_5/NaHCO_3$/dioxane. (iii) K_2CO_3/aqueous MeOH/20 °C.

3-butanoic acids. In each series, both acids and methyl esters were compared, and for each set of these compounds both the indole–NH and indole–NMe analogues were prepared, to give a basic set of 12 compounds (Table I). Because of the known ready oxidation of 2-indolinethiones to the 2,2′-dithiobis(1*H*-indole) compounds,[18] the 12 corresponding dimers were also prepared and evaluated (Table II). Other compounds were made to test the effects of nuclear substitution on the indole (compounds **31–33**)

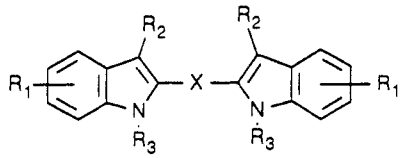
Table I. Physiochemical and Biological Properties of 2,3-Dihydro-2-thioxo-1*H*-indole-3-alkanoic Acids and Analogues



no.	R_1	R_2	R_3	mp (°C)	formula	analyses[a]	IC_{50}[b] (μM)
1	erbstatin						2.0
5	H	CH_2COOH	H	166–168	$C_{10}H_9NO_2S$	ref 23	14.9
6	H	CH_2COOH	Me	150–153	$C_{11}H_{11}NO_2S$	ref 23	>100
7	H	CH_2COOMe	H	150–152	$C_{11}H_{11}NO_2S$	C,H,N,S	ca. 100
8	H	CH_2COOMe	Me	68–70	$C_{12}H_{13}NO_2S$	C,H,N,S	>100
9	H	$(CH_2)_2COOH$	H	173–175	$C_{11}H_{11}NO_2S \cdot {}^1/_2H_2O$	C,H,N,S	1.62 ± 0.27[c]
10	H	$(CH_2)_2COOH$	Me	128–130	$C_{12}H_{13}NO_2S$	C,H,N,S	6.7
11	H	$(CH_2)_2COOMe$	H	95.5–98	$C_{12}H_{13}NO_2S$	C,H,N,S	>100
12	H	$(CH_2)_2COOMe$	Me	71–73	$C_{13}H_{15}NO_2S$	C,H,N,S	>100
13	H	$(CH_2)_3COOH$	H	132–134	$C_{12}H_{13}NO_2S$	C,H,[d]N,S	6.7
14	H	$(CH_2)_3COOH$	Me	144–146.5	$C_{13}H_{15}NO_2S \cdot H_2O$	C,H,N,S	>100
15	H	$(CH_2)_3COOMe$	H	109–110	$C_{13}H_{15}NO_2S$	C,H,N,S	>100
16	H	$(CH_2)_3COOMe$	Me	103–106	$C_{14}H_{17}NO_2S$	C,H,N,S	>100
17	H	$[(CH_2)_2COOH]_2$	H	214–218	$C_{14}H_{15}NO_4S$	C,H,N,S	>100
18	H	$[(CH_2)_2COOMe]_2$	H	122.5–125	$C_{16}H_{19}NO_4S$	C,H,N,S	>100
19	H	$[(CH_2)_2COOMe]_2$	$(CH_2)_2COOMe$	104–106	$C_{20}H_{25}NO_6S$	C,H,N,S	>100

[a] Analyses for all listed elements within ±0.4%. [b] IC_{50} stands for the concentration of the compound (μM) to inhibit cell growth by 50% (see text for details). Values represent the mean of at least two separate and duplicate determinations. Variation in IC_{50}'s between duplicate experiments was generally ±15%. [c] Mean and standard error for four separate determinations. [d] H out by 0.5%.

Table II. Physicochemical and Biological Properties of 2,2'-Dithiobis(1*H*-indole-3-alkanoic acids) and Analogues



no.	R_1	R_2	R_3	X	mp (°C)	formula	analyses[a]	IC_{50}[b] (μM)
1	erbstatin							2.0
20[c]	H	CH_2COOH	H	S_2	196–199	$C_{20}H_{16}N_2O_4S_2 \cdot {}^1/_2H_2O$	C,H,N,S	10.2 ± 4.9[d]
21[c]	H	CH_2COOH	Me	S_2	190–192.5	$C_{22}H_{20}N_2O_4S_2$	C,H,N,S	53
22[e]	H	CH_2COOMe	H	S_2	160–162	$C_{22}H_{20}N_2O_4S_2$	C,H,N,S	18
23	H	CH_2COOMe	Me	S_2	130–132.5	$C_{24}H_{24}N_2O_4S_2$	C,H,N,S	33
24	H	CH_2COOMe	Me	S	155–156	$C_{24}H_{24}N_2O_4S$	C,H,N,S	>100
25	H	CH_2COOH	H	S_3	199–202	$C_{20}H_{16}N_2O_4S_3$	C,H,N,S	9.3
26[e]	H	CH_2COOMe	H	S_3	130–132	$C_{22}H_{20}N_2O_4S_3$	C,H,N,S	35
27	H	$(CH_2)_2COOH$	H	S_2	118–120.5	$C_{22}H_{20}N_2O_4S \cdot H_2O$	C,H,N,S	4.2
28	H	$(CH_2)_2COOH$	Me	S_2	158.5–160	$C_{24}H_{24}N_2O_4S_2$	C,H,N,S	3.1
29	H	$(CH_2)_2COOMe$	H	S_2	162.5–164	$C_{24}H_{24}N_2O_4S_2$	C,H,N,S	21
30	H	$(CH_2)_2COOMe$	Me	S_2	139–141.5	$C_{26}H_{28}N_2O_4S_2$	C,H,N,S	>100
31	5-Me	$(CH_2)_2COOH$	H	S_2	91.5–95	$C_{24}H_{24}N_2O_4S_2$	HRMS[f]	8.4
32	6-Me	$(CH_2)_2COOH$	H	S_2	126–128	$C_{24}H_{24}N_2O_4S_2 \cdot {}^1/_2H_2O$	C,H,N,S	2.9
33	7-Me	$(CH_2)_2COOH$	H	S_2	172.5–175	$C_{24}H_{24}N_2O_4S_2$	C,H,N	1.5
34	H	$(CH_2)_3COOH$	H	S_2	141–143.5	$C_{24}H_{24}N_2O_4S \cdot {}^1/_2H_2O$	C,H,N,S	18
35	H	$(CH_2)_3COOH$	Me	S_2	106.5–109.5	$C_{28}H_{28}N_2O_4S_2 \cdot 2AcOH$	C,H,N,S	8.3
36	H	$(CH_2)_3COOMe$	H	S_2	91–93	$C_{26}H_{28}N_2O_4S_2$	C,H,N,S	>100
37	H	$(CH_2)_3COOMe$	Me	S_2	112–113	$C_{28}H_{32}N_2O_4S_2$	C,H,N,S	>100

[a,b] As for Table I. [c] Reference 23. [d] Average and standard error for four separate determinations. [e] Reference 24. [f] High-resolution mass spectrum molecular ion.

and the effect of preventing the thione–disulfide tautomerism (compounds 17–19).

Thiones (Monomers) versus Disulfides. Twelve pairs of compounds could be compared as thione monomers and the corresponding (oxidized) disulfides. The dimeric disulfides were the more active in 7/12 cases. For three of these pairs, where accurate IC_{50} values were available for both compounds, the disulfide was 2–5-fold more potent (e.g., **10** and **28**). There were a further four cases where the thione was inactive (defined as an IC_{50} of >100 μM), and the disulfide was more potent by from >2- to >10-fold. For two pairs of compounds (**9**,**27** and **13**,**34**; the propanoic and butanoic acids), the disulfides were about 3-fold less active than the thione. Compound **9** was the most potent inhibitor in the series, with an IC_{50} of 1.6 ± 0.3 μM, c.f. erbstatin (**1**), IC_{50} = 2.0 μM (Table I). Three pairs of compounds could not be compared, with both being inactive.

N-Methylation of the Indolinethione. There were 12 sets of compounds where the effect of N-methylation of the indolinethione could be evaluated, by comparing the corresponding NH and NMe derivatives. In 7/12 cases, the NH analogues were the more potent inhibitors. In the five cases where accurate IC_{50} values were available for both of the compounds, the differences varied from 2- to 5-fold (e.g., compounds **9** and **10**). In two of these cases (the propanoic and butanoic acid disulfides **27**,**28** and **34**,**35**) the NMe compound was slightly more potent, but the difference was not great. Three pairs of compounds could not be compared, since both were inactive.

Table III. Effect of Selected Compounds of Tables I and II on the Proliferation of Swiss 3T3 Mouse Fibroblasts

no.[a]	IC_{50}[b] (μM)	no.[a]	IC_{50}[b] (μM)
9	64	26	1.0
11	16	27	59
20	>50	29	7.4
21	36	30	5.2
22	2.3	31	>50
25	8.0	37	1.8

[a] Number of compound in Tables I and II. [b] IC_{50} stands for the concentration of the compound (μM) to inhibit cell growth by 50% (see text for further details). Values represent the mean of two separate and duplicate determinations.

Length of Alkanoic Acid Side Chain. There were eight sets of compounds (both thiones and disulfides) where acetic, propanoic, and butanoic acid side chains could be directly compared, but for only four of these could a complete ranking be obtained (i.e., where at least two compounds in the set were active). In all four of these cases, the propanoic acid analogue was either the best (3/4) or equal best (1/4). In two cases, the order was propanoic > butanoic > acetic, and in the other two (sets of disulfides), the propanoic and acetic acid analogues were essentially equipotent, with the butanoic acid inferior.

Acids versus Esters. Fourteen pairs of acids and their corresponding methyl esters were available. In 10/14 cases, the acids were more potent. In the only four sets (one trisulfide, one thione, two disulfides) which could be quantitated, the differences in potency were 2–7-fold. In another six sets where the esters were inactive (IC_{50} > 100 μM), the corresponding acids were from 5–70-fold more potent. The ester was more active in one case (compounds 21 and 23), but by less than 2-fold, and in 3/14 cases no comparisons could be made.

Other Structural Features. The results for compounds 9 and 17 suggest the requirement for a 3-proton; when C-3 is disubstituted as in 17, inhibitory activity is completely lost. The one dimeric monosulfide available 24 was inactive. The corresponding dimeric disulfide 23 showed only moderate activity (IC_{50} = 33 μM) and the thione monomer 8 was not active. Two examples of trisulfides were available (compounds 25 and 26). In both cases these were more active than the corresponding monomers (5 and 7) and were similar in potency to the corresponding disulfides (20 and 22). Very limited studies were done on nuclear substitution of the indole ring, looking at methyl substituents, to complement work being carried out in a related series.[30] Potencies for the 6- and 7-methyl propanoic acid derivatives 32 and 33 were similar to that of the parent 27, but the 5-methyl compound 31 was somewhat less active.

Growth Inhibitory Properties. Selected compounds were evaluated in a cellular growth inhibition assay to assess any relationship that might exist between inhibition of the isolated enzyme and effects on proliferation. Table III shows the growth inhibitory properties of selected compounds from Table II. Whereas all of the compounds were growth inhibitory, there was clear distinction in potency between free acids and their corresponding methyl esters. Thus, comparing compound 25 with 26, and compound 20 with 22, there was an 8- and >20-fold increase in potency, respectively, when the carboxyl group was esterified, with the acetic acid ester 26 being the most growth inhibitory of all the compounds studied (IC_{50} = 1.0 μM; Table III). To further evaluate this relationship a direct comparison was made between the monomer and dimer, and acid and ester pairs of a single structure (Table III). The methyl esters were in each case more potent than the acids (e.g., compound 9 versus 11, and 27 versus 29). As was the case with enzyme inhibition, the dimers were more growth inhibitory than the monomers.

Discussion and Conclusions

Following the initial observation of the potent inhibitory activity of 2,3-dihydro-2-thioxo-1*H*-indole-3-alkanoic acids against the tyrosine kinase activity of the EGFR complex (noncompetitive with respect to both ATP and peptide substrate), we prepared the series of compounds reported here to evaluate preliminary structure–activity relationships for this activity. Key questions to be answered included the dependence of activity on the length of the 3-alkanoic acid side chain, on N-alkylation of the indoline, and on substitution at other nuclear positions. The study was complicated by the known[18] thione–thiol tautomerism undergone by 2-indolinethiones and the facile oxidation of the thione monomers to the dimeric disulfide species. (Preliminary experiments show half-lives for the monomer with respect to oxidative dimerization of only a few minutes in dilute aqueous solutions; unpublished results, this laboratory.)

However, some conclusions can be made about the SAR for the inhibitory activity of these compounds against isolated EGFR-TK (Tables I and II). The propanoic acid is clearly the preferred side chain, in both the thione and disulfide series. The acids are significantly more potent than the corresponding esters (again both in the thiones and the disulfides). It is not clear whether this is due purely to effects on solubility, or whether the acids do have preferred site binding (perhaps to a metal). N-Methylation is generally deleterious to activity, suggesting bulk intolerance at the N-1 position (although electronic effects cannot be ruled out). In the disulfide series, nuclear substitution on the indole ring is sterically tolerated, but no more than that can be deduced from results from only three compounds.

The importance of the thione–disulfide exchange is difficult to determine at this stage. When compared pairwise with the corresponding thiones, the disulfides were generally more potent. Removal of both C-3 protons, blocking the ability of the compounds to undergo the thione–thiol tautomerism necessary for dimerization, abolished activity. Preliminary work (unpublished results, this laboratory) on the kinetics of oxidation of selected thiones suggest a significant degree of dimerization can occur over the time scale of the enzyme inhibition assay (10 min). The argument that the active form in each case is the disulfide, with the apparent activity of the thiones being due to varying degrees of conversion to the disulfide during the assay is therefore persuasive. On the other hand, the monosulfide 24, which is incapable of being reduced to the thione was inactive, whereas both di- and trisulfides (which can be so reduced) were active. Additionally, in some cases the thiones were more potent inhibitors than the disulfides. Therefore, the question as to which (if either) of these two forms is the active species cannot be decided at this point.

Several conclusions can be drawn from the (limited) growth inhibition data available (Table III). Generally, this class of compounds is growth inhibitory, with all examples tested inhibiting proliferation to some degree. The esters are more potent than their corresponding

carboxylic acids, and the dimers are more potent than the monomers. Comparing these data with inhibition of EGF receptor tyrosine kinase, it appears that enzyme inhibition does not correlate well with potency of growth inhibition. In particular, there exists an almost perfect reciprocal relationship between these two parameters, in that esters are inactive against the enzyme, whereas carboxylic acids are highly active. The reason for this is unclear. One possibility might be that esters are more stable in tissue-culture medium than the acids, but this seems unlikely. A more attractive hypothesis is that esters are transported more efficiently into the cell, where they may be hydrolyzed back to the active acid form by one of a number of intracellular esterases. Experiments are currently underway to investigate this possibility, and to further evaluate the mechanism of action of these compounds.

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. NMR spectra were measured on a Bruker AM-400 spectrometer (Me_4Si). Mass spectra were recorded on a VG 7070 spectrometer at nominal 5000 resolution.

Methyl 2,3-Dihydro-2-thioxo-1*H*-indole-3-acetate (7) and Dimethyl 2,2′-Dithiobis(1*H*-indole-3-acetate) (22). P_2S_5 (0.09 g, 0.41 mmol) and $NaHCO_3$ (0.11 g, 1.31 mmol) were added to a solution of methyl 2,3-dihydro-2-oxo-1*H*-indole-3-acetate[13] (**50**, 0.13 g, 0.63 mmol) in dry dioxane (10 mL), and the mixture was then stirred under N_2 at 90 °C for 40 min. The resulting solution was evaporated under reduced pressure, and the residue was diluted with water (50 mL). Extraction with $CHCl_3$ (3 × 50 mL), workup of the organic layer, and crystallization from MeOH gave methyl 2,3-dihydro-2-thioxo-1*H*-indole-3-acetate (**7**): 50 mg; 36%; mp 150–152 °C; ^{1}H NMR ($CDCl_3$) δ 10.36 (s, 1 H, NH), 7.29 (d, J = 7.6 Hz, 1 H, ArH), 7.27 (t, J = 7.8 Hz, 1 H, ArH), 7.11 (t, J = 7.6 Hz, 1 H, ArH), 7.00 (d, J = 7.8 Hz, 1 H, ArH), 4.14 (dd, J = 8.4, 4.2 Hz, 1 H, H-3), 3.72 (s, 3 H, OCH_3), 3.35 (dd, J = 17.0, 4.2 Hz, 1 H, 3-CH), 2.88 (dd, J = 17.0, 8.5 Hz, 1 H, 3-CH); ^{13}C NMR ($CDCl_3$) δ 206.59 (s, CSNH), 171.53 (s, $COOCH_3$), 143.10, 133.53 (2 × s, Ar), 128.45, 124.20, 124.12, 110.07 (4 × d, Ar), 53.53 (d, C-3), 52.02 (q, OCH_3), 37.94 (t, 3-CH_2). Anal. ($C_{11}H_{11}NO_2S$) C, H, N, S.

Chromatography of the mother liquor on silica gel, eluting with CH_2Cl_2, gave dimethyl 2,2′-dithiobis(1*H*-indole-3-acetate) (**22**): 30 mg; 22%; mp (CH_2Cl_2/light petroleum) 161-162 °C; ^{1}H NMR ($CDCl_3$) δ 8.69 (s, 1 H, NH), 7.52 (dd, J = 8.2, 0.6 Hz, 1 H, ArH), 7.21 (ddd, J = 8.8, 6.6, 1.1 Hz, 1 H, ArH), 7.12 (m, 2 H, ArH), 3.83 (s, 2 H, 3-CH_2), 3.71 (s, 3 H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 172.54 (s, $COOCH_3$), 137.20, 127.19, 127.03 (3 × s, Ar), 124.26, 120.31, 119.45 (3 × d, Ar), 116.23 (s, Ar), 111.41 (d, Ar), 52.25 (q, OCH_3), 30.51 (t, 3-CH_2). Anal. ($C_{22}H_{20}N_2O_4S_2$) C, H, N, S. This compound was also prepared by dissolving **7** (0.10 g) in benzene/light petroleum (1:1) and allowing it to stand in air for two days, when **22** crystallized in essentially quantitative yield.

2,2′-Dithiobis(1*H*-indole-3-acetic acid) (20) and 2,2′-Trithiobis(1*H*-indole-3-acetic acid) (25). A solution of purified S_2Cl_2 (0.50 mL) in THF (20 mL) was added dropwise to a stirred, ice-cooled solution of 1*H*-indole-3-acetic acid (**42**, 2.20 g) in dry THF (30 mL).[23] After 30 min at 20 °C, the solvent was removed under reduced pressure and the residue was crystallized successively from aqueous AcOH, aqueous MeOH, and EtOAc/benzene to give 2,2′-trithiobis(1*H*-indole-3-acetic acid) (**25**): 80 mg; 3%; mp 199–202 °C; ^{1}H NMR [$(CD_3)_2CO$] δ 10.18 (s, 1 H, NH), 7.59 (m, 1 H, ArH), 7.06 (m, 2 H, ArH), 6.82 (m, 1 H, ArH), 3.99 (s, 2 H, 3-CH_2); ^{13}C NMR [$(CD_3)_2CO$] δ 173.30 (s, COOH), 138.82, 128.26, 127.03 (3 × s, Ar), 124.76, 120.60, 120.33 (3 × d, Ar), 116.97 (s, Ar), 112.16 (d, Ar), 30.89 (t, 3-CH_2). Anal. ($C_{20}H_{16}N_2O_4S_3$) C, H, N, S. Further crystallization of mother liquor fractions from CH_2Cl_2 gave 2,2′-dithiobis(1*H*-indole-3-acetic acid) (**20**): 0.19 g; 7%; mp 196–199 °C (lit.[23] mp 208 °C); ^{1}H NMR [$(CD_3)_2CO$] δ 10.62 (s, 1 H, NH), 7.58 (dt, J = 8.1, 0.6 Hz, 1 H, ArH), 7.42 (dt, J = 8.2, 0.8 Hz, 1 H, ArH), 7.23 (ddd, J = 8.2, 7.1, 1.1 Hz, 1 H, ArH), 7.09 (ddd, J = 8.0, 7.1, 0.9 Hz, 1 H, ArH), 3.55 (s, 2 H, 3-CH_2); ^{13}C NMR [$(CD_3)_2CO$] δ 172.67 (s, COOH), 138.78, 128.33, 127.86 (3 × s, Ar), 124.79, 120.72, 120.56 (3 × d, Ar), 117.78 (s, Ar), 112.41 (d, Ar), 30.67 (t, 3-CH_2). Anal. ($C_{20}H_{16}N_2O_4S_2$·0.5H_2O) C, H, N, S.

Dimethyl 2,2′-Trithiobis(1*H*-indole-3-acetate) (26). An ethereal solution of excess diazomethane was added dropwise to a stirred, ice-cooled solution of crude **25** (0.32 g) in Et_2O (10 mL). After 30 min at 20 °C the solvent was removed under reduced pressure, and the residue was chromatographed on silica gel to give dimethyl 2,2′-trithiobis(1*H*-indole-3-acetate) (**26**): 0.16 g; 47%; mp (CH_2Cl_2/light petroleum) 130–132 °C; ^{1}H NMR ($CDCl_3$) δ 8.76 (s, 1 H, NH), 7.40 (d, J = 8.0 Hz, 1 H, ArH), 6.99 (ddd, J = 8.0, 7.1, 0.9 Hz, 1 H, ArH), 6.88 (ddd, J = 8.2, 7.1, 0.9 Hz, 1 H, ArH), 6.41 (d, J = 8.2 Hz, 1 H, ArH), 3.93 (s, 2 H, 3-CH_2), 3.78 (s, 3 H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 172.93 (s, $COOCH_3$), 137.66, 127.02, 125.80 (3 × s, Ar), 124.29, 120.06, 118.46 (3 × d, Ar), 114.61 (s, Ar), 111.15 (d, Ar), 52.40 (q, OCH_3), 30.30 (t, 3-CH_2). Anal. ($C_{22}H_{20}N_2O_4S_2$) C, H, N, S.

2,3-Dihydro-2-thioxo-1*H*-indole-3-acetic Acid (5). $NaBH_4$ (0.15 g) was added to a stirred solution of **20** (0.17 g) and K_2CO_3 (57 mg) in MeOH (8 mL) at 20 °C. After 5 min, the mixture was quenched with water (100 mL), adjusted to pH 2 with dilute HCl, and extracted with EtOAc (2 × 100 mL). This extract was washed with water, evaporated, and crystallized from EtOAc/light petroleum to give 2,3-dihydro-2-thioxo-1*H*-indole-3-acetic acid (**5**): 58 mg; 34%; mp 166–168 °C (lit.[23] mp 170–171 °C); ^{1}H NMR [$(CD_3)_2CO$] δ 11.51 (s, 1 H, NH), 7.39 (d, J = 7.9 Hz, 1 H, ArH), 7.29 (td, J = 7.7, 0.8 Hz, 1 H, ArH), 7.11 (m, 2 H, ArH), 4.02 (dd, J = 8.4, 3.9 Hz, 1 H, H-3), 3.36 (dd, J = 17.2, 3.9 Hz, 1 H, 3-CH), 2.83 (dd, J = 17.2, 8.4 Hz, 1 H, 3-CH).

Dimethyl 2,2′-Thiobis(1-methyl-1*H*-indole-3-acetate) (24) and Dimethyl 2,2′-Dithiobis(1-methyl-1*H*-indole-3-acetate) (23). Methyl 1-methyl-1*H*-indole-3-acetate[14] (**58**) (1.18 g) was treated with S_2Cl_2 (0.25 mL) as above, and the product was chromatographed on silica gel. Elution with CH_2Cl_2/light petroleum (2:1) and CH_2Cl_2 followed by slow crystallization from EtOAc/light petroleum gave dimethyl 2,2′-thiobis(1-methyl-1*H*-indole-3-acetate) (**24**): 0.17 g; 13%; mp 155–156 °C; ^{1}H NMR ($CDCl_3$) δ 7.54 (d, J = 8.0 Hz, 1 H, ArH), 7.22 (m, 2 H, ArH), 7.11 (ddd, J = 8.0, 4.9, 3.0 Hz, 1 H, ArH), 3.96 (s, 2 H, 3-CH_2), 3.61 (s, 3 H, OCH_3), 3.48 (s, 3 H, NCH_3); ^{13}C NMR ($CDCl_3$) δ 171.54 (s, $COOCH_3$), 137.80, 126.80, 126.24 (3 × s, Ar), 123.03, 119.92, 118.96 (3 × d, Ar), 112.95 (s, Ar), 109.37 (d, Ar), 51.85 (q, OCH_3), 31.04 (t, 3-CH_2), 30.38 (q, NCH_3). Anal. ($C_{24}H_{24}N_2O_4S$) C, H, N, S.

Further crystallization of mother liquor fractions from benzene/light petroleum gave dimethyl 2,2′-dithiobis(1-methyl-1*H*-indole-3-acetate) (**23**): 0.16 g; 13%; mp 130–132.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.51 (dt, J = 8.0, 0.8 Hz, 1 H, ArH), 7.29 (m, 2 H, ArH), 7.12 (ddd, J = 8.0, 6.0, 2.0 Hz, 1 H, ArH), 3.57 (s, 3 H, OCH_3), 3.48 (s, 3 H, NCH_3), 3.33 (s, 2 H, 3-CH_2); ^{13}C NMR ($CDCl_3$) δ 171.44 (s, $COOCH_3$), 138.42, 128.13, 126.38 (3 × s, Ar), 124.37, 120.13, 120.08 (3 × d, Ar), 117.48 (s, Ar), 109.94 (d, Ar), 51.79 (q, OCH_3), 30.57 (q, NCH_3), 29.96 (t, 3-CH_2). Anal. ($C_{24}H_{24}N_2O_4S_2$) C, H, N, S. Treatment of the remaining mother liquor successively with $NaBH_4$ as above (in order to reduce traces of the trisulfide), then with excess $FeCl_3$ in EtOAc at 20 °C, gave a further 0.36 g (26%) of **23**.

Methyl 2,3-Dihydro-1-methyl-2-thioxo-1*H*-indole-3-acetate (8). Treatment of **23** with $NaBH_4$ as above gave methyl 2,3-dihydro-1-methyl-2-thioxo-1*H*-indole-3-acetate (**8**): 0.23 g; 61%; mp (benzene/light petroleum) 68–70 °C; ^{1}H NMR ($CDCl_3$) δ 7.34 (m, 2 H, ArH), 7.16 (td, J = 7.5, 0.9 Hz, 1 H, ArH), 7.01 (d, J = 7.8 Hz, 1 H, ArH), 4.15 (dd, J = 8.7, 4.1 Hz, 1 H, H-3), 3.71 (s, 3 H, OCH_3), 3.65 (s, 3 H, NCH_3), 3.40 (dd, J = 17.0, 4.1 Hz, 1 H, 3-CH), 2.83 (dd, J = 17.0, 8.7 Hz, 1 H, 3-CH); ^{13}C NMR ($CDCl_3$) δ 204.24 (s, $CSNCH_3$), 171.68 (s, $COOCH_3$), 145.74, 132.95 (2 × s, Ar), 128.47, 124.40, 123.96, 109.54 (4 × d, Ar), 53.41 (d, C-3), 51.96 (q, OCH_3), 38.46 (t, 3-CH_2), 31.57 (q, NCH_3). Anal. ($C_{12}H_{13}NO_2S$) C, H, N, S.

2,2′-Dithiobis(1-methyl-1*H*-indole-3-acetic acid) (21). 1-Methyl-1*H*-indole-3-acetic acid[14,21] (**57**, 1.05 g) was treated with S_2Cl_2 as above,[23] and the product was chromatographed on silica

gel. Elution with EtOAc/light petroleum (1:1) gave an oil, which was crystallized successively from EtOAc/light petroleum, AcOH, and Me_2CO/light petroleum to yield 2,2′-dithiobis(1-methyl-1*H*-indole-3-acetic acid) (**21**): 0.10 g; 8%; mp 190–192.5 °C (lit.[23] mp 190–191 °C); ^{1}H NMR [$(CD_3)_2CO$] δ 7.56 (dt, J = 8.1, 0.9 Hz, 1 H, ArH), 7.44 (dt, J = 8.3, 0.9 Hz, 1 H, ArH), 7.31 (ddd, J = 8.2, 7.0, 1.2 Hz, 1 H, ArH), 7.11 (ddd, J = 8.0, 7.0, 0.9 Hz, 1 H, ArH), 3.65 (s, 3 H, NCH_3), 3.23 (s, 2 H, 3-CH_2); ^{13}C NMR [$(CD_3)_2CO$] δ 172.21 (s, COOH), 139.52, 128.56, 127.45 (3 × s, Ar), 125.21, 120.91, 120.74 (3 × d, Ar), 119.38 (s, Ar), 111.04 (d, Ar), 30.81 (t, 3-CH_2), 30.31 (q, NCH_3). Anal. ($C_{22}H_{20}N_2O_4S_2$) C, H, N, S.

2,3-Dihydro-1-methyl-2-thioxo-1*H*-indole-3-acetic Acid (6). A stirred solution of **21** (104 mg) and K_2CO_3 (36 mg) in MeOH (8 mL) was treated with $NaBH_4$ (0.10 g). Crystallization of the product from CH_2Cl_2/light petroleum gave 2,3-dihydro-1-methyl-2-thioxo-1*H*-indole-3-acetic acid (**6**): 62 mg; 60%; mp 150–153 °C (lit.[23] mp 149–150 °C); ^{1}H NMR ($CDCl_3$) δ 7.37 (m, 2 H, ArH), 7.18 (t, J = 7.5 Hz, 1 H, ArH), 7.02 (d, J = 7.8 Hz, 1 H, ArH), 4.14 (dd, J = 8.6, 3.9 Hz, 1 H, H-3), 3.65 (s, 3 H, NCH_3), 3.48 (dd, J = 17.5, 4.0 Hz, 1 H, 3-CH), 2.86 (dd, J = 17.5, 8.7 Hz, 1 H, 3-CH); ^{13}C NMR ($CDCl_3$) δ 203.88 (s, CSNCH$_3$), 176.31 (s, COOH), 145.67, 132.64 (2 × s, Ar), 128.57, 124.52, 124.00, 109.59 (4 × d, Ar), 53.07 (d, C-3), 38.33 (t, 3-CH_2), 31.59 (q, NCH_3).

Methyl 2,3-Dihydro-2-thioxo-1*H*-indole-3-propanoate (11) and Dimethyl 2,2′-Dithiobis(1*H*-indole-3-propanoate) (29). A stirred solution of 1*H*-indole-3-propanoic acid (**43**) (0.93 g) in DMSO (3.25 mL) was treated dropwise with concentrated HCl (7.86 mL) over 5 min at 20 °C.[31] After 30 min the mixture was diluted with water (100 mL) and extracted with EtOAc (4 × 100 mL). The combined extracts were washed with water (200 mL) and worked up to give crude 2,3-dihydro-2-oxo-1*H*-indole-3 propanoic acid (**46**) as an oil (1.00 g), which was esterified with diazomethane. Chromatography on silica gel gave methyl 2,3-dihydro-2-oxo-1*H*-indole-3-propanoate (**51**, 0.89 g, 89%) as an oil (lit.[25] mp 79–80 °C): ^{1}H NMR ($CDCl_3$) δ 8.75 (s, 1 H, NH), 7.22 (m, 2 H, ArH), 7.03 (ddd, J = 7.8, 7.1, 1.1 Hz, 1 H, ArH), 6.91 (dd, J = 7.3, 1.3 Hz, 1 H, ArH), 3.63 (s, 3 H, OCH_3), 3.54 (t, J = 5.8 Hz, 1 H, H-3), 2.61–2.20 (m, 4 H, 3-CH_2CH_2); HREIMS m/z calcd for $C_{12}H_{13}NO_3$ 219.0895 (M^+), found 219.0898.

Treatment of **51** (0.89 g) with P_2S_5/Na_2CO_3 in THF,[20] followed by chromatography on silica gel, eluting with EtOAc/light petroleum (3:1), gave dimethyl 2,2′-dithiobis(1*H*-indole-3-propanoate) (**29**): 61 mg; 6%; mp (MeOH) 162.5–164 °C; ^{1}H NMR ($CDCl_3$) δ 8.21 (s, 1 H, NH), 7.55 (dd, J = 8.0, 0.7 Hz, 1 H, ArH), 7.25 (m, 2 H, ArH), 7.12 (ddd, J = 8.0, 5.4, 2.6 Hz, 1 H, ArH), 3.56 (s, 3 H, OCH_3), 2.98, 2.47 (2 × t, J = 7.9 Hz, 2 × 2 H, 3-CH_2-CH_2); ^{13}C NMR ($CDCl_3$) δ 173.38 (s, $COOCH_3$), 137.25, 127.21, 125.80 (3 × s, Ar), 124.30 (d, Ar), 122.79 (s, Ar), 120.10, 119.59, 111.21 (3 × d, Ar), 51.56 (q, OCH_3), 34.97 (t, CH_2CO), 20.27 (t, 3-CH_2). Anal. ($C_{24}H_{24}N_2O_4S_2$) C, H, N, S.

Crystallization of the mother liquors from benzene/light petroleum gave methyl 2,3-dihydro-2-thioxo-1*H*-indole-3-propanoate (**11**): 0.24 g; 25%; mp (CH_2Cl_2/light petroleum) 96–98 °C; ^{1}H NMR ($CDCl_3$) δ 9.83 (s, 1 H, NH), 7.29 (m, 2 H, ArH), 7.16 (td, J = 7.5, 0.9 Hz, 1 H, ArH), 6.99 (d, J = 7.8 Hz, 1 H, ArH), 3.91 (t, J = 5.4 Hz, 1 H, H-3), 3.60 (s, 3 H, OCH_3), 2.52 (m, 2 H, 3-CH_2), 2.42, 2.11 (2 × m, 2 × 1 H, CH_2CO); ^{13}C NMR ($CDCl_3$) δ 207.26 (s, CSNH), 173.37 (s, $COOCH_3$), 143.24, 133.08 (2 × s, Ar), 128.43, 124.35, 124.09, 110.01 (4 × d, Ar), 56.45 (d, C-3), 51.68 (q, OCH_3), 29.33, 28.19 (2 × t, 3-CH_2CH_2). Anal. ($C_{12}H_{13}NO_2S$) C, H, N, S.

2,3-Dihydro-2-thioxo-1*H*-indole-3-propanoic Acid (9) and 2,2′-Dithiobis(1*H*-indole-3-propanoic acid) (27). A solution of **11** (0.12 g) in MeOH (10 mL) and K_2CO_3 (0.28 g) in water (3 mL) was stirred at 20 °C for 18 h. The mixture was then diluted with water (100 mL) and extracted with CH_2Cl_2 (100 mL). The aqueous portion was adjusted to pH 2 with dilute HCl and then extracted with EtOAc (3 × 100 mL). This extract was washed with water, and the solvent was removed under vacuum to give an oil (0.10 g), which was purified by chromatography on silica gel, and then treated with $NaBH_4$ as above and crystallized from CH_2Cl_2/isopropyl ether/light petroleum to give 2,3-dihydro-2-thioxo-1*H*-indole-3-propanoic acid (**9**): 25 mg; 22%; mp 173–175 °C; ^{1}H NMR [$(CD_3)_2CO$] δ 11.48 (s, 1 H, NH), 7.43 (d, J = 7.4 Hz, 1 H, ArH), 7.30 (t, J = 7.7 Hz, 1 H, ArH), 7.15 (t, J = 7.4 Hz, 1 H, ArH), 7.11 (d, J = 7.8 Hz, 1 H, ArH), 3.90 (t, J = 5.3 Hz, 1 H, H-3), 2.49, 2.37, 2.11 (3 × m, 4 H, 3-CH_2CH_2); ^{13}C NMR [$(CD_3)_2CO$] δ 208.48 (s, CSNH), 174.14 (s, COOH), 145.18, 134.55 (2 × s, Ar), 129.05, 125.08, 124.30, 110.87 (4 × d, Ar), 57.18 (d, C-3), 29.86, 29.25 (2 × t, 3-CH_2CH_2). Anal. ($C_{11}H_{11}NO_2S$·0.5H_2O) C, H, N, S.

Hydrolysis of **11** in EtOH/water/NaOH for 25 min at 20 °C, followed by chromatography of the product on silica gel and elution with light petroleum/EtOAc/AcOH (66:33:1), gave 1*H*-indole-3-propanoic acid (**43**) [35%, mp (CH_2Cl_2/light petroleum) 125–130 °C (lit.[32] mp 133–134 °C)] followed by 2,3-dihydro-3-hydroxy-2-thioxo-1*H*-indole-3-propanoic acid (**55**) (32%) as an unstable oil: ^{1}H NMR (CD_3OD) δ 7.42 (dd, J = 7.4, 0.7 Hz, 1 H, ArH), 7.32 (td, J = 7.7, 1.2 Hz, 1 H, ArH), 7.15 (td, J = 7.5, 0.9 Hz, 1 H, ArH), 7.00 (d, J = 7.8 Hz, 1 H, ArH), 2.33 (ddd, J = 12.9, 11.6, 5.0 Hz, 1 H, 3-CH), 2.18 (ddd, J = 13.0, 11.5, 5.0 Hz, 1 H, 3-CH), 2.10 (ddd, J = 16.0, 11.6, 5.1 Hz, 1 H, CHCO), 1.83 (ddd, J = 16.0, 11.5, 5.0 Hz, 1 H, CHCO); ^{13}C NMR (CD_3OD) δ 211.91 (s, CSNH), 176.36 (s, COOH), 144.35, 135.84 (2 × s, Ar), 130.84, 125.84, 124.92, 111.48 (4 × d, Ar), 84.69 (s, C-3), 37.01, 29.50 (2 × t, 3-CH_2CH_2); HRFABMS m/z calcd for $C_{11}H_{12}NO_3S$ 238.0538 ($M + H^+$), found 238.0556.

A solution of **9** (150 mg) in MeOH (10 mL) was stirred at 20 °C for 12 days and then diluted with water (5 mL) and cooled. Recrystallization of the resulting precipitate gave 2,2′-dithiobis(1*H*-indole-3-propanoic acid) (**27**): 30 mg; 20%; mp (MeOH/H_2O) 118–120.5 °C; ^{1}H NMR (CD_3OD) δ 7.47 (dt, J = 8.0, 0.8 Hz, 1 H, ArH), 7.30 (dt, J = 8.1, 0.8 Hz, 1 H, ArH), 7.15 (ddd, J = 8.1, 7.1, 1.0 Hz, 1 H, ArH), 7.00 (ddd, J = 8.0, 7.1, 0.9 Hz, 1 H, ArH), 2.74, 2.24 (2 × t, J = 8.0 Hz, 2 × 2 H, 3-CH_2CH_2); ^{13}C NMR (CD_3OD) δ 176.95 (s, COOH), 139.26, 128.26, 126.65 (3 × s, Ar), 124.69 (d, Ar), 123.66 (s, Ar), 120.36, 120.20, 112.41 (3 × d, Ar), 36.29 (t, CH_2CO), 21.22 (t, 3-CH_2). Anal. ($C_{22}H_{20}N_2O_4S_2$·H_2O) C, H, N, S.

Methyl 2,3-Dihydro-1-methyl-2-thioxo-1*H*-indole-3-propanoate (12) and Dimethyl 2,2′-dithiobis(1-methyl-1*H*-indole-3-propanoate) (30). A solution of 18-crown-6 (0.44 g), potassium *tert*-butoxide (2.20 g), and methyl 1*H*-indole-3-propanoate (**38**) (prepared from 1*H*-indole-3-propanoic acid with CH_2N_2; 3.24 g) in dry benzene (20 mL) was stirred at room temperature for 15 min and then cooled in ice. A solution of MeI (3.42 g) in benzene (10 mL) was added, the flask was then sealed, and the mixture was stirred at 20 °C for 1 day.[14] The resulting solution was filtered to remove salts and washed with CH_2Cl_2, and the combined filtrates were washed with water and the solvents were removed. Chromatography of the residue on silica gel, eluting with CH_2Cl_2/light petroleum (1:1), gave methyl 1-methyl-1*H*-indole-3-propanoate (**40**) (1.90 g, 52%) as a colorless oil (lit.[33] oil, $bp_{0.25}$ 180–190 °C): ^{1}H NMR ($CDCl_3$) δ 7.58 (dt, J = 7.7, 0.9 Hz, 1 H, ArH), 7.28 (dt, J = 7.9, 1.3 Hz, 1 H, ArH), 7.21 (ddd, J = 8.1, 6.7, 1.3 Hz, 1 H, ArH), 7.10 (ddd, J = 7.9, 6.5, 1.5 Hz, 1 H, ArH), 6.86 (s, 1 H, H-2), 3.73, 3.67 (2 × s, 2 × 3 H, NCH_3, OCH_3), 3.09, 2.70 (2 × t, J = 7.6 Hz, 2 × 2 H, 3-CH_2CH_2); HREIMS m/z calcd for $C_{13}H_{15}NO_2$ 217.1103 (M^+), found 217.1101.

A stirred solution of **40** (1.85 g) in DMSO (6.05 mL) was treated dropwise with concentrated HCl (14.6 mL) over 30 min at 20 °C.[31] After 3 h, the mixture was diluted with water (150 mL) and extracted with EtOAc (3 × 150 mL). These combined extracts were washed with water (150 mL), and then the solvent was removed under reduced pressure to give crude 2,3-dihydro-1-methyl-2-oxo-1*H*-indole-3-propanoic acid (**48**) (2.08 g) as a colorless oil: ^{1}H NMR (CD_3OD) δ 7.31 (m, 2 H, ArH), 7.09 (td, J = 8.0, 1.0 Hz, 1 H, ArH), 6.98 (d, J = 7.6 Hz, 1 H, ArH), 3.56 (t, J = 6.1 Hz, 1 H, H-3), 3.20 (s, 3 H, NCH_3), 2.41–2.15 (m, 4 H, 3-CH_2CH_2); ^{13}C NMR (CD_3OD) δ 179.64 (s, COOH), 176.55 (s, $CONCH_3$), 145.52, 129.73 (2 × s, Ar), 129.39, 125.00, 123.93, 109.64 (4 × d, Ar), 45.79 (d, C-3), 31.01, 26.91 (2 × t, 3-CH_2CH_2), 26.44 (q, NCH_3); HREIMS m/z calcd for $C_{12}H_{13}NO_3$ 219.0895 (M^+), found 219.0897. This was esterified with diazomethane as above, and then the product was chromatographed on silica gel. Elution with EtOAc/light petroleum (1:2) gave methyl 2,3-dihydro-1-methyl-2-oxo-1*H*-indole-3-propanoate (**53**) (1.40 g, 70%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 7.27 (m, 2 H, ArH), 7.06 (td, J = 7.5, 0.8 Hz, 1 H, ArH), 6.83 (d, J = 7.7 Hz, 1 H, ArH), 3.62 (s, 3 H, OCH_3), 3.50 (t, J = 6.0 Hz, 1 H, H-3), 3.20 (s, 3 H, NCH_3), 2.52–2.18 (m, 4 H, 3-CH_2CH_2); ^{13}C NMR ($CDCl_3$) δ 177.23 (s, $CONCH_3$), 173.38 (s, $COOCH_3$), 144.36 (s, Ar), 128.20 (d, Ar),

128.11 (s, Ar), 123.92, 122.48, 108.06 (3 × d, Ar), 51.64 (q, OCH_3), 44.36 (d, C-3), 30.12 (t, CH_2CO), 26.14 (q, NCH_3), 25.64 (t, 3-CH_2); HREIMS *m/z* calcd for $C_{13}H_{15}NO_3$ 233.1052 (M^+), found 233.1055.

Treatment of **53** (1.38 g) with $P_2S_5/NaHCO_3$ in dioxane, followed by chromatography on silica gel, eluting with CH_2Cl_2/light petroleum (3:2), gave methyl 2,3-dihydro-1-methyl-2-thioxo-1*H*-indole-3-propanoate (**12**): 1.40 g; 95%; mp (benzene/light petroleum) 71–73 °C; ^1H NMR ($CDCl_3$) δ 7.35 (m, 2 H, ArH), 7.19 (td, *J* = 7.5, 0.9 Hz, 1 H, ArH), 7.00 (d, *J* = 7.7 Hz, 1 H, ArH), 3.92 (t, *J* = 5.4 Hz, 1 H, H-3), 3.63, 3.58 (2 × s, 2 × 3 H, NCH_3, OCH_3), 2.53 (m, 2 H, 3-CH_2), 2.34, 2.03 (2 × m, 2 × 1 H, CH_2CO); ^{13}C NMR ($CDCl_3$) δ 204.77 (s, *C*SCH$_3$), 173.32 (s, *C*OOCH$_3$), 145.89, 132.37 (2 × s, Ar), 128.40, 124.31, 123.99, 109.51 (4 × d, Ar), 56.26 (d, C-3), 51.61 (q, OCH_3), 31.35 (q, NCH_3), 29.31, 28.46 (2 × t, 3-CH_2CH_2). Anal. ($C_{13}H_{15}NO_2S$) C, H, N, S.

Oxidation of **12** (0.70 g) with $FeCl_3$ (0.70 g) in EtOAc/CH_2Cl_2 as above, followed by chromatography on silica gel and elution with CH_2Cl_2, gave dimethyl 2,2′-dithiobis(1-methyl-1*H*-indole-3-propanoate) (**30**): 0.38 g; 54%; mp (CH_2Cl_2/MeOH) 139–141.5 °C; ^1H NMR ($CDCl_3$) δ 7.25 (d, *J* = 8.0 Hz, 1 H, ArH), 7.27 (ddd, *J* = 8.3, 6.1, 0.9 Hz, 1 H, ArH), 7.25 (d, *J* = 8.1 Hz, 1 H, ArH), 7.09 (ddd, *J* = 8.0, 6.1, 1.9 Hz, 1 H, ArH), 3.59, 3.53 (2 × s, 2 × 3 H, NCH_3, OCH_3), 2.76, 2.21 (2 × t, *J* = 7.8 Hz, 2 × 2 H, 3-CH_2-CH_2); ^{13}C NMR ($CDCl_3$) δ 173.17 (s, *C*OOCH$_3$), 138.49, 127.00, 126.09 (3 × s, Ar), 124.14 (d, Ar), 123.77 (s, Ar), 119.68, 119.65, 109.87 (3 × d, Ar), 51.39 (q, OCH_3), 35.09 (t, CH_2CO), 29.86 (q, NCH_3), 20.50 (t, 3-CH_2). Anal. ($C_{26}H_{28}N_2O_4S_2$) C, H, N, S.

2,3-Dihydro-1-methyl-2-thioxo-1*H*-indole-3-propanoic Acid (10) and 2,2′-Dithiobis(1-methyl-1*H*-indole-3-propanoic acid) (28). Hydrolysis of **12** with 2 N aqueous NaOH in EtOH at 20 °C for 80 min gave a crude product, which was treated with excess $NaBH_4$ in MeOH/H_2O/NaOH as above to give 2,3-dihydro-1-methyl-2-thioxo-1*H*-indole-3-propanoic acid (**10**): 60% yield; mp (CH_2Cl_2/light petroleum) 128–130 °C; ^1H NMR ($CDCl_3$) δ 7.35 (m, 2 H, ArH), 7.18 (td, *J* = 7.5, 0.9 Hz, 1 H, ArH), 7.00 (d, *J* = 7.8 Hz, 1 H, ArH), 3.93 (t, *J* = 5.3 Hz, 1 H, H-3), 3.63 (s, 3 H, NCH_3), 2.51 (m, 2 H, 3-CH_2), 2.38 (ddd, *J* = 16.1, 9.3, 6.7 Hz, 1 H, CHCO), 2.06 (ddd, *J* = 16.0, 9.8, 6.1 Hz, 1 H, CHCO); ^{13}C NMR ($CDCl_3$) δ 204.61 (s, *C*SCH$_3$), 178.41 (COOH), 145.88, 132.24 (2 × s, Ar), 128.50, 124.38, 123.96, 109.57 (4 × d, Ar), 56.05 (d, C-3), 31.37 (q, NCH_3), 29.16, 28.16 (2 × t, 3-CH_2CH_2). Anal. ($C_{12}H_{13}NO_2S$) C, H, N, S.

Similar hydrolysis of (**30**) gave 2,2′-dithiobis(1-methyl-1*H*-indole-3-propanoic acid) (**28**): 73 mg; 20%; mp (AcOH) 158.5–160 °C; ^1H NMR ($(CD_3)_2CO$) δ 7.59 (d, *J* = 8.1 Hz, 1 H, ArH), 7.39 (d, *J* = 8.0 Hz, 1 H, ArH), 7.27 (ddd, *J* = 8.2, 7.1, 0.9 Hz, 1 H, ArH), 7.07 (ddd, *J* = 8.1, 7.1, 0.8 Hz, 1 H, ArH), 3.60 (s, 3H, NCH_3), 2.79, 2.31 (2 × t, *J* = 7.9 Hz, 2 × 2 H, 3-CH_2CH_2); ^{13}C NMR ($(CD_3)_2CO$) δ 173.75 (s, COOH), 139.61, 127.54, 127.06 (3 × s, Ar), 125.08 (d, Ar), 125.02 (s, Ar), 120.55, 120.53, 110.03 (3 × d, Ar), 35.56 (t, CH_2CO), 30.13 (q, NCH_3), 21.32 (t, 3-CH_2). Anal. ($C_{24}H_{24}N_2O_4S_2$) C, H, N, S. Chromatography of the mother liquors on silica gel and then treatment with $NaBH_4$ as above also gave **10** (32% yield).

2,2′-Dithiobis(5-methyl-1*H*-indole-3-propanoic acid) (31). Condensation of 5-methyl-2-indolinone[26] (**59**) with diethyl oxalate in NaOEt/EtOH using the published method[25] gave ethyl 3-(2,3-dihydro-5-methyl-2-oxo-1*H*-indol-3-ylidene)-3-hydroxypropanoate (**62**): 48% yield; mp (benzene) 159–162 °C; ^1H NMR ($CDCl_3$) δ 8.85 (s, 1 H, NH), 7.14 (s, 1 H, H-4), 6.99 (d, *J* = 7.9 Hz, 1 H, H-6), 6.88 (d, *J* = 7.9 Hz, 1 H, H-7), 4.24 (q, *J* = 7.1 Hz, 2 H, C*H*$_2$CH$_3$), 3.76 (s, 2 H, CH_2CO_2), 2.36 (s, 3 H, $ArCH_3$), 1.28 (t, *J* = 7.1 Hz, 3 H, CH_2CH_3); ^{13}C NMR ($CDCl_3$) δ 172.9 (CO_2Et), 167.2, 167.1 (C-2 and C=*C*(OH)), 134.4, 131.7, 121.8 (C-5,8,9), 128.3, 126.7, 120.8 (C-4, 6, 7), 103.9 (*C*=C(OH)), 61.8 (O*C*H$_2$-CH$_3$), 40.3 (*C*H$_2$CO$_2$), 21.4 (Ar*C*H$_3$), 14.1 (OCH$_2$*C*H$_3$). Anal. ($C_{14}H_{15}NO_4$) C, H, N.

Hydrogenation of **62** in glacial AcOH containing concentrated H_2SO_4 and 5% Pd/C catalyst[25] gave ethyl 2,3-dihydro-5-methyl-2-oxo-1*H*-indole-3-propanoate (**65**) as a pale yellow oil: 84% yield; ^1H NMR ($CDCl_3$) δ 9.03 (br s, 1 H, NH), 7.08–6.96 (m, 2 H, ArH), 6.86–6.75 (m, 1 H, ArH), 4.09 (q, *J* = 7.1 Hz, 2 H, C*H*$_2$CH$_3$), 3.56–3.45 (m, 1 H, C*H*CH$_2$CH$_2$CO$_2$), 2.64–1.90 (m, 4 H, CHC*H*$_2$C*H*$_2$CO$_2$), 2.32 (s, 3 H, $ArCH_3$), 1.23 (t, *J* = 7.1 Hz, 3 H, CH_2CH_3); ^{13}C NMR ($CDCl_3$) δ 180.1 (C-2), 173.0 (CO_2Et), 139.2, 131.9, 128.8 (C-5,8,9), 128.4, 125.0, 109.6 (C-4,6,7), 60.5 (O*C*H$_2$-CH$_3$), 45.0 (*C*HCH$_2$CH$_2$CO$_2$), 30.3, 25.5 (CH*C*H$_2$*C*H$_2$CO$_2$), 21.1 (Ar*C*H$_3$) 14.2 (OCH$_2$*C*H$_3$); MS *m/z* 247 (18, M^+), 201 (30, M – EtOH), 159 (100, M – $C_4H_8O_2$); HREIMS *m/z* calcd for $C_{14}H_{17}NO_3$ 247.12084 (M^+), found 247.12039.

Thiation of **65** with P_2S_5/Na_2CO_3,[20] followed by oxidation of the crude product with $FeCl_3$ as described above, then gave diethyl 2,2′-dithiobis(5-methyl-1*H*-indole-3-propanoate) (**68**): 48% yield; mp (benzene/petroleum ether) 138.5–139 °C; ^1H NMR ($CDCl_3$) δ 8.10 (s, 1 H, NH), 7.32 (d, *J* = 0.6 Hz, 1 H, H-4), 7.15 (d, *J* = 8.3 Hz, 1 H, H-7), 7.06 (dd, *J* = 8.3, 1.4 Hz, 1 H, H-6), 4.03 (q, *J* = 7.2 Hz, 2 H, C*H*$_2$CH$_3$), 3.02–2.85 (m, 2 H, $CH_2CH_2CO_2$), 2.51–2.36 (m, 2 H, $CH_2CH_2CO_2$), 2.43 (s, 3 H, $ArCH_3$), 1.18 (t, *J* = 7.2 Hz, 3 H, CH_2CH_3); ^{13}C NMR ($CDCl_3$) δ 173.1 (CO_2Et), 135.6, 129.3, 127.4, 125.9, 122.3 (C-2,3,5,8,9), 126.0, 119.1, 110.9 (C-4,6,7), 60.4 (O*C*H$_2$CH$_3$), 35.2 (*C*H$_2$CH$_2$CO$_2$), 21.5 (Ar*C*H$_3$), 20.3 (CH$_2$*C*H$_2$CO$_2$), 14.1 (OCH$_2$*C*H$_3$). Anal. ($C_{28}H_{32}N_2O_4S_2$·0.5C_6H_6) C, H, N, S. Hydrolysis of **68** with LiOH in aqueous EtOH gave 2,2′-dithiobis(5-methyl-1*H*-indole-3-propanoic acid) (**31**): 12% yield, mp (CH_2Cl_2/petroleum ether) 91.5–95 °C; ^1H NMR ($CDCl_3$) δ 7.98 (s, 1 H, NH), 7.33 (s, 1 H, H-4), 7.14 (d, *J* = 8.4 Hz, 1 H, H-7), 7.07 (dd, *J* = 8.4, 1.3 Hz, 1 H, H-6), 2.98 (t, *J* = 7.5 Hz, 2 H, $CH_2CH_2CO_2$), 2.56 (t, *J* = 7.5 Hz, 2 H, $CH_2CH_2CO_2$), 2.43 (s, 3 H, $ArCH_3$); HREIMS *m/z* calcd for 0.5.($C_{24}H_{24}N_2O_4S_2$) 235.0664, found 235.0667.

2,2′-Dithiobis(6-methyl-1*H*-indole-3-propanoic acid) (32). Similar treatment of 6-methyl-2-indolinone[34] (**60**) gave ethyl 3-(2,3-dihydro-6-methyl-2-oxo-1*H*-indol-3-ylidene)-3-hydroxypropanoate (**63**): 50% yield; mp (EtOH) 142.5–145.5 °C; ^1H NMR ($CDCl_3$) δ 8.97 (s, 1 H, NH), 7.21 (d, *J* = 7.8 Hz, 1 H, H-4), 6.88 (d, *J* = 7.8 Hz, 1 H, H-5), 6.82 (s, 1 H, H-7), 4.23 (q, *J* = 7.1 Hz, 2 H, C*H*$_2$CH$_3$), 3.74 (s, 2 H, CH_2CO_2), 2.37 (s, 3 H, $ArCH_3$), 1.27 (t, *J* = 7.1 Hz, 3 H, CH_2CH_3); ^{13}C NMR ($CDCl_3$) δ 173.1 (CO_2Et), 167.2, 166.2 (C-2 and C=*C*(OH)), 136.9, 136.4, 119.0 (C-6,8,9), 123.1, 119.8, 111.3 (C-4,5,7), 104.0 (C=C(OH)), 61.9 (O*C*H$_2$CH$_3$), 40.2 (*C*H$_2$CO$_2$), 21.6 (Ar*C*H$_3$), 14.1 (OCH$_2$*C*H$_3$). Anal. ($C_{14}H_{15}NO_4$) C, H, N.

Hydrogenation as above gave ethyl 2,3-dihydro-6-methyl-2-oxo-1*H*-indole-3-propanoate (**66**): oil; 85% yield; ^1H NMR ($CDCl_3$) δ 8.77 (br s, 1 H, NH), 7.11 (d, *J* = 7.6 Hz, 1 H, H-4), 6.84 (d, *J* = 7.6 Hz, 1 H, H-5), 6.74 (s, 1 H, H-7) 4.09 (q, *J* = 7.2 Hz, 2 H, C*H*$_2$CH$_3$), 3.50 (t, *J* = 5.9 Hz, 1 H, C*H*CH$_2$CH$_2$CO$_2$), 2.59–2.21 (m, 4 H, CHC*H*$_2$C*H*$_2$CO$_2$), 2.33 (s, 3 H, $ArCH_3$), 1.22 (t, *J* = 7.2 Hz, 3 H, CH_2CH_3); ^{13}C NMR ($CDCl_3$) δ 180.2 (C-2), 172.9 (CO_2Et), 141.7, 138.3, 125.6 (C-6,8,9), 123.9, 122.9, 110.6 (C-4,5,7), 60.4 (O*C*H$_2$CH$_3$), 44.7 (*C*HCH$_2$CH$_2$CO$_2$), 30.3, 25.6 (CH*C*H$_2$*C*H$_2$CO$_2$), 21.6 (Ar*C*H$_3$), 14.1 (OCH$_2$*C*H$_3$); HREIMS *m/z* calcd for $C_{14}H_{17}NO_3$ 247.12084 (M^+), found 247.12155.

Thiation and oxidation of **66** gave diethyl 2,2′-dithiobis(6-methyl-1*H*-indole-3-propanoate) (**69**): 57% yield; mp (benzene/petroleum ether) 122–123.5 °C; ^1H NMR ($CDCl_3$) δ 8.06 (s, 1 H, NH), 7.43 (d, *J* = 8.2 Hz, 1 H, H-4), 7.02 (s, 1 H, H-7), 6.97–6.92 (m, 1 H, H-5), 4.02 (q, *J* = 7.2 Hz, 2 H, C*H*$_2$CH$_3$), 2.98–2.91 (m, 2 H, CH_2CH_2CO), 2.48–2.42 (m, 2 H, $CH_2CH_2CO_2$), 2.44 (s, 3 H, $ArCH_3$), 1.17 (t, *J* = 7.2 Hz, 3 H, CH_2CH_3); ^{13}C NMR ($CDCl_3$) δ 173.0 (CO_2Et), 137.7, 134.3, 125.2, 125.0, 122.9 (C-2, 3,6,8,9), 121.9, 119.3, 110.9 (C-4,5,7), 60.3 (O*C*H$_2$CH$_3$), 35.2 (*C*H$_2$CH$_2$-CO$_2$), 21.8 (Ar*C*H$_3$), 20.3 (CH$_2$*C*H$_2$CO$_2$), 14.1 (OCH$_2$*C*H$_3$). Anal. ($C_{28}H_{32}N_2O_4S_2$) C, H, N, S. Basic hydrolysis as above gave 2,2′-dithiobis(6-methyl-1*H*-indole-3-propanoic acid) (**32**): 12% yield, mp (CH_2Cl_2/petroleum ether) 126–128 °C; ^1H NMR [$(CD_3)_2CO$] δ 10.34 (br s, 1 H, NH), 7.49 (d, *J* = 8.2 Hz, 1 H, H-4), 7.19 (s, 1 H, H-7), 6.19 (dd, *J* = 8.2, 1.2 Hz, 1 H, H-5), 2.97–2.90 (m, 2 H, $CHCH_2CO_2$), 2.49–2.43 (m, 2 H, $CH_2CH_2CO_2$), 2.42 (s, 3 H, $ArCH_3$). Anal. ($C_{24}H_{24}N_2O_4S_2$·0.5H_2O) C, H, N.

2,2′-Dithiobis(7-methyl-1*H*-indole-3-propanoic acid) (33). Similar treatment of 7-methyl-2-indolinone[26,35] (**61**) gave ethyl 3-(2,3-dihydro-7-methyl-2-oxo-1*H*-indol-3-ylidene)-3-hydroxypropanoate (**64**): 32% yield; mp (benzene) 184–188 °C; ^1H NMR ($CDCl_3$) δ 9.67 (s, 1 H, NH), 7.21–7.15 (m, 1 H, ArH), 7.04–6.97 (m, 2 H, ArH), 4.24 (q, *J* = 7.1 Hz, 2 H, C*H*$_2$CH$_3$), 3.76 (s, 2 H, CH_2CO_2), 2.34 (s, 3 H, $ArCH_3$), 1.27 (t, *J* = 7.1 Hz, 3 H, CH_2CH_3); ^{13}C NMR ($CDCl_3$) δ 173.2 (CO_2Et), 167.5, 167.1 (C-2 and C=*C*(OH)), 135.7, 121.3, 120.0 (C-7,8,9), 127.5, 122.4, 117.7 (C-4,5,6), 104.5 (*C*=C(OH)), 61.9 (O*C*H$_2$CH$_3$), 40.2 (*C*H$_2$CO$_2$), 16.4 (Ar*C*H$_3$), 14.2 (OCH$_2$*C*H$_3$). Anal. ($C_{14}H_{15}NO_4$) C, H, N.

Hydrogenation of **64** as above gave ethyl 2,3-dihydro-7-methyl-2-oxo-1*H*-indole-3-propanoate (**67**): oil; 91% yield; ^{1}H NMR ($CDCl_3$) δ 9.38 (br s, 1 H, NH), 7.08 (d, J = 7.3 Hz, 1 H, ArH), 7.04 (d, J = 7.6 Hz, 1 H, ArH), 6.95 (t, J = 7.5 Hz, 1 H, H-5), 4.11 and 4.08 (2 × dq, J = 10.9, 7.1 Hz, 2 × 1 H, CH_2CH_3), 3.55 (t, J = 6.0 Hz, 1 H, $CHCH_2CH_2CO_2$), 2.53–2.22 (m, 4 H, $CHCH_2CH_2CO_2$), 2.30 (s, 3 H, $ArCH_3$), 1.22 (t, J = 7.1 Hz, 3 H, CH_2CH_3); ^{13}C NMR ($CDCl_3$) δ 180.4 (C-2), 172.9 (CO_2Et), 140.5, 128.3, 119.3 (C-7,8,9), 129.4, 122.3, 121.5 (C-4,5,6), 60.4 (OCH_2CH_3), 45.3 ($CHCH_2CH_2CO_2$), 30.3, 25.6 ($CHCH_2CH_2CO_2$), 16.4 ($ArCH_3$), 14.1 (OCH_2CH_3); HREIMS m/z calcd for $C_{14}H_{17}NO_3$ 247.12084 (M^+), found 247.12006.

Thiation and oxidation of **67** gave diethyl 2,2′-dithiobis(7-methyl-1*H*-indole-3-propanoate) (**70**): 25% yield; mp (benzene/petroleum ether) 120–122.5 °C; ^{1}H NMR ($CDCl_3$) δ 8.23 (s, 1 H, NH), 7.38 (d, J = 7.4 Hz, 1 H, ArH), 7.00 (t, J = 7.3 Hz, 1 H, H-5), 6.94 (d, J = 6.3 Hz, 1 H, ArH), 4.02 (q, J = 7.2 Hz, 2 H, CH_2CH_3), 3.16 (t, J = 7.5 Hz, 2 H, $CH_2CH_2CO_2$), 2.71 (t, J = 7.5 Hz, 2 H, $CH_2CH_2CO_2$), 1.96 (s, 3 H, $ArCH_3$), 1.23 (t, J = 7.2 Hz, 3 H, CH_2CH_3); ^{13}C NMR ($CDCl_3$) δ 173.6 (CO_2Et), 136.9, 127.0, 124.8, 122.9, 121.0 (C-2, 3, 7, 8, 9), 124.3, 120.0, 117.0 (C-4, 5, 6), 60.6 (OCH_2CH_3), 35.3 ($CH_2CH_2CO_2$), 20.9 ($CH_2CH_2CO_2$), 16.0 ($ArCH_3$), 14.1 (OCH_2CH_3). Anal. ($C_{28}H_{32}N_2O_4S_2$) C, H, N, S. Basic hydrolysis as above gave 2,2′-dithiobis(7-methyl-1*H*-indole-3-propanoic acid) (**33**): 20% yield; mp (AcOH/petroleum ether) 172.5–175 °C; ^{1}H NMR [$(CD_3)_2CO$] δ 10.37 (br s, 1 H, NH), 7.45 (d, J = 7.0 Hz, 1 H, ArH), 7.03–6.95 (m, 2 H, ArH), 3.01–2.94 (m, 2 H, $CH_2CH_2CO_2$), 2.50–2.42 (m, 2 H, $CH_2CH_2CO_2$), 2.49 (s, 3 H, $ArCH_3$). Anal. ($C_{24}H_{24}N_2O_4S_2$) C, H, N.

Methyl 2,3-Dihydro-2-thioxo-1*H*-indole-3-butanoate (15) and Dimethyl 2,2′-Dithiobis(1*H*-indole-3-butanoate) (36). A stirred solution of 1*H*-indole-3-butanoic acid (**44**) (2.00 g) in DMSO (7.0 mL) was treated dropwise with concentrated HCl (16.6 mL) over 5 min at room temperature.[31] After 15 min the reaction was quenched with water (80 mL) and worked up as above, to give 2,3-dihydro-2-oxo-1*H*-indole-3-butanoic acid (**47**): 2.07 g; 96%; mp (water) 169–171 °C (lit.[36] mp 170–171 °C). A stirred, ice-cooled solution of **47** (2.05 g) in dry MeOH (50 mL) was treated dropwise with CH_3COCl (10 mL), and the mixture was stirred at 20 °C for a further 18 h. Solvents were removed, and the residue was partitioned between $CHCl_3$ and water to yield crude product (2.2 g). A sample was chromatographed on silica gel, eluting with EtOAc/light petroleum (1:2), to give pure methyl 2,3-dihydro-2-oxo-1*H*-indole-3-butanoate (**52**): oil; ^{1}H NMR ($CDCl_3$) δ 8.82 (s, 1 H, NH), 7.24 (d, J = 7.7 Hz, 1 H, ArH), 7.21 (t, J = 7.8 Hz, 1 H, ArH), 7.03 (td, J = 7.6, 0.8 Hz, 1 H, ArH), 6.91 (d, J = 7.7 Hz, 1 H, ArH), 3.65 (s, 3 H, OCH_3), 3.49 (t, J = 6.0 Hz, 1 H, H-3), 2.34 (t, J = 7.5 Hz, 2 H, CH_2CO), 2.00, 1.72 (2 × m, 4 H, 3-CH_2CH_2); ^{13}C NMR ($CDCl_3$) δ 180.23 (s, CONH), 173.57 (s, $COOCH_3$), 141.54, 129.24 (2 × s, Ar), 127.97, 124.11, 122.37, 109.80 (4 × d, Ar), 51.53 (q, OCH_3), 45.74 (d, C-3), 33.83, 29.79, 21.18 (3 × t, $(CH_2)_3CO$). Anal. ($C_{13}H_{15}NO_3$) C, H, N.

Treatment of crude **52** (0.48 g) with $P_2S_5/NaHCO_3$ in dioxane as above, followed by chromatography on silica gel, eluting with CH_2Cl_2, gave a mixture of products as an oil (0.42 g, 82%). Crystallization from benzene/light petroleum gave methyl 2,3-dihydro-2-thioxo-1*H*-indole-3-butanoate (**15**): (0.18 g; 35%; mp 109–110 °C; ^{1}H NMR ($CDCl_3$) δ 10.59 (s, 1 H, NH), 7.31 (d, J = 7.4 Hz, 1 H, ArH), 7.27 (td, J = 7.7, 0.9 Hz, 1 H, ArH), 7.14 (td, J = 7.5, 0.9 Hz, 1 H, ArH), 7.02 (d, J = 7.7 Hz, 1 H, ArH), 3.85 (t, J = 5.5 Hz, 1 H, H-3), 3.64 (s, 3 H, OCH_3), 2.32 (t, J = 7.5 Hz, 2 H, CH_2CO), 2.26, 2.15, 1.67, 1.46 (4 × m, 4 H, 3-CH_2CH_2); ^{13}C NMR ($CDCl_3$) δ 207.80 (s, CSNH), 173.69 (s, $COOCH_3$), 143.27, 133.85 (2 × s, Ar), 128.19, 124.17, 124.02, 110.12 (4 × d, Ar), 57.36 (d, C-3), 51.61 (q, OCH_3), 33.92, 32.76, 20.41 (3 × t, $(CH_2)_3CO$). Anal. ($C_{13}H_{15}NO_2S$) C, H, N, S.

A methanolic solution of **15** was kept in air for 2 weeks, then worked up, and chromatographed on silica gel (elution with CH_2Cl_2) to give dimethyl 2,2′-dithiobis(1*H*-indole-3-butanoate) (**36**): 80% yield; mp (MeOH/dilute HCl) 91–93 °C; ^{1}H NMR ($CDCl_3$) δ 8.19 (s, 1 H, NH), 7.57 (d, J = 7.9 Hz, 1 H, ArH), 7.28 (d, J = 8.0 Hz, 1 H, ArH), 7.24 (ddd, J = 8.2, 7.1, 1.1 Hz, 1 H, ArH), 7.12 (ddd, J = 8.0, 6.9, 1.4 Hz, 1 H, ArH), 3.56 (s, 3 H, OCH_3), 2.67, 2.18 (2 × t, J = 7.4 Hz, 2 × 2 H, 3-$CH_2CH_2CH_2$), 1.85 (quintet, J = 7.4 Hz, 2 H, 3-$CH_2CH_2CH_2$); ^{13}C NMR ($CDCl_3$) δ 174.02 (s, $COOCH_3$), 137.29, 127.49, 125.99 (3 × s, Ar), 124.21 (d, Ar), 123.70 (s, Ar), 119.95, 119.88, 111.08 (3 × d, Ar), 51.42 (q, OCH_3), 33.45, 25.67, 23.95 (3 × t, $(CH_2)_3CO$). Anal. ($C_{26}H_{28}N_2O_4S_2$) C, H, N, S.

2,3-Dihydro-2-thioxo-1*H*-indole-3-butanoic Acid (13) and 2,2′-Dithiobis(1*H*-indole-3-butanoic acid) (34). A solution of **15** (0.26 g) in MeOH (10 mL) and K_2CO_3 (0.55 g) in water (3 mL) was stirred at 20 °C for 2 days. $NaBH_4$ (100 mg) was then added, and the mixture was stirred for 25 min, quenched with water (100 mL), and worked up to yield 2,3-dihydro-2-thioxo-1*H*-indole-3-butanoic acid (**13**): 30 mg; 12%; mp (CH_2Cl_2/light petroleum) 132–134 °C; ^{1}H NMR (CD_3OD) δ 7.34 (d, J = 7.4 Hz, 1 H, ArH), 7.26 (td, J = 7.7, 1.1 Hz, 1 H, ArH), 7.12 (td, J = 7.5, 0.8 Hz, 1 H, ArH), 7.00 (d, J = 7.8 Hz, 1 H, ArH), 2.25 (t, J = 7.5 Hz, 2 H, CH_2CO), 2.24, 2.10, 1.55, 1.33 (4 × m, 4 H, 3-CH_2CH_2). Anal. ($C_{12}H_{13}NO_2S$) C, H, N, S.

Similar hydrolysis of **36**, followed by slow crystallization from aqueous MeOH, gave 2,2′-dithiobis(1*H*-indole-3-butanoic acid) (**34**): 20% yield; mp 141–143.5 °C; ^{1}H NMR (CD_3OD) δ 7.48 (dt, J = 8.0, 0.8 Hz, 1 H, ArH), 7.32 (dt, J = 8.2, 0.7 Hz, 1 H, ArH), 7.16 (ddd, J = 8.1, 7.1, 1.0 Hz, 1 H, ArH), 7.00 (ddd, J = 8.0, 7.1, 0.8 Hz, 1 H, ArH), 2.42 (t, J = 7.6 Hz, 2 H, CH_2CO), 1.93 (t, J = 7.3 Hz, 2 H, 3-CH_2), 1.58 (quintet, J = 7.5 Hz, 2 H, 3-$CH_2CH_2CH_2$); ^{13}C NMR (CD_3OD) δ 177.52 (s, COOH), 139.31, 128.69, 126.69, 124.84 (4 × s, Ar), 124.67, 120.48, 120.27, 112.34 (4 × d, Ar), 34.39, 27.24, 24.82 (3 × t, $(CH_2)_3CO$). Anal. ($C_{24}H_{24}N_2O_4S_2 \cdot 0.5H_2O$) C, H, N, S.

Methyl 2,3-Dihydro-1-methyl-2-thioxo-1*H*-indole-3-butanoate (16) and Dimethyl 2,2′-Dithiobis(1-methyl-1*H*-indole-3-butanoate) (37). Methyl 1*H*-indole-3-butanoate (**39**) (prepared by treatment of 1*H*-indole-3-butanoic acid with CH_2N_2) was N-methylated, using 18-crown-6, potassium *tert*-butoxide, and MeI, as described above, to give methyl 1-methyl-1*H*-indole-3-butanoate (**41**) as a brown oil: 40% yield; ^{1}H NMR ($CDCl_3$) δ 7.58 (td, J = 7.9, 0.9 Hz, 1 H, ArH), 7.28 (d, J = 8.2 Hz, 1 H, ArH), 7.21 (ddd, J = 8.1, 7.0, 1.1 Hz, 1 H, ArH), 7.09 (ddd, J = 8.0, 7.0, 1.0 Hz, 1 H, ArH), 6.84 (s, 1 H, ArH), 3.74 (s, 3 H, NCH_3), 3.66 (s, 3 H, OCH_3), 2.79, 2.38 (2 × t, J = 7.4 Hz, 2 × 2 H, 3-$CH_2CH_2CH_2$), 2.03 (quintet, J = 7.4 Hz, 2 H, 3-$CH_2CH_2CH_2$); ^{13}C NMR ($CDCl_3$) δ 174.21 (s, $COOCH_3$), 137.08, 127.84 (2 × s, Ar), 126.34, 121.50, 118.98, 118.62 (4 × d, Ar), 114.07 (s, Ar), 109.13 (d, Ar), 51.44 (q, OCH_3), 33.68 (t, CH_2CO), 32.55 (q, NCH_3), 25.58, 24.41 (2 × t, 3-CH_2CH_2); HREIMS m/z calcd for $C_{14}H_{17}NO_2$ 231.1259 (M^+), found 231.1255. Acidification of the filtered precipitates recovered 1*H*-indole-3-butanoic acid: 1.04 g; 52%; mp 124–126 °C (lit.[37] mp 124 °C).

A stirred solution of **41** (0.92 g) in DMSO (2.79 mL) was treated dropwise with concentrated HCl (6.67 mL) over 25 min at 20 °C.[31] After 3 h, the mixture was diluted with water (90 mL) and worked up as above to give crude 2,3-dihydro-1-methyl-2-oxo-1*H*-indole-3-butanoic acid (**49**) as an oil: 0.84 g; 91%; ^{1}H NMR ($CDCl_3$) δ 7.28 (dt, J = 7.7, 0.9 Hz, 1 H, ArH), 7.25 (d, J = 7.7 Hz, 1 H, ArH), 7.06 (td, J = 7.5, 0.9 Hz, 1 H, ArH), 6.83 (d, J = 7.8 Hz, 1 H, ArH), 3.47 (t, J = 5.9 Hz, 1 H, H-3), 3.21 (s, 3 H, NCH_3), 2.37 (t, J = 7.4 Hz, 2 H, CH_2CO), 2.00, 1.69 (2 × m, 2 × 2 H, 3-CH_2CH_2). This was esterified with diazomethane as above to give, after chromatography on silica gel and elution with EtOAc/light petroleum (1:2), methyl 2,3-dihydro-1-methyl-2-oxo-1*H*-indole-3-butanoate (**54**): 72%; mp (EtOAc/light petroleum) 69–71 °C; ^{1}H NMR ($CDCl_3$) δ 7.28 (t, J = 7.8 Hz, 1 H, ArH), 7.26 (d, J = 7.6 Hz, 1 H, ArH), 7.05 (td, J = 7.6, 0.7 Hz, 1 H, ArH), 6.82 (d, J = 7.7 Hz, 1 H, ArH), 3.64 (s, 3 H, OCH_3), 3.44 (t, J = 6.0 Hz, 1 H, H-3), 3.20 (s, 3 H, NCH_3), 2.33 (t, J = 7.5 Hz, 2 H, CH_2CO), 1.98, 1.68 (2 × m, 2 × 2 H, 3-CH_2CH_2); ^{13}C NMR ($CDCl_3$) δ 177.52 (s, $CONCH_3$), 173.59 (s, $COOCH_3$), 144.38, 128.71 (2 × s, Ar), 128.00, 123.84, 122.40, 108.02 (4 × d, Ar), 51.54 (q, OCH_3), 45.26 (d, C-3), 33.89, 29.98 (2 × t, 3-$CH_2CH_2CH_2$), 26.15 (q, NCH_3), 21.30 (t, 3-CH_2CH_2). Anal. ($C_{14}H_{17}NO_3$) C, H, N.

Treatment of **54** (1.28 g) with P_2S_5 as above, followed by chromatography on silica gel, eluting with CH_2Cl_2/light petroleum (3:2), gave methyl 2,3-dihydro-1-methyl-2-thioxo-1*H*-indole-3-butanoate (**16**): 1.07 g; 79%; mp (benzene/light petroleum) 103–106 °C; ^{1}H NMR ($CDCl_3$) δ 7.34 (m, 2 H, ArH), 7.19 (td, J = 8.0, 0.9 Hz, 1 H, ArH), 7.00 (dd, J = 8.0, 0.9 Hz, 1 H, ArH), 3.84 (t, J = 5.5 Hz, 1 H, H-3), 3.63 (2 × s, 2 × 3 H, NCH_3, OCH_3), 2.30 (t, J = 7.6 Hz, 2 H, CH_2CO), 2.27, 2.16, 1.58, 1.39 (4 × m, 4 H, 3-CH_2CH_2); ^{13}C NMR ($CDCl_3$) δ 205.39 (s, $CSNCH_3$), 173.61 (s,

$COOCH_3$), 145.80, 133.14 (2 × s, Ar), 128.15, 124.25, 123.86, 109.51 (4 × d, Ar), 57.18 (d, C-3), 51.52 (q, OCH_3), 33.90, 33.13 (2 × t, 3-$CH_2CH_2CH_2$), 31.40 (q, NCH_3), 20.36 (t, 3-CH_2CH_2). Anal. ($C_{14}H_{17}NO_2S$) C, H, N, S.

Treatment of **16** with $FeCl_3$ in EtOAc as above, followed by chromatography on silica gel and elution with CH_2Cl_2, gave dimethyl 2,2'-dithiobis(1-methyl-1*H*-indole-3-butanoate) (**37**): 85%; mp (CH_2Cl_2/MeOH) 112–113 °C; ^1H NMR ($CDCl_3$) δ 7.52 (d, J = 8.0 Hz, 1 H, ArH), 7.28 (ddd, J = 8.2, 6.0, 1.0 Hz, 1 H, ArH), 7.25 (d, J = 8.0 Hz, 1 H, ArH), 7.09 (ddd, J = 8.0, 6.0, 1.9 Hz, 1 H, ArH), 3.59, 3.55 (2 × s, 2 × 3 H, NCH_3, OCH_3), 2.42, 2.07 (2 × t, J = 7.4 Hz, 2 × 2 H, 3-$CH_2CH_2CH_2CO$), 1.68 (quintet, J = 7.4 Hz, 2 H, 3-$CH_2CH_2CH_2$); ^{13}C NMR ($CDCl_3$) δ 173.82 (s, $COOCH_3$), 138.47, 127.23, 126.43, 124.74 (4 × s, Ar), 124.05, 119.90, 119.49, 109.72 (4 × d, Ar), 51.35 (q, OCH_3), 33.40 (t, CH_2CO), 29.82 (q, NCH_3), 25.83, 24.17 (2 × t, 3-CH_2CH_2). Anal. ($C_{28}H_{32}N_2O_4S_2$) C, H, N, S.

2,3-Dihydro-1-methyl-2-thioxo-1*H*-indole-3-butanoic Acid (14). Basic hydrolysis of **16**, followed by treatment with $NaBH_4$ in MeOH/H_2O/NaOH as above, gave 2,3-dihydro-1-methyl-2-thioxo-1*H*-indole-3-butanoic acid (**14**): 44% yield; mp (CH_2Cl_2/light petroleum) 144–146.5 °C; ^1H NMR ($CDCl_3$) δ 7.34 (m, 2 H, ArH), 7.18 (t, J = 7.6 Hz, 1 H, ArH), 7.00 (d, J = 7.7 Hz, 1 H, ArH), 3.85 (t, J = 5.5 Hz, 1 H, H-3), 3.63 (s, 3 H, NCH_3), 2.34 (t, J = 7.6 Hz, 2 H, CH_2CO), 2.28, 2.18, 1.59, 1.40 (4 × m, 4 × 1 H, 3-CH_2CH_2); ^{13}C NMR ($CDCl_3$) δ 205.31 (s, $CSNCH_3$), 178.62 (s, COOH), 145.81, 133.06 (2 × s, Ar), 128.20, 124.30, 123.86, 109.54 (4 × d, Ar), 57.14 (d, C-3), 33.77, 33.01 (2 × t, 3-$CH_2CH_2CH_2$), 31.42 (q, NCH_3), 20.11 (t, 3-CH_2CH_2). Anal. ($C_{13}H_{15}NO_2S \cdot H_2O$) C, H, N, S.

2,2'-Dithiobis(1-methyl-1*H*-indole-3-butanoic acid) (35). Similar hydrolysis of **37**, followed by chromatography on silica gel and elution with EtOAc/light petroleum (1:2) containing 1% AcOH, gave 2,2'-dithiobis(1-methyl-1*H*-indole-3-butanoic acid) (**35**): 42%; mp (AcOH) 106.5–109.5 °C; ^1H NMR ($CDCl_3$) δ 7.51 (d, J = 8.0 Hz, 1 H, ArH), 7.27 (m, 2 H, ArH), 7.08 (ddd, J = 8.0, 6.0, 2.0 Hz, 1 H, ArH), 3.55 (s, 3 H, NCH_3), 2.44, 2.12 (2 × t, J = 7.4 Hz, 2 × 2 H, 3-$CH_2CH_2CH_2CO$), 1.68 (quintet, J = 7.4 Hz, 2 H, 3-$CH_2CH_2CH_2$); ^{13}C NMR ($CDCl_3$) δ 179.32 (s, COOH), 138.49, 127.49, 126.43, 124.56 (4 × s, Ar), 124.14, 119.86, 119.62, 109.79 (4 × d, Ar), 33.37 (t, CH_2CO), 29.86 (q, NCH_3), 25.59, 24.13 (2 × t, 3-CH_2CH_2). Anal. ($C_{26}H_{28}N_2O_4S_2 \cdot 2CH_3COOH$) C, H, N, S.

Dimethyl 1,2-dihydro-2-thioxo-3*H*-indole-3,3-dipropanoate (18) and Trimethyl 1,2-Dihydro-2-thioxo-1*H*-indole-1,3,3-tripropanoate (19). 2-Indolinone (1.33 g) and ethyl acrylate (2.05 g) were added successively to a solution of Na (0.23 g) in dry EtOH (15 mL), and then the mixture was refluxed for 16 h.[27] The resulting mixture was diluted with water (100 mL), adjusted to pH 2 with dilute HCl, and then extracted with EtOAc (3 × 100 mL). Removal of the solvent under reduced pressure gave an oil, which was dissolved in MeOH (20 mL) and stirred with a solution of K_2CO_3 (6 g) in water (10 mL) for 2 days at 20 °C.[21] The resulting mixture was diluted with water (100 mL) and extracted with EtOAc, and the aqueous layer was then acidified and extracted into EtOAc to give a mixture of acids (3.11 g). This material was esterified with HCl/MeOH, and the esters were chromatographed on silica gel. Elution with CH_2Cl_2 and CH_2Cl_2/$CHCl_3$ mixtures gave first trimethyl 1,2-dihydro-2-oxo-3*H*-indole-1,3,3-tripropanoate (**71**): 1.09 g; 29%; mp (MeOH) 85–87 °C; ^1H NMR ($CDCl_3$) δ 7.28 (td, J = 7.7, 1.1 Hz, 1 H, ArH), 7.16 (d, J = 7.3 Hz, 1 H, ArH), 7.07 (t, J = 7.5 Hz, 1 H, ArH), 6.92 (d, J = 7.8 Hz, 1 H, ArH), 4.02 (t, J = 7.1 Hz, 2 H, NCH_2), 3.65 (s, 3 H, OCH_3), 3.53 (s, 6 H, 2 × OCH_3), 2.72 (t, J = 7.1 Hz, 2 H, NCH_2CH_2), 2.24 (ddd, J = 13.3, 10.8, 5.4 Hz, 2 H, 2 × 3-CH), 2.15 (ddd, J = 13.4, 10.6, 5.3 Hz, 2 H, 2 × 3-CH), 2.05 (ddd, J = 15.8, 10.5, 5.4 Hz, 2 H, 2 × CHCO), 1.82 (ddd, J = 15.8, 10.7, 5.2 Hz, 2 H, 2 × CHCO); ^{13}C NMR ($CDCl_3$) δ 178.44 (s, $CONCH_2$), 172.95 (s, 2 × $COOCH_3$), 171.54 (s, $COOCH_3$), 142.78, 129.90 (2 × s, Ar), 128.59, 123.51, 122.94, 108.42 (4 × d, Ar), 51.97 (q, OCH_3), 51.60 (q, 2 × OCH_3), 51.16 (s, C-3), 36.11 (t, NCH_2), 32.44 (t, 2 × 3-CH_2), 32.25 (t, CH_2CO), 29.02 (t, 2 × CH_2CO). Anal. ($C_{20}H_{25}NO_7$) C, H, N.

Further elution with $CHCl_3$ (1% EtOH) gave dimethyl 1,2-dihydro-2-oxo-3*H*-indole-3,3-dipropanoate[38] (**72**): 0.78 g; 29%; mp (benzene/light petroleum) 70–73 °C; ^1H NMR ($CDCl_3$) δ 8.66 (s, 1 H, NH), 7.23 (td, J = 7.7, 1.2 Hz, 1 H, ArH), 7.14 (d, J = 7.3 Hz, 1 H, ArH), 7.06 (td, J = 7.5, 0.9 Hz, 1 H, ArH), 6.92 (d, J = 7.7 Hz, 1 H, ArH), 3.54 (s, 6 H, 2 × OCH_3), 2.30–2.12 (m, 6 H, 2 × 3-CH_2CH), 1.94 (m, 2 H, 2 × CHCO); ^{13}C NMR ($CDCl_3$) δ 180.88 (s, CONH), 173.06 (s, 2 × $COOCH_3$), 141.07, 130.32 (2 × s, Ar), 128.56, 123.63, 122.87, 110.05 (4 × d, Ar), 51.91 (s, C-3), 51.63 (q, 2 × OCH_3), 32.36 (t, 2 × 3-CH_2), 29.14 (t, 2 × CH_2CO); HREIMS m/z calculated for $C_{16}H_{19}NO_5$, 305.1263 (M^+), found 305.1251.

Treatment of **72** with P_2S_5 as above, followed by chromatography on silica gel, gave dimethyl 1,2-dihydro-2-thioxo-3*H*-indole-3,3-dipropanoate (**18**): 41%; mp (benzene/light petroleum) 122.5–125 °C; ^1H NMR ($CDCl_3$) δ 10.50 (s, 1 H, NH), 7.29 (td, J = 7.6, 1.3 Hz, 1 H, ArH), 7.24 (d, J = 7.5 Hz, 1 H, ArH), 7.17 (td, J = 7.4, 0.9 Hz, 1 H, ArH), 7.04 (d, J = 7.8 Hz, 1 H, ArH), 3.52 (s, 6 H, 2 × OCH_3), 2.36 (ddd, J = 13.3, 10.3, 6.4 Hz, 2 H, 2 × 3-CH), 2.32 (ddd, J = 13.3, 10.1, 6.1 Hz, 2 H, 2 × 3-CH), 2.07 (ddd, J = 16.1, 9.6, 6.6 Hz, 2 H, 2 × CHCO), 1.64 (ddd, J = 16.1, 9.9, 6.3 Hz, 2 H, 2 × CHCO); ^{13}C NMR ($CDCl_3$) δ 209.58 (s, CSNH), 173.15 (s, 2 × $COOCH_3$), 142.86, 134.40 (2 × s, Ar), 128.77, 124.29, 124.08, 110.23 (4 × d, Ar), 62.18 (s, C-3), 51.64 (q, 2 × OCH_3), 35.66 (t, 2 × 3-CH_2), 28.93 (t, 2 × CH_2CO). Anal. ($C_{16}H_{19}NO_4S$) C, H, N, S.

Similar treatment of **71** gave trimethyl 1,2-dihydro-2-thioxo-3*H*-indole-1,3,3-tripropanoate (**19**): 18%; mp (MeOH) 104–106 °C; ^1H NMR ($CDCl_3$) δ 7.36 (td, J = 7.7, 1.2 Hz, 1 H, ArH), 7.27 (dd, J = 7.4, 1.3 Hz, 1 H, ArH), 7.20 (td, J = 7.4, 0.9 Hz, 1 H, ArH), 7.11 (d, J = 8.0 Hz, 1 H, ArH), 4.48 (t, J = 7.2 Hz, 2 H, NCH_2), 3.65 (s, 3 H, OCH_3), 3.50 (s, 6 H, 2 × OCH_3), 2.85 (t, J = 7.2 Hz, 2 H, NCH_2CH_2), 2.36 (ddd, J = 13.3, 10.7, 5.6 Hz, 2 H, 2 × 3-CH), 2.29 (ddd, J = 13.4, 10.6, 5.5 Hz, 2 H, 2 × 3-CH), 1.92 (ddd, J = 16.3, 10.5, 5.6 Hz, 2 H, 2 × CHCO), 1.50 (ddd, J = 16.1, 10.7, 5.4 Hz, 2 H, 2 × CHCO); ^{13}C NMR ($CDCl_3$) δ 207.18 (s, $CSNCH_2$), 172.96 (s, 2 × $COOCH_3$), 171.43 (s, $COOCH_3$), 144.61, 134.06 (2 × s, Ar), 128.78, 124.56, 123.87, 109.70 (4 × d, Ar), 61.73 (s, C-3), 52.03 (q, OCH_3), 51.57 (q, 2 × OCH_3), 40.48 (t, NCH_2), 36.01 (t, 2 × 3-CH_2), 30.79 (t, CH_2CO), 28.79 (t, 2 × CH_2CO). Anal. ($C_{20}H_{25}NO_6S$) C, H, N, S.

Hydrolysis of **18** with MeOH/H_2O/K_2CO_3 for 1 day, followed by crystallization from EtOAc/light petroleum, gave 1,2-dihydro-2-thioxo-3*H*-indole-3,3-dipropanoic acid (**17**): 34%; mp (EtOAc/light petroleum) 214–218 °C; ^1H NMR [$(CD_3)_2CO$] δ 11.65 (s, 1 H, NH), 7.46 (d, J = 7.5 Hz, 1 H, ArH), 7.35 (td, J = 7.7, 1.1 Hz, 1 H, ArH), 7.22 (td, J = 7.5, 0.8 Hz, 1 H, ArH), 7.18 (d, J = 7.8 Hz, 1 H, ArH), 2.33 (ddd, J = 13.1, 12.0, 4.8 Hz, 2 H, 2 × 3-CH), 2.25 (ddd, J = 13.2, 11.9, 4.9 Hz, 2 H, 2 × 3-CH), 1.97 (ddd, J = 16.3, 11.8, 4.8 Hz, 2 H, 2 × CHCO), 1.56 (ddd, J = 16.2, 11.7, 4.7 Hz, 2 H, 2 × CHCO); ^{13}C NMR [$(CD_3)_2CO$] δ 210.83 (s, CSNH), 173.69 (s, 2 × COOH), 144.77, 135.98 (2 × s, Ar), 129.46, 124.79, 124.72, 111.13 (4 × d, Ar), 62.71 (s, C-3), 36.42 (t, 2 × 3-CH_2), 29.15 (t, 2 × CH_2CO). Anal. ($C_{14}H_{15}NO_4S$) C, H, N, S.

Enzyme Assays. Epidermal growth factor receptor was prepared from human A431 carcinoma cell shed membrane vesicles as previously described.[28] The reactions were carried out in 96-well plates with a 0.65-μm pore size polyvinylidene fluoride membrane bottom. The tyrosine kinase activity was assessed by solubilizing the partially purified vesicles in a mixture of 4% Triton X-100 detergent and 10% glycerol. Total vesicle preparation protein (10 μg) was added to a final volume of assay buffer of 125 μL comprised of 20 mM HEPES buffer pH 7.4, 15 mM $MgCl_2$, 4 mM $MnCl_2$, 0.02% bovine serum albumin, 5 μM ATP containing 0.2 μCi of 0.5–3 Ci/mmol ^{32}P labeled ATP, 25 μg of a random copolymer of glutamate, alanine, and tyrosine in a ratio of 6:3:1, 250 ng of epidermal growth factor, and appropriate solvent controls or inhibitors. Both Mn^{2+} and Mg^{2+} were required in the reaction buffer to obtain inhibition of the kinase activity in these preparations by genistein and erbstatin.

The reaction was allowed to progress for 10 min at room temperature and stopped by the addition of 125 μL of cold 30% trichloroacetic acid containing 0.1 M sodium pyrophosphate for 5 min on ice. The precipitate (comprised of acid-insoluble proteins and copolymer) was washed in the 96-well filter plate with the membrane bottom with two 250-μL portions of 15% trichloroacetic acid containing 0.1 M sodium pyrophosphate. Incorporated label was assessed by scintillation counting of the filters (membrane bottoms of the wells) in an aqueous fluor.

Typically, 80% of the incorporated, precipitated ^{32}P was due to the presence of copolymer substrate. Autophosphorylation controls were performed in each experimental assay.

Cell Culture and Growth Inhibition Assays. Swiss 3T3 mouse fibroblasts were obtained from the American Type Culture Collection, Bethesda, MD. Cells were maintained 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 were placed in 24-well Linbro plates (1.7 × 1.6 cm, flat bottom) followed by the addition of cells (2×10^4) 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).

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