Tyrosine Kinase Inhibitors. 2. Synthesis of 2,2'-Dithiobis(1*H*-indole-3-alkanamides) and Investigation of Their Inhibitory Activity against Epidermal Growth Factor Receptor and pp60$^{v\text{-}src}$ Protein Tyrosine Kinases

Andrew M. Thompson,† David W. Fry,‡ Alan J. Kraker,‡ and William A. Denny*,†

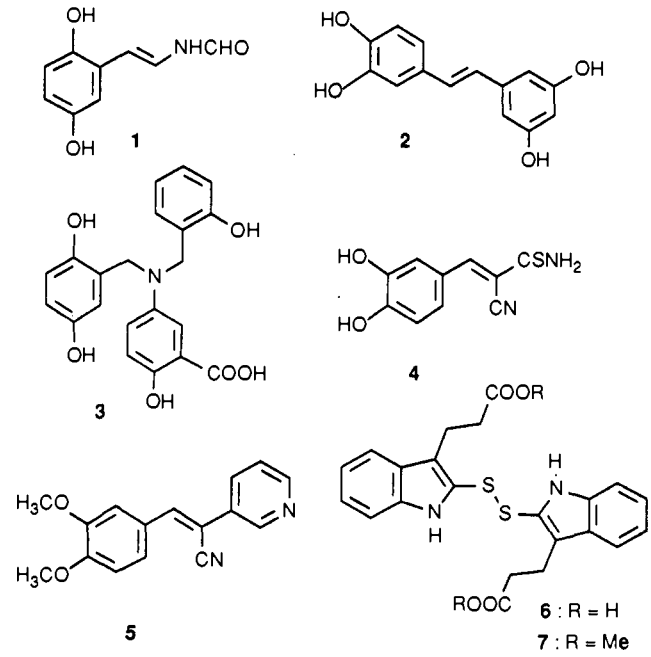
Cancer Research Laboratory, University of Auckland School of Medicine, Private Bag 92019, Auckland, New Zealand, and Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48106-1047

Received August 31, 1993•

A series of amide analogues of the 2,2'-dithiobis(1*H*-indole-3-alkanoic acid) class of tyrosine kinase inhibitors have been prepared, by reaction of 1*H*-indole-3-alkanamides (**8**) with S_2Cl_2, and separation of the desired disulfides from the initial mixtures of mono-, di-, and trisulfides formed. These amides were evaluated *in vitro* against epidermal growth factor receptor and pp60$^{v\text{-}src}$ protein tyrosine kinases. Inhibitory activity against EGF receptor tyrosine kinase was chain-length dependent, with the propanamides being the most effective. Hydrogen bond donor capabilities in the amide function did not appear to be necessary, with an *N*-benzylamide being the most potent ($IC_{50} = 0.85\ \mu M$). Further substitution on the benzyl ring did not increase potency, and substitution in the α-position of the propanamide side chain was acceptable. A water-soluble α-NH_2 derivative showed good inhibitory activity toward the enzyme, was a potent inhibitor of cell growth in fibroblasts, and selectively inhibited intracellular tyrosine phosphorylation patterns. The nonreceptor kinase pp60$^{v\text{-}src}$ was in general much more sensitive than EGF receptor kinase to inhibition by these compounds, but with less pronounced structure–activity relationships.

Several classes of small molecules have recently been reported to be potent inhibitors of the protein tyrosine kinase activity of a number of trans-membrane growth factor receptors and cellular oncogene products, particularly epidermal growth factor (EGF) receptor.[1,2] Such compounds include the phenolic natural products erbstatin (**1**),[3] piceatannol (**2**),[4] and lavendustin (**3**),[5] together with a number of synthetic compounds collectively known as the tyrphostins (e.g., **4** and **5**),[6] which were initially considered to be competitive inhibitors at the peptide (tyrosine) binding site. However, recent kinetic studies using the intracellular domain of EGF receptor expressed from a recombinant baculovirus have suggested that the picture is more complex, with erbstatin acting as a partial competitive inhibitor with respect to both the peptide and ATP binding sites.[7] Protein tyrosine kinases (particularly those associated with trans-membrane receptors)[8] play a fundamental role in the regulation of cell growth. Selective inhibitors of this function are of increasing interest as mediators of cell growth (e.g., in psoriasis[6]) and as potential anticancer drugs.[9]

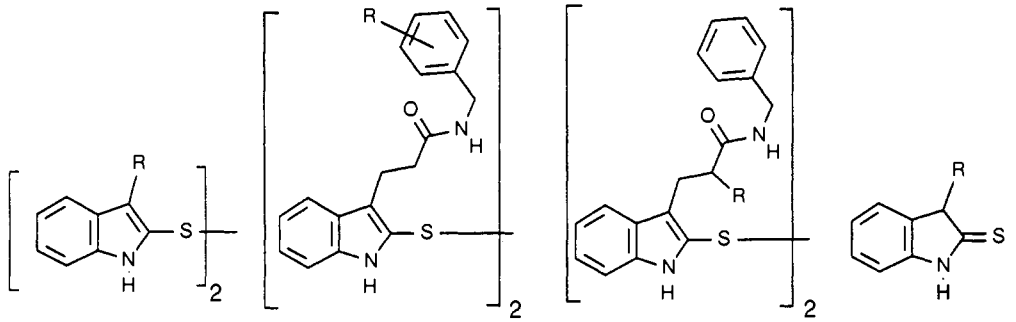
In a previous paper,[10] we reported the synthesis and structure–activity relationships for a novel class of inhibitors of the tyrosine kinase of the EGF receptor, the 2,3-dihydro-2-thioxo-1*H*-indole-3-alkanoic acids and their dimeric oxidation products, the 2,2'-dithiobis(1*H*-indole-3-alkanoic acids). These compounds appear to be noncompetitive inhibitors at the tyrosine substrate binding site.[11] The thiones were rapidly oxidized to the corresponding symmetrical disulfides, which were generally the more potent inhibitors. Within a small homologous series of 2,2'-dithiobis(1*H*-indole-3-alkanoic acids), activity was dependent on the length of the side chain, with the propanoic acid derivative (**6**) being the most potent inhibitor of the isolated enzyme obtained from A431 cells. The corresponding esters (e.g., **7**) were generally considerably less inhibitory toward the isolated enzyme but showed better cellular growth inhibition, possibly because of more efficient cell uptake.[10] In this paper we report the synthesis and structure–activity relationships of a series of amide analogues of **6** to see whether such neutral, hydrogen bond donor groups on the side chain can provide analogues with both potent enzyme inhibitory properties and cellular growth inhibition.



† University of Auckland School of Medicine.
‡ Warner-Lambert Company.
• Abstract published in *Advance ACS Abstracts*, February 1, 1994.

0022-2623/94/1837-0598$04.50/0

Table 1. Physicochemical Properties of 2,2′-Dithiobis(1*H*-indole-3-alkanamides) **10** and 2,3-Dihydro-2-thioxo-1*H*-indole-3-alkanamides **11**



A B C D

no.	type	R	mp (°C)	cryst solvent[a]	yield[b] (%)	formula	IC_{50} EGF-R[c]	IC_{50} SRC[d]
10a	A	$CH_2CONHCH_2Ph$	200.5–203.5	a, b	29	$C_{34}H_{30}N_4O_2S_2$	38	3.8
10b	A	CH_2CN[e]	168.5–170	h	52	$C_{20}H_{14}N_4S_2$	32	2.5
6	A	$(CH_2)_2COOH$[f]					4.8	51
7	A	$(CH_2)_2COOMe$[f]					21	3.5
10c	A	$(CH_2)_2CN$[e]	167–169	c	69	lit.[e] mp 165–167 °C	47	
10d	A	$(CH_2)_2NO_2$	153–154	d	73	$C_{20}H_{18}N_4O_4S_2 \cdot 0.5H_2O$	89	
10e	A	$(CH_2)_2CONH_2$	>101 dec	f	75	$C_{22}H_{22}N_4O_2S_2 \cdot 0.5H_2O$	16	1.5
10f	A	$(CH_2)_2CONHMe$	162.5–164	e	34	$C_{24}H_{26}N_4O_2S_2$	9.4	0.75
10g	A	$(CH_2)_2CONHOMe$	176–178	a	31	$C_{24}H_{26}N_4O_4S_2$	68	2.9
10h	A	$(CH_2)_2CONMe_2$	179–180	b	52	$C_{26}H_{30}N_4O_2S_2$	12	1.2
10i	A	$(CH_2)_2CONHPh$	114 dec	d	16[g]	$C_{34}H_{30}N_4O_2S_2 \cdot 0.5H_2O$	25	4.4
10j	A	$(CH_2)_2CONHCH_2Ph$	141–144	d	38	$C_{36}H_{34}N_4O_2S_2$	0.85	2.9
10k	B	4-COOMe	151–153	a	42	$C_{40}H_{38}N_4O_6S_2$	44	1.8
10l	B	4-COOH	136–139	f	26[h]	$C_{38}H_{34}N_4O_6S_2 \cdot H_2O$	7.4	1.5
10m	B	4-COOMe,3-OH	183–185	j	27[i]	$C_{40}H_{38}N_4O_8S_2$	ca. 100	ca. 100
10n	B	4-COOH,3-OH	160–163.5	f	27[h]	$C_{38}H_{34}N_4O_8S_2 \cdot H_2O$	8.5	
10o	A	$(CH_2)_2CONH(CH_2)_2Ph$	oil	–	61	$C_{38}H_{38}N_4O_2S_2$	24	14
10p	C	NHCOMe[j]	140–144	d	62[k]	$C_{40}H_{40}N_6O_4S_2 \cdot 0.5H_2O$	51	0.5
	C	NHCOMe[j]	154.5–157.5	a	62[k]	$C_{40}H_{40}N_6O_4S_2$		
10q	C	$NHCOCF_3$	160–164	i	44[l]	$C_{40}H_{34}F_6N_6O_4S_2 \cdot 0.5H_2O$	>100	0.7
10r	C	NH_2	147–150	d	22[m]	$C_{36}H_{38}N_6O_2S_2 \cdot 0.5H_2O$	7.6	1.5
10s	C	OAc	120–124	d	45[k]	$C_{40}H_{38}N_4O_6S_2$	28	
10t	C	OH	120–125	d	88[h]	$C_{36}H_{34}N_4O_4S_2$	14	
10u	A	$(CH_2)_3CONHCH_2Ph$	98.5–101	g, c	36	$C_{38}H_{38}N_4O_2S_2$	ca. 100	1.3
11a	D	$CH_2CONHCH_2Ph$	193–195	a	100[n]	$C_{17}H_{16}N_2OS$	ca. 100	3.2
11e	D	$(CH_2)_2CONH_2$	160–163	b	100[n]	$C_{11}H_{12}N_2OS$	22	2.0
11j	D	$(CH_2)_2CONHCH_2Ph$	149.5–151	c	100[n]	$C_{18}H_{18}N_2OS \cdot 0.5H_2O$	ca. 100	9.3

[a] Crystallizing solvents: a = EtOAc/petroleum ether; b = EtOAc; c = CH_2Cl_2; d = CH_2Cl_2/petroleum ether; e = EtOAc/benzene/petroleum ether; f = MeOH/dilute HCl; g = CH_2Cl_2/benzene; h = CH_2Cl_2/MeOH; i = EtOH; j = MeOH. [b] Percent yield overall from the indole amide after successive S_2Cl_2, $NaBH_4$, and H_2O_2 or $FeCl_3$ reactions, unless otherwise stated. [c,d] Values (μM) for the 50% inhibition respectively of the EGF receptor or the $pp60^{v\text{-}src}$ tyrosine kinases (see text). Values represent the mean of at least two separate and duplicate determinations. Variation in IC_{50}s between duplicate experiments was generally ±15%. [e] Reference 14. [f] Reference 10. [g] Via the acid disulfide; Scheme 3. [h] From hydrolysis of the corresponding, preceding ester. [i] Yield after successive S_2Cl_2, $NaBH_4$, H_2O_2, and $KHCO_3$ reactions (see text). [j] Diastereomer pair. [k] Combined yield for both diastereomer pairs. [l] Yield after S_2Cl_2 reaction only. [m] Yield from $NaBH_4$ reduction of **10q** and aerial oxidation (Scheme 2). [n] Percent yield obtained from purified disulfide (by $NaBH_4$ reduction).

Table 2. Effect of 2,2′-Dithiobis(1*H*-indole-3-alkanamides) on the Proliferation of Swiss 3T3 Mouse Fibroblasts

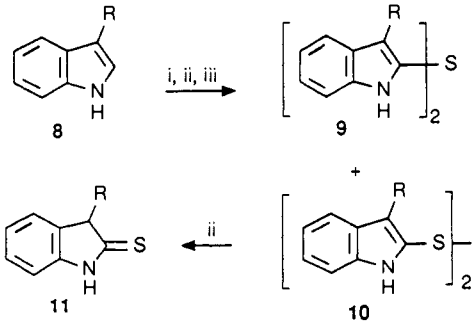
no.	IC_{50} (μM)[a]	no.	IC_{50} (μM)[a]
6	60	**10j**	5.9
7	7.4	**10r**	5.3
10a	2.7		

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

Chemistry

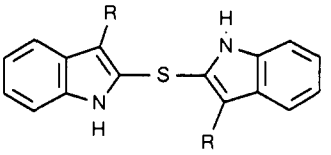
The 2,2′-dithiobis(1*H*-indole-3-alkanamides) (disulfides) (**10**) and some related 2,3-dihydro-2-thioxo-1*H*-indole-3-alkanamides (thiones) (**11**) (Table 1) were prepared by the synthetic methods outlined in Schemes 1–3. The disulfides were generally obtained by the treatment of 1*H*-indole-3-alkanamides **8** with freshly purified S_2Cl_2[12] in THF to give a mixture of corresponding mono-, di-, and trisulfides,[13,14] in which the disulfide is the major component (Scheme 1). This reaction, first applied by Wieland[15] to 3-alkyl- or 3-aryl-substituted indoles and to 1*H*-indole-3-acetic acid, has since been extended to various indole substrates, including tryptamine,[16] serotonin,[16]

Scheme 1[a]



[a] (i) S_2Cl_2/THF/0 °C; (ii) $NaBH_4$/EtOH/20 °C; (iii) H_2O_2 (or $FeCl_3$)/MeOH/20 °C.

Table 3. Physicochemical Properties of 2,2′-Thiobis(1*H*-indole-3-alkanamides)



no.	R	mp (°C)	cryst solvent[a]	yield[b] (%)	formula
9a	$CH_2CONHCH_2Ph$	222–225	b, c	22	$C_{34}H_{30}N_4O_2S$
9b	CH_2CN[c]	237–240	f	10	$C_{20}H_{14}N_4S{\cdot}0.5H_2O$
9c	$(CH_2)_2CN$[c]	204.5–207	d	4	lit.[c] mp 198–200 °C
9d	$(CH_2)_2NO_2$	134.5–136	d	3	$C_{20}H_{18}N_4O_4S$
9e	$(CH_2)_2CONH_2$	196.5–197.5	a	14	$C_{22}H_{22}N_4O_2S$
9f	$(CH_2)_2CONHMe$	120–123	b	16	$C_{24}H_{26}N_4O_2S{\cdot}C_8H_6$
9g	$(CH_2)_2CONHOMe$	157.5–158.5	a	11	$C_{24}H_{26}N_4O_4S$
9h	$(CH_2)_2CONMe_2$	189–190	a	14	$C_{26}H_{30}N_4O_2S$
9j	$(CH_2)_2CONHCH_2Ph$	218–219	d	6	$C_{36}H_{34}N_4O_2S{\cdot}0.5H_2O$
9k	$(CH_2)_2CONHCH_2Ph(4\text{-}COOMe)$	101–104.5	e	16	$C_{40}H_{38}N_4O_6S{\cdot}0.5H_2O$
9m	$(CH_2)_2CONHCH_2Ph(3\text{-}OH,4\text{-}COOMe)$	109–112	e	9[d]	$C_{40}H_{38}N_4O_6S$
9o	$(CH_2)_2CONH(CH_2)_2Ph$	120–121.5	a	23	$C_{38}H_{38}N_4O_2S$
9p	$CH_2CH(NHAc)CONHCH_2Ph$[e]	190–194	a	23	$C_{40}H_{40}N_6O_4S{\cdot}0.5H_2O$
9s	$CH_2CH(OAc)CONHCH_2Ph$[e]	105–109	e	13	$C_{40}H_{38}N_4O_6S$
9u	$(CH_2)_3CONHCH_2Ph$	105.5–108	d	10	$C_{38}H_{38}N_4O_2S$

[a] Crystallizing solvents: a = EtOAc/petroleum ether; b = EtOAc/petroleum ether/benzene; c = EtOAc/EtOH; d = CH_2Cl_2/petroleum ether; e = MeOH/dilute HCl; f = CH_2Cl_2. [b] Percent yield after successive S_2Cl_2, $NaBH_4$, and H_2O_2 (or $FeCl_3$) treatment. [c] Reference 14. [d] Yield after additional $KHCO_3$ hydrolysis. [e] Mixed diastereoisomers.

tryptophan,[17] and a 29 amino acid peptide containing trytophan.[18]

The crude product mixtures obtained from the S_2Cl_2 reaction were reduced with $NaBH_4$ to convert all polysulfides (except the monosulfide) into the corresponding thiones, as described previously.[10] Oxidation of this material with H_2O_2 (or $FeCl_3$) then gave a mixture of the disulfide (**10**) together with a small amount of the monosulfide **9**, which could be separated by chromatography and/or crystallization. Yields of the disulfides **10** varied from 30 to 75% and those of the corresponding monosulfides from 5 to 20% (Tables 1 and 3). Reduction of three of the purified disulfides **10a**, **10e**, and **10j** with $NaBH_4$ gave the corresponding pure thiones **11a**, **11e**, and **11j** in essentially quantitative yields (Scheme 1).

When successive S_2Cl_2, $NaBH_4$, and H_2O_2 reactions were performed on *N*-[[3-acetoxy-4-(methoxycarbonyl)phenyl]-methyl]-1*H*-indole-3-propanamide (**8m**), partial cleavage of the aromatic acetoxy group occurred. In this case, treatment of the crude product mixture with $KHCO_3$/aqueous methanol at 20 °C completed the selective hydrolysis of the acetoxy group. The resulting mixture of the corresponding 3-hydroxy monosulfide **9m** and disulfide **10m** were then separated in the usual manner. In contrast, the aliphatic acetoxy group of **8s** was stable toward successive S_2Cl_2, $NaBH_4$, and H_2O_2 reactions, enabling isolation of the α-acetoxy amide monosulfide **9s** and disulfide **10s**. However, the acetoxy group of **10s** was able to be readily cleaved by $KHCO_3$ in aqueous methanol at 20 °C to give the desired hydroxy amide **10t** in good yield. Hydrolysis of the aromatic methyl ester groups in **10k** and **10m** was achieved in moderate yield by treatment with K_2CO_3 in aqueous methanol at 50 °C under nitrogen.

The racemic α-substituted amides gave rise to mixtures of diastereoisomers which were separable in some cases by chromatography and/or crystallization. The purified disulfide diastereoisomers were stable as solids and racemized relatively slowly (over several hours at 20 °C) in nonpolar solvents such as CH_2Cl_2, $CHCl_3$, and EtOAc by disulfide exchange. However, in DMSO solution disulfide exchange was rapid, with racemization being complete within 3 min at 20 °C as shown by ^1H NMR spectroscopy.

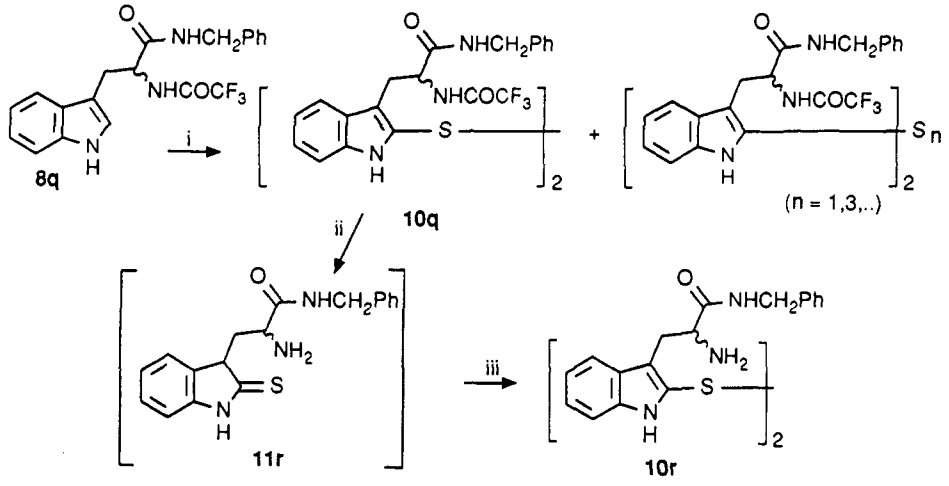
The α-(trifluoroacetyl)amino amide disulfide **10q** was obtained by direct chromatography of the S_2Cl_2 reaction products (Scheme 2). Reduction with $NaBH_4$ then gave the unstable α-amino amide thione **11r**, by cleavage of the trifluoroacetamide, as described by Weygand.[19] During the alkaline workup of this reaction, the thione **11r** was converted by aerial oxidation into the corresponding disulfide **10r**, which was purified by chromatography on alumina and crystallization.

Since the majority of compounds prepared were 2,2′-dithiobis(1*H*-indole-3-propanamides), an alternative route was investigated via amidation of 2,2′-dithiobis(1*H*-indole-3-propanoic acid) (**6**),[10] which was prepared from 1*H*-indole-3-propanoic acid (**12**) by successive treatment with S_2Cl_2, $NaBH_4$, and H_2O_2 as above (Scheme 3). However, addition of DEPC to a mixture of **6**, triethylamine, and aniline in THF at 0 °C[20] resulted in an immediate loss of the yellow color characteristic of the disulfides, suggesting a reaction of the coupling reagent with the disulfide bond. Treatment with aqueous base appeared to increase the proportion of disulfide present, but the desired 2,2′-dithiobis(*N*-phenyl-1*H*-indole-3-propanamide) (**10i**) was obtained in only 16% yield from a complex product mixture, following column chromatography on silica gel in two different solvent systems, and this route was not investigated further.

The starting 1*H*-indole-3-alkanamides (Table 4), required for Schemes 1–3, were prepared by the methods described in Schemes 4–8. The *N*-benzylamides **8a**, **8j**, and **8u** derived from the 1*H*-indole-3-alkanoic acids **12–14** were prepared in excellent yields by treating the corresponding methyl esters **15–17** with neat benzylamine at 140 °C, as described by Katritzky[21] (Scheme 4). Other amides of 1*H*-indole-3-propanoic acid (**12**) were prepared directly in good yield by treatment with the appropriate amine or amine hydrochloride in the presence of DEPC and triethylamine (Scheme 5).[20]

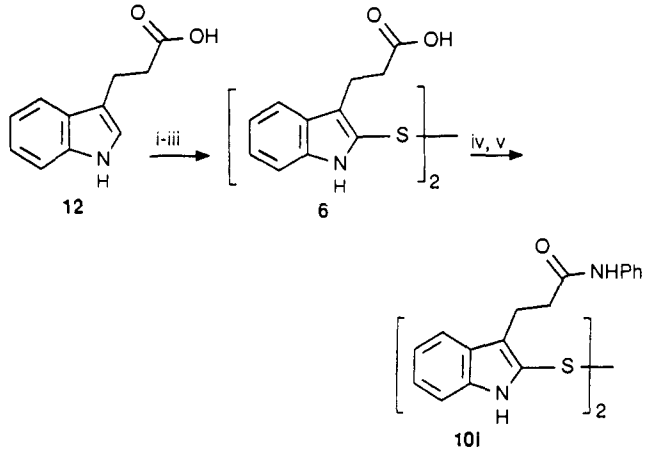
The *N*-[[4-(methoxycarbonyl)phenyl]methyl]- and *N*-[[3-hydroxy-4-(methoxycarbonyl)phenyl]methyl]a-

Scheme 2[a]



[a] (i) S_2Cl_2/THF/0 °C; (ii) $NaBH_4$/EtOH/20 °C; (iii) K_2CO_3/H_2O/20 °C.

Scheme 3[a]



[a] (i–iii) As for Scheme 1; (iv) $PhNH_2$/DEPC/Et_3N/THF/0–20 °C; (v) dilute KOH/20 °C.

mides (**8k** and **8v**, respectively) were prepared from reactions of **12** with methyl 4-(aminomethyl)benzoate hydrochloride[22] and methyl 4-(aminomethyl)-2-hydroxybenzoate hydrochloride (**20**), respectively. The latter amine was obtained from the reaction of methyl 2-acetoxy-4-(bromomethyl)benzoate (**18**) with hexamethylenetetramine, followed by hydrolysis of the aminal salt **19** with concentrated hydrochloric acid in methanol (Scheme 6), based on the procedure used by Meindl[23] to obtain the corresponding ethyl ester. The phenol group in the resulting amide (**8v**) was protected prior to the reaction with S_2Cl_2 by conversion into the acetate ester (using acetyl chloride and triethylamine in THF), giving derivative **8m** (Scheme 5).

Similarly, DL-*N*-acetyltryptophan (**24**) and DL-*N*-(trifluoroacetyl)tryptophan[24] (**25**) (the latter prepared *in situ* from DL-tryptophan (**22**), ethyl trifluoroacetate, and triethylamine in DMF[25]) were converted into the corresponding *N*-benzylamides according to Scheme 5. For these acids it was found that changing the solvent from THF to DMF significantly increased the yield of amide obtained (from 30% to 60% for DL-*N*-acetyltryptophan). However, when this method was applied to DL-α-acetoxy-1*H*-indole-3-propanoic acid (**23**) (prepared from DL-indole-3-lactic acid (**21**) using acetyl chloride and triethylamine in THF), the desired *N*-benzylamide (**8s**) was obtained in low yield (18%).

Recently, a novel, single-step method for the preparation of methyl *rac*-α-hydroxyindole-3-propanoate was reported, involving the $SnCl_4$-promoted coupling of indole with methyl *rac*-2,3-epoxypropanoate.[26] However, a similar reaction of indole with *rac*-*N*-benzyl-2,3-epoxypropanamide[27] (**26**) (Scheme 7) gave the expected α-hydroxy amide (**8t**) in only 5% yield after purification (7% based on recovered indole). The reaction temperature had little effect on the product distribution, except in the amount of indole recovered, and the identity of the remaining products could not by established by NMR spectroscopy. Acetylation of **8t** with pyridine/acetic anhydride at 20 °C gave a quantitative yield of **8s**, but the low yield in the previous step made this route less attractive.

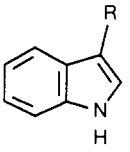
The previously-reported[14] sulfides and disulfides **9b**, **9c**, **10b**, and **10c**, derived from 1*H*-indole-3-acetonitrile (**8b**) and 1*H*-indole-3-propionitrile (**8c**), were also prepared for comparison with the primary amide **10e**. The 2-nitroethyl derivative **10d** was also prepared from 3-(2-nitroethyl)-1*H*-indole (**8d**),[28] which was unexpectedly obtained (instead of the α-nitro ester obtained by Lyttle[29]) from the reaction of gramine (**27**) with methyl nitroacetate (Scheme 8). This compound was of interest because the nitroethyl side chain is essentially isosteric with the propanoic acid side chain of **6**, but has different electronic and hydrogen bonding properties.

Results and Discussion

The compounds were assayed for their ability to inhibit the tyrosine kinase activity of both the EGF receptor and the product encoded by $pp60^{v\text{-}src}$. The EGF receptor was a native complex contained in plasma membrane vesicles shed from cultured A431 cells.[30] Inhibition of EGF-stimulated tyrosine kinase activity was measured as IC_{50} values (the concentration of drug necessary to reduce by 50% the incorporation of ^{32}P (from added [α-^{32}P]ATP) into a random copolymer of glutamate, alanine, and tyrosine used as the substrate. The $pp60^{v\text{-}src}$ oncogene product is a membrane-bound kinase lacking an extracellular domain, but closely-associated with a number of receptor tyrosine kinases, including that of the EGF receptor.[31] It plays an important role in signal transduction and is involved in a number of human malignant states.[32,33] For this work, it was incorporated in a baculovirus vector and expressed as described.[34]

A minimum of two independent and duplicate concentration–response curves were determined for each com-

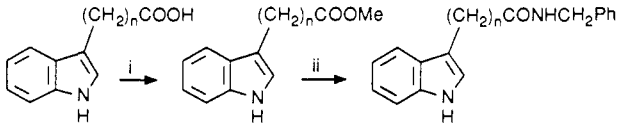
Table 4. Physicochemical Properties of 1*H*-Indole-3-alkanamides



no.	R	method[a]	mp (°C)	cryst. solvent[b]	yield (%)	formula	analyses/lit. mp (°C)
8a	$CH_2CONHCH_2Ph$[c]	A	152–153	a	80		lit.[21] mp 152.5–153.5
8d	$CH_2CH_2NO_2$[d]	D	57–59.5	d	48		lit.[28] mp 54–55
8e	$(CH_2)_2CONH_2$[e]	B	134–136	c	84		lit.[38] mp 131.5–133
8f	$(CH_2)_2CONHMe$[f]	B	97.5–99	a	69		lit.[37] mp 97–99
8g	$(CH_2)_2CONHOMe$[f]	B	116–118	a	62		lit.[37] mp 114–115
8h	$(CH_2)_2CONMe_2$[g]	B	141–142	a	76		lit.[41] mp 139–140.5
8j	$(CH_2)_2CONHCH_2Ph$[f]	A	125–126.5	b	88	$C_{18}H_{18}N_2O$	C,H,N
8k	$(CH_2)_2CONHCH_2Ph$(4-COOMe)	B	130–132	a	7	$C_{20}H_{20}N_2O_3$	C,H,N
8v	$(CH_2)_2CONHCH_2Ph$(3-OH,4-COOMe)	B	132–133	b	50	$C_{20}H_{20}N_2O_4$	C,H,N
8m	$(CH_2)_2CONHCH_2Ph$(3-OAc,4-COOMe)	B[h]	oil	–	94	$C_{22}H_{22}N_2O_5$	mass spectrum
8o	$(CH_2)_2CONH(CH_2)_2Ph$	B	88–89	b	54	$C_{19}H_{20}N_2O$	C,H,N
8p	$CH_2CH(NHAc)CONHCH_2Ph$	B	169–170	a	60	$C_{20}H_{21}N_3O_2$	C,H,N
8q	$CH_2CH(NHCOCF_3)CONHCH_2Ph$	B	181–183	b	50	$C_{20}H_{18}F_3N_3O_2$	C,H,N
8s	$CH_2CH(OAc)CONHCH_2Ph$	B[i]	oil	–	18	$C_{20}H_{20}N_2O_3$	mass spectrum
8t	$CH_2CH(OH)CONHCH_2Ph$	C	127–128.5	a	5	$C_{18}H_{18}N_2O_2 \cdot 0.25H_2O$	C,H,N
8u	$(CH_2)_3CONHCH_2Ph$	A	123–124	a	90	$C_{19}H_{20}N_2O$	C,H,N

[a] Methods (see Schemes 4–8): A, from acid via ester; B, from acid via DEPC coupling; C, from indole and epoxide; C, from gramine and methyl nitroacetate. [b] Crystallizing solvents: a = CH_2Cl_2/petroleum eher; b = EtOAc/petroleum ether; c = MeOH/H_2O; d = benzene/petroleum ether. [c] Reference 21. [d] Reference 28. [e] Reference 39. [f] Reference 38. [g] Reference 44. [h] By acetylation of 8v. [i] Acetylation of 8t gave a quantitative yield of 8s.

Scheme 4[a]

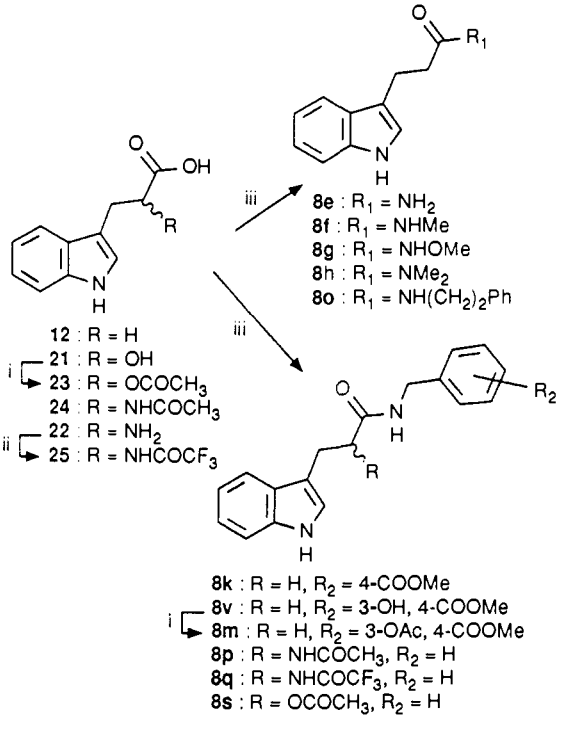


[a] (i) CH_2N_2/Et_2O/20 °C; (ii) $PhCH_2NH_2$/140 °C.

pound, and the results were averaged to produce the IC_{50} values recorded in Table 1. The previous study[10] showed that the highest inhibitory potency was seen with a free indole NH position and a side-chain length of three carbon atoms, with the propanoic acid **6** among the most active compounds seen. That this was also true for the corresponding amides is shown by comparing the homologous series of *N*-benzylamides **10a**, **10j** and **10u**; peak activity against the EGF receptor kinase (IC_{50} = 0.85 μM) is shown by the propanamide **10j**. As noted above, the amides discussed here were prepared primarily to explore the consequences of having a variety of neutral, hydrogen bond donor groups in the $(CH_2)_2CONHR$ side chain. Previous studies with nitrostyryl sulfonates have shown that modulation of sites in the inhibitor remote from the putative tyrosine mimic can have dramatic effects on inhibitory properties, possibly by recognizing further enzyme features such as bound metals.[35]

Compounds **10e–j** explore the consequences of varying N-substitution on the amide. There appears to be no requirement for hydrogen bond donor capability, since the primary, secondary, and tertiary amides **10e**, **10f**, and **10h**, respectively, differ little in their activity. However, the closely isosteric cyanoethyl and nitroethyl derivatives **10c** and **10d** and the *N*-methoxyamide (**10g**) are much less effective. The *N*-phenylamide **10i** also showed no improvement in activity, but the benzyl analogue **10j** was more than 10-fold more potent, with an IC_{50} for the EGF-receptor kinase of 0.85 μM. Extending the chain of this

Scheme 5[a]

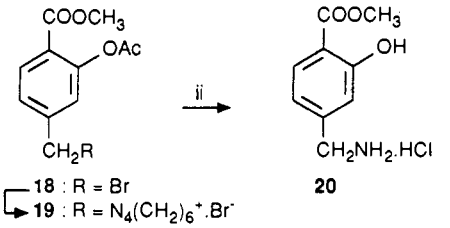


[a] (i) CH_3COCl/Et_3N/THF/0–20 °C; (ii) CF_3COOEt/Et_3N/DMF/20 °C; (iii) amine or amine hydrochloride/DEPC/Et_3N/THF or DMF/0–20 °C.

by one carbon atom to give the *N*-phenethylamide **10o** greatly reduced activity.

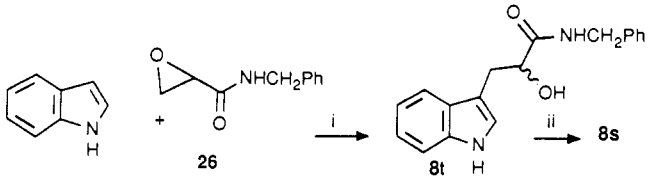
Compounds **10k–n** were prepared to study the effects of further substitution on the benzyl ring. The choice of substituents was guided both by previous studies of acid/ester pairs in the indolinethione series,[10] and by the structure–activity relationships observed in the nitrostyryl sulfonates.[35] Both ester derivatives **10k** and **10m** were very poor inhibitors, with IC_{50} values around 50–100 μM. This is somewhat surprising, given that these compounds

Scheme 6[a]



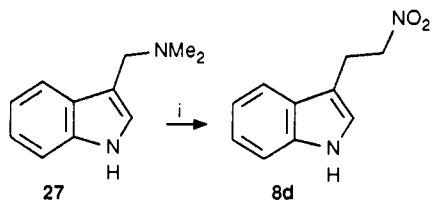
[a] (i) Hexamethylenetetramine/$CHCl_3$/reflux; (ii) concentrated HCl/MeOH/20 °C.

Scheme 7[a]



[a] (i) $SnCl_4/CCl_4$/−5 to 20 °C; (ii) Ac_2O/pyridine/20 °C.

Scheme 8[a]



[a] (i) O_2NCH_2COOMe/xylene/100 °C.

possess the requisite benzylpropanamide chain. As found previously,[10] the corresponding acids **10l** and **10n** were much more active than the esters, but there was no enhancement in potency for the 3-OH, 4-COOH derivative **10n**, as seen in the nitrostyryl sulfonates,[35] and both were less active than the parent **10j**.

Finally, compounds **10p–t** explore substitution in the α-position of the propanamide side chain. This was done primarily to improve water solubility, and the racemic α-NH_2 and α-OH compounds **10r** and **10t** were the primary goals, but the intermediate protected analogues were also evaluated. The *N*-acetate **10p** (from racemic DL-*N*-acetyltryptophan) was obtained as two separate pairs of diastereoisomers, but these equilibrated rapidly under physiological conditions (see above). Neither the *N*-acetate nor the *N*-trifluoroacetate **10q** were significantly active against EGF receptor kinase, but the corresponding racemic amine **10r** was an effective inhibitor of both the EGF receptor and $pp60^{v\text{-}src}$ kinases. However, both the *O*-acetate (**10s**) and the derived OH compound **10t** had only modest activity.

Since previous work[10] showed that the corresponding monomeric thiones of 2,2′-dithiobis(1*H*-indole-3-alkanoic acids) undergo rapid oxidative dimerisation, the monomers were not routinely tested. Those which were (**11a**, **11e**, and **11j**) showed generally lower inhibitory activities than the corresponding dimers (**10a**, **10e**, and **10j**). The dimeric monosulfide analogues **9** of the dithiobis compounds **10** (an inevitable byproduct of the S_2Cl_2 reaction) were also tested, but all were inactive (IC_{50} values > 100 μM; data not shown) as inhibitors of both the EGF receptor and $pp60^{v\text{-}src}$ tyrosine kinases, as expected from previous results.[10]

Inhibitors with high selectivity between different tyrosine kinase enzymes would be of value in studying the complex and interlocking pathways of phosphorylation-induced signal transduction in cells.[6] Therefore, the differential activity shown by some of these compounds between the two quite close-related[31] tyrosine kinases from the EGF receptor and the $pp60^{v\text{-}src}$ protein were of interest. Generally, the kinase activity of the $pp60^{v\text{-}src}$ protein proved more sensitive to the thioindoles than did that of the EGF receptor and was less modulated by structural differences. There was no difference with chain length among the homologous series of *N*-benzylamides (**10a**, **10j**, and **10u**), with all showing IC_{50}s of 1–4 μM. The different N-substituted propanamides **10e–j** also show similar and potent activity (below 5 μM). However, in contrast to the EGF receptor kinase, the $pp60^{v\text{-}src}$ kinase retains sensitivity to the ester (**10k**) (but not **10m**), and to the *N*-acetates (**10p** and **10q**), which show very low activity against the EGF receptor kinase.

Previously, we reported that ester-substituted derivatives were potent inhibitors of cellular growth in fibroblasts, whereas the corresponding free acids were much less inhibitory[10] (e.g., compounds **6** and **7**; Table 2). The alkanamides showed similar activity to the esters; the *N*-benzylamides **10a**, **10j**, and **10r** inhibited the growth of Swiss 3T3 fibroblasts with IC_{50}s in the range 2.7–5.9 μM (Table 2).

The effects of selected analogues on intracellular tyrosine phosphorylation was studed in Swiss 3T3 fibroblasts, by pretreating them with varying concentrations of compounds **10j** or **10r** for 2 h and then exposing them to different growth factors. Intracellular phosphotyrosine was assessed by western blotting with antiphosphotyrosine antibodies. Typically, when Swiss 3T3 cells are exposed to bFGF, a protein of approximately 85 kDa is phosphorylated on tyrosine. Both **10j** and **10r** inhibited the phosphorylation of this protein in a concentration-dependent manner, with IC_{50} values of 14 and 3 μM, respectively. Compound **10r** inhibited PDGF receptor autophosphorylation with an IC_{50} of 6 μM, whereas **10j** had no effect on this receptor. Surprisingly, neither compound inhibited EGF receptor autophosphorylation in cells. These results indicate that side-chain composition in this class of compounds influences inhibitory specificity among different kinases.

Conclusions

Reaction of 1*H*-indole-3-alkanamides **8** with S_2Cl_2 is an efficient route to the desired disulfides **10**. Although the reactions give mixtures of the mono-, di-, and trisulfides, the disulfide is the major component. Treatment of the crude product mixture successively with $NaBH_4$ then H_2O_2 (or $FeCl_3$) gave mixtures of the monosulfides **9** and the disulfides **10**, from which the latter could be separated by chromatography and/or crystallization in 30–75% yield. As with the previous series of acids and esters,[10] the inhibitory activity of the disulfides against EGF receptor tyrosine kinase was chain-length dependent, with the propanamides being the most effective. Hydrogen bond donor capabilities in the amide function did not appear to be necessary, with the *N*-benzylamide **10j** being the most potent inhibitor of EGF receptor tyrosine kinase (IC_{50} = 0.85 μM). However, further substitution on the benzyl ring of **10j** did not increase potency. Substitution in the α-position of the propanamide side chain of **10j** was acceptable and allowed the preparation of water-soluble derivative **10r**, which shows good inhibitory activity and

is undergoing *in vivo* evaluation. The nonreceptor kinase $pp60^{v\text{-}src}$ was in general much more sensitive than EGF receptor kinase to inhibition by these compounds, but with less pronounced structure–activity relationships. However, some individual compounds (e.g., **10g**, **10k**, **10p**, **10q**, and **10u**) showed strikingly different levels of effectiveness against these two enzymes. The *N*-benzylamides were potent inhibitors of cell growth, being comparable to the previously reported esters and much more potent than the free acids. They also selectively inhibit intracellular phosphorylation induced by different growth factors.

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. Melting points were determined using an Electrothermal Model 9200 digital melting point apparatus and are reported as read. NMR spectra were determined on a Bruker AM-400 spectrometer (Me_4Si). Mass spectra were recorded on a Varian VG 7070 spectrometer at nominal 5000 resolution.

Methyl 4-(Aminomethyl)-2-hydroxybenzoate Hydrochloride (20) (Scheme 6). A stirred solution of methyl 2-acetoxy-4-(bromomethyl)benzoate (18)[36] (10.7 g, 37 mmol) and hexamethylenetetramine (17.1 g, 122 mmol) in $CHCl_3$ (150 mL) was heated under reflux for 5 h, and then the solvent was removed under reduced pressure.[23] The residue of crude **19** was stirred with MeOH (60 mL) and concentrated HCl (30 mL) at 20 °C for 10 min, and the solvents were then removed under reduced pressure. The solid residue was similarly treated twice more with HCl/MeOH and then washed with CH_2Cl_2 and dissolved in saturated $KHCO_3$ solution. The base was back-extracted with EtOAc and CH_2Cl_2, and the resulting crude base was dissolved in Et_2O. Treatment with HCl gas gave the crude (ca. 70%) 4-(aminomethyl)-2-hydroxybenzoate hydrochloride (**20**) (5.30 g). A sample of the above base was purified by chromatography on silica gel, eluting with EtOAc/petroleum ether (1:2). Acidification gave pure **20**: mp (CH_2Cl_2/petroleum ether) 225–227 °C; ^{1}H NMR ($(CD_3)_2SO$) δ 10.56 (s, 1 H, OH), 8.58 (br s, 3 H, NH_3^+), 7.78 (d, J = 8.1 Hz, 1 H, H-6), 7.14 (s, 1 H, H-3), 7.05 (d, J = 8.1 Hz, 1 H, H-5), 4.01 (br s, 2 H, 4-CH_2), 3.88 (s, 3 H, OCH_3); ^{13}C NMR δ 168.81 (s, $COOCH_3$), 159.80 (s, C-2), 141.84 (s, C-4), 130.25 (d, C-6), 119.61 (d, C-5), 117.48 (d, C-3), 112.90 (s, C-1), 52.53 (q, OCH_3), 41.63 (t, 4-CH_2). Anal. ($C_9H_{11}NO_3$·HCl·0.5H_2O) C, H, N, Cl.

3-(2-Nitroethyl)-1*H*-indole (8d) (Scheme 8). A solution of gramine (**27**) (8.4 g, 48 mmol) and methyl nitroacetate (11.5 g, 97 mmol) in xylene (50 mL) was stirred under nitrogen at 90–100 °C for 5 h.[29] Evaporation gave an oil which was chromatographed on silica gel, eluting with CH_2Cl_2/petroleum ether (1:1), to give 3-(2-nitroethyl)-1*H*-indole (**8d**) (4.44 g, 48%): mp (benzene/petroleum ether) 57–59.5 °C (lit.[28] mp (MeOH) 54–55 °C); ^{1}H NMR ($CDCl_3$) δ 8.05 (br s, 1 H, NH), 7.57 (d, J = 7.9 Hz, 1 H, ArH), 7.37 (dt, J = 8.2, 0.8 Hz, 1 H, ArH), 7.22 (ddd, J = 8.1, 7.0, 1.1 Hz, 1 H, ArH), 7.16 (ddd, J = 7.9, 7.1, 0.9 Hz, 1 H, ArH), 7.04 (d, J = 2.4 Hz, 1 H, H-2), 4.66 (t, J = 7.3 Hz, 2 H, 3-CH_2CH_2), 3.49 (td, J = 7.3, 0.6 Hz, 2 H, 3-CH_2); ^{13}C NMR δ 136.25, 126.67 (2 s, Ar), 122.56, 122.54, 119.91, 118.13, 111.45 (5 d, Ar), 110.05 (s, Ar), 75.73 (t, 3-CH_2CH_2), 23.63 (t, 3-CH_2).

Preparation of *N*-(Phenylmethyl)-1*H*-indole-3-propanamide (8j): Example of Scheme 4. A suspension of 1*H*-indole-3-propanoic acid (**12**) (1.50 g) in Et_2O was treated with a solution of excess diazomethane in Et_2O for 1 h at 20 °C to give methyl 1*H*-indole-3-propanoate (**16**) (1.62 g, 100%), mp 81–82 °C (lit.[37] mp 79–80 °C). This was stirred with excess benzylamine (5 mL) at 140 °C for 4 h, dilute HCl (0.5 M, 100 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 100 mL). Evaporation followed by chromatography of the residue on silica gel, eluting with CH_2Cl_2 and then EtOAc, gave *N*-(phenylmethyl)-1*H*-indole-3-propanamide (**8j**) (1.81 g, 88%): mp (EtOAc/petroleum ether) 125–126.5 °C (lit.[38] mp 121 °C); ^{1}H NMR ($CDCl_3$) δ 8.05 (s, 1 H, NH), 7.59 (d, J = 7.8 Hz, 1 H, ArH), 7.34 (d, J = 7.9 Hz, 1 H, ArH), 7.24 (m, 3 H, ArH), 7.18 (dd, J = 7.9, 7.2 Hz, 1 H, ArH), 7.10 (dd, J = 7.9, 7.2 Hz, 1 H, ArH), 7.07 (m, 2 H, ArH), 6.93 (d, J = 1.9 Hz, 1 H, H-2), 5.64 (t, J = 5.7 Hz, 1 H, N*H*CH_2), 4.35 (d, J = 5.7 Hz, 2 H, NHCH_2), 3.13, 2.59 (2 t, J = 7.3 Hz, 2 × 2 H, 3-CH_2CH_2); ^{13}C NMR δ 172.54 (s, CONH), 138.20, 136.35 (2 s, Ar), 128.58, 127.66 (2 d, 2 × 2 C, Ar), 127.35 (d, Ar), 127.08 (s, Ar), 122.04, 121.88, 119.35, 118.68 (4 d, Ar), 113.79 (s, Ar), 111.21 (d, Ar), 43.51 (t, $NHCH_2$), 37.42 (t, 3-CH_2CH_2), 21.38 (t, 3-CH_2). Anal. ($C_{18}H_{18}N_2O$) C, H, N.

1*H*-Indole-3-propanamide (8e) (Scheme 5). DEPC (1.28 mL of 98%, 8.3 mmol) was added to a stirred solution of 1*H*-indole-3-propanoic acid (**12**) (1.30 g, 6.9 mmol) and Et_3N (1.15 mL, 8.3 mmol) in THF (15 mL) at 0 °C.[20] After 5 min the solution was saturated with ammonia gas and then stirred at 20 °C for 16 h. The reaction was quenched with water and extracted with EtOAc. Evaporation of solvent and chromatography of the product on silica gel, eluting with EtOAc, gave 1*H*-indole-3-propanamide (**8e**) (1.09 g, 84%): mp (MeOH/water) 134–136 °C (lit.[39] mp 131.5–133 °C); ^{1}H NMR ($(CD_3)_2CO$) δ 9.95 (s, 1 H, NH), 7.58 (dt, J = 8.2, 0.7 Hz, 1 H, ArH), 7.36 (dt, J = 8.1, 0.8 Hz, 1 H, ArH), 7.13 (m, 1 H, H-2), 7.08 (ddd, J = 8.1, 7.0, 1.1 Hz, 1 H, ArH), 7.00 (ddd, J = 8.0, 7.0, 1.0 Hz, 1 H, ArH), 6.75, 6.12 (2 br s, 2 × 1 H, $CONH_2$), 3.04 (m, 2 H, 3-CH_2), 2.05 (m, 2 H, 3-CH_2CH_2); ^{13}C NMR δ 174.87 (s, $CONH_2$), 137.75, 128.44 (2 s, Ar), 122.80, 122.02 (2 d, Ar), 119.30 (2 d, Ar), 115.67 (s, Ar), 112.08 (d, Ar), 37.05 (t, 3-CH_2CH_2), 21.87 (t, 3-CH_2).

Similar reactions of 1*H*-indole-3-propanoic acid (**12**), methyl 1*H*-indole-3-acetate (**15**),[21] or methyl 1*H*-indole-3-butanoate (**17**)[40] (obtained from the acid (**14**) using diazomethane) with commercial amines, methyl 4-(aminomethyl)benzoate hydrochloride,[22] or **20** gave the corresponding 1*H*-indole-3-alkanamides whose physicochemical properties are given in Table 4 (see supplementary material for details of ^{1}H and ^{13}C NMR spectra). For amine hydrochlorides, method B was modified by adding a second mole equivalent of Et_3N and stirring the mixture for 0.5–3 h at 20 °C prior to the addition of DEPC at 0 °C.

***N*-[[3-Acetoxy-4-(methoxycarbonyl)phenyl]methyl]-1*H*-indole-3-propanamide (8m).** A solution of CH_3COCl (0.42 mL, 5.9 mmol) in THF (5 mL) was added to a stirred solution of *N*-[[3-hydroxy-4-(methoxycarbonyl)phenyl]methyl]-1*H*-indole-3-propanamide (**8v**) (1.22 g, 3.5 mmol) and Et_3N (1.00 mL, 7.2 mmol) in THF (15 mL) at 0 °C. The mixture was stirred at 20 °C for 18 h, quenched with water (100 mL), and extracted with EtOAc (3 × 100 mL). Evaporation of solvent and chromatography of the residue on silica gel, eluting with EtOAc/petroleum ether (2:1), gave *N*-[[3-acetoxy-4-(methoxycarbonyl)phenyl]methyl]-1*H*-indole-3-propanamide (**8m**) (1.28 g, 94%) as an oil: ^{1}H NMR ($CDCl_3$) δ 8.18 (br s, 1 H, NH), 7.87 (d, J = 8.1 Hz, 1 H, ArH), 7.57 (d, J = 8.0 Hz, 1 H, ArH), 7.31 (dt, J = 8.1, 0.8 Hz, 1 H, ArH), 7.17 (ddd, J = 8.1, 7.0, 1.1 Hz, 1 H, ArH), 7.09 (ddd, J = 7.9, 7.0, 0.9 Hz, 1 H, ArH), 6.97 (dd, J = 8.1, 1.6 Hz, 1 H, ArH), 6.84 (d, J = 1.5 Hz, 1 H, ArH), 6.77 (d, J = 2.3 Hz, 1 H, H-2), 5.67 (br t, J = 5.8 Hz, 1 H, N*H*CH_2), 4.31 (d, J = 6.0 Hz, 2 H, NHCH_2), 3.87 (s, 3 H, $COOCH_3$), 3.11, 2.58 (2 t, J = 6.9 Hz, 2 × 2 H, 3-CH_2CH_2), 2.36 (s, 3 H, $OCOCH_3$); ^{13}C NMR δ 172.84 (s, CONH), 170.14 (s, $OCOCH_3$), 164.64 (s, $COOCH_3$), 150.82, 145.26, 136.33 (3 s, Ar), 132.04 (d, Ar), 126.85 (s, Ar), 125.42, 122.93, 122.31, 121.95 (4 d, Ar), 121.87 (s, Ar), 119.28, 118.52 (2 d, Ar), 114.08 (s, Ar), 111.36 (d, Ar), 52.23 (q, OCH_3), 42.62 (t, $NHCH_2$), 37.32 (t, 3-CH_2CH_2), 21.46 (t, 3-CH_2), 21.06 (q, $OCOCH_3$); HREIMS m/z calcd for $C_{22}H_{22}N_2O_5$ 394.1529 (M^+), found 394.1526.

(*R,S*)-α-(Acetylamino)-*N*-(phenylmethyl)-1*H*-indole-3-propanamide (8p). A stirred solution of DL-*N*-acetyltryptophan (**24**) (1.00 g, 4.1 mmol) and benzylamine (2.00 mL, 18.3 mmol) in DMF (10 mL) was treated with DEPC (0.72 mL of 98%, 4.7 mmol) at 0 °C. The mixture was stirred at 20 °C for 16 h, quenched with water, and extracted with EtOAc. Evaporation gave an oil which was chromatographed on silica gel. Elution with CH_2Cl_2 and EtOAc gave firstly foreruns, and then (*R,S*)-α-(acetylamino)-*N*-(phenylmethyl)-1*H*-indole-3-propanamide (**8p**) (0.82 g, 60%): mp (CH_2Cl_2/petroleum ether) 169–170 °C; ^{1}H NMR ($(CD_3)_2SO$) δ 10.80 (s, 1 H, NH), 8.47 (br t, J = 5.8 Hz, 1 H, N*H*CH_2), 8.08 (d, J = 8.1 Hz, 1 H, CHN*H*), 7.61 (d, J = 7.8 Hz, 1 H, ArH), 7.33 (d, J = 8.1 Hz, 1 H, ArH), 7.26 (dt, J = 7.1, 1.5 Hz, 2 H, ArH), 7.20 (dt, J = 7.2, 1.5 Hz, 1 H, ArH), 7.13 (m, 1 H, H-2), 7.12 (d, J = 7.2 Hz, 2 H, ArH), 7.06 (ddd, J = 7.9, 7.1, 0.9 Hz, 1 H, ArH), 6.97 (ddd, J = 7.9, 7.0, 0.9 Hz, 1 H, ArH), 4.57 (td, J = 8.3, 5.7 Hz, 1 H, 3-CH_2CH), 4.28, 4.24 (2 dd, J = 15.9,

5.9 Hz, 2 × 1 H, NHCH_2), 3.13 (dd, J = 14.4, 5.6 Hz, 1 H, 3-CH), 2.93 (dd, J = 14.4, 8.6 Hz, 1 H, 3-CH), 1.80 (s, 3 H, $COCH_3$); ^{13}C NMR δ 171.59 (s, $COCH_3$), 169.02 (s, CONH), 139.18, 135.99 (2 s, Ar), 128.06 (d, 2 C, Ar), 127.21 (s, Ar), 126.87 (d, 2 C, Ar), 126.49, 123.47, 120.75, 118.39, 118.10, 111.17 (6xd, Ar), 110.11 (s, Ar), 53.53 (d, CH), 41.91 (t, $NHCH_2$), 27.92 (t, 3-CH_2), 22.50 (q, CH_3). Anal. ($C_{20}H_{21}N_3O_2$) C, H, N.

Acidification of the aqueous portion with dilute HCl, extraction with EtOAc, and evaporation gave recovered DL-*N*-acetyltryptophan (0.30 g, 30%), mp (EtOAc/petroleum ether) 204–206 °C.

(*R,S*)-*N*-(Phenylmethyl)-α-[(trifluoroacetyl)amino]-1*H*-indole-3-propanamide (8q). Ethyl trifluoroacetate (1.7 mL, 14.3 mmol) was added to a stirred solution of DL-tryptophan (2.3 g, 11.3 mmol) and Et_3N (1.6 mL, 11.5 mmol) in DMF (5 mL), the flask was sealed and purged with nitrogen, and the mixture was stirred at 20 °C for 1 day.[25] Excess reagents were removed under reduced pressure, Et_3N (1.9 mL, 13.6 mmol) and DMF (10 mL) were added, and the mixture was cooled to 0 °C. DEPC (2.0 mL of 98%, 12.9 mmol) was added, followed by benzylamine (1.72 mL, 15.7 mmol), and the mixture was stirred under nitrogen at 20 °C for 24 h. The resulting solution was diluted with water (100 mL) and extracted with EtOAc (3 × 100 mL). Evaporation gave an oil which was purified by chromatography on silica gel, eluting with EtOAc/petroleum ether (1:1), to give (*R,S*)-*N*-(phenylmethyl)-α-[(trifluoroacetyl)amino]-1*H*-indole-3-propanamide (8q) (2.21 g, 50%): mp (EtOAc/petroleum ether) 181–183 °C; ^1H NMR ($(CD_3)_2SO$) δ 10.84 (s, 1 H, NH), 9.65 (br s, 1 H, CHNH), 8.79 (t, J = 5.5 Hz, 1 H, $NHCH_2$), 7.67 (d, J = 7.8 Hz, 1 H, ArH), 7.34 (d, J = 8.0 Hz, 1 H, ArH), 7.30 (t, J = 7.2 Hz, 2 H, ArH), 7.23 (t, J = 7.3 Hz, 1 H, ArH), 7.18 (d, J = 7.5 Hz, 2 H, ArH), 7.15 (d, J = 2.2 Hz, 1 H, H-2), 7.07 (ddd, J = 8.0, 7.1, 0.9 Hz, 1 H, ArH), 6.98 (dd, J = 7.8, 7.0 Hz, 1 H, ArH), 4.63 (br m, 1 H, 3-CH_2CH), 4.32 (d, J = 5.8 Hz, 2 H, $NHCH_2$), 3.25 (dd, J = 14.5, 5.0 Hz, 1 H, 3-CH), 3.12 (dd, J = 14.5, 9.9 Hz, 1 H, 3-CH); ^{13}C NMR δ 169.89 (s, CONH), 156.14 (q, J_{CF} = 36.5 Hz, $COCF_3$), 138.92, 135.97 (2 s, Ar), 128.17, 126.95 (2 d, 2 × 2 C, Ar), 126.95 (s, Ar), 126.68, 123.77, 120.86, 118.36, 118.17 (5 d, Ar), 115.69 (q, J_{CF} = 288 Hz, CF_3), 111.24 (d, Ar), 109.41 (s, Ar), 54.24 (d, 3-CH_2CH), 42.11 (t, $NHCH_2$), 27.08 (t, 3-CH_2). Anal. ($C_{20}H_{18}F_3N_3O_2$) C, H, N.

Acidification of the aqueous portion with dilute HCl, extraction with EtOAc (3 × 100 mL), and evaporation gave (*R,S*)-α-[(trifluoroacetyl)amino]-1*H*-indole-3-propanoic acid (25) (0.72 g, 21%): mp (water) 155–157 °C (lit.[24] mp 162–163 °C); ^1H NMR ($(CD_3)_2SO$) 10.86 (br s, 1 H, NH), 9.75 (br d, J = 8.0 Hz, 1 H, CHNH), 7.55 (d, J = 7.8 Hz, 1 H, ArH), 7.34 (d, J = 8.1 Hz, 1 H, ArH), 7.14 (d, J = 2.3 Hz, 1 H, H-2), 7.07 (ddd, J = 8.0, 7.1, 0.9 Hz, 1 H, ArH), 6.99 (ddd, J = 7.9, 7.0, 0.9 Hz, 1 H, ArH), 4.51 (ddd, J = 10.2, 8.0, 4.2 Hz, 1 H, 3-CH_2CH), 3.32 (dd, J = 14.8, 4.3 Hz, 1 H, 3-CH), 3.17 (dd, J = 14.8, 10.3 Hz, 1 H, 3-CH); ^{13}C NMR δ 171.64 (s, COOH), 156.23 (q, J_{CF} = 36.5 Hz, $COCF_3$), 136.01, 126.85 (2 s, Ar), 123.45, 120.93, 118.35, 117.90 (4 d, Ar), 115.66 (q, J_{CF} = 288 Hz, CF_3), 111.36 (d, Ar), 109.56 (s, Ar), 53.58 (d, 3-CH_2CH), 25.88 (t, 3-CH_2).

(*R,S*)-α-Acetoxy-*N*-(phenylmethyl)-1*H*-indole-3-propanamide (8s). (i) From DL-1*H*-Indole-3-lactic Acid (21) (Scheme 5). CH_3COCl (0.50 mL, 7.0 mmol) was added to a stirred solution of DL-1*H*-indole-3-lactic acid (21) (1.00 g, 4.9 mmol) and Et_3N (2 mL, 14.3 mmol) in THF (5 mL) at 0 °C. The mixture was stirred at 0 °C for 7 h and then at 20 °C for 15 h, quenched with water (100 mL), acidified with dilute HCl (to pH 2), and then extracted with EtOAc (3 × 100 mL). Evaporation gave crude (ca. 90%) (*R,S*)-α-acetoxy-1*H*-indole-3-propanoic acid (23) (1.30 g) as an oil which was used directly: ^1H NMR ($(CD_3)_2SO$) δ 10.88 (s, 1 H, NH), 7.54 (d, J = 7.8 Hz, 1 H, ArH), 7.33 (d, J = 8.0 Hz, 1 H, ArH), 7.17 (br s, 1 H, H-2), 7.06 (dd, J = 8.0, 7.1 Hz, 1 H, ArH), 6.99 (t, J = 7.4 Hz, 1 H, ArH), 5.06 (dd, J = 7.3, 4.9 Hz, 1 H, 3-CH_2CH), 3.22 (dd, J = 15.1, 4.5 Hz, 1 H, 3-CH), 3.16 (dd, J = 15.0, 7.7 Hz, 1 H, 3-CH), 2.00 (s, 3 H, $COCH_3$); ^{13}C NMR δ 170.87, 169.96 (2 s, COOH, $OCOCH_3$), 136.04, 127.28 (2 s, Ar), 123.84, 120.94, 118.43, 118.33, 111.39 (5 d, Ar), 108.90 (s, Ar), 72.70 (d, 3-CH_2CH), 26.75 (t, 3-CH_2), 20.54 (q, CH_3); HREIMS m/z calcd for $C_{13}H_{13}NO_4$ 247.0845 (M^+), found 247.0848.

The above crude α-*O*-acetate (23) (1.30 g of 90%, 4.4 mmol) and Et_3N (0.88 mL, 6.3 mmol) in DMF (10 mL) at 0 °C was treated sequentially with DEPC (0.91 mL of 98%, 5.9 mmol) and benzylamine (0.69 mL, 6.3 mmol), and the mixture was stirred under nitrogen at 20 °C for 18 h. Workup and chromatography on silica gel, eluting with EtOAc/petroleum ether (1:2 then 1:1), gave (*R,S*)-α-acetoxy-*N*-(phenylmethyl)-1*H*-indole-3-propanamide (8s) (0.29 g, 18%) as an oil: ^1H NMR ($CDCl_3$) δ 8.05 (s, 1 H, NH), 7.60 (d, J = 7.9 Hz, 1 H, ArH), 7.37 (dt, J = 8.1, 0.9 Hz, 1 H, ArH), 7.26–7.21 (m, 3 H, ArH), 7.20 (ddd, J = 8.1, 7.0, 1.1 Hz, 1 H, ArH), 7.12 (ddd, J = 8.0, 7.0, 1.0 Hz, 1 H, ArH), 6.97 (d, J = 2.4 Hz, 1 H, H-2), 6.94 (m, 2 H, ArH), 6.07 (t, J = 5.8 Hz, 1 H, $NHCH_2$), 5.47 (t, J = 5.4 Hz, 1 H, 3-CH_2CH), 4.38 (dd, J = 14.9, 6.1 Hz, 1 H, NHCH), 4.29 (dd, J = 14.9, 5.5 Hz, 1 H, NHCH), 3.41 (d, J = 5.5 Hz, 2 H, 3-CH_2), 2.06 (s, 3 H, $COCH_3$); ^{13}C NMR δ 169.63, 169.33 (2 s, CONH, $OCOCH_3$), 137.56, 136.05 (2 s, Ar), 128.55 (d, 2 C, Ar), 127.75 (s, Ar), 127.60 (d, 2 C, Ar), 127.40, 123.43, 122.08, 119.61, 118.92, 111.13 (6 d, Ar), 109.83 (s, Ar), 74.56 (d, 3-CH_2CH), 43.12 (t, $NHCH_2$), 27.42 (t, 3-CH_2), 21.09 (q, CH_3); HREIMS m/z calcd for $C_{20}H_{20}N_2O_3$ 336.1474 (M^+), found 336.1471.

Acidification of the aqueous portion with dilute HCl, extraction with EtOAc, and evaporation gave an oil. Chromatography on silica gel, eluting with EtOAc/petroleum (2:1) containing 1% AcOH, gave unreacted (*R,S*)-α-acetoxy-1*H*-indole-3-propanoic acid (0.68 g, 52%).

(ii) Via the α-Hydroxy Compound (8t) (Scheme 7). A solution of $SnCl_4$ (5.4 mL, 46 mmol) in CCl_4 (50 mL) was added dropwise to a stirred solution of 1*H*-indole (5.4 g, 46 mmol) and 2,3-epoxy-*N*-(phenylmethyl)propanamide (26)[27] (14 g of ca. 85%, 67 mmol) in CCl_4 (100 mL) at −5 °C.[26] The mixture was stirred at 20 °C for 16 h, diluted with $CHCl_3$ (100 mL) and 10% $NaHCO_3$ (250 mL), and stirred vigorously for 4 h. The aqueous portion was separated and extracted with CH_2Cl_2 (2 × 100 mL), and the combined organic extracts were washed with water and dried and the solvents removed. The resulting oil was chromatographed on silica gel, eluting with CH_2Cl_2/petroleum ether (1:1) to yield unreacted 1*H*-indole (1.27 g, 24%). Elution with CH_2Cl_2 gave a mixture of uncharacterized products, and elution with CH_2Cl_2/EtOAc (4:1) gave material which was crystallized successively from CH_2Cl_2/petroleum ether and then CH_2Cl_2/benzene/petroleum ether to give (*R,S*)-α-hydroxy-*N*-(phenylmethyl)-1*H*-indole-3-propanamide (8t) (0.70 g, 5%): mp 127–128.5 °C; ^1H NMR ($(CD_3)_2SO$) δ 10.79 (s, 1 H, NH), 8.20 (t, J = 6.2 Hz, 1 H, $NHCH_2$), 7.56 (d, J = 7.8 Hz, 1 H, ArH), 7.34 (d, J = 8.1 Hz, 1 H, ArH), 7.24 (m, 2 H, ArH), 7.19 (m, 1 H, ArH), 7.12 (d, J = 2.3 Hz, 1 H, H-2), 7.10 (m, 1 H, ArH), 7.05 (ddd, J = 8.0, 7.0, 1.0 Hz, 1 H, ArH), 6.96 (ddd, J = 7.9, 7.0, 0.9 Hz, 1 H, ArH), 5.54 (d, J = 5.7 Hz, 1 H, OH), 4.26 (d, J = 6.2 Hz, 2 H, $NHCH_2$), 4.19 (ddd, J = 7.5, 5.7, 4.3 Hz, 1 H, 3-CH_2CH), 3.14 (dd, J = 14.5, 4.1 Hz, 1 H, 3-CH), 2.91 (dd, J = 14.5, 7.6 Hz, 1 H, 3-CH); ^{13}C NMR δ 173.59 (s, CONH), 139.40, 135.93 (2 s, Ar), 128.00 (d, 2 C, Ar), 127.60 (s, Ar), 126.95 (d, 2 C, Ar), 126.42, 123.58, 120.56, 118.60, 117.97, 111.05 (6 d, Ar), 110.53 (s, Ar), 71.86 (d, 3-CH_2CH), 41.60 (t, $NHCH_2$), 30.33 (t, 3-CH_2). Anal. ($C_{18}H_{18}N_2O_2 \cdot 0.25H_2O$) C, H, N.

Acetylation of 8t (0.62 g) in pyridine (1.5 mL) with Ac_2O (1.7 mL) at 20 °C for 17 h, followed by dilution with water and extraction of the mixture with CH_2Cl_2 (3 × 100 mL), gave a quantitative yield of (*R,S*)-α-acetoxy-*N*-(phenylmethyl)-1*H*-indole-3-propanamide (8s), identical with that prepared above.

Preparation of 2,2′-Thiobis(1*H*-indole-3-propanamide) (9e), 2,2′-Dithiobis(1*H*-indole-3-propanamide) (10e), and 2,3-Dihydro-2-thioxo-1*H*-indole-3-propanamide (11e): Example of Scheme 1. A solution of freshly-purified[12] S_2Cl_2 (0.22 mL, 2.75 mmol) in dry THF (10 mL) was added dropwise to a stirred solution of 1*H*-indole-3-propanamide (8e) (1.03 g, 5.47 mmol) in THF (10 mL) at 0 °C. After 2 h at 20 °C, the mixture was diluted with water (100 mL), neutralized with dilute aqueous KOH, and extracted with EtOAc (4 × 100 mL). Evaporation under reduced pressure gave an oil. This was dissolved in EtOH (10 mL) and treated with an excess of $NaBH_4$ (0.40 g, 10.6 mmol). After 30 min at 20 °C, the reaction was quenched with water (100 mL), and the mixture was acidified to pH 2 with dilute HCl and then extracted with EtOAc (4 × 100 mL). Evaporation of solvent gave an oil which was dissolved in MeOH (10 mL) and stirred with 35% H_2O_2 (0.25 mL, 2.85 mmol) at 20 °C for 1 h. The reaction mixture was diluted with water (100 mL) and extracted with EtOAc (4 × 100 mL). The combined organic extracts were

washed with water, the solvent was evaporated, and the residue was chromatographed on silica gel. Elution with EtOAc/petroleum ether (3:1) gave 2,2′-thiobis(1*H*-indole-3-propanamide) (**9e**) (0.16 g, 14%): mp (EtOAc/petroleum ether) 196.5–197.5 °C; ^{1}H NMR ($(CD_3)_2SO$) δ 11.02 (s, 1 H, NH), 7.55 (d, *J* = 8.0 Hz, 1 H, ArH), 7.38 (s, 1 H, NH), 7.26 (d, *J* = 8.1 Hz, 1 H, ArH), 7.08 (ddd, *J* = 8.0, 7.1, 0.8 Hz, 1 H, ArH), 6.98 (dd, *J* = 7.8, 7.1 Hz, 1 H, ArH), 6.85 (s, 1 H, NH), 3.16, 2.46 (2 t, *J* = 7.7 Hz, 2 × 2 H, 3-CH_2CH_2); ^{13}C NMR δ 174.26 (s, $CONH_2$), 136.77, 126.82, 123.29 (3 s, Ar), 122.09, 118.82, 118.68 (3xd, Ar), 118.43 (s, Ar), 111.12 (d, Ar), 35.94 (t, 3-CH_2CH_2), 20.58 (t, 3-CH_2). Anal. ($C_{22}H_{22}N_4O_2S$) C, H, N, S.

Further elution with EtOAc and EtOAc/EtOH (9:1) gave 2,2′-dithiobis(1*H*-indole-3-propanamide) (**10e**) (0.90 g, 75%): mp (MeOH/dilute HCl) decomposition above 101 °C; ^{1}H NMR ($(CD_3)_2SO$) δ 11.37 (s, 1 H, NH), 7.55 (d, *J* = 8.0 Hz, 1 H, ArH), 7.32 (d, *J* = 8.2 Hz, 1 H, ArH), 7.16 (t, *J* = 7.6 Hz, 1 H, ArH), 7.00 (t, *J* = 7.5 Hz, 1 H, ArH), 6.94, 6.64 (2 s, 2 × 1 H, $CONH_2$), 2.72, 2.14 (2 m, 2 × 2 H, 3-CH_2CH_2); ^{13}C NMR δ 173.48 (s, $CONH_2$), 137.42, 126.58, 125.09 (3 s, Ar), 123.29 (d, Ar), 122.65 (s, Ar), 119.53, 118.91, 111.46 (3 d, Ar), 36.48 (t, 3-CH_2CH_2), 20.26 (t, 3-CH_2). Anal. ($C_{22}H_{22}N_4O_2S_2 \cdot 0.5H_2O$) C, H, N, S.

Reduction of **10e** with $NaBH_4$ as above gave 2,3-dihydro-2-thioxo-1*H*-indole-3-propanamide (**11e**) in essentially quantitative yield: mp (EtOAc) 160–163 °C; ^{1}H NMR ($(CD_3)_2SO$) δ 12.63 (s, 1 H, NH), 7.38 (d, *J* = 7.3 Hz, 1 H, ArH), 7.27 (t, *J* = 7.6 Hz, 1 H, ArH), 7.22 (s, 1 H, NH), 7.12 (t, *J* = 7.5 Hz, 1 H, ArH), 7.00 (d, *J* = 7.7 Hz, 1 H, ArH), 6.70 (s, 1 H, NH), 3.84 (t, *J* = 5.4 Hz, 1 H, H-3), 2.38 (m, 1 H, 3-CH_2CH_2), 2.16–1.96 (m, 2 H, 3-CH_2CH_2), 1.77 (ddd, *J* = 14.6, 10.3, 4.2 Hz, 1 H, 3-CH_2CH_2); ^{13}C NMR δ 206.83 (s, CSNH), 173.37 ($CONH_2$), 144.11, 133.81 (2 s, Ar), 127.95, 124.11, 123.21, 110.03 (4xd, Ar), 56.35 (d, C-3), 30.12, 28.32 (2 t, 3-CH_2CH_2). Anal. ($C_{11}H_{12}N_2OS$) C, H, N, S.

Similar reaction of the 1*H*-indole-3-alkanamides of Table 4, 1*H*-indole-3-acetonitrile, and 1*H*-indole-3-propionitrile[41] gave the thiones, sulfides, and disulfides listed in Tables 1 and 3. (See the supplementary material for details of ^{1}H and ^{13}C NMR spectra.)

2,2′-Dithiobis[α-(acetylamino)-*N*-(phenylmethyl)-1*H*-indole-3-propanamide]: Isolation of Diastereoisomer Pairs (10p). (*R,S*)-α-(Acetylamino)-*N*-(phenylmethyl)-1*H*-indole-3-propanamide (**24**) (1.25 g) was treated successively with S_2Cl_2, $NaBH_4$, and H_2O_2 as described above. The resulting oil was chromatographed on silica gel, eluting with CH_2Cl_2/EtOAc (2:1) to give firstly 2,2′-thiobis[α-(acetylamino)-*N*-(phenylmethyl)-1*H*-indole-3-propanamide] (**9p**) (0.30 g, 23%) as a mixture of diastereoisomers: mp (EtOAc/petroleum ether) 190–194 °C; ^{1}H NMR ($(CD_3)_2SO$) δ 10.97, 10.94 (2 s, 2 × 1 H, NH), 8.50, 8.48 (2 br t, *J* = 5.8 Hz, 2 × 1 H, N*H*CH_2), 8.17, 8.15 (2 d, *J* = 8.4 Hz, 2 × 1 H, CHN*H*), 7.63 (d, *J* = 7.7 Hz, 2 × 1 H, ArH), 7.3–6.9 (m, 2 × 8 H, ArH), 4.75 (m, 2 × 1 H, 3-CH_2CH), 4.27, 4.19 (2 dd, *J* = 16.1, 5.7 Hz, 2 × 2 H, NHCH_2), 3.44 (m, 2 × 1 H, 3-CH), 3.18 (m, 2 × 1 H, 3-CH), 1.79 (s, 2 × 3 H, $COCH_3$); ^{13}C NMR δ 171.20, 171.18 (2 s, $COCH_3$), 169.13 (s, 2 C, CONH), 138.83, 138.79 (2 s, Ar), 136.66 (s, 2 C, Ar), 128.03, 128.01 (2 d, 2 × 2 C, Ar), 127.42 (s, 2 C, Ar), 126.96, 126.91 (2 d, 2 × 2 C, Ar), 126.51, 126.48 (2 d, Ar), 124.58, 124.55 (2 s, Ar), 121.97 (d, 2 C, Ar), 119.02, 118.98 (2 d, Ar), 118.66 (d, 2 C, Ar), 115.01, 114.94 (2 s, Ar), 110.79 (d, 2 C, Ar), 53.66, 53.59 (2 d, 3-CH_2CH), 42.13 (t, 2 C, $NHCH_2$), 28.14, 28.07 (2 t, 3-CH_2), 22.52 (q, 2 C, CH_3). Anal. ($C_{40}H_{40}N_6O_4S \cdot 0.5H_2O$) C, H, N, S.

Elution with CH_2Cl_2/EtOAc (1:2) gave 2,2′-dithiobis[α-(acetylamino)-*N*-(phenylmethyl)-1*H*-indole-3-propanamide] (0.84 g, 62%) as a yellow oil (a mixture of diastereoisomers). Crystallization from CH_2Cl_2/petroleum ether gave a single pair of diastereoisomers: mp 140–144 °C dec (**10p**); ^{1}H NMR ($CDCl_3$) δ 9.16 (s, 1 H, NH), 7.51 (d, *J* = 8.1 Hz, 1 H, ArH), 7.2–7.0 (m, 6 H, ArH), 6.89 (m, 2 H, ArH), 6.76 (d, *J* = 7.2 Hz, 1 H, CHN*H*), 6.16 (t, *J* = 5.8 Hz, 1 H, N*H*CH_2), 4.64 (q, *J* = 7.2 Hz, 1 H, 3-CH_2CH), 4.20, 4.12 (2 dd, *J* = 14.8, 5.9 Hz, 2 × 1 H, NHCH_2), 3.13 (dd, *J* = 14.0, 7.1 Hz, 1 H, 3-CH), 2.96 (dd, *J* = 14.0, 7.3 Hz, 1 H, 3-CH), 1.84 (s, 3 H, $COCH_3$). Anal. ($C_{40}H_{40}N_6O_4S_2 \cdot 0.5H_2O$) C, H, N, S.

Crystallization from EtOAc/petroleum ether gave the other pair of diastereoisomers (**10p**; mp 154.5–157.5 °C dec): ^{1}H NMR ($CDCl_3$) δ 9.27 (s, 1 H, NH), 7.42 (d, *J* = 8.0 Hz, 1 H, ArH), 7.28–7.12 (m, 5 H, ArH), 7.04 (dd, *J* = 7.8, 7.0 Hz, 1 H, ArH), 6.75 (m, 2 H, ArH), 6.45 (br d, *J* = 7.1 Hz, 1 H, CHN*H*), 5.90 (br s, 1 H, N*H*CH_2), 4.41 (q, *J* = 7.4 Hz, 1 H, 3-CH_2CH), 4.17 (dd, *J* = 14.8, 6.0 Hz, 1 H, NHC*H*), 4.08 (dd, *J* = 14.8, 5.0 Hz, 1 H, NHC*H*), 2.99 (dd, *J* = 14.0, 6.9 Hz, 1 H, 3-CH), 2.93 (dd, *J* = 13.9, 7.6 Hz, 1 H, 3-CH), 1.82 (s, 3 H, $COCH_3$); ^{13}C NMR δ 170.74 (s, $COCH_3$), 169.92 (s, CONH), 137.42, 137.28 (2 s, Ar), 128.58 (d, 2 C, Ar), 127.59 (s, Ar), 127.51 (d, 2 C, Ar), 127.40 (d, Ar), 126.26 (s, Ar), 124.39, 120.37, 119.51 (3 d, Ar), 118.96 (s, Ar), 111.51 (d, Ar), 54.63 (d, 3-CH_2CH), 43.70 (t, $NHCH_2$), 28.87 (t, 3-CH_2), 23.23 (q, CH_3). Anal. ($C_{40}H_{40}N_6O_4S_2$) C, H, N, S.

However, in DMSO both pairs of diastereoisomers of **10p** reverted to a 1:1 mixture of diastereoisomers within 3 min by disulfide exchange.

2,2′-Dithiobis[*N*-[(4-carboxyphenyl)methyl]-1*H*-indole-3-propanamide] (10l). A suspension of 2,2′-dithiobis[*N*-[[4-(methoxycarbonyl)phenyl]methyl]-1*H*-indole-3-propanamide] (**10k**) (0.24 g, 0.33 mmol) in 30% aqueous MeOH (10 mL) containing K_2CO_3 (0.38 g, 0.27 mmol) was stirred at 30 °C for 24 h and then at 50 °C for 1 h under nitrogen. The solution was then diluted with water (100 mL), acidified (pH 2) with dilute HCl, and extracted with EtOAc (4 × 100 mL). Evaporation gave an oil, which was chromatographed on silica gel. Elution with EtOAc/petroleum ether (1:1) containing 1% AcOH gave 2,2′-dithiobis[*N*-[(4-carboxyphenyl)methyl]-1*H*-indole-3-propanamide] (**10l**) (60 mg, 26%): mp (MeOH/dilute HCl) 135.5–138.5 °C dec; ^{1}H NMR ($(CD_3)_2SO$) δ 11.41 (s, 1 H, NH), 8.03 (t, *J* = 5.8 Hz, 1 H, N*H*CH_2), 7.79 (d, *J* = 8.2 Hz, 2 H, ArH), 7.55 (d, *J* = 8.0 Hz, 1 H, ArH), 7.33 (d, *J* = 8.2 Hz, 1 H, ArH), 7.16 (t, *J* = 7.6 Hz, 1 H, ArH), 7.09 (d, *J* = 8.1 Hz, 2 H, ArH), 6.99 (t, *J* = 7.5 Hz, 1 H, ArH), 4.18 (d, *J* = 5.8 Hz, 2 H, NHCH_2), 2.73, 2.23 (2 t, *J* = 7.5 Hz, 2 × 2 H, 3-CH_2CH_2); ^{13}C NMR δ 171.44 (s, CONH), 167.10 (s, COOH), 144.46, 137.37 (2 s, Ar), 129.14 (d, 2 C, Ar), 129.05 (s, Ar), 126.87 (d, 2 C, Ar), 126.53, 125.18 (2 s, Ar), 123.23 (d, Ar), 122.40 (s, Ar), 119.58, 118.85, 111.37 (3 d, Ar), 41.65 (t, $NHCH_2$), 36.42 (t, 3-CH_2CH_2), 20.37 (t, 3-CH_2). Anal. ($C_{38}H_{34}N_4O_6S_2 \cdot H_2O$) C, H, N, S.

2,2′-Dithiobis[*N*-[[3-hydroxy-4-(methoxycarbonyl)phenyl]methyl]-1*H*-indole-3-propanamide] (10m) and 2,2′-Dithiobis[*N*-[(4-carboxy-3-hydroxyphenyl)methyl]-1*H*-indole-3-propanamide] (10n). *N*-[(3-Acetoxy-4-(methoxycarbonyl)-phenyl]methyl]-1*H*-indole-3-propanamide (**8m**) (1.47 g) was treated successively with S_2Cl_2, $NaBH_4$, and H_2O_2 as above. The crude product was then treated with excess $KHCO_3$ in 10% aqueous MeOH (10 mL) at 20 °C to hydrolyze the acetoxy group. Usual workup and chromatography on silica gel, eluting with EtOAc/petroleum ether (1:2), gave firstly 2,2′-thiobis[*N*-[[3-hydroxy-4-(methoxycarbonyl)phenyl]methyl]-1*H*-indole-3-propanamide] (**9m**) (0.12 g, 9%): mp (MeOH/dilute HCl) 109–112 °C dec; ^{1}H NMR ($CDCl_3$) δ 10.50 (s, 1 H, OH), 10.17 (s, 1 H, NH), 7.49 (d, *J* = 7.9 Hz, 1 H, ArH), 7.31 (d, *J* = 8.2 Hz, 1 H, ArH), 7.19 (d, *J* = 8.1 Hz, 1 H, ArH), 7.07 (ddd, *J* = 8.0, 7.1, 0.8 Hz, 1 H, ArH), 6.97 (ddd, *J* = 7.8, 7.2, 0.6 Hz, 1 H, ArH), 6.32 (d, *J* = 1.1 Hz, 1 H, ArH), 5.98 (dd, *J* = 8.2, 1.5 Hz, 1 H, ArH), 5.72 (t, *J* = 5.7 Hz, 1 H, N*H*CH_2), 4.22 (d, *J* = 5.7 Hz, 2 H, NHCH_2), 3.86 (s, 3 H, OCH_3), 3.50, 2.88 (2 m, 2 × 2 H, 3-CH_2CH_2); ^{13}C NMR δ 173.77 (s, CONH), 170.06 (s, $COOCH_3$), 161.36, 145.57, 137.16 (3 s, Ar), 130.02 (d, Ar), 126.62, 125.16 (2 s, Ar), 122.69, 119.13, 118.43 (3 d, Ar), 117.65 (s, Ar), 117.40, 115.51, 111.53 (3 d, Ar), 111.07 (s, Ar), 52.18 (q, OCH_3), 43.19 (t, $NHCH_2$), 36.32 (t, 3-CH_2CH_2), 21.22 (t, 3-CH_2). Anal. ($C_{40}H_{38}N_4O_6S$) C, H, N, S.

Elution with EtOAc/petroleum ether (2:3) gave 2,2′-dithiobis[*N*-[[3-hydroxy-4-(methoxycarbonyl)phenyl]methyl]-1*H*-indole-3-propanamide] (**10m**) (0.38 g, 27%): mp (MeOH) 183–185 °C; ^{1}H NMR ($CDCl_3$) δ 10.80 (s, 1 H, OH), 8.65 (s, 1 H, NH), 7.67 (d, *J* = 8.1 Hz, 1 H, ArH), 7.52 (d, *J* = 8.0 Hz, 1 H, ArH), 7.27 (d, *J* = 7.7 Hz, 1 H, ArH), 7.15 (ddd, *J* = 8.1, 7.2, 0.9 Hz, 1 H, ArH), 7.01 (ddd, *J* = 7.9, 7.2, 0.7 Hz, 1 H, ArH), 6.55 (d, *J* = 1.5 Hz, 1 H, ArH), 6.52 (dd, *J* = 8.2, 1.5 Hz, 1 H, ArH), 5.10 (t, *J* = 5.9 Hz, 1 H, N*H*CH_2), 4.13 (d, *J* = 6.0 Hz, 2 H, NHCH_2), 3.94 (s, 3 H, OCH_3), 2.88, 1.94 (2 t, *J* = 7.7 Hz, 2 × 2 H, 3-CH_2CH_2); ^{13}C NMR δ 172.12 (s, CONH), 170.39 (s, $COOCH_3$), 161.55, 146.95, 137.29 (3 s, Ar), 130.09 (d, Ar), 127.01, 125.87 (2 s, Ar), 124.39 (d, Ar), 123.79 (s, Ar), 120.16, 119.86, 118.34, 115.69, 111.37 (5 d, Ar),

111.20 (s, Ar), 52.31 (q, OCH_3), 42.82 (t, $NHCH_2$), 37.09 (t, 3-CH_2CH_2), 20.54 (t, 3-CH_2). Anal. ($C_{40}H_{38}N_4O_8S_2$) C, H, N, S.

Hydrolysis of **10m** with K_2CO_3 in aqueous MeOH at 50 °C for 5 h and then chromatography on silica gel as above gave 2,2′-dithiobis[*N*-[(4-carboxy-3-hydroxyphenyl)methyl]-1*H*-indole-3-propanamide] (**10n**) (72 mg, 27%): mp (MeOH/dilute HCl) 160–163.5 °C dec; ^1H NMR ($(CD_3)_2SO$) δ 11.39 (s, 1 H, NH), 8.03 (t, *J* = 5.9 Hz, 1 H, N*H*CH_2), 7.65 (d, *J* = 8.1 Hz, 1 H, ArH), 7.54 (d, *J* = 8.0 Hz, 1 H, ArH), 7.32 (d, *J* = 8.2 Hz, 1 H, ArH), 7.16 (ddd, *J* = 8.1, 7.1, 1.0 Hz, 1 H, ArH), 6.99 (ddd, *J* = 7.8, 7.1, 0.7 Hz, 1 H, ArH), 6.72 (d, *J* = 1.3 Hz, 1 H, ArH), 6.57 (dd, *J* = 8.2, 1.4 Hz, 1 H, ArH), 4.13 (d, *J* = 5.9 Hz, 2 H, NHC*H*$_2$), 2.75, 2.24 (2 t, *J* = 7.8 Hz, 2 × 2 H, 3-CH_2CH_2); ^{13}C NMR δ 171.70 (s, CONH), 171.47 (s, COOH), 161.04, 147.83, 137.37 (3 s, Ar), 130.08 (d, Ar), 126.51, 125.11 (2 s, Ar), 123.25 (d, Ar), 122.42 (s, Ar), 119.49, 118.86, 117.73, 115.09, 111.41 (5 d, Ar), 111.21 (s, Ar), 41.67 (t, $NHCH_2$), 36.63 (t, 3-CH_2CH_2), 20.41 (t, 3-CH_2). Anal. ($C_{38}H_{34}N_4O_8S_2 \cdot H_2O$) C, H, N, S.

2,2′-Dithiobis[α-hydroxy-*N*-(phenylmethyl)-1*H*-indole-3-propanamide] (10t). Hydrolysis of **10s** with excess $KHCO_3$ in aqueous MeOH at 20 °C for 2 h gave 2,2′-dithiobis[α-hydroxy-*N*-(phenylmethyl)-1*H*-indole-3-propanamide] (**10t**) as an oil (mixture of diastereoisomers) in essentially quantitative yield. Crystallization from CH_2Cl_2/petroleum ether gave a single pair of diastereoisomers (88% yield): mp 120–125 °C; ^1H NMR ($CDCl_3$) δ 8.53 (s, 1 H, NH), 7.61 (d, *J* = 8.0 Hz, 1 H, ArH), 7.33–7.17 (m, 5 H, ArH), 7.12 (dd, *J* = 7.8, 1.5 Hz, 2 H, ArH), 7.09 (ddd, *J* = 8.1, 5.4, 2.7 Hz, 1 H, ArH), 6.80 (t, *J* = 5.8 Hz, 1 H, N*H*CH_2), 4.33, 4.27 (2 dd, *J* = 14.8, 5.9 Hz, 2 × 1 H, NHC*H*$_2$), 3.78 (ddd, *J* = 9.5, 5.4, 3.4 Hz, 1 H, 3-CH_2CH), 3.30 (d, *J* = 5.4 Hz, 1 H, OH), 3.24 (dd, *J* = 14.4, 3.4 Hz, 1 H, 3-CH), 2.88 (dd, *J* = 14.3, 9.5 Hz, 1 H, 3-CH). Anal. ($C_{38}H_{34}N_4O_4S_2$) C, H, N, S.

2,2′-Dithiobis[*N*-(phenylmethyl)-α-[(trifluoroacetyl)amino]-1*H*-indole-3-propanamide] (10q) and 2,2′-Dithiobis[α-amino-*N*-(phenylmethyl)-1*H*-indole-3-propanamide] (10r) (Scheme 2). (*R*,*S*)-*N*-(Phenylmethyl)-α-[(trifluoroacetyl)amino]-1*H*-indole-3-propanamide (**8q**) (2.15 g) was treated with S_2Cl_2 (only) as above, and the product obtained on workup was chromatographed directly on silica gel. Elution with CH_2Cl_2 and CH_2Cl_2/EtOAc (19:1) gave foreruns including mono- and trisulfides, followed by 2,2′-dithiobis[*N*-(phenylmethyl)-α-[(trifluoroacetyl)amino]-1*H*-indole-3-propanamide] (**10q**) (1.01 g, 44%) as an oil (mixture of diastereoisomers). A subsample crystallized from EtOH as a single pair of diastereoisomers: mp 160–164 °C dec; ^1H NMR ($CDCl_3$) δ 8.76 (s, 1 H, NH), 7.57 (d, *J* = 8.0 Hz, 1 H, CHN*H*), 7.43 (d, *J* = 7.9 Hz, 1 H, ArH), 7.3–7.0 (m, 6 H, ArH), 6.75 (m, 2 H, ArH), 5.49 (t, *J* = 5.2 Hz, 1 H, N*H*CH_2), 4.26 (td, *J* = 7.9, 6.4 Hz, 1 H, 3-CH_2CH), 4.14 (dd, *J* = 14.8, 5.8 Hz, 1 H, NHCH_2), 4.00 (dd, *J* = 14.5, 4.9 Hz, 1 H, NHCH_2), 2.99 (dd, *J* = 14.0, 8.4 Hz, 1 H, NHCH_2), 4.00 (dd, *J* = 14.5, 4.9 Hz, 1 H, NHCH_2), 2.99 (dd, *J* = 14.0, 8.4 Hz, 1 H, 3-CH), 2.77 (dd, *J* = 14.0, 5.9 Hz, 1 H, 3-CH); ^{13}C NMR δ 168.87 (s, CONH), 156.81 (q, J_{CF} = 36.5 Hz, $COCF_3$), 137.25, 136.61 (2 s, Ar), 128.73 (d, 2 C, Ar), 127.71 (d, 3 C, Ar), 126.96, 126.11 (2 s, Ar), 124.97, 120.95, 119.25 (3 d, Ar), 118.14 (s, Ar), 115.62 (q, J_{CF} = 288 Hz, CF_3), 111.49 (d, Ar), 54.67 (d, 3-CH_2CH), 44.02 (t, $NHCH_2$), 28.22 (t, 3-CH_2). Anal. ($C_{40}H_{34}F_6N_6O_4S_2 \cdot 0.5H_2O$) C, H, N, S.

A solution of **10q** (0.80 g, 0.95 mmol) in EtOH (10 mL) was treated with $NaBH_4$ (0.65 g, 17.2 mmol) at 20 °C for 30 min.[19] The reaction was quenched with water (100 mL), acidified to pH 2 with dilute HCl, and extracted with EtOAc (2 × 100 mL). The aqueous layer was adjusted to pH 10 with K_2CO_3 solution and then extracted with EtOAc (4 × 100 mL). The latter combined extracts were washed with water, the solvent was evaporated, and the residue was chromatographed on alumina. Elution with $CHCl_3$/EtOH (99:1) gave foreruns, and then elution with $CHCl_3$/EtOH (98:2) gave 2,2′-dithiobis[α-amino-*N*-(phenylmethyl)-1*H*-indole-3-propanamide] (**10r**) (0.14 g, 22%): mp (CH_2Cl_2/petroleum ether) 147–150 °C dec; ^1H NMR ($(CD_3)_2SO$) δ 11.56 (s, 1 H, NH), 8.18 (t, *J* = 5.8 Hz, 1 H, N*H*CH_2), 7.61 (d, *J* = 7.8 Hz, 1 H, ArH), 7.36 (d, *J* = 8.1 Hz, 1 H, ArH), 7.33–6.95 (m, 7 H, ArH), 4.23, 4.13 (2 dd, *J* = 15.3, 5.9 Hz, 2 × 1 H, NHC*H*$_2$), 3.41 (br m, 1 H, 3-CH_2CH), 2.93 (dd, *J* = 13.7, 4.9 Hz, 1 H, 3-CH), 2.64 (br m, 1 H, 3-CH), 1.7 (br s, 2 H, NH_2); ^{13}C NMR δ 174.12 (s, CONH), 139.13, 137.38 (2 s, Ar), 128.06, 127.02 (2 d, 2 × 2 C, Ar), 126.95, 126.71 (2 s, Ar), 126.51, 123.19, 119.62 (3 d, Ar), 119.18 (s, Ar), 118.87, 111.39 (2 d, Ar), 55.57 (d, 3-CH_2CH), 41.90 (t, $NHCH_2$), 30.58 (t, 3-CH_2). Anal. ($C_{36}H_{36}N_6O_2S_2 \cdot 0.5H_2O$) C, H, N.

2,2′-Dithiobis(*N*-phenyl-1*H*-indole-3-propanamide) (10i) (Scheme 3). Treatment of 1*H*-indole-3-propanoic acid (**12**) (0.95 g) successively with S_2Cl_2, $NaBH_4$, and H_2O_2 as described above gave crude 2,2′-dithiobis(1*H*-indole-3-propanoic acid)[10] (**6**) (1.12 g) as an oil. This was reacted with excess aniline using DEPC and Et_3N as described above. However, TLC indicated that very little of the yellow disulfide was in the product mixture (suggesting a reaction of the disulfide bond with the coupling reagent). Therefore the product mixture was stirred with dilute KOH (0.1 M, 100 mL) at 20 °C for 30 min to cleave the adduct and reform the disulfide. Following usual workup, the mixture was chromatographed on silica gel, eluting with EtOAc/petroleum ether (2:1) and collecting only the yellow disulfide. This was rechromatographed on silica gel, eluting with CH_2Cl_2 and then $CHCl_3$/EtOH (99:1), to give 2,2′-dithiobis(*N*-phenyl-1*H*-indole-3-propanamide) (**10i**) (0.23 g, 16%), mp (CH_2Cl_2/benzene) 181–182.5 °C. An analytical sample recrystallized from CH_2Cl_2/petroleum ether decomposed above 114 °C: ^1H NMR ($(CD_3)_2CO$) δ 10.52 (s, 1 H, NH), 8.88 (s, 1 H, NHPh), 7.64 (d, *J* = 8.0 Hz, 1 H, ArH), 7.56 (dd, *J* = 7.5, 0.9 Hz, 2 H, ArH), 7.37 (d, *J* = 8.2 Hz, 1 H, ArH), 7.24 (dd, *J* = 8.4, 7.5 Hz, 2 H, ArH), 7.16 (ddd, *J* = 8.1, 7.1, 1.1 Hz, 1 H, ArH), 7.02 (m, 2 H, ArH), 3.04, 2.54 (2 m, 2 × 2 H, 3-CH_2CH_2); ^{13}C NMR δ 171.48 (s, CONH), 140.24, 138.80 (2 s, Ar), 129.37 (d, 2 C, Ar), 128.17, 126.81 (2 s, Ar), 124.57, 124.02 (2 d, Ar), 123.86 (s, Ar), 120.62, 120.36 (2 d, Ar), 120.23 (d, 2 C, Ar), 112.38 (d, Ar), 38.97 (t, 3-CH_2CH_2), 21.39 (t, 3-CH_2). Anal. ($C_{34}H_{30}N_4O_2S_2 \cdot 0.5H_2O$) C, H, N, S.

Enzyme Assays. Epidermal growth factor receptor was prepared from human A431 carcinoma cell shed membrane vesicles as previously described.[10,30] As previously reported, both Mn^{2+} and Mg^{2+} were added to the assay to assure inhibition by various classes of inhibitors.[7,10] Mn^{2+} is known to be required to observe inhibition by erbstatin,[7] and Mg^{2+} is necessary for the observation of inhibition by genistein. The presence of 4 mM Mn^{2+} did not reduce the activity of uninhibited enzyme, and the indolinethiones were inhibitory in the absence of Mn^{2+} (data not shown). The reactions were carried out in 96-well plates as previously described,[10] using a random copolymer of glutamate, alanine, and tyrosine in a ratio of 6:3:1, 250-ng epidermal growth factor, and appropriate solvent controls or inhibitors. Following precipitation of the polypeptide, incorporated label was assessed by scintillation counting the filters in an aqueous fluor. Autophosphorylation controls were performed for each experiment.

Protein from v-src baculovirus-infected insect cells was purified from lysates as follows. Latex beads of 0.65-μm diameter coated with Protein A/G (Interfacial Dynamics Corporation, Portland, OR) were coupled to monoclonal antibody 2-17[42,43]) by a carbodiimide linker. Washed beads were incubated with insect cell lysates containing $pp60^{v\text{-}src}$ at 4 °C for 4 h, washed with lysis buffer (150 mM NaCl, 50 mM Tris pH 7.5, 1% NP-40, 2 mM EGTA, 1 mM sodium orthovanadate, 1 mM PMSF, 1 μg/mL leupeptin, 1 μg/mL aprotinin, 1 μg/mL pepstatin, 10% glycerol, 1 mM dithiothreitol) and frozen at −90 °C until use. At the time of use, the beads containing bound v-src kinase were washed in assay buffer (40 mM Tris pH 7.5, 5 mM $MgCl_2$) and then assayed in final volume of 125 μL containing 25 μg of $polyGlu_4Tyr_1$ as substrate, 5 μM ATP containing 0.2 μCi/well ^{32}P and DMSO or inhibitors in DMSO in a 96-well plate with a 0.65-μm polyvinylidine membrane bottom. The reaction was begun by the addition of the labeled ATP and quenched after 10 min at 25 °C by the addition of 125 μL of 30% cold trichloroacetic acid and 0.1 M sodium pyrophosphate. The precipitates were incubated on ice for 15 min, filtered, and washed by successive aliquots of 15% TCA/pyrophosphate. The precipitated material was then counted in a liquid scintillation counter and percent inhibition calculated from the resulting data.

Autophosphorylation (incorporation of ^{32}P label into kinase protein itself) for the EGF receptor membrane vesicle preparation comprised less than 6% of incorporated label, as assessed by laser densitometer quantitation of autoradiographic determination of label distribution of vesicle preparations electrophoretically resolved on polyacrylamide gels. For the v-src kinase assay, autophosphorylation comprised less than 10% of incorporated

label. Therefore the contribution of this incorporated label to the calculation of percent inhibition was sufficiently low to be ignored in IC_{50} calculations.

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).

Immunoprecipitation and Western Blotting. Cells were grown to 100% confluency in 100-mm Petri dishes (Corning). After the designated treatments described in the results section, the medium was removed and the monolayer was scraped into 1 mL of ice-cold lysis buffer (50 mM Hepes, pH 7.5, 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1 mM EDTA, 1 mM EGTA, 10 mM sodium pyrophosphate, 30 mM 4-nitrophenyl phosphate, 1 mM orthovanadate, 50 mM sodium fluoride, 1 mM phenylmethanesulfonyl fluoride, 10 μg/mL of aprotinin, and 10 μg/mL of leupeptin). The lysate was transferred to a microfuge tube, kept on ice 15 min, and then centrifuged for 5 min at 10000*g*. The supernatant was transferred to a clean microfuge tube and, depending on whether mouse monoclonals or rabbit antiserum was to be used for the immune step, either 5 μg of mouse IgG + 25 μL of hydrated packed protein A sepharose or 25 μg of sepharose coated with preimmune rabbit serum was added to the tubes. These were then rotated at 4 °C for 2 h. After the tubes were centrifuged to bring down the sepharose, the supernatant was transferred to a clean tube, and either 5 μg of monoclonal antibody or 10 μL of antisera was added to designated samples. The tubes were rotated for 2 h at 4 °C, after which 25 μL of protein A sepharose was added, and then rotation continued for at least a further 2 h. The protein A sepharose was washed five times with 50 mM Hepes, pH 7.5, 150 mM NaCl, 10% glycerol, and 0.02% sodium azide. The precipitates were resuspended with 30 μL of Laemmli buffer, heated to 100 °C for 5 min, and centrifuged to obtain the supernatant. The entire supernatant was loaded onto a polyacrylamide gel (4–20%) and electrophoresced. 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 the primary antibody (1 μg/mL in blocking buffer) and then washed twice sequentially in TNA, TNA containing 0.05% each of the detergents Tween-20 and 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 then washed again as above. When the blots were dry they were loaded into a film cassette and exposed to X-AR X-ray film for 1–7 days. Bands were quantified by scanning densitometry.

Acknowledgment. The authors thank Dr. Ellen Dobrusin for helpful discussions, C. W. Moore, J. Jones, and M. E. Schemmel for technical support, and Wendy Hodgson for preparation of the manuscript. This work was partially supported by the Auckland Division of the Cancer Society of New Zealand.

Supplementary Material Available: ^{1}H and ^{13}C NMR data for the compounds of Tables 1, 3, and 4 (18 pages). Ordering information is given on any current masthead page.

References

(1) Burke, T. R. Protein-tyrosine kinase inhibitors. *Drugs Future* **1992**, *17*, 119–131.
(2) Chang, C.-J.; Geahlen, R. L. Protein-tyrosine kinase inhibition: mechanism-based discovery of antitumor agents. *J. Nat. Prod.* **1992**, *55*, 1529–1560.
(3) Umezawa, K.; Hori, T.; Tajima, H.; Imoto, M.; Isshiki, K.; Takeuchi, T. Inhibition of epidermal growth factor-induced DNA synthesis by tyrosine kinase inhibitors. *FEBS Lett.* **1990**, *260*, 198–200.
(4) Geahlen, R. L.; McLaughlin, J. L. Piceatannol (3,4,3′,5′-tetrahydroxy-*trans*-stilbene) is a naturally occurring protein-tyrosine kinase inhibitor. *Biochem. Biophys. Res. Commun.* **1989**, *165*, 241–245.
(5) Hsu, C.-Y. J.; Persons, P. E.; Spada, A. P.; Bednar, R. A.; Levitzki, A.; Zilberstein, A. Kinetic analysis of the inhibition of the EGF receptor tyrosine kinase by lavendustin A and its analogue. *J. Biol. Chem.* **1991**, *266*, 21105–21112.
(6) Levitzki, A. Tyrphostins: tyrosine kinase blockers as novel antiproliferative agents and dissectors of signal transduction. *FASEB J.* **1992**, *6*, 3275–3282.
(7) Hsu, C.-Y. J.; Jacoski, M. V.; Maguire, M. P.; Spada, A. P.; Zilberstein, A. Inhibition kinetics and selectivity of the tyrosine kinase inhibitor erbstatin and a pyridone-based analogue. *Biochem. Pharmacol.* **1992**, *43*, 2471–2477.
(8) Yarden, Y.; Ullrich, A. Growth factor receptor tyrosine kinases. *Annu. Rev. Biochem.* **1988**, *57*, 443–478.
(9) Dobrusin, E. M.; Fry, D. W. Protein tyrosine kinases and cancer. *Annu. Rep. Med. Chem.* **1992**, *27*, 169–178.
(10) Thompson, A. M.; Rewcastle, G. W.; Tercel, M.; Dobrusin, E. M.; Fry, D. W.; Kraker, A. J.; Denny, W. A. 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). *J. Med. Chem.* **1993**, *36*, 2459–2469.
(11) Kraker, A. J.; Jones, J. A.; Schemmel, M. E.; Moore, C. W. Inhibition of epidermal growth factor receptor tyrosine kinase by 2-thioindoles, a new structural class of tyrosine kinase inhibitor. *Proc. Am. Assoc. Cancer Res.* **1993**, *34*, 408.
(12) Hino, T.; Suzuki, T.; Takeda, S.; Kano, N.; Ishii, Y.; Sasaki, A.; Nakagawa, M. Preparation of 3-substituted-2-indolinethiones via diindolyl disulfides. The reaction of 3-substituted indoles with sulfur monochloride. *Chem. Pharm. Bull.* **1973**, *21*, 2739–2748.
(13) Palmisano, G.; Brenna, E.; Danieli, B.; Lesma, G.; Vodopivec, B.; Fiori, G. Selectivity in the thiocyanation of 3-alkylindoles: an unexpectedly easy access to 2-isothiocyano derivatives. *Tetrahedron Lett.* **1990**, *31*, 7229–7232.
(14) Piotrowska, H.; Serafin, B.; Wejroch-Matacz, K. New sulfide and disulfide derivatives of indole. *Rocz. Chem.* **1975**, *49*, 635–638.
(15) Wieland, T.; Weiberg, O.; Fischer, E.; Horlein, G. Preparation of sulfur-containing indole derivatives. *Annalen* **1954**, *587*, 146–161.
(16) Freter, K.; Weissbach, H.; Redfield, B.; Udenfriend, S.; Witkop, B. Oxindole analogs of (5-hydroxy)-tryptamine and -tryptophan, as inhibitors of the biosynthesis and breakdown of serotonin. *J. Am. Chem. Soc.* **1958**, *80*, 983–987.
(17) Wieland, T.; Weiberg, O.; Dilger, W.; Fischer, E. Synthesis of 2-oxy- and 2-thiotryptophans. *Annalen* **1955**, *591*, 69–80.
(18) Fontana, A.; Veronese, F. M.; Boccu, E. Labelling of the indole nucleus of tryptophan at the 2-position. *Acta Vitamin Enzymol. (Milano)* **1975**, *29*, 270.
(19) Weygand, F.; Frauendorfer, E. *N*-(Trifluoroacetyl)amino acids. XXI. Reductive elimination of the *N*-trifluoroacetyl and *N*-trichloroacetyl groups by sodium boron hydride and applications in peptide chemistry. *Chem. Ber.* **1970**, *103*, 2437–2449.
(20) Yamada, S.; Kasai, Y.; Shioiri, T. Diethylphosphoryl cyanide. A new reagent for the synthesis of amides. *Tetrahedron Lett.* **1973**, 1595–1598.
(21) Katritzky, A. R. An attempt to simulate the biogenesis of strychnine. Part I. Experiments with 4-2′-aminoethylpyridine. *J. Chem. Soc.* **1955**, 2581–2586.
(22) Nair, M. G.; Baugh, C. M. The synthesis of pteridine-6-carboxamides. 9-Oxofolic acid and 9-oxoaminopterin. *J. Org. Chem.* **1973**, *38*, 2185–2189.
(23) Meindl, W.; v. Angerer, E.; Ruckdeschel, G.; Schonenberger, H. Antimycobacterial *N*-alkylbenzylamines: synthesis and evaluation of 4-aminomethyl-2-hydroxybenzoic acid and its homologues. *Arch. Pharm.* **1982**, *315*, 941–946.
(24) Weygand, F.; Geiger, R. *N*-Trifluoroacetyl-aminoacids. IV. *N*-Trifluoroacetylation of amino acids in water-free trifluoroacetic acid. *Chem. Ber.* **1956**, *89*, 647–652.
(25) Curphey, T. J. Trifluoroacetylation of amino acids and peptides by ethyl trifluoroacetate. *J. Org. Chem.* **1979**, *44*, 2805–2807.
(26) Entzeroth, M.; Kunczik, T.; Jaenicke, L. Preparation of 3-(3-indolyl)lactic acids. A new synthesis of *rac*-blepharismone, the low molecular conjugation gamone of *Blepharisma japonicum*. *Liebigs Ann. Chem.* **1983**, 226–230.
(27) Dolzani, L.; Tamaro, M.; Monti-Bragadin, C.; Cavicchioni, G.; Vecchiati, G.; D'Angeli, F. Selective mutagenic activity of 2-bromopropanamides on *Salmonella typhimurium*. A structure-activity study. *Mutat. Res.* **1986**, *172*, 29–36.
(28) Somei, M.; Karasawa, Y.; Kaneko, C. Selective monoalkylation of carbon nucleophiles with gramine. *Heterocycles* **1981**, *16*, 941–949.
(29) Lyttle, D. A.; Weisblat, D. I. The chemistry of nitroacetic acid and its esters. I. The alkylation of alkylnitroacetates with gramine. *J. Am. Chem. Soc.* **1947**, *69*, 2118–2119.
(30) Cohen, S.; Ushiro, H.; Stoscheck, C.; Chinkers, M. A native 170,000 epidermal growth factor receptor-kinase complex from shed plasma membrane vesicles. *J. Biol. Chem.* **1982**, *257*, 1523–1531.

(31) Jove, R.; Hanafusa, H. Cell transformation by the viral *src* oncogene. *Annu. Rev. Cell Biol.* **1987**, *3*, 31–56.
(32) Bolen, J. B.; Rosen, N.; Israel, M. A. Increased pp60$^{c\text{-}src}$ tyrosyl kinase activity in human neuroblastomas is associated with amino-terminal tyrosine phosphorylation of the *src* gene product. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 7275–7279.
(33) Kwon, H. J.; Yoshida, M.; Fukui, Y.; Horinouchi, S.; Beppu, T. Potent and specific inhibition of pp60$^{v\text{-}src}$ protein kinase both *in vivo* and *in vitro* by radicicol. *Cancer Res.* **1992**, *52*, 6926–6930.
(34) Park, S.; Marshall, M. S.; Gibbs, J. B.; Jove, R. Reconstitution of interactions between the *src* tyrosine kinases and *ras* GTPase-activating protein using a baculovirus expression system. *J. Biol. Chem.* **1992**, *267*, 11612–11618.
(35) Traxler, P. M.; Wacker, O.; Bach, H. L.; Geissler, J. F.; Kump, W.; Meyer, T.; Regenass, U.; Roesel, J. L.; Lydon, N. Sulfonylbenzoyl-nitrostyrenes: potential bisubstrate type inhibitors of the EGF-receptor tyrosine protein kinase. *J. Med. Chem.* **1991**, *34*, 2328–2337.
(36) Regnier, G.; Canevari, R.; Le Douarec, J.-C. Acid-phenols. I. Synthesis of piperazine derivatives of salicylic acid. *Bull. Soc. Chim. Fr.* **1966**, 2821–2827.
(37) Manske, R. H. F.; Robinson, R. The decomposition of β-3-indolylpropionic azide. *J. Chem. Soc.* **1927**, 240–242.
(38) Kononova, V. V.; Vereshchagin, A. L.; Polyachenko, V. M.; Semenov, A. A. Synthesis and cytotoxic properties of α-isocarbolines. *Khim.-Farm. Zh.* **1978**, *12*, 30–33.
(39) Crosby, D. G.; Boyd, J. B.; Johnson, H. E. Indole-3-alkanamides. *J. Org. Chem.* **1960**, *25*, 1826–1827.
(40) Jackson, R. W.; Manske, R. H. The synthesis of indolylbutyric acid and some of its derivatives. *J. Am. Chem. Soc.* **1930**, *52*, 5029–5035.
(41) Reppe, W.; Ufer, H. Ger. Pat. 698,273, 1940.
(42) Illum, L.; Jones, P. D. E. Attachment of monoclonal antibodies to microspheres. *Meth. Enzymol.* **1985**, *112*, 67–84.
(43) Staros, J. V.; Wright, R. W.; Swingle, D. M. Enhancement by *N*-hydroxysulfosuccinimide of water soluble carbodiimide-mediated coupling reactions. *Anal. Biochem.* **1986**, *156*, 221–222.
(44) Avramenko, V. G.; Suvorov, N. N.; Mashkovskii, M. D.; Mushulov, P. I.; Eryshev, B. Ya.; Fedorova, V. S.; Orlova, I. A.; Trubitsyna, T. K. Indole derivatives. LXII. Synthesis and pharmacological properties of tryptamine homologs. *Khim.-Farm. Zh.* **1970**, *4*, 10–14.