Tyrosine Kinase Inhibitors. 3. Structure–Activity Relationships for Inhibition of Protein Tyrosine Kinases by Nuclear-Substituted Derivatives of 2,2′-Dithiobis(1-methyl-*N*-phenyl-1*H*-indole-3-carboxamide)

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A series of indole-substituted 2,2′-dithiobis(1-methyl-*N*-phenyl-1*H*-indole-3-carboxamides) were prepared and evaluated for their ability to inhibit the tyrosine kinase activity of both the epidermal growth factor receptor (EGFR) and the nonreceptor $pp60^{v\text{-}src}$ tyrosine kinase. The compounds were synthesized by conversion of appropriate 1-methyloxindoles to 1-methyl-2-indolinethiones with P_2S_5 followed by subsequent reaction with NaH and phenyl isocyanate and oxidative dimerization of the resulting 2,3-dihydro-*N*-phenyl-2-thioxo-1*H*-indole-3-carboxamides. The parent compound and many of the substituted analogues were moderately potent inhibitors of both kinase enzymes, but no clear relationships were seen between substitution on the indole ring and inhibitory activity. While 4-substituted compounds were generally inactive, 5-substituted derivatives with electron-withdrawing groups showed inhibitory activity. However, none of the substituted compounds showed significantly better activity than the unsubstituted parent compound. There was generally a good correlation between activity against the EGFR and $pp60^{v\text{-}src}$ kinases, but several compounds did show some specificity (>20-fold) of inhibition; 5-Cl and 5-Br derivatives preferentially inhibited $pp60^{v\text{-}src}$, while the 5-CF_3 compound preferentially inhibited EGFR. Selected compounds from the series were found to inhibit the growth of Swiss 3T3 fibroblasts with IC_{50}s in the range 2–25 μM, the most active being 4-substituted derivatives. The compounds inhibited bFGF-mediated protein tyrosine phosphorylation in intact cells more effectively than EGFR- or PDGF-mediated phosphorylation.

The recent elucidation[1,2] of many of the steps in the pathway by which external cellular growth signals are transmitted to the nucleus provides a new mechanism-based approach to the development of anticancer drugs.[3,4] The primary molecular mechanism of this signal transduction in normal and transformed cells is protein phosphorylation,[5] by the action of both membrane-spanning and cytoplasmic protein tyrosine kinase enzymes which catalyze the transfer of the terminal phosphate from ATP to the phenolic hydroxyl group of tyrosine in substrate proteins. Many of these enzymes are encoded by proto-oncogenes,[6–8] and their transformation or overexpression is considered in many cases to be the cause of malignant transformation. For example, human mammary, ovarian, and squamous cell head and neck carcinomas have been linked to overproduction of the epidermal growth factor receptor (EGFR) tyrosine kinase[9] or the closely homologous *c-erbB2* oncogene.[6] The nonreceptor *v-src* family of oncogene products, membrane-bound kinases lacking extracellular domains but closely associated with a number of receptor tyrosine kinases including the EGFR,[10] are believed to be involved in a number of human malignant states and other proliferative disorders.[11]

A number of compounds of diverse structure, but particularly polyphenols (e.g., 1), have recently been reported as inhibitors of protein tyrosine kinases, with most of the work to date using EGFR protein isolated from A431 human epidermoid carcinoma cells as a screening system.[4,6,10,12] These compounds can be divided mechanistically into a number of different classes.[4] Despite the high degree of homology known to exist among the kinase domains of different protein tyrosine kinases,[13,14] some compounds considered to bind to the substrate site show considerable selectivity between different enzymes.[15,16] This gives rise to the possibility that highly specific tyrosine kinase inhibitors can be developed,[6] especially when more structural data become available. Although no three-dimensional structure is yet available for a protein tyrosine kinase, an X-ray crystal structure of the cyclic AMP enzyme, a serine–threonine kinase which has a closely homologous kinase domain, shows the ATP-binding site to be in a deep cleft beneath a β-sheet, with the substrate-binding site probably lying close to the protein surface.[4,13,14]

On the basis of this concept, Burke *et al.*[17,18] have suggested that an important design motif for inhibitors is extended planarity and have shown that conformationally constrained bicyclic analogues (3, 4) of the cinnamamide class (2) show highly specific activity against the cytoplasmic $p56^{lck}$ kinases. While the unconstrained analogue 2 has an IC_{50} of 22 μM, the 6,7-dihydroxy compound 3 is much less active (IC_{50} 1900 μM), while the 7,8-dihydroxy isomer 4 is very potent (IC_{50} 0.5 μM).[18] The difference in potency with ring positional substitution in this series is striking.

We have previously shown that 2,2′-dithiobis(1*H*-indole-3-alkanoic acids) and their corresponding esters and amides (6; R_1 = $(CH_2)_nCOOH$), which form readily from the corresponding 2-thiones (5), are potent inhibitors of both EGFR and $pp60^{v\text{-}src}$ tyrosine kinases[19,20] by noncompetitive inhibition at the tyrosine substrate-binding site.[21] Changes

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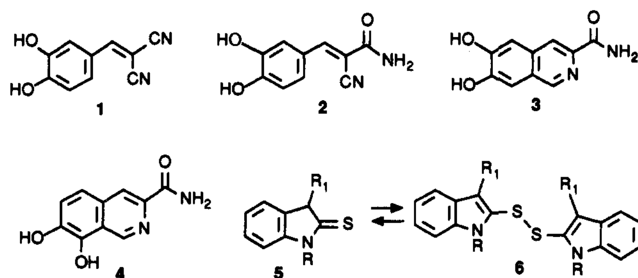


in the 3-alkyl side chain of the disulfides (**6**) markedly affected activity, with the propanoic acids, esters, and amides being the most effective; the benzyl derivative (**6**; R_1 = $(CH_2)_2CONHCH_2Ph$) has an IC_{50} of 0.85 μM for inhibition of EGFR kinase activity.[20] Substitution in the indole ring of the propanoic acid analogue (**6**; R_1 = $(CH_2)_2$-COOH) did not lead to marked effects on inhibitory potency, but this study was extremely limited.[19] We have now found that the 1-methyl-3-(*N*-phenyl carboxamide) analogue (**10a**; R = H) also shows potent tyrosine kinase inhibitory activity and report here structure–activity relationships for indole substitution in this series.

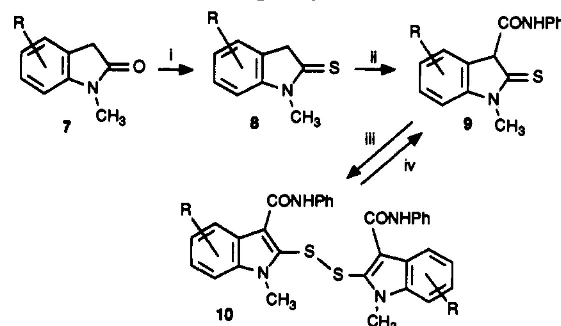
Chemistry

The substituted 2,2'-dithiobis(1-methyl-*N*-phenyl-1*H*-indole-3-carboxamides) **10** of Table 1 were synthesized as shown in Scheme 1. The required substituted *N*-methyloxindoles (1,3-dihydro-1-methyl-2*H*-indol-2-ones **7**) were prepared by a variety of methods, the choice of which was dictated by a number of considerations. Although a number of general routes are available for the synthesis of *N*-unsubstituted oxindoles,[22] the preparation of *N*-alkyl derivatives is less straightforward. The commonly used $AlCl_3$-catalyzed ring closure of *N*-alkyl-α-haloacetanilides (Stollé synthesis; Scheme 2, method A)[23,24] involves closure onto the *ortho* position of an *N*-alkylaniline derivative, so that *meta*-substituted anilines give mixtures of regioisomers that are difficult to separate. In addition, both dealkylation of alkoxy groups[25] and isomerization of alkyl substituents[26] can occur under the strongly acidic reaction conditions. Thus, preparation of 1,7-dimethyloxindole (**7v**)[27] by this method in the present study was accompanied by some isomerization of the aromatic methyl group, as demonstrated by ^1H and ^{13}C NMR spectroscopy. However, in cases where these complications do not occur, the method was successfully employed for the synthesis of substituted *N*-methyloxindoles (Table 5).

A related synthetic method via the preparation of isatins has the advantage that the mixtures of 4- and 6-isomers obtained from ring closure of *meta*-substituted isonitrosoacetanilides are readily separable.[28] *N*-Methylation of such isatins with Me_2SO_4/K_2CO_3 was readily achieved, but reduction to the corresponding *N*-methyloxindoles was not general. Hydrogenation led to dimeric byproducts as a result of coupling at C-3, and reaction via the 3-hydrazones gave much lower yields than those with *N*-unsubstituted isatins.[29] Reduction with zinc–copper couple in acetic acid (Scheme 2, method B) was successful in certain cases but was not general (for example, 4-chloro-1-methylisatin could be converted cleanly to the analogous oxindole, but the isomeric 6-chloro compound could not).

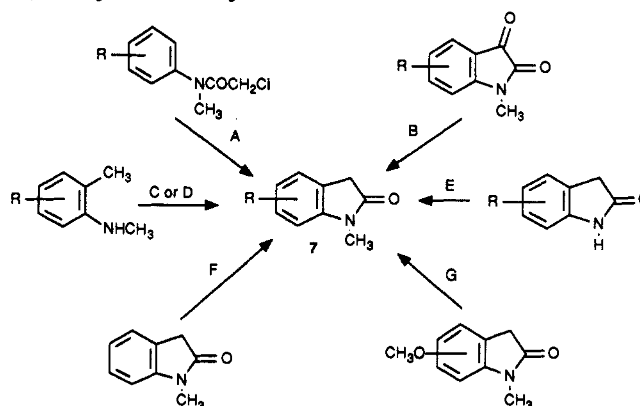
N-Unsubstituted oxindoles have been synthesized via lithiation of *N*-(*tert*-butoxycarbonyl)-2-alkylanilines[30] and *N*-methylindoles via lithiation of *N*-carboxyl derivatives of *N*,2-dimethylanilines.[31] A combination of these two

Scheme 1. Synthesis of 1-Methyl-*N*-phenyl-1*H*-indole-3-carboxamides and 2,2'-Dithiobis(1-methyl-*N*-phenyl-1*H*-indole-3-carboxamides)[a]



[a] (i) P_2S_5/Na_2CO_3; (ii) NaH, then PhNCO, then H^+; (iii) air, H_2O_2, or $NaBO_3$; (iv) $NaBH_4$.

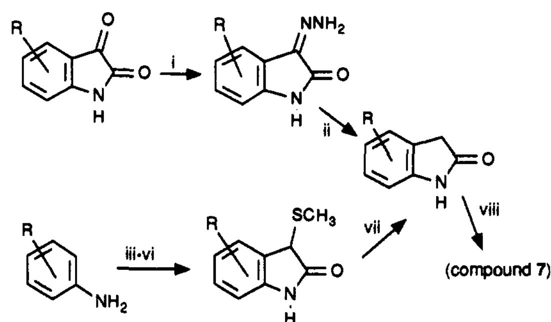
Scheme 2. Syntheses of Substituted 1,3-Dihydro-1-methyl-2*H*-indol-2-ones[a]



[a] (A) $AlCl_3$; (B) Zn:Cu/AcOH; (C) i. *n*-BuLi, ii. CO_2, iii. *n*-BuLi; iv. CO_2, v. H^+; (D) i. *n*-BuLi; ii. CO_2, iii. H^+; (E) Me_2SO_4/aqueous NaOH; (F) HNO_3 or Br_2; (G) (i.) HBr/AcOH, ii. Ac_2O/pyridine.

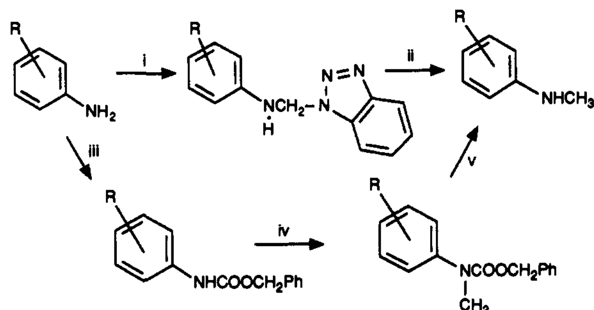
methods was found to be broadly useful for the preparation of the desired *N*-methyloxindoles. By using *N*-carboxyl protection,[31] a variety of *N*,2-dimethylaniline derivatives were successfully lithiated on the 2-methyl group and subsequently converted to the analogous oxindoles after quenching with CO_2 and treatment with aqueous acid (Scheme 2, method C). In one case, protection of the nitrogen was not necessary, and 2-(methylamino)-3-methylpyridine was converted directly to 7-azaoxindole (1,3-dihydro-1-methyl-2*H*-pyrrolo[2,3-*b*]pyridin-2-one, **7z**) via a *C,N*-dianion intermediate[32] (Scheme 2, method D). However, lithiation procedures were not compatible with a few substituent groups, such as *m*-chloro.[32] In these cases, *N*-methylation of the corresponding *N*-unsubstituted oxindoles with dimethyl sulfate and NaOH under aqueous conditions (Scheme 2, method E) proceeded cleanly, with no C-3 alkylation being observed. The *N*-unsubstituted oxindoles required were prepared either by reduction of the appropriately substituted isatin-3-hydrazones[29] or by cyclization of azasulfonium salts via 3-(methylthio)oxindoles (Gassman method)[33] (Scheme 3). In the latter cases, it was necessary to remove the 3-methylthio groups prior to the Me_2SO_4/NaOH alkylation step, since 3-(methylthio)oxindole gave the analogous 1,3-dimethyl derivative when subjected to this procedure. Reduction to the C-3-unsubstituted derivatives was readily achieved with zinc dust in refluxing acetic acid in less than 1 h. While the Gassman procedure has been used to prepare *N*-methyloxindoles directly,[33] we found reaction

Scheme 3. Synthesis of Substituted 1,3-Dihydro-2H-indol-2-ones[a]



[a] (i) NH_2NH_2; (ii) NaOEt/EtOH; (iii) t-BuOCl; (iv) $MeSCH_2CO_2Et$; (v) Et_3N; (vi) H^+; (vii) Zn/AcOH; (viii) Me_2SO_4/aqueous NaOH.

Scheme 4. Synthesis of Substituted N-Methylanilines[a]



[a] (i) CH_2O/benzotriazole; (ii) $NaBH_4$; (iii) $PhCH_2OCOCl$; (iv) NaH/MeI; (v) H_2/Pd/C.

yields to be lower than those with N-unsubstituted anilines. As an extreme example, in our hands, 4-(methylamino)benzonitrile gave only 3-chloro-4-(methylamino)benzonitrile and none of the desired oxindole derivative.

Finally, modification of preexisting 1-methyloxindoles was also used where appropriate, with both the 5-bromo and 5-nitro derivatives (**7h,o**) being prepared by direct electrophilic substitution of 1-methyloxindole (**7a**) (Scheme 2, method F). The 4-, 5-, 6-, and 7-acetoxy-1-methyloxindoles (**7e,k,s,x**) were obtained from the analogous methoxy compounds via demethylation and acetylation (Scheme 2, method G).

The substituted N-methylanilines required as starting materials for many of these syntheses were normally prepared by Mannich reaction of the appropriately substituted anilines with formaldehyde and benzotriazole[34] followed by reduction of the resulting N-(1H-benzotriazol-1-ylmethyl)anilines (**15–23**) (Table 7) with $NaBH_4$[35] (Scheme 4). When this route was unsuccessful, the desired compounds were prepared by alkylation and hydrogenation of phenyl-substituted benzyl N-phenylcarbamates (**24–29**) (Table 8; Scheme 4).

The 1-methyloxindoles were converted in good yields to the corresponding 1-methyl-2-indolinethiones (1,3-dihydro-1-methyl-2H-indole-2-thiones **8**) of Table 6 with P_2S_5/Na_2CO_3 in THF.[36] Deprotonation of these using NaH in THF followed by reaction with phenyl isocyanate gave good yields of the corresponding 2,3-dihydro-N-phenyl-2-thioxo-1H-indole-3-carboxamides **9** (Scheme 1). However, owing to their facile air oxidation to the resulting 2,2′-dithiobis(1-methyl-N-phenyl-1H-indole-3-carboxamides) **10**[19,37], they were not generally isolated but were oxidized to the corresponding disulfides by exposure of dilute methanolic solutions to air or by treatment with dilute H_2O_2 or sodium perborate. The acetoxy disulfides (**10k,s,x**) were hydrolyzed to the corresponding phenol disulfides (**10l,t,y**) by prior reduction ($NaBH_4$) to the thiones, treatment with 3 N KOH, and reoxidation. The mixed disulfides (**13, 14**) were prepared by reaction of the dimer **10a** with phenyl or benzyl mercaptan under acid or base catalysis. Methylation of **9a** (MeI/K_2CO_3) gave the corresponding S-methyl compound **12**.

Results and Discussion

The compounds were tested for their ability to inhibit the tyrosine kinase activity of both the native EGFR complex, contained in plasma membrane vesicles shed from cultured A431 epidermoid carcinoma cells[38] as described previously,[19] and the pp60$^{v\text{-}src}$ protein, obtained from v-src baculovirus-infected insect cells and coupled to 0.65-μm-diameter latex beads via a monoclonal antibody.[39,40] IC_{50} values are defined as the concentration of drug necessary to reduced by 50% incorporation of ^{32}P (from added [γ-^{32}P]ATP) into a substrate (a 6:3:1 random copolymer of glutamate, alanine, and tyrosine for EGFR and polyglu$_4$tyr$_1$ for v-src). The results are recorded in Table 1.

Previous work[19,20] with the 3-alkyl disulfides (**6**) showed that these and the corresponding monomeric 3-alkyl thiones (**5**) underwent facile redox interconversion but that the disulfides were the more potent inhibitors and were likely to be the relevant species. The 3-carboxamides likewise underwent ready, but less rapid, interconversion. Thus, whereas the 3-alkylindolinethione (**5**; $R_1 = (CH_2)_2COOH$) had a half-life of only a few minutes in oxygenated buffer at pH 7, the half-life of **9a** was about 5 h (unpublished results, this laboratory).

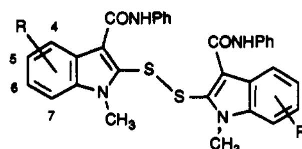
In the present series, three sets of monomer–dimer pairs were studied, and while the monomer was somewhat more potent in the unsubstituted case (compounds **9a** and **10a**), in two other examples (compounds **9g** and **10g**, and **9z** and **10z**), the dimers were substantially more potent. In addition, the 2-SMe indole derivative **12** and the 3-methyloxindole derivative **11**, neither of which can convert to the disulfide dimer, were both inactive. The two mixed disulfides **13** and **14** were also inactive, which was somewhat surprising since both slowly convert to the symmetrical dimer **10a**. Thus, a study of the effects of nuclear substitutions in this series was conducted using only the disulfide dimers (compounds **10a–z**).

The primary aim was to examine structure–activity relationships for substitution in the indole ring. A secondary goal was to determine whether these could be interpreted according to the tentative model of Burke, who proposed that extended planar structures are preferred and showed that 6,7-dihydroxy substitution in bicyclic isoquinolines of somewhat similar topology to the indolinethiones was much less effective than corresponding 7,8-dihydroxy substitution (for activity against the nonreceptor src family p56^{lck} kinase).[17,18]

In the previous study[19] of 2,2′-dithiobis(1H-indole-3-alkanoic acids), analogues substituted in the indole ring by methyl groups retained activity, but no other substituents were studied; thus, no conclusions could be drawn about substituent steric, lipophilic, or electronic effects. In the present work, initial studies focused on -Cl, -Me, and -OMe substituents at all four available ring positions. We have discussed previously[41] (in another context) the justification for using these (approximately isosteric) substituents for preliminary analysis of local electronic

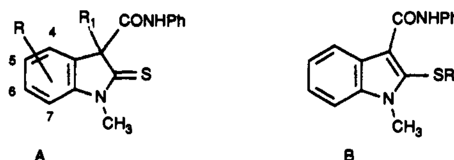
Table 1

a. Physicochemical and Biological Properties of Substituted 2,2'-Dithiobis(1-methyl-*N*-phenyl-1*H*-indole-3-carboxamides)



no.	R	yield[a] (%)	mp (°C)	formula	anal.[b]	IC50EGF[c] (μM)	IC50src[d] (μM)
10a	H	33	187–188	$C_{32}H_{26}N_4O_2S_2$	C, H, N, S	10	3.2
10b	4-Cl	21	225–228	$C_{32}H_{24}Cl_2N_4O_2S_2$	C, H, N, Cl	ca.100	>100
10c	4-Me	29	237–239	$C_{34}H_{30}N_4O_2S_2$	C, H, N, S	>100	>100
10d	4-OMe	38	225–228	$C_{34}H_{30}N_4O_4S_2$	C, H, N, S	>100	>100
10e	4-OAc	31	194	$C_{36}H_{30}N_4O_6S_2$	HRMS[e]	20	0.65
10f	5-F	74	205–207	$C_{32}H_{24}F_2N_4O_2S_2$	C, H, N, S	>100	6.0
10g	5-Cl	27	214–216	$C_{32}H_{24}Cl_2N_4O_2S_2$	C, H, N, S	4.3	>100
10h	5-Br	68	219–221	$C_{32}H_{24}Br_2N_4O_2S_2$	C, H, N, S	11.4	ca. 100
10i	5-Me	34	231–234	$C_{34}H_{30}N_4O_2S_2$	C, H, N, S	>100	>100
10j	5-OMe	41	161–164	$C_{34}H_{30}N_4O_4S_2$	C, H, N, S	>100	>100
10k	5-OAc	45	147–150	$C_{36}H_{30}N_4O_6S_2\cdot0.5H_2O$	C, H, N, S	5.3	7.7
10l	5-OH	*f*	185–187	$C_{32}H_{26}N_4O_4S_2\cdot H_2O$	C, H, N	40.5	3.9
10m	5-CF3	71	214–216	$C_{34}H_{24}F_6N_4O_2S_2$	C, H, N, S	>100	5.8
10n	5-CN	47	221–224	$C_{34}H_{24}N_6O_2S_2\cdot0.5H_2O$	C, H, N, S	54.1	4.5
10o	5-NO2	52	236–240	$C_{32}H_{24}N_6O_2S_2\cdot0.5H_2O$	C, H, N	5.0	7.2
10p	6-Cl	61	243–245	$C_{32}H_{24}Cl_2N_4O_2S_2$	C, H, N, S	>100	>100
10q	6-Me	33	192–195	$C_{34}H_{30}N_4O_2S_2$	C, H, N, S	>100	ca. 100
10r	6-OMe	41	197–200	$C_{34}H_{30}N_4O_4S_2$	C, H, N, S	3.6	3.6
10s	6-OAc	53	219–222	$C_{36}H_{30}N_4S_2O_6$	C, H, N, S	>100	3.5
10t	6-OH	*f*	182–183	$C_{32}H_{27}N_4O_4S_4$	HRMS	44	5.1
10u	7-Cl	27	232–234	$C_{32}H_{24}Cl_2N_4O_2S_2$	C, H, N, Cl	>100	>100
10v	7-Me	31	221–223	$C_{34}H_{30}N_4O_2S_2$	C, H, N, S	>100	>100
10w	7-OMe	44	205–206	$C_{34}H_{30}N_4O_4S_2$	C, H, N, S	6.5	3.6
10x	7-OAc	52	212–214.5	$C_{36}H_{30}N_4S_2O_6\cdot0.5H_2O$	C, H, N, S	9.9	11.1
10y	7-OH	*f*	207 dec	$C_{32}H_{26}N_4O_4S_2$	C, H, N, S	ca.100	>50
10z	7-aza	*g*	162–164	$C_{30}H_{24}N_6O_2S_2\cdot2H_2O$	C, H, N, S	22.3	5.2

b. Physicochemical and Biological Properties of Substituted 2,3-Dihydro-1-methyl-*N*-phenyl-2-thioxo-1*H*-indole-3-carboxamides and Related Thioenol Derivatives



no.	form	R	R1	mp (°C)	formula	anal.[b]	IC50EGF[c] (μM)	IC50src[d] (μM)
9a	A	H	H	149–151	$C_{16}H_{14}N_2OS$	C, H, N, S	1.0	9.7
9g	A	5-Cl	H	312–320	$C_{16}H_{13}ClN_2OS$	HRMS	>100	>100
9z	A	7-aza	H	162–164	$C_{15}H_{13}N_3OS\cdot CH_3OH$	C, H, N, S	>100	30.7
11	A	H	CH3	106–109	$C_{17}H_{16}N_2SO$	C, H, N, S	>100	>50
12	B	Me		116–118	$C_{17}H_{16}N_2OS$	C, H, N, S	>100	>50
13	B	SPh		131–134	$C_{22}H_{18}N_2OS_2$	C, H, N, S	>100	>100
14	B	SCH2Ph		146–148	$C_{23}H_{30}N_2OS_2$	C, H, N, S	>100	>100

[a] Yield from the indolinethiones (8) without isolation of the intermediate thiones (9; see text. [b] Analyses for all listed elements are within ±0.4%. [c,d] Drug concentrations (μM) to cause 50% inhibition of tyrosine phosphorylation activities of the EGF receptor and pp60$^{v\text{-}src}$ tyrosine kinases, respectively; see text. [e] High-resolution FAB mass spectrum molecular ion. [f] Prepared by hydrolysis of corresponding acetoxy compound; see text. [g] From dimerization of pure indolinethione 9z.

and lipophilic factors. In addition, the OH (and intermediate OCOMe) compounds were prepared and evaluated because of the activity associated with ring OH groups in other series of tyrosine kinase inhibitors (*e.g.*, analogues of 1).[7,42]

The results (Table 1) show that any substitution at the 4-position is not favorable for activity, with three of the four compounds being inactive at 100 μM in both assays. Ten 5-substituted disulfides were prepared, using substituents which spanned a wide range of σ and π values. While no statistically significant correlation was observed, all of the five analogues with higher activity than the parent possessed electron-withdrawing groups, while two of the three inactive compounds had electron-donating ones (Figure 1). Among the 6- and 7-substituted compounds, no patterns could be observed. Cl and Me groups at both positions abolished activity, while both OMe analogues (**10r,w**) were highly active. The more hydrophilic 7-OAc and 7-aza compounds (**10x,z**) were active, but the 7-OH derivative (**10y**) was not.

Over the whole series, there was a good correlation between activity against the EGFR and pp60$^{v\text{-}src}$ kinases (Table 1). Exceptions were the 5-Cl and 5-Br compounds (**10g,h**) which showed some specificity for the EGFR kinase and the 5-CF$_3$ compound (**10m**) which had the opposite specificity. Since the Br and CF$_3$ groups are practically identical in size, lipophilicity, and electronic properties,

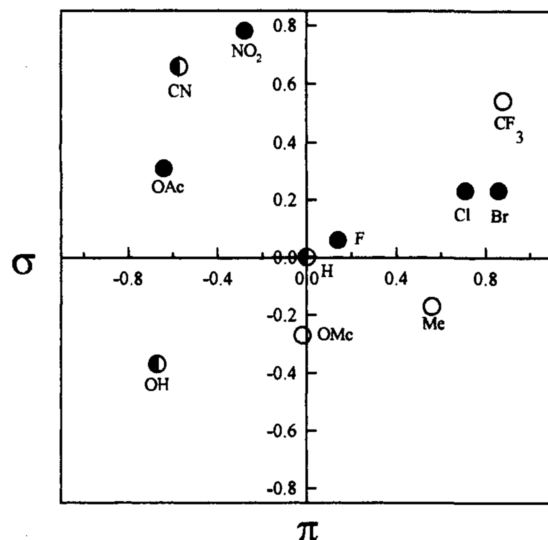


Figure 1. Relationship between substituent electronic and lipophilic properties and EGFR tyrosine kinase inhibition for 5-substituted 2,2′-dithiobis(1-methyl-*N*-phenyl-1*H*-indole-3-carboxamides: (●) $IC_{50} < 10$ μM, (◐) IC_{50} 10–100 μM, (○) $IC_{50} > 100$ μM.

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

no.	R	IC_{50} (μM)[a]
10a	H	12
10c	4-Me	4.0
10i	5-Me	22
10q	6-Me	96
10d	4-OMe	1.9
10j	5-OMe	20
10r	6-OMe	17
10w	7-OMe	24
10g	5-Cl	9.3
10z	7-aza	10

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

these "global" substituent properties appear to have little effect on specific activities.

The mechanism of inhibition of **10a,z** has been examined by characterizing the enzyme inhibition in the presence of varying concentrations of ATP and peptide substrate and assessing K_i values (manuscript in preparation). As has been previously reported for other compounds in this series,[21] compounds **10a,z** are noncompetitively inhibitory with respect to peptide substrate and are also noncompetitive with respect to ATP.[43] Thus, although cells contain much higher concentrations of ATP than those employed in the enzyme assays, this is not (from a mechanistic standpoint, at least) an obstacle to them retaining kinase inhibitory activity in cellular systems.

Indeed, selected compounds showed an ability to inhibit the growth of Swiss 3T3 mouse fibroblasts with IC_{50}s in the low micromolar range (Table 2). In contrast to the enzyme inhibition data, methyl or methoxy groups at the 4-position enhance potency (IC_{50}s of 2–4 μM, 5 times lower than that of the parent **10a**) whereas substitution at the 5- or 6-position was detrimental. Table 3 shows the response of several human tumor lines to the parent compound **10a** and the corresponding monomer **9a**; all the lines showed approximately equal sensitivity to **10a** (IC_{50}s 12–18 μM), with the monomer **9a** being much less effective.

Table 3. Effect of Compounds **9a** and **10a** on the Proliferation of Different Tumor Cell Lines

cell line	cell type	IC_{50} (μM)[a] **9a**	IC_{50} (μM)[a] **10a**
Swiss 3T3	mouse fibroblast	64	12
A431	human epidermoid carcinoma	15	14
K562	chronic myelogenous leukemia	32	18
KG1A	acute myelogenous leukemia	38	12

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

Table 4. Effect of 2,2′-Dithiobis(1-methyl-*N*-phenyl-1*H*-indole-3-carboxamides) on Growth Factor-Mediated Tyrosine Phosphorylation in Swiss 3T3 Fibroblasts[a]

no.	IC_{50} (μM)[b] EGF receptor	PDGF receptor	bFGF-mediated
9a	>100	17	3
10a	>100	>100	3
10r	>100	>100	37
10w	>100	>100	25
10z[c]	>25	>25	12

[a] Cells were treated for 2 h with varying concentrations of compound and then stimulated for 5 min with either EGF, PDGF, or bFGF. Procedures for making cell cell extracts and Western blotting are described in the Experimental Section. [b] Concentration of compound necessary to inhibit by 50% either EGF or PDGF receptor autophosphorylation of bFGF-mediated tyrosine phosphorylation of a 85-kDa protein. [c] Concentrations of **10z** greater than 25 μM could not be evaluated since it independently caused excessive tyrosine phosphorylation in cells.

Finally, the effects of selected compounds on tyrosine phosphorylation in cellular systems were examined by pretreating Swiss 3T3 fibroblasts with varying concentrations of drugs for 2 h, before exposure to different growth factors (Table 4). Intracellular phosphotyrosine levels were then 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. All of the evaluated compounds inhibited phosphorylation of this protein in a concentration-dependent manner, with IC_{50} values ranging from 3 to 37 μM (Table 4). Surprisingly, none of the disulfides inhibited EGF or PDGF receptor autophosphorylation in cells, suggesting that this structural class of compounds specifically inhibits bFGF-mediated tyrosine phosphorylation in intact cells.

Conclusions

The above results show the 2,2′-dithiobis(1-methyl-*N*-phenyl-1*H*-indole-3-carboxamide) (**10a**) is a moderately potent inhibitor of both isolated EGFR and $pp60^{v\text{-}src}$ tyrosine kinases. However, no clear relationships were seen between nuclear substitution on the indole ring of **10a** and inhibitory potency. Among the 5-substituted compounds, where the largest number of examples were available, electron-withdrawing groups appeared to retain activity. However, none of the substituted compounds showed significantly better activity against the isolated enzymes than did the parent compound. A previous (much more limited) study[19] of 2,2′-dithiobis(1*H*-indole-3-alkanoic acids) also showed that methyl substitution in the indole ring had no effect on biological activity. Selected compounds inhibited the growth of Swiss 3T3 cells at the low micromolar level, but these data did not correlate with the isolated enzyme results, suggesting that other mechanisms of cytotoxicity apart from EFGR inhibition are

operative. The compounds were shown to selectively inhibit bFGF-mediated (rather than EGFR-mediated) protein tyrosine phosphorylation, suggesting that the mechanism of cellular growth inhibition may involve the preferential inhibition of kinases other than EGFR.

Experimental Section

Where analyses are indicated by symbols of the elements, results were within ±0.4% of the theoretical value and were performed by the Microchemical Laboratory, University of Otago, Dunedin, NZ. 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 and referenced to tetramethylsilane. Mass spectra were recorded on a Varian VG 7070 spectrometer at nominal 5000 resolution. FAB mass spectra for the symmetrical disulfides were obtained from a 3-nitrobenzyl alcohol matrix.

Preparation of *N*,2,5-Trimethylaniline: Example of the Benzotriazole Method of Scheme 4.[34] A mixture of 2,5-dimethylaniline (27.4 g, 0.2 mol) and benzotriazole (23.8 g, 0.2 mol) in EtOH (300 mL) was stirred at 20 °C as 37% aqueous formaldehyde (16.1 g, 0.2 mol) was added gradually. After 30 min, the white solid which precipitated was collected and washed with EtOH to give *N*-(1*H*-benzotriazol-1-ylmethyl)-2,5-dimethylaniline (21) (33.9 g, 67%), mp (EtOH) 147–149 °C. ^{1}H NMR ($CDCl_3$): δ 6.85–8.10 (m, 7 H, ArH), 6.56 (minor isomer) and 6.13 (major isomer) (2m, 2 H, CH_2), 5.08 (minor) and 4.70 (major) (2m, 1 H, NH), 2.24 (s, 3 H, CH_3), 2.12 (s, 3 H, CH_3). Anal. ($C_{15}H_{16}N_4$) C, H, N.

Similar preparations gave the (benzotriazolylmethyl)anilines 15–23 of Table 7 (see the supplementary material for details of NMR spectra).

A suspension of 21 (33 g, 0.13 mol) and $NaBH_4$ (5 g) in dioxane (400 mL) was heated under reflux for 5 h, and the solution was concentrated. After the solution was cooled, water was added and the resulting mixture was extracted with EtOAc. The organic layer was washed twice with aqueous K_2CO_3 and water and dried (Na_2SO_4). Removal of the solvent gave *N*,2,5-trimethylaniline[44] (17.6 g, 99%) as an oil, which was used directly. ^{1}H NMR ($CDCl_3$): δ 6.93 (d, *J* = 7.4 Hz, 1 H, H-3), 6.49 (d, *J* = 7.6 Hz, 1 H, H-4), 6.44 (s, 1 H, H-6), 3.72 (s, 1 H, NH), 2.88 (s, 3 H, NCH_3), 2.31 (s, 3 H, CH_3), 2.09 (s, 3 H, CH_3).

Preparation of *N*,2,6-Trimethylaniline: Example of the Carbamate Method of Scheme 4. A solution of 2,6-dimethylaniline (12.12 g, 0.1 mol) and pyridine (8.7 g, 0.11 mol) in toluene (100 mL) was treated dropwise with benzyl chloroformate (16.5 mL of 95%, 0.11 mol) at 0 °C. After stirring at 20 °C for 15 h, volatiles were removed under reduced pressure and the residue was diluted with water to give benzyl *N*-(2,6-dimethylphenyl)carbamate (24) (14.57 g, 57%), mp (aqueous EtOH) 126.5–129 °C. ^{1}H NMR ($CDCl_3$): δ 7.36 (m, 5 H, phenyl), 7.08 (m, 3 H, H-3′,4′,5′), 6.09 (m, 1 H, NH), 5.19 (s, 2 H, CH_2), 2.25 (s, 6 H, CH_3). Anal. ($C_{16}H_{17}NO_2$) C, H, N.

A solution of 24 (11.4 g, 45 mmol) in dry THF (100 mL) was treated with NaH (1.6 g of 80% dispersion in oil, 54 mmol). When H_2 evolution ceased, MeI (7.7 g, 55 mmol) was added and the mixture was stirred for a further 30 min at 20 °C before the reaction was quenched with AcOH. Chromatography of the crude product on alumina, eluting with CH_2Cl_2/hexane, gave benzyl *N*-(2,6-dimethylphenyl)-*N*-methylcarbamate (25) (11.4 g, 95%) as an oil (mixture of two conformers). ^{1}H NMR ($CDCl_3$): δ 7.45–7.32 (m, 1 H), 7.21 (m, 2 H), 7.17–7.04 (m, 5 H), 5.23 (s, CH_2, minor conformer), 5.09 (s, CH_2, major conformer), 3.15 (s, NCH_3, minor conformer), 3.14 (s, NCH_3, major conformer), 2.20 (s, CH_3, minor conformer), 2.14 (s, CH_3, major conformer). HREIMS: found, M^+ 269.1418. $C_{17}H_{19}NO_2$ requires M^+ 269.1416. Hydrogenation of 25 in MeOH with Pd/C gave *N*,2,6-trimethylaniline as an oil (lit.[45] oil), which was used directly. ^{1}H NMR ($CDCl_3$): δ 7.44 (d, *J* = 7.4 Hz, 2 H, H-3,5), 6.81 (t, *J* = 7.4 Hz, 1 H, H-4), 2.78 (s, 3 H, NCH_3), 2.63 (br s, 1 H, NH), 2.29 (s, 6 H, $2CH_3$).

Similar preparations gave the benzyl *N*-phenylcarbamates and benzyl *N*-methyl-*N*-phenylcarbamates of Table 8 (see the supplementary material for details of NMR spectra).

4-Chloro-1,3-dihydro-1-methyl-2*H*-indol-2-one (7b): Example of General Method B of Scheme 2. 4-Chloroisatin[28] was alkylated with Me_2SO_4/K_2CO_3 in MeOH to give 4-chloro-1-methylisatin, mp (MeOH) 191.5–193 °C (lit.[46] mp 192 °C). ^{1}H NMR ($(CD_3)_2SO$): δ 7.64 (t, *J* = 8.1 Hz, 1 H, 6-H), 7.12 and 7.10 (2d, *J* = 8.0 and 8.2 Hz, 2 H, 5,7-H), 3.14 (s, 3 H, CH_3). A solution of this isatin (4.88 g, 25 mmol) in AcOH (100 mL) was heated under reflux with Zn/Cu couple (30 g) for 48 h. The cooled mixture was diluted with EtOAc and filtered to remove inorganic solids. After dilution with water, the organic layer was separated, washed twice with water, and worked up and the residue was chromatographed on Al_2O_3. Elution with CH_2Cl_2 gave 4-chloro-1,3-dihydro-1-methyl-2*H*-indol-2-one (7b) (1.15 g, 25%), mp (aqueous Me_2CO) 139.5–142 °C. ^{1}H NMR ($CDCl_3$): δ 7.23 (t, *J* = 8.0 Hz, 1 H, 6-H), 7.02 (d, *J* = 8.2 Hz, 1 H, 5-H), 6.72 (d, *J* = 7.8 Hz, 1 H, 7-H), 3.52 (s, 2 H, CH_2), 3.21 (s, 3 H, CH_3). Anal. (C_9H_8ClNO) C, H, N, Cl.

1,3-Dihydro-1,6-dimethyl-2*H*-indol-2-one (7q): Example of General Method C of Scheme 2. A solution of *N*,2,6-trimethylaniline (6.86 g, 5 mmol) in dry THF (100 mL) under an atmosphere of N_2 was cooled to −78 °C and *n*-butyllithium (21 mL, 2.5 M solution in hexanes) was added dropwise. The mixture was allowed to warm to 0 °C, and dry CO_2 gas was bubbled in for 2–3 min. The excess CO_2 was removed under vacuum, and after the addition of further THF to replace that lost by evaporation, the solution was recooled to −78 °C. *n*-Butyllithium (22 mL, 2.5 M solution in hexanes) was again added dropwise, and the temperature was then allowed to rise slowly to −10 °C, where a deep red-colored solution was obtained. After a further 30 min at that temperature, the mixture was again recooled to −78 °C and CO_2 gas was bubbled in until the red color disappeared. The reaction mixture was allowed to warm to room temperature, and after removal of the solvent, 0.1 M HCl (50 mL) was added to initiate both deprotection of the nitrogen and ring closure. The resulting mixture was extracted with EtOAc, and this was then washed successively with 0.1 M HCl, water, and dilute aqueous Na_2CO_3. After drying (Na_2SO_4), the solvent was removed under vacuum to leave an oil which was purified by chromatography on Al_2O_3 to give 1,3-dihydro-1,6-dimethyl-2*H*-indol-2-one (7q) (3.37 g, 42%), mp (hexane) 94.5–96 °C. ^{1}H NMR ($CDCl_3$): δ 7.11 (d, *J* = 7.5 Hz, 2 H, H-4), 6.85 (d, *J* = 7.5 Hz, 2 H, H-5), 6.65 (s, 1 H, H-7), 3.47 (s, 2 H, CH_2), 3.19 (s, 3 H, 1-CH_3), 2.38 (s, 3 H, 6-CH_3). Anal. ($C_{10}H_{11}NO$) C, H, N.

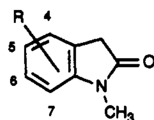
Similar preparations gave the other 1,3-dihydro-1-methyl-2*H*-indol-2-ones of Table 5 (see the supplementary material for details of NMR spectra).

1-Methyl-5-(trifluoromethyl)-1,3-dihydro-2*H*-indol-2-one (7m): Example of Method E of Scheme 2. A suspension of 5-(trifluoromethyl)-1,3-dihydro-2*H*-indol-2-one (5.03 g, 25 mmol) in water (100 mL) containing NaOH (1.5 g) was treated with Me_2SO_4 (4.7 g, 37 mmol). The mixture was warmed to 100 °C for 10 min and cooled, a further portion of Me_2SO_4 and NaOH was added, and the solution was warmed again briefly. After thorough cooling, the solid was collected and chromatographed on alumina. Elution with CH_2Cl_2/hexane (7:3) gave 1-methyl-5-(trifluoromethyl)-1,3-dihydro-2*H*-indol-2-one (7m) (3.5 g, 65%), mp (hexane) 127.5–129 °C. ^{1}H NMR ($CDCl_3$): δ 7.58 (d, *J* = 8.2 Hz, 1 H, H-6), 7.50 (s, 1 H, H-4), 6.89 (d, *J* = 8.2 Hz, 1 H, H-7), 3.58 (s, 2 H, CH_2), 3.25 (s, 3 H, CH_3). Anal. ($C_{10}H_8F_3NO$) C, H, N.

Similar methylations gave the other 1,3-dihydro-1-methyl-2*H*-indol-2-ones of Table 5 (see the supplementary material for details of NMR spectra).

6-Chloro-1,3-dihydro-1-methyl-2*H*-indol-2-one (7p): Method of Scheme 3a. A mixture of 6-chloroisatin[28] (11 g, 60 mmol) and hydrazine hydrate (20 mL) in EtOH (500 mL) was heated under reflux for 30 min to give 6-chloroisatin 3-hydrazone, mp (EtOH) 249–251 °C (lit.[47] mp 247–248 °C). ^{1}H NMR ($(CD_3)_2SO$): δ 10.85 (m, 1 H, H-1), 10.58 (d, *J* = 13.3 Hz, 1 H, NH), 9.75 (d, *J* = 13.2 Hz, 1 H, NH), 7.36 (d, *J* = 8.0 Hz, 1 H, H-4), 7.01 (dd, *J* = 2.0, 8.2 Hz, 1 H, H-5), 6.88 (d, *J* = 1.9 Hz, 1 H, H-7). The powdered hydrazone was added slowly in portions to a solution of Na (4 g) in EtOH (100 mL), and the mixture was heated under reflux gently until N_2 evolution ceased. The solution was concentrated and diluted with water, and the product was extracted with EtOAc. The residue was chromatographed on Al_2O_3, eluting with CH_2Cl_2/EtOAc (4:1), to give 6-chloro-1,3-dihydro-2*H*-indol-2-one (4.32 g, 43%), mp (aqueous EtOH) 190.5–

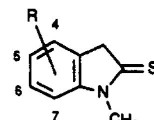
Table 5. Synthesis and Physicochemical Data for 1,3-Dihydro-1-methyl-2*H*-indol-2-ones



no.	R	method[a]	mp (°C)	C_9H_9NO	anal./lit. mp
7a	H[b]	A	87–88	C_9H_9NO	88
7b	4-Cl	B	139.5–142	C_9H_8ClNO	C, H, N, Cl
7c	4-Me[c]	C	123.5–125.5	$C_{10}H_{11}NO$	119.5–121.5
7d	4-OMe[d]	C	138–139.5	$C_{10}H_{11}NO_2$	137–138
7e	4-OAc	G	109–111	$C_{11}H_{11}NO_3$	C, H, N
7f	5-F[e]	A	130.5–132	C_9H_8FNO	128–130
7g	5-Cl[e]	A	115–117	C_9H_8ClNO	107–109
7h	5-Br[e]	F	133–135.5	C_9H_8BrNO	133.5–135
7i	5-Me[f]	C	102–104.5	$C_{10}H_{11}NO$	104–105
7j	5-OMe[g]	C	98–100	$C_{10}H_{11}NO_2$	98
7k	5-OAc	G	104–106	$C_{11}H_{11}NO_3$	C, H, N
7m	5-CF3	E	127.5–129	$C_{10}H_8F_3NO$	C, H, N
7n	5-CN	E	201–203	$C_{10}H_8N_2O$	C, H, N
7o	5-NO2[h]	F	196	$C_9H_8N_2O_3$	191–192
7p	6-Cl	E	119.5–122	C_9H_8ClNO	C, H, N, S
7q	6-Me	C	94.5–96	$C_{10}H_{11}NO$	C, H, N
7r	6-OMe[g]	C	110–113	$C_{10}H_{11}NO_2$	99–100
7s	6-OAc	G	119–121	$C_{10}H_{10}NO_3$	C, H, N
7u	7-Cl[f]	A	125–126.5	C_9H_8ClNO	120–123
7v	7-Me[f]	C	121.5–124.5	$C_{10}H_{11}NO$	119–120
7w	7-OMe[d]	C	102.5–104.5	$C_{10}H_{11}NO_2$	101–102
7x	7-OAc	G	95–97	$C_{10}H_{10}NO_3$	C, H, N
7z	7-aza	D	94–96	$C_8H_8N_2O$	C, H, N

[a] See Scheme 2 and text. [b] Reference 23. [c] Reference 50. [d] Reference 25. [e] Reference 51. [f] Reference 52. [g] Reference 48. [h] Reference 53.

Table 6. Physicochemical Data for 1,3-Dihydro-1-methyl-2*H*-indole-2-thiones



no.	R	yield[a]	mp (°C)	formula	anal./lit. mp
8a	H[b]	71	108–109	C_9H_9NS	109–111
8b	4-Cl	92	147.5–149.5	C_9H_8ClNS	C, H, N, S
8c	4-Me	81	160–162	$C_{10}H_{11}NS$	C, H, N, S
8d	4-OMe[c]	92	141–144	$C_{10}H_{11}NOS$	C, H, N, S (144–145)
8e	4-OAc	94	156	$C_{11}H_{11}NO_2S$	C, H, N, S
8f	5-F	93	155–157	C_9H_8FNS	C, H, N, S
8g	5-Cl[d]	94	163–165	C_9H_8ClNS	C, H, N, S (153–155)
8h	5-Br	95	137–139	C_9H_8BrNS	C, H, N, S
8i	5-Me[d]	86	143–145	$C_{10}H_{11}NS$	132–133
8j	5-OMe[e]	86	148–150	$C_{10}H_{11}NOS$	C, H, N, S (142–144)
8k	5-OAc	86	134–135.5	$C_{11}H_{11}NO_2S$	C, H, N, S
8m	5-CF3	96	126	$C_{10}H_8F_3NS$	C, H, N, S
8n	5-CN	41	185–187	$C_{10}H_8N_2S$	C, H, N, S
8o	5-NO2	68	>300	$C_9H_8N_2O_2S$	C, H
8p	6-Cl	87	162–165	C_9H_8ClNS	C, H, N, S
8q	6-Me	91	141–143.5	$C_{10}H_{11}NS$	C, H, N, S
8r	6-OMe[c]	87	133–136	$C_{10}H_{11}NOS$	C, H, N (135–136)
8s	6-OAc	91	131–133	$C_{11}H_{11}NO_2S$	C, H, N, S
8u	7-Cl[f]	90	126–128	C_9H_8ClNS	C, H, N, S (125–127)
8v	7-Me	86	138–139	$C_{10}H_{11}NS$	C, H, N, S
8w	7-OMe[c]	82	124–126	$C_{10}H_{11}NOS$	C, H, N, S (114–116)
8x	7-OAc	85	133–135	$C_{11}H_{11}NO_2S$	C, H, N, S
8z	7-aza	73	130–133	$C_8H_8N_2S$	C, H, N, S

[a] Yields were not optimized. [b] Reference 54. [c] Reference 55. [d] Reference 56. [e] References 55 and 56. [f] Reference 52.

193.5 °C (lit.[22] mp 196–198 °C). ^{1}H NMR (($(CD_3)_2SO$)): δ 10.49 (s, 1 H, H-1), 7.21 (d, *J* = 7.8 Hz, 1 H, H-4), 6.97 (dd, *J* = 2.0, 7.9 Hz, 1 H, H-5), 6.81 (d, *J* = 1.9 Hz, 1 H, H-7), 3.47 (s, 2 H, CH_2). Methylation of this with Me_2SO_4 in dilute NaOH as above gave 6-chloro-1,3-dihydro-1-methyl-2*H*-indol-2-one (**7p**), mp (hexane) 119.5–122 °C. ^{1}H NMR ($CDCl_3$): δ 7.15 (d, *J* = 7.8 Hz, 1 H, H-4), 7.01 (dd, *J* = 7.8 and 1.8 Hz, 1 H, H-5), 6.82 (d, *J* = 1.7 Hz, 1 H, H-7), 3.49 (s, 2 H, CH_2), 3.19 (s, 3 H, CH_3). Anal. (C_9H_8ClNO) C, H, N.

Preparation of 1,3-Dihydro-1,6-dimethyl-2*H*-indole-2-thione (8q): Example of the General Method of Scheme 1. Powdered Na_2CO_3 (0.70 g, 6.61 mmol) was added to a suspension of P_2S_5 (2.93 g, 6.61 mmol) in THF (40 mL), and the mixture was stirred vigorously at room temperature until homogeneous and gas evolution had ceased (15 min). A solution of 1,3-dihydro-1,6-dimethyl-2*H*-indol-2-one (**7q**) (0.80 g, 5.50 mmol) in THF (10 mL) was added, and stirring was continued for 18 h. After being poured into brine, the mixture was extracted into EtOAc, worked up, and chromatographed on silica gel. Elution with EtOAc/petroleum ether (1:4) gave 1,3-dihydro-1,6-dimethyl-2*H*-indole-2-thione (**8q**) (0.89 g, 91%), mp 141–143.5 °C. ^{1}H NMR ($CDCl_3$): δ 7.17 (d, *J* = 7.5 Hz, 1 H, H-4), 6.97 (d, *J* = 7.5 Hz, 1 H, H-5), 6.79 (br, 1 H, H-7), 4.05 (s, 2 H, H-3), 3.61 (s, 3 H, NCH_3), 2.42 (s, 3 H, $ArCH_3$). ^{13}C NMR ($CDCl_3$): δ 201.49 (C-2), 146.80, 138.16, 126.15 (3s), 124.92, 123.58, 110.44 (3d), 48.79 (C-3), 31.22 (NCH_3), 21.68 ($ArCH_3$). Anal. ($C_{10}H_{11}NS$) C, H, N, S.

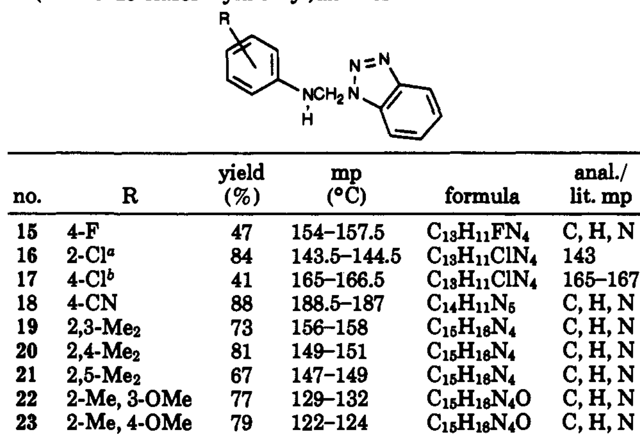
Similar reactions with other substituted 1,3-dihydro-1-methyl-2*H*-indol-2-ones gave the 1,3-dihydro-1-methyl-2*H*-indole-2-thiones of Table 6 (see the supplementary material for details of NMR spectra).

Preparation of 5-Acetoxy-1,3-dihydro-1-methyl-2*H*-indole-2-thione (8k): Example of the General Method. A solution of 1,3-dihydro-5-methoxy-1-methyl-2*H*-indol-2-one (**7i**)[48] (1.20 g, 6.77 mmol) in 48% HBr/glacial AcOH (40 mL) was heated under reflux for 6 h and then poured into water. The precipitate of crude phenol was filtered off, washed well with water, and dried and then acetylated with Ac_2O/pyridine for 1 h at 20 °C. Solvents were removed under reduced pressure, and the residue was partitioned between EtOAc and 3 N HCl. Chromatography of the organic residue on silica gel, eluting with EtOAc/petroleum ether, gave 5-acetoxy-1,3-dihydro-1-methyl-2*H*-indol-2-one (**7k**) (0.98 g, 70%), mp (EtOAc/petroleum ether) 104–106 °C. ^{1}H NMR ($CDCl_3$): δ 7.01 (br s, 1 H, H-4), 7.00 (dd, *J* = 9.1 and 2.4 Hz, 1 H, H-6), 3.53 (s, 2 H, H-3), 3.20 (s, 3 H, NCH_3), 2.30 (s, 3 H, $OCOCH_3$). ^{13}C NMR: δ 174.79 (C-2), 169.96 ($OCOCH_3$), 146.08, 142.96, 125.50 (3s), 120.84, 118.54, 108.25 (3d), 35.89 (C-3), 26.30 (NCH_3), 21.04 ($OCOCH_3$). Anal. ($C_{11}H_{11}NO_3$) C, H, N. Thiation with P_2S_5/Na_2CO_3 as above gave 5-acetoxy-1,3-dihydro-1-methyl-2*H*-indole-2-thione (**8k**) (86%), mp 134–135.5 °C. ^{1}H NMR ($CDCl_3$): δ 7.06 (br s, 2 H, H-4,6), 6.93 (d, *J* = 8.6 Hz, 1 H, H-7), 4.08 (s, 2 H, H-3), 3.60 (s, 3 H, NCH_3), 2.31 (s, 3 H, $OCOCH_3$). ^{13}C NMR: δ 200.86 (C-2), 169.62 ($OCOCH_3$), 147.62, 144.14, 130.10 (3s), 120.97, 117.99, 109.62 (3d), 48.79 (C-3), 31.24 (NCH_3), 20.94 ($OCOCH_3$). Anal. ($C_{11}H_{11}NO_2S$) C, H, N, S.

Similar reactions gave the isomeric acetoxy-1-methyl-2*H*-indol-2-ones and acetoxy-1-methyl-2*H*-indole-2-thiones of Tables 5 and 6 (see the supplementary material for details of NMR spectra).

2,2′-Dithiobis(1-methyl-*N*-phenyl-1*H*-indole-3-carboxamide) (10a): Example of the General Method of Scheme 1. A solution of 1,3-dihydro-1-methyl-2*H*-indole-2-thione (**8a**) (5.00 g, 31 mmol) in dry THF (20 mL) was added dropwise under N_2 to a stirred suspension of NaH (1.49 g of a 60% dispersion in mineral oil, 37 mmol) in THF (30 mL). After gas evolution had ceased, phenyl isocyanate (3.44 g, 32 mmol) was added and stirring was continued for 1 h before the reaction was quenched with 3 N HCl (30 mL). Extraction with EtOAc gave an oil which was adsorbed directly onto silica gel and chromatographed. Elution with EtOAc/light petroleum (1:1) gave foreruns, while EtOAc/MeOH (19:1) gave crude 2,3-dihydro-1-methyl-*N*-phenyl-2-thioxo-2*H*-indole-3-carboxamide (**9a**) as a green oil. This was dissolved in MeOH (30 mL) and filtered, and the solution was allowed to stand at 20 °C for several days with free access to oxygen, during which time yellow crystals of 2,2′-dithiobis(1-methyl-*N*-phenyl-1*H*-indole-3-carboxamide) (**10a**) (2.7 g, 31%) were deposited. A sample recrystallized from benzene had mp

Table 7. Physicochemical Data for *N*-(1*H*-Benzotriazol-1-ylmethyl)anilines



no.	R	yield (%)	mp (°C)	formula	anal./lit. mp
15	4-F	47	154–157.5	$C_{13}H_{11}FN_4$	C, H, N
16	2-Cl[a]	84	143.5–144.5	$C_{13}H_{11}ClN_4$	143
17	4-Cl[b]	41	165–166.5	$C_{13}H_{11}ClN_4$	165–167
18	4-CN	88	188.5–187	$C_{14}H_{11}N_5$	C, H, N
19	2,3-Me2	73	156–158	$C_{15}H_{16}N_4$	C, H, N
20	2,4-Me2	81	149–151	$C_{15}H_{16}N_4$	C, H, N
21	2,5-Me2	67	147–149	$C_{15}H_{16}N_4$	C, H, N
22	2-Me, 3-OMe	77	129–132	$C_{15}H_{16}N_4O$	C, H, N
23	2-Me, 4-OMe	79	122–124	$C_{15}H_{16}N_4O$	C, H, N

[a] Reference 57. [b] Reference 34.

187–188 °C. ^{1}H NMR (CDCl$_3$): δ 8.21 (s, 1 H, NH), 8.01 (d, *J* = 8.1 Hz, 1 H, H-4), 7.19 (ddd, *J* = 8.1, 7.1, and 0.9 Hz, 1 H, ArH), 7.13 (d, *J* = 4.3 Hz, 4 H, Ph), 7.09 (ddd, *J* = 8.1, 7.1, and 0.9 Hz, 1 H, ArH), 7.05 (d, *J* = 8.1 Hz, 1 H, ArH), 6.98 (quintet, *J* = 4.3 Hz, 1 H, Ph), 3.77 (s, 3 H, NCH$_3$). ^{13}C NMR: δ 161.57 (CO), 138.55, 137.94 (2s), 128.64 (d), 127.41, 126.07 (2s), 125.54, 123.56, 122.28, 122.00 (4d), 119.76 (s), 119.27, 110.14 (2s), 30.33 (NCH$_3$). Anal. ($C_{32}H_{26}N_4O_2S_2$) C, H, N, S.

A vigorously stirred suspension of the disulfide (**10a**) (1.00 g, 1.78 mmol) in MeOH (20 mL) was treated with a solution of $NaBH_4$ (0.20 g, 5.33 mmol) in MeOH (20 mL) at 20 °C for 10 min. The solution was then diluted with water, extracted with EtOAc, and worked up rapidly to give 2,3-dihydro-1-methyl-*N*-phenyl-2-thioxo-2*H*-indole-3-carboxamide (**9a**) (1.0 g, 100%), mp (N_2-purged CHCl$_3$/light petroleum) 149–151 °C. ^{1}H NMR (CDCl$_3$): δ 10.36 (s, 1 H, NH), 7.87 (d, *J* = 7.4 Hz, 1 H, ArH), 7.60 (d, *J* = 7.9 Hz, 2 H, ArH), 7.41 (t, *J* = 7.5 Hz, 2 H, ArH), 7.31 (m, 2 H, ArH), 7.11 (t, *J* = 7.3 Hz, 1 H, ArH), 7.03 (d, *J* = 7.8 Hz, 1 H, ArH), 3.73 (s, 3 H, NCH$_3$). Anal. ($C_{16}H_{14}N_2OS$) C, H, N, S.

In a similar manner, reaction of appropriate 1,3-dihydro-1-methyl-2*H*-indole-2-thiones with phenyl isocyanate followed by aerial oxidation gave the symmetrical disulfides of Table 1 (see the supplementary material for details of NMR spectra).

In instances where aerial oxidation was too slow, H_2O_2 or sodium perborate was used. For example, reaction of 1,3-dihydro-1-methyl-2*H*-pyrrolo[2,3-*b*]pyridine-2-thione (**8z**) with phenyl isocyanate as above gave 1,3-dihydro-1-methyl-*N*-phenyl-2-thioxo-2*H*-pyrrolo[2,3-*b*]pyridine-3-carboxamide (**9z**), mp 162–164 °C. ^{1}H NMR ((CD$_3$)$_2$SO): δ 12.46 (s, 1 H, CONH), 8.68 (dd, *J* = 7.7 and 1.0 Hz, 1 H, H-6), 8.02 (dd, *J* = 6.0 and 1.0 Hz, 1 H, H-4), 7.72 (d, *J* = 8.4 Hz, 2 H, H-2′,6′), 7.31 (m, 4 H, H-3,5,3′,5′), 7.01 (t, *J* = 7.3 Hz, 1 H, H-4′), 3.80 (s, 3 H, NCH$_3$). ^{13}C NMR: δ 166.96 (C-2), 129.28 (CONH), 140.77, 139.81 (2s), 129.28, 128.84 (2d), 127.21 (s), 126.84, 122.17, 118.65, 115.92 (4d), 48.57 (C-3), 29.19 (NCH$_3$). Anal. ($C_{15}H_{13}N_3OS{\cdot}CH_3OH$) C, H, N, S. This compound was quite stable to aerial oxidation. Therefore, a suspension of **9z** (0.50 g, 1.76 mmol) in glacial AcOH (50 mL) was treated with a solution of $NaBO_3$ (0.50 g, 5 mmmol) in water (25 mL), and the mixture was stirred vigorously for 1 h. The solid was then filtered off, washed with Et$_2$O, and dried to give a quantitative yield of 2,2′-dithiobis(1,3-dihydro-1-methyl-*N*-phenyl-2*H*-pyrrolo[2,3-*b*]pyridine-3-carboxamide) (**10z**), mp 197–198 °C. ^{1}H NMR ((CD$_3$)$_2$SO): δ 9.49 (s, 1 H, CONH), 8.36 (dd, *J* = 4.5 and 1.5 Hz, 1 H, H-6), 8.14 (dd, *J* = 7.9 and 1.5 Hz, 1 H, H-4), 7.19 (dd, *J* = 7.9 and 4.5 Hz, 1 H, H-5), 7.14 (m, 4 H, H-2′,3′,5′,6′), 6.99 (m, 1 H, H-4′), 3.75 (s, 3 H, NCH$_3$). ^{13}C NMR: δ 160.42 (CONH), 147.58 (s), 145.99 (d), 138.29, 129.86 (2s), 128.25, 123.05, 119.23 (3d), 118.09 (s), 117.76 (d), 117.57 (s), 28.61 (NCH$_3$). Anal. ($C_{30}H_{24}N_6O_2S_2{\cdot}2H_2O$) C, H, N, S.

2,2′-Dithiobis(5-hydroxy-1-methyl-*N*-phenylindole-3-carboxamide) (10l). A stirred suspension of the 5-acetoxy disulfide **10k** (0.25 g, 0.37 mmol) in MeOH (15 mL) was treated with $NaBH_4$ (0.05 g, 1.32 mmol) at 20 °C for 10 min. Aqueous 3 N KOH (2 mL) was then added, and after a further 15 min, the solution was diluted with water and extracted with CH_2Cl_2. The resulting oil was immediately dissolved in MeOH (3 mL) and mixed with H_2O_2 (0.10 mL of 35%). The solution was chilled at −30 °C for 48 h and then filtered to yield 2,2′-dithiobis(5-hydroxy-1-methyl-*N*-phenylindole-3-carboxamide) (**10l**) (41 mg, 19%), mp 185–187 °C. ^{1}H NMR ((CD$_3$)$_2$SO): δ 9.50 (s, 1 H, CONH), 9.15 (br, 1 H, OH), 7.32 (d, *J* = 7.8 Hz, 2 H, H-2′,6′), 7.27 (d, *J* = 8.9 Hz, 1 H, H-7), 7.19 (d, *J* = 2.3 Hz, 1 H, H-4), 7.18 (dd, *J* = 7.8 and 7.4 Hz, 2 H, H-3′,5′), 6.99 (t, *J* = 7.4 Hz, 1 H, H-4′), 6.83 (dd, *J* = 8.9 and 2.3 Hz, 1 H, H-6), 3.51 (s, 3 H, NCH$_3$). Anal. ($C_{32}H_{26}N_4O_4S_2{\cdot}H_2O$) C, H, N.

Similar reaction of the 6- and 7-acetoxy compounds (**10s,x**) gave the corresponding hydroxy compounds (**10t,y**) (Table 1) (see the supplementary material for details of NMR spectra).

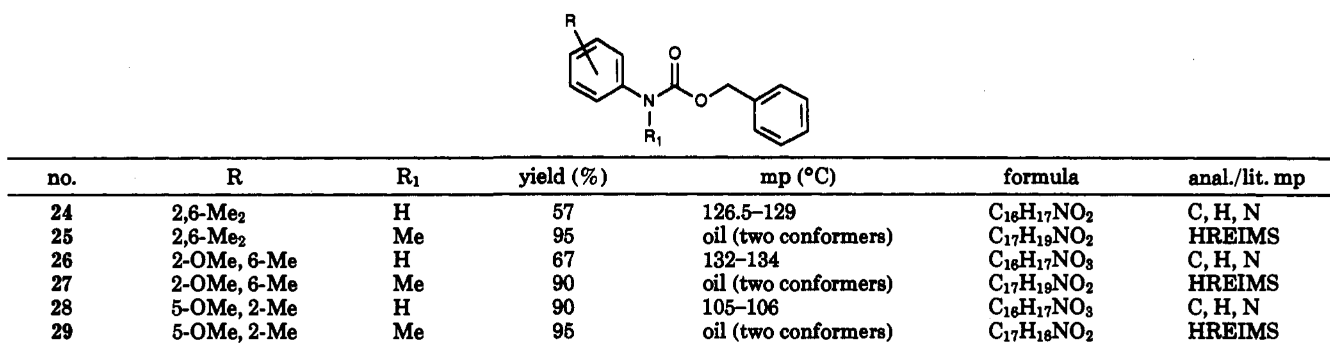
1,3-Dihydro-1,3-dimethyl-*N*-phenyl-2-thioxo-2*H*-indole-3-carboxamide (11). Reaction of 1,3-dihydro-1,3-dimethyl-2*H*-indole-2-thione with phenyl isocyanate and NaH as above gave 1,3-dihydro-1,3-dimethyl-*N*-phenyl-2-thioxo-2*H*-indole-3-carboxamide (**11**) (31%), mp 106–109 °C. ^{1}H NMR (CDCl$_3$): δ 10.02 (br, 1 H, CONH), 7.90 (d, *J* = 7.4 Hz, 1 H, H-4), 7.55 (d, *J* = 7.9 Hz, 2 H, CONH*Ph*), 7.40 (t, *J* = 7.7 Hz, 1 H, CONH*Ph*), 7.34–7.27 (m, 3 H, H-5,6,7), 7.09 (t, *J* = 7.7 Hz, 2 H, CONH*Ph*), 3.72 (s, 3 H, NCH$_3$), 1.88 (s, 3 H, 3-CH$_3$). ^{13}C NMR: δ 203.69 (C-2), 165.58 (CONH), 143.39, 137.69 (2q), 134.61 (q), 128.86, 128.70, 126.83, 125.11, 124.40, 119.62 (6d), 109.61 (C-7), 65.22 (C-3), 31.96 (NCH$_3$), 29.32 (3-CH$_3$). HREIMS: found, M$^+$ 296.0982. $C_{17}H_{16}N_2SO$ requires M$^+$ 296.0983. Anal. ($C_{17}H_{16}N_2SO$) C, H, N.

1-Methyl-2-(methylthio)-*N*-phenyl-1*H*-indole-3-carboxamide (12). A solution of **9a** (200 mg) in Me$_2$CO (20 mL) was treated with K_2CO_3 (0.12 g) and MeI (0.14 g) at 20 °C for 1 h and then diluted with CH_2Cl_2 (100 mL). Filtration and evaporation of the filtrate gave a brown oil which was chromatographed on silica gel. Elution with CH_2Cl_2 gave 1-methyl-2-(methylthio)-*N*-phenyl-1*H*-indole-3-carboxamide (**12**) (200 mg, 95%), mp (MeOH/CH$_2$Cl$_2$) 116–118 °C. ^{1}H NMR (CDCl$_3$): δ 9.99 (s, 1 H, NH), 8.58 (d, *J* = 8.0 Hz, 1 H, ArH), 7.75 (d, *J* = 7.6 Hz, 2 H, ArH), 7.38 (m, 4 H, ArH), 7.29 (quin, *J* = 4.3 Hz, 1 H, ArH), 7.12 (t, *J* = 7.4 Hz, 1 H, ArH), 3.95 (s, 3 H, NCH$_3$), 2.47 (s, 3 H, SCH$_3$). ^{13}C NMR (CDCl$_3$): δ 162.59 (s, CONH), 138.80, 137.46, 131.43 (3s, Ar), 129.03 (2d, Ar), 127.35 (s, Ar), 124.14, 123.67, 123.02, 122.24 (4d, Ar), 119.86 (2d, Ar), 114.04 (s, Ar), 109.69 (Ar), 30.23 (NCH$_3$), 20.50 (SCH$_3$). Anal. ($C_{17}H_{16}N_2OS$) C, H, N, S.

1-Methyl-*N*-phenyl-2-(phenyldithio)-1*H*-indole-3-carboxamide (13). Thiophenol (0.11 mL, 1.07 mmol) was added to a suspension of **10a** (0.30 g, 0.53 mmol) and Et$_3$N (2 drops) in CH_2Cl_2 (15 mL). After stirring at 20 °C for 4 days, the solution was adsorbed onto Florisil. Elution with CH$_2$Cl$_2$/light petroleum mixtures gave foreruns, while elution with CH_2Cl_2 gave 1-methyl-*N*-phenyl-2-(phenyldithio)-1*H*-indole-3-carboxamide (**13**) (0.27 g, 65%), mp (CH$_2$Cl$_2$ at −30 °C) 131–134 °C. ^{1}H NMR (CDCl$_3$): δ 8.70 (br s, 1 H, CONH$_2$), 8.49 (d, *J* = 8.0 Hz, 1 H, H-4), 7.42–7.12 (m, 12 H, ArH), 7.05 (t, *J* = 7.2 Hz, 1 H, H-4′), 3.79 (s, 3 H, NCH$_3$). ^{13}C NMR: δ 161.92 (CONH), 138.21, 138.18 (2s), 134.09 (d), 134.0 (s), 130.33 (d), 129.96 (s), 129.59, 128.65 (2d), 127.09 (s), 124.97, 123.63, 123.34, 122.34, 119.85 (5d), 116.39 (s), 109.93 (d), 30.63 (NCH$_3$). Anal. ($C_{22}H_{18}N_2S_2O$) C, H, N, S.

2-(Benzyldithio)-1-methyl-*N*-phenyl-1*H*-indole-3-carboxamide (14). Benzyl mercaptan (0.02 mL, 0.178 mmol) was added to a suspension of **10a** (50 mg, 89 mmol) and BF$_3$–etherate (1 drop) in CH_2Cl_2 (1 mL). After stirring at 20 °C for 3 h, the homogeneous mixture was poured into saturated aqueous $NaHCO_3$, diluted with CH_2Cl_2, and worked up, and the residue was chromatographed on silica gel. Elution with CH$_2$Cl$_2$/petroleum ether (1:1) gave foreruns, while CH_2Cl_2 eluted 2-(benzyldithio)-1-methyl-*N*-phenyl-1*H*-indole-3-carboxamide (**14**) (39 mg, 54%), mp (CHCl$_3$/petroleum ether) 146–148 °C. ^{1}H NMR (CDCl$_3$): δ 8.95 (br s, 1 H, CONH), 8.47 (dd, *J* = 7.7 and 1.3 Hz, 1 H, ArH-4), 7.66 (dd, *J* = 7.5 and 1.2 Hz, 2 H, Ph), 7.40–7.07 (m, 11 H, ArH-5,-6,-7 and Ph), 3.90 (s, 3 H, NMe). ^{13}C NMR: δ 162.31 (*C*ONHPh), 138.31, 138.04, 135.13, 130.00 (4s), 129.15, 129.06, 128.69, 127.83 (4d), 126.83 (s), 124.79, 123.94, 122.80, 122.36, 119.90, 109.92 (6d), 42.51 (*C*H$_2$Ph), 30.73 (NCH$_3$). Anal. ($C_{23}H_{20}N_2OS_2$) C, H, N, S. HRFABMS: found, (M + H)$^+$ 404.1013. $C_{23}H_{20}N_2OS_2$ requires (M + H)$^+$ 404.1017.

Table 8. Physicochemical Data for Benzyl *N*-Phenylcarbamates and Benzyl *N*-Methyl-*N*-phenylcarbamates



no.	R	R_1	yield (%)	mp (°C)	formula	anal./lit. mp
24	2,6-Me_2	H	57	126.5–129	$C_{16}H_{17}NO_2$	C, H, N
25	2,6-Me_2	Me	95	oil (two conformers)	$C_{17}H_{19}NO_2$	HREIMS
26	2-OMe, 6-Me	H	67	132–134	$C_{16}H_{17}NO_3$	C, H, N
27	2-OMe, 6-Me	Me	90	oil (two conformers)	$C_{17}H_{19}NO_2$	HREIMS
28	5-OMe, 2-Me	H	90	105–106	$C_{16}H_{17}NO_3$	C, H, N
29	5-OMe, 2-Me	Me	95	oil (two conformers)	$C_{17}H_{18}NO_2$	HREIMS

Enzyme Assays. Epidermal growth factor receptor was prepared from human A431 carcinoma cell-shed membrane vesicles as previously described.[19,38] The assays were carried out in 96-well plates as previously described,[19] using as substrate 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. As previously reported,[20] selected indolinethiones were examined in the absence of Mn^{2+} and found to retain inhibitory activity (data not shown). 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 (Interfacial Dynamics Corporation, Portland, OR) were coupled to monoclonal antibody (2–17)[39,40] 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 a final volume of 125 μL containing 25 μg polyglu$_4$tyr$_1$ 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 poly(vinylidine) 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 TCA 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.

Cell Culture and Growth Inhibition Assays. Swiss 3T3 mouse fibroblasts, A431 human epidermoid carcinoma, K562 human chronic myelogenous leukemia, and KG-1A human acute myelogenous leukemia were obtained from the American Type Culture Collection, Bethesda, MD. All cells were maintained in dMEM/F12 (50:50) (Gibco, Grand Island NY) supplemented with 10% fetal bovine serum and 50 μg/mL gentimycin. For growth inhibition assays, dilutions of compounds in 10 μL of DMSO were placed in 24-well Linbro plates (1.7 × 1.6 cm^2, 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).

Cell Extracts and Western Blot Protocols. Cells were grown to 100% confluency in 6-well plates. After the designated treatments described in the results section, the medium was removed and the monolayer scraped into 0.2 mL of boiling Laemmli buffer (50 mM Tris, pH 7, 2% sodium dodecylsulfate, 30 mM β-mercaptoethanol). The extracts were transferred to a microfuge tube and heated to 100 °C for 5 min. Thirty-five microliters of the extract was loaded onto a poly(acrylamide) gel (4–20%) and electrophoresis carried out by the method of Laemmli.[49] 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, and 0.01% azide (TNA) and blocked overnight in TNA containing 5% bovine serum albumin and 1% ovalbumin. The membrane was blotted for 2 h with antiphosphotyrosine antibody from UBI, Lake Placid, NY (1 μg/mL in blocking buffer), and then washed two times sequentially in TNA, TNA containing 0.05% Tween-20 and 0.05% nonidet P-40, and TNA. The membranes were then incubated for 2 h in blocking buffer containing 0.1 μCi/mL [^{125}I]protein A and washed again as above. The dried blots were loaded into a film cassette and exposed to X-AR X-ray film for 1–7 days. Bands were quantified by scanning densitometry.

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Supplementary Material Available: ^1H and ^{13}C NMR data for the compounds of Tables 1 and 5–8 (17 pages). Ordering information is given on any current masthead page.

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