Synthesis of C-5 Benzoyl and Azido Functionalized 2,2'-Dithiobis- and 2,2'-Diselenobis(1*H*-indoles)

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Novel methods for the synthesis of C-5 benzoyl and azido analogues of 2,2'-dithiobis(1*H*-indole), **1**, and 2,2'-diselenobis(1*H*-indole), **2**, are described to further explore the structure activity relationships in this region of the molecule. Analogues **3**-**6** displayed inhibitory activity (IC_{50} = 0.45-2.03 μM) toward the catalytic domain of the epidermal growth factor receptor tyrosine kinase that was equivalent to or better than that of unsubstituted compounds **1** and **2**. The regiochemistry of Friedel-Crafts benzoylation onto **1** was determined by X-ray crystallography. To test the potential for compounds of this class to interact with the epidermal growth factor receptor tyrosine kinase *via* a sulfhydryl exchange mechanism, reaction of a 2,2'-dithiobis(1*H*-indole) with glutathione was carried out and the product characterized.

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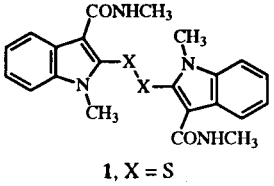
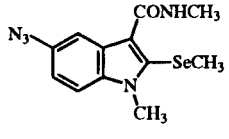
Previous reports from our laboratories have detailed the synthesis and tyrosine kinase inhibitory activity of a large series of 3-substituted 2,2'-dithiobis(1*H*-indoles) [1] and a smaller series of selenium congeners [2], both against the epidermal growth factor receptor and the nonreceptor $pp60^{v\text{-}src}$ tyrosine kinases. These two classes are exemplified by compounds **1** and **2**, two of the most potent mem-



1, X = S
2, X = Se



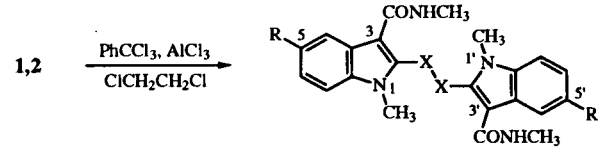
14

bers of the series. The mechanism of inhibition toward both kinases by this class has been shown to be noncompetitive with respect to both ATP and peptide substrate, and thiol reversal studies suggest that one of the possible mechanisms of inhibition is via reversible sulfhydryl exchange with a thiol-containing residue(s) within or adjacent to the catalytic domain [2].

Our prior synthetic efforts within these two series were devoted principally to the synthesis of compounds in which the benzenoid ring of the indole template was unsubstituted. In order to further explore the structure activity relationships in this region of the molecule and to lay the groundwork for potential photoaffinity labeled probes [3], we decided to target the synthesis of selected analogues possessing electron withdrawing moieties at the C-5 position. Thus, compounds **3** and **4** with the benzophenone substituent (Scheme 1) and **5** and **6** with the azide substituent (Scheme 2) became our targets.

The synthesis of benzophenone analogues **3** and **4** is shown in the Scheme 1. Starting from the known 2,2'-dithiobis(1*H*-indole) and 2,2'-diselenobis(1*H*-indole)carboxamides, **1** [1] and **2** [2], respectively, Friedel-Crafts acylation with α,α,α-trichlorotoluene under mild conditions [4] gave the desired products **3** and **4** in moderate yields. Fast atom bombardment mass spectrometry and elemental analyses confirmed the incorporation of two benzoyl moieties into **3** and **4**. However, the regiochemistry of acylation could not be unambiguously assigned by ^1H nmr NOE studies. Thus, an X-ray analysis was performed on the sulfur dimer **3**. The X-ray structure is illustrated *via* the ORTEP diagram in Figure 1, and shows that the benzoyl moieties are attached to the C-5 position of the indole ring. Crystal and refinement parameters for

Scheme 1



3, X = S, R = PhCO
4, X = Se, R = PhCO

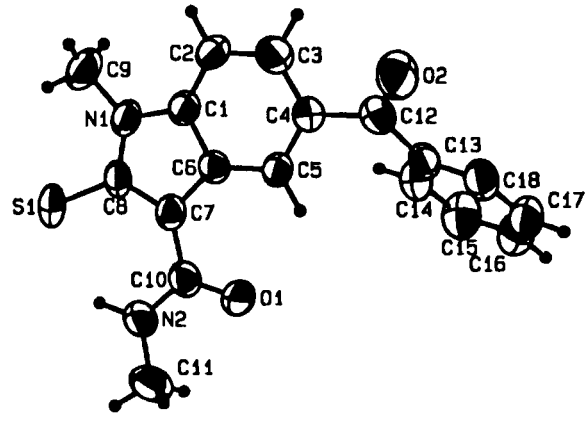


Figure 1. ORTEP plot of assymetric portion of compound **3**. The second half is generated by crystal symmetry.

Table 1
Crystal and Refinement Data for 3

formula	$C_{36}H_{30}N_4O_4S_2$
formula weight	646.79
crystal system	monoclinic
space group	C2/c (#15)
a, Å	19.250(9)
b, Å	13.644(5)
c, Å	13.602(7)
ß, deg.	117.70(4)
V, Å^3	3162.3(3)
Z	4
F(000)	1352
density (calc), g/cm^3	1.36
crystal size (mm)	0.2 x 0.2 x 0.3
&, absorbtion coef., mm	18.6
2 theta (max), deg.	148.7
reflections collected	3501
independent reflections	3219
parameters refined	221
final R indices	R1 = 0.060, wR2 = 0.071
largest diff. peak, e/Å^3	0.56

Table 2
Positional Parameters and Their Estimated Standard Deviations for 3

Atom	x	y	z	B(A2)
S1	0.97697(7)	0.38193(8)	0.16296(9)	4.76(3)
O1	0.9160(2)	0.7170(2)	0.0908(3)	6.60(9)
O2	1.1912(2)	0.8846(2)	0.0306(2)	6.86(9)
N1	1.0980(2)	0.4761(2)	0.1542(2)	4.28(9)
N2	0.8627(2)	0.5719(3)	0.0902(3)	5.0(1)
C1	1.1217(2)	0.5671(3)	0.1354(3)	4.0(1)
C2	1.1903(2)	0.5941(3)	0.1355(3)	4.7(1)
C3	1.1990(2)	0.6908(3)	0.1148(3)	4.8(1)
C4	1.1412(2)	0.7613(3)	0.1012(3)	4.0(1)
C5	1.0726(2)	0.7334(3)	0.1023(3)	3.9(1)
C6	1.0609(2)	0.6337(3)	0.1178(3)	3.7(1)
C7	0.9981(2)	0.5810(3)	0.1210(3)	3.9(1)
C8	1.0241(2)	0.4834(3)	0.1422(3)	4.0(1)
C9	1.1468(3)	0.3890(3)	0.1843(4)	6.0(1)
C10	0.9232(2)	0.6272(3)	0.1006(3)	4.4(1)
C11	0.7888(2)	0.6125(4)	0.0682(4)	6.5(1)
C12	1.1541(2)	0.8656(3)	0.0806(3)	4.5(1)
C13	1.1250(2)	0.9453(3)	0.1264(3)	4.1(1)
C14	1.1176(2)	0.9346(3)	0.2222(3)	5.0(1)
C15	1.0969(3)	1.0144(4)	0.2661(4)	6.3(1)
C16	1.0831(3)	1.1047(4)	0.2132(4)	6.6(1)
C17	1.0883(3)	1.1145(3)	0.1188(4)	5.8(1)
C18	1.1102(2)	1.0369(3)	0.0749(3)	4.9(1)

compound **3** and positional parameters and their estimated standard deviations are shown in Tables 1 and 2, respectiviely. Bond distances and angles of **3** are tabulated in Tables 3 and 4, respectively.

The synthesis of bisazides **5** and **6**, as shown in Scheme 2, required that we develop a general method for the synthesis of 5-azido substituted analogues of **1** and **2**. Reaction of 1-methyl-1,3-dihydroindol-2-one (**7**) [5] with fuming nitric acid in trifluoroacetic acid at 0° [6] furnished the 5-nitrooxindole **8**. The position of nitration on the oxindole was determined by ^{1}H nmr NOE studies. The ^{1}H nmr showed a pattern of a doublet of doublets at δ 8.27 ppm (J = 8.7, 2.4 Hz) and two additional doublets at δ 7.20 ppm (J = 8.7, assigned to H-7) and δ 8.15 ppm (J =

Scheme 2

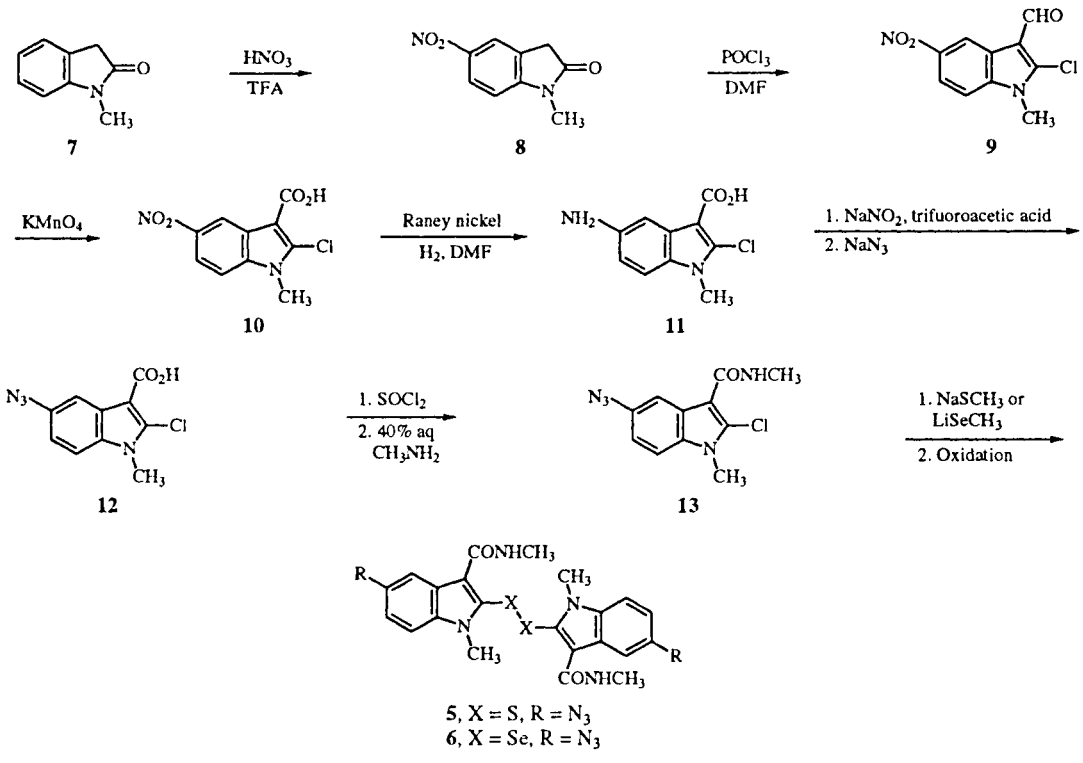


Table 3
Bond Distances (Å) for 3 [a]

Atom 1	Atom 2	Distance	Atom 1	Atom 2	Distance
S1	C8	1.749(4)	C4	C12	1.493(6)
O1	C10	1.233(5)	C5	C6	1.410(5)
O2	C12	1.221(6)	C6	C7	1.426(6)
N1	C1	1.387(5)	C7	C8	1.404(5)
N1	C8	1.357(6)	C7	C10	1.477(6)
N1	C9	1.451(5)	C12	C13	1.485(6)
N2	C10	1.338(6)	C13	C14	1.384(7)
N2	C11	1.423(6)	C13	C18	1.397(6)
C1	C2	1.370(6)	C14	C15	1.388(7)
C1	C6	1.410(6)	C15	C16	1.386(7)
C2	C3	1.375(6)	C16	C17	1.340(8)
C3	C4	1.416(6)	C17	C18	1.376(7)
C4	C5	1.381(6)			

[a] Numbers in parentheses are estimated standard deviations in the least significant digits.

Table 4
Bond Angles (deg) for 3 [a]

Atom 1	Atom 2	Atom 3	Angle	Atom 1	Atom 2	Atom 3	Angle
C1	N1	C8	109.1(3)	C8	C7	C10	131.9(4)
C1	N1	C9	124.1(4)	S1	C8	N1	121.0(3)
C8	N1	C9	126.8(4)	S1	C8	C7	128.4(4)
C10	N2	C11	122.6(4)	N1	C8	C7	110.4(4)
N1	C1	C2	129.8(4)	O1	C10	N2	120.1(4)
N1	C1	C6	106.9(4)	O1	C10	C7	119.7(4)
C2	C1	C6	123.3(4)	N2	C10	C7	120.2(4)
C1	C2	C3	117.8(4)	O2	C12	C4	119.9(4)
C2	C3	C4	121.0(4)	O2	C12	C13	120.7(4)
C3	C4	C5	120.5(4)	C4	C12	C13	119.3(4)
C3	C4	C12	118.8(4)	C12	C13	C14	122.8(4)
C5	C4	C12	120.6(4)	C12	C13	C18	118.2(4)
C4	C5	C6	119.1(4)	C14	C13	C18	118.8(4)
C1	C6	C5	118.1(4)	C13	C14	C15	119.9(4)
C1	C6	C7	108.5(3)	C14	C15	C16	119.8(5)
C5	C6	C7	133.4(4)	C15	C16	C17	120.4(5)
C6	C7	C8	104.9(4)	C16	C17	C18	120.9(4)
C6	C7	C10	123.1(3)	C13	C18	C17	120.1(5)

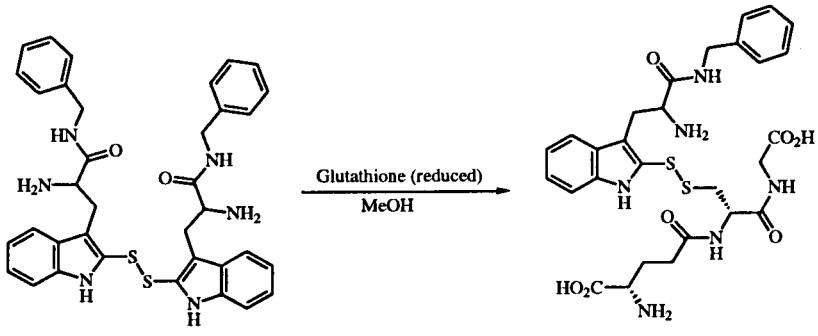
[a] Numbers in parentheses are estimated standard deviations in the least significant digits.

2.4 Hz), indicating either C-5- or C-6 substitution. An NOE between the *N*-methyl protons of the indole and the doublet (J = 8.7 Hz) at δ 7.20 ppm assigns the nitro substituent to C-5. Vilsmeier formylation of **8**, carried out under previously described conditions [1] provided **9** in quantitative yield. Oxidation of this with potassium permanganate proceeded smoothly to provide acid **10** in 60-70% yield. Catalytic hydrogenation of **10** with Raney nickel afforded amine **11**, which upon standard diazotization followed by reaction with sodium azide yielded the highly functionalized azido acid **12**. Amidation of **12** under Schotten-Bauman conditions with 40% aqueous methylamine then gave the key intermediate **13**. Addition of solid **13** to a room temperature solution of three equivalents of sodium thiomethoxide in *N,N*-dimethylacetamide followed by heating at 60° for 1 hour, then oxidative dimerization with sodium perborate, provided the target 2,2'-dithiobis(1*H*-indole)bisazide **5** in 67% yield. However, similar reaction of **13** with lithium thiomethoxide, lithium selenomethoxide, or sodium selenomethoxide was unsuccessful, perhaps due to a greater proclivity of these nucleophiles toward azide reduction [7]. To introduce the diselenium moiety, we found it necessary to conduct the initial displacement reaction at -20° with three equivalents of freshly prepared lithium selenomethoxide. Warming the reaction slowly to -5° completed the reaction and provided the target 2,2'-diselenobis(1*H*-indole)bisazide **6** in 44% yield after workup. If one carries out the displacement with 1.5 equivalents of selenomethoxide and works up the reaction at -20°, then the monomeric intermediate, 5-azido-2-methylselanyl-1*H*-indole-3-carboxylic acid methylamide (**14**), can be isolated. These same low temperature conditions work equally well for displacements with lithium thiomethoxide. The structural assignments of **5** and **6** are fully supported by ^{1}H nmr and infrared spectroscopy, and by microanalysis.

The inhibitory activity of compounds **3-6** was determined against the epidermal growth factor receptor catalytic domain. The IC_{50} values for the 2,2'-dithiobis(1*H*-indoles) are 2.03 μ*M* for benzophenone **3** and 0.97 μ*M* for azide **5**. The IC_{50} for the corresponding C-5 unsubstituted compound **1** is 6.9 μ*M* [1]. The respective IC_{50} values for the 2,2'-diselenobis(1*H*-indoles) are 0.70 μ*M* for **4**, 0.45 μ*M* for **6**, and 6.1 μ*M* for **2** [2]. Thus, these results reveal that the benzoyl and azide substitutents at C-5 of the indole ring enhance inhibitory activity within this class of compounds and confirm previous structure-activity conclusions which show diselenides to be more potent epidermal growth factor receptor epidermal growth factor receptor inhibitors than the corresponding disulfides.

Our prior studies have shown that compounds of the diselenium series generally display about 10-fold greater potency toward the epidermal growth factor receptor tyrosine kinase than direct congeners of the disulfur series [2]. We attribute this in part to the greater stability of the diselenide bond relative to the disulfide moiety. Accordingly, one would expect disulfur series compounds to be more reactive toward sulfhydryl exchange mechanisms. To test this out, we looked at the reaction of glutathione with one of the most potent members, **15**, [2] of the 2,2'-dithiobis(1*H*-indole) series, which had been derived from tryptophan. Thus reaction of a methanolic solution of **15** with an excess of reduced glutathione at room temperature for three days led to a slow sulfhydryl exchange to provide the mixed disulfide **16** in 81% yield (Scheme 3). However, similar reaction of the diselenium congener of **15** [2] under the same conditions gave only recovered starting materi-

Scheme 3



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als. This finding lends support to a putative mechanism of more facile inactivation of compounds of the disulfur series by intracellular glutathione prior to interaction with the receptor where inhibition likely occurs *via* sulfhydryl exchange with one or more of the thiol residues located within or near the catalytic domain. Our results here are corroborated by prior reversal studies with dithiothreitol which caused a general decrease in inhibition of the epidermal growth factor receptor tyrosine kinase by both the diselenium and disulfur series. However, the reversal of enzyme inhibition occurred less readily within the diselenium series [2]. Compound **15** weakly inhibited the epidermal growth factor receptor tyrosine kinase catalytic domain with an IC_{50} = *ca.* 50 μ*M*.

In summary, we have developed novel methods for the synthesis of C-5 benzoyl and azido analogues of 2,2'-dithiobis- and 2,2'-diselenobis(1*H*-indoles). These showed inhibitory activity toward the catalytic domain of the epidermal growth factor receptor tyrosine kinase that is equivalent to or better than that of their unsubstituted compounds. We also tested the potential for compounds of this class to interact with the epidermal growth factor receptor tyrosine kinase *via* a sulfhydryl exchange mechanism *via* reaction of a 2,2'-dithiobis(1*H*-indole) with glutathione.

EXPERIMENTAL

Melting points were measured with a Gallenkamp melting point apparatus and are uncorrected. Infrared (ir) spectra were recorded on a Mattson Cygnus 100 FTIR spectrometer. Proton and carbon nuclear magnetic resonance (^1H nmr and ^{13}C nmr) spectra were measured on a Varian Unity 400, Varian XL300, or Gemini 200 NMR spectrometer. Chemical shifts are reported as δ values (parts per million) downfield from internal tetramethylsilane. The following abbreviations are used to describe peak patterns when appropriate: br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Mass spectra were obtained on a Finnigan TSQ-70 (fast atom bombardment), Fisons VG Trio-2A (chemical ionization), or Fisons VG Trio-2000 (electrospray) mass spectrometer. High resolution spectra were obtained on a Finnigan MAT-900Q electrospray instrument operating in the positive ionization mode. The uv spectra were recorded in methanol on a Varian Cary 3E uv-visible spectrophotometer. High pressure liquid chromatography (hplc) was performed on a Varian Vista 5500 instrument. Combustion analyses were carried out on a CEC 440 Elemental Analyzer (CHN analysis) or by Robertson Microlit Laboratories, Inc., Madison, NJ. Amino acid analysis was carried out on an Applied Biosystems 420A/H instrument. Column chromatography was carried out in the flash mode utilizing E. Merck 230-400-mesh silica gel. Analytical tlc was carried out on E. Merck silica gel 60 F_{254} plates with detection by uv light. All reaction solvents were reagent grade or distilled-in-glass, and were stored over activated 3Å (for lower alcohols) or 4Å molecular sieves. Following normal workup procedures, organic extracts were dried over anhydrous sodium sulfate prior to concentration.

2,2'-Dithiobis[5-benzoyl-*N*,1-dimethyl-1*H*-indole-3-carboxamide] (3).

To a 0-5° solution of 912 mg (6.8 mmoles) of anhydrous aluminum chloride in 10 ml of 1,2-dichloroethane was added dropwise 0.33 ml (2.3 mmoles) of α,α,α-trichlorotoluene over 5 minutes. The mixture was stirred for 15 minutes at 0°, then 500 mg (1.1 mmoles) of 2,2'-dithiobis[*N*,1-dimethyl-1*H*-indole-3-carboxamide] (1) [1] was added over 5 minutes. The cooling bath was removed and the mixture was stirred at room temperature for 5 hours. The dark solution was poured onto crushed ice and the mixture was stirred at 75° for 30 minutes. The cooled mixture was extracted with dichloromethane, and the combined organic phases were washed with brine, dried, and concentrated to a yellow residue that was crystallized from dichloromethane. The solids were collected to give 360 mg (50%) of **3** as fine needles, mp 225-227° dec; ^1H nmr (dimethyl sulfoxide-d_6): δ 8.27 (s, 1H), 7.80-7.53 (m, 7H), 7.38 (br, exchangeable, 1H), 3.81 (s, 3H), 2.12 (d, J = 4.4 Hz, 3H); ^{13}C nmr (dimethyl sulfoxide-d_6): δ 195.6, 162.5, 139.7, 138.0, 132.1, 130.2, 130.0, 129.4, 128.4, 125.5, 125.1, 124.5, 120.7, 111.2, 30.4, 25.4; ir (potassium bromide): 3406, 1649, 1606 cm^{-1}; uv (methanol): λ (log ε) 255 nm (4.78), 293 (4.64); ms: (fast atom bombardment) m/z 647 (M^+, 100).

Anal. Calcd. for $C_{36}H_{30}O_4N_4S_2 \cdot 0.2CH_2Cl_2$: C, 65.83; H, 4.63; N, 8.49; S, 9.72; Cl, 1.61. Found: C, 65.91; H, 4.87; N, 8.38; S, 9.74; Cl, 1.55.

2,2'-Diselenobis[5-benzoyl-*N*,1-dimethyl-1*H*-indole-3-carboxamide] (4).

Benzoylation of 2,2'-diselenobis[*N*,1-dimethyl-1*H*-indole-3-carboxamide] (2) [2] was carried out as described above for **1** to

give **4** in 33% yield following column chromatography on silica gel, eluting with 1:1:0.05 toluene:ethyl acetate:methanol, then crystallization from dichloromethane: mp 247-250° dec; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 8.26 (s, 1H), 7.74-7.55 (m, 8H), 3.77 (s, 1H), and 2.25 (d, J = 13.8 Hz, 3H); ^{13}C nmr (dimethyl sulfoxide-d_6): δ 195.8, 163.3, 139.9, 138.1, 132.1, 129.7, 129.5, 128.4, 127.8, 125.0, 124.9, 124.8, 120.4, 111.1, 32.1, 25.5; ir (potassium bromide): 3391, 1647, 1604 cm^{-1}; uv (methanol): λ (log ε) 254 nm (4.75), 298 (4.58).

Anal. Calcd. for $C_{36}H_{30}O_4N_4Se_2$•0.5H_2O: C, 57.68; H, 4.17; N, 7.47. Found: C, 57.52; H, 3.95; N, 7.20.

1-Methyl-5-nitro-1,3-dihydroindol-2-one (**8**).

To a stirred ice-cold solution of 7.0 g (48 mmoles) of 1-methyl-1,3-dihydroindol-2-one (**7**) [5] in 70 ml of trifluoroacetic acid was added 4.0 g (64 mmoles) of fuming nitric acid over 10 minutes. Following addition, the ice bath was removed and the mixture was stirred at room temperature for 5 minutes, then poured carefully into ice water. The precipitate was collected, washed with water until *p*H 7, and dried to give a crude solid that was chromatographed on silica gel eluting with 1:1 ethyl acetate:hexanes. The product fractions were concentrated to a solid that was crystallized from ethyl acetate:hexanes. The solids were collected to give 6.3 g (70%) of **8**, mp 195-195.5° (lit [8] mp 198°); ^{1}H nmr (dimethyl sulfoxide-d_6): δ 8.27 (dd, J = 8.7, 2.4 Hz, 1H), 8.15 (d, J = 2.4 Hz, 1H), 7.20 (d, J = 8.7 Hz, 1H), 3.71 (s, 2H), 3.19 (s, 3H); ir (potassium bromide): 3080, 1720, 1616 cm^{-1}; ms: (chemical ionization) m/z 193 (MH^+, 100).

Anal. Calcd. for $C_9H_8O_3N_2$: C, 56.27; H, 4.27; N, 14.41. Found: C, 56.25; H, 4.20; N, 14.58.

2-Chloro-1-methyl-5-nitro-1*H*-indole-3-carbaldehyde (**9**).

A solution of 8.0 ml of phosphorus oxychloride in 15 ml of dichloromethane was slowly added to a ice-cold solution of 8 ml of *N,N*-dimethylformamide and 15 ml of dichloromethane. The bath was removed and the mixture was stirred at room temperature for 10 minutes. To the solution of Vilsmeier reagent was added portionwise 6.0 g (32 mmoles) of solid nitro oxindole **8**. The resultant bright red suspension was heated at 50° for 18 hours, cooled, and carefully poured into ice water. The suspension was treated portionwise with 30 g of 50% aqueous sodium hydroxide. The resultant precipitate was collected, washed with water until *p*H 7, and dried to leave 7.9 g (100%) of **9**, mp 202.5-203.5°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 10.05 (s, 1H), 8.88 (d, J = 2.2 Hz, 1H), 8.22 (dd, J = 9.2, 2.2 Hz, 1H), 7.90 (d, J = 9.2 Hz, 1H), 3.91 (s, 3H); ir (potassium bromide): 3113, 1660, 1618, 1587 cm^{-1}; ms: (chemical ionization) m/z 239 (MH^+, 100).

Anal. Calcd. for $C_{10}H_7O_3N_2Cl$: C, 50.37; H, 2.95; N, 11.67; Cl, 14.69. Found: C, 50.33; H, 2.96; N, 11.74; Cl, 14.86.

2-Chloro-1-methyl-5-nitro-1*H*-indole-3-carboxylic Acid (**10**).

To a room temperature solution of 7.9 g (33 mmoles) of aldehyde **9** in 200 ml of acetone was added in one charge a solution of 7.8 g (49.5 mmoles) of potassium permanganate in 100 ml of water. The mixture was stirred at room temperature for 5 hours, then treated with 30% hydrogen peroxide to decompose excess permanganate. The resultant suspension was basified with 50% aqueous sodium hydroxide until *p*H 13 and filtered through Celite® with the pad washed well with water. The filtrate was acidified to *p*H 4 with 6 *N* aqueous hydrochloric acid. The solids were collected, washed with water, and dried to leave 5.5 g (65%) of **10**: mp 200.5-201.5°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 13.02 (s, 1H, exchangeable), 8.86 (d, J = 2.3 Hz, 1H), 8.18 (dd, J = 9.2, 2.4 Hz, 1H), 7.84 (d, J = 9.2 Hz, 1H), 3.88 (s, 3H); ir (potassium bromide): 3097, 1674, 1658, 1618, 1585, 1516 cm^{-1}; ms: (chemical ionization) m/z 255 (MH^+, 100).

Anal. Calcd. for $C_{10}H_7O_4N_2Cl$: C, 47.48; H, 2.89; N, 10.86; Cl, 13.59. Found: C, 47.17; H, 2.77; N, 11.00; Cl, 13.92.

5-Amino-2-chloro-1-methyl-1*H*-indole-3-carboxylic Acid (**11**).

A mixture of 5.6 g (21.9 mmoles) of nitro acid **10**, 100 ml of *N,N*-dimethylformamide, and 2.0 g of Raney nickel was hydrogenated at 50 psi for 4.5 hours. The mixture was filtered, and the filtrate was concentrated to 50 ml then poured into 1.5 liters of ice water. The precipitated solids were collected and washed with water to leave 4.4 g (89%) of **11**, mp 278.0-279.0°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 7.24 (d, J = 8.8 Hz, 1H), 7.21 (s, 1H), 6.61 (dd, J = 8.8, 2.0 Hz, 1H), 3.69 (s, 3H); ir (potassium bromide): 3433, 3350, 1633, 1500 cm^{-1}; ms: (chemical ionization) m/z 225 (MH^+, 100).

Anal. Calcd. for $C_{10}H_9O_2N_2Cl$: C, 53.47; H, 4.04; N, 12.47; Cl, 15.78. Found: C, 53.37; H, 3.83; N, 12.42; Cl, 15.58.

5-Azido-2-chloro-1-methyl-1*H*-indole-3-carboxylic Acid (**12**).

To a -5 to 0° solution of 4.06 g (18.1 mmoles) of amine **11** in 85 ml of trifluoroacetic acid was added 4.6 g (67 mmoles) of sodium nitrite over 30 minutes. The resultant dark red solution maintained at -5 to 0° was treated with 10.5 g (161.5 mmoles) of sodium azide. The mixture was then poured into ice water and the precipitated solids were collected, washed with water, and dried to leave a dark residue that was chromatographed on silica gel, eluting with 1:1 ethyl acetate:hexanes, to give 3.85 g (85%) of **12** following crystallization from ethyl acetate:hexanes, mp 159.5° dec; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 12.6 (br, exchangeable, 1H), 7.70 (d, J = 2.2 Hz, 1H), 7.66 (d, J = 8.8 Hz, 1H), 7.04 (dd, J = 8.8, 2.2 Hz, 1H), 3.81 (s, 3H); ir (potassium bromide): 2106, 1649, 1620 cm^{-1}.

Anal. Calcd. for $C_{10}H_7O_2N_4Cl$•0.1H_2O: C, 47.58; H, 2.87; N, 22.19; Cl, 14.04. Found: C, 47.92; H, 2.66; N, 22.07; Cl, 13.71.

5-Azido-2-chloro-1-methyl-1*H*-indole-3-carboxylic Acid Methylamide (**13**).

Thionyl chloride (1.5 ml) was slowly added to a room temperature suspension of 1.2 g (4.9 mmoles) of azido acid **12** in 10 ml of dichloromethane. The reaction mixture was heated to 70° for 1 hour then concentrated to a solid residue that was cooled in a ice bath. To the cold residue was added 13 ml of 40% aqueous methylamine in one portion. The mixture was stirred for 30 minutes and the solids were collected, washed with water, and dried to give crude product that was chromatographed on silica gel, eluting with 1:1 ethyl acetate:hexanes, to give 960 mg (74%) of **13** following crystallization from ethyl acetate:hexanes, mp 153.5-154.5°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 7.68 (m, 1H, exchangeable), 7.64 (d, J = 2.2 Hz, 1H), 7.60 (d, J = 9.7 Hz, 1H), 7.01 (dd, J = 9.7, 2.2 Hz, 1H), 3.78 (s, 3H), 2.81 (d, J = 4.6 Hz, 3H); ir (potassium bromide): 3315, 2116, 1624 cm^{-1}; ms: (chemical ionization) m/z 264 (MH^+, 100).

Anal. Calcd. for $C_{11}H_{10}ON_5Cl$: C, 49.92; H, 3.77; N, 26.42; Cl, 13.57. Found: C, 50.11; H, 3.82; N, 26.56; Cl, 13.45.

2,2'-Dithiobis[5-azido-*N*,1-dimethyl-1*H*-indole-3-carboxamide] (**5**).

To an ice-cold solution of 167 mg (2.38 mmoles) of sodium thiomethoxide in *N,N*-dimethylacetamide was added 92 mg

(0.34 mmole) of amide **13** in one charge. The mixture was heated to 60° for 1 hour then quenched with 4 ml of 5% aqueous hydrochloric acid. The resultant suspension was extracted twice with dichloromethane. The combined organic phases were washed with brine, dried, and concentrated. The orange solids were suspended in 2 ml of 1:1 acetic acid:water and treated with 163 mg (1.0 mmole) of sodium perborate tetrahydrate. The suspension was stirred at room temperature for 30 minutes then the yellow solids were collected, washed with water, and dried to give 59 mg (67%) of **5**, mp 182-184° dec; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 7.64 (d, J = 9.0 Hz, 1H), 7.60 (d, J = 1.9 Hz, 1H), 7.32 (m, 1H, exchangeable), 7.09 (dd, J = 9.0, 1.9 Hz, 1H), 3.69 (s, 3H), 2.21 (d, J = 4.5 Hz, 3H); ir (potassium bromide): 3404, 3346, 3284, 2116, 1629 cm^{-1}; uv (methanol): λ (log ε) 250 nm (4.76), 319 (4.09).

Anal. Calcd. for $C_{22}H_{20}O_2N_{10}S_2 \cdot 0.1H_2O$: C, 50.58; H, 3.90; N, 26.81; S, 12.27. Found: C, 50.55; H, 3.85; N, 26.42; S, 12.19.

2,2'-Diselenobis[5-azido-*N*,1-dimethyl-1*H*-indole-3-carboxamide] (**6**).

To suspension of 75 mg (0.95 mmole) of elemental selenium in 2 ml of tetrahydrofuran was added 0.7 ml of methyllithium:lithium bromide complex (1.5 *M* in ether) at room temperature. A stream of nitrogen was blown into the reaction mixture at 80° to evaporate the tetrahydrofuran. The resultant white semisolid of lithium selenomethoxide was diluted with 2 ml of *N*,*N*-dimethylacetamide and cooled to -20°. Then 100 mg (0.4 mmole) of amide **13** in 2 ml of *N*,*N*-dimethylacetamide was added slowly over 10 minutes to the solution maintained at -20 to -10°. The resultant mixture was stirred at -15 to -10° for 1 hour, -10 to -5° for 1 hour, and -5 to 0° for 16 hours. The solution was treated with 4 ml of ice-cold 2:1 acetic acid:water, then stirred at room temperature for 30 minutes. The resultant yellow precipitate was collected, washed sequentially with water and ether, and dried at 40° overnight to leave 50 mg (44%) of **6**: mp 184-186° dec; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 7.61 (d, J = 9.0 Hz, 1H), 7.59 (br, 1H), 7.49 (m, 1H), 7.04 (dd, J = 9.0, 2.2 Hz, 1H), 3.66 (s, 3H), 2.37 (d, J = 4.6 Hz, 3H); ir (potassium bromide): 3383, 3340, 3275, 2114, 1624, 1539 cm^{-1}.

Anal. Calcd. for $C_{22}H_{20}O_2N_{10}Se_2$: C, 43.01; H, 3.28; N, 22.80. Found: C, 43.21; H, 3.25; N, 22.41.

2-Amino-4-[2-[3-(2-amino-2-benzylcarbamoyl-ethyl)-1*H*-indol-2-yldisulfanyl]-1-(carboxymethylcarbamoyl)ethylcarbamoyl]-butyric Acid, (*RS*);L,L (**16**).

A solution of 204 mg (0.3 mmole) of 2,2'-dithiobis[α-amino-*N*-(phenylmethyl)-1*H*-indole-3-propanamide] (**15**) [2] and 114 mg (0.7 mmole) of glutathione (reduced) in 40 ml of methanol was stirred at room temperature for one day. An additional 110 mg (0.7 mmole) of glutathione was added and the solution was stirred for two more days. The solution was concentrated and the residue was purified by preparative hplc eluting with 0.1% trifluoroacetic acid in water and acetonitrile to yield 164 mg (81%) of the desired product, mp 171-173°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 1.98 (m, 2H), 2.34 (m, 2H), 2.95 (m, 1H), 3.20 (m, 1H), 3.29-3.38 (m, br, 2H), 3.76 (d, J = 6.0 Hz, 2H), 3.82-3.93 (m, 2H), 4.05-4.15 (m, 2H), 4.68 (m, 1H), 6.77 (m, 1H), 6.86 (m, 1H), 7.07 (t, J = 7.5 Hz, 1H), 7.16-7.22 (m, 4H), 7.40 (d, J = 8.2 Hz, 1H), 7.58 (t, J = 8.2 Hz, 1H), 8.45-8.61 (m, 9H, exchangeable), and 11.65 (d, J = 1.7 Hz, 1H, exchangeable); ir (potassium bromide): 3404, 3394, 3300, 3284, 3063, 3030, 2928, 1730, 1672, 1539, and 1201 cm^{-1}; hrms: m/z Calcd. for $C_{28}H_{34}N_6O_7S_2 \cdot H^+$: 631.2008. Found: 631.2012. Amino acid analysis: Gly and Glu/Gln; hplc (mobile phase of 7:3 0.1% aqueous trifluoroacetic acid: 0.1% trifluoroacetic acid in acetonitrile): >99% purity.

Crystal Structure Determination of **3**.

The title compound crystallized from acetonitrile as amber rods. The crystals are monoclinic, space group C2/c, with unit cell dimensions; a = 19.250(9), b = 13.644(5), c = 13.602(7) Å, ß = 117.730(4)°, z = 4; V= 3162.3(3) Å^3. Lattice constants and intensity data were measured at room temperature using graphite monochomated CuKα radiation, lambda = 1.54184 Å, on an Enraf-Nonius CAD-4 diffractometer. A total of 3501 reflections were measured, of which 3219 were unique and not systematically absent. The structure was determined using MULTAN and refined by full matrix least squares using 1726 refections with I/sigma(I)>3.0. Heavy atoms were refined using anisotropic temperature factors. Calculated positions and isotropic temperature factors for hydrogen atoms were included in structure factor calculations, but were not refined. The final unweighted and weighted R-factors were 0.060 and 0.071 respectively. The highest peak in the final difference map was 0.56 e/Å^3.

Enzyme Assay.

Most of the chemicals used in this assay were purchased from Sigma. For other reagents, trichloroacetic acid was purchased from E. Merck, [γ-^{32}P]ATP with a specific activity of 3 Ci/mmol (2 mCi/ml) in aqueous solution from Amersham Life Sciences, and the scintillation fluid from Beckman. The epidermal growth factor receptor catalytic domain (ser671-phe973) was derived from human cDNA and expressed in SF9 insect cells [9] and purified with M2 affinity gel from Eastman Chemical Company as described by Fry *et al.* [10] with a modification in which the DNA constructs were placed under the transcriptional control of the polyhedrin promoter only of the baculovirus transfer vector. Counts were recorded on a Pharmacia 1450 Microbeta Liquid Scintillation Counter. The assay was performed as previously described [1,2].

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