# Tyrosine Kinase Inhibitors. 6. Structure−Activity Relationships among *N*- and 3-Substituted 2,2′-Diselenobis(1*H*-indoles) for Inhibition of Protein Tyrosine Kinases and Comparative *in Vitro* and *in Vivo* Studies against Selected Sulfur Congeners

H. D. Hollis Showalter,*,† Anthony D. Sercel,† Boguslawa M. Leja,† Craig D. Wolfangel,† Linda A. Ambroso,† William L. Elliott,† David W. Fry,† Alan J. Kraker,† Curtis T. Howard,† Gina H. Lu,† Charles W. Moore,† James M. Nelson,† Bill J. Roberts,† Patrick W. Vincent,† William A. Denny,‡ and Andrew M. Thompson‡

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

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A small series of 2,2′-diselenobis(1*H*-indoles) was synthesized as redox-modified congeners of our earlier reported 2,2′-dithiobis(1*H*-indole) series. Utilizing chemistry similar to that developed earlier for the disulfur series, compounds were made from 2-halogeno-3-indolecarboxylic acid precursors bearing various polar functionality at the C-3 position and small alkyl substituents at the N-1 position of the indole nucleus. Additional compounds were derived from (*R*)- or (*S*)-tryptophan via a novel application of diselenium dichloride as an electrophilic source of diselenium, and a much improved process to a 2,2′-dithiobis(1*H*-indole) congener was developed utilizing disulfur dichloride as a source of disulfur. Against isolated epidermal growth factor receptor (EGFr), platelet-derived growth factor receptor (PDGFr), and v-src tyrosine kinases, compounds in this series displayed broad inhibitory activity with $IC_{50}$ = 0.9 to >100 $\mu$M vs EGFr, 3.4 to >50 $\mu$M vs PDGFr, and 0.4−6.7 $\mu$M vs v-src. In general, compounds derived from tryptophan displayed the greatest potency against EGFr and those from 2-halogeno-3-indolecarboxylic acids greater potency against PDGFr and v-src. Enzyme kinetics studies showed that both classes of compounds display primarily noncompetitive inhibition with respect to either ATP or peptide substrate. The sulfhydryl reducing agent dithiothreitol (DTT) caused a general decrease in inhibition of the EGFr and v-src tyrosine kinases by both the diselenium and disulfur series with the reversal of enzyme inhibition occurring less readily within the diselenium series. In whole cell studies, compounds of this class were growth inhibitory against Swiss 3T3 mouse fibroblasts with $IC_{50}$ values from 0.5 to 19.5 $\mu$M, and the observed SAR was different from that of the 2,2′-dithiobis(1*H*-indoles). A comparative study in the same cell line on the effects of the 2,2′-diselenobis(1*H*-indole) derived from (*R*)-tryptophan vs its disulfur congener on growth factor mediated tyrosine phosphorylation showed that this compound significantly inhibited EGFr and PDGFr (in response to its ligand) autophosphorylation with complete suppression at 25 and 5 $\mu$M, respectively. Tyrosine phosphorylation of an 85 kDa protein typically phosphorylated in response to bFGF was also exquisitely sensitive to this compound, and it displayed inhibitory effects on DNA, RNA, and protein synthesis at submicromolar concentrations.The disulfur congener exhibited a qualitatively similar pattern; however, its potency was 10-fold less. This same diselenium/disulfur pair was evaluated *in vivo* against the B16 melanoma, colon carcinoma 26, and M5076 sarcoma murine tumors, and the A431 epidermoid, and C6 glioma human tumor xenografts. At maximum tolerated doses (1.8 and 5.0 mg/kg/injection, respectively), neither the diselenium nor disulfur congener was effective against the C6 glioma when administered intraperitoneally on a d1-9 schedule. Studies were also carried out against the A431 epidermoid xenograft to evaluate the same pair of compounds via continuous subcutaneous infusion from Alzet miniosmotic pumps. The maximum dose that could be administered daily was limited by compound solubility. Neither compound produced an antitumor effect in a 7-day continuous infusion study. In the 27-day study, the disulfur compound was inactive whereas the diselenium compound produced a 10.8-day growth delay without appreciable treatment related weight loss. The *in vitro* and *in vivo* findings offer a mechanistic rationale as to why the 2,2′-diselenobis(1*H*-indoles) are more potent inhibitors than their disulfur congeners.

## Introduction

Protein phosphorylation plays a major role in the response of cells to various regulatory signals.[1] A number of pathways by which such signaling is initiated by external growth factors and transmitted to the nucleus via protein kinases are now known.[2−4] Binding of the appropriate growth factor to the extracellular domain of the receptor results in its activation and ultimately leads to cell proliferation primarily via a

* Author to whom correspondence should be addressed. Phone: 313-996-7028. Fax: 313-996-7879. E-mail: showalh@aa.wl.com.
† Warner-Lambert Company.
‡ University of Auckland School of Medicine.
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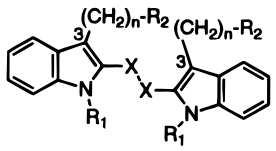
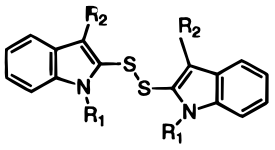
cascade of receptor and nonreceptor protein tyrosine kinases.[5−8] Thus, these enzymes play a crucial role in the normal functioning and development of cells. However, in a number of diseases including cancer, atherosclerosis, and psoriasis, the generation of mitogenic signals from abnormally expressed protein tyrosine kinases is believed to play a major role in the loss of growth control.[9,10] The concept that deregulated signal transduction can drive cellular transformation is supported by many experimental findings,[6−8] such as studies on the role of the epidermal growth factor receptor (EGFr) and p185$^{c-erbB2}$ protein tyrosine kinases in epithelial proliferation.[11−14]

A comparison of the primary amino acid sequence within the kinase domain of members of the tyrosine kinase gene family has revealed a high degree of homology.[15] Despite this and the lack until recently of precise structural data concerning the active site conformation of the enzyme,[16] numerous small molecule inhibitors have emerged with many showing good selectivity between different enzymes.[17−20] Hence, this has lent credence to a paradigm that the selective interruption of signal transduction by specific tyrosine kinase inhibitors could have therapeutic potential in the control of certain proliferative diseases.[21−24]

Previous reports from our laboratories have detailed the synthesis and inhibitory activity of a large series of 3-substituted 2,2′-dithiobis(1*H*-indoles), generalized by **1**, both against the EGFr and the nonreceptor pp60$^{v-src}$ tyrosine kinases.[25−28] This class possesses low micromolar potency both against isolated enzyme and in cells after relatively short times of treatment. Structure−activity relationship (SAR) studies on this class of compounds have shown that biological activity is affected to some extent by the overall nature of the indole substitution pattern, but more markedly by variation of the 3-substituent. The mechanism of inhibition toward the above 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 thiol−disulfide exchange with a thiol-containing residue(s) within the catalytic site (*vide infra*).




**1**: X = S; 2,2'-dithiobis(1*H*-indoles)
**2**: X = Se; 2,2'-diselenobis(1*H*-indoles)




**3**: $R_1$=$CH_3$; $R_2$=$CONHCH_3$
**4**: $R_1$=H; $R_2$=$CH_2CH(NH_2)CONHCH_2Ph$

In an attempt to broaden our understanding of the mechanism of action of the 2,2′-dithiobis(1*H*-indoles), we decided to synthesize a smaller series of closely related congeners, namely the 2,2′-diselenobis(1*H*-indoles), generalized by **2**. We anticipated that replacement of the bridging S−S bond with the longer Se−Se bond would have an effect on (a) the reduction potential of the connector moiety (and thus interaction of **2** with endogenous thiols), (b) the relative conformation of the connecting indole rings, and (c) metal binding since divalent magnesium and manganese are required for catalysis. In this paper, we detail the synthesis and SAR of this class of compounds against a range of protein tyrosine kinases including the epidermal growth factor receptor (EGFr), platelet-derived growth factor receptor (PDGFr), and v-src, and for selected compounds compare their inhibitory effects in cellular and receptor thiol reversal studies against direct congeners of the sulfur series, namely compounds **3** and **4**. Lastly, we present the first results of *in vivo* studies for selected compounds from both of these series.

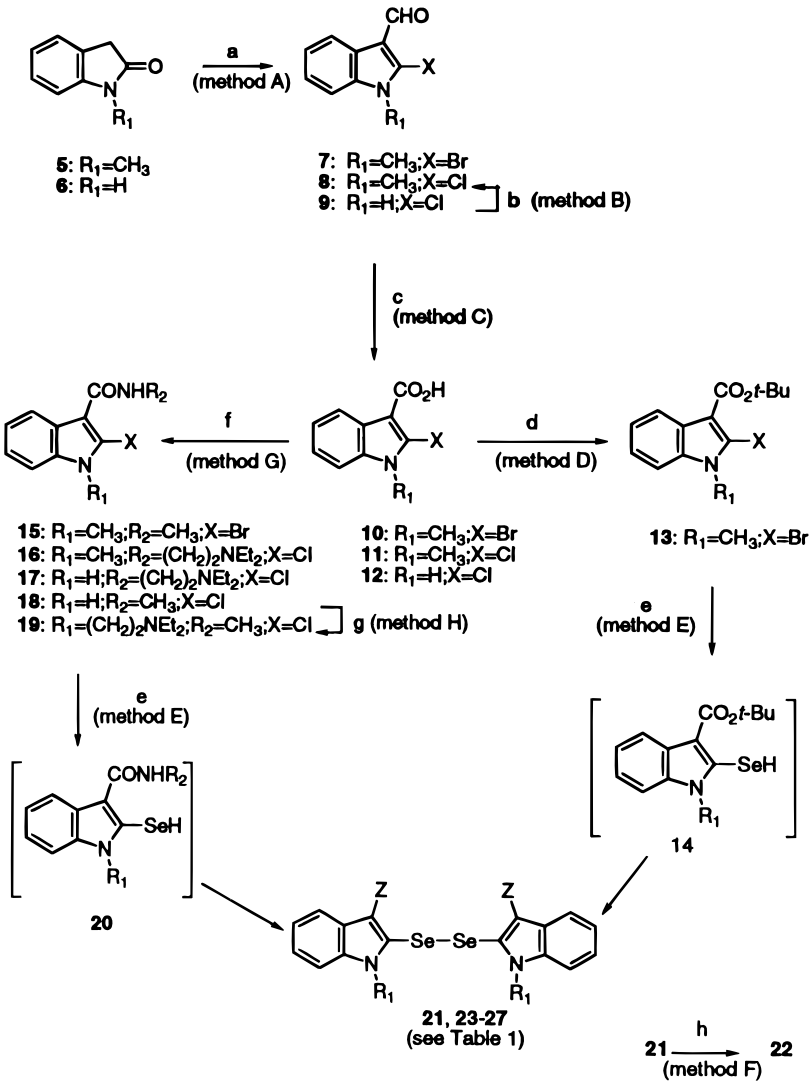
## Chemistry

A common method of 2,2′-dithiobis(1*H*-indole) synthesis is the direct thiation of suitably functionalized oxindole precursors with $P_2S_5$ followed by oxidative dimerization of the derived thione.[25−28] However, this was not applicable to compounds of the diselenium series since the analogous selenium reagent ($P_2Se_5$) is unknown. Instead, we utilized two other methods that had also previously been applied to the disulfur series. The first utilized chemistry in which a selenium nucleophile attacked a 2-halogeno-3-indole precursor activated by an electron-withdrawing carbonyl group at the 3-position.[28] The second proceeded from a C-3 alkylindole substrate in which installation of the diselenium connector at the 2-position was carried out by electrophilic chemistry utilizing diselenium dichloride ($Se_2Cl_2$).[25,26]

Most of the target 2,2′-diselenobis(1*H*-indoles) were derived from a 2-halogeno-3-indolecarboxylic acid precursor and were synthesized as outlined in Scheme 1. Thus, starting from either 1-methyloxindole (**5**)[29] or oxindole (**6**), the synthesis of intermediates **7**−**9** was achieved under Vilsmeier conditions similar to those previously described for **9** (method A).[30] Modification of this procedure utilizing the bromide salt of the Vilsmeier reagent provided **7**. The known aldehyde **9**[30,31] was made in a much improved yield with a simple modification of the workup. Alternatively, **8** could be made from **9** in near quantitative yield via a modification of the known alkylation conditions (method B).[32] The carboxylic acids **10**−**12** were initially prepared by $KMnO_4$ oxidation of the 3-carboxaldehydes **7**−**9** by a literature procedure.[31] However, yields generally did not exceed 50%. A better procedure was to employ sodium chlorite oxidation conditions[33] which provided the acids **10**−**12** in 62−78% yield (method C). Esterification of **10** with *tert*-butyl alcohol was difficult to achieve and was unsuccessful by a number of common methodologies. However, utilization of bis(2-oxo-3-oxazolindinyl)phosphinic chloride (BOP chloride) as the condensing agent provided **13** in 63% yield (method D).

On the basis of Moody's work in which a range of standard nucleophiles had been introduced into the 2-position of a suitably functionalized 2-chloroindole-3-carboxaldehyde,[34] we extended this chemistry toward the introduction of a selenium moiety. After evaluating a number of different selenium nucleophiles, we eventually resorted to the chemistry of Tiecco[35] who had evaluated the displacement of a range of inactivated aryl

**Scheme 1**

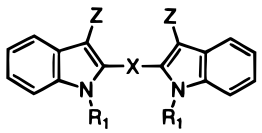



[a] (a) $POX_3$−DMF, $CH_2Cl_2$, 25−42 °C; (b) $K_2CO_3$, $CH_3I$, acetone, 25 °C; (c) $NaClO_2$, $NaH_2PO_4$, 2-methyl-2-butene, aqueous *p*-dioxane, 25 °C; (d) *tert*-butyl alcohol, BOPCl, $NEt_3$, 1,2-DCE, reflux; (e) $CH_3SeLi$, DMA, 0−25 °C; $H_2O_2$ or $NaBO_3$, 0−25 °C; (f) $SOCl_2$, 1,2-DCE, reflux; aqueous $NH_2R_2$, 0 °C or $NH_2R_2$, $CH_2Cl_2$ or THF, 0°C; (g) $Cl(CH_2)_2NEt_2$·HCl, $Cs_2CO_3$, 3A molecular sieves, acetone, 25 °C; (h) TFA, 0 °C.

halides with alkane thiolate or selenolate anions. Thus, treatment of ester **13** with *in situ* generated lithium selenomethylate in DMA at 25 °C followed by workup led to the bis-seleno ester **21** in 77% yield (method E). A 2-fold excess of the selenium nucleophile was used so that the nascent 2-methylseleno substituent would be further demethylated to the arylselenide anion. In contrast to the corresponding reaction with the methanethiolate anion, as in the 2,2′-dithiobis(1*H*-indole) series,[28] it was not possible to isolate the resultant arylselenol **14**. Instead, upon acidification during workup, it spontaneously dimerized to the diselenide diester **21**. Simple TFA hydrolysis of **21** gave diacid **22** in 78% yield (method F). The carboxamide target compounds (**23**−**27**) shown in Table 1 were derived from simple Schotten−Bauman acylation of acids **10**−**12** to provide monomeric amides **15**−**18** (method G). The addition of an aqueous solubilizing amine side chain onto the indole nitrogen of **18** to give **19** was carried out by alkylation with 2-(*N*,*N*-diethylamino)ethyl chloride in acetone utilizing $Cs_2CO_3$ as base (method H). Nucleophilic displacement of the C-2 halogen of **15**−**19** with lithium selenomethylate proceeded as described above to the give target amide dimers **23**−**27** in 20−68% yields. The physicochemical properties of compounds **21**−**27** are listed in Table 1.

Compounds **30a**, **32a**, **32b**, **33**, and **34** of Table 1 were synthesized starting from either (*R*)- or (*S*)-tryptophan as outlined in Scheme 2 for the [*R*-(*R**,*R**)]-stereoisomer **33**. Trifluoroacetylation of (*R*)-tryptophan utilizing the literature procedure[36] gave the known trifluoroacetamide **28a**,[37] which was then coupled with benzylamine via DCC/HBT condensation to provide **29a** in 66% yield (method I). Introduction of selenium into the 2-position was accomplished with $Se_2Cl_2$, a reagent previously used as an electrophilic source of selenium in reactions with olefins[38] and alkynes.[39] We hoped that this reagent would react with **29a** analogous to the reaction of **29a** with $S_2Cl_2$.[26] Thus, reaction of **29a** with $Se_2Cl_2$ in THF at 0−5 °C provided a mixture of three products by TLC: the diaryl selenide **30a**, the presumed diaryl triselenide **31a**, and **32a** as the major component (method J). This mixture was purified by treatment with $NaBH_4$ in cold methanol to leave a mixture of **30a** and a sodium aryl selenide monomer resulting from reduction of the di- and triselenides **31a** and **32a**,

**Table 1.** Physicochemical Properties of 2,2′-Diselenobis(1*H*-indole-3-carboxamides) and Analogues



| no. | $R_1$ | Z | X | method | yield,[a] % | mp, °C | molecular formula | analyses[b] |
|---|---|---|---|---|---|---|---|---|
| **21** | $CH_3$ | $CO_2t$-Bu | $Se_2$ | E | 77 | 187−189 | $C_{28}H_{32}N_2O_4Se_2 \cdot 0.2H_2O$ | C, H, N |
| **22** | $CH_3$ | $CO_2H$ | $Se_2$ | F | 78 | 174[c] | $C_{20}H_{16}N_2O_4Se_2 \cdot 0.1H_2O$ | C, H, N |
| **23** | $CH_3$ | $CONHCH_3$ | $Se_2$ | E | 67 | 225−230[c] | $C_{22}H_{22}N_4O_2Se_2 \cdot 0.9H_2O$ | C, H, N |
| **24** | $CH_3$ | $CONH(CH_2)_2NEt_2$ | $Se_2$ | E | 68 | 160−164[d] | $C_{32}H_{44}N_6O_2Se_2 \cdot 2.0HCl \cdot 1.7H_2O$ | C, H, N, Cl |
| **25** | H | $CONHCH_3$ | $Se_2$ | E | 20 | 272−275[c] | $C_{20}H_{18}N_4O_2Se_2 \cdot 0.9H_2O$ | C, H, N |
| **26** | H | $CONH(CH_2)_2NEt_2$ | $Se_2$ | E | 44 | 257−259[c,e] | $C_{30}H_{40}N_6O_2Se_2 \cdot 2.0\ HCl \cdot H_2O$ | C, H, N |
| **27** | $(CH_2)_2NEt_2$ | $CONHCH_3$ | $Se_2$ | E | 63 | 156−157 | $C_{32}H_{44}N_6O_2Se_2 \cdot 0.5H_2O$ | C, H, N |
| **30a** | H | $CH_2CH(NHTFA)CONHCH_2Ph[R\text{-}(R^*,R^*)]$[f,g] | Se | J | | 134−136 | $C_{40}H_{34}N_6O_4F_6Se \cdot 0.3H_2O$ | C, H, N |
| **32a** | H | $CH_2CH(NHTFA)CONHCH_2Ph[R\text{-}(R^*,R^*)]$[f,g] | $Se_2$ | J | 45 | 181−185 | $C_{40}H_{34}N_6O_4F_6Se_2 \cdot H_2O$ | C, H, N |
| **32b** | H | $CH_2CH(NH\text{-}t\text{-}Boc)CONHCH_2Ph[R\text{-}(R^*,R^*)]$[f,g] | $Se_2$ | J | 6 | 154−156 | $C_{46}H_{54}N_6O_6Se_2 \cdot H_2O$ | C, H, N |
| **33** | H | $CH_2CH(NH_2)CONHCH_2Ph[R\text{-}(R^*,R^*)]$[g] | $Se_2$ | K | 57 | 172−174[h] 178−180[i] | $C_{36}H_{36}N_6O_2Se_2 \cdot 1.5H_2O$ | C, H, N[h] |
| **34** | H | $CH_2CH(NH_2)CONHCH_2Ph[S\text{-}(R^*,R^*)]$[j] | $Se_2$ | K | 50 | 173−175 | $C_{36}H_{36}N_6O_2Se_2 \cdot 0.3H_2O \cdot 0.2C_6H_{14}$[k] | C, H, N |

[a] Yields were not optimized. [b] The analyses were within ±0.4% of the theoretical values. [c] With decomposition. [d] Free base, mp = 128−130 °C. [e] Free base, mp = 225−226 °C. [f] TFA = trifluoroacetyl. [g] Derived from (*R*)-tryptophan. [h] Derived from **32a**. [i] Derived from **32b**. [j] Derived from (*S*)-tryptophan. [k] *n*-Hexane detected by $^1H$ NMR.

respectively. While carefully maintaining a nitrogen environment, the mixture was diluted with water and then extracted with diethyl ether to selectively remove the neutral diaryl selenide **30a**. Acidification of the aqueous phase then resulted in the spontaneous dimerization of the selenide to the dimer **32a** in an overall yield of 45%. To the best of our knowledge, this is the first application of $Se_2Cl_2$ for the synthesis of diaryl diselenides. The cleavage of the trifluoroacetate protecting group to give target compound **33** was then carried out by $NaBH_4$ in refluxing ethanol by a literature procedure[40] (method K). While this reductive procedure claims to be racemization free, we observed 10−15% of another diastereomer (due to epimerization of one of the centers of chirality) by chiral HPLC. Compound **33** is stable when stored as a solid in the cold, but is sensitive to slow decomposition on standing in the light at room temperature. The same sequence of reactions was carried out starting from (*S*)-tryptophan to provide the [*S*-(*R**,*R**)]-enantiomer **34** in a comparable overall yield and chiral purity.

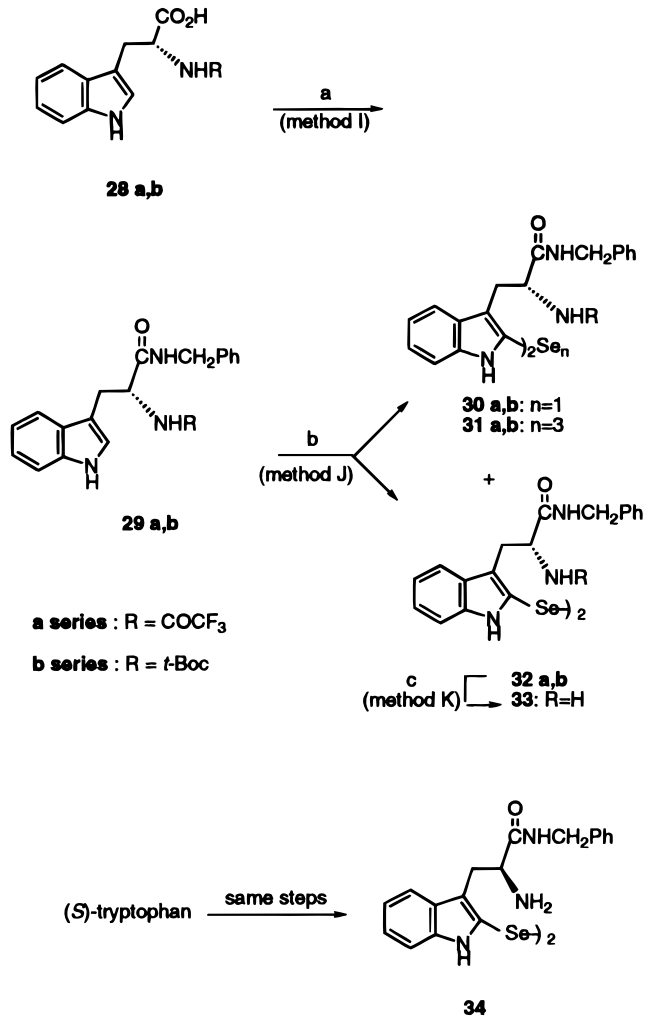
Since we were observing some apparent inversion of one of the centers of chirality in the $NaBH_4$ cleavage of **32a** to **33**, we decided to synthesize **33** by an alternate procedure in which a more easily cleavable nitrogen protecting group would be utilized. Thus, reaction of *N*-*t*-Boc derivative **29b**, made in a manner similar to the literature procedure,[41] with $Se_2Cl_2$ as described above (method J) followed by a similar extractive purification provided the dimer **32b** in only a 6% yield. This reduced yield relative to **32a** can likely be attributed to the instability of the *t*-Boc protecting group to the strongly acidic conditions of the $Se_2Cl_2$ reaction. The cleavage of the *N*-*t*-Boc protecting group to give target compound **33** was then carried out in 58% yield under the mild conditions of trimethylsilyl triflate in dichloromethane buffered with 2,6-lutidine at 0 °C[42] (method K). Standard methods of *N*-*t*-Boc cleavage (TFA in dichloromethane or HCl/methanol) resulted in impure product along with the deposition of some elemental selenium. Compound **33** obtained by this procedure was shown to be 98.5% pure by chiral HPLC.

The reported synthesis[26] of the 2,2′-dithiobis(1*H*-indole) **4** was substantially improved to provide multigram quantities necessary for the *in vivo* studies. Each step in the original route was examined, achieving an improvement in the overall yield of **4** from (*R*,*S*)-tryptophan from 5% to 50%. The optimum route is shown in Scheme 3 (method L). In the diethylphosphoryl cyanide (DEPC) coupling of the *N*-trifluoroacetamide of (*R*,*S*)-tryptophan (**28a**) with benzylamine, the yield of racemic **35** was increased from 50% to 83% by adding DEPC directly to the protected tryptophan acid, followed by benzylamine, with the base added last. Presumably the amine initially acts as the base, forming the amine salt and the carboxylate anion. The DMF was then removed at 60 °C prior to aqueous workup, which may have also helped by driving the reaction to completion. The yield of dimer **35** from the amide **29a** via reaction with $S_2Cl_2$ was also markeldy improved (from 44% to 81%), primarily by purification of the $S_2Cl_2$ by rapid distillation immediately prior to the reaction. Maintaining strictly anhydrous conditions (dry apparatus, solvent, and substrate and protection from atmospheric moisture during the reagent addition and the reaction) was also important. Finally, a major improvement (from 22% to 75% yield) was achieved in the reductive cleavage of the TFA group in **35** to give **4**, by employing a longer reaction time and higher temperature for the reduction (3 h at 80 °C) and using $H_2O_2$ at pH 8 for the reoxidation of the acid soluble material, instead of relying on aerial oxidation at pH 10. Compound **4** obtained by this sequence of reactions was shown to be a *ca.* 1:2:1 mixture of [*S*-(*R**,*R**)]:[*R*-(*R**,*S**)]:[*R*-(*R**,*R**)] stereoisomers by chiral HPLC.

## SAR vs Isolated Tyrosine Kinases

For the synthesized 2,2′-diselenobis(1*H*-indoles) listed in Table 1, compounds **21**−**27** were derived from 2-halogeno-3-indolecarboxylic acid precursors whereas **30**,

**Scheme 2**




[a] (a) HOBT, DCC, benzylamine, THF, 25 °C; (b) $Se_2Cl_2$, THF, 5 °C; $NaBH_4$, MeOH, 5 °C; $H_3O^+$; (c) For **32a**, $NaBH_4$, EtOH, 80 °C; for **32b**, TMS triflate, 2,6-lutidine, $CH_2Cl_2$, 5 °C.

**32a**, **32b**, **33**, and **34** were derived from (*R*)- or (*S*)-tryptophan. The C-3 side chain in compounds **21**−**27** has either acid, ester, or amide functionality, whereas the C-3 side chain in compounds **30a**, **32a**, **32b**, **33**, and **34** possesses a terminal amine that is either free or trifluoroacetylated and a benzyl amide moiety. In all cases the substituent on the indole nitrogen is either hydrogen or methyl except for **27**, which has the 2-(*N*,*N*-diethylamino)ethyl substituent. Two compounds (**24**, **26**) possess this same tertiary amine functionality at the 3-position whereas compounds **33** and **34** possess the primary $NH_2$ moiety in the side chain. All compounds that were evaluated biologically possess the Se−Se connector except for **30a**, which was isolated as a side product and has a single Se connector.

For activity against all kinases evaluated in Table 2, there was a requirement for a diselenium connector (compare **30a** vs other congeners). Against the EGFr, compounds **21**−**27** derived from 2-halogeno-3-indolecarboxylic acid precursors showed generally equivalent potency ($IC_{50}$ 3.5−6.1 $\mu$M) regardless of the C-3 or N-1 substitution pattern except for **21** ($IC_{50}$ >100 $\mu$M) with the bulky *tert*-butyl ester moiety and **25** ($IC_{50}$ 13 $\mu$M) which is unsubstituted on N-1 and has a small amide moiety at C-3. Compounds **33** and **34**, derived from (*R*)- and (*S*)-tryptophan, respectively, and each possessing a basic side chain nitrogen, showed the greatest potency ($IC_{50}$ *ca.* 1 $\mu$M). Against PDGFr, the pattern of inhibition differed from that of EGFr in that the greatest potency was associated with compounds derived from 2-halogeno-3-indolecarboxylic acid precursors, especially **23** and **24** which have amide functionality at C-3 and methyl substitution at N-1. Compounds derived from (*R*)- or (*S*)-tryptophan were less potent, although **33** derived from (*R*)-tryptophan again displayed greater potency than its enantiomer **34**. The pattern of inhibition against the cytoplasmic tyrosine kinase v-src was markedly different from the above two receptor kinases. Within the 2,2′-diselenobis(1*H*-indoles), there was about a 10-fold range in potencies ($IC_{50}$ 0.4−6.2 $\mu$M) with the highest potency again associated with analogues derived from 2-halogeno-3-indolecarboxylic acid precursors (*e.g.*, **21**, **23**, **24**, **27**).

A comparison of two pairs of diselenium−disulfur congeners in Table 2 (**3** vs **23**; **4** vs **32** or **33**) against the three kinases demonstrates a 5−10-fold increase in potency for diselenium compound **23** against PDGFr and v-src and equivalent potency vs EGFr. Its indole N-H congener **25** demonstrated a similar trend relative to its sulfur congener[28] (data not shown). For the diselenium compound **33** (**34**) vs the disulfur congener **4**, the opposite pattern was observed vs EGFr and v-src.

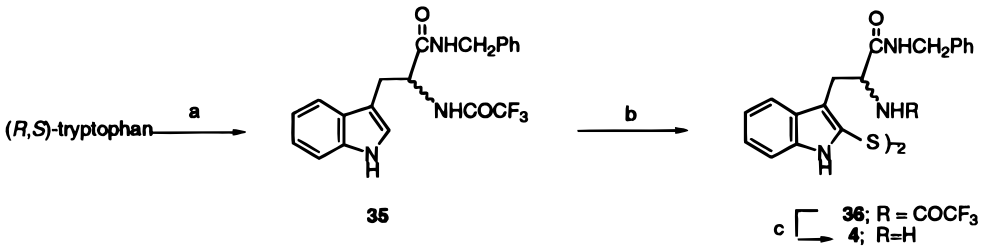
## Enzyme Kinetics

The inhibition kinetics of the **3**/**23** and **4**/**34** pairs of disulfur/diselenium congeners are shown in Table 3. For the **3** vs **23** pair, the potency of the diselenium compound **23** was greater in the v-src assay ($K_i$ = 0.8 and 0.6 $\mu$M, respectively) than that of the disulfur compound ($K_i$ = 8 and 1.4 $\mu$M, respectively) with respect to varying ATP or the peptide polymer substrate, whereas in the EGFr assay, little if any difference in the inhibition kinetics was observed. In contrast, in the EGFr assay for the **4**/**34** pair, the potency of the diselenium compound **34** was markedly greater ($K_i$ = 2.7 and 5.6 $\mu$M, respectively) than that of the disulfur congener **4** ($K_i$ = 19.4 and 21.2 $\mu$M, respectively) with respect to varying the same substrates. A curve-fitting analysis of the inhibition (Grafit, Erithicus Software, Ltd.) showed primarily noncompetitive inhibition by both classes of compounds with respect to either ATP or peptide substrate. Double reciprocal plots (not shown) confirm this inhibition mechanism.

## Thiol Reversal Studies

The effect of the sulfhydryl reducing agent dithiothreitol (DTT) on the inhibition of tyrosine kinases by various compounds has been previously described.[43−45] Figures 1 and 2 show the effect of increasing DTT on the activity of diselenium compound **33** and disulfur congener **4** in the EGFr kinase assay. For a given inhibitor concentration, increasing concentrations of DTT caused a general decrease in kinase inhibition. This effect may be indirect (*i.e.*, not via a direct disulfide or seleno-sulfide exchange) since the stoichiometry of DTT to inhibitor is much greater than 1:1 at 0% inhibition. The reversal of enzyme inhibition by DTT occurs somewhat less readily with the diselenium compound **33** (Figure 1) than with the disulfur compound **4** (Figure 2), both against the EGFr tyrosine kinase and the v-src kinase (data not shown). This

**Scheme 3**




[a] (a) $CF_3CO_2Et$, $NEt_3$, DMF, 20 °C, then DEPC, benzylamine, $NEt_3$, DMF, 0−20 °C; (b) $S_2Cl_2$, THF, 20 °C; (c) $NaBH_4$, EtOH, 80 °C, then 35% $H_2O_2$.

**Table 2.** Inhibition of Tyrosine Kinases by 2,2′-Selenobis(1*H*-indoles) *in Vitro* and the Ability to Inhibit the Proliferation of Swiss 3T3 Mouse Fibroblasts ($IC_{50}$, $\mu$M)[a]

| no. | EGFr | PDGFr | v-src | Swiss 3T3 cells |
|---|---|---|---|---|
| **3** | 6.9 | 28.1 | 6.7 | 1.8[b] |
| **4** | 7.4 | ND[c] | 1.5 | 5.3[d] |
| **21** | >100 | >31 | 1.5 | ND |
| **22** | 3.5 | 17.4 | 2.4 | 8.0 |
| **23** | 6.1 | 4.7 | 0.4 | 5.5 |
| **24** | 4.7 | 3.4 | 1.8 | 19.5 |
| **25** | 13 | 8.0 | 2.8 | 1.0 |
| **26** | 4.6 | 12.8 | 3.6 | 8.0 |
| **27** | 6.9 | *ca.* 50 | 1.7 | ND |
| **30a** | >50 | >50 | >50 | ND |
| **32a** | 7.0 | >50 | 6.2 | ND |
| **33** | 0.9 | 14.1 | 2.0 | 0.5 |
| **34** | 1.3 | 25.5 | 3.8 | 0.6 |

[a] $IC_{50}$ values reported for kinase and cellular inhibition are means of duplicates from at least two separate experiments with typical variation less than 30% between values. The $IC_{50}$ vs Swiss 3T3 cells represents the concentration of compound required to inhibit cell growth rate by 50%. [b] Reference 26. [c] ND, not determined. [d] Reference 28.

**Table 3.** Inhibition Kinetics of Selected 2,2′-Diselenobis(1*H*-indoles) and Sulfur Congeners in EGFr and v-Src Tyrosine Kinases Varying ATP and Peptide Polymer Substrate[a]

| no. | EGFr $K_{i\ ATP}$ | v-src $K_{i\ ATP}$ | EGFr $K_{i\ peptide}$ | v-src $K_{i\ peptide}$ |
|---|---|---|---|---|
| **3** | 2.3 | 8 | 3.9 | 1.4 |
| **23** | 3.1 | 0.8 | 3.9 | 0.6 |
| **4** | 19.4 | ND[b] | 21.2 | ND[b] |
| **34** | 2.7 | ND[b] | 5.6 | ND[b] |

[a] Values given are micromolar and were determined by a nonlinear regression analysis curve-fitting program (Grafit) of the inhibition data using Cleland's equations for enzyme inhibition. The type of inhibition was determined to be substantially noncompetitive with respect to both ATP and peptide polymer substrate based on comparisons of $K_i$ values calculated from slope and intercept determinations. Double-reciprocal plots of the inhibitor data confirmed the curve-fitting analysis (data not shown). [b] ND, not determined.

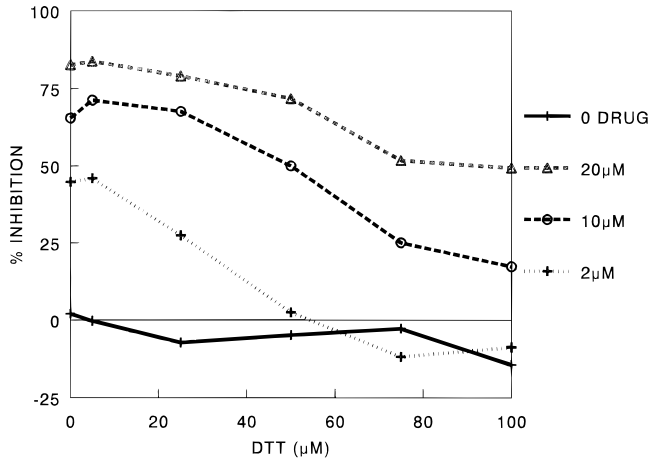



**Figure 1.** Effect of DTT on the inhibition of EGFr tyrosine kinase by compound **33**. Data shown are duplicate determinations in a representative experiment.

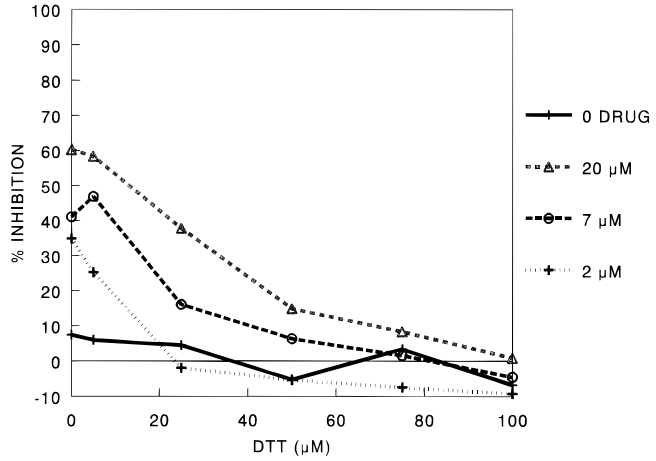



**Figure 2.** Effect of DTT on the inhibition of EGFr tyrosine kinase by compound **4**. Data shown are duplicate determinations in a representative experiment.

difference may be related to the relatively more stable nature of the diselenide bond compared to the disulfide moiety.[46] The retention of inhibitory activity by **33** in the presence of thiol suggested better cellular activity for the 2,2′-diselenobis(1*H*-indoles) compared to their sulfur congeners (*vide infra*).

## Cellular Studies

The 2,2′-diselenobis(1*H*-indoles) were also evaluated in whole cell studies. Shown in Table 2 is the ability of several compounds to inhibit the proliferation of Swiss 3T3 mouse fibroblasts. Compounds of this class were growth inhibitory with $IC_{50}$ values ranging from 0.5 $\mu$M for **33** to 19.5 $\mu$M for **24**, with potency highly dependent on the substitution pattern on the indole ring. It appears that methyl substitution on the indole nitrogen is detrimental to growth inhibition (compare **23** vs **25** and **24** vs **26**). Surprisingly, the carboxylic acid **22** was nearly as potent as methyl amide **23**. This is a different pattern than that found for the 2,2′-dithiobis(1*H*-indoles) where carboxylic acid substituted derivatives were much less potent than alkyl amides or esters.[26] The most potent compounds were **33** and **34**. Since both isomers were essentially equipotent, the [*R*-(*R**,*R**)] isomer, **33**, was selected for further testing. Figure 3 is an antiphosphotyrosine western blot showing the effect of compound **33** on growth factor-mediated tyrosine phosphorylation in Swiss 3T3 murine fibroblasts. The results indicate that this compound significantly

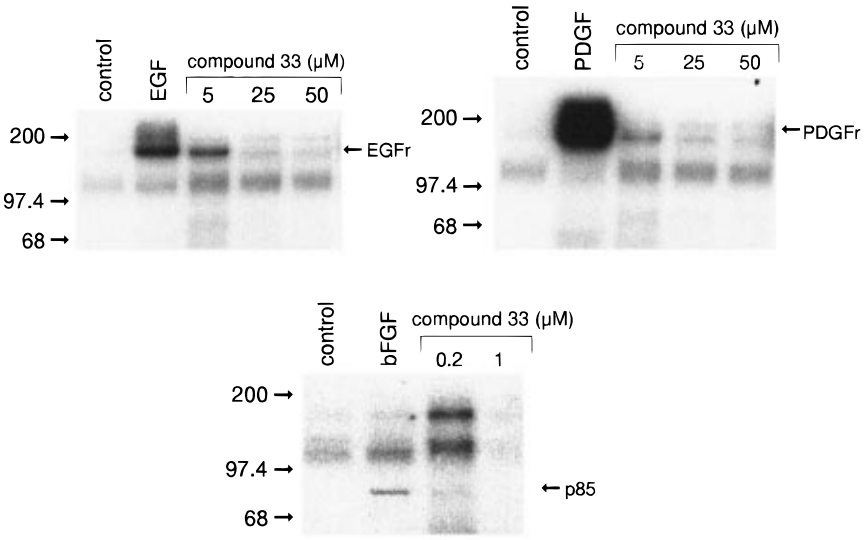



**Figure 3.** Effect of compound **33** on growth factor mediated tyrosine phosphorylation in Swiss 3T3 murine fibroblasts. Cells were treated for 2 h with varying concentrations of compound and then exposed to the indicated growth factor for 5 min. Extraction and western blotting procedures are described in the Experimental Section.

**Table 4.** Comparison of 2,2′-Diselenobis(1*H*-indole) **33** and Sulfur Congener **4** in Selected Cellular Assays[a]

| | $IC_{50}$ (μM) | |
|---|---|---|
| cellular process | **4** | **33** |
| bFGF-mediated tyrosine phosphorylation[b] | 2.3 | 0.2 |
| PDGFr autophosphorylation[b] | 6.2 | 0.57 |
| EGFr autophosphorylation[b] | >100 | 4.2 |
| proliferation[b] | 5.3 | 0.54 |
| DNA synthesis[c] | 2.5 | 0.18 |
| RNA synthesis[c] | 2.0 | 0.35 |
| protein synthesis[c] | 0.6 | 0.17 |

[a] $IC_{50}$ values reported for kinase inhibition are means of duplicates from at least two separate experiments with typical variation less than 30% between values. [b] See ref 27 for details of experimental procedures. [c] See the Experimental Section for details.

inhibits EGF receptor autophosphorylation at 5 μM with complete suppression at 25 μM. Autophosphorylation of the PDGF receptor in response to its ligand was even more susceptible and was completely suppressed at 5 μM. Tyrosine phosphorylation of an 85 kDa protein typically phosphorylated in response to bFGF was also exquisitely sensitive to compound **33**, and a significant effect was seen as low as 0.2 μM.

Table 4 summarizes data from several cellular assays for the diselenium compound **33** and compares this with its disulfur congener **4**. $IC_{50}$ values generated from quantitating the results of western blots similar to Figure 3 indicate that compound **33** is indeed most active against bFGF- and PDGF-mediated tyrosine phosphorylation and produces a 50% inhibition at less than 1 μM. The disulfur congener **4** exhibited a qualitatively similar pattern; however, its potency was 10-fold less. Likewise, the inhibitory effects of **33** on cellular proliferation and DNA, RNA, and protein syntheses also occurred at submicromolar concentrations. Compound **4** again produced similar effects but was consistently 10-fold less potent.

### *In Vivo* Studies

The murine tumors B16 melanoma, colon carcinoma 26, and M5076 sarcoma were selected for an initial evaluation of disulfur compound **4** based upon their growth properties and the response of similar histotypes to growth factors *in vitro*. Compound **4** was ineffective against all three tumor models (Table 5). This was the case against B16 and C26 even though the administration sites for tumor implant and compound **4** were both intraperitoneal. A q4h × 3 treatment schedule was used in an attempt to maximize tumor cell exposure to **4** on each treatment day. This rigorous treatment schedule did not improve the antitumor effectiveness based upon either % T/C (B16 and C26) or tumor growth delay (M5076). The maximum tolerated dose of **4** was approximately 3.0 mg/kg/day for the daily treatment schedule for 9 days and 9.0 mg/kg/day for the q4h × 3 daily for 9 days treatment schedule. It should be noted that the maximum tolerated doses in the intraperitoneal tumor implant studies were not necessarily the doses producing the optimal (largest) % T/C values that are shown in Table 5. The % T/C values against the B16 and C26 tumor models were in the range 85−95% when **4** was administered at a maximum tolerated dose.

The human tumor xenografts A431 epidermoid, C6 glioma, and MCF-7 used in these studies were selected based upon the *in vitro* responsiveness of these tumors to growth factors. Compound **4** was ineffective against the MCF-7 breast xenograft on both of the administration schedules tested. At maximum tolerated doses, neither **4** nor **33** was effective against the C6 glioma when administered intraperitoneally on a daily treatment schedule for 9 days. The maximum tolerated dose for **4** administered on a daily treatment schedule was >12.0 mg/kg/injection against the MCF-7 breast line, but only 5.0 mg/kg/injection against the C6 glioma.

We then carried out studies to evaluate the effectiveness of **4** and **33** administered by continuous subcutaneous infusion from Alzet miniosmotic pumps, based upon studies of a quinazoline EGFr tyrosine kinase inhibitor evaluated in our laboratories that showed the need for continuous drug exposure.[47] Compound solubility was the limiting factor for the dose that could be delivered to the animals. The doses in Table 5 for the infusion studies represent the maximum dose that could be administered daily without significant compound precipitation. The degree and amount of compound precipitation were reasonably consistent in the two studies

**Table 5.** *In Vivo* Anticancer Activity of Compounds **4** and **33** against Murine Tumors and Human Tumor Xenografts[a]

| tumor | no. | dose (mg/kg) | schedule | weight change (g) | T/C or T − C[b] | net cell kill ($log_{10}$)[c] |
|---|---|---|---|---|---|---|
| B16 melanoma | **4** | 0.75[d] | ip, q4h × 3; d1−9 | +1.1 | 99 | −1.3 |
| | | 3.0[d] | ip, d1−9 | +1.4 | 100 | −1.3 |
| colon 26 | **4** | 0.75[d] | ip, q4h × 3; d1−9 | +0.8 | 119 | −0.2 |
| | | 1.5[d] | ip, d1−9 | +1.8 | 115 | −0.3 |
| M5076 sarcoma | **4** | 3.0[e] | ip, q4h × 3; d1−9 | −0.5 | 0.7 | −0.8 |
| A431 epidermoid | **4** | 10[f] | sc Alzet d9−15 | −1.2 | 1.9 | −0.2 |
| | **33** | 0.5[f] | | −0.2 | 2.2 | −0.2 |
| | **4** | 9.0[f] | sc Alzet d1−27 | + | 1.0 | −0.6 |
| | **33** | 0.5[f] | | + | 10.8 | −0.4 |
| C6 glioma | **4** | 5[e] | ip, D1−9 | + | 0 | |
| | **33** | 1.8[e] | ip, D1−9 | −0.9 | 2.0 | −0.2 |
| MCF-7 | **4** | 3.0[e] | ip, q4h × 3; d5−11 | + | 1.0 | −1.2 |
| | **4** | 12.0[g] | ip, d5−11 | −0.4 | 0 | − |

[a] For B16 and colon 26 models $1.8 \times 10^6$ and $1.5 \times 10^6$ viable cells, respectively, were implanted ip on day 0. Tumor fragments of A431, C6, MCF-7, or M5076 were implanted sc into the right axilla of mice on day 0. [b] Endpoint for the B16 and C26 studies was % T/C, while the endpoint in all other evaluations was tumor growth delay (T − C). [c] The net reduction in tumor burden (in logs) between the first and last treatments. [d] Optimal dose. The dose producing the largest % T/C from the dose response. [e] Maximum tolerated dose, $\leq LD_{10}$ (mg/kg/injection), from a complete dose response. [f] Dose represents the maximum amount of compound (mg/kg/day) that could be delivered without significant precipitation after the test compound was extruded from the pump. [g] Highest dose tested.

conducted against the A431 epidermoid xenograft. Neither compound produced an antitumor effect in an initial 7-day infusion study. A second study evaluated the effect of a 27-day continuous infusion to determine if a prolonged exposure time would produce a greater effect. The miniosmotic pumps in this study were removed and replaced with full pumps each week to increase the infusion time to a total of 27 days. Treatment with **4** over this time period resulted in no antitumor effect. However, treatment with **33** on the same schedule resulted in a 10.8-day growth delay. The lack of any appreciable treatment-related weight loss in the 27-day study indicated that more **33** could have been delivered to the animals on a daily basis had solubility not been a limiting factor.

## Summary

We have synthesized a small series of 2,2′-diselenobis(1*H*-indoles) and have determined that their SAR pattern is similar to that of the congeneric 2,2′-dithiobis(1*H*-indoles) against selected isolated tyrosine kinases. However, against these kinases and in whole cells, compounds of the diselenium series are generally more potent (up to 10-fold). Additionally, the pattern of inhibition of cellular phosphorylation by the diselenium series is markedly different, and the reversal of inhibition by thiols such as DTT is markedly less for a given concentration of inhibitor. Similar effects have been observed in studies carried out in the presence of glutathione (data not shown). This retention of inhibitory activity by the diselenium series in the presence of added thiol might relate in part to the better cellular activity for this class compared to their disulfur congeners.

Two congeners from the disulfur and diselenium series (compounds **4** and **33**, respectively) have also been evaluated for antitumor effectiveness against murine and human tumor xenograft model systems. The modification from the S−S to Se−Se linkage appears to increase the dose potency as reflected in a 3-fold lower maximum tolerated dose for **33** compared to **4** in the C-6 glioma tumor model (Table 5). However, **33** was approximately 20-fold less soluble than **4**, which limited the amount of compound that could be continuously delivered from a subcutaneously implanted Alzet miniosmotic pump. The observation that **33** produced a 10.8-day tumor growth delay against the A431 epidermoid carcinoma compared with the inactivity of compounds **4** and **33** against the other models evaluated suggested that continuous exposure would be necessary for *in vivo* antitumor effectiveness. However, the reduced solubility of **33** prohibited the use of higher infusion doses which might have increased antitumor effectiveness. The weight loss data, as an indicator of toxicity, showed that with the infusion doses used in these studies there was little, if any, weight loss, indicating that a larger dose might be tolerated and contribute to greater antitumor effectiveness.

Our *in vitro* and *in vivo* findings offer a mechanistic rationale as to why the 2,2′-diselenobis(1*H*-indoles) are more potent inhibitors than their disulfur congeners. In the presence of physiological intracellular glutathione levels (*ca.* 1 mM), compounds of the diselenium series might be less susceptible to thiol reduction, and thus be more likely to be presented to a tyrosine kinase in dimeric form where they could interact with a sulfhydryl residue via an exchange mechanism. Our mechanistic studies show that both the 2,2′-diselenobis(1*H*-indoles) and 2,2′-dithiobis(1*H*-indoles) are noncompetitive inhibitors, with respect to both ATP and peptide substrate. Over a 2-h period it appears that binding is essentially irreversible, suggesting that these agents might act either as selenolating or sulfenolating agents on a sulfhydryl residue. Within the EGFr catalytic domain, there are six sulfhydryl residues.[15] Cysteine-272 near the catalytic site is conserved over a broad range of kinases and thus might be a site of selective covalent modification by compounds of this general class. Additional studies at the molecular level are necessary to support this proposal.

## Experimental Section

Melting points are uncorrected. Proton nuclear magnetic resonance ($^1$H NMR) spectra were measured at 300 or 400 MHz. Chemical shifts are reported as $\delta$ values (parts per million) downfield from internal $Me_4Si$. The following abbreviations are used to describe peak patterns when appropriate: br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Selenium nuclear magnetic resonance ($^{77}$Se NMR) spectra were recorded at a frequency of 76.3 MHz. Chemical shifts are referenced to neat $(CH_3)_2Se$ at 0 ppm with $(CD_3)_2SO$ in an external capillary. Mass spectra (MS) were recorded in either the APCI or FAB mode in a matrix of MeOH/

$CH_3CN$/DMSO or thioglycerol, respectively. 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. HPLC was carried out on a Vydac 218 TP54 column with a gradient mobile phase of 10−76% of 0.1% TFA in $CH_3CN$:0.1% TFA in $H_2O$ to assay for chemical purity, and on a 5 $\mu$M Chiralpak AD column (serial no. 15-42-01225) with a mobile phase of 5:30:65 MeOH:EtOH:hexanes containing 1.0 mL of diethylamine/L at a flow rate of 0.7 mL/min to assay for chiral purity. Combustion analyses were determined by Robertson Microlit Laboratories, Inc., Madison, NJ. All reaction solvents were reagent grade or distilled-in-glass and were stored over activated 3A (for lower alcohols) or 4A molecular sieves. For the scale-up synthesis of **4**, DMF, triethylamine, and benzylamine were additionally predried over molecular sieves. Following normal workup procedures, organic extracts were dried over anhydrous $Na_2SO_4$ or $Mg_2SO_4$ prior to concentration.

**Method A. 2-Bromo-1-methylindole-3-carboxaldehyde (7).** A three-neck round-bottomed flask was equipped with a mechanical stirrer, pressure equalizing dropping funnel, and $N_2$ inlet. To a stirred 25 °C solution of 55 mL (744 mmol) of DMF and 105 mL of $CH_2Cl_2$ was added dropwise a solution of 178 g (620 mmol) of $POBr_3$ in 125 mL of $CH_2Cl_2$ at such a rate to maintain a gentle reflux via the exothermic reaction (*ca.* 1 h). The resulting thick tan mixture was stirred vigorously for 10 min, and then 36.5 g (248 mmol) of *N*-methyloxindole (**5**)[29] was added portionwise over 20 min. The mixture was stirred at 25 °C for 60 h at which time TLC ($SiO_2$; 1:1 EtOAc:hexanes) showed clean conversion to product. The solution was poured *slowly* onto 600 mL of crushed ice. The resulting suspension was stirred for 30 min, and the phases were then separated. The golden aqueous phase was extracted with 200 mL of $CH_2Cl_2$ and then was allowed to stir at 25 °C for 18 h. The flocculent tan precipitate that had formed was collected by filtration and washed well with $H_2O$. The aqueous filtrate was stirred for 48 h during which additional precipitate formed. The combined crops were dried at 70 °C over $P_2O_5$ to leave 40.7 g (69%) of **7** as a light tan solid: mp 110−111.5 °C; $^1$H NMR ($CDCl_3$) $\delta$ 10.04 (s, 1H, CHO), 8.33−8.31 (m, 1H, H-4), 7.34−7.29 (m, 3H, ArH), 3.86 (s, 3H, $NCH_3$). Anal. ($C_{10}H_8BrNO$) C, H, N, Br.

**2-Chloroindole-3-carboxaldehyde (9).** The following is a modification of the procedure of Schulte.[30] A 3 L three-neck round-bottomed flask fitted with a pressure-equalizing addition funnel, an $N_2$ inlet, and an internal thermometer was charged with 1 L of $CH_2Cl_2$ and 233 mL of DMF. The solution was cooled to 0 °C with an ice bath and treated dropwise over 25 min with 233 mL (2.5 mol) of $POCl_3$. The solution was brought to 25 °C and maintained there for 2 h. The clear amber solution was cooled to 0 °C and charged over 35 min with 137.3 g (1 mol) of oxindole (**6**) via a solid addition funnel. The darkened mixture was stirred under $N_2$ at 25 °C for 38 h at which time TLC (1:1 hexanes:EtOAc) showed no starting material. The mixture was cautiously poured into 1.7 L of wet ice, and additional ice was kept available to control a subsequent exotherm. The mixture was transferred to a separatory funnel and extracted with 200 mL of $CH_2Cl_2$ (during the extraction the temperature of the aqueous layer rose and was controlled with ice addition). The organic extract was back-extracted with $H_2O$ (3 × 350 mL), and all of the aqueous layers were combined and stirred at 25 °C overnight. The resultant flocculent solids were collected by filtration, washed well with $H_2O$, air dried, and then dried over $P_2O_5$ to afford 131.8 g (73%) of **9**: mp 218−219 °C dec (lit. mp 232−235 °C[30], 223−225 °C[31]). The material was pure by TLC and used directly in the next reaction.

**Method B. 2-Chloro-1-methylindole-3-carboxaldehyde (8).** A solution of 10 g (55.7 mmol) of 2-chloroindole-3-carboxaldehyde (**9**) in 100 mL of dry acetone was treated with 14.61 g (111 mmol) of finely powdered anhydrous $K_2CO_3$, and the resulting suspension was stirred vigorously for 10 min under an $N_2$ atmosphere. Iodomethane (6.95 mL; 111 mmol) was then added, and stirring was continued for 16 h. The suspension was filtered and the filter cake washed with acetone (3 × 30 mL). The filtrate was concentrated to a solid residue that was stirred in 25 mL of $H_2O$ for 15 min. The solids were collected by filtration, washed well with $H_2O$, and dried over $P_2O_5$ to afford 10.41 g (97%) of **8** as an off-white solid: mp 97−98 °C (lit.[32] mp 89−91 °C).

**Method C. 2-Bromo-1-methylindole-3-carboxylic Acid (10).** To a vigorously stirred solution of 39 g (164 mmol) of 2-bromo-1-methylindole-3-carboxaldehyde (**7**), 164 mL of 2-methyl-2-butene, and 615 mL of *p*-dioxane at 25 °C was added dropwise over *ca.* 30 min a solution made up of 81.9 g (902 mmol) of $NaClO_2$ and 81.9 g (590 mmol) of $NaH_2PO_4 \cdot H_2O$ in 410 mL of $H_2O$. The mixture was treated with 20 g each of solid $NaClO_2$ and $NaH_2PO_4 \cdot H_2O$ after 2.5 h and then 10 g each of these reagents after 5 h. After a total reaction time of 6.5 h, the mixture was diluted with 1.1 L of EtOAc and stirred for *ca.* 45 min. The layers were separated, and the aqueous phase was extracted twice with EtOAc (2 × 300 mL). The combined organic extracts were concentrated to 1 L and then extracted with 1% aqueous NaOH (3 × 400 mL). The combined aqueous extracts were cooled and acidified to *ca.* pH 2 with 6 N aqueous HCl. The resultant solids were collected, washed well with $H_2O$, and dried over $P_2O_5$ to leave 33 g (78%) of **10** as a white solid: mp 198 °C dec; $^1$H NMR ($(CD_3)_2SO$) $\delta$ 12.45 (br s, 1H, $CO_2H$), 8.04 (d, $J$ = 7.9 Hz, 1H, H-4), 7.60 (d, $J$ = 7.9 Hz, 1H, H-7), 7.29−7.19 (m, 2H, ArH), 3.84 (s, 3H, indole $NCH_3$). Anal. ($C_{10}H_8BrNO_2$) C, H, N, Br.

**2-Chloroindole-3-carboxylic Acid (12).** Application of these reaction conditions to 15 g (83.5 mmol) of 2-chloroindole-3-carboxaldehyde (**9**) afforded 10.1 g (62%) of **12** [mp 181.5 °C (lit.[31] mp 160 °C)] in two crops following crystallization from 7:3 acetone:hexanes.

**Method D. 2-Bromo-1-methylindole-3-carboxylic Acid, *tert*-Butyl Ester (13).** A suspension of 2.54 g (10 mmol) of 2-bromo-1-methylindole-3-carboxylic acid (**10**), 2.54 g (10 mmol) of bis(2-oxo-3-oxazolidinyl)phosphinic chloride, 1.13 mL (12 mmol) of *tert*-butyl alcohol, 2.78 mL (20 mmol) of triethylamine, and 25 mL of 1,2-dichloroethane was heated at reflux for 1.5 h. The mixture was cooled, poured into 150 mL of 5% aqueous $NaHCO_3$, and stirred for 30 min. The mixture was extracted with $CH_2Cl_2$ (3×), and the combined organic phase was washed with $H_2O$ and brine, dried, and concentrated to leave a red oil that was triturated in EtOAc:hexanes and filtered. The filtrate was concentrated to a viscous oil that was dissolved into $CHCl_3$ and purified by column chromatography, eluting with 95:5 hexanes:EtOAc. Product fractions were pooled, concentrated, and triturated in isooctane to give 1.96 g (63%) of **13** as a white solid: mp 87−88 °C; $^1$H NMR ($CDCl_3$) $\delta$ 8.11 (dd, $J$ = 2.5, 6.5 Hz, 1 H, H-4), 7.40−7.19 (m, 3H, ArH), 3.84 (s, 3H, $NCH_3$), 1.67 (s, 9H, $C(CH_3)_3$). Anal. ($C_{14}H_{16}BrNO_2$) C, H, N, Br.

**Method E. 2,2′-Diselenobis[1-methyl-1*H*-indole-3-carboxylic acid, *tert*-butyl ester] (21).** An ice-cold suspension of 119 mg (1.5 mmol) of elemental Se in 2 mL of THF under $N_2$ was treated dropwise with 1.1 mL of $CH_3Li \cdot LiBr$ complex (1.5 M in ether). The flask was opened to the air, and with a brisk stream of $N_2$, the resultant white suspension was warmed to *ca.* 85 °C to distill off the ether and most of the THF. The residual semisolid was cooled in an ice bath and diluted with 1.5 mL of DMA followed by 155 mg (0.5 mmol) of 2-bromo-1-methylindole-3-carboxylic acid, *tert*-butyl ester (**13**). The resultant solution was stirred at room temperature for 24 h, cooled to 0 °C, and then treated with 2 mL of dilute HOAc. The mixture was diluted with $H_2O$ and extracted with $CHCl_3$ (3 × 10 mL). The combined extracts were washed with $H_2O$ (4×), dried, and concentrated to leave a golden solid. The solid was suspended in 2.3 mL of 2:1 v/v HOAc:$H_2O$, and the suspension was treated with 154 mg of $NaBO_3 \cdot 4H_2O$ and then stirred at 25 °C for 30 min. The solids were collected by filtration, washed with $H_2O$, and dried to leave 119 mg of pure **21**: $^1$H NMR ($CDCl_3$) $\delta$ 8.13 (dd, $J$ = 0.7, 7.9 Hz, 2 × 1 H, H-4), 7.31−7.19 (m, 2 × 3H, ArH), 3.63 (s, 2 × 3H, $NCH_3$), 1.44 (s, 2 × 9H, $C(CH_3)_3$). Anal. ($C_{28}H_{32}N_2O_4Se_2 \cdot 0.2\ H_2O$) C, H, N.

**2,2′-Diselenobis[*N*,1-dimethyl-1*H*-indole-3-carboxamide] (23).** An ice-cold solution of 3 mmol of $CH_3SeLi$ in 2 mL of DMA, made up as described above, was treated with 267 mg (1.0 mmol) of 2-bromo-1-methylindole-3-(*N*-methyl-

carboxamide) (**15**). The resultant solution was stirred at room temperature for 3.5 h, cooled to 0 °C and then treated with 5% aqueous HCl. The mixture was extracted with $CH_2Cl_2$ (2 × 10 mL), and the combined extracts were washed with $H_2O$ (2×) and then concentrated in vacuo to leave an oil that was dissolved in MeOH. The solution was ice-cooled and treated with 113 μL of 30% aqueous $H_2O_2$. After being stirred for 10 min, the resultant suspension was filtered, and the solids were washed with 2-propanol and dried to leave 183 mg of pure **23** as a yellow solid: $^1$H NMR ($CDCl_3$ + $(CD_3)_2SO$) δ 7.97 (d, $J$ = 7.9 Hz, 2 × 1H, H-4), 7.39−7.18 (m, 2 × 3H, ArH), 6.84 (s, 2 × 1H, N*H*CH$_3$), 3.85 (s, 2 × 3H, indole NCH$_3$), 2.12 (d, $J$ = 4.5 Hz, 2 × 3H, NHC*H*$_3$); FABMS *m*/*z* (relative intensity) 535 ($MH^+$, 10). Anal. ($C_{22}H_{22}N_4O_2Se_2$·0.9$H_2O$) C, H, N.

**2,2′-Diselenobis[*N*-[2-(diethylamino)ethyl]-1-methyl-1*H*-indole-3-carboxamide] (24)**: $^1$H NMR ($(CD_3)_2SO$) δ 10.13 (s, 2 × 1H, $^+$N*H*$(CH_2CH_3)_2$), 8.14−8.11 (m, 2 × 1H, CONH), 7.89 (d, $J$ = 8.2 Hz, 2 × 1H, H-4), 7.57 (d, $J$ = 8.4 Hz, 2 × 1H, H-7), 7.34−7.17 (m, 2 × 2H, ArH), 3.63 (s, 2 × 3H, NCH$_3$), 3.17−3.14 (m, 2 × 2H, CONHC*H*$_2$), 3.06−3.00 (m, 2 × 4H, N(C*H*$_2$CH$_3$)$_2$), 2.86 (t, $J$ = 6.5 Hz, 2 × 2H, CONHCH$_2$C*H*$_2$), 1.16 (t, $J$ = 7.2 Hz, 2 × 6H, N(CH$_2$C*H*$_3$)$_2$). Anal. ($C_{32}H_{44}N_6O_2Se_2$·2.0HCl·1.7$H_2O$) C, H, N, $Cl^-$.

**2,2′-Diselenobis[*N*-methyl-1*H*-indole-3-carboxamide] (25)**: $^1$H NMR ($(CD_3)_2SO$) δ 12.36 (s, 2 × 1H, indole NH), 7.83 (d, $J$ = 7.7 Hz, 2 × 1H, H-4), 7.79 (d, $J$ = 4.1, 2 × 1H, N*H*CH$_3$), 7.48 (d, $J$ = 7.7 Hz, 2 × 1H, H-7), 7.16−7.07 (m, 2 × 2H, ArH), 2.90 (d, $J$ = 4.1 Hz, 2 × 3H, NHC*H*$_3$). Anal. ($C_{20}H_{18}N_4O_2Se_2$·0.9$H_2O$) C, H, N.

**2,2′-Diselenobis[*N*-[2-(diethylamino)ethyl]-1*H*-indole-3-carboxamide] (26)**: $^1$H NMR ($(CD_3)_2SO$) δ 12.75 (s, 2 × 1H, indole NH), 10.08 (s, 2 × 1H, $^+$N*H*$(CH_2CH_3)_2$), 8.09 (t, $J$ = 5.7 Hz, 2 × 1H, CONH), 7.93 (d, $J$ = 8.9 Hz, 2 × 1H, H-4), 7.51 (d, $J$ = 6.8 Hz, 2 × 1H, H-7), 7.19−7.12 (m, 2 × 2H, ArH), 3.78−3.73 (m, 2 × 2H, CONHC*H*$_2$), 3.32 (t, $J$ = 6.5 Hz, 2 × 2H, CONHCH$_2$C*H*$_2$), 3.29−3.20 (m, 2 × 4H, N(C*H*$_2$CH$_3$)$_2$), 1.26 (t, $J$ = 7.2 Hz, 2 × 6H, N(CH$_2$C*H*$_3$)$_2$). Anal. ($C_{30}H_{40}N_6O_2Se_2$·2.0HCl·1.0$H_2O$) C, H, N, $Cl^-$.

**2,2′-Diselenobis[1-[2-(diethylamino)ethyl]-*N*-methyl-1*H*-indole-3-carboxamide] (27)**: $^1$H NMR ($CDCl_3$) δ 8.17 (d, $J$ = 8.2 Hz, 2 × 1H, H-4), 7.35−7.20 (m, 2 × 3H, ArH), 6.61 (d, $J$ = 4.6 Hz, 2 × 1H, CON*H*CH$_3$), 4.28 (t, $J$ = 7.0 Hz, 2 × 2H, 1-NC*H*$_2$), 2.62 (t, $J$ = 7.0 Hz, 2 × 2H, 1-NHCH$_2$C*H*$_2$), 2.45 (q, $J$ = 7.0 Hz, 2 × 4H, N(C*H*$_2$CH$_3$)$_2$), 2.39 (d, $J$ = 4.6 Hz, 2 × 3H, CONHC*H*$_3$), 0.90 (t, $J$ = 7.1 Hz, 2 × 6H, N(CH$_2$C*H*$_3$)$_2$). Anal. ($C_{32}H_{44}N_6O_2Se_2$·0.5$H_2O$) C, H, N.

**Method F. 2,2′-Diselenobis[1-methyl-1*H*-indole-3-carboxylic acid] (22)**. To an ice-cold solution of 4 mL of TFA under $N_2$ was added 420 mg (0.68 mmol) of 2,2′-diselenobis-[1-methyl-1*H*-indole-3-carboxylic acid, *tert*-butyl ester] (**21**). The suspension was maintained at 0 °C for 3 h and then poured into ice $H_2O$. The solids were collected by filtration, washed well with $H_2O$, and then suspended into 80 mL of 10% aqueous $NH_4OH$. The suspension was filtered, and filtrate was adjusted to pH 3 with 6 N aqueous HCl. The precipitated solids were collected by filtration, washed with $H_2O$, and dried to leave 268 mg of pure **22** as an orange solid: $^1$H NMR ($(CD_3)_2SO$) δ 12.35 (s, 2 × 1H, $CO_2H$), 8.04 (d, $J$ = 7.9 Hz, 2 × 1H, H-4), 7.56 (d, $J$ = 8.4 Hz, 2 × 1H, H-7), 7.31−7.20 (m, 2 × 2H, ArH), 3.63 (s, 2 × 3H, NCH$_3$). Anal. ($C_{20}H_{16}N_2O_4Se_2$·0.1$H_2O$) C, H, N.

**Method G. 2-Bromo-1-methylindole-3-(*N*-methylcarboxamide) (15).** A 25 °C suspension of 2.79 g (11 mmol) of 2-bromo-1-methylindole-3-carboxylic acid (**10**) in 13 mL of 1,2-dichloroethane was treated dropwise with 2.41 mL (33 mmol) of $SOCl_2$. The mixture was heated at 75 °C for 2 h. The solution was concentrated to a solid that was coevaporated once with $CH_2Cl_2$. The solid was ice cooled and treated rapidly with 26 mL of 40% aqueous methylamine. The bath was removed and the suspension was stirred at 25 °C for 2 h. The solids were collected by filtration, washed well with $H_2O$, and dried over $P_2O_5$ to leave 2.2 g (75%) of product, mp 154−157 °C. Recrystallization from MeOH provided 1.91 g of **15** as a beige solid in three crops: mp 159−160 °C; $^1$H NMR ($CDCl_3$) δ 8.25−8.14 (m, 1H, H-4), 7.31−7.18 (m, 3H, ArH), 3.78 (s, 3H, indole NCH$_3$), 3.05 (s, 3H, NHC*H*$_3$). Anal. ($C_{11}H_{11}N_2OBr$) C, H, N, Br.

**2-Chloro-1-methylindole-3-[*N*-(2-(diethylamino)ethyl)-carboxamide] (16).** Similar reaction of 2-chloro-1-methylindole-3-carboxylic acid **(11)**[31] with $SOCl_2$ and further reaction with 3 equiv of *N*,*N*-diethylethylenediamine in $CH_2Cl_2$ at 0 °C gave **16** as an oil in 68% yield, further characterized as the hydrochloride salt: mp 170−171 °C; $^1$H NMR ($(CD_3)_2SO$) δ 10.33 (br s, exchanges with $D_2O$, 1H, *H*N$^+$$(CH_2CH_3)_2$), 8.07 (t, $J$ = 5.7 Hz, exchanges with $D_2O$, 1H, CONH), 7.95 (d, $J$ = 7.9 Hz, 1H, H-4), 7.57 (d, $J$ = 8.2 Hz, 1H, H-7), 7.28 (t, $J$ = 7 Hz, 1H, ArH), 7.21 (t, $J$ = 7 Hz, 1H, ArH), 3.80 (s, 3H, indole NCH$_3$), 3.68 (q, $J$ = 6.3 Hz, 2H, CONHC*H*$_2$), 3.27−3.15 (m, 6H, C*H*$_2$N(C*H*$_2$CH$_3$)$_2$), 1.25 (t, $J$ = 7.2 Hz, 6H, N(CH$_2$C*H*$_3$)$_2$. Anal. ($C_{16}H_{22}ClN_3O$·HCl) C, H, N.

**2-Chloroindole-3-[*N*-(2-(diethylamino)ethyl)carboxamide] (17).** Similar reaction of 2-chloroindole-3-carboxylic acid (**12**) with $SOCl_2$ and further reaction with 3 equiv of *N*,*N*-diethylethylenediamine in ether gave **17** in 38% yield: mp 99−108 °C; $^1$H NMR ($CDCl_3$) δ 11.50 (s, 1H, indole NH), 8.19 (d, $J$ = 6.5 Hz, 1H, H-4), 7.33 (d, $J$ = 8.4 Hz, 1H, H-7), 7.21−7.15 (m, 3H, ArH and CONH), 3.54 (q, $J$ = 5.3 Hz, 2H, CONHC*H*$_2$), 2.69 (t, $J$ = 6.0 Hz, 2H, CONHCH$_2$C*H*$_2$), 2.59 (q, $J$ = 7.2 Hz, 4H, N(C*H*$_2$CH$_3$)$_2$, 1.05 (t, $J$ = 7.2 Hz, 6H, N(CH$_2$C*H*$_3$)$_2$). The product was further characterized as the HCl salt: mp 164−165 °C. Anal. ($C_{15}H_{20}ClN_3O$·HCl) C, H, N.

**2-Chloroindole-3-(*N*-methylcarboxamide) (18).** Similar reaction of 2-chloroindole-3-carboxylic acid (**12**) with $SOCl_2$ and further reaction with a saturated solution of anhydrous methylamine in THF at 0 °C gave **18** in 51% yield: mp 234−236 °C; $^1$H NMR ($CD_3)_2SO$) δ 7.85 (d, $J$ = 7.5 Hz, 1H, H-4), 7.59 (br d, $J$ = 4.3 Hz, exchanges with $D_2O$, 1H, CONH), 7.34 (d, $J$ = 8.7 Hz, 1H, H-7), 7.22−7.04 (m, 2H, ArH), 2.80 (d, $J$ = 4.6 Hz, collapses to s with $D_2O$ wash, 1H, CONHC*H*$_3$). Anal. ($C_{10}H_9N_2OCl$) C, H, N.

**Method H. 1-[2-(Diethylamino)ethyl]-*N*-methyl-1*H*-indole-3-carboxamide (19).** A mixture of 2.09 g (10 mmol) of 2-chloroindole-3-(*N*-methylcarboxamide) (**18**), 1.72 g (10 mmol) of 2-(*N*,*N*-diethylamino)ethyl chloride hydrochloride, 7.5 g (23 mmol) of anhydrous $Cs_2CO_3$, 3 g of activated 3A molecular sieves, and 20 mL of acetone was stirred under $N_2$ at 25 °C for 16 h. The mixture was filtered through Celite, and the filtrate was concentrated to a solid that was partitioned between $CHCl_3$ and $H_2O$. The organic phase was dried and concentrated to a residue that was crystallized from EtOAc:hexanes (5:8). The solid was collected and dried to leave 1.43 g (46%) of **19**: mp 103−104 °C; $^1$H NMR ($CDCl_3$) δ 8.24 (d, $J$ = 8.0 Hz, 1H, H-4), 7.33−7.21 (m, 3H, ArH), 6.35 (s, 1H, CON*H*CH$_3$), 4.27 (t, $J$ = 7.6 Hz, 2H, 1-NCH$_2$), 3.06 (d, $J$ = 4.8 Hz, 3H, CONHC*H*$_3$), 2.73 (t, $J$ = 7.5 Hz, 2H, 1-NHCH$_2$C*H*$_2$), 2.62−2.55 (m, 4H, N(C*H*$_2$CH$_3$)$_2$), 1.02 (t, $J$ = 7.0 Hz, 6H, N(CH$_2$C*H*$_3$)$_2$). Anal. ($C_{16}H_{22}N_3OCl$) C, H, N.

**Method I. (*R*)-*N*-(Phenylmethyl)-α-[(trifluoroacetyl)-amino]-1*H*-indole-3-propanamide (29a).** An ice-cold solution of 15 g (50 mmol) of the *N*-trifluoroacetamide of (*R*)-tryptophan (**28a**),[37] synthesized by the general literature procedure,[36] in 50 mL of THF under $N_2$ was treated sequentially with 7.1 g (52.5 mmol) of 1-hydroxybenzotriazole and then 10.83 g (52.5 mmol) of 1,3-dicyclohexylcarbodiimide. After 15 min, the solution was treated with 5.74 mL (52.6 mmol) of benzylamine. The solution was maintained at 0−5 °C for 1 h and then stirred at room temperature overnight. The mixture was filtered, and the collected solid was washed with EtOAc. The filtrate was concentrated to an oil that was dissolved in 250 mL of EtOAc. The solution was washed sequentially with 250 mL portions of 10% aqueous HOAc, $H_2O$, 5% aqueous $NaHCO_3$, $H_2O$, and brine, then dried, and concentrated to a solid. Crystallization from 170 mL of 65:35 2-propanol:petroleum ether afforded 12.81 g (66%) of **29a** as an off-white solid: mp 186−188 °C; $[\alpha]^{23}_D$ −5.6° (*c* = 1.0, MeOH). The $^1$H NMR was essentially identical to that reported previously for the racemate.[26] Anal. ($C_{20}H_{18}F_3N_3O_2$) C, H, N.

**(*R*)-[1-(1*H*-Indol-3-ylmethyl)-2-oxo-2-[(phenylmethyl)-amino]ethyl]carbamic Acid, 1,1-Dimethylethyl Ester**

**(29b).** To a −8 °C mechanically stirred solution of 60 g (197 mmol) of the *N*-BOC derivative of (*R*)-tryptophan (**28b**) in 200 mL of THF under $N_2$ was added sequentially 28 g (207 mmol) of HOBT and 42.7 g (207 mmol) of DCC. After 0.5 h, the solution was treated with 22.6 mL (207 mmol) of benzylamine, and then the mixture was allowed to warm to 25 °C. After 3 d of stirring, the mixture was filtered and the collected solid was washed with EtOAc (3 × 50 mL). The filtrate was concentrated to an oil that was dissolved in 250 mL of EtOAc. The organic phase was washed sequentially with 300 mL portions of 5% aqueous HOAc (2×), $H_2O$, 5% aqueous $NaHCO_3$, $H_2O$, and brine, dried, and concentrated to a solid. Crystallization from 350 mL of 6:1 EtOAc:hexanes afforded 69.2 g (89%) of **29b** as a white solid: mp 136−139 °C (lit.[41] mp 142.5−143.5 °C); $[\alpha]^{23}_D$ +0.02° (*c* = 1.0, MeOH) [lit.[41] $[\alpha]^{23}_D$ +8° (*c* = 0.25, 1:1 DMF:MeOH)]; $^1$H NMR ($CDCl_3$) $\delta$ 8.00 (br s, 1H, NH), 7.67 (d, *J* = 8 Hz, 1H, ArH), 7.36 (d, *J* = 8.2 Hz, 1H, ArH), 7.23−7.19 (m, 4H, ArH), 7.15 (t, *J* = 7.0 Hz, 1H, ArH), 7.00−6.95 (m, 3H, ArH), 5.96 (br s, 1H, CHN*H*), 5.16 (br s, 1H, N*H*$CH_2$), 4.46−4.40 (m, 1H, 3-$CH_2CH$), 4.38−4.22 (m, 2H, NHC$H_2$), 3.35 (dd, *J* = 14.6, 5.4 Hz,1H, 3-CH), 3.17 (dd, *J* = 14.4, 7.7 Hz, 1H, 3-CH), 1.41 (s, 9H, $C(CH_3)_3$). The mother liquor was processed to leave 5.2 g (7%) of a second crop after two crystallizations: mp 132−136 °C.

**Method J. [*R*-(*R*,R**)]-2,2′-diselenobis[*N*-(phenylmethyl)-α-[(trifluoroacetyl)amino]-1*H*-indole-3-propanamide] (32a) and [*R*-(*R*,R**)]-2,2′-selenobis[*N*-(phenylmethyl)-α-[(trifluoroacetyl)amino]-1*H*-indole-3-propanamide] (30a).** To an ice-cold solution of 10 g (25.7 mmol) of (*R*)-*N*-(phenylmethyl)-α-[(trifluoroacetyl)amino]-1*H*-indole-3-propanamide (**29a**) in 70 mL of THF under $N_2$ was added dropwise 1.1 mL (13.1 mmol) of $Se_2Cl_2$. The resultant deep red suspension was stirred at 0−5 °C for 4 h, and then quenched with 300 mL of $H_2O$. The solid was collected by filtration, washed well with $H_2O$, and air dried to leave 12 g of impure product as an orange solid. A portion of this material (10.7 g) was dissolved in 100 mL of MeOH, and the solution under $N_2$ was cooled in an ice bath. $NaBH_4$ (*ca.* 1 g) was added portionwise until there was no more color discharge. The mixture was poured immediately into a $N_2$-purged separatory funnel containing 200 mL of ether. The mixture was diluted with 200 mL of $H_2O$, the mixture was shaken, and the phases were separated. The aqueous layer was treated with a small portion of additional $NaBH_4$, extracted again with ether, ice cooled, and then acidified to pH 1 with concentrated HCl. The aqueous phase was extracted with EtOAc (2×), and then the combined extracts were dried and filtered through a pad of $SiO_2$. The filtrate was concentrated to leave 5.91 g of a foam that was dissolved in *ca.* 40 mL of absolute EtOH. The solution was kept at 25 °C for several hours to initiate crystallization and then stored at 5 °C. The solid was collected by filtration, washed with 2-propanol, and dried to leave 4.23 g of pure **32a** as a powdery yellow solid: $^1$H NMR ($CDCl_3$) $\delta$ 8.68 (s, 2 × 1H, indole NH), 7.49 (d, *J* = 7.9 Hz, 2 × 1H, CHN*H*), 7.38 (d, *J* = 7.9 Hz, 2 × 1H, ArH), 7.24−7.18 (m, 2 × 5H, ArH), 7.11 (t, *J* = 7.9 Hz, 2 × 1H, ArH), 6.79−6.76 (m, 2 × 2H, ArH), 5.25 (t, *J* = 5.2 Hz, 2 × 1H, N*H*$CH_2$), 4.33 (m, 2 × 1H, 3-$CH_2CH$), 4.17 (dd, *J* = 14.8, 5.8 Hz, 2 × 1H, NHC$H_2$), 4.05 (dd, *J* = 14.5, 4.9 Hz, 2 × 1H, NHC$H_2$), 2.99 (dd, *J* = 14.0, 8.4 Hz, 2 × 1H, 3-CH), 2.88 (dd, *J* = 14.0, 5.9 Hz, 2 × 1H, 3-CH); FABMS *m*/*z* (relative intensity) 937 ($MH^+$, 52); $[\alpha]^{23}_D$ −132° (*c* = 0.94, MeOH). Anal. ($C_{40}H_{34}N_6O_4F_6Se_2 \cdot H_2O$) C, H, N. Further processing of the filtrate by column chromatography eluting first with 100:0 and then 93:7 $CH_2Cl_2$:EtOAc provided an additional 671 mg of **32a** following crystallization: mp 180−183 °C.

The ether layer from above was concentrated to leave a solid residue that was crystallized from EtOAc:petroleum ether to give pure **30a**: $^1$H NMR [$(CD_3)_2SO$; 60 °C] $\delta$ 10.84 (s, 2 × 1H, NH), 9.48 (d, *J* = 7.7 Hz, 2 × 1H,CHN*H*COF$_3$), 8.42 (t, *J* = 5.3 Hz, 2 × 1H, N*H*$CH_2$), 7.61 (d, *J* = 7.9 Hz, 2 × 1H, ArH), 7.24−7.15(m, 2 × 4H, ArH), 7.06−7.01 (m, 2 × 3H, ArH), 6.95 (t, *J* = 7.2 Hz, 2 × 1H, ArH), 4.79−4.73 (m, 2 × 1H, 3-$CH_2CH$), 4.28−4.14 (m, 2 × 1H, NHC$H_2$Ph), 3.55 (dd, *J* = 14.1, 7.5 Hz, 2 × 1H, dd, 3-C*H*$CH_2$), 3.29 (dd, *J* = 14.2, 7.5 Hz, 2 × 1H, 3-C*H*$CH_2$). Anal. ($C_{40}H_{34}N_6O_4F_6Se \cdot 0.3H_2O$) C, H, N.

**[*R*-(*R*,R**)]-[Diselenobis[1*H*-indol-2,3-ylidene[1-[[(phenylmethyl)amino]carbonyl]-2,1-ethanediyl]]]biscarbamic Acid, Bis(1,1-dimethylethyl) Ester (32b).** To a −5 °C solution of 33 g (84 mmol) of (*R*)-[1-(1*H*-indol-3-ylmethyl)-2-oxo-2-[(phenylmethyl)amino]ethyl]carbamic acid, 1,1-dimethylethyl ester (**29b**) in 170 mL of THF under $N_2$ was added rapidly 3.52 mL (42 mmol) of $Se_2Cl_2$. The resultant deep red suspension was stirred at −5 °C for 2 h, and then quenched with 160 mL of 1 N aqueous NaOH prechilled to −5 °C. The mixture was filtered through a pad of $SiO_2$, and the filter pad was washed with 1.4 L of EtOAc. The filtrate was diluted with 700 mL of $H_2O$, the phases were separated, and the aqueous phase was extracted again with EtOAc. The combined organic phases were washed with brine, dried, and concentrated to leave 32 g of an orange oil. The crude material was dissolved in 120 mL of MeOH, and the solution under $N_2$ was cooled in an ice bath. $NaBH_4$ (*ca.* 1.2 g) was added portionwise until there was no more color discharge. The mixture was poured immediately into a $N_2$-purged separatory funnel containing 250 mL of ether. A small charge of additional $NaBH_4$ was added, and the solution was then diluted with 250 mL of $H_2O$. The funnel was shaken, the phases were separated, and the aqueous phase was extracted again with a small portion of ether. The aqueous phase was ice-cooled and carefully acidified to pH 3.7 with 6 N aqueous HCl. The resultant yellow suspension was stirred at 0−5 °C for 2 h, and then the solid was collected, washed well with $H_2O$, and dried to leave a yellow solid that was slightly impure by TLC (6:1 $CH_2Cl_2$:EtOAc). The solid was dissolved in a minimum volume of hot $CHCl_3$, and the solution was purified by column chromatography, eluting with *ca.* 500 mL of $CH_2Cl_2$ and then with 4:1 $CH_2Cl_2$:EtOAc until all **32b** had been stripped off. The combined product fractions were concentrated to leave 2.8 g of a viscous orange oil. A small drop of this was scratched on a watch glass with some *tert*-butylmethyl ether. Upon standing, crystallization commenced. The bulk oil was seeded and then diluted with MeOH. The precipitated solid was collected, washed with MeOH, and dried to leave 2.28 g of pure **32b** as a yellow-orange powder: $^1$H NMR ($CDCl_3$) $\delta$ 9.39 (br s, 2 × 1H, indole NH), 7.61 (d, *J* = 6.3 Hz, 2 × 1H, ArH), 7.40−6.90 (m, 2 × 8H, ArH), 6.05 (br s, 2 × 1H, CHN*H*), 5.49 (d, *J* = 8.4 Hz, 2 × 1H, N*H*$CH_2$), 4.75 (br m, 2 × 1H, 3-$CH_2CH$), 4.41 (br m, 2 × 2H, NHC$H_2$), 3.48 (d, *J* = 12.3 Hz, 2 × 1H, 3-CH), 3.22 (d, *J* = 8.7 Hz, 2 × 1H, 3-CH), 1.23 (s, 2 × 9H, $C(CH_3)_3$); APCIMS *m*/*z* (relative intensity) 944 ($M^+$, 10); $[\alpha]^{23}_D$ −1.0° (*c* = 1.05, MeOH). Anal. ($C_{46}H_{52}N_6O_6Se_2 \cdot H_2O$) C, H, N. Processing of the mother liquor gave 97 mg of a second crop: mp 145−153 °C.

**Method K. [*R*-(*R*,R**)]-2,2′-Diselenobis[α-amino-*N*-(phenylmethyl)-1*H*-indole-3-propanamide] (33). (a) From 32a.** A suspension of 233.5 mg (0.25 mmol) of [*R*-(*R**,*R**)]-2,2′-diselenobis[*N*-(phenylmethyl)-α-[(trifluoroacetyl)amino]-1*H*-indole-3-propanamide] **(32a)** in 4.5 mL of dry absolute EtOH was treated with 95 mg (2.5 mmol) of $NaBH_4$. The mixture was heated to reflux and then treated with 95 mg of additional portions of $NaBH_4$ after 15 min and 1.25 h. After being heated for a total of 2 h, the mixture was cooled to 25 °C, diluted with MeOH, and poured into an ice-cold stirring mixture of 5% aqueous HCl and EtOAc. The resultant mixture was stirred vigorously for 15 min and filtered, the phases were separated, and the aqueous layer was extracted once more with EtOAc. The combined EtOAc phases were then back-extracted with $H_2O$ (2×). The aqueous phases were combined and diluted with an equal volume of EtOAc. While the pH was carefully monitored, the stirred solution was treated carefully with 10% aqueous NaOH to pH 9.5. The resultant yellow precipitate was collected by filtration, washed well with $H_2O$, and dried to leave 90 mg of pure **33** as a yellow powder: $^1$H NMR ($(CD_3)_2SO$) $\delta$ 11.62 (s, 2 × 1H, NH), 8.23 (t, *J* = 5.1 Hz, 2 × 1H, N*H*$CH_2$), 7.61 (d, *J* = 8.0 Hz, 2 × 1H, ArH), 7.38 (d, *J* = 8.2 Hz, 2 × 1H, ArH), 7.35−6.95 (m, 2 × 7H, ArH), 4.20, 4.17 (2 × dd, *J* = 15.2, 5.8 Hz, 4 × 1H, NHC$H_2$), 3.46−3.40 (br m, 2 × 1H, Ar-$CH_2CH$), 3.04−2.98 (br m, 2 × 1H, Ar-CH), 2.75−2.68 (br m, 2 × 1H, Ar-CH), 1.70 (br s, 2 × 2H, $NH_2$); $^{77}$Se NMR ($CD_3OD$) $\delta$ 383.8 (s); FABMS *m*/*z* (relative intensity) 743 [$(M - 1)^+$, 6]; $[\alpha]^{23}_D$ +0.42° (*c* = 1.0, DMF); HPLC

100% chemical purity and 90% chiral purity. Anal. ($C_{36}H_{36}N_6O_2Se_2 \cdot 1.5H_2O$) C, H, N. Processing of the EtOAc layer from the base treatment provided 15 mg of additional **33**, mp 165−171 °C.

**From 32b.** To a 0−5 °C suspension of 3.63 g (3.85 mmol) of [*R*-(*R**,*R**)]-[diselenobis[1*H*-indol-2,3-ylidene[1-[[(phenylmethyl)amino]carbonyl]-2,1-ethanediyl]]]biscarbamic acid, bis-(1,1-dimethylethyl) ester (**32b**) and 1.35 mL (11.6 mmol) of 2,6-lutidine in 31 mL of $CH_2Cl_2$ was added dropwise 2.25 mL (11.6 mmol) of trimethylsilyl triflate. The solution was stirred under $N_2$ at 0 °C for 15 h and concentrated at 25 °C. The residue was diluted with MeOH and the solution poured into ice-cold 5% aqueous HCl. The aqueous phase was washed with $CH_2Cl_2$, then with cooling and rapid stirring layered with EtOAc and carefully adjusted to pH 9.5 with aqueous NaOH. The precipitate was collected, washed with 2-propanol, and dried to give 2.24 g of a deep orange solid, mp 262−264 °C. The solid was further purified by suspension in 50 mL of ice-cold MeOH and then treatment with portionwise addition of $NaBH_4$ until the color had discharged. The resultant solution was poured slowly into a rapidly stirring solution of 300 mL of ice-cold 1:1 5% aqueous HCl:EtOAc. The precipitate was collected, washed well with $H_2O$, and dried to leave 1.74 g of pure **33** as a deep yellow solid in two crops: HPLC 100% chemical purity and 98.5% chiral purity.

**Method L. (*R,S*)-*N*-(Phenylmethyl)-α-[(trifluoroacetyl)amino]-1*H*-indole-3-propanamide (35).** To a stirred solution of 50.0 g (0.245 mol) of (*R,S*)-tryptophan (previously dried at 60 °C/1 d) and 45 mL (0.32 mol) of dry triethylamine in 80 mL of dry DMF was added 37 mL (0.31 mol) of ethyl trifluoroacetate (dried over $P_2O_5$ and freshly distilled). The flask was sealed and purged with $N_2$, and the mixture was stirred at 20 °C for 2 d. Excess reagents were removed under reduced pressure, and then the derived racemic trifluoroacetamide (**28a**) was diluted with 100 mL of DMF and cooled to 0 °C (ice bath) under $N_2$. Diethylphosphoryl cyanide (DEPC) (46 mL of 93%, 0.28 mol) was added to the stirred solution, followed by 56 mL (0.51 mol) of benzylamine and then 84 mL (0.60 mol) of triethylamine. The resulting mixture was allowed to warm to 20 °C over several hours and then stirred at 20 °C for 3 d. The solution was concentrated under reduced pressure at 60 °C (oil pump), then diluted with 300 mL of $H_2O$, and extracted with EtOAc (3 × 100 mL), back-washing the combined extracts with dilute aqueous $NaHCO_3$ and $H_2O$. The combined extracts were dried and evaporated to leave 116 g of a solid that was recrystallized first from EtOAc/$CH_2Cl_2$ and then $CH_2Cl_2$/petroleum ether to give 76.5 g (80%) of pure **35**: mp 181−183 °C (lit.[26] mp 181−183 °C). Chromatography of the liquors, eluting with $CH_2Cl_2$/petroleum ether (1:2) and then $CH_2Cl_2$, followed by crystallization from $CH_2Cl_2$/petroleum ether, gave 2.9 g (3%) of additional **35**.

**2,2′-Dithiobis[*N*-(phenylmethyl)-α-[(trifluoroacetyl)-amino]-1*H*-indole-3-propanamide] (36).** A solution of 2.06 mL (25.8 mmol) of freshly purified[48] $S_2Cl_2$ in 30 mL of dry THF was added dropwise over 10 min to a stirred, ice-cold solution of 20.0 g (51.4 mmol) of (*R,S*)-*N*-(phenylmethyl)-α-[(trifluoroacetyl)amino]-1*H*-indole-3-propanamide (**35**) in 150 mL of dry THF. After 1 d at 20 °C, the mixture was diluted with 200 mL of $H_2O$, neutralized with $NaHCO_3$, and extracted with $CH_2Cl_2$ (3 × 200 mL). Evaporation under reduced pressure gave a yellow oil that was crystallized directly from EtOH and EtOH/$H_2O$ to leave 17.4 g (76%) of pure **36** as a single pair of diastereoisomers solvated with 1 equiv of EtOH: mp 176.5−178.5 °C (lit.[26] mp 160−164 °C). Chromatography of the liquors, eluting with $CH_2Cl_2$ and then 5% EtOAc/$CH_2Cl_2$, followed by crystallization (EtOH/$H_2O$) gave 1.04 g (5%) of additional **36**.

**2,2′-Dithiobis[α-amino-*N*-(phenylmethyl)-1*H*-indole-3-propanamide] (4).** A suspension of 10.0 g (11.3 mmol) of 2,2′-dithiobis[*N*-(phenylmethyl)-α-[(trifluoroacetyl)amino]-1*H*-indole-3-propanamide] (**36**) in 200 mL of absolute EtOH was treated cautiously with 4.5 g (0.12 mol) of $NaBH_4$, and then the mixture was stirred at 80 °C for 15 min. Further $NaBH_4$ (4.5 g, 0.12 mol) was added, and the mixture was stirred at 80 °C for another 3 h. The reaction was quenched with 1.0 L of $H_2O$, and the mixture was acidified with concentrated HCl to pH 1 and extracted with EtOAc (3 × 500 mL), back-washing the combined extracts with 500 mL of 0.1 M aqueous HCl. The combined aqueous acid portions were neutralized with $NaHCO_3$ and treated with 0.90 mL (9.3 mmol) of 35% $H_2O_2$, followed by stirring at 20 °C for 10 min. The resulting solution was adjusted to pH 10 with $Na_2CO_3$ and then extracted with EtOAc (3 × 500 mL), back-washing the combined extracts with $H_2O$. Evaporation under reduced pressure gave a yellow oil that was crystallized from $CH_2Cl_2$/petroleum ether to give 5.52 g (75%) of **4**: mp 141 °C dec (lit.[26] mp 147−150 °C); chiral HPLC shows **4** to be a *ca.* 1:2:1 mixture of [*S*-(*R**,*R**)]:[*R*-(*R**,*S**)]:[*R*-(*R**,*R**)] stereoisomers.

**Enzyme Assays.** Epidermal growth factor receptor from human A431 carcinoma cell-shed membrane vesicles and protein from v-src baculovirus-infected insect cells were prepared as previously described.[27] A full-length cDNA for the mouse PDGF-β receptor tyrosine kinase was obtained from J. Escobedo (UCSF) and was used to overexpress the protein in baculovirus infected insect cells as previously described.[49,50] The assay was performed in 96-well plates using cell lysates. The kinase activity of the enzyme lysate was assayed by measuring the incorporation of $^{32}$P-labeled phosphate from ATP into trichloroacetic acid precipitable random copolymer of glutamate/tyrosine(4:1 ratio) *in vitro*.

**Cell Culture and Growth Inhibition Assays.** Studies in Swiss 3T3 mouse fibroblasts and A431 human epidermoid carcinoma were carried out as previously described.[27]

**Incorporation of Radioactive Precursors into Macromolecules.** The incorporation of radiolabeled precursors into whole-cell DNA, RNA, and protein was monitored by exposing drug-treated (2 h) or control logarithmically growing cells to [*methyl*-$^3$H]thymidine, [5-$^3$H]uridine or (*S*)-[4,5-$^3$H]leucine (all from ICN Biomedicals, Costa Mesa, CA) at a concentration of 1 μM and a specific activity of 1 μCi/nmol. After 2 h the cells were trypsinized and injected into 2 mL of ice cold 15% trichloroacetic acid (TCA). The resulting precipitate was collected on glass fiber filters, washed with 2 mL aliquots of ice-cold 15% TCA (5×), dried, and placed in scintillation vials along with 10 mL of Ready Gel (Beckman, Irvine, CA). Radioactivity was determined in a Beckman LS 6800 scintillation counter.

**Immunoprecipitation and Western Blotting.** These studies were conducted as previously described.[26]

***In Vivo* Chemotherapy.** Immune competent mice were housed in wire cages on automatic flush racks. Immune deficient mice were housed in microisolator cages within a barrier facility. All animals were maintained on a 12 h light/dark cycle and received food and $H_2O$ *ad libitum*. Animal housing was in accord with AAALAC guidelines. All experimental protocols involving animals were approved by the institutional animal care and use committee.

B16 melanoma, colon carcinoma 26, and M5076 sarcoma were maintained by serial transplantation of tumor fragments (~30 mg) every 2 weeks into naive mice. Passage mice for B16 melanoma and M5076 sarcoma were C57BL/6, while BALB/c mice were the host for colon carcinoma 26. F1 hybrid mice were used for *in vivo* anticancer agent evaluations; B6C3F1 for B16 and M5076; and CD2F1 for colon 26.

The A431 epidermoid carcinoma, C6 glioma, and MCF-7 breast carcinoma human tumor xenografts were maintained by serial passage as described above in nude mice (NCr nu/nu). Nude mice were also used as tumor hosts for anticancer agent evaluations against these human tumor xenografts. Estrogen-containing pellets (Innovative Research of America, 60 day release) were implanted into the left axilla region (side opposite the tumor) of animals bearing the MCF-7 breast xenograft to support its growth.

In each experiment for anticancer evaluation, test mice weighing 18−22 g were randomized and implanted intraperitoneally (ip) with counted numbers of tumor cells or subcutaneously (sc) in the region of the right axilla with tumor fragments on day 0. Animals were treated on the basis of average cage weight on the days indicated in Table 5. The vehicles for compounds **4** and **33** were distilled $H_2O$ and 10% DMA in 50 mM lactic acid buffer, pH 4.0, respectively. Compounds were administered as ip bolus injections or

continuously by an sc implanted Alzet miniosmotic pump (model 2001). General anesthesia, ketamine−xylazine, was used for the implant of miniosmotic pumps. Local anesthesia, lidocaine−epinephrine, was used for pump removal and replacement when a longer duration of therapy was desired. Host body weight change data are reported as the difference between mean group weights on the last and first days of treatment for the B16 and colon 26 life span assays, or as the maximum treatment related weight loss in the M5076, A431, C6, and MCF-7 growth delay assays. Calculation of the median day of death, % T/C (B15 and C26), T − C (M5076, A431, MCF-7, C6), and net logs of tumor cell kill was performed as described previously.[51−54] A positive net cell kill indicates that the tumor burden at the end of therapy was less than at the beginning of therapy. A negative net log cell kill indicates that the tumor grew during treatment.

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