Design, Synthesis, and Evaluations of Substituted 3-[(3- or 4-Carboxyethylpyrrol-2-yl)methylidenyl]indolin-2-ones as Inhibitors of VEGF, FGF, and PDGF Receptor Tyrosine Kinases

Li Sun,* Ngoc Tran, Congxin Liang, Flora Tang, Audie Rice, Randall Schreck, Kara Waltz, Laura K. Shawver, Gerald McMahon, and Cho Tang*

SUGEN, Inc., 230 East Grand Avenue, South San Francisco, California 94080-4811

Received August 23, 1999

Receptor tyrosine kinases (RTKs) have been implicated as therapeutic targets for the treatment of human diseases including cancers, inflammatory diseases, cardiovascular diseases including arterial restenosis, and fibrotic diseases of the lung, liver, and kidney. Three classes of 3-substituted indolin-2-ones containing propionic acid functionality attached to the pyrrole ring at the C-3 position of the core have been identified as catalytic inhibitors of the vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF) RTKs. Some of the compounds were found to inhibit the tyrosine kinase activity associated with isolated vascular endothelial growth factor receptor 2 (VEGF-R2) [fetal liver tyrosine kinase 1 (Flk-1)/kinase insert domain-containing receptor (KDR)], fibroblast growth factor receptor (FGF-R), and platelet-derived growth factor receptor (PDGF-R) tyrosine kinase with IC_{50} values at nanomolar level. Thus, compound **1** showed inhibition against VEGF-R2 (Flk-1/KDR) and FGF-R1 tyrosine kinase activity with IC_{50} values of 20 and 30 nM, respectively, while compound **16f** inhibited the PDGF-R tyrosine kinase activity with IC_{50} value of 10 nM. Structural models and structure−activity relationship analysis of these compounds for the target receptors are discussed. The cellular activities of these compounds were profiled using cellular proliferation assays as measured by bromodeoxyuridine (BrdU) incorporation. Specific and potent inhibition of cell growth was observed for some of these compounds. These data provide evidence that these compounds can be used to inhibit the function of these target receptors.

Introduction

Many growth factors and cytokines mediate cellular signaling through the activation of tyrosine kinases. In human disease, tyrosine kinases have been implicated as potential therapeutic targets for cancers, cardiovascular disease, inflammatory diseases, fibrotic diseases, and other diseases resulting from chronic tissue injury. More recently, synthetic compounds have been identified that can block the function of specific tyrosine kinases and preclinical data are emerging to support the use of these compounds in clinical studies. In the area of cancer, receptor tyrosine kinases play important roles in the process of tumor development and spread.[1−3] In this regard, RTKs have been involved in tumor growth, survival, metastasis, and angiogenesis.[4,5]

We previously reported a series of 3-substituted indolin-2-ones with potent and selective inhibitory activity toward different RTKs.[6] The selectivity of these compounds against particular RTKs depended on the substituents on the indolin-2-one core, especially at the C-3 position. Of particular interest, 3-[(substituted pyrrol-2-yl)methylidenyl]indolin-2-ones showed selective inhibitory activity against the VEGF-R2 (Flk-1/KDR) tyrosine autophosphorylation at the cellular level.[6] From this series of compounds, SU5402 (compound **1** in Table 1) and SU5416 (compound **2** in Table 3) have been utilized as specific inhibitors of the FGF-R and VEGF-R, respectively.

Compound **1** was cocrystallized with the catalytic domain of FGF-R1 (flg-1)[7] and was found to inhibit tyrosine phosphorylation of VEGF-R2 (Flk-1/KDR) and PDGF-R in NIH 3T3 cells with IC_{50} values of 0.4 and 60.9 μM, respectively.[6] The cocrystal structure revealed that the indolin-2-one core occupied the adenine binding site of ATP and that **1** induced receptor conformational changes as a result of interactions between the propionic acid moiety of **1** and Asn568 in the sugar binding region.[7] This study suggested that the substitution on the core might confer kinase selectivity of these ATP mimetic 3-substituted indolin-2-ones. The information deduced from this study is useful for further modification of 3-[(substituted pyrrol-2-yl)methylidenyl]indolin-2-ones as inhibitors against FGF-R1 and other highly homologous RTKs, such as VEGF-R2 (Flk-1/KDR).

In addition, compound **2** was derived from a previous chemical series and is currently under clinical evaluation for the treatment of human cancers.[8] Compound **2** was found to inhibit tyrosine autophosphorylation of VEGF-R2 (Flk-1/KDR) and PDGF-R tyrosine kinases (TKs) in NIH 3T3 mouse fibroblast cells with IC_{50} values of 1.04 and 20.26 μM, respectively.[6] Preclinical studies have indicated that **2** did not inhibit tumor cell growth in vitro but did specifically inhibit endothelial cell proliferation facilitated by VEGF (IC_{50} = 40 nM). Consistent with its anti-angiogenic properties, compound **2** also inhibited tumor growth in vivo in a wide range of tumor models.[8]

Our goal in the present study was to explore the

* To whom correspondence should be addressed.

10.1021/jm9904295 CCC: $18.00
Published on Web 11/19/1999

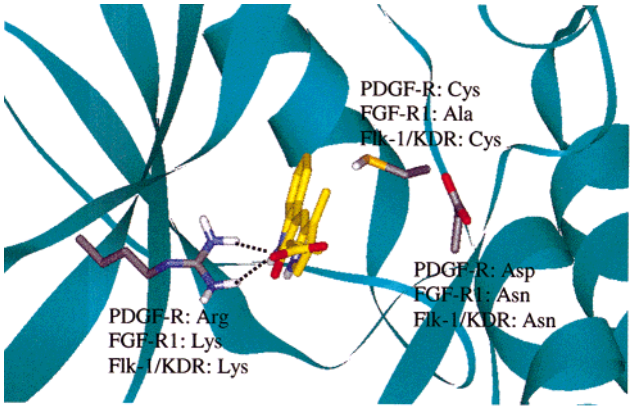


Figure 1. Compound **11a** is shown to be docked within a homology model of the PDGF-R. The receptor peptide backbone is represented by ribbons. Key nonconserved residues surrounding **11a** are shown with carbon atoms colored gray. Corresponding residues of the FGF-R1 and VEGF-R2 (Flk-1/KDR) are indicated. Compound **11a** is designated with carbon atoms colored yellow. Hydrogen bonds are denoted between **11a** and arginine (dotted lines).

influence of various chemical modifications of 3-[(substituted pyrrol-2-yl)methylindenyl]indolin-2-ones on their inhibitory activity and selectivity against VEGF, FGF, and PDGF RTKs. The structure−activity relationship of these compounds with respect to both kinase activity and ligand-dependent events in cells is discussed.

Design

All of the compounds included in this study were based, in part, on chemical features of **1**. The cocrystallographic structure of **1** and the catalytic domain of FGF-R1 revealed important ligand−receptor interactions as well as areas for further modifications.[7] First, it showed the presence of three crystallographic water molecules in proximity to the C-5 position of the indolin-2-one core, suggesting that there is more space in this area for hydrophilic substitutions (such as carboxylic acid and aminosulfonyl functionalities). Second, a comparison of a cocrystallographic study with FGF-R1 and PD173074[9] suggested that introduction of a lipophilic phenyl (or substituted phenyl) substituent at the C-6 position of the **1** core might enhance inhibitory activity against FGF-R1.

For VEGF-R and PDGF-R, homology models were built based on the FGF-R1 crystal structure. Since VEGF-R like FGF-R1 also has an asparagine residue in the sugar binding region (Figure 1), the propionic acid−asparagine interaction observed in the **1** cocrystal structure would likely be preserved upon binding to the VEGF-R. In the case of PDGF-R, the corresponding residue in the sugar-binding region is aspartic acid (Figure 1). Although favorable interaction between the propionic acid of **1** and the aspartic acid of PDGF-R could exist if one of the acids is deprotonated, such interaction would be less favorable than the propionic acid−asparagine interaction observed in the **1**/FGF-R1 cocrystal. On the other hand, PDGF-R has a basic arginine residue in proximaty to the C-4′ position on the pyrrole ring of **1**. Thus, to enhance PDGF-R inhibitory activities, the propionic acid side chain of **1** could be moved to the C-4′ position. In this manner, three types of 3-substituted indolin-2-ones containing propionic acid functionality were designed. First, modification of the substitutions at the C-4, C-5, or C-6 positions resulted in a series of **1** analogues (**9a**−**d** in Table 1). Second, swapping the propionic acid moiety at the C-3′ position with the methyl group at the C-4′ position of **1** gave the corresponding regioisomer, compound **11a** (Table 2). In addition, analogues of **11a** with different substitutions on the indolin-2-one core were also prepared (compounds **11b**−**h** in Table 2). Third, combining the structural features of **2** (a potent VEGF-R inhibitor) and compound **11a** yielded compound **16a** and its analogues (Table 3).

Chemistry

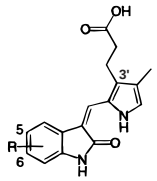
In general, compounds were prepared following condensation of substituted indolin-2-ones with aldehydes in the presence of base as described previously.[6] Indolin-2-one and 6-methoxy-indolin-2-one are commercially available, whereas 4-methyl-, 5-bromo-, 5-aminosulfonyl-, 6-aryl-, and 5-carboxyindolin-2-ones and all of the aldehydes were prepared using the following methods. 4-Methyl- and 5-bromoindolin-2-one were prepared by the method described previously.[6] 5-(Aminosulfonyl)-indolin-2-ones (compounds **4** in Scheme 1) were prepared by amidation of 5-(chlorosulfonyl)indolin-2-one (compound **3** in Scheme 1) that was prepared by sulfonylation of indolin-2-one with chlorosulfonic acid (Scheme 1). 6-Arylindolin-2-ones (**8a**−**e**) were prepared from 5-bromo-2-fluoronitrobenzene. Suzuki coupling of the commercially available aryl boronic acid with 5-bromo-2-fluoronitrobenzene gave 5-aryl-2-fluoronitrobenzene (compounds **5a**−**e** in Scheme 1). Displacement of *o*-fluoro substitution of **5a**−**e** with dimethyl malonate followed by hydrolytic decarboxylation with 6 N aqueous hydrochloric acid and reductive cyclization gave 6-arylindolin-2-ones (**8a**−**e**) (Scheme 1).[10] 5-Carboxyindolin-2-one was synthesized according to the reported method.[11]

Analogues of **1** (**9a**−**d**, Table 1) were prepared as depicted elsewhere.[12] Compound **11a** analogues (Scheme 2 and Table 2) were prepared by condensing the substituted indolin-2-ones with 4-carboxyethyl-3-methylpyrrol-2-carboxaldehyde (**10**), which was prepared as described previously (Scheme 2).[13] Analogues of compound **16a** (Scheme 3 and Table 3) were synthesized by condensing substituted indolin-2-ones and 3-(5-formyl-2,4-dimethyl-1*H*-pyrrol-3-yl)propionic acid (compound **15** in Scheme 3), which was prepared from 4-(2-methoxycarbonylethyl)-3,5-dimethyl-1-*H*-pyrrole-2-carboxylic acid benzyl ester via hydrogenolysis, decarboxylation, formylation, and hydrolysis (Scheme 3). As reported in our previous report, analogues of 3-[(substituted pyrrol-2-yl)methylidenyl]indolin-2-ones were shown to exist exclusively as cis isomers (*Z*-isomer).[6] We suggested that this may be due in part to intramolecular hydrogen bonding between the NH-1′ and C═O at the C-2 position of the indolin-2-one core. All of the compounds in this study are assumed to preferably exist as cis isomers.

Results and Discussion

Compounds were evaluated for their inhibitory activity toward tyrosine phosphorylation activity associated with isolated VEGF-R2 (Flk-1/KDR), FGF-R1, PDGF-R, and epidermal growth factor receptor (EGF-R) ty-

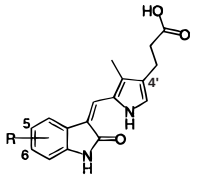
Table 1. Inhibition of Tyrosine Kinase Activities and Ligand-Dependent Cell Proliferation Using **1** Analogues



compounds		inhibition of tyrosine kinase activity (IC_{50}, μM)[a]				inhibition of cell proliferation (IC_{50}, μM)[b]			
ID	R	VEGF-R2	FGF-R1	PDGF-Rβ	EGF-R	VEGF	FGF	PDGF	EGF
1	H	0.02	0.03	0.51	>100	0.05	2.80	28.4	>50
9a	4-CH_3	0.20	0.03	2.11	>100	0.10	0.25	46.4	28.8
9b	5-Br	0.35	0.08	0.62	>100	0.04	0.49	6.70	>50
9c	6-(3-OCH_3phenyl)	28.3	1.2	0.45	>100	0.61	0.14	8.29	49.2
9d	6-(3-OC_2H_5phenyl)	1.0	4.5	2.38	>100	0.46	0.27	11.3	>50

[a] IC_{50} values for VEGF-R2 (Flk-1/KDR) and FGF-R1 were determined by at least two separate tests and reported as mean values. [b] IC_{50} values for PDGF- and EGF-dependent cell proliferations were determined by at least two separate tests and reported as mean values.

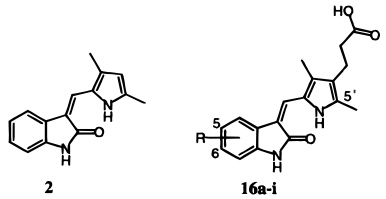
Table 2. Inhibition of Tyrosine Kinase Activities and Ligand-Dependent Cell Proliferation Using Compounds **11a**−**h**



compounds		inhibition of tyrosine kinase activity (IC_{50}, μM)[a]				inhibition of cell proliferation (IC_{50}, μM)[b]			
ID	R	VEGF-R2	FGF-R1	PDGF-Rβ	EGF-R	VEGF	FGF	PDGF	EGF
11a	H	2.14	3.68	0.14	>100	0.40	10.1	58.4	>50
11b	5-COOH	0.24	0.77	4.19	>100	>50	>50	>50	>50
11c	5-SO_2NH_2	0.92	0.46	5.98	>100	>50	>50	>50	>50
11d	6-OCH_3	1.35	3.89	0.14	>100	1.20	31.6	1.40	>50
11e	6-phenyl	0.30	1.05	0.16	>100	0.81	1.00	0.15	21.8
11f	6-(3-OCH_3phenyl)	0.09	0.36	0.17	>100	0.18	0.13	0.18	30.5
11g	6-(2-OCH_3phenyl)	1.47	2.13	1.42	>100	0.04	0.04	0.65	19.2
11h	6-(4-OCH_3phenyl)	0.45	1.54	3.08	>100	1.80	1.60	2.78	22.8

[a] IC_{50} values for VEGF-R2 (Flk-1) and FGF-R1 were determined by at least two separate tests and reported as mean values. [b] IC_{50} values for PDGF- and EGF-dependent cell proliferations were determined by at least two separate tests and reported as mean values.

Table 3. Inhibition of Tyrosine Kinase Activities and Ligand-Dependent Cell Proliferation Using Compounds **16a**−**i**



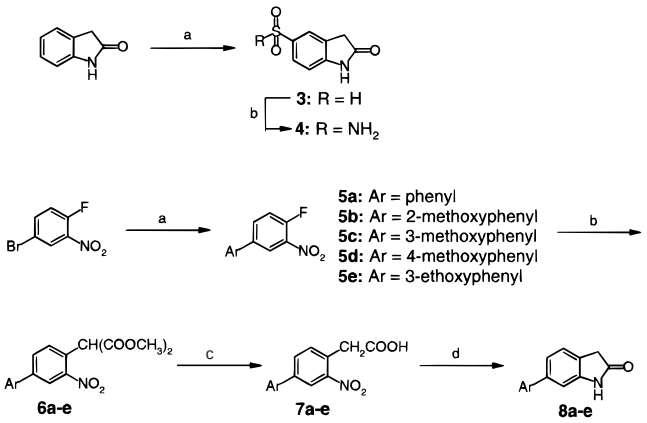
2 **16a-i**

compounds		inhibition of tyrosine kinase activity (IC_{50}, μM)[a]				inhibition of cell proliferation (IC_{50}, μM)[b]			
ID	R	VEGF-R2	FGF-R1	PDGF-Rβ	EGF-R	VEGF	FGF	PDGF	EGF
2		0.70	7.08	10.50	>100	0.04	50.0	4.54	>50
16a	H	2.43	3.04	0.06	>100	0.41	9.30	16.5	>50
16b	5-Br	1.73	2.05	0.06	>100	0.07	3.60	3.65	>50
16c	5-COOH	0.07	0.28	1.21	>100	>50	>50	>50	>50
16d	5-SO_2NH_2	1.26	0.28	1.53	84.8	42.8	>50	>50	>50
16e	6-OCH_3	8.29	5.40	0.55	>100	0.67	25.1	2.35	>50
16f	6-phenyl	0.14	2.29	0.01	>100	10.0	10.0	0.11	21.9
16g	6-(3-OCH_3phenyl)	0.30	1.40	0.10	>100	0.99	1.50	0.23	24.1
16h	6-(2-OCH_3phenyl)	4.37	7.33	2.22	>100	3.0	4.00	0.94	31.3
16i	6-(4-OCH_3phenyl)	0.52	5.76	1.0	>100	4.20	5.64	0.68	>50

[a] IC_{50} values for VEGF-R2 (Flk-1) and FGF-R1 were determined by at least two separate tests and reported as mean values. [b] IC_{50} values for PDGF- and EGF-dependent cell proliferations were determined by at least two separate tests and reported as mean values.

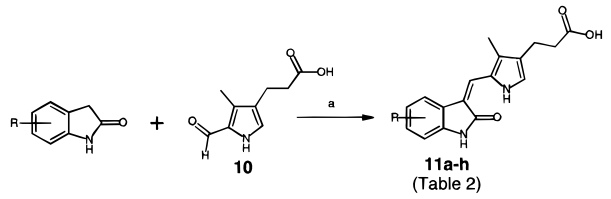
rosine kinases. Tyrosine autophosphorylation assays were used to assess the inhibitory activities of compounds toward PDGF-R and EGF-R tyrosine kinases, whereas peptide−base transphosphorylation assays were performed for VEGF-R2 (Flk-1/KDR) and FGF-R1 kinase activity. Compounds were also evaluated in ligand-stimulated cell proliferation assays using human umbilical vein endothelial cells (HUVECs) and NIH3T3

Scheme 1. Synthesis of Substituted Indolin-2-ones[a,b]



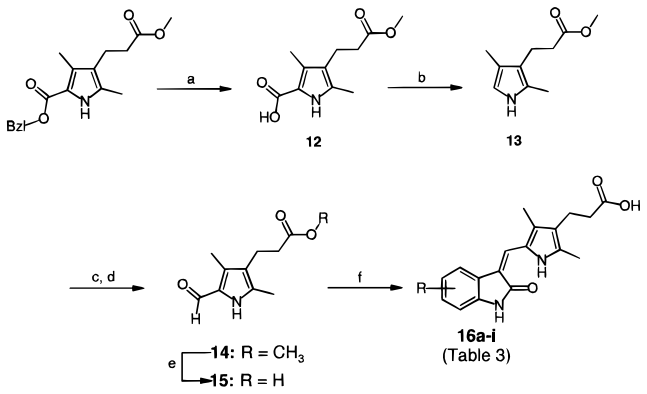
[a] **4**: (a) HSO_3Cl, room temperature to 68 °C, 2.5 h. (b) NH_4OH, ethanol, room temperature, overnight. [b] **8a**−**e**: (a) $ArB(OH)_2$, $(Ph_3P)_4Pd$, $NaHCO_3$, toluene−ethanol, reflux, 2 h. (b) $CH_2(COOCH_3)_2$, NaH, DMSO, 100 °C, 2 h. (c) 6 N HCl_{aq}, overnight. (d) Fe, HOAc, reflux, 2 h.

Scheme 2. Synthesis of Compounds **11a**−**h**[a]



[a] (a) Piperidine, ethanol, reflux, 4 h, then 2 N HCl_{aq}.

Scheme 3. Synthesis of Compounds **16a**−**i**[a]



[a] (a) Pd/C, H_2, methanol, room temperature, 2 h. (b) NaOAc, 100 °C, 3 days. (c) $ClCH{=}N^+(CH_3)_2Cl^-$, $ClCH_2CH_2Cl$, room temperature, 2 h. (d) $NaOH_{aq}$, room temperature. (e) $NaOH_{aq}$, reflux, 2 h. (f) Substituted indolin-2-ones, piperidine, ethanol, reflux, overnight, then 2 N HCl_{aq}.

mouse fibroblast cells. IC_{50} values were defined as the concentration of a compound required to achieve 50% inhibition of tyrosine kinase activity or the ligand-stimulated growth compared to vehicle-treated controls (DMSO). Compounds with IC_{50} values greater than 50 μM were considered inactive. The results from these assays are summarized in Tables 1−3. The SAR analyses are discussed separately for each class of compounds in this report.

1. Analogues of Compound 1. Compound **1** is a prototype compound that exhibits good potency against VEGF-R2 (Flk-1/KDR), FGF-R1, and PDGF-R tyrosine kinases with IC_{50} values of 0.02, 0.03, and 0.51 μM, respectively (Table 1). Since VEGF-R2 (Flk-1) also has an Asn at the position similar to Asn568 in FGF-R1, it is not surprising that **1** has been shown to exhibit similar potency for this receptor kinase as well (Figure 1). In the case of PDGF-R, the corresponding residue is either aspartic acid or aspartate, depending on the ionization status of **1** (Figure 1). Nonetheless, the interaction between the propionic acid of **1** and aspartic acid would be less favorable than that between this propionic acid and asparagine. The reduced potency of **1** toward PDGF-R tyrosine kinase is consistent with this model. Methylation at the C-4 position (**9a** in Table 1) of **1** retained potency against FGF-R1 but led to 10- and 4-fold decrease in potency toward VEGF-R2 (Flk-1/KDR) and PDGF-R, respectively. Bromination at the C-5 position of **1** had little effect on inhibitory activity against either PDGF-R or FGF-R, but considerably decreased the potency toward VEGF-R2 (Flk-1) (**9b** in Table 1). These results have supported a model where FGF-R1 can better accommodate substitutions at both the C-4 and C-5 positions of the indolin-2-one core than either VEGF-R2 (Flk-1) or PDGF-R. In this model, FGF-R1 contains an alanine residue in the vicinity of the C-5 position of indolin-2-one, whereas VEGF-R2 (Flk-1) and PDGF-R contain larger cysteine residues at the comparable position (Figure 1).

Aryl substitutions at the C-6 position of the **1** core retained or slightly decreased the potency against PDGF-R but led to a significant decrease in potency toward VEGF-R2 (Flk-1) and FGF-R1 (compounds **9c** and **9d** in Table 1). The decreased activity of **9c** and **9d** against FGF-R1 was unexpected since superposition of **1** with PD173074, which is a highly potent and selective inhibitor of FGF-R1,[9] suggested that such phenyl substitution might enhance FGF-R1 inhibitory activity by increasing further side chain interactions.

All of these **1** analogues showed potent inhibitory activity against VEGF- and FGF-induced cell proliferation but weak potency toward PDGF-induced cell proliferation. However, these results were not correlated to the data from kinase assays. For instance, compound **9a** was found to be 141- and 40-fold more potent than **9c** when tested against VEGF-R2 (Flk-1) and FGF-R1, respectively. However, both compounds showed comparable potencies in the cell proliferation assays. This is probably due to other factors such as cell membrane penetration, chemical stability, metabolic stability, or the ability to affect other cellular targets including protein kinases. Therefore, advance of these compounds for in vivo assessment will largely rely on clarifying the role of the above factors in the cellular inhibitory activity for these inhibitors. Compound **9b** was the best compound from this series that showed high potency against all three targets in both kinase and cell proliferation assays when compared to EGF-R.

2. Analogues of Compound 11a. The regioisomer of **1**, **11a** (Table 2), was found to be 107- and 123-fold less potent than **1** toward VEGF-R2 (Flk-1) and FGF-R1, respectively, and 4-fold more potent when tested against the PDGF-R (IC_{50} = 0.14 μM, Table 2). Modeling studies have suggested that differences in the inhibitory target profiles of **11a** and **1** may be attributed to different compound−receptor interactions. Specifically, the propionic acid moiety of **1** can interact with asparagine within the catalytic domain of VEGF-R2 (Flk-1)

or FGF-R1 as has been observed previously in the **1**/FGF-R1 cocrystal structure.[7] Also it may interact, though less favorably, with the aspartic acid/aspartate of PDGF-R as discussed previously. On the other hand, the propionic acid moiety of **11a**, attached to the C-4′ position of the core, might interact with a lysine within the catalytic domain of VEGF-R2 (Flk-1) or FGF-R1 and an arginine residue of PDGF-R (Figure 1). In particular, the guanidine group of the arginine residue in PDGF-R is aligned to interact with the carboxylate of **11a** with hydrogen bond distance of about 2 Å. For VEGF-R2 (Flk-1) and FGF-R1, the lysine residue exhibiting a shorter side chain than arginine in PDGF-R would be in a less favorable position to interact with the carboxylate of **11a** (the hydrogen bond distance would be about 2.8 Å). These considerations may help to explain, in part, the finding that **11a** was found to be more potent against PDGF-R than against VEGF-R or FGF-R.

Hydrophilic substituents at the C-5 position of **11a** (i.e., carboxyl or aminosulfonyl group in **11b** or **11c**, respectively) were shown to lead to increased potency toward both VEGF-R2 (Flk-1) and FGF-R1 and decreased the potency against the PDGF-R tyrosine kinase (Table 2). It is conceivable that these substitutions may displace water molecules in the vicinity of the C-5 position that were observed in the **1**/FGF-R1 cocrystal structure[7] and hence might lead to increased potencies toward these RTKs.

In addition, lipophilic substituents at the C-6 position on the indolin-2-one core maintained or reduced the inhibitory activity against the PDGF-R as in the case of **1** series. In this regard, methoxy, phenyl, and 3-methoxyphenyl groups at the C-6 position of the indolin-2-one core (compounds **11d**−**f**) retained the potency against the PDGF-R, whereas C-6 substituents of **11g** and **11h** led to 10- and 22-fold decreased potency, respectively, when compared to **11a**. However, the effect of a C-6 aryl substitution in the **11a** series with respect to potency toward the VEGF-R2 (Flk-1) and FGF-R1 kinases (compounds **11e**−**h**) was found to be opposite that observed for the **1** series. In this regard, C-6 aryl substitutions in the **11a** series increased the inhibitory activity as it relates to VEGF-R2 (Flk-1) and FGF-R1 (Table 2) whereas C-6 aryl substituents in the **1** series reduced the potency (Table 1). We explain these findings by suggesting that the C-6 aryl substitutions may move the indolin-2-one core slightly toward the entrance of the ATP binding site. This movement may weaken the propionic acid/asparagine interaction in **1** series, but strengthen the propionic acid/lysine interaction in the **11a** series. Compound **11f** was found to be the most potent inhibitor of this series as it relates to inhibition of all three of these RTKs with IC_{50} values of 0.09, 0.36, and 0.17 μM for VEGF-R2 (Flk-1), FGF-R1, and PDGF-R, respectively.

Most of the compounds from the **11a** series were also found to show potent inhibitory activity toward VEGF-, FGF-, and PDGF-dependent cell proliferation. The exceptions to this are compounds **11b** and **11c**, which were shown to be inactive using these cell-based assays while exhibiting potency in the kinase assays. This result may be due to poor cell permeability since these compounds are highly polar. A similar lack of correlation between kinase inhibition and inhibition of cell proliferation was observed for the **11a** series as for the **1** series. For example, **11g** was over 31- and 790-fold more potent than **11d** when tested against VEGF- and FGF-dependent cell proliferation, respectively, despite their similar kinase inhibitory profile on these RTKs. In addition, **11g** is slightly more potent against PDGF-dependent cell proliferation but 10-fold less potent in the kinase assay than **11d**. The compound **11f** was the only compound of this series that showed potent inhibitory activity against both kinase and ligand-dependent cell proliferation. In this regard, it inhibited VEGF-, FGF-, and PDGF-dependent cell proliferation with IC_{50} values of 0.18, 0.13, and 0.18 μM, respectively (Table 2).

3. Analogues of Compound 16a. Combining the chemical structural features of compounds **11a** (propionic acid at the C-4′ position of the pyrrole ring) and **2** (3,5-dimethylpyrrole side chain), the **16a** series (Table 3) was prepared. Compound **16a** showed equal potency compared to **11a** when tested against VEGF-R2 (Flk-1) and FGF-R1 but was found to be slightly more potent than **11a** toward PDGF-R (IC_{50} = 0.06 μM) (Table 3). This result suggested that the extra methyl substituent at the C-5′ position on the pyrrole ring of **16a** might provide an extra interaction to PDGF-R. In general, analogues of **16a** shared some structure−activity relationships with the **11a** series. In this regard, a small lipophilic bromo substituent at the C-5 position of **16b** did not change the kinase profile when compared to the parent compound **16a**. On the other hand, the hydrophilic substituents (i.e., carboxyl or aminosulfonyl group in **16c** and **16d**, respectively) increased the inhibitory potency against both VEGF-R2 (Flk-1/KDR) and FGF-R, but decreased the potency toward PDGF-R. Phenyl and 3-methoxyphenyl substitution at the C-6 position on the indolin-2-one core (**16f** and **16g** in Table 3) increased potency toward VEGF-R2 (Flk-1) and FGF-R1 and retained or slightly decreased the potency against PDGF-R. Compound **16f** represented the most potent inhibitor of PDGF-R in this study (IC_{50} = 0.01 μM). In addition, 2-methoxyphenyl substitution in compound **16h** exhibited decreased inhibitory activity against all three RTKs when compared to **16a**.

Lastly, most of the compounds from the **16a** series were found to have inhibitory activity against ligand-dependent cell proliferation stimulated by VEGF, FGF, and PDGF. This is in contrast to their low activity when tested against EGF-dependent cell proliferation. A comparison of this series to the corresponding analogues of **11a** indicated that the **16a** series showed decreased potency toward VEGF- and FGF-dependent cell proliferation and retained similar inhibitory activity against PDGF-dependent cell proliferation. Compounds **16c** and **16d** were inactive in all cell−base assays due to their poor membrane permeability. Similarly, there is no correlation between the kinase and cell proliferation results.

Conclusion

In this report, we described a series of 3-substituted indolin-2-ones containing a propionic acid functionality and their inhibitory activities toward VEGF-R2 (Flk-1/KDR), FGF-R1, and PDGF-R tyrosine kinases. These in vitro results suggest potential utilities for the treat-

ment of cancers, inflammatory diseases, cardiovascular diseases, and fibrotic diseases of the lung, liver, and kidney. The chemical structural requirements for inhibiting these RTKs have been explored.

Our previous report has indicated that **2** showed good potency toward inhibition of VEGF-R2 (Flk-1), whereas **1**, unlike **2**, was also a potent inhibitor of FGF-R1.[6,7] In this case, potency of **1** toward FGF-R1 tyrosine kinase activity was attributed, in part, to the presence of a propionic acid side chain of **1** at the C-3′ position on the pyrrole ring.[7] In this report, we designed, synthesized, and evaluated a series of analogues possessing structural features contained in these two prototype compounds in order to assess the impact of these structural modifications on the profile of kinase inhibitory activities. As described above, the compounds were classified into three groups, **1**-related compounds (Table 1), analogues of **1** regioisomer, **11a** (Table 2), and hybrid compounds (**16a** series in Table 3) containing structure features of both **2** and **11a**.

The following chemical structural requirements for these 3-substituted indolin-2-ones to inhibit VEGF-R2 (Flk-1), FGF-R1, and PDGF-R tyrosine kinases were deduced from the enzymatic assay results and rationalized by protein kinase structural models. First, it was shown that a solubilizing propionic acid was found to be required for high potency against these RTKs. Structural models suggested that the propionic acid side chain could interact favorably with asparagine residues in VEGF-R2 (Flk-1) and FGF-R1 for **1** analogues or with basic residues (lysine or arginine) in all three target RTKs for the **11a** and **16a** analogues. Second, regarding the location of the propionic acid, FGF-R1 and VEGF-R2 (Flk-1) clearly favor substitution at the C-3′ position, while PDGF-R prefers the C-4′ position of the pyrrole ring. Third, the hydrophilic substituent (i.e., aminosulfonyl or carboxyl group) at the C-5 position of the indolin-2-one core is favorable for the inhibitory activity against both VEGF-R2 (Flk-1) and FGF-R1 (i.e., **11b** and **11c** vs **11a** and **16c** and **16d** vs **16a**). This is in agreement with the cocrystallographic study of **1**/FGF-R1, which indicated that three water molecules localized near the C-5 position of the indolin-2-one core. Therefore, hydrophilic substituent at the C-5 position should enhance the affinity of the inhibitor to FGF-R1 as well as VEGF-R2 (Flk-1), given the high homology existing between the two RTKs. Finally, The effect of C-6 aryl substitutions depends on the location of the propionic acid side chain. For the **1** series, it reduced the inhibitory activities against FGF-R1 and VEGF-R2 (Flk-1). However, for the **11a** and **16a** analogues, it increased potency toward these receptors, especially when the substituent is phenyl or 3-methoxyphenyl.

In summary, we have prepared a series of compounds that can inhibit VEGF-R2 (Flk-1/KDR), FGF-R1, and PDGF-R tyrosine kinases. Since all three RTKs play crucial roles in many disease processes such as cancer, arterial restenoesis, diabetic retinopathies, atherosclerosis, fibrosis, and rheumatoid arthritis, inhibitors of these RTKs may have therapeutic potential. For instance, inhibitors that selectively inhibit VEGF-R2 (Flk-1) and Flt-1 could be developed for the treatment of rheumatoid arthritis whereas compounds with selective inhibitory activity against PDGF-R may be useful for arterial restenosis. On the other hand, multiple signal transduction pathways and their respective RTKs could be involved in a given disease (i.e., tumor angiogenesis). In this case, compounds inhibiting all three RTKs discussed in this report (i.e., **11f** in Table 2) may be advantageous as long as such inhibition does not compromise safety. Further evaluation of compounds from these series' for in vivo efficacy in various disease models is ongoing. In vivo efficacy of these compounds in animal models will also depend on pharmacokinetic properties including aqueous solubility, relative oral bioavailability, metabolic stability, and tissue distribution.

Experimental Section

^{1}H NMR spectra were recorded by Acorn NMR using a Nicolet NT300 or a Nicolet NT360. Tetramethylsilane (TMS) was used as an internal standard, and chemical shifts are reported in parts per million (δ) downfield from TMS. Coupling constants are reported in hertz. Mass spectra (electron spray) were recorded by SYNPEP CORP, using an API I PLUS spectrometer. Elemental analyses were performed by Galbraith Laboratories, Inc. Elemental analysis results are within +0.4% of the theoretical values.

5-Aminosulfonyl-2-indolin-2-one (4). To a 100 mL flask charged with 27 mL of chlorosulfonic acid was added slowly 13.3 g (100 mmol) of indolin-2-one. The reaction temperature was maintained below 30 °C during the addition. After the addition, the reaction mixture was stirred at room temperature for 1.5 h, heated to 68 °C for 1 h, cooled, and poured into water. The precipitate was washed with water and dried in a vacuum oven to give 11.0 g of 5-chlorosulfonyl-2-indolin-2-one (**3**) (50% yield), which was used without further purification. Compound **3** (2.1 g, 9.1 mmol) was added to 10 mL of ammonium hydroxide in 10 mL of ethanol and stirred at room temperature overnight. The mixture was concentrated and the solid collected by vacuum filtration to give 0.4 g (20% yield) of 5-aminosulfonyl-2-indolin-2-one as an off-white solid (**4**): ^{1}H NMR (360 MHz, DMSO-d_6) δ 10.67 (s, 1H, NH-1), 7.63−7.66 (m, 2H, H-4,6), 7.13 (s, 2H, H_2NSO_2-5), 6.91 (d, J = 8.04 Hz, 1H, H-7), and 3.56 (s, 2H, CH_2-3); MS *m/z* (relative intensity, %) 211 ($[M - 1]^+$, 100).

6-Phenyl-2-indolin-2-one (8a). Tetrakis(triphenylphosphine)palladium (0.8 g, 0.7 mmol) was added to a mixture of 3.1 g (25.4 mmol) of benzeneboronic acid, 5 g (22.7 mmol) of 5-bromo-2-fluoronitrobenzene and 22 mL of 2 M sodium carbonate solution in 50 mL of toluene and 50 mL of ethanol. The mixture was refluxed for 2 h, concentrated, and the residue extracted twice with ethyl acetate. The ethyl acetate layer was washed with water and brine, dried, and concentrated to give a yellow oil. The oil was chromatographed on silica gel eluting with 5% ethyl acetate in hexane to give 4.75 g (96% yield) of 4-fluoro-3-nitrobiphenyl (**5a**) as a yellow oil.

Dimethyl malonate (10 mL, 87.5 mmol) in 25 mL of dimethyl sulfoxide was added dropwise to 3.5 g (145.8 mmol) of sodium hydride suspended in 25 mL of dimethyl sulfoxide and the mixture was heated at 100 °C for 10 min. The mixture was cooled to room temperature and 4.7 g (21.6 mmol) of **5a** in 25 mL of dimethyl sulfoxide was added. The mixture was heated at 100 °C for 2 h, cooled, and quenched with 300 mL of saturated ammonium chloride solution. The mixture was extracted three times with ethyl acetate and the combined organic layers washed with water and brine and evaporated to give crude dimethyl-3-nitrobiphenyl-4-malonate (**6a**) as a yellow oil.

Crude **6a** was refluxed in 30 mL of 6 N hydrochloric acid for 24 h. The precipitate was collected by filtration, washed with water, and dried to give 4.5 g (80% based on **6a**) of 3-nitrobiphenyl-4-acetic acid (**7a**) as a cream-colored solid.

Iron chips (2.6 g, 46.6 mmol) was added all at once to 4.5 g (17.5 mmol) of **7a** in 40 mL of acetic acid. The mixture was refluxed for 2 h, concentrated to dryness, and taken up in ethyl

acetate. The solids were removed by filtration and the filtrate was washed twice with 1 N hydrochloric acid and brine and dried over anhydrous sodium sulfate. The filtrate was concentrated to give 3.4 g (93% yield) of 6-phenyl-2-indolin-2-one (**8a**) as a light brown solid: ^{1}H NMR (360 MHz, DMSO-d_6) δ 10.40 (s, br, 1H, NH-1), 7.57−7.60 (m, 2H, H-2′,6′), 7.42−7.46 (m, 2H, H-3′,5′), 7.34 (dt, J = 1.96, 7.23 Hz, 1H, H-4′), 7.27 (d, J = 7.69 Hz, 1H, H-4), 7.19 (dd, J = 1.59, 7.69 Hz, 1H, H-5), 7.01 (d, J = 1.59 Hz, 1H, H-7), 3.49 (s, 2H, CH_2-3); MS *m*/*z* (relative intensity, %) 210 ([M + 1]$^+$, 100).

6-(2-Methoxyphenyl)-2-indolin-2-one (8b). Tetrakis-(triphenylphosphine)palladium (1 g, 0.9 mmol) was added to a mixture of 5 g (32.9 mmol) of 2-methoxyphenylboronic acid, 6.6 g (30 mmol) of 5-bromo-2-fluoronitrobenzene, and 30 mL of 2 M sodium carbonate solution in 50 mL of toluene and 50 mL of ethanol. The mixture was refluxed for 2 h, concentrated, and the residue extracted twice with ethyl acetate. The ethyl acetate layer was washed with water and brine, dried, and concentrated to give dark green oil which solidified on standing to give 6 g of crude 4-fluoro-2′-methoxy-3-nitrobiphenyl (**5b**).

Dimethyl malonate (14 mL, 122.5 mmol) was added dropwise to 2.9 g (120.8 mmol) of sodium hydride suspended in 50 mL of dimethyl sulfoxide. The mixture was heated at 100 °C for 15 min and cooled to room temperature. Crude **5b** in 60 mL of dimethyl sulfoxide was added, and the mixture was heated at 100 °C for 2 h. The reaction mixture was cooled and quenched with 300 mL of saturated ammonium chloride solution and extracted twice with ethyl acetate. The extracts were combined, washed with saturated ammonium chloride, water, and brine, dried over anhydrous sodium sulfate, and concentrated to give crude dimethyl 2′-methoxy-3-nitrobiphenyl-4-malonate (**6b**) as a yellow oil.

Crude **6b** was heated at 100 °C in 50 mL of 6 N hydrochloric acid for 24 h and cooled. The precipitate was collected by filtration, washed with water and hexane, and dried to give 9.8 g of 2′-methoxy-3-nitrobiphenyl-4-acetic acid (**7b**) as a light tan solid.

Iron chips (5 g, 89.6 mmol) was added in one portion to 9.8 g (34.1 mmol) of **7b** in 50 mL of glacial acetic acid was heated to 100 °C for 3 h. The reaction mixture was concentrated to dryness, sonicated in ethyl acetate and filtered to remove the insolubles. The filtrate was washed twice with 1 N hydrochloric acid, water, brine, dried over anhydrous sodium sulfate and concentrated. The residue was chromatographed on silica gel eluting with ethyl acetate−hexane (1:2) to give 5.4 g (75% yield based on 5-bromo-2-fluoronitrobenzene) of 6-(2-methoxyphenyl)-2-indolin-2-one (**8b**) as a rose-colored solid: ^{1}H NMR (360 MHz, DMSO-d_6) δ 10.32 (s, br, 1H, NH-1), 7.31 (dt, J = 1.86, 7.39 Hz, 1H, H-4′), 7.23 (dd, J = 1.86, 7.39 Hz, 1H, H-6′), 7.28 (d, J = 7.65 Hz, 1H, H-4), 7.08 (dd, J = 1.16, 7.39 Hz, 1H, H-3′), 6.70 (dt, J = 1.16, 7.39 Hz, 1H, H-5′), 6.98 (dd, J = 1.69, 7.65 Hz, 1H, H-5), 6.91 (d, J = 1.69 Hz, 1H, H-7), 3.74 (s, 3H, OCH_3-2′), 3.47 (s, 2H, CH_2-3); MS *m*/*z* (relative intensity, %) 240 ([M + 1]$^+$, 100).

6-(3-Methoxyphenyl)-2-indolin-2-one (8c). Tetrakis-(triphenylphosphine)palladium (0.7 g, 0.6 mmol) was added to a mixture of 5 g (32.9 mmol) of 3-methoxyphenylboronic acid, 3.8 g (17.3 mmol) of 5-bromo-2-fluoronitrobenzene and 11 mL of 2 M sodium carbonate solution in 100 mL of toluene. The mixture was refluxed for 2 h, diluted with water, and extracted with ethyl acetate. The ethyl acetate was washed with saturated sodium bicarbonate, brine, dried, and concentrated to give an oily solid. The solid was chromatographed on silica gel eluting with ethyl acetate−hexane to give 4.3 g of crude 4-fluoro-3′-methoxy-3-nitrobiphenyl (**5c**).

Dimethyl malonate (9.7 mL, 84.9 mmol) was added dropwise to 2.0 g (83.3 mmol) of sodium hydride suspended in 50 mL of dimethyl sulfoxide. The mixture was heated to 100 °C for 35 min and cooled to room temperature. Compound **5c** (4.2 g, 17.0 mmol) in 50 mL of dimethyl sulfoxide was added and the mixture was heated at 100 °C for 1 h. The reaction mixture was cooled and quenched with 300 mL of saturated ammonium chloride solution and extracted twice with ethyl acetate. The extracts were combined, washed with brine, dried over anhydrous sodium sulfate, and concentrated to give crude dimethyl 3′-methoxy-3-nitrobiphenyl-4-malonate (**6c**) as a pale yellow solid.

Crude **6c** was heated at 110 °C in 45 mL of 6 N hydrochloric acid for 4 days and cooled. The precipitate was collected by filtration, washed with water and hexane, and dried to give 5.3 g of crude 3′-methoxy-3-nitrobiphenyl-4-acetic acid (**7c**) as a light tan solid.

Compound **7c** (5.2 g, 18.1 mmol) was dissolved in methanol and hydrogenated over 0.8 g of 10% palladium on carbon for 3 h at room temperature. The catalyst was removed by filtration and washed with methanol, and the filtrates were combined and concentrated to give a brown solid. The solid was chromatographed on silica gel eluting with ethyl acetate−hexaneacetic acid to give 3.0 g (73% yield based on **5c**) of 6-(3-methoxypheny)-2-indolin-2-one (**9c**) as a pink solid: ^{1}H NMR (360 MHz, DMSO-d_6) δ 10.39 (s, br, 1H, NH-1), 7.35 (t, J = 7.99 Hz, 1H, H-5′), 7.26 (d, J = 7.79 Hz, 1H, H-4), 7.19 (dd, J = 1.30, 7.79 Hz, 1H, H-5), 7.51 (td, J = 0.79, 7.99 Hz, 1H, H-4′), 7.09 (t, J = 2.34 Hz, 1H, H-2′), 7.01 (d, J = 1.30 Hz, 1H, H-7), 6.92 (ddd, J = 0.79, 2.34, 7.99 Hz, 1H, H-6′), 3.80 (s, 3H, OCH_3-3′), 3.49 (s, 2H, CH_2-3); MS *m*/*z* (relative intensity, %) 240 ([M + 1]$^+$, 100).

6-(4-Methoxyphenyl)-2-indolin-2-one (8d). Tetrakis-(triphenylphosphine)palladium (1 g, 0.9 mmol) was added to a mixture of 5 g (32.9 mmol) of 4-methoxyphenylboronic acid, 6.6 g (30 mmol) of 5-bromo-2-fluoronitrobenzene, and 30 mL of 2 M sodium carbonate solution in 50 mL of toluene and 50 mL of ethanol. The mixture was refluxed for 2 h, concentrated, and the residue extracted twice with ethyl acetate. The ethyl acetate layer was washed with water and brine, dried, and concentrated to give a brown oily solid. The solid was chromatographed on silica gel eluting with 5% ethyl acetate in hexane to give crude 4-fluoro-4′-methoxy-3-nitrobiphenyl (**5d**) as a pale yellow solid.

Dimethyl malonate (10 mL, 87.5 mmol) was added dropwise to 2.0 g (83.3 mmol) of sodium hydride suspended in 60 mL of dimethyl sulfoxide. The mixture was heated to 100 °C for 10 min and cooled to room temperature. Crude **5d** (5.2 g, 21.0 mmol) in 50 mL of dimethyl sulfoxide was added and the mixture was heated at 100 °C for 2 h. The reaction mixture was cooled and quenched with 300 mL of saturated ammonium chloride solution and extracted three times with ethyl acetate. The extracts were combined, washed with saturated ammonium chloride, water, and brine, dried over anhydrous sodium sulfate, and concentrated to give crude dimethyl 4′-methoxy-3-nitrobiphenyl-4-malonate (**6d**) as a yellow oil.

Crude **6d** was heated at 100 °C in 60 mL of 6 N hydrochloric acid for 15 h and cooled. The precipitate was collected by filtration, washed with water and hexane, and dried to give 7.2 g of crude 4′-methoxy-3-nitrobiphenyl-4-acetic acid (**7d**) as a light tan solid.

Iron chips (3.6 g, 62.1 mmol) was added in one portion to 7.2 g (25.1 mmol) of **7d** in 50 mL of glacial acetic acid and heated at 100 °C overnight. The reaction mixture was concentrated to dryness, sonicated in ethyl acetate, and filtered to remove the insolubles. The filtrate was washed twice with 1 N hydrochloric acid, brine, dried over anhydrous sodium sulfate, and concentrated to give 2.7 g (38% yield based on 5-bromo-2-fluoronitrobenzene) of 6-(4-methoxyphenyl)-2-indolin-2-one (**8d**) as a rose-colored solid: ^{1}H NMR (360 MHz, DMSO-d_6) δ 10.38 (s, br, 1H, NH-1), 7.50−7.54 (m, 2H, H-2′6′), 7.23 (d, J = 7.72 Hz, 1H, H-4), 7.14 (dd, J = 1.38, 7.72 Hz, 1H, H-5), 6.98−7.02 (m, 2H, H-3′,5′), 6.97 (d, J = 1.38 Hz, 1H, H-7), 3.78 (s, 3H, OCH_3-4′), 3.47 (s, 2H, CH_2-3); MS *m*/*z* (relative intensity, %) 240 ([M + 1]$^+$, 100].

6-(3-Ethoxyphenyl)-2-indolin-2-one (8e). Tetrakis(triphenylphosphine)palladium (0.8 g. 0.7 mmol) was added to a mixture of 4.2 g (25.3 mmol) of 3-ethoxyphenylboronic acid, 5.0 g (22.7 mmol) of 5-bromo-2-fluoronitrobenzene and 22 mL of 2 M sodium carbonate solution in 50 mL of toluene and 50 mL of ethanol. The mixture was refluxed for 2 h and concentrated, water was added, and the mixture was extracted twice with ethyl acetate. The ethyl acetate layer was washed with

water and brine, dried, and concentrated. The residue was chromatographed on silica gel eluting with 5% ethyl acetate in hexane to give 5.3 g of crude 4-fluoro-3′-ethoxy-3-nitrobiphenyl (**5e**) as a yellow oil.

Dimethyl malonate (11.4 mL, 100 mmol) was added dropwise to 4.0 g (167 mmol) of sodium hydride suspended in 20 mL of dimethyl sulfoxide. The mixture was heated to 100 °C for 10 min and cooled to room temperature. Crude **5e** (5.3 g, 20.2 mmol) in 25 mL of dimethyl sulfoxide was added, and the mixture was heated at 100 °C for 2 h. The reaction mixture was cooled and quenched with 300 mL of saturated ammonium chloride solution and extracted three times with ethyl acetate. The extracts were combined, washed with water and brine, dried over anhydrous sodium sulfate, and concentrated to give crude dimethyl 3′-ethoxy-3-nitrobiphenyl-4-malonate (**6e**) as yellow oil.

Crude **6e** was heated at 100 °C in 60 mL of 6 N hydrochloric acid for a total of 4 days and cooled. The precipitate was collected by filtration, washed with water and hexane, and dried to give 4.7 g (77% yield based on 5-bromo-2-fluoronitrobenzene) of crude 3′-ethoxy-3-nitrobiphenyl-4-acetic acid as a light tan solid (**7e**).

Iron chips (2.4 g, 41 mmol) was added in one portion to 4.6 g (15.3 mmol) of **7e** in 40 mL of glacial acetic acid and refluxed for 2 h. The reaction mixture was concentrated to dryness, treated repeatedly with ethyl acetate, and filtered to remove the insoluble. The filtrate was washed twice with 1 N hydrochloric acid, brine, dried over anhydrous sodium sulfate, and concentrated to give 3.5 g (91% yield) of **8e** as a light brown solid: ^{1}H NMR (360 MHz, DMSO-d_6) δ 10.40 (s, br, 1H, NH-1), 7.33 (t, J = 7.92 Hz, 1H, H-5′), 7.25 (d, J = 7.70 Hz, 1H, H-4), 7.19 (dd, J = 1.34, 7.70 Hz, 1H, H-5), 7.13 (d, br, J = 7.92 Hz, 1H, H-4′), 7.07 (t, J = 2.62 Hz, 1H, H-2′), 7.00 (d, J = 1.34 Hz, 1H, H-7), 6.90 (dd, J = 2.62, 7.92 Hz, 1H, H-6′), 4.08 (q, J = 7.00 Hz, 2H, OCH_2CH$_3$-3′), 3.49 (s, 2H, CH$_2$-3) 1.34 (t, J = 7.00 Hz, OCH$_2$CH_3-3′); MS *m*/*z* (relative intensity, %) 254 ([M + 1]$^+$, 100).

3-[4-Methyl-5-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrol-3-yl]propionic Acid (11a). 3-(5-Formyl-4-methyl-1*H*-pyrrol-3-yl)propionic acid (**10**) (9.0 g, 50 mmol), which was prepared as previously reported,[13] and 6.0 g (45 mmol) of indolin-2-one in 50 mL of ethanol were heated to 70 °C for 4 h. Acetic acid (12 mL) was slowly added, resulting in a copious precipitate. The mixture was refluxed for 5 min and cooled to room temperature, and the precipitate was collected by vacuum filtration and washed with 30 mL of ethanol. The precipitate was slurry-washed at reflux in 30 mL of ethanol, cooled to room temperature, collected by vacuum filtration, washed with 20 mL of ethanol, and dried under vacuum to give 11.9 g (89% yield) of 3-[4-methyl-5-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrol-3-yl]propionic acid as an orange solid: ^{1}H NMR (360 MHz, DMSO-d_6) δ 13.29 (s, br, 1H, NH-1′), 12.05 (s, br, 1H, CH$_2$CH$_2$COO*H*-4′), 10.78 (s, br, 1H, NH-1), 7.73 (d, J = 7.43 Hz, 1H, H-4), 7.61 (s, 1H, H-vinyl), 7.13 (s, 1H, H-5′), 7.10 (t, J = 7.43 Hz, 1H, H-6), 6.97 (t, J = 7.43 Hz, 1H, H-5), 6.85 (d, J = 7.43 Hz, 1H, H-7), 2.65 (t, J = 7.30 Hz, 2H, CH_2CH$_2$COOH-4′), 2.46 (t, J = 7.30 Hz, 2H, CH$_2$CH_2-COOH-4′), 2.25 (s, 3H, CH$_3$-3′); MS *m*/*z* (relative intensity, %) 297 (M$^+$, 100). Anal. (C$_{17}$H$_{16}$N$_2$O$_3$·0.25H$_2$O) C, H, N.

3-[4-(2-Carboxyethyl)-3-methyl-1*H*-pyrrol-2-ylmethylene]-2-oxo-2,3-dihydro-1*H*-indole-5-carboxylic Acid (11b). Indolin-2-one (6.7 g, 50 mmol) was added to a stirred suspension of 23 g (172.5 mmol) of aluminum chloride in 30 mL of dichloroethane in an ice bath. Chloroacetyl chloride (11.3 g, 100 mmol) was slowly added and hydrogen chloride gas was evolved. After 10 min of stirring, the reaction was warmed to 40−50 °C for 1.5 h. The mixture was cooled to room temperature and poured into ice water. The precipitate was collected by vacuum filtration, washed with water, and dried under vacuum to give 10.3 g (98%) of 5-chloroacetylindolin-2-one as an off-white solid. A suspension of 9.3 g (44.4 mmol) of 5-chloroacetylindolin-2-one was stirred in 90 mL of pyridine at 80−90 °C for 3 h and then cooled to room temperature. The precipitate was collected by vacuum filtration and washed with 20 mL of ethanol. The solid was dissolved in 90 mL of 2.5 N sodium hydroxide and stirred at 70−80 °C for 3 h. The mixture was cooled to room temperature and acidified to pH 2 with 0.5 N hydrochloric acid. The precipitate was collected by vacuum filtration and washed thoroughly with water to give crude 5-carboxyindolin-2-one as a dark brown solid. After standing overnight, the filtrate yielded 2 g of 5-carboxy-indolin-2-one as a yellow solid. The crude dark brown product was dissolved in hot methanol, the insoluble material was removed by filtration, and the filtrate was concentrated to give another 5.6 g of 5-carboxyindolin-2-one as a brown solid. The combined yield was 85%. ^{1}H NMR (360 MHz, DMSO-d_6) δ 12.56 (s, br, 1H, COOH-5), 10.70 (s, 1H, NH-1), 7.81 (dd, J = 0.68. 8.23 Hz, 1H, H-6), 7.74 (s, br, 1H, H-4), 6.87 (d, J = 8.23 Hz, 1H, H-7), and 3.53 (s, 2H, CH$_2$-3).

The reaction mixture of 88.6 mg (0.5 mmol) of 5-carboxyindolin-2-one, 90.6 mg (0.5 mmol) of **10**, and 75 μL (0.75 mmol) of piperidine in 2.0 mL of ethanol was heated at 95 °C for 5 h and cooled to room temperature. The precipitate was filtered, washed with cold ethanol, 2 N hydrochloric acid, and water, and dried to give 42 mg (25%) of 3-[4-(2-carboxyethyl)-3-methyl-1*H*-pyrrol-2-ylmethylene]-2-oxo-2,3-dihydro-1*H*-indole-5-carboxylic acid (**11b**) as a dark yellow solid: ^{1}H NMR (360 MHz, DMSO-d_6) δ 13.27 (s, br, 1H, NH-1′), 12.28 (s, br, 2H, COOH-5 and CH$_2$CH$_2$COO*H*-4′), 11.11 (s, 1H, NH-1), 8.34 (d, J = 1.36 Hz, 1H, H-4), 7.78 (s, 1H, H-vinyl), 7.75 (dd, J = 1.36, 8.20 Hz, 1H, H-6), 7.19 (d, J = 3.07 Hz, 1H, H-5′), 6.93 (d, J = 8.20 Hz, 1H, H-7), 2.65 (t, J = 7.56 Hz, 2H, CH_2CH$_2$COOH-4′), 2.46 (t, J = 7.56 Hz, 2H, CH$_2$CH_2COOH-4′), 2.29 (s, 3H, CH$_3$-3′); MS *m*/*z* (relative intensity, %) 341 ([M + 1]$^+$, 7). Anal. (C$_{18}$H$_{16}$N$_2$O$_5$·0.5H$_2$O) C, H, N.

3-[4-Methyl-5-(2-oxo-5-sulfamoyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrol-3-yl]propionic Acid (11c). The reaction mixture of 106 mg (0.5 mmol) of 5-aminosulfonylindolin-2-one (**4**), 90.6 mg (0.5 mmol) of **10**, and 75 μL (0.75 mmol) of piperidine in 2.0 mL of ethanol was heated at 95 °C for 5 h and cooled to room temperature. The precipitate was filtered, washed with cold ethanol, 2 N hydrochloric acid, and water, and dried to give 132 mg (70%) of 3-[4-methyl-5-(2-oxo-5-sulfamoyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrol-3-yl]-propionic acid (**11c**): ^{1}H NMR (360 MHz, DMSO-d_6) δ 13.28 (s, br, 1H, NH-1′), 12.03 (s, br, 1H, CH$_2$CH$_2$COO*H*-4′), 11.15 (s, br, 1H, NH-1), 8.20 (d, J = 1.60 Hz, 1H, H-4), 7.73 (s, 1H, H-vinyl), 7.59 (dd, J = 1.60, 8.17 Hz, 1H, H-6), 7.23 (d, J = 2.85 Hz, 1H, H-5′), 7.10 (s, 2H, NH$_2$SO$_2$-5), 6.99 (d, J = 8.17 Hz, 1H, H-7), 2.66 (t, J = 7.41 Hz, 2H, CH_2CH$_2$COOH-4′), 2.47 (t, J = 7.41 Hz, 2H, CH$_2$CH_2COOH-4′), 2.29 (s, 3H, CH$_3$-3′). Anal. (C$_{17}$H$_{17}$N$_3$O$_5$S·H$_2$O) C, H, N.

3-[5-(6-Methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1*H*-pyrrol-3-yl]propionic Acid (11d). This compound was prepared using the same procedure described in the preparation of **11c** with a yield of 43%: ^{1}H NMR (360 MHz, DMSO-d_6) δ 13.10 (s, br, 1H, NH-1′), 12.04 (s, br, 1H, CH$_2$CH$_2$COO*H*-4′), 10.76 (s, br, 1H, NH-1), 7.63 (d, J = 8.29 Hz, 1H, H-4), 7.46 (s, 1H, H-vinyl), 7.07 (d, J = 3.03 Hz, 1H, H-5′), 6.55 (dd, J = 2.32, 8.29 Hz, 1H, H-5), 6.43 (d, J = 2.32 Hz, 1H, H-7), 3.74 (s, 3H, OCH$_3$-6), 2.63 (t, J = 7.31 Hz, 2H, CH_2CH$_2$COOH-4′), 2.45 (t, J = 7.31 Hz, 2H, CH$_2$CH_2-COOH-4′), 2.23 (s, 3H, CH$_3$-3′); MS *m*/*z* (relative intensity, %) 327 ([M + 1]$^+$, 100). Anal. (C$_{18}$H$_{18}$N$_2$O$_4$·0.5H$_2$O) C, H, N.

3-[4-Methyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrol-3-yl]propionic Acid (11e). This compound was prepared using the same procedure described in the preparation of **11c** with a yield of 78%: ^{1}H NMR (360 MHz, DMSO-d_6) δ 13.29 (s, br, 1H, NH-1′), 12.01 (s, vbr, 1H, CH$_2$-CH$_2$COO*H*-4′), 10.89 (s, br, 1H, NH-1), 7.83 (d, J = 7.92, 1H, H-4), 7.65 (s, 1H, H-vinyl), 7.63 (d, J = 7.51 Hz, 2H, H-2″,6″), 7.45 (t, J = 7.51 Hz, 2H, H-3″,5″), 7.33 (t, J = 7.51 Hz, 1H, H-4″), 7.28 (dd, J = 1.93, 7.92 Hz, 1H, H-5), 7.16 (d, J = 1.93 Hz, 1H, H-7), 7.09 (d, J = 1.71 Hz, 1H, H-5′), 2.66 (t, J = 7.47 Hz, 2H, CH_2CH$_2$COOH-4′), 2.46 (t, J = 7.47 Hz, 2H, CH$_2$CH_2-COOH-4′), and 2.27 (s, 3H, CH$_3$-3′); MS *m*/*z* (relative intensity, %) 373 (M$^+$, 100). Anal. (C$_{23}$H$_{20}$N$_2$O$_3$·H$_2$O) C, H, N.

3-{5-[6-(3-Methoxy-phenyl)-2-oxo-1,2-dihydroindol-3-

ylidenemethyl]-4-methyl-1*H*-pyrrol-3-yl}propionic Acid (11f). This compound was prepared using the same procedure as for preparation of **11c** with a yield of 97%: ^{1}H NMR (360 MHz, DMSO-d_6) δ 13.29 (s, br, 1H, NH-1′), 12.07 (s, br, 1H, CH_2CH_2COOH-4′), 10.88 (s, br, 1H, NH-1), 7.82 (d, J = 7.77, 1H, H-4), 7.65 (s, 1H, H-vinyl), 7.36 (t, J = 8.06 Hz, 1H, H-5″), 7.29 (dd, J = 1.40, 7.77 Hz, 1H, H-5), 7.20 (d, J = 8.06 Hz, 1H, H-6″), 7.16 (d, J = 2.23 Hz, 1H, H-2″), 7.14−7.15 (m, br, 1H, H-5′), 7.09 (d, J = 1.40 Hz, 1H, H-7), 6.91 (dd, J = 2.23, 8.06 Hz, 1H, H-4″), 3.82 (s, 3H, OCH_3-3″), 2.65 (t, J = 7.55 Hz, 2H, CH_2CH_2COOH-4′), 2.57 (t, J = 7.55 Hz, 2H, CH_2CH_2-COOH-4′), 2.27 (s, 3H, CH_3-3′); MS *m*/*z* (relative intensity, %) 401 (M^+, 7). Anal. ($C_{24}H_{22}N_2O_4 \cdot 0.75H_2O$) C, H, N.

3-{5-[6-(2-Methoxy-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-4-methyl-1*H*-pyrrol-3-yl}propionic Acid (11g). This compound was prepared using the same procedure as for preparation of **11c** with a yield of 86%: ^{1}H NMR (360 MHz, DMSO-d_6) δ 13.29 (s, br, 1H, NH-1′), 11.59 (s, br, 1H, CH_2CH_2COOH-4′), 10.78 (s, br, 1H, NH-1), 7.76 (d, J = 8.13 Hz, 1H, H-4), 7.62 (s, 1H, H-vinyl), 7.29−7.34 (m, 2H), 6.99−7.15 (m, 6H), 3.76 (s, 3H, OCH_3-2″), 2.66 (t, J = 7.46 Hz, 2H, CH_2CH_2COOH-4′), 2.45 (t, J = 7.46 Hz, 2H, CH_2CH_2COOH-4′), 2.27 (s, 3H, CH_3-3′); MS *m*/*z* (relative intensity, %) 401 ([M − 1]$^+$, 100). Anal. ($C_{24}H_{22}N_2O_4 \cdot H_2O$) C, H, N.

3-{5-[6-(4-Methoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-4-methyl-1*H*-pyrrol-3-yl}propionic Acid (11h). This compound was prepared using the same procedure as for preparation of **11c** with a yield of 59%: ^{1}H NMR (360 MHz, DMSO-d_6) δ 13.26 (s, br, 1H, NH-1′), 10.83 (s, br, 1H, NH-1), 7.78 (d, J = 8.07 Hz, 1H, H-4), 7.61 (s, 1H, H-vinyl), 7.57 (d, J = 8.95 Hz, 2H, H-2″,6″), 7.22 (dd, J = 1.44, 8.07 Hz, 1H, H-5), 7.13 (d, J = 3.09 Hz, 1H, H-5′), 7.04 (d, J = 1.44 Hz, 1H, H-7), 7.00 (d, J = 8.95 Hz, 2H, H-3″,5″), 3.79 (s, 3H, OCH_3-4″), 2.65 (t, J = 7.65 Hz, 2H, CH_2CH_2COOH-4′), 2.44 (t, J = 7.65 Hz, 2H, CH_2CH_2COOH-4′), 2.27 (s, 3H, CH_3-3′); MS *m*/*z* (relative intensity, %) 403 ([M + 1]$^+$, 100). Anal. ($C_{24}H_{22}N_2O_4 \cdot 0.5H_2O$) C, H, N.

3-[2,4-Dimethyl-5-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrol-3-yl]propionic Acid (16a). 4-(2-Methoxycarbonylethyl)-3,5-dimethyl-1*H*-pyrrole-2-carboxylic acid benzyl ester (2.0 g, 6.3 mmol) was hydrogenated over 0.2 g of 10% palladium on carbon in 40 mL of methanol for 2 h at room temperature. The catalyst was removed by filtration and washed with 40 mL of methanol. The filtrate was evaporated to dryness and dried overnight in a vacuum oven to give 1.3 g (92% yield) of 4-(2-methoxycarbonylethyl)-3,5-dimethyl-1*H*-pyrrole-2-carboxylic acid (**12**).

Compound **12** (1.3 g, 5.8 mmol) was ground with 0.5 g of anhydrous sodium acetate and then heated at 100 °C for 3 days. The mixture was dissolved in water, extracted with ethyl acetate, and chromatographed on a column of silica gel in ethyl acetate−hexane to give 0.4 g (38% yield) of 3-(2,4-dimethyl-1*H*-pyrrol-3-yl)propionic acid methyl ester (**13**) as a thick, pale yellow oil.

Compound **13** (0.35 g, 1.9 mmol) and 0.5 g (3.9 mmol) of chloromethylenedimethylammonium chloride in 10 mL of dichloroethane were stirred at room temperature under nitrogen for 2 h. The reaction mixture was concentrated and treated with 10 mL of 6 M sodium hydroxide solution and then extracted three times with 10 mL of ethyl acetate. The ethyl acetate extracts were combined, dried over anhydrous sodium sulfate, and evaporated to dryness to give 0.24 g (60% yield) of 3-(5-formyl-2,4-dimethyl-1*H*-pyrrol-3-yl)propionic acid methyl ester (**14**) as a brown oil.

Compound **14** (0.23 g, 1.1 mmol) in 6 N sodium hydroxide (10 mL) was heated at 100 °C for 2 h. The reaction mixture was cooled to room temperature, acidified with 6 N hydrochloric acid, and extracted three times with 10 mL of ethyl acetate. The combined organic layers were washed with 10 mL of water and 5 mL of brine, dried over anhydrous sodium sulfate, and evaporated to dryness to give 230 mg (100%) of crude 3-(5-formyl-2,4-dimethyl-1*H*-pyrrol-3-yl)propionic acid (**15**) as a brown oil.

Compound **15** (0.22 g, 1.1 mmol), 0.15 g (1.1 mmol) of indolin-2-one, and 0.05 g (0.6 mmol) of piperidine were refluxed in 5 mL of ethanol overnight. The mixture was concentrated to dryness and chromatographed on a silica gel column eluting with ethyl acetate−hexane−acetic acid to give 80 mg (23% yield) of 3-[2,4-dimethyl-5-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrol-3-yl]propionic acid (**16a**) as a mustard yellow solid: ^{1}H NMR (360 MHz, DMSO-d_6) δ 13.38 (s, br, 1H, NH-1′), 12.05 (s, br, 1H, CH_2CH_2COOH-4′), 10.70 (s, br, 1H, NH-1), 7.69 (d, J = 7.39 Hz, 1H, H-4), 7.53 (s, 1H, H-vinyl), 7.06 (t, J = 7.39 Hz, 1H, H-6), 6.95 (t, J = 7.39 Hz, 1H, H-5), 6.85 (d, J = 7.39 Hz, 1H, H-7), 2.63 (t, J = 7.45 Hz, 2H, CH_2CH_2-COOH-4′), 2.34 (t, J = 7.45 Hz, 2H, CH_2CH_2COOH-4′), 2.28 (s, 3H, CH_3), 2.24 (s, 3H, CH_3); MS *m*/*z* (relative intensity, %) 311 ([M + 1]$^+$, 100). Anal. ($C_{18}H_{18}N_2O_3$) C, H, N.

3-[5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrol-3-yl]propionic Acid (16b). The reaction mixture of 106.0 mg (0.5 mmol) of 5-bromo-indolin-2-one, 97.5 mg (0.5 mmol) of **15**, and 75 μL (0.75 mmol) of piperidine in 2.0 mL of ethanol was heated at 95 °C for 5 h and cooled to room temperature. The precipitate was filtered, washed with cold ethanol, 2 N hydrogen chloride, and water, and dried to give 171 mg (88%) of 3-[5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrol-3-yl]-propionic acid as a dark yellow solid: ^{1}H NMR (360 MHz, DMSO-d_6) δ 12.04 (s, br, 1H, CH_2CH_2COOH-4′), 10.80 (s, br, 1H, NH-1), 8.00 (d, J = 2.06 Hz, 1H, H-4), 7.67 (s, 1H, H-vinyl), 7.19 (dd, J = 2.06, 8.50 Hz, 1H, H-6), 6.79 (d, J = 8.50 Hz, 1H, H-7), 2.64 (t, J = 7.63 Hz, 2H, CH_2CH_2COOH-4′), 2.35 (t, J = 7.63 Hz, 2H, CH_2CH_2COOH-4′), 2.29 (s, 3H, CH_3), 2.27 (s, 3H, CH_3); MS *m*/*z* (relative intensity, %) 389 (M^+, 100). Anal. ($C_{18}H_{17}BrN_2O_3$) C, H, N.

3-[4-(2-Carboxyethyl)-3,5-dimethyl-1*H*-pyrrol-2-yl methylene]-2-oxo-2,3-dihydro-1*H*-indole-5-carboxylic Acid (16c). This compound was prepared using the same procedure as for preparation of **16b** with a yield of 65%: ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.39 (s, 1H, NH-1′), 12.31 (s, br, 2H, COOH-5 and CH_2CH_2COOH-4′), 11.07 (s, 1H, NH-1), 8.04 (s, 1H, H-4), 7.72 (d, J = 8.07 Hz, 1H, H-6), 7.70 (s, 1H, H-vinyl), 6.93 (d, J = 8.07 Hz, 1H, H-7), 2.64 (t, J = 7.37 Hz, 2H, CH_2-CH_2COOH-4′), 2.34 (t, J = 7.37 Hz, 2H, CH_2CH_2COOH-4′), 2.30 (s, 3H, CH_3), 2.28 (s, 3H, CH_3); MS *m*/*z* (relative intensity, %) 354 (M^+, 100). Anal. ($C_{19}H_{18}N_2O_5 \cdot 0.5H_2O$) C, H, N.

3-[2,4-Dimethyl-5-(2-oxo-5-sulfamoyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrol-3-yl]propionic Acid (16d). This compound was prepared using the same procedure as for preparation of **16b** with a yield of 60%: ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.40 (s, 1H, NH-1′), 12.09 (s, br, 1H, CH_2CH_2-$COOH$-4′), 11.10 (s, 1H, NH-1), 8.16 (s, 1H, H-4), 7.65 (s, 1H, H-vinyl), 7.55 (d, J = 8.21 Hz, 1H, H-6), 7.10 (s, br, 2H, H_2-NSO_2-5), 6.98 (d, J = 8.21 Hz, 1H, H-7), 2.64 (t, J = 7.22 Hz, 2H, CH_2CH_2COOH-4′), 2.38 (t, J = 7.22 Hz, 2H, CH_2CH_2-COOH-4′), 2.31 (s, 3H, CH_3), 2.28 (s, 3H, CH_3); MS *m*/*z* (relative intensity, %) 389 (M^+, 100). Anal. ($C_{18}H_{19}N_3O_5S \cdot 0.8H_2O$) C, H, N.

3-[5-(6-Methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrol-3-yl]propionic Acid (16e). This compound was prepared using the same procedure as for preparation of **16b** with a yield of 76%: ^{1}H NMR (360 MHz, DMSO-d_6) δ 13.15 (s, br, 1H, NH-1′), 11.70 (s, br, 1H, CH_2-CH_2COOH-4′), 10.65 (s, br, 1H, NH-1), 7.58 (d, J = 8.27 Hz, 1H, H-4), 7.39 (s, 1H, H-vinyl), 6.54 (dd, J = 2.26, 8.27 Hz, 1H, H-5), 6.43 (d, J = 2.26 Hz, 1H, H-7), 3.74 (s, 3H, OCH_3-6), 2.62 (t, J = 7.67 Hz, 2H, CH_2CH_2COOH-4′), 2.30 (t, J = 7.67 Hz, 2H, CH_2CH_2COOH-4′), 2.26 (s, 3H, CH_3), and 2.22 (s, 3H, CH_3); MS *m*/*z* (relative intensity, %) 341 (M^+, 100). Anal. ($C_{19}H_{20}N_2O_4 \cdot 0.5H_2O$) C, H, N.

3-[2,4-Dimethyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrol-3-yl]propionic Acid (16f). This compound was prepared using the same procedure as for preparation of **16b** with a yield of 71%: ^{1}H NMR (360 MHz, DMSO-d_6) δ 13.38 (s, br, 1H, NH-1′), 12.05 (s, br, 1H, CH_2-CH_2COOH-4′), 10.81 (s, br, 1H, NH-1), 7.79 (d, J = 7.84 Hz, 1H, H-4), 7.62 (d, J = 7.51 Hz, 2H, H-2″,6″), 7.58 (s, 1H, H-vinyl), 7.44 (t, J = 7.51 Hz, 2H, H-3″,5″), 7.32 (t, br, J =

7.51 Hz, 1H, H-4″), 7.26 (dd, J = 1.13, 7.84 Hz, 1H, H-5), 7.09 (d, J = 1.13 Hz, 1H, H-7), 2.64 (t, J = 7.71 Hz, 2H, CH_2CH$_2$-COOH-4′), 2.35 (t, J = 7.71 Hz, 2H, CH$_2$CH_2COOH-4′), 2.30 (s, 3H, CH$_3$), 2.26 (s, 3H, CH$_3$); MS m/z (relative intensity, %) 387 ([M + 1]$^+$, 100). Anal. ($C_{24}H_{22}N_2O_3$·0.5H_2O) C, H, N.

3-{5-[6-(3-Methoxy-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrol-3-yl}propionic Acid (16g). This compound was prepared using the same procedure as for preparation of **16b** with a yield of 91%: ^{1}H NMR (360 MHz, DMSO-d_6) δ 13.38 (s, br, 1H, NH-1′), 12.04 (s, br, 1H, CH$_2$CH$_2$COOH-4′), 10.79 (s, br, 1H, NH-1), 7.77 (d, J = 8.05, 1H, H-4), 7.58 (s, 1H, H-vinyl), 7.35 (t, J = 8.11 Hz, 1H, H-5″), 7.27 (dd, J = 1.49, 8.05 Hz, 1H, H-5), 7.19 (d, br, J = 8.11 Hz, 1H, H-6″), 7.14 (t, J = 2.31 Hz, 1H, H-2″), 7.09 (d, J = 1.49 Hz, 1H, H-7), 6.90 (dd, J = 2.31, 8.11 Hz, 1H, H-4″), 3.81 (s, 3H, OCH$_3$-3″), 2.65 (t, J = 7.62 Hz, 2H, CH_2CH$_2$COOH-4′), 2.35 (t, J = 7.62 Hz, 2H, CH$_2$CH_2COOH-4′), 2.30 (s, 3H, CH$_3$), 2.27 (s, 3H, CH$_3$); MS m/z (relative intensity, %) 417 ([M + 1]$^+$, 72). Anal. ($C_{25}H_{24}N_2O_4$·0.25H_2O) C, H, N.

3-{5-[6-(2-Methoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrol-3-yl}propionic Acid (16h). This compound was prepared using the same procedure as for preparation of **16b** with a yield of 44%: ^{1}H NMR (360 MHz, DMSO-d_6) δ 13.38 (s, br, 1H, NH-1′), 12.00 (s, br, 1H, CH$_2$CH$_2$COOH-4′), 10.70 (s, br, 1H, NH-1), 7.71 (d, J = 7.81 Hz, 1H, H-4), 7.55 (s, 1H, H-vinyl), 7.28−7.33 (m, 2H, H-4″,6″), 7.09 (d, J = 7.60 Hz, 1H, H-3″), 7.05 (dd, J = 1.27, 7.81 Hz, 1H, H-5), 7.01 (t, J = 7.60 Hz, 1H, H-5″), 7.00 (d, J = 1.27 Hz, 1H, H-7), 3.76 (s, 3H, OCH$_3$-2″), 2.66 (t, J = 7.60 Hz, 2H, CH_2CH$_2$COOH-4′), 2.35 (t, J = 7.60 Hz, 2H, CH$_2$CH_2COOH-4′), 2.30 (s, 3H, CH$_3$), 2.26 (s, 3H, CH$_3$); MS m/z (relative intensity, %) 415 ([M − 1]$^+$, 100). Anal. ($C_{25}H_{24}N_2O_4$·0.2H_2O) C, H, N.

3-{5-[6-(4-Methoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrol-3-yl}propionic Acid (16i). This compound was prepared using the same procedure as for preparation of **16b** with a yield of 57%: ^{1}H NMR (360 MHz, DMSO-d6) δ 13.35 (s, br, 1H, NH-1′), 12.02 (s, br, 1H, CH$_2$CH$_2$COOH-4′), 10.75 (s, br, 1H, NH-1), 7.74 (d, J = 6.75 Hz, 1H, H-4), 7.56 (d, J = 9.01 Hz, 2H, H-2″,6″), 7.54 (s, 1H, H-vinyl), 7.21 (dd, J = 1.57, 6.75 Hz, 1H, H-5), 7.04 (d, J = 1.57 Hz, 1H, H-7), 7.00 (d, J = 9.01 Hz, 2H, H-3″,5″), 3.79 (s, 3H, OCH$_3$-4″), 2.65 (t, J = 7.54 Hz, 2H, CH_2CH$_2$COOH-4′), 2.35 (t, J = 7.54 Hz, 2H, CH$_2$CH_2COOH-4′), 2.29 (s, 3H, CH$_3$), 2.26 (s, 3H, CH$_3$); MS m/z (relative intensity, %) 417 ([M + 1]$^+$, 100). Anal. ($C_{25}H_{24}N_2O_4$·0.25H_2O) C, H, N.

Molecular Modeling. Homology models for the catalytic domains of VEGF-R2 (Flk-1/KDR) and PDGF-R were generated using the Modeler program in InsightII 95.5 released from Molecular Simulations, Inc. The "open form" of FGF-R1 cocrystallized with SU4984 (PDB code: 1agw) was used as reference, and t he sequence alignment was based on previous study[15] with slight modifications. Since the sequence homologies between FGF-R1 and VEGF-R2 (Flk-1/KDR) (62%) and between FGF-R1 and PDGF-R (51%) are very high, the overall structures were similar. The VEGF-R2 (Flk-1/KDR) and PDGF-R models were superimposed with the FGF-R1 crystal structures based on the Cα trace. Docking of substituted indolin-2-ones to these receptors were done manually based on the FGF-R1/SU4984 crystal structure followed by simple energy minimization.

PDGF-R and EGF-R Kinase Assays. Solubilized membranes derived from NIH3T3 mouse fibroblasts overexpressing human PDGF-Rβ or EGF-R were added to polystyrene 96-well microtiter plates which had been coated with a monoclonal antibody that recognized either the PDGF-R or EGF-R.[14] After a 30 min incubation with lysate, the plates were washed to remove unbound material, and serial dilutions of chemical inhibitors were added to the immunolocalized receptor. The kinase reaction was started by the addition of ATP to the wells at a final concentration of 20 μM for PDGF-Rβ and 3 μM for EGF-R, twice the experimentally determined K_m values for ATP. The kinase reaction was allowed to proceed for 30 min for PDGF-Rβ and 5 min for EGF-R at room temperature and then was stopped by addition of EDTA. The amount of phosphotyrosine present on the receptors in the individual wells was determined by incubating the immunolocalized receptor with a biotinylated monoclonal antibody directed against phosphotyrosine. After removal of the unbound anti-phosphotyrosine antibody, avidin-conjugated horseradish peroxidase H was added to the wells. A stabilized form of 3,3′,5,5′-tetramethylbenzidine dihydrochloride (TMB) and hydrogen peroxide (H_2O_2) was added to the wells. The color readout of the assay was allowed to develop for approximately 30 min and the reaction was stopped with sulfuric acid (H_2SO_4).

FGF-R1 and Flk-1/KDR Kinase Assays. The catalytic portion of FGF-R1 and Flk-1/KDR were expressed as GST fusion proteins following infection of *Spodoptera frugiperda* (sf9) cells with engineered baculoviruses. GST-FGFR1 and GST-Flk1 were purified to homogeneity from infected sf9 cell lysates by glutathione sepharose chromatography. The assays were performed in 96-well microtiter plates that had been coated overnight with 2.0 μg of a polyGlu-Tyr peptide (4:1) (Sigma P-0275) in 0.1 mL of PBS per well. The purified kinases were diluted in kinase assay buffer (100 mM Hepes pH 7.5, 100 mM NaCl, and 0.1 mM sodium orthovanadate) and added to all test wells at 5 ng of GST fusion protein per 0.05 mL volume buffer. Test compounds were diluted in 4% DMSO and added to test wells (0.025 mL/well). The kinase reaction was initiated by the addition of 0.025 mL of 40 μM ATP/40 mM $MnCl_2$, and plates were shaken for 10 min before stopping the reactions with the addition of 0.025 mL of 0.5 M EDTA. The final ATP concentration was 10 μM, which is twice the experimentally determined K_m value for ATP. Negative control wells received $MnCl_2$ alone without ATP. The plates were washed three times with 10 mM Tris pH 7.4, 150 mM NaCl, and 0.05% Tween-20 (TBST). Rabbit polyclonal anti-phosphotyrosine antiserum was added to the wells at a 1:10000 dilution in TBST for 1 h. The plates were then washed three times with TBST. Goat anti-rabbit antiserum conjugated with horseradish peroxidase was then added to all wells (Biosource Cat. No. ALI0404; 1:10000 dilution in TBST) for 1 h. The plates were washed three times with TBST, and the peroxidase reaction was detected with the addition of 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) (Sigma A1888). The color readout of the assay was allowed to develop for 20−30 min and read on a Dynatech MR5000 ELISA plate reader using a 410 nM test filter.

PDGF and EGF-Dependent BrdU Incorporation Assays. NIH 3T3 cells overexpressing human EGF receptors (8000/well) were added to a 96-well microtiter plate in DMEM containing 10% calf serum (Rockville, MD). The plates were incubated overnight at 37 °C, and then deprived of serum for 24 h by incubation with DMEM containing 1% BSA, followed by stimulation with PDGF-β (3.8 nM) or EGF (4 nM) in the presence of various test compounds. After 20 h of incubation, BrdU (Roche Molecular Biochemicals, Indianapolis, IN) was added for a 2 h labeling period, and the cells were fixed with Fix/Denat solution (Roche Molecular Biochemicals, Indianapolis, IN). The amount of BrdU incorporation was then determined using anti-BrdU/POD and the horseradish peroxidase substrate, ABTS (both from Roche Molecular Biochemicals, Indianapolis, IN). All experiments were performed three times, and the IC_{50} values were determined by nonlinear regression using the data analysis program PRISM (Graphpad Inc., San Diego, CA).

VEGF and FGF Cell BrdU Incorporation Assays. These cellular assays were preformed as previously described.[8]

Acknowledgment. The authors thank Robert Blake, Danny Tam, James Rodda, and Kim Lach for providing data on the enzymatic activities of the inhibitors, Zhaoyang Wen for preparing compounds **16c** and **16d**, and Kenneth E. Lipson for organizing the BrdU data.

References

(1) Kolibaba, K. S.; Druker, B. J. Protein tyrosine kinases and cancer. *Biochim. Biophys. Acta* **1997**, *1333*, F217−F248.

(2) Cantley, L. C.; Auger, K. R.; Carpenter, C.; Duckworth, B.; Graziani, A.; Kapeller, R.; Soltoff, S. Oncogenes and signal transduction. *Cell* **1991**, *64*, 281−302.

(3) Merenmies, J.; Parada, L. F.; Henkemeyer, M. Receptor tyrosine kinase signaling in vascular development. *Cell Growth Differ.* **1997**, *8*, 3−310.

(4) Shawver, L. K.; Lipson, K. E.; Fong, T. A. T.; McMahon, G.; Plowman, G. D.; Strawn, L. M. Receptor tyrosine kinases as targets for inhibition of angiogenesis. *Drug Discovery Today* **1997**, *2* (2), 50−63.

(5) Plowman, G. D.; Ullrich, A.; Shawver, L. K. Receptor tyrosine kinases as targets for drug intervention. *DN&P* **1994**, *7* (6), 334−339.

(6) Sun, L.; Tran, N.; Tang, T.; App, H.; Hirth, P.; McMahon, G.; Tang, C. Synthesis and biological evaluations of 3-substituted indolin-2-ones: a novel class of tyrosine kinase inhibitors that exhibit selectivity towards particular receptor tyrosine kinases. *J. Med. Chem.* **1998**, *41* (14), 2588−2603.

(7) Mohammadi, M.; McMahon, G., Sun, L.; Tang, P. C.; Hirth, P.; Yeh, B. K.; Hubbard, S. R.; Schlessinger, J. Structures of the tyrosine kinase domain of fibroblast growth factor receptor in complex with inhibitors. *Science* **1997**, *276*, 955−960.

(8) Fong, A. T. T.; Shawver, L. K.; Sun, L.; Tang, C.; App. H.; Powell, T. J.; Kim, Y. H.; Schreck, R.; Wang, X. Y.; Risau, W.; Ullrich, A.; Hirth, K. P.; McMahon, G. SU5416 is a potent and selective inhibitor of the vascular endothelial growth factor receptor (Flk-1/KDR) that inhibits tyrosine kinase catalysis, tumor vascularization, and growth of multiple tumor types. *Cancer Res.* **1999**, *59*, 99−106.

(9) Mohammadi, M.; Froum, S.; Hamby, J. M.; Schroeder, M. C.; Panek, R. L.; Lu, G. H.; Eliseenkova, A. V.; Green, D.; Schlessinger, J.; Hubbard, S. R. Crystal structure of an angiogenesis inhibitor bound to the FGF receptor tyrosine kinase domain. *EMBO J.* **1998**, *17* (20), 5896−5904.

(10) Quallich, G. J.; Morrissey, P. M. A general oxindole synthesis. *Synthesis* **1993**, *1*, 51−53.

(11) Ogawa, H.; Tamada, S.; Fujioka, T.; Teramoto, S.; Kondo, K.; Yamashita, S.; Yabuuchi, Y.; Tominaga, M.; Nakagawa, K. Studies on Positive Inotropic Agents. V. Synthesis of 1-Heteroaroylpiperazine Derivatives. *Chem. Pharm. Bull.* **1988**, *36*, 2253−2258.

(12) Tang, C.; Nematalla, A.; Sun, L.; Andrews, S.; Garst, M. Improved synthesis of potent tyrosine kinase inhibitor. Manuscript in preparation.

(13) Woodward, R. B.; Ayer, W. A.; Beaton, J. M.; Bickelhaupt, F.; Bonnett, R.; Buchschacher, P.; Closs, G. L.; Dutler, H.; Hannah, J.; Hauck, F. P.; Ito, S.; Langemann, A.; Goff, E. L.; Leimgruber, W.; Lwowski, W.; Sauer, J.; Valenta, Z.; Volz, H. *Tetrahedron* **1990**, *46* (22), 7599−7659.

(14) Strawn, L. M.; McMahon, G.; App, H.; Schreck, R.; Kuchler, W. R.; Longhi, M. P.; Hui, T. H.; Tang, C.; Levitzki, A.; Gazit, A.; Chen, I.; Keri, G.; Orfi, L.; Risau, W.; Flamme, I.; Ullrich, A.; Hirth, K. P.; Shawver, L. K. Flk-1/KDR as a target for tumor growth inhibition. *Cancer Res.* **1996**, *56*, 3540−3545.

(15) Hanks, S.; Quinn, A. M. Protein kinase catalytic domain sequence database: Identification of conserved features of primary structure and classification of family members. *Methods Enzymol.* **1991**, *200*, 38−62.

JM9904295