# Design, Synthesis, and Evaluation of Indolinones as Triple Angiokinase Inhibitors and the Discovery of a Highly Specific 6-Methoxycarbonyl-Substituted Indolinone (BIBF 1120)<sup>†</sup>

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Inhibition of tumor angiogenesis through blockade of the vascular endothelial growth factor (VEGF) signaling pathway is a new treatment modality in oncology. Preclinical findings suggest that blockade of additional pro-angiogenic kinases, such as fibroblast and platelet-derived growth factor receptors (FGFR and PDGFR), may improve the efficacy of pharmacological cancer treatment. Indolinones substituted in position 6 were identified as selective inhibitors of VEGF-, PDGF-, and FGF-receptor kinases. In particular, 6-methoxycarbonyl-substituted indolinones showed a highly favorable selectivity profile. Optimization identified potent inhibitors of VEGF-related endothelial cell proliferation with additional efficacy on pericyctes and smooth muscle cells. In contrast, no direct inhibition of tumor cell proliferation was observed. Compounds 2 (BIBF 1000) and 3 (BIBF 1120) are orally available and display encouraging efficacy in in vivo tumor models while being well tolerated. The triple angiokinase inhibitor 3 is currently in phase III clinical trials for the treatment of nonsmall cell lung cancer.

## Introduction

The concept of tumor angiogenesis inhibition, interfering with tumor blood vessel formation, as a new approach for treating cancer was first proposed almost four decades ago.<sup>1</sup> Several drugs targeting the tumor vasculature, notably the monoclonal antibody to vascular endothelial growth factor (VEGF) bevacizumab,<sup>2</sup> as well as the small-molecule VEGF receptor (VEGFR<sup>*a*</sup>) inhibitors sorafenib<sup>3</sup> and sunitinib,<sup>4</sup> have been successfully introduced into clinical practice during the past years. The pivotal role of VEGFR signaling in the formation of blood vessels and its role in disease-associated angiogenesis is well documented.<sup>5</sup> Other signaling molecules, including platelet-derived growth factor (PDGF) and its receptors (PDGFR), are additional mediators of blood vessel formation and stabilization via pathways that control the proliferation and survival of perivascular cells such as pericytes and smooth muscle cells.<sup>6</sup> A potential escape mechanism, whereby tumor cells switch from VEGF to FGF (fibroblast growth factor) signaling upon sustained VEGFR inhibition has been described recently.<sup>7</sup> Although tumor angiogenesis is an essential mechanism in all solid cancers, a

wide variability in response to treatment with antiangiogenic drugs has been observed, both between patients and between tumor types. Clearly, the development of drugs that simultaneously target several pro-angiogenic receptors (angiokinases) at the same time, while retaining a favorable overall kinase selectivity profile, represents a promising avenue toward a more effective and well tolerated cancer treatment.

In this paper, we report the identification of substituted indolinones as inhibitors of VEGFR-2 kinase. Optimization of an initial lead 1 led to 2 (BIBF 1000) and 3 (BIBF 1120) (Chart 1), compounds targeting VEGFR, FGFR, and PDGFR receptors with low cross-reactivity in the human kinome. The triple angiokinase inhibitor 3 is currently in phase III clinical trials in nonsmall cell lung cancer.<sup>8</sup>

#### Chemistry

The compounds described in Tables 1 and 2 were typically synthesized starting from the corresponding indolinones and aromatic amines as building blocks. Because indolinones substituted in position 6 are rarely described in literature, different routes of synthesis had to be developed by adapting known procedures, as exemplified for compounds 14-17 in Scheme 1. The particular synthetic path was based on the accessibility of the starting materials in each case. Aromatic nitration of phenyl-acetic acids followed by reduction of the nitro group in acidic media with subsequent ring closure offered an easy approach, as shown for compound 14. Alternatively, the acetic acid moiety could be introduced by vicarious nucleophilic substitution<sup>9</sup> followed by reduction, optional removal of protecting groups and subsequent ring closure, as seen for compounds 15 and 16. Should the respective 2-nitro halo benzenes be available, introduction of malonic ester equivalents followed by decarboxylation was

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<sup>&</sup>lt;sup>†</sup> The atomic coordinates for the X-ray structure of compound **3** have been deposited in the Protein Data Bank: PDB code 3C7Q.

<sup>&</sup>lt;sup>*a*</sup>Abbreviations: VEGFR, vascular endothelial growth factor receptor; FGFR, fibroblast growth factor receptor; PDGFR, plateletderived growth factor recptor; CDK, cyclin-dependent kinase; HUVEC, human umbilical vein endothelial cell; IGFR, insulin-like growth factor receptor; InsR, insulin receptor; EGFR, epidermal growth factor receptor; HER, human epidermal growth factor receptor; Plk, polo-like kinase; HNSCC, head and neck squamous cell carcinoma; TBTU, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium tetrafluoroborate; HOBT, hydroxy-benzotriazole.

Chart 1. VEGFR-2 Hit Compound 1 and Clinical Candidates 2 (BIBF 1000) and 3 (BIBF 1120)



the method of choice,<sup>10</sup> as exemplified for compound **17**. The anilines or cycloalkyl amines used as building blocks in Scheme 3 were available by standard chemistry summarized in Scheme 2 starting from commercially available precursors.

The majority of the final compounds were synthesized using a two- or three-step sequence: N-acetylation of the corresponding indolinones<sup>11</sup> (22-25) activated the 3-position for subsequent condensation with aryl ortho-esters.<sup>12</sup> Both steps could be combined in a practical one-pot sequence using acetic anhydride as a solvent (26-28). For large-scale synthesis, however, a stepwise procedure is recommended, facilitating workup due to the cleaner reaction progress (29-33). The amino side chains were introduced in the final step by addition and subsequent elimination of alcohol,<sup>13</sup> followed by in situ acetyl cleavage (2, 3, 34-39, 49-63). The double-bond geometry in the final compounds is locked in a Z-conformation due to an intramolecular hydrogen bond, as could clearly be detected in the respective NMR spectra (upfield shift of the proton in position 4 of the indolinone core due to magnetic shielding by the central aryl group, see also ref 13). Because no shielding was visible in the NMR spectra of intermediates 26-33, the double bond seemed to adopt the *E*-conformation in these compounds.

Several additional analogues were prepared with modifications at the C-6 position (Scheme 4). Amides and esters 1 and 41-45 were obtained by ester cleavage of 34 and subsequent amide coupling or ester formation. Compounds 47 and 48 were accessible by acetylation or condensation reactions of the 6-amino substituted indolinone 46, available by reduction of 35 with Raney nickel.

In general, most compounds with basic side chains displayed good aqueous solubility at pH 4.0 and moderate solubility at higher pH values (e.g., for compound **2**: 690 mg/mL at pH 4.0 and 22 mg/mL at pH 6.0, free base). Table 1. VEGFR-2/HUVEC Inhibition of 6-Substituted Indolinones



compd	<b>P</b> <sup>1</sup>	VEGFR-2 IC $(pM)^{a}$	HUVEC/VEGF EC $(nM)^{a}$
compa	K	1C <sub>50</sub> (IIIvI)	$LC_{50}$ (IIIVI)
35	$NO_2$	$7\pm9$	$60 \pm 10$
45	COOMe	$36 \pm 36$	$103 \pm 11$
34	COOEt	$109 \pm 3$	$47 \pm 21$
36	Cl	$129\pm78$	$49 \pm 54$
46	$NH_2$	$132 \pm 34$	$414 \pm 323$
37	CN	$248 \pm 121$	$281\pm98$
1	CONH <sub>2</sub>	$763 \pm 198$	$342 \pm 176$
48	pyrrol-1-yl	$791\pm349$	$585 \pm 156$
41	CONH <i>i</i> Pr	$1230\pm620$	$1070 \pm 565$
42	CONMe <sub>2</sub>	$1312\pm719$	$542 \pm 310$
43	CONEtMe	$1447 \pm 1751$	$718 \pm 165$
38	COCH <sub>3</sub>	$1752 \pm 433$	NT <sup>b</sup>
44	CONHCH <sub>3</sub>	$2099 \pm 1647$	$1907\pm806$
39	Н	$2112\pm1215$	NT <sup>b</sup>
40	СООН	>1000	NT <sup>b</sup>
47	NHCOCH <sub>3</sub>	> 3000	NT <sup>b</sup>

<sup>*a*</sup> Values are averages  $\pm$  SD of at least three independent determinations. Values "greater than" indicate that half-maximum inhibition was not achieved at the highest concentration tested. <sup>*b*</sup> Not tested.

#### **Results and Discussion**

When evaluating compounds from our CDK4 kinase inhibitor project in a set of kinase selectivity assays, the 6amido-substituted indolinone **1** was identified as a nanomolar inhibitor of VEGFR-2 (IC<sub>50</sub> = 763 nM). Interestingly, this compound was completely devoid of CDK4 inhibition, in contrast to the related 5-amido substituted derivatives.<sup>14</sup> In addition, no other kinase included in the selectivity evaluation was inhibited (IGF1R, InsR, CDK1, CDK2, CDK4, EGFR, HER2, Plk1: IC<sub>50</sub> > 10  $\mu$ M).<sup>15</sup> Compound **1** was therefore chosen as an interesting starting point, complementing activities from a VEGFR-2 HTS campaign.

In a first attempt to evaluate the suitability of this structural class for further investigation, a couple of derivatives with modified basic groups were synthesized by introducing a diverse set of anilines. Unfortunately, all initial derivatives were less active than the hit compound (structures not shown). showing that the 6-amido-substituted indolinone core might not yet be optimal for improving the potency. Compound 1 was, however, more potent than the corresponding unsubstituted indolinone 39 and showed a favorable selectivity profile when compared with 39, which inhibits CDK4, InsR, and IGF1R in the same selectivity panel.<sup>15</sup> We decided to use computational modeling to explore the binding mode of 1 and especially the role of the 6-amido group in more detail.<sup>16</sup> Because indolinone-type kinase inhibitors had been reported as ATP pocket binders before,<sup>10,13</sup> 1 was positioned in a similar way in the homology model of the VEGFR-2 kinase domain showing the typical canonical hydrogen bonds between the lactam moiety and the hinge region (Figure 1A).

## Table 2. Inhibitory Profile of 6-Methoxycarbonyl-Substituted Indolinones



				$IC_{50}\left( nM ight)$			EC50 (nM)
compd	$R^2$	VEGFR-2 <sup>a</sup>	FGFR-1 <sup>b</sup>	PDGFR $\alpha^b$	VEGFR-1 <sup>b</sup>	VEGFR-3 <sup>b</sup>	HUVEC/VEGF a
3	4-(NCH <sub>3</sub> )COCH <sub>2</sub> -(4-methyl-piperazin-1-yl)	$5\pm 2$	$38\pm4$	$18 \pm 0.1$	$104 \pm 14$	$5 \pm 1$	$10 \pm 13$
49	4-(NCH <sub>3</sub> )COCH <sub>2</sub> -(imidazol-1-yl)	$6 \pm 4$	$66 \pm 1$	$8 \pm 0.1$	$55 \pm 10$	$7 \pm 1$	$52 \pm 19$
50	4-(NCOCH <sub>3</sub> )CH <sub>2</sub> CONMe <sub>2</sub>	$8 \pm 4$	$54 \pm 4$	$9 \pm 1$	$61 \pm 6$	$6 \pm 1$	$37 \pm 16$
51	4-(NCOCH <sub>3</sub> )(CH <sub>2</sub> ) <sub>3</sub> NMe <sub>2</sub>	$9\pm 6$	$17 \pm 4$	$7 \pm 1$	$28 \pm 1$	$3 \pm 1$	$15 \pm 5$
52	4-(NCOCH <sub>3</sub> )(CH <sub>2</sub> ) <sub>2</sub> NMe <sub>2</sub>	$12 \pm 4$	$18 \pm 3$	$8 \pm 0.3$	$35\pm8$	$5 \pm 0.3$	$15 \pm 9$
53	4-(NSO <sub>2</sub> CH <sub>3</sub> )(CH <sub>2</sub> ) <sub>2</sub> NMe <sub>2</sub>	$23 \pm 10$	$25\pm5$	$12 \pm 1$	$69 \pm 11$	$10 \pm 1$	$14 \pm 9$
54	4-CO(NCH <sub>3</sub> )(CH <sub>2</sub> ) <sub>2</sub> NMe <sub>2</sub>	$24 \pm 17$	$88\pm8$	$11 \pm 0.2$	$325\pm45$	$59 \pm 4$	$25\pm 8$
45	4-CH <sub>2</sub> (piperidin-1-yl)	$36\pm36$	$71 \pm 20$	$54 \pm 9$	$146 \pm 13$	$32\pm5$	$103 \pm 11$
2	4-(NCH <sub>3</sub> )COCH <sub>2</sub> NMe <sub>2</sub>	$61 \pm 17$	$50 \pm 4$	$20 \pm 2$	$82 \pm 7$	$10 \pm 0.4$	$22 \pm 11$
55	4-CH <sub>2</sub> NMe <sub>2</sub>	$64 \pm 44$	$82\pm 6$	$28\pm3$	$173 \pm 73$	$73 \pm 26$	$28 \pm 11$
56	4-NHCOCH <sub>2</sub> -(4-methyl-piperazin-1-yl)	$83 \pm 42$	$219 \pm 3$	$164 \pm 13$	$278\pm50$	$24 \pm 5$	$83 \pm 45$
57	4-NHCOCH <sub>2</sub> NMe <sub>2</sub>	$119 \pm 82$	NT <sup>c</sup>	NT <sup>c</sup>	NT <sup>c</sup>	NT <sup>c</sup>	$88 \pm 64$
58	4-(NCH <sub>3</sub> )SO <sub>2</sub> Me	$150 \pm 11$	NT <sup>c</sup>	NT <sup>c</sup>	NT <sup>c</sup>	NT <sup>c</sup>	NT <sup>c</sup>
59	4-CH <sub>2</sub> (2-oxo-pyrrolidin-1-yl)	$245\pm43$	NT <sup>c</sup>	NT <sup>c</sup>	NT <sup>c</sup>	NT <sup>c</sup>	NT <sup>c</sup>
60	3-CO(NCH <sub>3</sub> )(CH <sub>2</sub> ) <sub>2</sub> NMe <sub>2</sub>	$248 \pm 61$	NT <sup>c</sup>	NT <sup>c</sup>	NT <sup>c</sup>	NT <sup>c</sup>	$172 \pm 43$
61	Н	$\sim 8000$	NT <sup>c</sup>	NT <sup>c</sup>	NT <sup>c</sup>	NT <sup>c</sup>	NT <sup>c</sup>
62		>10000	NT <sup>c</sup>	NT <sup>c</sup>	NT <sup>c</sup>	NT <sup>c</sup>	NT <sup>c</sup>
63		>10000	NT <sup>c</sup>	NT <sup>c</sup>	NT <sup>c</sup>	NT <sup>c</sup>	NT <sup>c</sup>

<sup>*a*</sup> Values are averages  $\pm$  SD of at least three independent determinations. Values "greater than" indicate that half-maximum inhibition was not achieved at the highest concentration tested. <sup>*b*</sup> Selectivity values are averages  $\pm$  SD of at least two independent determinations. <sup>*c*</sup> Not tested.

Scheme 1. Synthesis of 6-Substituted Indolinones<sup>a</sup>



 $^{a}$ (a) NH<sub>4</sub>NO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, -5 °C; (b) H<sub>2</sub>, Pd/C, AcOH, rt; (c) methyl chloroacetate, KOtBu, DMF, -10 °C, then **6**, -2 °C; (d) *tert*-butyl chloroacetate, KOtBu, DMF, then **8**, -10 °C; (e) H<sub>2</sub>, Ra–Ni, MeOH, rt; (f) 1 N HCl, MeOH, reflux; (g) dimethyl malonate, KOtBu, DMSO, rt, then **10**, 100 °C; (h) LiCl, DMSO, H<sub>2</sub>O, 100 °C; (i) H<sub>2</sub>, Lindlar catalyst, MeOH, rt.

Scheme 2. Synthesis of Aniline or Cycloalkylamine Building Blocks<sup>a</sup>



<sup>*a*</sup>(a) *N*-Methylpiperazine or imidazole or dimethylamine·HCl,  $K_2CO_3$ , acetone, then **64** or **68**, rt; (b) H<sub>2</sub>, Pd/C, MeOH, rt; (c) Ac<sub>2</sub>O, 140 °C; (d) 2-chloro-*N*,*N*-dimethylethylamine·HCl,  $K_2CO_3$ , NaI, acetone, 50 °C; (e) MeI, KOtBu, DMSO, rt; (f) *N*,*N*,*N'*-trimethylpropane-1,3-diamine, NEt<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, then **79** or **80**, rt; (g) chloroacetyl chloride, NEt<sub>3</sub>, THF, rt; (h) dimethylamine·HCl,  $K_2CO_3$ , acetone, rt; (i) trifluoroacetic acid, CH<sub>2</sub>Cl<sub>2</sub>, rt.

The 6-amido moiety points toward the VEGFR-2 specificity pocket flanked by the gatekeeper Val<sup>916</sup> and Lys<sup>868</sup>, which may explain the favorable selectivity profile of **1**. In addition, the carbonyl oxygen of the amido group can form an additional hydrogen bond with Lys<sup>868</sup>, probably accounting for the higher potency of **1** compared with **39**. The basic side chain points toward the water phase of the enzyme. The specificity pocket is defined by mainly hydrophobic amino acids (Figure 1B), suggesting that more lipophilic substituents on the indolinone core than an amido moiety might have the potential for improved potency while retaining high selectivity.

Various 6-substituted derivatives of **1** were synthesized to test this hypothesis (Table 1). For clarity of discussion, only a limited set of representatives is discussed within this paper, sufficient to explain structure-activity relationships. To slightly increase lipophilicity while conserving the amide hydrogen bond, substituted amides **41–44** were tested. Disappointingly, all compounds were slightly less active, probably due to steric hindrance within the specificity pocket. In addition, 6-acetyl substituted **38** did not show any improvement. Inversion of the amido moiety, as in 47, led to complete loss of activity. The significantly more lipophilic 6-ethoxycarbonyl- and 6-methoxycarbonyl-indolinones 34 and 45, however, were considerably more active than 1. By far the most active compound in the whole series was the 6nitro substituted indolinone 35, showing single-digit nanomolar activity. Surprisingly, several substituents without carbonyl moieties displayed high activities, too. The 6-chloro, 6amino, and 6-cyano indolinones 36, 46, and 37 were representatives of this group of compounds. In contrast, attaching a pyrrol-1-yl, as in 48, led to loss of activity. Taken together, the structure-activity relationships in this series are complex. A subtle interplay between steric requirements, polarity, and hydrogen-bonding capability seemed to be decisive for good potency. Not too bulky, preferably lipophilic substituents with the ability to form an additional hydrogen bond, such as in compounds 34, 35, and 45, represented the best combination. The electronic influence of the substituent on the indolinone core, modifying the ability of the lactam to form hydrogen bonds to the hinge region, may also play

#### Scheme 3. Synthesis of Final Compounds<sup>a</sup>



<sup>a</sup> (a) Ac<sub>2</sub>O, 130 °C; (b) (EtO)<sub>3</sub>CPh, Ac<sub>2</sub>O, 130 °C; (c) (EtO)<sub>3</sub>CPh, Ac<sub>2</sub>O, 120 °C; (d) R<sup>3</sup>NH<sub>2</sub>, DMF, 80-100 °C, then piperidine, rt.

Scheme 4. Synthesis of Specific 6-Substituted Indolinones<sup>a</sup>



<sup>*a*</sup>(a) 1 N NaOH, EtOH, 80 °C; (b) R<sup>4</sup>NH<sub>2</sub>, TBTU, HOBT, NEt<sub>3</sub>, or EtN(iPr)<sub>2</sub>, DMF, rt; (c) CDI, DMF, 80 °C, then MeOH, 50 °C; (d) H<sub>2</sub>, Ra–Ni, CH<sub>2</sub>Cl<sub>2</sub>/MeOH, rt; (e) AcCl, NEt<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, rt; (f) 2,5-dimethoxy tetrahydrofuran, AcOH, 100 °C.

a role. However, because electron-withdrawing as well as electron-donating substituents as in **46** can display high activities, this influence is probably minor.

The more potent compounds were also evaluated for their ability to inhibit the VEGF-stimulated proliferation of human

umbilical vein endothelial cells (HUVEC). In general, the trend for inhibition correlated with the biochemical activity (Table 1). This demonstrated that the cellular inhibition is specifically dependent on VEGFR-2 mediated signaling rather than on a general cytostatic or cytotoxic effect. To additionally



**Figure 1.** Proposed binding mode of **1** in VEGFR-2. (A) The hydrogen bonds to the hinge region residues and the proposed additional hydrogen bond of the amide moiety at the indolinone scaffold to  $Lys^{868}$  are shown. The gatekeeper Val<sup>916</sup> and Phe<sup>1047</sup> from the DFG motif are additionally highlighted. (B) Zoom into the hydrophobic kinase selectivity pocket of the ATP pocket that binds to the back part of the indolinone scaffold and the amide substituent. The coloring of the Connolly surface is shown as follows: hydrophobic (brown), hydrophilic (blue), in between (white).

verify this important issue, we tested the effects of compounds **34**, **35**, and **45** on the proliferation of the human epithelial cancer cell line HeLa, which does not express detectable amounts of VEGFR. None of the compounds inhibited proliferation of HeLa cells when tested at similar concentrations to those tested in HUVEC cells (EC<sub>50</sub> > 1  $\mu$ M).

Although we managed to improve the potency of the indolinones by modifying the substituent in position 6 in general, the most active compounds identified were disappointingly not ideal candidates for further optimization at first glance, with 35 containing a nitro group (mutagenic potential) and 45 displaying an ester moiety usually prone to metabolic degradation. On the other hand, selectivity testing revealed that 45 did not inhibit any of 23 kinases tested on a larger kinase panel, whereas 35 was not as selective.<sup>17</sup> The angiokinases FGFR-1 and PDGFRa were additionally targeted by 45 (Table 2), contributing to what we considered an overall very favorable selectivity profile. We were therefore very pleased to learn that despite its ester moiety, 45 showed significant plasma levels after single oral administration in nude mice ( $c_{max}$  952 ng/mL,  $t_{1/2}$  3.5 h, AUC 5246 ng·h/mL, dose 50 mg/kg po). Compound 45 was therefore chosen as a lead for further optimization.

To further explore structure-activity relationships, the basic side chain pointing toward the water phase of the enzyme was chosen for modification (Table 2). A wide variety of linkers between the aniline core and the basic moiety was tolerated, as seen in **51**–**55**. Various basic groups with different  $pK_a$  values were tolerated (**3**, **49**, **45**). In general, basicity was not a prerequisite for high activity, as shown in **50** and **59**. Nevertheless, neutral compounds were inferior to charged compounds in terms of low solubility and therefore not followed up further. Anilines with smaller substituents (**58**) or without any substitution (**61**) were clearly less active. Shifting the substituent from the 4- into the 3-position was also detrimental to activity (**60** vs **54**). Side chains positioned in an orthogonal position to the aniline appeared to be more active than side chains that adopt a coplanar geometry

 Table 3. Blood Levels After Single Oral Administration to Mice<sup>a</sup>

compd	dose (mg/kg)	$c_{\text{plasma}}$ (nM) (2 h)	$c_{\text{plasma}}$ (nM) (24 h)
2	40	$1252\pm177$	$16\pm7$
3	50	$923\pm313$	$8\pm5$

<sup>*a*</sup> For pharmacokinetic analysis, blood was isolated at indicated points in time from the retroorbital venous plexus of five- to six-week-old athymic NMRI-nu/nu female mice and plasma was analyzed using HPLC-MS/MS methodology. The compounds were administered in 0.5% (w/v) hydroxypropyl methylcellulose solution (free base). Characteristic pharmacokinetic parameters have been evaluated for compound **3** (CL 173 mL/min/kg,  $V_d = 32 1/\text{kg}$ , F45%,  $c_{max}$  (po) 1091 nM,  $t_{1/2}$  (po) 5.0 h, AUC (po) 2746 nM·h, dose: 50 mg/kg po, 10 mg/kg iv).

(compare 56 and 57 with 3 and 2). As an alternative explanation, the additional methyl groups in 3 and 2 may increase the potency by picking up lipophilic contacts to the enzyme. The aromatic core of the aniline was essential for activity, as seen when comparing 63 with 57. Although structure–activity relationships were generally shallow in this region, additional potency could be gained by special substituents. Compounds 3 and 49–51, for instance, were active in the single-digit nanomolar region. As revealed by an X-ray structure,<sup>8</sup> compound 3 forms an additional hydrogen bond between the methyl-piperazinyl moiety and Glu<sup>850</sup>, which can explain the excellent IC<sub>50</sub>.

All derivatives tested additionally inhibited the angiokinases FGFR-1 and PDGFR $\alpha$  as well as the homologous kinases VEGFR-1 and -3 in the same range as VEGFR-2 (Table 2), contributing to an overall attractive antiangiogenic target profile. By contrast, selected compounds such as **2** and **3** did not inhibit the 23 kinases<sup>17</sup> mentioned before, neither did they inhibit IGF1R, InsR, CDK1, CDK2, CDK4, EGFR, HER2, or Plk1 (IC<sub>50</sub> > 10  $\mu$ M),<sup>8,15</sup> confirming the low risk of off-target effects for 6-methoxycarbonyl-substituted indolinones in general. Again, correlation of VEGF-stimulated HUVEC proliferation with biochemical data followed the same trend as before (Table 2). As a consequence, our efforts to modify the side chain furnished several highly potent inhibitors of endothelial cell proliferation.



Figure 2. Compound 2 inhibits human tumor xenograft growth in a model of human head and neck SCC (FaDu). Effect of 2 ( $\blacksquare$  100 mg/kg qd,  $\triangle$  2 × 50 mg/kg bid, and  $\blacklozenge$  50 mg/kg qd) or vehicle ( $\bigcirc$ ) on growth of tumor xenografts. Xenografts were established sc in athymic NMRI-nu/nu female mice and allowed to reach a volume of ~50 mm<sup>3</sup> before treatment. Once or twice daily, oral administration of compound 2 or vehicle then commenced and was continued for the duration of the experiment. Data points represent the mean of five mice, bars represent the SD. The compound was administered in 0.5% (w/v) hydroxypropyl methylcellulose solution.

Table 4. Efficacy of 3 in Various Tumor Models

model	derivation	dose [mg/kg/d]	T/C value [%] <sup>a</sup>
FaDu	HNSCC	100	11
		50	$14/27^{b}$
		$2 \times 50$	15
		25	46
		10	82
Caki-1	kidney	100	16
		50	25
		10	71
HT-29	colon	100	16
SKOV-3	ovary	50	19
Calu-6	lung	50	24
PAC-120	prostate	100	34

<sup>*a*</sup>Nude mice bearing established human xenografts  $(0.05-0.1 \text{ cm}^3 \text{ volume})$  were treated once or twice daily with compound **3** po or vehicle control. For *T/C* values, median tumor volumes of each treatment group were compared with the median of the control group at the end of the experiment, depending on tumor growth kinetics. The compound was administered in 0.5% (w/v) hydroxypropyl methylcellulose solution. <sup>*b*</sup> Results of two separate experimental series.

Because of their cellular potency and attractive selectivity profiles, compounds **2** and **3** were selected for in vivo testing. Both compounds yielded good plasma levels 2 h after oral administration to mice and were almost completely cleared from plasma 24 h after administration (Table 3). As shown for the lead structures, none of the compounds inhibited the proliferation of VEGF-independent cell lines at similar concentrations to those tested in HUVEC cells (EC<sub>50</sub> > 1  $\mu$ M), particularly HeLa, Calu-6, and FaDu tumor cell lines.

In mice with established human head and neck FaDu tumor xenografts, once or twice daily po treatment with compound **2** resulted in significant inhibition of tumor growth after 21 days (Figure 2). Compound **2** was well tolerated, with no obvious weight loss over the whole treatment period. Ratios of tumor volumes of treated versus control animals (T/C) in this

experiment were 31% (50 mg/kg qd), 13% (100 mg/kg qd), and 11% (50 mg/kg bid).

Compound 2 was initially chosen as a preclinical candidate and extensively profiled.<sup>18</sup> It was later replaced by 3. The efficacy of 3 was tested in a wide variety of tumor types, summarized in Table 4.

A detailed pharmacological evaluation of 3 has been described elsewhere.<sup>8</sup> Compound 3 inhibited growth-dependent proliferation in a wide range of endothelial cells, pericytes, and smooth muscle cells. Inhibition usually resulted in apoptosis. The Src-family of kinases (IC50 Src 156 nM, Lck 16 nM, Lyn 195 nM) and Flt-3 (IC50 26 nM) were identified as additional targets of 3. Flt-3 inhibition might be an interesting addition to the compound profile, opening up therapeutic options in acute myelogenous leukemia (AML).<sup>19</sup> Compound 3 yielded significant plasma levels after oral administration in various species but is rapidly cleared from plasma by ester cleavage at later points in time. Part of the in vivo efficacy may be attributed to the sustained inhibition of VEGF receptor phosphorylation by 3, lasting up to 32 h after compound exposure.8 The mechanistic reason for this prolonged inhibition is currently under investigation. It is intriguing to speculate that prolonged receptor blockade in combination with fast in vivo clearance after drug exposition<sup>20</sup> may explain the excellent efficacy of 3, being at the same time well tolerated in different species.

## Conclusions

6-Methoxycarbonyl-substituted indolinones are potent inhibitors of VEGFR-1/2/3, PDGFRa, and FGFR-1, with low cross-reactivity against a panel of other kinases. This attractive antiangiogenic target profile translates well into cellular efficacy, exemplified by VEGF-specific inhibition of HUVEC proliferation, as well as inhibition of pericytes and smooth muscle cells. At similar concentrations, no direct effects on the proliferation of tumor cell lines can be observed. Typical methoxycarbonyl-substituted compounds are orally available but almost completely cleared from plasma 24 h after administration in mice. Oral application in tumor xenograft experiments leads to complete inhibition of tumor growth after continuous dosing. The triple angiokinase inhibitory pattern in combination with good efficacy in vivo defines a unique pharmacological target profile of this compound class, with potential for improved efficacy in cancer treatment. Compound 3 (BIBF 1120) is currently being evaluated in phase III clinical trials in the treatment of nonsmall cell lung cancer<sup>21</sup> and is in clinical development for other tumor types. Since it was shown that 2 and 3 are also highly effective in an animal model of lung fibrosis,<sup>22</sup> clinical development of 3 in idiopathic pulmonary fibrosis was recently initiated.

#### **Experimental Section**

All starting materials and reagents were either commercially available or their synthesis had been described in the literature before. All purchased chemicals and solvents were used without further purification. Reaction progresses were usually monitored by TLC using Merck silica gel 60  $F_{254}$  plates and UV light at 254 nm. All chromatographic purifications were conducted as MPLC using DAVISIL LC60A silica gel (35–70  $\mu$ m) unless otherwise noted. Yields refer to purified products and were not optimized. <sup>1</sup>H NMR (400 MHz) spectra were recorded on a Bruker DPX 400 spectrometer using DMSO-*d*<sub>6</sub> as solvent and Si (CH<sub>3</sub>)<sub>4</sub> as an internal standard. Low resolution mass spectra (MS) were run on a Micromass platform mass spectrometer.

High resolution masses (HRMS) were determined on a Micromass Q-Tof-2 mass spectrometer. Combustion analyses were performed by InfraServ Knapsack on an Elementar Vario Macro System and are within  $\pm 0.4\%$  of theoretical values. HPLC retention times were recorded on a Waters 1515 apparatus using a X-terra MS C18 column (2.5  $\mu$ m, 4.6 mm × 30 mm) eluted with a 3.1 min gradient from 5% to 98% B, wherein A = water/0.1% formic acid and B = acetonitrile/0.1% formic acid.

**4-Carboxymethyl-3-nitrobenzoic** Acid Ethyl Ester (5). 4-Carboxymethylbenzoic acid ethyl ester<sup>23</sup> **4** (20.8 g, 100 mmol) was dissolved in conc sulfuric acid (200 mL) and ammonium nitrate (8.80 g, 110 mmol) was slowly added in a way that the reaction temperature did not exceed -5 °C. Stirring was continued for 2 h at -5 °C. After that time, the mixture was carefully poured into ice water. The precipitate was filtered off, washed with water, and dried at 80 °C to give 22.9 g (91%) of **5**. <sup>1</sup>H NMR:  $\delta$  1.38 (t, 3H), 4.10 (s, 2H), 4.38 (q, 2H), 7.76 (d, 1H), 8.21 (d, 1H), 8.50 (s, 1H), 12.50 (br, 1H). MS: m/z 276 [M + Na]<sup>+</sup>.

**4-Methoxycarbonylmethyl-3-nitrobenzoic Acid Methyl Ester** (7). Potassium *tert*-butylate (78.5 g, 700 mmol) was dissolved in dimethylformamide (600 mL) and a solution of methyl chloro-acetate (29.0 mL, 330 mmol) and 3-nitrobenzoic acid methyl ester **6** (54.3 g, 300 mmol) in dimethylformamide (100 mL) was slowly added at -10 °C. Stirring was continued for 10 min at -10 °C. After that time, the mixture was poured into a mixture of ice water (1.0 L) and conc hydrochloric acid (350 mL). The precipitate was filtered off and washed with water. The residue was recrystallized from methanol and dried at 40 °C in vacuum to give 47.8 g (63%) of **7**. <sup>1</sup>H NMR:  $\delta$  3.61 (s, 3H), 3.92 (s, 3H), 4.18 (s, 2H), 7.74 (d, 1H), 8.26 (d, 1H), 8.52 (s, 1H). MS: *m/z* 276 [M + Na]<sup>+</sup>.

[4-(2-Methyl-[1,3]dioxolan-2-yl)-2-nitrophenyl]acetic Acid tert-Butyl Ester (9). Potassium tert-butylate (7.0 g, 62 mmol) was dissolved in dimethylformamide (50 mL) and a solution of tertbutyl chloroacetate (3.7 mL, 26 mmol) and 2-methyl-2-(3-nitrophenyl)-[1,3]dioxolane<sup>24</sup> (8) (5.0 g, 24 mmol) in dimethylformamide (15 mL) was slowly added at -5 °C. Stirring was continued for 10 min at -2 °C. After that time, the mixture was poured into a mixture of ice water (1.6 L) and conc hydrochloric acid (100 mL). Methylene chloride was added, and the organic layer was separated and washed with water. After drying over sodium sulfate, the solvent was evaporated and the residue was purified by column chromatography (silica gel), eluting with methylene chloride/cyclohexane (4/1) to give 2.4 g (31%) of 9. <sup>1</sup>H NMR:  $\delta$  1.40 (s, 9H), 1.62 (s, 3H), 3.74 (t, 2H), 3.98 (s, 2H), 4.13 (t, 2H), 7.53 (d, 1H), 7.73 (d, 1H), 8.04 (s, 1H). MS: m/z 324 [M + H]<sup>+</sup>.

**2-(4-Cyano-2-nitrophenyl)malonic Acid Dimethyl Ester (11).** Potassium *tert*-butylate (42.0 g, 112 mmol) was dissolved in dimethyl sulfoxide (140 mL) at 20 °C, and dimethyl malonate (44.0 mL, 385 mmol) was slowly added. Stirring was continued for 1 h at ambient temperature. After that time, 4-cyano-2-nitrobenzonitrile **10** (20.0 g, 110 mmol) was slowly added and the mixture was stirred at 100 °C for 1 h. After being cooled, the mixture was poured into water (1 L) and neutralized with 1 N hydrochloric acid (24 mL). The precipitate was filtered off and washed with water. The residue was taken up in methylene chloride, extracted with water, and dried over sodium sulfate to give 27.0 g (88%) of **11**. <sup>1</sup>H NMR:  $\delta$  3.72 (s, 6H), 5.65 (s, 1H), 7.80 (d, 1H), 8.27 (d, 1H), 8.66 (s, 1H). MS: m/z 301 [M + Na]<sup>+</sup>.

[2-Amino-4-(2-methyl-[1,3]dioxolan-2-yl)phenyl]acetic Acid tert-Butyl ester (12). [4-(2-Methyl-[1,3]dioxolan-2-yl)-2-nitrophenyl]acetic acid tert-butyl ester 9 (3.1 g, 9.6 mmol) was dissolved in methanol (50 mL) and hydrogenated (50 psi) at room temperature for 4.5 h using 1.6 g Raney nickel as catalyst. After that time, the catalyst was filtered off and the solvent was removed by evaporation to give 2.6 g (92%) of 12. <sup>1</sup>H NMR:  $\delta$  1.40 (s, 9H), 1.51 (s, 3H), 3.37 (s, 2H), 3.67 (t, 2H), 3.94 (t, 2H), 6.56 (d, 1H), 6.73 (s, 1H), 6.89 (d, 1H). MS: m/z 294 [M + H]<sup>+</sup>. **2-(4-Cyano-2-nitrophenyl)acetic Acid Methyl Ester (13).** 2-(4-Cyano-2-nitrophenyl)malonic acid dimethyl ester **11** (20.0 g, 71.9 mmol) was dissolved in dimethyl sulfoxide (350 mL), and lithium chloride (6.10 g, 143 mmol) and water (1.3 mL) were added. The mixture was stirred for 3.5 h at 100 °C. After being cooled, the mixture was poured into ice water (1.8 L) and stirred for 10 min. The precipitate was filtered off and washed with water. The residue was triturated with water, filtered off, and dried at 60 °C to give 7.77 g (49%) of **13**. <sup>1</sup>H NMR:  $\delta$  3.55 (s, 3H), 4.21 (s, 2H), 7.80 (d, 1H), 8.22 (d, 1H), 8.64 (s, 1H). MS: m/z 219 [M-H]<sup>-</sup>.

**2-***oxo***-2**,**3**-**Dihydro**-1*H*-**indole**-**6**-**carboxylic** Acid Ethyl Ester (14). 4-Carboxymethyl-3-nitrobenzoic acid ethyl ester 5 (2.50 g, 10.0 mmol) was dissolved in acetic acid (50 mL) and hydrogenated (50 psi) at room temperature for 1 h using 0.50 g palladium on charcoal (10%) as catalyst. After that time, the catalyst was filtered off and the solvent was removed by evaporation. The residue was triturated with diethyl ether, filtered off, and dried at 100 °C under vacuum to give 1.70 g (83%) of 14. <sup>1</sup>H NMR:  $\delta$  1.46 (t, 3H), 3.59 (s, 2H), 4.30 (q, 2H), 7.30 (s, 1H) superimposed on 7.32 (d, 1H), 7.58 (d, 1H), 10.55 (s, 1H). MS: m/z 205 [M]<sup>+</sup>.

**2-***oxo***-2**,**3-Dihydro-1***H***-indole-6-carboxylic Acid Methyl Ester (15).** 4-Methoxycarbonylmethyl-3-nitrobenzoic acid methyl ester 7 (38.7 g, 153 mmol) was dissolved in acetic acid (800 mL) and hydrogenated (50 psi) at room temperature for 2.5 h using 5.0 g palladium on charcoal (10%) as catalyst. After that time, the catalyst was filtered off and the solvent was removed by evaporation. The residue was triturated with to-luene, filtered off, and dried at 100 °C under vacuum to give 28.6 g (98%) of **15**. <sup>1</sup>H NMR:  $\delta$  3.56 (s, 2H), 3.84 (s, 3H), 7.33 (s, 1H) superimposed on 7.34 (d, 1H), 7.57 (d, 1H), 10.53 (s, 1H). MS: m/z 190 [M – H]<sup>-</sup>.

**6-Acetylindolin-2-one (16).** [2-Amino-4-(2-methyl-[1,3]dioxolan-2-yl)phenyl]acetic acid *tert*-butyl ester **12** (780 mg, 2.66 mmol) was dissolved in a mixture of methanol (3 mL) and 1 N hydrochloric acid (15 mL). The mixture was stirred at reflux for 1 h. After being cooled, methylene chloride was added. The organic layer was separated and extracted with water. After drying over sodium sulfate, the solvent was removed by evaporation to give 390 mg (84%) of **16**. <sup>1</sup>H NMR:  $\delta$  2.55 (s, 3H), 3.58 (s, 2H), 7.28 (s, 1H), 7.33 (d, 1H), 7.59 (d, 1H), 10.55 (s, 1H). MS: m/z 176 [M + H]<sup>+</sup>.

**6-Cyanoindolin-2-one (17).** 2-(4-Cyano-2-nitrophenyl)acetic acid methyl ester **13** (18.7 g, 85.0 mmol) was dissolved in methanol (500 mL) and hydrogenated (44 psi) at room temperature for 4.0 h using 7.0 g Lindlar catalyst. After that time, the catalyst was filtered off and the solvent was removed by evaporation. The residue was taken up in acetic acid (300 mL) and stirred for 0.5 h at 100 °C. After that time, the solvent was removed by evaporation. The residue was washed with water and dried at 70 °C to give 11.5 g (86%) of **17**. <sup>1</sup>H NMR:  $\delta$  3.60 (s, 2H), 7.12 (s, 1H), 7.39 (2xs, 2 × 1H), 10.30 (s, 1H). MS: m/z 157 [M – H]<sup>-</sup>.

**6-Nitroindolin-2-one (19).** 3,3-Bis(methoxycarbonyl)-6-nitroindolin-2-one<sup>25</sup> **18** (5.40 g, 18.4 mmol) was dissolved in a mixture of formic acid (100 mL) and methanesulfonic acid (100 mL) and refluxed at 110 °C for 2.5 h. After that time, the solvents were evaporated and water was added. The precipitate was filtered off, dissolved in dichloromethane/methanol (1:1), and washed with water. The aqueous phase was extracted with dichloromethane/ methanol (1:1) and ethyl acetate, and all combined organic layers were dried over sodium sulfate. After filtration over celite, the solvent was removed by evaporation to give 2.30 g (70%) of **19**. <sup>1</sup>H NMR:  $\delta$  3.65 (s, 2H), 7.45 (d, 1H), 7.51 (s, 1H), 7.83 (d, 1H), 10.70 (s, 1H). MS: m/z 178 [M]<sup>+</sup>.

**1-Acetyl-6-chloroindolin-2-one (22).** 6-Chloroindolin-2-one **20** (100 g, 168 mmol) was suspended in acetic anhydride (200 mL) and refluxed at 130 °C for 7 h. After that time, the mixture was allowed to cool and the precipitate was filtered off and washed with petroleum ether to give 109 g (87%) of **22.** <sup>1</sup>H NMR:  $\delta$  2.50

(s, 3H), 3.80 (s, 2H), 7.25 (d, 1H), 7.35 (d, 1H), 8.05 (s, 1H). MS: *m*/*z* 210 [M + H]<sup>+</sup>.

**1,6-Diacetylindolin-2-one (23).** Compound **23** was prepared using the same procedure as described for the synthesis of **22** by substituting **16** for 6-chloroindolin-2-one **20**. <sup>1</sup>H NMR:  $\delta$  2.50 (s, 3H), 2.60 (s, 3H), 3.91 (s, 2H), 7.50 (d, 1H), 7.86 (d, 1H), 8.61 (s, 1H). MS: m/z 240 [M + Na]<sup>+</sup>.

**1-Acetylindolin-2-one (24).** Compound **24** was prepared using the same procedure as described for the synthesis of **22** by substituting indolin-2-one **21** for 6-chloroindolin-2-one **20**. <sup>1</sup>H NMR:  $\delta$  2.50 (s, 3H), 3.81 (s, 2H), 7.20 (t, 1H), 7.31 (1xd/1xt, 2 × 1H), 8.07 (d, 1H). MS: *m/z* 176 [M + H]<sup>+</sup>.

6-Acetyl-2-*oxo*-2,3-dihydro-1*H*-indole-6-carboxylic Acid Methyl Ester (25). 2-*oxo*-2,3-Dihydro-1*H*-indole-6-carboxylic acid methyl ester 15 (11.5 g, 60.2 mmol) was suspended in acetic anhydride (100 mL) and refluxed at 130 °C for 8 h. After that time, the mixture was allowed to cool and the precipitate was filtered off and washed with petroleum ether to give 10.2 g (73%) of 25. <sup>1</sup>H NMR:  $\delta$  2.56 (s, 3H), 3.87 (s, 3H), 3.88 (s, 2H), 7.47 (d, 1H), 7.80 (d, 1H), 8.62 (s, 1H). MS: *m/z* 256 [M + Na]<sup>+</sup>.

(*E*)-1-Acetyl-3-(ethoxy-phenyl-methylene)-2-oxo-2,3-dihydro-1*H*-indole-6-carboxylic Acid Ethyl Ester (26). 2-oxo-2,3-Dihydro-1*H*-indole-6-carboxylic acid ethyl ester 14 (12.9 g, 62.9 mmol) was dissolved in acetic anhydride (130 mL) and orthobenzoic acid triethyl ester (42.7 mL, 189 mmol) was added. The mixture was stirred at 110 °C for 3.5 h. After that time, the solvent was removed by evaporation. The residue was triturated with petroleum ether, filtered off, and dried at 50 °C under vacuum to give 18.2 g (76%) of 26. <sup>1</sup>H NMR:  $\delta$  1.32 (m, 2 × 3H), 2.48 (s, 3H), 4.02 (q, 2H), 4.32 (q, 2H), 7.40–7.60 (m, 5H), 7.88 (d, 1H), 8.08 (d, 1H), 8.76 (s, 1H). MS: m/z 380 [M + H]<sup>+</sup>.

(*E*)-1-Acetyl-3-(ethoxy-phenyl-methylene)-6-nitroindolin-2-one (27). Compound 27 was prepared using the same procedure as described for the synthesis of 26 by substituting 19 for 14. <sup>1</sup>H NMR:  $\delta$  1.43 (t, 3H), 2.55 (s, 3H), 4.11 (q, 2H), 7.50–7.70 (m, 5H), 8.20 (m, 2 × 1H), 8.93 (s, 1H). MS: *m/z* 353 [M + H]<sup>+</sup>.

(*E*)-1-Acetyl-6-cyano-3-(ethoxy-phenyl-methylene)-indolin-2one (28). Compound 28 was prepared using the same procedure as described for the synthesis of 26 by substituting 17 for 14. <sup>1</sup>H NMR:  $\delta$  1.35 (t, 3H), 2.43 (s, 3H), 4.04 (q, 2H), 7.45–7.60 (m, 5H), 7.73 (d, 1H), 8.12 (d, 1H), 8.42 (s, 1H). MS: *m*/*z* 355 [M + Na]<sup>+</sup>.

(*E*)-1-Acetyl-6-chloro-3-(ethoxy-phenyl-methylene)-indolin-2one (29). 1-Acetyl-6-chloroindolin-2-one 22 (41.9 g, 200 mmol) was dissolved in acetic anhydride (150 mL), and *ortho*-benzoic acid triethyl ester (136 mL, 600 mmol) was added. The mixture was stirred at 120 °C for 6 h. After that time, the solvent was removed by evaporation. The residue was triturated with petroleum ether (100 mL), filtered off, and dried at 80 °C under vacuum to give 38.0 g (56%) of 29. <sup>1</sup>H NMR:  $\delta$  1.31 (t, 3H), 2.42 (s, 3H), 3.96 (q, 2H), 7.29 (d, 1H), 7.45–7.60 (m, 5H), 7.93 (d, 1H), 8.14 (s, 1H). MS: *m*/*z* 342 [M + H]<sup>+</sup>.

(*E*)-1,6-Diacetyl-3-(ethoxy-phenyl-methylene)-indolin-2-one (30). Compound 30 was prepared using the same procedure as described for the synthesis of 29 by substituting 23 for 22. <sup>1</sup>H NMR:  $\delta$  1.36 (t, 3H), 2.45 (s, 3H), 2.60 (s, 3H), 4.02 (q, 2H), 7.45-7.55 (m, 5H), 7.95 (d, 1H), 8.10 (d, 1H), 8.70 (s, 1H). MS: m/z 350 [M + H]<sup>+</sup>.

(*E*)-3-(Ethoxy-phenyl-methylene)-indolin-2-one (31). Compound 31 was prepared using the same procedure as described for the synthesis of 29 by substituting 24 for 22. <sup>1</sup>H NMR:  $\delta$  1.33 (t, 3H), 2.43 (s, 3H), 3.94 (q, 2H), 7.25 (m, 2H), 7.45–7.55 (m, 5H), 7.98 (d, 1H), 8.17 (d, 1H). MS: *m/z* 330 [M + Na]<sup>+</sup>.

(*E*)-1-Acetyl-3-(ethoxy-phenyl-methylene)-2-*oxo*-2,3-dihydro-1*H*-indole-6-carboxylic Acid Methyl Ester (32). Compound 32 was prepared using the same procedure as described for the synthesis of 29 by substituting 25 for 22. <sup>1</sup>H NMR:  $\delta$  1.46 (t, 3H), 2.48 (s, 3H), 3.88 (s, 3H), 4.03 (q, 2H), 7.45–7.60 (m, 5H), 7.88 (d, 1H), 8.09 (d, 1H), 8.74 (s, 1H). MS: *m*/*z* 366 [M + H]<sup>+</sup>. (*E*)-1-Acetyl-3-(methoxy-phenyl-methylene)-2-oxo-2,3-dihydro-1*H*indole-6-carboxylic Acid Methyl Ester (33). Compound 33 was prepared using the same procedure as described for the synthesis of 29 by substituting 25 for 22 and ortho-benzoic acid triethyl ester for ortho-benzoic acid trimethyl ester. <sup>1</sup>H NMR:  $\delta$  2.44 (s, 3H), 3.75 (s, 3H), 3.87 (s, 3H), 7.45–7.60 (m, 5H), 7.86 (d, 1H), 8.06 (d, 1H), 8.73 (s, 1H). MS: m/z 374 [M + Na]<sup>+</sup>.

(Z)-2-oxo-3-[Phenyl-(4-piperidin-1-ylmethyl-phenylamino)-methylene]-2,3-dihydro-1H-indole-6-carboxylic Acid Ethyl Ester (34). (E)-1-Acetyl-3-(ethoxy-phenyl-methylene)-2-oxo-2,3-dihydro-1Hindole-6-carboxylic acid ethyl ester (26) (1.50 g, 3.95 mmol) and 4-piperidin-1-ylmethyl-phenylamine<sup>26</sup> (1.13 g, 5.93 mmol) were dissolved in dimethylformamide (15 mL). The mixture was stirred at 100 °C for 0.5 h. After that time, heating was suspended and piperidine (5 mL) was added at room temperature and stirring was continued for 3.0 h. After that time, the solvent was removed by evaporation. The residue was purified by column chromatography (aluminum oxide, activity 2-3) eluting with methylene chloride/ethanol (100/3) to give 1.10 g (58%) of **34**. <sup>1</sup>H NMR: δ 1.28 (t, 3H), 1.30–1.50 (m, 6H), 2.23 (m, 4H), 3.30 (s, 2H), 4.22 (q, 2H), 5.80 (d, 1H), 6.80 (d, 2H), 7.07 (d, 2H), 7.18 (d, 1H), 7.40-7.65 (m, 6H), 10.92 (s, 1H), 12.22 (s, 1H). MS: m/z 481 [M]<sup>+</sup>. Anal. (C<sub>30</sub>H<sub>31</sub>N<sub>3</sub>O<sub>3</sub>) C. H. N.

(*Z*)-6-Nitro-3-[phenyl-(4-piperidin-1-ylmethyl-phenylamino)methylene]-indolin-2-one (35). Compound 35 was prepared using the same procedure as described for the synthesis of 34 by substituting 27 for 26. <sup>1</sup>H NMR:  $\delta$  1.30–1.50 (m, 6H), 2.20 (m, 4H), 3.30 (s, 2H), 5.80 (d, 1H), 6.84 (d, 2H), 7.08 (d, 2H), 7.45–7.70 (m, 7H), 11.18 (s, 1H), 12.39 (s, 1H). MS: *m*/*z* 455 [M + H]<sup>+</sup>. Anal. (C<sub>27</sub>H<sub>26</sub>N<sub>4</sub>O<sub>3</sub>·0.25H<sub>2</sub>O) C, H, N.

(*Z*)-6-Chloro-3-[phenyl-(4-piperidin-1-ylmethyl-phenylamino)methylene]-indolin-2-one (36). Compound 36 was prepared using the same procedure as described for the synthesis of 34 by substituting 29 for 26. <sup>1</sup>H NMR:  $\delta$  1.30–1.50 (m, 6H), 2.20 (m, 4H), 3.18 (s, 2H), 5.70 (d, 1H), 6.57 (d, 1H), 6.77 (d, 2H), 6.85 (s, 1H), 7.05 (d, 2H), 7.40–7.60 (m, 5H), 10.82 (s, 1H), 11.98 (s, 1H). MS: *m/z* 444 [M + H]<sup>+</sup>. HPLC: >95% (*t*<sub>R</sub>=2.3 min). HRMS (ES<sup>+</sup>) calcd for C<sub>27</sub>H<sub>26</sub>ClN<sub>3</sub>O [M + H]<sup>+</sup> *m/e* 444.1843, found *m/e* 444.1839.

(*Z*)-6-Cyano-3-[phenyl-(4-piperidin-1-ylmethyl-phenylamino)methylene]-indolin-2-one (37). Compound 37 was prepared using the same procedure as described for the synthesis of 34 by substituting 28 for 26. <sup>1</sup>H NMR:  $\delta$  1.30–1.50 (m, 6H), 2.20 (m, 4H), 3.28 (s, 2H), 5.77 (d, 1H), 6.79 (d, 2H), 6.98 (d, 1H), 7.08 (d, 2H), 7.17 (s, 1H), 7.45–7.65 (m, 5H), 11.03 (s, 1H), 12.28 (s, 1H). MS: *m/z* 435 [M + H]<sup>+</sup>. HPLC: >95% (*t*<sub>R</sub>=2.2 min). HRMS (ES<sup>+</sup>) calcd for C<sub>28</sub>H<sub>26</sub>N<sub>4</sub>O [M + H]<sup>+</sup> *m/e* 435.2185, found *m/e* 435.2178.

(*Z*)-6-Acetyl-3-[phenyl-(4-piperidin-1-ylmethyl-phenylamino)methylene]-indolin-2-one (38). Compound 38 was prepared using the same procedure as described for the synthesis of 34 by substituting 30 for 26. <sup>1</sup>H NMR:  $\delta$  1.30–1.80 (m, 6H), 2.43 (s, 3H), 2.75 (m, 2H), 3.18 (m, 2H), 4.12 (s, 2H), 5.82 (d, 1H), 6.88 (d, 2H), 7.23 (d, 1H), 7.40–7.70 (m, 8H), 11.02 (s, 1H), 12.26 (s, 1H). MS: *m/z* 451 [M]<sup>+</sup>. Anal. (C<sub>29</sub>H<sub>29</sub>N<sub>3</sub>O<sub>2</sub>·0.25H<sub>2</sub>O) C, H, N.

(*Z*)-3-[Phenyl-(4-piperidin-1-ylmethyl-phenylamino)-methylene]indolin-2-one (39). Compound 39 was prepared using the same procedure as described for the synthesis of 34 by substituting 31 for 26. <sup>1</sup>H NMR:  $\delta$  1.30–1.50 (m, 6H), 2.10–2.30 (m, 4H), 3.38 (m, 2H), 5.76 (d, 1H), 6.51 (t, 1H), 6.72 (d, 2H), 6.86 (m, 2H), 7.04 (d, 2H), 7.40–7.60 (m, 5H), 10.69 (s, 1H), 12.03 (s, 1H). MS: m/z 409 [M]<sup>+</sup>. Anal. (C<sub>27</sub>H<sub>27</sub>N<sub>3</sub>O) C, H, N.

(Z)-2-oxo-3-[Phenyl-(4-piperidin-1-ylmethyl-phenylamino)-methylene]-2,3-dihydro-1*H*-indole-6-carboxylic Acid (40). (Z)-2-oxo-3-[Phenyl-(4-piperidin-1-ylmethyl-phenylamino)-methylene]-2,3dihydro-1*H*-indole-6-carboxylic acid ethyl ester (34) (800 mg, 1.66 mmol) was dissolved in ethanol (30 mL) and 1 N NaOH (8.3 mL) was added. The mixture was stirred at 80 °C for 1 h. After that time, heating was suspended and the mixture was neutralized using 1 N HCl. The precipitate was filtered off, washed with water, ethanol, and diethyl ether, and dried at 100 °C under vacuum to give 670 mg (89%) of **40**. <sup>1</sup>H NMR:  $\delta$  1.25–1.50 (m, 6H), 2.15–2.30 (m, 4H), 3.30 (s, 2H), 5.78 (d, 1H), 6.77 (d, 2H), 7.05 (d, 2H), 7.14 (d, 1H), 7.40–7.60 (m, 6H), 10.88 (s, 1H), 12.22 (s, 1H). MS: m/z 453 [M]<sup>+</sup>. HPLC: >95% ( $t_{\rm R}$  = 2.0 min). HRMS (ES<sup>+</sup>) calcd for C<sub>28</sub>H<sub>27</sub>N<sub>3</sub>O<sub>3</sub> [M + H]<sup>+</sup> m/e 454.2131, found m/e 454.2123.

(Z)-2-oxo-3-[Phenyl-(4-piperidin-1-ylmethyl-phenylamino)-methylene]-2,3-dihydro-1H-indole-6-carboxylic Acid Amide (1). (Z)-2-oxo-3-[Phenyl-(4-piperidin-1-ylmethyl-phenylamino)-methylene]-2,3dihydro-1H-indole-6-carboxylic acid (40) (907 mg, 2.00 mmol), O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate (TBTU, 771 mg, 2.40 mmol), and hydroxy-benzotriazole (HOBT, 367 mg, 2.40 mmol) were suspended in dimethylformamide (25 mL) and triethylamine (1.0 mL) was added. The mixture was stirred at room temperature for 0.25 h. After that time, the mixture was cooled to 0 °C and ammonia was bubbled through the solution for 15 min in a way that the reaction temperature did not exceed 15 °C. Stirring was continued for 1.5 h at room temperature. The precipitate was filtered off, washed with water, ethanol, and diethyl ether and dried at 100 °C under vacuum to give 578 mg (64%) of 1. <sup>1</sup>H NMR:  $\delta$  1.30–1.50 (m, 6H), 2.15–2.30 (m, 4H), 3.30 (s, 2H), 5.72 (d, 1H), 6.76 (d, 2H), 7.05 (d, 2H) superimposed on 7.08 (d, 1H), 7.38 (s, 1H), 7.45-7.75 (m, 5H), 10.88 (s, 1H), 12.13 (s, 1H). MS: m/z 452 [M]<sup>+</sup>. Anal. (C<sub>28</sub>H<sub>28</sub>N<sub>4</sub>O<sub>2</sub>· 0.25H<sub>2</sub>O) C, H, N.

(Z)-2-oxo-3-[Phenyl-(4-piperidin-1-ylmethyl-phenylamino)-methylene]-2,3-dihydro-1H-indole-6-carboxylic Acid Isopropylamide (41). (Z)-2-oxo-3-[Phenyl-(4-piperidin-1-ylmethyl-phenylamino)-methylene]-2,3-dihydro-1H-indole-6-carboxylic acid (40) (454 mg, 1.00 mmol), O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate (TBTU, 385 mg, 1.20 mmol), and hydroxy-benzotriazole (HOBT, 184 mg, 1.20 mmol) were suspended in dimethylformamide (10 mL) and N-ethyl diisopropylamine (2.8 mL) was added. Isopropylamine (128 mL, 1.50 mmol) was added, and the mixture was stirred at room temperature for 1 h. After that time, water was added, the precipitate was filtered off, washed with water, acetone, and diethyl ether, and dried at 100 °C under vacuum to give 368 mg (74%) of 41. <sup>1</sup>H NMR: δ 1.10 (d, 6H), 1.30-1.50 (m, 6H), 2.15-2.25 (m, 4H), 3.23 (s, 2H), 4.03 (sept, 1H), 5.70 (d, 1H), 6.78 (d, 2H), 7.04 (s, 1H) superimposed on 7.06 (d, 2H), 7.32 (s, 1H), 7.40-7.60 (m, 5H), 10.88 (s, 1H), 12.10 (s, 1H). MS: m/z 494 [M]<sup>+</sup>. Anal. (C<sub>31</sub>H<sub>34</sub>N<sub>4</sub>O<sub>2</sub>·0.5-H<sub>2</sub>O) C. H. N.

(*Z*)-2-oxo-3-[Phenyl-(4-piperidin-1-ylmethyl-phenylamino)-methylene]-2,3-dihydro-1*H*-indole-6-carboxylic Acid Dimethylamide Hydrochloride (42). Compound 42 was prepared using the same procedure as described for the synthesis of 41 by substituting dimethylamine hydrochloride for isopropylamine. <sup>1</sup>H NMR:  $\delta$  1.30–1.80 (m, 6H), 2.90 (s, 6H) superimposed on 2.60–2.90 (m, 2H), 3.10–3.30 (m, 2H), 4.12 (s, 2H), 5.78 (d, 1H), 6.62 (d, 1H), 6.82 (d, 2H) superimposed on 6.86 (s, 1H), 7.25 (d, 2H), 7.45–7.65 (m, 5H), 9.10 (br, 1H), 10.88 (s, 1H), 12.15 (s, 1H). MS: *m*/*z* 480 [M]<sup>+</sup>. HPLC: >95% (*t*<sub>R</sub>=2.0 min). HRMS (ES<sup>+</sup>) calcd for C<sub>30</sub>H<sub>32</sub>N<sub>4</sub>O<sub>2</sub> [M + H]<sup>+</sup> *m*/*e* 481.2604, found *m*/*e* 481.2595.

(*Z*)-2-oxo-3-[Phenyl-(4-piperidin-1-ylmethyl-phenylamino)-methylene]-2,3-dihydro-1*H*-indole-6-carboxylic Acid Ethylmethylamide (43). Compound 43 was prepared using the same procedure as described for the synthesis of 41 by substituting *N*-methyl-ethylamine for isopropylamine. <sup>1</sup>H NMR:  $\delta$  1.04 (t, 3H), 1.35–1.45 (m, 6H), 2.22 (m, 4H), 2.85 (s, 3H), 3.28 (q, 3H) superimposed on 3.29 (m, 2H), 5.77 (d, 1H), 6.57 (d, 1H), 6.75 (d, 2H), 6.85 (s, 1H), 7.04 (d, 2H), 7.45–7.55 (m, 5H), 10.78 (s, 1H), 12.10 (s, 1H). MS: *m*/*z* 494 [M]<sup>+</sup>. Anal. (C<sub>31</sub>H<sub>34</sub>N<sub>4</sub>O<sub>2</sub>· 0.25H<sub>2</sub>O) C, H, N.

(*Z*)-2-oxo-3-[Phenyl-(4-piperidin-1-ylmethyl-phenylamino)-methylene]-2,3-dihydro-1*H*-indole-6-carboxylic Acid Methylamide Hydrochloride (44). Compound 44 was prepared using the same procedure as described for the synthesis of 41 by substituting methylamine hydrochloride for isopropylamine. <sup>1</sup>H NMR:  $\delta$  1.30–1.80 (m, 6H), 2.75 (d, 3H) superimposed on 2.60–2.90 (m, 2H), 3.10–3.30 (m, 2H), 4.09 (m, 2H), 5.74 (d, 1H), 6.83 (d, 1H), 6.82 (d, 2H), 7.15 (d, 1H), 7.30–7.70 (m, 7H), 8.20 (q, 1H), 10.35 (br, 1H), 10.98 (s, 1H), 12.14 (s, 1H). MS: *m*/*z* 467 [M+H]<sup>+</sup>. Anal. (C<sub>29</sub>H<sub>30</sub>N<sub>4</sub>O<sub>2</sub>·1.0HCL·0.75H<sub>2</sub>O) C, H, N.

(Z)-2-oxo-3-[Phenyl-(4-piperidin-1-ylmethyl-phenylamino)-methylene]-2,3-dihydro-1H-indole-6-carboxylic Acid Methyl Ester (45). (Z)-2-oxo-3-[Phenyl-(4-piperidin-1-ylmethyl-phenylamino)-methylene]-2,3-dihydro-1H-indole-6-carboxylic acid (40) (900 mg, 1.98 mmol) was suspended in dimethylformamide (35 mL), and N, N'-carbonyldiimidazole (385 mg, 2.38 mmol) was added. The mixture was stirred at 80 °C for 14 h. After that time, methanol (20 mL) was added and stirring was continued for 3 h at 50 °C. After that time, the solvent was removed by evaporation. The residue was purified by column chromatography (aluminum oxide, activitiy 2-3) eluting with methylene chloride/methanol (3/1) to give to give 450 mg (49%) of 45.  $^{1}$ H NMR: δ 1.30–1.50 (m, 6H), 2.20 (m, 4H), 3.23 (m, 2H), 3.78 (s, 3H), 5.79 (d, 1H), 6.79 (d, 2H), 7.03 (d, 2H), 7.19 (d, 1H), 7.39 (s, 1H) superimposed on 7.40-7.65 (m, 5H), 10.94 (s, 1H), 12.24 (s, 1H). MS: m/z 468 [M + H]<sup>+</sup>. Anal. (C<sub>29</sub>H<sub>29</sub>N<sub>3</sub>O<sub>3</sub>·0.25H<sub>2</sub>O) C, H, N.

(*Z*)-6-Amino-3-[phenyl-(4-piperidin-1-ylmethyl-phenylamino)methylene]-indolin-2-one (46). (*Z*)-6-Nitro-3-[phenyl-(4-piperidin-1-ylmethyl-phenylamino)-methylene]-indolin-2-one (35) (850 mg, 1.87 mmol) was dissolved in methylene chloride (50 mL) and methanol (50 mL) and hydrogenated at room temperature and atmospheric pressure for 24 h using 0.05 g Raney nickel as catalyst. After that time, the catalyst was filtered off and the solvent was removed by evaporation to give 675 mg (85%) of 46. <sup>1</sup>H NMR:  $\delta$  1.25–1.65 (m, 6H), 2.40–2.70 (m, 4H), 3.30 (s, 2H), 5.55 (d, 1H), 5.78 (dd, 1H), 6.14 (d, 1H), 6.63 (d, 2H), 7.12 (m, 2H), 7.40–7.60 (m, 5H), 10.43 (s, 1H), 11.55 (s, 1H). MS: m/z 425 [M + H]<sup>+</sup>. HPLC: >95% ( $t_{\rm R}$  = 1.6 min). HRMS (ES<sup>+</sup>) calcd for C<sub>27</sub>H<sub>28</sub>N<sub>4</sub>O [M + H]<sup>+</sup> m/e 425.2341, found m/e 425.2335.

(Z)-N-{2-oxo-3-[Phenyl-(4-piperidin-1-ylmethyl-phenylamino)methylene]-2,3-dihydro-1H-indol-6-yl}-acetamide Hydrochloride (47). (Z)-6-Amino-3-[phenyl-(4-piperidin-1-ylmethyl-phenylamino)-methylene]-indolin-2-one (46) (500 mg, 1.18 mmol) was dissolved in methylene chloride (20 mL), and triethylamine (0.2 mL) was added. Acetylchloride (0.1 mL, 1.2 mmol) was added, and the mixture was stirred for 5 h at ambient temperature. After that time, the solvent was removed by evaporation and the residue was purified by column chromatography (silica gel) eluting with methylene chloride/methanol (4/1) to give 360 mg (66%) of 47. <sup>1</sup>H NMR:  $\delta$  1.40–1.80 (m, 6H), 1.98 (s, 3H), 2.60-2.90 (m, 2H), 3.30-3.40 (m, 2H), 3.97 (m, 2H), 5.67 (d, 1H), 6.62 (d, 1H), 6.74 (d, 2H), 7.30 (d, 2H), 7.45-7.65 (m, 6H), 9.90 (s, 1H), 10.50 (br, 1H), 10.73 (s, 1H), 11.86 (s, 1H). MS: m/z 467  $[M + H]^+$ . HPLC: >95% ( $t_R = 2.1 \text{ min}$ ). HRMS (ES<sup>+</sup>) calcd for C<sub>29</sub>H<sub>30</sub>N<sub>4</sub>O<sub>2</sub> [M + H]<sup>+</sup> m/e 467.2447, found m/e467.2441

(Z)-3-[Phenyl-(4-piperidin-1-ylmethyl-phenylamino)-methylene]-6pyrrol-1-yl-indolin-2-one (48). (Z)-6-Amino-3-[phenyl-(4-piperidin-1-ylmethyl-phenylamino)-methylene]-indolin-2-one (46) (500 mg, 1.18 mmol) were dissolved in acetic acid (20 mL) and 2,5-dimethoxy tetrahydrofuran (160 mL, 1.18 mmol) were added. The mixture was refluxed for 1 h. After that time, the solvent was removed by evaporation. The residue was taken up in water (20 mL) and stirred for 2.0 h at ambient temperature. After that time, the mixture was neutralized with 1 N sodium hydroxide solution. Methylene chloride was added, and the organic layer was separated and dried over sodium sulfate. The solvent was removed by evaporation, and the residue was purified by column chromatography (silica gel) eluting with methylene chloride/methanol (4/1) to give 200 mg (36%) of **48**. <sup>1</sup>H NMR:  $\delta$  1.25–1.55 (m, 6H), 2.20–2.30 (m, 4H), 3.30 (m, 2H), 5.78 (d, 1H), 6.18 (s, 2H), 6.72 (s, 1H), 6.76 (s, 2H), 6.95 (s, 1H), 7.04 (d, 2H), 7.16 (s, 2H), 7.40–7.65 (m, 5H), 10.87 (s, 1H), 11.94 (s, 1H). MS: *m*/*z* 475 [M + H]<sup>+</sup>. Anal. (C<sub>31</sub>H<sub>30</sub>N<sub>4</sub>O·0.25H<sub>2</sub>O) C, H, N.

(Z)-3-[(4-{Methyl-[2-(4-methylpiperazin-1-yl)acetyl]amino}phenylamino)-methylene|-2-oxo-2,3-dihydro-1H-indole-6-carboxylic Acid Methyl Ester (3). (E)-1-Acetyl-3-(methoxy-phenyl-methylene)-2-oxo-2,3-dihydro-1H-indole-6-carboxylic acid methyl ester (33) (10.5 g, 30.0 mmol) and N-(4-aminophenyl)-N-methyl-2-(4-methylpiperazin-1-yl)acetamide (84, 8.60 g, 33.0 mmol) were dissolved in dimethylformamide (80 mL). The mixture was stirred at 80 °C for 1 h. After that time, heating was suspended and piperidine (6.5 mL) was added at room temperature and stirring was continued for 2.0 h. After that time, water was added and the precipitate was filtered off and washed with water. The residue was triturated with methanol, filtered off, washed with cold methanol and diethyl ether, and dried at 110 °C under vacuum to give 12.4 g (77%) of 3. <sup>1</sup>H NMR: δ 2.00-2.35 (m, 11H), 2.70 (s, 2H), 3.06 (s, 3H), 3.76 (s, 3H), 5.82 (d, 1H), 6.87 (d, 2H), 7.11 (d, 2H), 7.17 (d, 1H), 7.40-7.60 (m, 6H), 10.94 (s, 1H), 12.22 (s, 1H). MS: m/z 540  $[M + H]^+$ . Anal.  $(C_{31}H_{33}N_5O_4 \cdot 0.25H_2O)$  C, H, N.

(*Z*)-3-({4-[(2-Imidazol-1-yl-acetyl)methylamino]-phenylamino}phenyl-methylene)-2-oxo-2,3-dihydro-1*H*-indole-6-carboxylic Acid Methyl Ester (49). Compound 49 was prepared using the same procedure as described for the synthesis of 3 by substituting 85 for 84. <sup>1</sup>H NMR:  $\delta$  3.10 (s, 3H), 3.79 (s, 3H), 4.50 (s, 2H), 5.84 (d, 1H), 6.80 (s, 1H), 6.92 (m, 3H), 7.18 (d, 1H) superimposed on 7.24 (m, 2H), 7.41 (m, 2H), 7.50–7.70 (m, 5H), 11.00 (s, 1H), 12.29 (s, 1H). MS: m/z 508 [M + H]<sup>+</sup>. HPLC: >95% ( $t_R$  = 2.4 min). HRMS (ES<sup>+</sup>) calcd for C<sub>29</sub>H<sub>25</sub>N<sub>5</sub>O<sub>4</sub> [M + H]<sup>+</sup> m/e508.1985, found m/e 508.1974.

(*Z*)-3-{[4-(*N*-Acetyl-dimethylcarbamoylmethyl-amino)-phenylamino]-phenyl-methylene}-2-*oxo*-2,3-dihydro-1*H*-indole-6-carbo-xylic Acid Methyl Ester (50). Compound 50 was prepared using the same procedure as described for the synthesis of 3 by substituting 88 for 84. <sup>1</sup>H NMR:  $\delta$  1.71 (s, 3H), 2.78 (s, 3H), 2.92 (s, 3H), 3.78 (s, 3H), 4.33 (s, 2H), 5.82 (d, 1H), 6.84 (d, 2H), 7.19 (d, 1H), 7.41 (s, 1H), 7.50–7.70 (m, 5H), 10.98 (s, 1H), 12.27 (s, 1H). MS: *m*/*z* 535 [M + Na]<sup>+</sup>. Anal. (C<sub>29</sub>H<sub>28</sub>N<sub>4</sub>O<sub>5</sub>) C, H, N.

(Z)-3-({4-[N-Acetyl-(3-dimethylaminopropyl)-amino]-phenylamino}-phenyl-methylene)-2-oxo-2,3-dihydro-1*H*-indole-6-carboxylic Acid Methyl Ester (51). Compound 51 was prepared using the same procedure as described for the synthesis of 3 by substituting **89** for **84**. <sup>1</sup>H NMR:  $\delta$  1.42 (m, 2H), 1.63 (s, 3H), 1.99 (s, 6H), 2.09 (t, 2H), 3.52 (t, 2H), 3.78 (s, 3H), 5.84 (d, 1H), 6.90 (d, 2H), 7.10 (d, 2H), 7.20 (d, 1H), 7.41 (s, 1H), 7.50–7.65 (m, 5H), 10.98 (s, 1H), 12.25 (s, 1H). MS: m/z 513 [M + H]<sup>+</sup>. Anal. (C<sub>30</sub>H<sub>32</sub>N<sub>4</sub>O<sub>4</sub>·0.25H<sub>2</sub>O) C, H, N.

(Z)-3-({4-[N-Acetyl-(2-dimethylaminoethyl)-amino]-phenylamino}-phenyl-methylene)-2-oxo-2,3-dihydro-1*H*-indole-6-carboxylic Acid Methyl Ester (52). Compound 52 was prepared using the same procedure as described for the synthesis of 3 by substituting 90 for 84. <sup>1</sup>H NMR:  $\delta$  1.62 (s, 3H), 2.02 (s, 6H), 2.13 (t, 2H), 3.58 (t, 2H), 3.78 (s, 3H), 5.86 (d, 1H), 6.88 (d, 2H), 7.10 (d, 2H), 7.18 (d, 1H), 7.41 (s, 1H), 7.50–7.70 (m, 5H), 10.93 (s, 1H), 12.22 (s, 1H). MS: m/z 499 [M+H]<sup>+</sup>. Anal. (C<sub>29</sub>H<sub>30</sub>N<sub>4</sub>O<sub>4</sub>-0.25H<sub>2</sub>O) C, H, N.

(*Z*)-3-({4-[(2-Dimethylaminoethyl)-methanesulfonyl-amino]phenylamino}-phenyl-methylene)-2-*oxo*-2,3-dihydro-1*H*-indole-6-carboxylic Acid Methyl Ester (53). Compound 53 was prepared using the same procedure as described for the synthesis of 3 by substituting 91 for 84. <sup>1</sup>H NMR:  $\delta$  2.04 (s, 6H), 2.13 (t, 2H), 2.92 (s, 3H), 3.58 (t, 2H), 3.78 (s, 3H), 5.81 (d, 1H), 6.87 (d, 2H), 7.16 (d, 2H) superimposed on 7.19 (d, 1H), 7.41 (s, 1H), 7.50– 7.65 (m, 5H), 11.00 (s, 1H), 12.15 (s, 1H). MS: *m/z* 535 [M + H]<sup>+</sup>. Anal. (C<sub>28</sub>H<sub>30</sub>N<sub>4</sub>O<sub>5</sub>S·1.0H<sub>2</sub>O) C, H, N. (*Z*)-3-({4-[(3-Dimethylaminopropyl)-methyl-carbamoyl]-phenylamino}-phenyl-methylene)-2-*oxo*-2,3-dihydro-1*H*-indole-6-carboxylic Acid Methyl Ester (54). Compound 54 was prepared using the same procedure as described for the synthesis of 3 by substituting 93 for 84. <sup>1</sup>H NMR (2 rotamers):  $\delta$  1.52/1.68 (m, 2H), 1.95/2.10 (s, 6H) superimposed on 1.90–2.20 (t, 2H, incompletely resolved), 2.80/2.86 (s, 3H), 3.10/3.35 (t, 2H), 3.79 (s, 3H), 5.87 (d, 1H), 6.68 (d, 2H), 7.19 (d, 2H) superimposed on 7.21 (d, 1H), 7.42 (s, 1H), 7.45–7.65 (m, 5H), 11.00 (s, 1H), 12.25 (s, 1H). MS: *m*/*z* 513 [M + H]<sup>+</sup>. HPLC: >95% ( $t_{\rm R}$  = 2.2 min). HRMS (ES<sup>+</sup>) calcd for C<sub>30</sub>H<sub>32</sub>N<sub>4</sub>O<sub>4</sub> [M + H]<sup>+</sup> *m*/*e* 513.2502, found *m*/*e* 513.2490.

(*Z*)-3-({4-[(2-Dimethylamino-acetyl)-methyl-amino]-phenylamino}-phenyl-methylene)-2-*oxo*-2,3-dihydro-1*H*-indole-6-carboxylic Acid Methyl Ester (2). Compound 2 was prepared using the same procedure as described for the synthesis of 3 by substituting 86 for 84. <sup>1</sup>H NMR:  $\delta$  2.03 (s, 6H), 2.68 (s, 2H), 3.33 (s, 3H), 3.78 (s, 3H), 5.82 (d, 1H), 6.89 (d, 2H), 7.12 (d, 2H), 7.19 (d, 1H), 7.41 (s, 1H), 7.45–7.65 (m, 5H), 10.98 (s, 1H), 12.22 (s, 1H). MS: *m*/*z* 485 [M + H]<sup>+</sup>. Anal. (C<sub>28</sub>H<sub>28</sub>N<sub>4</sub>O<sub>4</sub>) C, H, N.

(Z)-3-[(4-Dimethylaminomethyl-phenylamino)-phenyl-methylene]-2-*oxo*-2,3-dihydro-1*H*-indole-6-carboxylic Acid Methyl Ester (55). Compound 55 was prepared using the same procedure as described for the synthesis of 3 by substituting 95 for 84. <sup>1</sup>H NMR:  $\delta$  2.05 (s, 6H), 3.22 (s, 2H), 3.77 (s, 3H), 5.82 (d, 1H), 6.90 (d, 2H), 7.06 (d, 2H), 7.19 (d, 1H), 7.41 (s, 1H), 7.45–7.65 (m, 5H), 10.95 (s, 1H), 12.25 (s, 1H). MS: *m*/*z* 428 [M + H]<sup>+</sup>. Anal. (C<sub>26</sub>H<sub>25</sub>N<sub>3</sub>O<sub>3</sub>) C, H, N.

(*Z*)-3-({4-[2-(4-Methylpiperazin-1-yl)acetylamino]-phenylamino}phenyl-methylene)-2-*oxo*-2,3-dihydro-1*H*-indole-6-carboxylic Acid Methyl Ester (56). Compound 56 was prepared using the same procedure as described for the synthesis of 3 by substituting 87 for 84. <sup>1</sup>H NMR:  $\delta$  2.13 (s, 3H), 2.30–2.55 (m, 8H), 3.13 (s, 2H), 3.78 (s, 3H), 5.82 (d, 1H), 6.84 (d, 2H), 7.19 (d, 1H), 7.40–7.65 (m, 8H), 9.60 (s, 1H), 10.92 (s, 1H), 12.18 (s, 1H). MS: *m*/*z* 526 [M+H]<sup>+</sup>. Anal. (C<sub>30</sub>H<sub>31</sub>N<sub>5</sub>O<sub>4</sub>·1.0H<sub>2</sub>O) C, H, N.

(*Z*)-3-{[4-(2-Dimethylaminoacetylamino)-phenylamino]-phenylmethylene}-2-*o.xo*-2,3-dihydro-1*H*-indole-6-carboxylic Acid Methyl Ester (57). Compound 57 was prepared using the same procedure as described for the synthesis of 3 by substituting *N*-(4-aminophenyl)-2-dimethylamino-acetamide<sup>27</sup> for 84. <sup>1</sup>H NMR:  $\delta$  2.20 (s, 6H), 3.00 (s, 2H), 3.78 (s, 3H), 5.81 (d, 1H), 6.83 (d, 2H), 7.19 (d, 1H), 7.40–7.65 (m, 8H), 9.70 (s, 1H), 10.96 (s, 1H), 12.18 (s, 1H). MS: *m*/*z* 470 [M]<sup>+</sup>. Anal. (C<sub>27</sub>H<sub>26</sub>N<sub>4</sub>O<sub>4</sub>·0.25H<sub>2</sub>O) C, H, N.

(Z)-3-{[4-(N-Methanesulfonyl-methylamino)-phenylamino]-phenylmethylene}-2-oxo-2,3-dihydro-1*H*-indole-6-carboxylic Acid Methyl Ester (58). Compound 58 was prepared using the same procedure as described for the synthesis of 3 by substituting 92 for 84. <sup>1</sup>H NMR:  $\delta$  2.85 (s, 3H), 3.13 (s, 3H), 3.80 (s, 3H), 5.82 (d, 1H), 6.86 (d, 2H), 7.20 (d, 2H) superimposed on 7.22 (d, 1H), 7.42 (s, 1H), 7.50–7.70 (m, 5H), 10.96 (s, 1H), 12.28 (s, 1H). MS: m/z 478 [M + H]<sup>+</sup>. Anal. (C<sub>25</sub>H<sub>23</sub>N<sub>3</sub>O<sub>5</sub>S) C, H, N.

(Z)-2-oxo-3-{[4-(2-oxo-Pyrrolidin-1-ylmethyl)-phenylamino]-phenylmethylene}-2,3-dihydro-1*H*-indole-6-carboxylic Acid Methyl Ester (59). Compound 59 was prepared using the same procedure as described for the synthesis of 3 by substituting 1-(4-aminobenzyl)-pyrrolidin-2-one<sup>28</sup> for 84. <sup>1</sup>H NMR:  $\delta$  1.88 (m, 2H), 2.26 (t, 2H), 3.17 (t, 2H), 3.78 (s, 3H), 4.23 (s, 3H), 5.81 (d, 1H), 6.82 (d, 2H), 7.03 (d, 2H), 7.19 (d, 1H), 7.42 (s, 1H), 7.50–7.65 (m, 5H), 10.98 (s, 1H), 12.24 (s, 1H). MS: *m/z* 468 [M + H]<sup>+</sup>. Anal. (C<sub>29</sub>H<sub>26</sub>N<sub>2</sub>O<sub>4</sub>·0.5H<sub>2</sub>O) C, H, N.

(Z)-3-({3-[(3-Dimethylaminopropyl)-methyl-carbamoyl]-phenyl amino}-phenyl-methylene)-2-oxo-2,3-dihydro-1H-indole-6-carbo-xylic Acid Methyl Ester (60). Compound 60 was prepared using the same procedure as described for the synthesis of 3 by substituting 94 for 84 and 32 for 33. <sup>1</sup>H NMR (2 rotamers):  $\delta$  1.45/1.65 (m, 2H), 1.98/2.17 (s, 6H) superimposed on 2.00–2.30 (t, 2H, incompletely resolved), 2.58/2.88 (s, 3H) superimposed

on 2.90/3.20 (t, 2H, incompletely resolved), 3.78 (s, 3H), 5.83 (d, 1H), 6.40–7.30 (m, 5H), 7.42 (s, 1H), 7.40–7.65 (m, 5H), 11.00 (s, 1H), 12.14 (s, 1H). MS: m/z 513 [M + H]<sup>+</sup>. Anal. (C<sub>30</sub>H<sub>32</sub>N<sub>4</sub>O<sub>4</sub>·1.5H<sub>2</sub>O) C, H, N.

(*Z*)-2-oxo-3-(Phenyl-phenylamino-methylene)-2,3-dihydro-1*H*indole-6-carboxylic Acid Methyl Ester (61). Compound 61 was prepared using the same procedure as described for the synthesis of 3 by substituting aniline for 84. <sup>1</sup>H NMR:  $\delta$  3.78 (s, 3H), 5.82 (d, 1H), 6.86 (d, 2H), 7.14 (m, 1H), 7.19 (m, 3H), 7.42 (s, 1H), 7.45–7.65 (m, 5H), 10.94 (s, 1H), 12.24 (s, 1H). MS: *m*/*z* 371 [M + H]<sup>+</sup>. Anal. (C<sub>23</sub>H<sub>18</sub>N<sub>2</sub>O<sub>3</sub>·0.25H<sub>2</sub>O) C, H, N.

(*Z*)-3-[(1-Methylpiperidin-4-ylamino)-phenyl-methylene]-2-*oxo*-2,3dihydro-1*H*-indole-6-carboxylic Acid Methyl Ester (62). Compound 62 was prepared using the same procedure as described for the synthesis of 3 by substituting 1-methylpiperidin-4-ylamine for 84 and 32 for 33. <sup>1</sup>H NMR:  $\delta$  1.55 (m, 2H), 1.75–1.85 (m, 4H), 2.08 (s, 3H), 2.57 (m, 2H), 3.11 (m, 1H), 3.78 (s, 3H), 5.46 (d, 1H), 7.16 (d, 1H), 7.38 (s, 1H), 7.50–7.70 (m, 5H), 10.62 (d, 1H) superimposed on 10.66 (s, 1H). MS: *m*/ *z* 392 [M + H]<sup>+</sup>. Anal. (C<sub>23</sub>H<sub>25</sub>N<sub>3</sub>O<sub>3</sub>) C, H, N.

(*Z*)-3-{[4-(2-Dimethylaminoacetylamino)-cyclohexylamino]-phenylmethylene}-2-*oxo*-2,3-dihydro-1*H*-indole-6-carboxylic Acid Methyl Ester (63). Compound 63 was prepared using the same procedure as described for the synthesis of 3 by substituting 99 for 84. <sup>1</sup>H NMR:  $\delta$  1.10 (m, 2H), 1.48 (m, 2H), 1.70–1.90 (m, 4H), 2.13 (s, 6H), 2.76 (s, 2H), 3.03 (m, 1H), 3.57 (m, 1H), 3.76 (s, 3H), 5.45 (d, 1H), 7.09 (d, 1H), 7.34 (s, 1H) superimposed on 7.35 (d, 1H), 7.45–7.70 (m, 5H), 10.50 (d, 1H), 10.69 (s, 1H). MS: *m*/*z* 477 [M + H]<sup>+</sup>. Anal. (C<sub>27</sub>H<sub>32</sub>N<sub>4</sub>O<sub>4</sub>·0.25H<sub>2</sub>O) C, H, N.

*N*-Methyl-2-(4-methylpiperazin-1-yl)-*N*-(4-nitrophenyl)-acetamide (65). 1-Methylpiperazine (14.8 mL, 133 mmol) and potassium carbonate (37.6 g, 205 mmol) were dissolved in acetone (500 mL), and 2-bromo-*N*-methyl-*N*-(4-nitrophenyl) acetamide<sup>29</sup> 64 (28.0 g, 103 mmol) was gradually added. The mixture was stirred for 3 h at ambient temperature. After that time, the precipitates were filtered off and the solvent was evaporated from the filtrate. The residue was taken up in ethyl acetate and extracted with water. After drying over sodium sulfate, the solvent was removed by evaporation to give 29.5 g (98%) of 65. <sup>1</sup>H NMR:  $\delta$  2.00–2.40 (m, 11H), 3.12 (s, 2H), 3.31 (s, 3H), 7.64 (d, 2H), 8.26 (d, 2H). MS: m/z 293 [M + H]<sup>+</sup>.

**2-Imidazol-1-yl-***N***-methyl***N***-(4-nitrophenyl)acetamide (66).** Compound **66** was prepared using the same procedure as described for the synthesis of **65** by substituting imidazole for 1-methylpiperazine. <sup>1</sup>H NMR:  $\delta$  3.33 (s, 3H), 4.92 (s, 2H), 6.85 (s, 1H), 7.05 (s, 1H), 7.51 (s, 1H), 7.72 (d, 2H), 8.31 (d, 2H). MS: *m*/*z* 261 [M + H]<sup>+</sup>.

**2-Dimethylamino-***N***-methyl-***N***-(4-nitrophenyl)acetamide (67).** Compound **67** was prepared using the same procedure as described for the synthesis of **65** by substituting dimethylamine hydrochloride for 1-methylpiperazine. <sup>1</sup>H NMR:  $\delta$  2.18 (s, 6H), 3.09 (s, 2H), 3.32 (s, 3H), 7.63 (d, 2H), 8.25 (d, 2H). MS: m/z 238 [M + H]<sup>+</sup>.

**2-(4-Methylpiperazin-1-yl)**-*N*-(4-nitrophenyl)acetamide (69). Compound 69 was prepared using the same procedure as described for the synthesis of 65 by substituting 2-bromo-*N*-(4-nitrophenyl)acetamide<sup>30</sup> (68) for 2-bromo-*N*-methyl-*N*-(4-nitrophenyl)acetamide (64). <sup>1</sup>H NMR:  $\delta$  2.13 (s, 3H), 2.30–2.55 (m, 8H), 3.18 (s, 2H), 7.90 (d, 2H), 8.22 (d, 2H), 10.32 (s, 1H).

*N*,*N*-Dimethyl-2-(4-nitrophenylamino)acetamide (70). 2-(4-Nitrophenylamino)-acetic acid (5.30 g, 27.0 mmol), *O*-(benzotriazol-1-yl)-*N*,*N*,*N'*,*N'*-tetramethyluronium tetrafluoroborate (TBTU, 10.4 g, 32.4 mmol), hydroxy-benzotriazole (HOBT, 4.97 g, 32.4 mmol), and dimethylamine hydrochloride (13.2 g, 162 mmol) were suspended in dimethylformamide (50 mL), and triethylamine (37.5 mL) was added at 0 °C. The mixture was stirred at room temperature for 8 h. After that time, the precipitate was filtered off, washed with water, and dried at 100 °C to give 4.70 g (78%) of **70**. <sup>1</sup>H NMR:  $\delta$  2.88 (s, 3H), 3.03 (s, 3H), 4.10 (d, 2H), 6.74 (d, 2H), 7.20 (t, 1H), 8.00 (d, 2H). MS: m/z 224 [M + H]<sup>+</sup>.

**2-[Acetyl-(4-nitrophenyl)amino]**-*N*,*N*-dimethylacetamide (73). *N*,*N*-Dimethyl-2-(4-nitrophenylamino)acetamide (70, 450 mg, 2.00 mmol) was dissolved in acetic anhydride (5 mL). The mixture was stirred at 140 °C for 1.5 h. After that time, the solvent was removed by evaporation. The residue was triturated with diethyl ether, filtered off, and dried at 60 °C under vacuum to give 464 mg (88%) of 73. <sup>1</sup>H NMR:  $\delta$  1.96 (s, 3H), 2.83 (s, 3H), 2.98 (s, 3H), 4.58 (s, 2H), 7.61 (d, 2H), 8.28 (d, 2H). MS: *m/z* 288 [M + Na]<sup>+</sup>.

*N*-(3-Dimethylaminopropyl)-*N*-(4-nitrophenyl)acetamide (74). Compound 74 was prepared using the same procedure as described for the synthesis of 73 by substituting *N*,*N*-dimethyl-*N'*-(4-nitrophenyl)-propane-1,3-diamine<sup>31</sup> 71 for 70. <sup>1</sup>H NMR:  $\delta$  1.54 (m, 2H), 1.90 (s, 3H), 2.04 (s, 6H), 2.16 (t, 2H), 3.73 (t, 2H), 7.63 (d, 2H), 8.27 (d, 2H). MS: *m/z* 266 [M + H]<sup>+</sup>.

*N*-(2-Dimethylaminoethyl)-*N*-(4-nitrophenyl)acetamide (75). Compound 75 was prepared using the same procedure as described for the synthesis of 73 by substituting *N*,*N*-dimethyl-N'-(4-nitrophenyl)-ethane-1,2-diamine<sup>31</sup> (72) for 70. <sup>1</sup>H NMR:  $\delta$  1.88 (s, 3H), 2.09 (s, 6H), 2.28 (t, 2H), 3.79 (t, 2H), 7.62 (d, 2H), 8.28 (d, 2H). MS: m/z 252 [M + H]<sup>+</sup>.

*N*-(2-Dimethylaminoethyl)-*N*-(4-nitrophenyl)methanesulfonamide (77). *N*-(4-Nitrophenyl)methanesulfonamide<sup>32</sup> (76) (21.6 g, 100 mmol) was dissolved in acetone (1.0 L), and 2-chloro-*N*,*N*-dimethylethylamine hydrochloride (21.6 g, 150 mmol), potassium carbonate (35.9 g, 260 mmol), and sodium iodide (3.0 g, 20 mmol) were added. The mixture was stirred at 50 °C for 3 days. After that time, the precipitates were filtered off and the solvent was evaporated from the filtrate. The residue was taken up in ethyl acetate (300 mL) and extracted with water (3 × 200 mL). The organic layer was separated and dried over sodium sulfate. The solvent was removed by evaporation, and the residue was recrystallized from 2-propanol to give 15.2 g (53%) of 77. <sup>1</sup>H NMR:  $\delta$  2.08 (s, 6H), 2.29 (t, 2H), 3.12 (s, 3H), 3.84 (t, 2H), 7.66 (d, 2H), 8.27 (d, 2H). MS: *m*/z 288 [M + H]<sup>+</sup>.

*N*-Methyl-*N*-(4-nitrophenyl)methanesulfonamide (78). *N*-(4-Nitrophenyl)methanesulfonamide<sup>32</sup> 76 (4.32 g, 20.0 mmol) was dissolved in dimethylsulfoxide (40 mL), and potassium *tert*-butyrate (2.50 g, 22.0 mmol) was added. The mixture was stirred at ambient temperature for 1 h. After that time, a solution of methyl iodide (4.20 g, 18.0 mmol) in dimethylsulfoxide 10 mL) was slowly added. Stirring was continued for 8 h at ambient temperature. After that time, the mixture was poured onto ice water (120 mL) and ethyl acetate was added. The organic layer was separated and extracted with water (3×). Finally, the organic layer was dried over sodium sulfate. The solvent was removed by evaporation, and the residue was triturated with diethyl ether and filtered off to give 4.20 g (91%) of 78. <sup>1</sup>H NMR:  $\delta$  3.18 (s, 3H), 3.35 (s, 3H), 7.64 (d, 2H), 8.28 (d, 2H). MS: m/z 229 [M – H]<sup>-</sup>.

*N*-(3-Dimethylaminopropyl)-*N*-methyl-4-nitrobenzamide (81). *N*,*N*,*N'*-Trimethylpropane-1,3-diamine (4.80 g, 41.3 mmol) was dissolved in methylene chloride (150 mL), and triethylamine (11.5 mL, 82.6 mmol) was added. 4-Nitrobenzoyl chloride **79** (7.67 g, 41.3 mmol) was added at 0 °C. The mixture was stirred for 5 min at 0 °C and for 20 min at room temperature. After that time, the precipitates were filtered off and the filtrate was extracted three times with water. The organic layer was separated and dried over sodium sulfate. The solvent was removed by evaporation to give 10.33 g (94%) of **81**. <sup>1</sup>H NMR (2 rotamers):  $\delta$  1.61/1.73 (m, 2H), 1.98/2.06 (s, 6H), 2.04/2.28 (t, 2H), 2.86/3.00 (s, 3H), 3.16/3.48 (t, 2H), 7.78 (d, 2H), 8.29 (d, 2H). MS: *m*/*z* 266 [M + H]<sup>+</sup>.

*N*-(3-Dimethylaminopropyl)-*N*-methyl-3-nitrobenzamide (82). Compound 82 was prepared using the same procedure as described for the synthesis of 81 by substituting 3-nitrobenzoyl chloride 80 for 4-nitrobenzoyl chloride 79. <sup>1</sup>H NMR (2 rotamers):  $\delta$  1.62/1.78 (m, 2H), 2.00/2.23 (s, 6H), 2.10/2.43 (t, 2H), 2.88/2.98 (s, 3H), 3.19/3.49 (t, 2H), 7.60-7.90 (m, 2H, incompletely resolved), 8.15-8.35 (m, 2H, incompletely resolved). MS: m/z 266 [M + H]<sup>+</sup>.

**Dimethyl-(4-nitrobenzyl)amine (83).** A solution of dimethylamine in water (40%, 114 mL, 900 mmol) was dissolved in methyl-*iso*-butylketone (600 mL) and a solution of 4-nitrobenzyl chloride (52.0 g, 300 mmol) in methyl-*iso*-butylketone (300 mL) was slowly added over 30 min. The mixture was stirred at ambient temperature for 2.0 h and at 35 °C for 0.5 h. After that time, ethyl acetate (100 mL) was added and the organic layer was separated. The organic layer was extracted with brine (4×), separated, and dried over sodium sulfate. The solvent was removed by evaporation, The residue was triturated with diethyl ether, filtered, and the solvent was removed by evaporation to give 55.1 g (99%) of **83**. <sup>1</sup>H NMR:  $\delta$  2.17 (s, 6H), 3.52 (s, 2H), 7.57 (d, 2H), 8.18 (d, 2H). MS: m/z 181 [M + H]<sup>+</sup>.

*N*-(4-Aminophenyl)-*N*-methyl-2-(4-methylpiperazin-1-yl)acetamide (84). *N*-Methyl-2-(4-methylpiperazin-1-yl)-*N*-(4-nitrophenyl)acetamide (65, 29.5 g, 101 mmol) was dissolved in methanol (350 mL) and hydrogenated (50 psi) at room temperature for 1.5 h using 3.0 g palladium on charcoal (10%) as catalyst. After that time, the catalyst was filtered off and the solvent was removed by evaporation. The residue was triturated with diethyl ether, filtered off, and dried at 80 °C under vacuum to give 22.1 g (83%) of 84. <sup>1</sup>H NMR:  $\delta$  2.00–2.40 (m, 11H), 2.79 (s, 2H), 3.04 (s, 3H), 5.20 (s, 2H), 6.55 (d, 2H), 6.90 (d, 2H). MS: *m*/*z* 263 [M + H]<sup>+</sup>.

*N*-(4-Aminophenyl)-2-imidazol-1-yl-*N*-methylacetamide (85). Compound 85 was prepared using the same procedure as described for the synthesis of 84 by substituting 66 for 65. <sup>1</sup>H NMR:  $\delta$  3.12 (s, 3H), 4.54 (s, 2H), 5.33 (s, 2H), 6.62 (d, 2H), 6.82 (s, 1H), 7.01 (s, 1H), 7.07 (d, 2H), 7.46 (s, 1H). MS: m/z 231 [M + H]<sup>+</sup>.

*N*-(4-Aminophenyl)-2-dimethylamino-*N*-methylacetamide (86). Compound 86 was prepared using the same procedure as described for the synthesis of 84 by substituting 67 for 65. <sup>1</sup>H NMR:  $\delta$  2.21 (s, 6H), 2.78 (s, 2H), 3.14 (s, 3H), 5.25 (s, 2H), 6.54 (d, 2H), 6.91 (d, 2H). MS: *m/z* 208 [M + H]<sup>+</sup>.

*N*-(4-Aminophenyl)-2-(4-methylpiperazin-1-yl)acetamide (87). Compound 87 was prepared using the same procedure as described for the synthesis of 84 by substituting 69 for 65. <sup>1</sup>H NMR:  $\delta$  2.16 (s, 3H), 2.30–2.55 (m, 8H), 3.04 (s, 2H), 4.80 (br, 2H), 6.51 (d, 2H), 7.24 (d, 2H), 9.19 (s, 1H). MS: *m/z* 249 [M + H]<sup>+</sup>.

**2-[Acetyl-(4-aminophenyl)-amino]**-*N*,*N*-dimethylacetamide (88). Compound 88 was prepared using the same procedure as described for the synthesis of 84 by substituting 73 for 65. <sup>1</sup>H NMR:  $\delta$  1.73 (s, 3H), 2.80 (s, 3H), 2.91 (s, 3H), 4.30 (s, 2H), 5.20 (s, 2H), 6.53 (d, 2H), 7.02 (d, 2H). MS: *m*/*z* 258 [M + Na]<sup>+</sup>.

*N*-(4-Aminophenyl)-*N*-(3-dimethylaminopropyl)acetamide (89). Compound 89 was prepared using the same procedure as described for the synthesis of 84 by substituting 74 for 65. <sup>1</sup>H NMR:  $\delta$  1.50 (m, 2H), 1.68 (s, 3H), 1.94 (s, 6H), 2.15 (t, 2H), 3.49 (t, 2H), 5.20 (br, 2H), 6.57 (d, 2H), 6.86 (d, 2H). MS: *m*/*z* 236 [M + H]<sup>+</sup>.

*N*-(4-Aminophenyl)-*N*-(2-dimethylaminoethyl)acetamide (90). Compound 90 was prepared using the same procedure as described for the synthesis of 84 by substituting 75 for 65. <sup>1</sup>H NMR:  $\delta$  1.68 (s, 3H), 2.10 (s, 6H), 2.23 (t, 2H), 3.58 (t, 2H), 5.20 (br, 2H), 6.56 (d, 2H), 6.88 (d, 2H). MS: *m/z* 222 [M + H]<sup>+</sup>.

*N*-(4-Aminophenyl)-*N*-(2-dimethylaminoethyl)methanesulfonamide (91). Compound 91 was prepared using the same procedure as described for the synthesis of 84 by substituting 77 for 65. <sup>1</sup>H NMR:  $\delta$  2.08 (s, 6H), 2.24 (t, 2H), 2.91 (s, 3H), 3.56 (t, 2H), 5.25 (br, 2H), 6.53 (d, 2H), 6.99 (d, 2H). MS: m/z 258 [M + H]<sup>+</sup>.

*N*-(4-Aminophenyl)-*N*-methylmethanesulfonamide (92). Compound 92 was prepared using the same procedure as described for the synthesis of 84 by substituting 78 for 65. <sup>1</sup>H NMR:  $\delta$  2.85 (s, 3H), 3.10 (s, 3H), 5.22 (s, 2H), 6.54 (d, 2H), 7.02 (d, 2H). MS: m/z 200 [M]<sup>+</sup>.

**4-Amino-***N***-(3-dimethylaminopropyl)-***N***-methylbenzamide (93).** Compound **93** was prepared using the same procedure as described for the synthesis of **84** by substituting **81** for **65**. <sup>1</sup>H NMR:  $\delta$  1.66 (m, 2H), 2.08 (s, 6H), 2.17 (t, 2H), 2.91 (s, 3H), 3.30 (t, 2H), 5.40 (s, 2H), 6.53 (d, 2H), 7.10 (d, 2H). MS: *m/z* 236 [M+H]<sup>+</sup>.

**3-Amino-***N***-(3-dimethylaminopropyl)**-*N***-methylbenzamide (94).** Compound **94** was prepared using the same procedure as described for the synthesis of **84** by substituting **82** for **65**. <sup>1</sup>H NMR (2 rotamers):  $\delta$  1.55/1.65 (m, 2H), 2.00/2.12 (s, 6H) superimposed on 2.00–2.30 (t, 2H, incompletely resolved), 2.88/3.00 (s, 3H), 3.20–3.40 (t, 2H, incompletely resolved), 5.35 (br, 2H), 6.40–6.60 (m, 3H, incompletely resolved), 7.0–7.10 (m, 1H, incompletely resolved). MS: *m*/*z* 236 [M + H]<sup>+</sup>.

**4-(Dimethylaminomethyl)phenylamine (95).** Compound **95** was prepared using the same procedure as described for the synthesis of **84** by substituting **83** for **65** and Raney nickel for palladium on charcoal. <sup>1</sup>H NMR:  $\delta$  2.07 (s, 6H), 3.16 (s, 2H), 4.88 (s, 2H), 6.49 (d, 2H), 6.89 (d, 2H). MS: m/z 151 [M + H]<sup>+</sup>.

*trans*-[4-(2-Chloroacetylamino)cyclohexyl]carbamic Acid *tert*-Butyl Ester (97). *trans*-(4-Aminocyclohexyl)carbamic acid *tert*butyl ester (96) (2.50 g, 11.7 mmol) was suspended in tetrahydrofuran (500 mL) and triethylamine (2.43 mL, 17.5 mmol) was added. After stirring for 2 h at ambient temperature, chloro acetyl chloride (0.93 mL, 11.7 mmol) was added. The mixture was stirred for 8 h at ambient temperature. After that time, the solvent was removed by evaporation and the residue was taken up in methylene chloride. The organic layer was extracted with 2 N hydrochloric acid and a saturated solution of sodium hydrogen carbonate. The organic layer was separated and dried over sodium sulfate. Finally, the solvent was removed by evaporation to give 3.40 g (99%) of 97. <sup>1</sup>H NMR:  $\delta$  1.21 (m, 4H), 1.37 (s, 9H), 1.76 (m, 4H), 3.20 (m, 1H), 3.45 (m, 1H), 3.98 (s, 2H), 6.71 (d, 1H), 8.05 (d, 1H). MS: m/z 289 [M – H]<sup>-</sup>.

*trans*-{4-[2-(Dimethylamino)acetylamino]-cyclohexyl}-carbamic Acid *tert*-Butyl Ester (98). *trans*-[4-(2-Chloroacetylamino) cyclohexyl]carbamic acid *tert*-butyl ester 97 (2.62 g, 9.00 mmol) was suspended in acetone (50 mL) and potassium carbonate (3.73 mL, 27.0 mmol), and dimethylamine hydrochloride (1.10 g, 13.5 mmol) were added. The mixture was stirred for 8 h at ambient temperature. After that time, the precipitates were filtered off and the solvent was removed by evaporation to give 1.65 g (61%) of 98. <sup>1</sup>H NMR:  $\delta$  1.20 (m, 4H), 1.36 (s, 9H), 1.74 (m, 4H), 2.17 (s, 6H), 2.73 (s, 2H), 3.18 (m, 1H), 3.50 (m, 1H), 6.67 (d, 1H), 7.44 (d, 1H). MS: *m*/*z* 300 [M + H]<sup>+</sup>.

*trans-N*-(4-Aminocyclohexyl)-2-(dimethylamino)acetamide (99). *trans*-{4-[2-(Dimethylamino)acetylamino]cyclohexyl}carbamic acid *tert*-butyl ester 98 (1.80 g, 6.01 mmol) was dissolved in methylene chloride (50 mL), and trifluoroacetic acid (20.0 mL) was added. The mixture was stirred for 0.5 h at ambient temperature. After that time, the solvent was removed by evaporation and the residue was taken up in methylene chloride/methanol (1:1). The solution was neutralized using a basic MP resin. The solvent was removed by evaporation to give 1.20 g (100%) of 99. <sup>1</sup>H NMR:  $\delta$  1.34 (m, 4H), 1.70–1.90 (m, 4H), 2.28 (s, 6H), 2.95 (m, 1H) superimposed on 2.98 (s, 2H), 3.17 (br, 2H), 3.53 (m, 1H), 7.62 (d, 1H). MS: m/z 200 [M + H]<sup>+</sup>.

**Computational Methods.** All binding mode models have been built using the MOE (MOE revision 2008.10, Chemical Computing Group Inc., Montreal, Quebec, 2008) software package. The X-ray structure of **3** in complex with VEGFR-2<sup>8</sup> served as a template for all initial binding conformations (PDB code 3C7Q). The final binding mode has been calculated using the MMFF94x force field and a distance dependent dielectric constant. The ligand and a shell of 4 Å around the ligand has been free to move, a second shell of 4 Å has been tethered by a force constant of 100, and the rest of the VEGFR-2 protein has been fixed. All pictures have been rendered using PyMol 1.1r1 (DeLano Scientific LLC).

In Vitro VEGFR-2 Kinase Assay. The cytoplasmic kinase domain of VEGFR-2 (residues 797 to 1335 according to sequence

deposited in databank SWISS-PROT P35968) was cloned into pFastBac fused to Glutathion-S-transferase (GST). The GSTfusion protein was expressed in SF-9 insect cells and extracted with HEPEX (20 mM HEPES pH 7.4, 100 mM NaCl, 10 mM ss-glycerophosphate, 10 mM para-nitro-phenylphosphate, 30 mM NaF, 5 mM EDTA, 5% glycerol, 1% Triton X-100, 1 mM Na<sub>3</sub>VO<sub>4</sub>, 0.1% SDS, 0.5 µg/mL pepstatin A, 2.5 µg/mL 3,4dichloroisocoumarin, 2.5 µg/mL trans-epoxysuccinyl-L-leucyl-L-amido butane, aprotinin 20 KIU/mL, leupeptin 2  $\mu$ g/mL, benzamidine 1 mM and 0.002% PMSF). Enzyme activity was assayed in the presence or absence of serial dilutions of the inhibitor performed in 25% DMSO. Each microtiter plate contained internal controls such as blank, maximum reaction, and historical reference compound. All incubations were conducted at room temperature on a rotation shaker. Ten  $\mu$ L of each inhibitor dilution was added to 10 µL of diluted kinase (0.8 µg/mL VEGFR-2, 10 mM Tris pH 7.5, 2 mM EDTA, 2 mg/mL BSA) and preincubated for 1 h. The reaction was started by addition of 30  $\mu$ L of substrate mix containing 62.4 mM Tris pH 7.5, 2.7 mM DTT, 5.3 mM MnCl<sub>2</sub>, 13.3 mM Mg-acetate, 0.42 mM ATP, 0.83 mg/mL Poly-Glu-Tyr(4:1), and 1.7 µg/mL Poly-Glu-Tyr(4:1)-biotin and incubated for 1 h. The reaction was stopped by addition of 50  $\mu$ L of 250 mM EDTA, 20 mM HEPES, pH 7.4. Then 90  $\mu$ L of stopped solution was transferred to a streptavidin plate and incubated for 1-2 h. After three washes with PBS the EU-labeled antibody, PY20 was added (recommended dilution 1:2000 of 0.5 mg/mL labeled antibody in DELFIA assay buffer). Excessive detection antibody was removed by three washes of DELFIA washing buffer. Then 10 minutes before measurement on the multilabel reader VICTOR, each well was incubated with 100  $\mu$ L of DELFIA enhancement solution. IC<sub>50</sub> values were calculated by using a sigmoidal curve analysis program (Graph Pad Prism) using the nonlinear regression analysis with variable slope.

In Vitro VEGFR-1, VEGFR-3, FGFR-1, PDGFR $\alpha$  Kinase Assay. IC<sub>50</sub> in these assays were determined by Invitrogen Ltd., United Kingdom. For details please refer to www.invitrogen. com.

**Cellular Assays.** All cellular assays (HUVEC, HeLa, Calu-6, FaDu) were conducted as previously described in ref 8.

In Vivo Tumor Models. All in vivo experiments were conducted as previously described in ref 8 and complied with the Declaration of Helsinki and European Policy Legislations (FELASA and GV-SOLAS) on the Care and Use of Laboratory Animals.

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- (15) Assay conditions as described in ref 8.
- (16) Computational modeling was originally performed using a homology model based on the X-ray structure of FGFR-1. For better accuracy, the representations shown in this paper have been retrospectively modeled using the X-ray structure of VEGFR-2 recently reported in ref (8).
- (17) Twenty-three kinases were analyzed at 10 μmol/L with 100 μmol/L ATP as described in ref 8: GSK3β, ROCKII, DYRK1A, PKCα, MAPK2ERK2, MSK1, PDK1, CHK1, MAPKAPK2, SAP-K2AP38, S6K1, SGK, CK1, CK2, PKA, SAPK2BP38β2, SAPK3P38γ, JNK1A1, SAPK4P38δ, PHK, PKBα, CSK, PRAK. In contrast to 45, 35 inhibited CHK1, DYRK1A, MSK1, and PHK in the same panel significantly (<10% CTRL at 10 μmol/L).</p>
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