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Novel Potent Orally Active Multitargeted Receptor Tyrosine Kinase Inhibitors: Synthesis, Structure−Activity Relationships, and Antitumor Activities of 2-Indolinone Derivatives

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The inhibition of receptor tyrosine kinases (RTKs) has become a successful approach in the development of anticancer agents. Many potent small-molecule kinase inhibitors have been discovered. We report herein a series of pyrrolo-fused-heterocycle-2-indolinone analogues as inhibitors of vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), and c-Kit. Among them, some pyrrolo-fused six- and seven-membered-heterocycle derivatives such as **9**, **15**, **23**, and **25** are potent inhibitors of VEGFR, PDGFR, and c-Kit both enzymatically (< 50 nM) and cellularly (< 50 nM). Furthermore, compounds **9** and **25** possess favorable pharmacokinetic profiles and demonstrate good efficacies against human HT-29 cell colon tumor xenografts in nude mice. Further evaluations are in progress.

Introduction

Angiogenesis is essential for solid tumor growth and metastasis.[1] Inhibition of angiogenesis is an attractive therapeutic approach for the treatment of tumors. A subset of growth factors (VEGF, FGF, and PDGF)[a] and their receptor tyrosine kinases have been proven to be key signaling molecules during angiogenesis. In addition, some tumors have been found to overexpress the receptors to VEGF, PDGF, and FGF, which allows them to attain additional growth advantages and promote tumor survival.[2,3] Therefore, the interruption of VEGF, FGF, and PDGF signaling by binding of an ATP competitive small molecule inhibitor to the receptor kinase domain has been shown to inhibit tumor growth. This has been further validated by the FDA's approval of two small multitargeted kinase inhibitors, **1** (Bay43-9006) and **2** (SU11248) (Figure 1), as new agents for cancer treatment.[4−7]

In the search for novel and potent multitargeted kinase inhibitors, our programs began with the third-generation pyrrole indolinone derivative **2**, a potent VEGFR and PDGFR inhibitor,[8−10] with more favorable inhibition spectrum and better overall drug properties pharmacokinetics than other two earlier clinical candidates (*Z*)-3-((3,5-dimethyl-1*H*-pyrrol-2-yl)methylene)indolin-2-one (SU5416) and (*Z*)-3-[2,4-dimethyl-5-(2-oxo-2,3-dihydro-1*H*-indol-3-ylidenemethyl)-1*H*-pyrrol-3-yl]-propionic acid (SU6668).[11−13] The aims of our efforts were to improve both the potency and physicochemical properties. Tetrahydroindole indolinones were reported to have better VEGF activity than SU5416.[10] Maintaining the amino side chain as seen in **2** would increase the water solubility for favorable pharmacokinitics. Closing the 5′-1*H*-pyrrolemethyl onto the 4′-amide in **2** will give a series of molecules based on a pyrrolo-fused-heterocycle-2-indolinone scaffold **I**, which possessed similar conformations compared to **2** by overlapping the minimized conformations in Sybyl 7.3.[14] The larger ring size would reduce the rigidity of the bicyclo pyrrole system which may result in better solubility.

Herein we reported the synthesis of pyrrolo-fused six-, seven-, and eight-heterocycle derivatives. The targeted compounds were obtained by condensation of various substituted indolin-2-ones **II** with substituted pyrrole aldehydes **III** as shown in Figure 2. Pyrrolo-fused six- and seven-heterocycle derivatives were found to be potent inhibitors against the members of the VEGFR, PDGFR, and c-Kit kinases. A number of these compounds were also found to possess attractive pharmacokinetic characteristics and efficacies in tumor growth models. Herein, we describe our progress on optimization of this series of compounds, including chemical synthesis, structure−activity relationship, and in vivo activities. From this series, four candidates, **9**, **15**, **23**, and **25**, were selected for further preclinical development.

Chemistry

As shown in Scheme 1 ($n = 1$), selective base hydrolysis of the ethyl ester group at the C5-acetic acid side chain in pyrrole ester **3** led to pyrrole acetic acid **4**.[15,16] The compound was treated with TFA to give decarboxylated pyrrole **5**, which was condensed with different amines to obtain pyrrole amides.

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[a] Abbreviations: RTKs, receptor tyrosine kinases; VEGFR, vascular endothelial growth factor receptor; PDGFR, platelet-derived growth factor receptor; VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; FGF, fibroblast growth factor; ATP, adenosine triphosphate; FDA, Food and Drug Administration; HUVEC, human umbilical vein endothelial cells; PDB, Protein Data Bank; $t_{1/2}$, half-life; CL, clearance; V_d, volume of distribution; *F*, oral bioavailability; TGI, tumor growth inhibition.

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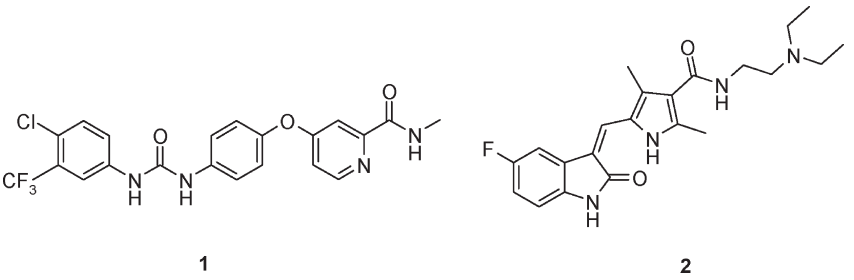

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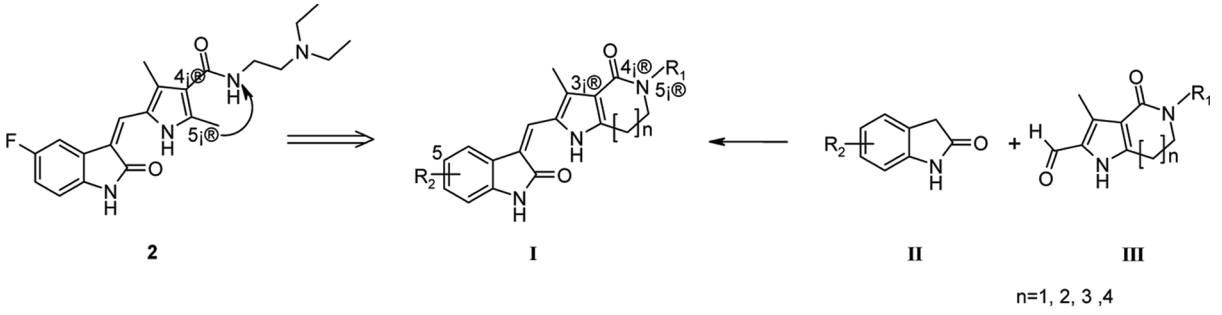
Figure 1. Multitargeted kinase inhibitors.



2 I II III

n=1, 2, 3 ,4

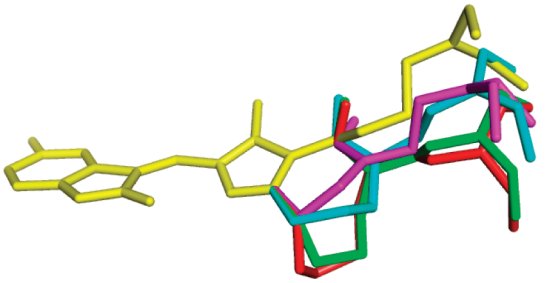


Figure 2. Design of pyrrolo-fused-heterocycles 2-indolinone derivatives **I**. The structure figures were created by use of Pymol: compound **2**, yellow; $n = 1$, magenta; $n = 2$, cyan; $n = 3$, green; $n = 4$, red.

The amides were then reduced to different pyrrole amines **6** with borane−tetrahydrofuran complex. Treatment of **6** with lithium hydroxide in ethylene glycol gave compounds **7** via intramolecular ring-closing reaction. Vilsmeier formylation followed by condensation with different indolin-2-ones afforded the desired series of compounds **9**−**15**.

As shown in Scheme 2 ($n = 2$), selective oxidation of pyrrole diester **16**[17] followed by Wittig reaction at the pyrrole aldehyde **17** and hydrogenation with H_2 over Pd/C in ethanol afforded pyrrole ethyl propionate **18**.[18] Selective saponification of the more exposed ethyl propionate at C5 under basic conditions produced pyrrole propanoic acid, which was further reduced to alcohol **19**. The primary alcohol of **19** was mesylated in the presence of triethylamine and converted to the key intermediates **20** with different amines under nucleophilic substitution reaction conditions. Trimethylaluminium-promoted lactamizations of compounds **20** was found to be a better method than LiOH ($n = 1$) to provide compounds **21** in refluxing toluene.[19] Hydrolytic decarboxylation and formylation of compounds **21** with triethyl orthoformate in TFA led to aldehydes **22**, which condensed with 5-fluoro-indolin-2-one to afford target compounds **23**−**25**.

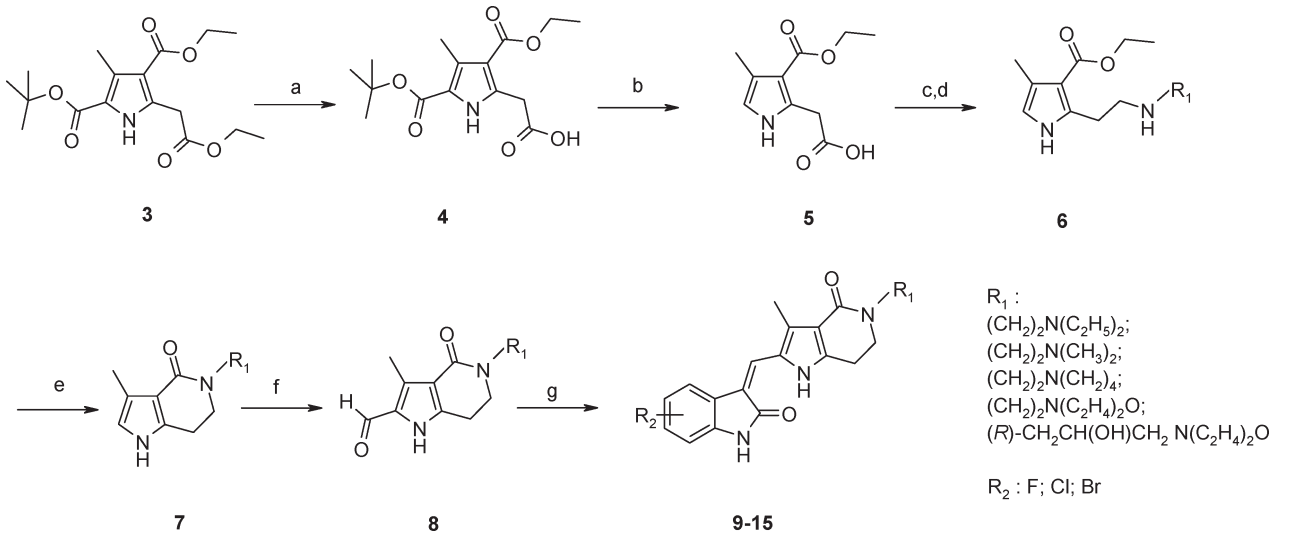
As shown in Scheme 3 ($n = 3$), the pyrrole aldehyde **17** reacted with cyclopropylmagnesium bromide to give the pyrrole cyclopropylcarbinol **26** in 40% yield.[20,21] Hydrobromic acid in water (40%) initiated the homoallylic rearrangement of **26** to give the corresponding butenyl bromide **27**, which was first hydrogenated and displaced with *N*,*N*-diethylethylenediamine to give the desired pyrrole amine **29**. Analogue **32** was synthesized in the same manner as shown in Figure 2.

Results and Discussion

Kinase and Cellular Inhibitory Activities. The compounds shown in Table 1 were evaluated for their abilities to inhibit VEGFR-2, PDGFRβ, and c-Kit in vitro. Tetrahydropyrrolo-[3,2-*c*]pyridin-4-one derivatives **9**, **10**, and **11** exhibited very potent inhibitory activities against VEGFR-2, PDGFRβ, and c-Kit in biochemical assays. The SAR showed that different halogen substitutions had little effect on the biochemical activities but affected the cellular activities. When compared with the F analogue, the Cl and Br derivatives showed much lower activities in HUVECs.

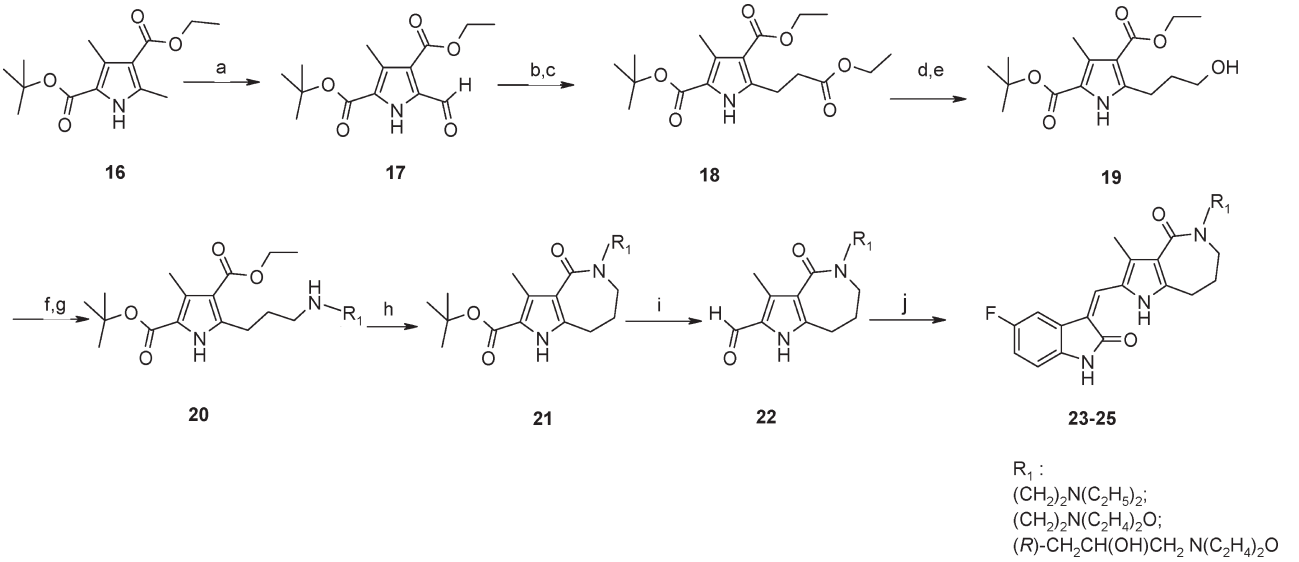
Replacing the diethylamine in **9** by dimethylamine, pyrrolidine, morphine, or hydroxyl morphine (**12**−**15**) retained the activities in the biochemical assay but significantly decreased activities in the cell assay. This observation may be related to their solubility and cell permeability. Compared to **14** (HUVEC $IC_{50} = 5.640\ \mu M$), compound **15** (HUVEC $IC_{50} = 0.019\ \mu M$) is remarkably more potent against VEGFR-2 induced proliferation in cellular assays. Introduction of hydroxyl group could improve both aqueous solubility and cell potency.[22]

Scheme 1. Synthesis of Tetrahydropyrrolo[3,2-*c*]pyridin-4-one Derivatives ($n = 1$)[a]



[a] Reagents and conditions: (a) LiOH, H_2O, THF, CH_3OH, 72%; (b) TFA, CH_2Cl_2, reflux, 86%; (c) EDC, HOBt, R_1NH_2, CH_2Cl_2, >90% ; (d) B_2H_6, THF; (e) LiOH, ethylene glycol, 97%; (f) DMF, $POCl_3$, NaOH, 38%; (g) piperidine, indolin-2-ones, EtOH, 54%.

Scheme 2. Synthesis of Tetrahydro-1*H*-pyrrolo[3,2-*c*]azepin-4-one Derivatives ($n = 2$)[a]



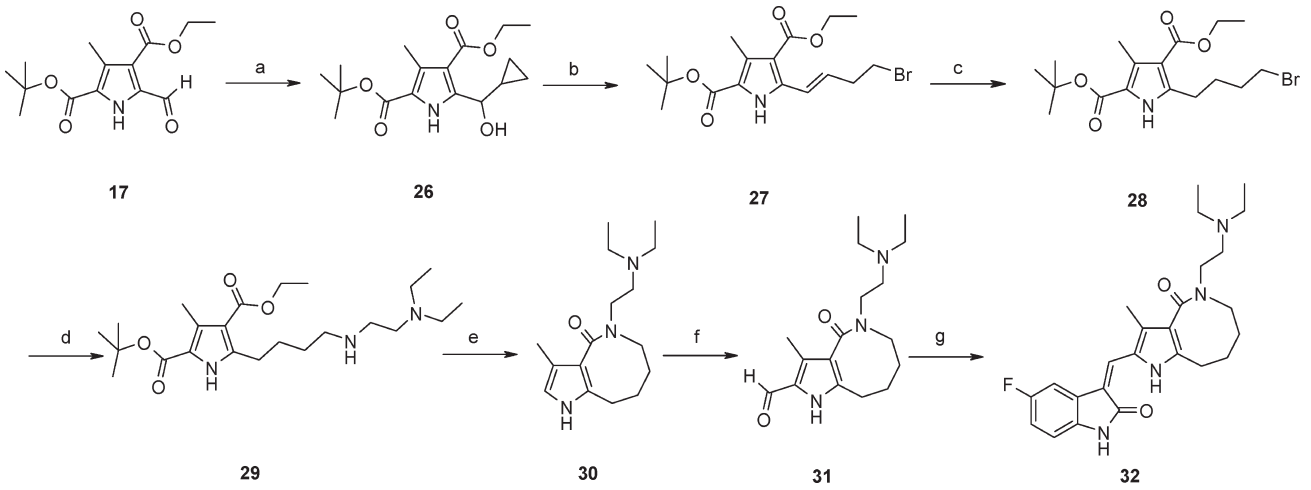
[a] Reagents and conditions: (a) CAN, THF, HOAc, H_2O, 98%; (b) $Ph_3P{=}CHCO_2C_2H_5$, THF, room temp, 80%; (c) H_2, 10% Pd/C, C_2H_5OH, 95%; (d) LiOH, THF, CH_3OH; (e) B_2H_6, THF; (f) CH_3SO_2Cl, Et_3N, CH_2Cl_2; (g) R_1NH_2; (h) $AlMe_3$, toluene, 76%; (i) $HC(OC_2H_5)_3$, TFA, 55%; (j) piperidine, 5-fluoroindolin-2-one, EtOH, 81%.

As an extension of the tetrahydropyrrolo[3,2-*c*]pyridin-4-one scaffold, tetrahydro-1*H*-pyrrolo[3,2-*c*]azepin-4-one derivatives and tetrahydro-1*H*,5*H*-1,5-diazacyclopentacycloocten-4-one derivatives (**23**−**25**, **32**) were also evaluated. 1*H*-Pyrrolo[3,2-*c*]azepin-4-one derivatives **23** and **25** showed a slight decrease in bioassay when compared with **9**. Interestingly, compound **24** (VEGFR-2 $IC_{50} = 0.001$ μM) exhibited roughly 5-fold higher potency than **9** against VEGFR-2. Fortunately, all of them retained moderate cellular potency. The conclusion that introduction of a hydroxyl could benefit the cellular potency is further supported. 1*H*,5*H*-1,5-Diazacyclopentacycloocten-4-one derivative **32** showed poor activities against VEGFR-2 in both biochemical and cellular assays.

Molecular Modeling. To investigate potential binding modes, compound **9** was docked into the ATP-binding site of VEGFR-2 (PDB entry 1Y6A)[23] and c-Kit (PDB entry 3G0E) using Surflex-Dock.[14,24] The predicted binding mode of **9** to VEGFR-2 is shown in Figure 3, in which the indolinone scaffold forms two hydrogen bonds to the backbone amide of Cys917 and the backbone carbonyl oxygen of Glu915 in the hinge region, while the diethylamine tail group is exposed to solvent without specific contacts with VEGFR-2. Similar binding mode was observed when **9** docked to c-Kit, which is nearly the same with **2** (Figure 4a). The tail group of **9** is also exposed to solvent showing no specific interaction with the c-Kit receptor (Figure 4b). These results provide the explanation that most of the indolinone type compounds exhibited good activities in biochemical assay.

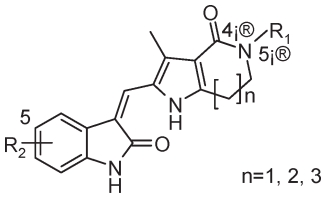
Pharmacokinetics Properties. The pharmacokinetic profiles of selected analogues were evaluated (Table 2). Derivatives **9**, **15**, **23**, **25** were found to have desirable pharmacokinetic behaviors. Compound **23** exhibited the highest oral

Scheme 3. Synthesis of Tetrahydro-1*H*,5*H*-1,5-diazacyclopentacycloocten-4-one Derivatives ($n = 3$)[a]



[a] Reagents and conditions: (a) cyclopropylmagnesium bromide, THF, 40%; (b) 40% HBr, C_2H_5OH, 90%; (c) H_2, 5% Pd/C, C_2H_5OH, 70%; (d) $(C_2H_5)_2NCH_2CH_2NH_2$, CH_2Cl_2, 78% ; (e) $AlMe_3$, toluene, then 2 M HCl, 27%; (f) DMF, $POCl_3$, NaOH, 51%; (g) piperidine, EtOH, 49%.

Table 1. SAR of Pyrrolo-Fused-Heterocycles-2-Indolinone Analogues **I**



I

				IC_{50} (μM)[a]			
				biochemical			
compd	R_1	R_2	n	VEGFR-2	PDGFRβ	c-Kit	cellular HUVEC
9	$(CH_2)_2N(C_2H_5)_2$	5-F	1	0.005	0.004	0.002	0.002
10	$(CH_2)_2N(C_2H_5)_2$	5-Cl	1	0.010	0.002	0.004	0.410
11	$(CH_2)_2N(C_2H_5)_2$	5-Br	1	0.016	0.003	0.004	0.150
12	$(CH_2)_2N(CH_3)_2$	5-F	1	0.002	0.003	0.001	0.410
13	$(CH_2)_2N(CH_2)_4$	5-F	1	0.008	0.004	0.003	ND
14	$(CH_2)_2N(C_2H_4)_2O$	5-F	1	0.025	0.010	0.001	5.640
15	(*R*)-$CH_2CH(OH)CH_2N(C_2H_4)_2O$	5-F	1	0.001	0.008	0.001	0.019
23	$(CH_2)_2N(C_2H_5)_2$	5-F	2	0.014	0.010	0.004	0.090
24	$(CH_2)_2N(C_2H_4)_2O$	5-F	2	0.001	0.005	0.004	0.036
25	(*R*)-$CH_2CH(OH)CH_2N(C_2H_4)_2O$	5-F	2	0.027	0.011	0.008	0.026
32	$(CH_2)_2N(C_2H_5)_2$	5-F	3	0.112	0.013	0.009	0.215

[a] Average values (at least two experiments). In vitro activities of **2**: VEGFR $IC_{50} = 0.015\,\mu M$; PDGFRβ $IC_{50} = 0.009\,\mu M$; c-Kit $IC_{50} = 0.035\,\mu M$; HUVEC $IC_{50} = 0.139\,\mu M$. ND: not determined.

bioavailability (65.8%). The substituent R_1 is very important to pharmacokinetic properties. Replacement of the *N*′,*N*′-diethyl-1,2-diaminoethane in **9** (or **23**) by (*R*)-2-hydroxy-3-morpholin-4-ylpropylamine **15** (or **25**) markedly improved the overall exposure (AUC at an oral dose of 40 mg/kg to Sprague−Dawley rats: 10.96 μg/(mL·h) for **9** vs 41.18 μg/(mL·h) for **15**; 27.61 μg/(mL·h) for **23** vs 49.21 μg/(mL·h) for **25**). The trend in reducing volume of distribution (V_d) and shortening terminal half-life ($t_{1/2}$) had also been observed in the four compounds. Although the other three compounds had a higher bioavailability, the terminal half-life of **9** was the longest.

In Vivo Antitumor Activities of Compound 9 and Compound 25. The in vivo antitumor activities of **9** and **25** were evaluated in human HT-29 colon tumor xenografts assay in BALB/cA-nude mice along with **2** (Figure 5). Once daily administration of compounds **9**, **25**, and **2** for 14 days led to antitumor efficacies in a dose-dependent manner. At 10 mg/kg dose, significant reductions in tumor size were observed, with a percent tumor growth inhibition (%TGI) of 49.75% and 51.67%, respectively. By comparison, **2** was found to be equally efficacious at 30 mg/kg dose with a percent tumor growth inhibition of 53.83%. When compound **9** was at a high dose of 30 mg/kg , 74.21% tumor growth inhibition was achieved. No overt toxicity as measured by weight loss or morbidity was observed at any dose level throughout the dosing regimen.

Conclusion

We have developed a novel series of new pyrrolo-fused-heterocycles-2-indolinone derivatives as multitargeted kinase

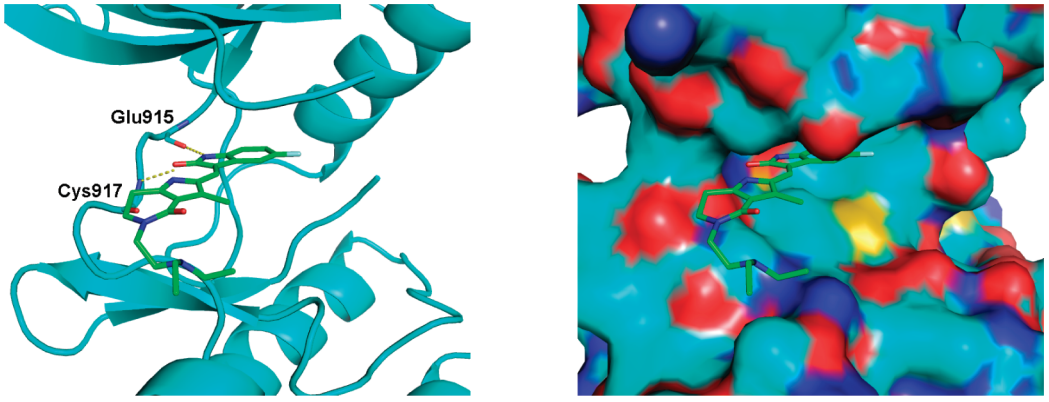


Figure 3. Binding models of compound **9** (green) bound to VEGFR-2 kinase (PDB entry 1Y6A). (a) Hydrogen bonds in yellow are shown between the indolinone and the Glu915 backbone carbonyl and between the indolinone and the Cys917 N−H. (b) Surface representation is shown, with the surface colored by atom type (red = oxygen, blue = nitrogen, yellow = sulfur, cyan = hydrogen, carbon). The donor−acceptor−donor motif is shown to interact with the hinge region, while the diethylamine group is exposed to solvent. The structure figures were created by use of Pymol.[14]

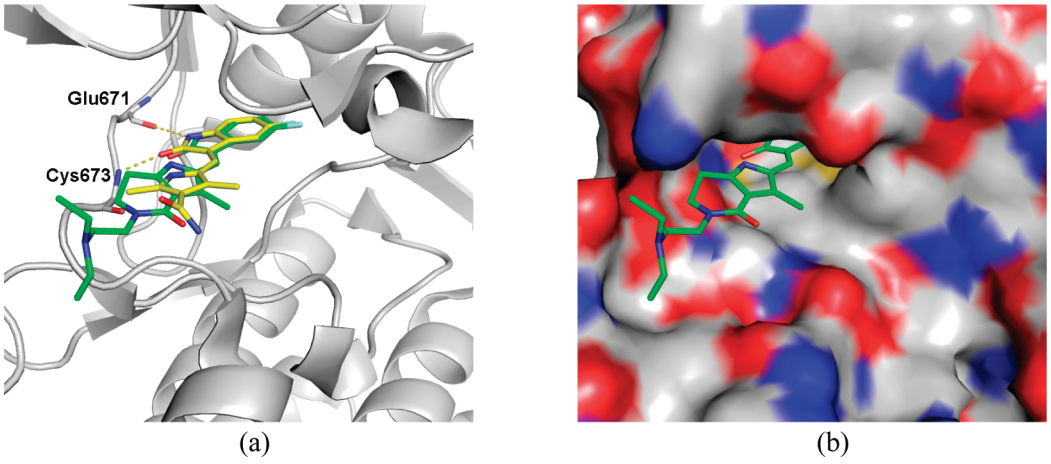


Figure 4. Binding models of compounds **9** (green) and **2** (yellow) bound to c-Kit kinase (PDB entry 3G0E). (a) Hydrogen bonds in yellow are shown between the indolinone and the Glu671 backbone carbonyl and between the indolinone and the Cys673 N−H. (b) Surface representation is shown, with the surface colored by atom type (red = oxygen, blue = nitrogen, yellow = sulfur, white = hydrogen, carbon). The donor−acceptor−donor motif is shown to interact with the hinge region, while the diethylamine group is exposed to solvent. The structure figures were created by use of Pymol.[14]

Table 2. Pharmacokinetic Profiles of Selected Compounds[a]

compd	C_{max} (μg/mL)	AUC (μg/(mL·h))	$t_{1/2}$ (h)	CL ((mL/min)/kg)	V_d (L/kg)	F (%)
9	0.45 ± 0.10	10.96 ± 3.20	18.02 ± 4.55	3.87 ± 0.94	97.39 ± 19.90	36.7
15	6.11 ± 2.60	41.18 ± 23.30	3.41 ± 1.76	1.02 ± 0.48	4.13 ± 0.90	50.7
23	2.41 ± 0.65	27.61 ± 8.63	9.65 ± 1.60	1.37 ± 0.61	18.79 ± 7.85	65.8
25	9.03 ± 3.15	49.21 ± 33.40	3.76 ± 1.98	1.26 ± 0.82	5.10 ± 1.38	55.2

[a] $t_{1/2}$, half-life; CL, clearance; V_d, volume of distribution; F, oral bioavailability. In vivo experiments were carried out with Sprague−Dawley rats (n = 6).

inhibitors and demonstrated that ring closure of the C4 amide to C5-methyl group of **2** indeed produced more potent indolinones. The optimized fused ring sizes were found to be six and seven. The preference of the fluoro substituent, diethylaminoethyl, and morpholino hydroxy side chains was similarly observed. Compounds **9**, **15**, **23**, and **25** were found to have potent enzymatic and cellular activities and possess good pharmacokinetic characteristics. Compounds **9** and **25** deomonstrated dose dependent oral antitumor efficacies against human HT-29 cell colon tumor xenografts in nude mice. When compared with **2**, compound **9** was found to be equally efficaous as **2** at $^1/_3$ of the dosage in this model. Compounds **9** and **25** were chosen for further evaluation.

Experimental Section

All purchased starting materials were used without further purification. ^{1}H NMR spectra were acquired on a Bruker Avance-400 spectrometer (400 MHz), with tetramethylsilane (TMS) as an internal standard; chemical shifts are expressed in parts per million (ppm, δ units). Mass spectra were obtained on a Finnigan LCQAd instrument (ESI). Most masses were reported as those of the protonated parent ions. Preparative column chromatography was performed using Yantai Huanghai 200−300

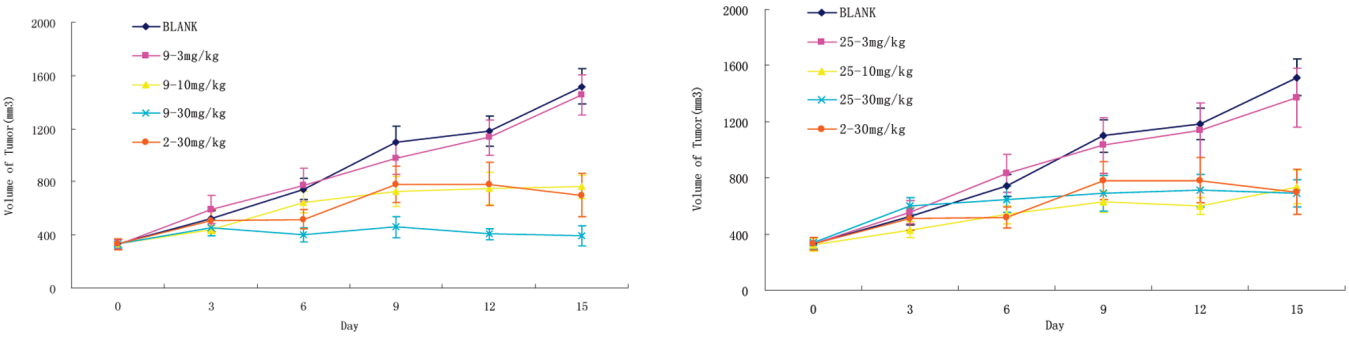


Figure 5. Antitumor activity upon treatment with **9**, **25**, **2** or vehicle control in HT29 colon tumor xenograft in BALB/cA-nude mice (n = 8 animals per group). Each compound was administered through once-daily oral treatments for 14 days from the 11th day after implantation. The used vehicle was 5% DMSO in distilled water. Compounds **9** and **25** were administrated at 3, 10, 30 mg/kg, respectively. Compound **2** was administrated at 30 mg/kg.

mesh silica. High-resolution mass spectra (HRMS) were recorded on an Apex III 7.0 T FTMS from Bruker Daltonics, Inc. (U.S.). Elemental analyses were performed on an Elementar VARIO EL3 analyzer, and the results obtained were within ±0.4% of the theoretical values. Where noted, compounds were determined to be >95% pure by analytical reverse-phase HPLC. The retention time (t_R) was determined using the following HPLC conditions: An isocratic program using 60−80% methanol, 40−20% water, and 0.1% aqueous ammonia was employed on a Gemini C18 column (250 mm × 4.6 mm). The flow rate was 1.0 mL/min, and UV detection was at 214 and 254 nm.

5-Ethoxycarbonylmethyl-3-methyl-1*H*-pyrrole-2,4-dicarboxylic Acid 2-*tert*-Butyl Ester 4-Ethyl Ester (3). A stirred solution of 3-oxobutyric acid *tert*-butyl ester (32 g, 0.2 mol) in glacial acetic acid was added dropwise with an aqueous solution of sodium nitrite (13.8 g, 0.2 mol of sodium nitrite in 20 mL of water) while maintaining the temperature at 0−5 °C. Upon completion of the addition, the reaction mixture was stirred in an ice−water bath for 1 h and at room temperature for another 3 h. The solution of 2-hydroxyimino-3-oxobutyric acid *tert*-butyl ester obtained was used without further purification. A mixture of 3-oxoglutaric acid diethyl ester (40 g,0.2 mol) in 90 mL of glacial acetic acid was added to the above solution of 2-hydroxyimino-3-oxobutyric acid *tert*-butyl ester and zinc dust (26 g, 0.4 mol) alternately at a rate to maintain the reaction temperature below 65 °C. Upon completion of the addition, the mixture was stirred at 75 °C for 2 h, cold water (100 mL) was added, and the mixture was stirred at room temperature for another 1 h. The zinc dust was removed by filtration, and the filtrate was extracted with ethyl acetate (100 mL × 3). The combined organic layers were washed with water (100 mL × 3), saturated sodium bicarbonate solution (100 mL × 4), and brine (100 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated to give the title compound (60 g, yield 88.5%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.84 (s, 1H, NH), 4.30 (q, J = 7.2 Hz, 2H, CH_2), 4.22 (q, J = 7.2 Hz, 2H, CH_2), 4.06 (s, 2H, CH_2), 2.57 (s, 3H, CH_3), 1.60 (s, 9H, 3 × CH_3), 1.37 (t, J = 7.2 Hz, 3H, CH_3), 1.31 (t, J = 7.2 Hz, 3H, CH_3). MS m/z (ESI): 340 $[M + H]^+$.

5-Carboxymethyl-3-methyl-1*H*-pyrrole-2,4-dicarboxylic Acid 2-*tert*-Butyl Ester 4-Ethyl Ester (4). To a solution of 5-ethoxycarbonylmethyl-3-methyl-1*H*-pyrrole-2,4-dicarboxylic acid 2-*tert*-butyl ester 4-ethyl ester (**3**) (30 g, 88.5 mmol) in tetrahydrofuran (268 mL) and methanol (134 mL) was added aqueous lithium hydroxide solution (11.1 g lithium hydroxide in 197 mL of water). Upon completion of the addition, the mixture was stirred for 1.5 h at room temperature. The resulting mixture was concentrated under vacuum. The residue was diluted with water (200 mL) and extracted with ethyl ether. The aqueous phase was adjusted to pH 1−3 with 20% hydrochloric acid solution. The resulting solid was filtered, washed with ethyl ether, and dried in vacuo to give the title compound (20 g, yield 72.3%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.40 (s, 1H, COOH), 11.71 (s, 1H, NH), 4.14 (q, J = 7.2 Hz, 2H, CH_2), 3.83 (s, 2H, CH_2), 2.50 (s, 3H, CH_3), 1.53 (s, 9H, 3 × CH_3), 1.25 (t, J = 7.2 Hz, 3H, CH_3). MS m/z (ESI): 312 $[M + H]^+$.

2-Carboxymethyl-4-methyl-1*H*-pyrrole-3-carboxylic Acid Ethyl Ester (5). A solution of 5-carboxymethyl-3-methyl-1*H*-pyrrole-2,4-dicarboxylic acid 2-*tert*- butyl ester 4-ethyl ester (**4**) (6.3 g, 20 mmol) in dichloromethane (600 mL) and trifluoroacetic acid (120 mL, 1.55 mol) was heated to reflux under a nitrogen atmosphere for 2.5 h. The reaction mixture was cooled in a dry ice−ethanol bath diluted with aqueous sodium hydroxide solution (1.55 mol of sodium hydroxide in 100 mL of water) while maintaining the temperature at −30 °C. Upon completion of the addition, the mixture was extracted with dichloromethane (100 mL × 7). The combined organic layers were washed with brine (100 mL), dried over anhydrous sodium sulfate, and evaporated to give the title compound (3.6 g, yield 85.7%) as a white solid.

2-[2-(2-Diethylamino-ethylamino)ethyl]-4-methyl-1*H*-pyrrole-3-carboxylic Acid Ethyl Ester (6). To a stirred solution of 2-carboxymethyl-4-methyl-1*H*-pyrrole-3-carboxylic acid ethyl ester (**5**) (2.11 g, 10 mmol) in *N*,*N*-dimethylformamide (5 mL) and dichloromethane (50 mL) was added *N*′,*N*′-diethylethane-1,2-diamine (1.23 g, 11 mmol), *N*-ethyl-*N*′-(dimethylaminopropyl)-carbodiimide hydrochloride (5 g, 26 mmol), and 1-hydroxybenzotriazole (1.5 g, 11 mmol) in an ice−water bath. Upon completion of the addition, the mixture was stirred at room temperature overnight. The mixture was poured into cold water (50 mL) and extracted with dichloromethane (50 mL × 3). The combined organic layers were washed with saturated sodium bicarbonate (50 mL), water (50 mL), and brine (50 mL), dried over anhydrous sodium sulfate, and evaporated to give 2-[(2-diethylaminoethylcarbamoyl)methyl]-4-methyl-1*H*-pyrrole-3-carboxylic acid ethyl ester (28.1 g, yield 90.9%) as a colorless oil. To the resulting oil (310 mg, 1 mmol) in anhydrous tetrahydrofuran (2 mL) was slowly added (dropwise) 1 M borane−tetrahydrofuran complex in tetrahydrofuran (3 mL, 3 mmol) under an argon atmosphere. Upon completion of the addition, the mixture was stirred at room temperature for 1 h and heated to reflux for another 5 h. The reaction mixture was diluted with water (5 mL) and 1 N hydrochloric acid (2 mL) dropwise, stirred for 5 min, adjusted to pH 10 with 10% aqueous sodium hydroxide solution, and extracted with ethyl acetate (10 mL × 5). The combined organic layers were washed with brine (15 mL), dried with anhydrous sodium sulfate, and evaporated to give the title compound (300 mg) as a brown oil which was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.02 (s, 1H, NH), 6.41 (s, 1H, pyrrole ArH), 4.30 (q, J = 7.2 Hz, 2H, CH_2), 3.17 (t, J = 6.0 Hz, 2H, CH_2), 3.01 (t, J = 5.6 Hz, 2H, CH_2), 2.78 (t, J = 5.6 Hz, 2H, CH_2), 2.60−2.54 (m, 6H, 3 × CH_2), 2.52 (s, 3H, CH_3), 1.57 (t, J = 6.4 Hz, 3H, CH_3), 1.07 (t, J = 6.4 Hz, 6H, 2 × CH_3). MS m/z (ESI): 296 $[M + H]^+$.

5-(2-Diethylaminoethyl)-3-methyl-1,5,6,7-tetrahydropyrrolo-[3,2-*c*]pyridine-4-one (7). To a stirred solution of 2-[2-(2-diethylaminoethylamino)ethyl]-4-methyl-1*H*-pyrrole-3-carboxylic acid

ethyl ester (**6**) (295 mg, 1 mmol) in 5 mL of toluene was slowly added (dropwise) 2 M trimethylaluminum in toluene (1 mL, 2 mmol) under an argon atmosphere. The mixture was stirred for 1 h at room temperature and heated to reflux for another 4 h. The reaction mixture was cooled to 0 °C and diluted with 1 N hydrochloric acid solution (3 mL). The mixture was adjusted to pH 12 with 10% aqueous sodium hydroxide solution and extracted with dichloromethane (30 mL × 4). The combined organic layers were filtered through a pad of Celite. The filtrate was dried over anhydrous sodium sulfate and evaporated to afford the title compound (242 mg, yield 97%) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (s, 1H, NH), 6.40 (s, 1H, pyrrole ArH), 3.64 (t, J = 6.8 Hz, 2H, CH_2), 3.55 (t, J = 6.4 Hz, 2H, CH_2), 2.84 (t, J = 7.2 Hz, 2H, CH_2), 2.68−2.61 (m, 6H, 3 × CH_2), 2.23 (s, 3H, CH_3), 1.07 (t, J = 6.4 Hz, 6H, 2 × CH_3). MS m/z (ESI): 250 $[M + H]^+$.

5-(2-Diethylaminoethyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carbaldehyde (8). To a stirred solution of *N*,*N*-dimethylformamide (2 mL) was slowly added 104 μL of phosphorus oxychloride under an argon atmosphere while maintaining the temperature at 0 °C. Upon completion of the addition, the mixture was stirred for 15 min at room temperature and cooled to 0−5 °C in an ice−water bath. A mixture of **7** (249 mg, 1 mmol) in 2 mL of *N*,*N*-dimethylformamide was added dropwise to the above solution. Upon completion of the addition, the mixture was stirred for 2 h at 0 °C and diluted with cold water (15 mL). The resulting mixture was adjusted to pH 12 with 10% aqueous sodium hydroxide solution and extracted with dichloromethane (15 mL × 6). The combined organic layers were washed with brine (15 mL), dried with anhydrous sodium sulfate, and evaporated. The residue was purified by silica gel column chromatography with triethylamine/methanol/dichloromethane (1:20:500) as eluents to afford the title compound (105 mg, yield 38%) as a pink oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.73 (s, 1H, NH), 9.59 (s, 1H, CHO), 3.68 (t, J = 7.2 Hz, 2H, CH_2), 3.60 (t, J = 7.2 Hz, 2H, CH_2), 2.97 (t, J = 6.8 Hz, 2H, CH_2), 2.75 (t, J = 6.8 Hz, 2H, CH_2), 2.67−2.63 (m, 4H, 2 × CH_2), 2.63 (s, 3H, CH_3), 1.09 (t, J = 7.2 Hz, 6H, 2 × CH_3). MS m/z (ESI): 278 $[M + H]^+$.

(*Z*)-2-(5-Fluoro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-5-(2-diethylaminoethyl)-3-methyl-1,5,6,7-tetrahydropyrrolo-[3,2-*c*]pyridin-4-one (9). To a suspension of 5-(2-diethylaminoethyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carbaldehyde (**8**) (74 mg, 0.237 mmol) and 5-fluoro-1,3-dihydroindol-2-one (40 mg, 0.267 mmol, commercially available from Aldrich) in 0.66 mL of ethanol was added dropwise anhydrous piperidine (0.1 mL). The mixture was stirred at room temperature overnight. The resulting solid was filtered under reduced pressure and rinsed with anhydrous ethanol (1 mL × 3) to afford the title compound (60 mg, yield 54.8%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.77 (s, 1H, NH), 10.94 (s, 1H, indole NH), 7.79−7.76 (m, 1H, ArH), 7.75 (s, 1H, C=CH), 6.97−6.92 (m, 1H, ArH), 6.87−6.84 (m, 1H, ArH), 3.62 (t, J = 6.8 Hz, 2H, CH_2), 3.45 (t, J = 7.2 Hz, 2H, CH_2), 3.00 (t, J = 6.8 Hz, 2H, CH_2), 2.56−2.46 (m, 9H, 3 × CH_2, CH_3), 0.99 (t, J = 7.2 Hz, 6H, 2 × CH_3).

(*Z*)-2-(5-Fluoro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-5-(2- diethylaminoethyl)-3-methyl-1,5,6,7-tetrahydropyrrolo[3,2-*c*]pyridin-4-one L-Malate. To a stirred yellow suspension of **9** (27 mg, 0.066 mmol) in 1 mL of methanol was added L-malic acid (9 mg, 0.069 mmol). The resulting solution was stirred for 5 min. The solvent was evaporated under reduced pressure, and the residue was treated with 1 mL of acetonitrile. The resulting suspension was heated to reflux for 1 h, cooled to room temperature, and filtered. The filter cake was washed with cold acetonitrile (0.5 mL × 3) and ethanol (0.5 mL × 3) and dried in vacuo to afford the title compound (35 mg, yield 95%) as a yellow solid. MS m/z (ESI): 411 $[M + H]^+$. HRMS calcd for $C_{23}H_{28}FN_4O_2$ $[M + H]^+$: 411.219 08. Found: 411.218 52.

Compounds **10**−**15** were similarly prepared using the appropriate raw materials. In most cases, the products deposited as the malate salts or lactate salts.

5-Formyl-3-methyl-1*H*-pyrrole-2,4-dicarboxylic Acid 2-*tert*-Butyl Ester 4-Ethyl Ester (17). 3,5-Dimethyl-1*H*-pyrrole-2,4-dicarboxylic acid 2-*tert*-butyl ester 4-ethyl ester **16** (30 g, 0.113 mol) was dissolved in 300 mL of tetrahydrofuran. To the mixture was added 360 mL of acetic acid and 300 mL of water under stirring at room temperature. Upon completion of the addition, the mixture was treated with with ammonium ceric nitrate (246 g, 0.449 mol), then stirred at room temperature for 0.5 h as the color of the solution changed from orange to pale yellow. The reaction mixture was poured into 800 mL of ice−water, and a light yellow precipitate formed. The mixture was stirred for another 0.5 h. The precipitate was filtered and dried in vacuo to afford the title compound (31.13 g, yield 98%) as a light yellow solid. MS m/z (ESI): 282 $[M + H]^+$

5-(2-Ethoxycarbonylethyl)-3-methyl-1*H*-pyrrole-2,4-dicarboxylic Acid 2-*tert*-Butyl Ester 4-Ethyl Ester (18). Compound **17** (23 g, 81.7 mmol) and (ethoxycarbonylmethylene)triphenylphosphorane (34.66 g, 99.4 mmol) were dissolved in 450 mL of tetrahydrofuran. The mixture was stirred at room temperature overnight under an argon atmosphere. After TLC showed the starting material disappeared, the mixture was concentrated under vacuum to afford a yellow oil. The residue was purified by column chromatography with *n*-hexane/ethyl acetate (20:1) as eluents to afford 5-(2-ethoxycarbonylvinyl)- 3-methyl-1*H*-pyrrole-2,4-dicarboxylic acid 2-*tert*-butyl ester 4-ethyl ester (23 g, yield 80.1%) as a yellow solid. MS m/z (ESI): 352 $[M + H]^+$. The resulting solid was dissolved in anhydrous ethanol (180 mL). Palladium on activated carbon (2.44 g, 10%) was added, and the mixture was stirred at room temperature overnight under a hydrogen atmosphere. After TLC showed the starting material disappeared, the reaction mixture was filtered and washed with ethanol (10 mL). The filtrate was concentrated under reduced pressure to give **18** (21.9 g, yield 95%) as a white solid. MS m/z (ESI): 354 $[M + H]^+$.

5-(2-Diethylaminoethyl)-3-methyl-4-oxo-1,4,5,6,7,8-hexahydropyrrolo[3,2-*c*]azepine-2-carboxylic Acid *tert*-Butyl Ester (21). 5-[3-(2-Diethylaminoethylamino)propyl]-3-methyl-1*H*-pyrrole-2,4-dicarboxylic acid 2-*tert*-butyl ester 4-ethyl ester **20** (3.547 g, 8.67 mmol) was dissolved in 70 mL of toluene at room temperature under an argon atmosphere. The mixture was treated with trimethylaluminum in toluene (5.6 mL, 2 mol/L, 11.27 mmol) and stirred for another 30 min at room temperature until no white smoke was released. The reaction mixture was heated to reflux for 4 h in an oil bath. After TLC showed the starting material had disappeared, the mixture was allowed to cool to room temperature, quenched with ethanol (10 mL, 95%), and diluted with anhydrous ethanol (60 mL). The resulting mixture was filtered through a pad of Celite, washed with anhydrous ethanol (200 mL × 4), and concentrated under vacuum. The residue was purified by silica gel column chromatography to afford the title compound (0.413 g, yield 75.7%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80 (s, 1H, NH), 3.57 (t, J = 7.2 Hz, 2H, CH_2), 3.31 (t, J = 6.0 Hz, 2H, CH_2), 2.75 (t, J = 7.2 Hz, 2H, CH_2), 2.65 (t, J = 7.2 Hz, 2H, CH_2), 2.57 (q, J = 7.2 Hz, 4H, 2 × CH_2), 2.42 (s, 3H, CH_3), 2.01−1.98 (m, 2H, CH_2), 1.50 (s, 9H, 3 × CH_3), 1.00 (t, J = 7.2 Hz, 6H, 2 × CH_3). MS m/z (ESI): 364 $[M + H]^+$.

5-(2-Diethylaminoethyl)-3-methyl-4-oxo-1,4,5,6,7,8-hexahydropyrrolo[3,2-*c*]azepine-2-carbaldehyde (22). Compound **21** (0.413 g, 1.14 mmol) was dissolved in trifluoroacetic acid (1.5 mL, 20 mmol). The resulting solution was cooled to −5 °C in an ice−salt bath under stirring, and triethoxymethane (0.34 mL, 1.7 mmol) added. The mixture was allowed to warm to room temperature and stirred for another 2 h. After TLC showed the starting material had disappeared, the mixture was diluted with 3 mL of cold water and 10 mL of dichloromethane, adjusted to pH 11 with aqueous sodium hydroxide solution (2 mol/L), and extracted with dichloromethane (10 mL × 3). The combined organic layers were dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure to give a yellow oil. The residue was purified by silica gel column chromatography

to afford the title compound (0.271 g, yield 55%) as a light brown oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.92 (s, 1H, CHO), 9.59 (s, 1H, NH), 3.50 (t, J = 6.4 Hz, 2H, CH_2), 3.31 (t, J = 6.4 Hz, 2H, CH_2), 2.81 (t, J = 7.2 Hz, 2H, CH_2), 2.60−2.44 (m, 6H, 3 × CH_2), 2.41 (s, 3H, CH_3), 2.01−1.95 (m, 2H, CH_2), 1.01 (t, J = 6.4 Hz, 6H, 2 × CH_3). MS m/z (ESI): 292 $[M + H]^+$.

(*Z*)-5-(2-Diethylaminoethyl)-2-(5-fluoro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-5,6,7,8-tetrahydro-1*H*-pyrrolo[3,2-*c*]azepin-4-one (23). Compound **22** and 5-fluoro-1,3-dihydroindol-2-one (0.127 g, 0.84 mmol) were dissolved in 1.4 mL of anhydrous ethanol. The resulting mixture was stirred for 10 min in the dark and treated with piperidine (0.15 mL, 1.49 mmol). The mixture was refluxed at 70 °C for about 1.5 h in an oil bath under an argon atmosphere, and a large orange precipitate formed. After TLC showed the starting material had disappeared, the reaction mixture was allowed to cool to room temperature. The precipitate was filtered and dried to give the title compound (0.288 g, yield 80.76%) as an orange solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.70 (s, 1H, NH), 10.90 (s, 1H, indole NH), 7.78−7.75 (m, 1H, ArH), 7.74 (s, 1H, CH═C), 6.96−6.91 (m, 1H, ArH), 6.86−6.83 (m, 1H, ArH), 3.51−3.48 (t, J = 6.8 Hz, 2H, CH_2), 3.36−3.28 (m, 4H, 2 × CH_2), 2.92 (t, J = 7.2 Hz, 2H, CH_2), 2.57−2.52 (m, 4H, 2 × CH_2), 2.45 (s, 3H, CH_3), 2.07−2.03 (m, 2H, CH_2), 0.97 (t, J = 6.8 Hz, 6H, 2 × CH_3). MS m/z (ESI): 425 $[M + H]^+$. Elemental analysis ($C_{24}H_{29}FN_4O_2$) calcd: C, 67.90; H, 6.89; N, 13.20; F, 4.48. Found: C, 67.81; H, 6.90; N, 13.11; F, 4.47. HRMS calcd for $C_{24}H_{30}FN_4O_2$ $[M + H]^+$: 425.234 73. Found: 425.234 17.

Compounds **23**−**25** were similarly prepared using the appropriate raw materials. In most cases, the products deposited as the malate salts.

5-(Cyclopropylhydroxymethyl)-3-methyl-1*H*-pyrrole-2,4-dicarboxylic Acid 2-*tert*-Butyl Ester 4-Ethyl Ester (26). To a stirred solution of cyclopropylmagnesium bromide (15 mL, 0.5 mol/L) cooled to −10 °C in an ice−salt bath under an argon atmosphere was slowly added a solution of 5-formyl-3-methyl-1*H*-pyrrole-2,4-dicarboxylic acid 2-*tert*-butyl ester 4-ethyl ester **17** (1.26 g, 4.5 mmol) in tetrahydrofuran (10 mL) . Upon completion of the addition, the ice−salt bath was removed. The mixture was stirred for 1 h at room temperature until TLC indicated that all starting materials had been consumed. The reaction mixture was quenched with water, diluted with 10% sulfuric acid solution (20 mL), and stirred for a further 30 min. The residue was extracted with ethyl acetate (50 mL × 3). The combined organic layers were washed with water and brine (50 mL each), dried over anhydrous magnesium sulfate, and evaporated to give a yellow solid. The resulting solid was purified by silica gel column chromatography to give the title compound (0.576 g, 39.6%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.47 (s, 1H, NH), 4.56 (d, J = 7.6 Hz, 1H, CH), 4.29 (q, J = 7.2 Hz, 2H, CH_2), 2.51 (s, 3H, CH_3), 1.53 (s, 9H, 3 × CH_3), 1.35 (t, J = 6.8 Hz, 3H, CH_3), 0.62−0.61 (m, 1H, CH), 0.61−0.58 (m, 2H, CH_2), 0.55−0.30 (m, 2H, CH_2). MS m/z (ESI): 322 $[M - H]^-$.

5-(4-Bromobut-1-enyl)-3-methyl-1*H*-pyrrole-2,4-dicarboxylic Acid 2-*tert*-Butyl Ester 4-Ethyl Ester (27). 5-(Cyclopropylhydroxymethyl)-3-methyl-1*H*-pyrrole-2,4-dicarboxylic acid 2-*tert*-butyl ester 4-ethyl ester **26** (323 mg, 1 mmol) was dissolved in 4 mL of ethanol. To the mixture was added 40% hydrobromic acid (2.8 mL), and the solution was stirred for 30 min at room temperature. After TLC showed the starting material disappeared, the reaction mixture was extracted with ethyl acetate (10 mL × 5). The combined organic extracts were washed with saturated brine (15 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (345 mg, yield 89.5%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.00 (s, 1H, NH), 7.14 (d, J = 16.4 Hz, 1H, CH═C), 6.05 (td, J = 16.4 Hz, J = 6.8 Hz, 1H, C═CH), 4.31 (q, J = 7.2 Hz, 2H, CH_2), 3.47 (t, J = 6.8 Hz, 2H, CH_2), 2.83−2.78 (m, 2H, CH_2), 2.52 (s, 3H, CH_3), 1.58 (s, 9H, 3 × CH_3), 1.37 (t, J = 7.2 Hz, 3H, CH_3). MS m/z (ESI): 329 $[M + H]^+$.

5-(4-Bromobutyl)-3-methyl-1*H*-pyrrole-2,4-dicarboxylic Acid 2-*tert*-Butyl Ester 4-Ethyl Ester (28). 5-(4-Bromobut-1-enyl)-3-methyl-1*H*-pyrrole-2,4-dicarboxylic acid 2-*tert*-butyl ester 4-ethyl ester **27** (30 mg, 0.08 mmol) was dissolved in 3 mL of ethanol. Then palladium on activated carbon (6 mg, 5%) was added to the solution at room temperature. The reaction mixture was stirred for 45 min under a hydrogen atmosphere. After TLC showed the starting material had disappeared, the reaction mixture was filtered and concentrated under reduced pressure to give the title product (21 mg, 70%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80 (s, 1H, NH), 4.21 (q, J = 7.2 Hz, 2H, CH_2), 3.45 (t, J = 6.8 Hz, 2H, CH_2), 2.87 (t, J = 7.6 Hz, 2H, CH_2), 2.46 (s, 3H, CH_3), 1.85−1.80 (m, 2H, CH_2), 1.76−1.70 (m, 2H, CH_2), 1.51 (s, 9H, 3 × CH_3), 1.27 (t, J = 7.2 Hz, 3H, CH_3). MS m/z (ESI): 388 $[M + H]^+$.

5-[4-(2-Diethylaminoethylamino)butyl]-3-methyl-1*H*-pyrrole-2,4-dicarboxylic Acid 2-*tert*-Butyl Ester 4-Ethyl Ester (29). 5-(4-Bromobutyl)-3-methyl-1*H*-pyrrole-2,4-dicarboxylic acid 2-*tert*--butyl ester 4-ethyl ester **28** (220 mg, 0.57 mmol) was dissolved in 5 mL of dichloromethane. The mixture was treated with *N*,*N*-diethylethylenediamine (164 μL, 1.13 mmol) and refluxed for 30 min in an oil bath. The reaction mixture was concentrated to remove most of the solvent and refluxed for another 1 h. After TLC showed the starting material had disappeared, the reaction mixture was concentrated under reduced pressure and purified by silica gel column chromatography to give the title compound as a white solid (187 mg, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.21 (q, J = 7.2 Hz, 2H, CH_2), 2.86 (t, J = 7.2 Hz, 2H, CH_2), 2.63−2.58 (m, 4H, 2 × CH_2), 2.52−2.47 (m, 2H, CH_2), 2.45 (s, 3H, CH_3), 2.45−2.44 (m, 4H, 2 × CH_2), 1.64−1.61 (m, 2H, CH_2), 1.53−1.47 (m, 2H, CH_2), 1.47 (s, 9H, 3 × CH_3), 1.28 (t, J = 7.2 Hz, 3H, CH_3), 0.94 (t, J = 7.2 Hz, 6H, 2 × CH_3). MS m/z (ESI): 424 $[M + H]^+$.

5-(2-Diethylaminoethyl)-3-methyl-6,7,8,9-tetrahydro-1*H*,5*H*-1,5-diazacyclopentacycloocten-4-one (30). Trimethylaluminum (489 μL, 2 mol/L) was dissolved in 3 mL of toluene. Then a solution of 5-(4-diethylaminobutyl)-3-methyl-1*H*-pyrrole-2,4-dicarboxylic acid 2-*tert*-butyl ester 4-ethyl ester **29** (345 mg, 0.82 mmol) in 6 mL of toluene was added at room temperature. Upon completion of the addition, the reaction mixture was stirred for 30 min at room temperature and heated to reflux for 2 h in an oil bath. After TLC showed the starting material had disappeared, the reaction mixture was quenched with water, diluted with hydrochloric acid solution (1 mL, 2 mol/L), and stirred for 30 min at room temperature. The mixture was adjusted to pH 10 with 10% aqueous sodium hydroxide solution and extracted with ethyl acetate (25 mL × 3). The combined organic extracts were washed with saturated brine (25 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound as a white solid (60 mg, 26.7%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (s, 1H, NH), 6.25 (s, 1H, pyrrole ArH), 2.68−2.64 (m, 4H, 2 × CH_2), 2.51 (q, J = 7.2 Hz, 4H, 2 × CH_2), 2.04 (s, 3H, CH_3), 1.66 (br s, 4H, 2 × CH_2), 1.24−1.18 (m, 4H, 2 × CH_2), 0.96 (t, J = 7.2 Hz, 6H, 2 × CH_3). MS m/z (ESI): 278 $[M + H]^+$.

5-(2-Diethylaminoethyl)-3-methyl-4-oxo-4,5,6,7,8,9-hexahydro-1*H*-1,5-diazacyclopentacyclooctene-2-carbaldehyde (31). To a stirred solution of *N*,*N*-dimethylformamide (88 μL) in dichloromethane was slowly added 35 μL of phosphorus oxychloride under an argon atmosphere while maintaining the temperature at 0 °C. Upon completion of the addition, the mixture was stirred for 15 min at room temperature and cooled to 0−5 °C in an ice−water bath. A mixture of **30** (70 mg, 0.25 mmol) in 2 mL of *N*,*N*-dimethylformamide was added dropwise to the above solution. Upon completion of the addition, the mixture was stirred for 2 h at 0 °C and diluted with cold water (5 mL). The resulting mixture was adjusted to pH 12 with 10% aqueous sodium hydroxide solution and extracted with dichloromethane (15 mL × 3). The combined organic layers were washed

with brine (15 mL), dried with anhydrous sodium sulfate, and evaporated. The residue was purified by silica gel column chromatography with triethylamine/methanol/dichloromethane (1:20:500) as eluents to afford the title compound (40 mg, yield 51%) as a pale yellow solid. MS m/z (ESI): 306 $[M + H]^+$.

(*Z*)-5-(2-Diethylaminoethyl)-2-(5-fluoro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-6,7,8,9-tetrahydro-1*H*,5*H*-1,5-diazacyclopentacycloocten-4-one (32). 5-(2-Diethylaminoethyl)-3-methyl-4-oxo-4,5,6,7,8,9-hexahydro-1*H*-1,5-diazacyclopentacyclooctene-2-carbaldehyde **31** (20 mg, 0.066 mmol) was dissolved in 1 mL of ethanol, and 5-fluoro-1,3-dihydroindol-2-one (10 mg, 0.066 mmol) was added to the solution at room temperature. The reaction mixture was stirred in the dark until dissolved, treated with 0.1 mL of piperidine, and heated to reflux for 2 h. After TLC showed the starting material disappeared, the reaction mixture was allowed to cool to room temperature and filtered to give the title compound (14 mg, yield 48.8%) as a yellow solid. ^{1}H NMR(400 MHz, DMSO-d_6) δ 13.51 (s, 1H, pyrrole NH), 10.90 (s, 1H, indole NH), 7.77−7.74 (m, 1H, ArH), 7.73 (s, 1H, CH=C), 6.97−6.92 (m, 1H, ArH), 6.87−6.84 (m, 1H, ArH), 4.06−4.03 (m, 1H, CH), 3.58−3.56 (m, 2H, CH_2), 3.54−3.51 (m, 2H, CH_2), 2.99 (br s, 2H, CH_2), 2.92−2.90 (m, 6H, 3 × CH_2), 2.39−2.35 (m, 2H, CH_2), 2.34 (s, 3H, CH_3), 1.74 (s, 4H, 2 × CH_2), 1.15 (t, J = 7.2 Hz, 6H, 2 × CH_3). MS m/z (ESI): 439 $[M + H]^+$. HRMS calcd for $C_{25}H_{32}FN_4O_2$ $[M + H]^+$: 439.250 38. Found: 439.249 79.

In Vitro Kinase Assays. An enzyme linked immunosorbent assay (ELISA) was conducted to measure the kinase activity of VEGFR-2, PDGFRβ, and c-Kit in vitro. The assay was performed in 96-well plates (PerkinElmer Life Sciences no. AAAND-0005).

VEGFR2 Bioassay. An amount o f22 ng of hVEGFR-2 was used to phosphorylate 1.5 μM biotin-gastrin precursor (Tyr87) peptide in the presence of 10 μM ATP, 5 mM $MgCl_2$, 5 mM $MnCl_2$, 3 μM Na_3VO_4, 1.25 mM DTT, and 60 mM HEPES (pH 7.5). A 30 μL portion of 50 mM EDTA was added to the reactions as negative controls. The 30 μL kinase reaction with or without inhibitors in 5% DMSO was carried out at room temperature for 30 min and then stopped by 30 μL of 50 mM EDTA. Then 100 μL portion of phosphotyrosine mAb (P-Tyr-100) (1:1000) was added to each well and incubated at room temperature for 60 min. Then a 100 μL portion of dilute europium labeled anti-mouse IgG was added to each well and incubated at room temperature for 30 min. A 100 μL portion of DELFIA enhancement solution was added to the well. The plate was incubated at room temperature for 5 min and read on the BMG Nova star in the time-resolved fluorescence mode by exciting at 340 nm and reading the emission at 615 nm.

PDGFRβ Bioassay. An amount of 45 ng of hPDGFRβ was used to phosphorylate 1.5 μM FLT3 (Tyr589) biotinylated peptide in the presence of 20 μM ATP, 5 mM $MgCl_2$, 5 mM $MnCl_2$, 3 μM Na_3VO_4, 1.25 mM DTT, and 60 mM HEPES (pH 7.5). A 30 μL portion of 50 mM EDTA was added to the mixture as negative control. The 30 μL kinase reaction with or without inhibitors in 5% DMSO was carried out at room temperature for 30 min and then stopped by 30 μL of 50 mM EDTA. Then a 100 μL portion of phosphotyrosine mAb (P-Tyr-100) (1:1000) was added to each well and incubated at room temperature for 60 min. Then a 100 μL portion of dilute europium labeled anti-mouse IgG was added to each well and incubated at room temperature for 30 min. A 100 μL portion of DELFIA enhancement solution was added to the well. The plate was incubated at room temperature for 5 min and read on the BMG Nova star in the time-resolved fluorescence mode by exciting at 340 nm and reading the emission at 615 nm.

c-Kit Bioassay. A HTScan c-Kit kinase assay kit (no. 7755, Cell Signaling) was used to measure the kinase activity of c-Kit in vitro. An amount of 30 ng c-Kit was used to phosphorylate 1.5 μM biotin-gastrin precursor (Tyr589) peptide in the presence of 25 μM ATP, 5 mM $MgCl_2$, 5 mM $MnCl_2$, 3 μM Na_3VO_4, 1.25 mM DTT, and 60 mM HEPES (pH 7.5). A 30 μL portion of 50 mM EDTA was added to the reactions as negative controls. The 30 μL kinase reaction with or without inhibitors in 5% DMSO was carried out at room temperature for 30 min and then stopped by 30 μL of 50 mM EDTA. A 100 μL portion of phosphotyrosine mAb (P-Tyr-100) (1:1000) was added to each well and incubated at room temperature for 60 min. Then a 100 μL portion of dilute europium labeled anti-mouse IgG was added to each well and incubated at room temperature for 30 min. A 100 μL portion of DELFIA enhancement solution was added to the well. The plate was incubated at room temperature for 5 min and read on the BMG Nova Star in the time-resolved fluorescence mode by exciting at 340 nm and reading the emission at 615 nm.

Cell Proliferation Assays. HUVECs (human umbilical vein endothelial cells) were purchased from Institute of Biochemistry and Cell Biology. Cells were grown as monolayers in media (M199, supplemented with 20% FBS) and maintained at 37 °C with 5% CO_2. HUVECs were plated at a density of 50 000 cells/mL in M199 medium containing 20% FBS and 10 ng/mL human recombinant VEGF in a Corning 96-well plate. The plate was incubated at 37 °C overnight. The medium was removed by aspiration, and test compounds were added to each well in a volume of 0.18 mL/well in M199 medium containing 5% FBS. Compound concentrations ranged from 1.0 nM to 100 μM. The plate was incubated at 37 °C for 72 h. The inhibition effects of HUVEC cells were observed by the sulforhodamine B (SRB) method. IC_{50} values were calculated by the data of inhibition rates of serial concentrations of the tested compounds.

Pharmacokinetics in the Rat. 9, **15**, **23**, and **25** were administered orally to Sprague−Dawley rats (n = 6) via gavage at a dose volume of 10 mg/kg and by intravenous injection to rats at a dose volume of 10 mg/kg. Plasma was collected after centrifugation. Plasma samples were stored frozen at −20 °C until the time of analysis. The plasma samples were prepared by liquid−liquid extraction after basification. Samples were analyzed by high performance liquid chromatography (HPLC)/mass spectrometric analysis. Pharmacokinetic parameters were determined by DAS 2.0.

Antitumor Avtivity against HT29 Colon Tumor Xenograft Model. The HT29 cell line was originally obtained from ATCC. The cells were maintained at 37 °C in DMEM high glucose/5% FBS, 1× NEAA, and 2 mM L-glutamine (Gibco/BRL, Grand Island, NY) under standard tissue culture condition. The antitumor activities of **9**, **25**, and **2** were evaluated in human HT-29 cell colon tumor xenografts in BALB/cA-nude mice in vivo. BALB/cA-nude mice (female, 5−6 weeks old, SCXK (Shanghai) 2008-0016) were obtained from Shanghai SIPPR/BK Laboratory Animal Co. Ltd. After the adaptation period, the female BALC/cA-nude mice were implanted subcutaneously with HT-29 cells (1×10^7 cells per mouse). When the tumors grew to a size of approximately 300 mm^3, drug treatment began. The tumor-bearing mice were randomized into groups (8 mice/groups) and administered either vehicle or **9** or **25** (3, 10, and 30 (mg/kg)/d) or **2** (30 (mg/kg)/d) once daily by oral gavage for continuous 14 days. Tumor volumes were measured twice per week, along with body weight as an index of toxicity. Data are expressed as mean values ± standard errors as a function of time. Compound **2** was synthesized in-house.

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Supporting Information Available: Additional experimental details and NMR, elemental analysis, HRMS, and HPLC data

for **9**−**15**, **23**−**25**, and **32**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Carmeliet, P.; Jain, R. K. Angiogenesis in cancer and other diseases. *Nature* **2000**, *407*, 249–257.
(2) Ellis, L. M.; Fidler, I. J. Angiogenesis and metastasis. *Eur. J. Cancer* **1996**, *32A*, 2451–2460.
(3) Folkman, J. Angiogenesis dependent diseases. *Semin. Oncol.* **2001**, *28*, 536–542.
(4) Chow, L. Q.M.; Eckhardt, S. G. Sunitinib: from rational design to clinical efficacy. *J. Clin. Oncol.* **2007**, *25*, 884–896.
(5) Wilhelm, S. M.; Carter, C.; Tang, L.; Wilkie, D.; McNabola, A.; Rong, H.; et al. BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. *Cancer Res.* **2004**, *64*, 7099–7109.
(6) Zhu, Z.; Bohlen, P.; Witte, L. Clinical development of angiogenesis inhibitors to vascular endothelial growth factor and its receptors as cancer therapeutics. *Curr. Cancer Drug Targets* **2002**, *2*, 135–156.
(7) Schenone, S.; Bondavalli, F.; Botta, M. Antiangiogenic agents: an update on small molecule VEGFR ihibitors. *Curr. Med. Chem.* **2007**, *14*, 2495–2516.
(8) Schlessinger, J. SU11248: genesis of a new cancer drug. The development of a promising RTK inhibitor. *Scientist* **2005**, *19*, 17–18.
(9) Li, X. Y.; Huang, P.; Cui, J. R.; Zhang, J.; Tang, P. C. Novel pyrrolyllactone and pyrrolyllactam indolinones as potent cyclin-dependent kinase 2 inhibitors. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1939.
(10) Sun, L.; Tran, N.; Liang, C. X.; Hubbard, S.; Tang, F.; Lipson, K.; Schreck, R.; Zhou, Y.; McMahon, G.; Tang, P. C. Identification of substituted 3-[(4,5,6,7-tetrahydro-1*H*- indol-2-yl)methylene]- 1,3-dihydroindol-2-ones as growth factor receptor inhibitors for VEGF-R2(Flk-1/KDR), FGF-R1, and PDGF-Rβ tyrosine kinases. *J. Med. Chem.* **2000**, *43*, 2655–2633.
(11) Sun, L.; Tran, N.; Tang, F.; 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 toward particular receptor tyrosine kinases. *J. Med. Chem.* **1998**, *41*, 2588–2603.
(12) Naparat, K.; Chantana, B.; Satoshi, T.; Hiroaki, S.; Ikuo, S.; Grierson, D. S.; Opa, V. Novel inhibitor for fibroblast growth factor receptor tyrosine kinase. *Bioorg. Med. Chem. Lett.* **2007**, *7*, 4812–4818.
(13) Patyna, S.; Laird, A. D.; Mendel, D. B.; O'Farrell, A. M.; Liang, C.; Guan, H. P.; Vojkovsky, T.; Vasile, S.; Wang, X. Y.; Chen, J.; Grazzini, M.; Yang, C. Y.; Haznedar, J. O.; Sukbuntherng, J.; Zhong, W. Z.; Cherrington, J. M.; Hu-Lowe, D. SU14813: a novel multiple receptor tyrosine kinase inhibitor with potent antiangiogenic and antitumor activity. *Mol. Cancer Ther.* **2006**, *5*, 1774–1782.
(14) (a) Tripos, headquartered in St. Louis, MO; http://www.tripos.com. (b) DeLano, W. L. *The PyMOL Molecular Graphics System*; DeLano Scientific LLC: Palo Alto, CA, 2008.
(15) Fabiano, E.; Golding, B. T. On the mechanism of pyrrole formation in the Knorr pyrrole synthesis and by porphobilinogen synthase. *J. Chem. Soc., Perkin Trans. 1* **1991**, *12*, 3371–3375.
(16) Sun, L.; Liang, C.; Sheri, S.; Zhou, Y.; Miller, T.; Cui, J.; Fukuda, J. Y.; Chu, J. Y.; Nematalla, A.; Wang, X. Y.; Chen, H.; Sistla, A.; Luu, T. C.; Tang, F.; Wei, J.; Tang, C. Discovery of 5-[5-fuoro-2-oxo-1,2-dihydroindol-(3*Z*)-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl) amide, a novel tyrosine kinase inhibitor targeting vascular endothelial and platelet-derived growth factor receptor tyrosine kinase. *J. Med. Chem.* **2003**, *46*, 1116–1119.
(17) Thyrann, T.; Lightner, D. A. Oxidation of pyrrole α-methyl to formyl with ceric ammonium nitrate. *Tetrahedron Lett.* **1995**, *36*, 4345–4348.
(18) Bit, R. A.; Davis, P. D.; Hill, C. H.; Keech, E.; Vesey, D. R. A Deckmann/ring expansion approach to tetrahydropyrido- and tetrahydroazepino-[1,2-*a*]indoles. *Tetrahedron* **1991**, *47*, 4645–4664.
(19) Park, Y. S.; Yum, E. K.; Basu, A.; Beak, P. Dynamic thermodynamic resolution: solvent effects, mechanism, and an asymmetric 3,4,5-substituted benzazepine synthesis. *Org. Lett.* **2006**, *8*, 2667–2670.
(20) Zanirato, V.; Pollini, G. P.; Risi, C. D.; Valente, F.; Melloni, A.; Fusi, S.; Barbetti, G.; Olivucci, M. Synthesis of biomimetic light-driven molecular switches via a cyclopropyl ring-opening/nitrilium ion ring-closing tandem reaction. *Tetrahedron* **2007**, *63*, 4975–4982.
(21) Hoegberg, T.; Ross, S. B.; Stroem, P.; Grunewald, G. L.; Creese, M. W.; Bunce, J. D. Homoallylic amines related to zimeldine. Comparative study on neuronal serotonin and norepinephrine reuptake based on conformational analysis. *J. Med. Chem.* **1988**, *31*, 913–919.
(22) Guan, H. P.; Liang, C. X.; Sun, L.; Tang, P. C.; Wei, C. C.; Mauragis, M. A.; Vojkovsky, T.; Jin, Q. W.; Herrinton, P. M. 3-(4-Amidopyrrol-2-ylmethylidene)-2-indolinone derivatives as protein kinase inhibitors. WO 2002066463A1, 2002.
(23) Harris, P. A.; Cheung, M.; Hunter, R. N.; Brown, M. L.; Veal, J. M.; Nolte, R. T.; Wang, L.; Liu, W.; Crosby, R. M.; Johnson, J. H.; Epperly, A. H.; Kumar, R.; Luttrell, D. K.; Stafford, J. A. Discovery and evaluation of 2-anilino-5-aryloxazoles as a novel class of VEGFR2 kinase inhibitors. *J. Med. Chem.* **2005**, *48*, 1610–1619.
(24) Jain, A. N. Surflex: fully automatic flexible molecular docking using a molecular similarity-based search engine. *J. Med. Chem.* **2003**, *46*, 499–511.