Discovery of 5-[5-Fluoro-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

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Abstract: To improve the antitumor properties and optimize the pharmaceutical properties including solubility and protein binding of indolin-2-ones, a number of different basic and weakly basic analogues were designed and synthesized. 5-[5-Fluoro-2-oxo-1,2-dihydroindol-(3*Z*)-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide (**12b** or SU11248) has been found to show the best overall profile in terms of potency for the VEGF-R2 and PDGF-Rβ tyrosine kinase at biochemical and cellular levels, solubility, protein binding, and bioavailability. **12b** is currently in phase I clinical trials for the treatment of cancers.

Introduction. Vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) receptors have been well validated as targets for the treatment of cancers because of their critical roles in tumor growth and suvival via autocrine and paracrine loops.[1−4] In this regard, both receptor tyrosine kinases (RTKs) have been found to be expressed on the tumor cells and to directly affect tumor cell proliferation (e.g., VEGF receptor in melanoma and PDGF receptor in gliomas).[1] In addition, both RTKs have been found to play prominent roles in tumor angiogenesis by participating in the transmission of proliferation, migration, differentiation, and survival signals between tumor cells and endothelial cells.[2−4] Thus, simultaneous inhibition of both endothelial growth factor receptor-2 (VEGF-R2) and platelet-derived growth factor receptor-β (PDGF-Rβ) might be expected to show better antitumor activity than by inhibiting only one of these RTKs. In the past several years, we have taken two indolin-2-ones, **5a** (SU5416) (VEGF-R selective inhibitor[5]) and **5b** (SU6668) (a potent and selective PDGF-Rβ tyrosine kinase inhibitor) (Table 1), to clinical trials for the treatment of cancers.[6,7] However, their low solubility and/or high protein binding properties were considered to be potential liabilities. In this study, efforts have been focused on finding an indolin-2-one analogue that shows potent and broad inhibitory activity against both VEGF-R2 and PDGF-Rβ and has good pharmaceutical properties with regard to solubility and protein binding. We report here the design, synthesis, and structure−activity relationship that led to the discovery of **12b** (Table 1),[8] a potent tyrosine kinase inhibitor targeting VEGF-R2 and PDGF-Rβ.

Chemistry. To broaden the kinase selectivity spectrum of indolin-2-one for both VEGF-R2 and PDGF-Rβ and to optimize the pharmaceutical properties including solubility and protein binding, diversification at the C-4′ position on the pyrrole ring of **5a** has been explored. We learned from the past structure−activity relationship studies on indolin-2-ones that modifications at the C-4′ position could lead to compounds with different kinase inhibition profiles for VEGF-R2 and PDGF-Rβ. In this regard, neutral **5a**[12] has been found to be a potent and selective inhibitor for VEGF-R2 while acidic **5b**[6] was found to be such for PDGF-Rβ. On the other hand, the cocrystal structure of **5b** in the catalytic domain of the FGF-R1 kinase,[6] a kinase that has high amino acid sequence homology for the ATP-binding pocket to VEGF-R2, revealed that the substitution at the C-4′ position on the pyrrole ring is positioned close to the opening of the binding pocket and could be exposed to solvent. Thus, substitution at this position might serve as a handle for improving pharmaceutical properties of the indolin-2-ones. In this study, various basic side chains have been introduced at the C-4′ position of **5a** to broaden the kinase inhibition spectrum and to improve pharmaceutical properties (particularly solubility) of indolin-2-ones. First, we introduced the aminoalkyl functionality in **5a** (**5c** in Scheme 1). Starting from **1**,[7] **5c** was synthesized by amidation with *N*-methylpiperazine, reduction of the amide functionality, formylation, and condensation with the indolin-2-one. Further diversification at the C-4′ position on the pyrrole ring of **5a** was also explored by introducing soluble aminoalkylamide functionalities as depicted in Scheme 2 (**12a**−**j**). The key intermediate **10** was prepared from commercially available 3-oxobutyric acid *tert*-butyl ester (**6**) by condensing with sodium nitrite in acetic acid, reductive cyclization with 3-oxobutyrate ethyl ester, hydrolytic decarboxylation and formylation, and base hydrolysis of the ethyl ester of **9**. Condensation of **10** with different indolin-2-ones afforded **11**, which upon amidation with different amines was converted to **12a**−**j**. Most of the amines used are commercially available except the amines (Scheme 3) for synthesizing **12g** and **12j**.

Results and Discussion. The biological activities of the synthesized compounds were first evaluated in biochemical assays measuring tyrosine phosphorylation of VEGF-R2, PDGF-Rβ, fibroblast growth factor receptor-1 (FGF-R1), and epidermal growth factor receptor (EGF-R) as described previously.[6,7] Potent and selective inhibitors against VEGF-R2 and PDGF-Rβ were subsequently tested for their kinase inhibitory activity in 3T3 cells, inhibitory activity against ligand-induced cellular proliferation (as measured by BrdU incorporation in 3T3 cells), cellular cytotoxicity, and solubility at pH 2 and 6 (Table 1).

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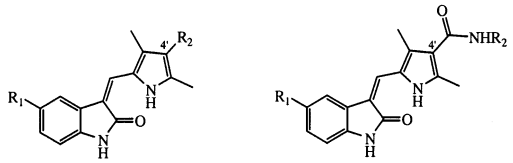
Table 1. In Vitro Kinase Inhibitory Activities and Solubility

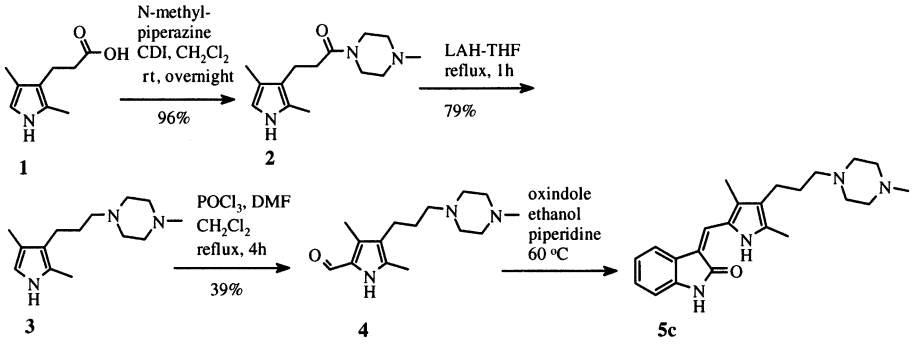


5 **12**

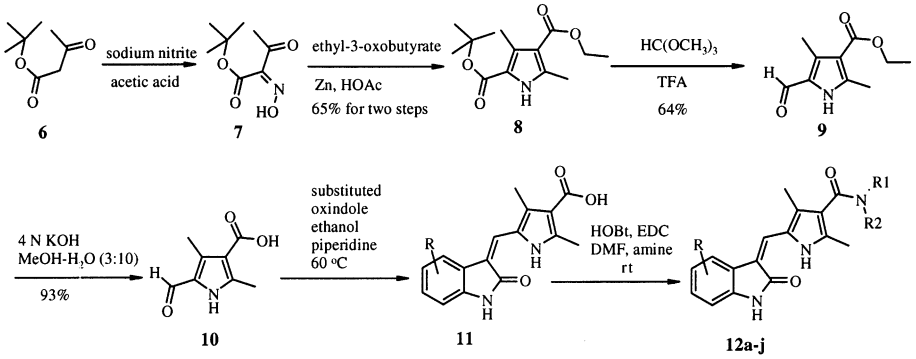
			biochemical activity against kinases IC_{50},[a] μM				cellular kinase activity in 3T3 cell IC_{50},[a] μM		PDGF-induced BrdU incorporation		solubility,[b] μg/mL	
compd	R1	R2	VEGF-R2	PDGF-Rβ	FGF-R1	EGF-R	VEGF	PDGF	IC_{50},[a] μM	cytotoxicity LD_{50},[a] μM	pH 2	pH 6
5a	H	H	1.23	22.9	>100	>100	1.04	20.3	4.05	>50	<1	<1
5b	H	$(CH_2)_2COOH$	2.4	0.060	3.00	>20	1−2	0.1−1.0	16	>50	<5	18
5c	H	$(CH_2)_3N(CH_2CH_2)_2NCH_3$	0.3	0.060	4.20	>20	*d*	*d*	0.20	>50	*d*	*d*
12a	H	$(CH_2)_2N(C_2H_5)_2$	0.050	0.017	0.88	>20	0.05−0.5	0.1−1.0	<0.07	>50	3022	511
12b	F	$(CH_2)_2N(C_2H_5)_2$	0.080	0.0020	2.90	>20	0.005−0.05	0.01	0.008	48.9	2582	364
12c	Cl	$(CH_2)_2N(C_2H_5)_2$	0.027	0.0030	0.17	>20	0.005−0.05	0.01−0.1	<0.07	15.6	3259	186
12d	Br	$(CH_2)_2N(C_2H_5)_2$	0.032	0.0050	0.73	10	0.005−0.05	0.01−0.1	<0.07	16.3	1299	101
12e	F	$(CH_2)_2N(CH_3)_2$	0.080	0.0005	1.60	>20	0.005−0.05	0.01−0.1	0.015	48.5	3012	75
12f	F	$(CH_2)_2$-pyrrolidin-1-yl	0.060	0.0010	3.90	>20	0.005−0.05	0.01−0.1	<0.07	49	3319	9
12g	F	$CH_2CH(CH_2CH_2)_2N-CH_3$	0.025	0.0030	0.20	*d*	0.04	0.02	0.030	38	>250	>250
12h	F	$(CH_2)_2$-morpholin-4-yl	0.090	0.0020	2.60	>20	0.05	*d*	0.037	>50	2945	0.3
12i	F	CH_2-pyridin-4-yl	<0.16	0.0010	3.10	>20	0.005−0.05	0.01−0.1	0.080	>50	486	<LD[c]
12j	F	$(CH_2)_2$-triazol-1-yl	0.085	0.010	17.1	>20	0.05−0.5	0.1−1.0	0.19	>50	2	6

[a] IC_{50} and LD_{50} values were determined by at least two separate tests and reported as mean values. [b] Solubility of the compounds was determined in 20 mM buffered solutions (pH 2, KCl/HCl; pH 6, phosphate) after shaking for 24 h at 22 °C. Data presented are from a single determination or an average of two determinations. [c] LD = limit for detection. [d] Not tested.

Scheme 1. Synthesis of **5c**



Scheme 2. Synthesis of 4′-Carboxamide Analogues of **5a**



It is interesting to note that converting the carboxylate group of **5b** into an amino group (**5c**) enhances the inhibitory activity for VEGF-R2 (8-fold) while retaining the potency against PDGF-Rβ kinase (Table 1). The difference in potency observed between inhibition of PDGF-induced BrdU incorporation and inhibition of cellular kinase activity for **5b** might be related to its poor solubility (Table 1). The SAR implied that a basic analogue of **5a** has shown broader kinase inhibitory activity against both VEGF-R2 and PDGF-Rβ than neutral (i.e. **5a**-VEGF-R-selective inhibitor[9]) and an acid analogue (i.e., **5b**-PDGF-Rβ-selective inhibitor), confirming that the kinase selectivity could be affected by a substitent at the C-4′ position.

Being encouraged by the above results, we extensively explored other possibilities for diversification at the C-4′ position and found that the readily accessible **12** showed the most promise. **12a**−**d** exhibited even more potent

Scheme 3. Synthesis of Amines for **12g** and **12j**

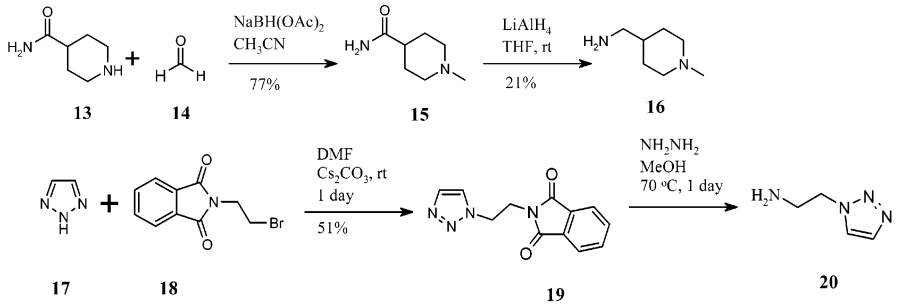


Table 2. Effects of Protein Binding on Cellular Potency[a]

	PDGF-induced BrdU incorporation in 3T3 cells, IC_{50} (μM)				
compd	at 0% BSA	at 0.1% BSA	at 0.5% BSA	at 1% BSA	at 5% BSA
5b	0.3	9.5	16	36	49
12b	<0.007	<0.007	<0.007	0.008	0.083

[a] BSA = bovine serum albumin.

inhibitory activity against VEGF-R2 and PDGF-Rβ in biochemical and cellular assays (Table 1). Specifically, compared to **5b**, **12b** is about 30-fold more potent against VEGF-R2 and PDGF-Rβ in biochemical assays, over 10-fold more potent in cellular kinase assays, and significantly more soluble under neutral (20-fold) and acidic (>500-fold) conditions. Among **12a**−**d**, **12a** is markedly less potent in inhibiting PDGF-Rβ phosphorylation in biochemical and cellular assays. Different halogen substitutions had little effect on the biochemical activities against VEGF-R2 and PDGF-Rβ but affected the cytotoxicity profiles. For example, the bulkier Cl and Br substitutions (**12c** and **12d**) show some extent of cytotoxicity when compared to the F analogue (**12b**) (Table 1). Therefore, diversification on **12b** has been further explored.

Surprisingly, replacing the diethylamine in **12b** by either dimethylamine (**12e**) or pyrrolidine (**12f**) markedly reduced solubility at pH 6 (from 364 to 75 or 9 μg/mL, respectively), while the in vitro activities were similar (Table 1). The more basic *N*-methylpiperidine group (which significantly increased solubility of the quinazoline-based VEGF-R2/EGF-R inhibitor (4-bromo-2-fluorophenyl)[6-methoxy-7-(1-methylpiperidin-4-ylmethoxy)quinazolin-4-yl]amine or ZD6474)[10] also improved the solubility of **12g**. However, this substitution increased cytotoxicity and decreased metabolic stability because of N-demethylation (data not shown). The much less basic morpholino analogue **12h** is also much less soluble at pH 6, though it has good solubility at pH 2. The basic heteroaromatic analogues **12i** and **12j** have generally poor solubility and sometimes reduced cellular potency (**12j** in Table 1).

To assess the protein binding properties for **5b** and **12b**, cellular IC_{50} values were measured in the presence or absence of serums. Compared to **5b**, the cellular potency of **12b** against PDGF-induced proliferation is less likely to be affected by the presence of serum protein (Table 2). In this regard, **12b** was still a very potent inhibitor of PDGF-Rβ while **5b** became inactive in the presence of a high level of serum.

In conclusion, **12b** possesses the best overall profile in terms of inhibitory potency against the VEGF-R2 and PDGF-Rβ targets in biochemical and cellular assays, solubility under both neutral and acidic conditions, and protein binding properties. Additional kinase selectivity study revealed that **12b** is also a good inhibitor of KIT and FLT-3.[13] Furthermore, **12b** also had very good oral bioavailability, was highly efficacious in a number of preclinical tumor models, and was well tolerated at efficacious doses.[8] It is currently in clinical phase I trials for the treatment of cancers.

Supporting Information Available: Synthesis of **12b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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