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Novel C-3 *N*-urea, amide, and carbamate dihydroindazolo[5,4-*a*]pyrrolo[3,4-*c*]carbazole analogs as potent TIE-2 and VEGF-R2 dual inhibitors

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Abstract—A novel series of C-3 urea, amide, and carbamate fused dihydroindazolocarbazole (DHI) analogs are reported as highly potent dual inhibitors of TIE-2 and VEGF-R2 receptor tyrosine kinases with excellent cellular potency. Structure–activity relationship (SAR) studies indicate the optimal N-13 alkyl substitutions are *n*-propyl and *i*-butyl. The isopropyl carbamate **39** displayed good dual enzyme, cell potency, and rat pharmacokinetic properties for advancement to in vivo evaluation.

Antiangiogenic therapy—inhibition of the generation and growth of new blood vessels from the endothelium of an existing vascular, an essential process required to support solid tumor growth and metastasis—remains an area of intense drug discovery research.[1] Vascular endothelial growth factor (VEGF) and its corresponding receptor tyrosine kinases, principally VEGF-R2, plays a key role in regulating vascular endothelial cells during embryonic development and tumor angiogenesis.[1] A significant body of clinical evidence has now accumulated demonstrating the association of tumor VEGF expression with disease progression in a wide range of solid malignancies. As a result, anti-angiogenesis therapies directed against VEGF-R kinases have been under active evaluation in clinical trials.[2,3] In addition to VEGF, the angiopoietins (Ang-1 and Ang-2) with its receptor tyrosine kinases, particularly TIE-2, also play an important role in angiogenesis and in vascular maintenance and stabilization.[4–6] A body of published literature provides evidence in support of a dynamic interaction between VEGF and Ang mediated signaling events in normal physiological angiogenesis and tumor-associated angiogenesis, vessel regression and remodeling.[6,7] Thus, optimal anti-angiogenic kinase therapy may require blocking multiple targets such as VEGFR-2 and TIE-2 concurrently. A combination approach simultaneously inhibiting both mechanisms may result in more dramatic effects on the integrity and stability of both the normal vasculature and the tumor-associated vasculature than inhibition of either pathway alone.[4,8]

The dual inhibitor strategy against VEGF-R2 and TIE-2 has been under active evaluation in clinical trials and in pre-clinical development (Fig. 1). The Pfizer thiazole compound **1** (TIE-2 IC_{50} = 48 nM, VEGF-R2 IC_{50} = 11 nM) is currently in phase II clinical trial.[9] Pre-clinical compounds include the Abbott pyrrolo[2,3-*d*]pyrimidine **2** (TIE-2 IC_{50} = 3 nM, VEGF-R2 IC_{50} = 340 nM),[10] and the GlaxoSmithKline furo[2,3-*d*]pyrimidine **3** (TIE-2 IC_{50} = 2 nM, VEGF-R2 IC_{50} = 3 nM).[11] Previously we reported on our first-generation pan-VEGF-R anti-angiogenic clinical candidate **4** (CEP-7055).[3] The objective for a second-generation compound superior to CEP-7055 (in terms of biochemical, pharmacokinetic, pharmacodynamic, and anti-tumor efficacy profiles) was to apply the dual TIE-2/VEGF-R2 approach by building in TIE-2 activity.

Our goal was to identify a dual TIE-2/VEGF-R2 inhibitor with IC_{50} values less than 50 nM with good cell

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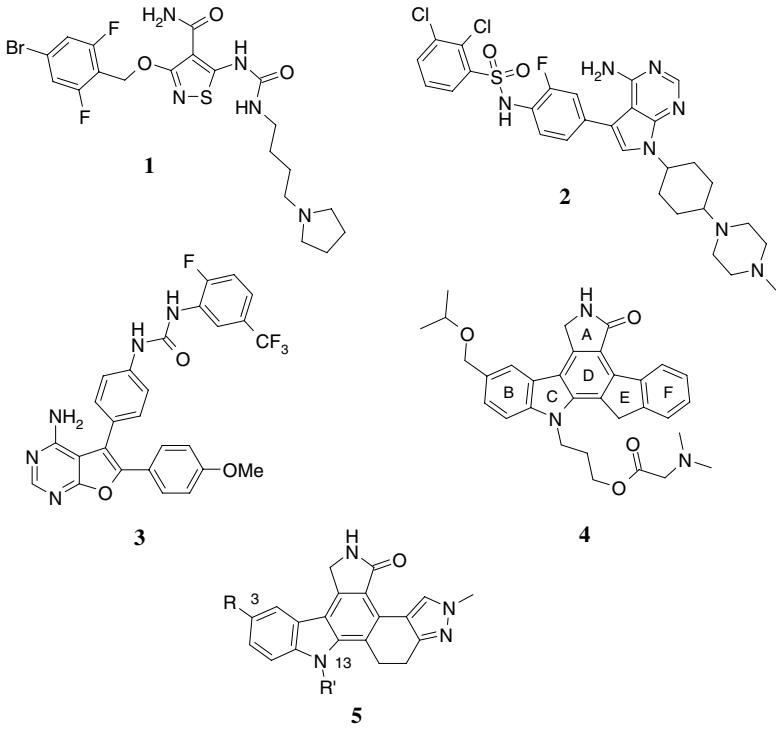
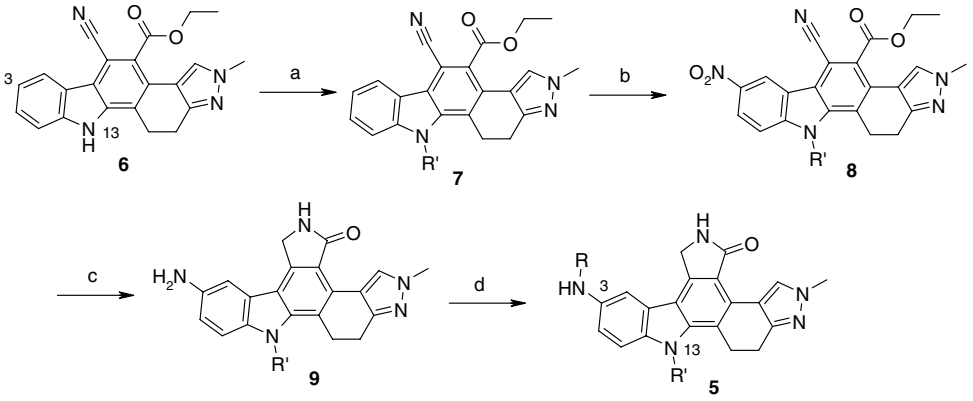


Figure 1.

potency and pharmacokinetic properties for in vivo evaluation. As a starting point, structural modification to the E- and F-rings identified the dihydroindazole core **5** (DHI) with improved TIE-2 activity (R, R′ = H; IC_{50} = 1.3 μM) and pharmacokinetic properties compared to the indenocarbazole core. This paper describes optimization of the C-3 and N-13 positions and the identification of potent *N*-amide, urea, and carbamate derivatives as potent dual inhibitors.

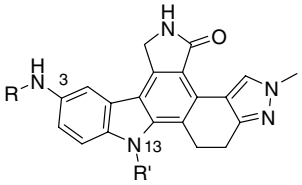
Synthesis of dihydroindazole scaffold **5** is shown in Scheme 1. The cyano-ester carbazole intermediate **6** was prepared utilizing our published patented procedure.[12] N-Alkylation of carbazole **6** with alkyl iodides and NaOH as base produced the N-alkylated intermediates **7** in good yield. Selective C-3 nitration to **8** was accomplished by careful control of the reaction conditions (2 equiv HNO_3, AcOH, 80 °C, 1 h). The aminolactam **9** was obtained in a one-pot reductive cyclization of **8** (Raney-Ni/H_2/DMF/MeOH). Reaction of the intermediate amines **9** with various isocyanates and carbonyl chlorides gave the target *N*-amide, urea, and carbamate compounds **5**.

Introduction of the 3-amino group dramatically improved the TIE-2 activity (Table 1). The *n*-propyl derivative **10** and *i*-butyl derivative **11** showed excellent dual inhibitory activity with IC_{50} values <50 nM. The *n*-butyl compound **12** displayed dual inhibition potencies that were 5- and 8-fold weaker against TIE-2 and VEGF-R2 kinases, respectively. Incorporation of a 4-methoxyphenyl urea substituent did not have a dramatic effect on the VEGF-R2 activity with the *n*-butyl group being the weakest compound (compare **10**–**11** with **13**–**15**). However, single digit nanomolar activity for TIE-2 was obtained regardless of the N-alkyl substitution. Compounds **13**–**15** met the program requirement for dual IC_{50} values <50 nM, however the cellular inhibition score for the ethyl analog **13** was 3 compared to a 4 for the *n*-propyl and *i*-butyl analogs. The cell activity was assessed using a VEGF-R autophosphorylation assay and scored as described previously (see footnote Table 1 for a description of scoring system).[12a,13] Thus, from these data the optimal alkyl groups for the N-13 nitrogen (R′) appeared to be *n*-propyl or *i*-butyl. Although compounds **14** and **15** displayed excellent enzyme and cellular inhibition, their poor pharmacokinetic (PK) properties (low oral AUC values, high clearance, and short half-life) prevented them from further advancement. Replacement of 4-methoxyphenyl (**14**) with 4-*S*-methylphenyl (**17**), 4-*N*,*N*-dimethylphenyl (**18**), 4-methylphenyl (**19**), or 2-thienyl urea (**20**) retained potent dual TIE-2/VEGF-R2 potencies, however their cellular activities were poor possibly due to low cell permeability resulting from the urea N–H group. Methylation of the outer N–H in phenyl urea **23** to give **24** improved the cell score from 2 to 4 while retaining the dual TIE-2/VEGF-R2 potency. The disubstituted 2-fluoro-5-methylphenyl urea derivative **21** displayed good dual TIE-2/VEGF-R2 activity with excellent cellular activity; however changing the methyl to a CF_3 (**22**) resulted in a 5-fold decrease in VEGF-R2 activity. Both steric and electronic effects on cellular activity could be observed in the phenyl urea SAR. An electron-donating *p*-methoxy group provided good cellular activity (**14**; cell score of 4). However, the cell activity decreased to a score of 1 as the methoxy group was moved from the *para*- to the *ortho*-position (**14**, **25**, and **26**). The reverse



Scheme 1. Reagents and conditions: (a) alkyl iodide, NaOH, acetone, reflux, 19 h, 87–93%; (b) NHO_3, AcOH, 80 °C, 1 h 70–87%; (c) H_2, Raney-Ni, DMF/MeOH 81–97% ; (d) carbonyl chloride or isocyanate, pyridine, CH_2Cl_2, 58 °C, 51–99%.

Table 1. TIE-2 and VEGF-R2 kinase inhibition data



Compound	R	R′	TIE-2[a] IC_{50} (nM)	VEGF-R2[a] IC_{50} (nM)	VEGF-R2[b] cell score
10	H	$CH_2CH_2CH_3$	36	9	N/D
11	H	$CH_2CH(CH_3)_2$	48	11	N/D
12	H	$CH_2CH_2CH_2CH_3$	230	88	N/D
13	4-OMe–phenylNHCO	CH_2CH_3	4	11	3
14	4-OMe–phenylNHCO	$CH_2CH_2CH_3$	2	16	4
15	4-OMe–phenylNHCO	$CH_2CH(CH_3)_2$	3	18	4
16	4-OMe–phenylNHCO	$CH_2CH_2CH_2CH_3$	7	101	N/D
17	4-SMe–phenylNHCO	$CH_2CH_2CH_3$	3	14	1
18	4-NMe_2–phenylNHCO	$CH_2CH_2CH_3$	6	24	2
19	4-Me–phenylNHCO	$CH_2CH_2CH_3$	3	8	2
20	2-ThienylNHCO	$CH_2CH_2CH_3$	19	21	0
21	2-F-5-Me–phenylNHCO	$CH_2CH_2CH_3$	1	29	4
22	2-F-5-CF_3–phenylNHCO	$CH_2CH_2CH_3$	4	159	N/D
23	PhenylNHCO	$CH_2CH_2CH_3$	5	16	2
24	Phenyl(Me)NCO	$CH_2CH_2CH_3$	2	7	4
25	3-OMe–phenylNHCO	$CH_2CH(CH_3)_2$	4	25	2
26	2-OMe–phenylNHCO	$CH_2CH(CH_3)_2$	8	41	1
27	4-F-phenylNHCO	$CH_2CH_2CH_3$	2	12	2
28	3-F-phenylNHCO	$CH_2CH_2CH_3$	2	12	2
29	2-F-phenylNHCO	$CH_2CH_2CH_3$	2	9	4
30	4-Cl-phenylNHCO	$CH_2CH_2CH_3$	6	48	2
31	2-Cl-phenylNHCO	$CH_2CH_2CH_3$	3	9	4
32	2-Br-phenylNHCO	$CH_2CH_2CH_3$	4	10	4

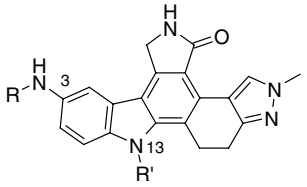
[a] The IC_{50} values were reported as the average of at least two separate determinations.

[b] The data were scored based on decrease in protein band density at 50 nM concentration compared to VEGF-stimulated control (no inhibitor) as follows: 0 = no decrease; 1 = 1–25%; 2 = 26–50%; 3 = 51–75%; 4 = 76–100%.[13]

trend was observed for the electron-withdrawing fluoro group where the cellular inhibition increased to a score of 4 as the fluoro group was moved from the *para*- to the *ortho*-position (**27**–**29**). Good cell activity was also observed for the 2-chloro (**31**) and the 2-bromo (**32**) ureas. Unfortunately, dual inhibitors **29**, **31**, and **32** displayed unacceptable pharmacokinetic properties in the rat.

To further address the PK issues, additional C-3 functionality was investigated. The SAR provided by **24** suggested that the outer urea N–H may not be required for dual TIE-2/VEGF-R2 activity. Moreover, since the cellular activity was greatly enhanced by blocking the outer N–H, additional series such as amides and carbamates were prepared and evaluated (Table 2). The 2-thienyl (**33**–**34**) and 2-furanyl (**35**–**36**) amides displayed good

Table 2. TIE-2 and VEGF-R2 kinase inhibition of the C-3 amino analogs of DHI



Compound	R	R′	TIE-2[a] IC_{50} (nM)	VEGF-R2[a] IC_{50} (nM)	VEGF-R2[b] cell score
33	2-ThienylCO	$CH_2CH_2CH_3$	12	4	4
34	2-ThienylCO	$CH_2CH(CH_3)_2$	10	7	4
35	2-FuranylCO	$CH_2CH_2CH_3$	29	3	4
36	2-FuranylCO	$CH_2CH(CH_3)_2$	20	5	4
37	4-OMe–phenylOCO	$CH_2CH_2CH_3$	5	12	4
38	4-F-phenylOCO	$CH_2CH_2CH_3$	11	46	4
39	*i*-PrOCO	$CH_2CH_2CH_3$	11	5	4
40	EtOCO	$CH_2CH_2CH_3$	18	2	4
41	PrOCO	$CH_2CH_2CH_3$	7	3	4

[a] The IC_{50} values were reported as the average of at least two separate determinations.
[b] The data were scored based on decrease in protein band density at 50 nM concentration compared to VEGF-stimulated control (no inhibitor) as follows: 0 = no decrease; 1 = 1–25%; 2 = 26–50%; 3 = 51–75%; 4 = 76–100%.[13]

dual TIE-2/VEGF-R2 activity along with excellent cellular activity (cell score of 4). Similarly, aryl (**37**–**38**) and alkyl (**39**–**41**) carbamates also displayed excellent dual inhibition and cellular activity.

Among these compounds, the isopropyl carbamate **39** showed good PK properties in the rat with good oral bioavailability (Table 3). The iv $t_{1/2}$ was 2.8 h with an $AUC_{0-\infty}$ of 1710 ng h/mL and a low clearance of 10.5 mL/min/kg. The oral bioavailability in the rat was estimated to be greater than 44% (based on 6 h AUC). Compound **39** was evaluated for dose-related oral anti-tumor efficacy against murine SVR angiosarcomas in nude mice. A significant anti-tumor efficacy was observed at oral doses of 0.3 mg/kg and 1.0 mg/kg BID beginning at day 4 and extending to day 10 of the study with a maximum inhibition of 62%. However, at higher doses toxicity was observed precluding **39** from further studies and advancement.[14] Based on this finding, chemistry efforts were prioritized to other series to address the toxicity and metabolic stability of the side-chain, which will be reported in due course.

Table 3. PK data for compound **39**

iv 1 mg/kg	
$t_{1/2}$ (h)	2.84
AUC_{0-t} (ng h/mL)	1423
$AUC_{0-\infty}$ (ng h/mL)	1710
V (L/kg)	2.34
CL (mL/min/kg)	10.5
po 5 mg/kg	
t_{max} (h)	4.00
AUC_{0-t} (ng h/mL)	2416
$AUC_{0-\infty}$ (ng h/mL)	3769
C_{max} (ng/mL)	552
Bioavailability (estimated)	>44%

In conclusion, we report on a novel class of C-3 urea, amide, and carbamate fused dihydroindazolocarbazole analogs as highly potent dual inhibitors of TIE-2 and VEGF-R2 receptor tyrosine kinases with excellent cellular potency. The SAR of the urea compounds revealed that optimal alkyl N-13 substitution was *n*-propyl or *i*-butyl. The outer urea N–H function was not required for dual potency, leading to the synthesis of a series of amide and carbamate analogs, also displaying potent dual activity. The isopropyl carbamate **39** was identified as a potent dual inhibitor (VEGF-R2 IC_{50} = 5 nM; TIE-2 IC_{50} = 11 nM) with good cell potency and rat pharmacokinetic properties for in vivo evaluation.

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13. Compounds with IC_{50} values $\leqslant$50 nM in both TIE-2 and VEGF-R2 enzyme inhibition were further tested for their cellular inhibition against VEGF-R2. Score = 1: 0–25%; 2: 26–50%; 3: 51–75%; 4: 76–100% inhibition.
14. Gastrointestinal hemorrhaging through the entire tract was observed at 10 mg/kg dose.