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Optimization of the indolyl quinolinone class of KDR (VEGFR-2) kinase inhibitors: effects of 5-amido- and 5-sulphonamido-indolyl groups on pharmacokinetics and hERG binding

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Abstract—Modifications to the basic side-chain of early lead structures of the indolyl quinolinone class of KDR kinase inhibitors resulted in improved pharmacokinetic and ancillary profiles. Specifically, compounds bearing 5-amido- and 5-sulphonamido-indolyl substituents exhibited lower plasma clearance and weaker binding affinity for the I_{Kr} potassium channel hERG. ©2003 Elsevier Science Ltd. All rights reserved.

Angiogenesis, the formation of new capillaries from established blood vessels, is an essential process for normal growth and development that has also been implicated in the pathogenesis of several diseases including diabetic retinopathy,[1] rheumatoid arthritis,[2] osteoarthritis,[3] psoriasis,[4] and cancer.[5] Specifically, the growth and metastasis of solid tumors has been shown to be dependent on angiogenesis at an early stage,[6] while tumors that lack adequate vascularization become necrotic or apoptotic and do not grow beyond a limited size.[7] Interest in inhibition of angiogenesis as a new approach for the treatment of cancer has led to the elucidation of key underlying molecular mechanisms that control the angiogenic process. Angiogenesis is regulated by the expression of a variety of growth factors including vascular endothelial growth factor (VEGF), a selective mitogen for endothelial cells whose mitogenic signaling is mediated through the receptor tyrosine kinase KDR (VEGFR-2).[8] Several lines of evidence indicate that expression and signaling of VEGF are critical for tumor angiogenesis. Among these, antibodies against VEGF[9] and its receptor KDR,[10] as well as small molecule inhibitors of KDR kinase activity,[11] have been shown to inhibit angiogenesis in tumor xenograft models. Importantly, bevacizumab (Avastin; Genentech, Inc.), a chimeric anti-VEGF monoclonal antibody, prolonged the survival of patients with colorectal cancer when administered in combination with standard chemotherapy, providing valuable proof-of-concept in a clinical setting.[12] KDR kinase inhibitors derived from a number of different structural classes, including indolinones (SU5416 and SU11248), quinazolines (ZD4190 and ZD6474), phthalazines (PTK-787), and isothiazoles (CP-547,632), have progressed to the clinical evaluation stage.[13]

We recently reported the discovery and evolution of the indolyl quinolinone class of KDR kinase inhibitors (Fig. 1).[14] Briefly, the benzoannulenone core of screening lead **1** was replaced with the more chemically stable 2-quinolinone ring system of **2** to provide an 8-fold improvement in KDR kinase activity,[15] a gain that we attributed to the additional hydrogen bonding interaction formed between the backbone and the quinolinone NH. Potency was further optimized by substitution of the benzimidazole moiety with an indolyl nucleus.

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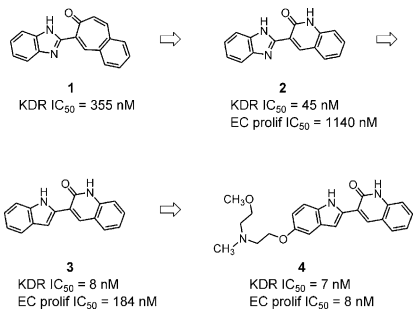
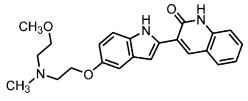


Figure 1. Evolution of the indolyl quinolinone class.

Introduction of a basic side chain via an ether linkage at the 5-position of the indolyl ring system, as in **4**, provided compounds that exhibited improved physical properties and excellent cellular activity.[16]

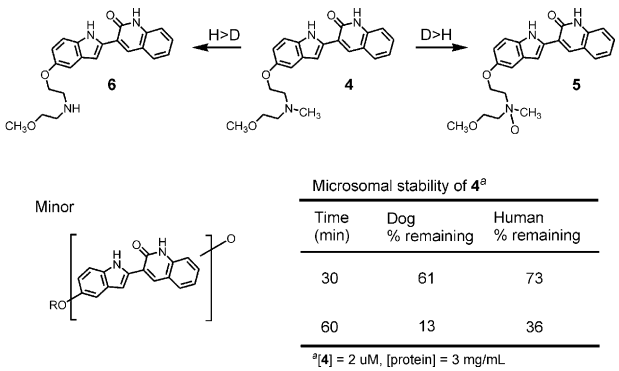
Compound **4** served as an assay and target validation tool for our program. Oral dosing of **4** to HT1080 xenografted mice inhibited tumor growth in a dose-proportional manner.[17] Satisfyingly, tumor growth inhibition correlated with systemic exposure, in vivo inhibition of KDR phosphorylation in mouse lung, and inhibition of tumor angiogenesis. Thus, with the identification of **4** as a potent and efficacious in vivo inhibitor of KDR kinase, we turned our attention to the optimization of pharmacokinetics as well as the identification of ancillary activity that might limit chronic oral dosing.

Table 1. Pharmacokinetics of **4** in rat and dog



Species	Cl (mL/min/kg)	$t_{1/2}$ (h)	Vdss (L/kg)	C_{max} (nM)	%F
Rat[a]	56	1.4	5.0	470	20
Dog[b]	> 50	< 1.0	N.D.	N.D.	N.D.

N.D., not determined
[a] Dosed 2 mpk iv (DMSO), 10 mpk po (1.0% methyl cellulose, pH 5).
[b] Dosed 0.25 mpk iv (DMSO) in a cocktail of six compounds.



Microsomal stability of **4**[a]

Time (min)	Dog % remaining	Human % remaining
30	61	73
60	13	36

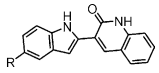
[a][4] = 2 uM, [protein] = 3 mg/mL

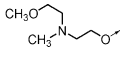
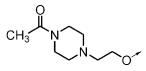
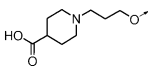
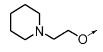
Figure 2. Metabolic profile of **4** in human and dog liver microsomes.

Compound **4** exhibited high plasma clearance in rat and dog (Table 1). Human and dog liver microsome stability studies indicated that although the relative stability between the two species was similar, the metabolic profile in each system was distinct (Fig. 2). In dog liver microsomes, *N*-oxidation predominated furnishing **5**, while in the human preparation *N*-demethylation was the major metabolic pathway giving rise to **6**. Encouragingly, metabolism appeared primarily limited to the side chain with only minor amounts of core oxidation products detected. These results suggested that the core was stable relative to the side chain and that modifications to the side chain might lead to reductions in plasma clearance.

With regard to ancillary activity, **4** exhibited moderate binding to the I_{Kr} potassium channel hERG (human *Ether-a-go-go* Related Gene) with an inflection point (IP) of 1.9 uM.[18] Blockade of hERG has been implicated in the drug-induced alteration of cardiac ventricular repolarization that is typically manifested in prolongation of

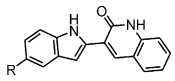
Table 2. Properties of selected compounds of the ether series

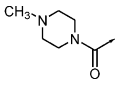
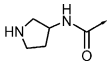


Compd	R	KDR IC_{50} (nM)/ Cell Prolif IC_{50} (nM)	LogP	Dog Cl (mL/min/kg)	$t_{1/2}$ (h)	hERG IP (nM)/ QTc EC_{10} (nM)
4[a]		7/8	3.1	> 50	< 1.0	1950/1900
7[b]		4/4	3.2	7.8	2.2	520
8[b]		8/41	0.9	7.7	3.2	3050/5200
9[a]		4/17	3.7	> 50	1.3	980/640

[a] Dosed 0.25 mpk iv (DMSO) in a cocktail of six compounds.
[b] Dosed 1.0 mpk iv (DMSO) as a single.

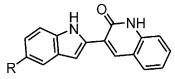
Table 3. Properties of selected compounds of the 5-amido series

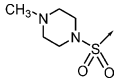
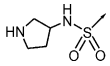


Compd	R	KDR IC_{50} (nM)/ cell IC_{50} (nM)	LogP	Dog Cl (mL/min/kg)	$t_{1/2}$ (h)	hERG IP (nM)
10[b]		5/4	2.6	19	2.4	10,800
11[a]		5/4	1.7	27	4.2	> 10,000
12[a] *(rac)*		5/4	N.D.	26	1.1	> 10,000
13[a] *(rac)*		6/53	N.D.	38	1.7	13,700

[a] Dosed 0.25 mpk iv (DMSO) in a cocktail of six compounds.
[b] Dosed 1.0 mpk iv (DMSO) as a single.

Table 4. Properties of selected compounds of the 5-sulphonamido series



Compd	R	KDR IC_{50} (nM)/ cell IC_{50} (nM)	LogP	Dog Cl (mL/min/kg)	$t_{1/2}$ (h)	hERG IP (nM)
14[b]		10/31	3.0	0.5	6.4	3440
15[b]		11/30	2.3	3.5	12.7	2450
16[a] *(rac)*		16/27	N.D.	11	3.4	2650
17[a] *(rac)*		14/97	N.D.	0.4	9.5	2835

[a] Dosed 0.25 mpk iv (DMSO) in a cocktail of six compounds.
[b] Dosed 1.0 mpk iv (DMSO) as a single.

the heart rate-corrected QT interval (QT_c).[19] The ability of **4** to bind to hERG was corroborated by an electrophysiologic, rising-dose infusion study in anesthetized dogs which demonstrated that **4** caused 10% QT_c interval prolongation at a plasma concentration of 1.9 uM. Because prolongation of the QTc interval may potentiate cardiac arrhythmia in patients and lead to torsades de points or sudden death, we closely monitored hERG binding affinity during our optimization efforts.

Attempts to optimize both plasma clearance and I_{Kr} profile simultaneously in the ether series yielded only limited success (Table 2). For example, although compound **7** exhibited improved plasma clearance, the level of hERG binding remained a potential issue for this compound. Interestingly, compound **8** showed lower clearance and hERG activity compared to analogue **9**, perhaps as a result of its greater polarity.[20] We hypothesized at this juncture that further optimization of pharmacokinetics and reduction in hERG binding affinity would require a design strategy focused on increasing the polarity of this inhibitor class. Initially, we chose to investigate a 5-amido group as a linkage between the indolyl core and requisite basic amine. Gratifyingly, this modification resulted in more polar, equipotent compounds that exhibited reduced binding affinity for hERG (Table 3).

While compounds **10**–**13** showed moderate to high plasma clearance in dogs, the corresponding analogues in the 5-sulphonamido series exhibited low clearance and long half-lives (Table 4). However, the improve-

Table 5. Pharmacokinetics of **10** and **14** in rat, dog, and monkey

Compd	Species	Cl (mL/min/kg)	$t_{1/2}$ (h)	Vdss (L/kg)	C_{max} (nM)	%F
10	rat[a]	3.8	2.3	0.7	5790	31
14	rat[a]	29	1.1	0.9	4800	54
10	dog[b]	19	2.4	2.9	160	15
14	dog[b]	0.5	6.4	0.3	5200	63
10	monkey[b]	3.4	2.8	0.9	1250	46
14	monkey[b]	12	3.4	2.1	630	40

[a] Mesylate salt dosed 2 mg/kg iv (DMSO), 10 mg/kg po (0.05 M citric acid).
[b] Mesylate salt dosed 1 mg/kg iv (DMSO), 1 mg/kg po (0.05 M citric acid).

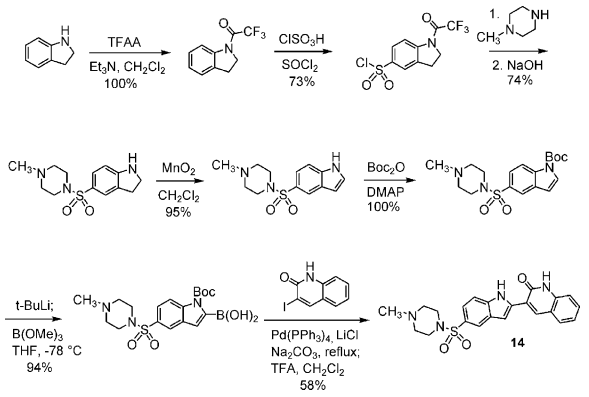


Figure 3. Synthesis of **14**.

ment in pharmacokinetics with the 5-sulphoamido linker came at the expense of decreased KDR kinase activity and greater binding affinity for hERG. The difference in hERG activity between the two series may be related, in part, to their relative lipophilicity; the logP values of sulphonamides **14** and **15** measured approximately one-half log unit higher than their direct amide counterparts **10** and **11**.

The oral pharmacokinetic profiles for compounds **10** and **14** in rat, dog, and monkey are found in Table 5. Sulphonamide **14** showed greater bioavailability in rat and dog than did amide **10**. That amide **10** exhibited moderate plasma clearance relative to hepatic blood flow in the dog suggested that the low bioavailability in this species was due to a combination of incomplete absorption and first-pass hepatic elimination.

The synthesis of **14** is depicted in Figure 3. Key steps included the sulfonylation reaction of 1-(trifluoroacetyl)indoline[21] and the Suzuki cross-coupling reaction between the intermediate 2-indolyl boronic acid and 3-iodo-2-quinolinone[22] to furnish the indolyl quinolinone ring system.

In summary, exchange of the ether linkage in our first generation KDR kinase inhibitors of the indolyl quinolinone class for 5-amido and 5-sulfonamido tethers provided potent compounds that exhibited more favorable pharmacokinetic properties and potentially safer ancillary profiles with lower binding affinity for the I_{Kr} potassium channel hERG.

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