Bioorganic & Medicinal Chemistry Letters 18 (2008) 4054–4058

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of novel 1,2,3,4-tetrahydroisoquinolines and 3,4-dihydroisoquinoline-1(2*H*)-ones as potent and selective inhibitors of KDR: Synthesis, SAR, and pharmacokinetic properties

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ARTICLE INFO

Article history:
Received 21 April 2008
Revised 29 May 2008
Accepted 29 May 2008
Available online 4 June 2008

Keywords:
KDR
VEGFR-2
1,2,3,4-Trahydroisoquinolines
3,4-Dihydroisoquinoline-1(2*H*)-ones

ABSTRACT

1,2,3,4-Tetrahydroisoquinolines and 3,4-dihydroisoquinoline-1(2*H*)-ones were identified as potent and selective inhibitors of KDR. The discovery, synthesis, and structure–activity relationships of these novel inhibitors are reported. In vitro metabolism and pharmacokinetic profiles of the most interesting compounds are discussed.

Inhibition of the vascular endothelial growth factor (VEGF) pathway has proven to be a valuable approach to reduce tumor growth by limiting the formation of new capillaries from existing vasculature (angiogenesis).[1] Endothelial cell proliferation and migration, two critical steps in angiogenesis, are mediated through a specific VEGF receptor, VEGFR-2, also referred to as the Kinase insert Domain containing Receptor (KDR). Inhibition of KDR kinase activity by small molecules offers an effective therapeutic approach to treating human tumors in the clinic.[2]

We have previously reported the design, synthesis, and evaluation of a series of *N*-aryl naphthamides.[3] This scaffold afforded low nanomolar inhibitors of KDR and led to the discovery of **1** (Fig. 1). Although efficacious in xenograft tumor models, the physicochemical properties of **1** such as low aqueous solubility and high lipophilicity (Table 1) were not optimal.

Having identified compound **1** as a potent and selective inhibitor of KDR, our primary goal was to design an alternative scaffold that would simultaneously increase the overall polarity of the molecule and occupy the same chemical space as the naphthoyl group. Our strategy was based on the incorporation of a solubilizing group within the core of the molecule, thereby preserving the relatively small size of the inhibitor while imparting more favorable physicochemical properties. To implement this strategy, we chose to modify the A-ring of the naphthoyl core (Fig. 1).

Herein, we describe the synthesis, structure–activity relationships, and pharmacokinetic properties of 1,2,3,4-tetrahydroisoquinolines (**2**, Fig. 1) and 3,4-dihydroisoquinoline-1(2*H*)-ones (**3**, Fig. 1), two novel classes of potent inhibitors of KDR.

A convergent and scalable synthesis for the construction of the 1,2,3,4-tetrahydroisoquinoline and 3,4-dihydroisoquinoline-1(2*H*)-one carboxamides was developed which allowed efficient analoging of the amide portion of these molecules.

The general synthesis for the preparation of the 1,2,3,4-tetrahydroisoquinoline carboxamides is described in Scheme 1. Penultimate intermediate **8** was prepared from commercially available

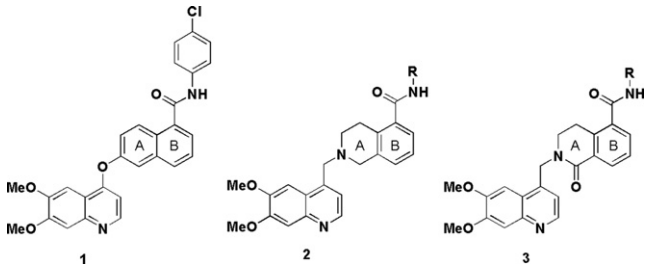

Figure 1. Generation of 1,2,3,4-tetrahydroisoquinoline and 3,4-dihydroisoquinoline-1(2*H*)-one leads.

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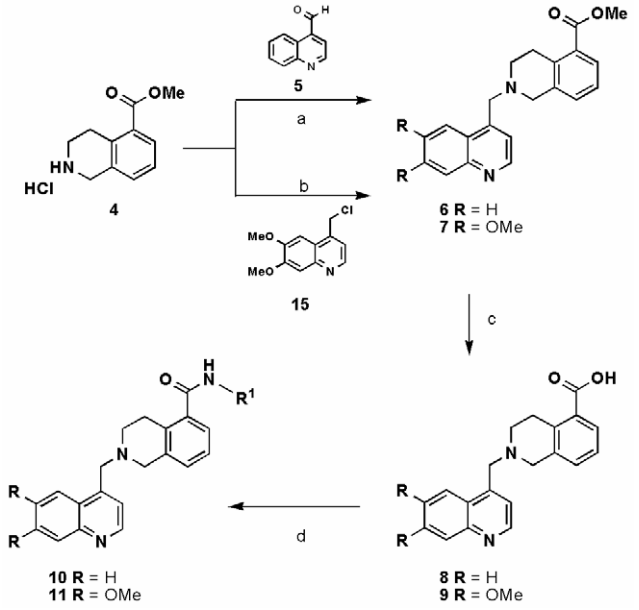
doi:10.1016/j.bmcl.2008.05.114

Table 1
Potency and physicochemical properties of **1**

Compound	IC_{50} (nM)			*c*Log*P*[a]	SIF sol.[b] (μg/mL)
	KDR	HUVEC (VEGF)	HUVEC (FGF)		
1	0.5	7.6	>1140	7.02	1.4

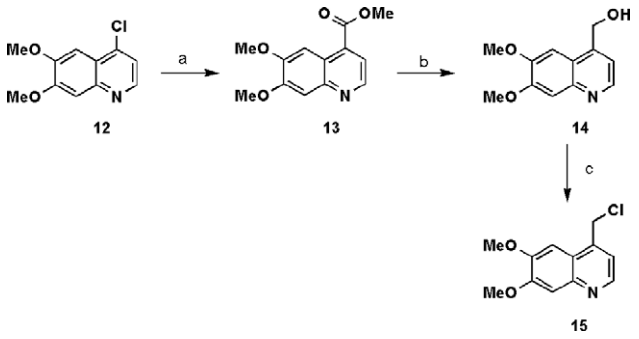
[a] Determined using ACDLabs 8.0 (daylight *c*Log*P*).
[b] SIF sol: simulated intestinal fluid solubility assay.[4]



Scheme 1. Synthesis of 1,2,3,4-tetrahydroisoquinoline carboxamides. Reagents: (a) $NaBH(OAc)_3$, Et_3N, AcOH, THF, 56–82%; (b) NaI, Et_3N, DMF, 89%; (c) 2 N aq NaOH, dioxane/H_2O, 91%; (d) R^1NH_2, HOAt, EDCI, *i*-Pr_2EtNH, DMF, 41–92%.

methyl 1,2,3,4-tetrahydroisoquinoline-5-carboxylate hydrochloride **4** and quinoline-4-carbaldehyde **5** via standard reductive amination conditions followed by saponification of methyl ester **6**. In the case of analogous intermediate **9**, the preparation was achieved via alkylation of **4** with 4-chloromethyl-6,7-dimethoxyquinoline **15** in the presence of sodium iodide followed by saponification with aqueous sodium hydroxide in 81% yield over two steps. Subsequent coupling of acids **8** and **9** with various amines afforded carboxamides **10** and **11** in moderate to good yield.

The 4-chloromethyl-6,7-dimethoxyquinoline intermediate **15** was prepared as described in Scheme 2.[5] Commercially available 4-chloro-6,7-dimethoxyquinoline **12** was converted in high yield



Scheme 2. Synthesis of 4-chloromethyl-6,7-dimethoxyquinoline. Reagents: (a) $Pd(OAc)_2$, 1,3-bis(diphenylphosphino)propane, Et_3N, MeOH, CO (atm), DMF, 85 °C, 62–88%; (b) LAH, THF, −78 °C, 87%; (c) thionyl chloride, 97%.

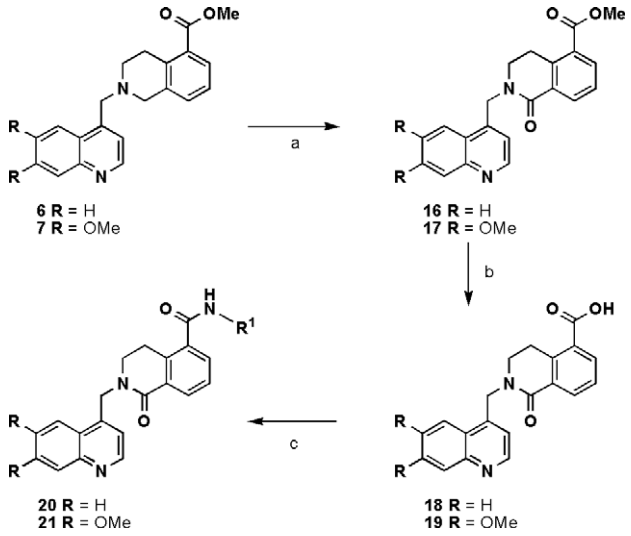
to the corresponding methyl 6,7-dimethoxyquinoline-4-carboxylate **13** through palladium-catalyzed carbonylation. Subsequent reduction of methyl ester **13** with lithium aluminum hydride followed by reaction with thionyl chloride afforded the key 4-chloromethyl-6,7-dimethoxyquinoline intermediate **15** in 84% yield over two steps.

The corresponding 3,4-dihydroisoquinoline-1(2*H*)-ones were prepared from advanced intermediates **6** and **7** as illustrated in Scheme 3. Regioselective oxidation of **6** and **7** was achieved in good yield by reaction with potassium permanganate in the presence of 18-crown-6 to afford 3,4-dihydroisoquinoline-1(2*H*)-ones **16** and **17**.[6] Subsequent saponification to generate key acid intermediates **18** and **19** followed by coupling with various amines provided final compounds **20** and **21**.

Initial investigation of the 1,2,3,4-tetrahydroisoquinoline core as a replacement for the naphthoyl moiety was accomplished using the commercially available unsubstituted quinoline **5**.

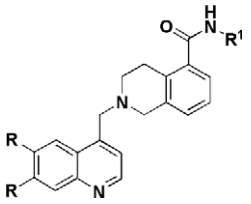
Analogs **22** and **23** (Table 2) exemplify compounds in this series and exhibited encouraging enzyme and cell (VEGF-driven HUVEC proliferation) potency. Additionally, both compounds demonstrated good selectivity at the cellular level as indicated by the lack of activity in the FGF proliferation assay.[7] Having established that the 1,2,3,4-tetrahydroisoquinoline core was tolerated and provided a significant increase in aqueous solubility (SIF > 15×)[4] over compound **1**, we incorporated the 6,7-dimethoxyquinoline moiety as a means to further increase enzyme and cellular potency, a strategy successfully exploited earlier in our KDR program.[3,8,9]

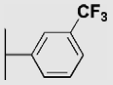
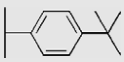
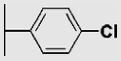
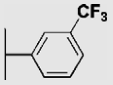
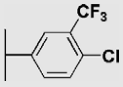
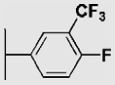
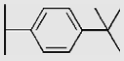
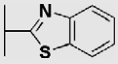
Indeed, as indicated in Table 2, compounds **25** and **28** exhibited superior enzyme and cell potency relative to compounds **22** and **23**. Analysis of the SAR revealed that the more potent compounds had the highest lipophilicity (*c*Log*P* > 6) values. Based on the reported X-ray structure of **1** bound to KDR,[3] we hypothesized that the 1,2,3,4-tetrahydroisoquinoline core was less effective in occupying the mostly hydrophobic pocket of the ATP binding cavity due to its generally lower lipophilicity. As a result, introduction of hydrophobicity into the amide residue which binds to the extended lipophilic pocket was important in maximizing inhibitor efficiency. Attempts to introduce smaller and more polar amide substituents (**31** and **32**) led to significant decreases in enzyme potency. Heteroarylamides were also introduced (**29** and **30**) in an attempt to more subtly incorporate polarity within this group.



Scheme 3. Synthesis of 3,4-dihydroisoquinoline-1(2*H*)-one carboxamides. Reagents: (a) 18-crown-6, $KMnO_4$, CH_2Cl_2, 70–76%; (b) 2 N aq NaOH, dioxane/H_2O, 89%; (c) R^1NH_2, HOAt, EDCI, *i*-Pr_2EtNH, DMF, 57–92%.

Table 2
SAR of amide group variations in the 4-((3,4-dihydroisoquinolin-2(1*H*)-yl)methyl)quinolines and 4-((3,4-dihydroisoquinolin-2(1*H*)-yl)methyl)-6,7-dimethoxyquinolines[a]



Compound	R	R^1	IC_{50} (nM)			*c*Log*P*[b]	SIF sol.[c] (μg/mL)
			KDR	HUVEC (VEGF)	HUVEC (FGF)		
22	H		4	21	>1140	5.76	26
23	H		8	50	>1140	6.25	15
24	OMe		165	—	—	5.63	12
25	OMe		1	7	>1140	6.00	14
26	OMe		1	12	>1140	6.60	36
27	OMe		1	5	>1140	6.23	55
28	OMe		1	6	>1140	6.49	34
29	OMe		13	68	>1140	5.49	24
30	OMe		225	—	—	3.99	—
31	OMe		92	—	—	3.23	173
32	OMe		3000	—	—	2.93	>200

[a] IC_{50} values were averaged values determined by at least two independent experiments.
[b] Determined using ACDLabs 8.0 (daylight *c*Log*P*).
[c] SIF sol: simulated intestinal fluid solubility assay.[4]

Nevertheless, these compounds had moderate enzyme and cell potency.

Having identified 1,2,3,4-tetrahydroisoquinoline compounds with good enzyme and cell potency, we examined the in vitro metabolic stability of a few select inhibitors. Analysis of the data indicated that compound **27** had reasonable stability in both rat and human liver microsomes (Table 3).

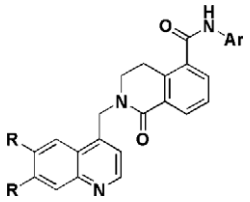
In an effort to better understand and ultimately improve the metabolic stability of these inhibitors, metabolite identification studies were performed on compound **22**.[11] In both rat and human liver microsomes, the major metabolite observed was derived from oxidation of the piperidine A-ring of the 1,2,3,4-tetrahydroisoquinoline core. Minor metabolites resulting from oxidation on the quinoline ring and the aryl ring of the amide were also observed.

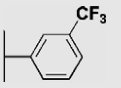
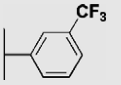
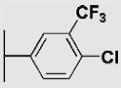
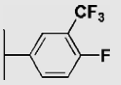
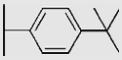
With the insight gained from the metabolite identification study, we incorporated additional polarity into the piperidine A-ring of the 1,2,3,4-tetrahydroisoquinoline core and simultaneously blocked one potential site of oxidation with the preparation of the 3,4-dihydroisoquinoline-1(2*H*)-ones (Table 4). The introduction of a carbonyl group into the piperidine A-ring was generally well tolerated. In particular, the 3-trifluoromethylphenyl amides **34** and **36** maintained good potency especially at the cellular level. Additionally, the 3,4-dihydroisoquinoline-1(2*H*)-ones

Table 3
In vitro metabolic stability of selected 1,2,3,4-tetrahydroisoquinolines and 3,4-dihydroisoquinoline-1(2H)-ones in rat (RLM) and human liver microsomes (HLM)[10]

Compound	CL_{int} (μL/min/mg)	
	RLM	HLM
22	284	432
27	120	279
33	350	439
36	115	298

Table 4
SAR of aryl variations on the amide group in the 3,4-dihydroisoquinoline-1(2*H*)-one series[a]



Compound	R	Ar	IC_{50} (nM)			*c*Log*P*[b]	SIF sol.[c] (μg/mL)
			KDR	HUVEC (VEGF)	HUVEC (FGF)		
33	H		18	15	>1140	4.99	27
34	OMe		3	6	>1140	5.23	43
35	OMe		3	22	>1140	5.78	24
36	OMe		2	7	>1140	5.41	13
37	OMe		8	19	>1140	5.96	39

[a] IC_{50} values were averaged values determined by at least two independent experiments.
[b] Determined using ACDLabs 8.0 (daylight *c*Log*P*).
[c] SIF sol: simulated intestinal fluid solubility assay.[4]

Table 5
Pharmacokinetic parameters for compounds **27** and **36** following iv and oral dosing in male Sprague–Dawley rats

Compound	iv pk[a]			po pk[b]			
	Cl (L/h kg)	V_{ss} (L/kg)	$T_{1/2}$ (h)	$AUC_{0-\infty}$ (ng h/mL)	C_{max} (ng/mL)	$T_{1/2}$ (h)	*F* (%)
27	1.4	10.3	7.4	1456	204	7.7	103
36	2.0	4.6	2.8	235	53	2.1	24

[a] Dosed at 0.5 mg/kg as a solution in 100% DMSO.
[b] Dosed at 2 mg/kg as a solution in 100% OraPlus.

demonstrated excellent selectivity in the cellular HUVEC proliferation assay[7] and maintained the improved aqueous solubility observed in the 1,2,3,4-tetrahydroisoquinoline series.

With potent compounds in hand, the in vitro metabolic stability of select 3,4-dihydroisoquinoline-1(2*H*)-ones (**33** and **36**) was assessed in both rat and human liver microsomes (Table 3). Despite increased polarity and removal of a possible site of oxidation, the rate of metabolism in rat and human liver microsomes did not improve.

Given the modest in vitro metabolism observed for compounds **27** and **36**, we chose to evaluate the in vivo pharmacokinetic profiles of both compounds (Table 5). When dosed intravenously (iv) in rats, **27** exhibited moderate clearance and a high volume of distribution resulting in a long half-life. Compound **27** also demonstrated excellent bioavailability and good exposure levels when dosed orally. Conversely, compound **36** had elevated clearance and much lower exposure levels, indicating that addition of the carbonyl group was not an effective strategy for improving the pharmacokinetic parameters.

In summary, we have reported the discovery of novel 1,2,3,4-tetrahydroisoquinolines and 3,4-dihydroisoquinoline-1(2*H*)-ones as potent inhibitors of KDR. From this set of compounds, we further identified 1,2,3,4-tetrahydroisoquinoline **27** as a potent and selective[12] inhibitor of KDR with improved solubility (SIF sol 55 μg/mL) over naphthamide **1** (SIF sol 1.4 μg/mL). Compound **27** exhibited reasonable pharmacokinetic properties in rodents which would allow for further evaluation of this class of inhibitors in in vivo pharmacology studies. Moreover, compound **27** serves as a lead compound suitable for additional structural optimization, primarily to improve metabolic stability both in vitro and in vivo.

Acknowledgments

We are grateful to our colleagues Loren Berry for generating in vitro metabolism data, Nicholas Doerr for generating enzyme and cell data, and Van Luu for conducting aqueous solubility studies.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.05.114.

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4. A Symyx solubility system (Santa Clara, CA) was used for this measurement. The solubility media were fasted state simulated intestinal fluid (SIF), pH 6.8, containing 5 mM sodium taurochol, 1.5 mM lecithin, 2.9 mM KH_2PO_4, and 0.22 M KCl.
5. See Supplemental material for experimental details regarding the preparation of 4-chloromethyl-6,7-dimethoxyquinoline **15**.
6. See Supplemental material for experimental details regarding the regioselective oxidation of **6** and **7**.
7. Cellular potency was evaluated by determining the inhibition of human umbilical vein endothelial cell (HUVEC) proliferation induced by vascular endothelial growth factor (VEGF). In addition, the HUVEC proliferation assay was used to assess selectivity at the cellular level comparing on-target inhibition of VEGF driven proliferation and off-target fibroblast growth factor (FGF) driven proliferation. Although both growth factors, VEGF and FGF, induce HUVEC proliferation, each operates through a different signaling pathway. For a detailed description of the KDR enzyme and cellular assays, see Ref. 3.
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9. Weiss, M. M.; Harmange, J.-C. ; Polverino, A. J.; Bauer, D.; Berry, L.; Berry, V.; Borg, G.; Bready, J.; Chen, D.; Choquette, D.; Coxon, A.; DeMelfi, T.; Doerr, N.; Estrada, J.; Flynn, J.; Graceffa, R. F.; Harriman, S. P.; Kaufman, S.; La, D.; Long, A.; Neervannan, S.; Patel, V.; Potashman, M.; Regal, K.; Roveto, P.; Schrag, M.; Starnes, C.; Tasker, A.; Teffera, Y.; Whittington, D.; Zanon, R. *J. Med. Chem.* **2008**, *51*, 1668.
10. Intrinsic clearance values were determined using 0.25 mg microsomal protein/mL, 1 mM NADPH, and 1 μM test compound in 50 mM potassium phosphate buffer. Samples were collected at 0, 10, 20, 30, and 40 min. Intrinsic clearance was determined from the half-life.
11. Liver microsomal (HLM, RLM) incubations were conducted using 1 mg/mL protein, 10 μM of test compound at 37 °C for 60 min, with or without NADPH (1 mM).
12. Compound **27** exhibits >100× selectivity against the following kinases: Aurora 1, Aurora 2, BTK, c-Met, JAK3, and PI3Kδ. In addition, compound **27** demonstrates >10× selectivity against c-FMS, TIE2, and LCK.