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Discovery of a novel and potent series of dianilinopyrimidineurea and urea isostere inhibitors of VEGFR2 tyrosine kinase

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Abstract—A series of dianilinopyrimidineureas demonstrate potency as VEGFR2 kinase inhibitors.
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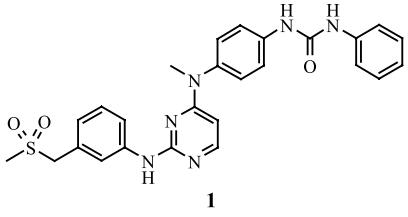
1. Introduction

Angiogenesis is the process of forming new blood vessels from existing ones and is tightly controlled by the balance of a number of angiogenic growth factors and endogenous inhibitors.[1] Disruption of this balance is thought to have implications for a number of diseases including diabetic retinopathy and macular degeneration,[2] rheumatoid arthritis,[3] coronary artery disease[4], and stroke.[5] Furthermore, angiogenesis is required for the establishment and maintenance of solid tumors.[6] It has been postulated that inhibition of angiogenesis through the blockade of the tyrosine kinase VEGFR2 (FLK-1/KDR) signaling pathway could starve developing cancers of required blood flow by limiting vasculature to the growth site.[7] This approach is supported by recent phase III clinical data from combination studies with Avastin™, the monoclonal antibody to VEGF, and standard chemotherapy.[8]

Clinical validation for VEGF as an antitumor target has generated considerable interest in developing inhibitors of the VEGFR2 kinase.[9] We have investigated a series of dianilinopyrimidine ureas and urea isosteres[10] (Fig. 1). These compounds are low nanomolar inhibitors of the VEGFR2 enzyme and have anti-proliferative activity on human umbilical vein endothelial cells[11] (HUVECs). In addition, we have used homology modeling to identify key residues in the ATP-binding site that we propose are responsible for optimal interaction with our inhibitors. This urea series was discovered during our comprehensive exploration of the dianilinopyrimidine scaffold as a kinase inhibitor.[12] In this paper, we report SAR for our urea and urea isostere series.



1

Figure 1.

All of the ureas and isosteres presented here were synthesized by acylation of aniline **6** (see Scheme 1). The synthesis of **6** started with the displacement of the 4-chloro group of 2,4-dichloropyrimidine with the acetamide-protected dianiline **2**. The resulting aminopyrimidine **3** was methylated with methyl iodide and cesium carbonate. The methylated adduct **4** was heated to reflux in isopropanol with substituted aniline **10a** and a catalytic amount of hydrochloric acid to produce the protected dianilinopyrimidine intermediate **5**. Aniline **6** was revealed by heating **5** in 6 N hydrochloric acid.

Keywords: VEGFR2; FLK-1; KDR; Kinase inhibitor.
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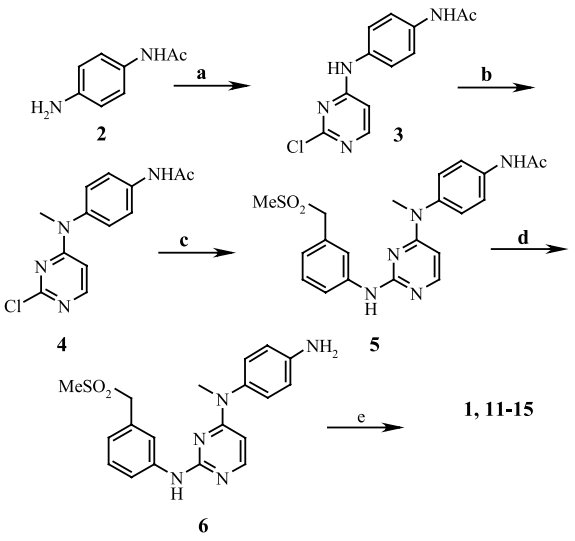
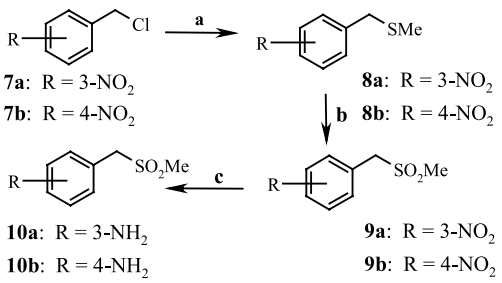


Scheme 1. Reagents and conditions: (a) 2, 4-dichloropyrimidine, $NaHCO_3$, 1:3 THF/EtOH, 75 °C, 95%; (b) MeI, Cs_2CO_3, DMF, 84%; (c) **10a**, $HCl_{(cat.)}$, *i*-PrOH, 80 °C; (d) 6 N HCl, 90 °C, 4 h, 67% over steps (c and d); (e) PhNCO, DMF, 26%; phenylacetylchloride, DMF, 21%; phenylchloroformate, DMF, 69%; phenylthioisocyanate, 33%; α-toluenesolfonylchloride, DMF, 21%; **13**, ammonium hydroxide (30% in water), sodium periodate, 80 °C, 39%.

Compounds **1** and **11**–**14** were prepared by acylation of **6** with phenylisocyanate, phenylacetylchloride, phenylchloroformate, phenylthioisocyanate, and α-toluenesulfonyl chloride, respectively. Guanidine derivative **15** was prepared by heating **13** with aqueous ammonium hydroxide and sodium periodate.[13]

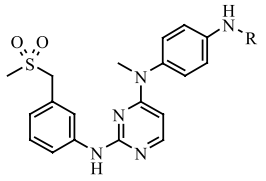
Synthesis of the sulfone aniline tails was achieved starting from the commercially available 3- or 4-nitrobenzyl chloride (Scheme 2). Chloromethylnitrobenzene **7** was treated with sodium methylsulfide to afford the methyl thioether **8**. The thioether was oxidized to sulfone **9** with 3-chloroperbenzoic acid. Reduction of the nitro group by hydrogenation with 10% palladium on carbon produced the sulfone aniline tail **10**.

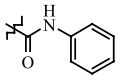
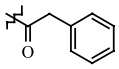
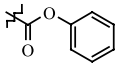
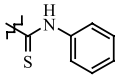
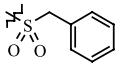
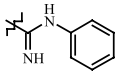
The SAR for a series of urea isosteres is presented in Table 1. No improvement in enzyme or cell potency was observed with the urea isosteres examined. In this series, urea **1** gave the best enzyme and cell potency. Based on these data, we chose to continue our investigation on the urea class of compounds.



Scheme 2. Reagents and conditions: (a) NaSMe, EtOH, 85%; (b) 3-chloroperbenzoic acid, CH_2Cl_2, 0 °C, 63%; (c) 10% Pd/C, H_2 (55 psi), EtOAc, 79%.

Table 1. SAR of urea isosteres



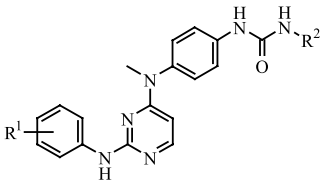
Compound	R	VEGFR2 IC_{50}[a] (nM)	HUVEC-v cell IC_{50}[b] (nM)
1		18	0.2
11		176	49
12		95	224
13		117	6
14		269	350[c]
15		324	596[c]

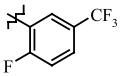
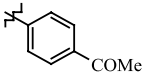
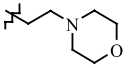
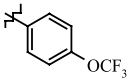
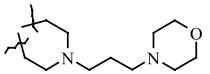
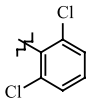
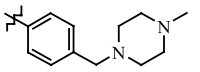
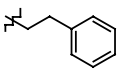
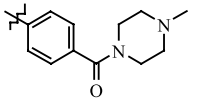
[a] All values represent the average of two or more experiments except where noted.
[b] Inhibition of VEGF induced proliferation of HUVECs.
[c] Value reported from one experiment.

The SAR for a representative sample of our urea compounds is presented in Table 2.[14] The data suggest that a variety of groups are accommodated at the urea. Examples of straight chain alkyl (**27**–**29**), cycloalkyl (**30**) and phenyl (**1** and **16**–**25**) substituted ureas all demonstrated low nanomolar potency against the VEGFR2 enzyme. The most potent compounds in this series appear to be the diphenylureas and the data indicate tolerance for a wide range of substituents and positions around the distal ring (**1** and **16**–**25**). Furthermore, of the examples modified at the end opposite the urea, those with a sulfonamide substituent at the 3- or 4-position (**16**, **19**, **20**, **23**, and **24**) exhibited superior enzyme inhibition to those presenting the corresponding sulfone substituents (**1**, **17**, **18**, and **22**). However, in most cases, the sulfonamides were less potent in the HUVEC assay, which may be due to reduced cell permeability of the hydrogen bond donating the sulfonamide group. This SAR is consistent with our understanding of the VEGFR2 enzyme binding site. X-ray crystallographic work in our laboratories[15] and work by others[16] suggest that binding orientation for our compounds projects the urea substituent into the back pocket of the enzyme and the opposite end is directed toward the solvent interface.

Although lipophilic residues dominate the back pocket of VEGFR2 ATP-binding site,[17] Schindler and coworkers have reported that the Abelson (Abl) tyrosine kinase inhibitor STI-571 (Gleevec^{TM}) benefits from protein backbone hydrogen bonding interactions with the

Table 2. SAR of urea derivatives



Compound	R^1	R^2	VEGFR2 IC_{50}[a] (nM)	HUVEC-v IC_{50}[b] (nM)	Compound	R^1	R^2	VEGFR2 IC_{50}[a] (nM)	HUVEC-v IC_{50}[b] (nM)
1	3-$MeSO_2CH_2$–	–Ph	18	0.2	**29**	3-$MeSO_2CH_2$–	–*n*-Bu	29	2
16	3-H_2NSO_2–		5[c]	2					
					30	3-$MeSO_2CH_2$–		31	1
17	3-$MeSO_2CH_2$–		35	7[c]					
18	4-$MeSO_2CH_2$–		2	2	**31**	3-$MeSO_2CH_2$–		681	530[c]
19	3-H_2NSO_2–		1[c]	10	**32**	4-$MeSO_2CH_2$–		3114	2982
20	4-H_2NSO_2–		1[c]	8					
21	3-$MeSO_2CH_2$–		3	2	**33**	4-$MeSO_2CH_2$–		990	471[c]
22	3-$MeSO_2CH_2$–		6	7					
23	3-H_2NSO_2–		2	5	**34**	4-$MeSO_2CH_2$–		4490	850[c]
24	4-H_2NSO_2–		2[c]	37					
25	3-$MeSO_2CH_2$–		65	29	**35**	4-$MeSO_2CH_2$–		4[c,d]	2
26	3-$MeSO_2CH_2$–		11[c]	10[c]	**36**	4-$MeSO_2CH_2$–		150	516[c]
27	3-$MeSO_2CH_2$–	–Et	17	3					
28	4-$MeSO_2CH_2$–		26[c]	27[c]					

[a] All values represent the average of two or more experiments except where noted.
[b] Inhibition of VEGF induced proliferation of HUVECs.
[c] Value reported from one experiment.
[d] K_i = 11 nM.

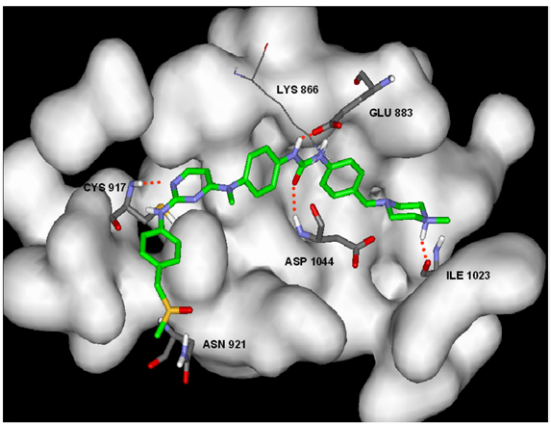


Figure 2. The VEGFR2 kinase inhibitor **35** bound in the enzyme active site as proposed based on homology modeling.

back of the related Abl kinase pocket.[18] In light of this finding, we were encouraged to pursue finding an analogous interaction in the VEGFR2 back pocket. A number of compounds were synthesized that tethered polar functionalities at the end of extended aryl and alkyl linkers (**31**–**36**). Generally, the SAR did not reward this exercise. However, it appears that in the case of methylpiperazine **35** a stabilizing interaction may have been discovered, as the enzyme potency is maintained in the range of the best compounds in this series. The same benzylic methylpiperazine is found on the Bcr-Abl, kinase inhibitor STI-571, and based on our modeling appears to be picking up a similar hydrogen bond interaction with a backbone carbonyl at the back of the pocket.

Our modeling of the urea series inhibitor is exemplified by the methylpiperazine example **35** (Fig. 2). The model illustrates that the pyrimidine core nitrogen is supported by hydrogen bonding to the enzyme backbone residue CYS917. This interaction with the β strand 'hinge' region is flanked by a hydrogen bond between the inhibitor tail sulfone and the backbone nitrogen of ASN921, occurring close to the solvent interface of the protein. Extending toward the back pocket of the enzyme, the inhibitor pushes through a narrow passage lined with the residues GLU883, which accepts a hydrogen bond from a urea nitrogen, and ASP1044, which makes a backbone interaction with the urea carbonyl. In the case of the methylpiperazine, one additional point interaction is proposed between the distal piperazine nitrogen and the backbone carbonyl of residue ILE1023.

2. Conclusion

We have reported a SAR for a potent series of VEGFR2 kinase inhibitors that demonstrate potency against both the enzyme and the HUVEC line. Based on published X-ray crystal analysis and homology modeling of inhibitors bound to the VEGFR2 enzyme, we have proposed a likely binding mode for a representative compound from our urea series.

Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmcl.2005.05.096.

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