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Isothiazolopyrimidines and isoxazolopyrimidines as novel multi-targeted inhibitors of receptor tyrosine kinases

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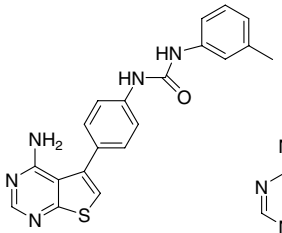
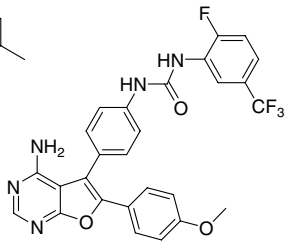
Abstract—A series of isothiazolopyrimidines and isoxazolopyrimidines were synthesized and identified as potent KDR inhibitors. SAR studies led to isothiazolopyrimidine urea analogs that potently inhibit VEGFR tyrosine kinases (KDR enzymatic and cellular IC_{50} values below 10 nM) as well as cKIT and TIE2. The selected compounds **8** and **13** display 56% and 48% oral bioavailability in mice, respectively.

Receptor tyrosine kinases (RTKs), a family of transmembrane proteins, have been shown to be important mediators of signal transduction in cells.[1–3] Overactivation of RTKs is associated with various human cancers.[4] A major sub-class of the RTKs is the vascular endothelial growth factor receptor (VEGFR) tyrosine kinases family which includes FLT1 (VEGFR1), KDR (VEGFR2), and FLT4 (VEGFR3). VEGF-mediated KDR signaling in particular plays a central role in angiogenesis through induction of proliferation, migration, and survival of endothelial cells.[5–7] TIE2, another endothelium-specific tyrosine kinase, promotes tumor angiogenesis through interaction with angiopoietin,[8] playing an important role in stabilizing the immature endothelial cell network, attracting pericytes, and maintaining biochemical interactions and vessel integrity.[9] The PDGFRs, another sub-class of RTKs consisting of PDGFRβ, cKIT, CSF1R, and FLT3, are believed to contribute to tumor angiogenesis and tumor growth as well.[10–12]



I



II

The development of VEGFR inhibitors has been the subject of intense research.[13] The first generation of clinical candidates, such as SU5416,[14] PTK787,[15] and CP-547632[16] focused on selective KDR inhibition. However in recent years, the focus has shifted toward multi-targeted inhibitors in an effort to overcome redundancies in signaling pathways and thus more effectively inhibit tumor growth. The recent approval of the multi-targeted agents Sutent™[17] and Nexavar™[18] demonstrates that clinical benefit with manageable side effects is possible with broad-acting kinase inhibitors.

As part of our continuing efforts to identify novel RTK inhibitors with improved efficacy and tolerability, we have identified a series of thienopyrimidine-based ureas, exemplified by **I** as potent multi-targeted inhibitors.[19]

Keywords: Isothiazolopyrimidine; Isoxazolopyrimidine; Multi-targeted RTK inhibitor.
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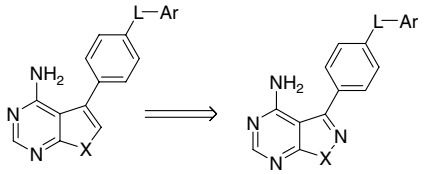
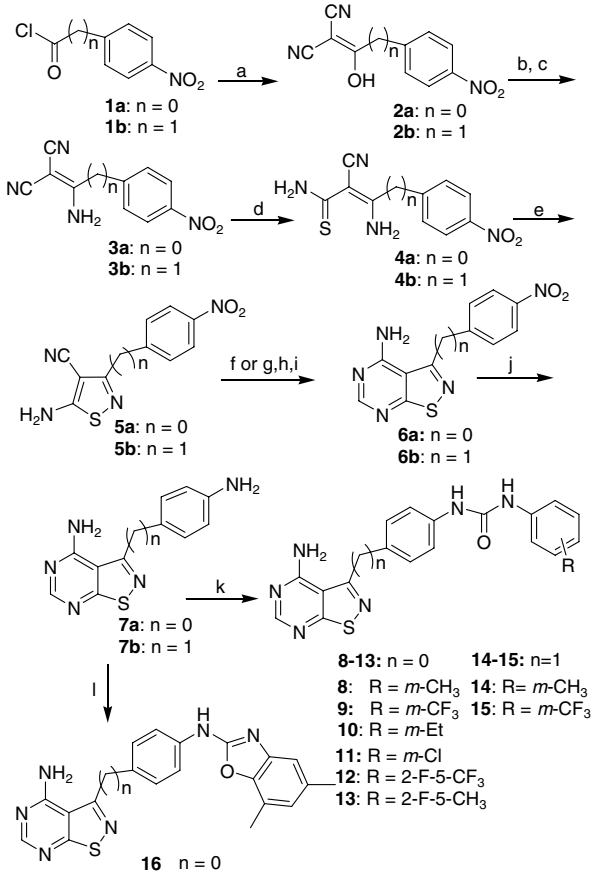
A closely related series of furopyrimidines (e.g., **II**) have also been recently reported as dual inhibitors of KDR and TIE2.[20]

We now wish to report the results of our expanded SAR studies on five-membered fused pyrimidine heterocycles covering the related isothiazolopyrimidines and isoxazolopyrimidines (**III**).



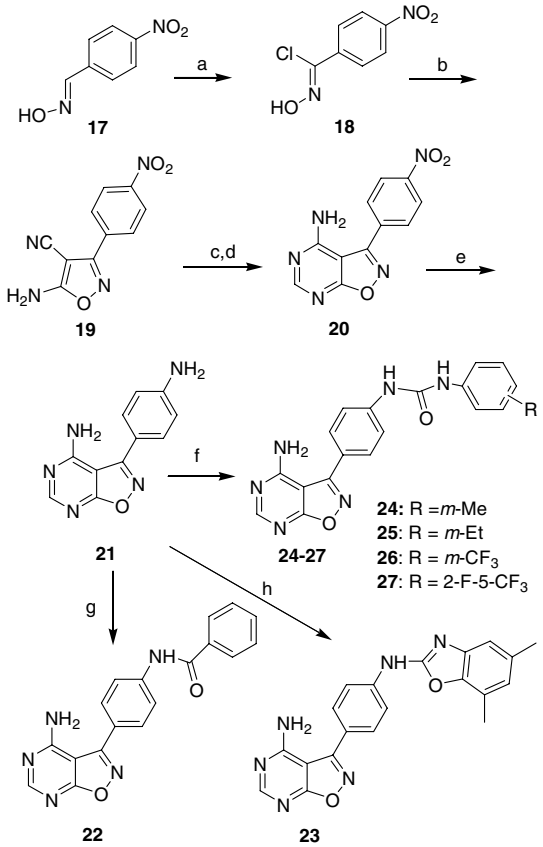
The general synthetic strategy for the preparation of isothiazolopyrimidines is described in Scheme 1. Condensation of **1** with malononitrile in the presence of NaOH and a phase transfer reagent gave rise to **2**, which in turn was converted to **3** via sequential treatment with PCl_5 and ammonium hydroxide. Treating **3** with diethyl dithiophosphate formed the desired intermediate **4**, which was cyclized to **5** with H_2O_2. Heating **5a** with formamide at 210 °C under microwave conditions led to isothiazolopyrimidine **6a**. Compound **6b** was prepared in a stepwise fashion by first refluxing **5b** with $(EtO)_3CH$ then treating with ammonia followed by lithium methoxide-mediated cyclization. Reduction of the nitro group of **6** afforded aniline **7**, which was converted to the ureas **8–15** and benzoxazole **16**.



Scheme 1. Reagents and conditions: (a) malononitrile, 10 N NaOH, $PhCH_2NEt_3Cl$, CH_2Cl_2, 0–5 °C, 76–82%; (b) PCl_5, CH_2Cl_2, reflux 24 h; (c) NH_4OH, EtOH, rt, b and c two-step yield 53–58%; (d) diethyl dithiophosphate, EtOH/H_2O, reflux 24 h, 85–95%; (e) H_2O_2, EtOH, 90–96%; (f) for **6a**: $HCONH_2$, microwave, 210 °C, 30 min, 60%; g–i for **6b**: (g) $(EtO)_3CH$, $(NH_4)_2SO_4$, reflux; (h) NH_3 in EtOH, 35%; (i) LiOMe, MeOH, reflux, 40%; (j) Fe/NH_4Cl, EtOH/H_2O 50–60 °C, 90–97%; (k) ArNCO, DMF, 0–5 °C, 30–40%; (l) 2,4-dimethyl-6-aminophenol, 1,1′-thiocarbonyldiimidazole, EDC, DMF, 10%.

The synthesis of the isoxazolopyrimidines was accomplished by the route illustrated in Scheme 2. Treating **17** with sodium hypochlorite gave compound **18**, which was then condensed with malononitrile to form oxazole **19**. Refluxing **19** with $(EtO)_3CH$ followed by treatment with ammonia gave the desired isoxazolopyrimidine **20**. Reduction of the nitro group of **20** afforded aniline **21**, which was converted to products **22–27**. The corresponding furano-linked compounds **28** and **29** (Table 2) were prepared by substituting **17** with 5-nitro-furan-2-carboxaldehyde oxime in Scheme 2 except using Al–Hg–THF in place of $SnCl_2$ for the nitro group reduction.

Our initial SAR studies focused on KDR potency. The inhibitory activity was measured in the presence of a



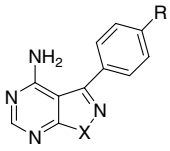
Scheme 2. Reagents and conditions: (a) NaOCl, HCl, dioxane, 70%; (b) malononitrile, NaOMe, THF, 75%; (c) $HC(OEt)_3$, reflux; (d) NH_3 in EtOH, rt, c and d two-step yield 54%; (e) $SnCl_2$/HCl, 80%; (f) RNCO, Py, DMF, 40–55%; (g) benzoyl chloride, pyridine, 40%; (h) 2,4-dimethyl-6-aminophenol, 1,1′-thiocarbonyldiimidazole, EDC, DMF, 8%.

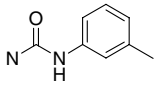
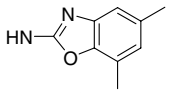
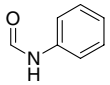
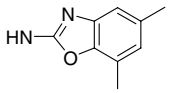
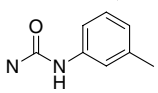
high concentration of ATP (1.0 mM) as described in the previous paper.[19] Table 1 summarizes the SAR of the northern part of the molecule. In general, the SAR mirrors that seen in the thienopyrimidine series[19] with the ureas being most potent. Both the isothiazole **8** and isoxazole **24** exhibit IC_{50} values of less than 100 nM, although the isoxazole was ca. 7-fold less active. Replacement of the urea with aminobenzoxazole (cf. **16**, **23**) led to a more dramatic drop in potency than that observed with the thienopyrimidine template.[19] The amide analog **22** also had drastically diminished inhibitory activity.

The linkage between isothiazolopyrimidine or isoxazolopyrimidine core and urea moiety also has profound impact on potency as shown in Table 2, with a 1,4-phenyl group being the optimal linker (**9**, **26**). Replacing the phenyl linkage with benzyl (**14**, **15**) or furan (**28**, **29**) results in a markedly reduced potency.

A model of **9** in the active site of KDR, constructed according to our previous protocol,[19] is shown in Figure 1. The isothiazolopyrimidine forms the hinge binding unit and the trifluoromethylphenyl portion occupies a pocket allosteric to the ATP-binding site and found in the inactive (DFG-out) conformation of kinases. The five-membered ring of the furan-linked compounds, illustrated in Figure 1 with a model of **29**, gives a projection vector of approximately 140°, in contrast with the 180° of the para-substitution of **9**, necessitating significant re-alignment of the binding interactions. The skewed fit and sub-optimal hydrogen bonding of **29** within the active site provides a rationale for the decrease in inhibition strength. Similarly, the additional methylene group of the benzyl-linked compounds necessitates a 109° bend in the overall trajectory of compounds **14** and **15**, and leads to a binding mode with less optimal interactions (model not shown).

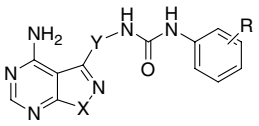
Table 1. KDR inhibitory activity of isothiazolopyrimidine and isoxazolopyrimidine analogs

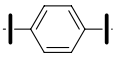
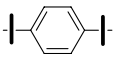
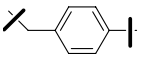
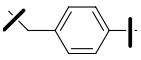
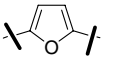
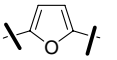


Compound	X	R	KDR IC_{50}[a] (μM)
6a	S	NO_2	>50
7a	S	NH_2	46
8	S		0.013
16	S		43
22	O		>50
23	O		>50
24	O		0.097

[a] Each IC_{50} determination was performed with seven concentrations, and each assay point was determined in duplicate.

Table 2. KDR inhibitory activity of isothiazolopyrimidines and isoxazolopyrimidines with varying links between the heterocycle core and urea moiety



Compound	X	Y	R	KDR IC_{50}[a] (μM)
9	S		*m*-CF_3	0.004
26	O		*m*-CF_3	0.070
14	S		*m*-CH_3	8.6
15	S		*m*-CF_3	2.1
28	O		*m*-CH_3	22
29	O		*m*-CF_3	16

[a] Each IC_{50} determination was performed with seven concentrations, and each assay point was determined in duplicate.

The effect of varying the terminal phenyl substitution of the urea moiety on KDR enzymatic and cellular activity is shown in Table 3. The isothiazolopyrimidines are consistently more potent than the corresponding isoxazolo-

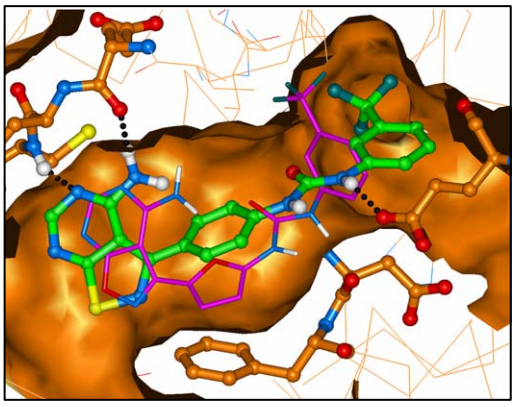
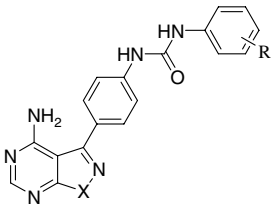


Figure 1. Models of **9** (green, thick) and **29** (magenta, thin) bound to KDR kinase using a homology model of the active site.[19] Hydrogen bonds in black are shown between the urea and Glu885 carboxylate, between the exocyclic amine and Glu917 backbone carbonyl, and between the ring nitrogen and Cys919 N–H. Also in thick bonds are residues Asp1046 and Phe1047 of the DFG motif in the 'inactive' conformation (DFG-out).

Table 3. Enzymatic and cellular activities of isothiazolopyrimidines and isoxazolopyrimidines



Compound	X	R	KDR IC_{50} (μM)	
			Enzymatic[a]	Cellular[b]
8	S	*m*-Me	0.012	0.001
9	S	*m*-CF_3	0.004	0.005
10	S	*m*-Et	0.005	0.005
11	S	*m*-Cl	0.019	0.050
12	S	2-F-5-CF_3	0.016	NA
13	S	2-F-5-Me	0.008	0.021
24	O	*m*-Me	0.097	0.10
25	O	*m*-Et	0.024	0.25
26	O	*m*-CF_3	0.070	0.17
27	O	2-F-5-CF_3	0.225	0.385
I			0.003	0.001

[a] Each IC_{50} determination was performed with seven concentrations, and each assay point was determined in duplicate.

[b] Each cellular IC_{50} determination was performed with five concentrations and each assay point was determined in duplicate.

pyrimidines and have comparable activity to the thienopyrimidines (cf. **8** vs **I**) in both enzymatic and cellular activity.

The isothiazolopyrimidines were further investigated for their ability to inhibit TIE2, cKIT, and the other members of the VEGFR family, FLT1 and FLT4 (Table 4). The compounds tested had comparable activity against the VEGFR family and cKIT. Additionally significant TIE2 activity was observed with the fluoro-containing analogs **9**, **12**, and **13**, a trend previously seen in the thieno and furopyrimidine series.

Compounds **8** and **13** were evaluated in a mouse pharmacokinetic study (Table 5). Data for the thienopyrimidine **I** as a comparator are also included. Both compounds display good oral bioavailability (56% and 48%), that is comparable to the thienopyrimidine **I**. Compound **8** has a similar iv profile to the corresponding thienopyrimidine with a half-life of 0.65 h, while the 2-fluoro-5-methyl analog **13** has a shorter half-life and considerably higher plasma clearance rate.

Table 4. Inhibitory activity against a series of kinases

Compound	IC_{50} (μM)[a]				
	KDR	TIE2	FLT1	FLT 4	CKIT
8	0.013	0.415	0.079	0.004	0.011
9	0.004	0.028	0.039	<0.003	0.018
12	0.001	0.022	0.075	0.023	0.028
13	0.008	0.019	0.010	0.068	0.007
I[19]	0.003	0.730	0.002	0.013	0.006

[a] Each IC_{50} determination was performed with seven concentrations, and each assay point was determined in duplicate.

Table 5. Mouse PK profiles of **8** and **13**[a]

Compound	$t_{1/2}$ (h)	V_{dss} (L/kg)	Cl (L/h·kg)	F (%)	AUC (po, μM·h)
8	0.6	1.36	1.6	56	9.1
13	0.2	1.19	4.5	48	2.7
I[19]	0.7	1.30	1.2	65	14.0

[a] Dosed intravenously at 3 mg/kg and orally at 10 mg/kg.

In summary, potent VEGFR and PDGFR inhibitory activity can be obtained with compounds using an isothiazolopyrimidine template, while the corresponding isoxazolopyrimidines have weaker inhibitory activity. A comparison of a limited set of compounds showed that the pharmacokinetic profile of isothiazolopyrimidines is comparable to the corresponding thienopyrimidines.

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