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Discovery of dual Axl/VEGF-R2 inhibitors as potential anti-angiogenic and anti-metastatic drugs for cancer chemotherapy

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ABSTRACT

Axl tyrosine kinase has been shown to be involved in multiple pathways contributing to tumor development, angiogenesis, and metastasis. High Axl expression has been observed in many human tumors where it appears to confer aggressive tumor behavior. Here we present several series of dual Axl-VEGF-R2 kinase inhibitors based on extensive optimization of an acyl diaminotriazole. It was hypothesized that dual inhibition of these two receptor tyrosine kinases may have a synergistic affect in inhibiting tumor angiogenesis and metastasis. One of these molecules, **R916562** showed comparable activity to Sunitinib in two mouse tumor xenograft models and a mouse corneal micropocket model.

Axl is a receptor tyrosine kinase (RTK) originally identified from patients with chronic myelogenous leukemia.[1] By use of retroviral screening technology, Axl was shown to regulate endothelial cell migration.[2] Axl is also necessary for in vivo angiogenesis and tumor development in mouse models.[2] Axl stimulation triggers several signaling pathways involved in cellular migration, invasiveness, transformation, and proliferation as well as angiogenesis and resistance to chemotherapeutic agents.[3,4] High Axl expression is observed in many human tumors where it appears to confer aggressive tumor behavior, leading to tumor dissemination and mortality from metastasis.[5] We therefore sought to further validate and characterize Axl as a therapeutic oncology target by the development of small molecule inhibitors.[6]

We describe the development of a series of dual Axl-VEGF-R2 kinase inhibitors culminating in **R916562**, a diaminotriazole which exhibits antitumor activity comparable to Sunitinib in a mouse xenograft model. Initial goals were to achieve good Axl potency as measured by an in-cell western blot (ICWB) assay. Selectivity over the insulin receptor (INSR) and the closely related RTK Mer were desired.[7,8] Non specific anti-proliferative or cytotoxic activity (PAD assay) was undesirable.[9] Compounds were also screened against vascular endothelial growth factor receptor 2 (VEGF-R2) due to the known antiangiogenic and antitumor effects of targeting this receptor.[10] We hypothesized that dual Axl/VEGF inhibitors might be very effective oncology therapeutics since multiple biological pathways involved with angiogenesis and tumorogenesis would be targeted. An initial high throughput screen generated an acyl diaminotriazole as a starting point. Lead optimization led to compound **1**. The donor-acceptor-donor triad provided by the diaminotriazole as well as a basic nitrogen optimally spaced 4–5 atoms away from the left-hand side aryl ring were essential to the pharmacophore (Fig. 1).

Compound **1** showed a 47 nM cellular EC_{50} against Axl, 2.75 μM against INSR and 1 μM cytotoxicity. Unfortunately **1** had poor aqueous solubility and <1% oral bioavailability in the SD rat making it unsuitable for testing in a xenograft model.

A logical next step was to replace the acyl carbonyl with a pyridyl nitrogen (Fig1). The dashed line in Fig, 1 indicates a potential intramolecular hydrogen bond between the triazole amino group and the pendant carbonyl group of compound **1** or pyridyl nitrogen of compound **2**. Kinase inhibitors designed to contain pseudo six membered rings formed by intramolecular hydrogen bonding have been reported.[11] Indeed, this hypothesis proved correct as replacing the 4-morpholinophenylcarboxamide of compound **1** with a 2-pyridyl gave compound **2** which showed an Axl EC_{50} of 334 nM and a comparable selectivity profile. Since synthesis of a series of substituted 2-pyridyl analogues did not result in improved Axl potency, a series of bicyclic analogues was prepared.

A variety of quinazoline or fused pyrimidine-substituted diaminotriazoles showed sub-100 nM inhibition of Axl (Table 1). Diaminotriazoles similarly substituted with quinolines, isoquinolines and benzothiazoles also showed potent Axl activity (data not shown), but generally exhibited potent cytotoxicity and INSR

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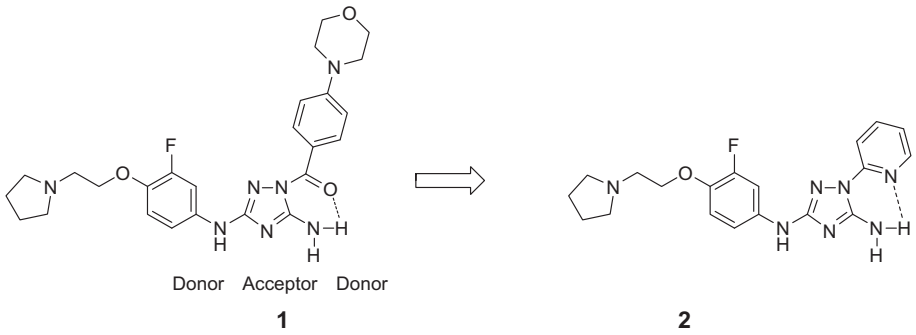
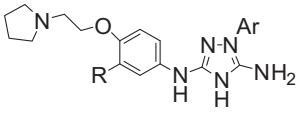
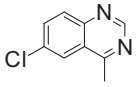
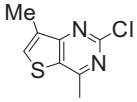
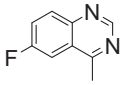
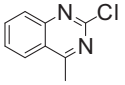
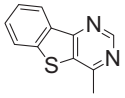
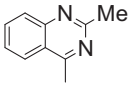
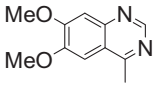


Fig. 1. Evolution of the Acyl Diaminotriazole Series to a 2-Pyridyl Diaminotriazole. The dashed line shows a potential hydrogen bond to form a pseudo six-membered ring.

Table 1
Axl, Insulin Receptor and PAD Activity for bicyclic diaminotriazoles.



Compound	Ar	R	Axl (ICWB) EC_{50}(μM)	Axl (in vitro) IC_{50}(μM)	INSR (ICWB) EC_{50}(μM)	Cytotoxicity EC_{50}(μM)
3		H	0.004	0.003	0.014	0.040
4		H	0.012	0.002	0.582	0.040
5		H	0.022	0.003	0.126	0.040
6		H	0.023	0.014	1.005	0.040
7		H	0.027	0.007	0.241	0.040
8		H	0.028	0.004	0.300	0.026
9		H	0.052	0.032	0.320	0.040
10		Cl	0.062	0.016	4.17	1.44

activity. An exception was compound **10**, which contained a 6, 7-dimethoxyquinazoline on the right hand side. Data for some analogues containing the 6,7-dimethoxyquinazoline moiety is shown in Table 2.

The desired potency and selectivity for Axl over the INSR and PAD shown by compound **10** was maintained, although Mer selectivity was not achieved. These compounds were also potent inhibitors of VEGF-R2. Unfortunately, oral rat pharmacokinetics showed high clearance and volume of distribution for this series.

A breakthrough came when a constrained norbornyl piperazine side chain on the left hand side was combined with bicyclic heterocycles on the right hand side (Table 3). Thienopyrimidine **17** showed much-improved clearance (11.5 mL/min/kg), reduced volume of distribution and a promising half life (4.9 h) in the SD rat. Comparison of compounds **3** and **21**, **4** and **17**, and **5** and **18** established that replacement of the left-hand side 2-pyrrolidinoethoxy group with the norbornylpiperazine significantly reduced cytotoxicity. Interestingly, compounds **9** and **20** did not show this trend.