



Design and chemical synthesis of [1,2,4]triazol[1,5-c]pyrimidin-5-yl amines, a novel class of VEGFR-2 kinase inhibitors

Alexander S. Kiselyov^{a,*}, Eugene L. Piatnitski Chekler^b, Natalia B. Chernisheva^c, Lev K. Salamandra^c, Victor V. Semenov^c

^a deCODE Chemistry, 2501 Davey Road, Woodridge, IL 60517, USA

^b Department of Chemistry, ImClone Systems Inc., 180 Varick Street, New York, NY 10014, USA

^c N. D. Zelinsky Institute of Organic Chemistry, RAN, 47 Leninsky Prospekt, 117918, Russia

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ABSTRACT

The Letter describes a facile approach to 7,8-dihydro[1,2,4]triazol[1,5-c]pyrimidin-5-yl amines, a novel class of potent inhibitors of vascular endothelial growth factor receptor II (VEGFR-2). The synthetic sequence is centered around preparation of the key 3(5)-cyanomethyl-1,2,4-triazole intermediates and their Knoevenagel condensation with aromatic aldehydes. A subsequent three-step conversion of Knoevenagel adducts involving a reduction of vinyl nitriles followed by the reaction of the resulting amines with aryl isothiocyanates and cyclization of the respective thioureas yielded targeted heterocycles as a 1:1 mixture of tautomers. A representative molecule featured sound activity against VEGFR-2 in both enzymatic and cellular assays.

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Angiogenesis, or formation of new blood vessels, is involved in embryonic development^{1,2} and wound healing as well as in pathological conditions such as tumor growth and degenerative eye conditions.^{3–6} The family of vascular endothelial growth factors (VEGFs) has been implicated in regulation of angiogenesis in vivo. VEGFs mediate their biological effect through high-affinity receptors (VEGFRs).^{7–11} Modulation of VEGF/VEGFR signaling activity offers an attractive target for inhibition of an aberrant angiogenesis and suppression of tumor growth.^{12–14} A number of small-molecule inhibitors are known to affect VEGF/VEGFR signaling. These include PTK787 (*Vatalanib*[™] **A**),^{15–17} its analogue BAY579352 (**B**), and the isosteric anthranil amide derivatives **C** and AMG-706 (**D**).¹² Intra-molecular hydrogen bonding in **C** and **D** was suggested to be responsible for the optimal spatial orientation of the pharmacophore pieces, similar to that of the parent PTK787 (Fig. 1).¹⁸

The essential pharmacophore elements^{19,20} for the VEGFR-2 activity of phthalazine-based molecules and their analogues (**A–D**) include (i) [6,6]fused (or related) aromatic system; (ii) *para*-, *meta*-, or 3,4-di-substituted aniline functionality in position 1 of the phthalazine core (**A**, Fig. 1); (iii) hydrogen bond acceptor (Lewis' base: lone pairs of nitrogen- or oxygen atoms) attached to po-

sition 4 via an appropriate linker (aryl or fused aryl group).^{21–23} In this research article, we report a synthetic approach towards 7,8-dihydro[1,2,4]triazol[1,5-c]pyrimidin-5-yl amines as inhibitors of VEGFR-2 kinase. We reasoned that this template could provide a proper pharmacophore arrangement relevant to compounds **A–D** (Fig. 2). MMFF94 Force Field minimization studies suggested good overlap between our proposed chemical series and the development candidate *Vatalanib*[™] **A** (Fig. 2a and b).²⁴

Our synthetic effort was based on the development of expeditious route to the key intermediate nitriles **6a** and **6b** (Scheme 1). We reasoned that the thiourea derivatives **8a–8d** resulting from the reduction of Knoevenagel products **6a** and **6b** to furnish the respective amines **7a** and **7b**, would yield the targeted molecules **9a–9d** upon ring closure reaction. In the synthesis of unsubstituted triazoles (R = H), we initially prepared **2** in 84% yield by reacting ethyl ethoxycarbonylacetimidate **1** with formyl hydrazine (Scheme 1). It was subsequently heated in vacuo to afford a cyclization product **3** in 78% yield. The triazole derivative **3** was treated with ammonia to result in the intermediate triazolyl acetamide that was further dehydrated using P₂O₅/dry sand/absolute pyridine to furnish the targeted heteroaryl acetonitrile **5a** in 33% combined yield. The respective 5-methyl-3-cyanomethyl-1,2,4-triazole **5b** was prepared in 60% yield by reaction of commercially available cyanoacetic acid hydrazide and acetic acid iminoester prepared

* Corresponding author. Tel.: +1 630 783 4600.

E-mail address: akiselyov@decode.com (A.S. Kiselyov).

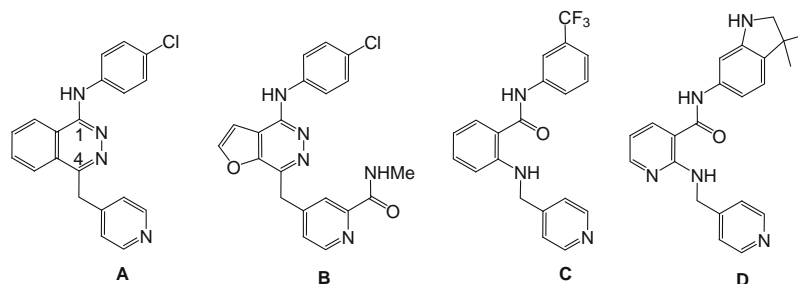


Figure 1. Selected inhibitors of vascular endothelial growth factor receptor II (VEGFR-2) based on phthalazine core (A) and its isosteric analogues.

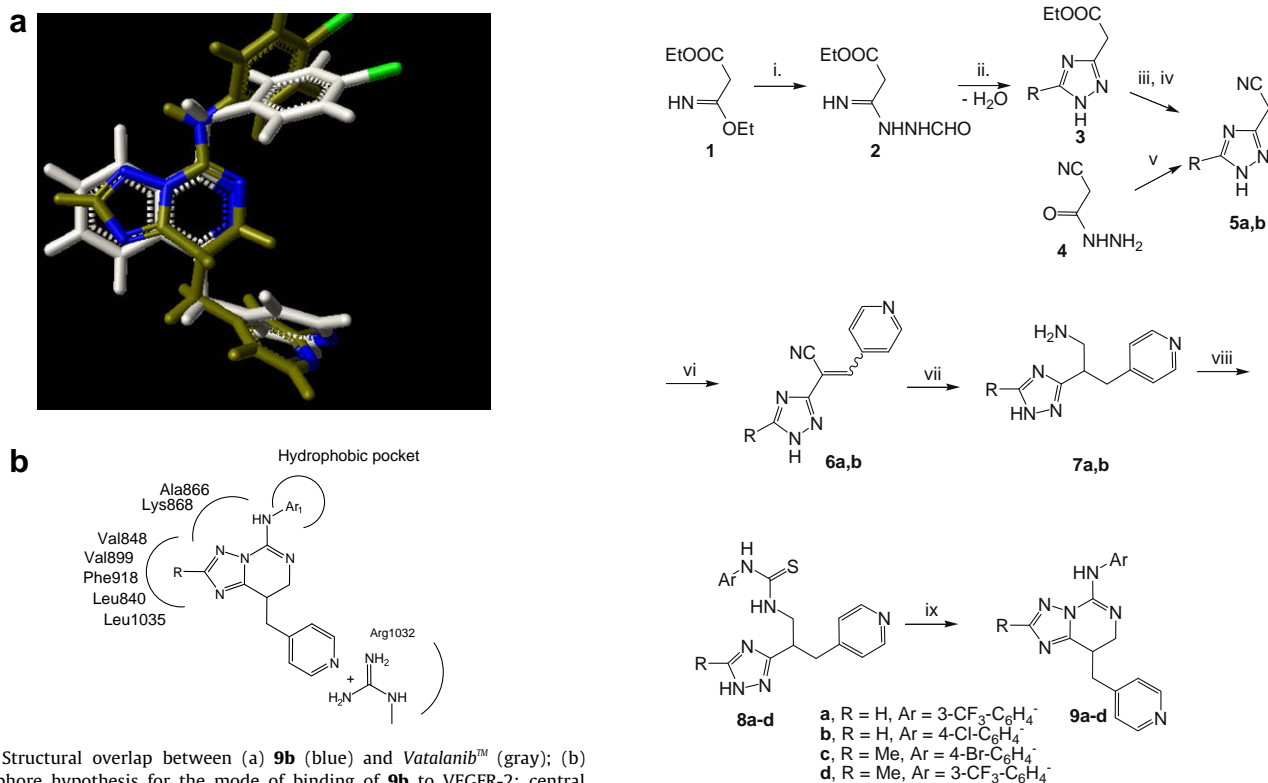


Figure 2. Structural overlap between (a) **9b** (blue) and *Vatalanib*[™] (gray); (b) Pharmacophore hypothesis for the mode of binding of **9b** to VEGFR-2: central phenyl ring is surrounded by *Leu840*, *Val848*, *Val899*, *Phe918*, and *Leu1035*; the triazole ring is in close proximity with the *Ala866* and *Lys868*, and pyridine nitrogen is near *Arg1032*.

in situ from acetonitrile.²⁵ Presence of the nitrile group was confirmed by both mass spectrometry and infrared spectrometry (strong IR band at 2256 cm⁻¹). Knoevenagel condensation of nitriles **5a** and **5b** with 4-pyridylaldehyde afforded the key intermediate nitriles **6a** and **6b** predominantly as *trans*-isomers in 71–75% yield. These intermediates were hydrogenated in the presence of Raney-Ni to give amines **7a** and **7b** that were used in the next step without purification. Specifically, **7a** and **7b** were reacted with corresponding aryl isothiocyanates to furnish respective thioureas **8a–8d** in 20–95% yield. Notably, thioureas eliminate corresponding isothiocyanate fragments in the mass spectrometer (direct electron impact). The final step of the synthesis was thermal cyclization of thioureas in the presence of yellow HgO to afford heterocycles **9a–9d** in 39–79% yields. We were not able to convert molecules **9a–9d** to the respective [6.5]fused aromatic derivatives under a variety of experimental conditions (DDQ or MnO₂-mediated oxidation, Pd-catalyzed aromatization, etc.).

Chemical structure of the [6.5]-fused triazoles was unambiguously assigned using NMR spectroscopy. The N1 versus N4 triazole

Scheme 1. Reagents and conditions: (i) H₂NN-CHO, MeOH, rt, 4 days (84%); (ii) 125–130 °C at 29 mm Hg, 20 min (78%); (iii) aqueous NH₃, 17 h; (iv) P₂O₅, dry pyridine, reflux, 5 h (33% for iii and iv); (v) MeCN, HCl (gas), MeOH, –4 °C, 4 days; MeOH, 40 °C–55 °C, 9.5 h (60%); (vi) 4-PyCHO, piperidine, *i*-PrOH, 4 Å molecular sieves, reflux, 6 h; (vii) NH₃, *i*-PrOH; H₂/Raney-Ni, 50 atm, 50 °C, 20 h (71–75%); (viii) ArNCS, *t*-BuOH, reflux, 10 min (20–95%); (ix) HgO (yellow), *i*-PrOH, reflux, 3 h (39–79%).

regiochemistry of the cyclization is well documented.²⁶ In a representative example (Fig. 3), upon cyclization of **8c**, one NH-triazole (13.2–13.7 Hz) and both thiourea NH-protons signals (7.76 (d, *J* = 8.6 Hz, 1H, NH), 9.6 (s, 1H, NH)) of the starting material disappeared to afford **9c**. Tautomers **9c** were confirmed by ¹³C NMR, 1H–1H NOESY, and 1H–¹³C HSQC. Notably, the resulting 7,8-dihydro[1,2,4]triazolo[1,5-*c*]pyrimidin-5-amines exist as ca. 1:1 mixture of tautomers in DMSO. For example, proton NMR spectrum of **9c** features a double set of signals for both methyl substituent in the triazole ring and the Ar group protons.

7,8-Dihydro[1,2,4]triazolo[1,5-*c*]pyrimidin-5-yl amines **9a–9d** were screened in vitro for kinase activity. The synthesized analogues were subjected to biological evaluation in VEGFR-2 enzymatic and cellular assays. Compound **9a** demonstrated the highest activity in both VEGFR-2 kinase enzymatic assay^{19–23}

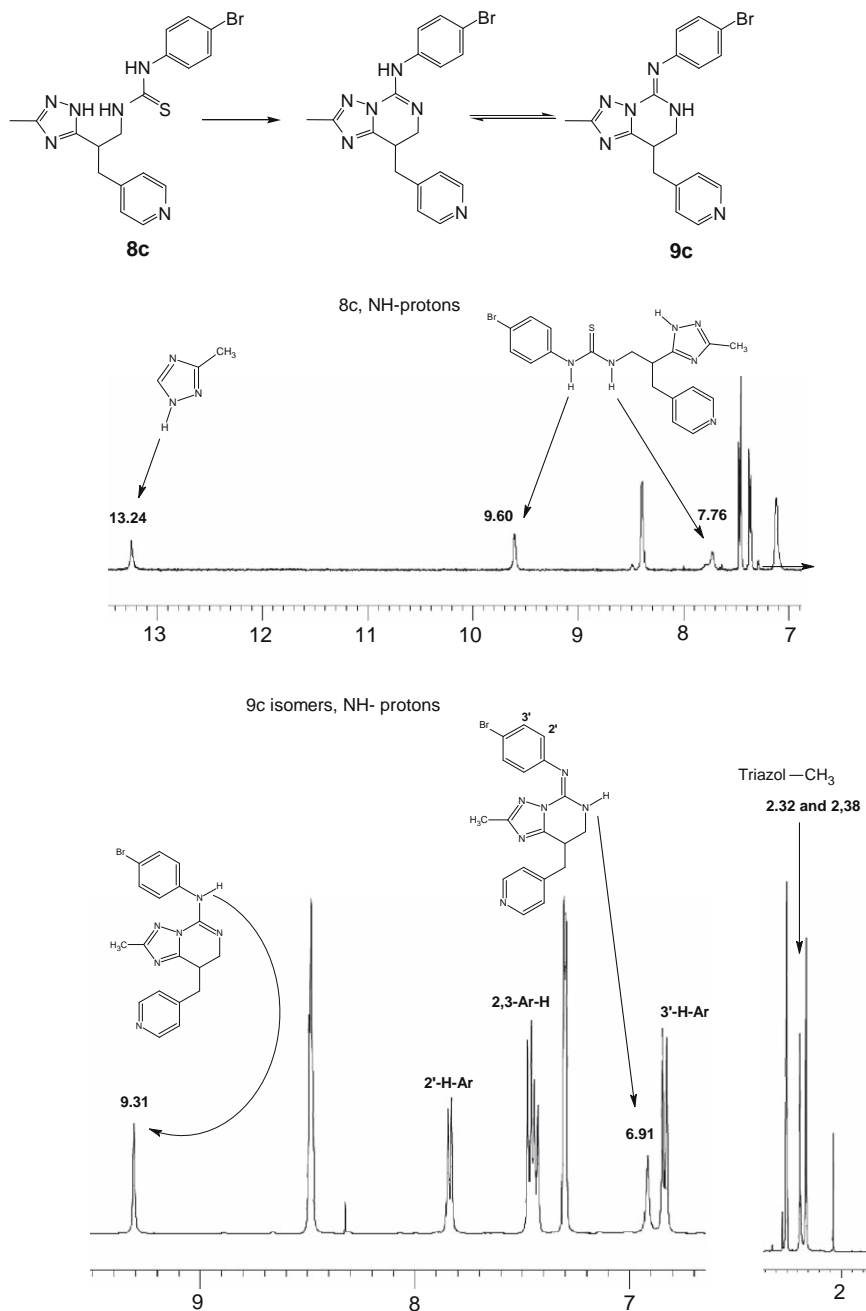


Figure 3. ¹H NMR spectra of the starting thiourea **8c** and the product **9c** (1:1 mixture of tautomers).

(IC₅₀ = 62 nM) and cellular phosphorylation assay^{19–23} (IC₅₀ = 78 nM). In comparison, clinical candidate PTK787 (*Vatalanib*[™] **A**) showed IC₅₀ values of 54 nM and 21 nM in these assays, respectively. These results confirm our previous observations^{19–23} that the interaction of nitrogen lone pair of the 4-pyridyl moiety with Arg1032 (Fig. 2) is critical for VEGFR-2 kinase activity.

In summary, we have achieved a convenient synthesis of 7,8-dihydro[1,2,4]triazol[1,5-*c*]pyrimidin-5-yl amines, a novel class of kinase inhibitors. Representative compound **9a** exhibited nanomolar activity for VEGFR-2 kinase. The discovery of a novel chemical series active against VEGFR-2 may yield additional derivatives with affinity for other kinases. Furthermore, the scaffold's resistance to aromatization under a variety of conditions suggests potential for its metabolic stability in vivo. Currently, we continue with evaluation of the active compounds in a series of biological assays. These data will be reported elsewhere.

Supplementary data

Experimental details, proton NMR spectra, mass spectrometry data and elemental analysis data. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.04.062.

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