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

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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.

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 anthranyl amide derivatives **C** and AMG-706 (**D**).[12] Intramolecular 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 position 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 P_2O_5/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

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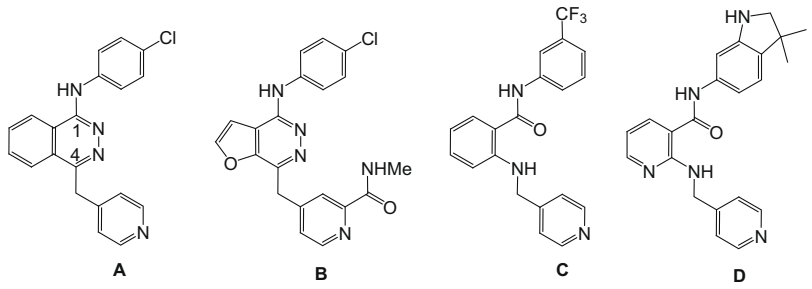


Figure 1. Selected inhibitors of vascular endothelial growth factor receptor II (VEGFR-2) based on phthalazine core (**A**) and its isosteric analogues.

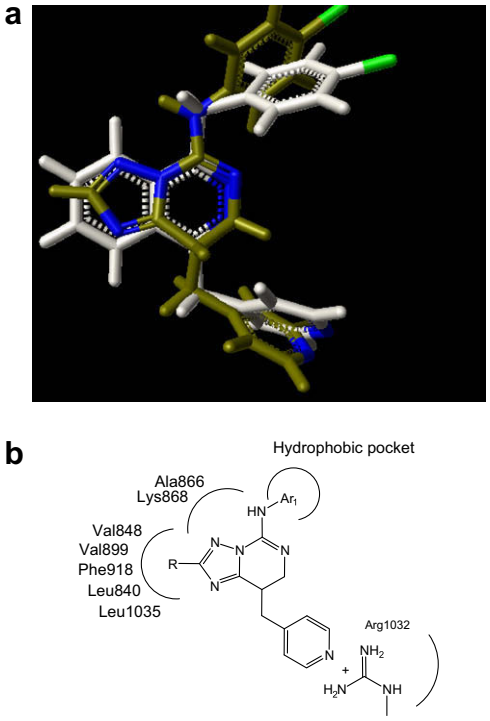
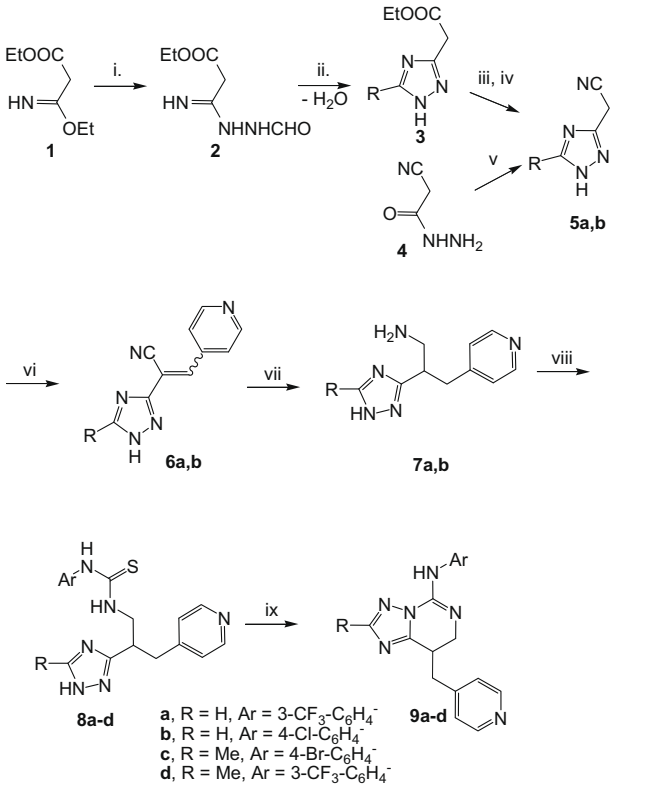


Figure 2. Structural overlap between (a) **9b** (blue) and *Vatalanib*™ (gray); (b) Pharmacophore hypothesis for the mode of binding of **9b** to VEGFR-2: central phenyl ring is surrounded by *Leu840*, *Val848*, *Val899*, *Phe918*, and *Leu1035*; the triazole ring is in close proximity with the *Ala*866 and *Lys*868, and pyridine nitrogen is near *Arg1032*.



Scheme 1. Reagents and conditions: (i) H_2NN–CHO, MeOH, rt, 4 days (84%); (ii) 125–130 °C at 29 mm Hg, 20 min (78%); (iii) aqueous NH_3, 17 h; (iv) P_2O_5, 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) NH_3, *i*-PrOH; H_2/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%).

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 MnO_2-mediated oxidation, Pd-catalyzed aromatization, etc.).

Chemical structure of the [6.5]-fused triazoles was unambiguously assigned using NMR spectroscopy. The *N*1 versus *N*4 triazole regiochemistry of the cyclization is well documented.[26] In a representative example (Fig. 3), upon cyclization of **8c**, one *N*H-triazole (13.2–13.7 Hz) and both thiourea *N*H-protons signals (7.76 (d, *J* = 8.6 Hz, 1H, *N*H), 9.6 (s, 1H, *N*H)) of the starting material disappeared to afford **9c**. Tautomers 9c were confirmed by ^{13}C 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]triazol[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]