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2-Aminobenzimidazoles as potent ITK antagonists: *trans*-stilbene-like moieties targeting the kinase specificity pocket

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ABSTRACT

Based on the information from molecular modeling and X-ray crystal structures, the kinase specificity pocket of ITK could be occupied upon extension of the right-hand-side of the 2-benzimidazole core of the inhibitors. 2-Aminobenzimidazoles with a *trans*-stilbene-like extension were designed and synthesized as novel ITK antagonists. Significant improvement on binding affinity and cellular activity were obtained through the *trans*-stilbene-like antagonists. Several compounds showed inhibitory activity in an IL-2 functional assay.

Interleukin-2-inducible T-cell kinase (ITK) is a tyrosine kinase from the Tec kinase family.[1] ITK is expressed in T-cells, NK cells and mast cells.[2] Studies using ITK deficient murine $CD4^+$ T-cells showed reduced IL-2, IL-4, IL-5, and IL-13 production upon T-cell receptor stimulation.[3–5] The hypothesis that a selective ITK antagonist would reduce the production of T-cell cytokines, which contribute to inflammation, is attractive for the treatment of inflammatory diseases such as rheumatoid arthritis and allergic asthma.[6]

Recently, we have discovered that 2-aminobenzimidazoles are potent ITK inhibitors.[7] Lead compounds such as **1** showed significant inhibitory activity in an ITK DELFIA screening assay (IC_{50} = 25 nM). However, their potency was severely reduced in a cell assay measuring Ca^+ flux using DT40/ITK cells (IC_{50} = 2.4 μM). Furthermore, compounds such as **1** had a low selectivity over insulin receptor kinase (IRK) (IC_{50} = 145 nM). Therefore, we embarked in a synthetic effort to improve potency and selectivity.

The binding mode of compound **1** was predicted by molecular modeling and confirmed by X-ray crystallography of the enzyme–inhibitor complex. (Fig. 1).[8] Interestingly, the aminobenzimidazole **1** adopts a tautomeric form that allows the establishment of a key hydrogen bonding with the backbone Met^{438}. On the right-hand side, the thiophene group points toward an unoccupied

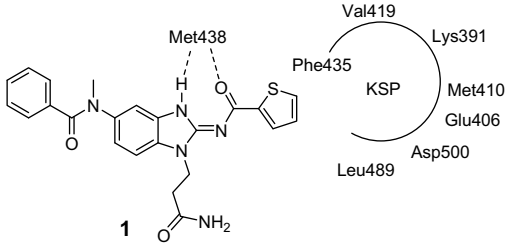

Figure 1. The structure and binding mode of **1** in ITK.

kinase specificity pocket (KSP). The KSP spans a 7.3-Å space from the 'gate-keeper' Phe^{435} to Met^{410}. It contains hydrophobic (Leu^{418}, Leu^{489}, and Val^{419}) as well as hydrophilic residues (Lys^{391}, Glu^{406}, Met^{410}, and Asp^{500}). It was envisioned that appropriate substitutions of the thiophene ring could occupy the KSP, and hence improve binding affinity for ITK while enhancing selectivity over IRK. To extend into the KSP, the 'gate-keeper' residue Phe^{435}, which resides at the entrance of the KSP, had to be circumvented. We designed a *trans*-stilbene-like extension, predicted by molecular modeling to present the correct orientation necessary for occupying the KSP, while avoiding Phe^{435} (Fig. 2).

The preparation of various *trans*-stilbene-like analogs is illustrated in Schemes 1 and 2. In order to synthesize a number of analogs efficiently, a parallel synthesis approach was used. Common precursors such as **5**, **6**, and **7** were prepared as the divergent

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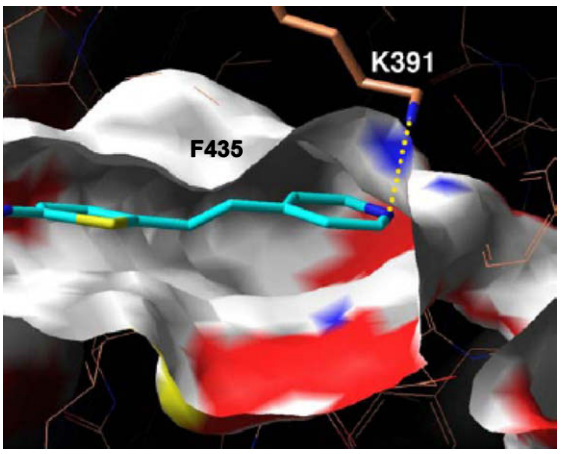
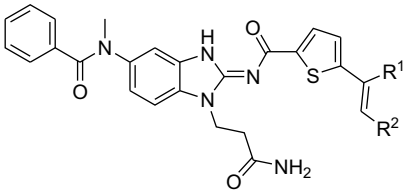




General formula

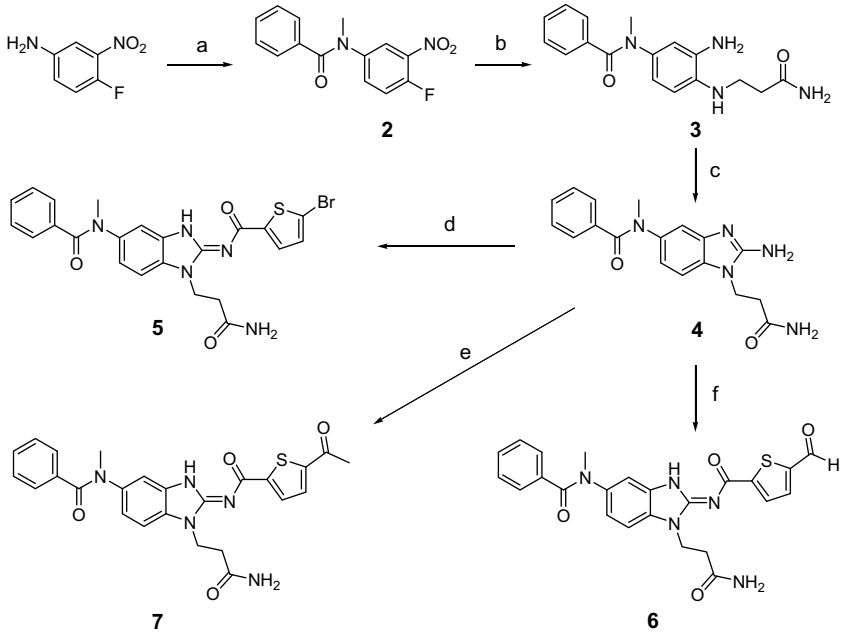
Figure 2. Molecular modeling of **8d** (R^1 = H, R^2 = 4-pyridine). The 4-pyridine ring resides in the KSP may provides hydrogen bonding interactions with amino acid residues such as Lys^{391}.

points for the analogs' syntheses (Scheme 1). Simple acylation of commercially available fluoronitroaniline with benzoyl chloride, followed by methylation of the corresponding amide, gave tri-substituted amide **2** in good yield. SNAr displacement of the fluorine in **2** with β-alanine amide, followed by reduction of nitro group led to aniline **3**. Formation of benzimidazole **4** from amide **3** was accomplished with cyanogen bromide without incident. With benzimidazole **4** in hand, precursors **5**, **6**, and **7** were prepared efficiently by a standard amide coupling reaction with the corresponding acid partners, (5-bromothiophene-2-carboxylic acid, 5-formyl-2-thiophenecarboxylic acid, and 5-acetylthiophene-2-carboxylic acid), respectively.

Starting from intermediates **5**, **6**, and **7**, various types of derivatives featuring different olefinic linkers could be prepared. The syntheses of some representative examples are described in Scheme 2. For the analogs carrying a di-substituted olefinic linker and aromatic end-moieties such as pyridine **8a**, a Heck-type coupling protocol was adopted, using precursor **5** as the starting material, and the corresponding vinyl aromatic system as the coupling partners.[9] For mono-substituted olefin analogs such as **8b**, a Stille-type coupling was performed with precursor **5** and tributylvinyl tin as the starting materials. A Wittig reaction was used for the efficient preparation of nitrile **8c** from precursor **6**. Compounds bearing a tri-substituted olefin linker, such as **9a** and **9b**, were synthesized from precursor **7**, starting with a Wittig protocol to yield **9a**. Bromide **9a** could then become the precursor for the preparation of several compounds featuring aromatic moieties, such as **9b**, In this case, palladium-catalyzed coupling reactions with the corresponding boronic acids were carried out.

The analogs were tested in the ITK DELFIA assay[10a] to determine the intrinsic binding affinity, a Ca^+ flux assay with the DT40/ITK cell line[10b] to determine the cell activity, and also in an IRK DELFIA assay for selectivity. The results are summarized in Tables 1 and 2.

For di-substituted olefinic type of inhibitors (Table 1), introduction of the olefinic linker with small substitutions such as **8b**, **8c**, and **8e**, showed more than 5-fold improvement on molecular and cellular activity of ITK and superior selectivity over IRK compared with the lead compound **1**. Phenyl substitution analog **8f** however only maintained potency and selectivity profile as **8b**, though we had hypothesized that the aromatic system should had occupied the KSP and further enhanced the compound profile. Extensive studies on the substitution pattern on the aromatic ring were initiated. Electronic effect on the binding was the first factor to be investigated. Compounds with an electron donating group (**8h**, **8i**, and **8j**) or an electron withdrawing group (**8k**, **8l**, **8m**, and **8r**) on all positions on the aromatic ring were synthesized. All of these



Scheme 1. General synthetic route for common precursors. Reagents and conditions: (a) i—benzoyl chloride, K_2CO_3, EtOAc, rt, 98%; ii—NaH, MeI, THF, 0 °C to rt, 80%; (b) i—β-alanine amide, *i*-Pr_2NEt, DMF, 80 °C, 75%; ii—10% Pd/C, H_2, EtOH, 100%; (c) cyanogen bromide, EtOH, rt, 88%; (d) 5-bromothiophene-2-carboxylic acid, EDCI, HOBt, *i*-Pr_2NEt, DMF, rt, 93%; (e) 5-acetylthiophene-2-carboxylic acid, EDCI, HOBt, *i*-Pr_2NEt, DMF, rt, 90%; (f) 5-formyl-2-thiophenecarboxylic acid, EDCI, HOBt, *i*-Pr_2NEt, DMF, rt, 83%.