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2-Aminobenzimidazoles as potent ITK antagonists: de novo design of a pyrrole system targeting additional hydrogen bonding interaction

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ABSTRACT

Based on information from molecular modeling, a series of 2-aminobenzimidazoles with pyrrole moieties were designed and synthesized as ITK antagonists. Results showed that a significant improvement of intrinsic and cell-based potency was achieved. X-ray crystallographic analysis of an inhibitor complex with ITK confirmed the prediction from the de novo design that the pyrrole moiety of the inhibitor would form an additional hydrogen bonding interaction with Glu436 in the catalytic domain, and hence improve overall binding affinity of the inhibitor.

During the quest for novel anti-inflammatory agents and mechanisms which could be used on the treatment of inflammatory disease, such as allergic asthma, rheumatoid arthritis, and multiple sclerosis, we pursued Interleukin-2 inducible T-cell kinase (ITK)[1] as a promising target.

In our recent search for novel small molecule inhibitors for ITK,[2] we discovered a series of potent, selective ITK antagonists featuring 2-aminobenzimidazole scaffold as a key pharmacophore. Lead compound such as **1** (Fig. 1) exhibited a good activity profile: IC_{50}'s were determined to be 140 nM in an ITK DELFIA assay[3a] for the intrinsic binding affinity and 780 nM in an Insulin Receptor Kinase (IRK) DELFIA assay for the selectivity. However, compound **1** was not active ($IC_{50} > 3\ \mu M$) in a DT40/ITK cell assay,[3b] which might be due to the relatively low intrinsic potency of the compound.

Based on an X-ray co-crystal structure of a structurally related ITK inhibitor bound to the human ITK kinase domain,[4] we realized that beside the double 'backbone' hydrogen bonding interactions between the amide carbonyl group of Met^{438} and the N–H of the benzimidazole core, Glu^{436} might be able to provide an additional hydrogen bonding interaction by its amide moiety.

Next to Glu^{436}, there was an unoccupied kinase specificity pocket (KSP). The KSP is about 7.3 Å deep (measuring from the 'gate-keeper' Phe^{435} to Met^{410}) and contains hydrophobic (Leu^{418}, Leu^{489} and Val^{419}) and hydrophilic residues (Lys^{391}, Glu^{406}, Met^{410} and Asp^{500}). In order to access the KSP from the thiophene side of the inhibitor effectively, the design had to take into consideration structural modifications that would avoid creating steric interactions with Phe^{435}. Molecular modeling suggested that a compound such as **2** which features a pyrrole moiety off the 2-amide position could potentially act as a hydrogen bonding donor through interactions with the carbonyl group of Glu^{436} as shown in Figure 2. Furthermore, an additional hydrogen bond could also be achieved through interactions with Lys^{391} and the carbonyl group off the 3-position of the pyrrole ring. Thirdly, the 'pyrrole-carbonyl-aromatic' substitution pattern might provide the correct angle necessary to avoid Phe^{435} and occupy the KSP. This would provide an opportunity for additional interactions with various amino acids (Ser^{499}, Asp^{500} and Glu^{406}) within the pocket.

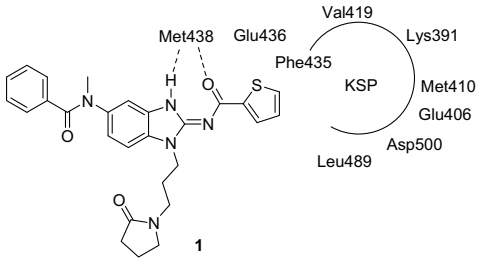

Figure 1. Illustration of the binding mode of **1** in ITK.

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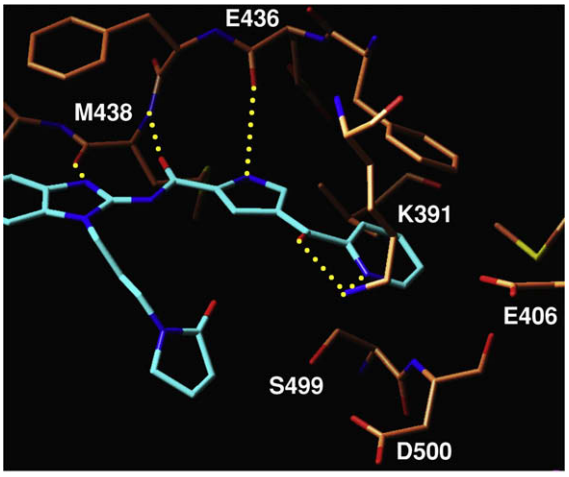


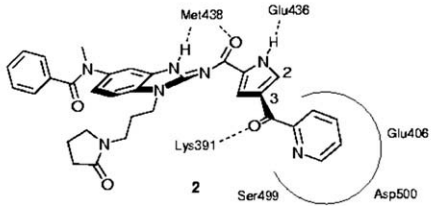
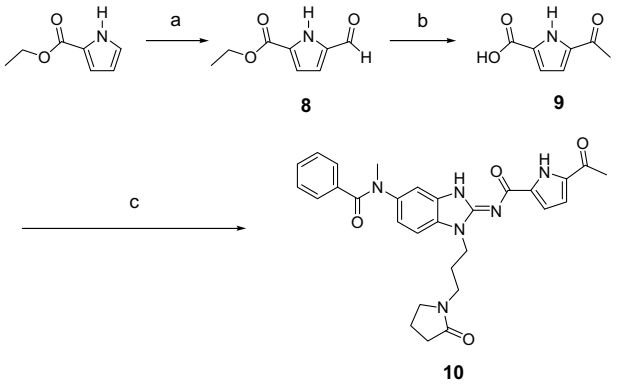


Figure 2. Molecular modeling and diagram show the binding mode of **2** in ITK (orange: ITK; grey: **2**).

Successful design of this pyrrole type of compounds was anticipated to improve binding affinity for ITK and selectivity over IRK.

The synthetic routes toward the 2-aminobenzimidazole-pyrrole type of compounds were shown in Schemes 1 and 2. For the synthesis of 3-substituted pyrrole analogs, such as **7** (Scheme 1), commercially available 4-fluoro-3-nitroaniline was used as the starting material. Acylation of 4-fluoro-3-nitroaniline with benzoyl chloride followed by methylation of the resulting amide gave amide **3** in good yield. SNAr displacement of the fluorine in **3** with 1-(3-aminopropyl)-2-pyrrolidinone followed by transition metal reduction of the nitro group provided diamine **4**. The formation of the 2-aminobenzimidazole core **5** was carried out with cyanogen bromide in good yield. Pyrrole moiety **6** was prepared by firstly performing a Friedel–Craft acylation[5] of ethyl pyrrole-2-carboxylate with benzoyl chloride, followed by acid hydrolysis of the corresponding ester. With both 2-aminobenzimidazole **5** and pyrrole **6** in hands, the amide coupling reaction gave final target **7** in modest yield.

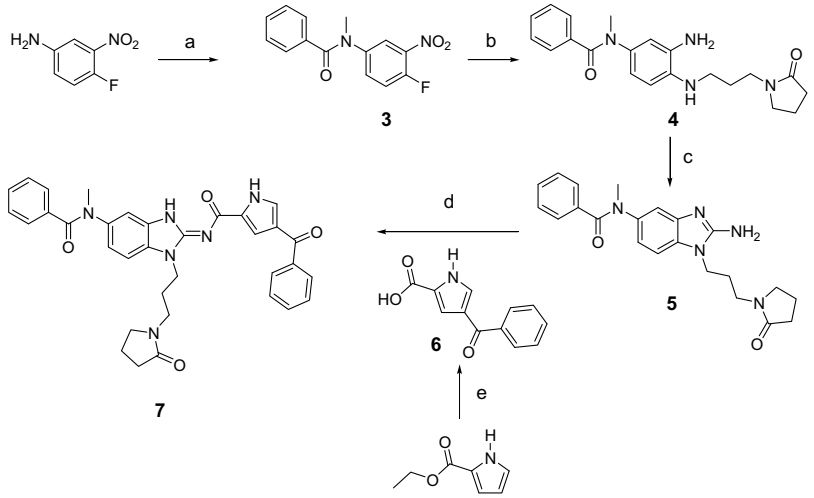


Scheme 2. Synthetic route for the preparation of 2-substituted pyrrole type of compound. Reagents and conditions: (a) $POCl_3$, DMF, DCE, 70%; (b) (i) MeMgBr, THF, 91%; (ii) Dess–Martin periodinane, 96%; (iii) 1 N NaOH, MeOH, 74%; (c) **5**, EDCI, HOBt, iPr$_2$NEt, DMF, 60%.

For 2-substituted pyrrole analogs, such as **10** (Scheme 2), the preparation of the pyrrole moiety started from α-formylation of ethyl pyrrole-2-carboxylate to give aldehyde **8**. The addition of a methyl group with the use of Grignard reagent followed by oxidation and acid hydrolysis gave 3-acetylpyrrole-carboxylic acid **9** in excellent yield. Simple amide coupling reaction between **9** and 2-aminobenzimidazole **5** provided the target 2-substituted acetyl pyrrole **10**.

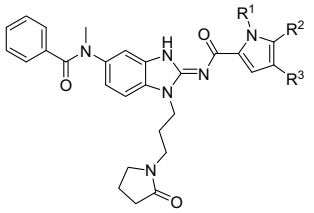
The analogs were tested in an ITK DELFIA assay to determine the intrinsic binding affinities; an IRK DELFIA assay to determine the selectivity; and a DT40/ITK Ca^+ flux cell assay to determine the cell activities. Selected compounds from the series were also tested in a functional assay[6] measuring IL-2 inhibition using human $CD4^+$ T-cells stimulated with anti-CD-3 and anti-CD-28 mAbs. The results were shown in Table 1.

As predicted from the modeling study, the introduction of a pyrrole moiety proved to be beneficial in terms of binding affinity. 3-Nitropyrrole **11** and bromopyrrole **13** exhibited significant improvements in both molecular and cellular activities compared



Scheme 1. Synthetic route for 3-substituted pyrrole analogs. Reagents and conditions: (a) (i) benzoyl chloride, K_2CO_3, EtOAc, rt, 98%; (ii) NaH, MeI, THF, 0 °C to rt, 80%; (b) (i) 1-(3-aminopropyl)-2-pyrrolidinone, iPr$_2$NEt, DMF, 80 °C, 70%; (ii) Pd/C, H_2, EtOH, 100%; (c) cyanogen bromide, EtOH, rt, 80%; (d) **6**, EDCI, HOBt, iPr$_2$NEt, DMF, rt, 60%; (e) (i) benzoyl chloride, $AlCl_3$, CH_2Cl_2, 0 °C, 76%; (ii) NaOH, MeOH, 100%.

Table 1
Results of selected 2-aminobenzimidazole-pyrrole analogs



Compounds	R^1	R^2	R^3	ITK IC_{50}[a] (μM)	IRK IC_{50}[a] (μM)	Ca^+flux IC_{50}[a] (μM)	IL-2 inhibition IC_{50}[a] (μM)
1	—	—	—	0.140	0.78	>3	
11	H	H	NO_2	0.024	0.89	0.77	
12	CH_3	H	NO_2	3.9	>5	>5	
13	H	H	Br	0.018	0.28	0.89	
14	CH_3	H	H	>5	>5	>5	
15	H	H	Acetyl	0.026	>5	1.6	
10	H	Acetyl	H	0.077	>5	>5	
7	H	H	Benzoyl	0.021	4.1	0.19	0.97
16	H	H	2-Fluorobenzoyl	0.025	4	0.19	1.5
17	H	H	2-Methylbenzoyl	0.1	>5	>5	

[a] Values are means of three experiments.

to the lead compound **1**. Unfortunately, direct comparison with **1** could not be accomplished due to the chemical instability of the corresponding unsubstituted pyrrole analog. In general, electron-withdrawing group had to be incorporated into the pyrrole ring in order to increase the overall chemical stability of the inhibitor.

To test our hypothesis that the pyrrole N–H could indeed act as a donor for the hydrogen bonding, *N*-methyl pyrrole analogs such as **12** and **14** were prepared as a counter proof. As the modeling predicted, both compounds showed a significant loss in binding affinity compared to **11** and **1**, respectively. Additionally, the methyl group might induce an unfavorable steric hindrance, which would be anticipated to lead to a loss of activity.

Next, the opportunity for additional hydrogen bonding by the carbonyl substitution on either 2- or 3-position of the pyrrole ring was investigated. Introduction of an acetyl group in the 2- and 3-position of the pyrrole ring gave acetylpyrrole compounds **10** and **15,** respectively. Both compounds showed comparable molecular potency with 3-substitution leading to slightly more potent inhibitors (26 nM vs 77 nM). However, there was no significant improvement of potency by the introduction of this additional carbonyl group compared to the bromo-substituted pyrrole **13**. Incorporation of benzoyl group in the 3-position of the pyrrole ring (**7**) retained molecular potency and a 4-fold improvement in the cell activity compared to bromopyrrole **13**.

To investigate the possibility of occupying the KSP by the phenyl group of compound **7** (IC_{50} = 21 nM), substitution patterns on the benzene ring were explored. Selected from all of the analogs that were synthesized for this purpose, the *o*-substituted phenyl analogs provided the most interesting results. First of all, an electron-withdrawing group such as fluoro in **16** did not alter the binding affinity of the inhibitor. However, with the introduction of steric hindrance, such as methyl group in **17,** the binding affinity of the inhibitor diminished significantly. This result pointed to the direction that the benzene ring in **7** was positioned in a very confined environment that even a small change in the torsional angle between the carbonyl and the benzene ring would affect the binding affinity of the inhibitor. With the promising result of benzoyl pyrrole **7** in a functional assay (IC_{50} = 0.97 μM), compared to the lack of activity (>5 μM) of lead compound **1**, along with an improved IRK selectivity, the occupation of the KSP seemed to be a very possible scenario.

To verify the binding mode of benzoyl pyrrole **7**, an X-ray co-crystal structure was generated. As shown in Figure 3, the benzimidazole core resided near Met^{438} and formed two backbone hydrogen bonds with the kinase as predicted. Encouragingly, the crystal structure showed that the carbonyl group in Glu^{436} was within hydrogen bonding distance (3.1 Å) to the pyrrole nitrogen, indicating that an additional hydrogen bond had indeed been established. This accounts for the improvements in the potencies and IRK selectivity over lead compound **1**. However, to our disappointment, the benzoyl group in the crystal structure did not occupy the KSP (region around Leu^{418}, Met^{410}, Phe,^{501} and Asp^{500} in the figure). The benzene ring rotated away from the KSP. Also, LYS^{391} was disordered and could not possibly form extra hydrogen bond with the carbonyl group on the inhibitor. This result explained the lack of further improvement in binding affinity of the inhibitors upon incorporation of carbonyl substitutions.

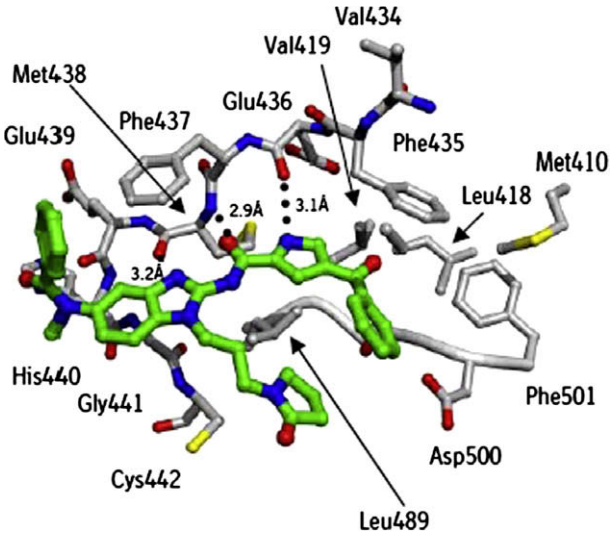


Figure 3. Crystal structure of **7** in ITK: (green: **7**; grey: ITK; only in the key amino acid moieties in the KSP were shown).

In conclusion, a series of 2-benzimidazole-pyrrole type ITK inhibitors were developed by de novo design aiming for additional hydrogen bonding interactions. Experimental results demonstrated a significant improvement of binding affinity (7-fold increase compared to lead compound **1**. Finally, an X-ray co-crystal

structure of one of the most potent compounds in the series confirmed the predicted binding mode, revealing that an additional hydrogen bonding interaction was indeed established with the introduction of pyrrole moiety. However, the KSP was not occupied by the design. Further refinement of the modeling and synthetic efforts in the program will allow better design for the occupation of the KSP and hence will provide superior ITK inhibitors in the future.

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5. Representative procedure for Friedel–Craft acylation: To a solution of ethyl pyrrole-2-carboxylate (200 mg, 1.4 mmol) in CH_2Cl_2 (10 mL), were added benzoyl chloride (0.3 mL, 2.8 mmol) and $AlCl_3$ (375 mg, 2.8 mmol) at 0 °C under nitrogen atmosphere. The solution was warmed to room temperature for 12 h. The solution was cooled back to 4 °C and saturated $NaHCO_3$ solution (10 mL) was added. The solution was extracted with CH_2Cl_2, and the organic layer was collected and dried with $MgSO_4$. The solution was filtered and concentrated. The residue was then dissolved in MeOH/H_2O (10 mL/2 mL). 1 N NaOH (2 mL) was added, and the solution was stirred at room temperature for 12 h. The pH of the solution was adjusted to 6 by the addition of 0.1 N HCl. The solution was then concentrated, and the residue was purified by column chromatography to afford benzoyl pyrrole **6** (230 mg, 76%) as a white solid.
6. CD4+ T-cells are isolated from whole blood by positive selection. Purified T-cells are activated through the TCR and CD28 via anti-CD3 and anti-CD28 mAbs. Compounds are diluted in 10% DMSO to a final concentration of .25% DMSO at every dose. 50,000 cells are added in 100 μL of media/well followed by 100 μL of compound or DMSO alone. Cells are incubated overnight at 37 °C, and then the supernatants are analyzed for IL-2 with the R&D Systems IL-2 ELISA kit (Cat.#D2050) following a 1:10 dilution. IC_{50}'s are calculated by SAS.