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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

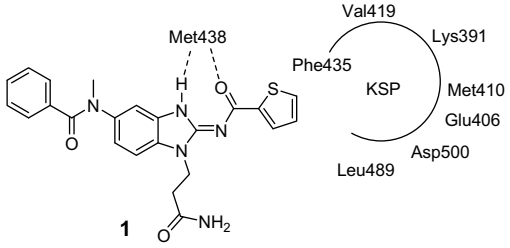

Figure 1. The structure and binding mode of **1** in ITK.

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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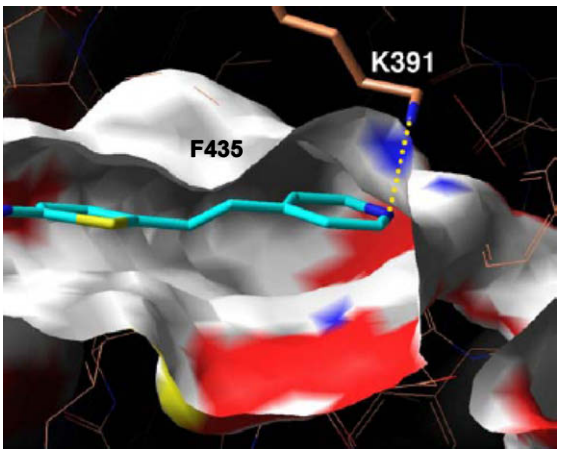
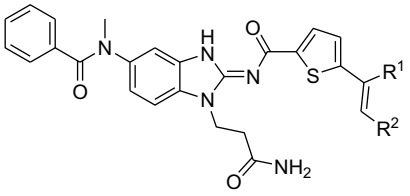




General formula

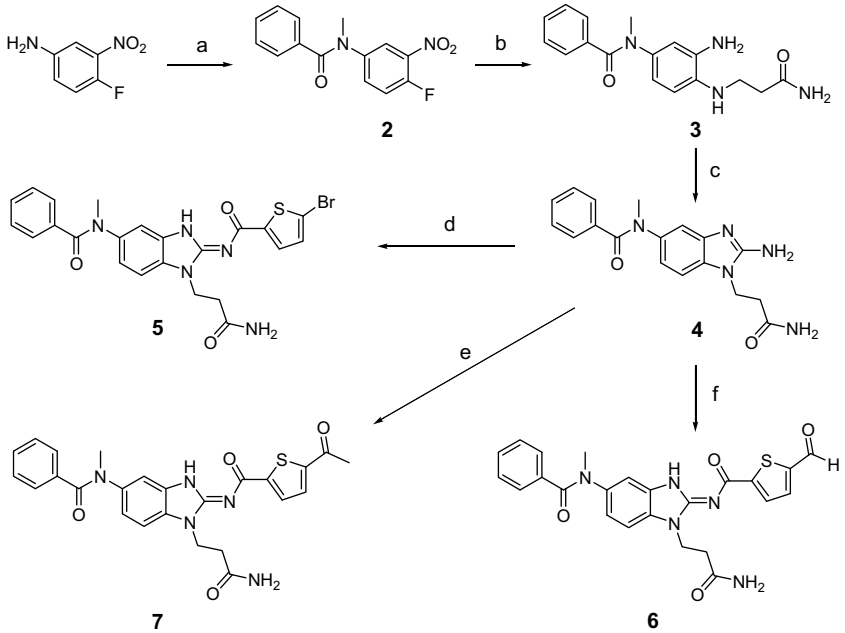
Figure 2. Molecular modeling of **8d** (R^1 = H, R^2 = 4-pyridine). The 4-pyridine ring resides in the KSP may provides hydrogen bonding interactions with amino acid residues such as Lys^{391}.

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.

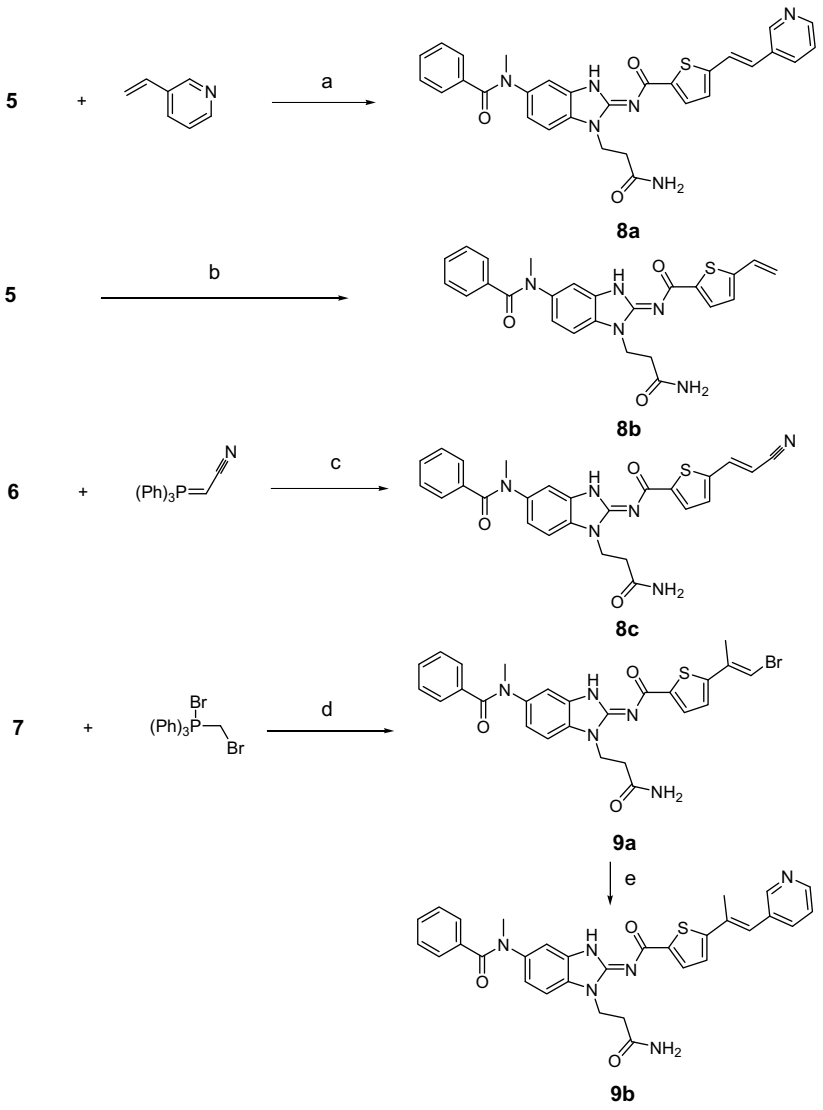
Starting from intermediates **5**, **6**, and **7**, various types of derivatives featuring different olefinic linkers could be prepared. The syntheses of some representative examples are described in Scheme 2. For the analogs carrying a di-substituted olefinic linker and aromatic end-moieties such as 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%.



Scheme 2. Synthetic routes for representative targets. Reagents and conditions: (a) $Pd_2(dba)_3$, (tBu)$_3$P, *N*-methyl-dicyclohexylamine, Et_4NCl, DMF, 100 °C, 12 h, 30%; (b) Tributylvinyl tin, $Pd(PPh_3)_4$, 100 °C microwave, 1 h, 54%; (c) THF, rt, 12 h, 50%; (d) nBuLi, THF, 0 °C, 60%; (e) pyridine-3-boronic acid, Cs_2CO_3, 120 °C, 12 h, 54%.

compounds showed inferior molecular potency compared with parent phenyl analog **8f,** and there was no significant difference between compounds with electron-donating groups and electron-withdrawing groups. We hypothesized that the loss of activity was mainly due to steric hindrance imposed by the substitutions. The next modification evaluated was the effect of introducing hydrogen bond donors or acceptors separately at each position on the phenyl ring. A hydrogen bonding acceptor such as pyridine (**8g**, **8a**, and **8d**) offered no advantage over phenyl analog **8f**, and the 4-pyridine analog **8d** showed a 10-fold decrease in molecular potency compared with **8g** and **8a**. A hydrogen bonding donor such as amine (**8n**, **8p**, and **8q**) showed a similar profile as the pyridine analogs. There is no obvious impact on the binding affinity with either hydrogen bonding donors or acceptors in that region of the molecule.

Different heterocycles bearing hydrogen bond donors were also introduced to fine tune the affinity of the inhibitors. Compounds such as aminopyridine **8w** and aminopyrimidine **8x** offered no improvement over the parent aminophenyl analogs **8f**. However, introduction of smaller heterocycles such as imidazole and pyrazole provided an advantage. Significant improvement in cell activity was achieved with pyrazole **8v** (0.28 μM), which was also the most potent compound in the series in terms of cell activity; though with only moderate selectivity over IRK.

The idea of a tri-substituted olefinic linker was based on the hypothesis that the presence of an extra methyl group could provide additional steric hindrance between the olefinic linker and the thiophene ring, which in turn would generate a different turning angle toward the KSP. Representative compounds of this type were synthesized and tested. The results are summarized in Table 2. To our disappointment, the 'tri-substituted' analogs were generally weaker in terms of binding affinity toward ITK when compared with to their 'di-substituted' partners. The reason behind the loss of binding affinity might be due to the steric hindrance between the newly introduced methyl group and the gatekeeper Phe^{435}.

Some of the of potent compounds in the 'di-substituted' series were tested in a functional assay [11], in which IL-2 production was measured using human CD4$^+$ T-cells stimulated with anti-CD3 and anti-CD28 mAbs. These compounds (**8v** and **8x**) had IC_{50} values in the low-micromolar range. Compared with the lead compound **1**, which had no inhibitory activity ($IC_{50} > 5$ μM) when tested, the improvement of molecular activity by the series did translate to the cellular functional assay.

Table 1
Results of di-substituted inhibitors' activities with different R^2 (refer to general formula in Fig. 2)

Compound	R^1	R^2	ITK IC_{50}[a] (μM)	IRK IC_{50}[a] (μM)	Ca^+ flux IC_{50}[a] (μM)	IL-2 inhibition IC_{50} (μM)
1	—	—	0.025	0.145	2.4	>5
8b	H	H	0.007	0.48	0.38	
8c	H	CN	0.005	3.25	0.39	
8e	H	COOMe	0.010	>5	0.54	
8f	H	Ph	0.011	0.97	0.62	
8g	H	2-Pyridine	0.006	4.00	0.76	
8a	H	3-Pyridine	0.005	>5	0.69	
8d	H	4-Pyridine	0.065	>5	0.99	
8h	H	2-MeO-Ph	0.067	>5	>5	
8i	H	3-MeO-Ph	0.013	>5	1.90	
8j	H	4-MeO-Ph	0.058	>5	>5	
8k	H	2-CF_3-Ph	0.270	>5	>5	
8l	H	3-CF_3-Ph	0.030	>5	>5	
8m	H	4-CF_3-Ph	0.130	>5	>5	
8n	H	2-NH_2-Ph	0.006	1.95	0.62	
8p	H	3-NH_2-Ph	0.009	4.90	1.40	
8q	H	4-NH_2-Ph	0.006	3.30	0.57	
8r	H	3-NO_2-Ph	0.042	4.30	>5	
8s	H	4-OH-Ph	0.009	2.20	0.74	
8t	H	2-Pyrazine	0.008	>5	0.81	
8u	H	1-Imidazole	0.012	>5	2.2	
8v	H	4-(1*H*)-Pyrazole	0.009	0.33	0.28	3
8w	H	6-Pyridine-2-ylamine	0.007	3.8	2	
8x	H	5-Pyrimidin-2-ylamine	0.005	1.5	0.41	1.6

[a] Values are means of three experiments.

Table 2
Results of tri-substituted inhibitors' activities with different R^2 (refer to general formula in Fig. 2)

Compound	R^1	R^2	ITK IC_{50}[a] (μM)	IRK IC_{50}[a] (μM)	Ca^+ flux IC_{50}[a] (μM)
9c	Me	H	0.037	>5	0.29
9d	Me	CN	0.063	4.50	0.29
9e	Me	COOMe	0.039	3.60	1.10
9f	Me	Ph	0.075	>5	3
9b	Me	3-Pyridine	0.039	>5	1.6
9h	Me	4-Pyridine	0.059	>5	0.83

[a] Values are means of three experiments.

Concerning the selectivity over other Tec kinase family, compound **8x** showed high activity on BTK (5 nM) and moderate selectivity over TXK (700 nM). However the implication of the selectivity over other Tec kinase on the indication (inflammation) is unclear at this point.

In conclusion, a series of *trans*-stilbene-like 2-aminobenzimidazole inhibitors were designed and synthesized. The intrinsic potencies, cellular activities, and selectivity of the compounds were improved when compared with the lead compound. Furthermore, some of the top compounds showed activity in the IL-2 functional assay. It is predicted that the improvement in activity is due to partial occupancy of the KSP. Further studies in this area are underway.

References and notes

1. Smith, C. I.; Islam, T. C.; Mattsson, P. T.; Mohamed, A. J.; Nore, B. F.; Vihinen, M. *Bioessays* **2001**, *23*, 436.
2. Forssell, J.; Sideras, P.; Eriksson, C.; Malm-Erjefält, M.; Rydell-Törmänen, K.; Ericsson, P.; Erjefält, J. S. Am. *J. Respir. Cell. Mol. Biol* **2005**, *32*, 511.
3. Schaeffer, E. M.; Yap, G. S.; Lewis, C. M.; Czar, M. J.; McVicar, D. W.; Cheever, A. W.; Sher, A.; Schwartzberg, P. L. *Nat. Immune* **2001**, *2*, 1183.
4. Schaeffer, E. M.; Debnath, J.; Yap, G.; McVicar, D.; Liao, X. C.; Littman, D. R.; Sher, A.; Varmus, H. E.; Lenardo, M. J.; Schwartzberg, P. L. *Science* **1999**, *284*, 638.
5. Fowell, D. J.; Shinkai, K.; Liao, X. C.; Beebe, A. M.; Coffman, R. L.; Littman, D. R.; Locksley, R. M. *Immunity* **1999**, *11*, 399.
6. Kanner, S. B.; Perez-Villar, J. J. *Trends Immunol.* **2003**, *24*, 249.
7. (a) Snow, R. J.; Abeywardane, A.; Cywin, C. L.; Lord, J.; Kashem, M. A.; Khine, H. H.; Kowalski, J. A.; Pullen, S. S.; Roth, G. P.; Sarko, C. R.; Wilson, N. S.; Winters, M.; Wolak, J. P. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3660; (b) Winters, M. P.; Robinson, D. J.; Khine, H. H.; Pullen, S. S.; Woska, J. R., Jr.; Raymond, E. L.; Sellati, R.; Cywin, C. L.; Snow, R. J.; Kashem, M. A.; Wolak, J. P.; King, J.; Kaplita, P. V.; Liu, L. H.; Farrell, T. M.; DesJarlais, R.; Roth, G. P.; Takahashi, H.; Moriaty, K. J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5541; (c) Moriarty, K. J.; Takahashi, H.; Pullen, S. S.; Khine, H. H.; Sallati, R. H.; Raymond, E. L.; Woska, J. R., Jr.; Jeanfavre, D. D.; Roth, G. P.; Winters, M. P.; Qiao, L.; Ryan, D.; DesJarlais, R.; Robinson, D.; Wilson, M.; Bobko, M.; Cook, B. N.; Lo, H. Y.; Nemoto, P. A.; Kashem, M. A., et al *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5545; (d) Moriarty, K. J.; Winters, M.; Qiao, L.; Ryan, D.; DesJarlis, R.; Robinson, D.; Cook, B. N.; Kashem, M. A.; Kaplita, P. V.; Liu, L. H.; Farrell, T. M.; Khine, H. H.; King, J.; Pullen, S. S.; Roth, G. P.; Magolda, R.; Takahashi, H. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5537.
8. White, A.; Abeywardane, A.; Cook, B. N.; Fuschetto, N.; Gautschi, E.; John, A.; Kroe, R. R.; Kronkaitis, A.; Li, Xiang; Pullen, S. S.; Roma, T.; Moriarty, K. J.; Roth, G. P.; Snow, R. J.; Studts, J. M.; Takahashi, H.; Farmer, B.T. II.; *J. Biol. Chem*, submitted for publication.
9. *Representative procedure for the Heck type coupling reactions:* To a solution of bromothiophene 5 (50 mg, 0.095 mmol) in DMF (5 mL) were added 2-vinyl pyridine (0.02 mL, 0.19 mmol), $Pd_2(dba)_3$ (9 mg, 0.01 mmol), tri-*tert*-butylphosphine (21 mg, 0.095 mmol), *N*-methyl-dicyclohexylamine (0.02 mL, 0.095 mmol) and Et_4NCl (16 mg, 0.095 mmol). The solution was heated to 100 °C in a sealed tube for 12 h. The reddish-brown solution was cooled down and 3-mercaptopropyl-functionalized silica gel (100 mg) was added. The mixture was stirred for 10 min and was filtered. The filtrate was washed with water and extracted with EtOAc. The organic layer was concentrated and the residue was purified by column chromatography with 5% MeOH in CH_2Cl_2 as the eluent to afford vinyl pyridine 8 g (39 mg, 75%) as colorless oil.
10. (a) Kashem, M. A.; Nelson, R. M.; Yingling, J. D.; Pullen, S. S.; Prokopowicz, A. S., III; Jones, J. W.; Wolak, J. P.; Rogers, G. R.; Morelock, M. M.; Snow, R. J.; Homon, C. A.; Jakes, S. J. *Biomol. Screening* **2007**, *12*, 70; (b) Takata, R.; Kurosaki, T. *J. Exp. Med.* **1996**, *184*, 31.
11. $CD4^+$ 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. Fifty thousand cells are added in 100 μL of media/well followed by 100 μL of Compound or DMSO alone. Cells incubate overnight at 37 °C and then the supernatants are analyzed for IL-2 with the R&D Systems IL-2 ELISA kit (Cat. No. D2050) following a 1:10 dilution. IC_{50}s are calculated by SAS.