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Discovery of pyrimidine benzimidazoles as Src-family selective Lck inhibitors. Part II

Guobao Zhang *, Pingda Ren, Nathanael S. Gray, Taebo Sim, Xia Wang, Yi Liu, Jianwei Che, Weitong Dong, Shin-Shay Tian, Mark L. Sandberg, Tracy A. Spalding, Russell Romeo, Maya Iskandar, Zhiliang Wang, H. Martin Seidel, Donald S. Karanewsky, Yun He *,†

Genomics Institute of the Novartis Research Foundation (GNF), 10675 John Jay Hopkins Drive, San Diego, CA 92121, USA

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ABSTRACT

A series of 4-amino-6-benzimidazole-pyrimidines was designed to target lymphocyte-specific tyrosine kinase (Lck), a member of the Src-family kinases (SFKs). These type II inhibitors were optimized using a cellular Lck-dependent proliferation assay and are capable of inhibiting Lck at single-digit nanomolar concentrations. This scaffold is likely to serve a valuable template for developing potent inhibitors of a number of SFKs.

Lymphocyte-specific kinase (Lck) is a member of the Src-family of protein tyrosine kinases. Lck plays a critical role in the initial steps of T-cell receptor (TCR) signaling.[1] Activation of TCR signaling by Lck triggers a cascade of downstream signaling pathways, leading to the production of cytokines such as interleukin-2 (IL-2) and interferon-γ.[2,3] Lck is an attractive drug target because of its restricted expression in T-cells and natural killer (NK) cells.[4] Selective inhibitors of Lck would be expected to have an improved safety profile over current immunosuppressive agents, which invariably have non-lymphocyte related toxicities. Therefore, development of selective Lck inhibitors offers a promising approach for treating T-cell mediated autoimmune diseases and chronic transplantation rejection.[5] Selective Lck inhibitors have been shown to prolong the survival of major histocompatibility mismatched allografts in preclinic animal models of solid organ transplantation [6].

Protein kinase inhibitors are generally classified into three types based on their binding modes.[7] Type I kinase inhibitors bind only to the ATP binding region. Type II protein kinase inhibitors bind to both ATP and an adjacent hydrophobic pocket, whereas type III protein kinase inhibitors bind only to an allosteric binding site. Both type I and type II Lck inhibitors have been reported in the literature.[6,8] In our previous communication, we reported the design and synthesis of pyrimidine benzimidazoles as type I Lck inhibitors.[9] To take advantage of the known ability of Lck to be inhibited by ATP-competitive inhibitors that bind to the 'DFG-out' conformation of the activation loop (type II), we extended our effort to exploit this binding conformation. Herein we report the design, synthesis, SAR, and kinase selectivity profilings of a series of 4-amino-6-benzimidazole-pyrimidines as type II Lck inhibitors.

To develop type II Lck inhibitors, we followed the strategies described by Okram et al.[10] Briefly, a R^2 group was introduced to target the Lck hydrophobic binding site (Fig. 1). Compounds **7a**–**7n** were synthesized according to Scheme 1. First, nucleophilic substitution of 4,6-dichloropyrimidine (**1**) with 2-chlorobenzimidazole (**2**) was carried out in *N*,*N*-dimethylforamide in the presence of so-

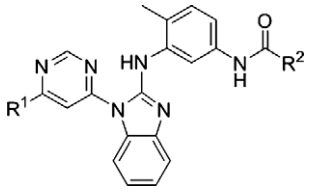

Figure 1. General structure of type II Lck inhibitors based on pyrimidine benzimidazoles.

* Corresponding authors. Tel.: +1 858 812 1614; fax: +1 858 812 1684 (G.Z.); tel.: +86 10 80768611; fax: +86 10 80703150 (Y.H.).
E-mail addresses: gzhang@gnf.org (G. Zhang), yun.he@bioduro.com (Y. He).
† Present address: Bioduro (Beijing) Co. Ltd, Building E, 29 Life Science Park Road, Changping District, Beijing 102206, China.

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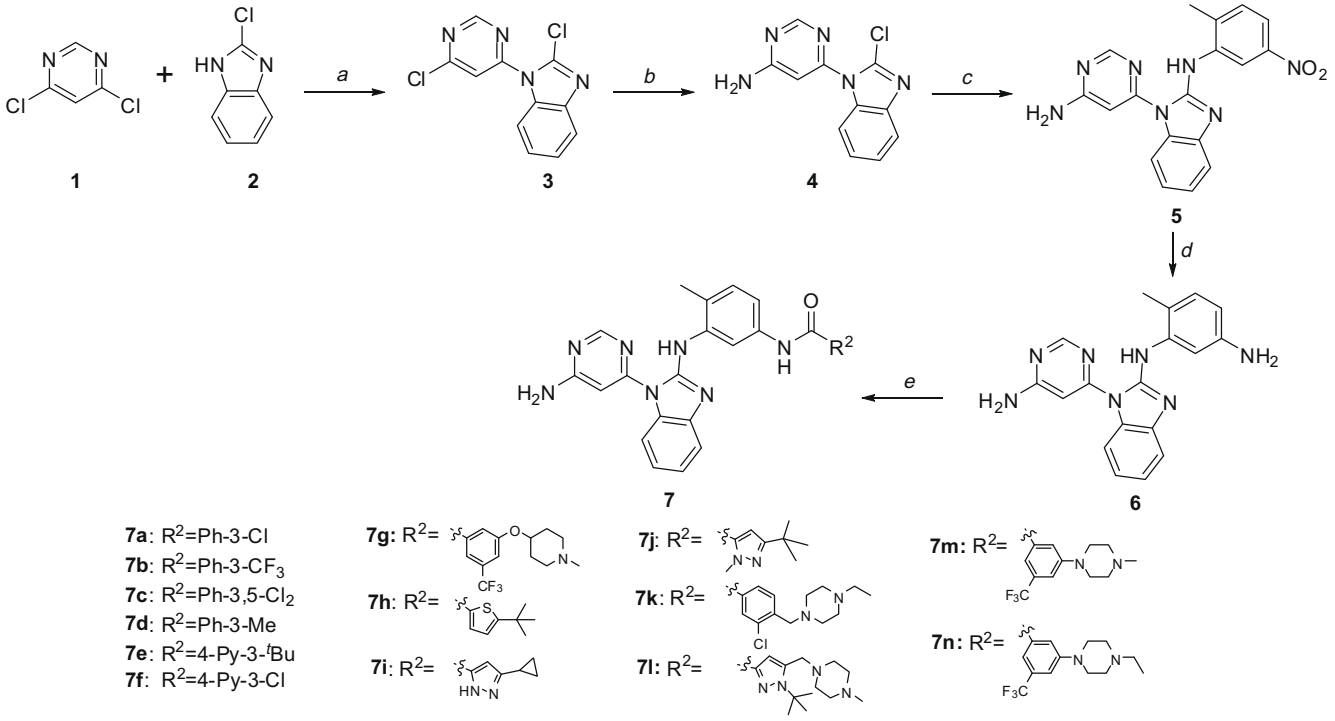


Scheme 1. Synthesis of compounds **7a**–**7n**. Reagents and conditions: (a) NaH (1.5 equiv), DMF, 0–25 °C, 12 h, 56%; (b) NH_3 (excess), isopropanol, 50 °C, 12 h, 92%; (c) 2-methyl-5-nitroaniline (1.5 equiv), CH_3SO_3H (2.0 equiv), *N*,*N*-dimethylimidazolinone, 90 °C, 2 h, 83%; (d) H_2, Pd/C, ethanol, 25 °C, 4 h, 89%; (e) RCO_2H (1.2 equiv), HATU (1.2 equiv), DIEA (3.0 equiv), DMF, 25 °C, 2 h, 60–95%.

dium hydride to give compound **3** in 56% yield. Selective amination of the chloropyrimidine ring was readily achieved by reacting compound **3** with excess ammonia in isopropanol at 50 °C to give compound **4** in 92% yield. The 2-chlorobenzimidazolegroup in compound **4** was then reacted with 2-methyl-5-nitroaniline in the presence of methanesulfonic acid to give compound **5** 83% yield. The nitro group of compound **5** was subsequently reduced under hydrogenation conditions to give compound **6** in 89% yield. Compound **6** was then reacted with various commercial and in-house made benzoic acids in the presence of HATU and diisopropylethylamine to give the target compounds **7a–7n**.

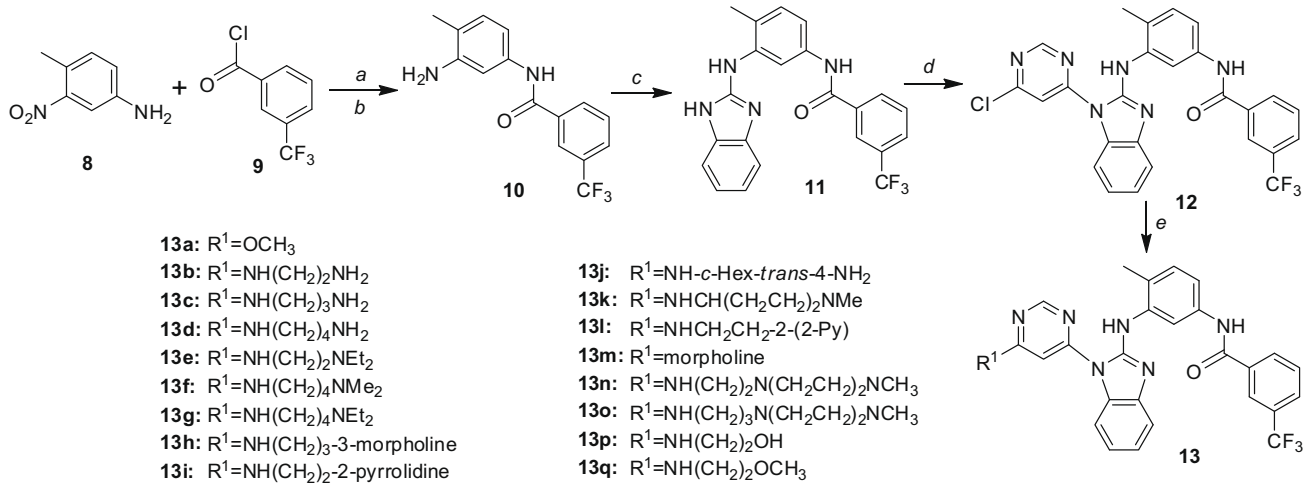
Compounds **7a–7n** were tested for their Lck inhibitory activities using both biochemical (LcK Lance) and cellular (BaF3/Tel-Lck) assays as previously described.[9] Activity against several other protein tyrosine kinases was also measured and the data are summarized in Table 1. In general, these pyrimidine benzimidazole compounds show strong inhibition of Lck in biochemical and cellular assays. Large hyrophobic substituents, for example, *t*-Butyl (**7e**, **7h**, and **7i**), can be tolerated in the 'DFG-out' binding pocket. The Lck inhibition potency is improved as the size of the substituent becomes bigger (**7a, 7d, 7b, 7e,** and **7f**). Heterocycles are also tolerated in the allosteric binding site (**7e, 7f, 7h, 7i**, and **7l**). The discrepancy between biochemical and cellular data of compound **7c** is likely due to its poor cell permeability because it has high *C*log *P* (6.56). The apparent cellular activity of compound **7h** is due to its general cytotoxicity (IC_{50} = 6 nM in Ba/F3 parental cell line) presumably due to engagement of additional kinases. Hydrophilic substitutions on the right phenyl ring are also well tolerated (**7g**, **7k–7n**).

Most of these compounds also display good selectivity over Src in both biochemical and cellular assays. However, their selectivity over Hck is very low. Inhibition of Hck might have undesirable effects on myeloid cell function because it is expressed broadly in hematopoietic cells.[11] Among them, compound **7b** shows very

Table 1
Biochemical and cellular kinase inhibition activity (IC_{50} nM) of compounds **7a–7n**

Compounds	Lck Lance	Hck Lance	Src Lance	BaF3/Tel-LCK	BaF3/Tel-LYN	BaF3/Tel-SRC	BaF3/Tel-KDR	BaF3/Tel-InsR
7a	132	475	1650	84	133	650	576	1988
7b	11	43	450	7	14	78	36	661
7c	58	121	530	1074	3608	9693	6365	>10,000
7d	70	245	1539	32	82	490	280	1493
7e	10	46	ND*	7	7	56	ND*	ND*
7f	715	>2500	ND*	890	595	>10,000	ND*	ND*
7g	24	27	233	7	5	39	66	746
7h	43	84	916	4	5	4	4	4
7i	120	446	7359	50	96	56	48	75
7j	316	844	7188	224	332	959	451	1961
7k	4	9	32	5	10	47	30	63
7l	8	11	177	18	88	249	63	818
7m	11	20	130	3	6	47	43	409
7n	11	16	71	4	9	57	5	588

* ND = not determined.



Scheme 2. Synthesis of compounds **12**–**13q**. Reagents and conditions: (a) DIEA (1.5 equiv), DCM, 25 °C, 3 h; (b) H_2, Pd/C, ethanol, 25 °C, 4 h, 85% in two steps; (c) 2-chlorobenzimidazole (1.0 equiv), CH_3SO_3H (2.0 equiv), *N*,*N*-dimethylimidazolinone, 90 °C, 2 h, 88%; (d) 4,6-dichloropyrimidine (2.5 equiv), NaH (1.5 equiv), DMF, 0 °C to 80 °C, 1 h, 80%; e) RNH_2 (3.0 equiv), isopropanol, 50 °C, 1 h, 80–95%.

potent Lck inhibition in both biochemical and cellular assays. This compound also displays 40-fold selectivity against Src and 4-fold selectivity against Hck in the biochemical (Lance) assay. Therefore, compound **7b** was chosen for further optimization.

To explore the SAR on R^1 (Fig. 1), with a focus on improving aqueous solubility and selectivity against Hck, we fixed R^2 as 3-(trifluoromethyl)phenyl and synthesized a series of analogues **13a–13q** as outlined in Scheme 2. Briefly, aniline **10** was prepared by coupling 4-methyl-3-nitro-aniline (**8**) with 3-(trifluoromethyl)benzoyl chloride (**9**) in the presence of base followed by Pd/C catalyzed hydrogenation. Reaction of 2-chlorobenzimidazole with **10** under acid catalysis gave aminoimidazole **11** in excellent yield. Compound **11** was then coupled to 4,6-dichloro-pyrimidine (**1**) in the presence of sodium hydride to provide key intermediate **12** for derivatization. Compound **12** was then reacted with various amines or methanol to give compounds **13a–13q**.

Table 2 summarizes the SAR at the R^1 position. Not surprisingly, compounds **12**, **13a**, and **13m** are inactive or have very weak Lck inhibition because they lack the hydrogen bond-donating NH group at the pyrimidine 6 position, which is important for binding to the Lck hinge region. In the Lck Lance assay, compounds **13f**, **13g**, and **13o** are similar in potency to compound **7b**. However, all the other compounds display weaker Lck inhibition. The potency drop is consistent with our previous results and can be explained by the conformational analysis described in our previous communication.[9] Molecular modeling studies (Fig. 2) suggest that the basic nitrogen in compounds **13f**, **13g**, and **13o** can participate in an electrostatic interaction with the carboxylic acid of Glu320 (Lck numbering) on Lck. Sequence alignment shows that Glu320 is not conserved across other Scr-family protein tyrosine kinases. Therefore, compounds **13f**, **13g**, and **13o** display better Src-family kinase selectivity compared to compound **7b**. In fact, most of the compounds with R^1 bearing a basic nitrogen atom display better selectivity over Hck (Lance data) compared to compound **7b**. It is also interesting to note that the electrostatic interaction weakens as the alkyl spacer is shortened from four carbons to two carbons (**13b–13d**, Lck lance data). These data suggest that this non-conserved Glu320 can provide a valuable handle for improving the selectivity over Hck.

Unfortunately, all of the basic amine compounds **13a–13q** showed relatively weak cellular activity in comparison to compound **7b**. We believe this is due to decreased cellular permeability, since the compounds with the largest discrepancy between enzymatic and cellular activity have polar substituents; for example, –OH in compound **13p** and $–NH_2$ in compounds **13c**, **13d**, and **13j** (Table 2).

Table 2
Kinase inhibition activity (IC_{50} nM) of compounds **12**–**13q**

Compounds	Lck Lance	Hck Lance	Src Lance	BaF3/Tel-LCK	BaF3/Tel-LYN	BaF3/Tel-SRC	BaF3/Tel-KDR	BaF3/Tel-InsR
7b	11	43	450	7	14	78	36	661
12	1162	3826	16,507	2702	3296	1220	9069	3332
13a	2030	>2500	>2500	>10,000	>10,000	>10,000	>10,000	>10,000
13b	230	>2500	ND	470	1471	1288	1748	1573
13c	23	1960	ND	387	1117	1322	1472	2768
13d	13	345	ND	366	457	1661	599	3760
13e	111	378	>2500	418	642	398	1726	1141
13f	5	65	139	108	399	1211	1365	3109
13g	12	430	97	41	217	438	885	3012
13h	26	49	165	40	67	600	318	4156
13i	38	805	>2500	238	818	903	941	1087
13j	71	450	1722	392	547	739	609	1443
13k	168	>2500	>2500	881	1422	1378	1712	1613
13l	204	917	2370	171	259	524	549	>10,000
13m	>2500	>2500	>2500	>10,000	>10,000	>10,000	>10,000	>10,000
13n	29	69	305	56	204	918	605	1435
13o	6	31	128	25	149	601	582	3162
13p	27	192	1920	229	400	1698	590	4401
13q	135	405	1688	132	126	606	296	>10,000

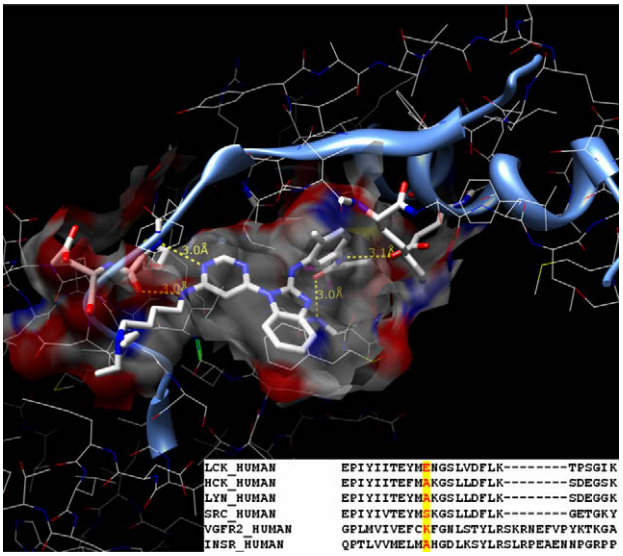
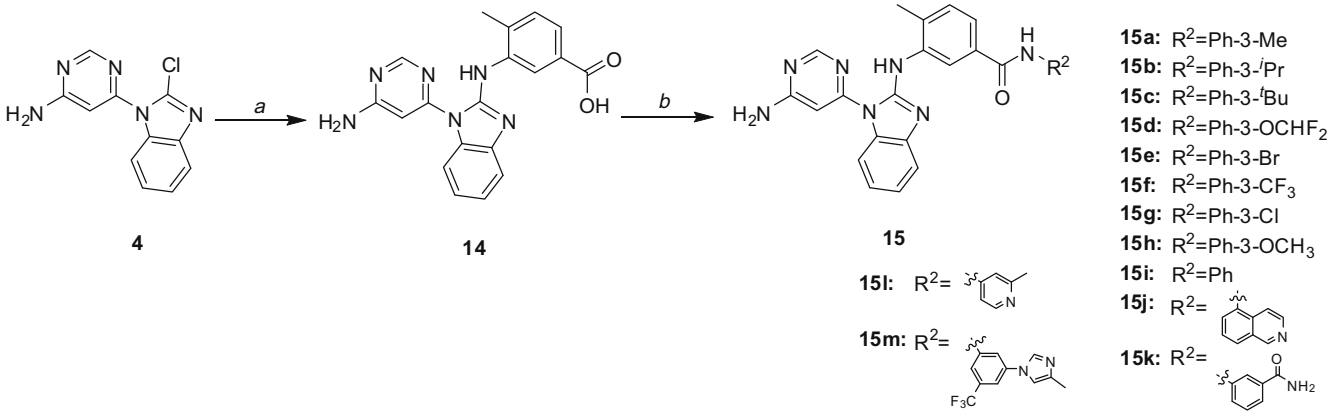


Figure 2. Binding mode of compound **13g** to Lck based on modeling studies. The sequences of several other protein tyrosine kinases of interest are aligned with Lck and partial sequences around the protein kinase hinge region are shown. The non-conserved Glu320 in Lck and its corresponding residues in other protein tyrosine kinases are highlighted.

In mouse pharmacokinetics (PK) studies, compounds **13g** and **13j** showed very low plasma exposure, with AUC value of 368 h nM and 0 h nM, respectively, following 20 mg/kg PO dosing, despite having good in vitro metabolic stability, with mouse extraction ratio of <0.3 (**13g**) and 0.383 (**13j**). However, compound **13q** displayed much improved plasma exposure (AUC = 2687 h*nM) in a mouse PK study following the same dosing. These data further support the hypothesis that compounds bearing a basic nitrogen atom have low cell permeability. Thus, despite the success in achieving enzymatic Hck selectivity through inclusion of a basic amine as part of the R^1 group, we were forced to seek an alternative approach to improving Hck selectivity.

By using the versatile intermediate **4**, we were able to quickly synthesize compounds **15a–15k** (Scheme 3) to explore how a 'reversed' amide bond affects Lck inhibition potency and kinase selectivity. These compounds are hereafter referred to as reverse amides and the previous compounds as normal amides. The Lck inhibition data of these reverse amides are shown in Table 3. Generally, reverse amides have comparable or slightly weaker Lck inhibition than the normal amides. However, the kinase selectivity profiles are considerably improved (**15a** vs **7d**, **15g** vs **7a**, **15f** vs **7b**). Compound **15a** is especially interesting because of its superior kinase selectivity. The SAR on the right phenyl ring substitutions is quite similar to that of normal amides, and hydrophobic substitutions are preferred for inhibitory activity (**15b–c** vs **15a**, **15d** vs **15h**, **15e** vs **15g**). The compound without substitution on the right phenyl ring (**15i**) has very weak Lck inhibition while analogs **15j** and **15k** which bear hydrophilic substituents, lose Lck inhibitory activity significantly.

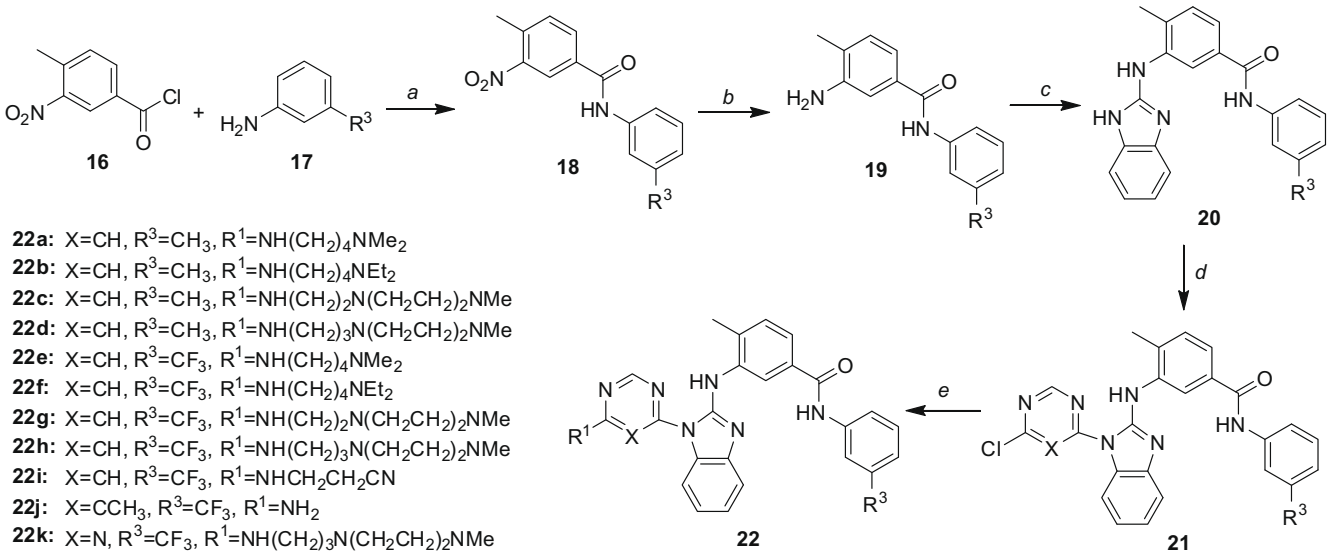
With R^3 fixed as methyl or trifluoromethyl, compounds **22a–22k** (Scheme 4) were synthesized in a similar fashion to compounds **13a–13q** except that the amide bond direction was reversed. With R^3 fixed as methyl, compounds **22a–22d** showed



Scheme 3. Synthesis of reverse amides **15a–15m**. Reagents and conditions: (a) 3-amino-4-methyl-benzoic acid (1.2 equiv), CH_3SO_3H (2.0 equiv), *N*,*N*-dimethylimidazolinone, 80 °C, 12 h, 82%; (b) RNH_2 (2.0 equiv), DIEA (3 equiv), DMF, 25–60 °C, 2 h, 40–90%.

Table 3
Kinase inhibition activity (IC_{50} nM) of compounds **15a–15m**

Compounds	Lck Lance	Hck Lance	Src Lance	BaF3/Tel-LCK	BaF3/Tel-LYN	BaF3/Tel-SRC	BaF3/Tel-KDR	BaF3/Tel-InsR
15a	52	>2500	ND	83	1079	7173	1015	7121
15b	30	151	ND	7	15	21	17	29
15c	22	139	ND	16	16	202	52	1224
15d	17	>2500	ND	47	100	>10,000	92	>10,000
15e	43	>2500	ND	52	272	5375	464	5629
15f	59	>2500	ND	20	15	>10,000	55	>10,000
15g	63	>2500	ND	76	>10,000	>10,000	260	>10,000
15h	90	>2500	ND	157	437	4389	516	5435
15i	1080	>2500	ND	2280	5512	6168	8817	5935
15j	616	>2500	ND	3241	7836	7613	10,577	5708
15k	>2500	>2500	ND	>10,000	>10,000	>10,000	>10,000	>10,000
15l	177	>2500	ND	702	>10,000	>10,000	135	>10,000
15m	238	904	ND	14	28	107	20	7631



Scheme 4. Synthesis of compounds **22a–22k**. Reagents and conditions: (a) DIEA (1.5 equiv), DCM, 25 °C, 3 h; (b) H_2, Pd/C, ethanol, 25 °C, 4 h, 85–90% in two steps; (c) 2-chloro-benzimidazole (1.0 equiv), CH_3SO_3H (2.0 equiv), *N*,*N*-dimethylimidazolinone, 90 °C, 2 h, 85–90%; (d) 4,6-dichloropyrimidine or 2,4-dichloro-1,3,5-triazine (2.5 equiv), NaH (1.5 equiv), DMF, 0–80 °C, 1 h, 60–71%; (e) RNH_2 (3.0 equiv), isopropanol, 50 °C, 1 h, 80–95%.

Table 4
Kinase inhibition activity (IC_{50} nM) of compounds **22a–22k**

Compounds	Lck Lance	Hck Lance	Src Lance	BaF3/Tel-LCK	BaF3/Tel-LYN	BaF3/Tel-SRC	BaF3/Tel-KDR	BaF3/Tel-InsR
22a	22	>2500	ND	531	1384	1268	1497	2628
22b	20	1810	ND	406	1315	1065	1364	2263
22c	38	>2500	ND	762	1658	1537	2094	3486
22d	19	1875	ND	337	1228	1409	1113	3194
22e	15	56	485	38	134	556	249	1221
22f	18	475	274	52	140	461	243	1808
22g	11	130	458	45	113	527	185	1506
22h	18	260	494	23	84	364	117	1856
22i	1367	>2500	ND	275	468	1223	245	1092
22j	2400	>2500	>2500	4456	8012	>10,000	8181	>10,000
22k	12	54	ND	20	43	117	133	1365

improved Lck enzymatic inhibition activity compared to compound **15a** (Table 4). However, their cellular potencies are decreased significantly. This SAR is consistent with the normal amides series and has been rationalized in a previous publication.[9] Compound **22i** showed very weak Lck inhibition, while compounds **22e–22h** showed improved Lck inhibitory activity over compound **15f** in both the enzymatic assay and the cellular assay, suggesting that the basic amine in the left side is very important for Lck inhibition. Also, compound **22f** showed very good Lck inhibition potency as well as protein kinase selectivity.

Finally, we conducted a brief investigation of pyrimidine ring variants. Triazine **22k** has slightly improved Lck inhibition activity compared with the corresponding pyrimidine **22h**. However, the kinase selectivity of compound **22k** is decreased. The apparent cellular activity of compound **22k** is partially due to its general cytotoxicity (IC_{50} = 1.33 μM in Ba/F3 parental cell line). Introduction of a methyl group at the C5 position of the pyrimidine (**22j**) essentially eradicates inhibition activity at Lck and all other kinases assayed. This phenomenon is consistent with our previous results and has been explained by conformational analysis.[9]

In summary, a series of very potent type II Lck inhibitors was discovered through rational design. Highly selective Lck inhibitors were prepared through SAR-guided optimization and structure-based drug design. Some of these Lck inhibitors (e.g., **15a** and **22f**) display good selectivity over non-Src-family kinases as well as Src-family kinases.

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