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Imidazo[1,5-*a*]quinoxalines as irreversible BTK inhibitors for the treatment of rheumatoid arthritis

Kyung-Hee Kim [a,*], Andreas Maderna [a], Mark E. Schnute [a], Martin Hegen [b], Shashi Mohan [b], Joy Miyashiro [b], Laura Lin [b], Evelyn Li [b], Sean Keegan [b], Jennifer Lussier [b], Christopher Wrocklage [b], Cheryl L. Nickerson-Nutter [b], Arthur J. Wittwer [b], Holly Soutter [c], Nicole Caspers [c], Seungil Han [c], Ravi Kurumbail [c], Kyri Dunussi-Joannopoulos [b], John Douhan III [b], Allan Wissner [a]

[a] *Medicinal Chemistry, Pfizer, 200 Cambridge Park Drive, Cambridge, MA 02140, United States*
[b] *Inflammation and Autoimmunity, Pfizer, 200 Cambridge Park Drive, Cambridge, MA 02140, United States*
[c] *Structural Biology and Biophysics, Pfizer, 100 Eastern Point Road, Groton, CT 06340, United States*

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ABSTRACT

Imidazo[1,5-*a*]quinoxalines were synthesized that function as irreversible Bruton's tyrosine kinase (BTK) inhibitors. The syntheses and SAR of this series of compounds are presented as well as the X-ray crystal structure of the lead compound **36** in complex with a gate-keeper variant of ITK enzyme. The lead compound showed good in vivo efficacy in preclinical RA models.

Bruton's tyrosine kinase (BTK), a nonreceptor cytoplasmic tyrosine kinase belonging to the Tec kinase family, has been shown to be critical for B-cell proliferation, differentiation, and signaling.[1] In human, defects in the BTK gene lead to X-linked agammaglobulinemia (XLA), which is characterized by a profound decrease in B cell numbers and in serum immunoglobulin levels. BTK mutation in mice resulted in X-linked immunodeficiency (xid), a similar though less severe phenotype than XLA. Since B cells play an essential role in regulating immune response, inhibitors of BTK may be effective in treating autoimmune diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). The evidence that B cells play a central role in immune dysregulation in RA is substantiated by the observation of clinical improvements in RA patients receiving B-cell depleting antibody therapies such as Rituximab. Currently, there are no small molecule BTK inhibitors in clinical use for the treatment of autoimmune diseases. In this communication, we will describe the discovery of a series of small molecule inhibitors of BTK having efficacy in in vivo preclinical models of RA.

Since the ATP binding site in kinases is highly conserved, it is often difficult to find an ATP competitive inhibitor having sufficient selectivity. In the present case, the ATP binding site of BTK shows a close homology to that of other Src-family kinases, such as lymphocyte-specific protein tyrosine kinase (LCK) and LYN. A lack of selectivity of an inhibitor for BTK over these kinases could potentially have negative consequences. BTK is a member of a group of eleven tyrosine kinases (the Tec family kinases, EGFR, Jak3, ErbB2, ErbB4, and BLK) that contain a conserved cysteine residue adjacent to the ATP-binding site.[2] This cysteine (Cys 481 in BTK) is a potential nucleophilic site which could form a covalent adduct with an electrophilic inhibitor. As the inhibitory activity of such an inhibitor is dependent on the covalent interaction, this cysteine residue could provide a handle for achieving the desired degree of selectivity.[2a] Previously PCI-32765 (Fig. 1) has been reported to be a potent and selective irreversible BTK inhibitor that binds covalently to Cys 481 and is efficacious in animal cancer models. It is currently under clinical development in patients with B cell non-Hodgkin lymphoma.[3]

Our series of irreversible BTK inhibitors is based on the imidazo[1,5-*a*]quinoxaline scaffold (Fig. 1). Some compounds of this type have been previously reported as LCK inhibitors.[4] Using molecular modeling, we predicted that a Michael acceptor attached via an amide linkage to the C8 position of this core structure

* Corresponding author. Tel.: + 1 617 665 5647; fax: +1 617 665 5682.
E-mail addresses: khkim87@hotmail.com, kyung-hee.kim@pfizer.com (K.-H. Kim).

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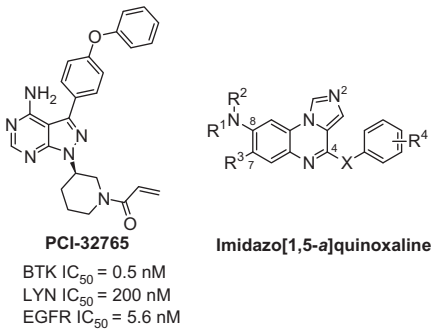
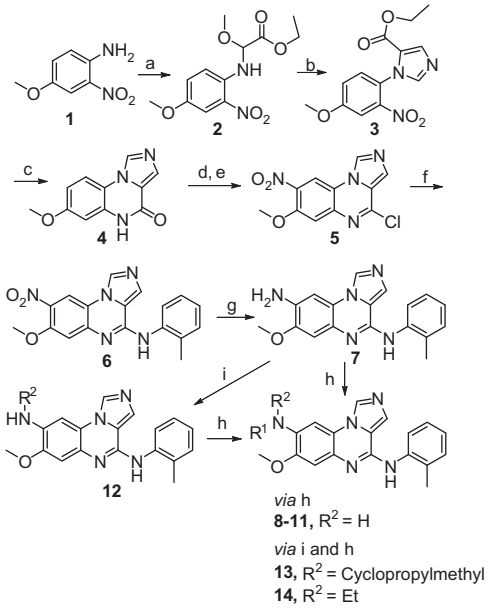


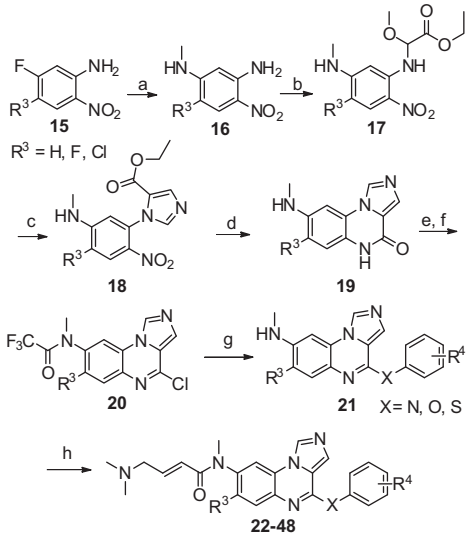
Figure 1. The structure of the BTK inhibitor PCI-32765 and the proposed imidazoquinoxaline BTK inhibitor series.

is properly oriented to allow a covalent interaction with Cys 481 of the BTK enzyme. With this in mind, we decided to pursue the synthesis of members of this series. The synthesis resulted in irreversible BTK inhibitors having high potency, good selectivity relative to the Src-family kinases, and good in vivo efficacy in models of RA.

The syntheses of this class of compounds are illustrated in Schemes 1 and 2 where the core structure was prepared using a method previously reported.[5] As shown in Scheme 1, the reaction of 4-methoxy-2-nitroaniline **1** with ethyl glyoxylate provided compound **2** which was then reacted with Tosmic to give the imidazole **3**. Imidazole **3** was reduced with sodium hydrosulfite and cyclized to give imidazo quinoxalinone **4**. Selective nitration at the C8-position was achieved using ammonium nitrate in sulfuric acid; in this reaction, it is important to use no more than 1 equiv of nitrate in order to avoid over nitration. The carbonyl group was converted to chloride to give chloro derivative **5**. The reaction of this imidazo quinoxaline **5** with *ortho*-toluidine provided the nitrogen linked arylated compound **6**. The nitro group on compound **6** was reduced to amino group to obtain compound **7** which was acylated to install a Michael acceptor providing the final compounds **8**–**11**. In order to prepare compounds with an alkyl group on the nitrogen at C8 position, compound **7** was reacted



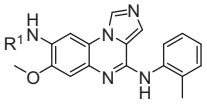
Scheme 1. Reagents: (a) 50% ethyl glyoxylate in toluene, methanol, reflux; (b) Tosmic, K_2CO_3, abs ethanol, reflux; (c) $Na_2S_2O_4$, acetic acid/water, reflux; (d) NH_4NO_2, H_2SO_4; (e) $POCl_3$, reflux; (f) *o*-toluidine, pyridine–HCl (cat.), amyl alcohol, reflux; (g) H_2, Pd/C, methanol; (h) acid chloride or mixed anhydride; (i) R^2Br, Et_3N, DMF.

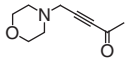
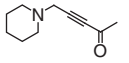
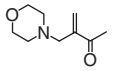
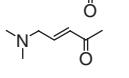


Scheme 2. Reagents: (a) CH_3NH_2, 40% in water, THF, reflux; (b) 50% ethyl glyoxylate in toluene, methanol, reflux; (c) Tosmic, K_2CO_3, abs ethanol, reflux; (d) $Na_2S_2O_4$, acetic acid/water, reflux; (e) Hunig's base, TFA-anhydride, rt; (f) $POCl_3$, reflux; (g) Method A: anilines, pyridine–HCl (cat.), amyl alcohol reflux, and then, KOH, water/methanol, Method B: phenols, Cs_2CO_3 (3 equiv), DMF, or Method C: thiols, Cs_2CO_3 (3 equiv), DMA; (h) Method A: (*E*)-4-(dimethylamino)but-2-enoic acid HCl (1.0 equiv), $POCl_3$ (1.0 equiv), −15 °C, DMA or Method B: (*E*)-4-(dimethylamino)but-2-enoic acid HCl, oxalyl chloride, DMF(trace amount), acetonitrile/DMF.

with corresponding alkyl bromides to give intermediates **12** and then subjected to acylation with the Michael acceptor to produce the compounds **13** and **14**. An alternative synthetic route for additional compounds is outlined in Scheme 2. As shown in Scheme 2, three different starting materials **15** were used which differed depending on the substituent on C7 position at the final compounds. The methyl amino group was installed from the beginning using methylamine to provide compounds **16**. The later steps were similar to the corresponding steps shown on Scheme 1 except for the introduction of a trifluoroacetyl protecting group at the step e to provide intermediates **20** after chlorination. Various nitrogen, oxygen, or sulfur linked aryl group were coupled to provide compounds **21**. The methods used to incorporate these substituents at the C4 position also resulted in deprotection of the trifluoroacetamide group. Finally, the Michael acceptor was introduced to produce the final compounds **22**–**48**.

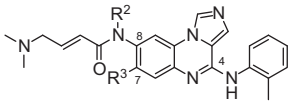
Table 1
Activities of imidazoquinoxaline compounds **8**–**11**



Compound	R^1	BTK IC_{50}[a] (nM)	LYN IC_{50}[a] (nM)
8		0.847	46.6
9		0.847	21.3
10		2.52	297
11		0.884	10.9

For experimental details regarding assay conditions see Supplementary data.
[a] Enzyme IC_{50} values are averages of two or more experiments.

Table 2
Activities of imidazoquinoxaline compounds **11**, **13**, **14**, and **22**



Compound	R^2	R^3	BTK IC_{50}[a] (nM)	LYN IC_{50}[a] (nM)	BTK B cell IC_{50}[a,b] (nM)
11	H	OCH_3	0.884	10.9	NA[c]
13	Cyclopropyl-methyl	OCH_3	45.3	1980	43.15
14	ethyl	OCH_3	19.1	533	16.15
22	methyl	H	3.99	623	3.165

For experimental details regarding assay conditions see Supplementary data.
[a] IC_{50} values are averages of two or more experiments.
[b] IC_{50} for inhibition of B cell proliferation.
[c] Hyper-proliferation was observed.

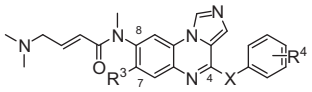
Subsequent to completion of this work, an internally-developed in silico model noted compounds **6**–**14** and **21**–**48** to have some structural similarity to chloracnegenic agents.[6] A representative sample of these compounds was selected for further screening. Compounds **25**, **36**, **42**, and **47** were tested (Xenobiotic Detection Systems, Inc), in an in vitro CALUX assay, which is a sensitive test that uses a genetically engineered mouse cell line containing the firefly luciferase gene under transactivational control of the Aryl-hydrocarbon receptor to screen for potential chloracnegens, such as dioxin and dioxin-like compounds. The compounds tested failed to induce activation of the Ah receptor, and were therefore considered negative for agonist activity (EC_{50} >10 μM compared to TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) with EC_{50} = 0.03 nM).

Our initial efforts were focused on finding a proper Michael acceptor for covalent binding with the BTK enzyme. On the basis of our previous experience with irreversible inhibitors of EGFR/Her2, which have the same conserved Cys residue, various Michael acceptors were examined for covalent binding to Cys 481.[7] As shown in Table 1, compounds **8**–**11** each showed excellent BTK inhibitory activity with good selectivity versus LYN kinase. Compound **11** showed the best combination of properties such as potency, potential reactivity, and physical properties, therefore, the (*E*)-4-(dimethylamino)-*N*-methylbut-2-enamide group was chosen for the further investigation.[7a,c]

As expected, most of the compounds synthesized inhibited the in vitro proliferation of human B cells following stimulation through the B cell receptor with anti-IgM. However, some of the earliest compounds we prepared in this series, for example compound **11**, while functioning as a potent irreversible inhibitor of BTK, did not inhibit B cell proliferation in cell culture, but, on the contrary, appeared to stimulated B cell proliferation. We found that we could overcome this issue simply by placing a small alkyl group on the C8 amide nitrogen (see Table 2). A simple methyl group was effective in eliminating the hyper-proliferation and increased the BTK inhibitory potency. Consequently, all subsequent compounds that we prepared in the series retained the methylated C8 amide nitrogen. We also observed that compounds lacking a substituent at the C7 position retained inhibitory activity (Table 2, compound **22**).

We now turn our attention to modifications of the aryl group on C4 position since we observed that the aryl group was very important for the BTK activity. Compounds lacking an aryl group at this position lost BTK and LYN inhibitory activity nearly completely (Table 3, compound **23**). The goal in optimizing the C4 aryl

Table 3
Activities of imidazoquinoxaline compounds **22**–**48**



Compound	R^3	X	R^4	BTK IC_{50}[a] (nM)	LYN IC_{50}[a] (nM)	BTK B cell IC_{50}[a,b] (nM)
22	H	N	2-Me	3.99	623	3.165
23	H	Cl	No aryl group	4290	>50,000	NA
24	H	N	2-Me, 5-$NHCOC_6H_4$-*m*-CF_3	16.8	1.55	NA
25	H	N	2-Me, 5-F	3.33	1420	4.88
26	H	N	2-Me, 4-F	2.96	716	2.00
27	H	N	2-Me, 3-F	1.89	327	3.17
28	H	N	2-Me, 6-Cl	10.3	44.4	NA
29	H	N	2-Me, 5-Cl	13.2	1670	53.7
30	H	N	2-Me, 4-Cl	7.56	378	4.12
31	H	N	2-Me, 3-Cl	60.6	2320	NA
32	H	N	2-Me, 6-OH	10.4	167	44.9
33	H	N	2-Me, 5-OH	1.55	10.9	2.56
34	H	N	2-Me, 4-OH	12.2	350	1.02
35	H	N	2-Me, 3-OH	76.5	490	NA
36	F	N	2-Me	1.93	624	3.41
37	F	N	2-Cl	1.82	2130	6.75
38	F	N	2-Me, 5-F	6.76	3080	23.5
39	F	N	2-Me, 4-F	5.35	3450	8.08
40	Cl	N	2-Me	4.83	1300	2.17
41	Cl	N	2-Cl	5.03	2000	4.40
42	Cl	N	2-Me, 5-F	3.98	3390	5.84
43	Cl	N	2-Me, 4-F	9.53	1590	2.41
44	F	O	2-Me	5.51	6020	17.4
45	F	O	2-Me, 5-F	13.0	7400	144
46	F	O	2-Me, 4-F	9.53	10,400	32.3
47	H	O	2-Me, 4-F	6.58	3730	228
48	H	S	2-Me	283	19,600	651

For experimental details regarding assay conditions see Supplementary data.
[a] IC_{50} values are averages of two or more determinations.
[b] IC_{50} for inhibition of B cell proliferation.

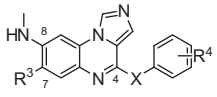
substituent was to identify irreversible BTK inhibitors that would retain high potency in inhibiting the enzyme and in inhibiting B cell proliferation while showing good selectivity with respect to LYN inhibition (Table 3). The initial investigation was on the C7 unsubstituted series. Molecular modeling of our inhibitors having smaller groups attached to the C4 aryl ring suggested that they bound to the enzyme in the DFG-in configuration. Since we were interested in investigating if compounds in our series could also bind to BTK in the DFG-out configuration, we prepared the compound **24** containing a well-known DFG-out type substituent at the C4 position.[8] While this compound retained BTK inhibitory activity, it was found to be more potent for LYN inhibition. As a consequence of this observation, we concentrated our efforts in designing compounds that would only bind in the DFG-in configuration. Molecular modeling predicted that only smaller substituents on the C4 aryl ring would be compatible with the DFG-in binding mode and it was important that at least one of these substituents be located *ortho* to the C4 hetero-atom. Consequently, we retained a methyl or chloro substituent in the *ortho*-position and introduced fluoro, chloro, and hydroxy groups elsewhere on the C4 aryl ring. In general, fluoro substituted compounds (**25**–**27**) showed better BTK activity and selectivity over LYN compared to the chloro or hydroxyl substituted compounds (**28**–**35**). Within the fluoro substituted compounds, 5- or 4-substituents (**25** and **26**) showed better selectivity compared to the 3-F substituent (**27**). A similar trend was observed with the chloro and hydroxyl substituted compounds. Only fluoro substituted compounds **25** and **26** displayed better activity and selectivity relative to mono-substituted compound **22**.

Introduction of fluoro or chloro substituents at C7 position resulted in compounds that retained good BTK inhibitory activity and sometimes improved selectivity over LYN inhibition relative to the C7 unsubstituted analogs (**36**–**43**). We also examined changes to the hetero-atom that linked the C4 aryl ring to the core structure. In general, the oxygen linked compounds showed better selectivity but decreased BTK potency compared to the nitrogen linked compounds (**36** vs **44**, **38** vs **45**, **39** vs **46**, and **26** vs **47**). The sulfur linked compound (**48**) was much less active than oxygen or nitrogen linked compounds.

While our focus is on the identification of an irreversible inhibitor of BTK, we were interested in the potency of the original reversible scaffold. As shown in Table 4 the reversible compounds without a Michael acceptor showed some BTK potency, but they were much less potent than irreversible compounds and, in addition, they were significantly less selective against LYN. This shows the significant impact of the covalent interaction on the BTK inhibitory potency and selectivity.

In order to understand the molecular basis for the observed SAR, we pursued crystallographic studies of a few compounds in the series with a gate-keeper variant (Phe435Thr) of ITK. The ATP-site of BTK shares ~70% sequence identity with ITK which is another TEC family member kinase with ~58% sequence identify across the kinase domain. The crystal structure (Fig. 2) shows that compound **36** binds at the active site by forming two direct hydrogen bonds at the kinase hinge region.[9] The imidazo nitrogen (N2) forms a hydrogen bond with the main chain amide nitrogen of Met 438 in the hinge while the C4-amino group makes another hydrogen bond with side chain oxygen of the gatekeeper Thr 435. In addition, the carbonyl oxygen adjacent to the Michael acceptor forms a water-mediated hydrogen bond with Asp 445. The toluene substituent binds into a hydrophobic pocket formed

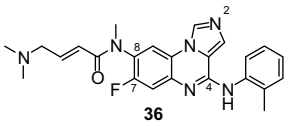
Table 4
Activities of reversible imidazoquinoxaline compounds **21**



21

Compound	R^3	X	R^4	BTK IC_{50}[a] (nM)	LYN IC_{50}[a] (nM)
21a	H	N	2-Me, 5-F	72	40
21b	H	O	2-Me, 4-F	1220	560
21c	F	N	2-Me	40	19
21d	F	O	2-Me	670	130

For experimental details regarding assay conditions see Supplementary data.
[a] IC_{50} values are averages of two or more determinations.



36

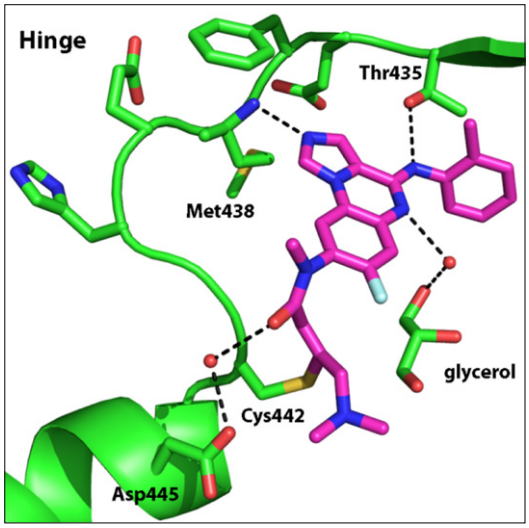


Figure 2. X-ray structure of **36** in complex with a gate-keeper variant of ITK enzyme in the ATP binding site.

Table 5
Selectivity data for compounds **36** and **42**

Compound		BTK IC_{50} (nM)	LYN IC_{50} (nM)	LCK IC_{50} (nM)	SRC IC_{50} (nM)
36	No PI	1.93	624	772	298
	W/ PI	0.7	353	755	84
42	No PI	3.98	3390	NA	1620
	W/ PI	2.6	3020	2790	410

PI = pre-incubation.

Table 6
EGFR Mechanistic differentiation of selected BTK inhibitors

Compound	BTK No PI IC_{50} (nM)	BTK W/PI IC_{50} (nM)	BTK k_{inact}/K_I (1/(M·s))	EGFR No PI IC_{50} (nM)	EGFR W/PI IC_{50} (nM)	EGFR k_{inact}/K_I (1/(M·s))
36	1.93	0.70	53,200	17.2	1.4	101,000
42	3.98	2.6	11,900	68	3.35	22,500

PI = pre-incubation.

Table 7
Pharmacokinetic parameters of **36** and **42**

Compound	**36**		**42**	
(mouse)	iv dose	po dose	iv dose	po dose
Dose (mg/Kg)	2	10	2	10
$AUC_{0-\infty}$ (h ng/mL)	510	876	1125	3622
$T_{1/2}$ (h)	1.5	2.0	2.2	2.7
CL (mL/min/kg)	63		26.6	
V_{ss} (L/kg)	6.2		4.51	
T_{max} (h)		3.0		3.0
C_{max} (ng/ml)		220		454
F (%)		34		58

by Lys 391, Thr 435 and Leu 433 in the DFG-in mode. Also, we observe a tightly-bound glycerol molecule (from the crystallographic cryo-solution) adjacent to the tricyclic core which forms a water mediated hydrogen bond to N5 of the inhibitor. Most significantly, Cys 442 (corresponding to Cys 481 in BTK) makes a covalent interaction with the Michael acceptor as predicted. This was subsequently confirmed by LC-Mass spectrometric studies which showed the formation of a 1:1 stoichiometric complex formed by **36** with BTK.

Unlike reversible inhibitors, irreversible inhibitors typically display a time-dependent inhibition profile due to different enzyme kinetics caused by covalent bond formation or protein conformational changes.[10] In order to shed further light on the kinetics of inhibition, we performed enzymatic assays whereby the compounds were pre-incubated with the enzyme. The compounds in our imidazo[1,5-*a*]quinoxaline series generally show good LYN/LCK/Src selectivities versus BTK in the presence or absence of pre-incubation, suggesting that the observed selectivities are not solely the consequence of the covalent interaction (see Table 5 for selected compounds).

We also monitored cross-over activity of compounds to EGFR which also possesses a Cys residue corresponding to Cys 481 of BTK. Off-target inhibition of EGFR has been observed during clinical use of inhibitors of the ErbB kinase family (which are generally dosed at the maximum tolerated dose (MTD) in treating cancer) and could raise a concern for potential toxicity. We have found that members of imidazoquinoxaline series show less selectivity for EGFR when pre-incubated with the enzyme compared to studies where there is no pre-incubation (see Table 6 for selected compounds). Poor selectivity with respect to EGFR under pre-incubation conditions and a high k_{inact}/K_I ratio[11], which is similar or greater in magnitude to that of BTK, indicates that this series could potentially inhibit EGFR in cells and tissues. However, at this time it is not clear what dose would be needed to show efficacy in treating RA and whether this dose will be high enough for any of the EGFR side effects to be evident in patients treated with such inhibitors. One could speculate that the dose needed to treat RA could be significantly lower than the MTD used to treat cancer and under these conditions the undesirable side effects due to EGFR inhibition might be minimized.

The inhibition of BTK autophosphorylation at pY 223 in Ramos cell by compound **36** was observed (IC_{50} = 48 nM), which is the evidence that the inhibition of B-cell growth is due to the inhibiting BTK.[12] The pharmacokinetic properties of **36** and **42** were evaluated following intravenous and oral delivery in mice. These studies demonstrated that **36** and **42** exhibit favorable pharmacokinetic properties with $T_{1/2}$ of 1.5 h and 2.2 h (iv dose), AUC of 876 and 3622 h ng/mL (po dose), and oral bioavailability (%*F*) of 34 and 58, respectively (Table 7). While the $T_{1/2}$ of these compounds in circulation is relatively short, since these compounds are functioning as irreversible inhibitors, the $T_{1/2}$ in circulation may not be a good measure of the effective half-life, which will be largely determined by the rate of receptor turn over.

In vivo efficacy of **36** was examined in the NP-Ficoll model.[13] NP-Ficoll is a T-independent type 2 (TI-2) antigen which can activate B-cells in the absence of T cell help. BTK-deficient mice are unable to mount TI-2 responses. Female C57BL/6 mice at 8–10 weeks of age were immunized ip with NP(109)-Ficoll.[14] Mice were dosed with compound **36** as indicated or vehicle daily po for 7 days, beginning 1 day prior to immunization. Serum was collected 6 days after immunization and NP-specific IgM and IgG3 antibody titers were measured by ELISA. It is clear that compound **36** demonstrates oral efficacy in the NP-Ficoll model in a dose responsive manner (Fig. 3).

The efficacy of compound **36** was evaluated in the mouse collagen-induced arthritis (CIA) model, an autoimmune disease model which shares pathogenic similarities to human rheumatoid arthritis. The model was performed as previously described.[15] Compound **36** was examined in a semi-therapeutic model, which involved assignment to treatment groups (n = 14/group) when >10% of all the mice exhibited disease. Mice were scored in a blinded manner for disease and dosed orally once each day for 22 days.[16]

Treatment with compound **36** significantly inhibited the progression of the disease compared to the vehicle control at 10 and 3 mg/kg from day 7 and 5, respectively, to the end of the study. The 1 mg/kg regimen was only significant at the end of the study from day 19–22 post-treatment (Fig. 4).

In summary, we have described the synthesis and SAR of a series of imidazo[1,5-*a*]quinoxalines, which function as potent and

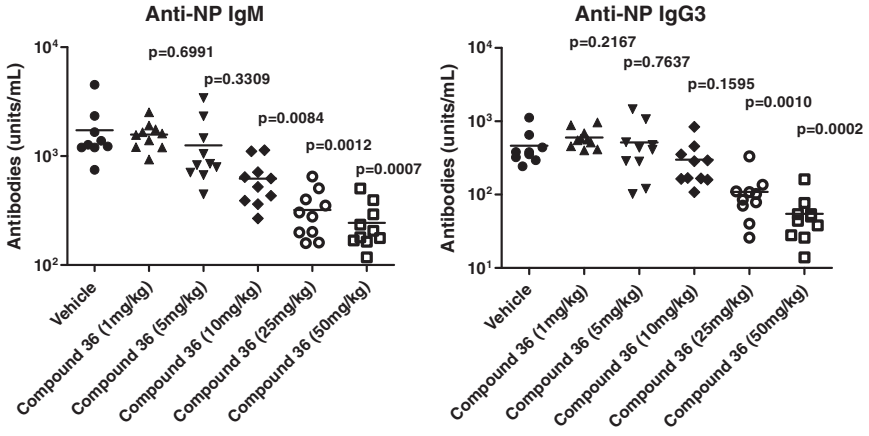


Figure 3. Inhibition of TI-2 antibody responses to NP-Ficoll of **36**.

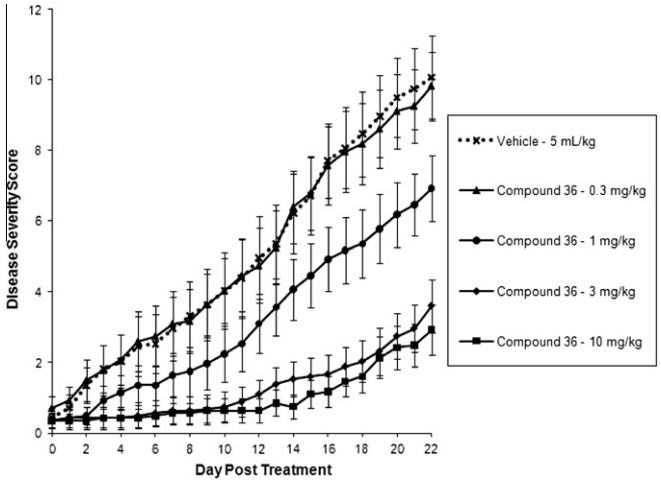


Figure 4. Inhibition of BTK prevents disease in the murine collagen-induced arthritis (CIA) model.[16]

selective irreversible BTK inhibitors. Members of this series show good efficacy in preclinical in vivo models of RA after oral dosing.

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Supplementary data

Supplementary data (experimental details for the biological assays, BTK autophosphorylation data, mass spectroscopic data of binding of compound **36** with BTK, and kinase screening data for compound **36**) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.09.008.

References and notes

1. (a) Niiro, H.; Clark, E. A. *Nat. Rev. Immunol.* **2002**, *2*, 945; (b) Uckun, F. M.; Tibbles, H. E.; Vassilev, A. O. *Anti-Cancer Agents Med. Chem.* **2007**, *7*, 624; (c) Di Paolo, J. A.; Huang, T.; Balazs, M.; Barbosa, J.; Barck, K. H.; Bravo, B. J.; Carano, R. A. D.; Darrow, J.; Davies, D. R.; DeForge, L.; Diehl, L.; Ferrando, R.; Gallion, S. L.; Giannetti, A. M.; Gribling, P.; Hurez, V.; Hymowitz, S. G.; Jones, R.; Kropf, J. E.; Lee, W. P.; Maciejewski, P. M.; Mitchell, S. A.; Rong, H.; Staker, B. L.; Whitney, J. A.; Yeh, S.; Young, W. B.; Yu, C.; Zhang, J.; Reif, K.; Currie, K. S. *Nat. Chem. Biol.* **2011**, *7*, 41; (d) Chang, B. Y.; Huang, M. M.; Francesco, M.; Chen, J.; Sokolove, J.; Magadala, P.; Robinson, W. H.; Buggy, J. J. *Arthritis Res. Ther.* **2011**, *13*, R115. doi:10.1186/ar3400.
2. (a) Cohen, M. S.; Zhang, C.; Shokat, K. M.; Taunton, K. *Science* **2005**, *308*, 1318; (b) Leproult, E.; Barluenga, S.; Moras, D.; Wurtz, J. M.; Winssinger, N. *J. Med. Chem.* **2011**, *54*, 1347.
3. (a) Pan, Z.; Scheerens, H.; Li, S.-J.; Schultz, B. E.; Sprengeler, P. A.; Burrill, L. C.; Mendonca, R. V.; Sweeney, M. D.; Scott, K. C. K.; Grothaus, P. G.; Jeffery, D. A.; Spoerke, J. M.; Honigberg, L. A.; Young, P. R.; Dalrymple, S. A.; Palmer, J. T. *ChemMedChem* **2007**, *2*, 58; (b) Honigberg, L. A.; Smith, A. M.; Sirisawad, M.; Verner, E.; Loury, D.; Chang, B.; Li, S.; Pan, Z.; Thamm, D. H.; Miller, R. A.; Buggy, J. J. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 13075.
4. Chen, P.; Doweyko, A. M.; Norris, D.; Gu, H. H.; Spergel, S. H.; Das, J.; Moquin, R. V.; Lin, J.; Wityak, J.; Iwanowicz, E. J.; McIntyre, K. W.; Shuster, D. J.; Behnia, K.; Chong, S.; de Fex, H.; Pang, S.; Pitt, S.; Shen, D. R.; Thrall, S.; Stanley, P.; Kocy, O. R.; Witmer, M. R.; Kanner, S. B.; Schieven, G. L.; Barrish, J. C. *J. Med. Chem.* **2004**, *47*, 4517.
5. Chen, B.-C.; Zhao, R.; Bednarz, M. S.; Wang, B.; Sundeen, J. E.; Barrish, J. C. *J. Org. Chem.* **2004**, *69*, 977.
6. Gawkrodger, D. J.; Harris, G.; Bojar, R. A. *Br. J. Dermatol.* **2009**, *161*, 939.
7. (a) Wissner, A.; Mansour, T. S. *Arch. Pharm. Chem. Life Sci.* **2008**, *341*, 465; (b) Tsou, H.-R.; Overbeek-Klumpers, E. G.; Hallett, W. A.; Reich, M. F.; Floyd, B.; Johnson, B. D.; Michalak, R. S.; Nilakantan, R.; Discafani, C.; Golas, J.; Rabindran, S. K.; Shen, R.; Shi, X.; Wang, Y.-F.; Upeslacis, J.; Wissner, A. *J. Med. Chem.* **2005**, *48*, 1107; (c) Wissner, A.; Overbeek, E.; Reich, M. F.; Floyd, B.; Johnson, B. D.; Mamuya, N.; Rosfjord, E. C.; Discafani, C.; Davis, R.; Shi, X.; Rabindran, S. K.; Gruber, B. C.; Ye, F.; Hallett, W. A.; Nilakantan, R.; Shen, R.; Wang, Y.-F.; Greenberger, L. M.; Tsou, H.-R. *J. Med. Chem.* **2003**, *46*, 49.
8. Okram, B.; Nagle, A.; Adrian, F. J.; Lee, C.; Ren, P.; Wang, X.; Sim, T.; Xie, Y.; Wang, X.; Xia, G.; Spraggon, G.; Warmuth, M.; Liu, Y.; Gray, N. S. *Chem. Biol.* **2006**, *13*, 779.
9. PDB code: 3T9T.
10. (a) Gierse, J. K.; Koboldt, C. M.; Walker, M. C.; Seibert, K.; Isakson, P. C. *Biochem. J.* **1999**, *339*, 607; (b) Singh, J.; Dobrusin, E. M.; Fry, D. W.; Haske, T.; Whitty, A.; McNamara, D. J. *J. Med. Chem.* **1997**, *40*, 1130; (c) Wang, Z.; Watt, W.; Brooks, N. A.; Harris, M. S.; Urban, J.; Boatman, D.; McMillan, M.; Kahn, M.; Heinrikson, R. L.; Finzel, B. C.; Wittwer, A. J.; Blinn, J.; Kamtekar, S.; Tomasselli, A. G. *Biochim. Biophys. Acta* **2010**, *1804*, 1817.
11. The efficiency of covalent inhibitors could be expressed as a function of the k_{inact}/K_I ratio, see Ref. 10 for more detail.

$$E + I \overset{K_I}{\rightleftarrows} [EI] \overset{K_{inact}}{\rightarrow} EI^*$$

[EI] initial non covalent complex
EI^* final covalent complex
12. Park, H.; Wahl, M. I.; Afar, D. E. H.; Turck, C. W.; Rawlings, D. J.; Tam, C.; Scharenberg, A. M.; Kinet, J.-P.; Witte, O. N. *Immunity* **1996**, *4*, 515.
13. Ellmeier, W.; Jung, S.; Sunshine, M. J.; Hatam, F.; Xu, Y.; Baltimore, D.; Mano, H.; Littman, D. R. *J. Exp. Med.* **2000**, *192*, 1611.
14. The Pfizer institutional animal care and use committee reviewed and approved the animal use in these studies. The animal care and use program is fully accredited by the association for assessment and accreditation of laboratory animal care, international.
15. Lin, T. H.; Hegen, M.; Quadros, E.; Nickerson-Nutter, C. L.; Appell, K. C.; Cole, A. G.; Shao, Y.; Tam, S.; Ohlmeyer, M.; Wang, B.; Goodwin, D. G.; Kimble, E. F.; Quintero, J.; Gao, M.; Symanowicz, P.; Wrocklage, C.; Lussier, J.; Schelling, S. H.; Hewet, A. G.; Xuan, D.; Krykbaev, R.; Togias, J.; Xu, X.; Harrison, R.; Mansour, T.; Collins, M.; Clark, J. D.; Webb, M. L.; Seidl, K. J. *Arthritis Rheum.* **2010**, *62*, 2283.
16. Arthritis was induced by immunizing DBA/1JBomTac mice with bovine type II collagen (CII) emulsified in complete Freund's adjuvant and by a boost 21 days later with CII emulsified in incomplete Freund's adjuvant, as described previously.[15] Disease severity was evaluated by scoring all four paws for each animal, with a maximum possible score being 16. 0: No arthritis, 1: One or two swollen digits, 2: Three or more swollen digits or mild to moderate swelling of the entire paw, 3: Extensive swelling of the entire paw, 4: Resolution of swelling, ankylosis of the paw. Values are the mean ± SEM. Mice were dosed orally with compound **36** at 0.3, 1, 3, or 10 mg/kg QD. Disease progression was significantly inhibited by **36** compared to the vehicle control (P <0.05, by Student's t-test) at 10 mg/kg (day 7–22), 3 mg/kg (day 5–22) and, at the end of the study, 1 mg/kg (day 19–22).