Potent and Selective Bruton's Tyrosine Kinase Inhibitors: Discovery of GDC-0834

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ARTICLE INFO

Article history:

Received

Revised

Accepted

Available online

Keywords:

Kinase inhibitor, Bruton's tyrosine kinase, Btk, Rheumatoid arthritis, GDC-0834, Amide hydrolysis, single dose IND

ABSTRACT

SAR studies focused on improving the pharmacokinetic (PK) properties of the previously reported potent and selective Btk inhibitor CGI-1746 (**1**) resulted in the clinical candidate GDC-0834 (**2**), which retained the potency and selectivity of CGI-1746, but with much improved PK in preclinical animal models. Structure based design efforts drove this work as modifications to **1** were investigated at both the solvent exposed region as well as "H3 binding pocket." However, *in vitro* metabolic evaluation of **2** revealed a non CYP-mediated metabolic process that was more prevalent in human than preclinical species (mouse, rat, dog, cyno), leading to a high-level of uncertainty in predicting human pharmacokinetics. Due to its promising potency, selectivity, and preclinical efficacy, a single dose IND was filed and **2** was taken in to a single dose phase I trial in healthy volunteers to quickly evaluate the human pharmacokinetics. In human, **2** was found to be highly labile at the exo-cyclic amide bond that links the tetrahydrobenzothiophene moiety to the central aniline ring, resulting in insufficient parent drug exposure. This information informed the back-up program and discovery of improved inhibitors.

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Bruton's tyrosine kinase (Btk) plays a critical role in the development, differentiation and proliferation of B-lineage cells, making it an attractive target for the treatment of immunological disorders such as (rheumatoid arthritis (RA), lupus, multiple sclerosis (MS))¹⁻⁴ as well as B-cell malignancies.⁵ It has been reported that 1 in 150,000 males is born with a deficiency in the Btk gene,⁶ a condition called X-linked agammaglobulinemia (XLA), manifested by lack of immunoglobulins and B cells and first reported by Ogden Bruton in 1952.⁷⁻⁸ A related but less severe X-linked immunodeficiency (*xid*) in mice is also known.⁹⁻¹⁰ Importantly, these *xid* mice are resistant to acquiring lupus¹¹ and collagen induced arthritis.¹² In addition to B cells, Btk also plays an important role in myeloid cell inflammatory cytokine production.²⁻⁴ Thus, there have been significant efforts from the pharmaceutical community with the goal of identifying Btk inhibitors for clinical development.¹³⁻¹⁶ Of these, the most advanced compound in oncology indications is ibrutinib/Imbruvica®,¹⁷⁻¹⁸ which was recently approved for the treatment of mantle cell lymphoma (MCL) and chronic lymphocytic leukemia (CLL)¹⁹ and remains under evaluation in

additional indications.²⁰ The most advanced Btk inhibitor in non-oncology indications is CC-292, which is currently in Phase IIa studies in RA patients.²¹

Figure 1 Btk inhibitors CGI-1746 (**1**) and GDC-0834 (**2**)

We previously reported on the discovery of CGI-1746 (**1**),^{3, 22-23} a potent, highly selective, non-covalent, reversible inhibitor of Btk and an ideal tool molecule to investigate the biological role that Btk signaling plays in rheumatoid arthritis and other B cell and immune complex-mediated disorders. CGI-1746 (**1**) was shown to be efficacious in several animal models of rheumatoid arthritis.³ Although **1** was not considered to be a viable drug candidate based on its poor ADME properties, it was an appealing scaffold by virtue of its exquisite selectivity profile, due to an induced 'H3' specificity pocket. For this reason, we chose to pursue further development of this scaffold. In this report, we describe the optimization of **1** to molecules with improved drug-like properties, culminating in our first Btk inhibitor clinical candidate, GDC-0834, **2**.

As reported by Di Paolo, *et. al.*³ the CGI-1746 (**1**) scaffold binds in an extended conformation to Btk as illustrated in **Figure 2**. This binding event induces a conformational change in Btk relative to the Apo structure (pdb 3P08) resulting in a Src-like inactive conformation of the kinase domain. This includes rearrangement of Y551 by ~18Å from an extended, solvent exposed position to a buried conformation. The t-butyl phenyl group of CGI-1746 (**1**) binds into a lipophilic pocket (termed the H3 pocket). The pyrazinone HN-C-C-CO binds to the hinge M477 while the morpholine-amide extends into solvent.

Figure 2 X-ray crystal structure of CGI-1746 (**1**) bound in Btk (pdb code 3OCS³) illustrating the location of key regions of Btk and important interactions with CGI-1746.

Our initial SAR studies evaluated the effect of varying the substitution on the terminal phenyl ring of **1** as well as replacing this ring with alternate arenes on binding interactions in the H3 pocket. Over 100 analogs were synthesized with representative examples shown in **Table 1**. We assessed inhibition against the target enzyme (Btk) as well as the downstream effect on anti-IgM-stimulated cell surface CD86 expression. Since the binding pocket is relatively confined and comprised of a series of lipophilic residues, only a small subset of these analogs bound well. For instance, the unsubstituted phenyl **3** had poor binding affinity with an IC₅₀ of 4.6 uM but addition of *para* substituents, as in **1** and **4-11**, all offered increased potency as compared to **3**. The thiophene isostere, **12**, was fairly potent but still inferior to **1**. Indeed, the original lead CGI-1746 (**1**), possessing a *para*-t-butyl group, was the best from this initial series. Unfortunately, this compound suffered from poor pharmacokinetic properties in rat (clearance (CL) = 87 mL/min/kg; oral bioavailability (F%) <5%), so an early goal was to improve the oral exposure of this compound. Adding polarity to the aryl ring, as in examples **13-16**, caused a decrease in binding affinity in all cases, presumably due to clashes with lipophilic residues in the binding pocket. We were able to identify tetrahydrobenzothiophene **17** that did maintain equivalent potency to **1**, although related analogs **18-21** failed to offer any improvement in affinity. Importantly, from this set, **17**, represented the only example with improved

clearance and appreciable oral bioavailability in rat (CL=6.0 mL/min/kg; F = 29%). At this point, we sought to optimize the other parts of the molecule while maintaining the tetrahydrobenzothiophene moiety in the H3 pocket. Thiophenes can be problematic from a medicinal chemistry viewpoint given that they are known to undergo bioactivation to yield reactive metabolites,²⁵⁻²⁶ despite there being examples of marketed, oral and chronically dosed drugs featuring this motif.²⁷ In this case, we reasoned that the risk was mitigated given the steric bulk (tri-substituted thiophene) and electron withdrawing effect of the adjacent acyl group.

Table 1. Btk activity SAR for H3 group variations.

Compound ID	R	IC ₅₀ (uM) ^a	
		Btk ^a	CD86 ^a
3		4.60	--
4		0.86	--
5		0.225	--
CGI-1746 (1)		0.008	0.15
6		0.23	0.41
7		0.04	0.08
8		0.11	--
9		0.03	0.58
10		0.15	2.8
11		0.09	1.1
12		0.06	0.49
13		0.27	3.7
14		0.05	--
15		0.13	3.5
16		0.07	0.89
17		0.008	0.23
18		0.15	1.3
19		0.34	--
20		0.19	--
21		0.03	--

a) assay conditions in supplementary materials

Table 2 SAR for H2 group variations

Compound ID	R	IC ₅₀ uM		Rat CL ^a / F ^b
		Btk	CD86	
17		0.008	0.23	6.0 / 29%
22		0.23	--	--
23		0.050	5.0	9.2 / 53%
24		0.49	--	--
25		0.65	--	5.4 / 37%
26		0.043	1.1	--
27		0.21	--	--
28		0.054	1.3	--
29		0.01	0.06	111 / < 5%
30		0.06	0.80	--
31		0.05	23.9	--
32		0.02	0.16	11 / 41%
2		0.006	0.06	4.4 / 35%

^aclearance (CL)=mL/min/kg ^boral bioavailability (F)

Our next efforts focused on determining the scope of moieties that would be tolerated in the partially solvent-exposed H2 region. Over 300 compounds were prepared in this endeavor, with a range of these shown in **Table 2**. Inhibitors with unsubstituted mono aryl groups **22-25** lost significant potency. Bicyclic analogs such as **26-28** likewise failed to match the enzyme potency of CGI-1746 (**1**). Ultimately, extending moieties from the para position of the aryl ring appeared to offer the best balance of biochemical and cellular potency while retaining acceptable pharmacokinetic properties in rat. *N*-Methylpiperidine-containing example **29** proved to be highly potent but suffered from high *in vivo* CL in rat (111 mL/min/kg). Lower *in vivo* CL was found with the dimethyl-3-oxopiperazin-2-yl enantiomers, **32** and **2**. The absolute configuration of **2** was determined by a single crystal X-ray. Accordingly, **32** was then assigned as the opposite enantiomer. Between **2** and **32**, a 3-fold

potency difference was observed between the enantiomers *in vitro*, and thus compound **2** was selected for further studies and ultimately was nominated as the clinical candidate, GDC-0834 (**2**).

GDC-0834 (**2**) inhibits Btk *in vitro* with an IC₅₀ of 0.006 uM and has an EC₅₀ of 0.060 uM in the cell based CD86 assay. In human whole blood, **2** demonstrated potent inhibition of both anti-IgE stimulated CD63 expression (basophils) and anti-IgD stimulated CD69 expression (B-cells) with EC₅₀'s of 0.35 and 0.38 uM, respectively (**Table 3**). Additionally, GDC-0834 (**2**) has shown efficacy in a rat collagen-induced arthritis (CIA) model, causing a dose-dependent decrease of ankle swelling and reduction of morphologic pathology.²⁸

Table 3 Human Potency of GDC-0834 (**2**)

Enzyme	B-cell	whole blood	
IC ₅₀	EC ₅₀	EC ₅₀	
Btk	CD86	basophils	B-cell
		CD63	CD69
0.006 uM	0.06 uM	0.35 uM	0.38 uM

Importantly, GDC-0834 (**2**) retained the same selectivity profile as CGI-1746 (**1**) demonstrating excellent biochemical selectivity against a panel of 331 kinases (**Figure 3**). The sole exception was the TEC-family kinase Bmx²⁹ for which there was only a 3-fold preference for Btk based on biochemical IC₅₀ values. Additionally, GDC-0834 (**2**) had minimal to no off-target receptor activity when screened against a panel of 30 targets (all < 35% at 10 uM), minimal inhibition of the hERG channel (27% at 10 uM), and was negative in a gene-tox profile Ames (TA98/100 strains; +/- S9) and micronucleus tests (+/- S9).

Figure 3 Kinase selectivity profile of GDC-0834 (**2**) vs. a panel of 331 kinases at 1 uM concentration.

We obtained a 1.95 Å resolution crystal structure of GDC-0834 (**2**) in complex with Btk,³⁰ confirming a near-identical binding configuration to CGI-1746 (**Figure 4**). The conformation of the Btk kinase domain is also very similar between the two structures with an r.m.s.d. of 0.12 Å over 1838 atoms. The tetrahydrobenzothiophene moiety binds in the H3 pocket created by residues L542, V546, S543, and Y551 of the fully ordered activation loop. The side chains of the Gly-rich loop residues Q412 and F413, the backbone of residues T410 and

G411, as well as the catalytic loop residues D521 and N526, and the DFG residue D539 also contribute to the specificity. The amide linking the central aryl to the tetrahydrobenzothiophene makes interactions identical to those observed in the CGI-1746-Btk co-crystal structure, including a non-classical hydrogen bond to K430. The pyrazinone HN-C-C-CO binds to the hinge M477 while the dimethyl-3-oxopiperazin-2-yl extends into the solvent region where it can likely access more than one conformation.

Figure 4: a) Crystal structure of GDC-0834 (**2**) in Btk; b) Comparison of the binding modes of CGI-1746 and GDC-0834.

Scheme 1: Synthesis of GDC-0834

The original synthesis of GDC-0834 (**2**) has been previously reported.³¹ Here, in **Scheme 1** (with full details in Supplemental Material), we outline an improved method for larger scale production. Carboxylic acid **I** was transformed to the acid chloride **II** via the action of oxalyl chloride in DCM. Thereafter, reaction of acid chloride **II** with amino-boronate **III** afforded **IV** in 80% yield from **I**. Aniline **V** (single stereoisomer) underwent coupling with dibromopyrazinone **VI** to afford **VII**. Final coupling of bromo-pyrazine **VII** and boronate **IV** catalyzed by tetrakis(triphenylphosphine)palladium provided GDC-0834 (**2**).

GDC-0834 (**2**) exhibited low clearance in rats, moderate clearance in mice and dogs, and high clearance in cynomolgus monkeys (**Table 4**). Oral bioavailability was acceptable in all species and showed that oral exposures were achievable following crystalline suspension. The rat oral bioavailability of crystalline freebase suspension was slightly lower compared to solution, presumably due to low solubility limiting its oral absorption (mp = 212 °C; solubility = 0.007 mg/mL in fasted-state simulated intestinal fluid pH 6.8, 37 °C). However, data from dogs, suggested otherwise since oral bioavailability was similar when comparing solution and crystalline freebase suspension dosing.

In vitro studies in liver microsomes and hepatocytes were run with GDC-0834 (**2**) in order to aid in an estimation of human clearance. Of note, the *in vitro* to *in vivo* correlation was variable among the preclinical species, and was particularly poor in the rat which suggested a large overestimation of *in vivo* clearance by *in vitro* methodologies (**Table 4**). *In vitro* liver microsome studies were run in the presence and absence of NADPH in order to characterize the extent of non cyp-mediated metabolism that had not previously been observed. The *in vitro* clearance of **2** in the absence of NADPH was identical to that measured in the presence of NADPH in human microsomes, suggesting significant non cyp-mediated metabolism. Non CYP-mediated metabolism was less pronounced in other species (mouse, rat, dog, and cyno). As previously reported²⁴, the clearance of GDC-0834 (**2**) in human was predicted to be low to moderate when projected from preclinical species using allometric scaling of *in vivo* data, but high from human microsomes and hepatocytes (CL_{hep} 19, 19 mL/min/kg, respectively). Overall, the predicted human clearance ranged from low to high.

Table 4 GDC-0834 Preclinical Data

Preclinical DMPK Data				
species	Clearance mL/min/kg			Oral Bioavailability sol. ^a (susp.) ^b
	<i>in vitro</i>		<i>in vivo</i>	
	Liver microsomes (+/-NADPH)	Liver hepatocytes		
human	19 / 18	19		
mouse	79 / 25	63	43	18% ^a
rat	39 / 8	25	5	35% ^a (16%) ^b
dog	28 / 7	19	14	35% ^c (32%) ^b
cyno	36 / 2	32	33	16% ^c

^aSolution in 20% (v/v) Cremophor EL, 10% (v/v) ethanol in water;

^bSuspension of crystalline freebase in 0.5% (w/v) methylcellulose, 0.2% (w/v) polysorbate 80 in water; ^cSolution in 60% polyethylene glycol 400 in water

There was little confidence in our human clearance predictions for GDC-0834 since there appeared to be species differences in the metabolic route of elimination with GDC-0834 being metabolized primarily by non-CYP mediated pathways. At the time, there was considerably less known about the *in vitro* to *in vivo* correlation of non-CYP mediated metabolism compared to CYP metabolism.²⁴ Some reassurance was gained when **2** was studied in PXB chimeric mice with humanized livers and low clearance was observed.²⁴ Due to the risks associated with the projected human PK of this molecule, we chose to file a single dose investigational new drug (IND) application.³² A single dose IND allowed us to expedite the understanding of the disposition of GDC-0834 (**2**) in human while reducing initial risk and cost. Furthermore, these human data would serve to inform the back-up program.

Under the single dose IND, healthy volunteers received GDC-0834 (**2**) orally (35 mg or 105 mg) and plasma concentrations were monitored. Parent compound **2** was not observed in plasma, rather only the product of amide hydrolysis **M1** (Figure 5) and, in turn, its metabolites were detected. We later learned, perhaps surprisingly, that aldehyde oxidase (AO) and/or carboxy esterase, is the enzyme most likely responsible for the amide cleavage.³³ AO is known to have higher expression in human as compared to dog, rat, mouse and cyno. In light of this, we focused our subsequent efforts on addressing this metabolic pathway with the goal of identifying a compound predicted to have improved metabolic stability in human.

Figure 5 Cleavage of **2** in human to generate aniline metabolite **M1**

In conclusion, GDC-0834, a highly potent and selective Btk inhibitor with acceptable preclinical *in vivo* profile was identified as the first clinical candidate from this series. Due to the large range of human *in vivo* predictions based on varying metabolism in preclinical species, we chose to take GDC-0834 into a single dose phase I healthy volunteer trial to rapidly learn the human PK profile and to inform potential back-up efforts. In human, **2**, was found to be highly labile at the amide bond linking the H3-moieity to the aniline ring, with this being a particular liability in human. Subsequent efforts have shown that this metabolic liability can be minimized by substitution of the aniline ring, through steric blockage of amide hydrolysis or cyclization of the amide –NH onto the H3-group. Our efforts in these areas will be described in upcoming communications.

Acknowledgments

We thank the Btk team members at Gilead (formerly CGI Pharmaceuticals Inc.), Genentech, Chem Partners, and AMRI for their contributions in generating the data for this manuscript. Use of the Advanced Light Source is supported by the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.

References and notes

- Satterthwaite, A. B.; Witte, O. N. *Immunol. Rev.* **2000**, 175, 120.
- Brunne, R. C.; Mueller, B.; Wirth, T. *Histol. Histopathol.* **2005**, 20, 945.
- Di Paolo, J. A.; Huang, T.; Balazs, M.; Barbosa, J.; Barck, K. H.; Bravo, B. J.; Carano, R. A.; Darrow, J.; Davies, D. R.; DeForge, L. E.; Diehl, L.; Ferrando, R.; Gallion, S. L.; Giannetti, A. M.; Gribbling, 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. *Nat. Chem. Biol.* **2011**, 7, 41.
- Puri, K. D.; Di Paolo, J.; Gold, M. R. *Int. Rev. Immunol.* **2013**, 32, 397.
- Uckun FM, Qazi S. *Expert Opin Ther Pat.* 2010, 20, 1457.
- Smith, C. I. E.; Islam, K.B.; Vorechovsky, I.; Olerup, O.; Wallin, E.; Rabbani, H.; Baskin, B.; Hammarstrom, L. *Immunol. Rev.* **1994**, 138, 159.
- Bruton, O. C. *Pediatrics* **1952**, 9, 722.
- Howard, V.; Greene, J.M.; Pahwa, S.; Winkelstein, J. A.; Boyle, J. M.; Kocak, M.; Conley, M. E. *Clin. Immunol.* **2006**, 118, 201.
- Satterthwaite, A.B.; Witte, O.N. *Immunol. Rev.* **2000**, 175, 120.
- Tsukada, S.; Rawlings, D.J.; Witte, O.N. **1994** *Curr. Opin. Immunol.* 6, 623.
- Steinberg, D. D. *J. Clin. Invest.* **1982**, 70, 587.
- Jansson, L.; Holmdahl, R. *Clin. Exp. Immunol.* **1993**, 94, 459.
- Lou, Y.; Owens, T. D.; Kuglstat, A.; Kondru, R. K.; Goldstein, D. M. *J. Med. Chem.* **2012**, 55, 4539.
- Pan, Z. *Drug News Perspect.* **2008**, 21, 357.
- Rokosz, L. L.; Beasley, J. R.; Carroll, C. D.; Lin, T.; Zhao, J.; Appell, K. C.; Webb, M. L. *Expert Opin. Ther. Targets* **2008**, 12, 883.
- Uckun, F. M.; Tibbles, H. E.; Vassilev, A. O. *Med. Chem.* **2007**, 7, 624.
- Chavez, J. C.; Sahakian, E.; Pinilla-Ibarz, J. *Core Evidence*, **2013**, 8, 37.
- Akinleye, A.; Chen, Y. I.; Mukhi, N.; Song, Y.; Liu, D. *J. Hematol. Oncol.* **2013**, 6, 59.
- <http://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm374857.htm>
- <http://www.clinicaltrial.gov/ct2/results?term=ibrutinib&Search=Search>
- <http://clinicaltrials.gov/show/NCT01975610>
- Brittelli, D. R. *et al.* WO/2006/099075 2006.
- Currie, K. S. Brussels, Belgium; 2010 EFMC 21st International Symposium on Medicinal Chemistry, Brussels, Belgium
- Liu, L.; Halladay, J.; Shin, Y.; Wong, S.; Coraggio, M.; La, H.; Baumgardner, M.; Le, H.; Gopaul, S.; Boggs, J.; Kuebler, P.; Davis, J.; Liao, C.; Lubach, J.; Deese, A.; Sowell, G.; Currie, K.; Young, W.; Khojasteh, C.; Hop, C.; Wong, H. *Drug Metab. Dispos.*, **2011**, 39, 1840-9.
- Kalgutkar, A. S.; Gardner, I.; Obach, R. S.; Shaffer, C. L.; Callegari, E.; Henne, K. R.; Mutlib, A. E.; Dalvie, D. K.; Lee, J. S.; Nakai, Y.; O'Donnell, J. P.; Boer, J.; Harriman, S. P. *Curr Drug Metab.* **2005**, 3, 161.
- Rademacher, P. M.; Woods, C. M.; Huang, Q.; Szklarz, G. D.; Nelson, S. D. *Chem. Res. in Toxicol.*, **2012**, 25, 895.
- Recent example: InvokanaTM (canagliflozin) <http://www.invokanahcp.com/prescribing-information.pdf>
- Liu, L.; Di Paolo, J.; Barbosa, J.; Rong, H.; Reif, K.; Wong, H. *J. Pharmacol. Exp. Ther.* **2011**, 338, 154.
- Muckelbauer, J.; Sack, J. S.; Ahmed, N.; Burke, B.; Chang, C. Y.; Gao, M.; Tino, J.; Xie, D.; Tebben, A. *Chem Biol. Drug Des.* **2011**, 78, 793.
- See the [Supplementary data](#) for the experimental details associated with the co-crystal structure of BTK with GDC-834. The coordinates and structure factors have been deposited with the protein data bank and assigned the accession code 4OTF.
- Blomgren, P.A. *et al.* U.S. Patent 7,888,108.
- <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm078933.pdf>
- Sodhi, J.; Wong, S.; Liu, L.; Khojasteh, S. C.; Hop, C. E. C. A.; Barr, J. T.; Jones, J. P.; Kirkpatrick D. S.; Halladay, J. *Drug Metab. Dispos.*, submitted.

Graphical Abstract

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