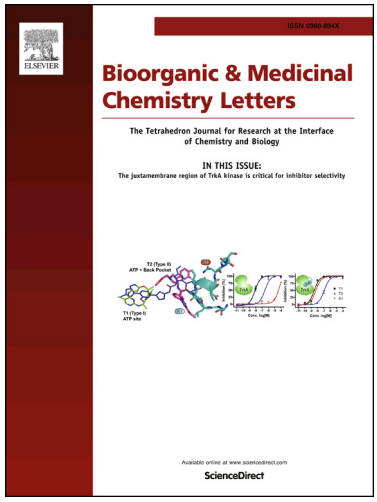

Discovery of 3-morpholino-imidazole[1,5-a]pyrazine BTK inhibitors for rheumatoid arthritis

Sobhana Babu Boga, Abdul-Basit Alhassan, Jian Liu, Deodial Guiadeen, Arto Krikorian, Xiaolei Gao, James Wang, Younong Yu, Rajan Anand, Shilan Liu, Chundao Yang, Hao Wu, Jiaqiang Cai, Hugh Zhu, Jagdish Desai, Kevin Maloney, Ying-Duo Gao, Thierry O. Fischmann, Jeremy Presland, My Mansueto, Zangwei Xu, Erica Leccese, Ian Knemeyer, Charles G. Garlisi, Nathan Bays, Peter Stivers, Philip E. Brandish, Alexandra Hicks, Alan Cooper, Ronald M. Kim, Joseph A. Kozlowski

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Graphical Abstract

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Discovery of 3-morpholino-imidazole[1,5-a]pyrazine BTK inhibitors for rheumatoid arthritis

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Discovery of 3-morpholino-imidazole[1,5-a]pyrazine BTK inhibitors for rheumatoid arthritis[#]

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[#] *Dedicated to Professor Léon Ghosez, Emeritus Professor UCL, Belgium & visiting scientist IECB, France on the occasion of his 80th birthday*

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ABSTRACT

8-Amino-imidazo[1,5-a]pyrazine-based Bruton's tyrosine kinase (BTK) inhibitors, such as **6,** exhibited potent inhibition of BTK but required improvements in both kinase and hERG selectivity.[1] In an effort to maintain the inhibitory activity of these analogs and improve their selectivity profiles, we carried out SAR exploration of groups at the 3-position of pyrazine compound **6**. This effort led to the discovery of the morpholine group as an optimized pharmacophore. Compounds **13**, **23** and **38** displayed excellent BTK potencies, kinase and hERG selectivities, and pharmacokinetic profiles.

Bruton's tyrosine kinase (BTK) is a TEC family kinase expressed in B cells, mast cells and macrophages, but not in T cells or natural killer cells[2]. BTK is important in cell signaling pathways such as B cell antigen receptor (BCR) and Fc receptor (FcR) signaling cascades[3]. Mutations in the human BTK gene cause X-linked agammaglobulinemia (XLA), a disease which is characterized by a deficit of peripheral B cells and low levels of serum Ig[4]. In mice, point mutation or deletion of BTK causes X-linked immunodeficiency (xid) where fewer B cells and reduced serum Ig levels are observed[5]. Xid mice have been shown to be resistant to collagen-induced arthritis (CIA). Thus, a connection between the pathogenesis of rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) and BTK has been postulated as RA is characterized, in part, as being an outcome of autoreactive B cell activation, which in turn gives rise to expansion of B cells and the subsequent production of autoantibodies. Given that rituximab, a therapeutic anti-CD20 antibody, impacts B cell levels of patients with autoimmune diseases such as RA, multiple sclerosis (MS) and SLE, a significant interest in pursuing small molecule inhibitors of BTK has emerged[5]. Extensive research efforts[6] have focused on the development of both reversible (i.e. **4, 5)** and irreversible BTK inhibitors (i.e. **1-3)** as represented in Figure-1. The irreversible inhibitors form a covalent bond with cysteine-481 of the BTK enzyme. Several irreversible BTK inhibitors have progressed to the clinic and Ibrutinib was approved.[7]

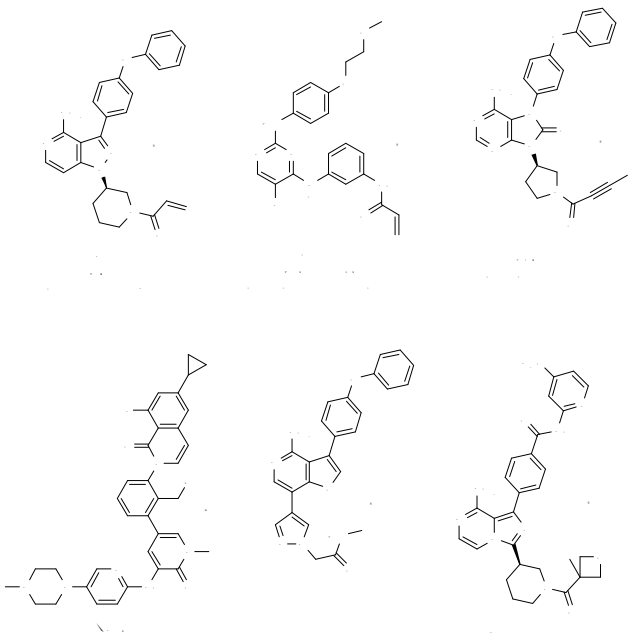


Figure 1. Representative structures of BTK inhibitors.

We recently described reversible BTK inhibitors, such as 8-amino-imidazo[1,5-a]pyrazines **6,** with good kinase selectivity and acceptable oral bioavailability.[1] In an effort to further optimize potency, selectivity and PK parameters of this series we now report the SAR and optimization surrounding 3-substitution of the 8-amino-imidazo[1,5-a]pyrazine series.

Early SAR indicated that substitution off the 3-position significantly impacted BTK inhibitory activity and kinase selectivity. Therefore we focused our efforts on expanding SAR in this region. To that end, as compared to compound **6,** tolerability for various substitutions was probed (Table 1). Initial effort focused upon replacing the chiral piperidine ring with an achiral methyl morpholine group, compound **7,** caused significant loss in BTK inhibitory activity compared to lead compound **6**.

We next systematically modified the ring size of the groups appended at the 3-position of the pyrazine. Evaluation of the ring size was initially tested by maintaining a common pendant group, the methyl oxetane amide as in the lead compound **6**. The 4-membered azetadine **8,** as well as the 5-membered pyrrolidine **9** maintained BTK inhibitory activity but unfortunately lost 5-20 fold in hPBMC (human peripheral blood mononuclear cell)[1] cellular potency. Significant loss of BTK inhibitory activity was also observed with the 6-membered rings, (e.g., piperidine analog **10** with *para*-orientation, and the cyclohexyl amine analog **11**). Increasing the ring as exemplified with 7-membered heptane analogue **12**, suffered ten-fold loss of BTK inhibitory activity compared to the lead piperidine analog **6**.

Next we explored replacement of the 6-membered piperidine motif of **6** with a morpholine, compound **13**, which resulted in BTK inhibitory activity comparable to **6** while the cell based activity was slightly weaker but showed improvement in hERG inhibition. Further modifications of morpholine analogue **14** such as thiomorpholine analog **15**, sulfone morpholine analog **16,** and piperazine analog **17** resulted in reduced BTK inhibitory activity.

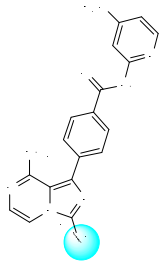
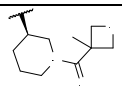
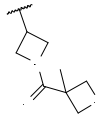
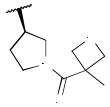
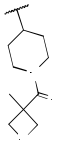
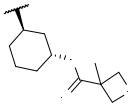
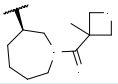
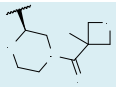
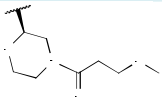
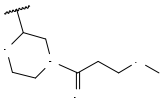
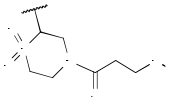
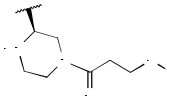


Table 1. Exploration at the 3-position of 8-Amino-imidazo[1,5-a]pyrazine towards BTK, hPBMC and hERG.

#.	R^1	BTK IC_{50} (nM)	hPBMC IC_{50} (nM)	hERG/ikr[a] IC_{50} (μM)
6		0.3	8	3.5
7		132	1225	ND
8		5.9	167	0.4
9		1.2	37	2.5
10		3.4	80	ND
11		10.7	ND	ND
12		5.3	ND	ND
13		0.9	48	14.4
14		3.3	58	5.8
15		6.6	ND	1.8
16		294	ND	ND
17		377	ND	ND

[a] ikr channel or MK-499 assay[11]; [ND] Not Determined

While several compounds in Table **1** emerged more similar to or slightly weaker than **6** for either enzyme or cell potency, morpholine analogue **13** showed greater than four-fold improvement in hERG selectivity. We then focused on the morpholine compound **13** for further exploration. Compound **13** was modeled in the BTK active site using the X-ray crystal structure of **6**[1] and the BTK complex (PDB Code 5FBO). One hundred conformations of compound **13** were generated using the method described by Feuston *et al.*[8] and docked into the BTK active site using the SQ procedure developed by Miller *et al.*[9] The binding mode of **13** is predicted to be very similar to the conformation of compound **6** depicted in the crystal structure. Similarly, **13** bound in the catalytic subunit of BTK showed typical binding of 8-amino-imidazo[1,5-a]pyrazine to the hinge region via amino pyridine hydrogen bonding with Ser538 and Asp539, trifluoromethyl pyridine chain extending deep into the hydrophobic back pocket while the morpholine ring demonstrated close binding interactions with the ribose pocket. (Figure-**2**). With respect to the water molecule that binds to the pyrazine core, the morpholine oxygen is approximately 3.7 Å from the water and is not believed to be making any hydrogen bond interactions. Our model indicates that the morpholine oxygen does not provide additional interactions for better activity through a water bridge, but rather enhances desolvation. Hence the BTK potency drops approximately 2-3 fold compared with piperidine analog **6**.

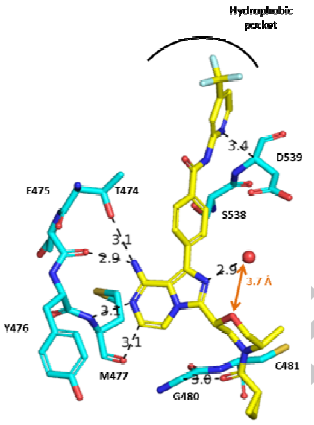


Figure 2. Morpholine compound **13** modeled in the BTK active site using the X-Ray crystal structure of **6** and BTK complex (PDB code: 5FBO). The hydrogen bond interactions with the key residues are indicated.

It was reported in our previous communication[1] that 2-F substitution on the middle phenyl ring, of piperidine compound **18** improved BTK inhibition and cellular hPBMC potency. We explored an analogous approach in the morpholine series in an effort to optimize the back pocket region. As depicted in Table **2**, morpholine compounds **19**, **21**, **23**, and **25** displayed comparable or slightly decreased BTK inhibition compared to the corresponding piperidine analogs **18**, **20**, **22**, and **24**, respectively. However, it was observed that morpholine series of compounds **19**, **21**, **23**, and **25** consistently offered an improvement in hERG inhibition ranging from 3-6 fold, depending upon the pendant group attached to it compared to the piperidine analogues. Interestingly, the *N*-alkyl morpholine analog **27** was slightly more potent in the cellular functional assay, but also showed significant improvement in hERG activity over the piperidine analog **26**.

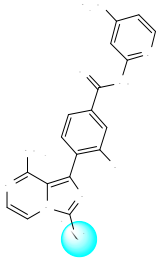
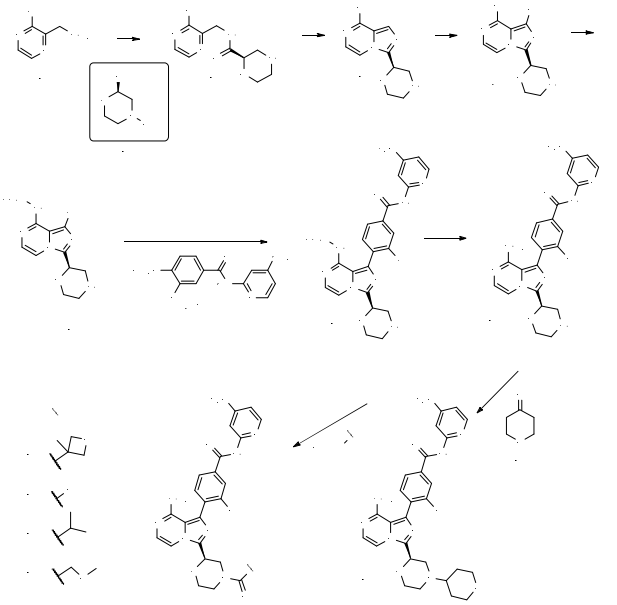


Table 2. Comparison of Piperidine and morpholine analogs of 8-Amino-imidazo[1,5-a]pyrazine series towards BTK, hPBMC and hERG.

#.	R[1]	BTK IC_{50} (nM)	hPBMC IC_{50} (nM)	hERG/ikr[b] IC_{50} (μM)
18		X=CH_2; 0.3	7	ND
19		X=O; 0.7	35	14
20		X=CH_2; 0.1	11	2.4
21		X=O; 0.4	6	19
22		X=CH_2; 0.3	5	2.8
23		X=O; 0.4	26	15
24		X=CH_2; 0.2	13	4.5
25		X=O; 0.4	14	24
26		X=CH_2; 0.8	65	0.1
27		X=O; 0.6	13	4.6

[b] ikr channel or MK-499 assay[11]; [ND] Not Determined

The synthesis of morpholine based BTK inhibitors is similar to the synthetic routes described in an earlier report[1] which involves preparation of bromide **33** from commercially available (*R*)-4-(*tert*-butoxycarbonyl)morpholine-2-carboxylic acid **29** as shown in Scheme-**1**. Coupling of boronate **34** with **33** lead to the common morpholine intermediate **36** after deprotection using TFA. The final morpholine based BTK inhibitors were synthesized using HATU as coupling reagent for the amide formation with the corresponding pendant carboxylic groups to give compounds **19**, **21**, **23**, and **25**. Reductive amination with tetrahydro-4H-pyran-4-one **37** using $Na(OAc)_3BH$ gave the *N*-alkyl morpholine analog **27**. The corresponding piperidine analogs were synthesized using commercially available (R)-1-(tert-butoxycarbonyl)-piperidine-3-carboxylic acid replacing (*R*)-4-(*tert*-butoxycarbonyl)morpholine-2-carboxylic acid **29** in the scheme **1**.[12]



Scheme **1**. Reagents. i. HATU, TEA, CH_2Cl_2,; ii $POCl_3$, CH_3CN, 57% two steps; iii. NBS, DMF, rt, 95%; iv. 2,4-DMB, DIEA, RT, 97%; vi. $PdCl_2(dppf).CH_2Cl_2$, 2M K_3PO_4 (aq.), DMF, 80 ^oC, ON, 80%; vi. Pd/C(10%), H_2 (atom), EtOH, 95%; vii. HATU, DIEA, DMF, 0 ^oC, 1hr, 60-70%; viii. $Na(OAc)_3BH$, MeOH, 90%

The morpholine class of BTK inhibitors were further evaluated across a battery of Tec and Src family kinases and compared to piperidine analogs. Kinase selectivity was assessed relative to BTK inhibitory activity and summarized in Table **3**. In general, the kinase selectivity of morpholine analogs **23**, **25** and **27** trended higher than the corresponding piperidine analogs **22**, **24**, and **26** respectively.

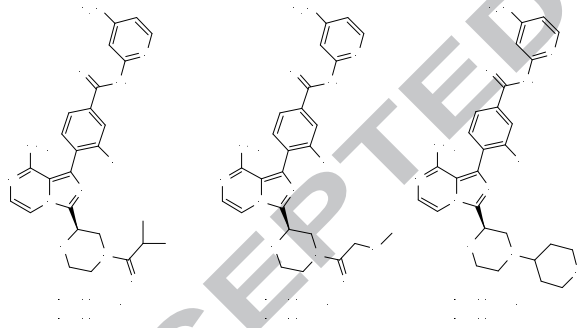


Table 3. Comparison of piperidine and morpholine BTK analogs towards selectivity over SRC family kinases.

Kinase	Compound # IC_{50} (nM) (fold selectivity)		Compound # IC_{50} (nM) (fold selectivity)		Compound # IC_{50} (nM) (fold selectivity)	
	22	**23**	**24**	**25**	**26**	**27**
BTK	0.25	0.44	0.22	0.37	0.83	0.59
BLK	53 (212)	280 (636)	9 (39)	78 (212)	29 (35)	50 (84)
BMX	83 (332)	703 (1598)	20 (93)	331 (895)	127 (153)	200 (339)
CSK	1154 (4616)	2145 (4875)	261 (1185)	626 (2232)	455 (548)	665 (1466)
ERBB4	230 (920)	514 (1168)	71 (322)	169 (457)	59 (71)	76 (128)
FGR	203 (812)	268 (610)	28 (127)	69 (187)	22 (26)	39 (66)
FRK	472 (1888)	706 (1605)	151 (687)	214 (578)	85 (102)	132 (224)
FYN	894	1904	188	571	105	248
	(3576)	(4327)	(853)	(1544)	(127)	(420)
LCK	74 (296)	100 (227)	11 (51)	28 (75)	7 (9)	21 (35)
LYNB	646 (2584)	1045 (2375)	74 (335)	268 (723)	115 (138)	399 (676)
PTK6	350 (1400)	173 (393)	60 (273)	77 (209)	48 (58)	113 (191)
SRC	265 (1060)	512 (1164)	55 (251)	123 (333)	77 (93)	112 (189)
SRMS	84 (336)	103 (234)	19 (86)	76 (205)	50 (60)	65 (110)
TEC	378 (1512)	428 (973)	63 (287)	232 (626)	170 (205)	191 (323)
TXK	132 (528)	464 (1055)	45 (203)	256 (691)	231 (278)	282 (478)
YES1	189 (756)	266 (605)	37 (167)	73 (198)	32 (38)	60 (102)

Based on their potency and improved kinase selectivity profiles compounds **23**, **25,** and **27** were further profiled in rat and dog pharmacokinetic studies as summarized in Table-**4**. Morpholine compound **23** displayed low clearance and good bioavailability in both rat and dog. Replacing the amide group with methoxy acetyl **25** and *N*-alkyl morpholine **27** resulted in bioavailability of 42% and 75%, and decreased $t_{½}$ of 0.8 and 1.1 hour in rats respectively. For comparison, compound **38** was also synthesized (scheme-**2**), which included an alpha methyl substitution in the morpholine ring and with cyclopropyl amide. Compound **38** maintained excellent potency (BTK IC_{50} = 0.4 nM; hPBMC IC_{50}= 5.3 nM) and resulted in an acceptable overall PK profile both in rat with total clearance of 4.2 mL/min/kg, $t_{½}$ of 3.9 h, bioavailability 40% and in dog with total clearance of 1.6 mL/min/kg, $t_{½}$ of 8.8 h, bioavailability 88%.

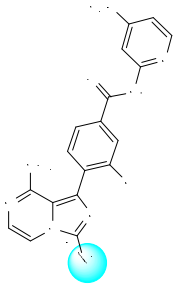


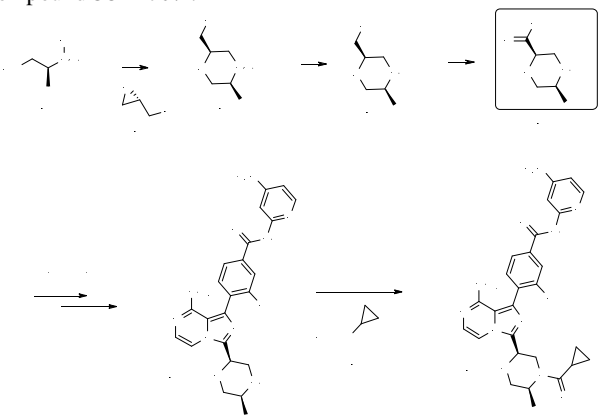
Table 4. Pharmacokinetic parameters of morpholine based BTK inhibitors, **23**, **25, 27** & **38**

R^1 =	 **23**	 **25**	 **27**	 **38**
Rat PK @ 10 mpk				
Clp (mL/min/kg)	1.4	8.2	23	4.2
t½ (h)	2.3	0.8	1.1	3.9
F %	87	42	75	40
Dog PK @ 2 mpk				
Clp (mL/min/kg)	4.8	13.8[a]	5.9[a]	1.6
t½ (h)	3.9	2.5[a]	3.1[a]	8.8
F %	89	ND	ND	88

[a] Dog cassette PK study at 0.5 mpk ; [ND] Not Determined

Synthesis of enantiomerically pure morpholine BTK inhibitor **38** involves the preparation of key intermediate **43** in accordance to the literature report[10] by the addition of (*S*)-2-((4-methoxybenzyl)amino)propan-1-ol **39** to (*S*)-(+)-epichlorohydrin **40** in the presence of $LiClO_4$, subsequent deprotection and protection of PMB to Boc and oxidation with TEMPO (Scheme-

2). Morpholine intermediate **44** was then synthesized following the steps described as depicted in scheme **1** and final amidation using HATU with cyclo propyl carboxylic acid **45** gave the compound **38** in 70%.[12]



Scheme **2**. Reagents. i. $LiClO_4$, NaOMe, rt, 3d, 84%; ii. BOC_2O, 10% Pd/C, 1-methyl-1,4-cyclohexadiene/BHT, EtOH, reflux, 3h, 80%; iii. TEMPO, $PhI(OAc)_2$, CH_2Cl_2; iv. HATU, DIEA, DMF, 0 ^oC, 1hr, 70%

In summary, exploration of the 3-position of the 8-amino-imidazo[1,5-a]pyrazine series **6** led to the discovery of the morpholine moiety as a replacement for piperidine. These morpholine based BTK inhibitors (**23**, **25** and **27**) displayed good BTK inhibitory activity and moderate hPBMC cellular potency, and offered advantages over the corresponding piperidine series with respect to kinase selectivity and hERG ion channel activity. Additionally the morpholines **23** and **38** demonstrated acceptable pharmacokinetic profiles. This demonstrates the utility of the morpholine 8-amino-imidazo[1,5-a]pyrazine series as BTK inhibitors and will be reported in due course.

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