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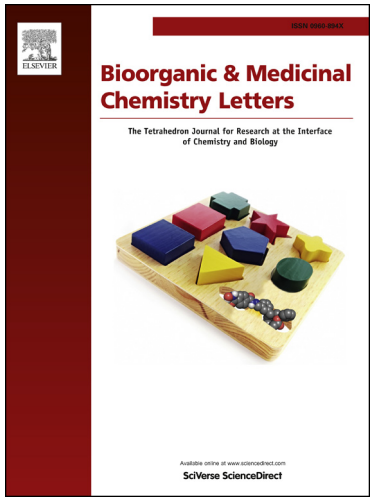

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Design and Synthesis of Carbazole Carboxamides as Promising Inhibitors of Bruton's Tyrosine Kinase (BTK) and Janus Kinase 2 (JAK2)

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Abstract—Four series of disubstituted carbazole-1-carboxamides were designed and synthesized as inhibitors of Bruton's tyrosine kinase (BTK). 4,7- and 4,6-Disubstituted carbazole-1-carboxamides were potent and selective inhibitors of BTK, while 3,7- and 3,6-disubstituted carbazole-1-carboxamides were potent and selective inhibitors of Janus kinase 2 (JAK2).

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Bruton's tyrosine kinase (BTK) is a member of the TEC family of protein tyrosine kinases which is predominantly expressed in B lymphocytes. The expression and activity of BTK are critical to several key steps in the life cycle of B-lineage cells including proliferation, development, differentiation, survival, and apoptosis.[1,2] Following B cell receptor ligation, BTK is recruited to a macromolecular complex at the cell membrane where it is activated by phosphorylation on two key tyrosine residues. Activated BTK then phosphorylates PLCγ2, leading to the generation of inositol-1,4,5-triphosphate and diacylglycerol, which in turn cause calcium mobilization and protein kinase C (PKC) activation.[3,4]

Mutations in the human BTK gene are known to cause X-linked agammaglobulinemia (XLA), a male immune deficiency disorder characterized by a lack of mature, immunoglobulin-producing B cells.[5] There is strong evidence that B cells can play a critical role in the pathogenesis of rheumatoid arthritis and other autoimmune diseases. Rituximab (Rituxan®), a chimeric monoclonal antibody, was the first approved B cell targeted therapy. Besides utility in the treatment of non-Hodgkin's lymphoma, clinical experience has shown that B cell depletion with Rituximab is an effective and generally well tolerated treatment option for rheumatoid arthritis.[6] Evidence for utility in chronic graft-vs.-host disease, relapsing-remitting multiple sclerosis, and systemic lupus erythematosis has also been reported.[7-9] Inhibitors of BTK may thus provide selective modulation of B cell activity, and may be useful in treating inflammatory and autoimmune conditions.

Researchers at Bristol-Myers Squibb have disclosed a series of disubstituted nicotinamides which exhibit potent inhibition of BTK (single-digit nanomolar IC_{50}).[10,11] An x-ray crystal structure of one example, (R)-6-(3-(4-tert-butylbenzamido)-piperidin-1-yl)-2-(4-(morpholine-4-carbonyl)phenyl-amino)nicotinamide, bound in the catalytic subunit of human BTK showed typical binding to the hinge region in the ATP pocket (PDB ID 5BPY) (Figure 1).[11,12] The primary amide NH formed a hydrogen bond with the backbone carbonyl of Glu 475, the carbonyl oxygen interacted with the backbone NH of Met 477, and the aniline NH formed a weak hydrogen bond with the carbonyl of Met 477. The morpholine amide was solvent-exposed, suggesting toleration of a variety of substituents. A noteworthy feature of this structure is the nearly co-planar arrangement of the pyridine and aminophenyl rings. The importance of this spatial arrangement is demonstrated by the significant loss of BTK inhibitory potency resulting from replacement of the pyridyl nitrogen with

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carbon, which would be expected to distort the near co-planarity through steric hinderance.

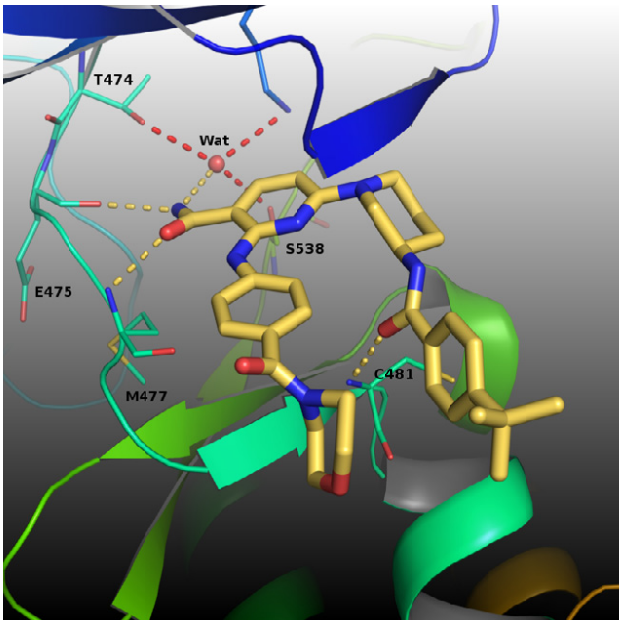

Figure 1. X-ray crystal structure of (R)-6-(3-(4-tert-butylbenzamido)-piperidin-1-yl)-2-(4-(morpholine-4-carbonyl)phenylamino)nicotinamide bound to BTK. (PDB ID 5BPY) [12]

Unfortunately, compounds of this series have generally shown poor metabolic stability. For the compound shown in Figure 1, less than 3% was recovered after 10 minute incubation with human, rat or mouse liver microsomes, and closely related compounds gave poor oral exposure in mouse pharmacokinetic studies (AUC generally <1 μM·h at 10 mg/kg). Seeking to address these issues, we designed a carbazole series, constraining the pyridine and aniline phenyl rings of the nicotinamides (Figure 2).[13] This tricyclic core would lock the required co-planarity of the rings without disturbing the hinge-binding motif, and also offer a variety of substitution possibilities for exploration of SAR. The substituents R and R' of the nicotinamide series could be mimicked by either R^3 or R^4, and by either R^6 or R^7, respectively, on the carbazole core.

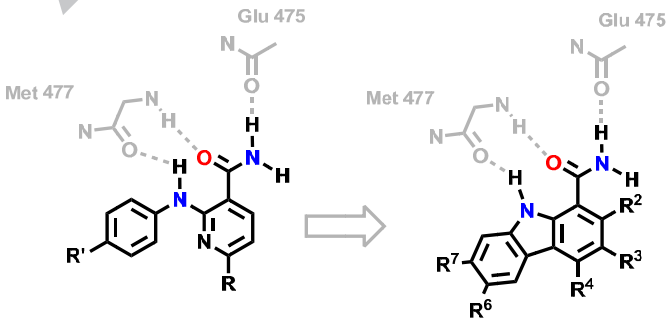

Figure 2. Constraining the nicotinamide to form a carbazole.

A few carbazoles bearing a substituted phenyl at the 4-positon, and N-methyl-piperazine amide at either the 6- or 7- positions, were prepared (Table 1**,** entries **1-10**). Encouragingly, all of these initial compounds showed good BTK inhibition. A pharmacokinetic study with compound **6** demonstrated good oral exposure in the mouse (AUC 25.6 μM·h at 10 mg/kg), a promising improvement over the nicotinamide series.

Table 1. 4,6- and 4,7-Disubstituted carbazole-1-carboxamides.

Compds	X	Amide Position	BTK IC_{50}, nM[14]	JAK2[15]/ BTK
1	2-F	6	16	1
2	3-OMe	6	28	1
3	3-NHCOMe	6	88	1
4	4-OMe	6	29	1
5	4-NHCOMe	6	39	1
6	**2-F**	**7**	**15**	**3**
7	3-OMe	7	32	1
8	3-NHCOMe	7	30	8
9	4-OMe	7	55	1
10	4-NHCOMe	7	15	4
11	2-Me	7	24	3
12	2-OMe	7	144	4
13	**2-Cl**	**7**	**15**	**3**
14	3-Cl	7	38	2
15	3-CONHMe	7	71	2
16	4-Cl	7	59	2
17	4-t-butyl	7	103	2
18	4-CONHMe	7	44	1
19	4-OPh	7	127	3
20	3,4-di-Cl	7	101	2
21	2,3-di-Cl	7	13	7
22	**2,3-di-F**	**7**	**6.9**	3
23	2-Me, 3-Cl	7	32	6
24	2-Me, 3-NH2	7	19	5
25	**2-Me, 3-NHCOMe**	**7**	16	**13**
26	2,4-di-F	7	22	2
27	2,5-di-F	7	16	3
28	2,6-di-F	7	12	2

Unfortunately, selectivity of these compounds with respect to inhibition of several other kinases was low (1- to 8-fold). Notably, selectivity against JAK2, a member of the Janus kinase family, was very poor. Activation of JAK2, which functions as a mediator of cytokine receptor signaling, stimulates cell proliferation, differentiation, migration and apoptosis. These cellular events are critical to hematpoiesis, immune development and other biological functions. A mutation in JAK2 has been identified in most patients with

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polycythaemia vera, essential thrombocythaemia and primary myelofibrosis. While JAK2 has emerged as an interesting target for treatment of myeloproliferative disorders,[16-19] concomitant inhibition of JAK2 would be undesirable for the treatment of B-cell mediated disorders with a BTK inhibitor. We therefore needed to improve the selectivity of BTK inhibition over that of other kinases, particularly JAK2.

Paired compounds with either 6- (**1-5**) or 7-amide substituents (**6-10**) showed similar BTK potency and JAK2 selectivity. However, 7-substituted compounds generally exhibited better metabolic stability than the corresponding 6-substituted analogs. For example, incubation of compound **6** (0.5 μM) with human, rat and mouse liver microsomes for 10 minutes at 37 °C gave 78%, 59%, and 49% recoveries, respectively, of unchanged compound, while the recoveries from similar incubation of compound **1** were only 34%, 7%, and 8%. Therefore, 4,7-disubstituted carbazole-1-carboxamides were selected for further exploration of the SAR. Additional variation of the substitution on the 4-phenyl ring was examined in detail (Table 1, entries **11**-**28**).

Compounds with small *para*-substituents (OMe, Cl, NHCOMe, and CONHMe; entries **9**, **16**, **10**, and **18**) showed similar BTK potency, with IC_{50} values in the range of 15-59 nM, but without significant selectivity over JAK2. Bulky *para* substituents (t-butyl and phenoxy; entries **17** and **19**) decreased BTK potency to 100 nM or greater.

Small *ortho*-substituents (F, Cl, and Me) on the 4-phenyl group were well tolerated, with BTK IC_{50} values of 15-24 nM (entries **6**, **11**, **13**) while the larger *ortho*-methoxy (entry **12**) had somewhat reduced potency. Compounds with *meta*-substituents (OMe, Cl, CONHMe, and NHCOMe; entries **7**, **14**, **15**, and **8**) all showed similar BTK potencies (30-71 nM). However, the *meta*-acetamide **8** was more selective relative to JAK2 than the others. Better JAK2 selectivities were also realized when *ortho*-Me, Cl, or F was combined with a *meta*-substituent. The most interesting example is compound **25** (*ortho*-Me with *meta*-acetamide), which showed 13-fold selectivity for BTK over JAK2. A similar effect was observed in other 2,3-disubstituted phenyl compounds (entries **21** through **24**). Among these, the 2,3-difluoro analog **22** had the most potent BTK activity (7 nM). Other difluoro analogs (2,4-, 2,5-, and 2,6-difluoro; entries **26 – 28**) were 2-3 fold less potent. This SAR suggested that potency and selectivity could be potentially increased by optimization of the *ortho*- and *meta*- substituents.

An x-ray crystal structure of compound **13** bound to the active site of BTK was obtained (PDB ID 5BQ0), and showed the carbazole amide bound to the hinge region as expected, with the piperazine amide projecting toward solvent (Figure 3).[20] The carbazole NH is hydrogen bonded to the backbone carbonyl of Met 477, an interaction not observed in the nicotinamide structure. The conformation of the chlorophenyl substituent in compound **13** differs significantly from the aminopiperidine of the nicotinamide series (Figure 1). The chlorophenyl ring is nearly orthogonal to the carbazole core and forms hydrophobic interactions with Val 416 and Cys 481. These interactions, stabilized by hindered rotation due to the *ortho* chloro substituent on the 4-phenyl ring, may be responsible for improved potency relative to analogs lacking an *ortho*-substituent. This conformation may also contribute to selectivity over other kinases, although reasons for this are not apparent. (Note that the *ortho*-chloro group of compound **13** was resolved in the x-ray structure into at least 2 conformations. The two rotomers that best fit the observed density are shown as yellow and orange below. Both are free of steric clashes in the refined structure.)

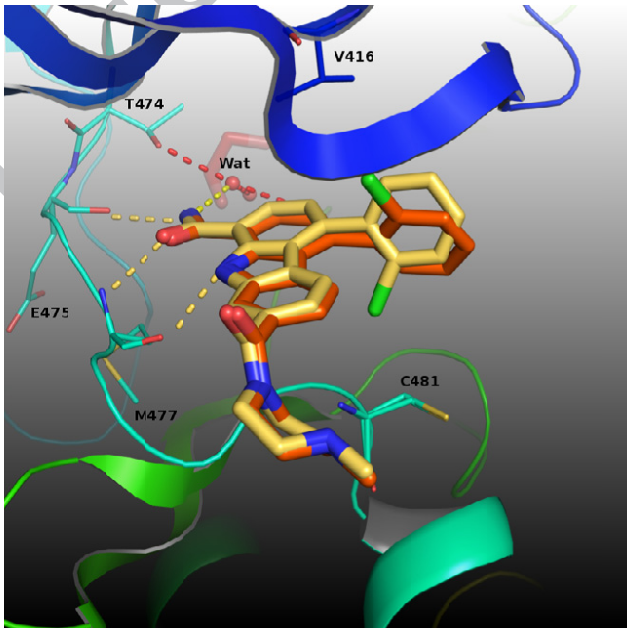

Figure 3. X-ray structure of compound **13** bound to BTK. (PDB ID 5BQ0) [20] Both resolved rotamersof the 2-chlorophenyl group are shown.

The x-ray structure of **13** suggested the pendant phenyl would be tolerant to diverse substitution. To follow up on the early SAR described above, small *ortho*-substituents (F, Me) were paired with a wide variety of *meta*-substituents.[13] As part of this effort, a small set of carbazoles with *meta*-benzamide substituents was synthesized (Table 2). Potency was maintained or improved, and good to excellent selectivity over JAK2 was achieved.

The simple 2-methyl-3-benzamide compound **29** was potent (BTK IC_{50} 7 nM) with good (28-fold) JAK2

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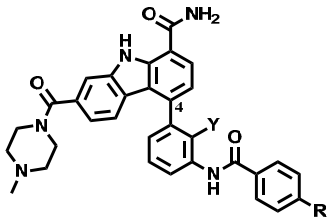
selectivity. *Para*-substitution of the benzamide (entries **30** and **31**) gave a 2-fold improvement in BTK potency, but increased JAK2 selectivity dramatically (58- and 114- fold, respectively). The bulky *tert*-butyl group (entry **32**) maintained good potency but further improved JAK2 selectivity to 140-fold. Similar trends were observed in compounds **33** and **34**, bearing *ortho*-F in place of *ortho*-Me. The dimethylamino analog **34** showed not only improved BTK potency (IC_{50} 3 nM) but also more than 300-fold JAK2 selectivity. In contrast, compound **35** (lacking an *ortho*-substituent) suffered a 4- to 10-fold loss in potency, and 4- to 6-fold loss in JAK2 selectivity, relative to **30** and **33**.

Table 2. Potent and selective BTK inhibitors.



Compds	Y	R	BTK IC_{50}, nM[13]	JAK2/BTK[14]
29	Me	H	7.2	28
30	Me	F	3.8	58
31	Me	$N(Me)_2$	3.5	114
32	Me	t-Butyl	12	141
33	F	F	9.4	29
34	**F**	**$N(Me)_2$**	**3.0**	**339**
35	H	F	39	7

Activities for three example compounds against selected kinases are shown in Table 3. Selectivity against other additional kinases (20-25 enzymes examined) were generally >100-fold, with greatly reduced potencies (IC_{50} values > 1 μM) against most of them.

The reason for the enhanced selectivity of compounds bearing a *meta*-carboxamide on the 4-phenyl substituent is not obvious. Although the 4-substituents of compounds **29**-**35** are similar to substituents in previously reported BTK inhibitors,[21-23] it is doubtful that these substituents play the same role in enzyme potency and selectivity in the carbazole series. In the earlier-reported cases, BTK potency is exquisitely sensitive to the presence of the *tert*-butylphenyl (or a similar group): replacement by a simple unsubstituted benzamide[22] or acetamide[21] leads to dramatic loss of potency (575-fold and 1420-fold, respectively). However, analogous changes in the carbazole series gave compounds with potencies within 3-fold of each other (compare compounds **32**, **29** and **16**), suggesting that the innate binding affinity of the carbazole core is greater than in the other cases. Indeed, complete removal of the benzamide moiety (compound **11**) causes only a 2-fold change in potency compared to **32**.

Table 3. Kinase selectivity for three BTK inhibitors.[a]

Kinase	Compd 6	Compd 25	Compd 34
BTK	15	16	3.0
JAK2	50 (3.3)	212 (13)	1020 (339)
BMX	119 (7.9)	193 (12)	109 (36)
ITK	39 (2.6)	55 (3.4)	115 (38)
TEC	116 (7.7)	162 (10)	41 (14)
TXK	119 (7.9)	172 (11)	95 (32)
AURa	444 (30)	>2000 (>125)	493 (164)
CDK2	610 (41)	2700 (169)	>50000
cKIT	41 (2.7)	471 (29)	1050 (350)
LCK	81 (5.4)	108 (6.8)	569 (190)
LYNa	68 (4.5)	204 (13)	824 (275)
TYK2	753 (50)	>2000 (>125)	14300 (4770)

[a] IC_{50} (nM). Numbers in parentheses are selectivities for BTK over the indicated kinase.

Additionally, the extended conformation reported for the imidazopyrazine, pyrazinone and pyridinone series,[21-23] where the *tert*-butylphenyl occupies the so-called H3 pocket, is thought to be important for both potency and selectivity, but this conformation is unlikely in the carbazole series. The presence of the tricyclic ring system would destabilize this extended conformation through steric clash between the carbazole core and the 4-phenyl substituent, as suggested by the near-orthogonal arrangement observed in the X-ray structure of **13**. Further, the potency of compounds **29**-**34** suggests that for these compounds, a conformation analogous to that shown in yellow in Figure 3, with the *ortho*-substituent pointing toward Cys 481, is the rotamer responsible for BTK inhibiton. The other rotamer (comparable to that shown in orange) would be expected to introduce a serious steric clash between the benzamide and the P-loop of the enzyme (shown as a blue ribbon in Figure 3).

Notably, compounds of this series were less selective against the other kinases in the TEC family (TEC, BMX, ITK and TXK), of which BTK is a member, where selectivities ranged from less than 3-fold to about 40-fold (Table 3). This could be due to the presence of a cysteine residue (homologous to Cys 481 of BTK) in the other members of the TEC family, since a hydrophobic interaction with this residue was suggested by the X-ray structure of **13** with BTK, particularly with the conformation shown in yellow (Figure 3). Cysteine in this position is rare in the kinome, found in only 10 known kinases. (In Jak2 and LCK, for example, Cys 481 is replaced by the less lipophilic serine.)

In addition to 4,7- and 4,6-disubstituted carbazole-1-carboxamides, 3,7- and 3,6-disubstituted isomers were also synthesized (Table 4). Surprisingly, these proved to

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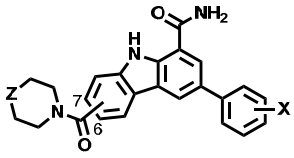
be very potent and selective inhibitors of JAK2. Compounds **36**-**40**, bearing a morpholine amide at the 6-position, were over 100 times less potent against BTK than against JAK2. Replacement of the morpholine with N-methylpiperazine to give **41** increased both the BTK and JAK2 potencies but failed to alter the selectivity.

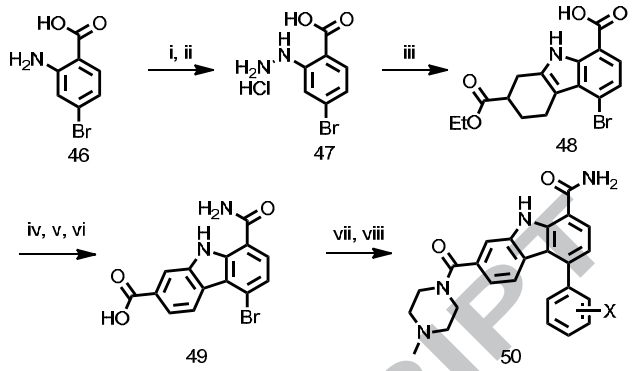
Table 4. 3,6- and 3,7-Disubstituted-carbazole-1-carbozamides.



Compds	X	Amide Position, Z	BTK IC_{50}, nM[13]	JAK2 IC_{50}, nM[14]
36	3-OMe	6, O	360	2.8
37	3-Cl	6, O	189	1.7
38	4-OMe	6, O	327	2.0
39	4-Cl	6, O	308	2.6
40	3,4-Cl_2	6, O	461	2.4
41	4-Cl	6, N-Me	110	1.2
42	3-OMe	7, N-Me	404	4.3
43	4-OMe	7, N-Me	436	5.5
44	3,4-Cl_2	7, N-Me	812	6.2
45	3-NHCOMe	7, N-Me	379	27

Moving the amide to C-7 (**42-45**) showed similar potency and selectivity except for the 4-acetanilide **45**, which was less potent and less selective for JAK2. The selectivity of the 3-arylcarbazole-1-carboxamides for JAK2 over BTK, and also the dramatic selectivity of these compounds with respect to other JAK family members (JAK1 and JAK3, data not shown) have prompted extensive exploration of this isomeric series for oncology indications.[15,24]

The synthesis of the 4,7-disubstituted carbazole-1-carboxamides is summarized in Scheme **1**. The commercially available 2-amino-4-bromobenzoic acid (**46**) was treated with sodium nitrite in hydrochloric acid to form the diazonium intermediate, which was then reduced with tin (II) chloride to the hydrazine **47**.[25] The tetrahydrocarbazole acid **48** was prepared in good yield by Fischer indole synthesis[26] from **47** and ethyl 3-oxocyclohexanecarboxylate in boiling acetic acid. The carboxylic acid of **48** was converted to the primary amide, and the tricyclic core was aromatized by treatment with DDQ to give the bromocarbazole-1-carboxamide ester. Saponification provided the carboxylic acid **49**, which was coupled with N-methyl piperazine in the presence of EDC and HOBT to provide the corresponding amide. Suzuki coupling[27,28] then gave the final products **50**. The isomeric 4,6-, 3,6- and 3,7-disubstituted compounds were prepared analogously.



Scheme 1. Reagents and conditions: i. $NaNO_2$, H_2O, conc. HCl, -10 ^oC to -5 ^oC, 10 min; ii. $SnCl_2$, conc. HCl, -10 ^oC to rt, 1 h, 82% in two steps; iii. ethyl 3-oxocyclohexanecarboxylate, AcOH, reflux, 4 h, 64%; iv. NH_4OH, EDC, HOBT, in THF/CH_2Cl_2, rt, 16 h, 98%; v. DDQ, toluene, reflux, 2.5 h, 91%; vi. LiOH in THF/EtOH/H_2O, reflux, 3 h, 90%; vii. N-methyl piperazine, EDC, HOBT, rt, 16 h, 76%; viii. boronic esters or boronic acids, $PdCl_2(dppf)_2$ and K_2CO_3 or $Pd(Ph_3P)_4$ and Na_2CO_3, toluene/EtOH, 90 ^oC, 10 h, 15–80%.

In conclusion, we have designed and synthesized novel 4,7- and 4,6-disubstituted carbazole-1-carboxamides as potent and selective BTK inhibitors. The 4-position substituents had a significant effect on potency and selectivity for BTK relative to other kinases, particularly JAK2. The 3,7- and 3,6-disubstituted carbazole-1-carboxamides have also been identified as highly potent and selective JAK2 inhibitors with potential utility in oncology. Further exploration of the intriguing BTK activity and SAR of the carbazole carboxamides will be reported in due course.

Acknowledgment

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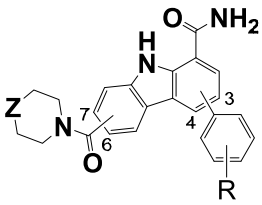

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