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Non-hinge-binding pyrazolo[1,5-*a*]pyrimidines as potent B-Raf kinase inhibitors

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ABSTRACT

As part of our research effort to discover B-Raf kinase inhibitors, we prepared a series of C-3 substituted *N*-(3-(pyrazolo[1,5-*a*]pyrimidin-7-yl)phenyl)-3-(trifluoromethyl)benzamides. X-ray crystallography studies revealed that one of the more potent inhibitors (**10n**) bound to B-Raf kinase without forming a hinge-binding hydrogen bond. With basic amine residues appended to C-3 aryl residues, cellular activity and solubility were enhanced over previously described compounds of this class.

The Ras-Raf-MEK-ERK signal transduction pathway (ERK pathway) plays a key role in cellular growth and proliferation. Mutations of either Ras or B-Raf lead to a sustained and constitutive activation of the ERK pathway, resulting in increased cell division and cell survival, and are associated with a variety of human cancers. Mutant B-Raf (most commonly V600E) is associated with melanoma[1] (66%), colorectal cancer[2] (12%), and papillary thyroid cancers[3] (~45%). B-Raf (mutant and wild-type) inhibitors therefore have the potential to treat cancers associated with dysregulation of the ERK pathway. A number of inhibitors of the Ras-Raf-MEK-ERK pathway have been discovered and some of these have now advanced into clinical trials.[4]

In our effort to discover novel and selective B-Raf kinase inhibitors, we have explored the SAR of a series of C-2 and C-3 substituted pyrazolo[1,5-*a*] pyrimidines.[5–7] As shown in Figure 1, the initial lead in this series was compound **1** (B-Raf IC_{50}: 1.5 μM, B-Raf mutant cell HT29 and WM266 IC_{50}s: 7.0 μM and 6.2 μM, respectively). We now report additional SAR information for this pyrazolo[1,5-*a*]pyrimidine series, focusing on the incorporation of substituents on the central phenyl ring (Fig. 1, R^1) and a survey of replacements for the ester group on the pyrazole ring (Fig. 1, R^2). Specifically, a series of substituted alkyne, five- and six-membered aryl and heteroaryl substituents were explored in order to optimize the B-Raf kinase inhibitory activity of these compounds. In order to improve the aqueous solubility of these compounds, a number of them were prepared with basic amine water-solubilizing groups attached.

For all of the target compounds, the optimized trifluorobenzamide group was retained at the *meta*-position of the C-7 phenyl residue.[5] The preparation of intermediates **6a–d** were carried out as outlined in Scheme 1. Coupling of 3-aminoacetophenones **3a–c** with 3-(trifluoromethyl)benzoyl chloride followed by reaction with *N,N*-dimethylformamide dimethyl acetal afforded enaminone intermediates **4a–c**. Condensation of **4b** and **4c** with 5-aminopyrazole **5a** in refluxing acetic acid afforded pyrazolo[1,5-*a*]pyrimidines **6a** and **6b**. Under similar conditions, **6d** was prepared from the reaction of **4a** with **5b**. A slightly different route was required for

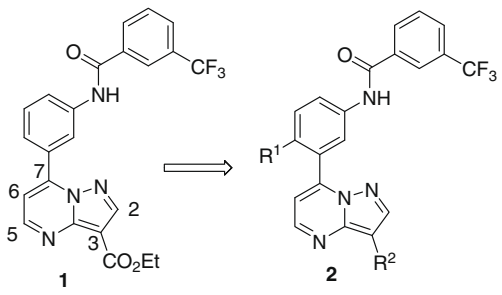

Figure 1. B-Raf inhibitors.

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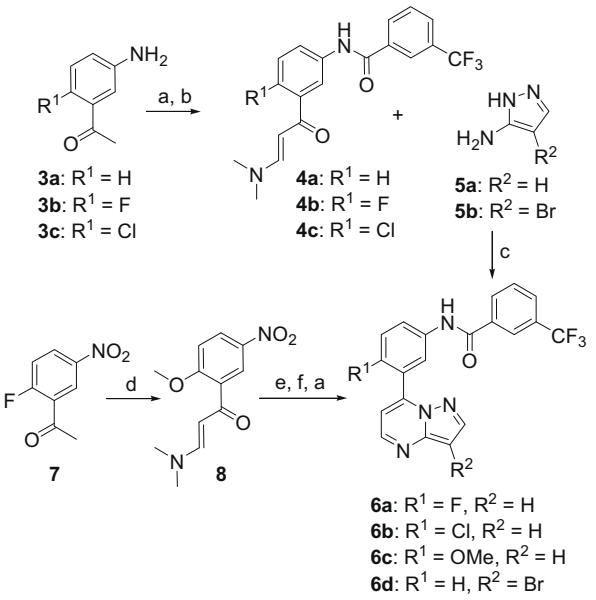


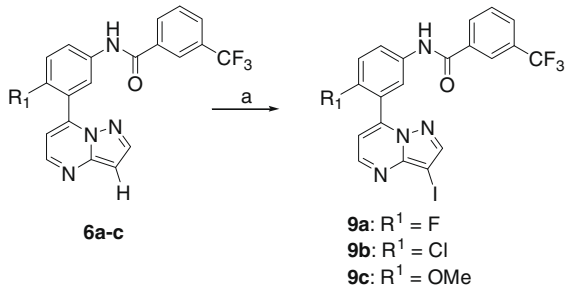
Scheme 1. Reagents and conditions: (a) 3-(trifluoromethyl)benzoyl chloride, Et_3N, CH_2Cl_2, 0 °C to rt, 2–12 h, 80–95%; (b) DMF–DMA, reflux, 6–12 h, 90–100%; (c) acetic acid, 80 °C, 12–24 h, 60–85%; (d) DMF–DMA, reflux, 12 h, 70%; (e) **5a**, acetic acid, 80 °C, 12 h, 82%; (f) H_2, 10% Pd/C, EtOAc, rt, 48 h, 96%.

the synthesis of the methoxy substituted analog **6c**. Thus, reaction of 3-nitro-6-fluoro-acetophenone **7** with *N,N*-dimethylformamide dimethyl acetal produced the methoxy substituted aromatic enaminone **8**. Condensation of **8** with 5-aminopyrazole **5a**, followed by hydrogenation over 10% palladium on carbon and subsequent acylation with 3-(trifluoromethyl)benzoyl chloride gave **6c**.

Intermediates **9a–c** were prepared through the reaction of **6a–c** with *N*-iodosuccinimide, as shown in Scheme 2.

Final products **10a** and **10b** were prepared by Sonogashira et al.[8] coupling of **6d** with substituted alkynes. The preparation of the majority of the target compounds **10c–e, 10i–v, 10x, 10aa–dd** (Scheme 3) was carried out by Suzuki[9] chemistry, where the appropriately substituted boronic acids or esters were coupled with **6d, 9a–c**. For certain analogs, it was desirable to further elaborate the C-3 substituents after the coupling reaction (Scheme 3). Thus, following the preparation of **11a** and **11b** under Suzuki coupling conditions, the resulting anilines were converted to the amide-substituted targets **10f–h** in the presence of benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (Pybop).

Additional target compounds were prepared as shown in Scheme 4. Compound **10v** was converted to **10w** by reaction with 1-(2-chloroethyl)pyrrolidine under basic conditions. The reaction of **6d** with 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)furan-2-carbaldehyde under microwave conditions provided aldehyde



Scheme 2. Reagents and conditions: (a) *N*-iodosuccinimide, CH_2Cl_2, rt, 12–24 h, 45–60%.

intermediate **12**. Reductive amination of aldehyde **12** with pyrrolidine and *N*-ethylpiperidine provided target compounds **10y** and **10z**, respectively.

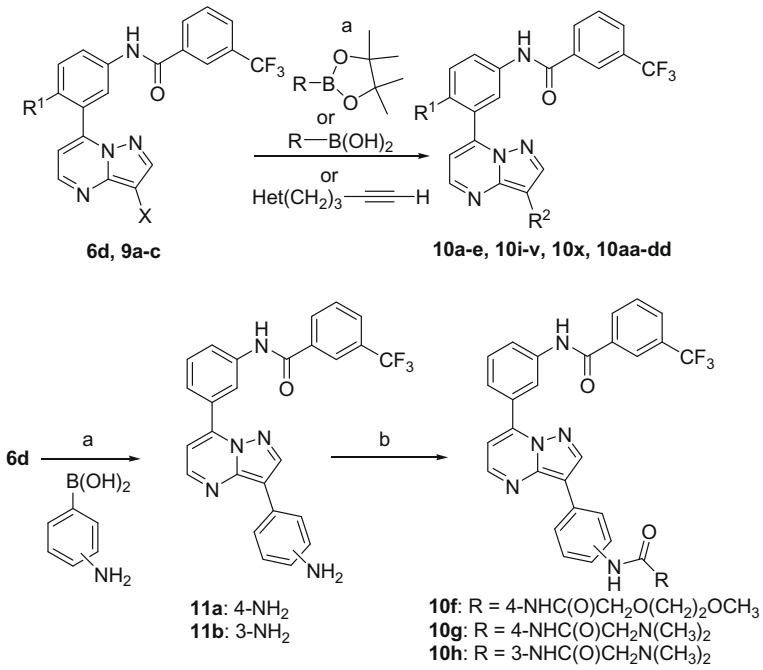
The in vitro activity of the 30 compounds prepared is shown in Tables 1 and 2. Several trends are evident from the data presented. The C-3 alkyne substituted analogs **10a** and **10b** were modest B-Raf kinase inhibitors (IC_{50}s 0.552 μM and 0.842 μM, respectively), in contrast to C-3 aryl substituted analogs such as **10p** (B-Raf IC_{50}: 0.038 μM). Of the compounds lacking strongly basic amine substituents on the C-3 phenyl group listed in Table 1, the *meta*-amide-substituted analog **10e** and pyrazole-substituted **10v** were the most potent (B-Raf IC_{50}s: 0.057 μM and 0.084 μM, respectively), raising the possibility of an additional interaction with the protein. Molecular modeling of these two analogs (not shown) indicates that both can potentially act as hydrogen bond donors to the hinge region Cys532 backbone carbonyl group. Consistent with this postulate, the substituted pyrazole analog **10w** was a modest B-Raf enzyme inhibitor (B-Raf IC_{50}: 0.393 μM) in comparison to **10v**. Despite the good enzyme activity of **10e** and **10v**, cellular activity for these compounds was modest (**10e**: WM266 IC_{50}: 3.7 μM, HT29 IC_{50}: 3.2 μM, **10v**: WM266 IC_{50}: 3.7 μM, HT29 IC_{50}: 2.8 μM). The *para*-phenylsulfonamide-substituted compound **10d** was a significantly weaker B-Raf kinase inhibitor (B-Raf IC_{50}: >10 μM).

The impact of a basic amine moiety on in vitro activity was demonstrated by comparing compounds **10f** and **10g**. Thus, compound **10g** was approximately 3.6-fold more potent than **10f** against B-Raf enzyme (IC_{50}s 0.034 μM vs 0.139 μM, respectively), and had submicromolar activity in the cellular assays (WM266 IC_{50}: 0.88 μM, HT29 IC_{50}: 0.77 μM). A small decrease in activity was observed for the *meta*-phenyl-substituted analog **10h** compared to **10g**, demonstrating that *para*-substitution on the C-3 aryl residue provided optimal potency. This was further confirmed by preparation of analogs **10i–n**, wherein the best potency is observed for the *para*-substituted compounds **10k** and **10n**, with slightly decreased activity for the corresponding *meta*-substituted analogs **10j** and **10m**. A more significant loss in activity is seen for the *ortho*-substituted compounds **10i** and **10l**. Similar activity was observed for the pyrrolidine and methylpiperazine substituted **10o** and **10p** as compared to the dimethylamine-substituted **10n**.

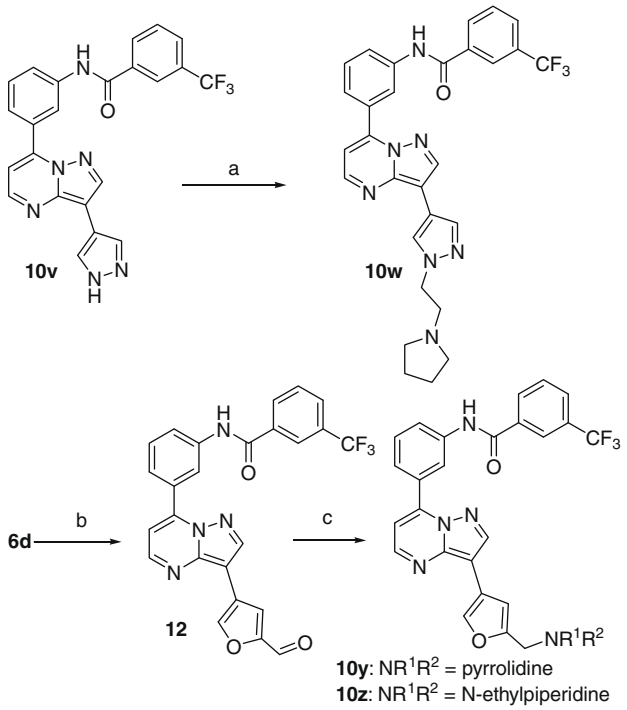
The C-3 pyridine substituted analogs **10s** and **10t**, with water-solubilizing groups, have activity comparable to the corresponding phenyl-substituted analogs **10n–p**. The HT29 cellular potencies of **10s** and **10t** are particularly noteworthy, with IC_{50}s of 0.46 μM and 0.31 μM, respectively. In contrast, the 3-pyridyl substituted analogs **10q** and **10r**, which lack strongly basic amine substituents, were less active in both enzyme and cellular assays. The morpholine-substituted pyrimidyl analog **10u** was modestly active in the enzyme assay (B-Raf IC_{50}: 0.941 μM).

The 3-furyl analog **10x**, and substituted 3-furyl analogs **10y** and **10z** all had modest activity against B-Raf. It appears that compounds containing five-membered ring linkers to the water-solubilizing groups (**10w**, **10y**, and **10z**) were generally less potent than the corresponding six-membered ring analogs. This decrease in activity is most likely due to a reduction in hydrophobic contact with the enzyme when compared to the six-membered ring analogs, as the C-3 *meta*- and *para*-substituted phenyl compounds were potent enzyme inhibitors.

In Table 2 is listed a set of compounds exploring R^1 substitution on the central phenyl ring. It is noteworthy that both the fluoro and chloro substituted compounds **10aa** and **10bb**, had better enzyme activity than the parent **10v**, while the methoxy substituted compound **10cc** was somewhat less active than **10v**. The fluoro analog **10aa** was an 18 nM inhibitor of B-Raf kinase and the most active of the compounds in the pyrazolo[1,5-*a*]pyrimidine series. However, the cellular activities of both **10aa** and **10bb** did not show a corre-



Scheme 3. Reagents and conditions: (a) $Pd(PPh_3)_4$ or $Pd(dppf)Cl_2$, Na_2CO_3, aq DME, μW, 175 °C, 1000–2000 s, 10–54%, or DME, 80–100 °C, 24 h, 15–18%, or CuI, $(PPh_3)_2PdCl_2$, Et_3N, PPh_3, NMP, 80 °C, 3 h, 18% (**10a**), 25% (**10b**); (b) Pybop, diisopropylethylamine, DMF, 25 °C, 15 h, 66–80%.



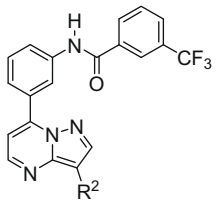
Scheme 4. Reagents and conditions: (a) 1-(2-chloroethyl)pyrrolidine·HCl, Cs_2CO_3, Bu_4NI, DMF, 65 °C, 24 h, 43%; (b) 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)furan-2-carbaldehyde, $Pd(PPh_3)_4$, Na_2CO_3, DME, μW, 175 °C, 1000 s, 33%; (c) NHR^1R^2, $Na(OAc)_3BH$, HOAc, NMP, CH_2Cl_2, 25 °C, 15 h, 38% (**10y**), 89% (**10z**).

sponding improvement over compound **10v**. Based on the enhanced enzyme activity of the C-7 fluorophenyl-substituted **10aa** versus phenyl-substituted **10v**, compound **10dd** was prepared in an effort to further boost B-Raf activity versus the relatively potent compound **10t**. In this case, both compounds had comparable activity in both enzyme and cellular assays. It appears that appropriate R^1 substituents can boost the B-Raf enzyme activity of certain pyrazolo[1,5-*a*]pyrimidine analogs, possibly by forming a favorable interaction within a hydrophobic pocket. Unfortunately, this did not provide a corresponding improvement in cellular potency for the analogs prepared.

While most of the compounds lacking a basic amine substituent were insoluble in a pH 7.4 aqueous buffer (e.g., solubility of **10d**, **10r**, and **10u**: 0 μg/mL), the basic amine groups modestly enhanced solubility. For example, the C-3 phenyl-substituted **10m** was slightly soluble in pH 7.4 aqueous buffer (4 μg/mL). The C-3 alkyne substituted analog **10b** was somewhat more soluble (8 μg/mL at pH 7.4), but far less potent in vitro. Thus, while a significant amount of ligand–protein hydrophobic contact is required to provide the observed level of potency, this considerably reduces the aqueous solubility of these inhibitors, even with attached basic amine residues. An issue generally encountered with analogs substituted with dimethylamine or *N*-methylpiperazine groups was poor microsomal stability (for example, rat microsome t1/2 for **10b**, **10n**, and **10p** was 3, 6, and 3 min, respectively). In contrast, analogs substituted with cyclic amines morpholine or pyrrolidine were considerably more stable (rat microsome t1/2 for **10a**, **10o**, and **10w** was >30, 27, and 26 min, respectively).

Several attempts were made to co-crystallize these analogs with B-Raf wild-type enzyme. Of the compounds examined, **10n** was successfully co-crystallized with B-Raf at a resolution of 2.9 Å (PDB code: 3II5). A depiction of **10n** bound to B-Raf, based on the crystallographic coordinates is shown in Figure 2. As shown, compound **10n** is bound to the inactive (DFG-out) conformation of the enzyme. Two hydrogen bonds are formed, one from the amide carbonyl of **10n** to Asp593, the other from the amide NH group to Glu500. There is no hydrogen bond to the hinge region. Hydrophobic binding interactions of the trifluoromethylbenzamide moiety with the enzyme appear to contribute significantly to overall binding affinity of this analog, since it fits well in a hydrophobic pocket consisting of Ile 512, Leu 566, His 573, and Ile 591. Additionally, the C-3 phenyl ring makes significant hydrophobic interactions with the residues of Ile 462 and Leu 596 of the enzyme. The information

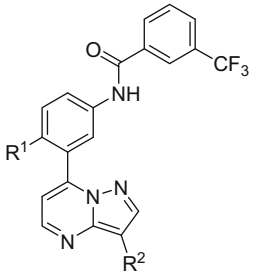
Table 1
B-Raf kinase and cellular activity of pyrazolopyrimidine analogs **1**, **10a–z**



Compd	R^2	B-Raf IC_{50}[a,b] (μM)	WM 266-4 IC_{50}[a] (μM)	HT29 IC_{50}[a] (μM)
1	CO_2Et	1.50	6.2	7.0
10a	$C{\equiv}C(CH_2)_3$-morpholine	0.552	5.5	3.5
10b	$C{\equiv}C(CH_2)_3$-*N*-methylpiperazine	0.842	1.4	2.6
10c	4-Ph-$N(CH_3)_2$	0.308	>10	ND
10d	4-Ph-$SO_2N(CH_3)_2$	>10	ND	ND
10e	3-Ph-$CONH_2$	0.057	3.7	3.2
10f	4-Ph-$NHC(O)CH_2OCH_2CH_2OCH_3$	0.139	>10	3.9
10g	4-Ph-$NHC(O)CH_2N(CH_3)_2$	0.034	0.88	0.77
10h	3-Ph-$NHC(O)CH_2N(CH_3)_2$	0.040	2.46	0.8
10i	2-Ph-CH_2CH_2–$N(CH_3)_2$	0.318	2.6	0.84
10j	3-Ph-CH_2CH_2–$N(CH_3)_2$	0.041	0.74	0.65
10k	4-Ph-CH_2CH_2–$N(CH_3)_2$	0.026	0.74	0.73
10l	2-Ph-CH_2–$N(CH_3)_2$	0.117	2.9	2.9
10m	3-Ph-CH_2–$N(CH_3)_2$	0.044	1.3	0.8
10n	4-Ph-CH_2–$N(CH_3)_2$	0.024	0.92	0.78
10o	4-Ph-CH_2-pyrrolidine	0.020	2.1	0.65
10p	4-Ph-CH_2-*N*-methylpiperazine	0.038	0.82	0.7
10q	3-Pyridyl	0.113	3.7	ND
10r	3-Pyridinyl-6-NH_2	0.112	3.4	2.4
10s	3-Pyridinyl-6-$NHCH_2CH_2N(CH_3)_2$	0.030	0.92	0.46
10t	3-Pyridinyl-6-*N*-methylpiperazine	0.044	0.74	0.31
10u	5-Pyrimidyl-2-morpholine	0.941	ND	ND
10v	4-Pyrazolyl	0.084	3.7	2.8
10w	4-Pyrazolyl-1-CH_2CH_2-pyrrolidine	0.393	3.3	2.8
10x	3-Furyl	0.326	ND	ND
10y	3-Furyl-5-CH_2-pyrrolidine	0.362	3.2	2.2
10z	3-Furyl-5-CH_2-*N*-ethylpiperazine	0.219	2.9	1.7

[a] Values are averages of more than two experiments (ND = not done).
[b] B-Raf IC_{50}s were determined as previously described.[5]

Table 2
B-Raf kinase and cellular activity of pyrazolopyrimidine analogs **10v**, **10aa–dd**



Compd	R^1	R^2	B-Raf IC_{50}, μM[a,b]	WM 266-4 IC_{50}, μM[a]	HT29 IC_{50}, μM[a]
10v	H	4-Pyrazolyl	0.084	3.7	2.8
10aa	F	4-Pyrazolyl	0.018	6.6	2.8
10bb	Cl	4-Pyrazolyl	0.042	ND	2.9
10cc	OMe	4-Pyrazolyl	0.098	ND	2.9
10dd	F	3-Pyridinyl-6-*N*-methylpiperazine	0.030	0.81	0.63

[a] Values are averages of more than two experiments (ND = not done).
[b] B-Raf IC_{50}s were determined as previously described.[5]

provided by this crystal structure indicates that the *meta*- and *para*-basic amine substituted C-3 aromatics can readily reach the aqueous environment around the enzyme, accounting for their

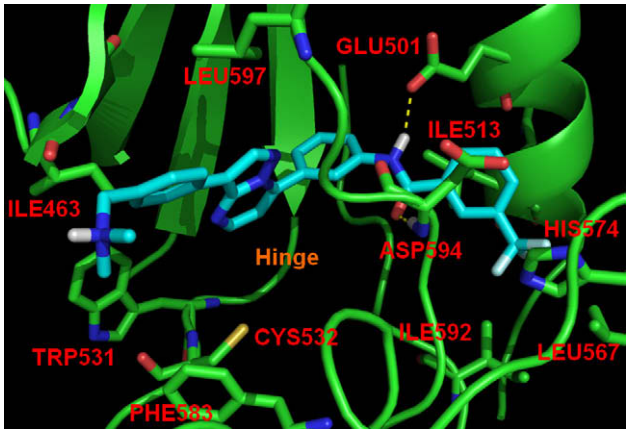


Figure 2. Representation of compound **10n** bound to wild-type B-Raf based on the crystallographic data obtained at 2.9 Å resolution.

greater activity versus the *ortho*-substituted analogs. Thus, the observed binding mode of **10n** is consistent with the SAR of this class of compounds.

Having determined the nature of the key interactions of compound **10n** with B-Raf kinase through the crystallographic data, this compound was profiled against a panel of 16 additional kinases. Compound **10n** was equipotent against c-Raf (IC_{50}: 0.025 μM) Moderate selectivity was observed for compound **10n**

versus p38α (IC_{50}: 0.216 μM) and CAMKII (IC_{50}: 0.822 μM), while high selectivity was observed versus CDK2, CDK4, PKCα, IKKβ, JNK1, MK2, PKA, Src, MKK6, PLK1, p70S6K, PI3 Kα, and PDK1 (IC_{50}s: >2 μM).

In conclusion, a series of pyrazolo[1,5-*a*]pyrimidines was prepared that showed good activity as B-Raf inhibitors, despite lacking a hinge-binding interaction. Optimization of these compounds provided potent analogs **10s** and **10t**, which effectively inhibited proliferation of HT29 cells at submicromolar concentrations. It is noteworthy that the potency achieved for this series is similar to that observed for pyrazolo[1,5-*a*]pyrimidine analogs with hinge region binding residues.[6] Further optimization of B-Raf inhibitory activity could potentially be achieved by incorporating hinge-binding groups into these structures to enhance potency. Our continued efforts to enhance the binding potencies and physical properties of these B-Raf inhibitors will be described in future publications.

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References and notes

1. Davies, H.; Bignell, G. R.; Cox, C.; Stephens, P.; Edkins, S.; Clegg, S.; Teague, J.; Woffendin, H.; Garnett, M. J.; Bottomley, W.; Davis, N.; Dicks, E.; Ewing, R.; Floyd, Y.; Gray, K.; Hall, S.; Hawes, R.; Hughes, J.; Kosmidou, V.; Menzies, A.; Mould, C.; Parker, A.; Stevens, C.; Watt, S.; Hooper, S.; Wilson, R.; Jayatilake, H.; Gusterson, B. A.; Cooper, C.; Shipley, J.; Hargrave, D.; Pritchard-Jones, K.; Maitland, N.; Chenevix-Trench, G.; Riggins, G. J.; Bigner, D. D.; Palmieri, G.; Cossu, A.; Flanagan, A.; Nicholson, A.; Ho, J. W. C.; Leung, S. Y.; Yuen, S. T.; Weber, B. L.; Seigler, H. F.; Darrow, T. L.; Paterson, H.; Marais, R.; Marshall, C. J.; Wooster, R.; Stratton, M. R.; Futreal, P. A. *Nature* **2002**, *417*, 949.
2. Rajagopalan, H.; Bardelli, A.; Lengauer, C.; Kinzler, K. W.; Vogelstein, B.; Velculescu, V. E. *Nature* **2002**, *418*, 934.
3. Xing, M. *Endocr. Relat. Cancer* **2005**, *12*, 245.
4. McCubrey, J. A.; Milella, M.; Tafuri, A.; Martelli, A. M.; Lunghi, P.; Bonati, A.; Cervello, M.; Lee, J. T.; Steelman, L. S. *Curr. Opin. Invest. Drugs* **2008**, *9*, 614.
5. Gopalsamy, A.; Ciszewski, G.; Hu, Y.; Lee, F.; Feldberg, L.; Frommer, E.; Kim, S.; Collins, K.; Wojciechowicz, D.; Mallon, R. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2735.
6. Shi, M.; Gopalsamy, A.; Berger, D. M.; Dutia, M.; Hu, Y.; Lee, F.; Feldberg, L.; Frommer, E.; Kim, S.; Collins, K.; Wojciechowicz, D.; Mallon, R. *Abstracts of Papers*, 235th ACS National Meeting, New Orleans, LA, United States, April 6–10, 2008; MEDI-081.
7. Ciszewski, G.; Gopalsamy, A.; Levin, J. I.; Kim, K.-H.; Hu, Y.; Lee, F.; Feldberg, L.; Frommer, E.; Kim, S.; Collins, K.; Wojciechowicz, D.; Mallon, R. *Abstracts of Papers*, 235th ACS National Meeting, New Orleans, LA, United States, April 6–10, 2008; MEDI-080.
8. Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tetrahedron Lett.* **1975**, *50*, 4467.
9. Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *50*, 4467.