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## Boronic acid-containing aminopyridine- and aminopyrimidinecarboxamide CXCR1/2 antagonists: Optimization of aqueous solubility and oral bioavailability

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## ABSTRACT

The chemokine receptors CXCR1 and CXCR2 are important pharmaceutical targets due to their key roles in inflammatory diseases and cancer progression. We have previously identified 2-[5-(4-fluoro-phenyl-carbamoyl)-pyridin-2-ylsulfanylmethyl]-phenylboronic acid (SX-517) and 6-(2-boronic acid-5-trifluoromethoxy-benzylsulfanyl)-N-(4-fluoro-phenyl)-nicotinamide (SX-576) as potent non-competitive boronic acid-containing CXCR1/2 antagonists. Herein we report the synthesis and evaluation of aminopyridine and aminopyrimidine analogs of SX-517 and SX-576, identifying (2-((benzyl)((5-boronic acid-2-pyridyl)methyl)amino)-5-pyrimidinyl)(4-fluorophenylamino)formaldehyde as a potent chemokine antagonist with improved aqueous solubility and oral bioavailability.

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The chemokine receptors CXCR1 and CXCR2 are major targets of drug development in the pharmaceutical industry<sup>1,2</sup> due to their key role in neutrophil chemotaxis and its association with inflammation.<sup>3</sup> The endogenous ligands for these G-protein coupled receptors (GPCRs) include the CXCR2-specific growth-related oncogene  $\alpha$  (GRO  $\alpha$ , or CXCL1) and interleukin-8 (IL8, or CXCL8), which binds to both receptors.<sup>4</sup> Experiments with animals lacking CXCR1 and/or CXCR2 activity have demonstrated that their inhibition could be beneficial in the treatment of several diseases, including asthma,<sup>5</sup> chronic obstructive pulmonary disease (COPD),<sup>6</sup> and inflammatory bowel disease<sup>7</sup>, cancer (melanoma,<sup>8</sup> pancreatic,<sup>9,10</sup> and colon<sup>11,12</sup> cancer), Alzheimer's disease,<sup>13</sup> and traumatic brain injury.<sup>14</sup> Several previously reported CXCR1 and/or CXCR2 inhibitors representing the key structural classes are summarized in Figure 1. The diaryl urea CXCR2 antagonist SB656933 was evaluated in clinical trials for the treatment of COPD<sup>15,16</sup> and cystic fibrosis,<sup>17</sup> and GlaxoSmithKline has recently advanced into the

clinic a new structurally-similar inhibitor danirixin **1**,<sup>18</sup> with trials currently recruiting patients for COPD and respiratory syncytial virus infections. The CXCR2 antagonist navarixin (SCH527123, **2**)<sup>19</sup> from Merck utilized a cyclic urea bioisostere 3,4-diaminoclobut-3-ene-1,2-dione instead of the diaryl urea to generate a potent inhibitor that was evaluated in clinical trials for the treatment of COPD,<sup>20,21</sup> asthma,<sup>22</sup> and psoriasis. Their Phase 2 trial demonstrated safety and efficacy in moderate to severe COPD patients.<sup>21</sup> AstraZeneca's pyrimidine-based CXCR1/2 inhibitors AZD8309,<sup>23</sup> AZD5069 **3**,<sup>24</sup> and AZD4721 (structure undisclosed) have been clinically evaluated to treat COPD<sup>25,26</sup> and asthma.<sup>2</sup> Dompé's ketoprofen derivative reparixin **4**, an inhibitor of CXCL8 receptor CXCR1 and CXCR2 activation,<sup>27,28</sup> is being explored for the reduction of post-surgical inflammation after transplantation surgeries.<sup>2</sup> Novartis<sup>29,30</sup> and Pfizer<sup>23,31</sup> also have active CXCR2 programs.

We have previously reported a novel class of dual allosteric CXCR1/2 antagonists that utilize an aromatic boronic acid moiety on a nicotinamide core.<sup>32,33</sup> Our first-generation inhibitor **5** (SX-517) exhibited anti-inflammatory activity in vivo, but its preclinical development was halted due to its metabolic instability. A focused SAR effort to increase metabolic stability led to the discovery of our second-generation inhibitor **6** (SX-576), which was less susceptible to oxidation of the boronic acid.<sup>32</sup> Modeling of the

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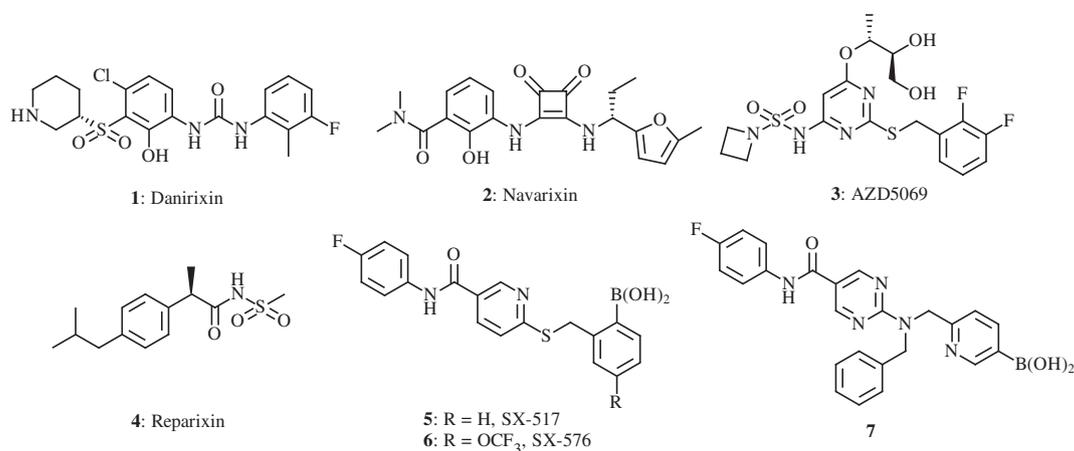
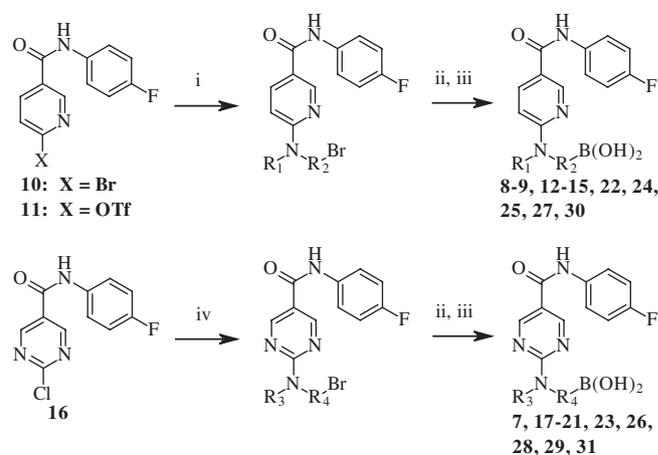


Figure 1. Chemokine antagonists.

binding of **6** with CXCR2, constructed from the recently solved structure of CXCR1,<sup>34</sup> revealed that this class of antagonists has a different binding model than previously described for other classes of compounds. However, the poor aqueous solubility of **6** led us to examine whether heteroatom replacement of the sulfur could improve its oral bioavailability. Herein, we describe our focused SAR studies that led to the identification of a third-generation compound **7**, which exhibited improved aqueous solubility and oral bioavailability while maintaining activity in an animal model of pulmonary inflammation.

Starting from our first- (**5**) and second- (**6**) generation inhibitors, we synthesized compounds that maintained a boronic acid in the 2-position of the phenyl ring, replacing the sulfur in **5** with secondary (**8**) and tertiary (**9**) amines, utilizing either a bromonicotinamide<sup>35</sup> or triflatenicotinamide<sup>36</sup> intermediate (**10**, **11**), depending on the reactivity of the amine. Generally, primary and *N*-methyl secondary amines were coupled with the bromonicotinamide intermediate, and more sterically-hindered secondary amines were coupled with the triflatenicotinamide intermediate. The general synthesis of the inhibitors is shown in Scheme 1. Shifting the position of the boronic acid to the 3- (**12**, **13**) and 4-positions (**14**, **15**) was also explored. The corresponding pyrimidine compounds were also prepared from a chloropyrimidinamide<sup>37</sup> intermediate (**16**), with the boronic acid in the 2- (**17**), 3- (**18**, **19**), and 4-positions (**20**, **21**). The corresponding pyrimidine analog to **8** could not be prepared using our standard synthetic methods, because the palladium-catalyzed conversion of the halogen to the boronic acid pinacol ester instead led to reduction of the halogen to an unsubstituted benzyl ring. Alternate routes to the desired product also proved unsuccessful.

The compounds were screened for their ability to inhibit calcium flux in a previously described cell-based assay.<sup>33,38</sup> Briefly, the synthesized compounds were incubated for 30 min with rat basophilic leukemia (RBL) cells stably transfected with either CXCR1 or CXCR2. After the addition of IL8, the release of intracellular calcium was measured via FLUO-4AM detection in a fluorescent microplate reader. The activity of the compounds against CXCR1 and CXCR2 is summarized in Table 1. In contrast to what was observed with our sulfur-containing compounds, the 2-position for the boronic acid was not well tolerated. Instead, the 4-position appeared to yield the most potent compounds, such as **21**. The lack of activity for the 2-position compounds was likely due to neighboring group interactions between the boronic acid and the amine.<sup>39</sup> Due to these observations, the 4-boronic acid, 2-pyridinyl analogs (**22**, **23**) were prepared and found to maintain inhibitory activity at CXCR2. These compounds also exhibited improved



Scheme 1. Reagents and conditions: (i) primary or secondary amine, DIPEA, anhyd DMF or NMP, 120–160 °C, 16–72 h; (ii) PdCl<sub>2</sub>(dppf), bis(pinacolato)diboron, potassium acetate, anhyd. DMF, 80 °C, 3–6 h; (iii) KHF<sub>2</sub>, then TMS-Cl/H<sub>2</sub>O or HCl/dioxane or aq formic acid; (iv) primary or secondary amine, triethylamine, anhyd NMP, rt, 16–72 h.

aqueous solubility, making them good scaffolds for further substitution.

The addition of a phenyl ring (**24**, **7**) at the tertiary amine significantly improved potency of the inhibitors, in contrast to the addition of another pyridine ring (**25**, **26**). Additions of a tetrahydropyran ring (**27**), carboxylic acid (**28**), or amine (**29**) in efforts to further improve aqueous solubility, yielded inactive compounds. However, the addition of a furan ring (**30**, **31**) yielded compounds with similar potency to the phenyl compounds. The third generation inhibitors are summarized in Table 2. Interestingly, aminopyrimidine-based **7** was significantly more potent than its aminopyridine analog **24**, while the aminopyridine-based **30** was significantly more potent than its aminopyrimidine analog **31**. It was somewhat surprising that changing a single atom between the paired compounds made such a difference in their activity. Since they were the most potent in the cell-based assays, **7**<sup>40</sup> and **30**<sup>41</sup> were selected for further evaluation.

Compared with **6**, **7** and **30** were more potent inhibitors of IL8-mediated calcium flux in RBL cells stably transfected with CXCR1 or CXCR2, but they were not as potent at inhibiting calcium flux in isolated neutrophils, and **7** (185 ± 52 nM) was more potent than **30** (321 ± 96 nM). Due to its higher activity, **7** was selected for further testing.

**Table 1**

Inhibition of IL8-mediated intracellular calcium release in RBL cells stably transfected with CXCR1 or CXCR2

Compd	R <sub>1</sub>	R <sub>2</sub>	CXCR1 IC <sub>50</sub> <sup>a</sup> (nM)	CXCR2 IC <sub>50</sub> <sup>a</sup> (nM)	Compd	R <sub>3</sub>	R <sub>4</sub>	CXCR1 IC <sub>50</sub> <sup>a</sup> (nM)	CXCR2 IC <sub>50</sub> <sup>a</sup> (nM)
<b>8, 9</b>	H, Me		>5000	>5000	<b>17</b>	Me		>5000	>5000
<b>12</b>	H		611 ± 62	733 ± 85	<b>18</b>	H		1340 ± 90	918 ± 114
<b>13</b>	Me		4100 ± 500	410 ± 40	<b>19</b>	Me		2500 ± 200	2500 ± 400
<b>14</b>	H		4300 ± 400	2200 ± 400	<b>20</b>	H		1700 ± 300	2600 ± 200
<b>15</b>	Me		1400 ± 200	1100 ± 200	<b>21</b>	Me		681 ± 80	121 ± 30
<b>22</b>	Me		1700 ± 200	81 ± 19	<b>23</b>	Me		1700 ± 100	933 ± 54

<sup>a</sup> Experiments performed in triplicate, reported as mean ± SE. Compound **6** had IC<sub>50</sub> values of 31 ± 4 nM and 21 ± 6 nM versus CXCR1 and CXCR2, respectively.**Table 2**

Inhibition of IL8-mediated intracellular calcium release in RBL cells stably transfected with CXCR1 or CXCR2

Compd	R <sub>1</sub>	R <sub>2</sub>	CXCR1 IC <sub>50</sub> <sup>a</sup> (nM)	CXCR2 IC <sub>50</sub> <sup>a</sup> (nM)	Compd	R <sub>3</sub>	R <sub>4</sub>	CXCR1 IC <sub>50</sub> <sup>a</sup> (nM)	CXCR2 IC <sub>50</sub> <sup>a</sup> (nM)
<b>22</b>	Me		1700 ± 200	81 ± 19	<b>28</b>			>5000	>5000
<b>24</b>			326 ± 39	298 ± 45	<b>7</b>			7 ± 2	4 ± 1
<b>25</b>			>5000	4850 ± 250	<b>26</b>			2050 ± 430	4310 ± 290
<b>27</b>			>5000	2300 ± 400	<b>29</b>			>5000	>5000
<b>30</b>			3.3 ± 0.4	21 ± 3	<b>31</b>			444 ± 58	655 ± 101

<sup>a</sup> Experiments performed in triplicate, reported as mean ± SE.

To evaluate stability *in vitro*, **6** and **7** were incubated separately in human plasma at a concentration of 1 μM for 24 h at 37 °C. Samples were analyzed by LC–MS/MS at 0, 1, 4, and 24 h, and as shown in **Table 3** and **7** was found to be significantly more stable than **6**, suggesting it might have improved bioavailability. However, there was no significant difference in their stability in rat plasma (data not shown). Unlike **6**, **7** was soluble in 0.1 N HCl at 0.5 mg/mL, suggesting it would be soluble in stomach acid. In order to evaluate whether the improved aqueous solubility of **7**

would lead to improved systemic exposure *in vivo*, the pharmacokinetics of both **6** and **7** was evaluated in the rat. Compounds **6** and **7** were administered intravenously<sup>42</sup> and orally<sup>43</sup> at 1 mg/kg, and the plasma concentrations of both compounds were determined by LC–MS/MS analysis, and calculated using a calibration curve of the test compound spiked in rat plasma, which was run concurrently with the samples. While orally-dosed **6** gave plasma levels below the limit of quantification (LLOQ) for our system,<sup>44</sup> **7** was found to have a bioavailability of 24% from the aqueous oral

**Table 3**  
Comparison of compounds **6** and **7**

Compd	RBL cell line IC <sub>50</sub> <sup>a</sup> (nM)		Isolated human PMNs IC <sub>50</sub> <sup>b</sup> (nM)	Human plasma stability (% remaining)			Solubility in 0.1 N HCl (mg/mL)	Rat PK AUC @ 1 mg/kg (μmol-h/L)	
	CXCR1 <sup>b</sup>	CXCR2 <sup>b</sup>	CXCR1/2 <sup>b</sup>	1 h	4 h	24 h		IV	Oral
<b>6</b>	31 ± 4	21 ± 6	22 ± 3	62	34	2	Insoluble	5.40	<LLOQ
<b>7</b>	7 ± 2	4 ± 1	185 ± 52	90	71	62	0.5	4.37	1.15

<sup>a</sup> Experiments performed in triplicate, reported as mean ± SE.<sup>b</sup> IL8 used as agonist.

dose, despite having a slightly lower intravenous AUC (5.40 for **6** vs 4.37 for **7** μmol-h/L).

To determine whether its improved aqueous solubility, plasma stability, and PK parameters improved its in vivo characteristics, **7** was compared side-by-side with **6** in an ozone rat model of pulmonary inflammation and found to be twice as effective at reducing neutrophil influx, when both compounds were given via intravenous injection at a dose of 1 mg/kg (Fig. 2). In this model, Sprague–Dawley rats ( $n = 4$  per cohort) were given a single intravenous dose at  $t = 0$  of either vehicle (negative and positive groups), **6**, or **7**. The rats were then placed in either air (negative) or 1 ppm ozone (positive, **6**, and **7** groups) for 4 h. The rats were sacrificed at  $t = 24$  h, and the bronchoalveolar lavage fluid (BALF) was collected. The cells were spun down, stained with Wright–Giemsa and counted. In the negative group, no neutrophils were observed when stained. Whereas **6** only produced a modest reduction in neutrophil influx, treatment with **7** led to a significant reduction of neutrophil influx. This suggests that the improved aqueous solubility of **7** may have led to increased systemic exposure of the compound to circulating neutrophils.

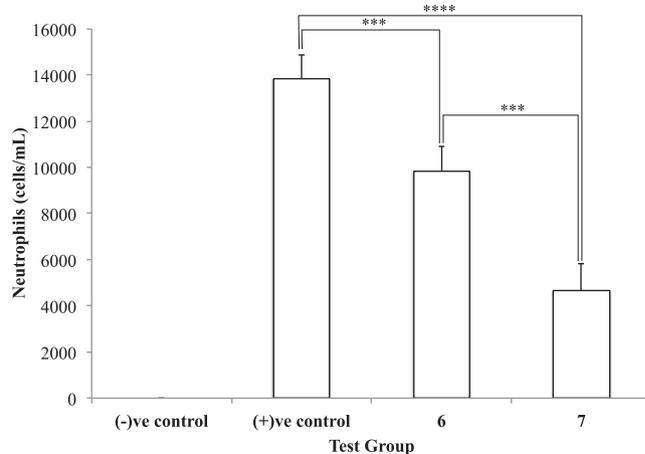
In conclusion, **7** is a potent CXCR1 and CXCR2 antagonist identified from a focused SAR effort to improve the aqueous solubility and in vivo characteristics of our previous lead compounds. Compound **7** is soluble in 0.1 N HCl, has improved plasma stability, and is orally bioavailable in the rat. These improvements over our prior lead compound **6** were further demonstrated in a head-to-head comparison in a rat ozone model of pulmonary inflammation, where **7** exhibited a more durable inhibitory effect than **6** after a single intravenous dose. Compound **7** represents an improved lead candidate for the treatment of inflammatory diseases, cancer, and other diseases associated with CXCR1/2 activation. Further evaluation of the biological activity and properties of **7** are currently underway.

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**Figure 2.** Ozone rat model of pulmonary inflammation. \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ ,  $t$ -test of **6** or **7** versus positive control and **6** versus **7**.

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35. 6-Bromonicotinic acid (1 equiv) was dissolved in anhydrous dimethylformamide (1.25 mL/mmol) under a nitrogen atmosphere. *N*-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (1 equiv) was added, followed by 4-fluoroaniline (1 equiv), and the reaction was stirred at room temperature for 18 h. The product was precipitated by dilution into water (24:1 v/v), filtered, and washed to yield **10** as a white solid. ESI-MS *m/z* 294.9/296.9 [M+H]<sup>+</sup>. Calcd for C<sub>12</sub>H<sub>8</sub>BrFN<sub>2</sub>O: C, 48.84; H, 2.73; N, 9.49. Found: C, 48.75; H, 2.57; N, 9.40. <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 10.54 (s, 1H), 8.92 (d, 1H), 8.25–8.23 (m, 1H), 7.86–7.85 (m, 1H), 7.79 (d, 2H), 7.24–7.23 (m, 2H).
36. 6-Hydroxynicotinic acid (1 equiv) was suspended in acetonitrile (0.6 mL/mmol). Pyridine (0.005 equiv) was added, and the suspension was heated to 80 °C under nitrogen gas. Sulfonyl chloride (1.05 equiv) was added dropwise, then stirred for 45 min before cooling to room temperature. A precipitate formed that was collected by filtration and washed with cold acetonitrile before drying under vacuum to yield the acid chloride as a tan solid, which was added under nitrogen gas to a solution of 4-fluoroaniline (1.1 equiv) in pyridine (1.5 mL/mmol). The reaction was stirred overnight at room temperature and precipitated from water (20:1 v/v). The hydroxynicotinamide was collected by filtration, washed with water, and dried to constant weight at 90 °C overnight to yield an off-white solid. The solid (1 equiv) was suspended in ethyl acetate (3 mL/mmol) with *N*-phenylbis(trifluoromethyl sulfonimide) (1.5 equiv) and dimethylaminopyridine (0.1 equiv). Diisopropylethylamine (3 equiv) was added, and the reaction was heated to reflux for 1 h. It was cooled to room temperature, diluted with ethyl acetate, and washed with water, saturated aqueous sodium bicarbonate, water, and 1 N hydrochloric acid. The organic layer was concentrated and purified by flash chromatography to yield **11** as a tan solid. ESI-MS *m/z* 365.3 [M+H]<sup>+</sup>. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 10.65 (s, 1H), 8.96 (d, 1H), 8.62–8.58 (m, 1H), 7.81–7.76 (m, 3H), 7.27 (t, 2H).
37. 2-Chloropyrimidine-5-carboxylic acid (1 equiv) was dissolved in anhydrous dimethylformamide (0.8 mL/mmol) under a nitrogen atmosphere. *N*-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (1 equiv) was added, followed by 4-fluoroaniline (1 equiv), and the reaction was stirred at room temperature for 40 h. The product was partitioned between ethyl acetate and water. The aqueous layer was washed with ethyl acetate, and combined organic layers were dried over sodium sulfate, filtered, and dried under vacuum. The crude product was purified by flash chromatography to yield **16** as a white solid. ESI-MS *m/z* 252.1 [M+H]<sup>+</sup>. <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 10.67 (s, 1H), 9.23 (s, 2H), 7.78–7.75 (m, 2H), 7.25 (t, 2H).
38. Maeda, D. Y.; Quinn, M. T.; Schepetkin, I. A.; Kirpotina, L. N.; Zebala, J. A. *J. Pharmacol. Exp. Ther.* **2010**, *332*, 145.
39. Hall, D. G. *Boronic Acids: Preparation and Applications in Organic Synthesis and Medicine*; WILEY-VCH GmbH & Co. KGaA: Weinheim, 2005.
40. 5-Bromo-2-formylpyridine (1 equiv) and benzylamine (1 equiv) were dissolved in methanol (2 mL/mmol) in a round bottom flask and stirred at room temperature for 4 h. Solid sodium borohydride (1.2 equiv) was to the solution, and it was stirred for an additional 2.5 h. The methanol was removed via rotary evaporation, and the residual yellow oil was partitioned between ethyl acetate and saturated aqueous sodium bicarbonate, and the aqueous layer was extracted three additional times with ethyl acetate. The combined ethyl acetate extracts were dried over sodium sulfate, filtered, and concentrated under vacuum to yield (benzyl)[(5-bromo-2-pyridyl)methyl]amine. Without purification, the amine (1 equiv) was combined with the chloropyrimidinamide intermediate (**16**, 1.5 equiv) in anhydrous *N*-methyl-2-pyrrolidone (2 mL/mmol) in a scintillation vial. Triethylamine (2.0 equiv) was added and the reaction was left to stand at room temperature for 38 h, before the solvent was removed via rotary evaporation. The yellow oil was partitioned between ethyl acetate and water, and the aqueous layer was re-extracted with ethyl acetate. The combined ethyl acetate layers were dried over sodium sulfate, filtered, and concentrated under vacuum. The crude product was purified via flash chromatography. The purified product (1 equiv) was dissolved in anhydrous dimethylformamide (6 mL/mmol) and degassed under vacuum. The solution was transferred to a pressure bottle containing PdCl<sub>2</sub>(dppf) (0.08 equiv), bis(pinacolato)diboron (3 equiv), and potassium acetate (3.2 equiv) and heated to 80 °C for 4.5 h, then cooled to room temperature. The reaction was filtered through Celite, rinsing with dimethylformamide, dried under vacuum, diluted with ethyl acetate, dried over sodium sulfate, gravity filtered, and dried under vacuum. The crude product was purified by flash chromatography to yield a white foam, which was dissolved in methanol and diluted with water to form a thick white suspension, which was concentrated on a rotary evaporator and filtered to yield a white solid. The boronic acid pinacol ester (1 equiv) was dissolved in methanol (18 mL/mmol) and formic acid (10 equiv) was added, followed by dilution with water to yield a fine white precipitate. The water and methanol were removed via lyophilization. The white powder obtained was suspended in water with minimal methanol and cooled in the fridge. It was collected by vacuum filtration and dried in a vacuum desiccator to yield compound **7**. ESI-MS *m/z* = 458.1 [M+H]<sup>+</sup>. Calcd for C<sub>24</sub>H<sub>21</sub>BFN<sub>3</sub>O<sub>3</sub>: C, 63.04; H, 4.63; N, 15.32. Found: C, 63.30; H, 4.65; N, 15.22. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 10.17 (s, 1H), 8.91–8.86 (m, 2H), 8.82 (s, 1H), 8.29 (s, 2H), 8.04 (d, 1H), 7.75–7.71 (m, 2H), 7.36–7.27 (m, 5H), 7.21–7.16 (m, 3H), 5.03 (s, 2H), 4.96 (s, 2H).
41. 5-Bromo-2-formylpyridine (1 equiv) and 2-furfurylamine (1.5 equiv) were dissolved in methanol (2 mL/mmol) in a round bottom flask and stirred at room temperature for 3 h. Solid sodium borohydride (1.2 equiv) was to the solution, and it was stirred for an additional 2 h. The methanol was removed via rotary evaporation, and the residual yellow oil was partitioned between ethyl acetate and saturated aqueous sodium bicarbonate, and the aqueous layer was extracted twice with ethyl acetate. The combined ethyl acetate extracts were dried over sodium sulfate, filtered, and concentrated under vacuum to yield [(5-bromo-2-pyridyl)methyl](furfuryl)amine. Without purification, the amine (1.5 equiv) was combined with the triflatenicotinamide intermediate (**11**, 1 equiv) in anhydrous *N*-methyl-2-pyrrolidone (5 mL/mmol) in a pressure bottle, layered with nitrogen. Diisopropylethylamine (2.0 equiv) was added and the reaction was heated to 150 °C for 16 h, then cooled to room temperature before the solvent was removed via rotary evaporation. The dark oil was diluted with ethyl acetate and dried over sodium sulfate, filtered through a silica plug (ethyl acetate), and concentrated under vacuum. The crude product was purified via flash chromatography. The purified product (1 equiv) was dissolved in anhydrous dimethylformamide (6 mL/mmol) and degassed under vacuum. The solution was transferred to a pressure bottle containing PdCl<sub>2</sub>(dppf) (0.08 equiv), bis(pinacolato)diboron (3 equiv), and potassium acetate (3 equiv) and heated to 80 °C for 4.5 h, then cooled to room temperature. The reaction was dried under vacuum, diluted with ethyl acetate, dried over sodium sulfate, filtered through a silica plug (ethyl acetate, then 10% methanol in ethyl acetate), and dried under vacuum. The crude product was purified by C18 flash chromatography and lyophilized to yield compound **30** as a white fluffy solid. ESI-MS *m/z* = 447.1 [M+H]<sup>+</sup>. Calcd for C<sub>23</sub>H<sub>20</sub>BFN<sub>4</sub>O<sub>4</sub>: C, 61.91; H, 4.52; N, 12.56. Found: C, 61.64; H, 4.65; N, 12.21. <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 10.05 (s, 1H), 8.83 (s, 1H), 8.70 (d, 1H), 8.29 (s, 2H), 8.03–7.99 (m, 2H), 7.76–7.74 (m, 2H), 7.59 (s, 1H), 7.19 (t, 2H), 7.08 (d, 1H), 6.86 (d, 1H), 6.40–6.38 (m, 2H), 4.96 (s, 2H), 4.91 (s, 2H).
42. Intravenous injections were performed using DPS (dimethylacetamide/PEG400/0.9% saline 40:40:20) as the vehicle.
43. Oral gavage of **6** was performed using Labrasol as the vehicle as previously described. Oral gavage of **7** was performed using 0.1 N HCl as the vehicle. The use of this vehicle for oral gavage in rats has been previously described (Huang, Y. T.; Liu, T. B.; Lin, H. C.; Yang, M. C.; Hong, C. Y. *Pharmacology* **1997**, *54*, 225).
44. The limit of detection on our LC-MS/MS was approximately 20 nM for compound **6**.