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Tri- and tetrasubstituted imidazoles as p38α mitogen-activated protein kinase inhibitors

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ABSTRACT

The synthesis of 2,4,5-trisubstituted and 1,2,4,5-tetrasubstituted imidazoles as potent p38α mitogen-activated protein kinase inhibitors is described. The trisubstituted imidazole series was found to be more potent than the tetrasubstituted imidazole series. Many of these compounds show low-nanomolar activities in the isolated p38α MAP kinase inhibition assay. The structure–activity relationships between these two series are different and not comparable.

The p38α mitogen-activated protein (MAP) kinase, a serine/threonine kinase, is a key enzyme of the cascade leading to pro-inflammatory cytokines such as interleukin-1β and tumor necrosis factor-α.[1] These cytokines are known to be involved in the pathogenesis of inflammatory disorders such as rheumatoid arthritis, inflammatory bowel disease, and psoriasis.[2–4] In peripheral mononuclear blood cells, the p38α MAP kinase pathway is activated by a variety of external stress stimuli like osmotic shock, heat, lipopolysaccharide and other cytokines.[5] Inhibition of p38α MAP kinase is therefore a promising therapeutic strategy to block the biosynthesis of these pro-inflammatory cytokines.

As part of our lead optimization program, different 1,2,4,5-tetrasubstituted imidazoles **(A)** were identified which can be regarded as open chain analogues of the prototypical dihydrothiazoline SKF86002 (Fig. 1).[6,7] These pyridinylimidazoles inhibit the kinase by competing for the binding site of the substrate ATP. For optimal interaction with the kinase, this class of inhibitors must possess a 4-pyridinyl moiety, a 4-fluorophenyl group, and an unsubstituted N-atom in the imidazole ring adjacent to the 4-fluorophenyl group. The 4-pyridinyl moiety interacts with the amino group on the main chain of Met109 of the hinge region via a hydrogen bond while the 4-fluorophenyl ring is located at the hydrophobic region I (selectivity pocket) which is mediated by the gatekeeper amino acid Thr106 (Fig. 2). The imidazole N-3 accepts a hydrogen bond from the ε-amino group of Lys53 side chain. In contrast to SKF86002, the

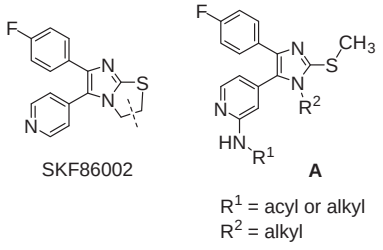

Figure 1. Vicinal 4-fluorophenyl/pyridin-4-yl-substituted imidazoles A, derived from the early lead SKF86002 [IC_{50}(p38α): 0.26 μM],[8] under investigation as inhibitors of p38α MAP kinase.

1-alkyl-4-(4-fluorophenyl)-2-methylsulfanyl-5-pyridin-4-ylimidazoles **A** possess an acylated or alkylated amino function at the pyridine-C2 position which can act as a hydrogen bond donor to the carbonyl function of Met109 and the moiety R^1 addresses the hydrophobic region II.

We recently reported that the carbonyl function of 2-acylaminopyridin-4-ylimidazoles contributes to the metabolic stability of this structural class of compounds, in contrast to the analogous 2-alkylaminopyridin-4-ylimidazoles.[7]

Our initial approach was to investigate the structure–activity relationship (SAR) of 2-acylaminopyridin-4-ylimidazoles (**A**, R^1 = acyl) targeting the moieties R^1 and R^2.

The general synthesis to the 1,2,4,5-tetrasubstituted imidazoles **A** (R^1 = acyl) has been already described by our group.[7,9] As shown in Scheme 1, the putative inhibitors **7–29** and **37–51** were

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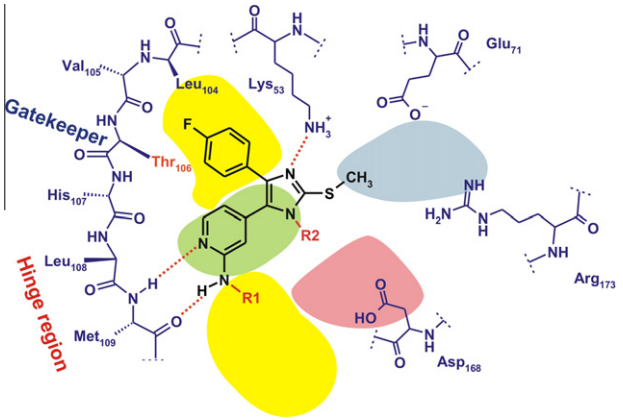
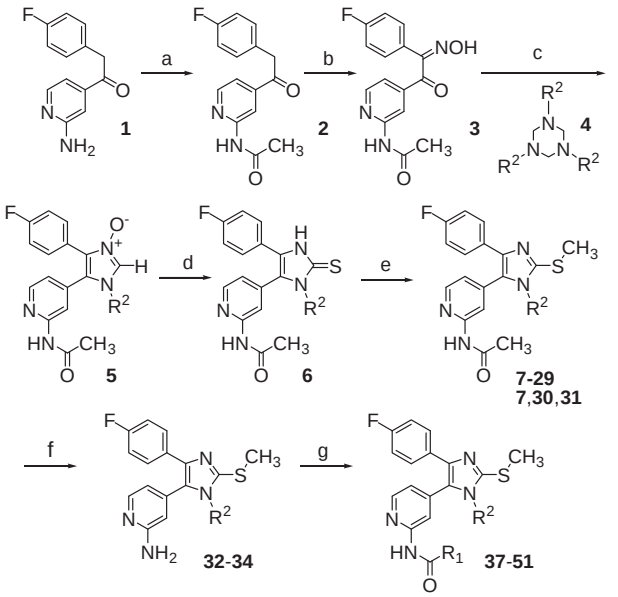


Figure 2. p38α MAP kinase binding schematic for tetrasubstituted imidazoles **A** (hydrogen bonds are drawn in dashed lines).



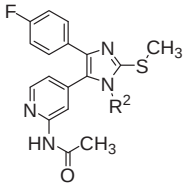
Scheme 1. Synthetic pathway toward 1,2,4,5-tetrasubstituted imidazoles **7**–**29** and **37**–**51**. Reagents and conditions: (a) Ac_2O, DMAP, reflux; (b) isoamyl nitrite, NaOMe, MeOH, rt; (c) EtOH, reflux; (d) 2,2,4,4-tetramethylcyclobutane-1,3-dithione, DCM, rt; (e) iodomethane, Na_2CO_3, EtOH, rt; (f) 10% HCl aq, reflux; (g) R^1-COCl **35**, NEt_3, THF, 0 °C or R^1-COOH **36**, CDI, *N*-methylpyrrolidinone, rt, then 120 °C.

synthesized starting from substituted ethanone **1**. The amino function at the pyridine-C2 position was protected with an acetyl group by heating **1** in acetic anhydride to reflux temperature. Compound **3** was prepared by reacting **2** with isoamyl nitrite and sodium methoxide in methanol at room temperature. Different functional groups at the imidazole-N1 position (R^2) were introduced by using several substituted triazinanes **4** which were synthesized according to literature procedures.[10,11] The imidazole *N*-oxides **5** were converted into the thiones **6** by using 2,2,4,4-tetramethylcyclobutane-1,3-dithione as the sulfur donor. The target compounds **7**–**29** were obtained by *S*-methylation with iodomethane. To vary the acyl moiety (R^1), the acetyl protecting group of compounds **7**, **30,31** (R^2 = methyl, ethyl, or methoxyethyl) was removed using an excess of 10% aqueous hydrochloric acid at reflux temperature to generate compounds **32**–**34**.

Starting from these compounds, different coupling methods yielded the 2-acylaminopyridin-4-ylimidazole derivatives **37**–**51**. The target compounds were synthesized using the carboxylic acid chlorides **35** or the corresponding carboxylic acids **36** after activation with *N*,*N*′-carbonyldiimidazole (CDI).

For all compounds prepared in this Letter, we evaluated inhibitory potency of p38α MAP kinase with the immunosorbent-based assay of Laufer et al.[12] The p38α MAP kinase activities for compounds **7**–**29** are summarized in Table 1. Since R^2 is directed

Table 1
Tetrasubstituted imidazoles: SAR of modifications to R^2



Compd	R^2	p38α IC_{50} (μM)[a]
7		0.11 ± 0.01
8		0.11 ± 0.04
9		0.15 ± 0.05
10		0.14 ± 0.01
11		1.49 ± 0.64
12		6.28 ± 1.50
13		6.14 ± 1.59
14		2.26 ± 0.87
15		0.44 ± 0.07
16		0.33 ± 0.08
17		1.62 ± 0.28
18		0.77 ± 0.17
19		1.14 ± 0.12
20		1.20 ± 0.15
21		2.24 ± 0.69
22		1.57 ± 0.19
23		0.060 ± 0.006
24		0.28 ± 0.03
25		0.78 ± 0.25
26		0.32 ± 0.09
27		0.79 ± 0.35
28		0.40 ± 0.04
29		0.57 ± 0.07

[a] Mean ± SEM of three experiments.

toward the sugar pocket of the ATP binding site, we especially chose flexible hydroxylated aliphatic moieties (**8**–**13**), which could imitate the ribose part of ATP. The hydroxylated moieties (**8**–**10**) show no improvement in inhibitory activity compared to **7**. Modification of R^2 to α-branched diols **12**–**13** resulted in only modest activity with IC_{50} values in the micromolar range. Docking results[13] of compound **13** show that although the hydroxy groups can form hydrogen bonds with the carbonyl functions of Ala111 and Ser154, the bulky moiety at the imidazole-N1 position causes a steric hindrance within the ligand (Fig. 3). The pyridine moiety becomes twisted, which may interfere with the entry of the inhibitor molecule into the binding cleft of p38α MAP kinase and lead to weaker enzyme–ligand interactions.

Substitution of the imidazole-N1 position with propanoic acid or propanoic acid derivatives (esters and amides) led to decreased p38α MAP kinase activity compared to compound **7**. The ester derivatives **15** and **16** were superior to the carboxylic acid **14** in terms of p38α MAP kinase potency. The introduction of cyclic moieties (compounds **25**–**28**) at this position resulted in a weaker inhibition compared to **7**. The most potent inhibitor of this series, compound **23**, featured a one-carbon spacer between the *N*,*N*-disubstituted amide group and the imidazole-N1 atom and possess no substituent in α-position to the imidazole-N1 position. Inhibitor **23** is the only compound of this series with an IC_{50} value in the double-digit nanomolar range.

We then synthesized compounds **52**–**57** which bear an alkylhalide substituent at the imidazole-N1 position and tested their ability to inhibit p38α MAP kinase. These compounds can serve as intermediates for further derivatives and may interact via halogen bonding[14] with the enzyme. The synthetic pathway to this series is depicted in Scheme 2. Compounds **8**–**10**, which all bear a terminal hydroxy group at the imidazole-N1 moiety but differ in chain length, were used as starting materials for the synthesis of test compounds **52**–**57**. The introduction of a chlorine atom (compounds **52**–**54**) was achieved using a modified Appel reaction.[15] Compounds **8**–**10** were treated with triphenylphosphine and hexachloroacetone. The bromo compound **55** was synthesized using the classic Appel reaction,[16] using tetrabromomethane as the bromine source. Compound **56** was synthesized by fluorination of **9** using *N*,*N*-diethylaminosulfur trifluoride[17] in dichloromethane. Finkelstein-reaction[18] of **55** yielded the iodo compound **57**.

The chlorine compounds **52**–**54** were first used to define the favored chain length (Table 2). The inhibitory potency improved

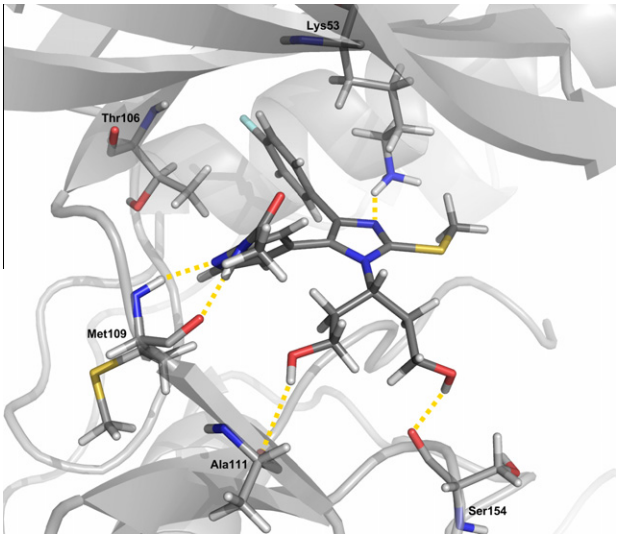
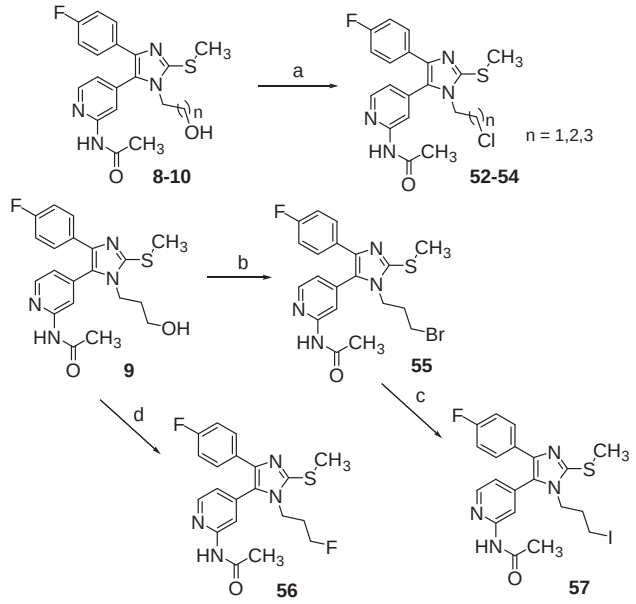
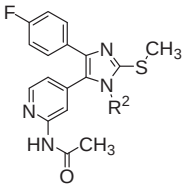


Figure 3. Proposed binding mode of **13** in the ATP binding site of p38α MAP kinase. As the protein model, the X-ray structure 1YWR.pdb was used.



Scheme 2. Synthetic pathway toward 1,2,4,5-tetrasubstituted imidazoles **52**–**57**. Reagents and conditions: (a) PPh_3, hexachloroacetone, DCM; (b) CBr_4, PPh_3; (c) NaI, acetone; (d) *N*,*N*-diethylaminosulfur trifluoride, DCM.

Table 2
Tetrasubstituted imidazoles: SAR of modifications to R^2



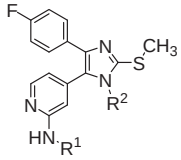
Compd	R^2	p38α IC_{50}[a] (μM)
52	Cl	0.15 ± 0.04
53	Cl	0.017 ± 0.002
54	Cl	0.18 ± 0.002
55	Br	0.027 ± 0.012
56	F	0.18 ± 0.09
57	I	0.019 ± 0.009

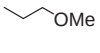
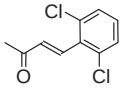
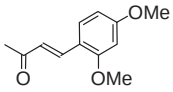
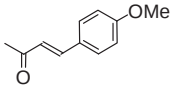
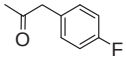
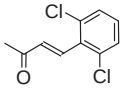
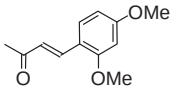
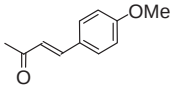
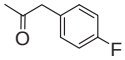
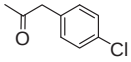
[a] Mean ± SEM of three experiments.

when R^2 was modified to incorporate three carbons. In the next step, the influence of the halogen atom was investigated. However, there were no significant differences in inhibitory activity among the chloro, iodo, and bromo compounds. The fluoro derivative **56** showed a clear decrease in inhibitory activity compared to **53**, **55**, and **57**.

The SARs of compounds **37**–**51** are listed in Table 3 and show the influence of modifications at R^1 and R^2. The acetyl moiety of **7** was replaced in compounds **37**–**40** by an acrylamide, a propionylamide, a trifluoropropionylamide, and a thiophene-2-carboxamide, respectively. Introduction of a larger moiety at the pyridine-C2 position (R^1) resulted in a decrease of inhibitory activity, compare **7** versus **37**–**40**. The methoxyethyl moiety at R^2 may cause a steric hindrance with larger moieties at R^1. Thus, we shortened the moiety at the imidazole-N1 position. Compounds **41**–**51** bear only a methyl or an ethyl group at this position. Previous experiments in a similar series identified moieties at R^1 with a carbon spacer between the aminoacyl group and a phenyl ring as favorable substituents at this position.[7] Therefore, we chose

Table 3
Tetrasubstituted imidazoles: SAR of modifications to R^1 and R^2

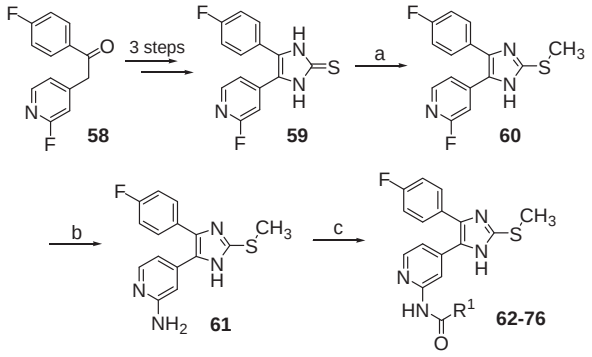


Compd	R^1	R^2	p38α IC_{50}[a] (μM)
37			0.17 ± 0.05
38			0.18 ± 0.04
39			0.49 ± 0.03
40			0.24 ± 0.04
41			0.10 ± 0.01
42			0.038 ± 0.010
43			0.075 ± 0.015
44			0.093 ± 0.023
45			0.17 ± 0.04
46			0.28 ± 0.06
47			0.018 ± 0.006
48			0.13 ± 0.03
49			0.022 ± 0.002
50			0.17 ± 0.02
51			0.15 ± 0.01

[a] Mean ± SEM of three experiments.

different substituted cinnamide and 2-phenylactamide moieties at the pyrdine-C2 position.

The introduction of different substituted 2-phenylamides and cinnamides was well tolerated. Within the series of compounds bearing an ethyl moiety at the imidazole-N1 position (**41**–**44**), compound **42** with an (*E*)-3-(2,4-dimethoxyphenyl)acrylamide at the pyridine-C2 position exhibited an IC_{50} value of 38 nM. In the series of N1-methylated compounds (**45**–**51**), two compounds could be identified with IC_{50} values in the low double-digit nanomolar range. Compound **47** having a (*E*)-3-(4-methoxyphenyl)acrylamide and **49** having a 2-(4-chlorophenyl)acetamide moiety at the pyridine-C2 position exhibit IC_{50} values of 18 and 22 nM, respectively.

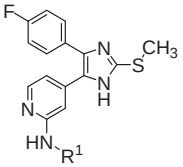


Scheme 3. Synthetic pathway to 2,4,5-trisubstituted imidazoles **62**–**76**. Reagents and conditions: (a) CH_3I, Na_2CO_3, EtOH, rt; (b) NH_4OH, 180 °C; (c) R^1-COOH, CDI, *N*-methylpyrrolidinone, rt, then 120 °C.

However, attempts to clarify the role of substituents at the pyridine-C2 position (R^1) in p38α MAP kinase inhibition through SAR comparisons between the imidazole N1-ethyl (**41**–**44**) and the imidazole N1-methyl (**45**–**51**) series were not so straightforward. While the dichloro, dimethoxy, and fluoro pyridine-C2 analogues in the N1-ethyl series were generally better inhibitors than their counterparts in the N1-methyl series, the aforementioned *p*-methoxy compound **47** of the methyl series contradicted this trend.

In the next step of optimization, we synthesized compounds **62**–**76**, which bear no substituent at the imidazole-N1 position. The general synthesis to the 2,4,5-trisubstituted imidazole derivatives **62**–**76** is depicted in Scheme 3. Methylation of the exocyclic

Table 4
Trisubstituted imidazoles: SAR of modifications to R^1



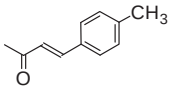
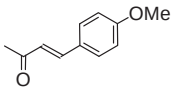
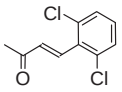
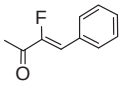
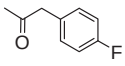
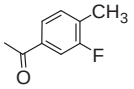
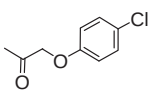
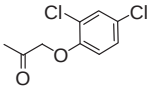
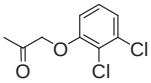
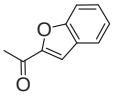
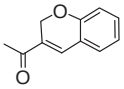
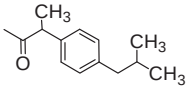
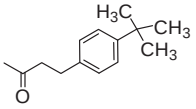
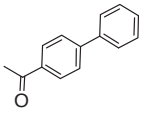
Compd	R^1	p38α IC_{50}[a] (μM)
62		0.032 ± 0.008
63		0.010 ± 0.003
64		0.003 ± 0.0004
65		0.027 ± 0.002
66		0.009 ± 0.0004
67		0.014 ± 0.002
68		0.10 ± 0.01

Table 4 (*continued*)

Compd	R^1	p38α IC_{50}[a] (μM)
69		0.16 ± 0.01
70		0.16 ± 0.03
71		0.053 ± 0.017
72		0.027 ± 0.004
73		0.090 ± 0.014
74		0.13 ± 0.04
75		0.013 ± 0.005
76		0.10 ± 0.01

[a] Mean ± SEM of three experiments.

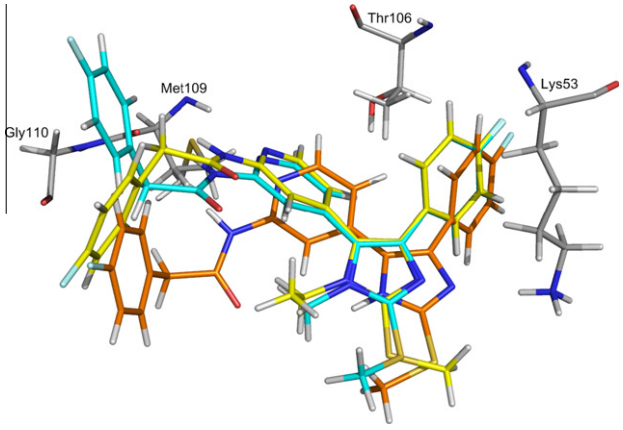


Figure 4. Overlay of the proposed binding modes of inhibitors **44** (yellow), **48** (cyan) and **66** (gold) in the ATP binding site of p38α MAP kinase. As the protein model, the X-ray structure 1YWR.pdb was used.

sulfur atom of imidazol-2-thione **59**[9] furnished the 2-methylsulfanylimidazole derivative **60**. The amino function at the pyridine-C2 position was introduced by nucleophilic replacement with ammonium hydroxide. The final compounds **62**–**76** were obtained by coupling with the corresponding carboxylic acids after its activation with CDI.

As shown in Table 4, the trisubstituted imidazoles were potent inhibitors with IC_{50} values as low as the single-digit nanomolar range. Compounds bearing a substituted 2-phenoxyacetamide moiety (**68**–**70**) or a 3-phenylpropionamide moiety (**74**) at the pyridine-C2 position show less inhibitory activity in comparison to compounds having the rigid cinnamide moieties (**62**–**65**). Of particular interest were compounds **64** and **66** bearing an (*E*)-3-(2, 6-dichlorophenyl)acrylamide and a 2-(4-fluorophenyl)acetamide moiety at the pyridine-C2 position, respectively. Both compounds inhibited the p38α MAP kinase with IC_{50} values in the single-digit nanomolar range.

In Figure 4 an overlay of the proposed binding modes of compounds **44**, **48** and **66** is depicted. All these compounds bear a 2-(4-fluorophenyl)acetamide moiety at the pyridine-C2 position but differ in the substitution pattern of the imidazole-N1 position. The substituent at the imidazole-N1 position causes a rotation of the pyridine moiety compared to the imidazole-N1 unsubstituted compound. This may result in a weaker enzyme–inhibitor interaction of the pyridine-C2 moiety and therefore in a decrease of inhibitory activity of the tetrasubstituted imidazoles.

In summary, we have reported p38α MAP kinase inhibition data for different series of 1,2,4,5-tetrasubstituted and 2,4,5-trisubstituted imidazoles. The SARs between these two compound classes are different and not readily comparable. The trisubstituted imidazole series was found to be more potent than the tetrasubstituted imidazole series. Potent inhibitors with IC_{50} values down to 3 nM were identified.

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