Design, Synthesis, and Biological Evaluation of Aminoboronic Acids as Growth-Factor Receptor Inhibitors of EGFR and VEGFR-1 Tyrosine Kinases

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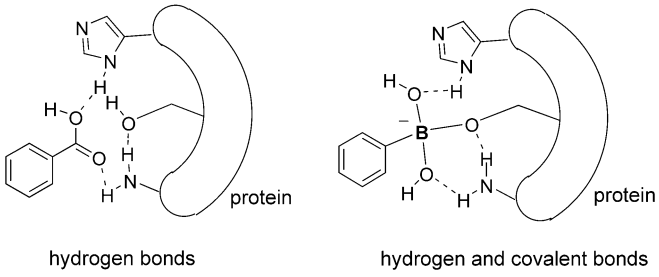
*A series of aminoboronic acids was synthesized based on the structure of lavendustin pharmacophore **1**. Their inhibitory activities against the epidermal growth-factor receptor (EGFR) and vascular endothelial growth-factor receptor-1 (VEGFR-1, Flt-1) protein tyrosine kinases, and various protein kinases, PKA, PKC, PTK, and eEF2K were evaluated. Selective inhibition activities were observed in a series of aminoboronic acids. 4-Methoxy-3-((2-methoxyphenylamino)methyl)phenylboronic acid **10** inhibited EGFR tyrosine kinase, whereas 4-(2,5-dihydroxybenzylamino)phenylboronic acid **12** inhibited Flt-1 protein kinase, although lavendustin pharmacophore **1** inhibited both EGFR and Flt-1 kinases at a compound concentration of 1.0 $\mu g\,mL^{-1}$. The selective inhibition of EGFR by **10** is considered to be due to the substitution of the dihydroxy groups on the benzyl moiety for a boronic acid group at the para position, whereas the selective inhibition of Flt-1 by **12** is due to the substitution of the carboxyl group on the aniline moiety in the lavendustin pharmacophore **1** for a boronic acid group.*

Introduction

The use of boron atoms in pharmaceutical drug design possesses a high potential for the discovery of new biological activity.[1] A boron atom has a vacant orbital and interconverts with ease between the neutral sp^2 and the anionic sp^3 hybridization states; this generates a new stable interaction between the boron atom and a donor molecule through a covalent bond.[2] Therefore, it is expected that the boron atoms introduced into biologically active molecular frameworks would interact with a target protein not only through hydrogen bonds but also through covalent bonds, and this interaction would produce potent biological activity (Scheme 1).[3] Among various boron compounds synthesized, much attention has recently been paid to boronic acid-containing peptides.[4] Fevig and co-workers have developed boropeptides as thrombin inhibitors,[5,6] and Adams and co-workers have developed dipeptidyl boronic acids as proteasome inhibitors.[7] In those boropeptides a carboxyl group has been replaced by a boronic acid group.



Scheme 1. *Interaction of a carboxylic acid through hydrogen bonds (left) and interaction of a boronic acid through both hydrogen and covalent bonds (right) with a certain protein.*

Our strategy for the design of boron compounds is based on their unique properties, which are different from conventional biologically active compounds. Boron compounds presumably interact with a target protein through both hydrogen and covalent bonds, as mentioned above. The distribution of boron compounds in the body can be easily traced by using α-autoradiography and inductively coupled plasma atomic-emission spectroscopy (ICP-AES),[8] since boron atoms are not normally found in the living body. Furthermore, the nuclear reaction of the ^{10}B isotope and a thermal neutron produces an alpha particle and recoils a ^{7}Li ion bearing approximately 2.4 MeV. The alpha particle and lithium ion dissipate their kinetic energy before traveling one cell diameter; this affords the potential for precise cell killing (boron neutron capture therapy).[9] Therefore, a boron-containing tyrosine kinase inhibitor[10] would be converted from a cytostatic agent into a cytotoxic agent by irradiation of the target tissue with thermal neutrons.

We focused on the active pharmacophore **1** of lavendustin A[11,12] (Scheme 2). Lavendustin A is the EGFR protein tyrosine

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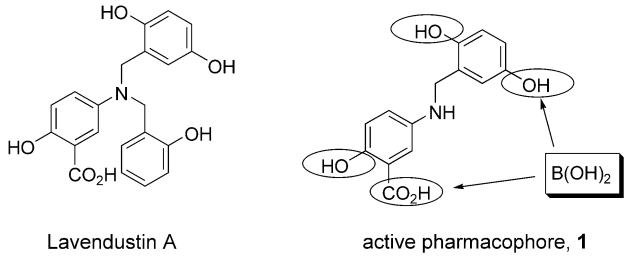
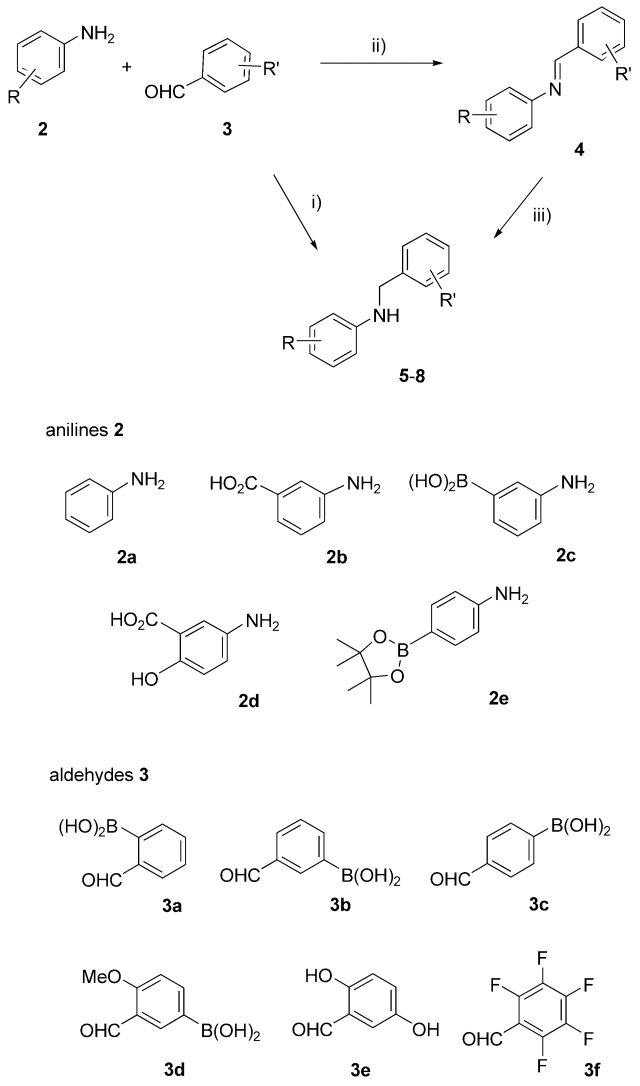


Scheme 2. *Introduction of a boronic acid group into the active pharmacophore of lavendustin A.*

kinase (PTK) inhibitor isolated from a butyl acetate extraction of a *Streptomyces griseolavendus* culture filtrate.[11] The active pharmacophore **1** is a secondary amine containing three phenolic hydroxy groups and a carboxyl group. It is considered to interact with EGFR-PTK through hydrogen bonds formed by these functional groups. Inhibition activities would be expected to be higher by replacing those hydroxy and carboxyl groups with boronic acid groups (Scheme 2). In this paper, we report the synthesis of aminoboronic acids and the evaluation of inhibition activities toward various protein kinases.

Results

The active pharmacophore **1** of lavendustin is composed of two aromatic rings linked by a methyleneamino chain and it was previously synthesized from 3-aminosalicylic acid and 2,5-dihydroxybenzaldehyde by reductive amination.[12b] A series of aminoboronic acid analogues **5**–**8** was synthesized from various anilines **2a**–**d** and aldehydes **3a**–**e** in the presence of $NaCNBH_3$ in MeOH at room temperature or $NaBH_4$ in EtOH after the formation of the imine **4** (Scheme 3). Structures and yields of the aminoboronic acids **5**–**8** are summarized in Table 1.



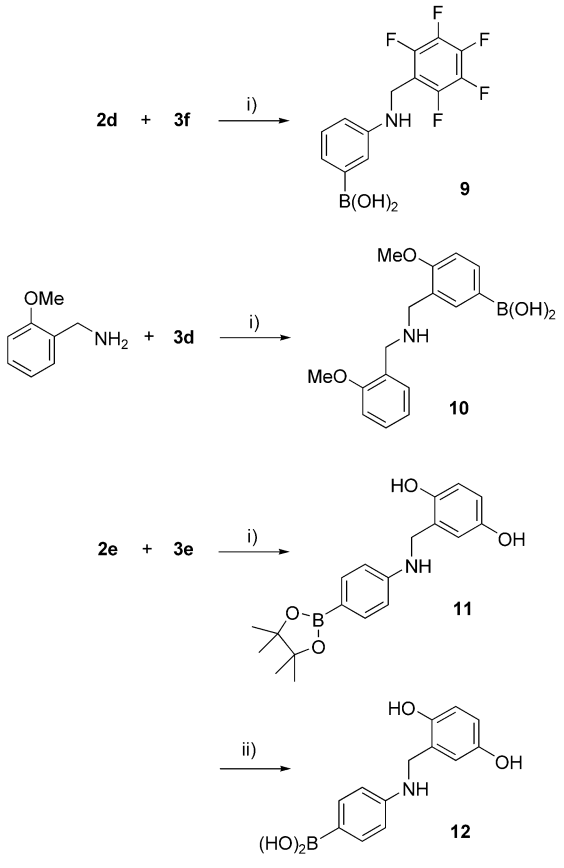
Scheme 3. *General synthesis of aminoboronic acids. Reagents and conditions: i) $NaCNBH_3$, MeOH, room temperature, ii) MeOH or EtOH, iii) $NaBH_4$, EtOH.*

Table 1. *Structures and yields of the aminoboronic acids **5**–**8**.*

aniline R =	$(HO)_2B$	$B(OH)_2$	$B(OH)_2$	MeO, $B(OH)_2$	HO, OH
NHR	**5a**: 86%	**5b**: 81%	**5c**: 39%	**5d**: 16%	–[a]
HO_2C, NHR	**6a**: 20%	**6b**: 31%	**6c**: 74%	**6d**: 15%	**6e**: 73%b
$(HO)_2B$, NHR	**7a**: 58%	**7b**: 28%	**7c**: 39%	**7d**: 23%	**7e**: 90%
HO_2C, NHR, HO	–[a]	**8b**: >99%	**8c**: >99%	**8d**: 47%	**1**: 50%[b]

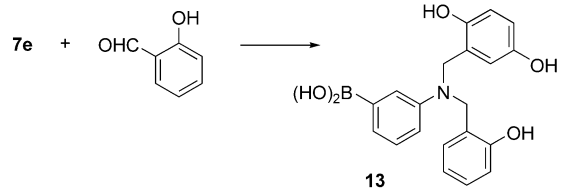
[a] Not synthesized. [b] Already reported in ref. [11].

Pentafluorophenylmethyl-3-boronophenylamine **9** and 5′-borono-2′-methoxyphenylmethyl-2-methoxybenzylamine **10** were also synthesized by reductive amination from the anilines **2d** or 2-methoxybenzylamine, and the aldehydes **3f** or **3d**, respectively. Furthermore, the aminoboronic acid **12**, in which a boronic acid group is introduced at the *para*-position of an aniline moiety, was synthesized from aniline **2e** and the aldehyde **3e**. The reaction of **2e** and **3e** proceeded under reductive amination conditions to afford the corresponding pinacol boron ester **11** in 60% yield. The deprotection of the pinacol group was not an easy task. Although the oxidative cleavage of the pinacol ester **11** with sodium metaperiodate was not effective,[13] the transesterification of the pinacol from **11** to phenylboronic acid under acidic conditions gave 43% of **12** (Scheme 4). This yield was increases to 81% by using KHF_2 an acid.[14] As an analogue of lavendustin A, the tertiary amine **13** was synthesized from **7e** and salicylaldehyde in 19% yield by using $NaCNBH_3$ in MeOH (Scheme 5).



	Condition	Yield of **12** [%]
1	$NaIO_4$ / NH_4OAc / acetone	recovery
2	$NaIO_4$ / THF / H_2O	0
3	HCl / $PhB(OH)_2$	43
4	KHF_2 / HCl	63
5	KHF_2 / $PhB(OH)_2$	81

Scheme 4. *Synthesis of **9**–**12** and yields of the various routes to aminoboronic acid 12. Reaction conditions i) $NaCNBH_3$, MeOH, room temperature.*



Scheme 5. *Synthesis of **13**, an analogue of lavendustin A.*

Discussion

The lavendustin pharmacophore **1** and the aminoboronic acids **5a**–**d**, **6a**–**d**, **7a**–**e**, **8b**–**d**, **9**, **10**, **12**, and **13** were tested for the inhibition of EGF-stimulated EGFR tyrosine kinase phosphorylation according to assay conditions described in the literature.[11,15] As a preliminary experiment, the inhibition assay of EGFR tyrosine kinase was carried out at a compound concentration of 10 μg mL^{-1}. The compounds **1**, **6b**–**c**, **6e**, **10**, and **12**, which exhibited inhibitory activities higher than 50% at this concentration, were tested at lower concentrations (10–0.1 μg mL^{-1}). The results are summarized in Figure 1. EGF(+) corresponds to the maximum phosphorylation of the peptide substrate by the EGF-stimulated EGFR tyrosine kinase and it is plotted as 100%. EGF(−) corresponds to the phosphorylation activity of EGFR tyrosine kinase without EGF stimulation. The lavendustin pharmacophore **1** and the analogue **6e**, which have already been reported,[11,12a] showed high inhibitory activities against EGFR tyrosine kinase. The phosphorylation activities were on a level similar to that of EGF(−) at 10 μg mL^{-1}, and 50% inhibition was observed even at 0.1 μg mL^{-1}. Compounds **5a**–**d**, derived from aniline **2a** and boronic aldehydes **3a**–**d**, did not show significant tyrosine kinase inhibition at a concentration of 10 μg mL^{-1}. Although compounds **6a** and **6d** did not show inhibitory activity at 10 μg mL^{-1}, moderate inhibition was observed in the case of the compounds **6b**–**c** and **8c**–**d**. Very interestingly, compound **10**, which has a methoxy group at the *ortho* position on the aniline moiety, showed a high inhibitory activity similar to the lavendustin pharmacophore **1** at 10 μg mL^{-1}. Although the inhibitory activity at 1.0 μg mL^{-1} was a bit lower than that of **1**, still more than 50% inhibitory activity was observed. The introduction of a boronic acid group into the *para* position of the aniline moiety increased the tyrosine kinase inhibition, and compound **12** exhibited a high inhibitory activity similar to the lavendustin pharmacophore **1** at 10 μg mL^{-1}. However, **7e**, which has a boronic acid group at the *meta* position on the aniline moiety, did not show the expected tyrosine kinase inhibition. In case of the tertiary amine **13**, synthesized as an analogue of lavendustin A, no inhibition was observed at a concentration of 10 μg mL^{-1}. Roques and co-workers had previously reported that the third aromatic ring of lavendustin A is not necessarily important for inhibition of the tyrosine kinase activity.[12b]

Inhibition specificity of the compounds was investigated semi-quantitatively by using PKA, PKC, PTK, eEF2K, EGFR, and Flt-1 (Table 2).[16,17] The lavendustin pharmacophore **1**, the aminoboronic acid **6b**, and the analogue **6e** showed selective kinase inhibition of both EGFR and Flt-1. The selective inhibi-

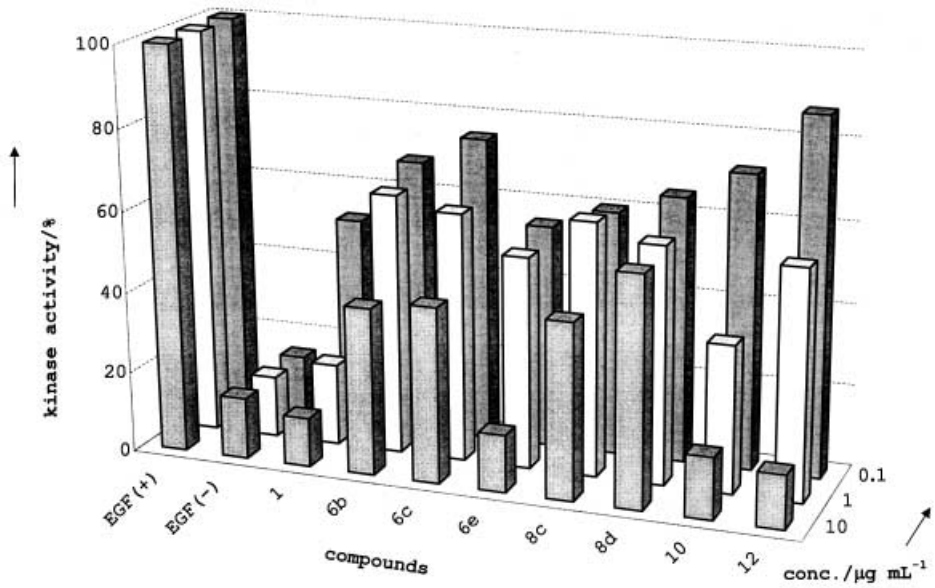


Figure 1. *Inhibition of EGF-stimulated EGFR phosphorylation. Activities are expressed as a percentage of the maximum phosphorylation induced by EGF.*

Table 2. *Inhibition specificity of compounds* ***1***, ***6b***, ***6c***, ***6e***, ***7e***, ***8c***, ***10***, *and* ***12***.

compounds	c [μmL^{-1}]	PKA	PKC	PTK	eEF2K	EGFR	Flt-1
1	10	−	−	−	−	++	++
	1	−	−	−	−	++	++
	0.1	−	−	−	−	−	−
6b	10	−	−	−	−	+	+
	1					−	−
6c	10	−	−	−	−	+	−
	1		−		−	−	−
6e	10	−	−	−	−	++	++
	1					−	−
7e	10	−	−	−	−	−	++
	1					−	−
8c	10	−	−	−	−	+	−
	1	−	−	−	−	−	−
10	10	+	−	−	−	++	+
	1	−	−	−	−	+	−
	0.1					−	
12	10	+	+	+	+	+	++
	1	−	−	−	−	−	+
	0.1						−

[a] The indications ++, +, and − mean >80%, 50–80%, and <50% kinase inhibition activities in each kinase assay, respectively.

tion of EGFR tyrosine kinase was observed in the case of the aminoboronic acids **6c**, **8c**, and **10**. Presumably the substitution of the dihydroxy groups of the benzyl moiety in **1** for a boronic acid group at the *para* position enhances the specific inhibition of EGFR tyrosine kinase. Although compound **7e** did not show inhibition of EGFR kinase, specific inhibition activity was observed in the Flt-1 kinase assay at a concentration of 10 μg mL^{-1}. Compound **12** exhibited nonspecific inhibition activity toward various protein kinases at a concentration of 10 μg mL^{-1}, but specific inhibition activity of Flt-1 kinase was observed at 1.0 μg mL^{-1}. Since the compounds **6e**, **7e**, and **12** contain a 2,5-dihydroxybenzyl group, it is considered that the selective inhibition of Flt-1 by **7e** and **12** is due to the boronic acid group on the aniline ring in the molecules, the *para*-substituted boronic acid also increased the inhibitory activity.

Conclusion

We have synthesized a novel class of EGFR and Flt-1 protein kinase inhibitors by the introduction of a boronic acid group into the framework of the lavendustin pharmacophore **1** in order to allow dual interactions with protein kinases through both hydrogen and covalent bonds. Among the aminoboronic acids synthesized, selective inhibition of EGFR and Flt-1 protein kinases by aminoboronic acids **10** and **12**, respectively, was observed at a concentration of 1.0 μg mL^{-1}. It is clear that a boronic acid introduced into a known interaction site of the structural framework of **1** plays a key role in inhibiting kinase activity. According to this, compounds **7e**, **10**, and **12** exhibit significantly high inhibition activities, although they are not higher than those of the lavendustin pharmacophore itself.

In vivo cytotoxicity studies of the aminoboronic acids **7e**, **10**, and **12** by using irradiation of neutrons as well as structure–activity relationship studies are now in progress in our laboratory.

Experimental Section

Materials and methods: ^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL JNM-AL 300 (300 MHz), JEOL GSX 270 (270 MHz), or VARIAN UNITY-INOVA 400 (400 MHz) spectrometer. ^{1}H NMR chemical shifts are reported in parts per million downfield from $CDCl_3$ ($\delta = 7.24$), CD_3OD ($\delta = 3.35$) or $(CD_3)_2SO$ ($\delta = 2.49$) as internal standard. ^{13}C NMR chemical shifts are reported in ppm downfield from $CDCl_3$ ($\delta = 77.0$), CD_3OD ($\delta = 49.3$) or $(CD_3)_2SO$ ($\delta = 39.7$) as internal standard. IR spectra were recorded on a Shimadzu FTIR-8200A spectrometer. Analytical thin layer chromatography (TLC) was performed on glass plates (Merck Kieselgel 60 F_{254}, layer thickness 0.2 mm or RP-18 F_{254s}, layer thickness 0.2 mm). Samples were visualized by UV light (254 nm) with $FeCl_3$ and $KMnO_4$. Column chromatography was performed on silica gel (Merck Kieselgel 70–230 mesh). All reactions were carried out under argon atmosphere by using standard Schlenk techniques. Most chemicals and solvents were of analytical grade and used without further purification.

2-(*N*-Phenylaminomethyl)phenylboronic acid (5a): 2-Formylphenylboronic acid (**3a**) (150 mg, 1 mmol) was added to aniline (**2a**) (0.09 mL, 1 mmol) in methanol (10 mL). Sodium cyanoborohydride $NaBH_3CN$ (124 mg, 2 mmol) was added with stirring, and the reaction mixture was stirred at room temperature for 24 h. Afterwards a saturated solution of NaCl (50 mL) was added to the reaction mixture, which was then extracted with ethyl acetate(3×50 mL). The combined organic layers were washed with brine (30 mL), dried over sodium sulfate and concentrated to furnish the crude product, which was further purified by column chromatography with ethyl acetate/hexane (1:1). The product **5a** (195 mg, 86%) was isolated as a white solid. M.p. 127–130 °C; ^{1}H NMR (300 MHz, CD_3OD): $\delta = 7.29$–6.58 (m, 9H), 4.28 (s, 2H); ^{13}C NMR (75 MHz, CD_3OD): $\delta = 156.6$, 150.1, 129.8, 128.9, 126.8, 120.4, 118.5, 115.9, 114.7, 44.7; IR (KBr): 3047, 2964, 1600, 1500, 1460, 1251, 798, 754, 727, 690 cm^{-1}; HRMS (ESI): $[M+H]^+$ calcd for $C_{13}H_{14}BNO_2$: 228.1198, found: 228.1120; elemental analysis calcd (%) for $C_{13}H_{14}BNO_2$ ($-H_2O$): C 74.62, H 5.79, N 6.70, found: C 74.65, H 5.84, N 6.59.

3-(*N*-Phenylaminomethyl)phenylboronic acid (5b): By using a similar procedure as for **5a**, **2a** (0.09 mL, 1 mmol), **3b** (150 mg, 1 mmol) and $NaBH_3CN$ (124 mg, 2 mmol) gave pure **5b** (183 mg, 81%) as a slightly yellow solid. M.p. 152–154 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.21$–8.14 (m, 2H), 7.65–7.52 (m, 2H), 7.28–7.21 (m, 2H), 6.81–6.72 (m, 3H) 4.45 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta =$ 147.8, 138.7, 134.5, 131.8, 129.2, 128.2, 117.7, 112.9, 48.2; IR (KBr): 3408, 3005, 1600, 1506, 1429, 1350, 1269, 1201, 1066, 750, 723, 692, 586 cm^{-1}; HRMS (ESI): calcd for $C_{13}H_{14}BNO_2$: 228.1198 $[M+H]^+$, found: 228.1139; elemental analysis calcd (%) for $C_{13}H_{14}BNO_2$ ($-\frac{4}{5}H_2O$): C 73.36, H 5.88, N 6.59, found: C 73.60, H 5.79, N 6.13.

4-(*N*-Phenylaminomethyl)phenylboronic acid (5c): By using a similar procedure as for **5a**, **2a** (0.09 mL, 1 mmol), **3c** (150 mg, 1 mmol) and $NaBH_3CN$ (124 mg, 2 mmol gave pure **5c** (88.55 mg, 39%) as a white solid. M.p. 151–154 °C; ^{1}H NMR (300 MHz, CD_3OD): $\delta = 7.67$–7.54 (m, 2H), 7.35 ($J = 7.5$ Hz, 2H), 7.07–6.55 (m, 5H), 4.30 (s, 2H); ^{13}C NMR (75 MHz, CD_3OD): $\delta = 150.0$, 134.9, 130.0, 129.9, 127.5, 119.3, 117.9, 116.6, 114.0, 48.7; IR (KBr): 3440, 1602, 1508, 1409, 1348, 750, 692 cm^{-1}; HRMS (ESI): $[M+H]^+$ calcd for $C_{13}H_{14}BNO_2$: 228.1198, found: 228.1310.

3-Methoxy-4-(*N*-phenylaminomethyl)phenylboronic acid (5d): By using a similar procedure as for **5a**, **2a** (0.09 mL, 1 mmol), **3d** (180 mg, 1 mmol) and $NaBH_3CN$ (124 mg, 2 mmol) gave pure **5d** (41 mg, 16%) as a white solid. M.p. 93–95 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.04$ (d, $J = 5.4$ Hz, 2H), 7.20 (t, $J = 7.2$ Hz, 8.7 Hz, 3H), 6.96 (d, $J = 8.7$ Hz), 1H), 6.71 (d, $J = 8.1$ Hz, 3H), 4.37 (s, 2H), 3.91(s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 161.0$, 148.4, 136.6, 129.1, 126.6, 122.2, 117.5, 113.2, 109.8, 55.3, 43.6; IR (KBr): 3410, 1602, 1504, 1340, 1249, 1136, 1026, 748, 692 cm^{-1}; HRMS (ESI): $[M+H]^+$ calcd for $C_{14}H_{16}BNO_3$: 258.1303, found: 228.1216; elemental analysis calcd (%) for $C_{13}H_{14}BNO_2$ ($-\frac{2}{3}H_2O$): C 68.54, H 6.03, N 5.71, found: C 68.50, H 6.27, N 5.79.

2-(*N*-3-Corboxyphenylaminomethyl)-2-phenylboronic acid (6a): By using a similar procedure as for **5a**, **2b** (137 mg, 1 mmol), **3a** (150 mg, 1 mmol) and $NaBH_3CN$ (124 mg, 2 mmol) gave pure **6a** (54 mg, 20%) as a white solid. M.p. >300 °C; ^{1}H NMR (300 MHz, CD_3OD): $\delta = 7.39$–7.15 (m, 7H), 6.89–6.87 (m, 1H), 4.35 (s, 2H); ^{13}C NMR (67 MHz, CD_3OD): $\delta = 170.1$, 149.6, 145.2, 132.2, 129.7, 129.4, 127.4, 127.1, 120.8, 119.9, 116.5, 50.2; IR (KBr): 3064, 1685, 1602, 1375, 1292, 756 cm^{-1}; HRMS (ESI): $[M+H]^+$ calcd for $C_{14}H_{14}BNO_4$: 272.1096, found: 272.1086; elemental analysis calcd (%) for $C_{14}H_{14}BNO_4$ ($-\frac{1}{2}H_2O$): C 64.099, H 5.000, N 5.343, found: C 63.838, H 5.322, N 5.038.

3-(*N*-3-Carboxyphenylaminomethyl)-2-phenylboronic acid (6b): By using a similar procedure as for **5a**, **2b** (137 mg, 1 mmol), **3b** (150 mg, 1 mmol) and $NaBH_3CN$ (124 mg, 2 mmol) gave pure **6b** (84 mg, 31%) as a white solid. M.p. >300 °C; ^{1}H NMR (300 MHz, CD_3OD): $\delta = 7.71$–7.56 (m, 1H), 7.40–7.12 (m, 6H), 6.81 (d, $J = 7.2$ Hz, 1H), 4.35 (s, 2H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 169.6$, 148.3, 141.0, 133.0, 131.0, 128.0, 128.0, 125.6, 118.7, 118.4, 117.2, 116.3, 112.8, 47.9; IR (KBr): 3367, 1683, 1608, 1398, 1373, 754 cm^{-1}; HRMS (ESI): $[M+H]^+$ calcd for $C_{14}H_{14}BNO_4$: 272.1096, found: 272.1046.

4-(*N*-3-Carboxyphenylaminomethyl)-2-phenylboronic acid (6c): By using a similar procedure as for **5a**, **2b** (137 mg, 1 mmol), **3c** (150 mg, 1 mmol) and $NaBH_3CN$ (124 mg, 2 mmol) gave pure **6c** (201 mg, 74%) as a white solid. M.p. >300 °C; ^{1}H NMR (300 MHz, CD_3OD): $\delta = 7.59$–7.23 (m, 5H), 7.14 (t, $J = 7.8$ Hz, 1H), 6.79 (d, $J = 8.1$ Hz, 1H), 4.33 (s, 2H); ^{13}C NMR (75 MHz, CD_3OD): $\delta = 174.2$, 149.9, 143.4, 134.8, 130.3, 129.6, 129.5, 127.5, 127.0, 124.7, 119.1, 117.0, 114.8, 61.3; IR (KBr): 3396, 3055, 1695, 1606, 1429, 1379, 1274, 761, 711 cm^{-1}; HRMS (ESI): $[M+H]^+$ calcd for $C_{14}H_{14}BNO_4$, 272.1096, found: 272.1104.

3-Methoxy-4-(*N*-3-carboxyphenylaminomethyl)-2-phenylboronic acid (6d): By using a similar procedure as for **5a**, **2b** (137 mg, 1 mmol), **3d** (180 mg, 1 mmol) and $NaBH_3CN$ (124 mg, 2 mmol) gave pure **6d** (45 mg, 15%) as a white solid. M.p. 154–156 °C; ^{1}H NMR (300 MHz, CD_3OD): $\delta = 7.52$–7.47 (m, 2H), 7.24–7.17 (m, 2H), 7.09 (t, $J = 7.5$, 8.1 Hz, 1H), 6.78 (d, $J = 7.8$ Hz, 1H), 4.30 (s, 2H), 3.85 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 165.8$, 159.1, 137.0, 135.2, 132.3, 129.6, 126.3, 123.2, 117.1, 109.3, 54.3, 51.2; IR (KBr): 3396, 1568, 1494, 1242, 758 cm^{-1}; HRMS (ESI): $[M+H]^+$ calcd for $C_{15}H_{16}BNO_5$: 302.1202, found: 302.1226; elemental analysis calcd (%) for $C_{16}H_{18}BNO_5$ ($-\frac{1}{3}H_2O$): C 62.111, H 5.651, N 4.530, found: C 62.138, H 5.850, N 4.556.

3-(2-(dihydroxyboranyl)benzylamino)phenylboronic acid (7a): By using a similar procedure as for **5a**, **2c** (155 mg, 1 mmol), **3a** (150 mg, 1 mmol) and $NaBH_3CN$ (124 mg, 2 mmol) gave pure **7a** (157 mg, 58%) as a white solid. M.p. >300 °C; ^{1}H NMR (300 MHz, CD_3OD): $\delta = 7.31$–6.75(m, 8H), 4.35 (s, 2H); ^{13}C NMR (75 MHz, CD_3OD): $\delta = 147.5$, 145.3, 132.3, 131.7, 129.3, 129.1, 127.5, 126.5, 124.9, 121.9, 118.4, 51.5; IR (KBr): 3308, 1446, 1373, 1338, 1271, 754, 709 cm^{-1}; HRMS (ESI): $[M+H]^+$ calcd for $C_{13}H_{16}B_2NO_4$: 272.1267, found: 272.1271.

3-(3-(dihydroxyboranyl)benzylamino)phenylboronic acid (7b): By using a similar procedure as for **5a**, **2c** (155 mg, 1 mmol), **3b** (150 mg, 1 mmol) and $NaBH_3CN$ (124 mg, 2 mmol) gave pure **7b** (76 mg, 28%) as a white solid. M.p. >300°C; ^1H NMR (300 MHz, CD_3OD): $\delta = 7.75–6.66$ (m, 8H), 4.32 (s, 2H); ^{13}C NMR (68 MHz, CD_3OD): $\delta = 149.1, 140.2, 133.8, 133.1, 130.2, 129.1, 128.5, 123.7, 119.6, 116.1$; IR (KBr): 3405, 3309, 1602, 1429, 1361, 707 cm^{-1}; HRMS (ESI): $[M]^+$ calcd for $C_{13}H_{15}B_2NO_4$: 271.1187, $[M+H]^+$ calcd for $C_{13}H_{16}B_2NO_4$: 286.1427, found: 286.2705.

3-(4-(Dihydroxyboranyl)-benzylamino)phenylboronic acid (7c): By using a similar procedure as for **5a**, **2c** (155 mg, 1 mmol), **3c** (150 mg, 1 mmol) and $NaBH_3CN$ (124 mg, 2 mmol) gave pure **7c** (106 mg, 39%) as a white solid. M.p. >300°C; ^1H NMR (300 MHz, CD_3OD): $\delta = 7.73–6.58$ (m, 8H), 4.23 (s, 2H). ^{13}C NMR (68 MHz, CD_3OD): $\delta = 149.1, 143.5, 134.8, 129.1, 127.4, 126.9, 123.7, 122.9, 119.6, 118.9, 116.0, 115.4, 65.1$; IR (KBr): 3306, 1610, 1481, 1409, 1342, 1112, 1018, 705 cm^{-1}; HRMS (ESI): $[M]^+$ calcd for $C_{13}H_{15}B_2NO_4$: 271.1187, $[M+MeOH\text{-}H_2O]^+$ calcd for $C_{14}H_{18}B_2NO_4$: 286.1427, found: 286.2705.

3-(3-(Dihydroxyboranyl)-6-methoxybenzylamino)phenylboronic acid (7d): By using a similar procedure as for **5a**, **2c** (155 mg, 1 mmol), **3d** (180 g, 1 mmol) and $NaBH_3CN$ (124 mg, 2 mmol) gave pure **7d** (69 mg, 23%) as a white solid. M.p. >300°C; ^1H NMR (300 MHz, CD_3OD): $\delta = 7.66–6.67$ (m, 7H), 4.23 (s, 2H), 3.81 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD): $\delta = 168.8, 148.4, 133.7, 133.2, 130.5, 127.9, 127.2, 125.5, 117.3, 116.7, 112.9, 108.7, 53.8, 41.5$; IR (KBr): 3393, 1604, 1456, 1338, 1249, 1136, 1026, 700 cm^{-1}; HRMS (ESI): $[M]^+$ calcd for $C_{14}H_{17}B_2NO_5$: 301.1293, $[M+H]^+$ calcd for $C_{14}H_{18}B_2NO_5$: 302,1373, found: 302.1400.

3-(2,5-Dihydroxybenzylamino)phenylboronic acid (7e): By using a similar procedure as for **5a**, **2c** (155 mg, 1 mmol), **3e** (138 mg, 1 mmol) and $NaBH_3CN$ (124 mg, 2 mmol) gave pure **7e** (233 mg, 90%) as a white solid. M.p. >300°C; ^1H NMR (300 MHz, CD_3OD): $\delta = 7.11–6.49$ (m, 7H), 4.24 (s, 2H); ^{13}C NMR (68 MHz, CD_3OD): $\delta = 150.9, 149.2, 148.9, 129.1, 127.7, 124.0, 120.0, 116.7, 116.6, 116.3, 115.1, 115.0, 45.0$; IR (KBr): 3390, 1496, 1456, 1224, 754 cm^{-1}; HRMS (ESI): $[M]^+$ calcd for $C_{13}H_{14}BNO_4$: 259.1016, $[M+H]^+$ calcd for $C_{14}H_{18}B_2NO_5$: 260.1096, found: 260.1098.

5-[3-(Dihydroxyboranyl)benzylamino]-2-hydroxybenzoic acid (8b): By using a similar procedure as for **5a**, **2d** (153 mg, 1 mmol), **3b** (150 mg, 1 mmol) and $NaBH_3CN$ (124 mg, 2 mmol) gave pure **8b** (103 mg, 36%) as a white solid. M.p. >300°C; ^1H NMR (300 MHz, $[D_6]DMSO$): $\delta = 7.55–6.61$ (m, 7H), 4.14 (d, $J = 5.7$ Hz, 2H); ^{13}C NMR (100 MHz, $[D_6]DMSO$): $\delta = 171.1, 155.6, 142.1, 138.6, 132.8, 132.0, 127.8, 126.7, 124.2, 119.9, 117.2, 116.8, 113.8, 51.4$; IR (KBr): 3500, 3000, 1654, 1398, 1161, 835, 796 cm^{-1}; HRMS (ESI): $[M+H]^+$ calcd for $C_{14}H_{14}BNO_5$: 288.1045, found: 288.1085.

5-(4-(dihydroxyboranyl)benzylamino)-2-hydroxybenzoic acid (8c): By using a similar procedure as for **5a**, **2d** (153 mg, 1mmol), **3c** (150 mg, 1 mmol) and $NaBH_3CN$ (124 mg, 2 mmol) gave pure **8c** (63 mg, 21%) as a white solid. M.p. >300°C; ^1H NMR (300 MHz, $[D_6]DMSO$): $\delta = 7.96–6.75$ (m, 7H), 4.20 (s, 2H); ^{13}C NMR (100 MHz, $[D_6]DMSO$): $\delta = 162.9, 134.0, 133.1, 131.1, 127.7, 125.9, 119.1, 117.6, 116.1, 113.7, 64.9$; IR (KBr): 3000, 1670, 1500, 1340, 1190, 980, 833, 797 cm^{-1}; HRMS (ESI): $[M+H]^+$ calcd for $C_{14}H_{14}BNO_5$: 288.1045, found: 288.1161.

2-Hydroxy-5-(3-(dihydroxyboranyl)-6-methoxybenzylamino)benzoic acid (8d): By using a similar procedure as for **5a**, **2d** (153 mg, 1 mmol), **3d** (180 g, 1 mmol) and $NaBH_3CN$ (124 mg, 2 mmol) gave pure **8d** (41 mg, 13%) as a yellow solid. M.p. 191–195°C; ^1H NMR (300 MHz, $[D_6]DMSO$): $\delta = 7.25–7.18$ (m, 2H), 6.98–6.70 (m, 4H), 4.16 (s, 2H), 3.81 (s, 3H); ^{13}C NMR (100 MHz, $[D_6]DMSO$): $\delta = 172.1, 156.9, 152.9, 141.2, 127.9, 127.2, 121.9, 120.2, 117.4, 112.7, 111.2, 110.5, 55.3, 41.9$; IR (KBr): 2800, 1606, 1496, 1340, 1253, 1026, 758 cm^{-1}; HRMS (ESI): $[M+H]^+$ calcd for $C_{15}H_{17}BNO_6$: 318.1180, found: 318.0824.

3-(Pentafluorobenzylamino)phenylboronic acid (9): By using a similar procedure as for **5a**, **2c** (155 mg, 1 mmol), **3f** (123 mL, 1 mmol) and $NaBH_3CN$ (124 mg, 2 mmol) gave pure **9** (231 mg, 73%) as a white solid. M.p. 136–140°C; ^1H NMR (300 MHz, CD_3OD): $\delta = 7.23–6.71$ (m, 4H), 4.42 (s, 2H); ^{13}C NMR (68 MHz, CD_3OD): $\delta = 154.3, 150.6, 149.2, 146.3, 145.5, 142.5, 135.1, 134.9, 129.9, 124.9, 121.3, 42.3$; IR (KBr): 3375, 1606, 1587, 1504, 1407, 1361, 1234, 1118, 1024, 935, 785, 736, 709 cm^{-1}; HRMS (ESI): $[M]^+$ calcd for $C_{13}H_9BF_5NO_2$: 317.0646, $[M+H]^+$ calcd for $C_{13}H_{10}BF_5NO_2$: 318.0726, found: 318.0723.

4-Methoxy-3-((2′-methoxybenzylamino)methyl)phenylboronic acid (10): By using a similar procedure as for **5a**, 2-methoxybenzylamine (219 mL, 1 mmol), **3d** (180 mg, 1 mmol) and $NaBH_3CN$ (124 mg, 2 mmol) gave pure **10** (265 mg, 88%) as a white liquid; ^1H NMR (400 MHz, CD_3OD): $\delta = 7.59–6.9$ (m, 6H), 3.99 (s, 4H) 3.91–3.87 (m, 9H); ^{13}C NMR (125 MHz, CD_3OD): $\delta = 159.1, 158.8, 137.0, 136.3, 131.9, 131.2, 130.3, 121.6, 121.5, 111.6, 111.4, 110.0, 56.0, 55.8, 55.7, 49.3$; IR (KBr): 3000, 2349, 1593, 1439, 1115, 1026, 754 cm^{-1}.

***N*-(2′′,5′′-dihydroxybenzyl)-4-(4′,4′,5′,5′-tetramethyl-1′,3′,2′-dioxaborolan-2′-yl)aniline (11)**: By using a similar procedure as for **5a**, **2e** (219 mL, 1 mmol), **3e** (138 mg, 1 mmol) and $NaBH_3CN$ (124 mg, 2 mmol) gave pure **11** (205 mg, 60%) as a white solid. M.p. 186–190°C; ^1H NMR (300 MHz, CD_3OD): $\delta = 7.23–6.71$ (d, $J = 8.8$ Hz, 2H), 6.75–6.54 (m, 5H) 4.31 (s, 2H), 1.34 (s, 12H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 153.0, 151.1, 149.1, 137.1, 129.9, 127.8, 116.7, 116.1, 115.0, 113.0, 84.4, 43.3, 25.1$; IR (KBr): 3350, 1604, 1359, 1267, 1190, 1087, 854, 821, 651 cm^{-1}; HRMS (ESI): $[M]^+$ calcd for $C_{19}H_{24}BNO_4$: 341.1798, $[M+H]^+$ calcd for $C_{19}H_{25}BNO_4$: 342.1878, found: 342.1877.

4-(2′,5′-dihydroxybenzylamino)phenylboronic acid (12): The phenylboranate **11** (171 mg, 0.5 mmol) was dissolved in methanol (4.5 mL). Excess saturated KHF_2 (78 mL, ~4.5 M solution, 3.5 mmol) was added slowly under vigorous stirring. After 2 h, methanol was evaporated on a rotary evaporator, and phenylboronic acid was added during concentration. Acetonitrile (4 mL) and pentane (11 mL) were added to the aqueous solution, and phenyl boronic ester was separated in pentane phase. The aqueous acetonitrile solution was concentrated on a rotary evaporator. Hot acetonitrile (10 mL) was added, and excess solid KHF_2 was filtered off. The filtrate was concentrated to furnish the crude product, which was further purified by silica gel TLC (chloroform/methanol (5:1)). The product **12** (138 mg, 81%) was isolated as a brown solid. M.p. 113–118°C; ^1H NMR (400 MHz, CD_3OD): $\delta = 7.02–7.00$ (m, 2H), 6.69–6.48 (m, 5H), 4.19 (s, 2H); ^{13}C NMR (75 MHz, CD_3OD): $\delta = 151.0, 149.8, 149.2, 130.4, 129.9, 127.8, 122.1, 118.6, 116.7, 116.3, 115.1, 114.7, 44.7$; IR (KBr): 3519, 3343, 3251, 1600, 1506, 1446, 1261, 1234, 754, 696 cm^{-1}; HRMS (ESI): $[M]^+$ calcd for $C_{13}H_{14}BNO_4$: 259.1016, $[M+H]^+$ calcd for $C_{13}H_{15}BNO_4$: 260.1096, found: 260.1127.

3-((2′,5′-Dihydroxybenzyl)-(2′′-hydroxybenzyl)amino)phenylboronic acid (13): Salicylic aldehyde (213 mL, 2 mmol) was added to the aniline **7e** (518 mg, 2 mmol) in methanol (20 mL). While stirring, $NaBH_3CN$ (248 mg, 4 mmol) was added, and the reaction mixture was stirred at room temperature for 4 h. A saturated solution of NaCl (100 mL) was added to the reaction mixture, which was then extracted with ethyl acetate (3×100 mL). The combined or-

ganic layers were washed with brine (60 mL), dried over sodium sulfate, and concentrated to furnish the crude product, which was further purified by silica gel TLC (chloroform/methanol (4:1)). The product **13** (138 mg, 19%) was isolated as a brown solid. M.p. $>300\,^{\circ}C$; ^{1}H NMR (300 MHz, CD_3OD): $\delta = 7.05$–6.45 (m, 11H), 4.50 (ds, 4H); ^{13}C NMR (68 MHz, CD_3OD): $\delta = 156.3$, 151.0, 149.1, 129.2, 126,5, 125.4, 123.6, 120.3, 116.8, 115.7, 114.9, 52.3; IR (KBr): 3302, 1508, 1458, 1228, 750 cm^{-1}; HRMS (ESI): $[M]^+$ calcd for $C_{20}H_{20}BNO_5$: 365.1435, $[M+H]^+$ calcd for $C_{20}H_{21}BNO_5$: 366.1515, found: 366.1523; elemental analysis calcd (%) for $C_{20}H_{20}BNO_5$: C 65.780, H 5.520, N 3.840, found: C 65.742, H 5.924, N 3.862.

EGFR tyrosine kinase assay: Tyrosine kinase reactions were carried out in a final volume of 20 μL containing HEPES buffer (20 mm, pH 7.4), $MnCl_2$ (5 mM), EGF (20 μg mL^{-1}), the membrane fraction of A431 cells (10 μg), angiotensin II (20 μg), and [γ-^{32}P]ATP (2.5 μCi). The kinase activities were measured by a slight modification of the method described by Onoda et al.[11] The compounds were dissolved in 10% DMSO at 1 mg mL^{-1} concentration and diluted with distilled water. The A431 membrane fraction (EGF receptor) was incubated with the compound, angiotensin II, and EGF at 25 °C for 30 min. In the case of EGF(−), the A431 membrane fraction was incubated with angiotensin II and bovine serum albumin (BSA, 0.1% in PBS, 2 μL). The kinase reactions were initiated by the addition of [γ-^{32}P]ATP and cold ATP (total 5 μL), and the reaction mixture was incubated at 0 °C for 15 min. The reaction was stopped by addition of TCA (20%, 10 μL) and BSA (5 mg mL^{-1}, 10μL). Precipitated proteins were removed by centrifugation (15000 rpm, 10 min), and the supernatants (35 μL) were spotted on Whatman P81 phosphocellulose papers (2 cm × 2 cm). The papers were washed with phosphoric acid (75 mM) for 10 min (4×), with EtOH for 5 min, and then dried at room temperature. The radioactivity was counted with a liquid scintillation counter.

VEGFR tyrosine kinase assay: VEGFR tyrosine kinase activity was measured by using membrane fractions derived from Flt-1 overexpressing Tn5 cells. Briefly, membrane fractions (2 μg) were incubated with kinase assay buffer containing HEPES (50 mM, pH 7.4), Triton X-100 (0.1%), $MnCl_2$ (10 mM), $MgCl_2$ (2 mM), DTT (1 mM), NaF (1 mM), Na_3VO_4 (0.1 mM), and [γ-^{32}P]ATP (2.5 μCi, 10 μM) at 25 °C for 10 min and the reaction was terminated by addition of SDS loading buffer. After being boiled for 3 min, the reaction mixture was fractionated by SDS-PAGE (7.5% polyacrylamide gel), and bands of phosphorylated Flt-1 were visualized by autoradiography.

Multiple protein kinase assay: Kinase activities of PKA, PKC, PTK, and eEF2K were assessed as described.[16] NIH 3T3 cells transformed with v-src were suspended in a hypotonic buffer containing 1 mM HEPES-NaOH (pH 7.4), 5 mM $MgCl_2$, and 25 μg mL^{-1} each of antipain, leupeptin and pepstatin A and allowed to stand on ice for 10 min. After vigorous mixing for 1 min with a vortex mixer, the concentration of HEPES-NaOH (pH 7.4) was brought to 20 mM. Then the lysate was centrifuged at 500 *g* for 5 min at 4 °C to obtain the supernatant postnuclear fraction, which included protein kinases and their substrate proteins. Additions were made to the supernatant to give final concentrations of 1 mg mL^{-1} of protein, 20 mM HEPES-NaOH (pH 7.4), 10 mM $MgCl_2$, 0.1 mM Na_3VO_4, 1 mM NaF, 20 μM cAMP, 100 μM $CaCl_2$. Solutions of protein kinase inhibitors (3 μL) in DMSO were added to the postnuclear fraction (22 μL), and the mixture was incubated for 10 min on ice. The kinase reaction was initiated by adding [γ-^{32}P]ATP (5 μL, 75 μM, 10 μCi), and the mixture was incubated for 20 min at 25 °C. At the end of the reaction fourfold concentrated SDS-PAGE sample buffer (10 μL) was added to the mixture, and phosphorylated protein was separated by SDS-PAGE (9% polyacrylamide gel). To detect PTK activity, the gel was further treated with NaOH (1 N) for 2 h at 55 °C. The results were visualized by autoradiography. The position of the substrate protein band of each kinase was identified according to the sensitivity to various protein kinase inhibitors or activators and phosphoamino acid analysis as described previously.[16]

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