Polycyclic *N*-Heterocyclic Compounds, Part 67: Reaction of 6,7-Substituted *N*-(Quinazolin-4-yl)amidine Derivatives with Hydroxylamine Hydrochloride: Formation of *in Vitro* Inhibitors of Pentosidine

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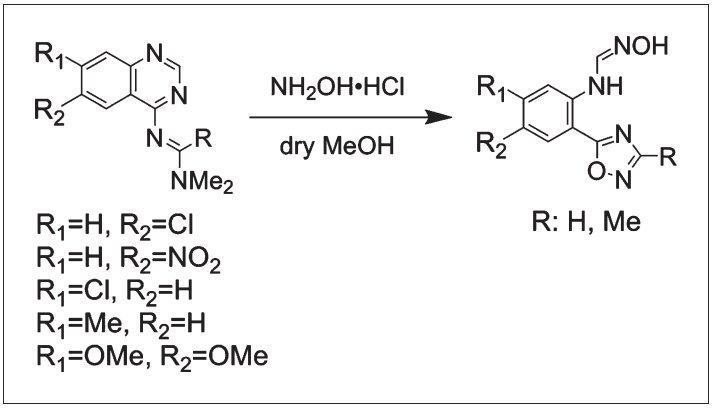

Reactions of *N*-(quinazolin-4-yl)amidines and their amide oximes with hydroxylamine hydrochloride gave cyclization products that were formed by an initial ring cleavage of the pyrimidine component followed by a ring closure formation of 1,2,4-oxadiazole to give *N*-[2-([1,2,4]oxadiazol-5-yl)phenyl]formamide oximes. All isolated products were evaluated for *in vitro* inhibitory activity on the formation of pentosidine, which is one of representative advanced glycation end products. Some products exhibited significant inhibitory activity against pentosidine formation.

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INTRODUCTION

Hyperglycemia plays a major role in the complicated pathogenesis of diabetes by increasing protein glycation through nonenzymatic reaction of amino groups (particularly the lysine residue) with reducing sugars (Maillard reaction) [1]. This glycation then leads to the formation of Amadori products *via* reversible Schiff-base adducts. Through subsequent oxidation and dehydration steps, a broad range of heterogeneous fluorescent and brown products form that are termed advanced glycation end products (AGEs) [2]. The gradual accumulation of AGEs in body tissues is a physiological phenomenon associated with normal aging. However, they are formed at an accelerated rates in diabetes as a result of the associated hyperglycemia and oxidative stress. In addition to diabetes, AGEs are also important causative factors in the pathogenesis of neurological diseases such as Alzheimer's disease and they also play a major role in vascular stiffening, atherosclerosis, osteoarthritis, inflammatory arthritis and cataracts [3]. Accordingly, the discovery and development of inhibitors of AGEs formation represents a new approach for treatment of diabetic or other pathogenic complications [3,4]. Pimagedine (aminoguanidine hydrochloride) was the first AGEs inhibitor explored in clinical trials and initially appeared to be promising for treatment of overt nephropathy of type 1 diabetics [5]. However, clinical trial results in overt nephropathy of type 2 diabetics were vague and the drug ultimately was not approved for commercial production. In our own approach to developing AGEs inhibitors we have initiated development and random screening of compound libraries.

Recently, we have reported that reaction of *N*-(quinazolin-4-yl)formamidines (**1**) with hydroxylamine hydrochloride results in a pyrimidine ring opening reaction accompanied by formation of the 1,2,4-oxadiazole ring to give *N*-[2-([1,2,4]oxadiazol-5-yl)phenyl]formamide oximes (**2**) (Fig. 1) [6]. This reaction is applicable to several substituted quinazoline derivatives for the synthesis of potential pharmaceutics and we found some of the products showed some inhibitory effects on the

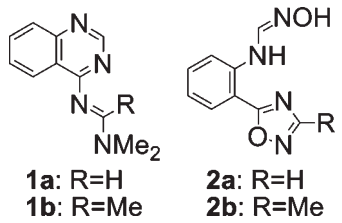


Figure 1. Substrates (**1**) and their rearranged products (**2**).

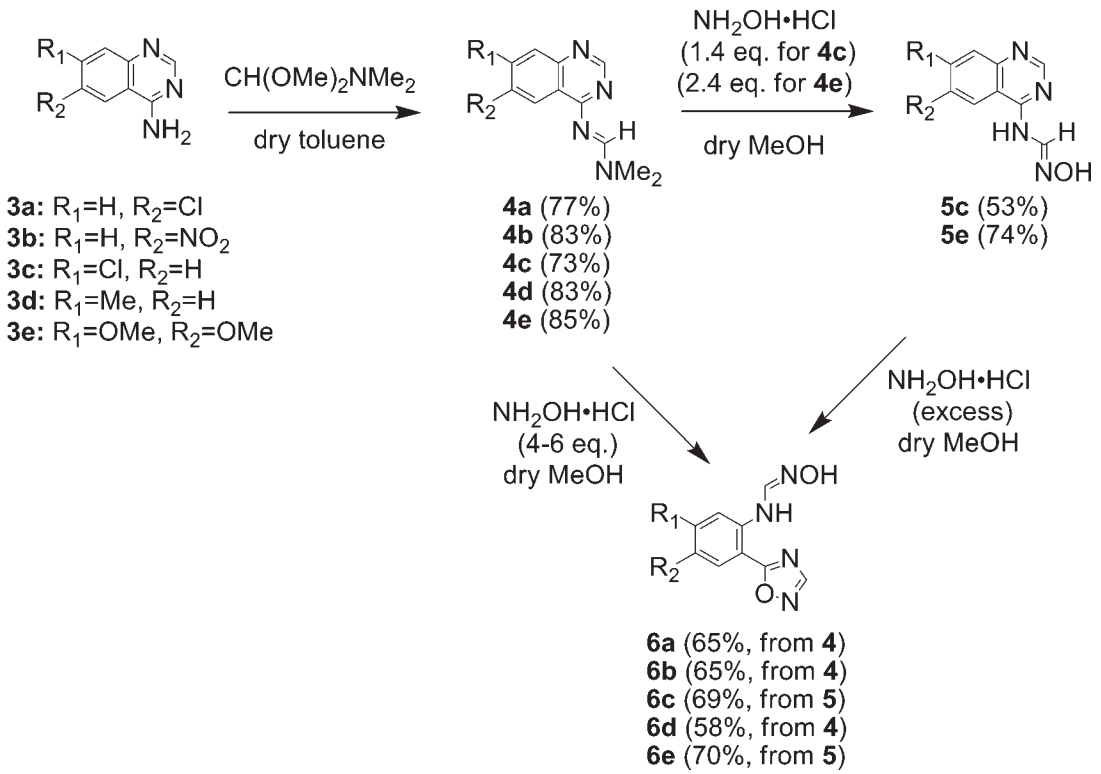
formation of pentosidine, which is one of representative AGEs. Here we report these results in detail.

RESULTS AND DISCUSSION

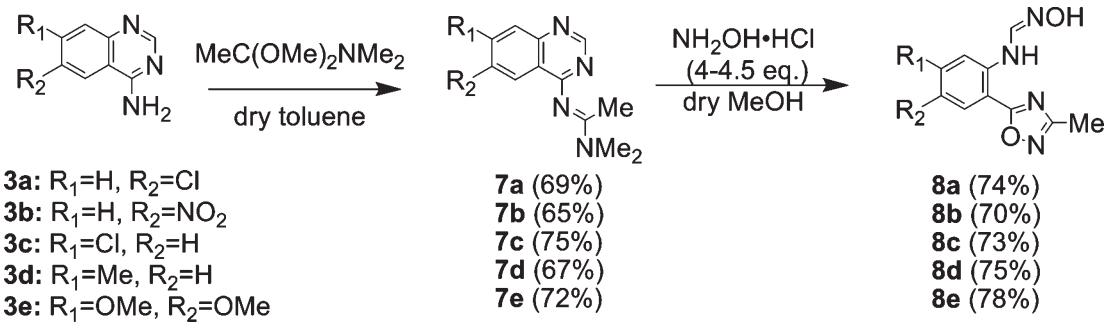
First, we studied reactions of the formamidines (**4**). The requisite 4-aminoquinazolines (**3**) were prepared from 2-aminobenzonitriles and formamidine acetate. The amidines **4** then were prepared by the reaction of **3** with *N*,*N*-dimethylformamide dimethyl acetal in refluxing toluene (Scheme 1). The reactions of **4** with hydroxylamine hydrochloride in dry methanol were then investigated. In the case of **4c**, the reaction with 1.4 equivalents of hydroxylamine hydrochloride at room temperature gave amide oxime **5c** (53%). Reaction of **5c** with 4 equivalents of hydroxylamine hydrochloride at room temperature for 4h produced the 1,2,4-oxadiazole derivative **6c**. In the case of **4e**, 2.4 equivalents of hydroxylamine hydrochloride at room temperature for 4 h was necessary to yield amide oxime **5e** (74%). Reflux conditions were required for the reaction of **5e** with hydroxylamine hydrochloride (3.6 equivalents) to form **6e**. In contrast, in the reactions of **4a**, **4b**, and **4d** with 1.2 equivalents hydroxylamine hydrochloride at room temperature, we were unable isolate amide oxime **5**. In those cases, thin layer chromatography (TLC) analysis indicated that amide oxime **5** and 1,2,4-oxadiazole derivative **6** were present in the reaction mixture, even though two equivalent of hydroxylamine hydrochloride was necessary to give the 1,2,4-oxadiazole derivatives [7]. Therefore we added 4–6 equivalents of hydroxylamine hydrochloride for the reactions of **4a**, **4b**, and **4d** at room temperature and obtained **6a**, **6b**, and **6d**, respectively, without isolation of intermediate oximes. The reason that **5e** was quite resistant to reaction with hydroxylamine hydrochloride and needed reflux condition compared to **5c** could be explained as follows. Compound **5e** has two methoxy groups, which are strong electron donating groups. As we have already discussed [6], LUMO energy level of amide oxime **5** govern the reactivity of these intermediates. The calculated LUMO energy of **5e** is –1.3181 eV, whilst that of **5c** is –1.7516 [8]. Therefore, it is reasonable that **5e** would be more resistant to nucleophilic attack of amide oxime than **5c**.

Next, we examined reactions of the acetamidines (**7**). The amidines **7** were prepared by the reaction of **3** with

Scheme 1. Synthesis of **6**.



Scheme 2. Synthesis of **8**.



N,*N*-dimethylacetamide dimethyl acetal in refluxing toluene (Scheme 2). In all attempts, the reaction of **7** with 1.2 equivalents of hydroxylamine hydrochloride at room temperature did not completely consume **7** as evidenced by TLC analysis. Reaction of **7** with the 4–4.5 equivalents of hydroxylamine hydrochloride gave the oxadiazoles **8** directly (70–78%) instead of amide oximes.

Finally, all isolated products in the present study as well as parent compounds (**2a** and **2b**) [6] were evaluated for their ability to inhibit the formation of pentosidine (Table 1). Aminoguanidine hydrochloride [3,9] was used as a positive control. Amidine derivatives (**4** and **7**) did not show any significant inhibitory activity (<20%, data not shown). In contrast, some formamide oximes (**2**, **5**, **6**, and **8**) showed significant inhibitory activity. Among them, **2a** exhibited the most potent inhibitory activity against pentosidine formation. Compound **6b**, **6c**, and **6d** also showed some significant inhibitory activity. We are currently exploring their structure-activity relationships for further elucidation of anti-AGEs compounds.

EXPERIMENTAL

All melting points were determined on a Yanagimoto micro-melting point apparatus, and are uncorrected. Elemental analyses were performed on a Yanagimoto MT-5 CHN Corder elemental analyzer. The fast atom bombardment (FAB) mass spectra were obtained on a VG 70 mass spectrometer and *m*-nitrobenzyl alcohol was used as the matrix. The IR spectra were recorded on a Japan Spectroscopic FT/IR-200 spectrophotometer with potassium bromide and frequencies are expressed in cm^{-1}. The ^1H NMR spectra were recorded on a Varian VXR-200 instrument operating at 200 MHz with tetramethylsilane as an internal standard. Chemical shifts are given in ppm (δ) and *J* values in Hz, and the signals are designated as follows: s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; br, broad; m, multiplet. TLC was carried out on Kieselgel 60F254 (Merck).

4-Amino-6-chloroquinazoline (3a). To a solution of 2-amino-5-chlorobenzonitrile (2.00 g, 13.1 mmol) in dry 2-ethoxyethanol (20 mL) was added formamidine acetate (7.00 g, 67.2 mmol) and the reaction mixture was refluxed for 4 h. After cooling to room temperature, water was added. The precipitate was filtered, washed with 10% aqueous sodium chloride solution, water, and then recrystallized from DMF to give **3a** (2.00 g, 85%) as colorless feathers, mp >300°C (lit [10] >310°C); IR (potassium bromide): 3270, 3350 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.66 (d, 1H, J = 9.0 Hz, H8), 7.78 (dd, 1H, J = 9.0, 2.2 Hz, H7), 7.88 (br s, 2H, deuterium oxide exchangeable, NH_2), 8.37 (d, 1H, J = 2.2 Hz, H5), 8.40 (s, 1H, H2); FAB-ms: *m*/*z* 180 (MH^+), 182 (MH^++2).

4-Amino-6-nitroquinazoline (3b). To a solution of 2-amino-5-nitrobenzonitrile (2.00 g, 12.3 mmol) in dry 2-ethoxyethanol (20 mL) was added formamidine acetate (9.60 g, 92.2 mmol) and the reaction mixture was refluxed for 5 h. After cooling to room temperature, water was added. The precipitate was filtered, washed with 10% aqueous sodium chloride solution, water, and then recrystallized from DMF to give **3b** (1.73 g, 74%) as colorless feathers, mp >300°C (lit [11] 320–320.5°C); IR (potassium bromide): 3340, 3440 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.81 (d, 1H, J = 9.2 Hz, H8), 8.40 (br s, 2H, deuterium oxide exchangeable, NH_2), 8.48 (1H, dd, J = 9.2, 2.5 Hz, H7), 8.52 (s, 1H, H2), 9.33 (d, 1H, J = 2.5 Hz, H5); FAB-ms: *m*/*z* 191 (MH^+).

Table 1

Effects of **2, 5, 6,** and **8** on pentosidine formation *in vitro*.

Compd.	% inhibition	Compd	% inhibition	Compd.	% inhibition
2a	72.0	**2b**	11.1	**5c**	28.9
6a	21.2	**8a**	−0.4	**5e**	−11.5
6b	41.5	**8b**	3.6	Aminoguanidine	46.0
6c	51.7	**8c**	3.6		
6d	44.8	**8d**	5.9		
6e	−22.8	**8e**	−23.7		

Data represent % inhibition of the vehicle control group.

4-Amino-7-chloroquinazoline (3c). To a solution of 2-amino-4-chlorobenzonitrile (1.80 g, 11.8 mmol) in dry 2-ethoxyethanol (20 mL) was added formamidine acetate (6.40 g, 61.5 mmol) and the reaction mixture was refluxed for 6 h. After cooling to room temperature, water was added. The precipitate was filtered, washed with 10% aqueous sodium chloride solution, water, and then recrystallized from methanol to give **3c** (1.60 g, 76%) as colorless feathers, mp >300°C (lit [12] 305–310°C); IR (potassium bromide): 3270, 3370 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.54 (dd, 1H, J = 8.8, 2.0 Hz, H6), 7.71 (d, 1H, J = 2.0 Hz, H8), 7.94 (br s, 2H, deuterium oxide exchangeable, NH_2), 8.25 (d, 1H, J = 8.8 Hz, H5), 8.40 (s, 1H, H2); FAB-ms: *m/z* 180 (MH^+), 182 (MH^++2).

4-Amino-7-methylquinazoline (3d). To a solution of 2-amino-4-methylbenzonitrile (500 mg, 3.78 mmol) in dry 2-ethoxyethanol (5.0 mL) was added formamidine acetate (2.00 g, 19.2 mmol) and the reaction mixture was refluxed for 1 h. After cooling to room temperature, water was added. The precipitate was filtered, washed with 10% aqueous sodium chloride solution, water, and then recrystallized from methanol to give **3d** (473 mg, 79%) as colorless feathers, mp 288–289°C (lit [13] 286–289°C); IR (potassium bromide): 3240, 3330 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.46 (s, 3H, 7-Me), 7.32 (d, 1H, J = 8.4 Hz, H6), 7.46 (s, 1H, H8), 7.65 (br s, 2H, deuterium oxide exchangeable, NH_2), 8.09 (d, 1H, J = 8.4 Hz, H5), 8.34 (s, 1H, H2); FAB-ms: *m/z* 160 (MH^+).

4-Amino-6,7-dimethoxyquinazoline (3e). To a solution of 2-amino-4,5-dimethoxybenzonitrile (500 mg, 2.81 mmol) in dry 2-ethoxyethanol (5.0 mL) was added formamidine acetate (1.40 g, 13.4 mmol) and the reaction mixture was refluxed for 2 h. After cooling to room temperature, water was added. The precipitate was filtered, washed with 10% aqueous sodium chloride solution, water, and then recrystallized from ethanol to give **3e** (435 mg, 76%) as colorless feathers, mp 208–210°C (lit [14] 204–206°C); IR (potassium bromide): 3320, 3390 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.87 and 3.89 (each s, each 3H, 2 x OMe), 7.06 (s, 1H, H8), 7.40 (br s, 2H, deuterium oxide exchangeable, NH_2), 7.56 (s, 1H, H5), 8.25 (s, 1H, H2); FAB-ms: *m/z* 206 (MH^+).

***N*^{1},*N*^{1}-Dimethyl-*N*^{2}-(6-chloroquinazolin-4-yl)formamidine (4a).** To a suspension of **3a** (300 mg, 1.67 mmol) in dry toluene (30 mL) was added *N*,*N*-dimethylformamide dimethyl acetal (275 mg, 2.31 mmol), and the mixture was refluxed for 3 h. After evaporation of solvent *in vacuo*, the residue was recrystallized from cyclohexane to give **4a** (300 mg, 77%) as a pale yellow powder, mp 128–130°C; ^1H NMR (DMSO-d_6): δ 3.24 and 3.26 (each s, each 3H, 2 x NMe), 7.77 (d, 1H, J = 9.0 Hz, H8), 7.84 (dd, 1H, J = 9.0, 2.4 Hz, H7), 8.40 (d, 1H, J = 2.4 Hz, H5), 8.69 (s, 1H, H2), 8.94 (s, 1H, N=C*H*NMe_2); FAB-ms: *m/z* 235 (MH^+), 237 (MH^++2). *Anal*. Calcd. for $C_{11}H_{11}ClN_4$: C, 56.30; H, 4.72; N, 23.87. Found: C, 56.03; H, 4.79; N, 24.11.

***N*^{1},*N*^{1}-Dimethyl-*N*^{2}-(6-nitroquinazolin-4-yl)formamidine (4b).** To a suspension of **3b** (500 mg, 2.63 mmol) in dry toluene (50 mL) was added *N*,*N*-dimethylformamide dimethyl acetal (440 mg, 3.47 mmol), and the mixture was refluxed for 2 h. After evaporation of solvent *in vacuo*, the residue was recrystallized from benzene-cyclohexane to give **4b** (536 mg, 83%) as yellow needles, mp 174–176°C (lit [15] 153°C). ^1H NMR (DMSO-d_6): δ 3.23 and 3.26 (each s, each 3H, 2 x NMe), 7.89 (d, 1H, J = 9.2 Hz, H8), 8.50 (dd, 1H, J = 9.2, 2.7 Hz, H7), 8.78 (s, 1H, H2), 9.02 (s, 1H, N=C*H*NMe_2), 9.20 (d, 1H, J = 2.7 Hz, H5); FAB-ms: *m/z* 246 (MH^+). *Anal*. Calcd. for $C_{11}H_{11}N_5O_2$: C, 53.87; H, 4.52; N, 28.56. Found: C, 53.53; H, 4.67; N, 28.49.

***N*^{1},*N*^{1}-Dimethyl-*N*^{2}-(7-chloroquinazolin-4-yl)formamidine (4c).** To a suspension of **3c** (500 mg, 2.78 mmol) in dry toluene (50 mL) was added *N*,*N*-dimethylformamide dimethyl acetal (550 mg, 4.62 mmol), and the mixture was refluxed for 3.5 h. After evaporation of solvent *in vacuo*, the residue was recrystallized from cyclohexane to give **4c** (476 mg, 73%) as colorless needles, mp 133–135°C. ^1H NMR (DMSO-d_6): δ 3.23 and 3.26 (each s, each 3H, 2 x NMe), 7.57 (dd, 1H, J = 8.8, 2.2 Hz, H6), 7.80 (d, 1H, J = 2.2 Hz, H8), 8.45 (d, 1H, J = 8.8 Hz, H5), 8.70 (s, 1H, H2), 8.95 (s, 1H, N=C*H*NMe_2); FAB-ms: *m/z* 235 (MH^+), 237 (MH^++2). *Anal*. Calcd. for $C_{11}H_{11}ClN_4$: C, 56.30; H, 4.72; N, 23.87. Found: C, 56.45; H, 4.58; N, 23.91.

***N*^{1},*N*^{1}-Dimethyl-*N*^{2}-(7-methylquinazolin-4-yl)formamidine (4d).** To a suspension of **3d** (100 mg, 0.628 mmol) in dry toluene (10 mL) was added *N*,*N*-dimethylformamide dimethyl acetal (105 mg, 0.881 mmol), and the mixture was refluxed for 1 h. After evaporation of solvent *in vacuo*, the residue was recrystallized from cyclohexane to give **4d** (112 mg, 83%) as a white powder, mp 92–93°C. ^1H NMR (DMSO-d_6): δ 2.50 (s, 3H, 7-Me), 3.21 and 3.24 (each s, each 3H, 2 x NMe), 7.38 (d, 1H, J = 8.4 Hz, H6), 7.55 (s, 1H, H8), 8.33 (d, 1H, J = 8.4 Hz, H5), 8.64 (s, 1H, H2), 8.90 (s, 1H, N=C*H*NMe_2); FAB-ms: *m/z* 215 (MH^+). *Anal*. Calcd. for $C_{12}H_{14}N_4$: C, 67.27; H, 6.59; N, 26.15. Found: C, 66.95; H, 6.65; N, 26.27.

***N*^{1},*N*^{1}-Dimethyl-*N*^{2}-(6,7-dimethoxyquinazolin-4-yl)formamidine (4e).** To a suspension of **3e** (500 mg, 2.44 mmol) in dry toluene (50 mL) was added *N*,*N*-dimethylformamide dimethyl acetal (378 mg, 3.17 mmol) and then the mixture was refluxed for 2 h. After evaporation of solvent *in vacuo*, the residue was recrystallized from toluene to give **4e** (537 mg, 85%) as a white powder, mp 159–161°C. ^1H NMR (DMSO-d_6): δ 3.21 and 3.22 (each s, each 3H, 2 x NMe), 3.90 and 3.93 (each s, each 3H, 2 x OMe), 7.16 (s, 1H, H8), 7.69 (s, 1H, H5), 8.55 (s, 1H, H2), 8.86 (s, 1H, N=C*H*NMe_2); FAB-ms: *m/z* 261 (MH^+). *Anal*. Calcd. for $C_{13}H_{16}N_4O_2$: C, 59.99; H, 6.20; N, 21.52. Found: C, 60.03; H, 6.07; N, 21.62.

***N*-(7-Chloroquinazolin-4-yl)formamide oxime (5c).** To a solution of **4c** (200 mg, 0.852 mmol) in dry methanol (20 mL) was added hydroxylamine hydrochloride (80.0 mg, 1.15 mmol), and the reaction mixture was stirred at room temperature for 4 h. Water was added, and then the solution was made basic with saturated aqueous sodium bicarbonate solution. The precipitate was filtered, washed with water and then recrystallized from methanol to give **5c** (100 mg, 53%) as a white powder, mp 155–157°C (dec); IR (potassium bromide): 3070, 3370 (NH and OH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.64 (dd, 1H, J = 9.0, 2.2 Hz, H6), 7.89 (d, 1H, J = 2.2 Hz, H8), 8.14 (br s, 1H, changed to sharp singlet after addition of deuterium oxide, NC*H*=NOH), 8.70 (d, 1H, J = 9.0 Hz, H5), 8.73 (s, 1H, H2), 9.98 (br, 1H, deuterium oxide exchangeable, NH), 10.90 (br s, 1H, deuterium oxide exchangeable, OH); FAB-ms: *m/z* 223 (MH^+), 225 (MH^+ + 2). *Anal*. Calcd. for $C_9H_7ClN_4O$: C, 48.55; H, 3.17; N, 25.17. Found: C, 48.19; H, 3.55; N, 25.28.

***N*-(6,7-Dimethoxyquinazolin-4-yl)formamide oxime (5e).** To a solution of **4e** (100 mg, 0.384 mmol) in dry methanol (10 mL) was added hydroxylamine hydrochloride (64.0 mg, 0.921 mmol), and the reaction mixture was stirred at room

temperature for 4 h. Water was added, and then the solution was made basic with saturated aqueous sodium bicarbonate solution. The precipitate was filtered, washed with water and then recrystallized from DMF-ethanol to give **5e** (71.0 mg, 74%) as a white powder, mp 217–219°C (dec); IR (potassium bromide): 3080, 3410 (NH and OH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.94 and 3.96 (each s, each 3H, 2 x OMe), 7.22 (s, 1H, H8), 7.91 (s, 1H, H5), 8.12 (d, 1H, J = 9.0 Hz, changed to singlet after addition of deuterium oxide, NC*H*=NOH), 8.56 (s, 1H, H2), 9.81 (d, 1H, J = 9.0 Hz, deuterium oxide exchangeable, NH), 10.72 (br s, 1H, deuterium oxide exchangeable, OH); FAB-ms: *m/z* 249 (MH^+). *Anal*. Calcd. for $C_{11}H_{12}N_4O_3$: C, 53.22; H, 4.87; N, 22.57. Found: C, 53.16; H, 5.04; N, 22.48.

***N*-[5-Chloro-2-([1,2,4]oxadiazol-5-yl)phenyl]formamide oxime (6c).** To a solution of **5c** (50.0 mg, 0.225 mmol) in dry methanol (5.0 mL) was added hydroxylamine hydrochloride (63.0 mg, 0.907 mmol), and the reaction mixture was stirred at room temperature for 4 h. Water was added, and then the solution was made basic with saturated aqueous sodium bicarbonate solution. The precipitate was filtered, washed with water then recrystallized from methanol to give **6c** (37.0 mg, 69%) as colorless feathers, mp 190–192°C; IR (potassium bromide): 3130, 3240 (NH and OH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.12 (dd, 1H, J = 8.6, 1.9 Hz, H4), 7.81 (d, 1H, J = 1.9 Hz, H6), 8.00 (d, 1H, J = 10.0 Hz, changed to singlet after addition of deuterium oxide, NC*H*=NOH), 8.07 (d, 1H, J = 8.6 Hz, H3), 9.30 (s, 1H, H3'), 10.56 (s, 1H, deuterium oxide exchangeable, OH), 10.65 (d, 1H, J = 10.0 Hz, deuterium oxide exchangeable, NH); FAB-ms: *m/z* 239 (MH^+), 241 (MH^+ + 2). *Anal*. Calcd. for $C_9H_7ClN_4O_2$: C, 45.30; H, 2.96; N, 23.48. Found: C, 45.10; H, 3.35; N, 23.58.

***N*-[4,5-Dimethoxy-2-([1,2,4]oxadiazol-5-yl)phenyl]formamide oxime (6e).** To a solution of **5e** (50.0 mg, 0.201 mmol) in dry methanol (5.0 mL) was added hydroxylamine hydrochloride (50.0 mg, 0.720 mmol), and the reaction mixture was refluxed for 4 h. After cooling, water was added, and then the solution was made basic with saturated aqueous sodium bicarbonate solution. The precipitate was filtered, washed with water then recrystallized from DMF-ethanol to give **6e** (37.0 mg, 70%) as colorless feathers, mp 197–200°C; IR (potassium bromide): 3110, 3210 (NH and OH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.81 and 3.92 (each s, each 3H, 2 x OMe), 7.13 (s, 1H, H6), 7.43 (s, 1H, H3), 8.00 (d, 1H, J = 10.0 Hz, changed to singlet after addition of deuterium oxide, NC*H*=NOH), 9.18 (s, 1H, H3'), 10.33 (s, 1H, deuterium oxide exchangeable, OH), 10.46 (d, 1H, J = 10.0 Hz, deuterium oxide exchangeable, NH); FAB-ms: *m/z* 265 (MH^+). *Anal*. Calcd. for $C_{11}H_{12}N_4O_2$: C, 50.00; H, 4.58; N, 21.20. Found: C, 49.89; H, 4.75; N, 21.23.

General procedure for the reaction of 4a, 4b, and 4d with hydroxylamine hydrochloride to give 6. To a solution of amidine **4a**, **4b**, and **4d** in dry methanol was added hydroxylamine hydrochloride, and the reaction mixture was stirred at room temperature. Water was added, and then the solution was made basic with saturated aqueous sodium bicarbonate solution. The precipitate was filtered, washed with water then recrystallized from methanol to give **6a**, **6b**, and **6d**.

***N*-[4-Chloro-2-([1,2,4]oxadiazol-5-yl)phenyl]formamide oxime (6a).** Compound **4a** (200 mg, 0.852 mmol) was allowed to react with hydroxylamine hydrochloride (354 mg, 5.09 mmol) in dry methanol (20 mL) for 7 h. Compound **6a** (133 mg, 65%) was isolated as pale yellow feathers, mp 187–189°C; IR (potassium bromide): 3120, 3240 (NH and OH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.64 (dd, 1H, J = 9.0, 2.2 Hz, H5), 7.70 (d, 1H, J = 9.0 Hz, H6), 7.93 (d, 1H, J = 10.0 Hz, changed to singlet after addition of deuterium oxide, NC*H*=NOH), 8.05 (d, 1H, J = 2.2 Hz, H3), 9.53 (s, 1H, H3'), 10.54 (s, 1H, deuterium oxide exchangeable, OH), 10.55 (d, 1H, J = 10.0 Hz, deuterium oxide exchangeable, NH); FAB-ms: *m/z* 239 (MH^+), 241 (MH^+ + 2). *Anal*. Calcd. for $C_9H_7ClN_4O_2$: C, 45.30; H, 2.96; N, 23.48. Found: C, 45.15; H, 3.30; N, 23.60.

***N*-[4-Nitro-2-([1,2,4]oxadiazol-5-yl)phenyl]formamide oxime (6b).** Compound **4b** (100 mg, 0.408 mmol) was allowed to react with hydroxylamine hydrochloride (120 mg, 1.73 mmol) in dry methanol (10 mL) for 5 h. Compound **6b** (66.0 mg, 65%) was obtained as yellow feathers, mp 195–197°C; IR (potassium bromide): 3110, 3230 (NH and OH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.86 (d, 1H, J = 9.6 Hz, H6), 8.11 (d, 1H, J = 9.6 Hz, changed to singlet after addition of deuterium oxide, NC*H*=NOH), 8.38 (dd, 1H, J = 9.6, 2.6 Hz, H5), 8.82 (d, 1H, J = 2.6 Hz, H3), 9.39 (s, 1H, H3'), 10.95 (s, 1H, deuterium oxide exchangeable, OH), 11.10 (d, 1H, J = 9.6 Hz, deuterium oxide exchangeable, NH). FAB-ms: *m/z* 250 (MH^+). *Anal*. Calcd. for $C_9H_7N_5O_4$: C, 43.38; H, 2.83; N, 28.11. Found: C, 43.16; H, 3.15; N, 28.10.

***N*-[5-Methyl-2-([1,2,4]oxadiazol-5-yl)phenyl]formamide oxime (6d).** Compound **4d** (100 mg, 0.467 mmol) was allowed to react with hydroxylamine hydrochloride (130 mg, 1.87 mmol) in dry methanol (10 mL) for 4 h. Compound **6d** (59.0 mg, 58%) was obtained as colorless feathers, mp 177–179°C; IR (potassium bromide): 3120, 3260 (NH and OH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.38 (s, 3H, Me), 6.91 (d, 1H, J = 8.0 Hz, H4), 7.50 (s, 1H, H6), 7.91 (d, 1H, J = 10.2 Hz, changed to singlet after addition of deuterium oxide, NC*H*=NOH), 7.94 (d, 1H, J = 8.0 Hz, H3), 9.24 (s, 1H, H3'), 10.41 (1H, s, deuterium oxide exchangeable, OH), 10.49 (d, 1H, J = 10.2 Hz, deuterium oxide exchangeable, NH). FAB-ms: *m/z* 219 (MH^+). *Anal*. Calcd. for $C_{10}H_{10}N_4O_2$: C, 55.04; H, 4.62; N, 25.68. Found: C, 54.73; H, 4.54; N, 25.97.

***N*^{1},*N*^{1}-Dimethyl-*N*^{2}-(6-chloroquinazolin-4-yl)acetamidine (7a).** To a suspension of **3a** (200 mg, 1.11 mmol) in dry toluene (20 mL) was added *N*,*N*-dimethylacetamide dimethyl acetal (247 mg, 1.85 mmol), and the mixture was refluxed for 5 h. After evaporation of solvent *in vacuo*, the residue was recrystallized from cyclohexane to give **7a** (192 mg, 69%) as a pale yellow powder, mp 93–95°C. ^1H NMR (DMSO-d_6): δ 2.30 (s, 3H, Me), 3.21 and 3.27 (each s, each 3H, 2 x NMe), 7.67 (dd, 1H, J = 8.8, 2.4 Hz, H7), 7.81 (d, 1H, J = 8.8 Hz, H8), 8.20 (d, 1H, J = 2.4 Hz, H5), 8.80 (s, 1H, H2); FAB-ms: *m/z* 249 (MH^+), 251 (MH^++2). *Anal*. Calcd. for $C_{12}H_{13}ClN_4$: C, 57.95; H, 5.27; N, 22.53. Found: C, 57.97; H, 5.17; N, 22.35.

***N*^{1},*N*^{1}-Dimethyl-*N*^{2}-(6-nitroquinazolin-4-yl)acetamidine (7b).** To a suspension of **3b** (500 mg, 2.63 mmol) in dry toluene (50 mL) was added *N*,*N*-dimethylacetamide dimethyl acetal (800 mg, 6.01 mmol), and the mixture was refluxed for 3 h. After evaporation of solvent *in vacuo*, the residue was recrystallized from benzene-cyclohexane to give **7b** (440 mg, 65%) as yellow needles, mp 173–174°C. ^1H NMR (DMSO-d_6): δ 2.38 (s, 3H, Me), 3.25 and 3.28 (each s, each 3H, 2 x NMe), 7.88 (d, 1H, J = 9.2 Hz, H8), 8.48 (dd, 1H, J = 9.2, 2.8 Hz, H7), 8.75 (s, 1H, H2), 9.23 (d, 1H, J = 2.8 Hz, H5);

FAB-ms: *m/z* 260 (MH^+). *Anal*. Calcd. for $C_{12}H_{13}N_5O_2$: C, 55.59; H, 5.05; N, 27.01. Found: C, 55.49; H, 5.01; N, 26.86.

***N*^{1},*N*^{1}-Dimethyl-*N*^{2}-(7-chloroquinazolin-4-yl)acetamidine (7c).** To a suspension of **3c** (500 mg, 2.78 mmol) in dry toluene (50 mL) was added *N*,*N*-dimethylacetamide dimethyl acetal (880 mg, 6.61 mmol), and the mixture was refluxed for 8 h. After evaporation of solvent *in vacuo*, the residue was recrystallized from cyclohexane to give **7c** (520 mg, 75%) as pale brown needles, mp 138–140°C; ^{1}H NMR (DMSO-d_6): δ 2.28 (s, 3H, Me), 3.20 (s, 6H, NMe_2), 7.52 (dd, 1H, J = 8.7, 2.2 Hz, H6), 7.78 (d, 1H, J = 2.2 Hz, H8), 8.22 (d, 1H, J = 8.7 Hz, H5), 8.68 (s, 1H, H2); FAB-ms: *m/z* 249 (MH^+), 251 (MH^++2). *Anal*. Calcd. for $C_{12}H_{13}ClN_4$: C, 57.95; H, 5.27; N, 22.53. Found: C, 57.74; H, 5.25; N, 22.22.

***N*^{1},*N*^{1}-Dimethyl-*N*^{2}-(7-methylquinazolin-4-yl)acetamidine (7d).** To a suspension of **3d** (200 mg, 1.26 mmol) in dry toluene (20 mL) was added *N*,*N*-dimethylacetamide dimethyl acetal (282 mg, 2.12 mmol), and the mixture was refluxed for 1 h. After evaporation of solvent *in vacuo*, the residue was recrystallized from cyclohexane/*n*-hexane to give **7d** (192 mg, 67%) as pale brown needles, mp 89–91°C; ^{1}H NMR (DMSO-d_6): δ 2.21 (s, 3H, Me), 2.49 (s, 3H, Me), 3.17 (s, 6H, NMe_2), 7.34 (d, 1H, J = 8.4 Hz, H6), 7.54 (s, 1H, H8), 8.06 (d, 1H, J = 8.4 Hz, H5), 8.64 (s, 1H, H2); FAB-ms: *m/z* 229 (MH^+). *Anal*. Calcd. for $C_{13}H_{16}N_4$: C, 68.39; H, 7.06; N, 24.54. Found: C, 68.03; H, 7.08; N, 24.41.

***N*^{1},*N*^{1}-Dimethyl-*N*^{2}-(6,7-dimethoxyquinazolin-4-yl)acetamidine (7e).** To a suspension of **3e** (300 mg, 1.46 mmol) in dry toluene (50 mL) was added *N*,*N*-dimethylacetamide dimethyl acetal (331 mg, 2.49 mmol) and then the mixture was refluxed for 3 h. After evaporation of solvent *in vacuo*, the residue was chromatographed on silica gel. The eluate of ethyl acetate was evaporated and the residue was recrystallized from toluene-cyclohexane to give **7e** (290 mg, 72%) as a white powder, mp 125–127°C; ^{1}H NMR (DMSO-d_6): δ 2.20 (s, 3H, Me), 3.17 (s, 6H, NMe_2), 3.86 and 3.92 (each s, each 3H, 2 x OMe), 7.15 (s, 1H, H8), 7.42 (s, 1H, H5), 8.56 (s, 1H, H2); FAB-ms: *m/z* 275 (MH^+). *Anal*. Calcd. for $C_{14}H_{18}N_4O_2$: C, 61.30; H, 6.61; N, 20.42. Found: C, 61.34; H, 6.57; N, 20.56.

General procedure for the reaction of 7 with hydroxylamine hydrochloride to give 8. To a solution of amidine **7** in dry methanol was added hydroxylamine hydrochloride, and the reaction mixture was stirred at room temperature. Water was added, and then the solution was made basic with saturated aqueous sodium bicarbonate solution. The precipitate was filtered, washed with water and then recrystallized from methanol (except **8e**) to give **8**.

***N*-[4-Chloro-2-(3-methyl[1,2,4]oxadiazol-5-yl)phenyl]formamide oxime (8a).** Compound **7a** (100 mg, 0.402 mmol) was allowed to react with hydroxylamine hydrochloride (111 mg, 1.60 mmol) in dry methanol (10 mL) for 3 h. Compound **8a** (75.0 mg, 74%) was obtained as colorless feathers, mp 210–212°C; IR (potassium bromide): 3150, 3210 (NH and OH) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.46 (s, 3H, Me), 7.62 (dd, 1H, J = 9.1, 2.2 Hz, H5), 7.68 (d, 1H, J = 9.1 Hz, H6), 7.91 (d, 1H, J = 10.0 Hz, changed to singlet after addition of deuterium oxide, NC*H*=NOH), 7.99 (d, 1H, J = 2.2 Hz, H3), 10.49 (s, 1H, deuterium oxide exchangeable, OH), 10.54 (d, 1H, J = 10.0 Hz, deuterium oxide exchangeable, NH); FAB-ms: *m/z* 253 (MH^+), 255 (MH^+ + 2). *Anal*. Calcd. for $C_{10}H_9ClN_4O_2$: C, 47.54; H, 3.59; N, 22.18. Found: C, 47.20; H, 3.78; N, 21.95.

***N*-[4-Nitro-2-(3-methyl[1,2,4]oxadiazol-5-yl)phenyl]formamide oxime (8b).** Compound **7b** (100 mg, 0.386 mmol) was allowed to react with hydroxylamine hydrochloride (120 mg, 1.73 mmol) in dry methanol (10 mL) for 4 h. Compound **8b** (71.0 mg, 70%) was obtained as yellow feathers, mp 200–201°C. IR (potassium bromide): 3160, 3220 (NH and OH) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.48 (s, 3H, Me), 7.84 (d, 1H, J = 9.6 Hz, H6), 8.12 (d, 1H, J = 9.6 Hz, changed to singlet after addition of deuterium oxide, NC*H*=NOH), 8.37 (dd, 1H, J = 9.6, 2.8 Hz, H5), 8.76 (d, 1H, J = 2.8 Hz, H3), 10.93 (s, 1H, deuterium oxide exchangeable, OH), 11.10 (d, 1H, J = 9.6 Hz, deuterium oxide exchangeable, NH). FAB-ms: *m/z* 264 (MH^+). *Anal*. Calcd. for $C_{10}H_9N_5O_4$: C, 45.63; H, 3.45; N, 26.61. Found: C, 45.38; H, 3.70; N, 26.59.

***N*-[5-Chloro-2-(3-methyl[1,2,4]oxadiazol-5-yl)phenyl]formamide oxime (8c).** Compound **7c** (200 mg, 0.804 mmol) was allowed to react with hydroxylamine hydrochloride (222 mg, 3.20 mmol) in dry methanol (20 mL) for 3 h. Compound **8c** (148 mg, 73%) was obtained as colorless feathers, mp 195–197°C; IR (potassium bromide): 3050, 3190 (NH and OH) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.44 (s, 3H, Me), 7.01 (dd, 1H, J = 8.6, 1.8 Hz, H4), 7.79 (d, 1H, J = 1.8 Hz, H6), 7.97 (d, 1H, J = 10.0 Hz, changed to singlet after addition of deuterium oxide, NC*H*=NOH), 8.15 (d, 1H, J = 8.6 Hz, H3), 10.50 (1H, s, deuterium oxide exchangeable, OH), 10.60 (d, 1H, J = 10.0 Hz, deuterium oxide exchangeable, NH); FAB-ms: *m/z* 253 (MH^+), 255 (MH^+ + 2). *Anal*. Calcd. for $C_{10}H_9ClN_4O_2$: C, 47.54; H, 3.59; N, 22.18. Found: C, 47.24; H, 3.85; N, 22.31.

***N*-[5-Methyl-2-(3-methyl[1,2,4]oxadiazol-5-yl)phenyl]formamide oxime (8d).** Compound **7d** (144 mg, 0.631 mmol) was allowed to react with hydroxylamine hydrochloride (178 mg, 2.56 mmol) in dry methanol (15 mL) for 1 h. Compound **8d** (110 mg, 75%) was obtained as a white powder, mp 221–224°C; IR (potassium bromide): 3100, 3190 (NH and OH) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.37 (s, 3H, Me), 2.43 (s, 3H, Me), 6.89 (d, 1H, J = 8.2 Hz, H4), 7.47 (s, 1H, H6), 7.88 (d, 1H, J = 10.2 Hz, changed to singlet after addition of deuterium oxide, NC*H*=NOH), 7.89 (d, 1H, J = 8.2 Hz, H3), 10.35 (s, 1H, deuterium oxide exchangeable, OH), 10.48 (d, 1H, J = 10.2 Hz, deuterium oxide exchangeable, NH); FAB-ms: *m/z* 233 (MH^+). *Anal*. Calcd. for $C_{11}H_{12}N_4O_2$: C, 56.89; H, 5.21; N, 24.12. Found: C, 56.89; H, 5.37; N, 24.12.

***N*-[4,5-Dimethoxy-2-(3-methyl[1,2,4]oxadiazol-5-yl)phenyl]-formamide oxime (8e).** Compound **7e** (100 mg, 0.365 mmol) was allowed to react with hydroxylamine hydrochloride (101 mg, 1.45 mmol) in dry methanol (10 mL) for 2 h. Compound **8e** (79.0 mg, 78%) was obtained as colorless feathers from DMF-ethanol, mp 235–237°C; IR (potassium bromide): 3150, 3290 (NH and OH) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.41 (s, 3H, C-Me), 3.80 and 3.91 (each s, each 3H, 2 x OMe), 7.11 (s, 1H, H6), 7.38 (s, 1H, H3), 7.97 (d, 1H, J = 10.2 Hz, changed to singlet after addition of deuterium oxide, NC*H*=NOH), 10.27 (s, 1H, deuterium oxide exchangeable, OH), 10.43 (d, 1H, J = 10.2 Hz, deuterium oxide exchangeable, NH); FAB-ms: *m/z* 279 (MH^+). *Anal*. Calcd. for $C_{12}H_{14}N_4O_2$: C, 51.80; H, 5.07; N, 20.13. Found: C, 51.89; H, 5.16; N, 20.03.

Determination of pentosidine formation *in vitro*. Test compound stock solutions were prepared at 100 mM concentration except for **5c**, **5e**, and **6e** (50 mM) in DMSO and

diluted to 100 mM sodium phosphate buffer (pH 7.4) to be 2.0 mM final concentration. The assay was performed based on a modified method of Vinson and Howard III [16]. In brief, the reaction mixture (total volume 100 μL) consisting of 30 mg/mL bovine serum albumin, 10 mM L-(+)-arabinose, and 2.0 mM test compounds in 100 mM sodium phosphate buffer (pH 7.4) was incubated at 37°C for 14 d. Then 10% trichloroacetic acid (100 μL) was added to precipitate proteins. After collecting the precipitate by centrifugation and drying under vacuum, hydrolysis was performed at 110°C for 16 h in 6N hydrochloric acid. After neutralization by 5N aqueous sodium hydroxide solution and filtration by ''Millipore DIMEX" membrane (pore size 0.22 μm), liberated pentosidine was analyzed by HPLC (1.0 mL/min, Waters, Puresil C18 column (4.6 x 250 mm). The fluorescence (excitation: 335 nm, emission: 385 nm) was monitored for detection with the gradient from water containing 0.1% trifluoroacetic acid (0 min) to 80% acetonitrile 20% water 0.1% trifluoroacetic acid (30 min). The inhibitory activity was calculated using the equation: % inhibition = [1−{pentosidine formation (pmol/mL) with test compound/pentosidine formation (pmol/mL) without test compound (DMSO blank)}] x 100. Aminoguanidine hydrochloride was used as a reference compound.

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